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Department of Chemistry and Chemical Biology

DISSERTATION

for the achievement of the academic degree of the
Doctor in Natural Sciences (Dr. rer. nat.)

**Structural and Cellular Characterization of Covalent-Allosteric Akt
Inhibitors as a Framework for Isoform-Selective Drug Design**

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Disclaimer

The research presented in this dissertation was conducted in close collaboration with Kosmas Alexandros Pervanidis, MSc., a PhD candidate in the Rauh Lab. My contributions to the projects included the biological and structural characterization of the developed Akt inhibitors. In particular, I performed cellular target-engagement and functional assays, including NanoBRET, CellTiter-Glo (CTG), and Western blot experiments, as well as the biological evaluation and interpretation of compound activity profiles. Furthermore, I carried out protein expression and purification for protein mass spectrometry studies and performed the crystallographic experiments associated with the projects. All X-ray crystal structures presented in this dissertation were solved, refined, and structurally analyzed by me.

Kosmas Alexandros Pervanidis was primarily responsible for structural modeling and molecular docking, as well as for the rational design and chemical synthesis of the inhibitor series. HTRF-based biochemical assays were performed by Dr. Jörn Weisner. The interpretation of the combined structural, biochemical, and cellular datasets was carried out collaboratively.

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Furthermore, I supervised the bachelor's theses of Vladimir Lukianchikov and Fabian Palz during my doctoral studies.

Artificial intelligence (AI)-based tools were used in the preparation of this dissertation exclusively for language refinement, stylistic revision, and editorial support. The scientific content, study design, data analysis, interpretation of results, and conclusions were developed independently by me. All intellectual contributions and scientific responsibility remain solely mine.

List of Publications

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List of Abbreviations

α C-helix	Alpha C-helix
3D	Three-dimensional
ATP	Adenosine triphosphate
CAAls	Covalent-allosteric Akt inhibitors
cryo-EM	Cryo-electron microscopy
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CTG	CellTiter-Glo
DFG	Aspartate-Phenylalanine-Glycine motif
DOI	Digital Object Identifier
FDA	Food and Drug Administration
HER2	Human Epidermal Growth Factor Receptor 2
HR	Hormone Receptor
HTRF	Homogeneous Time-Resolved Fluorescence
HHT	Hereditary Hemorrhagic Telangiectasia
IC ₅₀	Half maximal Inhibitory Concentration
K _d	Dissociation Constant
MEK	Mitogen-Activated Protein Kinase Kinase
mTOR	Mechanistic Target of Rapamycin
mTORC2	Mammalian Target of Rapamycin Complex 2
NanoBRET	Nano Bioluminescence Resonance Energy Transfer
PKD1	Phosphoinositide-Dependent Kinase-1
PH domain	Pleckstrin Homology Domain
PI3K	Phosphoinositide 3-kinase
PIP3	Phosphatidylinositol-3,4,5-trisphosphate
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PLA	Physician Lesion Assessment
PROTACs	PROteolysis TArgeting Chimeras
RNA	Ribonucleic acid

SAR	S tructure- a ctivity r elationship
SBDD	S tructure- b ased d rug d esign
SK	S eborrheic K eratoses
X-ray	X-ray crystallography

Abstract

Akt kinases are central regulators of cell proliferation, survival, metabolism, and migration within the PI3K/Akt/mTOR signaling pathway. Dysregulation of Akt signaling is strongly associated with cancer, metabolic disorders, and neurological diseases, making Akt an important therapeutic target. Over the past decades, extensive efforts have been dedicated to the development of Akt inhibitors; however, achieving high selectivity and minimizing adverse effects remain major challenges, particularly due to the strong structural homology among the three Akt isoforms (Akt1, Akt2, and Akt3). Since these isoforms exert distinct physiological and pathological functions, isoform-selective inhibition is increasingly recognized as a critical objective for next-generation targeted therapies.

The work presented in this cumulative thesis began with a comprehensive review of current Akt inhibitor strategies, including ATP-competitive, allosteric, and covalent-allosteric inhibitors (CAAls). The review highlighted both the therapeutic potential and the limitations of existing Akt-targeting approaches, emphasizing the clinical relevance of Akt inhibition as illustrated by the FDA approval of the ATP-competitive inhibitor capivasertib for breast cancer treatment. Particular focus was placed on covalent-allosteric inhibitors as a promising strategy to achieve improved potency, prolonged target engagement, and enhanced selectivity. Furthermore, the review identified unresolved challenges in the field, including the need for isoform-selective inhibitors and the development of more precise therapeutic approaches. Building on these insights, the subsequent studies focused on the rational design and optimization of isoform-selective covalent-allosteric Akt inhibitors. Using structure-based drug design approaches integrating X-ray crystallography, molecular modeling, and structure-activity relationship analyses, subtle structural differences within the allosteric pockets of Akt isoforms were identified and exploited. This enabled the development of novel inhibitors selectively targeting Akt2 and Akt3 while sparing Akt1. Pyrazole-containing compounds emerged as potent and selective Akt2 inhibitors, whereas pyridine-based scaffolds preferentially targeted Akt3. Biological characterization, including cellular target-engagement assays and evaluation in patient-derived colorectal cancer organoids, demonstrated the functional relevance and translational potential of these compounds.

The final stage of this work focused specifically on Akt3, an isoform with important functions in neurodevelopment and tumor progression. Through iterative structure-

guided optimization supported by multiple high-resolution co-crystal structures, highly potent and selective covalent Akt3 inhibitors were developed. Mechanistic investigations demonstrated that linker geometry and the precise positioning of isoform-discriminating elements within the allosteric pocket are key determinants of selectivity. These compounds provide valuable chemical probes for investigating Akt3-specific signaling pathways and establish a foundation for future therapeutic exploration in oncology and neurodevelopmental disorders.

Overall, this thesis establishes a coherent progression from the systematic analysis of Akt inhibition strategies to the successful development of isoform-selective covalent-allosteric Akt inhibitors. The presented findings advance the understanding of isoform-specific Akt biology and demonstrate how covalent-allosteric approaches can overcome intrinsic limitations of conventional kinase inhibitors, thereby contributing to the development of more selective and effective targeted therapies.

Kurzfassung

Akt-Kinasen sind zentrale Regulatoren von Zellproliferation, Zellüberleben, Metabolismus und Migration innerhalb des PI3K/Akt/mTOR-Signalwegs. Eine Fehlregulation der Akt-Signalübertragung ist eng mit Krebs, metabolischen Erkrankungen und neurologischen Störungen verbunden, wodurch Akt ein bedeutendes therapeutisches Ziel darstellt. Trotz intensiver Forschungsaktivitäten zur Entwicklung von Akt-Inhibitoren bleiben eine hohe Selektivität sowie die Minimierung unerwünschter Nebenwirkungen zentrale Herausforderungen, insbesondere aufgrund der starken strukturellen Homologie der drei Akt-Isoformen (Akt1, Akt2 und Akt3). Da die einzelnen Isoformen unterschiedliche physiologische und pathologische Funktionen erfüllen, wird die Entwicklung isoformselektiver Inhibitoren zunehmend als entscheidender Schritt für zukünftige zielgerichtete Therapien angesehen.

Die in dieser kumulativen Dissertation präsentierten Arbeiten begannen mit einer umfassenden Übersichtsarbeit zu aktuellen Strategien der Akt-Inhibition, einschließlich ATP-kompetitiver, allosterischer und kovalent-allosterischer Inhibitoren (CAAls). Die Übersichtsarbeit beleuchtete sowohl das therapeutische Potenzial als auch die Limitationen bestehender Ansätze zur Akt-Hemmung und hob die klinische Relevanz der Akt-Inhibition hervor, unter anderem durch die FDA-Zulassung des ATP-kompetitiven Inhibitors capivasertib zur Behandlung von Brustkrebs. Besonderes Augenmerk wurde auf kovalent-allosterische Inhibitoren als vielversprechende Strategie gelegt, da diese eine hohe Potenz, verlängerte Zielproteinbindung und verbesserte Selektivität ermöglichen. Darüber hinaus identifizierte die Übersichtsarbeit weiterhin bestehende Herausforderungen des Forschungsfeldes, insbesondere den Bedarf an isoformselektiven Inhibitoren und präziseren therapeutischen Ansätzen.

Aufbauend auf diesen Erkenntnissen konzentrierten sich die nachfolgenden Arbeiten auf das rationale Design und die Optimierung isoform-selektiver kovalent-allosterischer Akt-Inhibitoren. Mithilfe strukturgeleiteter Wirkstoffdesign-Strategien, die Röntgenkristallographie, molekulare Modellierung und Struktur-Wirkungs-Beziehungsanalysen kombinierten, konnten subtile strukturelle Unterschiede innerhalb der allosterischen Bindungstaschen der Akt-Isoformen identifiziert und gezielt ausgenutzt werden. Dadurch wurden neuartige Inhibitoren entwickelt, die selektiv Akt2 und Akt3 hemmen, während Akt1 nicht adressiert wird. Pyrazolhaltige Verbindungen erwiesen sich als potente und selektive Akt2-Inhibitoren, wohingegen Pyridin-basierte Strukturen bevorzugt Akt3 adressierten. Die biologische

Charakterisierung, einschließlich zellulärer Target-Engagement-Assays und Untersuchungen in patientenabgeleiteten kolorektalen Krebsorganoiden, bestätigte die funktionelle Relevanz und das translationale Potenzial dieser Verbindungen.

Der abschließende Teil der Arbeit fokussierte sich speziell auf Akt3, eine Isoform mit zentraler Bedeutung für Neuroentwicklung und Tumorprogression. Durch iterative strukturgeleitete Optimierung auf Grundlage mehrerer hochauflösender Co-Kristallstrukturen konnten hochpotente und selektive kovalente Akt3-Inhibitoren entwickelt werden. Mechanistische Untersuchungen zeigten, dass insbesondere die Geometrie des Linkers sowie die präzise Positionierung isoformdiskriminierender Strukturelemente innerhalb der allosterischen Tasche entscheidende Faktoren für die Selektivität darstellen. Die entwickelten Verbindungen liefern wertvolle chemische Werkzeuge zur Untersuchung Akt3-spezifischer Signalwege und schaffen eine Grundlage für zukünftige therapeutische Anwendungen in der Onkologie und bei neuroentwicklungsbedingten Erkrankungen.

Insgesamt zeigt diese Dissertation einen zusammenhängenden wissenschaftlichen Fortschritt von der systematischen Analyse bestehender Strategien der Akt-Inhibition bis hin zur erfolgreichen Entwicklung isoformselektiver kovalent-allosterischer Akt-Inhibitoren. Die vorgestellten Ergebnisse erweitern das Verständnis isoformspezifischer Akt-Biologie und demonstrieren, wie kovalent-allosterische Ansätze intrinsische Limitationen konventioneller Kinaseinhibitoren überwinden können. Dadurch leisten sie einen wichtigen Beitrag zur Entwicklung selektiverer und effektiverer zielgerichteter Therapeutika.

1. Introduction

1.1. Cancer as a Genomic Disease and the Hallmarks Framework

Cancer represents a multifactorial and dynamic group of diseases fundamentally rooted in the progressive accumulation of genetic and epigenetic alterations that disrupt the tightly regulated balance between cellular proliferation, differentiation, and programmed cell death.¹⁻² These alterations arise through diverse mechanisms, including point mutations, gene amplifications, and chromosomal rearrangements, ultimately leading to the transformation of normal cells into malignant counterparts.² On a global scale, the burden of cancer continues to increase, reflecting both demographic changes and the intrinsic complexity of the disease. At the molecular level, tumorigenesis is largely driven by the dysregulation of oncogenes and tumor suppressor genes. Proto-oncogenes can become constitutively activated through mechanisms such as gene amplification, activating mutations, or chromosomal translocations, thereby promoting uncontrolled cell growth and survival.³ Conversely, the functional loss of tumor suppressor genes, including PTEN and p53, removes essential regulatory constraints on cell cycle progression and apoptosis, enabling malignant transformation.⁴

To conceptualize the remarkable biological complexity underlying cancer, Douglas Hanahan and Robert Weinberg introduced the “Hallmarks of Cancer” framework,⁵ which defines a set of fundamental functional capabilities acquired during tumor development. The original model described six core hallmarks, including sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, replicative immortality, induction of angiogenesis, and activation of invasion and metastasis.⁵ This framework has since been widely adopted as a unifying conceptual model to interpret the diverse genetic and phenotypic features observed across human cancers. Importantly, it has not remained static but has evolved in response to advances in cancer research. Subsequent refinements incorporated additional hallmark capabilities, such as deregulated cellular metabolism and the ability to evade immune destruction, as well as enabling characteristics including genomic instability and tumor-promoting inflammation.⁶⁻⁷

More recent updates have further expanded this conceptualization, reflecting an increasingly nuanced understanding of cancer as a complex and adaptive system. In addition to cell-intrinsic alterations, emphasis has been placed on the role of cellular plasticity, non-genetic mechanisms of tumor evolution, and the dynamic interactions

between cancer cells and their microenvironment.⁸ The contribution of heterotypic cell populations within the tumor milieu, as well as systemic interactions with the host organism, are now recognized as critical determinants of disease progression and therapeutic response. Although these developments have increased the conceptual complexity of the Hallmarks framework, they also reinforce their value as an integrative tool for organizing current knowledge and identifying key biological processes that can be therapeutically targeted.⁸ A central insight emerging from this framework is that many hallmark capabilities are driven by aberrant activation of intracellular signaling pathways that regulate cell growth, survival, and metabolism. Among these, the phosphoinositide 3-kinase PI3K/Akt signaling axis represents one of the most frequently dysregulated pathways in human cancer.⁹ Hyperactivation of Akt signaling contributes to multiple hallmark traits, including sustained proliferative signaling, resistance to apoptosis, and metabolic reprogramming, thereby promoting tumor initiation and progression.¹⁰⁻¹¹ Consequently, targeting key nodes within such signaling networks has emerged as a central strategy in modern cancer therapy. Understanding the molecular basis of these processes, and how they can be selectively modulated, is therefore of fundamental importance and provides a strong rationale for the research presented in this thesis.

The recognition that dysregulated signaling pathways drive hallmark capabilities in cancer has directly enabled the development of targeted therapies, marking a fundamental shift from non-specific cytotoxic treatments to approaches focused on the molecular drivers of tumor progression. Central to this paradigm is the concept of “oncogene addiction,”¹² whereby tumor cells become highly dependent on a single hyperactivated pathway, such that its inhibition can induce profound apoptotic responses, often described as “oncogenic shock”.¹³ Within this framework, protein kinases have emerged as a particularly important class of therapeutic targets, acting as molecular switches that control cell growth, survival, and metabolism across diverse tumor types.

1.2. Protein Kinases: Structure-Function Relationships and Non-Catalytic Roles

Protein kinases constitute a large and highly conserved family of enzymes, with the human kinome comprising more than 500 members.¹⁴ They form a complex network of molecular switches that regulate essential cellular processes, including proliferation, survival, metabolism, and apoptosis. At the molecular level, kinases function as phosphoryltransferases, catalyzing the transfer of the γ -phosphate group from ATP to serine, threonine, or tyrosine residues on substrate proteins, a process typically coordinated by divalent metal ions such as Mg^{2+} .¹⁵ This reversible post-translational modification induces conformational and functional changes in target proteins, modulating their activity, subcellular localization, stability, and protein-protein interactions, thereby controlling the flow of intracellular signaling.¹⁶

Despite their sequence diversity, protein kinases share a conserved bilobal catalytic architecture. The N-terminal lobe, primarily composed of β -sheets and containing the regulatory α C-helix, is involved in ATP binding, while the larger C-terminal lobe mediates substrate recognition and catalysis. These lobes are connected by a flexible hinge region that anchors the adenine moiety of ATP. Key structural elements, including the glycine-rich loop, the DFG motif, and the activation loop, regulate kinase activity by controlling nucleotide positioning, metal coordination, and conformational transitions between active and inactive states.¹⁷⁻¹⁹ The gatekeeper residue further modulates access to a hydrophobic back pocket adjacent to the ATP-binding site, thereby influencing both substrate recognition and ligand binding.¹⁸ Importantly, the intrinsic conformational flexibility of these elements generates not only the canonical ATP-binding site but also transient or less conserved pockets that can be exploited for selective targeting.

Beyond their catalytic activity, kinases frequently perform non-enzymatic functions that are essential for signal transduction. Through scaffolding interactions, assembly of multiprotein complexes, and regulated subcellular localization, they can coordinate signaling outputs independently of phosphorylation.²⁰ These non-catalytic roles highlight that kinase function extends beyond enzymatic activity and that both catalytic and structural contributions must be considered in understanding their role in disease. Dysregulation of kinase activity through mutations, gene amplifications, or chromosomal rearrangements represents a major driver of oncogenesis.

1.3. Kinase Inhibition Strategies and Drug Design Approaches

Building on this structural and functional complexity, multiple strategies have been developed to pharmacologically modulate kinase activity (Fig. 1A). The largest class of compounds comprises ATP-competitive inhibitors, which target the catalytic cleft. Within this group, Type I inhibitors bind the active (DFG-in) conformation of the kinase (Fig. 1B),²¹ whereas Type I½ inhibitors exploit an active-like conformation, often associated with displacement of the α C-helix (Fig. 1C).²¹⁻²² In contrast, Type II inhibitors recognize inactive (DFG-out) conformations and extend into a hydrophobic back pocket that is accessible only in this state, thereby often achieving improved selectivity by stabilizing specific inactive conformations (Fig. 1D).^{21,23-24} Complementary to ATP-site binders, alternative strategies target less conserved regulatory regions of the kinase domain. Type III inhibitors bind to allosteric pockets adjacent to the ATP-binding site (Fig. 1E), while Type IV inhibitors engage more distal allosteric regions that modulate kinase activity through conformational coupling (Fig. 1A). Type V inhibitors are bivalent molecules capable of simultaneously interacting with two distinct regions of the kinase, thereby enhancing affinity and specificity. In contrast to these reversible mechanisms, Type VI inhibitors form covalent bonds with the target enzyme, resulting in prolonged target engagement. (Fig. 1F)^{21, 25-26} Covalent inhibition exploits the so-called “cysteinome,” referring to the subset of proteins that contain suitably positioned and reactive cysteine residues.²⁷ Although cysteine accounts for a relatively small proportion of aminoacids in proteins, its unique nucleophilicity and strategic localization within or near binding pockets enable highly selective targeting. Covalent inhibitors can therefore combine high binding affinity with irreversible engagement, often overcoming resistance mechanisms that impair reversible inhibitor binding. More recently, this concept has been extended beyond cysteine to other nucleophilic residues, such as lysine or arginine, further expanding the scope of covalent targeting strategies.²⁸ Additional modalities, including bivalent inhibitors and targeted protein degradation approaches such as PROteolysis Targeting Chimeras (PROTACs),²⁹ broaden the pharmacological landscape by enabling either simultaneous engagement of multiple binding regions or complete removal of the kinase rather than simple inhibition.

The discovery and optimization of kinase inhibitors have increasingly relied on a rational, structure-guided approach. Structure-based drug design (SBDD) integrates high-resolution structural information, primarily obtained through protein X-ray

crystallography, with computational modeling and medicinal chemistry to define the three-dimensional features of kinase active sites and allosteric pockets. This enables the precise design of ligands that complement target geometry, allowing detailed analysis of binding modes, conformational states, and interaction networks. Through iterative optimization, SBDD supports the development of compounds with improved potency, selectivity, and pharmacological properties, transforming kinase drug discovery into a predictive and mechanism-driven discipline. In this context, modern strategies increasingly exploit less conserved regions and nucleophilic amino acid residues to achieve selective and durable target engagement. This approach provides the conceptual foundation for the inhibitor design strategies explored in this thesis.

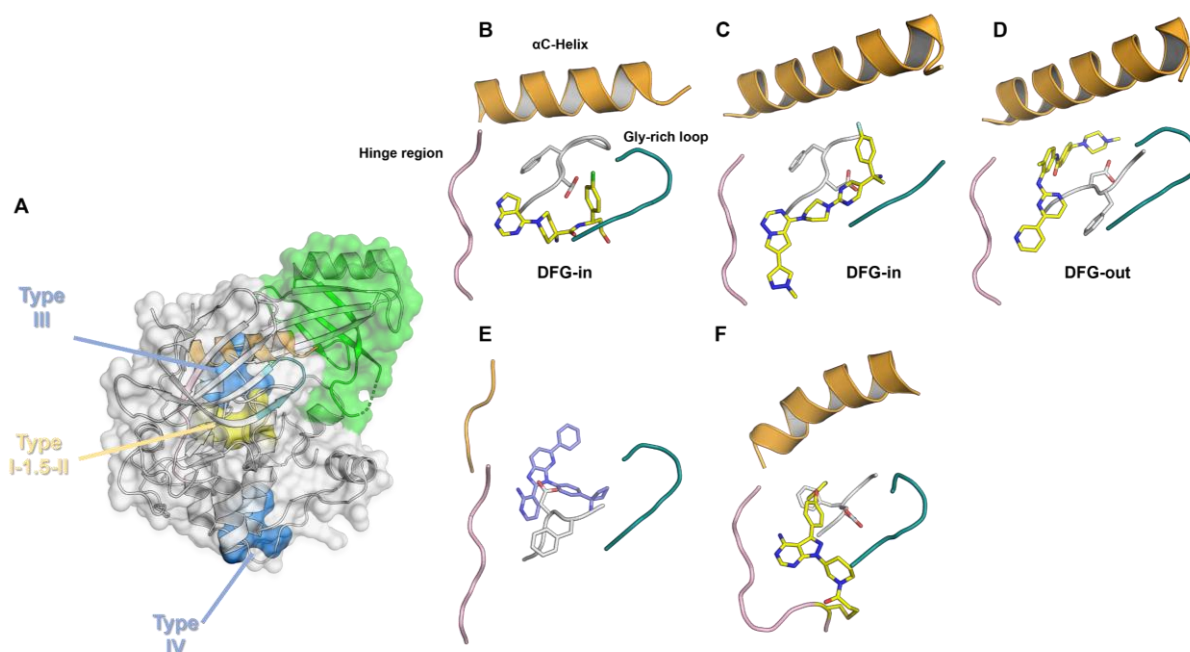


Figure 1. Schematic representation of a protein kinase highlighting the major inhibitor binding modes. (A) Overview of a protein kinase and binders of the ATP binding pocket indicated in yellow (PDB ID: 4GV1) and of two distinct allosteric sites (Type III and Type IV, indicated in blue, PDB IDs: 5KCV and 5MO4, respectively). (B) Type I inhibitors bind and stabilize an active kinase (DFG-in conformation). Capivasertib (PDB ID: 4GV1) is shown as an example. (C) Type I½ inhibitors bind and stabilize an active kinase (DFG-in conformation). Avapritinib (PDB ID: 8PQ9) is shown as an example. (D) Type II inhibitors bind and stabilize an inactive kinase (DFG-out conformation). Imatinib (PDB ID: 1T46) is shown as an example. (E) Type III inhibitors bind to an allosteric pocket. Miransertib (PDB ID: 5KCV) is shown as an example. (F) Type VI inhibitors bind forming a covalent bond. Ibrutinib (PDB ID: 5P9J) is shown as an example.

1.3.1. Structural Biology in the Post-Genome Era

The advent of genomic technologies has provided an extensive catalog of protein sequences and potential therapeutic targets; however, understanding protein function and enabling rational drug design requires detailed knowledge of three-dimensional structure. Structural biology bridges this gap by revealing the spatial organization of proteins, their conformational dynamics, and the molecular basis of their interactions with ligands. In the context of kinase-targeted drug discovery, such structural insight is essential for identifying binding pockets, characterizing conformational states, and guiding the design of selective inhibitors. Over the past decade, structural biology has undergone a profound “resolution revolution”³⁰ driven by major technological advances.³¹⁻³² Cryo-electron microscopy (cryo-EM) has enabled the visualization of large macromolecular assemblies and highly dynamic protein systems at near-atomic resolution, thereby overcoming several limitations associated with traditional X-ray crystallography.³³ In parallel, computational approaches, most notably AlphaFold, have transformed the field by enabling highly accurate prediction of protein structures directly from amino acid sequences.³⁴ Collectively, these developments have markedly expanded structural coverage across the proteome and provided unprecedented insights into protein architecture and domain organization. Despite these advances, a detailed and experimentally validated understanding of protein-ligand interactions remains essential for structure-guided drug discovery. In this context, X-ray crystallography continues to play a central role.³⁵ By measuring X-ray diffraction patterns from protein crystals, this technique enables the determination of atomic coordinates with high precision, frequently at resolutions in the range of 1-2 Å. Such high resolution allows for accurate characterization of side-chain conformations, hydrogen-bonding networks, ordered solvent molecules, and, critically, ligand binding modes.³⁵⁻³⁶ These features are indispensable for elucidating the molecular determinants of affinity and selectivity.

Importantly, X-ray crystallography provides direct experimental access to ligand-induced conformational changes and subtle structural rearrangements that govern binding and activity. While cryo-EM offers powerful insights into large and flexible systems, its application to small-molecule binding in relatively small proteins remains limited by achievable resolution.³⁷ Similarly, computationally predicted models, including those generated by AlphaFold,³⁴ do not inherently capture ligand-induced

effects, conformational equilibria, or solvent contributions, and therefore cannot replace experimentally determined protein-ligand structures.

This limitation is particularly relevant in kinase systems, where small conformational differences, such as DFG-in/DFG-out transitions or α C-helix positioning, play a decisive role in determining inhibitor binding modes and selectivity profiles.³⁸ Consequently, X-ray crystallography remains uniquely suited for the detailed characterization of kinase-inhibitor complexes and continues to represent a cornerstone methodology in rational drug design. For these reasons, it constitutes a central methodological pillar of the work presented in this thesis.

1.3.2. From Structural Design to Biological Activity: Potency, Selectivity, and Functional Validation

The structural insights provided by modern techniques form the basis of structure-based drug design (SBDD),³⁹ enabling the rational development of inhibitors with defined binding modes and optimized interactions within the target protein. However, achieving a favorable binding mode at the structural level does not necessarily translate into effective biological activity. In the context of kinase inhibitors, two fundamental parameters must be considered: potency and selectivity.⁴⁰ Potency describes the concentration required to inhibit a target, typically expressed as IC_{50} or K_d , and directly influences the dose required to achieve a therapeutic effect. High potency is desirable as it allows effective target inhibition at low concentrations, thereby reducing systemic exposure. In contrast, selectivity refers to the ability of a compound to discriminate between closely related kinases within the highly conserved kinome. Given the structural similarity of ATP-binding sites, insufficient selectivity can result in off-target inhibition, leading to adverse effects and limiting clinical applicability.

The evaluation of these parameters is typically initiated using *in vitro* biochemical assays, including enzymatic activity measurements, fluorescence-based assays such as Homogeneous Time-Resolved Fluorescence (HTRF),⁴¹ or mass spectrometry-based binding analyses.⁴² These approaches provide quantitative information on inhibitor affinity, kinetics, and mechanism of action under controlled conditions, allowing direct comparison between compounds. However, such assays are performed using purified proteins and therefore do not account for the complexity of the intracellular environment, where factors such as membrane permeability, intracellular ATP concentration, protein-protein interactions, and subcellular localization can

significantly influence inhibitor performance.⁴⁰ As a consequence, compounds with high biochemical potency may fail to demonstrate comparable activity in cellular systems. To bridge this gap, cellular target engagement assays have become an essential component of modern drug discovery. Techniques such as NanoBRET enable the direct measurement of inhibitor binding to the target protein in living cells by monitoring energy transfer between a luminescent donor fused to the kinase and a fluorescent tracer.⁴³⁻⁴⁴ Displacement of the tracer by the inhibitor provides a quantitative readout of intracellular target engagement, thereby integrating both binding affinity and cellular accessibility. In parallel, functional assays such as Western blotting allow the assessment of downstream signaling by measuring phosphorylation levels of kinase substrates or of the kinase itself, providing insight into pathway modulation.⁴⁵ Phenotypic assays, including CellTiter-Glo (CTG),⁴⁶ further extend this analysis by linking target inhibition to cellular outcomes such as proliferation or survival.⁴⁷ Beyond conventional two-dimensional cell culture systems, increasing attention is being directed toward more physiologically relevant models that better capture the complexity of human tissues. Three-dimensional (3D) cultures and organoid systems recapitulate key features of the *in vivo* environment, including cell-cell interactions, spatial organization, and gradients of nutrients and oxygen. These models provide a more predictive platform for evaluating drug response, resistance mechanisms, and therapeutic efficacy, bridging the gap between *in vitro* studies and *in vivo* applications.⁴⁸⁻⁴⁹ Together, these multi-level approaches, from biochemical assays to cellular target engagement and advanced model systems, enable a comprehensive evaluation of inhibitor performance. By integrating structural design with functional validation across increasing levels of biological complexity, it becomes possible to identify compounds that are not only potent and selective *in vitro* but also effective within the dynamic environment of living systems.

This integrated strategy is essential for the development of kinase inhibitors with improved therapeutic profiles and underpins the experimental framework applied throughout this thesis.

1.4. Chemical Biology in Kinase Research and Drug Discovery

Chemical biology lies at the interface of chemistry, biology, and medicine, providing a framework in which chemical tools are used to interrogate and manipulate biological systems at the molecular level. Rather than passively describing cellular processes, this approach enables direct intervention in signaling networks, transforming structural and biochemical knowledge into functional insight. In this context, small molecules serve not only as therapeutic agents but also as versatile probes that allow the controlled perturbation of protein function within complex cellular environments.⁵⁰

Compared to genetic approaches, such as RNA interference or CRISPR-mediated knockouts, small-molecule probes offer distinct advantages, including rapid, reversible, and dose-dependent modulation of protein activity. This temporal and quantitative control allows researchers to fine-tune biological responses and monitor dynamic changes in signaling pathways over time.⁵¹ Importantly, such approaches can help discriminating whether a protein's role in a given process depends on its catalytic activity or on non-enzymatic, scaffolding functions, an aspect that is often difficult to resolve using genetic perturbation alone.²⁰ In addition, chemical biology strategies can uncover previously inaccessible regulatory sites, enabling the targeting of proteins that were historically considered undruggable.⁵²⁻⁵³ Beyond functional perturbation, chemical biology provides tools to investigate the spatial and temporal organization of signaling pathways. Localization studies based on fluorescence microscopy enable visualization of protein distribution and translocation in response to stimuli or pharmacological intervention. By employing fluorescently tagged constructs or probes, it is possible to monitor how kinases such as Akt dynamically redistribute between cellular compartments, including the plasma membrane, cytoplasm, and nucleus, and how these processes are influenced by inhibitor binding. These approaches complement biochemical and structural analyses by revealing where signaling events occur within the cellular context. Chemical biology is also deeply integrated into modern drug discovery, supporting an iterative cycle in which biological hypotheses guide compound design, followed by structural, biochemical, and cellular evaluation.⁵⁴ High-resolution structural techniques inform the design of molecules with defined binding modes, while cellular assays provide critical validation of target engagement and pathway modulation.⁵⁵ Additional approaches, such as activity-based protein profiling, extend these analyses to native cellular environments, enabling the direct investigation of active enzyme populations and contributing to the assessment of

selectivity and off-target effects. In the context of cancer research, these strategies are particularly important for the development of precision therapies that selectively target the molecular drivers of tumor progression.⁵⁶ By disrupting key signaling dependencies, small-molecule probes can induce selective cytotoxic effects in cancer cells while sparing normal tissues. Moreover, chemical biology approaches facilitate the identification of resistance mechanisms and support the design of next-generation inhibitors capable of overcoming adaptive changes in the target. Emerging concepts, including targeted protein degradation and bioorthogonal chemistry, further expand the scope of this field by enabling novel modes of intervention beyond classical inhibition.⁵⁷⁻⁵⁸ Within the context of Akt signaling, these chemical biology strategies are especially powerful, as isoform-specific activity is closely linked to subcellular localization, dynamic regulation, and pathway context. The integration of structural, biochemical, and cellular approaches provides a comprehensive framework for understanding inhibitor mechanisms and for guiding the development of selective compounds.

In this context, the work presented in this thesis addresses key challenges in Akt-targeted drug discovery, including selectivity, resistance, and the development of novel inhibitory strategies. The first part provides a comprehensive overview of Akt inhibitors, their mechanisms of action, and their clinical progression. Building on this foundation, subsequent studies focus on the design and characterization of selective covalent inhibitors, with particular emphasis on isoform selectivity and the exploration of structure-activity relationships. These investigations include the development of Akt2- and Akt3-selective compounds, as well as the systematic evaluation of linker strategies to modulate selectivity and potency. Together, these studies aim to advance the understanding of Akt inhibition and to contribute to the development of more selective and effective therapeutic strategies.

2. Motivation and Aim

Despite significant advances in kinase-targeted therapies, the development of selective and effective inhibitors remains a major challenge. In the case of Akt, this difficulty arises from the high structural conservation of the kinase domain, the presence of multiple isoforms, and complex regulatory mechanisms. As a result, achieving both potency and isoform selectivity remains problematic, while current inhibitor classes are often limited by off-target effects, resistance mechanisms, and incomplete pathway modulation.

Akt represents a central node in signaling pathways that regulate proliferation, survival, metabolism, and migration, and its dysregulation is frequently associated with cancer progression and therapeutic resistance. Although extensive pharmacological efforts over the past two decades have led to the development of multiple classes of Akt inhibitors, clinical translation has been only partially successful. The recent approval of the ATP-competitive inhibitor capivasertib provides important validation of Akt as a druggable target; however, ATP-site targeting inherently relies on interaction with a highly conserved pocket, which limits isoform selectivity and is often associated with dose-limiting toxicities. These limitations highlight the need for alternative strategies capable of achieving more precise and sustained modulation of Akt signaling. In this context, allosteric and covalent approaches offer significant potential by targeting less conserved regulatory regions and enabling prolonged target engagement. The work presented in this thesis aims to address these challenges through an integrated strategy combining structural biology, chemical biology, and cellular approaches. In particular, this study focuses on the design and characterization of covalent allosteric inhibitors targeting the regulatory pocket located at the interface between the kinase domain and the PH domain. By combining covalent engagement with allosteric modulation, this approach seeks to enhance selectivity while maintaining potent and sustained inhibition. A central objective of this work is the investigation of isoform selectivity, with particular emphasis on Akt2 and Akt3, whose distinct biological roles remain incompletely understood. To this end, the thesis integrates structure-based drug design, protein engineering, and X-ray crystallography with cellular target engagement assays, including NanoBRET, as well as functional readouts such as Western blotting and cell viability assays. In addition, systematic exploration of linker strategies is employed to fine-tune compound properties and optimize selectivity profiles.

The first publication associated with this thesis (DOI: 10.1021/acs.jmedchem.4c00075)⁵⁹ provides a comprehensive overview of Akt inhibitors, including their structural features, mechanisms of action, and clinical development. This analysis establishes the conceptual framework for the development of covalent and isoform-selective inhibitors described in the following chapters.

3. Akt Inhibitor Advancements: From Capivasertib Approval to Covalent-Allosteric Promises

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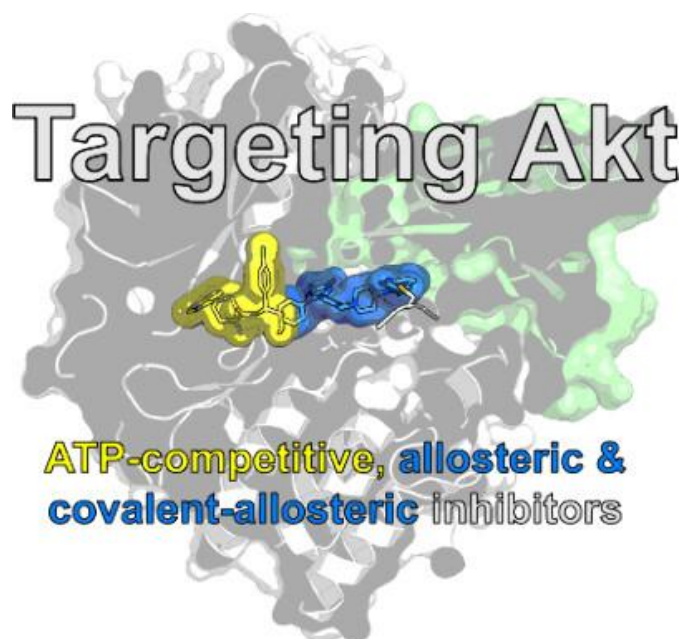
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Graphical Abstract



3.1. Abstract

Akt kinase is vital in cell growth, survival, metabolism, and migration. Dysregulation of Akt signaling is implicated in cancer and metabolic disorders. In the context of cancer, overactive Akt promotes cell survival and proliferation. This has spurred extensive research into developing Akt inhibitors as potential therapeutic agents to disrupt aberrant Akt signaling. Akt inhibitors are classified into three main types: ATP-competitive, allosteric, and covalent-allosteric inhibitors (CAAls). ATP-competitive inhibitors compete with ATP for binding to Akt, allosteric inhibitors interact with the Pleckstrin homology (PH) domain, and covalent-allosteric inhibitors form covalent bonds, making them more potent and selective. Notably, capivasertib (AZD5363), a potent ATP-competitive Akt inhibitor, received FDA approval in November 2023 for use in combination with the estrogen receptor degrader fulvestrant to treat breast cancer. Challenges remain, including improving selectivity, identifying biomarkers to tailor treatments, and enhancing therapeutic efficacy while minimizing adverse effects. Particularly covalent-allosteric inhibitors hold promise for future more effective and personalized treatments.

3.2. Significance

This Perspective highlights the pivotal role of Akt kinase in cancer and metabolic diseases. It focuses on the landmark FDA approval of capivasertib, a first-generation Akt inhibitor, for the treatment of breast cancer. It heralds the emergence of next generation Akt inhibitors, allosteric and covalent-allosteric, that promise improved selectivity and potency, ushering in a new era of therapeutic strategies and personalized, more effective interventions.

3.3. Introduction

Akt, referred to as protein kinase B (PKB), is a significant serine/threonine kinase involved in diverse cellular activities such as cell proliferation, survival, metabolism, and migration. Dysregulation of Akt signaling has been linked to numerous diseases, including cancer and metabolic disorders.¹ In cancer, hyperactivation of Akt is common and contributes to increased cell survival, proliferation, and invasion. In metabolic disorders like diabetes, Akt dysregulation can lead to insulin resistance and abnormal glucose metabolism.^{2,3} Therefore, Akt has been the target of intense research for potential therapeutic interventions for over 20 years. Various Akt inhibitors are currently

being developed and tested as anticancer drugs, either as monotherapy or in combination studies.

The Akt protein consists of several distinct structural domains that play essential roles in its function. These domains are common among the three isoforms of Akt (Akt1/2/3): the N-terminal Pleckstrin homology (PH) domain, the catalytic kinase domain, and the C-terminal hydrophobic motif. The PH domain is responsible for binding to phosphoinositides, specifically phosphatidylinositol-3,4,5-trisphosphate (PIP3), produced by phosphoinositide 3-kinase (PI3K) when growth factor receptors are activated. In the absence of PIP3, the PH domain interacts with the kinase domain in a closed conformation, preventing the interaction with phospholipids.^{4,5} When PIP3 binds to the PH domain of Akt, this interaction induces a change in the spatial arrangement of Akt's structural domains. Importantly, it results in the dissociation of the PH domain from the kinase domain.⁶ The PH domain is likely to fully detach from the kinase domain, thereby enhancing the flexibility and accessibility of the kinase domain. This, in turn, enables the activation of the kinase domain through phosphorylation, allowing it to subsequently phosphorylate its downstream targets.^{2,7} Upon membrane recruitment by PIP3, Akt can interact with phosphoinositide-dependent kinase-1 (PDK1), another PH domain comprising protein kinase, and undergoes phosphorylation at Thr308 in the activation loop of the kinase domain. This phosphorylation is essential for the partial activation of Akt.⁸ The central region of Akt contains the kinase domain, which mediates the phosphorylation of target proteins with roles in the DNA damage response, such as CHK1, and in cell cycle regulation, such as FOXO1A, FOXO3A, p21, and p27.¹ The catalytically active kinase domain is highly conserved among the three isoforms of Akt and across other members of the AGC kinase family to which Akt belongs. The C-terminal region of Akt contains a hydrophobic motif and a turn motif. These motifs, particularly Ser473 in the C-terminal region, are phosphorylated by other kinases, including mammalian target of rapamycin complex 2 (mTORC2), to fully activate or stabilize Akt. Dual phosphorylation of both Thr308 and Ser473 is required for Akt to reach its fully activated state (Figure 1A).^{9,10} The complex architecture of Akt, comprising several structural elements, coupled with its intricate regulation and dynamic behavior, makes it a promising target for designing inhibitors exhibiting different modes of action (MOAs) (Figure 1B).¹¹ The mounting evidence implicating Akt dysregulation in disease pathogenesis provides a compelling rationale for the pursuit of kinase inhibitor development. By specifically targeting Akt's

kinase activity, these inhibitors hold the potential to disrupt downstream signaling pathways responsible for promoting abnormal cell growth and survival. Thus, Akt inhibitors can offer precise and effective treatment strategies for diseases driven by Akt hyperactivation. Akt inhibitors comprise a diverse array of molecules that act through distinct mechanisms to modulate Akt signaling. In the early 2000s, research endeavors to develop both ATP-competitive and allosteric inhibitors were initiated to interfere with aberrant Akt signaling in cancer cells. Subsequently, substantial work has been invested in formulating Akt inhibitors to unravel Akt's function in cellular processes and explore its potential in cancer therapeutics.¹² It is noteworthy that some Akt inhibitors have managed to advance in clinical trials (Table 1) despite the significant challenges faced in Akt inhibitor research over the past two decades, including issues with developing selective inhibitors and minimizing off-target effects¹³ and the emergence of resistance mechanisms during treatment: *in vitro* studies employing LnCaP prostate cancer cells elucidated PIM signaling as a potential resistance mechanism in response to prolonged exposure to the ATP-competitive inhibitor ipatasertib. Additionally, the genetic lesion W80C was detected in Akt1, conferring resistance to the allosteric inhibitor MK-2206.¹⁴ However, Akt inhibitors provide therapeutic benefits for various diseases, including cancer, as shown in clinical trials. Capivasertib (AZD5363), a potent panAkt inhibitor, has achieved a major breakthrough in the Akt field. In November 2023, capivasertib, in a combination therapy with fulvestrant, an anti-estrogen drug, was granted FDA approval for treating adult patients with hormone receptor positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) breast cancer.¹⁵ This remarkable milestone marks a significant advancement in Akt-targeted precision medicine and offers new hope for patients with these specific cancer types. In this Perspective, we will focus on the development of such inhibitors and delve into the complex crystal structures of some representative inhibitors. The intricate structure and regulation of Akt and its isoforms make this kinase a fascinating target for rational inhibitor design with diverse MOAs, providing a rich landscape for scientific exploration and therapeutic innovation.

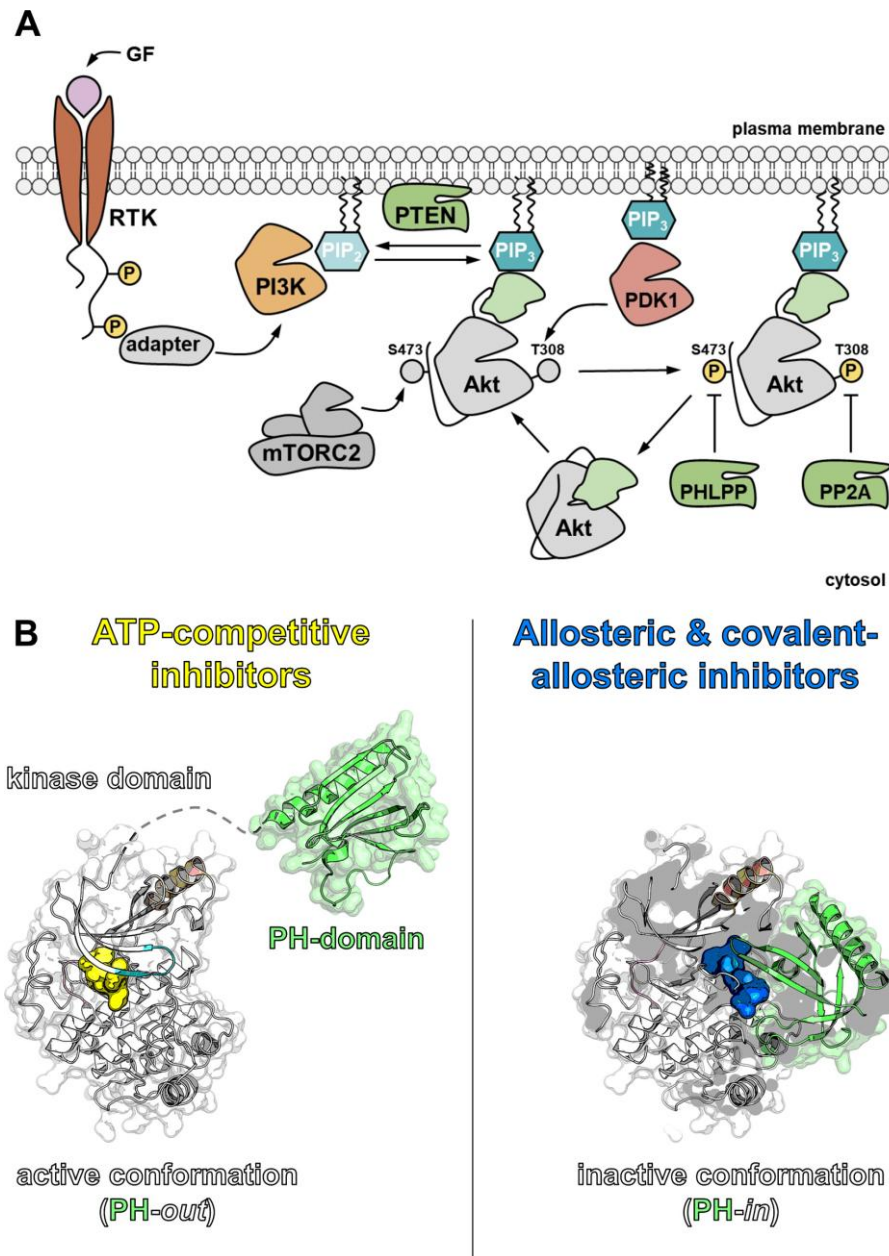
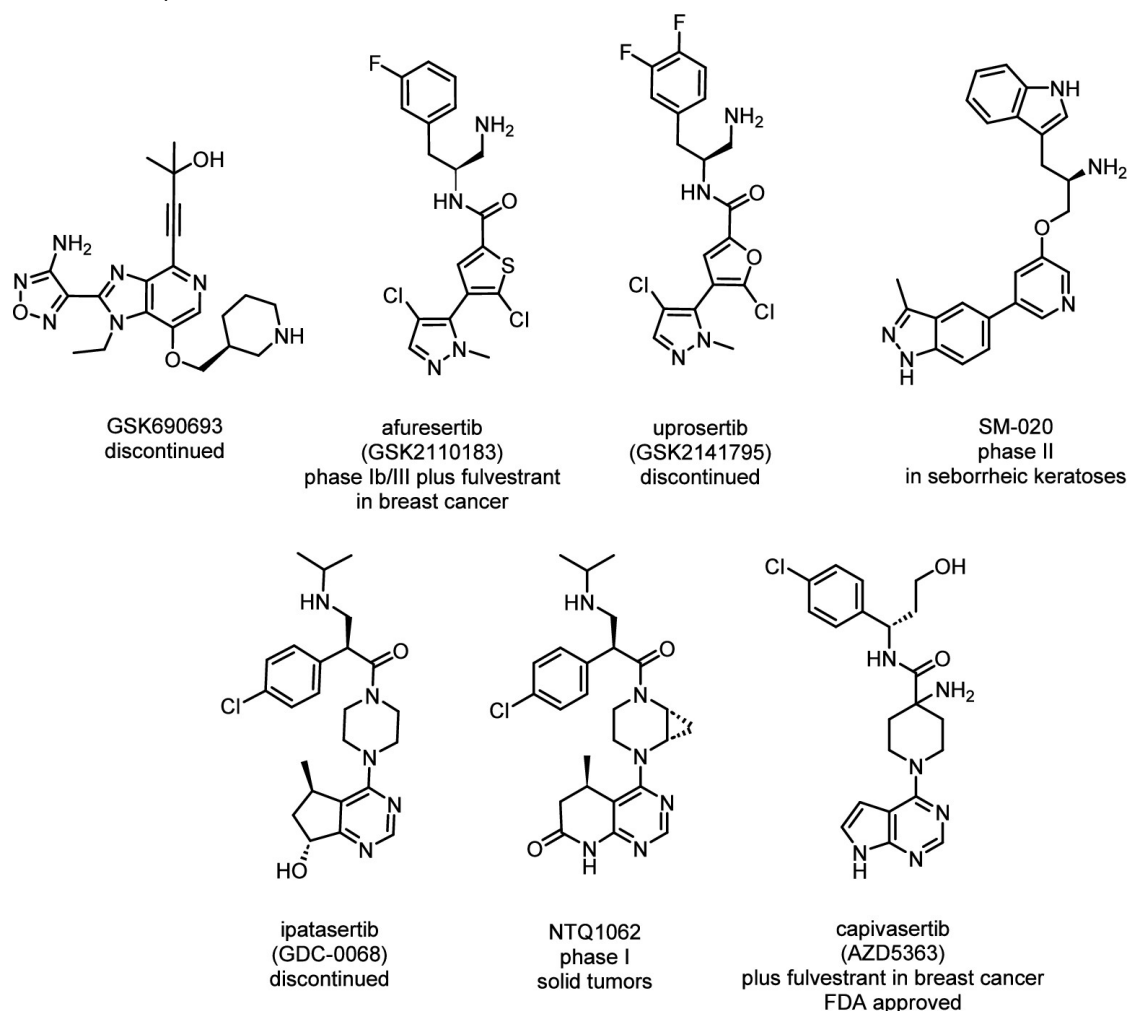


Figure 1. (A) Schematic representation of Akt activation and regulation. PI3K is activated, for example, by stimulation of RTKs by extracellular growth factors (GFs). PI3K catalyzes the phosphorylation of PIP₂, resulting in increased accumulation of PIP₃ at the plasma membrane. Cytosolic inactive Akt is recruited to the plasma membrane by the interaction of the PH domain with PIP₃, resulting in phosphorylation of the activation loop at Thr308 by PDK1 and at Ser473 of the hydrophobic motif by mTORC2, leading to full activation of Akt. Dephosphorylation of Akt by the protein phosphatases PP2A and PHLPP terminates the downstream signaling of Akt. (B) Crystal structures of protein kinase Akt. Depiction of the kinase domain (gray, PDB: 4GV1) and the regulatory PH domain (green, PDB: 1UNQ) in the active PH-out conformation (left) and the autoinhibitory (PH-in) conformation (right, PDB: 5KCV). The linker region is shown as a gray dashed line and the α C-helix colored in orange, the hinge region in pink, and the Gly-rich loop in cyan. ATP-competitive inhibitors (yellow) bind to the enzymatically active (PH-out) conformation. Allosteric and covalentallosteric inhibitors (blue) bind to and stabilize an allosteric pocket that is formed in the enzymatically inactive (PH-in) conformation.

Table 1. Summary of Akt Inhibitors That Have Progressed to Clinical Trials, Including Their Indications, Combination Agents, and Current Status, Categorized by Their Respective Mechanisms of Action

Mechanism of action	Name (Company)	Indication	Combination agent	Status
ATP competitive inhibitors	GSK690693 (GlaxoSmithKline)	hematologic malignancies	–	discontinued
	Afuresertib (GlaxoSmithKline)	breast cancer	fulvestrant	active (phase Ib/III)
	Uprosertib (GlaxoSmithKline)	melanoma cancer	dabrafenib, trametinib	discontinued
	SM-020 (Dermbion)	seborrheic keratoses	–	active (phase II)
	Ipatasertib (Genetech/Roche)	prostate and breast cancer	chemotherapeutic agents	discontinued
	NTQ-1062 (Nanjing Chia-Tai Tianqing Pharmaceutical)	breast cancer	fulvestrant	active (Phase Ib//II)
	Capivasertib (AstraZeneca)	breast cancer	fulvestrant	FDA approved
Allosteric inhibitors	Miransertib (Merck)	Proteus syndrome	–	discontinued
	Vevorisertib (Merck)	solid tumors	paclitaxel, fulvestrant	discontinued
	MK-2206 (Merck)	solid tumors	chemotherapeutic agents	discontinued
	Pifusertib (Taiho Pharmaceutical)	solid tumors	–	under development
	VAD-004 (Vaderis Therapeutics)	Osler–Weber–Rendu syndrome	–	active (phase I)

Chart 1. ATP-Competitive Inhibitors That Have Advanced to Clinical Trials and Their Current Status



3.4. ATP-Competitive Akt Inhibitors

ATP-competitive inhibitors represent one strategy to target Akt. These inhibitors engage the ATP-binding site within the kinase domain, where they compete with ATP for binding. This MOA sets them apart from allosteric Akt inhibitors, which instead interact with the PH domain. The binding of these competitive inhibitors hinders the transfer of a phosphate group from ATP to Akt's substrate proteins, leading to the inhibition of Akt kinase activity and downstream signaling cascades. Early development of these inhibitors faced challenges due to the high homology in the ATP-binding pocket between PKA, Akt (PKB), PKC, and other related protein kinases. Numerous ATP-competitive Akt inhibitors have been designed and synthesized using structural biology and medicinal chemistry approaches, effectively inhibiting Akt *in vitro* and *in vivo*. However, only a few of these inhibitors have entered clinical trials in the past two decades. The first ATP-competitive Akt inhibitor evaluated in the clinic was GSK690693 (Chart 1), derived from a structure–activity relationship (SAR) study on pyridylimidazole-oxadiazolyl amines. This potent pan-Akt inhibitor with modest selectivity for Akt isoforms over other related kinases¹⁶ showed encouraging preclinical data.^{16,17} Clinical development of this molecule was suspended due to significant adverse events, including hyperglycemia.¹⁸ Efforts have been made to improve the selectivity, safety, and tolerability of Akt inhibitors that contain different chemical cores, which led to the development of uprosertib (GSK 2141795), afuresertib (GSK2110183), ipatasertib (GDC-0068), capivasertib (AZD5363), SM020, and NTQ1062 that have advanced to clinical trials (Chart 1, Table 1). Afuresertib (GSK2110183) was discovered through lead optimization of a 2-pyrimidyl-5-amidothiophene derivative,¹⁹ wherein the pyrimidine ring was replaced with a substituted pyrazole ring (Chart 1). Uprosertib (GSK2141795) is a structural analog of afuresertib, with the thiophene core replaced by a bioisosteric furan ring (Chart 1). Both compounds, originally developed by GlaxoSmithKline, are orally available and potent Akt inhibitors with nanomolar potencies for all three Akt isoforms.²⁰ In a Phase I study, afuresertib demonstrated a favorable safety profile and clinical activity against hematologic malignancies, particularly multiple myeloma. Common adverse effects included nausea, diarrhea, and dyspepsia.²¹ A Phase Ib/ III study is currently ongoing to evaluate the safety and efficacy of afuresertib plus fulvestrant in patients with locally advanced or metastatic HR+/HER2– breast cancer who failed standard of care therapies (LAE205INT3101/NCT04851613).

Uprosertib (Chart 1) has also advanced into clinical trials, showing a safe and well-tolerated profile in a Phase I study in patients with solid tumors.²² Unfortunately, limited clinical efficacy and poor tolerability were observed when it was used in combination with the oral MEK1/MEK2 inhibitor trametinib.^{23–26} A Phase I/II study (NCT01902173) evaluating uprosertib in combination with dabrafenib and trametinib for the treatment of patients with stage IIIC–IV metastatic melanoma with BRAF mutation was discontinued due to lack of supply of the compound. One of the earliest ATP-competitive inhibitors was SM-020 (developed by Abbott Laboratories and previously known as A443654), a potent and reversible Akt inhibitor (Chart 1). This compound is equally potent against all isoforms and has 40-fold selectivity for Akt over PKA.²⁷ Combination with paclitaxel in PC-3 and with rapamycin in MiaPaCa-2 xenograft models increased efficacy and tumor growth inhibition compared to monotherapy.²⁷ In a Phase II clinical trial (NCT05136144), the safety and efficacy were investigated for a topical formulation of SM-020 when administered to patients with seborrheic keratoses (SK). The results of the study indicate the efficacy of SM-020 to reduce SK lesions, and over 80% of SKs treated for 14 to 28 days with SM-020 gel 1.0% had at least a one-point improvement in PLA score at the last visit. Currently, another Phase II study is ongoing to treat facial and intertriginous SKs (NCT06108024). Ipatasertib (GDC-0068), developed by Genentech in collaboration with Array BioPharma, is an orally available and potent ATP-competitive Akt inhibitor derived from a SAR study using the 6,7-dihydro-5H-cyclopenta[d]pyrimidine scaffold (Chart 1, Figure 2F). *In vitro* experiments demonstrated potent Akt inhibition and reduced cell proliferation, while *in vivo* results in murine xenografts showed tumor growth inhibition, leading to further clinical development.²⁸ Ipatasertib advanced into clinical trials and has been combined with chemotherapeutics such as paclitaxel and docetaxel, and monoclonal antibodies such as atezolizumab and trastuzumab, but according to the Genentech/Roche pipelines, the development of this compound was terminated. The discontinuation of ipatasertib is significant in the context of early generation Akt inhibitors, as it was one of the more advanced candidates in this class. The difficulties encountered with ipatasertib underscore the challenges faced in developing effective Akt inhibitors despite the initial high expectations for this class of drugs in cancer therapy. However, ipatasertib's efficacy and selectivity have laid the foundation for seeking out new ATP-competitive inhibitors with improved pharmacodynamic and pharmacokinetic features. This quest has led to the development of NTQ1062 (developed by Nanjing Chia-Tai

Tianqing Pharmaceutical Co.) through refined optimization approaches. NTQ1062 introduces a novel chemical scaffold, (R)-5-methyl-5,8-dihydropyrido[2,3-d]-pyrimidin-7(6H)-one, which binds to the hinge region of the kinase domain, and bears an optimized piperazine linker compared to ipatasertib (Chart 1). Extensive studies have showcased that NTQ1062 demonstrates favorable oral properties and exhibits dose-dependent pharmacodynamic effects in xenograft models exhibiting constitutive activation of the PI3K/Akt-mTOR pathway.²⁹ Presently, it is undergoing evaluation in a Phase I clinical trial for the treatment of advanced solid tumors (NCT06172309) and in a Phase Ib/II trial in combination with fulvestrant in patients with advanced HR+/HER2– breast cancer (NCT06172322). Capivasertib (AZD5363), developed by AstraZeneca in the early 2000s, is a potent, ATP-competitive Akt inhibitor with significant results as a therapeutic agent. Capivasertib contains a pyrrolopyrimidine scaffold (Chart 1) that enables binding to the hinge region of Akt and effectively targets all isoforms with remarkable potency, demonstrating low nanomolar IC₅₀ values of 3 nM, 8 nM, and 8 nM, respectively.³⁰ The analysis of the crystal structure of capivasertib and Akt1 (PDB 4GV1) unveiled crucial molecular interactions and structural characteristics. The active form of the protein reveals an arrangement with the C-terminal tail folding back over the N-terminal lobe to position Phe469 and Phe472 within a crucial hydrophobic pocket responsible for regulating Akt1 activity.³⁰ Notably, the pyrrolopyrimidine ring establishes two hydrogen bonds with the kinase hinge through interactions with Ala230 and Glu228 (Figure 2E). Equally interesting is the adoption of an axial conformation by the central piperidine ring concerning both the pyrrolopyrimidine hinge group and the Gly-rich loop aryl group. This conformational preference is influenced by the presence of an ortho-sp² nitrogen in the pyrrolopyrimidine core, which strategically places the basic amino group within an acidic hole formed by Glu234 and Glu278. Additionally, the chlorophenyl group finds itself comfortably situated in a hydrophobic pocket below the Gly-rich loop, surrounded by the side chains of Lys179, Leu181, and Val164, as well as the backbone atoms of Lys158 and Gly162.³⁰ These structural details shed light on the compound's high affinity for Akt1 and its potential implications for regulatory control. Initial studies by Davies et al. in 2012 showcased capivasertib's activity as a monotherapy against breast cancer cells, particularly those with HER2– subtypes, especially those harboring PIK3CA mutations or PTEN function loss.³¹ Subsequent investigations revealed encouraging antitumor activity, leading to disease stabilization or tumor regression.³²

However, its efficacy as a monotherapy showed limitations, prompting exploration of combination therapies to enhance its effectiveness, overcome resistance, and target multiple pathways simultaneously. In fact, when capivasertib was administered as a monotherapy, at therapeutically effective doses *in vitro*, capivasertib primarily exhibited an antiproliferative mechanism rather than a proapoptotic one. Combining capivasertib with other compounds in early stage clinical trials resulted in promising synergistic effects. Notably, when administered with fulvestrant, capivasertib showed remarkable improvements in progression-free and overall survival compared to fulvestrant alone in the Phase II FAKTION trial.³³ The Phase III CAPItello-291 trial further validated capivasertib's potential by evaluating its combination with fulvestrant for HR+/HER2- advanced breast cancer that had progressed on prior endocrine therapy. The trial demonstrated a significant improvement in progression-free survival among patients who had progressed during or after previous endocrine therapy, with manageable adverse events.³⁴

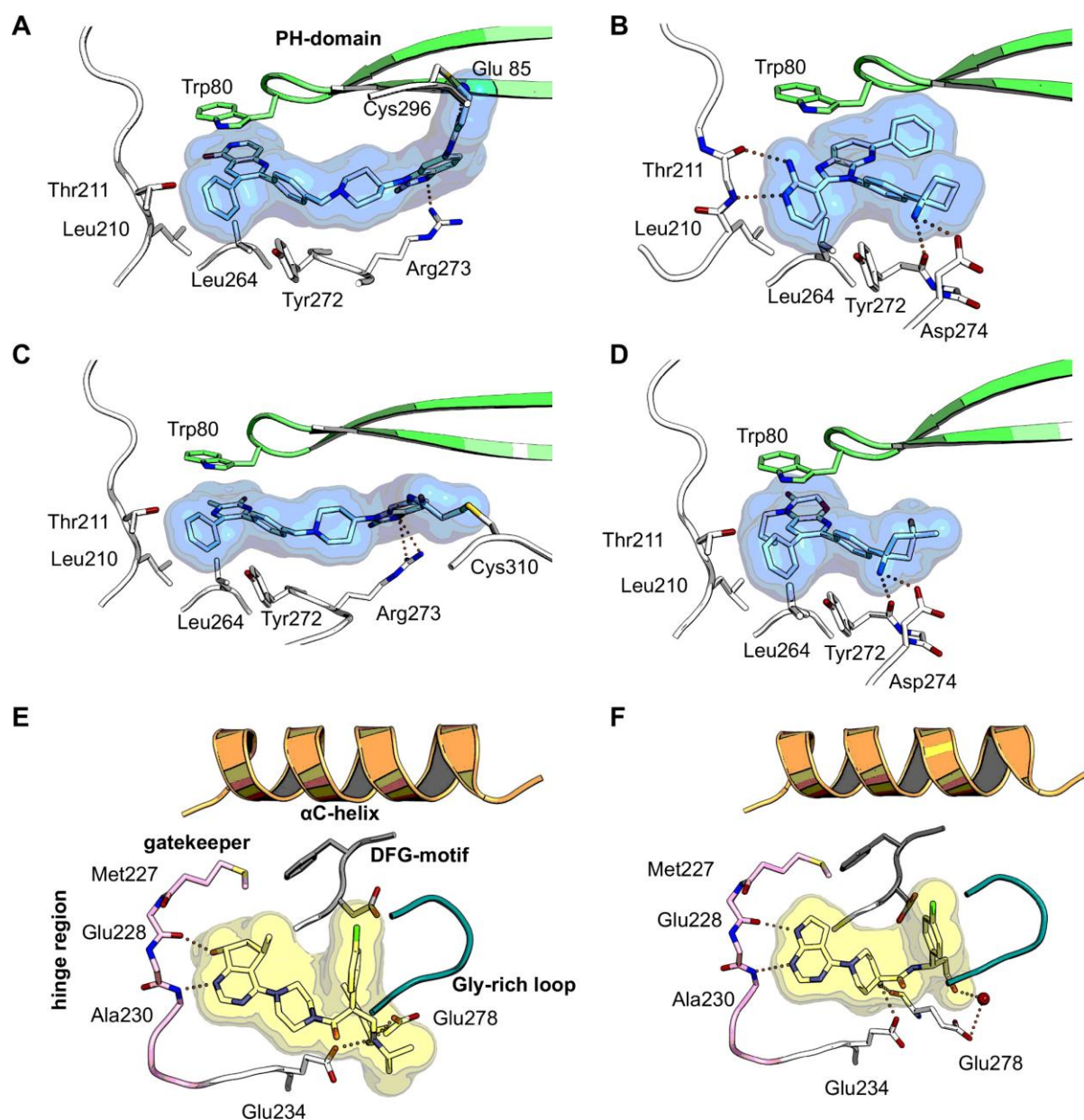


Figure 2. Crystal structures of Akt1 in complex with inhibitors displaying different modes of action: (A) the CAAI borussertib (PDB: 6HHF), (B) the allosteric miransertib (PDB: 5KCV), (C) an Akt1-selective CAAI (PDB: 6S9W), and (D) Akt2 with a VAD-044 analog (PDB: 8Q61) in the allosteric binding site. Also depicted are the crystal structure complexes of Akt1 with ATP-competitive inhibitors (E) capivasertib (PDB: 4GV1) and (F) ipatasertib (PDB: 4EKL). The kinase domain is colored in white and the PH domain in green. In the ATP binding pocket, key structural elements are colored: hinge region in pink, Gly-rich loop in dark cyan, DFG-motif in gray, and α C-helix in orange. Key hydrogen bonds between the inhibitors and the protein are shown by dashed lines.

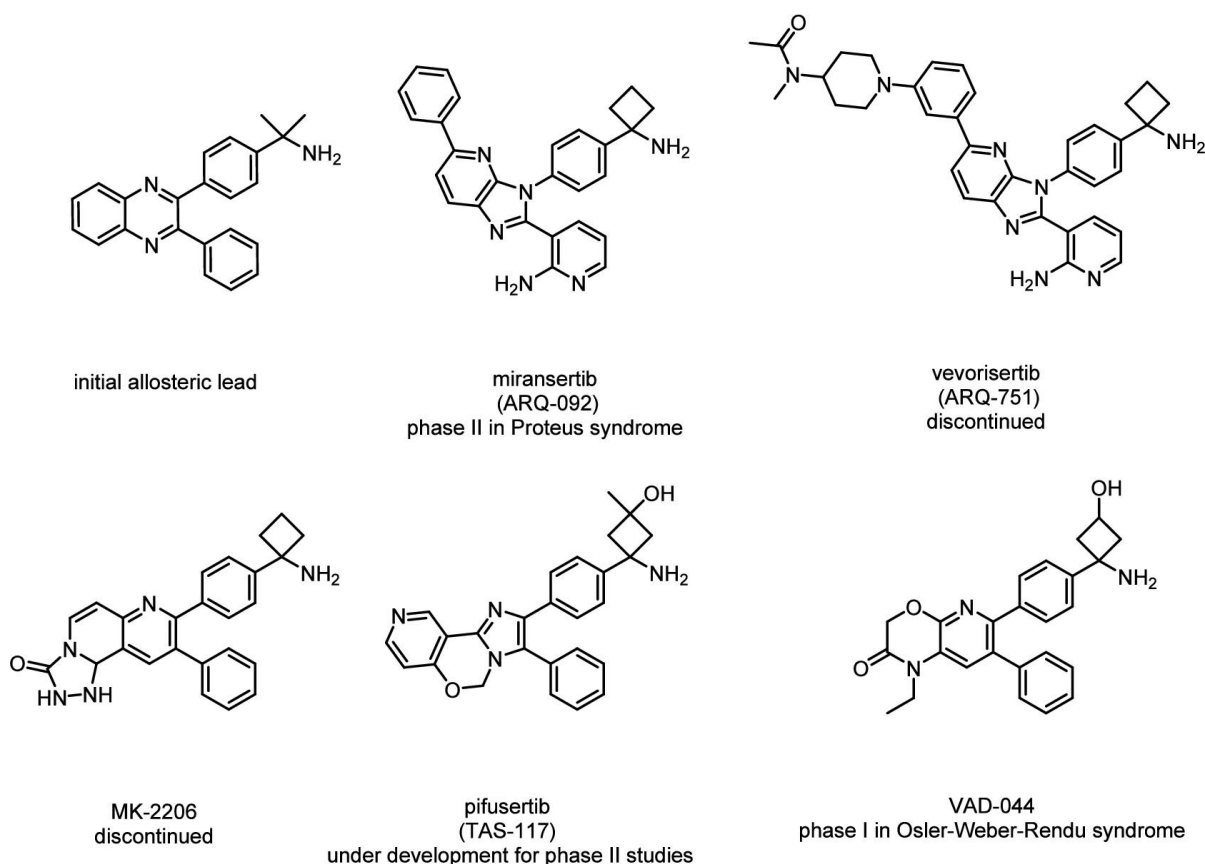
3.5. Allosteric Akt Inhibitors

Inhibition of kinases through ATP competition is the most commonly used MOA, although it suffers from major drawbacks. These shortcomings include limited selectivity due to closely related kinases and, in the case of Akt, difficulty in distinguishing among the three isoforms because of their homologous catalytic domains. Allosteric modulators offer significant advantages over conventional orthosteric ligands. These benefits include enhanced specificity for particular subtypes over closely related proteins and, consequently, reduced off-target related adverse effects. The key to these advantages lies in the unique binding mechanism of allosteric modulators, which target distinct sites in the protein, separate from the active site. In the case of Akt, these inhibitors bind to the interface between the PH domain and the kinase domain, stabilizing the inactive PH-in conformation (Figure 1B). While ATP-competitive inhibitors often act as pan-Akt inhibitors, allosteric inhibitors can be designed to more selectively address the individual isoforms. To fully comprehend the functions of each Akt isoform and their implications in cancer, there is a dedicated effort to identify isoform-selective allosteric Akt inhibitors.^{35,36} Merck (MSD) identified an allosteric Akt inhibitor, specifically a 2,3-diphenylquinoxaline derivative (Chart 2), following a screening initiative. This inhibitor demonstrated selectivity for Akt1 and Akt2 isoforms but also only to Akt when compared to other AGC kinases (PKA, PKC, SGK).³⁷ This compound's discovery was particularly intriguing: it was initially identified as an inhibitor using full-length Akt in high throughput screening. However, a subsequent shift to an experimental approach using a truncated version of Akt revealed that the initial hit was inactive, leading to the realization of its allosteric MOA.^{38,39} Once this unique mechanism was understood, it spurred efforts by other companies in the development of several chemical scaffolds, resulting in potent allosteric Akt inhibitors. Notable among these is Merck's own MK-2206, which along with miransertib and vevorisertib (developed by ArQule and later acquired by Merck) and pifusertib (developed by Taiho Pharmaceuticals) has advanced into clinical trials (Chart 2). Substituting the original Akt allosteric lead's 1,4-quinoxaline core with a naphthyridine scaffold in a tricyclic structure resulted in a substantial improvement in potency and cellular activity (Chart 2).⁴⁰ The derivative MK-2206 is a triazolo[3,4-f][1,6]naphthyridin-3(2H)-one (Chart 2) and serves as an orally active, highly potent, and selective inhibitor of all three Akt isoforms.⁴¹ In Phase I trials, this compound was well tolerated as monotherapy and showed acceptable side effects when combined

with other anticancer agents. However, in numerous Phase II studies, MK-2206 failed to demonstrate significant clinical success, both as a monotherapy and in combination approaches targeting various cancer types,⁴²⁻⁴⁷ so that the development of MK-2206 was also discontinued. During the optimization process of the 3-phenyl-3H-imidazo-[4,5-b]pyridine scaffold, ArQule developed a series of orally bioavailable and highly potent allosteric Akt inhibitors. Among those, miransertib (ARQ092/MK-7075, Figure 2B)⁴⁸ and vevorisertib (ARQ751/MK-4440) (Chart 2) emerged as the most promising compounds, functioning as highly selective Akt inhibitors. Miransertib has shown promising therapeutic results in an 18-year-old male with Proteus syndrome, a rare genetic disorder characterized by overgrowth of various tissues in the body. The syndrome is characterized by tissues and organs that grow at different rates and to different degrees, often resulting in significant physical deformities and complications. The use of miransertib in this case was notable for providing pain relief and slowing the progression of tissue overgrowth, offering a potential new avenue for treating this complex and challenging condition. This successful use of miransertib also highlights the role of Akt signaling in the pathogenesis of Proteus syndrome, suggesting that targeting this pathway may be a key strategy in managing and potentially treating the disease.^{49,50} This has led to an ongoing Phase II trial (NCT04316546) to assess its effectiveness in treating Proteus syndrome. On the other hand, vevorisertib was evaluated in a Phase Ib study involving advanced solid tumors with PIK3CA/Akt/PTEN mutations, either as monotherapy or in combination with paclitaxel or fulvestrant. The trial results indicated a manageable safety profile and minimal to modest antitumor activity of vevorisertib, both alone and in combination with paclitaxel, in patients who had previously received treatment for advanced solid tumors with PIK3CA/Akt/PTEN mutations.⁵¹ Due to business reasons, the development of vevorisertib has been officially terminated (NCT02761694). Pifusertib (TAS-117), developed by Taiho Pharmaceutical in the late 2000s, emerged as a promising allosteric Akt inhibitor with exceptional potency and selectivity against all Akt isoforms (Chart 2). *In vitro* studies demonstrated growth inhibition in various cancer cell lines, including breast, lung, and ovarian. Encouragingly, mouse model data affirmed its dose-dependent antitumor potential.⁵² Human trials revealed its efficacy against solid tumors, particularly in patients with PIK3CA or Akt mutations. Manageable safety has been noted, though maculopapular rash and hyperglycemia were reported. Pifusertib's potential, as illuminated by clinical and preclinical data, makes it a significant contender in cancer

therapy.⁴³ A Phase II trial of pifusertib in advanced solid tumors with PI3K/AKT gene aberration is already completed (NCT03017521), and another Phase II trial in patients with advanced solid tumors harboring germline PTEN mutations was terminated due to an insufficient rate of accrual of patients (NCT04770246). Pifusertib remains under development for Phase II clinical trials in Taiho's pipeline. Using medicinal chemistry and scaffold hopping approaches, VAD-044 (previously known as ALM301, developed by Almac Discovery in 2012) contains a bicyclic pyridine and oxazidone core (Chart 2, Figure 2D). This compound showed potent *in vitro* inhibition of Akt1/2 in the high nanomolar range, exhibited selectivity across the kinome, and demonstrated powerful antiproliferative effects in cancer cell lines. *In vivo* experiments further demonstrated VAD-044's efficacy as a single agent and in combination with tamoxifen in an MCF7 breast cancer xenograft model.⁵³ Based on promising preclinical results, considering the mechanism of action, ADME, and pharmacokinetic features, VAD-044 is currently undergoing Phase I studies to assess its safety and tolerability in patients with hereditary hemorrhagic telangiectasia (HHT), also known as Osler–Weber–Rendu syndrome (NCT05406362).

Chart 2. Chemical Structures of Allosteric Akt Kinase Inhibitors, Screening Lead for the Development of MK-2206, and Compounds That Advanced to Clinical Trials

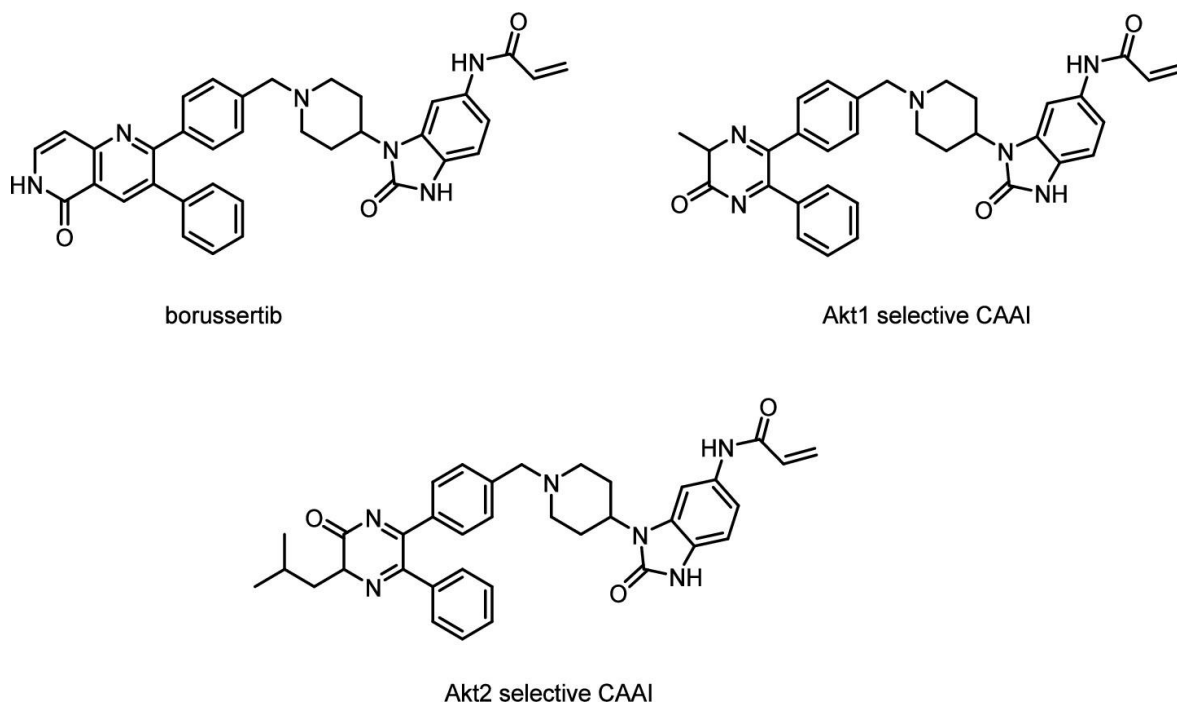


3.6. Covalent-Allosteric Akt Inhibitors (CAAls)

The clinically evaluated and well-described ATP-competitive inhibitors mentioned in this Perspective are pan-Akt inhibitors and lack selectivity for Akt isoforms.³⁹ In the past decade, a promising new class of inhibitors has been introduced to address selectivity challenges, which includes covalent binding and allosteric Akt inhibitors (CAAls).⁵⁴ These CAAls offer a distinct advantage, particularly in the context of Akt's relatively slow turnover rate, typically above 24 h.⁵⁵ This slow protein turnover makes Akt an ideal target for covalent inhibition, as the enduring binding of CAAls can effectively exploit this kinetic feature, potentially resulting in prolonged and more consistent therapeutic effects. Such sustained inhibition aligns well with the need for persistent blockade of Akt signaling in cancer cells, where Akt's role in promoting survival and growth is often upregulated. Unlike traditional noncovalent inhibitors, covalent-allosteric inhibitors offer a novel perspective on targeting key proteins involved in disease-associated pathways. By selectively binding to allosteric sites and forming stable covalent bonds, these inhibitors induce profound conformational changes in their target proteins, leading to the disruption of their function. The unique mechanism of action and potential for enhanced specificity make covalent-allosteric inhibitors an intriguing and dynamic area of research, holding great promise for the development of more effective and durable therapeutic interventions. Indeed, due to their remarkable selectivity, CAAls have drawn attention for their potential application in targeted therapy for patients afflicted with tumors exhibiting aberrantly activated Akt isoforms.⁵⁴ Borussertib is the first-in-class covalent-allosteric inhibitor that has shown superior inhibitory properties compared to reversible ATP-competitive and allosteric Akt inhibitors (Chart 3). It was synthesized following a structure-based design approach based on the 1,6-naphthyridinone core, and it bears a Michael acceptor that targets cysteines at position 296 and 310 in Akt1. Binding to these two noncatalytic cysteines achieves an irreversible stabilization of the inactive conformation of Akt. The crystal structure reveals its precise interactions with key residues, such as Trp80, Tyr272, and Cys296, enabling irreversible stabilization (Figure 2A). Cellular studies demonstrated that borussertib exhibited remarkable antiproliferative activities, with EC₅₀ values in the single-digit nanomolar range, making it up to 43 times more potent than other inhibitors (miransertib, MK-2206, ipatasertib, capivasertib) *in vitro*. Mouse pharmacokinetic studies assessed borussertib administration in various ways (2 mg/kg i.v., 20 mg/kg oral, 20 mg/kg i.p.) and showed it had limited oral bioavailability (<5%) but improved

intraperitoneal absorption (39.6%).⁵⁶ The promising data on CAAs such as borussertib are now the basis for the development of next-generation CAAs with improved pharmacokinetic properties. These efforts are aimed at overcoming the challenges of oral bioavailability while maintaining high potency and selectivity, paving the way for more effective and patient-friendly Akt-targeted therapies in the future.

Chart 3. First-in-Class CAAI, Borussertib,⁵⁴ and Akt1- and Akt2-Selective CAAs³⁶



3.7. Therapeutic Challenges and Future Perspective

While some ATP-competitive and allosteric Akt inhibitors have shown promising results and progressed to clinical trials, only capivasertib has recently become the first to be granted FDA approval, and specifically in combination with other antitumor drugs. The dose-limiting side effects along with the lack of efficacy are the most common challenges for ATP-competitive inhibitors. The ATP-binding site of Akt is highly conserved among the kinome, which leads to selectivity issues. Allosteric Akt inhibitors can contribute to selectivity due to their unique mechanism of binding to Akt's allosteric pocket which is formed at the interface of the kinase domain and the PH domain when Akt is in its inactive, PH-in conformation. Until now, however, no allosteric Akt inhibitor has yet achieved regulatory approval for clinical use. CAAs, with their covalent MOA, are an innovative approach to tackle the afore mentioned selectivity issues. It is worth noting that the distinctive role of each Akt isoform is not completely understood.^{57–59}

Evidence suggests hyperactivation of certain Akt isoforms in particular tumors, indicating Akt isoform-specificity in cell transformation. This specificity may arise from gene-dependent or -independent Akt isoform amplification.⁶⁰ Currently, insights into the functions of each Akt isoform are primarily derived from murine knockout studies.⁶¹ Also, the distinct isoforms of Akt play varying and sometimes contrasting roles in tumorigenesis.^{58,62} However, the development of Akt isoform-specific small molecules (Chart 3, Figure 2C) offers an alternative approach to gain more insights.³⁶ Patient heterogeneity and the diverse responses of tumors further complicate clinical outcomes. Identifying reliable biomarkers to predict responsiveness is essential for effective patient stratification and for tailoring treatments.⁶³ Elevated levels of proteins or variations in their phosphorylation states, whether involving Akt itself or downstream targets, indicate a disruption in the signaling pathway. This disruption is not only attributed to genomic changes in PTEN or PIK3CA genes. Recent evidence highlights the significance of the Akt1^{E17K} mutation as a notable oncogenic site.⁶⁴ But more molecular profiling and genetic studies are needed to find effective biomarkers for different kinds of cancer and for tissue specificity. Amino acid sequence variations among Akt isoforms lead to distinct characteristics in their allosteric binding pockets, despite their generally high homology. Hence, efforts are being made to develop compounds targeting these differences. Reversible and covalent inhibitors with pyridine, pyrazinone, and pyridazine cores have shown selectivity for certain Akt isoforms.^{35–37,65} Covalent modification by these inhibitors can contribute to selectivity and potency toward Akt across the kinome. Isoform selective Akt inhibitors hold significant promise as potential therapeutic agents, providing the opportunity for targeted treatments with enhanced specificity and reduced off-target effects. This progress paves the way for more effective and safer therapies in various diseases associated with Akt dysregulation. Looking ahead, the future of Akt inhibitors in cancer therapy holds promise, especially through combination treatments and the avenue of covalent inhibition. This approach offers superior specificity and potency by forming enduring covalent bonds with Akt kinases, ensuring a prolonged and highly effective suppression of Akt signaling. The development of isoform selective CAAls will also give an insight into the role of each Akt isoform in various cancer types. The sustained action of covalent inhibition may lead to longer-lasting antitumor effects, potentially allowing for reduced dosing frequency and enhancing overall patient compliance. These advancements in targeting Akt through covalent inhibition signify a significant

step forward in the development of more efficacious and individualized treatments for Akt-related diseases.

3.8. Conclusions

The pursuit of Akt inhibitors as potential therapeutic interventions has been motivated by the critical role of Akt in various cellular processes and its implications in diseases like cancer and metabolic disorders. Akt consists of several structural domains, and its dysregulation can lead to disease pathogenesis. Over the years, researchers have developed ATP-competitive and allosteric Akt inhibitors to target this critical kinase. ATP-competitive inhibitors, despite facing challenges, have made progress in clinical trials. Among them, capivasertib, a pan-Akt inhibitor, in combination with fulvestrant, was FDA-approved for the treatment of HR-positive, HER2-negative breast cancer, marking a significant advancement not only in the Akt field but also in precision medicine. Allosteric Akt inhibitors, which offer enhanced specificity, have also made their way into clinical trials, although some have been discontinued due to efficacy concerns. A novel class of covalent-allosteric Akt inhibitors has emerged, offering improved selectivity and durable suppression of Akt signaling. Borussertib has demonstrated remarkable antiproliferative properties, offering a potential breakthrough in targeting Akt isoforms more effectively. The FDA's approval of capivasertib in November 2023 marks a historic milestone in the field of Akt inhibitors, representing a significant breakthrough that sets the pace for future developments. As the first Akt inhibitor to receive regulatory approval, capivasertib not only validates the potential of targeting the Akt pathway but also opens doors to a new era of precision medicine. Furthermore, the identification of Akt isoform-specific inhibitors and reliable biomarkers for patient stratification introduces a personalized approach that could significantly enhance the effectiveness of treatments for Akt-related diseases. The future of Akt inhibitors appears very promising, offering the prospect of tailored and more efficacious therapies in the ongoing battle against diseases associated with Akt dysregulation.

3.9. References

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4. Breaking the Boundaries of Akt Selectivity: Covalent-Allosteric Inhibitors Targeting Akt Isoforms

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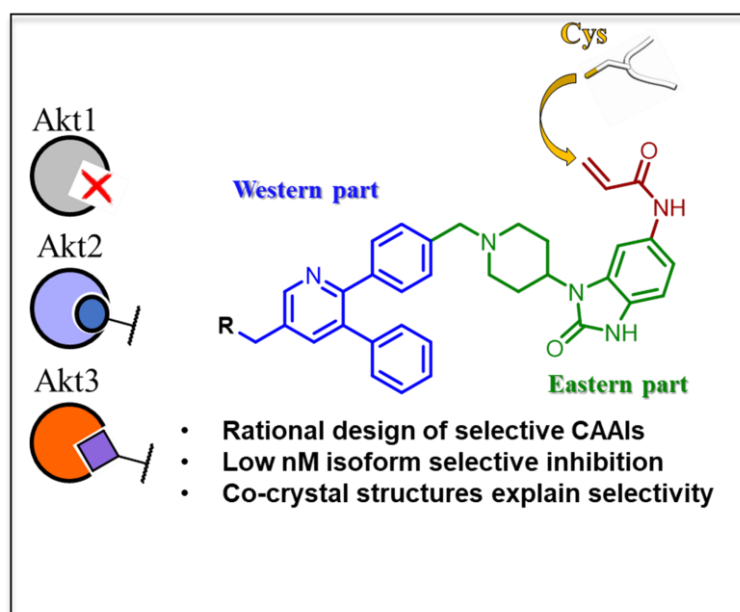
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Graphical Abstract



4.1. Abstract

The Akt family of serine/threonine kinases plays a crucial role in various cellular processes, including proliferation, survival, and metabolism. Three Akt isoforms (Akt1, Akt2, and Akt3) have distinct physiological roles, and while individual isoform dysregulation is disease-linked, unselective Akt inhibition leads to side effects. Here, we report the development of selective covalent-allosteric Akt inhibitors (CAAls) targeting Akt2 and Akt3 while sparing Akt1. Guided by protein X-ray crystallography and molecular modeling, key structural differences within the allosteric pockets of the isoforms were identified and exploited in a structure-based design strategy. By stabilizing the inactive kinase conformation, CAAls overcome the intrinsic selectivity limitations of ATP-competitive inhibitors. After biological characterization, the pyrazole-containing inhibitors emerged as the most potent and selective Akt2 inhibitors, while inhibitors with pyridines as an isoform-selective element were predominantly targeting Akt3 selectively. Importantly, these new inhibitors were also evaluated in patient-derived colorectal cancer organoids. Co-crystal structures of inhibitors bound to an engineered construct mimicking the Akt2 allosteric pocket elucidated the molecular basis of isoform selectivity, guiding further optimization. This work not only establishes a framework for the development of isoform-selective therapeutics but also highlights the potential for unraveling isoform-specific functions in signaling pathways relevant to cancer biology.

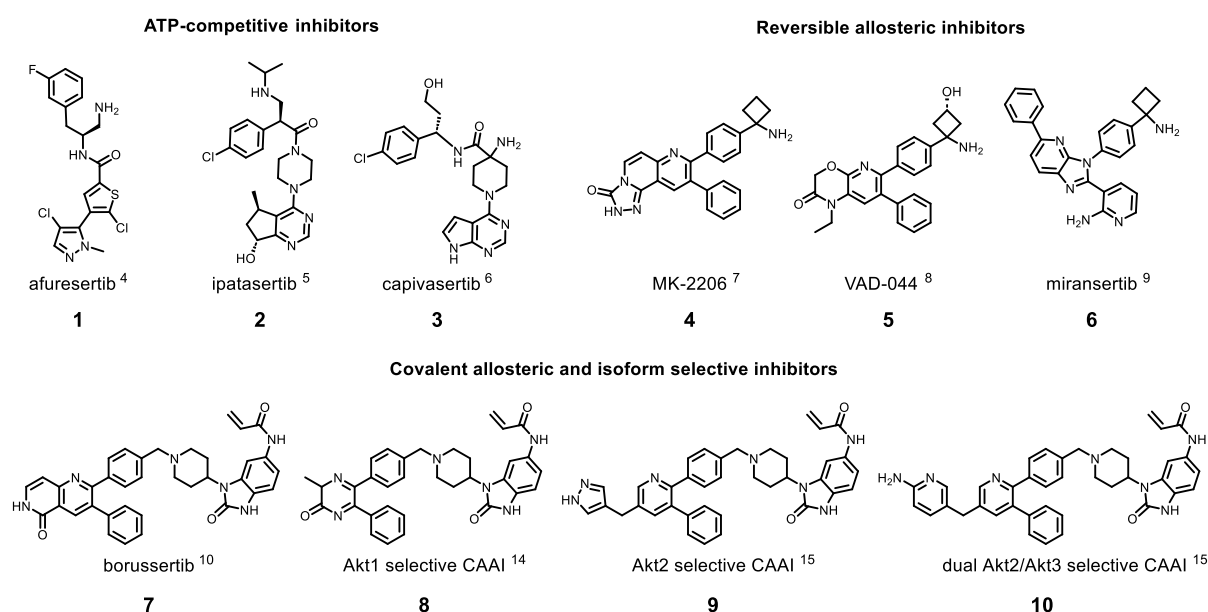
4.2. Introduction

The serine/threonine kinase Akt (protein kinase B, PKB) is a central effector of the PI3K/Akt/mTOR signaling cascade and regulates essential cellular processes, including proliferation, survival, metabolism, and apoptosis.¹ The human Akt family consists of three isoforms, Akt1, Akt2, and Akt3, that, despite sharing high sequence identity, exhibit non-redundant physiological functions and tissue-specific expression patterns.² Knockout studies have demonstrated that Akt1 is primarily involved in cell growth and survival, Akt2 in insulin signaling and glucose homeostasis, and Akt3 in neuronal development.³ These functional distinctions underscore the need for isoform-selective modulation strategies to better interrogate the individual contributions of each isoform to signaling and disease. While ATP-competitive Akt inhibitors have progressed into the clinic, for example, afuresertib (**1**)⁴, ipatasertib (**2**)⁵ and the recent FDA approval of the pan-Akt inhibitor capivasertib (**3**)⁶, their utility is limited by poor

isoform selectivity due to the highly conserved nature of the ATP-binding pocket (Scheme 1). In contrast, allosteric inhibitors engage regulatory sites outside the active site and stabilize Akt in an inactive conformation, thereby offering enhanced selectivity potential. These inhibitors bind to a pocket formed at the interface between the N-terminal pleckstrin homology (PH) domain and the kinase domain, reinforcing the autoinhibited, closed conformation of Akt. Several allosteric Akt inhibitors have entered clinical trials, including MK-2206 (**4**)⁷, VAD-004 (Engasertib, previously known as ALM301 **5**)^[8], miransertib (previously known as ARQ092, **6**)⁹, but none have yet achieved clinical approval, often due to suboptimal potency, cellular permeability, or context-dependent activity (Scheme 1).⁶

Covalent-allosteric Akt inhibitors (CAAls) represent a promising alternative, combining selective allosteric engagement with sustained target occupancy through covalent bond formation to one of the two conserved cysteines within the allosteric binding pocket. Borussertib (**7**) was the first in class CAAI that achieved very potent and selective inhibition of the Akt isoforms. This dual mechanism increases selectivity, potency, and target residence time.^[10] Covalent inhibitors have shown clinical success in the field of oncology, such as the approval of afatinib and osimertinib for EGFR-driven non-small cell lung cancer (NSCLC)^{11, 12} and ibrutinib for mantle cell lymphoma and chronic lymphocytic leukemia.¹³ Recent studies have shown that subtle structural differences within the allosteric pockets of Akt isoforms can be exploited to achieve differential binding profiles in biochemical and engineered cellular models. The methyl pyrazinone **8**¹⁴ showed Akt1 selectivity, while the pyridine-based pyrazole **9**¹⁵ and the amino-pyridine **10**¹⁵ showed Akt2 and dual Akt2/3 inhibition, respectively. These findings suggest that isoform-selective covalent modulation is feasible, although challenges remain in translating these principles into robust and broadly applicable chemical tools. Akt isoform-selective inhibitors represent a future opportunity, as they could provide a means to unravel the unique contributions of each isoform to cellular signaling and disease progression, while at the same time laying the groundwork for more refined therapeutic strategies. In this way, an isoform-targeted approach has the potential to retain the proven clinical efficacy of existing orthosteric and allosteric Akt inhibitors, while mitigating the adverse effects often associated with broad pan-Akt inhibition.¹⁶ Here, we report a structure-guided strategy for the development of isoform-selective covalent-allosteric Akt inhibitors. By exploiting isoform-specific features within the allosteric pocket, we achieved potent engagement of Akt2 or Akt3 while sparing

Akt1. Structural insights from protein X-ray crystallography of an engineered Akt1 variant, designed to mimic the allosteric pocket of Akt2, elucidate the molecular basis of selectivity. Isoform-selective engagement was further validated in cells and organoids using western blot analysis of phospho-Akt levels, protein mass spectrometry, and NanoBRET-based target engagement assays. Together, these findings establish a blueprint for covalent-allosteric modulation of Akt isoforms and pave the way toward selective chemical tools to interrogate isoform-specific signaling in complex biological systems.



Scheme 1: Different categories of Akt inhibitors, including ATP-competitive, reversible allosteric, covalent-allosteric and isoform-selective covalent-allosteric inhibitors (Akt1-, Akt2- and dual Akt2/Akt3 selective CAAls), highlighting distinct mechanisms of inhibition.

4.3. Results and Discussion

4.3.1. Structure-Guided Design and Synthesis of Isoform-Selective Covalent Inhibitors

Despite the high sequence conservation among the Akt isoforms, our structure-guided design approach took advantage of small but functionally significant structural differences within the allosteric binding pocket to achieve isoform selectivity, locking Akt in an inactive conformation (Figure 1A).¹⁷ While the allosteric sites of Akt1, Akt2, and Akt3 appear highly conserved at the sequence level, co-crystal structures and molecular modeling revealed distinct spatial differences. A key feature of Akt2 and Akt3 is the absence of a glutamate residue found in Akt1, influencing the local electrostatic

environment, as well as the deletion of one amino acid, which results in a truncated adjacent helix as observed in Figure 1C and 1D.¹⁷ This shortening expands the allosteric pocket, creating a wider and deeper binding cavity that accommodates ligands in a more extended conformation. In contrast, the presence of the glutamate in Akt1 maintains the full length of the helix, which protrudes further into the binding pocket, reducing its size and limiting the accommodation of bulkier substituents. As a result, ligands that should bind efficiently to Akt2 and Akt3 might experience steric clashes in Akt1. These structural differences are further modulated by key isoform-specific residue variations within the binding pocket. Specifically, Lys268 in Akt1, Arg269 in Akt2, and Gly265 in Akt3, which alter the local hydrogen bonding landscape and electrostatic environment (Figure 1E). Specifically, in Akt3, the presence of Ile276 instead of the smaller Val residue found in Akt1 and Akt2 appears to introduce a steric hindrance that interferes with the binding of compounds bearing bicyclic fused ring systems in their core scaffold, especially those designed to interact with Trp80. This larger residue seems to hinder the binding of such compounds to the binding site and likely explains their reduced potency in Akt3 compared to the other isoforms.¹⁸

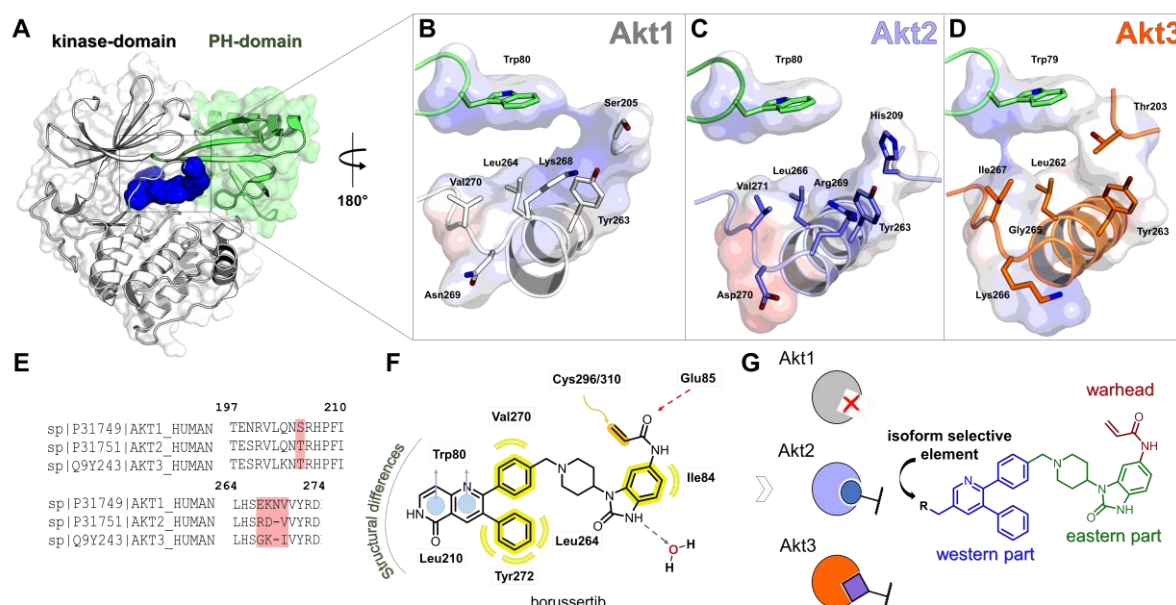
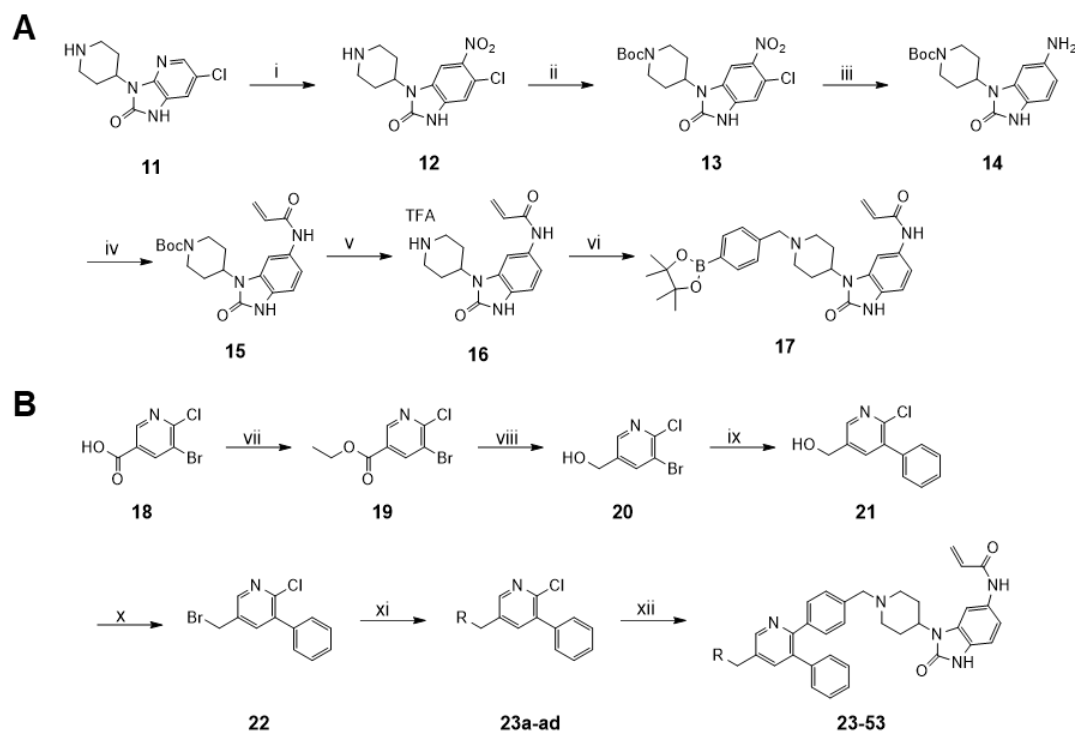


Figure 1. Structural overview of Akt isoforms, allosteric inhibition, and design strategy. **(A)** Inactive conformation of Akt1 bound to borussertib **7** (PDB: 6HHF); kinase domain in white, PH domain in green, ligand in blue, stabilizing the PH-in state. **(B-D)** Allosteric pockets of Akt1 (**B**, white, PDB: 6HHF), Akt2 (**C**, blue, PDB: 8Q61), and Akt3 (**D**, orange, SWISS-MODEL), with PH domains in green. Key amino acid differences affecting pocket size and electrostatics are labeled. **(E)** Sequence alignment showing allosteric site differences among Akt isoforms with a focus on the allosteric pocket. **(F)** Schematic binding mode of borussertib (PDB: 6HHF), showing π - π stacking (turquoise), hydrophobic interactions (yellow), hydrogen bonds (green/red), and nucleophilic attack site by Cys296/Cys310 (orange). **(G)** Design concept of new inhibitors, indicating retained features from borussertib and isoform-selective modifications. Western, eastern, and covalent warhead regions are highlighted in blue, green, and red, respectively.

To take advantage of these structural differences, we designed a new series of covalent-allosteric inhibitors that combine the selective isoform targeting through different chemical derivatives with the covalent modification of two conserved cysteine residues within the allosteric site.¹⁹

Unlike the surrounding regions that vary between isoforms, these cysteines are located in a more conserved area close to the ligand in the allosteric pocket, providing a stable anchor point for covalent binding while allowing selectivity to be tuned through interactions with the more variable regions nearby. Building on known allosteric scaffolds, we employed a pyridine-based core designed to maintain the π - π stacking interaction with Trp80, inspired by borussertib **7** (Figure 1F), with strategic modifications at the preferred 5'-position, as mentioned in previous studies, to fine-tune isoform selectivity based on steric compatibility.¹⁵ Substituents were linked to the

central pyridine via a methylene group, chosen for its favorable orientation within the expanded Akt2/3 allosteric pockets. The new inhibitors incorporated heteroaromatic rings of varying sizes, systematically decorated with substituents to probe isoform-specific binding and identify favorable interactions with key pocket residues.



Scheme 2: General synthesis of covalent-allosteric Akt inhibitors (CAAls): (A) Synthesis of the eastern part building block **17**: *i*) HNO₃, *o*-xylene, 60 °C, 2 h, 99 %, *ii*) di-*tert*-butyldicarbonate, TEA, DCM, rt, 2 h, 63%, *iii*) Pd/C, NH₄HCO₂, MeOH, 90 °C, 24 h, 85 %, *iv*) prop-2-enoylchloride, DIPEA, THF, rt, 16 h, 87 %, *v*) TFA/DCM (3:1), rt, 2 h, 99 %, *vi*) 4-Bromomethylphenylboronic acid pinacol ester, K₂CO₃, DMF, rt, 1 h, 81 %. (B) Synthesis of western part building blocks **23a-ad** and the final compounds **24-53**: *vii*) Ethyl iodide, K₂CO₃, DMF, 78 %, *viii*) DIBAL, DCM, rt, 2 h, 55 %, *ix*) phenyl boronic acid, 1,4-dioxane/H₂O (5:1), 70 °C, 16 h, 80 %, *x*) PBr₃, 1,4-dioxane, rt, 2 h, 84 %, *xi*) arylboronic acid/pinacol ester, K₃PO₄, Pd(dppf)Cl₂, 1,4-dioxane/H₂O, 100 °C, 1 h, 48-75 %. *xii*) **17**, K₂CO₃, Pd(PPh₃)₄, 1,4-dioxane/H₂O (5:1), 130 °C, 2 h, 10-45 %.

To support the structure-guided structure-activity relationship (SAR) study, a modular synthetic route was followed to access a library of covalent Akt inhibitors. The trisubstituted pyridine core, previously associated with isoform-selective binding, was equipped with a benzimidazolone linker on the eastern part for attachment of the electrophilic warhead (Figure 1G). The synthesis begins with nitration and Boc-protection of benzimidazolone **11**, followed by reduction of the nitro group and acylation with acryloyl chloride to introduce the covalent handle in compound **15**.¹⁵ After

deprotection and a nucleophilic substitution of compound **16** with 4-bromomethylphenylboronic acid pinacol ester, the boronic ester **17** was obtained as the final intermediate of the eastern part. In parallel, the dihalogenated nicotinic acid derivative **18** was esterified and then reduced to the corresponding alcohol **20**. After a Suzuki cross-coupling, a phenyl group was introduced to the 3-position of the pyridine scaffold (compound **21**) and the alcohol was converted to the corresponding bromide **22** with a Wolf-Ziegler bromination. This served as an important intermediate that was used for the introduction of the different residues via another Suzuki coupling to afford the substituted intermediates **23a-ad**. After sequential microwave-assisted Suzuki couplings, systematic variation of the 5'-position was achieved to further lead to the final target compounds (**24-53**). This route enabled efficient exploration of pocket-specific steric constraints and warhead positioning across Akt isoforms. The chemical structures of the intermediates **23a-ad** are reported in Supplementary Information.

4.3.2. Isoform Profiling of Novel Akt Inhibitors by Live-Cell NanoBRET Target Engagement Assay, Proliferation Assays and Protein-MS experiments

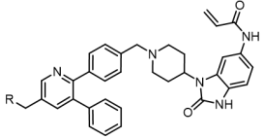
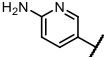
To evaluate the potency and isoform selectivity of our Akt inhibitors, we determined EC₅₀ values against full-length, active Akt1, Akt2, and Akt3 using the NanoBRET assay in HEK293 cells. The position and length of substituents on the pyrazole and pyridine cores, that were used as isoform-selective elements, significantly influenced the compounds' selectivity profiles. To extend these findings to functional cellular outcomes, we evaluated compound efficacy with the CellTiter-Glo viability assay in the ZR-75-1 breast cancer cell line, a luminal B subtype known to be highly dependent on Akt1 signaling for proliferation and survival. ZR-75-1 cells harbor wild-type PI3K and PTEN, but exhibit elevated basal Akt1 activity, making them a well-established model for probing Akt1-driven oncogenic signaling.²⁰ Importantly, Akt2 and Akt3 are expressed at significantly lower levels in this line and contribute minimally to the survival phenotype.²⁰ Since the synthesized compounds of this study were designed to target Akt2 and Akt3 and therefore should not target Akt1, this cell line serves as a negative control to further validate the selectivity of these compounds over Akt1. To establish a reference framework for the NanoBRET assay, we profiled clinically advanced Akt inhibitors. The ATP-competitive inhibitors afuresertib (**1**), ipatasertib (**2**), and capivasertib (**3**) showed comparable activity across Akt1-3, with a modest

preference for Akt3. In contrast, the allosteric inhibitors MK-2206 (**4**), VAD-044 (**5**), and miransertib (**6**) preferentially engaged Akt1 and Akt2, with reduced Akt3 activity. To our knowledge, NanoBRET target-engagement data for these compounds have not been reported previously, making them a useful benchmark for evaluating our covalent-allosteric inhibitors. In ZR-75-1 cells, the pan-Akt inhibitors miransertib (**6**) and borussertib (**7**) showed potent antiproliferative activity (EC_{50} = 2 nM and 22 nM), consistent with strong Akt1 inhibition and its dominant role in cell survival. In contrast, capivasertib (**3**) exhibited reduced potency (EC_{50} = 175 nM), reflecting its ATP-competitive binding mode. Accordingly, additional orthosteric inhibitors showed only micromolar NanoBRET potency, markedly weaker than the nanomolar activity consistently observed for allosteric and covalent-allosteric inhibitors. Reference compounds **9** and **10**, previously characterized biochemically, were also evaluated in the NanoBRET assay. In this cellular context, compound **9** showed comparable potency toward Akt2 and Akt3, whereas compound **10** was more potent against Akt3 than Akt2. Both compounds exhibited reduced engagement of Akt1 relative to the other isoforms, indicating that in a cellular setting, structural features and substituent properties influence cellular uptake and, consequently, potency. We next evaluated our first set of designed CAAs. Concerning the NanoBRET evaluation, within the pyrazole series (**24-37**), the inhibitors exhibited pronounced Akt2/Akt3 selectivity, achieving low nanomolar potency and their activity toward Akt1 varied substantially (100-1000 nM) depending on the nature and position of the pyrazole substituent. Building on compound **9**, which previously showed the highest Akt2 activity and selectivity in biochemical assays, we synthesized a first set of 1,4- and 1,5-substituted pyrazoles with varied N-substitution patterns to further probe the structure-activity relationships.¹⁵ Within the 1,4-substituted series, compounds **24** and **25** showed equipotent Akt2/Akt3 activity with reduced Akt1 engagement.

N-ethyl substitution (**26**) increased Akt3 potency but did not improve Akt1 selectivity, whereas compounds **27** and **28** displayed potent dual Akt2/Akt3 inhibition with strongly diminished Akt1 activity. Notably, compound **28** achieved 34-fold Akt1 selectivity. Increasing steric bulk at the N-position, as in the benzyl analogue **29**, reduced Akt2/3 potency, while the N-trifluoromethyl compound **30** maintained balanced Akt2/3 activity with moderate Akt1 selectivity. In the 1,5-substituted series, compound **31** already favored Akt2/3 over Akt1 and methyl substitutions in **32** and **33** further enhanced potency while retaining selectivity. N-substitution studies identified compound **34** as a

potent Akt2/3 inhibitor with an EC₅₀ around 25-30 nM, with X-ray analysis revealing opportunities for further pocket engagement. Introduction of bulkier N-substituents (**35-37**) improved even further the Akt2 potency while maintaining Akt2/3 selectivity, with reduced Akt1 activity attributed to steric exclusion. Consistent with NanoBRET data, pyrazole compounds exhibited low cellular potency in the Akt1-dependent ZR-75-1 line, with an EC₅₀ greater than 0.5 micromolar in most cases, and compounds **27** and **28** being the least active.

Table 1: Cellular evaluation of synthesized covalent-allosteric Akt inhibitors with NanoBRET assay in the HEK293 cell line and with a CTG assay in the ZR-75-1. The mean EC₅₀ values $\pm$ s.d. were calculated from experimental points measured in duplicates for each plate and were replicated in n = 3 biologically independent experiments.

		Biological evaluation EC ₅₀ (nM)			
		NanoBRET HEK293			CTG ZR-75-1
#	R	Akt1	Akt2	Akt3	
1	afuresertib	1151 $\pm$ 38	3430 $\pm$ 233	865 $\pm$ 323	n.d.
2	ipatasertib	3145 $\pm$ 265	7488 $\pm$ 859	1024 $\pm$ 201	n.d.
3	capivasertib	10557 $\pm$ 2561	10569 $\pm$ 1928	2381 $\pm$ 461	175 $\pm$ 65
4	MK-2206	26 $\pm$ 9	33 $\pm$ 14	88 $\pm$ 44	n.d.
5	VAD-044	169 $\pm$ 83	41 $\pm$ 13	877 $\pm$ 373	n.d.
6	miransertib	2 $\pm$ 1	8 $\pm$ 7	31 $\pm$ 22	21 $\pm$ 5
7	borussertib	22 $\pm$ 7	48 $\pm$ 20	53 $\pm$ 5	2 $\pm$ 1
9		140 $\pm$ 30	41 $\pm$ 13	53 $\pm$ 9	182 $\pm$ 42
10		520 $\pm$ 31	108 $\pm$ 46	37 $\pm$ 11	417 $\pm$ 49
1st set					
24		199 $\pm$ 33	39 $\pm$ 20	37 $\pm$ 16	770 $\pm$ 180
25		435 $\pm$ 98	57 $\pm$ 29	52 $\pm$ 34	566 $\pm$ 134
26		107 $\pm$ 54	43 $\pm$ 18	17 $\pm$ 8	360 $\pm$ 50
27		697 $\pm$ 75	37 $\pm$ 20	29 $\pm$ 4	2833 $\pm$ 760
28		1048 $\pm$ 18	30 $\pm$ 10	30 $\pm$ 23	3757 $\pm$ 1202
29		1078 $\pm$ 206	289 $\pm$ 199	245 $\pm$ 310	1007 $\pm$ 218
30		290 $\pm$ 52	49 $\pm$ 7	54 $\pm$ 6	336 $\pm$ 38
31		494 $\pm$ 332	118 $\pm$ 82	64 $\pm$ 24	592 $\pm$ 138
32		474 $\pm$ 206	82 $\pm$ 74	46 $\pm$ 20	553 $\pm$ 115
33		322 $\pm$ 100	45 $\pm$ 29	41 $\pm$ 3	1103 $\pm$ 319

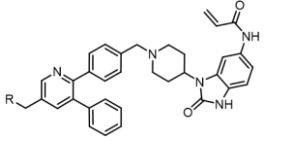
34		308 ± 39	29 ± 15	23 ± 8	988 ± 304
2nd set					
35		260 ± 50	18 ± 7	21 ± 3	211 ± 58
36		215 ± 12	11 ± 9	34 ± 31	223 ± 30
37		575 ± 289	31 ± 8	21 ± 2	127 ± 26

n.d.: not determined

The previously reported aminopyridine compound **10** exhibited dual preference for Akt2 and Akt3 in biochemical assays.¹⁵ To exploit the larger Akt3 allosteric pocket, a series of six-membered pyridine analogs was designed to increase steric bulk relative to pyrazoles. In the NanoBRET assay, all pyridine derivatives showed markedly reduced Akt1 potency, consistent with steric constraints imposed by the smaller Akt1 pocket. Accordingly, these Akt2/3-selective compounds exhibited weak activity in the CTG assay, with most EC₅₀ values in the micromolar range, underscoring the limited impact of Akt2/3 inhibition in an Akt1-dependent cell line. Inspired by the structure of compound **10**, a bioisosteric alcohol group was incorporated into the selective scaffold, yielding compound **38**, which demonstrated a clear preference for Akt3 over both Akt1 and Akt2. Replacing the alcohol with a methyl group in compound **39** markedly increased NanoBRET potency across all isoforms, yielding an EC₅₀ of 16 nM for Akt3 with strong selectivity (26-fold over Akt1, 10-fold over Akt2). This prompted further evaluation of hydrophobic substitutions on the pyridine core. Different substitution patterns revealed that the 2,3-substituted derivative **40** shifted selectivity toward Akt2 over Akt3, while remaining weak against Akt1. In contrast, the 3,4-substituted analogue **41** showed equipotent Akt2/Akt3 inhibition with around 30-fold selectivity over Akt1, supporting the 2,5-substitution pattern as optimal for selective Akt3 targeting. Incorporation of a -CF₃ group (**42**) maintained Akt3 selectivity (48-fold over Akt1, 11-fold over Akt2), while introduction of a -CF₂ group (**43**) increased potency across all isoforms, achieving single-digit nanomolar activity against Akt3. Comparatively, its ethyl analogue compound **44** exhibited better potency in Akt1 than the fluorinated substituents, while retaining comparable activity for Akt2 and Akt3 as compound **43**. Compound **45** showed good Akt3 potency with marked selectivity over the other isoforms.

To further probe spatial effects in the Akt3 allosteric site, compounds **46** and **47** were synthesized by combining the aminopyridine motif of compound **10** with additional methyl substitutions. Whereas compound **46** retained high Akt3 potency and selectivity, compound **47** increased Akt2 potency 3-fold, resulting in loss of Akt3 selectivity, highlighting the importance of substitution pattern and spatial orientation for isoform discrimination. Following the biological evaluation of the pyridine series, a second set of analogs was designed. To explore the effect of increased bulk, an indole was incorporated in compound **48** and its methylated analogue **49**. Both compounds displayed pronounced selectivity for Akt3, with 50-fold and 68-fold selectivity over Akt1, respectively. The methylated derivative compound **49** was also 2-fold less potent in Akt2 compared to its non-methylated counterpart, further enhancing its Akt3 selectivity. Inspired by compounds **45** and **49**, compound **50** was synthesized bearing a dimethylamino substituent. This compound exhibited an EC₅₀ in the micromolar range towards Akt1 while exhibiting single digit nanomolar EC₅₀ towards Akt3, making it the most potent and selective Akt3 inhibitor in this series with a selectivity of 288-fold for Akt1 and around 15-fold for Akt2. To probe the role of pyridine in isoform selectivity, pyrimidine analogs with favored substituents were synthesized. Compounds **51** and **52** inhibited Akt1 and Akt2 equally, with **52** showing distinct Akt3 potency. Introducing a dimethyl-amino group in **53** favored Akt3, but overall Akt2/3 potency was lower than the dimethyl-amino-pyridine **50**, indicating that the pyridine scaffold better supports high-affinity Akt2/3 binding. The evaluation of the pyridine series in the CTG assay in the ZR-75-1 cell line resulted in a decreased cellular potency, which is consistent with their design, which prioritized selectivity for Akt2 and Akt3 over Akt1, and it is further supported by the NanoBRET assay, indicating reduced potency towards Akt1 relative to the other isoforms. The indole-containing compounds **48** and **49** also displayed low activity in the ZR-75-1 cell line. The methylated analogue, compound **49**, was the least potent, with an EC₅₀ exceeding 1 μ M, consistent with its designed selectivity for Akt3 and reduced affinity for Akt1.

Table 2: Cellular evaluation of synthesized covalent-allosteric Akt inhibitors with NanoBRET assay in the HEK293 cell line and with a CTG assay in the ZR-75-1. The mean EC₅₀ values $\pm$ s.d. were calculated from experimental points measured in duplicates for each plate and were replicated in n = 3 biologically independent experiments.

 # R		Biological evaluation EC ₅₀ (nM)			
		NanoBRET HEK293			CTG ZR-75-1
		Akt1	Akt2	Akt3	
1st set					
38		2914 $\pm$ 428	406 $\pm$ 194	124 $\pm$ 106	10517 $\pm$ 2031
39		419 $\pm$ 282	44 $\pm$ 27	16 $\pm$ 5	795 $\pm$ 163
40		325 $\pm$ 71	24 $\pm$ 16	50 $\pm$ 7	1291 $\pm$ 201
41		602 $\pm$ 211	24 $\pm$ 10	21 $\pm$ 10	2385 $\pm$ 652
42		3012 $\pm$ 442	266 $\pm$ 113	63 $\pm$ 47	3834 $\pm$ 501
43		534 $\pm$ 102	67 $\pm$ 7	9 $\pm$ 4	413 $\pm$ 49
44		370 $\pm$ 37	92 $\pm$ 33	6 $\pm$ 5	384 $\pm$ 72
45		711 $\pm$ 439	59 $\pm$ 19	19 $\pm$ 2	467 $\pm$ 99
46		472 $\pm$ 128	67 $\pm$ 10	20 $\pm$ 2	545 $\pm$ 91
47		324 $\pm$ 89	21 $\pm$ 4	20 $\pm$ 13	532 $\pm$ 99
2nd set					
48		1846 $\pm$ 703	67 $\pm$ 4	37 $\pm$ 12	608 $\pm$ 189
49		1954 $\pm$ 1234	117 $\pm$ 57	29 $\pm$ 7	1317 $\pm$ 550
50		1153 $\pm$ 675	60 $\pm$ 18	4 $\pm$ 2	571 $\pm$ 174
51		240 $\pm$ 78	57 $\pm$ 20	44 $\pm$ 5	229 $\pm$ 53
52		284 $\pm$ 62	45 $\pm$ 22	9 $\pm$ 5	195 $\pm$ 20
53		1366 $\pm$ 750	211 $\pm$ 116	44 $\pm$ 29	1422 $\pm$ 203

Protein mass spectrometry was employed as an orthogonal approach to confirm covalent target engagement. Following treatment with our CAAls, all three Akt isoforms exhibited distinct mass shifts relative to the apo proteins, consistent with single-site covalent modification by the ligands (Figure 2). Compounds **27**, **28**, **45**, **49** and **50** did not exhibit any labeling of Akt1 after 3 hours of incubation, suggesting a preference for modification of Akt2 and Akt3 over Akt1. Among the tested compounds, **35** and **36** showed faster covalent labeling in Akt1, consistent with their higher activity observed in the NanoBRET assay. The results from the covalent labeling studies provide additional evidence for isoform-selective binding by these compounds and are consistent with the cellular NanoBRET findings, further supporting the Akt isoform selectivity of these new compounds.

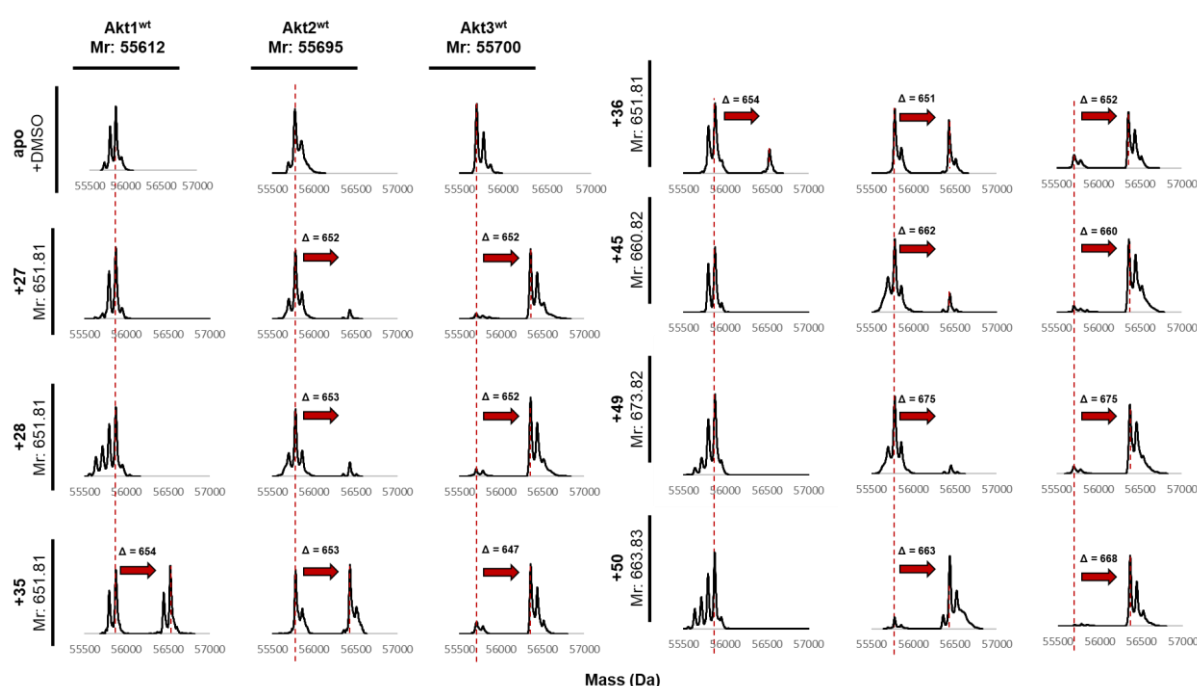


Figure 2: Deconvoluted mass spectra in Akt1^{wt}, Akt2^{wt} and Akt3^{wt} following treatment with DMSO (apo form) or of the newly synthesized isoform-selective CAAls. Under denaturing conditions, all compounds induced mass shifts corresponding to the addition of a single ligand per protein molecule, indicative of mono-covalent modification after 3h of incubation. The extent of labeling correlated with the compounds' cellular potency toward the respective Akt isoform.

To further validate isoform selectivity, Western blot analyses were conducted in PANC-1 cells, which overexpress Akt2, allowing direct assessment of compound effects on Akt2 phosphorylation. Compounds **35**, **36**, and **50**, each of which demonstrated high potency and selectivity in the NanoBRET assay were chosen for this analysis. The compounds induced a concentration-dependent decrease in phosphorylated Akt2 (pAkt2) levels. No substantial change was detected at 10 nM, whereas a clear reduction was observed at 50 nM. Treatment at 250 nM resulted in an almost complete loss of the pAkt2 signal, indicating robust inhibition (Figure S71). In contrast, phosphorylation of Akt1 remained largely unaffected, even at higher compound concentrations (> 6 μ M). Notably, compound **50**, which demonstrated selectivity against Akt3 in the NanoBRET assay, did not induce a significant reduction in pAkt2 except at concentrations exceeding 6 μ M. These findings are consistent with NanoBRET and MS data, further confirming the compounds' selectivity for Akt2 at the protein level. Altogether, these results validate the isoform-selectivity of our covalent inhibitors at both biochemical and functional levels. While mass spectrometry confirms that covalent labeling can occur across all three Akt isoforms, only selective cellular engagement translates into biological efficacy. This emphasizes the importance of coupling biochemical profiling with orthogonal functional assays to accurately assess inhibitor selectivity. Our data support the utility of these inhibitors as precise tools for dissecting isoform-specific signaling pathways and as starting points for the development of isoform-targeted therapies that avoid the liabilities of pan-Akt inhibition. Interestingly, despite robust biochemical inhibition, the compounds had a limited effect on cell viability in PANC-1 cells, with anti-proliferative effects observed only at micromolar concentrations. This likely reflects the intrinsic low dependence of PANC-1 cells on PI3K/Akt signaling for survival rather than insufficient target engagement, as this model was selected primarily for its high Akt2 expression rather than pathway dependence. Consequently, Akt2 inhibition alone is not expected to result in potent cytotoxicity in this context, underscoring that combination strategies or more Akt-dependent models may be required to unlock the full therapeutic potential of isoform-selective inhibition.

4.3.3. Selective Akt Inhibitors Exhibit Potent Activity in Tumor-Derived Organoids

While SAR studies using NanoBRET and CTG assays provide detailed insight into the binding affinity and cytotoxic effects of selective Akt inhibitors in 2D cell cultures, these simplified systems often fail to recapitulate the complex architecture and microenvironmental cues that shape drug responses in tumors. Patient-derived organoids preserve key aspects of cellular organization, lineage heterogeneity, and signaling context, providing a more physiologically relevant framework for assessing inhibitor efficacy and selectivity.²¹ Despite the central role of Akt signaling in cancer biology, selective Akt inhibition has not been systematically explored in organoid models. Motivated by our SAR findings and the limitations of conventional 2D assays, we evaluated our lead covalent-allosteric Akt inhibitors across a panel of tumor-derived organoids from primary colorectal cancers²². We initially performed dose-titration experiments using the reference compounds capivasertib and miransertib (Figure S72). 3 of 4 organoid lines studied showed resistance to capivasertib (IC_{50} values of over 100 μ M), while all lines showed sensitivity to miransertib (IC_{50} values between 0.1 and 4 μ M), indicating limited potency and selectivity, respectively. In contrast, our covalent-allosteric Akt inhibitors displayed differential potency among the organoid lines, likely reflecting intrinsic differences in Akt isoform dependence, feedback regulation, and genetic background among the organoid lines: in O05 and O18, IC_{50} values of allosteric Akt inhibitors were over 5 μ M, while in O23 and O28, the majority of compounds showed IC_{50} values below 5 μ M (Figure S72). This improvement arises from newly established structure-activity relationships and the optimized chemical features of our covalent-allosteric scaffold, which together enable sustained target occupancy and fine-tuned isoform engagement. These next-generation inhibitors thereby overcome the response variability characteristic of earlier Akt inhibitors, delivering robust and durable pathway inhibition across diverse organoid models.

4.3.4. Structural Insights into Isoform Selectivity via Engineered Akt1-Mutant

To understand the origins of isoform selectivity observed with our covalent inhibitors, we employed an engineered Akt1-mutant construct carrying Akt2-specific mutations (S205T, D262E, E267del, K268R, and N269D). This approach introduces key features of the Akt2 allosteric site into the structurally tractable Akt1 scaffold, enabling direct structural analysis of the Akt2 binding environment. Such mimicry strategies have been successfully applied in other systems to study proteins that resist crystallization and provided a suitable framework for probing isoform-specific interactions in our case.²³ In Akt1, the spatial restriction not only limits the conformational freedom of the ligand but also precludes the formation of stabilizing interactions observed in Akt2. Superimposition of ligand-bound Akt2 models with Akt1 further confirmed that Akt2-selective inhibitors would not be accommodated without significant structural rearrangement (Figure S81). This steric incompatibility explains both the lower labeling efficiency observed by intact protein MS and the poor cellular engagement in Akt1. Within the binding site, the pyridine core of the compounds is oriented to engage in π - π stacking interactions with Trp80, while the phenyl groups contribute to hydrophobic contacts with Leu210, Leu264, and Ile290. The benzimidazolone linker interacts with structured water molecules within the pocket and bridges the pyridine main core to Cys296, facilitating covalent bond formation. Because these inhibitors were designed to exploit isoform-specific differences in the western region of the allosteric site, the binding modes of the most potent Akt2-selective compounds were examined using the mutant construct. Crystal structures revealed that the pyrazole core fits well within the allosteric pocket, with substituents occupying defined regions of the isoform-specific sub-pocket depending on their size and orientation. In addition, selected pyridine inhibitors with strong Akt2 activity were co-crystallized with the Akt1 mutant, providing insight into how their bulkier substituents are accommodated within the allosteric site.

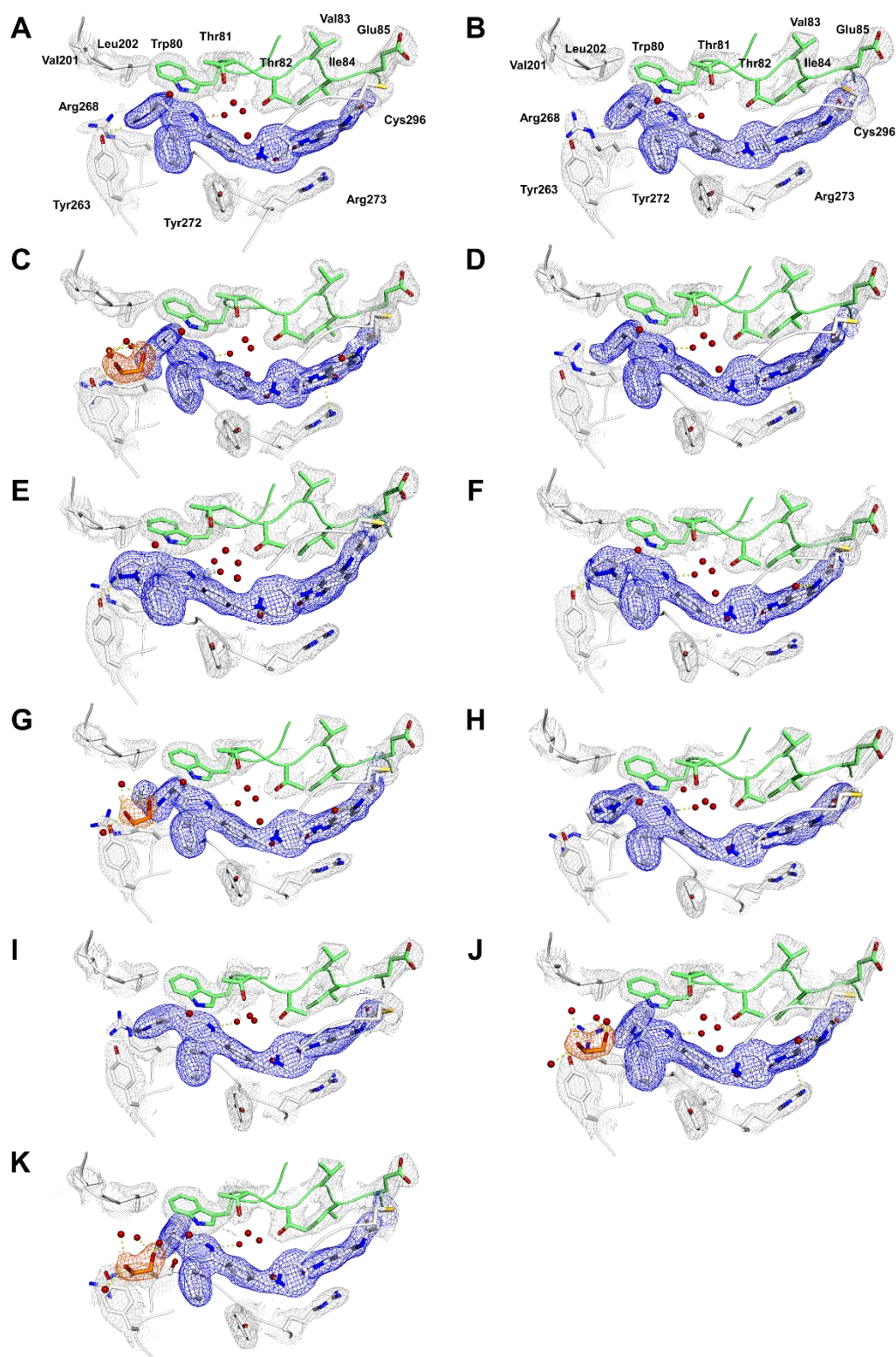


Figure 3: Co-crystal structures of Akt1 mutant with: (A) compound **25** (PDB: 9TMT), (B) compound **27** with EG (PDB: 9TMU), compound **28** with EG (C, PDB: 9TMV) and without (D, PDB: 9TMW), (E) compound **34** (PDB: 9TMY), (F) compound **35** (PDB: 9TMZ), (G) compound **36** (PDB: 9TN0), (H) compound **37** (PDB: 9TN2), (I) compound **40** without EG (PDB: 9TN3), (J) compound **40** with EG (PDB: 9TN1) and (K) compound **47** (PDB: 9TN4). The amino acid labels shown in panels A and B are not shown in panels C-K for clarity. The $|2F_o - F_c|$ -electron density map is shown at r.m.s.d. = 1.0.

The co-crystal structures obtained with selected inhibitors (Figures 3 and S72) show that the compounds adopt a conformation within the allosteric pocket that positions them for covalent bond formation with Cys296, while simultaneously orienting the isoform-selective substituents toward the region where the Akt isoforms diverge. The methylene linker between the core scaffold and the isoform-selective substituent plays a crucial role in directing the substituent into the region of the pocket where amino acid differences among Akt isoforms are located. For most compounds of the pyrazole series that demonstrated potent Akt2 inhibition in cellular assays, a consistent hydrogen bond was observed between one of the pyrazole nitrogens and the hydroxyl group of Tyr263, supporting binding affinity (Figure 4). However, both the pyrazole and pyridines that were crystallized also interacted with the sidechain of Arg268. Some of these crystals were harvested and flash-cooled both in the presence and absence of ethylene glycol (EG), used as a cryoprotectant. Interestingly, when EG was present as cryo-protectant, it could also be observed in the binding site near the ligand and the isoform-selective substituent adopted an alternative orientation compared to structures where EG is not present, likely due to rearrangements in the local water network within the allosteric site. Figure 4L shows an overlay of important residues within the vicinity of the selectivity-determining region. Together, these observations underscore the influence of the binding-site environment on the orientation of isoform-selective substituents and their interactions with neighboring residues. Furthermore, the distinct orientations observed with and without EG provide useful insights for the future optimization of this compound series.

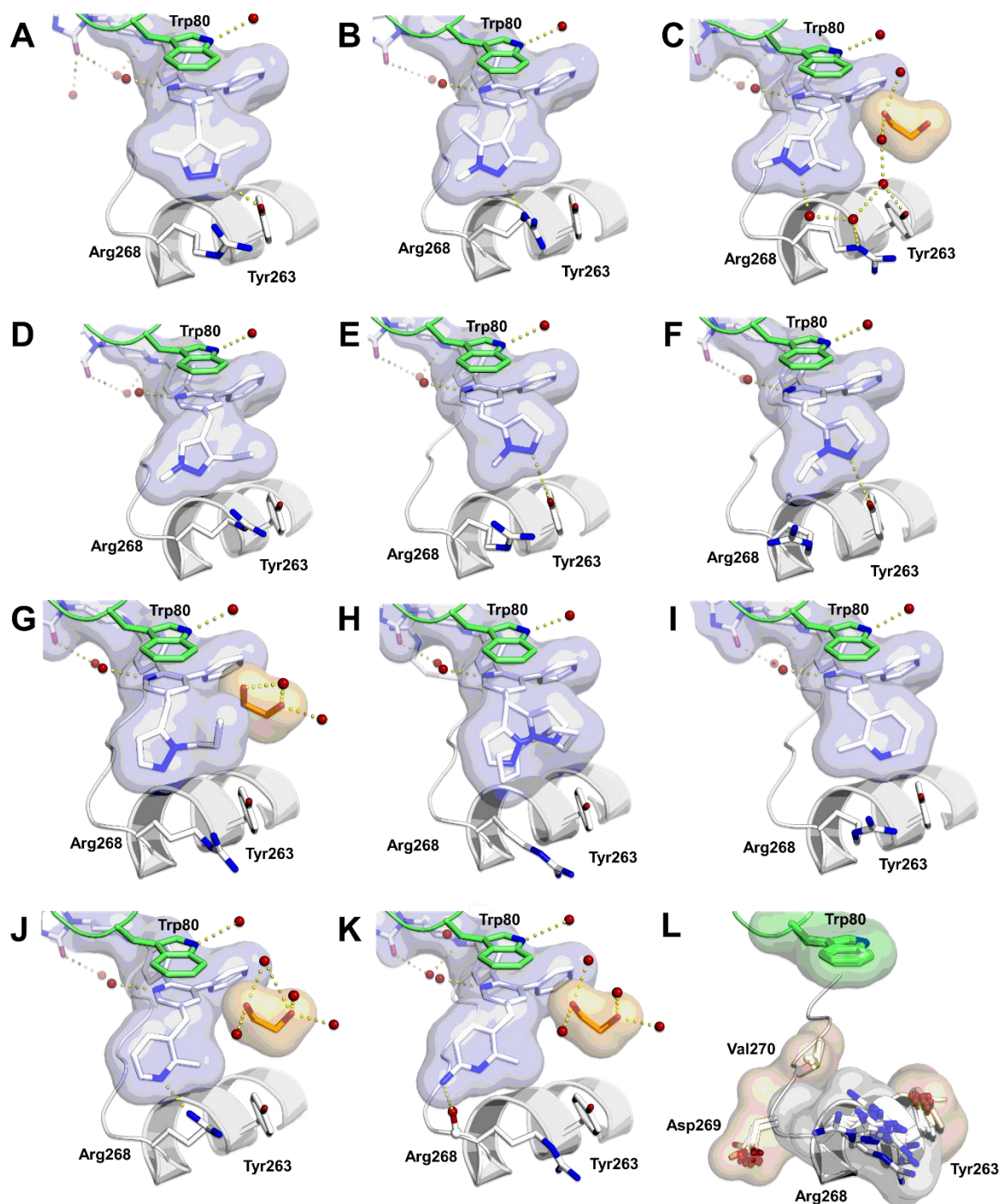


Figure 4: Close-up views of the isoform-selective residues highlighting the orientation of the pyrazole and pyridine motifs within the allosteric pocket of the Akt1 mutant, their key interactions with surrounding amino acids, and the conformations adopted by their substituents. Shown are co-crystal structures of the Akt1 mutant with: (A) compound **25** (PDB: 9TMT), (B) compound **27** with EG (PDB: 9TMU), compound **28** with EG (C, PDB: 9TMV) and without (D, PDB: 9TMW), (E) compound **34** (PDB: 9TMY), (F) compound **35** (PDB: 9TMZ), (G) compound **36** (PDB: 9TN0), (H) compound **37** (PDB: 9TN2), (I) compound **40** without EG (PDB: 9TN3), (J) compound **40** with EG (PDB: 9TN1) and (K) compound **47** (PDB: 9TN4). (L) Overlay of all crystal structures (A-K), revealing greater arginine flexibility relative to nearby residues.

One notable structural difference that sets Akt2 apart from the other isoforms is the presence of Arg268, where Akt1 and Akt3 instead feature a lysine and a glycine, respectively. Interestingly, in most of the crystal structures obtained with the Akt1 mutant containing the Akt2-specific pocket, Arg268 does not appear to form direct interactions with the inhibitors. Compound **27** forms a hydrogen bond with the NE nitrogen of Arg268 (Figure 4B) while compound **28** interacts with Arg268 through a water network in the presence of EG (Figure 4C). Pyrazoles **34** and **35** instead interact with Tyr263. In the case of compound **47** with the presence of EG, a hydrogen bond is observed between the pyridine nitrogen and the guanidine group of Arg268 (Figure 4J), while in the amino-pyridine **47**, a hydrogen bond is observed between the amine of the ligand and the carbonyl group of the backbone (Figure 4K). In the case of compound **28**, the presence of EG only affected the terminal carbon of the ethyl substituent on the pyrazole ring (Figure 4C, 4D), whereas in compound **47**, the residue was flipped (Figure 4I, 4J), leading to a markedly different orientation of the substituted pyridine within the pocket. For pyrazoles bearing longer substituents, such as compounds **28**, **36**, and **37**, no hydrogen bond with Tyr263 was observed (Figure 4C, 4G, 4H), suggesting steric accommodation overrides this interaction. In particular, compound **37** displayed electron density consistent with a dual conformation of the cyclobutyl-pyrazole moiety, occupying substantially more space than the other analogs. Where the electron density permitted modeling of the side chain, the arginine adopted multiple conformations, indicating substantial flexibility in this region of the pocket, as further supported by the elevated B factors of the corresponding atoms. While Arg268 interacts with only some of the inhibitors, its exclusive occurrence in Akt2 still represents a promising element to target in future efforts toward enhancing isoform selectivity. Despite this flexibility, Arg268 can form interactions with the isoform-selective residues either directly, as seen with the NE nitrogen, the guanidine group of the side chain, or its backbone (Figure 4B, 4J, 4K), or indirectly through a water-mediated network (Figure 4C). Targeting Arg268 specifically could help push isoform selectivity even further by taking advantage of an amino acid that is absent in the corresponding region of Akt1 and Akt3. Also, the flexibility of the linker allows the residues and their substitutions to adopt different conformations according to their environment, such as the presence or absence of EG, as observed in Figure 4C, 4D, 4I, 4J, to form favorable interactions. Taken together, these findings illustrate that ligand selectivity arises not from direct interactions with unique residues per se, but from a

spatial opportunity that only Akt2 presents, an opportunity that our inhibitors are specifically designed to exploit. This highlights the potential of targeting subtle conformational or volumetric differences in homologous protein families to achieve isoform selectivity, even in highly conserved pockets.

4.4. Conclusion

This study outlines a structure-based strategy for the design of isoform-selective covalent-allosteric Akt inhibitors. By exploiting subtle sequence and structural differences within the allosteric pockets of the three Akt isoforms, we established a clear SAR that translate from molecular design to cellular target engagement. NanoBRET and CTG profiling revealed distinct potency patterns driven by isoform-selective substitutions, with most compounds potently inhibiting Akt2 and Akt3 while sparing Akt1. In the five-membered ring series, 1,5-substituted pyrazoles enhanced Akt2 potency and selectivity over Akt1, whereas substituted pyridines and aza-indoles preferentially targeted Akt3. This demonstrates that isoform bias can be rationally tuned through scaffold and substituent selection even in the absence of Akt3 crystal structures. Insights from crystal structures of an engineered Akt1 construct mimicking the Akt2 allosteric pocket clarified the structural basis of Akt2 selectivity and provided a framework for further optimization of isoform-directed interactions. These compounds also demonstrated activity in patient-derived organoid models with varying Akt isoform expression, highlighting their utility as chemical probes to dissect isoform-specific signaling dependencies in more physiologically relevant systems. Although the compounds bound Akt2 at nanomolar levels in NanoBRET assays, Akt2-overexpressing PANC-1 cells showed limited sensitivity, with viability effects observed only at micromolar concentrations. This likely reflects the low intrinsic dependence of PANC-1 cells on PI3K/Akt signaling rather than insufficient target inhibition, as this model served primarily as a high-Akt2 expression system rather than a pathway-addicted context. These findings suggest that isoform-selective Akt inhibition alone may not be sufficient to achieve potent antiproliferative effects in certain tumor settings and instead may be most effective in rational combination strategies. This concept is consistent with clinical experience, such as the approval of the pan-Akt inhibitor capivasertib in combination with fulvestrant. Taken together, our results provide a blueprint for the design of isoform-selective Akt inhibitors and support their future use

both as precision chemical biology tools and as components of combination-based therapeutic approaches.

4.5. References

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4.6. Supporting information

4.6.1. Editorial Assistance

Language-editing and AI tools were used to improve grammar, phrasing, clarity and readability during the preparation of this manuscript.

4.6.2. Akt3 Homology Model

The homology model for Akt3 was generated with the SWISS-MODEL homology modelling server based on an Akt1 full-length co-crystal structure (PDB: 6HHJ).

4.6.3. Cell Culture

HEK239 and PANC1 cell lines were cultured in DMEM medium GlutaMAX (Gibco), supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin/streptomycin (Gibco). Cells were cultured in a humidified incubator at 37 °C, 5% CO₂. Cells were split upon reaching approximately 80% confluency and were trypsinized for 5 min prior to passaging using 0.25% Trypsin-EDTA (Gibco).

4.6.3.1. Cell Viability Assay

Cell Viability analysis was conducted using CellTiter-Glo assay (Promega). ZR-75-1 cells were plated on day 0 into white 384-well cell culture plates (Greiner Bio-One) using a Multidrop reagent dispenser (Thermo) at cell numbers that ensure linear and optimal luminescent signal intensity (400 cells/well). Then the cells were incubated for 24 h in a humidified atmosphere at 37 °C/5% CO₂. The cells were treated with inhibitors in serial dilutions ranging from 30 µM down to 0.1 nM using an Echo 650 acoustic liquid handler (Beckman Coulter GmbH). Cell viability was measured on day 5 and luminescence signal was recorded by an EnVision Multilabel 2104 Plate Reader (PerkinElmer) with 500 ms integration time. All data were normalized to the plate positive control (30 µM staurosporine) and negative control (DMSO). Finally, the data was processed with the Quattro Software Suite using a four-parameter logistic model. As quality control, the Z'-factor was calculated from 16 positive and negative control values. Only assay results showing a Z'-factor ≥ 0.5 were used for further analysis. All experimental points were measured in duplicates for each plate and were replicated three times.

4.6.3.2. Cellular Characterization of the Inhibitors Using NanoBRET Target Engagement

For NanoBRET experiments, cells were transiently transfected in suspension using a lipid-based transfection reagent together with plasmid DNA encoding the respective NanoLuc-Akt constructs (Akt1-NanoLuc (NV2411), Akt2-NanoLuc (NV1031), or Akt3-NanoLuc) (Promega). The transfection mixture was prepared in OPTIMEM reduced-serum medium (Gibco) at defined reagent-to-DNA ratios of 30:1 and incubated for 20 minutes at room temperature to allow complex formation. The complexes were then combined with a cell suspension adjusted to a density of approximately 2×10^5 cells/mL. Following transfection, the cell suspension was dispensed into 384-well plates (Greiner Bio-One), with each well containing a final volume of 25 μ L and approximately 5,000 cells using a Multidrop reagent dispenser (Thermo). After 24 hours (day 1), cells were incubated with the test inhibitors across a concentration range spanning 20 μ M to 1.2 nM, together with 0.5 μ M NanoBRET tracer K10 (Promega). After 3 h incubation, the NanoGlo substrate and the extracellular inhibitor (N2162, Promega) were prepared in OPTIMEM to generate the working detection solution. This mixture was then dispensed onto the assay plates (final addition corresponding to 10 μ L per well). Following addition of the detection solution, NanoBRET signals were recorded on the EnVision plate reader using the dual-emission configuration specific for NanoBRET assays, collecting donor emission in the ~450 nm range and acceptor emission in the ~600 nm range. Following acquisition, all data were normalized to the plate positive control (30 μ M staurosporine) and negative control (DMSO). Raw donor and acceptor emissions used to calculate the BRET ratio, expressed as the acceptor signal divided by the donor signal. For inhibitor titrations, BRET ratios were normalized to vehicle-treated controls to obtain fractional occupancy or tracer displacement values. Dose-response curves were generated by plotting the normalized BRET signal against inhibitor concentration, and data were fitted using a four-parameter logistic model in Origin (OriginLab) to derive potency values. All experimental points were measured in duplicates for each plate and were replicated three times.

4.6.3.3. Western Blot Analysis.

PANC-1 cells were seeded in 6-well plates at a density of 5×10^5 cells per well (day 0). On the following day (day 1), cells were treated with increasing concentrations of inhibitors or with DMSO as vehicle control. After 4 h incubation, cells were washed

twice with ice-cold PBS and lysed by addition of 100 μ L lysis buffer (PBS containing 1% Triton X-100) supplemented with protease and phosphatase inhibitor cocktails (Roche). Lysates were incubated on ice for 20 min, after which cells were scraped, collected into microcentrifuge tubes, and centrifuged for 20 min to remove insoluble material. The resulting supernatants were collected, and protein concentrations were determined using a BCA protein assay. Equal amounts of protein (10 μ g per sample, adjusted to 1 mg/mL) were resolved by SDS-PAGE and subsequently transferred onto Immobilon-FL PVDF membranes (Merck Millipore) using Pierce™ 1-Step Transfer Buffer and the Pierce™ Power Blotter system (Thermo Fisher Scientific). Following transfer, membranes were washed for 5 min with ddH₂O, then 5 min in TBS-T (T (50 mM Tris, 150 mM NaCl, 0.05% Tween-20, pH 7.4)) and then blocked for 1 h at room temperature using Odyssey® Blocking Buffer TBS (LI-COR). Membranes were then incubated overnight at 4 °C with primary antibodies diluted in TBS-T with gentle agitation. The next day, membranes were washed three times for 5 min with TBS-T and incubated with the appropriate secondary antibodies diluted in TBS-T for 1 h at room temperature with gentle agitation. After three additional washes with TBS-T, membranes were scanned using an Odyssey® CLx infrared imaging system (LI-COR).

4.6.4. Protein Expression, Purification and Crystallization

Synthetic genes encoding Akt1 (2-480,) Akt2 (2-481), Akt3 (2-479) and Akt1 mutant (S205T, D262E, E267del, K268R, and N269D) were designed to include an N-terminal hexahistidine tag followed by a TEV protease cleavage site and were obtained from GeneArt (Regensburg, Germany). The constructs were subcloned into the pLex/Bac3 expression vector (Merck Millipore) using the NcoI and BamHI restriction sites. Recombinant baculoviruses were generated and amplified in *Spodoptera frugiperda* (Sf9) insect cells (Thermo Fisher Scientific) according to the BacMagic expression system (Merck Millipore). Protein expression was performed in suspension cultures grown in Erlenmeyer flasks at 27 °C with agitation (120 rpm) for 72 h following infection. Cells were collected by centrifugation (3,000 $\times$ g, 15 min), washed once with PBS, and flash-frozen in liquid nitrogen. Frozen cell pellets were thawed and resuspended in lysis buffer containing 50 mM Tris, 500 mM NaCl, 1 mM DTT, 10 % glycerol, 0.1 % Triton X-100 (pH 8.0) supplemented with an EDTA-free protease inhibitor cocktail (Sigma). Cell disruption was achieved using a microfluidizer, and insoluble material was removed by high-speed centrifugation (40,000 $\times$ g for 1 h).

The clarified lysate was applied to a Ni-NTA Superflow affinity column (Qiagen), and bound proteins were eluted using an imidazole-containing buffer (50 mM Tris, 500 mM NaCl, 500 mM imidazole, 1 mM DTT, 10 % glycerol, pH 8.0). To remove the His₆ tag, TEV protease was added to the pooled elution fractions, followed by overnight dialysis at 4 °C against buffer containing 25 mM Tris, 50 mM NaCl, 1 mM DTT, and 5 % glycerol (pH 8.0). The cleaved proteins were further purified by size-exclusion chromatography on a Superdex 75 column (GE Healthcare) equilibrated in 50 mM HEPES, 200 mM NaCl, 1 mM DTT, 10 % glycerol (pH 7.3). For long-term storage, purified proteins were exchanged into storage buffer (25 mM Tris, 100 mM NaCl, 1 mM DTT, 10 % glycerol, pH 7.5) using a Superdex 75 10/300 GL column, concentrated as required, and stored at -80 °C. For crystallization, purified protein at a concentration of 3 mg/mL was incubated with 3 eq. of every inhibitor on ice for 180 min. The samples were centrifuged at 20,000 x g for 10 min at 4°C before hanging drops were prepared in 15-well crystallization plates (EasyXtal Tool, Qiagen) by mixing protein-ligand complex with reservoir solution containing 5-8 % PEG 1500, 0.1 M MMT pH 5.0-5.3 and seed stock solution (1:1:0.3). Seed stocks were generated from crystals obtained in the presence of inhibitor **27** using crystallization conditions consisting of 10 % PEG 1500 and 0.1 M MMT buffer at pH 5.3. For compound **28**, **34**, **35**, **36**, **37**, **40**, **47**, crystals were cryoprotected using 20 % ethylene glycol before they were flash cooled in liquid nitrogen. X-ray diffraction data were collected at The European Synchrotron Radiation Facility (ESRF, Grenoble, France, proposal MX2580, DOI 10.15151/ESRF-ES-1580701169, DOI 10.15151/ESRF-ES-1581727707, DOI 10.15151/ESRF-ES-1899699669, DOI 10.15151/ESRF-ES-1899511612, DOI 10.15151/ESRF-ES-1975525258, and proposal MX-2681, DOI 10.15151/ESRF-ES-2025026666 and DOI 10.15151/ESRF-ES-2025026699) The diffraction data were integrated with XDS and scaled using the program XSCALE. The crystal structure was solved by molecular replacement with PHASER using PDB 7NH4. The manual modification of the molecule of the asymmetric unit was performed using the program COOT.¹ For multiple cycles of refinement PHENIX.refine² was employed and the final structure was evaluated using MolProbity and the PDB validation server. Crystal structures were visualized using PyMOL³

4.6.5. Protein-Mass Spectroscopy

Akt1, Akt2, Akt3 wild-type protein solution was diluted with MS buffer (50 mM HEPES, 200 mM NaCl, 2 % glycerol, pH 7.3) to a final concentration of 0.5 mg/mL. Subsequently, each sample was incubated with three molar equivalents of compound. The mixtures were incubated at 4 °C for up to 24 hours to allow complex formation. Following incubation, the samples were centrifuged for 10 minutes at 21,000 × g and 4 °C to remove potential aggregates or precipitates. The samples were analyzed by mass spectrometry using a Thermo Fisher Scientific Ultimate 3000 HPLC system connected with a Thermo Fisher Scientific Velos Pro (2D ion trap). The sample (5 µL) was injected onto an AdvanceBio Desalting-RP Cartridge (Agilent Technologies) starting at 80 % solvent A (0.1 % formic acid in water) and 20 % solvent B (0.1 % formic acid in acetonitrile) for 30 seconds, followed by a linear gradient over 30 seconds up to 90 % solvent B and an isocratic elution for 4.5 minutes. A mass range of 700-2000 m/z was scanned and raw data were deconvoluted and analyzed using XCALIBUR and MAGTRAN software to obtain the molecular weight profiles of the protein-ligand complexes. The analysis of the masses was conducted through extraction of the mass list from MAGTRAN and analysis in OriginPro 2023 software.

4.6.6. Chemistry

All reagents and solvents were used as received from various suppliers such as abcr, Activate Scientific, Alfa Aesar, BLDpharm, Merck, Sigma-Aldrich, or VWR manufacturer for the synthesis and used without further purification. Dry solvents were purchased as anhydrous reagents from commercial suppliers. Air- and/or humidity-sensitive reactions were performed in heated glassware and under inert gas, using argon atmosphere. Reactions were monitored by thin-layer chromatography (TLC) and liquid chromatography-mass spectrometry (LC-MS). Analytical TLC was performed on Merck 60 F254 aluminum-backed silica gel plates and visualized under UV light (λ = 254 and 366 nm). Compounds were purified by column chromatography using VWR silica gel (40 - 63 µm particle size) or flash chromatography on a Biotage Isolera One using Büchi Reveleris Silica Cartridges (12 - 40 g) monitored by UV at λ = 210 nm and 280 nm. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance AV500 (500 MHz and 126 MHz), AV600 (600 MHz and 151 MHz) and AV700 (700 MHz and 176 MHz). ^1H chemical shifts are reported in λ (ppm) as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), dt (doublet of triplets), td (triplet of doublets), tt (triplet of triplets), q

(quartet), hept (heptet), m (multiplet) and are referenced to the residual solvent signal: DMSO-*d*₆ (2.50). ¹³C spectra are referenced to residual solvent signal: DMSO-*d*₆ (39.52). High-resolution electrospray ionization mass spectra (ESI-FTMS) were recorded on a Thermo LTQ Orbitrap (high-resolution mass spectrometer from Thermo Electron) coupled to an Accela HPLC system supplied with a Hypersil GOLD column (Thermo Electron). LC-MS (ESI-MS) analysis was performed using Agilent HPLC system (1200 series) with CC 125/4 Nucleodur C18 gravity column (3 μm) from Macherey Nagel coupled to an AGILENT G6160A InfinityLab iQ LC/MSD Single Pole Mass Spectrometer. Compounds were purified by flash chromatography on a Biotage Isolera One Silica Cartridges (12 - 40 g) monitored by UV at λ = 210 nm and 254 nm. All final compounds were purified to > 95 % purity as determined by LC-MS and NMR analysis.

4.6.7. Organoid Culture and Organoid Drug Treatments

Resection samples from patients with colorectal cancer were provided by the University Cancer Center Frankfurt (UCT). All materials were collected as part of the interdisciplinary Biobank and Database Frankfurt (iBDF), with prior written informed consent from patients. The study received approval from the institutional review board of the UCT and the Ethical Committee at the University Hospital Frankfurt (ethics vote: 4/09; project number: SGI-10-2025). Patient-derived organoids were cultured as described (PMID: 37489084). Organoids were individually transduced with a Luciferase2-P2A-EGFP lentivirus, as described by (PMID: 31036555), and stable expression was selected by expansion in the presence of 1 μg/mL puromycin. For drug testing, organoids were dissociated enzymatically using StemPro Accutase (cat: A1110501, Thermo Fisher Scientific), filtered (40 μm, cat: 542040, Greiner), embedded in 15 μL of 70% Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract (BME, Bio-Techne, cat: BME001-05), and seeded using a MANTIS Liquid Dispenser (Formulatrix) in 96-well round-bottom plates (cat: 83.3925.500, Sarstedt) in duplicate plates. In each well 100 μL of full culture medium was added, and the plates were sealed with Breath-Easy membranes (cat: Z380059, Sigma-Aldrich). On day 3, the medium was changed to reduced medium containing advanced DMEM/F12 with 10 mM HEPES, 1× GlutaMAX and 1× penicillin/streptomycin (all from Thermo Fisher Scientific), using an automatic multichannel pipette (MINI 96, INTEGRA Biosciences), and the drugs were dispensed in triplicate wells using a D300e digital dispenser

(Tecan) in 7-point dilutions: DMSO (control), 0.00192 μM , 0.0096 μM , 0.048 μM , 0.24 μM , 1.2 μM , 6 μM and 30 μM . The DMSO concentration was normalized in all wells, ensuring it did not exceed 1% of the final volume. The cells were treated for six days in total, with repeated treatment after three days. Morphological images of the entire 96-well plate were captured with a Cytation C10 confocal imaging reader (Agilent), and cell viability was assessed using the ONE-Glo EX Luciferase Assay (cat: E8120, Promega) according to the manufacturer's instructions. Luminescence was measured with a SpectraMax iD3 Microplate Reader (Molecular Devices) and the raw data was normalized to DMSO control. Viability curves and IC_{50} values were calculated in GraphPad Prism 10 following log10 transformation of X values and nonlinear fit ([Inhibitor] vs. normalized response - variable slope).

4.6.8. Data Availability

The obtained structures of Akt1 in complex with some of the newly synthesized inhibitors have been deposited in the Protein Data Bank under PDB-ID 9TMT for **25**, PDB-ID 9TMU for **27**, PDB-ID 9TMV and 9TMW for **28**, PDB-ID 9TMY for **34**, PDB-ID 9TMZ for **35**, PDB-ID 9TN0 for **36**, PDB-ID 9TN2 for **37**, PDB-ID 9TN3 and 9TN1 for **40**, PDB-ID 9TN4 for **47**.

4.6.9. Results and Discussion

4.6.9.1. Synthetic Procedures and Compound Characterization:

General procedure A: Benzylic Suzuki cross-coupling reaction

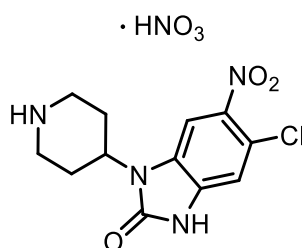
In a microwave vessel under argon atmosphere, the boronic acid/ester (1.0 eq.) and the bromo-pyridine (1.2 eq.) were dissolved in a mixture of 1,4-dioxane/ H_2O (5:1). The reaction mixture was degassed for 10 minutes, after which K_3PO_4 (2.0 eq.) and $\text{Pd}(\text{dppf})\text{Cl}_2$ (0.1 eq.) were added sequentially. The suspension was stirred under microwave irradiation at 100 $^\circ\text{C}$ for 1 hour. After completion, the mixture was filtered over Celite and extracted with DCM. The combined organic layers were washed with saturated NaCl solution, dried over Na_2SO_4 , and concentrated under reduced pressure. The pure product was obtained after silica gel column chromatography (1-4% MeOH/DCM + 1% NH_3).

General procedure B: Synthesis of final products via Suzuki cross-coupling reaction

In a microwave vessel under argon atmosphere, the pyridine-derivative (1.0 eq.) and compound **17** (1.1 or 1.2 eq.) were dissolved in a mixture of 1,4-dioxane/ H_2O (5:1).

The reaction mixture was degassed for 10 minutes, after which K_2CO_3 (2.0 eq.) and $Pd(PPh_3)_4$ (0.1 eq.) were added sequentially. The suspension was stirred under microwave irradiation at 130 °C for 2 hours. Upon completion of the reaction, the mixture was filtered over Celite and extracted with DCM. The combined organic fractions were washed with saturated NaCl solution, dried over Na_2SO_4 and concentrated in vacuo. The pure product was obtained after silica gel column chromatography (6-10% MeOH/DCM + 1% NH_3) followed by a reverse phase column chromatography (20-25% AcCN/ H_2O + 1% TFA).

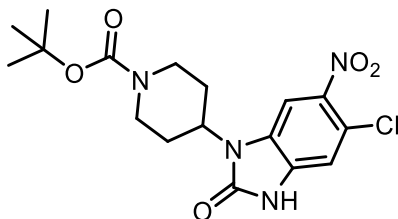
Synthesis of 5-chloro-6-nitro-1-(piperidin-4-yl)-1,3-dihydro-2Hbenzo[d]imidazol-2-one (12)



Compound **11** (3.0 g, 11.9 mmol, 1.0 eq.) was suspended in 100 mL o-xylene and heated up at 60°C. Then, concentrated nitric acid (3.2 mL, 71.5 mmol, 6 eq.) was added dropwise and the reaction mixture was heated for 1 h. After let to cool down, the reaction mixture was filtered under vacuum and the residual crude product was washed with MeOH, yielded the title compound **12** as pale-yellow solid (3.30 g, 11.15 mmol, 95%).

1H NMR (600 MHz, $DMSO-d_6$) δ = 11.75 (s, 1H), 8.57 (d, J =10.1, 1H), 8.39 - 8.30 (m, 1H), 8.06 (s, 1H), 7.27 (s, 1H), 4.58 (tt, J =12.3, 4.1, 1H), 3.45 (d, J =12.3, 2H), 3.07 (q, J =11.3, 2H), 1.92 (d, J =11.7, 2H). **^{13}C NMR** (151 MHz, $DMSO$) δ = 153.80, 140.65, 133.06, 128.13, 119.33, 110.60, 106.07, 47.68, 43.06, 25.19. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 297.07; found: 297.0.

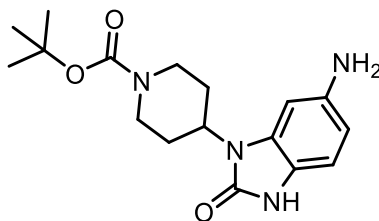
Synthesis of tert-butyl 4-(5-chloro-6-nitro-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidine-1-carboxylate (13**)**



Compound **12** (2.6 g, 7.2 mmol, 1.0 eq.) was dissolved in 26 mL of DCM followed by the addition of triethylamine (3 eq., 3 mL, 21.7 mmol). Subsequently, Boc₂O (1.8 mL, 8.0 mmol, 1.1 eq.) was prepared as a solution in 6 mL of DCM and added dropwise to the stirred reaction mixture. The reaction was allowed to proceed at room temperature for 16 hours. Upon completion, water was added to the mixture, and the aqueous phase was extracted with DCM. The combined organic layers were washed with a NaHCO₃, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (1-2% MeOH/DCM containing 1% NH₃), affording the desired compound **13** as a yellow solid (2.64 g, 6.6 mmol, 92%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 11.66 (s, 1H), 8.03 (s, 1H), 7.21 (s, 1H), 4.41 (tt, *J*=12.2, 4.0, 1H), 4.08 (s, 2H), 2.86 (s, 2H), 2.18 (qd, *J*=12.5, 4.4, 2H), 1.71 (d, *J*=10.0, 2H), 1.42 (s, 9H). **¹³C NMR** (151 MHz, DMSO) δ = 153.89, 153.83, 140.85, 132.73, 128.48, 118.80, 110.20, 106.03, 78.83, 50.73, 28.08. **LC-MS (ESI)**: *m/z* calculated for [M+H-Boc]⁺: 297.07; found: 297.1

Synthesis of tert-butyl 4-(6-amino-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidine-1-carboxylate (14**)**



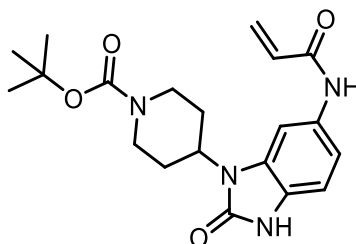
Compound **13** (2.0 g, 5.0 mmol, 1.0 eq.) was dissolved in methanol (20 mL), followed by the addition of 5% Pd/C (0.1 g, moistened with water) and ammonium formate (3.48 g, 50.4 mmol, 10 eq.). The reaction mixture was allowed to stir at 90 °C overnight. Upon

completion, the mixture was filtered over Celite, and the filtrate was concentrated under reduced pressure. The crude residue was dissolved in DCM and washed with a sodium bicarbonate solution. After drying over Na₂SO₄, filtration and evaporation of the solvents, the crude material was purified by silica gel column chromatography (3-4% MeOH/DCM containing 1% NH₃), affording the titled compound **14** as a pale pink solid (1.43 g, 4.3 mmol, 86%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 10.31 (s, 1H), 6.64 (d, *J*=8.2, 1H), 6.48 (d, *J*=1.8, 1H), 6.22 (dd, *J*=8.2, 1.9, 1H), 4.68 (s, 2H), 4.21 (tt, *J*=12.2, 3.9, 1H), 4.15 - 4.05 (m, 2H), 2.84 (s, 2H), 2.14 (qd, *J*=12.6, 4.4, 2H), 1.65 (d, *J*=10.4, 2H), 1.43 (s, 9H). **¹³C NMR** (126 MHz, DMSO) δ = 153.91, 153.83, 143.12, 129.92, 119.08, 109.15, 106.87, 95.59, 78.83, 49.62, 28.11.

LC-MS (ESI): *m/z* calculated for [M+Na]⁺: 355.39; found: 355.1

Synthesis of tert-butyl 4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidine-1-carboxylate (**15**)

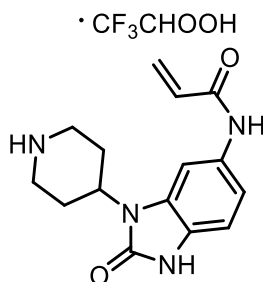


Compound **14** (1.5 g, 4.5 mmol, 1.0 eq.) was dissolved in 45 mL of dry THF, followed by the addition of DIPEA (1.6 mL, 9.0 mmol, 2 eq.), and the mixture was stirred for 15 minutes at 0 °C under an argon atmosphere. A solution of acryloyl chloride (0.4 mL, 5.0 mmol, 1.1 eq.) in 5 mL of THF was then added dropwise to the reaction mixture. Stirring was continued overnight at room temperature. Upon completion, the reaction was quenched with a saturated solution of NaHCO₃. The aqueous layer was extracted with DCM, and the combined organic layers were washed with saturated NaCl solution and dried over Na₂SO₄. After filtration and evaporation of the solvent, purification by silica gel column chromatography (3-4% MeOH/DCM containing 1% NH₃) afforded the desired product **15** as a white solid (1.37 g, 3.54 mmol, 83%).

¹H NMR (400 MHz, DMSO-*d*₆) δ = 10.81 (s, 1H), 10.05 (s, 1H), 7.71 (d, *J*=1.4, 1H), 7.18 (dd, *J*=8.4, 1.7, 1H), 6.92 (d, *J*=8.3, 1H), 6.40 (dd, *J*=16.9, 10.1, 1H), 6.22 (dd, *J*=16.9, 2.1, 1H), 5.72 (dd, *J*=10.0, 2.1, 1H), 4.28 (ddd, *J*=12.3, 8.4, 4.0, 1H), 4.11 (d,

$J=11.3$, 2H), 2.88 (s, 2H), 2.14 (qd, $J=12.6$, 4.3, 2H), 1.70 (d, $J=10.2$, 2H), 1.44 (s, 9H). **^{13}C NMR** (101 MHz, DMSO) δ = 162.75, 153.96, 153.89, 132.60, 131.99, 128.96, 126.33, 124.49, 112.33, 108.71, 100.92, 78.93, 49.86, 28.10. **LC-MS (ESI)**: m/z calculated for $[\text{M}+\text{H}-\text{Boc}]^+$: 287.14; found: 287.1.

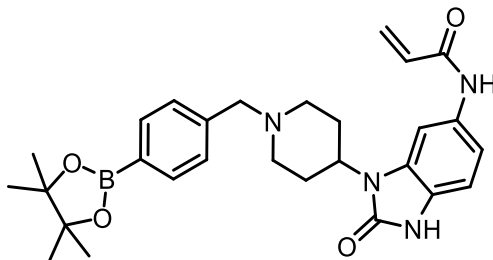
Synthesis of N-(2-oxo-3-(piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (16)



Compound **15** (1.6 g, 4.1 mmol, 1.0 eq.) was dissolved in a mixture of DCM (15 mL) and TFA (5 mL), and the reaction was stirred at room temperature for 2 hours. Afterwards, the solvents and excess TFA were removed under reduced pressure, and the resulting residue was resuspended in H_2O (50 mL) and subsequently lyophilized. The titled compound **16** was obtained as a white salt (1.66 g, 4.1 mmol, 99%).

^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ = 10.88 (s, 1H), 10.11 (s, 1H), 8.96 - 8.73 (m, 2H), 8.54 (d, $J=7.5$, 1H), 7.84 (s, 1H), 7.01 (dd, $J=8.3$, 1.6, 1H), 6.95 (d, $J=8.3$, 1H), 6.45 (dd, $J=17.0$, 10.2, 1H), 6.24 (dd, $J=17.0$, 1.9, 1H), 5.74 (dd, $J=10.1$, 1.9, 1H), 4.43 (tt, $J=12.3$, 4.0, 1H), 3.44 (s, 2H), 3.12 (q, $J=12.8$, 12.4, 2H), 2.56 (td, $J=13.1$, 4.0, 2H), 1.88 (d, $J=12.2$, 2H). **^{13}C NMR** (151 MHz, DMSO) δ = 162.85, 158.42, 158.21, 158.00, 157.80, 153.95, 132.38, 131.94, 128.99, 126.36, 124.72, 113.32, 108.83, 101.65, 47.51, 43.04, 25.64. **LC-MS (ESI)**: m/z calculated for $[\text{M}+\text{H}]^+$: 287.14; found: 287.1.

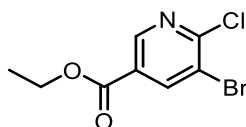
Synthesis of N-(2-oxo-3-(1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (17)



Compound **16** (1.0 g, 2.49 mmol, 1.0 eq.) was dissolved in 10 mL of DMF, followed by the addition of K_2CO_3 (1.72 g, 12.46 mmol, 5 eq.), and the mixture was stirred at room temperature for 30 minutes. Then, (4-(bromomethyl)phenyl)boronic acid pinacol ester (739.97 mg, 2.49 mmol, 1.0 eq.) was added, and the reaction mixture was stirred for 1 h. Upon completion, the reaction was quenched with a saturated solution of $NaHCO_3$, and the aqueous phase was extracted with DCM. The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (3-4% MeOH/DCM containing 1% NH_3) afforded the desired compound **17** as a light-yellow solid.

1H NMR (600 MHz, $DMSO-d_6$) δ = 10.79 (s, 1H), 10.08 (s, 1H), 7.71 (d, $J=2.0$, 1H), 7.66 (d, $J=8.0$, 2H), 7.37 (d, $J=8.0$, 2H), 7.26 (dd, $J=8.4$, 1.8, 1H), 6.91 (d, $J=8.4$, 1H), 6.42 (dd, $J=16.9$, 10.1, 1H), 6.25 (dd, $J=17.0$, 2.0, 1H), 5.73 (dd, $J=10.1$, 2.0, 1H), 4.10 (tt, $J=12.5$, 4.2, 1H), 3.55 (s, 2H), 2.95 (d, $J=11.4$, 2H), 2.31 (qd, $J=12.4$, 3.9, 2H), 2.09 (t, $J=10.8$, 2H), 1.29 (s, 12H). **^{13}C NMR** (151 MHz, $DMSO$) δ = 162.79, 154.01, 141.89, 134.42, 132.64, 132.04, 129.02, 128.41, 126.35, 124.49, 112.14, 108.65, 100.90, 83.56, 61.95, 52.66, 50.19, 28.69, 24.68. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 503.28; found: 503.2.

Synthesis of ethyl 5-bromo-6-chloronicotinate (19)

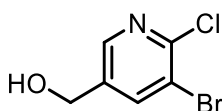


Compound **18** (4.0 g, 16.9 mmol, 1.0 eq.) was dissolved in DMF (34 mL), followed by the addition of K_2CO_3 (5.38 g, 38.9 mmol, 2.5 eq.) and ethyl iodide (1.6 mL, 20.3 mmol, 1.2 eq.). The reaction mixture was stirred at room temperature for 2 h. Upon

completion, the solvent was removed under reduced pressure, and the resulting residue was dissolved in DCM and extracted with a saturated NaCl solution. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Purification by silica gel column chromatography (1-3% EtOAc/PE) yielded the titled compound **19** as a white solid (3.6 g, 13.6 mmol, 80%).

¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.88 (d, *J*=2.0, 1H), 8.58 (d, *J*=2.1, 1H), 4.36 (q, *J*=7.1, 2H), 1.34 (t, *J*=7.1, 3H). **¹³C NMR** (101 MHz, DMSO) δ = 162.85, 153.44, 148.71, 142.84, 126.59, 119.75, 61.82, 13.97. **LC-MS (ESI)**: *m/z* calculated for [M+H]⁺: 265.50; found: 265.9.

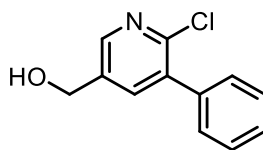
Synthesis of (5-bromo-6-chloropyridin-3-yl)methanol (**20**)



Compound **19** (4.2 g, 15.9 mmol, 1.0 eq.) was dissolved in dry DCM (60 mL), and DIBAL (48 mL, 48.0 mmol, 3.0 eq.) was added dropwise at 0 °C. The reaction mixture was then stirred at room temperature for 2 hours. Subsequently, Rochelle salt (Na⁺,K⁺ tartrate) was carefully added, and the mixture was stirred overnight at room temperature. The organic layer was separated and washed with a saturated NaCl solution. The combined organic fractions were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (4-5% MeOH/DCM containing 1% NH₃) afforded the desired compound **20** as a white solid (1.96 g, 8.8 mmol, 55%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 8.36 (d, *J*=2.0, 1H), 8.14 (d, *J*=2.3, 1H), 5.50 (t, *J*=5.7, 1H), 4.54 (d, *J*=5.7, 2H). **¹³C NMR** (176 MHz, DMSO) δ = 147.40, 146.66, 140.93, 139.40, 118.92, 59.19. **LC-MS (ESI)**: *m/z* calculated for [M+H]⁺: 223.47; found: 223.9.

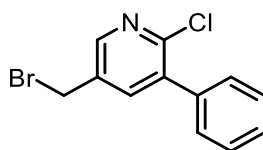
Synthesis of (6-chloro-5-phenylpyridin-3-yl)methanol (**21**)



Compound **20** (1.3 g, 5.8 mmol, 1.0 eq.) and phenylboronic acid (783.8 mg, 6.4 mmol, 1.1 eq.) were dissolved in a mixture of 1,4-dioxane and water (5:1) under an argon atmosphere. The mixture was degassed for 10 minutes, after which K_3PO_4 (2.48 g, 11.7 mmol, 2.0 eq.) and [1,1'-bis(diphenylphosphino)ferrocene]palladium(II)dichloride (427.2 mg, 0.6 mmol, 0.1 eq.) were added sequentially. The suspension was then stirred at 65 °C. Upon completion of the reaction, the mixture was filtered over Celite and extracted with DCM. The combined organic layers were washed with saturated NaCl solution, dried over Na_2SO_4 , filtered, and concentrated. Purification by silica gel column chromatography (4-5% MeOH/DCM containing 1% NH_3) yielded the desired product **21** as a white solid (902 mg, 4.1 mmol, 70%).

1H NMR (500 MHz, $DMSO-d_6$) δ = 8.37 (s, 1H), 7.77 (s, 1H), 7.48 (m, J =8.8, 6.5, 5H), 4.59 (s, 2H). **^{13}C NMR** (126 MHz, DMSO) δ = 146.80, 146.61, 138.54, 137.69, 137.15, 135.53, 129.15, 128.42, 128.30, 59.73. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 220.05; found: 220.0.

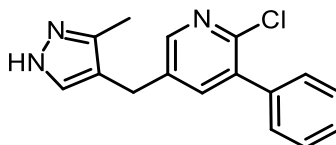
Synthesis of 5-(bromomethyl)-2-chloro-3-phenylpyridine (**22**)



To a solution of compound **21** (900 mg, 4.10 mmol, 1.0 eq.) in anhydrous 1,4-dioxane (10 mL), phosphorus tribromide (1.2 mL, 12.3 mmol, 3.0 eq.) was added dropwise under an ice bath, and the reaction was stirred at room temperature for 2 hours. Upon completion, water was added, and the mixture was extracted with EtOAc. The organic phase was washed with a saturated NaCl solution, dried over Na_2SO_4 , and concentrated under reduced pressure. The pure product **22** (975 mg, 3.5 mmol, 84%) was obtained after purification by silica gel column chromatography (1-3% EtOAc/PE) as a white solid.

¹H NMR (600 MHz, DMSO-*d*₆) δ = 8.53 (d, *J*=2.4, 1H), 7.97 (d, *J*=2.4, 1H), 7.64 - 7.27 (m, 5H), 4.79 (s, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 148.83, 147.90, 140.83, 136.54, 136.05, 134.19, 129.15, 128.53, 128.47, 29.33. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 283.57; found: 284.0.

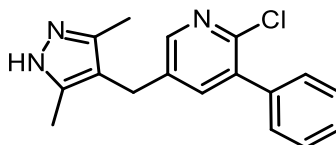
Synthesis of 2-chloro-5-((3-methyl-1H-pyrazol-4-yl)methyl)-3-phenylpyridine (23a)



4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-3-methyl-1H-pyrazole (37 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **22** (60 mg, 0.21 mmol, 1.2 eq.), K₃PO₄ (75 mg, mmol, 2.0 eq.) and Pd(dppf)Cl₂ (13 mg, mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23a** as a white solid (31 mg, 0.11 mmol, 62%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 8.31 (d, *J*=2.3, 1H), 7.76 (d, *J*=2.1, 1H), 7.73 (d, *J*=2.3, 1H), 7.52 - 7.42 (m, 6H), 6.04 (d, *J*=2.1, 1H), 5.34 (s, 2H), 2.13 (s, 3H). **¹³C NMR** (126 MHz, DMSO) δ = 147.94, 147.73, 147.51, 139.57, 136.74, 135.81, 133.35, 131.16, 129.12, 128.46, 105.23, 50.88, 13.33. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 284.09; found: 284.1.

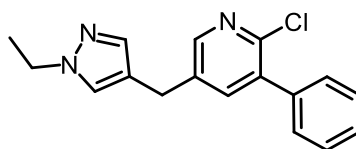
Synthesis of 2-chloro-5-((3,5-dimethyl-1H-pyrazol-4-yl)methyl)-3-phenylpyridine (23b)



3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (39 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **22** (60 mg, 0.21 mmol, 1.0 eq.), K₃PO₄ (75 mg, 0.35 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (13 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23b** as a white solid (35 mg, 0.12 mmol, 66%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 8.24 (d, *J*=2.4, 1H), 7.60 (d, *J*=2.4, 1H), 7.50 - 7.46 (m, 2H), 7.45 - 7.41 (m, 3H), 3.84 (s, 2H), 2.19 (s, 3H). **¹³C NMR** (151 MHz, DMSO) δ = 148.13, 146.08, 142.18, 139.81, 136.98, 135.84, 135.71, 129.19, 128.41, 128.33, 113.69, 24.16, 10.09. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 298.10; found: 298.1.

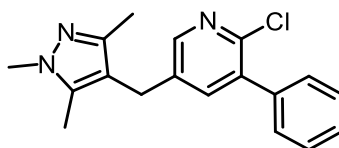
Synthesis of 2-chloro-5-((1-ethyl-1H-pyrazol-4-yl)methyl)-3-phenylpyridine (**23c**)



1-ethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (39 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **22** (60 mg, 0.21 mmol, 1.2 eq.), K₃PO₄ (75 mg, 0.35 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (13 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23c** as a white solid (39 mg, 0.13 mmol, 74%).

¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.32 (d, *J*=2.4, 1H), 7.70 (d, *J*=2.4, 1H), 7.58 (s, 1H), 7.47 (m, 5H), 7.33 (s, 1H), 4.04 (q, *J*=7.2, 2H), 3.84 (s, 2H), 1.31 (t, *J*=7.2, 3H). **¹³C NMR** (101 MHz, DMSO) δ = 148.26, 145.90, 140.11, 137.92, 137.20, 137.05, 135.69, 129.20, 128.36, 128.27, 127.81, 118.46, 46.04, 26.09, 15.48. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 298.10; found: 298.1.

Synthesis of 2-chloro-3-phenyl-5-((1,3,5-trimethyl-1H-pyrazol-4-yl)methyl)pyridine (**23d**)

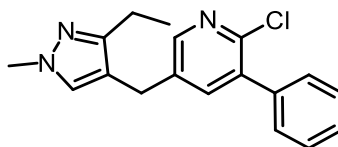


1,3,5-trimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (50 mg, 0.21 mmol, 1.0 eq.) was reacted with compound **22** (72 mg, 0.25 mmol, 1.2 eq.), K₃PO₄ (90 mg, 0.42 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (15.5 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23d** as a white solid (47 mg, 0.15 mmol, 71%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 8.21 (d, *J*=2.4, 1H), 7.54 (d, *J*=2.4, 1H), 7.50 - 7.46 (m, 2H), 7.45 - 7.40 (m, 3H), 3.78 (s, 2H), 3.65 (s, 3H), 2.18 (s, 3H), 2.05 (s, 3H). **¹³C NMR**

NMR (151 MHz, DMSO) δ = 148.03, 145.87, 143.80, 139.66, 137.57, 137.04, 136.58, 135.65, 129.17, 128.42, 128.31, 113.35, 35.56, 24.92, 11.38, 9.23. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 312.12; found: 312.1.

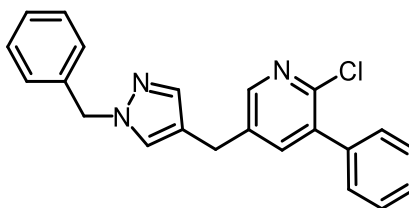
Synthesis of 2-chloro-5-((3-ethyl-1-methyl-1H-pyrazol-4-yl)methyl)-3-phenylpyridine (23e)



3-ethyl-1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (50 mg, 0.21 mmol, 1.0 eq.) was reacted with compound **22** (72 mg, 0.25 mmol, 1.2 eq.), K_3PO_4 (90 mg, 0.42 mmol, 2.0 eq.) and $Pd(dppf)Cl_2$ (15.5 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H_2O using general procedure A, yielding the titled product **23e** as a white solid (45 mg, 0.14 mmol, 68 %).

1H NMR (600 MHz, $DMSO-d_6$) δ = 8.28 (d, $J=2.4$, 1H), 7.64 (d, $J=2.4$, 1H), 7.51 - 7.45 (m, 2H), 7.47 - 7.42 (m, 3H), 7.40 (s, 1H), 3.78 (s, 2H), 3.69 (s, 3H), 2.45 (q, $J=7.6$, 2H), 1.07 (t, $J=7.6$, 3H). **^{13}C NMR** (151 MHz, DMSO) δ = 150.40, 148.22, 145.87, 140.03, 137.06, 136.94, 135.66, 130.17, 129.16, 128.39, 128.29, 115.42, 38.13, 25.32, 19.09, 13.60. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 312.12; found: 312.2.

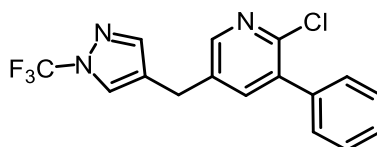
Synthesis of 5-((1-benzyl-1H-pyrazol-4-yl)methyl)-2-chloro-3-phenylpyridine (23f)



1-benzyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (50 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **22** (60 mg, 0.21 mmol, 1.2 eq.), K_3PO_4 (75 mg, 0.35 mmol, 2.0 eq.) and $Pd(dppf)Cl_2$ (13 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H_2O using general procedure A, yielding the titled product **23f** as a white solid (47 mg, 0.13 mmol, 74%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 8.32 (d, *J*=2.4, 1H), 7.69 (d, *J*=2.6, 2H), 7.50 - 7.47 (m, 2H), 7.46 - 7.43 (m, 3H), 7.38 (s, 1H), 7.30 (t, *J*=7.3, 2H), 7.26 (m, *J*=8.3, 6.3, 1H), 7.18 (d, *J*=7.1, 2H), 5.25 (s, 2H), 3.85 (s, 2H). **¹³C NMR** (176 MHz, DMSO) δ = 148.22, 145.91, 140.11, 138.67, 137.72, 137.11, 137.04, 135.68, 129.17, 128.96, 128.43, 128.36, 128.26, 127.50, 127.42, 119.04, 54.72, 25.99. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 360.12; found: 360.1.

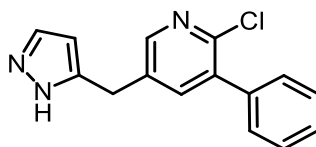
Synthesis of 2-chloro-3-phenyl-5-((1-(trifluoromethyl)-1H-pyrazol-4-yl)methyl)pyridine (**23g**)



4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(trifluoromethyl)-1H-pyrazole (50 mg, 0.19 mmol, 1.0 eq.) was reacted with compound **22** (65 mg, 0.23 mmol, 1.2 eq.), K₃PO₄ (81 mg, 0.38 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (14 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23g** as a white solid (39 mg, 0.12 mmol, 61%).

¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.37 (d, *J*=2.4, 1H), 8.36 (s, 1H), 7.93 (s, 1H), 7.79 (d, *J*=2.4, 1H), 7.53 - 7.41 (m, 5H), 3.93 (s, 2H). **¹³C NMR** (101 MHz, DMSO) δ = 148.39, 146.36, 144.58, 140.31, 136.97, 135.87, 135.70, 129.25, 128.36, 128.32, 128.14, 122.85, 25.47. **¹⁹F NMR** (377 MHz, DMSO) δ = -59.09. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 338.06; found: 338.1.

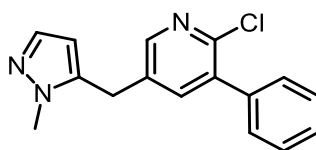
Synthesis of 5-((1H-pyrazol-5-yl)methyl)-2-chloro-3-phenylpyridine (**23h**)



3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (35 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **22** (61 mg, 0.22 mmol, 1.2 eq.), K₃PO₄ (77 mg, 0.35 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (13 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23h** as a white solid (29 mg, 0.11 mmol, 61%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 8.32 (d, *J*=2.4, 1H), 7.91 (d, *J*=2.3, 1H), 7.72 (d, *J*=2.3, 1H), 7.52 - 7.47 (m, 3H), 7.47 - 7.42 (m, 3H), 6.28 (t, *J*=2.1, 1H), 5.45 (s, 2H). **¹³C NMR** (126 MHz, DMSO) δ = 147.71, 147.56, 139.59, 139.56, 136.72, 135.84, 133.21, 130.49, 129.11, 128.46, 105.76, 51.06. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 270.07; found: 270.1.

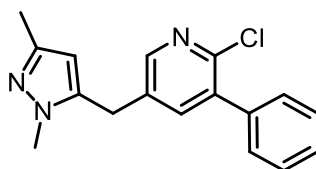
Synthesis of 2-chloro-5-((1-methyl-1H-pyrazol-5-yl)methyl)-3-phenylpyridine (23i)



1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (44 mg, 0.21 mmol, 1.0 eq.) was reacted with compound **22** (72 mg, 0.25, 1.2 eq.), K₃PO₄ (90 mg, 0.43 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (16 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23i** as a white solid (39 mg, 0.14 mmol, 65%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 8.33 (d, *J*=2.4, 1H), 7.71 (d, *J*=2.4, 1H), 7.62 - 7.41 (m, 5H), 7.30 (d, *J*=1.8, 1H), 6.03 (d, *J*=1.8, 1H), 4.12 (s, 2H), 3.75 (s, 3H). **¹³C NMR** (176 MHz, DMSO) δ = 148.43, 146.53, 140.36, 140.13, 137.54, 136.87, 135.87, 133.92, 129.17, 128.39, 128.35, 105.34, 36.17, 27.02. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 284.09; found: 284.1.

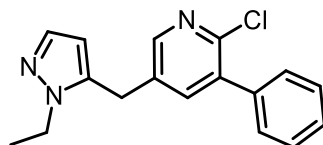
Synthesis of 2-chloro-5-((1,3-dimethyl-1H-pyrazol-5-yl)methyl)-3-phenylpyridine (23j)



(1,3-dimethyl-1H-pyrazol-5-yl)boronic acid (25 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **22** (60 mg, 0.21 mmol, 1.2 eq.), K₃PO₄ (75 mg, 0.35 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (13 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23j** as a white solid (34 mg, 0.11 mmol, 65%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 8.33 (d, *J*=2.4, 1H), 7.70 (d, *J*=2.4, 1H), 7.57 - 7.42 (m, 5H), 5.81 (s, 1H), 4.06 (s, 2H), 3.67 (s, 3H), 2.06 (s, 3H). **¹³C NMR** (176 MHz, DMSO) δ = 148.45, 146.53, 145.61, 141.04, 140.35, 136.89, 135.87, 133.93, 129.19, 128.40, 128.36, 104.93, 35.73, 27.06, 13.08. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 298.10; found: 298.1.

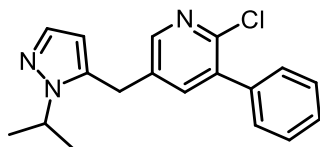
Synthesis of 2-chloro-5-((1-ethyl-1H-pyrazol-5-yl)methyl)-3-phenylpyridine (**23k**)



1-ethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (39 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **22** (60 mg, 0.2 mmol, 1.2 eq.), K₃PO₄ (75 mg, 0.35 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (13 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23k** as a white solid (29 mg, 0.10 mmol, 55%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 8.34 (d, *J*=2.4, 1H), 7.71 (d, *J*=2.4, 1H), 7.51 - 7.47 (m, 2H), 7.47 - 7.43 (m, 3H), 7.33 (d, *J*=1.8, 1H), 6.01 (d, *J*=1.8, 1H), 4.13 (s, 2H), 4.08 (q, *J*=7.2, 2H), 1.22 (t, *J*=7.2, 3H). **¹³C NMR** (176 MHz, DMSO) δ = 148.45, 146.51, 140.35, 139.43, 137.81, 136.87, 135.84, 134.14, 129.15, 128.42, 128.38, 105.30, 43.26, 26.84, 15.40. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 298.10; found: 298.1.

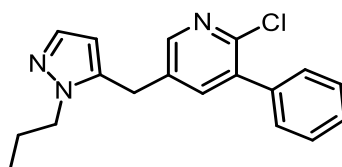
Synthesis of 2-chloro-5-((1-isopropyl-1H-pyrazol-5-yl)methyl)-3-phenylpyridine (**23l**)



1-isopropyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (38 mg, 0.16 mmol, 1.0 eq.) was reacted with compound **22** (55 mg, 0.19 mmol, 1.2 eq.), K₃PO₄ (69 mg, 0.32 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (12 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23l** as a white solid (31 mg, 0.10 mmol, 61%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 8.33 (d, *J*=2.4, 1H), 7.69 (d, *J*=1.9, 1H), 7.56 - 7.42 (m, 5H), 7.36 (d, *J*=1.8, 1H), 6.00 (d, *J*=1.8, 1H), 4.55 (hept, *J*=6.3, 1H), 4.15 (s, 2H), 1.28 (d, *J*=6.5, 6H). **¹³C NMR** (151 MHz, DMSO) δ = 148.42, 146.46, 140.27, 138.80, 137.71, 136.88, 135.81, 134.23, 129.11, 128.43, 128.38, 105.01, 48.84, 26.75, 22.61. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 312.12; found: 312.2.

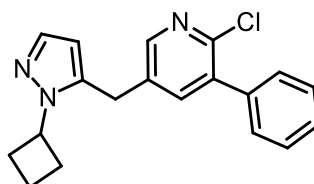
Synthesis of 2-chloro-3-phenyl-5-((1-propyl-1H-pyrazol-5-yl)methyl)pyridine (23m)



1-propyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (44 mg, 0.19 mmol, 1.0 eq.) was reacted with compound **22** (63 mg, 0.22 mmol, 1.2 eq.), K₃PO₄ (79 mg, 0.37 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (14 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23m** as a white solid (35 mg, 0.11 mmol, 60%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 8.34 (d, *J*=2.4, 1H), 7.72 (d, *J*=2.4, 1H), 7.52 - 7.46 (m, 2H), 7.47 - 7.42 (m, 3H), 7.34 (d, *J*=1.8, 1H), 6.02 (d, *J*=1.8, 1H), 4.13 (s, 2H), 4.00 (t, *J*=7.2, 2H), 1.66 (h, *J*=7.3, 2H), 0.77 (t, *J*=7.4, 3H). **¹³C NMR** (151 MHz, DMSO) δ = 148.45, 146.48, 140.37, 139.85, 137.82, 136.87, 135.82, 134.19, 129.12, 128.42, 128.36, 105.19, 49.73, 26.89, 23.15, 10.87. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 312.12; found: 312.2.

Synthesis of 2-chloro-5-((1-cyclobutyl-1H-pyrazol-5-yl)methyl)-3-phenylpyridine (23n)

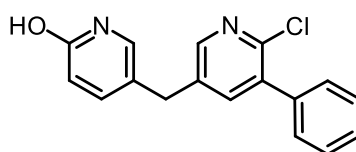


1-cyclobutyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (44 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **22** (60 mg, 0.21 mmol, 1.2 eq.), K₃PO₄ (75 mg, 0.35 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (13 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4

dioxane/ H₂O using general procedure A, yielding the titled product **23n** as a white solid (27 mg, 0.08 mmol, 47%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 8.30 (d, *J*=2.4, 1H), 7.64 (d, *J*=2.4, 1H), 7.52 - 7.40 (m, 5H), 7.40 (d, *J*=1.7, 1H), 6.05 (d, *J*=1.7, 1H), 4.86 (p, *J*=8.2, 1H), 4.12 (s, 2H), 2.50 - 2.41 (m, 2H), 2.17 (m, *J*=7.6, 2.9, 2H), 1.74 (m, *J*=15.8, 6.8, 2.9, 2H). **¹³C NMR** (126 MHz, DMSO) δ = 148.34, 146.45, 140.16, 139.28, 138.00, 136.88, 135.81, 134.21, 129.10, 128.44, 128.38, 105.52, 51.24, 29.67, 26.73, 14.17. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 324.12; found: 324.1.

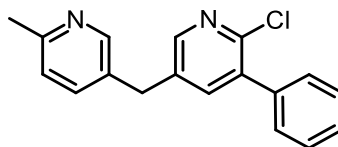
Synthesis of 5-((6-chloro-5-phenylpyridin-3-yl)methyl)pyridin-2-ol (**23o**)



5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-ol (47 mg, 0.21 mmol, 1.0 eq.) was reacted with compound **22** (72 mg, 0.21 mmol, 1.2 eq.), K₃PO₄ (90 mg, 0.42 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (16 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23o** as a white solid (21 mg, 0.07 mmol, 33%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 8.44 (d, *J*=2.4, 1H), 7.92 (dd, *J*=6.8, 2.1, 1H), 7.84 (d, *J*=2.4, 1H), 7.51 - 7.47 (m, 2H), 7.47 - 7.41 (m, 4H), 6.57 - 6.36 (m, 1H), 6.26 (td, *J*=6.7, 1.5, 1H), 5.16 (s, 2H). **¹³C NMR** (126 MHz, DMSO) δ = 161.42, 148.22, 147.55, 140.45, 140.00, 139.06, 136.73, 135.85, 132.84, 129.13, 128.45, 119.88, 105.90, 48.43. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 297.07; found: 297.1.

Synthesis of 2-chloro-5-((6-methylpyridin-3-yl)methyl)-3-phenylpyridine (**23p**)



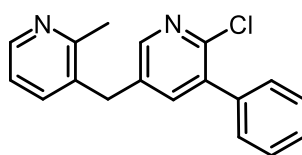
2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (42 mg, 0.19 mmol, 1.0 eq.) was reacted with compound **22** (65 mg, 0.23 mmol, 1.2 eq.), K₃PO₄ (82 mg, 0.39 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (14 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/

H₂O using general procedure A, yielding the titled product **23p** as a white solid (36 mg, 0.12 mmol, 63%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 8.43 (d, *J*=2.3, 1H), 8.37 (d, *J*=2.4, 1H), 7.76 (d, *J*=2.4, 1H), 7.59 (dd, *J*=8.0, 2.4, 1H), 7.49 - 7.46 (m, 2H), 7.45 - 7.42 (m, 3H), 7.17 (d, *J*=7.9, 1H), 4.01 (s, 2H), 2.41 (s, 3H). **¹³C NMR** (151 MHz, DMSO) δ = 155.93, 148.96, 148.44, 146.28, 140.34, 136.92, 136.53, 136.41, 135.85, 132.50, 129.20, 128.36, 128.31, 122.98, 33.67, 23.58.

LC-MS (ESI): *m/z* calculated for [M+H]⁺: 295.09; found: 295.1.

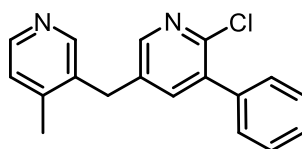
Synthesis of 2-chloro-5-((2-methylpyridin-3-yl)methyl)-3-phenylpyridine (**23q**)



2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (42 mg, 0.19 mmol, 1.0 eq.) was reacted with compound **22** (65 mg, 0.23 mmol, 1.2 eq.), K₃PO₄ (82 mg, 0.39 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (14 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/H₂O using general procedure A, yielding the titled product **23q** as a white solid (56 mg, 0.19 mmol, 98%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 8.32 (dd, *J*=4.8, 1.5, 1H), 8.29 (d, *J*=2.3, 1H), 7.66 (d, *J*=2.4, 1H), 7.57 (dd, *J*=7.7, 1.7, 1H), 7.49 - 7.46 (m, 1H), 7.47 - 7.40 (m, 2H), 7.18 (dd, *J*=7.6, 4.8, 1H), 4.08 (s, 1H), 2.45 (s, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 156.48, 148.57, 147.06, 146.33, 140.38, 136.99, 136.90, 135.83, 135.07, 133.06, 129.18, 128.39, 128.34, 121.61, 33.80, 22.35. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 295.09; found: 295.1.

Synthesis of 2-chloro-5-((4-methylpyridin-3-yl)methyl)-3-phenylpyridine (**23r**)

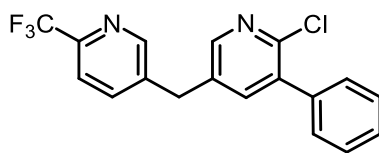


4-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (42 mg, 0.19 mmol, 1.0 eq.) was reacted with compound **22** (65 mg, 0.23 mmol, 1.2 eq.), K₃PO₄ (82 mg, 0.39 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (14 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/

H₂O using general procedure A, yielding the titled product **23r** as a white solid (29 mg, 0.10 mmol, 98%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 8.43 (s, 1H), 8.32 (d, *J*=4.8, 1H), 8.29 (d, *J*=2.4, 1H), 7.64 (d, *J*=2.4, 1H), 7.49 - 7.46 (m, 2H), 7.46 - 7.41 (m, 3H), 7.20 (d, *J*=4.9, 1H), 4.09 (s, 2H), 2.24 (s, 3H). **¹³C NMR** (151 MHz, DMSO) δ = 150.12, 148.44, 148.03, 146.27, 145.46, 140.21, 136.89, 135.82, 135.18, 133.78, 129.18, 128.39, 128.34, 125.35, 31.74, 18.57. **LC-MS (ESI)**: *m/z* calculated for [M+H]⁺: 295.09; found: 295.1.

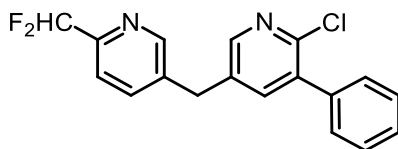
Synthesis of 2-chloro-3-phenyl-5-((6-(trifluoromethyl)pyridin-3-yl)methyl)pyridine (23s)



5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(trifluoromethyl)pyridine (53 mg, 0.19 mmol, 1.0 eq.) was reacted with compound **22** (65 mg, 0.23 mmol, 1.2 eq.), K₃PO₄ (89 mg, 0.39 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (14 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23s** as a white solid (44 mg, 0.13 mmol, 65%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 8.80 (d, *J*=2.1, 1H), 8.44 (d, *J*=2.4, 1H), 8.02 (dd, *J*=8.2, 2.2, 1H), 7.87 (d, *J*=2.4, 1H), 7.84 (dd, *J*=8.1, 0.8, 1H), 7.57 - 7.41 (m, 5H), 4.19 (s, 2H). **¹³C NMR** (126 MHz, DMSO) δ = 150.40, 148.67, 146.65, 140.63, 139.84, 138.26, 136.84, 136.01, 135.25, 129.24, 128.35, 120.76, 120.74, 120.71, 120.69, 33.75. **¹⁹F NMR** (565 MHz, DMSO) δ = -66.23. **LC-MS (ESI)**: *m/z* calculated for [M+H]⁺: 349.06; found: 349.1.

Synthesis of 2-chloro-5-((6-(difluoromethyl)pyridin-3-yl)methyl)-3-phenylpyridine (23t)

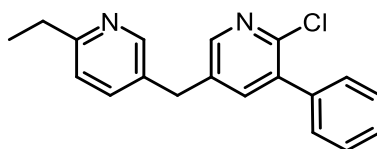


2-(difluoromethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (63 mg, 0.25 mmol, 1.0 eq.) was reacted with compound **22** (84 mg, 0.30 mmol, 1.2 eq.), K₃PO₄

(105 mg, 0.50 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (18 mg, 0.02 mmol, 0.1 eq.) in 2.5 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23t** as a white solid (39 mg, 0.12 mmol, 48%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 8.70 (d, *J*=2.1, 1H), 8.42 (d, *J*=2.4, 1H), 7.93 (dd, *J*=8.0, 2.2, 1H), 7.84 (d, *J*=2.4, 1H), 7.64 (d, *J*=8.0, 1H), 7.56 - 7.38 (m, 5H), 6.92 (t, *J*=55.0, 1H), 4.15 (s, 2H). **¹³C NMR** (126 MHz, DMSO) δ = 149.81, 148.61, 146.54, 140.55, 138.16, 137.79, 136.86, 135.97, 135.61, 129.23, 128.36, 128.34, 120.42, 120.39, 120.36, 115.69, 113.80, 111.91, 33.79. **¹⁹F NMR** (470 MHz, DMSO) δ = -114.90, -115.01. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 331.07; found: 331.0.

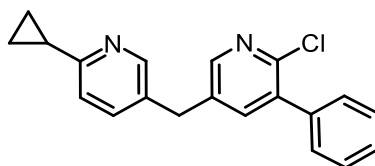
Synthesis of 2-chloro-5-((6-ethylpyridin-3-yl)methyl)-3-phenylpyridine (**23u**)



2-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (41 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **22** (60 mg, 0.21 mmol, 1.2 eq.), K₃PO₄ (75 mg, 0.35 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (13 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23u** as a white solid (35 mg, 0.11 mmol, 64%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 8.46 (d, *J*=2.4, 1H), 8.38 (d, *J*=2.4, 1H), 7.78 (d, *J*=2.3, 1H), 7.62 (dd, *J*=8.0, 2.4, 1H), 7.52 - 7.40 (m, 5H), 7.18 (d, *J*=8.0, 1H), 4.01 (s, 2H), 2.69 (q, *J*=7.6, 2H), 1.18 (t, *J*=7.6, 3H). **¹³C NMR** (126 MHz, DMSO) δ = 160.88, 148.96, 148.48, 140.37, 136.92, 136.61, 136.40, 135.87, 132.77, 129.21, 128.37, 128.32, 121.89, 33.72, 30.15, 13.74. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 309.11; found: 309.1.

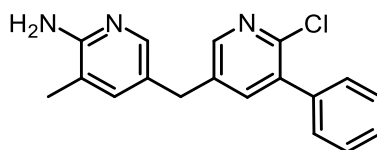
Synthesis of 2-chloro-5-((6-cyclopropylpyridin-3-yl)methyl)-3-phenylpyridine (23v)



2-cyclopropyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (52 mg, 0.21 mmol, 1.0 eq.) was reacted with compound **22** (72 mg, 0.25 mmol, 1.2 eq.), K₃PO₄ (90 mg, 0.42 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (16 mg, 0.02 mmol, 0.1 eq.) in 2.5 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23v** as a white solid (37 mg, 0.12 mmol, 54%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 8.38 (dd, *J*=2.3, 0.8, 1H), 8.37 (d, *J*=2.4, 1H), 7.77 (d, *J*=2.4, 0H), 7.56 (dd, *J*=8.0, 2.3, 1H), 7.50 - 7.46 (m, 1H), 7.46 - 7.41 (m, 2H), 7.20 (dd, *J*=8.0, 0.8, 1H), 3.98 (s, 1H), 2.03 (tt, *J*=8.0, 4.8, 1H), 0.89 (dt, *J*=8.1, 2.7, 1H), 0.85 (dt, *J*=4.6, 2.6, 1H). **¹³C NMR** (151 MHz, DMSO) δ = 160.17, 149.08, 148.44, 146.26, 140.34, 136.92, 136.45, 136.21, 135.86, 132.22, 129.20, 128.36, 128.31, 121.26, 33.68, 16.28, 9.53. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 321.11; found: 321.1.

Synthesis of 5-((6-chloro-5-phenylpyridin-3-yl)methyl)-3-methylpyridin-2-amine (23w)

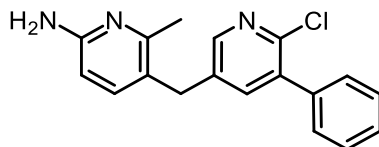


3-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine (53 mg, 0.23 mmol, 1.0 eq.) was reacted with compound **22** (77 mg, 0.27 mmol, 1.2 eq.), K₃PO₄ (104 mg, 0.45 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (17 mg, 0.02 mmol, 0.1 eq.) in 2.5 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23w** as a white solid (25 mg, 0.08 mmol, 36%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 8.31 (s, 1H), 7.76 (s, 1H), 7.69 (s, 1H), 7.45 (s, 8H), 7.15 (s, 1H), 5.55 (s, 2H), 3.80 (s, 2H), 1.99 (s, 3H). **¹³C NMR** (126 MHz, DMSO) δ = 157.03, 148.30, 145.96, 144.68, 140.15, 137.44, 137.02, 135.74, 129.20, 128.37,

128.28, 123.57, 115.94, 33.42, 17.00. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 310.10; found: 310.1.

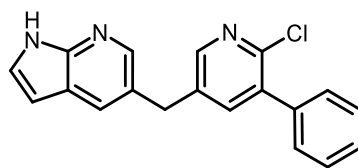
Synthesis of 5-((6-chloro-5-phenylpyridin-3-yl)methyl)-6-methylpyridin-2-amine (23x)



6-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine (55 mg, 0.24 mmol, 1.0 eq.) was reacted with compound **22** (81 mg, 0.29 mmol, 1.2 eq.), K_3PO_4 (110 mg, 0.48 mmol, 2.0 eq.) and $Pd(dppf)Cl_2$ (18 mg, 0.2 mmol, 0.1 eq.) in 2.5 mL 1,4 dioxane/ H_2O using general procedure A, yielding the titled product **23x** as a white solid (28 mg, 0.09 mmol, 38 %).

1H NMR (500 MHz, $DMSO-d_6$) δ = 8.24 (d, J =2.4, 1H), 7.57 (d, J =2.4, 1H), 7.52 - 7.46 (m, 2H), 7.46 - 7.41 (m, 3H), 7.21 (d, J =8.2, 1H), 6.24 (d, J =8.2, 1H), 5.68 (d, J =8.3, 2H), 3.86 (s, 2H), 2.21 (s, 3H). **^{13}C NMR** (126 MHz, $DMSO$) δ = 157.90, 153.85, 148.26, 145.90, 139.97, 138.88, 137.02, 136.60, 135.68, 129.15, 128.39, 128.28, 119.87, 105.54, 33.12, 21.82. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 310.10; found: 310.1.

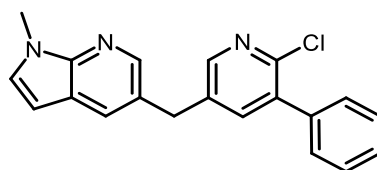
Synthesis of 5-((6-chloro-5-phenylpyridin-3-yl)methyl)-1H-pyrrolo[2,3-b]pyridine (23y)



5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-b]pyridine (47 mg, 0.19 mmol, 1.0 eq.) was reacted with compound **22** (65 mg, 0.23 mmol, 1.2 eq.), K_3PO_4 (82 mg, 0.39 mmol, 2.0 eq.) and $Pd(dppf)Cl_2$ (14 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H_2O using general procedure A, yielding the titled product **23y** as a white solid (34 mg, 0.11 mmol, 55%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 11.56 (s, 1H), 8.40 (d, *J*=2.4, 1H), 8.21 (d, *J*=2.1, 1H), 7.88 - 7.85 (m, 1H), 7.78 (d, *J*=2.4, 1H), 7.50 - 7.40 (m, 6H), 6.37 (dd, *J*=3.4, 1.9, 1H), 4.11 (s, 2H). **¹³C NMR** (176 MHz, DMSO) δ = 148.41, 147.46, 146.05, 143.13, 140.28, 137.52, 137.00, 135.80, 129.20, 128.36, 128.28, 127.87, 127.02, 126.57, 119.58, 99.53, 34.37. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 320.09; found: 320.1.

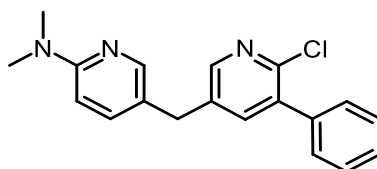
Synthesis of 5-((6-chloro-5-phenylpyridin-3-yl)methyl)-1-methyl-1H-pyrrolo[2,3-b]pyridine (**23z**)



1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-b]pyridine (50 mg, 0.19 mmol, 1.0 eq.) was reacted with compound **22** (65 mg, 0.23 mmol, 1.2 eq.), K₃PO₄ (82 mg, 0.39 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (14 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23z** as a white solid (42 mg, 0.13 mmol, 60%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 8.40 (d, *J*=2.4, 1H), 8.27 (d, *J*=2.0, 1H), 7.87 (d, *J*=2.1, 1H), 7.76 (d, *J*=2.4, 1H), 7.49 - 7.45 (m, 3H), 6.39 (d, *J*=3.4, 1H), 4.12 (s, 2H), 3.78 (s, 3H). **¹³C NMR** (176 MHz, DMSO) δ = 148.38, 146.47, 146.06, 143.01, 140.26, 137.48, 137.00, 135.80, 130.50, 129.19, 128.36, 128.28, 128.26, 127.06, 119.88, 98.53, 34.27, 30.83. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 334.10; found: 334.1.

Synthesis of 5-((6-chloro-5-phenylpyridin-3-yl)methyl)-N,N-dimethylpyridin-2-amine (**23aa**)

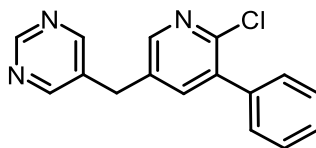


N,N-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine (53 mg, 0.21 mmol, 1.0 eq.) was reacted with compound **22** (72 mg, 0.25 mmol, 1.2 eq.), K₃PO₄ (98 mg, 0.42 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (16 mg, 0.02 mmol, 0.1 eq.) in 2.5 mL 1,4

dioxane/ H₂O using general procedure A, yielding the titled product **23aa** as a white solid (35 mg, 0.11 mmol, 51%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 8.33 (d, *J*=2.4, 1H), 8.05 (d, *J*=2.5, 1H), 7.70 (d, *J*=2.4, 1H), 7.51 - 7.39 (m, 6H), 6.57 (d, *J*=8.7, 1H), 3.87 (s, 2H), 2.96 (s, 6H). **¹³C NMR** (126 MHz, DMSO) δ = 157.97, 148.29, 147.24, 146.00, 140.13, 137.68, 137.34, 137.01, 135.76, 129.18, 128.37, 128.28, 122.56, 105.85, 37.69, 33.24. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 324.12; found: 324.1.

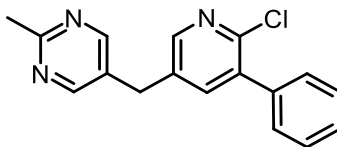
Synthesis of 5-((6-chloro-5-phenylpyridin-3-yl)methyl)pyrimidine (**23ab**)



5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (36 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **22** (60 mg, 0.21 mmol, 1.2 eq.), K₃PO₄ (75 mg, 0.35 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (13 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23ab** as a white solid (28 mg, 0.10 mmol, 56%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 9.06 (s, 1H), 8.81 (s, 2H), 8.43 (d, *J*=2.4, 1H), 7.86 (d, *J*=2.4, 1H), 7.50 - 7.42 (m, 5H), 4.08 (s, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 157.07, 156.77, 148.59, 146.64, 140.56, 136.86, 136.00, 135.18, 133.74, 129.26, 128.35, 31.50. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 282.07; found: 282.1.

Synthesis of 5-((6-chloro-5-phenylpyridin-3-yl)methyl)-2-methylpyrimidine (**23ac**)

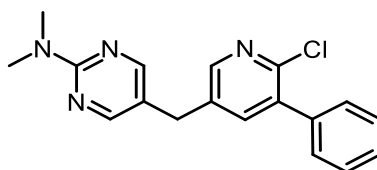


2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (47 mg, 0.21 mmol, 1.0 eq.) was reacted with compound **22** (72 mg, 0.25 mmol, 1.2 eq.), K₃PO₄ (90 mg, 0.42 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (16 mg, 0.02 mmol, 0.1 eq.) in 2.5 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23ac** as a white solid (40 mg, 0.14 mmol, 64%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 8.68 (s, 2H), 8.41 (d, *J*=2.4, 1H), 7.82 (d, *J*=2.4, 1H), 7.50 - 7.42 (m, 5H), 4.03 (s, 2H), 2.56 (s, 3H). **¹³C NMR** (151 MHz, DMSO) δ = 165.48, 157.07, 148.50, 146.54, 140.44, 136.87, 135.95, 135.51, 130.30, 129.24, 128.34, 128.32, 31.15, 25.21.

LC-MS (ESI): *m/z* calculated for [M+H]⁺: 296.09; found: 296.1.

Synthesis of 5-((6-chloro-5-phenylpyridin-3-yl)methyl)-N,N-dimethylpyrimidin-2-amine (23ad)

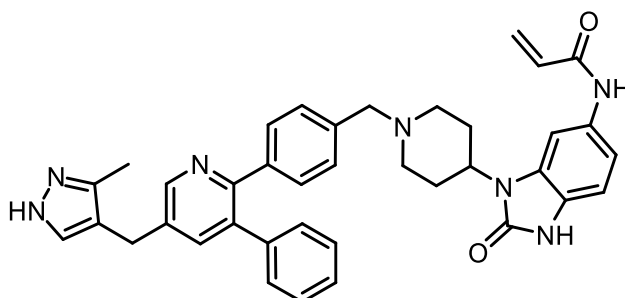


N,N-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-amine (58 mg, 0.23 mmol, 1.0 eq.) was reacted with compound **22** (78 mg, 0.28 mmol, 1.2 eq.), K₃PO₄ (106 mg, 0.46 mmol, 2.0 eq.) and Pd(dppf)Cl₂ (17 mg, 0.46 mmol, 0.1 eq.) in 2.5 mL 1,4 dioxane/ H₂O using general procedure A, yielding the titled product **23ad** as a white solid (43 mg, 0.13 mmol, 57%).

¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.36 (d, *J*=2.4, 1H), 8.32 (s, 2H), 7.75 (d, *J*=2.4, 1H), 7.50 - 7.41 (m, 5H), 3.85 (s, 2H), 3.07 (s, 6H). **¹³C NMR** (101 MHz, DMSO) δ = 160.95, 157.73, 148.28, 146.21, 140.15, 136.97, 136.70, 135.86, 129.22, 128.35, 128.30, 120.24, 36.65, 30.71.

LC-MS (ESI): *m/z* calculated for [M+H]⁺: 325.11; found: 325.1.

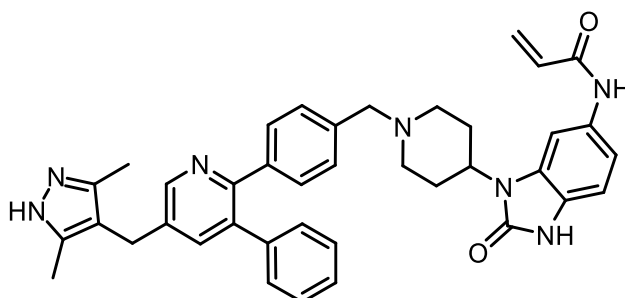
Synthesis of N-(3-(1-(4-(5-((3-methyl-1H-pyrazol-4-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (24)



Compound **23a** (20 mg, 0.07 mmol, 1.0 eq.) was reacted with compound **17** (42 mg, 0.08 mmol, 1.2 eq.), K₂CO₃ (20 mg, 0.14 mmol, 2.0 eq.) and Pd(PPh₃)₄ (8 mg, 0.007 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **24** as a white solid (12 mg, 0.004 mmol, 27%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.07 (s, 1H), 8.55 (d, *J*=1.6, 1H), 7.81 (d, *J*=2.1, 1H), 7.68 (s, 1H), 7.66 (d, *J*=2.1, 1H), 7.30 (q, *J*=4.5, 3H), 7.23 (q, *J*=9.3, 8.4, 3H), 7.17 - 7.12 (m, 1H), 6.91 (d, *J*=8.3, 1H), 6.41 (dd, *J*=16.9, 10.1, 1H), 6.24 (dd, *J*=16.9, 2.0, 1H), 6.05 (d, *J*=2.2, 1H), 5.73 (dd, *J*=10.0, 2.1, 1H), 5.37 (s, 1H), 4.11 (s, 1H), 3.50 (s, 1H), 2.93 (s, 2H), 2.31 - 2.25 (m, 2H), 2.14 (s, 2H), 2.07 (s, 2H), 1.65 (s, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.77, 155.43, 153.99, 147.79, 147.54, 147.08, 139.31, 138.44, 138.37, 137.84, 135.09, 132.63, 132.03, 131.13, 129.54, 129.25, 128.98, 128.42, 128.26, 127.41, 126.34, 124.50, 108.65, 105.51, 105.16, 100.94, 61.48, 52.60, 51.52, 28.69, 13.37. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 624.3009; found: 624.3088.

Synthesis of N-(3-(1-(4-(5-((3,5-dimethyl-1H-pyrazol-4-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (25**)**

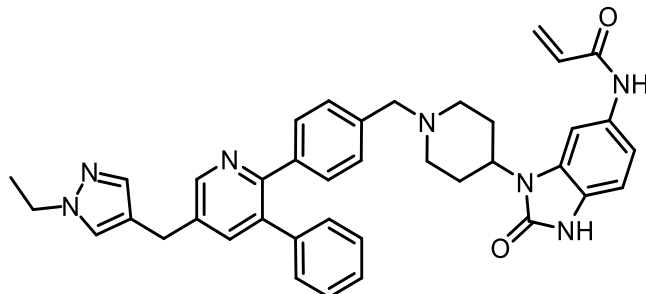


Compound **23b** (19 mg, 0.06 mmol, 1.0 eq.) was reacted with compound **17** (35 mg, 0.07 mmol, 1.1 eq.), K₂CO₃ (18 mg, 0.13 mmol, 2.0 eq.) and Pd(PPh₃)₄ (7 mg, 0.006 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **25** as a white solid (9 mg, 0.013 mmol, 22%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 12.02 (s, 1H), 10.80 (s, 1H), 10.07 (s, 1H), 8.46 (d, *J*=2.1, 1H), 7.69 (s, 1H), 7.44 (d, *J*=2.2, 1H), 7.32 - 7.26 (m, 4H), 7.23 (m, *J*=7.9, 4H), 7.16 - 7.09 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=17.0, 10.1, 1H), 6.24 (dd, *J*=16.9, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.12 (s, 1H), 3.79 (s, 2H), 3.51 (d, 2H), 2.95 (s, 2H), 2.30 (s, 2H), 2.11 (s, 8H), 1.66 (s, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.76, 153.98, 153.61, 147.96, 139.69, 137.78, 135.57, 134.93, 132.62, 132.03, 129.52, 129.23, 129.23, 128.97, 128.38, 127.24, 126.33, 124.49, 112.25, 108.64, 100.93, 69.78, 52.53, 50.07, 28.47, 25.17, 10.68.

HRMS (ESI): *m/z* calculated for [M+H]⁺: 638.3165; found 638.3226:.

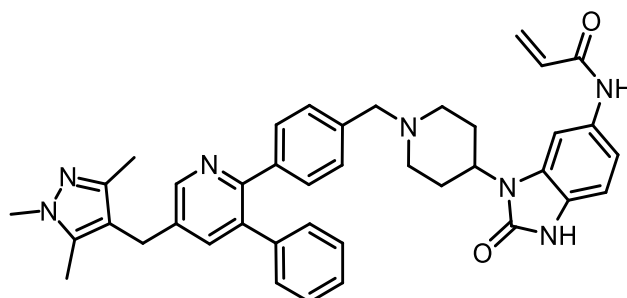
Synthesis of N-(3-(1-(4-(5-((1-ethyl-1H-pyrazol-4-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (26)



Compound **23c** (39 mg, 0.13 mmol, 1.0 eq.) was reacted with compound **17** (72 mg, 0.14 mmol, 1.1 eq.), K₂CO₃ (36 mg, 0.26 mmol, 2.0 eq.) and Pd(PPh₃)₄ (15 mg, 0.001 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **26** as a white solid (18 mg, 0.003 mmol, 22%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.08 (s, 1H), 8.55 (d, *J*=2.1, 1H), 7.68 (d, *J*=1.9, 1H), 7.63 (s, 1H), 7.62 (d, *J*=2.1, 1H), 7.36 (s, 1H), 7.32 - 7.27 (m, 4H), 7.22 (q, *J*=8.3, 4H), 7.16 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=16.9, 10.1, 1H), 6.24 (dd, *J*=17.0, 2.1, 1H), 5.76 - 5.72 (m, 1H), 4.11 (m, *J*=12.4, 4.1, 1H), 4.06 (q, *J*=7.2, 2H), 3.87 (s, 2H), 3.50 (s, 2H), 2.93 (d, *J*=11.0, 2H), 2.29 (qd, *J*=12.4, 3.9, 2H), 2.07 (td, *J*=11.9, 2.3, 2H), 1.64 (dd, *J*=11.7, 3.9, 2H), 1.32 (t, *J*=7.3, 3H). **¹³C NMR** (126 MHz, DMSO) δ = 162.77, 154.00, 153.85, 148.13, 139.66, 138.64, 138.21, 138.03, 138.03, 137.61, 135.83, 135.01, 132.65, 132.05, 129.50, 129.31, 128.98, 128.33, 128.20, 127.82, 127.23, 126.32, 124.49, 118.76, 112.11, 108.63, 100.91, 61.54, 52.63, 50.15, 46.04, 28.67, 26.58, 15.52. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 638.3165; found: 638.3240.

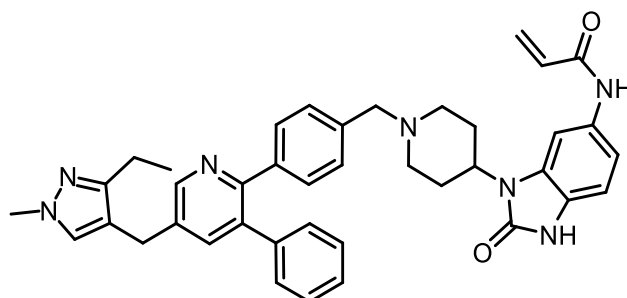
Synthesis of N-(2-oxo-3-(1-(4-(3-phenyl-5-((1,3,5-trimethyl-1H-pyrazol-4-yl)methyl)pyridin-2-yl)benzyl)piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (27**)**



Compound **23d** (35 mg, 0.11 mmol, 1.0 eq.) was reacted with compound **17** (68 mg, 0.13 mmol, 1.2 eq.), K₂CO₃ (31 mg, 0.22 mmol, 2.0 eq.) and Pd(PPh₃)₄ (13 mg, 0.01 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **27** as a white solid (12 mg, 0.02 mmol, 16%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.79 (s, 1H), 10.07 (s, 1H), 8.46 (s, 1H), 7.68 (s, 1H), 7.45 (s, 1H), 7.34 - 7.26 (m, 4H), 7.22 (d, *J*=15.5, 4H), 7.17 - 7.09 (m, 2H), 6.91 (d, *J*=8.3, 1H), 6.41 (dd, *J*=17.0, 10.1, 1H), 6.24 (dd, *J*=16.9, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.1, 1H), 4.10 (s, 1H), 3.79 (s, 2H), 3.62 (s, 3H), 3.50 (d, 1H), 2.93 (s, 2H), 2.29 (s, 2H), 2.05 (s, 5H), 1.64 (s, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.75, 153.97, 147.90, 144.10, 139.67, 137.74, 136.23, 134.94, 132.62, 132.02, 129.49, 129.24, 128.97, 128.38, 128.17, 127.24, 126.32, 124.49, 113.23, 108.63, 100.90, 69.77, 61.51, 52.60, 50.11, 35.65, 28.47, 25.56, 11.84, 9.32. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 652.3322; found: 652.3395.

Synthesis of N-(3-(1-(4-(5-((3-ethyl-1-methyl-1H-pyrazol-4-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (28)

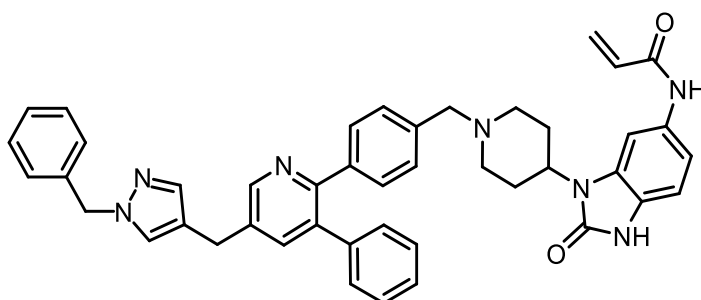


Compound **23e** (40 mg, 0.13 mmol, 1.0 eq.) was reacted with compound **17** (71 mg, 0.14 mmol, 1.1 eq.), K₂CO₃ (35 mg, 0.26 mmol, 2.0 eq.) and Pd(PPh₃)₄ (15 mg, 0.01 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **28** as a white solid (21 mg, 0.03 mmol, 25%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 10.79 (s, 1H), 10.07 (s, 1H), 8.52 (d, *J*=2.2, 1H), 7.69 (d, *J*=1.9, 1H), 7.55 (d, *J*=2.1, 1H), 7.44 (s, 1H), 7.31 - 7.27 (m, 4H), 7.22 (q, *J*=8.1, 3H), 7.15 - 7.13 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=16.9, 10.1, 1H), 6.24 (dd, *J*=16.9, 2.0, 1H), 5.78 - 5.68 (m, 1H), 4.11 (tt, *J*=13.2, 4.0, 1H), 3.82 (s, 2H), 3.70 (s, 3H), 3.51 (s, 2H), 2.94 (d, *J*=10.9, 2H), 2.50 (m, 2H) 2.29 (dt, *J*=12.1, 5.3, 2H), 2.08 (s, 2H), 1.71 - 1.60 (m, 2H), 1.10 (t, *J*=7.5, 3H). **¹³C NMR** (126 MHz, DMSO) δ = 162.77, 154.00, 153.78, 150.50, 148.12, 139.68, 138.13, 135.57, 134.98, 132.64, 132.04, 130.05, 129.51, 129.27, 128.98, 128.36, 127.24, 126.33, 124.49, 115.69, 112.14, 108.64, 100.91, 69.78, 52.59, 38.15, 28.64, 25.80, 19.18, 13.69.

HRMS (ESI): *m/z* calculated for [M+H]⁺: 652.3322; found: 652.3386.

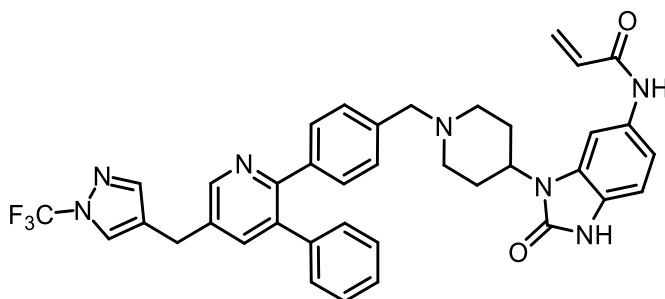
Synthesis of N-(3-(1-(4-(5-((1-benzyl-1H-pyrazol-4-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (29)



Compound **23f** (30 mg, 0.08 mmol, 1.0 eq.) was reacted with compound **17** (46 mg, 0.09 mmol, 1.1 eq.), K₂CO₃ (23 mg, 0.17 mmol, 2.0 eq.) and Pd(PPh₃)₄ (10 mg, 0.01 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **29** as a white solid (24 mg, 0.03 mmol, 41%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.07 (s, 1H), 8.55 (d, *J*=2.1, 1H), 7.73 (s, 2H), 7.68 (s, 1H), 7.60 (d, *J*=2.1, 1H), 7.42 (s, 1H), 7.33 - 7.27 (m, 7H), 7.26 - 7.18 (m, 6H), 7.16 - 7.13 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=16.9, 10.2, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 5.27 (s, 2H), 4.11 (s, 1H), 3.89 (s, 2H), 3.50 (s, 2H), 2.93 (d, *J*=8.4, 2H), 2.29 (d, *J*=11.3, 2H), 2.07 (t, *J*=9.2, 2H), 1.64 (d, *J*=9.3, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.76, 153.99, 148.10, 139.64, 138.80, 138.21, 137.79, 135.74, 135.01, 132.64, 132.04, 129.51, 129.28, 128.99, 128.45, 128.32, 128.20, 127.50, 127.44, 127.23, 126.32, 124.49, 119.30, 112.11, 108.63, 100.90, 61.53, 54.72, 52.61, 50.12, 28.67, 26.46. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 700.3322; found: 700.3391.

Synthesis of N-(2-oxo-3-(1-(4-(3-phenyl-5-((1-(trifluoromethyl)-1H-pyrazol-4-yl)methyl)pyridin-2-yl)benzyl)piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (30)

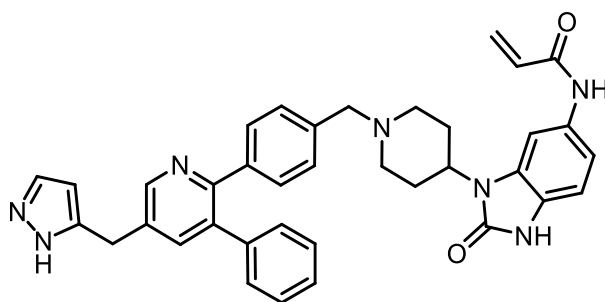


Compound **23g** (58 mg, 0.17 mmol, 1.0 eq.) was reacted with compound **17** (95 mg, 0.19 mmol, 1.1 eq.), K₂CO₃ (47 mg, 0.34 mmol, 2.0 eq.) and Pd(PPh₃)₄ (20 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **30** as a white solid (24 mg, 0.04 mmol, 21%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 10.79 (s, 1H), 10.07 (s, 1H), 8.60 (d, *J*=2.2, 1H), 8.42 (s, 1H), 7.98 (s, 1H), 7.70 (d, *J*=2.2, 1H), 7.68 (d, *J*=1.9, 1H), 7.30 (m, *J*=5.1, 1.8, 4H), 7.23 (q, *J*=8.3, 4H), 7.19 - 7.16 (m, 2H), 6.91 (d, *J*=8.3, 1H), 6.41 (dd, *J*=16.9, 10.1, 1H), 6.28 - 6.19 (m, 1H), 5.73 (dd, *J*=10.1, 2.1, 1H), 4.11 (tt, *J*=12.4, 4.2, 1H), 3.97 (s, 2H), 3.51 (s, 2H), 2.94 (d, *J*=10.6, 2H), 2.29 (qd, *J*=12.0, 3.8, 2H), 2.08 (m,

2H), 1.66 - 1.63 (m, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.77, 159.79, 154.25, 154.00, 148.18, 147.29, 144.73, 139.53, 138.59, 138.37, 135.16, 134.39, 132.64, 132.04, 129.51, 129.35, 128.98, 128.33, 128.24, 128.11, 127.29, 126.34, 124.49, 123.13, 118.77, 117.03, 115.29, 113.35, 112.13, 108.64, 108.02, 100.91, 61.50, 52.60, 50.11, 28.64, 25.92. **¹⁹F NMR** (565 MHz, DMSO) δ = -59.04. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 678.2726; found: 678.2805.

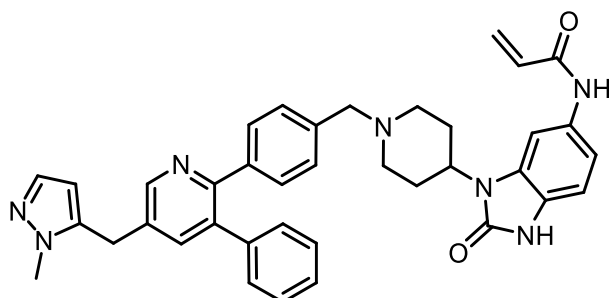
Synthesis of N-(3-(1-(4-(5-((1H-pyrazol-5-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (31)



Compound **23h** (30 mg, 0.11 mmol, 1.0 eq.) was reacted with compound **17** (61 mg, 0.12 mmol, 1.1 eq.), K_2CO_3 (31 mg, 0.22 mmol, 2.0 eq.) and $Pd(PPh_3)_4$ (13 mg, 0.01 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H_2O using general procedure B, yielding the titled product **31** as a white solid (11 mg, 0.026 mmol, 16%).

¹H NMR (600 MHz, $DMSO-d_6$) δ = 10.79 (s, 1H), 10.07 (s, 1H), 8.56 (d, $J=2.1$, 1H), 7.65 (dd, $J=16.0$, 2.0, 2H), 7.29 (tdd, $J=7.1$, 6.0, 1.5, 4H), 7.25 - 7.19 (m, 4H), 7.16 - 7.14 (m, 2H), 6.91 (d, $J=8.4$, 1H), 6.41 (dd, $J=16.9$, 10.1, 1H), 6.24 (dd, $J=17.0$, 2.0, 1H), 6.15 (d, $J=2.1$, 1H), 5.73 (dd, $J=10.1$, 2.0, 1H), 4.10 (t, $J=12.4$, 4.2, 1H), 4.05 (s, 2H), 3.50 (s, 3H), 2.93 (d, $J=12.6$, 1H), 2.29 (qd, $J=12.4$, 3.9, 2H), 2.07 (td, $J=11.8$, 2.3, 2H), 1.64 (dd, $J=11.9$, 4.3, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.74, 153.98, 148.23, 139.61, 138.59, 138.44, 137.64, 134.95, 132.63, 132.03, 129.48, 129.25, 128.96, 128.34, 128.20, 127.24, 126.31, 124.47, 112.09, 108.61, 103.68, 100.89, 69.78, 61.53, 52.62, 50.12, 28.67. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 610.2852; found: 610.2917.

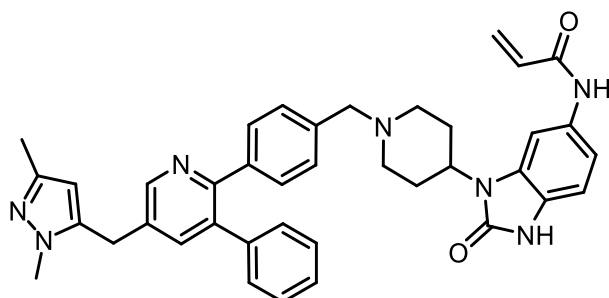
Synthesis of N-(3-(1-(4-(5-((1-methyl-1H-pyrazol-5-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (32)



Compound **23i** (35 mg, 0.12 mmol, 1.0 eq.) was reacted with compound **17** (68 mg, 0.14 mmol, 1.2 eq.), K₂CO₃ (34 mg, 0.25 mmol, 2.0 eq.) and Pd(PPh₃)₄ (14 mg, 0.01 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **32** as a white solid (12 mg, 0.026 mmol, 16%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.79 (s, 1H), 10.07 (s, 1H), 8.56 (d, *J*=2.1, 1H), 7.68 (s, 1H), 7.62 (s, 1H), 7.34 - 7.19 (m, 9H), 7.19 - 7.14 (m, 2H), 6.91 (d, *J*=8.3, 1H), 6.41 (dd, *J*=16.9, 10.1, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 6.09 (d, *J*=1.8, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.17 (s, 2H), 4.12- 4.09 (m, 1H), 3.78 (s, 3H), 3.51(s, 2H), 2.93 (d, *J*=9.5, 2H), 2.29 (d, *J*=12.4, 2H), 2.08 (d, *J*=11.7, 2H), 1.64 (d, *J*=9.4, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.76, 153.98, 148.25, 140.44, 139.46, 138.48, 137.75, 137.56, 135.15, 132.62, 132.53, 132.02, 129.52, 129.28, 128.97, 128.37, 128.21, 127.33, 126.33, 124.49, 112.10, 108.63, 105.36, 100.90, 69.78, 61.51, 52.61, 50.12, 36.22, 28.69, 27.45. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 624.3009; found: 624.3071.

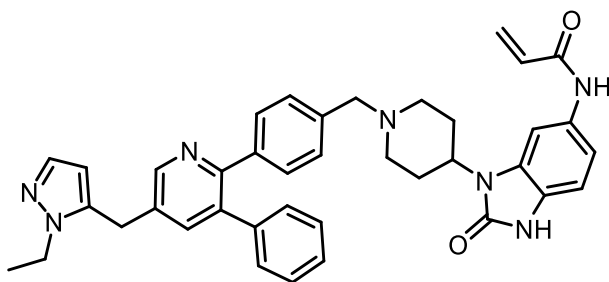
Synthesis of N-(3-(1-(4-(5-((1,3-dimethyl-1H-pyrazol-5-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (33**)**



Compound **23g** (18 mg, 0.06 mmol, 1.0 eq.) was reacted with compound **17** (33 mg, 0.066 mmol, 1.1 eq.), K₂CO₃ (17 mg, 0.12 mmol, 2.0 eq.) and Pd(PPh₃)₄ (7 mg, 0.006 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **33** as a white solid (7 mg, 0.011 mmol, 18%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.07 (s, 1H), 8.56 (s, 1H), 7.65 (d, 1H), 7.39 - 7.20 (m, 8H), 7.17 (dd, *J*=7.4, 2.1, 4H), 6.92 (d, *J*=8.4, 1H), 6.42 (dd, *J*=16.9, 10.2, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 5.84 (s, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.10 (s, 3H), 3.69 (s, 3H), 3.51 (d, *J*=1.9, 2H), 2.93 (s, 2H), 2.29 (s, 2H), 1.65 (s, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 170.81, 163.27, 154.45, 148.78, 146.13, 141.57, 138.97, 133.12, 132.47, 130.01, 129.79, 129.47, 128.88, 128.69, 127.83, 126.84, 124.99, 109.13, 105.29, 101.37, 70.26, 60.23, 53.09, 50.60, 36.28, 29.17, 28.00, 14.56, 13.70. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 638.3165; found: 638.3227.

Synthesis of N-(3-(1-(4-(5-((1-ethyl-1H-pyrazol-5-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (34**)**

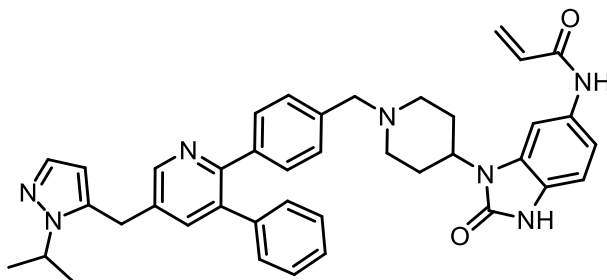


Compound **23k** (17 mg, 0.06 mmol, 1.0 eq.) was reacted with compound **17** (32 mg, 0.06 mmol, 1.1 eq.), K₂CO₃ (16 mg, 0.11 mmol, 2.0 eq.) and Pd(PPh₃)₄ (7 mg, 0.01

mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **34** as a white solid (10 mg, 0.016 mmol, 27%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.08 (s, 1H), 8.56 (d, *J*=2.1, 1H), 7.69 (s, 1H), 7.62 (d, *J*=2.2, 1H), 7.35 (d, *J*=1.8, 1H), 7.33 - 7.24 (m, 6H), 7.22 (d, *J*=8.0, 2H), 7.15 (dd, *J*=6.2, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=16.9, 10.1, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 6.07 (d, *J*=1.8, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.18 (s, 2H), 4.11 (q, 3H), 3.52 (s, 2H), 2.95 (s, 2H), 2.30 (d, *J*=10.7, 2H), 2.09 (s, 2H), 1.65 (d, *J*=9.9, 2H), 1.24 (t, *J*=7.2, 3H). **¹³C NMR** (151 MHz, DMSO) δ = 162.77, 154.34, 153.99, 148.25, 139.74, 139.48, 138.47, 137.82, 135.11, 132.75, 132.64, 132.04, 129.54, 129.25, 128.97, 128.40, 128.29, 127.34, 126.33, 124.48, 112.15, 108.64, 105.31, 100.92, 61.45, 52.57, 50.07, 43.29, 28.57, 27.29, 15.45. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 638.3165; found: 638.3225.

Synthesis of N-(3-(1-(4-(5-((1-isopropyl-1H-pyrazol-5-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (35)



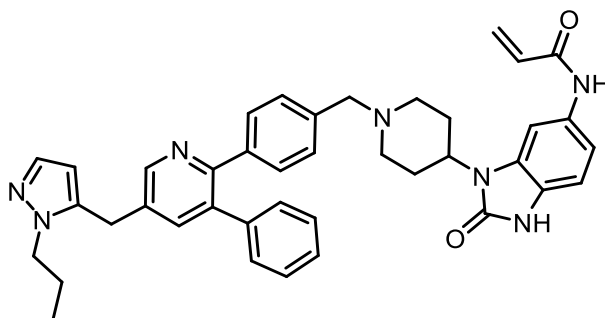
Compound **23I** (29 mg, 0.09 mmol, 1.0 eq.) was reacted with compound **17** (51 mg, 0.10 mmol, 1.1 eq.), K₂CO₃ (26 mg, 0.19 mmol, 2.0 eq.) and Pd(PPh₃)₄ (11 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **35** as a white solid (15 mg, 0.02 mmol, 25%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.07 (s, 1H), 8.56 (d, *J*=2.1, 1H), 7.69 (s, 1H), 7.60 (d, 1H), 7.37 (d, *J*=1.7, 1H), 7.33 - 7.20 (m, 8H), 7.14 (dd, *J*=7.8, 1.8, 2H), 6.91 (d, *J*=8.3, 1H), 6.41 (dd, *J*=16.9, 10.2, 1H), 6.24 (dd, *J*=16.9, 2.0, 1H), 6.05 (d, *J*=1.7, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.60 (hept, *J*=6.4, 1H), 4.18 (s, 2H), 4.11 (s, 1H), 3.50 (s, 2H), 2.93 (s, 2H), 2.29 (d, 2H), 2.07 (s, 1H), 1.64 (d, *J*=12.1, 2H), 1.30 (s, 3H), 1.29 (s, 3H). **¹³C NMR** (176 MHz, DMSO) δ = 162.77, 154.29, 153.99, 148.23, 139.50, 139.10, 138.39, 137.71, 135.07, 132.84, 132.63, 132.03, 130.83, 129.53,

129.20, 128.98, 128.41, 128.23, 127.95, 127.34, 126.67, 126.33, 125.62, 124.49, 112.12, 108.64, 105.00, 100.91, 61.51, 52.60, 50.12, 48.86, 28.66, 27.20, 22.65.

HRMS (ESI): m/z calculated for $[M+H]^+$: 652.3322; found: 652.3390.

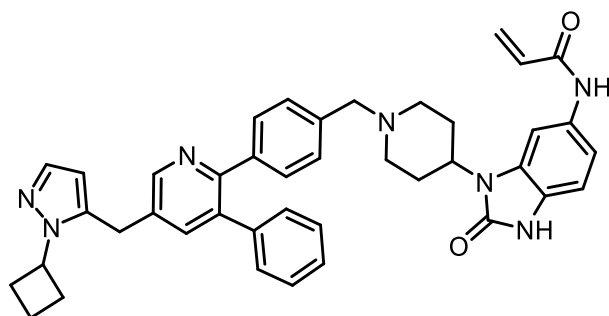
Synthesis of N-(2-oxo-3-(1-(4-(3-phenyl-5-((1-propyl-1H-pyrazol-5-yl)methyl)pyridin-2-yl)benzyl)piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (36)



Compound **23m** (35 mg, 0.11 mmol, 1.0 eq.) was reacted with compound **17** (68 mg, 0.13 mmol, 1.2 eq.), K_2CO_3 (31 mg, 0.22 mmol, 2.0 eq.) and $Pd(PPh_3)_4$ (13 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H_2O using general procedure B, yielding the titled product **36** as a white solid (12 mg, 0.02 mmol, 16%).

1H NMR (700 MHz, $DMSO-d_6$) δ = 10.80 (s, 1H), 10.07 (s, 1H), 8.56 (d, $J=2.1$, 1H), 7.69 (s, 1H), 7.63 (d, $J=2.2$, 1H), 7.36 (d, $J=1.7$, 1H), 7.31 - 7.21 (m, 8H), 7.17 - 7.13 (m, 3H), 6.91 (d, $J=8.3$, 1H), 6.41 (dd, $J=16.9$, 10.1, 1H), 6.24 (dd, $J=16.9$, 2.0, 1H), 6.07 (d, $J=1.8$, 1H), 5.73 (dd, $J=10.1$, 2.0, 1H), 4.17 (s, 2H), 4.11 (s, 1H), 4.03 (t, $J=7.2$, 2H), 3.50 (s, 2H), 2.93 (d, $J=10.8$, 2H), 2.29 (dd, $J=21.9$, 10.5, 2H), 2.08 (d, $J=12.0$, 2H), 1.66 (m, $J=19.7$, 12.0, 9.6, 4H), 0.78 (t, $J=7.4$, 3H). **^{13}C NMR** (176 MHz, $DMSO$) δ = 162.77, 154.35, 153.99, 148.27, 140.17, 139.49, 138.51, 137.83, 137.77, 135.10, 132.80, 132.64, 132.04, 129.52, 129.23, 128.98, 128.39, 128.24, 127.33, 126.33, 124.49, 112.12, 108.64, 105.21, 100.91, 61.52, 52.61, 50.13, 49.78, 28.67, 27.36, 23.20, 10.89. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 652.3322; found: 652.3390.

Synthesis of N-(3-(1-(4-(5-((1-cyclobutyl-1H-pyrazol-5-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (37**)**

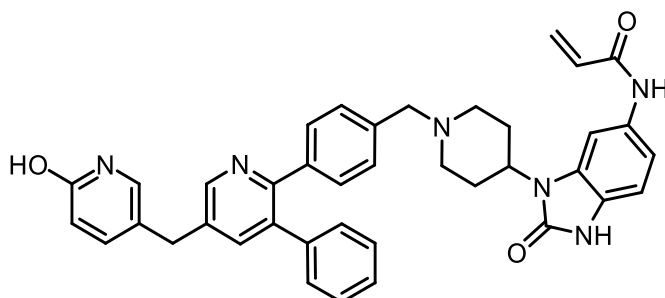


Compound **23n** (30 mg, 0.09 mmol, 1.0 eq.) was reacted with compound **17** (56 mg, 0.11 mmol, 1.2 eq.), K₂CO₃ (26 mg, 0.19 mmol, 2.0 eq.) and Pd(PPh₃)₄ (11 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **37** as a white solid (5 mg, 0.008 mmol, 8%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.07 (s, 1H), 8.53 (d, *J*=2.2, 1H), 7.68 (d, *J*=1.9, 1H), 7.54 (d, *J*=2.1, 1H), 7.42 (d, *J*=1.7, 1H), 7.29 (ddt, *J*=6.0, 3.7, 1.7, 4H), 7.25 (d, *J*=8.3, 2H), 7.21 (d, *J*=8.3, 2H), 7.13 (dd, *J*=7.7, 1.8, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=16.9, 10.2, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 6.10 (d, *J*=1.7, 1H), 5.83 - 5.70 (m, 1H), 4.90 (h, *J*=8.3, 1H), 4.16 (s, 2H), 4.10 (tt, *J*=12.4, 4.2, 1H), 3.50 (s, 2H), 2.93 (d, *J*=11.6, 3H), 2.29 (qd, *J*=12.3, 4.0, 2H), 2.23 - 2.14 (m, 2H), 2.07 (td, *J*=11.8, 2.3, 2H), 1.79 - 1.70 (m, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.76, 154.30, 154.00, 148.15, 139.62, 139.50, 138.44, 138.28, 138.02, 137.77, 135.07, 132.81, 132.64, 132.04, 129.50, 129.20, 128.97, 128.40, 128.22, 127.33, 126.32, 124.49, 112.10, 108.63, 105.48, 100.89, 61.52, 52.61, 51.28, 50.13, 29.74, 28.67, 27.16, 14.18.

HRMS (ESI): *m/z* calculated for [M+H]⁺: 664.3322; found: 664.3397.

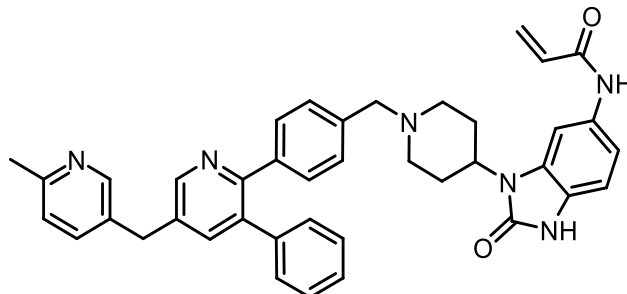
Synthesis of N-(3-(1-(4-(5-((6-hydroxypyridin-3-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (38)



Compound **23o** (19 mg, 0.06 mmol, 1.0 eq.) was reacted with compound **17** (35 mg, 0.07 mmol, 1.1 eq.), K₂CO₃ (18 mg, 0.13 mmol, 2.0 eq.) and Pd(PPh₃)₄ (7 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **38** as a white solid (6 mg, 0.01 mmol, 15%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.09 (s, 1H), 8.66 (d, *J*=2.1, 1H), 7.97 (dd, *J*=6.8, 2.1, 1H), 7.76 (d, *J*=2.1, 1H), 7.67 (d, *J*=1.9, 1H), 7.44 (ddd, *J*=8.9, 6.6, 2.1, 1H), 7.33 - 7.27 (m, 4H), 7.24 (d, *J*=8.3, 2H), 7.21 (d, *J*=8.3, 2H), 7.16 - 7.13 (m, 2H), 6.91 (d, *J*=8.3, 1H), 6.46 - 6.38 (m, 3H), 6.28 (td, *J*=6.7, 1.4, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 5.21 (s, 2H), 4.10 (tt, *J*=12.4, 4.2, 1H), 3.50 (s, 2H), 2.93 (d, *J*=12.2, 2H), 2.28 (qd, *J*=12.4, 3.9, 2H), 2.07 (td, *J*=11.9, 2.3, 2H), 1.64 (d, *J*=11.8, 2H). **¹³C NMR** (176 MHz, DMSO) δ = 162.77, 161.48, 155.48, 153.99, 147.91, 140.40, 139.32, 139.18, 138.34, 138.16, 137.93, 135.13, 132.65, 132.05, 131.57, 129.52, 129.25, 128.97, 128.42, 128.24, 127.41, 126.32, 124.49, 119.90, 112.11, 108.63, 105.86, 100.91, 61.52, 52.63, 50.13, 48.86, 28.67. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 637.2849; found: 637.2928.

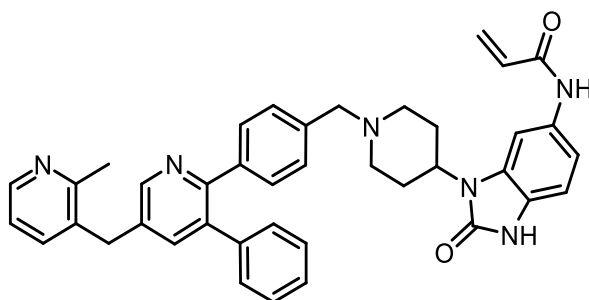
Synthesis of N-(3-(1-(4-(5-((6-methylpyridin-3-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (39)



Compound **23p** (36 mg, 0.12 mmol, 1.0 eq.) was reacted with compound **17** (67 mg, 0.13 mmol, 1.1 eq.), K₂CO₃ (34 mg, 0.24 mmol, 2.0 eq.) and Pd(PPh₃)₄ (14 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **39** as a white solid (18 mg, 0.03 mmol, 23%).

¹H NMR (500 MHz, DMSO-*d*₆) δ = 10.79 (s, 1H), 10.07 (s, 1H), 8.59 (d, *J*=2.1, 1H), 8.48 (d, *J*=2.2, 1H), 7.69 - 7.61 (m, 3H), 7.31 - 7.26 (m, 4H), 7.20 (dd, *J*=16.3, 8.5, 5H), 7.17 - 7.12 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=17.0, 10.1, 1H), 6.24 (dd, *J*=16.9, 2.1, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.10 (m, 1H), 4.04 (s, 2H), 3.50 (s, 2H), 2.93 (d, *J*=8.9, 2H), 2.42 (s, 3H), 2.29 (d, *J*=10.2, 2H), 2.06 (t, *J*=11.7, 2H), 1.64 (d, *J*=10.4, 2H). **¹³C NMR** (126 MHz, DMSO) δ = 155.84, 154.15, 153.99, 149.01, 148.27, 139.51, 138.45, 136.60, 135.15, 135.05, 132.88, 132.64, 132.04, 129.50, 129.30, 128.97, 128.33, 128.21, 127.27, 126.32, 124.49, 123.00, 112.12, 108.63, 100.91, 61.51, 52.60, 50.12, 34.24, 28.66, 23.60. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 635.3056; found: 635.3107.

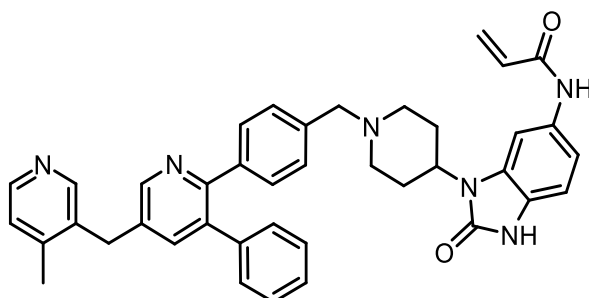
Synthesis of N-(3-(1-(4-(5-((2-methylpyridin-3-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (40)



Compound **23q** (20 mg, 0.07 mmol, 1.0 eq.) was reacted with compound **17** (38 mg, 0.07 mmol, 1.1 eq.), K₂CO₃ (19 mg, 0.14 mmol, 2.0 eq.) and Pd(PPh₃)₄ (9 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **40** as a white solid (13 mg, 0.2 mmol, 30%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.08 (s, 1H), 8.53 (d, *J*=2.2, 1H), 8.33 (dd, *J*=4.8, 1.7, 1H), 7.68 (d, *J*=1.9, 1H), 7.63 (dd, *J*=7.7, 1.7, 1H), 7.56 (d, *J*=2.1, 1H), 7.31 - 7.26 (m, 4H), 7.25 (d, *J*=8.3, 2H), 7.22 - 7.19 (m, 3H), 7.16 - 7.13 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=16.9, 10.2, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.11 (s, 2H), 4.08 (d, *J*=4.3, 1H), 3.50 (s, 2H), 2.93 (d, *J*=12.1, 2H), 2.49 (s, 3H), 2.29 (qd, *J*=12.4, 3.9, 2H), 2.07 (t, *J*=10.8, 2H), 1.64 (d, *J*=9.9, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.77, 156.52, 154.19, 154.00, 148.41, 147.00, 139.51, 138.51, 138.49, 137.73, 137.09, 135.11, 133.67, 133.42, 132.65, 132.05, 129.52, 129.28, 128.98, 128.37, 128.21, 127.31, 126.33, 124.49, 121.64, 112.12, 108.64, 100.90, 61.53, 52.63, 50.14, 34.33, 28.67, 22.41. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 635.3056; found: 635.3106.

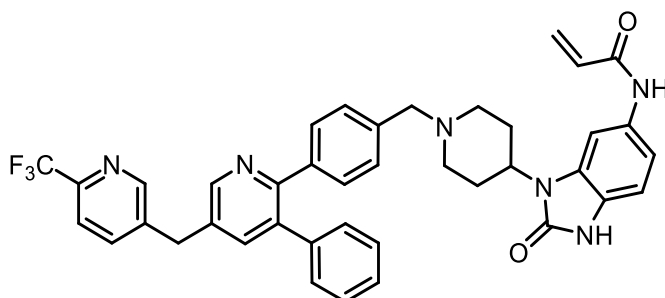
Synthesis of N-(3-(1-(4-(5-((4-methylpyridin-3-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (41)



Compound **23r** (54 mg, 0.18 mmol, 1.0 eq.) was reacted with compound **17** (101 mg, 0.20 mmol, 1.1 eq.), K₂CO₃ (51 mg, 0.37 mmol, 2.0 eq.) and Pd(PPh₃)₄ (21 mg, 0.02 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **41** as a white solid (6 mg, 0.01 mmol, 4%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.08 (s, 1H), 8.53 (d, *J*=2.1, 1H), 8.48 (s, 1H), 8.33 (d, *J*=4.9, 1H), 7.68 (s, 1H), 7.56 (d, *J*=2.1, 1H), 7.33 - 7.18 (m, 10H), 7.16 - 7.11 (m, 2H), 6.91 (d, *J*=8.3, 1H), 6.41 (dd, *J*=16.9, 10.1, 1H), 6.23 (dd, *J*=16.9, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.13 (s, 2H), 4.10 (s, 1H), 3.50 (s, 2H), 2.93 (s, 2H), 2.29 (s, 5H), 2.07 (s, 2H), 1.65 (s, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.77, 153.98, 150.20, 148.29, 147.93, 145.44, 139.47, 138.36, 137.71, 135.11, 134.14, 133.78, 132.63, 132.03, 129.53, 129.27, 128.97, 128.37, 128.21, 127.31, 126.32, 125.36, 124.49, 112.12, 108.64, 100.91, 61.50, 52.60, 50.10, 32.19, 28.69, 18.64. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 635.3056; found: 635.3136.

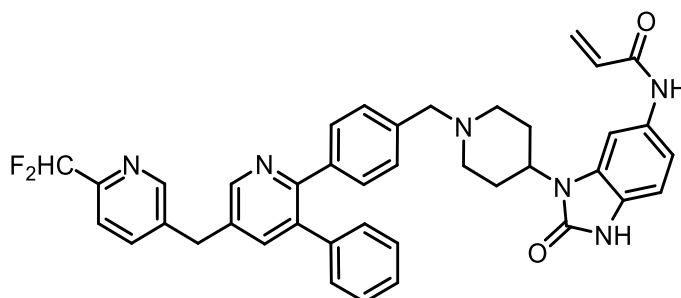
Synthesis of N-(2-oxo-3-(1-(4-(3-phenyl-5-((6-(trifluoromethyl)pyridin-3-yl)methyl)pyridin-2-yl)benzyl)piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (42**)**



Compound **23s** (34 mg, 0.10 mmol, 1.0 eq.) was reacted with compound **17** (54 mg, 0.11 mmol, 1.1 eq.), K₂CO₃ (27 mg, 0.19 mmol, 2.0 eq.) and Pd(PPh₃)₄ (11 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **42** as a white solid (13 mg, 0.02 mmol, 19%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.81 (s, 1H), 10.11 (s, 1H), 8.84 (d, *J*=2.1, 1H), 8.66 (d, *J*=2.2, 1H), 8.07 (dd, *J*=8.1, 2.2, 1H), 7.85 (d, *J*=8.1, 1H), 7.77 (d, *J*=2.2, 1H), 7.69 (d, *J*=1.9, 1H), 7.32 - 7.26 (m, 4H), 7.24 (d, *J*=8.3, 2H), 7.20 (d, *J*=8.2, 2H), 7.19 - 7.14 (m, 2H), 6.91 (d, *J*=8.3, 1H), 6.42 (dd, *J*=16.9, 10.1, 1H), 6.24 (dd, *J*=16.9, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.23 (s, 2H), 4.10 (tt, *J*=12.5, 4.3, 1H), 3.50 (s, 2H), 2.93 (d, *J*=11.1, 2H), 2.29 (qd, *J*=12.3, 3.9, 2H), 2.06 (t, *J*=11.2, 2H), 1.64 (d, *J*=9.9, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 154.52, 154.01, 150.46, 148.45, 144.99, 144.77, 144.54, 144.32, 140.29, 139.42, 138.74, 138.47, 138.31, 137.78, 135.30, 133.95, 132.67, 132.07, 129.51, 129.35, 128.98, 128.34, 128.23, 127.34, 126.32, 124.50, 122.69, 120.87, 120.79, 120.77, 112.13, 108.65, 100.92, 61.53, 52.63, 50.15, 34.32, 28.68. **¹⁹F NMR** (565 MHz, DMSO) δ = -66.19, -73.44. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 689.2774; found: 689.2833.

Synthesis of N-(3-(1-(4-(5-((6-(difluoromethyl)pyridin-3-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (43**)**

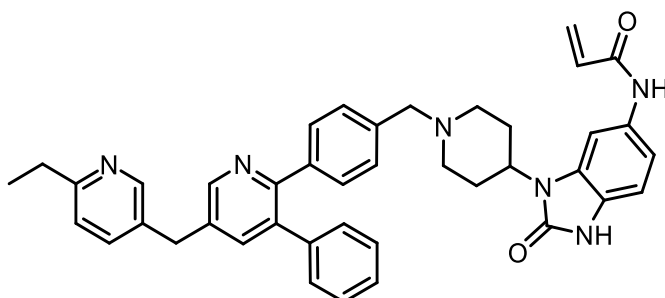


Compound **23t** (37 mg, 0.11 mmol, 1.0 eq.) was reacted with compound **17** (62 mg, 0.12 mmol, 1.2 eq.), K₂CO₃ (31 mg, 0.22 mmol, 2.0 eq.) and Pd(PPh₃)₄ (13 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **43** as a white solid (23 mg, 0.03 mmol, 31%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.82 (s, 1H), 10.09 (s, 1H), 8.77 - 8.73 (m, 1H), 8.65 (s, 1H), 7.98 (d, *J*=7.6, 1H), 7.76 (s, 1H), 7.70 (s, 1H), 7.66 (d, *J*=8.0, 1H), 7.31 - 7.20 (m, 8H), 7.17 - 7.15 (m, 2H), 6.92 (s, 1H), 6.91 (t, 1H), 6.42 (dd, *J*=16.9, 10.1, 1H), 6.24 (dd, *J*=17.0, 2.2, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.19 (s, 2H), 4.11 (s, 1H), 3.50 (s, 2H), 2.93 (s, 2H), 2.30 (s, 2H), 2.06 (s, 2H), 1.65 (s, 2H) **¹³C NMR** (151 MHz, DMSO) δ = 162.79, 158.06, 157.86, 157.66, 157.46, 154.33, 153.98, 150.31, 150.15, 149.98, 149.86, 148.42, 139.40, 138.67, 138.55, 137.84, 135.31, 134.30, 132.59, 132.01, 129.58, 129.35, 128.99, 128.35, 127.35, 126.35, 124.53, 120.45, 120.42, 120.40, 115.41, 113.84, 112.26, 108.69, 100.96, 66.35, 61.50, 54.91, 52.58, 50.11, 34.37, 28.69. **¹⁹F NMR** (565 MHz, DMSO) δ = -73.43, -114.84, -114.94.

HRMS (ESI): *m/z* calculated for [M+H]⁺: 671.2868; found: 671.2940.

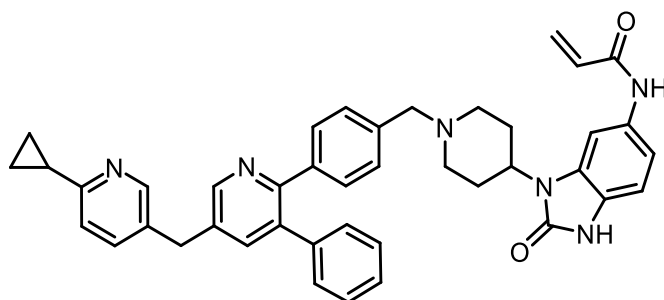
Synthesis of N-(3-(1-(4-(5-((6-ethylpyridin-3-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (44)



Compound **23u** (66 mg, 0.21 mmol, 1.0 eq.) was reacted with compound **17** (118 mg, 0.24 mmol, 1.1 eq.), K₂CO₃ (59 mg, 0.43 mmol, 2.0 eq.) and Pd(PPh₃)₄ (25 mg, 0.043 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **44** as a white solid (14 mg, 0.02 mmol, 10%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.81 (s, 1H), 10.07 (s, 1H), 8.61 (d, *J*=2.1, 1H), 8.51 (d, *J*=2.3, 1H), 7.69 (s, 2H), 7.67 (dd, *J*=8.0, 2.4, 1H), 7.33 - 7.18 (m, 9H), 7.17 - 7.14 (m, 2H), 6.92 (d, *J*=8.3, 1H), 6.41 (dd, *J*=16.9, 10.2, 1H), 6.24 (dd, *J*=16.9, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.1, 1H), 4.11 (s, 1H), 4.05 (s, 2H), 3.50 (s, 2H), 2.93 (s, 2H), 2.70 (q, *J*=7.6, 2H), 2.29 (s, 2H), 2.06 (s, 2H), 1.65 (s, 2H), 1.19 (t, *J*=7.6, 3H). **¹³C NMR** (151 MHz, DMSO) δ = 162.77, 160.79, 153.99, 149.01, 148.31, 139.49, 138.48, 137.68, 136.68, 135.19, 135.06, 133.14, 132.62, 132.03, 129.53, 129.32, 128.98, 128.34, 128.22, 127.29, 126.34, 124.50, 121.90, 112.14, 108.65, 100.93, 61.52, 52.60, 50.12, 34.29, 30.16, 28.67, 13.77. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 649.3213; found: 649.3283.

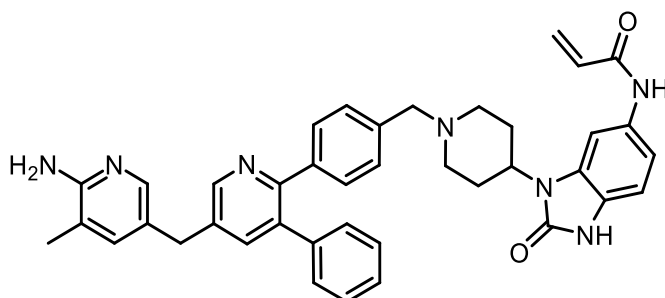
Synthesis of N-(3-(1-(4-(5-((6-cyclopropylpyridin-3-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (45)



Compound **23v** (49 mg, 0.15 mmol, 1.0 eq.) was reacted with compound **17** (84 mg, 0.17 mmol, 1.1 eq.), K₂CO₃ (42 mg, 0.31 mmol, 2.0 eq.) and Pd(PPh₃)₄ (18 mg, 0.02 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **45** as a white solid (14 mg, 0.02 mmol, 14%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.07 (s, 1H), 8.59 (d, *J*=2.1, 1H), 8.42 (d, *J*=2.3, 1H), 7.67 (d, *J*=2.2, 2H), 7.60 (dd, *J*=8.1, 2.3, 1H), 7.32 - 7.26 (m, 4H), 7.25 - 7.18 (m, 5H), 7.18 - 7.10 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=16.9, 10.2, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.1, 1H), 4.11 (s, 1H), 4.02 (s, 2H), 3.50 (s, 2H), 2.93 (s, 2H), 2.29 (d, 2H), 2.13 - 1.96 (m, 3H), 1.65 (s, 2H), 0.89 (dt, *J*=7.7, 2.6, 2H), 0.86 (dt, *J*=4.8, 2.6, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.76, 160.08, 154.14, 153.98, 149.13, 148.27, 139.50, 138.54, 138.44, 137.67, 136.28, 135.16, 135.10, 132.63, 132.59, 132.03, 129.51, 129.31, 128.97, 128.32, 128.20, 127.27, 126.32, 124.49, 121.27, 112.12, 108.63, 100.91, 61.52, 52.60, 50.12, 34.26, 28.67, 16.30, 9.52. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 661.3213; found: 661.3208.

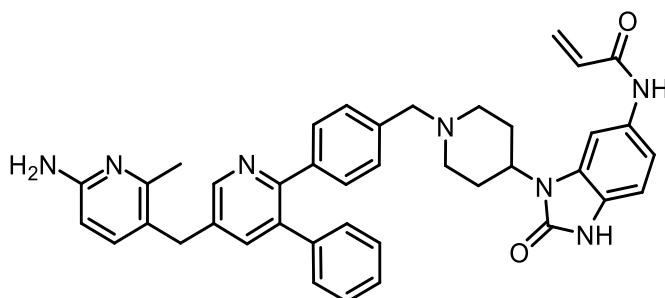
Synthesis of N-(3-(1-(4-(5-((6-amino-5-methylpyridin-3-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (46**)**



Compound **23w** (17 mg, 0.05 mmol, 1.0 eq.) was reacted with compound **17** (30 mg, 0.06 mmol, 1.1 eq.), K₂CO₃ (15 mg, 0.11 mmol, 2.0 eq.) and Pd(PPh₃)₄ (6 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **46** as a white solid (5 mg, 0.008 mmol, 14%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.07 (s, 1H), 8.55 (d, *J*=2.1, 1H), 7.80 (d, *J*=2.3, 1H), 7.68 (s, 1H), 7.61 (d, *J*=2.1, 1H), 7.32 - 7.27 (m, 4H), 7.23 (dd, *J*=14.2, 5.2, 5H), 7.15 (dd, *J*=7.7, 1.8, 2H), 6.91 (d, *J*=8.3, 1H), 6.41 (dd, *J*=16.9, 10.2, 1H), 6.24 (dd, *J*=16.9, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 5.59 (s, 2H), 4.12 (s, 1H), 3.85 (s, 2H), 3.53 (s, 2H), 2.95 (s, 2H), 2.30 (d, *J*=12.8, 2H), 2.09 (s, 2H), 2.00 (s, 3H), 1.65 (d, *J*=11.6, 2H). **¹³C NMR** (176 MHz, DMSO) δ = 162.76, 156.87, 153.98, 153.85, 148.18, 144.42, 139.60, 138.26, 137.67, 136.04, 135.07, 132.62, 132.03, 129.52, 129.31, 128.97, 128.34, 128.25, 127.25, 126.34, 124.49, 123.95, 116.08, 112.17, 108.64, 100.92, 61.42, 52.54, 50.01, 33.97, 28.54, 17.02. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 650.3165; found: 650.3232.

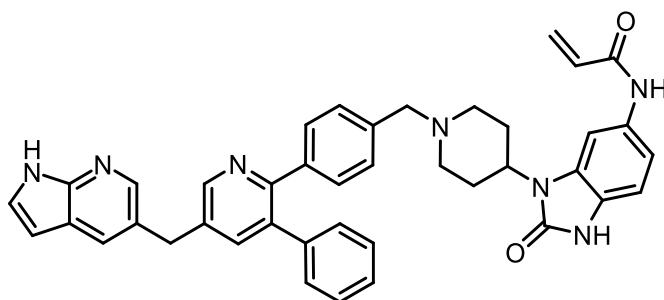
Synthesis of N-(3-(1-(4-(5-((6-amino-2-methylpyridin-3-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (47**)**



Compound **23x** (30 mg, 0.10 mmol, 1.0 eq.) was reacted with compound **17** (54 mg, 0.11 mmol, 1.1 eq.), K₂CO₃ (27 mg, 0.19 mmol, 2.0 eq.) and Pd(PPh₃)₄ (11 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **47** as a white solid (8 mg, 0.01 mmol, 13%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.08 (s, 1H), 8.48 (d, *J*=2.1, 1H), 7.67 (d, *J*=1.9, 1H), 7.49 (d, *J*=2.2, 1H), 7.32 - 7.26 (m, 4H), 7.26 - 7.22 (m, 3H), 7.20 (d, *J*=8.3, 2H), 7.14 - 7.12 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=16.9, 10.2, 1H), 6.27 - 6.22 (m, 2H), 5.73 (dd, *J*=10.1, 2.0, 1H), 5.67 (s, 2H), 4.10 (tt, *J*=12.4, 4.2, 1H), 3.90 (s, 2H), 3.50 (s, 2H), 2.93 (d, *J*=11.3, 2H), 2.29 (m, *J*=12.4, 3.9, 2H), 2.25 (s, 3H), 2.06 (td, *J*=11.8, 2.4, 2H), 1.64 (d, *J*=10.0, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.79, 157.87, 154.01, 153.86, 153.83, 148.19, 139.66, 138.99, 138.59, 138.12, 137.64, 135.18, 135.01, 132.65, 132.05, 129.51, 129.26, 128.99, 128.38, 128.21, 127.26, 126.36, 124.50, 120.27, 112.13, 108.65, 105.60, 100.93, 61.55, 52.63, 50.15, 33.64, 28.68, 21.91. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 650.3165; found: 650.3236.

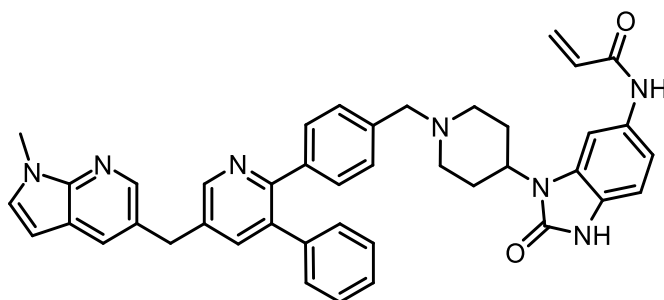
Synthesis of N-(3-(1-(4-(5-((1H-pyrrolo[2,3-b]pyridin-5-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (48**)**



Compound **23y** (40 mg, 0.13 mmol, 1.0 eq.) was reacted with compound **17** (69 mg, 0.14 mmol, 1.2 eq.), K₂CO₃ (35 mg, 0.25 mmol, 2.0 eq.) and Pd(PPh₃)₄ (15 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **48** as a white solid (9 mg, 0.014 mmol, 11%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 11.56 (s, 1H), 10.79 (s, 1H), 10.06 (s, 1H), 8.63 (d, *J*=2.1, 1H), 8.25 (d, *J*=2.1, 1H), 7.91 (d, *J*=2.0, 1H), 7.68 (d, *J*=2.1, 1H), 7.66 (d, *J*=1.9, 1H), 7.43 (dd, *J*=3.4, 2.5, 1H), 7.32 - 7.25 (m, 4H), 7.24 - 7.18 (m, 4H), 7.16 - 7.12 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.43 - 6.34 (m, 2H), 6.24 (dd, *J*=16.9, 2.0, 1H), 5.86 - 5.67 (m, 1H), 4.15 (s, 2H), 4.10 (tt, *J*=12.4, 4.2, 1H), 3.49 (s, 2H), 2.92 (d, *J*=12.5, 2H), 2.28 (qd, *J*=12.4, 3.9, 2H), 2.06 (t, *J*=11.7, 2H), 1.63 (d, *J*=10.0, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.75, 153.98, 153.96, 148.26, 147.46, 143.20, 139.60, 138.60, 138.37, 137.62, 136.14, 135.11, 132.64, 132.04, 129.49, 129.29, 128.97, 128.31, 128.19, 127.90, 127.39, 127.22, 126.52, 126.32, 124.48, 119.61, 112.10, 108.61, 100.90, 99.52, 61.52, 54.91, 52.61, 50.12, 34.95, 28.66. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 660.3009; found: 660.3068.

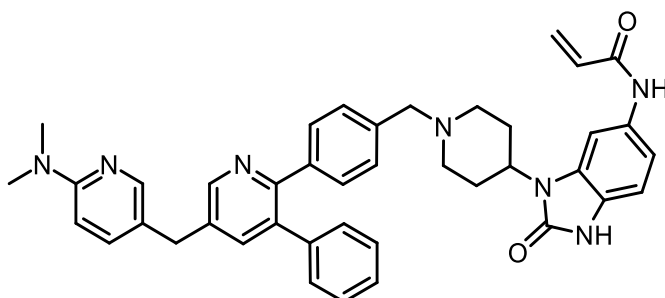
Synthesis of N-(3-(1-(4-(5-((1-methyl-1H-pyrrolo[2,3-b]pyridin-5-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (49**)**



Compound **23z** (45 mg, 0.13 mmol, 1.0 eq.) was reacted with compound **17** (75 mg, 0.15 mmol, 1.1 eq.), K₂CO₃ (37 mg, 0.27 mmol, 2.0 eq.) and Pd(PPh₃)₄ (16 mg, 0.01 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **49** as a white solid (17 mg, 0.03 mmol, 19%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.07 (s, 1H), 8.63 (d, *J*=2.1, 1H), 8.31 (d, *J*=2.0, 1H), 7.91 (d, *J*=2.0, 1H), 7.66 (d, *J*=2.1, 2H), 7.47 (d, *J*=3.4, 1H), 7.33 - 7.25 (m, 4H), 7.25 - 7.17 (m, 4H), 7.14 (dd, *J*=7.5, 2.0, 2H), 6.91 (d, *J*=8.3, 1H), 6.40 (q, *J*=3.4, 2H), 6.24 (dd, *J*=16.9, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H) 4.16 (s, 2H), 4.11 (s, 1H), 3.78 (s, 3H), 3.49 (s, 2H), 2.92 (s, 2H), 2.28 (d, *J*=11.9, 2H), 2.05 (s, 2H), 1.63 (d, *J*=11.9, 2H). **¹³C NMR** (176 MHz, DMSO) δ = 162.77, 153.99, 153.95, 148.25, 146.48, 143.09, 139.59, 138.59, 138.36, 137.62, 136.12, 135.12, 132.64, 132.04, 130.46, 129.51, 129.29, 128.98, 128.32, 128.29, 128.21, 127.42, 127.24, 126.34, 124.50, 119.92, 112.13, 108.64, 100.92, 98.52, 61.52, 54.92, 52.60, 50.12, 34.85, 30.84, 28.67. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 674.3165; found: 674,3227.

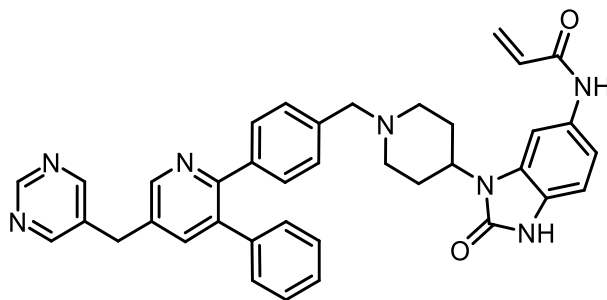
Synthesis of N-(3-(1-(4-(5-((6-(dimethylamino)pyridin-3-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (50**)**



Compound **23aa** (44 mg, 0.14 mmol, 1.0 eq.) was reacted with compound **17** (82 mg, 0.16 mmol, 1.2 eq.), K₂CO₃ (38 mg, 0.27 mmol, 2.0 eq.) and Pd(PPh₃)₄ (16 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **50** as a white solid (18 mg, 0.03 mmol, 20%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.08 (s, 1H), 8.56 (d, *J*=2.1, 1H), 8.13 - 8.04 (m, 1H), 7.68 (s, 1H), 7.61 (d, *J*=2.1, 1H), 7.46 (dd, *J*=8.7, 2.5, 1H), 7.31 - 7.25 (m, 4H), 7.26 - 7.18 (m, 4H), 7.17 - 7.11 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.59 (dd, *J*=8.7, 0.8, 1H), 6.41 (dd, *J*=16.9, 10.1, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.11 (s, 1H), 3.91 (s, 2H), 3.50 (s, 2H), 2.97 (s, 6H), 2.93 (s, 2H), 2.30 (d, *J*=12.8, 2H), 2.07 (s, 2H), 1.64 (d, *J*=12.1, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 162.77, 157.95, 153.99, 153.91, 148.17, 147.25, 139.61, 138.62, 138.24, 137.77, 137.57, 135.96, 135.09, 132.64, 132.04, 129.51, 129.29, 128.97, 128.32, 128.23, 127.23, 126.32, 124.49, 122.95, 112.15, 108.63, 105.87, 100.92, 61.49, 52.58, 50.09, 37.72, 33.80, 28.63. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 665.3274; found: 665.3424.

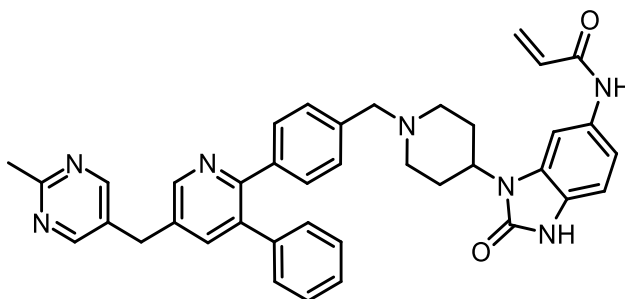
Synthesis of N-(2-oxo-3-(1-(4-(3-phenyl-5-(pyrimidin-5-ylmethyl)pyridin-2-yl)benzyl)piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (51)



Compound **23ab** (28 mg, 0.10 mmol, 1.0 eq.) was reacted with compound **17** (55 mg, 0.11 mmol, 1.2 eq.), K₂CO₃ (47 mg, 0.20 mmol, 2.0 eq.) and Pd(PPh₃)₄ (12 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **51** as a white solid (16 mg, 0.03 mmol, 26%).

¹H NMR (700 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.07 (s, 1H), 9.07 (s, 1H), 8.87 (s, 2H), 8.65 (d, *J*=2.1, 1H), 7.76 (d, *J*=2.1, 1H), 7.68 (s, 1H), 7.32 - 7.27 (m, 4H), 7.26 - 7.19 (m, 4H), 7.19 - 7.15 (m, 2H), 6.91 (d, *J*=8.3, 1H), 6.41 (dd, *J*=16.9, 10.2, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.12 (s, 3H), 3.50 (s, 2H), 2.93 (d, *J*=10.5, 2H), 2.34 - 2.25 (m, 2H), 2.06 (t, *J*=11.9, 2H), 1.70 - 1.57 (m, 2H). **¹³C NMR** (176 MHz, DMSO) δ = 162.76, 157.11, 156.74, 154.48, 153.99, 148.34, 139.41, 138.63, 138.47, 137.76, 135.29, 134.12, 133.88, 132.63, 132.03, 129.52, 129.35, 128.97, 128.32, 128.21, 127.32, 126.33, 124.49, 112.11, 108.63, 100.91, 61.52, 52.61, 50.12, 32.02, 28.67. **HRMS (ESI)**: *m/z* calculated for [M+H]⁺: 622.2852; found: 622.2922.

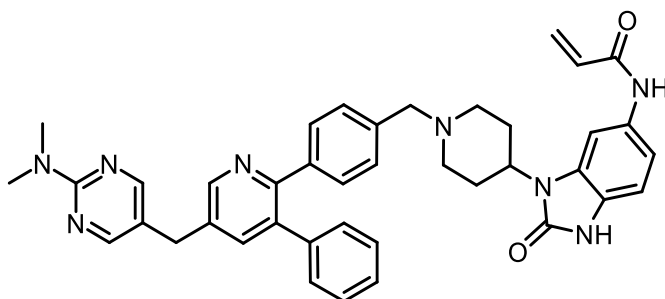
Synthesis of N-(3-(1-(4-(5-((2-methylpyrimidin-5-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (52)



Compound **23ac** (44 mg, 0.15 mmol, 1.0 eq.) was reacted with compound **17** (82 mg, 0.16 mmol, 1.1 eq.), K₂CO₃ (41 mg, 0.30 mmol, 2.0 eq.) and Pd(PPh₃)₄ (17 mg, 0.01 mmol, 0.1 eq.) in 2 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **52** as a white solid (16 mg, 0.03 mmol, 17%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.80 (s, 1H), 10.07 (s, 1H), 8.73 (s, 2H), 8.63 (d, *J*=2.2, 1H), 7.72 (d, *J*=2.2, 1H), 7.68 (s, 1H), 7.32 - 7.25 (m, 4H), 7.26 - 7.18 (m, 4H), 7.18 - 7.14 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.41 (dd, *J*=16.9, 10.1, 1H), 6.24 (dd, *J*=16.9, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.11 (t, *J*=9.7, 1H), 4.06 (s, 2H), 3.50 (s, 2H), 2.93 (d, *J*=11.0, 2H), 2.57 (s, 3H), 2.29 (d, *J*=12.9, 2H), 2.06 (s, 2H), 1.64 (d, *J*=11.2, 2H). **¹³C NMR** (151 MHz, DMSO) δ = 165.43, 162.76, 157.10, 154.39, 153.99, 148.27, 139.43, 138.51, 137.72, 135.25, 134.19, 132.63, 132.04, 130.66, 129.51, 129.33, 128.97, 128.31, 128.22, 127.29, 126.32, 124.48, 112.12, 108.63, 100.91, 61.50, 52.59, 50.11, 31.67, 28.65, 25.22. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 636.3009; found: 636.3082.

Synthesis of N-(3-(1-(4-(5-((2-(dimethylamino)pyrimidin-5-yl)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (53**)**



Compound **23ad** (34 mg, 0.10 mmol, 1.0 eq.) was reacted with compound **17** (63 mg, 0.13 mmol, 1.2 eq.), K₂CO₃ (29 mg, 0.21 mmol, 2.0 eq.) and Pd(PPh₃)₄ (12 mg, 0.01 mmol, 0.1 eq.) in 1 mL 1,4 dioxane/ H₂O using general procedure B, yielding the titled product **53** as a white solid (8 mg, 0.02 mmol, 12%).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.79 (s, 1H), 10.06 (s, 1H), 8.58 (d, *J*=2.2, 1H), 8.37 (s, 2H), 7.66 (dd, *J*=12.1, 2.0, 2H), 7.33 - 7.27 (m, 4H), 7.24 - 7.19 (m, 4H), 7.17 - 7.11 (m, 2H), 6.91 (d, *J*=8.4, 1H), 6.40 (djd, *J*=16.9, 10.1, 1H), 6.24 (dd, *J*=17.0, 2.0, 1H), 5.73 (dd, *J*=10.1, 2.0, 1H), 4.10 (tt, *J*=12.4, 4.1, 1H), 3.89 (s, 2H), 3.50 (s, 2H), 3.08 (s, 6H), 2.93 (d, *J*=11.1, 2H), 2.28 (qd, *J*=12.0, 3.4, 2H), 2.07 (t, *J*=11.6, 2H), 1.64

(d, $J=12.1$, 2H). **^{13}C NMR** (151 MHz, DMSO) δ = 162.76, 160.97, 157.76, 154.12, 153.98, 148.10, 139.56, 138.57, 138.22, 137.66, 135.32, 135.17, 132.63, 132.04, 129.49, 129.32, 128.97, 128.31, 128.20, 127.26, 126.33, 124.48, 120.60, 112.10, 108.62, 100.90, 61.52, 52.62, 50.12, 36.67, 31.22, 28.66. **HRMS (ESI)**: m/z calculated for $[\text{M}+\text{H}]^+$: 665.3274; found: 665.3360.

4.6.9.2. NMR Spectra

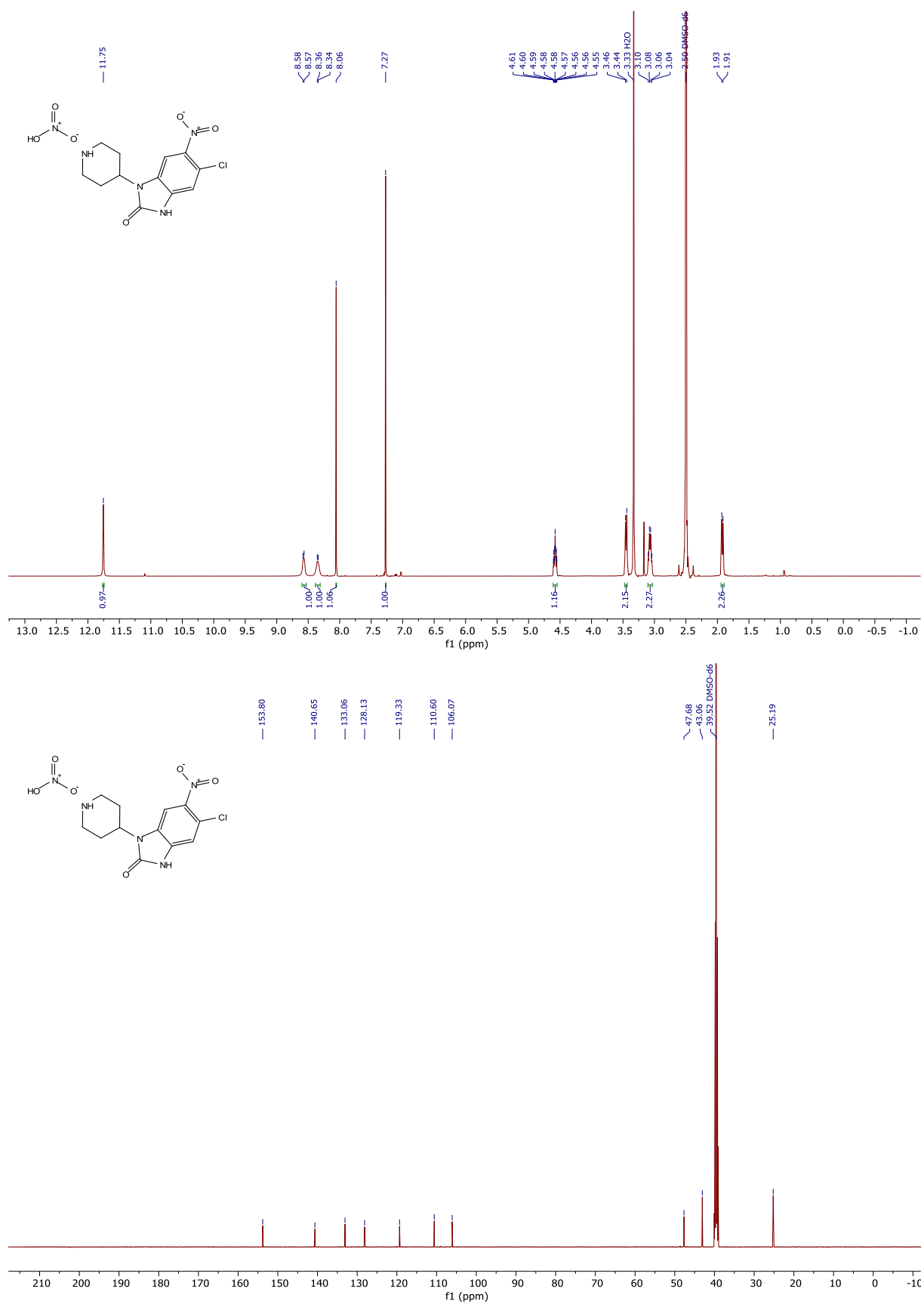


Figure S1. ¹H- and ¹³C-NMR spectra of compound 12.

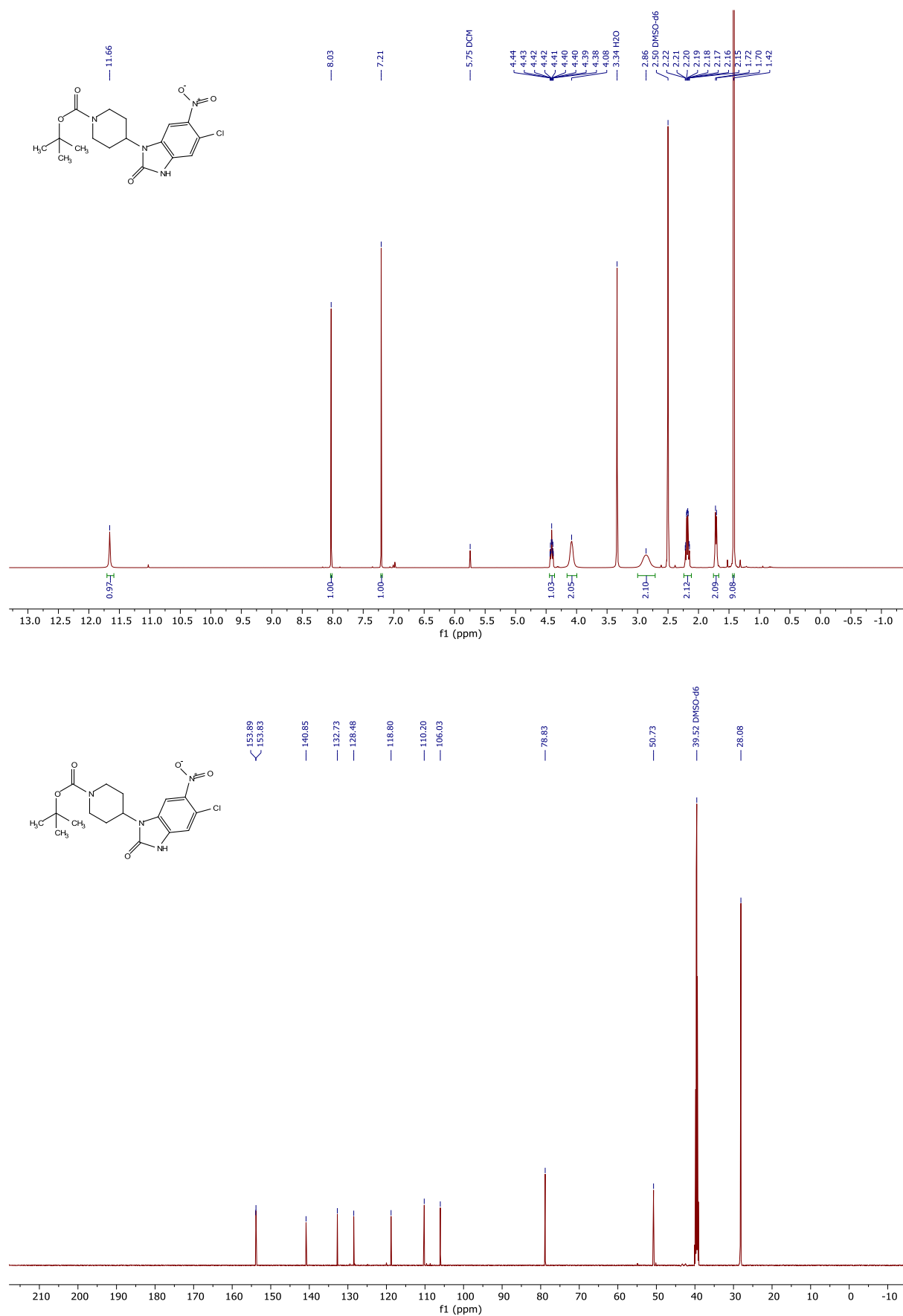


Figure S2. ^1H - and ^{13}C -NMR spectra of compound **13**.

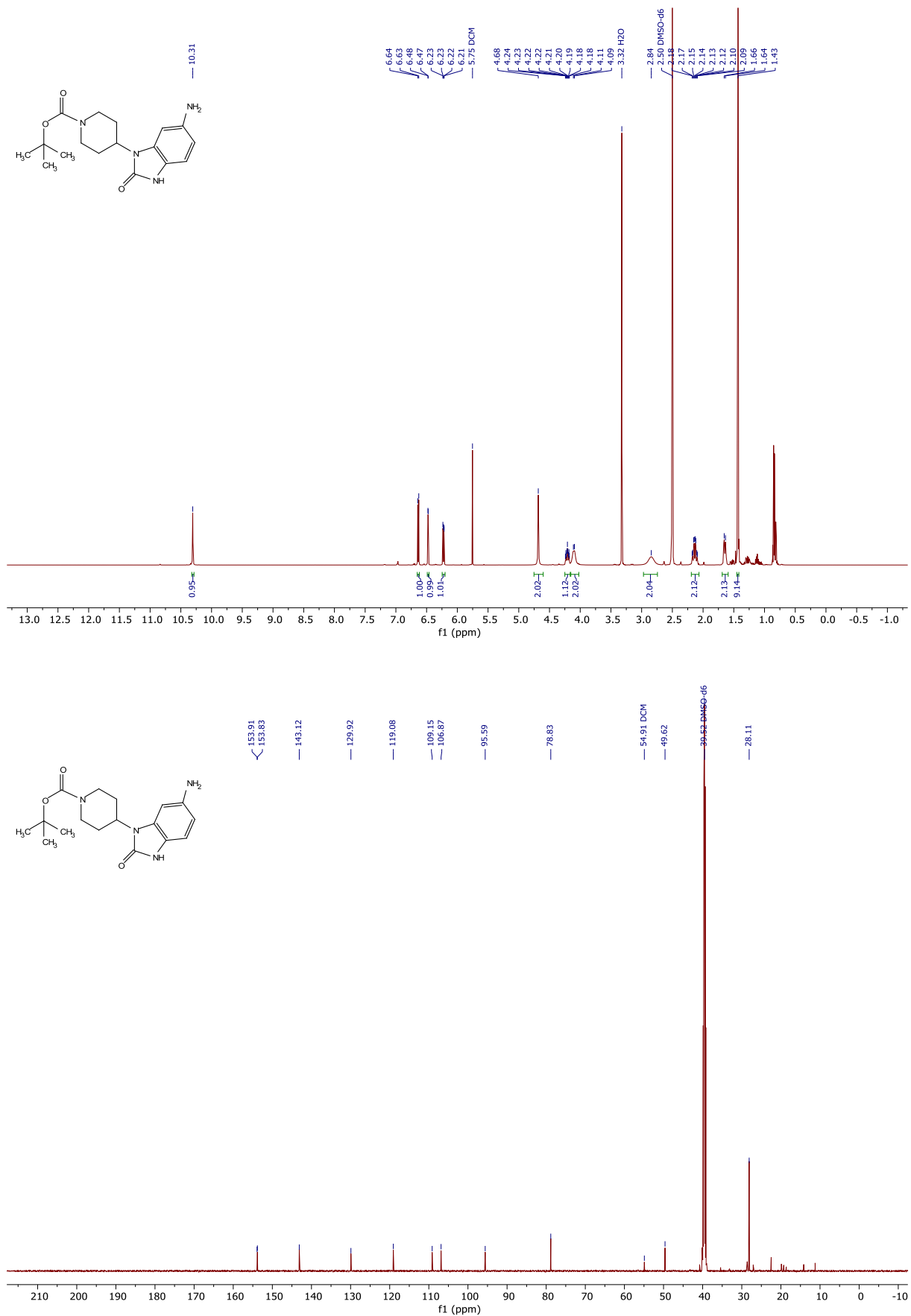


Figure S3. ¹H- and ¹³C-NMR spectra of compound 14.

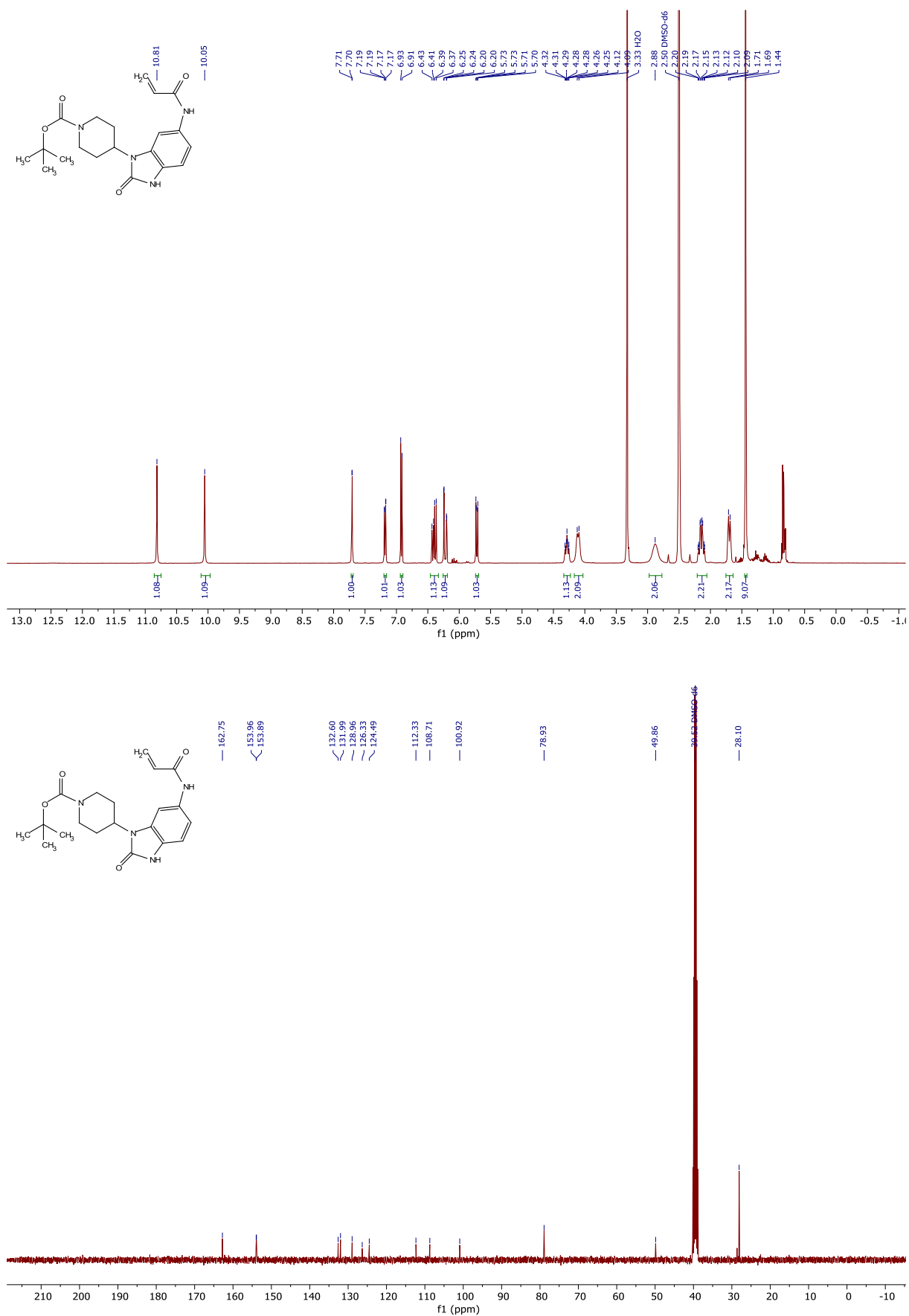


Figure S4. ^1H - and ^{13}C -NMR spectra of compound 15.

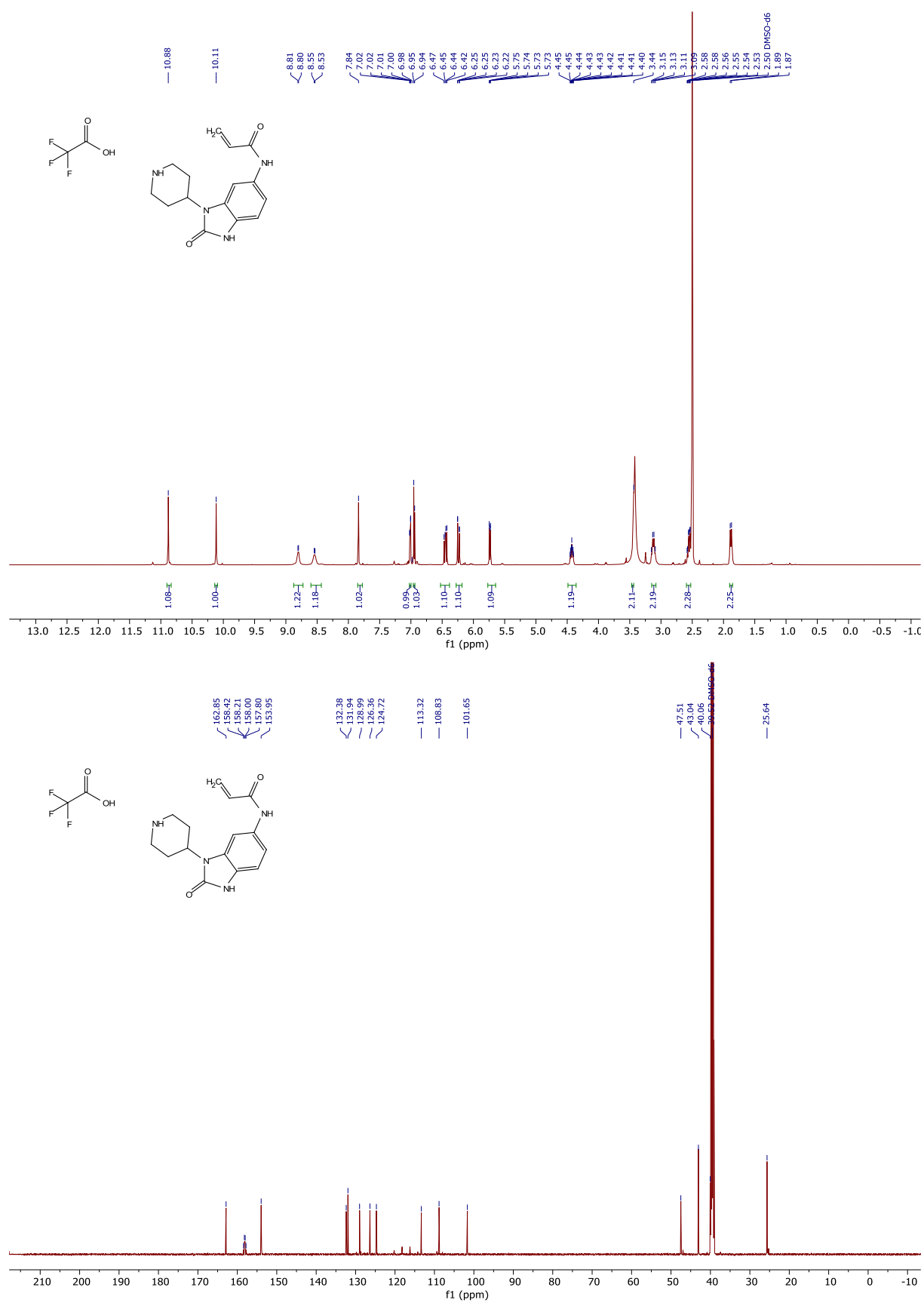


Figure S5. ¹H- and ¹³C-NMR spectra of compound 16.

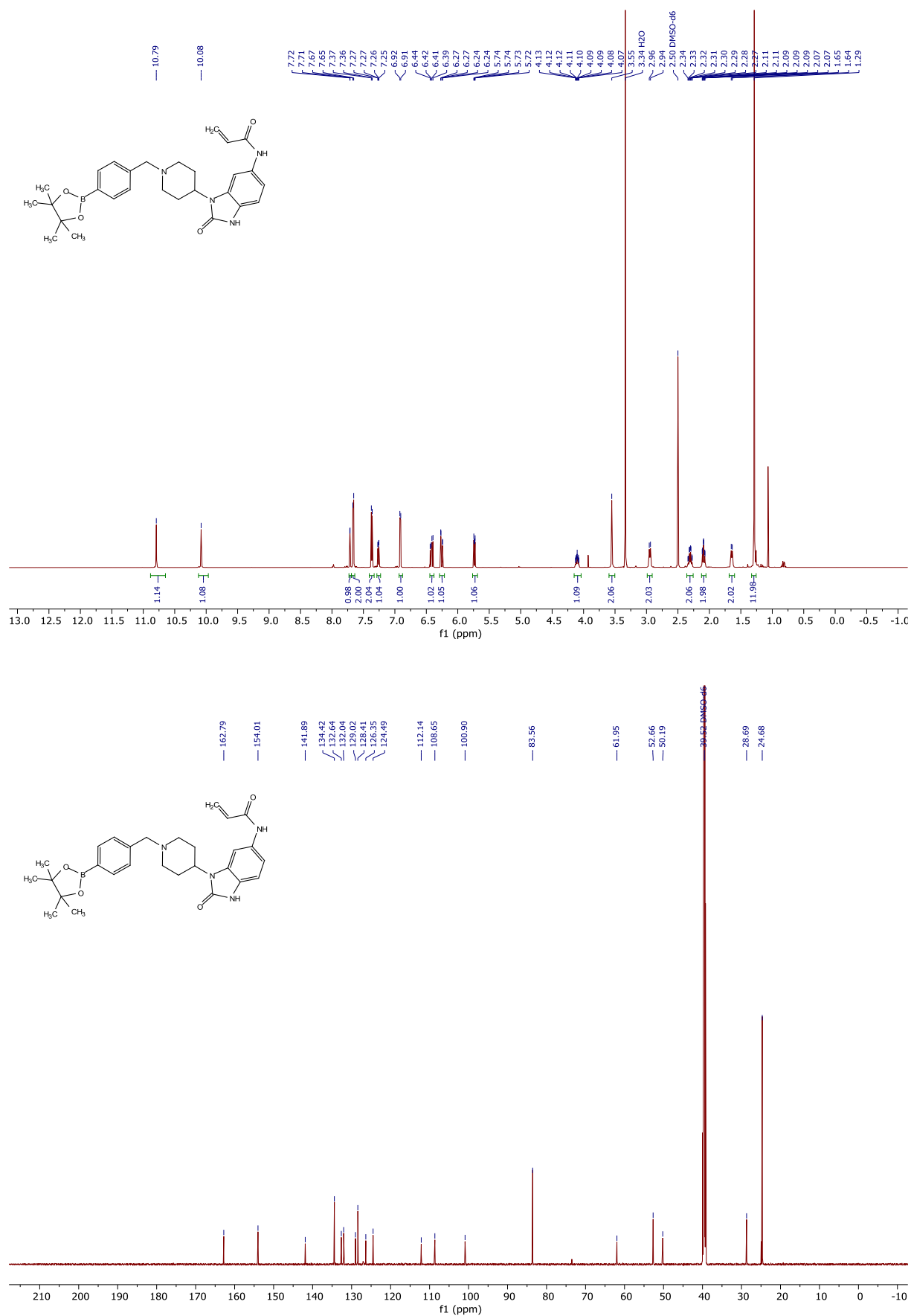


Figure S6. ¹H- and ¹³C-NMR spectra of compound 17.

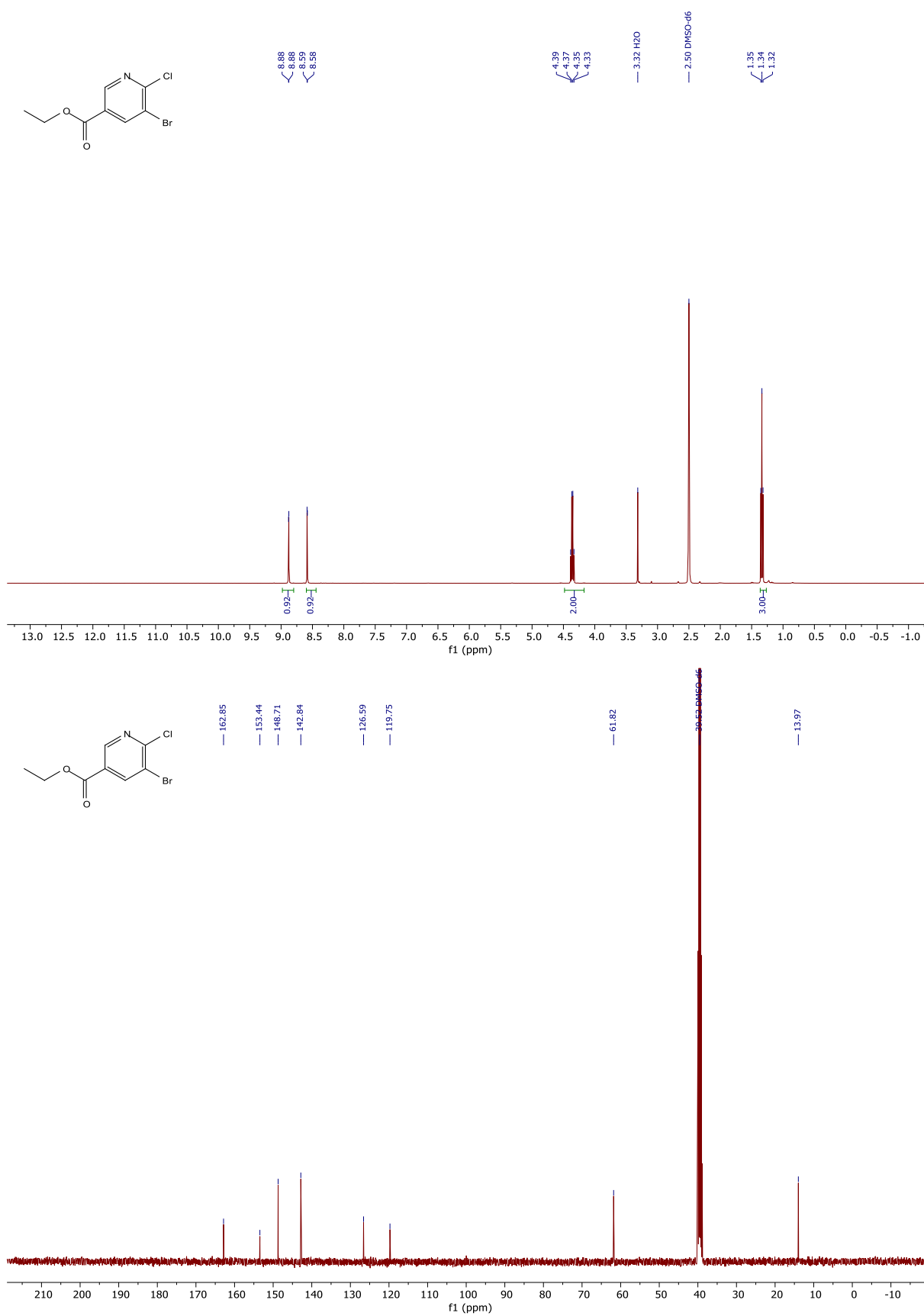


Figure S7. ¹H- and ¹³C-NMR spectra of compound 19.

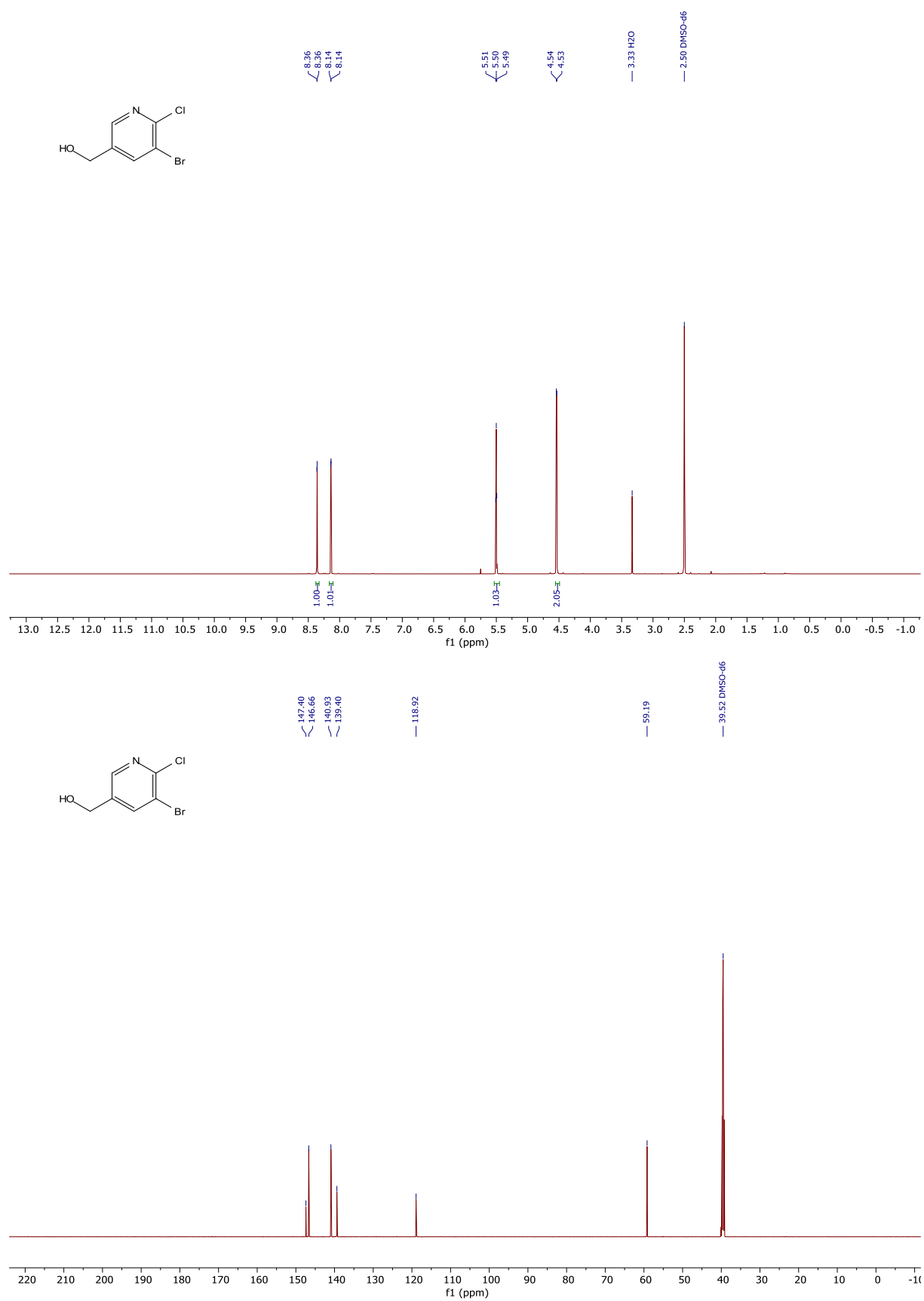


Figure S8. ¹H- and ¹³C-NMR spectra of compound 20.

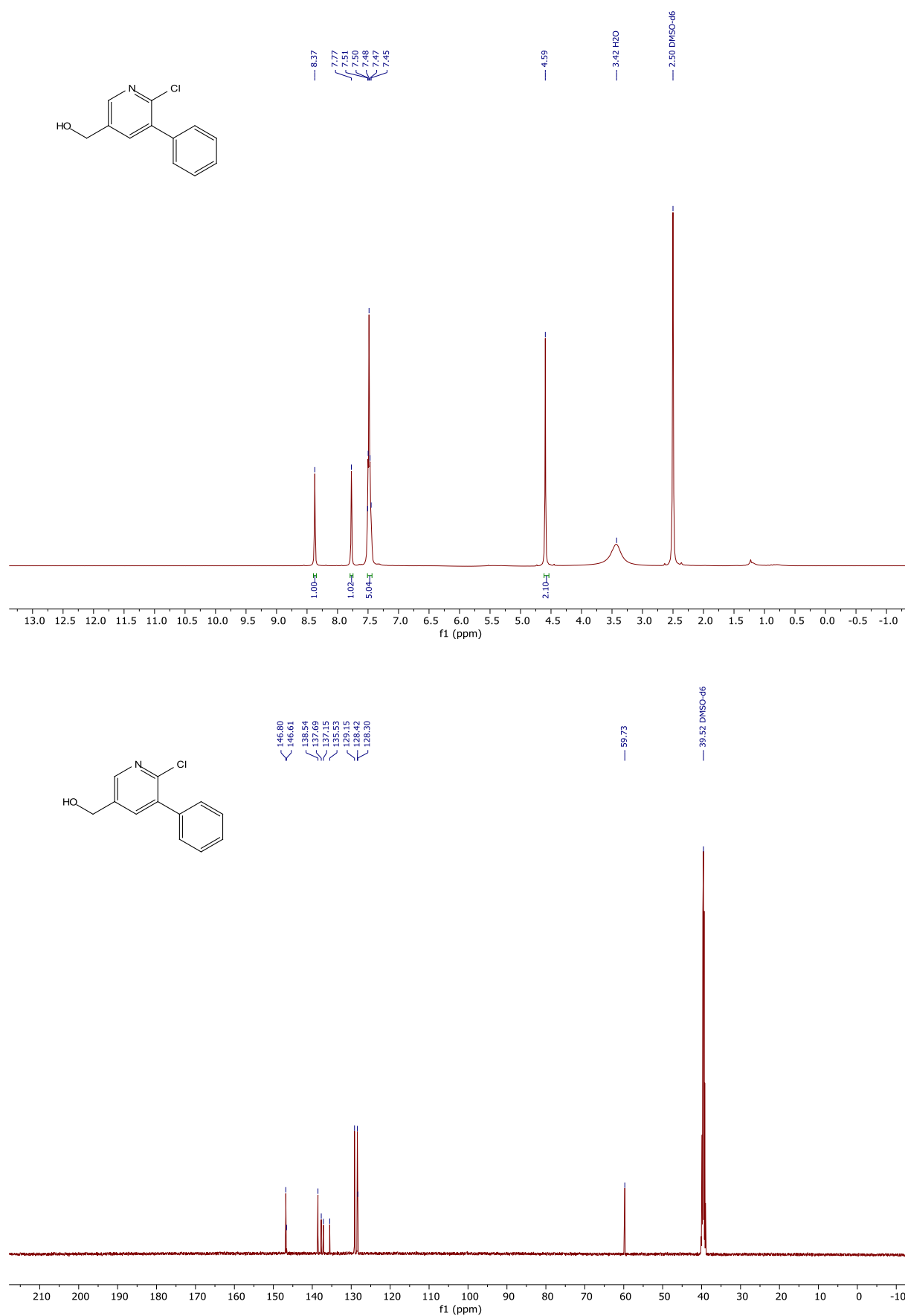


Figure S9. ¹H- and ¹³C-NMR spectra of compound **21**.

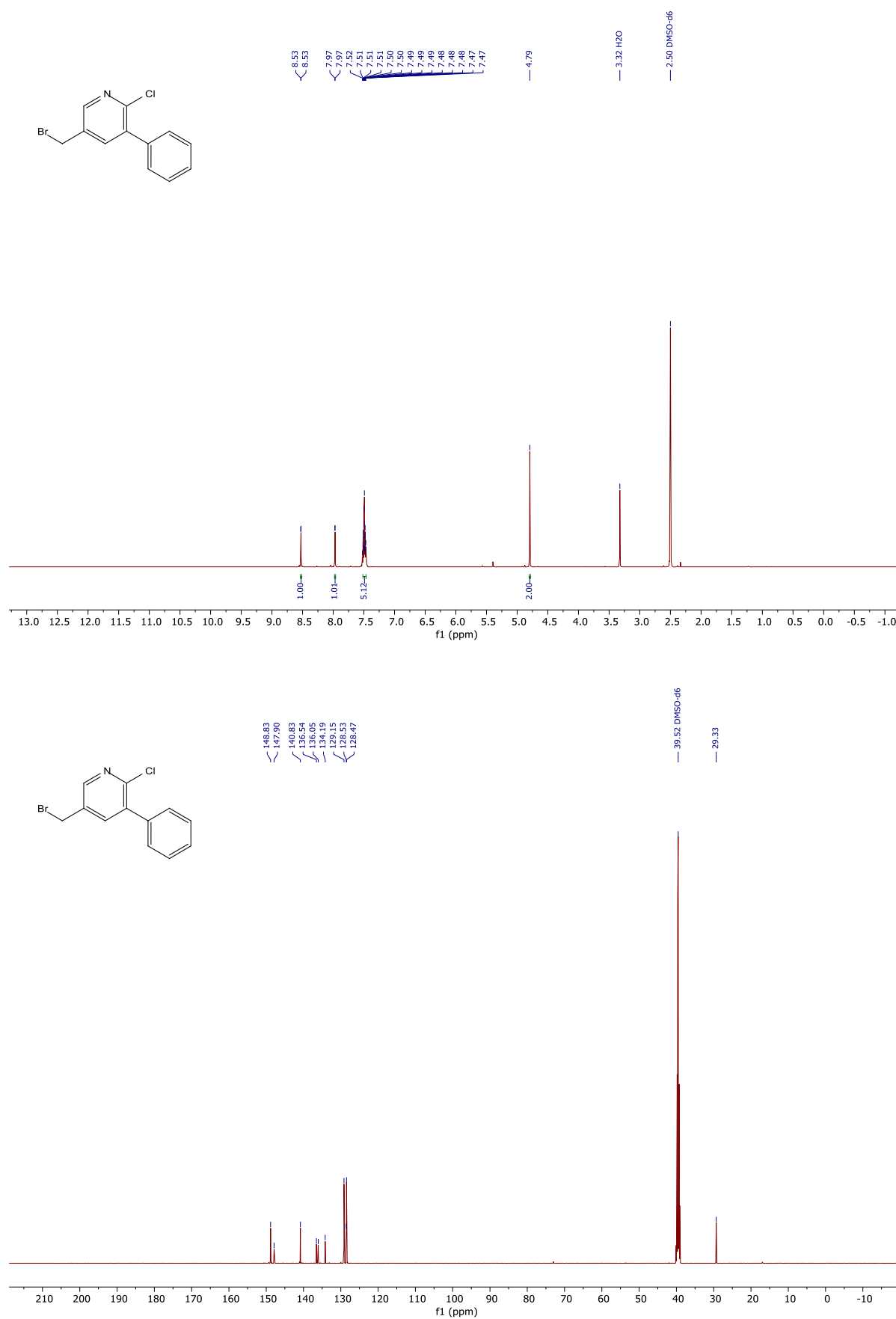


Figure S10. ¹H- and ¹³C-NMR spectra of compound **22**.

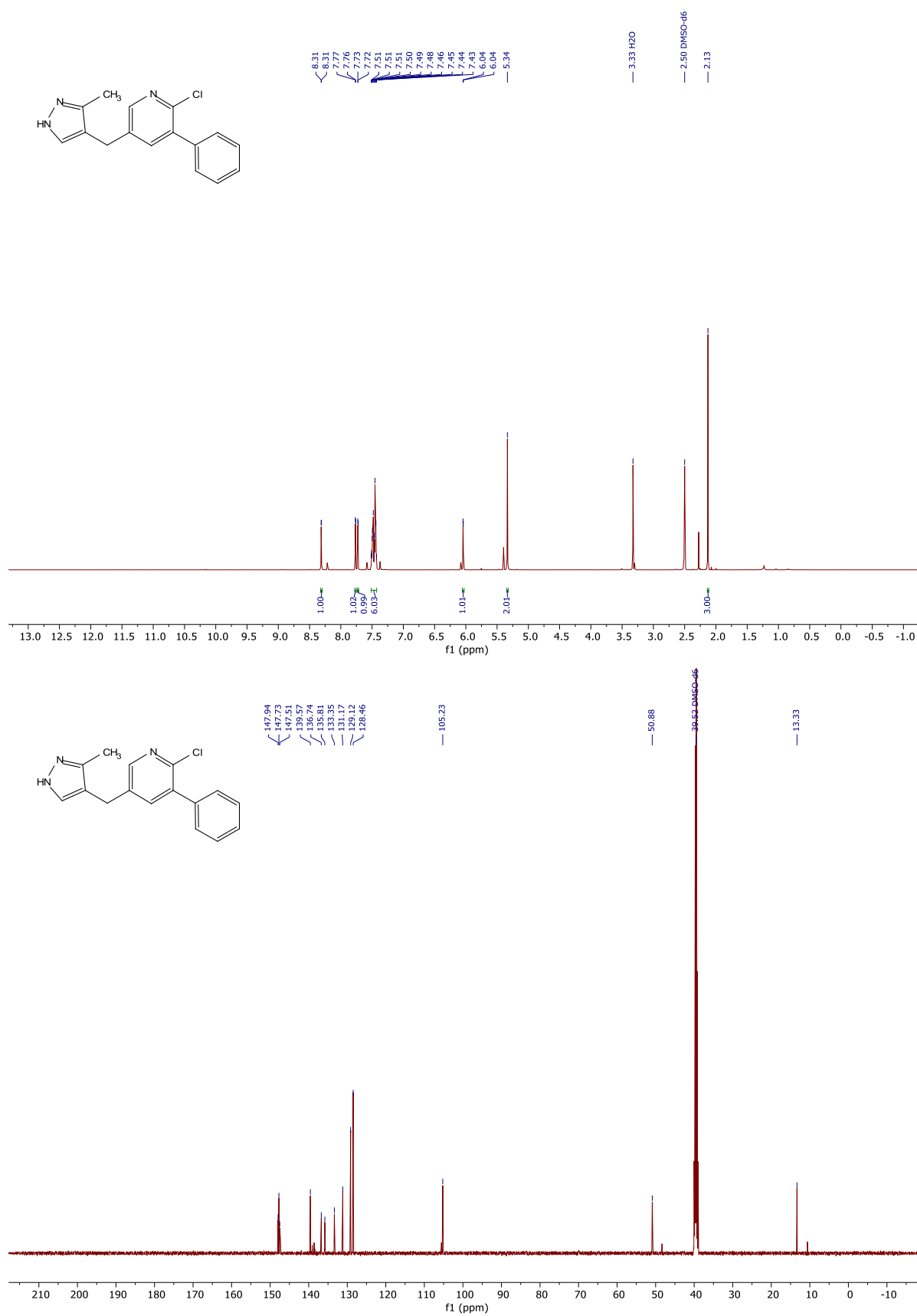


Figure S11. ¹H- and ¹³C-NMR spectra of compound **23a**.

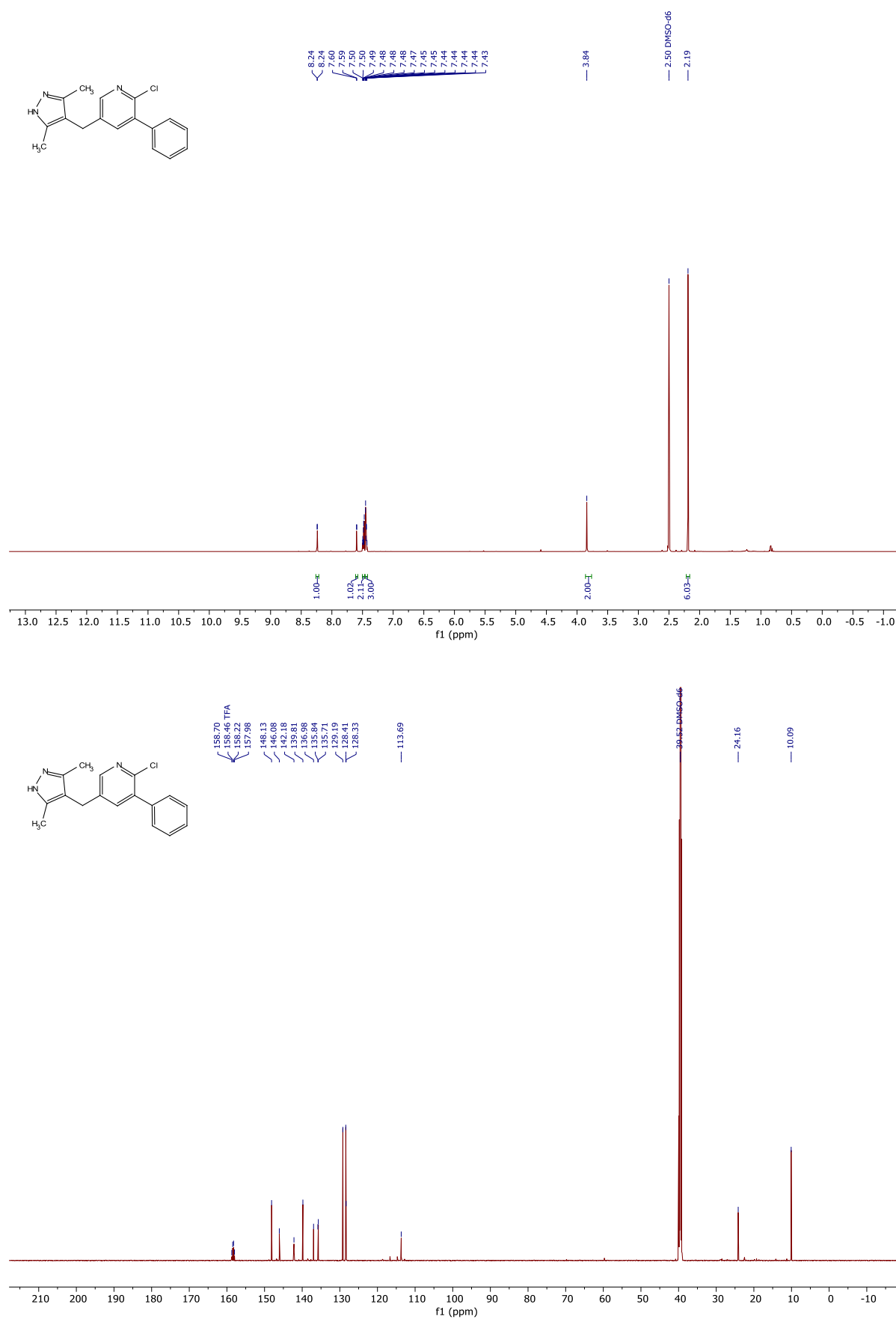


Figure S12. ^1H - and ^{13}C -NMR spectra of compound **23b**.

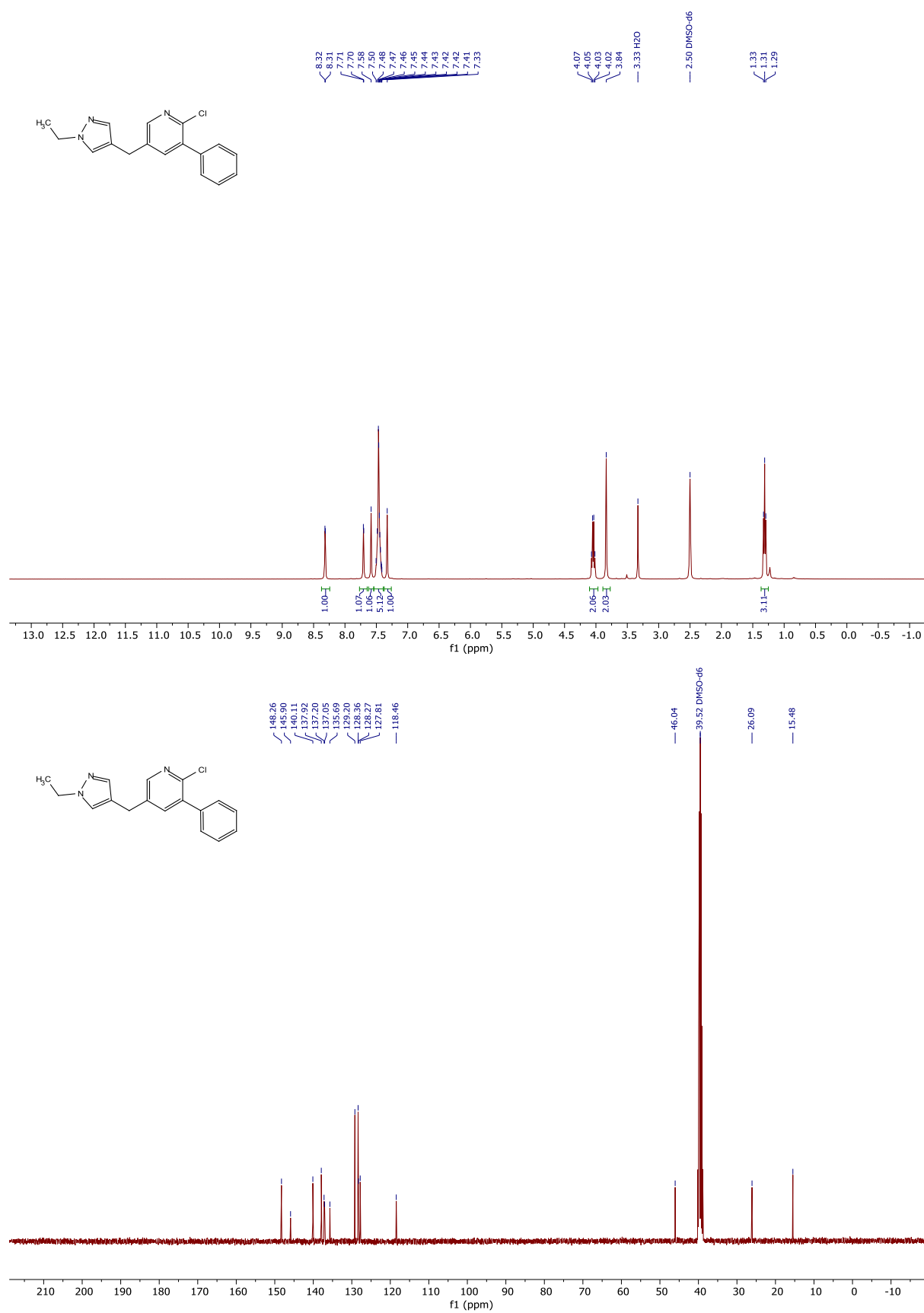


Figure S13. ¹H- and ¹³C-NMR spectra of compound **23c**.

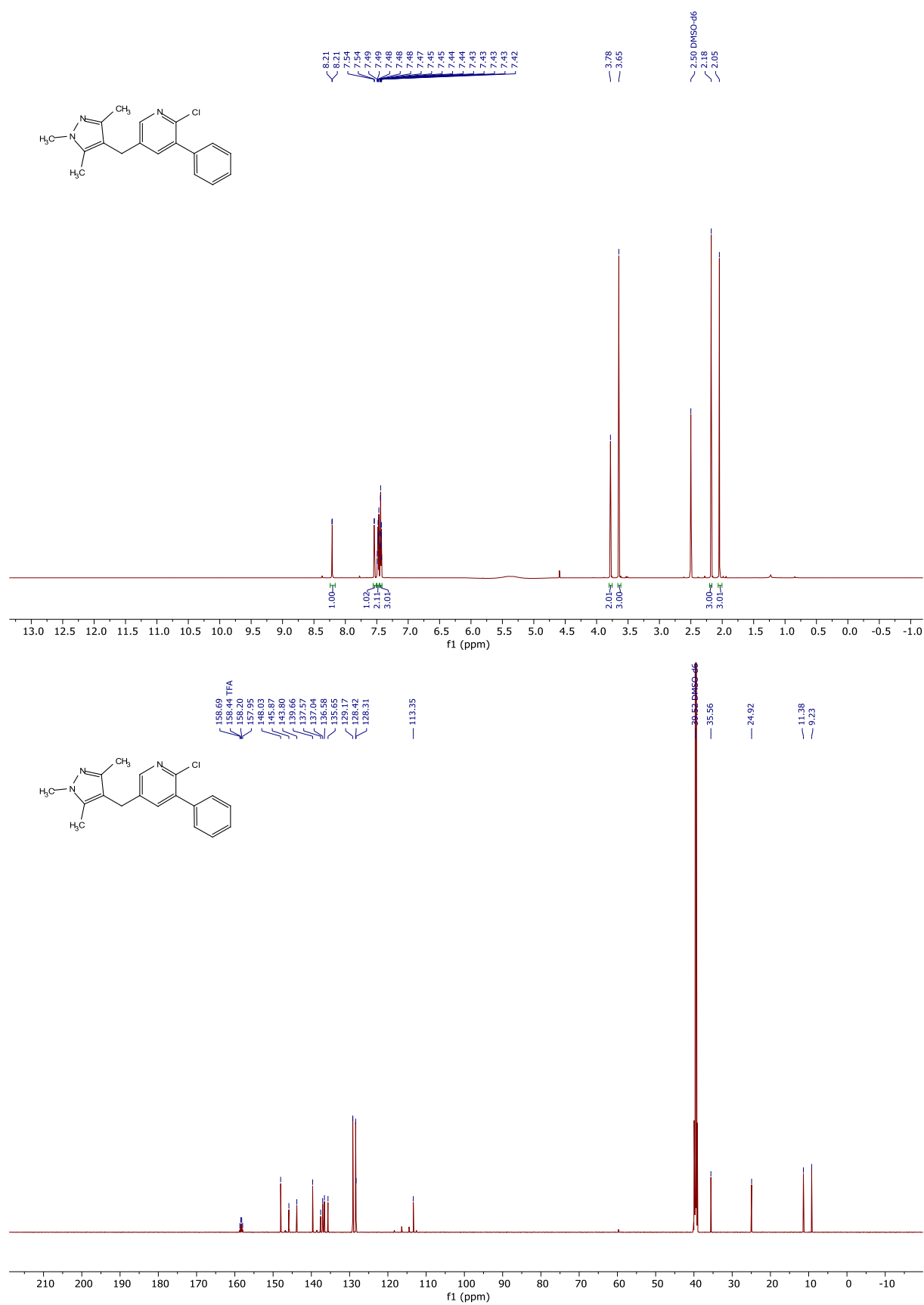


Figure S14. ^1H - and ^{13}C -NMR spectra of compound **23d**.

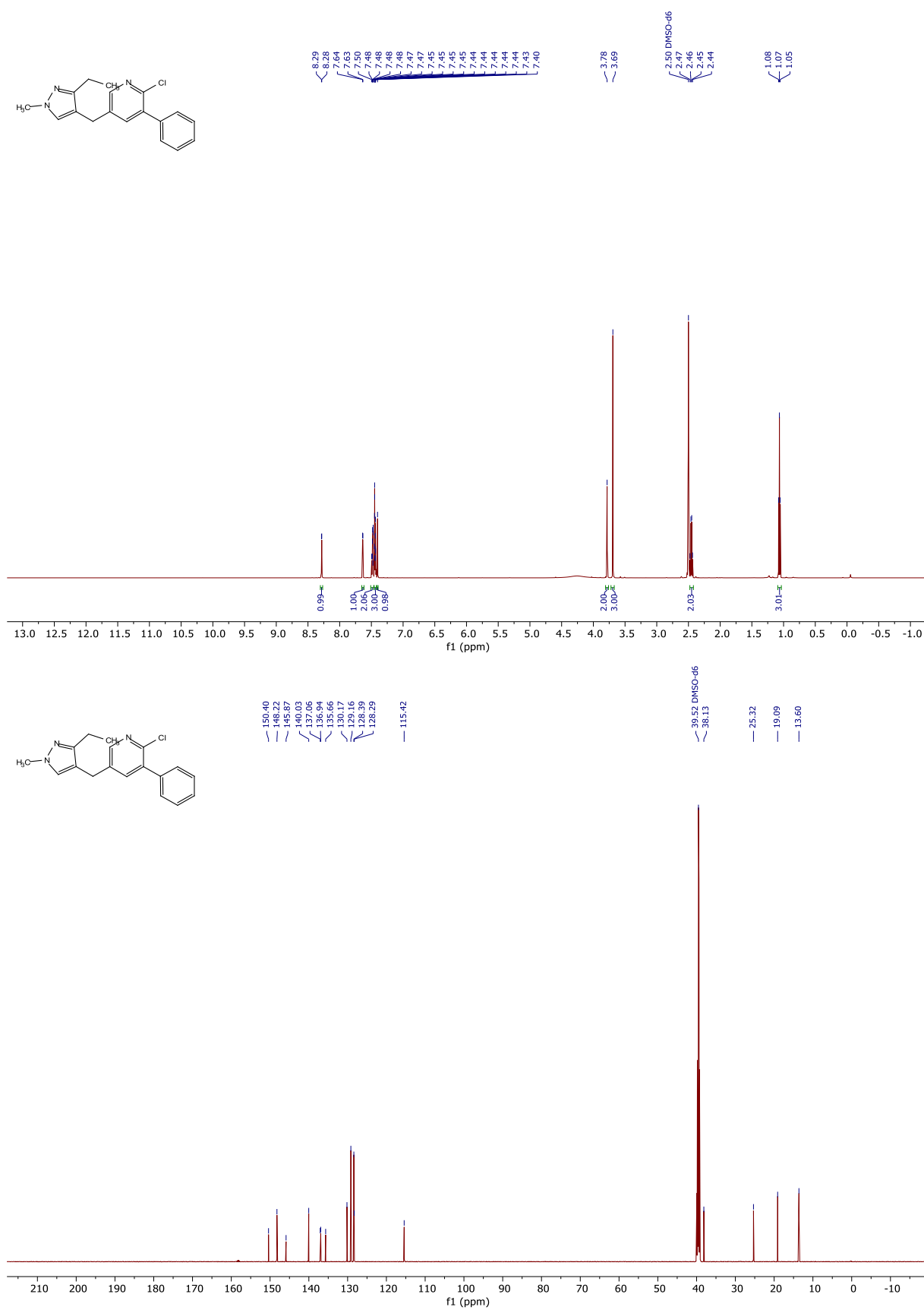


Figure S15. ^1H - and ^{13}C -NMR spectra of compound **23e**.

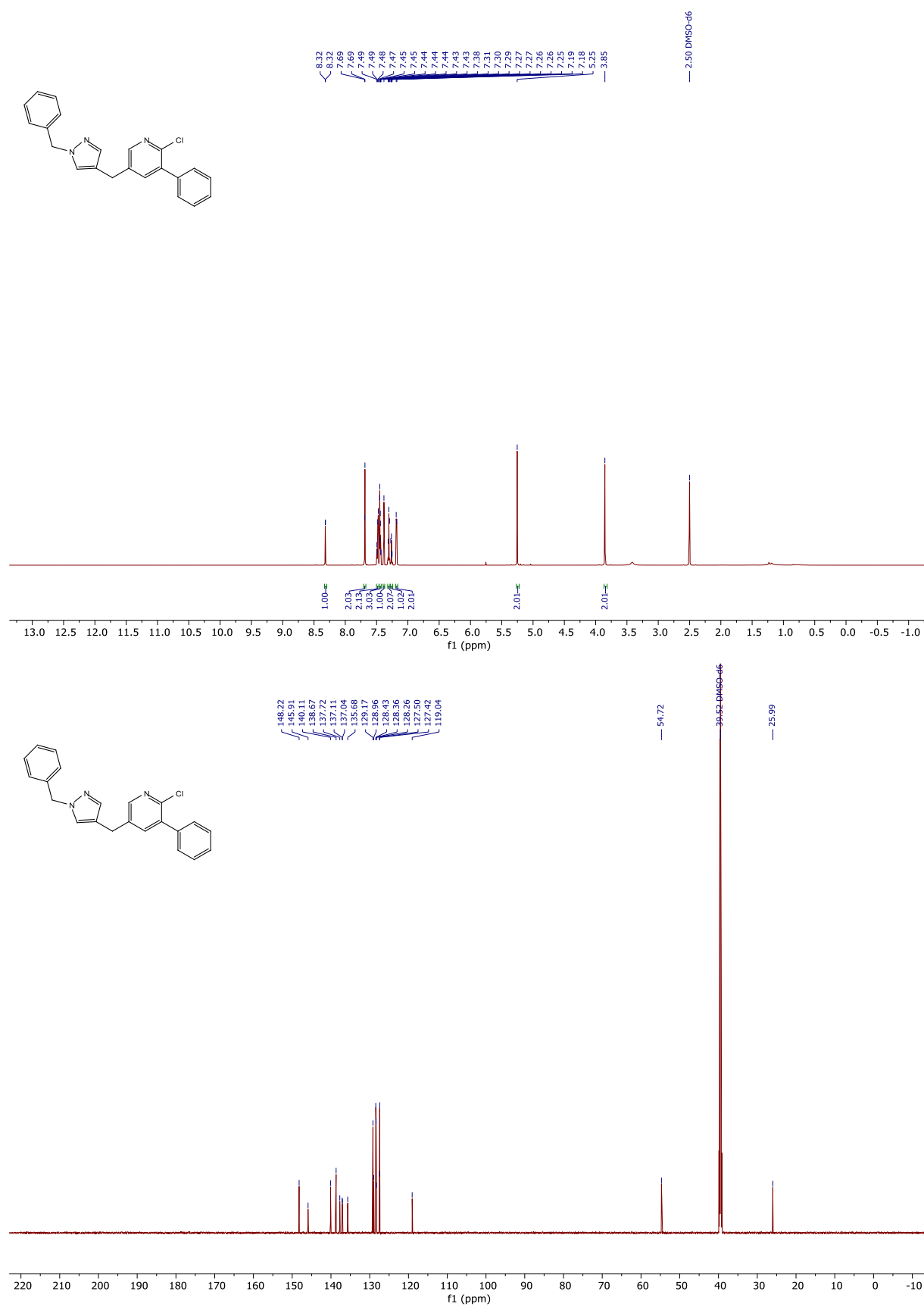
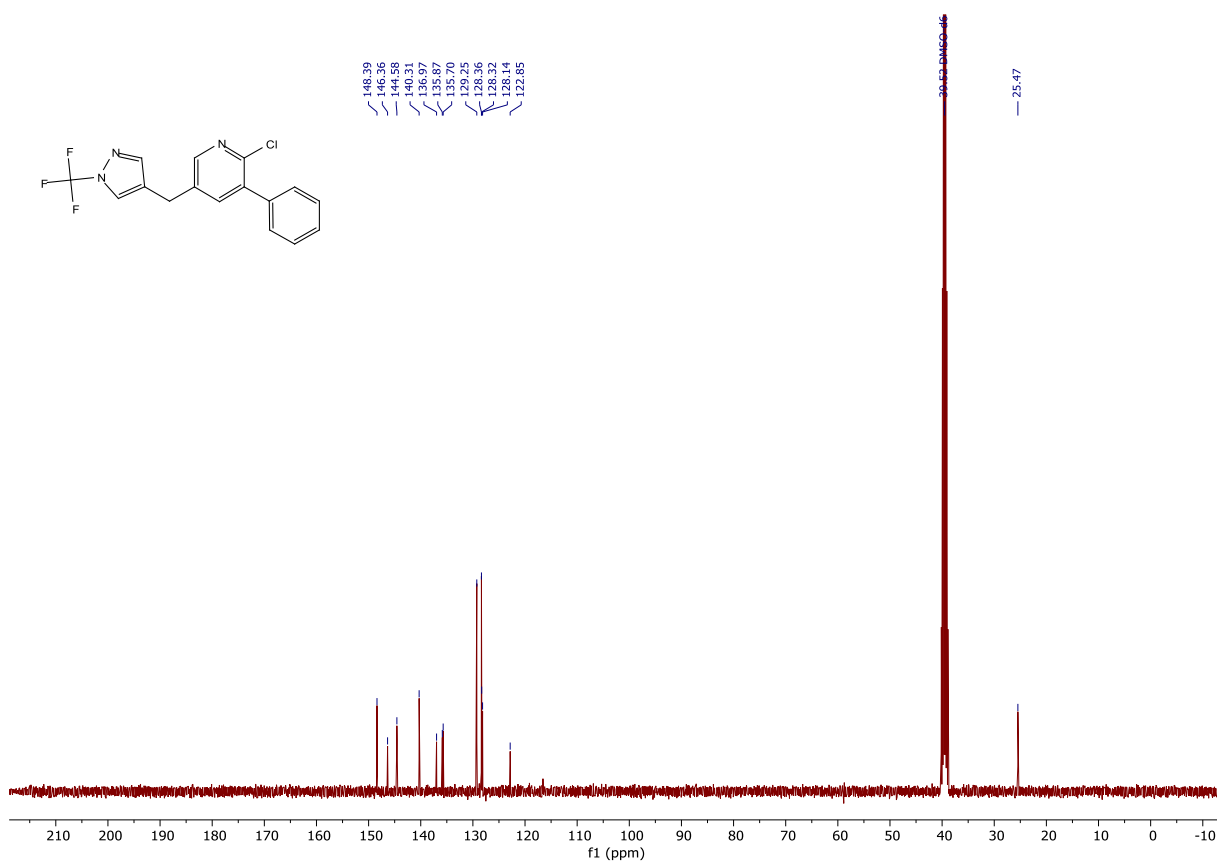
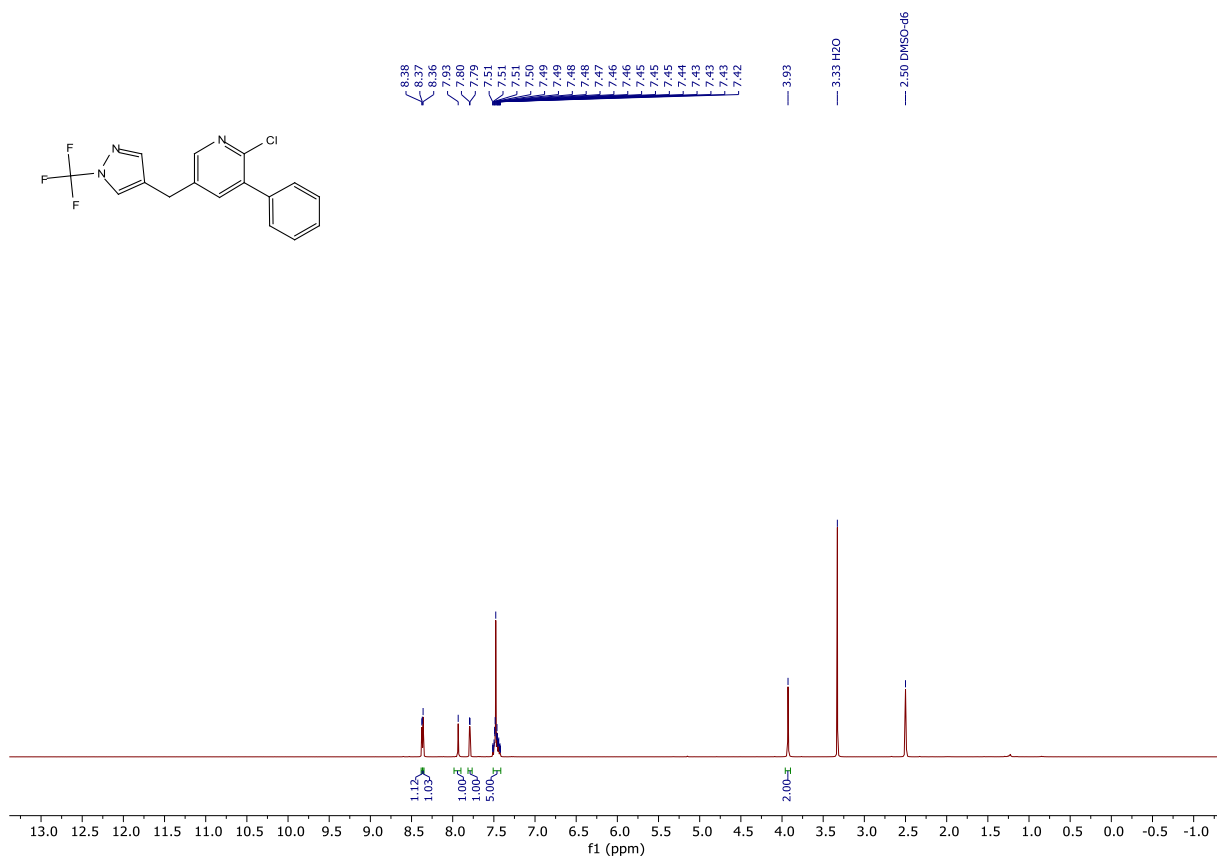


Figure S16. ¹H- and ¹³C-NMR spectra of compound **23f**.



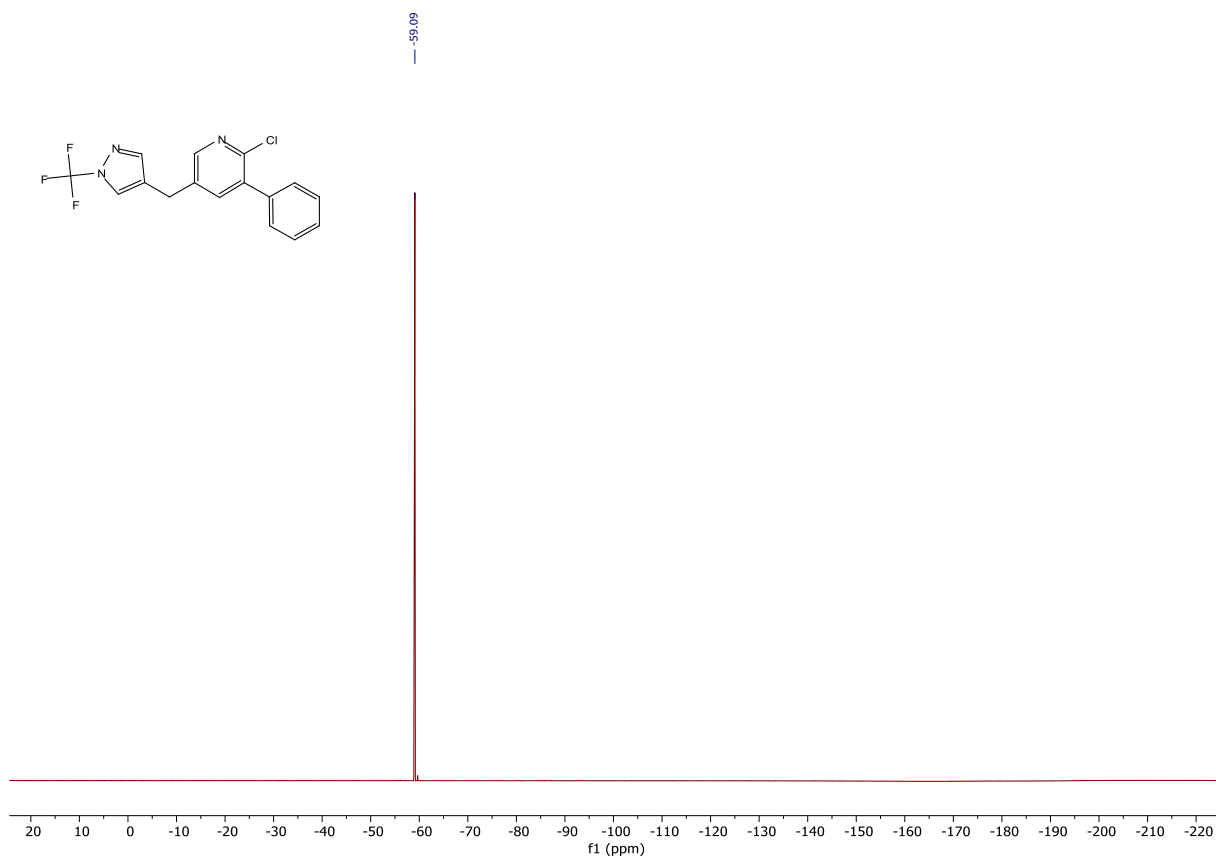


Figure S17. ^1H - and ^{13}C - and ^9F -NMR spectra of compound **23g**.

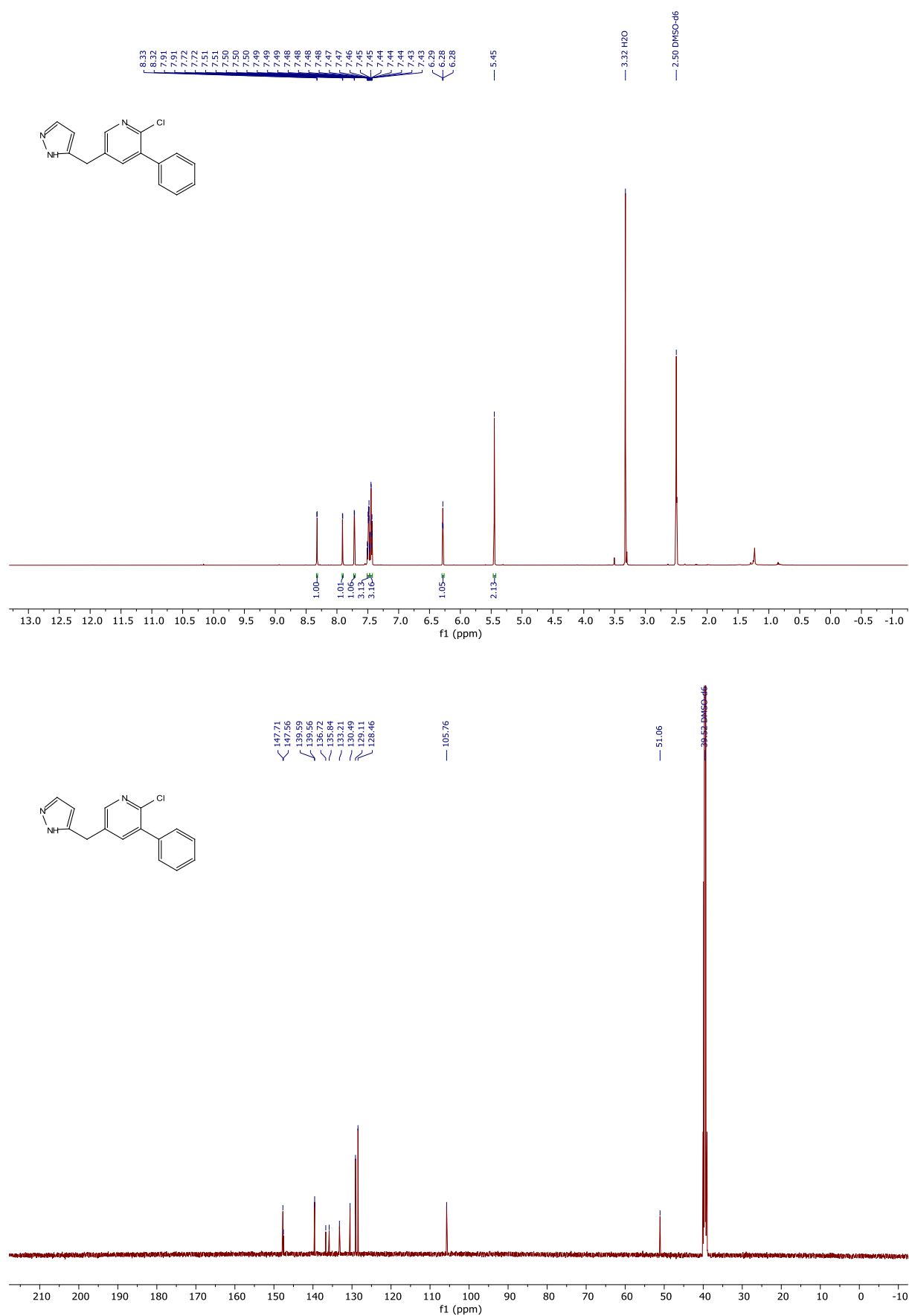


Figure S18. ¹H- and ¹³C-NMR spectra of compound **23h**.

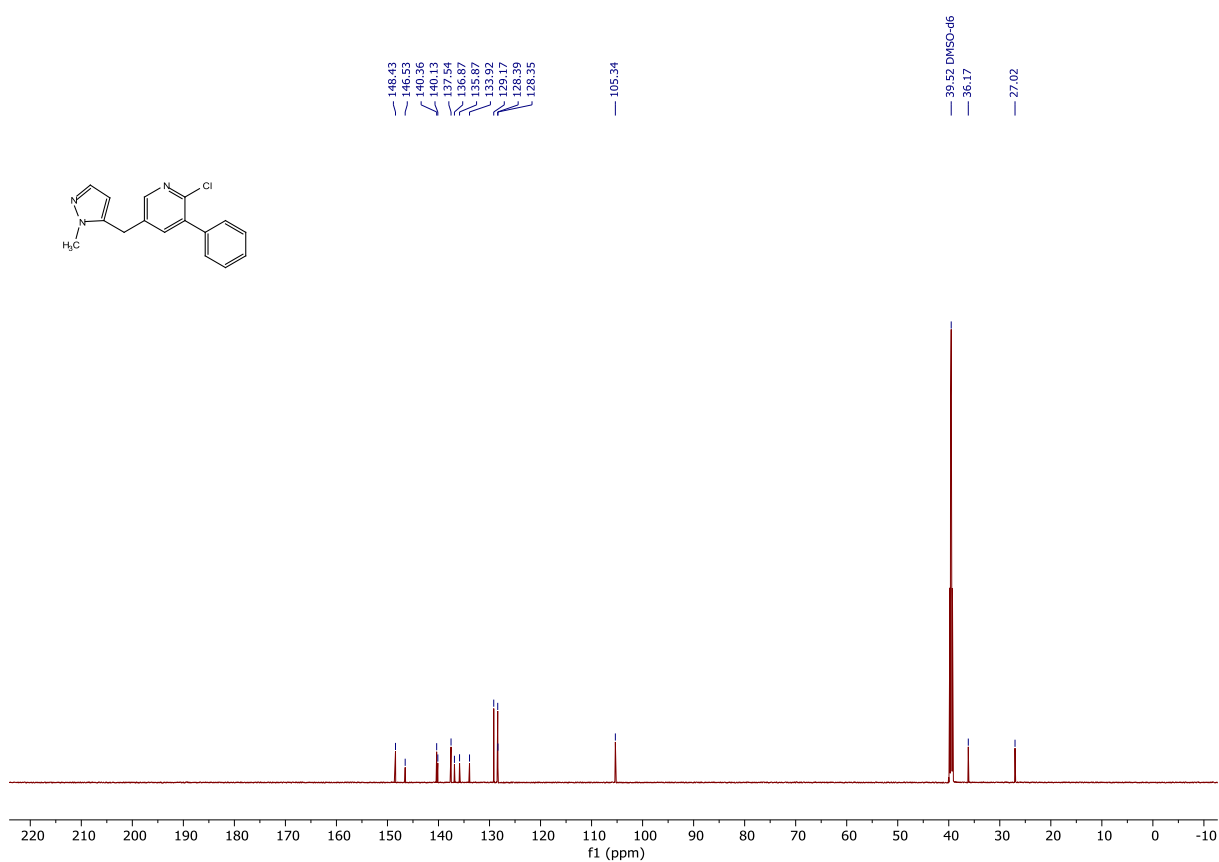
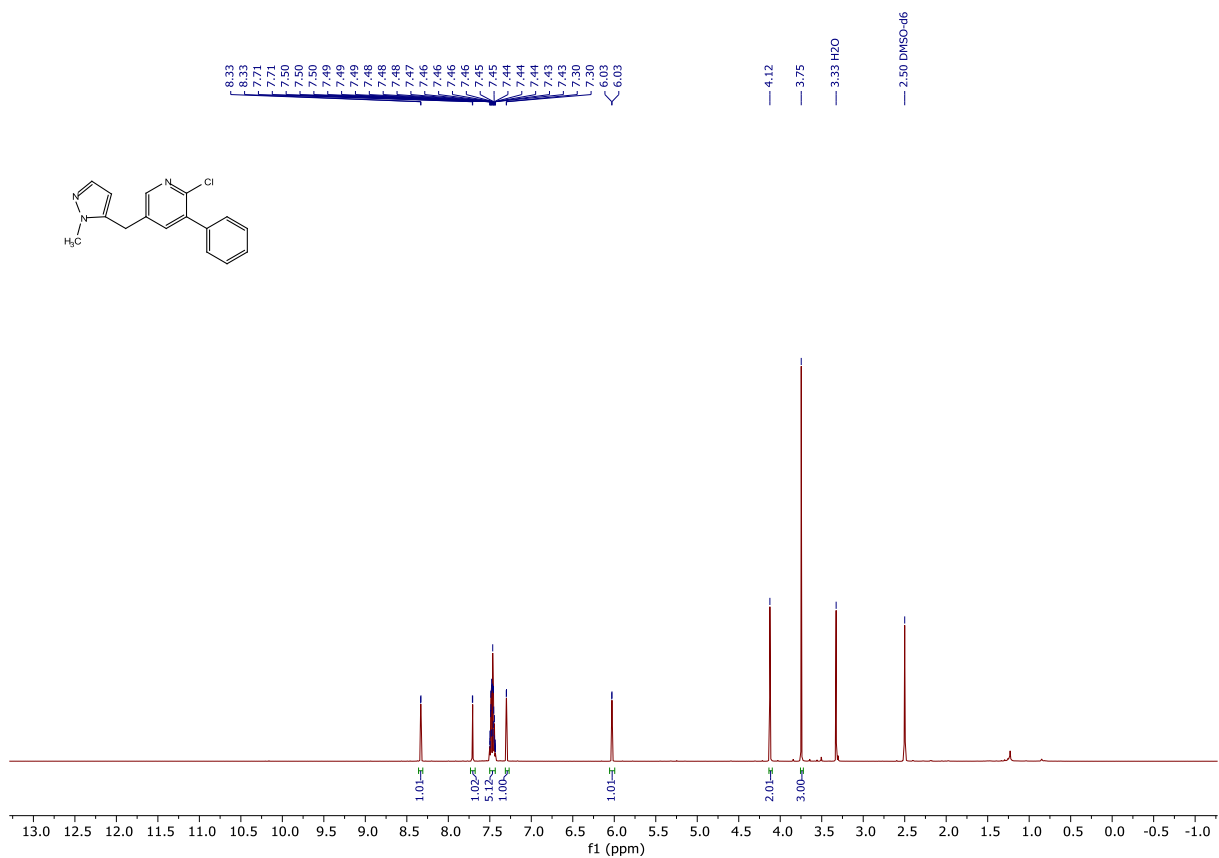


Figure S19. ^1H - and ^{13}C -NMR spectra of compound **23i**.

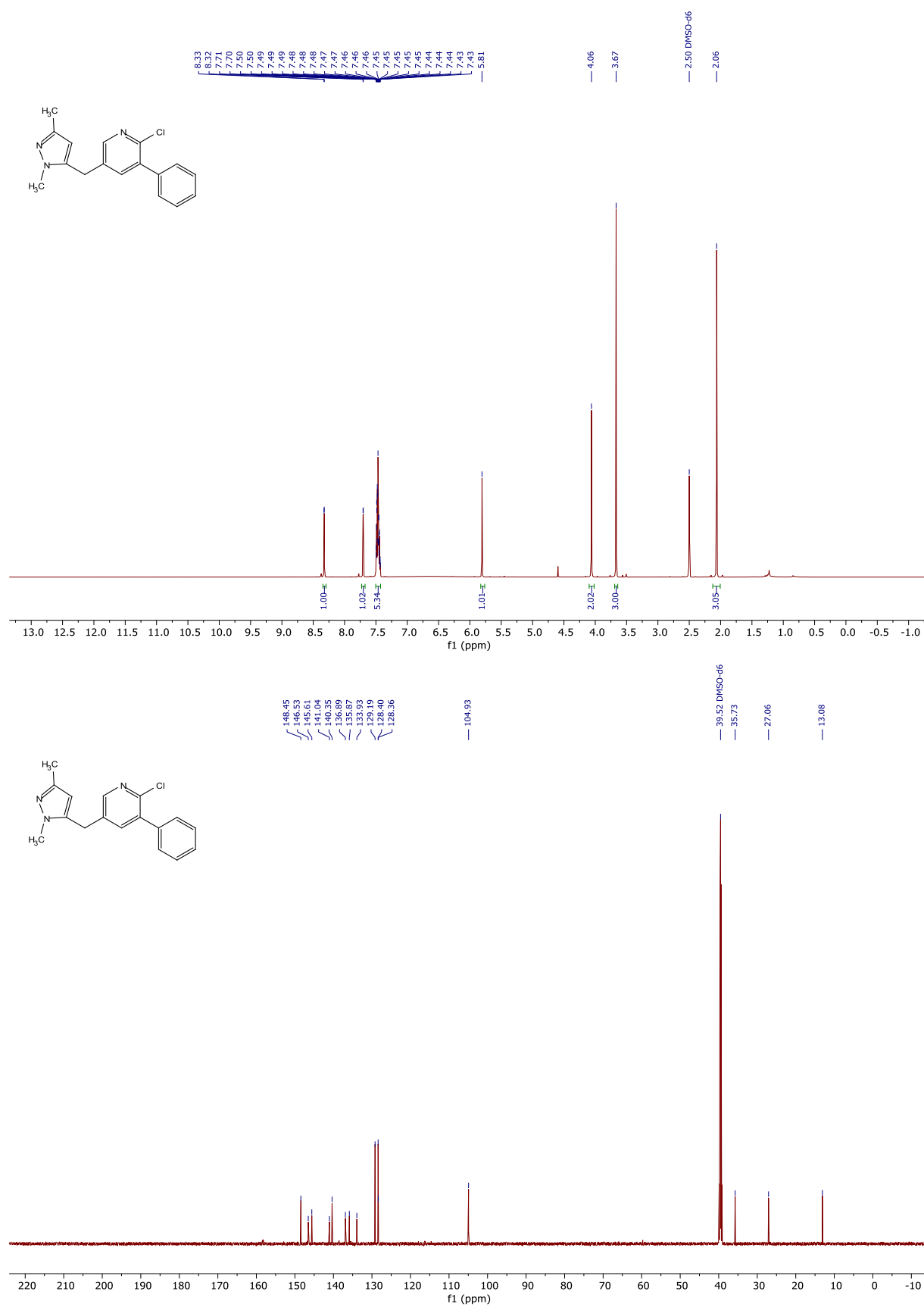


Figure S20. ^1H - and ^{13}C -NMR spectra of compound **23j**.

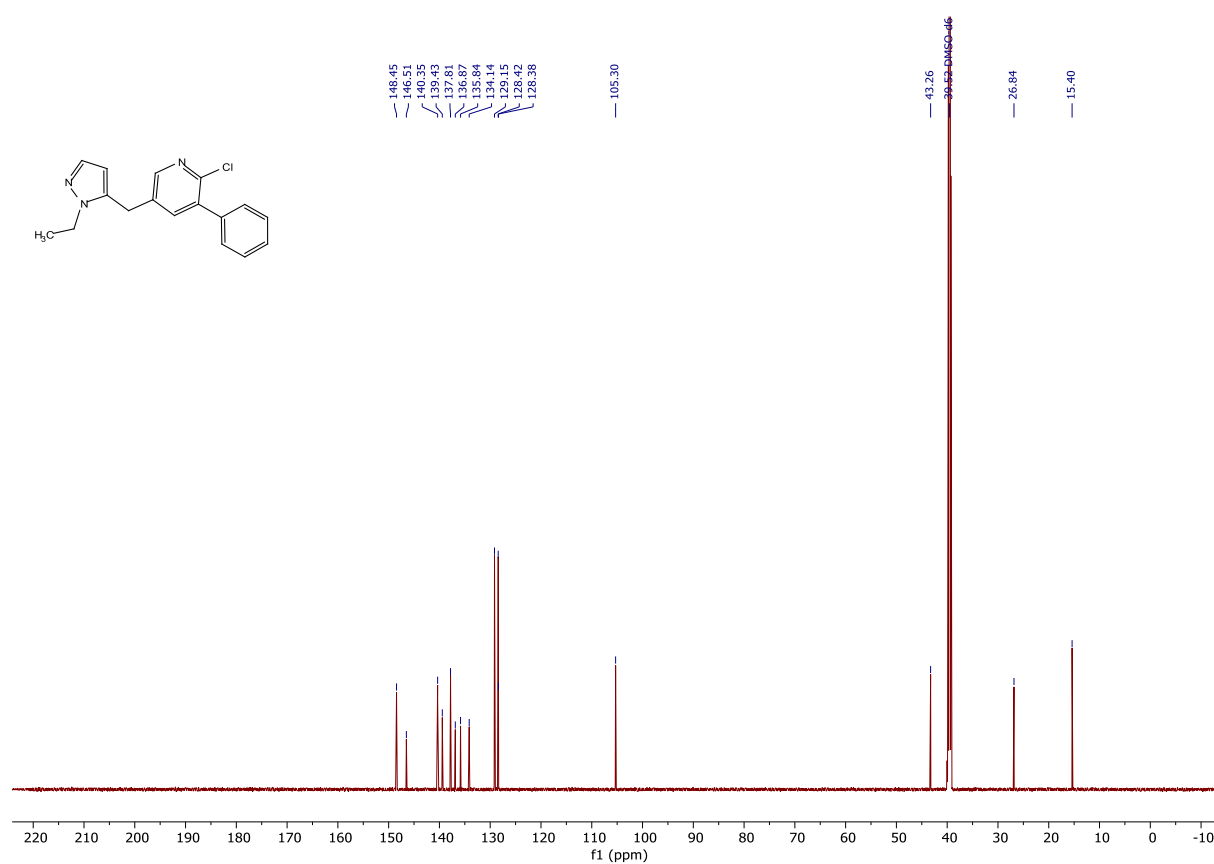
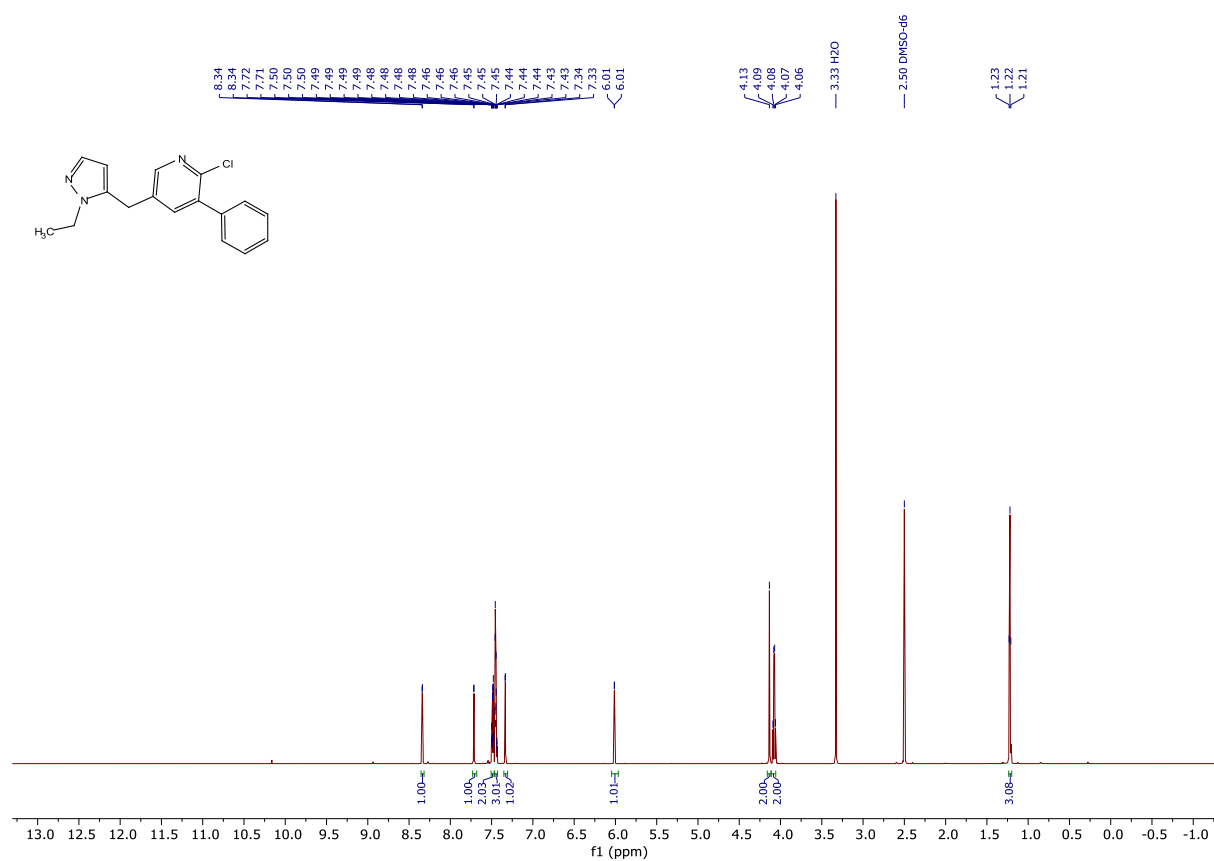


Figure S21. ¹H- and ¹³C-NMR spectra of compound **23k**.

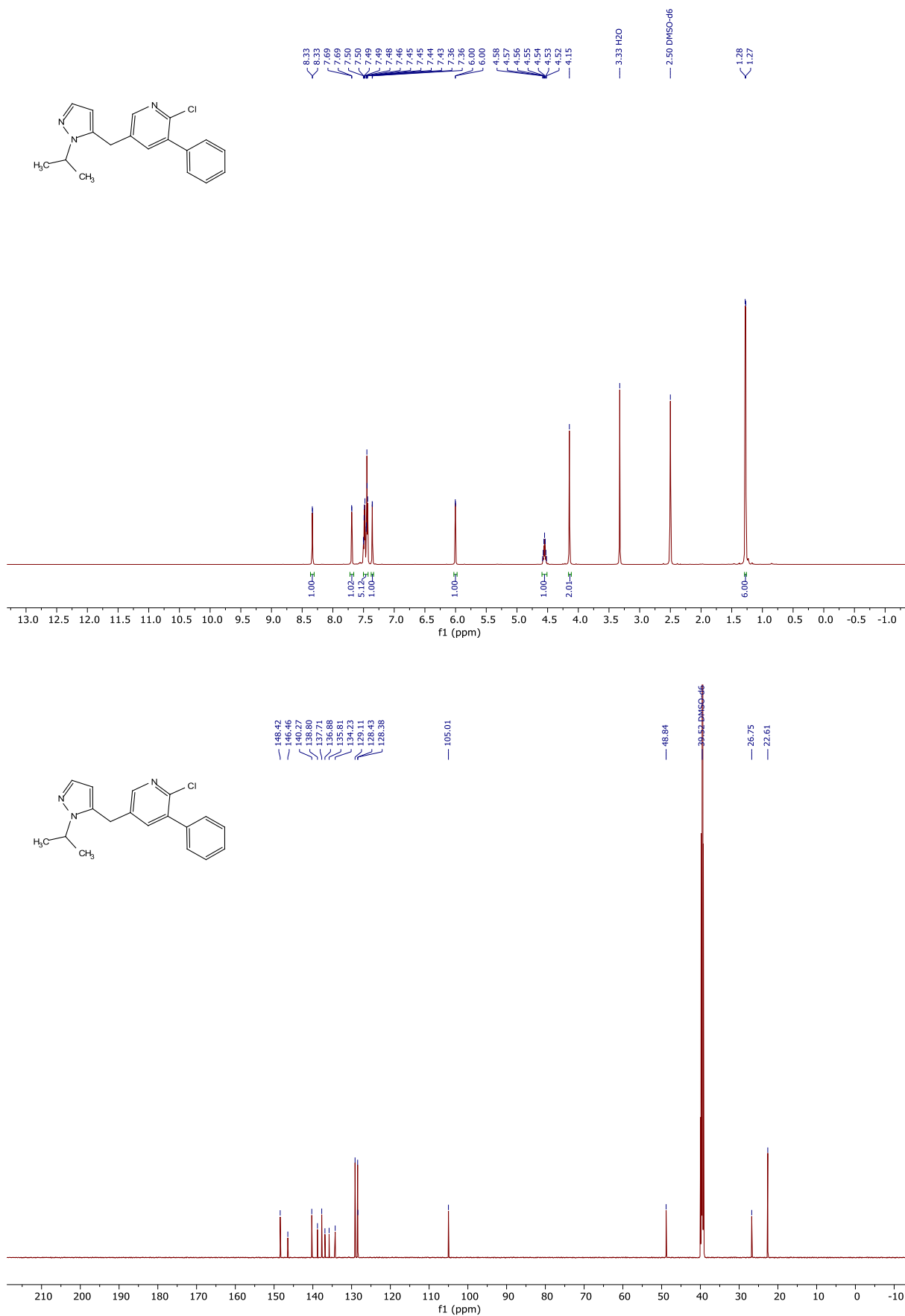


Figure S22. ¹H- and ¹³C-NMR spectra of compound **23I**.

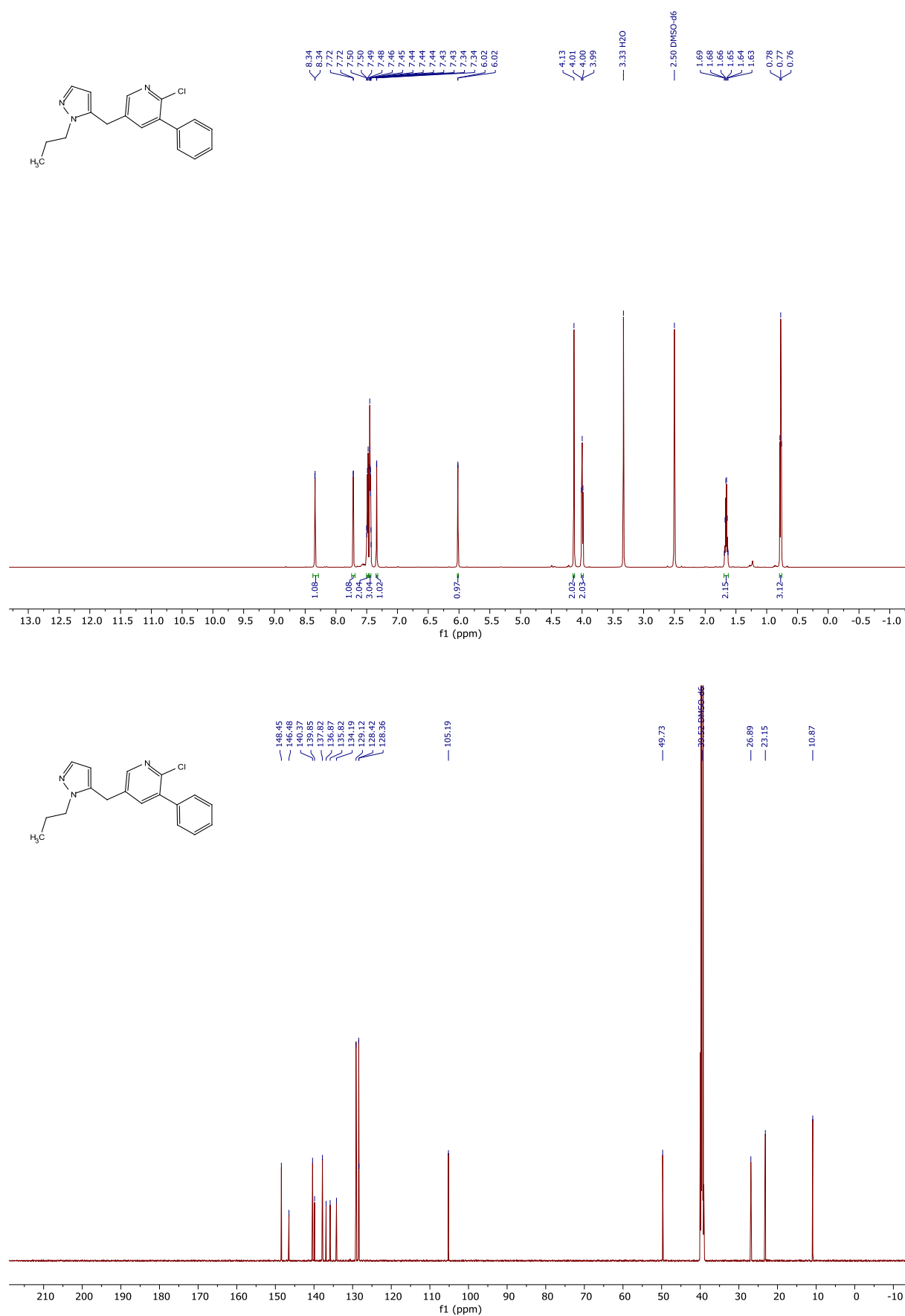


Figure S23. ^1H - and ^{13}C -NMR spectra of compound **23m**.

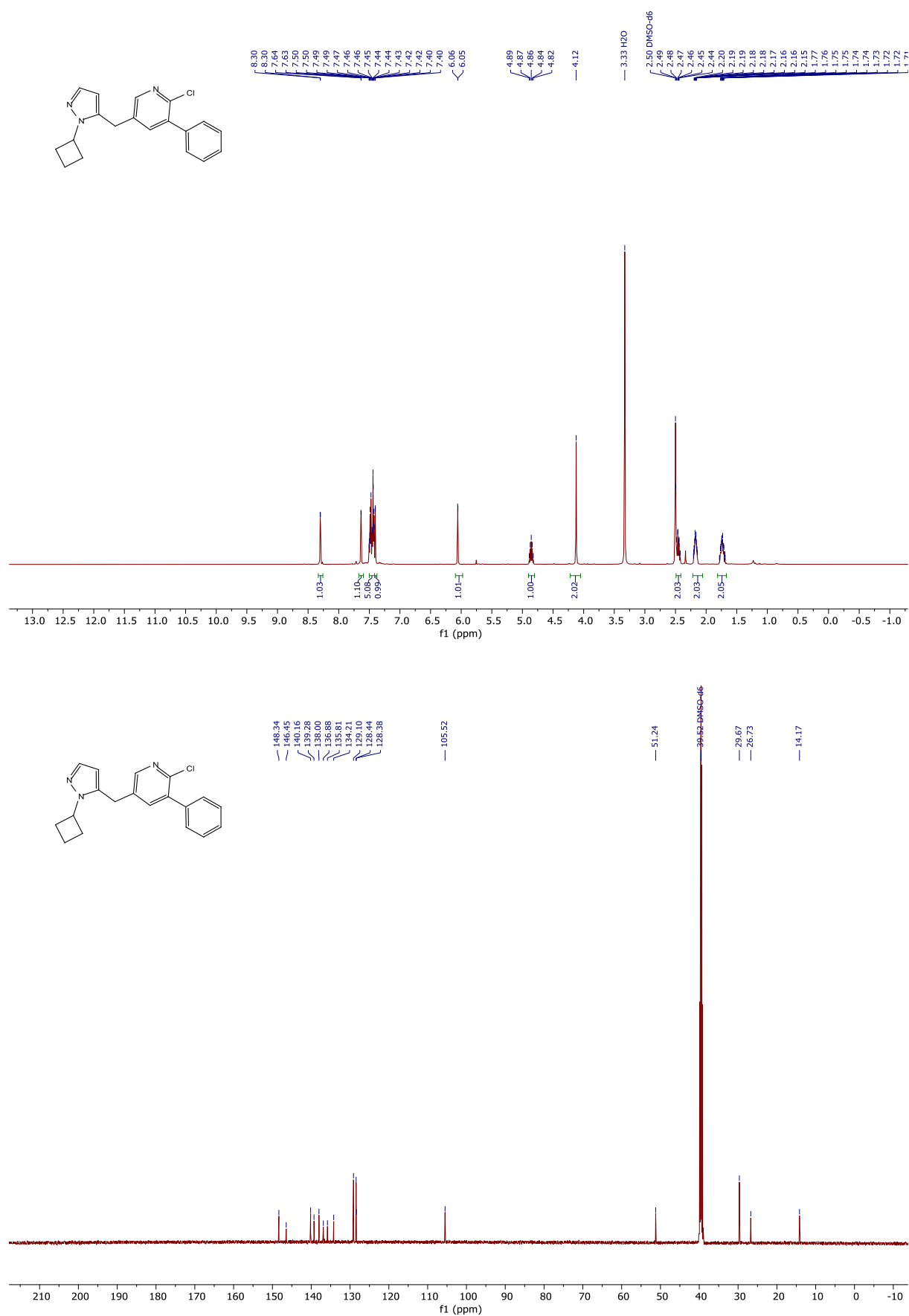


Figure S24. ¹H- and ¹³C-NMR spectra of compound **23n**.

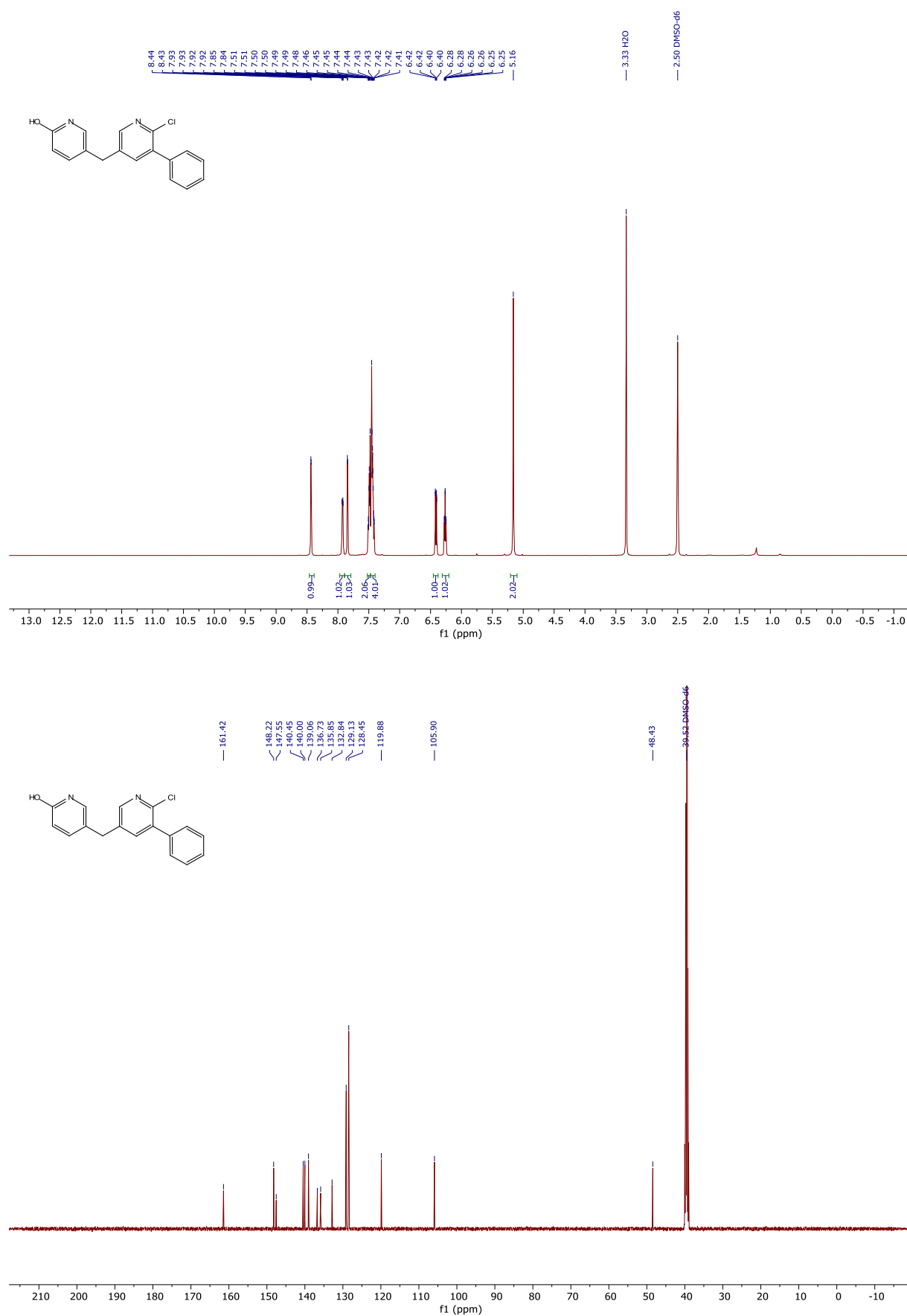


Figure S25. ¹H- and ¹³C-NMR spectra of compound **23o**.

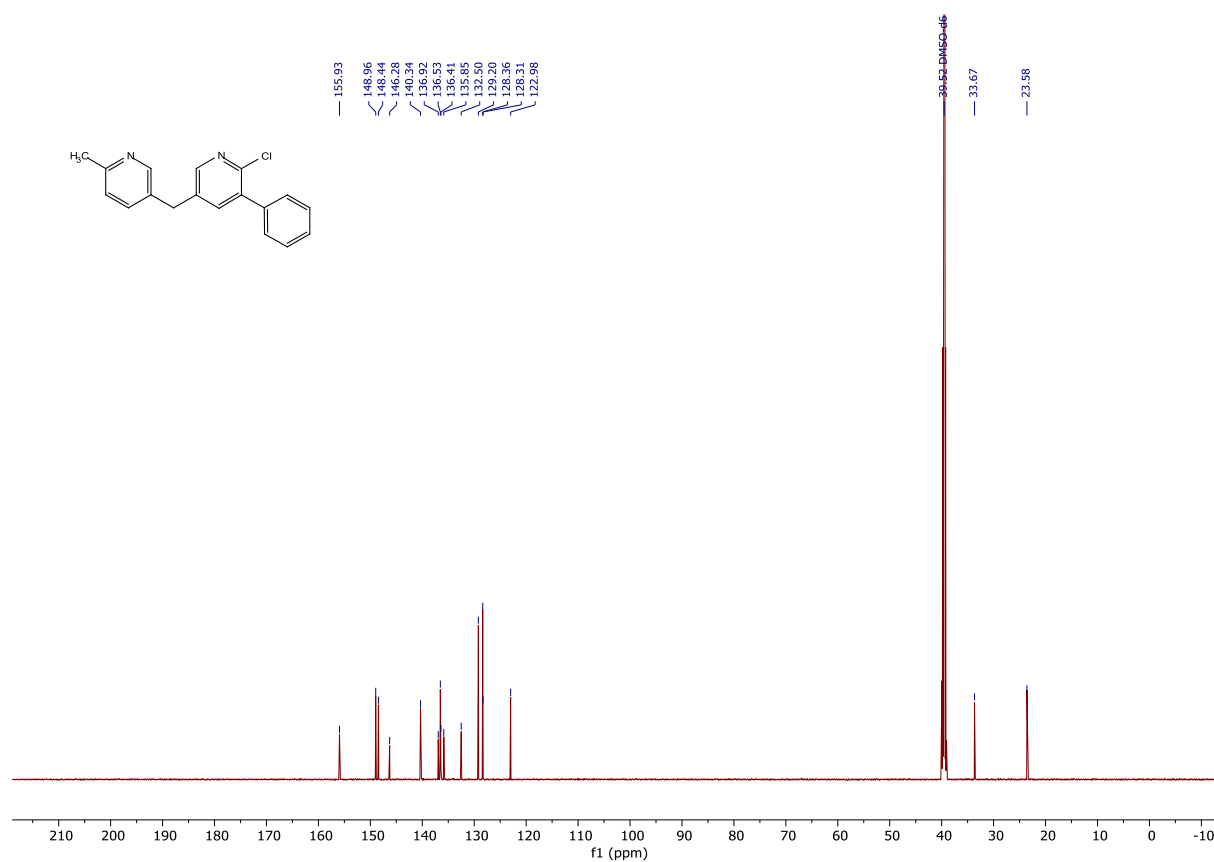
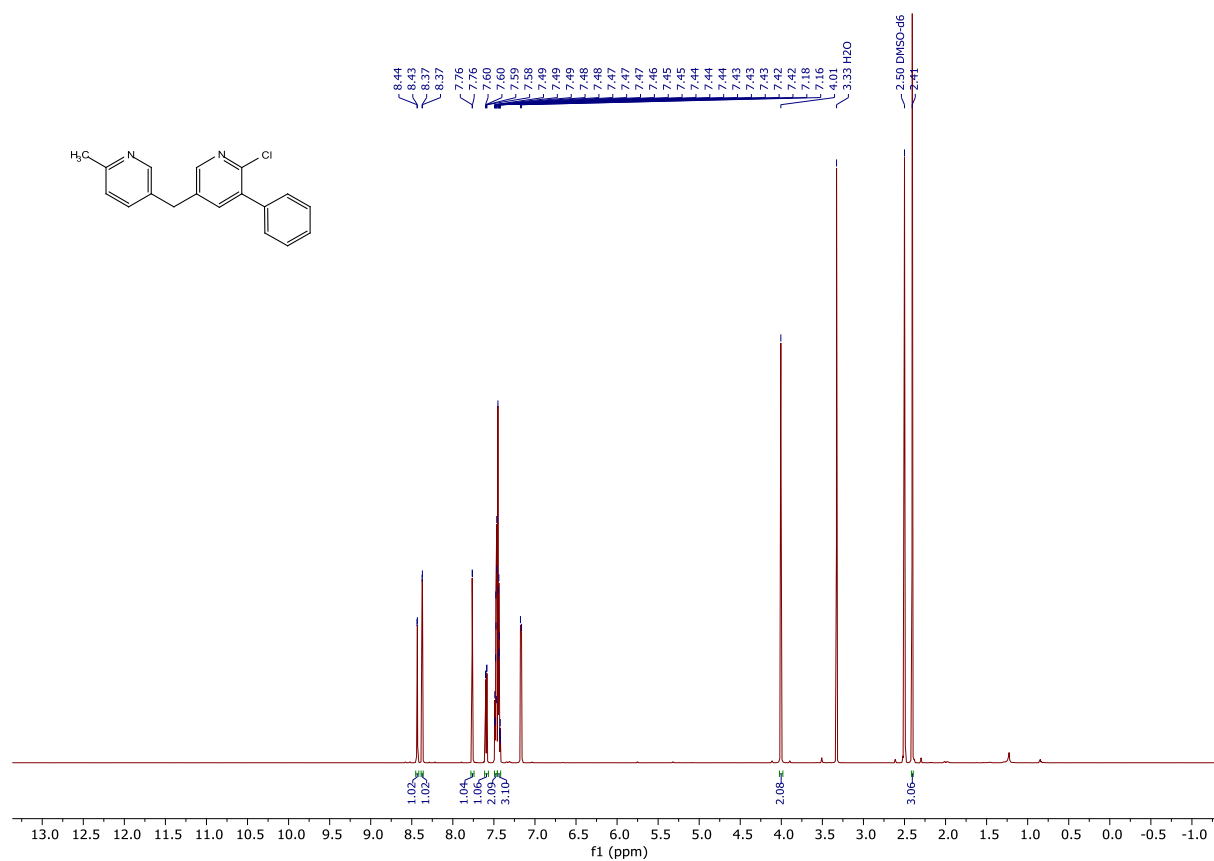


Figure S26. ¹H- and ¹³C-NMR spectra of compound **23p**.

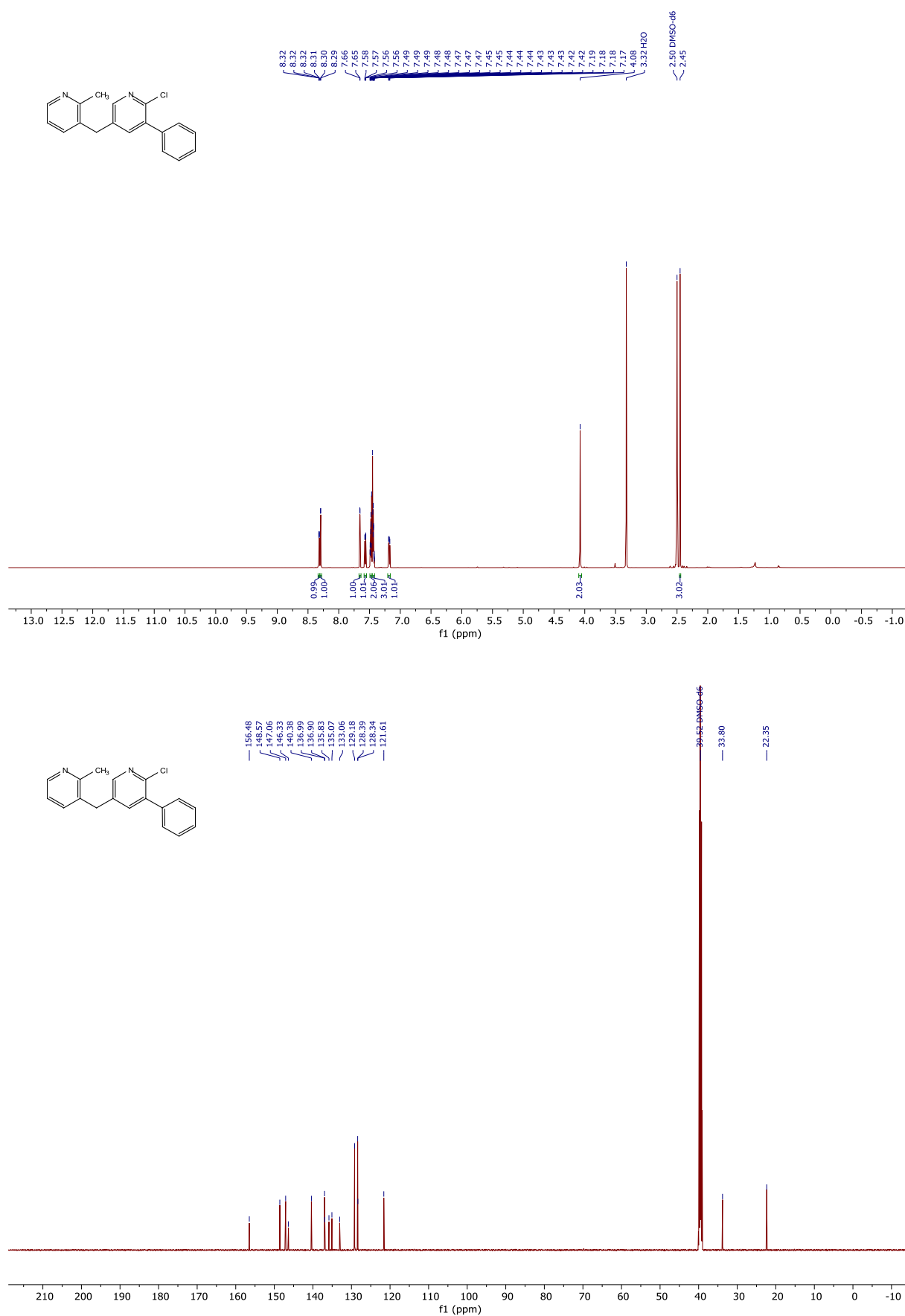


Figure S27. ¹H- and ¹³C-NMR spectra of compound **23q**.

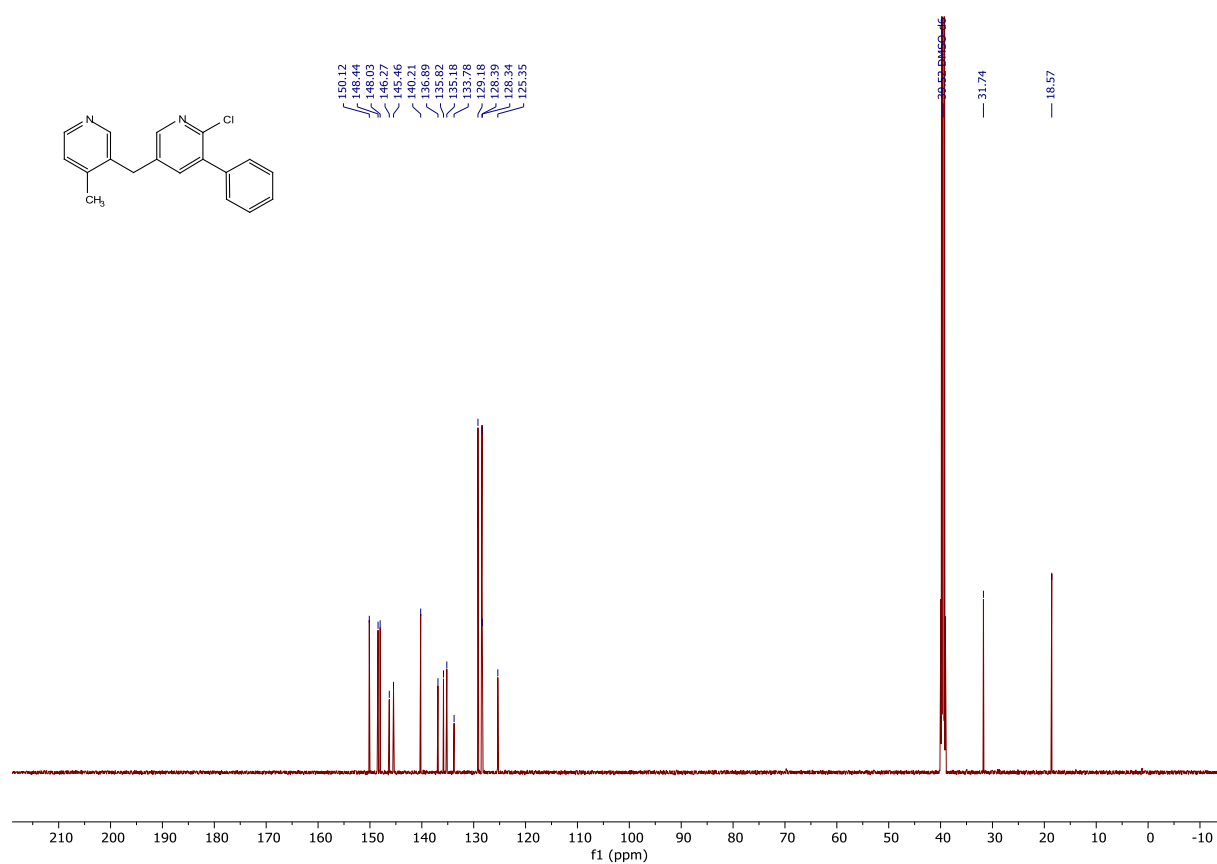
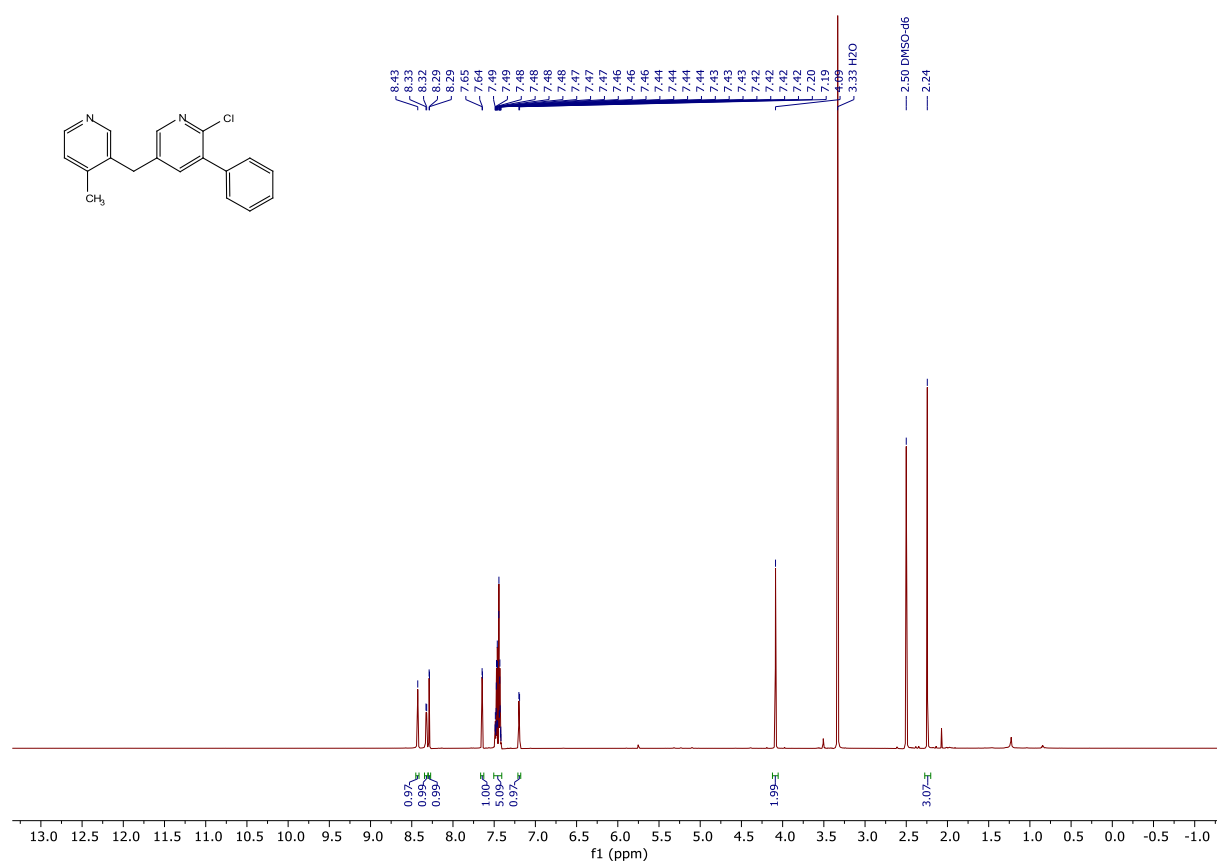
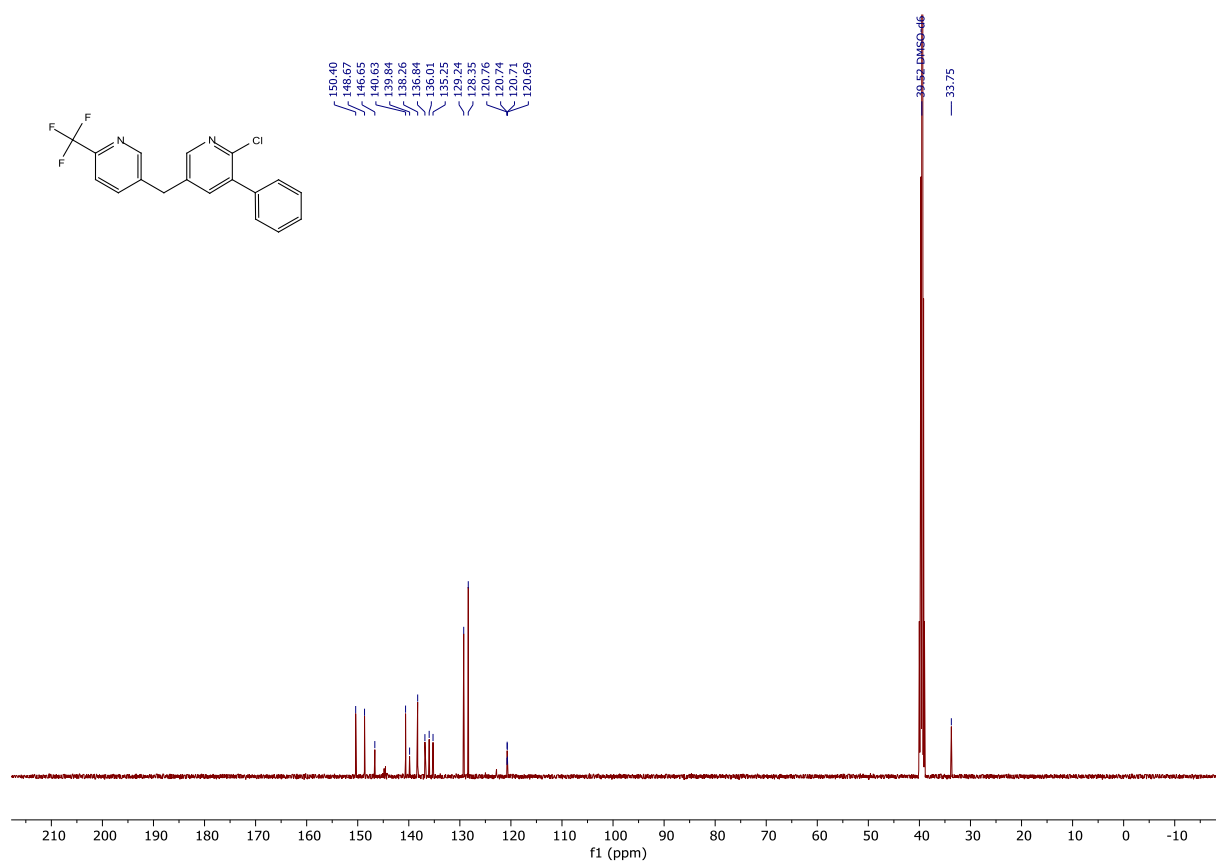
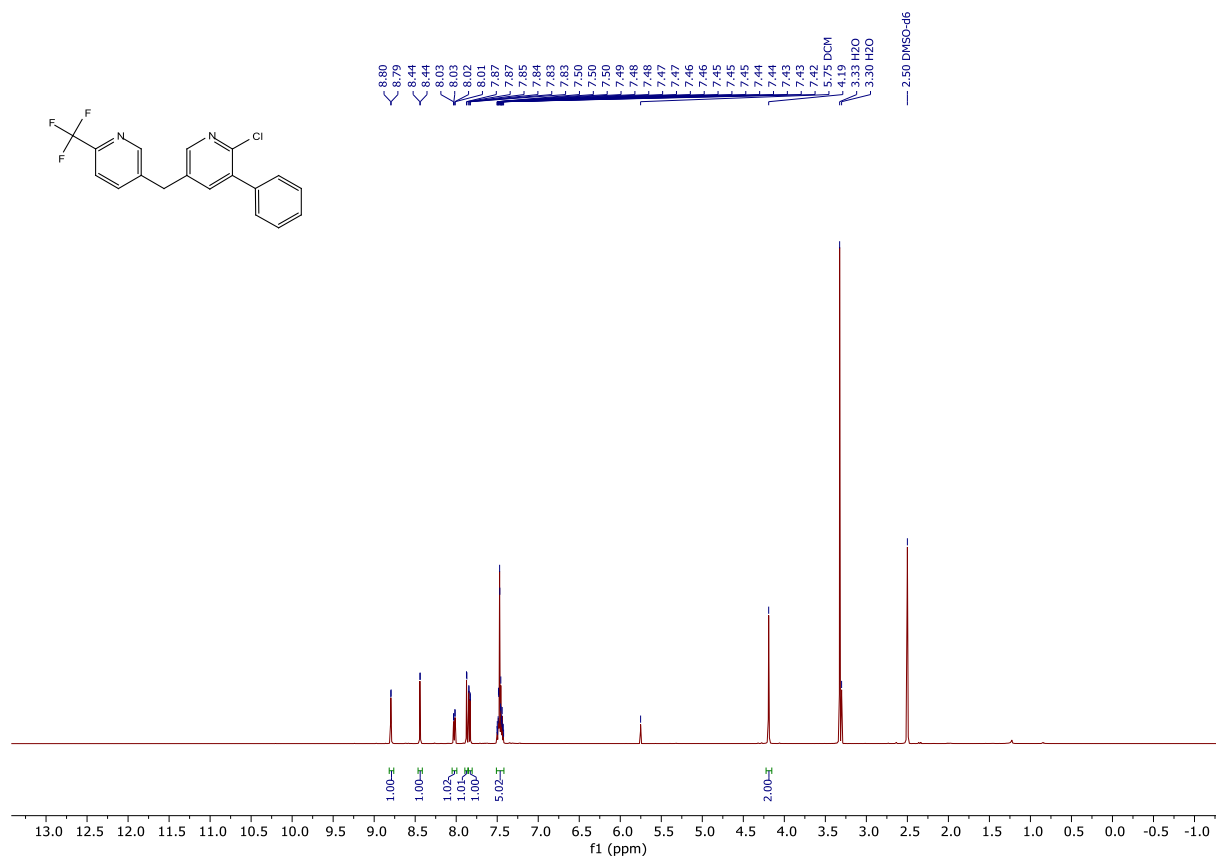


Figure S28. ¹H- and ¹³C-NMR spectra of compound **23r**.



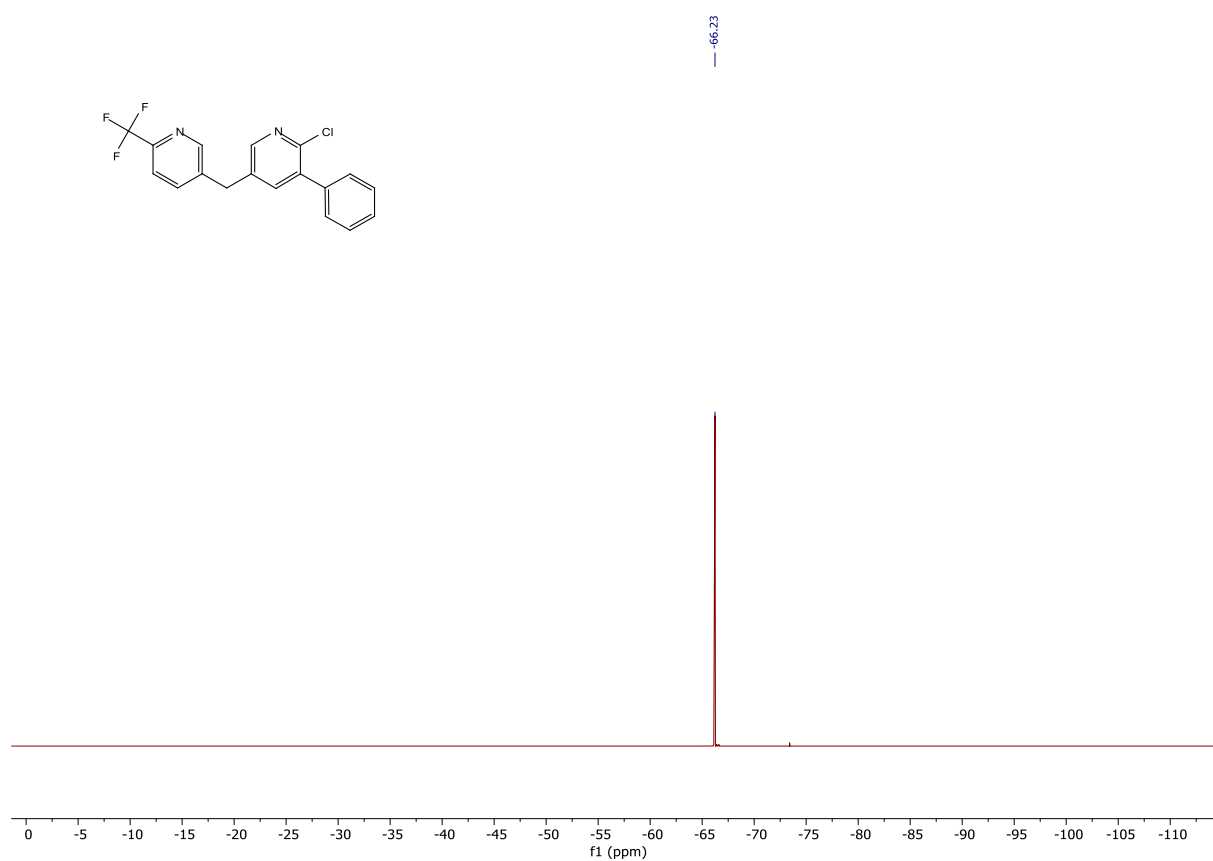
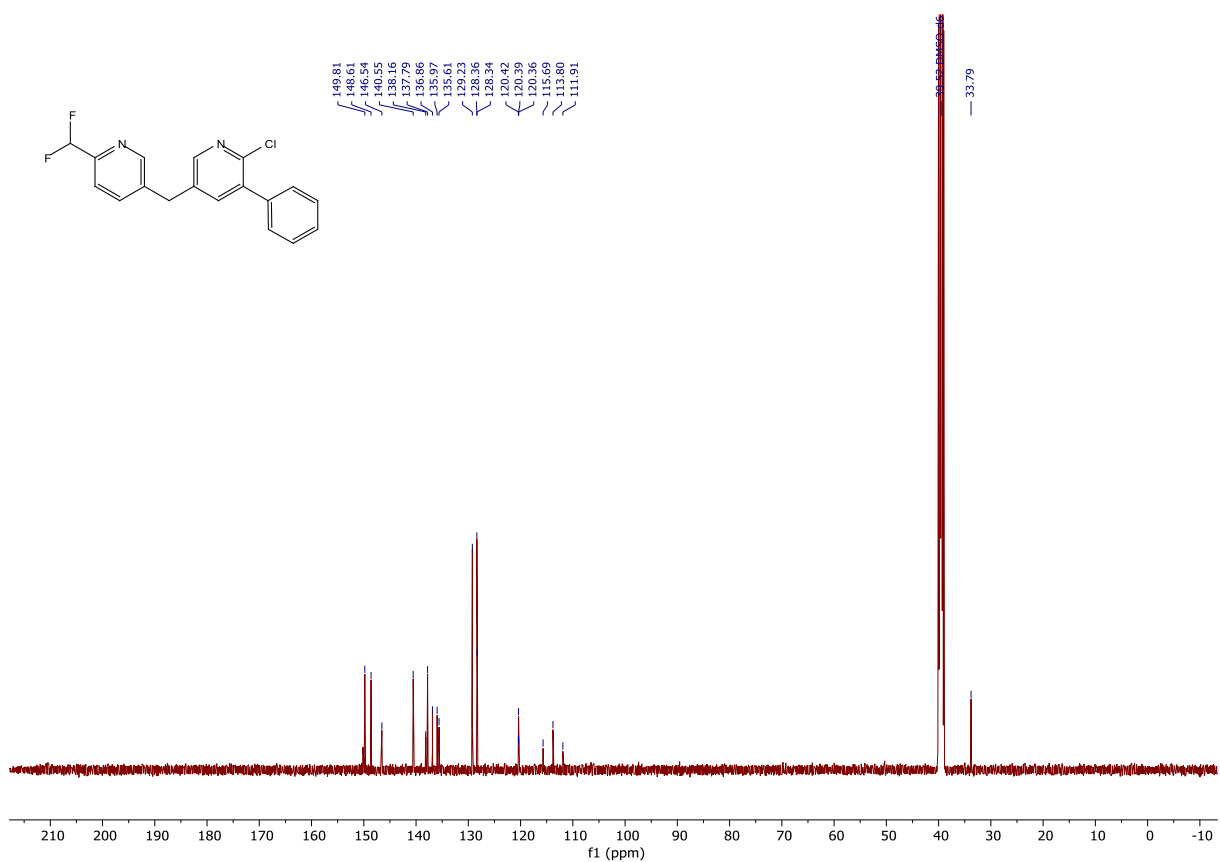
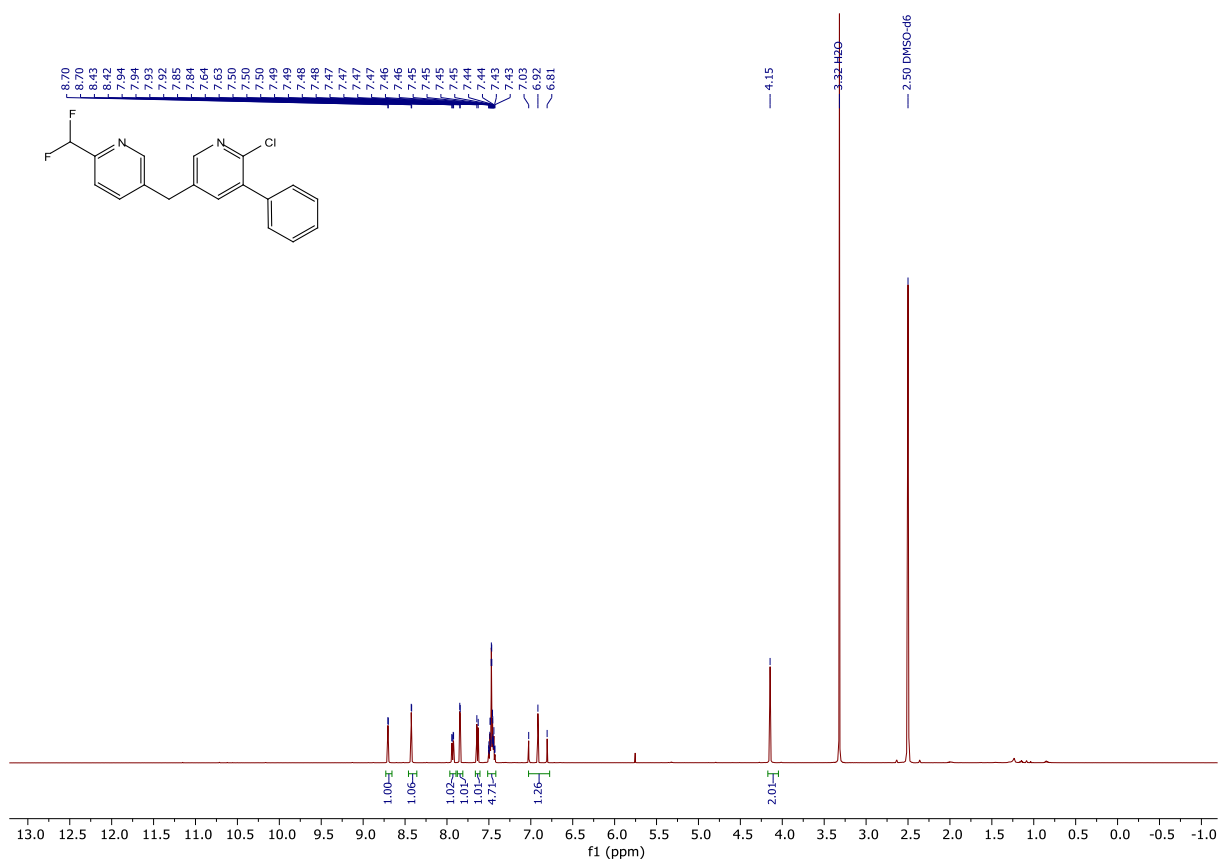


Figure S29. ^1H -, ^{13}C - and ^{19}F NMR spectra of compound **23s**.



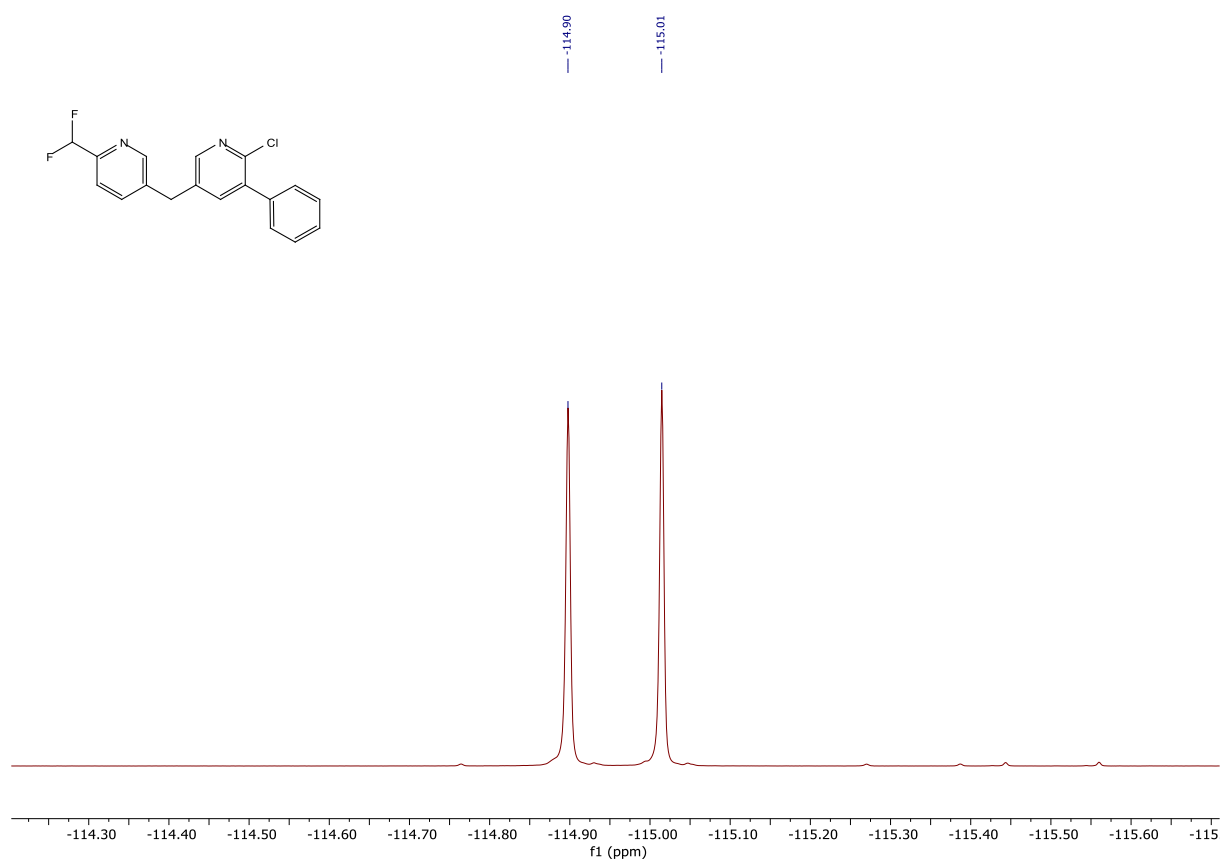


Figure S30. ^1H -, ^{13}C - and ^{19}F NMR spectra of compound **23t**.

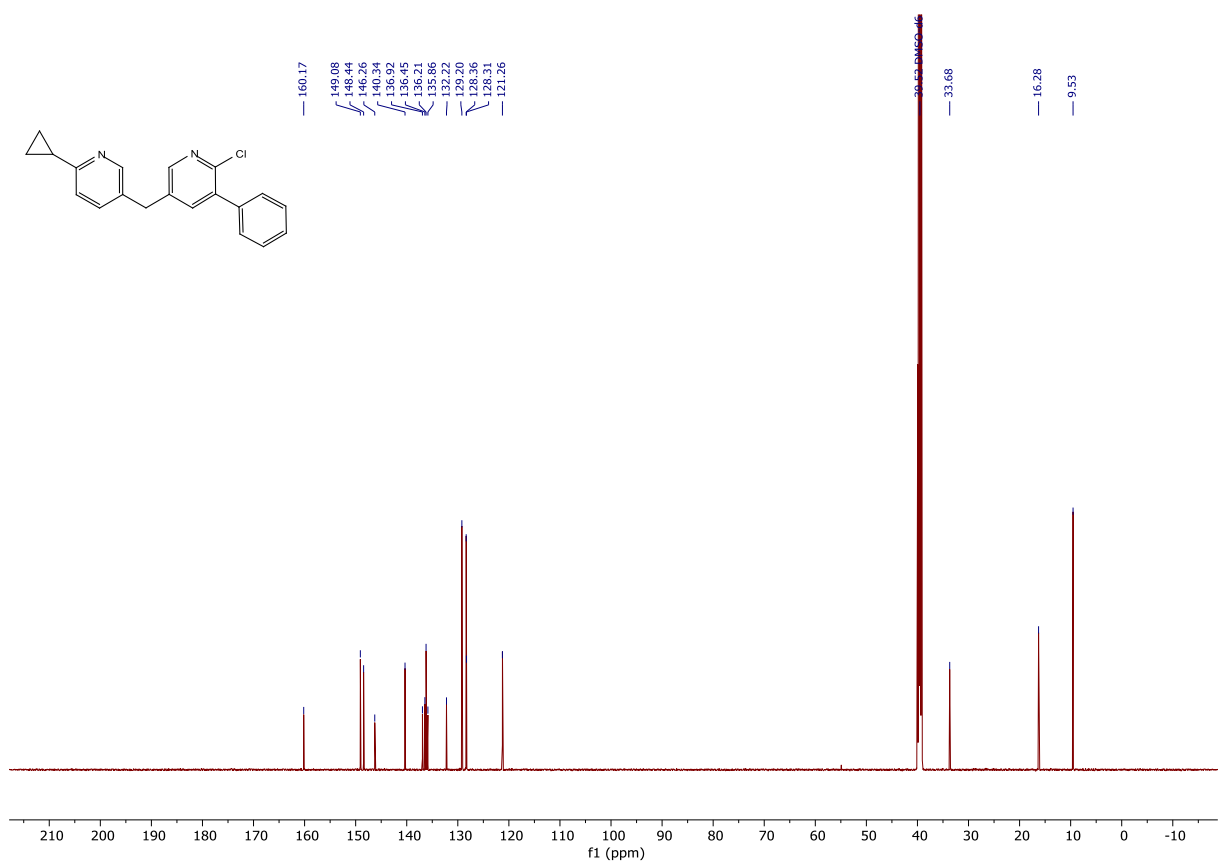
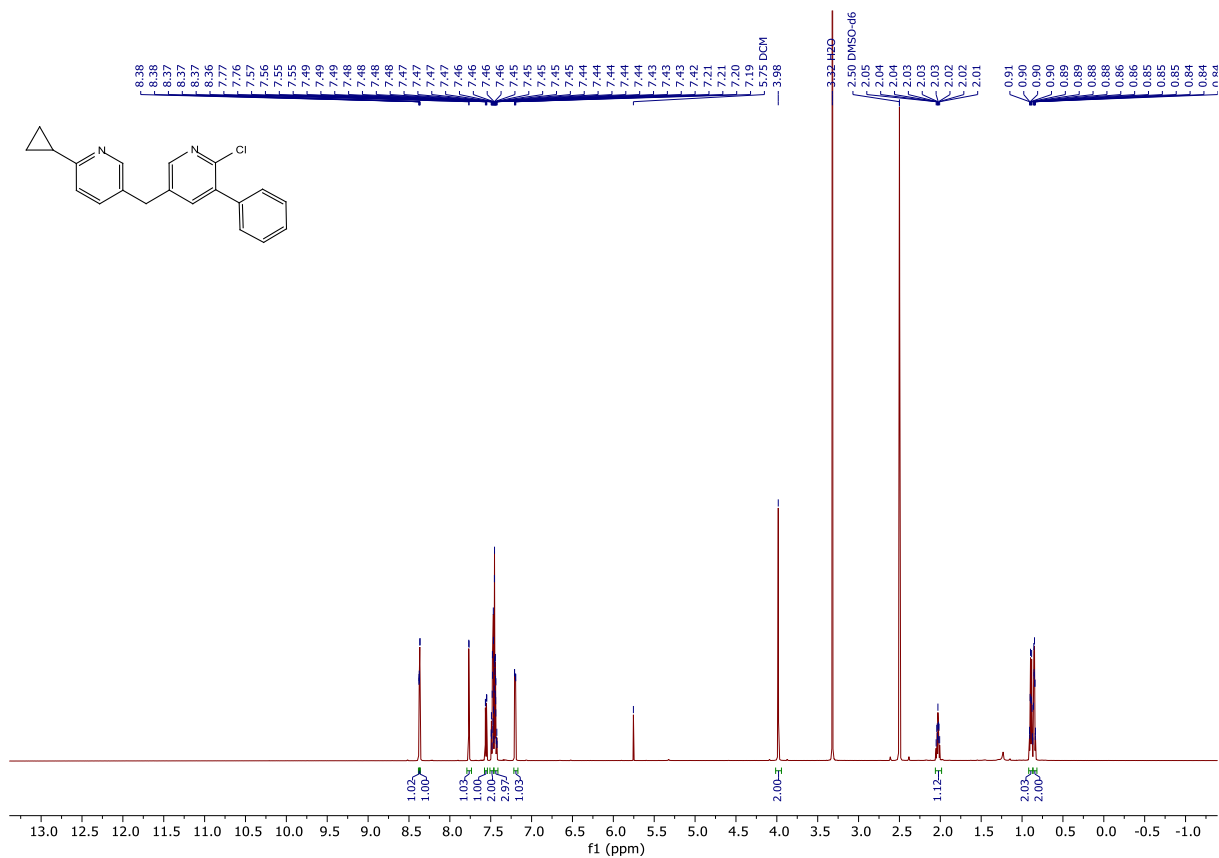


Figure S32. ¹H- and ¹³C-NMR spectra of compound **23v**.

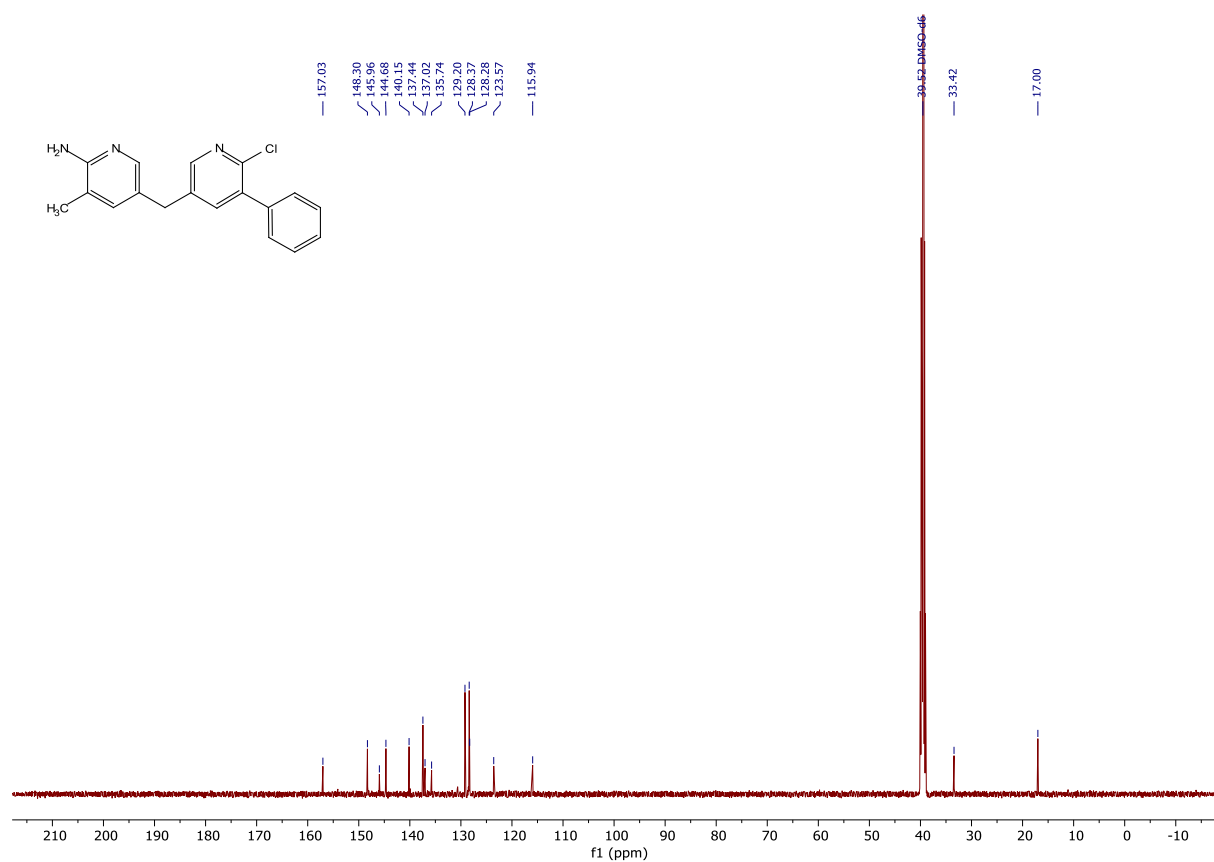
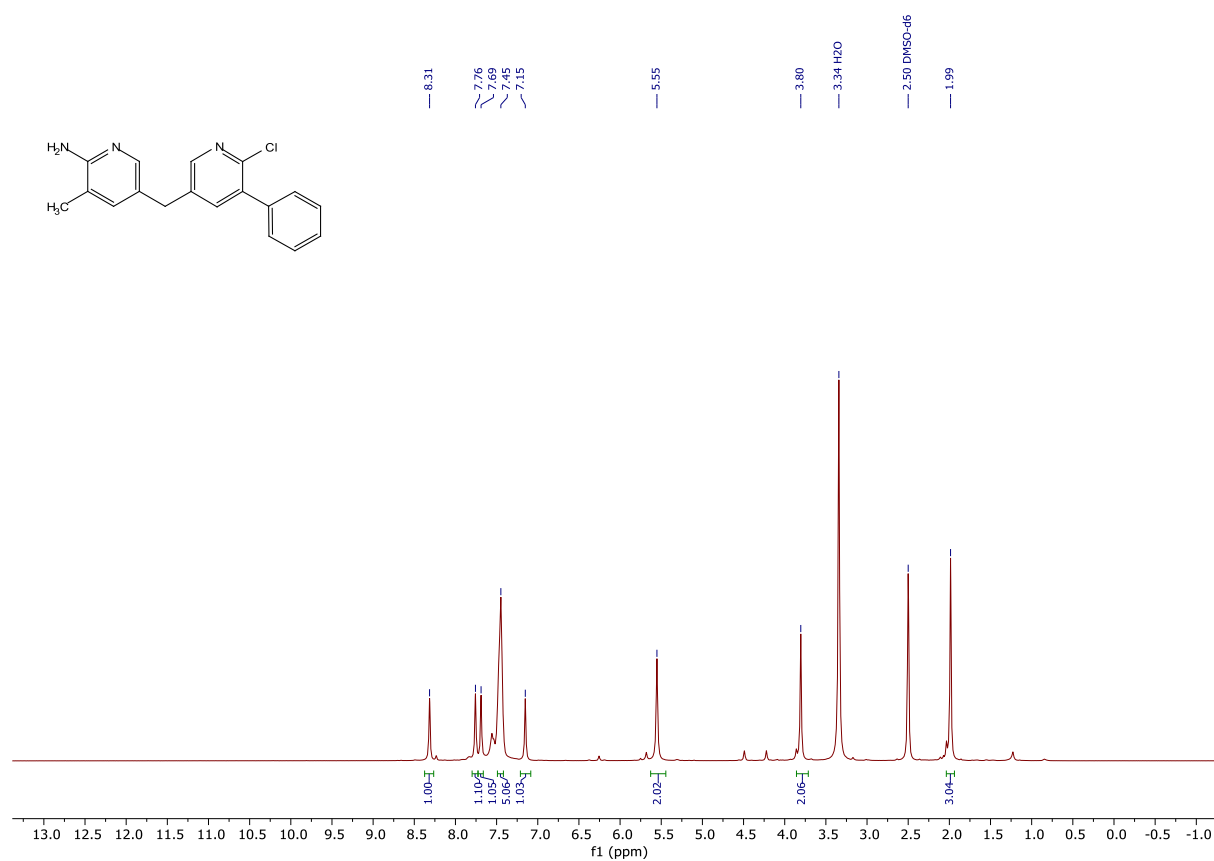


Figure S33. ^1H - and ^{13}C -NMR spectra of compound **23w**.

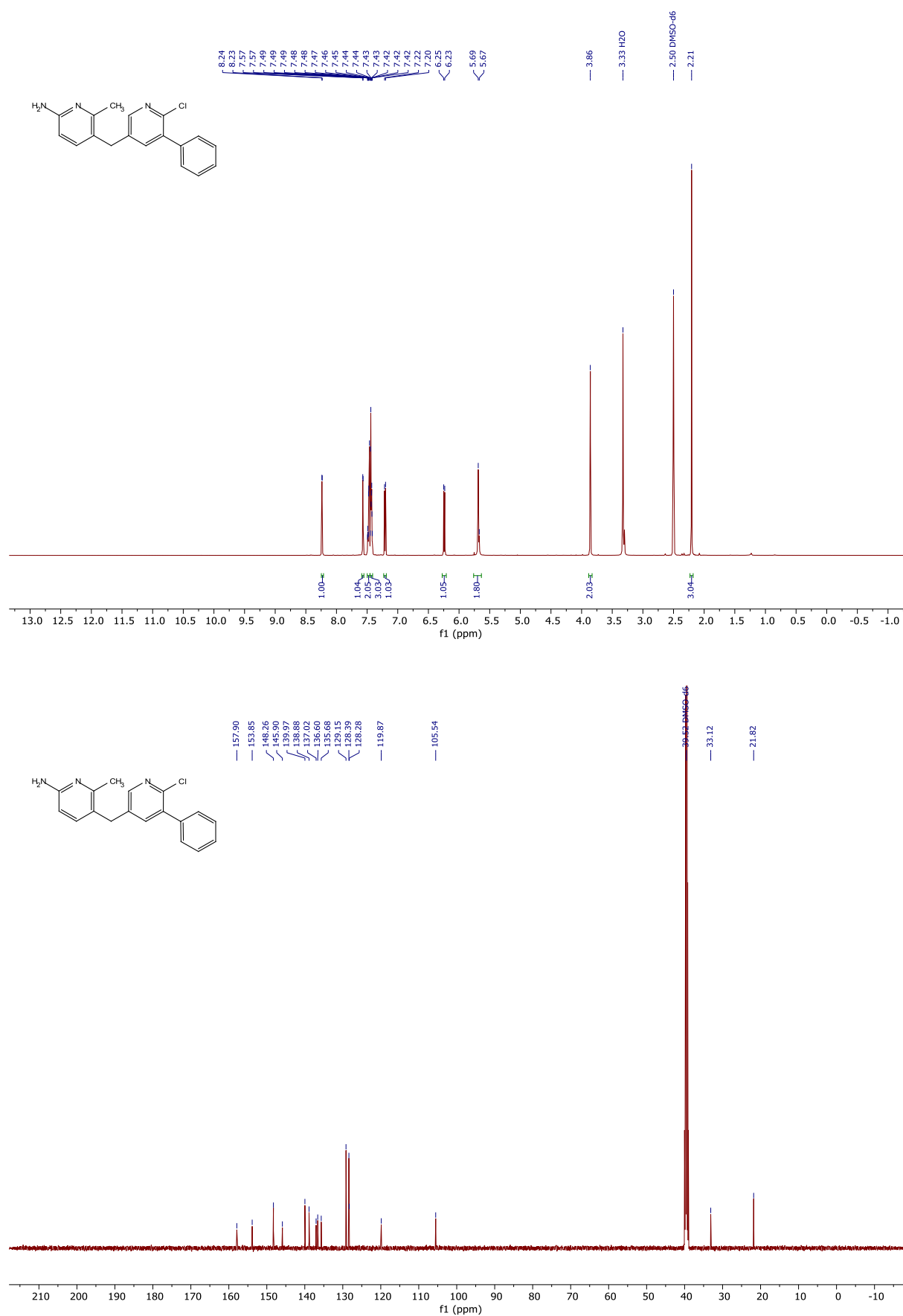


Figure S34. ¹H- and ¹³C-NMR spectra of compound **23x**.

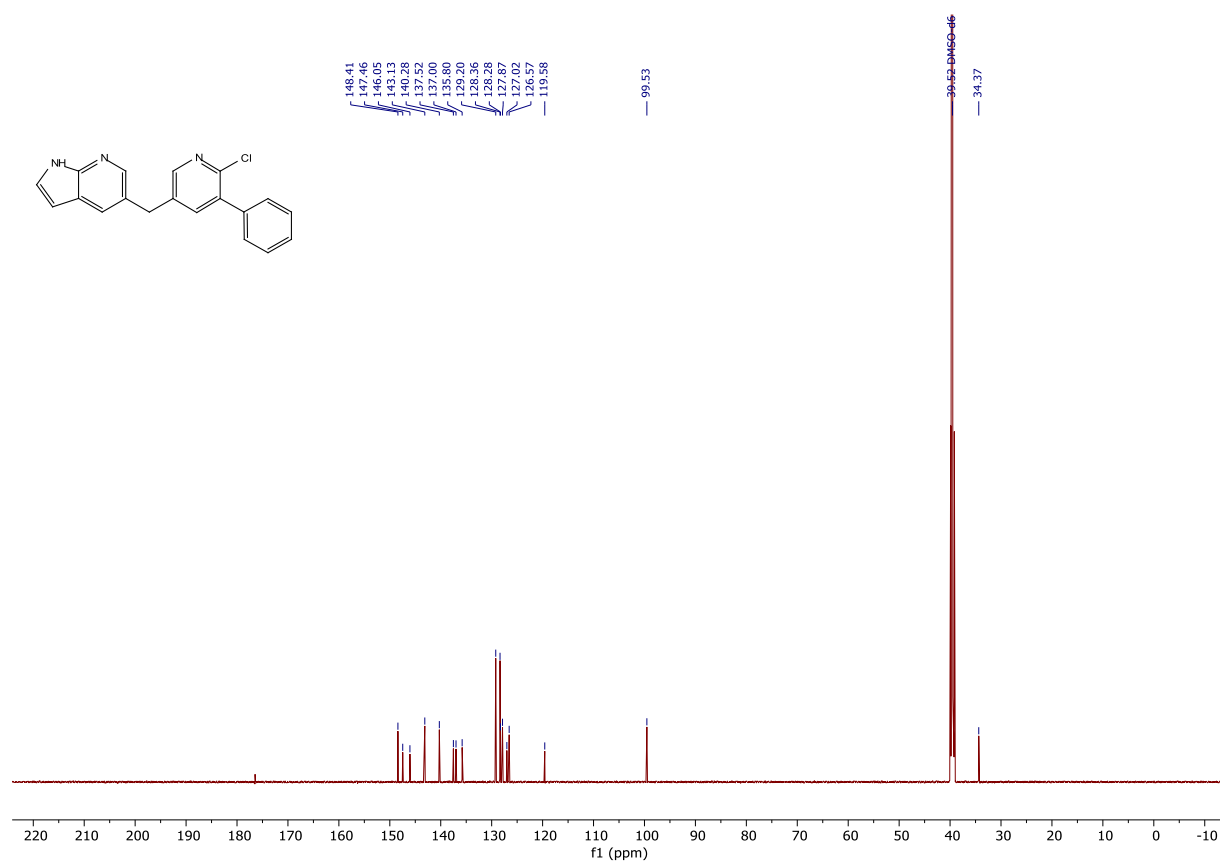
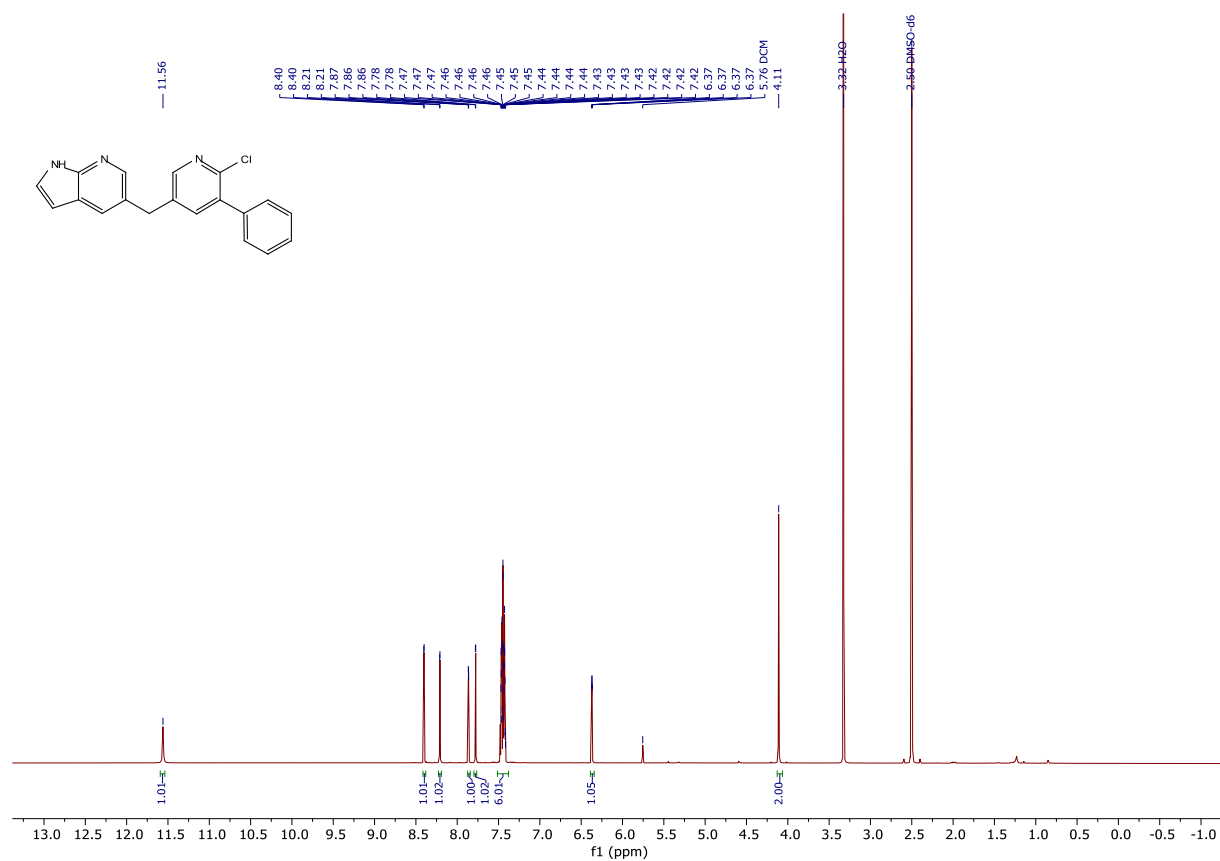


Figure S35. ¹H- and ¹³C-NMR spectra of compound **23y**.

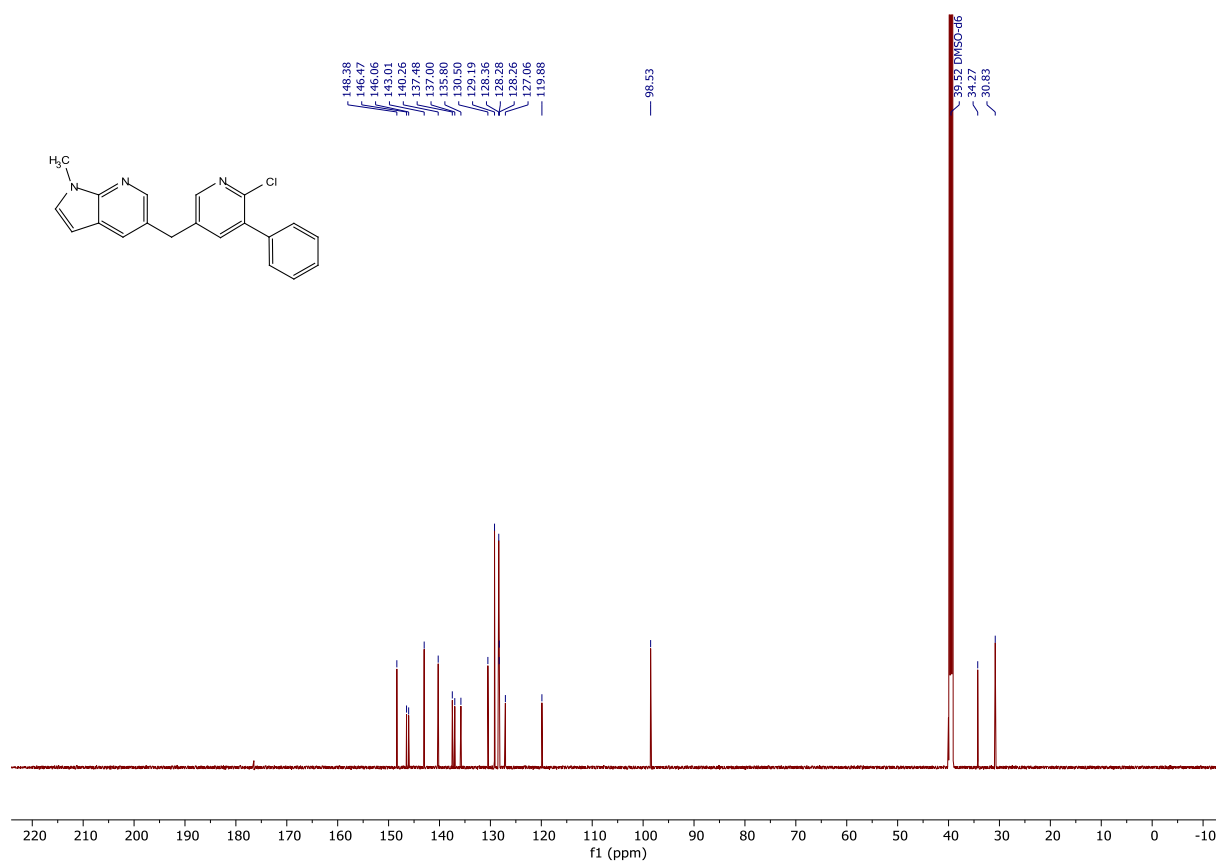
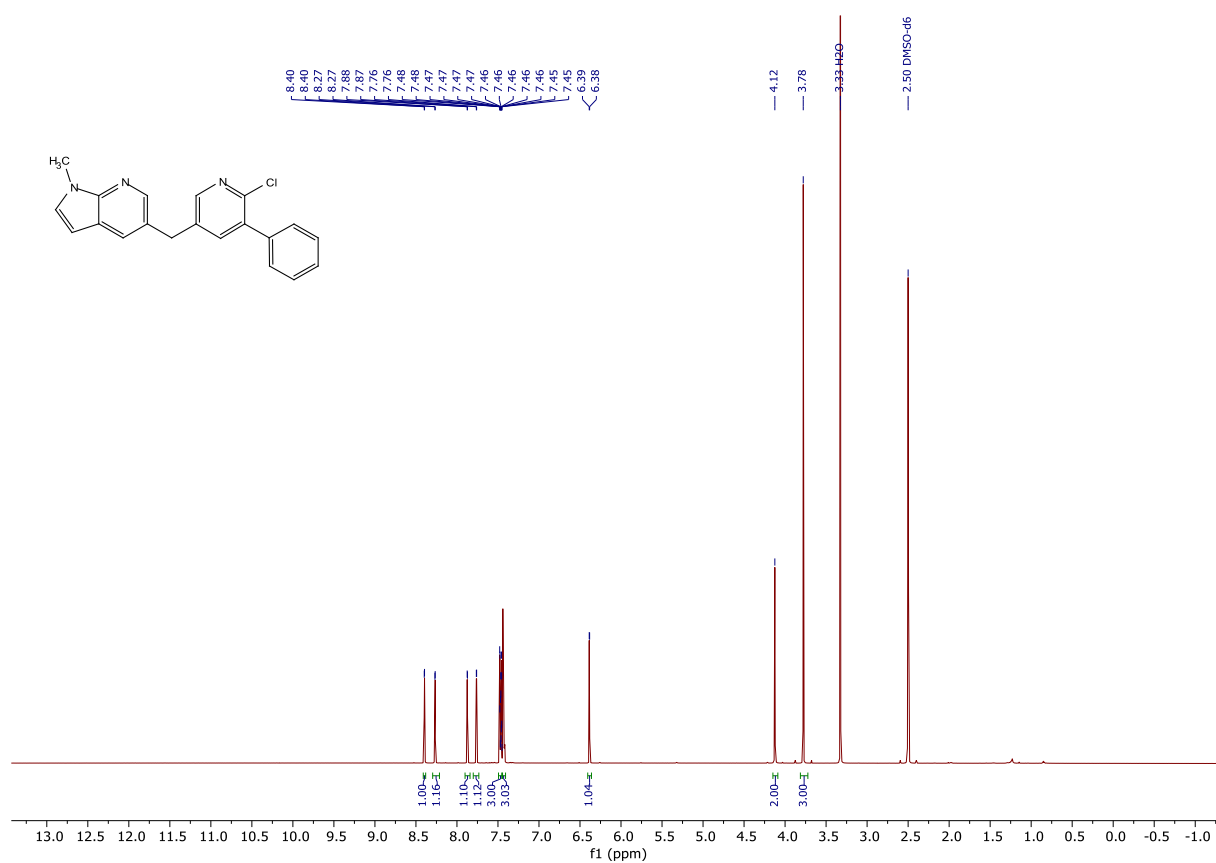


Figure S36. ¹H- and ¹³C-NMR spectra of compound **23z**.

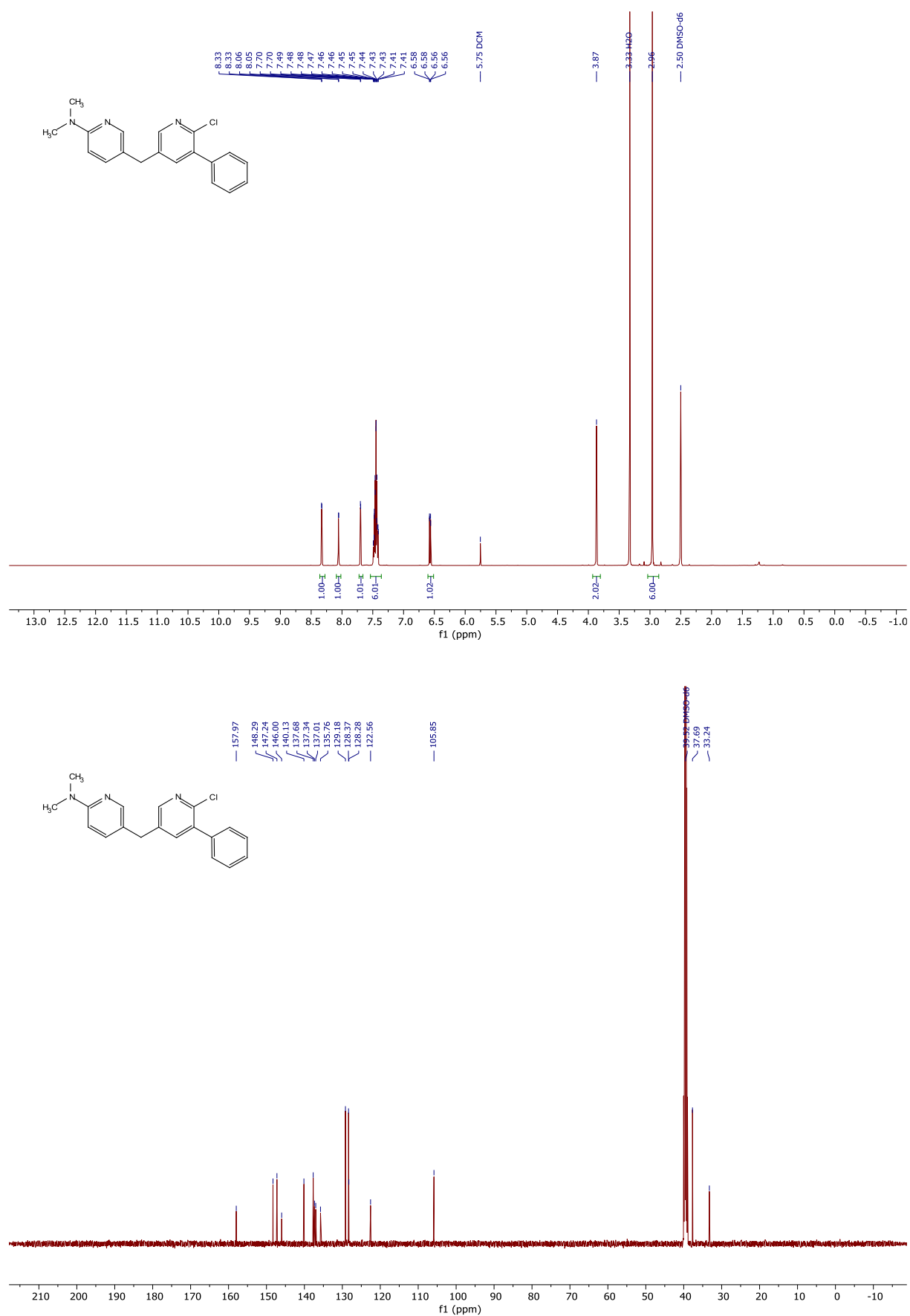


Figure S37. ¹H- and ¹³C-NMR spectra of compound 23aa.

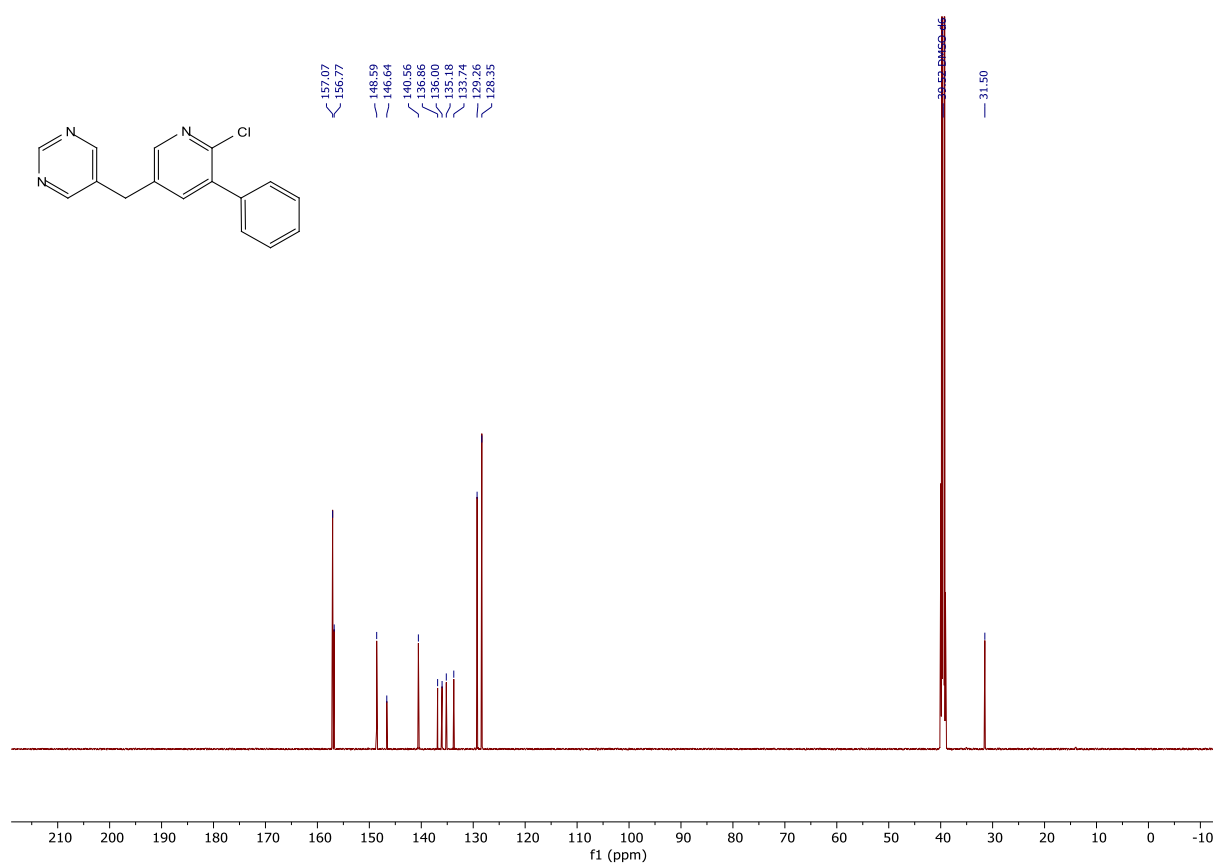
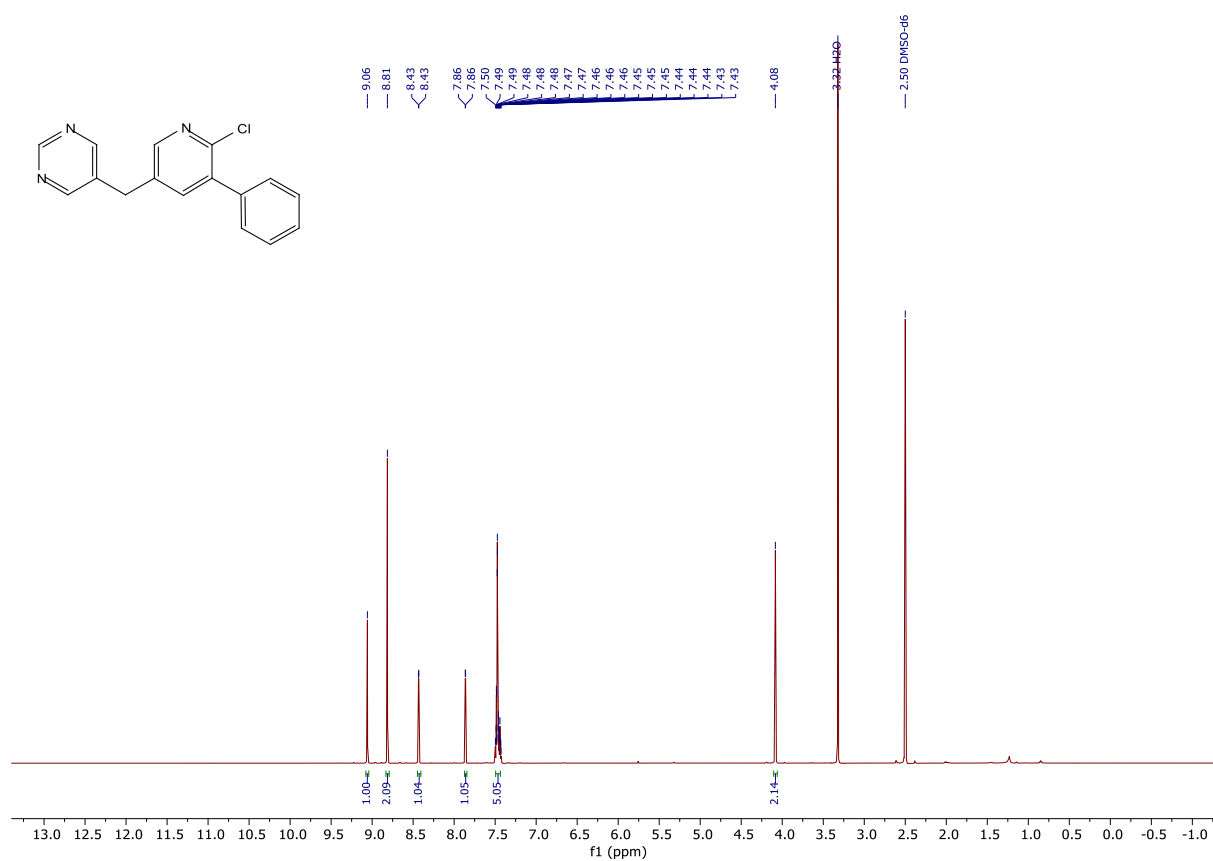


Figure S38. ¹H- and ¹³C-NMR spectra of compound **23ab**.

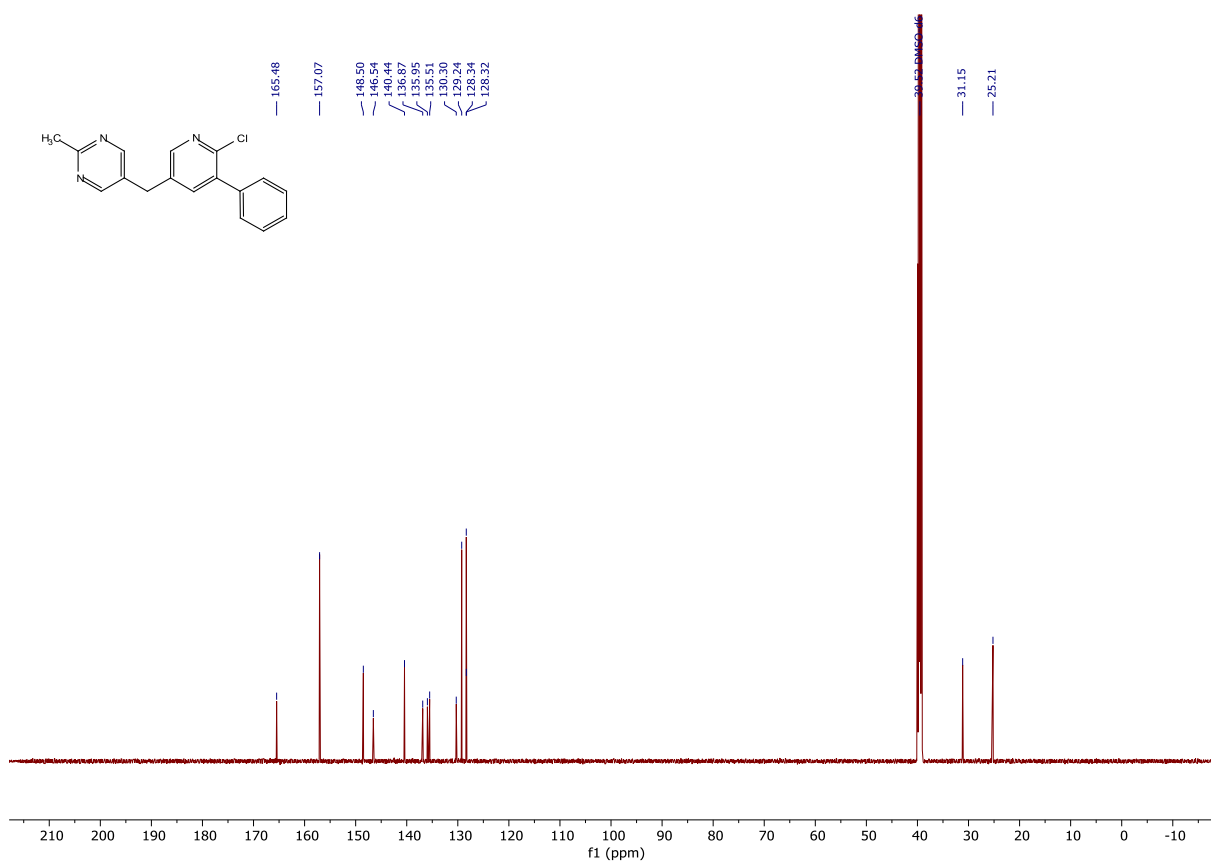
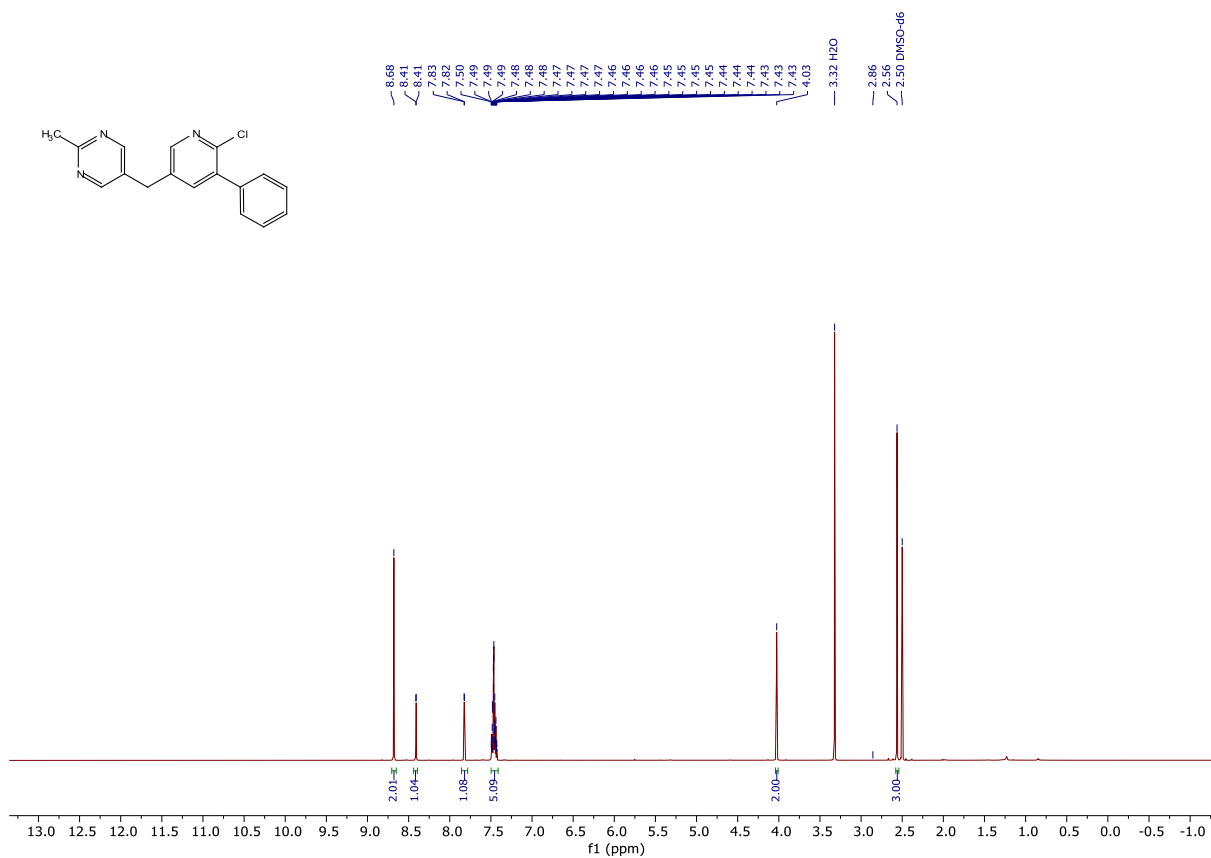


Figure S39. ^1H - and ^{13}C -NMR spectra of compound **23ac**.

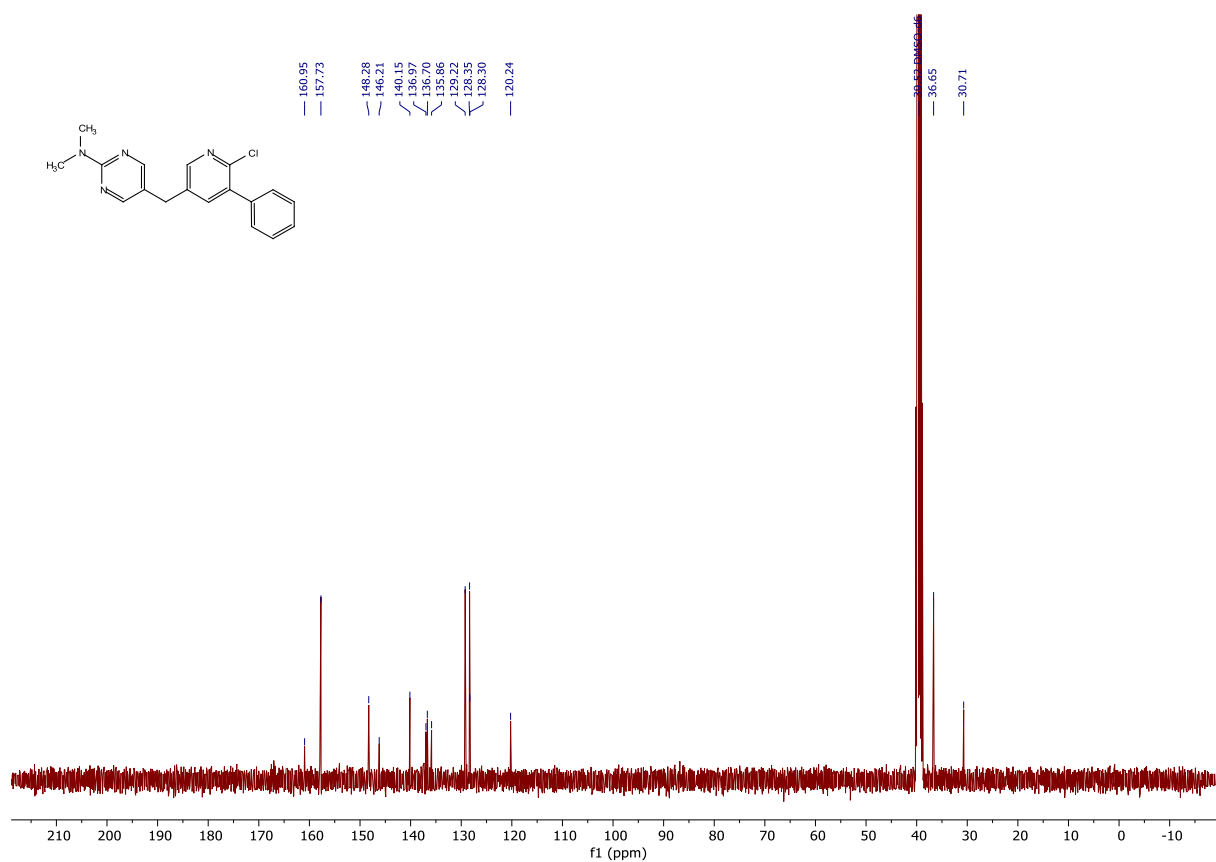
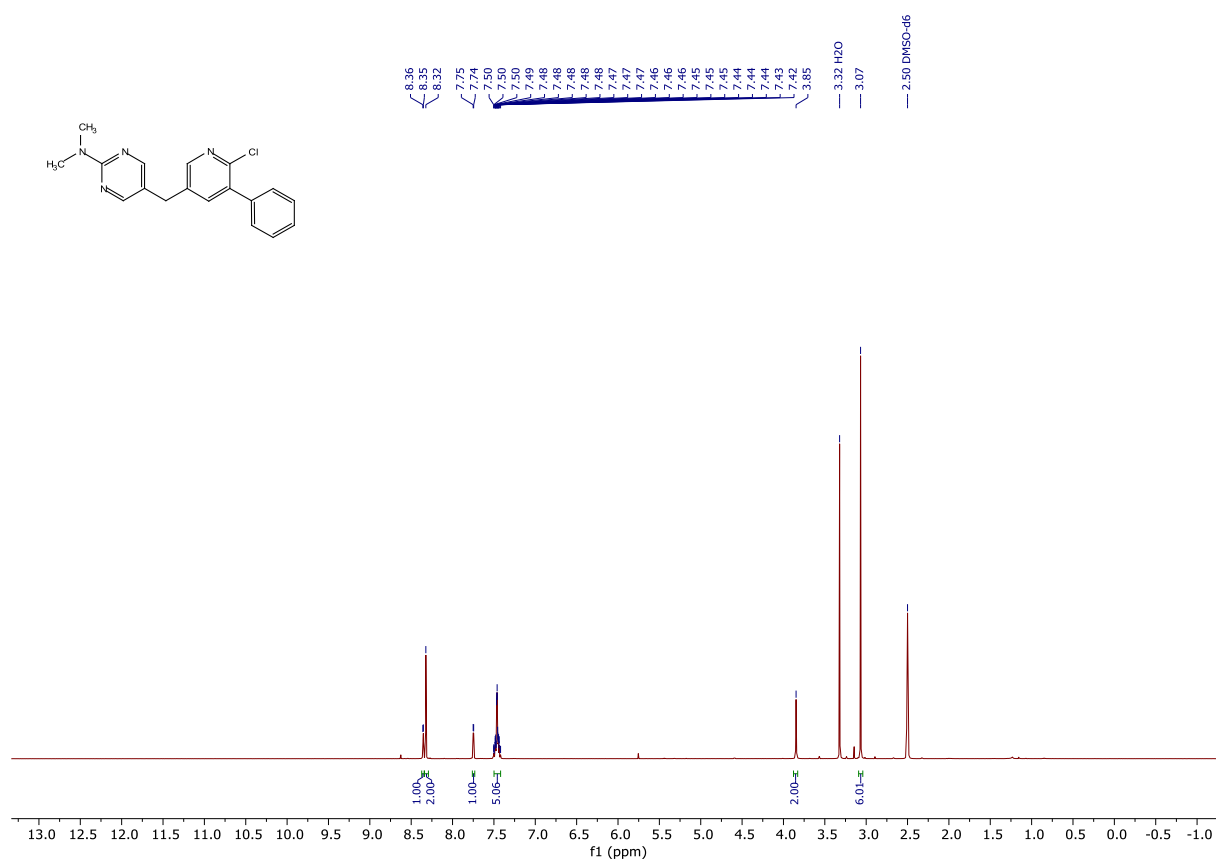


Figure S40. ¹H- and ¹³C-NMR spectra of compound **23ad**

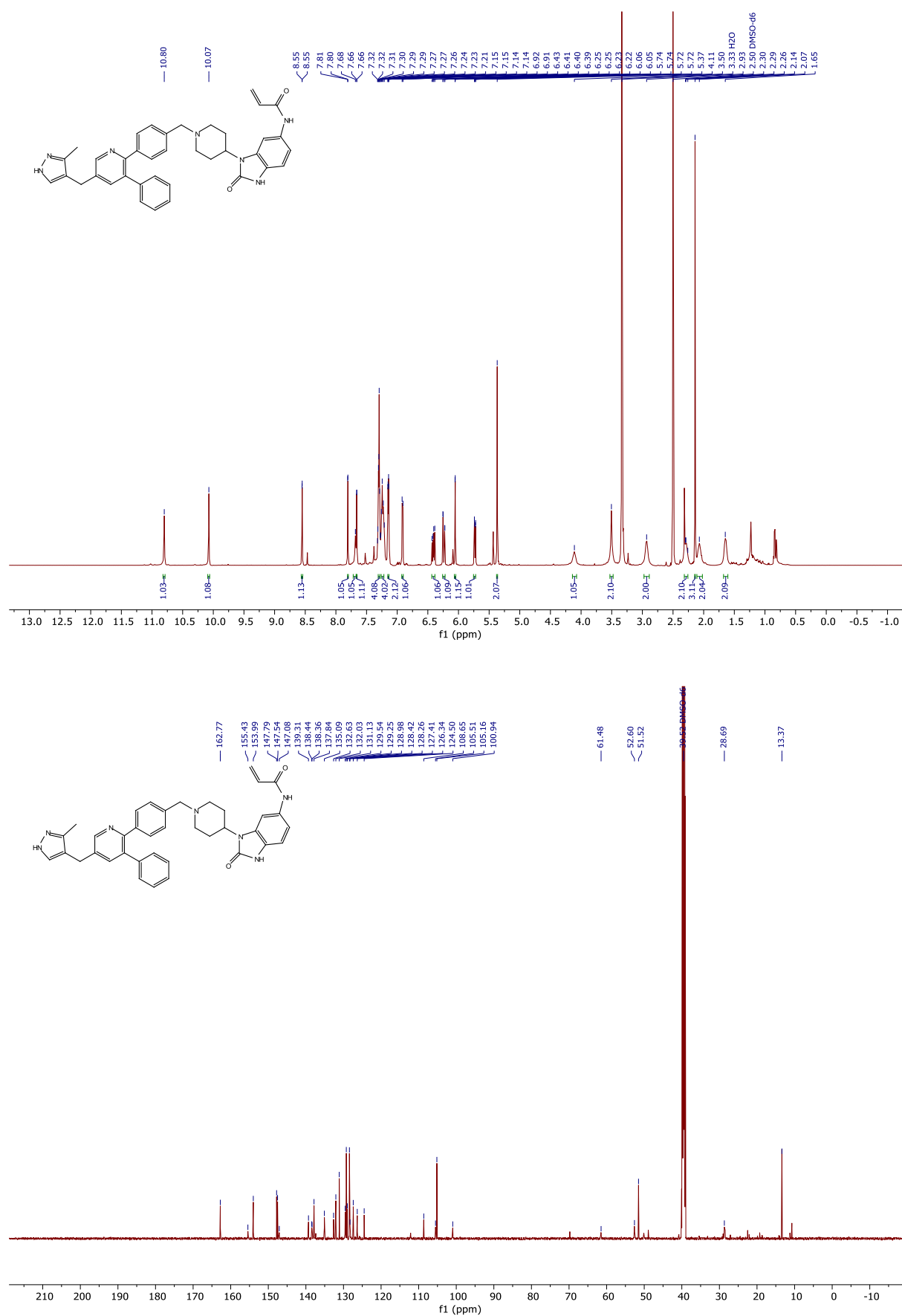


Figure S41. ¹H- and ¹³C-NMR spectra of compound 24.

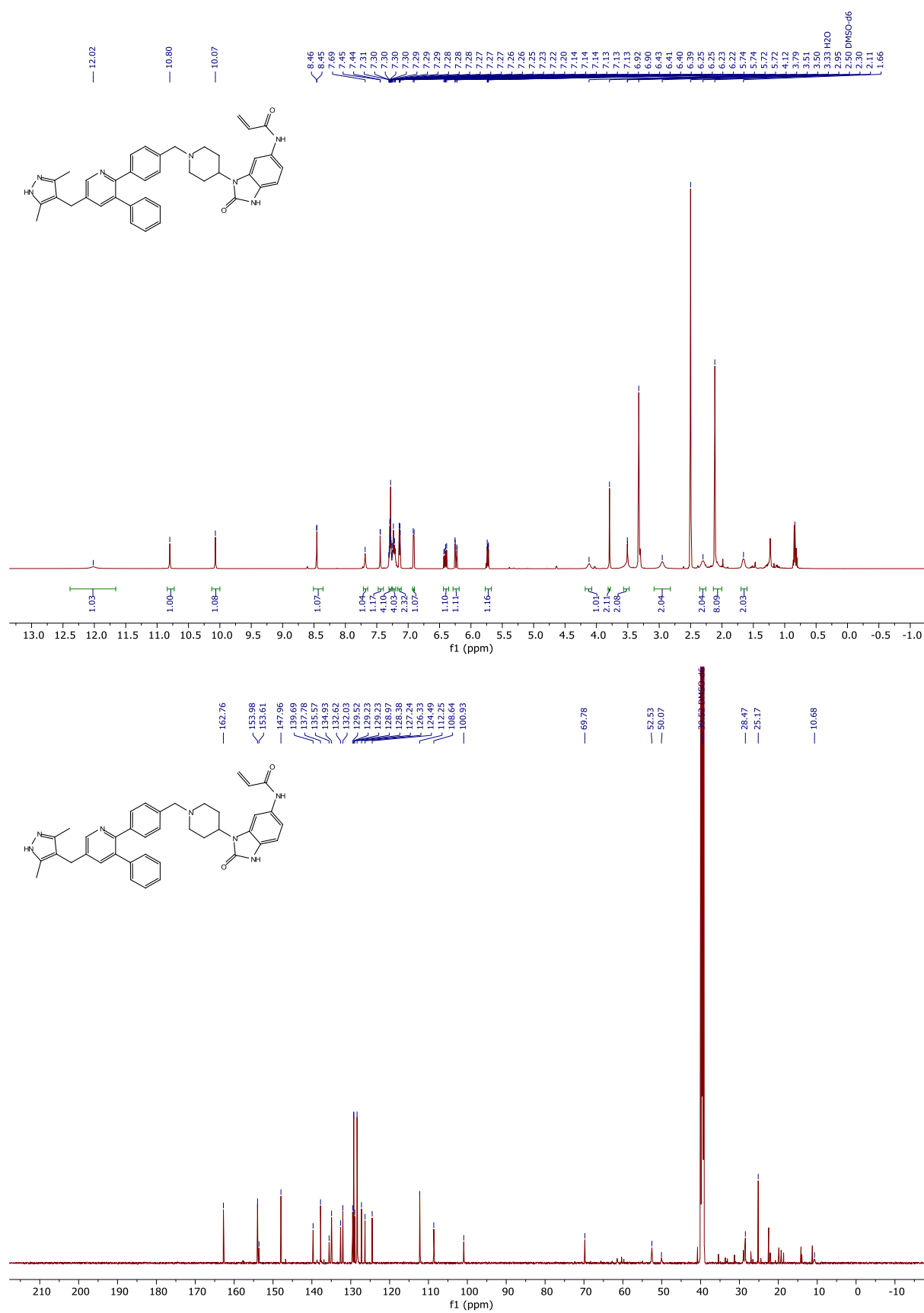


Figure S42. ¹H- and ¹³C-NMR spectra of compound 25.

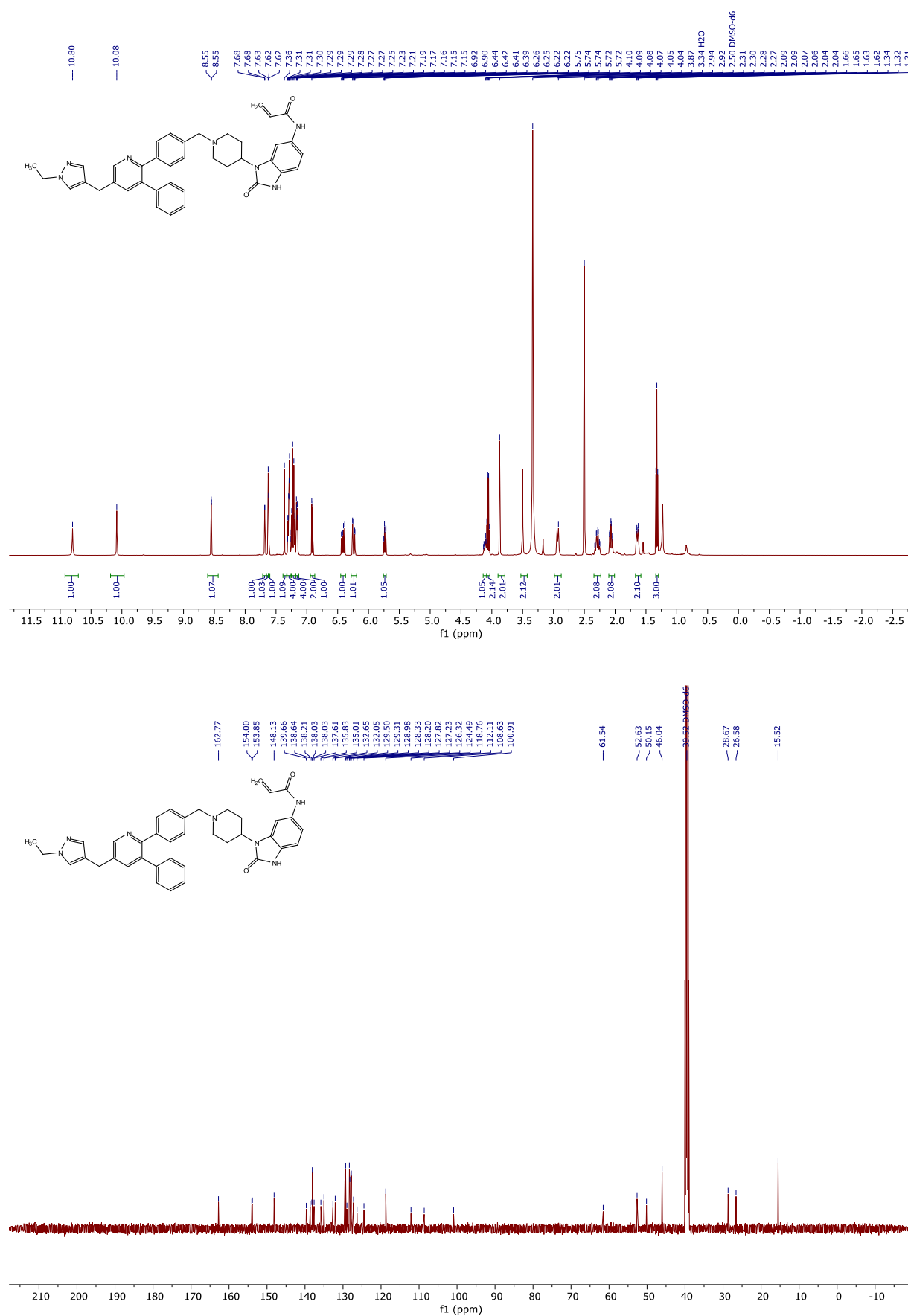


Figure S43. ¹H- and ¹³C-NMR spectra of compound **26**.

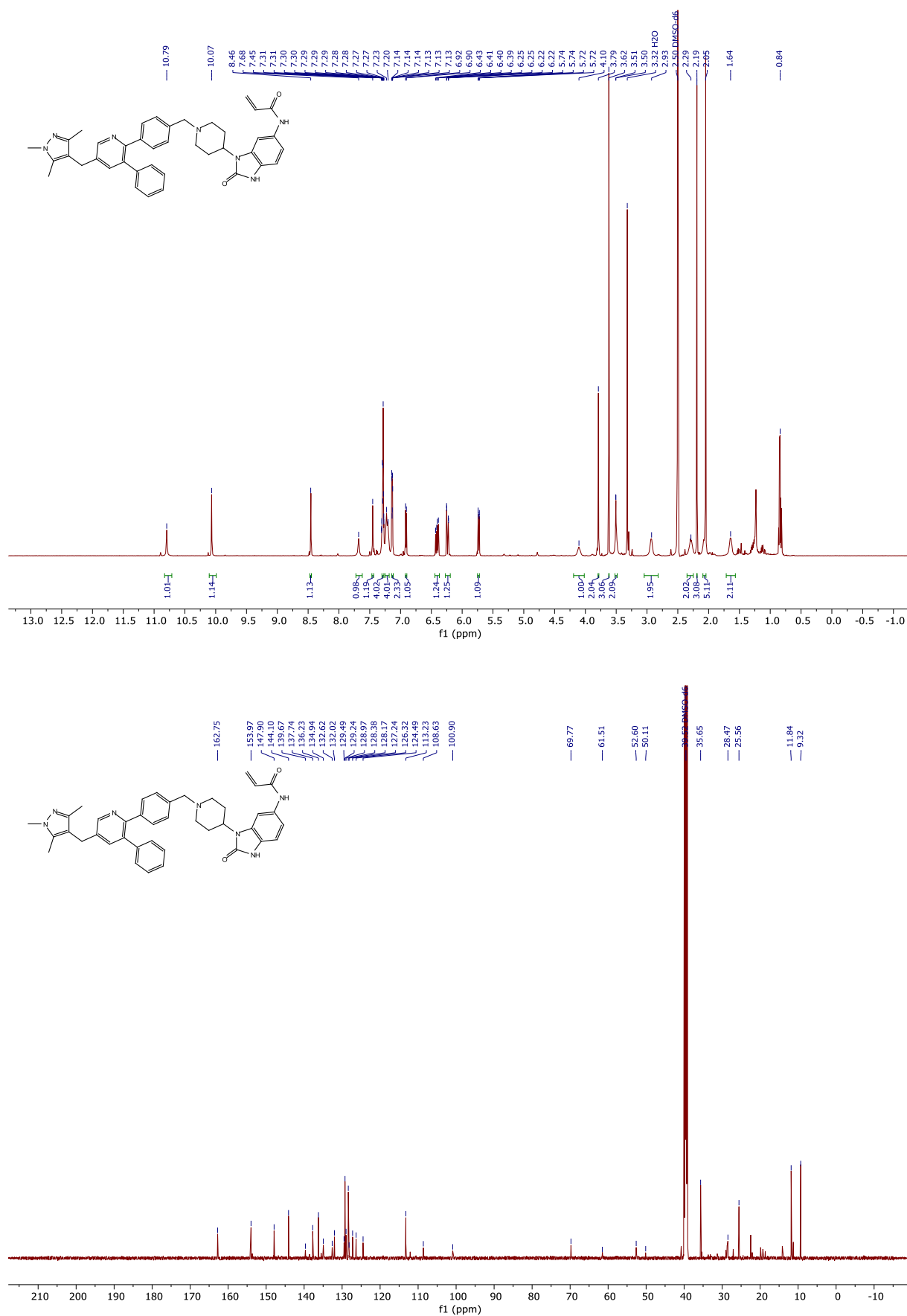


Figure S44. ¹H- and ¹³C-NMR spectra of compound **27**.

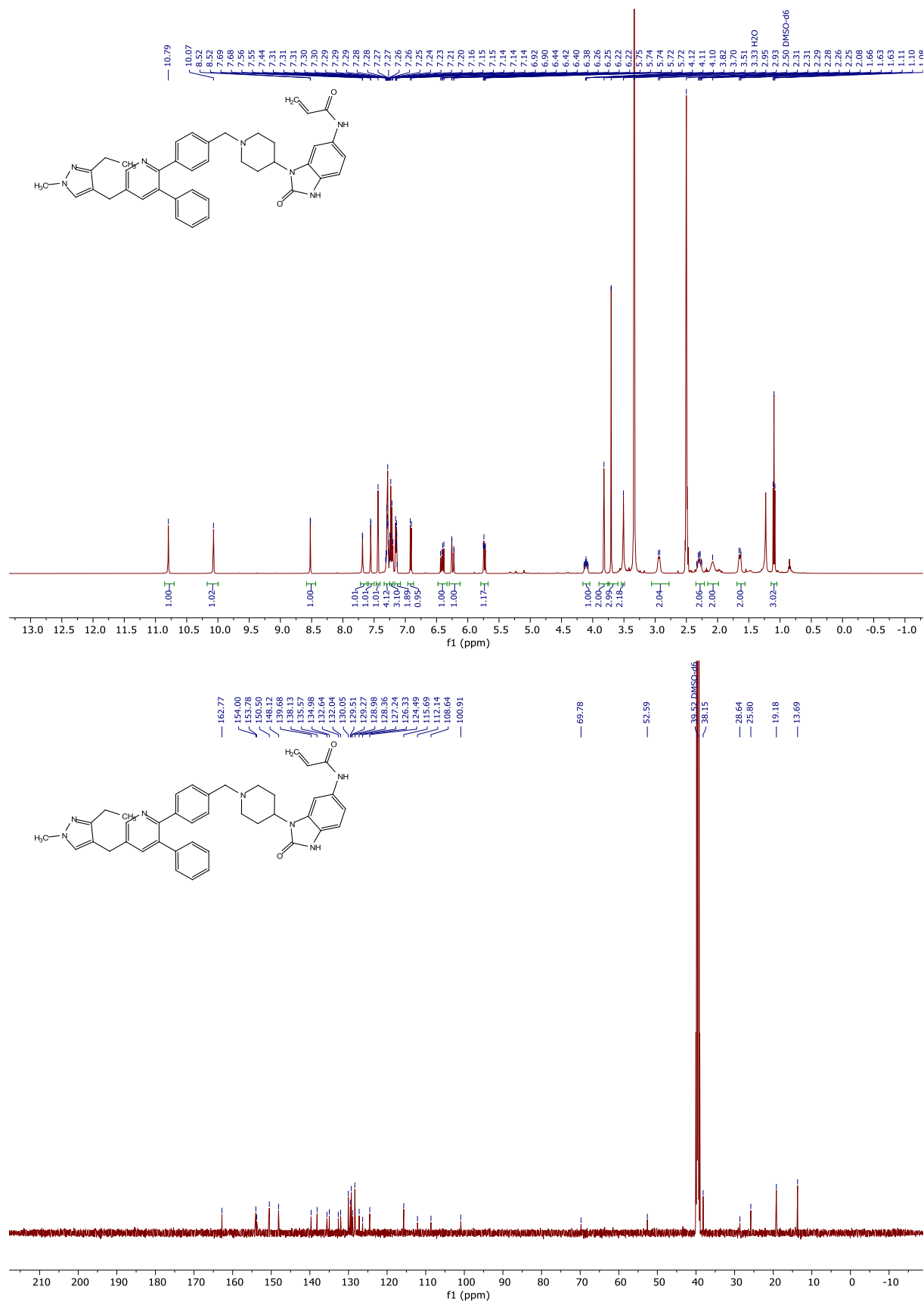


Figure S45. ¹H- and ¹³C-NMR spectra of compound 28.

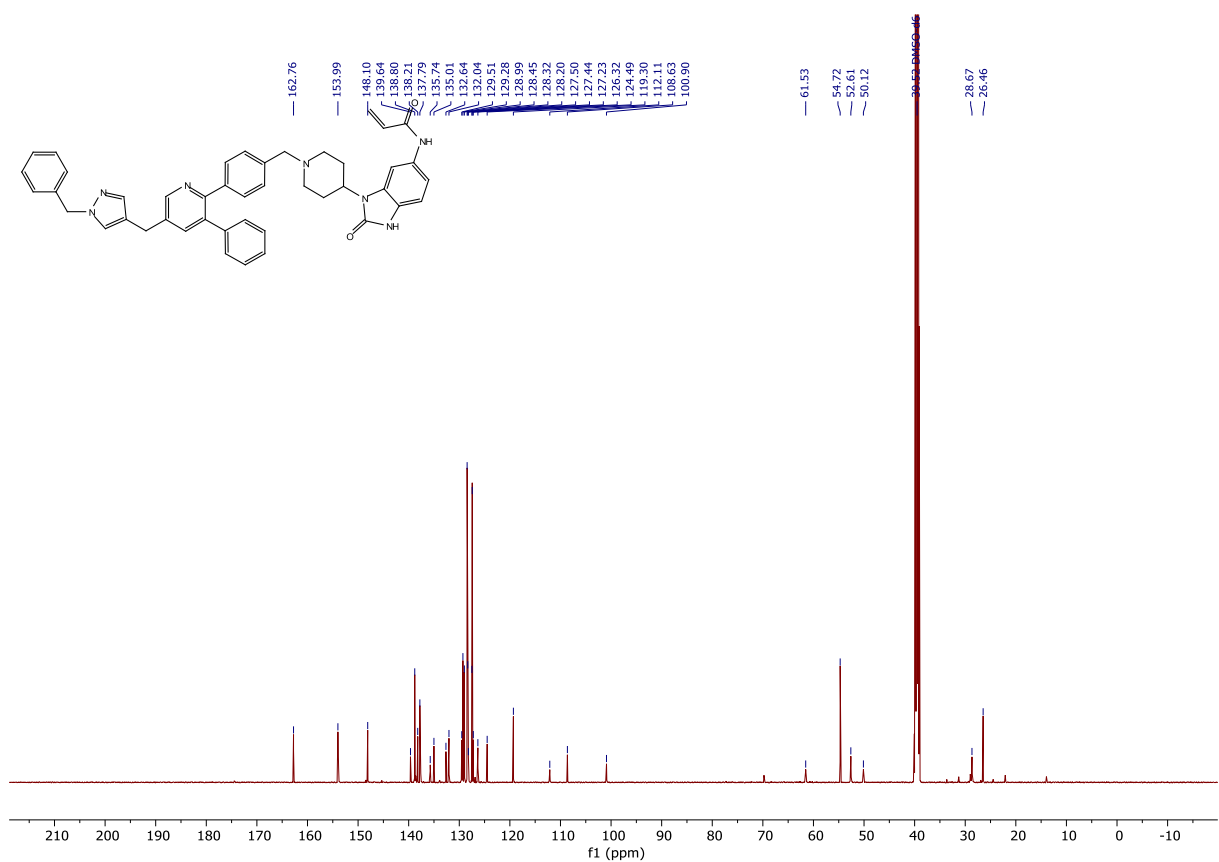
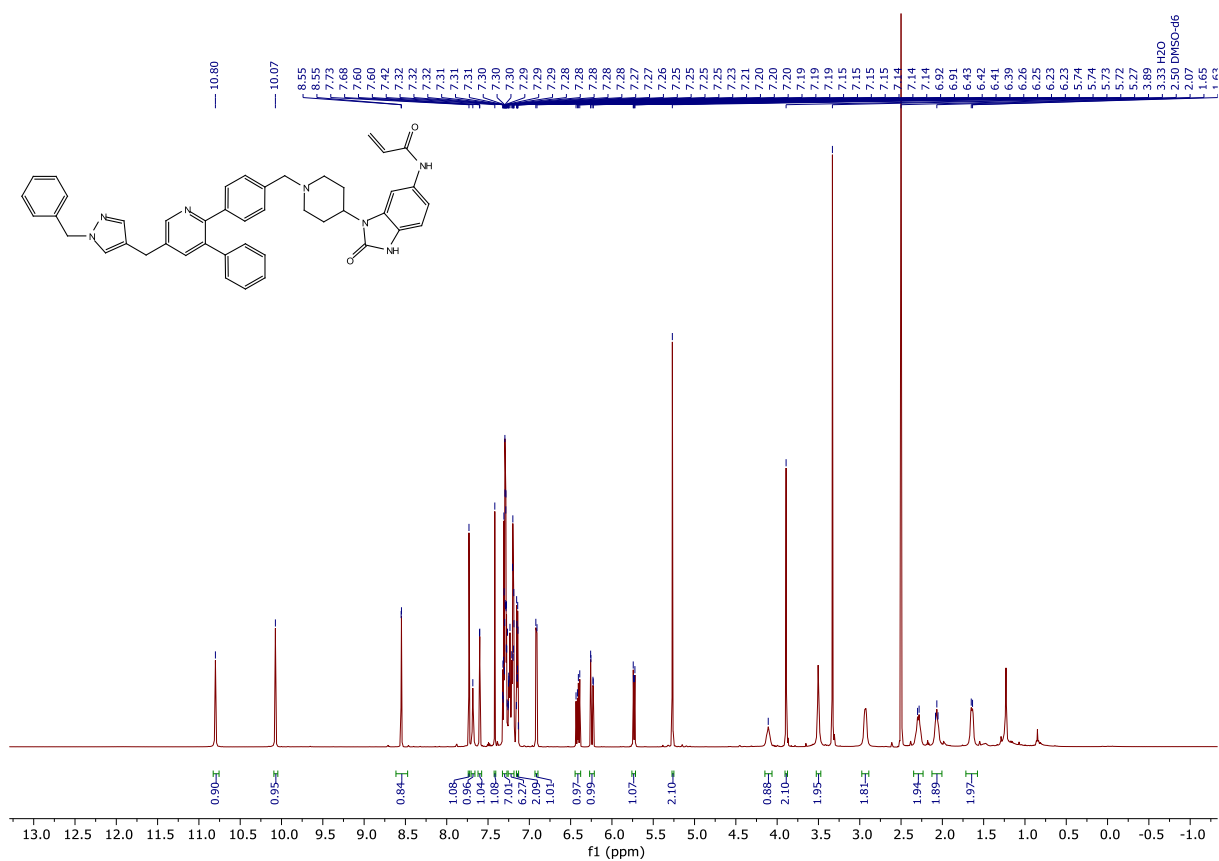
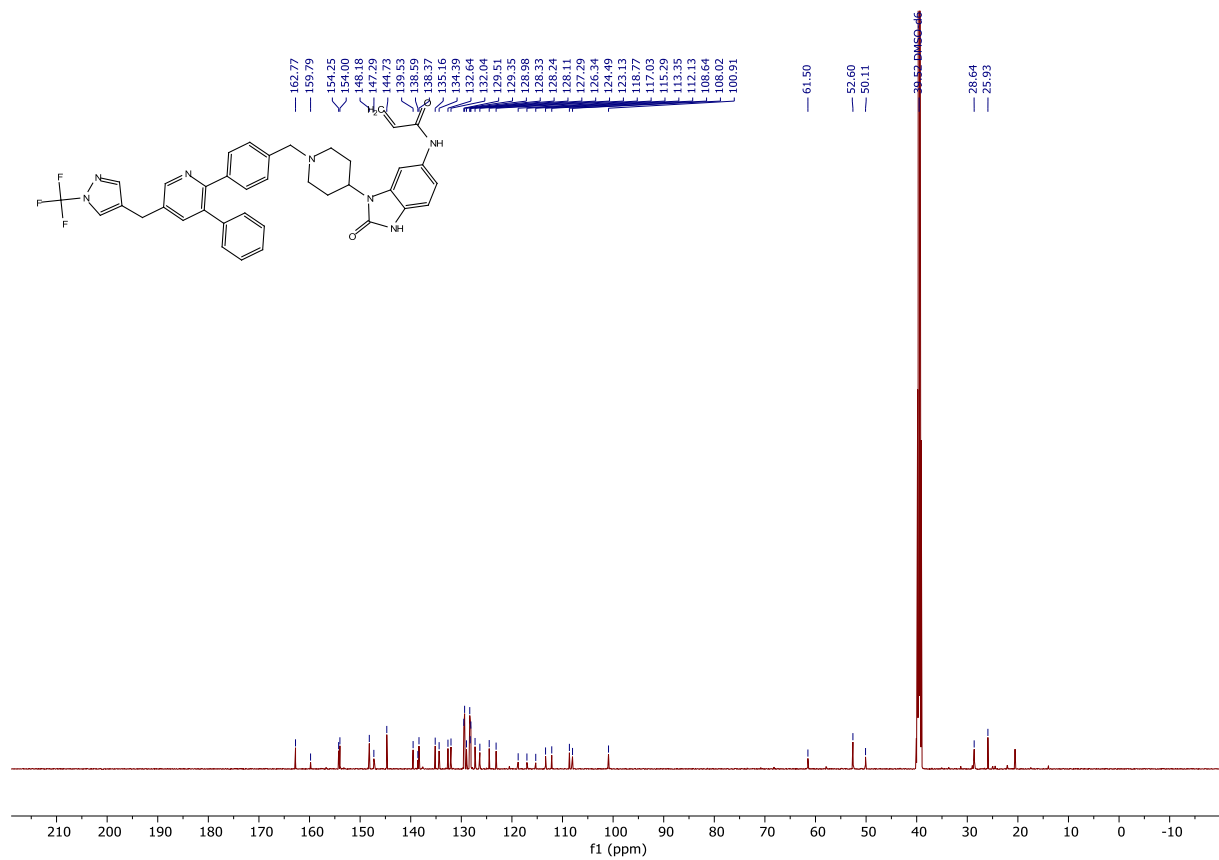
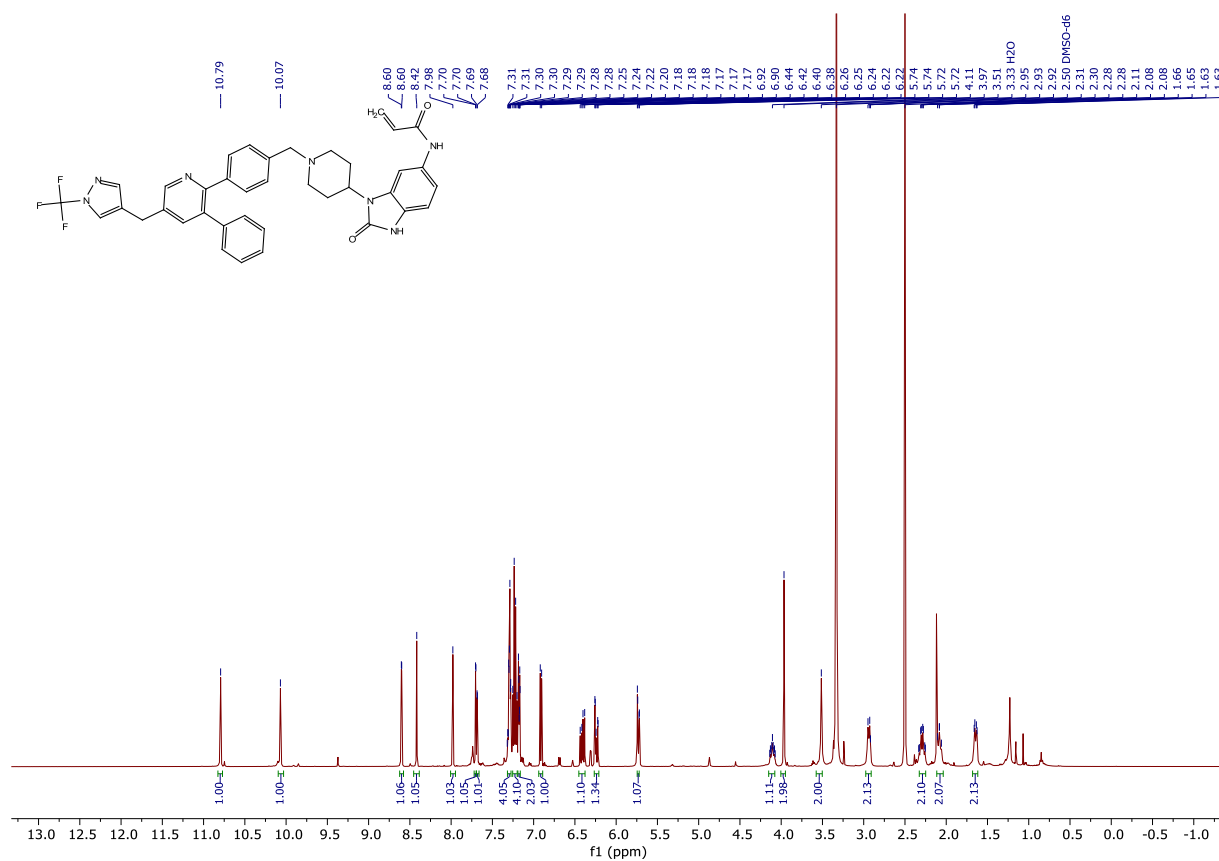


Figure S46. ^1H - and ^{13}C -NMR spectra of compound **29**.



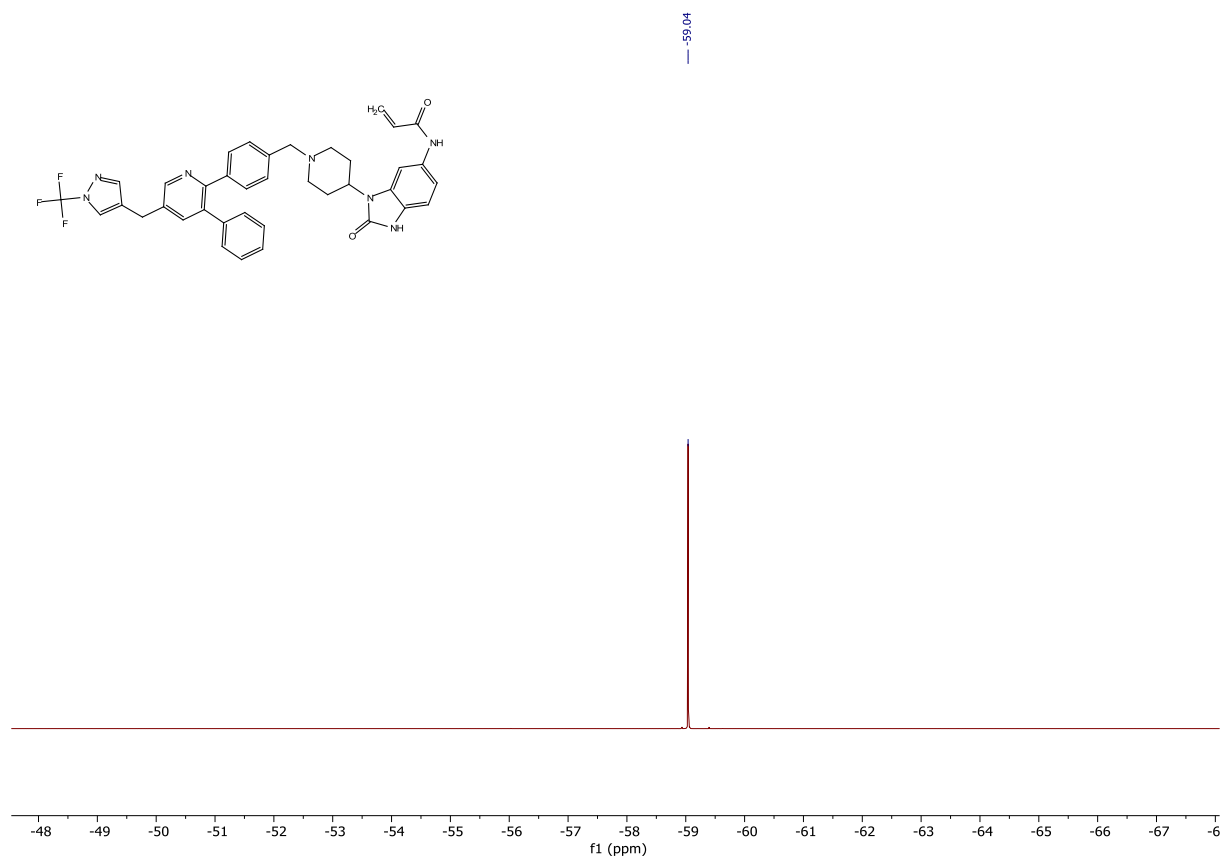


Figure S47. ^1H -, ^{13}C - and ^{19}F NMR spectra of compound 30.

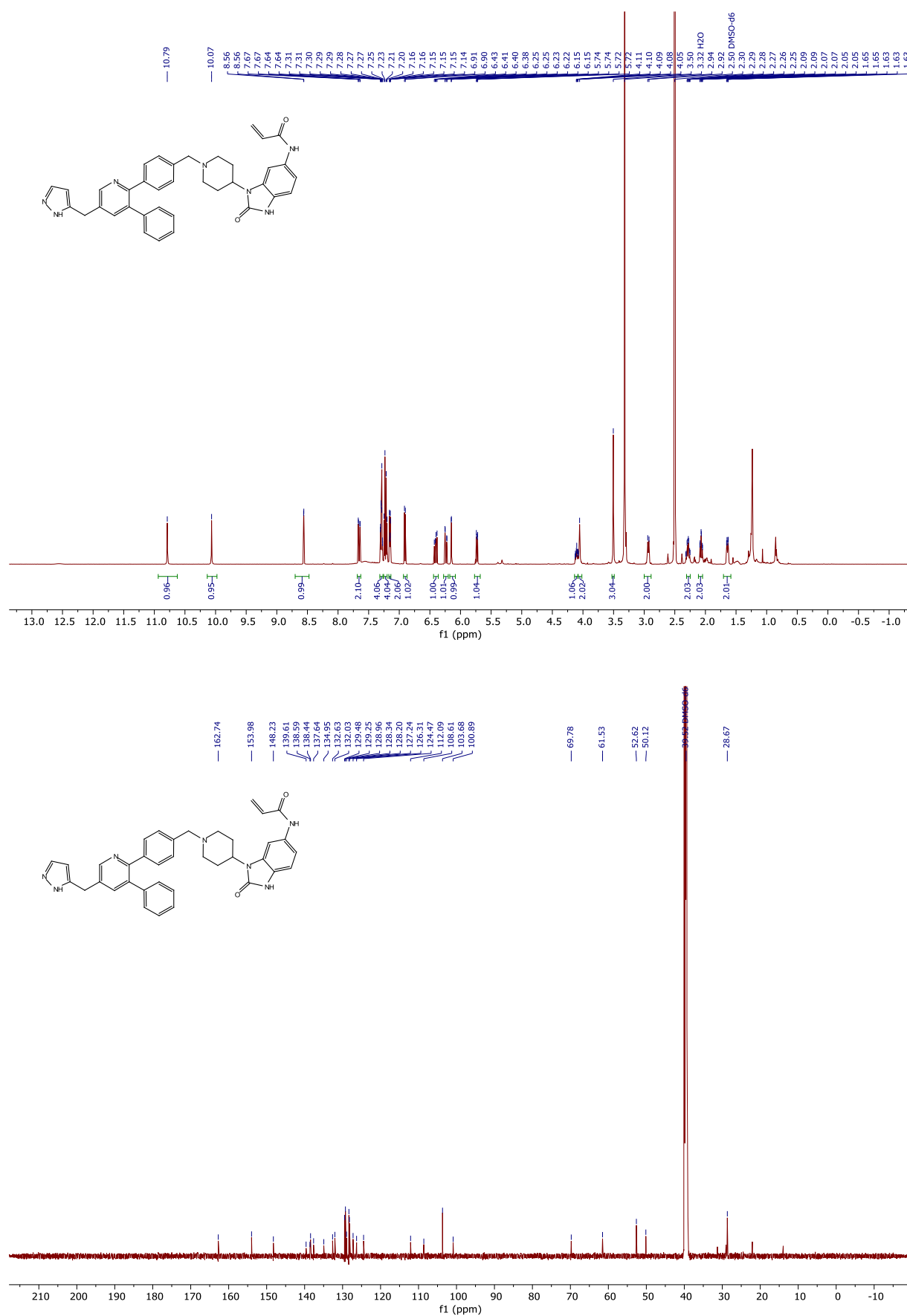


Figure S48. ¹H- and ¹³C-NMR spectra of compound 31.

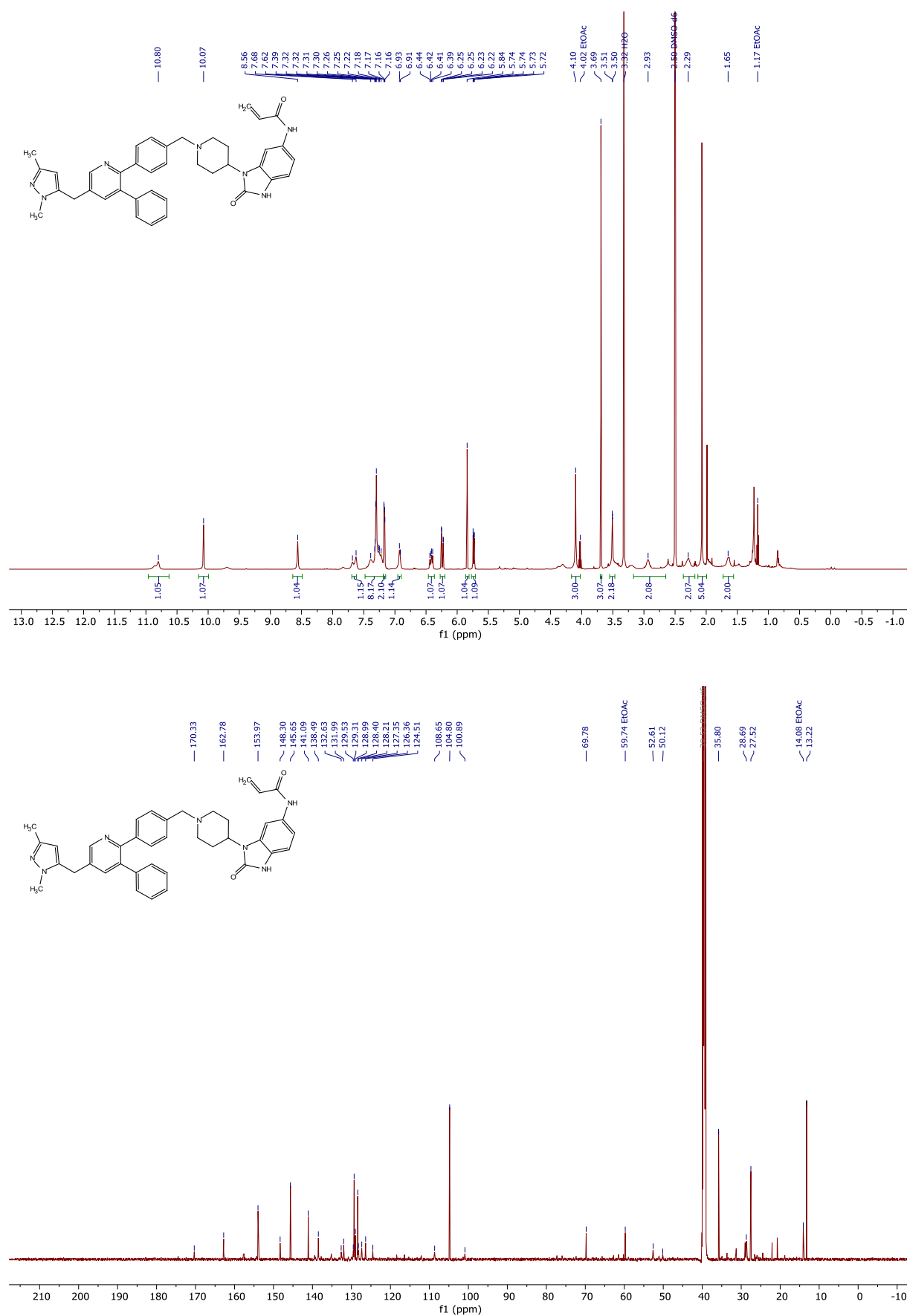


Figure S50. ¹H- and ¹³C-NMR spectra of compound **33**.

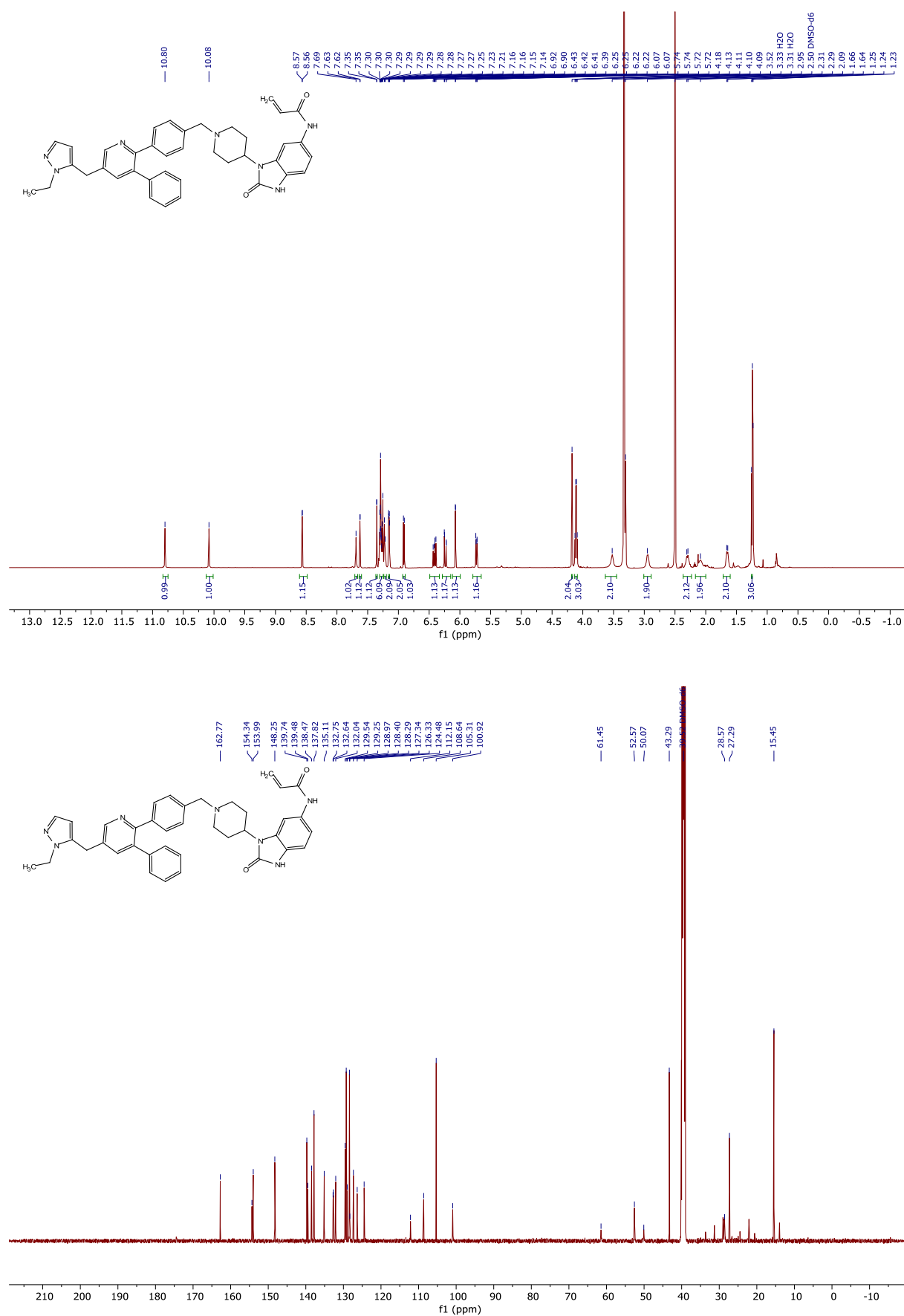


Figure S51. ¹H- and ¹³C-NMR spectra of compound **34**.

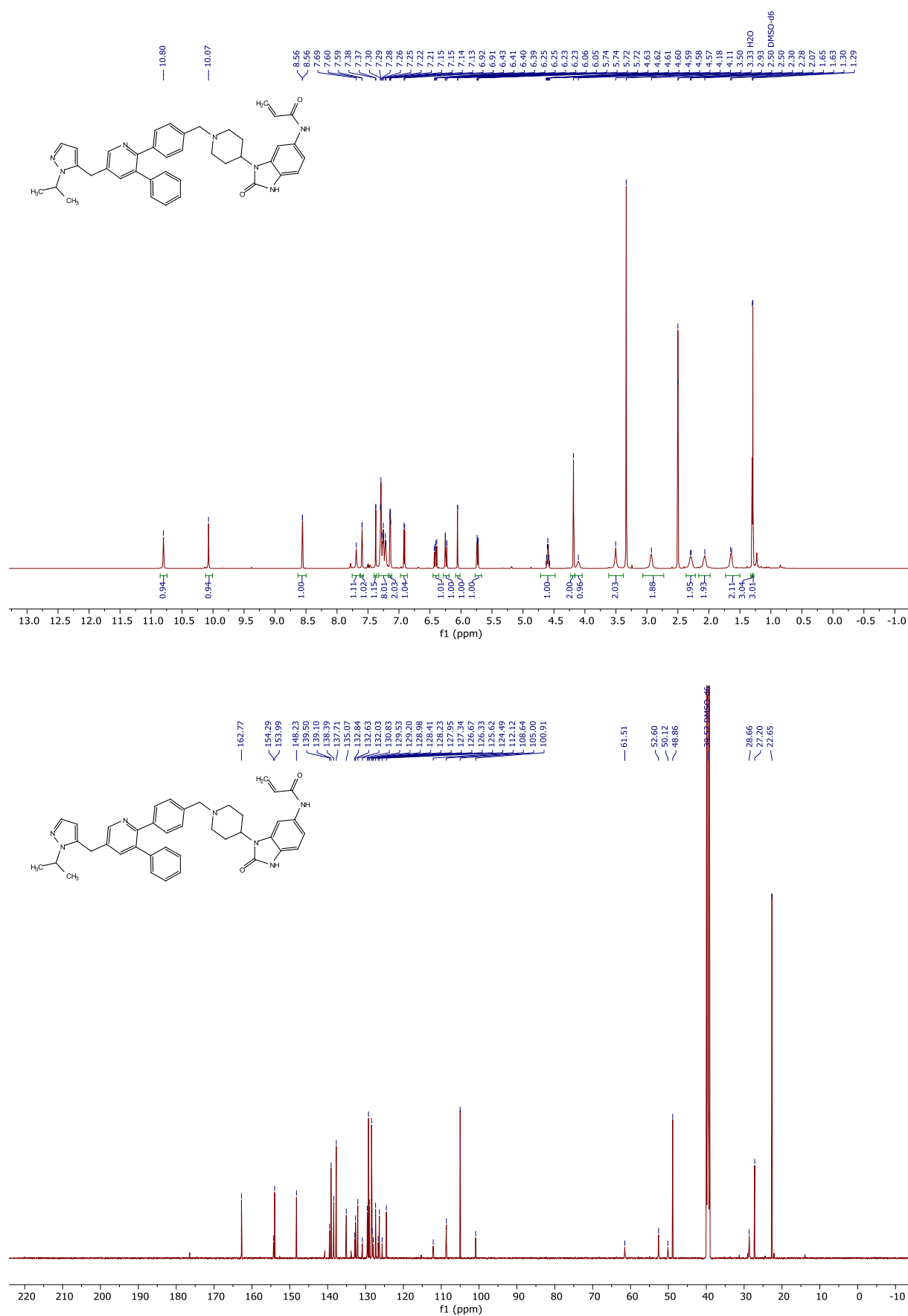


Figure S52. ¹H- and ¹³C-NMR spectra of compound **35**.

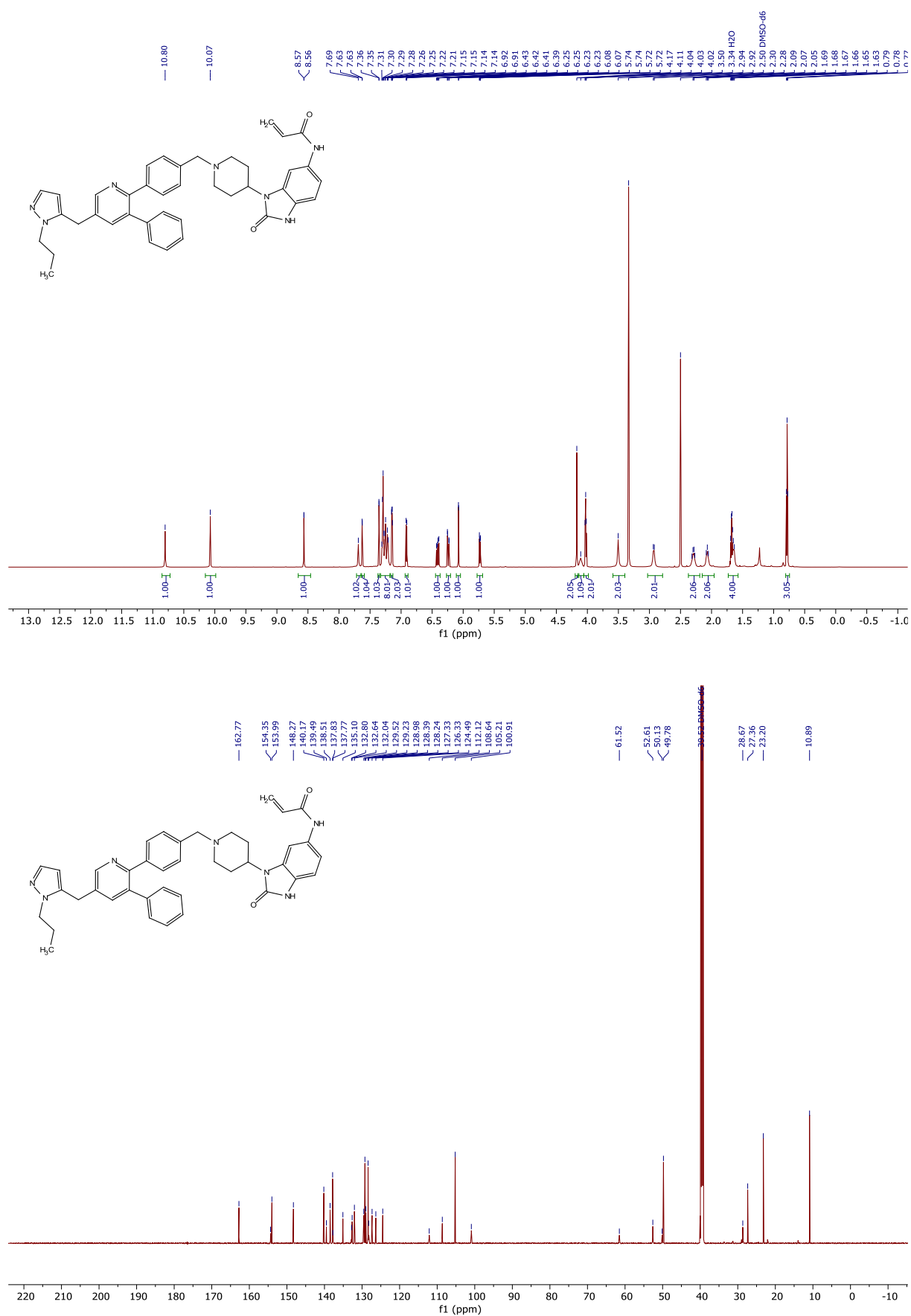


Figure S53. ¹H- and ¹³C-NMR spectra of compound **36**.

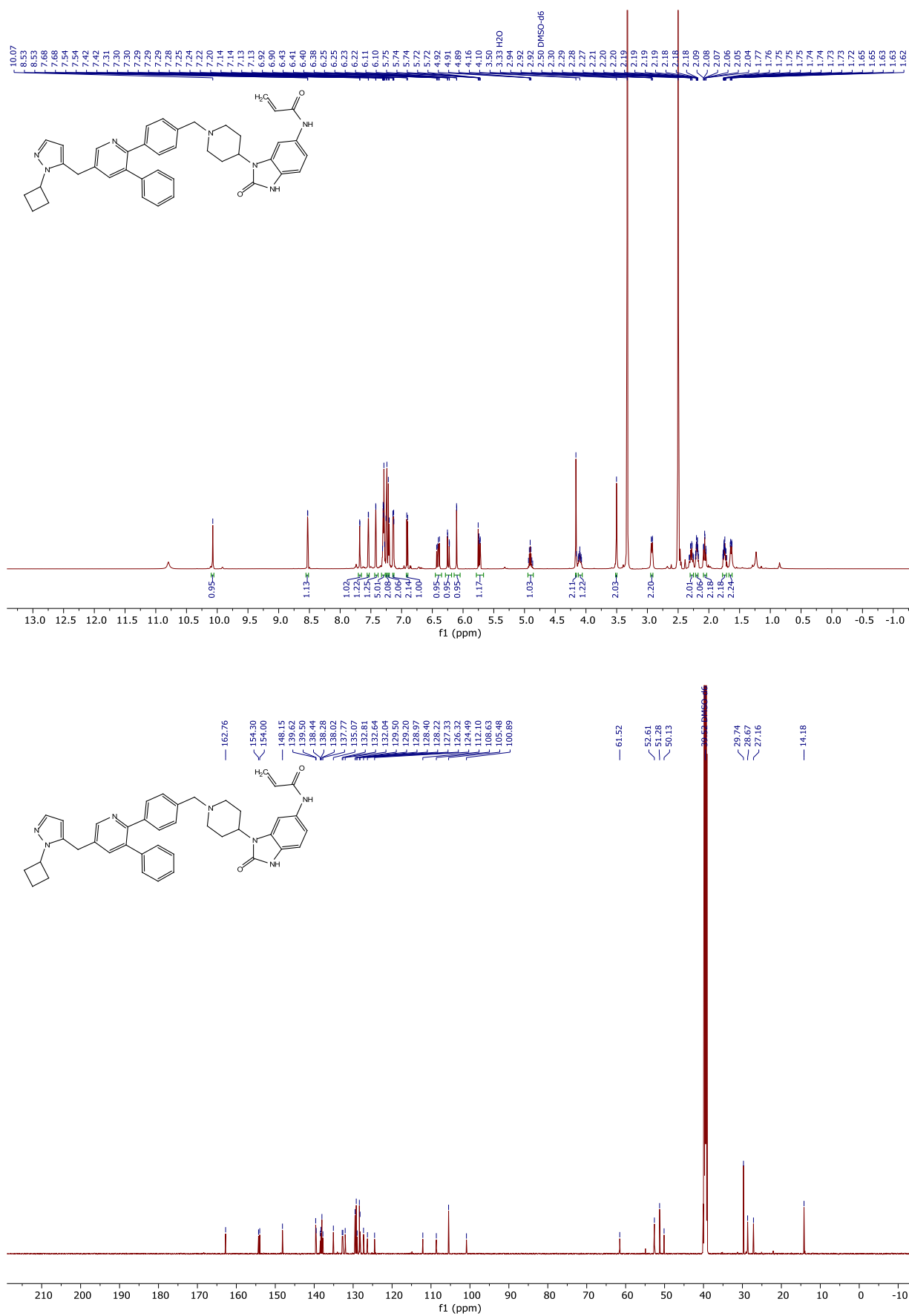


Figure S54. ¹H- and ¹³C-NMR spectra of compound 37.

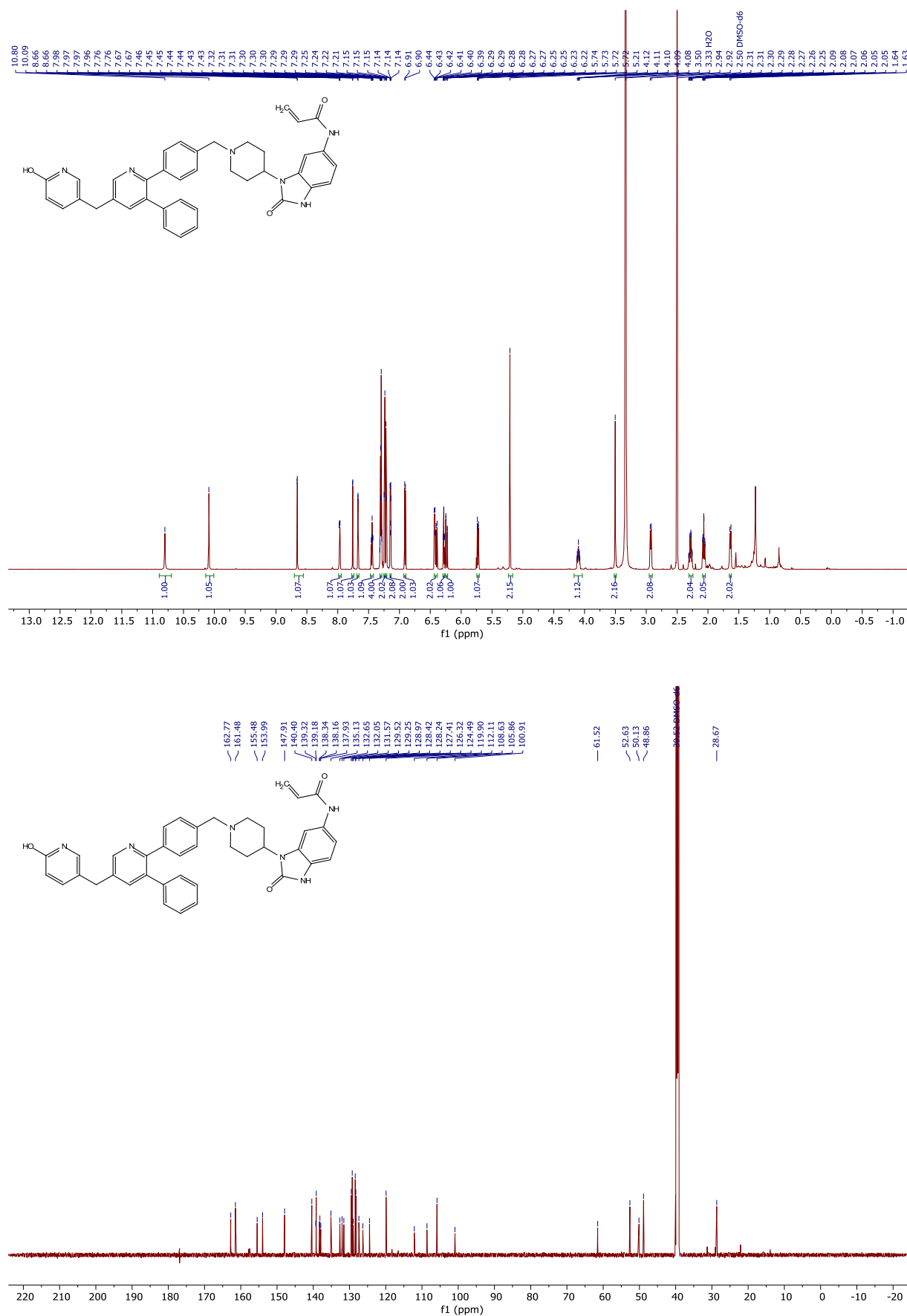


Figure S55. ¹H- and ¹³C-NMR spectra of compound **38**.

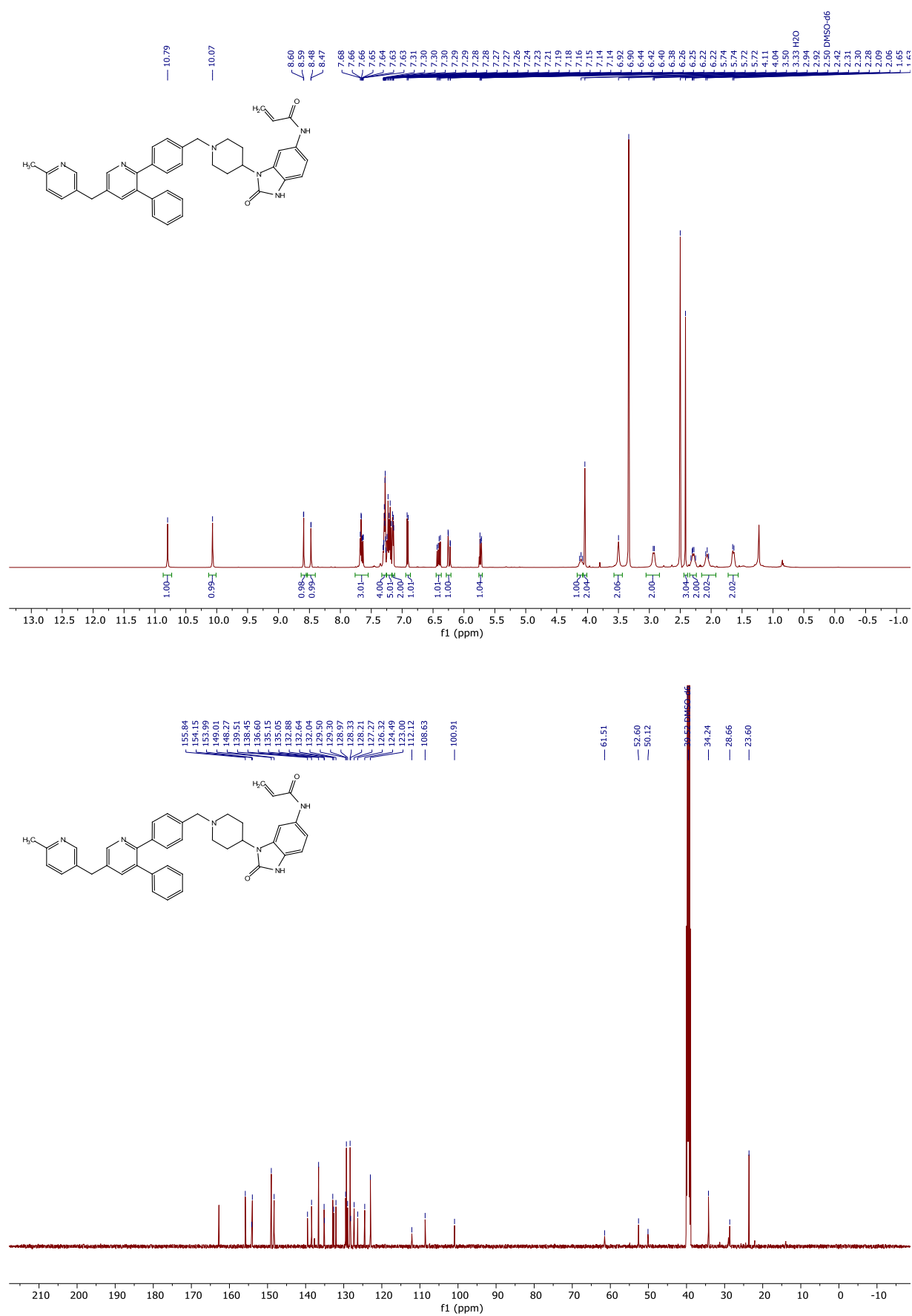


Figure S56. ¹H- and ¹³C-NMR spectra of compound **39**.

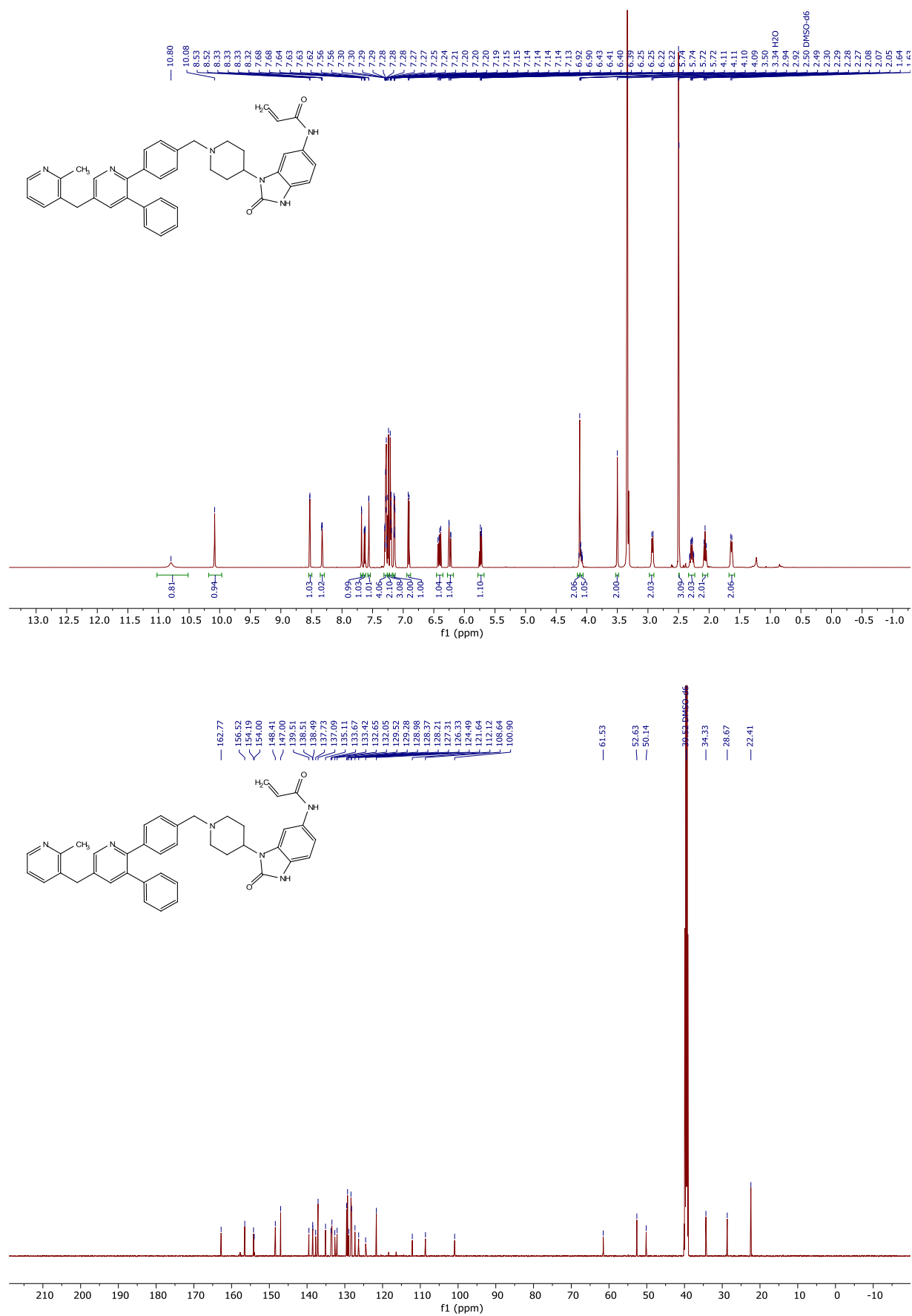


Figure S57. ¹H- and ¹³C-NMR spectra of compound 40.

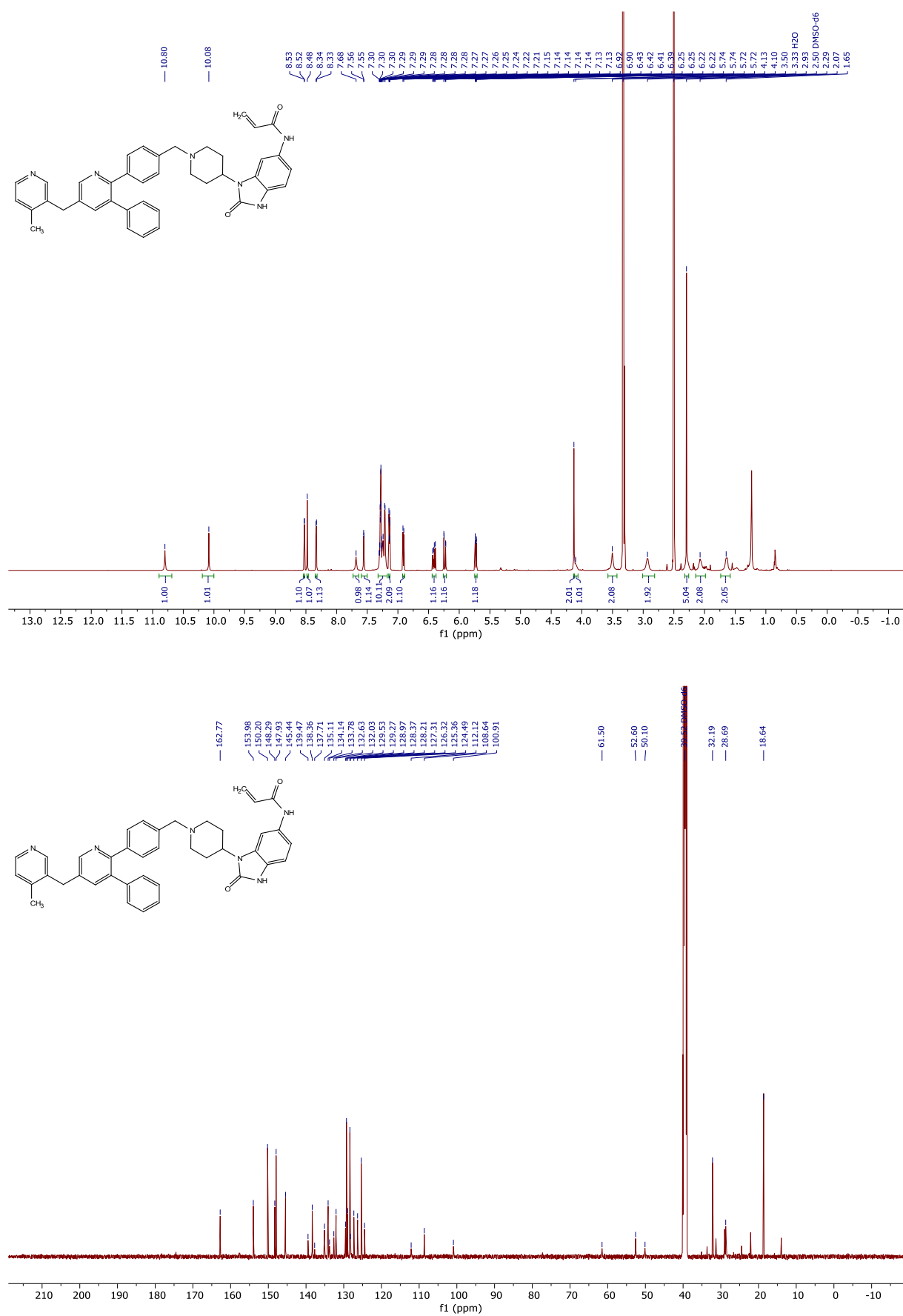
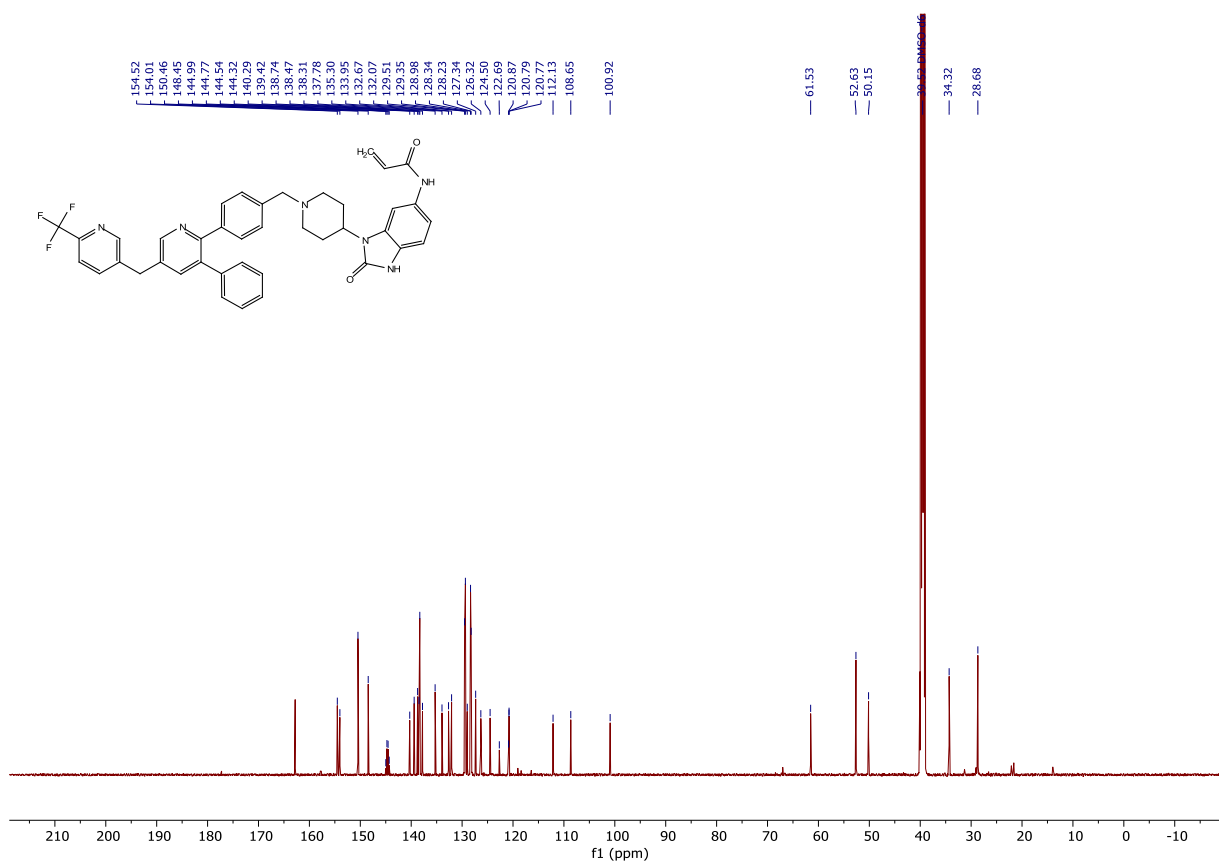
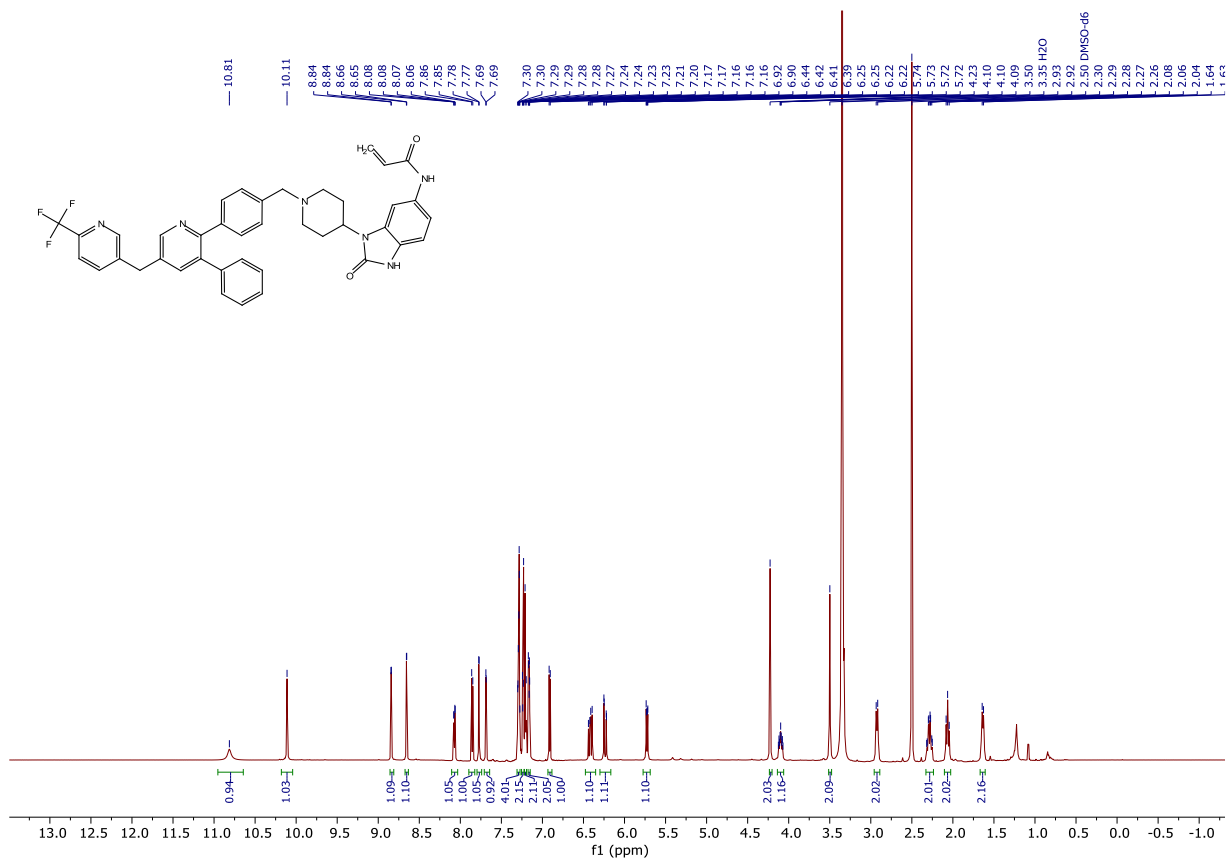
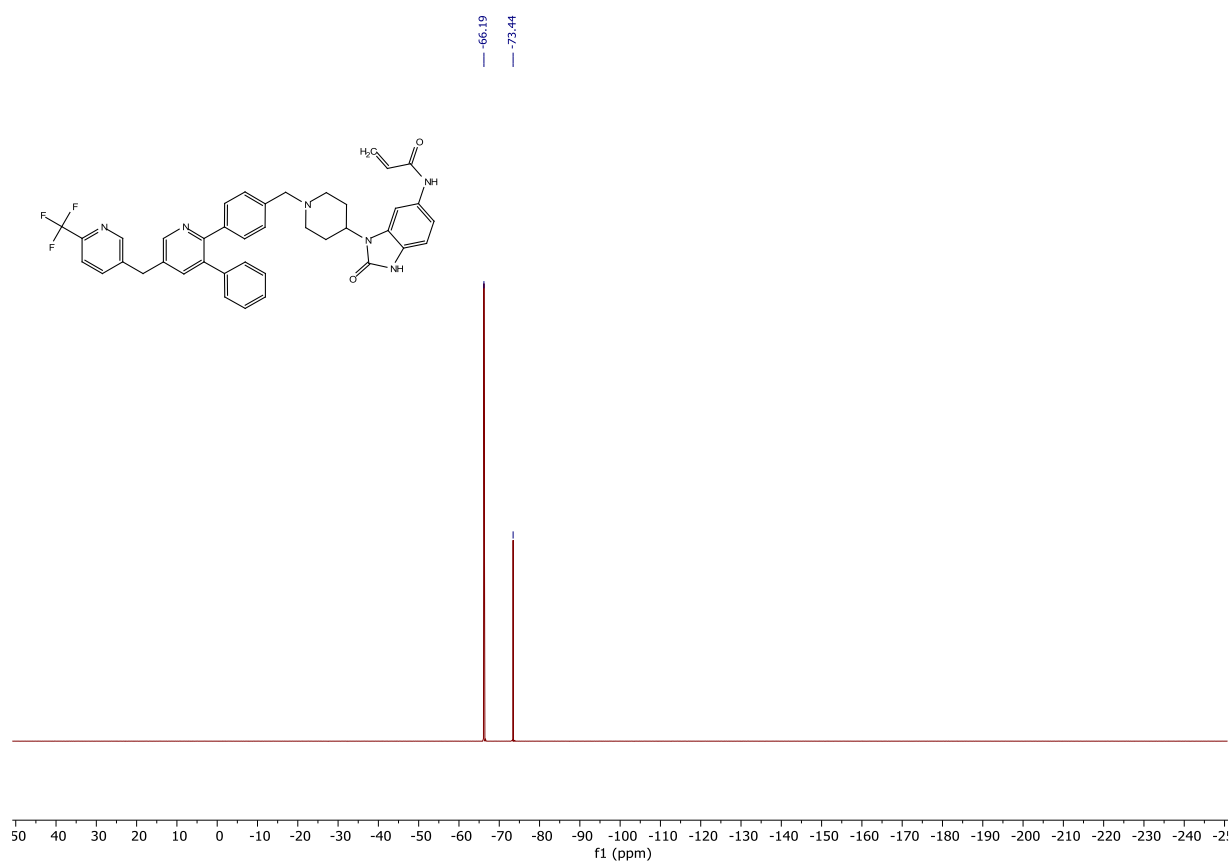
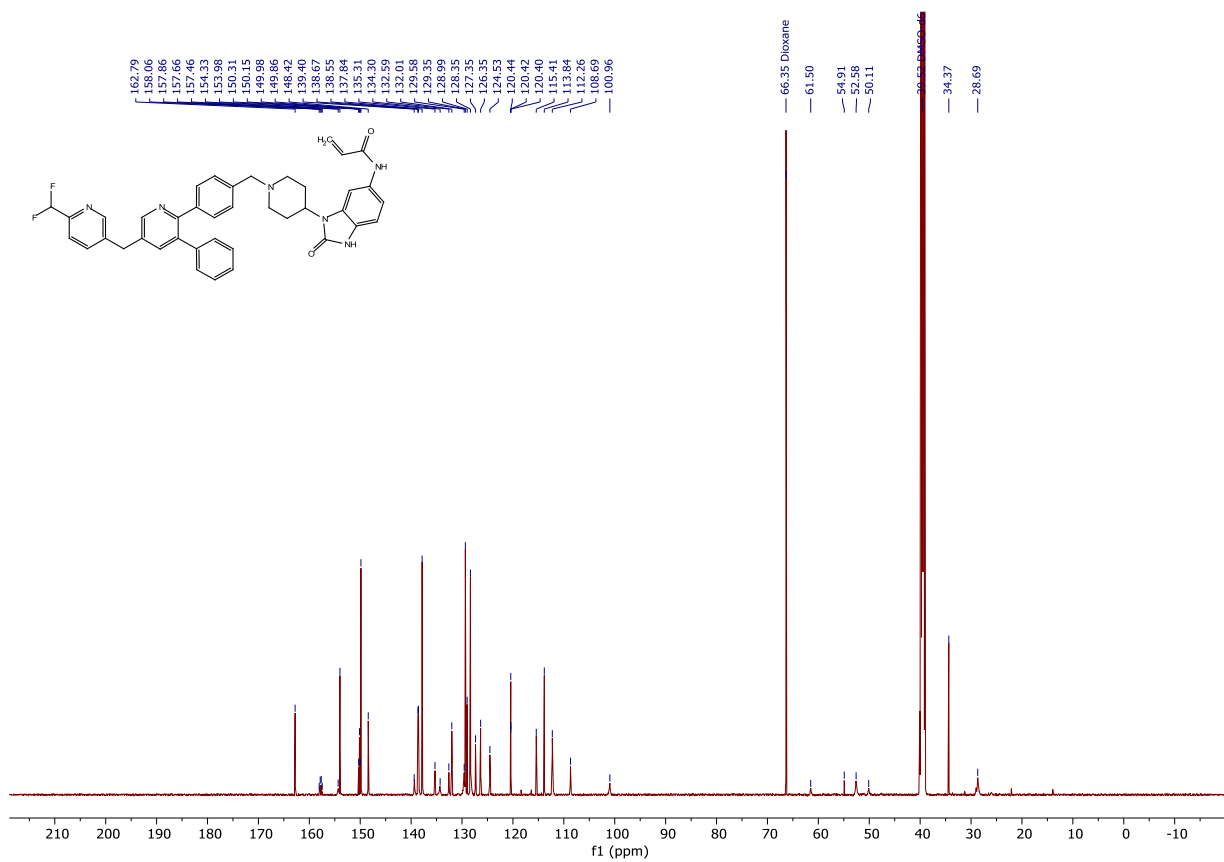
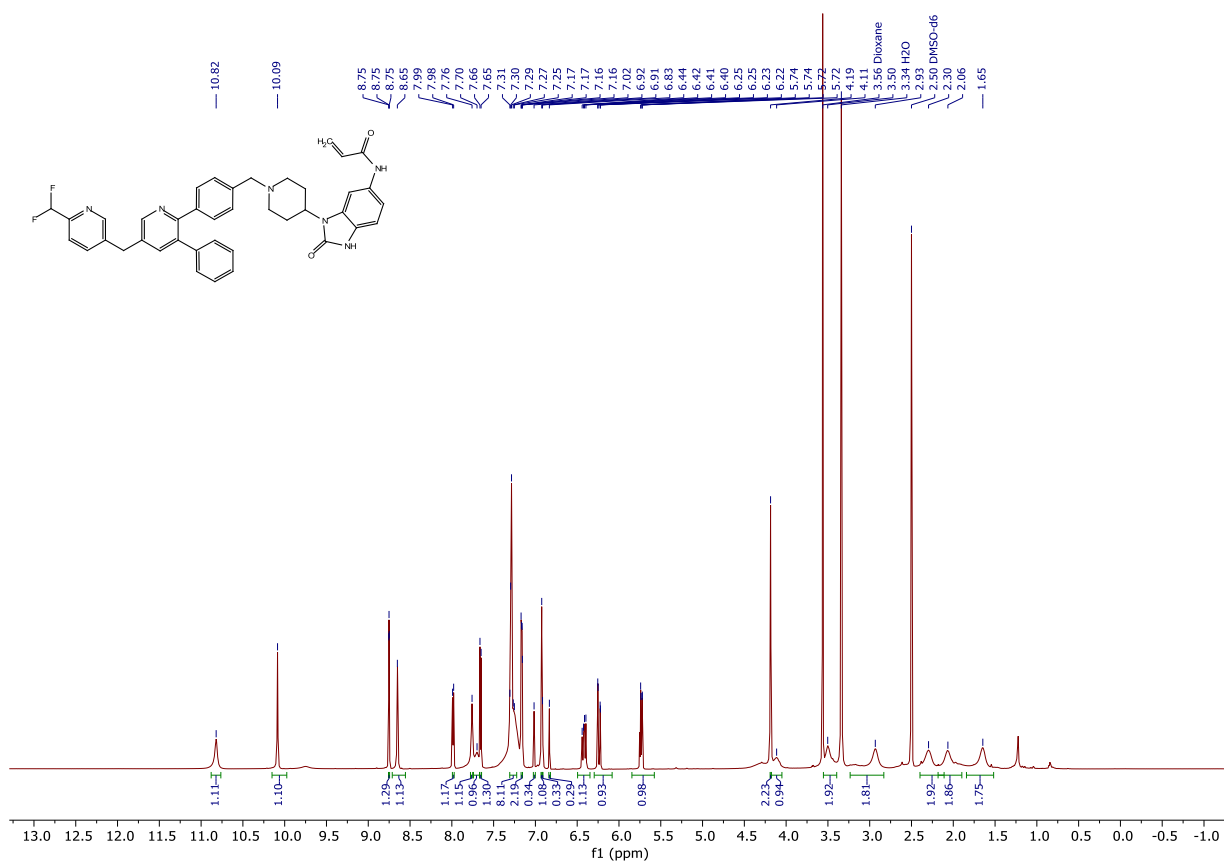


Figure S58. ¹H- and ¹³C-NMR spectra of compound 41.







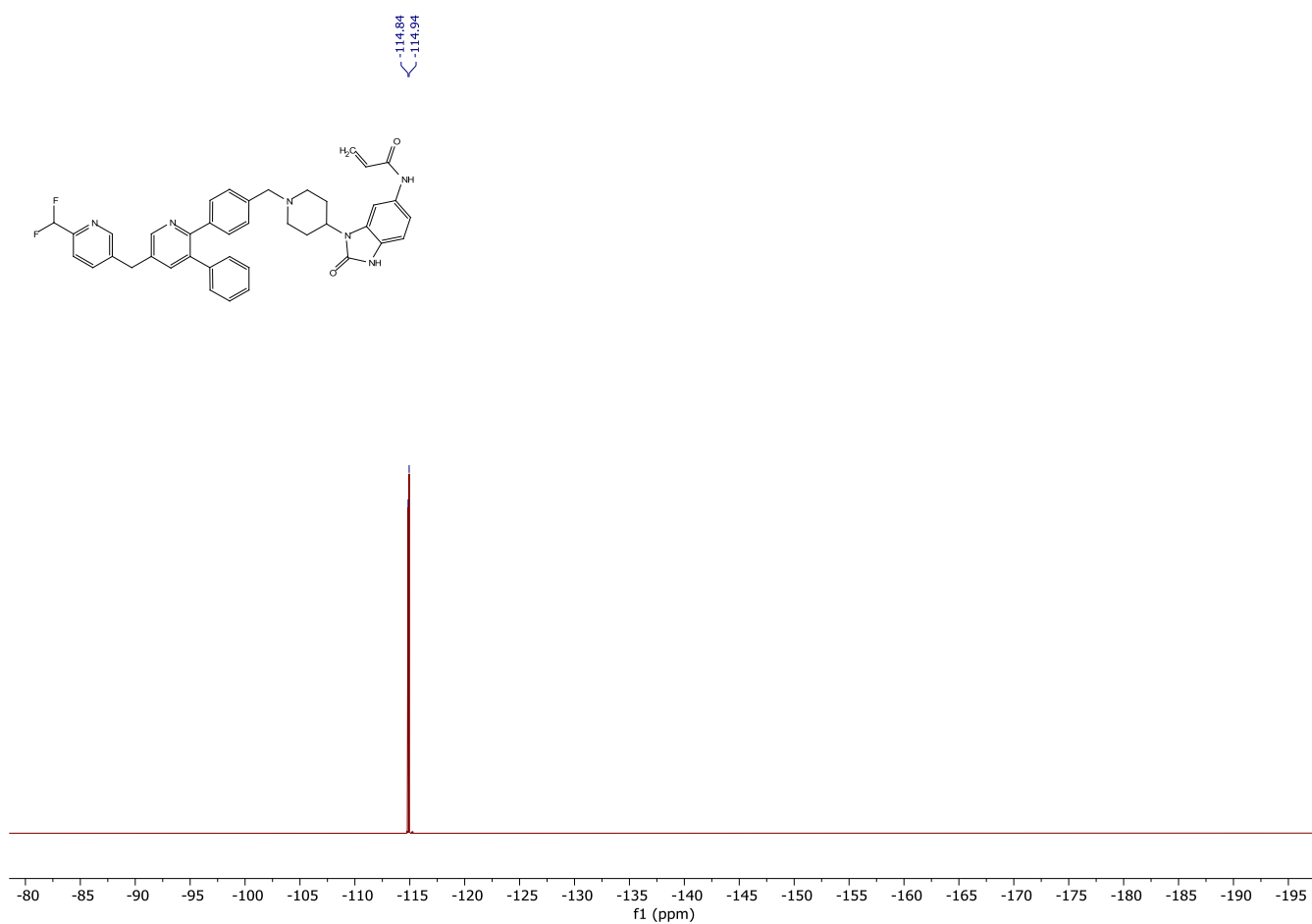


Figure S60. ^1H -, ^{13}C - and ^{19}F - NMR spectra of compound **43**.

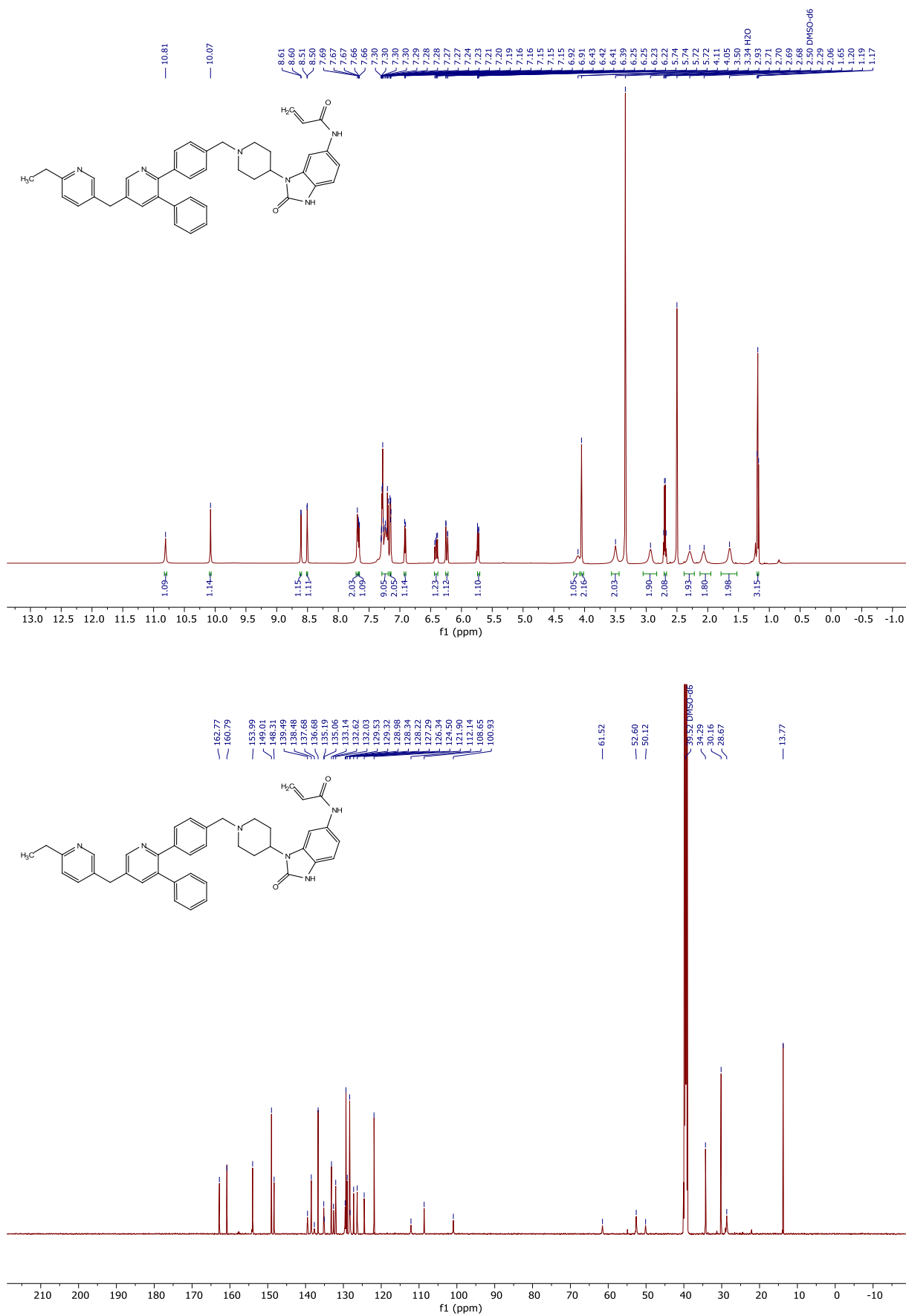


Figure S61. ¹H- and ¹³C-NMR spectra of compound **44**.

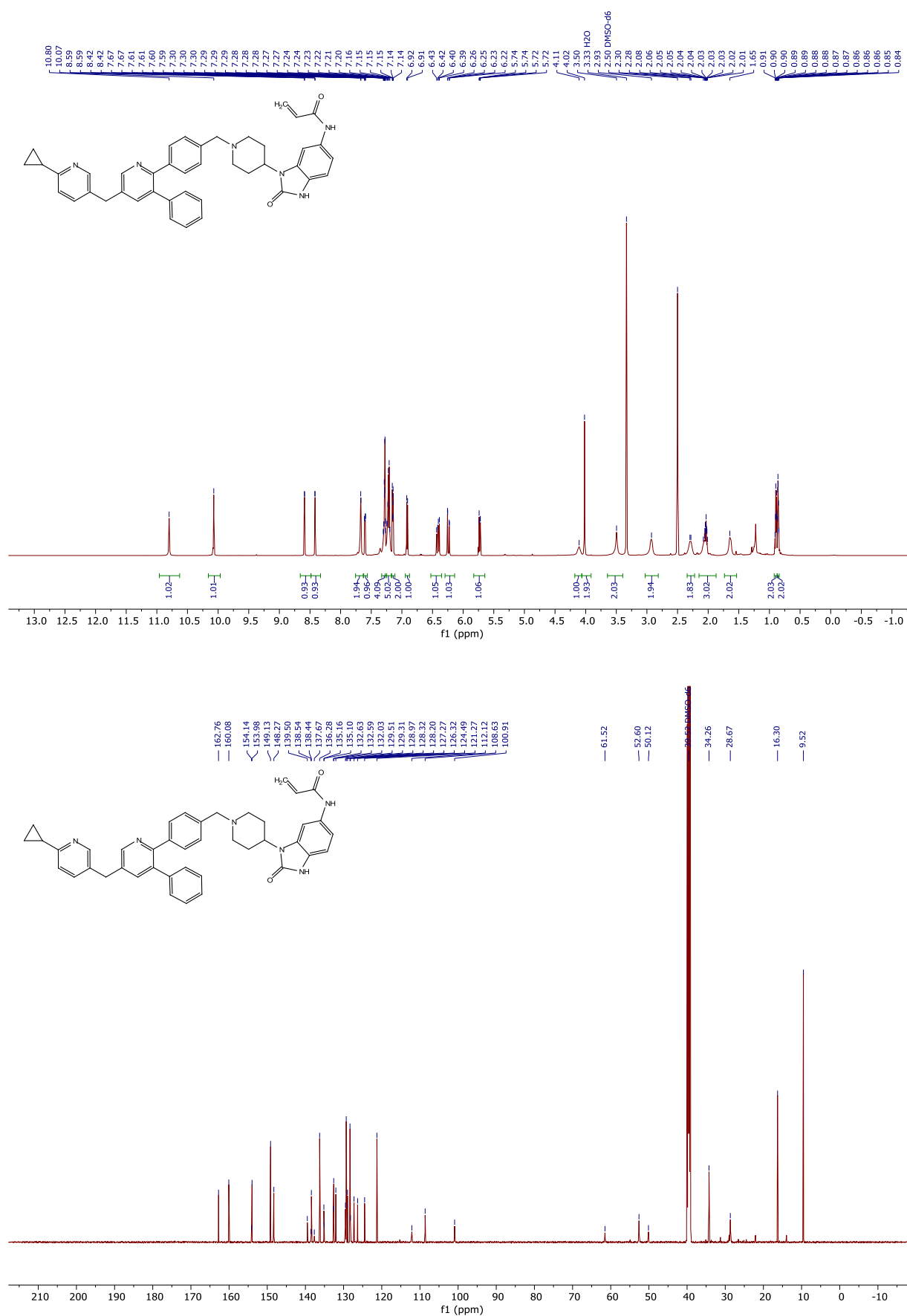


Figure S62. ¹H- and ¹³C-NMR spectra of compound 45.

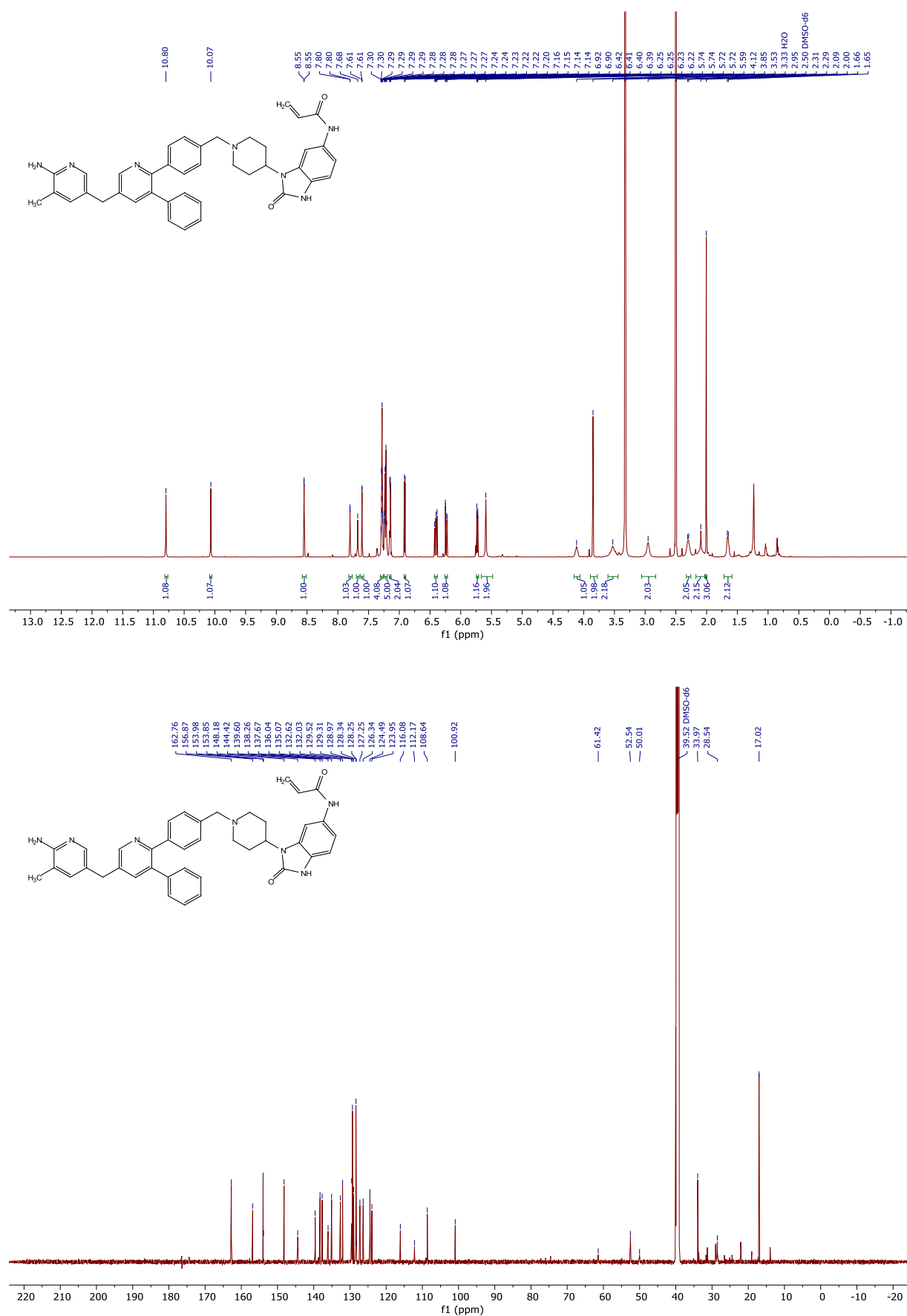


Figure S63. ¹H- and ¹³C-NMR spectra of compound **46**.

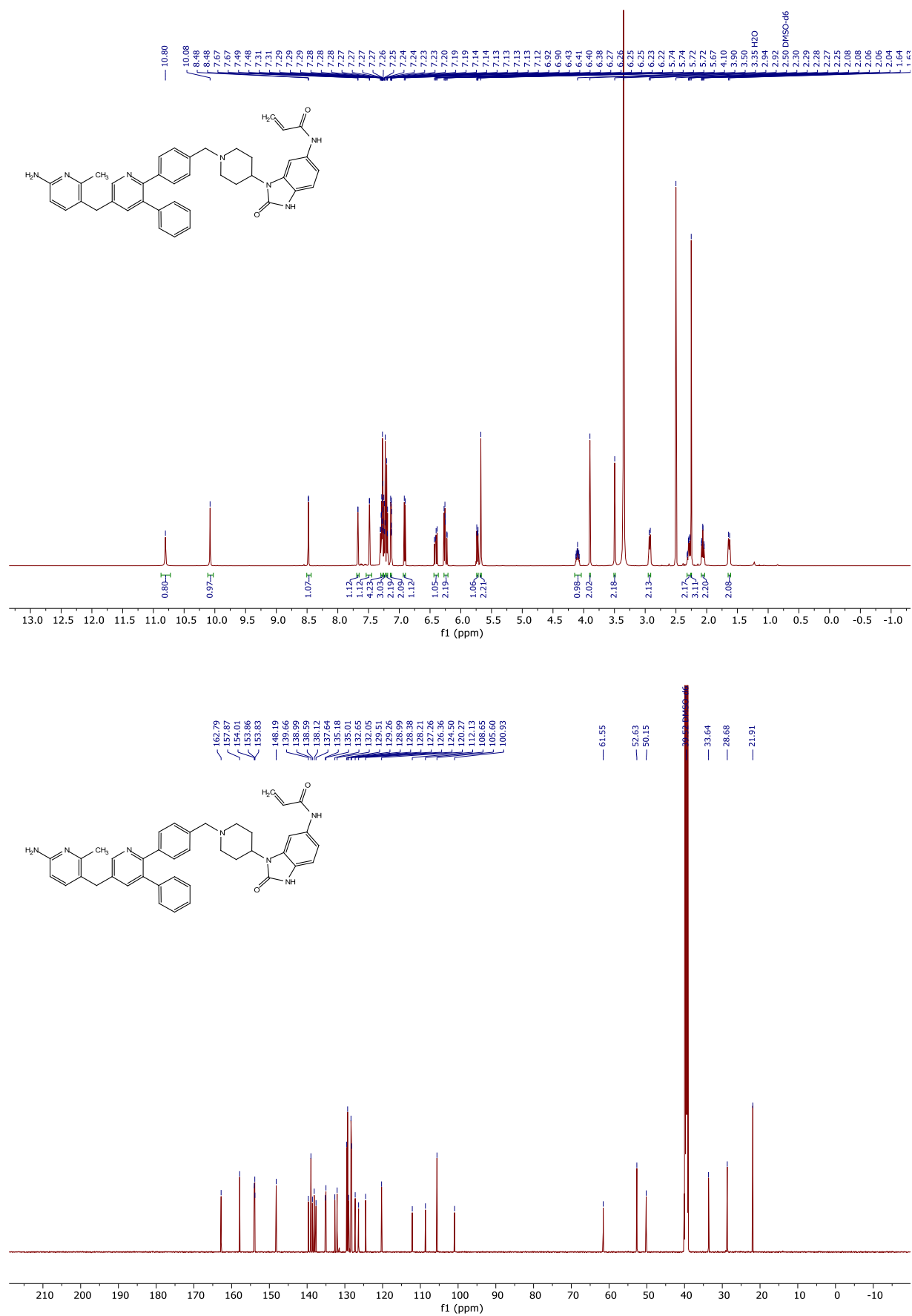


Figure S64. ¹H- and ¹³C-NMR spectra of compound 47.

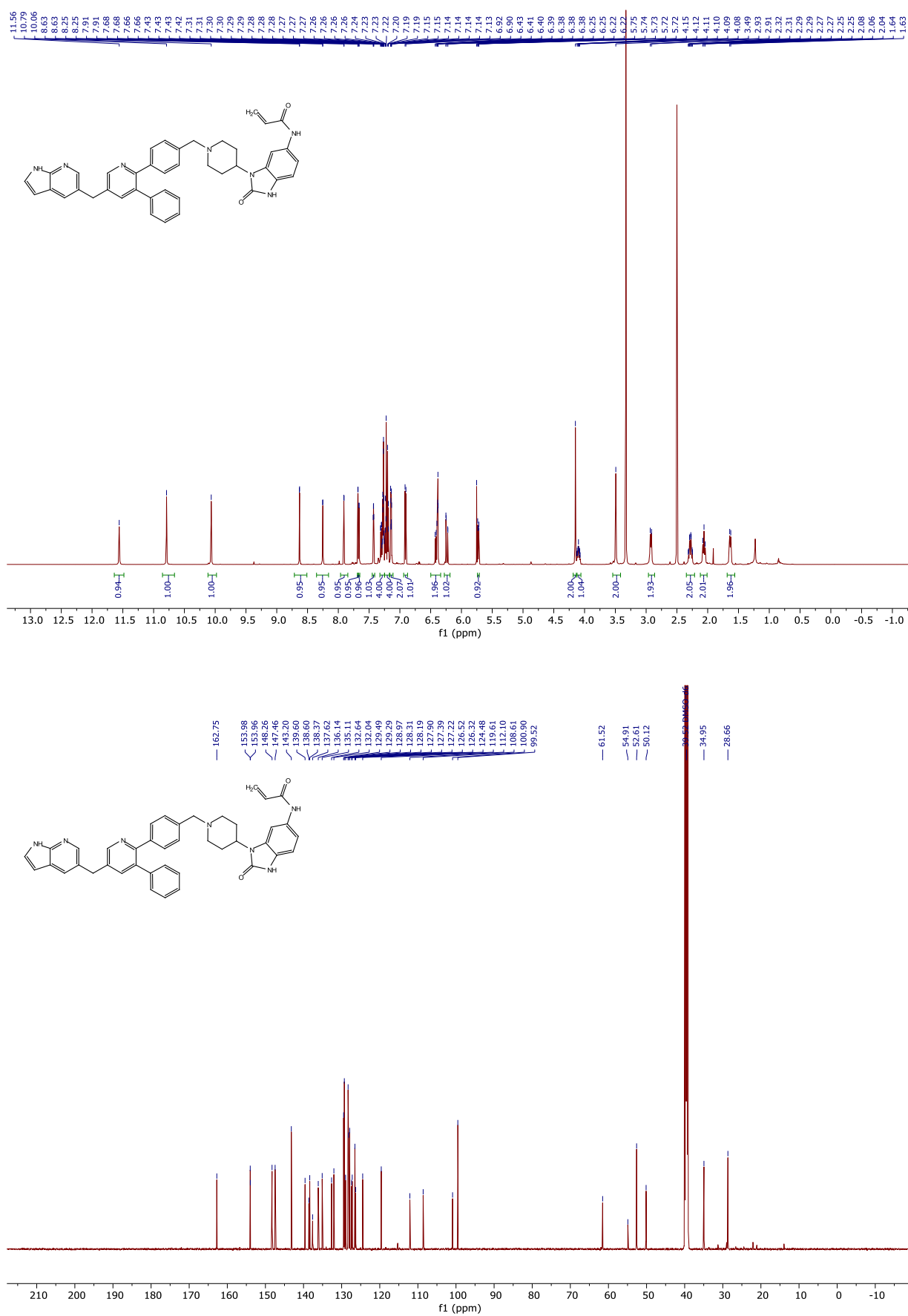


Figure S65. ¹H- and ¹³C-NMR spectra of compound 48.

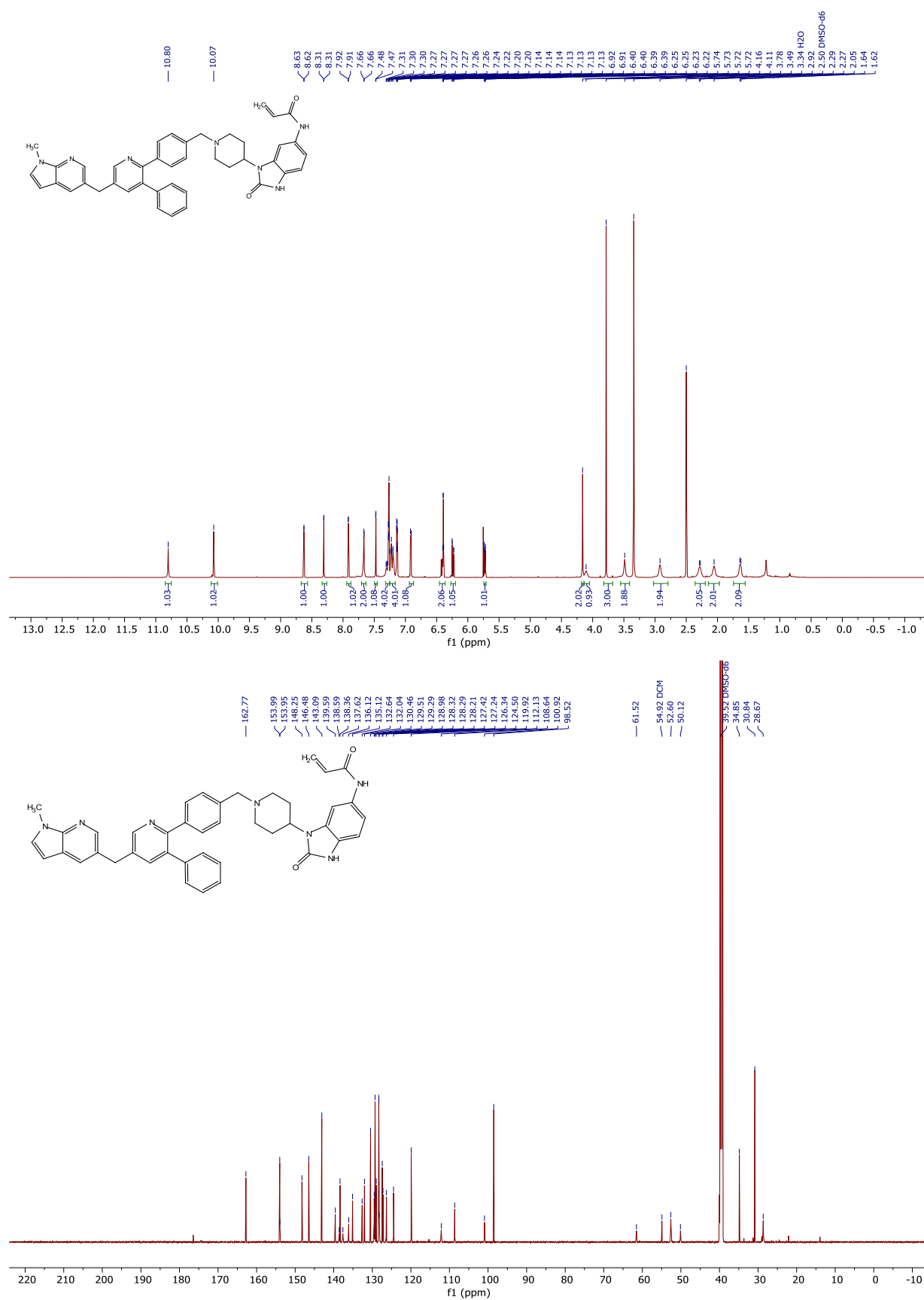


Figure S66. ¹H- and ¹³C-NMR spectra of compound **49**.

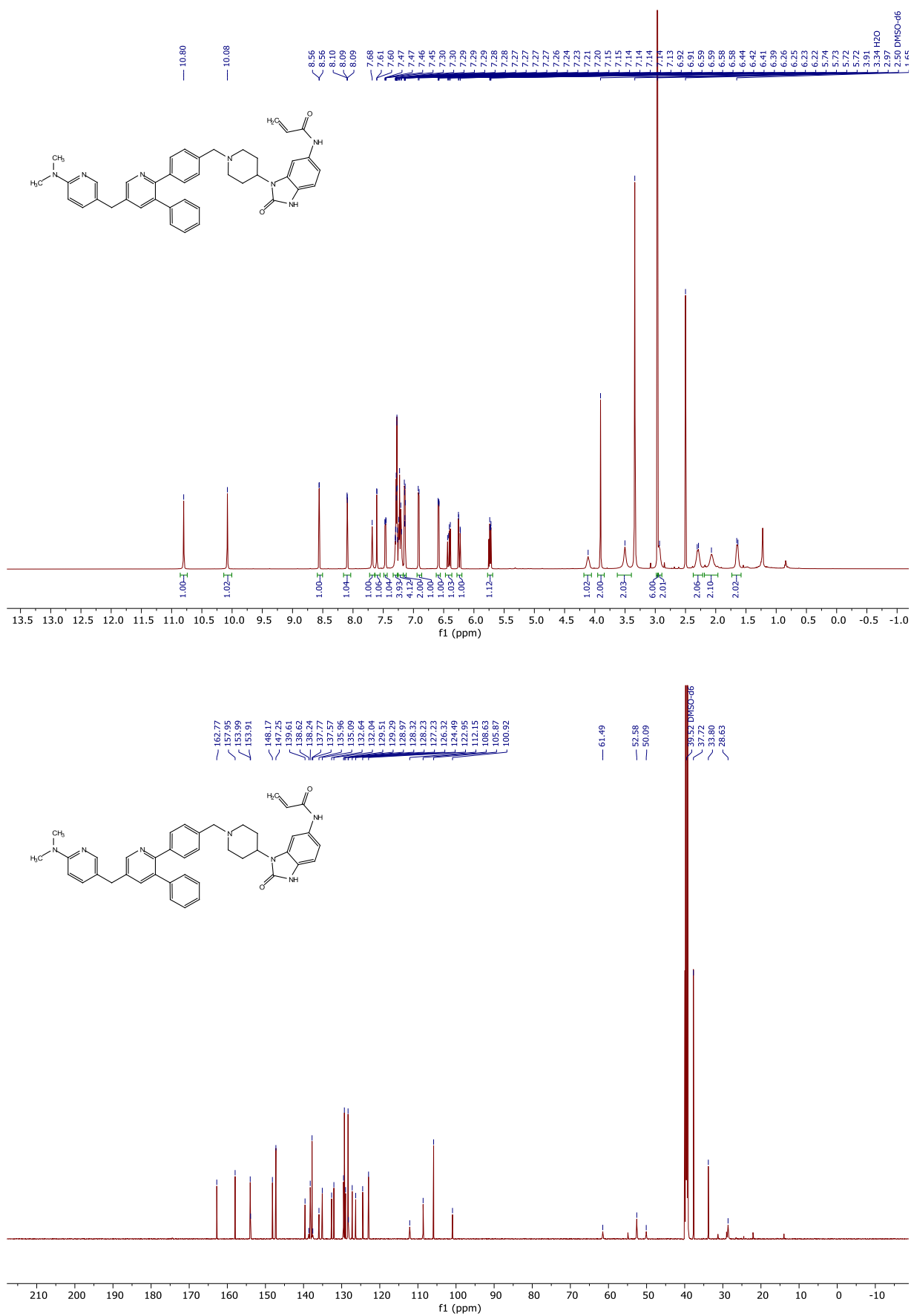


Figure S67. ¹H- and ¹³C-NMR spectra of compound **50**.

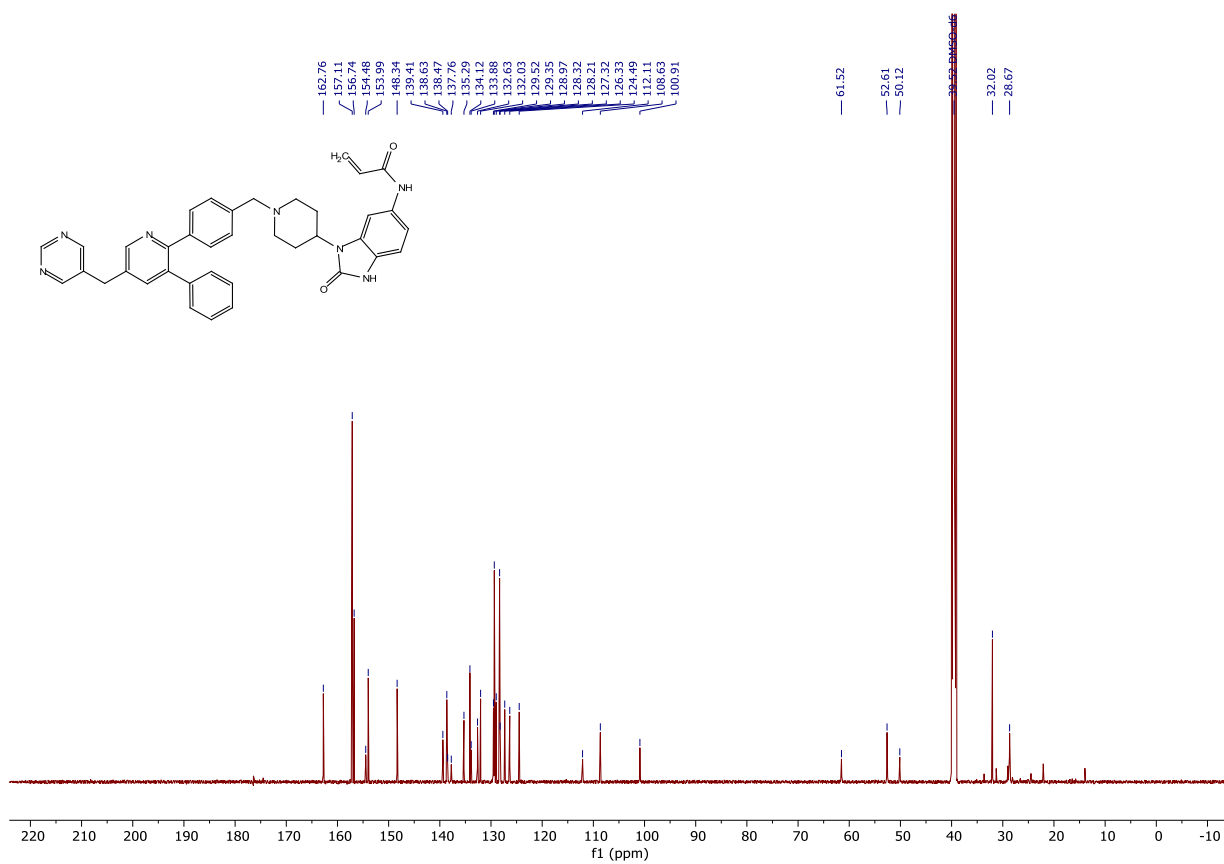
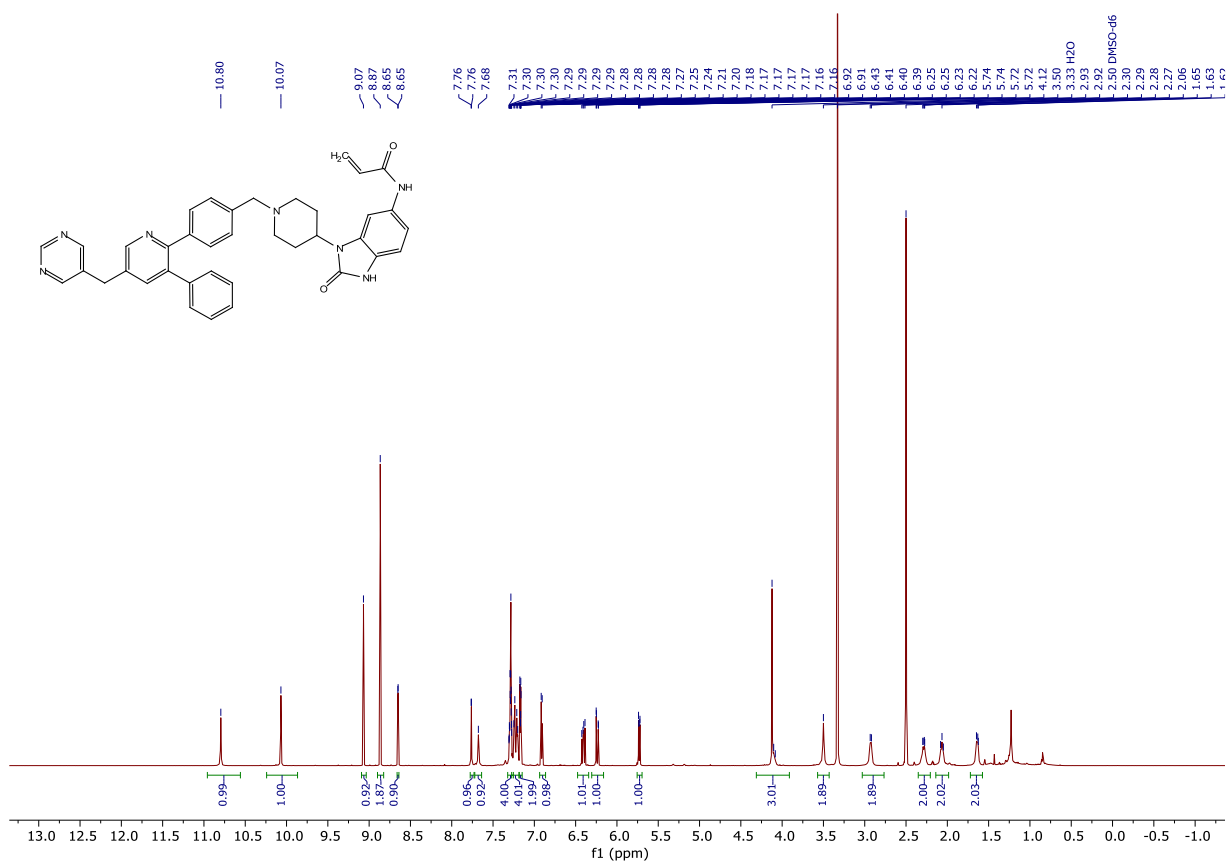


Figure S68. ^1H - and ^{13}C -NMR spectra of compound **51**.

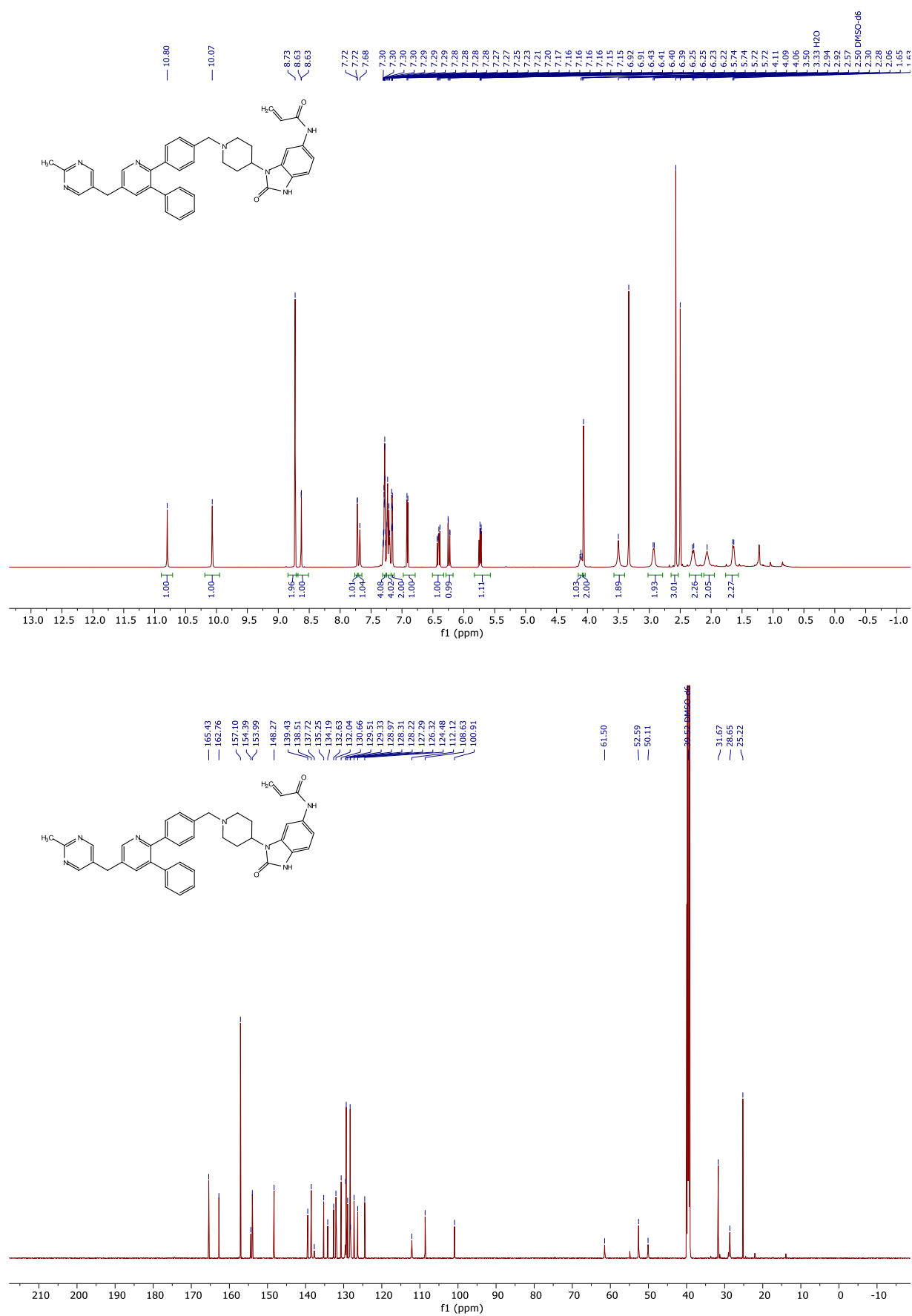


Figure S69. ¹H- and ¹³C-NMR spectra of compound **52**.

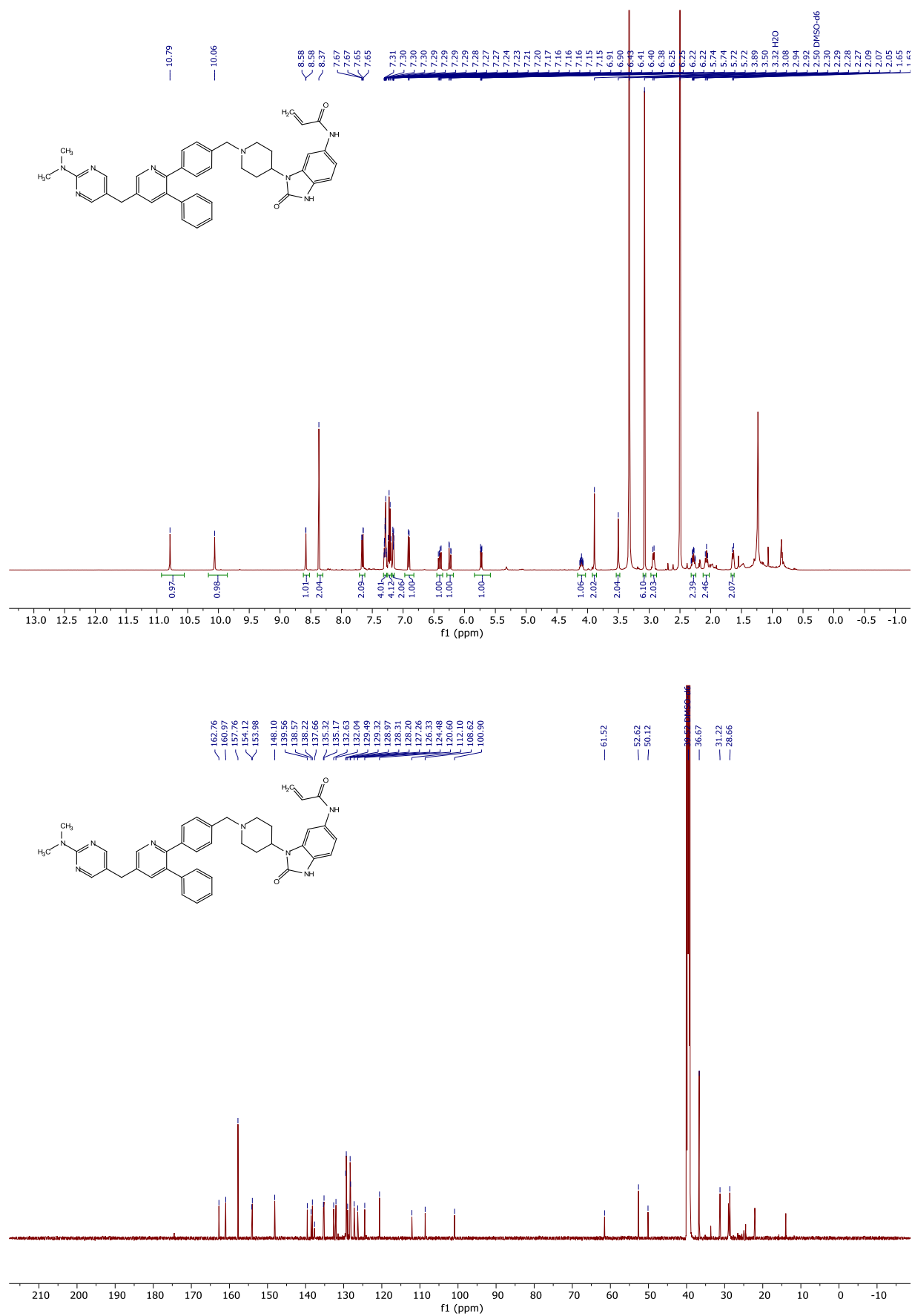
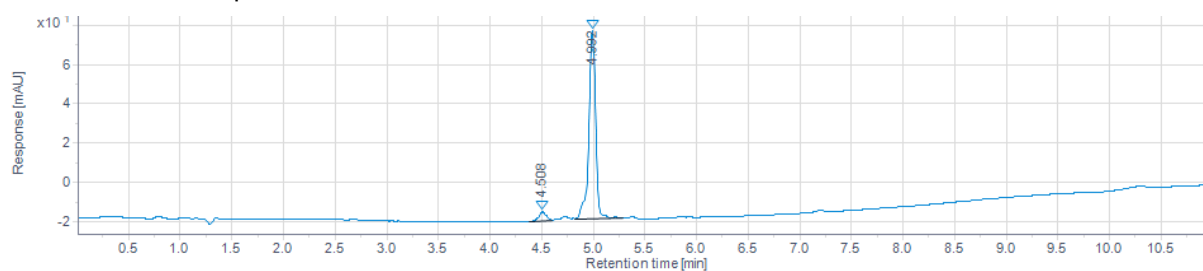


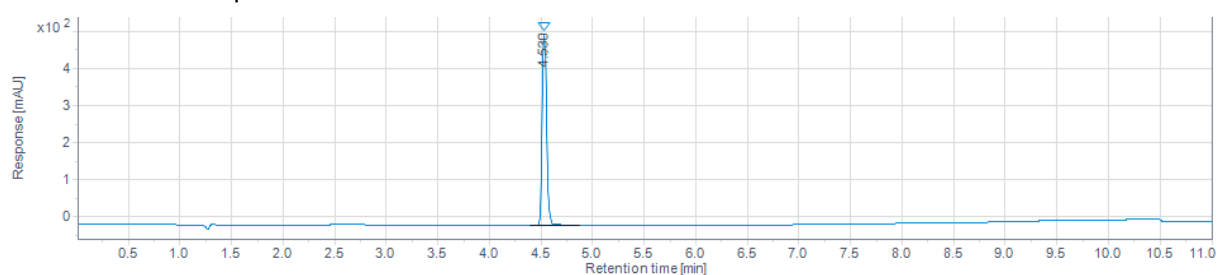
Figure S70. ¹H- and ¹³C-NMR spectra of compound **53**.

4.6.9.3. LC-MS Spectra of Final Compounds:

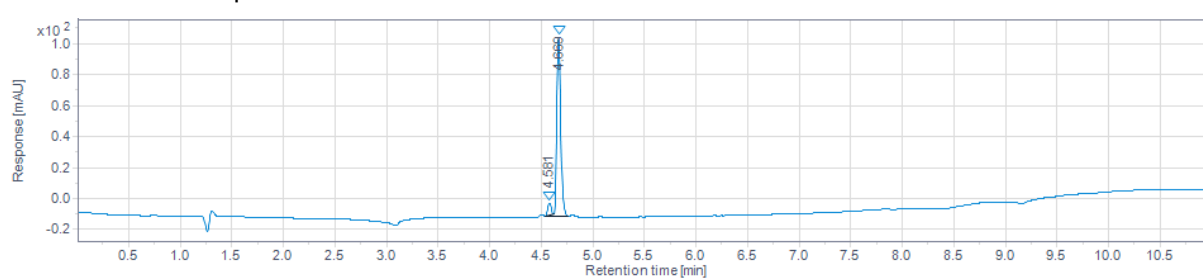
HPLC trace of compound **24**



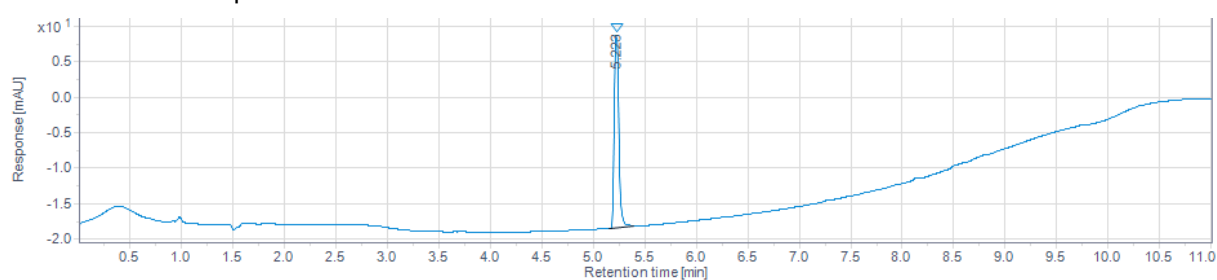
HPLC trace of compound **25**



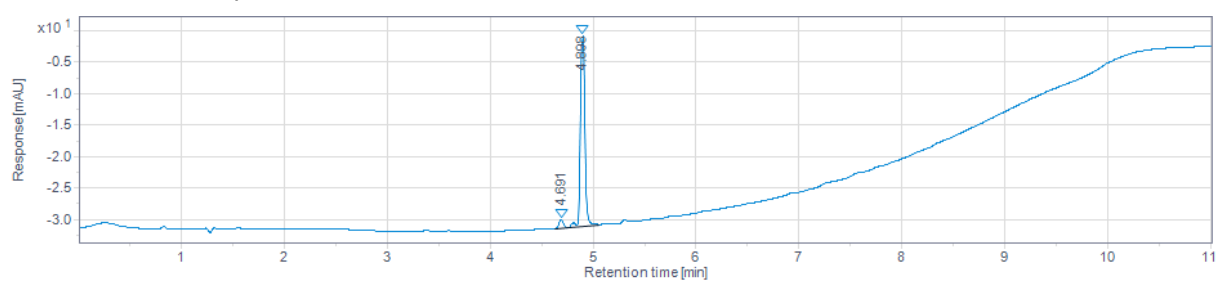
HPLC trace of compound **26**



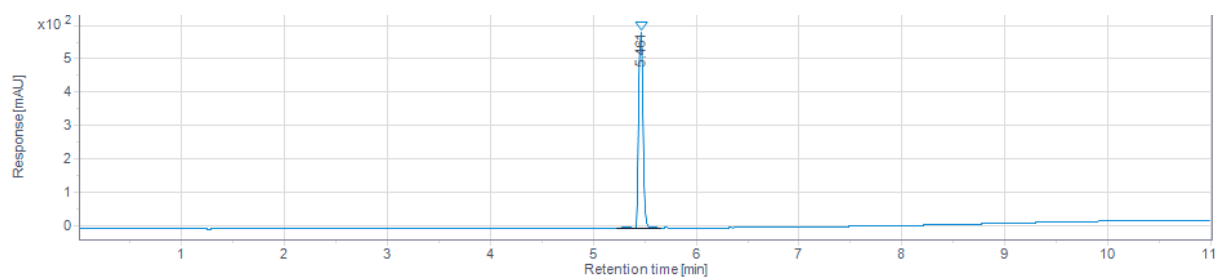
HPLC trace of compound **27**



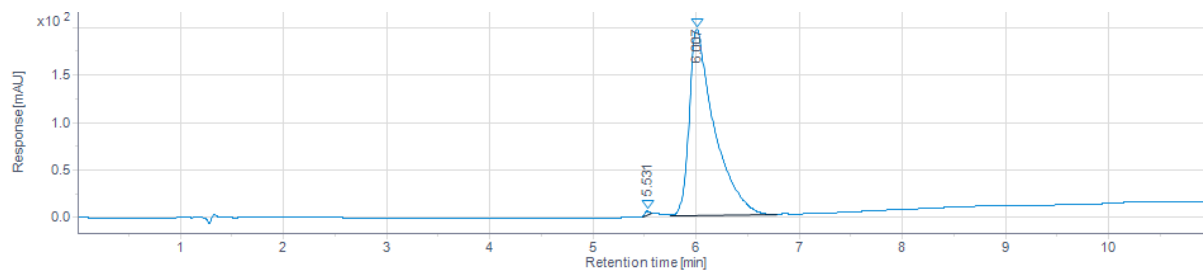
HPLC trace of compound **28**



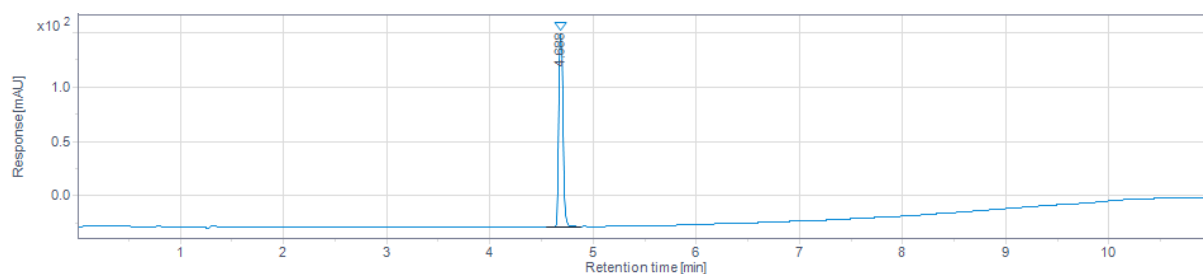
HPLC trace of compound **29**



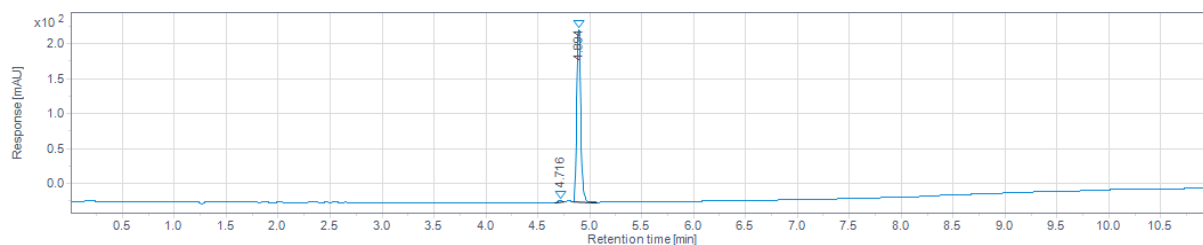
HPLC trace of compound **30**



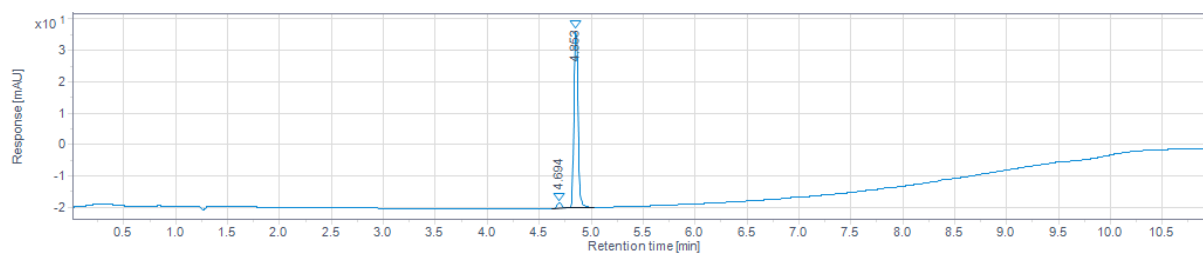
HPLC trace of compound **31**



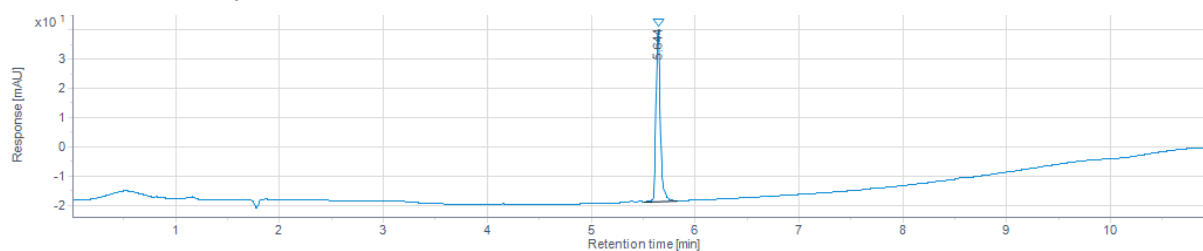
HPLC trace of compound **32**



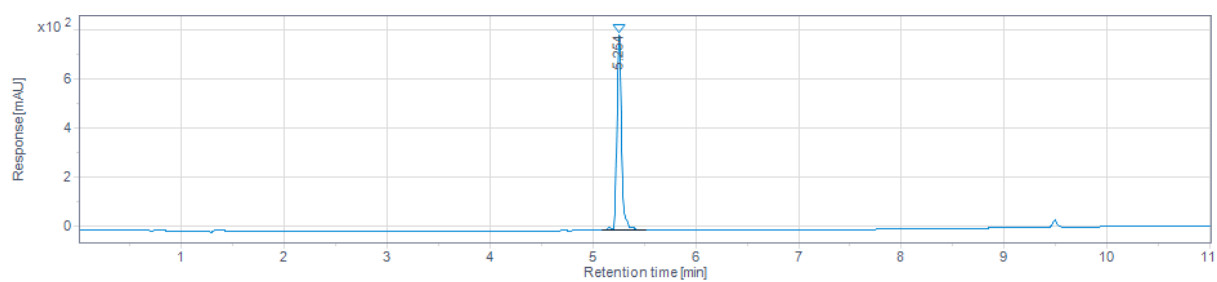
HPLC trace of compound **33**



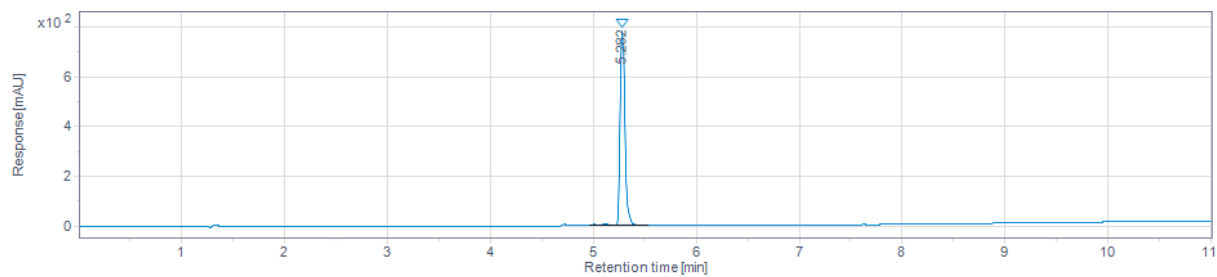
HPLC trace of compound **34**



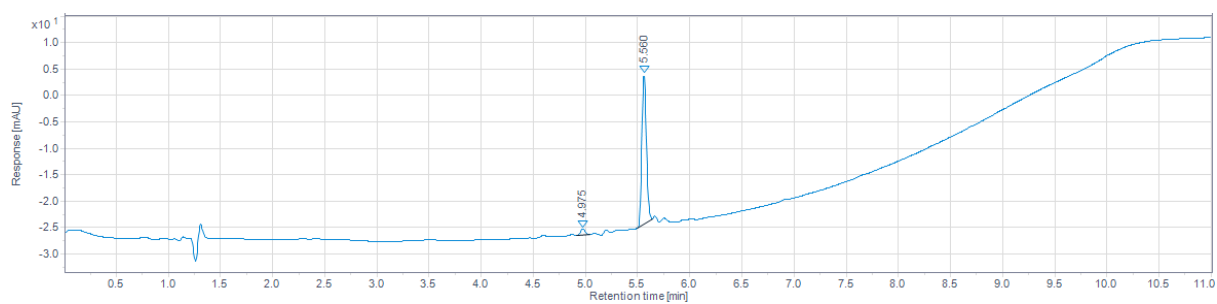
HPLC trace of compound **35**



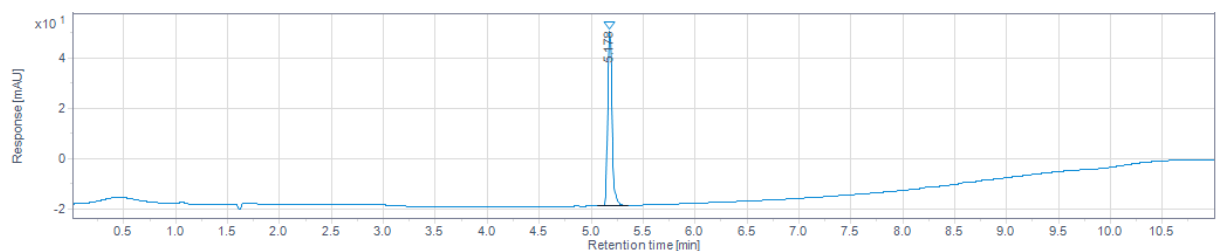
HPLC trace of compound **36**



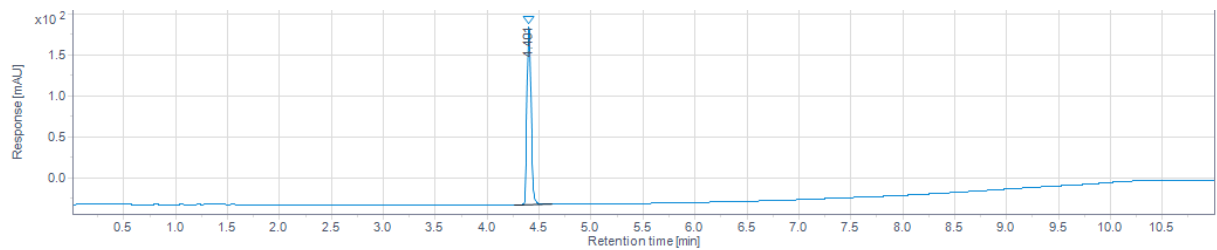
HPLC trace of compound **37**



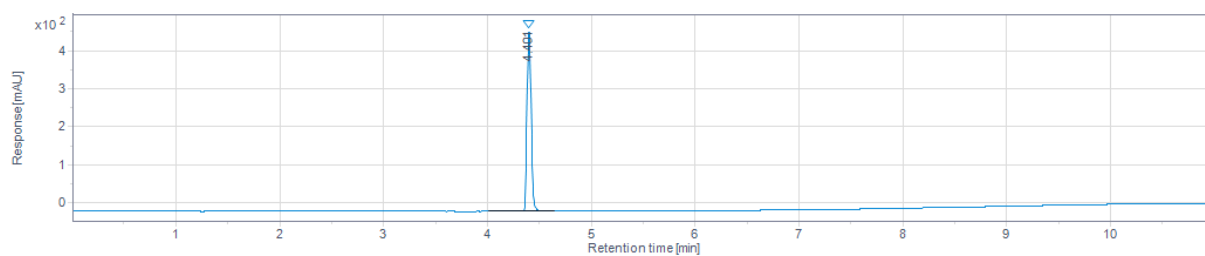
HPLC trace of compound **38**



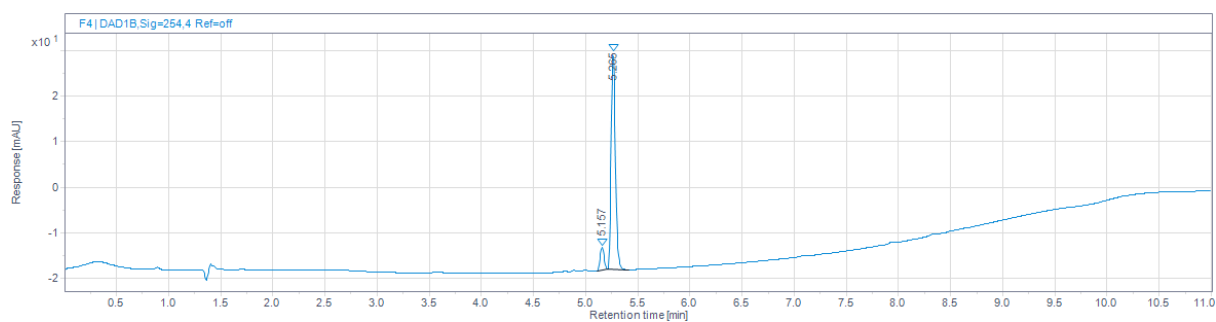
HPLC trace of compound **39**



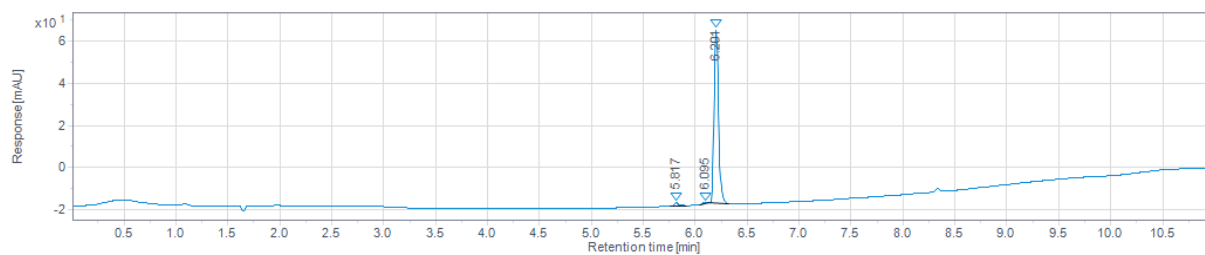
HPLC trace of compound **40**



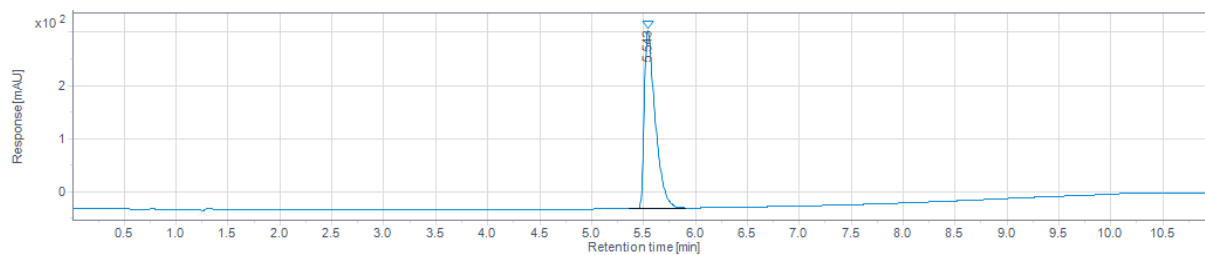
HPLC trace of compound 41



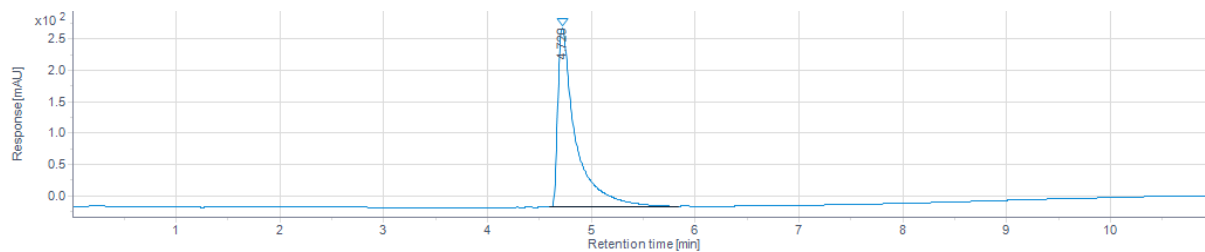
HPLC trace of compound 42



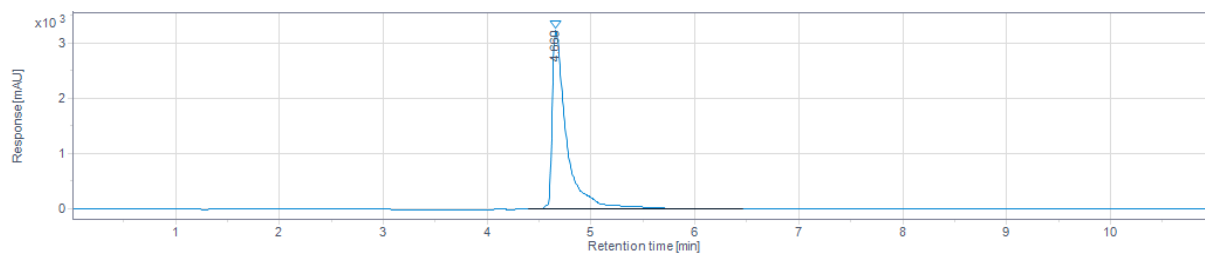
HPLC trace of compound 43



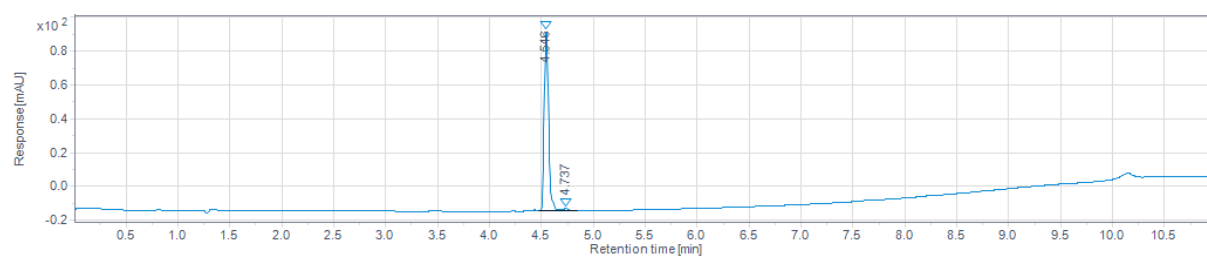
HPLC trace of compound 44



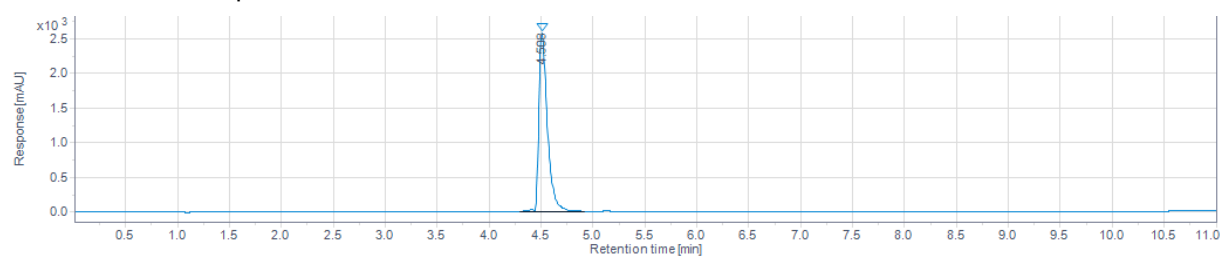
HPLC trace of compound 45



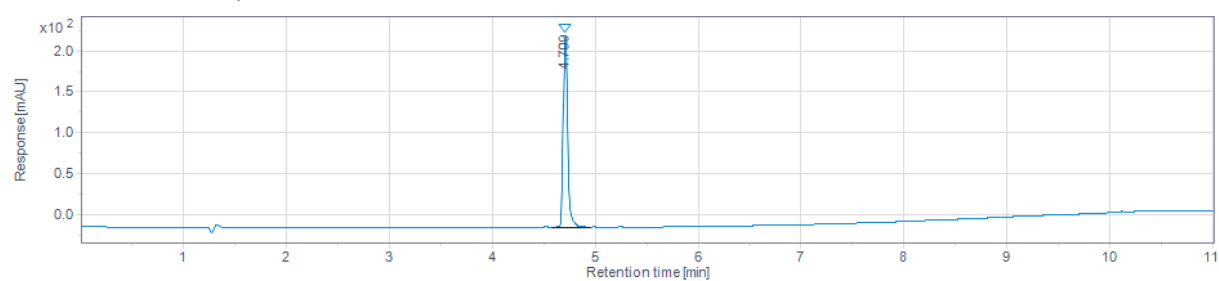
HPLC trace of compound **46**



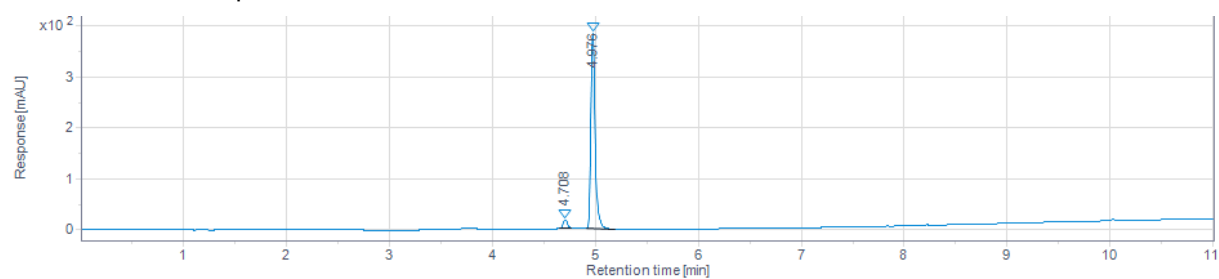
HPLC trace of compound **47**



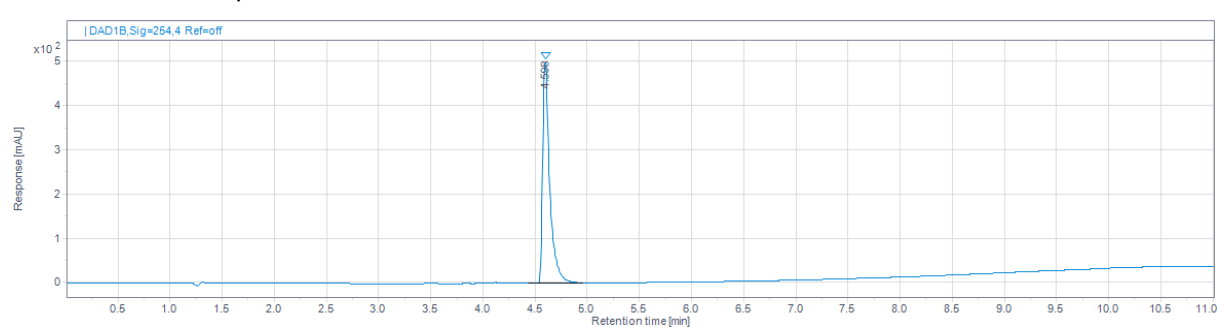
HPLC trace of compound **48**



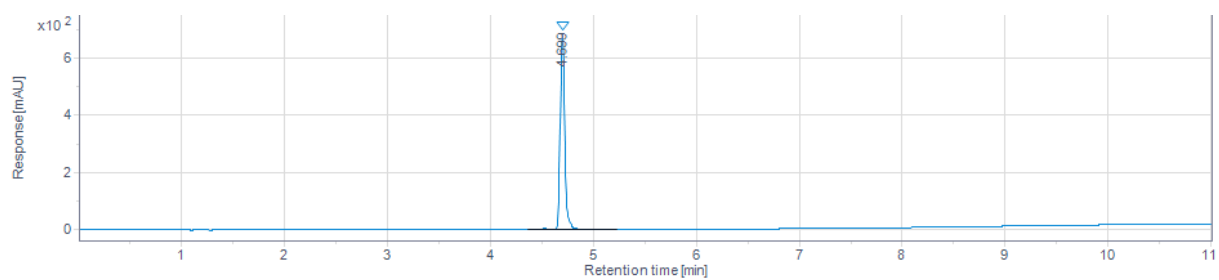
HPLC trace of compound **49**



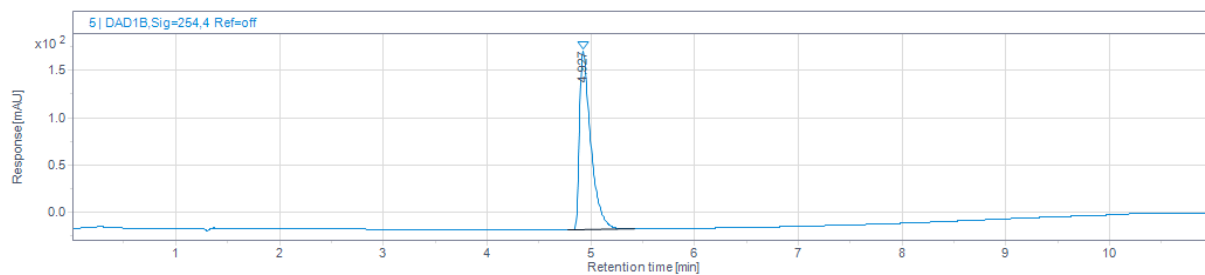
HPLC trace of compound **50**



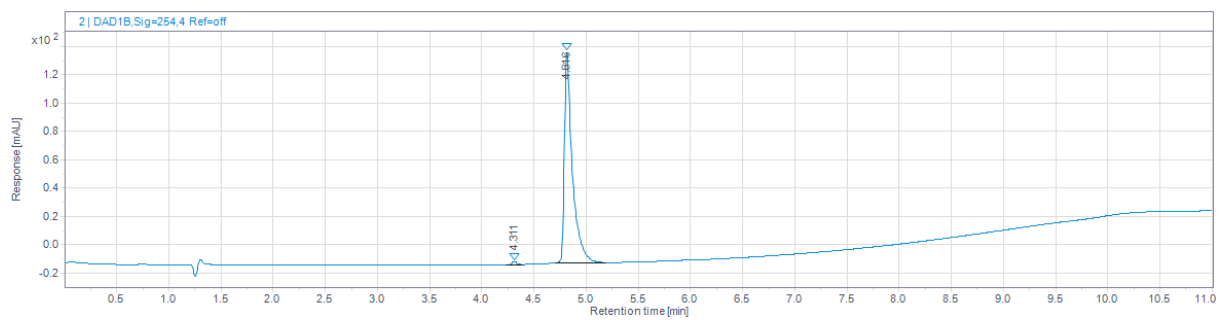
HPLC trace of compound **51**



HPLC trace of compound **52**



HPLC trace of compound **53**



4.6.10. X-ray Statistics

Table S1. Data collection and refinement statistics for full-length Akt1 in complex with different covalent-allosteric Akt inhibitors.

	Akt1 mutant in complex with compound 25	Akt1 mutant in complex with compound 27	Akt1 mutant in complex with compound 28
PDB-ID	9TMT	9TMU	9TMW
Data collection			
Space group	P12 ₁ 1	P12 ₁ 1	P12 ₁ 1
Cell dimensions			
a, b, c [Å]	50.01, 71.32, 68.01,	49.62, 71.33, 67.29,	49.74, 71.49 67.80,
α, β, γ [°]	90.00, 107.49, 90.00	90.00, 106.61, 90.00	90.00 107.56, 90.00
Resolution [Å]	47.7-2.10 (2.20-2.10)	47.83-2.40 (2.50-2.40)	47.95-2.00 (2.10-2.00)
R _{meas} [%]	16.0 (106.0)	11.5 (95.6)	18.9 (123.1)
<i>I</i> / σ <i>I</i>	9.55 (2.00)	11.38 (1.92)	7.44 (1.80)
Completeness [%]	99.5 (99.4)	99.6 (99.5)	98.8 (99.2)
CC _{1/2}	0.997 (0.668)	0.998 (0.638)	0.991 (0.644)
Redundancy	7.0 (7.0)	5.6 (5.5)	7.2 (7.4)
Refinement			
Resolution [Å]	47.70-2.10	47.83-2.40	39.52-2.00
No. reflections	26619/1332	17669/883	30392/1520
R _{work} / R _{free}	0.2357/0.1938	0.2262/0.2688	0.2028/0.2344
No. atoms	3094	2992	3155
Protein	2957	2904	2969
Ligands	48	49	49
Water	89	39	137
B-factors (Å ²)	31.11	49.32	39.18
Protein	37.84	49.40	39.29
Ligand	29.52	40.95	33.09
Water	36.16	53.56	39.14
RMS deviations			
Bond lengths [Å]	0.003	0.002	0.004
Bond angles [°]	0.646	0.57	0.66
Ramachandran [%]			
Favored	96.98	95.32	96.96
Allowed	3.02	4.68	3.04
Outliers	0.00	0.00	0.00

Statistics for the highest resolution shell are shown in parentheses.

Table S1 continued

	Akt1 mutant in complex with compound 28 and EG	Akt1 mutant in complex with compound 34	Akt1 mutant in complex with compound 35
PDB-ID	9TMV	9TMY	9TMZ
Data collection			
Space group	P12 ₁ 1	P12 ₁ 1	P12 ₁ 1
Cell dimensions			
a, b, c [Å]	49.61, 71.17, 67.74,	50.11, 71.76, 68.45,	49.88, 71.32, 68.01,
α , β , γ [°]	90.00, 107.27, 90.00	90.00, 107.52, 90.00	90.00, 107.26, 90.00
Resolution [Å]	47.14-1.90 (2.00-1.90)	48.29-2.20 (2.30-2.20)	48.02-2.20 (2.30-2.20)
R _{meas} [%]	15.7 (114.1)	10.5 (116.0)	17.0 (109.7)
<i>I</i> / σ <i>I</i>	12.8 (2.49)	8.67 (1.40)	8.69 (2.13)
Completeness [%]	99.0 (99.1)	98.4 (98.2)	99.0 (99.7)
CC _{1/2}	0.997 (0.707)	0.997 (0.549)	0.995 (0.723)
Redundancy	7.2 (7.3)	3.2 (3.2)	8.7 (2.1)
Refinement			
Resolution [Å]	45.14-1.90	48.29-2.20	48.02-2.20
No. reflections	35261/1764	23145/1157	23059/1153
R _{work} / R _{free}	0.1728/0.2103	0.2024/0.2419	0.1996/0.2429
No. atoms	3306	3063	3109
Protein	3020	2944	2959
Ligands	53	48	49
Water	233	71	101
B-factors (Å ²)	33.21	48.63	4030
Protein	32.93	48.86	40.54
Ligand	25.13	40.21	31.06
Water	38.67	45.00	37.72
RMS deviations			
Bond lengths [Å]	0.016	0.007	0.007
Bond angles [°]	1.356	0.95	0.961
Ramachandran			
[%] Favored	96.74	97.25	96.45
Allowed	3.26	2.75	3.55
Outliers	0.00	0.00	0.00

Statistics for the highest resolution shell are shown in parentheses.

Table S1 continued

	Akt1 mutant in complex with compound 36 and EG	Akt1 mutant in complex with compound 37	Akt1 mutant in complex with compound 40
PDB-ID	9TN0	9TN2	9TN3
Data collection			
Space group	P12 ₁ 1	P12 ₁ 1	P12 ₁ 1
Cell dimensions			
a, b, c [Å]	49.82, 71.22, 67.78,	50.44, 71.80, 68.43,	50.06, 71.78 68.40,
α, β, γ [°]	90.00, 107.22, 90.00	90.00, 107.23, 90.00	90.00, 107.53, 90.00
Resolution [Å]	47.91-2.40 (2.50-2.40)	48.33-2.30 (2.40-2.30)	39.75-2.40 (2.50-2.40)
R _{meas} [%]	17.4 (102.1)	11.4 (84.2)	17.7 (104.8)
<i>I</i> / σ <i>I</i>	10.74 (2.25)	12.11 (2.95)	8.66(1.75)
Completeness [%]	99.6 (99.4)	99.5 (99.5)	99.4 (99.5)
CC _{1/2}	0.995 (0.658)	0.998 (0.772)	0.995(0.654)
Redundancy	6.9 (6.8)	6.9 (7.0)	7.0 (7.2)
Refinement			
Resolution [Å]	47.91-2.40	45.80-2.30	39.75-2.40
No. reflections	17788/890	20758/1038	18119/906
R _{work} / R _{free}	0.1884/0.2389	0.1894/0.2354	0.1992/0.2368
No. atoms	3084	3060	3062
Protein	2960	2915	2952
Ligands	53	60	48
Water	71	85	62
B-factors (Å ²)	37.60	52.56	49.56
Protein	37.76	52.72	49.73
Ligand	32.16	42.94	41.76
Water	34.95	53.89	47.39
RMS deviations			
Bond lengths [Å]	0.007	0.002	0.002
Bond angles [°]	1.012	0.544	0.566
Ramachandran			
[%] Favored	95.60	95.34	95.33
Allowed	4.40	4.66	4.67
Outliers	0.00	0.00	0.00

Statistics for the highest resolution shell are shown in parentheses.

Table S1 continued

	Akt1 mutant in complex with compound 40 and EG	Akt1 mutant in complex with compound 47 and EG
PDB-ID	9TN1	9TN4
Data collection		
Space group	P12 ₁ 1	P12 ₁ 1
Cell dimensions		
a, b, c [Å]	49.85, 71.11, 67.88,	49.70, 70.930, 67.54,
α , β , γ [°]	90.00, 107.17, 90.00	90.00, 107.075, 90.00
Resolution [Å]	47.92-2.20 (2.30-2.20)	47.75-2.40 (2.50-2.40)
R _{meas} [%]	14.6 (195.3)	18.7 (96.6)
<i>I</i> / σ <i>I</i>	10.82 (2.71)	7.53 (1.84)
Completeness [%]	99.3 (99.5)	98.6 (99.8)
CC _{1/2}	0.997 (0.696)	0.991 (0.617)
Redundancy	7.0 (6.9)	5.9 (5.9)
Refinement		
Resolution [Å]	47.63-2.20	47.75-2.40
No. reflections	23014/1151	17446/873
R _{work} / R _{free}	0.2001/0.2282	0.2041/0.2406
No. atoms	3091	3090
Protein	2950	2963
Ligands	52	53
Water	89	74
<i>B</i> -factors (Å ²)	43.69	43.72
Protein	43.83	43.99
Ligand	38.25	35.37
Water	42.18	39.21
RMS deviations		
Bond lengths [Å]	0.002	0.002
Bond angles [°]	0.498	0.451
Ramachandran		
[%] Favored	96.17	96.43
Allowed	3.83	3.57
Outliers	0.00	0.00

Statistics for the highest resolution shell are shown in parentheses.

4.6.11. Western Blot Analysis

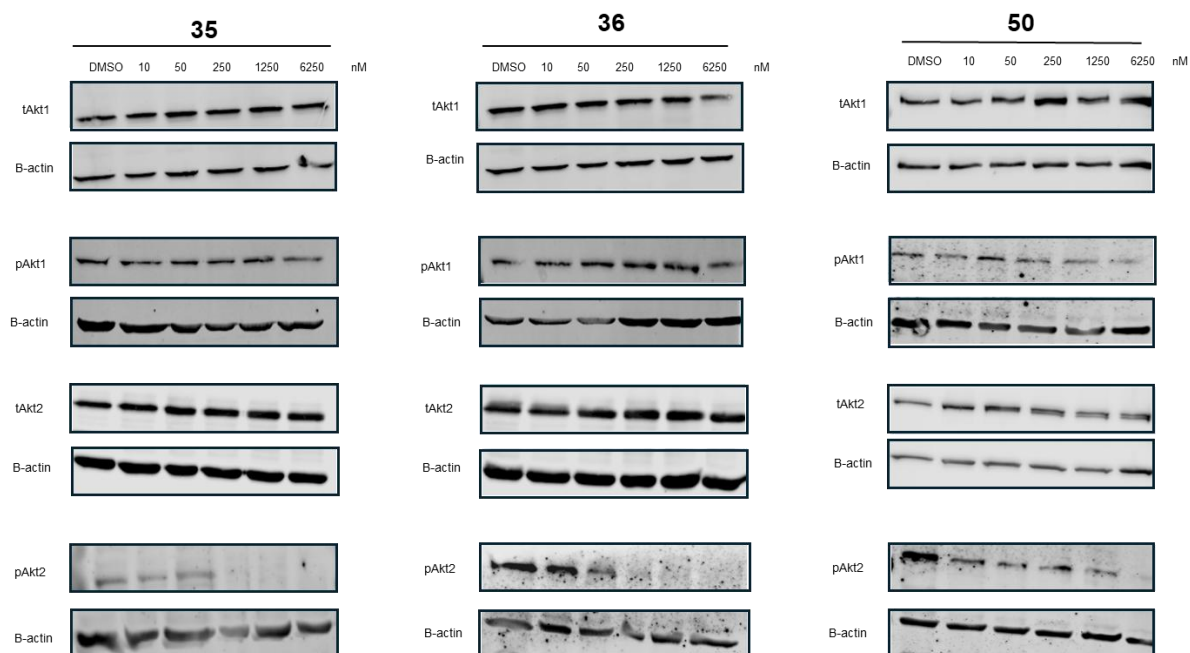


Figure S71: Effects of inhibitors **35**, **36** and **50** on Akt signaling in PANC-1 cells. PANC-1 pancreatic cancer cells were treated with Inhibitor 1, Inhibitor 2, or Inhibitor 3 at five increasing concentrations or with DMSO vehicle control for [4 h]. Whole-cell lysates were prepared and subjected to Western blot analysis. Membranes were probed with antibodies against phospho-Akt1 (Ser473), phospho-Akt2 (Ser474), total Akt1 (tAkt1), total Akt2 (tAkt2), and β -actin, which served as a loading control.

4.6.12. Selective Akt Inhibitors Exhibit Potent Activity in Tumor-Derived Organoids

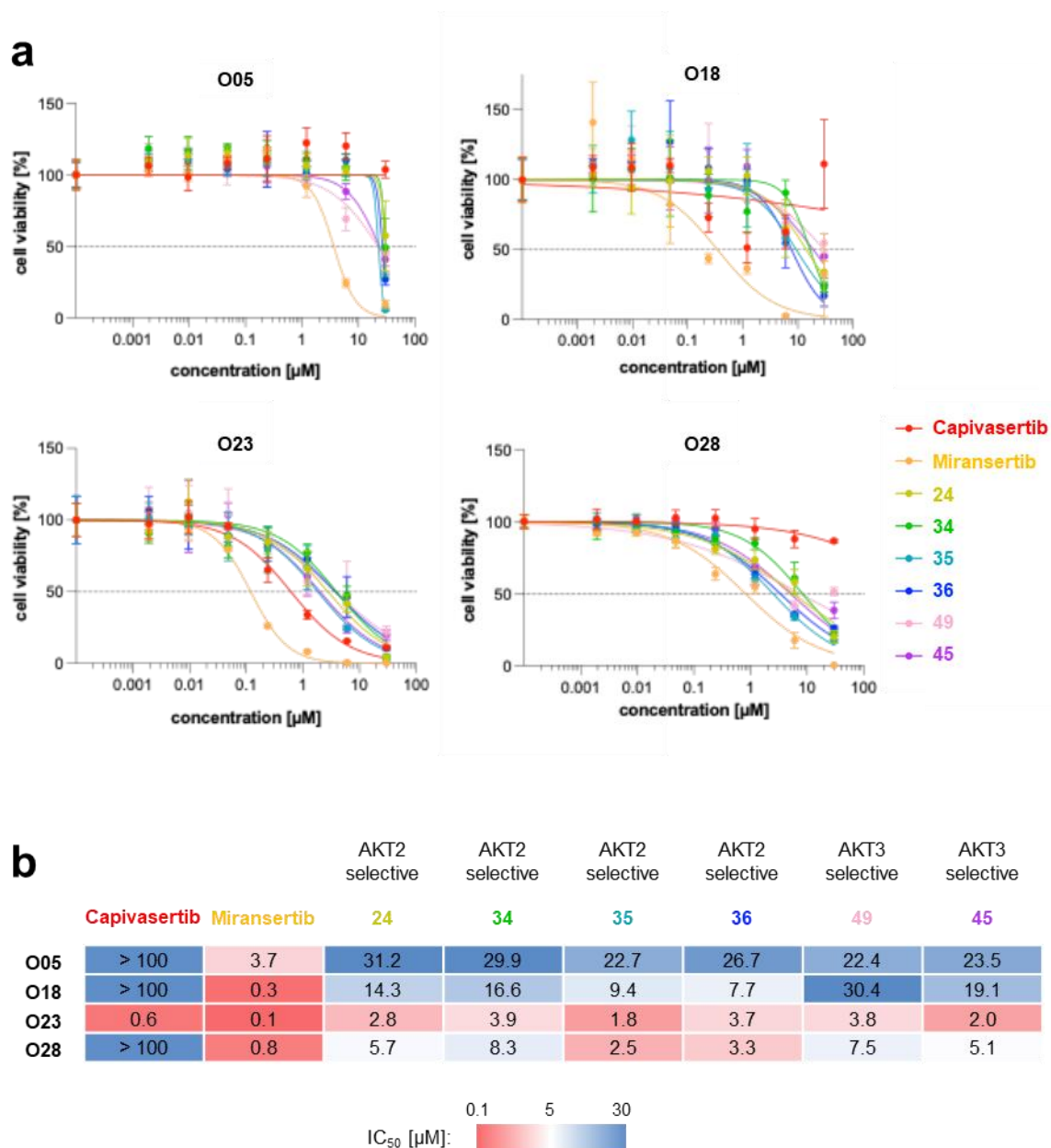


Figure S72: Cellular evaluation of synthesized covalent-allosteric Akt inhibitors in tumor-derived organoids. Cell viability in CRC organoids (O05, O18, O23 and O28) after 6 days in presence of the reference compounds (Capiasertib and Miransertib) or covalent allosteric inhibitors targeting Akt isoforms. Tumor cell viability was determined by luciferase measurement in luciferase-transgenic organoids (described in PMID: 31036555). a) Dose-titration curves. Mean cell viability $\pm$ SD in triplicate wells relative to DMSO is shown. b) Summary of IC₅₀ values from data in (a). Note that O05, O18 and O28 show pronounced resistance to Capiasertib, while all organoids were sensitive Miransertib. In contrast, the covalent allosteric inhibitors show consistent differential sensitivity (low in O05 and O18 and high in O23 and O28).

4.6.13. Additional Figures

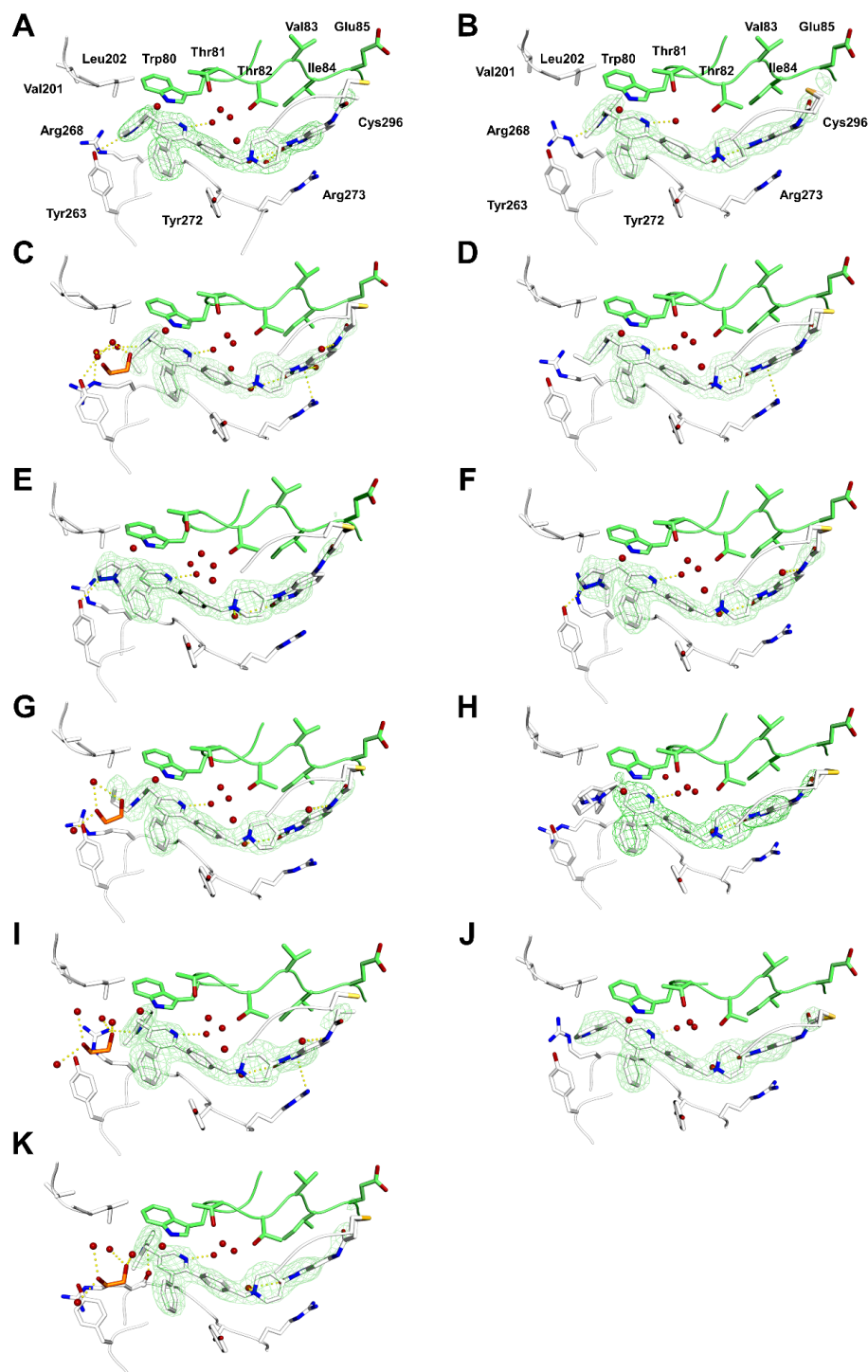


Figure S73: Co-crystal structures of Akt1 mutant with: **A**) compound **25** (PDB: 9TMT), **B**) compound **27** with EG (PDB: 9TMU), compound **28** with EG (**C**, PDB: 9TMV) and without (**D**, PDB: 9TMW), **E**) compound **34** (PDB: 9TMY), **F**) compound **35** (PDB: 9TMZ), **G**) compound **36** (PDB: 9TN0), **H**) compound **37** (PDB: 9TN2), **I**) compound **40** without EG (PDB: 9TN3), **J**) compound **40** with EG (PDB: 9TN1) and **K**) compound **47** (PDB: 9TN4). The amino acid labels shown in panels A and B are not shown in panels C-K for clarity. The simulated annealing omit $|F_o - F_c|$ -electron density map is shown at r.m.s.d. = 3.0

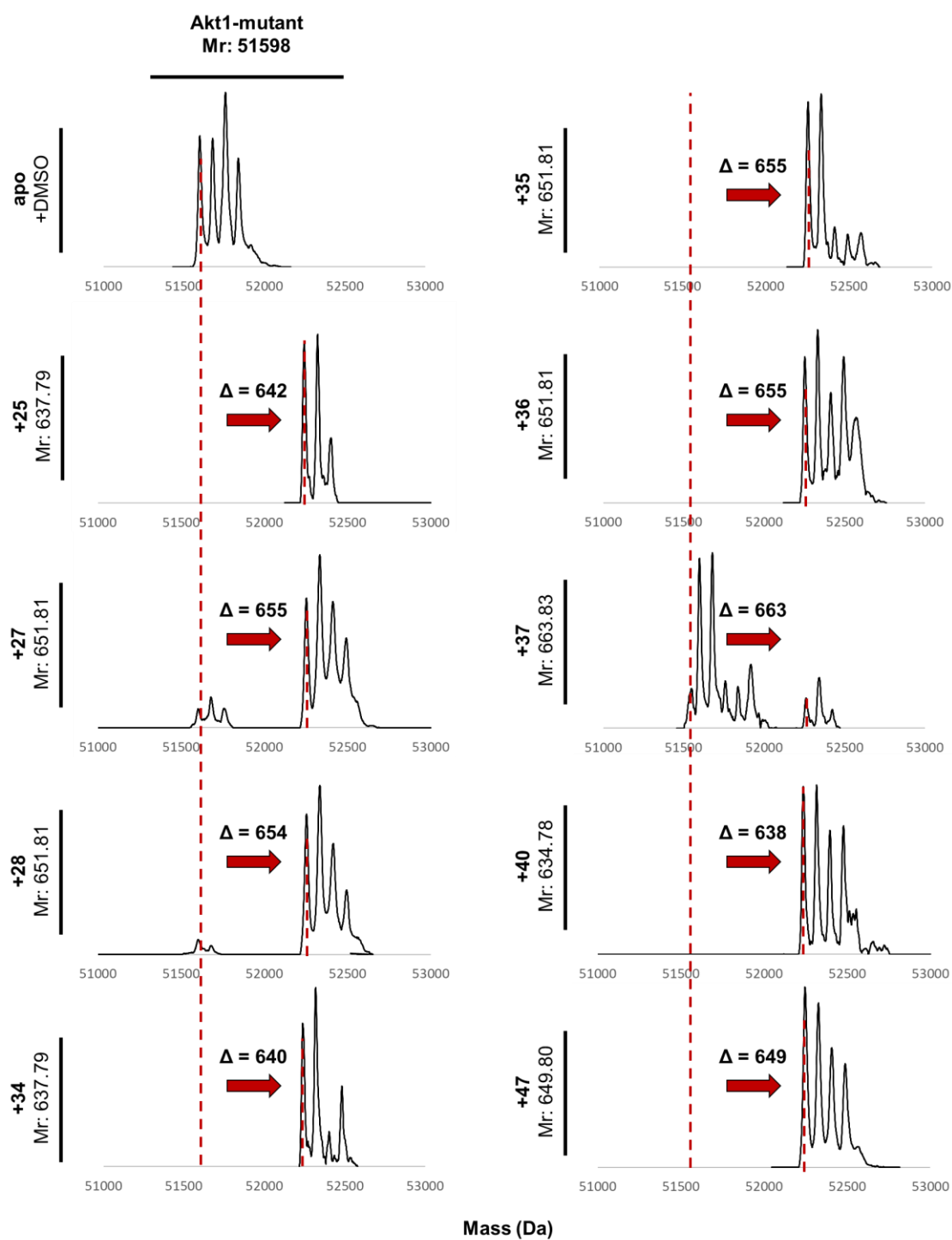


Figure S74: Deconvoluted mass spectra of Akt1-mutant following treatment with DMSO (apo) or selected isoform-selective CAAs that yielded crystal structures. Under denaturing conditions, all compounds induced mass shifts corresponding to the addition of a single ligand per protein molecule, indicative of single-covalent modification after 3 h of incubation. The extent of labeling correlated with the compounds' cellular potency toward the respective Akt isoform.

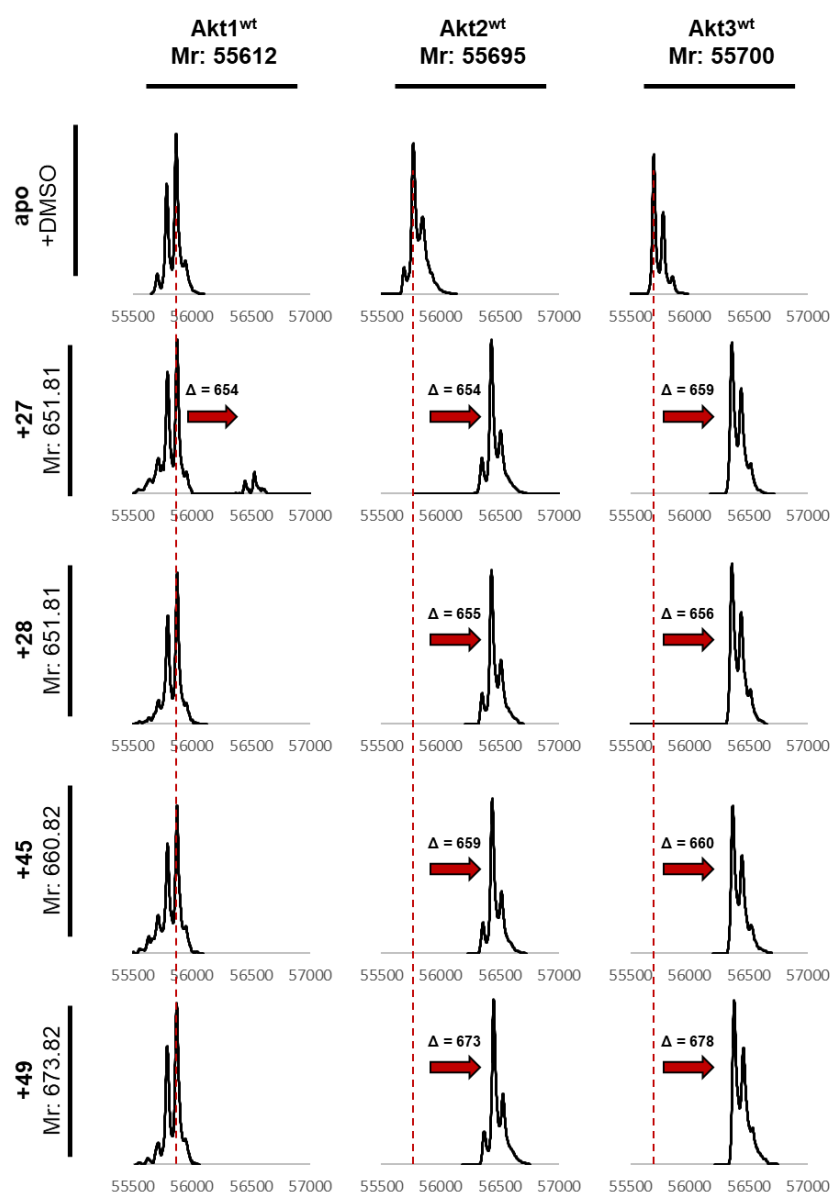


Figure S74: Deconvoluted mass spectra in Akt1^{wt}, Akt2^{wt} and Akt3^{wt} following treatment with DMSO (apo form) or of the newly synthesized isoform-selective CAIs. Under denaturing conditions, all compounds induced mass shifts corresponding to the addition of a single ligand per protein molecule, indicative of single-covalent modification after 24 h of incubation. The extent of labeling correlated with the compounds' cellular potency toward the respective Akt isoform.

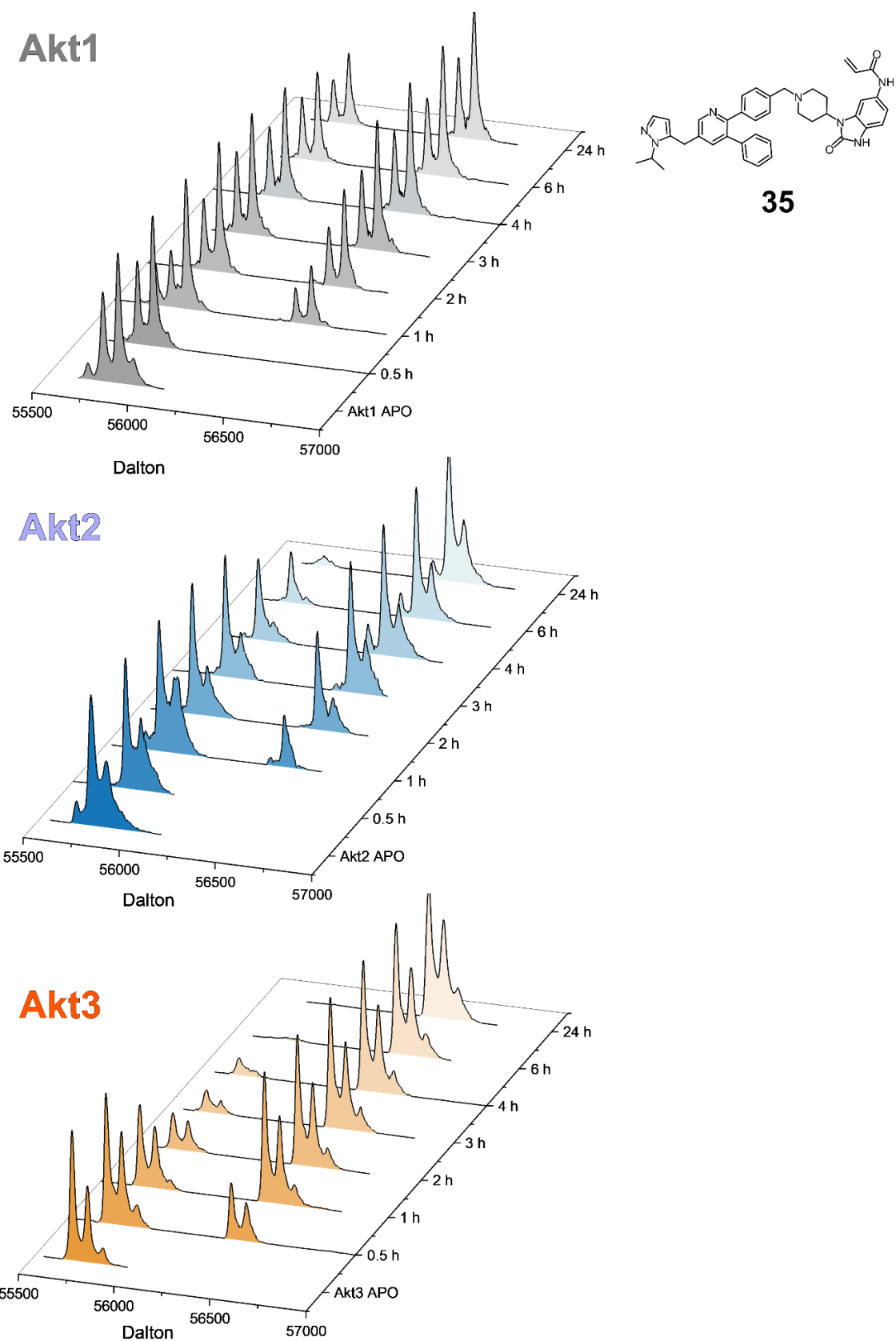
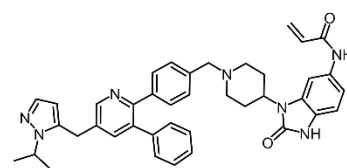
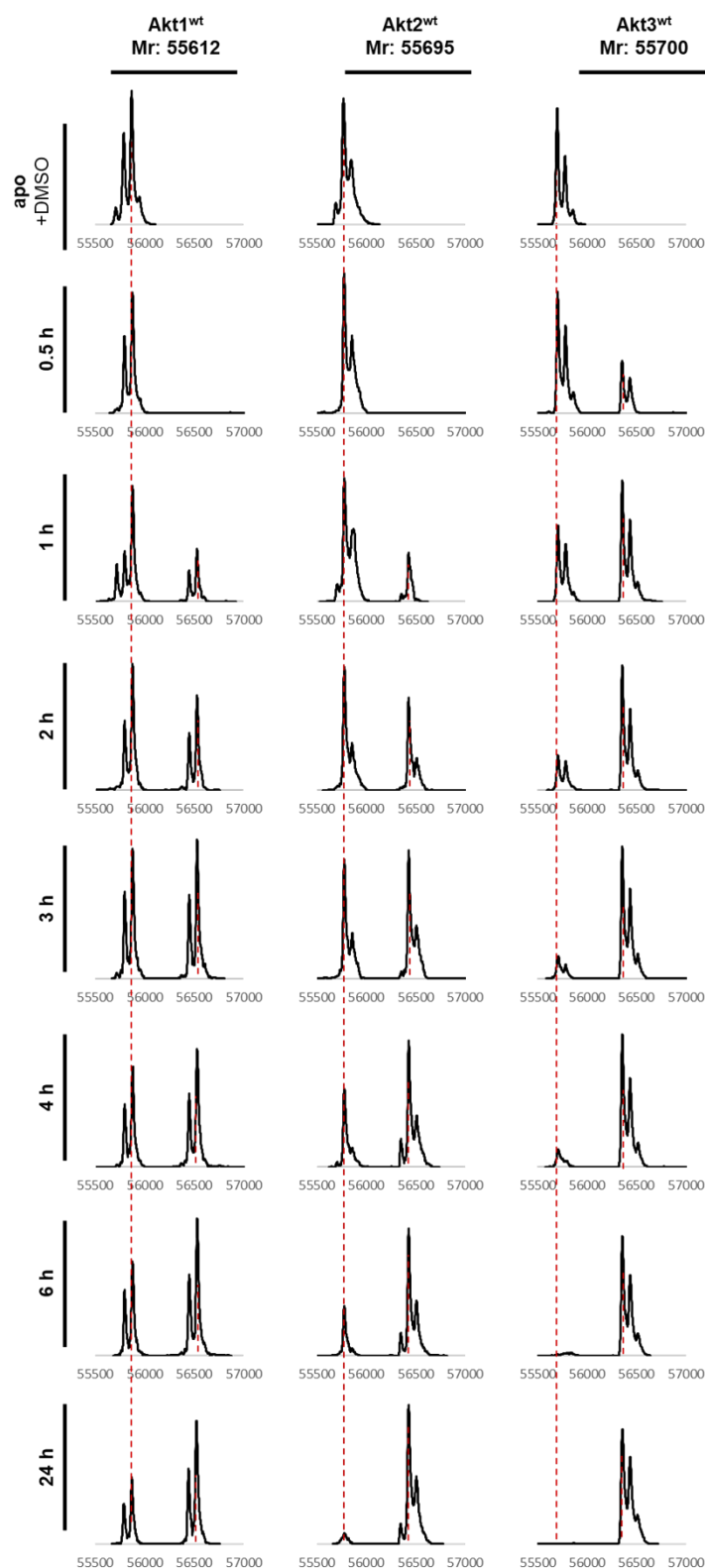


Figure S75: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt}, and Akt3^{wt} treated with DMSO and compound **35** show single covalent modification. Time-course labeling of compound **35** with Akt1^{wt} (gray), Akt2^{wt} (blue), and Akt3^{wt} (orange) is shown as a 3D plot (seven time points).



35

Figure S76: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt}, and Akt3^{wt} treated with DMSO and compound **35** show single-covalent modification. Time-course labeling of compound **35** with Akt1^{wt}, Akt2^{wt}, and Akt3^{wt} is shown over seven time-points.

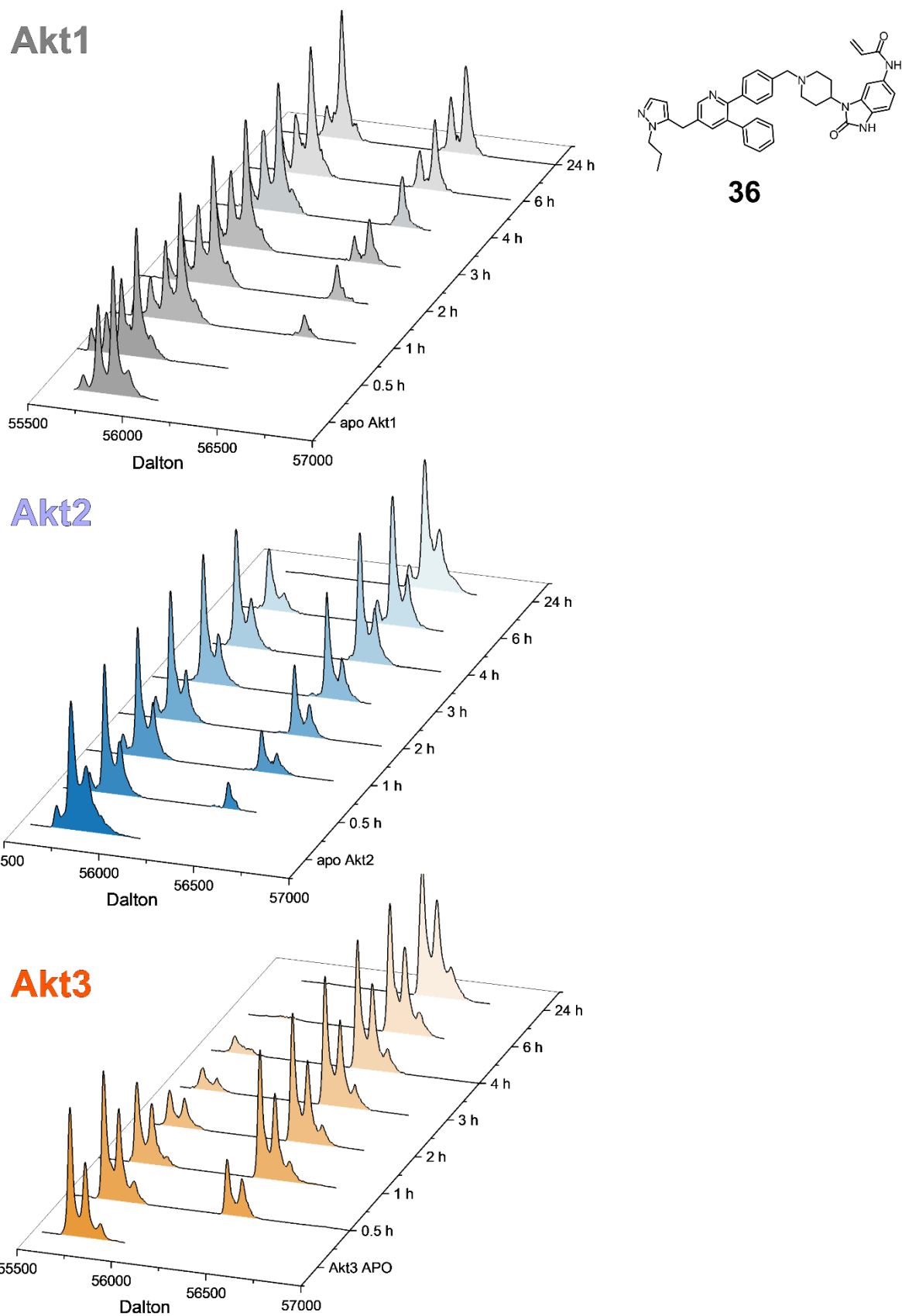
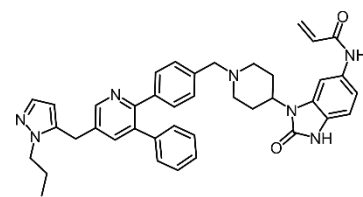
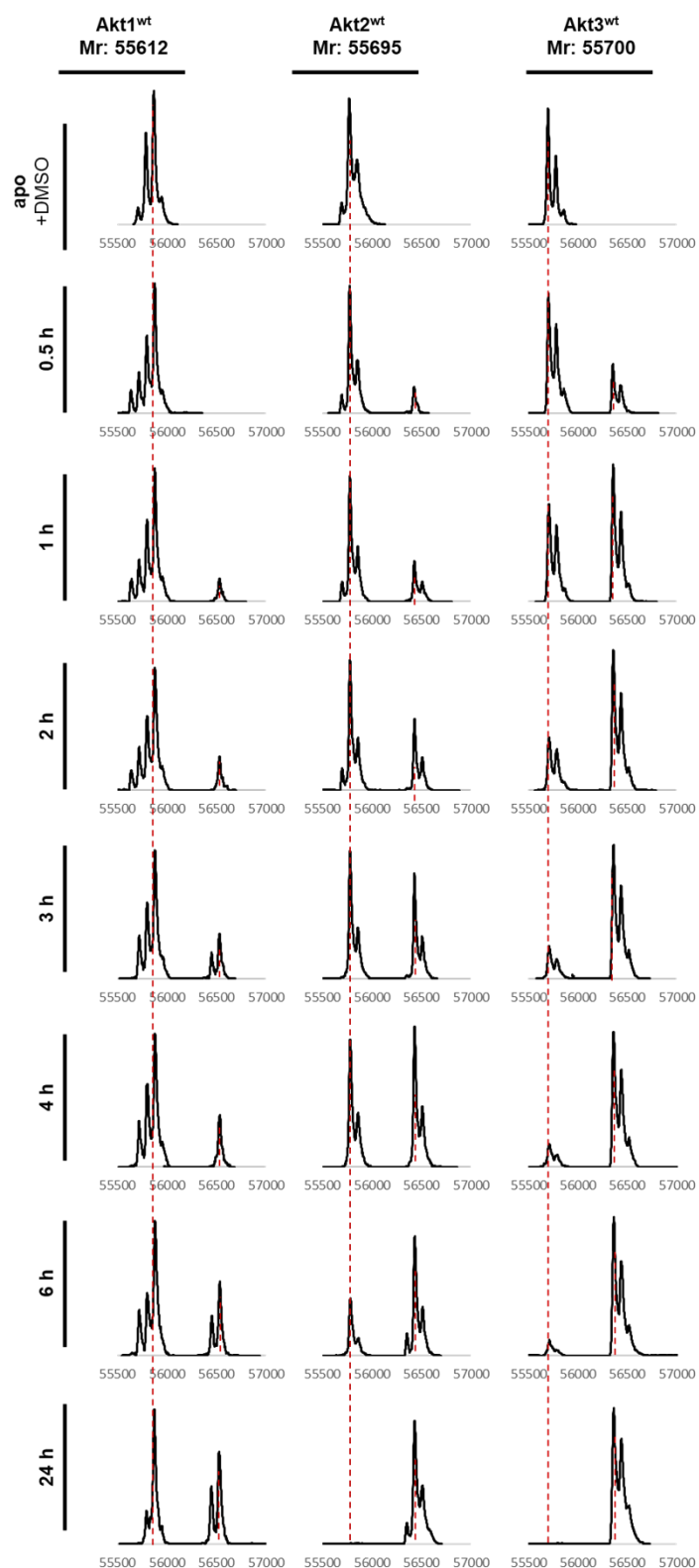


Figure S77: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt}, and Akt3^{wt} treated with DMSO and compound **36** show single-covalent modification. Time-course labeling of compound **36** with Akt1^{wt} (gray), Akt2^{wt} (blue), and Akt3^{wt} (orange) is shown as a 3D plot (seven time points).



36

Figure S78: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt}, and Akt3^{wt} treated with DMSO and compound **36** show single-covalent modification. Time-course labeling of compound **36** with Akt1^{wt}, Akt2^{wt}, and Akt3^{wt} is shown over seven time-points.

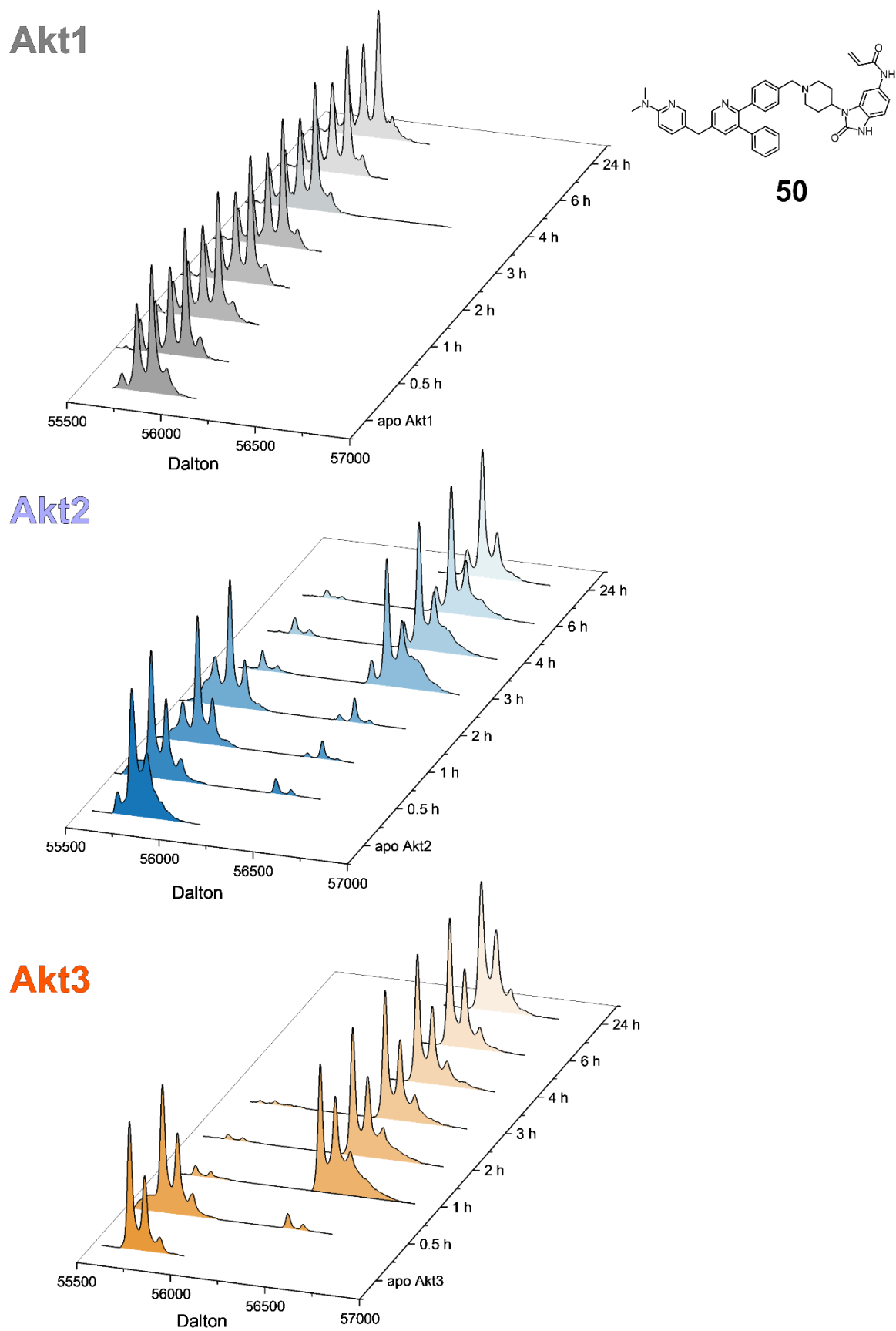


Figure S79: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt}, and Akt3^{wt} treated with DMSO and compound **50** show single-covalent modification. Time-course labeling of compound **50** with Akt1^{wt} (gray), Akt2^{wt} (blue), and Akt3^{wt} (orange) is shown as a 3D plot (seven time points).

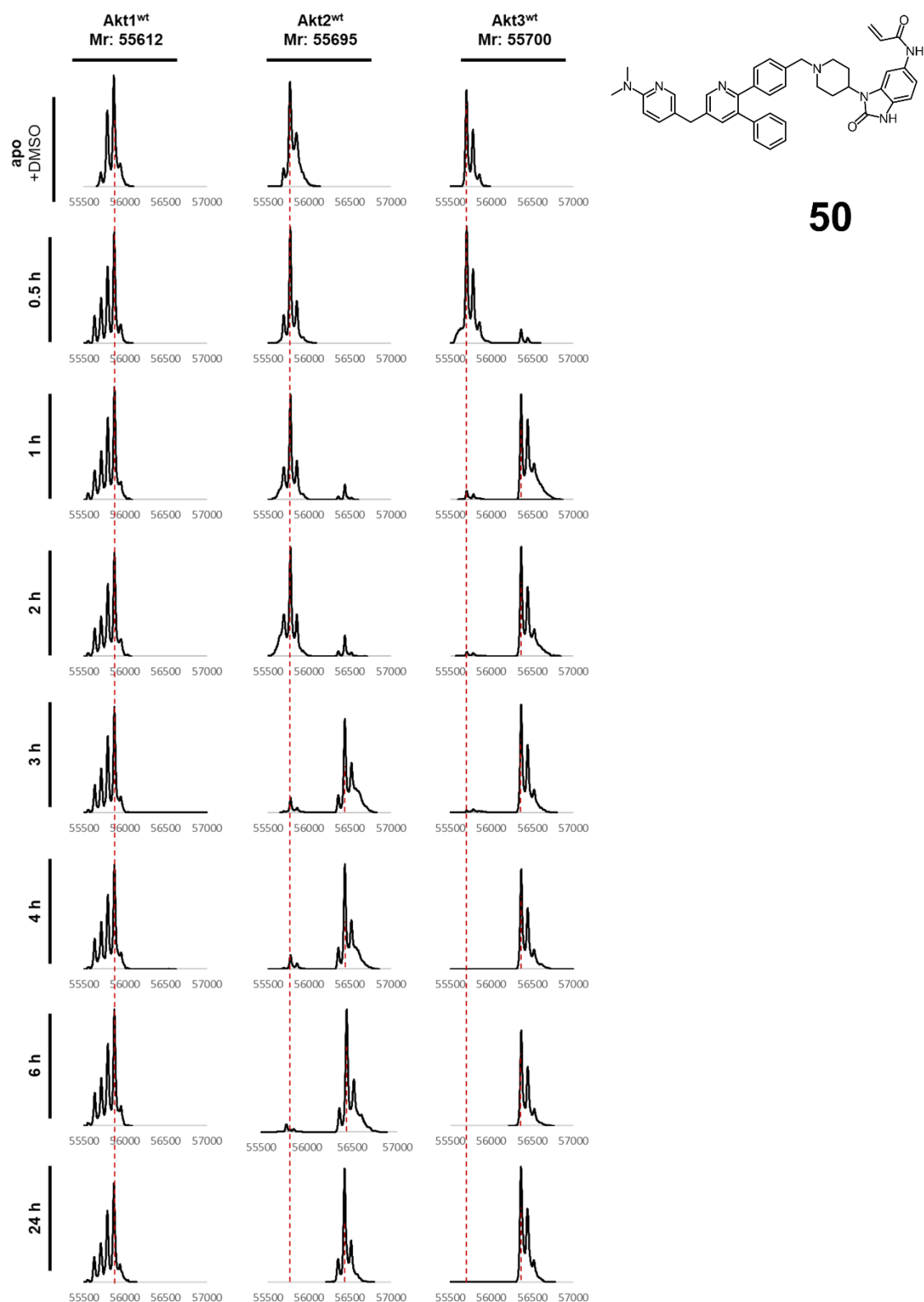


Figure S80: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt}, and Akt3^{wt} treated with DMSO and compound **50** show single-covalent modification. Time-course labeling of compound **50** with Akt1^{wt}, Akt2^{wt}, and Akt3^{wt} is shown over seven time-points.

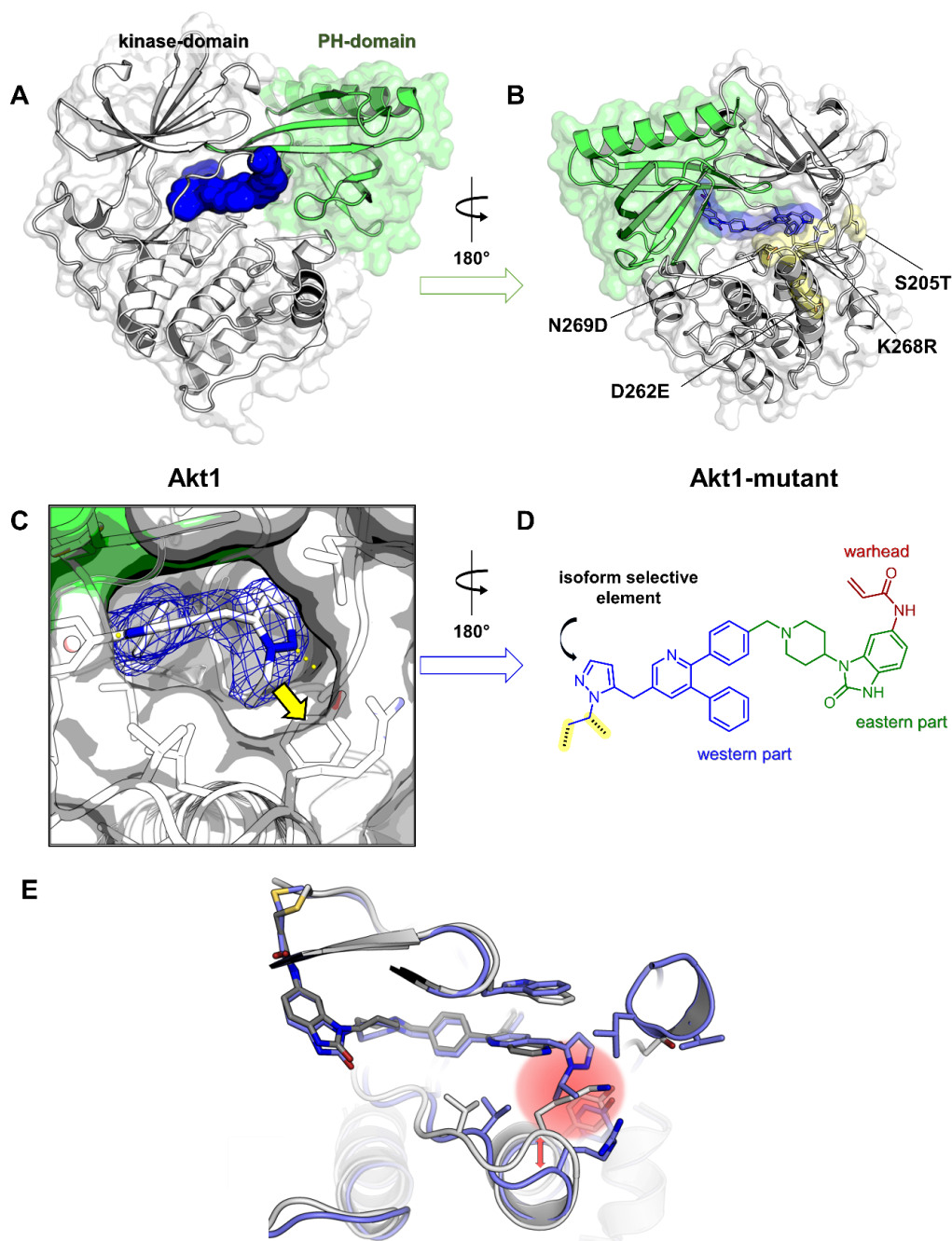


Figure S81: **A)** Representation of the inactive PH-in conformation of Akt1, showing an allosteric inhibitor bound at the interface between the PH domain (green) and the kinase domain (white) (PDB: 6HHF). **B)** Co-crystal structure of compound **34** bound to the Akt1 mutant containing the S205T, D262E, E267del, K268R, and N269D mutations (PDB: 9TMY). **C)** Close-up view of the isoform-selective moiety of compound **34**, with the corresponding electron density shown in blue, revealing potential vectors for extension and further derivatization. The $|2F_o - F_c|$ -electron density map is shown at r.m.s.d. = 1.0. **D)** Chemical structure of compound **34** illustrating additional derivatization opportunities at the ethyl substituent. **E)** Structural alignment of the optimized compound **35** in complex with the Akt1 mutant (PDB: 9TMZ) and borussertib bound to Akt1, highlighting that differences in the terminal region of the α -helix result in a steric clash between the pyrazole and Lys268, potentially rationalizing the observed selectivity of compound **35** for Akt2 over Akt1.

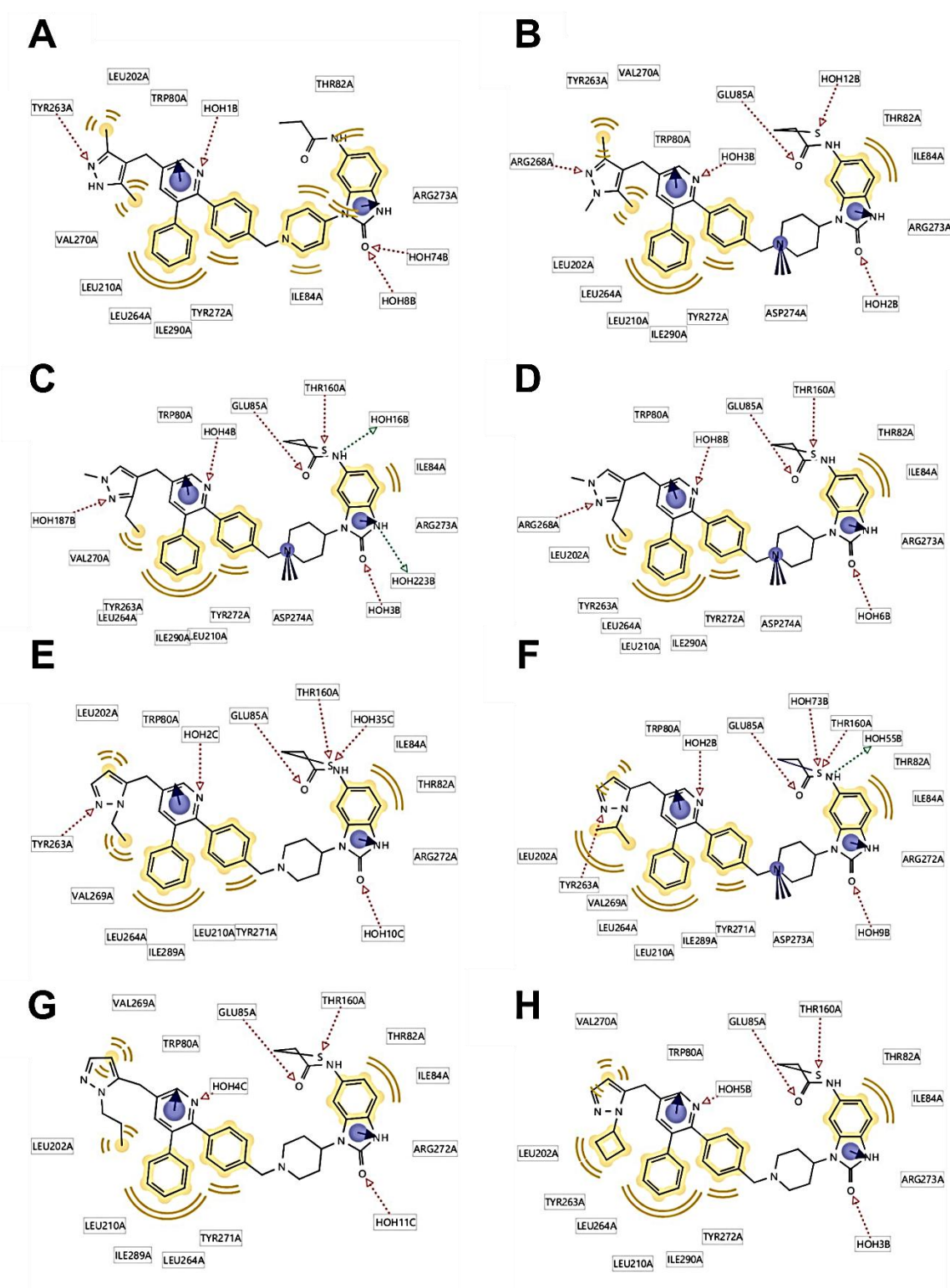


Figure S82: Pharmacophore model of: A) compound **25** (PDB: 9TMT), B) compound **27** (PDB: 9TMU), C) compound **28** (PDB: 9TMV), D) compound **28** (PDB: 9TMW), E) compound **34** (PDB: 9TMY), F) compound **35** (PDB: 9TMZ), G) compound **36** (PDB: 9TN0), H) compound **37** (PDB: 9TN2) in Akt1-mutant using LigandScout 4.4.3 [4].

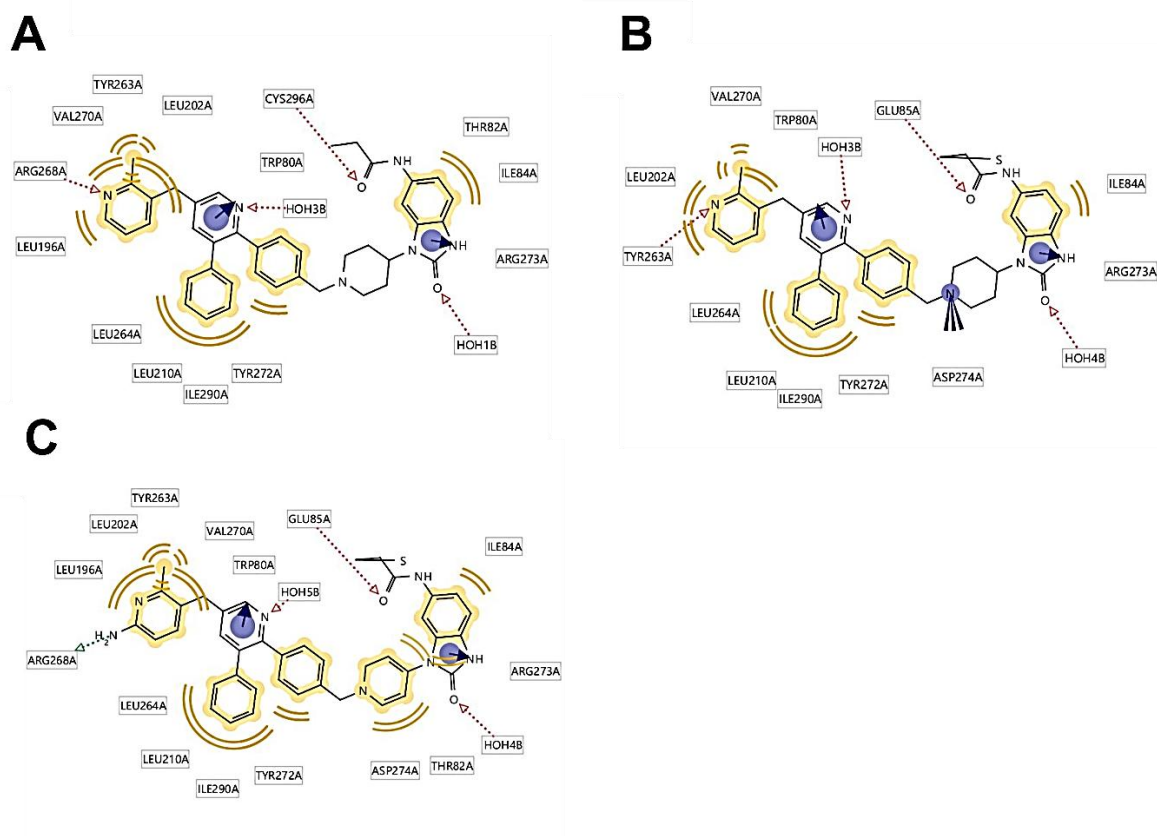


Figure S83: Pharmacophore model of: A) compound **40** (PDB: 9TN3), B) compound **40** (PDB: 9TN1), C) compound **47** (PDB: 9TN4) in Akt1-mutant using LigandScout 4.4.3 [4].

5. Covalent-Allosteric Akt Inhibitors: Sharpening Isoform Precision with Akt3 Focus

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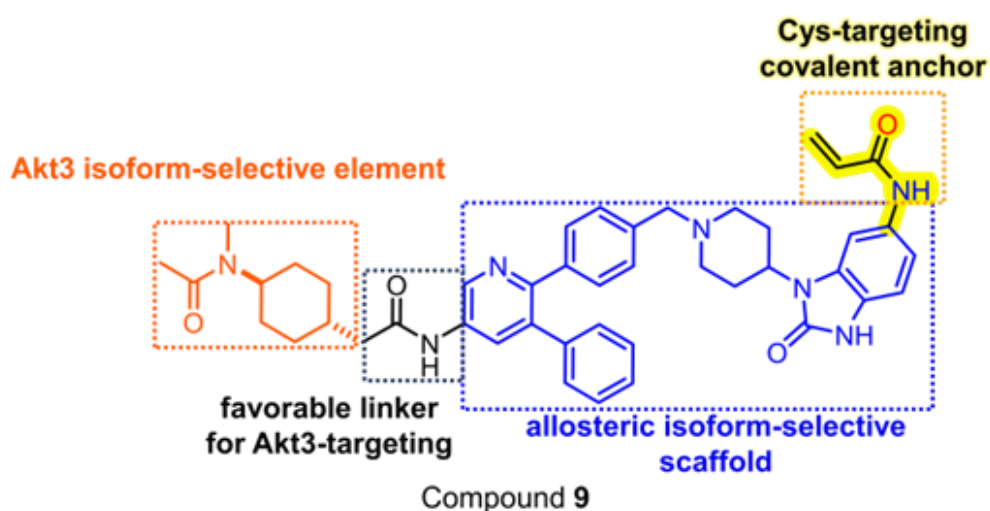
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Status: Revised

Graphical Abstract



- Potent cellular Akt3-targeting: $EC_{50} = 35$ nM
- Linker-driven SAR reveals a strong impact on allosteric pocket engagement
- Successful covalent Akt labelling
- Structural data from new isoform-selective CAAls explain Akt-isoform preferences

5.1. Abstract

Akt is a central serine/threonine kinase in the PI3K/Akt/mTOR signaling pathway that regulates cell survival, growth and metabolic homeostasis. Despite high sequence homology among the three isoforms (Akt1/2/3), they exert distinct biological functions. Akt3 is predominantly expressed in the central nervous system and plays a critical role in neurodevelopment, with dysregulation associated with epilepsy, cortical malformations and cognitive impairment. Aberrant Akt3 activation also contributes to tumor progression in cancers such as glioblastoma and melanoma, highlighting the need for isoform-selective chemical probes to dissect isoform-specific biology and therapeutic potential. Using an iterative structure-based design strategy guided by multiple high-resolution co-crystal structures, we combined Akt3-preferred structural features with the covalent pharmacophore of established covalent-allosteric Akt inhibitors. Structure-activity relationship studies revealed that precise linker geometry governs productive positioning of isoform-discriminating elements within the allosteric pocket. Cellular target-engagement assays identified compounds that function as potent, selective and covalent Akt3 inhibitors. Collectively, these data establish a structure-guided and mechanistically validated blueprint for isoform-selective Akt3 inhibition and provide a foundation for therapeutic exploration in oncology and neurodevelopmental disorders.

5.2. Introduction

Protein kinase B (Akt) is a central effector of the PI3K/Akt/mTOR pathway and regulates cell growth, survival and metabolism. Three highly homologous isoforms, Akt1, Akt2 and Akt3, perform distinct biological functions: Akt1 is primarily associated with proliferation, Akt2 with insulin-responsive metabolic signaling and Akt3 with neuronal development and brain function. Isoform-specific dysregulation contributes to cancer, metabolic disease and neurodevelopmental disorders, establishing Akt as an important therapeutic target. Early pharmacological efforts focused on ATP-competitive inhibitors such as the clinically approved capivasertib (**1**).¹ However, this strategy is limited by competition with intracellular ATP and poor isoform selectivity due to conservation of the kinase active site. Reversible allosteric inhibitors, including VAD044 (**2**)² and miransertib³⁻⁵ were subsequently developed to target the interdomain pocket formed between the pleckstrin homology and kinase domains (Figure 1B, D), stabilizing an inactive PH-in conformation of Akt. More recently, covalent-allosteric Akt

inhibitors (CAAls), exemplified by borussertib (**4**), have combined conformational selectivity with irreversible engagement of cysteine residues (Cys296 and Cys310 in Akt1) in the allosteric pocket to enhance target residence time (Figure 1C).⁶ Despite these advances, most Akt inhibitors inhibit all three isoforms with comparable potency. This lack of selectivity has been associated with dose-limiting toxicities, including hyperglycemia, likely reflecting Akt2 inhibition, highlighting the need for isoform-selective inhibition.⁷ Achieving selectivity is challenging because the Akt isoforms share high sequence conservation not only within the ATP-binding site but also within the interdomain allosteric pocket. Nevertheless, scaffold-dependent selectivity has been demonstrated. Methyl-pyrazinone-based compound **5** shows Akt1 preference,⁸ certain pyridine-derived inhibitors exhibit Akt2 (compound **6**) or Akt3 selectivity (like compound **7**)^{9, 10} and a reversible allosteric inhibitor series (similar to compound **8**) reported by BerGenBio selectively targets Akt3 in biochemical and cellular systems.¹¹ These studies suggest that subtle spatial and physicochemical differences between isoforms can be exploited through precise structural design (Figure 1A-C). Among the isoforms, Akt3 has emerged as a compelling therapeutic target. In triple-negative breast cancer, selective genetic depletion of Akt3 suppresses tumor growth in 3D culture and xenograft models.¹² Akt3 is also frequently overexpressed in melanoma,^{13, 14} particularly in the context of loss of PTEN,¹⁵ and activating mutations in Akt3 drive neurodevelopmental disorders characterized by megalencephaly and refractory epilepsy.¹⁶ Together, these findings underscore the need for Akt3-selective chemical probes and therapeutic agents.

Here, we report the design, synthesis and characterization of a new series of covalent-allosteric Akt inhibitors that combine Akt3-selective substituents on the west-part of the molecule with a pyridine-based CAAl core while systematically varying the linker connecting these moieties. Based on the reversible Akt3 inhibitor **8** and borussertib (**4**) a hybrid approach was followed for the design of compound **9** (Figure 1E). Additionally, replacement of the methylene linker present in earlier CAAls with amides, ether or amine functionalities was intended to modulate hydrogen-bonding capacity, electronics and rotational flexibility within the allosteric pocket. Biochemical assays, cellular NanoBRET engagement studies and X-ray crystallography reveal that subtle changes in linker chemistry markedly influence potency and isoform selectivity. These results identify linker composition as a key determinant of functional selectivity in covalent-

allosteric Akt inhibition and provide design principles for the development of Akt3-selective inhibitors.

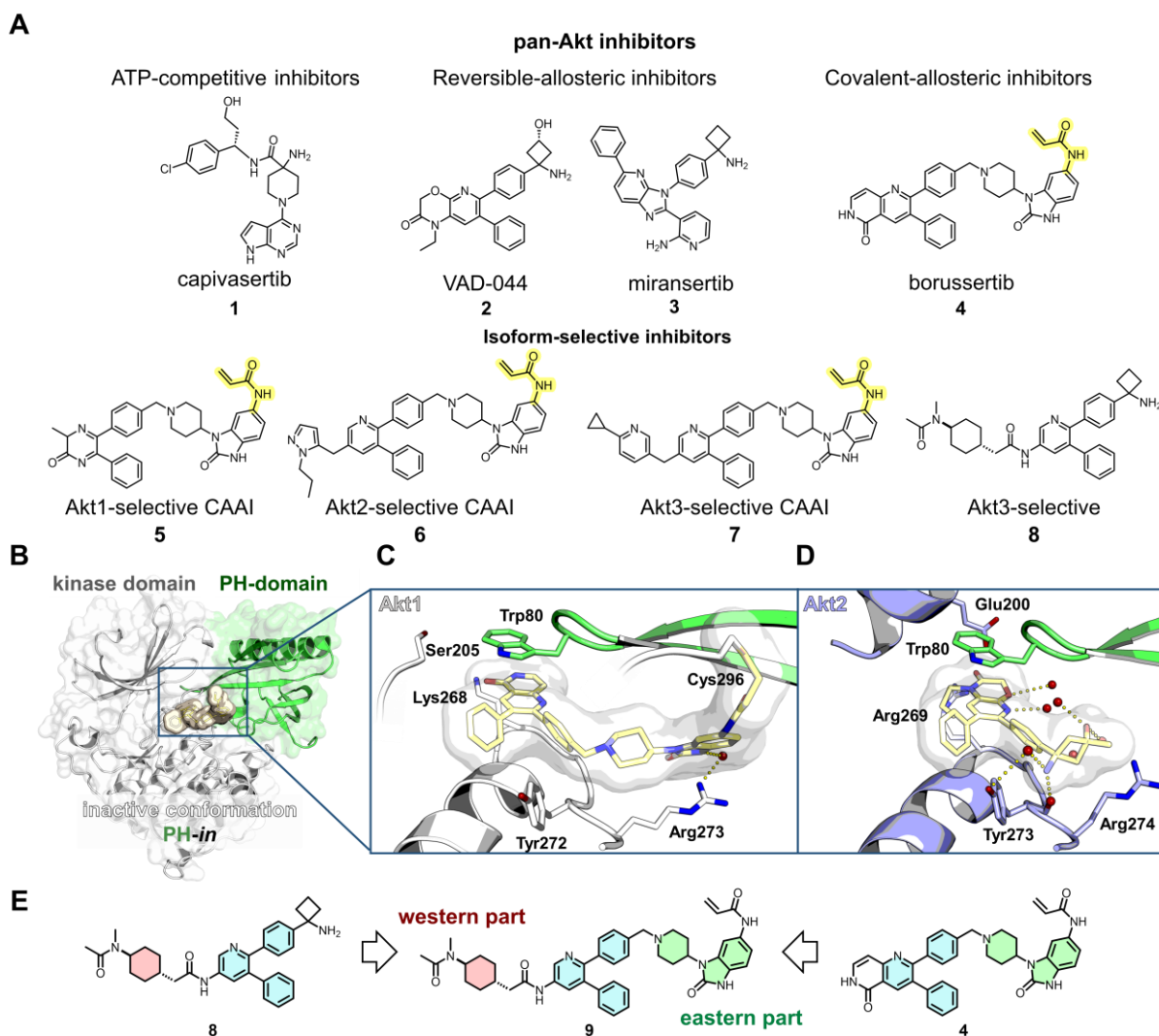


Figure 1: (A) Chemical structures of representative compounds from the different categories of Akt inhibitors. The covalent warhead is highlighted in yellow. (B) Crystal structure of borussertib (yellow) bound to Akt showing its position at the interface between the PH-domain (green) and the kinase domain (white) in the inactive PH-in conformation. Crystal structure of: (C) a covalent-allosteric inhibitor in Akt1 (borussertib, PDB: 6HHF) and (D) a reversible allosteric inhibitor in Akt2 (a derivative of VAD-044, PDB: 8Q61). (E) Hybrid inhibitor design strategy combining the western part of compound 8 with the eastern part of borussertib 4 to generate the covalent-allosteric Akt3-targeting compound 9.

5.3. Structure-Based Design of New Isoform Selective CAAls

The first step in the design of Akt3-selective CAAls was to elucidate possible reasons for the structural basis of the selectivity of existing Akt3-selective inhibitors. Docking of compound **8** to each Akt isoform, using known crystal structures for Akt1 and Akt2 (PDB: 6HHF and PDB:8Q61 respectively) and homology modeling for Akt3, Figure 2A-C) revealed putative isoform-specific interactions within the allosteric pocket. In Akt1, the bulky cyclohexyl-based substituent induces a shift of the central scaffold to avoid a steric clash with Lys268 (Figure 2A). In Akt2, the shorter α -helix provides additional space, but the compound needs to partially reorient to bypass Arg269 (Figure 2B). In contrast, Akt3 features Gly265 and a shortened α -helix, creating a more spacious pocket that accommodates the ligand without steric strain, which may explain its preferential binding (Figure 2C). These observations guided the design of subsequent covalent-allosteric inhibitors with a focus on Akt3 selectivity. Guided by these insights, hybrid compound **9** (Figure 1E) preserves the bulky Western group to maintain the clashes in Akt1 and Akt2, while introducing covalent engagement of Cys293/Cys307 in Akt3 to incorporate the advantages of covalent targeting (Figure 2D). Based on these findings, the series was designed to prioritize Akt3 selectivity by introducing motifs from previous Akt3-selective inhibitors, such as substituted pyridines. Moreover, pyridine residues with nitrogen atoms at alternate positions and amino-piperidine-containing compounds were designed.¹¹ Methyl- and ethyl-substituted pyrazoles were also incorporated to modulate Akt2 activity.¹⁰ The linker region was systematically varied to include amide and reverse amide linkages, with or without an additional methylene spacer and flexible motifs such as methylene amines and ethers were incorporated to explore effects on binding orientation and isoform preference (Figure 2E). Overall, this SAR strategy combined Akt3-selective scaffolds, auxiliary Akt2 motifs and linker modifications to test these modifications for optimization of both potency and isoform selectivity.

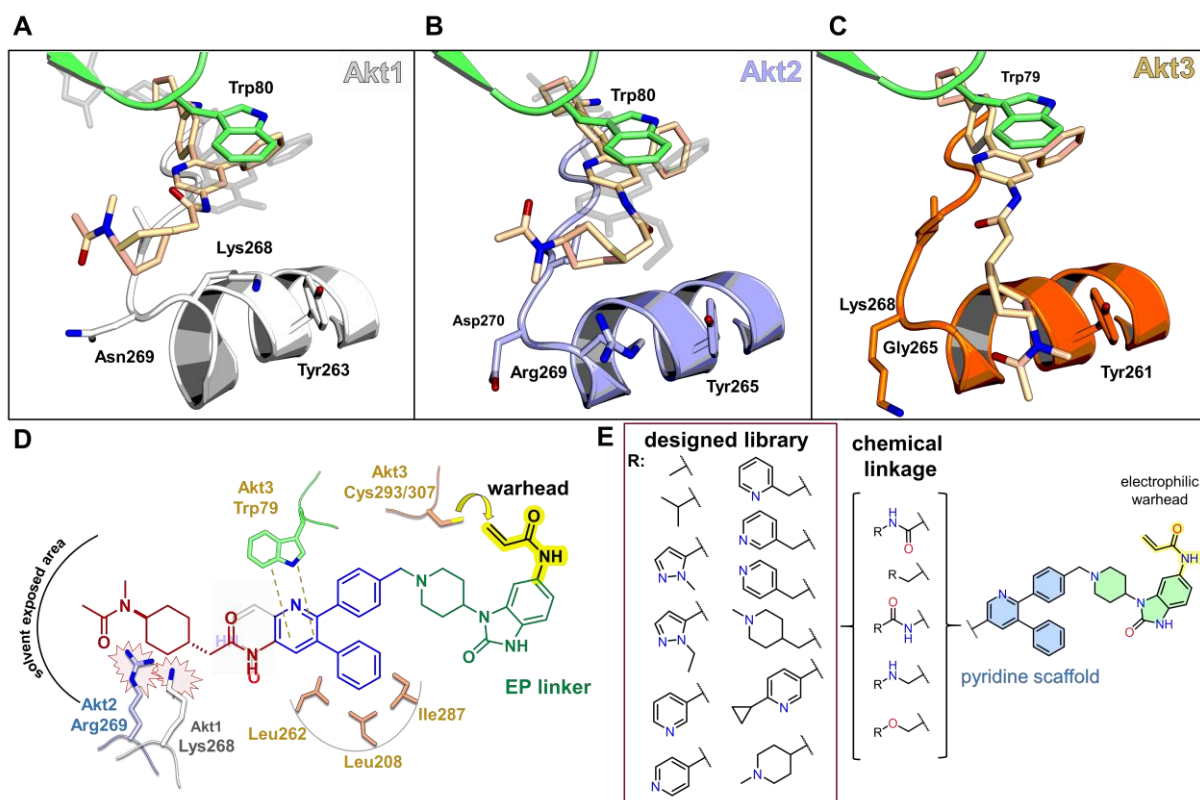
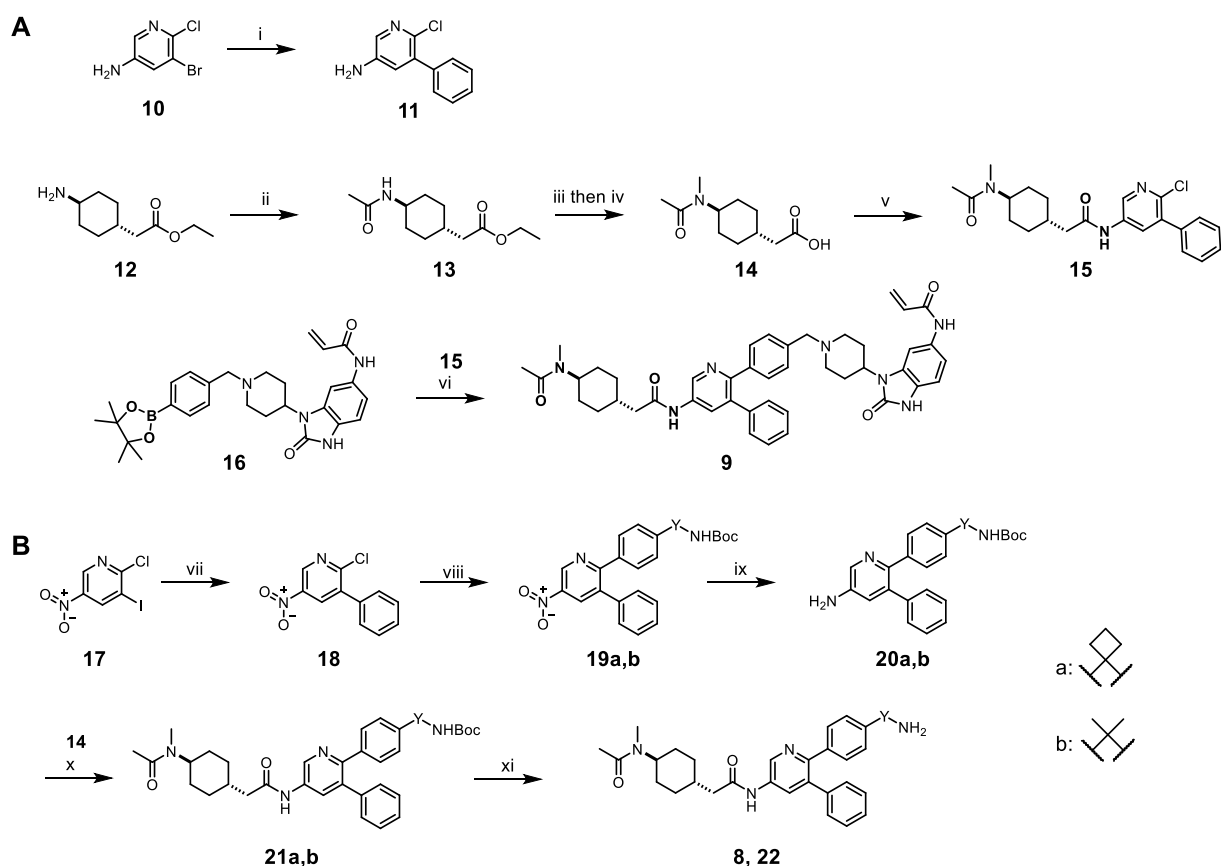


Figure 2: (A-C) Docking studies with compound **8** in (A) Akt1 (PDB: 6HHF), (B) Akt2 (PDB: 8Q61) and (C) Akt3 (homology model obtained from Swiss-model). The co-crystallized ligands in panels A and B are shown as faded gray structures to facilitate comparison. (D) Schematic depiction of key interactions of the hybrid compound **9** with Trp79 via π - π stacking and hydrophobic interactions with Leu262, Leu208 and Ile287 based on the docking pose of compound **8** in Figure 2C. The western part of hybrid compound **9** (dark red) extends toward the solvent-exposed region and is predicted to sterically clash with Lys268 in Akt1 and Arg269 in Akt2. In the eastern region of compound **9**, the central pyridine scaffold is linked via the piperidine-benzimidazolone moiety to the electrophilic warhead, which is directed to covalently react with Cys293/307 within the Akt3 allosteric pocket. (E) Overview of the designed library of new isoform-selective CAAls, incorporating aliphatic groups, aliphatic or aromatic ring systems, substituent sizes and alternative linker motifs, combined with the pyridine core and the eastern part bearing the electrophilic warhead.

5.4. Synthesis of a Small-Molecule Library of Isoform-Selective CAAls

Following previous efforts to design isoform-selective inhibitors, the pyridine core was retained as the central scaffold. Hybrid compound **9** was assembled by first generating the pyridine intermediate **11** via Suzuki coupling of commercially available **10**, providing a free amine for later derivatization. The western Akt3-selective moiety was generated from starting material **12** by acetylation to give **13**, followed by N-methylation with concomitant ester hydrolysis to give **14**. Amide coupling of **11** with **14** via T3P-mediated amide bond formation afforded intermediate **15**, which was subsequently

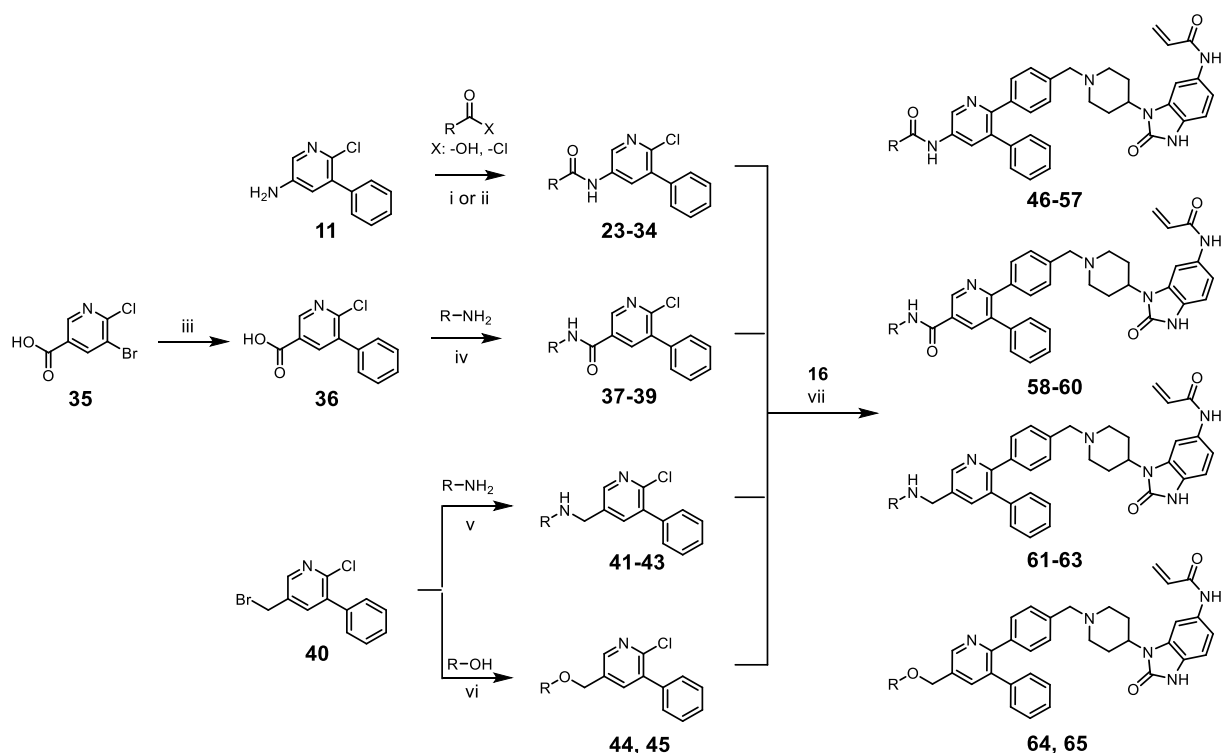
coupled with the benzimidazolone-based electrophilic warhead **16** via Suzuki coupling to afford hybrid compound **9**. For comparison, reversible analogues **8** and **22** were prepared from commercial **17**, which underwent Suzuki phenylation to produce **18**, followed by installation of the eastern portion via a second Suzuki coupling to yield **19a,b**. Nitro reduction afforded **20a,b** and amide coupling with **14** afforded **21a,b**. BOC deprotection as the final step afforded the reversible compounds **8** and **22**. All steps are summarized in Scheme 1.



Scheme 1: (A) Synthesis of hybrid compound **9**: i) K_3PO_4 , $\text{Pd}(\text{dppf})\text{Cl}_2$, phenyl boronic acid, 1,4-dioxane/ H_2O (5:1), 100 °C, 1 h, mw, 78 %, ii) Acetyl chloride, Et_3N , DCM, rt, 2 h, 84 % iii) CH_3I , NaH, THF, rt, 20 h then iv) NaOH aq., 40 °C, 18 h, 91 % over two steps, v) **11**, T3P, Et_3N , MeCN, 60 °C, 2 h, 18 %, vi) **15**, K_2CO_3 , $\text{Pd}(\text{PPh}_3)_4$, 1,4-dioxane/ H_2O (5:1), 130 °C, mw, 3 h, 21 %. (B) Synthesis of the positive controls **8** and **22**: vii) K_3PO_4 , $\text{Pd}(\text{dppf})\text{Cl}_2$, phenyl boronic acid, 1,4-dioxane/ H_2O (5:1), 130 °C, mw, 3 h, 92 %, viii) K_2CO_3 , $\text{Pd}(\text{PPh}_3)_4$, 1,4-dioxane/ H_2O (5:1), 130 °C, mw, 3 h, 80-86 %, ix) Fe, NH_4Cl , IPA/ H_2O (10:1), 50 °C, 1 h, 64-73 %, x) **22**, T3P, Et_3N , MeCN, 40 °C, 1 h then xi) HCl in 1,4-dioxane, 1 h, 10-25 % over two steps.

In order to diversify the CAAI library, key intermediates were selected to allow the introduction of various linkers.

Intermediate **11** was used to prepare to link the western part of the new inhibitors to the main pyridine scaffold through an amide bond: reaction of **11** with either acyl-chlorides or carboxylic acids gave amide derivatives **23-34**. For compounds containing the alternative amide position previously reported for CAAs, intermediate **36**, obtained by Suzuki coupling of nicotinic acid derivative **35** with phenylboronic acid, was coupled with the corresponding amines to give intermediates **37-39**. Secondary amine and ether linkers were prepared from the published intermediate **40** by nucleophilic substitution with appropriate amines or alcohols to give methylene-amine derivatives **41-43** and methylene-ethers **44-45**.¹⁰ In the final step, all linker-bearing intermediates were coupled with the eastern electrophilic moiety **16** to give the final CAAs **46-63**. The overall synthetic workflow is shown in Scheme 2.



Scheme 2: Synthesis of new isoform selective CAAs with different substituents and different linkers: *i*) acid, EDC, DMAP, HOBT, THF, 60 °C, 16-18 h, 19-65 %, *ii*) acyl halide, DIPEA, THF, rt, 2 h, 99 %, *iii*) K_3PO_4 , $Pd(dppf)Cl_2$, phenyl boronic acid, 1,4-dioxane/ H_2O (5:1), 70 °C, 18 h, 84 %, *iv*) amine, DIPEA, HATU, MeCN, rt, 3 h, 74-85 %, *v*) amine, K_2CO_3 , DMF, rt, 16-18 h, 75-84 %, *vi*) alcohol, K_2CO_3 , DMF, rt, 17-19 h, 54-67 %, *vii*) **14**, K_2CO_3 , $Pd(PPh_3)_4$, 1,4-dioxane/ H_2O (5:1), 130 °C, mw, 3 h, 2-54 %.

5.5. Biochemical Evaluation with Protein MS-Experiments

After synthesizing this focused library of inhibitors, the ability to form covalent bond with the Akt isoforms was investigated. Evaluation of the compounds by protein-MS experiments revealed isoform-dependent differences in the extent of covalent labeling across the CAAI series (Figure 3). After 3 h incubation, all compounds produced clear mass shifts consistent with the formation of a single covalent adduct for Akt1, Akt2 and Akt3, confirming mono-covalent modification. However, the relative intensity of the modified species varied among the three isoforms and across compounds bearing different linkers and isoform-selective substituents. The 24h measurements of all the covalent compounds are included in the Supporting information.

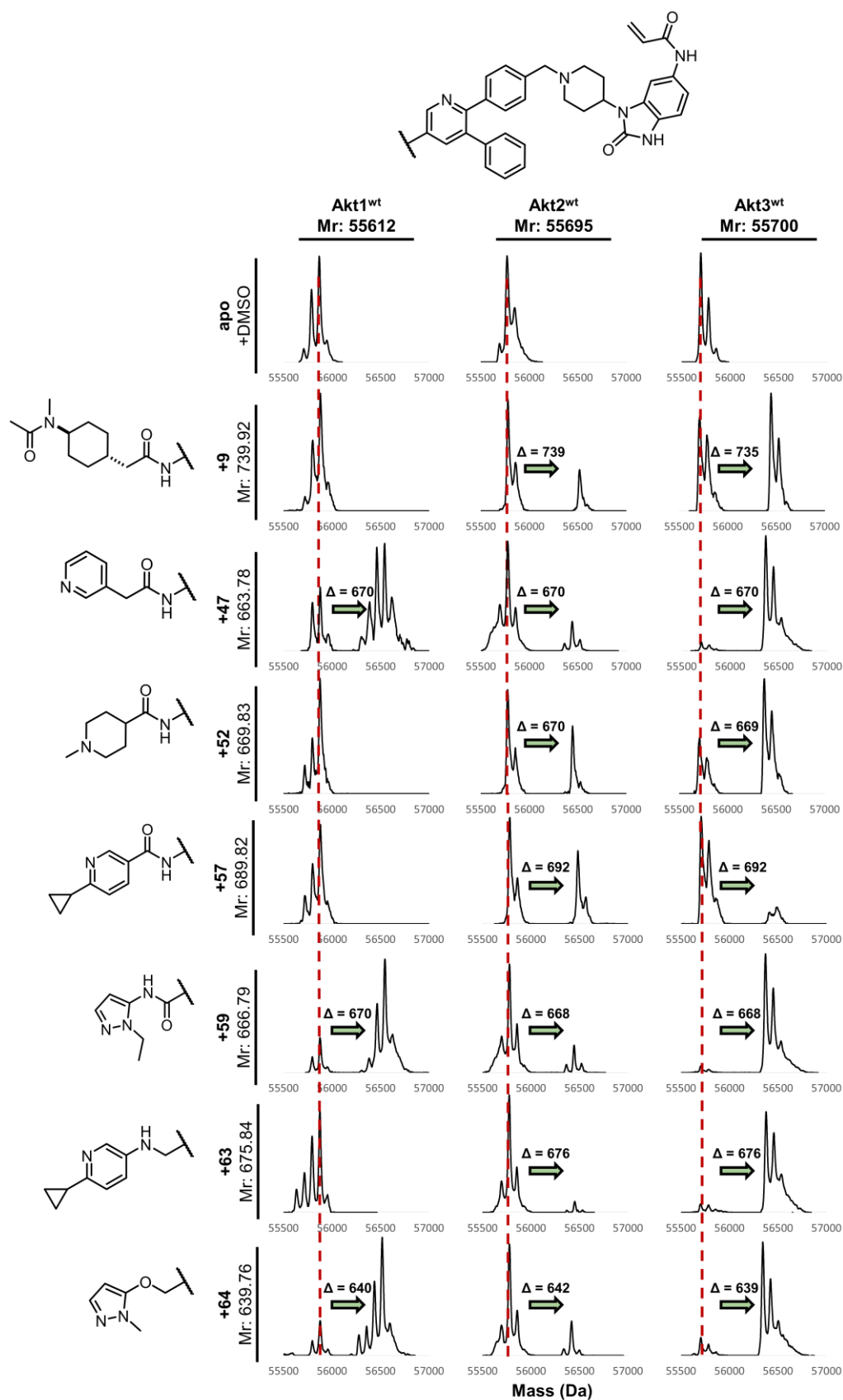


Figure 3: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt} and Akt3^{wt} after 3 h incubation with DMSO (apo) or the newly synthesized isoform-selective CAAIs **9**, **47**, **52**, **57**, **59**, **63** and **64**. All compounds produced mass increases consistent with the addition of a single ligand per protein, indicating mono-covalent modification.

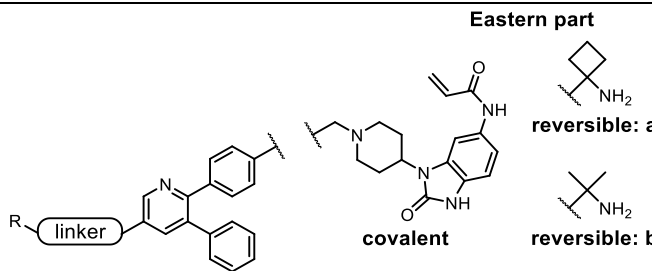
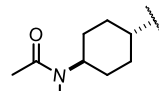
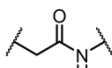
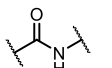
Compounds incorporating distinct linker lengths or degrees of flexibility exhibited different labeling profiles, with some designs showing reduced modification of one isoform while retaining detectable labeling of the others. Collectively, the MS data indicate that differences in labeling efficiency arise from isoform- and compound-dependent effects related to linker architecture, substituent positioning and subtle structural divergences among the Akt isoforms. Having established an initial indication of Akt isoform selectivity, the next step was to biologically evaluate the compound library in a more complex environment.

5.6. Cellular Target-Engagement

Following the biochemical protein-MS experiments, we evaluated their potency and isoform selectivity using the NanoBRET target-engagement assay (Table 1). This method, previously used in the Akt field to resolve isoform-specific inhibitor profiles, enabled the determination of EC₅₀ values for each compound against full-length Akt1, Akt2 and Akt3 in HEK293 cells.^{10, 17} Under the conditions of this assay, compounds **8** and **9** demonstrated robust and selective Akt3 targeting, with hybrid compound **9** exhibiting higher overall activity than its reversible analog **8** across all Akt isoforms. Although both compounds maintained a similar Akt2/Akt3 selectivity ratio, compound **9** exhibited reduced Akt3-over-Akt1 selectivity relative to **8**. These observations should be interpreted in light of the inherent mechanistic differences between reversible and covalent inhibitors, particularly the time-dependent nature of covalent bond formation and the contribution of local protein microenvironment to warhead reactivity. Among the amide-linked derivatives derived from compound **8**, a series of pyridine-containing isoform-selective residues were synthesized to investigate whether the position of the pyridine nitrogen influences Akt isoform selectivity.¹⁰ The pyridine residues bearing a methylene amide linker (**46-48**) were less potent on Akt1 and Akt2 compared to the direct amide linker (**50, 51**), but maintained or improved potency on Akt3, suggesting that deeper pocket engagement enhances Akt3 selectivity. In contrast, aliphatic tertiary amines were also investigated, such as compounds **49** and **52**, which showed significantly reduced activity on all isoforms. Among the simpler methyl and isopropyl amides (**53** and **54**), compound **54** exhibited significantly reduced Akt1 engagement compared to compound **53**, while showing approximately threefold increased potency towards Akt2. This observation suggests that even subtle changes in substituent size

can affect the steric compatibility of the allosteric pocket, resulting in distinct isoform-selective profiles.

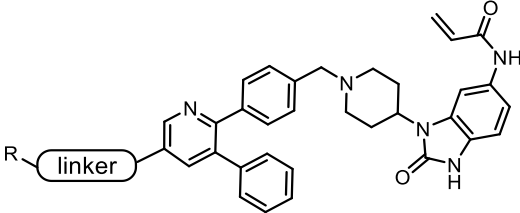
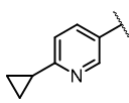
Table 1: Cellular evaluation of newly synthesized covalent-allosteric Akt inhibitors, together with the reversible reference compounds **8** and **22**, focusing on the amide linker connecting the western fragment to the central scaffold. EC₅₀ values (mean ± s.d.) were determined by NanoBRET target-engagement assays in HEK293 cells, with each value derived from duplicate measurements per plate and validated

 Eastern part reversible: a reversible: b covalent				NanoBRET EC₅₀ (nM) HEK293 cell line		
#	R	linker	EP	Akt1	Akt2	Akt3
3	miransertib			1 ± 0	6 ± 4	19 ± 6
4	borussertib			14 ± 4	25 ± 13	36 ± 25
9			cov	1184 ± 331	353 ± 191	35 ± 23
8			rev: a	10331 ± 973	1316 ± 414	90 ± 20
22			rev: b	20000	7176 ± 254	453 ± 81
46			cov	430 ± 110	427 ± 201	123 ± 26
47			cov	907 ± 163	435 ± 193	175 ± 54
48			cov	644 ± 163	314 ± 152	95 ± 11
49			cov	3252 ± 513	2444 ± 1062	1692 ± 956
50			cov	271 ± 21	230 ± 107	227 ± 120
51			cov	172 ± 25	79 ± 46	127 ± 29
52			cov	6188 ± 884	2764 ± 1536	1510 ± 316
53			cov	697 ± 75	697 ± 75	304 ± 99
54			cov	1922 ± 368	209 ± 115	426 ± 155

across n = 3 independent biological experiments.

Building on prior SAR, we incorporated the previously reported Akt2-selective 1,5-substituted pyrazoles and the Akt3-favoring cyclopropyl-pyridine motif, while systematically replacing the native methylene linker with two amide orientations.¹⁰ Also, aminomethylene and ether-methylene variants were also introduced to the new inhibitors to evaluate how linker geometry, electronics and flexibility influence isoform-selective inhibition. Concerning the evaluation of the new inhibitors bearing the 1,5-pyrazole as the isoform-selective residue, the amide-linked analog **55** showed comparable potency for Akt1 and Akt2 but was approximately 2-fold less selective for Akt3, whereas the inverse-amide analog **58** showed altered selectivity with little overall difference in potency between isoforms (Table 2). The methylene-amine linker in compound **61** shifted selectivity toward Akt3, whereas the methylene-ether linker in compound **64** completely abolished isoform selectivity and increased potency to the low nanomolar range across all isoforms, underscoring the sensitivity of isoform preference to linker's orientation or flexibility. In the ethyl-substituted pyrazole series, the amide linker in compound **56** was less potent than its ethyl analog **55**, while the inverse amide **59** improved potency. The methylene-amine linker in compound **62** again favored Akt3, mirroring the trend observed for compound **61**. Compound **65**, bearing a methylene ether linker, behaved similarly to compound **64**, losing selectivity and showing uniformly increased potency. Inspired by the previously reported Akt3-selective inhibitor **68**, replacing the methylene linker with an amide in compound **67** resulted in a slight shift toward Akt2 selectivity, whereas the inverse amide **60** favored Akt1 with sub-100 nM potency but lost isoform discrimination. Removal of the carbonyl group between compounds **60** and **63** restored both potency and Akt3 selectivity, increasing the selectivity for Akt3 over Akt1, highlighting the effect of the linker composition on the biological profile of the final inhibitors.

Table 2. Cellular NanoBRET evaluation of the newly synthesized covalent-allosteric Akt inhibitors in HEK293 cells, highlighting how linker variation influences isoform selectivity while maintaining the same western substituent. Previously reported compounds **66**, **67** and **68** were included for comparative analysis. EC₅₀ values (mean $\pm$ s.d.) were derived from duplicate measurements per plate and represent n = 3 independent biological experiments.

			NanoBRET EC₅₀ (nM) HEK293 cell line		
#	R	linker	Akt1	Akt2	Akt3
66			111 $\pm$ 37	17 $\pm$ 8	12 $\pm$ 7
55			60 $\pm$ 27	67 $\pm$ 34	126 $\pm$ 51
58			105 $\pm$ 18	86 $\pm$ 44	76 $\pm$ 35
61			132 $\pm$ 51	55 $\pm$ 28	24 $\pm$ 8
64			20 $\pm$ 5	14 $\pm$ 8	9 $\pm$ 3
67			215 $\pm$ 68	24 $\pm$ 14	12 $\pm$ 2
56			217 $\pm$ 28	109 $\pm$ 61	258 $\pm$ 125
59			125 $\pm$ 10	76 $\pm$ 42	42 $\pm$ 19
62			156 $\pm$ 26	39 $\pm$ 19	22 $\pm$ 7
65			15 $\pm$ 5	13 $\pm$ 8	6 $\pm$ 2
68			792 $\pm$ 430	125 $\pm$ 97	23 $\pm$ 6
57			124 $\pm$ 39	45 $\pm$ 22	292 $\pm$ 152
60			68 $\pm$ 10	56 $\pm$ 32	138 $\pm$ 24
63			480 $\pm$ 120	95 $\pm$ 78	40 $\pm$ 9

5.7. Crystallization of New Isoform-Selective CAAs In Akt1-Mutant

To evaluate the structural consequences of linker modification, co-crystal structures were determined using an engineered Akt1 construct incorporating Akt2-specific substitutions (Akt2-mimicry) within the allosteric pocket (S205T, D262E, E267del, K268R and N269D).¹⁰ Because crystallization conditions for Akt3 could not be established, this construct served as the closest structurally tractable surrogate to interrogate isoform-relevant interactions. Despite the introduction of an additional heteroatom in the ether- and amine-linked analogs relative to the previous methylene-linked series, the overall architecture of the allosteric pocket is preserved, with only localized rearrangements required to accommodate the modified linkers (Figure 4). The inhibitors adopt binding poses closely resembling those observed for the earlier series, maintaining the orientation required for covalent modification of Cys296 and preserving the key interactions of the central scaffold. Analysis of atomic displacement parameters reveals that, in both ether- and amine-linked structures, the pyrazole substituent exhibits substantially higher B-factors compared to the core scaffold, indicating increased positional disorder of this moiety within the structures. Consistent with this observation, the methyl-pyrazole substituent is not fully resolved in the corresponding Fo-Fc electron density map (Figure S61), further supporting enhanced local flexibility of this region. In contrast, the central scaffold remains well defined. A distinguishing feature of the new linker series is the presence of a water molecule coordinating the linker heteroatom, an interaction absent in the methylene-linked compounds published previously.¹⁰ Although this water molecule displays elevated B-factors relative to the surrounding solvent network, it may contribute to stabilization of the linker region through transient hydrogen-bonding interactions. The incorporation of this water-mediated contact, together with the increased positional disorder of the pyrazole substituent, suggests that linker modification subtly reshapes the microenvironment of the allosteric pocket while preserving the overall ligand pose. Collectively, these structural data indicate that the additional linker heteroatom is accommodated through localized ligand flexibility and solvent reorganization, while preserving the conserved covalent binding pose (Figure S62). This structural tolerance underscores the adaptability of the allosteric pocket but also reveals that subtle linker-dependent changes in local dynamics can influence isoform selectivity. Notably, the increased positional disorder observed for the ether-linked series may reduce the

energetic discrimination between closely related isoform-specific pocket environments, consistent with the loss of isoform selectivity observed in the cellular NanoBRET assay.

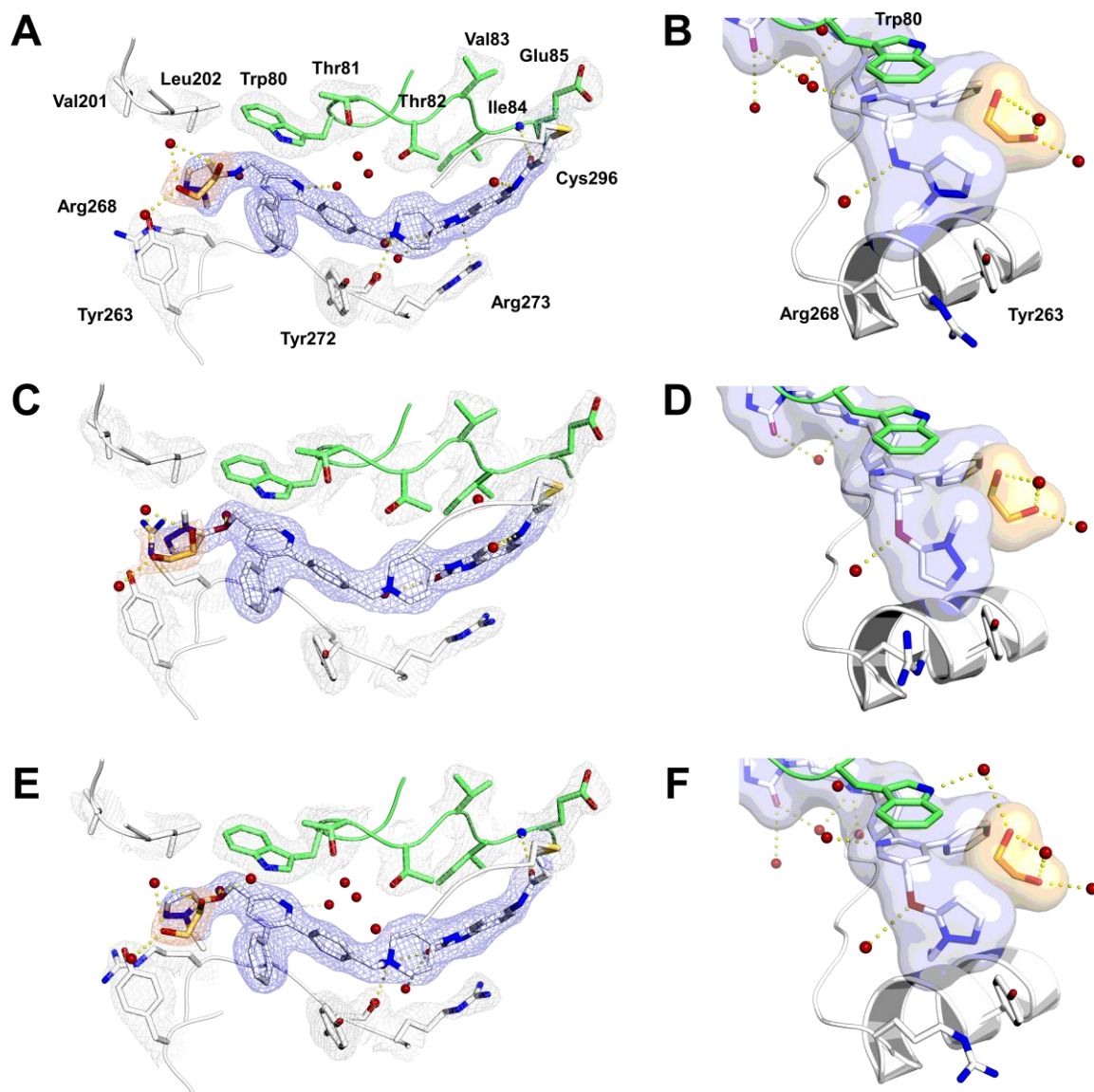


Figure 4: Co-crystal structures of Akt2-mimicry construct with: (A) compound **62** (PDB: 29BK), (C) compound **64** (PDB: 29BL) and (E) compound **65** (PDB: 29BM). The orientation of the substituted pyrazole with the amino and ether-containing linkers is depicted with a close-up view in panels B, D, and F, respectively. Amino acid labels are displayed in panels A and B but not shown in panels C-F for clarity. The $|2F_o - F_c|$ -electron density map is shown at r.m.s.d. = 1.0.

5.8. Conclusions

In this work, we designed and synthesized covalent-allosteric Akt inhibitors using an isoform-directed strategy focusing on Akt3, a kinase critically involved in metastasis and neurodevelopmental disorders. This approach identified the covalent hybrid compound **9**, which showed enhanced potency for Akt3 while maintaining selectivity over Akt1 and Akt2. The reported SAR revealed that Akt isoform-selectivity is highly sensitive to the nature of the isoform-selective residues and their substituents, as well as the geometry and composition of the linker connecting them to the scaffold. Specific combinations, such as cyclopropylpyridine motifs or 1,5-substituted pyrazoles as isoform-selective motifs with an amide or aminomethylene linkage to the main scaffold, can enhance Akt3 selectivity, whereas alternative linker designs, such as methylene ether variants, often abolish the isoform preference. These findings highlight that careful tuning of both the selective residues and their connecting linkers is critical for achieving potent and isoform-biased covalent-allosteric Akt inhibition. The compounds successfully labeled the Akt isoforms and structural data revealed that the Akt2 mimicking pocket preferentially accommodates aminomethylene and methylene-ether linkers, providing guidance for further compound optimization. These findings advance the understanding of the structural determinants of Akt isoform selectivity and provide a foundation for next-generation Akt2- and Akt3-targeted inhibitors with potential applications in cancer metastasis and Akt3-related neurological disorders.

5.9. Experimental Section

5.9.1. Synthetic Procedures

Safety statement: No unexpected or unusually high safety hazards have been encountered. All reagents and solvents were used as received from various suppliers, including ABCR, Activate Scientific, Alfa Aesar, BLD Pharm, Merck, Sigma-Aldrich, or VWR and were used without further purification for the synthesis. Dry solvents were purchased as anhydrous reagents from commercial suppliers. Air- and/or humidity-sensitive reactions were performed in heated glassware and under inert gas, using argon atmosphere. Reactions were monitored by TLC and LC-MS. Analytical TLC was performed on Merck 60 F254 aluminum-backed silica gel plates and visualized under UV light ($\lambda = 254$ and 366 nm). Compounds were purified by column chromatography using VWR silica gel ($40 - 63 \mu\text{m}$ particle size) or flash chromatography on a Biotage Isolera One using Büchi Reveleris Silica Cartridges ($12 - 40$ g) monitored by UV at $\lambda = 210$ nm and 280 nm. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance AV500 (500 MHz and 126 MHz), AV600 (600 MHz and 151 MHz) and AV700 (700 MHz and 176 MHz). ^1H chemical shifts are reported in λ (ppm) as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), dt (doublet of triplets), td (triplet of doublets), tt (triplet of triplets), q (quartet), hept (heptet), m (multiplet) and are referenced to the residual solvent signal: DMSO- d_6 (2.50). ^{13}C spectra are referenced to residual solvent signal: DMSO- d_6 (39.52). High-resolution electrospray ionization mass spectra (ESI-FTMS) were recorded on a Thermo LTQ Orbitrap (high-resolution mass spectrometer from Thermo Electron) coupled to an Accela HPLC system supplied with a Hypersil GOLD column (Thermo Electron). LC-MS (ESI-MS) analysis was performed using Agilent HPLC system (1200 series) with CC 125/4 Nucleodur C18 gravity column ($3 \mu\text{m}$) from Macherey Nagel coupled to an AGILENT G6160A InfinityLab iQ LC/MSD Single Pole Mass Spectrometer. Compounds were purified by flash chromatography on a Biotage Isolera One Silica Cartridges ($12 - 40$ g) monitored by UV at $\lambda = 210$ nm and 254 nm. All final compounds were purified to $> 95\%$ purity as determined by LC-MS and NMR analysis. NMR spectra and LC-MS traces for all the compounds are provided in the SI.

5.9.1.1. General Procedure A: Suzuki-Miyaura Reaction

The respective chlorides (1.0 eq) and boronic esters ($1.2 - 1.5$ eq) were dissolved in 1,4-dioxane/ H_2O ($5:1$) under an argon atmosphere. The mixture was degassed for 15 min and then K_2CO_3 (2.0 eq) and $\text{Pd}(\text{PPh}_3)_4$ (0.1 eq) were added.

Subsequently, the reaction mixture was heated up to 130 °C for 3 h in a microwave. The cooled reaction mixture was filtered over Celite and extracted with DCM and H₂O. The combined organic fractions were dried over Na₂SO₄ and concentrated in vacuo. The crude product was absorbed onto silica gel and purified by flash column chromatography (C18, 20 - 50 % H₂O/MeCN + 0.2 % TFA) and HPLC to give the desired product.

5.9.1.2. General Procedure B: Amide Coupling Using EDC

The corresponding acid (1.2 eq) was dissolved in dry THF (1-3 mL). Afterwards, EDC (1.2 eq), DMAP (1.2 eq) and HOBt (0.1 eq) were added. The mixture was stirred for 30 min. Then, the amine **11** (1.0 eq) was added to the above-prepared solution. The reaction mixture was stirred at 60 °C for 16-20 h. After the completion of the reaction, which was monitored via TLC and MS, the reaction mixture was concentrated in vacuo. The crude product was dissolved in DCM and washed with NaHCO₃ solution. The combined organic fractions were dried over Na₂SO₄, filtered and concentrated in vacuo. The crude product was absorbed onto silica gel and purified by flash column chromatography (8-10% MeOH/DCM), affording the desired product.

5.9.1.3. General Procedure C: Amide Coupling Using HATU

The acid **36** (1.0 eq) was suspended in MeCN (0.5 mL) and then DIPEA (3 eq) and HATU (1.5 eq) were added and stirred at rt for 10 minutes. Afterwards, the corresponding amine (1.3 eq) was added slowly in a prepared solution of MeCN (0.5 mL). After 3 h, the reaction mixture was extracted with DCM, brine and H₂O. The combined organic fractions were dried over Na₂SO₄, filtered and concentrated in vacuo. The crude product was absorbed onto silica gel and purified by flash column chromatography (4 - 8 % MeOH/DCM), affording the desired product.

5.9.1.4. General Procedure D: Amide Formation via Nucleophilic Addition-Elimination Reaction

The amine **11** (1.0 eq) was dissolved in dry solvent (1-3 mL) under argon atmosphere. Afterwards, DIPEA (2.0 eq) was added and the mixture was allowed to stir for 10 min. Then the corresponding halide (1.2 eq) was added to the above prepared solution. The reaction mixture was stirred for 2 h at rt. Afterwards, the crude product was dissolved

in EtOAc and washed with NaHCO₃ solution. The combined organic fractions were dried over Na₂SO₄, filtered and concentrated in vacuo to yield the desired product.

5.9.1.5. General Procedure E: Nucleophilic Substitution at the Benzylic Position

The corresponding amine/alcohol (1.1 eq) and K₂CO₃ (2-3 eq) were first dissolved in DMF (2 mL) and then the bromide **40** (1.0 eq) was added. The suspension was left stirring at rt for 16-18 h. After the completion of the reaction, the solution was extracted with DCM. The combined organic fractions were dried over Na₂SO₄, filtered and concentrated in vacuo. The combined organic fractions were dried over Na₂SO₄, filtered and concentrated in vacuo. The crude product was absorbed onto silica gel and purified by flash column chromatography (8-10% MeOH/DCM), affording the desired product.

5.9.2. Synthesis

Synthesis of 6-chloro-5-phenylpyridin-3-amine (**11**)

The commercially available 3-bromo-4-chloroaniline (**10**) (500.0 mg, 2.4 mmol, 1.0 eq) and phenylboronic acid (323.3 mg, 2.7 mmol, 1.1 eq) were dissolved in 1,4-dioxane/H₂O (5:1, 2 mL) under an argon atmosphere in a sealed vessel. The mixture was degassed for 15 min and then K₃PO₄ (1110.0 mg, 4.8 mmol, 2.0 eq) and Pd(dppf)Cl₂ (176.3 mg, 0.2 mmol, 0.1 eq) were added. Subsequently, the reaction mixture was heated up to 120 °C for 3 h in a microwave. The cooled reaction mixture was filtered over Celite and extracted with DCM and H₂O. The combined organic fractions were dried over Na₂SO₄ and concentrated in vacuo. The crude product was absorbed onto silica gel and purified by flash column chromatography (8-10% MeOH/DCM) to give the desired product **11** as a light-brown solid (386.2 mg, 78%). **¹H NMR** (700 MHz, DMSO-*d*₆) δ 7.73 (d, *J* = 2.9 Hz, 1H), 7.48 - 7.43 (m, 2H), 7.42 - 7.39 (m, 3H), 6.97 (d, *J* = 2.9 Hz, 1H), 5.58 (s, 2H). **¹³C NMR** (176 MHz, DMSO-*d*₆) δ 144.75, 137.87, 135.71, 134.17, 134.11, 128.97, 128.25, 127.94, 123.97. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 205.05; found: 205.0.

Synthesis of ethyl 2-((1*r*,4*r*)-4-acetamidocyclohexyl)acetate (**13**)

The commercially available ethyl 2-((1*r*,4*r*)-4-aminocyclohexyl)acetate (**12**) (1000.0 mg, 4.5 mmol, 1.0 eq) was dissolved in dry DCM (5 mL) under inert conditions and Et₃N (2.51 mL, 18.0 mmol, 4.0 eq) was added. Then, prediluted acetyl chloride (0.39 mL, 5.4 mmol, 1.2 eq) was slowly added. The reaction mixture was allowed to stir overnight at rt. After the completion of the reaction, which was monitored via TLC and MS, the reaction mixture was diluted with NaHCO₃ solution and extracted with DCM. The combined organic fractions were dried over Na₂SO₄, filtered and concentrated in vacuo. The crude product was absorbed onto silica gel and purified by reverse flash column chromatography (C18, 55 -60 % H₂O/MeCN + 0.2 % TFA) to yield the desired product **13** as a white solid. (871.0 mg, 84 %). ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.67 (d, *J* = 7.8 Hz, 1H), 4.04 (q, *J* = 7.1 Hz, 2H), 3.47 - 3.37 (m, 1H), 2.16 (d, *J* = 7.0 Hz, 2H), 1.81 - 1.71 (m, 5H), 1.67 (d, *J* = 12.3 Hz, 2H), 1.59 (ttt, *J* = 10.6, 7.0, 3.5 Hz, 1H), 1.17 (t, *J* = 7.1 Hz, 3H), 1.11 (td, *J* = 12.7, 3.1 Hz, 2H), 1.02 (td, *J* = 12.4, 3.1 Hz, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 172.07, 168.06, 59.63, 47.39, 40.75, 33.74, 32.09, 31.01, 22.75, 14.15. LC-MS (ESI): *m/z* calculated for [M+H]⁺: 228.15; found: 228.1.

Synthesis of 2-((1*r*,4*r*)-4-(*N*-methylacetamido)cyclohexyl)acetic acid (**14**)

Compound **13** (400.0 mg, 1.8 mmol, 1.0 eq) and NaH (351.9 mg, 8.8 mmol, 5.0 eq) were dissolved in dry THF (3 mL) before the mixture was cooled down to 0 °C and prediluted CH₃I (0.66 mL, 10.6 mmol, 6.0 eq) in THF was added. The reaction mixture was allowed to reach rt and left to stir overnight. When the starting material was completely consumed (TLC monitoring), aqueous NaOH (3 mL) was added and the mixture was stirred overnight at 40 °C. After completion, the reaction mixture was monitored by TLC and LC-MS, then extracted with DCM. The combined organic fractions were dried over Na₂SO₄, filtered and concentrated in vacuo. The crude product was absorbed onto silica gel and purified by reverse flash column chromatography (C18, 20 -25 % H₂O/MeCN + 0.2 % TFA) to yield the desired product **14** as an orange solid (341.1 mg, 91 %). ¹H NMR (600 MHz, DMSO-*d*₆) δ 4.19 (tt, *J* = 10.6, 6.3 Hz, conformer 1) and 3.51 (tt, *J* = 11.4, 4.2 Hz, conformer 2) for total of 1H, 2.77 (s, major) and 2.65 (s, minor) for total of 3H, 2.08 (dd, *J* = 7.0, 3.3 Hz, 2H), 1.99 (s, minor) and 1.95 (s, major) for total of 3H, 1.78 - 1.71 (m, 2H), 1.64 - 1.50 (m, 3H), 1.49 - 1.40 (m, 2H), 1.16 - 0.98 (m, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 169.19,

168.98, 56.09, 51.07, 48.58, 41.11, 33.44, 33.25, 31.43, 31.28, 29.85, 29.55, 28.59, 26.39, 22.33, 21.52. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 214.14; found: 214.1.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)-2-((1*r*,4*r*)-4-(N-methylacetamido)cyclohexyl) acetamide (15)

Compound **14** (69.5 mg, 0.3 mmol, 1.0 eq) and Et₃N (0.1 mL, 1.0 mmol, 3.0 eq) were dissolved in MeCN (1 mL) and T3P (0.2 mL, 0.7 mmol, 50 % w/w in MeCN, 2.0 eq) was added. The solution was left stirring for 10 minutes and then compound **11** (80.0 mg, 0.4 mmol, 1.2 eq) was added. The reaction mixture was left stirring at 60 °C. After 3 h, no further conversion of the starting material was observed via TLC and MS and the reaction mixture was diluted with NaHCO₃ solution and extracted with DCM. The combined organic fractions were dried over Na₂SO₄, filtered and concentrated in vacuo. The crude product was absorbed onto silica gel and purified by flash column chromatography (21 - 23 % MeOH/DCM) to yield the desired product **15** as an orange solid (24.1 mg, 18 %). **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.39 (s, 1H), 8.60 (d, *J* = 2.6 Hz, 1H), 8.13 (t, *J* = 2.3 Hz, 1H), 7.54 - 7.42 (m, 5H), 4.26 - 4.18 (m, conformer 1) and 3.53 (tt, *J* = 11.4, 4.0 Hz, conformer 2) for total of 1H, 2.77 (s, major) and 2.65 (s, minor) for total of 3H, 2.30 - 2.23 (m, 2H), 2.00 (s, minor) and 1.95 (s, major) for total of 3H, 1.81 - 1.68 (m, 3H), 1.64 - 1.51 (m, 2H), 1.50 - 1.42 (m, 2H), 1.20 - 1.02 (m, 2H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 171.33, 171.27, 169.27, 169.05, 141.37, 138.94, 137.07, 135.92, 135.69, 129.77, 129.11, 128.52, 128.48, 56.13, 51.11, 43.62, 43.54, 33.94, 33.74, 31.56, 31.39, 29.89, 29.61, 28.65, 26.46, 22.39, 21.59. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 400.17; found: 400.2.

Synthesis of N-(2-oxo-3-(1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (16) has been reported.¹⁰

Synthesis of N-(3-(1-(4-(5-(2-((1*r*,4*r*)-4-(N-methylacetamido)cyclohexyl)acetamido)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (9)

Compound **15** (22.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (41.5 mg, 0.1 mmol, 1.5 eq), K₂CO₃ (15.2 mg, 0.1 mmol, 2.0 eq) and Pd(PPh₃)₄ (6.4 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **9** (8.7 mg, 21 %)

as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.94 (d, *J* = 34.3 Hz, 1H), 10.69 (d, 1H), 10.39 (d, *J* = 51.8 Hz, 1H), 8.91 (ddd, *J* = 20.2, 9.9, 2.4 Hz, 1H), 8.44 (s, 1H), 8.17 (ddd, *J* = 13.8, 6.0, 2.4 Hz, 1H), 7.80 - 7.71 (m, 1H), 7.53 - 7.37 (m, 1H), 7.34 - 7.26 (m, 4H), 7.24 - 7.13 (m, 4H), 6.92 (t, *J* = 8.6 Hz, 1H), 6.54 - 6.47 (m, 1H), 6.23 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.70 (dd, *J* = 10.6, 2.0 Hz, 1H), 4.22 (ddd, *J* = 15.9, 9.4, 7.3 Hz, 1H), 4.09 (tt, *J* = 12.4, 4.2 Hz, 1H), 3.62 - 3.47 (m, 4H), 2.93 (d, *J* = 11.1 Hz, 2H), 2.78 (s, major) and 2.66 (s, minor) for total of 3H, 2.34 - 2.24 (m, 3H), 2.06 (t, *J* = 10.8 Hz, 1H), 2.01 (s, minor) and 1.95 (s, major) for total of 3H, 1.83 - 1.71 (m, 3H), 1.68 - 1.52 (m, 4H), 1.49 - 1.42 (m, 2H), 1.21 - 1.07 (m, 2H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 171.30, 171.26, 169.21, 169.01, 166.28, 162.82, 154.00, 150.41, 139.68, 139.18, 138.48, 137.43, 135.36, 135.14, 134.79, 132.76, 132.20, 129.42, 129.18, 128.45, 128.40, 128.17, 127.99, 127.35, 126.08, 124.47, 112.12, 108.61, 100.87, 61.89, 61.51, 56.15, 52.62, 51.15, 50.16, 48.56, 43.56, 43.45, 34.05, 33.87, 31.55, 31.37, 29.86, 29.63, 28.65, 26.39, 22.92, 22.33, 21.52. **¹H NMR** (700 MHz, Methanol-*d*₄) δ 8.85 (ddd, *J* = 16.7, 3.8, 2.4 Hz, 1H), 8.54 (s, 1H), 8.16 (ddd, *J* = 8.6, 4.8, 2.4 Hz, 1H), 7.73 (s, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.45 - 7.36 (m, 1H), 7.32 - 7.28 (m, 3H), 7.28 - 7.24 (m, 2H), 7.19 (ddd, *J* = 9.8, 6.7, 3.2 Hz, 2H), 7.02 (t, *J* = 8.5 Hz, 1H), 6.44 (td, *J* = 16.9, 10.1 Hz, 1H), 6.36 (ddd, *J* = 17.0, 13.3, 1.7 Hz, 1H), 5.76 (ddd, *J* = 12.1, 10.1, 1.8 Hz, 1H), 4.35 (tt, *J* = 11.8, 4.0 Hz, 1H), 4.29 (tt, *J* = 12.4, 4.2 Hz, conformer 1) and 3.61 - 3.51 (m, conformer 2) for total of 1H, 3.68 - 3.66 (m, 2H), 3.18 - 3.09 (m, 2H), 2.91 (s, major) and 2.80 (s, minor) for total of 3H, 2.54 (qd, *J* = 12.7, 3.9 Hz, 2H), 2.41 - 2.29 (m, 4H), 2.13 (s, minor) and 2.08 (s, major) for total of 3H, 1.99 - 1.83 (m, 3H), 1.80 - 1.68 (m, 4H), 1.67 - 1.55 (m, 4H), 1.23 (m, *J* = 12.4, 3.8 Hz, 2H). **¹³C NMR** (176 MHz, Methanol-*d*₄) δ 174.18, 174.09, 173.09, 172.82, 170.29, 166.06, 166.01, 156.56, 156.38, 153.25, 140.64, 140.54, 140.38, 140.27, 138.23, 138.14, 136.16, 133.71, 133.67, 133.64, 132.51, 131.03, 130.76, 130.65, 130.40, 129.56, 129.45, 128.67, 127.56, 126.59, 115.52, 110.39, 103.99, 63.98, 63.01, 58.72, 53.93, 53.87, 51.98, 45.06, 44.92, 35.85, 35.69, 33.02, 32.86, 30.97, 30.87, 30.06, 27.58, 24.53, 23.73, 22.32, 21.42. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 740.3846; found: 740.3913.

Synthesis of 2-chloro-5-nitro-3-phenylpyridine (18)

The commercially available 2-chloro-3-iodo-5-nitropyridine (**17**) (150.0 mg, 0.5 mmol, 1.0 eq) and phenylboronic acid (70.7 mg, 0.6 mmol, 1.1 eq) were dissolved in 1,4-dioxane/ H₂O (5:1, 2 mL) under an argon atmosphere in a sealed vessel. The mixture

was degassed for 15 min and then K₃PO₄ (242.9 mg, 1.1 mmol, 2.0) and Pd(dppf)Cl₂ (38.6, 0.1 mmol, 0.1 eq) were added. Subsequently, the reaction mixture was heated up to 120 °C for 3 h in a microwave. The cooled reaction mixture was filtered over Celite and extracted with DCM and H₂O. The combined organic fractions were dried over Na₂SO₄ and concentrated in vacuo. The crude product was absorbed onto silica gel and purified by flash column chromatography (8-10% MeOH/DCM) to give the desired product **18** as a white solid (113.4 mg, 92%). **¹H NMR** (600 MHz, DMSO-*d*₆) δ 9.26 (d, *J* = 2.7 Hz, 1H), 8.57 (d, *J* = 2.7 Hz, 1H), 7.61 - 7.49 (m, 5H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 153.55, 143.80, 143.71, 136.78, 135.17, 134.76, 129.31, 129.17, 128.63. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 235.02; found: 234.9.

Synthesis of tert-butyl (1-(4-(5-nitro-3-phenylpyridin-2-yl)phenyl)cyclobutyl)carbamate (19a)

Compound **18** (29.3 mg, 0.1 mmol, 1.0 eq) was reacted with tert-butyl (1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)cyclobutyl)carbamate (55.6 mg, 0.1 mmol, 1.2 eq), K₂CO₃ (34.3 mg, 0.2 mmol, 2.0 eq) and Pd(PPh₃)₄ (14.4 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **19a** (44.6 mg, 80 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 9.44 (d, *J* = 2.5 Hz, 1H), 8.49 (d, *J* = 2.5 Hz, 1H), 7.59 (s, 1H), 7.37 - 7.26 (m, 9H), 2.36 - 2.26 (m, 4H), 1.98 (s, 1H), 1.76 (s, 1H), 1.32 (s, 9H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 142.99, 142.71, 137.65, 135.87, 135.74, 133.40, 129.45, 129.30, 128.66, 128.15, 124.83, 77.58, 58.17, 33.56, 28.25, 15.19. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 446.20; found: 446.2.

Synthesis of tert-butyl (2-(4-(5-nitro-3-phenylpyridin-2-yl)phenyl)propan-2-yl)carbamate (19b)

Compound **18** (60 mg, 0.3 mmol, 1.0 eq) was reacted with tert-butyl (2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propan-2-yl)carbamate (110.9 mg, 0.3 mmol, 1.2 eq), K₂CO₃ (70.7 mg, 0.5 mmol, 2.0 eq) and Pd(PPh₃)₄ (29.5 mg, 0.03 mmol, 0.1 eq) using the general procedure A to get the final product **19b** (95.7 mg, 86 %) as a white solid. **¹H NMR** (700 MHz, DMSO-*d*₆) δ 9.43 (d, *J* = 2.5 Hz, 1H), 8.47 (d, *J* = 2.5 Hz, 1H), 7.56 (dd, *J* = 7.8, 4.3 Hz, 1H), 7.48 - 7.02 (m, 9H), 1.51 (s, 3H), 1.45 (s, 3H), 1.33 (d, 9H). **¹³C NMR** (176 MHz, DMSO-*d*₆) δ 161.28, 142.99, 142.67, 135.71, 135.66, 133.43, 129.49, 129.32, 129.29, 128.80, 128.69, 128.44, 128.43, 128.17, 125.99,

125.30, 124.44, 77.36, 54.13, 54.07, 29.65, 29.41, 28.26. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 434.20; found: 434.2.

Synthesis of tert-butyl (1-(4-(5-amino-3-phenylpyridin-2-yl)phenyl)cyclobutyl)carbamate (20a)

Compound **19a** (45.0 mg, 0.1 mmol, 1.0 eq), Fe powder (56.4 mg, 1.0 mmol, 10.0 eq) and NH_4Cl (8.1 mg, 0.2 mmol, 1.5 eq) were added to a mixture of IPA and H_2O (10:1, 0.5 mL) and left stirring and heated under reflux for 3 h. After completion of the reaction, the reaction mixture was filtered and the filter paper was washed with MeOH. The filtrate was concentrated in vacuo to afford the crude product, which was absorbed onto silica gel and purified by flash column chromatography (8 - 10 % MeOH/DCM) to give the product **20a** as a white solid (26.8 mg, 64 %). **^1H NMR** (600 MHz, $\text{DMSO}-d_6$) δ 8.02 (d, J = 2.6 Hz, 1H), 7.26 (s, 3H), 7.16 - 7.06 (m, 7H), 6.89 (d, J = 2.6 Hz, 1H), 5.47 (s, 2H), 2.29 (d, J = 6.7 Hz, 4H), 1.94 (s, 1H), 1.73 (s, 1H), 1.32 (s, 9H). **^{13}C NMR** (151 MHz, $\text{DMSO}-d_6$) δ 143.60, 140.65, 138.16, 135.38, 135.05, 129.06, 128.72, 128.20, 126.88, 124.24, 122.00, 77.41, 58.15, 33.65, 28.25, 15.21. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 416.23; found: 416.3.

Synthesis of tert-butyl (2-(4-(5-amino-3-phenylpyridin-2-yl)phenyl)propan-2-yl)carbamate (20b)

Compound **19b** (71.0 mg, 0.2 mmol, 1.0 eq), Fe powder (91.5 mg, 1.0 mmol, 10.0 eq) and NH_4Cl (13.1 mg, 0.2 mmol, 1.5 eq) were added in a mixture of isopropyl alcohol and H_2O (10:1, 1 mL) and left stirring and heated under reflux for 3 h. After completion of the reaction, the reaction mixture was filtered and the filter paper was washed with MeOH. The filtrate was concentrated in vacuo to afford the crude product was absorbed onto silica gel and purified by flash column chromatography (8 - 10 % MeOH/DCM) to give the product **20b** as a white solid (48.3 mg, 73 %). **^1H NMR** (600 MHz, $\text{DMSO}-d_6$) δ 8.02 (d, J = 2.6 Hz, 1H), 7.30 - 7.22 (m, 3H), 7.18 - 7.12 (m, 2H), 7.11 - 7.06 (m, 4H), 6.89 (d, J = 2.6 Hz, 1H), 5.47 (s, 2H), 1.43 (s, 6H), 1.32 (d, 9H). **^{13}C NMR** (151 MHz, $\text{DMSO}-d_6$) δ 143.63, 143.55, 140.69, 137.91, 135.36, 135.05, 129.05, 128.73, 128.22, 126.88, 123.80, 122.07, 77.24, 54.02, 29.59, 28.26. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 404.23; found: 404.3.

Synthesis of tert-butyl (1-(4-(5-(2-((1*r*,4*r*)-4-(*N*-methylacetamido)cyclohexyl)acetamido)-3-phenylpyridin-2-yl)phenyl)cyclobutyl)carbamate (21a)

Compound **15** (10.69 mg, 0.05 mmol, 1.0 eq) and Et₃N (0.02 mL, 0.15 mmol, 3.0 eq) were dissolved in MeCN (1 mL) and T3P (0.05 mL, 0.17 mmol, 50 % w/w in MeCN, 3.3 eq) was added. The solution was left stirring for 10 minutes and then compound **19a** (25.0 mg, 0.06 mmol, 1.2 eq) was added. The reaction mixture was left stirring at 60 °C. After 3 h, the reaction mixture was diluted with NaHCO₃ solution and extracted with DCM. The combined organic fractions were dried over Na₂SO₄, filtered and concentrated in vacuo. The crude product **21a** was directly used in the next step without additional purification. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 611.3519; found: 611.3593.

Synthesis of tert-butyl (2-(4-(5-(2-((1*r*,4*r*)-4-(*N*-methylacetamido)cyclohexyl)acetamido)-3-phenylpyridin-2-yl)phenyl)propan-2-yl)carbamate (21b)

Compound **15** (34.0 mg, 0.2 mmol, 1.0 eq) and Et₃N (0.07 mL, 0.5 mmol, 3.0 eq) were dissolved in MeCN (1 mL) and T3P (0.16 mL, 0.5 mmol, 50 % w/w in MeCN, 3.3 eq) was added. The solution was left stirring for 10 minutes and then compound **19b** (77.2 mg, 0.2 mmol, 1.2 eq) was added. The reaction mixture was left stirring at 60 °C. After 3 h, the reaction mixture was diluted with NaHCO₃ solution and extracted with DCM. The combined organic fractions were dried over Na₂SO₄, filtered and concentrated in vacuo. The crude product **21b** was directly used in the next step without additional purification. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 599.3519; found: 599.3581.

Synthesis of N-(6-(4-(1-aminocyclobutyl)phenyl)-5-phenylpyridin-3-yl)-2-((1*r*,4*r*)-4-(*N*-methylacetamido)cyclohexyl)acetamide (8)

The crude product **21a** was diluted in 1,4-dioxane and then a solution of HCl 4N in dioxane (3 mL) was added. After stirring for 30 minutes, the reaction was completed (TLC monitoring). The crude mixture was extracted with EtOAc (10% MeOH) and H₂O. The combined organic fractions were dried over Na₂SO₄ and concentrated in vacuo. The crude material was purified by reverse flash column chromatography (C18, 20 -30 % H₂O/MeCN + 0.2 % TFA) to afford compound **8** (6.4 mg, 25

% over two steps) as a monovalent TFA-salt (white solid). **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.30 (s, 1H), 8.84 - 8.78 (d, 1H), 8.13 - 8.09 (m, 1H), 7.38 - 7.26 (m, 7H), 7.23 - 7.18 (m, 2H), 4.27 - 4.19 (m, conformer 1), 3.58 - 3.50 (m, conformer 2) for total of 1H, 2.79 (s, major) and 2.66 (s, minor) for total of 3H, 2.43 (ddd, *J* = 12.1, 9.0, 6.1 Hz, 2H), 2.30 - 2.19 (m, 4H), 2.08 - 2.02 (m, 1H), 2.01 (s, minor) and 1.96 (s, major) for total of 3H, 1.90 (s, 1H), 1.85 - 1.44 (m, 7H), 1.21 - 1.03 (m, 2H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 171.22, 171.17, 169.21, 168.99, 150.04, 139.68, 139.18, 138.42, 135.25, 134.64, 129.46, 129.15, 128.50, 128.14, 127.46, 124.89, 58.25, 56.13, 51.12, 43.63, 43.55, 34.72, 34.00, 33.81, 31.55, 31.38, 29.85, 29.61, 28.64, 26.40, 22.33, 22.09, 21.52, 21.18, 13.95, 13.69. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 511.2995; found: 511.3057.

Synthesis of N-(6-(4-(2-aminopropan-2-yl)phenyl)-5-phenylpyridin-3-yl)-2-((1*r*,4*r*)-4-(N-methylacetamido)cyclohexyl)acetamide (22)

The crude product **21b** was diluted in 1,4-dioxane and then a solution of HCl 4N in dioxane (3 mL) was added. After stirring for 30 minutes, the reaction was completed (TLC monitoring). The crude mixture was extracted with EtOAc (10% MeOH) and H₂O. The combined organic fractions were dried over Na₂SO₄ and concentrated in vacuo. The crude material was purified by reverse-phase flash column chromatography (C18, 20-30% H₂O/MeCN + 0.2% TFA) to afford compound **22** (7.9 mg, 10% over two steps) as a monovalent TFA-salt (white solid). **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.28 (s, 1H), 8.80 (s, 1H), 8.10 (s, 1H), 7.39 - 7.30 (m, 5H), 7.27 - 7.23 (m, 2H), 7.22 - 7.19 (m, 2H), 4.32 - 4.17 (m, conformer 1) and 3.62 - 3.49 (m, conformer 2) for total of 1H, 2.79 (s, major) and 2.66 (s, minor) for total of 3H, 2.30 - 2.25 (m, 2H), 2.01 (s, minor) and 1.96 (s, major) for total of 3H, 1.90 (s, 1H), 1.83 - 1.70 (m, 3H), 1.66 - 1.52 (m, 2H), 1.53 - 1.45 (m, 2H), 1.43 (s, 6H), 1.22 - 1.07 (m, 2H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 171.20, 171.14, 169.20, 168.98, 150.05, 139.72, 139.16, 138.04, 135.22, 134.58, 129.29, 129.14, 128.49, 128.15, 127.45, 124.26, 56.12, 53.36, 51.12, 43.63, 43.55, 33.99, 33.81, 31.55, 31.38, 31.28, 30.52, 29.85, 29.61, 29.00, 28.69, 28.63, 26.40, 22.33, 22.09, 21.52. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 499.2995; found: 499.3067.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)-2-(pyridin-2-yl)acetamide (**23**)

The commercially available 2-(pyridin-2-yl)acetic acid (40.2 mg, 0.3 mmol, 1.2 eq), EDC (45.51 mg, 0.29 mmol, 1.2 eq), DMAP (35.8 mg, 0.3 mmol, 1.2 eq) and HOBt (3.3 mg, 0.02 mmol, 0.1 eq) and compound **11** (50.0 mg, 0.2 mmol, 1.0 eq) were reacted utilizing general procedure B to get the title product **23** (15.3 mg, 19 %) as a yellow solid. **¹H NMR** (600 MHz, Methanol-*d*₄) δ 8.61 (d, *J* = 2.6 Hz, 1H), 8.51 (dt, *J* = 5.0, 1.4 Hz, 1H), 8.13 (d, *J* = 2.6 Hz, 1H), 7.82 (td, *J* = 7.7, 1.8 Hz, 1H), 7.50 - 7.42 (m, 6H), 7.34 (dd, *J* = 7.3, 5.2 Hz, 1H), 3.96 (s, 2H). **¹³C NMR** (151 MHz, Methanol-*d*₄) δ 169.65, 154.85, 148.58, 142.91, 139.07, 137.43, 137.16, 136.99, 135.24, 130.71, 128.88, 128.17, 128.07, 124.52, 122.36, 44.78. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 324.08; found: 324.1.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)-2-(pyridin-3-yl)acetamide (**24**)

The commercially available 2-(pyridin-3-yl)acetic acid (40.2 mg, 0.3 mmol, 1.2 eq), EDC (45.5 mg, 0.3 mmol, 1.2 eq), DMAP (35.8 mg, 0.3 mmol, 1.2 eq) and HOBt (3.3 mg, 0.02 mmol, 0.1 eq) and compound **11** (50.0 mg, 0.2 mmol, 1.0 eq) were reacted utilizing general procedure B to get the title product **24** (41.7 mg, 53 %) as a white solid. **¹H NMR** (600 MHz, Methanol-*d*₄) δ 8.60 (d, *J* = 2.6 Hz, 1H), 8.53 (d, *J* = 1.8 Hz, 1H), 8.46 (dd, *J* = 4.9, 1.4 Hz, 1H), 8.11 (d, *J* = 2.6 Hz, 1H), 7.86 (dt, *J* = 7.9, 2.0 Hz, 1H), 7.50 - 7.41 (m, 6H), 3.81 (s, 2H). **¹³C NMR** (151 MHz, Methanol-*d*₄) δ 171.42, 150.75, 148.69, 144.37, 140.44, 139.23, 138.54, 138.42, 136.63, 133.02, 132.06, 130.28, 129.60, 129.49, 125.22, 41.16. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 324.08; found: 324.1.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)-2-(pyridin-4-yl)acetamide (**25**)

The commercially available 2-(pyridin-4-yl)acetic acid (40.2 mg, 0.3 mmol, 1.2 eq), EDC (45.5 mg, 0.3 mmol, 1.2 eq), DMAP (35.8 mg, 0.3 mmol, 1.2 eq) and HOBt (3.3 mg, 0.02 mmol, 1.2 eq) and compound **11** (50.0 mg, 0.2 mmol, 0.1 eq) were reacted utilizing general procedure B to get the title product **25** (51.2 mg, 65 %) as a yellow solid. **¹H NMR** (600 MHz, Methanol-*d*₄) δ 8.60 (d, *J* = 2.6 Hz, 1H), 8.50 - 8.49 (m, 2H), 8.11 (d, *J* = 2.7 Hz, 1H), 7.47 - 7.43 (m, 7H), 3.82 (s, 2H). **¹³C NMR** (151 MHz, Methanol-*d*₄) δ 170.67, 150.13, 146.78, 144.44, 140.44, 138.53, 138.43, 136.58, 132.07, 130.28, 129.61, 129.49, 126.36, 43.48. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 324.08; found: 324.1.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)-2-(1-methylpiperidin-4-yl)acetamide (**26**)

The commercially available 2-(1-methylpiperidin-4-yl)acetic acid (46.1 mg, 0.3 mmol, 1.2 eq), EDC (45.5 mg, 0.3 mmol, 1.2 eq), DMAP (35.8 mg, 0.3 mmol, 1.2 eq) and HOBt (3.30 mg, 0.02 mmol, 0.1 eq) and compound **11** (50.0 mg, 0.2 mmol, 1.0 eq) were reacted utilizing general procedure B to get the title product **26** (37.7 mg, 45 %) as a yellow solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.44 (s, 1H), 8.61 (d, *J* = 2.6 Hz, 1H), 8.13 (d, *J* = 2.6 Hz, 1H), 7.51 - 7.48 (m, 2H), 7.48 - 7.43 (m, 3H), 2.70 (d, *J* = 11.5 Hz, 2H), 2.27 (d, *J* = 7.2 Hz, 2H), 2.12 (s, 3H), 1.81 (td, *J* = 11.7, 2.5 Hz, 2H), 1.70 (dtp, *J* = 15.3, 7.7, 3.8 Hz, 1H), 1.33 - 1.15 (m, 4H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 171.18, 141.32, 138.94, 137.03, 135.65, 129.76, 129.05, 128.46, 55.20, 48.59, 46.20, 43.20, 31.63. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 344.15; found: 344.1.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)nicotinamide (**27**)

The commercially available nicotinoyl chloride (31.3 mg, 0.2 mmol, 1.2 eq), compound **11** (30.0 mg, 0.2 mmol, 1.0 eq) and DIPEA (37.9 mg, 0.3 mmol, 2.0 eq) were reacted utilizing general procedure D to get the title product **27** (45.0 mg, 99 %) as a yellow solid. **¹H NMR** (600 MHz, Methanol-*d*₄) δ 9.14 - 9.12 (m, 1H), 8.79 (d, *J* = 2.6 Hz, 1H), 8.75 (dd, *J* = 4.9, 1.6 Hz, 1H), 8.39 (dt, *J* = 8.0, 1.9 Hz, 1H), 8.27 (d, *J* = 2.6 Hz, 1H), 7.61 (ddd, *J* = 8.0, 4.9, 0.7 Hz, 1H), 7.52 - 7.48 (m, 4H), 7.48 - 7.44 (m, 1H). **¹³C NMR** (151 MHz, Methanol-*d*₄) δ 166.57, 153.21, 149.53, 144.88, 141.36, 138.56, 138.43, 137.46, 136.58, 133.01, 131.97, 130.34, 129.64, 129.53, 125.23. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 310.07; found: 310.1.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)isonicotinamide (**28**)

The commercially available isonicotinoyl chloride (31.3 mg, 0.2 mmol, 1.2 eq), compound **11** (30.0 mg, 0.2 mmol, 1.0 eq) and DIPEA (37.9 mg, 0.3 mmol, 2.0 eq) were reacted utilizing general procedure D to get the title product **28** (44.9 mg, 99 %) as a white solid. **¹H NMR** (600 MHz, Methanol-*d*₄) δ 8.79 (d, *J* = 2.6 Hz, 1H), 8.79 - 8.75 (m, 2H), 8.27 (d, *J* = 2.6 Hz, 1H), 7.93 - 7.92 (m, 2H), 7.53 - 7.47 (m, 4H), 7.49 - 7.43 (m, 1H). **¹³C NMR** (151 MHz, Methanol-*d*₄) δ 166.51, 151.16, 145.07, 143.67, 141.39, 138.52, 138.45, 136.40, 133.02, 130.33, 129.66, 129.54, 123.16. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 310.07; found: 310.1.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)-1-methylpiperidine-4-carboxamide (**29**)

The commercially available 1-methylpiperidine-4-carboxylic acid (30.0 mg, 0.2 mmol, 1.2 eq), EDC (32.5 mg, 0.2 mmol, 1.2 eq), DMAP (25.6 mg, 0.2 mmol, 1.2 eq) and HOBt (2.4 mg, 0.02 mmol, 0.1 eq) and compound **11** (35.7 mg, 0.2 mmol, 1.0 eq) were reacted utilizing general procedure B to get the title product **29** (22.69 mg, 38 %) as a white solid.

¹H NMR (600 MHz, DMSO-*d*₆) δ 10.55 (s, 1H), 8.60 (d, *J* = 2.6 Hz, 1H), 8.13 (d, *J* = 2.6 Hz, 1H), 7.54 - 7.49 (m, 2H), 7.48 - 7.45 (m, 3H), 3.50 (d, *J* = 12.6 Hz, 2H), 2.99 (t, *J* = 13.0 Hz, 2H), 2.79 (s, 3H), 2.65 - 2.56 (m, 1H), 2.05 (d, *J* = 14.0 Hz, 2H), 1.89 - 1.77 (m, 2H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 172.57, 141.67, 139.10, 136.94, 135.91, 135.44, 130.01, 129.02, 128.50, 128.48, 52.55, 42.64, 25.83. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 330.13; found: 330.1.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)acetamide (**30**)

Acetyl chloride (0.01 mL, 0.2 mmol, 1.2 eq), compound **11** (30.0 mg, 0.2 mmol, 1.0 eq) and DIPEA (0.05 mL, 0.3 mmol, 2.0 eq) were reacted utilizing general procedure D to get the title product **30** (36.1 mg, 99 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.38 (s, 1H), 8.57 (d, *J* = 2.6 Hz, 1H), 8.10 (d, *J* = 2.6 Hz, 1H), 7.52 - 7.45 (m, 5H), 2.09 (s, 3H). **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 247.06; found: 247.0. **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 169.14, 141.32, 138.86, 137.02, 135.86, 135.69, 129.64, 129.05, 128.48, 128.43, 23.89.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)isobutyramide (**31**)

Isobutyryl chloride (0.02 mL, 0.2 mmol, 1.2 eq), compound **11** (30.0 mg., 0.2 mmol, 1.0 eq) and DIPEA (0.05 mL, 0.3 mmol, 2.0 eq) were reacted utilizing general procedure D to get the title product **31** (40.3 mg, 99 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.27 (s, 1H), 8.62 (d, *J* = 2.6 Hz, 1H), 8.14 (d, 1H), 7.51 - 7.45 (m, 5H), 2.64 - 2.60 (m, 1H), 1.12 (d, *J* = 6.8 Hz, 6H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 176.00, 141.27, 138.98, 137.03, 135.84, 135.81, 129.83, 129.04, 128.46, 128.41, 34.98, 19.31. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 275.09; found: 275.1.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)-1-methyl-1H-pyrazole-5-carboxamide (**32**)

The commercially available 1-methyl-1H-pyrazole-5-carboxylic acid (30.0 mg, 0.2 mmol, 1.2 eq), EDC (36.9 mg, 0.2 mmol, 1.2 eq), DMAP (29.1 mg, 0.2 mmol, 1.2 eq) and HOBt (2.68 mg, 0.02 mmol, 0.1 eq) and compound **11** (40.6 mg, 0.2 mmol, 1.0 eq) were reacted utilizing general procedure B to get the title product **32** (26.8 mg, 43 %) as a light brown solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.58 (s, 1H), 8.78 (d, *J* = 2.6 Hz, 1H), 8.25 (d, *J* = 2.6 Hz, 1H), 7.57 (d, *J* = 2.1 Hz, 1H), 7.54 - 7.45 (m, 5H), 7.10 (d, *J* = 2.1 Hz, 1H), 4.09 (s, 3H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 158.43, 156.59, 142.27, 140.09, 137.35, 136.87, 135.85, 135.07, 134.53, 131.15, 129.07, 128.51, 108.57, 33.34. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 314.08; found: 314.0.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)-1-ethyl-1H-pyrazole-5-carboxamide (**33**)

The commercially available 1-ethyl-1H-pyrazole-5-carboxylic acid (33.3 mg, 0.2 mmol, 1.2 eq), EDC (36.9 mg, 0.2 mmol, 1.2 eq), DMAP (29.1 mg, 0.2 mmol, 1.2 eq) and HOBt (2.68 mg, 0.02 mmol, 0.1 eq) and compound **11** (40.6 mg, 0.20 mmol, 1.0 eq) were reacted utilizing general procedure B to get the title product **33** (19.6 mg, 30 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.58 (s, 1H), 8.77 (d, *J* = 2.6 Hz, 1H), 8.25 (d, *J* = 2.6 Hz, 1H), 7.59 (d, *J* = 2.1 Hz, 1H), 7.53 - 7.48 (m, 5H), 7.10 (d, 1H), 4.52 (q, *J* = 7.2 Hz, 2H), 1.33 (t, *J* = 7.2 Hz, 3H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 158.36, 142.28, 140.13, 137.53, 136.88, 135.85, 135.06, 133.87, 131.18, 129.08, 128.50, 108.69, 106.95, 46.18, 15.84. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 327.09; found: 327.1.

Synthesis of N-(6-chloro-5-phenylpyridin-3-yl)-6-cyclopropylnicotinamide (**34**)

The commercially available 6-cyclopropylnicotinic acid (38.8 mg, 0.2 mmol, 1.2 eq), EDC (36.9 mg, 0.2 mmol, 1.2 eq), DMAP (29.1 mg, 0.2 mmol, 1.2 eq) and HOBt (2.68 mg, 0.02 mmol, 0.1 eq) and compound **11** (40.6 mg, 0.2 mmol, 1.0 eq) were reacted utilizing general procedure B to get the title product **34** (23.5 mg, 34 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.69 (s, 1H), 8.96 (d, *J* = 2.2 Hz, 1H), 8.82 (d, *J* = 2.6 Hz, 1H), 8.27 (d, *J* = 2.6 Hz, 1H), 8.17 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.55 - 7.44 (m, 5H), 2.25 - 2.17 (m, 1H), 1.05 (dt, *J* = 5.4, 2.2 Hz, 2H), 1.01 (dt, *J* = 5.1, 2.7 Hz, 2H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 166.23, 164.59, 148.66, 142.08, 140.03, 136.94,

135.83, 135.50, 135.35, 130.98, 129.08, 128.51, 128.49, 126.52, 121.08, 17.01, 10.76.

LC-MS (ESI): m/z calculated for $[M+H]^+$: 350.10; found: 350.1.

Synthesis of 6-chloro-5-phenylnicotinic acid (**36**)

The commercially available 5-bromo-6-chloronicotinic acid (**35**) (1000 mg, 4.2 mmol, 1.0 eq) and phenylboronic acid (567.2 mg, 4.7 mmol, 1.1 eq) were dissolved in 1,4-dioxane/ H₂O (5:1, 20 mL) under an argon atmosphere. The mixture was degassed for 15 min and then K₃PO₄ (1795.5 mg, 8.5 mmol, 2.0 eq) and Pd(dppf)Cl₂ (309.4 mg, 0.4 mmol, 0.1 eq) were added. Subsequently, the reaction mixture was heated up to 60 °C for 16 h. After the reaction was completed, the cooled reaction mixture was filtered over Celite. The filtrate was extracted with DCM and H₂O. The obtained organic phase was basified with aqueous solution of NaOH until pH=9 and extracted with H₂O. Then concentrated HCl was added to the aqueous phase until a white, insoluble material formed, which was later filtered under reduced pressure. The solid was washed with H₂O and it was later lyophilized, affording the title product **36** (812.0 mg, 82 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 13.73 (s, 1H), 8.89 (d, J = 2.3 Hz, 1H), 8.18 (d, J = 2.3 Hz, 1H), 7.56 - 7.44 (m, 5H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 165.31, 152.14, 149.18, 140.37, 136.23, 136.11, 129.20, 128.70, 128.53, 126.44. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 234.02; found: 233.9.

Synthesis of 6-chloro-N-(1-methyl-1H-pyrazol-5-yl)-5-phenylnicotinamide (**37**)

The commercially available 1-methyl-1H-pyrazol-5-amine (52.0 mg, 0.5 mmol, 1.3 eq), compound **36** (96.2 mg, 0.4 mmol, 1.0 eq), DIPEA (0.22 mL, 1.2 mmol, 3.0 eq) and HATU (234.9 mg, 0.6 mmol, 1.5 eq) were reacted utilizing general procedure C to get the title product **37** (98.7 mg, 77 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.61 (s, 1H), 8.95 (d, J = 2.4 Hz, 1H), 8.40 (d, J = 2.4 Hz, 1H), 7.61 - 7.57 (m, 2H), 7.57 - 7.53 (m, 2H), 7.52 - 7.48 (m, 1H), 7.41 (d, J = 1.9 Hz, 1H), 6.27 (d, J = 2.0 Hz, 1H), 3.72 (s, 3H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 163.00, 151.22, 148.15, 139.37, 137.52, 136.31, 135.74, 135.56, 129.31, 128.72, 128.53, 100.40, 35.78. **LC-MS (ESI):** m/z calculated for $[M+H]^+$: 313.08; found: 313.1.

Synthesis of 6-chloro-N-(1-ethyl-1H-pyrazol-5-yl)-5-phenylnicotinamide (**38**)

The commercially available 1-ethyl-1H-pyrazol-5-amine (46.4 mg, 0.4 mmol, 1.3 eq), compound **36** (75.1 mg, 0.3 mmol, 1.0 eq), DIPEA (0.17 mL, 1.0 mmol, 3.0 eq) and HATU (183.3 mg, 0.5 mmol, 1.5 eq) were reacted utilizing general procedure C to get the title product **38** (89.1 mg, 85 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.54 (s, 1H), 8.94 (d, *J* = 2.4 Hz, 1H), 8.39 (d, *J* = 2.4 Hz, 1H), 7.61 - 7.57 (m, 2H), 7.57 - 7.53 (m, 2H), 7.52 - 7.48 (m, 1H), 7.45 (d, *J* = 1.9 Hz, 1H), 6.25 (d, *J* = 1.9 Hz, 1H), 4.03 (q, *J* = 7.2 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 3H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 163.28, 151.22, 148.12, 139.34, 137.76, 136.32, 135.75, 134.67, 129.31, 128.72, 128.69, 128.53, 100.84, 42.75, 14.94. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 327.09; found: 327.1.

Synthesis of 6-chloro-N-(6-cyclopropylpyridin-3-yl)-5-phenylnicotinamide (**39**)

The commercially available 6-cyclopropylpyridin-3-amine (52.0 mg, 0.4 mmol, 1.3 eq), compound **36** (69.7 mg, 0.3 mmol, 1.0 eq), DIPEA (0.16 mL, 0.9 mmol, 3.0 eq) and HATU (170.0 mg, 0.4 mmol, 1.5 eq) were reacted utilizing general procedure C to get the title product **39** (76.8 mg, 74 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.57 (s, 1H), 8.94 (d, *J* = 2.4 Hz, 1H), 8.70 (d, *J* = 2.5 Hz, 1H), 8.40 (d, *J* = 2.4 Hz, 1H), 8.02 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.60 - 7.57 (m, 2H), 7.57 - 7.53 (m, 2H), 7.53 - 7.48 (m, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 2.08 (tt, *J* = 8.1, 4.8 Hz, 1H), 0.93 (ddt, *J* = 8.0, 5.4, 2.6 Hz, 2H), 0.88 (dt, *J* = 4.7, 2.8 Hz, 2H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 162.80, 157.63, 150.93, 148.08, 141.58, 139.07, 136.40, 135.67, 132.53, 129.52, 129.32, 128.70, 128.53, 128.01, 120.95, 16.29, 9.49. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 350.10; found: 350.1.

Synthesis of 5-(bromomethyl)-2-chloro-3-phenylpyridine (40**) has been reported.¹⁰**

Synthesis of N-((6-chloro-5-phenylpyridin-3-yl)methyl)-1-methyl-1H-pyrazol-5-amine (**41**)

The commercially available 1-methyl-1H-pyrazol-5-amine (11.0 mg, 0.1 mmol, 1.1 eq), compound **40** (29.1 mg, 0.1 mmol, 1.0 eq) and K₂CO₃ (28.5 mg, 0.2 mmol, 2.0 eq) were reacted utilizing general procedure E to get the title product **41** (23.3 mg, 75 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 8.44 (d, *J* = 2.3 Hz, 1H), 7.88 (d, *J* = 2.3 Hz, 1H), 7.54 - 7.41 (m, 5H), 7.04 (d, *J* = 1.9 Hz, 1H), 6.11 (t, *J* = 6.1 Hz, 1H), 5.35

(d, J = 1.9 Hz, 1H), 4.27 (d, J = 6.0 Hz, 2H), 3.54 (s, 3H). **^{13}C NMR** (151 MHz, DMSO- d_6) δ 147.76, 147.71, 146.75, 139.55, 137.20, 137.02, 135.53, 135.26, 129.18, 128.43, 128.35, 87.11, 45.17, 34.36. **LC-MS (ESI):** m/z calculated for $[\text{M}+\text{H}]^+$: 299.10; found: 299.08.

Synthesis of N-((6-chloro-5-phenylpyridin-3-yl)methyl)-1-ethyl-1H-pyrazol-5-amine (42)

The commercially available 1-ethyl-1H-pyrazol-5-amine (23.5 mg, 0.2 mmol, 1.1 eq), compound **40** (54.2 mg, 0.2 mmol, 1.0 eq) and K_2CO_3 (53 mg, 0.4 mmol, 2.0 eq) were reacted utilizing general procedure E to get the title product **42** (50.3 mg, 84 %) as a white solid. **^1H NMR** (600 MHz, DMSO- d_6) δ 8.42 (d, J = 2.3 Hz, 1H), 7.87 (d, J = 2.3 Hz, 1H), 7.56 - 7.38 (m, 5H), 7.06 (d, J = 1.9 Hz, 1H), 6.12 (t, J = 6.0 Hz, 1H), 5.35 (d, J = 1.9 Hz, 1H), 4.27 (d, J = 6.0 Hz, 2H), 3.91 (q, J = 7.2 Hz, 2H), 1.20 (t, J = 7.2 Hz, 3H). **^{13}C NMR** (151 MHz, DMSO- d_6) δ 147.72, 146.89, 146.71, 139.52, 137.38, 137.02, 135.51, 135.37, 129.16, 128.44, 128.35, 87.16, 45.14, 41.42, 14.64. **LC-MS (ESI):** m/z calculated for $[\text{M}+\text{H}]^+$: 313.11; found: 313.1.

Synthesis of N-((6-chloro-5-phenylpyridin-3-yl)methyl)-6-cyclopropylpyridin-3-amine (43)

6-cyclopropylpyridin-3-amine (34.8 mg, 0.3 mmol, 1.1 eq), compound **40** (66.7 mg, 0.2 mmol, 1.0 eq) and K_2CO_3 (65.2 mg, 0.5 mmol, 2.0 eq) were reacted utilizing general procedure E to get the title product **43** (63.7 mg, 80 %) as a white solid. **^1H NMR** (600 MHz, DMSO- d_6) δ 8.41 (d, J = 2.3 Hz, 1H), 7.85 (dd, J = 6.0, 2.6 Hz, 2H), 7.55 - 7.41 (m, 5H), 6.96 (d, J = 8.4 Hz, 1H), 6.88 (dd, J = 8.4, 2.8 Hz, 1H), 6.27 (t, J = 6.3 Hz, 1H), 4.35 (d, J = 6.3 Hz, 2H), 1.87 (tt, J = 8.2, 4.9 Hz, 1H), 0.76 (dt, J = 8.0, 2.9 Hz, 2H), 0.70 (dq, J = 6.7, 3.6 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO- d_6) δ 149.48, 147.65, 146.70, 141.80, 139.46, 137.00, 135.62, 135.38, 134.80, 129.17, 128.42, 128.35, 121.04, 119.32, 43.03, 15.70, 8.33. **LC-MS (ESI):** m/z calculated for $[\text{M}+\text{H}]^+$: 336.12; found: 336.1.

Synthesis of 2-chloro-5-(((1-methyl-1H-pyrazol-5-yl)oxy)methyl)-3-phenylpyridine (44)

1-methyl-1H-pyrazol-5-ol (19.1 mg, 0.2 mmol, 1.1 eq), compound **40** (50.0 mg, 0.2 mmol, 1.0 eq) and K_2CO_3 (73.4 mg, 0.5 mmol, 3.0 eq) were reacted utilizing general

procedure E to get the title product **44** (28.7 mg, 54 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 8.56 (d, *J* = 2.4 Hz, 1H), 8.00 (d, *J* = 2.3 Hz, 1H), 7.54 - 7.43 (m, 5H), 7.22 (d, *J* = 2.0 Hz, 1H), 5.78 (d, *J* = 2.0 Hz, 1H), 5.25 (s, 2H), 3.56 (s, 3H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 153.26, 148.24, 148.02, 139.93, 137.23, 136.68, 135.90, 131.63, 129.23, 128.47, 128.45, 85.94, 69.46, 33.56. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 300.08; found: 300.1.

Synthesis of 2-chloro-5-(((1-ethyl-1H-pyrazol-5-yl)oxy)methyl)-3-phenylpyridine (**45**)

1-ethyl-1H-pyrazol-5-ol (43.7 mg, 0.4 mmol, 1.1 eq), compound **40** (100 mg, 0.4 mmol, 1.0 eq) and K₂CO₃ (146.7 mg, 1.1 mmol, 3.0 eq) were reacted utilizing general procedure E to get the title product **45** (74.7 mg, 67 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 8.56 (d, *J* = 2.3 Hz, 1H), 7.99 (d, *J* = 2.3 Hz, 1H), 7.53 - 7.44 (m, 5H), 7.23 (d, *J* = 2.0 Hz, 1H), 5.78 (d, *J* = 2.0 Hz, 1H), 5.26 (s, 2H), 3.91 (q, *J* = 7.2 Hz, 2H), 1.21 (t, *J* = 7.2 Hz, 3H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 152.63, 148.24, 148.02, 139.93, 137.27, 136.67, 135.90, 131.67, 129.21, 128.48, 128.46, 86.02, 69.43, 41.22, 14.74. **LC-MS (ESI):** *m/z* calculated for [M+H]⁺: 314.10; found: 314.1.

Synthesis of N-(2-oxo-3-(1-(4-(3-phenyl-5-(2-(pyridin-2-yl)acetamido)pyridin-2-yl)benzyl)piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (**46**)

Compound **23** (15.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (34.9 mg, 0.1 mmol, 1.5 eq), K₂CO₃ (26.8 mg, 0.2 mmol, 2.0 eq) and Pd(PPh₃)₄ (5.4 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **46** (5.91 mg, 19 %) as a white solid. **¹H NMR** (600 MHz, Methanol-*d*₄) δ 8.83 (d, *J* = 2.4 Hz, 1H), 8.52 (d, *J* = 5.6 Hz, 1H), 8.19 (d, *J* = 2.4 Hz, 1H), 7.84 (td, *J* = 7.7, 1.8 Hz, 1H), 7.72 (d, *J* = 1.8 Hz, 1H), 7.52 - 7.45 (m, 2H), 7.35 (ddd, *J* = 7.6, 5.0, 1.1 Hz, 1H), 7.32 - 7.23 (m, 7H), 7.18 (ddd, *J* = 5.4, 3.0, 1.5 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.44 (dd, *J* = 17.0, 10.0 Hz, 1H), 6.36 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.77 (dd, *J* = 10.0, 1.9 Hz, 1H), 4.28 (tt, *J* = 12.5, 4.3 Hz, 1H), 3.65 (s, 2H), 3.09 (d, *J* = 11.9 Hz, 2H), 2.53 (qd, *J* = 12.7, 3.8 Hz, 2H), 2.35 - 2.24 (m, 2H), 2.23 (t, *J* = 7.5 Hz, 2H), 1.81 - 1.75 (m, 2H). **¹³C NMR** (151 MHz, Methanol-*d*₄) δ 171.16, 166.07, 156.60, 156.34, 153.49, 150.00, 140.64, 140.41, 140.18, 138.84, 138.17, 136.13, 133.71, 132.49, 131.02, 130.67, 130.50,

130.34, 129.44, 128.67, 127.56, 126.63, 125.91, 123.78, 115.50, 110.37, 104.06, 63.12, 53.99, 52.09, 33.08, 30.78. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 664.2958; found: 664.3017.

Synthesis of N-(2-oxo-3-(1-(4-(3-phenyl-5-(2-(pyridin-3-yl)acetamido)pyridin-2-yl)benzyl)piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (47)

Compound **24** (30.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (69.8 mg, 0.1 mmol, 1.5 eq), K_2CO_3 (25.6 mg, 0.2 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (10.71 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **47** (21.9 mg, 36 %) as a white solid. **1H NMR** (600 MHz, Methanol- d_4) δ 8.84 - 8.81 (m, 1H), 8.56 (s, 1H), 8.48 - 8.45 (m, 1H), 8.19 - 8.16 (m, 1H), 7.90 - 7.87 (m, 1H), 7.78 - 7.76 (m, 1H), 7.44 (dd, J = 7.8, 5.0 Hz, 1H), 7.33 - 7.26 (m, 7H), 7.20 - 7.16 (m, 3H), 7.03 - 6.99 (m, 1H), 6.44 (dd, J = 17.0, 9.9 Hz, 1H), 6.37 (dd, J = 17.0, 1.9 Hz, 1H), 5.78 (dd, J = 9.9, 1.9 Hz, 1H), 4.32 (tt, J = 12.2, 4.1 Hz, 1H), 3.85 (s, 2H), 3.78 (s, 2H), 3.19 (d, J = 11.2 Hz, 2H), 2.58 (qd, J = 12.5, 3.1 Hz, 2H), 2.47 (t, J = 11.6 Hz, 2H), 1.84 (d, J = 10.6 Hz, 2H). **^{13}C NMR** (151 MHz, Methanol- d_4) δ 171.53, 166.06, 156.57, 153.32, 150.75, 148.68, 140.56, 140.43, 138.22, 136.18, 133.74, 133.16, 132.46, 131.18, 130.81, 130.66, 130.47, 129.48, 128.72, 127.61, 126.61, 125.25, 115.49, 110.44, 103.90, 62.70, 53.79, 51.57, 41.24, 29.04. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 664.2958; found: 664.3033.

Synthesis of N-(2-oxo-3-(1-(4-(3-phenyl-5-(2-(pyridin-4-yl)acetamido)pyridin-2-yl)benzyl)piperidin-4-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (48)

Compound **25** (30.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (69.8 mg, 0.1 mmol, 1.5 eq), K_2CO_3 (25.6 mg, 0.2 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (10.7 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **48** (8.7 mg, 14 %) as a white solid. **1H NMR** (600 MHz, Methanol- d_4) δ 8.85 (d, J = 2.4 Hz, 1H), 8.51 (d, J = 5.9 Hz, 2H), 8.18 (d, J = 2.4 Hz, 1H), 7.63 (s, 1H), 7.51 (d, J = 8.3 Hz, 2H), 7.50 - 7.46 (m, 2H), 7.40 (d, J = 8.3 Hz, 2H), 7.32 (dd, J = 8.4, 1.7 Hz, 1H), 7.31 - 7.27 (m, 3H), 7.22 - 7.17 (m, 2H), 7.03 (d, J = 8.4 Hz, 1H), 6.42 (dd, J = 17.0, 10.0 Hz, 1H), 6.34 (dd, J = 17.0, 1.9 Hz, 1H), 5.76 (dd, J = 10.0, 1.9 Hz, 1H), 4.56 (s, 2H), 4.52 (tt, J = 12.6, 4.5 Hz, 1H), 3.70 - 3.60 (m, 2H), 3.41 (d, J = 12.7 Hz, 2H), 3.12 (qd, J = 13.3, 3.8 Hz, 2H), 2.27 (t, J = 7.4 Hz, 2H), 1.85 (d, J = 13.7 Hz, 2H). **^{13}C NMR** (151 MHz, Methanol- d_4) δ 177.92, 170.83, 166.02, 156.40, 152.74, 150.14, 146.85, 142.66,

140.59, 140.43, 138.32, 136.31, 133.76, 133.73, 132.47, 131.24, 130.78, 130.68, 130.07, 129.59, 127.55, 126.69, 126.37, 116.02, 110.54, 103.96, 75.00, 63.77, 35.10, 33.08, 23.74. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 664.2958; found: 664.3035.

Synthesis of N-(3-(1-(4-(5-(2-(1-methylpiperidin-4-yl)acetamido)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (49)

Compound **26** (20.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (32.9 mg, 0.1 mmol, 1.5 eq), K_2CO_3 (12.1 mg, 0.2 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (5.0 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **49** (10.8 mg, 27 %) as a white solid. **1H NMR** (600 MHz, Methanol- d_4) δ 8.81 (d, J = 2.4 Hz, 1H), 8.15 (d, J = 2.3 Hz, 1H), 7.74 (d, J = 1.7 Hz, 1H), 7.33 - 7.26 (m, 7H), 7.23 (dd, J = 8.4, 1.8 Hz, 1H), 7.20 - 7.15 (m, 2H), 7.01 (d, J = 8.4 Hz, 1H), 6.44 (dd, J = 17.0, 10.0 Hz, 1H), 6.36 (dd, J = 17.0, 1.8 Hz, 1H), 5.77 (dd, J = 10.0, 1.8 Hz, 1H), 4.28 (tq, J = 12.4, 4.2 Hz, 1H), 3.64 (s, 2H), 3.08 (d, J = 11.8 Hz, 2H), 2.76 (t, J = 11.4 Hz, 2H), 2.69 (s, 3H), 2.57 - 2.49 (m, 2H), 2.45 (d, J = 7.0 Hz, 2H), 2.27 (t, J = 11.5 Hz, 2H), 2.14 - 2.07 (m, 1H), 2.00 (d, J = 13.7 Hz, 2H), 1.78 (dd, J = 12.3, 2.5 Hz, 2H), 1.58 - 1.49 (m, 2H), 1.39 - 1.30 (m, 2H). **^{13}C NMR** (151 MHz, Methanol- d_4) δ 172.96, 166.07, 156.60, 153.42, 140.63, 140.34, 140.11, 138.16, 136.05, 133.70, 132.49, 130.99, 130.78, 130.64, 130.52, 130.33, 129.46, 128.70, 127.92, 127.57, 126.80, 126.62, 115.49, 110.38, 104.05, 63.14, 54.00, 52.15, 43.46, 29.55. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 684.3584; found: 684.3652.

Synthesis of N-(6-(4-((4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)methyl)phenyl)-5-phenylpyridin-3-yl)nicotinamide (50)

Compound **27** (30.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (73.0 mg, 0.2 mmol, 1.5 eq), K_2CO_3 (26.8 mg, 0.2 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (11.2 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **50** (30.6 mg, 49 %) as a white solid. **1H NMR** (600 MHz, Methanol- d_4) δ 9.15 (d, J = 1.7 Hz, 1H), 9.00 (d, J = 2.4 Hz, 1H), 8.75 (dd, J = 4.9, 1.5 Hz, 1H), 8.41 (dt, J = 8.0, 1.9 Hz, 1H), 8.30 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 1.7 Hz, 1H), 7.63 - 7.58 (m, 1H), 7.32 - 7.25 (m, 7H), 7.24 - 7.18 (m, 3H), 7.00 (d, J = 8.4 Hz, 1H), 6.43 (dd, J = 17.0, 10.0 Hz, 1H), 6.36 (dd, J = 17.0, 1.8 Hz, 1H), 5.77 (dd, J = 10.0, 1.8 Hz, 1H), 4.28 (ddt, J = 12.4, 8.6, 4.2 Hz, 1H), 3.68 (s, 2H), 3.11 (d, J = 11.8 Hz, 2H), 2.52 (td, J = 12.6, 3.6 Hz, 2H), 2.33 (t, J =

11.5 Hz, 2H), 1.82 - 1.75 (m, 2H). **¹³C NMR** (151 MHz, Methanol-*d*₄) δ 166.61, 156.56, 153.80, 153.13, 149.55, 141.27, 140.57, 140.30, 138.12, 137.45, 136.04, 133.71, 132.48, 132.07, 131.71, 131.10, 130.70, 130.48, 130.45, 129.49, 128.72, 127.59, 126.59, 125.21, 115.48, 110.40, 103.96, 62.96, 53.91, 51.91, 29.36. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 650.2801; found: 650.2873.

Synthesis of N-(6-(4-((4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)methyl)phenyl)-5-phenylpyridin-3-yl)isonicotinamide (51)

Compound **28** (30.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (73.0 mg, 0.2 mmol, 1.5 eq), K₂CO₃ (26.8 mg, 0.2 mmol, 2.0 eq) and Pd(PPh₃)₄ (11.2 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **51** (7.7 mg, 12 %) as a white solid. **¹H NMR** (600 MHz, Methanol-*d*₄) δ 9.01 (d, *J* = 2.4 Hz, 1H), 8.80 - 8.75 (m, 2H), 8.32 (d, *J* = 2.4 Hz, 1H), 7.99 - 7.94 (m, 2H), 7.73 (d, *J* = 1.7 Hz, 1H), 7.33 - 7.28 (m, 7H), 7.27 - 7.20 (m, 3H), 7.02 (d, *J* = 8.4 Hz, 1H), 6.44 (dd, *J* = 17.0, 10.0 Hz, 1H), 6.37 (dd, *J* = 17.0, 1.9 Hz, 1H), 5.78 (dd, *J* = 10.0, 1.8 Hz, 1H), 4.28 (td, *J* = 8.2, 4.1 Hz, 1H), 3.63 (s, 2H), 3.08 (d, *J* = 11.8 Hz, 2H), 2.53 (qd, *J* = 12.5, 3.9 Hz, 2H), 2.26 (t, *J* = 11.4 Hz, 2H), 1.80 - 1.75 (m, 2H). **¹³C NMR** (151 MHz, Methanol-*d*₄) δ 166.61, 166.08, 156.61, 154.15, 151.15, 143.85, 141.28, 140.59, 140.03, 138.19, 135.86, 133.70, 132.49, 131.78, 131.02, 130.70, 130.52, 130.32, 129.50, 128.74, 127.57, 126.63, 123.19, 115.50, 110.37, 104.08, 63.20, 54.04, 52.20, 29.62. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 650.2801; found: 650.2871.

Synthesis of N-(6-(4-((4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)methyl)phenyl)-5-phenylpyridin-3-yl)-1-methylpiperidine-4-carboxamide (52)

Compound **29** (15.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (34.3 mg, 0.1 mmol, 1.5 eq), K₂CO₃ (12.6 mg, 0.1 mmol, 2.0 eq) and Pd(PPh₃)₄ (5.3 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **52** (14.9 mg, 48 %) as a white solid. **¹H NMR** (700 MHz, Methanol-*d*₄) δ 8.89 (d, *J* = 2.4 Hz, 1H), 8.16 (s, 1H), 7.92 (s, 1H), 7.49 (d, *J* = 8.1 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.33 - 7.24 (m, 3H), 7.24 - 7.12 (m, 3H), 7.02 (d, *J* = 8.4 Hz, 1H), 6.48 (dd, *J* = 16.9, 10.2 Hz, 1H), 6.35 (dd, *J* = 16.9, 1.5 Hz, 1H), 5.74 (dd, *J* = 10.2, 1.6 Hz, 1H), 4.55 (tt, *J* = 12.3, 4.3 Hz, 1H), 4.36 (s, 2H), 3.70 - 3.51 (m, 4H), 3.28 - 3.18 (m, 2H), 3.16 - 3.05 (m, 2H), 2.91 (s, 3H), 2.84 (h, *J* = 6.7 Hz, 2H), 2.36 - 2.25 (m, 1H), 2.25 - 2.16 (m, 2H), 2.08 - 2.03 (m,

4H). **¹³C NMR** (176 MHz, Methanol-*d*₄) δ 178.17, 174.40, 173.42, 166.01, 156.40, 152.47, 142.72, 140.38, 138.26, 136.38, 134.09, 132.60, 132.04, 131.85, 130.82, 130.68, 129.59, 128.86, 127.52, 126.48, 115.40, 110.58, 103.51, 59.79, 54.77, 44.00, 41.51, 39.44, 39.32, 30.75, 27.63, 20.71. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 670.3427; found: 670.3518.

Synthesis of N-(3-(1-(4-(5-acetamido-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (53)

Compound **30** (30.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (91.7 mg, 0.1 mmol, 1.5 eq), K₂CO₃ (33.6 mg, 0.2 mmol, 2.0 eq) and Pd(PPh₃)₄ (14.1 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **53** (1.3 mg, 2 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.79 (s, 1H), 10.31 (s, 1H), 10.07 (s, 1H), 8.78 (d, *J* = 2.4 Hz, 1H), 8.09 (d, *J* = 2.4 Hz, 1H), 7.68 (d, *J* = 1.9 Hz, 1H), 7.35 - 7.27 (m, 4H), 7.25 - 7.14 (m, 6H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.41 (dd, *J* = 16.9, 10.2 Hz, 1H), 6.24 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.73 (dd, *J* = 10.1, 2.0 Hz, 1H), 4.10 (tt, *J* = 12.5, 4.2 Hz, 1H), 3.50 (s, 2H), 3.00 - 2.82 (m, 2H), 2.29 (qd, *J* = 11.9, 3.5 Hz, 2H), 2.13 - 2.03 (m, 5H), 1.71 - 1.52 (m, 2H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 169.04, 162.76, 153.99, 150.53, 139.63, 139.08, 138.43, 137.45, 135.19, 134.68, 134.06, 132.64, 132.04, 129.42, 129.17, 128.97, 128.42, 128.19, 127.95, 127.39, 126.33, 124.48, 112.10, 108.62, 100.90, 61.53, 52.62, 50.14, 28.66, 23.92. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 587.2692; found: 587.2757.

Synthesis of N-(3-(1-(4-(5-isobutyramido-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (54)

Compound **31** (30.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (92.9 mg, 0.2 mmol, 1.5 eq), K₂CO₃ (30.2 mg, 0.2 mmol, 2.0 eq) and Pd(PPh₃)₄ (12.6 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **54** (28.4 mg, 42 %) as a white solid. **¹H NMR** (600 MHz, Methanol-*d*₄) δ 8.83 (d, *J* = 2.4 Hz, 1H), 8.17 (d, *J* = 2.4 Hz, 1H), 7.77 (d, *J* = 1.7 Hz, 1H), 7.32 (d, *J* = 5.9 Hz, 4H), 7.30 - 7.25 (m, 3H), 7.20 - 7.16 (m, 3H), 7.02 (d, *J* = 8.4 Hz, 1H), 6.44 (dd, *J* = 17.0, 9.9 Hz, 1H), 6.37 (dd, *J* = 16.9, 1.9 Hz, 1H), 5.78 (dd, *J* = 10.0, 1.9 Hz, 1H), 4.39 - 4.29 (m, 1H), 4.26 (dq, *J* = 11.0, 6.5 Hz, 1H), 3.80 (s, 2H), 3.21 (d, *J* = 11.8 Hz, 2H), 2.64 - 2.54 (m, 2H), 2.53 - 2.46 (m, 2H), 1.85 (d, *J* = 12.2 Hz, 2H), 1.24 (s, 3H), 1.23 (s, 3H). **¹³C NMR** (151 MHz, Methanol-*d*₄) δ 179.17, 166.05, 156.56, 152.96, 140.64, 140.49, 138.17, 136.47,

133.75, 132.46, 131.21, 130.84, 130.70, 130.67, 129.47, 128.69, 127.61, 126.60, 115.50, 110.45, 103.89, 62.64, 53.76, 51.48, 37.06, 28.97, 19.82. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 615.3005; found: 615.3073.

Synthesis of N-(6-(4-((4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)methyl)phenyl)-5-phenylpyridin-3-yl)-1-methyl-1H-pyrazole-5-carboxamide (55)

Compound **32** (19.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (45.8 mg, 0.1 mmol, 1.5 eq), K_2CO_3 (16.8 mg, 0.1 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (7.0 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **55** (6.2 mg, 16 %) as a white solid. **1H NMR** (600 MHz, Methanol- d_4) δ 8.97 (d, J = 2.4 Hz, 1H), 8.28 (d, J = 2.4 Hz, 1H), 7.75 (d, J = 1.7 Hz, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.32 (s, 4H), 7.29 (p, J = 3.2 Hz, 3H), 7.24 - 7.18 (m, 3H), 7.06 (d, J = 2.1 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 6.44 (dd, J = 17.0, 10.0 Hz, 1H), 6.37 (dd, J = 17.0, 1.9 Hz, 1H), 5.77 (dd, J = 10.0, 1.9 Hz, 1H), 4.30 (tt, J = 12.4, 4.2 Hz, 1H), 4.19 (s, 3H), 3.72 (s, 2H), 3.14 (d, J = 11.7 Hz, 2H), 2.56 (qd, J = 12.7, 3.5 Hz, 2H), 2.37 (t, J = 11.8 Hz, 2H), 1.84 - 1.78 (m, 2H). **^{13}C NMR** (151 MHz, Methanol- d_4) δ 166.05, 160.44, 156.58, 141.12, 140.60, 138.75, 138.13, 136.60, 133.73, 132.48, 131.59, 131.13, 130.70, 130.52, 130.48, 129.49, 128.72, 127.60, 126.61, 115.49, 110.41, 109.19, 103.96, 53.88, 51.83, 50.86, 39.64, 30.77, 29.28. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 653.2910; found: 653.2981.

Synthesis of N-(6-(4-((4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)methyl)phenyl)-5-phenylpyridin-3-yl)-1-ethyl-1H-pyrazole-5-carboxamide (56)

Compound **33** (15.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (34.6 mg, 0.1 mmol, 1.5 eq), K_2CO_3 (12.7 mg, 0.1 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (5.3 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **56** (12.0 mg, 39 %) as a white solid. **1H NMR** (600 MHz, Methanol- d_4) δ 9.03 (d, J = 2.4 Hz, 1H), 8.31 (d, J = 2.4 Hz, 1H), 7.90 (s, 1H), 7.57 (d, J = 2.1 Hz, 1H), 7.46 (dd, J = 5.8, 5.8 Hz, 4H), 7.34 - 7.29 (m, 3H), 7.24 (dd, J = 6.6, 3.0 Hz, 2H), 7.06 (d, J = 2.1 Hz, 1H), 7.06 - 7.02 (m, 2H), 6.46 (dd, J = 17.1, 10.2 Hz, 1H), 6.38 (dd, J = 16.9, 1.8 Hz, 1H), 5.79 (dd, J = 10.0, 1.9 Hz, 1H), 4.63 (q, J = 7.2 Hz, 2H), 4.53 (tt, J = 12.0, 3.9 Hz, 1H), 4.36 (s, 2H), 3.61 (d, J = 10.6 Hz, 2H), 3.28 - 3.18 (m, 2H), 2.79 (q, J = 11.5 Hz, 2H), 2.09 (d, J = 12.9 Hz, 2H), 1.45 (t, J = 7.2 Hz, 3H). **^{13}C NMR** (151 MHz, Methanol- d_4) δ

166.03, 160.37, 156.44, 152.68, 142.80, 141.34, 140.41, 138.95, 138.30, 136.31, 135.82, 132.42, 131.96, 131.88, 131.54, 130.74, 130.31, 129.61, 128.88, 127.70, 126.55, 110.66, 109.36, 103.41, 53.06, 47.83, 39.68, 39.40, 30.75, 16.25. **HRMS (ESI):** *m/z* calculated for $[M+H]^+$: 667.3067; found: 667.3140.

Synthesis of N-(6-(4-((4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)methyl)phenyl)-5-phenylpyridin-3-yl)-6-cyclopropylnicotinamide (57)

Compound **34** (20.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (43.1 mg, 0.1 mmol, 1.5 eq), K_2CO_3 (15.8 mg, 0.1 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (6.6 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **57** (12.2 mg, 31 %) as a white solid. **1H NMR** (600 MHz, Methanol- d_4) δ 9.02 (d, J = 2.4 Hz, 1H), 8.98 (d, J = 2.0 Hz, 1H), 8.31 (d, J = 2.4 Hz, 1H), 8.23 (dd, J = 8.2, 2.3 Hz, 1H), 7.65 (d, J = 1.5 Hz, 1H), 7.53 (d, J = 8.2 Hz, 2H), 7.45 - 7.37 (m, 4H), 7.31 (dq, J = 9.3, 2.7 Hz, 3H), 7.23 (dd, J = 6.6, 3.0 Hz, 2H), 7.03 (d, J = 8.4 Hz, 1H), 6.42 (dd, J = 17.0, 10.0 Hz, 1H), 6.34 (dd, J = 17.0, 1.8 Hz, 1H), 5.75 (dd, J = 10.0, 1.8 Hz, 1H), 4.58 (s, 2H), 4.52 (tt, J = 12.5, 4.3 Hz, 1H), 3.65 (td, J = 12.8, 3.0 Hz, 3H), 3.43 (d, J = 10.9 Hz, 2H), 3.13 (qd, J = 13.2, 3.7 Hz, 2H), 2.20 (ddd, J = 8.0, 5.6, 3.2 Hz, 1H), 1.85 (d, J = 12.8 Hz, 2H), 1.12 (dt, J = 8.0, 2.7 Hz, 2H), 1.09 (dt, J = 5.1, 2.8 Hz, 2H). **^{13}C NMR** (151 MHz, Methanol- d_4) δ 168.55, 167.04, 166.01, 156.40, 152.89, 149.48, 142.69, 141.49, 140.47, 138.25, 136.99, 136.42, 133.78, 132.47, 131.71, 131.28, 130.73, 130.08, 129.60, 128.86, 128.43, 127.56, 126.68, 122.14, 115.98, 110.54, 103.94, 74.99, 63.77, 24.45, 18.16, 11.37. **HRMS (ESI):** *m/z* calculated for $[M+H]^+$: 690.3114; found: 690.3183.

Synthesis of 6-(4-((4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)methyl)phenyl)-N-(1-methyl-1H-pyrazol-5-yl)-5-phenylnicotinamide (58)

Compound **37** (74.0 mg, 0.2 mmol, 1.0 eq) was reacted with compound **16** (142.6 mg, 0.3 mmol, 1.2 eq), K_2CO_3 (65.4 mg, 0.5 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (27.3 mg, 0.02 mmol, 0.1 eq) using the general procedure A to get the final product **58** (16.3 mg, 11 %) as a white solid. **1H NMR** (600 MHz, DMSO- d_6) δ 10.80 (s, 1H), 10.60 (s, 1H), 10.08 (s, 1H), 9.19 (d, J = 2.2 Hz, 1H), 8.37 (d, J = 2.2 Hz, 1H), 7.71 (s, 1H), 7.42 (d, J = 1.9 Hz, 1H), 7.39 - 7.31 (m, 5H), 7.31 - 7.25 (m, 5H), 6.92 (d, J = 8.4 Hz, 1H), 6.42 (dd, J

= 16.9, 10.2 Hz, 1H), 6.28 (d, J = 1.1 Hz, 1H), 6.25 (dd, J = 17.0, 2.0 Hz, 1H), 5.74 (dd, J = 10.1, 2.0 Hz, 1H), 4.11 (t, J = 13.7 Hz, 1H), 3.74 (s, 3H), 3.54 (s, 2H), 2.95 (d, J = 10.5 Hz, 2H), 2.31 (q, J = 12.1 Hz, 2H), 2.09 (t, J = 11.6 Hz, 2H), 1.66 (d, J = 12.2 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO- d_6) δ 163.88, 162.78, 158.87, 154.00, 147.62, 138.87, 138.67, 137.93, 137.88, 137.49, 135.81, 134.92, 132.64, 132.04, 129.65, 129.39, 128.99, 128.52, 128.37, 127.70, 127.36, 126.34, 124.50, 112.14, 108.65, 100.93, 100.46, 61.47, 54.91, 52.62, 50.12, 35.79, 28.67. **HRMS (ESI):** m/z calculated for $[\text{M}+\text{H}]^+$: 653.2910; found: 653.3107.

Synthesis of 6-(4-((4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)methyl)phenyl)-N-(1-ethyl-1H-pyrazol-5-yl)-5-phenylnicotinamide (59)

Compound **38** (54.0 mg, 0.2 mmol, 1.0 eq) was reacted with compound **16** (99.6 mg, 0.2 mmol, 1.2 eq), K_2CO_3 (45.7 mg, 0.3 mmol, 2.0 eq) and $\text{Pd}(\text{PPh}_3)_4$ (19.1 mg, 0.02 mmol, 0.1 eq) using the general procedure A to get the final product **59** (20.0 mg, 18 %) as a white solid. **^1H NMR** (600 MHz, DMSO- d_6) δ 10.81 (s, 1H), 10.54 (s, 1H), 10.08 (s, 1H), 9.19 (d, J = 2.2 Hz, 1H), 8.36 (d, J = 2.1 Hz, 1H), 7.72 (d, J = 1.9 Hz, 1H), 7.46 (d, J = 1.9 Hz, 1H), 7.39 - 7.32 (m, 5H), 7.31 - 7.25 (m, 5H), 6.92 (d, J = 8.4 Hz, 1H), 6.42 (dd, J = 16.9, 10.2 Hz, 1H), 6.28 - 6.23 (m, 2H), 5.74 (dd, J = 10.1, 2.0 Hz, 1H), 4.13 (td, J = 13.5, 5.9 Hz, 1H), 4.06 (q, J = 7.2 Hz, 2H), 3.54 (s, 2H), 2.95 (d, J = 10.8 Hz, 2H), 2.32 (q, J = 11.3 Hz, 2H), 2.09 (s, 2H), 1.66 (d, J = 12.1 Hz, 2H), 1.33 (t, J = 7.2 Hz, 3H). **^{13}C NMR** (151 MHz, DMSO- d_6) δ 164.16, 162.79, 158.88, 154.02, 147.60, 138.88, 137.91, 137.73, 134.94, 134.93, 132.66, 132.05, 129.66, 129.39, 129.00, 128.52, 128.39, 127.70, 127.36, 126.34, 124.51, 112.16, 108.66, 100.93, 61.46, 54.91, 52.61, 50.11, 42.81, 28.65, 14.96. **HRMS (ESI):** m/z calculated for $[\text{M}+\text{H}]^+$: 667.3067; found: 667.3128.

Synthesis of 6-(4-((4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)methyl)phenyl)-N-(6-cyclopropylpyridin-3-yl)-5-phenylnicotinamide (60)

Compound **39** (33.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (63.1 mg, 0.1 mmol, 1.2 eq), K_2CO_3 (28.9 mg, 0.2 mmol, 2.0 eq) and $\text{Pd}(\text{PPh}_3)_4$ (12.1 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **60** (12.7 mg, 18 %) as a white solid. **^1H NMR** (600 MHz, DMSO- d_6) δ 10.80 (s, 1H), 10.57 (s, 1H), 10.07

(s, 1H), 9.18 (d, $J = 2.2$ Hz, 1H), 8.74 (d, $J = 2.5$ Hz, 1H), 8.36 (d, $J = 2.2$ Hz, 1H), 8.05 (dd, $J = 8.5, 2.6$ Hz, 1H), 7.70 (s, 1H), 7.40 - 7.32 (m, 5H), 7.31 - 7.24 (m, 6H), 6.92 (d, $J = 8.3$ Hz, 1H), 6.41 (dd, $J = 16.9, 10.1$ Hz, 1H), 6.25 (dd, $J = 17.0, 2.0$ Hz, 1H), 5.74 (dd, $J = 10.1, 2.0$ Hz, 1H), 4.11 (tt, $J = 10.9, 3.9$ Hz, 1H), 3.53 (s, 2H), 2.94 (d, $J = 11.1$ Hz, 2H), 2.30 (qd, $J = 12.0, 3.6$ Hz, 2H), 2.14 - 2.03 (m, 3H), 1.65 (d, $J = 11.9$ Hz, 2H), 0.93 (dt, $J = 8.1, 2.8$ Hz, 2H), 0.89 (dt, $J = 5.0, 2.7$ Hz, 2H). **^{13}C NMR** (151 MHz, DMSO- d_6) δ 163.75, 162.77, 158.55, 157.43, 154.00, 147.55, 141.58, 138.97, 138.61, 137.91, 137.60, 134.87, 132.77, 132.64, 132.04, 129.65, 129.40, 128.98, 128.51, 128.34, 128.25, 127.97, 127.67, 126.34, 124.49, 120.91, 112.12, 108.64, 100.91, 61.48, 52.63, 50.13, 28.67, 16.30, 9.47. **HRMS (ESI):** m/z calculated for $[\text{M}+\text{H}]^+$: 690.3114; found: 690.3204.

Synthesis of N-(3-(1-(4-(5-(((1-methyl-1H-pyrazol-5-yl)amino)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (61)

Compound **41** (40.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (80.7 mg, 0.2 mmol, 1.2 eq), K_2CO_3 (37.0 mg, 0.3 mmol, 2.0 eq) and $\text{Pd}(\text{PPh}_3)_4$ (15.5 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **61** (35.2 mg, 41 %) as a white solid. **^1H NMR** (700 MHz, DMSO- d_6) δ 10.80 (s, 1H), 10.08 (s, 1H), 8.67 (d, $J = 2.1$ Hz, 1H), 7.82 (d, $J = 2.1$ Hz, 1H), 7.68 (d, $J = 1.9$ Hz, 1H), 7.33 - 7.26 (m, 4H), 7.25 (d, $J = 8.2$ Hz, 2H), 7.22 (d, $J = 8.2$ Hz, 2H), 7.19 - 7.15 (m, 2H), 7.06 (d, $J = 1.9$ Hz, 1H), 6.91 (d, $J = 8.3$ Hz, 1H), 6.41 (dd, $J = 16.9, 10.2$ Hz, 1H), 6.24 (dd, $J = 17.0, 2.0$ Hz, 1H), 6.13 (t, $J = 6.1$ Hz, 1H), 5.75 - 5.71 (m, 1H), 5.41 (d, $J = 1.9$ Hz, 1H), 4.30 (d, $J = 5.5$ Hz, 2H), 4.10 (tt, $J = 12.4, 4.2$ Hz, 1H), 3.56 (s, 3H), 3.51 (s, 2H), 2.93 (d, $J = 11.4$ Hz, 3H), 2.29 (qd, $J = 12.5, 3.9$ Hz, 2H), 2.07 (td, $J = 11.9, 2.4$ Hz, 2H), 1.64 (dd, 2H). **^{13}C NMR** (176 MHz, DMSO- d_6) δ 162.76, 154.82, 153.99, 148.00, 147.66, 139.63, 138.60, 137.81, 137.73, 137.22, 134.87, 133.78, 132.65, 132.05, 129.51, 129.29, 128.97, 128.38, 128.23, 127.28, 126.32, 124.48, 112.10, 108.63, 100.89, 87.04, 61.53, 52.63, 50.13, 45.76, 34.38, 28.67. **HRMS (ESI):** m/z calculated for $[\text{M}+\text{H}]^+$: 639.3118; found: 639.3186.

Synthesis of 6-(4-((4-(6-acrylamido-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-yl)methyl)phenyl)-N-(1-ethyl-1H-pyrazol-5-yl)-5-phenylnicotinamide (62)

Compound **42** (35.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (67.5 mg, 0.1 mmol, 1.2 eq), K₂CO₃ (30.9 mg, 0.2 mmol, 2.0 eq) and Pd(PPh₃)₄ (12.9 mg, 0.01 mmol, 0.1 eq) using the general procedure A to get the final product **62** (10.4 mg, 14 %) as a white solid. **¹H NMR** (700 MHz, DMSO-*d*₆) δ 10.82 (s, 1H), 10.11 (s, 1H), 8.66 (d, *J* = 2.1 Hz, 1H), 7.81 (d, *J* = 2.1 Hz, 1H), 7.68 (d, *J* = 1.9 Hz, 1H), 7.33 - 7.28 (m, 4H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 8.3 Hz, 2H), 7.18 - 7.15 (m, 2H), 7.07 (d, *J* = 1.9 Hz, 1H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.41 (dd, *J* = 16.9, 10.2 Hz, 1H), 6.24 (dd, *J* = 16.9, 2.0 Hz, 1H), 6.14 (t, *J* = 6.0 Hz, 1H), 5.73 (dd, *J* = 10.1, 2.0 Hz, 1H), 5.40 (d, *J* = 1.9 Hz, 1H), 4.31 (d, *J* = 6.0 Hz, 2H), 4.10 (tt, *J* = 12.5, 4.3 Hz, 1H), 3.93 (q, *J* = 7.2 Hz, 2H), 3.51 (s, 2H), 2.93 (d, *J* = 11.9 Hz, 2H), 2.29 (qd, *J* = 12.0, 3.7 Hz, 2H), 2.07 (td, *J* = 11.9, 2.3 Hz, 2H), 1.64 (dd, *J* = 12.1, 2.5 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 3H). **¹³C NMR** (176 MHz, DMSO-*d*₆) δ 162.76, 154.77, 154.01, 147.59, 147.18, 139.64, 138.59, 137.76, 137.73, 137.39, 134.85, 133.90, 132.65, 132.06, 129.50, 129.27, 128.98, 128.38, 128.22, 127.28, 126.30, 124.51, 112.10, 108.63, 100.89, 87.09, 61.53, 54.91, 52.63, 50.13, 45.73, 41.43, 28.67, 14.66. **HRMS (ESI):** *m/z* calculated for [M+H]⁺: 653.3274; found: 653.3341.

Synthesis of N-(3-(1-(4-(5-(((6-cyclopropylpyridin-3-yl)amino)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (63)

Compound **43** (56.0 mg, 0.2 mmol, 1.0 eq) was reacted with compound **16** (112.0 mg, 0.2 mmol, 1.2 eq), K₂CO₃ (51.4 mg, 0.4 mmol, 2.0 eq) and Pd(PPh₃)₄ (21.5 mg, 0.02 mmol, 0.1 eq) using the general procedure A to get the final product **63** (9.6 mg, 8 %) as a white solid. **¹H NMR** (600 MHz, DMSO-*d*₆) δ 10.79 (s, 1H), 10.07 (s, 1H), 8.65 (d, *J* = 2.1 Hz, 1H), 7.88 (d, *J* = 2.8 Hz, 1H), 7.79 (d, *J* = 2.1 Hz, 1H), 7.68 (s, 1H), 7.39 - 7.20 (m, 7H), 7.19 - 7.12 (m, 2H), 6.97 (d, *J* = 8.4 Hz, 1H), 6.94 - 6.88 (m, 2H), 6.41 (dd, *J* = 16.9, 10.2 Hz, 1H), 6.30 (t, *J* = 6.2 Hz, 1H), 6.24 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.73 (dd, *J* = 10.1, 2.0 Hz, 1H), 4.39 (d, *J* = 6.2 Hz, 2H), 4.11 (s, 1H), 3.51 (s, 2H), 2.94 (d, *J* = 10.9 Hz, 2H), 2.34 - 2.27 (m, 2H), 2.07 (s, 2H), 1.88 (tt, *J* = 8.1, 4.9 Hz, 1H), 1.65 (d, *J* = 12.0 Hz, 2H), 0.76 (dt, *J* = 8.1, 2.7 Hz, 2H), 0.71 (dt, *J* = 5.1, 2.8 Hz, 2H). **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 162.76, 154.74, 153.99, 149.29, 147.54, 142.07, 139.59, 137.67, 134.96, 134.72, 133.90, 132.63, 132.03, 129.52, 129.27, 128.97, 128.37, 128.23, 127.29, 126.33, 124.49, 121.05, 119.28, 112.13, 108.63, 100.91,

61.51, 52.60, 50.12, 43.55, 28.69, 15.71, 8.31. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 676.3322; found: 676.3397.

Synthesis of N-(3-(1-(4-(5-(((1-methyl-1H-pyrazol-5-yl)oxy)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (64)

Compound **44** (15.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (30.2 mg, 0.1 mmol, 1.2 eq), K_2CO_3 (13.8 mg, 0.1 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (5.8 mg, 0.005 mmol, 0.1 eq) using the general procedure A to get the final product **64** (17.3 mg, 54 %) as a white solid. **1H NMR** (600 MHz, $DMSO-d_6$) δ 10.80 (s, 1H), 10.07 (s, 1H), 8.78 (d, $J = 2.1$ Hz, 1H), 7.92 (d, $J = 2.1$ Hz, 1H), 7.69 (s, 1H), 7.35 - 7.26 (m, 6H), 7.26 - 7.18 (m, 5H), 6.91 (d, $J = 8.3$ Hz, 1H), 6.41 (dd, $J = 16.9, 10.2$ Hz, 1H), 6.24 (dd, $J = 17.0, 2.0$ Hz, 1H), 5.82 (d, $J = 2.0$ Hz, 1H), 5.73 (dd, $J = 10.1, 2.0$ Hz, 1H), 5.29 (s, 2H), 4.11 (ddd, $J = 16.5, 12.2, 4.0$ Hz, 1H), 3.57 (s, 3H), 3.52 (s, 2H), 2.94 (d, $J = 11.1$ Hz, 2H), 2.30 (qd, $J = 12.1, 3.7$ Hz, 2H), 2.13 - 2.03 (m, 2H), 1.70 - 1.57 (m, 2H). **^{13}C NMR** (151 MHz, $DMSO-d_6$) δ 162.76, 156.17, 153.99, 153.48, 147.85, 139.23, 138.35, 138.01, 137.25, 135.11, 132.64, 132.04, 130.21, 129.57, 129.33, 128.98, 128.42, 128.27, 127.44, 126.33, 124.49, 112.12, 108.63, 100.90, 85.90, 70.21, 61.50, 52.61, 50.12, 33.58, 28.66. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 640.2958; found: 640.3023.

Synthesis of N-(3-(1-(4-(5-(((1-ethyl-1H-pyrazol-5-yl)oxy)methyl)-3-phenylpyridin-2-yl)benzyl)piperidin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)acrylamide (65)

Compound **45** (18.0 mg, 0.1 mmol, 1.0 eq) was reacted with compound **16** (34.6 mg, 0.1 mmol, 1.2 eq), K_2CO_3 (15.9 mg, 0.1 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (6.6 mg, 0.006 mmol, 0.1 eq) using the general procedure A to get the final product **65** (13.1 mg, 35 %) as a white solid. **1H NMR** (600 MHz, $DMSO-d_6$) δ 10.79 (s, 1H), 10.07 (s, 1H), 8.77 (d, $J = 2.0$ Hz, 1H), 7.91 (d, $J = 2.0$ Hz, 1H), 7.69 (s, 1H), 7.37 - 7.26 (m, 6H), 7.26 - 7.18 (m, 5H), 6.91 (d, $J = 8.3$ Hz, 1H), 6.41 (dd, $J = 16.9, 10.1$ Hz, 1H), 6.24 (dd, $J = 17.0, 2.0$ Hz, 1H), 5.82 (d, $J = 2.0$ Hz, 1H), 5.73 (dd, $J = 10.1, 2.0$ Hz, 1H), 5.30 (s, 2H), 4.11 (td, $J = 11.8, 5.9$ Hz, 1H), 3.93 (q, $J = 7.2$ Hz, 2H), 3.52 (s, 2H), 2.94 (d, $J = 11.1$ Hz, 2H), 2.29 (qd, $J = 12.3, 3.8$ Hz, 2H), 2.08 (t, $J = 11.6$ Hz, 2H), 1.65 (d, $J = 12.0$ Hz, 2H), 1.23 (t, $J = 7.2$ Hz, 3H). **^{13}C NMR** (151 MHz, $DMSO-d_6$) δ 162.76, 156.15, 153.99,

152.84, 147.81, 139.22, 138.31, 137.28, 135.10, 132.63, 132.04, 130.25, 129.55, 129.30, 128.97, 128.42, 128.25, 127.44, 126.32, 124.48, 112.10, 108.62, 100.89, 86.00, 70.18, 61.51, 52.62, 50.13, 41.22, 28.67, 14.76. **HRMS (ESI):** m/z calculated for $[M+H]^+$: 654.3114; found: 654.3195.

5.9.3. Protein-Mass Spectroscopy

Akt1, Akt2, Akt3 wild-type protein solution was diluted with MS buffer (50 mM HEPES, 200 mM NaCl, 2 % glycerol, pH 7.3) to a final concentration of 0.5 mg/mL. Subsequently, each sample was incubated with three molar equivalents of compound. The mixtures were incubated at 4 °C for 3 up to 24 hours to allow complex formation. Following incubation, the samples were centrifuged for 10 minutes at 21,000 × g and 4 °C to remove potential aggregates or precipitates. The samples were analyzed by mass spectrometry using a Thermo Fisher Scientific Ultimate 3000 HPLC system connected with a Thermo Fisher Scientific Velos Pro (2D ion trap). The sample (5 µL) was injected onto an AdvanceBio Desalting-RP Cartridge (Agilent Technologies) starting at 80 % solvent A (0.1 % formic acid in H₂O) and 20 % solvent B (0.1 % formic acid in MeCN) for 30 seconds, followed by a linear gradient over 30 seconds up to 90 % solvent B and an isocratic elution for 4.5 minutes. A mass range of 700-2000 m/z was scanned and raw data were deconvoluted and analyzed using XCALIBUR and MAGTRAN software to obtain the molecular weight profiles of the protein-ligand complexes. The analysis of the masses was conducted through extraction of the mass list from MAGTRAN and analysis in OriginPro 2023 software.

5.9.4. Akt3 Homology-Model

The Akt3 homology model was generated with SWISS-MODEL using the human Akt3 UniProt sequence (Q9Y243-1) and PDB entry 6HHJ as the template structure.¹⁸⁻²²

5.9.5. Molecular Docking

Compound **8** was subjected to an energy minimization using the Merck Molecular Force Field 94 (MMFF94s) and prepared with LigandScout (4.4.3).^{23, 24} Docking studies were performed using SeeSAR (version 13.0.5, BioSolveIT GmbH). Crystal structures of Akt1 and Akt2 were retrieved from the Protein Data Bank (PDB IDs: 6HHF and 8Q61, respectively), while a homology model generated by SWISS-MODEL was used for Akt3. Protein structures were prepared using Protoss as implemented in

SeeSAR (BioSolveIT GmbH).²⁵ The binding sites for Akt1 and Akt2 were automatically defined based on the extracted co-crystallized ligand. For the Akt3 homology model, the ligand from the parent Akt1 structure (PDB: 6HHJ) was positioned in the allosteric pocket to define the binding site. Docking parameters were set to a maximum of 20 poses, standard clash tolerance and all possible ring conformations allowed. Standard and template docking were performed using the FlexX algorithm and poses were scored with the HYDE scoring function. Binding modes were visually inspected using torsional and HYDE scoring and the pose displaying the most favorable interactions was selected for further analysis and visualization.^{26, 27}

5.9.6. Cell Culture

HEK293T cells were maintained in DMEM GlutaMAX (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (Gibco). Cultures were kept at 37 °C in a humidified atmosphere containing 5% CO₂. Upon reaching approximately 80% confluence, cells were passaged following a 5 min incubation with 0.25% Trypsin-EDTA (Gibco).

5.9.6.1 Cellular Evaluation of Inhibitors by NanoBRET Target Engagement Assay

For NanoBRET assays, cells were transiently transfected in suspension using a lipid-based transfection reagent together with plasmid DNA encoding the respective NanoLuc-Akt fusion constructs (Akt1-NanoLuc (NV2411), Akt2-NanoLuc (NV1031), or Akt3-NanoLuc; Promega). Transfection complexes were prepared in OPTI-MEM reduced-serum medium (Gibco) at a reagent-to-DNA ratio of 30:1 and incubated at room temperature for 20 min to allow complex formation. The resulting complexes were mixed with a cell suspension adjusted to a concentration of $\sim 2 \times 10^5$ cells/mL. The transfected cells were seeded into 384-well plates (Greiner Bio-One) at a final volume of 25 μ L per well, corresponding to approximately 5,000 cells, using a Multidrop dispenser (Thermo). After 24 h, cells were treated with test compounds over a concentration range of 20 μ M to 1.2 nM in the presence of 0.5 μ M NanoBRET tracer K10 (Promega). Following a 3 h incubation, the NanoGlo substrate and extracellular NanoLuc inhibitor (N2162, Promega) were prepared in OPTI-MEM to generate a detection solution, which was added to the assay plates at 10 μ L per well. BRET signals were measured using an EnVision plate reader in dual-emission mode, detecting donor

emission at ~450 nm and acceptor emission at ~600 nm. Data were normalized to the positive control (30 μ M staurosporine) and the vehicle control (DMSO). The BRET ratio was calculated from raw donor and acceptor signals as the acceptor-to-donor emission ratio. For inhibitor titration experiments, BRET ratios were normalized to vehicle-treated samples to determine fractional target engagement or tracer displacement. Concentration-response curves were generated by plotting normalized BRET signals against inhibitor concentration and fitting the data with a four-parameter logistic equation in Origin (OriginLab) to determine potency values. Each condition was measured in duplicate per plate and independently repeated three times.

5.9.7 Protein Expression, Purification and Crystallization

Synthetic genes encoding Akt1 (2-480; UniProt P31749), Akt2 (2-481), Akt3 (2-479), and an Akt1 mutant (S205T, D262E, E267del, K268R, N269D) were designed with an N-terminal His₆ tag followed by a TEV protease cleavage site and purchased from GeneArt (Germany). The genes were cloned into the pLEx/Bac3 vector (Merck Millipore) via the NcoI and BamHI restriction sites. Recombinant baculoviruses were produced and amplified in Sf9 cells using the BacMagic system (Merck Millipore). Protein expression was carried out in Sf9 suspension cultures grown in Erlenmeyer flasks at 27 °C and 120 rpm for 72 h post-infection. Cells were harvested by centrifugation (3,000 $\times$ g, 15 min), washed with PBS, and flash-frozen in liquid nitrogen. Thawed pellets were resuspended in lysis buffer (50 mM Tris, 500 mM NaCl, 1 mM DTT, 10% glycerol, 0.1% Triton X-100, pH 8.0) supplemented with EDTA-free protease inhibitors (Sigma). Cells were lysed using a microfluidizer, and debris was removed by centrifugation at 40,000 $\times$ g for 1 h. The clarified lysate was loaded onto a Ni-NTA Superflow column (Qiagen), and bound protein was eluted with buffer containing 500 mM imidazole (50 mM Tris, 500 mM NaCl, 1 mM DTT, 10% glycerol, pH 8.0). The His₆ tag was cleaved by addition of TEV protease, followed by overnight dialysis at 4 °C against 25 mM Tris, 50 mM NaCl, 1 mM DTT, and 5% glycerol (pH 8.0). Proteins were further purified by size-exclusion chromatography on a Superdex 75 column (GE Healthcare) equilibrated in 50 mM HEPES, 200 mM NaCl, 1 mM DTT, and 10% glycerol (pH 7.3). For storage, proteins were exchanged into 25 mM Tris, 100 mM NaCl, 1 mM DTT, and 10% glycerol (pH 7.5) using a Superdex 75 10/300 GL column, concentrated as needed, and stored at -80 °C. For crystallization, purified protein (3 mg/mL) was incubated with a threefold molar excess of inhibitor on ice for 180 min. Samples were

centrifuged ($20,000 \times g$, 10 min, 4 °C) prior to preparing hanging drops in 15-well plates (EasyXtal Tool, Qiagen) by mixing protein-ligand complex with reservoir solution (5-8% PEG 1500, 0.1 M MMT buffer pH 5.0-5.3) and seed stock (1:1:0.3). Seed stocks were derived from crystals grown with inhibitor 62 under conditions of 10% PEG 1500 and 0.1 M MMT buffer (pH 5.3). Crystals of compounds 62, 64, and 65 were cryoprotected in 20% ethylene glycol before flash-cooling in liquid nitrogen. Diffraction data were collected at the European Synchrotron Radiation Facility (ESRF). Data processing was performed with XDS and scaling with XSCALE.²⁸ Structures were solved by molecular replacement using PHASER²⁹ with PDB entry 7NH4 as the search model. Model building was carried out in COOT,³⁰ followed by iterative refinement with PHENIX.refine.³¹ Final models were validated using MolProbity³ and the PDB validation server. Structural figures were generated with PyMOL.³²

5.10 References

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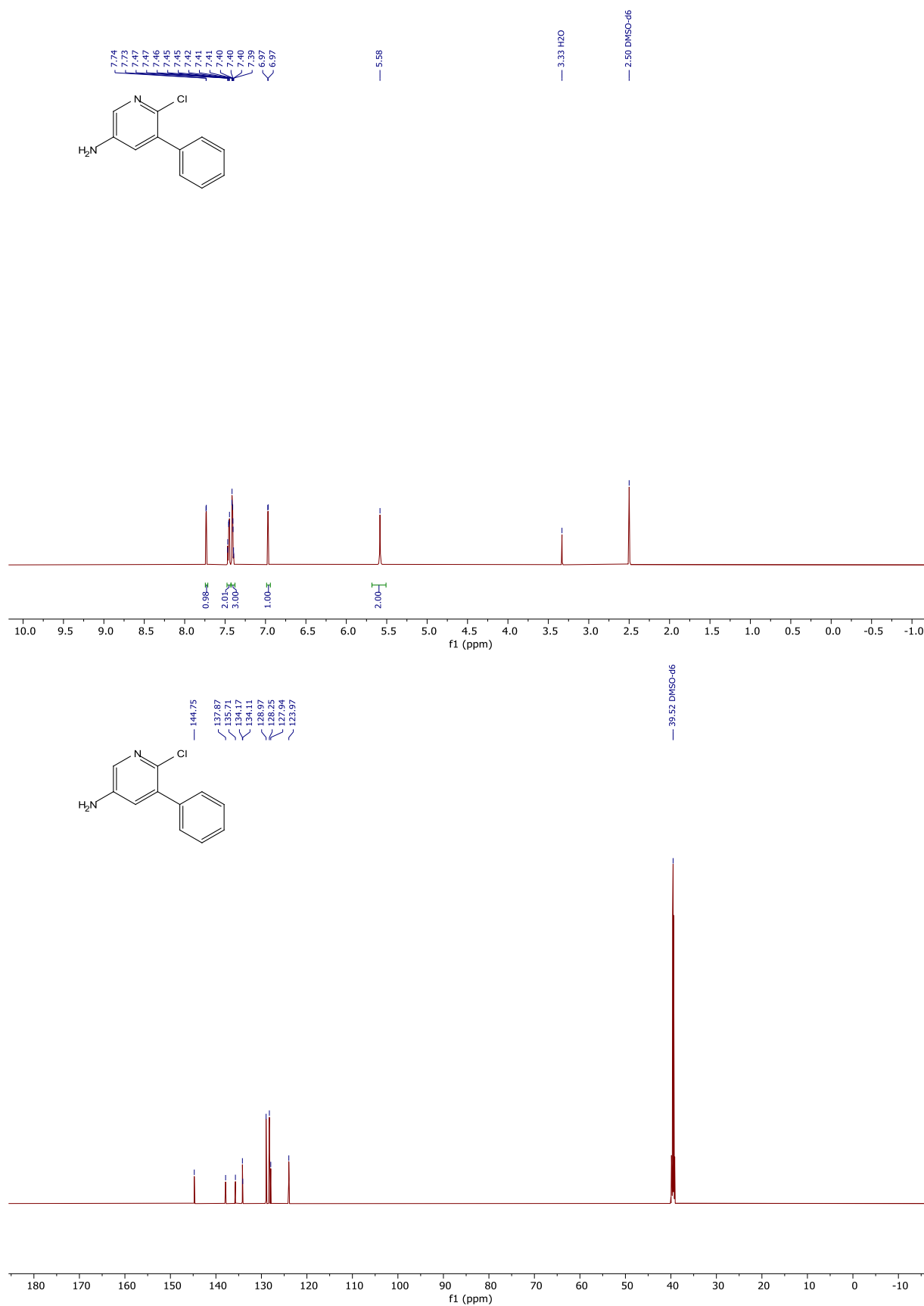
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5.11. Supporting Information

5.11.1. ^1H and ^{13}C NMR-Spectra for All the Compounds: S1-S54



FigureS1. ^1H - and ^{13}C -NMR spectra of compound 11.

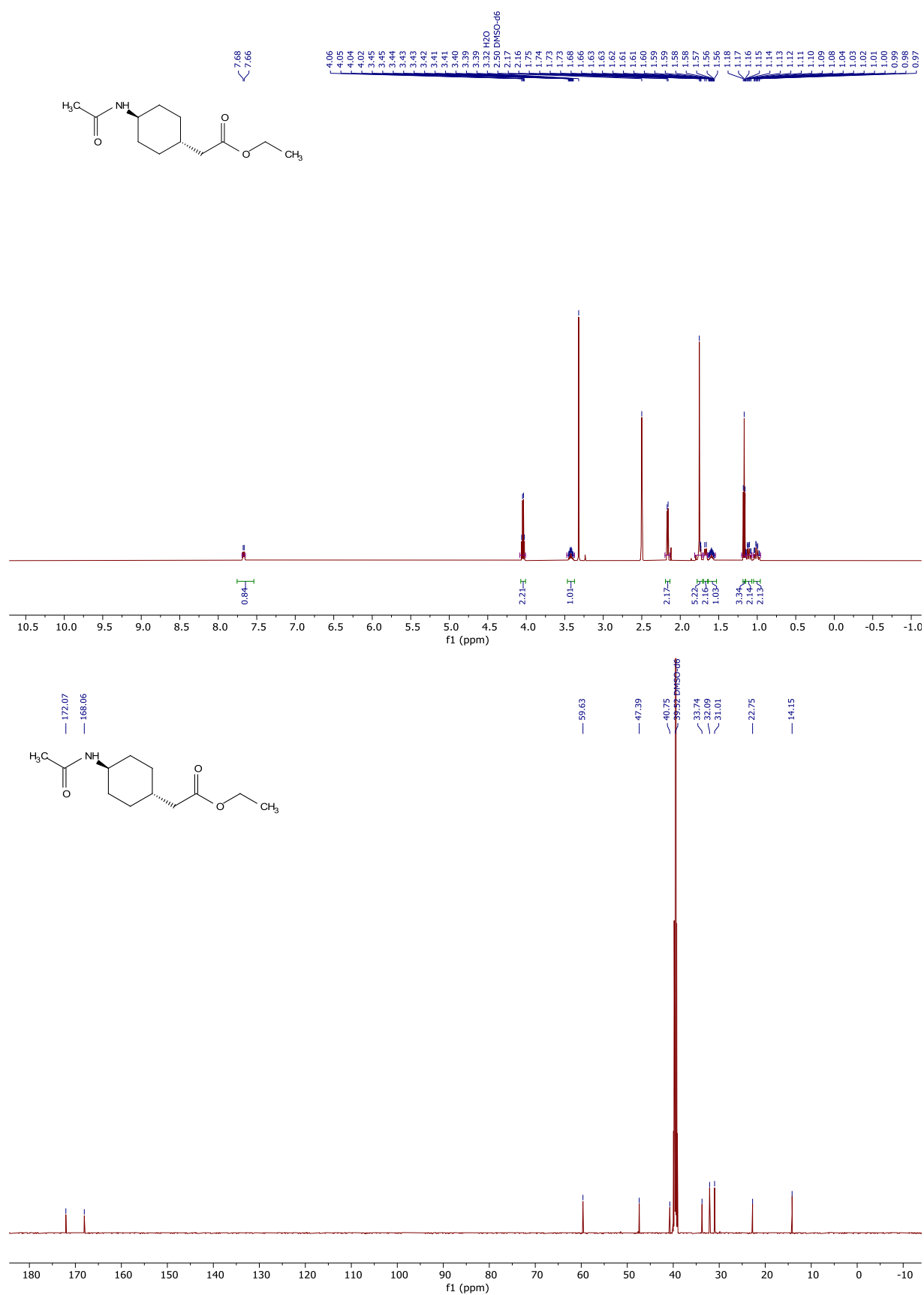


Figure S2. ¹H- and ¹³C-NMR spectra of compound 13.

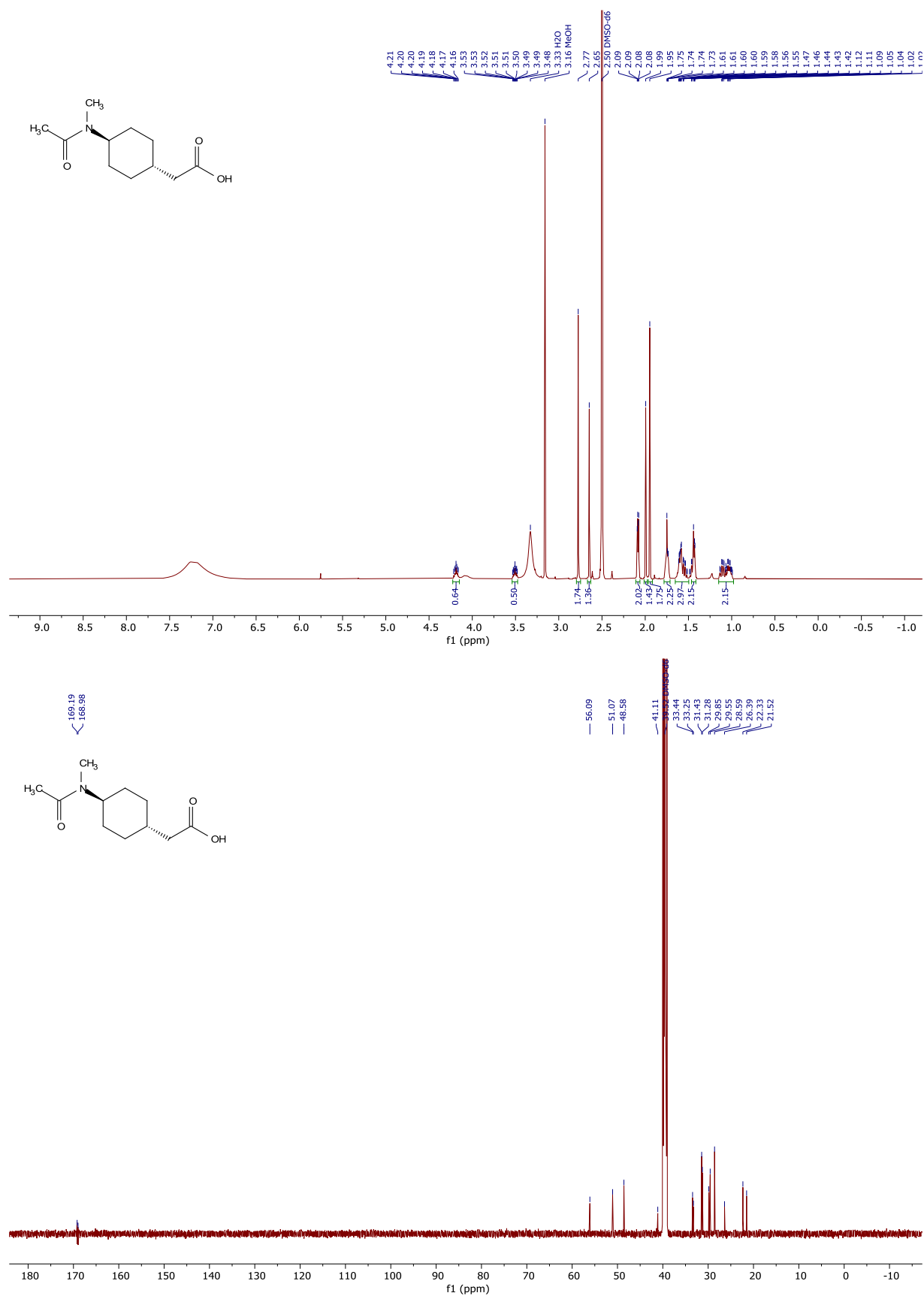


Figure S3. ¹H- and ¹³C-NMR spectra of compound 14.

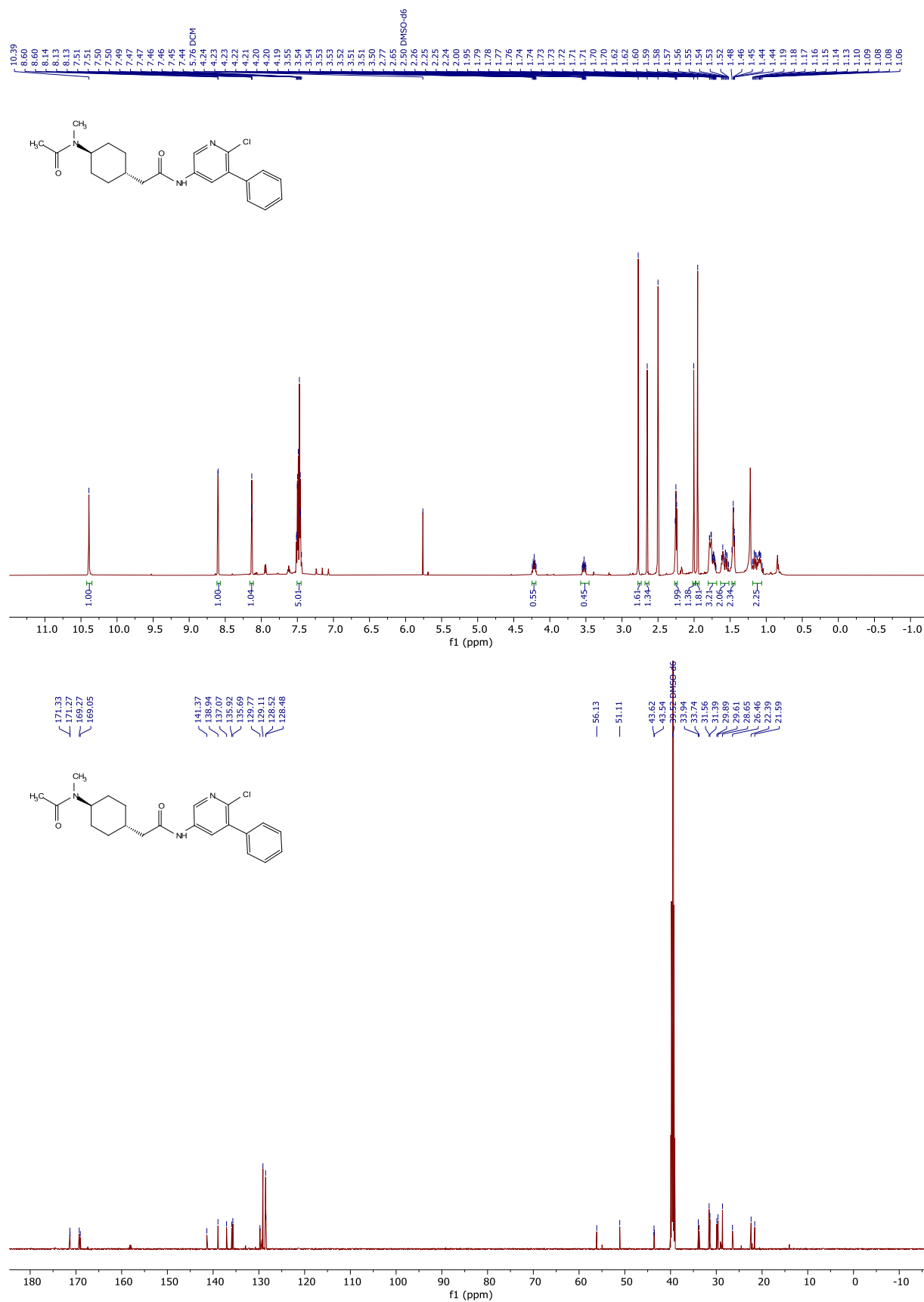


Figure S4. ¹H- and ¹³C-NMR spectra of compound 15.

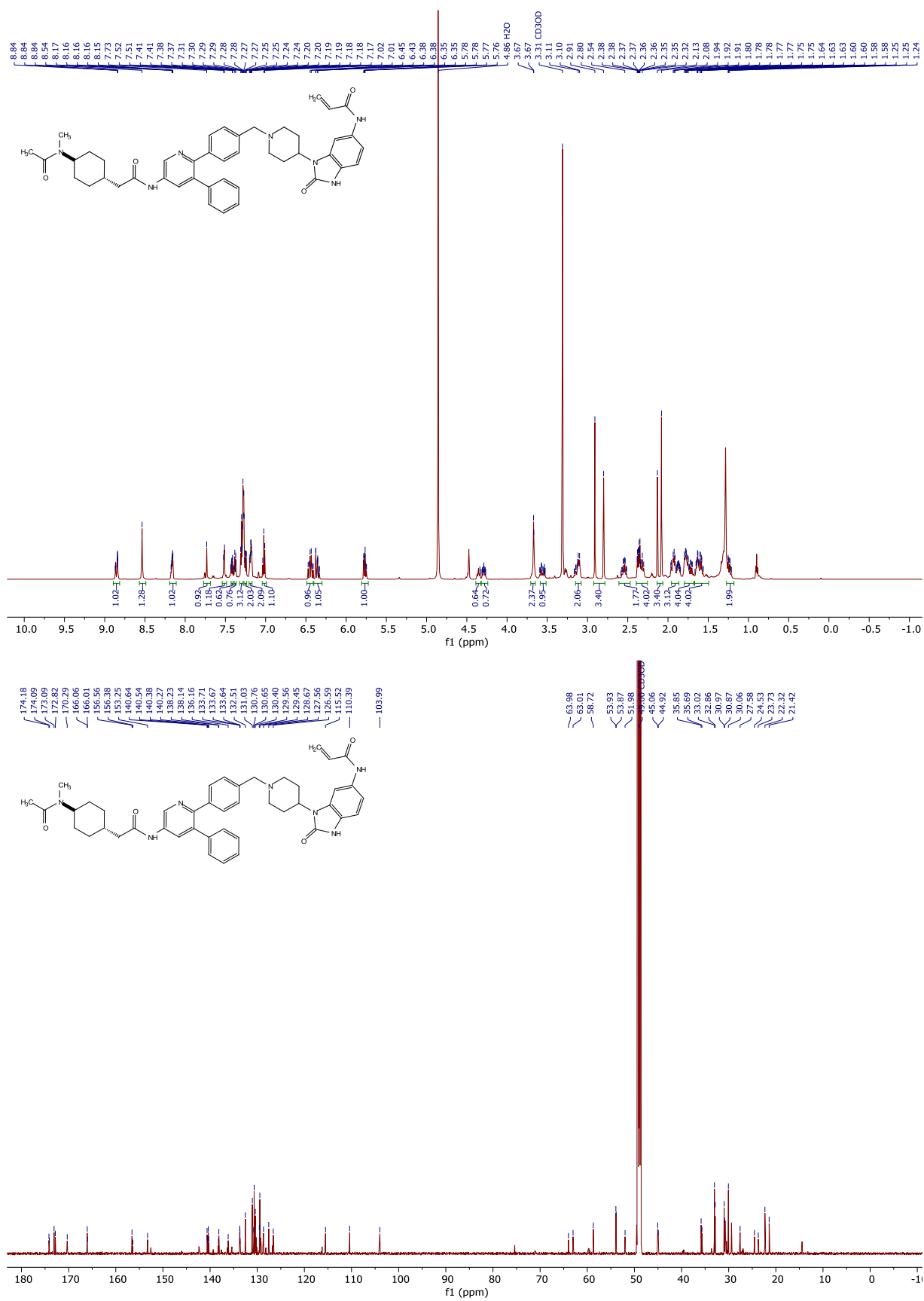


Figure S6. ^1H - and ^{13}C -NMR spectra of compound **9** in Methanol- d_6 .

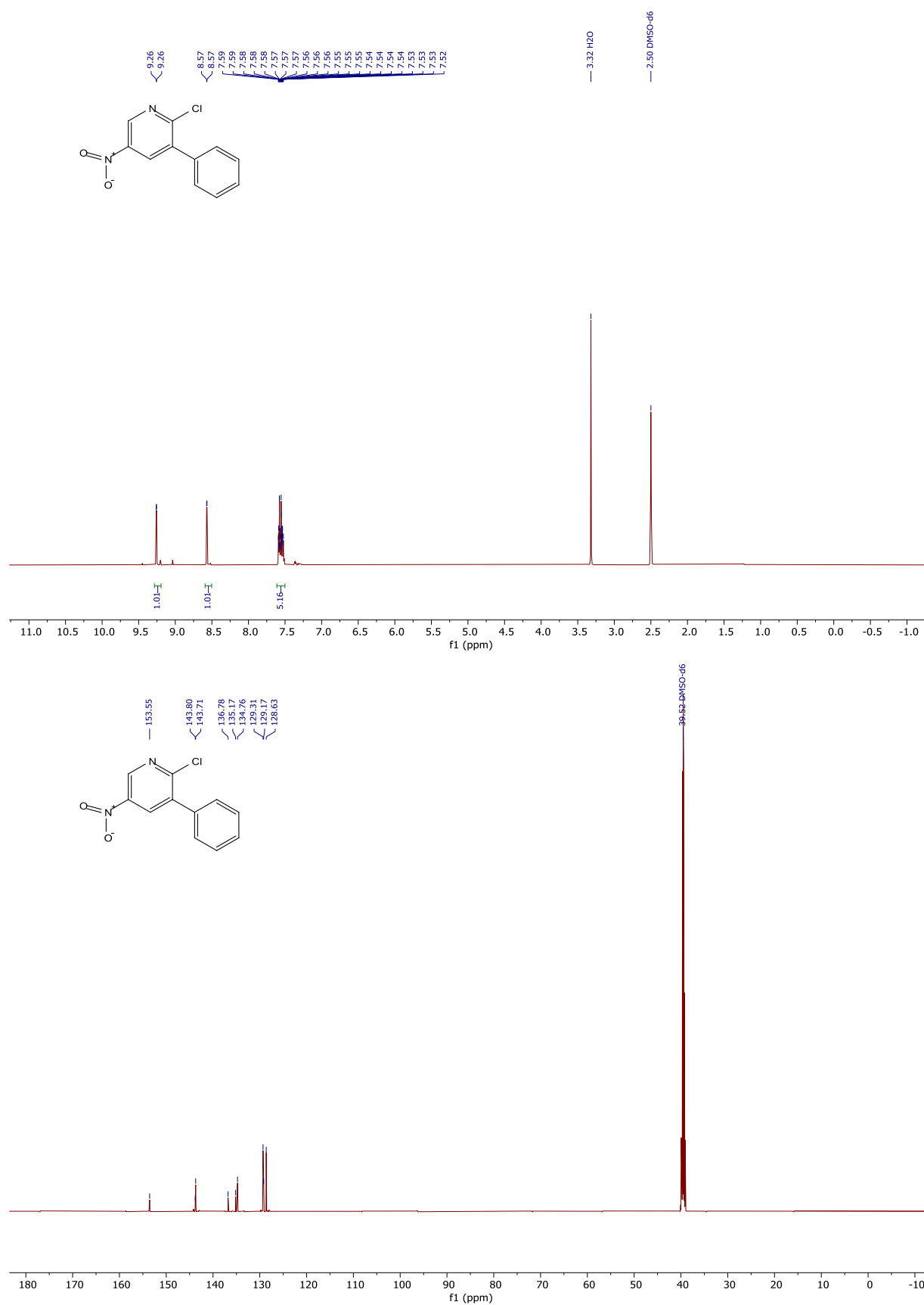


Figure S7. ¹H- and ¹³C-NMR spectra of compound **18**.

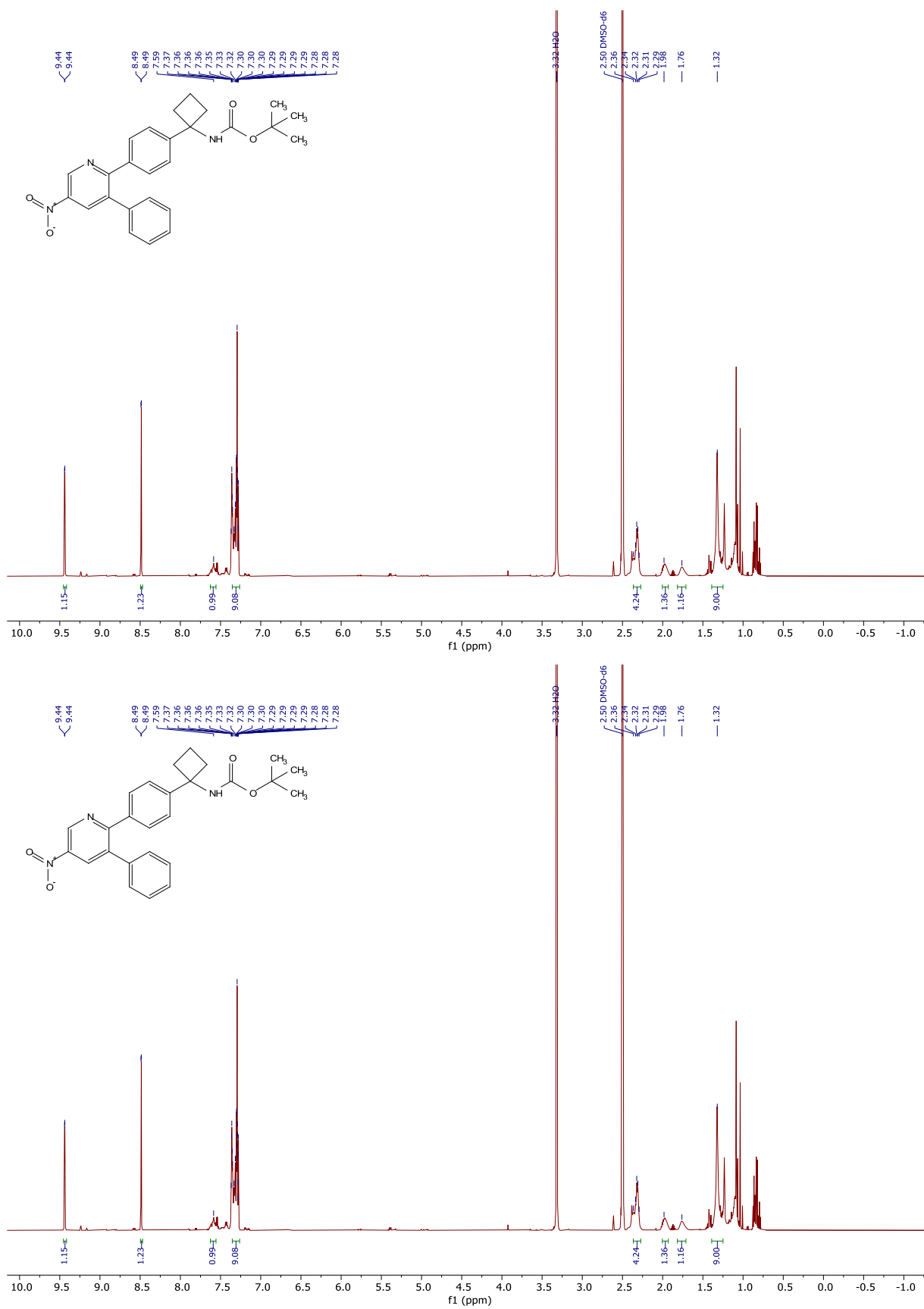


Figure S8. ¹H- and ¹³C-NMR spectra of compound 19a.

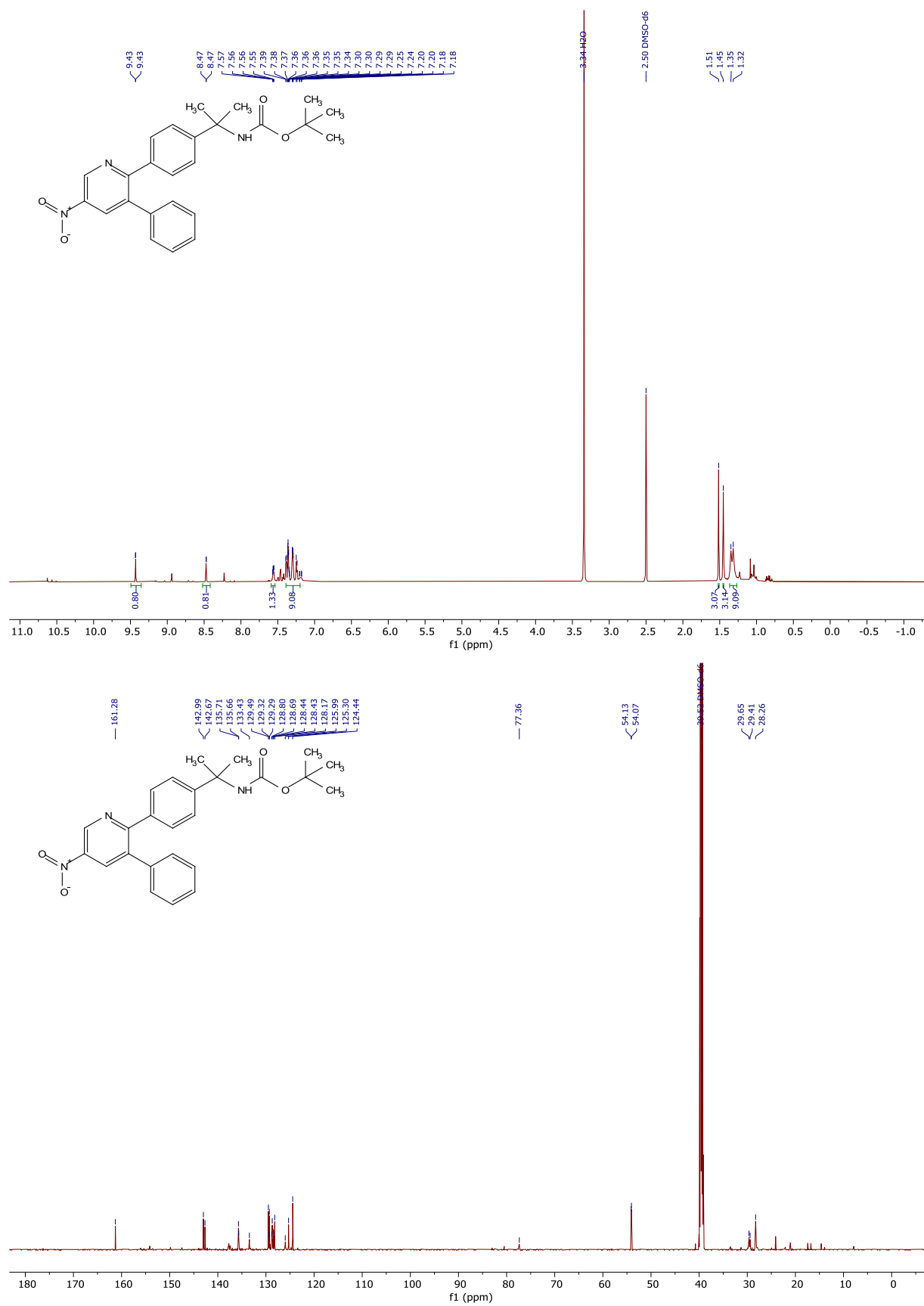


Figure S9. ¹H- and ¹³C-NMR spectra of compound 19b.

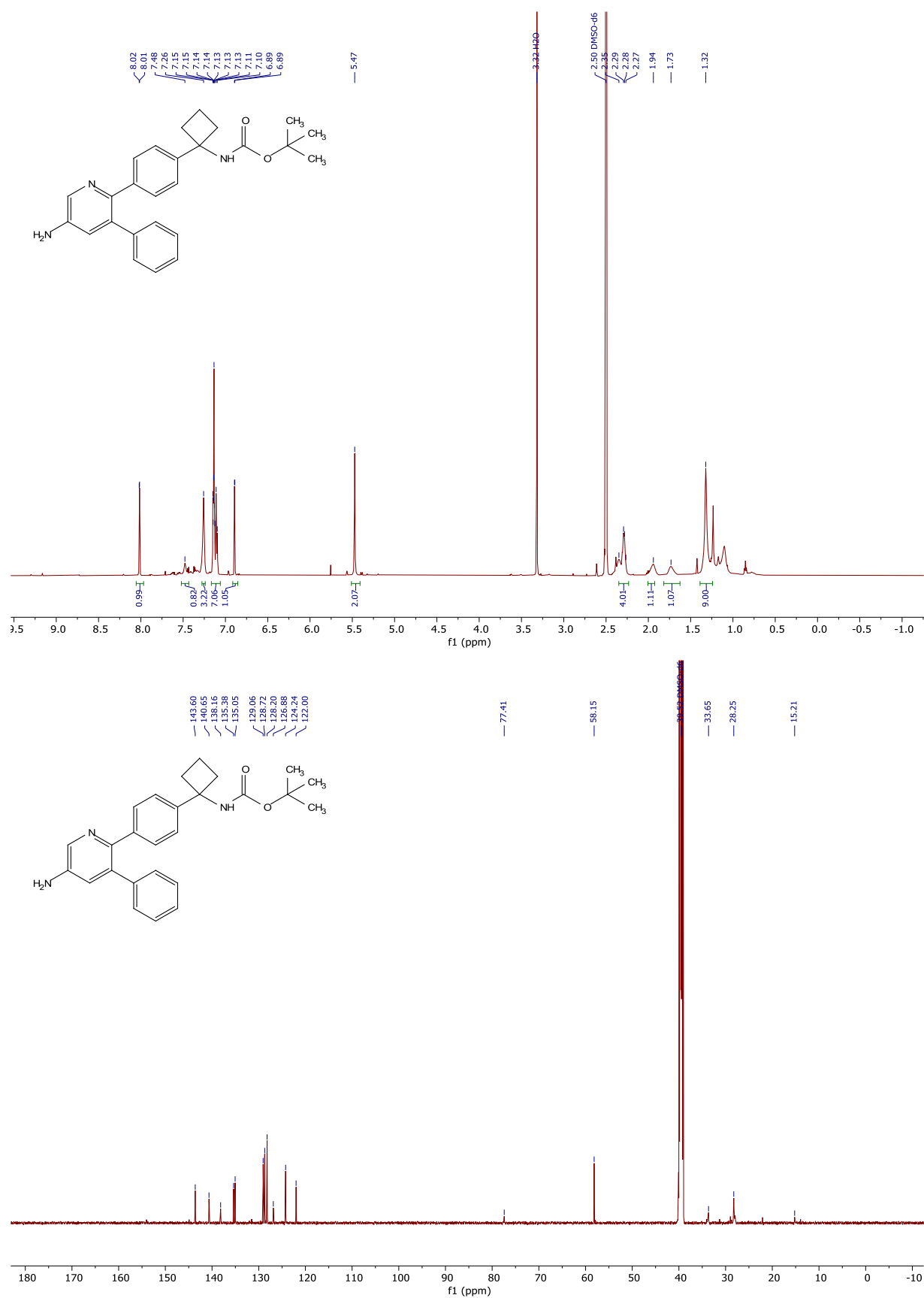


Figure S10. ^1H - and ^{13}C -NMR spectra of compound 20a.

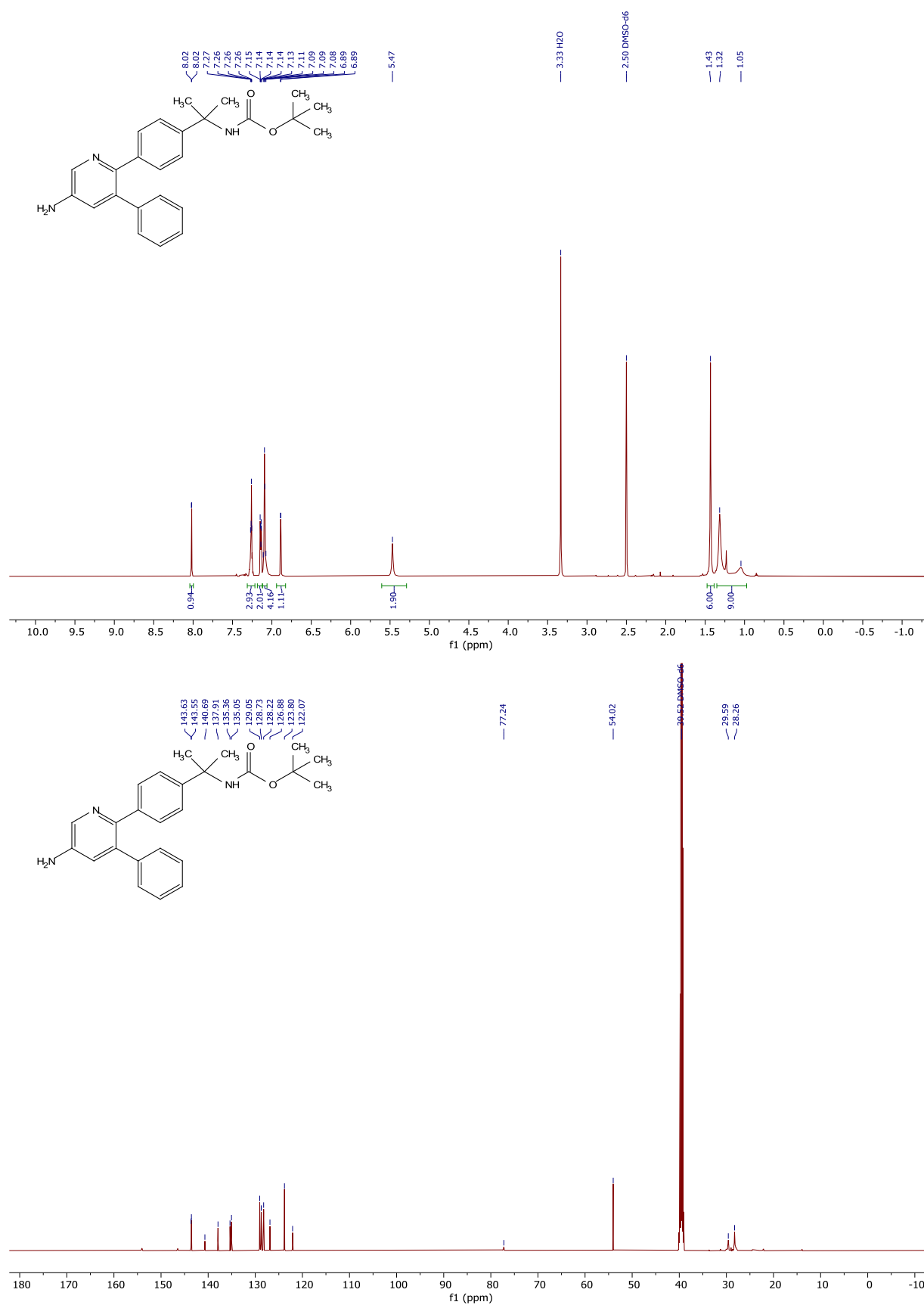


Figure S11. ^1H - and ^{13}C -NMR spectra of compound **20b**.

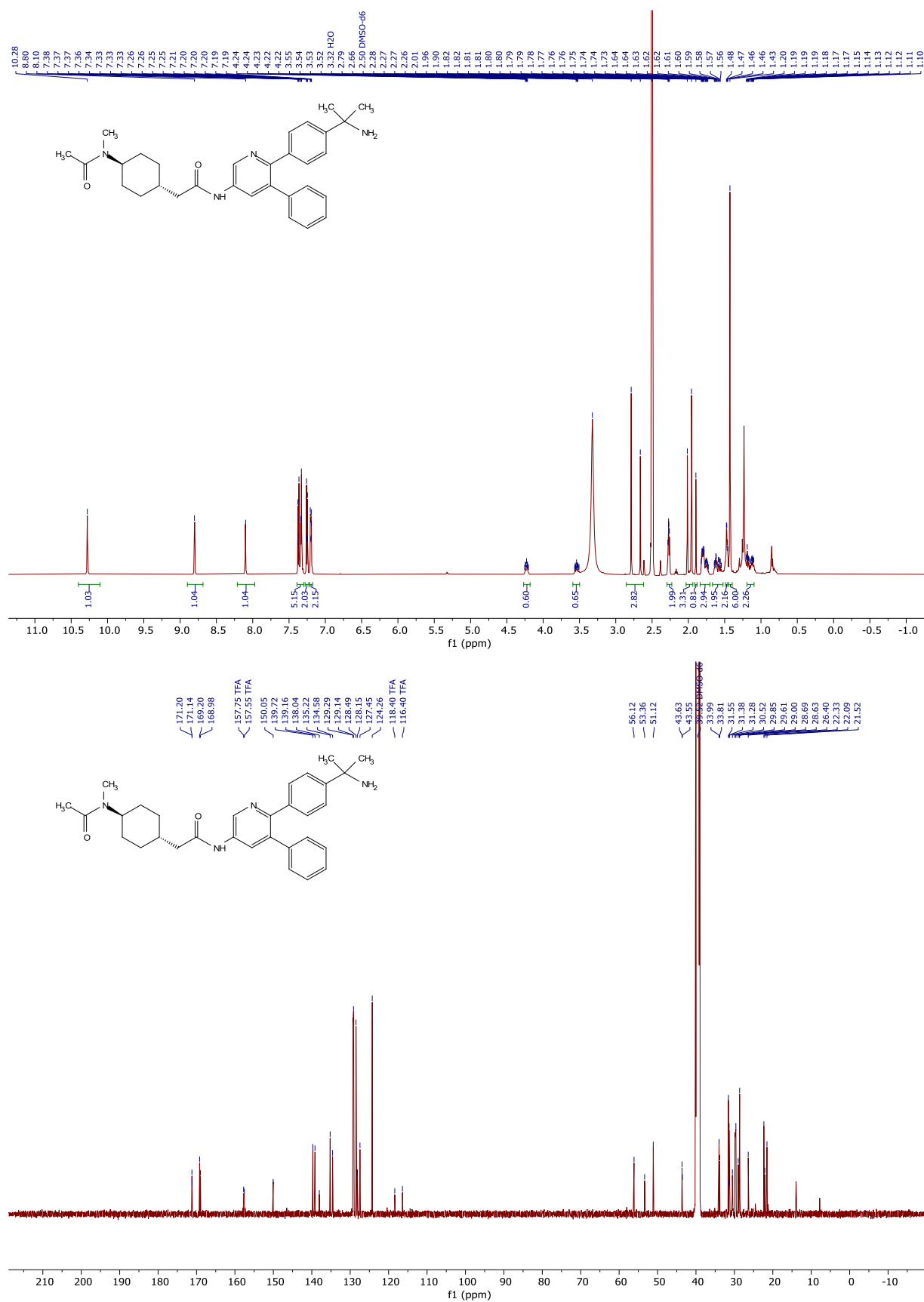


Figure S13. ^1H - and ^{13}C -NMR spectra of compound 22.

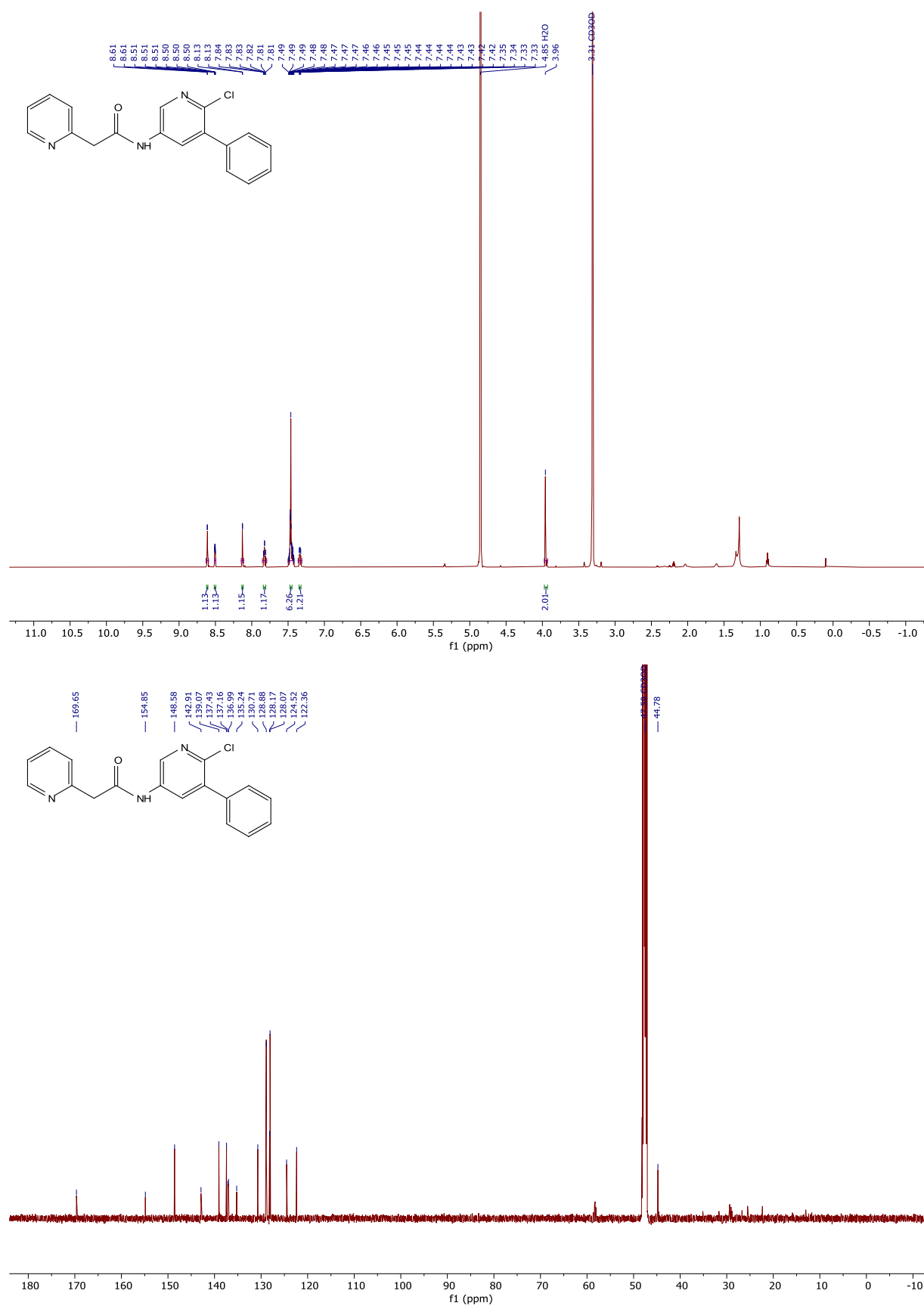


Figure S14. ¹H- and ¹³C-NMR spectra of compound 23.

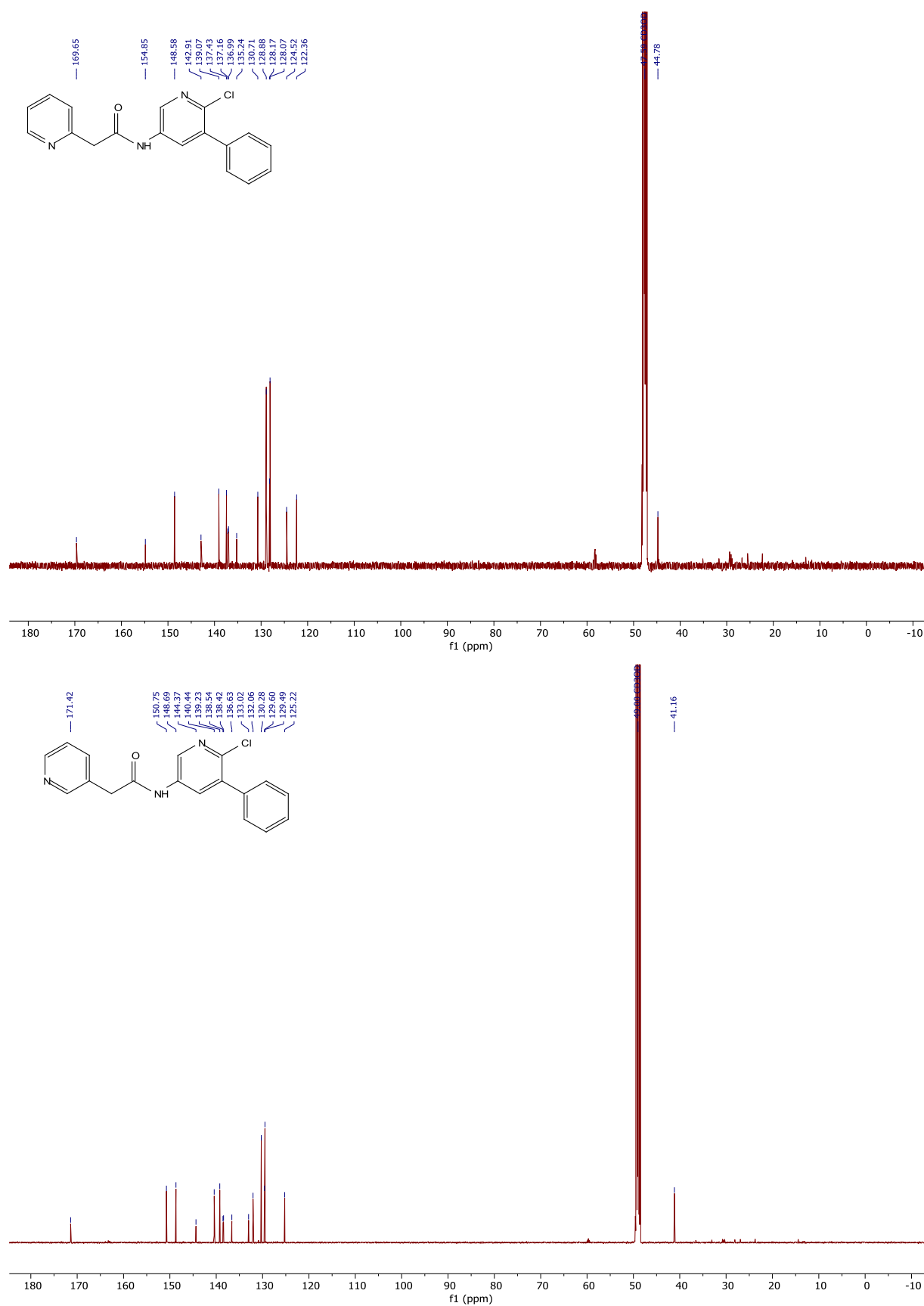


Figure S15. ¹H- and ¹³C-NMR spectra of compound **24**.

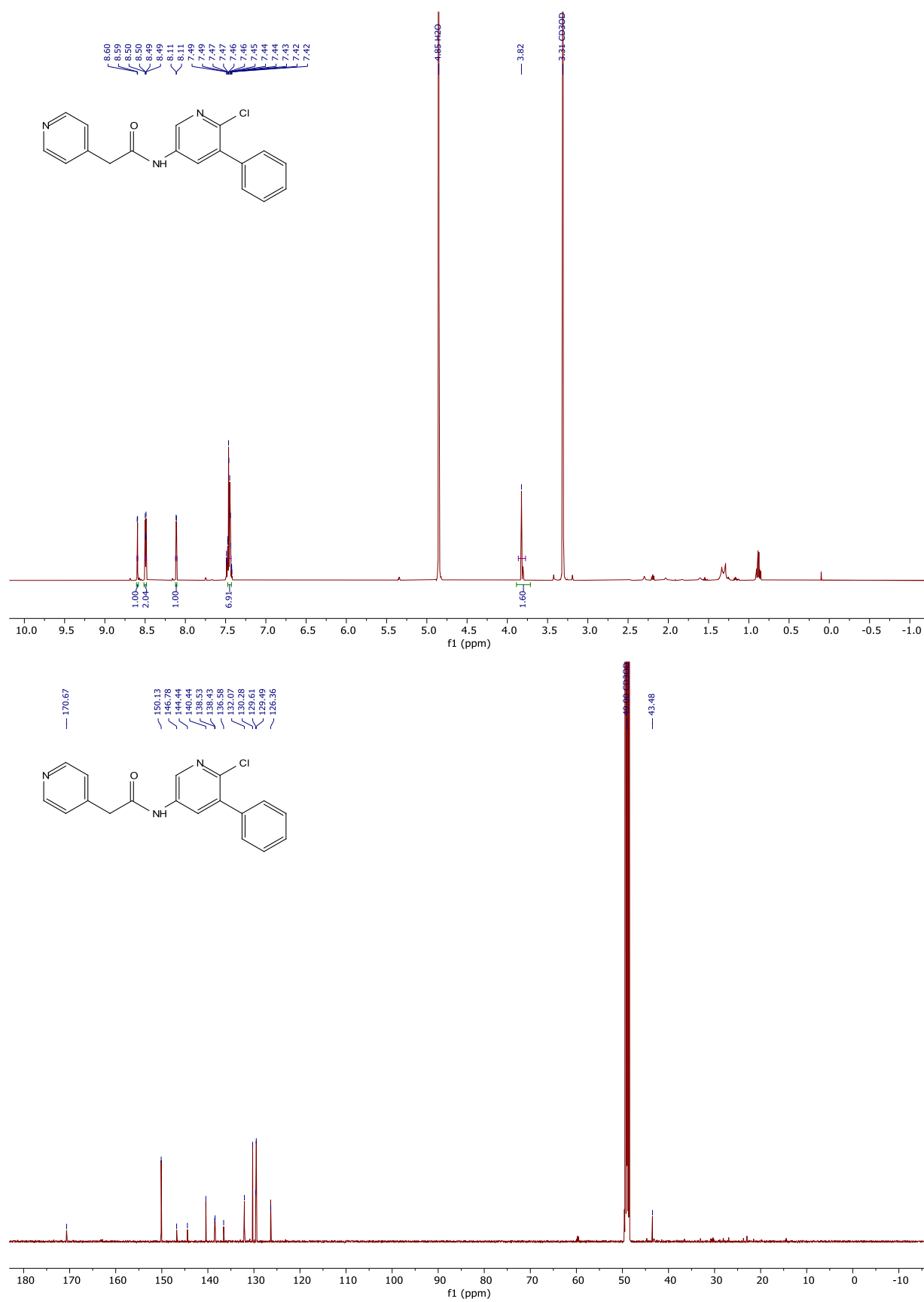


Figure S16. ¹H- and ¹³C-NMR spectra of compound 25.

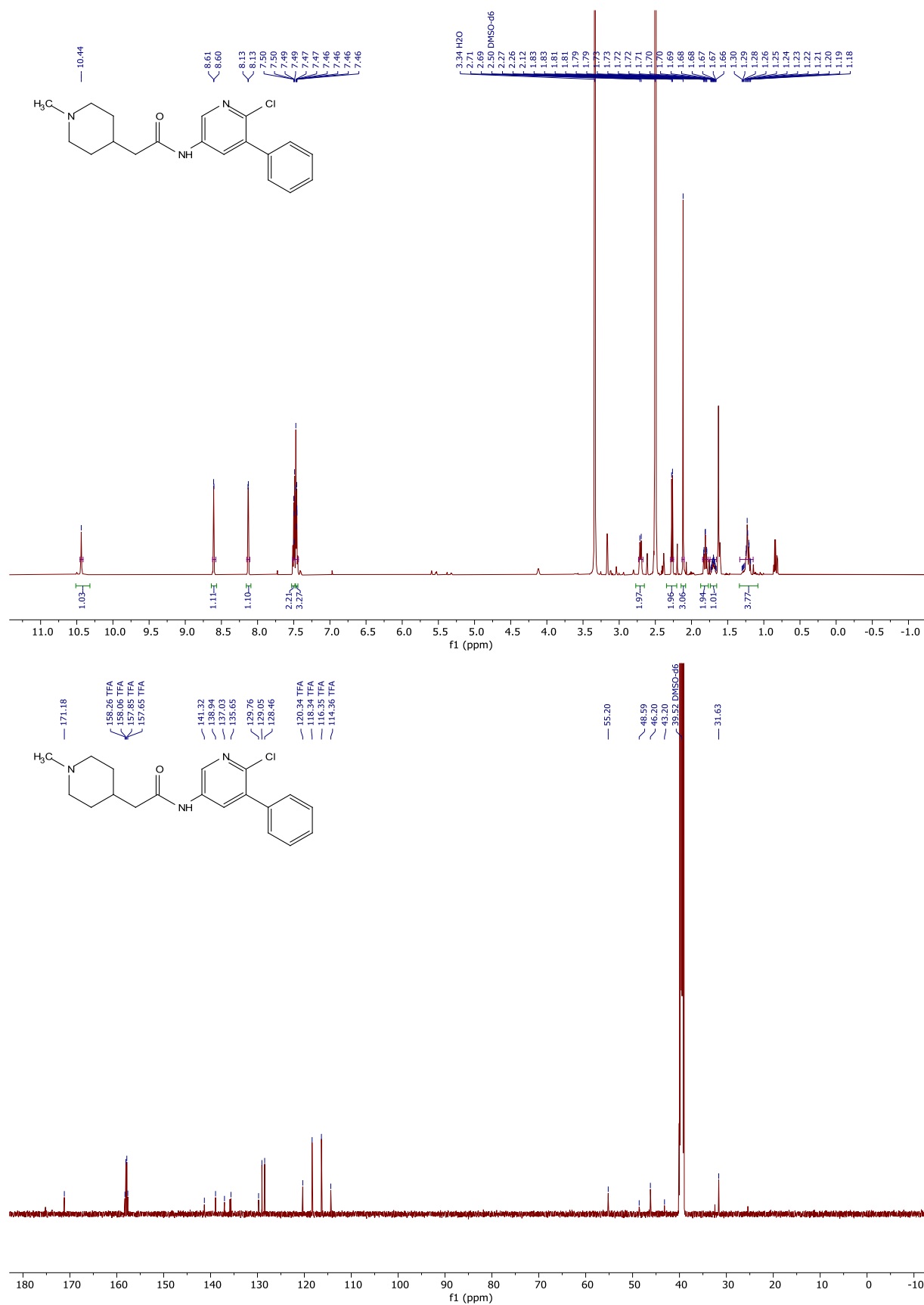


Figure S17. ¹H- and ¹³C-NMR spectra of compound **26**.

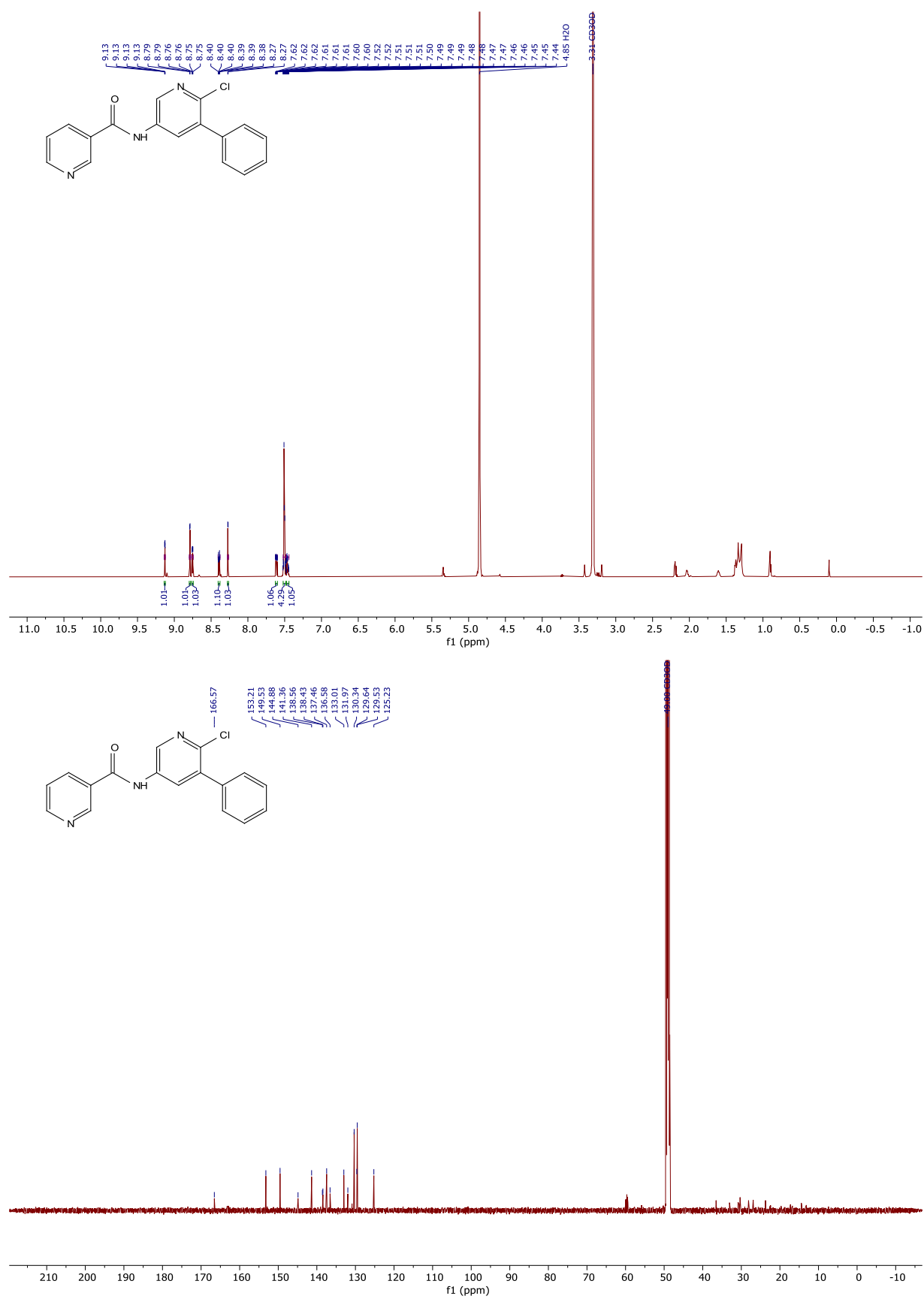


Figure S18. ^1H - and ^{13}C -NMR spectra of compound 27.

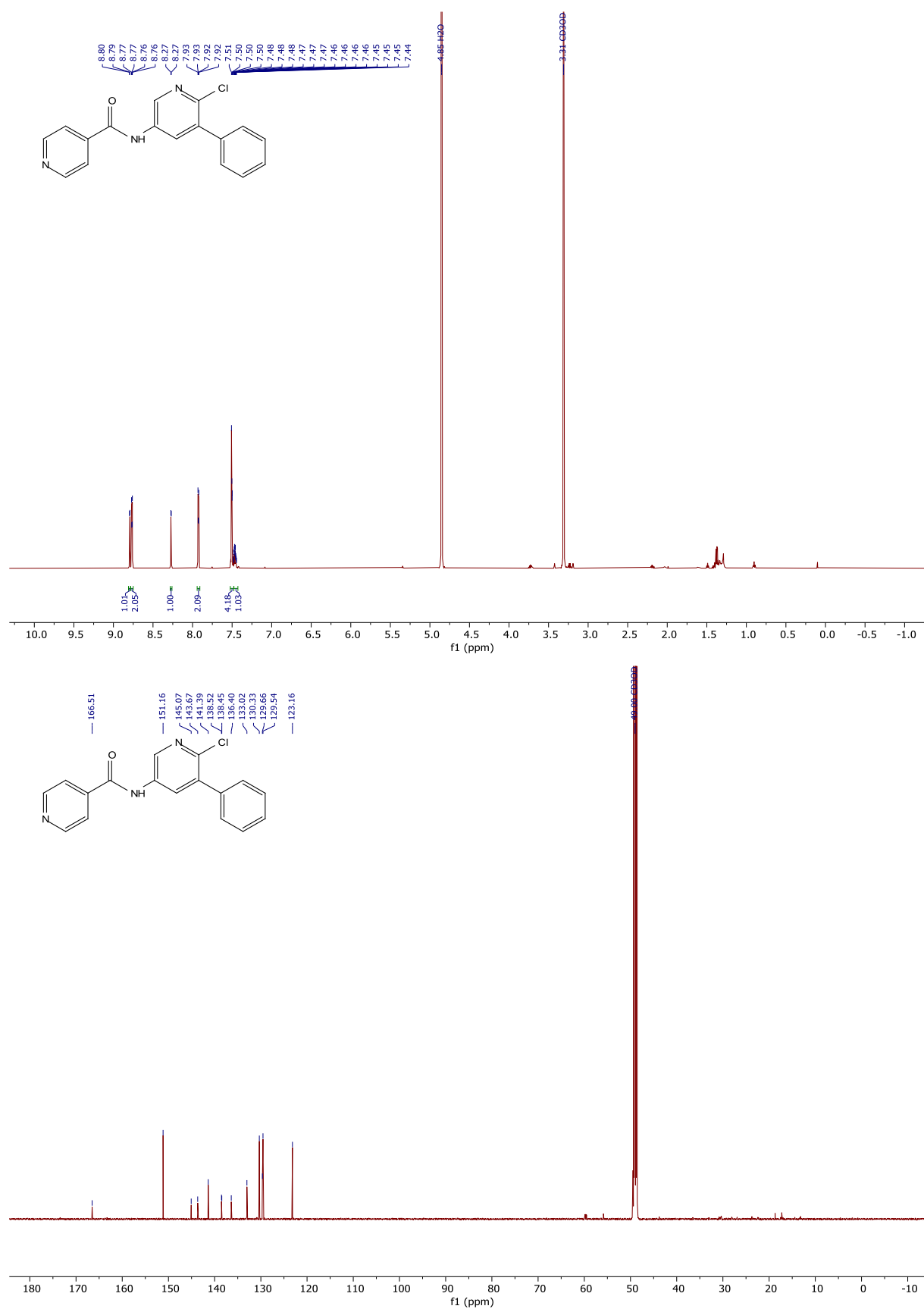


Figure S19. ^1H - and ^{13}C -NMR spectra of compound **28**.

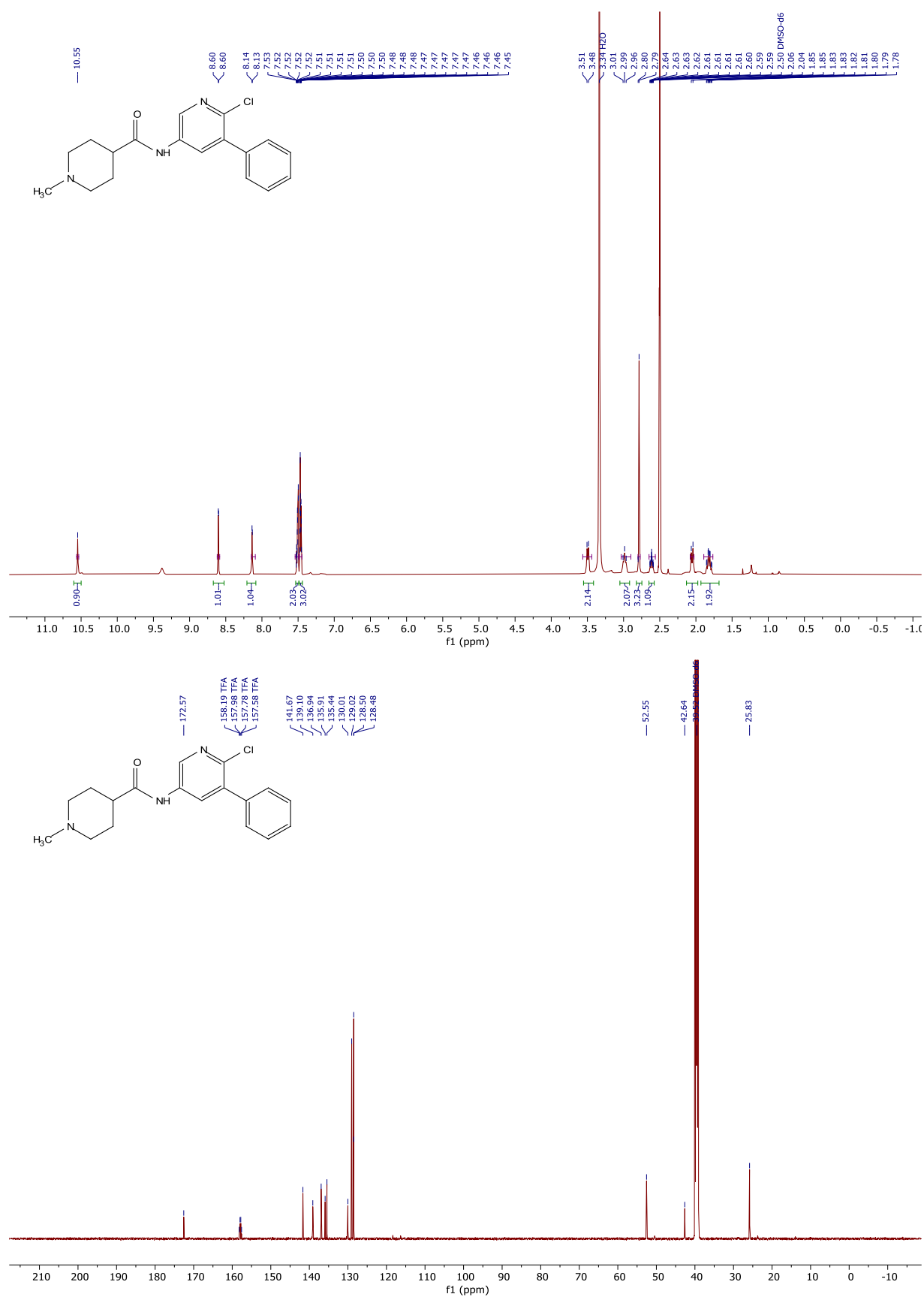


Figure S20. ¹H- and ¹³C-NMR spectra of compound 29.

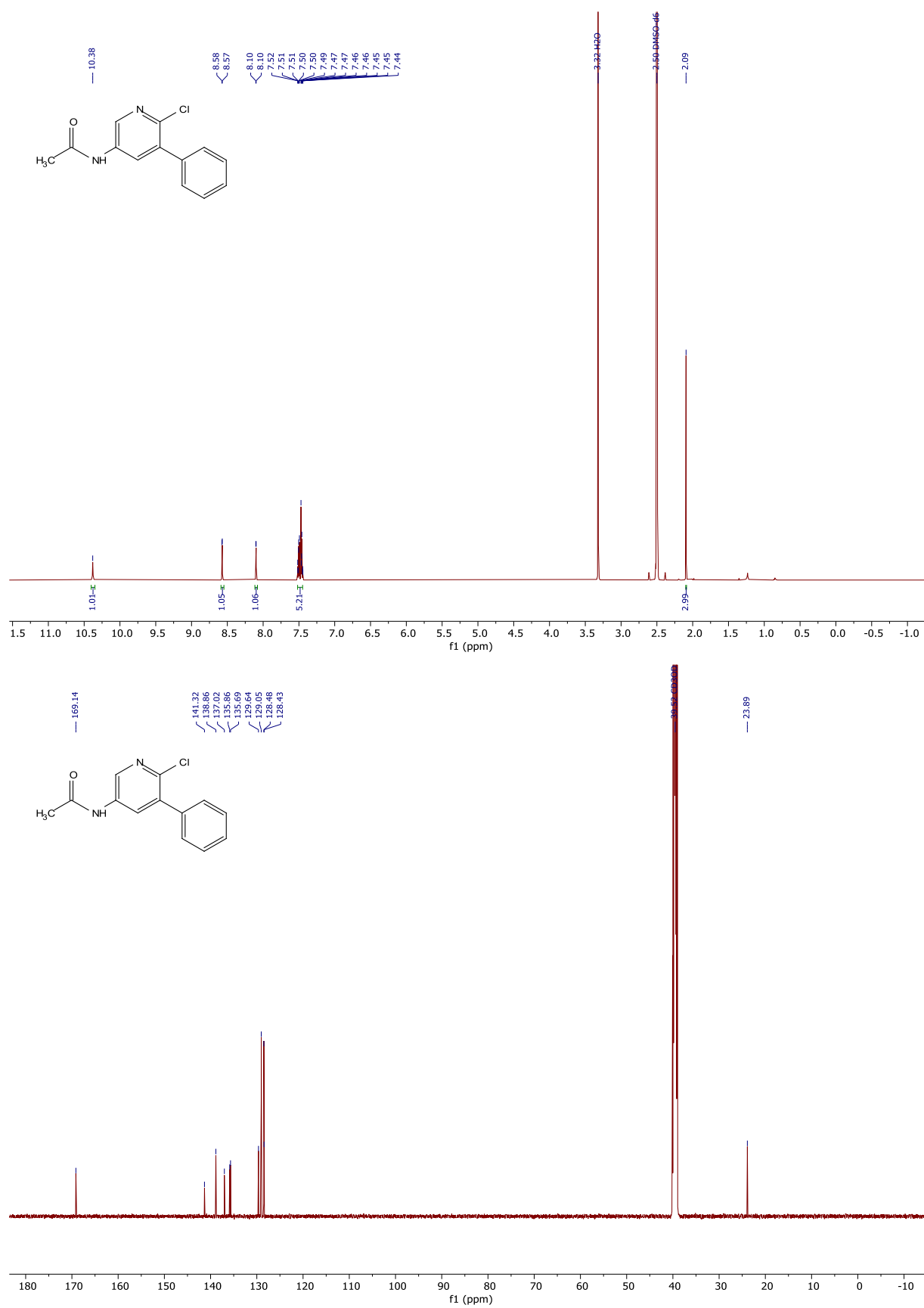


Figure S21. ¹H- and ¹³C-NMR spectra of compound **30**.

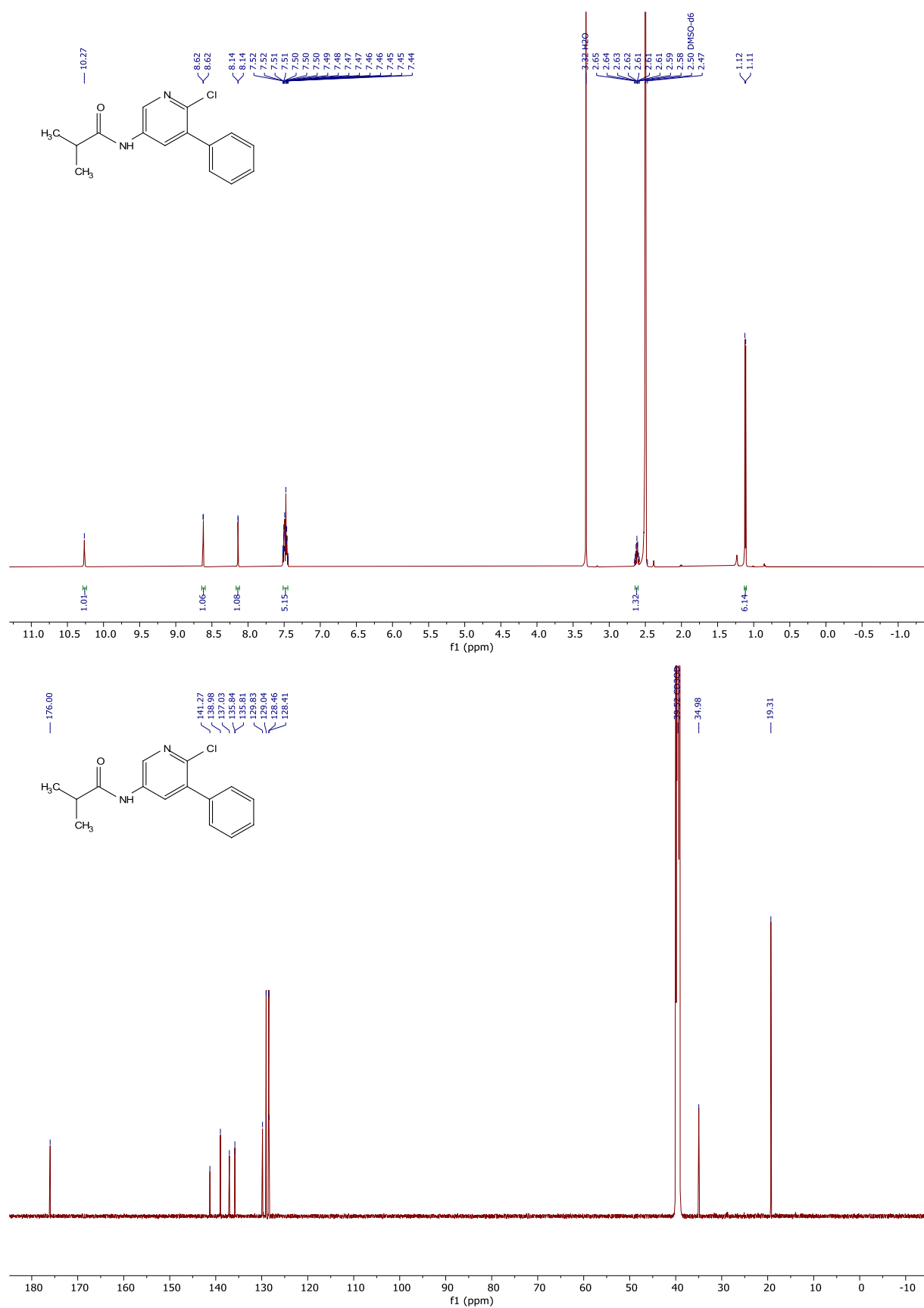


Figure S22. ^1H - and ^{13}C -NMR spectra of compound **31**.

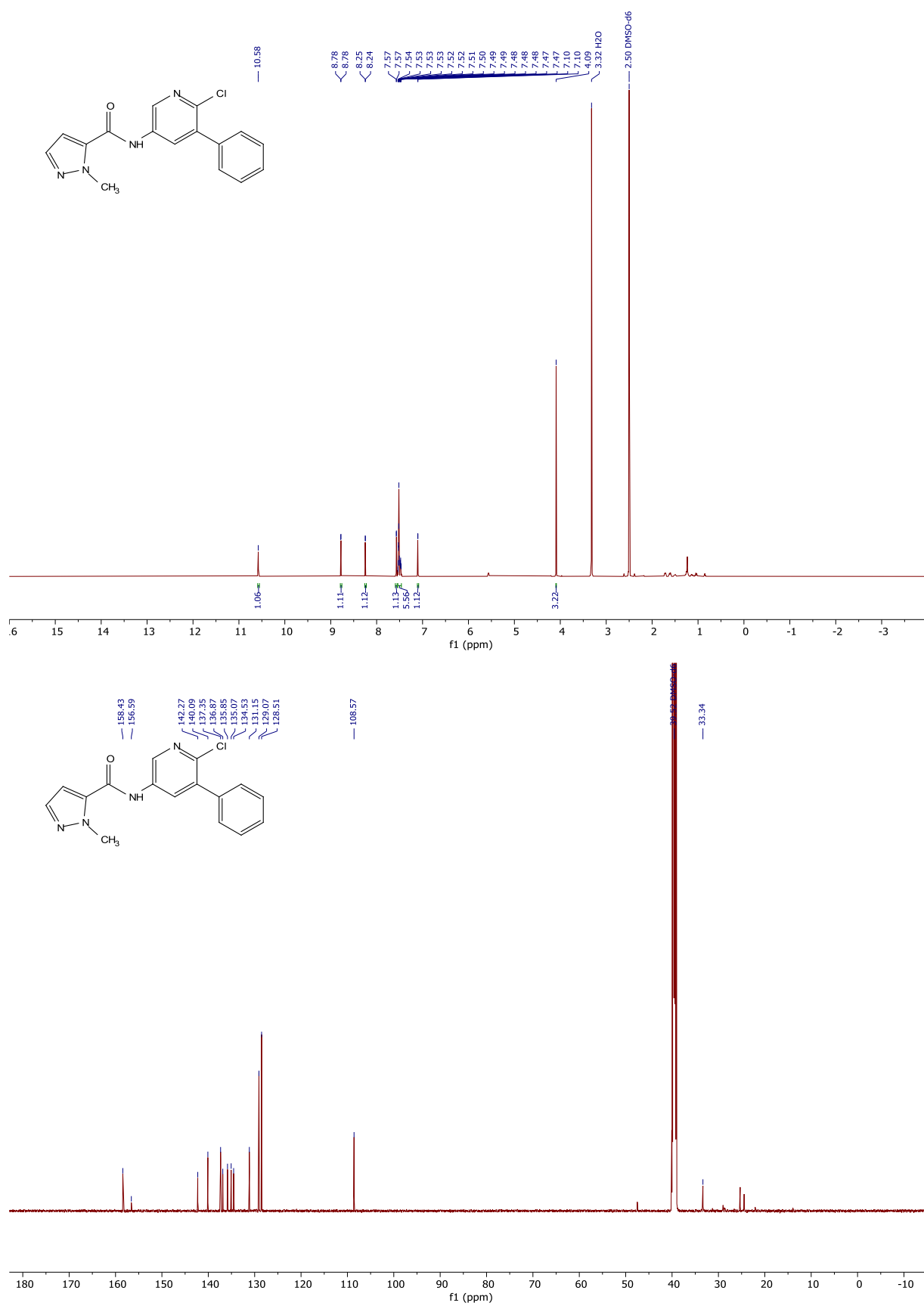


Figure S23. ^1H - and ^{13}C -NMR spectra of compound **32**.



Figure S24. ¹H- and ¹³C-NMR spectra of compound **33**.

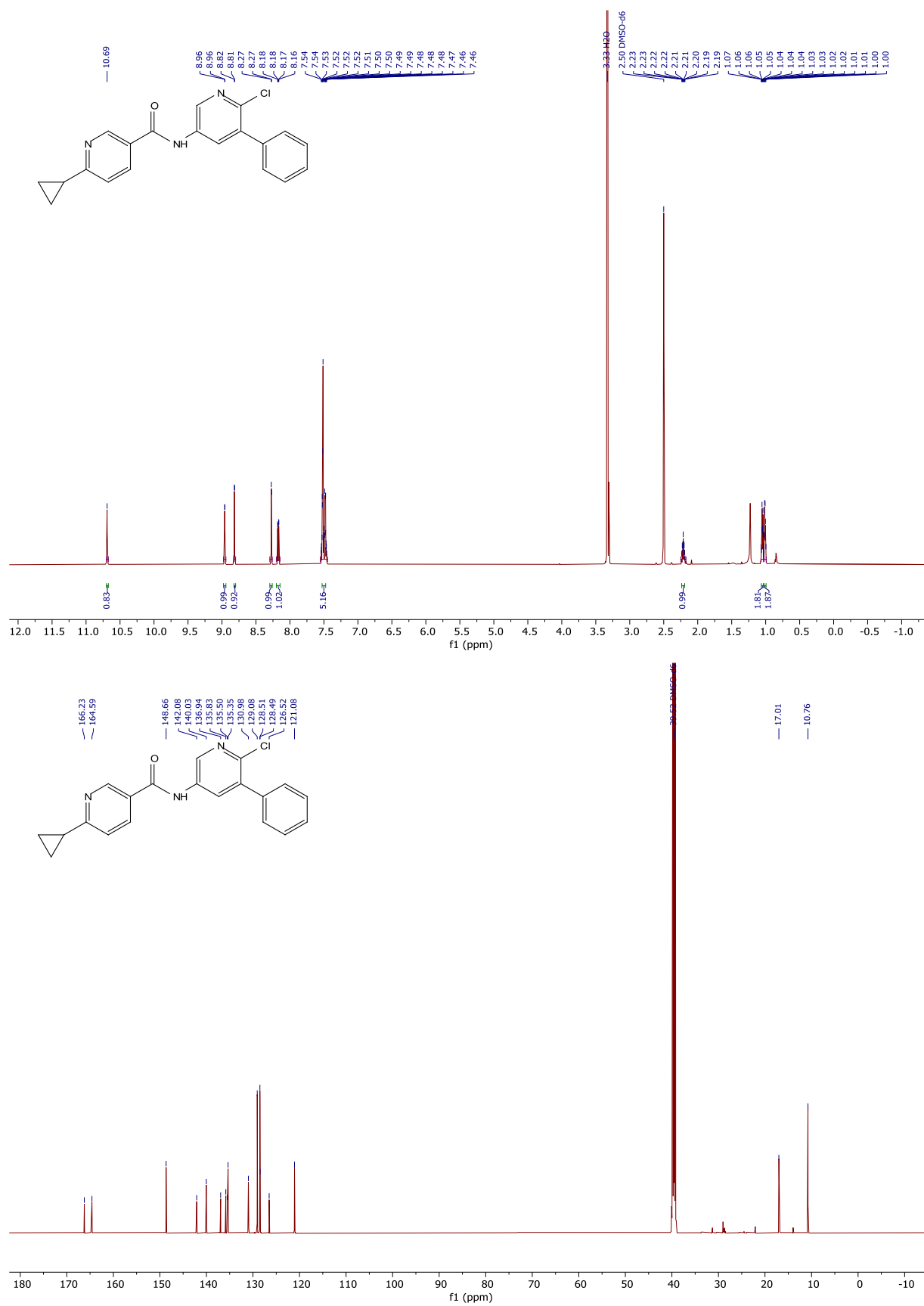


Figure S25. ¹H- and ¹³C-NMR spectra of compound **34**.

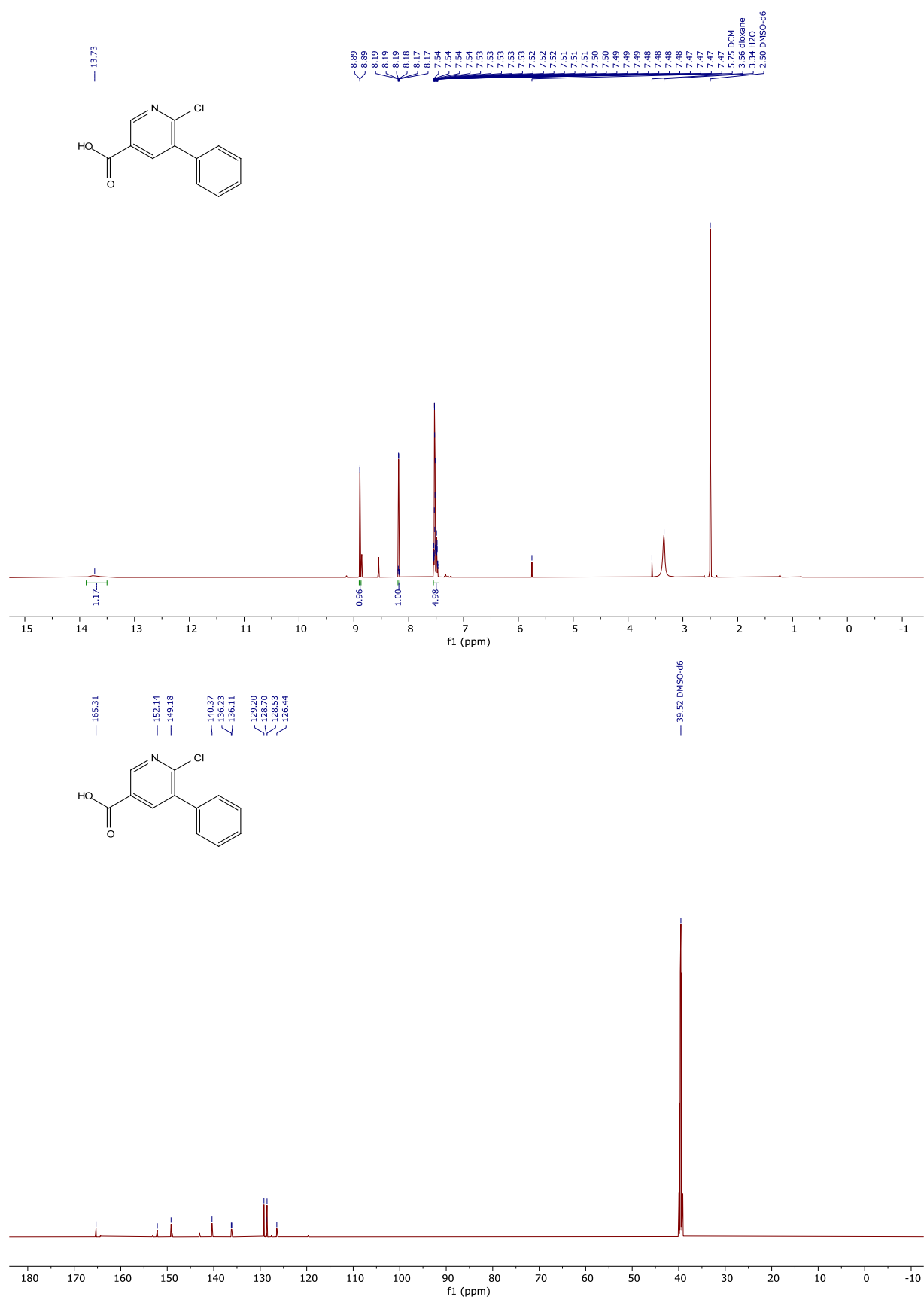


Figure S26. ^1H - and ^{13}C -NMR spectra of compound **36**.

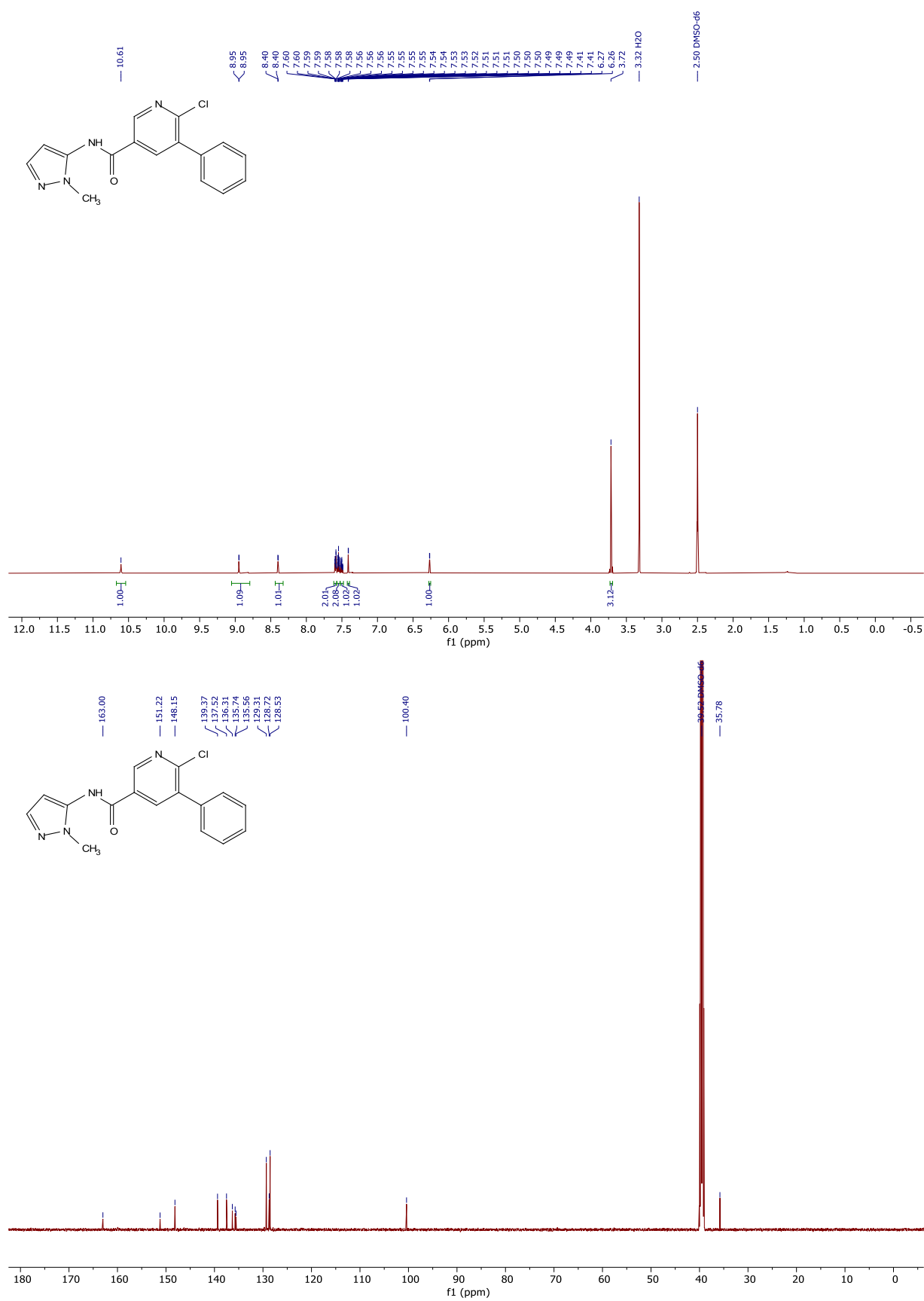


Figure S27. ¹H- and ¹³C-NMR spectra of compound **37**.

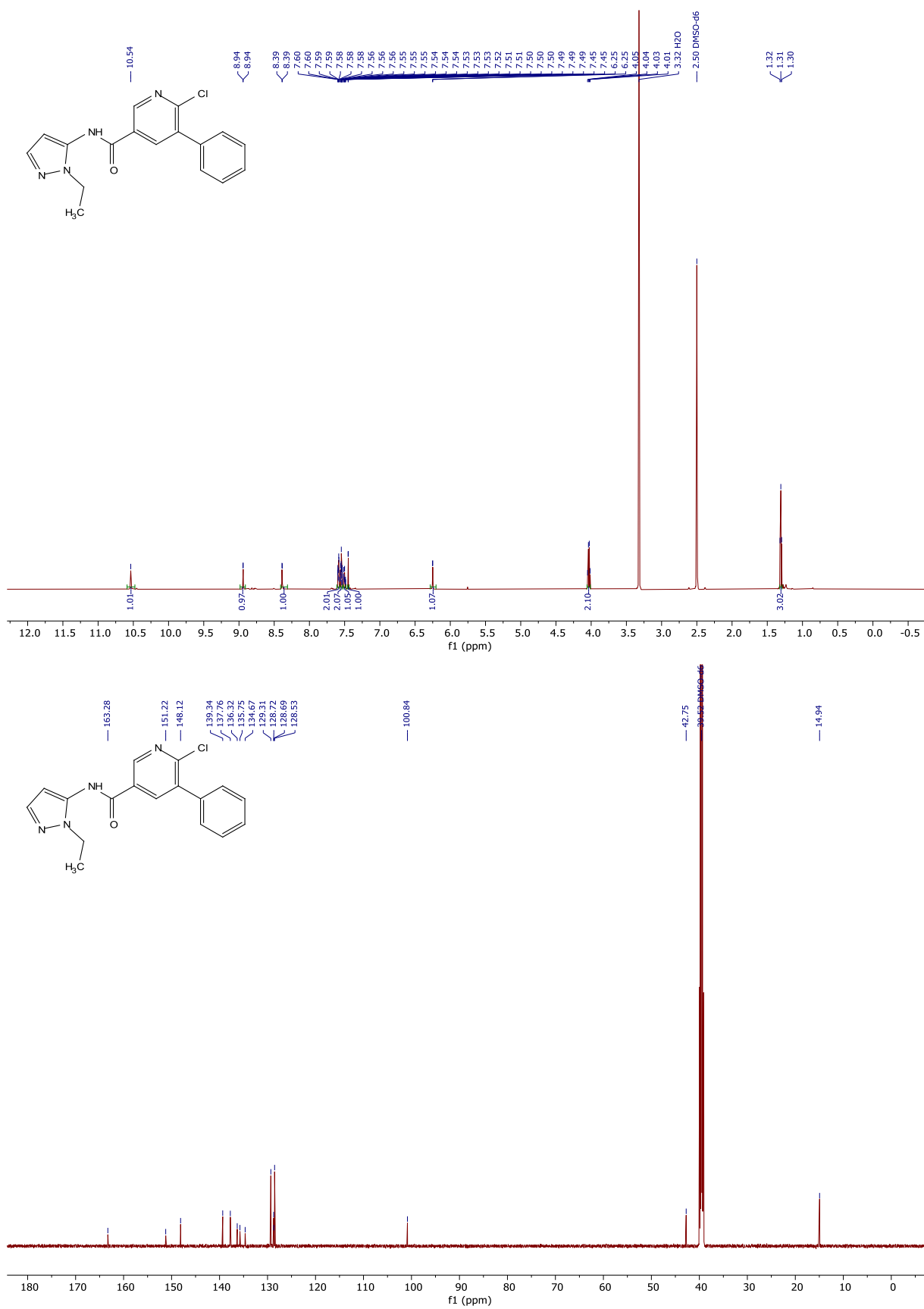
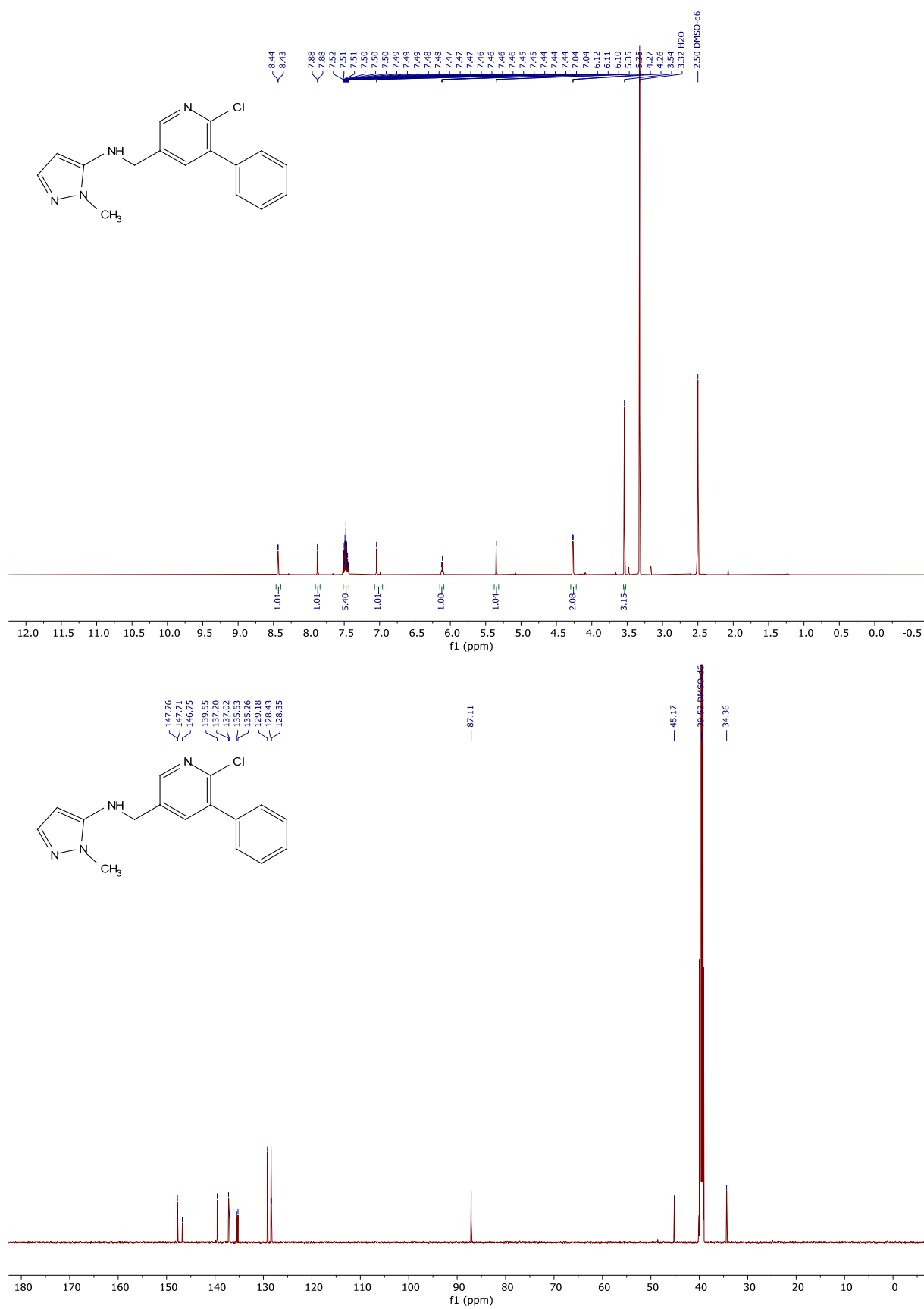




Figure S29. ¹H- and ¹³C-NMR spectra of compound **39**.



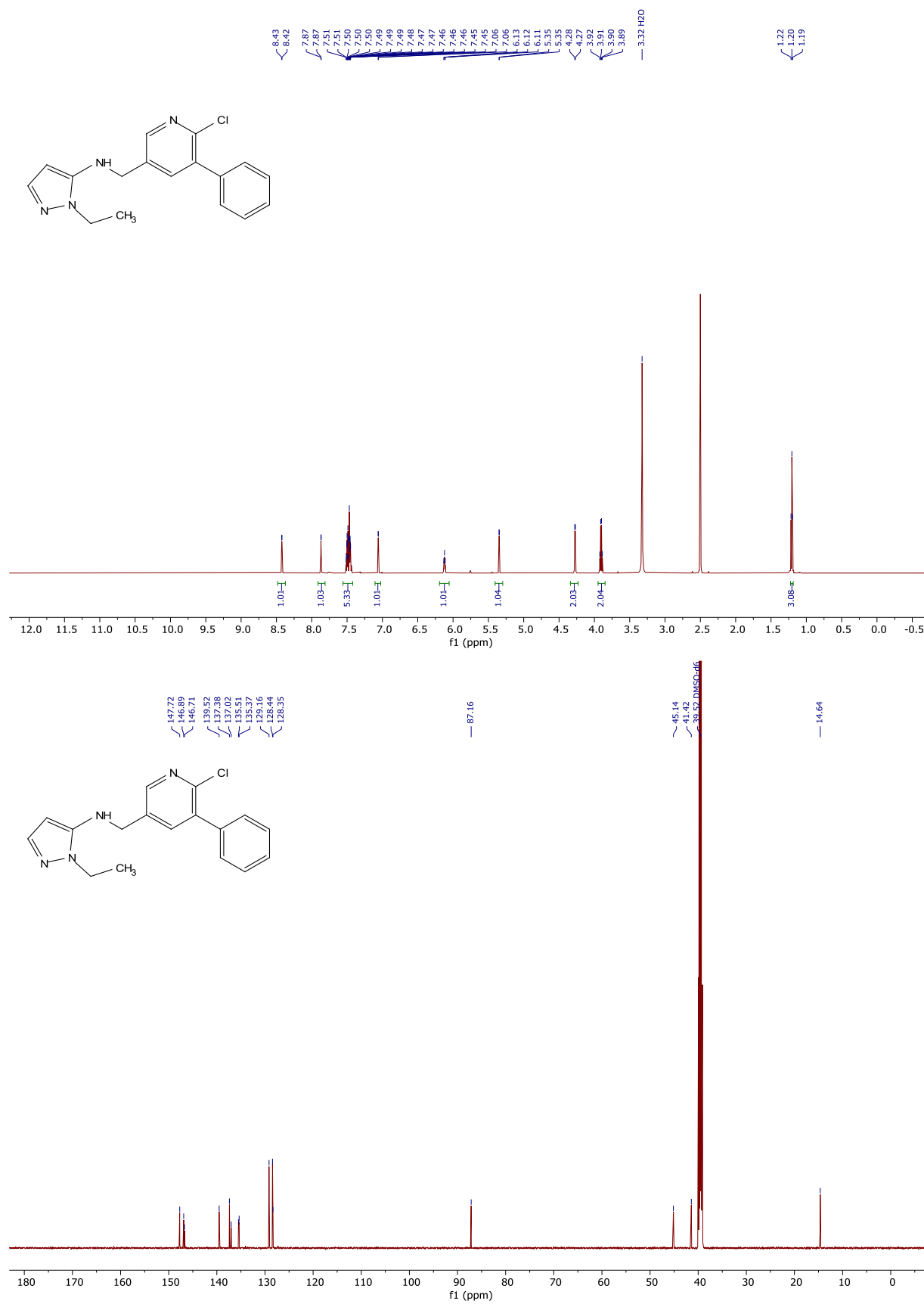


Figure S31. ¹H- and ¹³C-NMR spectra of compound 42.

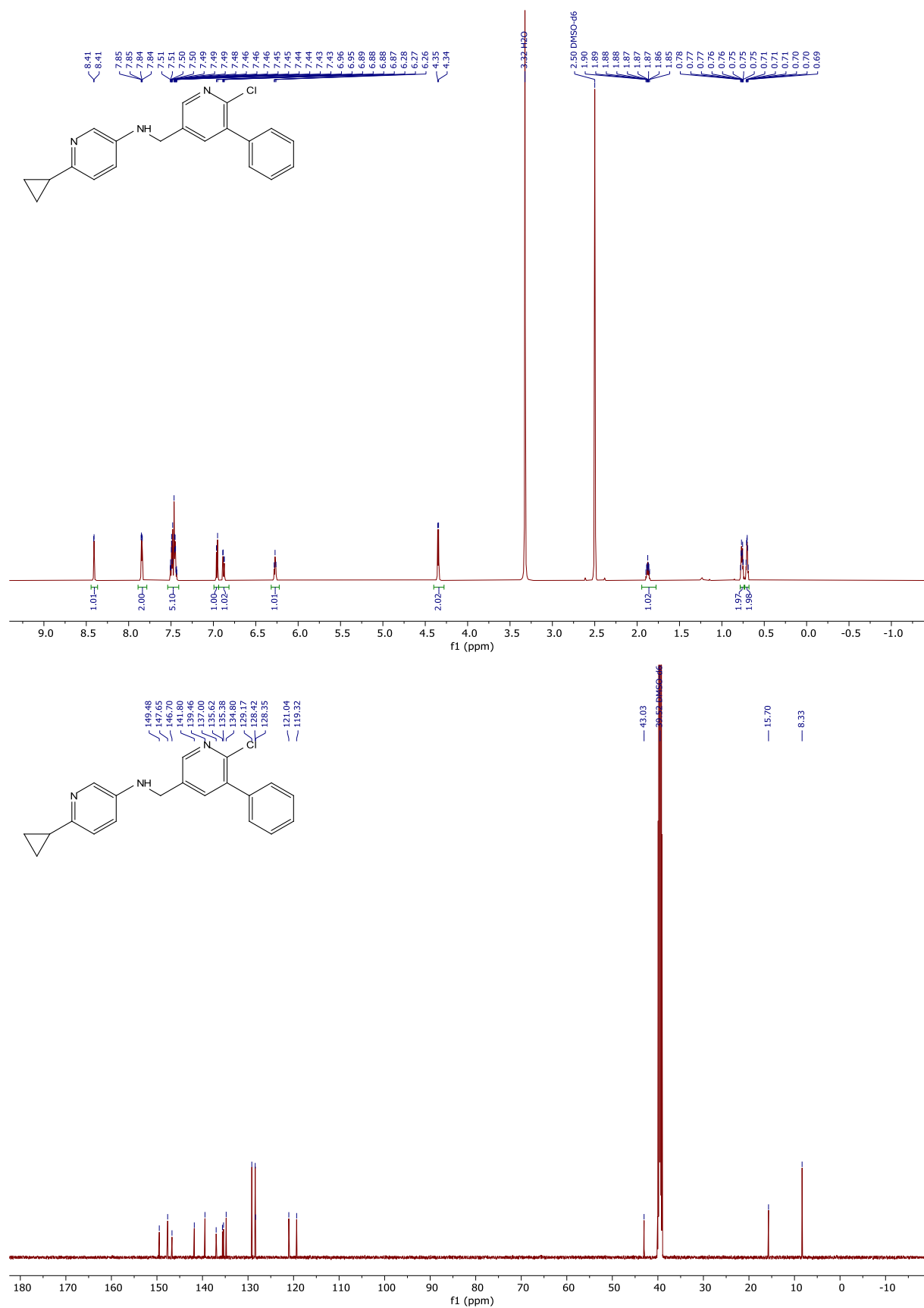


Figure S32. ¹H- and ¹³C-NMR spectra of compound 43.

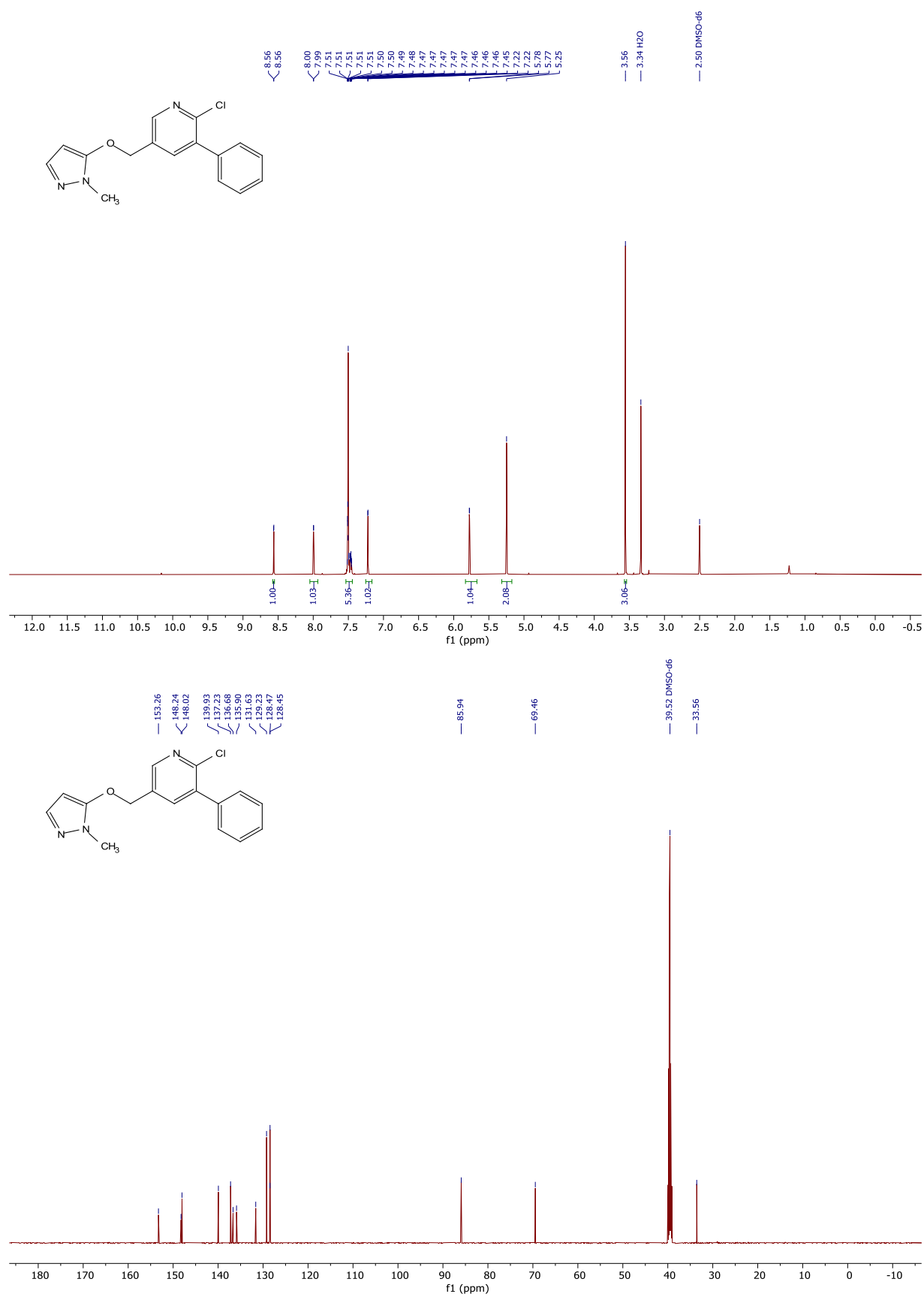


Figure S33. ¹H- and ¹³C-NMR spectra of compound **44**.

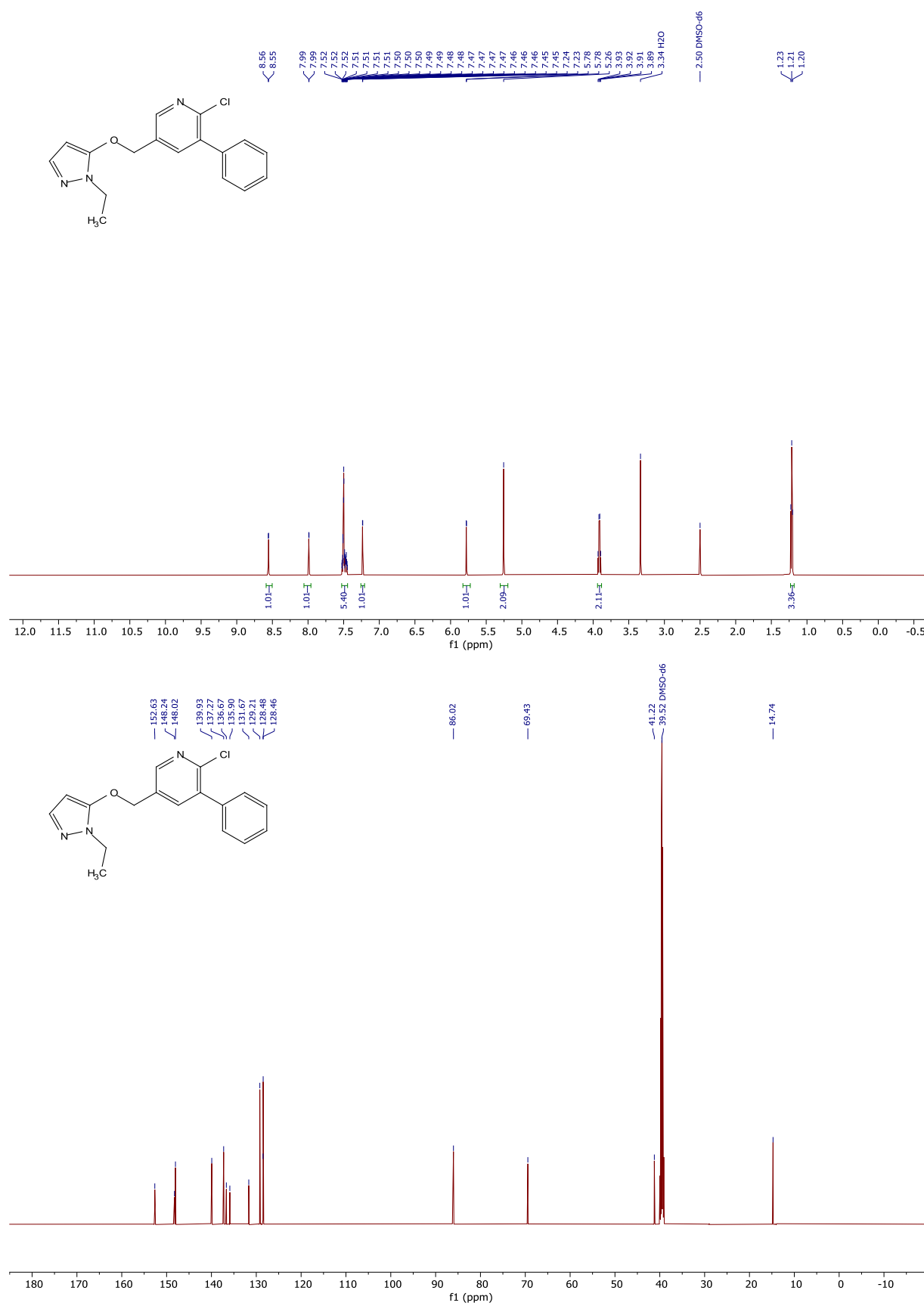


Figure S34. ¹H- and ¹³C-NMR spectra of compound **45**.

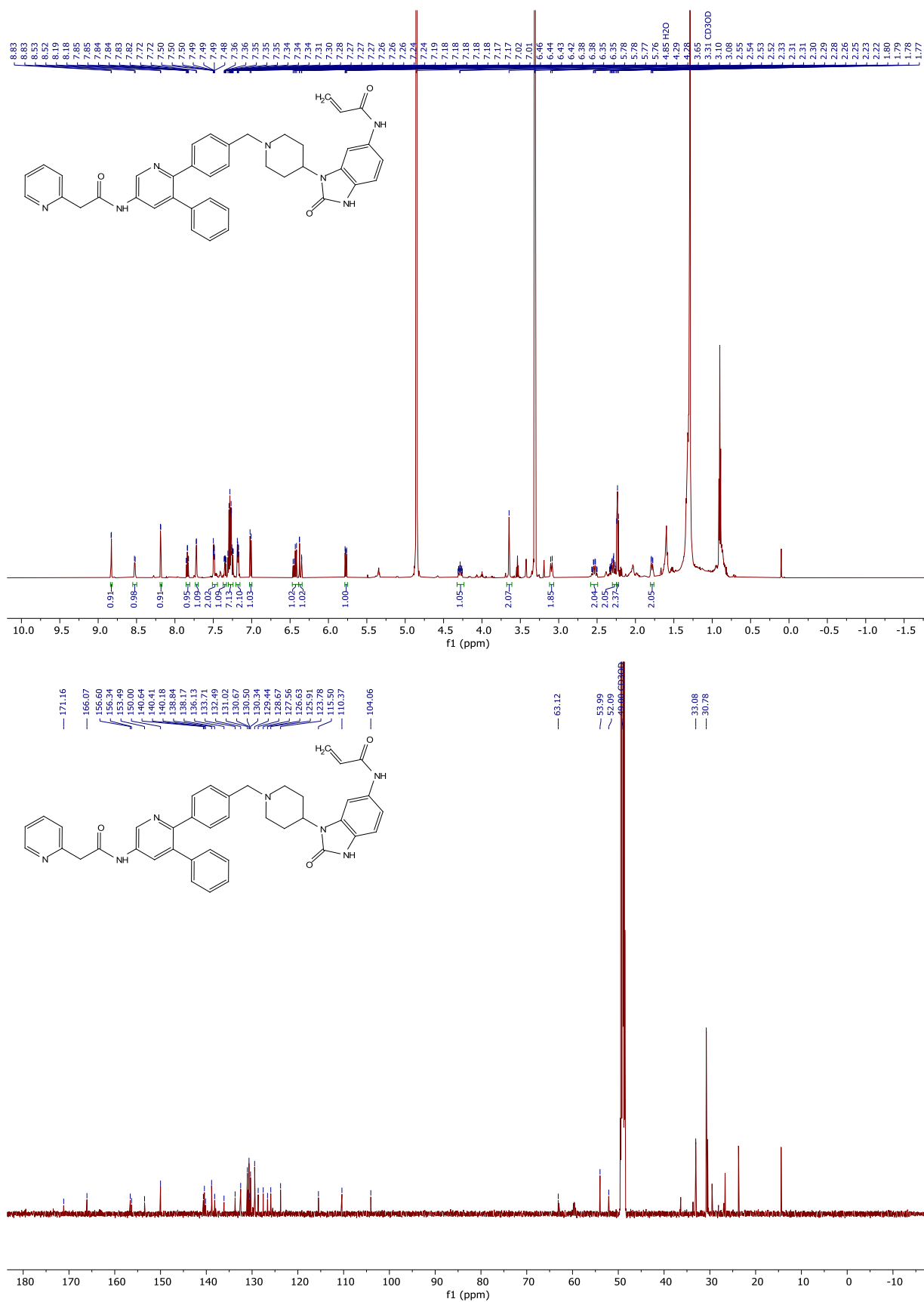


Figure S35. ¹H- and ¹³C-NMR spectra of compound 46.

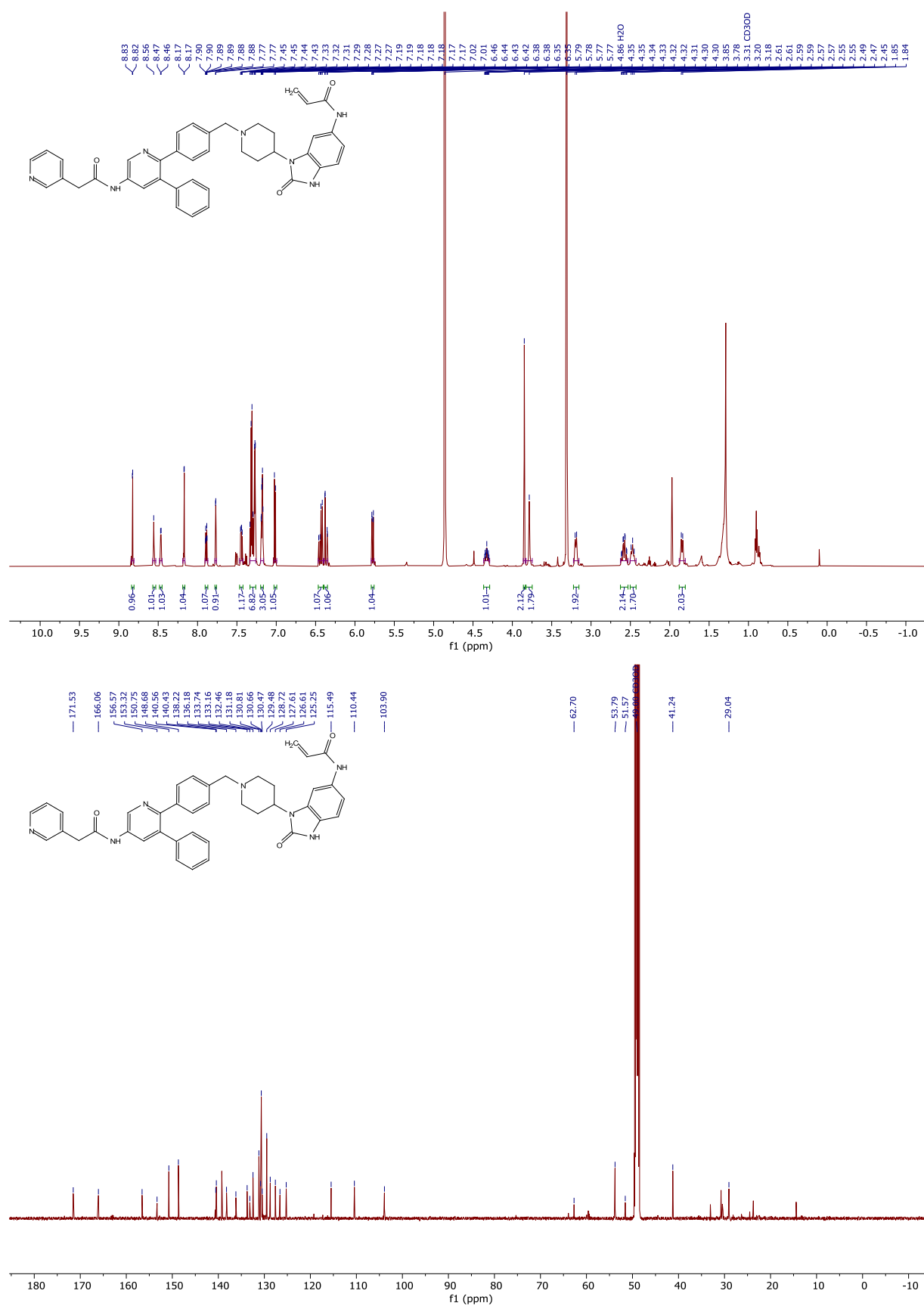


Figure S36. ^1H - and ^{13}C -NMR spectra of compound 47.

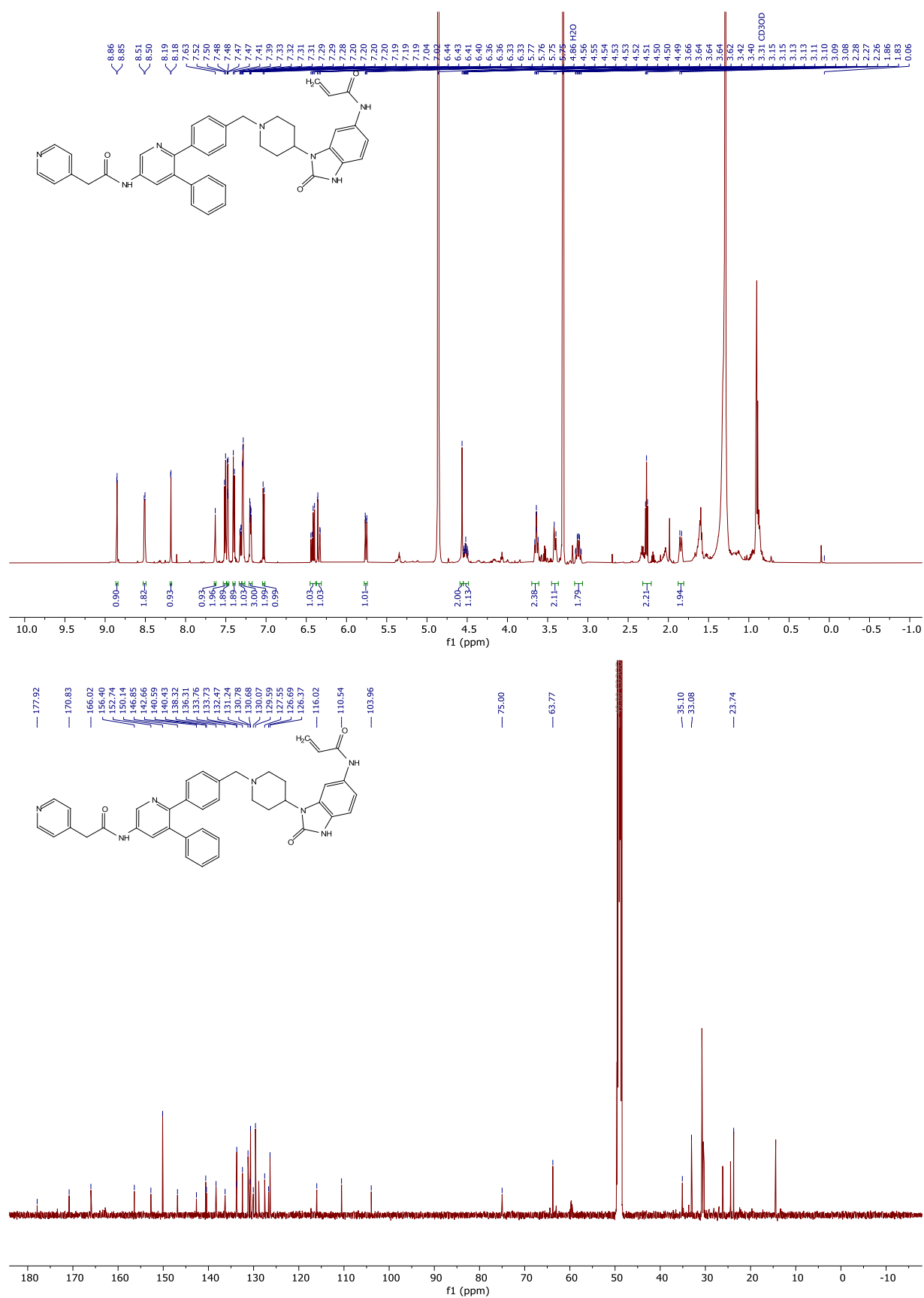


Figure S37. ¹H- and ¹³C-NMR spectra of compound 48.

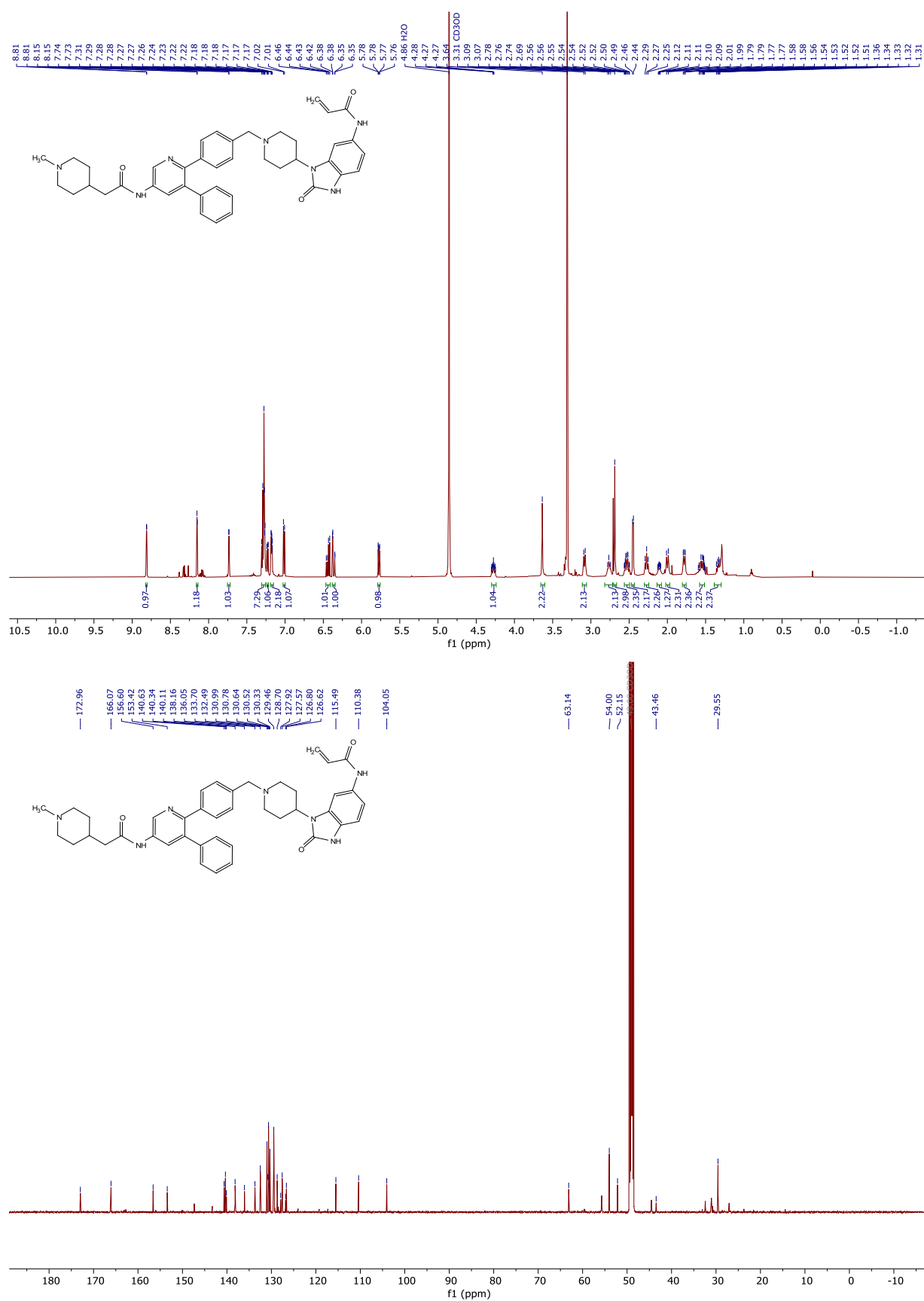


Figure S38. ¹H- and ¹³C-NMR spectra of compound 49.

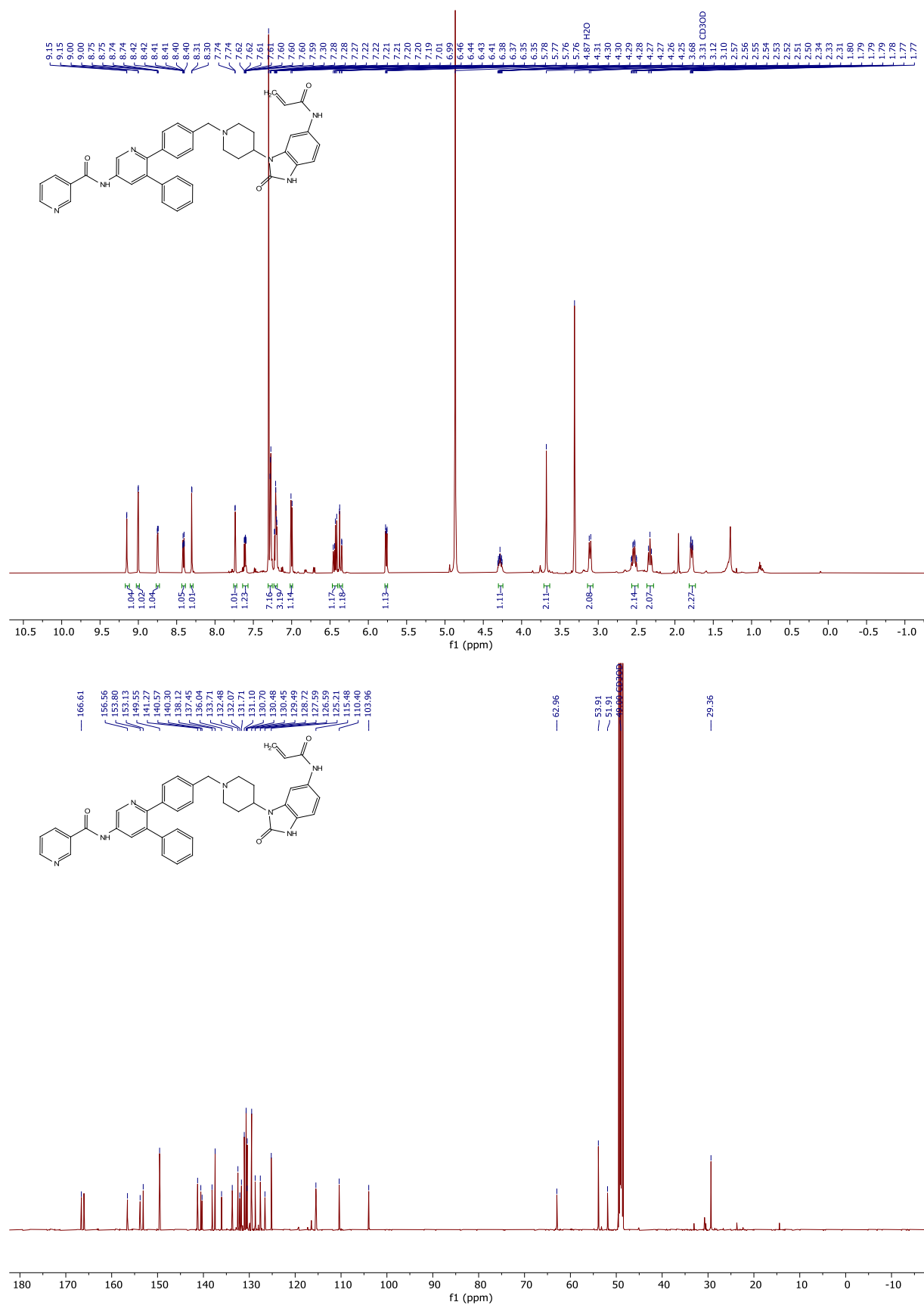


Figure S39. ¹H- and ¹³C-NMR spectra of compound **50**.

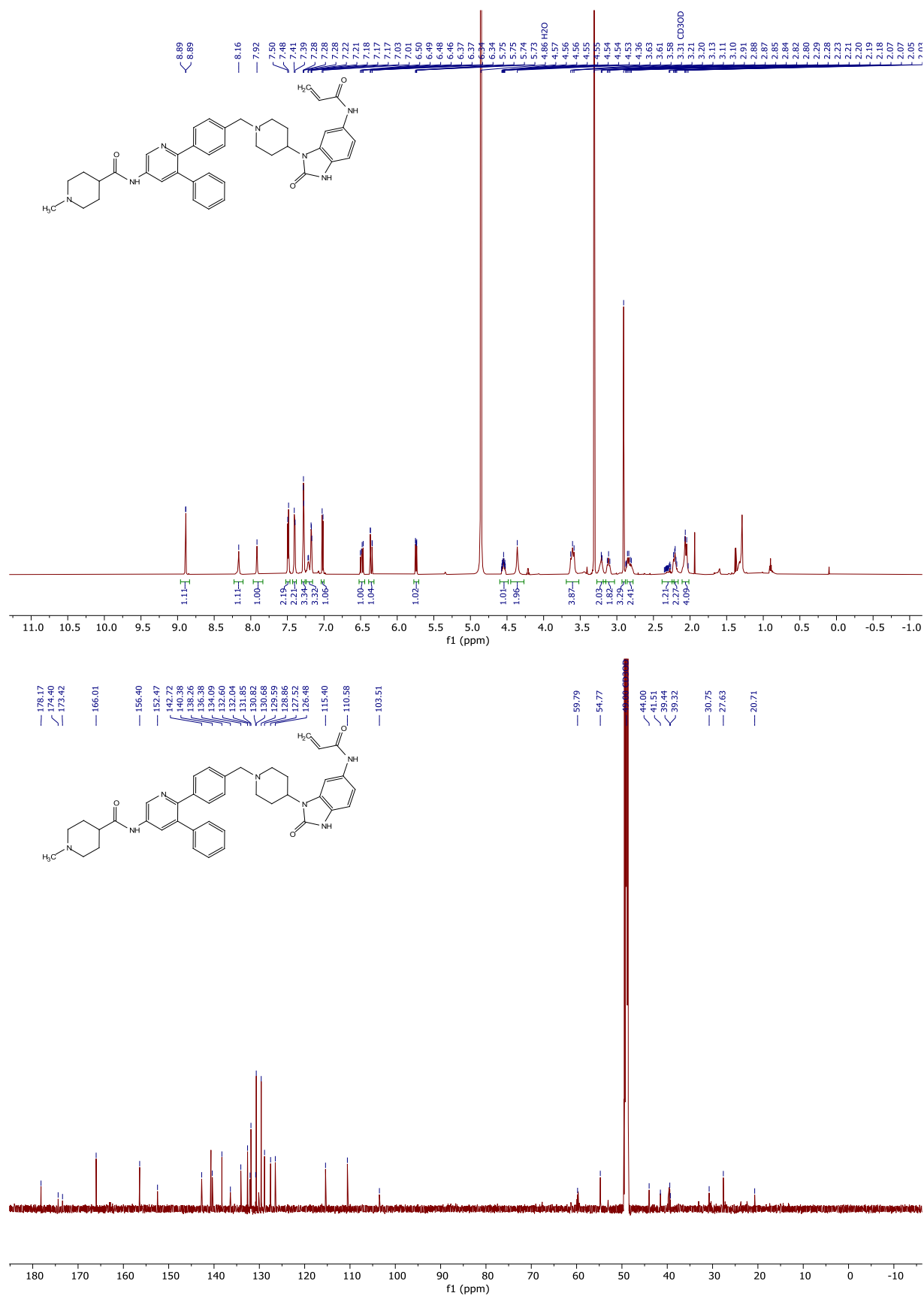
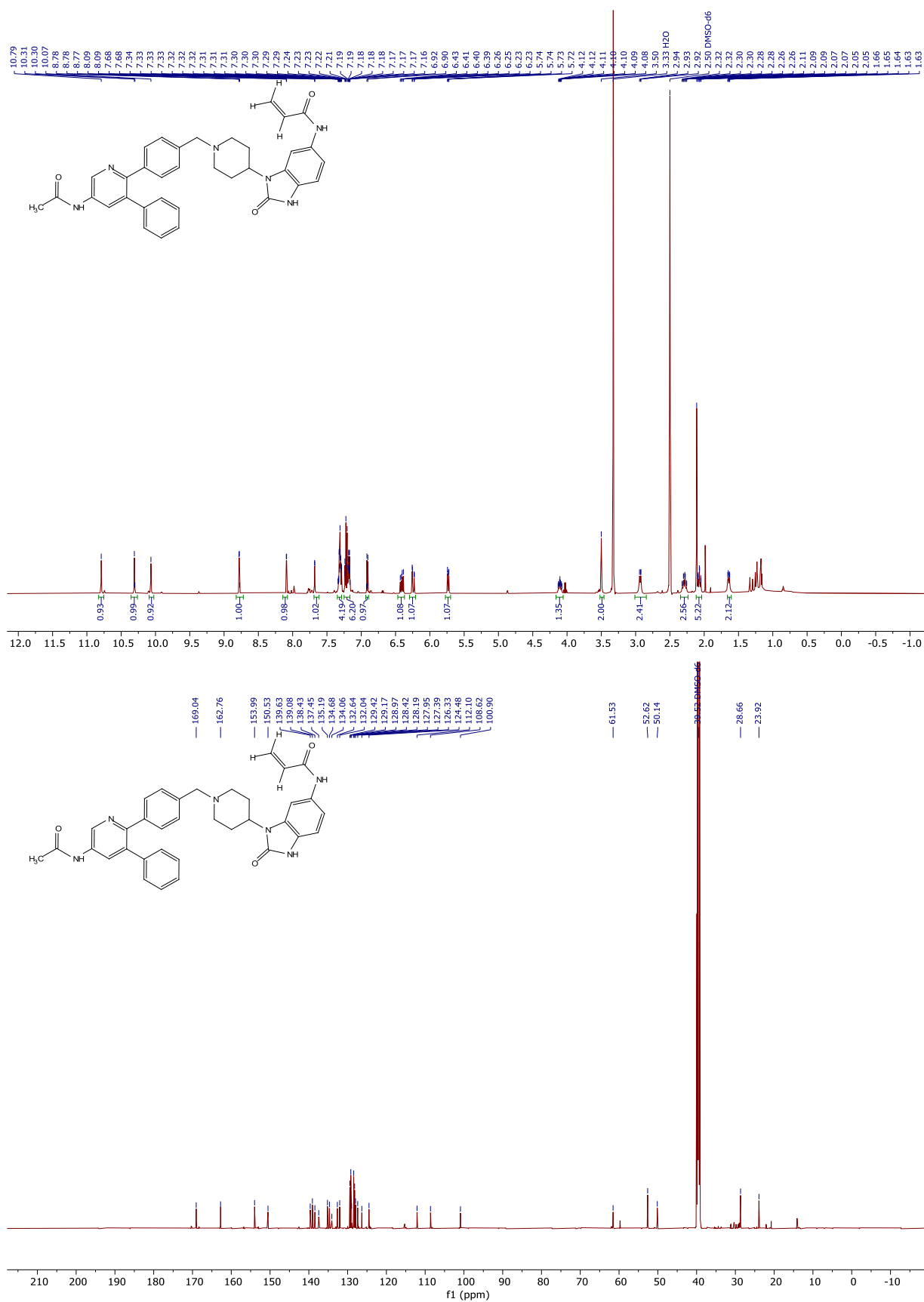


Figure S41. ¹H- and ¹³C-NMR spectra of compound 52.



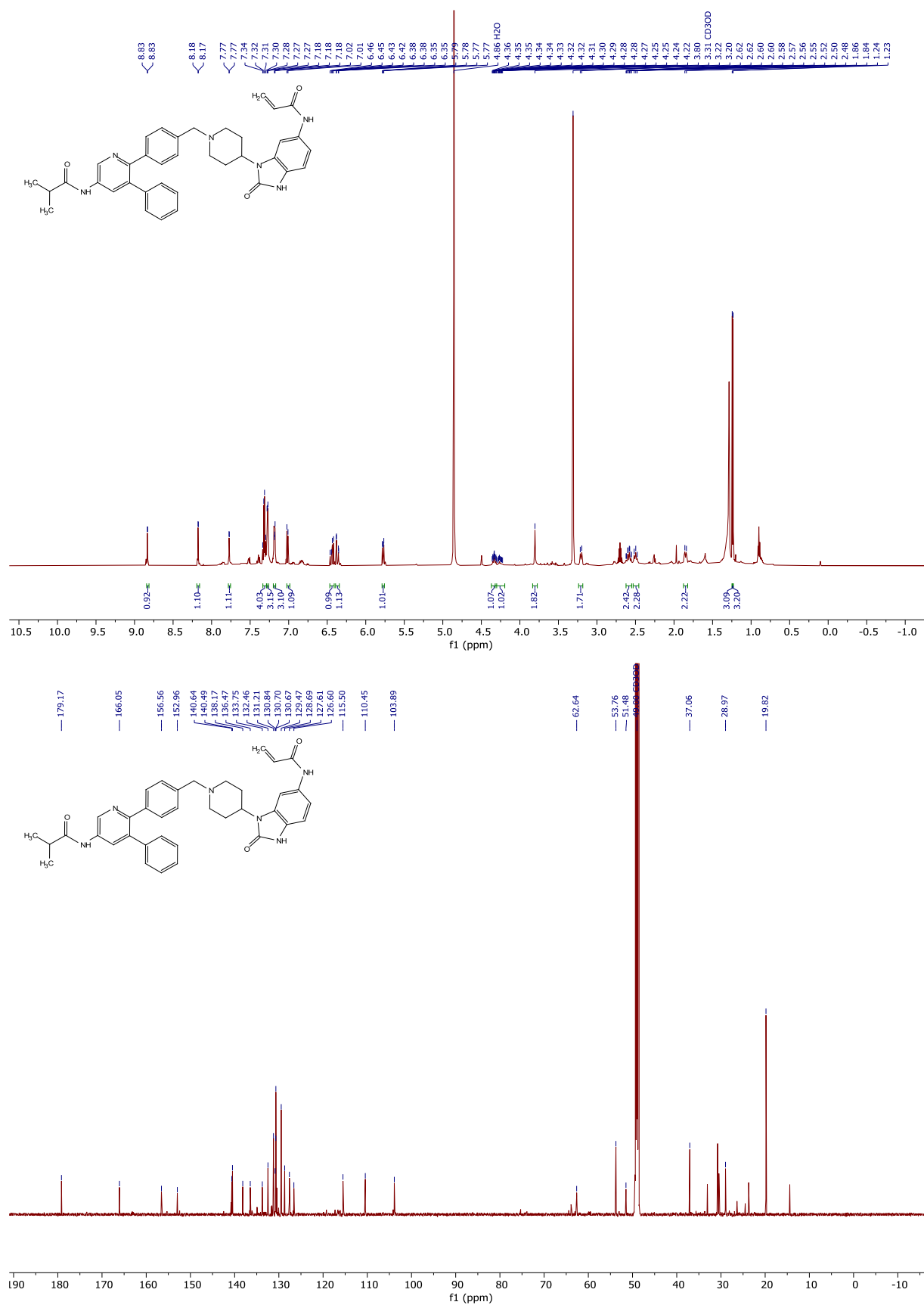


Figure S43. ¹H- and ¹³C-NMR spectra of compound **54**.

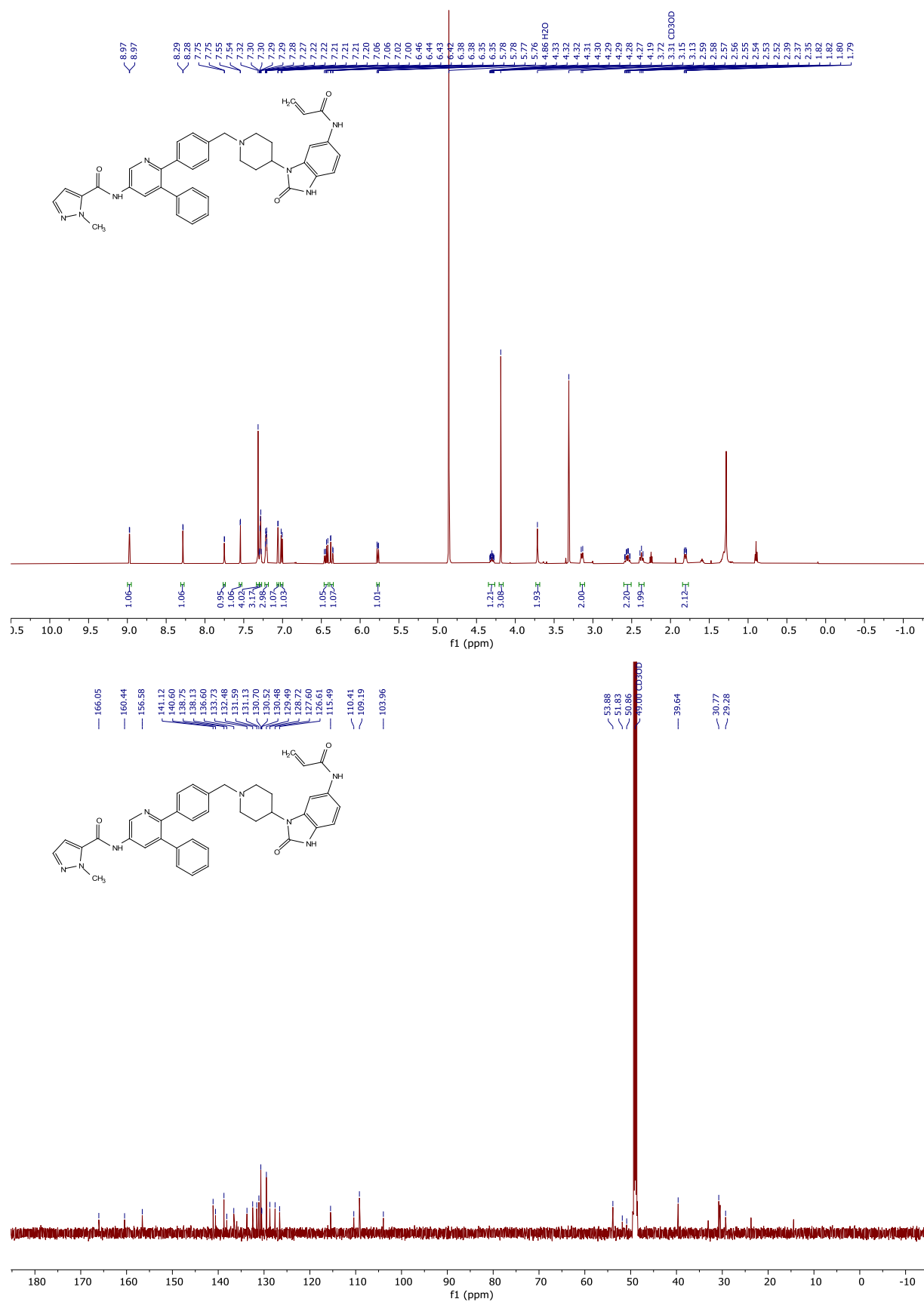


Figure S44. ¹H- and ¹³C-NMR spectra of compound **55**.

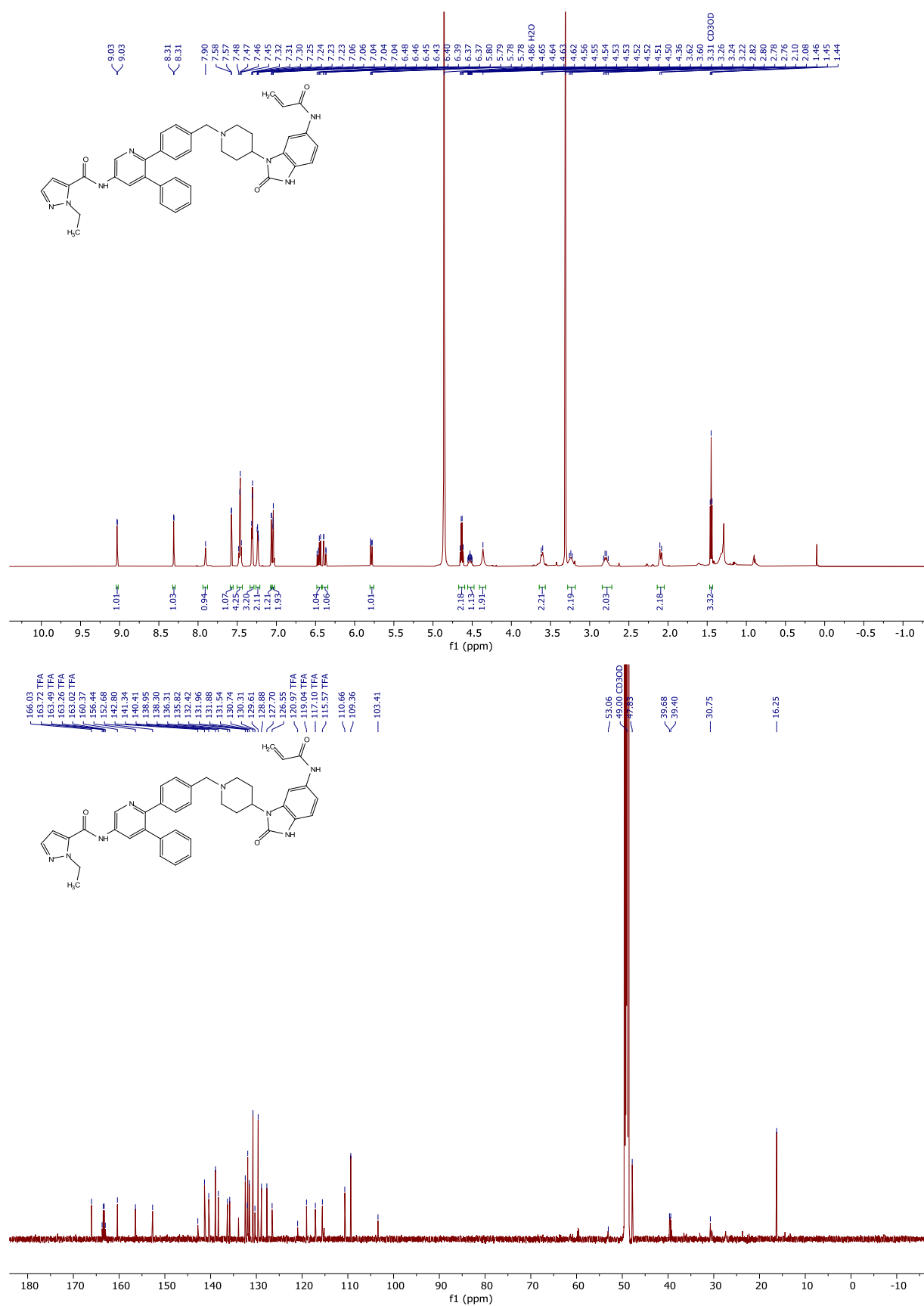


Figure S45. ^1H - and ^{13}C -NMR spectra of compound **56**.

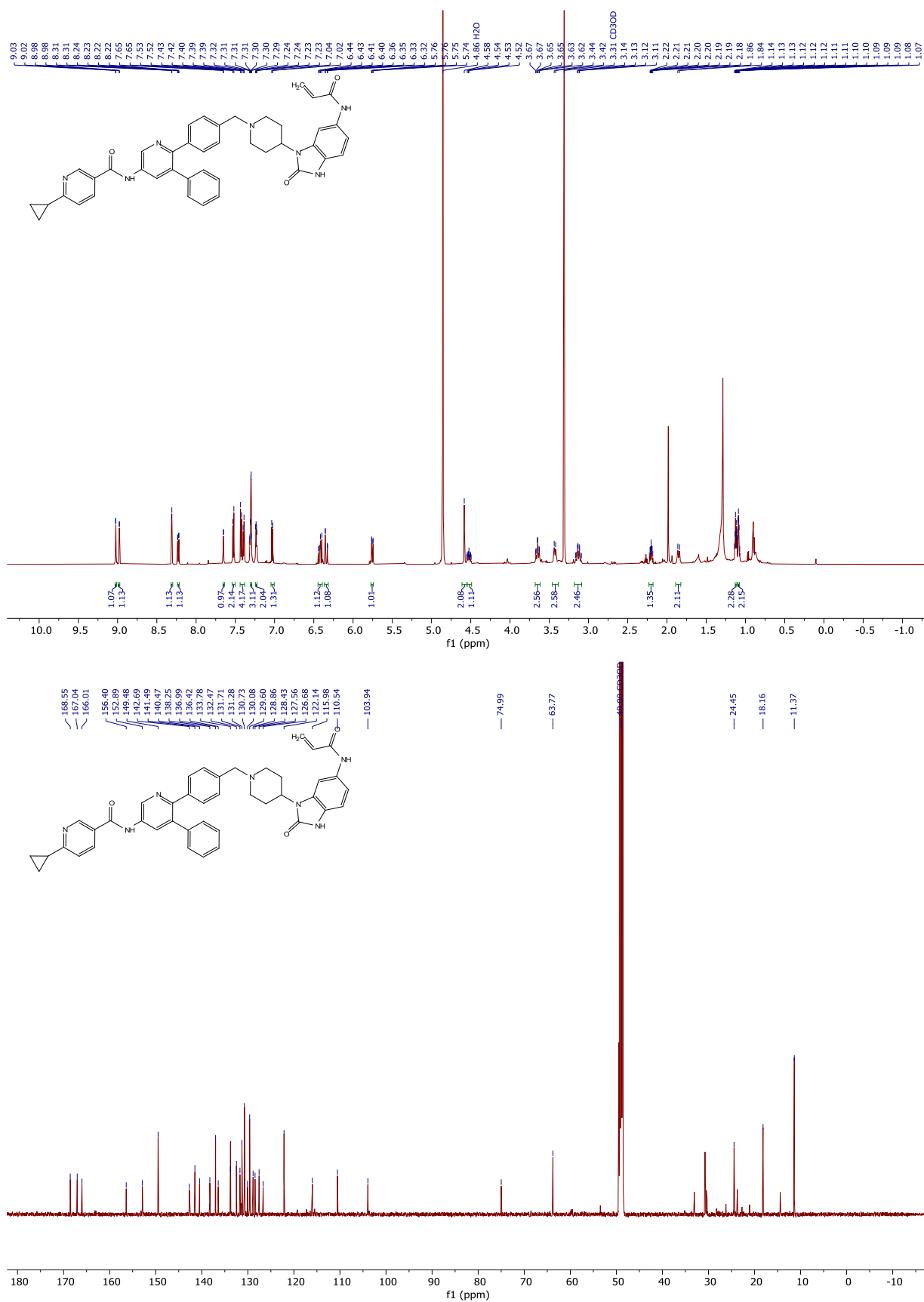


Figure S46. ^1H - and ^{13}C -NMR spectra of compound **57**.

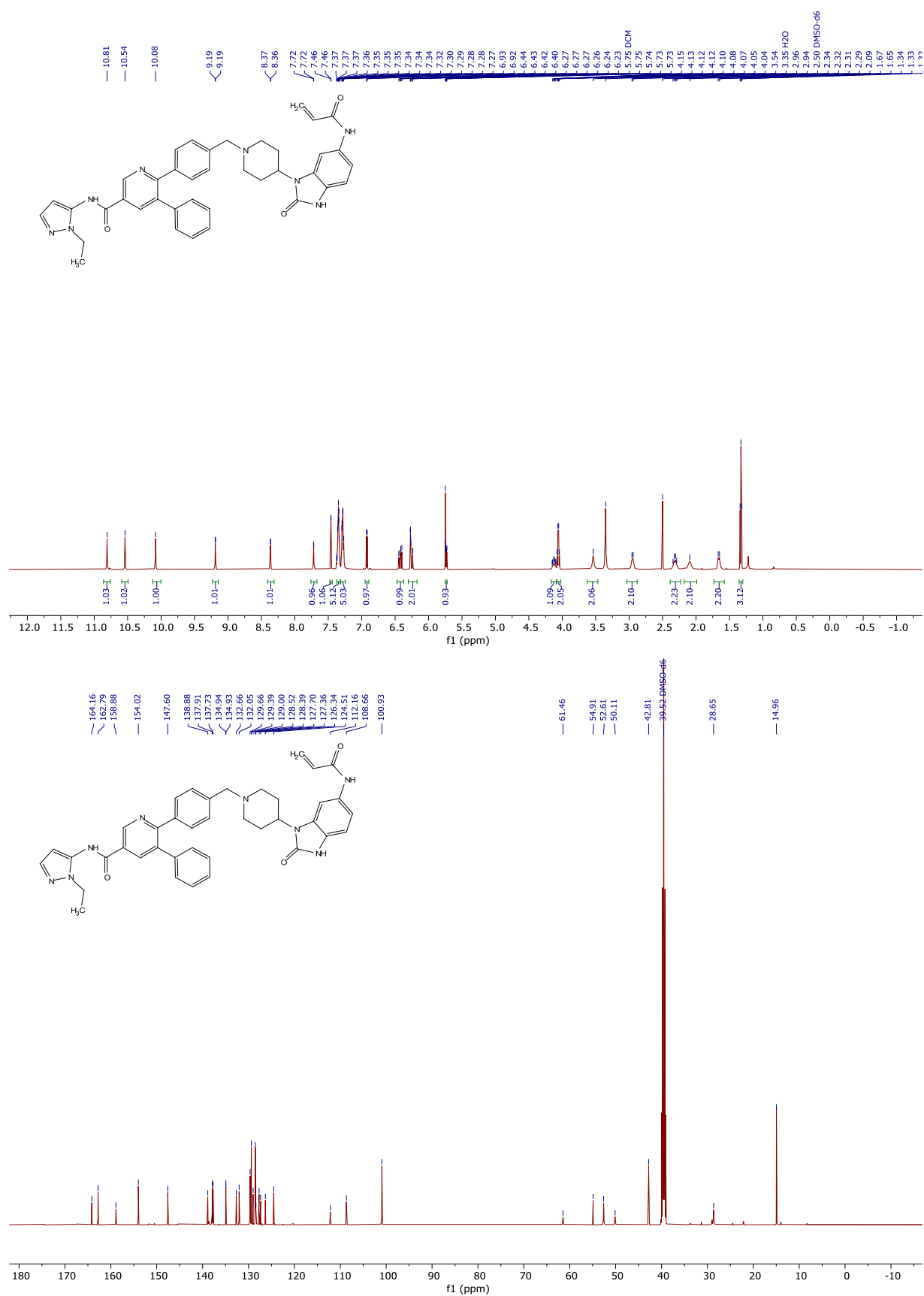


Figure S48. ¹H- and ¹³C-NMR spectra of compound **59**.

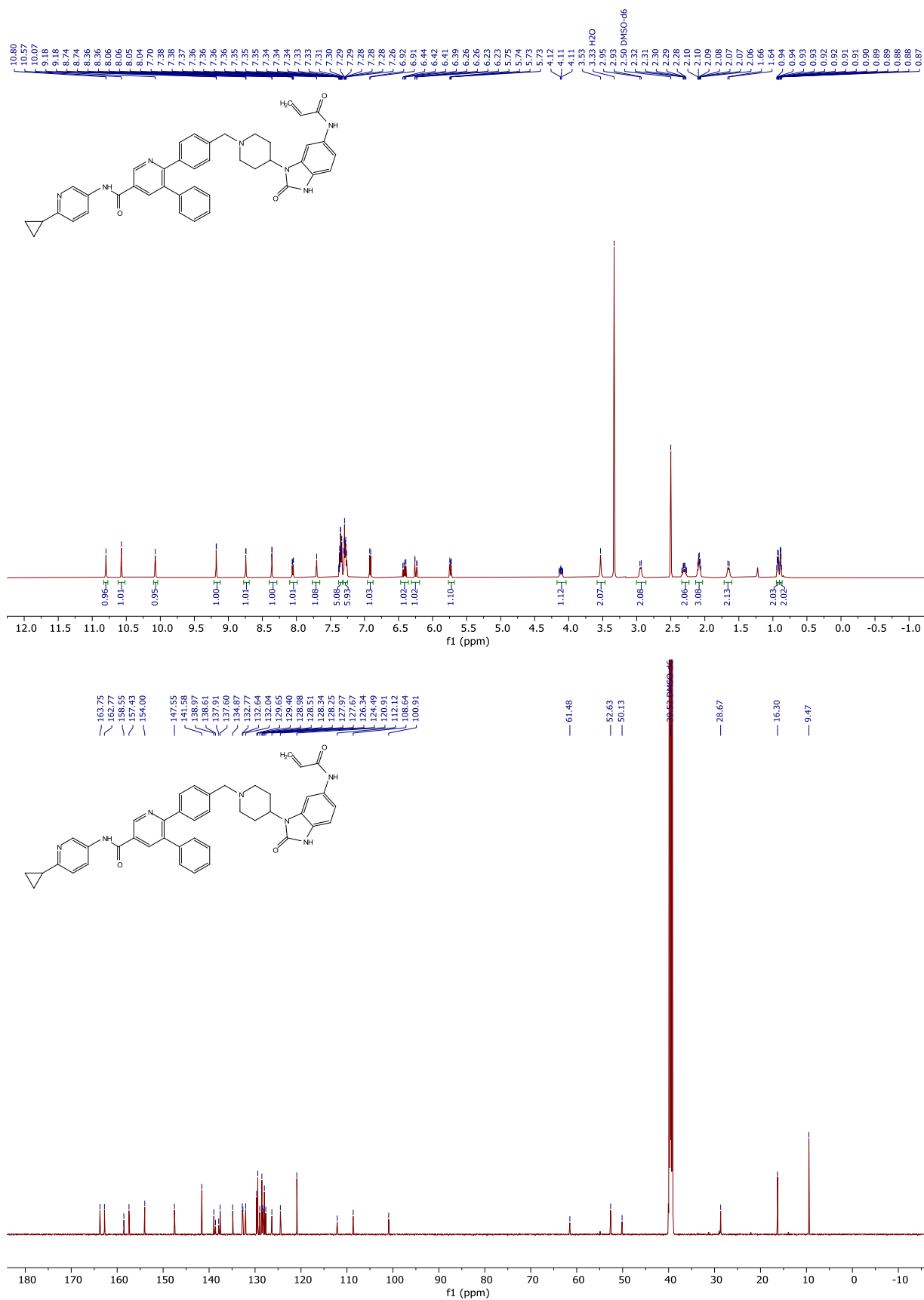


Figure S49. ¹H- and ¹³C-NMR spectra of compound **60**.

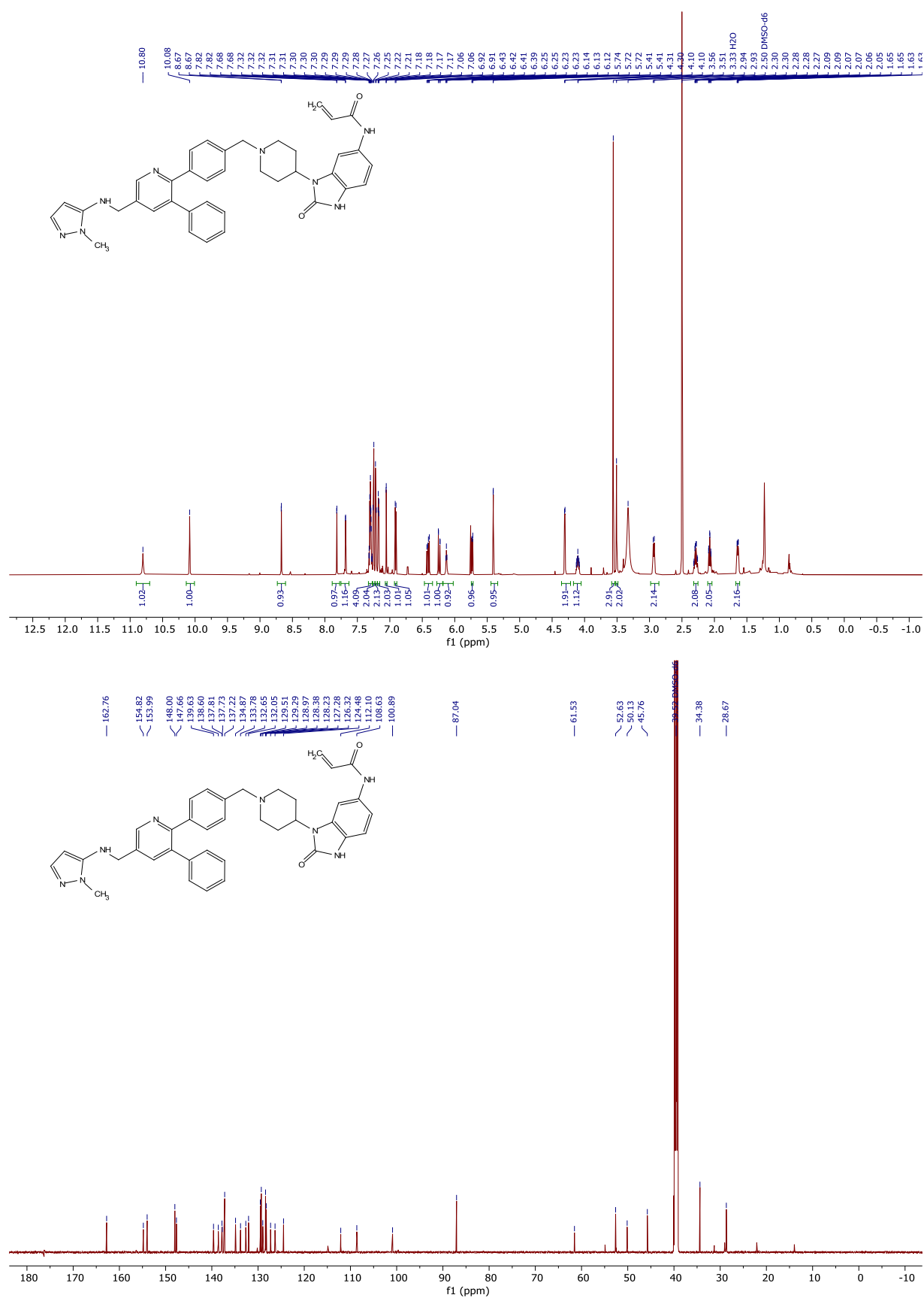


Figure S50. ¹H- and ¹³C-NMR spectra of compound **61**.

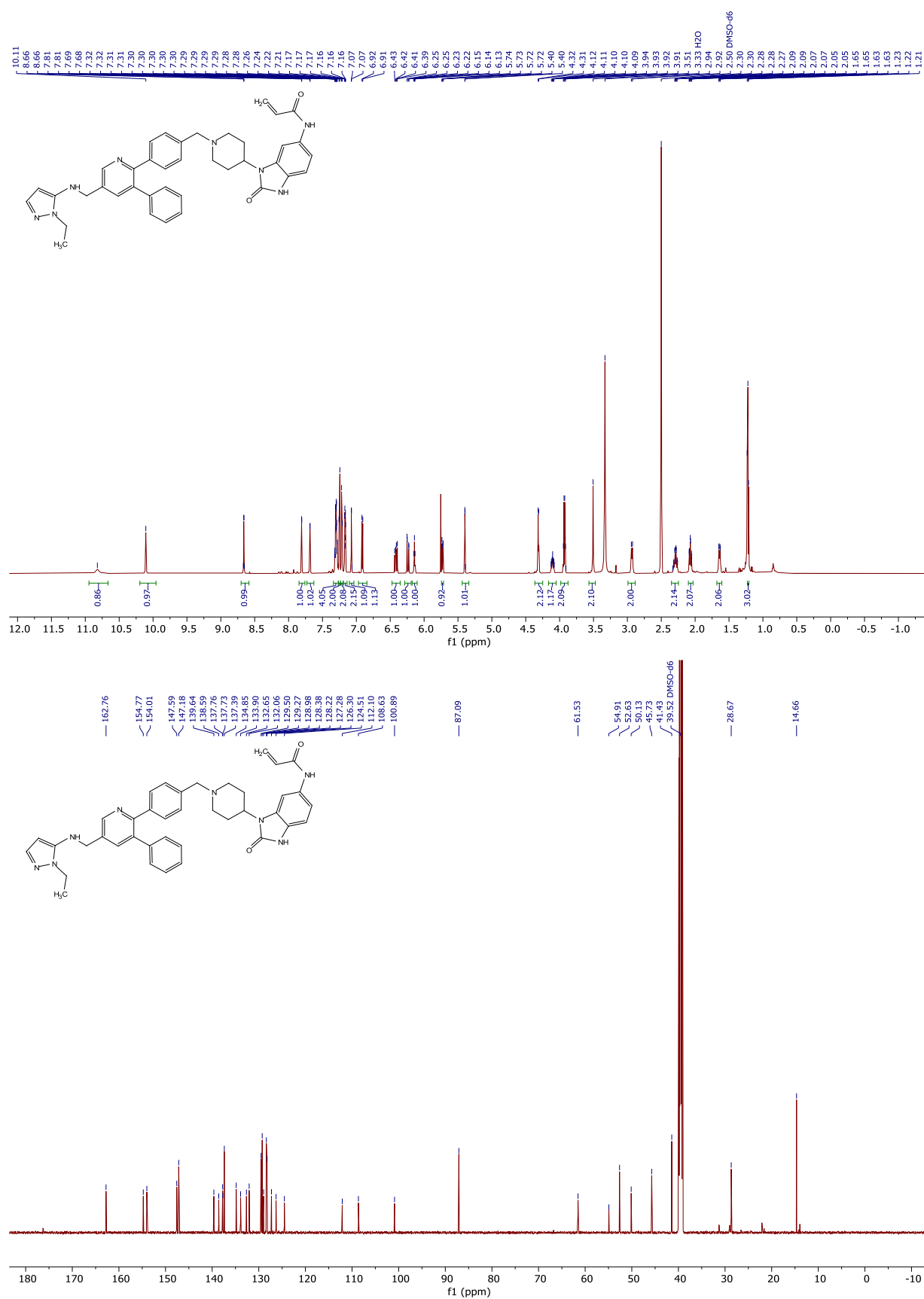


Figure S51. ¹H- and ¹³C-NMR spectra of compound **62**.

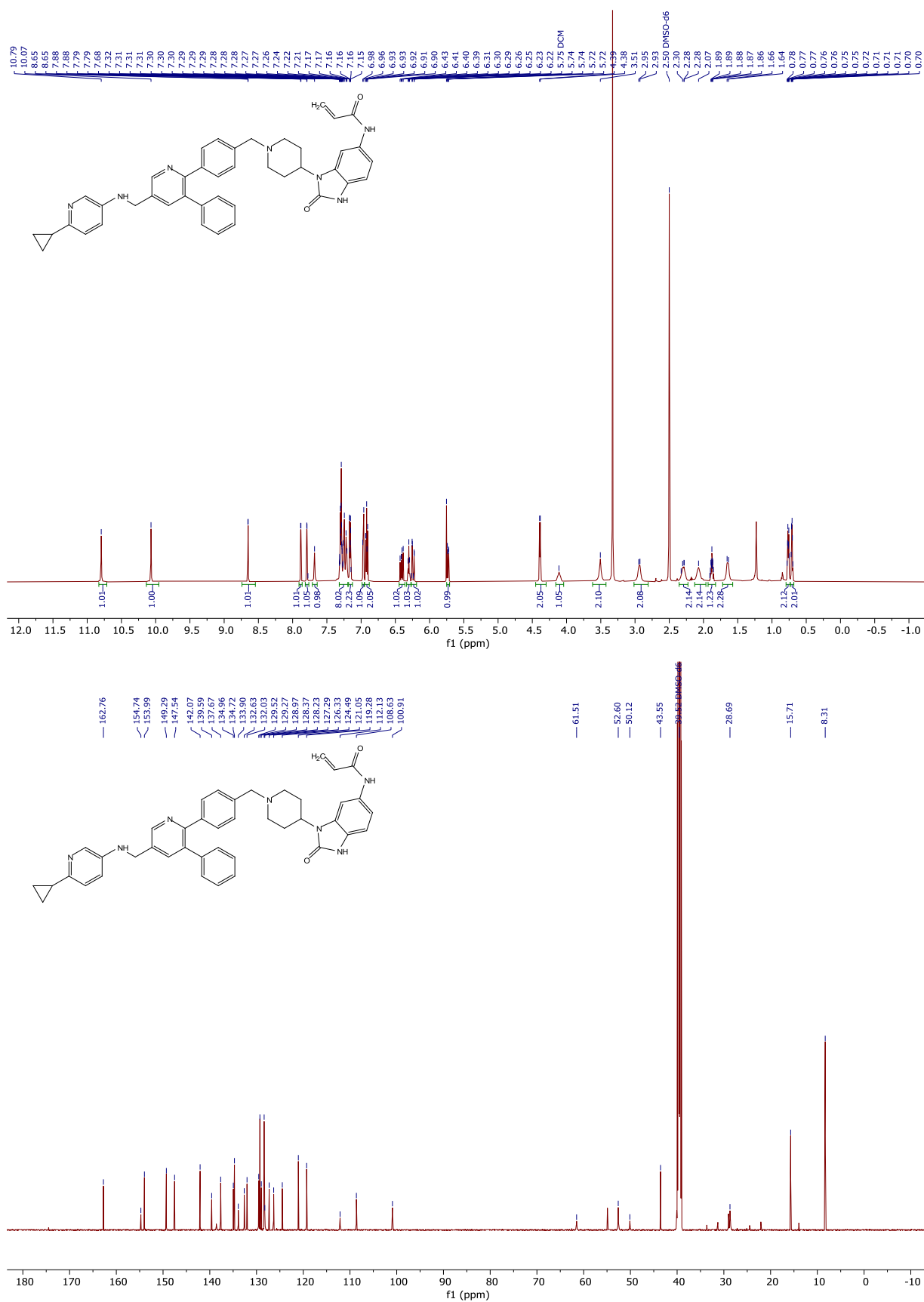


Figure S52. ^1H - and ^{13}C -NMR spectra of compound **63**.

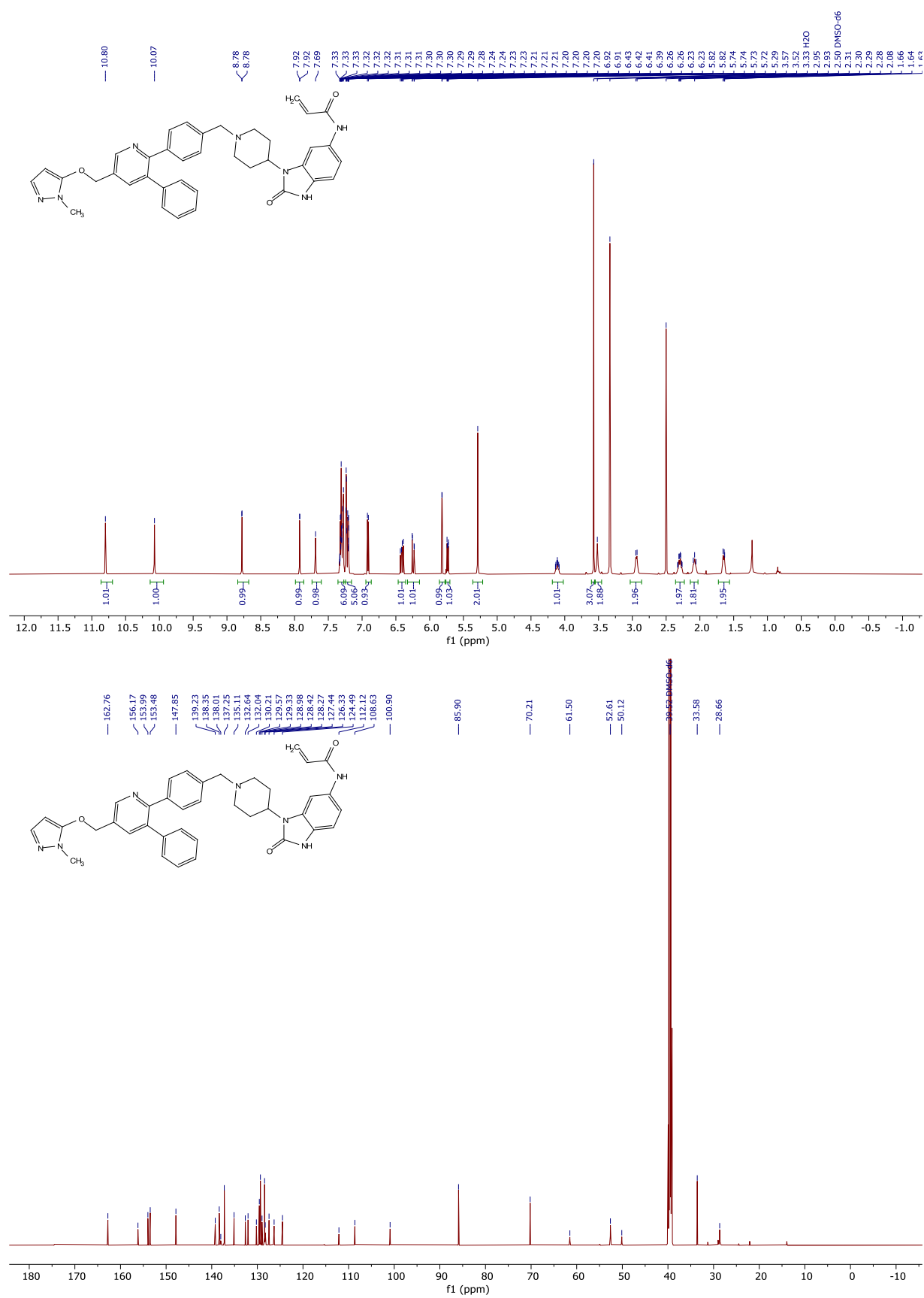
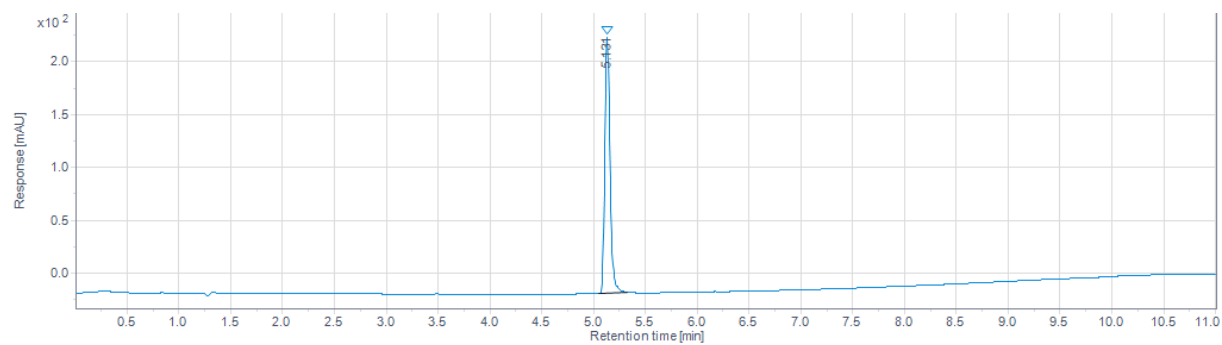


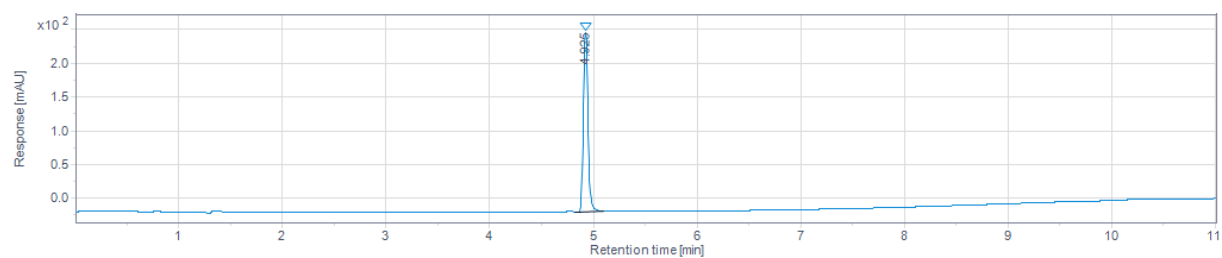
Figure S53. ¹H- and ¹³C-NMR spectra of compound **64**.

1. HPLC traces for all final compounds:

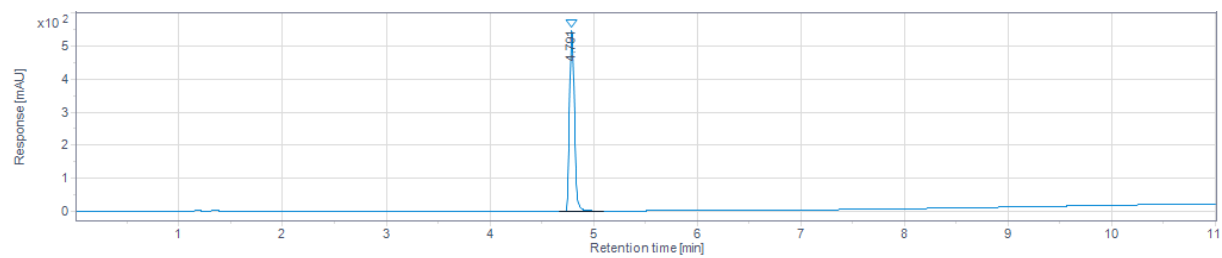
HPLC trace of compound **9**



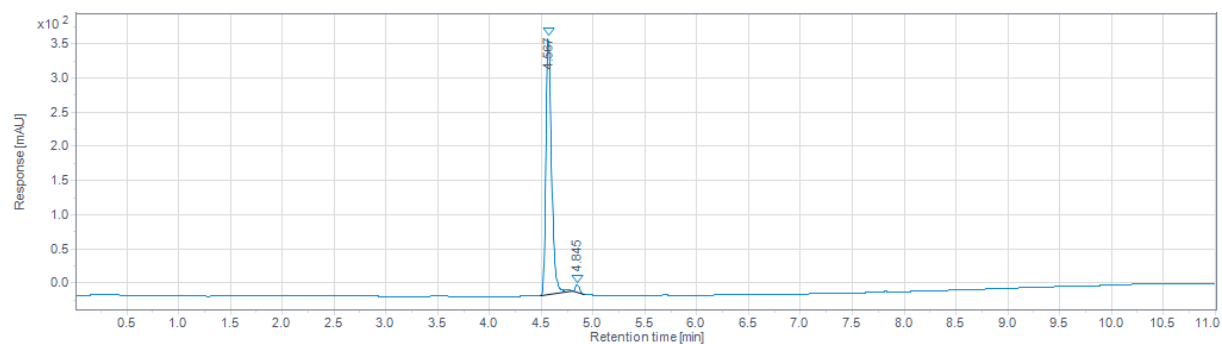
HPLC trace of compound **8**



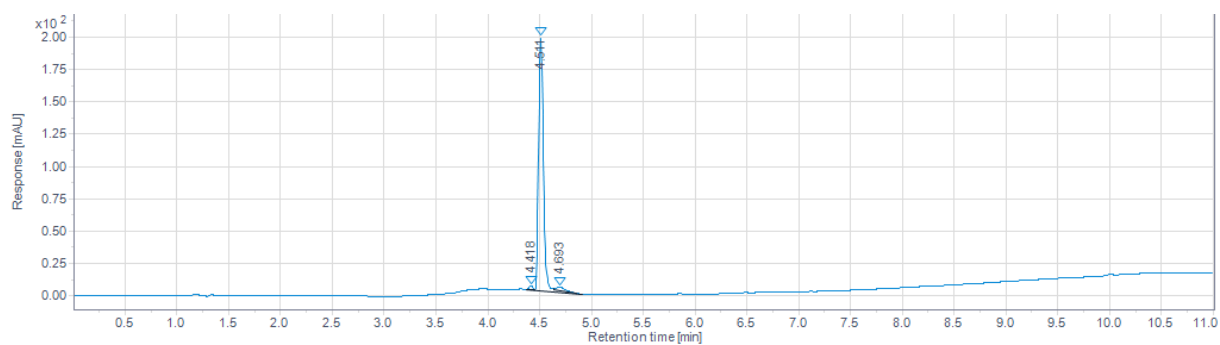
HPLC trace of compound **22**



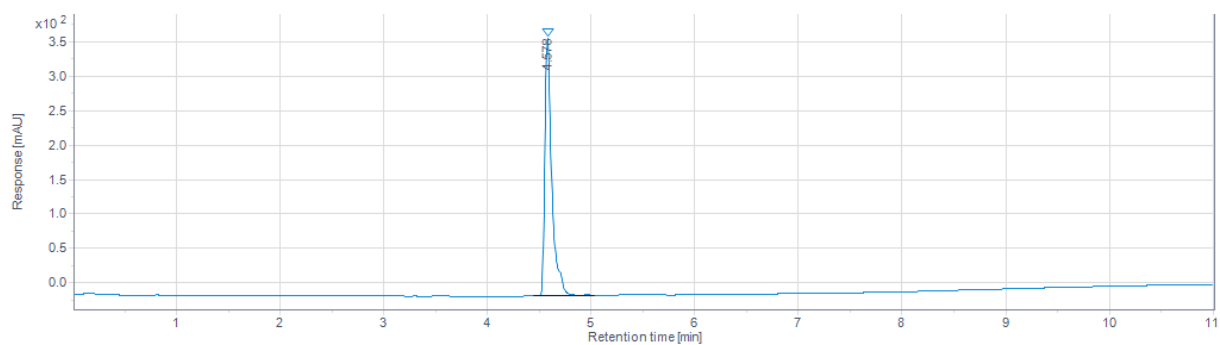
HPLC trace of compound **46**



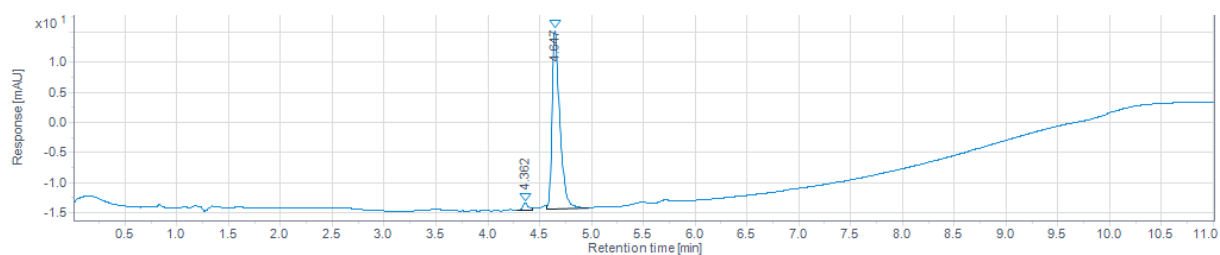
HPLC trace of compound **47**



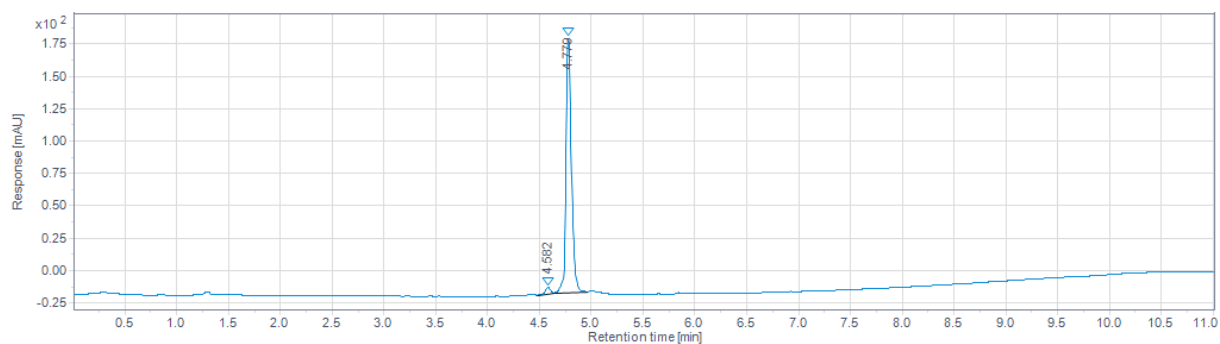
HPLC trace of compound **48**



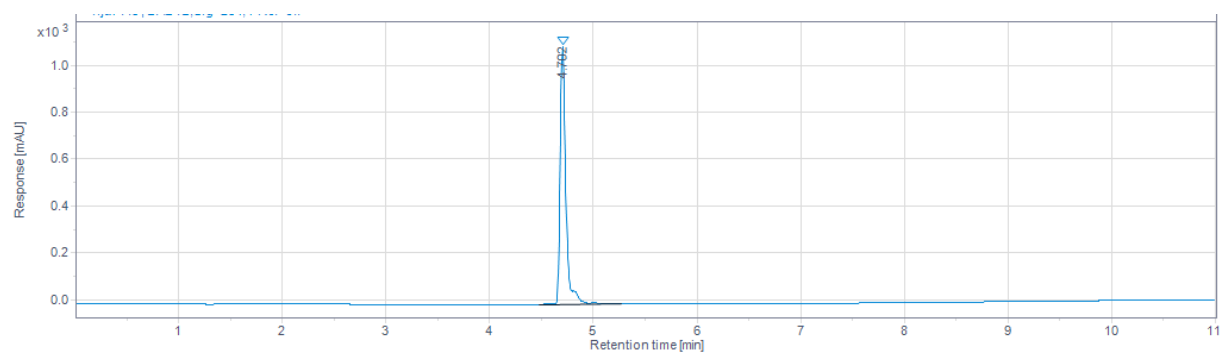
HPLC trace of compound **49**



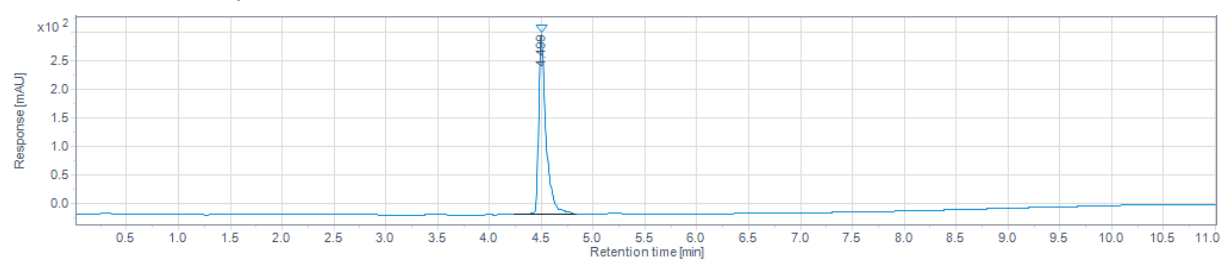
HPLC trace of compound **50**



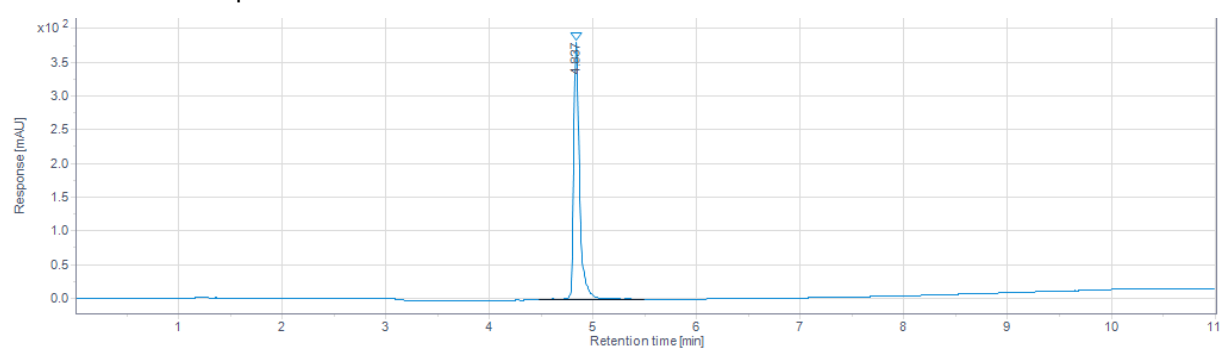
HPLC trace of compound **51**



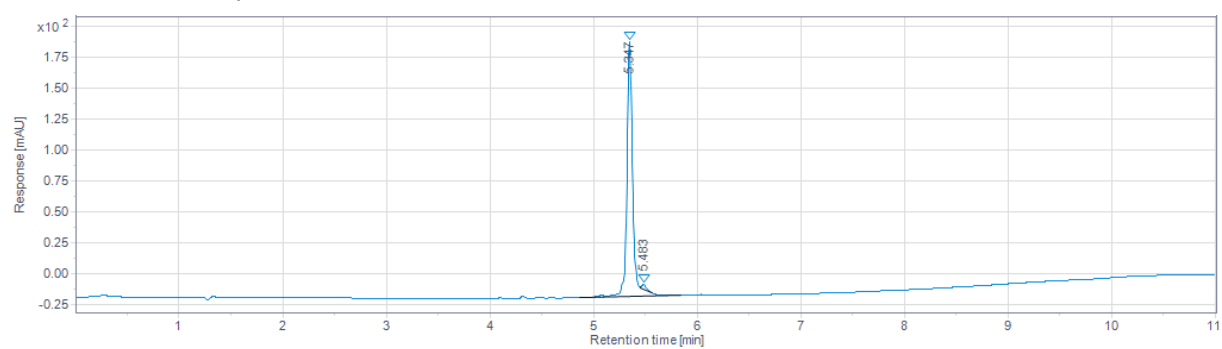
HPLC trace of compound **52**



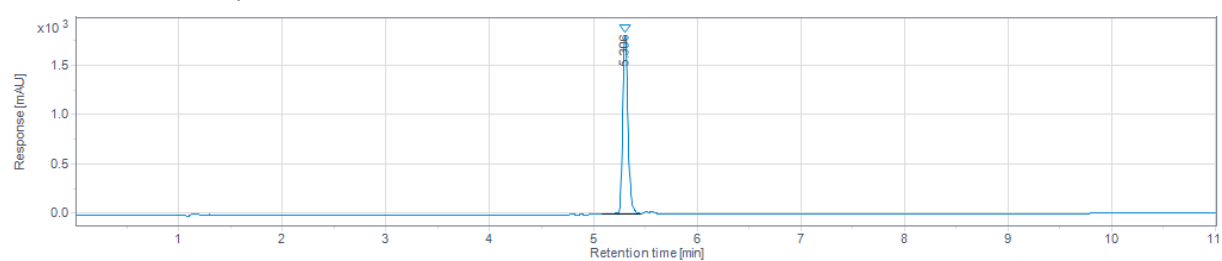
HPLC trace of compound **53**



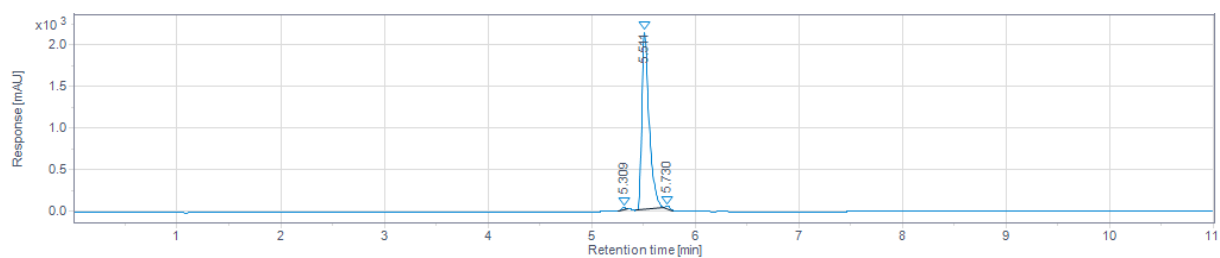
HPLC trace of compound **54**



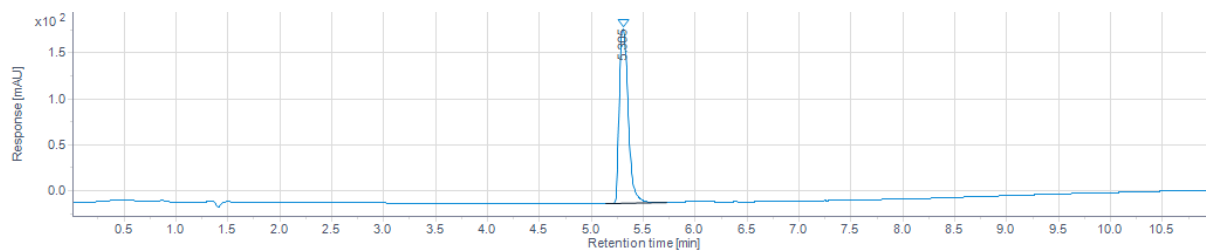
HPLC trace of compound **55**



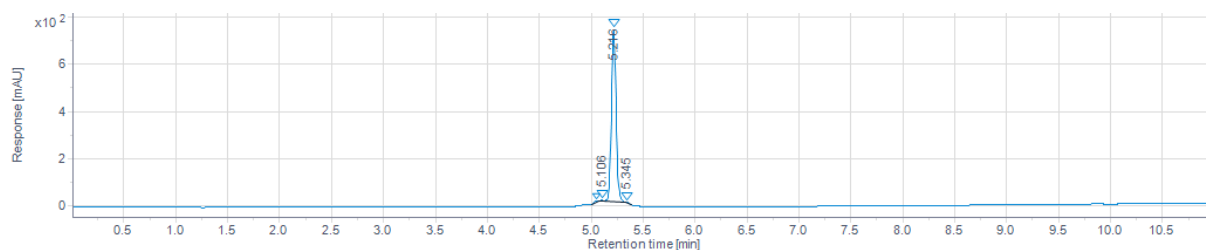
HPLC trace of compound **56**



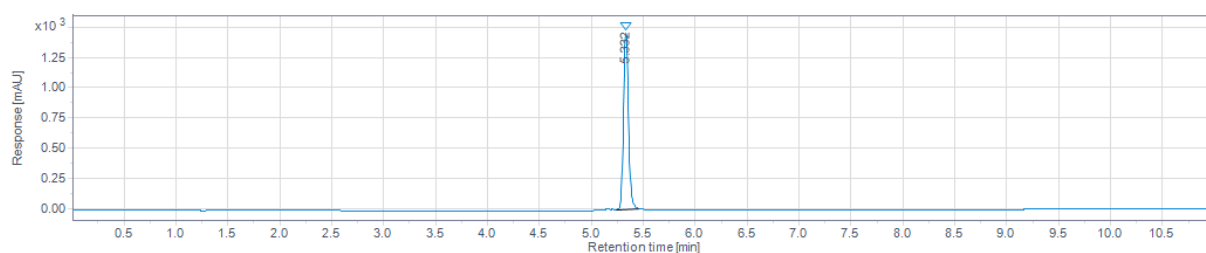
HPLC trace of compound **57**



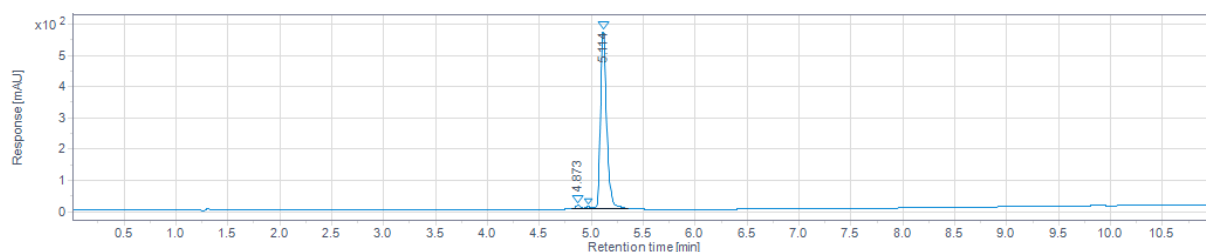
HPLC trace of compound **58**



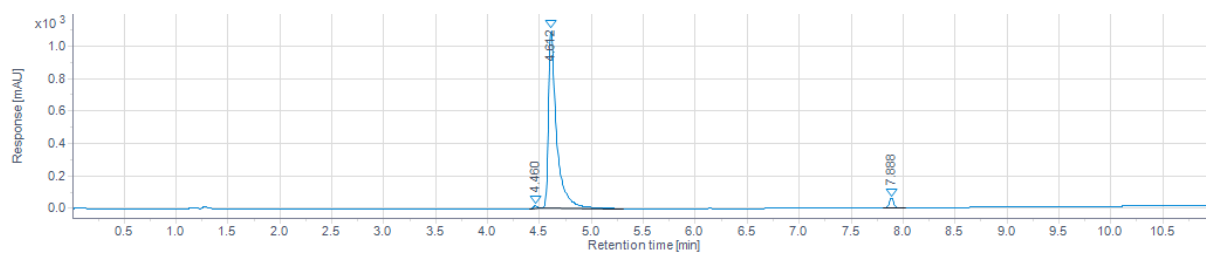
HPLC trace of compound **59**



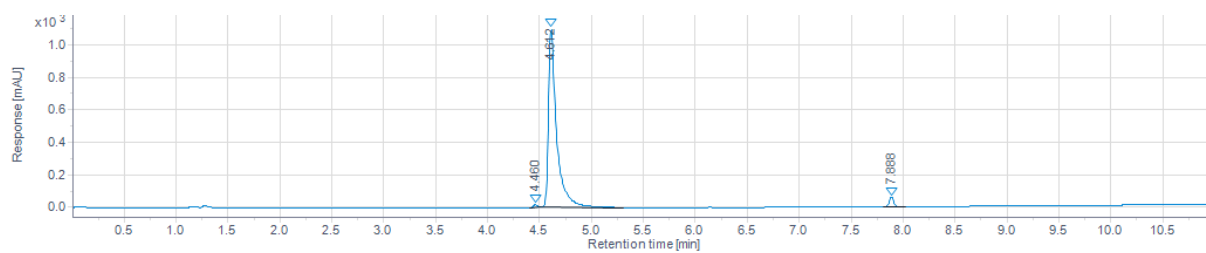
HPLC trace of compound **60**



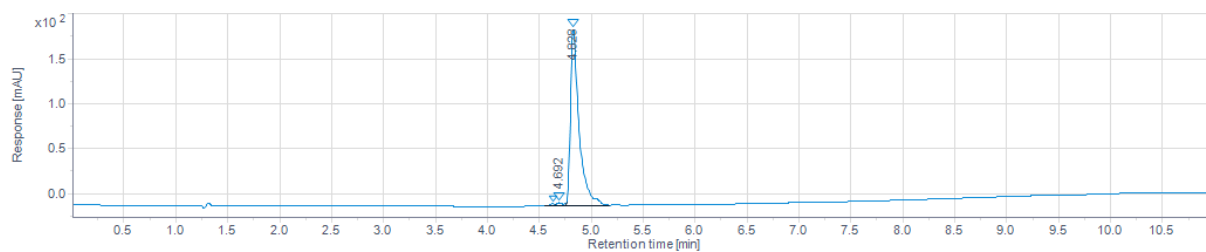
HPLC trace of compound **61**



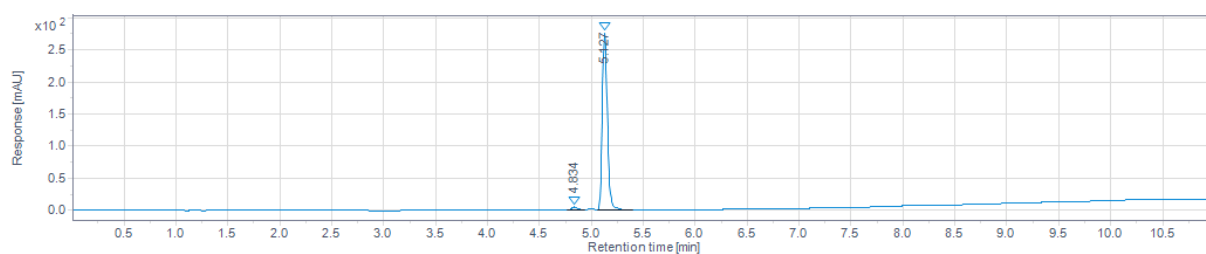
HPLC trace of compound **62**



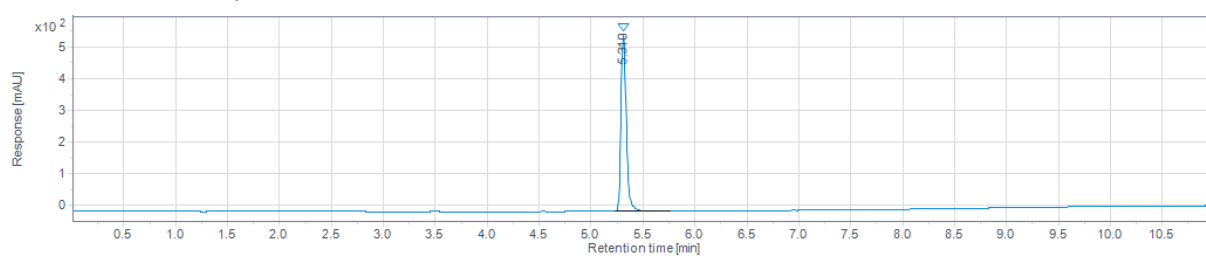
HPLC trace of compound **63**



HPLC trace of compound **64**



HPLC trace of compound **65**



2. Protein-MS Experiments for Compounds 9, 47-65.

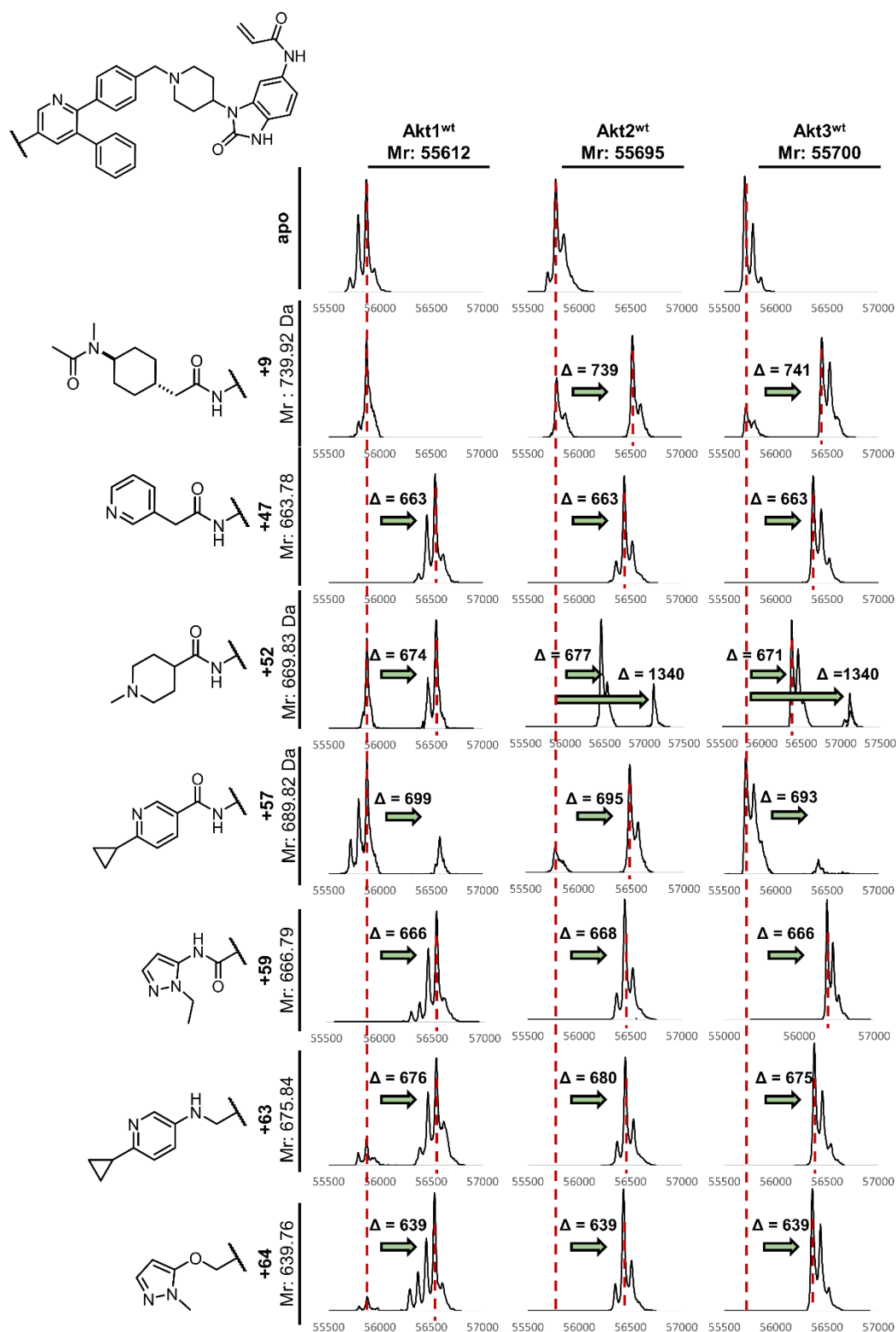


Figure S55: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt} and Akt3^{wt} after 24 h incubation with DMSO (apo) or the newly synthesized isoform-selective CAALs **9**, **47**, **52**, **57**, **59**, **63**, **64**. At this time point, most of the compounds produced mass increases consistent with the addition of a single ligand per protein, indicating mono-covalent modification, except compound **52** where a double-covalent modification was observed.

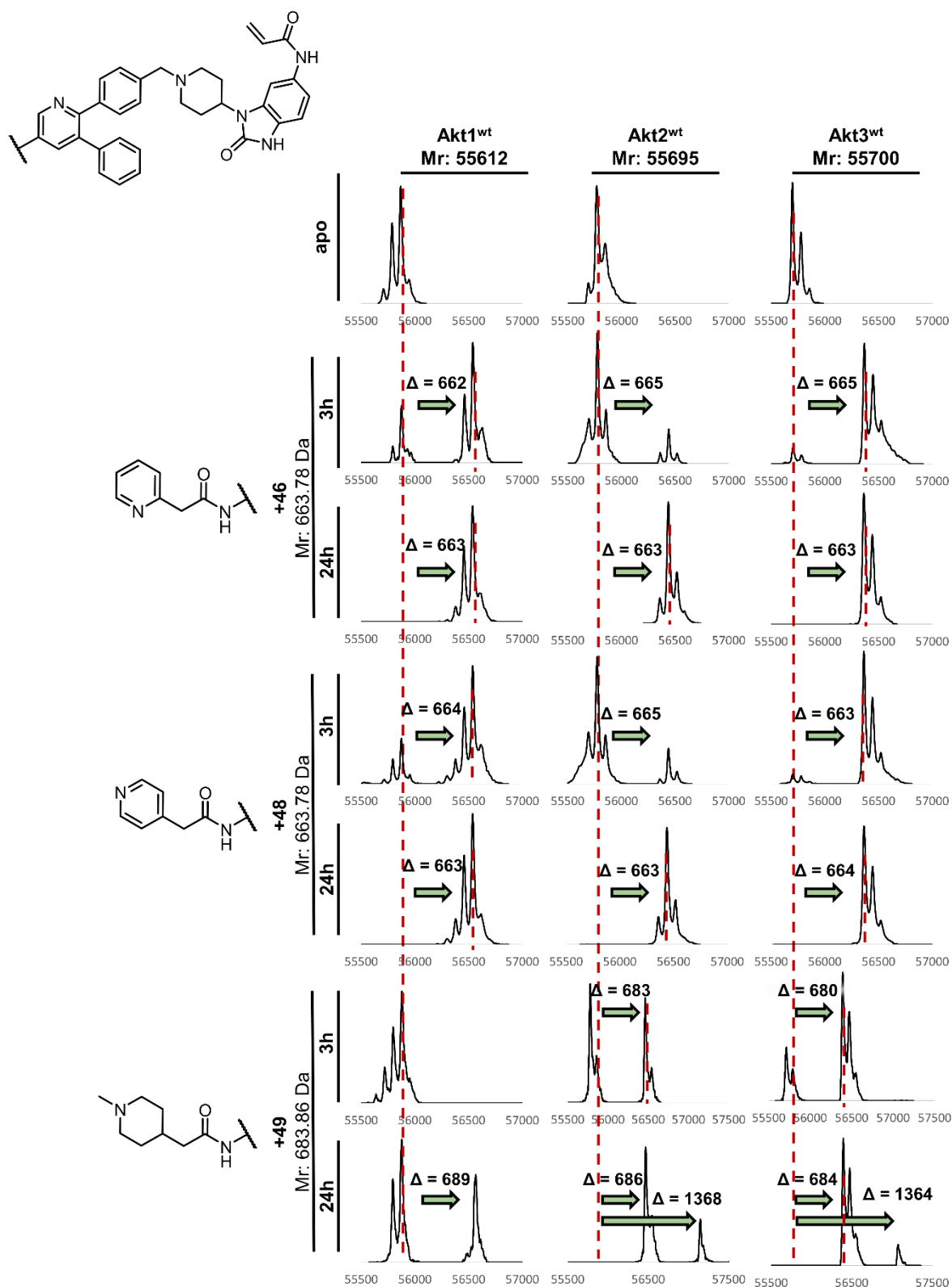


Figure S56: Deconvoluted mass spectra of Ak1^{wt}, Akt2^{wt} and Akt3^{wt} after 3 and 24 h incubation with DMSO (apo) or the newly synthesized isoform-selective CAAs **46**, **48**, **49**. At this time point, most of the compounds produced mass increases consistent with the addition of a single ligand per protein, indicating mono-covalent modification, except compound **49** where a double-covalent modification was observed.

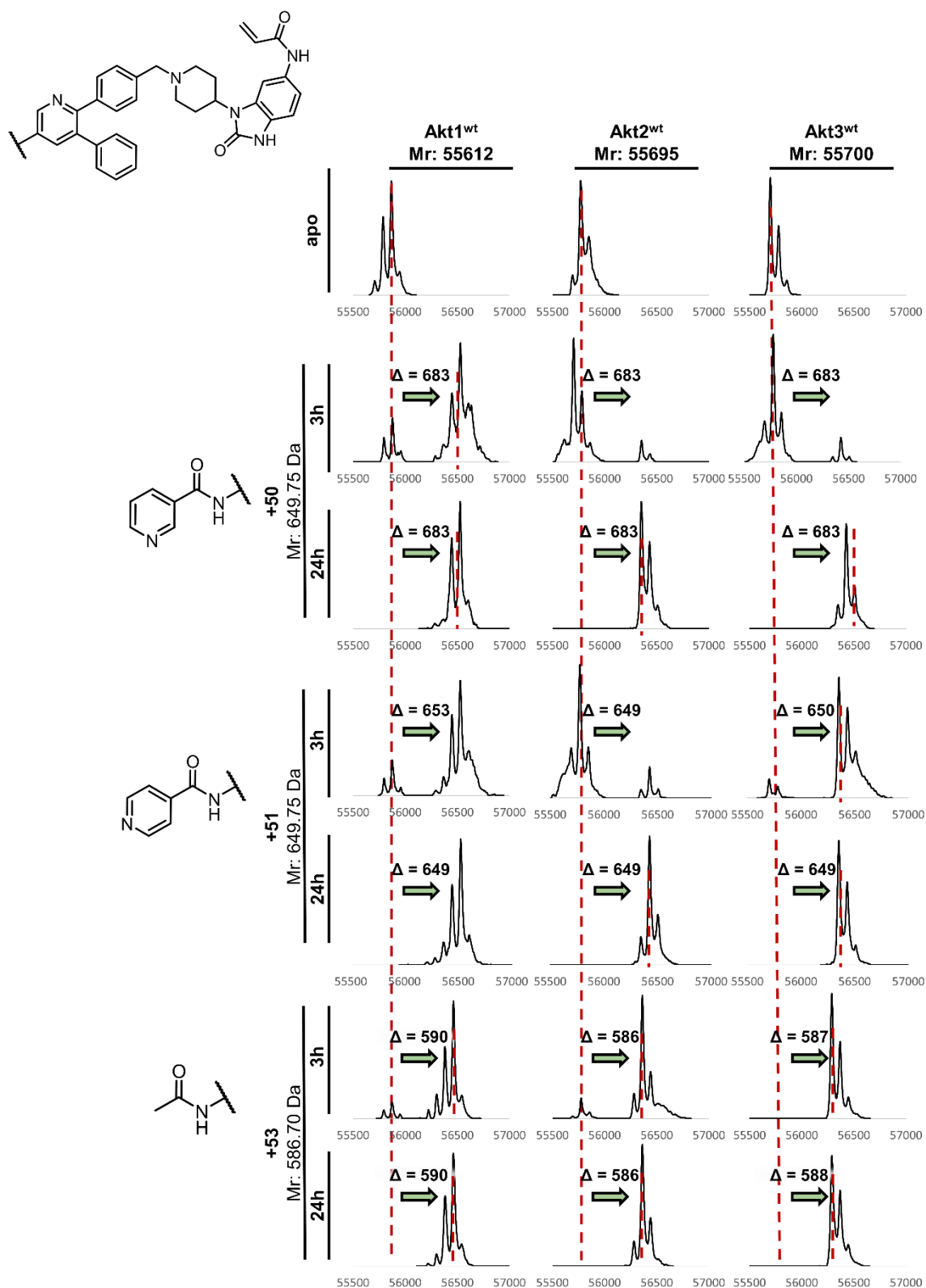


Figure S57: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt} and Akt3^{wt} after 3 and 24 h incubation with DMSO (apo) or the newly synthesized isoform-selective CAAs **50**, **51**, **53**. All the compounds produced mass increases consistent with the addition of a single ligand per protein, indicating mono-covalent modification.

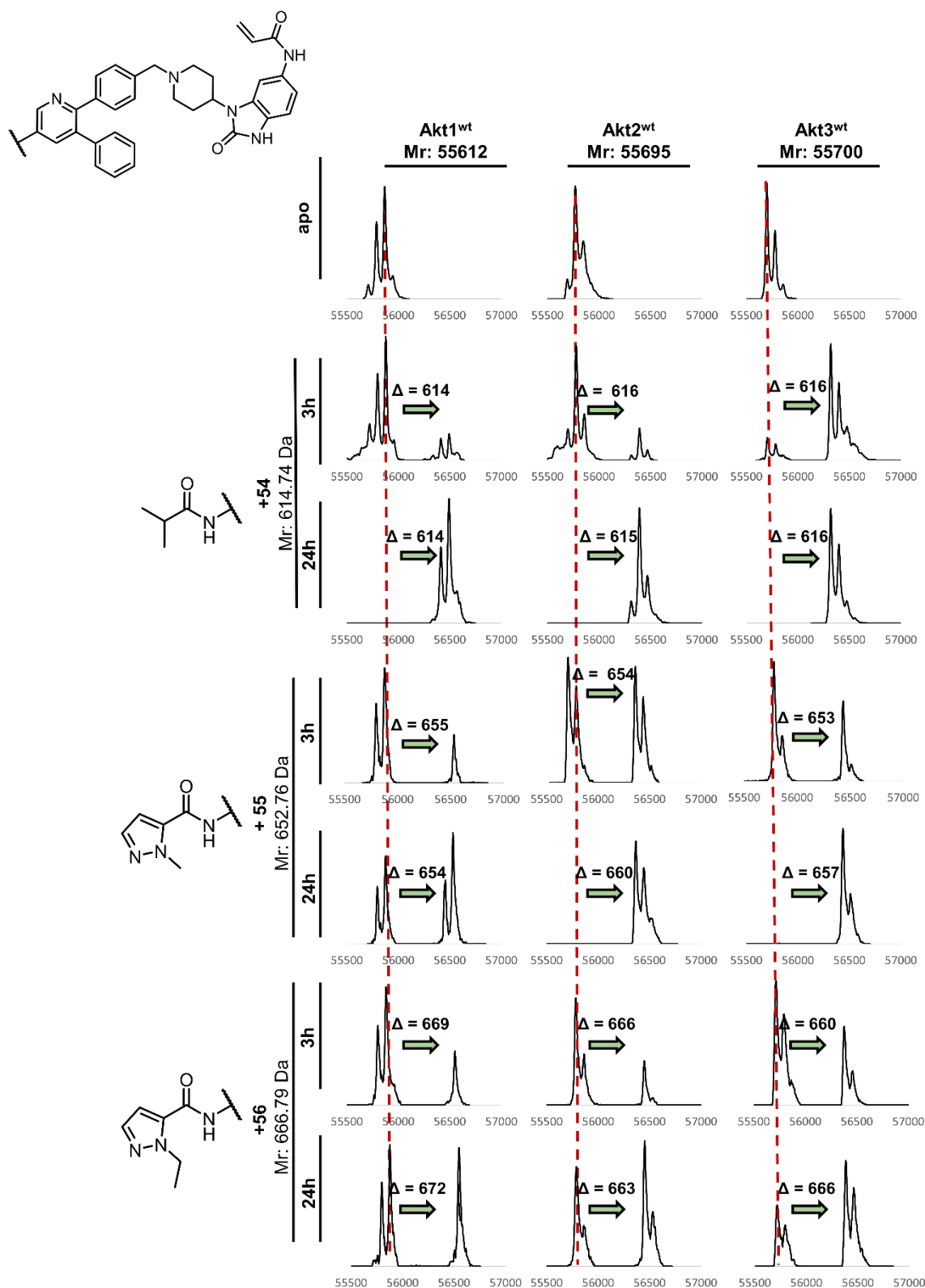


Figure S58: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt} and Akt3^{wt} after 3 and 24 h incubation with DMSO (apo) or the newly synthesized isoform-selective CAAs **54-56**. All the compounds produced mass increases consistent with the addition of a single ligand per protein, indicating mono-covalent modification.

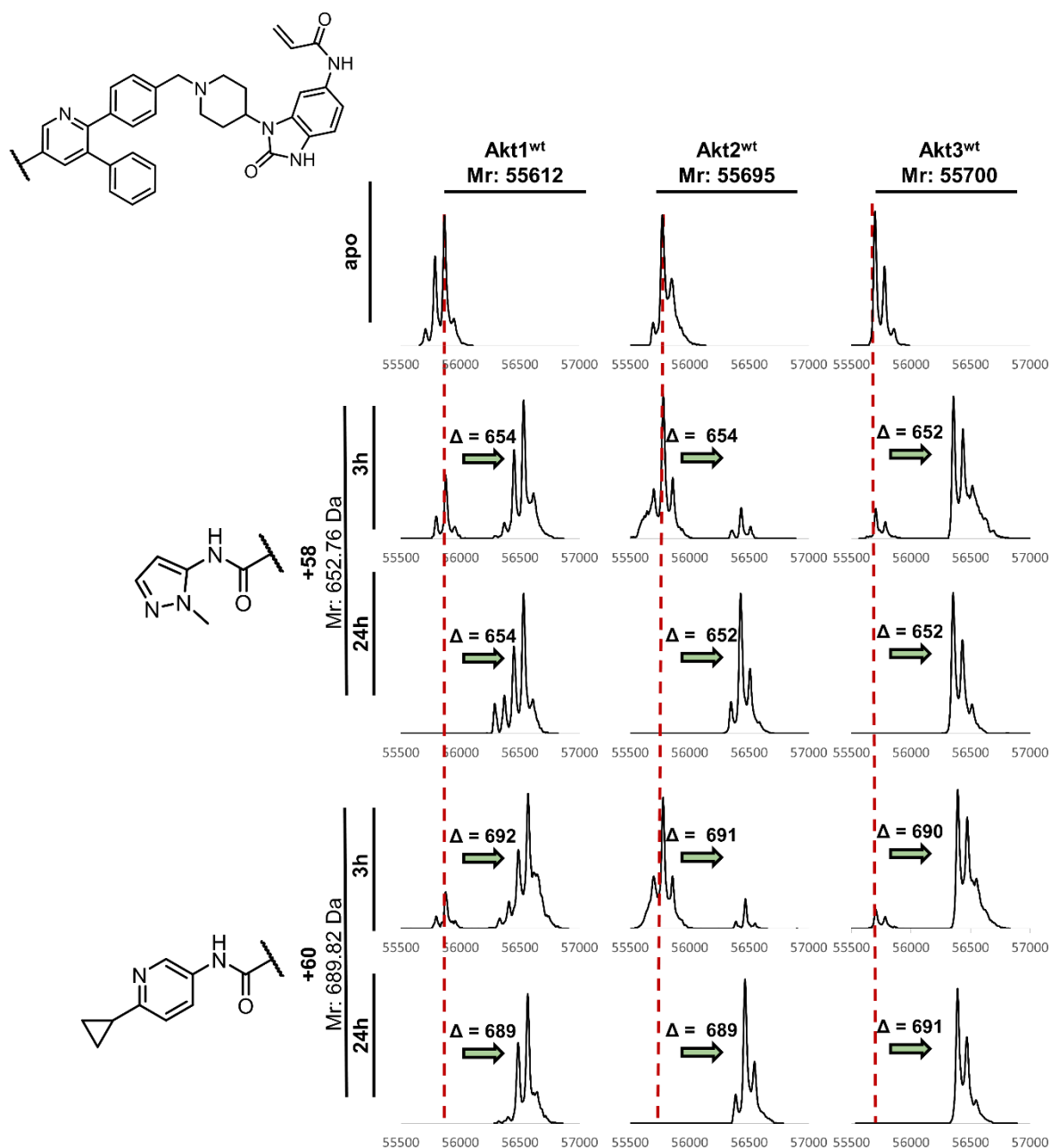


Figure S59: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt} and Akt3^{wt} after 3 and 24 h incubation with DMSO (apo) or the newly synthesized isoform-selective CAALs **58**, **60**. All the compounds produced mass increases consistent with the addition of a single ligand per protein, indicating mono-covalent modification.

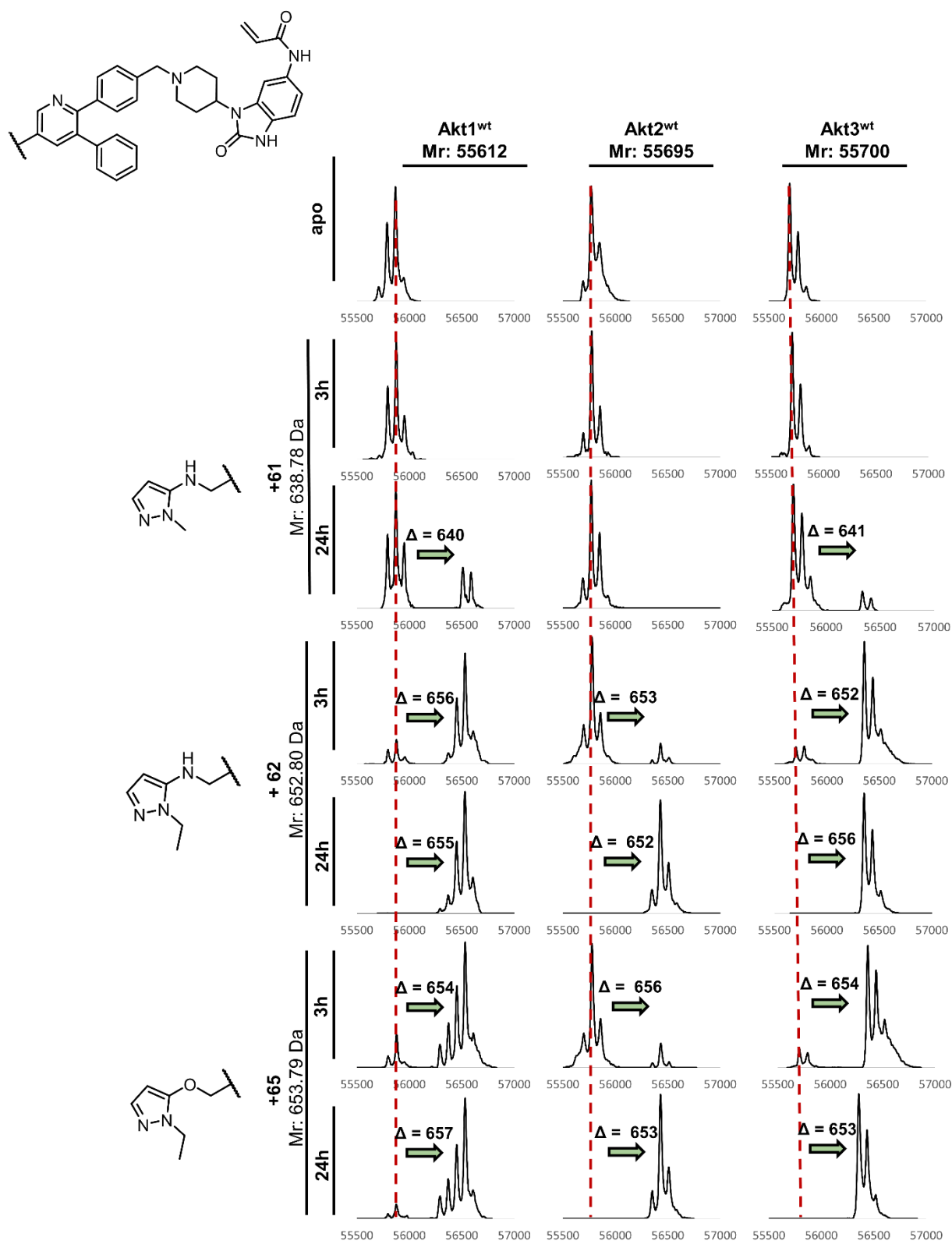


Figure S60: Deconvoluted mass spectra of Akt1^{wt}, Akt2^{wt} and Akt3^{wt} after 3 and 24 h incubation with DMSO (apo) or the newly synthesized isoform-selective CAAls **61**, **62**, **65**. All the compounds produced mass increases consistent with the addition of a single ligand per protein, indicating mono-covalent modification.

5.11.2. X-ray Statistics

Table S1. Data collection and refinement statistics for full-length Akt1 in complex with different covalent-allosteric Akt inhibitors.

	Akt1 mutant in complex with compound 64 and EG	Akt1 mutant in complex with compound 65 and EG	Akt1 mutant in complex with compound 62 and EG
PDB-ID	29BL	29BM	29BK
Data collection			
Space group	P12 ₁ 1	P12 ₁ 1	P12 ₁ 1
Cell dimensions a, b, c [Å] α , β , γ [°]	49.92, 71.38, 67.87, 90.0, 107.48, 90.00	49.56, 70.66, 67.45, 90.00, 107.02, 90.00	49.84, 71.25, 67.84, 90.00, 107.66, 90.00
Resolution [Å]	47.95-2.50 (2.60-2.50)	47.64-2.30 (2.40-2.30)	47.88-2.20 (2.30-2.20)
R _{meas} [%]	19.3 (133.0)	16.3 (86.4)	16.0 (131.1)
<i>I</i> / σ <i>I</i>	8.78 (2.05)	7.49 (2.32)	9.71 (1.62)
Completeness [%]	97.0 (97.9)	98.8 (99.5)	97.0 (97.9)
CC _{1/2}	0.992 (0.609)	0.990 (0.704)	0.996 (0.648)
Redundancy	5.6 (5.8)	4.2 (4.3)	7.3 (7.5)
Refinement			
Resolution [Å]	47.95-2.50	47.64-2.30	47.88-2.20
No. reflections	15691/785	19715/985	22447/1122
R _{work} / R _{free}	0.2031/0.2448	0.1915/0.2337	0.1965/0.2462
No. atoms	3109	3152	3180
Protein	3011	2995	3011
Ligands	52	53	53
Water	46	104	116
<i>B</i> -factors (Å ²)	44.36	46.76	43.31
Protein	44.49	46.79	43.47
Ligand	40.63	43.42	39.49
Water	47.33	47.71	41.05
RMS deviations Bond lengths [Å] Bond angles [°]	0.004 0.685	0.003 0.532	0.002 0.600
Ramachandran [%] Favored Allowed Outliers	 95.91 4.09 0.00	 96.71 3.29 0.00	 97.53 2.47 0.00

5.11.3. Additional Figures

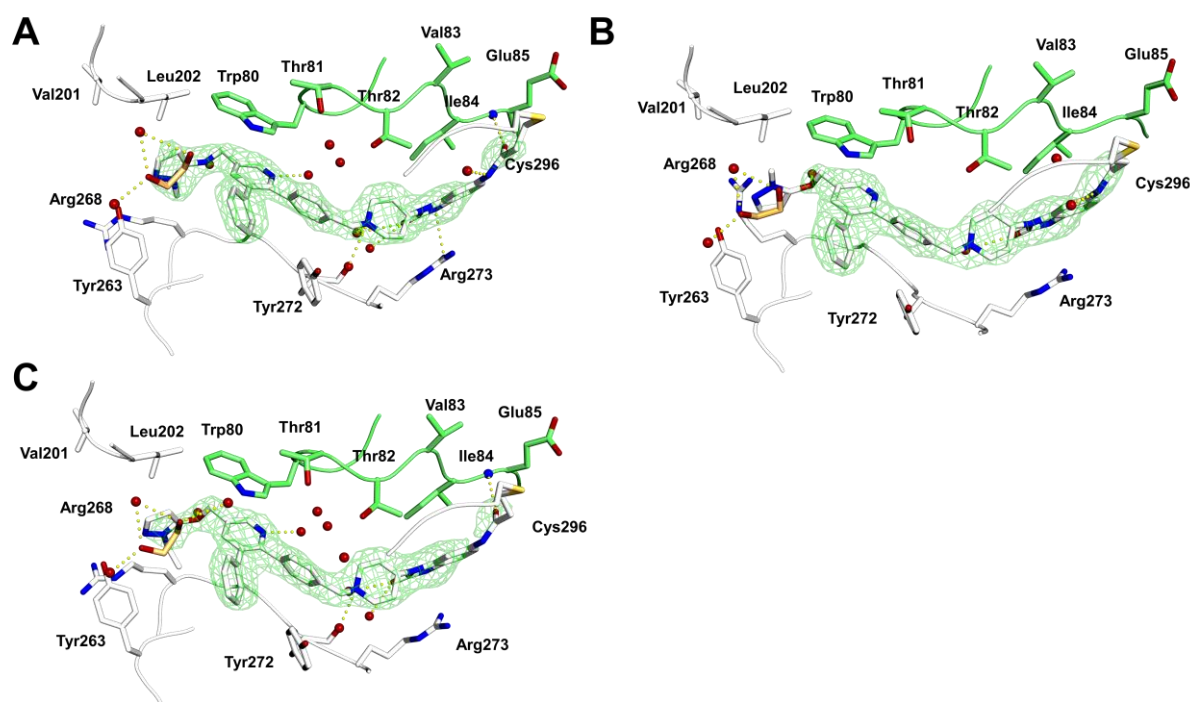


Figure S61: Co-crystal structures of Akt1 mutant with: (A) compound **62** (PDB: 29BK) (B) compound **64** (PDB: 29BL) and (C) compound **65** (PDB: 29BM). The simulated annealing omit $|F_o - F_c|$ -electron density map is shown at r.m.s.d. = 3.0.

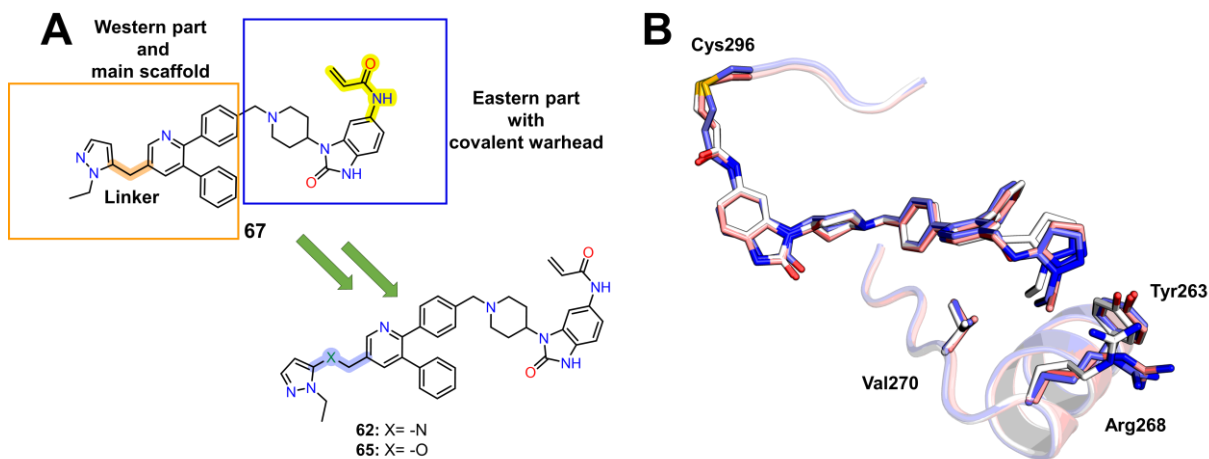


Figure S62: (A) Depiction of chemical structures **62**, **65** and **67** depicting the original idea about extension of the linker with incorporation of a heteroatom and highlighting important structural characteristics. (B) Overlay of co-crystal structures of compounds **62** (pink), **65** (blue) and **67** (white) in Akt1-mutant highlighting the similar accommodation of the ethyl-pyrazole in the allosteric pocket, despite the different linker length.

6. Summary and Outlook

Akt is a central serine/threonine kinase of the PI3K/Akt/mTOR signaling pathway and regulates key cellular processes including proliferation, survival, metabolism, and migration. Dysregulation of Akt signaling is associated with cancer and other human diseases, which has made Akt an important target for drug discovery for more than two decades. Considerable progress has been achieved in the development of ATP-competitive, allosteric, and covalent-allosteric inhibitors, culminating in the clinical approval of capivasertib. At the same time, the available data show that important challenges remain, particularly limited isoform selectivity, adverse effects associated with broad Akt inhibition, resistance mechanisms, and an incomplete understanding of the specific roles of Akt1, Akt2, and Akt3 in different biological contexts. These unresolved issues provided the starting point and the central motivation for this thesis. The overall aim of this work was to establish a structure-guided strategy for the development of selective covalent-allosteric Akt inhibitors targeting the interdomain pocket formed between the PH domain and the kinase domain. In contrast to the highly conserved ATP-binding site, this regulatory pocket offers a less conserved and conformationally informative environment and is therefore particularly attractive for selective inhibitor design. Building on the concept of covalent-allosteric inhibition established in the Akt field, this thesis focused on exploiting subtle structural differences between the Akt isoforms in order to achieve selective targeting of Akt2 and Akt3 and, in a subsequent step, to sharpen this selectivity further toward Akt3. A major result of this thesis is the demonstration that isoform-selective covalent-allosteric inhibition of Akt is feasible despite the high sequence homology of the three isoforms. Guided by structural analysis, molecular modeling, and X-ray crystallography, selective inhibitors were developed that preferentially target Akt2 and Akt3 while sparing Akt1. In particular, pyrazole-containing compounds emerged as potent Akt2-directed inhibitors, whereas pyridine-based designs shifted selectivity toward Akt3. These findings show that the allosteric pocket at the PH-kinase interface contains sufficient structural information to allow rational isoform discrimination and that selectivity can be deliberately tuned through medicinal chemistry. An important aspect of the work was the integration of structural, biochemical, and cellular evaluation. Structural insight into isoform-selective recognition was obtained using an engineered Akt1 construct that mimic isoform-specific features of the allosteric pocket of Akt2 and enabled direct analysis by X-ray crystallography.

These data were combined with NanoBRET target-engagement assays in living cells, protein mass spectrometry confirming mono-covalent labeling, and functional assays such as Western blotting and CellTiter-Glo measurements. In this way, the thesis established a direct connection between molecular design, target engagement, and cellular response. This integrated workflow was essential for distinguishing between biochemical binding and biologically meaningful selectivity. The cellular studies further showed that strong target engagement does not necessarily result in strong antiproliferative effects. Although several inhibitors showed potent and selective intracellular engagement, their effects on viability depended strongly on the biological context. In particular, the limited response observed in PANC-1 cells, despite efficient Akt2 engagement, indicated that target inhibition alone is insufficient if the respective model is not strongly dependent on PI3K/Akt signaling. This finding is highly relevant for the interpretation of selective Akt inhibitors, because it shows that these compounds should not only be considered as therapeutic leads, but also as chemical probes that can be used to dissect isoform-specific signaling dependencies. This concept was further supported by the experiments in patient-derived colorectal cancer organoids, where the developed covalent-allosteric inhibitors displayed differential activity profiles across models with distinct signaling backgrounds. A further central result of this thesis was the refinement of covalent-allosteric Akt inhibition toward Akt3 selectivity. Although the three Akt isoforms share a high degree of sequence homology, the structural analyses showed that the allosteric pocket contains subtle but functionally relevant differences that can be exploited for selective ligand design. In Akt1, bulky western substituents are disfavored because they induce steric clash with Lys268 and force a shift of the ligand scaffold. In Akt2, the shortened adjacent α -helix, due to the absence of Glu267, provides additional space, but the ligand must still partially reorient to avoid Arg269. In contrast, Akt3 combines a shortened α -helix with Gly265, thereby generating a more spacious and permissive pocket that can accommodate bulkier substituents without comparable steric strain. These differences provided the structural basis for the design of Akt3-directed covalent-allosteric inhibitors and explain why motifs derived from previously described Akt3-selective reversible inhibitors could be successfully transferred into the covalent-allosteric series.

Within this framework, the systematic variation of the linker region proved to be one of the most important mechanistic findings of the work. Replacement of the previously used methylene linker by amide, reverse amide, aminomethylene, or ether linkers

demonstrated that these elements do not merely connect two pharmacophores but actively determine how the isoform-discriminating western substituent is positioned within the allosteric pocket. In the cellular NanoBRET assay, some linker types shifted the activity profile toward Akt3, whereas others reduced or even abolished isoform selectivity. Aminomethylene linkers favored Akt3 engagement, while ether linkers produced highly potent but broadly active compounds across all three isoforms, indicating loss of isoform discrimination. Structural analysis showed that these changes were accompanied by altered ligand orientation and flexibility within the pocket. Importantly, the crystal structures revealed a coordinating water molecule in the binding site that was not observed in the earlier methylene-linker series, and some of the western substituents displayed increased positional disorder. These observations suggest that linker modification reshapes the local microenvironment of the allosteric pocket not only by changing steric presentation, but also by reorganizing solvent interactions. In this way, the water-mediated network appears to contribute to local stabilization and to influence how efficiently isoform-specific interactions can be translated into selectivity. An additional and conceptually important conclusion of this part of the thesis is that covalent bond formation is not automatically beneficial for selectivity. Comparison of the reversible Akt3-selective compound from BerGenBio with the corresponding covalent hybrid analogue showed that introduction of the Michael acceptor increased overall potency, but at the same time reduced the Akt3-over-Akt1 selectivity in cells. This suggests that when the initial reversible binding event is not sufficiently discriminating, subsequent covalent capture may broaden engagement across the isoforms rather than sharpen it. This interpretation is consistent with the protein mass spectrometry data, which showed mono-covalent labeling of all three Akt isoforms, although to different extents, and with the observation that linker modifications could markedly alter the selectivity profile without abolishing covalent engagement itself. In this context, covalency is most effective when it builds on a strongly isoform-selective reversible binding event; if the initial recognition is too permissive, irreversible bond formation may diminish the overall selectivity profile. An important future perspective emerging from these findings concerns the choice of the residue targeted for covalent bond formation. In the inhibitor series developed here, covalent bond formation relies on the conserved cysteine residues within the allosteric pocket, whereas isoform selectivity is primarily determined by how the variable western substituents and linker elements interact with the isoform-specific pocket environment.

The structural analyses, however, revealed that the variable region of this pocket contains isoform-distinct residues that critically influence ligand accommodation, most notably Lys268 in Akt1, Arg269 in Akt2, and Gly265 in Akt3. In the Akt2-focused study, the corresponding arginine-containing region already emerged as an important selectivity determinant and was shown to participate in local interaction networks, including indirect and solvent-mediated contacts. These observations suggest that future inhibitor design may not need to remain restricted to conserved cysteine-directed covalent targeting. Instead, it may become possible to exploit isoform-specific residues more directly, for example by designing tailored reactive motifs capable of engaging lysine in Akt1 or arginine in Akt2. Although such an approach remains challenging, it represents an attractive future direction because it could shift covalent targeting from a conserved anchoring principle toward a more intrinsically isoform-selective strategy.

7. References

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