

Synthesis of phospho-amino acid analogues as tissue adhesive cement additives

Christopher D. Spicer,^{*a,b,c} Michael Pujari-Palmer,^d H      Autefage,^a Gerard Insley,^d Philip Procter,^d H      Engqvist^d and Molly M. Stevens^{*a,e}

^aDepartment of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden.

^bDepartment of Chemistry, University of York, Heslington, YO10 5DD, UK.

^cYork Biomedical Research Institute, University of York, Heslington, YO10 5DD, UK.

^dApplied Material Science, Department of Engineering, Uppsala University, 75121 Uppsala, Sweden.

^eDepartment of Materials, Department of Bioengineering, and Institute of Biomedical Engineering, Imperial College London, London, United Kingdom.

* chris.spicer@york.ac.uk; m.stevens@imperial.ac.uk

ABSTRACT: In this paper we report the synthesis of a library of phospho-amino acid analogues, via a novel single step allyl-phosphoester protection/Pd-mediated deprotection strategy. These phosphoserine and phosphotyrosine analogues were then applied as additives to create adhesive calcium phosphate cements, allowing us to probe the chemical origins of the increased surface binding strength. We demonstrate the importance of multiple calcium binding motifs in mediating adhesion, as well as highlighting the crucial role played by substrate hydrophobicity and orientation in controlling binding strength.

Biomolecule phosphorylation is an essential process in biological systems.¹ Phosphorylated small molecules play diverse and crucial roles as building blocks for larger biomacromolecules,² intermediates during biosynthesis,^{3,4} core components of cellular metabolites,¹ and as agonists and inhibitors of intracellular signalling⁵. The biological potency and relevance of organic phosphates has led to increasing interest in their use as active agents in biomedical and biomaterial technologies.^{6–8} However, the routine synthesis of phosphorylated-biomolecule libraries remains challenging due to competition between multiple functional handles and the need for selective deprotection

of phosphate ester intermediates.⁹ Here, we report a straightforward approach to the synthesis of phospho-biomolecule analogues, via a novel strategy that combines single-step, allyl-phosphoester installation with palladium-mediated deprotection. Furthermore, we demonstrate the use of these analogues in the development of strongly adhesive bioceramics for tissue engineering.

Calcium phosphate cements (CPCs) are exciting candidates for the treatment of bone defects due to their osteoconductive and bioresorbable properties.¹⁰ However, while standard cements possess high bulk cohesive strength, they suffer from poor adherence to native tissues. This leads to the formation of a mechanically weak interface that in turn increases the risk of implant failure *in vivo*.¹¹ A cement capable of forming an interface with tissue with improved fixation strength would reduce the likelihood of mechanical loosening. To address this problem, Kirillova *et al* recently reported that phosphoserine (PSer, **1**) can provide CPCs with remarkable adhesive strength.¹² We have subsequently shown that PSer induces an organic/inorganic microstructure that templates calcium phosphate nucleation that may be responsible for the increased adhesion.^{13–15} PSer-modified cements (PMCs) are therefore able to produce strong biomaterial-tissue interfaces that enable their use within load-bearing environments.^{12,15} This has the advantage of enabling the use of CPCs as both fillers for bone defects and adhesive surfaces to form strong bridges between bone and alternative biomaterials such as metallic implants. Two commercial PMCs, Tetranite® and OsStic™ are currently undergoing advanced testing towards *in vivo* applications in dental and osteochondral applications. To investigate the ability of PSer to mediate adhesion, understand this phenomenon further, and potentially identify 2nd generation additives able to enhance mechanical properties further, we set out to synthesise a library of phospho-amino acid analogues. The physico-chemical properties of a cement are determined by the properties of the precursors, in the case of a PMC the organic amino acid and a basic calcium salt. We hypothesised that changes in the chemistry of the organic additive, for example through the removal of acidic or calcium chelating motifs, would directly affect the resulting adhesive strength.^{16,17} Mechanical (shear) testing would then be able to provide insight, at both the molecular- and macro-scale, into the *ex vivo* relationship between additive chemistry and cement adhesive strength.

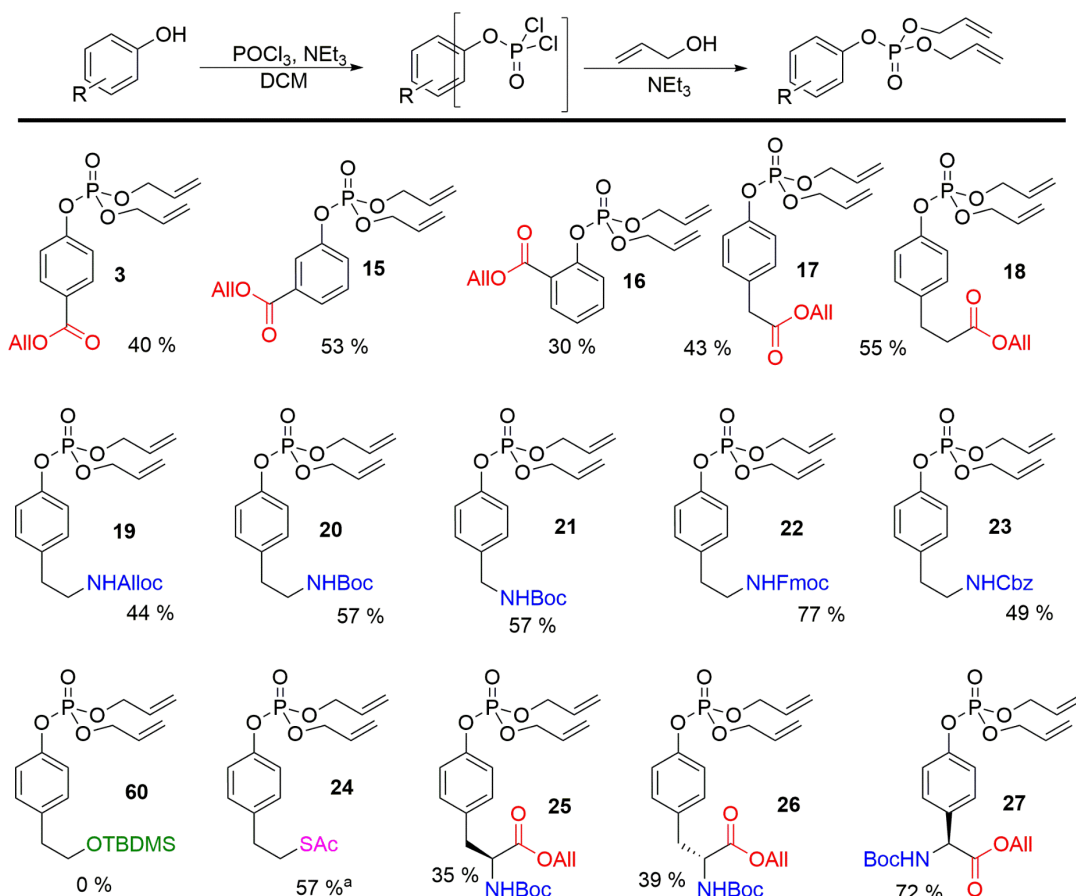
The synthesis of phospho-amino acids has previously been achieved via three main approaches, each of which has limitations for library synthesis. In a first strategy, the desired phosphate group can be installed directly using a suitable phosphate donor.^{18,19} However, the resultant phosphoric acid monoesters are typically difficult to purify.²⁰ Secondly, protected phosphonate esters can be installed through treatment with phosphorous (V) oxychloride

(POCl₃) and a sacrificial alcohol. The resultant phosphate esters are easily handled, but typically require subsequent deprotection via basic hydrolysis. This challenging step must be carefully controlled to avoid competing phosphate cleavage.²¹ Finally, phosphoramidite-based reagents can be used to install protected-phosphite esters that must be subsequently oxidised to the corresponding phosphate.⁹ While this process allows orthogonal phosphate ester protecting groups to be introduced, the need for a second oxidative step with the potential for side reactions may be limiting. We therefore aimed to identify a global strategy for the introduction and deprotection of phosphate esters, which would also allow parallel and straightforward manipulation of the amino- and carboxy-protecting groups common in biomolecule substrates.

We initially envisaged the use of an acid-labile protecting group strategy through the installation of *tert*-butyl phosphate esters.²² Although O*t*Bu-phosphoramidite reagents were effective for alcohol phosphorylation, yields were often poor due to the high bulk of the *t*Bu ester. Attempts to utilise *tert*-butanol as a sacrificial alcohol following substrate treatment with POCl₃ also proved ineffective. As an alternative, we reasoned that allyl protection would deliver an effective means to protect key functional groups during synthesis, and a convenient handle for palladium catalysed deprotection.²³ Furthermore, such an approach would be compatible with other commonly used protecting group strategies, including benzyl ethers and carbamates via orthogonal palladium-deprotection.^{24,25}

4-Hydroxybenzoic acid allyl ester **2** was used as a model substrate as an analogue of tyrosine. After addition of **2** to a small excess of POCl₃ and triethylamine (1.5 equiv. each) and stirring for 1 hr, the reaction was quenched by addition of an excess of allyl alcohol and triethylamine. Pleasingly, clean conversion to the diallyl phosphate ester **3** was observed (Scheme 1). This procedure proved successful on a multi-gram scale, leading to the isolation of **3** in a 40 % yield. The major impurities generated were found to be mixed allyl-hydroxy phosphates. Similar yields were obtained when the reaction was run under strictly anhydrous conditions or when no effort was made to exclude water or oxygen. This suggests that prior partial hydrolysis of the POCl₃ was responsible for the generation of these side products. The use of fresh reagent was therefore important to maximise yields, though even old bottles of POCl₃ (> 1 year, stored under ambient atmosphere) delivered acceptable yields of **3** (40-60 %). We next applied our allyl-phosphate protection strategy to a series of tyrosine-analogue phenols. Allyl-protected carbonyls (**2**, **4-7**) and alloc-protected amines (**8**) were well tolerated, as were alternative protecting groups including Boc- (**9**, **10**), Fmoc- (**11**), and benzyl- (**12**) amines. Ester-protected alcohols were stable under the reaction conditions, however

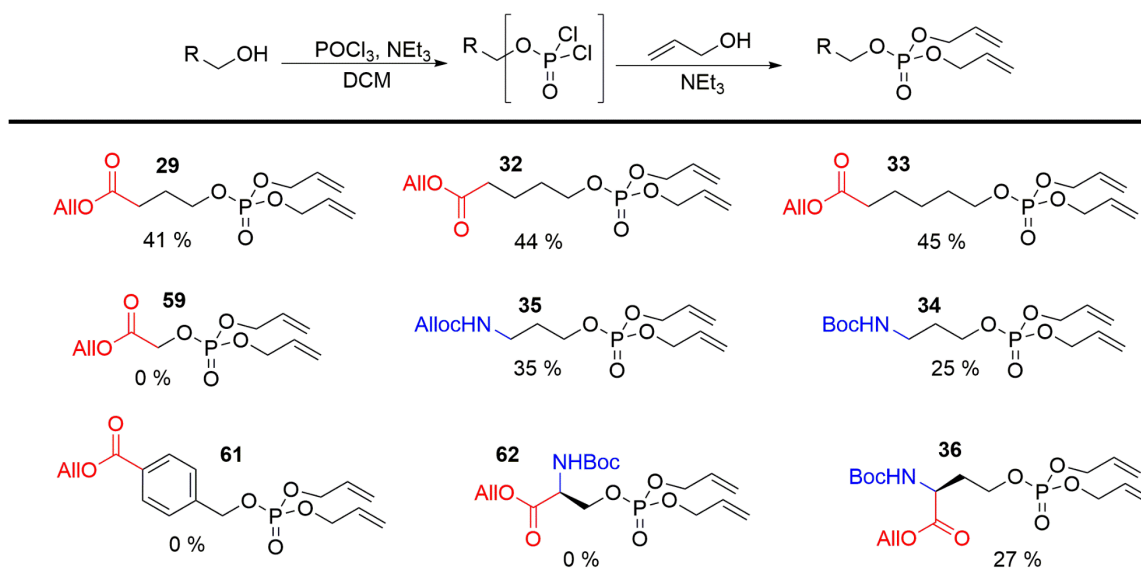
silyl ethers were poorly compatible (**13**). Protected thiols (**14**) were also compatible with the phosphorylation procedure, while *ortho*-functionalisation was tolerated (**5**), albeit with a reduction in isolated yield. A library of protected phosphotyrosine (PTyr) analogues (**3**, **15-27**) was therefore quickly generated from cheap and readily available reagents and intermediates.



Scheme 1: Synthesis of phospho-tyrosine analogues. ^aQuenched with stoichiometric allyl alcohol.

Having demonstrated the use of our approach for phenol derivatisation, we next switched our attention to the modification of alcohols. Due to the known propensity of β -hydroxyacids to undergo dehydration, particularly following phosphorylation,²⁶ 4-hydroxybutanoic acid allyl ester **28** was used as a model substrate, as the deaminated analogue of phospho-homoserine. Under our optimised phosphorylation conditions, allyl phosphate **29** was delivered in a yield of 41 % (Scheme 2). Boc- (**30**) and alloc- (**31**) protected amines were again well tolerated. No cyclisation to the carbamate amine was isolated during the modification of **30** and **31**, though *in situ* alcoholysis of an intermediate phosphoramidite cannot be discounted.²⁷ A library of protected alcohols was readily generated (**29**, **32-36**), including homoserine derivative **36**. The modifications of benzyl alcohol (**37**) and 2-hydroxyacetic acid (**38**)

were ineffective, with chloride substitution of the alcohols instead being isolated as the major products. Furthermore, attempts to modify serine derivative **39** proved unsuccessful, with dehydroalanine **40** being formed as a result of dehydration.



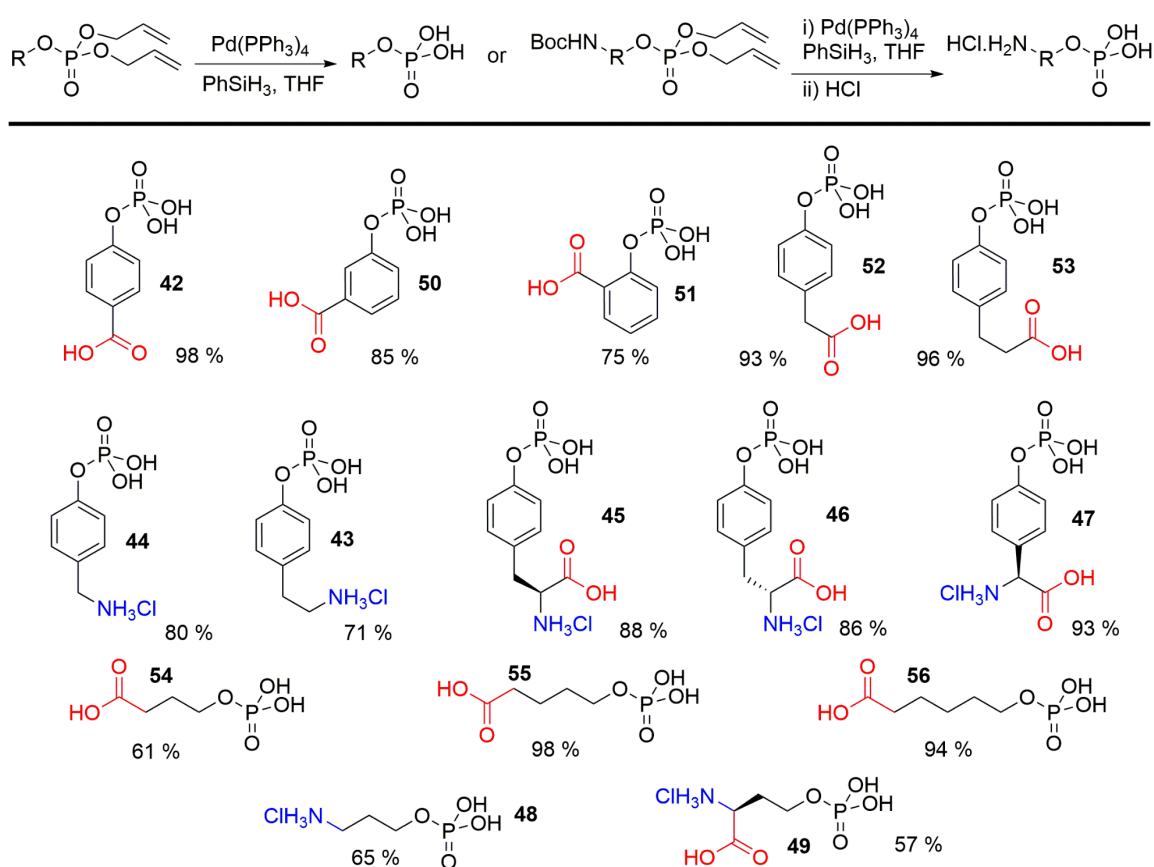
Scheme 2: Synthesis of phospho-serine analogues.

With a library of protected PTyr and PSer analogues in hand, we then attempted to undertake allyl deprotection to yield the free phospho-derivatives. Palladium catalysed deallylation is most commonly achieved in the presence of excess morpholine or a similar amine scavenger, that can react with the generated π -allyl palladium complex. The treatment of **3** with catalytic Pd(PPh₃)₄ and 10 equiv. of morpholine led to rapid deprotection of both the phosphate- and carboxy-allyl esters. However, the use of a basic amine led to the product being isolated as the tri-morpholine salt **41**. Phenylsilane was therefore used as a non-basic scavenger, leading to complete deprotection to form **42** in less than 20 minutes. Pure **42** could be easily isolated in acidic form by partitioning between water and diethyl ether, and lyophilisation of the aqueous fraction.

The developed deprotection conditions were able to deliver all members of the generated PTyr and PSer libraries (Scheme 3). Products commonly precipitated from solution as the reactions proceeded, however yields were found to be reduced for analogues containing alloc-protected amines. While carbonyl- and phosphate-deallylation proceeded rapidly, alloc-deprotection was significantly slower. This resulted in the precipitation of partially deprotected

impurities containing residual alloc-groups, lowering product yields. However, as these impurities were poorly soluble in water, purification could be achieved through simple filtration of the aqueous phase prior to lyophilisation.

As an alternative approach, we also considered a 2-step deprotection strategy exploiting *N*-Boc allylphosphates. Boc-protected amine **20** was first treated under acidic conditions to generate free amino intermediate **43**. Though subsequent deallylation was successful, the resultant amine was able to compete with phenylsilane as an allyl scavenger, leading to contamination of the final product with allyl-amine impurities. Reversing the order of deprotection, first undertaking deallylation and then acidic Boc removal proved more effective, generating the products **44-49** as their hydrochloride salts (Scheme 3).



Scheme 3: Deprotection of phospho-amino acid library to generate phospho-serine and phospho-tyrosine analogues.

Having successfully synthesised a library of PSer and PTyr analogues (**42**, **44-56**), they were next applied as additives within a CPC adhesion model, comparing adhesive (shear) strength as described in our previous report.^{13,14} Traditional CPCs display excellent bulk *cohesive* strength but poor *adhesive* strength, attaching to surfaces

via weak electrostatic and van der Waals forces, and mechanical interdigitation.²⁸ As recently reported by ourselves and Kirrilova *et al*, PSer additives act to greatly enhance adhesion to many tissues and biomaterial surfaces, with exciting opportunities for applications in biomedicine.^{12,13} However, the chemical origins of this effect remain unclear.

The adhesion of a 1 cm³ aluminium cube to a complementary cube was used to minimize substrate variation resulting from the use of heterogenous tissue samples. However, all results and trends subsequently discussed could be translated to the formation of bone-steel and bone-bone interfaces, as demonstrated through mechanical testing of a subset of analogues shown in SI Fig. S1.

We began by comparing the adhesion of PMCs to cements containing other phospho-amino acids (Fig. 1a). As previously observed, addition of PSer **1** to an α -tricalcium phosphate CPC led to a large increase in adhesive strength (211.6 ± 30.1 vs 0.2 ± 0.4 N). No significant difference was observed when either enantiopure or racemic PSer were used, supporting our hypothesis that adhesion is mediated by chemical rather than biological phenomena. PTyr **45** and phospho-threonine (PThr, **57**) were also able to induce a similar increase in adhesive strength, albeit at a slightly reduced level (Fig. 1a). Stereospecific conformational binding of PSer to the crystal surface of the CPC is therefore not essential to enable adhesion. Instead, the phospho-amino acid substrates share 4 chemical functionalities that may contribute to the observed adhesion: amine, carboxylic acid, and phosphate groups, and a carbon backbone. Furthermore, the steric arrangement of these functional groups is likely to play a critical role in dictating cement properties. We therefore set out to investigate the importance of each motif in isolation.

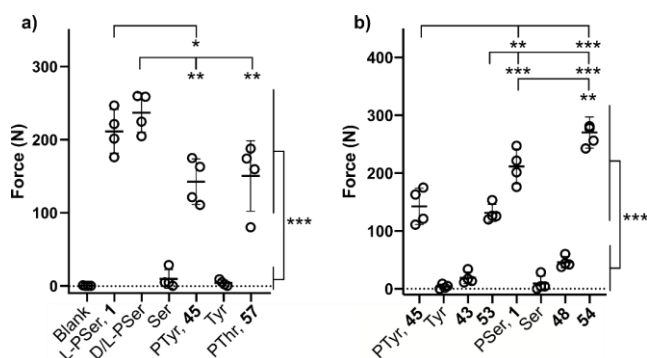


Figure 1: Comparison of cement adhesive strength in CPCs containing phospho-amino acid analogue additives. a) Comparison across amino acids; b) Comparison across analogues missing functional handles. Data are represented as scatter dot plots of each individual data point with the bars indicating the mean $\pm$

SD. * represents significant differences between groups (* $p < 0.05$, ** $p < 0.005$, * $p < 0.001$). Details of the statistical analysis are provided in the SI.**

We hypothesised that multiple calcium binding motifs, arranged in a suitable orientation, would be required to induce adhesion (Fig. 1b). Unsurprisingly, cements containing L-serine or L-tyrosine were poorly adhesive, highlighting the important role of the phosphate group. Similarly, decarboxylation of PTyr (analogue **43**) resulted in cements with poor adhesive strength. However, the similarity in cement adhesion resulting from the addition of PTyr **45** and deaminated analogue **53** to the cement demonstrated that the amino functionality was dispensable. This observation was mirrored for PSer analogues, with deaminated phospho-homoserine analogue **54** mediating strong adhesion, in contrast to the drastic drop off in strength observed following the addition of decarboxylated **48**. Cumulatively, these results support our hypothesis that monofunctional binding is insufficient for adhesion, with the amine motif being shown to not play an active role in bone binding.

We next investigated the effect of carbon backbone length within our PSer series (Fig. 2a). We anticipated that changes in both the balance of amphiphilicity and the organisation of the chelating motifs could lead to significant alterations in CPC properties. Indeed, we observed a gradual decrease in CPC adhesive strength when moving towards longer chain lengths (4-6 carbons, **54-56**). Disappointingly, the instability of the 3-carbon analogue **58** noted earlier, and our inability to generate the corresponding 2-carbon analogue **59**, prevented us from studying this effect further. However, the similarity in adhesive strength imparted by **54** and PSer suggests that no further increase would be induced by shorter chain lengths. Instead, the intriguing potential use of long chain phospho-

acids as plasticizers warrants further investigation. **56**, bearing a 6 carbon chain, was still able to provide notable adhesive strength when compared to L-serine.

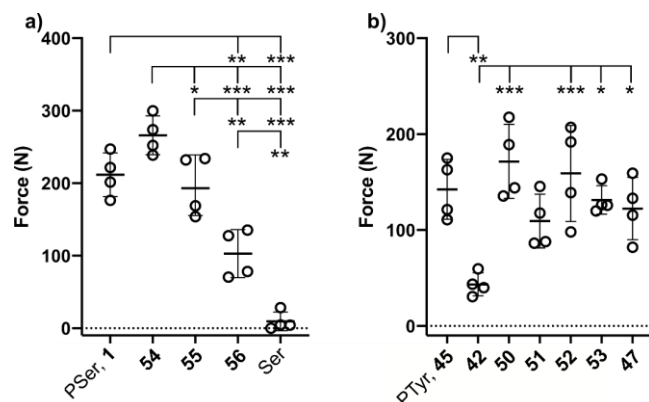


Figure 2: Comparison of cement adhesive strength in CPCs containing phospho-amino acid analogue additives. a) Differences in adhesive strength between PSer analogues of increasing carbon chain length; b) Differences in adhesive strength induced by phosphate-carboxylic acid orientation in PTyr analogues. Data are represented as scatter dot plots of each individual data point with the bars indicating the mean $\pm$ SD. * represents significant differences between groups (* $p < 0.05$, ** $p < 0.005$, * $p < 0.001$). Details of the statistical analysis are provided in the SI.**

Finally, we exploited the rigidity of the aromatic framework within our PTyr analogue library to investigate the role played by carboxyl-phosphate orientation (Fig. 2b). Positioning of the phosphate group ortho (**51**) or meta (**50**) to the carboxylic acid led to CPCs with comparable adhesive strength to PTyr. In contrast, para-presentation of the phosphate group led to a significant drop in adhesion (analogue **42**). This can be rationalised by the unfavourable orientation of the two calcium-binding motifs at opposite ends of the phenyl ring, suggesting that intra- rather than inter-molecular bidentate binding is necessary for adhesion. This is supported by the observation that adhesive strength could be restored by extension of the carboxylic acid away from the ring system by as little as one carbon (**52** and **53**), providing flexibility that enables a favourable bidentate arrangement to again be achieved. Selected compounds (**42**, **50**, **51**, and PTyr **45**) were also tested on freshly prepared bovine cortical bone cubes, with a higher sample size to account for large variability in tissue composition and surface roughness. The observed trends in bone (SI Fig. S1) matched those seen on metal substrates.

In conclusion, we have developed a novel allyl-protection strategy for phospho-amino acid synthesis, providing straightforward access to a library of PSer and PTyr derivatives. This strategy allows the challenges associated

with phosphate synthesis to be avoided, through late-stage global deprotection under mild and functional group-compatible Pd-mediated deallylation. The synthesised library could further be applied as additives in CPCs, to investigate the origins of PSer-induced cement adhesion. We have demonstrated that the presence of bidentate calcium binding motifs is necessary for high adhesive strength, with orientation and arrangement also playing a critical role. As this phenomenon does not require the presence of an amino motif, the possibility of condensation or polymerisation reactions occurring during cement curing can be discounted. Collectively, these results demonstrate that CPCs can be transformed into adhesives by a wide array of hetero-bifunctional organophosphates. The clear trends observed during macroscale mechanical testing support the use of such a system to investigate differences in chemical structure at the molecular scale. We are currently investigating an expanded library bearing analogues with multi-dentate functional handles in an effort to better understand and enhance adhesive strength. With the increased mechanistic understanding provided by this work, we are also continuing to apply our PSer-containing cements in clinically relevant models of bone defect treatment.¹⁴

ASSOCIATED CONTENT

Supporting Information

Synthesis details. Spectral data of all new compounds. Tabulated adhesive data and full statistical analysis of significance.

AUTHOR INFORMATION

Notes

M.P.-P., G.I., P.P., and H.E. declare partial ownership in a company that owns intellectual property related to PSer containing CPCs, GPBio LTD.

ACKNOWLEDGMENT

C.D.S. M.P.-P., H.A, H.E., and M.M.S. acknowledge support from the Swedish Foundation for Strategic Research (SSF 4-3713/2296 2016). C.D.S. acknowledges support from the 'Ulla och Gustaf af Ugglas Stiftelse'.

REFERENCES

- (1) Hunter, T. Why Nature Chose Phosphate to Modify Proteins. *Philos. Trans. R. Soc. B Biol. Sci.* **2012**, 367

(1602), 2513–2516.

- (2) Tsai, C. S. *Biomacromolecules: Introduction to Structure, Function and Informatics*; John Wiley & Sons, Inc.: Hoboken, New Jersey, New Jersey, 2006.
- (3) Helgadóttir, S.; Rosas-Sandoval, G.; Söll, D.; Graham, D. E. Biosynthesis of Phosphoserine in the Methanococcales. *J. Bacteriol.* **2007**, *189* (2), 575–582.
- (4) O'Leary, S. E.; Jurgenson, C. T.; Ealick, S. E.; Begley, T. P. O-Phospho-L-Serine and the Thiocarboxylated Sulfur Carrier Protein CysO-COSH Are Substrates for CysM, a Cysteine Synthase from Mycobacterium Tuberculosis. *Biochemistry* **2008**, *47* (44), 11606–11615.
- (5) Connick, J. H.; Heywood, G. C.; Smith, D. A. S.; Stone, T. W. O-Phosphohomoserine, a Naturally Occurring Analogue of Phosphonate Amino Acid Antagonists, Is an N-Methyl-d-Aspartate (NMDA) Antagonist in Rat Hippocampus. *Neurosci. Lett.* **1986**, *68* (2), 249–251.
- (6) Ma, C.; Tian, X.; Kim, J. P.; Xie, D.; Ao, X.; Shan, D.; Lin, Q.; Hudock, M. R.; Bai, X.; Yang, J. Citrate-Based Materials Fuel Human Stem Cells by Metabonegenic Regulation. *Proc. Natl. Acad. Sci. United States Am.* **2018**, *115* (50), E11741–E11750.
- (7) Liang, P.; Zheng, J.; Zhang, Z.; Hou, Y.; Wang, J.; Zhang, C.; Quan, C. Bioactive 3D Scaffolds Self-Assembled from Phosphorylated Mimicking Peptide Amphiphiles to Enhance Osteogenesis. *J. Biomater. Sci. Polym. Ed.* **2019**, *30* (1), 34–48.
- (8) Ramaswamy, J.; Nam, H. K.; Ramaraju, H.; Hatch, N. E.; Kohn, D. H. Inhibition of Osteoblast Mineralization by Phosphorylated Phage-Derived Apatite-Specific Peptide. *Biomaterials* **2015**, *73*, 120–130.
- (9) Jessen, H. J.; Ahmed, N.; Hofer, A. Phosphate Esters and Anhydrides-Recent Strategies Targeting Nature's Favoured Modifications. *Org. Biomol. Chem.* **2014**, *12* (22), 3526–3530.
- (10) Xu, H. H. K.; Wang, P.; Wang, L.; Bao, C.; Chen, Q.; Weir, M. D.; Chow, L. C.; Zhao, L.; Zhou, X.; Reynolds, M. A. Calcium Phosphate Cements for Bone Engineering and Their Biological Properties. *Bone Res.* **2017**, *5*, 17056.

- (11) Mann, K. A.; Miller, M. A.; Race, A.; Verdonschot, N. Shear Fatigue Micromechanics of the Cement-Bone Interface: An in Vitro Study Using Digital Image Correlation Techniques. *J. Orthop. Res.* **2009**, 27 (3), 340–346.
- (12) Kirillova, A.; Kelly, C.; Windheim, N. Von; Gall, K. Bioinspired Mineral – Organic Bioresorbable Bone Adhesive. *Adv. Healthc. Mater.* **2018**, 7, 1800467.
- (13) Pujari-Palmer, M.; Guo, H.; Wenner, D.; Autefage, H.; Spicer, C. D.; Stevens, M. M.; Omar, O.; Thomsen, P.; Edén, M.; Insley, G.; et al. A Novel Class of Injectable Bioceramics That Glue Tissues and Biomaterials. *Materials* **2018**, 11 (12), 2492.
- (14) Procter, P.; Pujari-Palmer, M.; Hulsart-Billström, G.; Wenner, D.; Insley, G.; Larsson, S.; Engqvist, H. A Biomechanical Test Model for Evaluating Osseous and Osteochondral Tissue Adhesives. *BMC Biomed. Eng.* **2019**, 1, 11.
- (15) Liu, X.; Pujari-Palmer, M.; Wenner, D.; Procter, P.; Insley, G.; Engqvist, H. Adhesive Cements That Bond Soft Tissue Ex Vivo. *Materials* **2019**, 12 (15), 2473.
- (16) González Garcia, F.; Leyva, M. E.; Oliveira, M. G.; De Queiroz, A. A. A.; Simões, A. Z. Influence of Chemical Structure of Hardener on Mechanical and Adhesive Properties of Epoxy Polymers. *J. Appl. Polym. Sci.* **2010**, 117 (4), 2213–2219.
- (17) Feitosa, V. P.; Sauro, S.; Ogliari, F. A.; Ogliari, A. O.; Yoshihara, K.; Zanchi, C. H.; Correr-Sobrinho, L.; Sinhoret, M. A.; Correr, A. B.; Watson, T. F.; et al. Impact of Hydrophilicity and Length of Spacer Chains on the Bonding of Functional Monomers. *Dent. Mater.* **2014**, 30 (12), e317–e323.
- (18) Dueymes, C.; Pirat, C.; Pascal, R. Facile Synthesis of Simple Mono-Alkyl Phosphates from Phosphoric Acid and Alcohols. *Tetrahedron Lett.* **2008**, 49 (36), 5300–5301.
- (19) Lira, L. M.; Vasilev, D.; Pilli, R. A.; Wessjohann, L. A. One-Pot Synthesis of Organophosphate Monoesters from Alcohols. *Tetrahedron Lett.* **2013**, 54 (13), 1690–1692.
- (20) Meziane, D.; Elias, A.; Guénin, E. Rapid and Efficient Synthesis of W-Alkylenediphosphoric Acids from

Phosphorus Oxychloride. *J. Chem.* **2016**, 2016, 8046893.

- (21) Engel, R. *Handbook of Organophosphorus Chemistry*; CRC Press, 1992.
- (22) Swarbrick, J. M.; Potter, B. V. L. Total Synthesis of a Cyclic Adenosine 5'-Diphosphate Ribose Receptor Agonist. *J. Org. Chem.* **2012**, 77 (9), 4191–4197.
- (23) Zhang, H. X.; Guibé, F.; Balavoine, G. Selective Palladium-Catalyzed Deprotection of the Allyl and Allyloxycarbonyl Groups in Phosphate Chemistry and in the Presence of Propargyl and Propargyloxycarbonyl Groups. *Tetrahedron Lett.* **1988**, 29 (6), 623–626.
- (24) Vutukuri, D. R.; Bharathi, P.; Yu, Z.; Rajasekaran, K.; Tran, M.-H.; Thayumanavan, S. A Mild Deprotection Strategy for Allyl-Protecting Groups and Its Implications in Sequence Specific Dendrimer Synthesis. *J. Org. Chem.* **2003**, 68 (3), 1146–1149.
- (25) Beugelmans, R.; Neuville, L.; Bois-Choussy, M.; Chastanet, J.; Zhu, J. Palladium Catalyzed Reductive Deprotection of Alloc: Transprotection and Peptide Bond Formation. *Tetrahedron Lett.* **2003**, 36 (18), 3129–3132.
- (26) Dadová, J.; Galan, S. R.; Davis, B. G. Synthesis of Modified Proteins via Functionalization of Dehydroalanine. *Curr. Opin. Chem. Biol.* **2018**, 46, 71–81.
- (27) Dujols, F.; Mulliez, M. The Influence of Ring Size on the Selectivity of Phosphorus Heterocycle Aminolysis in the Presence of Water or Alcohols - Case of 2-Oxo- or 2-Thioxo-3-Sulfonyl-1,3,2-Oxazaphosphorinanes. *European J. Org. Chem.* **2006**, 8, 1959–1964.
- (28) Grover, L. M.; Gbureck, U.; Farrar, D. F.; Barralet, J. E. Adhesion of a Novel Calcium Phosphate Cement to Cortical Bone and Several Common Biomaterials. *Key Eng. Mater.* **2006**, 309–311, 849–852.

Synthesis of phospho-amino acid analogues as tissue adhesive cement additives

Christopher D. Spicer,^{*a,b,c} Michael Pujari-Palmer,^d Hélène Autefage,^a Gerard Insley,^d
Philip Procter,^d Håkan Engqvist^d and Molly M. Stevens^{*a,e}

^aDepartment of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden.

^bDepartment of Chemistry, University of York, Heslington, YO10 5DD, UK.

^cYork Biomedical Research Institute, University of York, Heslington, YO10 5DD, UK.

^dApplied Material Science, Department of Engineering, Uppsala University, 75121 Uppsala, Sweden

^eDepartment of Materials, Department of Bioengineering, and Institute of Biomedical Engineering, Imperial College London, London, United Kingdom.

Table of contents

S2	Supplementary figures
S2	General considerations
S4	Substrate synthesis
S14	General phosphorylation procedure
S15	Phosphorylation – Tyrosine analogues
S20	Phosphorylation – Serine analogues
S22	Unsuccessful phosphorylations
S23	General deprotection procedure
S23	Deprotection – Tyrosine analogues
S24	Deprotection – Serine analogues
S25	Allyl-Boc sequential deprotection
S26	Mechanical testing
S27	Statistical analyses
S29	References
S31	NMR spectra of novel compounds

Supplementary figures and tables

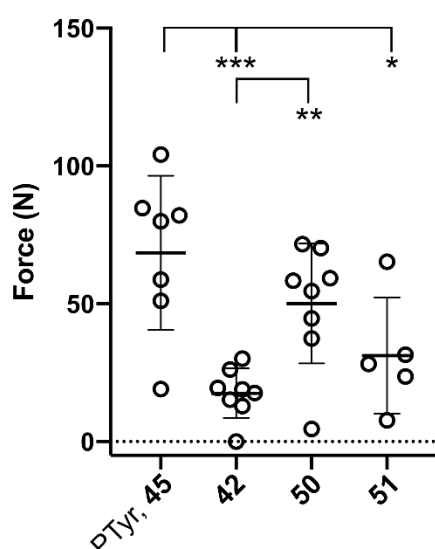


Figure S1: Comparison of cement adhesive strength when applied to bone cubes for representative compounds. Comparison between this graph and the data presented in Fig. 2b shows that trends match those observed for aluminium cube adhesion. Data are represented as scatter dot plots of each individual data point with the bars indicating the mean $\pm$ SD. * represents significant differences between groups (* $p < 0.05$, ** $p < 0.005$, * $p < 0.001$). Details of the statistical analysis are provided on page S25.**

Compound	CPC	Ser	Tyr	L-PSer, 1	D/L-PSer, 1	PTyr, 45	PThr, 57	42	43
Force (N)	0.2	9.4	3.9	211.6	237.2	142.5	150.6	43.4	19.1
Deviation	0.4	12.9	3.9	30.1	26.9	31.2	48.3	12.1	10.4

Compound	47	48	50	51	52	53	54	55	56
Force (N)	122.5	46.1	171.5	109.4	159.0	131.4	270.2	197.2	103.0
Deviation	32.4	9.8	38.6	28.1	50.1	14.9	26.8	41.8	33.2

Table S1: Tabulated mean adhesive strengths (aluminium-aluminium) and standard deviations for all compounds tested.

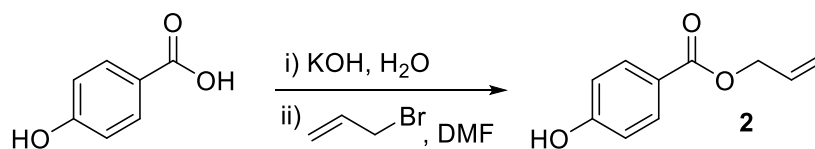
General considerations

Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Bruker AV-400 (400 MHz) spectrometer. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker AV400 (100 MHz) spectrometer. NMR shifts were assigned using COSY, HSQC and HMBC spectra. All chemical shifts are quoted on the δ scale in ppm using residual solvent as the internal standard (^1H NMR: $\text{CDCl}_3 = 7.26$; MeOD

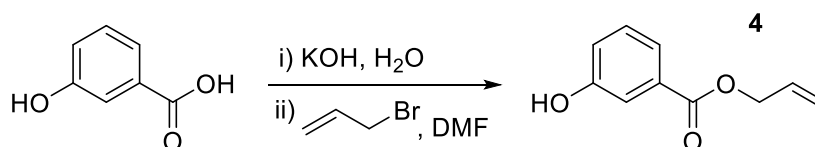
= 3.31; DMSO- d_6 = 2.50; D₂O = 4.79; and ¹³C NMR: CDCl₃ = 77.16, MeOD = 49.00, DMSO- d_6 = 39.52). Coupling constants (*J*) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, m = multiplet, app = apparent, br = broad. Melting points (m.p.) were recorded on a Zeiss Axio Imager: Z1M microscope equipped with a Linkam LTS 420 temperature controlled microscope stage and are uncorrected. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum 100 spectrophotometer with a Universal ATR Sampling Accessory. Absorption maxima ($\lambda_{\max}$) are reported in wavenumbers (cm⁻¹). Low resolution mass spectra (LRMS) were recorded on an Agilent 6130 Quadrupole mass spectrometer using electrospray ionization (ESI), connected to an Agilent 1260 Infinity liquid chromatography set-up with a Phenomenex Gemini-NX-C16 column. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier (ES-ToF) spectrometer connected to an Aquity-iClass UPLC. Matrix-assisted laser desorption-ionization (MALDI) spectra were recorded on a Micromass MALDI-ToF spectrometer. Nominal and exact *m/z* values are reported in Daltons. Thin layer chromatography (TLC) was carried out using aluminium backed sheets coated with 60 F254 silica gel (Merck). Visualization of the silica plates was achieved using a UV lamp ($\lambda_{\max}$ = 254, 302, or 366 nm), and/or ammonium molybdate (5 % in 2M H₂SO₄), and/or potassium permanganate (5 % KMnO₄ in 1M NaOH with 5 % potassium carbonate). Flash column chromatography was carried out using Geduran Si 60 (40-63 μ m) (Merck). Mobile phases are reported as % volume of more polar solvent in less polar solvent. Anhydrous solvents were purchased from Sigma-Aldrich and used as supplied. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Reagents were purchased from Sigma-Aldrich and used as supplied, unless otherwise indicated. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulfate (MgSO₄) was used as the drying agent after reaction workup unless otherwise stated.

Cortical bovine cubes were created by cutting freshly harvested adult humerus into cubes 1 cm³, with a water cooled, diamond coated, band saw blade (IMEB, California, USA). Each cube was cut in two to yield matching surfaces, and were stored frozen until use. Steel test cubes were made to a final dimension of 1 cm³ +/- 0.1 mm. The surfaces were polished with 80 grit polishing paper.

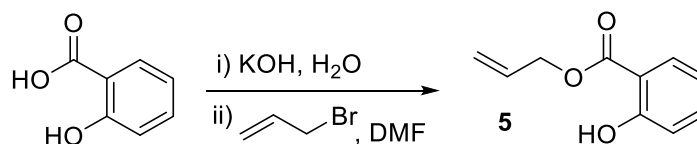
Substrate synthesis



4-Hydroxybenzoic acid (2.76 g, 20 mmol) and potassium hydroxide (1.23 g, 22 mmol) were dissolved in water (20 mL) and stirred for 10 min. The mixture was then concentrated *in vacuo*. The residue was suspended in DMF (20 mL) and allyl bromide (1.9 mL, 22 mmol) was added. After stirring for 18 hrs, the mixture was diluted with EtOAc (150 mL) and the organics washed with brine (2 x 100 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 20-30 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a white solid. A yield of 2.8 g, 15.7 mmol (79 %) was obtained. Data were consistent with those previously reported.¹ ¹H NMR (400 MHz, CDCl₃): δ = 8.01 (2H, d, *J* = 8.8 Hz, ArH₂), 6.89 (2H, d, *J* = 8.8 Hz, ArH₃), 5.88 (1H, ddt, *J* = 17.2, 10.4, 5.5 Hz, -CH=CH₂), 5.42 (1H, dd, *J* = 17.2, 1.6 Hz, -CH=CH₂), 5.30 (1H, ddt, *J*₁ = 10.4 Hz, *J*₂ = *J*₃ = 1.6 Hz, -CH=CH₂), 4.82 (2H, dt, *J* = 5.5, 1.6 Hz, -CH₂CH=CH₂) ppm;

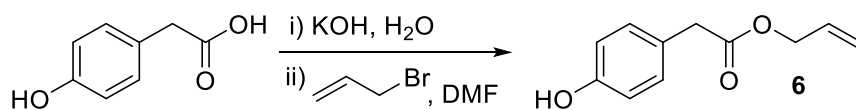


Run as described above on 3-hydroxybenzoic acid (2 g, 14.5 mmol). A yield of 2.3 g, 12.9 mmol (89 %) was obtained as a colourless oil. Data were consistent with those previously reported.¹ ¹H NMR (400 MHz, CDCl₃): δ = 7.60-7.68 (2H, m, ArH₂ and ArH₆), 7.32 (1H, dd, *J*₁ = *J*₂ = 7.9 Hz, ArH₅), 7.10 (1H, d, *J* = 7.9 Hz, ArH₄), 6.02 (1H, ddt, *J* = 17.2, 10.4, 5.5 Hz, -CH=CH₂), 5.42 (1H, d, *J* = 17.2, -CH=CH₂), 5.30 (1H, d, *J* = 10.4 Hz, -CH=CH₂), 4.84 (2H, d, *J* = 5.5, -CH₂CH=CH₂) ppm;

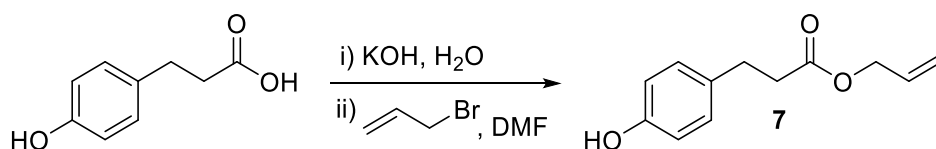


Run as described above on 2-hydroxybenzoic acid (2 g, 14.5 mmol). A yield of 2.2 g, 12.3 mmol (85 %) was obtained as a colourless oil. Data were consistent with those previously reported.¹ ¹H NMR (400 MHz, CDCl₃): δ = 10.78 (1H, s, -OH), 7.90 (1H, ddd, *J* = 7.6, 2.2, 0.4 Hz, ArH₆), 7.47 (1H, ddd, *J* = 7.1, 6.9, 1.4, 0.3 Hz, ArH₄), 7.00 (1H, ddd, *J* = 7.1, 0.8, 0.4 Hz, ArH₃), 6.9 (1H, ddd, *J* = 7.6, 7.1, 0.8 Hz, ArH₅), 6.05 (1H, ddt, *J* = 17.2, 10.4, 5.7 Hz, -CH=CH₂),

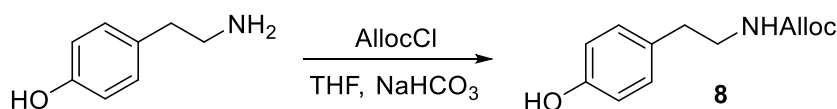
5.45 (1H, ddt, $J = 17.2, 1.5, 1.4$ Hz, $-\text{CH}=\text{CH}_2$), 5.30 (1H, ddt, $J = 10.4, 1.5, 1.4$ Hz, $-\text{CH}=\text{CH}_2$), 4.86 (2H, ddd, $J_1 = 5.5$ Hz, $J_2 = J_3 = 1.5$ Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$) ppm;



Run as described above on 4-hydroxyphenylacetic acid (2 g, 13.2 mmol). Purified by flash column chromatography eluting with 30 % EtOAc:Hexane. A yield of 2.3 g, 11.9 mmol (91 %) was obtained as a colourless oil. Data were consistent with those previously reported.² ^1H NMR (400 MHz, CDCl_3): $\delta = 7.13$ (2H, d, $J = 7.6$ Hz, ArH₂), 6.78 (2H, d, $J = 7.6$ Hz, ArH₃), 5.92 (1H, ddt, $J = 17.2, 10.4, 5.6$ Hz, $-\text{CH}=\text{CH}_2$), 5.29 (1H, d, $J = 17.2$ Hz, $-\text{CH}=\text{CH}_2$), 5.24 (1H, d, $J = 10.4$ Hz, $-\text{CH}=\text{CH}_2$), 4.62 (2H, d, $J = 5.6$ Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 3.59 (2H, s, $-\text{CH}_2\text{Ar}$) ppm;

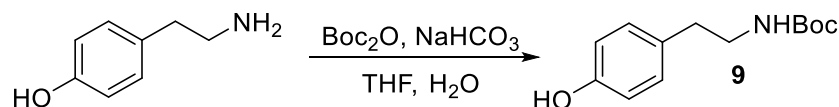


Run as described above on 3-(4-hydroxyphenyl)propionic acid (2 g, 12.0 mmol). Purified by flash column chromatography eluting with 30-40 % EtOAc:Hexane. A yield of 2.2 g, 10.6 mmol (88 %) was obtained as a colourless oil. Data were consistent with those previously reported.³ ^1H NMR (400 MHz, CDCl_3): $\delta = 7.08$ (2H, d, $J = 8.6$ Hz, ArH₂), 6.77 (2H, d, $J = 8.6$ Hz, ArH₃), 5.91 (1H, ddt, $J = 17.2, 10.4, 5.7$ Hz, $-\text{CH}=\text{CH}_2$), 5.29 (1H, ddt, $J_1 = 17.2$ Hz, $J_2 = J_3 = 1.3$ Hz, $-\text{CH}=\text{CH}_2$), 5.25 (1H, ddt, $J_1 = 10.4$ Hz, $J_2 = J_3 = 1.3$ Hz, $-\text{CH}=\text{CH}_2$), 4.59 (2H, dt, $J = 5.7, 1.3$ Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 2.91 (2H, t, $J = 15.4$ Hz, $-\text{CH}_2\text{Ar}$), 2.65 (2H, t, $J = 15.4$ Hz, $-\text{CH}_2\text{CO}_2\text{Allyl}$) ppm;

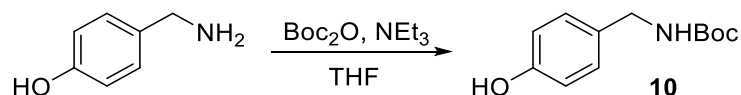


Tyramine (0.5 g, 3.6 mmol) and sodium bicarbonate (919 mg, 10.9 mmol) were dissolved in a mixture of water (10 mL) and THF (10 mL). Allyl chloroformate (422 μL , 4.0 mmol) was added dropwise, and the reaction stirred for 2 hrs. The mixture was then diluted with hydrochloric acid (0.5 M, 50 mL) and extracted with ethyl acetate (2 x 50 mL). The combined organics were washed with brine (50 mL), dried with MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 30 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a brown oil. A yield of 620 mg, 2.8 mmol (78 %) was obtained. ^1H NMR (400 MHz, CDCl_3): $\delta = 7.03$ (2H, d, $J = 8.4$ Hz, ArH₃), 6.80 (2H, d, $J = 8.4$ Hz, ArH₄), 5.91 (1H, ddt, $J = 17.2, 10.4, 5.5$ Hz, $-\text{CH}=\text{CH}_2$), 5.30 (1H, d, $J = 17.2$ Hz, $-\text{CH}=\text{CH}_2$), 5.22 (1H, d, $J = 10.4$ Hz, $-\text{CH}=\text{CH}_2$), 4.91 (1H, br s, $-\text{NH}$), 4.58 (2H, d, $J = 5.5$ Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 2.91 (2H, t, $J = 15.4$ Hz, $-\text{CH}_2\text{Ar}$), 2.65 (2H, t, $J = 15.4$ Hz, $-\text{CH}_2\text{CO}_2\text{Allyl}$) ppm;

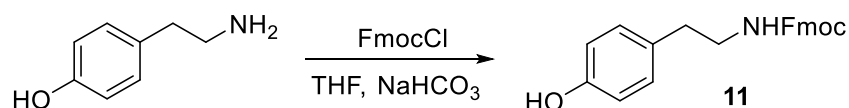
$\text{CH}_2\text{CH}=\text{CH}_2$), 3.42 (2H, td, $J = 7.0, 6.4$ Hz, $-\text{CH}_2\text{NHAlloc}$), 2.74 (2H, t, $J = 7.0$ Hz, $-\text{CH}_2\text{Ar}$) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 156.62$ ($-\text{CO}_2\text{All}$), 154.82 (ArC_4), 132.69 ($-\text{CH}=\text{CH}_2$), 130.09 (ArC_1), 129.82 (ArC_2), 117.88 ($-\text{CH}=\text{CH}_2$), 115.58 (ArC_3), 65.75 ($-\text{CH}_2\text{CH}=\text{CH}_2$), 42.46 ($-\text{CH}_2\text{NHAlloc}$), 35.17 ($-\text{CH}_2\text{Ar}$) ppm; IR (ν_{max} , film): 2939 (v br), 1695, 1614, 1596, 1509, 1441, 1365, 1219, 1170, 1134, 1106, 1053 cm^{-1} ; HRMS m/z (ESI+): Found: 222.1124 (M+H), Calc.: 222.1130 ($\text{C}_{12}\text{H}_{16}\text{NO}_3$).



Tyramine (1 g, 7.2 mmol) and sodium bicarbonate (725 mg, 8.6 mmol) were dissolved in a mixture of water (20 mL) and THF (30 mL). A solution of di-*tert*-butyl dicarbonate (1.56 g, 7.2 mmol) in THF (20 mL) was then added dropwise, and the reaction stirred for 4 hrs. The mixture was then diluted with ethyl acetate (150 mL), and the organics washed with water (100 mL) and brine (100 mL), dried with MgSO_4 , filtered, and concentrated *in vacuo* to give the DP as a colourless oil. A yield of 1.60 g, 6.7 mmol (93 %) was obtained. Data were consistent with those previously reported.⁴ ^1H NMR (400 MHz, CDCl_3): $\delta = 7.05$ (2H, d, $J = 7.3$ Hz, ArH_2), 6.79 (2H, d, $J = 7.3$ Hz, ArH_3), 5.49 (1H, br s, $-\text{OH}$), 4.59 (1H, br s, $-\text{NH}$), 3.30-3.40 (2H, m, $-\text{CH}_2\text{NHBoc}$), 2.70-2.78 (2H, m, $-\text{CH}_2\text{Ar}$), 1.46 (9H, s, Boc) ppm.

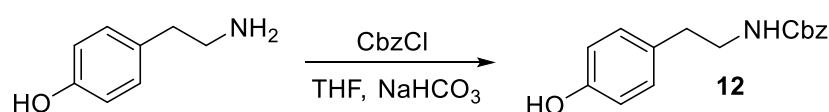


A solution of di-*tert*-butyl dicarbonate (2.1 g, 9.7 mmol) in THF (20 mL) was added dropwise to a mixture of 4-hydroxybenzylamine (1 g, 8.1 mmol) and trimethylamine (2.28 mL, 16.2 mmol) in THF (50 mL). After stirring for 18 hrs, the THF was removed *in vacuo* and the residue diluted with ethyl acetate (100 mL). The organics were washed with water (100 mL), hydrochloric acid (1 M, 100 mL), and brine (100 mL), dried with MgSO_4 , filtered, and concentrated *in vacuo* to give the DP as a colourless oil. A yield of 1.78 g, 7.9 mmol (98 %) was obtained. Data were consistent with those previously reported.⁵ ^1H NMR (400 MHz, CDCl_3): $\delta = 7.15$ (2H, d, $J = 8.1$ Hz, ArH_2), 6.80 (2H, d, $J = 8.1$ Hz, ArH_3), 5.67 (1H, br s, $-\text{OH}$), 4.82 (1H, br s, $-\text{NH}$), 4.24 (2H, d, $J = 5.4$ Hz, $-\text{CH}_2\text{NHBoc}$), 1.48 (9H, s, Boc) ppm;

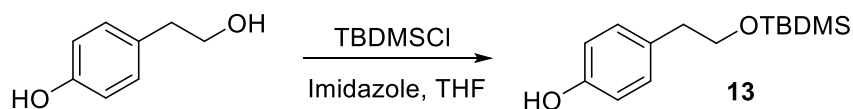


Tyramine (0.5 g, 3.6 mmol) and sodium bicarbonate (302 mg, 3.6 mmol) were dissolved in a mixture of water (20 mL) and THF (10 mL). A solution of fluorenylmethyloxycarbonyl chloride (1.03 g, 4.0 mmol) in THF (10 mL) was then added dropwise, and the reaction stirred for 4 hrs.

The mixture was then diluted with ethyl acetate (150 mL), and the organics washed with water (100 mL) and brine (100 mL), dried with MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 30 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a white solid. A yield of 1.07 g, 3.0 mmol (83 %) was obtained. Data were consistent with those previously reported.⁶ ^1H NMR (400 MHz, CDCl_3): δ = 7.75 (2H, d, J = 7.8 Hz, Fmoc), 7.52 (2H, d, J = 7.8 Hz, Fmoc), 7.38 (2H, dd, $J_1 = J_2$ = 7.8 Hz, Fmoc), 7.30 (2H, dd, $J_1 = J_2$ = 7.8 Hz, Fmoc), 7.02 (2H, d, J = 8.4 Hz, ArH₃), 6.75 (2H, d, J = 8.4 Hz, ArH₂), 4.71-4.79 (2H, m, -NH and -OH), 4.39 (2H, d, J = 6.9 Hz, Fmoc), 4.19 (1H, t, J = 6.9 Hz, Fmoc), 3.39 (2H, dt, $J_1 = J_2$ = 6.9 Hz, -CH₂NHFmoc), 2.72 (2H, t, J = 6.9 Hz, -CH₂Ar) ppm.

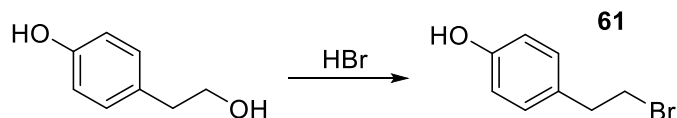


Tyramine (0.5 g, 3.6 mmol) and sodium bicarbonate (302 mg, 3.6 mmol) were dissolved in a mixture of water (20 mL) and THF (10 mL). A solution of benzyl chloroformate (575 μL , 4.0 mmol) in THF (10 mL) was then added dropwise, and the reaction stirred for 4 hrs. The mixture was then diluted with ethyl acetate (150 mL), and the organics washed with water (100 mL) and brine (100 mL), dried with MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 30 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil that solidified on standing. A yield of 861 mg, 3.2 mmol (88 %) was obtained. Data were consistent with those previously reported.⁷ ^1H NMR (400 MHz, CDCl_3): δ = 7.29-7.41 (5H, m, Cbz), 7.03 (2H, d, J = 7.8 Hz, ArH₃), 6.76 (2H, d, J = 7.8 Hz, ArH₂), 5.09 (2H, s, Cbz), 4.76 (1H, br s, -NH), 3.42 (2H, dt, $J_1 = J_2$ = 6.7 Hz, -CH₂NHCbz), 2.74 (2H, t, J = 6.7 Hz, -CH₂Ar) ppm.

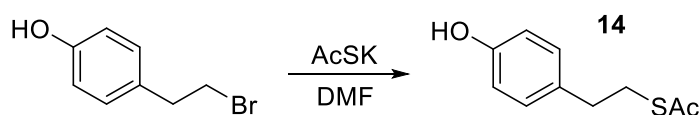


A solution of 2-(4-hydroxyphenyl)ethanol (1.38 g, 10 mmol) and imidazole (748 mg, 11 mmol) in THF (20 mL) was cooled to 0 °C. A solution of TBDMSCl (1.65 g, 11 mmol) in THF (5 mL) was added dropwise, the mixture warmed to room temperature, and stirring continued for 18 hrs. The solvent was then removed *in vacuo* and the residue redissolved in ethyl acetate (100 mL). The organics were washed with aqueous citric acid (10 %, 50 mL) and brine (50 mL), dried with MgSO_4 , filtered, and concentrated *in vacuo* to give the DP as a colourless oil that solidified on standing. A yield of 1.92 g, 7.6 mmol (76 %) was obtained. Data were consistent with those previously reported.⁸ ^1H NMR (400 MHz, CDCl_3): δ = 7.06 (2H, d, J = 8.2 Hz, ArH₃),

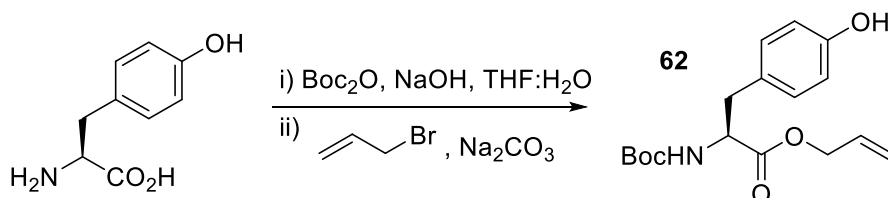
6.74 (2H, d, $J = 8.2$ Hz, ArH₂), 3.76 (2H, t, $J = 7.1$ Hz, -CH₂OR), 2.75 (2H, t, $J = 7.1$ Hz, -CH₂Ar), 0.88 (9H, s, TBDMS), 0.00 (6H, s, TBDMS) ppm.



2-(4-hydroxyphenyl)ethanol (3 g, 21.7 mmol) was heated to 85 °C for 18 hrs in hydrobromic acid (48 %, 15 mL). After cooling to room temperature the mixture was diluted with water (75 mL) and extracted with DCM (3 x 100 mL). The combined organics were dried with MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 20-30 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a light brown solid. A yield of 4.2 g, 21.0 mmol (96 %) was obtained. Data were consistent with those previously reported.⁹ ¹H NMR (400 MHz, CDCl₃): 7.08 (2H, d, $J = 7.2$ Hz, ArH₃), 6.79 (2H, d, $J = 7.2$ Hz, ArH₂), 4.75 (1H, br s, -OH), 3.52 (2H, t, $J = 7.6$ Hz, -CH₂Br), 3.09 (2H, t, $J = 7.6$ Hz, -CH₂Ar) ppm.



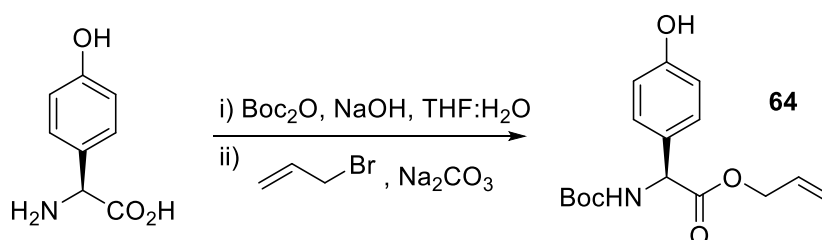
Potassium thioacetate (2.7 g, 24 mmol) was added to a solution of **61** (4 g, 20 mmol) in DMF (10 mL). After stirring for 18 hrs, the mixture was diluted with ethyl acetate (200 mL) and washed with brine (4 x 150 mL). The organics were dried with MgSO₄, filtered, and concentrated *in vacuo* to give the DP as a light brown oil. A yield of 3.6 g, 18.4 mmol (92 %) was obtained. ¹H NMR (400 MHz, CDCl₃): 7.06 (2H, d, $J = 7.3$ Hz, ArH₃), 6.77 (2H, d, $J = 7.3$ Hz, ArH₂), 5.63 (1H, s, -OH), 3.07 (2H, t, $J = 7.3$ Hz, -CH₂Ar), 2.78 (2H, t, $J = 7.3$ Hz, -CH₂SAc) ppm; ¹³C NMR (100 MHz, CDCl₃): $\delta = 196.77$ (-C(=O)Me), 154.32 (ArC₄), 131.87 (ArC₁), 129.67 (ArC₂), 115.31 (ArC₃), 34.81 (-CH₂Ar), 30.80 (-CH₂SAc), 30.67 (-C(=O)Me); IR (ν_{max} , film): 3375, 3023, 2928, 1687, 1660, 1613, 1596, 1514, 1444, 1354, 1262, 1219, 1172, 1130, 1100, 1016 cm⁻¹; HRMS m/z (ESI⁺): Found: 219.0454 (M+Na), Calc.: 219.0450 (C₁₀H₁₂NaO₂S).



A solution of di-*tert*-butyl dicarbonate (3.97 g, 18.2 mmol) in THF (20 mL) was added dropwise to a mixture of L-tyrosine (3 g, 16.6 mmol) and sodium hydroxide (728 mg, 18.2 mmol) in water (20 mL) and THF (20 mL). After stirring for 4 hrs, the THF was removed *in vacuo* and the

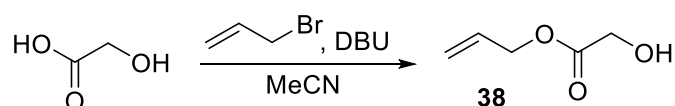
mixture washed with ethyl acetate (50 mL). The aqueous was then acidified with hydrochloric acid (2M, 50 mL), and extracted with ethyl acetate (3 x 100 mL). The combined were dried with MgSO_4 , filtered, and concentrated *in vacuo*. The residue was redissolved in DMF (30 mL), and potassium carbonate (2.4 g, 17.2 mmol) and allyl bromide (1.75 mL, 20.3 mmol) were sequentially added. After stirring for 18 hrs, the reaction was diluted with ethyl acetate (200 mL), and the organics washed with hydrochloric acid (1 M, 2 x 100 mL) and brine (100 mL), dried with MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 20-30 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil which solidified on standing. A yield of 3.5 g, 10.9 mmol (70 %) was obtained. Data were consistent with those previously reported.¹⁰ ^1H NMR (400 MHz, CDCl_3): δ = 6.99 (2H, d, J = 8.3 Hz, ArH₂), 6.74 (2H, d, J = 8.3 Hz, ArH₃), 5.89 (1H, ddt, J = 17.3, 10.4, 4.8 Hz, -CH=CH₂), 5.32 (1H, d, J = 17.3 Hz, -CH=CH₂), 5.26 (1H, d, J = 10.4 Hz, -CH=CH₂), 5.03 (1H, br d, J = 8.2 Hz, -NH), 4.62 (2H, d, J = 4.8 Hz, -CH₂CH=CH₂), 4.53-4.60 (1H, m, H _{α}), 3.06 (1H, dd, J = 13.9, 5.8 Hz, H _{β}), 3.00 (1H, dd, J = 13.9, 6.1 Hz, H _{β}), 1.43 (9H, s, Boc) ppm;

The use of D-tyrosine in the same procedure led to the production of enantiomer **63**.

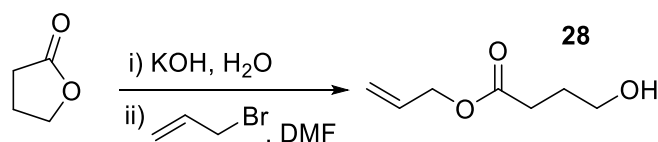


A solution of di-*tert*-butyl dicarbonate (2.4 g, 11 mmol) in THF (20 mL) was added dropwise to a mixture of L-phenylglycine (1.67 g, 10 mmol) and sodium hydroxide (440 mg, 11 mmol) in water (20 mL) and THF (20 mL). After stirring for 4 hrs, the THF was removed *in vacuo* and the mixture washed with ethyl acetate (50 mL). The aqueous was then acidified with hydrochloric acid (2M, 50 mL), and extracted with ethyl acetate (3 x 100 mL). The combined were dried with MgSO_4 , filtered, and concentrated *in vacuo*. The residue was redissolved in DMF (15 mL), and potassium carbonate (1.58 g, 11 mmol) and allyl bromide (1.03 mL, 12 mmol) were sequentially added. After stirring for 18 hrs, the reaction was diluted with ethyl acetate (200 mL), and the organics washed with hydrochloric acid (1 M, 2 x 100 mL) and brine (100 mL), dried with MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 20-30 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil which solidified on standing. A yield of 2.3 g, 7.5 mmol (75 %) was obtained. Data were consistent with those previously reported.¹⁰ ^1H NMR (400 MHz, CDCl_3): δ = 7.69 (1H, s, -OH), 7.07 (2H, d, J = 8.5 Hz, ArH₂), 6.67 (2H, d,

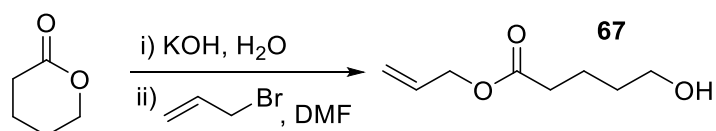
$J = 8.5$ Hz, ArH₃), 5.60-5.76 (2H, m, -CH=CH₂ & -NH), 5.14 (1H, d, $J = 7.1$ Hz, -CH=CH₂), 5.00-5.11 (2H, m, -CH=CH₂ & H_a), 5.03 (1H, br d, $J = 8.2$ Hz, -NH), 4.49 (2H, d, $J = 5.1$ Hz, -CH₂CH=CH₂), 4.53-4.60 (1H, m, H_a), 1.34 (9H, s, Boc) ppm; ¹³C NMR (100 MHz, CDCl₃): $\delta = 171.40$ (-CO₂All), 156.79 (ArC₄), 155.09 (-CO₂^tBu), 131.29 (-CH=CH₂), 128.47 (ArC₂), 127.54 (ArC₁), 118.52 (-CH=CH₂), 115.91 (ArC₃), 80.65 (-CMe₃), 66.12 (d, $J = 5.6$ Hz, -CH₂CH=CH₂), 57.31 (C_a), 28.31 (Boc) ppm; IR ($\nu_{\max}$, film): 3360, 2981, 1735, 1687, 1615, 1597, 1514, 1501, 1452, 1393, 1368, 1211, 1158, 1105, 1055, 1028 cm⁻¹; HRMS m/z (ESI⁺): Found: 330.1311 (M+Na), Calc.: 330.1312 (C₁₆H₂₁NaNO₅).



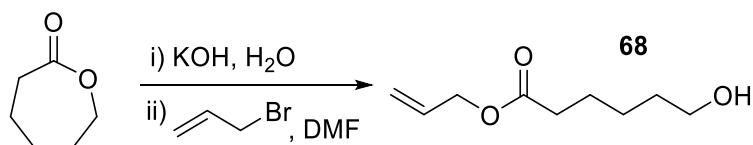
A mixture of glycolic acid (760 mg, 10 mmol), allyl bromide (951 μ L, 11 mmol) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (1.65 mL, 11 mmol) was heated to 60 °C for 18 hrs, then concentrated *in vacuo*. The residue was purified by column chromatography, eluting with 30-40 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil. A yield of 980 mg, 8.4 mmol (84 %) was obtained. ¹H NMR (400 MHz, CDCl₃): $\delta = 5.92$ (1H, ddt, $J = 17.2, 10.4, 5.8$ Hz, -CH=CH₂), 5.35 (1H, d, $J = 17.2$ Hz, -CH=CH₂), 5.29 (1H, d, $J = 10.4$ Hz, -CH=CH₂), 4.70 (2H, d, $J = 5.8$ Hz, -CH₂CH=CH₂), 4.20 (2H, d, $J = 5.5$ Hz, -CH₂OH), 2.70 (1H, t, $J = 5.5$ Hz, -OH) ppm; IR ($\nu_{\max}$, film): 3439 (br), 2945, 1738, 1649, 1435, 1382, 1272, 1199, 1089 cm⁻¹; HRMS m/z (ESI⁺): Found: 117.0549 (M+H), Calc.: 117.0546 (C₅H₉O₃).



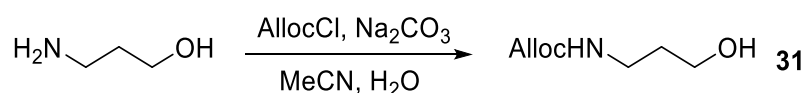
A mixture of γ -butyrolactone (2 mL, 26 mmol) and potassium hydroxide (1.6 g, 28.6 mmol) in water (30 mL) was stirred for 1 hr, then concentrated *in vacuo*. The residue was suspended in DMF (20 mL) and allyl bromide (2.5 mL, 28.6 mmol) was added. After stirring for 18 hrs, the mixture was diluted with EtOAc (150 mL) and the organics washed with hydrochloric acid (1 M, 100 mL) and brine (100 mL), dried with MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 30 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil. A yield of 3.5 g, 24.3 mmol (93 %) was obtained. Data were consistent with those previously reported.¹¹ ¹H NMR (400 MHz, CDCl₃): $\delta = 5.89$ (1H, ddt, $J = 17.2, 10.4, 5.7$ Hz, -CH=CH₂), 5.29 (1H, ddt, $J_1 = 17.2$ Hz, $J_2 = J_3 = 1.4$ Hz, -CH=CH₂), 5.22 (1H, ddt, $J_1 = 10.4$ Hz, $J_2 = J_3 = 1.4$ Hz, -CH=CH₂), 4.56 (2H, ddd, $J_1 = 5.7$ Hz, $J_2 = J_3 = 1.4$ Hz, -CH₂CH=CH₂), 3.65 (2H, t, $J = 5.8$ Hz, -CH₂OH), 2.45 (2H, t, $J = 7.2$ Hz, -CH₂CO₂All), 1.87 (2H, tt, $J = 7.2, 5.8$ Hz, -CH₂CH₂OH) ppm.



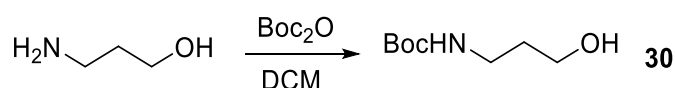
Run as described above on δ -valerolactone (2 g, 16.9 mmol). Purified by flash column chromatography eluting with 25-30 % EtOAc:Hexane. Pure fractions were dissolved in diethyl ether (100 mL), and the organics washed with brine (100 mL x 2), dried with MgSO₄, filtered, and concentrated *in vacuo* to give the DP as a colourless oil. A yield of 2 g, 12.6 mmol (75 %) was obtained. ¹H NMR (400 MHz, CDCl₃): δ = 5.89 (1H, ddt, J = 17.2, 10.4, 5.4 Hz, -CH=CH₂), 5.30 (1H, d, J = 17.2, -CH=CH₂), 5.23 (1H, d, J = 10.4 Hz, -CH=CH₂), 4.56 (2H, d, J = 5.4 Hz, -CH₂CH=CH₂), 3.59-3.67 (2H, m, -CH₂OH), 2.37 (2H, t, J = 7.2 Hz, -CH₂CO₂All), 2.16 (1H, br s, -OH), 1.65-1.77 (2H, m, -CH₂CH₂CO₂All) 1.54-1.64 (2H, m, -CH₂CH₂OH) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 173.43 (-CO₂All), 132.16 (-CH=CH₂), 118.21 (-CH=CH₂), 65.06 (-CH₂CH=CH₂), 62.08 (-CH₂OH), 33.81 (-CH₂CO₂All), 31.98 (-CH₂CH₂OH), 21.07 (-CH₂CH₂CO₂All) ppm; IR ($\nu_{\max}$, film): 3395 (br), 2941, 2873, 1734, 1649, 1453, 1420, 1379, 1272, 1156, 1063 cm⁻¹; HRMS m/z (ESI⁺): Found: 159.1017 (M+H), Calc.: 159.1016 (C₈H₁₅O₃).



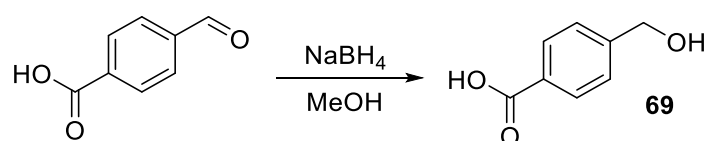
Run as described above on ϵ -caprolactone (2.21 mL, 21.5 mmol). Purified by flash column chromatography eluting with 50 % EtOAc:Hexane. Pure fractions were dissolved in diethyl ether (100 mL), and the organics washed with brine (100 mL x 2), dried with MgSO₄, filtered, and concentrated *in vacuo*, to give the DP as a light yellow oil. A yield of 2.5 g, 14.0 mmol (70 %) was obtained. ¹H NMR (400 MHz, CDCl₃): δ = 5.91 (1H, ddt, J = 17.2, 10.4, 5.7 Hz, -CH=CH₂), 5.31 (1H, ddt, J_1 = 17.2 Hz, J_2 = J_3 = 1.5 Hz, -CH=CH₂), 5.23 (1H, ddt, J_1 = 10.4 Hz, J_2 = J_3 = 1.5 Hz, -CH=CH₂), 4.57 (2H, ddd, J_1 = 5.7 Hz, J_2 = J_3 = 1.5 Hz, -CH₂CH=CH₂), 3.63 (2H, t, J = 6.4 Hz, -CH₂OH), 2.35 (2H, t, J = 7.4 Hz, -CH₂CO₂All), 1.85 (1H, br s, -OH), 1.62-1.71 (2H, m, -CH₂CH₂CO₂All) 1.53-1.61 (2H, m, -CH₂CH₂OH) 1.44-1.37 (2H, m, -CH₂CH₂CH₂OH) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 173.40 (-CO₂All), 132.22 (-CH=CH₂), 118.16 (-CH=CH₂), 65.00 (-CH₂CH=CH₂), 62.50 (-CH₂OH), 34.13 (-CH₂CO₂All), 32.26 (-CH₂CH₂OH), 25.26 (-CH₂CH₂CH₂OH), 24.60 (-CH₂CH₂CO₂All) ppm; IR ($\nu_{\max}$, film): 3373 (br), 2938, 2865, 1733, 1456, 1419, 1378, 1272, 1173, 1073, 1053 cm⁻¹; HRMS m/z (ESI⁺): Found: 173.1174 (M+H), Calc.: 173.1172 (C₉H₁₇O₃).



3-Amino-1-propanol (1.5 mL, 19.6 mmol) and sodium carbonate (2.3 g, 21.6 mmol) were dissolved in a mixture of acetonitrile (10 mL) and water (10 mL). Allyl chloroformate (2.3 mL, 21.6 mmol) was then added dropwise and the mixture stirred for 2 hrs. After removal of the acetonitrile *in vacuo*, the mixture was acidified slowly with 2 M hydrochloric acid and extracted with ethyl acetate (2 x 75 mL). The combined organics were dried with MgSO₄, filtered, and concentrated *in vacuo* to give the DP as a colourless oil. A yield of 1.9 g, 11.9 mmol (61 %) was obtained. Data were consistent with those previously reported.¹² ¹H NMR (400 MHz, CDCl₃): δ = 5.91 (1H, ddt, *J* = 17.2, 10.4, 5. Hz, -CH=CH₂), 5.31 (1H, dd, *J* = 17.2, 1.5 Hz, -CH=CH₂), 5.17-5.26 (2H, m, -CH=CH₂ and -NH), 4.56 (2H, d, *J* = 5.6 Hz, -CH₂CH=CH₂), 3.68 (2H, t, *J* = 5.7 Hz, -CH₂OH), 3.44 (2H, dt, *J*₁ = *J*₂ = 6.2 Hz, -CH₂NHAlloc), 1.71 (2H, tt, *J* = 6.2, 5.7 Hz, -CH₂CH₂OH) ppm.

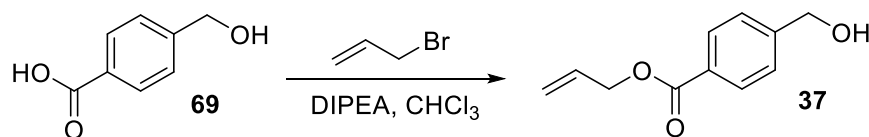


A solution of di-*tert*-butyl dicarbonate (2.4 g, 11 mmol) in DCM (5 mL) was added dropwise to a solution of 3-amino-1-propanol (740 mg, 10 mmol) in DCM (50 mL) and the mixture stirred for 4 hrs. The organics were then washed with water (2 x 50 mL), dried with MgSO₄, filtered, and concentrated *in vacuo* to give the DP as a colourless oil. A yield of 1.75 g, 10 mmol (98 %) was obtained. Data were consistent with those previously reported.¹³ ¹H NMR (400 MHz, CDCl₃): δ = 4.78 (1H, br s, -NH), 3.68 (2H, dt, *J* = 6.2, 5.9 Hz, -CH₂OH), 3.30 (2H, dt, *J*₁ = *J*₂ = 6.0 Hz, -CH₂NHBoc), 2.99 (1H, t, *J* = 6.2 Hz, -OH), 1.67 (2H, tt, *J* = 6.0, 5.9 Hz, -CH₂CH₂OH), 1.46 (9H, Boc) ppm.

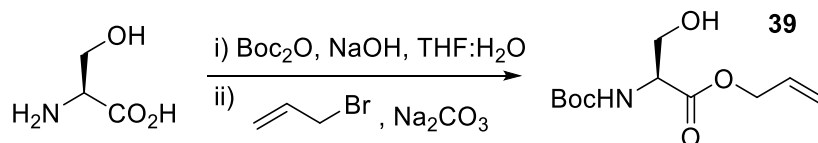


Sodium borohydride (1.26 g, 33.3 mmol) was added slowly over 10 min to a suspension of 4-carboxybenzaldehyde (2 g, 13.3 mmol) in methanol (50 mL) at 0 °C [CARE: GAS EVOLVED]. After stirring at room temperature for 1 hr, the mixture was acidified with hydrochloric acid (1 M, 150 mL) and extracted with ethyl acetate (150 mL). The organics were dried with MgSO₄, filtered, and concentrated *in vacuo*. The residue was triturated in hexane (100 mL) for 1 hr, collected by filtration, and dried *in vacuo* to give the DP as a white solid. A yield of 1.7 g, 11.1 mmol (83 %) was obtained. Data were consistent with those previously reported.¹⁴ ¹H NMR

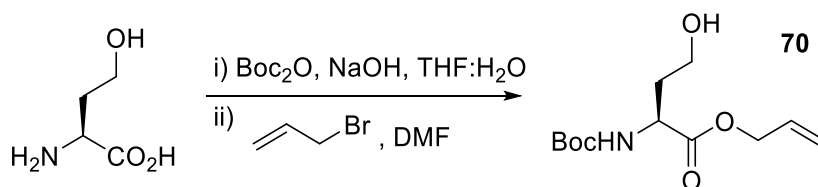
(400 MHz, DMSO- d_6): δ = 12.87 (1H, br s, -CO₂H), 7.91 (2H, d, J = 8.4 Hz, ArH₂), 7.43 (2H, d, J = 8.4 Hz, ArH₃), 4.58 (2H, s, -CH₂OH) ppm.



A mixture of **69** (1.6 g, 10.1 mmol), allyl bromide (1.3 mL, 15.1 mmol), and DIPEA (2.63 mmol, 15.1 mmol) in chloroform (50 mL) was refluxed for 2 hrs. After cooling to room temperature, the mixture was washed with water (50 mL), dried with MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with 30-40 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil. A yield of 740 mg, 3.85 mmol (38 %) was obtained. Data were consistent with those previously reported.¹⁵ ¹H NMR (400 MHz, CDCl₃): δ = 8.07 (2H, d, J = 7.8 Hz, ArH₂), 7.46 (2H, d, J = 7.8 Hz, ArH₃), 6.06 (1H, ddt, J = 17.2, 10.4, 5.7 Hz, -CH=CH₂), 5.43 (1H, ddt, J_1 = 17.2 Hz, J_2 = J_3 = 1.5 Hz, -CH=CH₂), 5.31 (1H, ddt, J_1 = 10.4 Hz, J_2 = J_3 = 1.5 Hz, -CH=CH₂), 4.84 (2H, ddd, J_1 = 5.7 Hz, J_2 = J_3 = 1.5 Hz, -CH₂CH=CH₂), 4.79 (2H, d, J = 5.8 Hz, -CH₂OH), 1.94 (1H, t, J = 5.8 Hz, -OH) ppm.

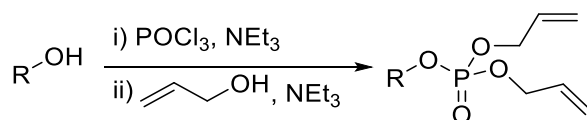


A solution of di-*tert*-butyl dicarbonate (6.85 g, 31.4 mmol) in THF (20 mL) was added dropwise to a solution of L-serine (3 g, 28.5 mmol) in THF (30 mL) and sodium hydroxide (1 M, 57 mL), and the mixture stirred for 4 hrs. After removing the THF *in vacuo* the mixture was acidified with 1 M hydrochloric acid and extracted with ethyl acetate (2 x 100 mL). The combined organics were dried with MgSO₄, filtered, and concentrated *in vacuo*. The residue was dissolved in DMF (30 mL) and potassium carbonate (4.7 g, 34.2 mmol) and allyl bromide (2.7 mL, 31.4 mmol) were sequentially added. After stirring for 18 hrs, the mixture was diluted with ethyl acetate (250 mL) and the organics washed with water (100 mL), hydrochloric acid (1 M, 100 mL), and brine (100 mL), dried with MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 30-40 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil. A yield of 2.7 g, 11.0 mmol (39 %) was obtained. Data were consistent with those previously reported.¹⁶ ¹H NMR (400 MHz, CDCl₃): δ = 5.93 (1H, ddt, J = 17.1, 10.5, 5.7 Hz, -CH=CH₂), 5.47 (1H, br s, -NH), 5.36 (1H, ddt, J_1 = 17.1 Hz, J_2 = J_3 = 1.5 Hz, -CH=CH₂), 5.28 (1H, ddt, J_1 = 10.5 Hz, J_2 = J_3 = 1.5 Hz, -CH=CH₂), 4.69 (2H, ddd, J_1 = 5.7 Hz, J_2 = J_3 = 1.5 Hz, -CH₂CH=CH₂), 4.39-4.46 (1H, m, H_α), 3.90-4.04 (2H, m, H_β), 1.47 (9H, s, Boc) ppm.



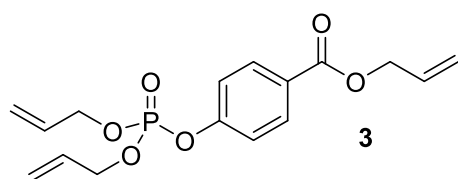
A solution of di-*tert*-butyl dicarbonate (4.8 g, 22 mmol) in THF (20 mL) was added dropwise to a solution of L-homoserine (2.38 g, 20 mmol) in THF (30 mL) and sodium hydroxide (1 M, 20 mL), and the mixture stirred for 4 hrs. After concentration *in vacuo*, the residue was dissolved in DMF (30 mL), allyl bromide (1.9 mL, 22 mmol) was added, and the mixture stirred for 18 hrs. Ethyl acetate (200 mL) was then added and the organics were washed with hydrochloric acid (1M, 2 x 150 mL) and brine (100 mL), dried with MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography, eluting with 30-40 % EtOAc:Hexane. Pure fractions were concentrated *in vacuo* to give the DP as a colourless oil. A yield of 3.9 g, 15.0 mmol (75 %) was obtained. Data were consistent with those previously reported.¹⁶ ^1H NMR (400 MHz, CDCl_3): δ = 5.91 (1H, ddt, J = 17.1, 10.4, 5.8 Hz, $-\text{CH}=\text{CH}_2$), 5.43 (1H, br d, J = 7.3 Hz, $-\text{NH}$), 5.35 (1H, ddt, J_1 = 17.1 Hz, $J_2 = J_3$ = 1.5 Hz, $-\text{CH}=\text{CH}_2$), 5.27 (1H, ddt, J_1 = 10.4 Hz, $J_2 = J_3$ = 1.5 Hz, $-\text{CH}=\text{CH}_2$), 4.65 (2H, ddd, J_1 = 5.8 Hz, $J_2 = J_3$ = 1.5 Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 4.47-4.55 (1H, m, H_α), 3.60-3.80 (2H, m, H_γ), 3.24 (1H, t, J = 3.0 Hz, $-\text{OH}$), 2.12-2.23 (1H, m, H_β), 1.60-1.73 (1H, m, H_β), 1.45 (9H, s, Boc) ppm.

General phosphorylation procedure



A solution of the specified alcohol (1 mmol) in DCM (5 mL) was added dropwise to a solution of phosphorous (V) oxychloride (1.5 mmol) and triethylamine (1.5 mmol) in DCM (10 mL). After stirring for 4 hrs, allyl alcohol (4 mmol) and triethylamine (4 mmol) were added sequentially, and the mixture stirred for 18 hrs. The volatiles were then removed *in vacuo*, and the residue suspended in THF (10 mL). After stirring for 15 min, the precipitated triethylamine hydrochloride was removed by filtration and washed with THF (10 mL), and the combined organics concentrated *in vacuo*. The residue was purified by flash column chromatography and pure fractions combined to give the specified product. Phosphorous NMR is provided for representative compounds.

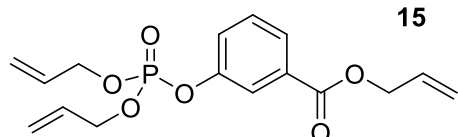
Phosphorylation – Tyrosine analogues



3

Run on **2** on a 5.6 mmol scale. Purified by flash column chromatography, eluting with 20-30 % EtOAc:Hexane. A yield of 0.74 g, 2.2 mmol (40 %) was obtained as a colourless oil. ^1H NMR (400 MHz, CDCl_3): δ = 8.06 (2H, dd, J = 8.4, 0.6 Hz, ArH₂), 7.29 (2H, dd, J = 8.4, 0.6 Hz, ArH₃), 5.89-6.09 (3H, m, -CH=CH₂), 5.35-5.44 (3H, m, -CH=CH₂), 5.25-5.32 (3H, m, -CH=CH₂), 4.81 (1H, ddd, J_1 = 5.6 Hz, J_2 = J_3 = 1.4 Hz, -CH₂CH=CH₂), 4.63-4.68 (2H, m, -CH₂CH=CH₂) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 165.34 (-CO₂Al), 154.26 (d, J = 6.7 Hz, ArC₄), 132.10 (-CH=CH₂), 131.89 (-CH=CH₂), 131.83 (-CH=CH₂), 131.62 (ArC₂), 127.02 (ArC₁), 119.92 (-CH=CH₂), 119.87 (-CH=CH₂), 118.92 (ArC₃), 118.34 (-CH=CH₂), 69.06 (-CH₂CH=CH₂), 69.00 (-CHCH=CH₂), 65.64 (-CH₂CH=CH₂) ppm; ^{31}P (160 MHz, CDCl_3): δ = -6.12 ppm; IR (ν_{max} , film): 3084, 2022, 2948, 2887, 1718, 1649, 1603, 1505, 1458, 1424, 1414, 1362, 1266, 1220, 1163, 1095, 1013 cm^{-1} ; HRMS m/z (ESI⁺): Found: 339.1002 (M+H), Calc.: 339.0998 (C₁₆H₂₀O₆P).

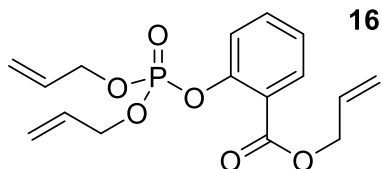
The reaction was amenable to synthesis on a 5 g scale (28 mmol), providing a yield of 4.5 g, 13.3 mmol (48 %). ^1H NMR (400 MHz, CDCl_3): δ = 8.03 (2H, dd, J = 8.5, 0.6 Hz, ArH₂), 7.25 (2H, dd, J = 8.5, 0.6 Hz, ArH₃), 5.84-6.06 (3H, m, -CH=CH₂), 5.31-5.40 (3H, m, -CH=CH₂), 5.22-5.29 (3H, m, -CH=CH₂), 4.80 (1H, ddd, J_1 = 5.6 Hz, J_2 = J_3 = 1.4 Hz, -CH₂CH=CH₂), 4.59-4.67 (2H, m, -CH₂CH=CH₂) ppm;



15

Run on **4** on a 10 mmol scale. Purified by flash column chromatography, eluting with 30 % EtOAc:Hexane. A yield of 1.8 g, 5.3 mmol (53 %) was obtained as a colourless oil. ^1H NMR (400 MHz, CDCl_3): δ = 7.85-7.91

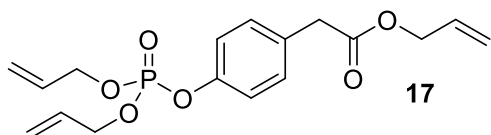
(2H, m, J = 8.4 Hz, ArH₂ and ArH₆), 7.37-7.49 (2H, m, ArH₄ and ArH₅), 5.87-6.07 (3H, m, -CH=CH₂), 5.33-5.43 (3H, m, -CH=CH₂), 5.24-5.31 (3H, m, -CH=CH₂), 4.82 (1H, ddd, J_1 = 5.6 Hz, J_2 = J_3 = 1.4 Hz, -CH₂CH=CH₂), 4.62-4.67 (2H, m, -CH₂CH=CH₂) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 165.16 (-CO₂Al), 150.60 (d, J = 6.8 Hz, ArC₃), 131.97 (-CH=CH₂), 131.89 (-CH=CH₂), 129.77 (ArC₅), 126.36 (ArC₁), 124.72 (d, J = 4.5 Hz, ArC₄), 121.30 (ArC₂/6), 121.24 (ArC₂/6), 68.95 (d, J = 5.9 Hz, -CH₂CH=CH₂), 65.81 (-CH₂CH=CH₂) ppm; ^{31}P (160 MHz, CDCl_3): δ = -5.66 ppm; IR (ν_{max} , film): 3084, 2947, 2890, 1721, 1649, 1587, 1486, 1443, 1362, 1292, 1266, 1202, 1097, 1021, 1006 cm^{-1} ; HRMS m/z (ESI⁺): Found: 361.0829 (M+Na), Calc.: 361.0817 (C₁₆H₁₉NaO₆P).



16

Run on **5** on a 1.1 mmol scale. Purified by flash column chromatography, eluting with 20-30 % EtOAc:Hexane. A yield of 110 mg, 0.32 mmol (30 %) was obtained as a colourless oil.

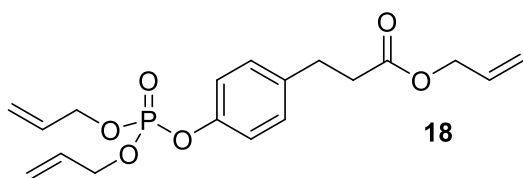
^1H (400 MHz, CDCl_3): δ = 7.83 (1H, dd, J = 7.8, 2.5 Hz, ArH₆), 7.36-7.46 (2H, m, ArH₄ and ArH₅), 7.16 (2H, dd, J = 7.0, 2.4 Hz, ArH₃), 5.80-6.02 (3H, m, -CH=CH₂), 5.25-5.37 (3H, m, -CH=CH₂), 5.13-5.23 (3H, m, -CH=CH₂), 4.73 (1H, ddd, J_1 = 5.6 Hz, J_2 = J_3 = 1.4 Hz, -CH₂CH=CH₂), 4.57-4.63 (2H, m, -CH₂CH=CH₂) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 164.46 (-CO₂All), 149.65 (d, J = 6.6 Hz, ArC₂), 133.60 (ArC₄), 132.21 (-CH=CH₂), 132.14 (-CH=CH₂), 132.05 (-CH=CH₂), 131.677 (ArC₆), 124.95 (ArC₃), 123.05 (d, J = 6.2 Hz ArC₁), 121.61 (d, J = 2.6 Hz, ArC₃), 118.47 (-CH=CH₂), 69.01 (-CH₂CH=CH₂), 68.95 (-CH₂CH=CH₂), 65.83 (-CHCH=CH₂) ppm; ^{31}P (160 MHz, CDCl_3): δ = -6.35 ppm; IR (ν_{max} , film): 3084, 3021, 2985, 2947, 2887, 1727, 1649, 1603, 1582, 1488, 1450, 1295, 1287, 1250, 1219, 1129, 1079, 1020 cm^{-1} ; HRMS m/z (ESI⁺): Found: 339.1002 (M+H), Calc.: 339.0998 (C₁₆H₂₀O₆P).



17

Run on **6** on a 11.9 mmol scale. Purified by flash column chromatography, eluting with 30-40 % EtOAc:Hexane. A yield of 1.8 g, 5.1 mmol (43 %) was

obtained as a colourless oil. ^1H NMR (400 MHz, CDCl_3): δ = 7.27 (2H, d, J = 9.0 Hz, ArH₂), 7.29 (2H, dd, J = 9.0, 1.1 Hz, ArH₃), 5.85-6.00 (3H, m, -CH=CH₂), 5.34-5.41 (2H, m, -CH=CH₂), 5.20-5.31 (4H, m, -CH=CH₂), 4.54-4.67 (6H, m, -CH₂CH=CH₂), 3.63 (2H, s, -CH₂CO₂All) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 170.94 (-CO₂All), 149.74 (d, J = 6.9 Hz, ArC₄), 132.11 (-CH=CH₂), 132.04 (-CH=CH₂), 131.91 (-CH=CH₂), 130.78 (ArC₁), 130.60 (ArC₂), 120.15 (-CH=CH₂), 120.10 (-CH=CH₂), 118.65 (ArC₃), 118.37 (-CH=CH₂), 68.14 (d, J = 5.4 Hz, -CH₂CH=CH₂), 65.54 (-CH₂CH=CH₂), 40.51 (-CH₂CO₂All) ppm; IR (ν_{max} , film): 3082, 2948, 2088, 1727, 1649, 1603, 1582, 1488, 1450, 1425, 1361, 1297, 1287, 1220, 1078, 1020 cm^{-1} ; HRMS m/z (ESI⁺): Found: 375.0969 (M+Na), Calc.: 375.0968 (C₁₇H₂₁NaO₆P).

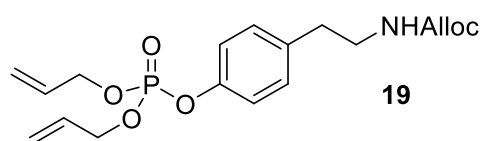


18

Run on **7** on a 8.7 mmol scale. Purified by flash column chromatography, eluting with 30 % EtOAc:Hexane. A yield of 1.7 g, 4.8 mmol (55 %) was obtained as a colourless oil. ^1H NMR (400

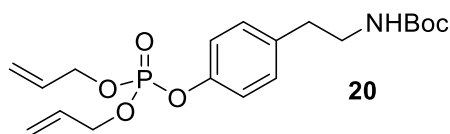
MHz, CDCl_3): δ = 7.11-7.20 (4H, m, ArH₂ & ArH₃), 5.83-6.00 (3H, m, -CH=CH₂), 5.30-5.41 (3H, m, -CH=CH₂), 5.20-5.29 (3H, m, -CH=CH₂), 4.61-4.67 (4H, m, -CH₂CH=CH₂), 4.58 (2H, ddd, J_1 = 5.7 Hz, J_2 = J_3 = 1.4 Hz, -CH₂CH=CH₂), 2.94 (2H, t, J = 7.7 Hz, -CH₂Ar), 2.64 (2H, d, J = 7.7 Hz, -CH₂CO₂All) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 172.31 (-CO₂All), 149.06 (d, J = 6.9 Hz, ArC₄), 137.32 (ArC₁), 132.16 (-CH=CH₂), 132.08 (-CH=CH₂), 129.55 (ArC₂), 120.06 (-CH=CH₂), 120.02 (-CH=CH₂), 118.59 (ArC₃), 118.32 (-CH=CH₂), 68.77 (d, J = 5.6 Hz, -

$\underline{\text{CH}_2\text{CH}=\text{CH}_2}$), 65.19 ($-\underline{\text{CH}_2\text{CH}=\text{CH}_2}$), 35.79 ($-\underline{\text{CH}_2\text{CO}_2\text{AlI}}$), 30.16 ($-\underline{\text{CH}_2\text{Ar}}$) ppm; IR (ν_{max} , film): 3080, 2984, 2943, 1733, 1649, 1608, 1507, 1454, 1424, 1377, 1277, 1214, 1165, 1099, 1016 cm^{-1} ; HRMS m/z (ESI+): Found: 389.1145 ($\text{M}+\text{Na}$), Calc.: 389.1130 ($\text{C}_{18}\text{H}_{23}\text{NaO}_6\text{P}$).



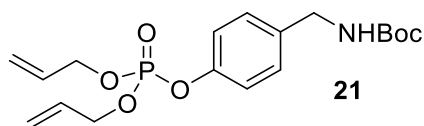
Run on **8** on a 0.7 mmol scale. Purified by flash column chromatography, eluting with 30-50 % EtOAc:Hexane.

A yield of 115 mg, 0.3 mmol (44 %) was obtained as a colourless oil. ^1H NMR (400 MHz, CDCl_3): δ = 7.13 (4H, app s, ArH_2 & ArH_3), 7.29 (2H, dd, J = 8.4, 0.6 Hz, ArH_3), 5.83-5.98 (3H, m, $-\underline{\text{CH}=\text{CH}_2}$), 5.16-5.39 (6H, m, $-\underline{\text{CH}=\text{CH}_2}$), 4.98 (1H, br t, J = 6.7 Hz, $-\underline{\text{NH}}$), 4.59-4.66 (4H, m, $-\underline{\text{CH}_2\text{CH}=\text{CH}_2}$), 4.53 (2H, d, J = 5.6 Hz, $-\underline{\text{CH}_2\text{CH}=\text{CH}_2}$), 3.38 (2H, dt, $J_1 = J_2 = 6.7$ Hz, $-\underline{\text{CH}_2\text{NHAlIoc}}$), 2.77 (2H, t, J = 6.7 Hz, $-\underline{\text{CH}_2\text{Ar}}$) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 156.91 ($-\underline{\text{NHCO}_2\text{AlI}}$), 149.19 (d, J = 6.9 Hz, ArC_4), 135.72 (ArC_1), 132.92 ($-\underline{\text{CH}=\text{CH}_2}$), 132.10 ($-\underline{\text{CH}=\text{CH}_2}$), 132.03 ($-\underline{\text{CH}=\text{CH}_2}$), 129.99 (ArC_2), 120.15 ($-\underline{\text{CH}=\text{CH}_2}$), 120.10 ($-\underline{\text{CH}=\text{CH}_2}$), 118.63 (ArC_3), 117.57 ($-\underline{\text{CH}=\text{CH}_2}$), 68.79 (d, J = 5.8 Hz, $-\underline{\text{CH}_2\text{CH}=\text{CH}_2}$), 65.43 ($-\underline{\text{CH}_2\text{CH}=\text{CH}_2}$), 42.14 ($-\underline{\text{CH}_2\text{NHAlIoc}}$), 35.36 ($-\underline{\text{CH}_2\text{Ar}}$) ppm; IR (ν_{max} , film): 3320, 3082, 2940, 2883, 1707, 1648, 1607, 1529, 1506, 1459, 1424, 1412, 1365, 1249, 1214, 1167, 1138, 1097, 1015 cm^{-1} ; HRMS m/z (ESI+): Found: 382.1412 ($\text{M}+\text{H}$), Calc.: 382.1410 ($\text{C}_{18}\text{H}_{25}\text{NO}_6\text{P}$).



Run on **9** on a 5.4 mmol scale. Purified by flash column chromatography, eluting with 20-40 % EtOAc:Hexane. A yield of 1.2 g, 3.0 mmol (57 %) was obtained.

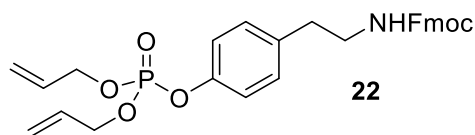
^1H NMR (400 MHz, CDCl_3): δ = 7.16 (4H, app s, ArH), 5.90-6.01 (2H, m, $-\underline{\text{CH}=\text{CH}_2}$), 5.38 (2H, ddt, $J_1 = 17.1$ Hz, $J_2 = J_3 = 1.5$ Hz, $-\underline{\text{CH}=\text{CH}_2}$), 5.24-5.30 (2H, m, $-\underline{\text{CH}=\text{CH}_2}$), 4.62-4.69 (4H, m, $-\underline{\text{CH}_2\text{CH}=\text{CH}_2}$), 4.52-4.60 (1H, m, $-\underline{\text{NH}}$), 3.35 (2H, td, J = 6.9, 6.4 Hz, $-\underline{\text{CH}_2\text{NHBoc}}$), 2.77 (2H, t, J = 6.9 Hz, $-\underline{\text{CH}_2\text{Ar}}$), 1.44 (9H, s, Boc) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 155.82 ($-\underline{\text{CO}_2\text{R}}$), 149.19 (d, J = 7.1 Hz, ArC_4), 135.91 (ArC_1), 132.11 (d, J = 6.9 Hz, $-\underline{\text{CH}=\text{CH}_2}$), 130.00 (ArC_2), 120.11 (d, J = 4.8 Hz, $-\underline{\text{CH}=\text{CH}_2}$), 118.61 (ArC_3), 79.27 ($-\underline{\text{CMe}_3}$), 68.79 (d, J = 5.7 Hz, $-\underline{\text{CH}_2\text{CH}=\text{CH}_2}$), 47.73 ($-\underline{\text{CH}_2\text{NHBoc}}$), 35.46 ($-\underline{\text{CH}_2\text{Ar}}$), 28.39 ($-\underline{\text{CMe}_3}$) ppm; IR (ν_{max} , film): 3336, 2977, 2935, 1708, 1608, 1507, 1458, 1391, 1365, 1273, 1252, 1215, 1167, 1099, 1014 cm^{-1} ; HRMS m/z (ESI+): Found: 415.1990 ($\text{M}+\text{H}$), Calc.: 415.1992 ($\text{C}_{19}\text{H}_{29}\text{NO}_6\text{P}$).



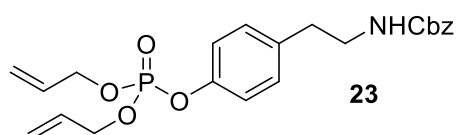
Run on **10** on a 4.5 mmol scale. Purified by flash column chromatography, eluting with 20-30 % EtOAc:Hexane. A yield of 0.98 g, 2.6 mmol (57 %) was obtained.

^1H NMR (400 MHz, CDCl_3): δ = 7.25 (2H, d, J = 8.5 Hz, ArH_2), 7.16-7.20 (2H, m, ArH_3), 5.88-6.01 (2H, m, $-\underline{\text{CH}=\text{CH}_2}$), 5.34-5.41 (2H, m, $-\underline{\text{CH}=\text{CH}_2}$), 5.24-5.29 (2H, m, $-\underline{\text{CH}=\text{CH}_2}$), 4.91 (1H, br s, $-\underline{\text{NH}}$), 4.52-4.67 (4H, m, $-\underline{\text{CH}_2\text{CH}=\text{CH}_2}$), 4.28 (2H, br d, J = 5.7 Hz, $-\underline{\text{CH}_2\text{NHBoc}}$), 1.46 (9H, s, Boc) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 155.84 ($-\underline{\text{CO}_2\text{R}}$), 149.79 (d, J = 6.4 Hz, ArC_4), 135.94

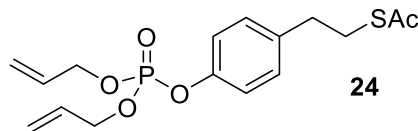
(ArC1), 132.53 (d, $J = 6.9$ Hz, $-\underline{\text{CH}}=\text{CH}_2$), 128.81 (ArC2), 120.14 (d, $J = 4.8$ Hz, $-\text{CH}=\underline{\text{CH}}_2$), 118.65 (ArC3), 79.65 ($-\underline{\text{C}}\text{Me}_3$), 68.81 (d, $J = 5.6$ Hz, $-\underline{\text{CH}}_2\text{CH}=\text{CH}_2$), 44.00 ($-\underline{\text{CH}}_2\text{NHBoc}$), 28.39 ($-\text{CMe}_3$) ppm; IR (ν_{max} , film): 3328, 2978, 2934, 1708, 1607, 1506, 1458, 1425, 1391, 1365, 1268, 1252, 1214, 1162, 1098, 1014 cm^{-1} ; HRMS m/z (ESI+): Found: 401.1839 (M+H), Calc.: 401.1836 ($\text{C}_{18}\text{H}_{27}\text{NO}_6\text{P}$).



Run on **11** on a 180 μmol scale. Purified by flash column chromatography, eluting with 20-50 % EtOAc:Hexane. A yield of 72 mg, 138 μmol (77 %) was obtained. ^1H NMR (400 MHz, CDCl_3): $\delta = 7.77$ (2H, d, $J = 7.3$ Hz, Fmoc), 7.57 (2H, d, $J = 7.3$ Hz, Fmoc), 7.40 (2H, dd, $J_1 = J_2 = 7.3$ Hz, Fmoc), 7.31 (2H, dd, $J_1 = J_2 = 7.3$ Hz, Fmoc), 7.11-7.24 (4H, m, ArH), 5.89-6.01 (2H, m, $-\underline{\text{CH}}=\text{CH}_2$), 5.37 (2H, ddt, $J_1 = 17.1$ Hz, $J_2 = J_3 = 1.5$ Hz, $-\text{CH}=\underline{\text{CH}}_2$), 5.26 (2H, ddt, $J_1 = 10.4$ Hz, $J_2 = J_3 = 1.5$ Hz $-\text{CH}=\underline{\text{CH}}_2$), 4.78 (1H, br t, $J = 7.1$ Hz, $-\text{NH}$), 4.50-4.69 (4H, m, $-\underline{\text{CH}}_2\text{CH}=\text{CH}_2$), 4.40 (2H, d, $J = 6.9$ Hz, Fmoc), 4.20 (1H, t, $J = 6.9$ Hz, Fmoc), 3.41 (2H, dt, $J = 7.1, 6.9$ Hz, $-\underline{\text{CH}}_2\text{NHFmoc}$), 2.77 (2H, t, $J = 6.9$ Hz, $-\underline{\text{CH}}_2\text{Ar}$) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 156.38$ ($-\text{CO}_2\text{R}$), 149.47 (ArC4), 144.02 (Fmoc), 141.43 (Fmoc), 132.19 (d, $J = 6.9$ Hz, $-\underline{\text{CH}}=\text{CH}_2$), 130.14 (ArC2), 127.78 (Fmoc), 127.13 (Fmoc), 125.11 (Fmoc), 120.28 (d, $J = 5.0$ Hz, ArC3), 120.07 ($-\text{CH}=\underline{\text{CH}}_2$), 118.75 (Fmoc), 118.38 (ArC1), 68.91 (d, $J = 5.7$ Hz, $-\underline{\text{CH}}_2\text{CH}=\text{CH}_2$), 66.63 (Fmoc), 47.38 (Fmoc), 42.30 ($-\underline{\text{CH}}_2\text{NHFmoc}$), 35.52 ($-\underline{\text{CH}}_2\text{Ar}$) ppm; IR (ν_{max} , film): 3318, 3066, 2942, 1716, 1608, 1537, 1506, 1450, 1424, 1251, 1216, 1167, 1138, 1101, 1017 cm^{-1} ; HRMS m/z (ESI+): Found: 542.1709 (M+Na), Calc.: 542.1703 ($\text{C}_{29}\text{H}_{30}\text{NaNO}_6\text{P}$).

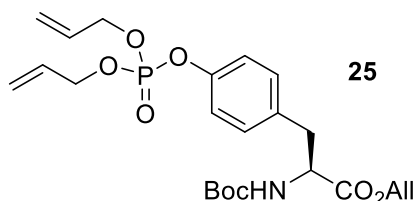


Run on **12** on a 180 μmol scale. Purified by flash column chromatography, eluting with 30-50 % EtOAc:Hexane. A yield of 38 mg, 88 μmol (49 %) was obtained. ^1H NMR (400 MHz, CDCl_3): $\delta = 7.31$ (5H, app s, Cbz), 7.10 (4H, app s, ArH), 5.84-5.97 (2H, m, $-\underline{\text{CH}}=\text{CH}_2$), 5.33 (2H, ddt, $J_1 = 17.1$ Hz, $J_2 = J_3 = 1.5$ Hz, $-\text{CH}=\underline{\text{CH}}_2$), 5.22 (2H, ddt, $J_1 = 10.4$ Hz, $J_2 = J_3 = 1.5$ Hz $-\text{CH}=\underline{\text{CH}}_2$), 5.05 (2H, s, Cbz), 4.92-4.99 (1H, br m, $-\text{NH}$), 4.60 (4H, ddd, $J_1 = 7.8$ Hz, $J_2 = J_3 = 1.5$ Hz $-\underline{\text{CH}}_2\text{CH}=\text{CH}_2$), 3.33-3.41 (2H, m, $-\underline{\text{CH}}_2\text{NHCbz}$), 2.75 (2H, t, $J = 6.9$ Hz, $-\underline{\text{CH}}_2\text{Ar}$) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 156.79$ ($-\text{CO}_2\text{R}$), 149.65 (d, $J = 7.2$ Hz, ArC4), 136.99 (ArC1), 136.13 (Cbz), 132.52 (d, $J = 7.0$ Hz, $-\underline{\text{CH}}=\text{CH}_2$), 130.46 (ArC2), 128.96 (Cbz), 128.56 (Cbz), 120.59 (d, $J = 4.8$ Hz, $-\text{CH}=\underline{\text{CH}}_2$), 119.10 (ArC3), 69.26 (d, $J = 5.8$ Hz, $-\underline{\text{CH}}_2\text{CH}=\text{CH}_2$), 67.06 (Cbz), 42.64 ($-\underline{\text{CH}}_2\text{NHR}$), 35.78 ($-\underline{\text{CH}}_2\text{Ar}$) ppm; IR (ν_{max} , film): 3315, 2940, 2885, 1713, 1607, 1528, 1506, 1455, 1425, 1366, 1249, 1214, 1167, 1135, 1098, 1015 cm^{-1} ; HRMS m/z (ESI+): Found: 432.1569 (M+H), Calc.: 432.1571 ($\text{C}_{22}\text{H}_{27}\text{NO}_6\text{P}$).



24

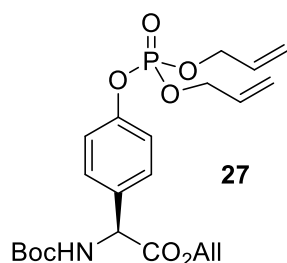
Run on **14** on a 1.5 mmol scale. The phosphorylation was quenched with 2.5 equiv allyl alcohol and triethylamine to minimise alcoholysis of the thioester. Purified by flash column chromatography, eluting with 20-30 % EtOAc:Hexane. A yield of 287 mg, 0.85 μ mol (57 %) was obtained. ^1H NMR (400 MHz, CDCl_3): δ = 7.06-7.18 (4H, m, ArH), 5.82-5.96 (2H, m, $-\text{CH}=\text{CH}_2$), 5.13-5.39 (4H, m, $-\text{CH}=\text{CH}_2$), 4.43-4.67 (4H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$), 2.99-3.07 (2H, m, $-\text{CH}_2\text{Ar}$), 2.80 (2H, t, J = 7.4 Hz, $-\text{CH}_2\text{SAc}$), 2.30 (3H, s, $-\text{SAc}$); ^{13}C NMR (100 MHz, CDCl_3): δ = 195.77 ($-\text{COMe}$), 149.23 (d, J = 6.6 Hz, ArC4), 136.91 (ArC1), 132.10 (d, J = 7.3 Hz, $-\text{CH}=\text{CH}_2$), 129.89 (ArC2), 120.19 (d, J = 4.5 Hz, $-\text{CH}=\text{CH}_2$), 118.67 (ArC3), 68.99 (d, J = 5.7 Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 35.19 ($-\text{CH}_2\text{Ar}$), 30.70 ($-\text{CH}_2\text{SAc}$), 30.50 ($-\text{COMe}$) ppm; IR (ν_{max} , film): 2926, 1689, 1507, 1425, 1355, 1216, 1169, 1134, 1100, 1028, 1017 cm^{-1} ; HRMS m/z (ESI+): Found: 357.0913 (M+H), Calc.: 357.0920 ($\text{C}_{16}\text{H}_{22}\text{O}_5\text{PS}$).



25

Run on **64** on a 3.5 mmol scale. Purified by flash column chromatography, eluting with 20-40 % EtOAc:Hexane. A yield of 570 mg, 1.2 mmol (35 %) was obtained as a colourless oil. ^1H NMR (400 MHz, CDCl_3): δ = 6.99-7.11 (4H, m, ArH2 & ArH3), 5.72-5.92 (3H, m, $-\text{CH}=\text{CH}_2$), 5.13-5.34 (6H, m, $-\text{CH}=\text{CH}_2$), 4.93 (1H, br d, J = 6.8 Hz, $-\text{NH}$), 4.43-4.60 (7H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$ & H_α), 2.91-3.08 (2H, m, H_β), 1.34 (9H, s, Boc) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 171.36 ($-\text{CO}_2\text{All}$), 155.09 ($-\text{CO}_2^t\text{Bu}$), 149.67 (d, J = 6.9 Hz, ArC4), 132.91 (ArC1), 132.07 (d, J = 6.9 Hz, $-\text{CH}=\text{CH}_2$), 131.42 ($-\text{CH}=\text{CH}_2$), 130.64 (ArC2), 120.05 (d, J = 4.6 Hz, $-\text{CH}=\text{CH}_2$), 119.07 ($-\text{CH}=\text{CH}_2$), 118.64 (ArC3), 79.98 ($-\text{CMe}_3$), 68.80 (d, J = 5.6 Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 65.98 ($-\text{CH}_2\text{CH}=\text{CH}_2$), 54.39 (C_α), 37.52 (C_β), 28.27 (Boc) ppm; IR (ν_{max} , film): 3304, 3078, 2978, 2935, 2168, 1742, 1711, 1608, 1507, 1454, 1425, 1391, 1366, 1344, 1272, 1215, 1163, 1099, 1016 cm^{-1} ; HRMS m/z (ESI+): Found: 504.1763 (M+Na), Calc.: 504.1758 ($\text{C}_{23}\text{H}_{32}\text{NaNO}_8\text{P}$).

The use of D-enantiomer **65** led to the production of **26**.

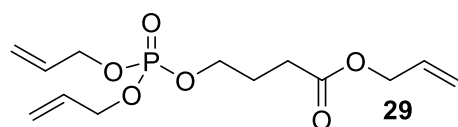


27

Run on **66** on a 5 mmol scale. Purified by flash column chromatography, eluting with 20-30 % EtOAc:Hexane. A yield of 1.7 g, 3.6 mmol (72 %) was obtained as a colourless oil. ^1H NMR (400 MHz, CDCl_3): δ = 7.36 (2H, d, J = 7.6 Hz, ArH2), 7.21 (2H, d, J = 7.6 Hz, ArH3), 5.77-6.00 (3H, m, $-\text{CH}=\text{CH}_2$), 5.59 (1H, br d, J = 7.0 Hz, $-\text{NH}$), 5.17-5.43 (7H, m, $-\text{CH}=\text{CH}_2$ & H_α), 4.53-4.68 (6H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$), 2.91-3.08 (2H, m, H_β), 1.44 (9H, s, Boc) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 170.55 ($-\text{CO}_2\text{All}$), 154.70 ($-\text{CO}_2^t\text{Bu}$), 150.55 (d, J = 6.7 Hz, ArC4), 133.90 (ArC1), 131.99 (d, J = 6.7 Hz, $-\text{CH}=\text{CH}_2$), 131.22 ($-\text{CH}=\text{CH}_2$), 128.58 (ArC2), 120.41 (d, J = 4.7 Hz, $-\text{CH}=\text{CH}_2$),

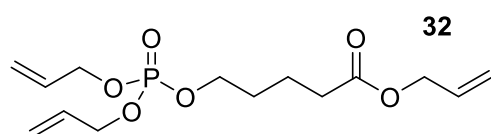
118.75 (ArC3), 80.27 ($-\underline{\text{C}}\text{Me}_3$), 68.88 (d, $J = 5.7$ Hz, $-\underline{\text{C}}\text{H}_2\text{CH}=\text{CH}_2$), 66.24 ($-\underline{\text{C}}\text{H}_2\text{CH}=\text{CH}_2$), 56.98 ($\underline{\text{C}}_\alpha$), 28.29 ($\underline{\text{Boc}}$) ppm; IR (ν_{max} , film): 2980, 1744, 1711, 1608, 1505, 1457, 1367, 1275, 1218, 1161, 1098, 1016 cm^{-1} ; HRMS m/z (ESI+): Found: 490.1606 (M+Na), Calc.: 490.1601 ($\text{C}_{22}\text{H}_{30}\text{NaNO}_8\text{P}$).

Phosphorylation – Serine analogues



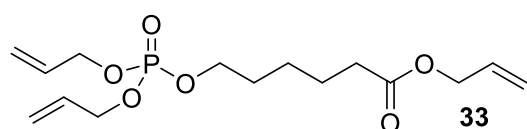
Run on **28** on a 13.8 mmol scale. Purified by flash column chromatography, eluting with 40 % EtOAc:Hexane. A yield of 1.7 g, 5.6 mmol (41 %) was

obtained as a colourless oil. ^1H NMR (400 MHz, CDCl_3): $\delta = 5.79\text{--}5.94$ (3H, m, $-\underline{\text{C}}\text{H}=\text{CH}_2$), 5.26–5.36 (3H, m, $-\text{CH}=\underline{\text{C}}\text{H}_2$), 5.15–5.25 (3H, m, $-\text{CH}=\underline{\text{C}}\text{H}_2$), 4.44–4.45 (6H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$), 4.01–4.08 (2H, m, $-\text{CH}_2\text{OPO}_3\text{All}_2$), 2.42 (2H, t, $J = 7.3$ Hz, $-\text{CH}_2\text{CO}_2\text{All}$), 1.92–2.00 (2H, m, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{All}$) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 172.26$ ($-\underline{\text{C}}\text{O}_2\text{All}$), 132.41 ($-\underline{\text{C}}\text{H}=\text{CH}_2$), 132.34 ($-\underline{\text{C}}\text{H}=\text{CH}_2$), 132.03 ($-\underline{\text{C}}\text{H}=\text{CH}_2$), 118.21 ($-\text{CH}=\underline{\text{C}}\text{H}_2$), 118.19 ($-\text{CH}=\underline{\text{C}}\text{H}_2$), 118.04 ($-\text{CH}=\underline{\text{C}}\text{H}_2$), 68.06 (d, $J = 5.5$ Hz, $-\underline{\text{C}}\text{H}_2\text{CH}=\text{CH}_2$), 66.61 (d, $J = 5.9$ Hz, $-\underline{\text{C}}\text{H}_2\text{OPO}_3\text{All}_2$), 65.11 ($-\underline{\text{C}}\text{H}_2\text{CH}=\text{CH}_2$), 30.00 ($-\underline{\text{C}}\text{H}_2\text{CO}_2\text{All}$), 25.48 ($-\text{CH}_2\text{CH}_2\text{CO}_2\text{All}$) ppm; IR (ν_{max} , film): 2877, 1727, 1607, 1508, 1438, 1206, 1158, 1104, 1053 cm^{-1} ; HRMS m/z (ESI+): Found: 305.1152 (M+H), Calc.: 305.1154 ($\text{C}_{13}\text{H}_{22}\text{O}_6\text{P}$).



Run on **67** on a 1.2 mmol scale. Purified by flash column chromatography, eluting with 40 % EtOAc:Hexane. A yield of 180 mg, 0.52 mmol (44 %) was

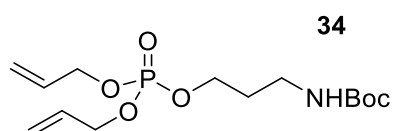
obtained as a colourless oil. ^1H NMR (400 MHz, CDCl_3): $\delta = 5.81\text{--}5.97$ (3H, m, $-\underline{\text{C}}\text{H}=\text{CH}_2$), 5.28–5.38 (3H, m, $-\text{CH}=\underline{\text{C}}\text{H}_2$), 5.17–5.27 (3H, m, $-\text{CH}=\underline{\text{C}}\text{H}_2$), 4.47–4.54 (6H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$), 3.99–4.08 (2H, m, $-\text{CH}_2\text{OPO}_3\text{All}_2$), 2.35 (2H, t, $J = 6.9$ Hz, $-\text{CH}_2\text{CO}_2\text{All}$), 1.64–1.76 (4H, m, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{All}$) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 172.74$ ($-\underline{\text{C}}\text{O}_2\text{All}$), 132.50 ($-\underline{\text{C}}\text{H}=\text{CH}_2$), 132.41 ($-\underline{\text{C}}\text{H}=\text{CH}_2$), 132.14 ($-\underline{\text{C}}\text{H}=\text{CH}_2$), 118.19 ($-\text{CH}=\underline{\text{C}}\text{H}_2$), 118.18 ($-\text{CH}=\underline{\text{C}}\text{H}_2$), 118.08 ($-\text{CH}=\underline{\text{C}}\text{H}_2$), 68.03 (d, $J = 5.6$ Hz, $-\underline{\text{C}}\text{H}_2\text{CH}=\text{CH}_2$), 67.21 (d, $J = 6.0$ Hz, $-\underline{\text{C}}\text{H}_2\text{OPO}_3\text{All}_2$), 65.01 ($-\underline{\text{C}}\text{H}_2\text{CH}=\text{CH}_2$), 33.45 ($-\underline{\text{C}}\text{H}_2\text{CO}_2\text{All}$), 29.56 (d, $J = 6.9$ Hz, $-\text{CH}_2\text{CH}_2\text{OPO}_3\text{All}_2$), 20.92 ($-\text{CH}_2\text{CH}_2\text{CO}_2\text{All}$) ppm; IR (ν_{max} , film): 3080, 2948, 1734, 1649, 1458, 1425, 1365, 1267, 1164, 1098, 1015 cm^{-1} ; HRMS m/z (ESI+): Found: 319.1316 (M+H), Calc.: 319.1311 ($\text{C}_{14}\text{H}_{24}\text{O}_6\text{P}$).



Run on **68** on a 10 mmol scale. Purified by flash column chromatography, eluting with 30–40 % EtOAc:Hexane. A yield of 1.5 g, 4.5 mmol (45 %) was

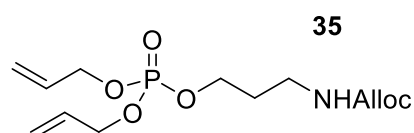
obtained as a colourless oil. ^1H NMR (400 MHz, CDCl_3): $\delta = 5.83\text{--}5.97$ (3H, m, $-\underline{\text{C}}\text{H}=\text{CH}_2$), 5.29–5.38 (3H, m, $-\text{CH}=\underline{\text{C}}\text{H}_2$), 5.17–5.27 (3H, m, $-\text{CH}=\underline{\text{C}}\text{H}_2$), 4.47–4.56 (6H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$), 3.99–4.06 (2H, m, $-\text{CH}_2\text{OPO}_3\text{All}_2$), 2.32 (2H, t, $J = 7.4$ Hz, $-\text{CH}_2\text{CO}_2\text{All}$), 1.59–1.72 (4H, m, -

$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{AlI}$), 1.35-1.45 (2H, m, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{AlI}$) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 173.01 ($-\text{CO}_2\text{AlI}$), 132.51 ($-\text{CH}=\text{CH}_2$), 132.46 ($-\text{CH}=\text{CH}_2$), 132.20 ($-\text{CH}=\text{CH}_2$), 118.14 ($-\text{CH}=\text{CH}_2$), 118.09 ($-\text{CH}=\text{CH}_2$), 118.08 ($-\text{CH}=\text{CH}_2$), 68.01 (d, J = 5.4 Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 67.52 (d, J = 6.0 Hz, $-\text{CH}_2\text{OPO}_3\text{AlI}_2$), 64.96 ($-\text{CH}_2\text{CH}=\text{CH}_2$), 33.97 ($-\text{CH}_2\text{CO}_2\text{AlI}$), 29.87 (d, J = 7.0 Hz, $-\text{CH}_2\text{CH}_2\text{OPO}_3\text{AlI}_2$), 24.49 ($-\text{CH}_2\text{CH}_2\text{CO}_2\text{AlI}$), 24.37 ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{AlI}$) ppm; ^{31}P (160 MHz, CDCl_3): δ = -0.15 ppm; IR (ν_{max} , film): 3085, 2943, 1734, 1649, 1459, 1425, 1380, 1267, 1161, 1100, 1024 cm^{-1} ; HRMS m/z (ESI+): Found: 333.1478 (M+H), Calc.: 333.1467 ($\text{C}_{15}\text{H}_{26}\text{NO}_6\text{P}$).



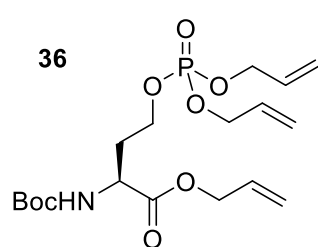
Run on **30** on a 5.7 mmol scale. Purified by flash column chromatography, eluting with 30-50 % EtOAc:Hexane. A yield of 470 mg, 1.4 mmol (25 %) was obtained as a colourless oil.

^1H NMR (400 MHz, CDCl_3): δ = 5.85-5.99 (2H, m, $-\text{CH}=\text{CH}_2$), 5.20-5.42 (4H, m, $-\text{CH}=\text{CH}_2$ and $-\text{NH}$), 4.98 (1H, br s, $-\text{NH}$), 4.46-4.56 (4H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$), 4.09 (2H, dt, J = 7.6, 6.0 Hz, $-\text{CH}_2\text{OPO}_3\text{AlI}_2$), 3.18-3.26 (2H, m, $-\text{CH}_2\text{NHBoc}$), 1.89-1.79 (2H, m, $-\text{CH}_2\text{CH}_2\text{OPO}_3\text{AlI}_2$), 1.41 (9H, s, Boc) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 155.96 ($-\text{CO}_2^t\text{Bu}$), 132.34 (d, J = 6.8 Hz, $-\text{CH}=\text{CH}_2$), 118.39 ($-\text{CH}=\text{CH}_2$), 79.14 ($-\text{CMe}_3$), 68.20 (d, J = 5.6 Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 65.35 (d, J = 5.8 Hz, $-\text{CH}_2\text{OPO}_3\text{AlI}_2$), 36.81 ($-\text{CH}_2\text{NHBoc}$), 30.32 (d, J = 5.9 Hz, $-\text{CH}_2\text{CH}_2\text{OPO}_3\text{AlI}_2$), 28.36 ($-\text{CMe}_3$) ppm; ^{31}P (160 MHz, CDCl_3): δ = 0.15 ppm; IR (ν_{max} , film): 3322, 2977, 2936, 1707, 1522, 1456, 1425, 1391, 1365, 1267, 1249, 1168, 1100, 1007 cm^{-1} ; HRMS m/z (ESI+): Found: 358.1387 (M+Na), Calc.: 358.1390 ($\text{C}_{14}\text{H}_{26}\text{NaNO}_6\text{P}$).



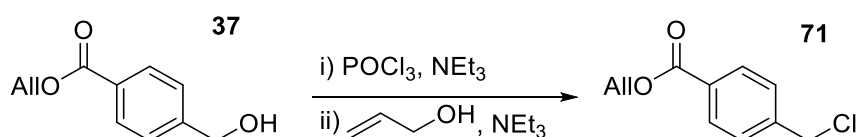
Run on **31** on a 1.25 mmol scale. Purified by flash column chromatography, eluting with 30-50 % EtOAc:Hexane. A yield of 140 mg, 0.44 mmol (35 %) was obtained as a

colourless oil. ^1H NMR (400 MHz, CDCl_3): δ = 5.85-5.99 (3H, m, $-\text{CH}=\text{CH}_2$), 5.17-5.42 (7H, m, $-\text{CH}=\text{CH}_2$ and $-\text{NH}$), 4.49-4.58 (6H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$), 4.13 (2H, dt, J = 7.6, 5.9 Hz, $-\text{CH}_2\text{OPO}_3\text{AlI}_2$), 3.31 (2H, dt, $J_1 = J_2 = 6.3$ Hz, $-\text{CH}_2\text{NHAlloc}$), 1.83-1.91 (2H, m, $-\text{CH}_2\text{CH}_2\text{OPO}_3\text{AlI}_2$) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 156.33 ($-\text{CO}_2\text{AlI}$), 132.93 ($-\text{CH}=\text{CH}_2$), 132.33 (d, J = 6.8 Hz, $-\text{CH}=\text{CH}_2$), 118.46 ($-\text{CH}=\text{CH}_2$), 117.54 ($-\text{CH}=\text{CH}_2$), 68.29 (d, J = 5.5 Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 65.44 ($-\text{CH}_2\text{CH}=\text{CH}_2$), 65.17 (d, J = 5.7 Hz, $-\text{CH}_2\text{OPO}_3\text{AlI}_2$), 37.20 ($-\text{CH}_2\text{NHAlloc}$), 30.18 (d, J = 6.1 Hz, $-\text{CH}_2\text{CH}_2\text{OPO}_3\text{AlI}_2$) ppm; IR (ν_{max} , film): 3309, 3084, 2947, 2897, 1702, 1648, 1533, 1460, 1425, 1245, 1145, 1094, 1067 cm^{-1} ; HRMS m/z (ESI+): Found: 320.1267 (M+H), Calc.: 320.1263 ($\text{C}_{13}\text{H}_{23}\text{NO}_6\text{P}$).

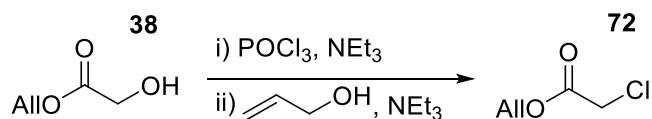


Run on **70** on a 3.8 mmol scale. Purified by flash column chromatography, eluting with 30-50 % EtOAc:Hexane. A yield of 430 mg, 1.1 mmol (27 %) was obtained as a colourless oil. ^1H NMR (400 MHz, CDCl_3): δ = 5.79-5.94 (3H, m, $-\text{CH}=\text{CH}_2$), 5.14-5.39 (6H, m, $-\text{CH}=\text{CH}_2$), 4.54-4.64 (3H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$ & $-\text{NH}$), 4.41-4.52 (4H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$), 4.32-4.40 (1H, m, H_α), 4.03-4.15 (2H, m, H_γ), 2.13-2.24 (1H, m, H_β), 1.98-2.09 (1H, m, H_β), 1.37 (9H, s, Boc) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ = 171.38 ($-\text{CO}_2\text{All}$), 155.30 ($-\text{CO}_2'\text{Bu}$), 132.37 (d, J = 6.9 Hz, $-\text{CH}=\text{CH}_2$), 131.51 ($-\text{CH}=\text{CH}_2$), 118.88 ($-\text{CH}=\text{CH}_2$), 118.35 ($-\text{CH}=\text{CH}_2$), 80.05 ($-\text{CMe}_3$), 68.22 (d, J = 5.5 Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 66.11 ($-\text{CH}_2\text{CH}=\text{CH}_2$), 63.88 (d, J = 5.5 Hz, C_γ), 50.68 (C_α), 32.64 (d, J = 7.3 Hz, C_β), 28.26 (Boc) ppm; IR (ν_{max} , film): 3287, 2978, 1737, 1710, 1649, 1510, 1456, 1366, 1250, 1158, 1102, 1003 cm^{-1} ; HRMS m/z (ESI+): Found: 442.1600 ($\text{M}+\text{H}$), Calc.: 442.1601 ($\text{C}_{18}\text{H}_{31}\text{NO}_8\text{P}$).

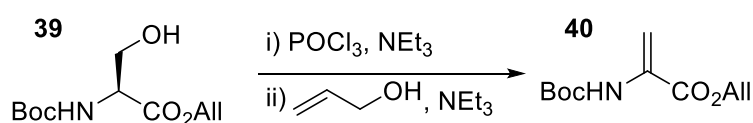
Unsuccessful phosphorylations



Run on **37** on a 3.8 mmol scale. The major product was purified by flash column chromatography, eluting with 10-20 % EtOAc:Hexane, and found to be the benzyl chloride product **71**. A yield of 705 mg, 3.33 mmol (87 %) was obtained as a colourless oil. Data were consistent with those previously reported.¹⁷ ^1H NMR (400 MHz, CDCl_3): δ = 7.97 (2H, d, J = 8.5 Hz, ArH_2), 7.38 (2H, d, J = 8.5 Hz, ArH_3), 5.95 (1H, ddt, J = 17.2, 10.4, 5.6 Hz, $-\text{CH}=\text{CH}_2$), 5.33 (1H, ddt, J_1 = 17.2 Hz, $J_2 = J_3$ = 1.5 Hz, $-\text{CH}=\text{CH}_2$), 5.21 (1H, ddt, J_1 = 10.4 Hz, $J_2 = J_3$ = 1.5 Hz, $-\text{CH}=\text{CH}_2$), 4.74 (2H, ddd, J_1 = 5.6 Hz, $J_2 = J_3$ = 1.5 Hz, $-\text{CH}_2\text{CH}=\text{CH}_2$), 4.53 (2H, s, $-\text{CH}_2\text{Cl}$) ppm.

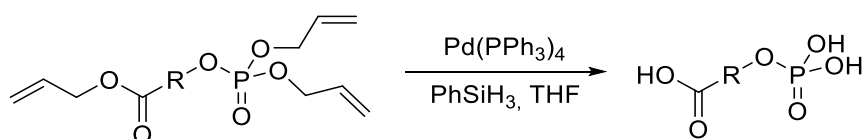


Run on **38** on a 1 mmol scale. NMR analysis of the crude mixture indicated formation of the alkyl chloride **72** as the major product. No further purification or characterisation was undertaken.



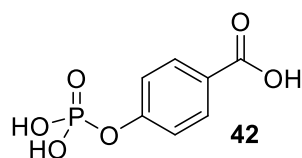
Run on **39** on a 1 mmol scale. The major product was purified by flash column chromatography, eluting with 20-40 % EtOAc:Hexane, and found to be the dehydroalanine derivative **40**, formed by dehydration. A yield of 193 mg, 0.86 mmol (86 %) was obtained as a colourless oil. Data were consistent with those previously reported.¹⁸ ¹H NMR (400 MHz, CDCl₃): δ = 6.95 (1H, br s, -NH), 6.10 (1H, br s, H _{β}), 5.87 (1H, ddt, J = 17.2, 10.4, 5.7 Hz, -CH=CH₂), 5.70 (1H, d, J = 1.4 Hz, H _{β}), 5.17-5.34 (2H, m, -CH=CH₂), 5.61 (2H, dd, J = 5.6, 1.5 Hz, -CH₂CH=CH₂), 1.41 (9H, s, Boc) ppm.

General deprotection procedure

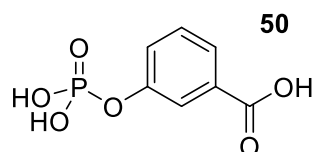


Allyl-protected phosphate (1 mmol) and phenylsilane (6 mmol) were dissolved in dry THF (10 mL) under nitrogen. Pd(PPh₃)₄ (0.05 mmol) was then added and the mixture was stirred for 2 hrs. During this time, the reaction often solidified. The mixture was then diluted with water (20 mL), and the aqueous washed with diethyl ether (2 x 20 mL) and lyophilised to provide the specified product. Phosphorous NMR is provided for representative compounds.

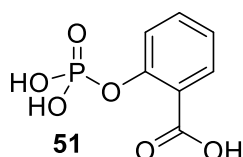
Deprotection - Tyrosine analogues



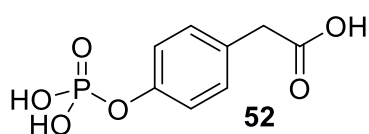
Run on **3** on a 1.5 mmol scale. A yield of 324 mg, 1.5 mmol (98 %) was obtained as a white solid. ¹H NMR (400 MHz, MeOD): δ = 7.96 (2H, d, J = 8.0 Hz, ArH₂), 7.31 (2H, d, J = 8.0 Hz, ArH₃) ppm; ¹³C NMR (100 MHz, MeOD): δ = 169.11 (-CO₂H), 156.65 (d, J = 6.3 Hz, ArC₄), 132.64 (ArC₂), 128.14 (ArC₁), 121.15 (d, J = 5.0 Hz, ArC₃) ppm; ³¹P (160 MHz, CDCl₃): δ = -4.15 ppm; m.p. = 187-191 ° C; IR (ν_{max} , solid): 2818 (v br), 2651, 2574, 2287 (br), 1683 (br), 1605, 1508, 1430, 1322, 1295, 1225, 1201, 1171, 1115, 1030 (br), 1016 cm⁻¹; HRMS m/z (ESI⁺): Found: 219.0053 (M+H), Calc.: 219.0053 (C₇H₈O₆P).



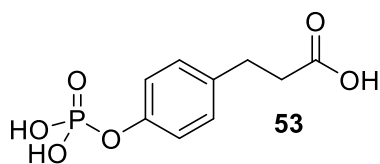
Run on **15** on a 0.34 mmol scale. A yield of 62 mg, 0.29 mmol (85 %) was obtained as a white solid. ¹H NMR (400 MHz, MeOD): δ = 7.93-7.90 (2H, m, ArH₂ and ArH₆), 7.43-7.51 (2H, m, ArH₄ and ArH₅) ppm; ¹³C NMR (100 MHz, MeOD): δ = 168.89 (-CO₂H), 152.93 (d, J = 6.5 Hz, ArH₃), 133.67 (ArC₁), 130.79 (ArC₅), 126.90 (d, J = 0.8 Hz, ArC₆), 126.05 (d, J = 4.7 Hz, ArC₄), 122.55 (d, J = 4.7 Hz, ArC₂) ppm; m.p. = 198-208 ° C; IR (ν_{max} , solid): 2658 (v br), 2554, 1682, 1585, 1486, 1451, 1416, 1295, 1271, 1215, 1156, 1105, 1046, 1012 cm⁻¹; HRMS m/z (ESI⁻): Found: 216.9897 (M-H), Calc.: 216.9902 (C₇H₆O₆P).



Run on **16** on a 0.35 mmol scale. A yield of 57 mg, 0.26 mmol (75 %) was obtained as a white solid. ^1H NMR (400 MHz, MeOD): δ = 7.75 (1H, dd, J = 7.8, 0.5 Hz, ArH6), 7.43 (1H, ddd, $J_1 = J_2 = 7.4$ Hz, $J_3 = 1.8$ Hz, ArH4), 7.31 (1H, ddd, $J_1 = 8.3$, $J_2 = J_3 = 1.1$ Hz, ArH3), 7.15 (1H, ddd, $J_1 = J_2 = 7.6$ Hz, $J_3 = 1.0$ Hz, ArH5) ppm; ^{13}C NMR (100 MHz, MeOD): δ = 169.09 ($-\text{CO}_2\text{H}$), 151.65 (d, J = 6.6 Hz, ArC2), 134.37 (d, J = 1.1 Hz, ArC4), 132.60 (ArC6), 125.62 (d, J = 0.9 Hz, ArC5), 125.53 (ArC6), 123.00 (d, J = 2.6 Hz, ArC3) ppm; m.p. = 183-186 ° C; IR (ν_{max} , solid): 2853 (v br), 2584, 2543, 2830 (br), 2171, 1687 (br), 1604, 1578, 1488, 1457, 1406, 1296, 1278, 1193 (br), 1159, 1096, 1087, 1020 cm^{-1} ; HRMS m/z (ESI+): Found: 233.0210 (M+MeOH), Calc.: 233.0210 ($\text{C}_8\text{H}_{11}\text{O}_7\text{P}$).

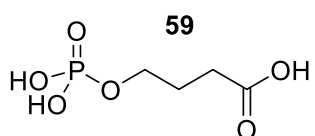


Run on **17** on a 4.2 mmol scale. A yield of 904 mg, 3.9 mmol (93 %) was obtained as a white solid. ^1H NMR (400 MHz, MeOD): δ = 7.25 (2H, d, J = 6.8 Hz, ArH3), 7.15 (2H, d, J = 6.8 Hz, ArH2), 3.62 (2H, s, $-\text{CH}_2\text{CO}_2\text{H}$) ppm; ^{13}C NMR (100 MHz, MeOD): δ = 173.91 ($-\text{CO}_2\text{H}$), 151.90 (d, J = 6.6 Hz, ArC4), 132.06 (ArC1), 131.57 (ArC2), 121.40 (d, J = 4.5 Hz, ArC3), 40.89 ($-\text{CH}_2\text{CO}_2\text{H}$) ppm; m.p. = 165-169 ° C; IR (ν_{max} , solid): 2955 (v br), 2919, 2737, 2655, 1694, 1609, 1509, 1408, 1343, 1291, 1214, 1196, 1133 cm^{-1} ; HRMS m/z (ESI-): Found: 231.0070 (M-H), Calc.: 231.0059 ($\text{C}_8\text{H}_8\text{O}_6\text{P}$).



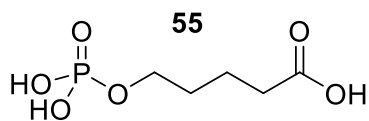
Run on **18** on a 1 mmol scale. A yield of 236 mg, 0.96 mmol (96 %) was obtained as a white solid. ^1H NMR (400 MHz, MeOD): δ = 7.23 (2H, d, J = 8.3 Hz, ArH3), 7.14 (2H, dd, J = 8.3, 1.2 Hz, ArH2), 2.90 (2H, t, J = 7.6 Hz, $-\text{CH}_2\text{Ar}$), 2.60 (2H, t, J = 7.6 Hz, $-\text{CH}_2\text{CO}_2\text{H}$) ppm; ^{13}C NMR (100 MHz, MeOD): δ = 176.58 ($-\text{CO}_2\text{H}$), 151.18 (d, J = 6.7 Hz, ArC4), 138.63 (ArC1), 130.49 (ArC2), 121.35 (d, J = 4.6 Hz, ArC3), 36.73 ($-\text{CH}_2\text{CO}_2\text{H}$), 31.25 ($-\text{CH}_2\text{Ar}$) ppm; ^{31}P (160 MHz, CDCl_3): δ = -3.38 ppm; m.p. = 148-152 ° C; IR (ν_{max} , solid): 2866 (v br), 2620, 2291, 1694, 1608, 1509, 1432, 1411, 1324, 1308, 1270, 1209, 1179, 1134, 1108 cm^{-1} ; HRMS m/z (ESI-): Found: 245.0223 (M-H), Calc.: 245.0215 ($\text{C}_9\text{H}_{10}\text{O}_6\text{P}$).

Deprotection - Serine analogues

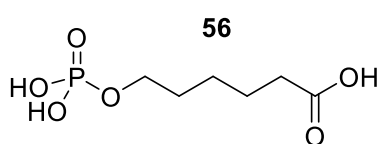


Run on **29** on a 2 mmol scale. A yield of 225 mg, 1.22 mmol (61 %) was obtained as a highly hydroscopic white solid. ^1H NMR (400 MHz, MeOD): δ = 4.03 (2H, dt, $J_1 = J_2 = 6.8$ Hz, $-\text{CH}_2\text{OPO}_3\text{H}_2$), 2.45 (2H, t, J = 7.4 Hz, $-\text{CH}_2\text{CO}_2\text{H}$), 1.92-2.00 (4H, m, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{OPO}_3\text{H}_2$) ppm; ^{13}C (100 MHz, MeOD): δ = 176.78 ($-\text{CO}_2\text{H}$), 66.80 (d, J = 5.6 Hz, $-\text{CH}_2\text{OPO}_3\text{H}_2$), 30.88 ($-\text{CH}_2\text{CO}_2\text{H}$), 26.90 (d, J = 7.5 Hz, $-\text{CH}_2\text{CH}_2\text{OPO}_3\text{H}_2$) ppm; m.p. = 98-104 ° C; IR (ν_{max} , solid): 2971, 2908 (v br), 2342

(br), 1690, 1508, 1483, 1430, 1421, 1401, 1209, 1131, 1099, 1077 cm^{-1} ; HRMS m/z (ESI⁻): Found: 185.0214 (M-H), Calc.: 185.0215 ($\text{C}_4\text{H}_8\text{O}_6\text{P}$).

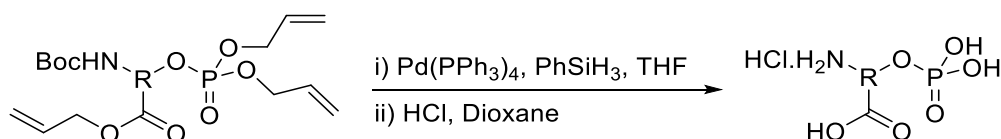


Run on **32** on a 1.1 mmol scale. A yield of 204 mg, 1.1 mmol (98 %) was obtained as a hygroscopic white solid. ^1H NMR (400 MHz, MeOD): δ = 3.97-4.03 (2H, m, $-\text{CH}_2\text{OPO}_3\text{H}_2$), 2.34-2.39 ($-\text{CH}_2\text{CO}_2\text{H}$), 1.69-1.77 (4H, m, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{OPO}_3\text{H}_2$) ppm; ^{13}C NMR (100 MHz, MeOD): δ = 177.29 ($-\text{CO}_2\text{H}$), 67.32 (d, J = 5.9 Hz, $-\text{CH}_2\text{OPO}_3\text{H}_2$), 34.33 ($-\text{CH}_2\text{CO}_2\text{H}$), 30.83 (d, J = 7.3 Hz, $-\text{CH}_2\text{CH}_2\text{OPO}_3\text{H}_2$), 22.69 ($-\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$) ppm; m.p. = 121-124 ° C; IR (ν_{max} , solid): 2945, 2909, 2870, 2695 (v br), 2287 (v br), 1692 (br), 1479, 1463, 1439, 1430, 1415, 1278, 1130, 1098, 996 (br) cm^{-1} ; HRMS m/z (ESI⁺): Found: 199.0366 (M+H), Calc.: 199.0372 ($\text{C}_5\text{H}_{12}\text{O}_6\text{P}$).

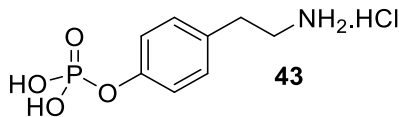


Run on **33** on a 0.37 mmol scale. A yield of 74 mg, 0.34 mmol (94 %) was obtained as a hygroscopic white solid. ^1H NMR (400 MHz, MeOD): δ = 3.87 (2H, dt, $J_1 = J_2 = 6.6$ Hz, $-\text{CH}_2\text{OPO}_3\text{H}_2$), 2.21 (2H, t, J = 7.4 Hz, $-\text{CH}_2\text{CO}_2\text{H}$), 1.49-1.64 (4H, m, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ and $-\text{CH}_2\text{CH}_2\text{OPO}_3\text{H}_2$), 1.30-1.40 (2H, m, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{OPO}_3\text{H}_2$) ppm; ^{13}C NMR (100 MHz, MeOD): δ = 177.54 ($-\text{CO}_2\text{H}$), 67.50 (d, J = 5.7 Hz, $-\text{CH}_2\text{OPO}_3\text{H}_2$), 34.83 ($-\text{CH}_2\text{CO}_2\text{H}$), 31.19 (d, J = 7.3 Hz, $-\text{CH}_2\text{CH}_2\text{OPO}_3\text{H}_2$), 26.26 ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{OPO}_3\text{H}_2$), 25.66 ($-\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$) ppm; m.p. = 83-87 ° C; IR (ν_{max} , solid): 2945, 2876, 2652 (v br), 2279 (v br), 1698 (v br), 1476, 1464, 1431, 1411, 1317, 1258, 1242, 1209, 1160, 1133, 1103, 1045, 1009 (br) cm^{-1} ; HRMS m/z (ESI⁺): Found: 213.0537 (M+H), Calc.: 213.0528 ($\text{C}_6\text{H}_{14}\text{O}_6\text{P}$).

Allyl-Boc sequential deprotection

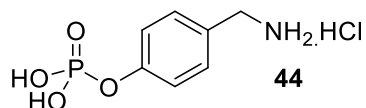


Allyl-protected phosphate (1 mmol) and phenylsilane (6 mmol) were dissolved in dry THF (10 mL) under nitrogen. $\text{Pd}(\text{PPh}_3)_4$ (0.05 mmol) was then added and the mixture was stirred for 3 hrs. The mixture typically stayed in solution during this time. The THF was then removed *in vacuo* and hydrochloric acid (4 M in dioxane, 15 mmol) was added. After stirring for 1 hr, the dioxane was removed *in vacuo*. The residue was diluted with water (20 mL), and the aqueous washed with diethyl ether (2 x 20 mL) and lyophilised to provide the specified product. Products were typically poorly soluble in a range of solvents, precluding analysis by ^{13}C NMR.



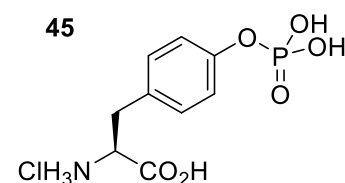
Run on **20** on a 2.77 mmol scale. A yield of 498 mg, 1.96 mmol (71 %) was obtained as an off white solid. ^1H NMR (400 MHz, D_2O): δ = 7.16 (2H, d, J = 8.4 Hz, ArH_2), 7.06 (2H, dd,

$J = 8.4$, ArH₃), 3.13 (2H, t, $J = 7.1$ Hz, -CH₂NH₃Cl), 2.84 (2H, t, $J = 7.1$ Hz, -CH₂Ar) ppm; ³¹P (160 MHz, CDCl₃): $\delta = -3.38$ ppm; m.p. = 240-245 ° C; IR ($\nu_{\max}$, solid): 2985, 2946, 2879, 2755, 1720, 1648, 1604, 1506, 1462, 1425, 1363, 1268, 1219, 1162, 1095, 1015 cm⁻¹; HRMS m/z (ESI+): Found: 218.0589 (M+H), Calc.: 218.0582 (C₈H₁₃NO₆P).



44

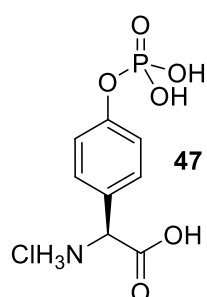
Run on **22** on a 1.56 mmol scale. A yield of 300 mg, 1.25 mmol (80 %) was obtained as an off white solid. ¹H NMR (400 MHz, D₂O): $\delta = 7.36$ (2H, d, $J = 8.6$ Hz, ArH₂), 7.17 (2H, dd, $J = 8.6$, 1.1 Hz, ArH₃), 4.08 (2H, s, -CH₂NH₃Cl) ppm; m.p. > 300 ° C; IR ($\nu_{\max}$, solid): 2990, 2877, 2754 (v br), 2302, 1715, 1608, 1507, 1380, 1217, 1154, 1072, 1017 cm⁻¹; HRMS m/z (ESI+): Found: 226.0232 (M+Na), Calc.: 226.0240 (C₇H₁₀NaNO₆P).



45

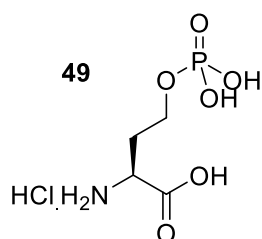
Run on **25** on a 1 mmol scale. A yield of 261 mg, 0.88 mmol (88 %) was obtained as an off white solid. Data were consistent with those previously reported.¹⁹ ¹H NMR (400 MHz, MeOD): $\delta = 7.04$ -7.22 (4H, m, ArH), 4.30-4.41 (1H, dd, $J = 8.0$, 5.2 Hz, H_α), 3.02-3.29 (1H, m, H_β), 3.13 (14.6, 8.0 Hz) ppm.

The use of D-enantiomer **26** led to the production of **46**.



47

Run on **27** on a 0.5 mmol scale. A yield of 132 mg, 0.46 mmol (93 %) was obtained as an off white solid. ¹H NMR (400 MHz, MeOD): $\delta = 7.47$ (2H, d, $J = 8.7$ Hz, ArH₂), 7.34 (2H, dt, $J = 8.7$, 1.1 Hz, ArH₃), 5.09 (1H, s, H_α) ppm; m.p. = 208-214 ° C; IR ($\nu_{\max}$, solid): 3338, 2970, 2884, 1734, 1638, 1467, 1379, 1369, 1341, 1305, 1160, 1128, 1107 cm⁻¹; HRMS m/z (ESI-): Found: 246.0171 (M-H), Calc.: 246.0173 (C₈H₉NO₆P).



49

Run on **36** on a 0.71 mmol scale. A yield of 95 mg, 0.42 mmol (57 %) was obtained as an hygroscopic white solid. ¹H NMR (400 MHz, D₂O): $\delta = 4.10$ -4.27 (3H, m, H_α & H_γ), 2.34-2.45 (1H, m, H_β), 2.14-2.26 (1H, m, H_β) ppm; IR ($\nu_{\max}$, solid): 2870 (v br), 1726, 1606, 1507, 1438, 1204, 1155, 1106, 1051 cm⁻¹; HRMS m/z (ESI-): Found: 198.0172 (M-H), Calc.: 198.0173 (C₄H₉NO₆P); m.p. not determined due to hygroscopic nature of product.

Mechanical testing

Powdered α-TCP (50 mg), prepared as described previously,^{19,20} was mixed with a solution of 25 % ethanol: 75 % ultrapure water containing the specified amino acid analogue to give a liquid to powder ratio (L/P) of 0.25 mL g⁻¹. Pre-dissolution of the amino acid was undertaken to avoid confounding effects related to solvation of the powder phase (e.g. dissolution rate, particle size, surface area). Amino acids were applied at a 1.13:1 molar ratio to α-TCP. After

mixing for approximately 10 sec, the mixture was applied to a 1 cm³ cube surface of aluminium or bone with a spatula and the opposing 1 cm³ steel cube adjoined by hand. Universal grips (Cocraft spring clamps) were applied to hold the cubes together at 37 °C, in a 100 % humidity sealed container for 24 hrs. Shear testing was then performed on the construct at a rate of 1 mm min⁻¹ with a 500 N load cell (Shimadzu) on an AGS-X Mechanical Testing System (Shimadzu). The peak force and shear modulus were recorded with Trapezium-Lite software (version 1.0.1) and the bond thickness and failure mode of each sample was recorded.

Statistical analyses

Aluminium adhesion tests were run with N = 4. For bone samples, where surface roughness is more varied N = 7/8 was used. Differences in adhesive strength between conditions were evaluated using a one-way ANOVA with Tukey's multiple comparison post-hoc test. Results with *p*-values lower than 0.05 were deemed significant.

Full statistical analysis of significance is provided below:

Fig. 1a

Tukey's multiple comparisons test	Summary	Adjusted P Value	
Blank vs. L-PSer, 1	***	<0.0001	A-B
Blank vs. D/L-PSer, 1	***	<0.0001	A-C
Blank vs. Ser	ns	0.9988	A-D
Blank vs. PTyr, 45	***	<0.0001	A-E
Blank vs. Tyr	ns	>0.9999	A-F
Blank vs. PThr, 57	***	<0.0001	A-G
L-PSer, 1 vs. D/L-PSer, 1	ns	0.8264	B-C
L-PSer, 1 vs. Ser	***	<0.0001	B-D
L-PSer, 1 vs. PTyr, 45	*	0.0231	B-E
L-PSer, 1 vs. Tyr	***	<0.0001	B-F
L-PSer, 1 vs. PThr, 57	ns	0.0563	B-G
D/L-PSer, 1 vs. Ser	***	<0.0001	C-D
D/L-PSer, 1 vs. PTyr, 45	**	0.0011	C-E
D/L-PSer, 1 vs. Tyr	***	<0.0001	C-F
D/L-PSer, 1 vs. PThr, 57	**	0.0029	C-G
Ser vs. PTyr, 45	***	<0.0001	D-E
Ser vs. Tyr	ns	>0.9999	D-F
Ser vs. PThr, 57	***	<0.0001	D-G
PTyr, 45 vs. Tyr	***	<0.0001	E-F
PTyr, 45 vs. PThr, 57	ns	0.9995	E-G
Tyr vs. PThr, 57	***	<0.0001	F-G

Fig. 1b

Tukey's multiple comparisons test	Summary	Adjusted P Value	
PTyr, 45 vs. Tyr	***	<0.0001	A-B
PTyr, 45 vs. 43	***	<0.0001	A-C
PTyr, 45 vs. 53	ns	0.9922	A-D

PTyr, 45 vs. PSer, 1	**	0.0012	A-E
PTyr, 45 vs. Ser	***	<0.0001	A-F
PTyr, 45 vs. 48	***	<0.0001	A-G
PTyr, 45 vs. 54	***	<0.0001	A-H
Tyr vs. 43	ns	0.9576	B-C
Tyr vs. 53	***	<0.0001	B-D
Tyr vs. PSer, 1	***	<0.0001	B-E
Tyr vs. Ser	ns	>0.9999	B-F
Tyr vs. 48	ns	0.099	B-G
Tyr vs. 54	***	<0.0001	B-H
43 vs. 53	***	<0.0001	C-D
43 vs. PSer, 1	***	<0.0001	C-E
43 vs. Ser	ns	0.9968	C-F
43 vs. 48	ns	0.5537	C-G
43 vs. 54	***	<0.0001	C-H
53 vs. PSer, 1	***	0.0002	D-E
53 vs. Ser	***	<0.0001	D-F
53 vs. 48	***	<0.0001	D-G
53 vs. 54	***	<0.0001	D-H
PSer, 1 vs. Ser	***	<0.0001	E-F
PSer, 1 vs. 48	***	<0.0001	E-G
PSer, 1 vs. 54	**	0.0075	E-H
Ser vs. 48	ns	0.2053	F-G
Ser vs. 54	***	<0.0001	F-H
48 vs. 54	***	<0.0001	G-H

Fig. 2a

Tukey's multiple comparisons test	Summary	Adjusted P Value	
PSer, 1 vs. 54	ns	0.0977	A-B
PSer, 1 vs. 55	ns	0.9596	A-C
PSer, 1 vs. 56	**	0.0012	A-D
PSer, 1 vs. Ser	***	<0.0001	A-E
54 vs. 55	*	0.0282	B-C
54 vs. 56	***	<0.0001	B-D
54 vs. Ser	***	<0.0001	B-E
55 vs. 56	**	0.0042	C-D
55 vs. Ser	***	<0.0001	C-E
56 vs. Ser	**	0.0045	D-E

Fig. 2b

Tukey's multiple comparisons test	Summary	Adjusted P Value	
PTyr, 45 vs. 42	**	0.0042	A-B
PTyr, 45 vs. 50	ns	0.8536	A-C
PTyr, 45 vs. 51	ns	0.7621	A-D
PTyr, 45 vs. 52	ns	0.9892	A-E
PTyr, 45 vs. 53	ns	0.9987	A-F
PTyr, 45 vs. 47	ns	0.9707	A-G
42 vs. 50	***	0.0002	B-C
42 vs. 51	ns	0.0985	B-D
42 vs. 52	***	0.0008	B-E
42 vs. 53	*	0.0127	B-F

42 vs. 47	*	0.0302	B-G
50 vs. 51	ns	0.1359	C-D
50 vs. 52	ns	0.9975	C-E
50 vs. 53	ns	0.579	C-F
50 vs. 47	ns	0.3535	C-G
51 vs. 52	ns	0.341	D-E
51 vs. 53	ns	0.955	D-F
51 vs. 47	ns	0.9969	D-G
52 vs. 53	ns	0.8785	E-F
52 vs. 47	ns	0.6755	E-G
53 vs. 47	ns	0.9996	F-G

Fig. S1

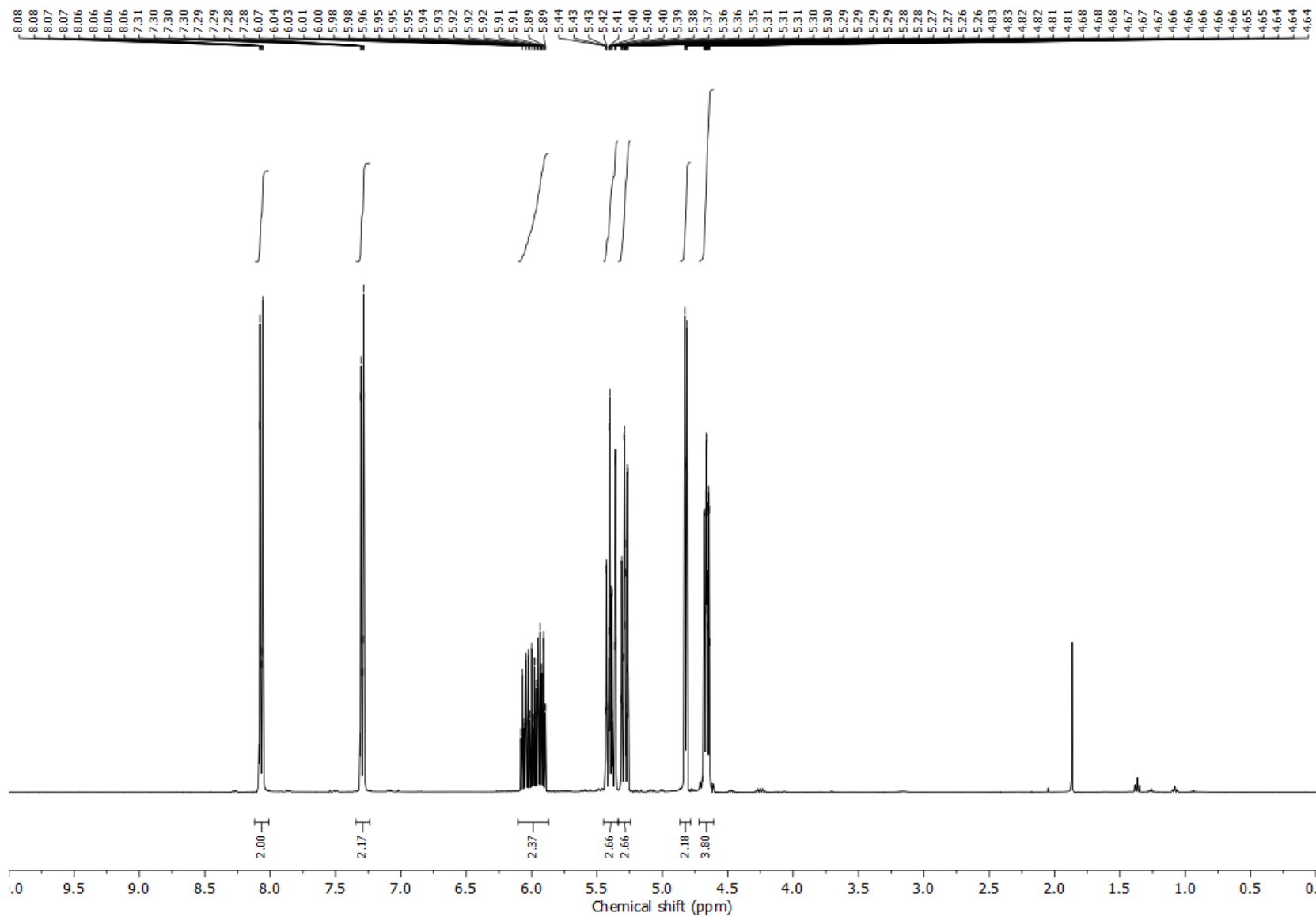
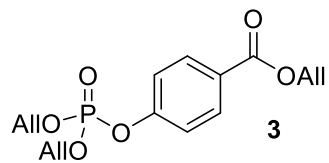
Tukey's multiple comparisons test	Summary	Adjusted P Value	
PTyr, 45 vs. 50	ns	0.3245	A-B
PTyr, 45 vs. 51	*	0.0251	A-C
PTyr, 45 vs. 42	***	0.0004	A-D
50 vs. 51	ns	0.4124	B-C
50 vs. 42	*	0.0227	B-D
51 vs. 42	ns	0.6566	C-D

References

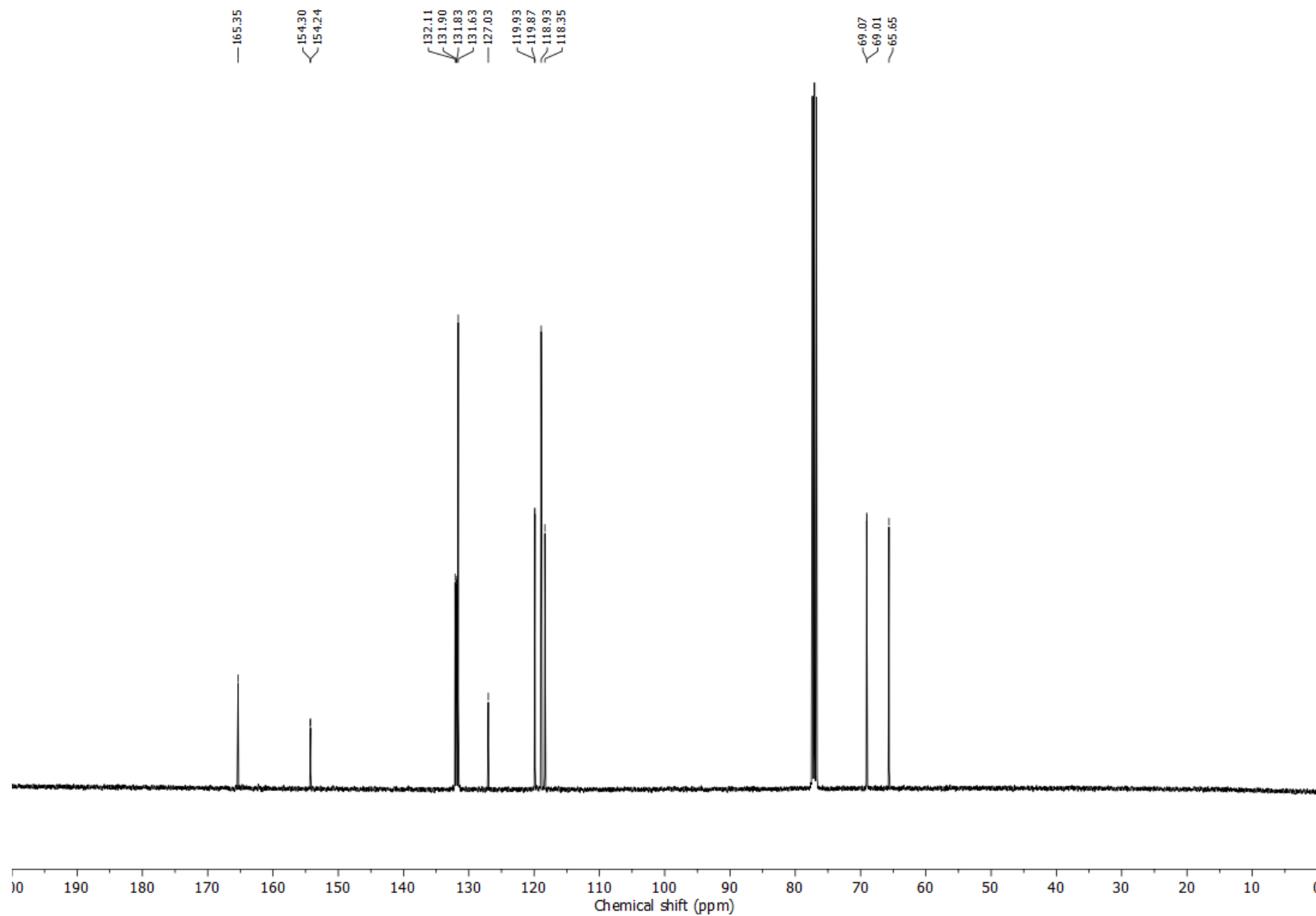
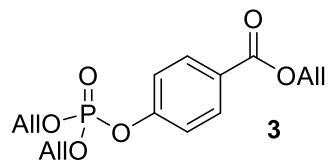
1. D. P. Brown, H. Q. Duong; *J. Heterocyclic Chem.*, **2008**, *45*, 435-443.
2. H. Garrido-Hernandez, K. D. Moon, R. L. Geahlen, R. F. Borch; *J. Med. Chem.*, **2006**, *49*, 3368-3376.
3. Y. Iso, H. Shindo, H. Hamana; *Tetrahedron*, **2000**, *56*, 5353-5361.
4. G. P. Wild, C. Wiles, P. Watts, S. J. Haswell; *Tetrahedron*, **2009**, *65*, 1618-1629.
5. T. Prakasam, M. Lusi, M. Elhabiri, C. Platas-Iglesias, J.-C. Olsen, Z. Asfari, S. Cianférani-Sanglier, F. Debaene, L. J. Charbonnière, A. Trabolsi; *Angew. Chem. Int. Ed.*, **2013**, *52*, 9956-9960.
6. G. Panigrahi, B. Zhao, J. J. Krepinsky, P. D. Sadowski; *J. Am. Chem. Soc.*, **1996**, *118*, 12004-12011.
7. A. P. Tamiz, S. X. Cai, Z.-L. Zhou, P.-W. Yuen, R. M. Schelkun, E. R. Whitemore, E. Weber, R. M. Woodward, J. F. W. Keana; *J. Med. Chem.*, **1999**, *42*, 3412-3420.
8. Z. You, A. H. Hoveyda, M. L. Snapper; *Angew. Chem. Int. Ed.*, **2009**, *48*, 347-550.
9. I. R. Baxendale, S. V. Ley, M. Nessi, C. Piutti; *Tetrahedron*, **2002**, *58*, 6285-6304.

10. J. Alsina, F. Rabanal, C. Chiva, E. Giralt, F. Albericio; *Tetrahedron*, **1998**, 54, 10125-10152.
11. K. Takagi, H. Fukuda, S. Shuto, A. Otaka, M. Arisawa; *Adv. Synth. Catal.*, **2015**, 357, 2119-2124.
12. G. Gellerman, S. Shitrit, T. Shalit, O. Ganot, A. Albeck; *Tetrahedron*, **2010**, 66, 878-886.
13. J. K. Kasper, J. E. Hitro, S. R. Fitzgerald, J. M. Schnitter, J. J. Rutkowski, J. A. Heck, J. L. Steinbacher; *J. Org. Chem.*, **2016**, 81, 8095-8103.
14. I. R. Marsh, M. Bradley; *Tetrahedron*, **1997**, 53, 17317-17334.
15. Celltech R & D Inc., *Patent: US2003/69305*, **2003**.
16. M. Carrasco, R. T. Brown; *J. Org. Chem.*, **2003**, 68, 8853-8858.
17. Nippon Terpene Chemicals Inc., *Patent: JP6222973*, **2017**.
18. S. Bregant, A. B. Tabor; *J. Org. Chem.*, **2005**, 70, 2430-2438.
19. C. M. Dreef-Tromp, A. W. M. Lefeber, G. A. van der Marel, J. H. van Boom; *Synthesis*, **1992**, 12, 1269-1272.
20. M. Pujari-Palmer, H. Guo, D. Wenner, H. Autefage, C. D. Spicer, M. M. Stevens, O. Omar, P. Thomsen, M. Edén, G. Insley, P. Procter, H. Engqvist; *Materials*, **2018**, 11, 2492.
21. P. Proctor, M. Pujari-Palmer, G. Hulsart-Billström, D. Wenner, G. Insley, S. Larsson, H. Engqvist; *BMC Biomed. Eng.*, **2019**, 1, 11.

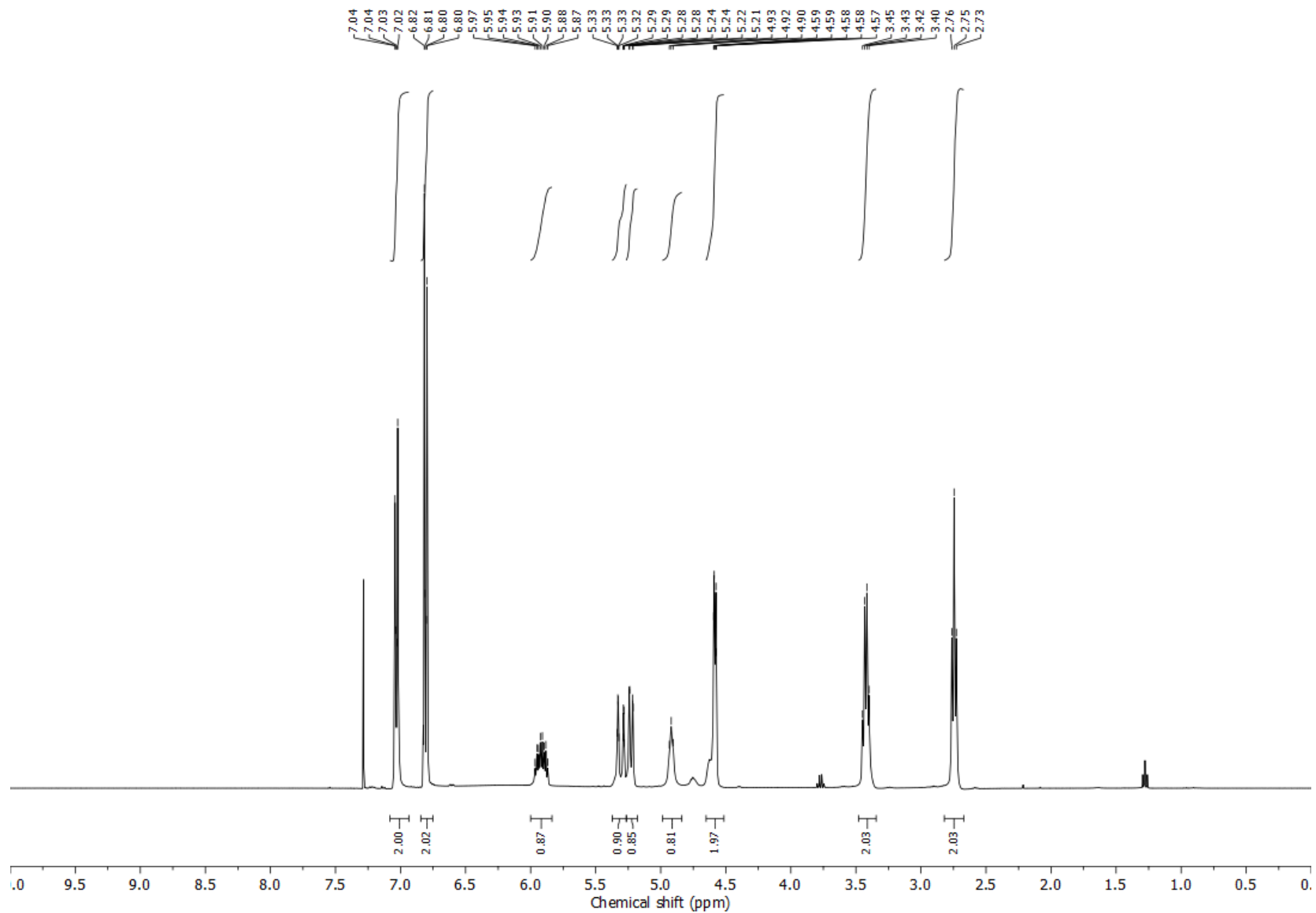
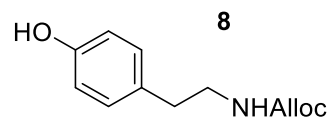
^1H NMR (400 MHz, CDCl_3)



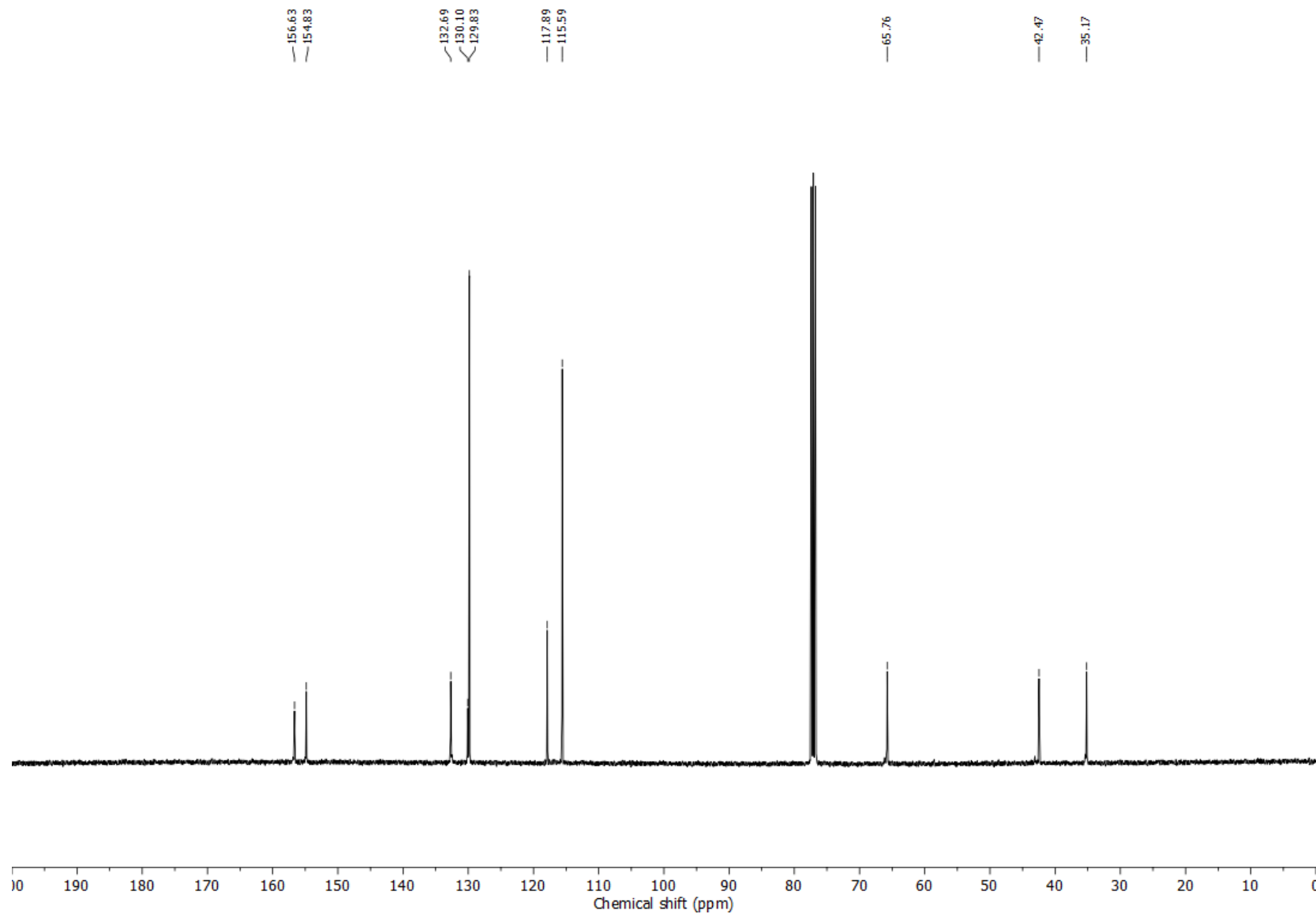
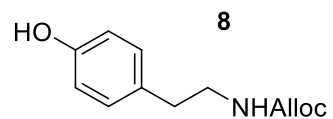
^{13}C NMR (100 MHz, CDCl_3)



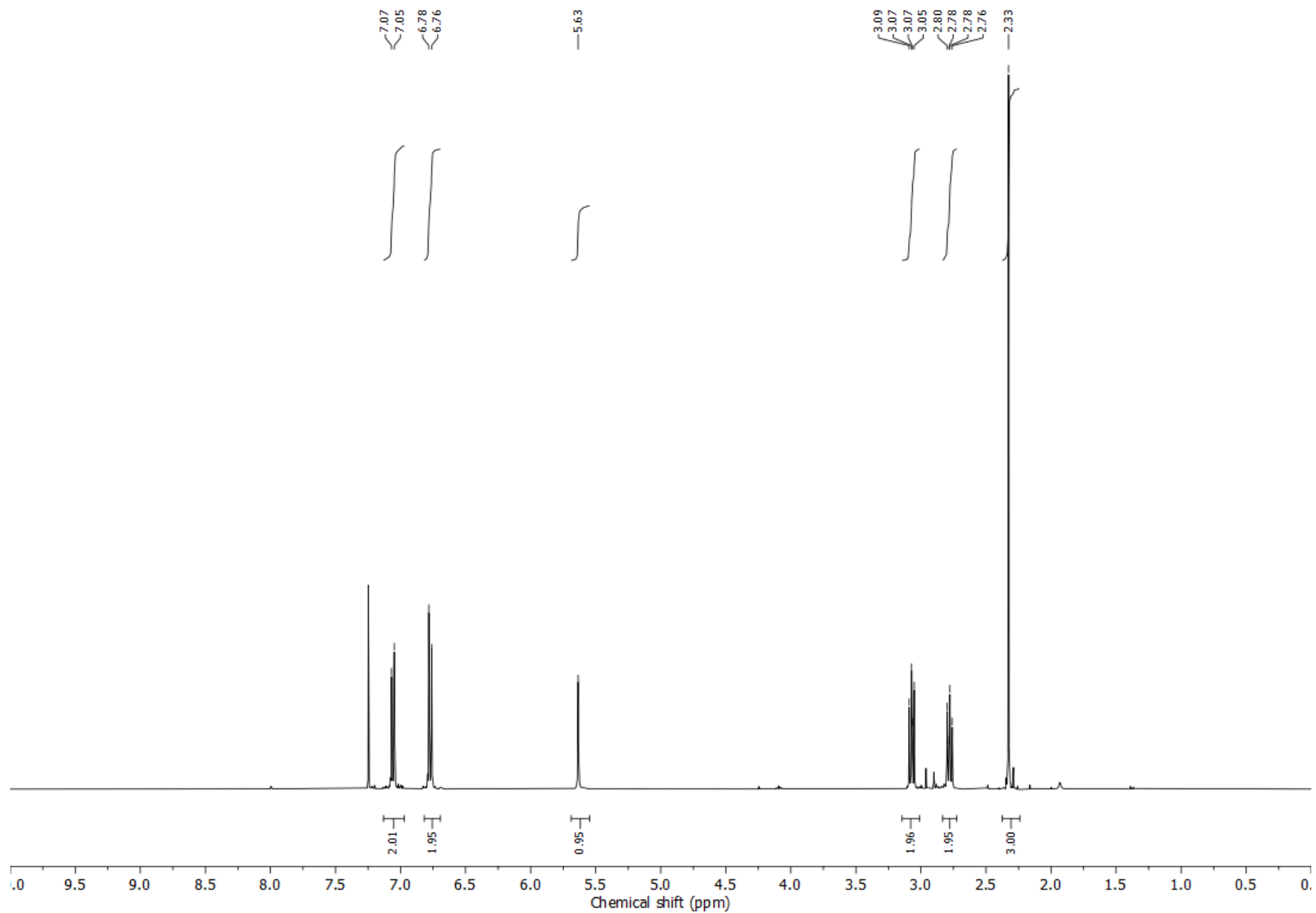
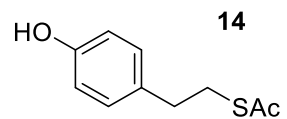
^1H NMR (400 MHz, CDCl_3)



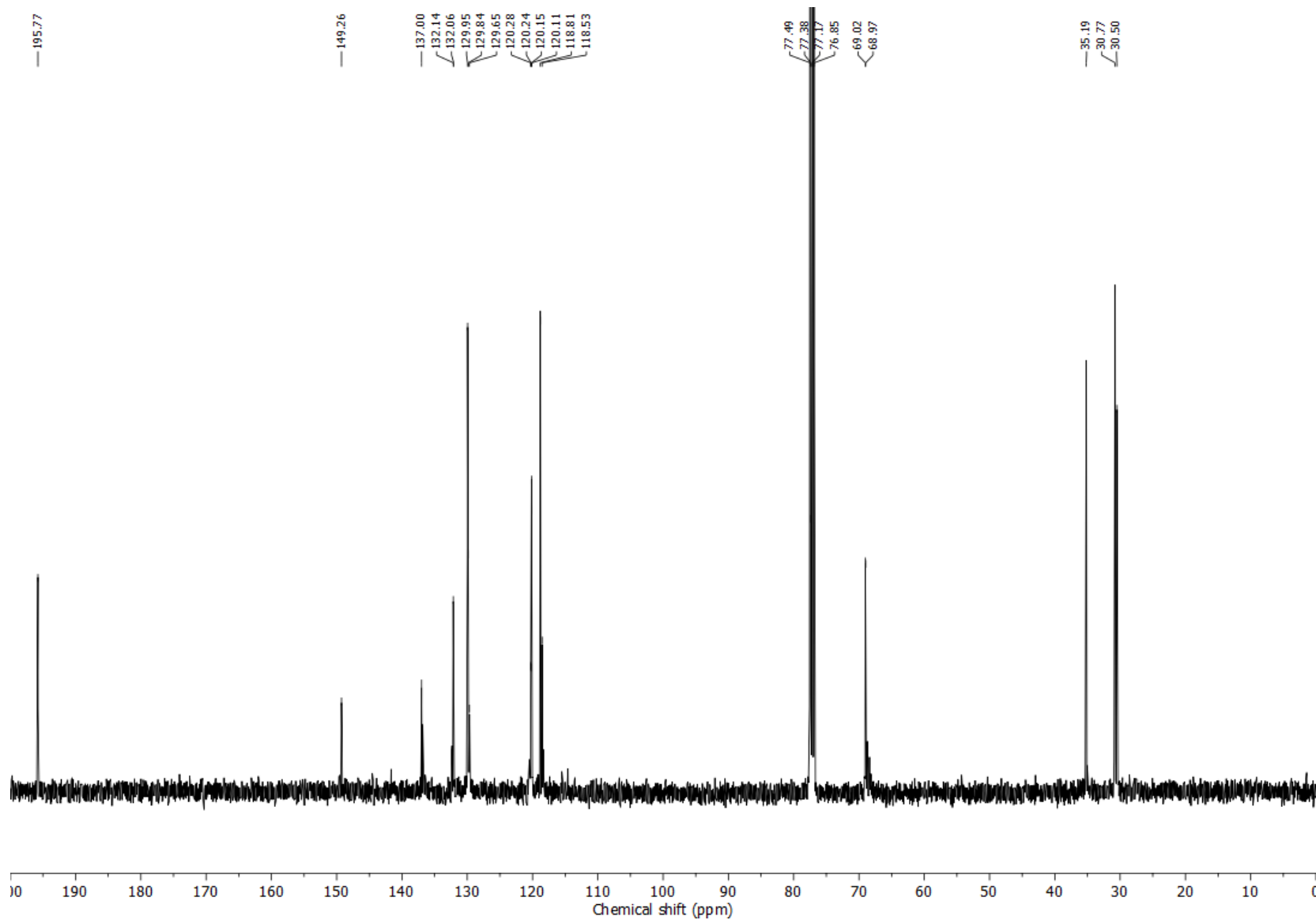
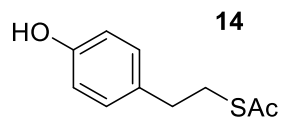
^{13}C NMR (100 MHz, CDCl_3)



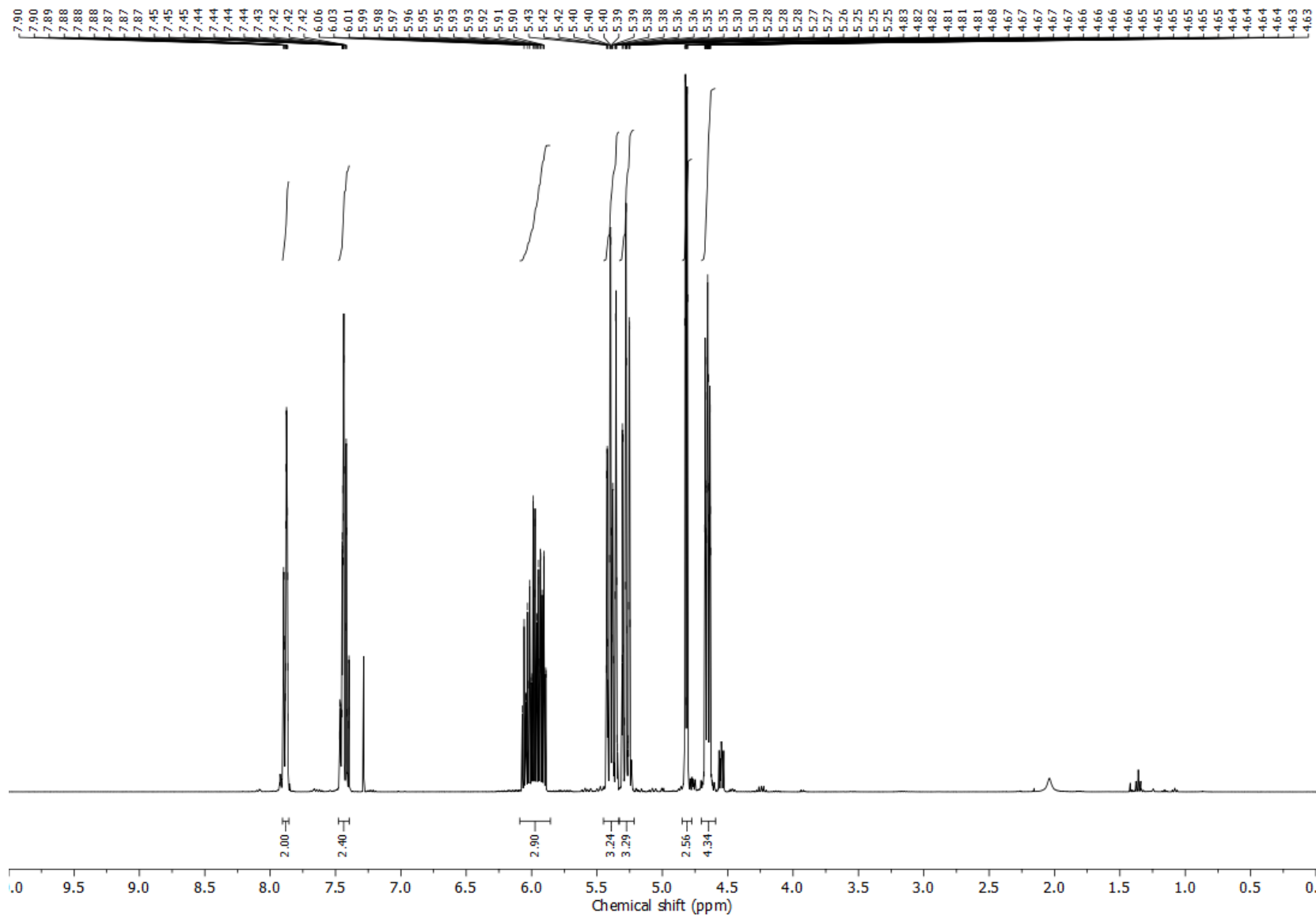
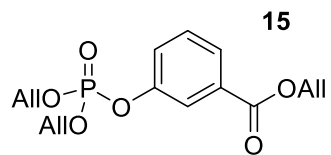
^1H NMR (400 MHz, CDCl_3)



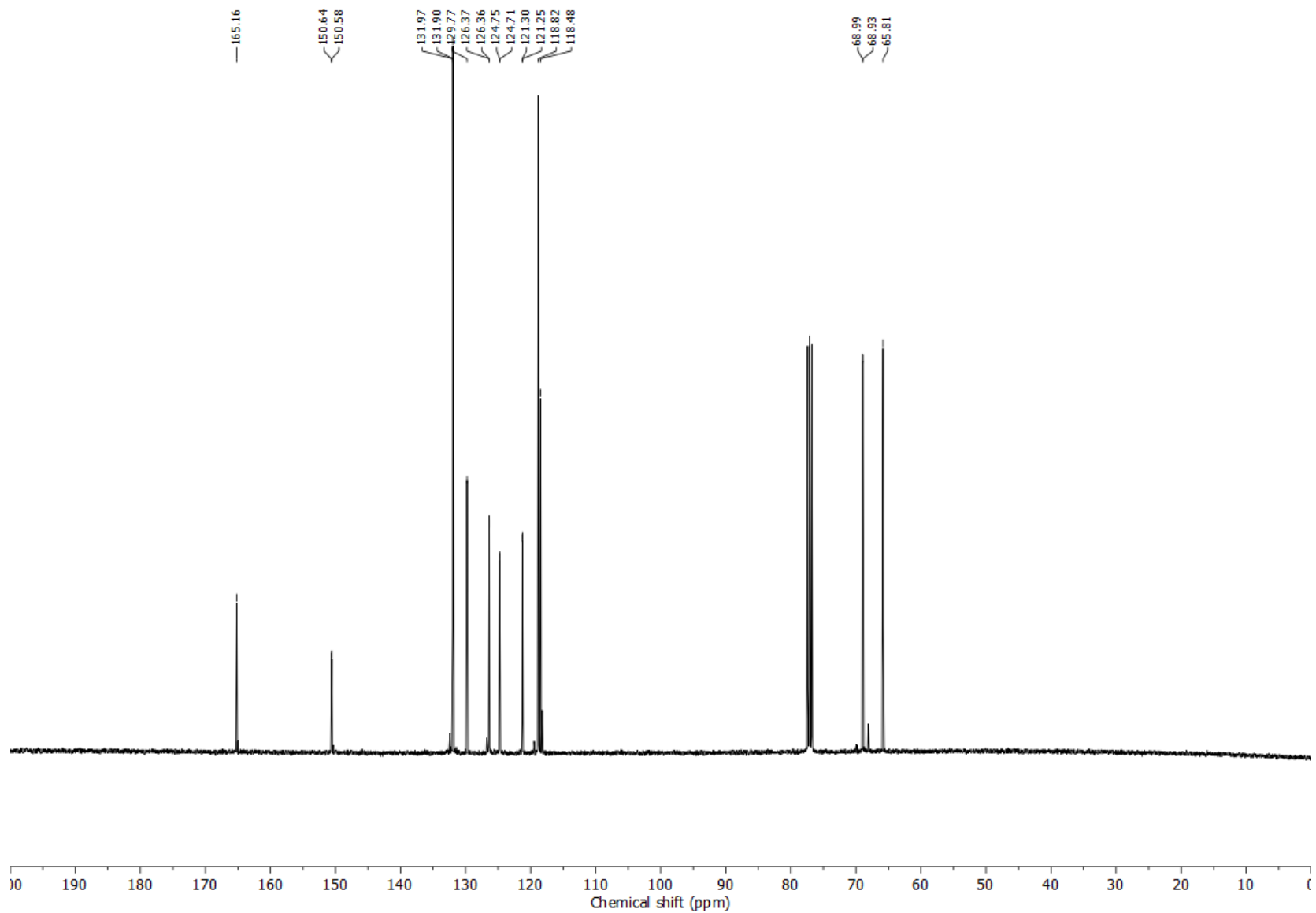
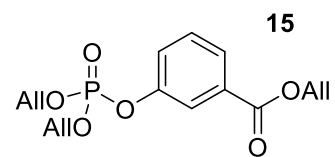
^{13}C NMR (100 MHz, CDCl_3)



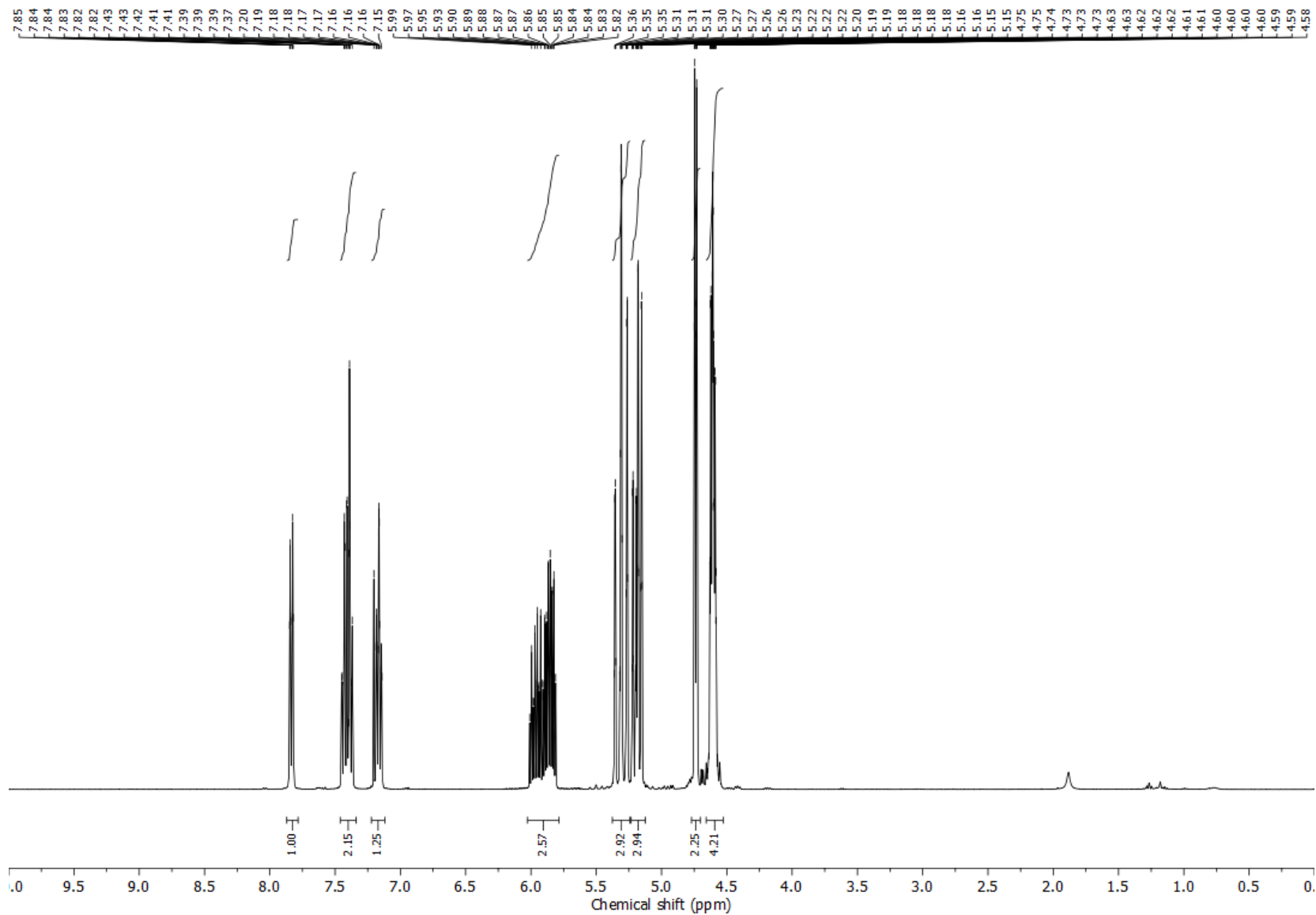
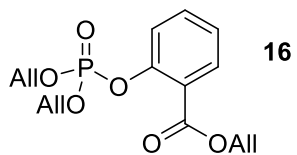
^1H NMR (400 MHz, CDCl_3)



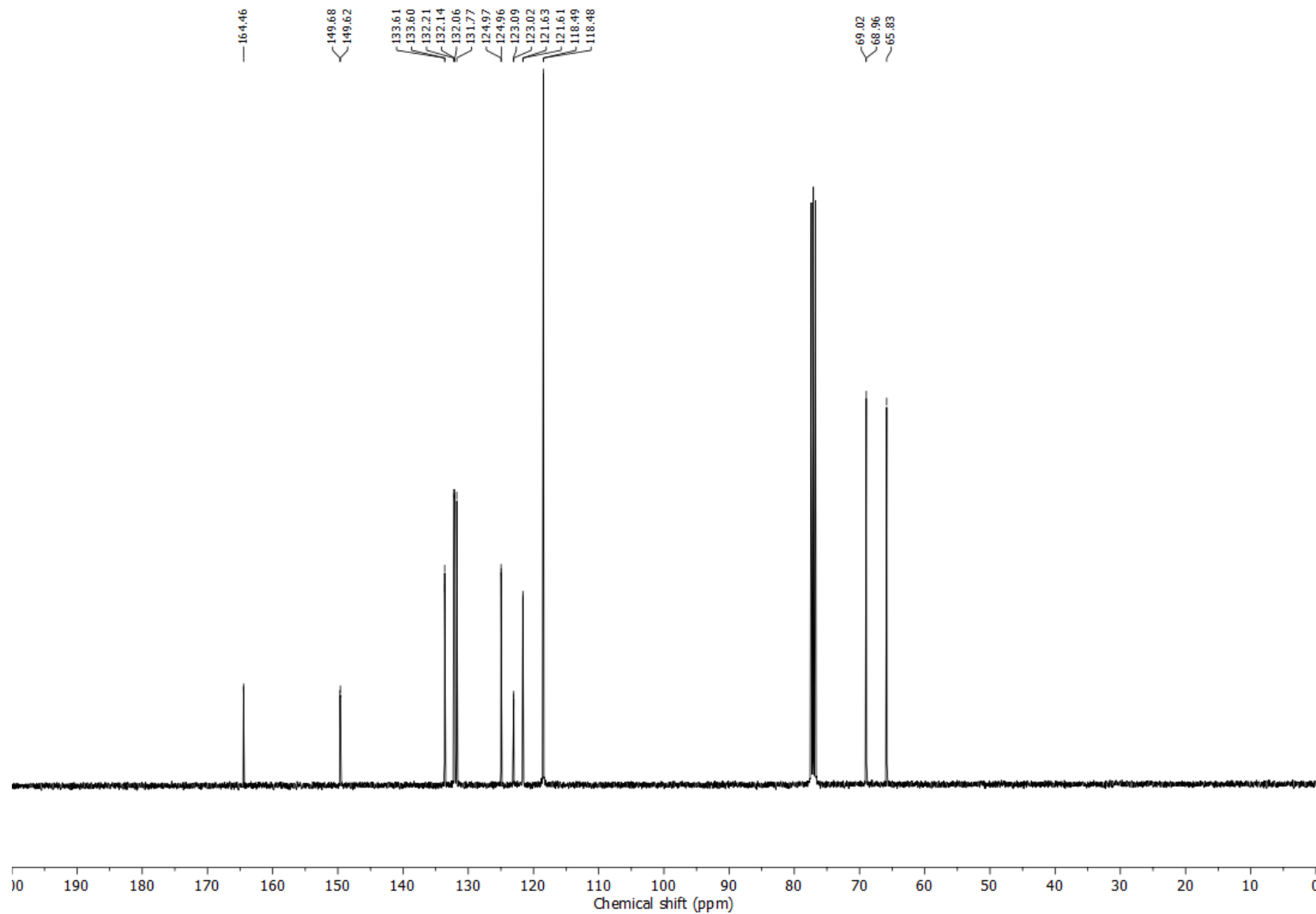
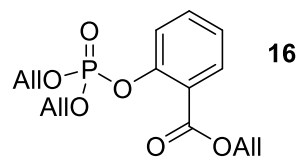
^{13}C NMR (100 MHz, CDCl_3)



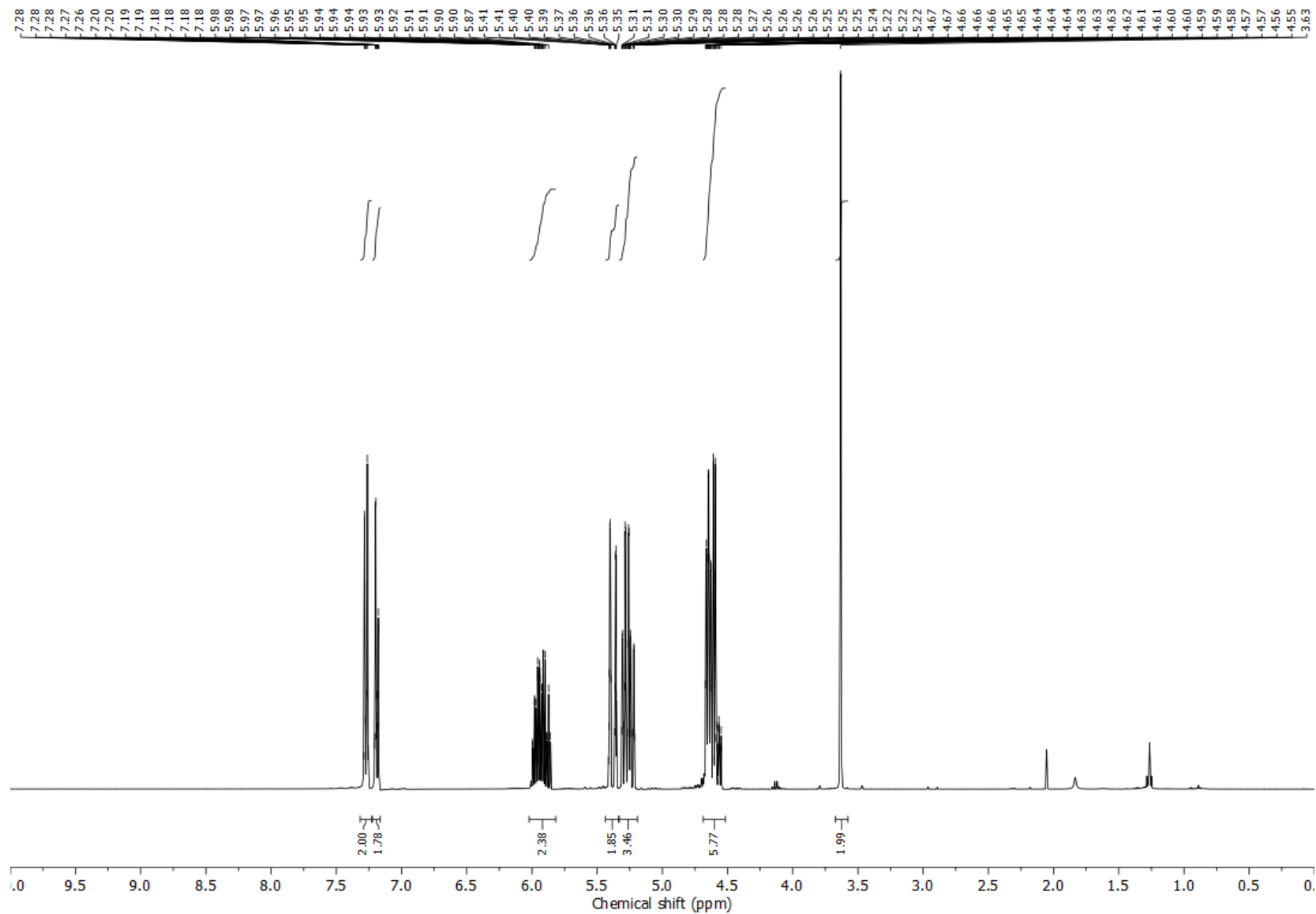
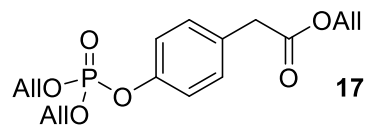
^1H NMR (400 MHz, CDCl_3)

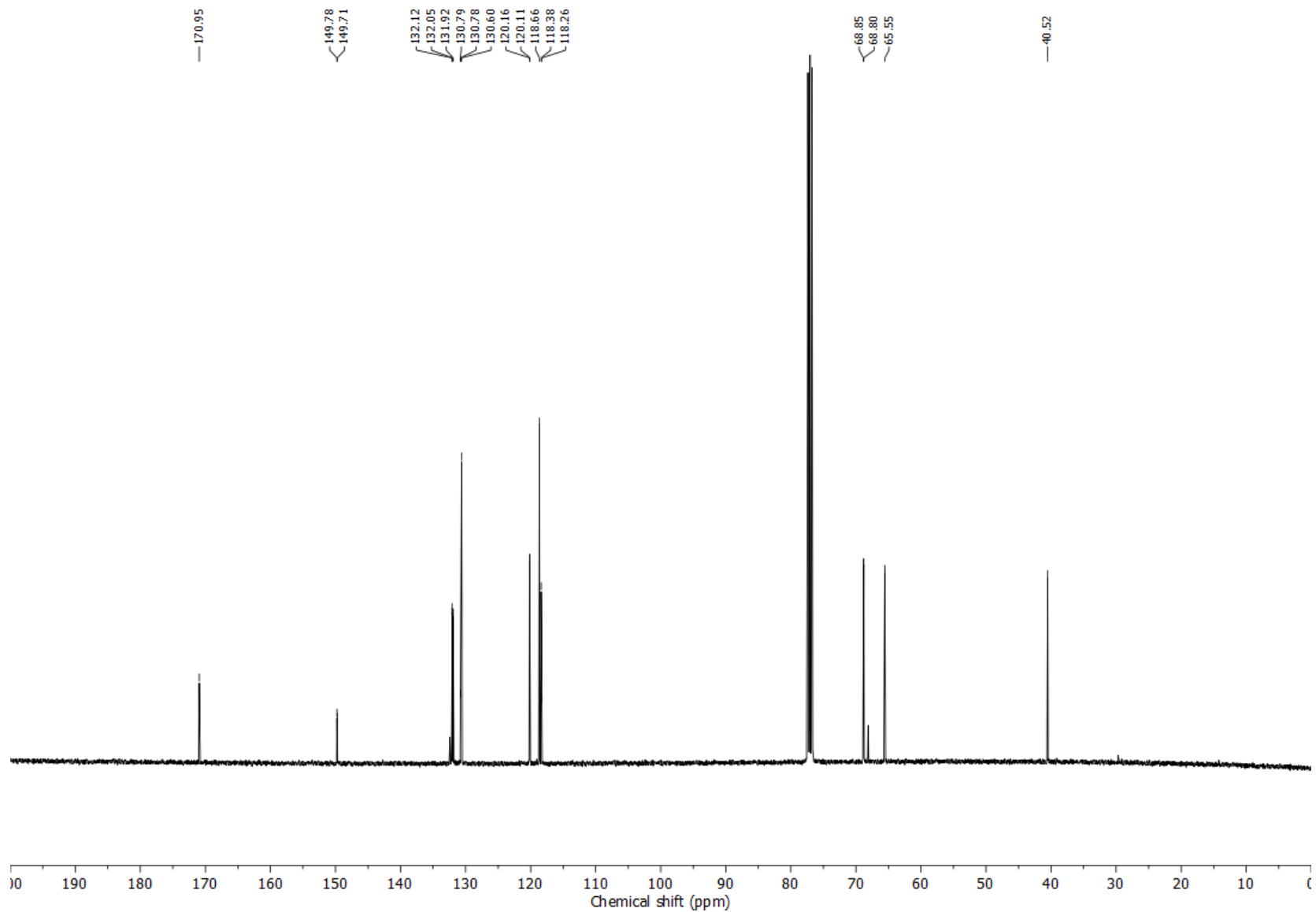
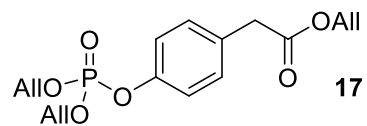


^{13}C NMR (100 MHz, CDCl_3)

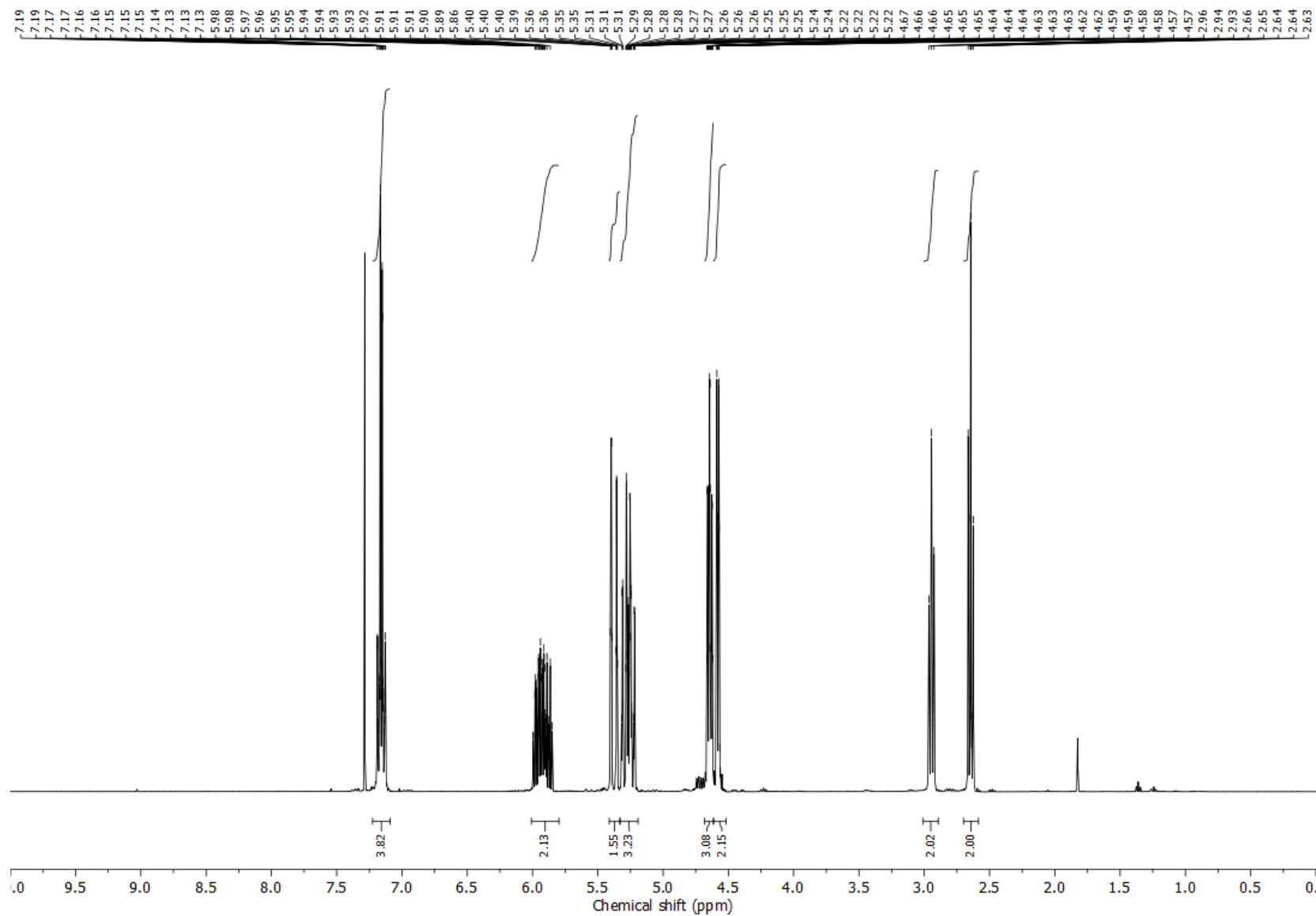
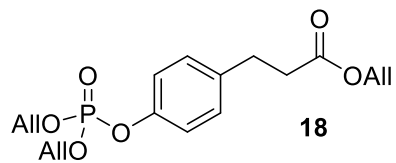


^1H NMR (400 MHz, CDCl_3)

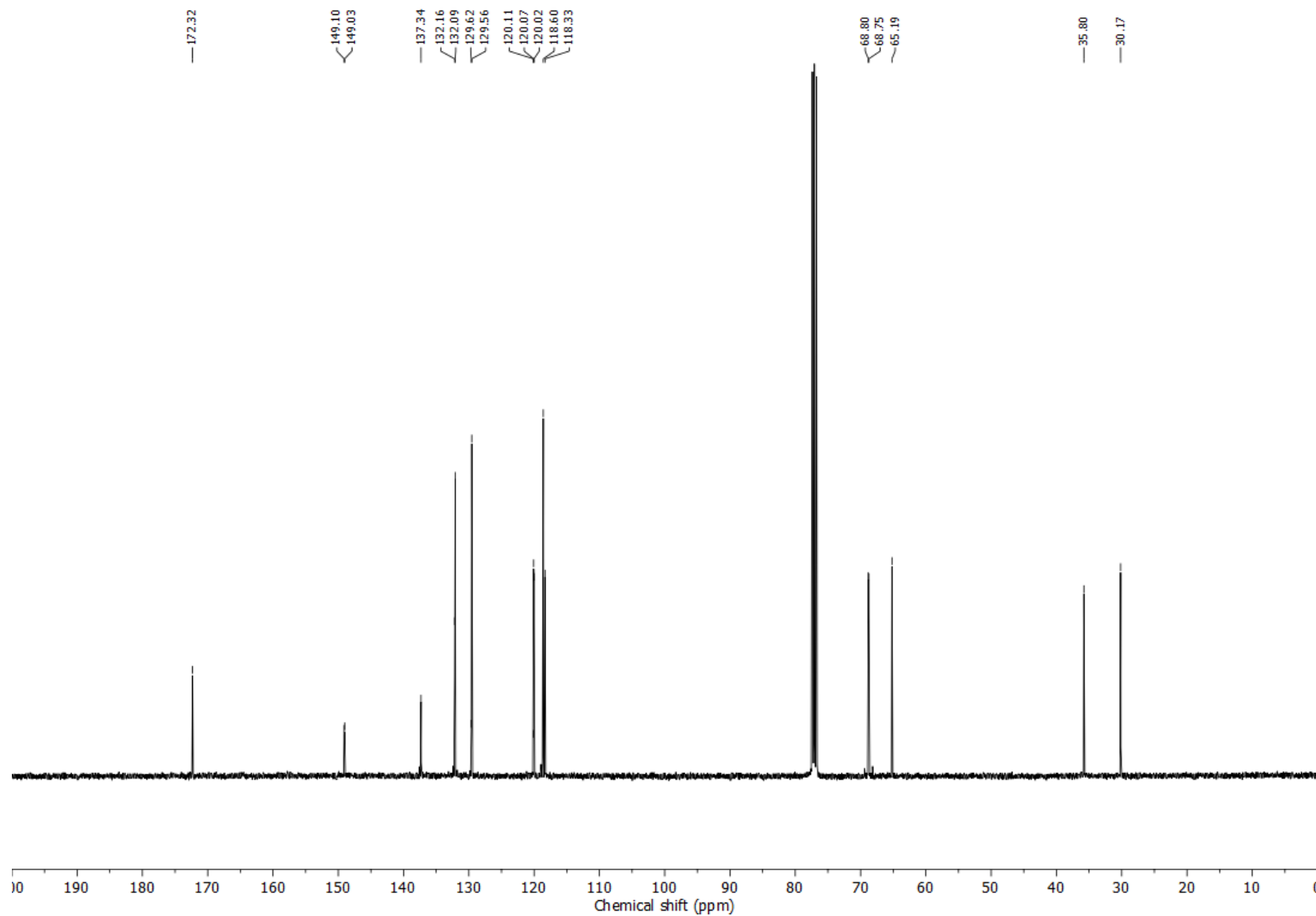
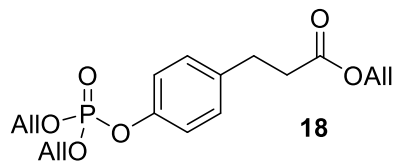


^{13}C NMR (100 MHz, CDCl_3)

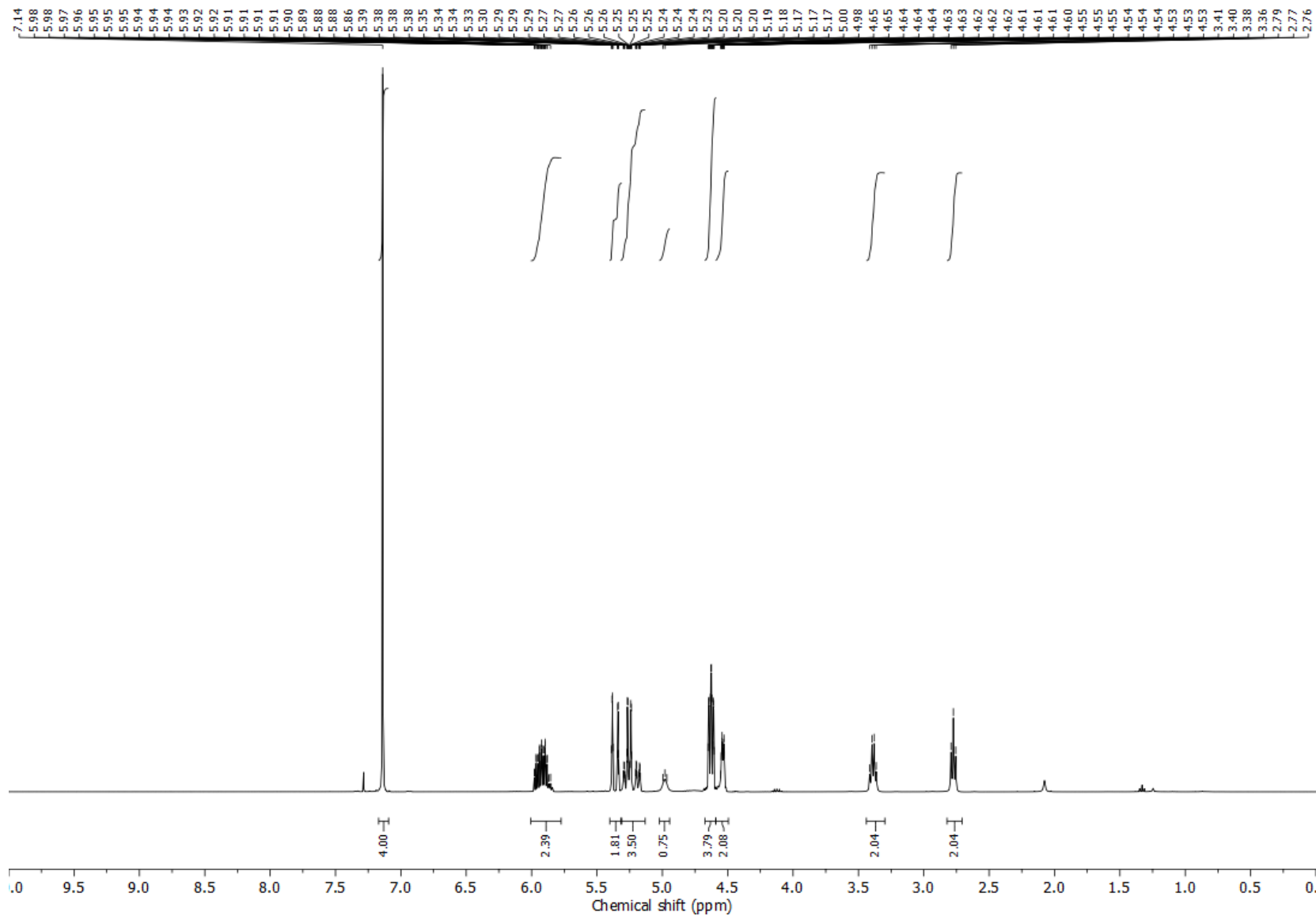
^1H NMR (400 MHz, CDCl_3)



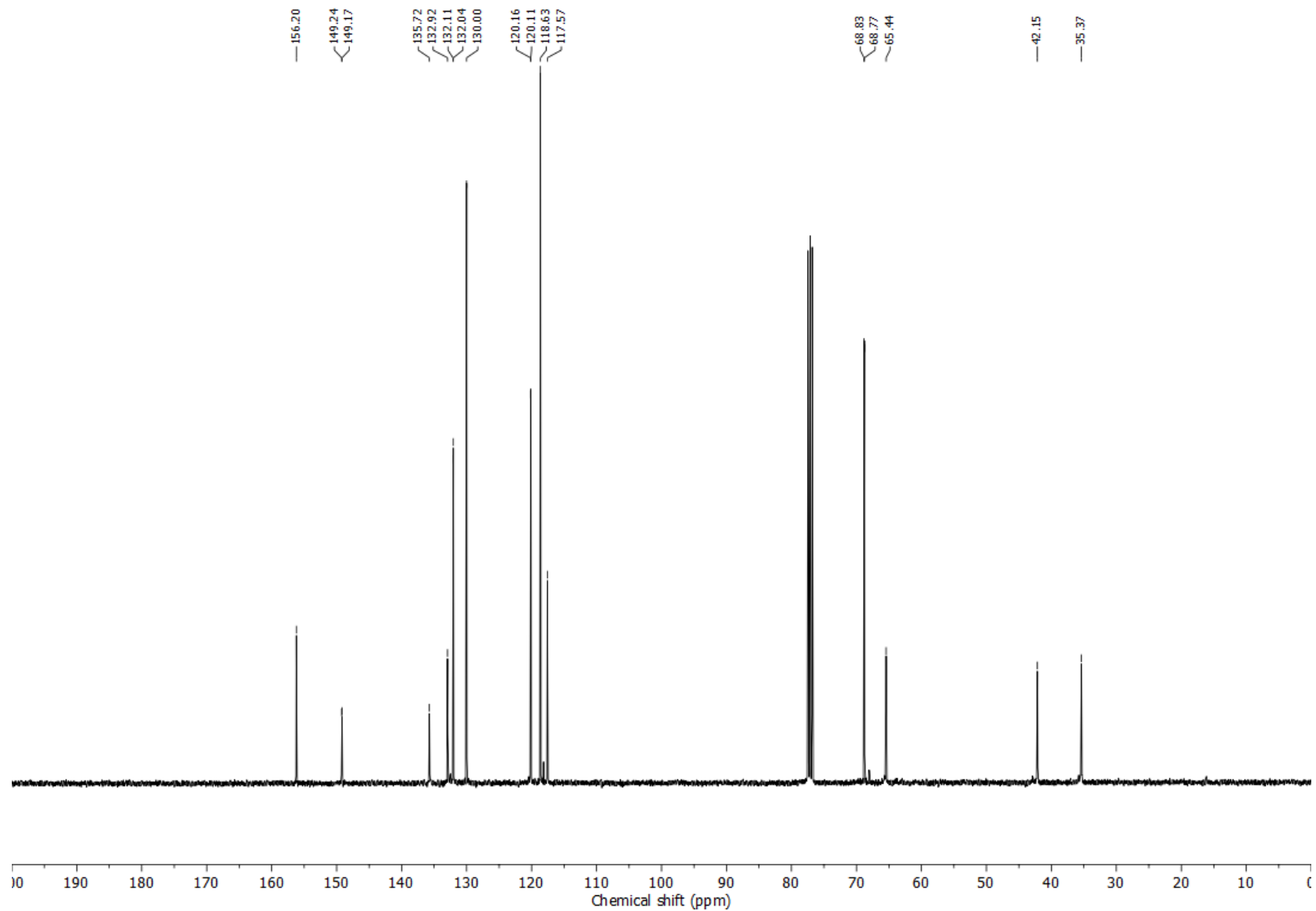
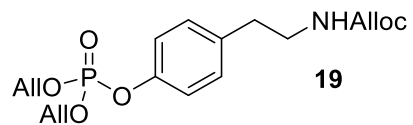
^{13}C NMR (100 MHz, CDCl_3)



19

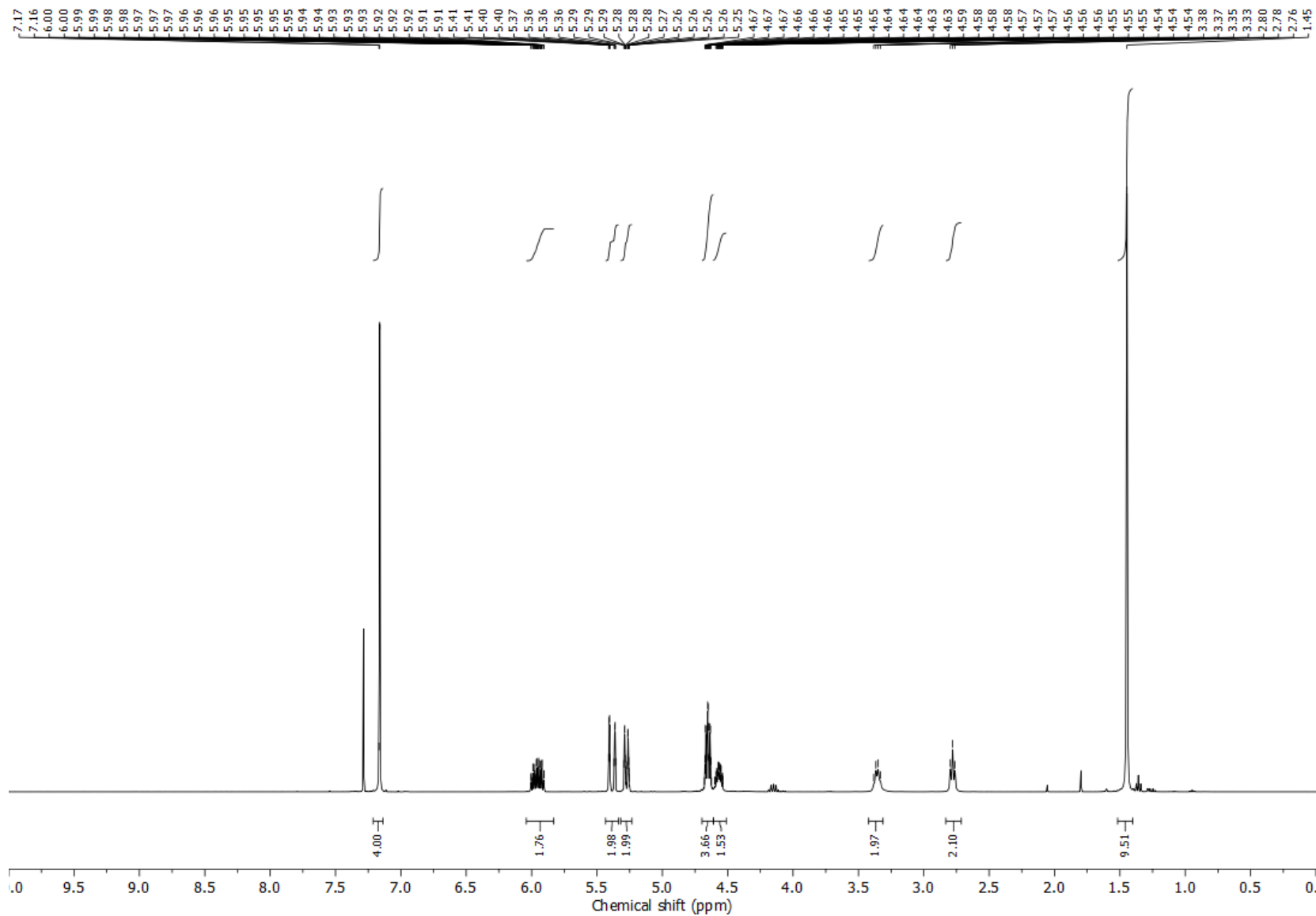


^{13}C NMR (100 MHz, CDCl_3)

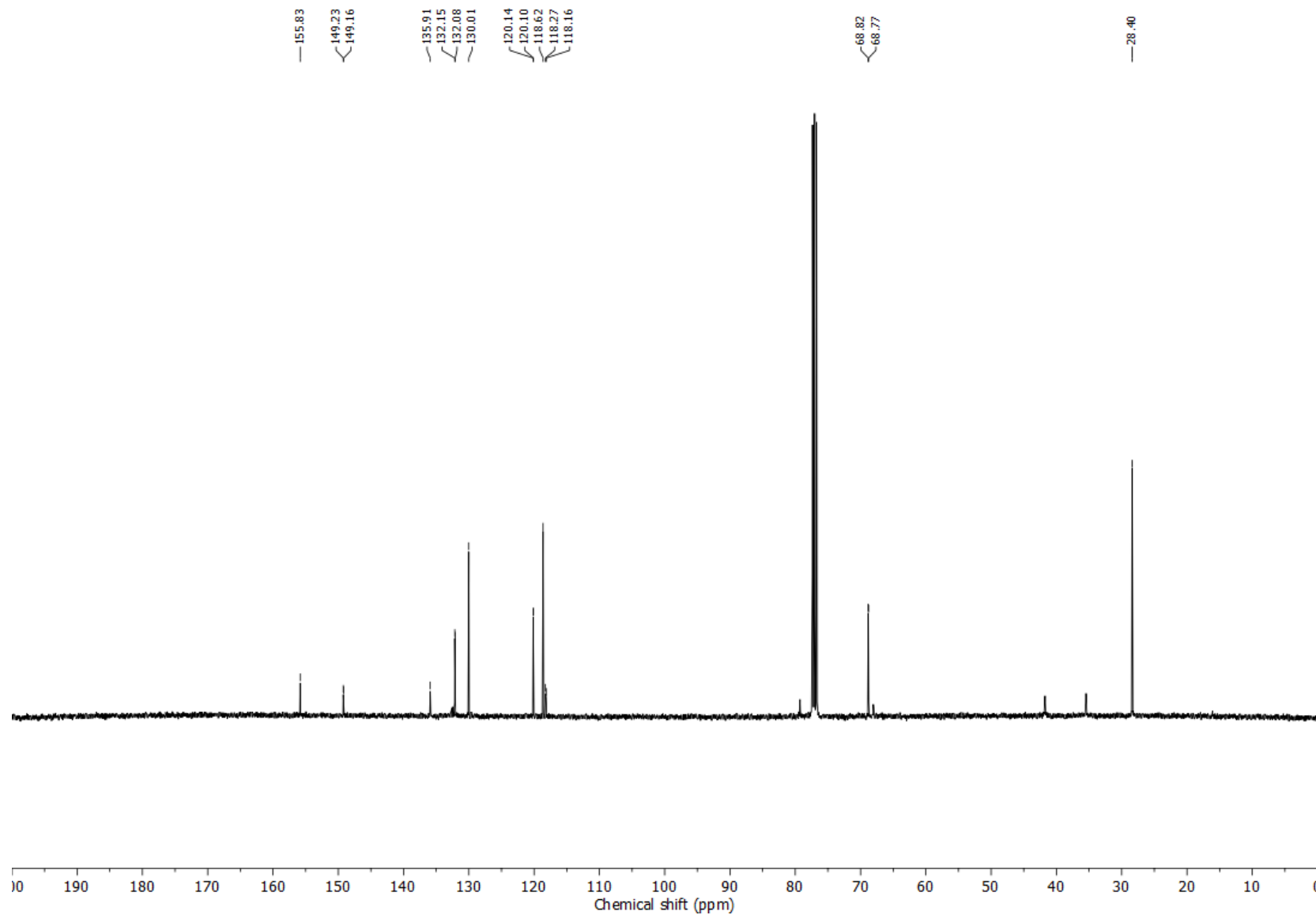
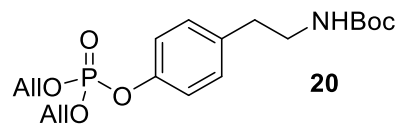


CCOC(=O)NCCCc1ccc(cc1)OP(=O)(O)O

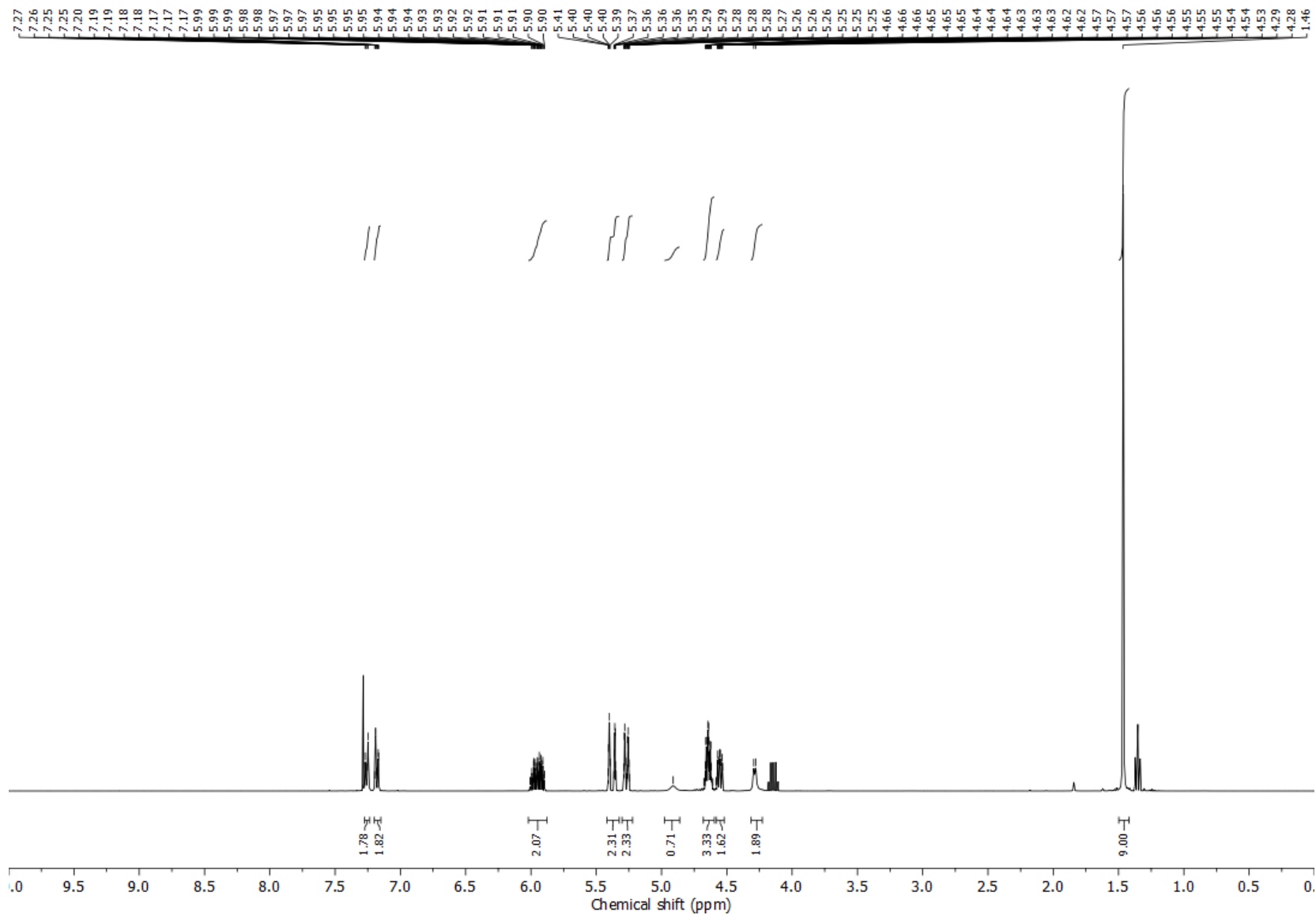
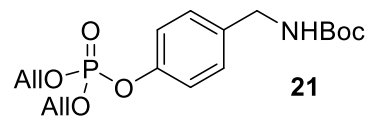
20



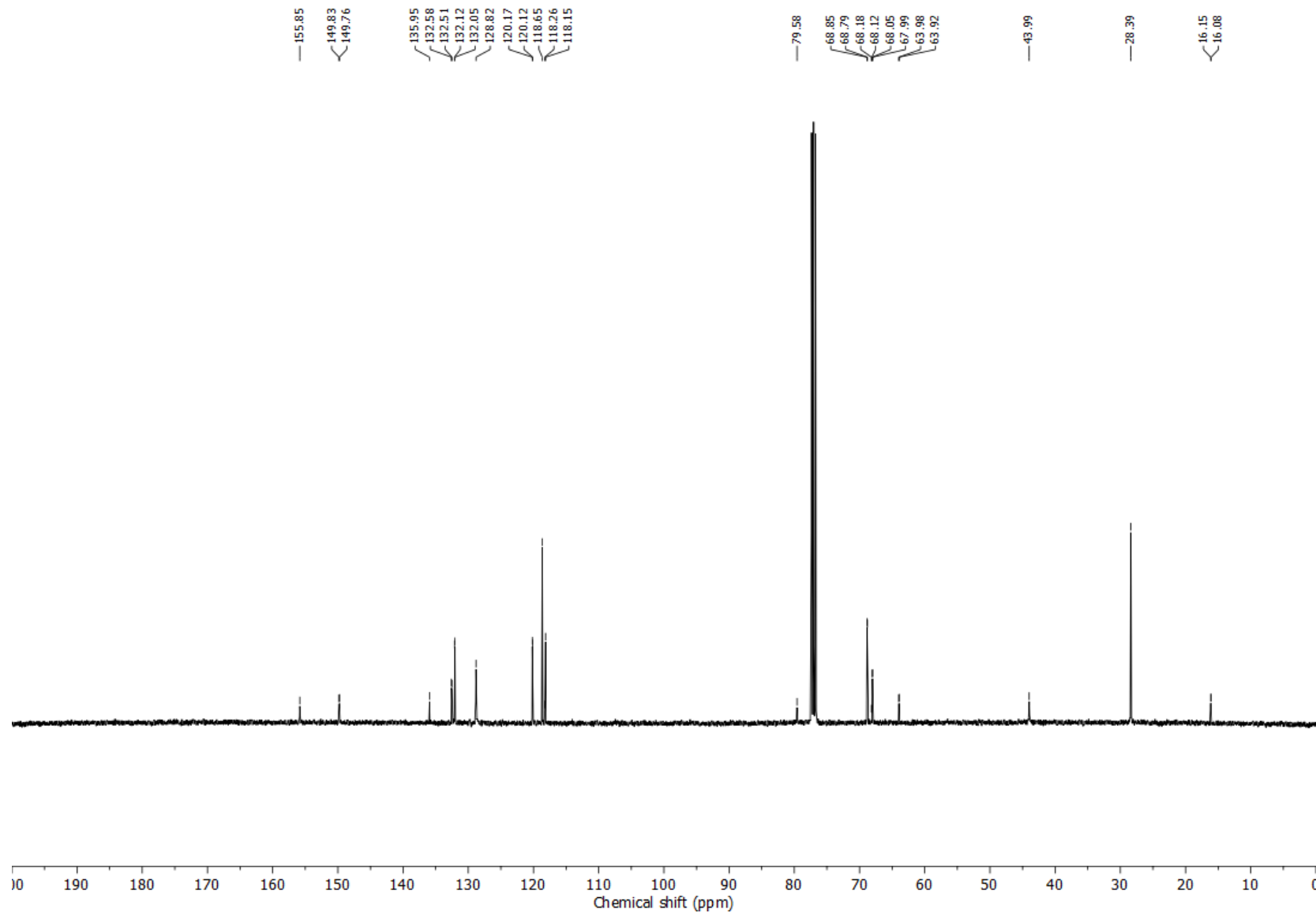
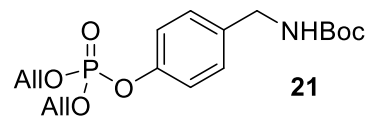
^{13}C NMR (100 MHz, CDCl_3)



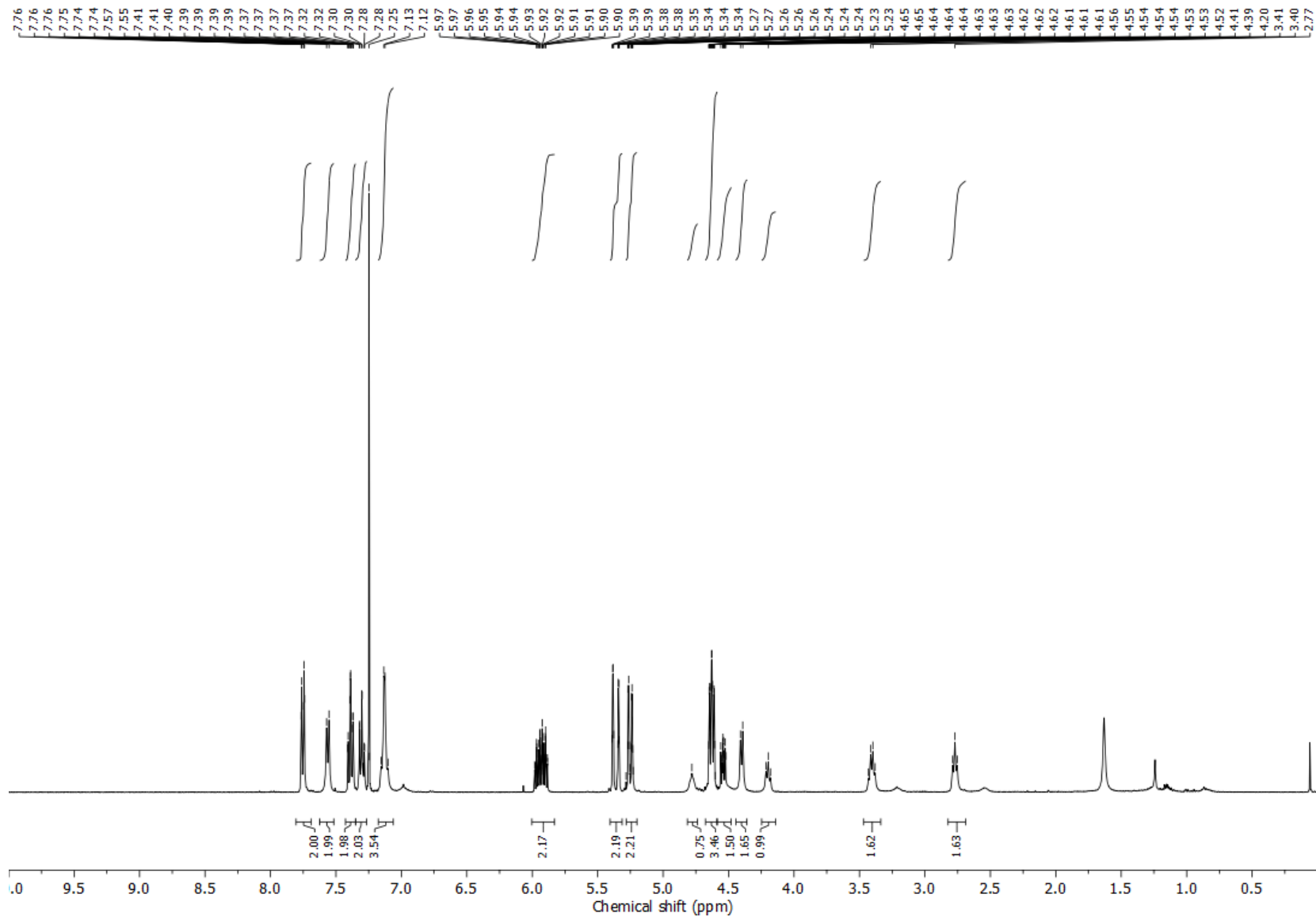
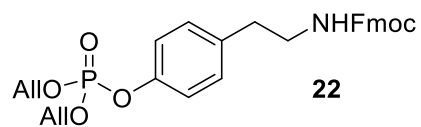
^1H NMR (400 MHz, CDCl_3)



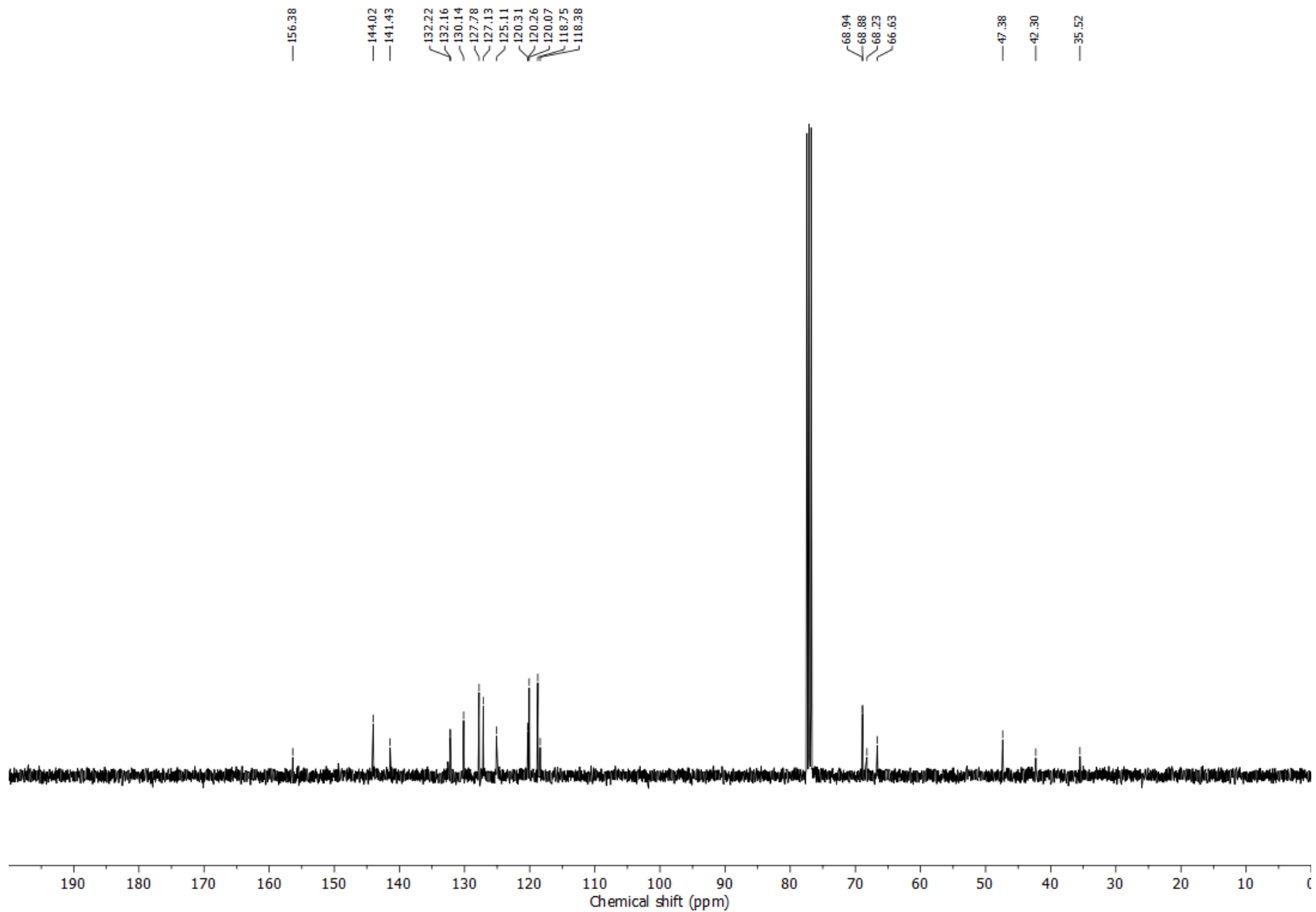
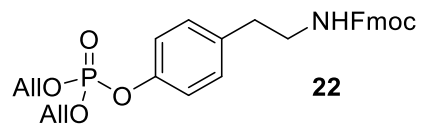
^{13}C NMR (100 MHz, CDCl_3)



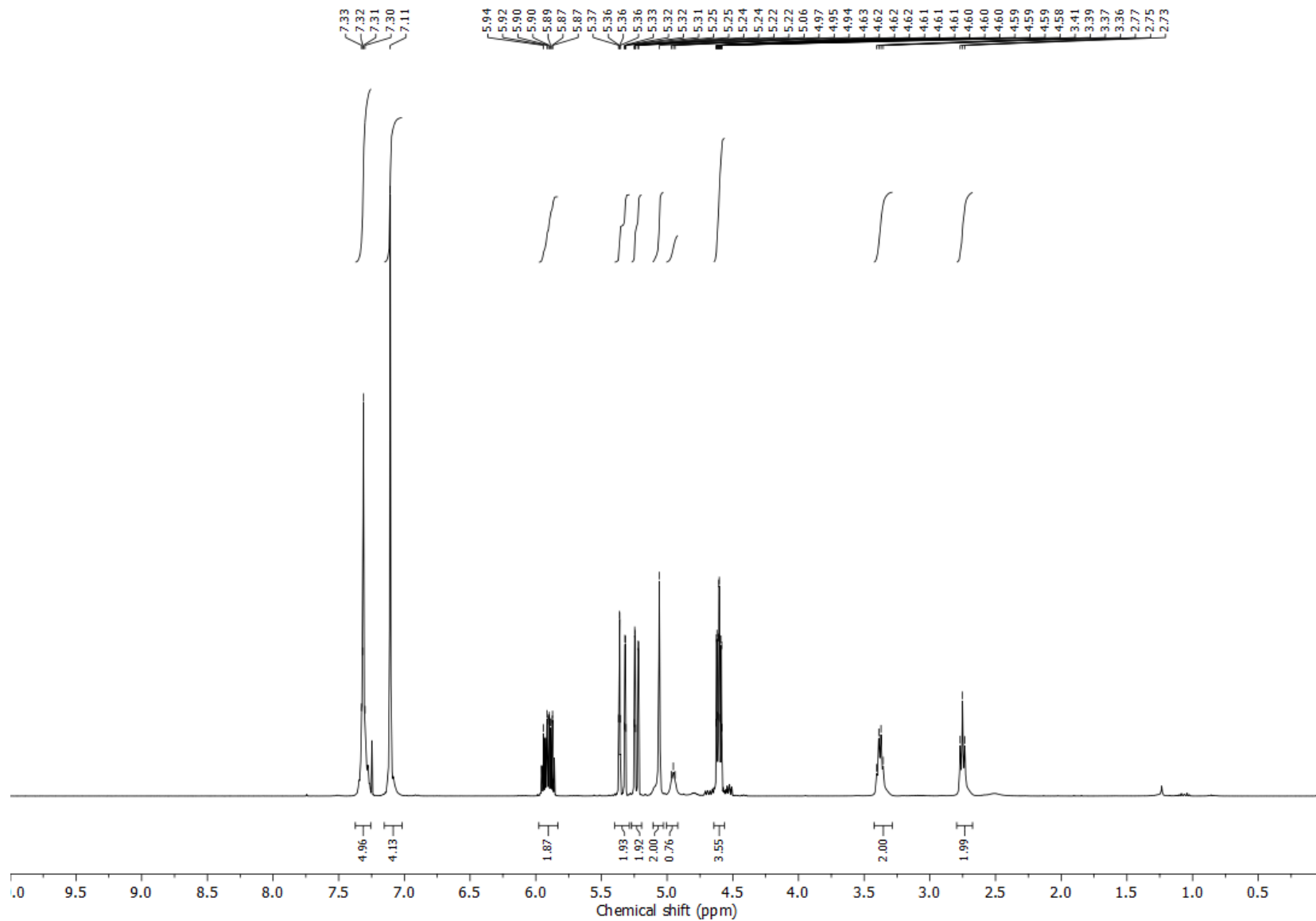
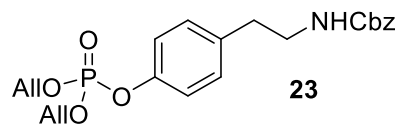
^1H NMR (400 MHz, CDCl_3)



^{13}C NMR (100 MHz, CDCl_3)

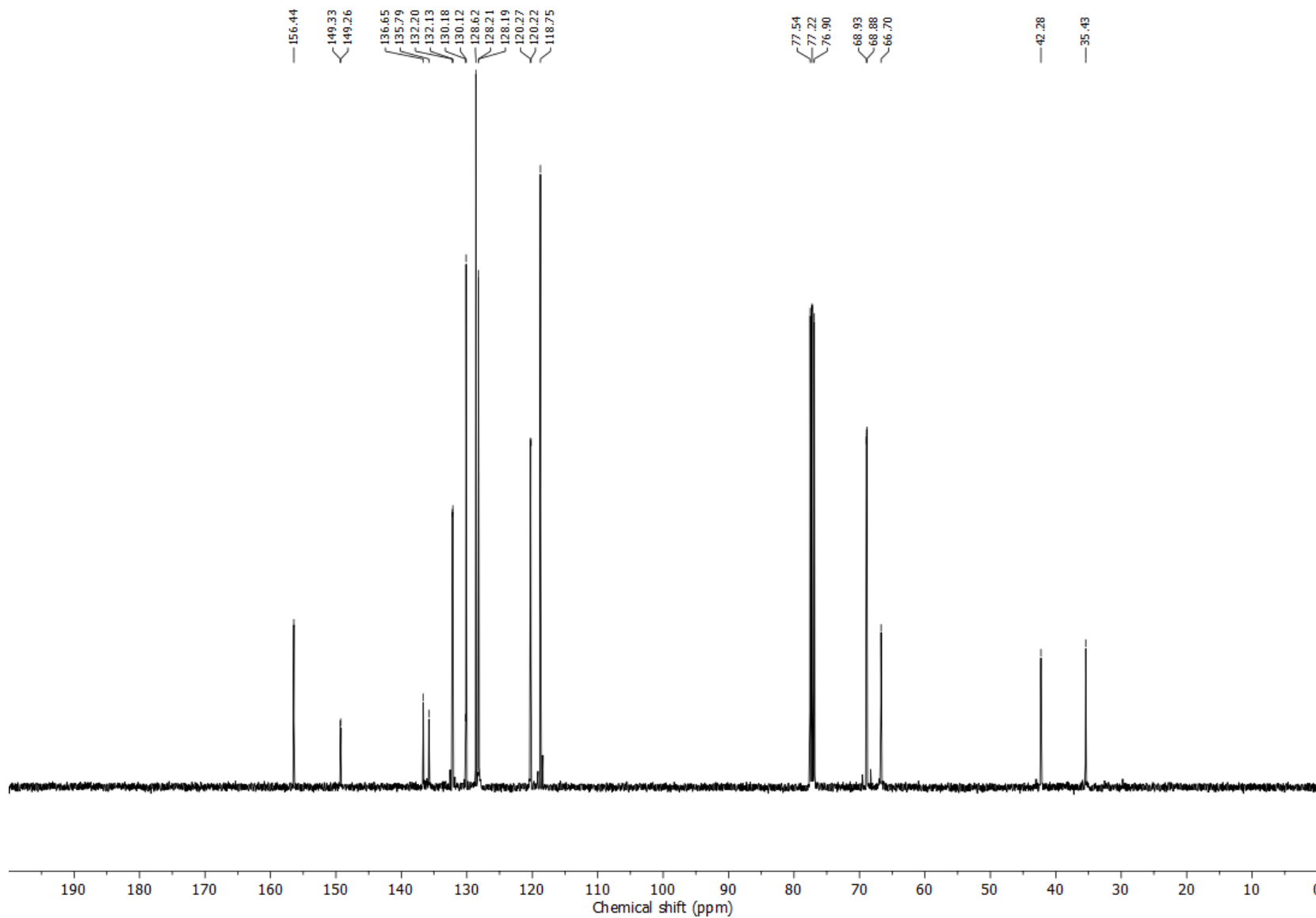


^1H NMR (400 MHz, CDCl_3)

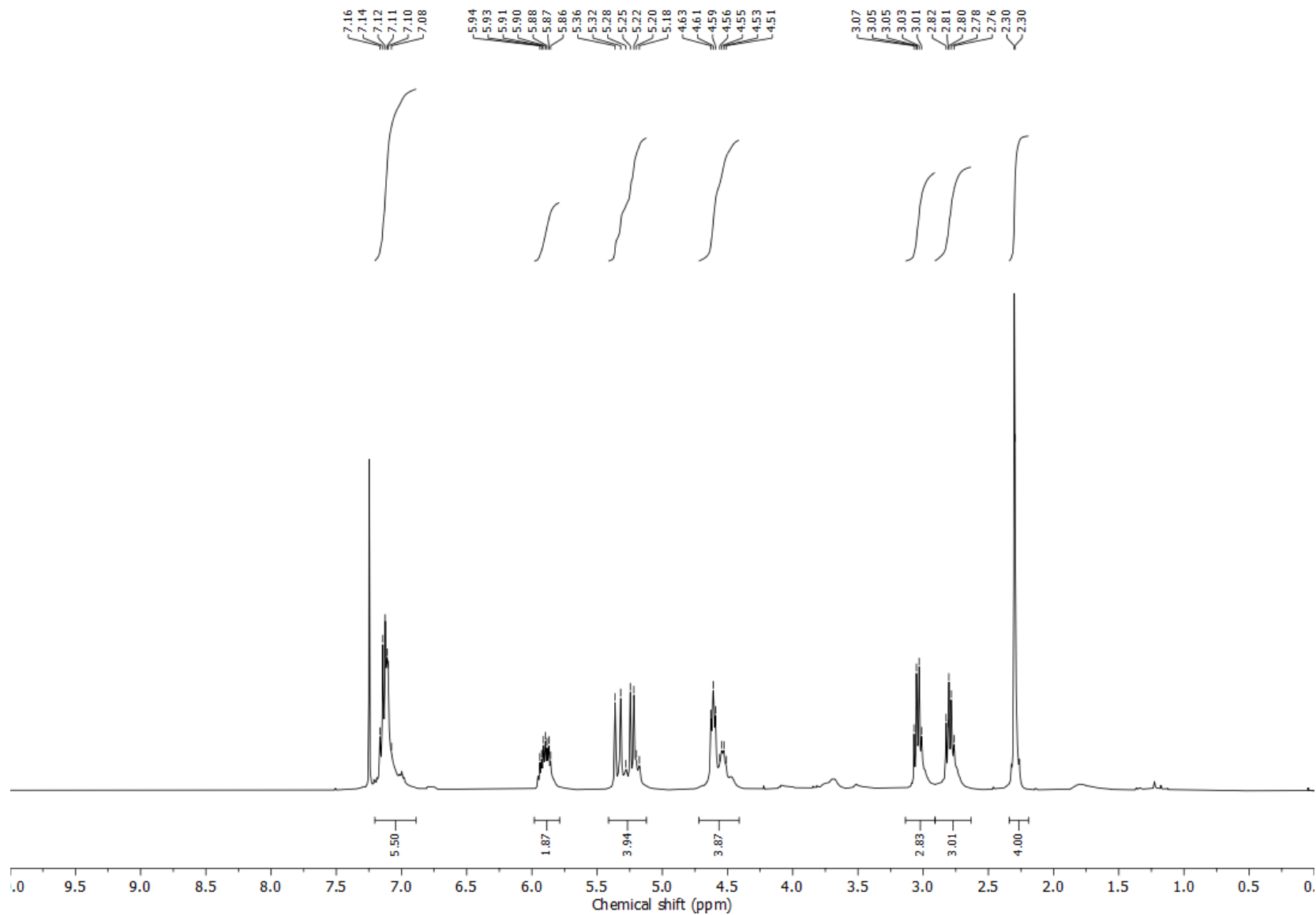
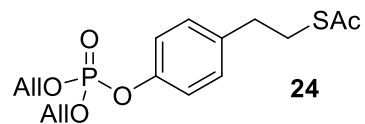


O=P(O)(O)c1ccc(cc1)CCNC(=O)c2ccccc2

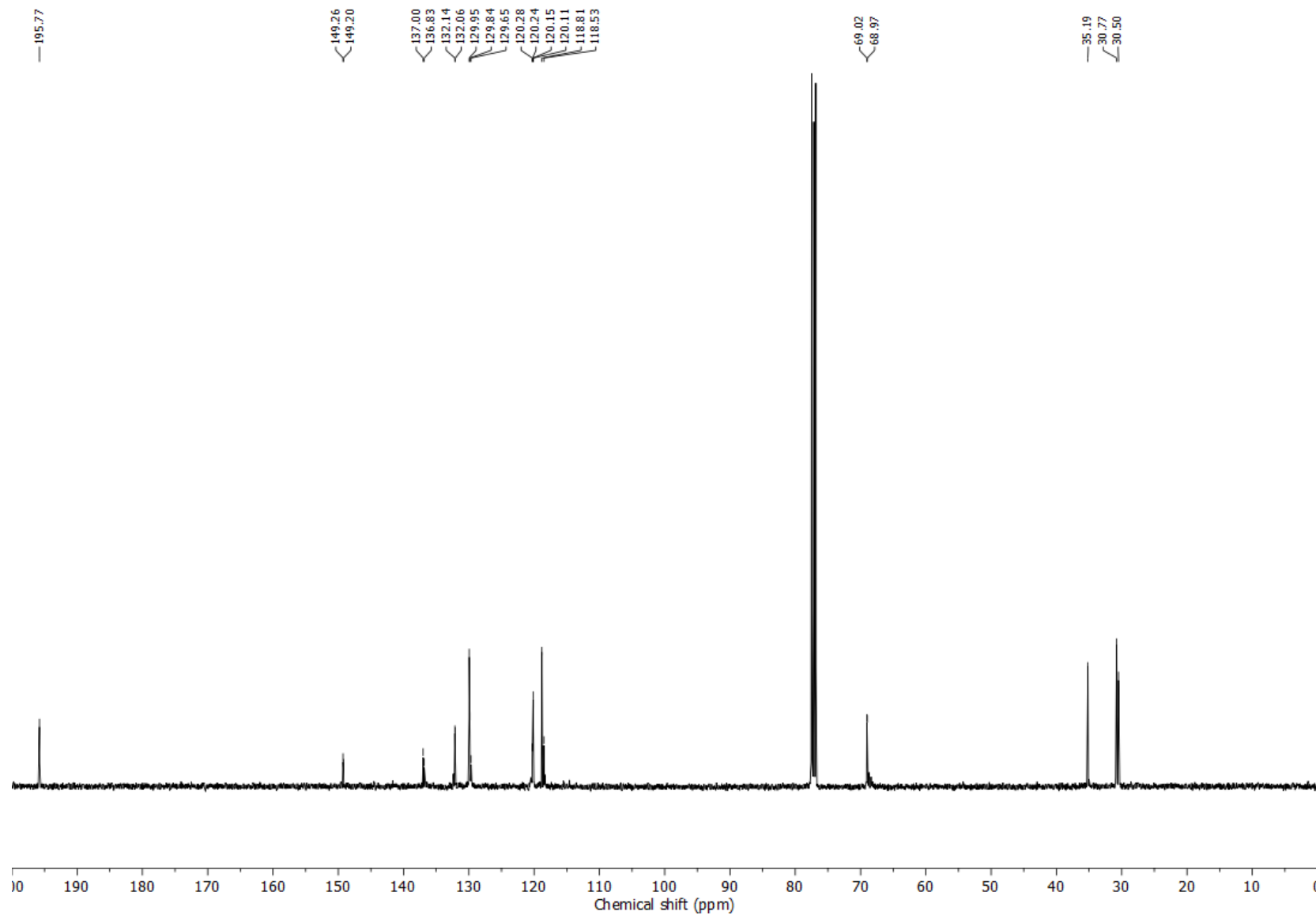
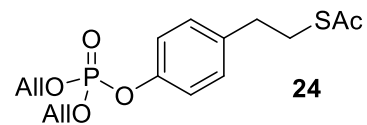
23



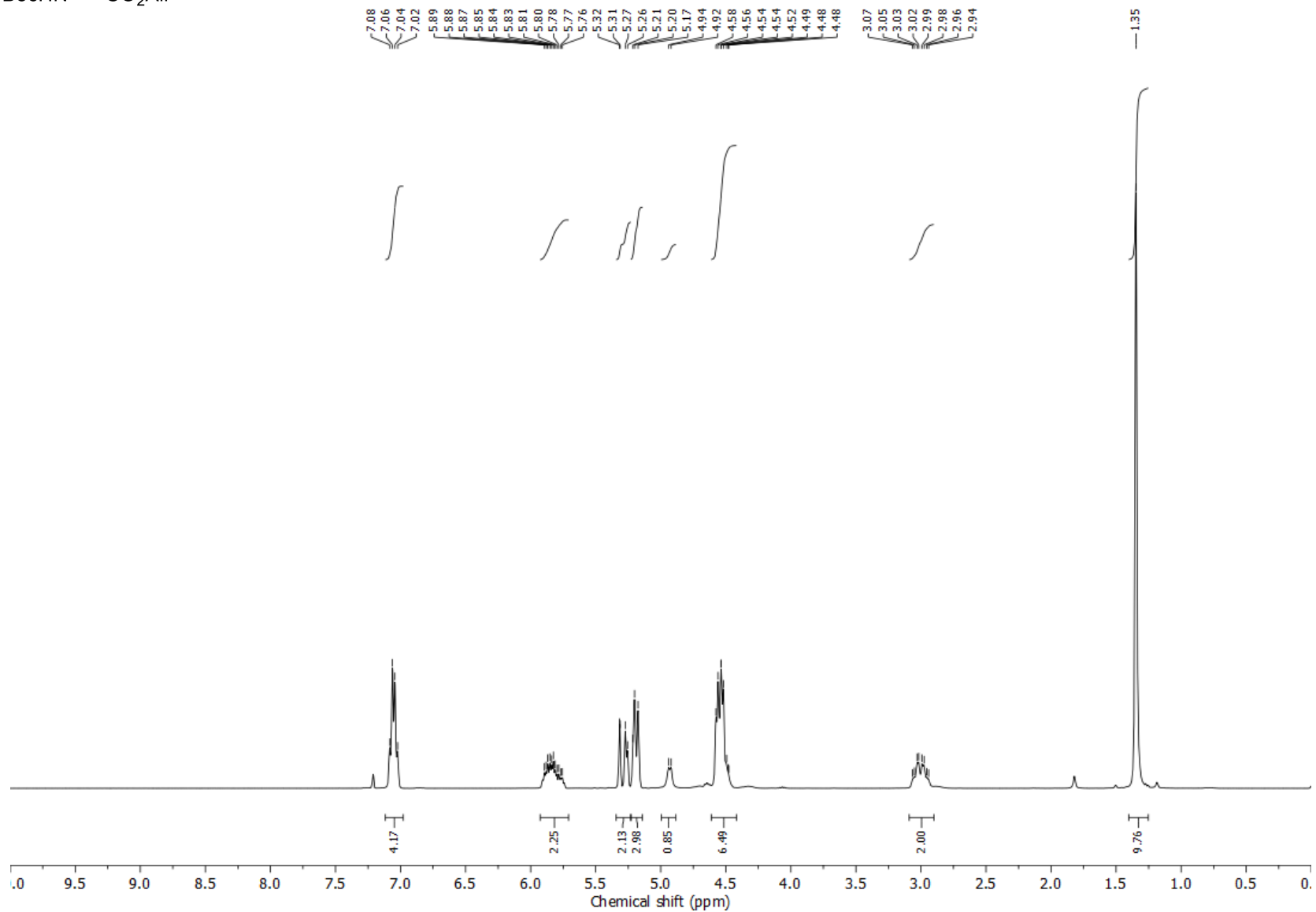
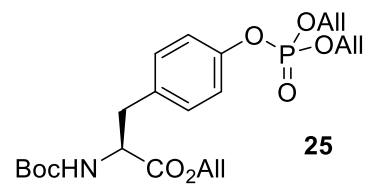
^1H NMR (400 MHz, CDCl_3)



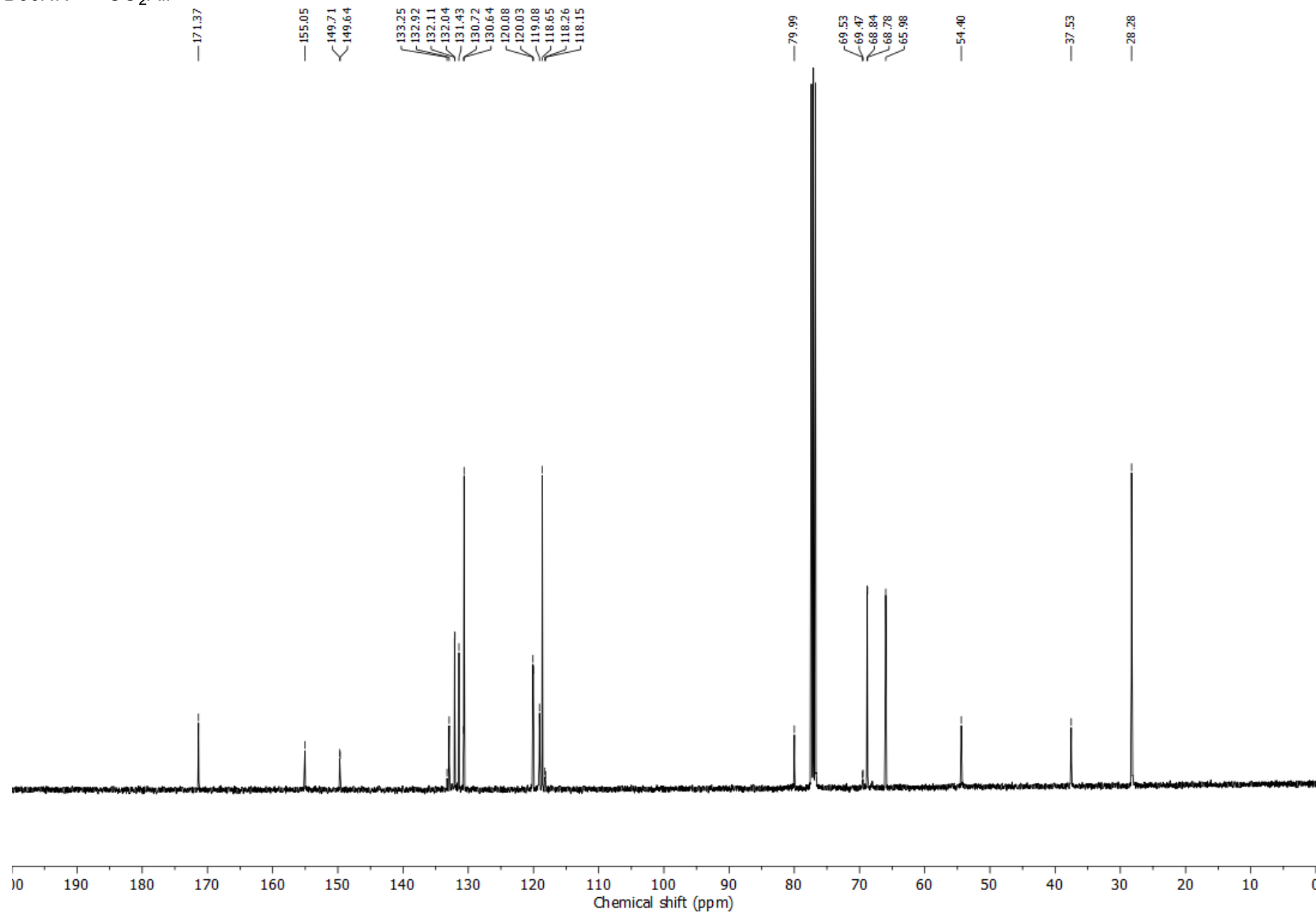
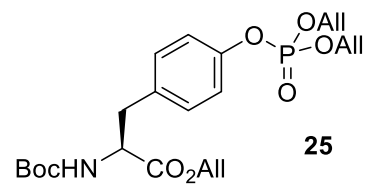
^{13}C NMR (100 MHz, CDCl_3)



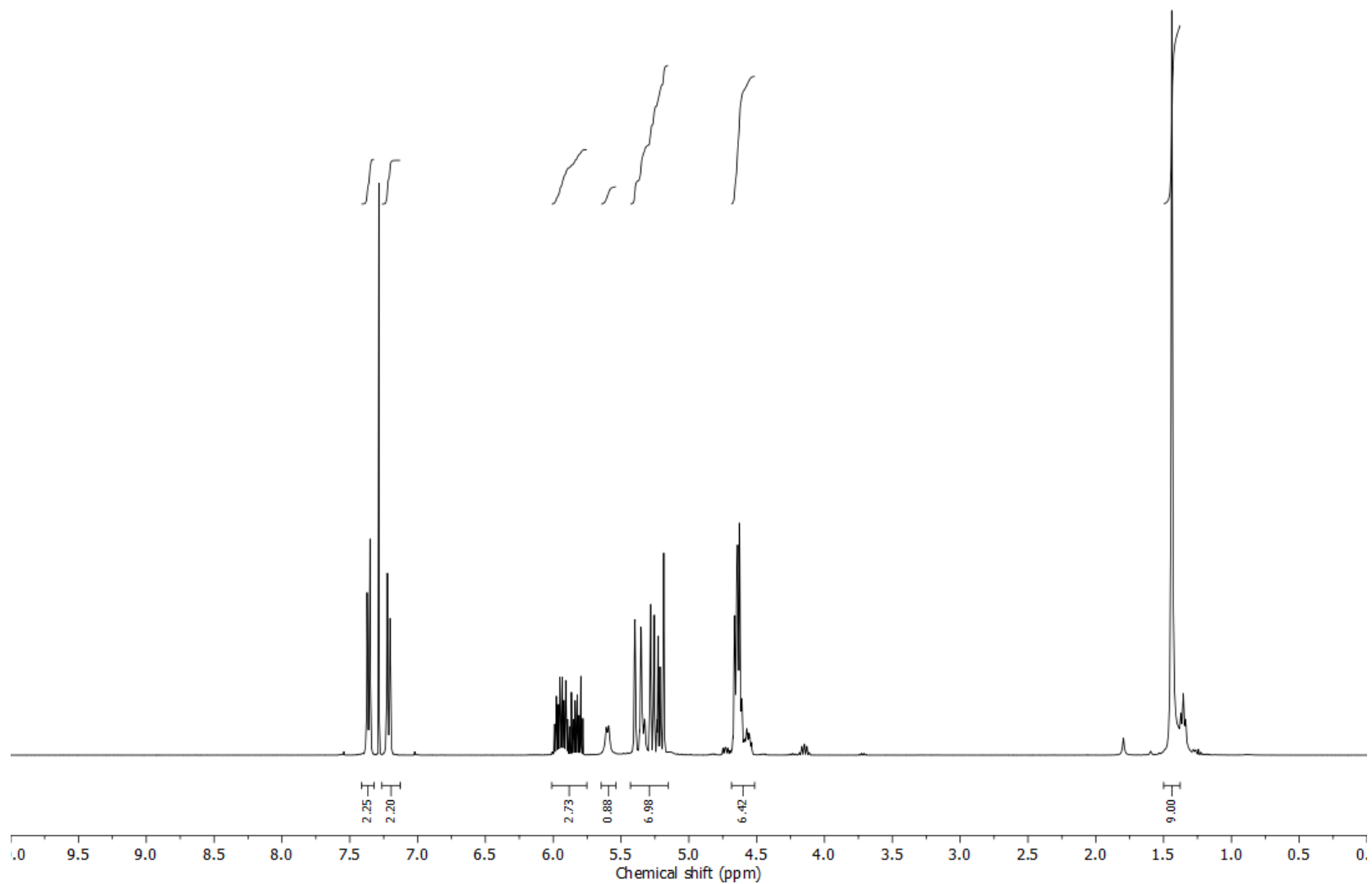
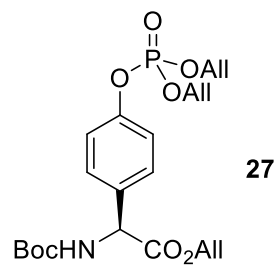
^1H NMR (400 MHz, CDCl_3)



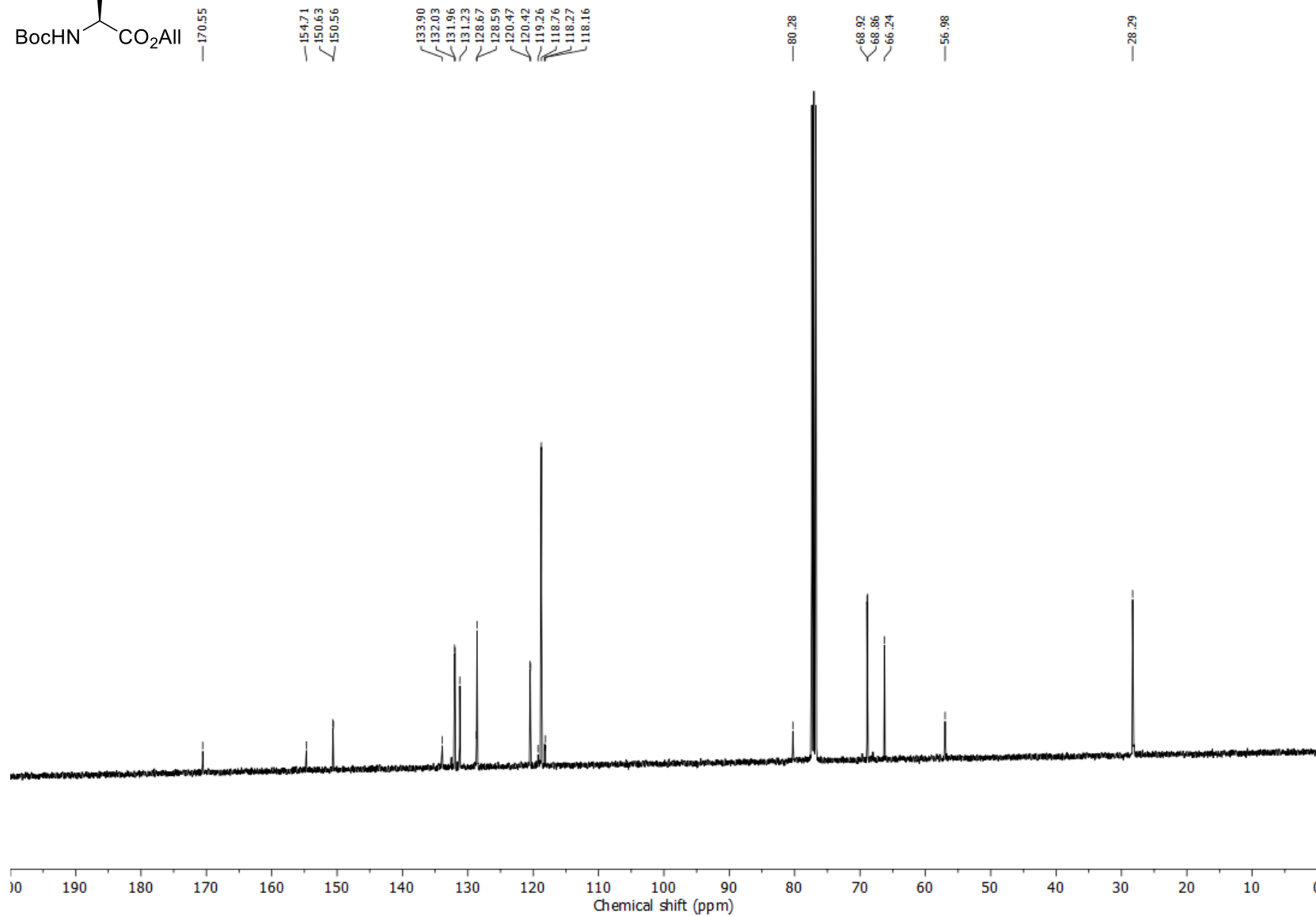
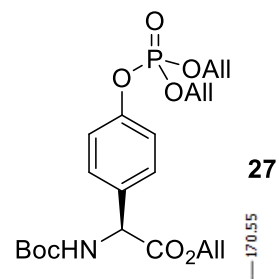
^{13}C NMR (100 MHz, CDCl_3)

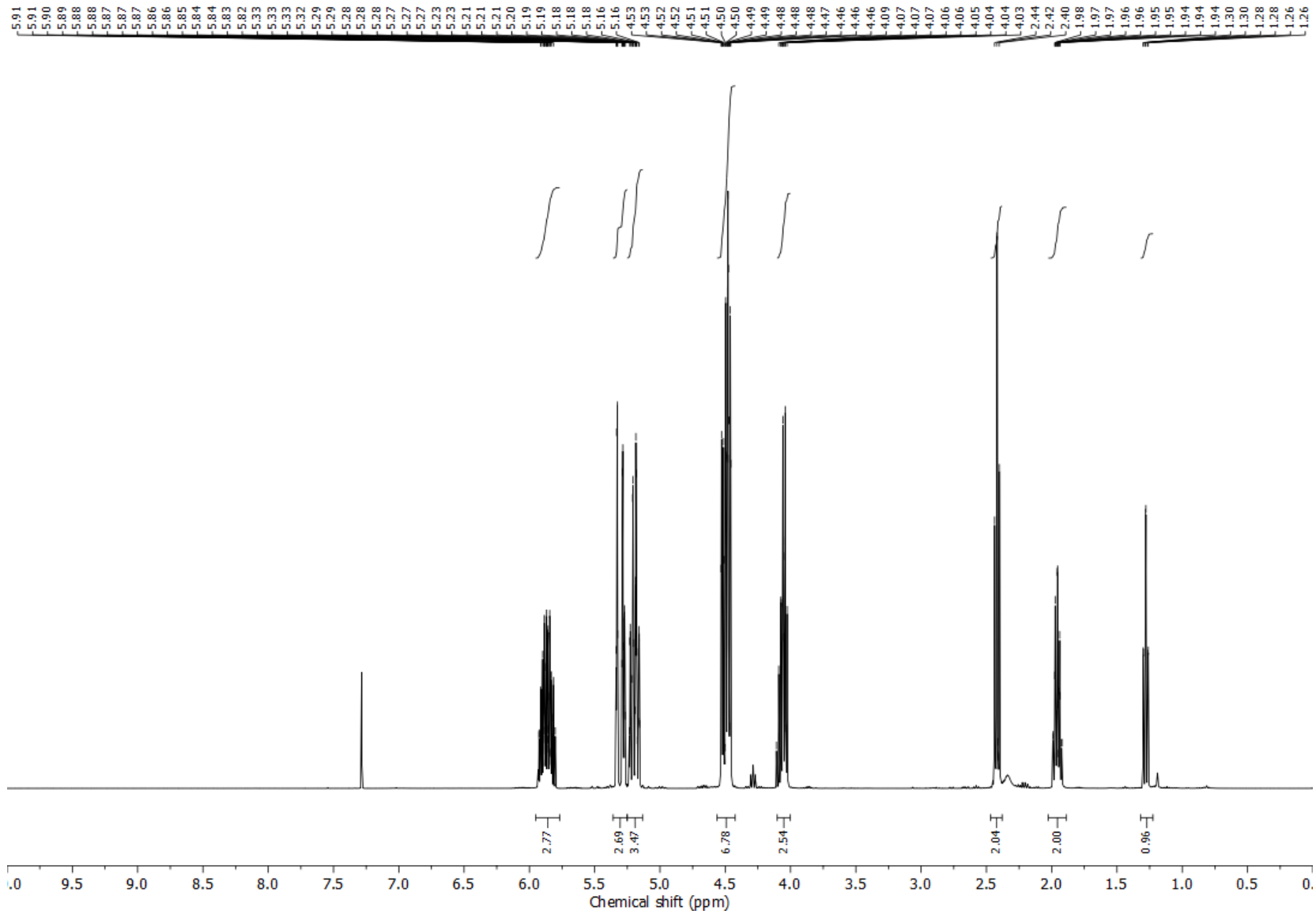
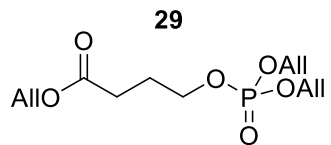


^1H NMR (400 MHz, CDCl_3)

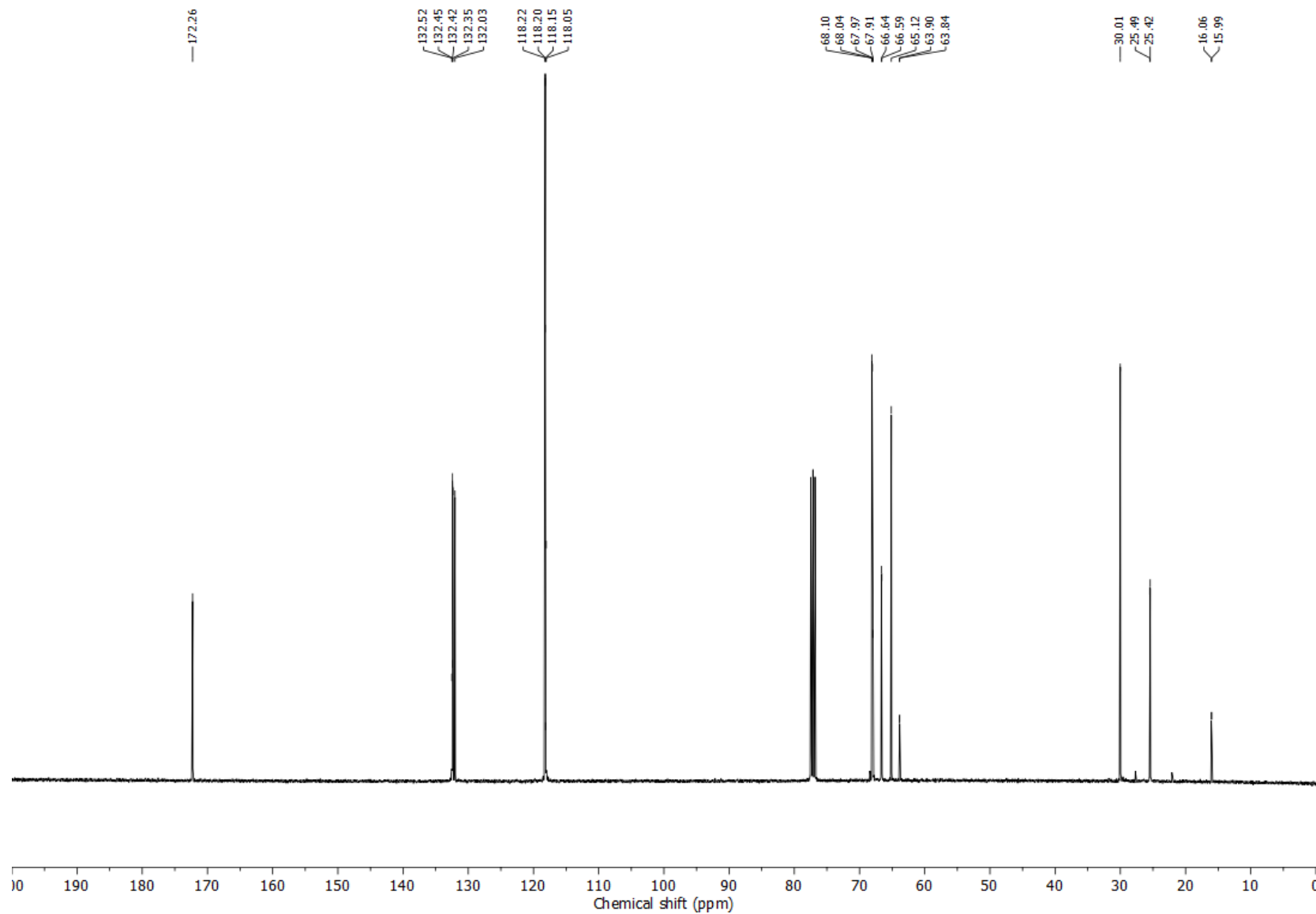
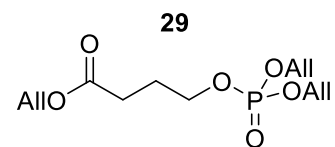


^{13}C NMR (100 MHz, CDCl_3)



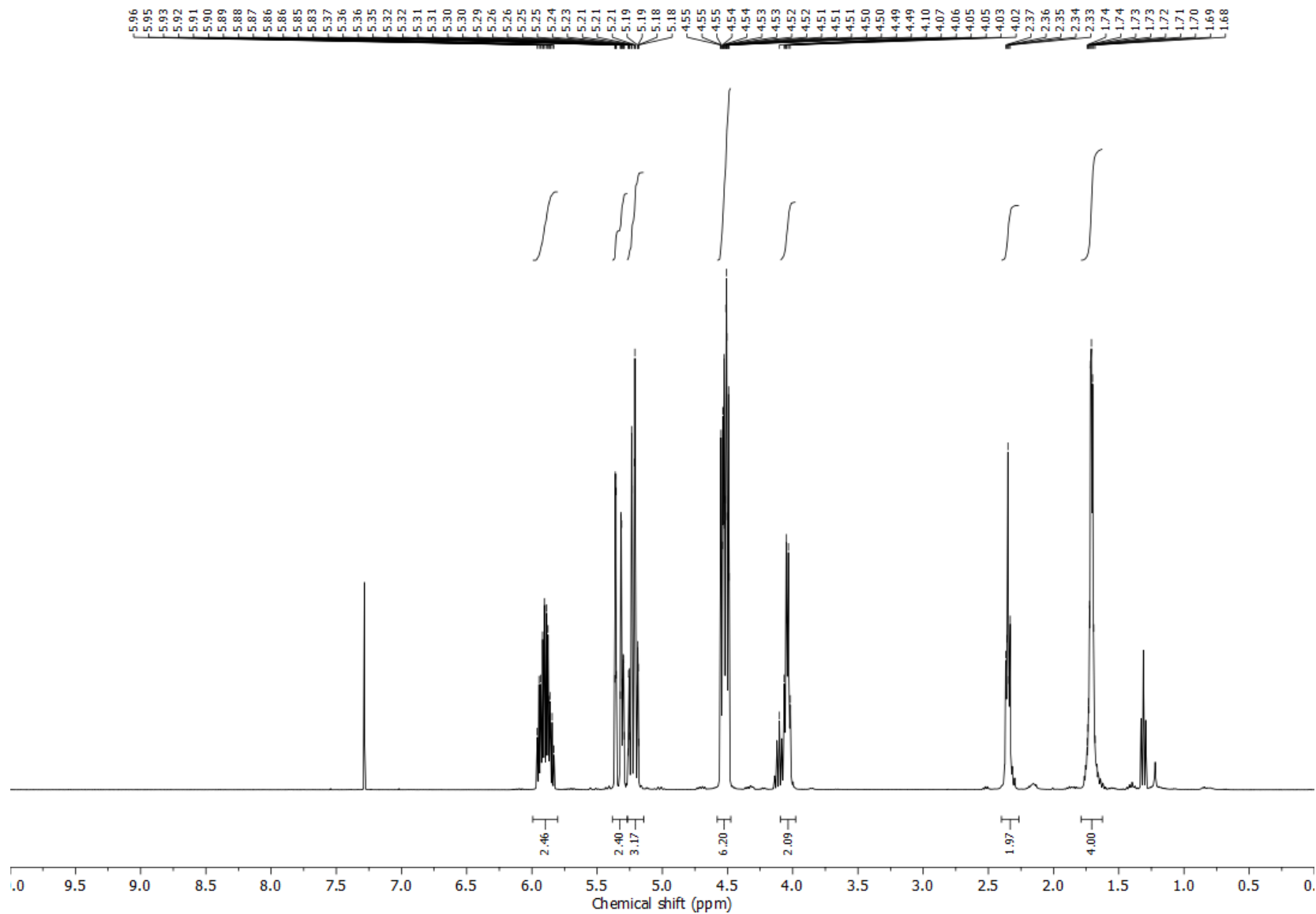
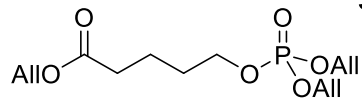
^1H NMR (400 MHz, CDCl_3)

^{13}C NMR (100 MHz, CDCl_3)

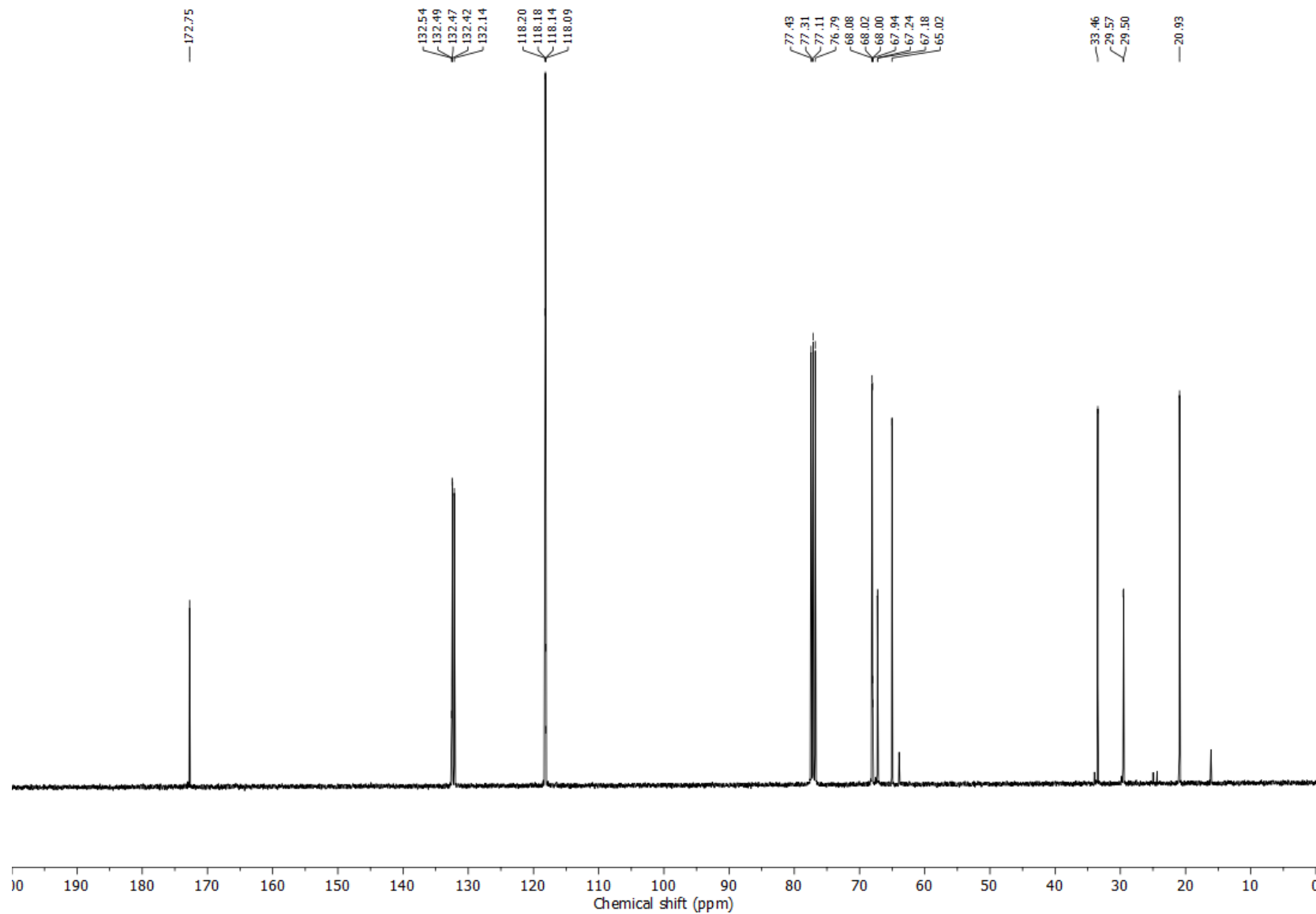
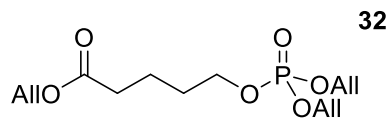


^1H NMR (400 MHz, CDCl_3)

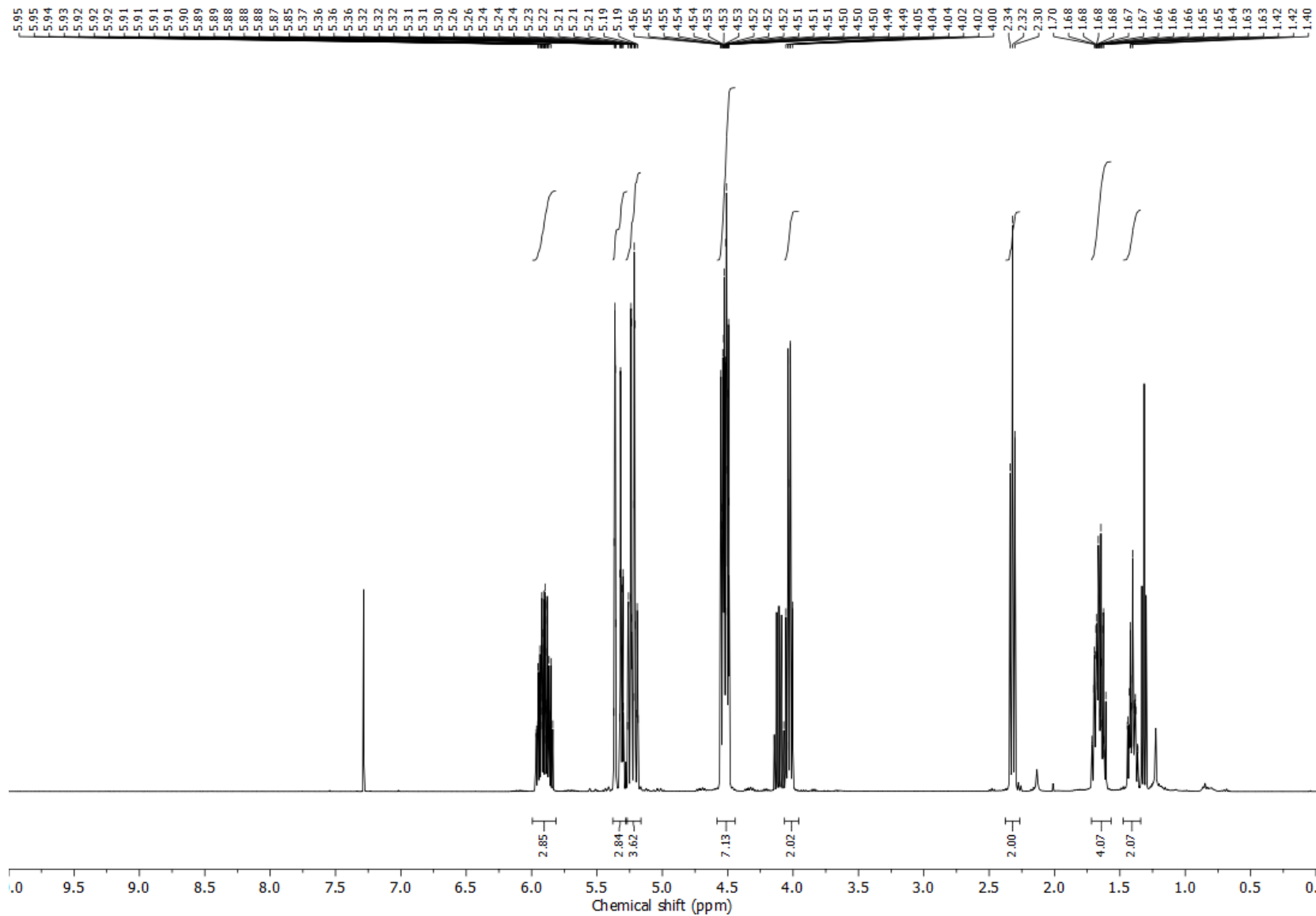
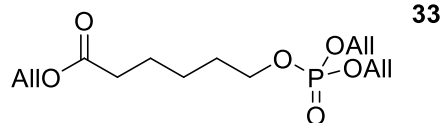
32



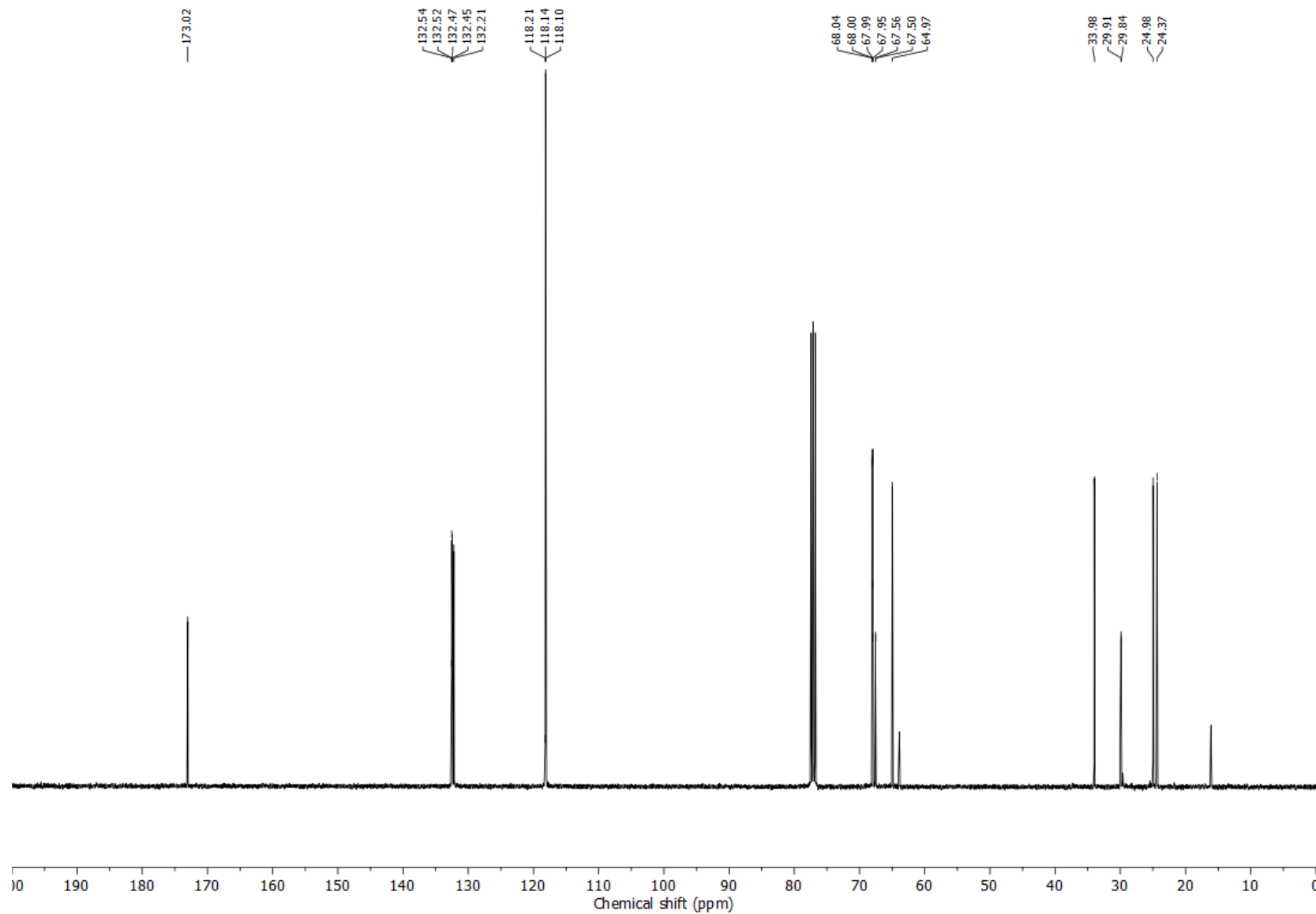
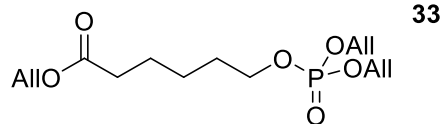
^{13}C NMR (100 MHz, CDCl_3)



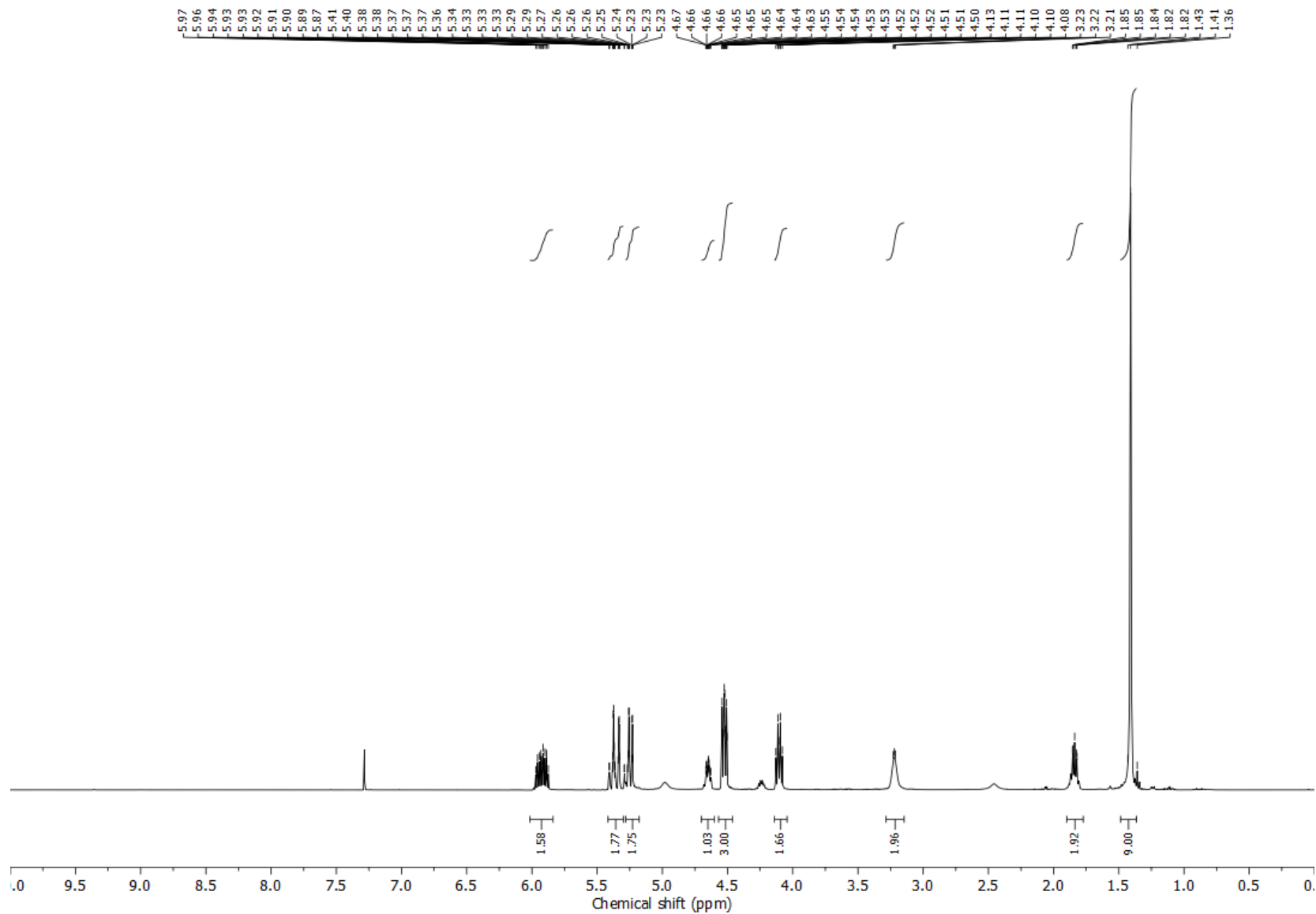
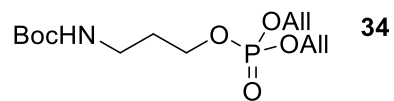
^1H NMR (400 MHz, CDCl_3)



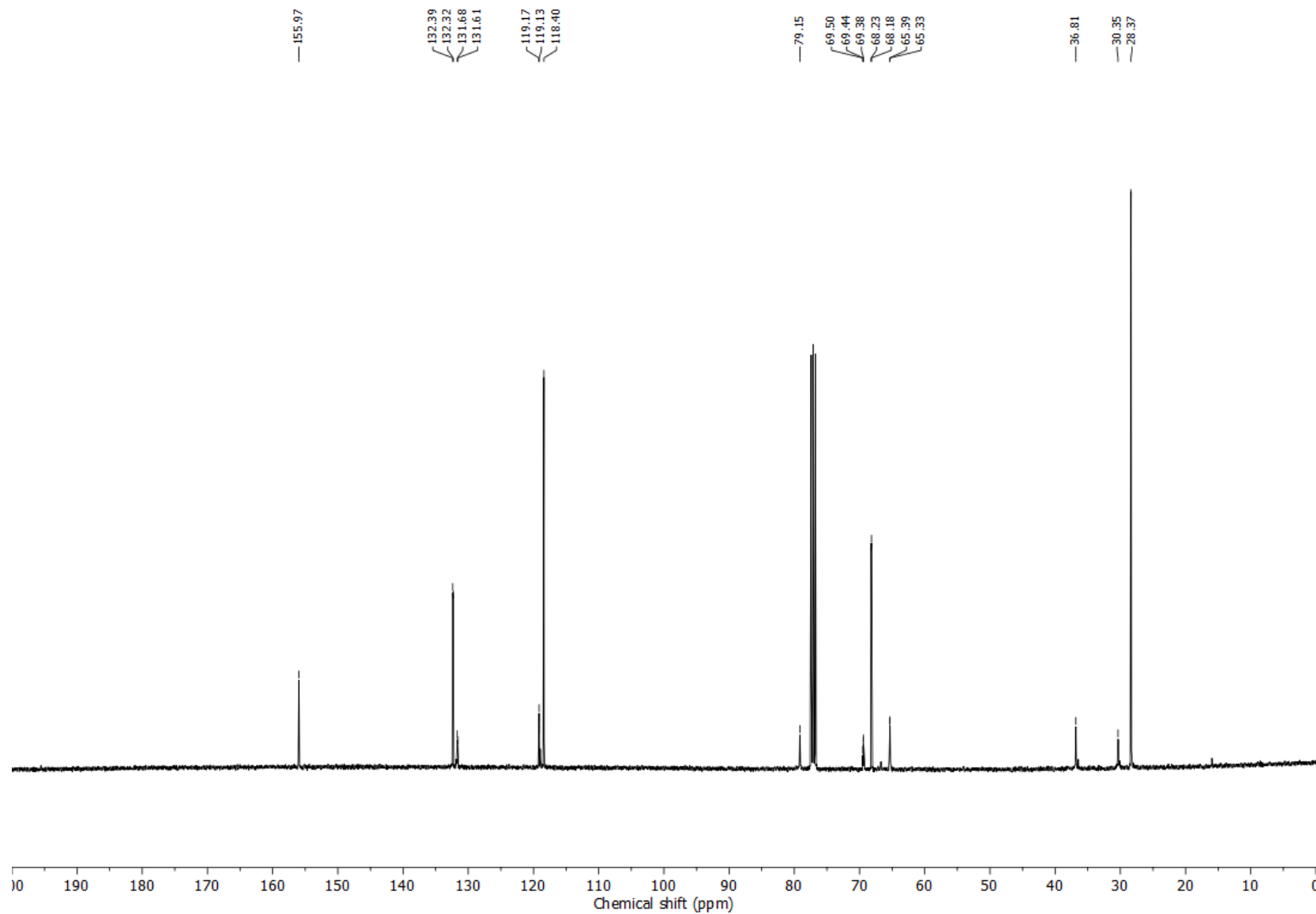
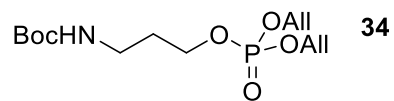
^{13}C NMR (100 MHz, CDCl_3)



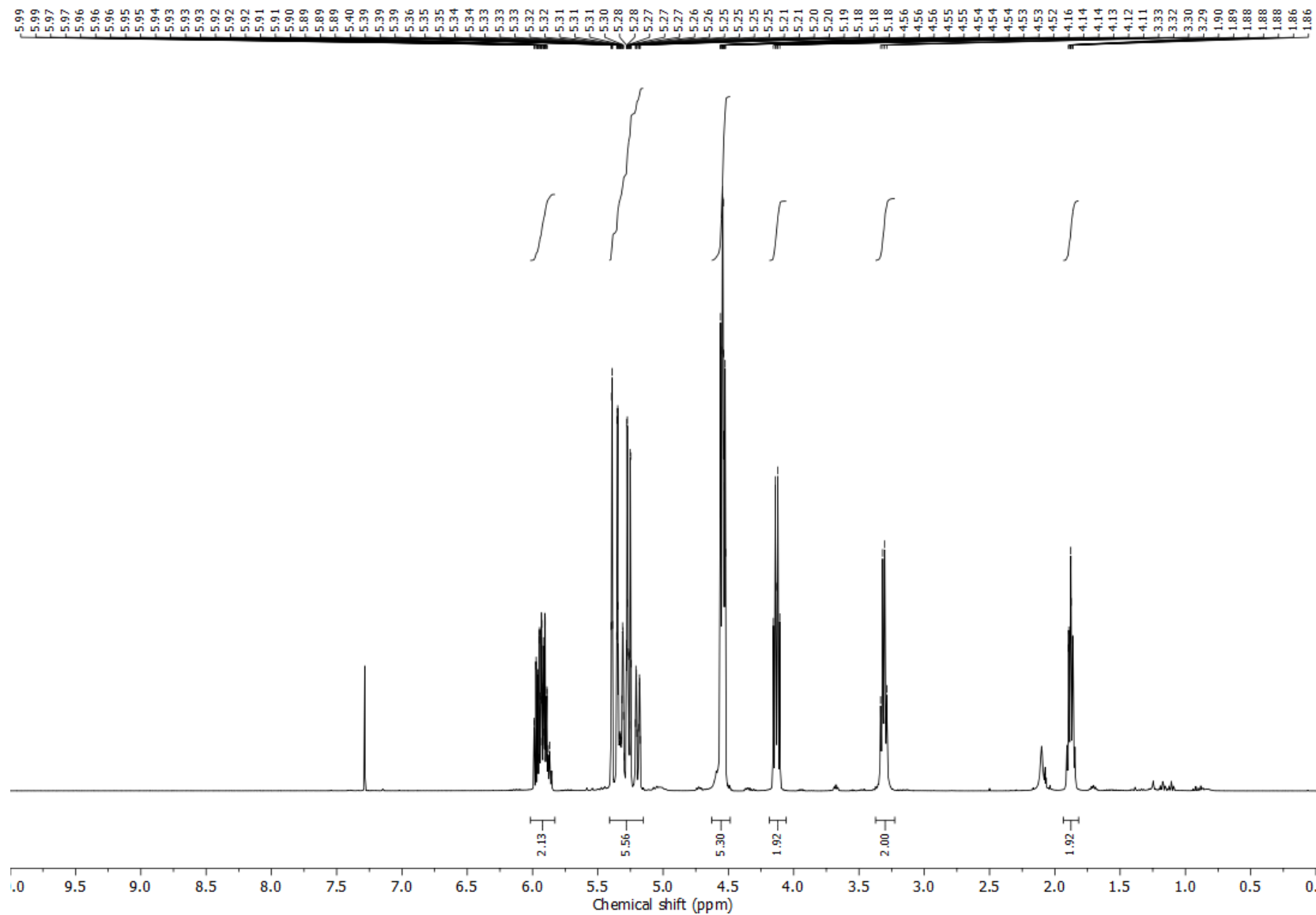
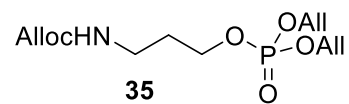
^1H NMR (400 MHz, CDCl_3)



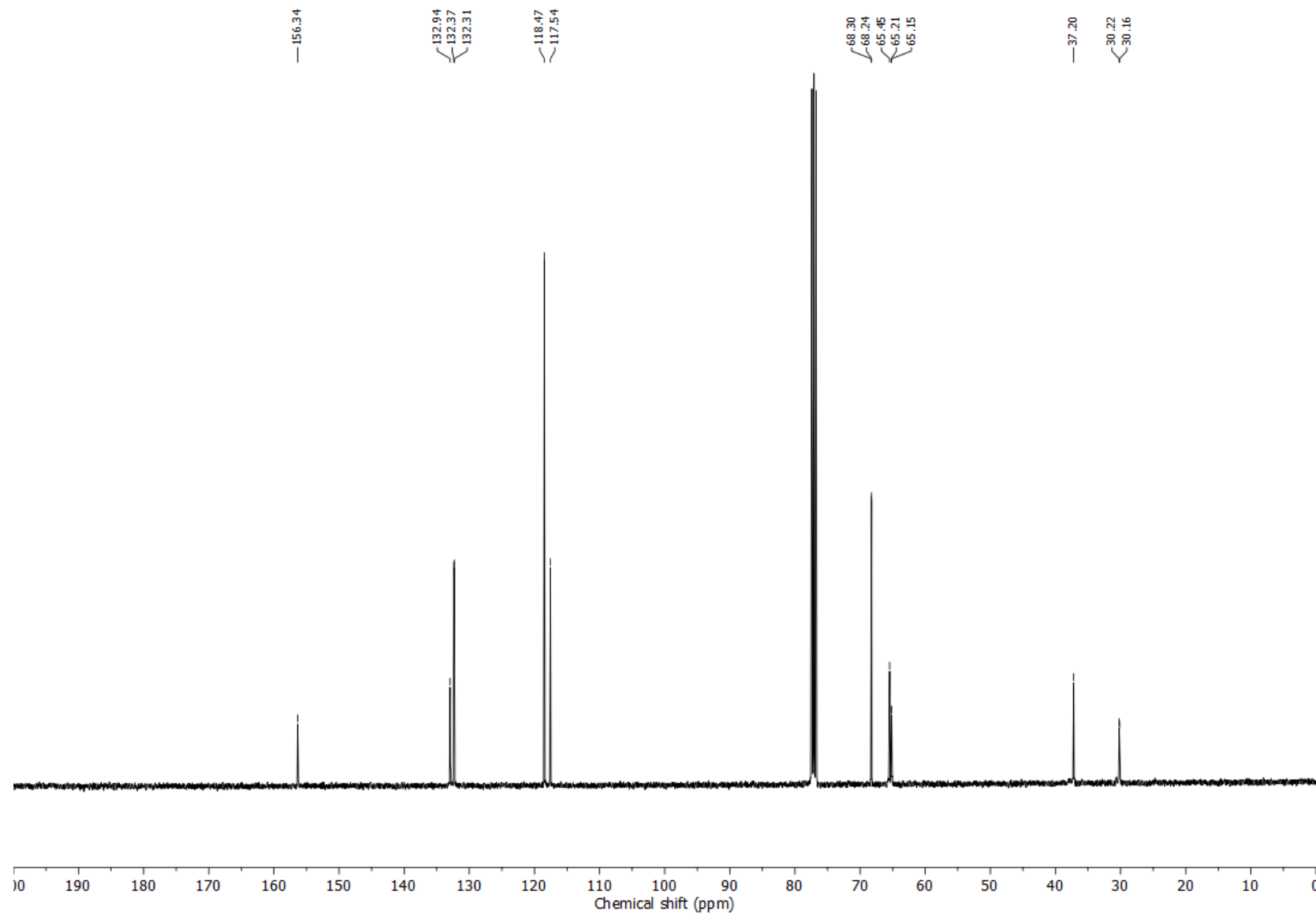
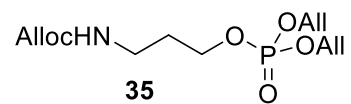
^{13}C NMR (100 MHz, CDCl_3)



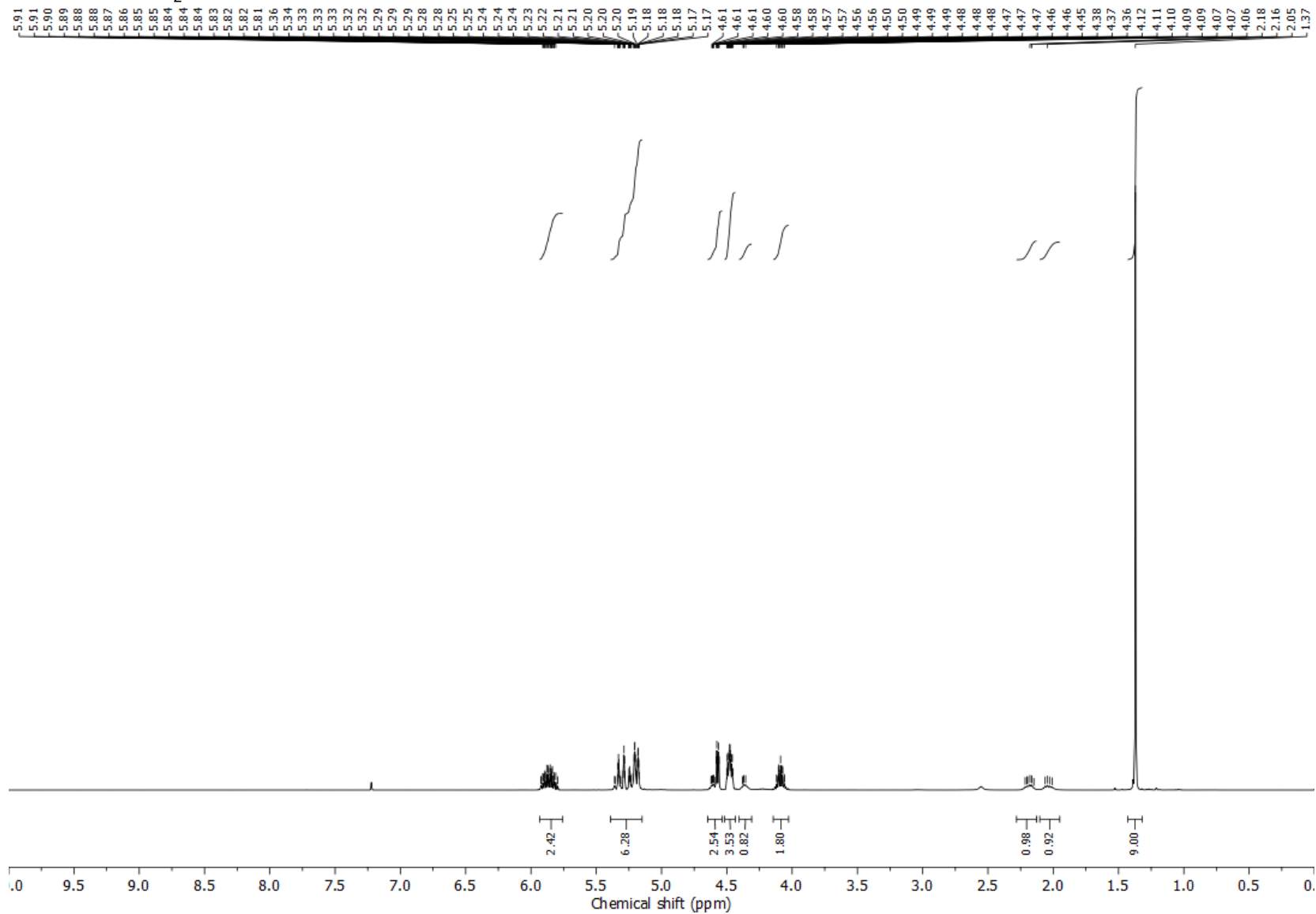
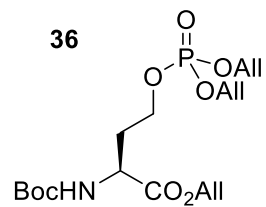
^1H NMR (400 MHz, CDCl_3)



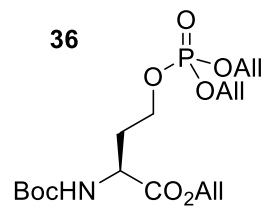
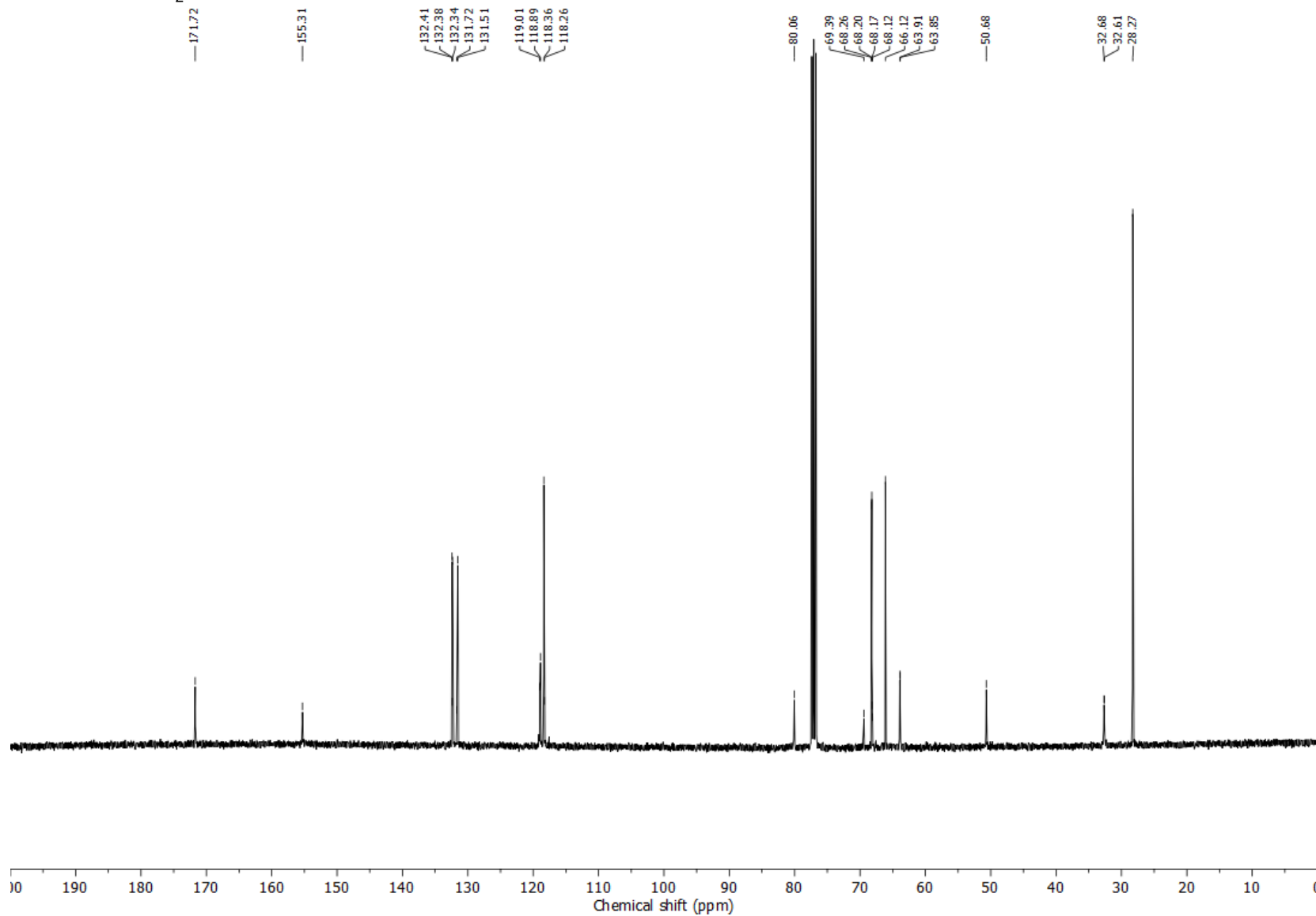
^{13}C NMR (100 MHz, CDCl_3)



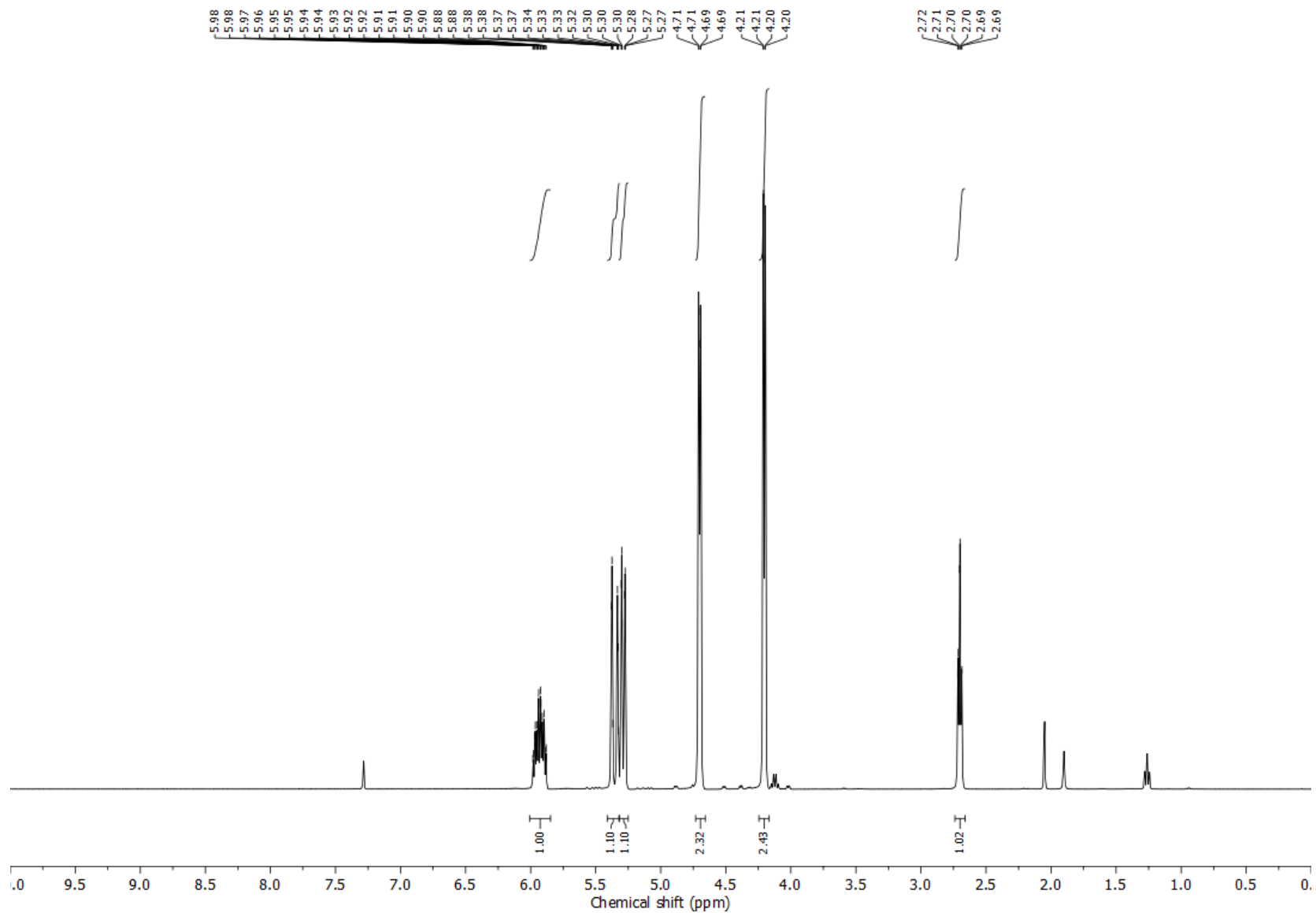
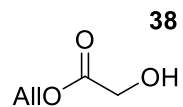
36

 ^1H NMR (400 MHz, CDCl_3)

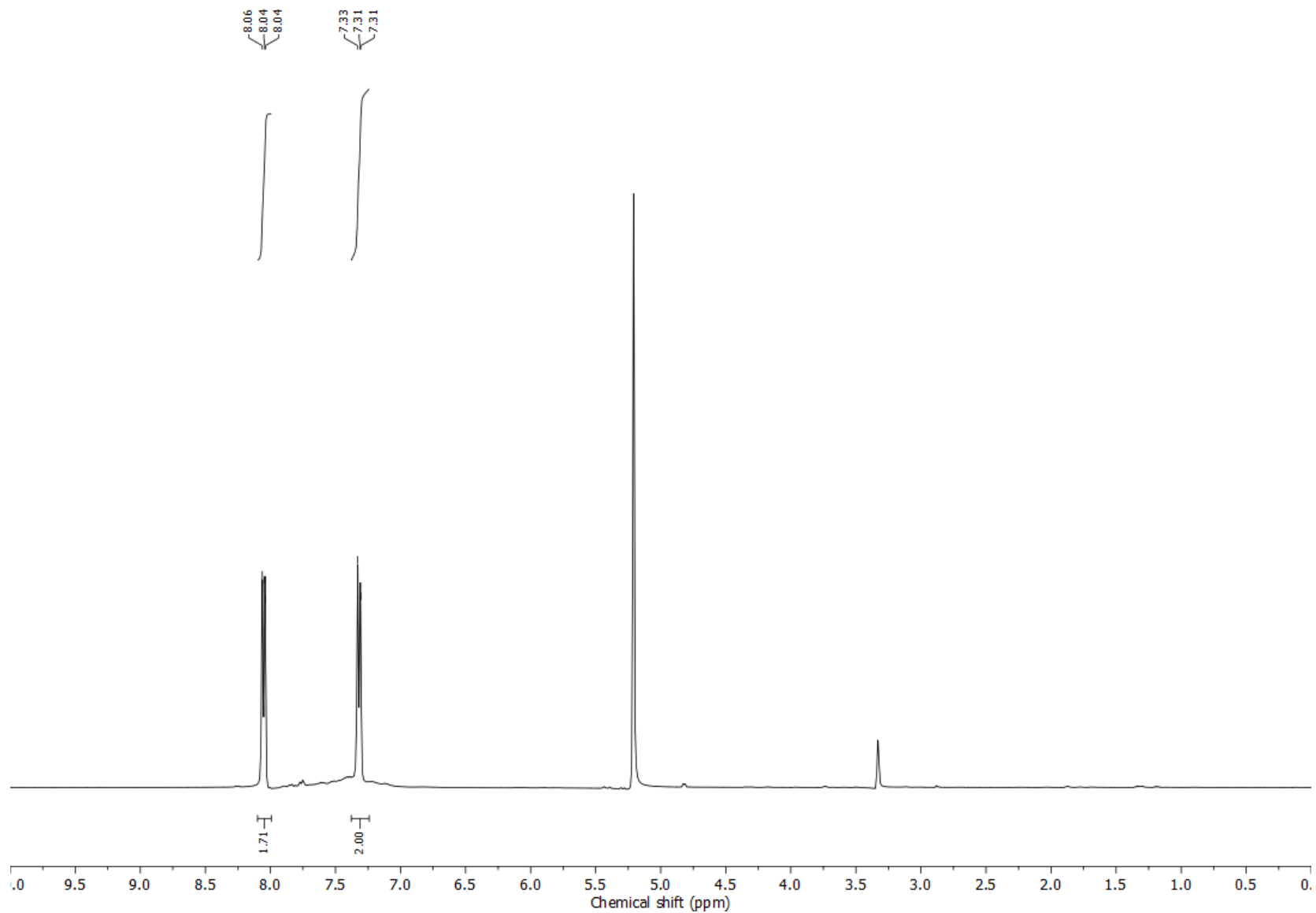
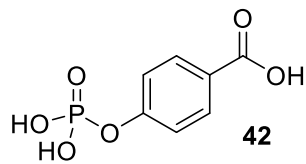
36

 ^{13}C NMR (100 MHz, CDCl₃)

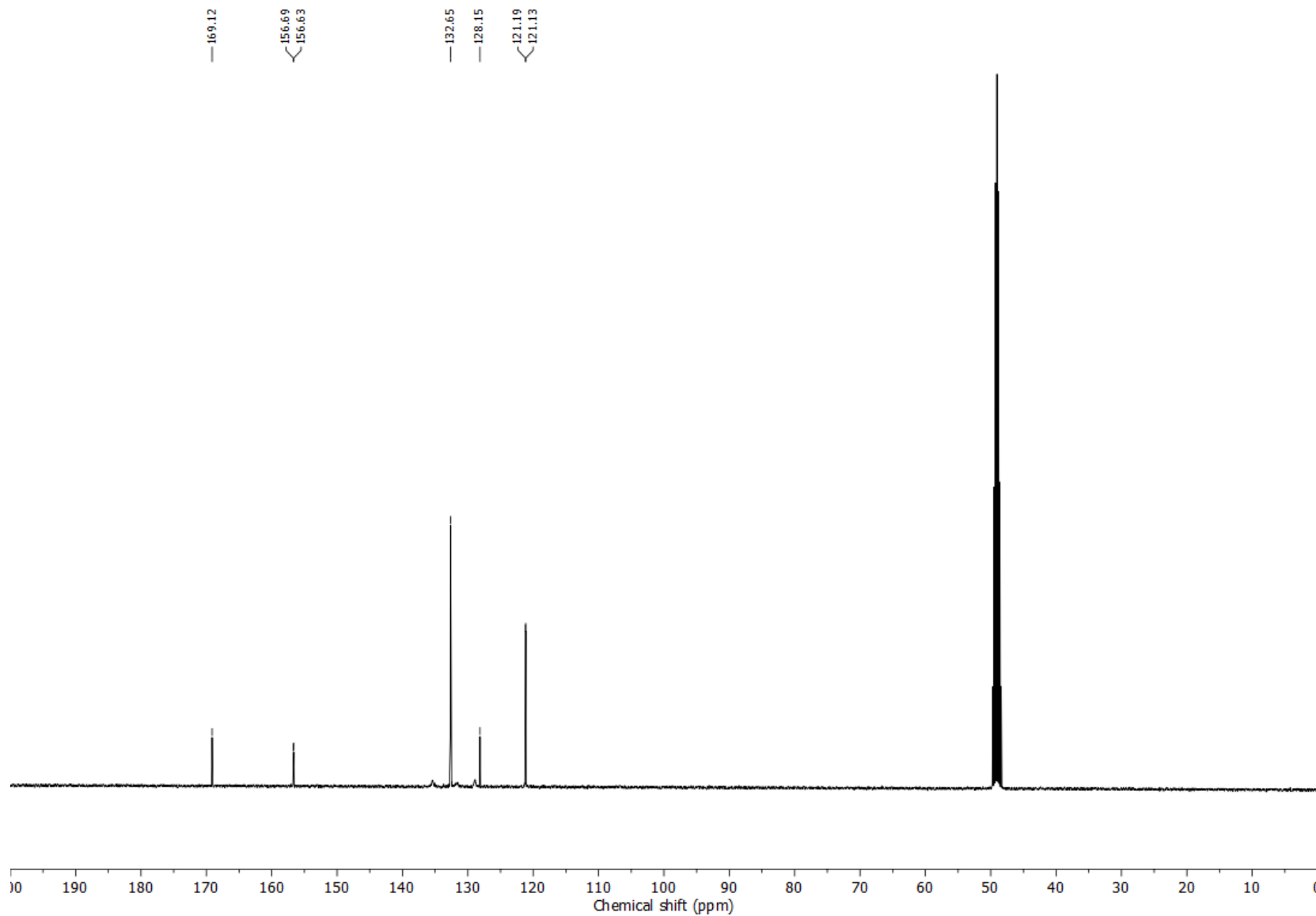
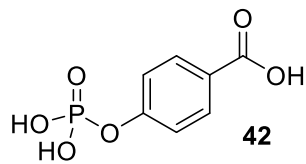
^1H NMR (400 MHz, CDCl_3)



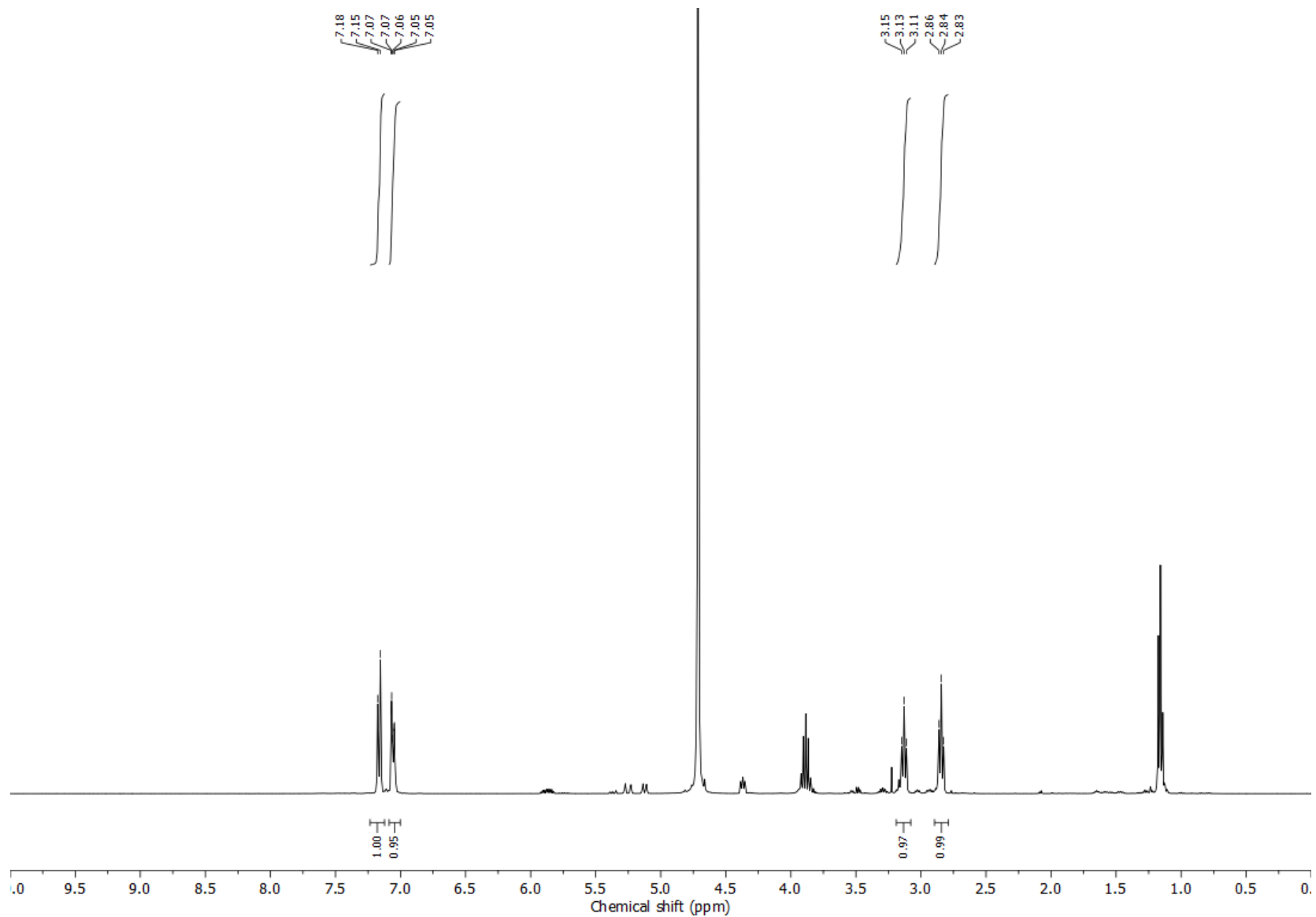
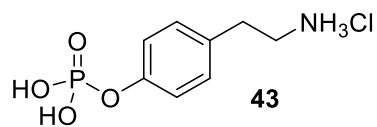
^1H NMR (400 MHz, MeOD)



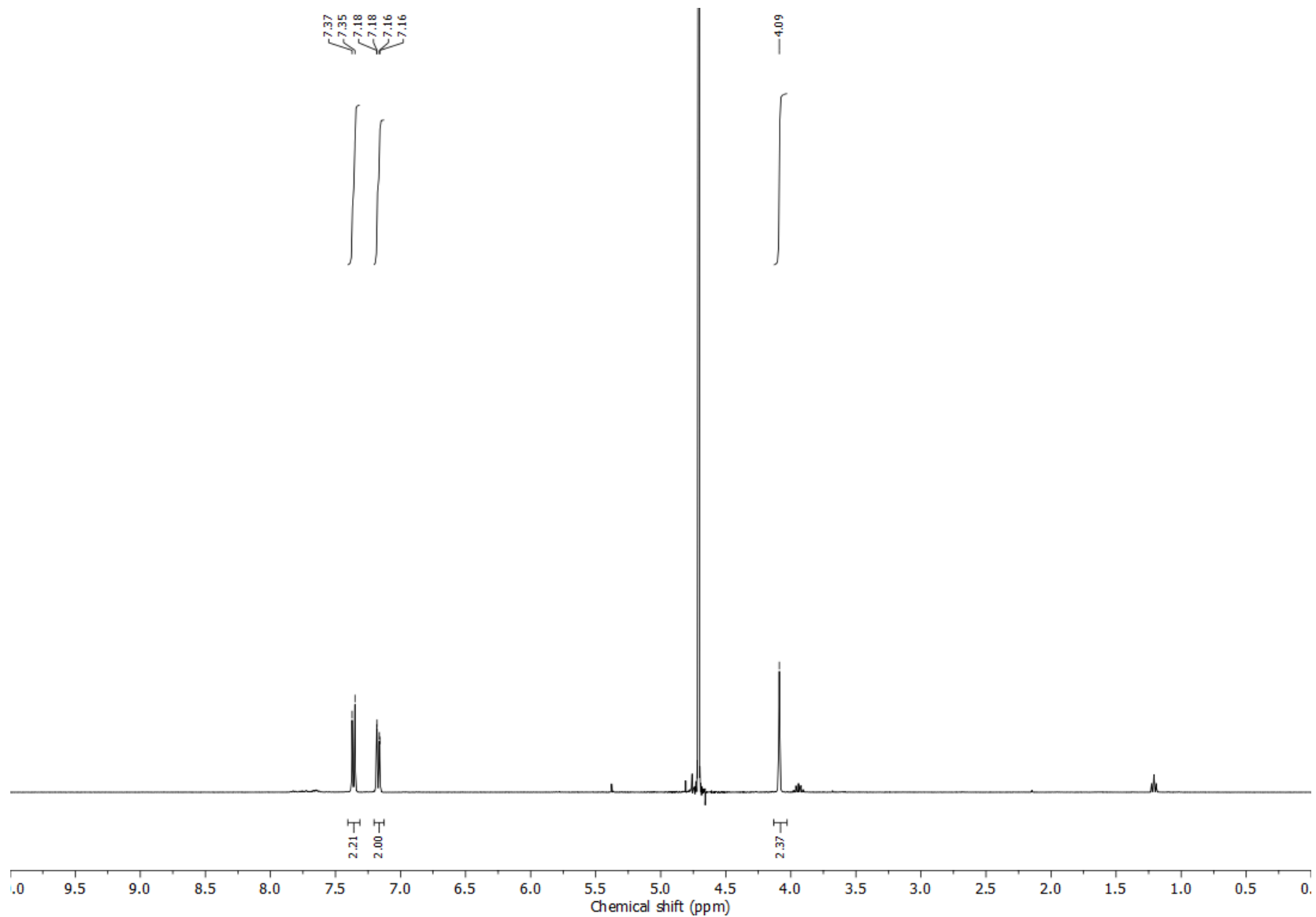
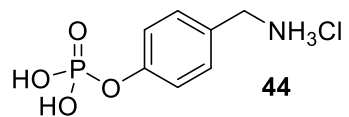
^{13}C NMR (100 MHz, MeOD)



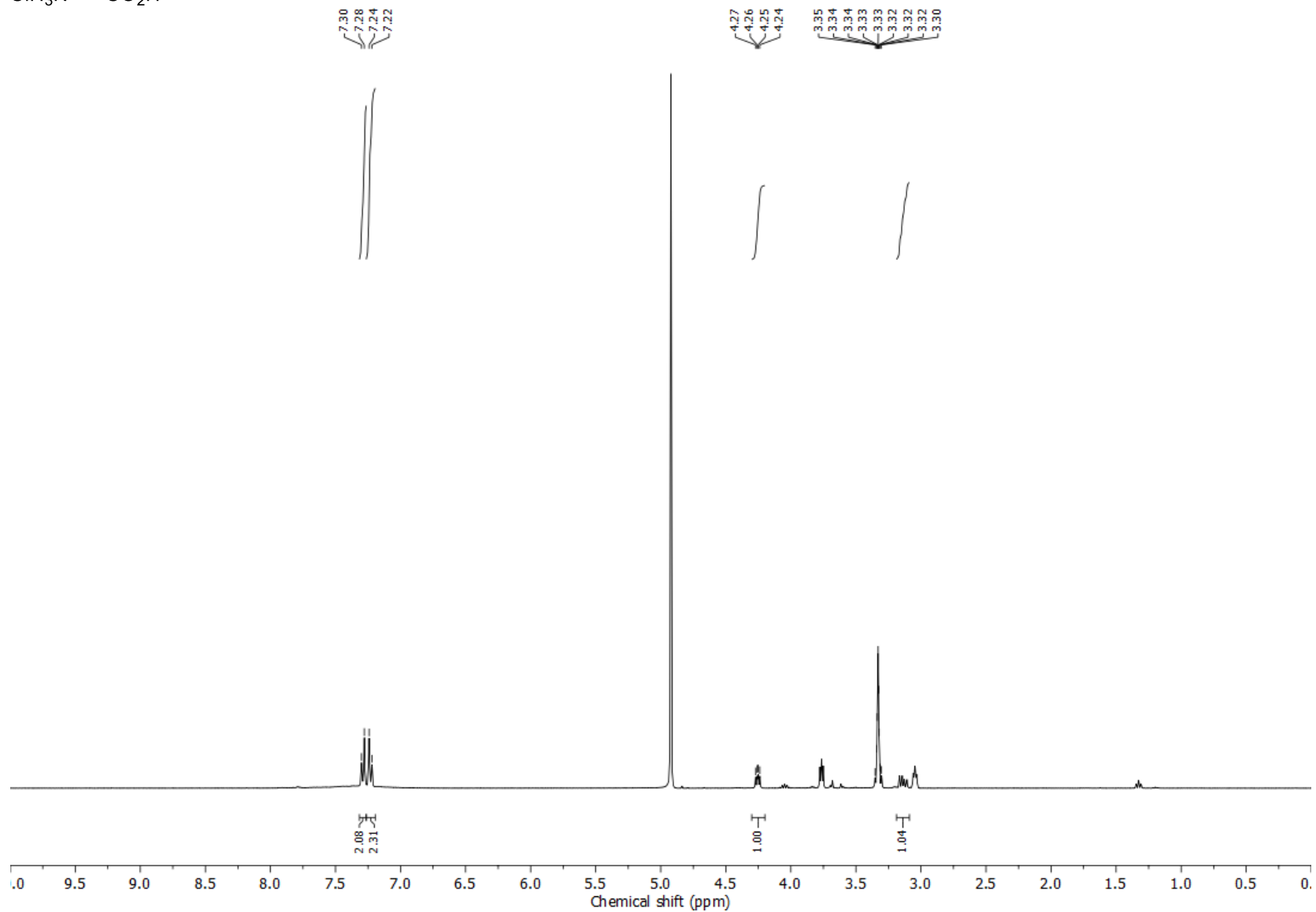
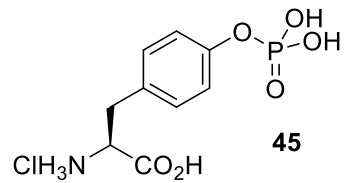
^1H NMR (400 MHz, D_2O)



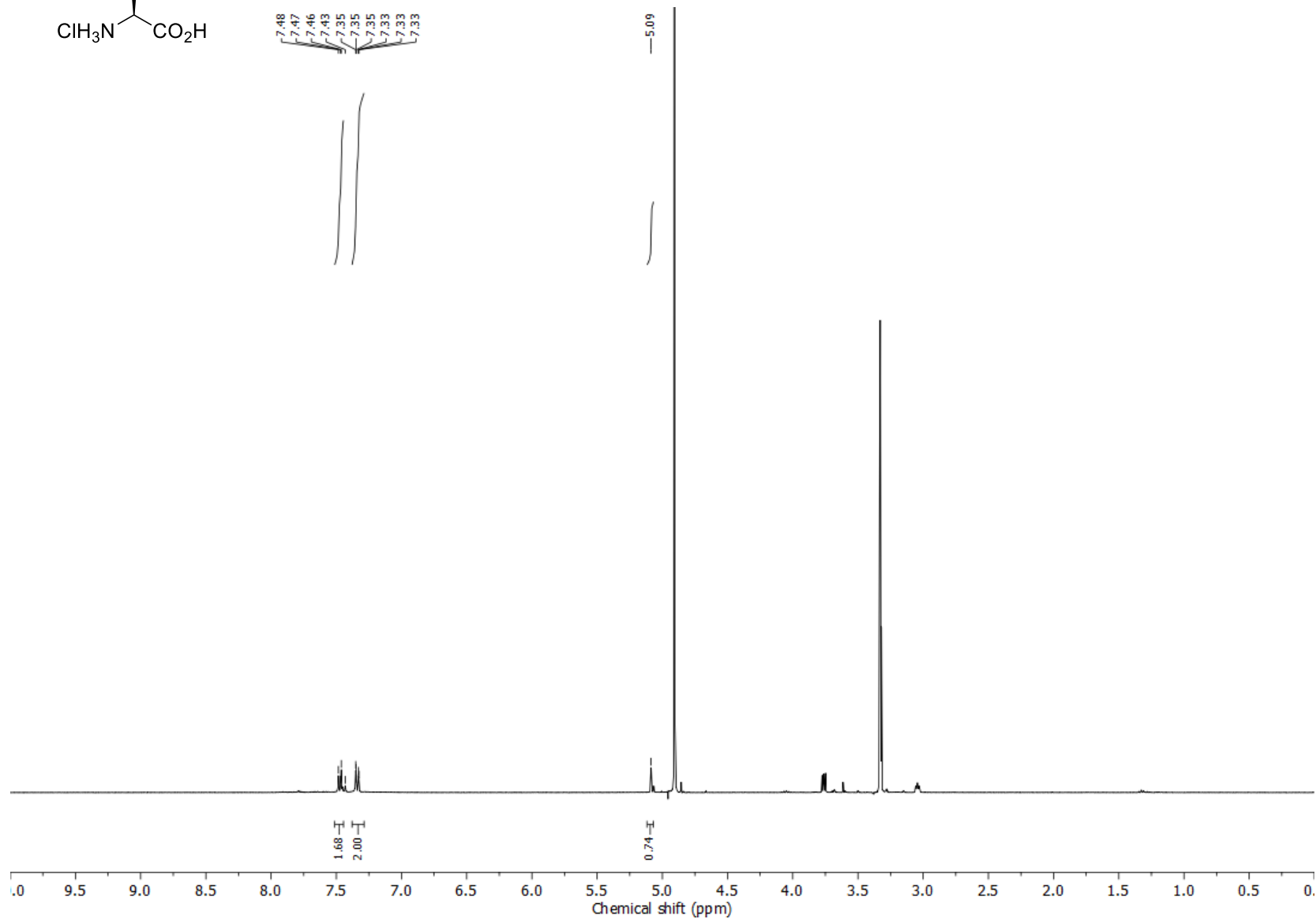
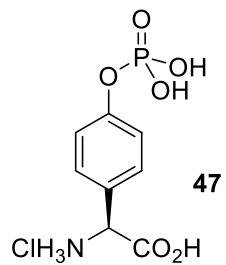
^1H NMR (400 MHz, D_2O)



^1H NMR (400 MHz, MeOD)

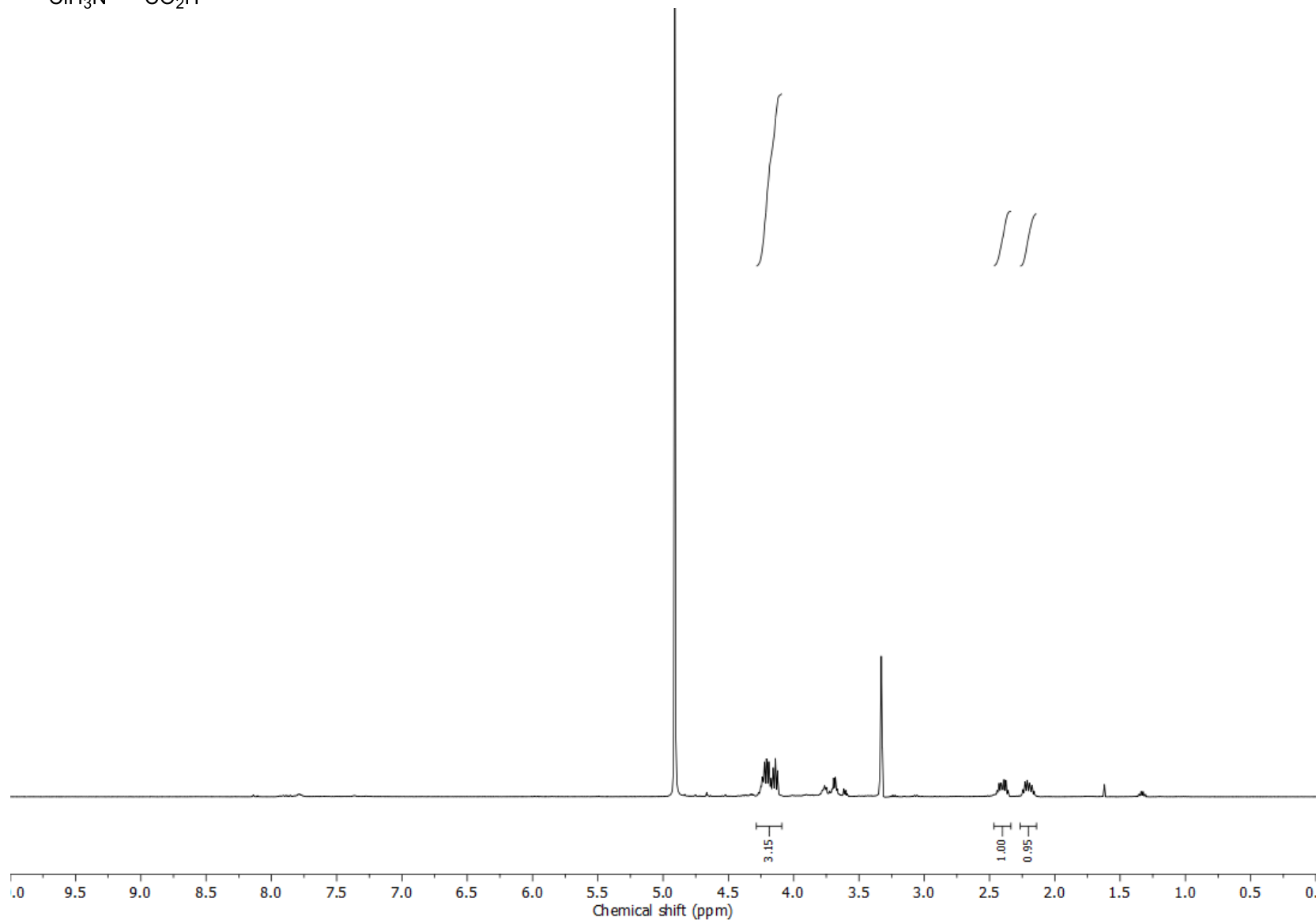
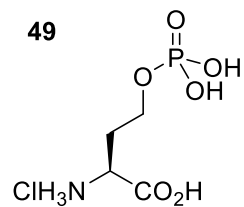


^1H NMR (400 MHz, MeOD)



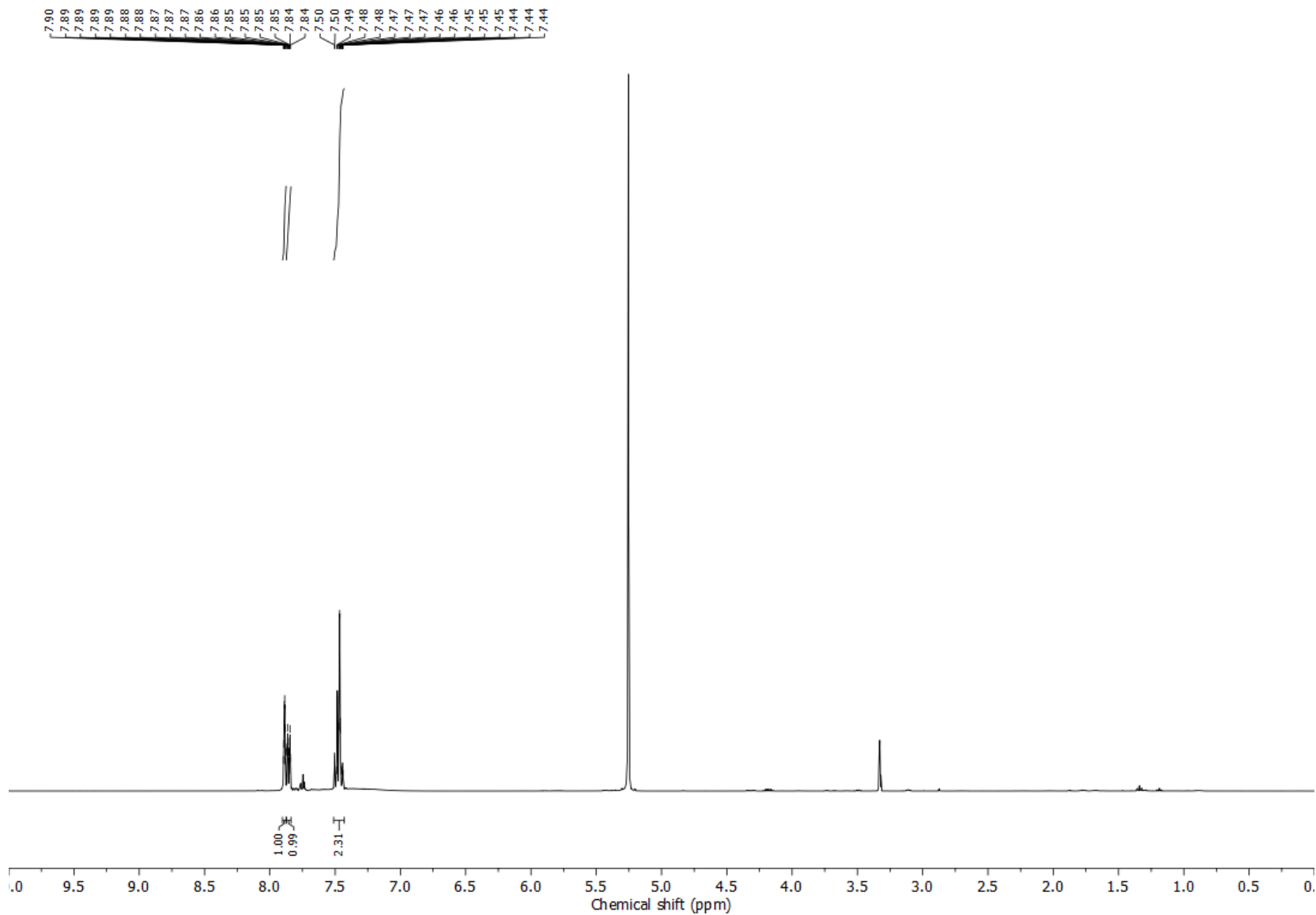
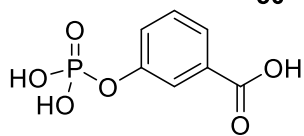
49

^1H NMR (400 MHz, D_2O)

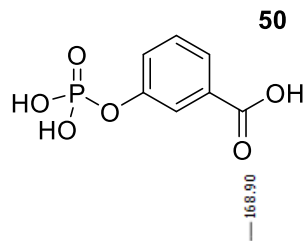


^1H NMR (400 MHz, MeOD)

50

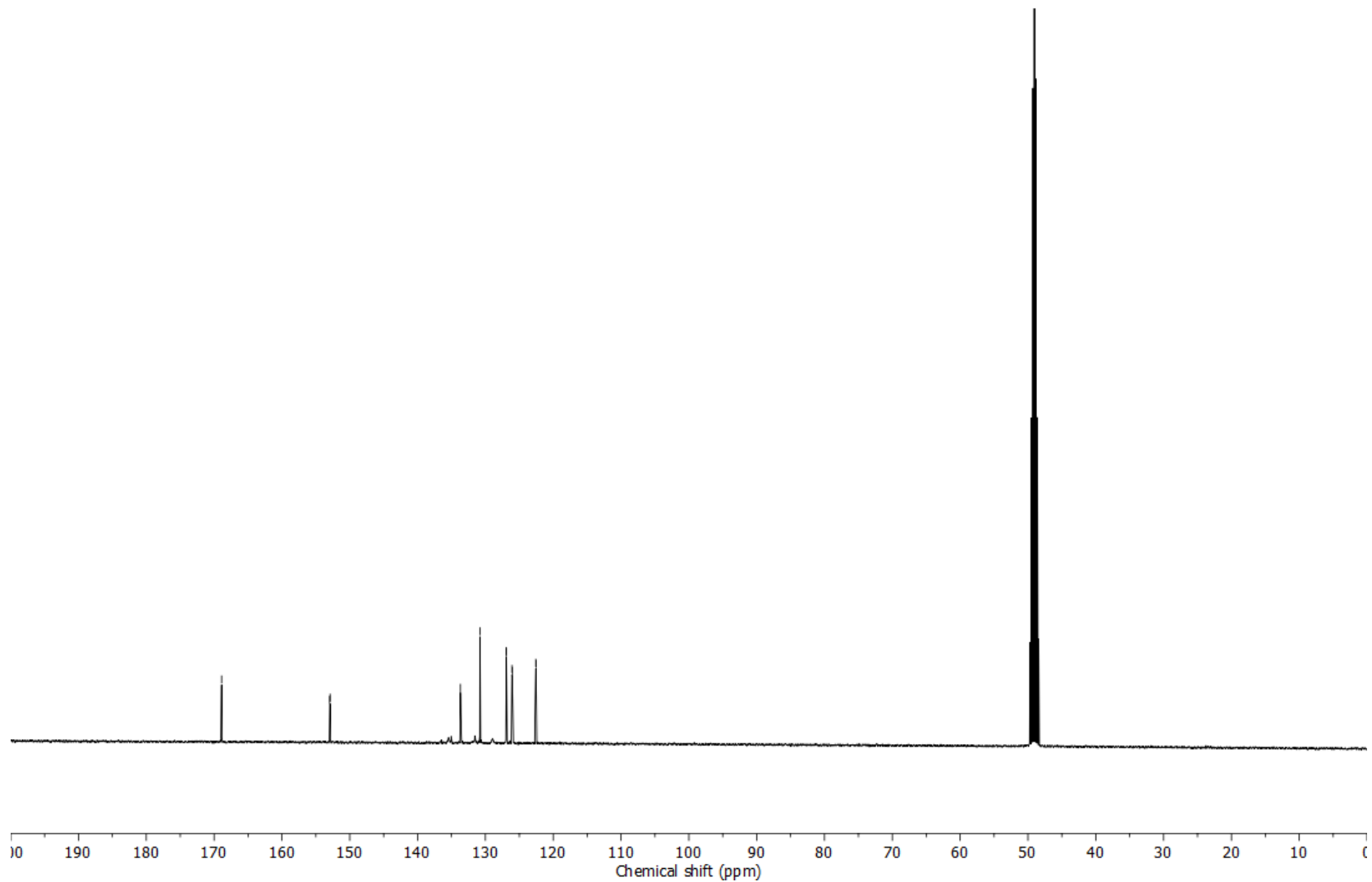


^{13}C NMR (100 MHz, MeOD)

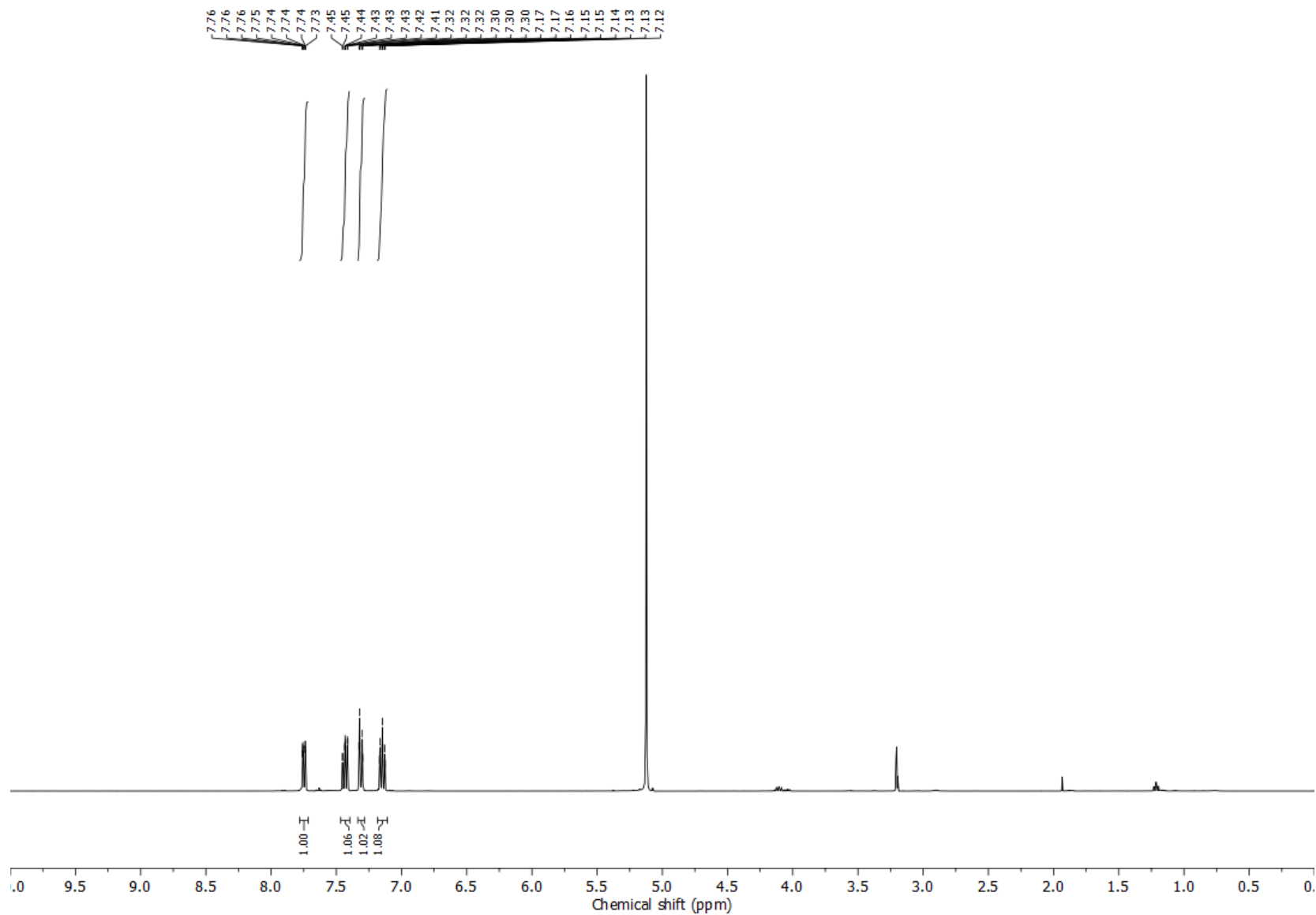
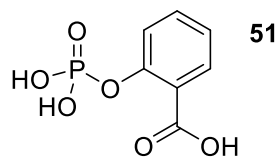


152.97
152.91

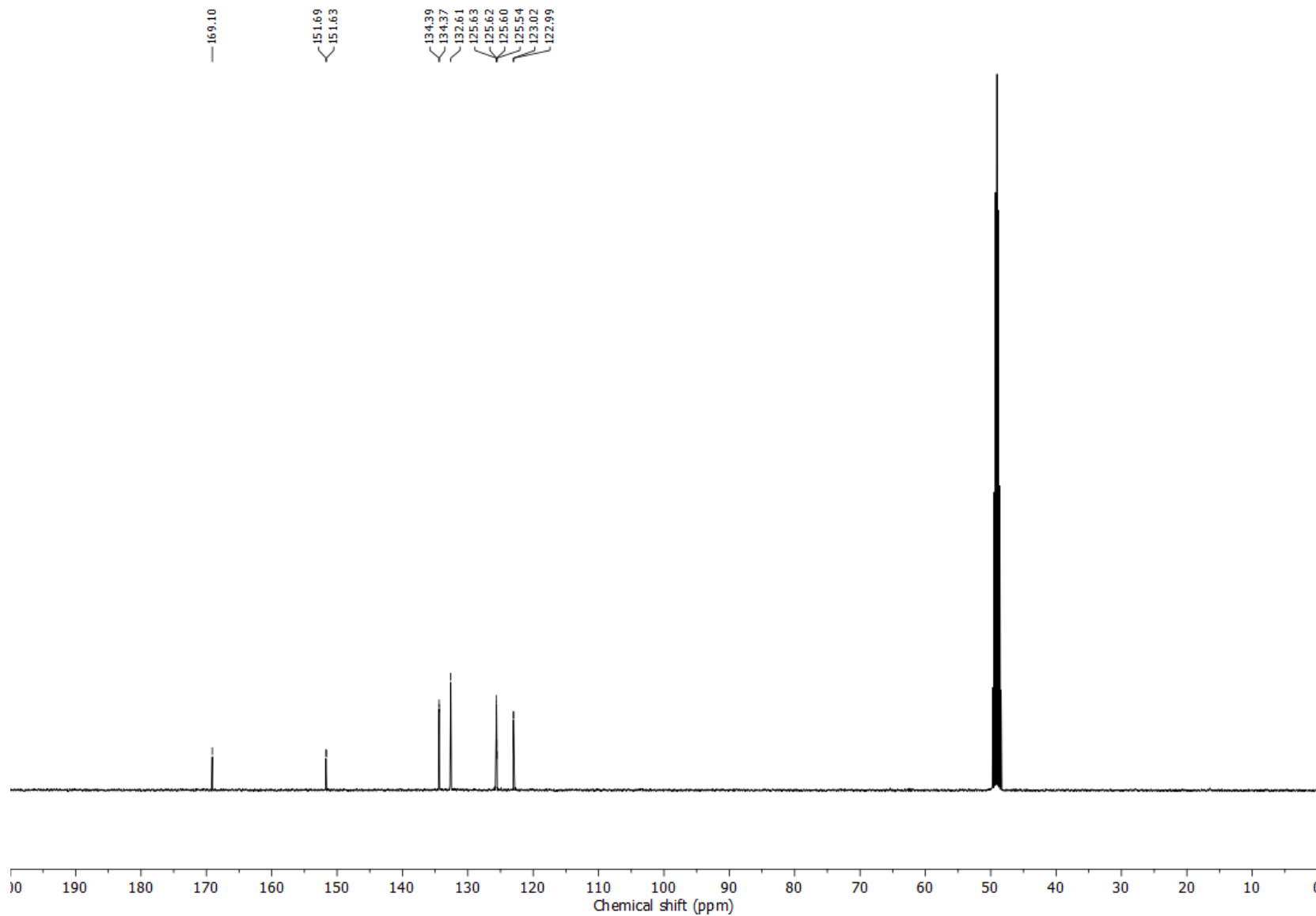
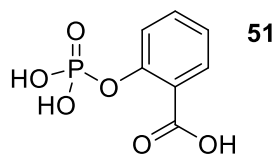
133.68
130.79
128.92
126.90
126.08
126.03
122.58
122.53



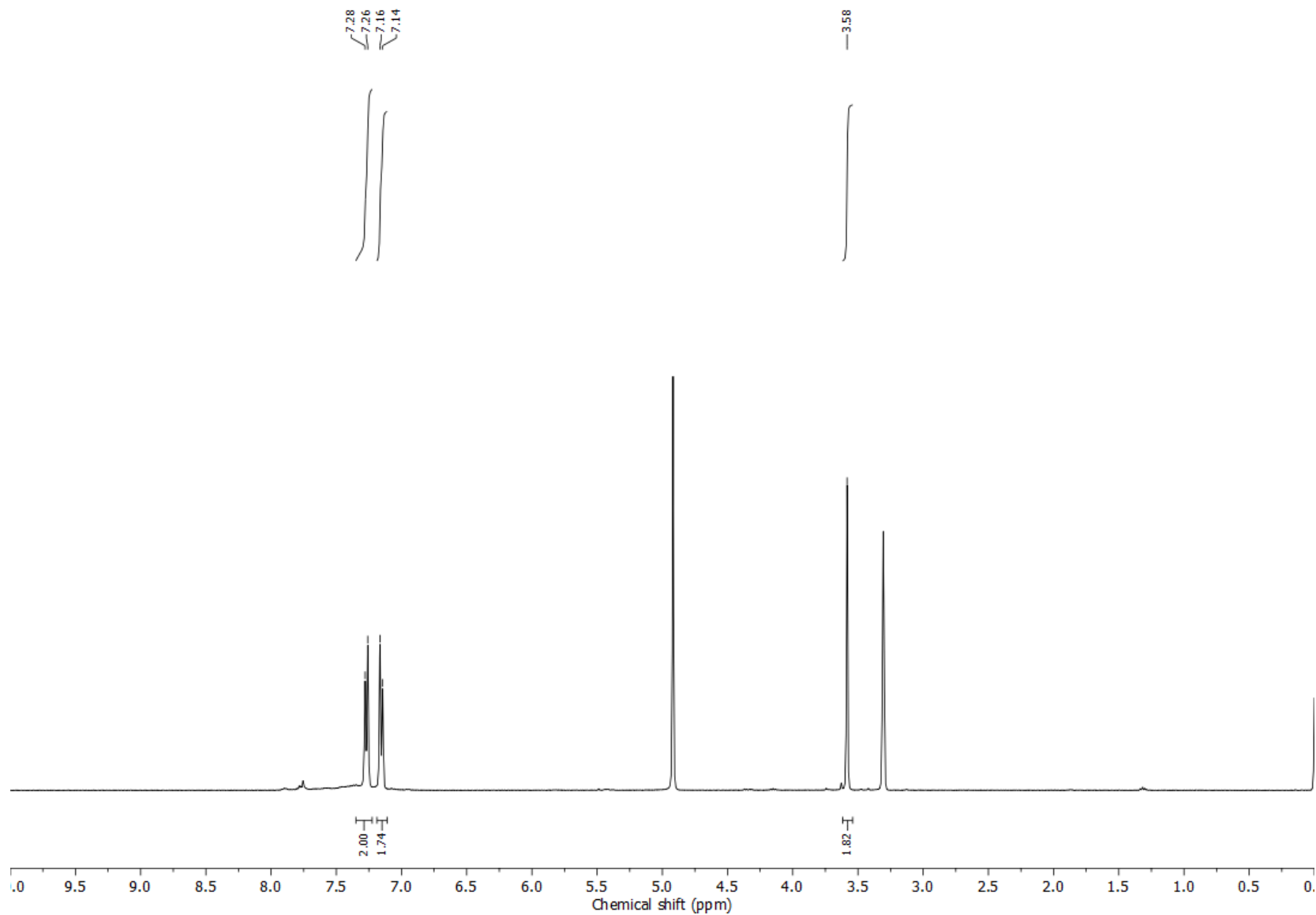
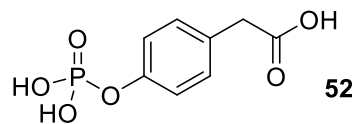
^1H NMR (400 MHz, MeOD)



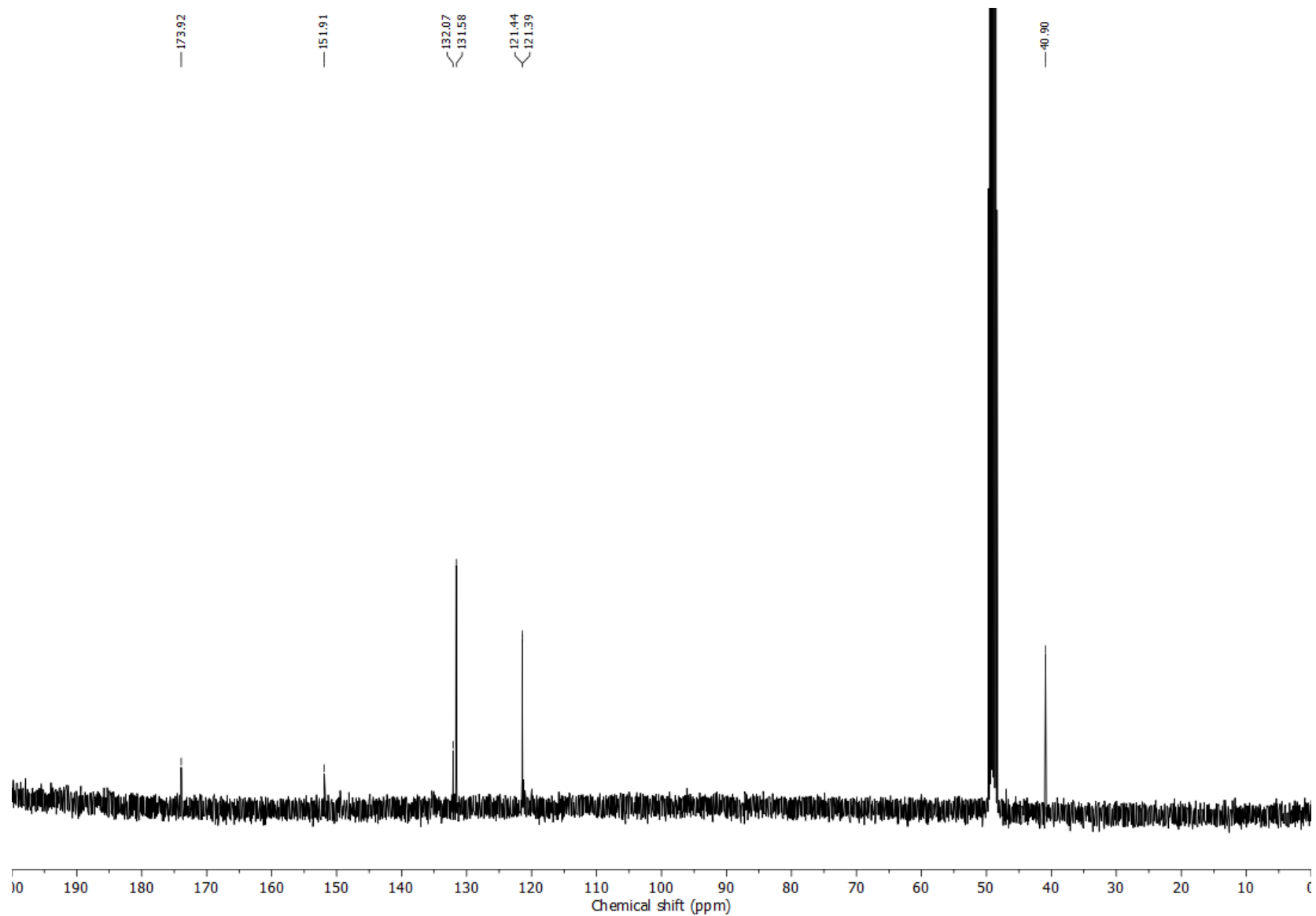
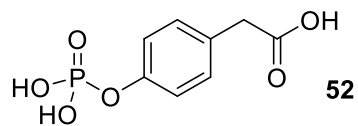
^{13}C NMR (100 MHz, MeOD)



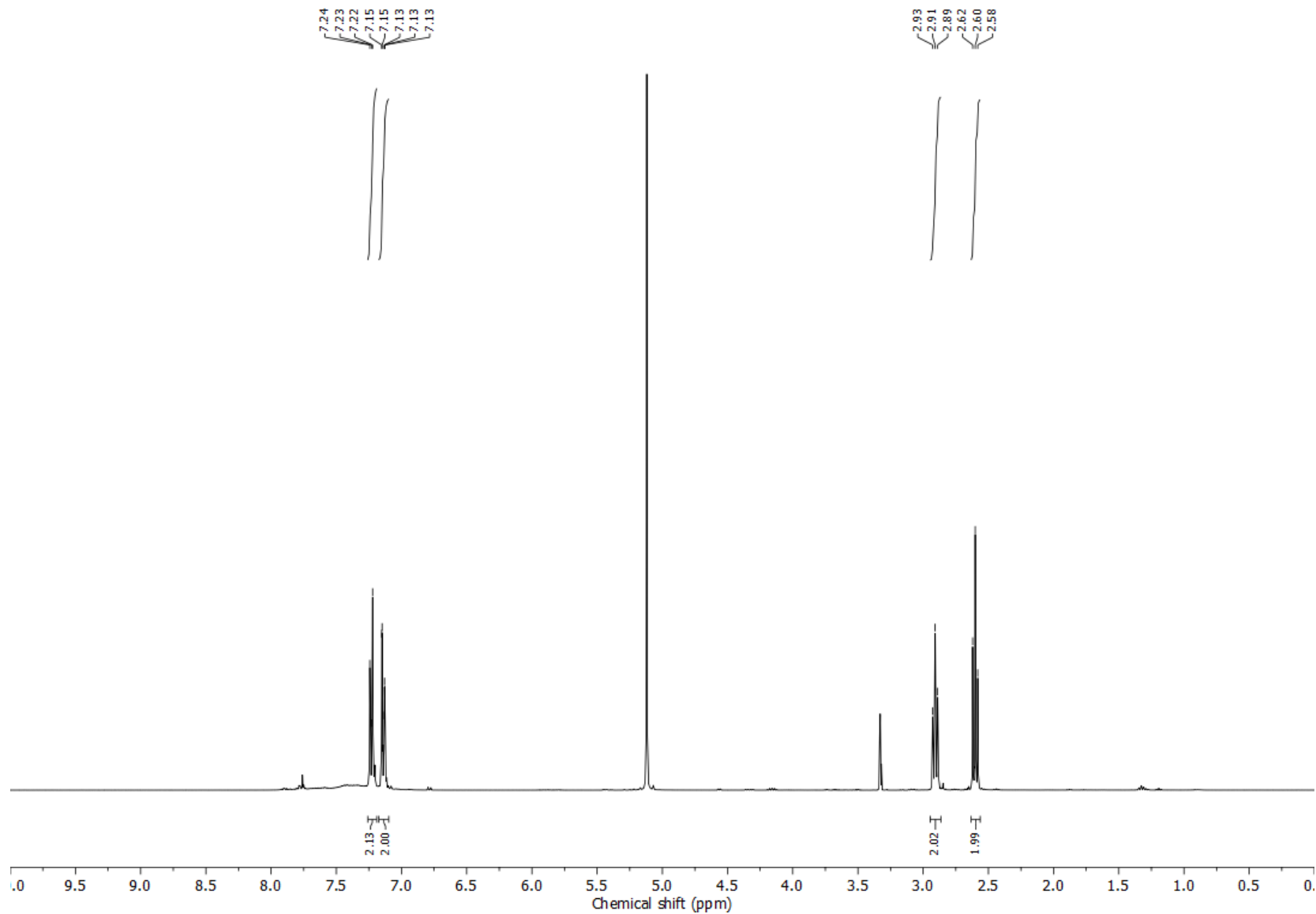
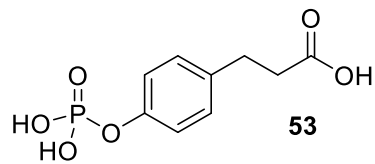
^1H NMR (400 MHz, MeOD)



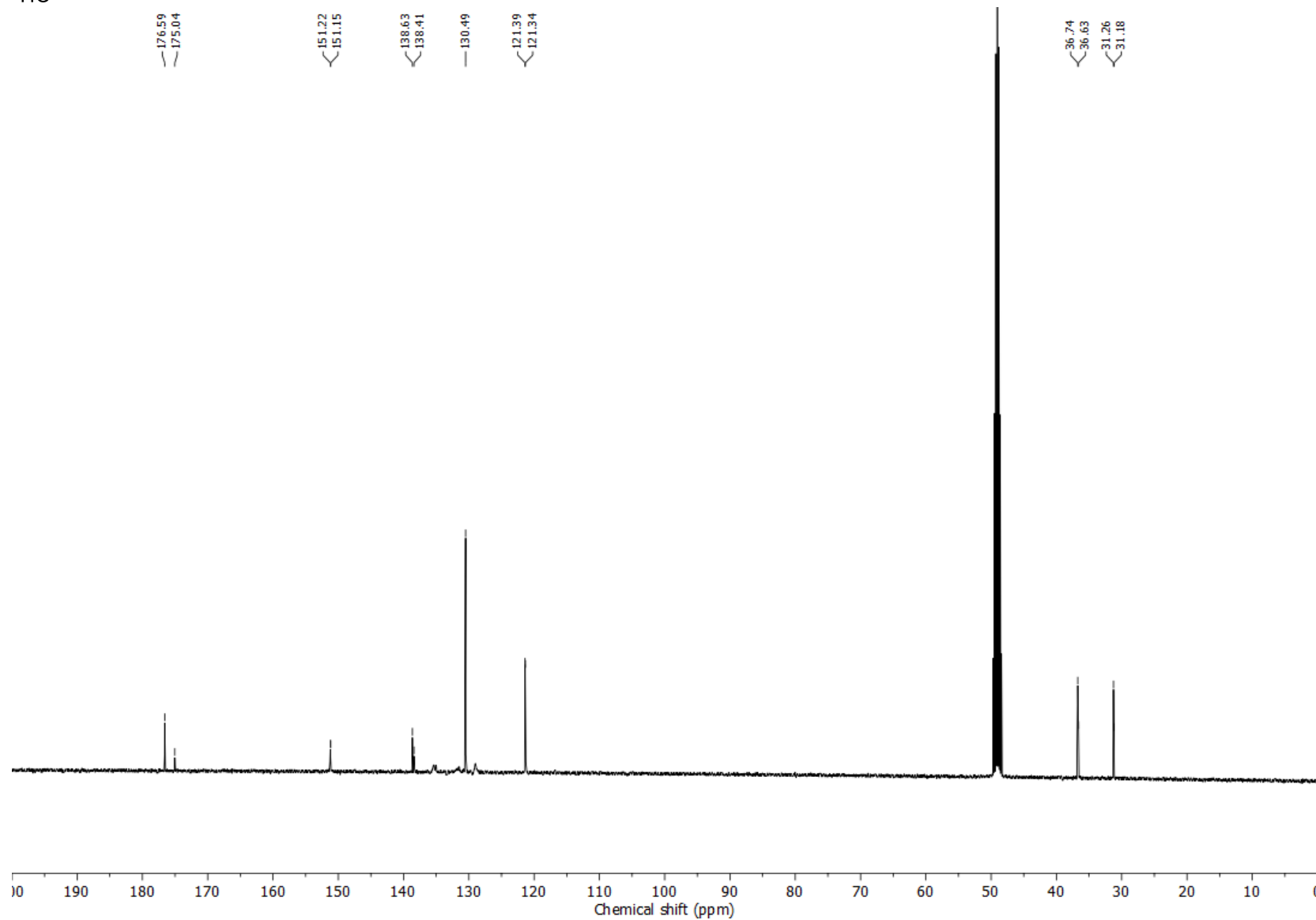
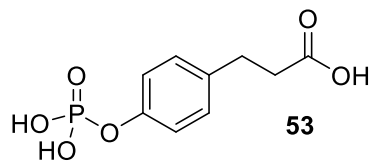
^{13}C NMR (100 MHz, MeOD)



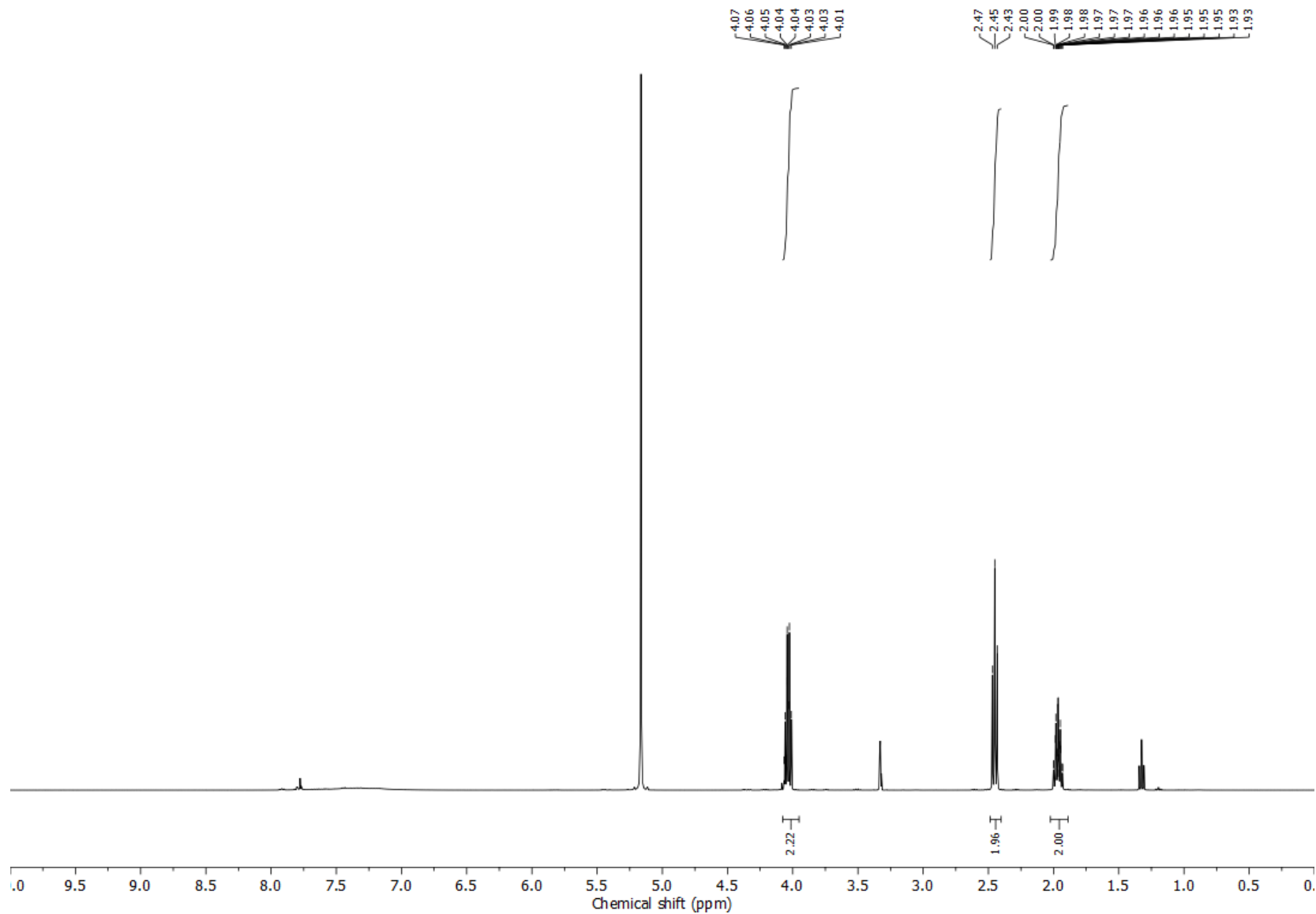
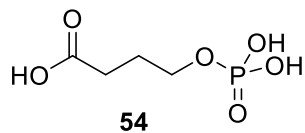
^1H NMR (400 MHz, MeOD)



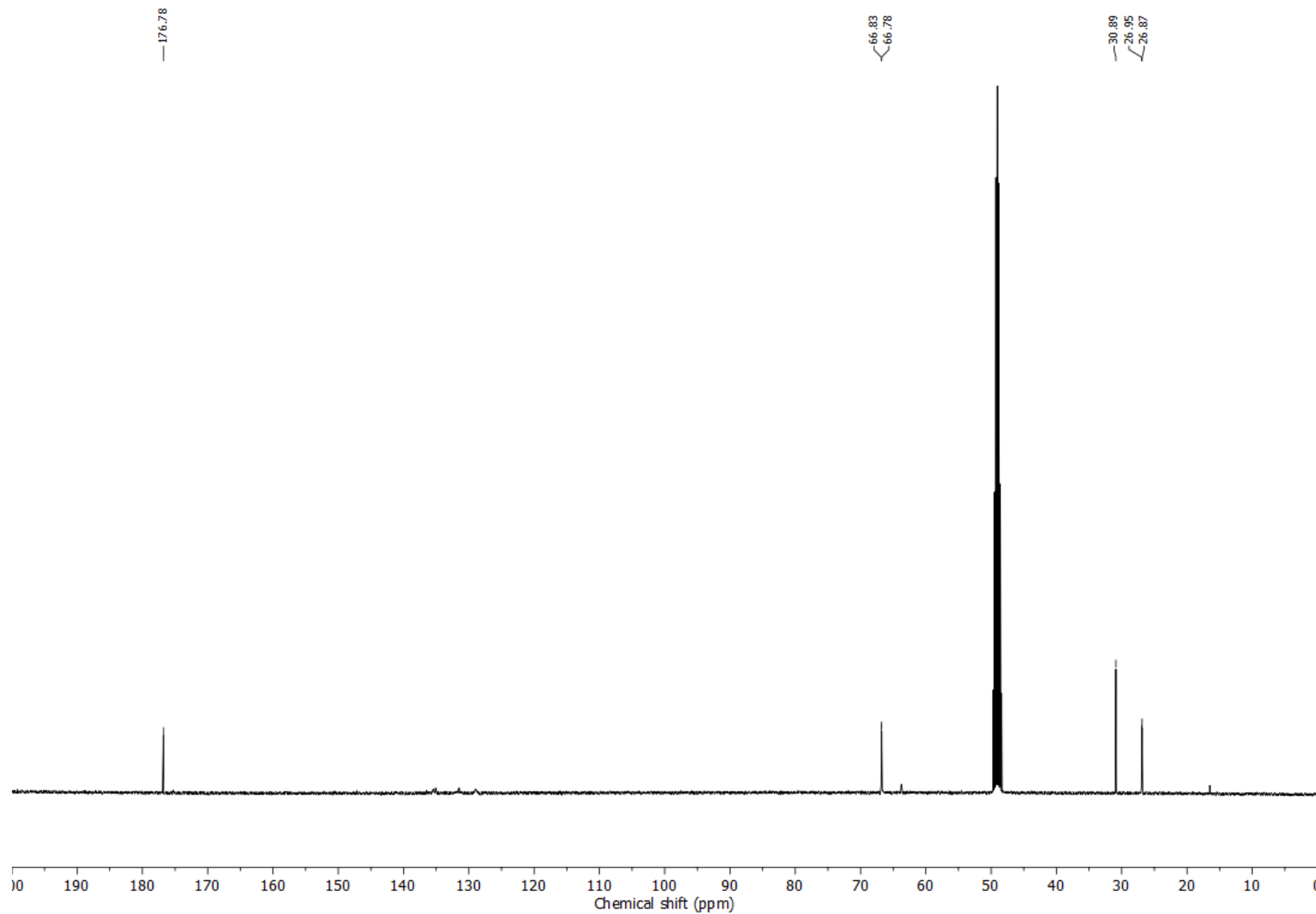
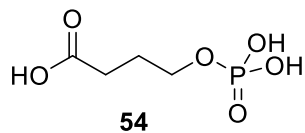
^{13}C NMR (100 MHz, MeOD)



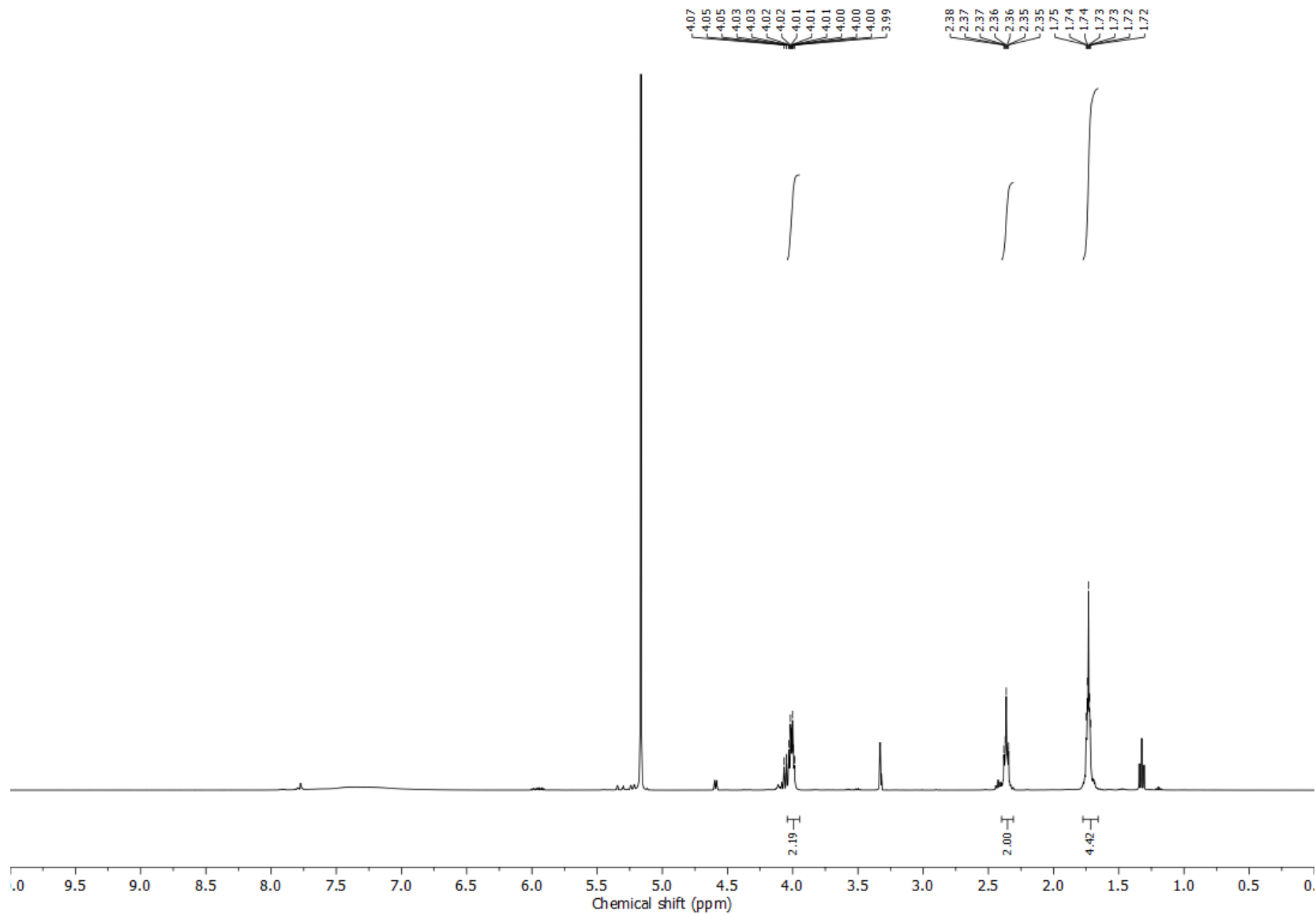
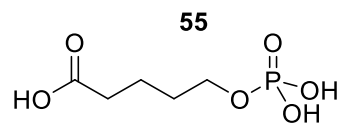
^1H NMR (400 MHz, MeOD)



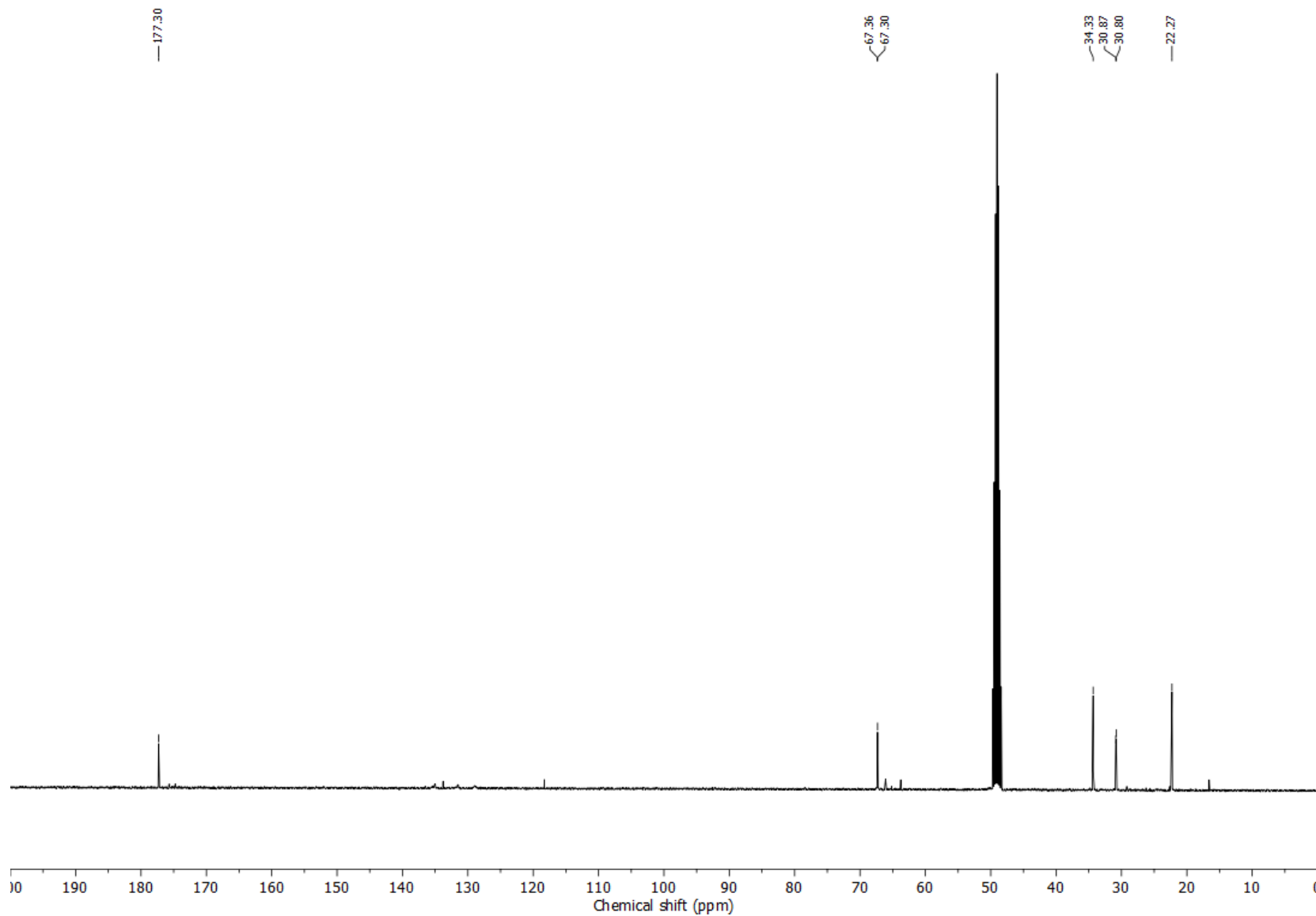
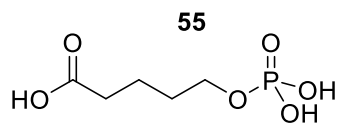
^{13}C NMR (100 MHz, MeOD)



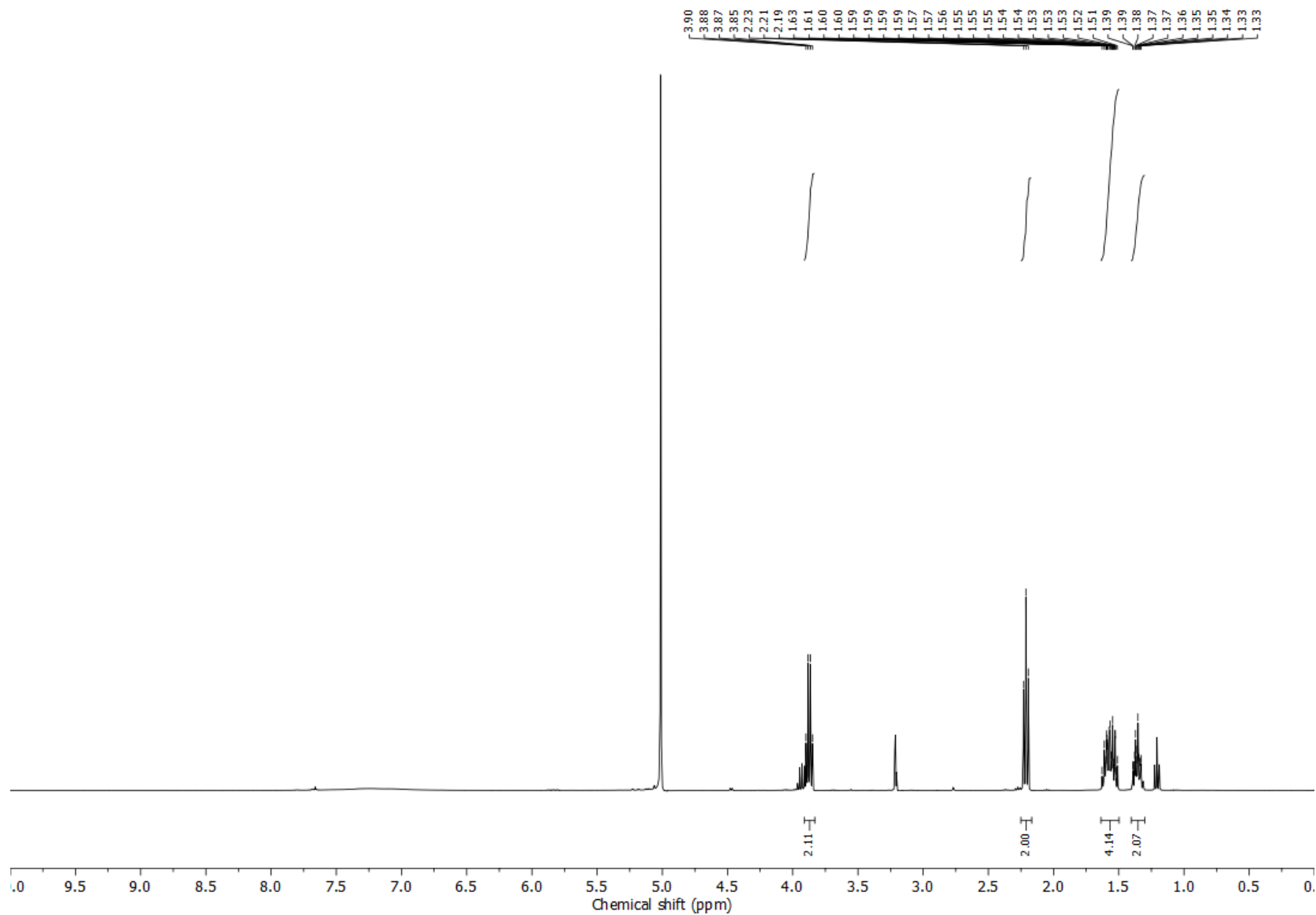
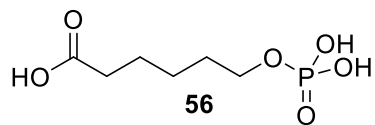
^1H NMR (400 MHz, MeOD)



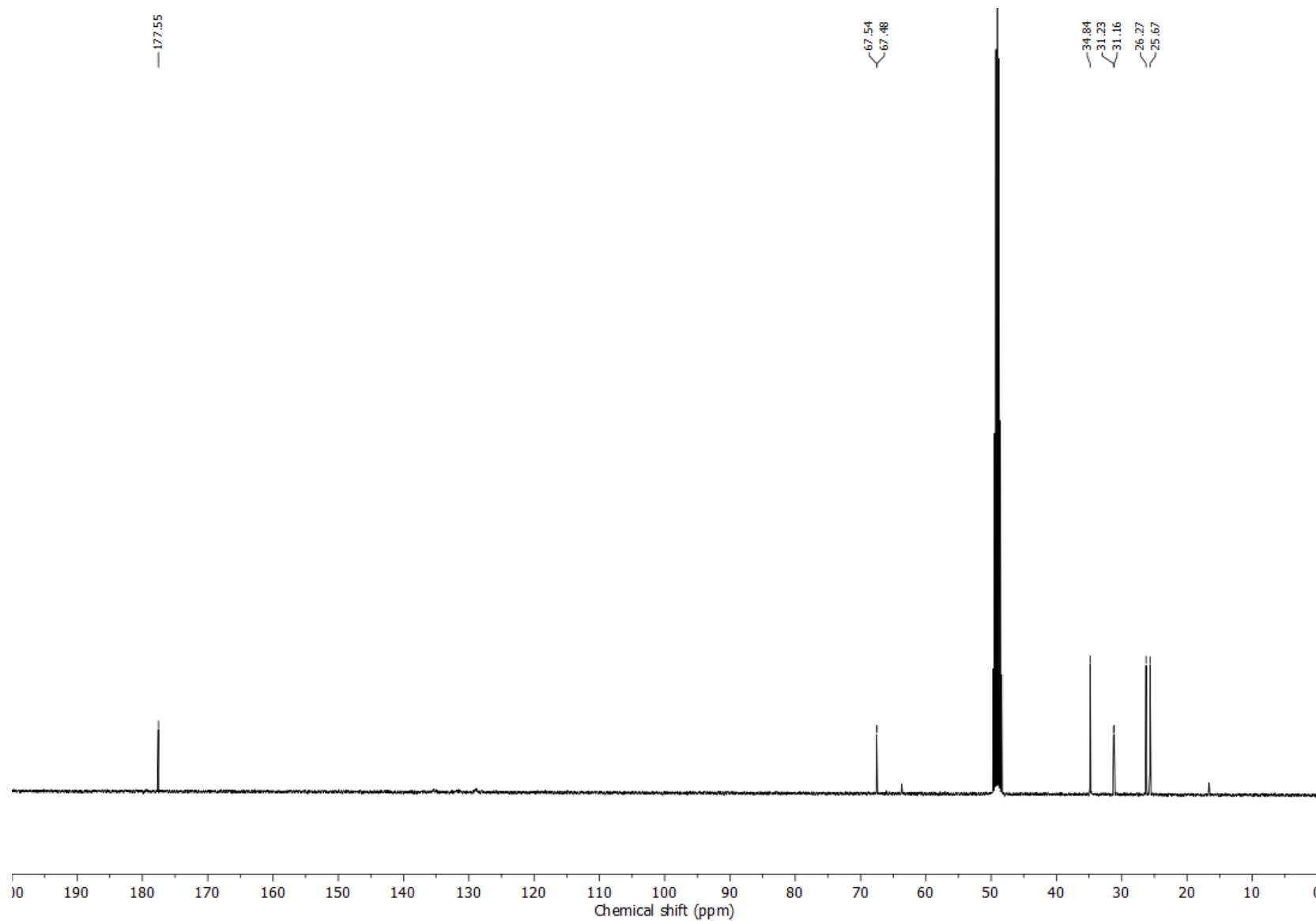
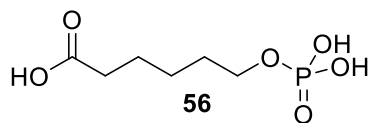
^{13}C NMR (100 MHz, MeOD)



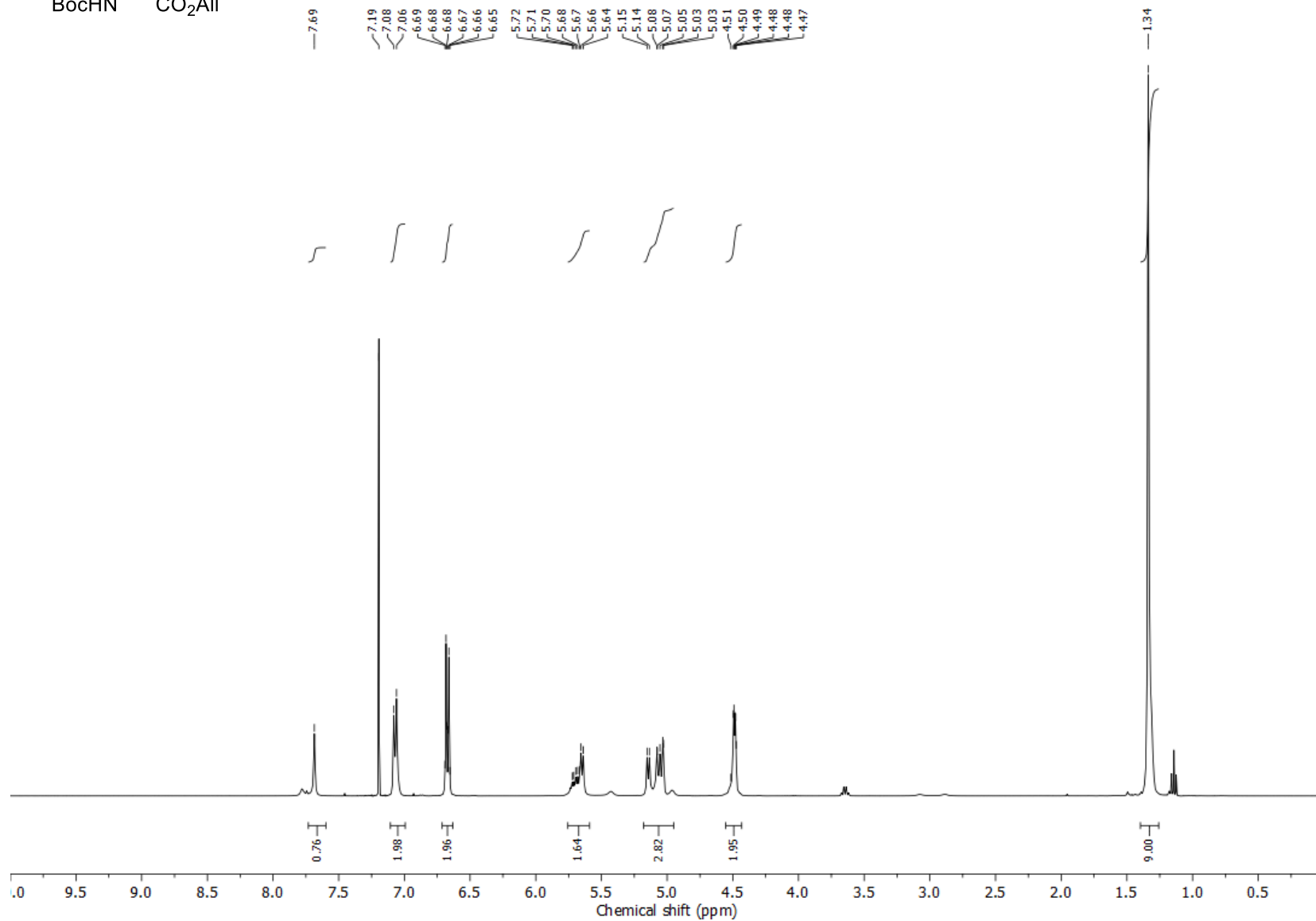
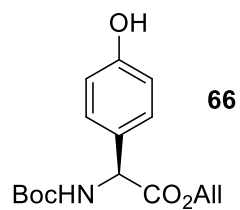
^1H NMR (400 MHz, MeOD)



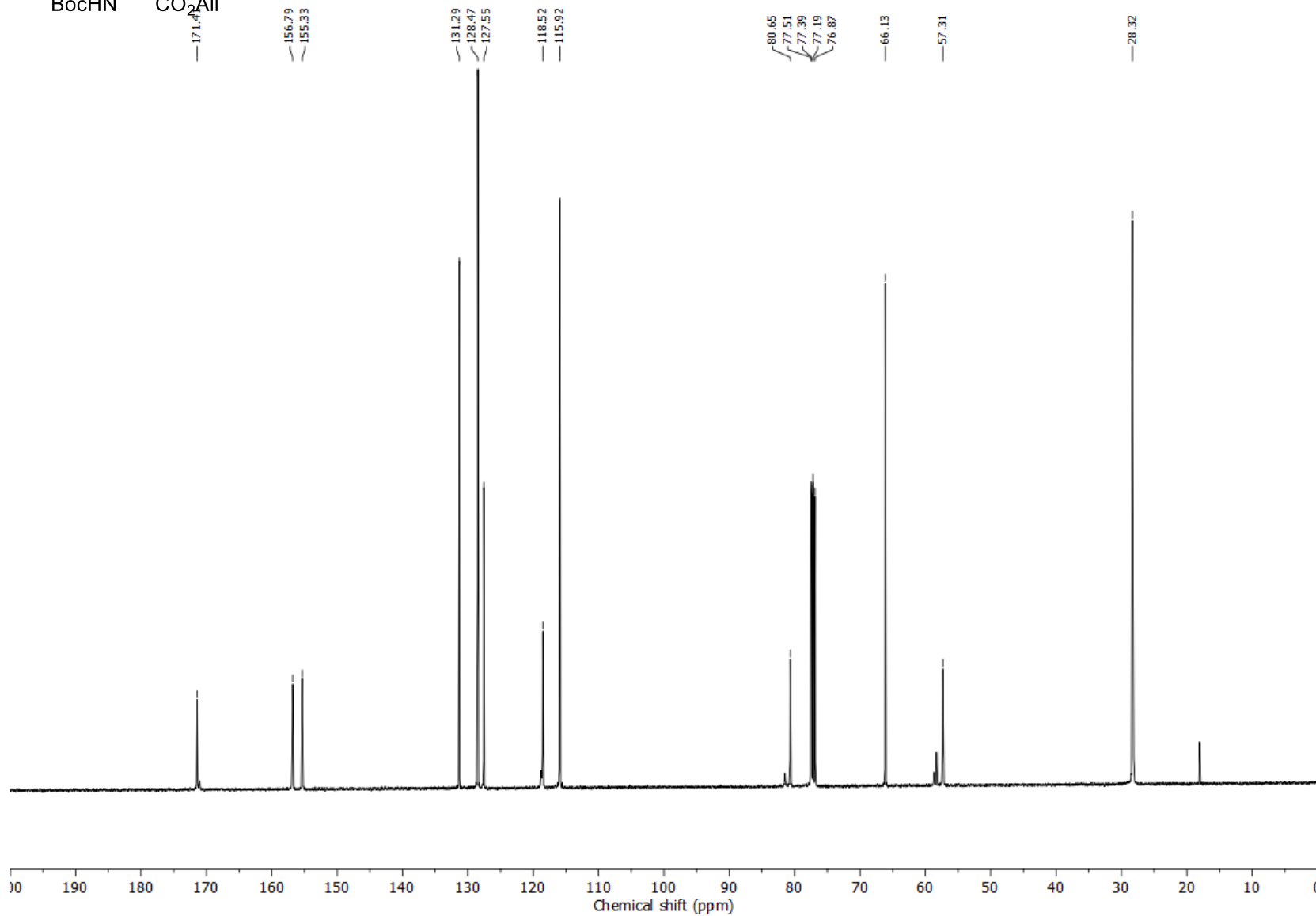
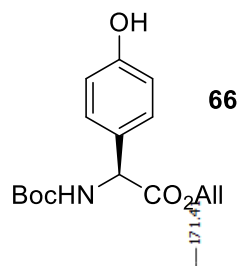
^{13}C NMR (100 MHz, MeOD)



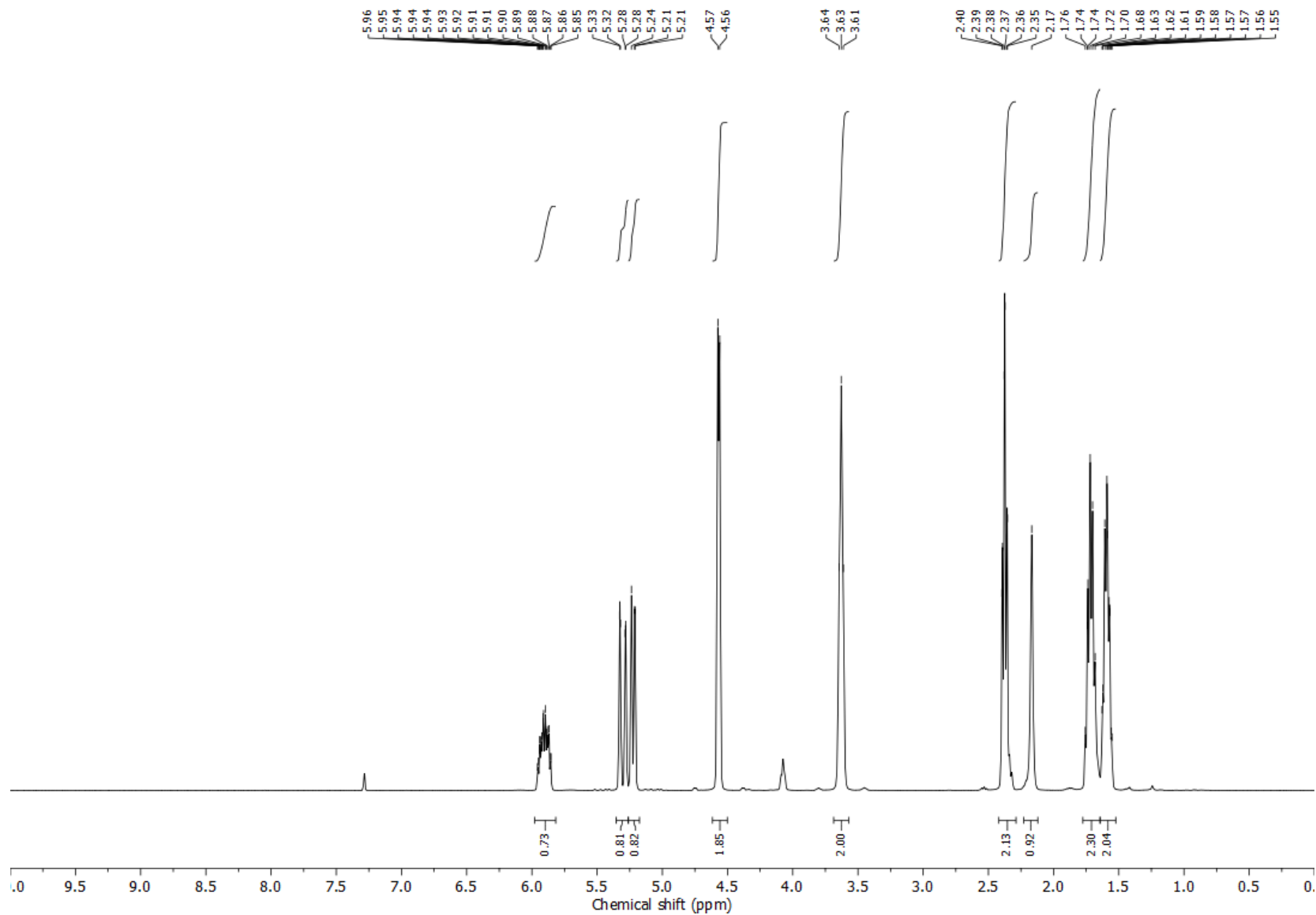
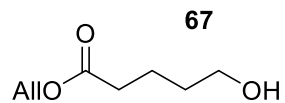
^1H NMR (400 MHz, CDCl_3)



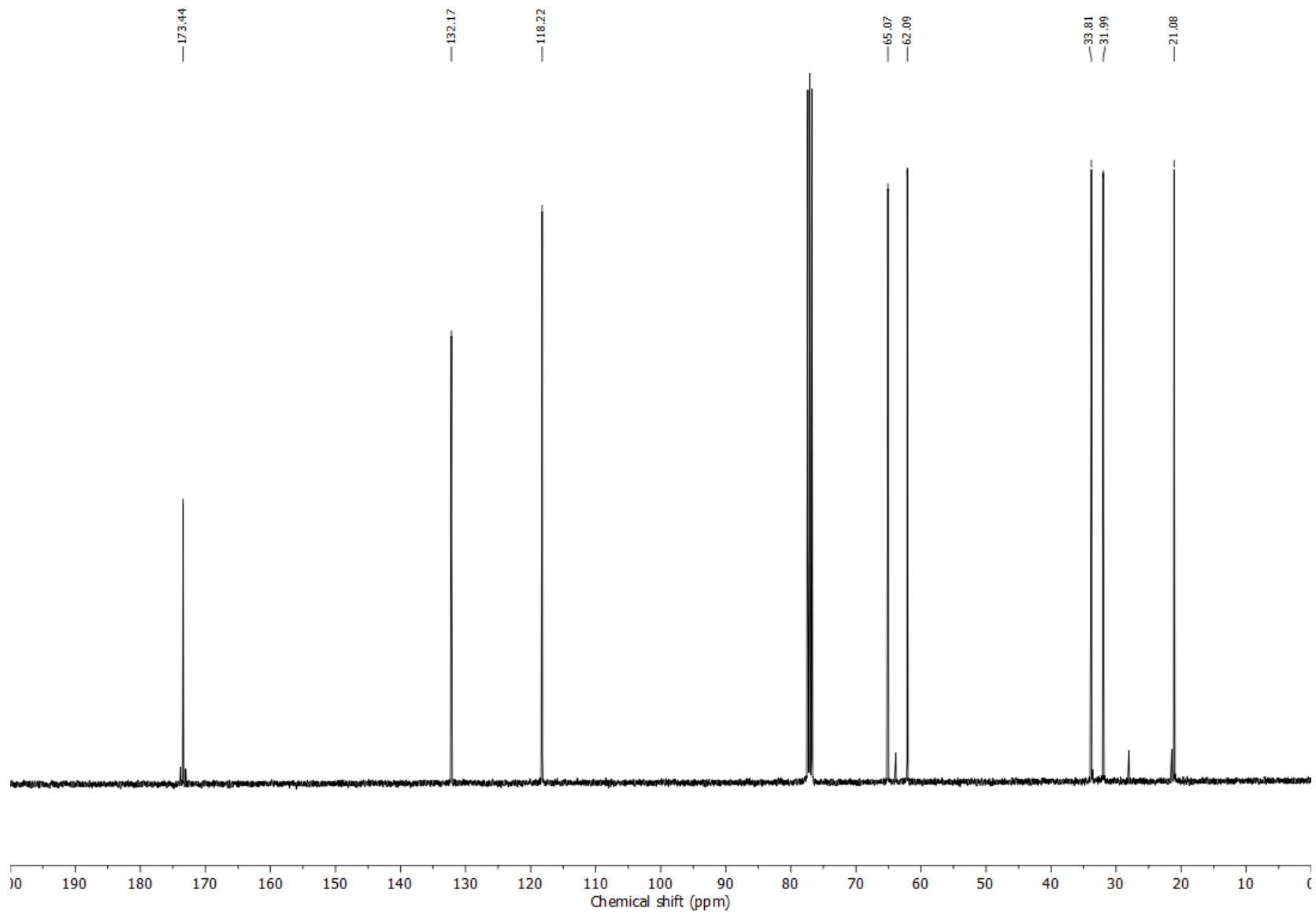
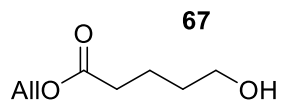
^{13}C NMR (100 MHz, CDCl_3)



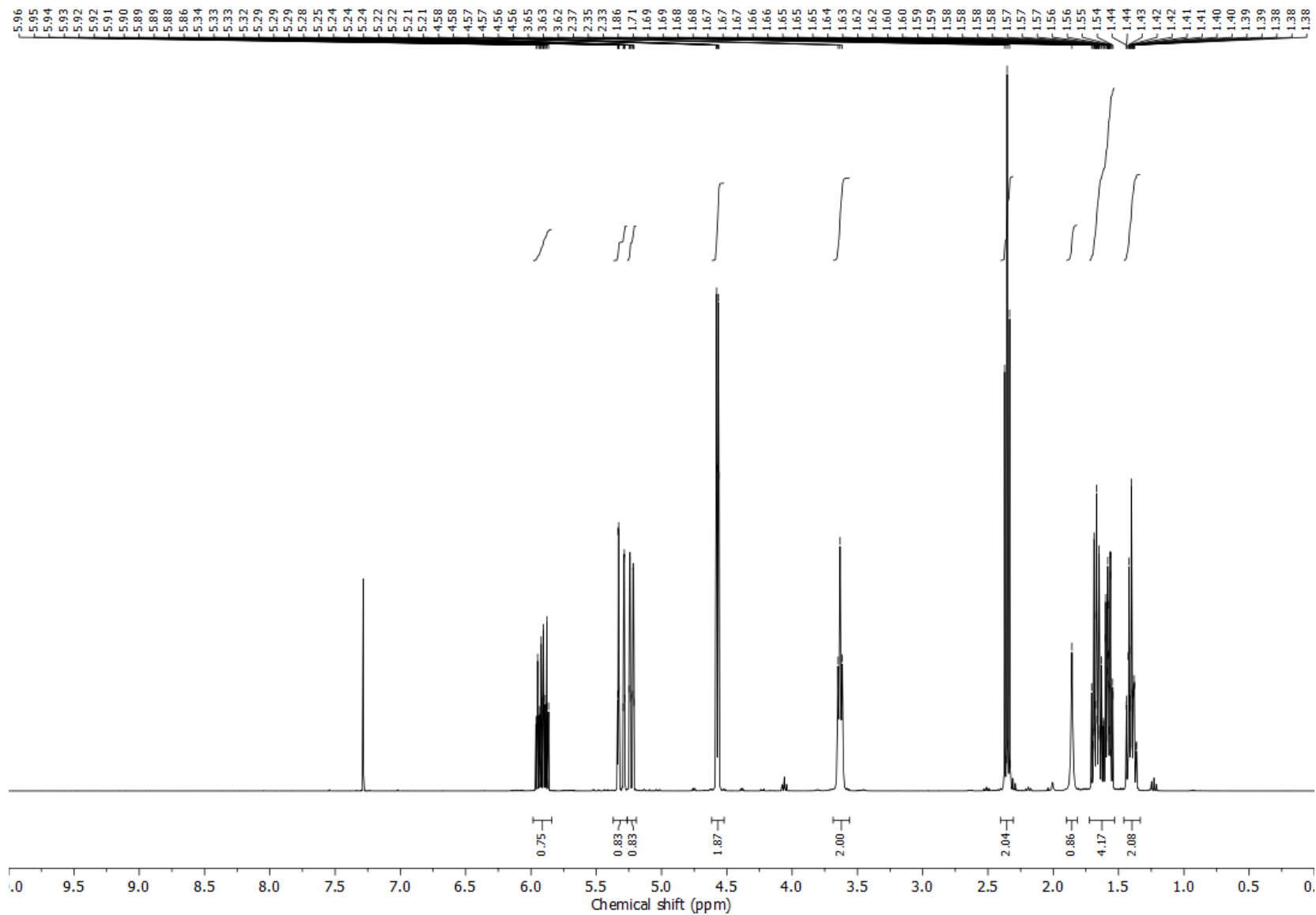
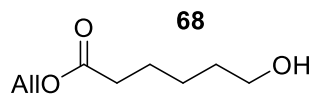
^1H NMR (400 MHz, CDCl_3)



^{13}C NMR (100 MHz, CDCl_3)



^1H NMR (400 MHz, CDCl_3)



^{13}C NMR (100 MHz, CDCl_3)

