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Synthesis of betulinic acid via Baylis-Hillman reaction

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SYNTHESIS OF BETULINIC ACID CONJUGATES VIA BAYLIS-HILLMAN REACTION

by

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A Thesis

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Thesis Chair: Subash C. Jonnalagadda, Ph.D.

Dedications

I dedicate this thesis to my dad Vallabha Rao and my mom Mani Mala for their unwavering support and for always believing in me.

Acknowledgments

Firstly, I would like to acknowledge my advisor Dr. Subash C. Jonnalagadda for his immense support, guidance and continued insights that guided me throughout this thesis work. He gave me the opportunity to explore and learn new skills. I would also like to express my sincere gratitude to Dr. Suman Pathi for his invaluable directions during my research work by helping me in learning new techniques and prompting me to focus on the quality of work. I will take this opportunity to acknowledge the lab members of Jonnalagadda research group for their help in completing this thesis and department of Chemistry and Biochemistry of Rowan university for providing the research facilities.

Abstract

Sai Krishna Kommineni
SYNTHESIS OF BETULINIC ACID DERIVATIVES VIA BAYLIS-HILLMAN REACTION
2018-2019
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Master of Science in Pharmaceutical Sciences

Betulin is readily isolated from the bark of birch trees using simple extraction techniques and this molecule as well as its derivatives (eg. betulinic acid) exhibit impressive levels of biological activity. While it is naturally available and shows selective toxicity towards certain cancers, betulin suffers from a general lack of solubility in aqueous conditions. In this regard, we took up a project involving the synthesis of conjugates of betulin with improved solubility characteristics and we were able to identify a series of compounds that showed cytotoxicity against breast and pancreatic cancer cells.

This thesis describes our efforts on the development of betulinic acid-derived second generation candidate compounds as potential anti-cancer agents. In our previous attempts, we coupled the betulinic acid template with Baylis-Hillman reaction based cinnamamides using click reaction-derived triazoles as the linker. We wanted to further explore the structure activity relationship profile for these conjugates. Accordingly, we synthesized two series of compounds (a) by removing the triazole linker and (b) by replacing the triazole with piperazine linker. The biological evaluation of these series of compounds is underway and the data obtained from this study would pave way for further functionalization and development.

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Chapter 1

Introduction

Betulin **1**, betulinic acid **2** and their derivatives exhibit impressive levels of biological activity.[1-4] Betulin is readily isolated from the bark of birch trees (10-15% w/w isolated yield) using simple extraction techniques.[5-6] While betulin is readily available in nature, the corresponding carboxylic acid derivative betulinic acid **2**, is known to exhibit more potent pharmacological activity. There have been several reports and reviews on the potential applications of betulinic acid as anti-cancer, anti-HIV, anti-micotic, anti-inflammatory, antioxidant, and anti-parasitic agents.[7-18]

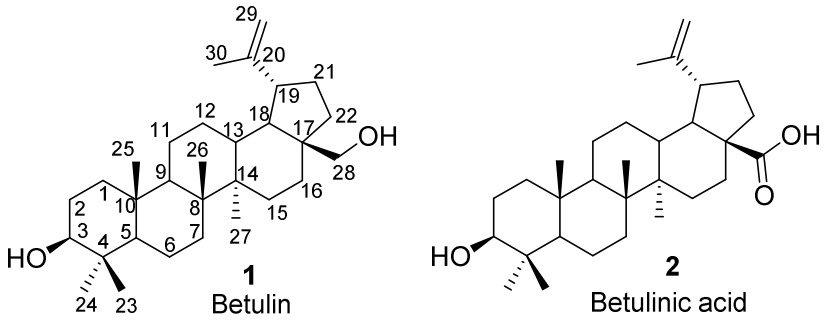


Figure 1. Betulin and Betulinic acid

Multi-component coupling reactions[19,20] such as Passerini reaction,[21-25] Ugi reaction,[26] Baylis-Hillman reaction,[27-34] click reaction, [35-38] etc. provide facile access to diverse densely functionalized chemical motifs and they are very useful tools in

medicinal and pharmaceutical chemistry toward drug design and development. For the past several years, we have been working on the development of novel small molecules using reactions such as Baylis-Hillman reaction, Passerini reaction, click reaction, aldol condensation, reductive amination, etc. for potential applications in drug discovery.[39-51] We have also been working on the isolation and chemical functionalization of betulin using some of these methodologies[11,18] particularly involving Baylis-Hillman and click reaction protocols.[40]

Synthesis of Betulin Conjugates via Click Reaction

Click reaction involves the coupling of alkynes **4** with azides **3** in the presence of copper catalyst to yield substituted triazoles **5** (**Figure 2**).[52-53] This cycloaddition reaction has been very thoroughly investigated and has found immense utility and applications in organic and medicinal chemistry.

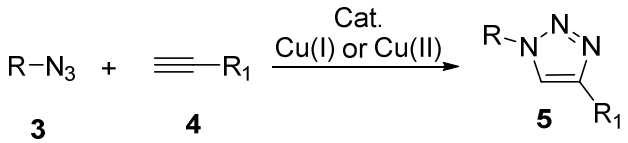


Figure 2. Click reaction.

Accordingly, several researchers have used click chemistry as a unique tool for the formation of several betulin and betulinic acid based triazole derivatives (**Figures 3-10**). Lee and coworkers described the reaction of betulin analog **7** and betulinic acid analog **10** with the drug azidothymidine yielding the compounds **8** and **11** respectively (**Figures 3-4**).[54]

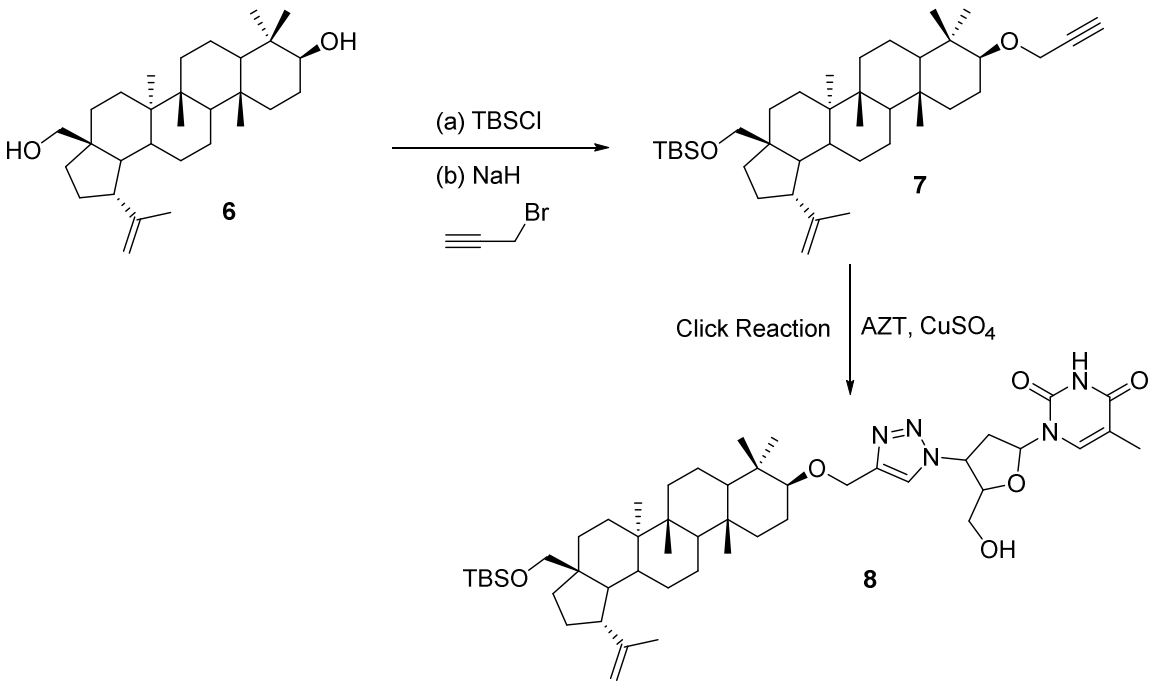


Figure 3. Betulin-AZT conjugates via click chemistry.

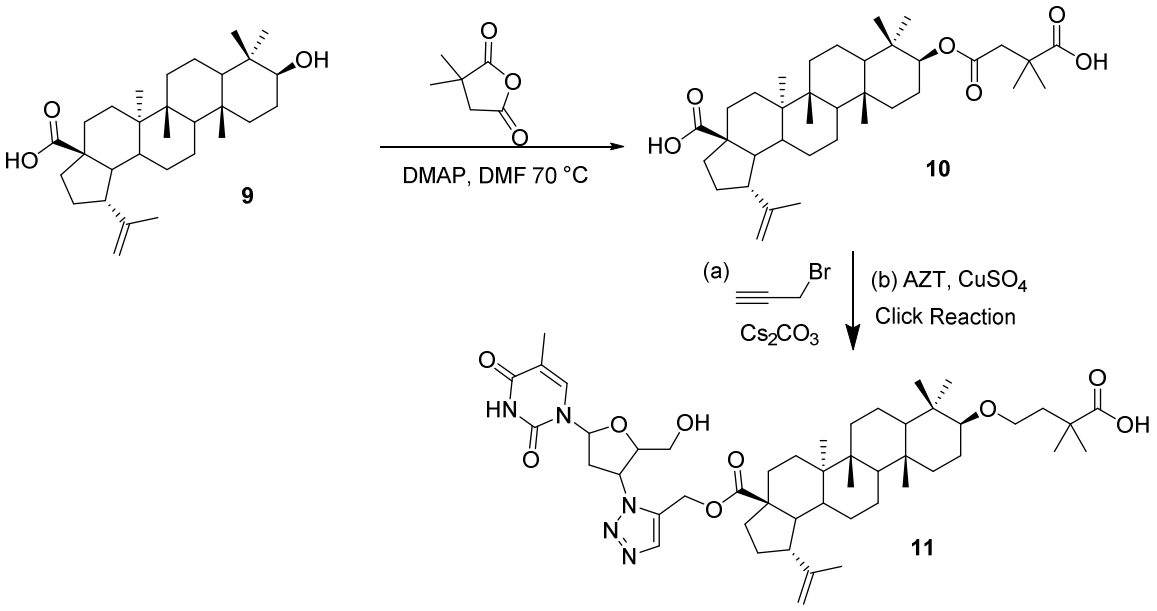


Figure 4. Betulinic acid-AZT conjugates via click chemistry

Khan et. al described the preparation of betulinic acid-triazole conjugates **13** via click reaction of propargyl ester of betulinic acid with aryl boronic acids in the presence of sodium azide and copper sulfate **(Figure 5)**. [55]

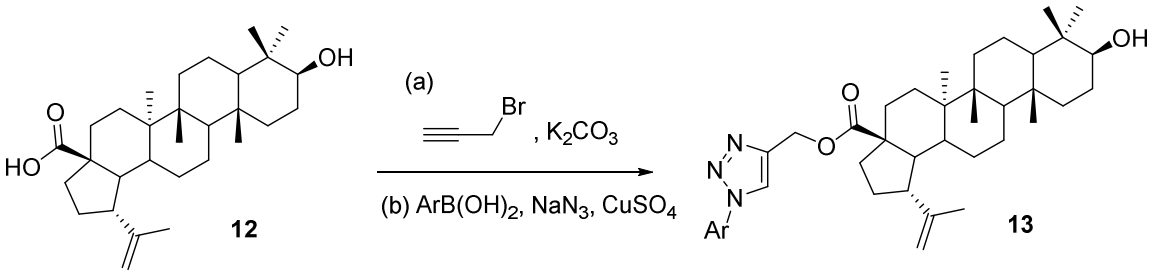


Figure 5. Betulinic acid-triazole conjugates via click chemistry.

Similarly, Thi and coworkers described the preparation of **16** via click coupling between the propargyl ester **15** and azidothymidine (**Figure 6**).[56]

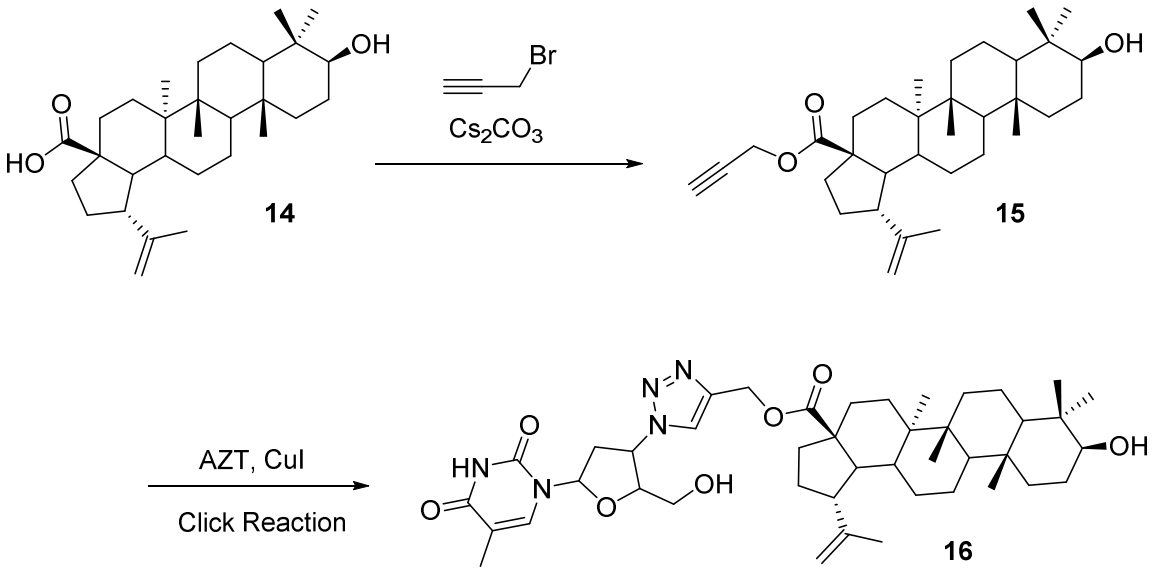


Figure 6. Betulinic acid-AZT conjugates as anti-cancer agents.

Csuk and coworkers described the reaction of betulin aldehyde **17** with lithium acetylide followed by Jones Oxidation to produce the diketone **18** which upon reaction with a series of azides furnished the triazoles **19** (**Figure 7**).[57]

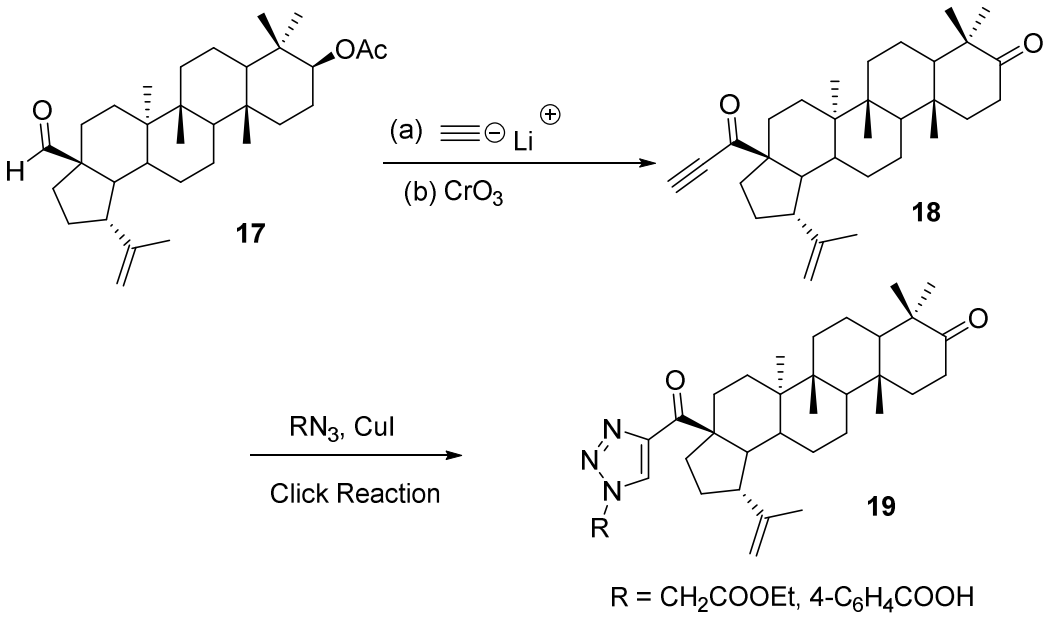


Figure 7. Betulonic acid-triazole conjugates via click chemistry.

Shi et. al described the synthesis of C30-triazole conjugates **22** starting from the allyl bromide **21**, which in turn was obtained from betulin via allylic halogenation (**Figure 8**).[58]

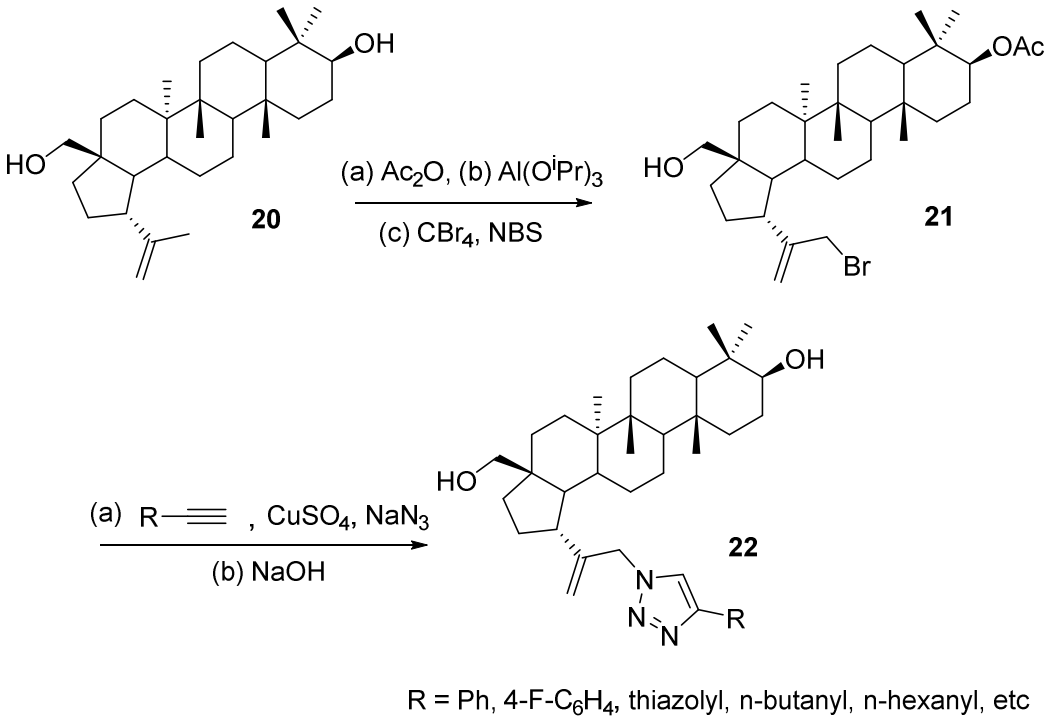


Figure 8. Betulin-C30 triazole conjugates via click chemistry.

Chakraborty and coworkers described the synthesis of C3-triazole esters **25** via coupling of betulinic acid with chloroacetyl chloride followed by nucleophilic substitution with sodium azide to furnish **24**, which was then subjected to click reaction with various alkynes (**Figure 9**).[59]

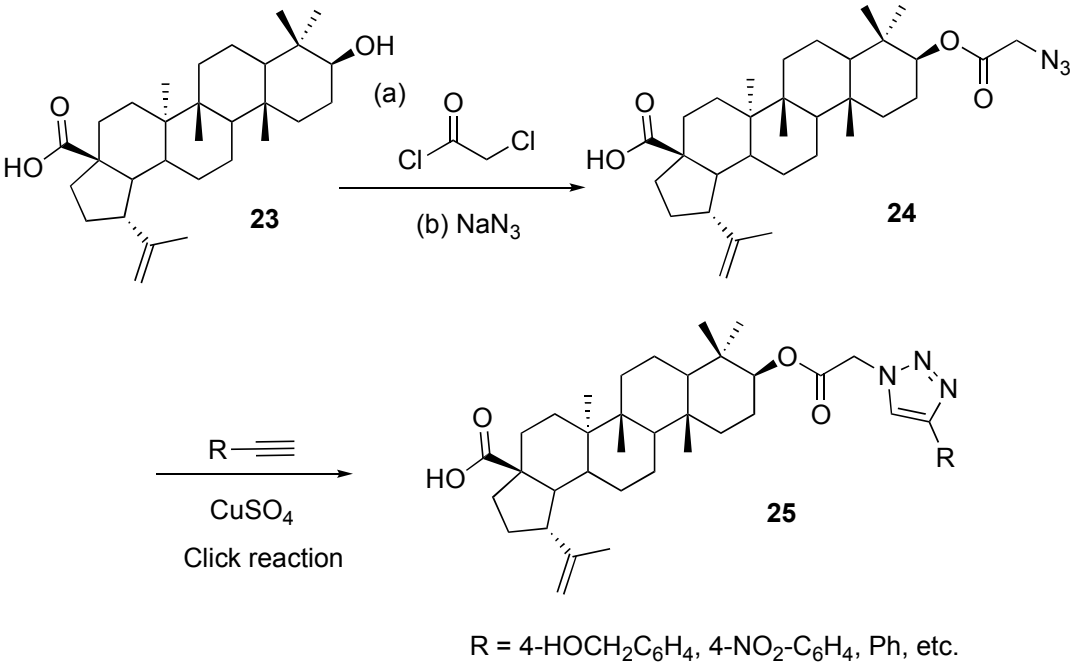


Figure 9. Betulinic acid-C3 triazole ester conjugates via click chemistry.

Betulinic acid C3-triazole conjugates **27** were also synthesized and evaluated for their biological efficacy (**Figure 10**).[60]

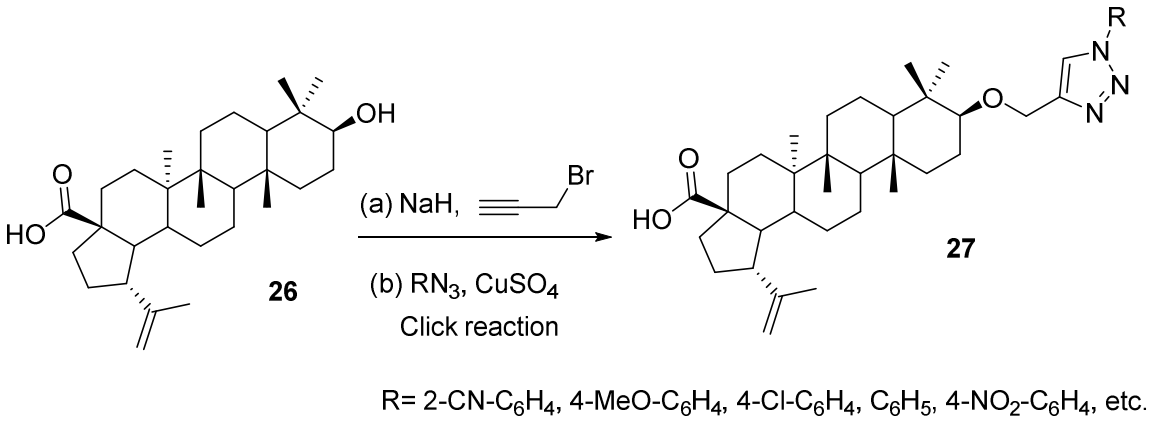


Figure 10. Betulinic acid-C3 triazole ether conjugates via click chemistry.

Chapter 2

Preparation of BH Reaction Derived Betulinic Acid Conjugates

Despite several reports on the synthesis of betulinic acid triazole derivatives using click reaction, limited success has been achieved in terms of identifying a potent lead compound for further development as a therapeutic agent. Owing to the importance of Baylis-Hillman and click reactions, we undertook a project involving the preparation of betulinic acid triazole conjugates using these two reactions as the key steps in our synthesis.[40] We initiated our synthesis with the acetylation of allylic alcohol **28** (obtained from Baylis-Hillman reaction of benzaldehyde with methyl acrylate) followed by treatment with N,N,N'-trimethylethylenediamine to furnish aminomethyl cinnamate **29**. Alkaline hydrolysis of **29** followed by the coupling of the resulting cinnamic acid with 2-azidoethylamine in the presence of 1-hydroxybenzotriazole (HOBt), and 1-ethyl-3-(-3-dimethylamino propyl) carbodimide hydrochloride (EDCI) furnished the azidoethylcinnamamide analog **30**. *N*-Propargyl betulinamide **31** was prepared in parallel by coupling betulinic acid with propargyl and was used for click reaction with azide **30**. The resulting betulinic acid triazole amine conjugate **32** was purified and rigorously characterized via proton and carbon NMR and mass spectral analysis. Using a similar protocol outlined in **Figure 11**, a series of compounds **32a-e** (**Figure 12**) were prepared and evaluated for biological efficacy as anti-cancer agents. These studies formed the basis for the synthesis of second generation of candidate compounds for further development.

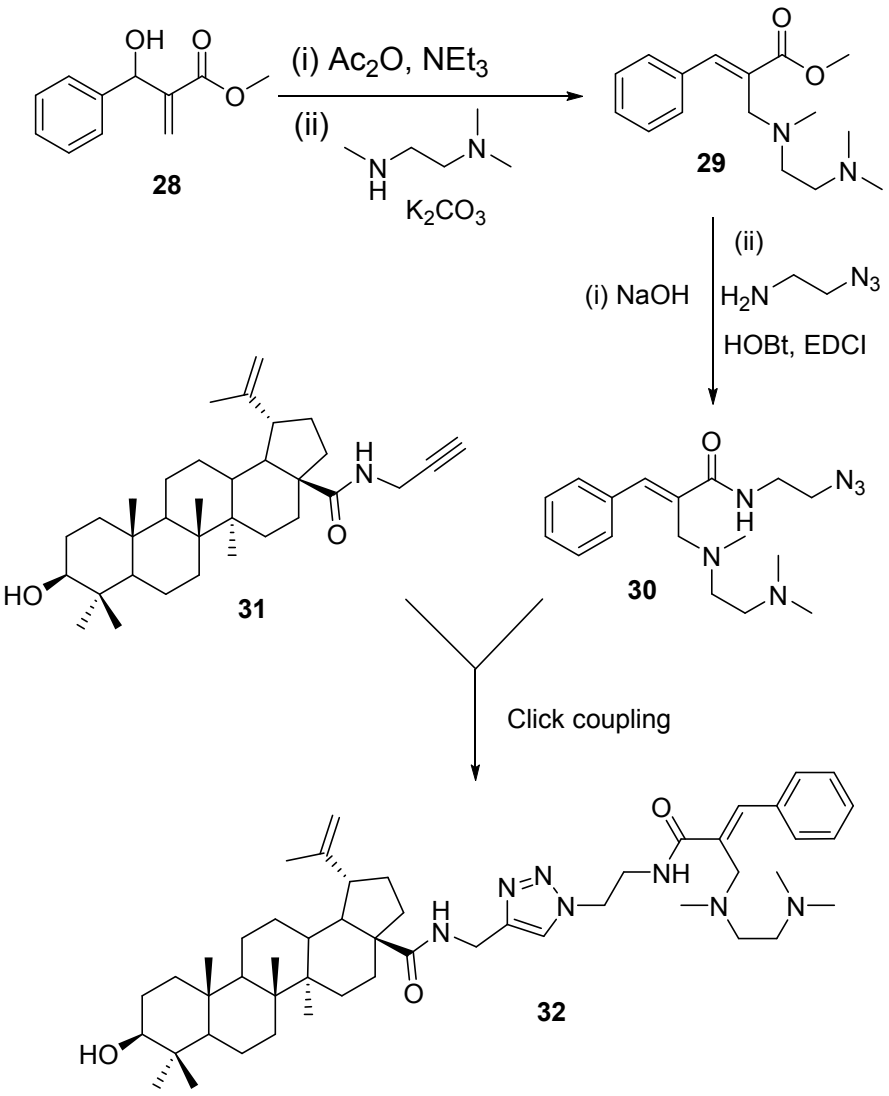


Figure 11. Preparation of BH reaction derived betulinic acid triazole conjugates.

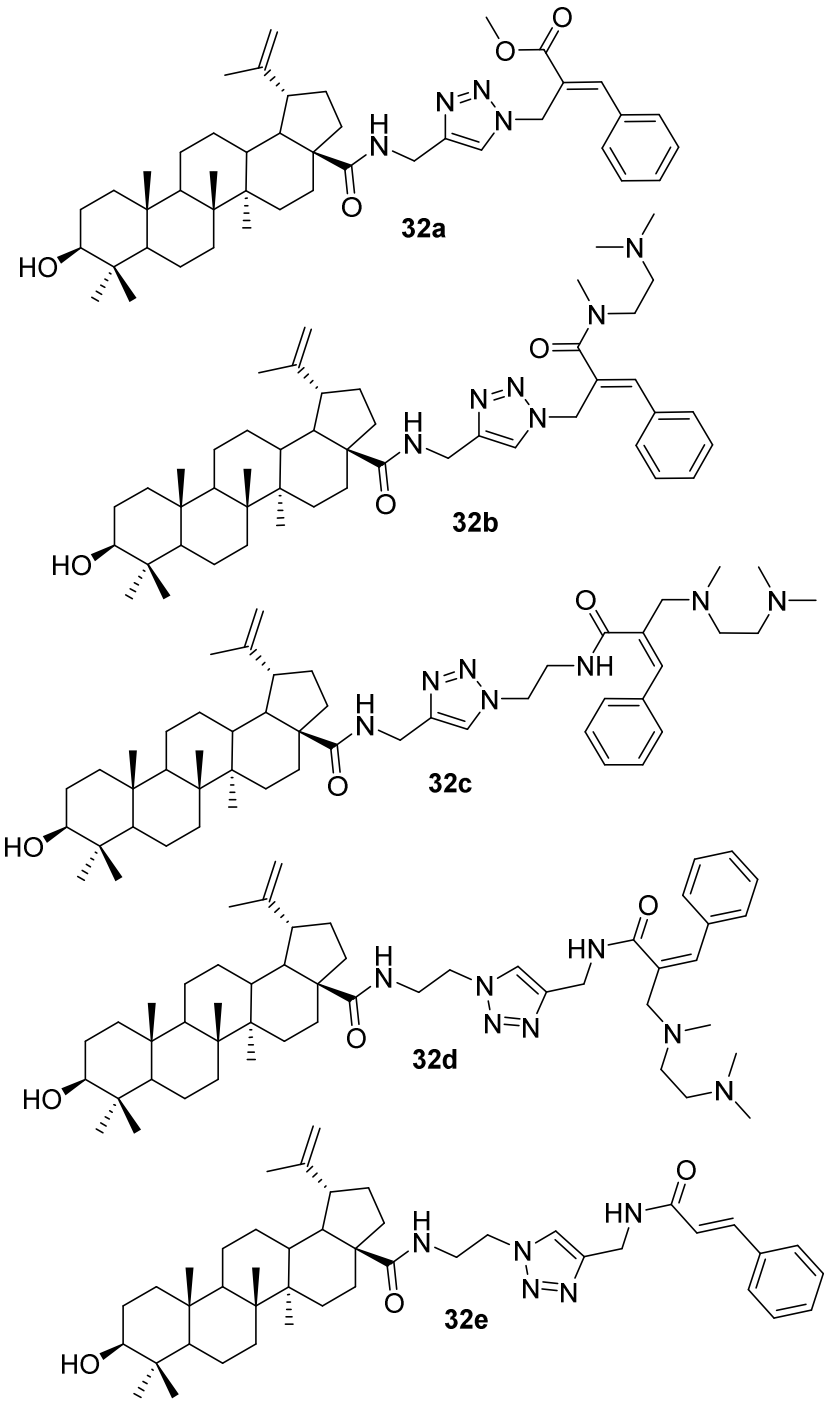


Figure 12. BH reaction derived betulinic acid triazole conjugates.

Having identified a potent lead derivative **33** based on our initial studies, we set forth on a rational investigation of the role of individual components in imparting biological activity for these conjugates. Accordingly, we took up a project involving the synthesis of betulinic acid conjugates of Baylis-Hillman template derived cinnamamides by either removing the triazole linker (**34**) or by replacing the triazole linker with piperazine linker (**35**) (**Figure 13**).

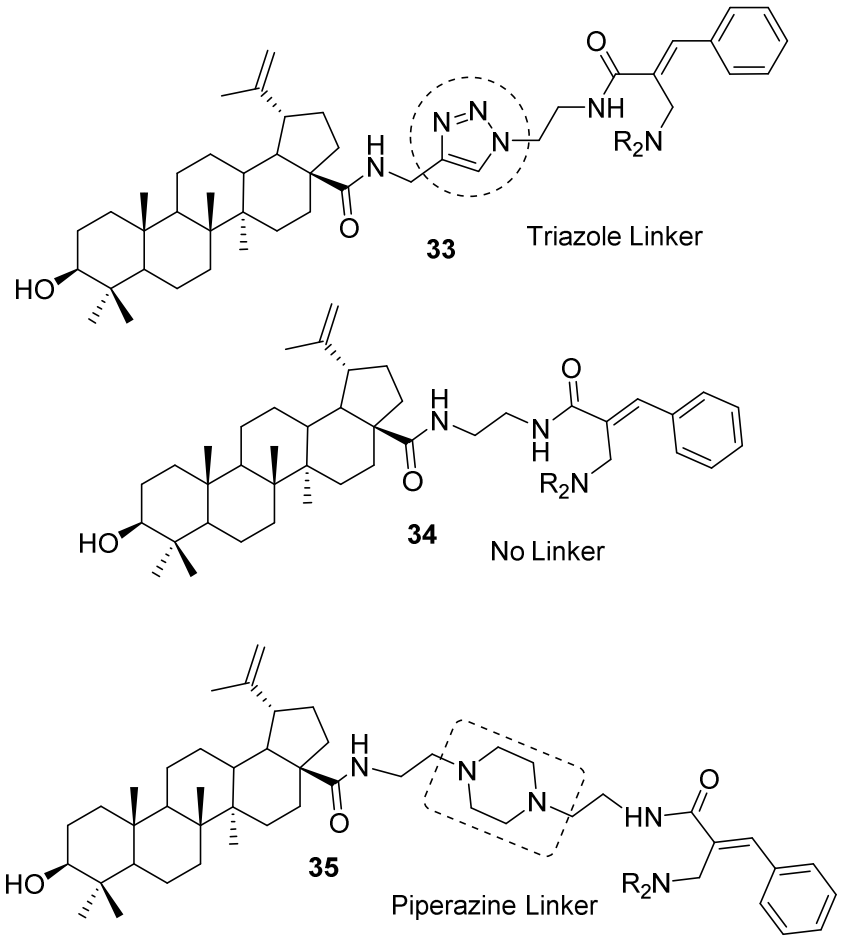


Figure 13. BH reaction derived betulinic acid conjugates.

Synthesis of *N*-2-Aminoethyl Cinnamamides 36-39

To understand the structural activity relationship of the previously identified lead molecules we designed a study to know the importance of triazole as a linker and Baylis-Hillman moiety in cytotoxic activity. Our primary choice for replacing triazole linker was piperazine/polyamines because of their well-established binding and solubility characteristics under pharmacological conditions. The two polyamines we utilized in our synthesis are 1,2-ethylenediamine and *N*,*N*'-bis(2-aminoethyl) piperazine. The ethylenediamine intermediates **36-39** (**Figure 14**) were hypothesized for final coupling with betulinic acid towards the goal of preparing the final target compound **34** without a linker.

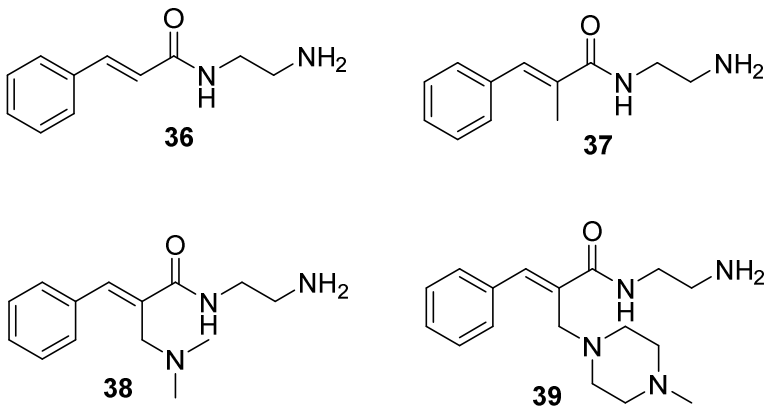


Figure 14. Amines used for coupling with betulinic acid.

Preparation of amine 36. The common intermediate required for the preparation of all four amines **36-39** was ^{t}butoxycarbonyl (BOC) protected ethylenediamine **42**. The synthesis of **42** was achieved by adding a solution of Boc anhydride **41** in chloroform dropwise to a solution of ethylenediamine **40** in chloroform over a period of 30 minutes and maintaining the temperature 0^{0}C during the entire addition. The resulting amine **42** was obtained in 78% yield (**Figure 15**).

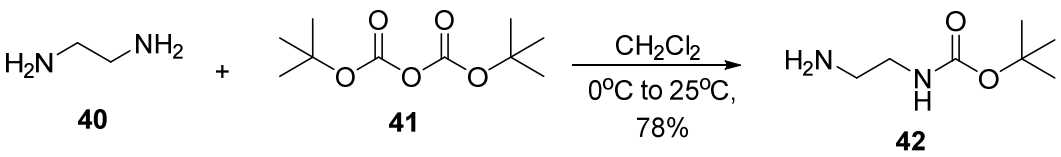


Figure 15. Preparation of *N*-Boc-ethylenediamine **42**.

The reaction of cinnamic acid **43** with *N*-Boc-ethylenediamine **42** in the presence of hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and *N*, *N*-diisopropylethylamine (DIPEA) in methylene chloride (CH_2Cl_2) for 14 hours resulted in the formation of *N*-Boc protected aminoethyl cinnamamide **44.** Deprotection of Boc grop was carried out in acidic environment using trifluoroacetic acid in methylene chloride, which yielded the corresponding amine **36** (**Figure 16**).

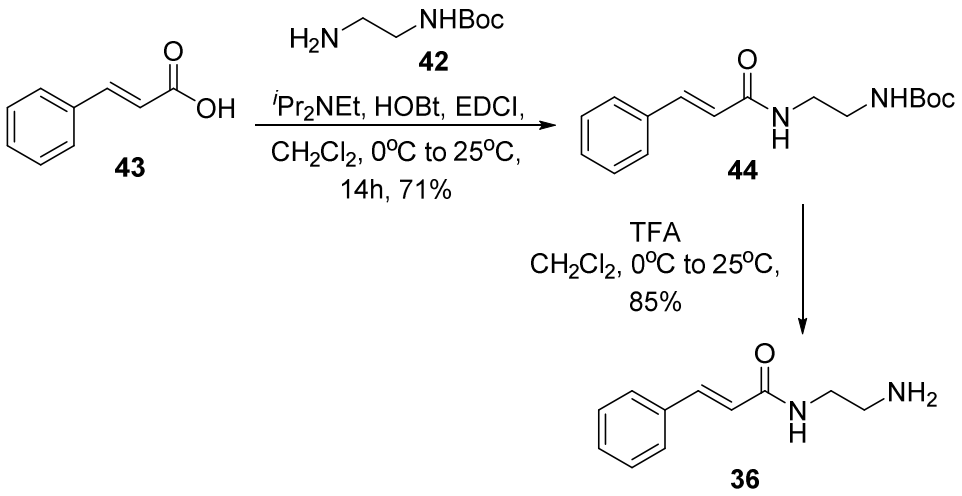


Figure 16. Preparation of amine **36**.

Preparation of amine 37. The α-substituted cinnamic acid **51** required for the preparation of amine **37** was synthesized by following the previously reported protocol using Baylis-Hillman reaction as a key step. We began the synthesis by reacting benzaldehyde **45** with methyl acrylate **46** in the presence of DABCO at room temperature to yield the corresponding allylic alcohol **28**. Acetylation of **28** was avhieved with acetic anhydride to furnish the acetate **49**. Reduction of **49** with sodium borohydride in methanol resulted in α-methylcinnamic acid methyl ester **50**, which was subsequently hydrolyzed into acid **51** using aqueous sodium hydroxide in methanol and tetrahydrofuran (1:9). This acid **51** was reacted with *N*-Boc ethylenediamine **42** in the presence of hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and *N*,*N*-diisopropylethylamine (DIPEA) in methylene chloride (CH_2Cl_2) for 13 hours resulting in the formation of **52.** Deprotection of Boc group was carried out in acidic

environment using trifluoroacetic acid in methylene chloride to yield the corresponding amine **37** (**Figure 17**)

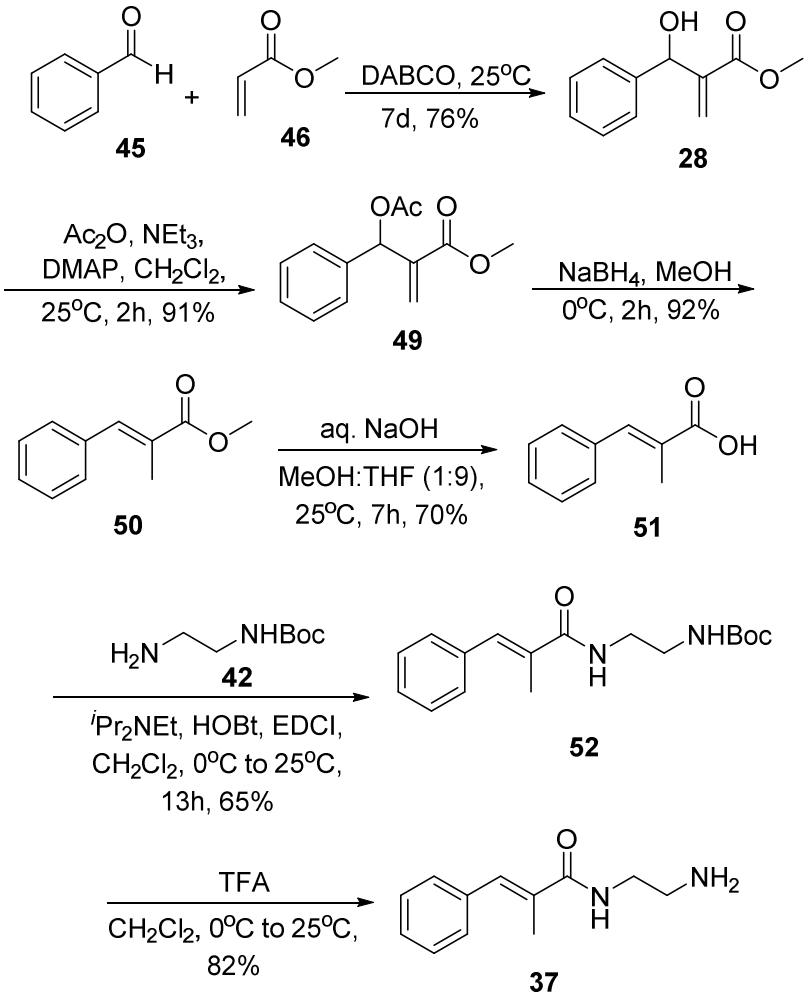


Figure 17. Preparation of amine **37**.

Preparation of amine 38. The Baylis-Hillman reaction derived alcohol **28** was also used for the synthesis of amine **38**. The reaction of alcohol **28** with dimethylformamide dimethyl acetal in DMF under reflux conditions yielded the dimethylaminomethyl cinnamate **53**. Conversion of ester **53** to acid **54** was accomplished via alkaline hydrolysis using NaOH. The final coupling of acid **54** with *N*-Boc protected ethylenediamine **42** was achieved under peptide coupling conditions using HOBt and EDCI to result in the formation of amine **38** (**Figure 18**).

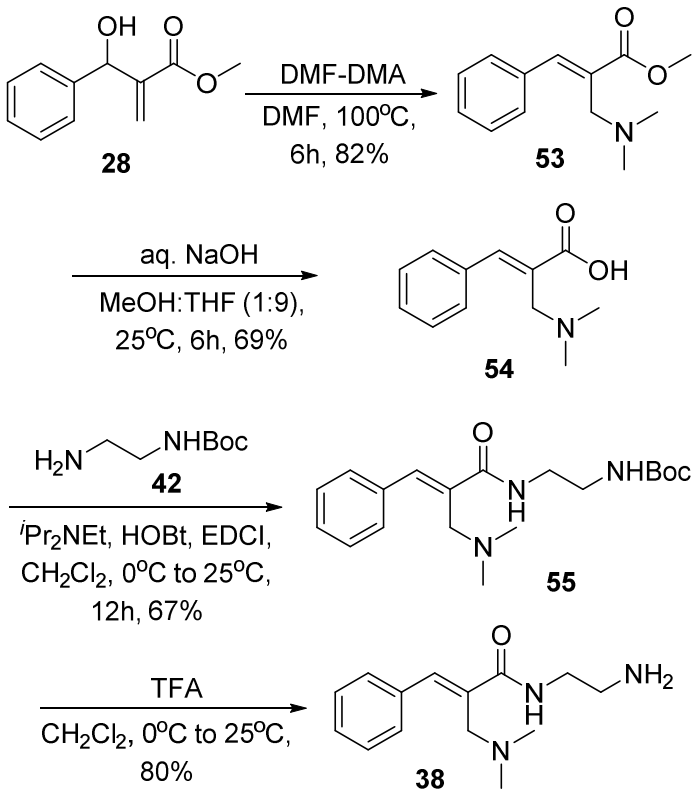


Figure 18. Preparation of amine **38**.

Preparation of amine 39. The allylic acetate **49** was treated with *N*-methyl piperazine and potassium carbonate in DMF to yield *N*-methylpiperazinylmethylcinnamate ester **57** via S_N2' substitution. Alkaline hydrolysis of ester **57** was realized upon reaction with 10% NaOH solution in THF:MeOH medium resulting in the formation of the acid **58**. The final target amine **39** was obtained in two steps from **58** by coupling with *N*-Boc ethylenediamine **42** in the presence of hydroxybenzotriazole (HOBt),1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and *N*,*N*-diisopropylethylamine (DIPEA) in methylene chloride (CH_2Cl_2) followed by deprotection of **59** with trifloroacetic acid (**Figure 19**).

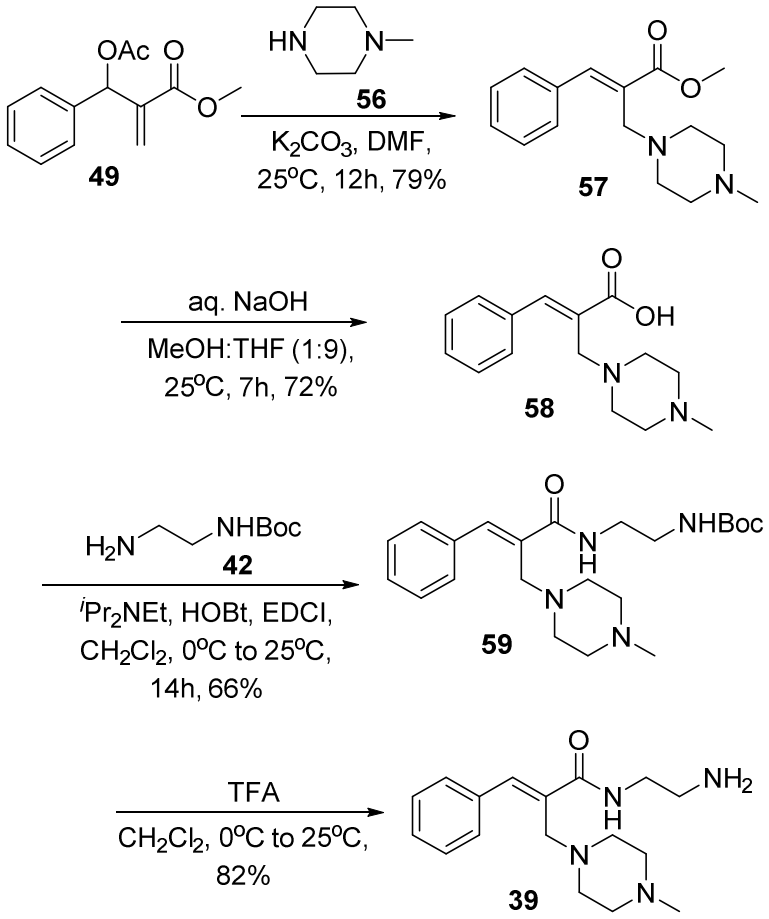


Figure 19. Preparation of amine **39**.

Preparation of Betulinic Acid Cinnamamide Conjugates 61a-d

The final target compounds **61a-d** were prepared in two steps starting from betulonic acid **60** involving sodium borohydride reduction followed by coupling with the four target amines **36-39** using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (TBTU) (**Figures 20-21**).

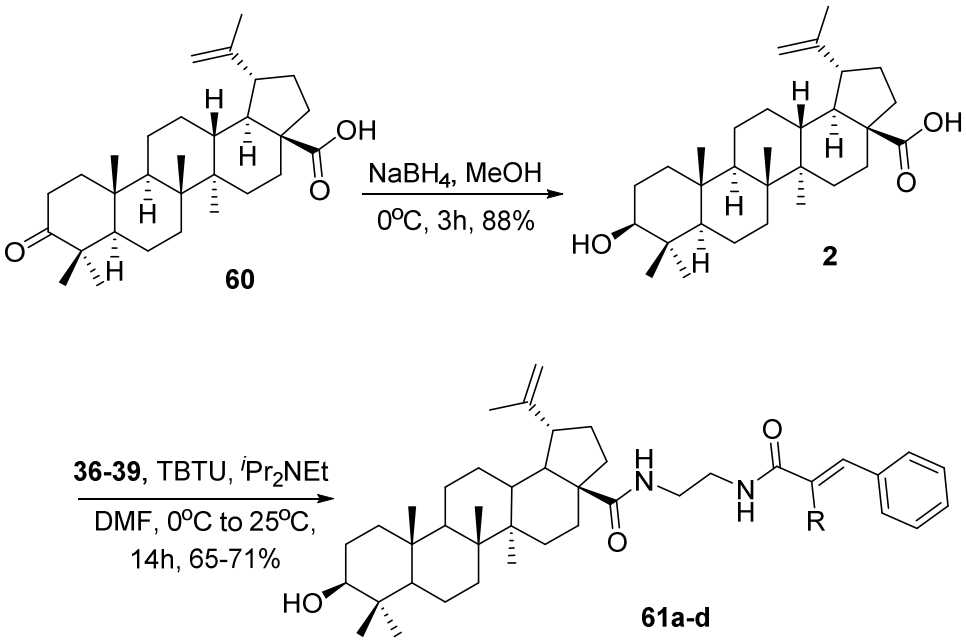


Figure 20. Preparation of betulinic acid-ethylenediamine conjugates **61a-d**.

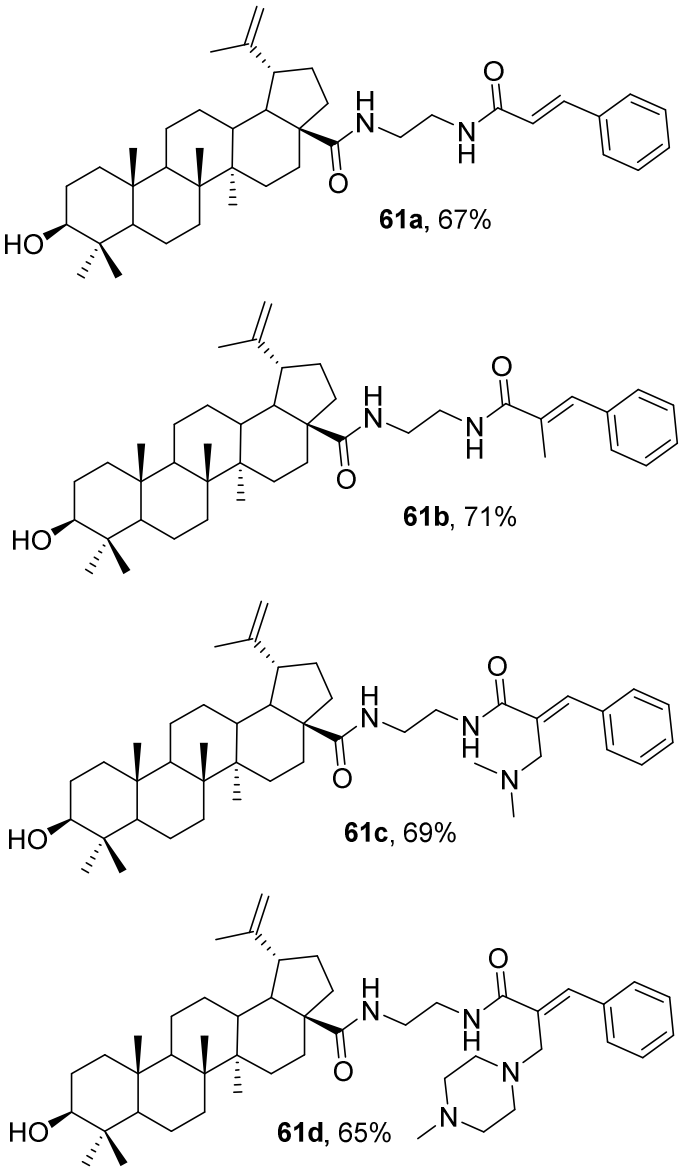


Figure 21. Betulinic acid-ethylenediamine conjugates **61a-d**.

Synthesis of Piperazinyl Cinnamamides

To further establish the structural activity relationship profile of the previously synthesized triazole based lead molecules we explored the possibility of replacing the triazole linker with piperazine. The piperazinyl cinnamamide intermediates **62a-d** (**Figure 22**) were hypothesized for final coupling with betulinic acid towards the goal of preparing the final target compound **35** with piperazine linker.

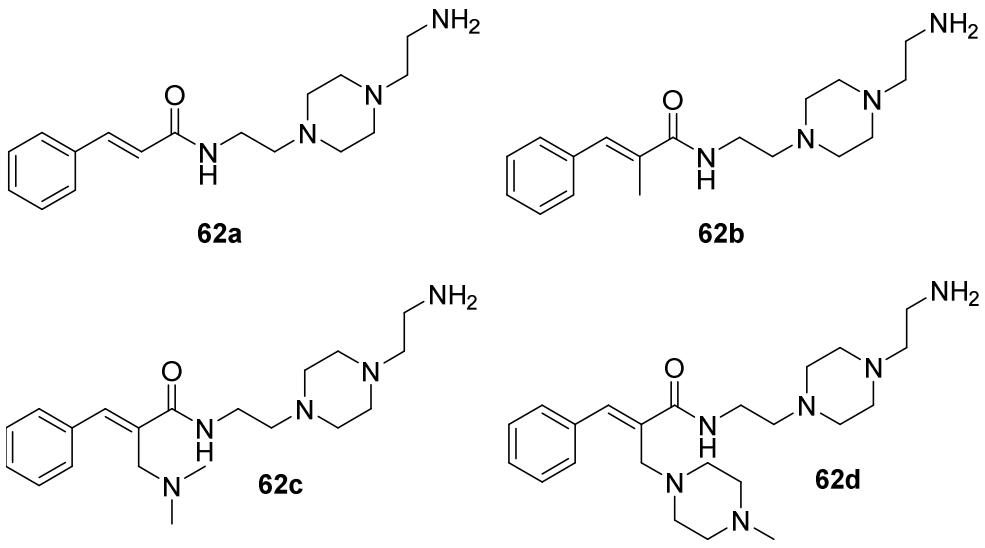


Figure 22. Piperazinyl-amines used for amide coupling.

Preparation of *N*-boc-piperazinyl amine 66. *N*-boc-piperazinyl amine **66** was a common intermediate required for the synthesis of all four cinnamamides **62a-d**. Thus, the synthesis of **66** was envisioned starting from piperazine **63** in three steps. Piperazine was initially reacted with chloroacetonitrile in the presence of anhydrous sodium carbonate in ethanol under reflux conditions giving 1,4-piperazinediacetonitrile **64**, which was dissolved in anhydrous tetrahydrofuran and added dropwise to a slurry of lithium aluminum hydride in anhydrous THF under nitrogen atmosphere while maintaining the temperature between 0-5 ^{o}C throughout the period of addition. The reaction mixture was then refluxed under nitrogen atmosphere for 4 hours. Finally, the quenching of excess lithium aluminum hydride was achieved via slow addition of 3 mL saturated sodium bicarbonate solution. The solution was filtered and concentrated to give a 1,4-bis(2-aminoethyl) piperazine **65**, which was monoprotected to yield the targe intermediate **66** by reacting with Boc anhydride in chloroform (**Figure 23**).

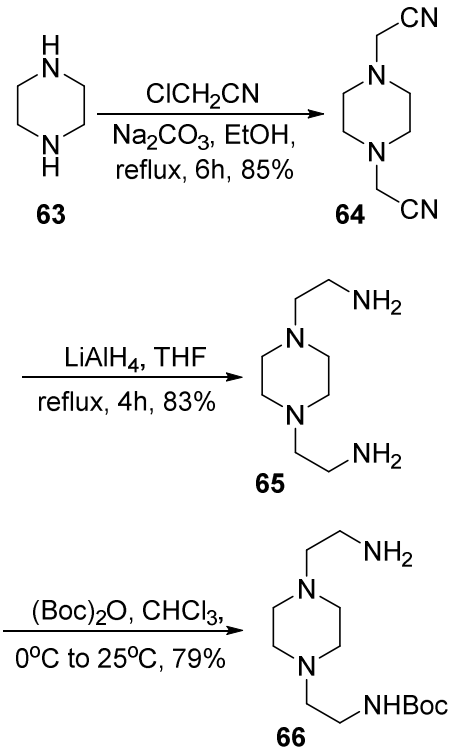


Figure 23. Preparation of *N*-boc-piperazinyl amine **66**.

Preparation of piperazinyl cinnamamides 62a-d. The piperazinyl cinnamamide intermediates **62a-d** were prepared starting from amine **66** by coupling with the four cinnamic acids **43** (**Figure 16**), **51** (**Figure 17**), **54** (**Figure 18**), and **58** (**Figure 19**). The coupling was achieved under hydroxybenzotriazole (HOBt), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) conditions to yield **67a-d**. Deprotection of Boc group was achieved upon reaction with trifluoroacetic acid in methylene chloride to produce the piperazinyl cinnamamides **62a-d** (**Figure 24**).

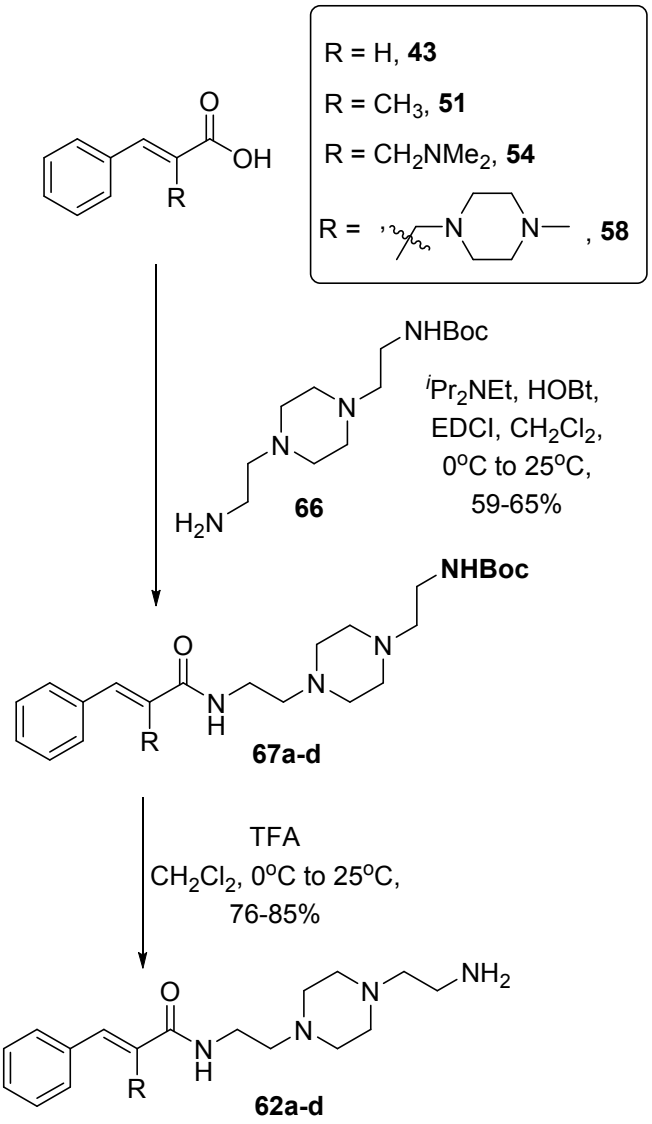


Figure 24. Preparation of piperazinyl cinnamamides **62a-d**.

Preparation of Betulinic Acid-Piperazine Conjugates 69a-d

The final target compounds **69a-d** were prepared starting from betulinic acid **2** coupling with the four target amines **62a-d** using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (TBTU) as the coupling reagent (**Figures 25-26**).

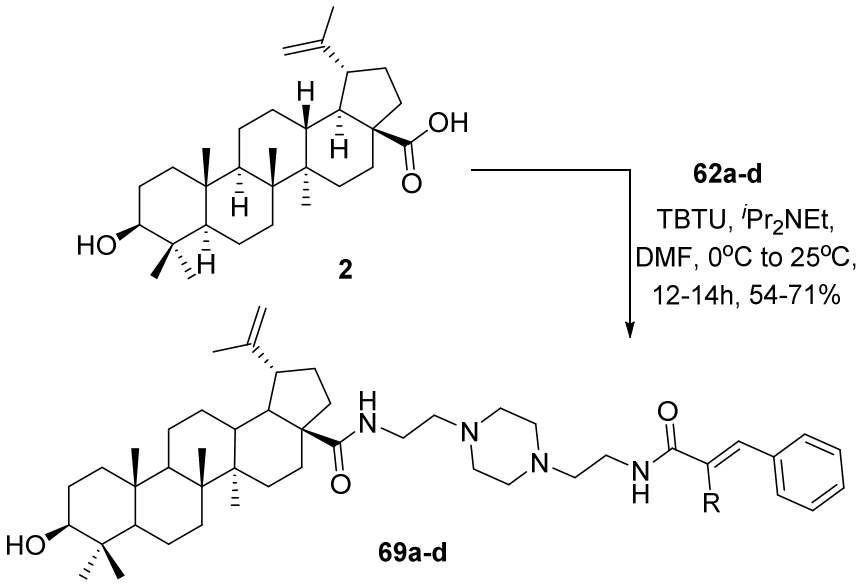


Figure 25. Preparation of betulinic acid-piperazine conjugates **69a-d**.

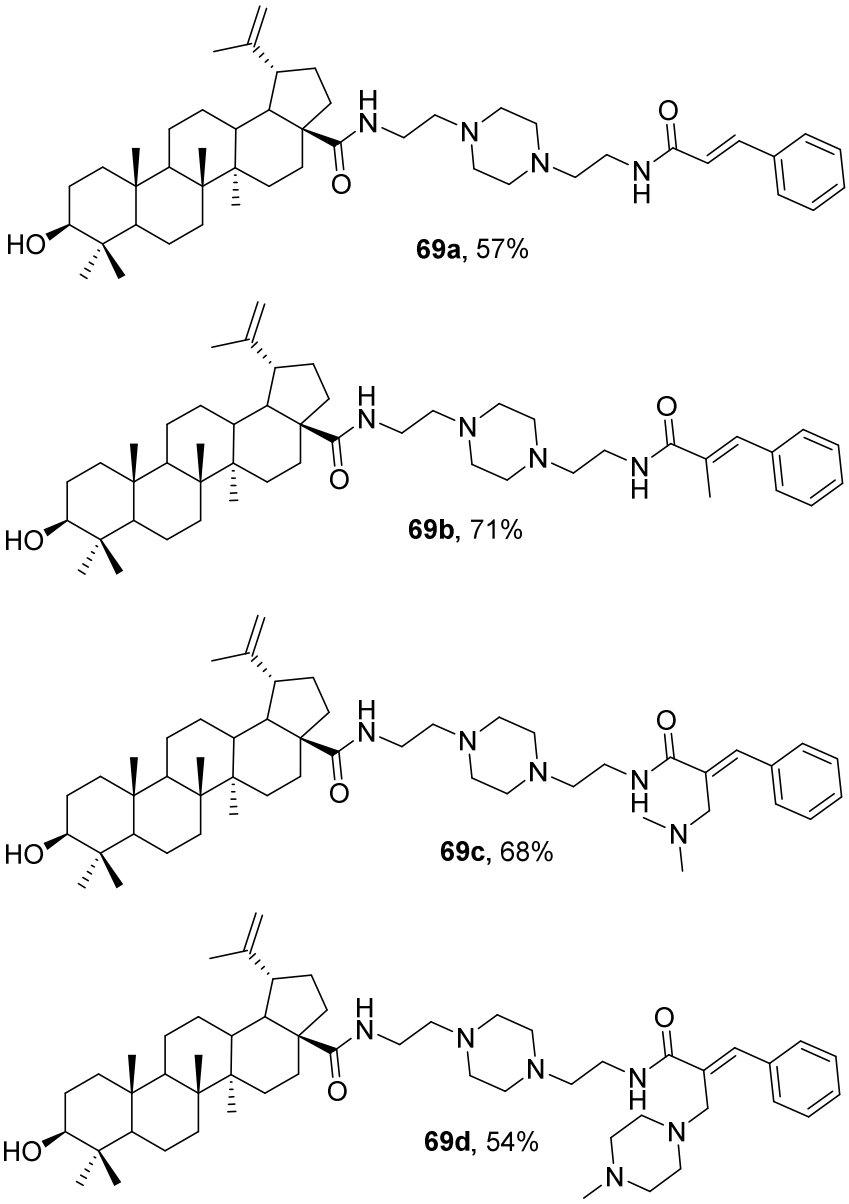


Figure 26. Betulinic acid-piperazine conjugates **69a-d**.

Conclusions

We have been working on the development of betulin based small molecules as potential anti-cancer agents. In this regard, we have identified few lead molecules (synthesized using Baylis-Hillman and click reactions as key steps) that exhibit potent cytotoxicity against breast cancer cells. The ample availability of betulin and betulinic acid from natural sources coupled with diverse functionalization opportunities possible with Baylis-Hillman reaction makes this project highly attractive for organic and medicinal chemistry community. The lead derivatives consisted of a betulinic acid moiety and a Baylis-Hillman reaction-derived cinnamamide template that were coupled via triazole using click cycloaddition reaction. In the second phase of this project, we decided to study the role of triazole linker in imparting biological activity. Accordingly, we designed two series of betulinic acid cinnamamide conjugates. In the first series of conjugates, betulinic acid was coupled with cinnamamide without a triazole linker and in the second series the two entities were connected via piperazine linker instead of triazole. The biological evaluation of these conjugates is underway and the results obtained from this study will provide future direction for further drug design.

Chapter 3

Experimental Procedures

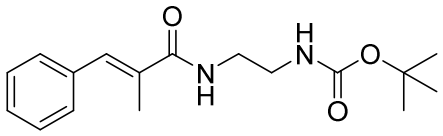
Materials and Methods

All the reactions were carried out under a N_2 atmosphere in oven-dried glassware. Flash column chromatography (FCC) was carried out with SiliaFlash F60, 230-400 mesh silica gel. Reactions and column chromatography were monitored with SiliaPlate F254 plates and visualized with potassium permanganate, or iodine stains. All the chemical reagents and solvents were used without further purification from commercial sources. Unless otherwise noted, melting points were obtained from material that solidified after chromatography.

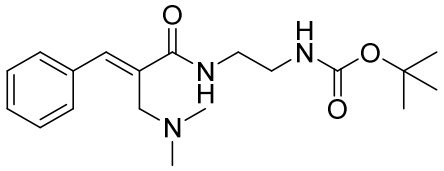
Instrumentation

Proton and carbon NMR spectra (1H NMR and 13C NMR) were recorded in deuterated chloroform ($CDCl_3$), unless otherwise noted on a Varian 400 MHz spectrometer with a quad-probe. Chemical shifts were reported in parts per million (ppm) and were calibrated according to residual protonated solvent. Low-resolution mass spectral analysis data was collected using an Agilent 1100-Series LC/MSD Trap LC-MS using acetonitrile with 0.1% formic acid in positive ionization mode. Purity of all final compounds were assessed on Agilent 1100 series HPLC equipped with a Sonoma 3µ (5cm x 2.1 mm) C18 column using a gradient of water to acetonitrile with 0.1% TFA.

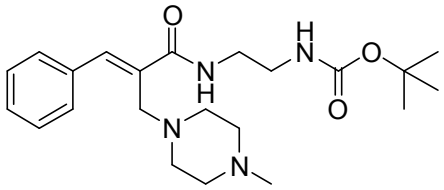
Procedures



Preparation of tert-butyl (E)-(2-(2-methyl-3-phenylacrylamido)ethyl)carbamate **52**: *N*,*N*-Diisopropylethylamine (1.34 mL, 7.70 mmol), HOBt (457 mg, 3.39 mmol), and EDCI (647 mg, 3.39 mmol) were added at 0°C to a stirred solution of the acid **51** (500 mg, 3.08 mmol) in dichloromethane (10.0 mL) and the reaction was stirred for 30 min. The appropriate amine (592 mg, 3.70 mmol) was added in one portion and the reaction was stirred overnight at room temperature. After completion of the reaction as indicated by TLC, the reaction mixture was quenched by the addition of saturated $NaHCO_3$ solution and extracted with dichloromethane (2 x 10.0 mL). The combined extracts were washed with brine (10.0 mL), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography (silica gel, hexanes:ethyl acetate) to yield 610 mg (65%) of **52** as cream solid. Mp 120 – 123 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.37 (s, 1H), 7.20 – 7.34 (m, 5H), 7.08 (br s, 1H), 5.36 (br s, 2H), 3.40 – 3.48 (m, 2H), 3.27 – 3.37 (m, 2H), 2.06 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 170.0, 157.4, 136.2, 134.1, 131.5, 129.3, 128.2, 127.7, 79.6, 41.8, 39.9, 28.3, 14.0.



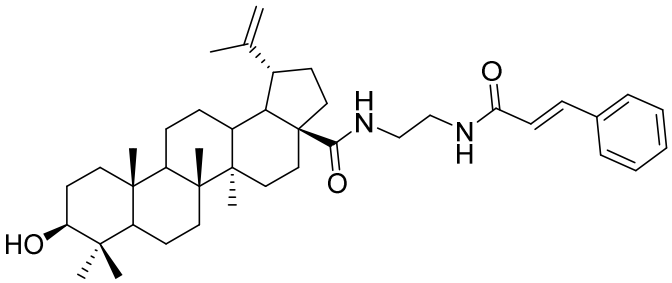
Preparation of tert-butyl (E)-(2-(2-((dimethylamino)methyl)-3-phenylacrylamido) ethylcarbamate **55**: Procedure similar to that of **52**. The reaction of acid **54** (200 mg, 0.97 mmol), and tert-butyl (2-aminoethyl) carbamate **42** (187 mg, 1.17 mmol), yielded 227 mg (67%) of **55** as pale cream solid. Mp 112 – 114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.75 (br s, 1H), 7.86 (s, 1H), 7.32 – 7.37 (m, 2H), 7.23 – 7.30 (m, 1H), 7.19 – 7.24 (m, 2H), 5.14 (br s, 1H), 3.40 – 3.47 (m, 2H), 3.34 (s, 2H), 3.25 – 3.32 (m, 2H), 2.19 (s, 6H), 1.41 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 168.9, 156.2, 139.7, 135.6, 130.5, 128.9(2C), 128.2, 127.8, 79.2, 55.9, 44.4, 40.8, 39.3, 28.4.



Preparation of tert-butyl(E)-(2-(2-((4-methylpiperazin-1-yl)methyl) -3-(phenylacrylamido)ethyl)carbamate **59**: Procedure similar to that of **52**. The reaction of

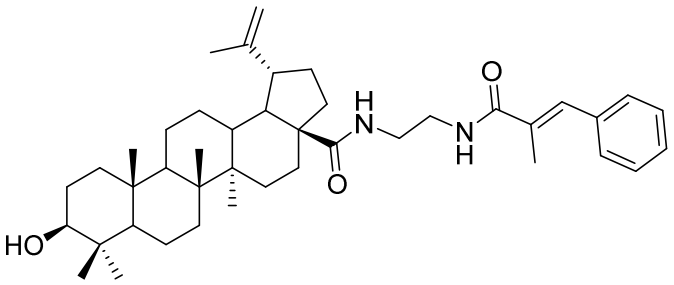
acid **58** (150 mg, 0.58 mmol), and tert-butyl (2-aminoethyl) carbamate **42** (110 mg, 0.69 mmol), yielded 153 mg (66%) of **59** as pale cream solid. Mp 135 – 137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.72 (br s, 1H), 7.93 (s, 1H), 7.27 – 7.40 (m, 3H), 7.17 – 7.27 (m, 2H), 4.98 (br s, 1H), 3.43 – 3.52 (m, 2H), 3.40 (s, 2H), 3.31 (q, J = 6.0 Hz, 2H), 2.31 – 2.70 (m, 8H), 2.29 (s, 3H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 168.7, 156.2, 140.2, 135.5, 129.7, 128.9, 128.2, 127.9, 79.1, 55.1, 54.7, 52.2, 45.8, 40.8, 39.2, 28.4.

Preparation of compounds **36-39**: To a stirred solution of appropriate Boc-protected amines (1.0 mmol) in dichloromethane (10.0 mmol) at 0°C, was added trifluoro acetic acid (2.0 mL) slowly dropwise. The reaction mixture was stirred at room temperature overnight. The solvent was removed *in vacuo,* the resulting slurry was diluted with cold water and adjusted pH to basic with 10% NaOH solution. The amine was extracted with ethyl acetate (2 x 10.0 mL), the combined extracts were extracted washed with saturated NaCl solution and dried over anhydrous Na2SO4. The solution was concentrated in *vacuo*, the resulting crude appropriate amines was utilized to react with betulinic acid without further purification.



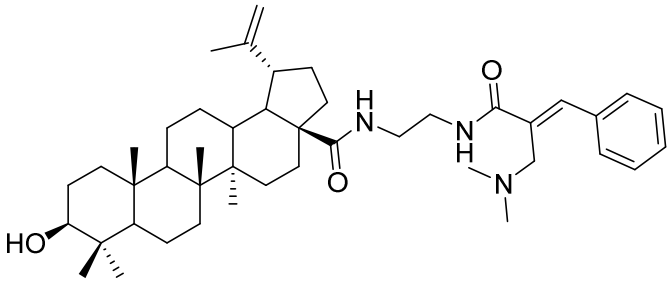
Preparation of compound **61a**: To a solution of betulinic acid **2** (250 mg, 0.55 mmol) in DMF (5.0 mL), were added *N*,*N*-diisopropylethylamine (191 μL, 1.10 mmol), and TBTU (194 mg, 0.60 mmol) at 0 °C. The solution was stirred for 30 min whereupon the appropriate amine **36** (157 mg, 0.82 mmol) was added and the mixture was stirred overnight at room temperature. Upon completion (TLC), the reaction mixture was quenched by the addition of saturated $NaHCO_3$ solution and extracted with ethyl acetate (2 x 10.0 mL). The combined extracts were washed with cold water (10.0 mL) and brine (10.0 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography (silica gel, hexanes:ethyl acetate) to afford 257 mg (67%) of **61a** as pale cream solid. Mp 112 – 114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.60 (d, *J* = 15.7 Hz, 1H), 7.47 – 7.53 (m, 2H), 7.32 – 7.38 (m, 3H), 6.62 (t, *J* = 4.7 Hz, 1H), 6.39 (d, *J* = 15.7 Hz, 1H), 6.25 (t, *J* = 5.3 Hz, 1H), 4.73 (d, *J* = 1.8 Hz, 1H), 4.58 (s, 1H), 3.49 – 3.63 (m, 3H), 3.34 – 3.41 (m, 1H), 3.06 – 3.17 (m, 2H),
2.43 (td, *J* = 3.6, 12.4 Hz, 1H), 1.86 – 2.00 (m, 2H), 0.59 – 1.76 (m, 21H), 1.67 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.87 (s, 3H), 0.68 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 178.4, 166.9, 150.8, 140.9, 134.8, 129.6, 128.8, 127.8, 120.6, 109.4, 78.9, 55.7, 55.3, 50.5,

50.1, 46.8, 42.4, 41.2, 40.7, 39.6, 38.8, 38.6, 38.4, 37.7, 37.1, 34.2, 33.6, 30.9, 29.5, 27.9, 27.4, 25.6, 20.9, 19.4, 18.1, 16.2, 15.9, 15.3, 14.6; ESIMS: m/z calculated for $C_{41}H_{60}N_2O_3$ $(M+H)^+$ 629.47, found 629.74; HPLC purity 95.7%.

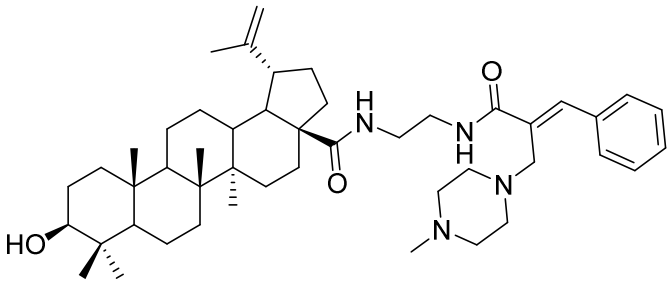


Preparation of compound **61b**: Procedure similar to that of **61a**. The reaction of betulinic acid **2** (120 mg, 0.26 mmol), and appropriate amine **37** (80 mg, 0.39 mmol), yielded 120 mg (71%) of **61b** as cream solid. Mp 125 – 127 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.27 – 7.42 (m, 6H), 7.09 (br s, 1H), 6.40 – 6.45 (m, 1H), 4.57 (s, 1H), 4.69 (s, 1H), 3.41 – 3.56 (m, 4H), 3.08 – 3.20 (m, 2H), 2.35 (dt, *J* = 3.6, 12.3 Hz, 1H), 2.11 (d, *J* = 1.4 Hz, 3H), 1.86 – 2.02 (m, 1H), 0.61 – 1.77 (m, 23H), 1.65 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.85 (s, 3H), 0.72 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 178.4, 170.2, 150.8, 136.1, 134.4, 131.3, 129.4, 128.3, 127.8, 109.4, 78.9, 55.7, 55.3, 50.5, 50.1, 46.9, 42.4, 41.8, 40.6, 39.6, 38.8, 38.6, 38.4, 37.9, 37.1, 34.3, 33.6, 30.9, 29.5, 27.9, 27.4, 25.5, 20.8, 19.4, 18.2, 16.1, 16.0, 15.4, 14.6, 14.2; ESIMS: m/z calculated for $C_{42}H_{62}N_2O_3$

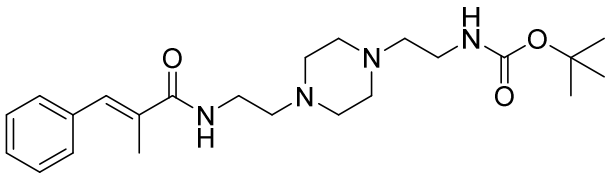
$(M+H)^+$ 643.49, found 643.71; HPLC purity 98.2%.



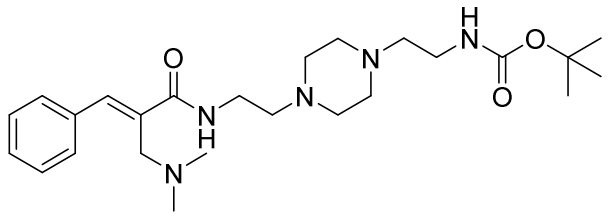
Preparation of compound **61c**: Procedure similar to that of **61a**. The reaction of acid **2** (100 mg, 0.22 mmol), and appropriate amine **38** (82 mg, 0.33 mmol), yielded 104 mg (69%) of **61c** as cream solid. Mp 115 – 117 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.99 (t, *J* = 5.9 Hz, 1H), 7.90 (s, 1H), 7.28 – 7.39 (m, 3H), 7.20 – 7.24 (m, 2H), 6.67 (t, *J* = 4.6 Hz, 1H), 4.73 (d, *J* = 2.4 Hz, 1H), 4.58 (d, *J* = 2.4 Hz, 1H), 3.35 – 3.58 (m, 4H), 3.32 (s, 2H), 3.12 – 3.20 (m, 2H), 2.41 (dt, *J* = 3.5, 12.8 Hz, 1H), 2.18 (s, 6H), 0.64 – 2.06 (m, 23H), 1.67 (s, 3H), 0.95 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H), 0.79 (s, 3H), 0.74 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.8, 169.7, 151.1, 140.0, 135.6, 130.4, 129.0, 128.2, 127.9, 109.2, 78.9, 56.2, 55.6, 55.3, 50.6, 50.2, 46.8, 44.5, 42.4, 41.3, 40.7, 38.8, 38.7, 38.5, 38.3, 37.8, 37.2, 34.4, 33.5, 30.9, 29.5, 27.9, 27.4, 25.6, 20.9, 19.5, 18.2, 16.2, 16.1, 15.4, 14.6; ESIMS: m/z calculated for $C_{44}H_{67}N_3O_3$ $(M+H)^+$ 686.53, found 686.75; HPLC purity 97.6%.



Preparation of compound **61d**: Procedure similar to that of **61a**. The reaction of acid **2** (125 mg, 0.27 mmol), and appropriate amine **39** (122 mg, 0.41 mmol), yielded 132 mg (65%) of **61d** as cream solid. Mp 121 – 123 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.82 (t, *J* = 5.9 Hz, 1H), 7.87 (s, 1H), 7.22 – 7.31 (m, 3H), 7.17 (d, *J* = 7.1 Hz, 3H), 6.76 (t, *J* = 4.5 Hz, 1H), 4.67 (s, 1H), 4.52 (s, 1H), 3.05 – 3.28 (m, 4H), 3.36 (s, 2H), 2.16 – 2.56 (m, 9H), 2.23 (s, 3H), 1.82 – 2.06 (m, 2H), 0.58 – 1.82 (m, 23H), 1.62 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.85 (s, 3H), 0.73 (s, 3H), 0.68 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.8, 169.5, 151.0, 140.7, 135.3, 129.4, 128.9, 128.3, 128.0, 109.2, 78.6, 55.6, 55.3, 55.1, 54.7, 52.1, 50.5, 50.1, 46.8, 45.8, 42.4, 41.5, 40.6, 38.8, 38.7, 38.5, 38.3, 37.7, 37.1, 34.3, 33.4, 30.9, 29.5, 28.0, 27.4, 25.6, 20.9, 19.4, 18.2, 16.2, 16.1, 15.4, 14.6; ESIMS: m/z calculated for $C_{47}H_{72}N_4O_3$ $(M+H)^+$ 741.57, found 741.73; HPLC purity 93.4%.

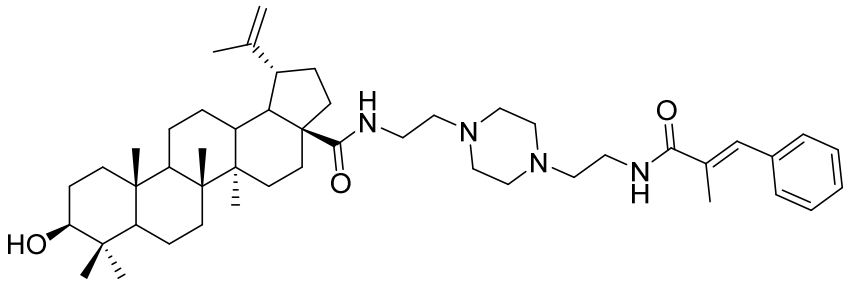


Preparation of compound **67b**: Procedure similar to that of **52**. The reaction of acid **51** (250 mg, 1.54 mmol), and amine **66** (630 mg, 2.31 mmol), yielded 392 mg (61%) of **67b** as white solid. Mp 107 – 109 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.26 – 7.40 (m, 6H), 6.54 (br s, 1H), 4.96 (s, 1H), 3.38 – 3.46 (m, 2H), 3.16 – 3.26 (m, 2H), 2.57 (t, *J* = 6.1 Hz, 2H), 2.38 – 2.53 (m, 8H), 2.45 (t, *J* = 6.1 Hz, 2H), 2.08 (d, *J* = 1.6 Hz, 3H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 169.3, 155.9, 136.2, 133.8, 131.9, 129.3, 128.3, 127.7, 79.1, 57.1, 56.2, 52.9, 52.7, 37.1, 36.3, 28.4, 14.2.



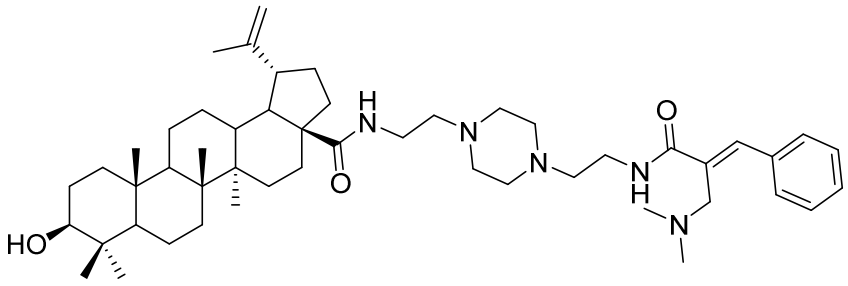
Preparation of compound **67c**: Procedure similar to that of **52**. The reaction of acid **54** (230 mg, 1.12 mmol), and amine **66** (458 mg, 1.68 mmol), yielded 304 mg (59%) of

67c as orange semi solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.83 (br s, 1H), 7.88 (s, 1H), 7.30 – 7.35 (m, 2H), 7.24 – 7.28 (m, 1H), 7.19 – 7.23 (m, 2H), 5.02 (s, 1H), 3.40 – 3.48 (m, 2H), 3.29 (s, 2H), 3.17 – 3.24 (m, 2H), 2.52 (t, *J* = 6.1 Hz, 2H), 2.44 (t, *J* = 6.0 Hz, 2H), 2.38 – 2.57 (m, 8H), (2.16 (s, 6H), 1.42 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 168.1, 155.9, 139.2, 135.9, 130.9, 129.0, 128.2, 127.6, 57.2, 56.8, 56.1, 53.1, 52.9, 44.6, 37.1, 36.6, 28.4.



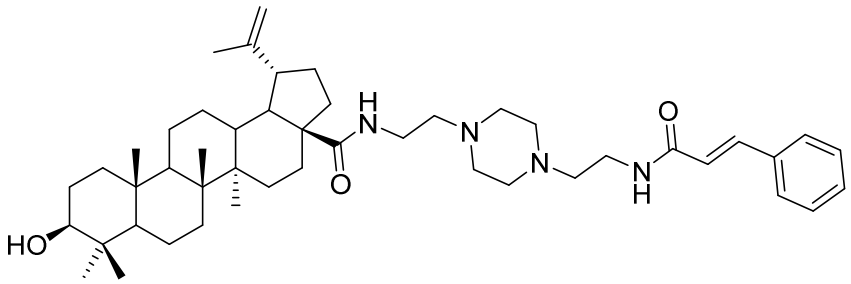
Preparation of compound **69b**: Procedure similar to that of **61a**. The reaction of acid **2** (90 mg, 0.19 mmol), and amine **62b** (94 mg, 0.29 mmol), yielded 106 mg (71%) of **69b** as cream solid. Mp 120 – 121 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.26 – 7.40 (m, 6H), 6.52 (t, *J* = 4.4 Hz, 2H), 6.22 (t, *J* = 4.8 Hz, 1H), 4.73 (s, 1H), 4.59 (s, 1H), 3.42 – 3.49 (m, 2H), 3.26 – 3.38 (m, 2H), 3.17 (dd, *J* = 5.0, 11.1 Hz, 1H), 3.07 (dt, *J* = 3.7, 11.0 Hz, 1H), 2.56 (t, *J* = 6.0 Hz, 2H), 2.49 (t, *J* = 6.1 Hz, 2H), 2.40 – 2.61 (m, 8H), 2.35 (dt, *J* = 3.6, 12.4 Hz, 1H), 2.09 (d, *J* = 1.5 Hz, 3H), 1.90 – 2.04 (m, 2H), 0.65 – 1.90 (m, 23H),

1.68 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H), 0.80 (s, 3H), 0.74 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.1, 169.4, 150.8, 136.2, 133.9, 131.9, 129.3, 128.3, 127.8, 109.4, 78.9, 56.3, 56.2, 55.8, 55.4, 52.9, 52.8, 50.5, 49.9, 47.0, 42.5, 40.7, 38.8, 38.7, 38.4, 37.9, 37.2, 36.3, 35.6, 34.4, 33.7, 30.9, 29.4, 27.9, 27.4, 25.6, 20.9, 19.4, 18.3, 16.2, 16.1, 15.4, 14.7, 14.2; ESIMS: m/z calculated for $C_{48}H_{74}N_4O_3$ $(M+H)^+$ 755.59, found 755.79; HPLC purity 95.7%.



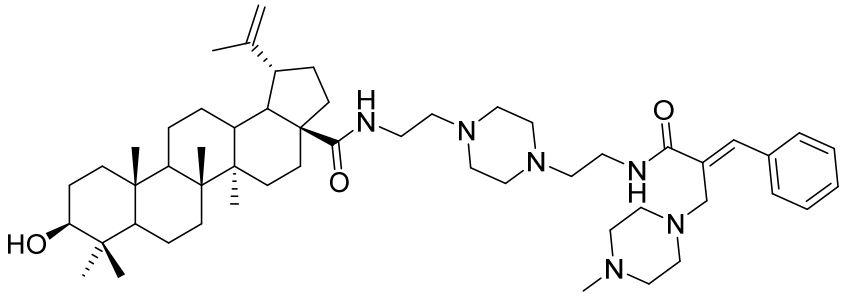
Preparation of compound **69c**: Procedure similar to that of **61a**. The reaction of acid **2** (125 mg, 0.27 mmol), and amine **62c** (148 mg, 0.41 mmol), yielded 95 mg (68%) of **69c** as cream solid. Mp 127 – 130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.86 (br s, 1H), 7.90 (s, 1H), 7.18 – 7.37 (m, 5H), 6.26 (br s, 1H), 4.73 (s, 1H), 4.58 (s, 1H), 3.40 – 3.51 (m, 2H), 3.26 – 3.38 (m, 2H), 3.31 (s, 2H), 3.16 (dd, *J* = 5.0, 11.1 Hz, 1H), 3.07 (dt, *J* = 3.8, 11.1 Hz, 1H), 2.41 – 2.56 (m, 12H), 2.30 – 2.39 (m, 1H), 2.18 (s, 6H), 1.88 – 2.05 (m, 1H), 0.74 – 1.68 (m, 23H), 1.68 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H), 0.79 (s,

3H), 0.74 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.2, 168.1, 150.9, 139.3, 135.9, 130.9, 129.0, 128.2, 127.7, 109.4, 78.9, 56.8, 56.4, 56.1, 55.9, 55.4, 53.0, 52.9, 50.5, 49.9, 47.0, 44.6, 42.5, 40.7, 38.8, 38.7, 38.4, 37.9, 37.2, 36.6, 35.6, 34.4, 33.7, 30.9, 29.4, 27.9, 27.4, 25.6, 20.9, 19.4, 18.3, 16.2, 16.1, 15.3, 14.7; ESIMS: m/z calculated for $C_{50}H_{79}N_5O_3$ $(M+H)^+$ 798.63 found 798.94; HPLC purity 96.2%.



Preparation of compound **69a**: Procedure similar to that of **61a**. The reaction of acid **2** (100 mg, 0.22 mmol), and amine **62a** (99 mg, 0.33 mmol), yielded 94 mg (57%) of **69a** as cream solid. Mp 110 – 113 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.62 (d, J = 15.6 Hz, 1H), 7.48 – 7.53 (m, 2H), 7.31 – 7.41 (m, 3H), 6.41 (d, J = 15.6 Hz, 1H), 6.16 – 6.25 (m, 2H), 4.59 (s, 1H), 4.73 (d, J = 2.3 Hz, 1H), 3.45 – 3.52 (m, 2H), 3.27 – 3.40 (m, 2H), 3.17 (dd, J = 5.0, 11.1 Hz, 1H), 3.08 (dt, J = 3.8, 11.1 Hz, 1H), 2.42 – 2.59 (m, 12H), 2.36 (dt, J = 3.6, 12.4 Hz, 1H), 1.90 – 2.06 (m, 2H), 0.62 – 1.80 (m, 22H), 1.68 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H), 0.79 (s, 3H), 0.74 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$):

δ (ppm) 176.1, 165.8, 150.9, 140.9, 134.9, 129.6, 128.8, 127.8, 120.7, 109.4, 78.9, 56.5, 56.3, 55.9, 55.4, 53.0, 52.7, 50.5, 49.8, 47.0, 42.5, 40.7, 38.8, 38.7, 38.4, 37.9, 37.2, 36.1, 35.6, 34.4, 33.7, 30.9, 29.4, 27.9, 27.4, 25.6, 20.9, 19.4, 18.3, 16.2, 16.1, 15.4, 14.7; ESIMS: m/z calculated for $C_{47}H_{72}N_4O_3$ $(M+H)^+$ 741.57, found 741.93; HPLC purity 97.4%.



Preparation of compound **69d**: Procedure similar to that of **61a**. The reaction of acid **2** (80 mg, 0.18 mmol), and amine **62d** (108 mg, 0.27 mmol), yielded 82 mg (54%) of **69d** as cream solid. Mp 131 – 133 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.56 (br s, 1H), 7.93 (s, 1H), 7.32 – 7.37 (m, 2H), 7.22 – 7.31 (m, 3H), 6.24 (br s, 1H), 4.74 (d, J = 2.4 Hz, 1H), 4.59 (s, 1H), 3.46 – 3.54 (m, 2H), 3.39 (s, 2H), 3.29 – 3.38 (m, 2H), 3.18 (dd, J = 4.9, 11.2 Hz, 1H), 3.08 (dt, J = 3.8, 11.1 Hz, 1H), 2.31 – 2.62 (m, 21H), 2.29 (s, 3H), 1.89 – 2.07 (m, 2H), 0.63 – 1.81 (m, 22H), 1.69 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.92 (s, 3H), 0.81 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.2, 168.2, 150.9,

140.1, 135.7, 129.9, 128.9, 128.2, 127.8, 109.4, 78.9, 57.3, 56.4, 55.9, 55.4, 55.1, 54.8, 53.2, 52.8, 52.3, 50.5, 49.9, 47.0, 45.9, 42.5, 40.7, 38.8, 38.7, 38.4, 37.9, 37.2, 36.6, 35.6, 34.4, 33.7, 30.9, 29.7, 29.4, 27.9, 27.4, 25.6, 20.9, 19.4, 18.3, 16.2, 16.1, 15.3, 14.7; ESIMS: m/z calculated for $C_{53}H_{84}N_6O_3$ $(M+H)^+$ 853.67, found 853.99; HPLC purity 98.7%.

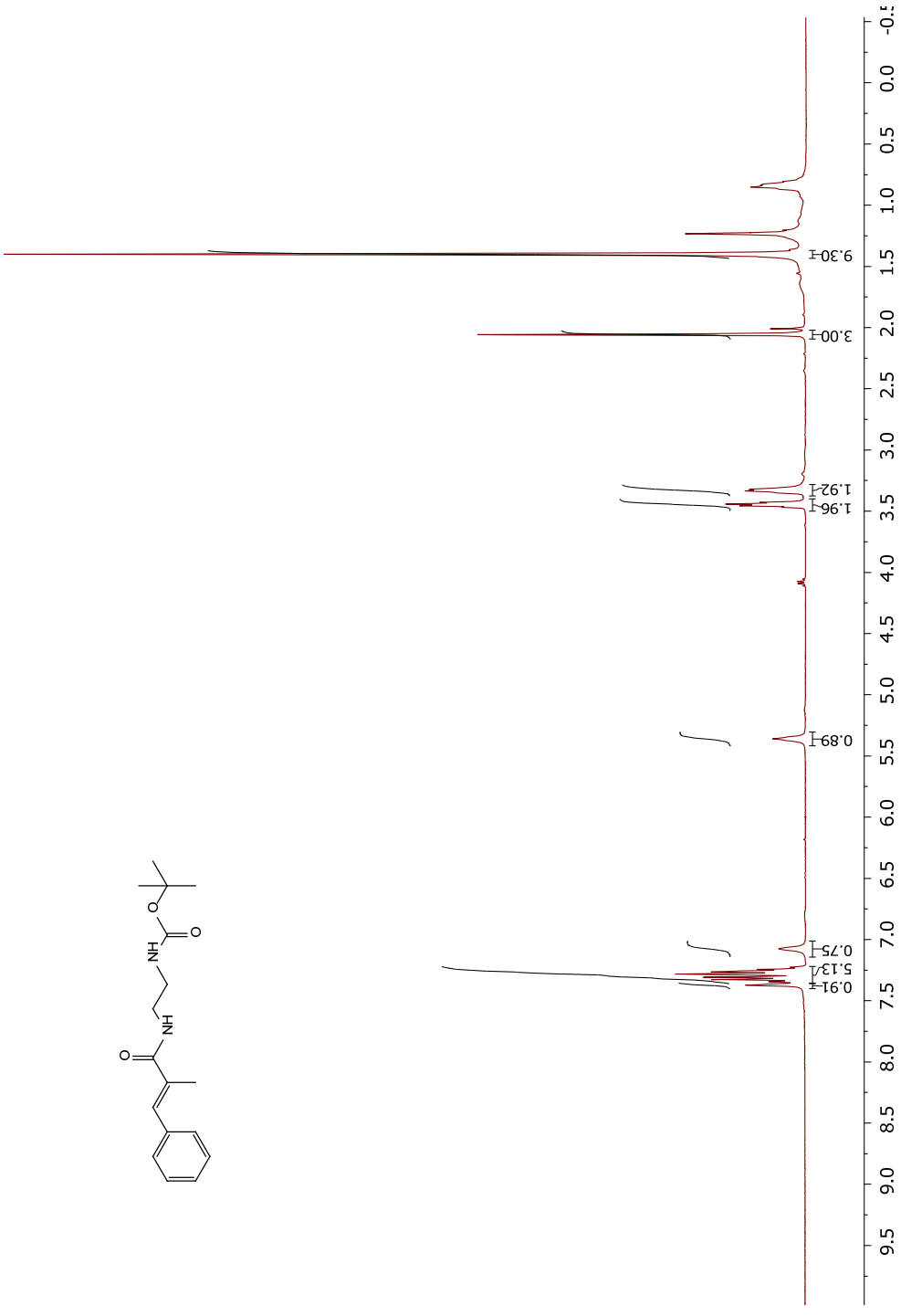


Figure 27. 400 MHz ^{1}H NMR of Compound **52** in $CDCl_3$

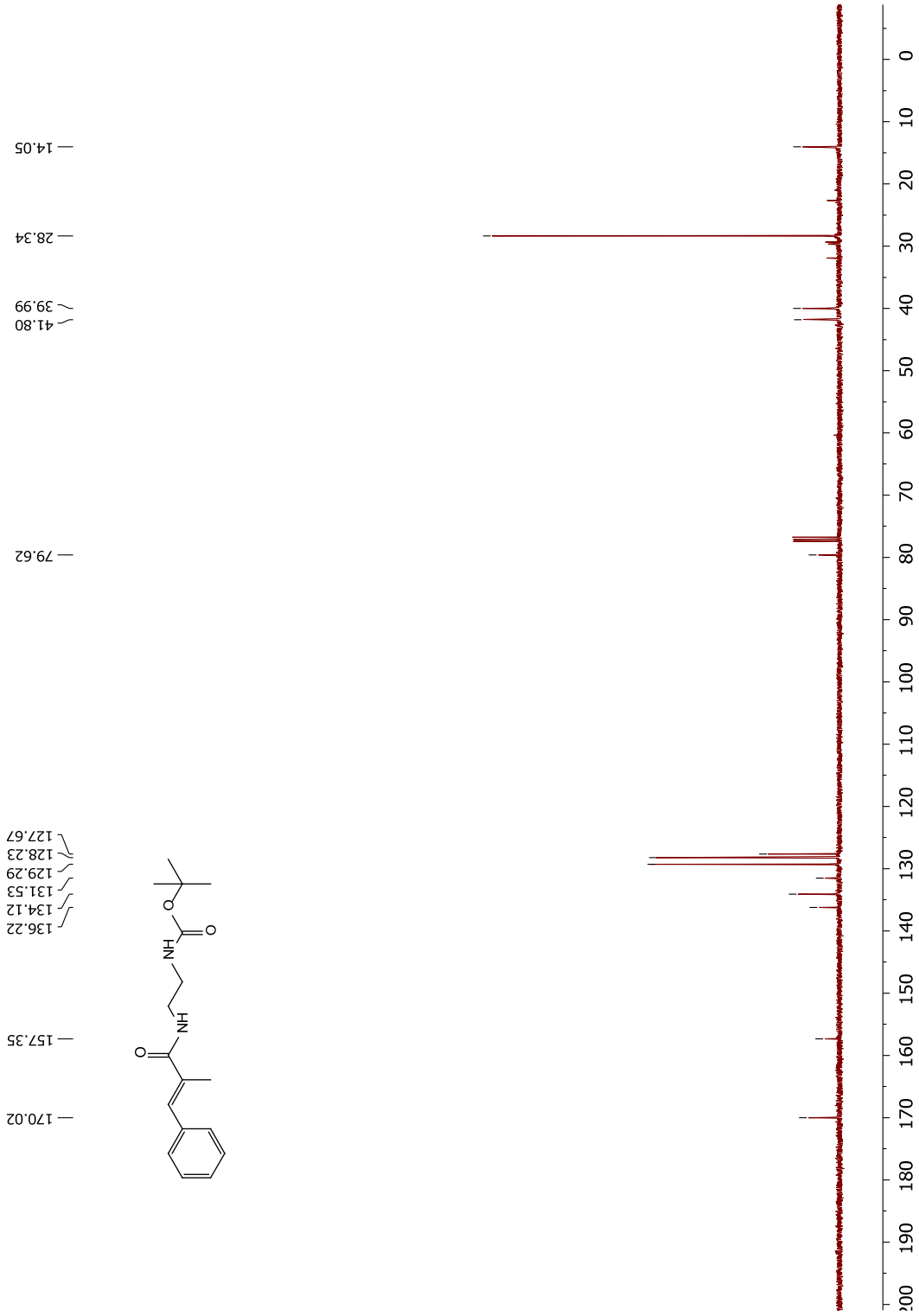


Figure 28. 101 MHz ^{13}C NMR of Compound **52** in $CDCl_3$

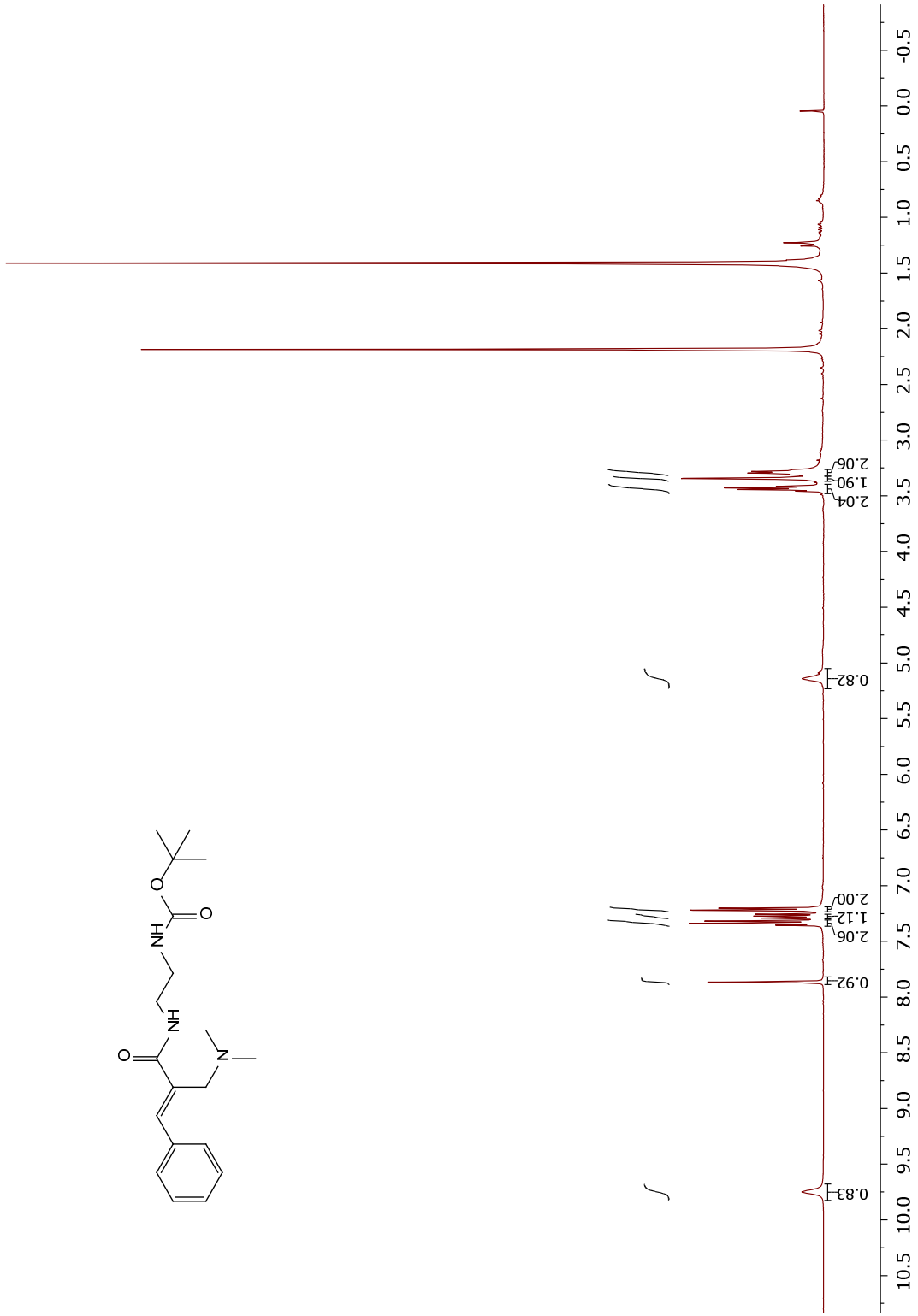


Figure 29. 400 MHz ^{1}H NMR of Compound **55** in $CDCl_3$

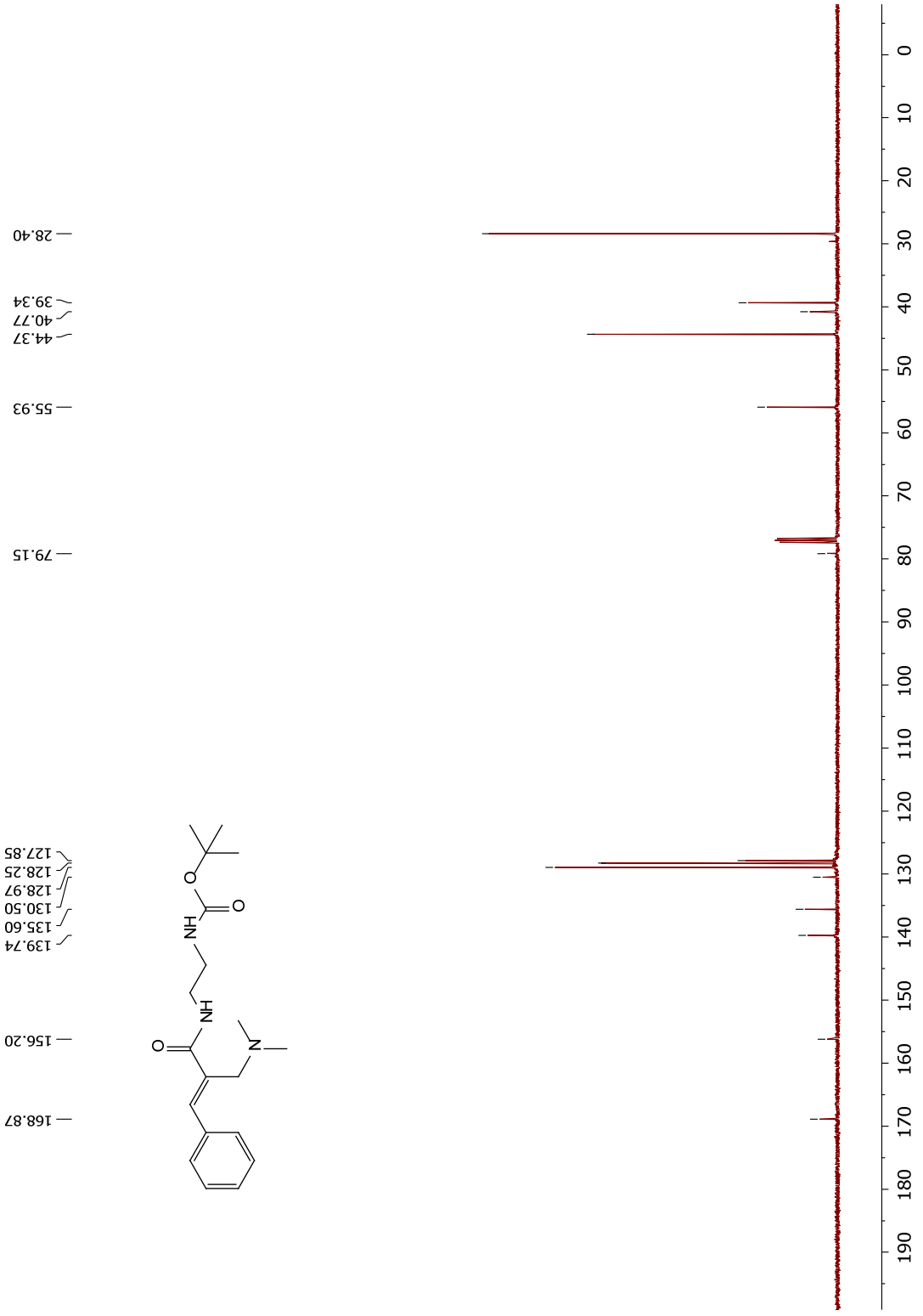


Figure 30. 101 MHz ^{13}C NMR of Compound **55** in $CDCl_3$

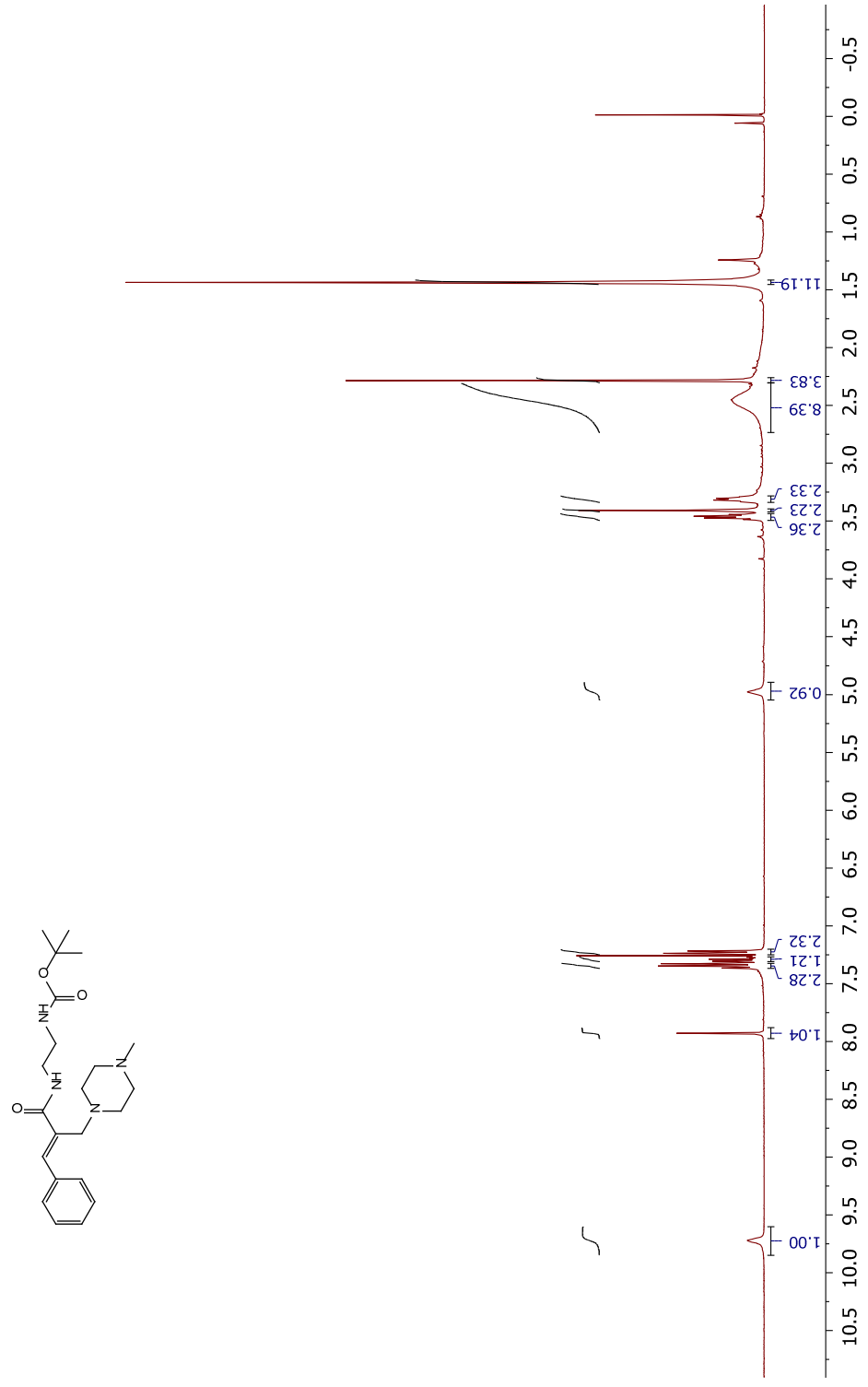


Figure 31. 400 MHz ^{1}H NMR of Compound **59** in $CDCl_3$

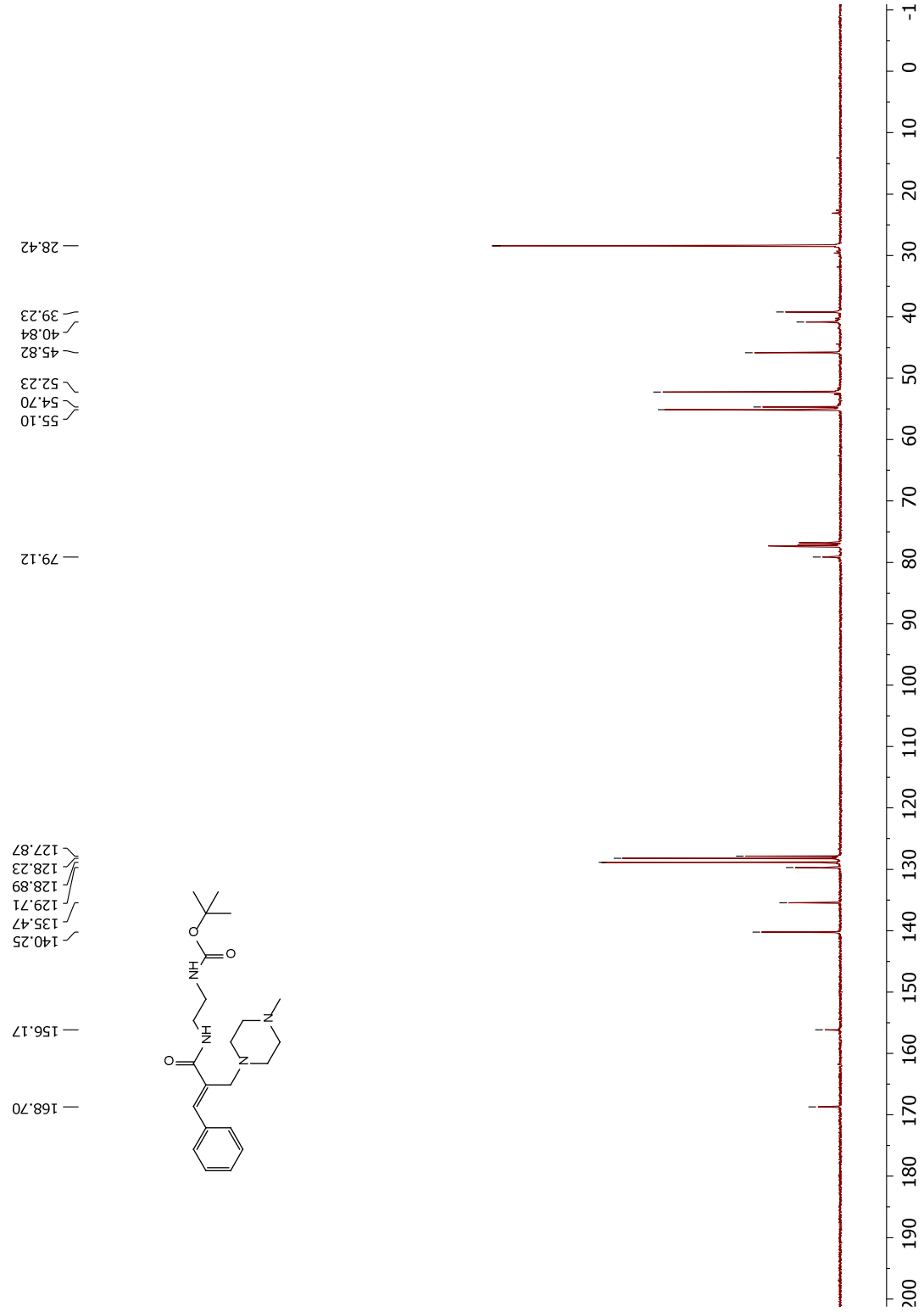


Figure 32. 101 MHz ^{13}C NMR of Compound **59** in $CDCl_3$

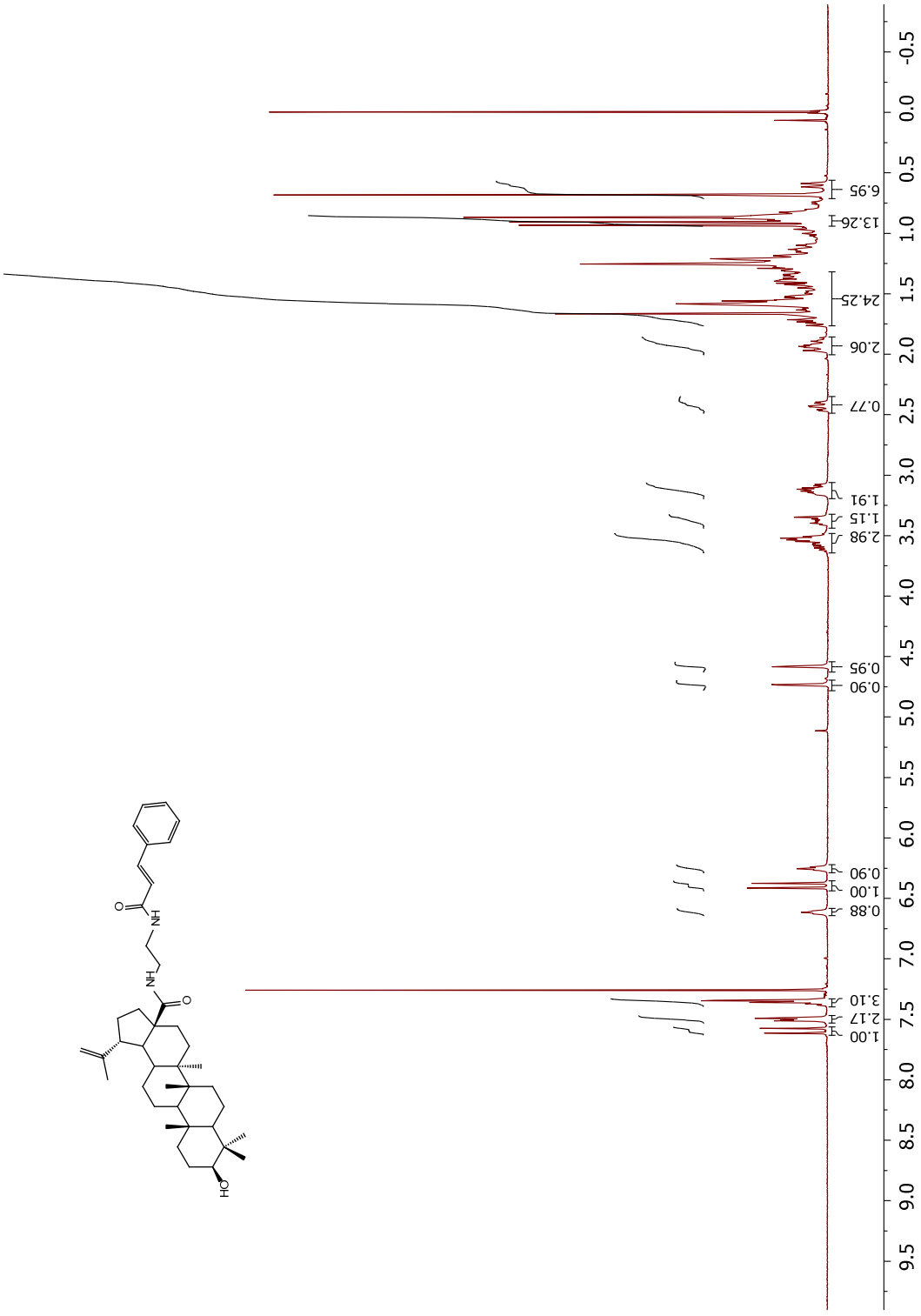


Figure 33. 400 MHz ^{1}H NMR of Compound **61a** in $CDCl_3$

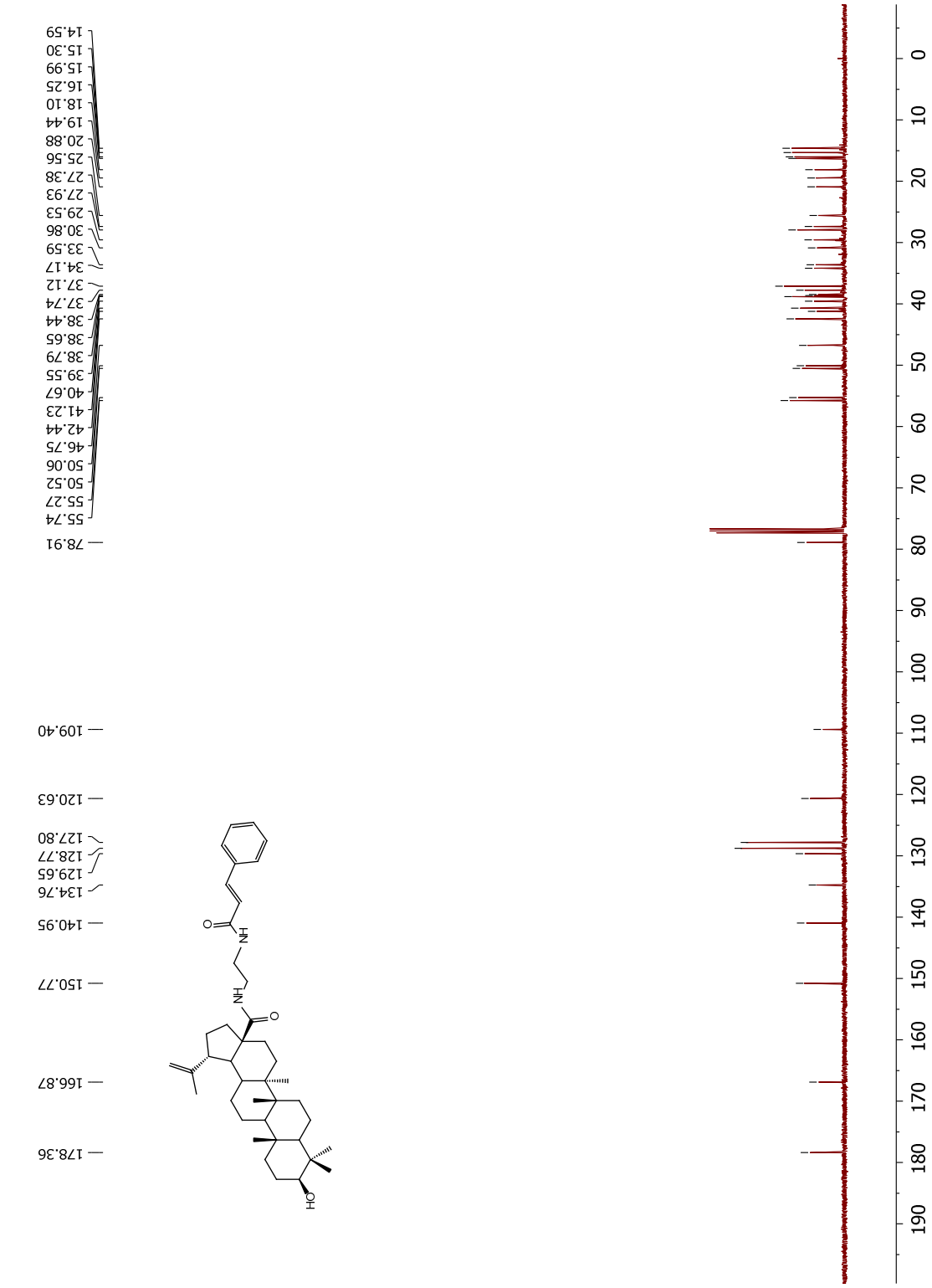


Figure 34. 101 MHz ^{13}C NMR of Compound **61a** in $CDCl_3$

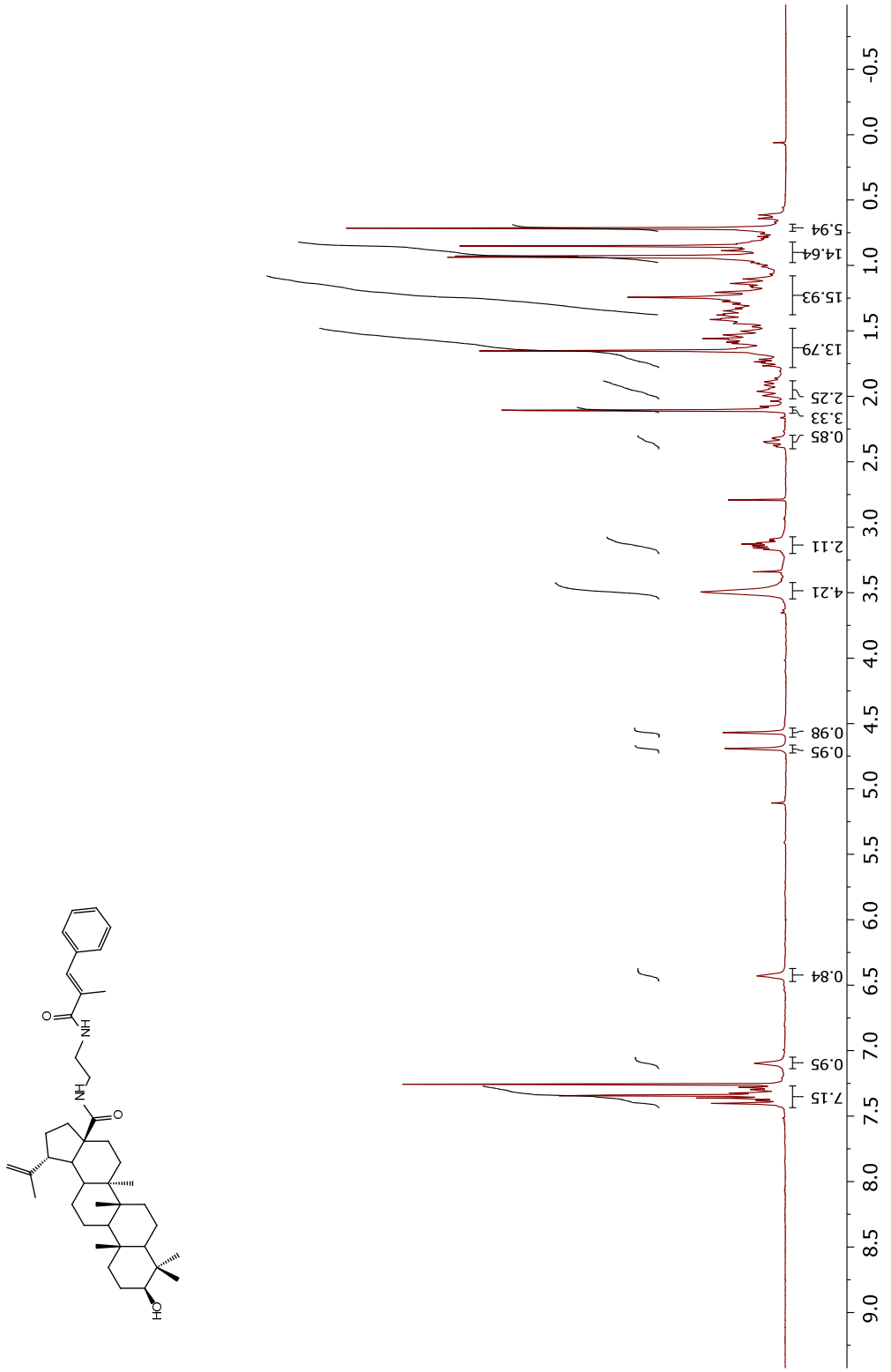


Figure 35. 400 MHz ^{1}H NMR of Compound **61b** in $CDCl_3$

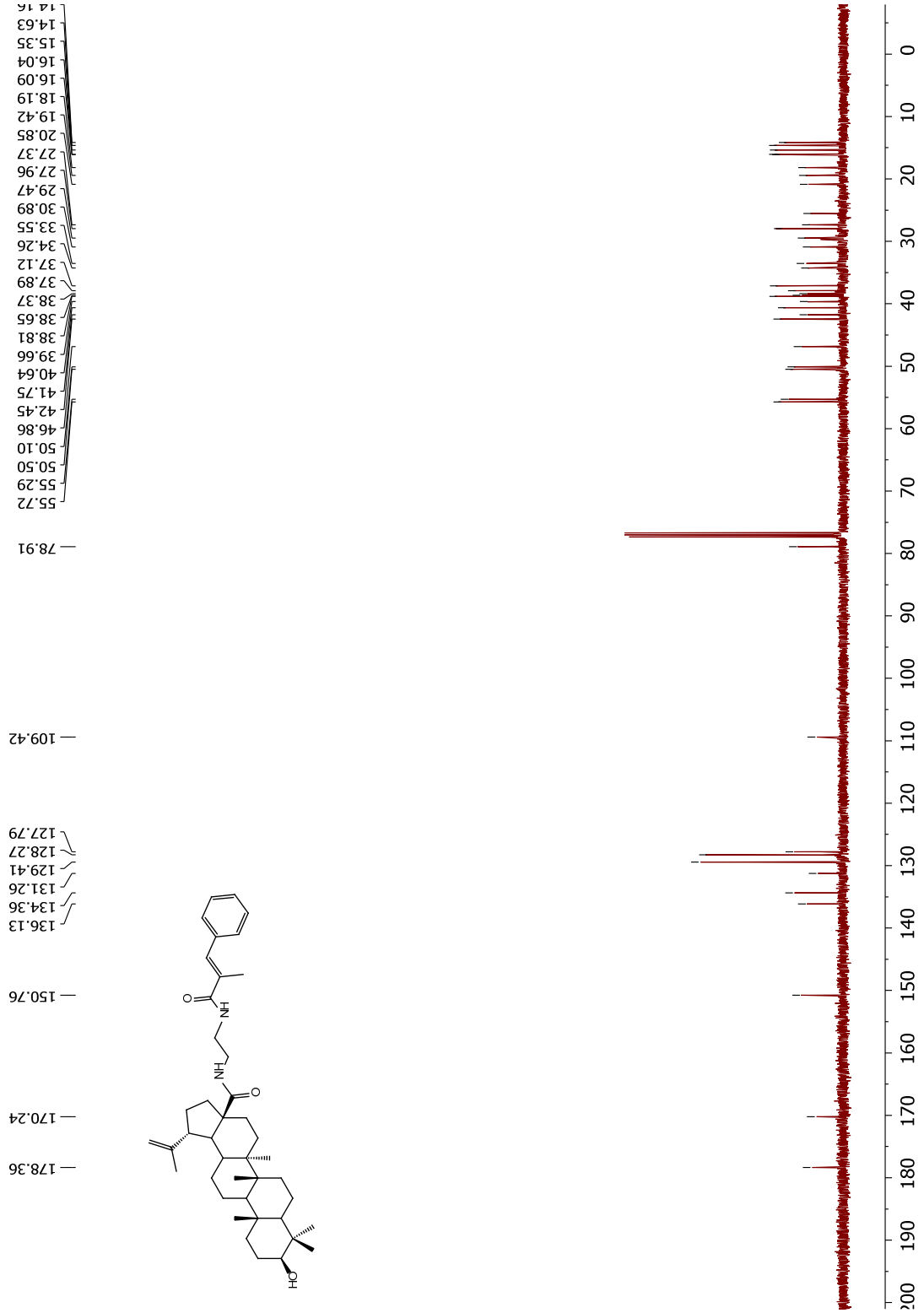


Figure 36. 101 MHz ^{13}C NMR of Compound **61b** in $CDCl_3$

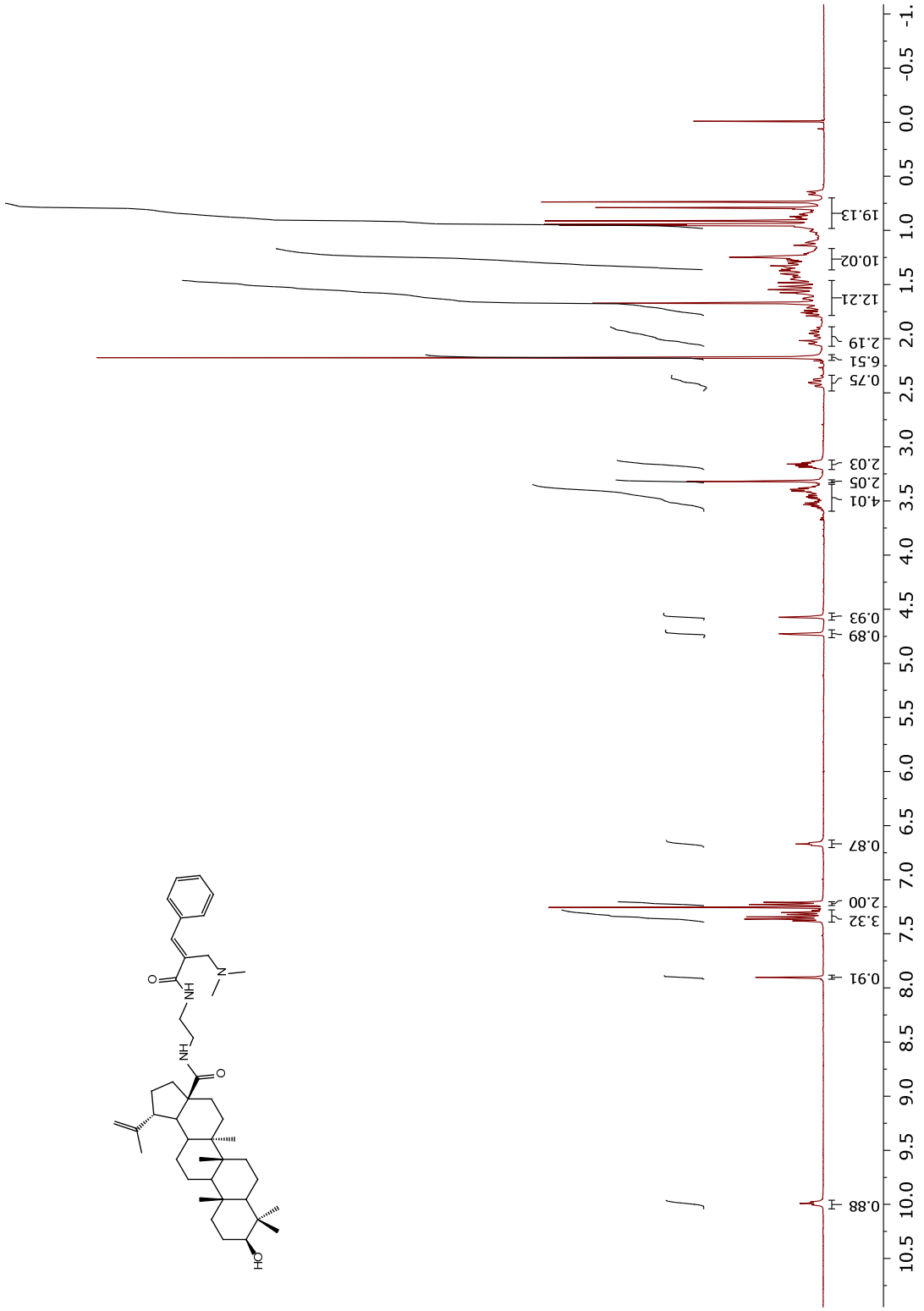


Figure 37. 400 MHz ^{1}H NMR of Compound **61c** in $CDCl_3$

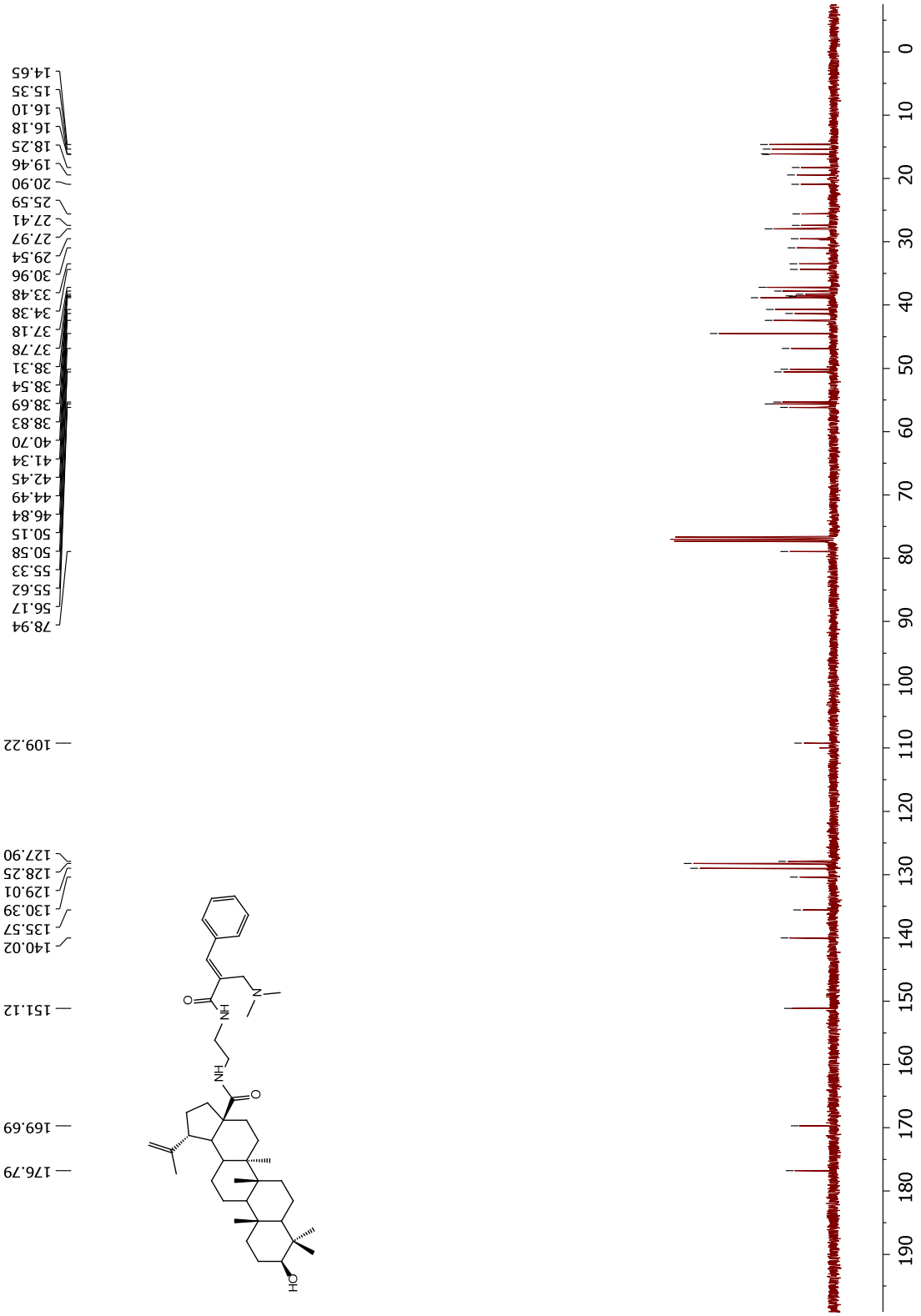


Figure 38. 101 MHz ^{13}C NMR of Compound **61c** in $CDCl_3$

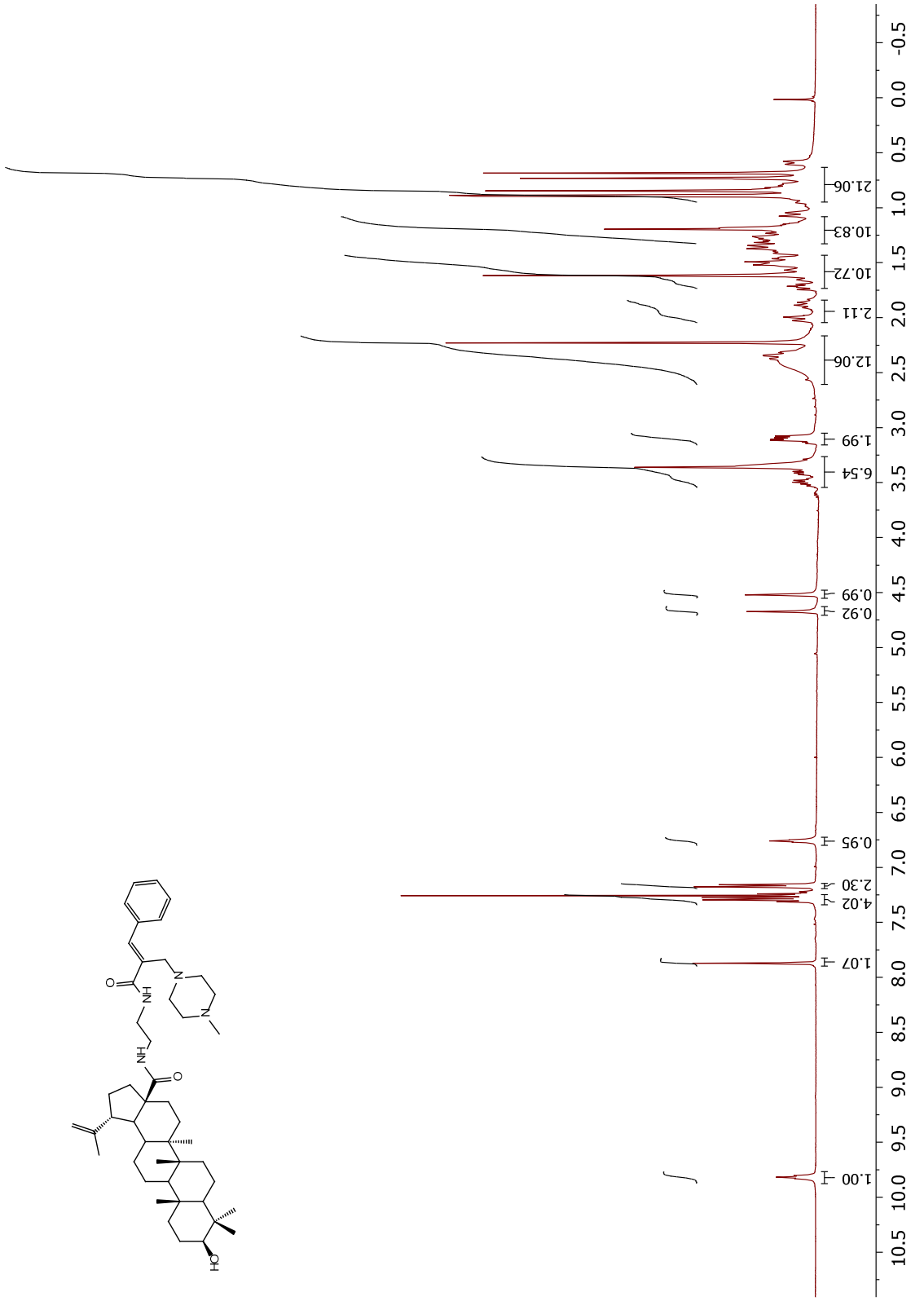


Figure 39. 400 MHz ^{1}H NMR of Compound **61d** in $CDCl_3$

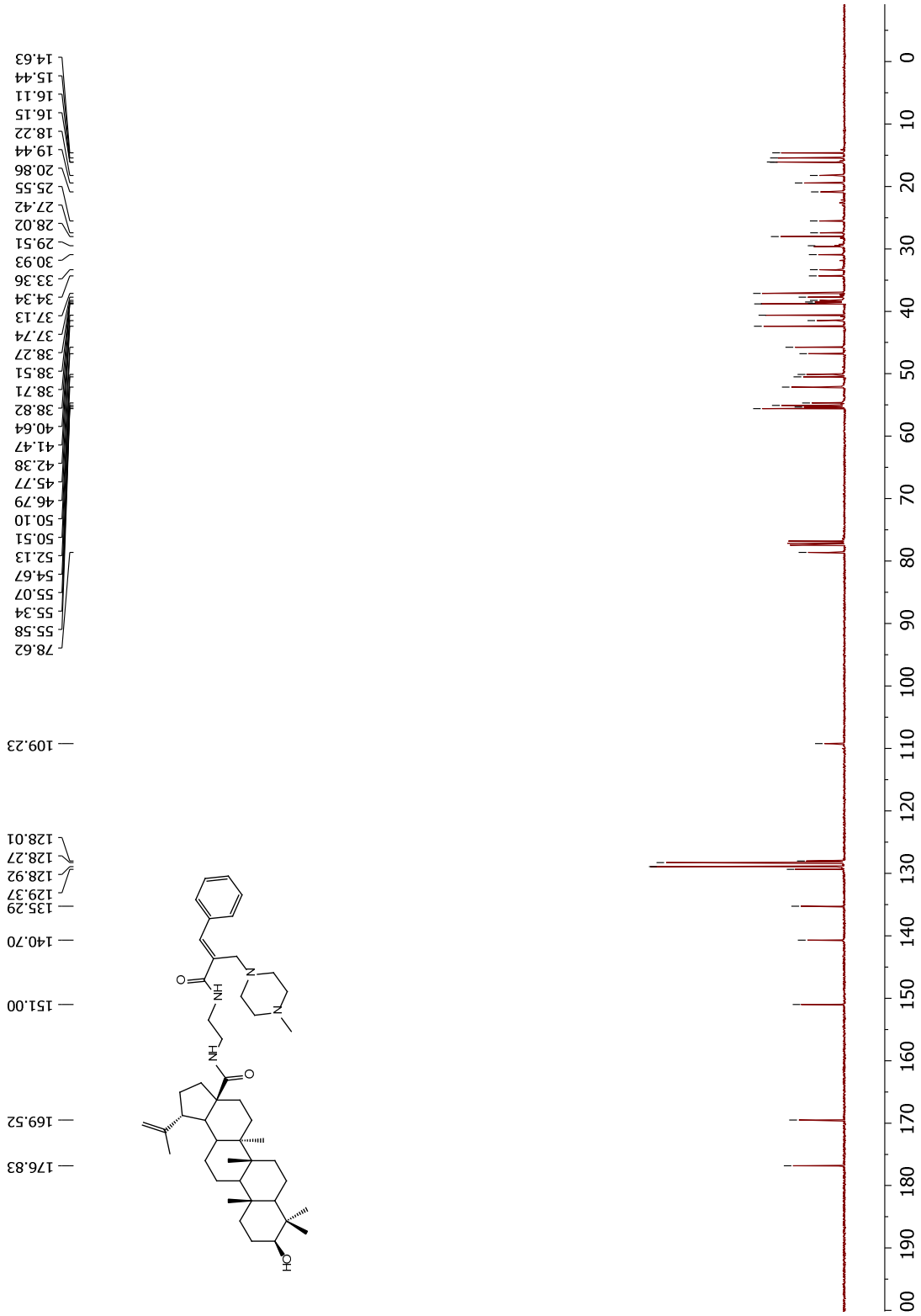


Figure 40. 101 MHz ^{13}C NMR of Compound **61d** in $CDCl_3$

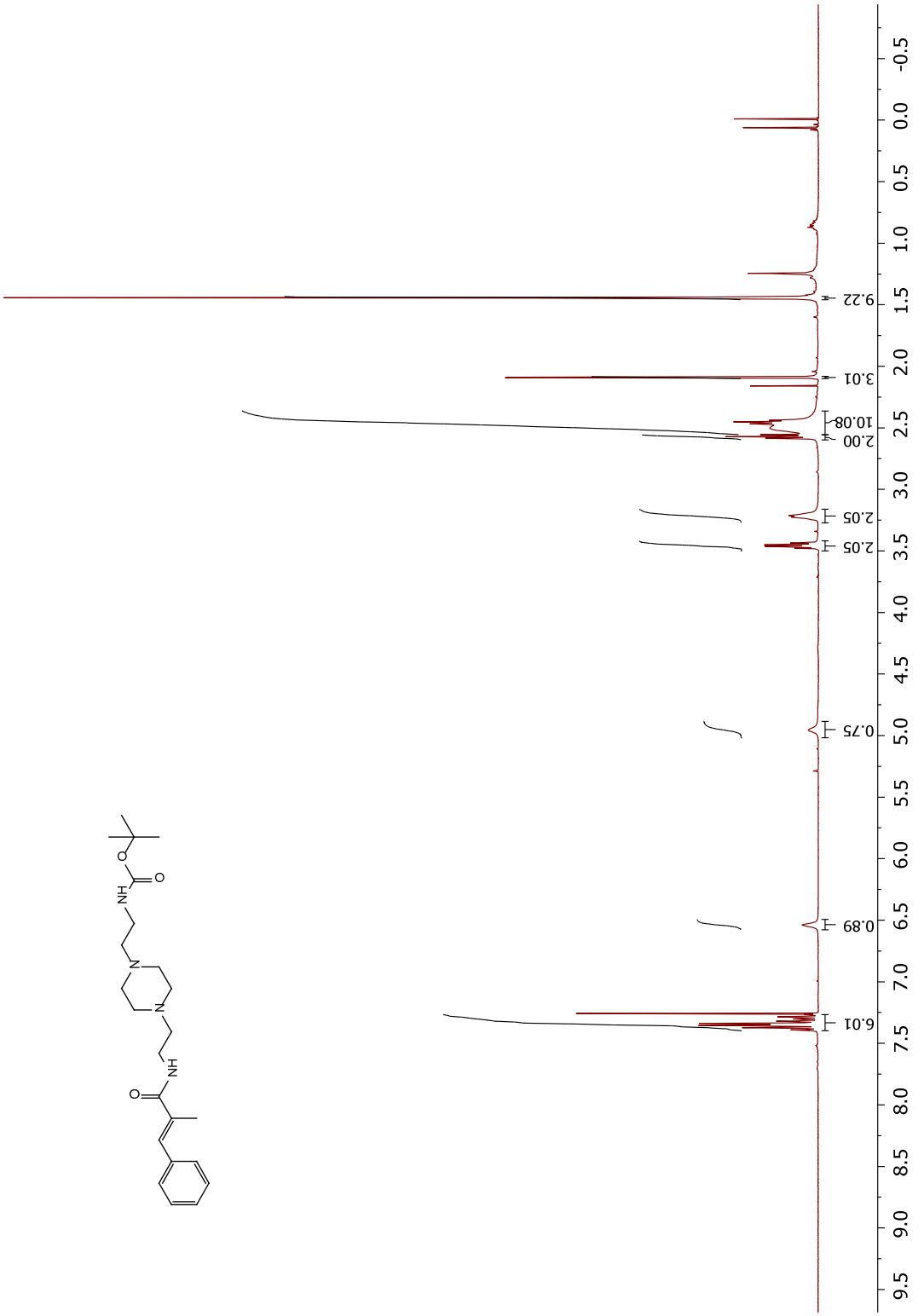


Figure 41. 400 MHz ^{1}H NMR of Compound **67b** in $CDCl_3$

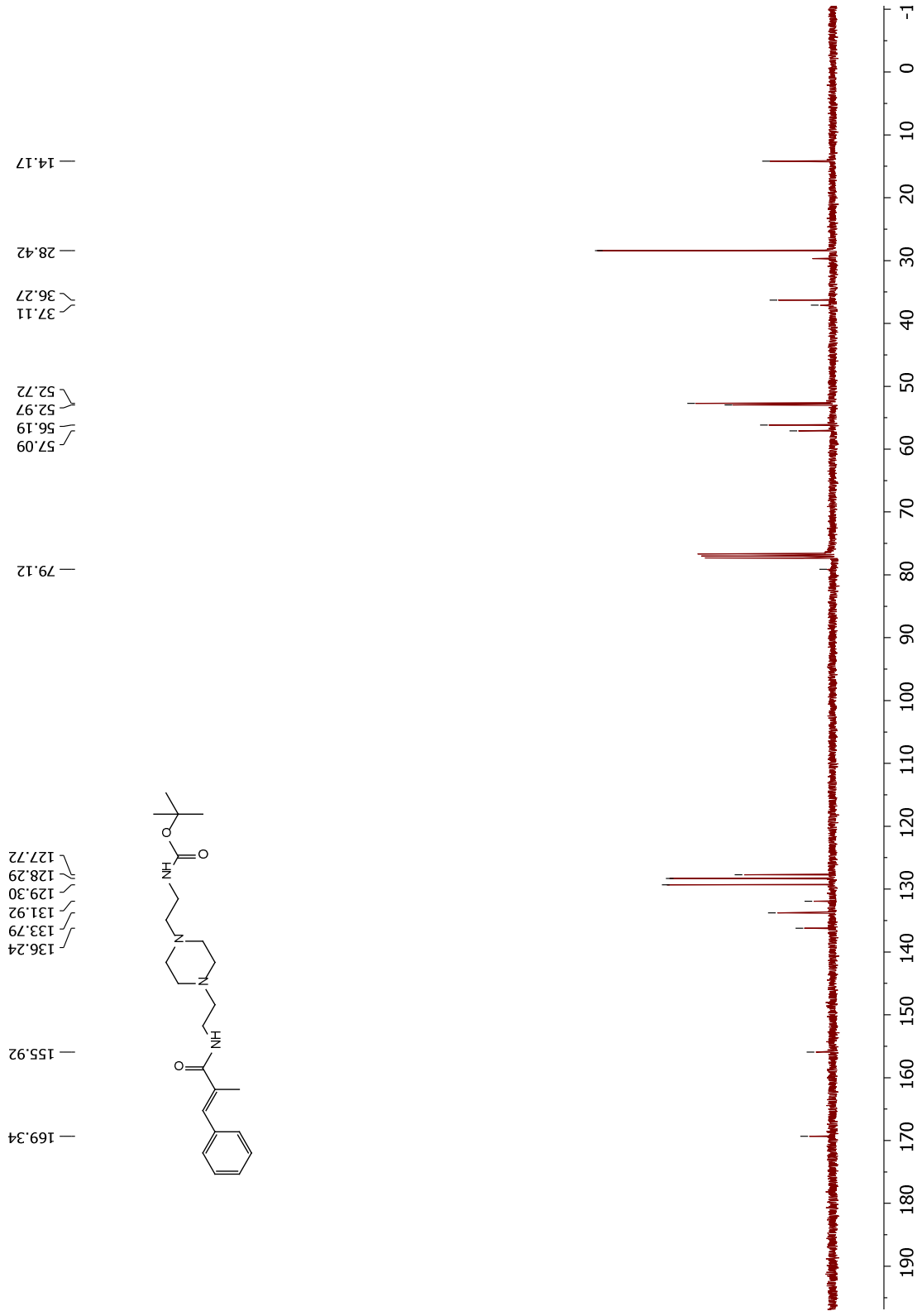


Figure 42. 101 MHz ^{13}C NMR of Compound **67b** in $CDCl_3$

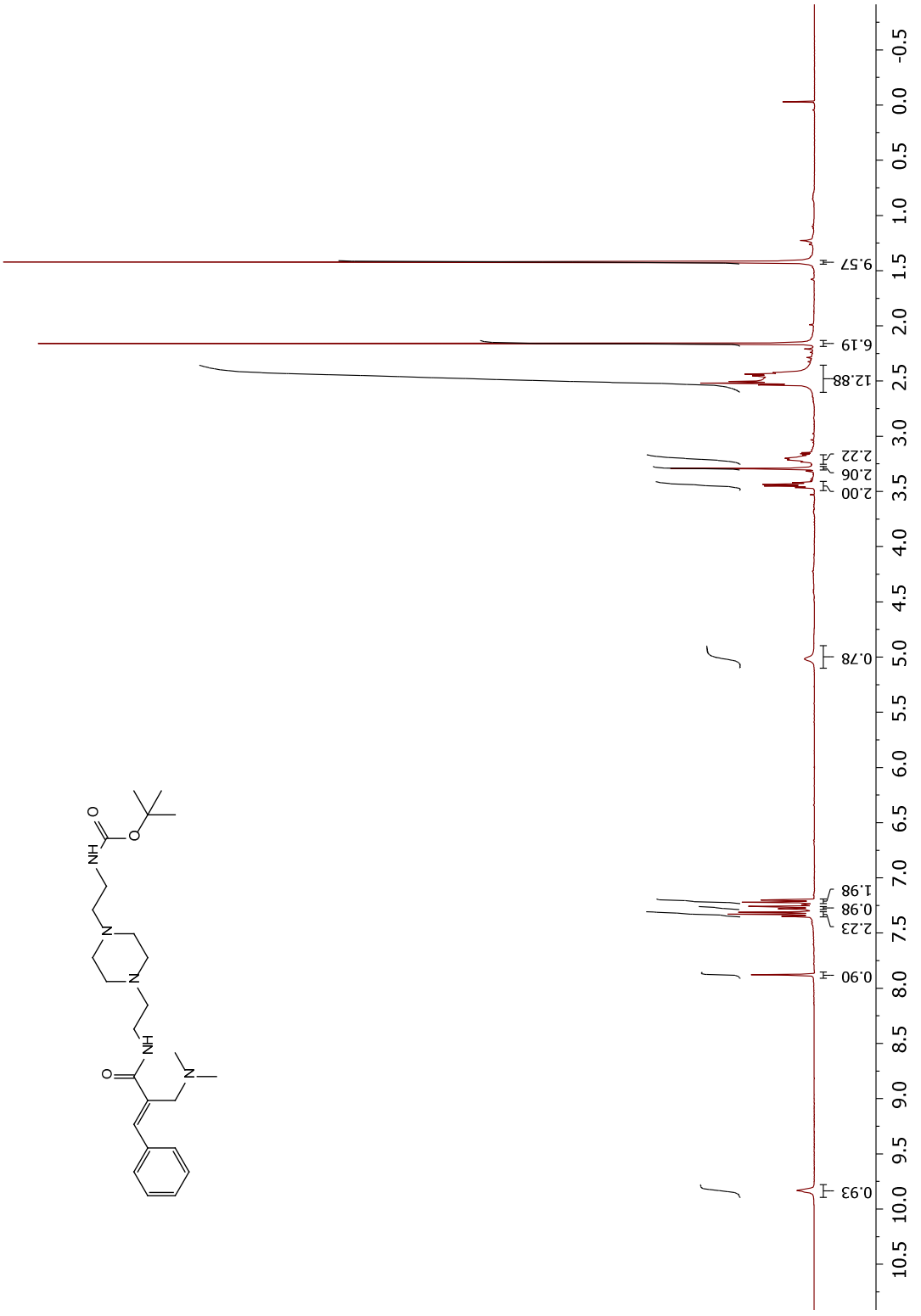


Figure 43. 400 MHz ^{1}H NMR of Compound **67c** in $CDCl_3$

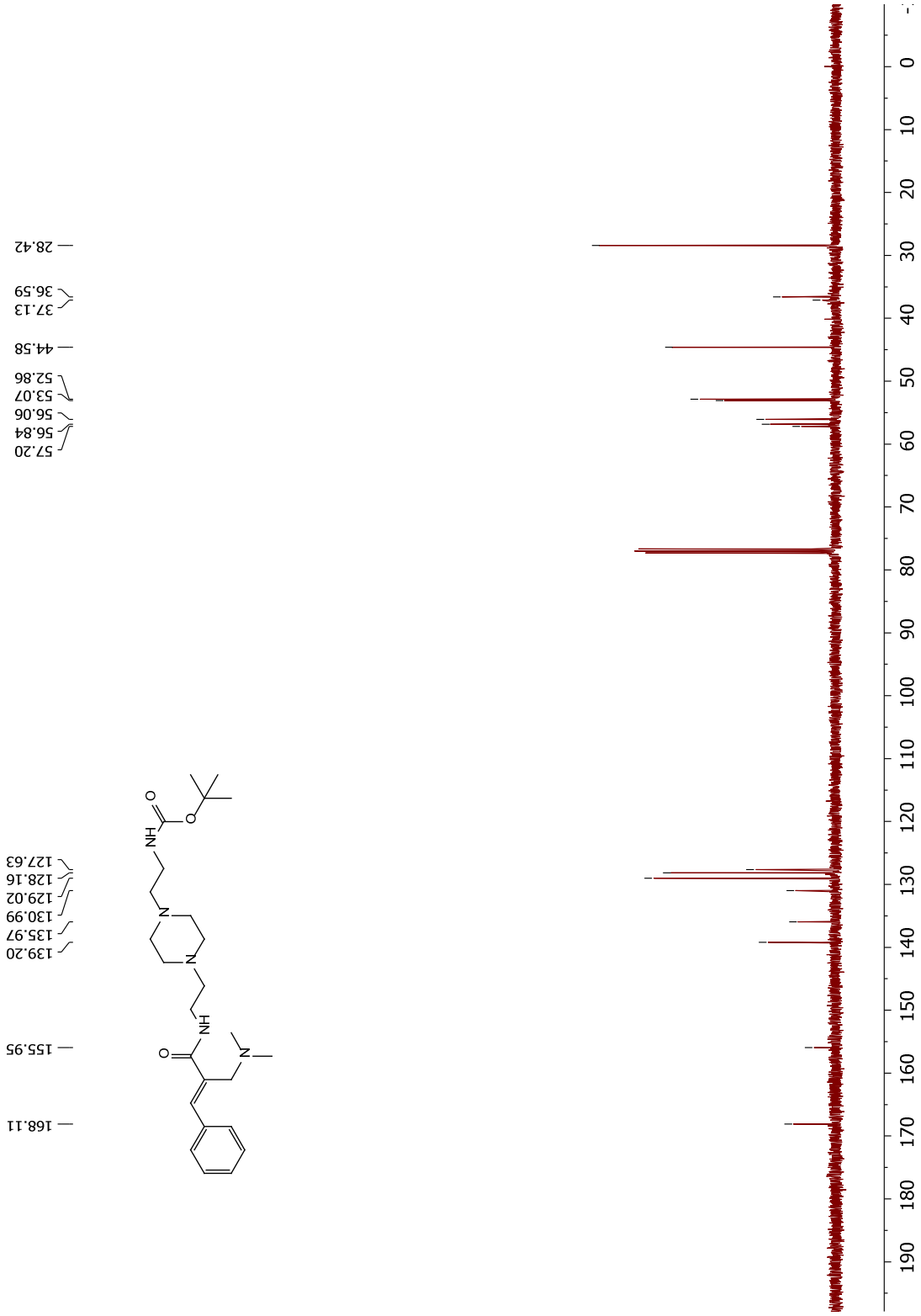


Figure 44. 101 MHz ^{13}C NMR of Compound **67c** in $CDCl_3$

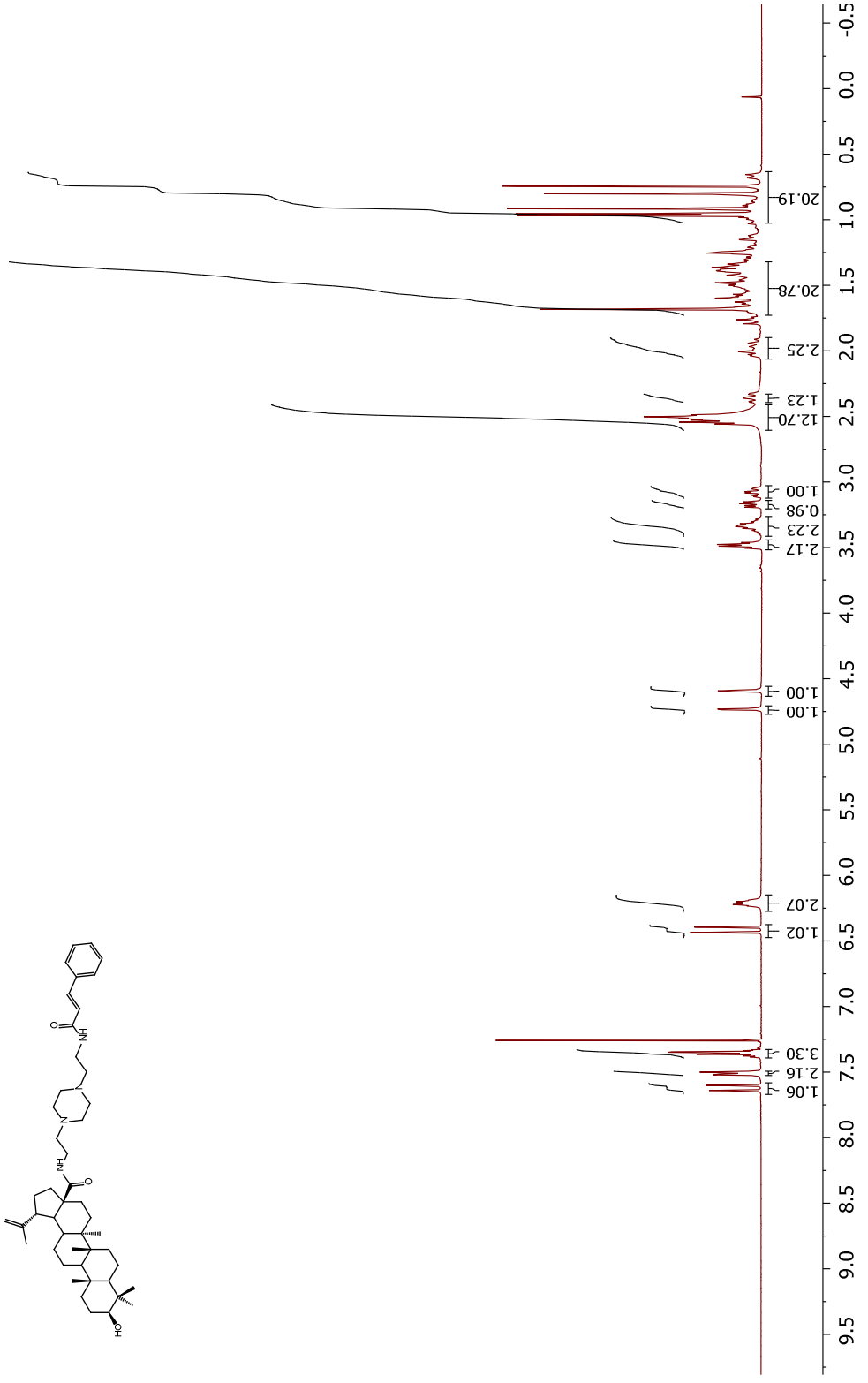


Figure 45. 400 MHz ^{1}H NMR of Compound **69a** in $CDCl_3$

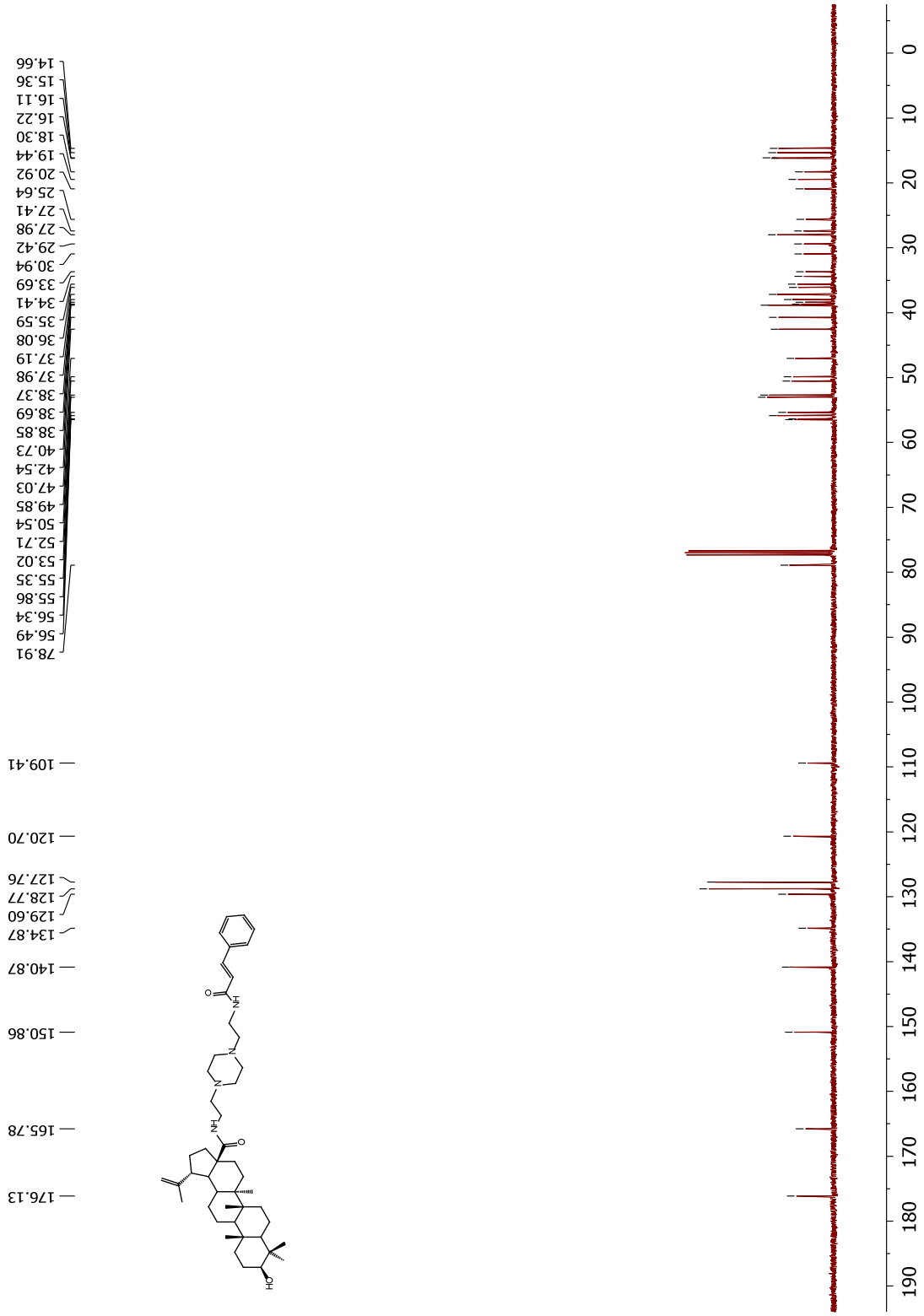


Figure 46. 101 MHz ^{13}C NMR of Compound **69a** in $CDCl_3$

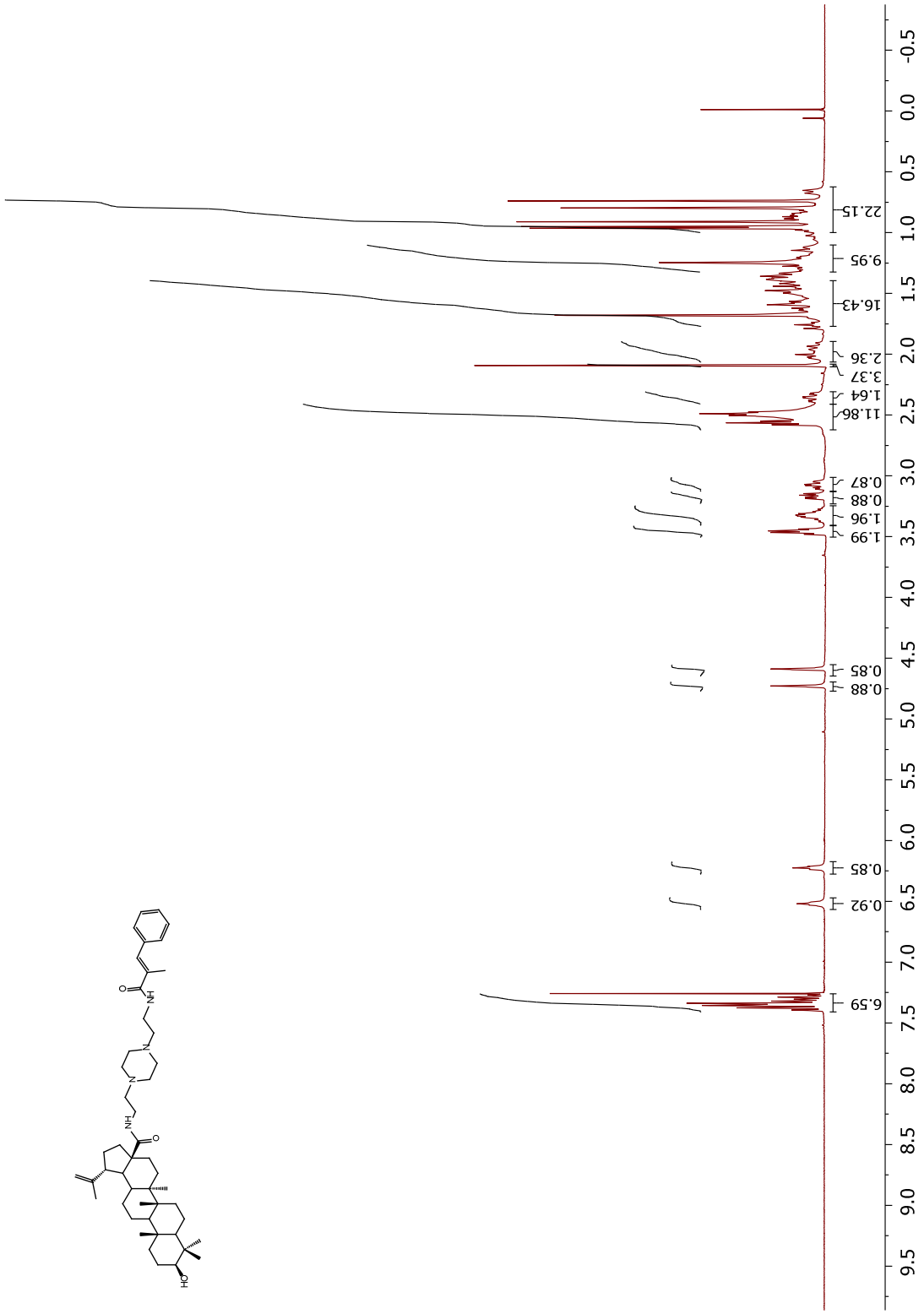


Figure 47. 400 MHz ^{1}H NMR of Compound **69b** in $CDCl_3$

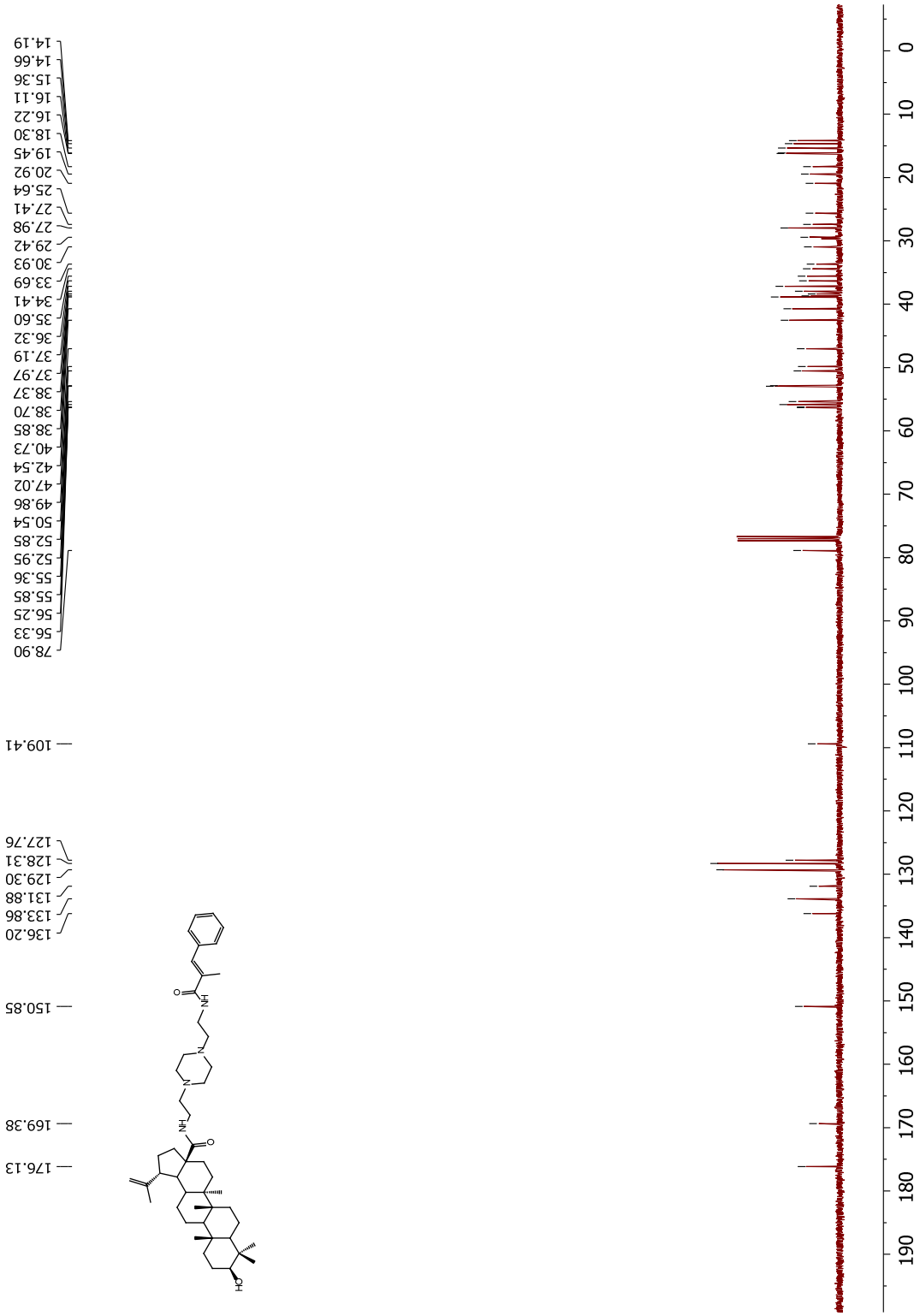


Figure 48. 101 MHz ^{13}C NMR of Compound **69b** in $CDCl_3$

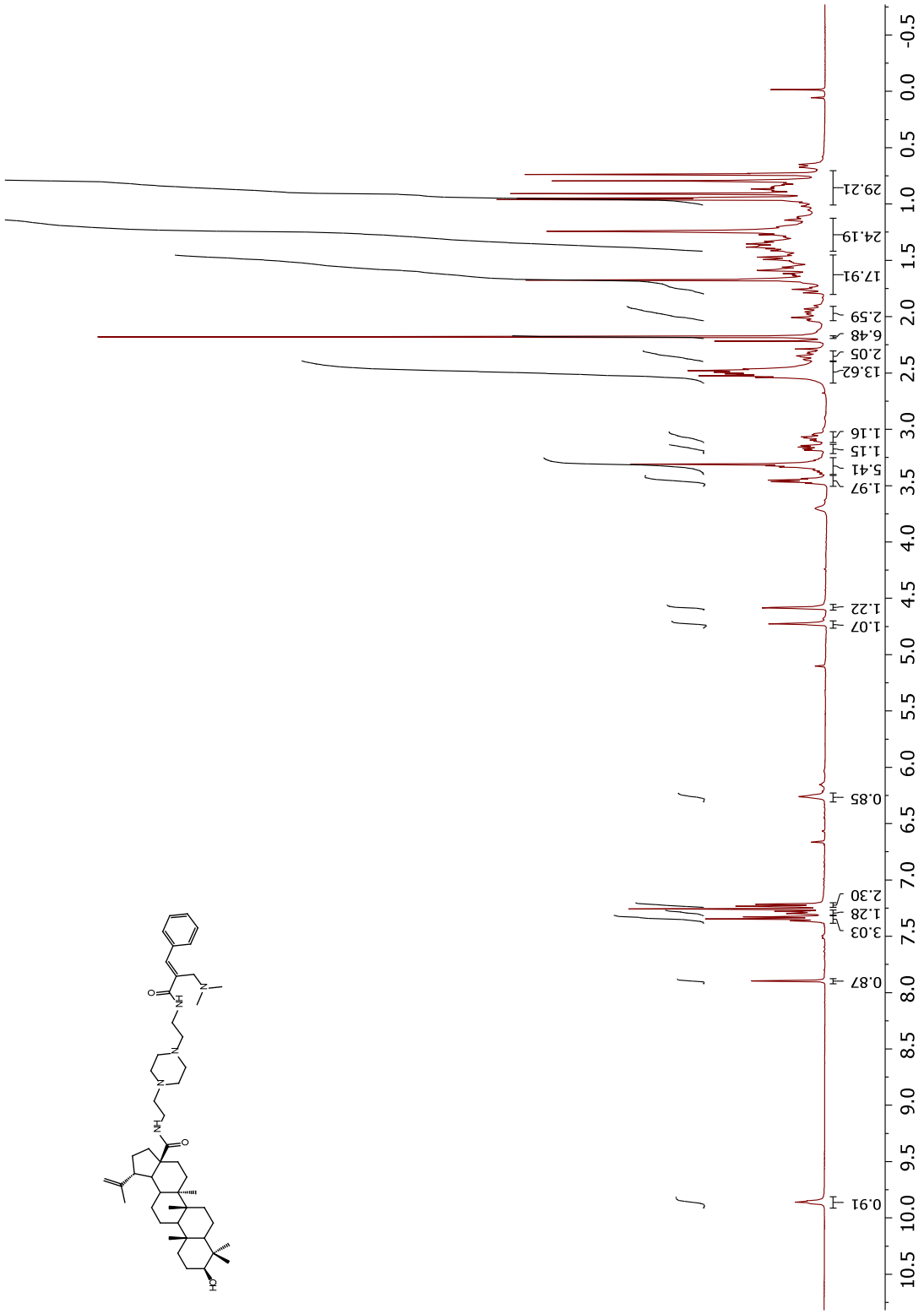


Figure 49. 400 MHz ^{1}H NMR of Compound **69c** in $CDCl_3$

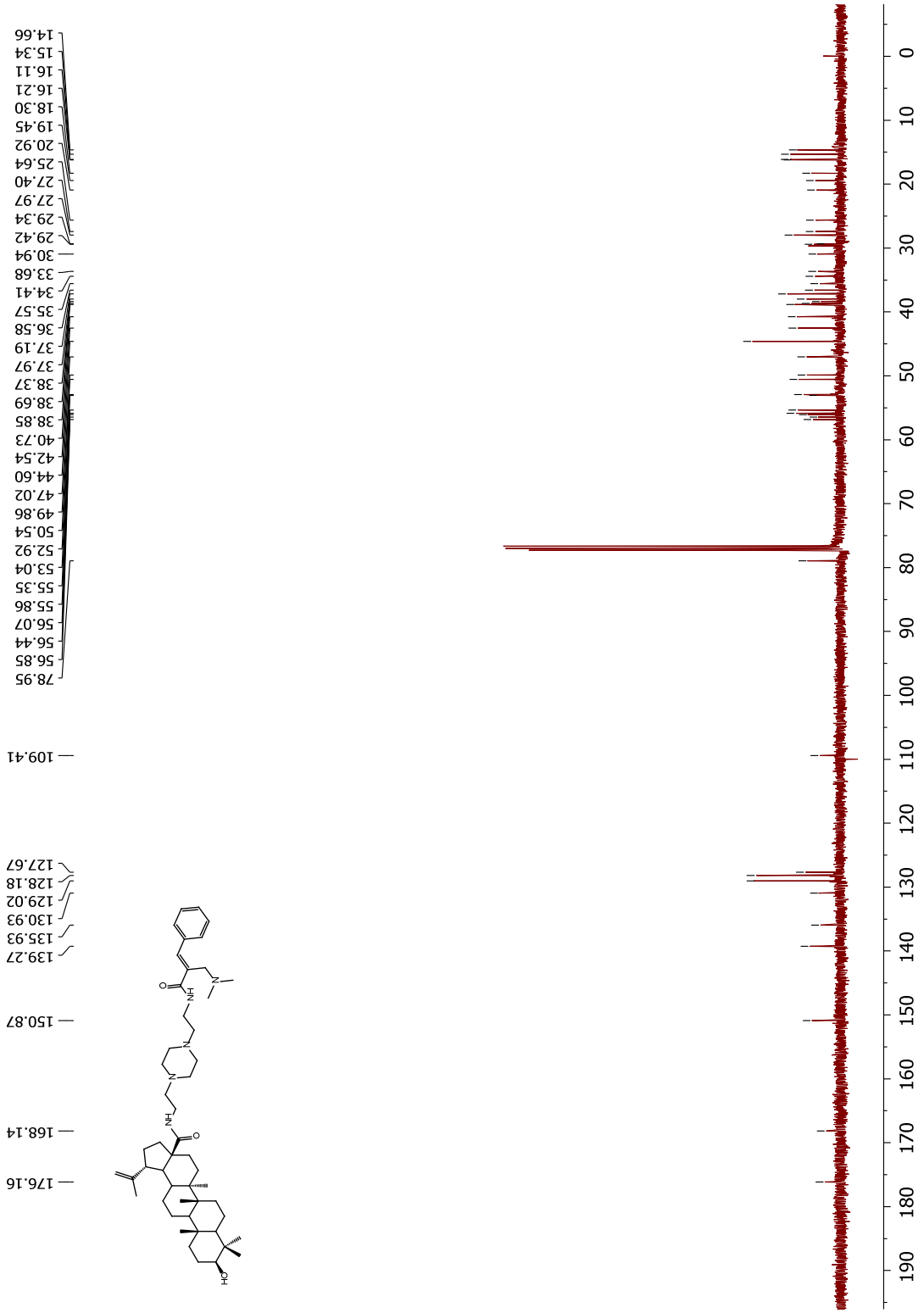


Figure 50. 101 MHz ^{13}C NMR of Compound **69c** in $CDCl_3$

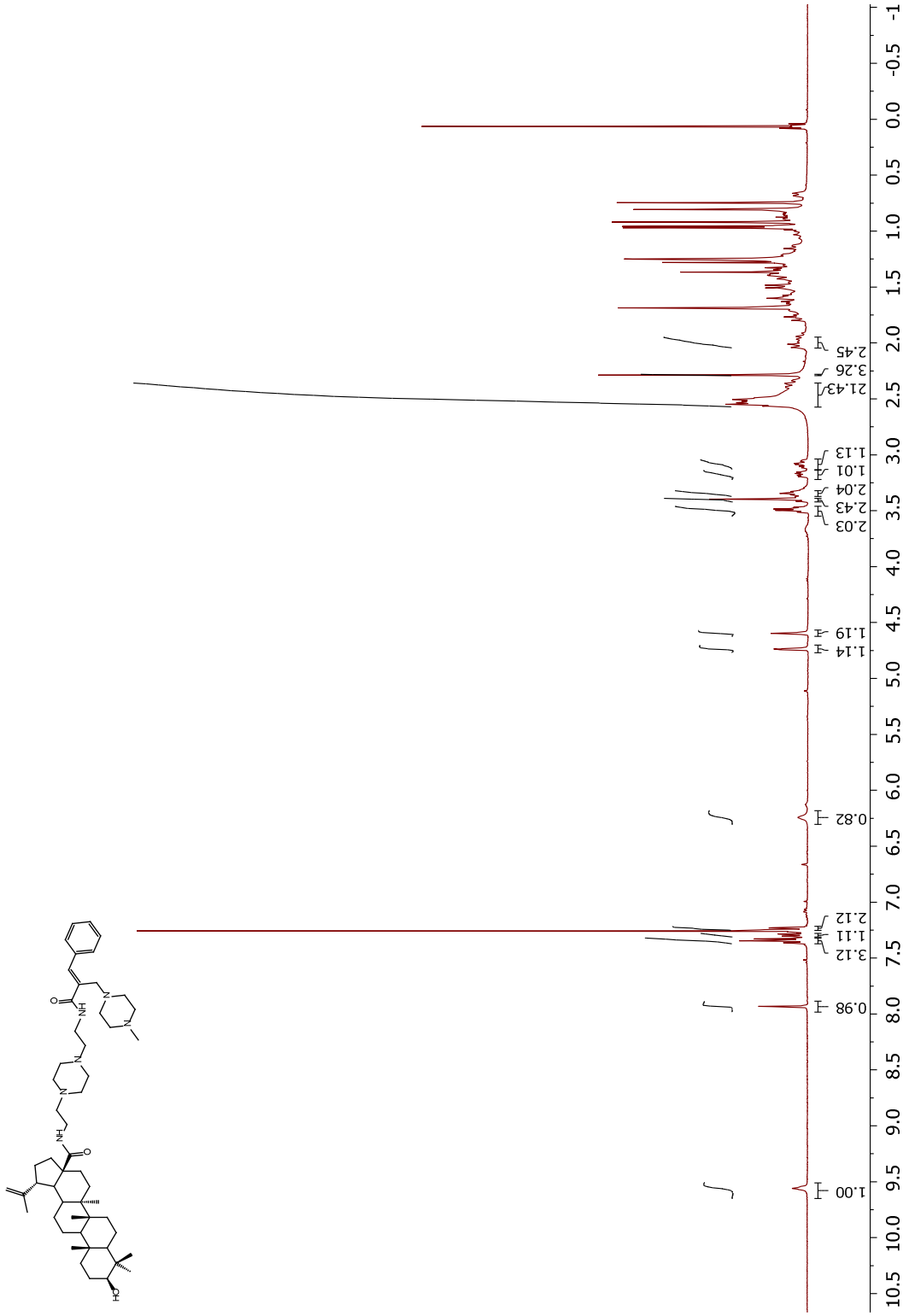


Figure 51. 400 MHz ^{1}H NMR of Compound **69d** in DMSO-d_6

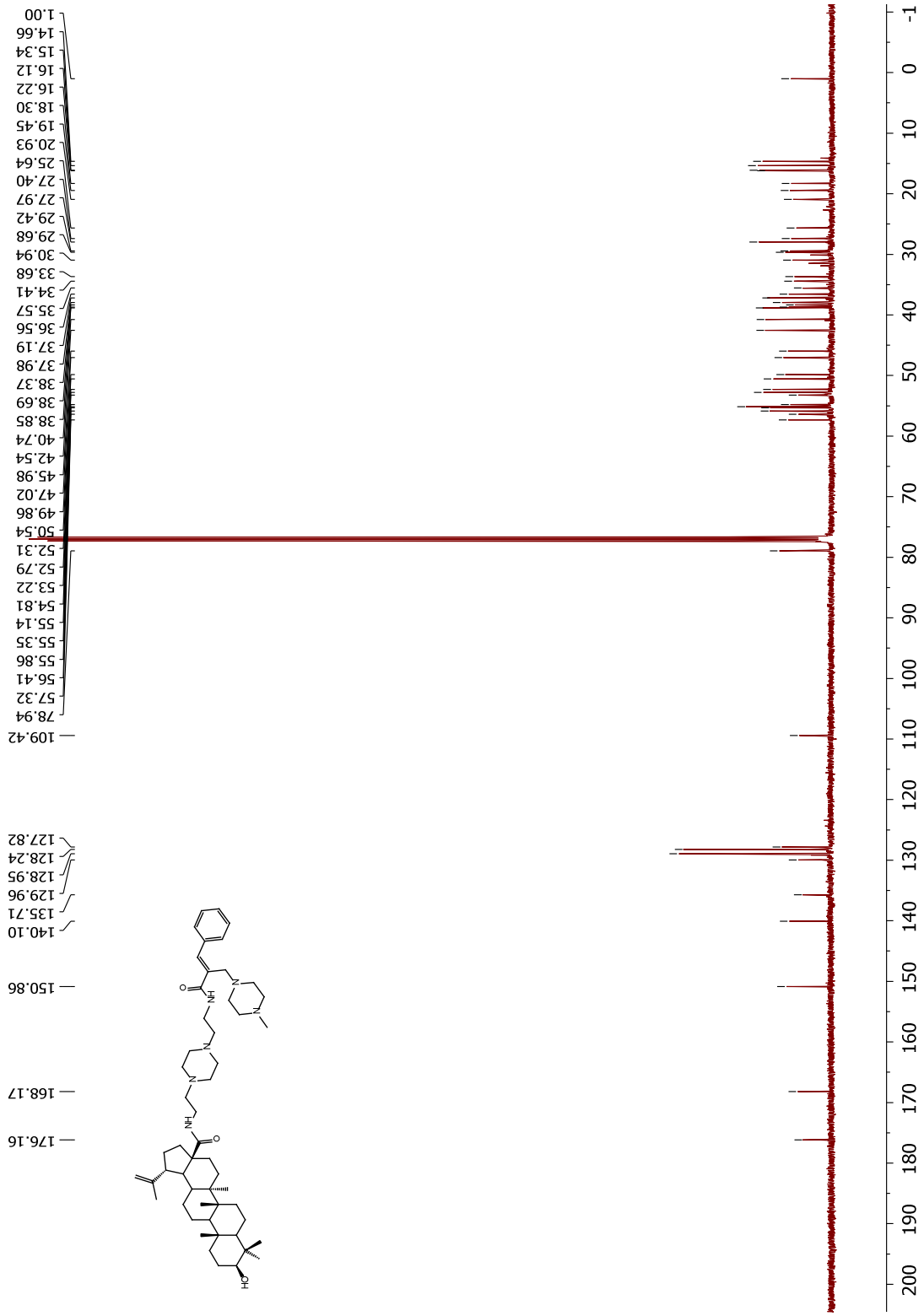


Figure 52. 101 MHz ^{13}C NMR of Compound **69d** in DMSO-d_6

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