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Development of betulinic acid triazole conjugates as potential anti-cancer agents

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DEVELOPMENT OF BETULINIC ACID TRIAZOLE CONJUGATES AS POTENTIAL ANTI-CANCER AGENTS

by

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Dedication

This thesis is dedicated to the memory of my Father, who supported me in good and bad times, his kindness and extensive support have been ever present, for which I am eternally grateful.

Acknowledgments

I would like to acknowledge my family and friends for their constant support throughout the course of this research and beyond. Additionally, I would like to thank my research advisor for the significant time and effort he devoted towards teaching me and honing me into the scientist that I am today. I would not be where I am today without the aid of all of these people.

Abstract

Gayathri Jampana
DEVELOPMENT OF BETULINIC ACID TRIAZOLE CONJUGATES AS POTENTIAL ANTI-CANCER AGENTS
2018-2019
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Master of Science in Pharmaceutical Sciences

Betulin and betulinic acid are pentacyclic triterpenoid natural product isolated from the bark of birch trees. While betulin is abundantly available in the bark, betulinic acid is present in scarce quantities in nature. However, betulinic acid can be conveniently synthesized from betulin via simple redox manipulations. Betulinic acid shows selective cytotoxicity profile against cancer cells *in vitro* while being relatively safe for normal cells. One of the challenges for the clinical development of betulinic acid is its lack of aqueous solubility. The three key structural features of betulin/betulinic acid that have been routinely exploited for structural modifications to enhance their chemical and biological properties include C_{28} primary alcohol, C_3 secondary alcohol, and a C_{20} alkene moiety.

We have been working on the functionalization of betulin for potential development as anti-cancer agents. Previously, we had reported the synthesis of betulin conjugates via reactions such as Passerini, reductive amination, and aldol condensation. Herein, we report the synthesis of betulinic acid triazole conjugates employing Baylis-Hillman and click reaction protocols as the key steps in our synthesis. The cytotoxicity evaluation of these conjugates demonstrated promising *anti*-cancer activity against two cancer cell lines.

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Chapter 1

Introduction

Betulin/Betulinic Acid

Betulin **1** and betulinic acid **2** (B/BA) are secondary metabolites isolated from a variety of plants. The bark of birch trees contains 10-15% of betulin and it can be readily isolated using simple extraction procedures.[1-2] While betulin is abundantly available, betulinic acid is present in trace quantities in the natural sources. However, betulinic acid can be conveniently prepared from betulin using sequential oxidation-reduction protocols. Betulinic acid exhibits significant cytotoxicity against variety of cancer cells.[3] Apart from the anti-cancer activity, betulinic acid is also known to possess anti-HIV, anti-micotic, anti-inflammatory, anti-parasitic, and antioxidant properties.[4-13] Bevirimat **3** is an analog of betulinic acid that was developed as a virus maturation inhibitor by Panacos Pharmaceuticals, and it reached Phase IIb clinical trials for the treatment of HIV. While the exact mechanism of action for the biological activity of betulinic acid is not fully understood, it is generally understood that BA causes apoptosis via direct regulation of mitochondrial pathways and increased production of Caspase-3.[4-14]

Betulin exhibits *in vivo* protective effect against colitis in mice[15] as well as on dexamethasone-induced thymocyte apoptosis.[16] Betulin inhibits TLR4/NF-kB pathway, which leads to reduced kidney,[17] liver, and lung[18] injuries in rats. Apart from the medicinal uses, there have been other applications for betulin/betulinic acid including their use as anti-feedants,[19] as a light stabilizer for cellulose and wood pulp, in the manufacture of

resins, emulsifiers, polyurethanes, and cosmetic products. Birch bark has been used in folk medicine as an *anti*-septic and for treating gout, rheumatism, and some skin diseases.[20]

Owing to the diverse range of applications, there have been several reports on the synthesis of a range of betulin derivatives. Several researchers have focused their attention on the preparation of the esters at C3 and C28 positions on betulin and betulinic acid. Some of the esters that have been prepared and tested for various biological applications include phthalate esters,[21] aryl and heteroaryl carboxylate esters,[22] aryl/aralkyl/alkyl/florinated estearrs,[23-24] artesunate esters,[25] mono and bidesmosidic saponin esters[26] and polyglycosides.[27] Other analogs such as amides,[28] carbamates,[29,30] ammonium salts,[31] sulfate esters,[32] oximes/hydrazones/imines,[33] nitriles,[34] sulfides,[35] amines,[36] and lactones[37] have also been synthesized. There have also been miscellaneous reports dealing with the cyanoethylation,[38] cyclopropanation,[39] and Prins reaction[40] on betulin template.

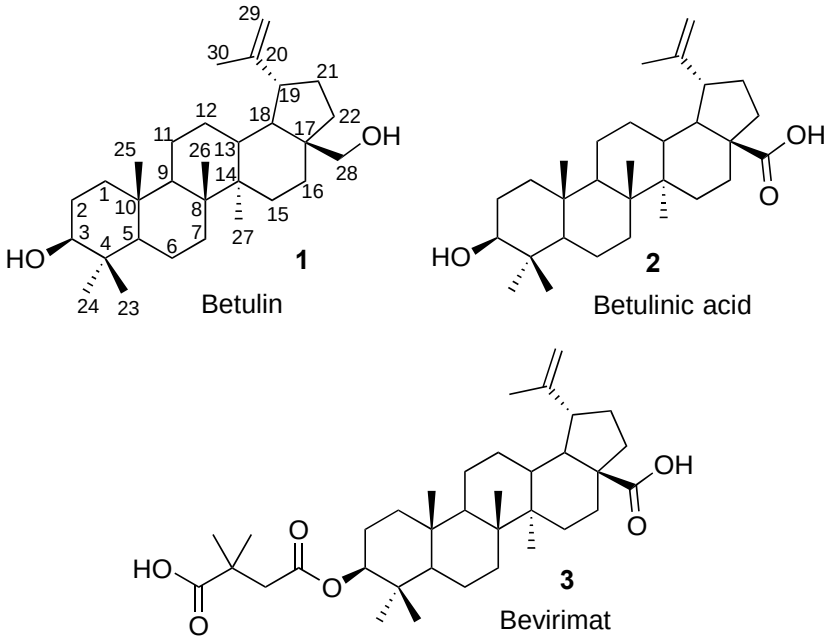


Figure 1. Betulinic acid derivatives.

Multicomponent Reactions

Multi-component coupling reactions offer an easy route for accessing structurally diverse chemical templates and are considered lucrative alternatives to linear or divergent multi-step organic synthesis.[41,42] Multicomponent coupling involves the addition of three or more components in a single pot reaction and it leads to the formation of a complex product. Isocyanides are quite useful synthons in organic chemistry because of strongly nucleophilic character and they have been routinely employed as the coupling agents in multicomponent reactions. Passerini reaction involves the combination of an aldehyde **4** with a carboxylic acid **5**, in the presence of an isocyanide **6**, and it results in the formation of α-acetoxyamides **7** in a one-pot three-component coupling transformation (**Figure 2**).[43]

Ugi reaction introduces a fourth component (amine, **8)** to this mixture and results in the formation of α-acylaminocarboxamide **9** (**Figure 2**).[44]

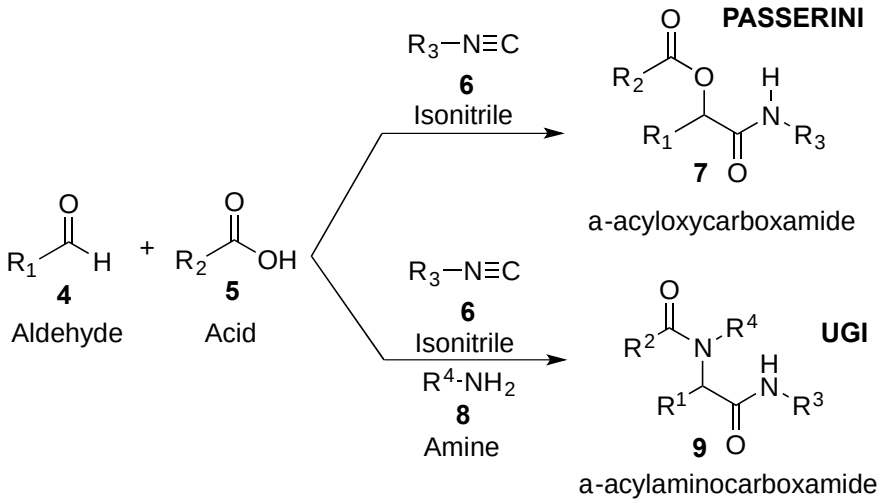


Figure 2. Isocyanide based multicomponent coupling reactions.

Functionalization of Betulin

Our research group has been working on the functionalization of betulin as potential anti-cancer agents. We have previously reported the synthesis of the chalcone **10**, the amide **11**, and the amine **12** via aldol condensation, Passerini reaction, and reductive amination respectively (**Figure 3**).[45]

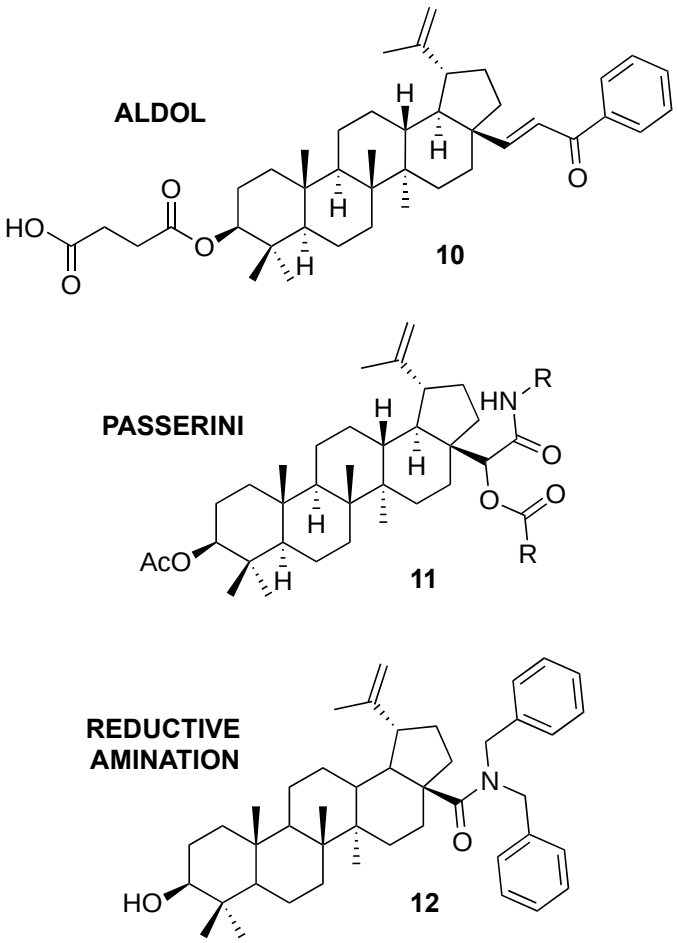


Figure 3. Functionalization of betulin.

Synthesis of Betulin Conjugates via Click Reaction

Click chemistry is a 1,3-dipolar cycloaddition reaction involving the coupling of alkynes **13** and azides **14** to form 1,4-disubstituted 1,2,3-triazoles **15** (**Figure 4**).[46] Click chemistry has been well recognized in organic synthesis and it finds applications in natural product drug discovery,[47] biopolymers,[48] nano-technology,[49-50] and other biomaterials.[51]

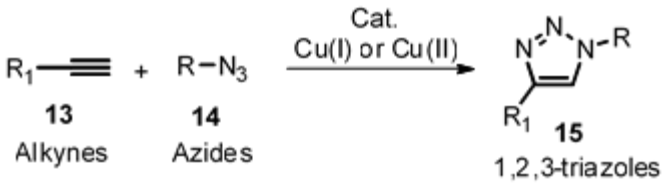


Figure 4. Click reaction.

There have been several reports on the synthesis of betulinic acid triazoles derivatives employing click chemistry as the key step in their synthesis. Bori *et al.* synthesized betulin conjugate **16,** and betulinic acid conjugate **17** by coupling the parent natural products with azidothymidine (AZT).[52] Some of these compounds showed efficacy (~0.1 μM EC_{50}) as *anti*-AIDS agents. Khan and co-workers synthesized *N*-aryl triazole conjugates **18** of betulinic acid under click reaction conditions. Some of these analogs showed better potency against four human cancer cell lines (HL-60, MiaPaCa-2, PC-3, and A549).[53] Dang Thi *et al* prepared azidothymidine conjugates **19** of betulinic acid which showed moderate cytotoxicity on two different cancer cells (**Figure 5**).[54]

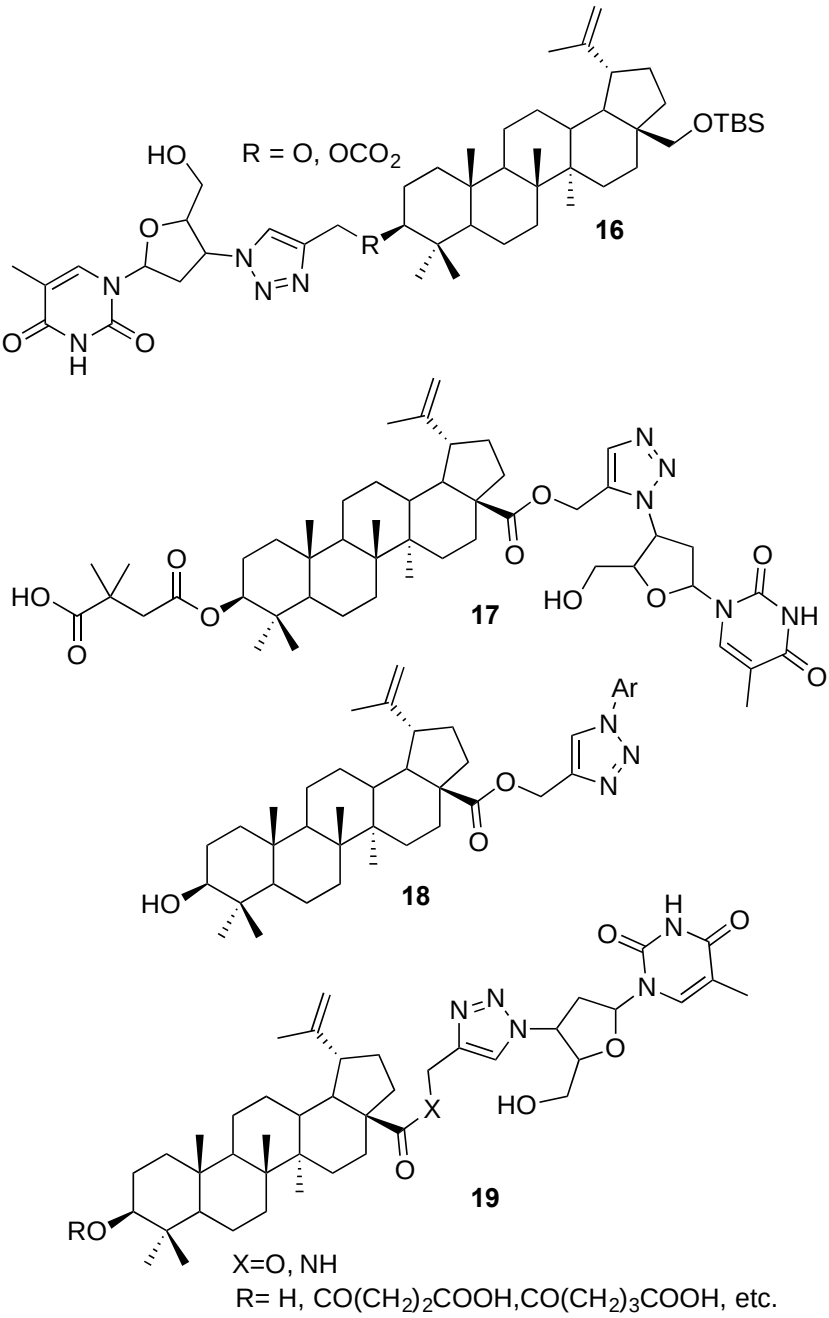


Figure 5. Betulin conjugates via click chemistry.

Csuk *et al* prepared the triazoles **20**, some of these compounds showed moderate cytotoxicity on diverse class of human cancer cell lines.[55] Shi *et al* prepared the triazoles

21 and **22** via modification at the C30 position of betulin and these conjugates showed *anti*-cancer activity against leukemia cell line HL60.[56] Chakrabarty *et al* [57] reported the betulinic acid conjugates **23** while Majeed and co-workers[58] synthesized betulinic acid conjugates **24** and both classes of these compounds showed limited potential as apoptotic agents (**Figure 6)**.

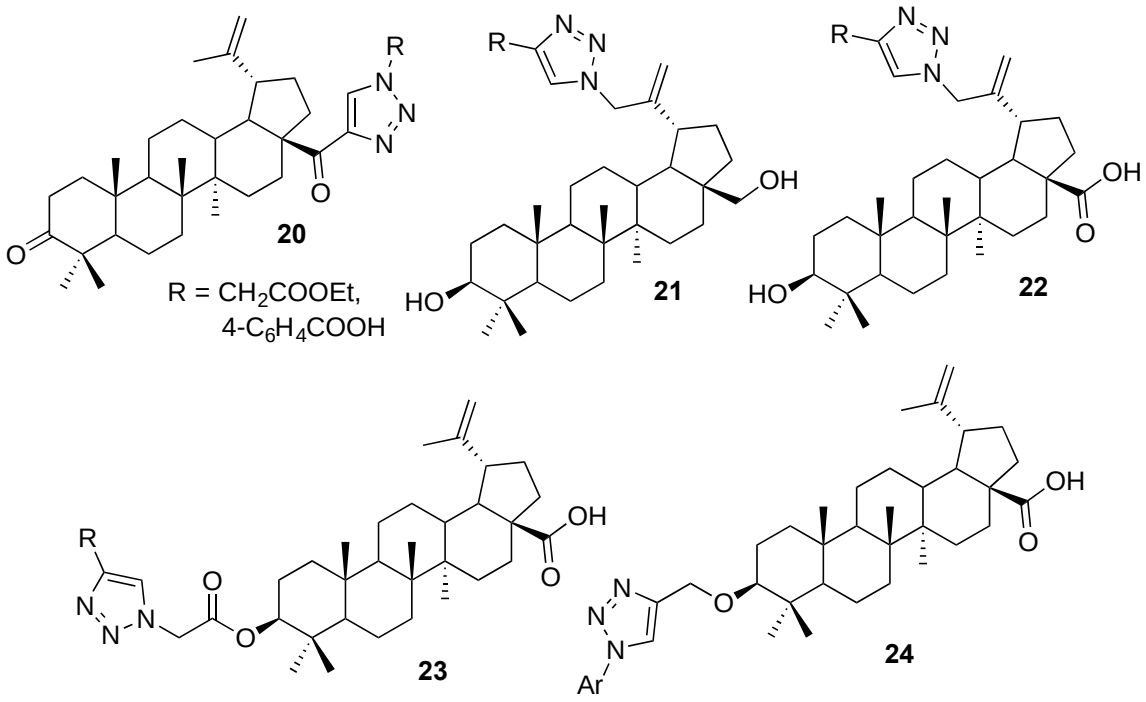


Figure 6. Betulinic acid derivatives via click chemistry.

Baylis-Hillman Reaction

Baylis-Hillman reaction is a carbon-carbon bond forming reaction involving the addition of carbonyl compounds and activated alkenes.[59] This reaction is quite versatile and a wide variety of substrates have been shown to undergo reaction under these

conditions to produce densely functionalized allylic alcohols and amines in high yields.[60] One of the pitfalls of this reaction involves the slow reaction times as the reaction typically requires one to two weeks for completion. There have been several efforts made towards accelerating the rate of this reaction including the use of reactive activated olefins/electrophiles,[61,62] microwave irradiation,[63] use of aqueous medium,[64] high pressures.[65,66,67] or choosing substrates with hydrogen bonding capabilities.[68] There have been several reports on the reaction of variety of aldehydes **25** with activated olefins **26** such as alkyl vinyl ketone,[69,70,71] acrylates,[72,73] acrylamides,[63] acrylonitriles, [70,74] acrolein,[65,75] vinyl sulfones,[76] vinyl sulfoxides,[77] vinyl phosphonates, and allenyl esters[78,79] and the corresponding allylic alcohols **28a-h** have been obtained in high yields (**Figure 7**).

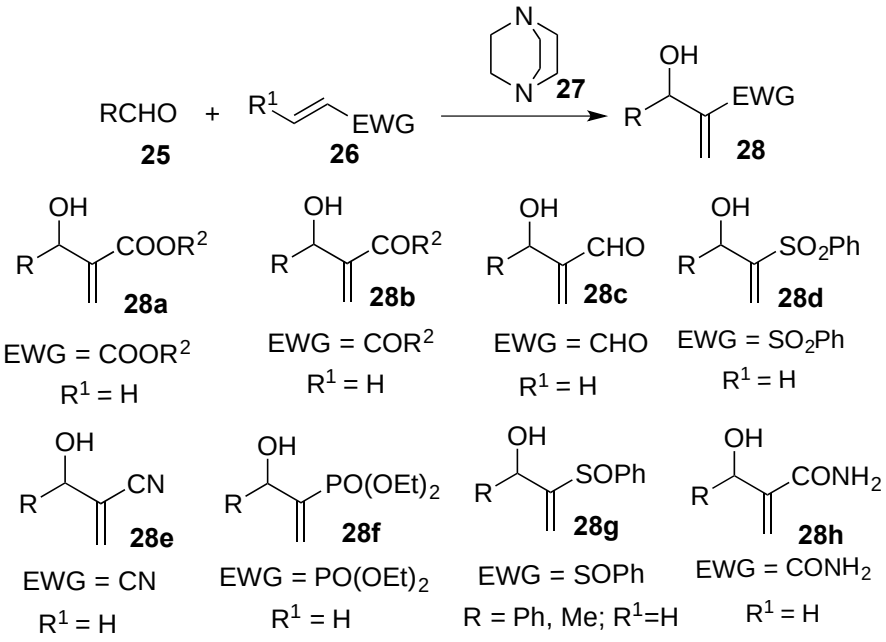


Figure 7. Baylis-Hillman reaction.

Chapter 2

Preparation of Betulin-Triazole Conjugates

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.[80] The two precursors for the click reaction namely the azides and alkynes were obtained starting with Baylis-Hillman reaction and/or the betulinic acid template.

Synthesis of Azides via Baylis-Hillman Reaction

We hypothesized the use of six azides **29a-f** using Baylis-Hillman reaction (**Figure 8**). As betulinic acid suffers from poor aqueous solubility, some of the azides **29b-e** were chosen with the intent of enhancing the polarity of the final compounds by the use of groups such as *N*,*N*,*N*'-trimethylethylenediamine and *N*-methylpiperazine.

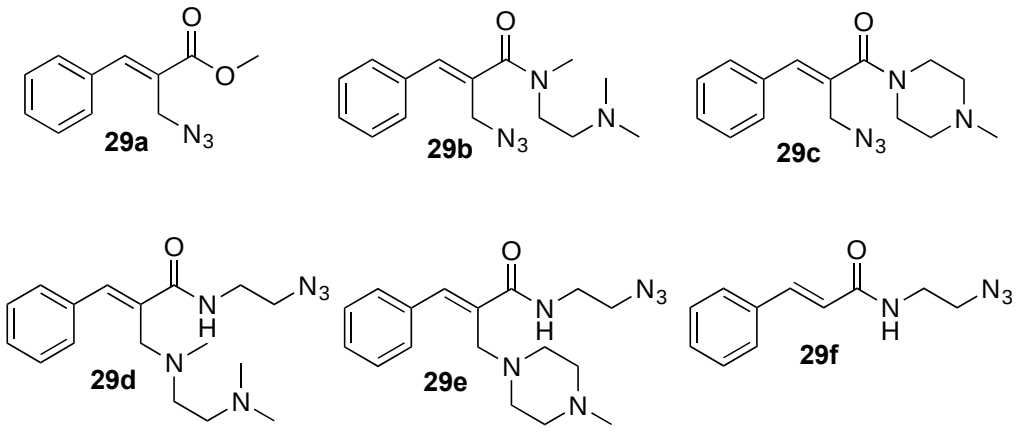


Figure 8. Azides used for click coupling.

The preparation of azides **29a-c** is shown in **Figure 9**. The synthesis was initiated by the reaction of benzaldehyde **30** with methyl acrylate **31** in the presence of 1,4-diazabicyclo[2.2.2]octane to yield the allylic alcohol **32** in 78% yield. Alcohol **32** was brominated with hydrobromic acid and sulfuric acid to yield the bromide **33**. Nucleophilic substitution of the bromide **33** with sodium azide in aqueous acetone resulted in the formation of one of the requisite azides **29a**. Hydrolysis of **29a** was accomplished using aqueous sodium hydroxide in methanol:THF medium to yield α-azidomethylcinnamic acid **34**. Coupling of acid **34** with *N*,*N*,*N*'-trimethylethylenediamine **35** and *N*-methylpiperazine **36** in the presence of TBTU and *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylformamide produced the azides **29b** and **29c** respectively (**Figure 9**).

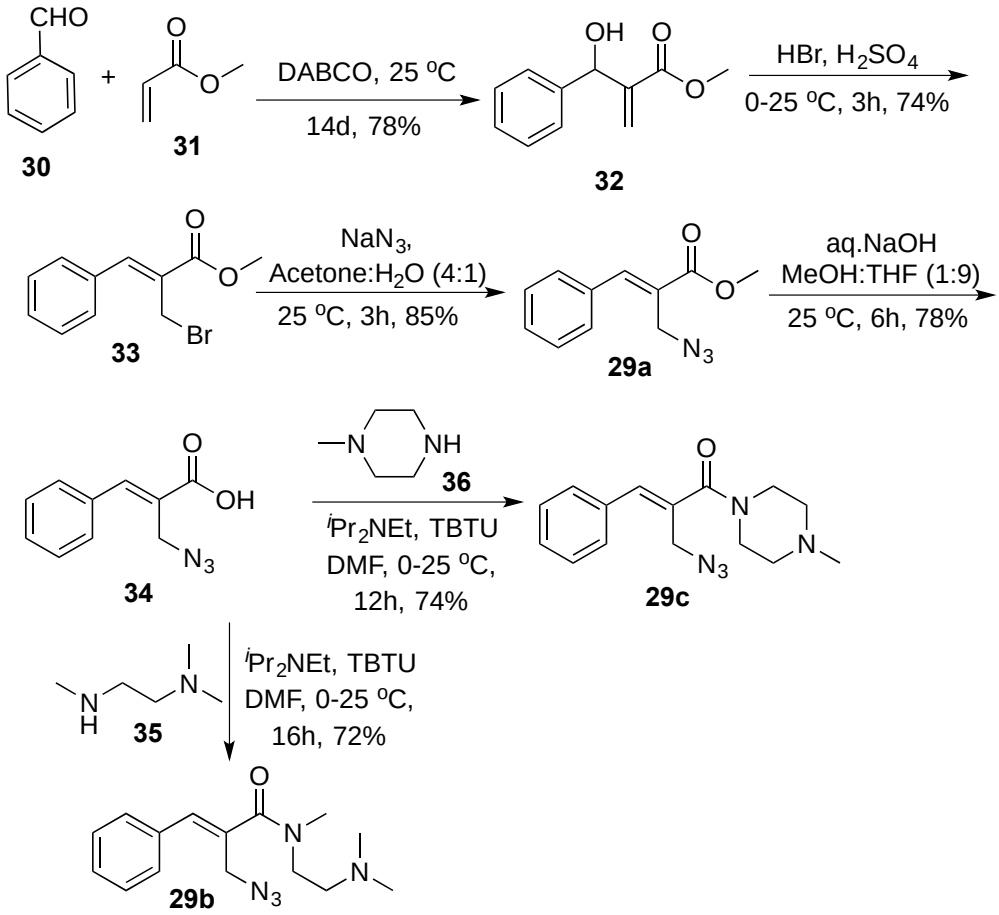


Figure 9. Preparation of azides 29a-c.

The azide **29d** was synthesized starting from Baylis-Hillman reaction-derived allylic alcohol **32**. Esterification of alcohol **32** with acetic anhydride in the presence of triethylamine and catalytic *N*,*N*-diisopropylethylamine yielded the acetate **37**. Substitution of acetate **37** with *N*,*N*,*N*'-trimethylethylenediamine **35** was achieved using potassium carbonate and DMF to generate the α-aminomethylcinnamate **38**. Hydrolysis of ester **38** followed by the amide coupling of the resulting acid **39** with 2-azidoethylamine yielded the azide **29d** (**Figure 10**).

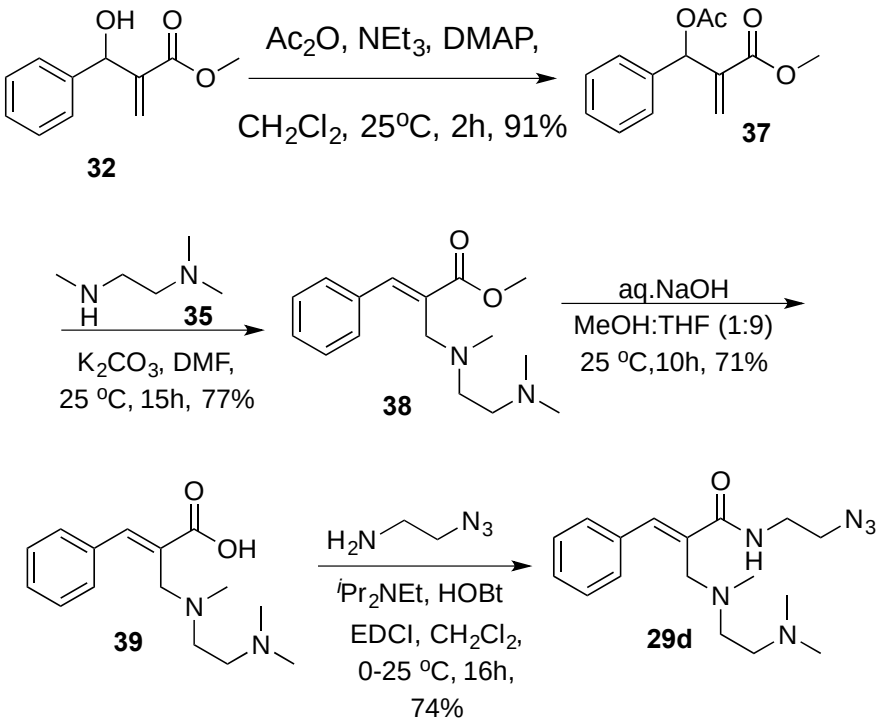


Figure 10. Preparation of azide 29d.

The azide **29e** was synthesized utilizing a very similar protocol as to that of **29d** by replacing *N*,*N*,*N*'-trimethylethylenediamine with *N*-methylpiperazine (**Figure 11**). The azide **29f** was obtained by a simple amide coupling of cinnamic acid **42** with 2-azidoethylamine (**Figure 12**).

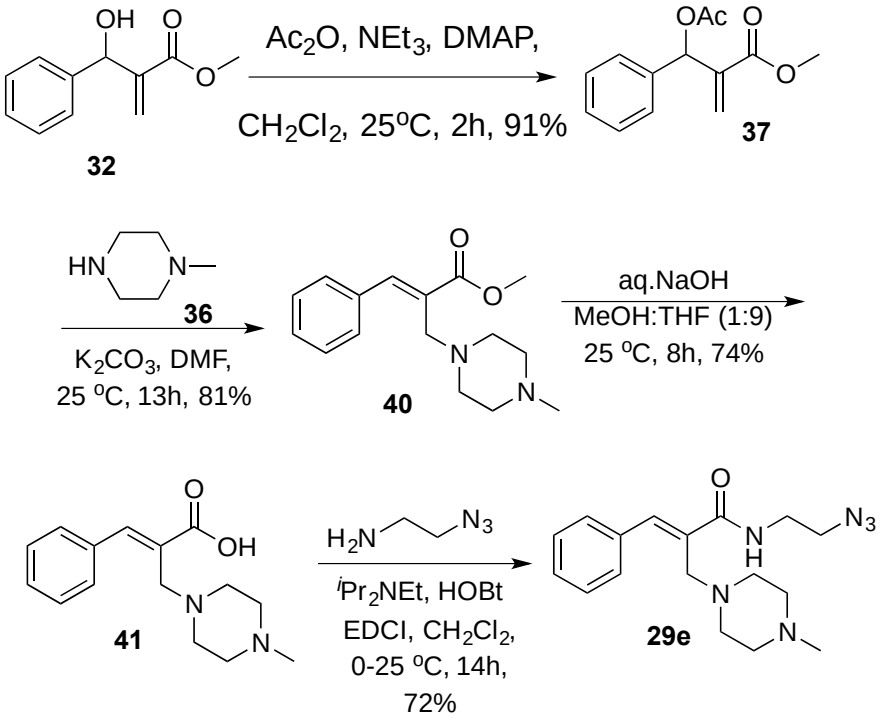


Figure 11. Preparation of azide 29e.

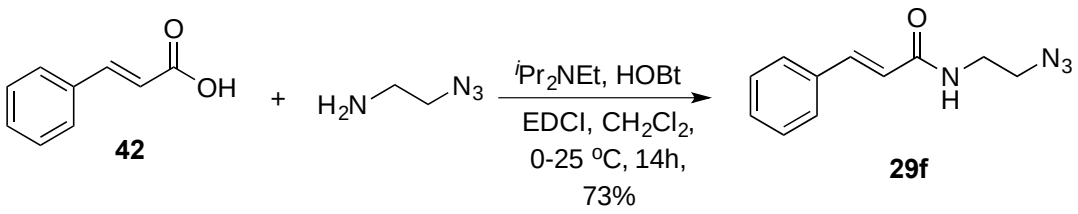


Figure 12. Preparation of azide 29f.

Synthesis of Pyrazinyl Triazoles

After having synthesized the azides **29a-f**, we ventured into the synthesis of the other click reaction partner namely the betulin derived-alkyne **45**. Jones oxidation of

betulin **1** with chromium trioxide in aqueous acetone furnished betulonic acid **43**, which underwent cycloaddition with ethylenediamine to yield the pyrazinylbetulinic acid derivative **44**. The acid **44** upon reaction with propargyl amine and TBTU produced the requisite alkyne *N*-propargyl pyrazinylbetulinamide **45.** The coupling of **45** with Baylis-Hillman motif derived azides **29a-f** in the presence of $CuSO_4$ and sodium ascorbate in tertiary butanol/water led to the formation of the corresponding pyazinyl betulinic acid-triazole derivatives **46a-f** in 82-88% yield (**Figures 13** & **14**). All the compounds were characterized using NMR and Mass spectrometry.

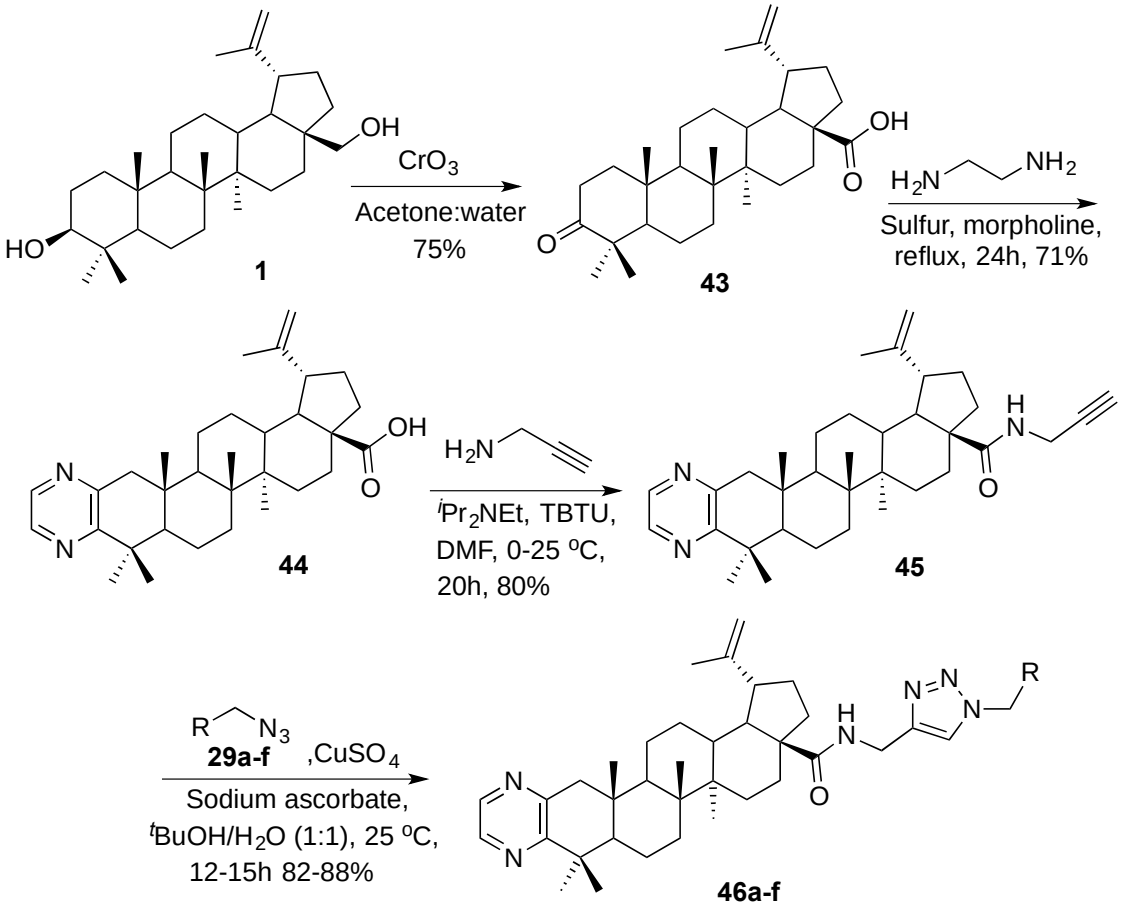


Figure 13. Preparation of pyrazinyl triazoles **46a-f**.

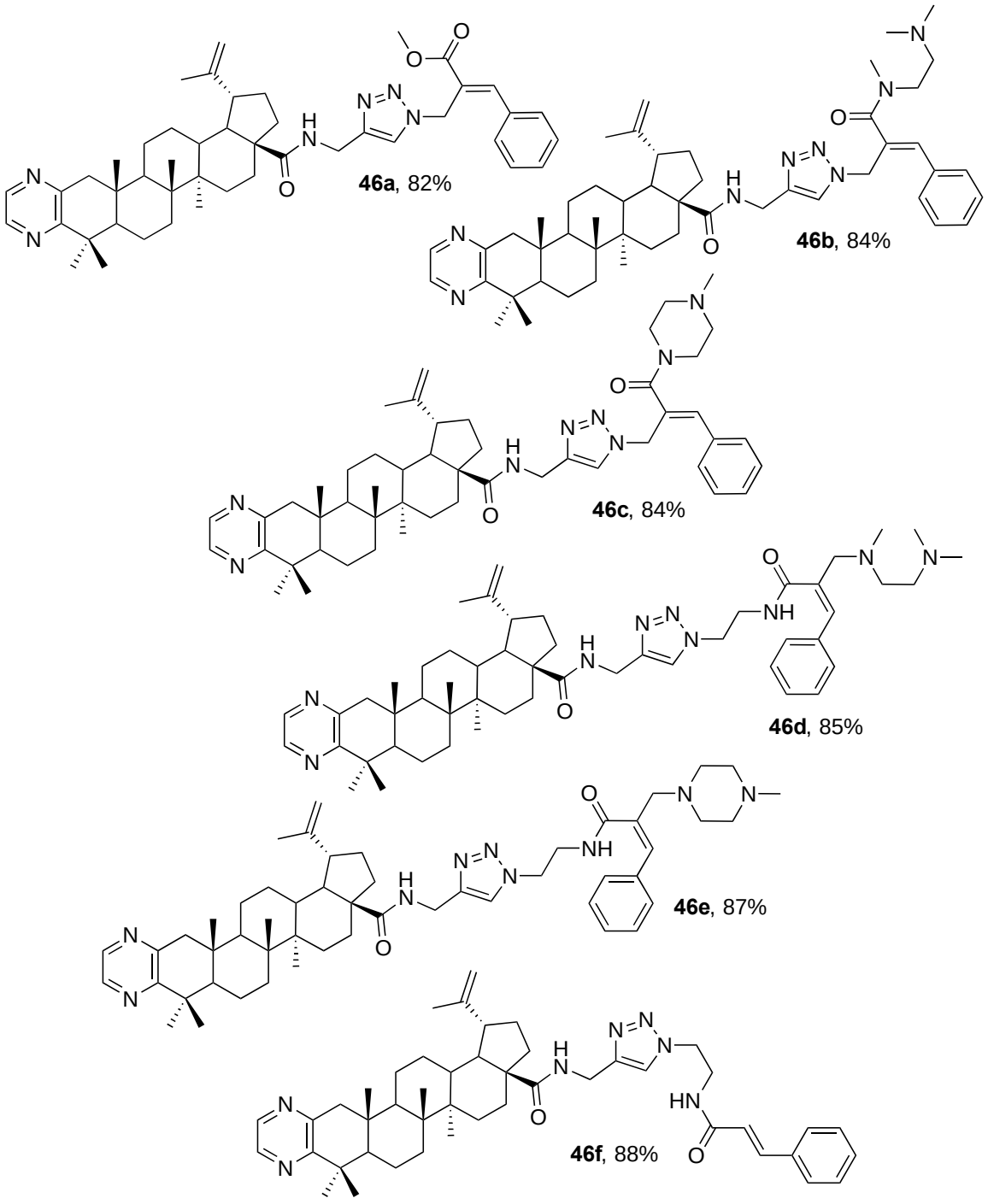


Figure 14. Pyrazinyl triazoles **46a-f**.

Synthesis of Indolyl Triazoles

Utilizing a similar strategy, we synthesized indolylbetulinic acid **47** via condensation of betulonic acid **43** with phenylhydrazine upon refluxing in acetic acid. Coupling of **47** with propargyl amine in the presence of TBTU and *N*,*N*-diisopropylethylamine yielded requisite alkyne *N*-propargyl indolylbetulinamide **48**. Cycloaddition of **48** with α-azido-methyl cinnamate **29a** and *N*-2-azidoethylcinnamamide **29f** under click reaction conditions using copper sulfate and sodium ascorbate yielded the indolylbetulinic acid-triazole conjugates **49a,f** (**Figures 15** & **16**). The resulting triazoles were purified by silica gel column chromatography and characterized using NMR and mass spectrometric analyses.

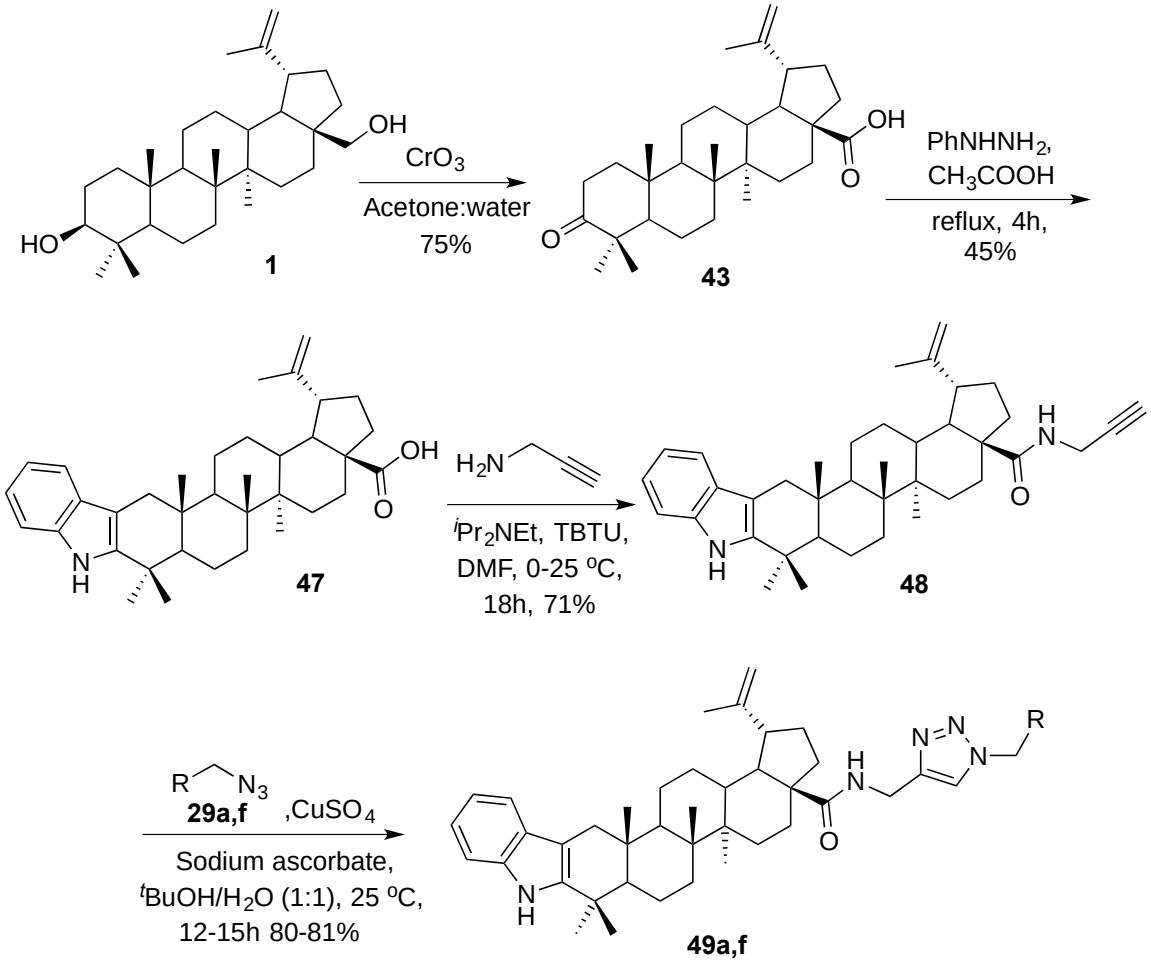


Figure 15. Preparation of indolyl triazoles **49a,f**.

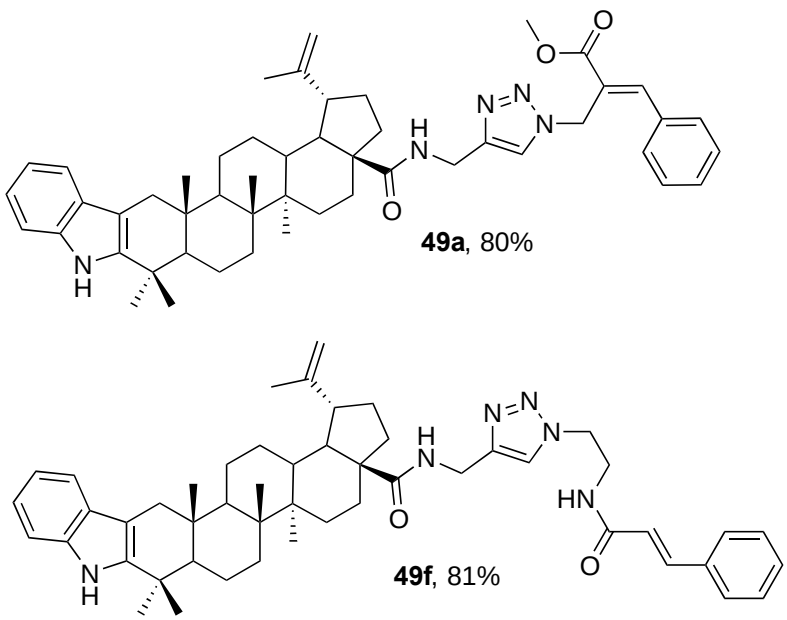


Figure 16. Indolyl triazoles **49a,f**.

Synthesis of *N*-propargylcinnamamides

After synthesizing the pyrazinyl betulinic acid-triazoles **46a-f** and indolyl betulinic acid-triazole conjugates **49a,f** we focused our attention on the synthesis of alkynes **50a-c** using Baylis-Hillman reaction for coupling with betulinic acid derived azides (**Figure 17**). The alkynes with *N*, *N*, *N*'-trimethylethylenediamine and *N*-methylpiperazine motifs were chosen to improve the polarity of the final compounds.

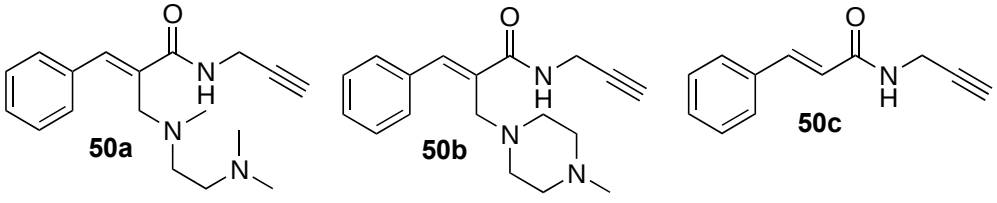


Figure 17. N-Propargyl cinnamamides.

The three alkynes **50a-c** were obtained under peptide coupling conditions by the reaction of propargyl amine with the previously synthesized acids **39**, **41**, and **42** respectively in the presence of HOBt and *N*,*N*-diisopropylamine (**Figure 18**).

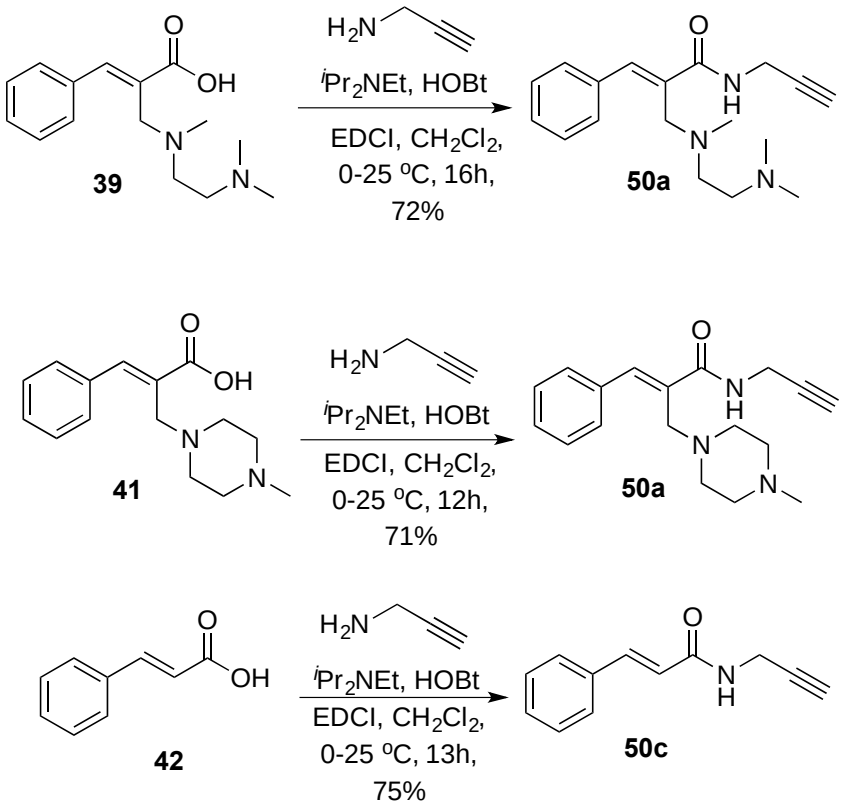


Figure 18. Preparation of *N*-propargylcinnamamides.

Synthesis of Betulonamide-Triazole Conjugates

After the synthesis of alkynes **50a-c**, the synthesis of azide **51** was undertaken starting from betulonic acid **43** by coupling with 2-azidoethylamine in the presence of TBTU and *N*,*N*-diisopropylethylamine. The coupling of **51** with *N*-propargyl α-

(dialkylaminomethyl)cinnamamides **50a-b** and *N*-propargyl cinnamamide **50c** proceeded smoothly to furnish triazoles **52a-c** in good yield (**Figures 19** & **20**). Finally, the reduction of betulonic acid-triazoles **52a-c** with $NaBH_4$ in methanol at room temperature furnished corresponding betulinic acid-triazole derivatives **53a-c** in 86-91% yield after trituration of the crude compounds with hexanes/$CHCl_3$ (**Figures 19** & **21**). All the compounds were well characterized using NMR and mass spectrometric analyses.

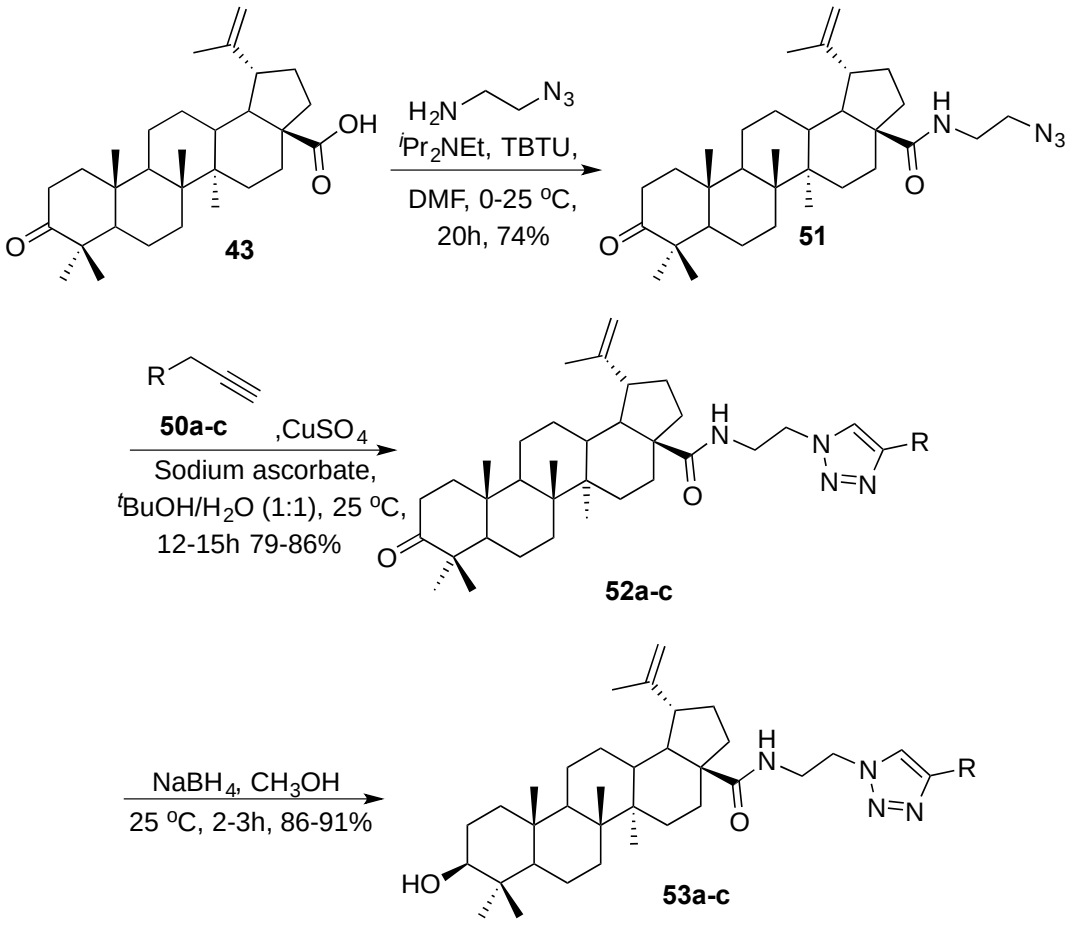


Figure 19. Preparation of betulinic acid triazoles via click chemistry.

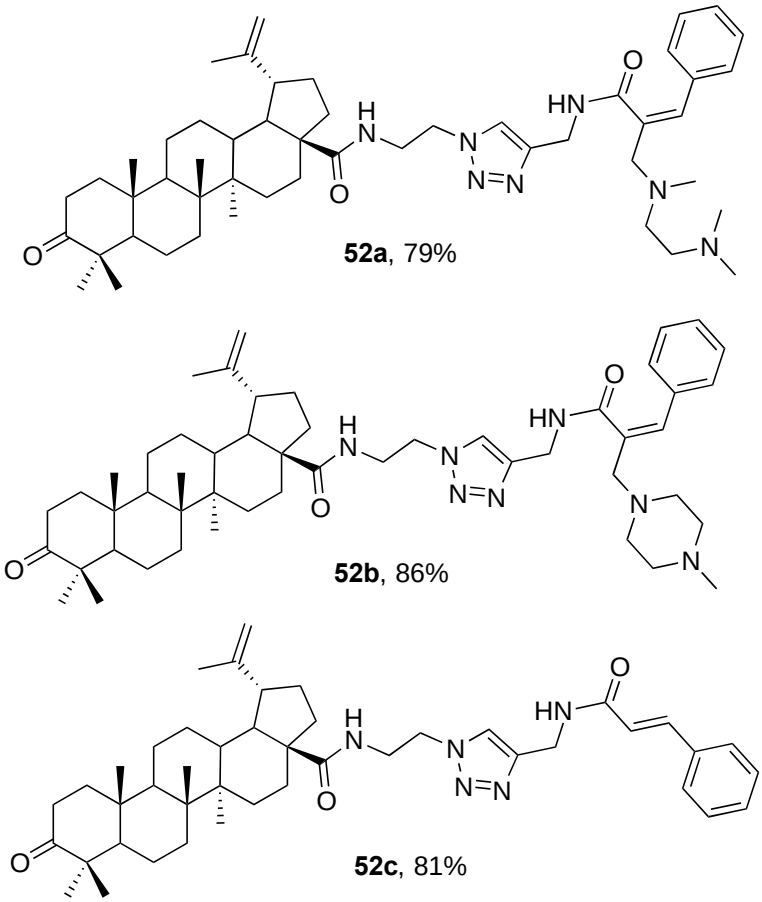


Figure 20. Betulonic acid triazoles **52a-c** obtained via click chemistry.

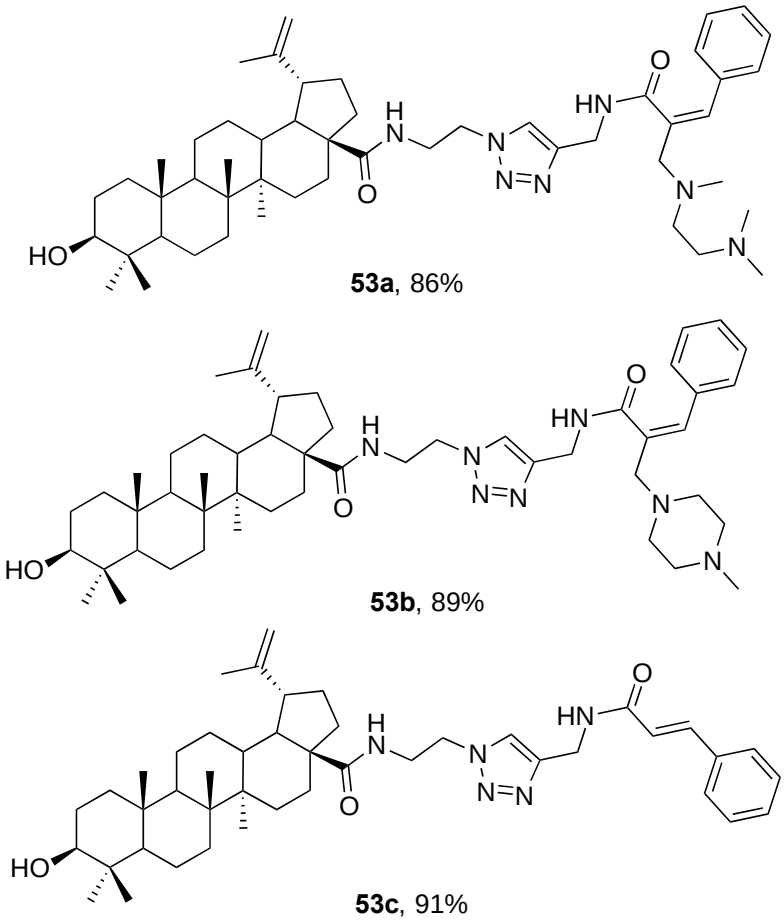


Figure 21. Betulinic acid triazoles **53a-c** obtained via reduction.

Biological Evaluation

After the synthesis of all the compounds, they were evaluated for their cytotoxicity against murine breast cancer (4T1) and pancreatic cancer (MIA PaCa-2) cell lines. The cells were obtained from ATCC, and standard conditions were used for the assays. The cells were seeded in 96 well plates, and the cell viability was determined using MTT assay. Initial screening was carried out at 50 μM, 12.5 μM and 1.5 μM concentrations on two tumor cell lines. Betulinic acid showed moderate activity (~60% inhibition of 4T1 and

~40% of MIAPaCa-2 cells) at 12.5 μM, and majority of betulinic acid-triazole conjugates that were assayed, also displayed promising activity at this concentration (>90% inhibition of 4T1 and ~80-90% MIA PaCa-2 cells). The IC_{50} values were determined for the compounds that displayed moderate to good cytotoxicity (Table 1). Efforts are ongoing for the determination of further structure activity relationship for development as anti-cancer agents.

Table 1

Cytotoxicity of Betulinic Acid-Triazole Conjugates.

#	Compound	4T1 [IC_{50} (μM)]	MIA PaCa-2 [IC_{50} (μM)]
1	**45**	39.33 ± 7.0	26.73 ± 3.70
2	**46a**	Not Toxic	Not Toxic
3	**46b**	5.14 ± 0.80	8.26 ± 0.91
4	**46c**	17.28 ± 5.00	15.21 ± 1.91
5	**46d**	2.88 ± 0.06	3.87 ± 0.56
6	**46e**	2.88 ± 0.04	4.36 ± 0.44
7	**46f**	13.47 ± 2.10	33.21 ± 2.80
8	**48**	Not Toxic	Not Toxic
9	**49a**	Not Toxic	Not Toxic
10	**49f**	Not Toxic	Not Toxic
11	**51**	Not Toxic	Not Toxic
12	**52a**	5.39 ± 0.40	5.02 ± 0.47
13	**52b**	4.74 ± 0.93	8.10 ± 2.42
14	**52c**	Not Toxic	Not Toxic
15	**53a**	***1.49 ± 0.06***	***1.34 ± 0.19***
16	**53b**	5.38 ± 0.22	3.86 ± 0.39
17	**53c**	***0.81 ± 0.03***	3.56 ± 0.38
18	Betulinic acid	6.29 ± 0.96	25.63 ± 3.79

Conclusions

Betulinic acid can be readily obtained from the abundantly available natural product betulin and exhibits wide range of medicinal properties. Accordingly, we have synthesized betulinic acid derivatives using Baylis-Hillman and click reaction protocol as the key steps in our synthesis. We have also prepared pyrazinyl and indolyl betulinic acid derivatives employing the above protocol. All the compounds were tested for their biological efficacy as anti-cancer agents in two cell lines namely 4T1 (breast) and MIAPaCa-2 (pancreatic). Some of the compounds tested showed significant cytotoxicity against these cell lines and efforts are underway for a thorough understanding of SAR.

Chapter 3

Experimental Procedures

Materials and Methods

All the reactants were of reagent grade, purchased from Acros Organics, Alfa Aesar or Sigma Aldrich, and used without further purification. All solvents were used without further drying or purification and were of ACS grade purchased from Fisher Scientific. All operations were carried out under an inert atmosphere of nitrogen. Glassware for all reactions was oven dried at 125 °C and cooled under nitrogen prior to use. Liquid reagents and solvents were introduced by oven-dried syringes or cannulas through septa sealed flasks under a nitrogen atmosphere.

Instrumentation

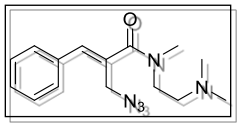
Nuclear Magnetic Spectroscopy (NMR) spectra were produced using a Varian 400 MHz spectrophotometer. The instrument was maintained at 25^{o} C operating at 400 MHz for ^{1}H NMR, and 101 MHz for ^{13}C NMR. The deuterated solvent ($CDCl_3$, DMSO-d_6) used for each respective spectrum is referenced to the appropriate literature peak shift.

Procedures

General amide-coupling procedure A. To a stirred solution of the appropriate acid (1.0 mmol) in dimethylformamide (10.0 mL), was added *N*,*N*-diisopropylethylamine (2.0 mmol) followed by TBTU (1.1 mmol) at 0 °C and stirred for 30 min. The appropriate amine (1.0 mmol) was then added in one portion and stirred overnight at room temperature. Upon completion (as indicated by TLC), the reaction mixture was quenched by the addition of saturated $NaHCO_3$ and extracted with dichloromethane (2 x 10.0 mL). The combined extracts were washed with cold water (10.0 mL) and brine (10.0 mL). The organic layer

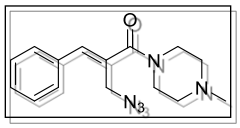
was dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography (silica gel, hexanes:ethyl acetate) to obtain pure amides.

General amide-coupling procedure B. *N*,*N*-diisopropylethylamine (2.0 mmol), HOBt (1.1 mmol), and EDCI (1.1 mmol) were added at 0 °C to a stirred solution of the appropriate acid (1.0 mmol) in dichloromethane (10.0 mL) and the reaction was stirred for 30 min. The appropriate amine (1.0 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 worked up 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 obtain pure amides in good yields.

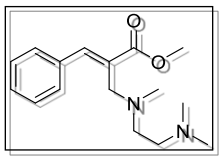


(E)-2-(azidomethyl)-N-(2-(dimethylamino)ethyl)-N-methyl-3-phenylacrylamide: The reaction of (*E*)-2-(azidomethyl)-3-phenylacrylic acid (4.5 mmol) with *N,N,N'*-trimethylethylenediamine (5.5 mmol) as per the general amide coupling ***procedure A*** yielded 72% of **29b** as a pale brown liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.31 – 7.44 (m, 3H), 7.26 – 7.31 (m, 2H), 6.78 (s, 1H), 4.30 (s, 2H), 3.61 (t, *J* = 7.1 Hz, 2H), 3.15 (s, 3H), 2.55 (t, *J* = 7.1 Hz, 2H), 2.28 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 169.5

134.4, 133.5, 131.6, 128.8, 128.6, 128.4, 49.4, 45.7. ESIMS: m/z calculated for $C_{15}H_{21}N_5O$ $(M+H)^+$ 288.18, found 288.38.

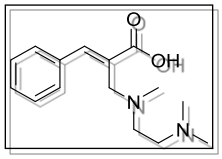


(E)-2-(azidomethyl)-1-(4-methylpiperazin-1-yl)-3-phenylprop-2-en-1-one: The reaction of (*E*)-2-(azidomethyl)-3-phenylacrylic acid (4.6 mmol) with *N*-methylpiperazine (5.6 mmol) as per the general amide coupling ***procedure A*** furnished 74% of **29c** as a pale brown liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.24 – 7.41 (m, 5H), 6.69 (s, 1H), 4.26 (s, 2H), 3.61 – 3.80 (m, 4H), 2.37 – 2.50 (m, 4H), 2.29 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 169.9, 134.3, 134.0, 131.2, 129.0, 128.9, 128.8, 55.0, 49.5, 46.2. ESIMS: m/z calculated for $C_{15}H_{19}N_5O$ $(M+H)^+$ 286.16, found 286.30.

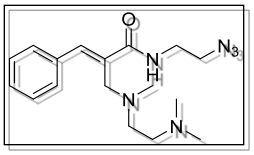


Methyl (E)-2-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)-3-phenylacrylate: *N,N,N'*-Trimethylethylenediamine (5.1 mmol) and K_2CO_3 (6.4 mmol) were added to a stirred solution of acetate (4.3 mmol) in *N,N*-dimethylformamide (10.0 mL) at room temperature and the reaction mixture was stirred overnight. Upon completion (TLC), the reaction was quenched with cold water and extracted with ethyl acetate (2 x 20.0 mL). The combined organic layers were washed thoroughly with cold water (2 x 10.0 mL), brine (2

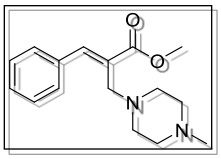
x 10.0 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate was concentrated *in vacuo* and purified by column chromatography (silica gel, hexanes: ethyl acetate, 1:4) to obtain the product ester as brown liquid (912 mg, 77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.80 (s, 1H), 7.57 – 7.61 (m, 2H), 7.29 – 7.41 (m, 3H), 3.82 (s, 3H), 3.39 (s, 2H), 2.48 – 2.54 (m, 2H), 2.37 – 2.44 (m, 2H), 2.21 (s, 6H), 2.18 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 168.9, 142.6, 135.3, 130.4, 130.2, 128.7, 128.3, 57.2, 55.5, 53.1, 51.9, 45.8, 41.9. ESIMS: m/z calculated for $C_{16}H_{24}N_2O_2$ $(M+H)^+$ 277.19, found 277.38.



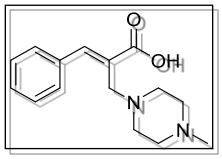
(E)-2-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)-3-phenylacrylic acid: *aq.* NaOH (2.5 M, 7.3 mmol) was added to a solution of ester (3.6 mmol) in THF:MeOH (9:1, 20.0 mL) at 0 °C and the reaction was stirred overnight at room temperature. Upon completion (TLC), the solution was acidified to pH 6 with 1N HCl. The solution was concentrated *in vacuo* and the resulting slurry was dissolved in isopropyl alcohol to effect the precipitation of sodium chloride. The reaction was then filtered and the filtrate was concentrated *in vacuo* to yield 71% of product as pale cream-colored semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.96 (s, 1H), 7.33 – 7.42 (m, 3H), 7.26 – 7.31 (m, 2H), 3.81 (s, 2H), 3.45 (m, 2H), 3.19 (m, 2H), 2.89 (s, 6H), 2.43 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 169.5, 142.9, 134.9, 130.4, 129.5, 129.1, 52.9, 52.7, 51.3, 42.6, 41.4. ESIMS: m/z calculated for $C_{15}H_{22}N_2O_2$ $(M+H)^+$ 263.17, found 263.37.



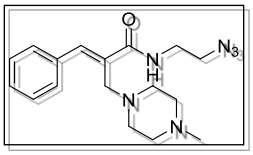
(E)-N-(2-azidoethyl)-2-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)-3-phenylacrylamide 7c: The reaction of acid (1.9 mmol) with 2-azidoethylamine (2.1 mmol) as per the general amide-coupling ***procedure B*** yielded 74% of **29d** as a pale orange liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.10 (s, 1H), 7.96 (s, 1H), 7.21 – 7.41 (m, 5H), 3.49 – 3.53 (m, 4H), 3.39 (s, 2H), 2.43 – 2.51 (m, 2H), 2.34 – 2.42 (m, 2H), 2.24 (s, 6H), 2.14 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 168.6, 140.1, 136.1, 131.0, 129.2, 128.4, 128.0, 56.8, 54.6, 53.1, 50.8, 45.6, 42.0, 39.7. ESIMS: m/z calculated for $C_{17}H_{26}N_6O$ $(M+H)^+$ 331.22, found 331.35.



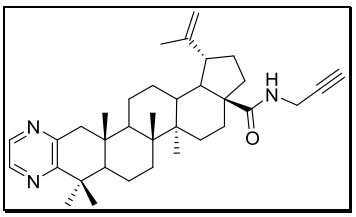
Methyl (E)-2-((4-methylpiperazin-1-yl)methyl)-3-phenylacrylate: The reaction of acetate (4.3 mmol) with *N*-methylpiperazine (5.2 mmol) and K_2CO_3 (6.4 mmol) provided 81% of ester as a pale cream liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.84 (s, 1H), 7.63 – 7.68 (m, 2H), 7.32 – 7.41 (m, 3H), 3.81 (s, 3H), 3.35 (s, 2H), 2.31 – 2.63 (m, 8H), 2.26 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 169.3, 143.6, 135.6, 130.7, 129.9, 129.1, 128.6, 55.5, 53.4, 52.8, 52.3, 46.3. ESIMS: m/z calculated for $C_{16}H_{22}N_2O_2$ $(M+H)^+$ 275.17, found 275.10.



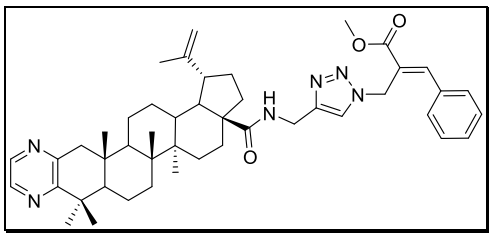
(E)-2-((4-methylpiperazin-1-yl)methyl)-3-phenylacrylic acid: The reaction of ester (3.6 mmol) and aq. NaOH (2.5 M, 7.3 mmol) yielded 74% of acid as a pale cream-colored solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.02 (s, 1H), 7.38 – 7.42 (m, 3H), 7.30 – 7.32 (m, 2H), 3.66 (s, 2H), 2.95 – 3.10 (m, 8H), 2.69 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 169.5, 142.9, 134.6, 129.5, 128.8, 128.3, 127.9, 53.2, 52.9, 49.4, 43.4. ESIMS: m/z calculated for $C_{15}H_{20}N_2O_2$ (M^+) 260.15, found 260.80.



(E)-N-(2-azidoethyl)-2-((4-methylpiperazin-1-yl)methyl)-3-phenylacrylamide: The reaction of acid (0.9 mmol) with 2-azidoethylamine (1.1 mmol) as per the general amide-coupling ***procedure B*** yielded 72% of **29e** as a pale orange liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.04 (s, 1H), 7.92 (s, 1H), 7.18 – 7.36 (m, 5H), 3.50 – 3.56 (m, 2H), 3.45 – 3.49 (m, 2H), 3.39 (s, 2H), 2.33 – 2.68 (m, 8H), 2.25 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 168.6, 140.7, 135.7, 129.8, 129.2, 128.5, 128.2, 55.3, 54.9, 52.5, 51.2, 46.2, 38.9. ESIMS: m/z calculated for $C_{17}H_{24}N_6O$ $(M+H)^+$ 329.20, found 329.33.

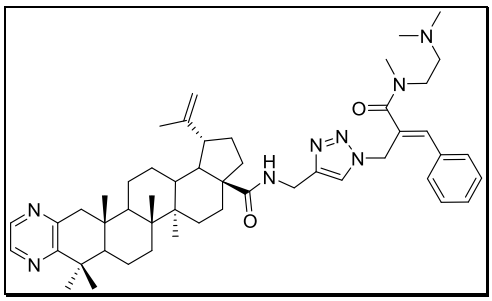


N-Propargyl pyrazinylbetulinamide **45**: The title compound was prepared by the reaction of compound **44** (0.9 mmol) and propargyl amine (1.1 mmol) as per the general amide-coupling ***procedure A*** to yield 80% of **45** as a white solid. Mp 122 – 125 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.38 (s, 1H), 8.25 (s, 1H), 5.77 – 5.89 (m, 1H), 4.74 (s, 1H), 4.60 (s, 1H), 3.93 – 4.10 (m, 2H), 3.11 – 3.16 (m, 1H), 3.01 (d, *J* = 16.6 Hz, 1H), 2.36 – 2.56 (m, 2H), 2.16 – 2.20 (m, 1H), 0.73 – 1.96 (m, 19H), 1.68 (s, 3H), 1.27 (s, 3H), 1.25 (s, 3H), 1.00 (s, 6H), 0.78 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.2, 159.8, 151.0, 150.8, 142.4, 141.6, 109.7, 80.5, 71.2, 55.8, 53.2, 50.3, 49.0, 48.8, 46.8, 42.7, 40.8, 39.7, 38.3, 37.9, 36.9, 33.7, 33.6, 31.7, 31.0, 29.6, 29.2, 25.8, 24.2, 21.7, 20.3, 19.8, 16.3, 15.9, 14.8; ESIMS: m/z calculated for $C_{35}H_{49}N_3O$ $(M+H)^+$ 528.81, found 528.60.



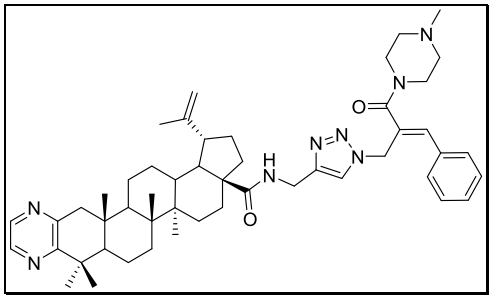
Preparation of triazole **46a**: To a stirred solution of alkyne **45** (0.9 mmol) and azide **29a** (0.9 mmol) in a mixture of *t*-butanol/water (1:1, 8.0 mL), was added $CuSO_4$ (0.1 mmol)

and sodium ascorbate (0.2 mmol). The reaction mixture was stirred overnight at room temperature. Upon completion (TLC), the reaction was concentrated *in vacuo* and diluted with water to effect precipitation. The resulting solid was filtered, washed with water, and further purified via column chromatography (silica gel, methanol:dichloromethane, 2:3) to obtain 1,2,3-triazole **46a** as a white solid. Yield: 82%; white solid; mp 127 – 129 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H), 8.20 (s, 1H), 7.98 (s, 1H), 7.68 (s, 1H), 7.49 – 7.60 (m, 2H), 7.45 – 7.29 (m, 3H), 6.30 (brs, 1H), 5.29 (s, 2H), 4.68 (s, 1H), 4.55 (s, 1H), 4.37 – 4.49 (m, 2H), 3.78 (s, 3H), 3.07 (dt, *J* = 4.3, 11.2 Hz, 1H), 2.93 (d, *J* = 16.4 Hz, 1H), 2.30 – 2.43 (m, 2H), 0.61 – 1.93 (m, 19H), 1.62 (s, 3H), 1.20 (s, 3H), 1.15 (s, 3H), 0.91 (s, 3H), 0.73 (s, 3H), 0.66 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.5, 167.2, 159.8, 151.1, 150.9, 146.2, 142.5, 141.7 (2C), 133.7 (2C), 130.2, 129.9, 129.2, 125.0, 109.7, 55.9, 53.2, 52.8, 50.3, 49.0, 48.9, 47.1, 46.9, 42.7, 40.8, 39.6, 38.4, 38.0, 36.9, 34.9, 33.7, 33.5, 31.7, 31.1, 29.6, 25.8, 24.2, 21.6, 20.3, 19.8, 16.3, 15.6, 14.8; ESIMS: m/z calculated for $C_{46}H_{60}N_6O_3$ $(M+H)^+$ 745.48, found 745.45.



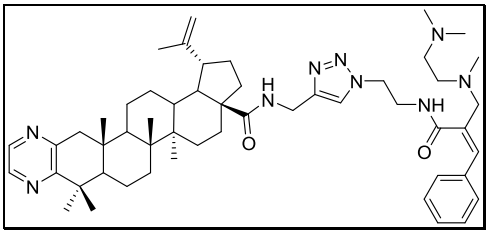
Preparation of triazole **46b**: Procedure similar to that of **46a**. This compound was prepared by the reaction of alkyne **45** with azide **29b**. Yield: 84%; white solid; mp 131 –

133 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (d, *J* = 2.4 Hz, 1H), 8.26 (d, *J* = 2.4 Hz, 1H), 7.63 (s, 1H), 7.29 – 7.48 (m, 5H), 6.95 (brs, 1H), 6.23 – 6.30 (m, 1H), 5.31 – 5.39 (m, 2H), 4.76 (s, 1H), 4.61 (s, 1H), 4.51 (dd, *J* = 5.4, 15.1 Hz, 1H), 4.44 (dd, *J* = 5.6, 15.2 Hz, 1H), 3.39 – 3.54 (m, 2H), 3.13 (dt, *J* = 4.2, 10.9 Hz, 1H), 2.92 – 3.05 (m, 4H), 2.33 – 2.51 (m, 4H), 2.12 – 2.31 (brs, 6H), 0.73 – 1.97 (m, 19H), 1.69 (s, 3H), 1.28 (s, 3H), 1.25 (s, 3H), 0.99 (s, 3H), 0.86 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.2, 159.6, 150.8, 150.7, 145.0, 142.2, 141.4 (2C), 134.8, 134.0, 130.2, 128.8, 128.7, 123.2, 109.4, 55.6, 53.0, 50.0, 48.8, 48.6, 48.6, 46.6, 45.6, 42.4, 40.6, 39.4, 38.2, 37.7, 36.7, 34.7, 33.4, 33.3, 31.4, 30.9, 29.4, 25.6, 24.0, 21.4, 20.0, 19.5, 16.0, 15.4, 14.6; ESIMS: m/z calculated for $C_{50}H_{70}N_8O_2$ $(M+H)^+$ 815.57, found 815.30.

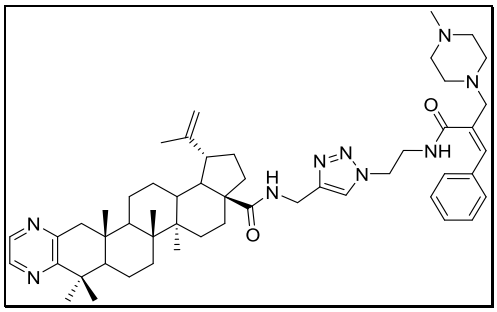


Preparation of triazole **46c**: Procedure similar to that of **46a**. This compound was prepared by the reaction of alkyne **45** with azide **29c**. Yield: 84%; white solid; mp 137 – 140 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.33 (d, *J* = 2.4 Hz, 1H), 8.20 (d, *J* = 2.4 Hz, 1H), 7.55 (s, 1H), 7.29 – 7.41 (m, 5H), 6.80 (s, 1H), 6.20 - 6.30 (m, 1H), 5.35 (s, 2H), 4.70 (s, 1H), 4.56 (s, 1H), 4.46 (dd, *J* = 5.5, 15.0 Hz, 1H), 4.36 (dd, *J* = 5.5, 15.0 Hz, 1H), 3.60 (brs, 4H), 3.12 (dt, *J* = 4.3, 11.2 Hz, 1H), 3.01 (d, *J* = 16.6 Hz, 1H), 2.05 – 2.39 (m,

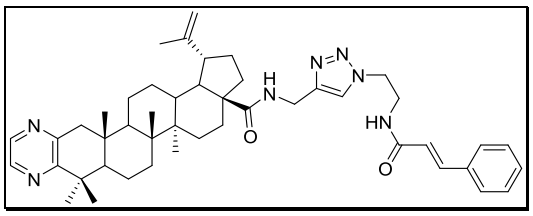
9H), 0.64 – 1.89 (m, 19H), 1.63 (s, 3H), 1.22 (s, 3H), 1.19 (s, 3H), 0.93 (s, 3H), 0.78 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.6, 169.0, 159.8, 151.0, 150.9, 145.3, 142.5, 141.7 (2C), 134.8, 134.1, 129.9, 129.0, 128.9, 123.7, 109.7, 55.8, 54.9, 53.2, 50.3, 49.0, 48.9, 48.8, 46.9, 46.2, 42.7, 40.8, 39.6, 38.4, 37.9, 36.9, 34.9, 33.7, 33.5, 31.7, 31.1, 29.6, 25.8, 24.2, 21.6, 20.3, 19.8, 16.3, 15.6, 14.8; ESIMS: m/z 835.75 [100%, $(M+Na)^+$], HRMS-ESI: calculated for $C_{50}H_{68}N_8O_2$ $[M+H]^+$ 813.5538, found 813.5503.



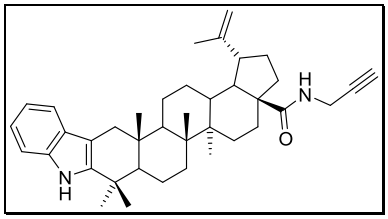
Preparation of triazole **46d**: Procedure similar to that of **46a**. This compound was prepared by the reaction of alkyne **45** with azide **29d**. Yield: 85%; white solid; mp 112 – 114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.96 (s, 1H), 8.39 (d, *J* = 2.3 Hz, 1H), 8.26 (d, *J* = 2.4 Hz, 1H), 7.95 (s, 1H), 7.59 (s, 1H), 7.28 – 7.39 (m, 3H), 7.21 – 7.25 (m, 2H), 6.37 (s, 1H), 4.75 (s, 1H), 4.61 (s, 1H), 4.60 – 4.55 (m, 2H), 4.50 (dd, *J* = 5.4, 15.1 Hz, 1H), 4.44 (dd, *J* = 5.4, 15.1 Hz, 1H), 3.82 – 3.65 (m, 2H), 3.33 (s, 2H), 3.13 (dt, *J* = 4.3, 11.2 Hz, 1H), 3.01 (d, *J* = 16.6 Hz, 1H), 2.51 – 2.26 (m, 6H), 2.14 (s, 6H), 2.06 (s, 3H), 0.76 – 1.97 (m, 19H), 1.69 (s, 3H), 1.28 (s, 3H), 1.26 (s, 3H), 0.98 (s, 3H), 0.88 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.4, 168.4, 159.6, 150.8, 150.7, 144.9, 142.2, 141.4, 140.1, 135.6, 130.5, 128.9, 128.2, 127.9, 122.9, 109.4, 56.1, 55.6, 53.9, 53.0, 52.5, 50.0, 49.2, 48.8, 48.6, 46.6, 45.0, 42.4, 41.6, 40.6, 39.9, 39.4, 38.2, 37.7, 36.7, 34.7, 33.4, 33.3, 31.4, 30.9, 29.3, 25.6, 24.0, 21.4, 20.0, 19.5, 16.1, 15.4, 14.7; ESIMS: m/z calculated for $C_{52}H_{75}N_9O_2$ $(M+H)^+$ 858.61, found 858.75.



Preparation of triazole **46e**: Procedure similar to that of **46a**. This compound was prepared by the reaction of alkyne **45** with azide **29e**. Yield: 87%; cream color solid; mp 132 – 135 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.79 (s, 1H), 8.33 (s, 1H), 8.20 (s, 1H), 7.85 (s, 1H), 7.58 (s, 1H), 7.15 – 7.30 (m, 5H), 6.37 (s, 1H), 4.69 (s, 1H), 4.55 (s, 1H), 4.34 – 4.51 (m, 4H), 3.69 – 3.91 (m, 2H), 3.28 (s, 2H), 3.00 – 3.14 (m, 1H), 2.95 (d, *J* = 16.4 Hz, 1H), 2.14 – 2.52 (m, 13H), 0.68 – 1.93 (m, 19H), 1.63 (s, 3H), 1.21 (s, 3H), 1.20 (s, 3H), 0.92 (s, 3H), 0.79 (s, 3H), 0.72 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.7, 168.9, 159.8, 150.9, 150.8, 145.6, 142.5, 141.7, 141.0, 135.4, 129.4, 129.2, 128.5, 128.3, 123.4, 109.8, 55.8, 54.9, 54.8, 53.2, 52.2, 50.2, 49.9, 48.9, 48.8, 46.9, 45.8, 42.7, 40.8, 39.7, 39.4, 38.4, 38.0, 36.9, 34.9, 33.7, 33.5, 31.7, 31.1, 29.6, 25.8, 24.3, 21.6, 20.3, 19.7, 16.4, 15.6, 14.8; ESIMS: m/z calculated for $C_{52}H_{73}N_9O_2$ $(M+H)^+$ 856.60, found 856.70.

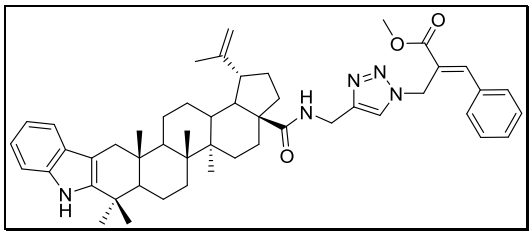


Preparation of triazole **46f**: Procedure similar to that of **46a**. This compound was prepared by the reaction of alkyne **45** with azide **29f**. Yield: 88%; white solid; mp 150 – 152 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.39 (s, 1H), 8.26 (s, 1H), 7.63 (d, *J* = 15.6 Hz, 1H), 7.61 (s, 1H), 7.45 – 7.52 (m, 2H), 7.30 – 7.39 (m, 3H), 6.26 – 6.42 (m, 3H), 4.74 (s, 1H), 4.60 (s, 1H), 4.37 – 4.56 (m, 4H), 3.84 – 3.95 (m, 2H), 3.05 – 3.17 (m, 1H), 3.00 (d, *J* = 16.5 Hz, 1H), 2.34 – 2.49 (m, 2H), 0.72 – 1.99 (m, 19H), 1.67 (s, 3H), 1.26 (s, 3H), 1.25 (s, 3H), 0.97 (s, 3H), 0.84 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.9, 166.7, 159.8, 150.9, 150.8, 145.7, 142.5, 141.9, 141.7, 134.8, 130.1, 129.0, 128.1, 123.7, 120.3, 109.8, 55.8, 53.2, 50.2, 49.9, 49.0, 48.8, 46.9, 42.7, 40.8, 39.8, 39.7, 38.4, 38.0, 36.9, 35.0, 33.6, 33.5, 31.7, 31.1, 29.6, 25.8, 24.3, 21.7, 20.2, 19.7, 16.4, 15.7, 14.8; ESIMS: m/z calculated for $C_{46}H_{61}N_7O_2$ $(M+H)^+$ 744.50, found 744.55.



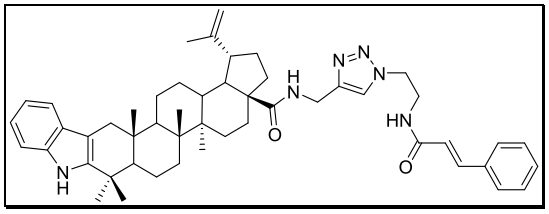
N-Propargyl indolylbetulinamide **48**: The title compound was prepared by the reaction of compound **47** (300 mg, 0.6 mmol) and propargyl amine (37.5 mg, 0.7 mmol) as per the general amide-coupling ***procedure A*** to yield 224 mg (71%) of **48** as a yellow

solid. Mp 251 – 254 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.64 (s, 1H), 7.98 (s, 1H), 7.26 (d, *J* = 7.7 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 6.81 – 6.98 (m, 2H), 4.68 (s, 1H), 4.55 (s, 1H), 3.65 – 3.92 (m, 2H), 2.99 – 3.12 (m, 1H), 2.73 (d, *J* = 15.2 Hz, 1H), 2.50 – 2.63 (m, 1H), 2.10 – 2.18 (m, 1H), 2.03 (d, *J* = 15.1 Hz, 1H), 0.67 – 1.77 (m, 19H), 1.64 (s, 3H), 1.24 (s, 3H), 1.13 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 176.1, 151.5, 141.9, 136.9, 128.3, 120.6, 118.4, 117.9, 111.1, 109.9, 105.5, 82.6, 72.5, 55.6, 53.8, 50.3, 49.6, 46.9, 42.6, 41.0, 40.8, 39.6, 38.5, 38.0, 37.6, 34.6, 33.9, 32.7, 31.1, 30.9, 29.6, 28.5, 26.1, 23.3, 21.8, 19.7, 19.5, 16.9, 16.3, 15.0; ESIMS: m/z calculated for $C_{39}H_{52}N_2O$ $(M-H)^+$ 563.40, found 563.50.

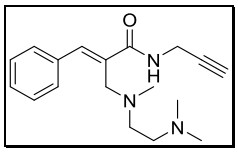


Preparation of triazole **49a**: Procedure similar to that of **46a**. This compound was prepared by the reaction of alkyne **48** with azide **29a**. Yield: 80%; tan color solid; mp 133 – 136 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.01 (s, 1H), 7.48 – 7.76 (m, 4H), 7.34 – 7.42 (m, 3H), 7.27 – 7.45 (m, 2H), 6.96 – 7.07 (m, 2H), 6.26 (m, 1H), 5.30 (s, 2H), 4.71 (s, 1H), 4.56 (s, 1H), 4.45 – 4.53 (m, 1H), 4.39 (dd, *J* = 5.9, 14.9 Hz, 1H), 3.79 (s, 3H), 3.12 (dt, *J* = 6.2, 12.1 Hz, 1H), 2.74 (d, *J* = 14.9 Hz, 1H), 2.33 – 2.43 (m, 1H), 2.05 (d, *J* = 14.8 Hz, 1H), 0.68 – 1.98 (m, 19H), 1.64 (s, 3H), 1.20 (s, 3H), 1.08 (s, 3H), 0.93 (s, 3H), 0.76 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 175.3, 165.9, 149.9, 144.9,

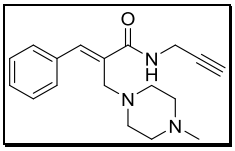
143.9, 139.8, 135.1, 132.5, 128.9, 128.7, 127.9, 127.3, 123.8, 122.1, 119.8, 117.8, 116.8, 109.3, 108.4, 105.9, 54.6, 52.2, 51.5, 49.1, 49.0, 48.4, 45.8, 41.4, 39.7, 37.2, 37.2, 36.9, 36.2, 33.8, 33.1, 32.5, 29.8, 29.8, 28.7, 28.4, 24.7, 22.1, 20.4, 18.4, 18.2, 15.3, 14.6, 13.6; ESIMS: m/z 782.85 [100%, $(M+H)^+$]; HRMS-ESI: calculated for $C_{50}H_{63}N_5O_3$ $[M+H]^+$ 782.5004, found 782.4986.



Preparation of triazole **49f**: Procedure similar to that of **46a**. This compound was prepared by the reaction of alkyne **48** with azide **29f**. Yield: 81%; tan color solid; mp 167 – 169 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.75 (s, 1H), 7.64 (d, *J* = 15.4 Hz, 1H), 7.61 (s, 1H), 7.44 - 7.54 (m, 2H), 7.33 – 7.41 (m, 4H), 7.28 – 7.32 (m, 1H), 7.00 – 7.13 (m, 2H), 6.38 (d, *J* = 15.7 Hz, 1H), 6.29 – 6.36 (m, 1H), 6.20 – 6.28 (m, 1H), 4.75 (s, 1H), 4.61 (s, 1H), 4.37 – 4.57 (m, 4H), 3.87 – 3.96 (m, 2H), 3.05 – 3.20 (m, 1H), 2.80 (d, *J* = 15.1 Hz, 1H), 2.33 – 2.50 (m, 1H), 2.11 (d, *J* = 15.1 Hz, 1H), 0.76 – 1.96 (m, 19H), 1.68 (s, 3H), 1.26 (s, 3H), 1.17 (s, 3H), 0.99 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 177.0, 166.7, 150.9, 145.7, 141.9, 141.2, 136.4, 134.8, 130.1, 129.1, 128.5, 128.1, 123.6, 121.1, 120.2, 119.0, 118.1, 110.6, 109.9, 107.0, 55.9, 53.5, 50.3, 49.9, 49.6, 47.0, 42.7, 41.0, 39.8, 38.5, 38.2, 37.5, 35.0, 34.4, 33.8, 33.7, 31.0, 29.7, 25.9, 23.4, 21.7, 19.6, 19.4, 16.6, 15.9, 14.9; ESIMS: m/z calculated for $C_{50}H_{64}N_6O_2$ $(M+Na)^+$ 803.50, found 803.55.

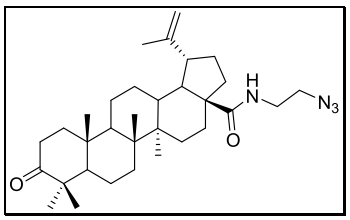


(E)-2-(((2-(Dimethylamino)ethyl)(methyl)amino)methyl)-3-phenyl-N-(prop-2-yn-1-yl) acrylamide **50a:** The reaction of acid (430 mg, 1.6 mmol) with propargyl amine (99 mg, 1.8 mmol) as per the general amide-coupling ***procedure B*** yielded 364 mg (72%) of **50a** as a pale orange liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.08 (s, 1H), 7.98 (s, 1H), 7.21 – 7.39 (m, 5H), 4.11 (dd, *J* = 2.5, 5.3 Hz, 2H), 3.38 (s, 2H), 2.36 – 2.50 (m, 4H), 2.24 (s, 6H), 2.17 (t, *J* = 2.6 Hz, 1H), 2.14 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 167.9, 140.3, 136.1, 130.9, 129.2, 128.4, 127.9, 80.9, 70.5, 56.8, 54.6, 52.8, 45.5, 42.1, 29.1. ESIMS: m/z calculated for $C_{18}H_{25}N_3O$ $(M+H)^+$ 300.20, found 300.41.

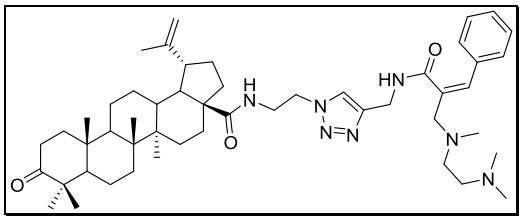


(E)-2-((4-methylpiperazin-1-yl)methyl)-3-phenyl-N-(prop-2-yn-1-yl)acrylamide **50b**: The reaction of acid (210 mg, 0.8 mmol) with propargyl amine (48 mg, 0.9 mmol) as per the general amide-coupling ***procedure B*** yielded 176 mg (71%) of **50b** as a pale orange liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.08 (s, 1H), 7.90 (s, 1H), 7.27 – 7.31 (m, 2H), 7.22 – 7.26 (m, 1H), 7.15 – 7.19 (m, 2H), 4.07 (dd, *J* = 2.6, 4.8 Hz, 2H), 3.35 – 3.38 (m, 2H), 2.25 – 2.60 (m, 8H), 2.21 (s, 3H), 2.18 (t, *J* = 2.6 Hz, 1H); ^{13}C NMR (101 MHz,

$CDCl_3$): δ (ppm) 167.9, 140.8, 135.6, 129.4, 129.2, 128.5, 128.2, 80.4, 71.4, 55.3, 54.8, 52.4, 46.2, 29.4. ESIMS: m/z calculated for $C_{18}H_{23}N_3O$ $(M+H)^+$ 298.19, found 298.40.

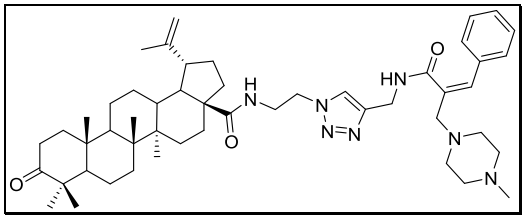


N-2-azidoethyl betulonamide **51**: The title compound was prepared by the reaction of betulonic acid (850 mg, 1.87 mmol) and 2-azidoethylamine (241 mg, 2.80 mmol) using the general amide-coupling ***procedure A*** to furnish the amide **51** (722 mg, 74%) as a white solid. Mp 82 – 84 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.94 (s, 1H), 4.72 (s, 1H), 4.58 (s, 1H), 3.32 – 3.48 (m, 4H), 3.03 – 3.15 (m, 1H), 2.35 – 2.49 (m, 3H), 0.81 – 1.95 (m, 21H), 1.66 (s, 3H), 1.05 (s, 3H), 1.00 (s, 3H), 0.96 (s, 6H), 0.91 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 218.4, 176.7, 150.9, 109.7, 55.9, 55.2, 51.4, 50.2, 50.2, 47.5, 46.9, 42.7, 40.9, 39.8, 38.9, 38.4, 38.0, 37.1, 34.4, 33.9, 31.0, 29.6, 26.8, 25.8, 21.7, 21.2, 19.8, 19.7, 16.2, 16.1, 14.8; ESIMS: m/z calculated for $C_{32}H_{50}N_4O_2$ $(M-H)^+$ 521.39, found 521.20.



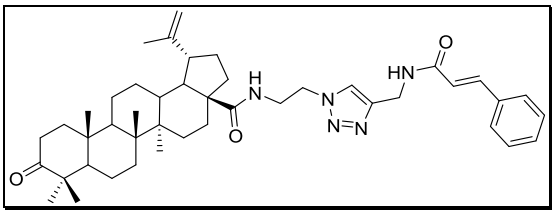
Preparation of triazole **52a**: Procedure similar to that of **46a**. This compound was prepared by the reaction of azide **51** with alkyne **50a**. Yield: 79%; off white solid; mp 108 – 111 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.86 (s, 1H), 7.61 (s, 1H), 7.20 – 7.40 (m,

5H), 6.40 (brs, 1H), 4.73 (s, 1H), 4.53 – 4.61 (m, 3H), 4.35 – 4.50 (m, 2H), 3.69 – 3.79 (m, 2H), 3.41 (s, 2H), 3.07 (m, 1H), 2.27 – 2.63 (m, 12H), 2.14 (s, 3H), 0.83 – 1.99 (m, 22H), 1.66 (s, 3H), 1.04 (s, 3H), 1.00 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 218.1, 176.9, 168.3, 150.7, 145.7, 139.5, 135.6, 131.3, 128.9, 128.3, 127.8, 123.4, 109.4, 56.1, 55.6, 54.9, 53.3, 53.1, 49.9, 49.8, 47.3, 46.5, 44.9, 42.4, 41.8, 40.7, 39.6, 39.3, 38.2, 37.6, 36.9, 35.3, 34.1, 33.7, 33.4, 30.8, 29.6, 29.4, 26.6, 25.6, 21.5, 20.9, 19.6, 19.4, 15.9, 15.9, 14.5; ESIMS: m/z 822.60 [100%, $(M+H)^+$]; HRMS-ESI: calculated for $C_{50}H_{75}N_7O_3$ $[M+H]^+$ 822.6004, found 822.6025.

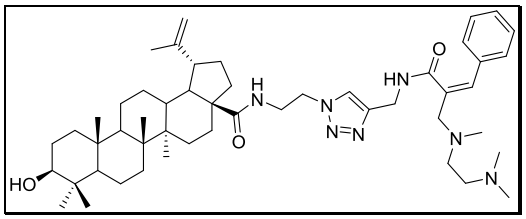


Preparation of triazole **52b**: Procedure similar to that of **46a**. This compound was prepared by the reaction of azide **51** with alkyne **50b**. Yield: 86%; cream color solid; mp 115 – 117 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.23 (m, 1H), 7.94 (s, 1H), 7.60 (s, 1H), 7.29 – 7.37 (m, 3H), 7.21 – 7.25 (m, 2H), 6.05 (t, *J* = 5.7 Hz, 1H), 4.73 (s, 1H), 4.59 (s, 2H), 4.57 (s, 1H), 4.38 – 4.52 (m, 2H), 3.78 (q, *J* = 5.7 Hz, 2H), 3.41 (s, 2H), 3.07 (dt, *J* = 4.3, 11.2 Hz, 1H), 2.31 – 2.62 (m, 10H), 3.69 (s, 3H), 0.87 – 1.66 (m, 22H), 1.66 (s, 3H), 1.04 (s, 3H), 1.00 (s, 3H), 0.95 (s, 6H), 0.91 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 218.1, 176.9, 168.2, 150.6, 145.3, 140.4, 135.3, 129.5, 128.9, 128.3, 128.0, 122.9, 109.5, 55.6, 54.9, 54.8, 54.6, 52.1, 49.9, 49.9, 49.6, 47.3, 46.5, 45.7, 42.4, 40.6, 39.6, 39.1, 38.2, 37.6, 36.9, 35.0, 34.1, 33.6, 33.4, 30.7, 29.4, 26.6, 25.6, 21.4, 20.9, 19.6, 19.4, 15.9,

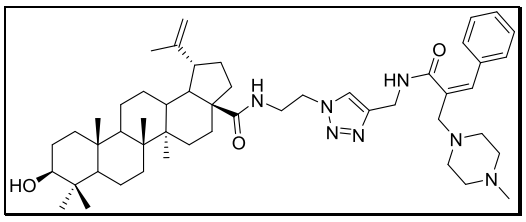
15.9, 14.5; ESIMS: m/z 820.60 [100%, $(M+H)^+$]; HRMS-ESI: calculated for $C_{50}H_{73}N_7O_3$ $[M+H]^+$ 820.5848, found 820.5855.



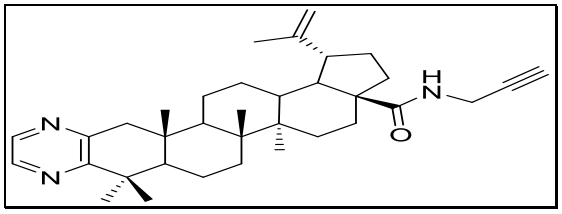
Preparation of triazole **52c**: Procedure similar to that of **46a**. This compound was prepared by the reaction of azide **51** with alkyne **50c**. Yield: 81%; white solid; mp 155 – 158 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.62 (d, *J* = 15.9 Hz, 1H), 7.60 (s, 1H), 7.44 – 7.51 (m, 2H), 7.31 – 7.39 (m, 3H), 6.55 – 6.66 (m, 1H), 6.43 (d, *J* = 15.6 Hz, 1H), 6.12 – 6.20 (m, 1H), 4.71 (s, 1H), 4.62 (d, *J* = 5.5 Hz, 2H), 4.57 (s, 1H), 4.39 – 4.53 (m, 2H), 3.68 – 3.80 (m, 2H), 2.97 – 3.09 (m, 1H), 2.29 – 2.53 (m, 3H), 0.82 – 1.90 (m, 21H), 1.64 (s, 3H), 1.03 (s, 3H), 0.99 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 218.5, 177.2, 166.4, 150.8, 145.2, 141.6, 134.8, 130.1, 129.0, 128.0, 123.5, 120.5, 109.8, 55.8, 55.2, 50.2, 50.1, 49.9, 47.5, 46.8, 42.7, 40.9, 39.8, 39.4, 38.4, 37.8, 37.1, 35.3, 34.4, 33.9, 33.6, 30.9, 29.6, 26.8, 25.8, 21.7, 21.2, 19.8, 19.6, 16.2, 16.2, 14.7; HRMS-ESI: calculated for $C_{44}H_{61}N_5O_3$ $[M+H]^+$ 708.4847, found 708.4840.



Preparation of **53a**: To a stirred solution of the appropriate ketone **52a** (0.2 mmol) in methanol at 0 °C, was added $NaBH_4$ (0.4 mmol), and stirred for 2 h at room temperature. Upon completion of reaction (as monitored by TLC), the reaction mixture was concentrated *in vacuo*, diluted with water and extracted with ethyl acetate (2 x 10.0 mL). The combined organic extracts were washed with brine (10.0 mL), dried over anhydrous Na_2SO_4, concentrated under vacuum and purified via column chromatography (silica gel, hexane:ethyl acetate, 1:2) to obtain pure alcohol **53a** as a white solid. Yield: 86%; pale yellow solid; mp 116 – 118 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.21 (brs, 1H), 7.92 (s, 1H), 7.61 (s, 1H), 7.33 – 7.43 (m, 2H), 7.28 – 7.32 (m, 1H), 7.19 – 7.25 (m, 2H), 6.21 (brs, 1H), 4.73 (s, 1H), 4.58 (s, 1H), 4.53 – 4.57 (m, 2H), 4.33 – 4.51 (m, 2H), 3.74 (q, *J* = 5.5 Hz, 2H), 3.38 (s, 2H), 3.16 (dd, *J* = 5.0, 11.2 Hz, 1H), 3.07 (dt, *J* = 3.7, 11.2 Hz, 1H), 2.36 – 2.51 (m, 5H), 2.25 (brs, 6H), 2.12 (s, 3H), 0.64 – 1.91 (m, 24H), 1.66 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.80 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.9, 168.2, 150.8, 145.9, 139.7, 135.7, 130.9, 128.9, 128.2, 127.8, 123.2, 109.4, 78.8, 56.2, 55.6, 55.4, 53.8, 52.8, 50.6, 50.0, 49.6, 46.6, 45.1, 42.4, 41.8, 40.7, 39.2, 38.8, 38.7, 38.2, 37.5, 37.2, 35.3, 34.4, 33.4, 30.8, 29.6, 29.4, 27.9, 27.4, 25.6, 20.9, 19.4, 18.3, 16.1, 15.4, 14.7; ESIMS: m/z 824.75 [100%, $(M+H)^+$]; HRMS-ESI: calculated for $C_{50}H_{77}N_7O_3$ $[M+Na]^+$ 846.5980, found 846.5954.



Preparation of **53b**: Procedure similar to that of **53a**. Yield: 89%; pale orange solid; mp 129 – 131 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.22 (t, *J* = 5.3 Hz, 1H), 7.95 (s, 1H), 7.60 (s, 1H), 7.33 – 7.37 (m, 2H), 7.28 – 7.31 (m, 1H), 7.21 – 7.24 (m, 2H), 6.00 – 6.10 (m, 1H), 4.73 (s, 1H), 4.56 – 4.60 (m, 3H), 4.39 – 4.52 (m, 2H), 3.77 (q, *J* = 5.7 Hz, 2H), 3.42 (s, 2H), 3.16 (dd, *J* = 5.0, 11.2 Hz, 1H), 3.06 (dt, *J* = 3.8, 11.1 Hz, 1H), 2.24 – 2.61 (m, 10H), 2.29 (s, 3H), 0.65 – 1.92 (m, 23H), 1.66 (s, 3H), 0.94 (s, 6H), 0.90 (s, 3H), 0.80 (s, 3H), 0.74 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.9, 168.1, 150.6, 145.2, 140.5, 135.3, 129.4, 128.9, 128.3, 128.0, 122.9, 109.5, 78.8, 55.7, 55.4, 54.7, 54.6, 51.9, 50.6, 49.9, 49.6, 46.6, 45.6, 42.4, 40.7, 39.1, 38.8, 38.7, 38.2, 37.6, 37.2, 35.0, 34.4, 33.4, 30.8, 29.6, 29.4, 27.9, 27.4, 25.6, 20.9, 19.4, 18.3, 16.1, 15.4, 14.6; ESIMS: m/z 822.80 [100%, $(M+H)^+$]; HRMS-ESI: calculated for $C_{50}H_{75}N_7O_3$ $[M+Na]^+$ 844.5824, found 844.5885.



Preparation of **53c**: Procedure similar to that of **53a**. Yield: 91%; white solid; mp 163 – 166 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.63 (d, *J* = 15.6 Hz, 1H), 7.59 (s, 1H), 7.46 – 7.50 (m, 2H), 7.33 – 7.39 (m, 3H), 6.50 (brs, 1H), 6.41 (d, *J* = 15.6 Hz, 1H), 6.10 (brs, 1H), 4.72 (s, 1H), 4.62 (d, *J* = 5.8 Hz, 2H), 4.57 (s, 1H), 4.37 – 4.52 (m, 2H), 3.76 (q, *J* = 5.6 Hz, 2H), 3.11 – 3.20 (m, 1H), 3.04 (dt, *J* = 4.0, 12.0 Hz, 1H), 2.42 (dt, *J* = 3.6, 12.7 Hz, 1H), 0.60 – 1.89 (m, 24H), 1.64 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.89 (s,

3H), 0.79 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 177.0, 166.2, 150.6, 144.9, 141.3, 134.6, 129.8, 128.8, 127.8, 123.2, 120.4, 109.5, 78.9, 55.6, 55.3, 50.6, 49.9, 49.7, 46.6, 42.4, 40.7, 39.2, 38.8, 38.7, 38.2, 37.6, 37.2, 34.9, 34.4, 33.4, 30.8, 29.4, 28.0, 27.4, 25.5, 20.9, 19.4, 18.3, 16.1, 15.4, 14.6; ESIMS: m/z 710.65 [100%, $(M+H)^+$]; HRMS-ESI: calculated for $C_{44}H_{63}N_5O_3$ $[M+Na]^+$ 732.4823, found 732.4836.

Chapter 4

Spectral Characterization

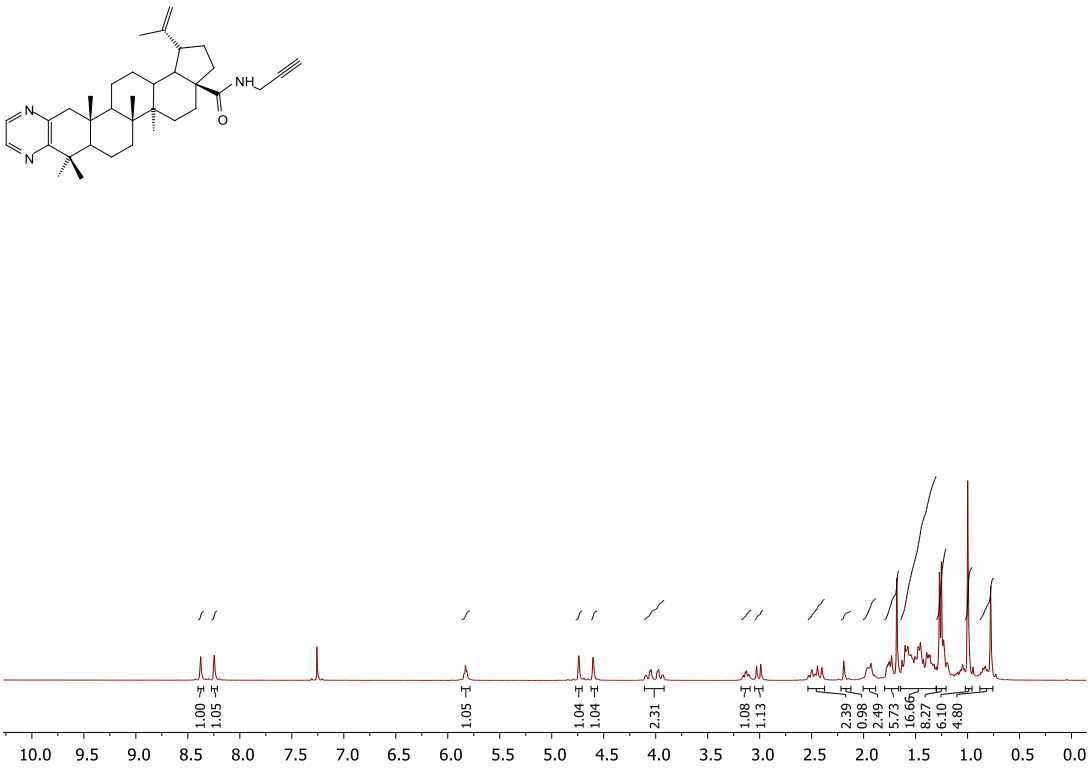


Figure 22. 400 MHz ^{1}H NMR of Compound **45** in $CDCl_3$

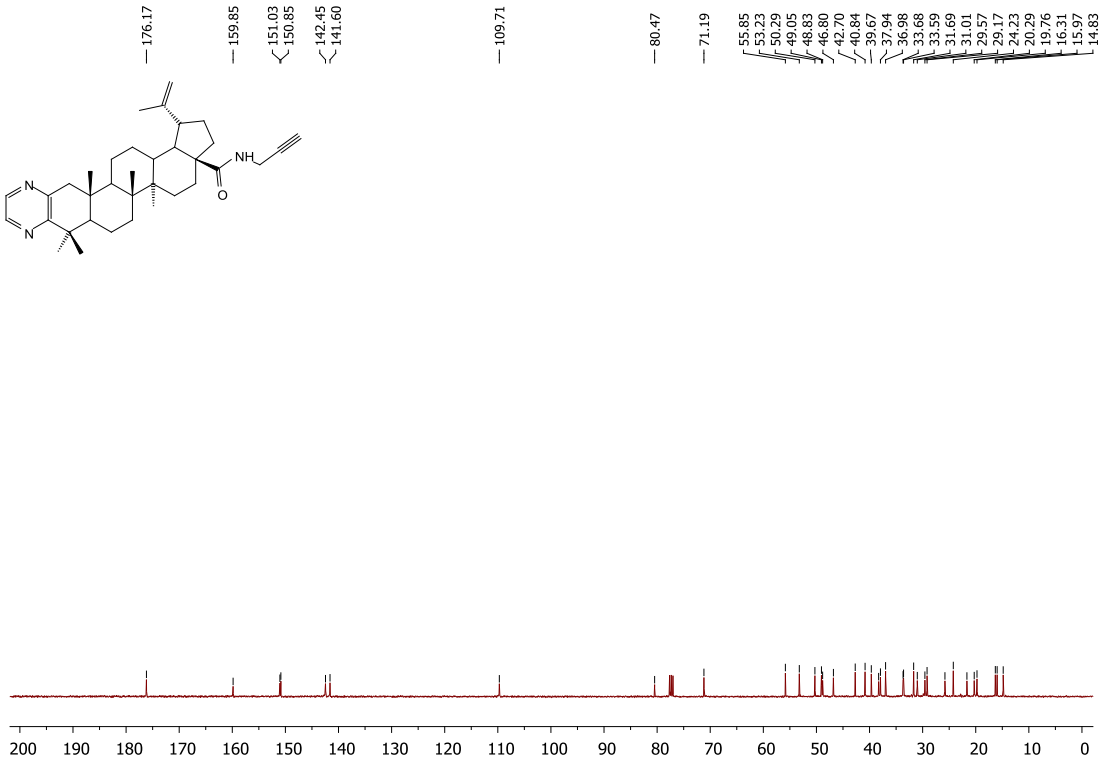


Figure 23. 101 MHz ^{13}C NMR of Compound **45** in $CDCl_3$

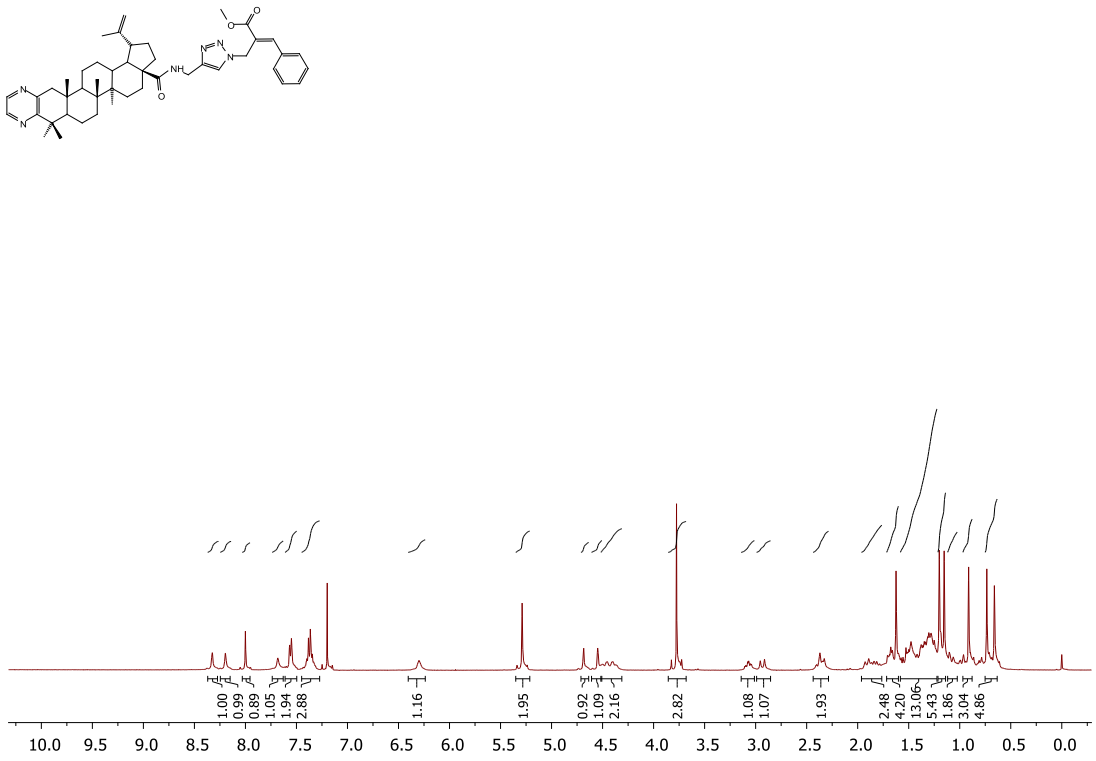


Figure 24. 400 MHz ^{1}H NMR of Compound **46a** in $CDCl_3$

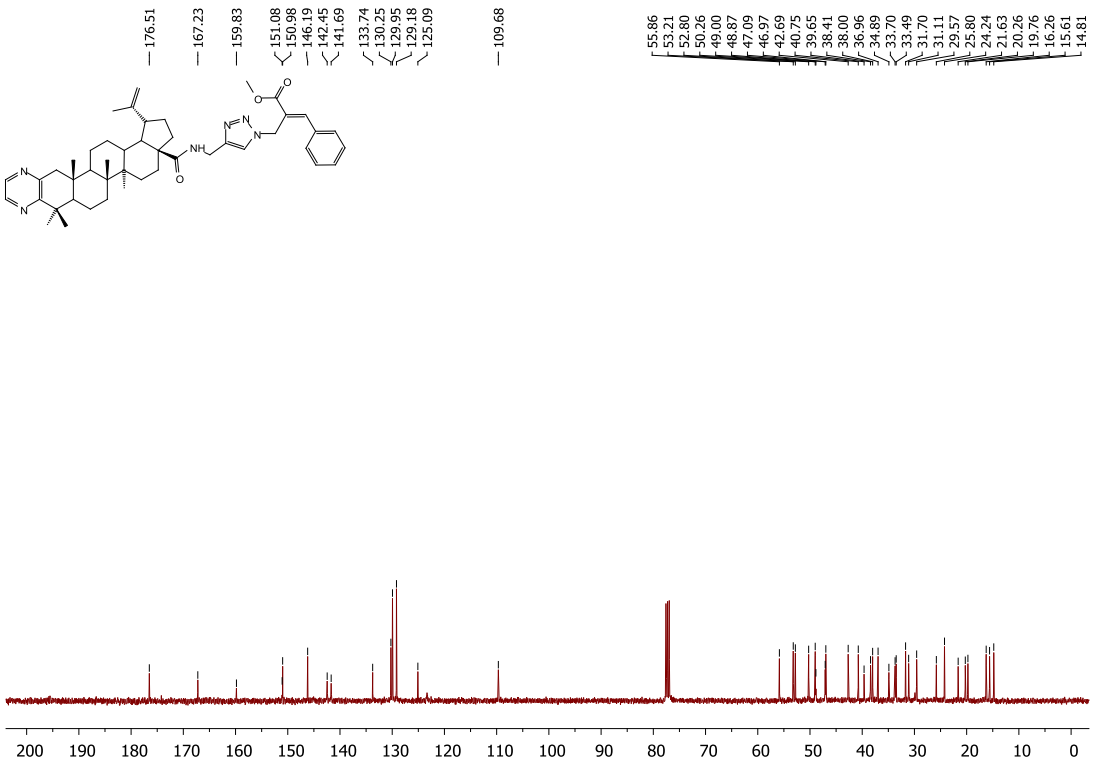


Figure 25. 101 MHz ^{13}C NMR of Compound **46a** in $CDCl_3$

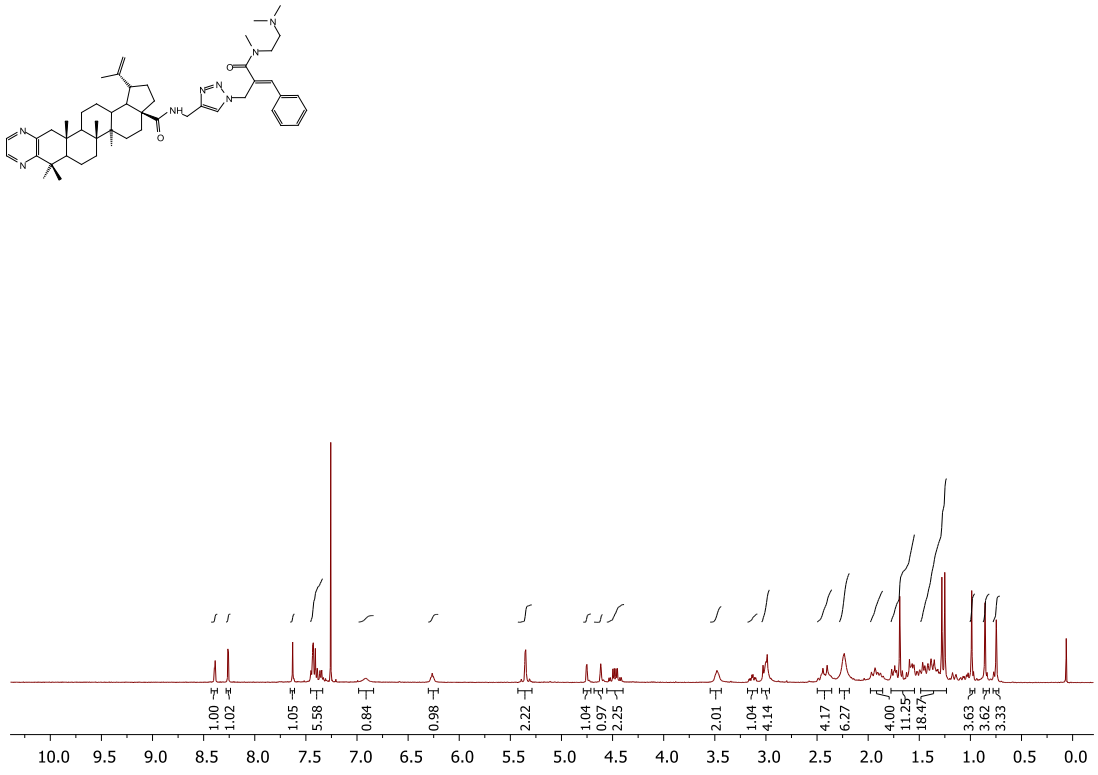


Figure 26. 400 MHz ^{1}H NMR of Compound **46b** in $CDCl_3$

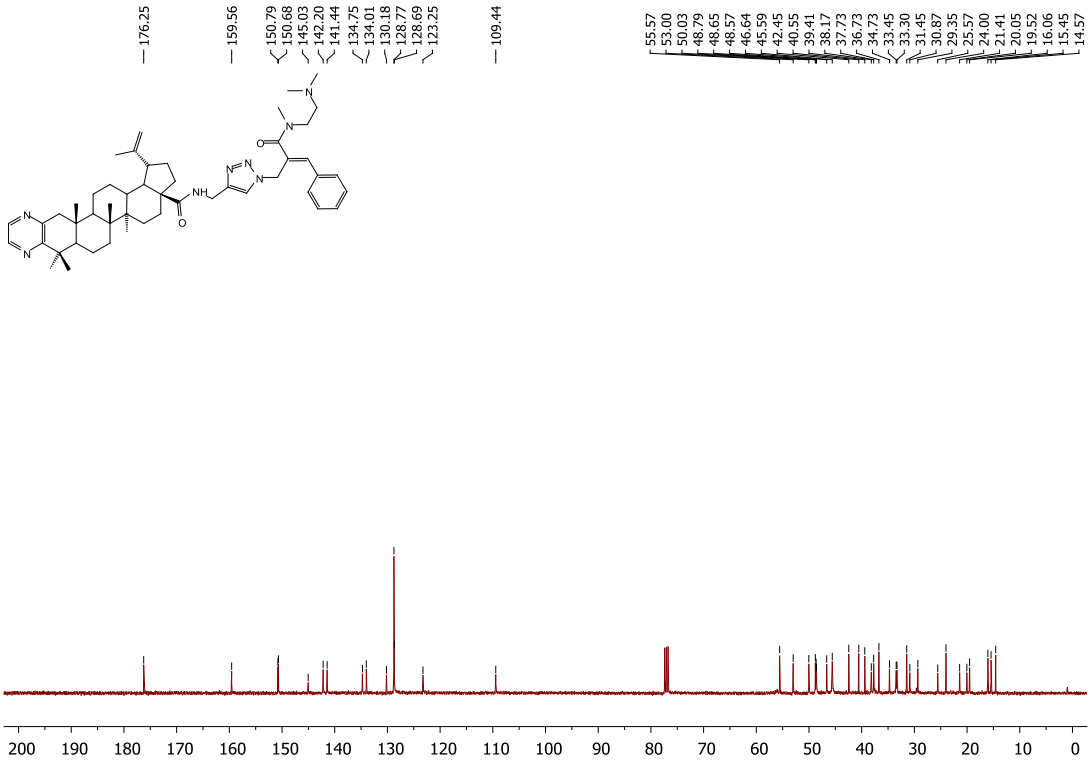


Figure 27. 101 MHz ^{13}C NMR of Compound **46b** in $CDCl_3$

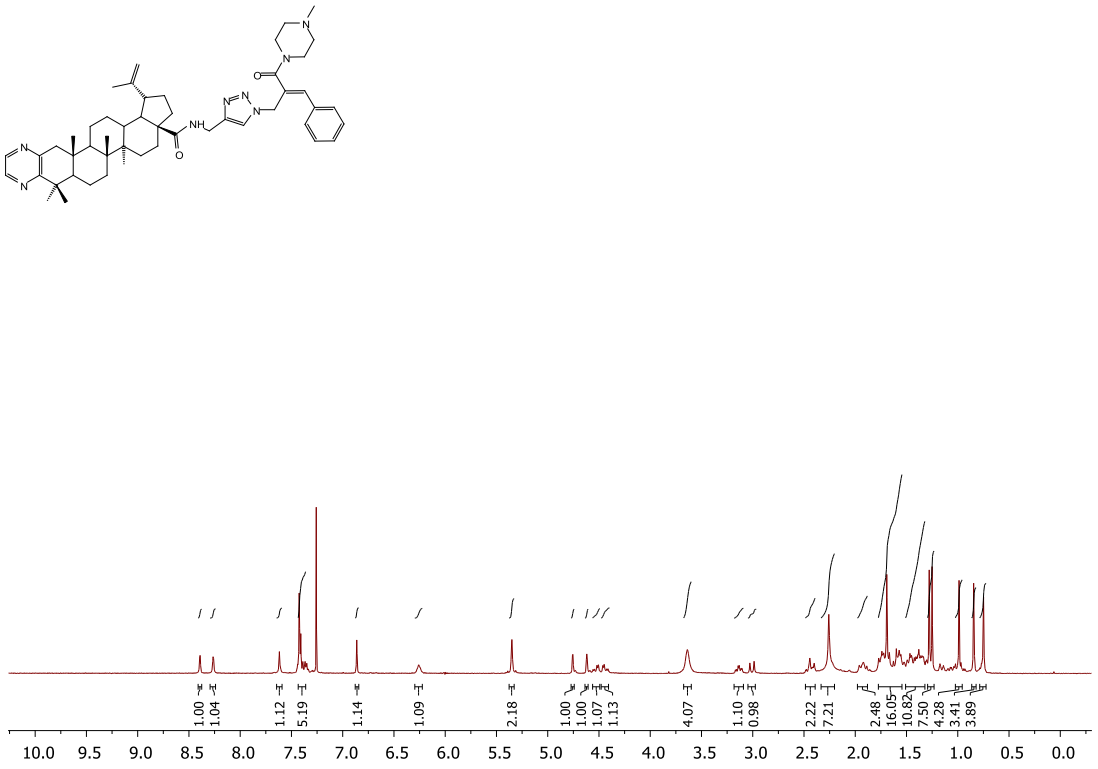


Figure 28. 400 MHz ^{1}H NMR of Compound **46c** in $CDCl_3$

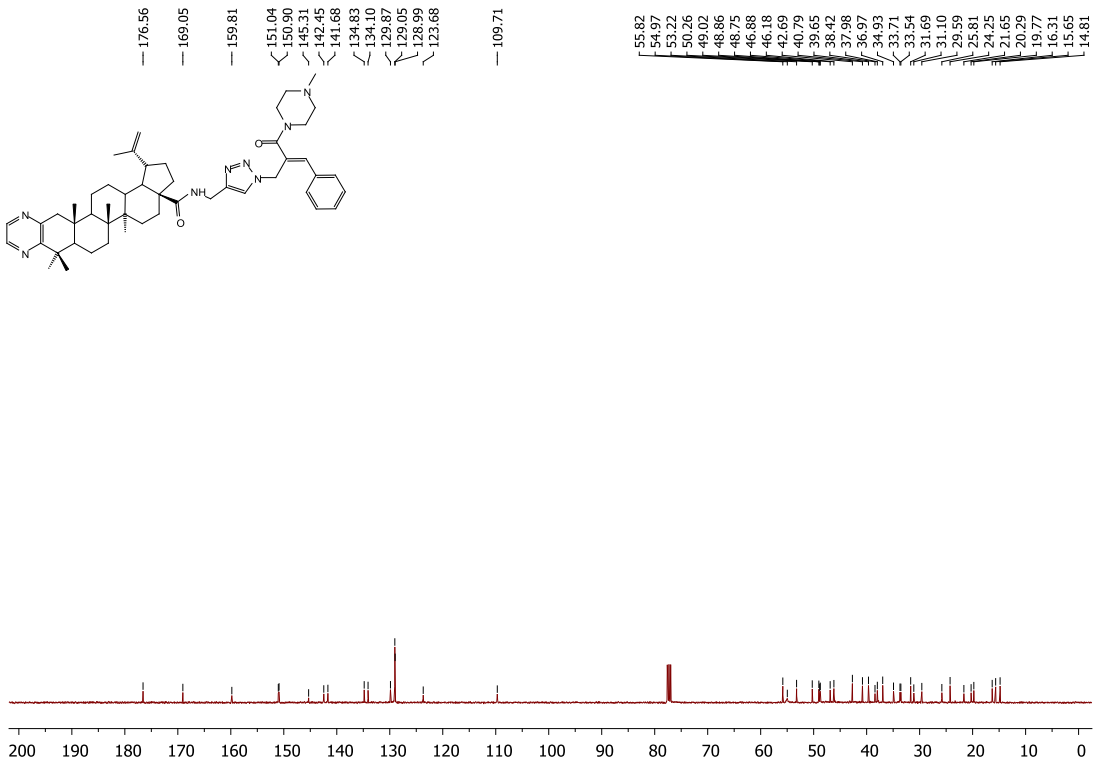


Figure 29. 101 MHz ^{13}C NMR of Compound **46c** in $CDCl_3$

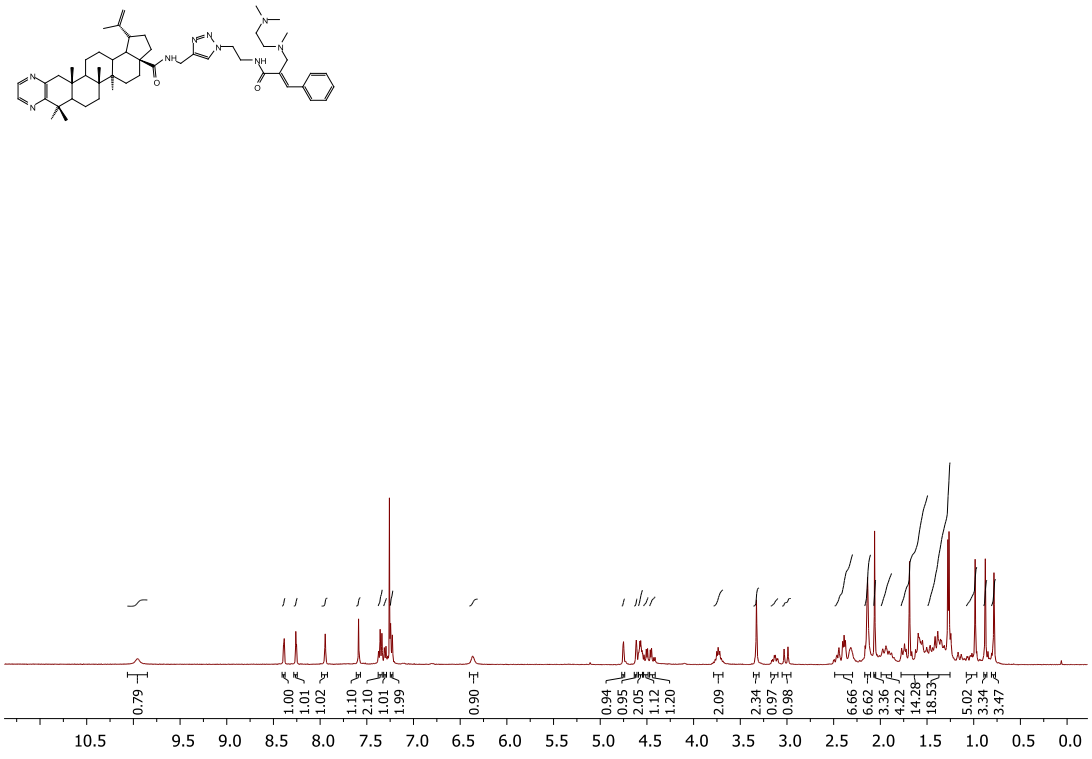


Figure 30. 400 MHz ^{1}H NMR of Compound **46d** in $CDCl_3$

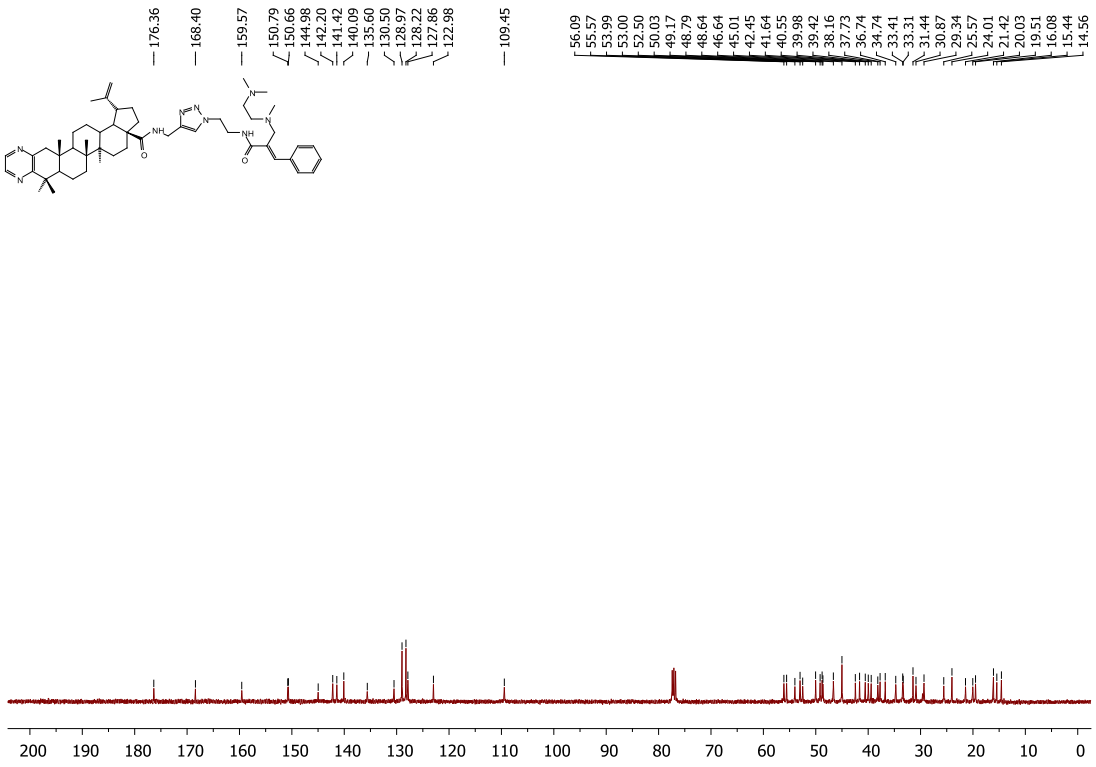


Figure 31. 101 MHz ^{13}C NMR of Compound **46d** in $CDCl_3$

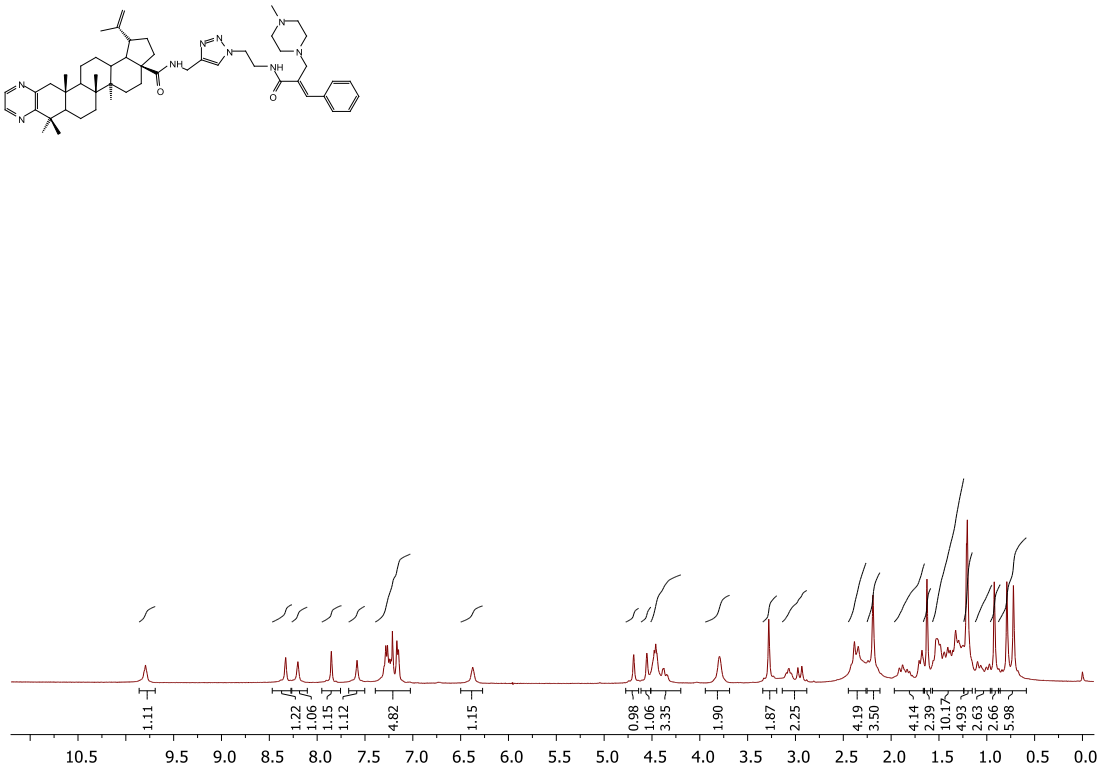


Figure 32. 400 MHz ^{1}H NMR of Compound **46e** in $CDCl_3$

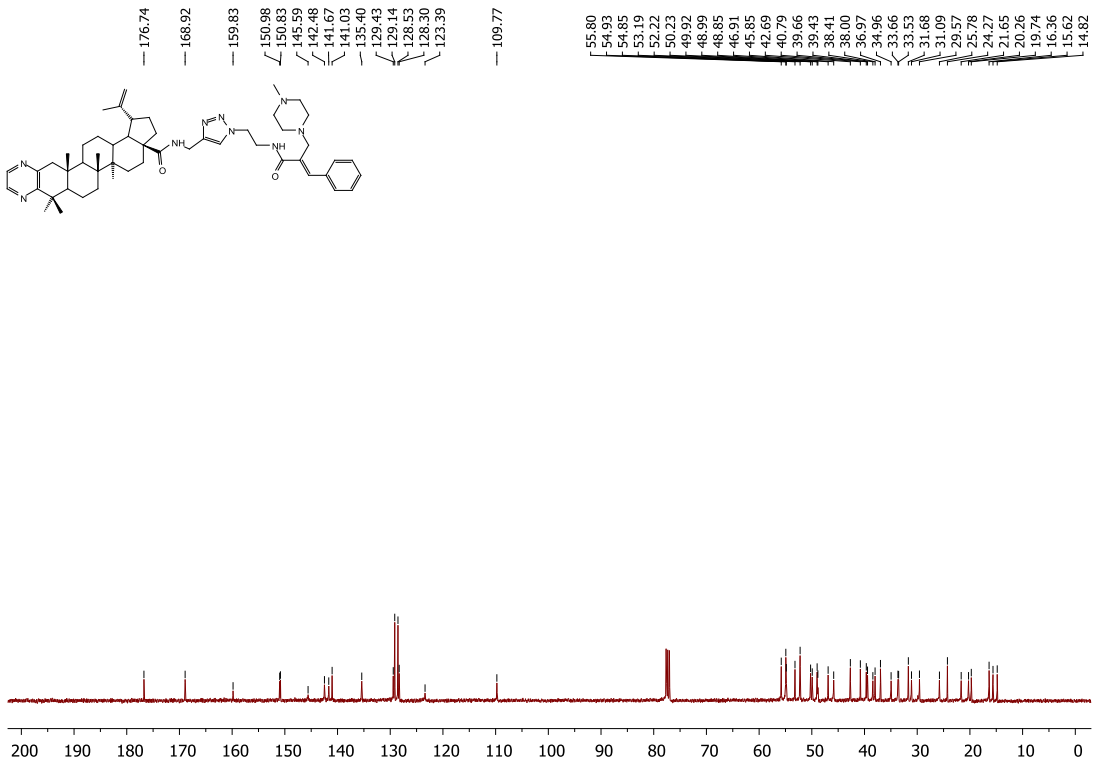
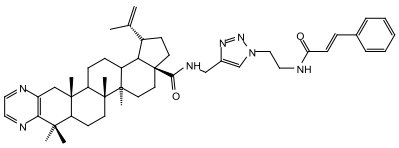


Figure 33. 101 MHz ^{13}C NMR of Compound **46e** in $CDCl_3$



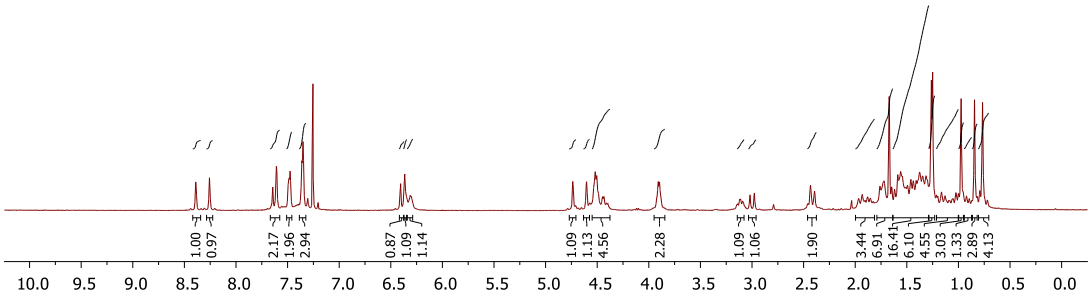


Figure 34. 400 MHz ^{1}H NMR of Compound **46f** in $CDCl_3$

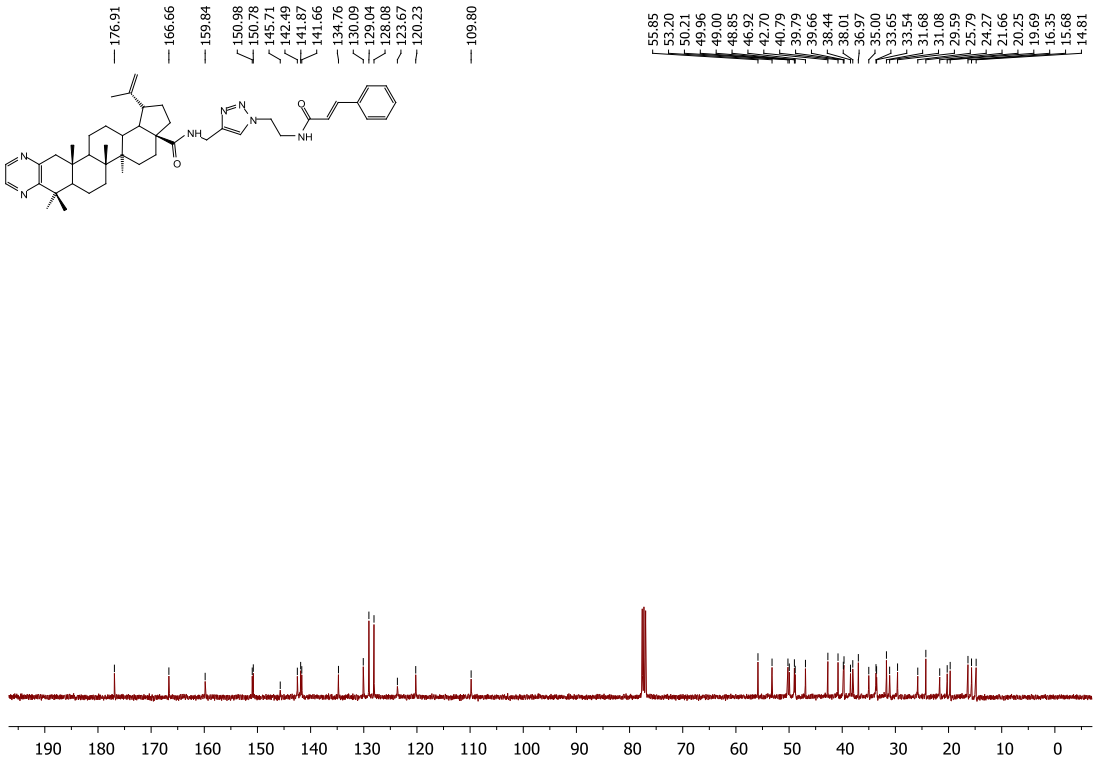
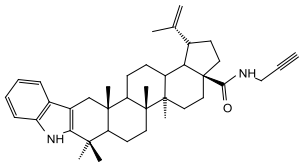


Figure 35. 101 MHz ^{13}C NMR of Compound **46f** in $CDCl_3$



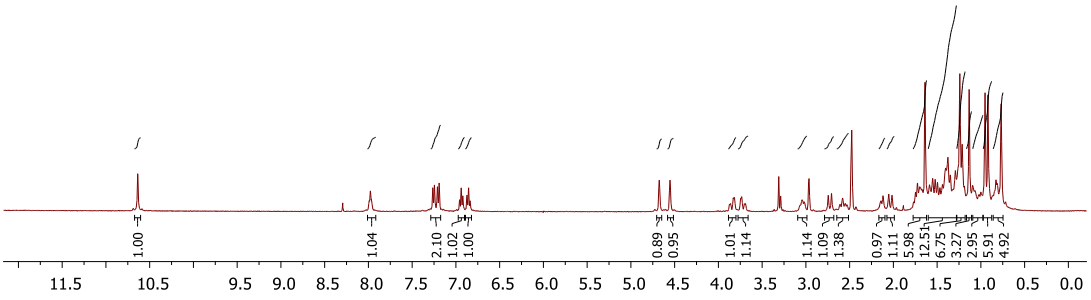


Figure 36. 400 MHz ^{1}H NMR of Compound **48** in DMSO-d_6

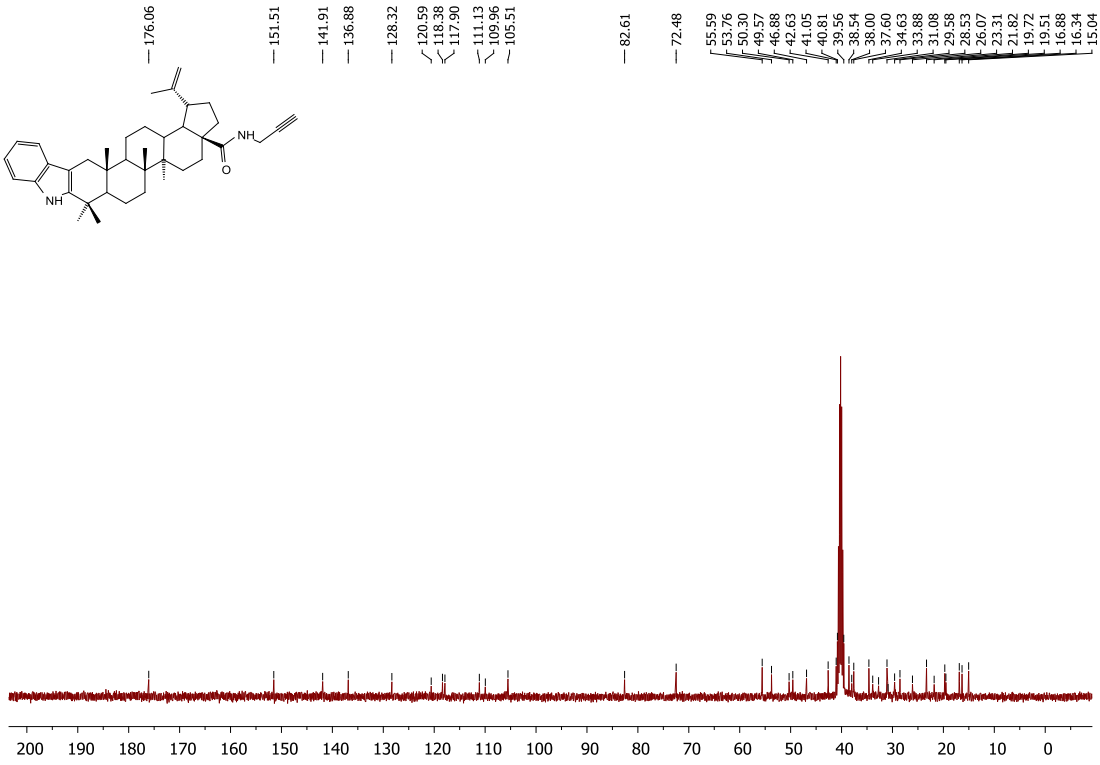
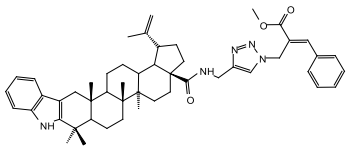


Figure 37. 101 MHz ^{13}C NMR of Compound **48** in DMSO-d_6



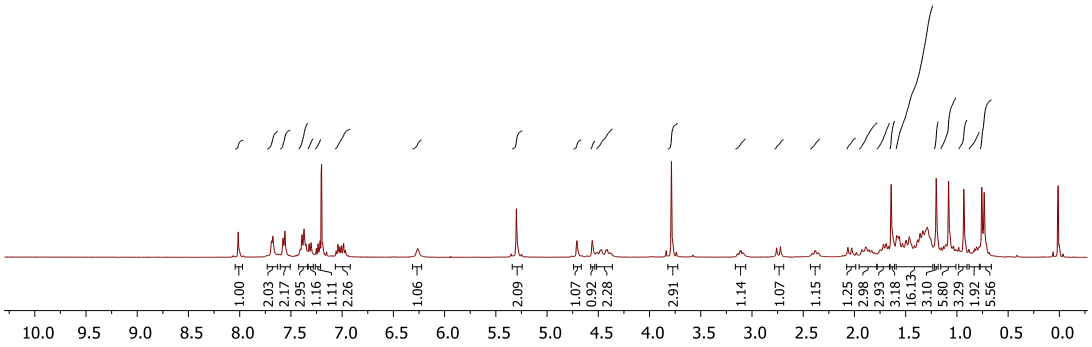


Figure 38. 400 MHz ^{1}H NMR of Compound **49a** in $CDCl_3$

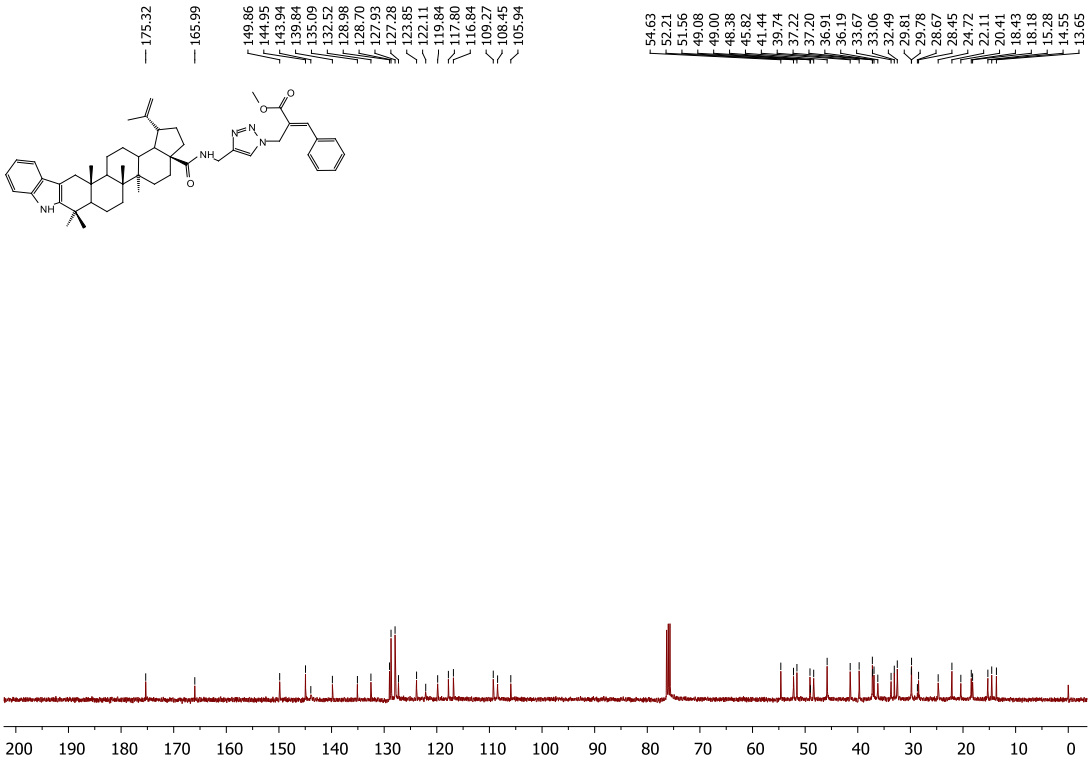


Figure 39. 101 MHz ^{13}C NMR of Compound **49a** in $CDCl_3$

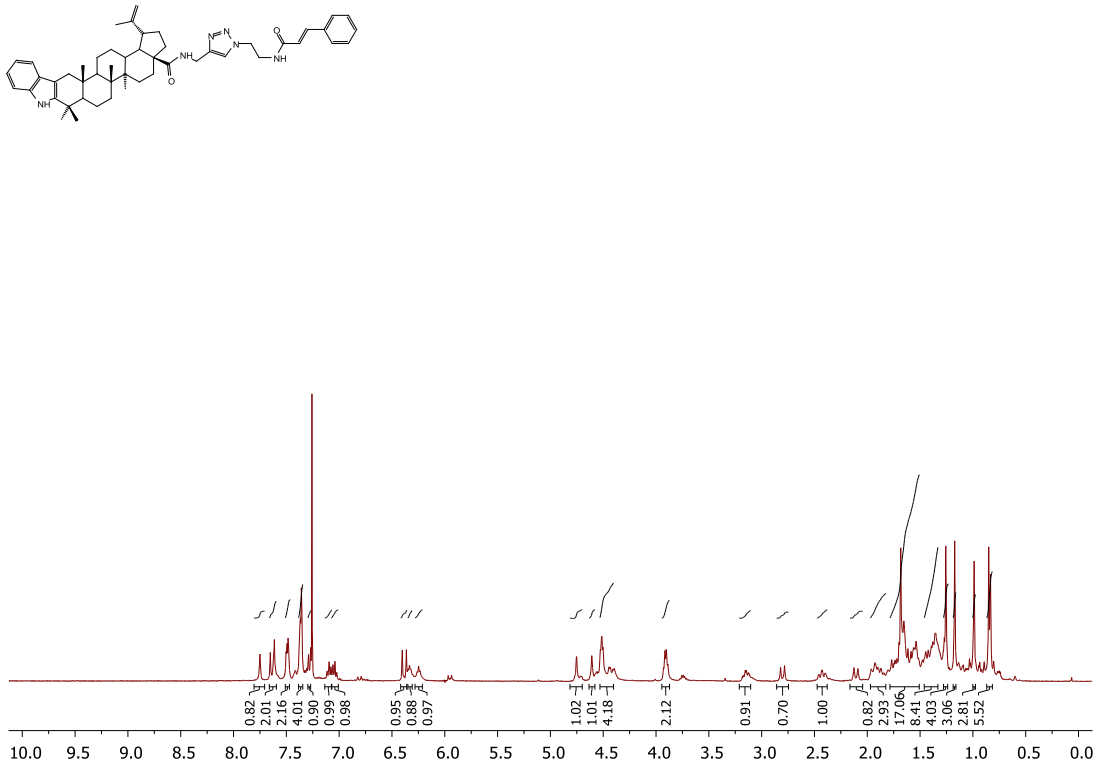


Figure 40. 400 MHz ^{1}H NMR of Compound **49f** in $CDCl_3$

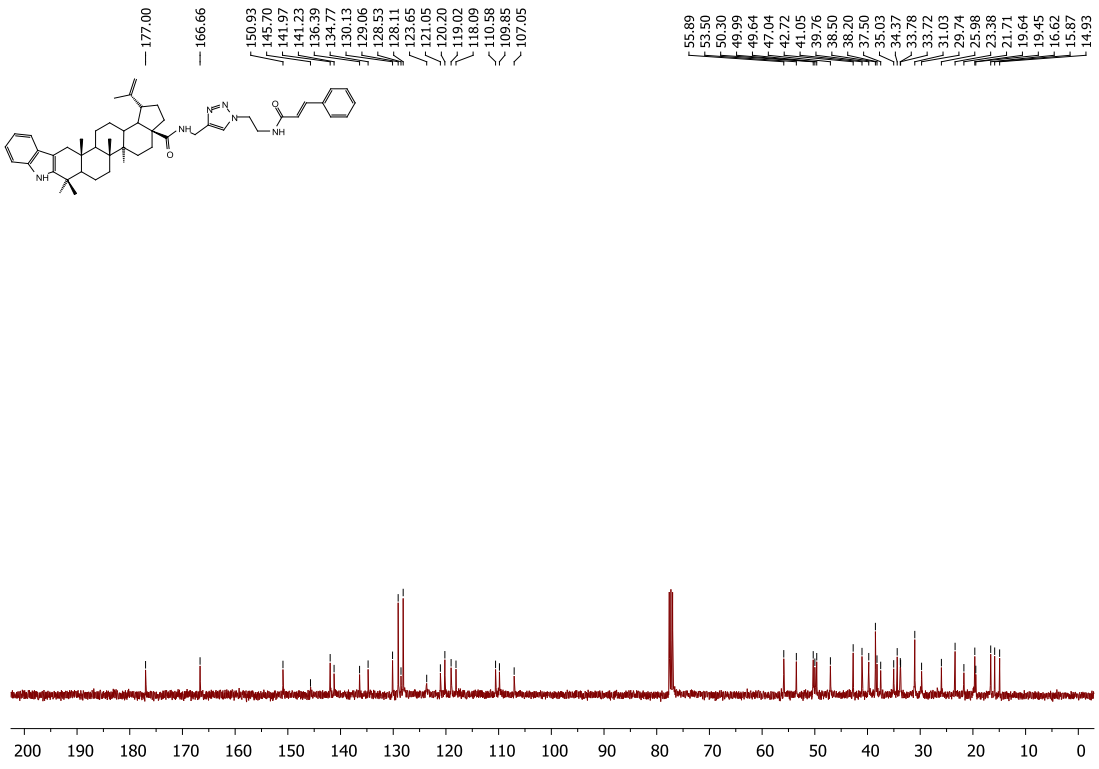
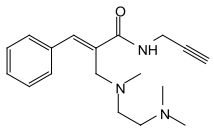


Figure 41. 101 MHz ^{13}C NMR of Compound **49f** in $CDCl_3$



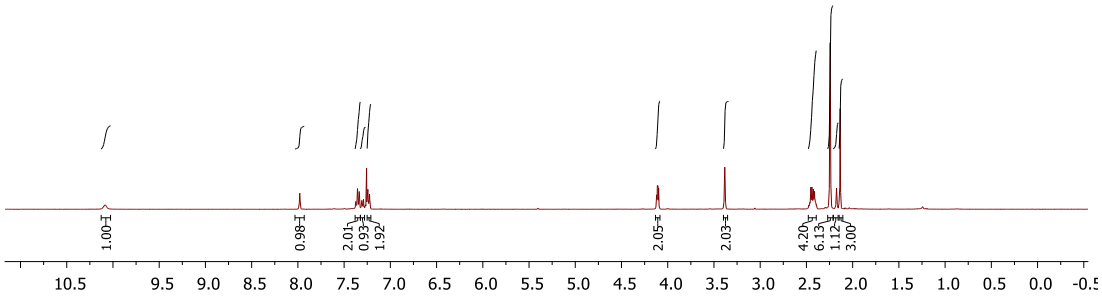


Figure 42. 400 MHz ^{1}H NMR of Compound **50a** in $CDCl_3$

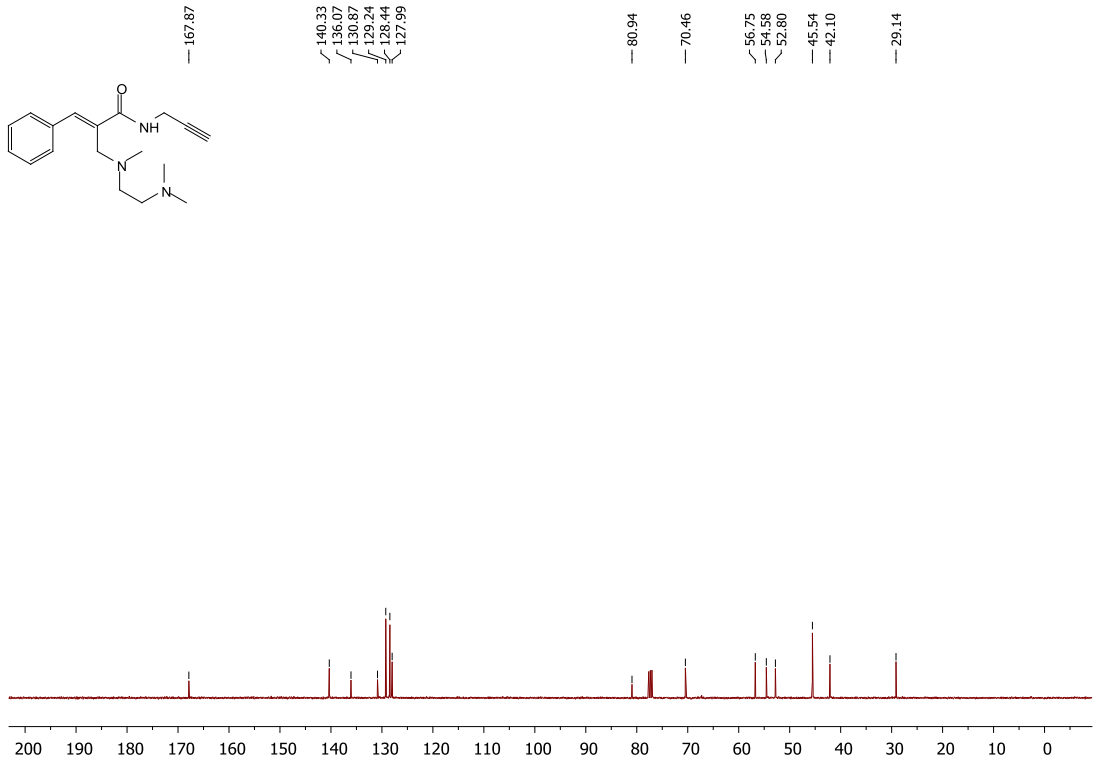


Figure 43. 101 MHz ^{13}C NMR of Compound **50a** in $CDCl_3$

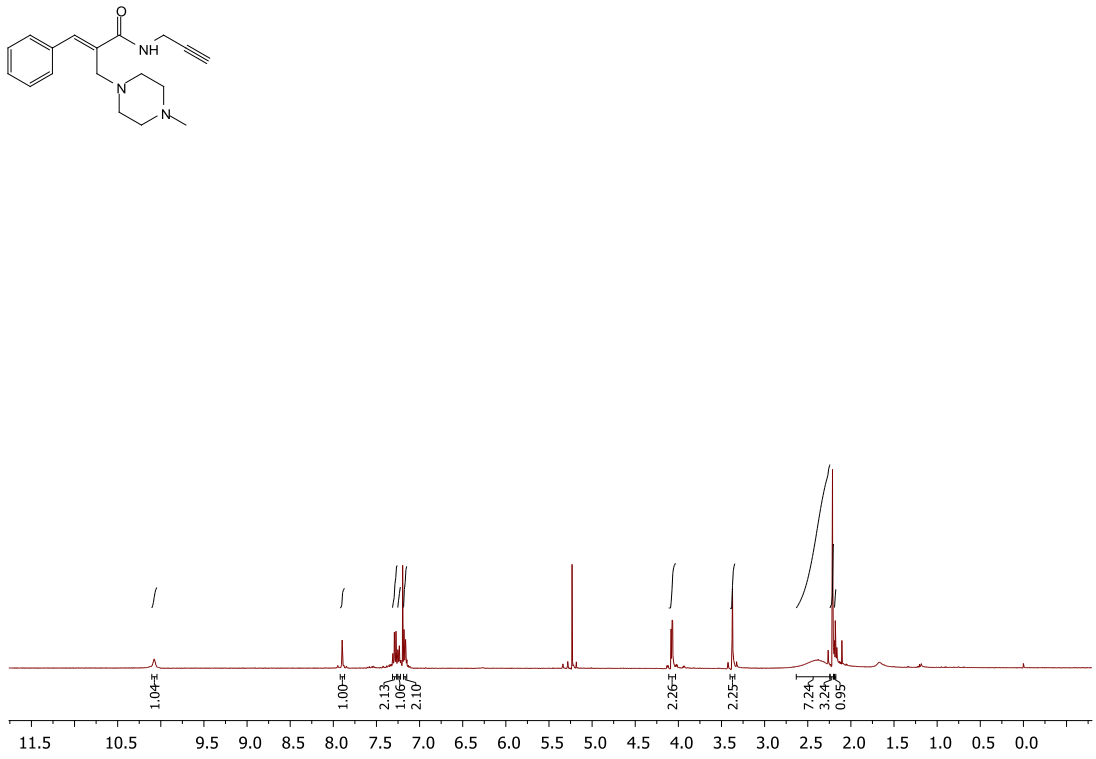


Figure 44. 400 MHz ^{1}H NMR of Compound **50b** in $CDCl_3$

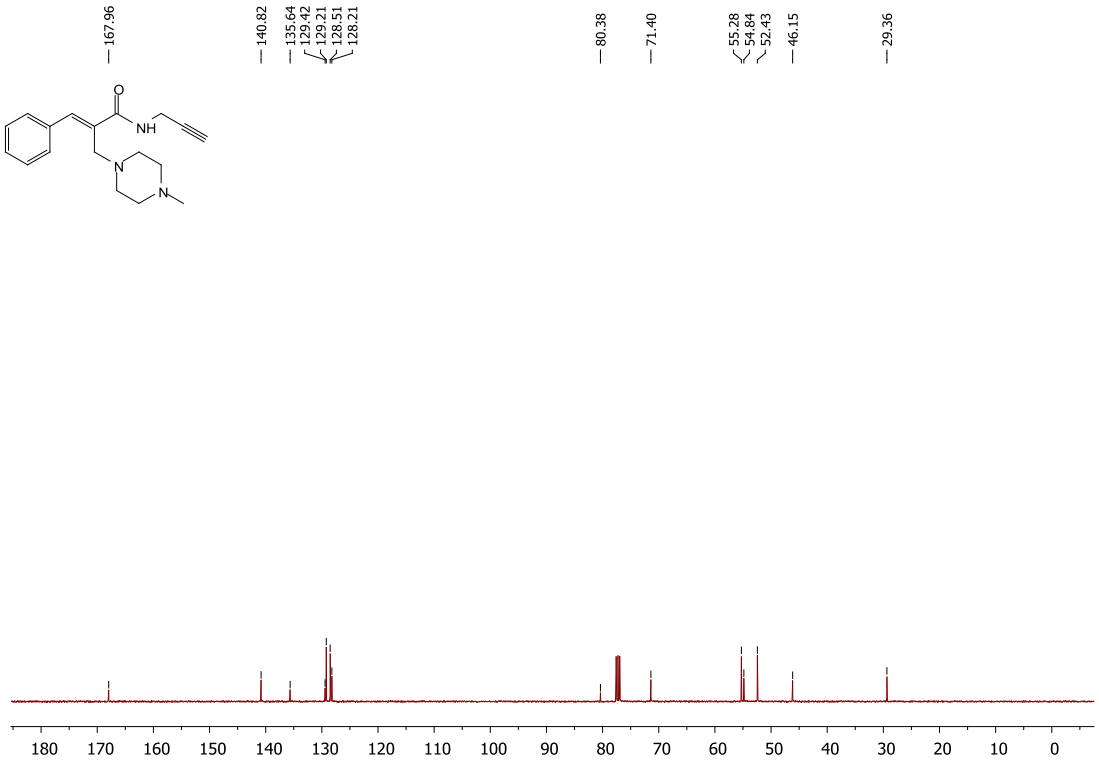
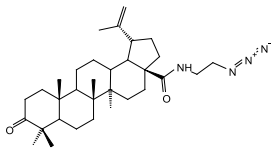


Figure 45. 101 MHz ^{13}C NMR of Compound **50b** in $CDCl_3$



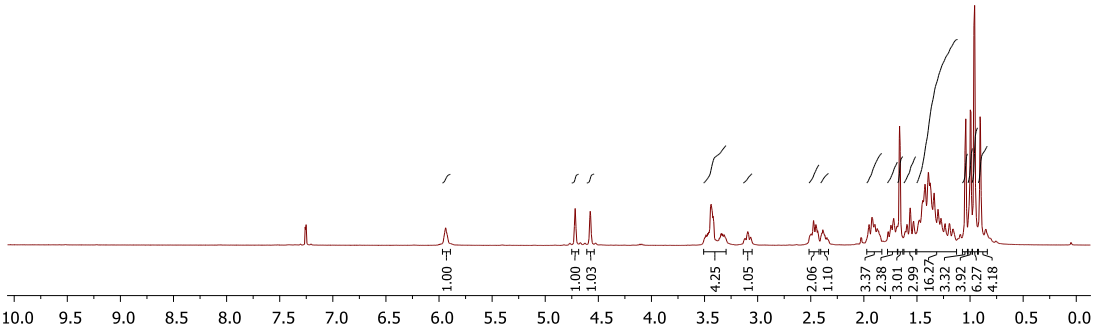


Figure 46. 400 MHz ^{1}H NMR of Compound **51** in $CDCl_3$

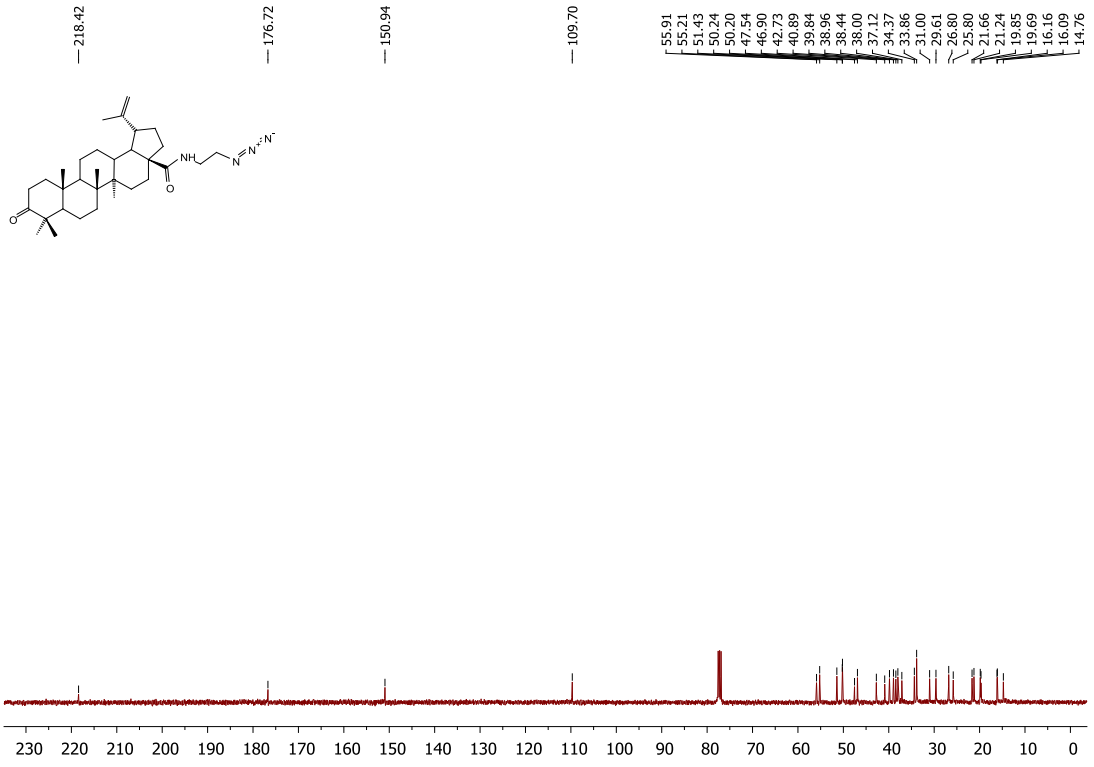


Figure 47. 101 MHz ^{13}C NMR of Compound **51** in $CDCl_3$

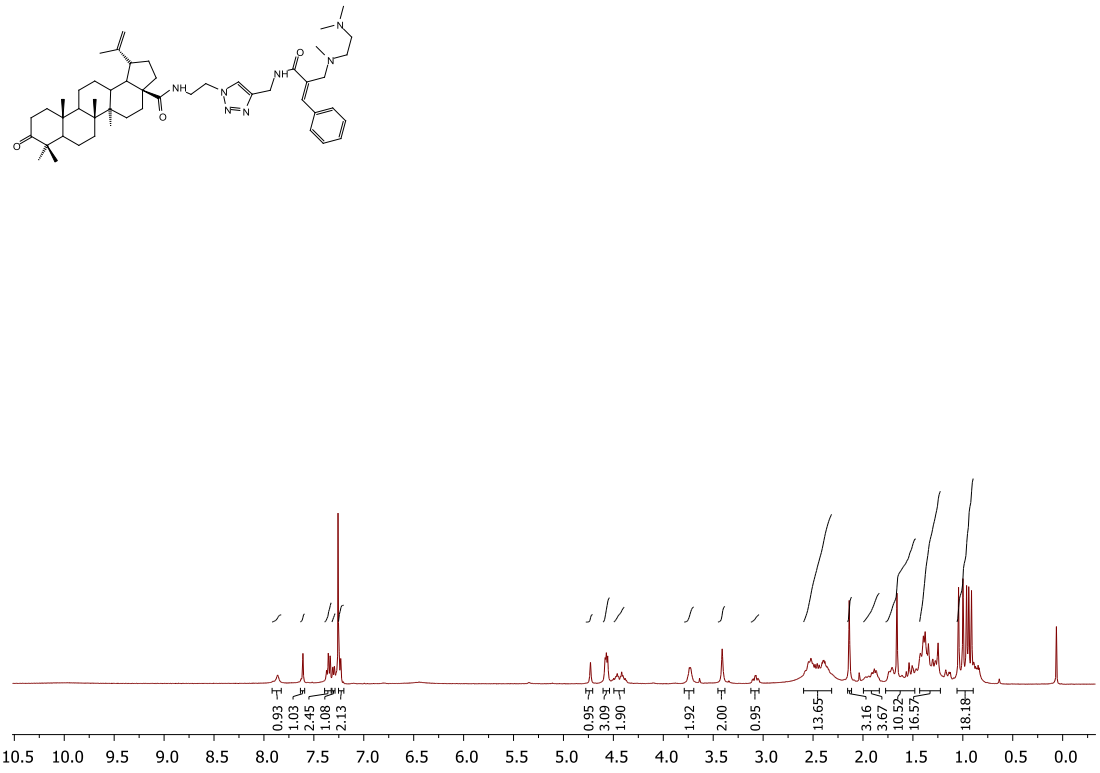


Figure 48. 400 MHz ^{1}H NMR of Compound **52a** in $CDCl_3$

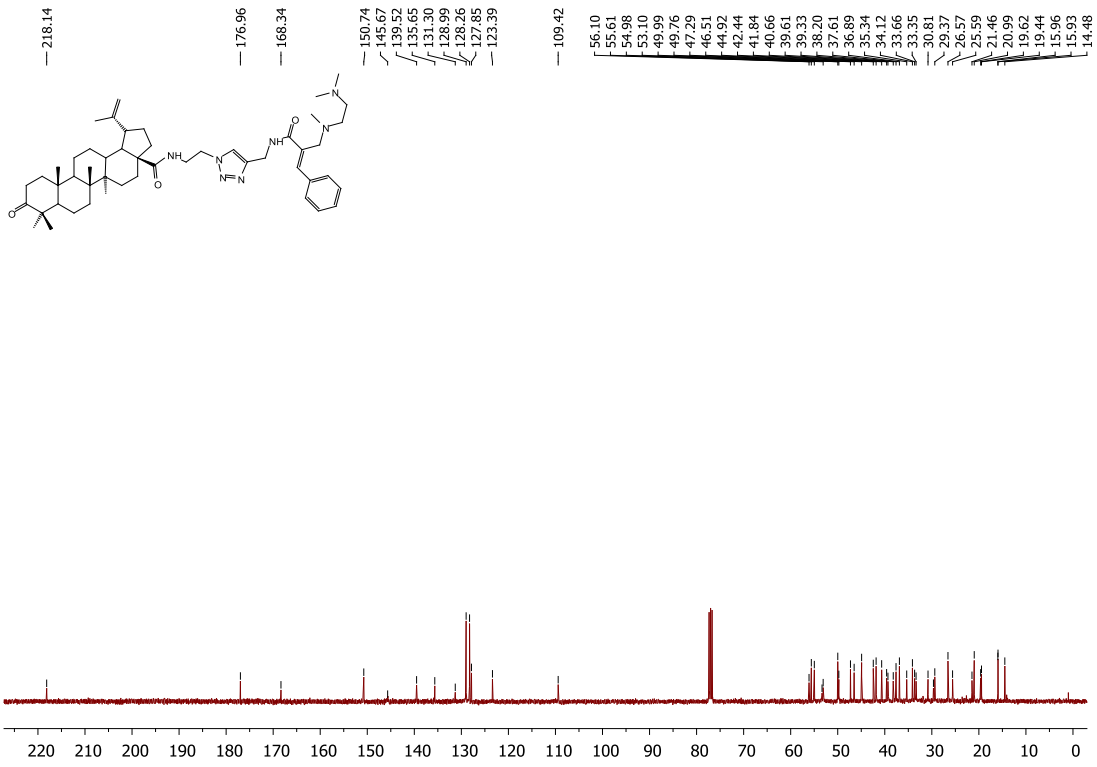


Figure 49. 101 MHz ^{13}C NMR of Compound **52a** in $CDCl_3$

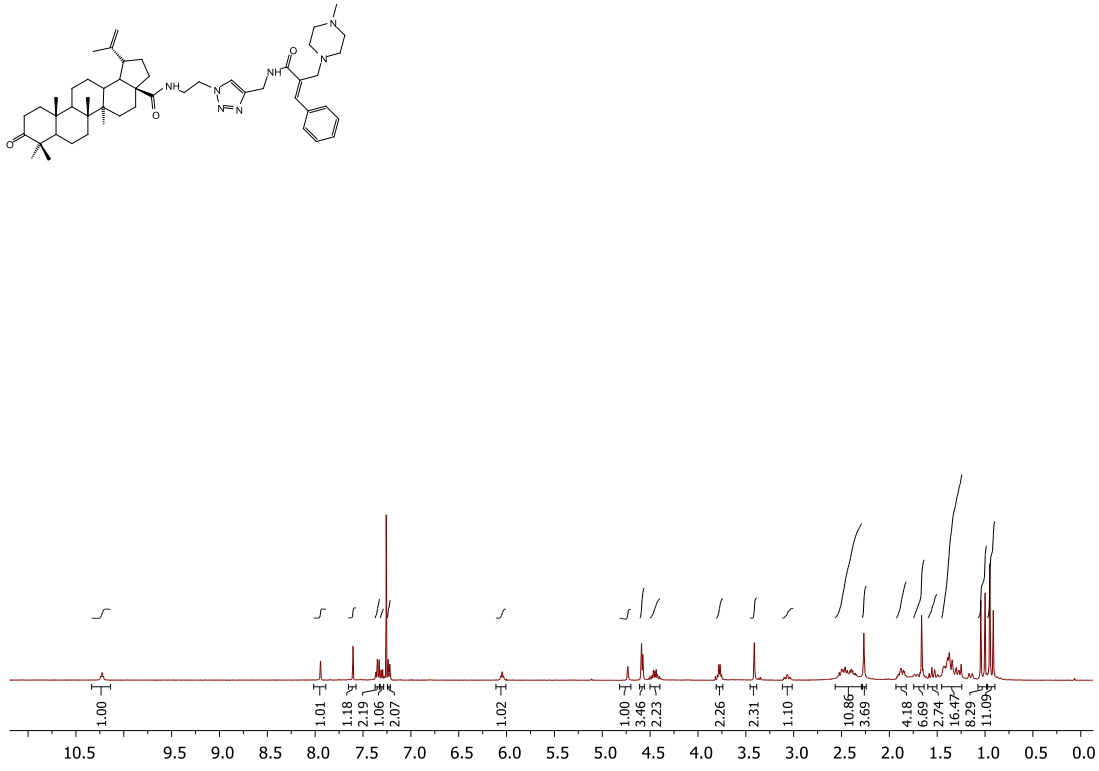


Figure 50. 400 MHz ^{1}H NMR of Compound **52b** in $CDCl_3$

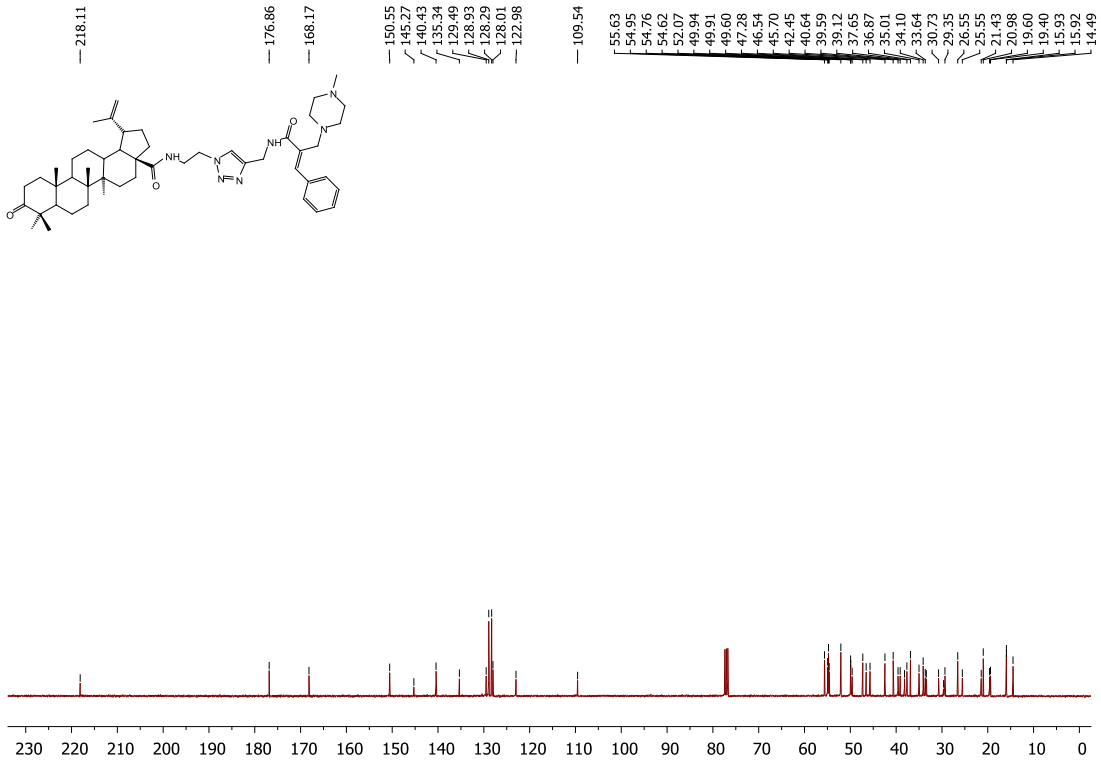
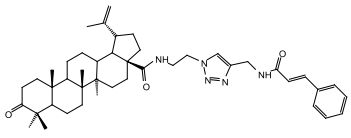


Figure 51. 101 MHz ^{13}C NMR of Compound **52b** in $CDCl_3$



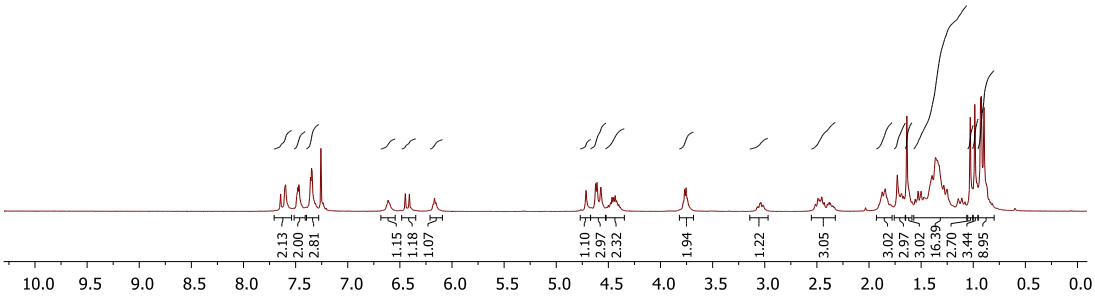


Figure 52. 400 MHz ^{1}H NMR of Compound **52c** in $CDCl_3$

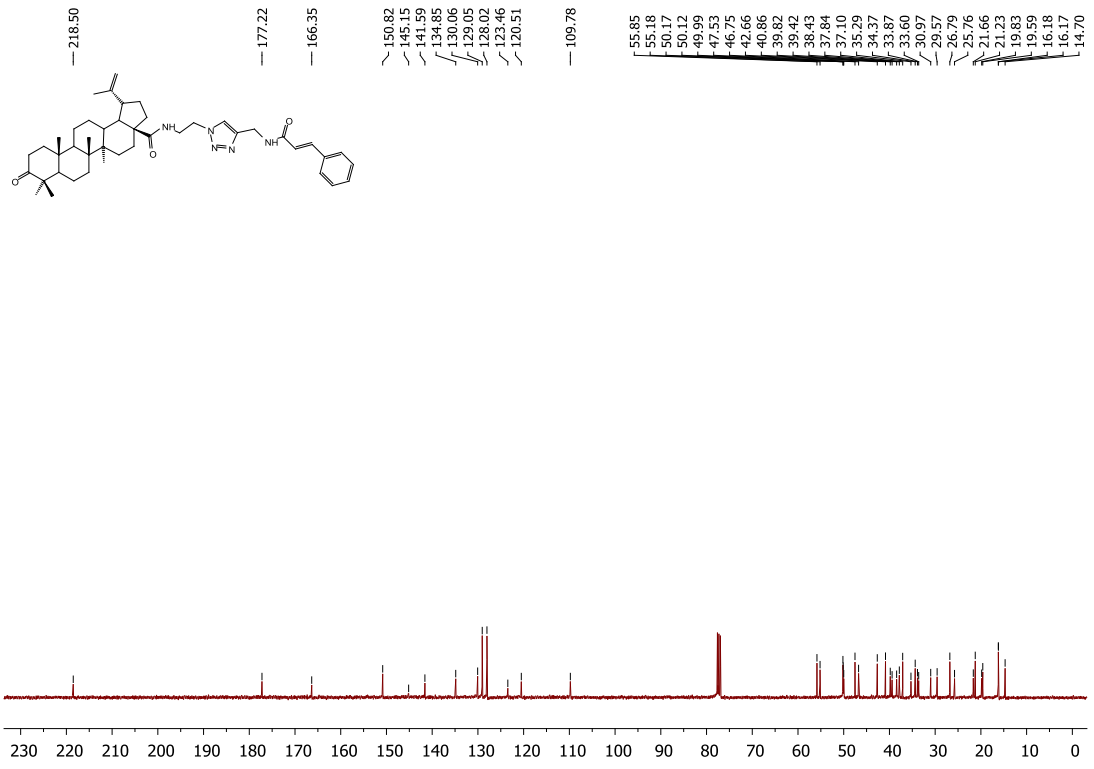


Figure 53. 101 MHz ^{13}C NMR of Compound **52c** in $CDCl_3$

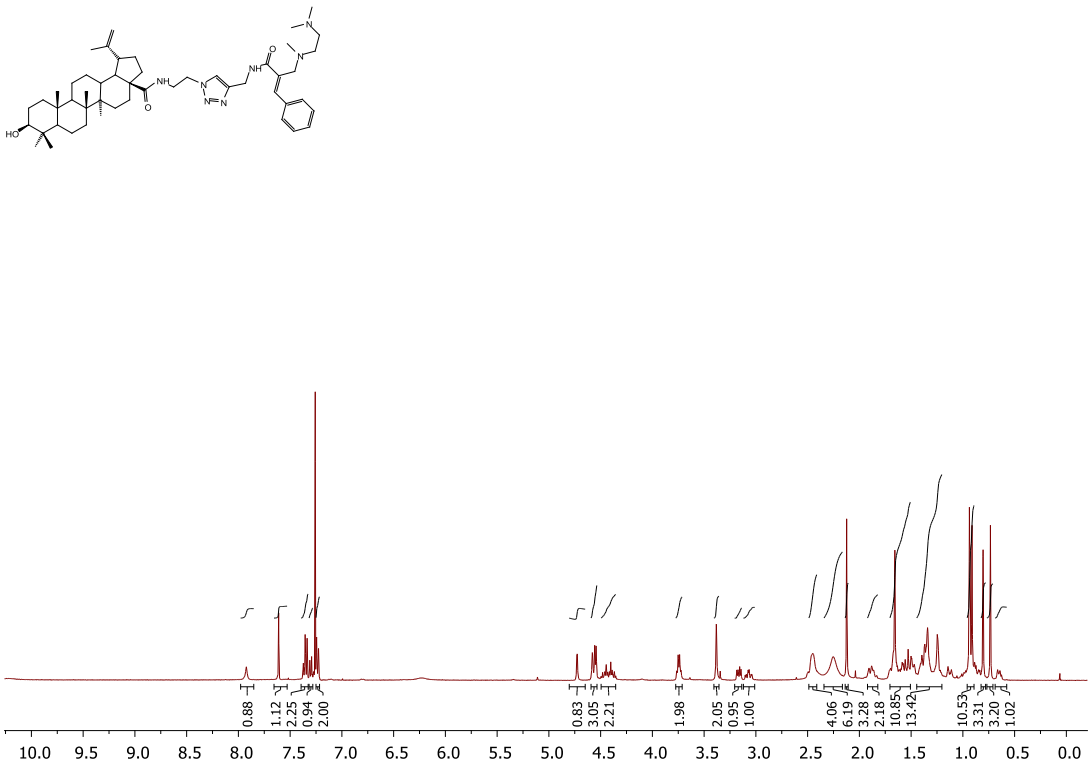


Figure 54. 400 MHz ^{1}H NMR of Compound **53a** in $CDCl_3$

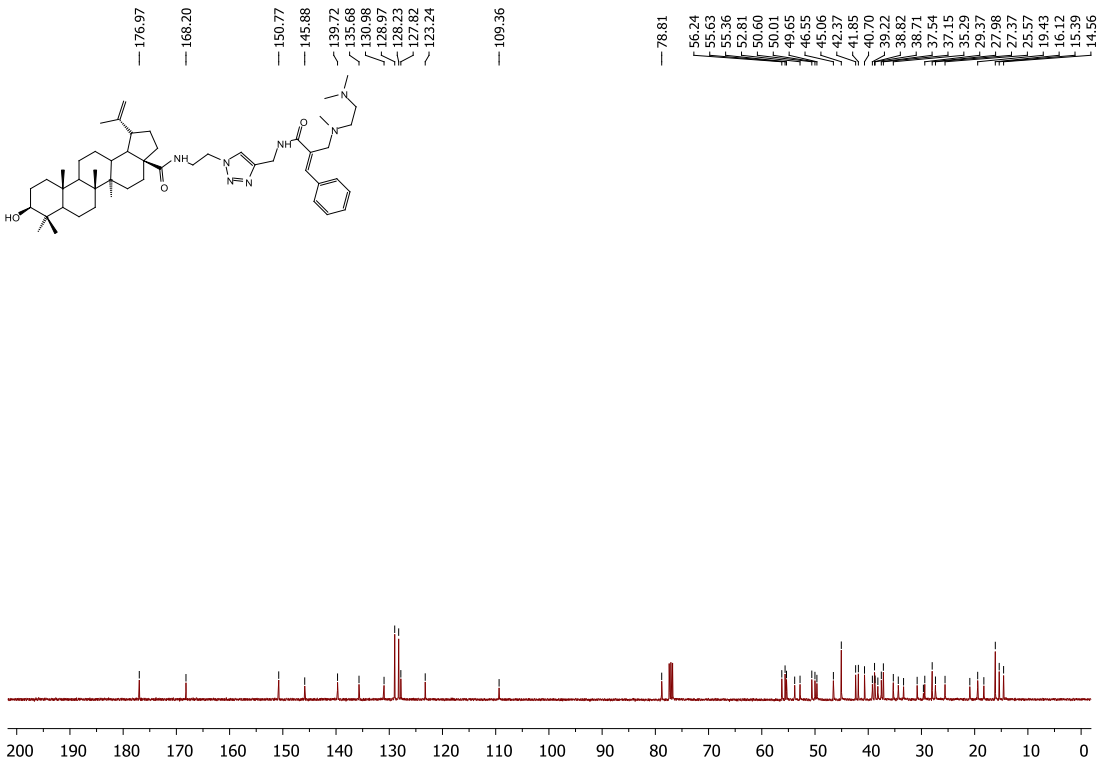


Figure 55. 101 MHz ^{13}C NMR of Compound **53a** in $CDCl_3$

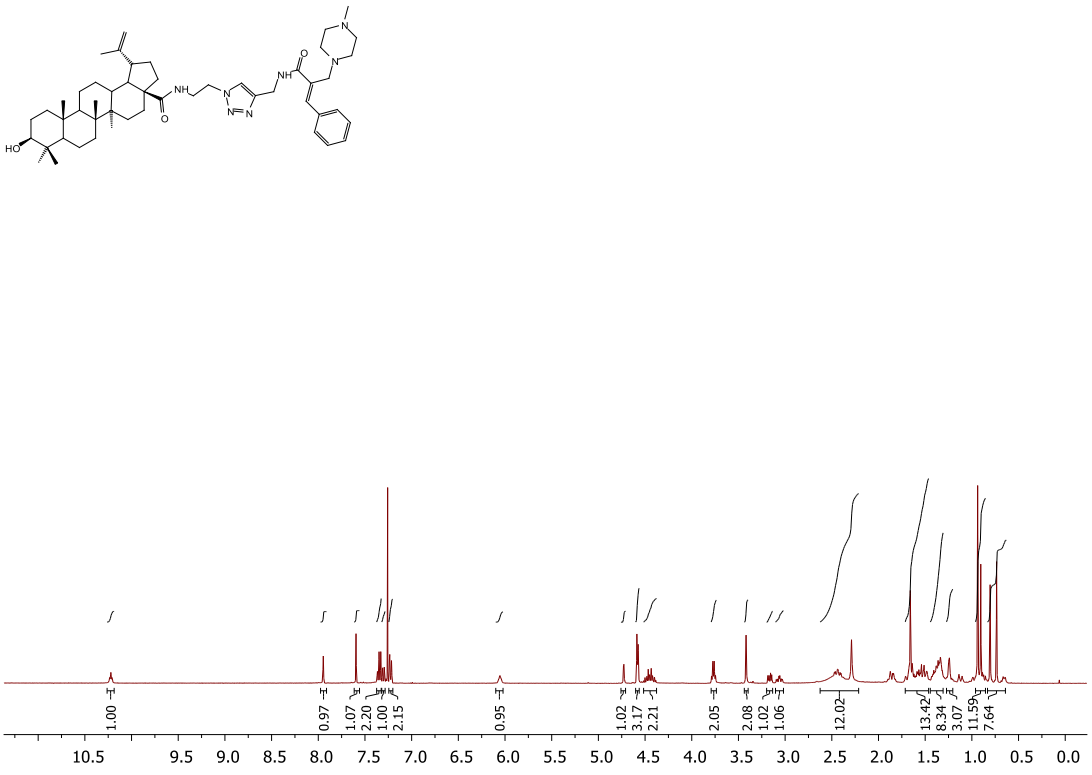


Figure 56. 400 MHz ^{1}H NMR of Compound **53b** in $CDCl_3$

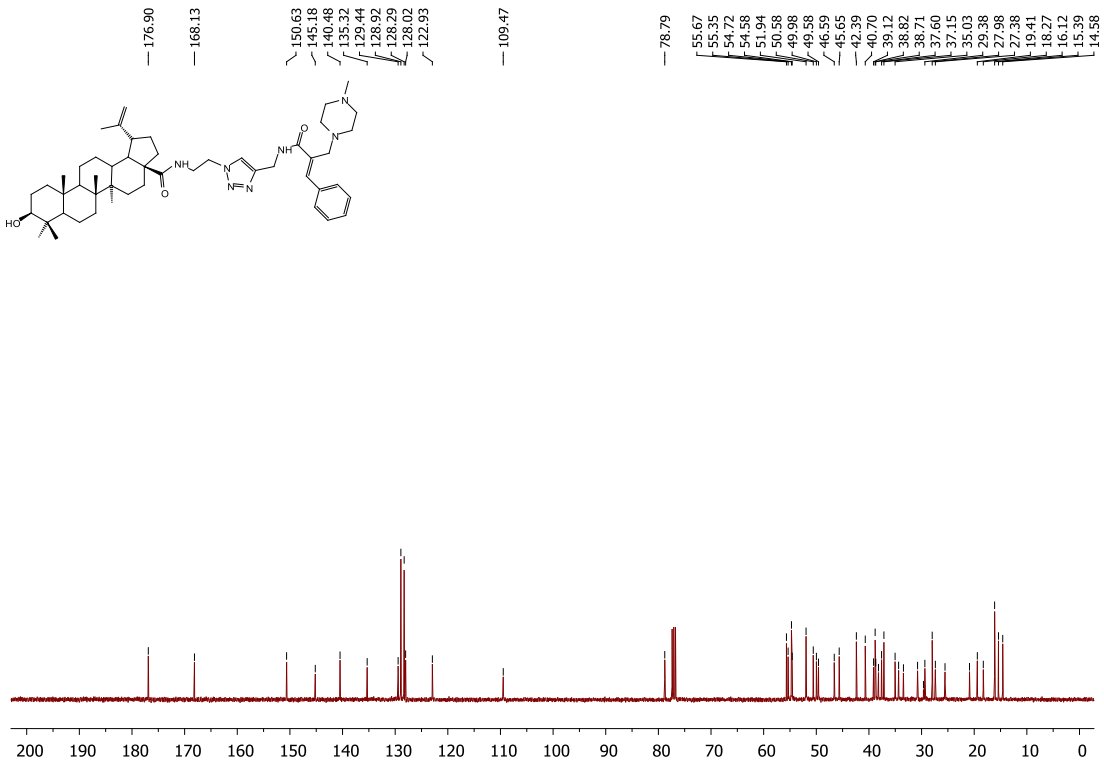


Figure 57. 101 MHz ^{13}C NMR of Compound **53b** in $CDCl_3$

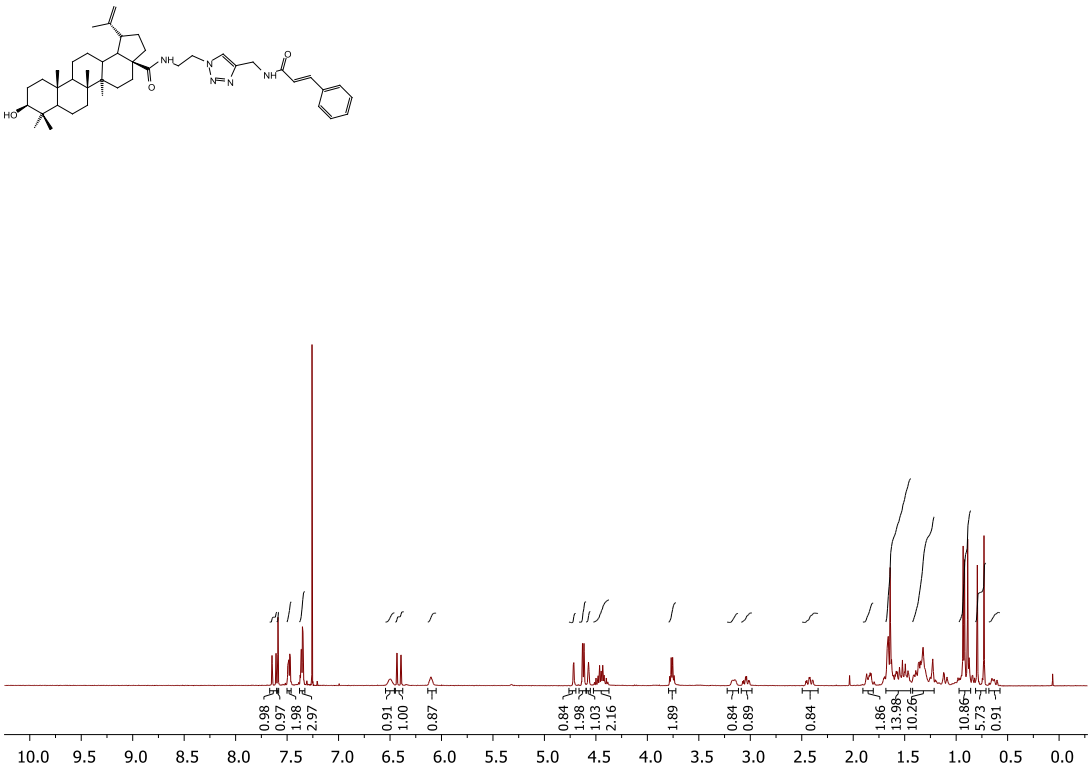


Figure 58. 400 MHz ^{1}H NMR of Compound **53c** in $CDCl_3$

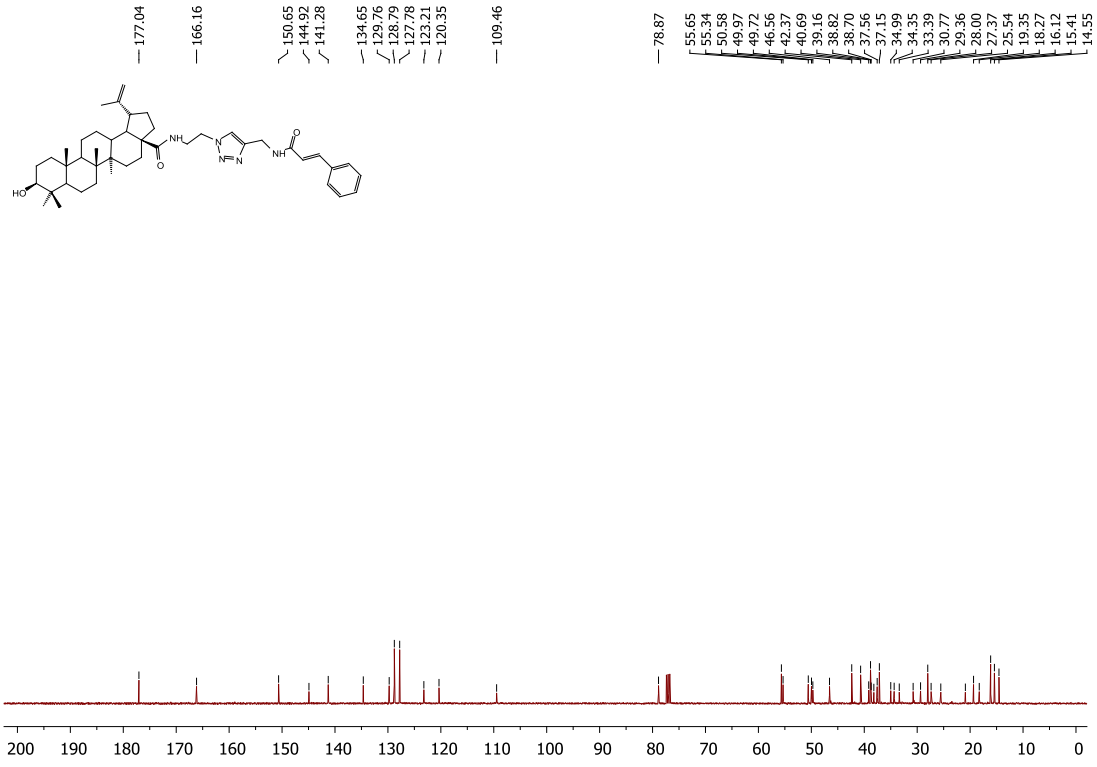


Figure 59. 101 MHz ^{13}C NMR of Compound **53c** in $CDCl_3$

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