Design and Synthesis of Enediyne-based Peptide with Selective Peptide-cleaving Activity

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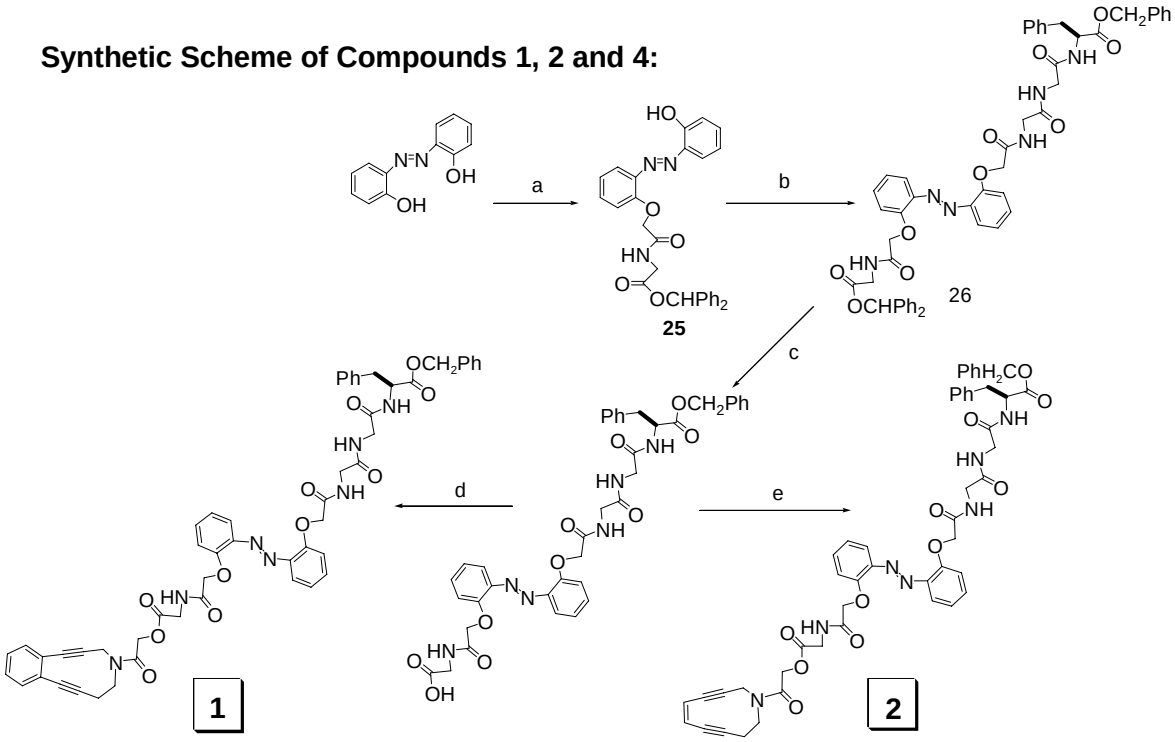
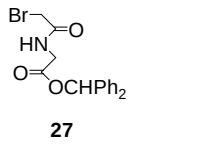
1. General Remarks:

All the reactions were monitored by TLC using polygramR SILG/UV254 precoated (0.25mm) silica gel TLC plates. Column chromatography was done with silica gel (60-120 or 230-400 mesh). NMR data were obtained with 200 MHz and 400 MHz Bruker NMR instruments. Proton and carbon spectra were referenced internally to solvent signals, using values of δ = 2.49 ppm for proton (middle peak) and δ = 39.5 ppm for carbon (middle peak) in d_6-DMSO and δ = 7.26 ppm for proton and δ = 77.0 for carbon (middle peak) in $CDCl_3$ and δ = 1.94 ppm for proton in CD_3CN and δ = 3.35, 4.78 ppm for proton and δ = 49.3 ppm for carbon (middle peak) in d_4-MeOH. The following abbreviations are used to describe peak patterns where appropriate: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app. = apparently and b = broad signal. All coupling constants (*J*) are given in Hz. FT-IR spectra were obtained as KBr discs. Mass spectra were recorded in ESI+ mode (70 eV). Melting points were determined in open capillary tubes and are uncorrected. MALDI-TOF-MS spectra were measured using α-Cyano-4-hydroxycinnamic acid as a matrix and a nitrogen laser (337 nm) on a PE Biosystems Voyager-DE PRO.

Synthetic Scheme of Compounds 1, 2 and 4:

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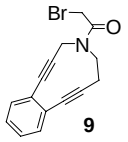
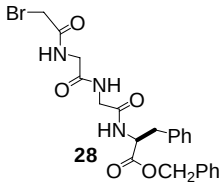
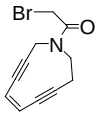
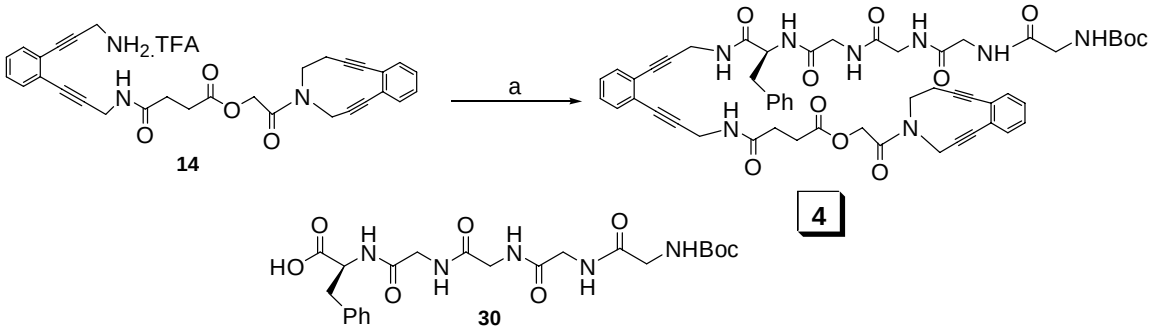

9


28


29

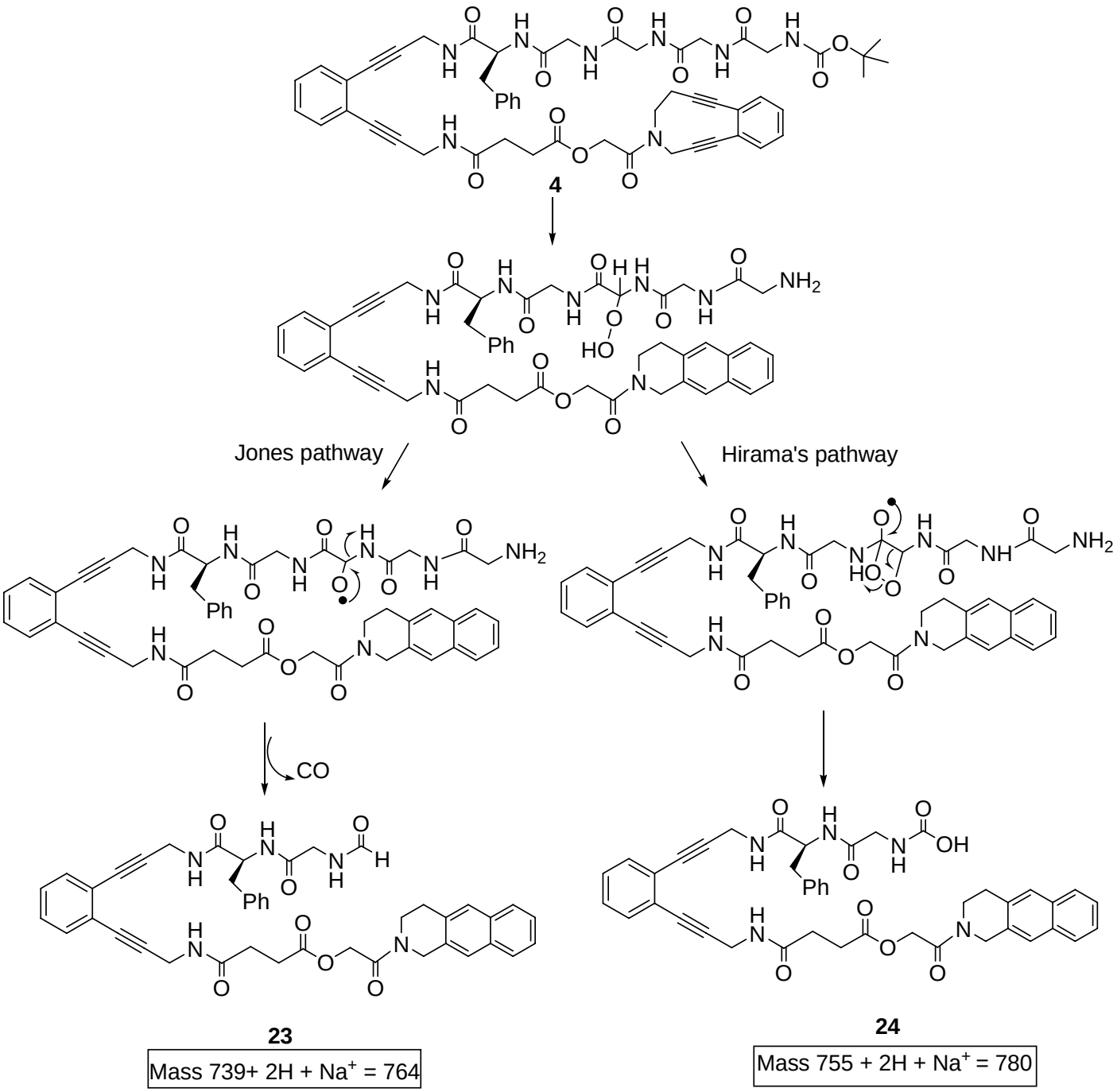
a) **27**, Cs_2CO_3, CH_3CN, r.t., 8 h, 56%; b) **28**, Cs_2CO_3, CH_3CN, r.t., 8 h, 60%; c) TFA, Anisole, DCM, 0 ^{O}C to r.t., 3 h; d) i) $NaHCO_3$, moist MeOH, r.t., 4 h; ii) **9**, DMF, r.t., 8 h, 58%; e) i) $NaHCO_3$, moist MeOH, r.t., 4 h; ii) **29,** DMF, r.t., 8 h, 36%.



30

a) **30**, EDC.HCl, DMAP, DCM, 0 ^{O}C to r.t., 12 h, 39%.

Abstraction of α -H from third glycine unit of the pentapeptide chain of compound 4



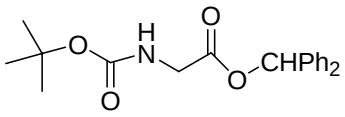
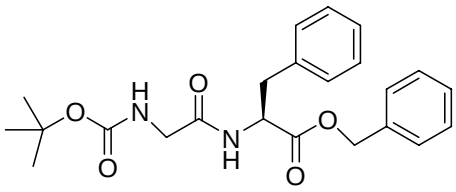
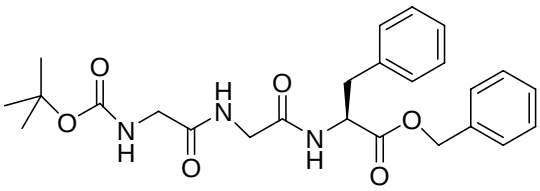
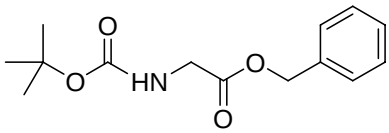
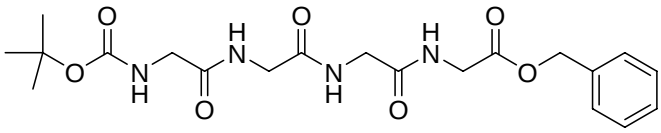
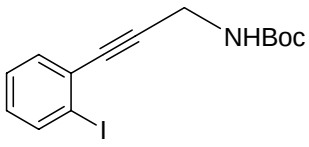
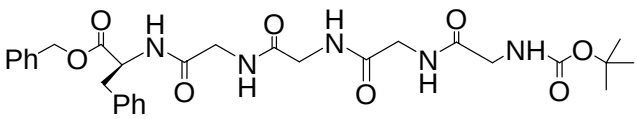
23

Mass 739+ 2H + Na^+ = 764

24

Mass 755 + 2H + Na^+ = 780

List of compounds not shown in the synthetic schemes:

Structures of the compounds	**Compound no.**
	**27a**
	**28a**
	**28b**
	**3a**
	**3b**
	**5a**
	**4a**

Experimental procedure

Synthesis of {2-[2-(2-Hydroxy-phenylazo)-phenoxy]-acetylamino}-acetic acid benzhydryl ester (25)

2, 2'-Dihydroxyazobenzene (154 mg, 0.71 mmol) was dissolved in dry acetonitrile under inert condition. Cesium carbonate (468 mg, 1.43 mmol) and (2-Bromo-acetylamino)-acetic acid benzhydryl ester (**27**) (260 mg, 0.71 mmol) were added in succession and left for 8hr under room temperature. The reaction mixture was then evaporated and the crude mass was taken in ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulphate, concentrated and subjected to column chromatography (Si-gel, 60-120 mesh, petroleum ether : ethyl acetate = 2:1 as eluent).

Synthesis of 2-(2-{2-[2-(2-{2-[(Benzhydryloxycarbonylmethyl-carbamoyl)-methoxy]-phenylazo}-phenoxy)-acetylamino]-acetylamino}-acetylamino)-3-phenyl-propionic acid benzyl ester (26)

{2-[2-(2-Hydroxy-phenylazo)-phenoxy]-acetylamino}-acetic acid benzhydryl ester (**25**) (100 mg, 0.20 mmol) was dissolved in dry acetonitrile under inert condition. Cesium carbonate (132 mg, 0.40 mmol) and compound **28** (99 mg, 0.20 mmol) were added in succession and left for 8hr under room temperature. The reaction mixture was then evaporated and the crude mass was taken in ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulphate, concentrated and subjected to column chromatography (Si-gel, 60-120 mesh, 10% MeOH in $CHCl_3$ as eluent).

Synthesis of Compound 1

2-(2-{2-[2-(2-{2-[(Benzhydryloxycarbonylmethyl-carbamoyl)-methoxy]-phenylazo}-phenoxy)-acetylamino]-acetylamino}-acetylamino)-3-phenyl-propionic acid benzyl ester **(26**) (54 mg, 0.06 mmol) was dissolved in dry DCM (10 mL) and kept in an ice bath at 0 ^{o}C. Trifluoroacetic acid (TFA) (0.12 mL, 1.50 mmol) was added dropwise slowly to it followed by the addition of anisole (10 μL, 0.09 mmol). The whole mixture was then stirred for 3 hr at 0 ^{o}C. The reaction mixture was concentrated under reduced pressure by liquid nitrogen while the excess TFA was evaporated out. The resulting reaction mass was then purified by column chromatography (Si-gel, 60-120 mesh, 15% MeOH in $CHCl_3$ as eluent). Then free acid (40

mg, 0.05 mmol) was then taken in moist MeOH (5 mL) and sodium bicarbonate (5.45 mg, 0.06 mmol) was added to it. The whole solution was left for 4 hr at room temperature. After evaporating MeOH, the reaction mass was lyophilized to solid sodium salt of the corresponding free acid which was taken in dry DMF (5 mL) and compound **9** (16 mg, 0.05 mmol) was added to it and left for 8-9 hr at room temperature. After completion of the reaction the reaction mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulphate, concentrated and subjected to column chromatography (Si-gel, 60-120 mesh, 5% MeOH in $CHCl_3$ as eluent).

Synthesis of [3-(2-Iodo-phenyl)-prop-2-ynyl]-carbamic acid *tert*-butyl ester (5a)

Tetrakis (triphenylphosphine) palladium (0) (35 mg, 0.03 mL) was added to degasified pyrrolidine (5 mL) followed by the addition of diiodobenzene (0.2 ml, 1.52 mmol). After 15 minutes stirring copper iodide (58 mg, 0.30 mmol) and *N*-*t*-butoxycarbonylprop-2-ynylamine (260 mg, 1.68 mmol) were added to it. The reaction mixture was heated to 40 °C for 18 h , then cooled to room temperature and concentrated under reduced pressure. To the resulting oil was added saturated aqueous NH_4Cl (15 mL), and the mixture was extracted with ethyl acetate (3×10 mL), dried and concentrated. The resulting oil was purified by column chromatography (Si-gel, 60-120 mesh, petroleum ether : ethyl acetate = 7:1 as eluent).

Synthesis of {3-[2-(3-Hydroxy-prop-1-ynyl)-phenyl]-prop-2-ynyl}-carbamic acid tert-butyl ester (5)

Tetrakis (triphenylphosphine) palladium (0) (17 mg, 0.01 mL) was added to the solution of [3-(2-Iodo-phenyl)-prop-2-ynyl]-carbamic acid *tert*-butyl ester **5a** (170 mg, 0.47 mmol) in degasified triethylamine (15 mL). After 15 minutes stirring copper iodide (27 mg, 0.14 mmol) and propargylalcohol (0.04 mL, 0.57 mmol) were added to it followed by further 8 h stirring at room temp. The reaction mixture was then concentrated under reduced pressure. To the reaction mixture saturated aqueous NH_4Cl (20 mL) was then added, and the mixture was extracted with ethyl acetate (3×15 mL), dried and concentrated. The resulting oil was purified by column chromatography (Si-gel, 60-120 mesh, petroleum ether : ethyl acetate = 3:1 as eluent).

Synthesis of Methanesulfonic acid 3-[2-(3-tert-butoxycarbonylamino-prop-1-ynyl)-phenyl]-prop-2-ynyl ester (6)

{3-[2-(3-Hydroxy-prop-1-ynyl)-phenyl]-prop-2-ynyl}-carbamic acid tert-butyl ester **5** (102 mg, 0.35 mmol) was dissolved in dry dichloromethane (10 mL) and it was cooled in ice bath. Then mesyl chloride (0.03 mL, 0.35 mmol) and triethylamine (0.08 mL, 0.53 mmol) were added at 0^0C. It was then stirred for 15 min. The reaction was monitored by TLC and on completion the reaction mixture was washed with brine solution. The organic part was dried and concentrated. The resulting brown colored oil was purified by column chromatography (Si-gel, 60-120 mesh, petroleum ether : ethyl acetate = 5:1 as eluent).

Synthesis of {3-[2-(3-Azido-prop-1-ynyl)-phenyl]-prop-2-ynyl}-carbamic acid tert-butyl ester (7)

Methanesulfonic acid 3-[2-(3-tert-butoxycarbonylamino-prop-1-ynyl)-phenyl]-prop-2-ynyl ester **6** (126 mg, 0.34 mmol) was taken in dry DMF (10 mL) and sodium azide (27 mg, 0.42 mmol) was added to it. The reaction mixture was stirred for 7 h at room temp. After the reaction was completed, the product was extracted with ethyl acetate, dried and concentrated. The resulting yellow colored oil was purified by column chromatography (Si-gel, 60-120 mesh, petroleum ether : ethyl acetate = 5:1 as eluent).

Synthesis of Succinic acid monobenzhydryl ester (10)

Succinic acid (1.10 g, 9.79 mmol) was dissolved in dry THF (20 mL). Then diphenyldiazomethane (1.9 g, 9.79 mmol) was added to it in portionwise at 0 ^{o}C. After the addition had completed the reaction mixture was allowed to stir at room temp for 45 min. The reaction mixture was concentrated under reduced pressure and purified by column chromatography (Si-gel, 60-120 mesh, petroleum ether : ethyl acetate = 3:1 as eluent).

Synthesis of Compound 11

Succinic acid monobenzhydryl ester **10** (28 mg, 0.10mmol) was taken in dry DMF (5 mL). To the solution cesium carbonate (36 mg, 0.10 mmol) was added and the whole mixture was allowed to stir for 30 mins at room temp. After that a solution of compound **9** (30 mg, 0.10 mmol) in dry DMF was added to it and left for overnight stirring. The completion of the

reaction was checked by TLC and was extracted with ethylacetate, dried and concentrated. Then it was purified by column chromatography (Si-gel, 60-120 mesh, petroleum ether : ethyl acetate = 1:1 as eluent).

Synthesis of Compound 12

Compound **11** (65 mg, 0.13 mmol) was dissolved in dry DCM (15 mL) and kept in an ice bath at 0 ^{o}C. Trifluoroacetic acid (TFA) (0.25 mL, 3.22 mmol) was added to it followed by the addition of anisole (0.02 mL, 0.19 mmol). The whole mixture was then stirred for 30 min at 0^{0}C. The reaction mixture was concentrated under reduced pressure by liquid nitrogen while the excess TFA was evaporated out. The resulting reaction mass was then purified by dry benzene wash.

Synthesis of Compound 13

Compound **12** (33.5 mg, 0.10 mmol), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC.HCl) (30 mg, 0.14 mmol) and DMAP (3 mg, 0.02 mmol) were dissolved in dry DCM (15 mL). The solution of compound **8** (27 mg, 0.12 mmol) in dry DCM was then added to it and stirred for overnight at room temp. The reaction mixture was then washed with saturated brine solution and the organic layer was dried, and concentrated. Then column purification (Si-gel, 60-120 mesh, 5% MeOH in $CHCl_3$ as eluent) was done which gave yellow colored pure viscous oil.

Synthesis of Compound 14

Compound **13** (30 mg, 0.05 mmol) was dissolved in dry DCM (10 mL). Then the solution was cooled to 0 ^{o}C. Trifluoroacetic acid (0.08 mL, 1.08 mmol) was slowly added to this reaction mixture and the temp was gradually allowed to reach to room temp. After 1.5 h stirring the reaction was found to be completed by TLC checking. Then the solution was concentrated under reduced pressure by liquid nitrogen. The resulting reaction mass was finally purified by dry benzene wash.

Synthesis of Compound 3

Compound **15** (18 mg, 0.05 mmol), EDC.HCl (12 mg, 0.06 mmol) and HOBt (8 mg, 0.06 mmol) were taken in dry DCM (8 mL). The solution was then cooled in ice bath and stirred for 1 h. Afterwards DCM solution of compound **14** (30 mg, 0.05 mmol) and DIPEA (8.83 μL, 0.05 mmol) were added to the reaction mixture. The stirring was continued for next 10 h at room temp. On completion, the reaction mixture was washed with saturated brine solution and the DCM layer was dried and concentrated. After column purification (Si-gel, 60-120 mesh, 8% MeOH in $CHCl_3$ as eluent) a creamish white solid was obtained as the pure final product.

Spectral Data

tert-Butoxycarbonylamino-acetic acid benzhydryl ester (27a) Colorless oil; Yield 800 mg, 47%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55-7.45 (m, 3H), 7.38-7.28 (m, 7H), 6.93 (s, 1H), 5.00 (bs, 1H), 4.04-4.03 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.6, 155.8, 139.7, 128.6, 128.3, 127.2, 80.0, 52.2, 42.8, 28.4.

(2-Bromo-acetylamino)-acetic acid benzhydryl ester (27) Yellow oil; Yield 260 mg, 94%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33-7.23 (bs, 10H), 7.05 (s, 1H), 6.90 (s, 1H), 4.15-4.13 (m, 2H), 3.84 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.6, 166.5, 139.4, 128.7, 128.3, 127.1, 76.6, 42.2, 28.5.

{2-[2-(2-Hydroxy-phenylazo)-phenoxy]-acetylamino}-acetic acid benzhydryl ester (25) Dark red viscous oil; Yield 200 mg, 56%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.12 (s, 1H), 7.94-7.90 (m, 2H), 7.48-7.44 (m, 1H), 7.34 -7.26 (m, 10H), 7.17-7.13 (m, 1H), 7.07-7.03 (m, 2H), 6.92-6.87 (m, 2H), 4.72 (s, 2H), 4.31-4.30 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.2, 167.8, 153.6, 153.4, 139.4, 138.6, 138.1, 133.3, 132.9, 132.8, 128.5, 128.0, 127.0, 122.5, 119.8, 118.5, 116.7, 113.4, 77.9, 67.7; HRMS: calcd. for $C_{29}H_{25}N_3O_5+H^+$ 496.1872 found 496.1860, for $C_{29}H_{25}N_3O_5+Na^+$ 518.1692 found 518.1685.

2-(2-tert-Butoxy carbonylamino-acetylamino)-3-phenyl-propionic acid benzyl ester (28a) Yellow oil; Yield 660 mg, 40%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.01 (m, 10H), 5.54 (bs, 1H), 5.12-5.03 (dd, *J* = 25.2 Hz, 12 Hz, 2H), 4.91-4.87 (m, 1H), 4.35 (bs, 1H), 3.75-3.72 (m, 2H), 3.09-3.06 (m, 2H), 1.42 (bs, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.3, 169.6, 156.1, 135.8, 135.1, 129.3, 128.6, 128.5, 127.0, 80.0, 67.2, 53.3, 37.9, 28.3.

2-[2-(2-tert-Butoxy carbonylamino-acetylamino)-acetylamino]-3-phenyl-propionic acid benzyl ester (28b) Colorless oil; Yield 413 mg, 46%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31-7.17 (m, 8H), 7.03-7.02 (m, 2H), 5.56 (bs, 1H), 5.12- 5.03 (dd, *J* = 24 Hz, 12.4 Hz, 2H), 4.87-4.82 (dd, *J* = 14 Hz, 6.8 Hz, 1H), 3.95-3.86 (m, 2H), 3.82-3.75 (m, 2H), 3.11-3.06 (dd, *J* = 14 Hz, 6.8 Hz, 1H), 3.04-2.99 (dd, *J* = 14 Hz, 6.8 Hz, 1H), 1.41 (bs, 9H); ^{13}C NMR (50 MHz,

$CDCl_3$): δ 171.3, 170.2, 168.7, 156.2, 135.8, 135.1, 129.3, 128.6, 128.5, 127.1, 80.3, 67.3, 53.5, 42.9, 37.8, 28.4; HRMS: calcd. for $C_{25}H_{31}N_3O_6$+H^+ 470.2291 found 470.2278.

2-{2-[2-(2-Bromo-acetylamino)-acetylamino]-acetylamino}-3-phenyl-propionic acid benzyl ester (28) White solid; Yield 265 mg, 61%; mp 156 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.22 (m, 8H), 7.12 (bs, 1H), 7.00 (bs, 2H), 6.62 (bs, 1H), 6.42-6.40 (m, 1H), 5.19-5.11 (dd, *J* = 21.6 Hz, 12 Hz, 2H), 4.93-4.88 (dd, *J* = 13.2 Hz, 6 Hz, 2H), 4.15-3.88 (m, 6H), 3.16-3.07 (m, 2H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 171.7, 169.3, 169.0, 166.8, 137.4, 136.2, 129.6, 128.8, 128.7, 128.5, 128.3, 127.1, 66.5, 54.2, 42.9, 42.0, 37.3, 29.8; HRMS: calcd. for $C_{22}H_{24}BrN_3O_5$+H^+ 490.0978 found 490.0990.

tert-Butoxycarbonylamino-acetic acid benzyl ester (3a) Light yellow colored viscous oil; Yield 186 mg, 70%; ^1H NMR (400 MHz, $CDCl_3$): δ 7.35 (s, 5H), 5.17 (s, 2H), 3.96-3.93 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.4, 156.1, 135.5, 128.5, 128.3, 128.2, 127.1, 126.8, 79.8, 66.8, 42.4, 28.3.

{2-[2-(2-tert-Butoxycarbonylamino-acetylamino)-acetylamiono]-acetylamino}-acetic acid benzyl ester (3b) White solid; Yield 247 mg, 54%; mp 176 ^oC, ^1H NMR (400 MHz, $CDCl_3$): δ 7.35-7.34 (s, 5H), 5.18-5.16 (m, 2H), 4.10-3.96 (m, 6H), 3.83-3.76 (m, 2H), 1.46-1.43 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$ and 4drops DMSO-d_6): δ 170.8, 169.8, 169.7, 169.6, 156.3, 135.7, 128.6, 128.3, 128.2, 79.4, 66.6, 43.0, 42.6, 41.1, 28.5; HRMS: calcd. for $C_{20}H_{28}N_4O_7$+H^+ 437.2036 found 437.2021, for $C_{20}H_{28}N_4O_7$+Na^+ 459.1856 found 459.1863.

2-(2-{2-[2-(2-{2-[(Benzhydryloxycarbonylmethyl-carbamoyl)-methoxy]-phenylazo}-phenoxy)-acetylamino]-acetylamino}-acetylamino)-3-phenyl-propionic acid benzyl ester (26) Red colored solid; Yield 110 mg, 60%; mp 134 ^oC, ^1H NMR (400 MHz, $CDCl_3$): δ 8.16-8.14 (t, *J* = 5.2 Hz, 1H), 8.09-8.06 (t, *J* = 5.2 Hz, 1H), 7.80-7.78 (m, 2H), 7.49-7.45 (m, 1H), 7.36- 7.18 (bm, 19H), 7.16-7.00 (m, 4H), 6.96 (s, 1H), 6.89-6.87 (m, 1H), 6.83-6.81 (m, 1H), 6.68-6.67 (m, 1H), 5.13-5.04 (dd, *J* = 20.8 Hz, 12 Hz, 2H), 4.85-4.82 (m, 1H), 4.76-4.69 (dd, *J* = 22.2Hz, 15 Hz, 2H), 4.52-4.50 (m, 1H), 4.45 (s, 2H), 4.23-6.17 (dd, *J* = 18.2 Hz, 6 Hz, 1H), 4.04-3.98 (dd, *J* = 18 Hz, 5.6 Hz, 1H), 3.94-3.89 (dd, *J* = 16.8 Hz, 5.6 Hz, 1H), 3.84-3.78

(dd, *J* = 16.8 Hz, 6 Hz, 1H), 3.72-3.67 (dd, *J* = 16.8 Hz, 5.2 Hz, 1H), 3.13-3.08 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 169.4, 169.3, 168.8, 168.5, 168.4, 154.4, 153.8, 142.7, 139.6, 139.4, 135.8, 135.0, 133.2, 132.9, 129.2, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 127.1, 126.9, 123.2, 123.1, 120.8, 119.8, 116.4, 116.1, 78.2, 69.9, 69.7, 67.1, 53.5, 42.9, 42.7, 40.8, 37.7; HRMS: calcd. for $C_{51}H_{48}N_6O_{10}+H^+$ 905.3510 found 905.3522, for $C_{51}H_{48}N_6O_{10}+Na^+$ 927.3330 found 927.3324.

Compound 1 Dark yellow colored oil; Yield 30 mg, 58%; ^{1}H NMR (400 MHz, CD_3CN): δ 7.85 (m, 1H), 7.76-7.69 (m, 3H), 7.47-7.43 (m, 2H), 7.36-7.12 (m, 14H), 7.04-7.00 (m, 3H), 6.96-6.91 (m, 1H), 6.83 (s, 1H), 6.68-6.66 (m, 1H), 5.08-5.07 (m, 2H), 4.84-4.54 (bm, 8H), 4.50-4.47 (m, 2H), 4.25-4.24 (bs, 2H), 4.12-4.05 (m, 2H), 3.89-3.62 (bm, 10H), 3.10-3.05 (m, 2H), 3.02-2.95 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4, 170.1, 169.7, 169.0, 168.9, 168.6, 168.5, 168.1, 165.9, 153.8, 153.4, 142.8, 142.7, 135.8, 135.1, 133.1, 133.0, 129.8, 129.7, 129.6, 129.2, 129.1, 128.5, 128.4, 128.3, 128.2, 128.1, 127.6, 127.5, 127.4, 127.0, 126.9, 123.3, 122.2, 122.1, 120.9, 119.1, 116.9, 116.6, 115.4, 114.6, 97.9, 92.4, 88.0, 82.5, 77.1, 70.3, 70.1, 67.1, 67.0, 62.2, 53.6, 53.4, 52.2, 51.4, 43.0, 42.8, 42.7, 40.9, 40.8, 40.7, 40.6, 37.6, 18.2, 18.1; IR (KBr, cm^{-1}): ν 2924, 2853, 2363, 2345, 1654, 1560; HRMS: calcd. for $C_{53}H_{49}N_7O_{11}+H^+$ 960.3568 found 960.3570.

Compound 2 Dark yellow colored viscous oil; Yield 15 mg, 36%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26-8.21 (m, 2H), 7.85-7.80 (m, 2H), 7.49-7.48 (m, 4H), 7.33-7.21(bm, 26H), 7.12-7.03 (m, 2H), 6.98-6.95 (m, 2H), 6.90-6.85 (m, 1H), 5.91-5.83 (m, 2H), 5.09-5.07 (m, 2H), 5.00-4.50 (bm, 14H), 4.22-3.56 (bm, 18H), 3.10-3.08 (m, 4H); IR (KBr, cm^{-1}): ν 2924, 2853, 2369, 2345, 1654, 1560; MS: m/z = 934.34 [MNa+], 910.33 [MH+], HRMS: calcd. for $C_{49}H_{47}N_7O_{11}+H^+$ 910.3412 found 910.3418.

[3-(2-Iodo-phenyl)-prop-2-ynyl]-carbamic acid *tert*-butyl ester (5a) Yellow colored oil; Yield 170 mg, 31%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84-7.82 (app. d, *J* = 8 Hz, 1H), 7.42-7.41 (m, 1H), 7.30-7.26 (m, 1H), 7.01-6.98 (m, 1H), 4.81 (bs, 1H), 4.21-4.13 (bs, 2H), 1.47-1.44 (bs, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.3, 138.6, 132.6, 129.4, 129.1, 128.4, 127.7, 100.8, 89.3, 85.2, 31.3, 28.3

{3-[2-(3-Hydroxy-prop-1-ynyl)-phenyl]-prop-2-ynyl}-carbamic acid tert-butyl ester (5) Brown colored oil; Yield 102 mg, 75%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (m, 2H), 7.26-7.20 (m, 2H), 5.20 (bs, 1H), 4.52 (s, 2H), 4.15-4.08 (bs, 2H), 1.45 (bs, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.7, 131.4, 128.1, 127.9, 125.9, 125.5, 92.1, 89.9, 83.8, 80.4, 61.5, 51.4, 29.7, 28.4.

Methanesulfonic acid 3-[2-(3-tert-butoxycarbonylamino-prop-1-ynyl)-phenyl]-prop-2-ynyl ester (6) Brown colored oil; Yield 126 mg, 96%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.43-7.40 (m, 2H), 7.31-7.26 (m, 2H), 5.12 (s, 2H), 4.19-4.17 (bm, 2H), 3.19 (s, 3H), 1.45 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.4, 132.2, 132.1, 129.1, 128.1, 126.0, 123.7, 90.5, 87.9, 84.5, 81.0, 80.1, 58.7, 58.4, 39.4, 38.9, 31.3, 28.4.

{3-[2-(3-Azido-prop-1-ynyl)-phenyl]-prop-2-ynyl}-carbamic acid tert-butyl ester (7) Yellow oil; Yield 72 mg, 67%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44-7.42 (m, 2H), 7.28-7.26 (m, 2H), 4.91 (bs, 1H), 4.20 (s, 4H), 1.46 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.3, 132.0, 128.5, 128.0, 125.5, 124.5, 90.1, 85.9, 84.9, 81.4, 79.9, 77.9, 77.2, 76.6, 40.7, 31.3, 28.3.

Succinic acid monobenzhydryl ester (10) Colorless solid; Yield 750 mg, 31%; mp 72 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.26 (m, 10H), 6.90 (s, 1H), 2.78-2.69 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 178.5, 171.4, 140.2, 128.7, 128.2, 127.3, 77.7, 29.3, 29.1.

Compound 11 Colorless oil; Yield 80 mg, 59%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.21 (m, 10H), 6.87 (s, 1H), 4.77 (s, 2H), 4.12 (s, 2H), 3.75-3.73 (t, J = 5.2 Hz, 2H), 2.93-2.90 (t, J = 5.2 Hz, 2H), 2.81-2.79 (bs, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.7, 171.1, 166.5, 140.0, 130.0, 128.5, 128.4, 128.3, 128.0,127.6, 127.4, 127.1, 98.2, 92.5, 88.1, 82.6, 77.3, 61.6, 51.4, 41.0, 29.4, 28.9, 18.3; HRMS: calcd. for $C_{32}H_{27}NO_5$+H^+ 506.1967 found 506.1970.

Compound 13 Yellowish viscous oil; Yield 240 mg, 50%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.21 (m, 8H), 6.96 (bs, 1H), 5.41 (bs, 1H), 4.84 (s, 2H), 4.28-4.27 (m, 2H), 4.16-4.12 (m, 4H), 3.71-3.69 (t, J = 5.2 Hz, 2H), 2.89-2.87 (t, J = 5 Hz, 2H), 2.83-2.80 (t, J = 6.4 Hz, 2H),

2.62-2.59 (t, J = 6.4 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.3, 171.4, 166.7, 155.8, 132.2, 132.0, 131.7, 129.9, 128.4, 128.2, 128.0, 127.7, 127.5, 125.7, 125.6, 98.2, 92.5, 90.1, 89.4, 88.0, 82.6, 81.3, 80.1, 61.6, 51.3, 40.9, 30.0, 29.7, 28.4, 22.7, 18.3, 14.2; HRMS: calcd. for $C_{36}H_{35}N_3O_6$+H^+ 606.2604 found 606.2609.

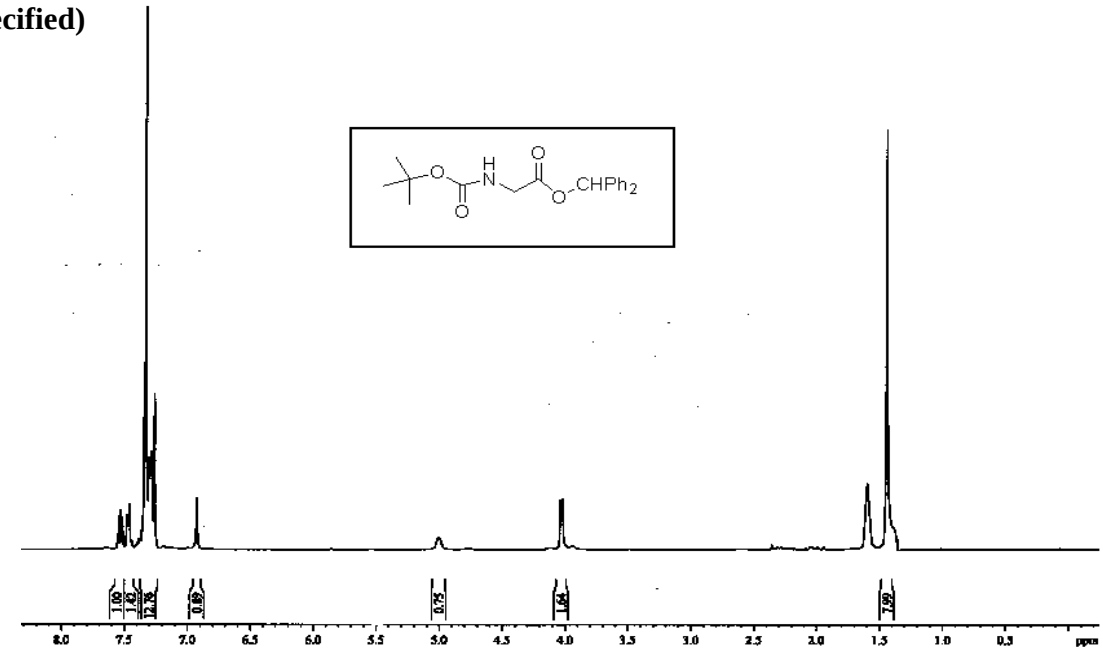
Compound 3 Creamish white oil; Yield 45 mg, 41%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.45-8.41 (m, 1H), 8.37 (m, 1H), 8.15-8.12 (m, 2H), 8.03 (m, 1H), 7.65-7.29 (bm, 8H), 7.00-6.97 (m, 1H), 4.84-4.83 (m, 2H), 4.47-4.40 (m, 2H), 4.17-4.14 (m, 4H), 3.84 (bs, 1H), 3.73-3.72 (bs, 7H), 3.63 (bs, 2H), 3.57-3.55 (m, 2H), 2.78-2.76 (bm, 2H), 2.61-2.58 (m, 2H), 2.43-2.40 (m, 2H); 1.36 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$ and 4drops DMSO-d_6): δ 172.5, 171.6, 171.2, 170.4, 169.8, 169.3, 166.7, 156.6, 132.0, 131.6, 129.6, 129.0, 128.2, 128.1, 128.0, 127.6, 127.4, 125.5, 125.4, 98.0, 92.6, 89.6, 89.4, 87.8, 82.4, 81.0, 80.8, 80.1, 61.6, 51.2, 44.3, 43.2, 43.1, 42.7, 40.8, 30.4, 29.5, 28.2, 22.5, 18.1, 14.0; IR (KBr, cm^{-1}): ν 2925, 2853, 2677, 1647, 1466; MS: m/z 856 [MNa^+], 834 [MH^+]; HRMS: calcd. for $C_{44}H_{47}N_7O_{10}$+H^+ 834.3463 found 834.3456.

2-(2-{2-[2-(2-tert-Butoxycarbonylamino-acetylamino)-acetylamino}-acetylamino)-3-phenyl-propionic acid benzyl ester (4a) White solid; Yield 72 mg, 34%; mp 154 ^{o}C -156 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$ and 2drops DMSO-d_6): δ 8.04-8.01 (t, J = 5.6 Hz, 1H), 7.91 (bs, 1H), 7.80 (bs, 1H), 7.71 (bs, 1H), 7.24-7.21 (m, 3H), 7.18-7.13 (m, 5H), 7.11-7.06 (m, 2H), 6.42 (bs, 1H), 5.01 (s, 2H), 4.66-4.61 (m, 1H), 3.87-3.81 (m, 3H), 3.77-3.76 (m, 2H), 3.72-3.66 (m, 3H), 3.05-2.93 (m, 2H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$ and 4drops DMSO-d_6): δ 169.7, 169.2, 168.7, 167.9, 167.5, 154.8, 134.8, 133.7, 127.6, 126.8, 126.7, 126.5, 126.4, 125.1, 77.8, 65.0, 52.1, 52.0, 42.5, 41.4, 41.3, 40.8, 35.8, 26.7; HRMS: calcd. for $C_{29}H_{37}N_5O_8$+H^+ 584.2720 found 584.2728.

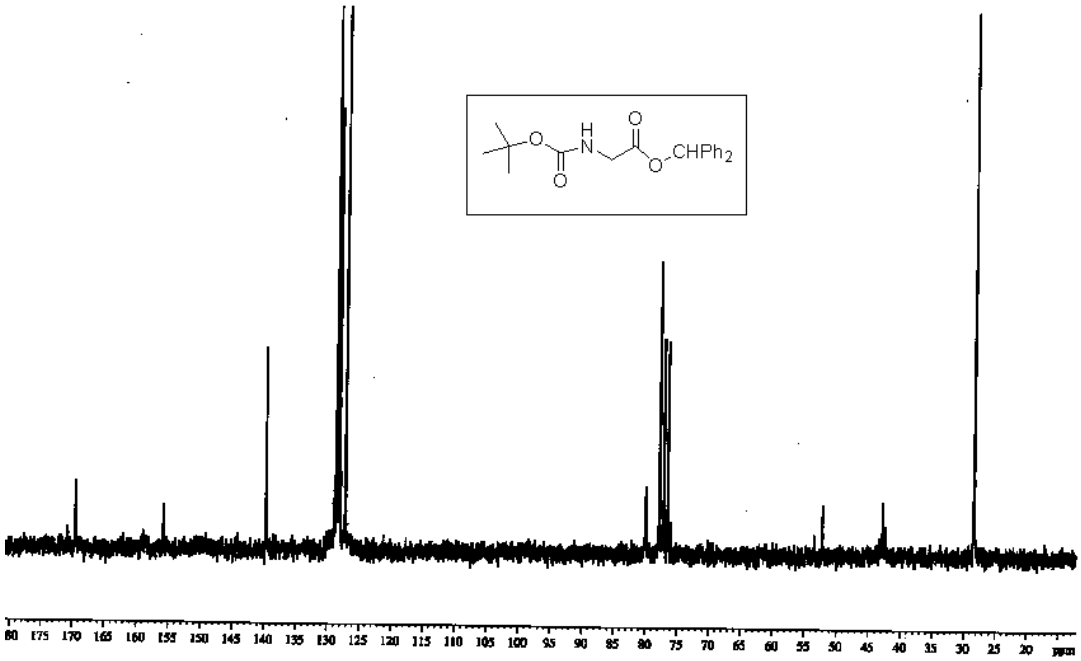
Compound 4 Cream colored white solid; Yield 31 mg, 39%; mp 124 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.56 (bs, 1H), 8.44-8.40 (bs, 1H), 8.14-8.10 (m, 2H), 8.00 (bs, 2H), 7.42-7.41 (m, 2H), 7.35-7.31 (m, 6H), 7.22-7.21 (m, 4H), 7.15 (bs, 1H), 6.97 (bs, 1H), 4.84 (s, 2H), 4.50-4.47 (m, 1.5H), 4.42-4.40 (m, 1.5H), 4.15-4.14 (m, 4H), 3.77-3.69 (m, 6H), 3.64-3.60 (m, 2H), 3.57-3.55 (m, 2H), 3.02-2.99 (m, 1H), 2.81-2.76 (m, 3H), 2.61-2.57 (m, 2H), 2.43-

2.39 (m, 2H), 1.35 (s, 9H); IR (KBr, cm^{-1}): ν 3752, 3676, 3301, 2926, 1656, 1526, 1368, 1166, 1029, 761; HRMS: calcd. for $C_{53}H_{56}N_8O_{11}+H^+$ 981.4147 found 981.4139.

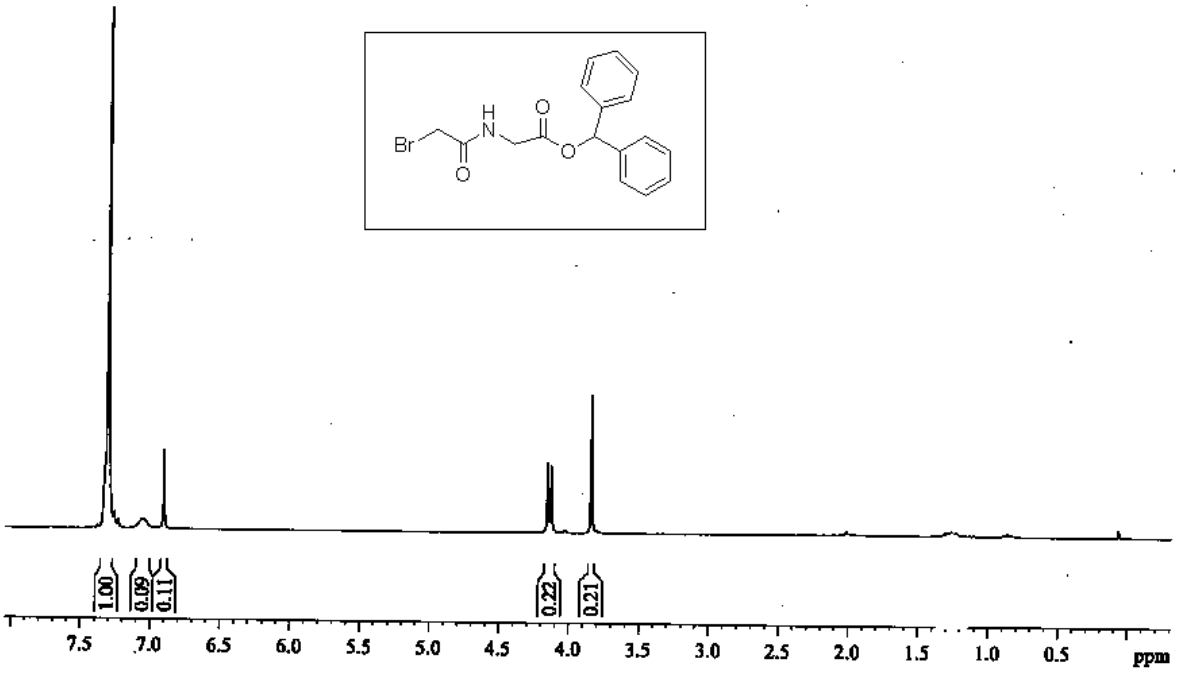
^{1}H and ^{13}C NMR spectra of all compounds (all were recorded in CDCl3 unless otherwise specified)



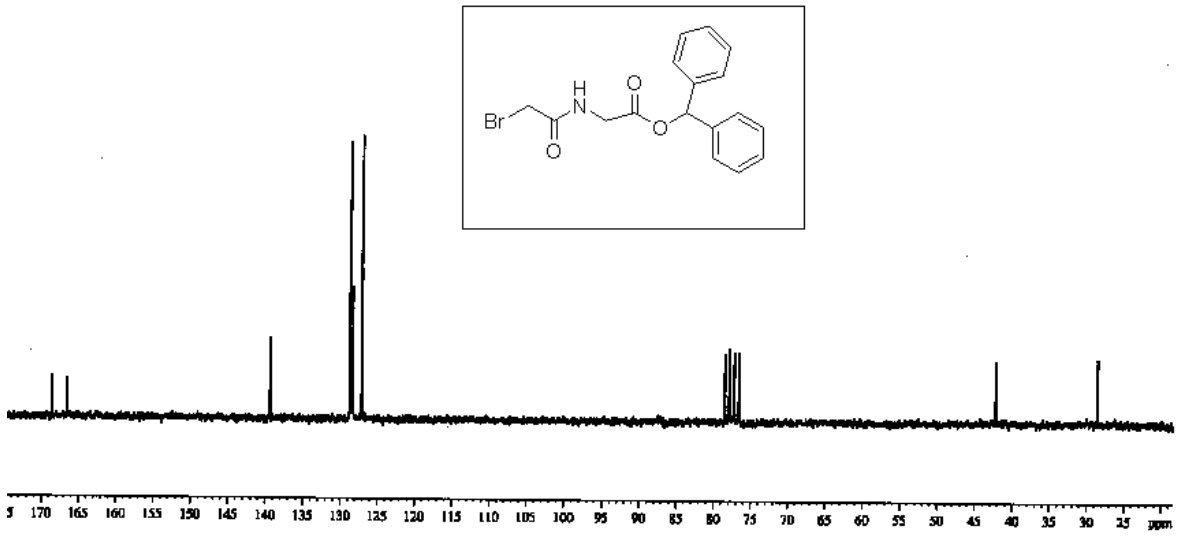
^{1}H NMR of Compound 27a (400 MHz)



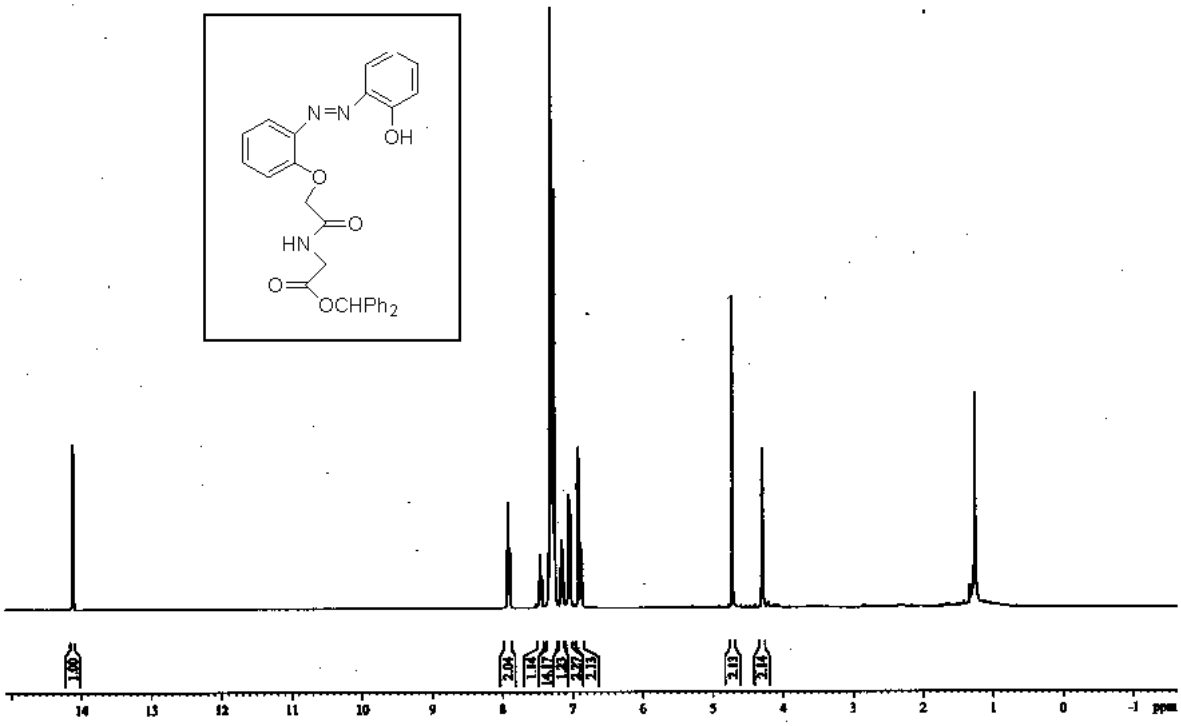
^{13}C NMR of Compound 27a (50 MHz)



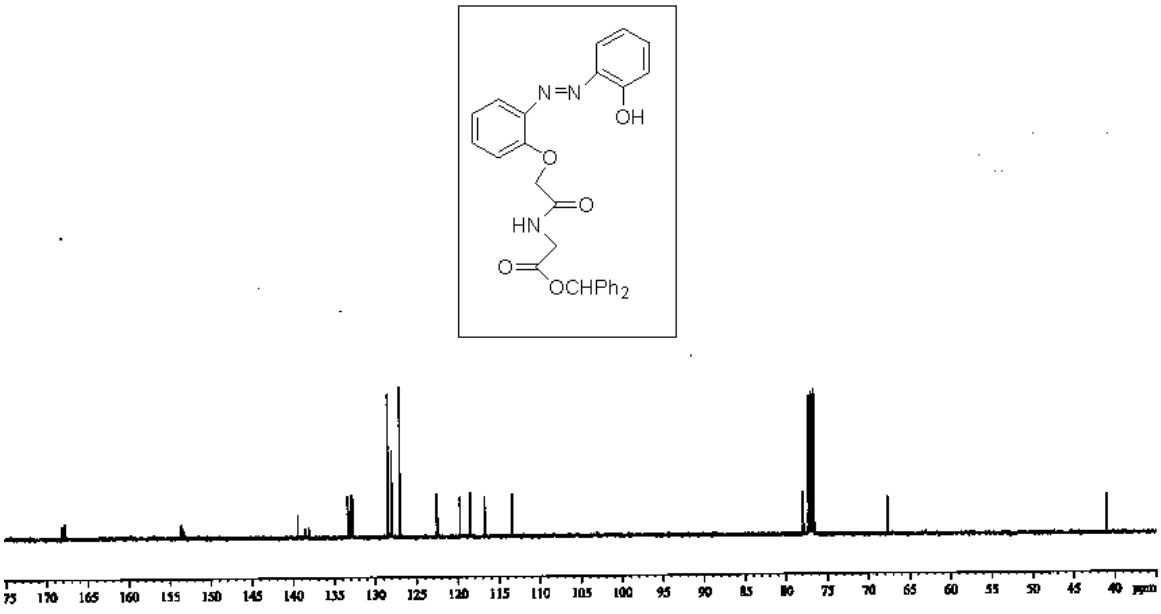
^{1}H NMR of Compound 27 (200 MHz)



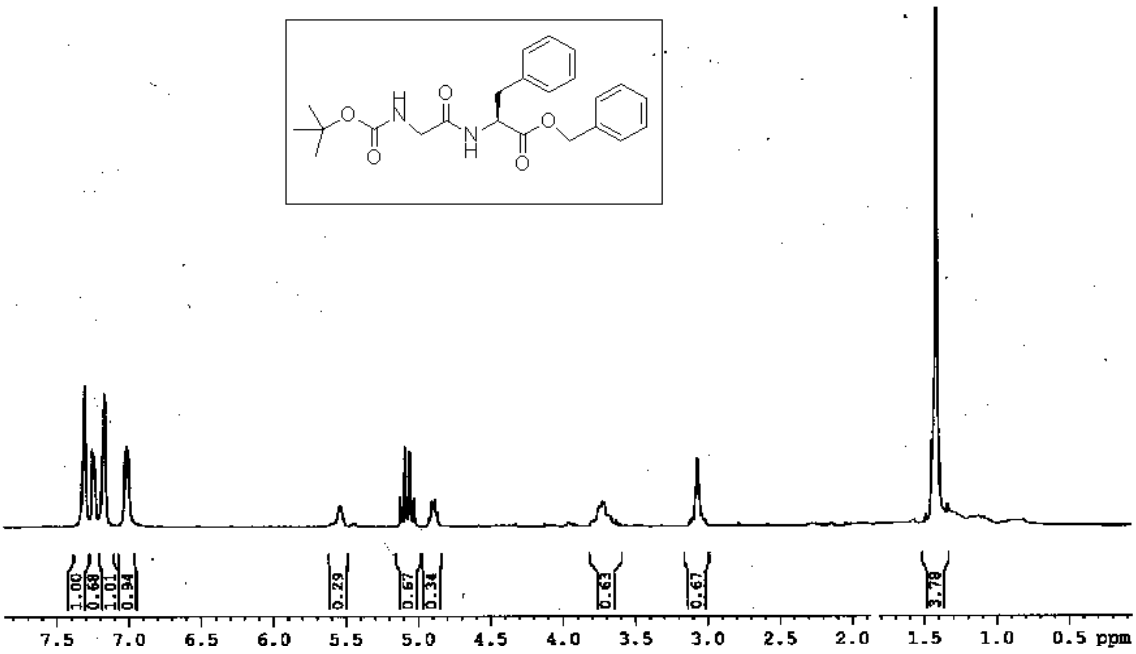
^{13}C NMR of Compound 27 (50 MHz)



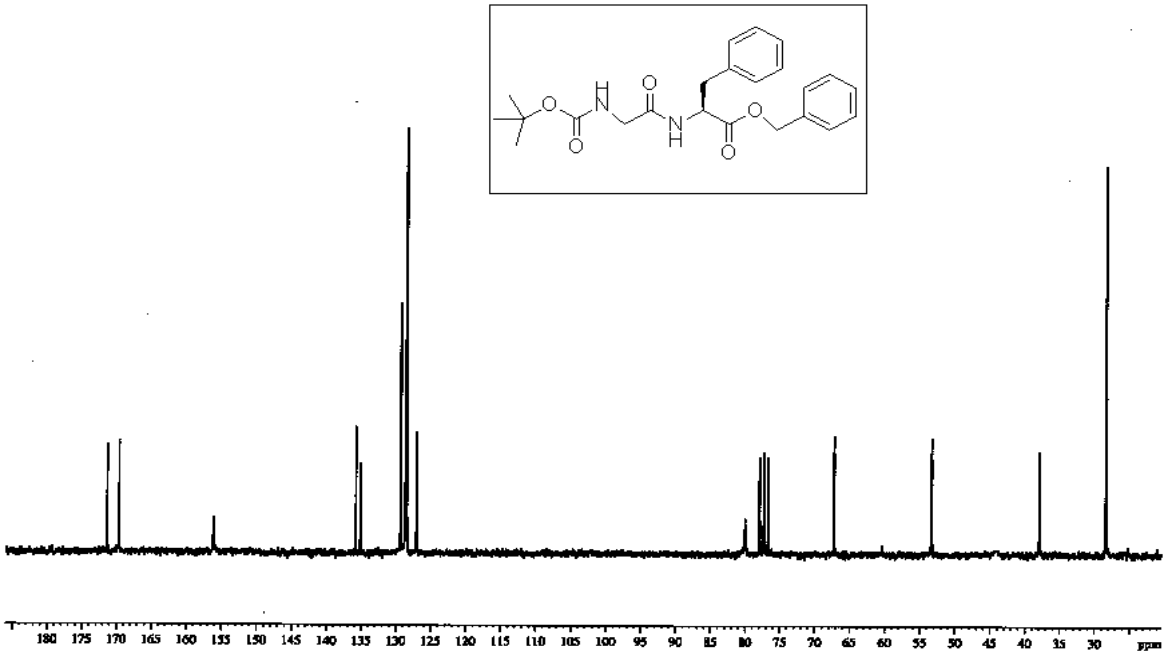
^{1}H NMR of Compound 25 (400 MHz)



^{13}C NMR of Compound 25 (100 MHz)

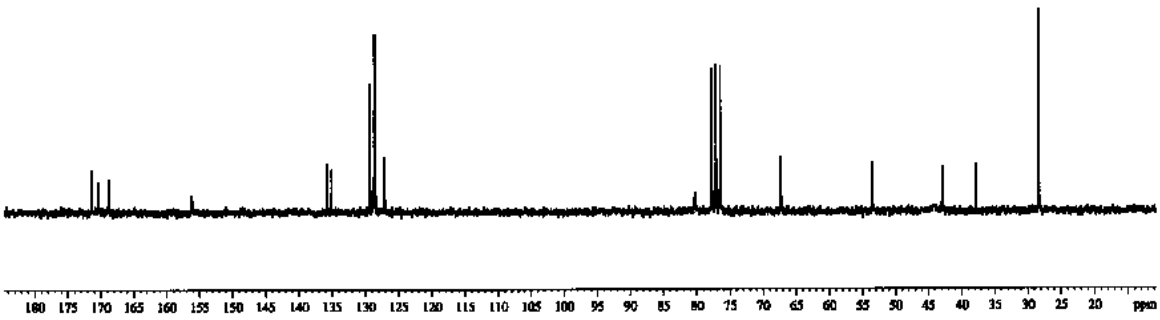


^{1}H NMR of Compound 28a (400 MHz)

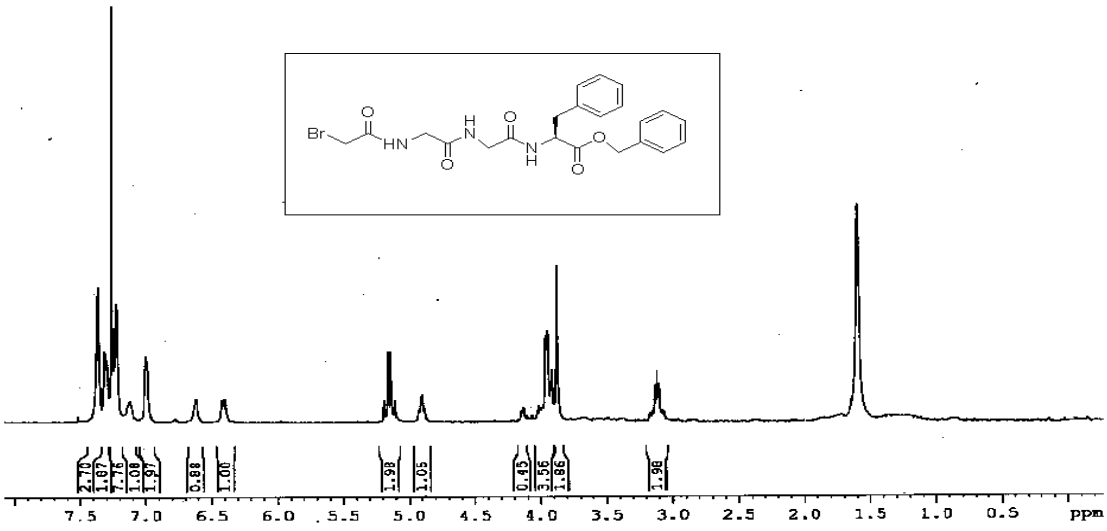


^{13}C NMR of Compound 28a (50 MHz)

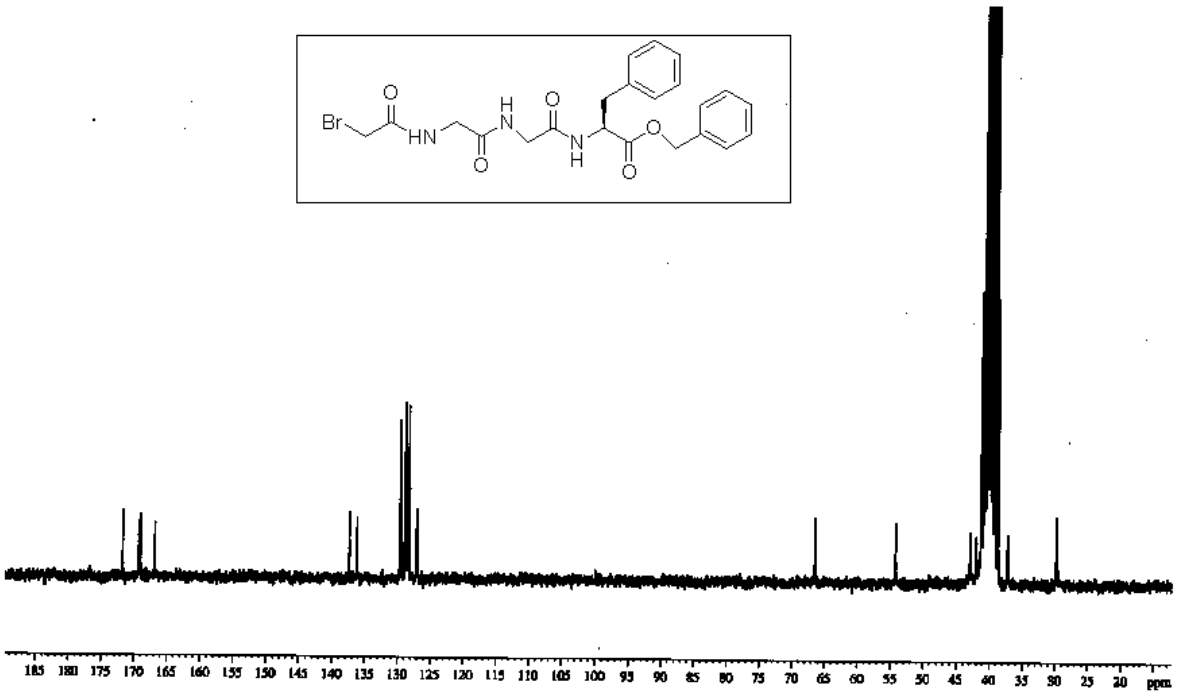
^{1}H NMR of Compound 28b (400 MHz)



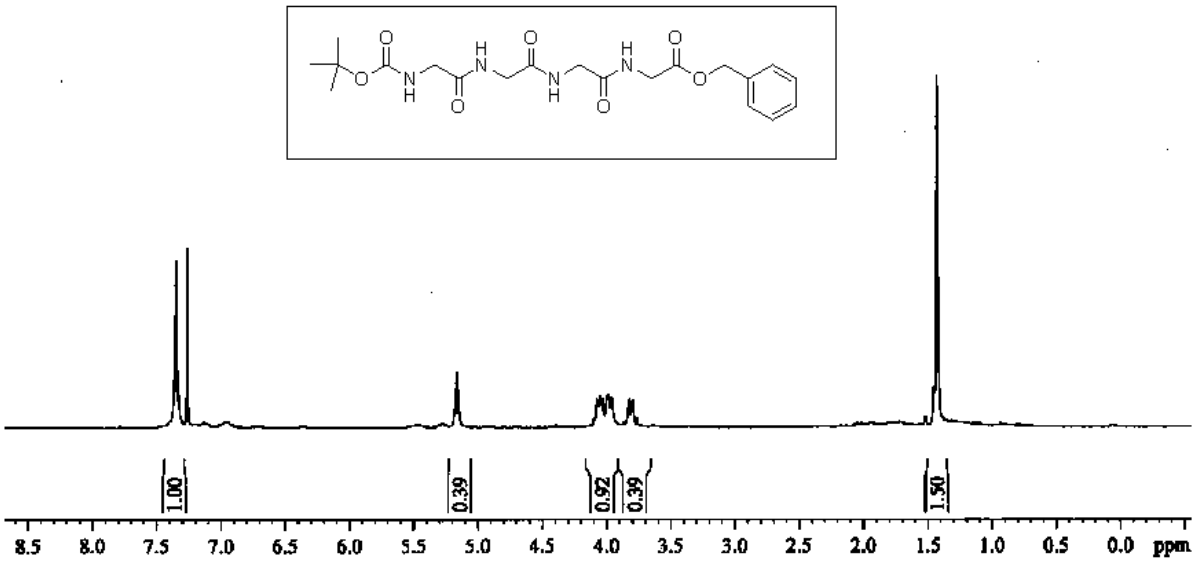
^{13}C NMR of Compound 28b (50 MHz)



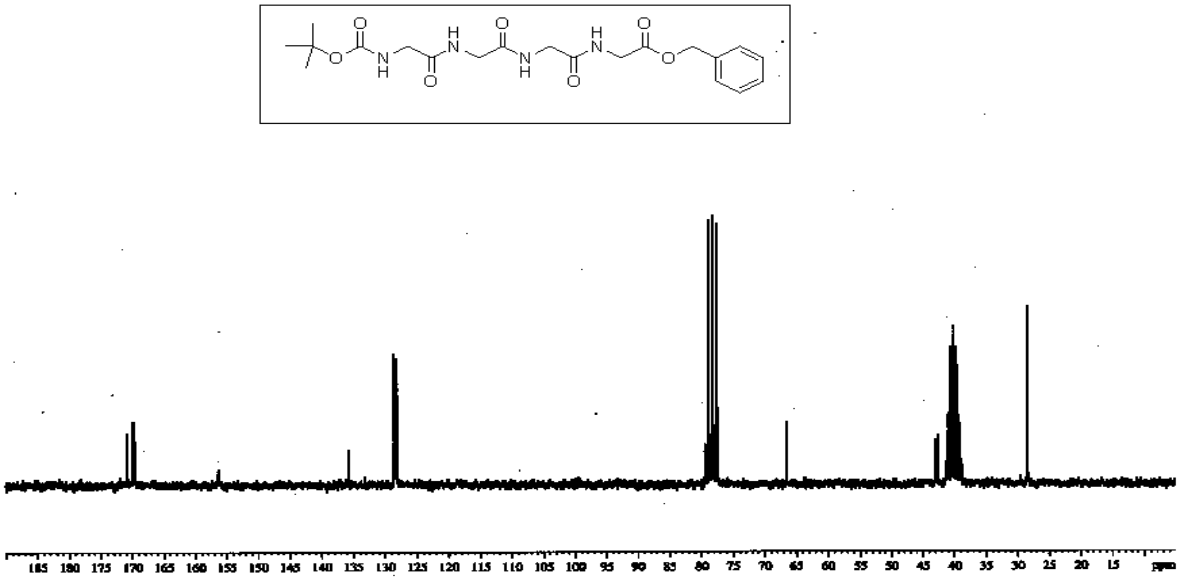
^{1}H NMR of Compound 28 (400 MHz)



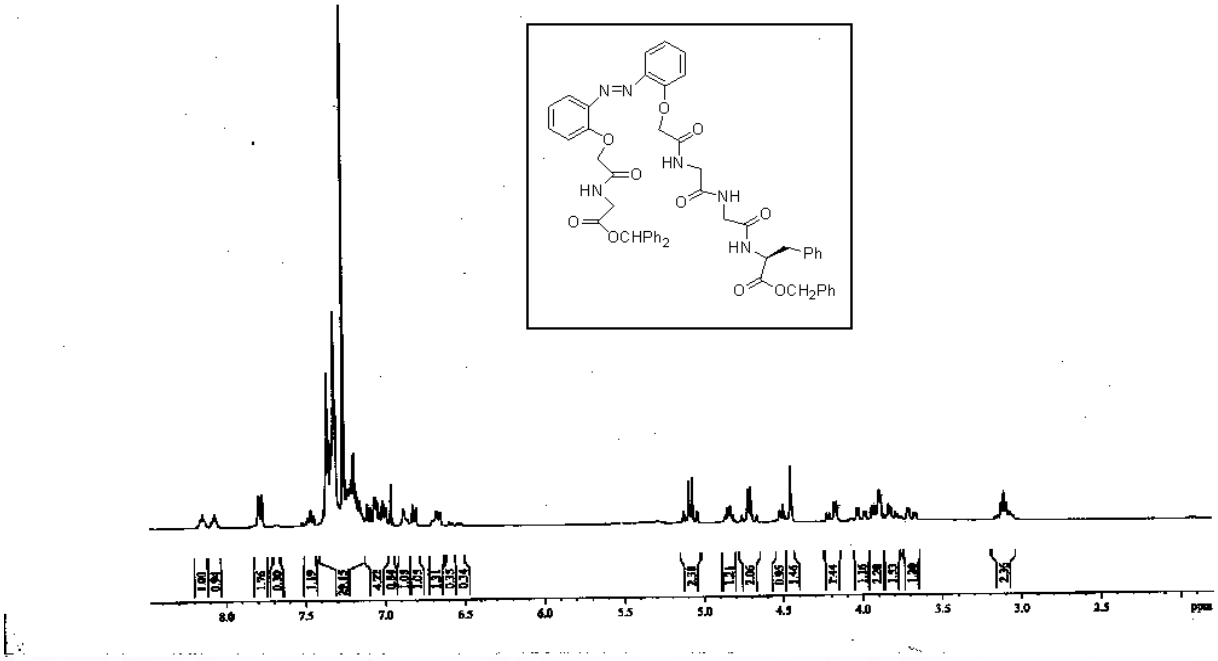
^{13}C NMR of Compound 28 (50 MHz)



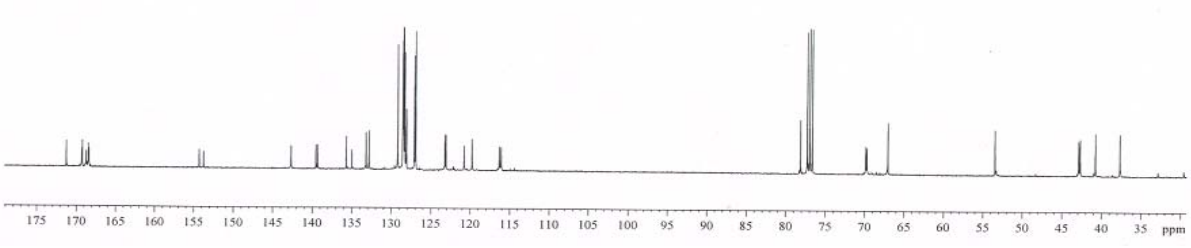
^{1}H NMR of Compound 3b (200 MHz)



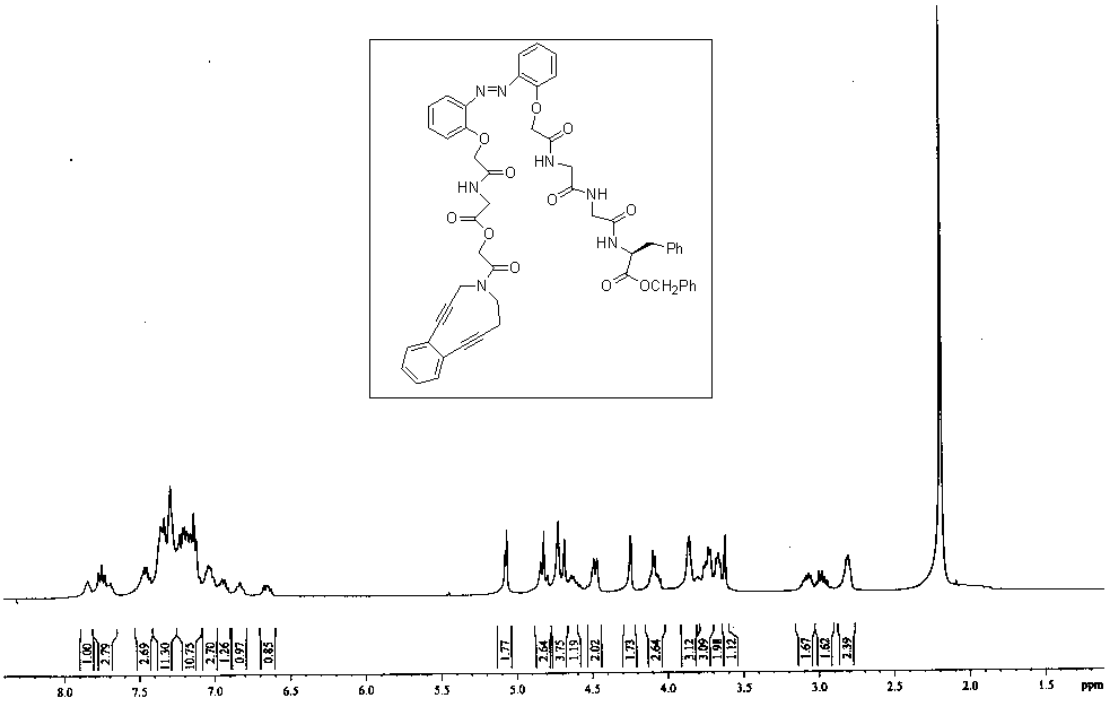
^{13}C NMR of Compound 3b (50 MHz)



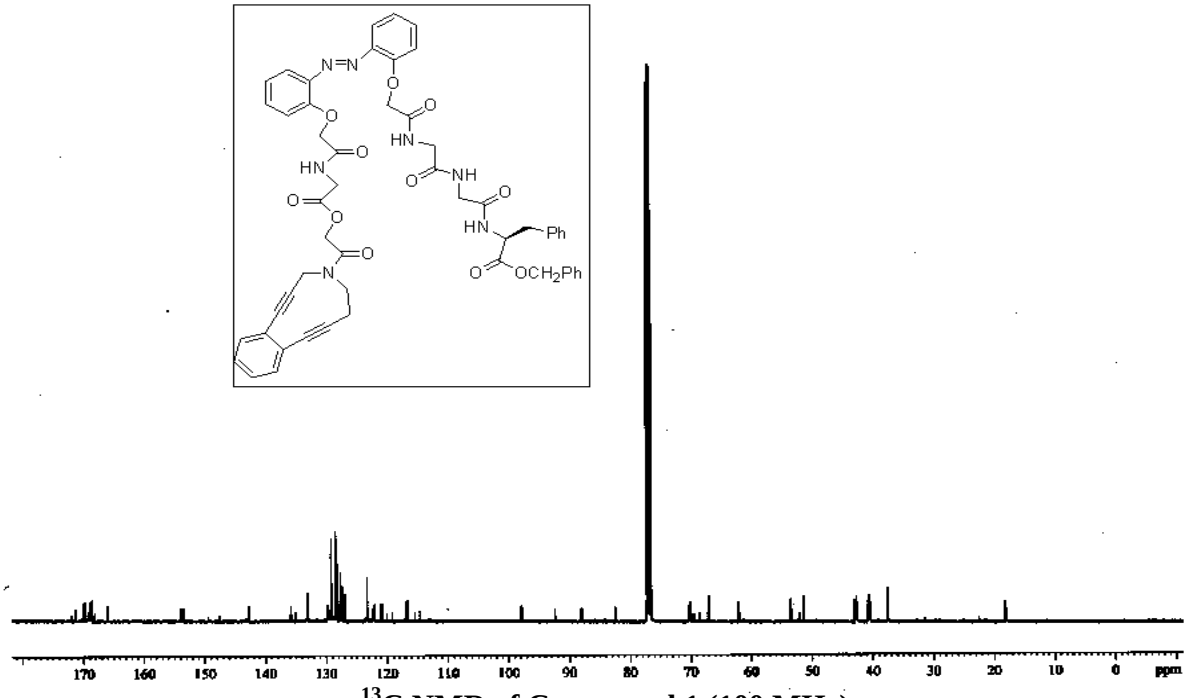
^{1}H NMR of Compound 26 (400 MHz)



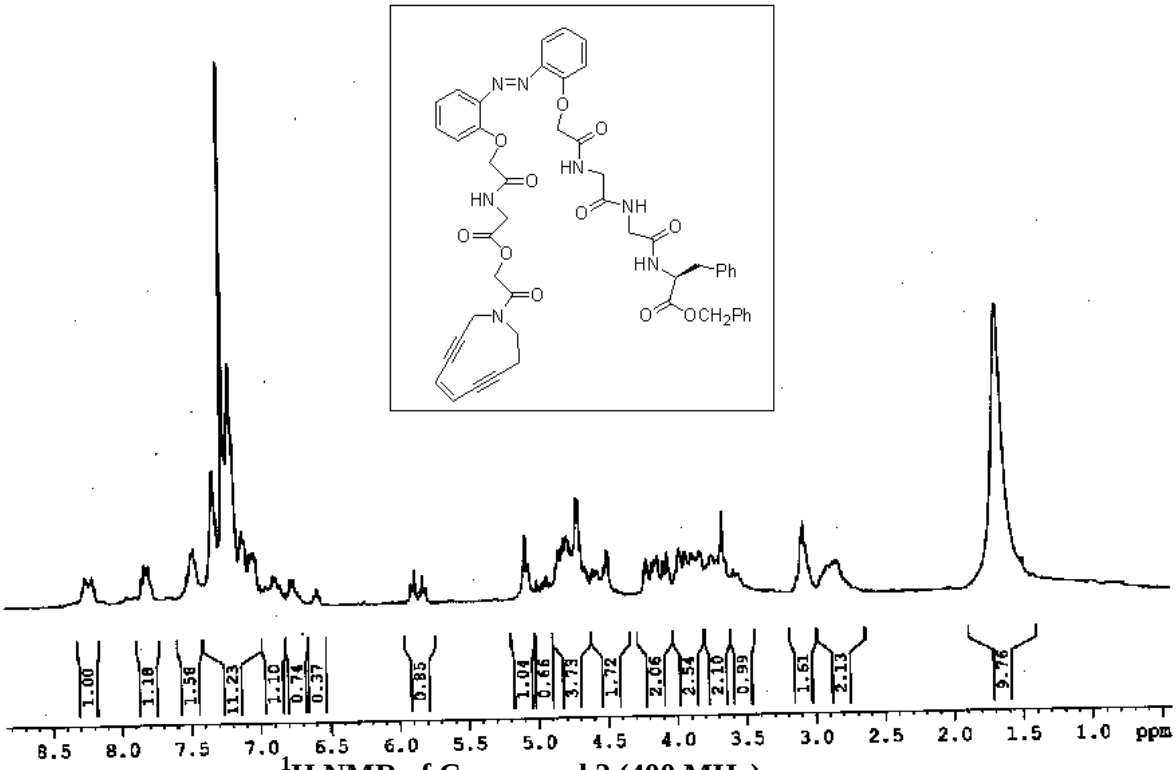
^{13}C NMR of Compound 26 (100 MHz)



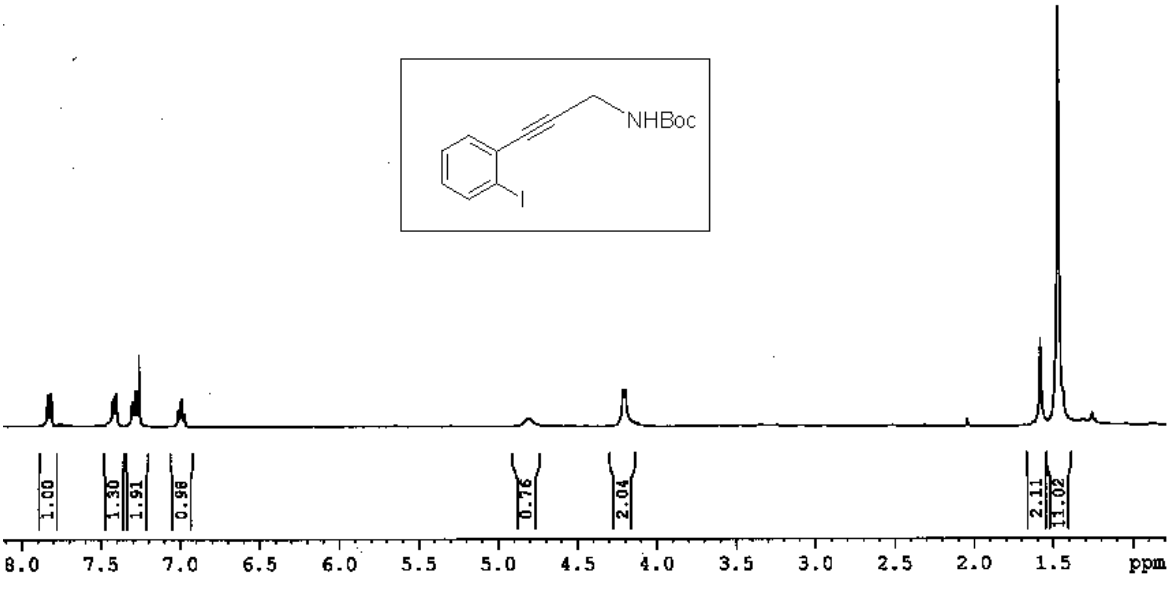
^{1}H NMR of Compound 1 (400 MHz)



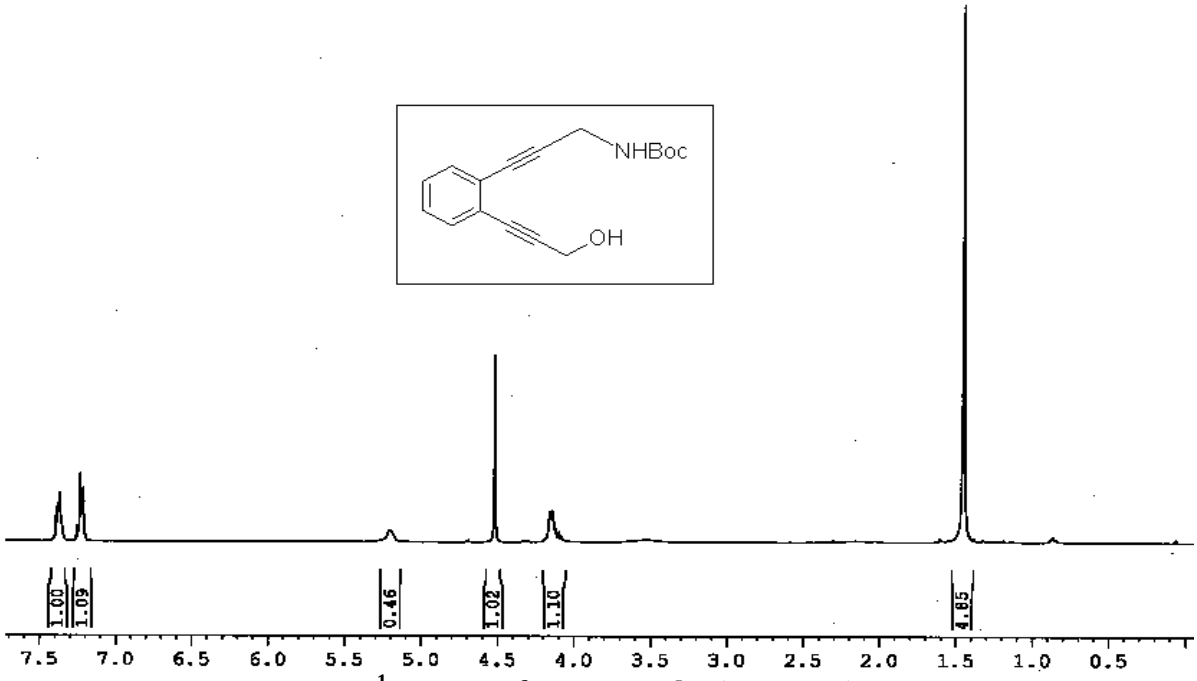
^{13}C NMR of Compound 1 (100 MHz)



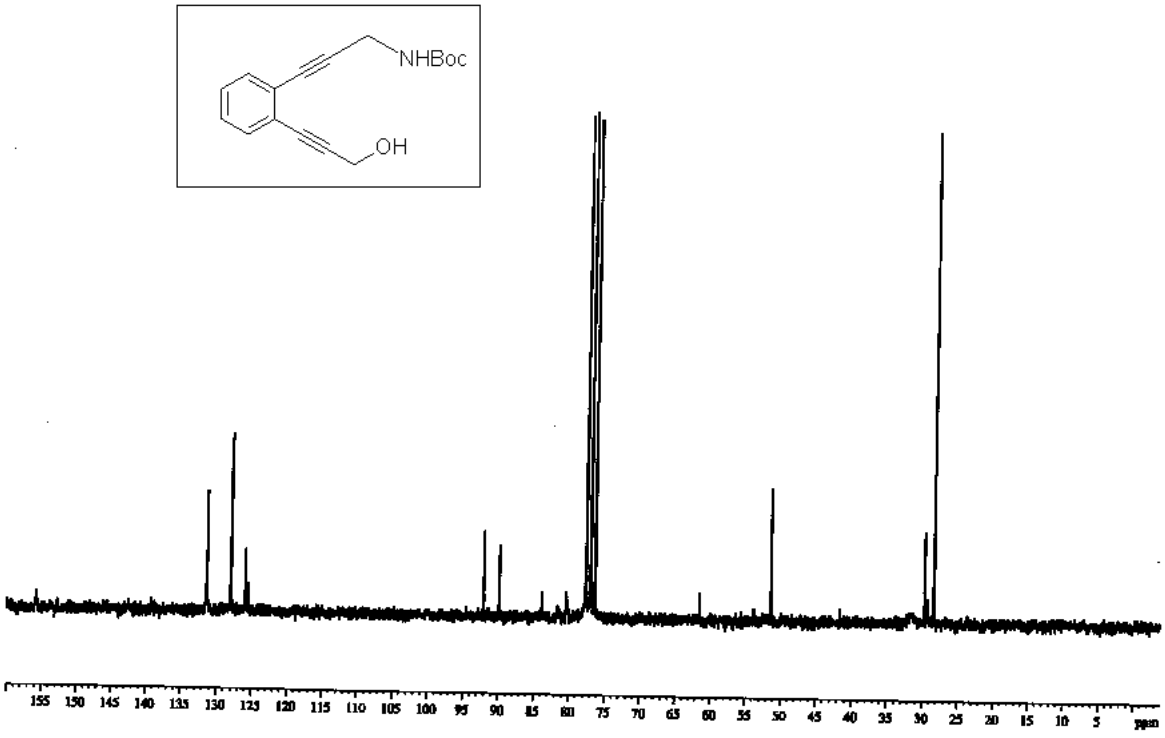
^{1}H NMR of Compound 2 (400 MHz)



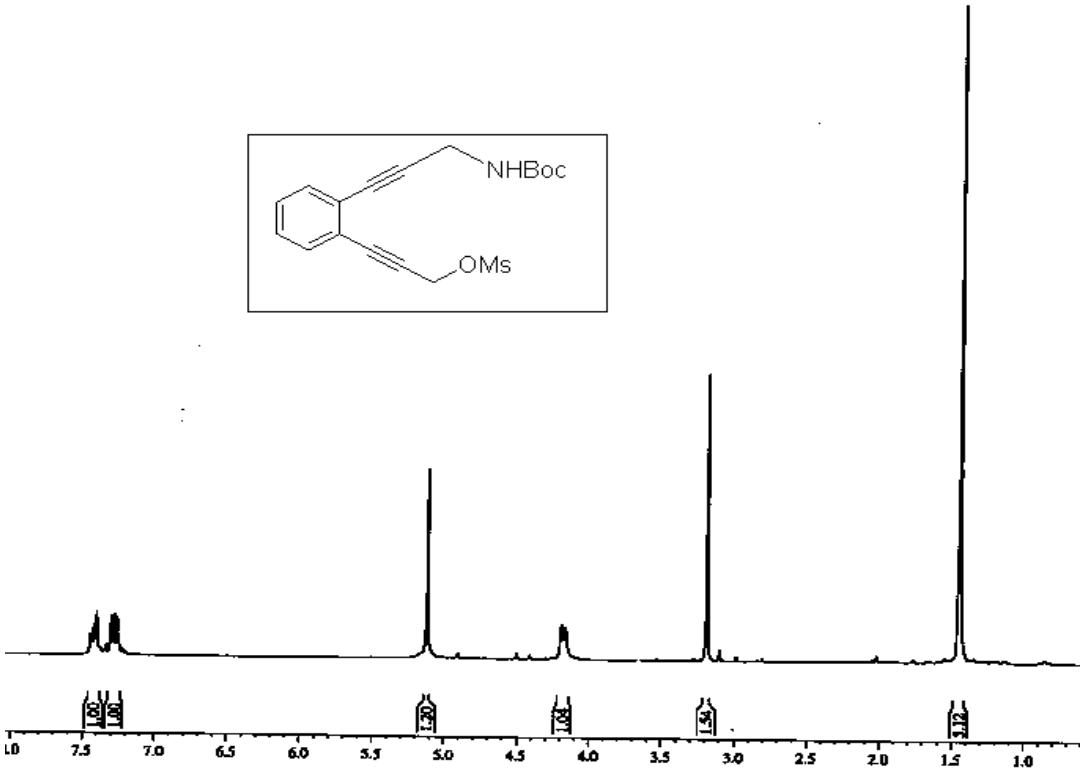
^{1}H NMR of Compound 5a (400 MHz)



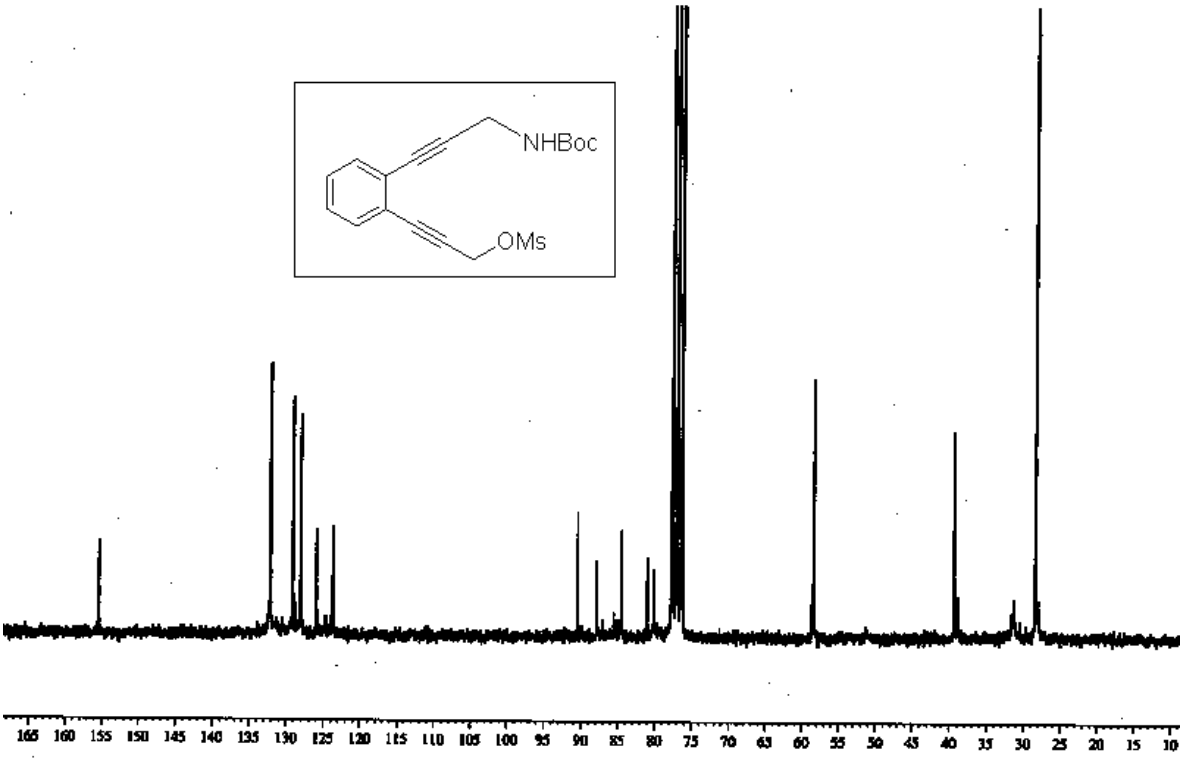
^{1}H NMR of Compound 5 (400 MHz)



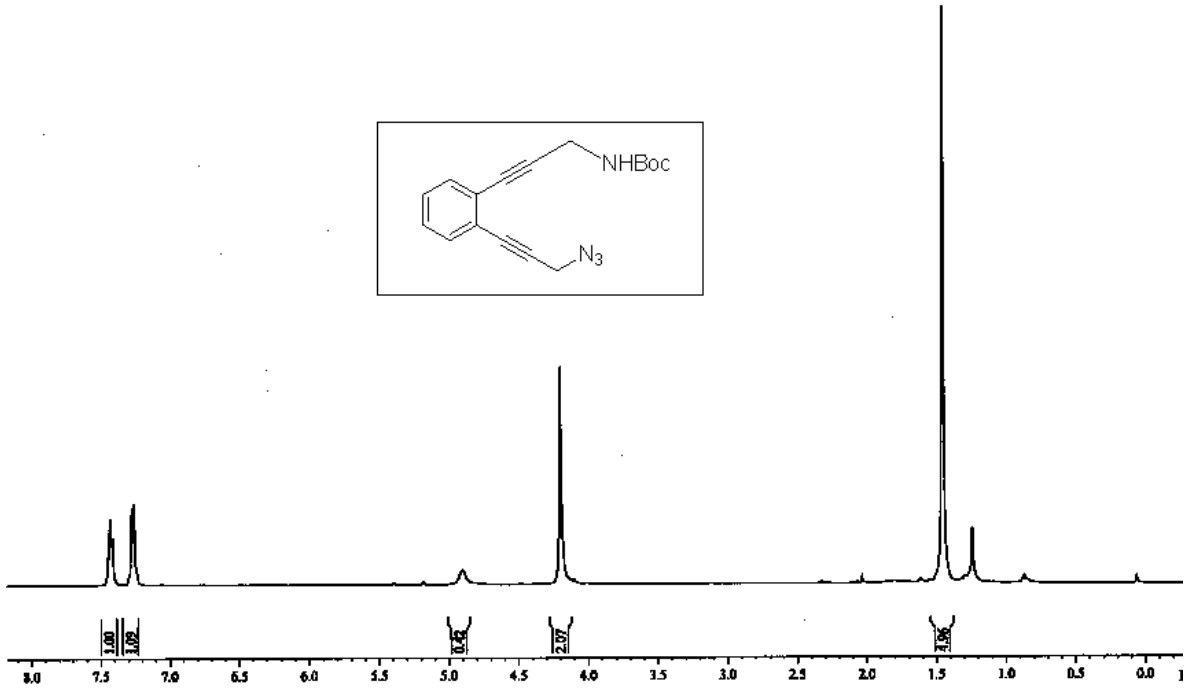
^{13}C NMR of Compound 5 (50 MHz)



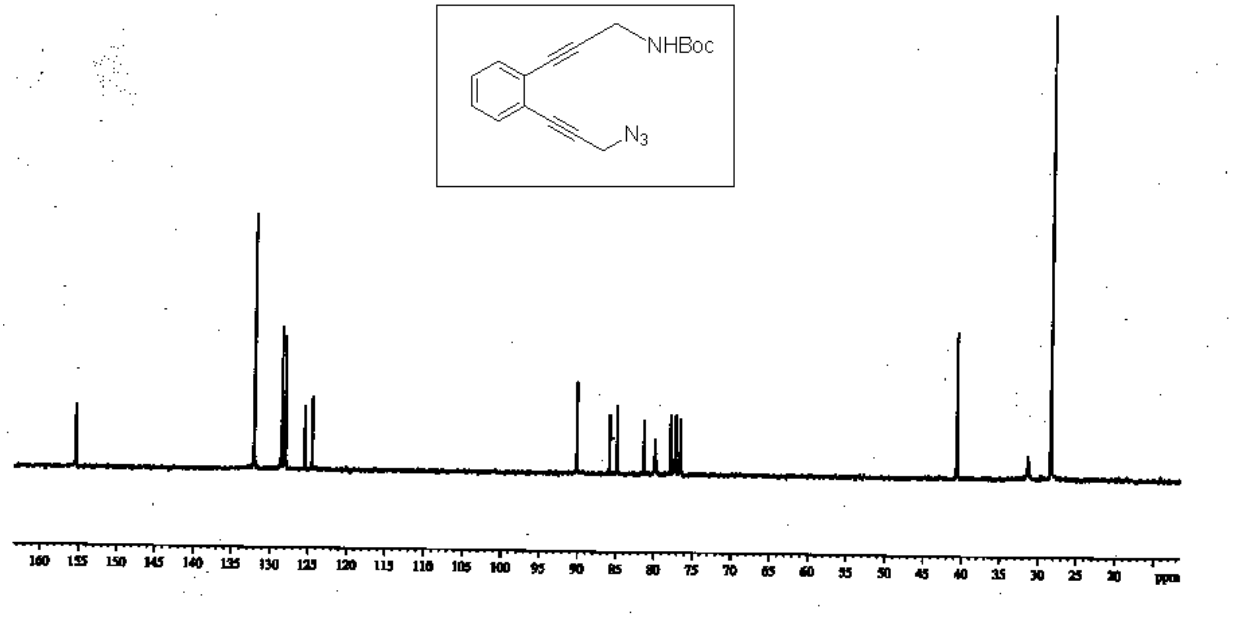
^{1}H NMR of Compound 6 (200 MHz)



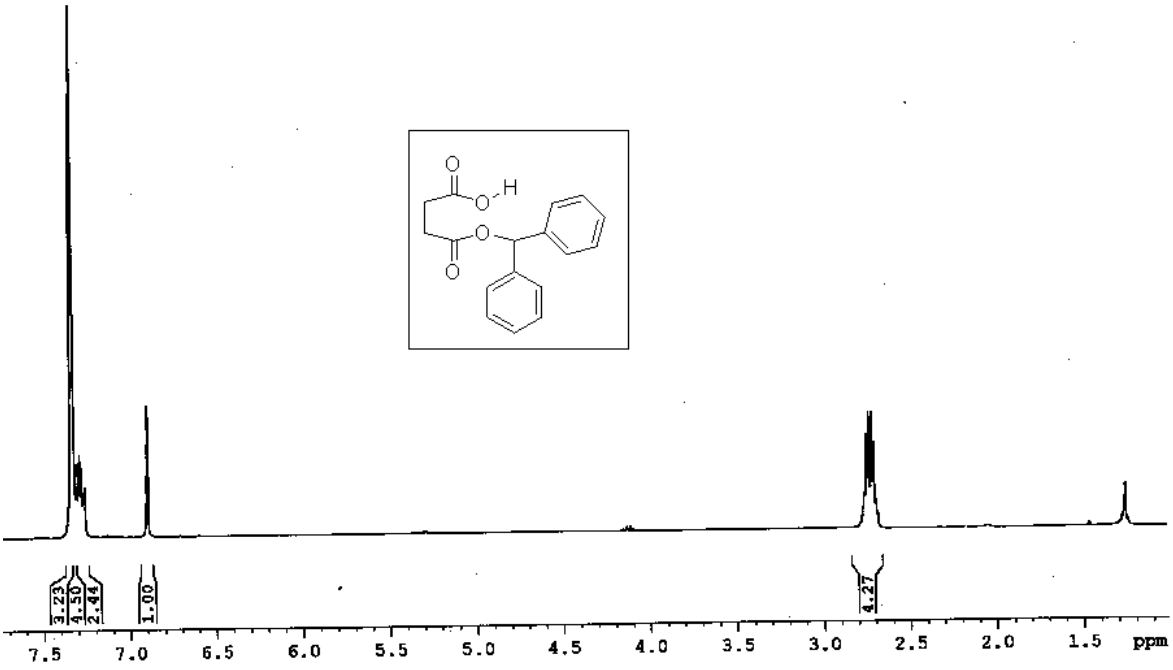
^{13}C NMR of Compound 6 (50 MHz)



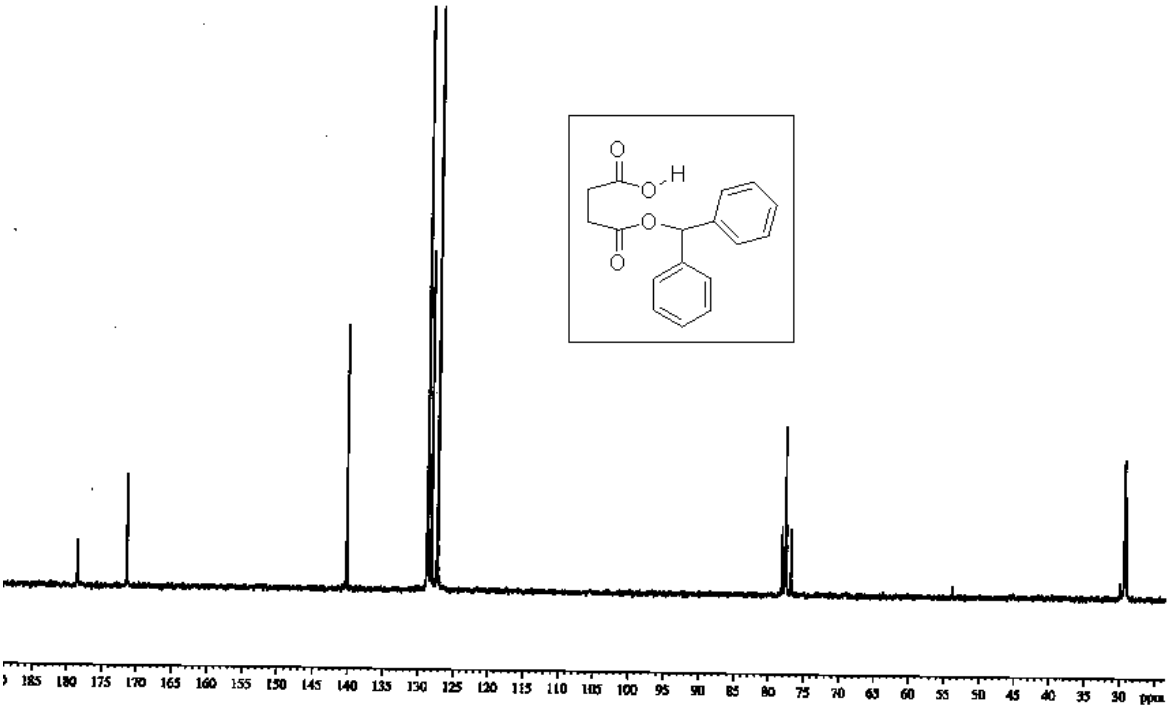
^{1}H NMR of Compound 7 (400 MHz)



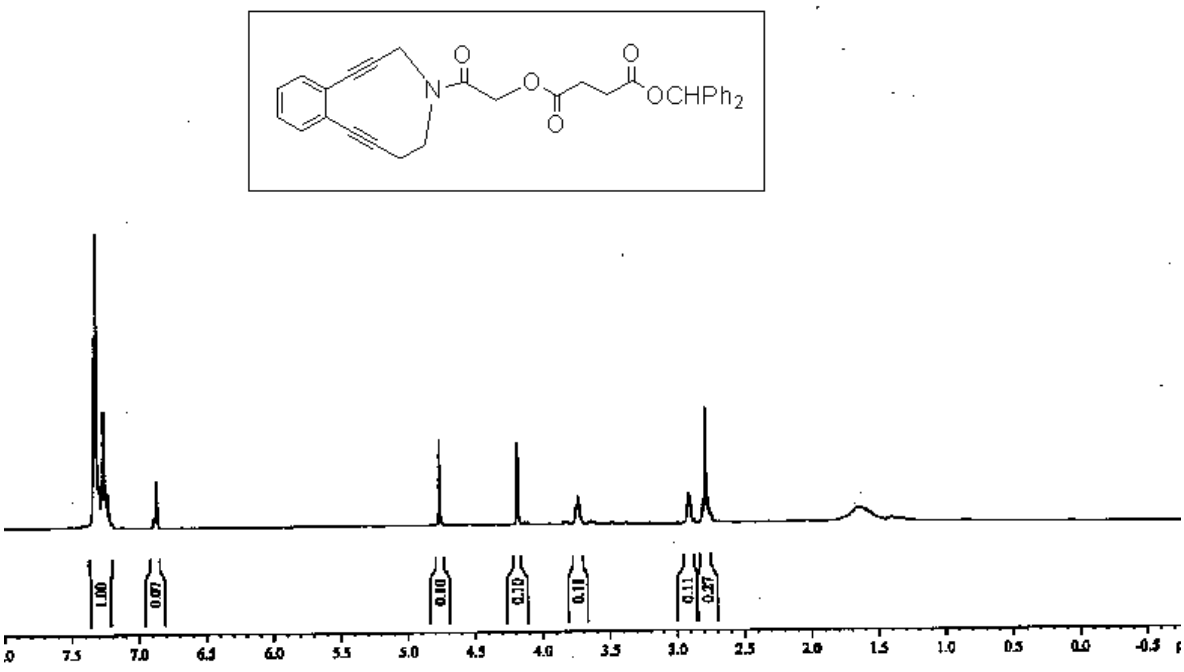
^{13}C NMR of Compound 7 (50 MHz)



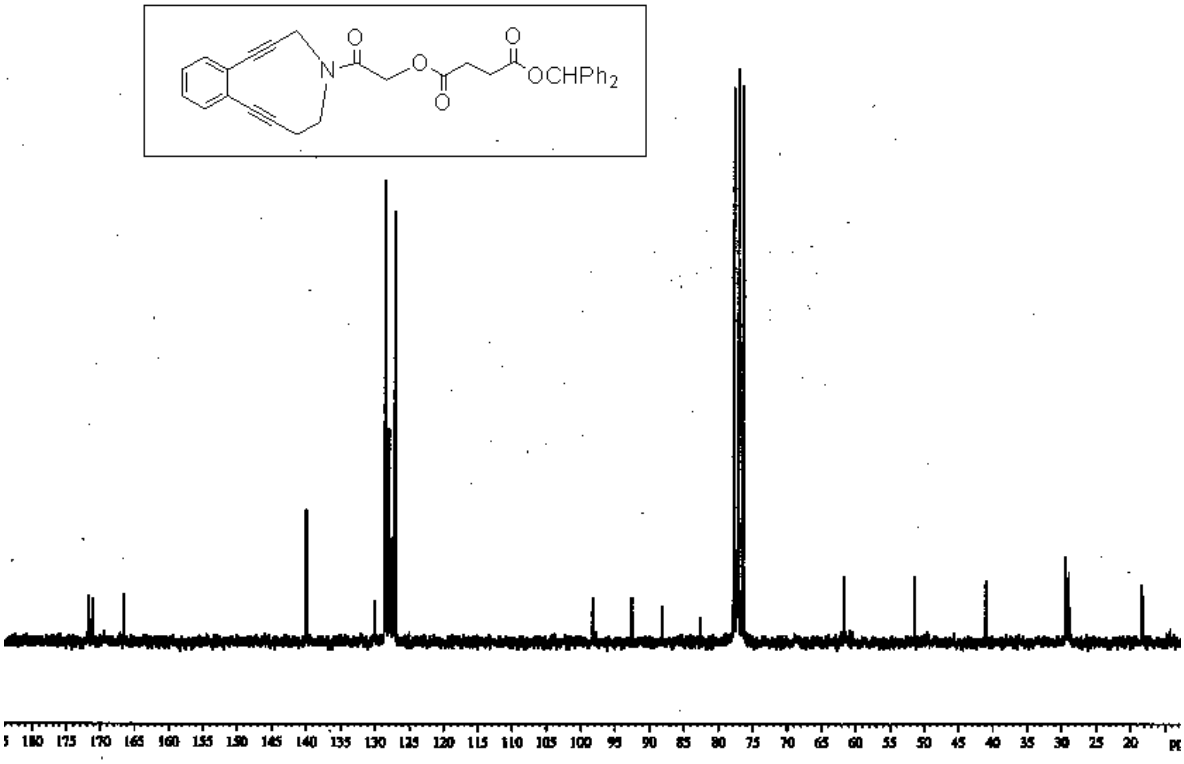
^{1}H NMR of Compound 10 (400 MHz)



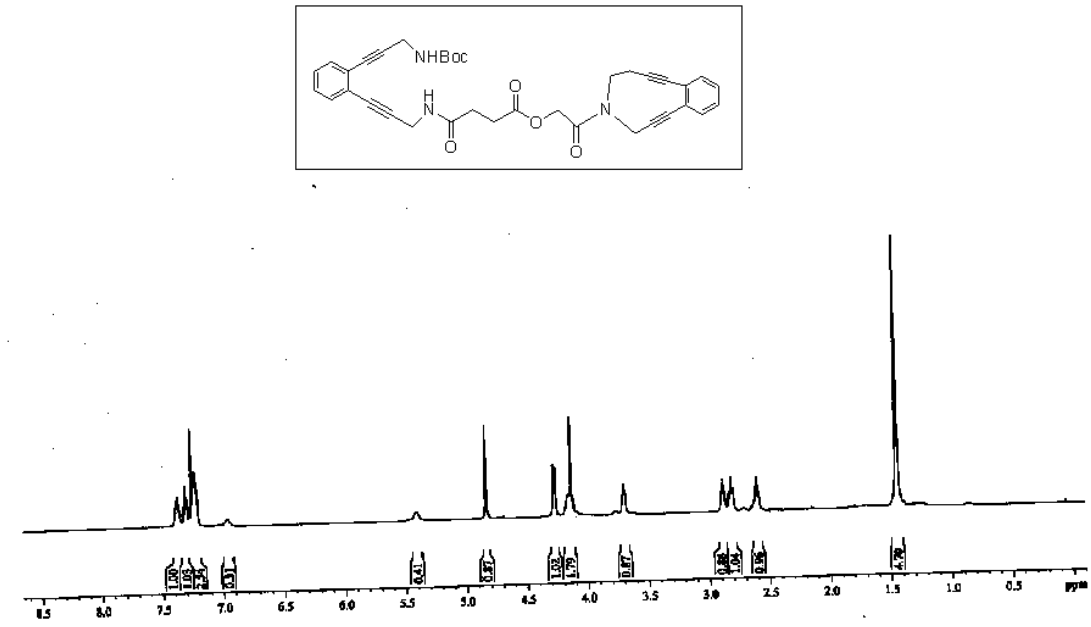
^{13}C NMR of Compound 10 (50 MHz)



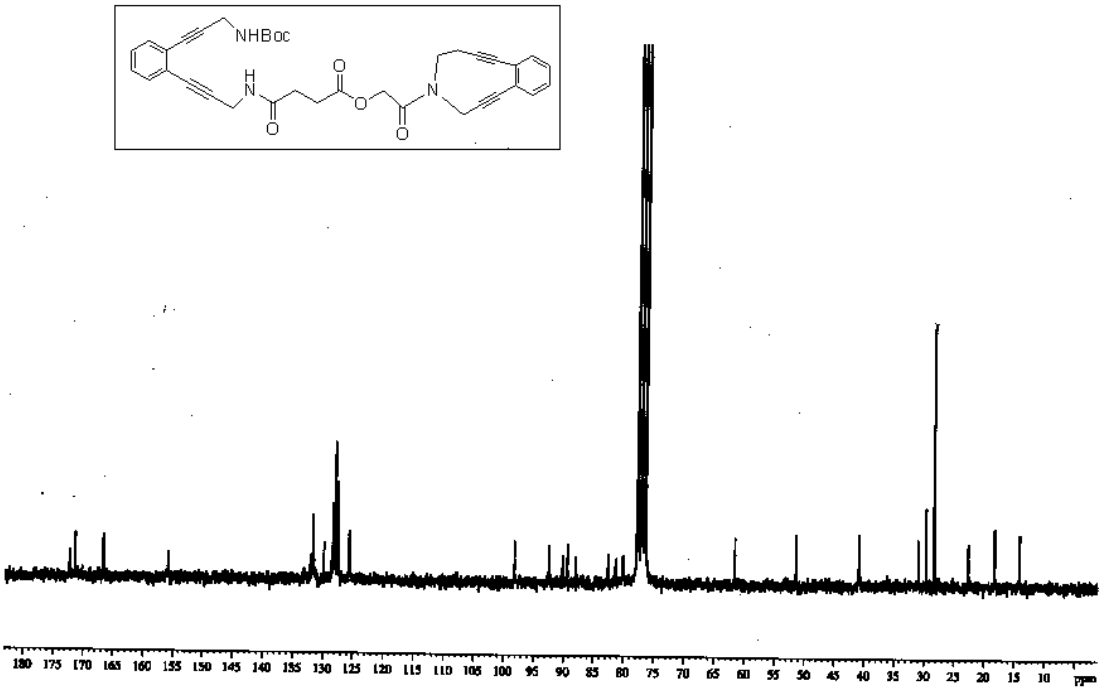
^{1}H NMR of Compound 11 (400 MHz)



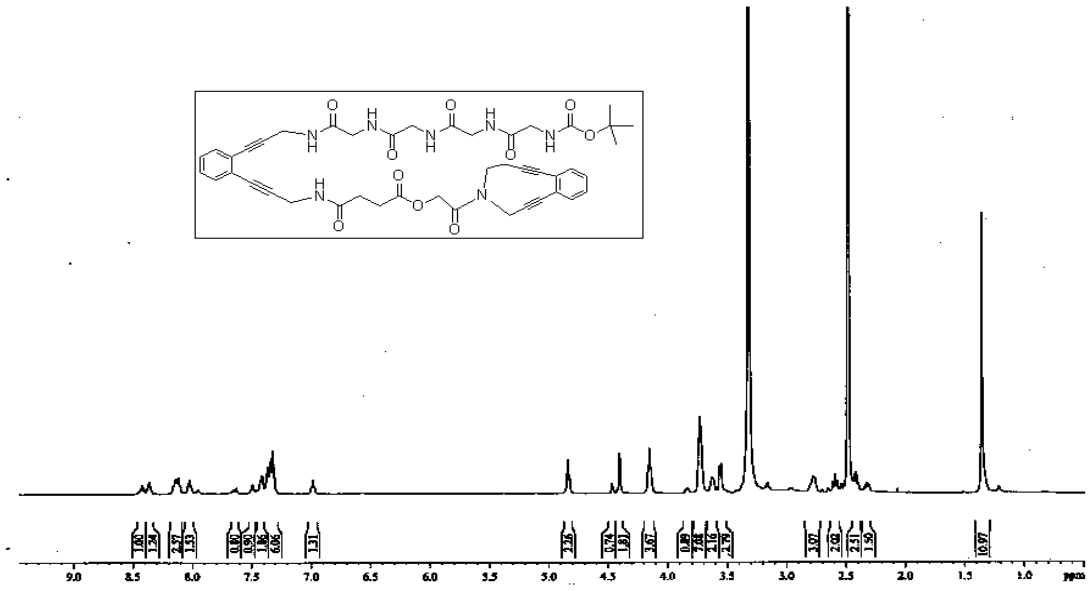
^{13}C NMR of Compound 11 (50 MHz)



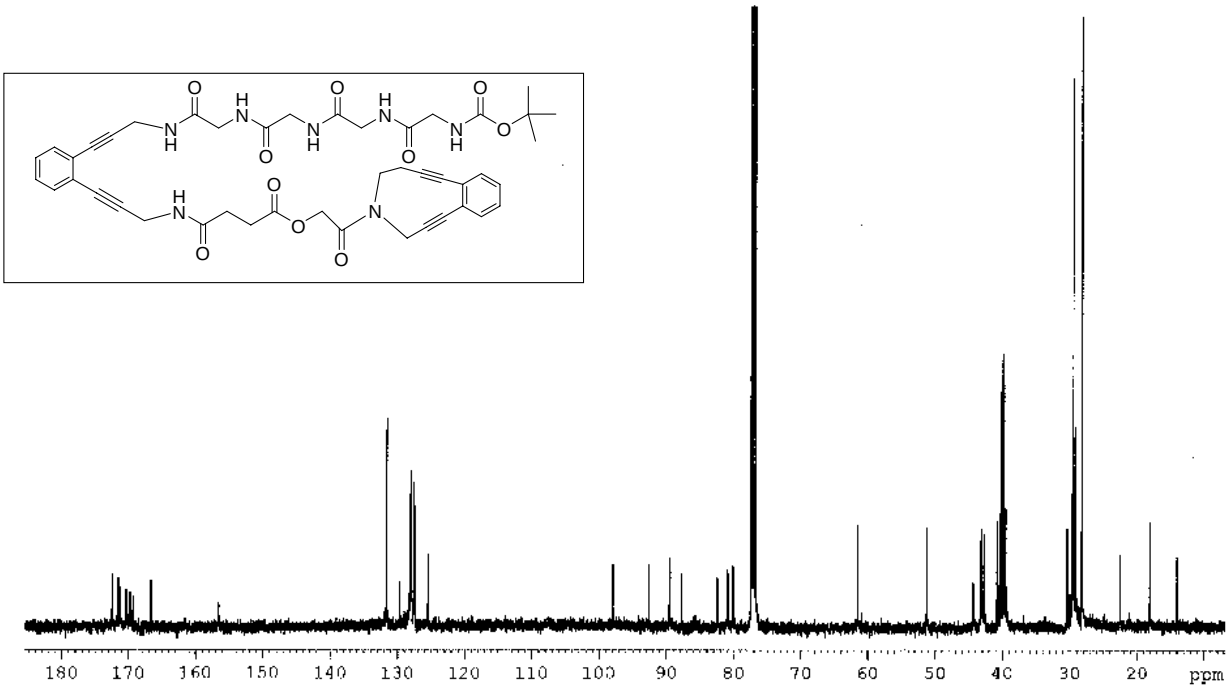
^{1}H NMR of Compound 13 (400 MHz)



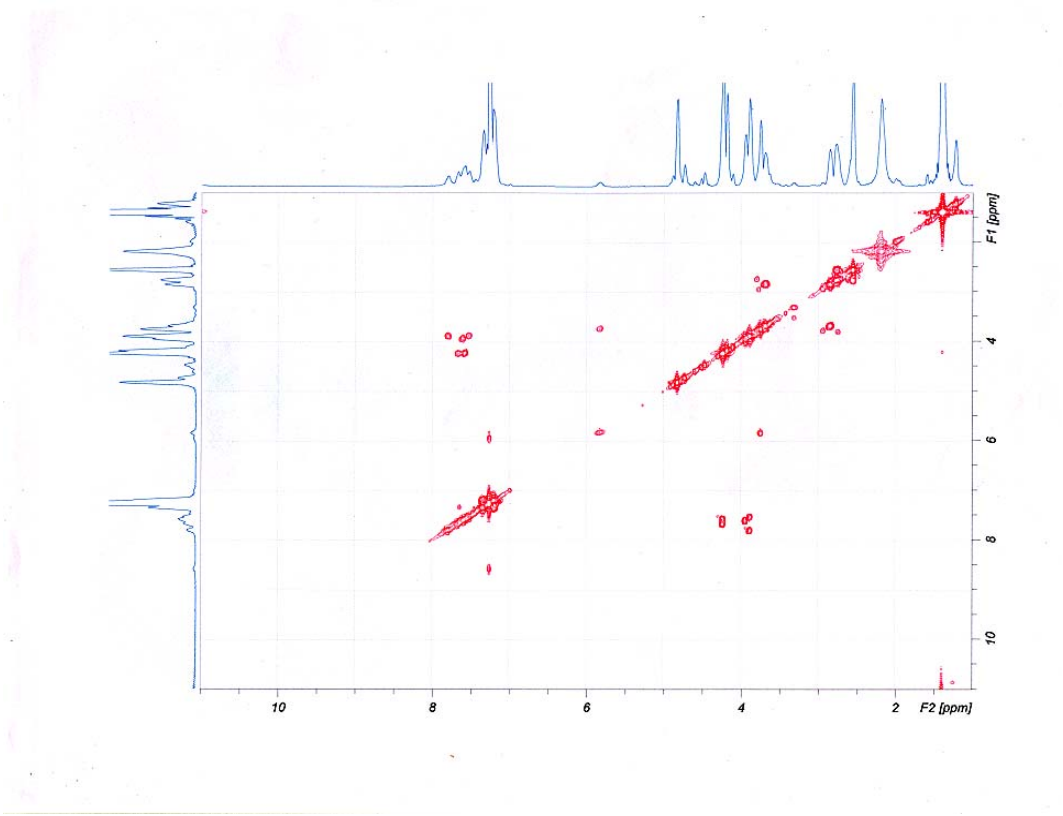
^{13}C NMR of Compound 13 (50 MHz)



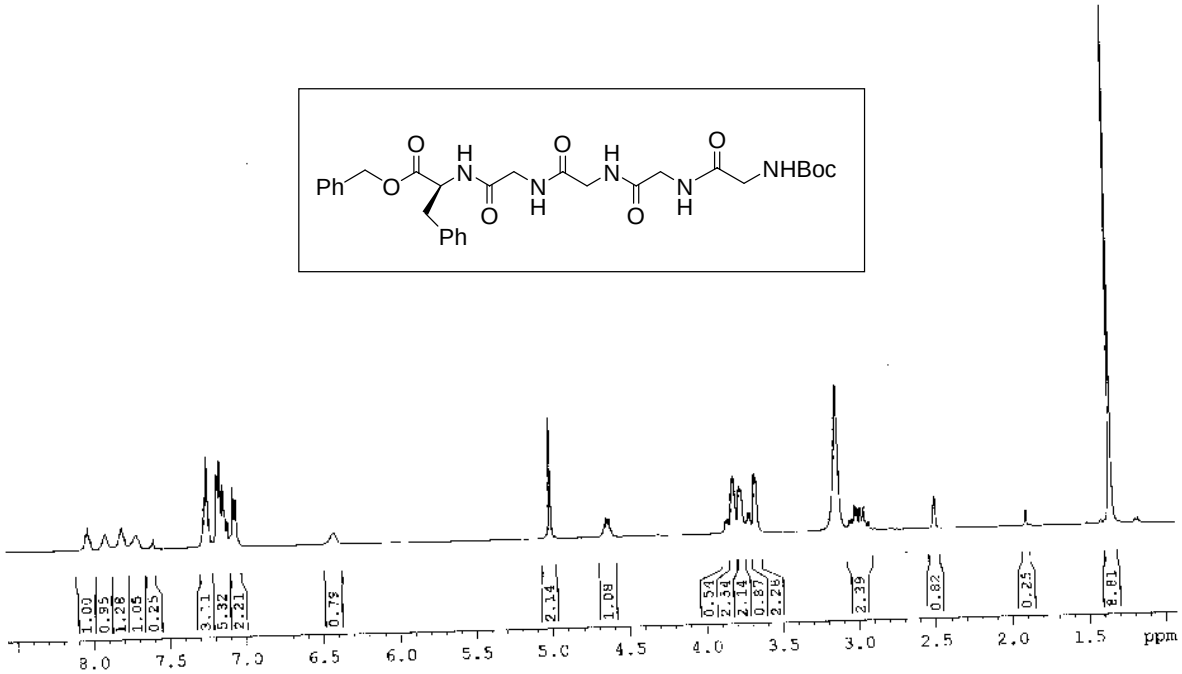
^{1}H NMR of Compound 3 (400 MHz)



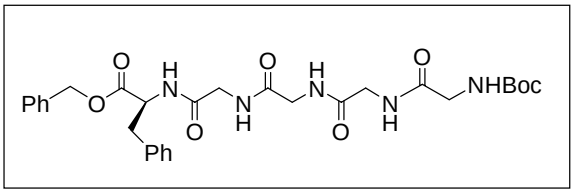
^{13}C NMR of Compound 3 (100 MHz)

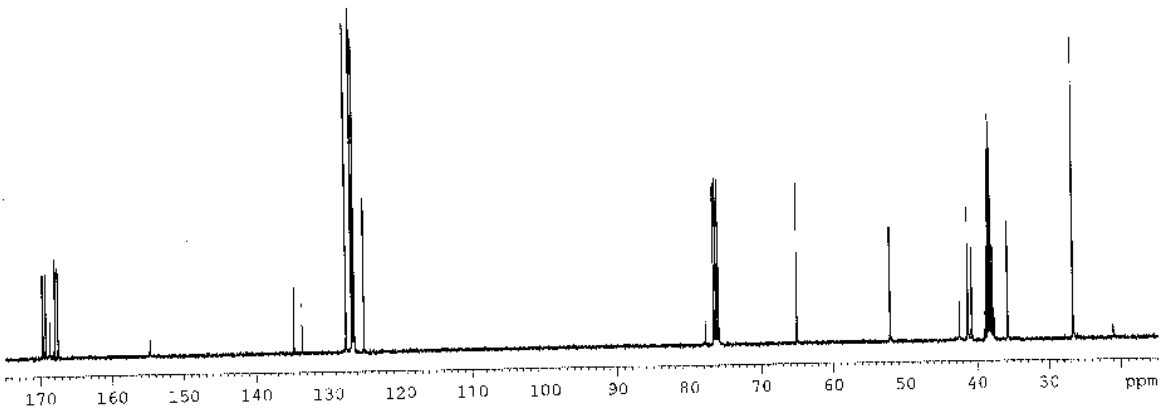


The ^{1}H COSY spectra of compounds 3:



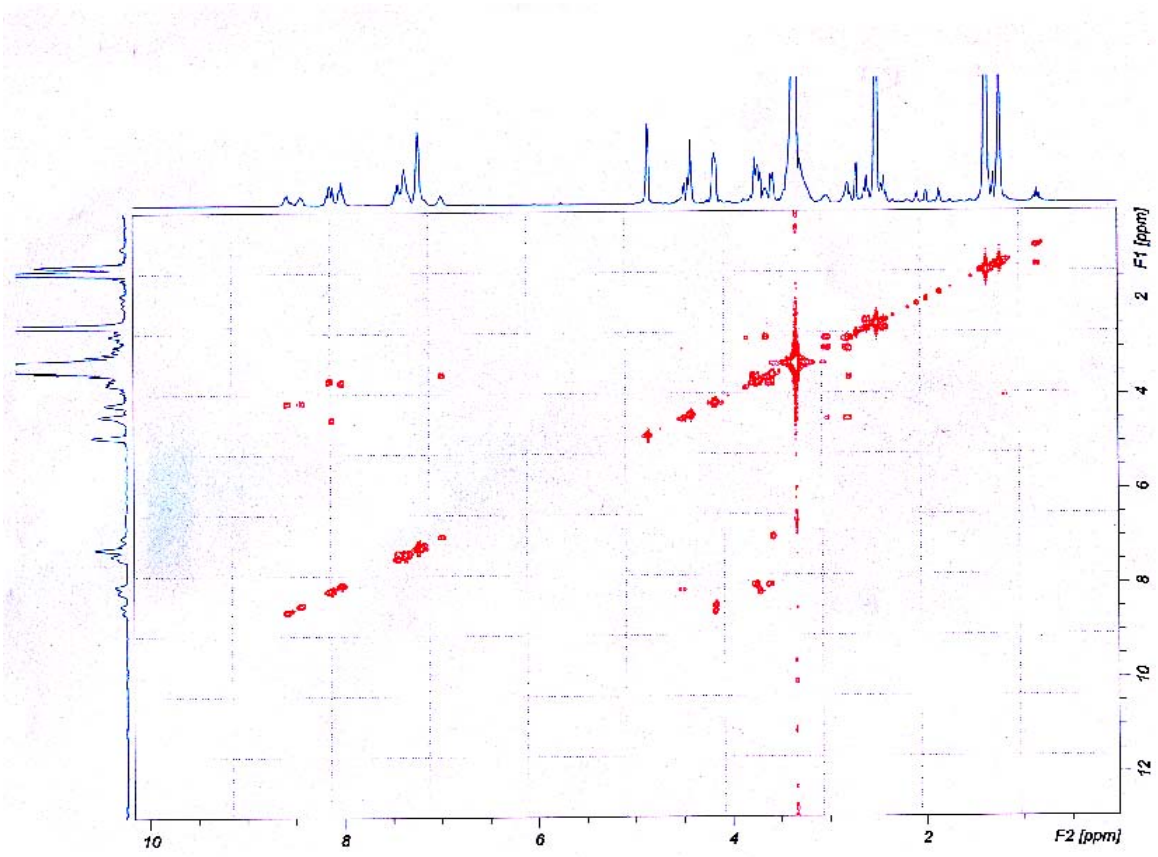
^{1}H NMR of Compound 4a (400 MHz)





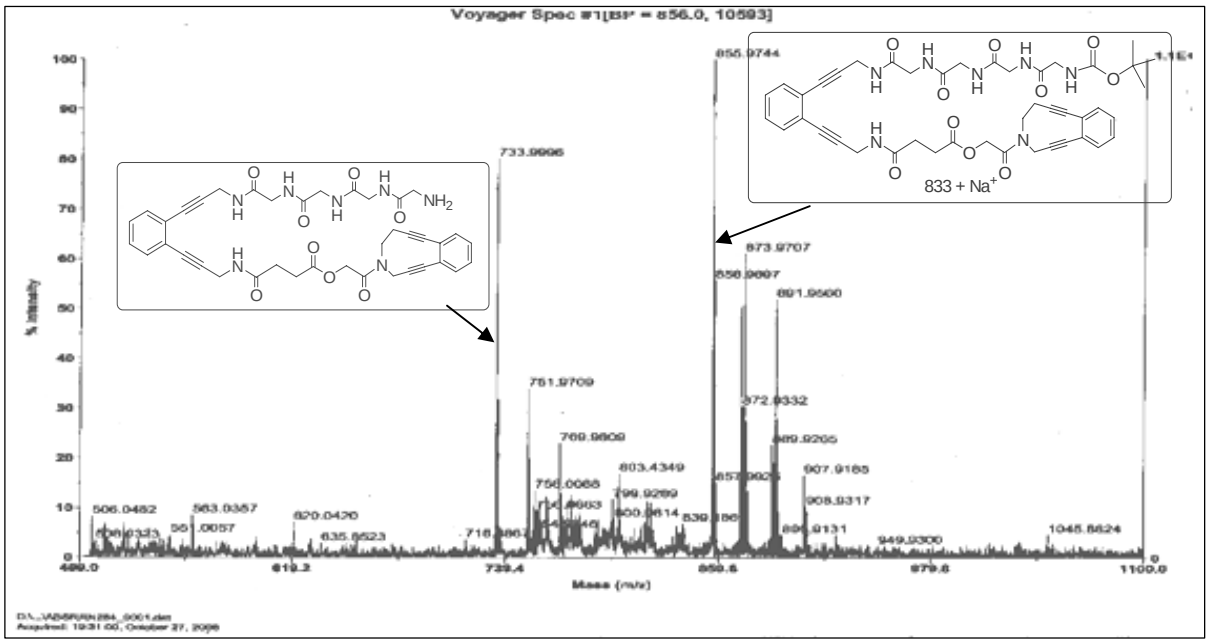
^{13}C NMR of Compound 4a (100 MHz)

^{1}H NMR of Compound 4 (400 MHz)

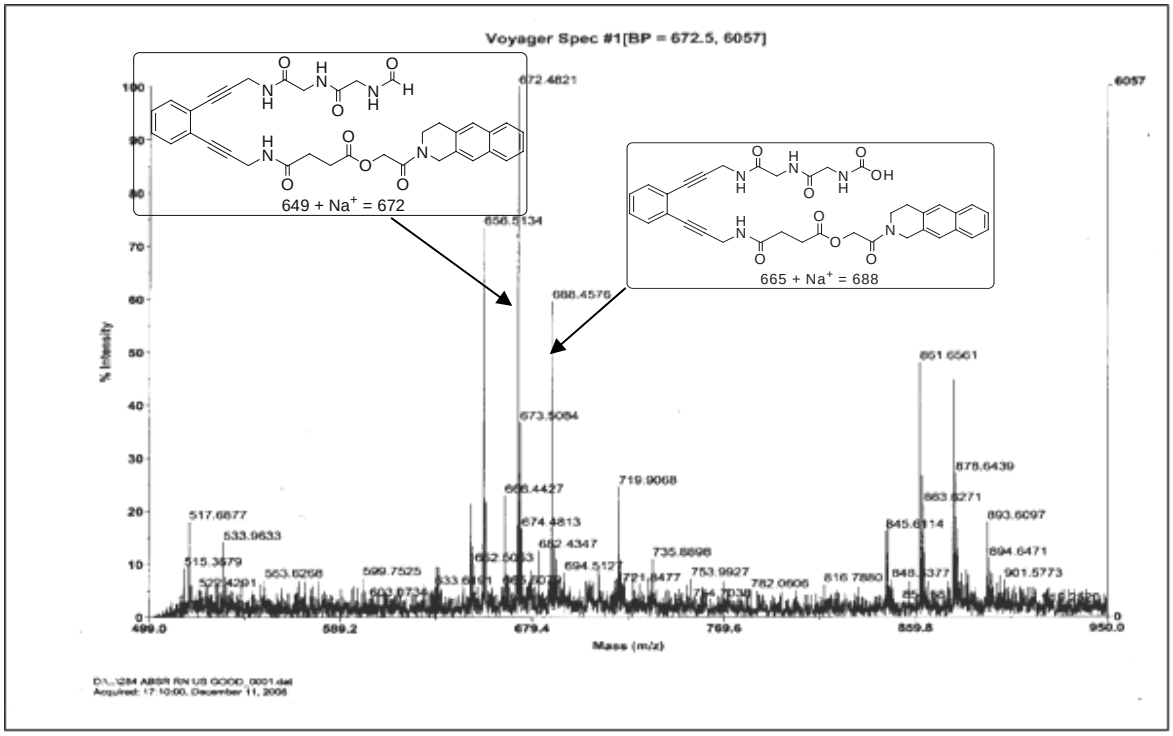


The ^{1}H COSY spectra of compounds 4:

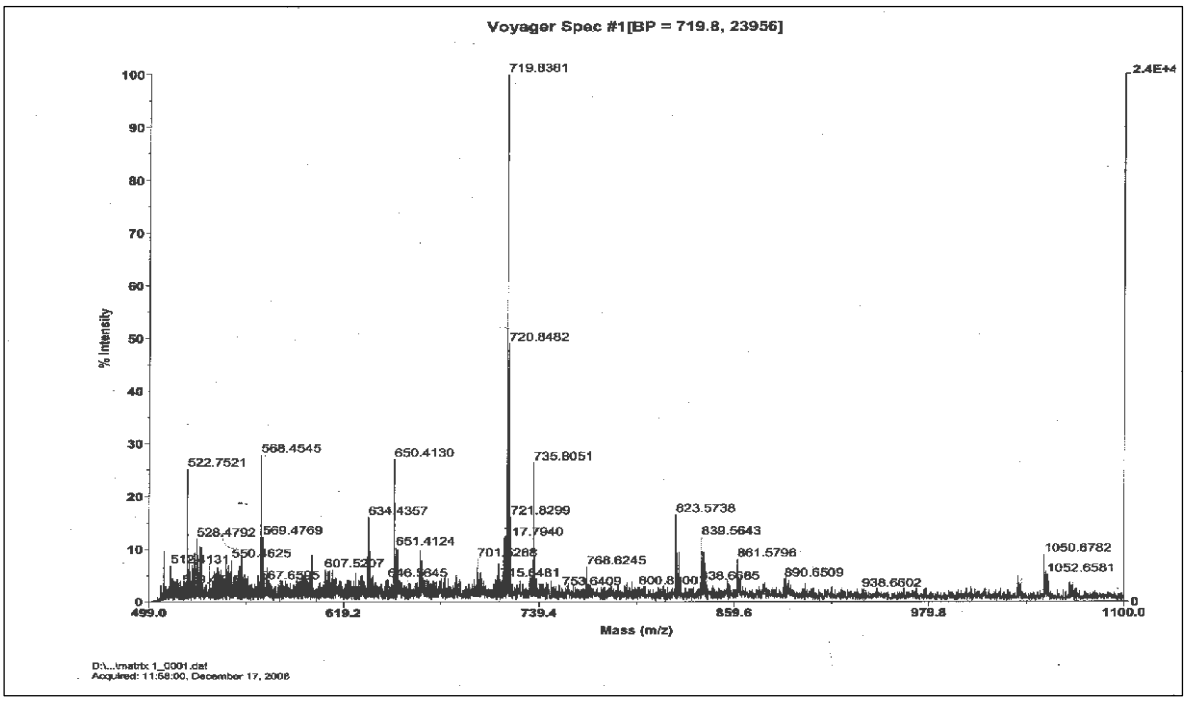
MALDI-TOF-MS of Compound 3 (Before incubation):



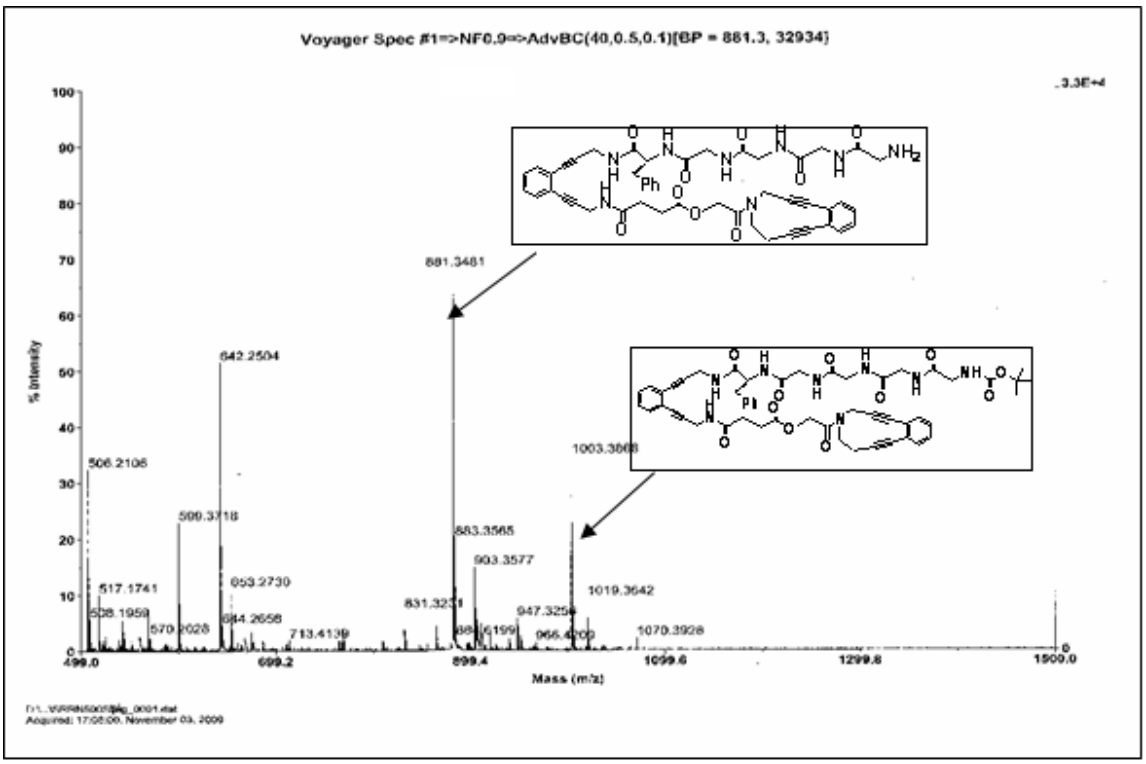
MALDI-TOF-MS of Compound 3 (After 6 days of incubation at 70 ^{o}C):



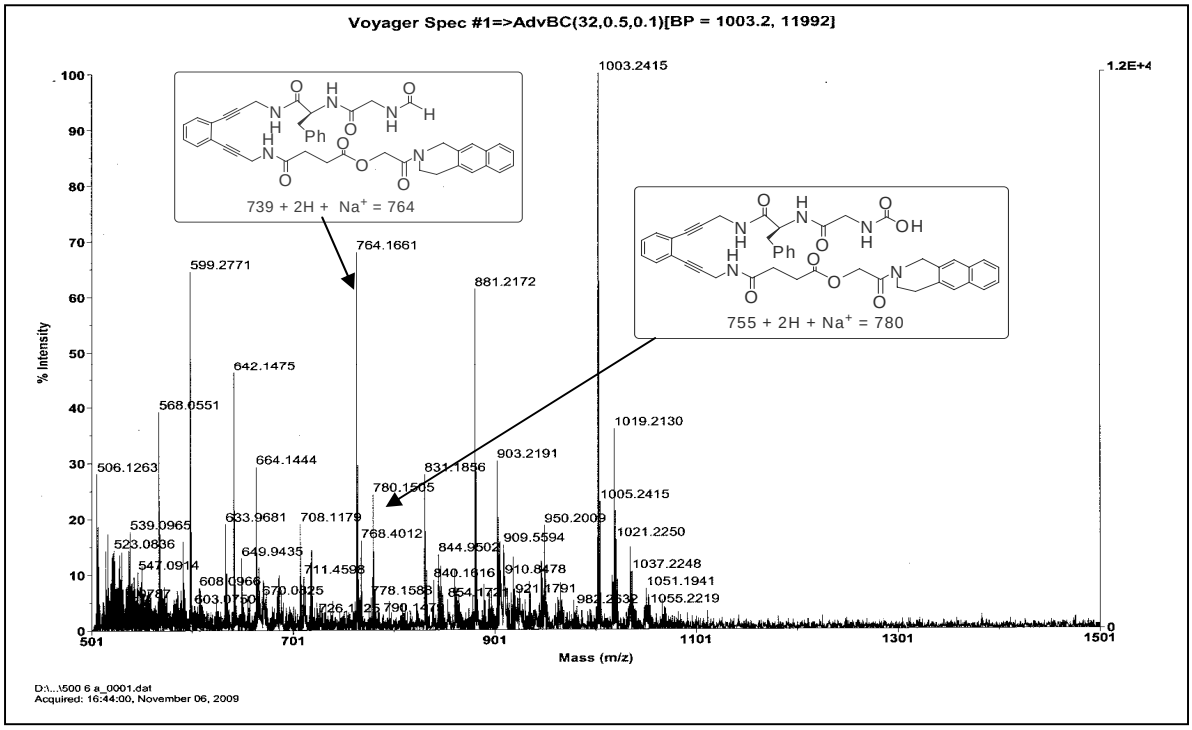
MALDI-TOF-MS of Matrix (CHCA):



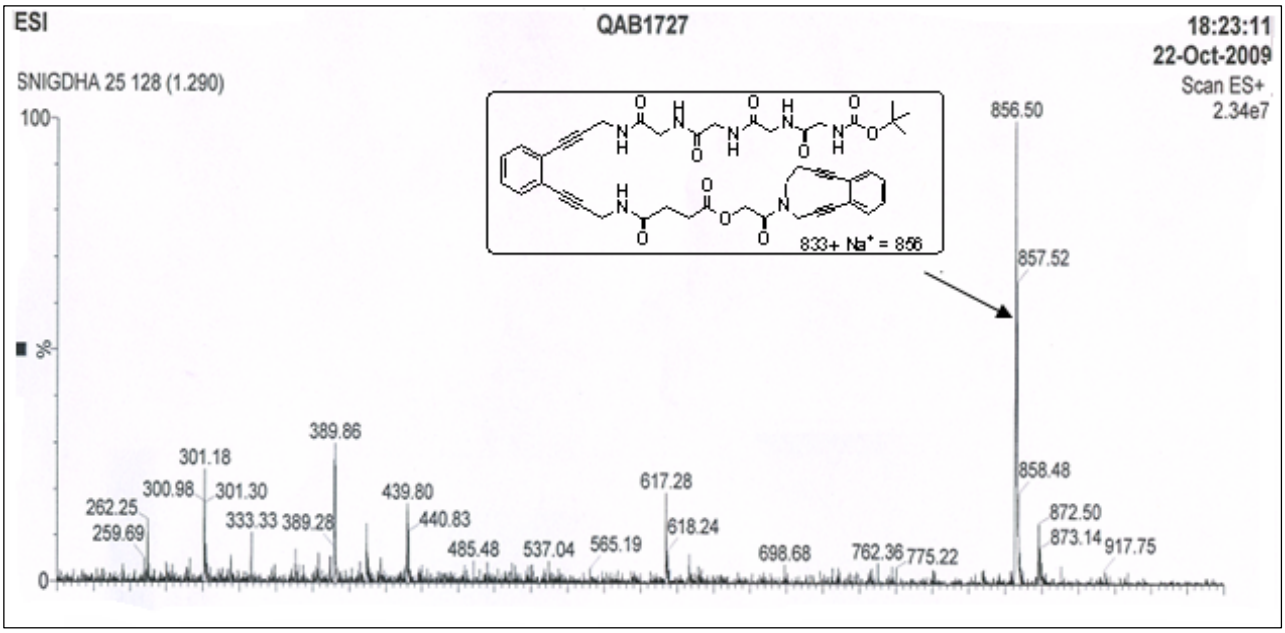
MALDI-TOF-MS of Compound 4 (Before incubation):



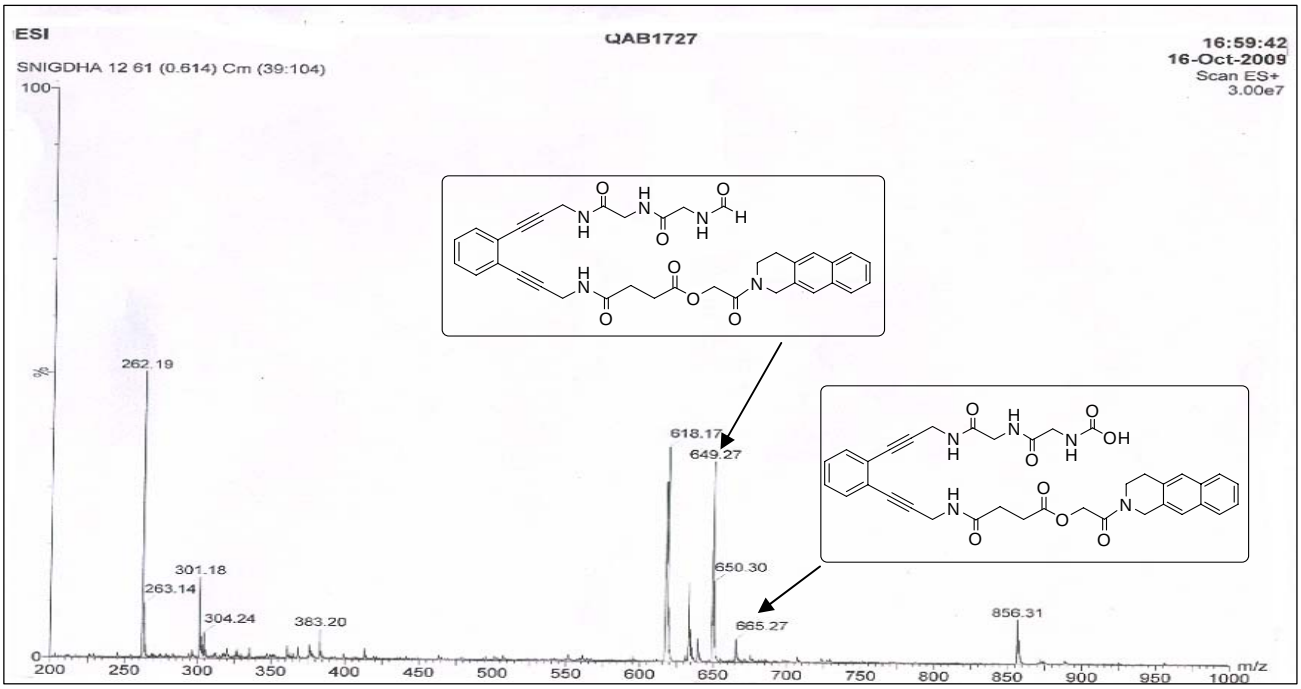
MALDI-TOF-MS of Compound 4 (After 6 days of incubation at 70 ^{o}C):



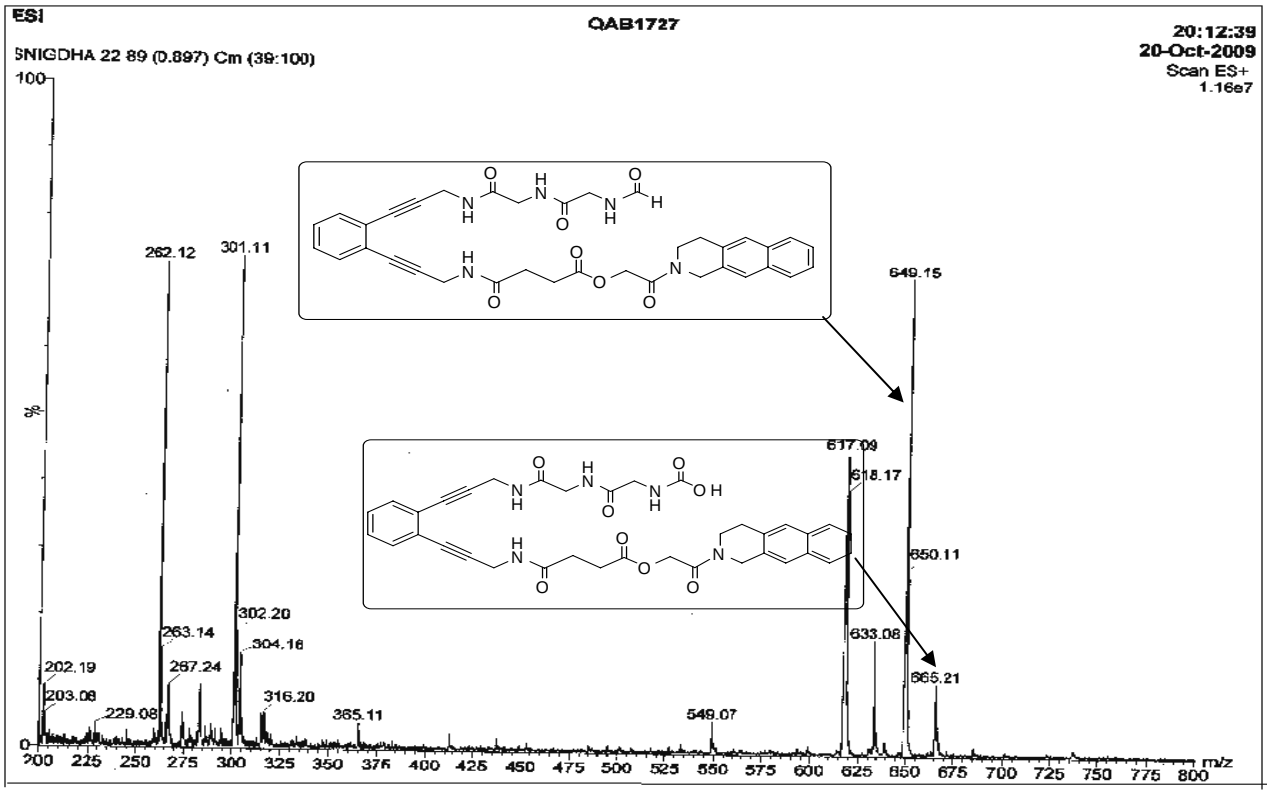
ESI mass spectra of compound 3:



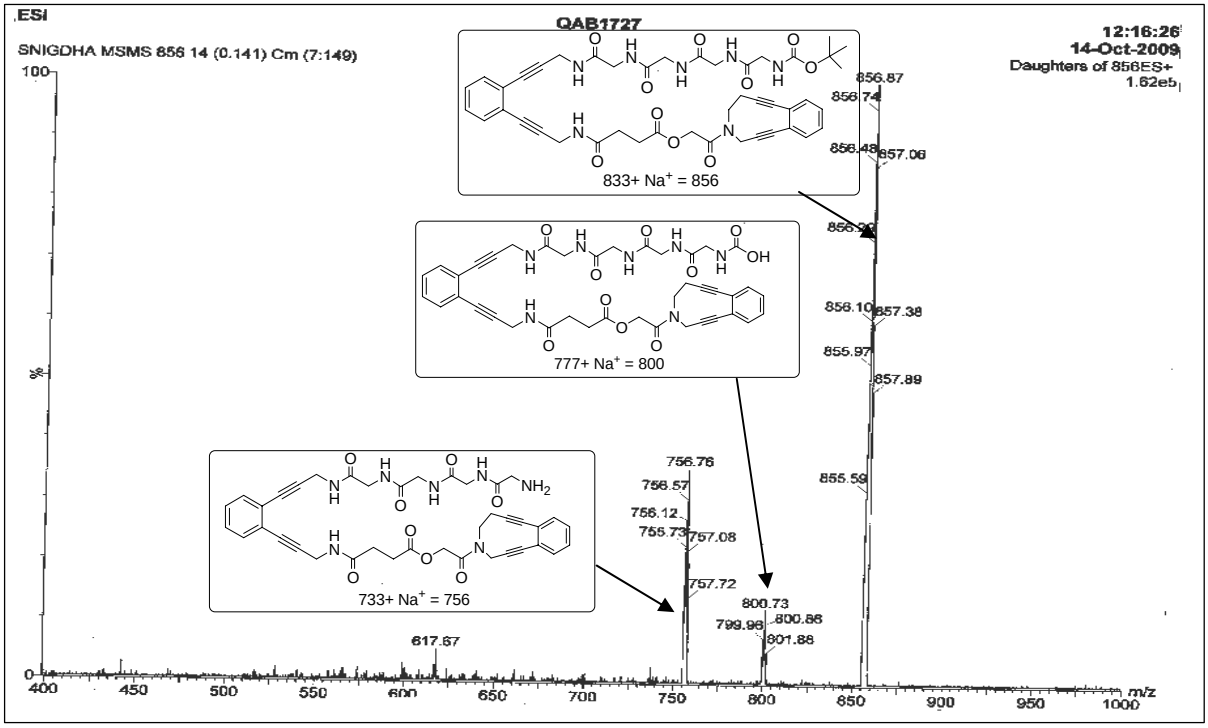
ESI mass spectra of compound 3 (After 3 days of incubation at 70 ^{o}C):



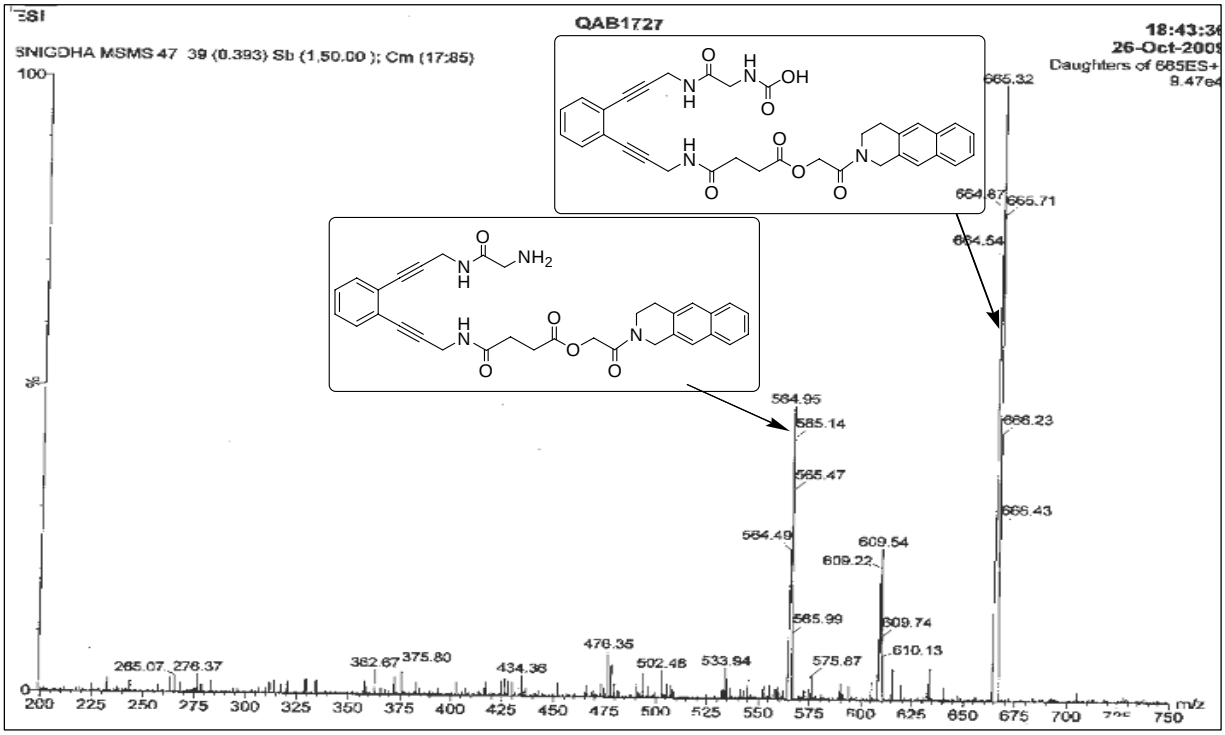
ESI mass spectra of compound 3 (After 6 days of incubation at 70 ^{o}C):



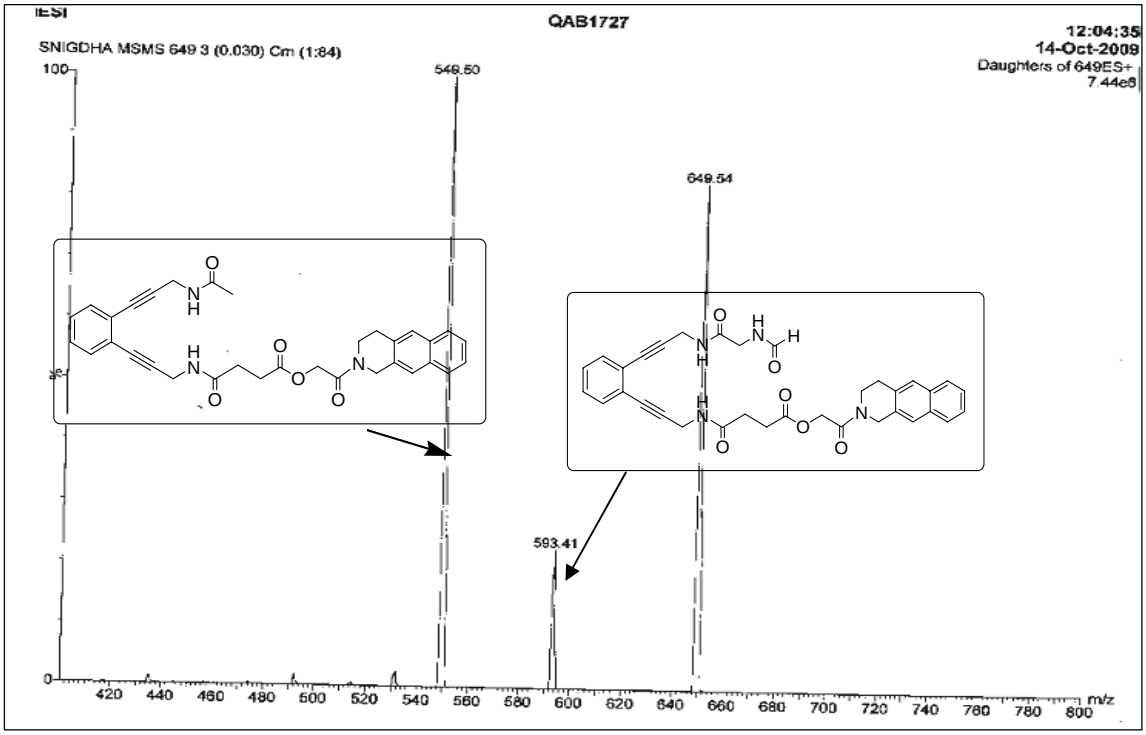
MSMS of peak at 856 mass value:



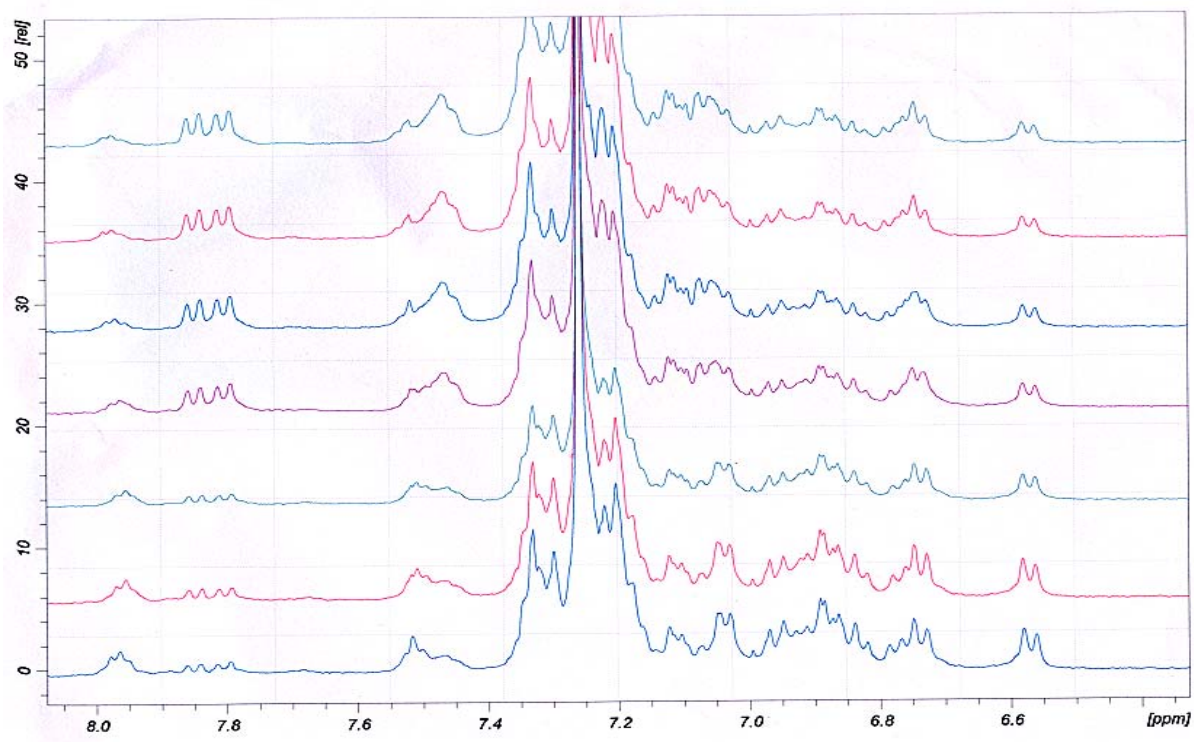
MSMS of peak at 665 mass value:



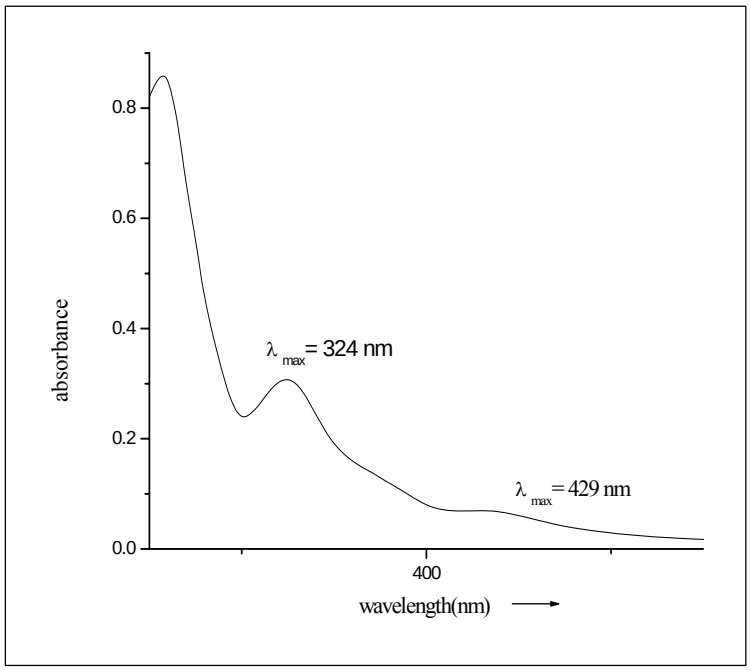
MSMS of peak at 649 mass value:



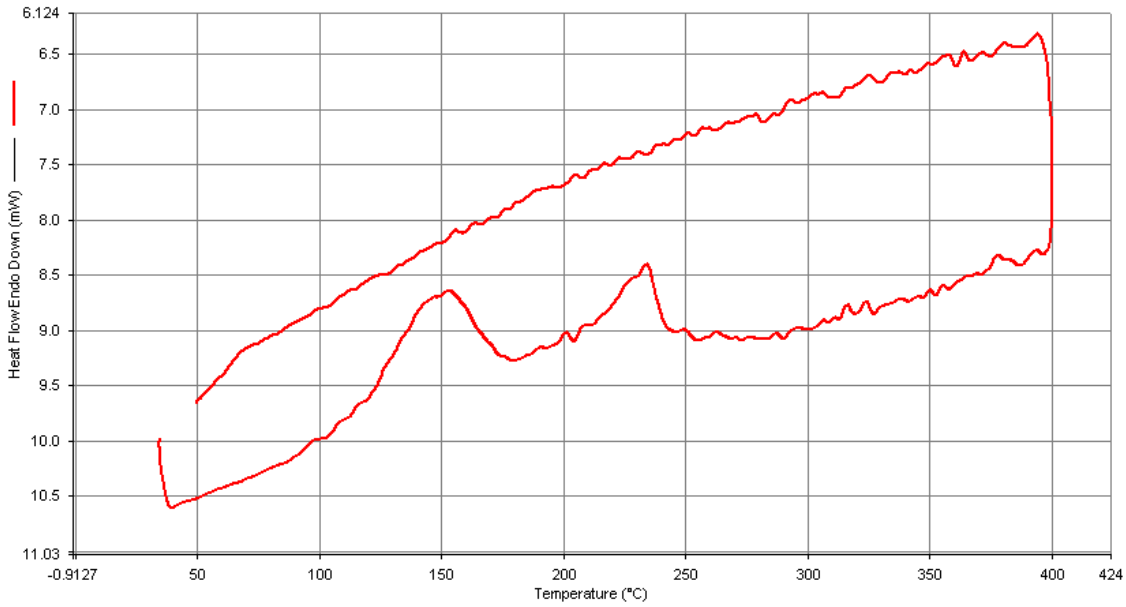
Reisomerisation Kinetic study of compound 1 after irradiation:



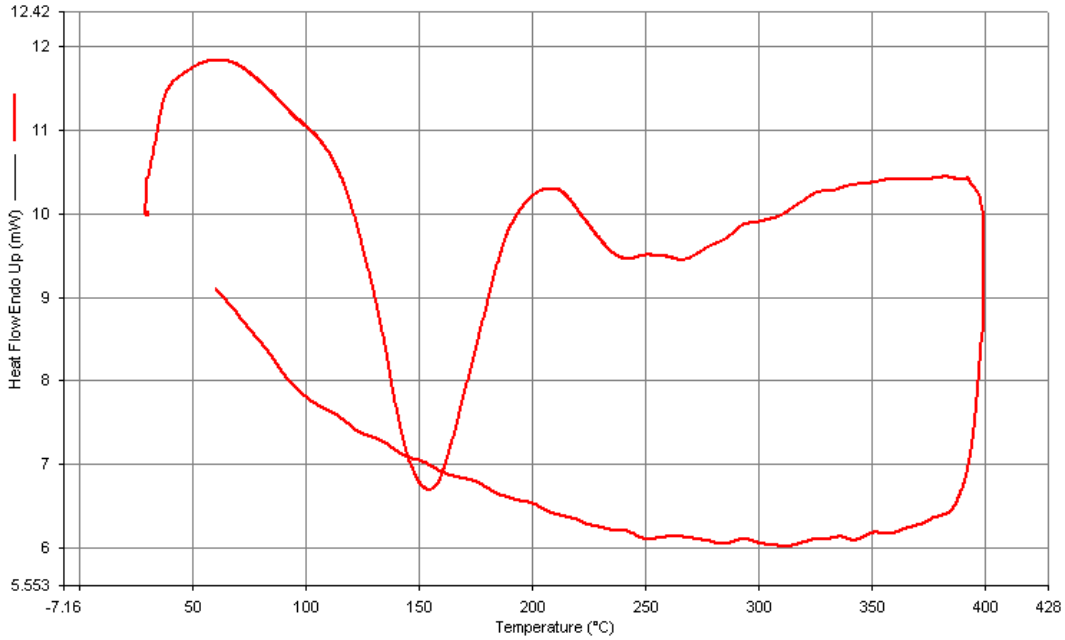
UV-vis absorption spectra of compound 1 in acetonitrile:



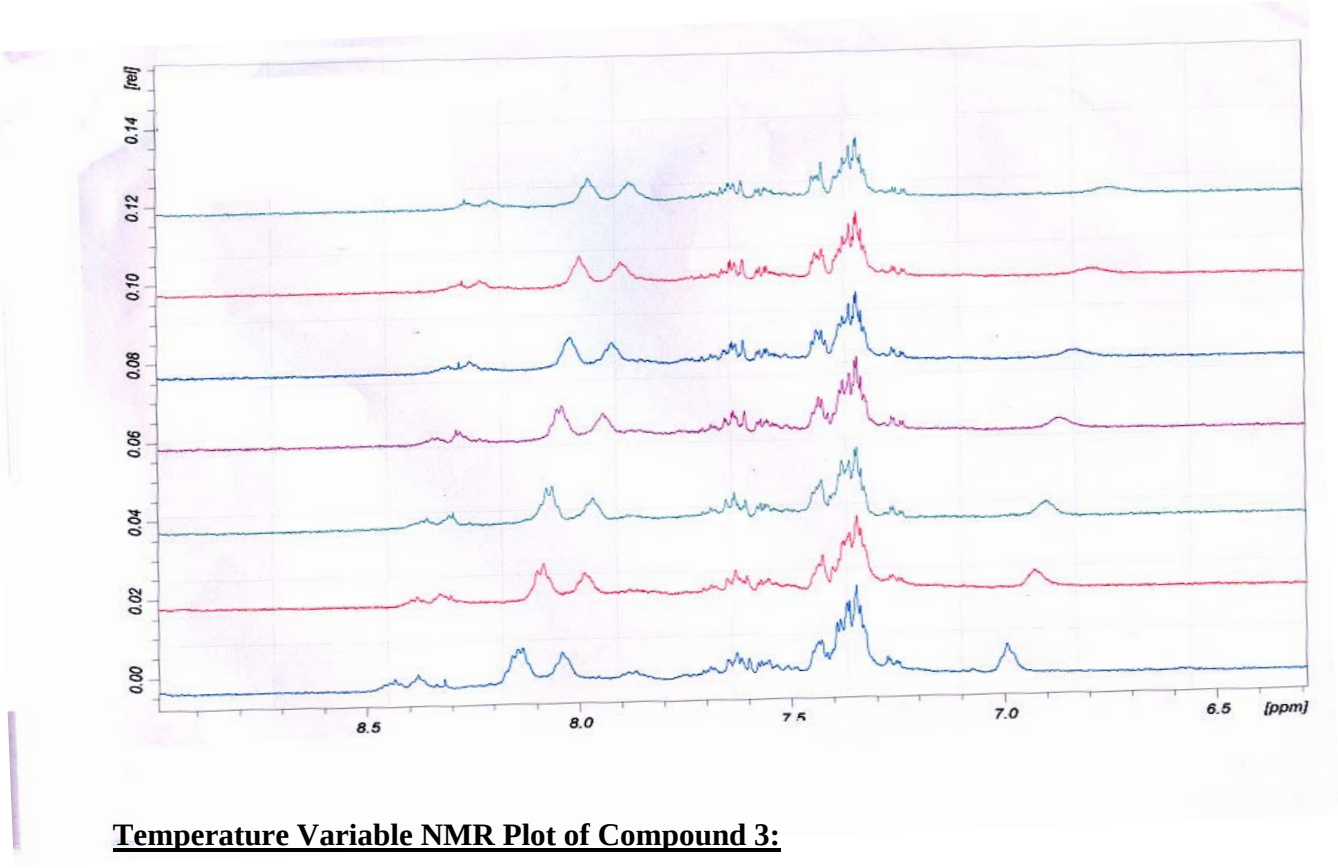
DSC Graph of compound 1:



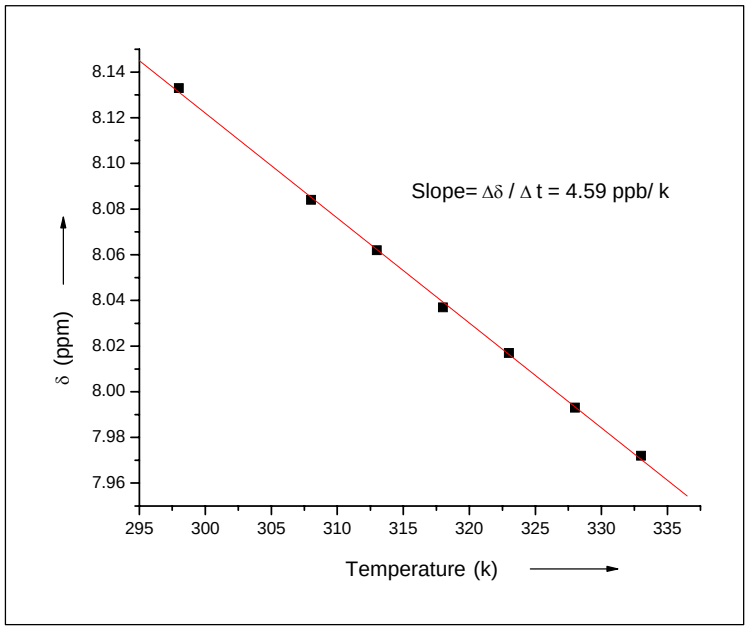
DSC Graph of compound 3:



Temperature variable NMR of compound 3 in DMSO-d_6:



Temperature Variable NMR Plot of Compound 3:



Energy Minimized Conformation of Compound 3 (ArgusLab 4.0.1):