

SUPPORTING INFORMATION

Negative Dendritic Effect on Enzymatic Hydrolysis of Dendrimer Conjugates

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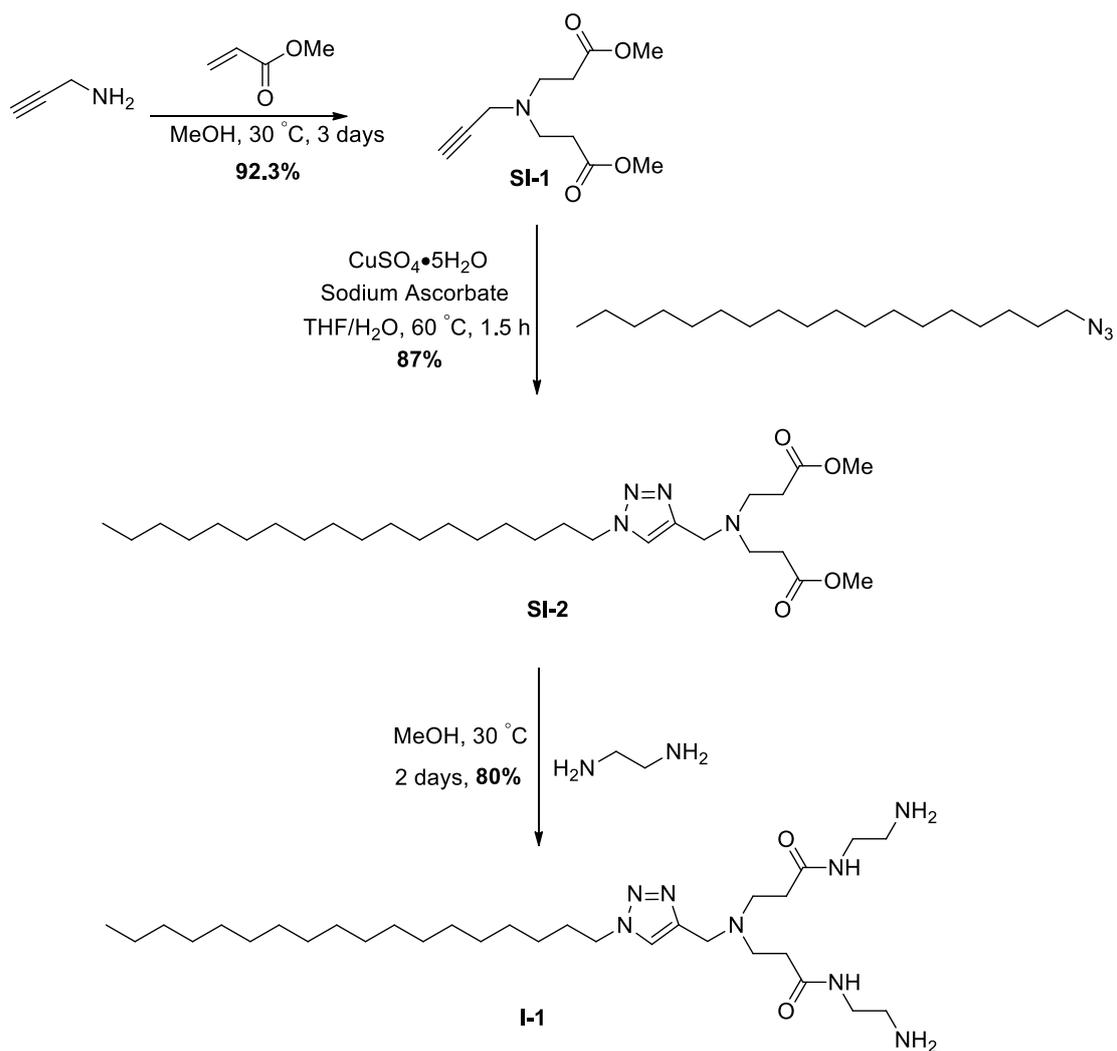
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Scheme S1: Synthesis of **I-1**.¹



Scheme S2: Synthesis of **II-1**.¹

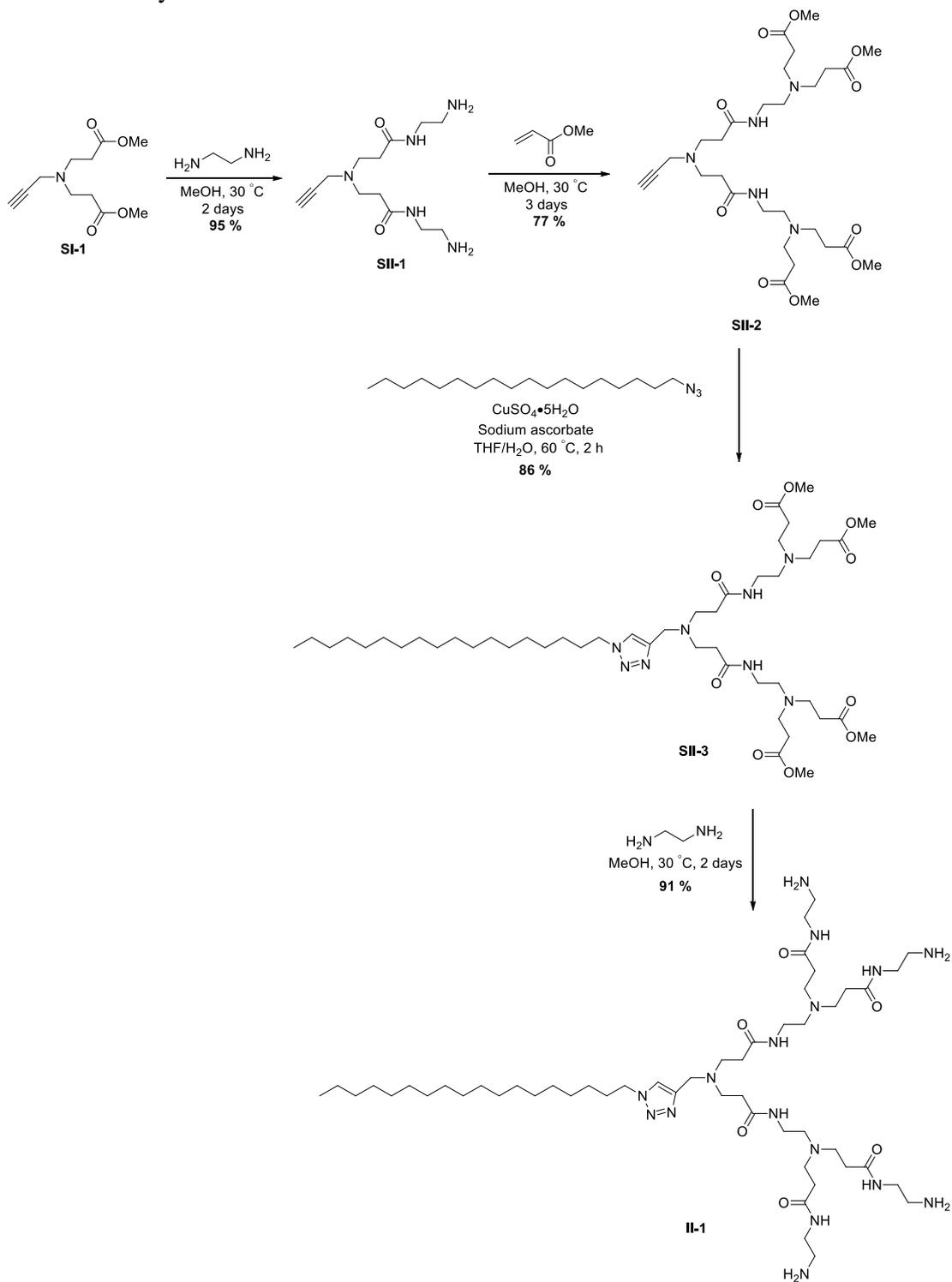
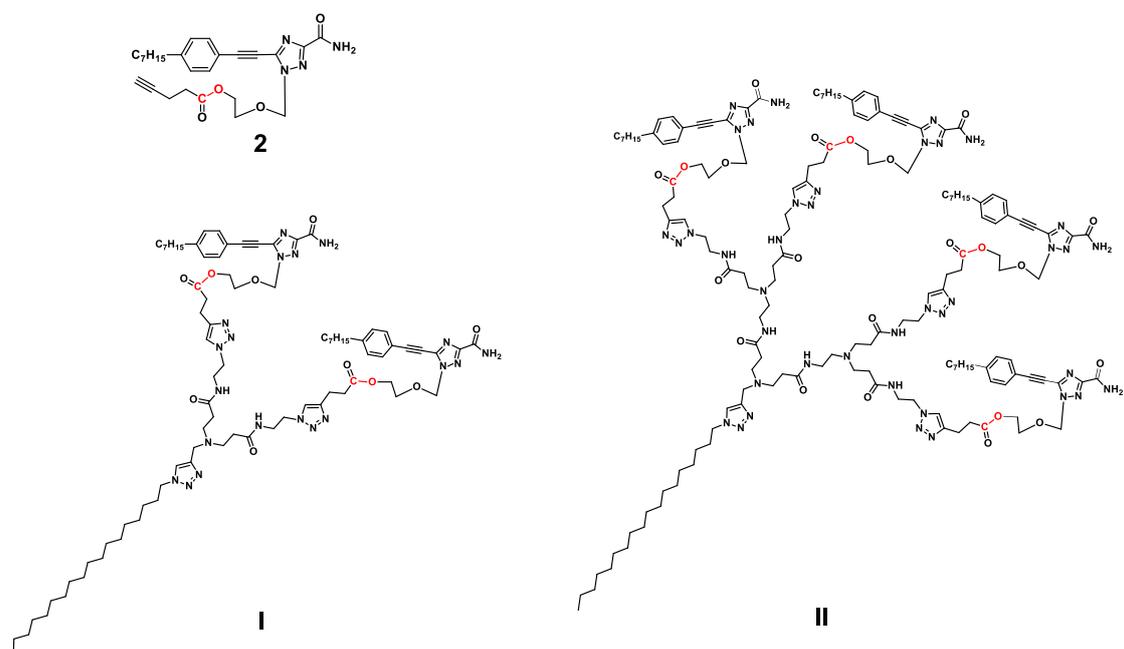


Table S1: The amount of released triazole nucleoside **1** from **2**, **I** and **II** in the presence of pig liver esterase in FBS, determined using HPLC analysis.

Time	1 released from 2 (%)	1 released from I (%)	1 released from II (%)
1 min	8.4	3.1	2.3
4h	23	8.8	4.6
8h	30	12	6.3
24h	38	24	9.6
48h	51	41	12

Table S2: Calculated average bond energy of the ester linkages in **2**, **I** and **II**.



Compound	Average Bond Energy (ester linkage) ΔE (kcal/mol)
2	-78.1015
I	-81.1663
	-80.2965
II	-84.8755
	-89.1693
	-84.1070
	-82.6549

Experimental section

General information

The chemical reagents used were purchased from Acros, Aldrich or Alfa Aesar. ^1H -NMR spectra were recorded at 400 MHz and ^{13}C -NMR spectra recorded at 100 MHz, on JEOL ECS 400 or Agilent DD2 400-MR spectrometers. High resolution MS and MS/MS experiments were performed using a QStar Elite mass spectrometer (Applied Biosystems SCIEX, Concord, ON, Canada) equipped with an electrospray ionization source operated in the positive mode. IR spectra were recorded with a Nicolet 380 spectrophotometer. Methyl acrylate, ethylenediamine and tetrahydrofuran were distilled before use. All other reagents and solvents were used without further purification from commercial sources. The alkyl azide compounds 1-azidooctadecane and azide transfer reagent azidosulfonyl imidazole were synthesized according to the literature.³⁶

Synthesis of **2**

To a mixture of **1** (0.20 mmol), 4-pentynoic acid (0.40 mmol) and DCC (0.40 mmol) was added 2.0 mL anhydrous CH_2Cl_2 . The stirring solution was as stirred at room temperature for 15 min followed by the addition of DMAP (0.10 mmol). The resulting mixture was stirred at room temperature and monitored by TLC until no reaction progress. Then the mixture was filtrated and washed with NaHCO_3 . The organic layer was combined and evaporated. The residue was purified by column chromatography on silica gel using the mixture of cyclohexane and ethyl acetate (1:1) as eluent, yielding **2**

(62 mg, 68%) as a white solid. ¹H-NMR (400 MHz, CDCl₃): δ 7.51 (d, 2H, J = 7.6 Hz, phenyl), 7.23 (d, 2H, J = 8.0 Hz, phenyl), 7.13 (br s, 1H, -C(O)NH), 6.52 (br s, 1H, -C(O)NH), 5.70 (s, 2H, -NCH₂O-), 4.24 (t, 2H, J = 4.2 Hz, -CH₂CH₂O-), 3.90 (t, 2H, J = 4.2 Hz, -CH₂CH₂O-), 2.65-2.45 (m, 6H, -CCH₂CH₂C-, -PhCH₂CH₂-), 1.94 (s, 1H, -CCH-), 1.60 (m, 2H, -PhCH₂CH₂CH₂-), 1.30-1.26 (m, 8H, -CH₂CH₂CH₂CH₂CH₃), 0.86 (t, 3H, J = 6.4 Hz, -CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ 171.6, 160.5, 156.3, 146.4, 141.4, 132.3, 129.0, 116.9, 99.1, 82.4, 78.2, 77.4, 77.1, 76.8, 73.7, 69.2, 68.2, 63.0, 36.1, 33.2, 31.8, 31.2, 29.2, 29.2, 22.7, 14.3, 14.1; HRMS: calcd. for C₂₆H₃₃N₄O₄⁺, [M+H]⁺ 465.2496, found 465.2495.

Synthesis of I-2:

To a solution of azidosulfonyl imidazole (35 mg, 0.18 mmol) in CH₃CN (3.0 mL) and CH₃OH (1.0 mL) were added compound **I-1** (35 mg, 0.060 mmol), CuSO₄·5H₂O (1.5 mg, 5.0 mol%) and K₂CO₃ (33 mg, 0.24 mmol). The resulting mixture was stirred at room temperature for 17 h. The solvent was evaporated under vacuum and followed by the addition of H₂O (10 mL). The resulting mixture was extracted with ethyl acetate (8.0 mL×3). Then the organic phase was combined, dried with MgSO₄, filtered and concentrated to get a crude product. The crude product was purified by column chromatography with CH₂Cl₂/CH₃OH = 25:1 to yield **I-2** as a pale yellow oil (26 mg, 68%). ¹H-NMR (400 MHz, CDCl₃): δ 7.46 (s, 1H, -NCHC-), 7.40 (br, 2H, -NH-), 4.34 (t, 2H, J = 7.4 Hz, CH₂), 3.80 (s, 2H, CH₂), 3.42-3.40 (m, 8H, CH₂), 2.78 (t, 4H, J = 6.2 Hz, CH₂), 2.46 (t, 4H, J = 6.2 Hz, CH₂), 1.91 (br, 2H, CH₂), 1.31-1.24 (br, 30H, CH₂),

0.87 (t, 3H, J = 6.8 Hz, CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ 172.4, 143.2, 122.4, 50.7, 50.4, 49.5, 47.4, 38.8, 33.6, 31.9, 30.3, 29.7, 29.5, 29.4, 29.3, 29.0, 26.5, 22.7, 14.1; IR-N₃ = 2099.26; HRMS: calcd. for C₃₁H₅₉N₁₃O₂⁺, [M+H]⁺ 631.4878, found 631.4880.

Synthesis of I

To a solution of compound **I-2** (25 mg, 0.040 mmol) in THF (2.4 mL) were added compound **2** (52 mg, 0.11 mmol), CuSO₄·5H₂O (27 mg, 0.10 mmol), and sodium ascorbate (43 mg, 0.20 mmol). The vessel was sealed and purged with argon for 5.0 min, H₂O (0.60 mL) was then added into the mixture. The reaction mixture was stirred at 60 °C until the reaction was completed indicated by TLC and IR analysis. The THF was evaporated under reduced pressure and the resulting residue was suspended in 7.0 mL saturated EDTA solution. The water phase was extracted with CH₂Cl₂ (7.0 mL) three times. The combined organic layers were dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure. The yielded residue was purified by column chromatography on silica gel using CH₂Cl₂/CH₃OH (20/1 then 10/1) as eluent, yielding **I** (24 mg, 40%) as a pale yellow solid. ¹H-NMR (400 MHz, CDCl₃): δ 8.12 (t, 2H, J = 5.2 Hz, -NH-), 7.55-7.49 (m, 7H, -NCHC- + NH₂ + phenyl), 7.22-7.20 (m, 6H, -NCHC- + phenyl), 6.74 (br s, 2H, NH₂), 5.68 (s, 4H, CH₂), 4.47 (t, 4H, J = 5.6 Hz, CH₂), 4.31 (t, 2H, J = 7.2 Hz, CH₂), 4.19-4.17 (m, 4H, CH₂), 3.89-3.87 (m, 4H, CH₂), 3.74-3.69 (m, 6H, CH₂), 2.91 (t, 4H, J = 7.6 Hz, CH₂), 2.69-2.60 (m, 12H, CH₂), 2.41 (t, 4H, J = 6.0 Hz, CH₂), 1.88-1.85 (m, 2H, CH₂), 1.62-1.58 (m, 4H, CH₂), 1.30-1.22 (m, 46H, CH₂), 0.88-0.85 (m, 9H, CH₃). ¹³C-NMR (100 MHz, CDCl₃): δ 173.0, 172.5,

160.6, 156.4, 146.4, 146.2, 141.5, 132.3, 129.0, 123.1, 122.2, 116.9, 99.1, 78.2, 73.8, 68.3, 63.0, 50.5, 49.5, 49.3, 47.3, 39.3, 36.1, 33.5, 32.0, 31.8, 31.2, 30.4, 29.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 26.6, 22.8, 22.7, 20.9, 14.2; HRMS: calcd. for $C_{83}H_{124}N_{20}O_{10}^{2+}$, $[M+2H]^{2+}$ 780.4899, found 780.4899.

Synthesis of II-2

To a solution of azidosulfonyl imidazole (105 mg, 0.60 mmol) in CH_3CN (3.0 mL) and CH_3OH (1.0 mL) were added compound **II-1** (78 mg, 0.075 mmol), $CuSO_4 \cdot 5H_2O$ (3.0 mg, 5.0 mol %) and K_2CO_3 (83 mg, 0.60 mmol). The resulting mixture was stirred at room temperature for 24 h. The solvent was evaporated under vacuum and followed by the addition of H_2O (10 mL). The resulting mixture was extracted with ethyl acetate (15 mL \times 3). Then the organic phase was combined, dried with $MgSO_4$, filtered and concentrated to get a crude product which was firstly purified by precipitation with CH_2Cl_2/Et_2O . The yielded mixture was further purified by column chromatography with $CH_2Cl_2/CH_3OH = 1:1$ to yield **II-2** as a pale yellow oil (34 mg, 40%). 1H -NMR (400 MHz, $CDCl_3$): δ 7.54-7.46 (m, 6H, -NH-), 7.24 (s, 1H, -NCHC-), 4.32 (t, 2H, J = 6.0 Hz, CH_2), 3.78 (s, 2H, CH_2), 3.42 (br, 16H, CH_2), 3.26-3.25 (m, 4H, CH_2), 2.74-2.73 (m, 12H, CH_2), 2.52-2.38 (m, 16H, CH_2), 1.88 (br, 2H, CH_2), 1.31-1.24 (br, 30H, CH_2), 0.87 (t, 3H, J = 6.2 Hz, CH_3); ^{13}C -NMR (100 MHz, $CDCl_3$): δ 173.0, 172.3, 143.1, 122.7, 52.5, 50.6, 50.4, 50.3, 49.1, 47.2, 38.8, 37.4, 34.1, 33.5, 31.9, 30.3, 29.6, 29.5, 29.4, 29.3, 29.0, 26.5, 22.6, 14.1; IR- $N_3 = 2098.47$; HRMS: calcd. for $C_{51}H_{95}N_{24}O_6^+$, $[M+H]^+$ 1139.7861, found 1139.7861.

Synthesis of II

To a solution of compound **II-2** (21 mg, 0.018 mmol) in THF (2.4 mL) were added compound **2** (41 mg, 0.088 mmol), CuSO₄·5H₂O (1.0 mg, 5.0 mol%), and sodium ascorbate (1.4 mg, 10 mol%). The vessel was sealed and purged with argon for 5.0 min, H₂O (0.60 mL) was then added into the mixture. The reaction mixture was stirred at 60 °C until the reaction was completed indicated by TLC and IR analysis. The THF was evaporated under reduced pressure and the resulting residue was suspended in 7.0 mL saturated EDTA solution. The water phase was extracted with CH₂Cl₂ (7.0 mL) three times. The combined organic layers were dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure. The yielded mixture was firstly purified by precipitation with CH₂Cl₂/Et₂O, and then further purified. The residue was purified by column chromatography on silica gel using ethyl acetate/CH₃OH (2/1 then 1/2) as eluent, yielding **II** (38 mg, 70%) as a pale yellow oil. ¹H-NMR (400 MHz, CDCl₃): δ 8.03-8.00 (m, 4H, -NH-), 7.78 (br s, 2H, NH), 7.58 (s, 1H, -NCHC-), 7.53-7.49 (m, 12H, phenyl + CH₂), 7.29 (s, 4H, -NH₂-), 7.23-7.21 (m, 8H, phenyl), 7.04 (s, 4H, -NH₂-), 5.69 (s, 22H, CH₂), 4.48 (t, 8H, J = 5.6 Hz, CH₂), 4.29 (t, 2H, J = 7.4 Hz, CH₂), 4.19 (t, 8H, J = 4.2 Hz, CH₂), 3.88 (t, 8H, J = 4.2 Hz, CH₂), 3.79 (s, 2H, CH₂), 3.72-3.70 (m, 8H, CH₂), 3.20 (t, 4H, J = 5.4 Hz, CH₂), 2.91 (t, 8H, J = 7.2 Hz, CH₂), 2.75-2.59 (m, 28H, CH₂), 2.49-2.32 (m, 16H, CH₂), 1.86-1.75 (m, 30H, CH₂), 1.62-1.57 (m, 8H, CH₂), 1.31-1.23 (m, 62H, CH₂), 0.89-0.86 (m, 15H, CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ 173.5, 172.7, 172.5, 160.7, 156.5, 146.4, 146.1, 143.2, 141.5, 132.3, 129.0,

122.3, 116.9, 99.0, 78.2, 77.3, 73.8, 68.3, 65.9, 63.0, 52.5, 50.4, 50.2, 49.4, 49.2, 39.4, 37.4, 36.1, 33.9, 33.7, 33.5, 32.0, 31.8, 31.2, 30.4, 29.8, 29.7, 29.6, 29.4, 29.3, 29.2, 26.7, 22.8, 22.7, 21.0, 15.4, 14.2; HRMS: calcd. for $C_{155}H_{225}N_{40}O_{22}^{3+}$, $[M+3H]^{3+}$ 999.5900, found 999.5902.

Synthesis of compound SI-1:

To a solution of propargylamine (0.22 g, 4.0 mmol) in methanol (10 mL) was added dropwise methyl acrylate (1.3 mL, 14 mmol). The reaction mixture was stirred under argon for 72 h at 30 °C. The reaction solution was evaporated, and the residue was purified by column chromatography on silica gel with cyclohexane/EtOAc (2/1), yielding compound **SI-1** (0.68 g, 75%) as a colorless oil. 1H -NMR (400 MHz, $CDCl_3$): δ 3.66 (s, 6H, CH_3), 3.41 (d, 2H, $J = 2.4$ Hz, CH_2), 2.82 (t, 4H, $J = 7.0$ Hz, CH_2), 2.45 (t, 4H, $J = 7.0$ Hz, CH_2), 2.18-2.17 (m, 1H, CH); ^{13}C -NMR (100 MHz, $CDCl_3$): δ 172.6, 78.1, 73.2, 51.5, 49.0, 41.9, 32.9.

Synthesis of compound SI-2:

To a solution of **SI-1** (52 mg, 0.23 mmol) in THF (3.0 mL) were added 1-azidooctadecane (67 mg, 0.23 mmol), $CuSO_4 \cdot 5H_2O$ (3.0 mg, 0.012 mmol), and sodium ascorbate (5.1 mg, 0.025 mmol). The vessel was sealed and purged with argon for 5.0 min and H_2O (0.75 mL) was then injected into the mixture. The reaction mixture was stirred at 60 °C for 1.5 h until the reaction was completed as indicated by TLC analysis. The THF was evaporated under reduced pressure and the resulting residue was

suspended in 7.0 mL saturated EDTA solution. The water phase was extracted with CH₂Cl₂ (7.0 mL) three times. The combined organic layers were dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with cyclohexane/ethyl acetate (2/1), yielding compound **SI-2** (0.1 g, 87%) as a white solid. ¹H-NMR (400 MHz, CDCl₃): δ 7.43 (s, 1H, CH), 4.32 (t, 2H, J = 7.4 Hz, CH₂), 3.80 (s, 2H, CH₂), 3.66 (s, 6H, CH₃), 2.80 (t, 4H, J = 7.0 Hz, CH₂), 2.49 (t, 4H, J = 7.0 Hz, CH₂), 1.89 (m, 2H, CH₂), 1.25 (m, 30H, CH₂), 0.88 (t, 3H, J = 6.8 Hz, CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ 173.0, 144.8, 122.4, 51.6, 50.4, 49.1, 48.8, 32.8, 32.0, 30.4, 29.8, 29.8, 29.7, 29.6, 29.5, 29.5, 29.1, 26.6, 22.8, 14.2.

Synthesis of compound **I-1**:

To a solution of **SI-2** (94 mg, 0.18 mmol) in methanol (2.0 mL) was added ethylenediamine (1.0 mL, 15 mmol). The reaction mixture was stirred under argon for 48 h at 30 °C until the IR analysis showed the complete consumption of **SI-2**. The reaction solution was evaporated, and the obtained residue was purified by precipitation with CH₃OH/Et₂O three times, yielding compound **I-1** (83 mg, 80%) as a white solid. ¹H-NMR (400 MHz, CDCl₃): δ 7.47 (br s, 3H, NH + CH), 4.32 (t, 2H, J = 7.2 Hz, CH₂), 3.76 (s, 2H, CH₂), 3.26-3.30 (m, 4H, CH₂), 2.76-2.83 (m, 8H, CH₂), 2.43 (t, 4H, J = 5.8 Hz, CH₂), 1.81-1.89 (m, 2H, CH₂), 1.25-1.31 (m, 30H, CH₂), 0.88 (t, 3H, J = 6.4 Hz, CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ 172.6, 143.7, 122.5, 50.8, 50.4, 49.9, 48.2, 42.0, 41.3, 34.3, 31.9, 30.3, 29.7, 29.5, 29.4, 29.3, 29.0, 26.5, 22.7, 14.1.

Synthesis of compound SII-1:

To a solution of **SI-1** (0.46 g, 2.0 mmol) in methanol (2.0 mL) was added ethylenediamine (2.5 mL, 37 mmol). The reaction mixture was stirred under argon at 30 °C and monitored by IR until the reaction reached completion. The reaction solution was evaporated, and then the residue was purified by precipitation with CH₃OH/Et₂O for three times, yielding amine terminated precursor of **SII-1** (0.54 g, 95%) as pale viscous oil. ¹H-NMR (400 MHz, CDCl₃): δ 7.22 (br, 2H, NH), 3.42 (d, 2H, J=2.1 Hz, CH₂), 3.28-3.30 (m, 4H, CH₂), 2.80-2.84 (m, 8H, CH₂), 2.38 (t, 4H, J =6.0 Hz, CH); 2.21 (t, 1H, CH), 1.38 (br, 4H, NH₂).; ¹³C-NMR (100 MHz, CDCl₃): δ 172.4, 77.6, 73.6, 50.0, 49.4, 41.9, 41.4, 41.3, 33.9.

Synthesis of compound SII-2:

To a solution of the amine terminated precursor **SII-1** (0.28 g, 1.0 mmol) in methanol (2.5 mL) was added dropwise methyl acrylate (1.5 mL, 17 mmol). The reaction mixture was stirred under argon for 72 h at 30 °C. The reaction solution was evaporated, and then the residue was purified by column chromatography on silica gel with EtOAc/CH₃OH (10/1), yielding 0.48 g (77 %) product **SII-2** as a pale yellow oil. ¹H-NMR (400 MHz, CDCl₃): δ 7.08 (br s, 2H, NH), 3.67 (s, 12H, CH₃), 3.46 (br s, 2H, CH₂), 3.28-3.30 (m, 4H, CH₂), 2.85 (t, 4H, J = 6.6 Hz, CH₂), 2.75 (t, 8H, J = 6.6 Hz, CH₂), 2.54 (t, 4H, J = 5.8 Hz, CH₂), 2.36-2.45 (m, 12H, CH₂), 2.19 (t, 1H, J = 2.4 Hz, CH); ¹³C-NMR (100 MHz, CDCl₃): δ 173.0, 171.8, 78.0, 73.3, 53.0, 51.6, 49.4, 49.3, 41.2, 37.1, 33.9, 32.8.

Synthesis of compound SII-3:

To a solution of compound **SII-2** (0.11 g, 0.17 mmol) in THF (3.0 mL) were added 1-azidooctadecane (53 mg, 0.18 mmol), CuSO₄·5H₂O (2.4 mg, 0.0096 mmol), and sodium ascorbate (3.6 mg, 0.018 mmol). The vessel was sealed and purged with argon for 5.0 min, H₂O (0.75 mL) was then added into the mixture. The reaction mixture was stirred at 60 °C until the reaction was completed indicated by TLC analysis. The THF was evaporated under reduced pressure and the resulting residue was suspended in 7.0 mL saturated EDTA solution. The water phase was extracted with CH₂Cl₂ (7.0 mL) three times. The combined organic layers were dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with CH₂Cl₂/ CH₃OH (20/1), yielding **SII-3** (0.69 g, 86%) as a pale yellow oil. ¹H-NMR (400 MHz, CDCl₃): δ 7.52 (s, 1H, CH), 7.13 (br s, 2H, NH), 4.30 (t, 2H, J = 7.2 Hz, CH₂), 3.83 (s, 2H, CH₂), 3.66 (br s, 12H, CH₃), 3.28-3.29 (m, 4H, CH₂), 2.73-2.80 (m, 12H, CH₂), 2.54 (t, 4H, J = 5.4 Hz, CH₂), 2.41-2.44 (m, 12H, CH₂), 1.88 (t, 2H, J = 6.0 Hz, CH₂), 1.24-1.30 (m, 30H, CH₂), 0.85-0.88 (t, 3H, J = 6.4 Hz, CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ 172.9, 171.9, 143.6, 122.4, 52.8, 51.5, 50.1, 49.1, 47.5, 37.0, 33.5, 32.6, 31.8, 30.2, 29.6, 29.4, 29.3, 29.2, 28.9, 26.4, 22.6, 14.0.

Synthesis of compound II-1:

To a solution of **SII-3** (100 mg, 0.11 mmol) in methanol (5.0 mL) was added

ethylenediamine (1.0 mL, 15 mmol). The reaction mixture was stirred under argon for 48 h at 30 °C until the IR analysis showed the complete consumption of **SH-3**. The reaction solution was evaporated, and the obtained residue was purified by precipitation with CH₃OH/Et₂O three times, yielding **II-1** (0.1 g, 91%) as a pale yellow oil. ¹H-NMR (400 MHz, CDCl₃): δ 8.01-8.04 (m, 2H, CH+NH), 7.51-7.62 (m, 4H, CH+NH), 7.26-7.28 (m, 2H, NH), 4.32 (t, 2H, J = 7.4 Hz, CH₂), 3.78 (s, 2H, CH₂), 3.22-3.31 (m, 12H, CH₂), 2.73-2.84 (m, 20H, CH₂), 2.35-2.51 (m, 16H, CH₂), 1.79-1.90 (m, 2H, CH₂), 1.25-1.31 (m, 30H, CH₂), 0.87 (t, 3H, J = 6.8 Hz, CH₃); ¹³C-NMR (100 MHz, CD₃OD + CDCl₃): δ 173.3, 173.2, 172.7, 172.6, 142.7, 122.9, 51.9, 51.9, 50.0, 49.7, 49.2, 49.0, 48.7, 48.5, 48.3, 48.1, 47.9, 47.7, 46.7, 41.5, 41.4, 40.6, 37.0, 33.5, 33.0, 31.5, 29.8, 29.2, 29.1, 29.0, 28.9, 28.6, 26.1, 22.2, 13.6.

Mass Spectrometry measurements

Prior to MS analysis, the compounds were dissolved in methanol doped with 1% formic acid (v/v) and afterwards, the sample solutions were introduced in the ionization source using a syringe pump (flow rate: 5.0 mL min⁻¹). High resolution MS and MS/MS experiments were performed using a QStar Elite mass spectrometer (Applied Biosystems SCIEX, Concord, ON, Canada) equipped with an electrospray ionization source operated in the positive mode. The capillary voltage was set at +5500 V and the cone voltage at +100 V. In this hybrid instrument, ions were measured using an orthogonal acceleration time-of-flight (oa-TOF) mass analyzer. A quadrupole was used for selection of precursor ions to be further submitted to collision-induced dissociation

(CID) in MS/MS experiments. Collision energy values are given in the laboratory frame. In MS, accurate mass measurements were performed using reference ions from a poly(propylene glycol) or a poly(ethylene glycol) internal standard. Air was used as the nebulizing gas (10 psi) while nitrogen was used as the curtain gas (20 psi) as well as the collision gas. Instrument control, data acquisition and data processing of all experiments were achieved using Analyst software (QS 2.0) provided by Applied Biosystems.

Bond energy calculation

The bond energy for the ester linkages in **2**, **I** and **II** was calculated using computer program AM1 in HyperChem7.5 and EDA (Energy Decomposition Analysis) on the basis of three different conformations of each compound.

Enzymatic hydrolysis assay

HPLC conditions

The products were analyzed by Agilent Technologies 1260 infinity high performance liquid chromatography (HPLC) system consisting of a G1322A 1260 Degasser, a G1312C 1260 Bin Pump VL, a G1316A 1260 TCC and a G1314B 1260 DAD VL. A Inertsil hypersil C18 silica-based RP-HPLC column (4.6*250 mm, made in Japan) was used for sample analysis. The mobile phase was consisted of a gradient elution of MeOH/H₂O. Elution system was the mixture of MeOH/H₂O from 10/90 to 100/0 in 20 min and then kept 100/0 in 25 min. The flow rate was 1.0 mL/min.

Temperature was 40 °C. All the compounds was detected at 280 nm.

Hydrolysis of 2

The stock solutions (6.1 mg in 0.20 mL DMSO) of **2** was prepared and 0.20 mL of the solution was diluted with 1.0 mL DMSO and 0.50 mL deionized water to obtain hydrolysis solution. The reaction was started by addition of 3.2 mg PLE and 1.5 mL FBS and the mixture was incubated at 37 °C in a thermomixer. At different time point (1.0 min, 4.0 h, 8.0 h, 24 h, 48 h), an aliquots (50 µL) were taken and the reaction was stopped by adding 0.10 mL MeOH. The mixture was kept for 5.0 min on ice followed by centrifugation for 5.0 min (13,000 rpm). Supernatant solution (20 µL) was subjected to HPLC analysis.

Hydrolysis of I

The stock solution (10 mg in 200 µL DMSO) of **I** was prepared and 200 µL of the solution was diluted with 1.0 mL DMSO and 0.50 mL deionized water to obtain hydrolysis solution. The reaction was started by addition of 3.2 mg PLE and 1.5 mL FBS and the mixture was incubated at 37 °C in the thermomixer. At different time point (1.0 min, 4.0 h, 8.0 h, 24 h, 48 h), an aliquot (50 µL) was taken and the reaction was stopped by adding 0.10 mL MeOH. The mixture was kept for 5.0 min on ice followed by centrifugation for 5.0 min (13,000 rpm). Supernatant solution (20 µL) was subjected to HPLC analysis.

Hydrolysis of II

The stock solution (9.9 mg in 0.20 mL DMSO) of **II** was prepared and 0.20 mL of the solution was diluted with 1.0 mL DMSO and 0.50 mL deionized water to obtain

hydrolysis solution. The reaction was started by addition of 3.2 mg PLE and 1.5 mL FBS and the mixture was incubated at 37 °C in the thermomixer. At different time point (1.0 min, 4.0 h, 8.0 h, 24 h, 48 h), an aliquot (50 µL) was taken and the reaction was stopped by adding 0.10 mL MeOH. The mixture was kept for 5.0 min on ice followed by centrifugation for 5.0 min (13,000 rpm). Supernatant solution (20 µL) was subjected to HPLC analysis.

Hydrolysis assay at pH = 5.0

HPLC conditions

The products were analyzed by Agilent Technologies 1260 infinity high performance liquid chromatography (HPLC) system consisting of a G1322A 1260 Degasser, a G1312C 1260 Bin Pump VL, a G1316A 1260 TCC and a G1314B 1260 DAD VL. A Inertsil hypersil C18 silica-based RP-HPLC column (4.6*250 mm, made in Japan) was used for sample analysis. The mobile phase was consisted of a gradient elution of MeOH/H₂O. Elution system was the mixture of MeOH/H₂O from 10/90 to 100/0 in 10 min and then kept 100/0 in 25 min. The flow rate was 1.0 mL/min. Temperature was 40 °C. All the compounds were detected at 280 nm.

*Hydrolysis of **I** and **II***

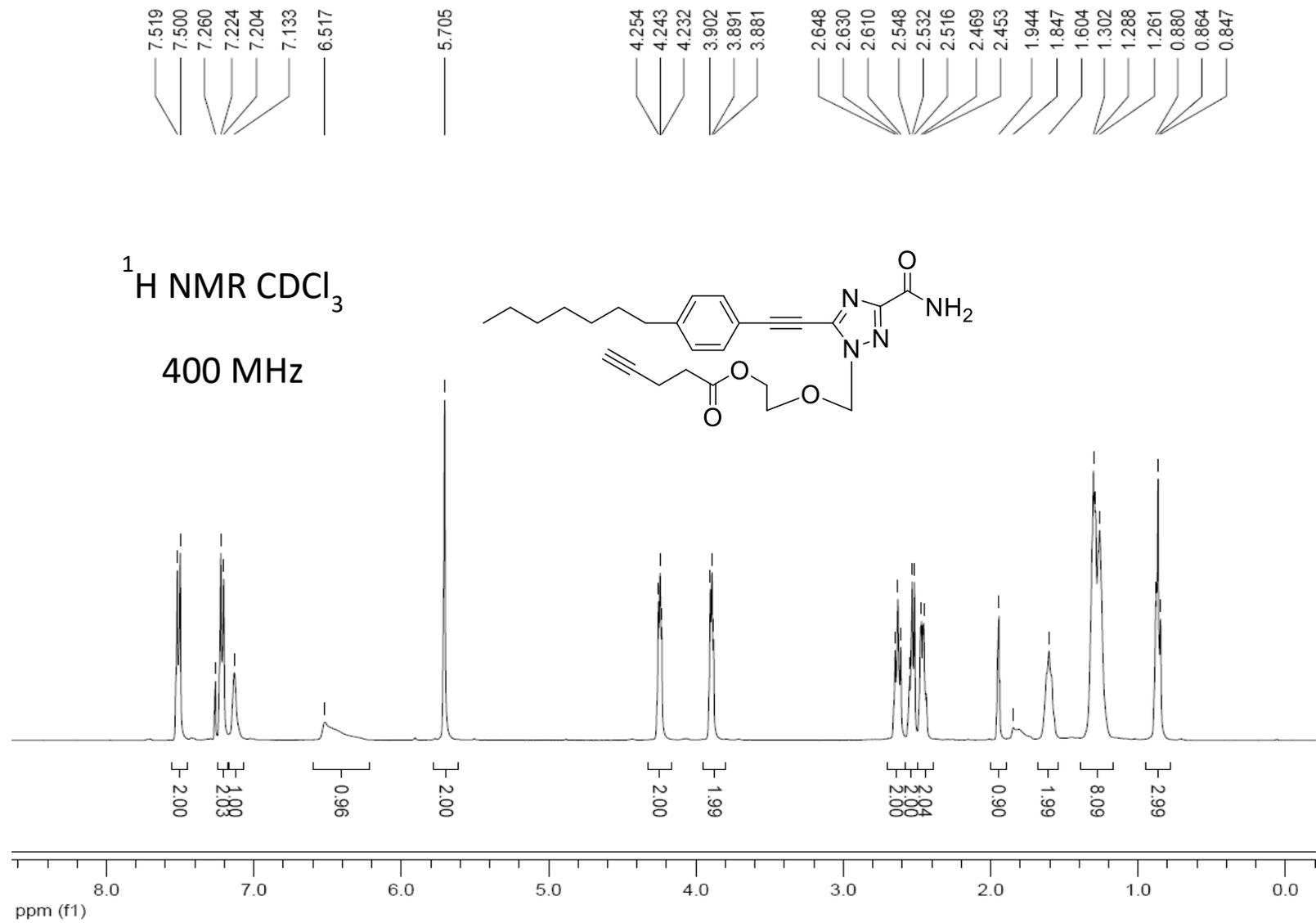
The compound (1.0 mg) was dissolved in 1.0 mL MeOH/H₂O (4/1) (pH = 5.0). The solution was incubated at 37 °C in a thermomixer. After 48h incubation, 20 µL reaction liquid were taken out and subjected to HPLC directly to analysis.

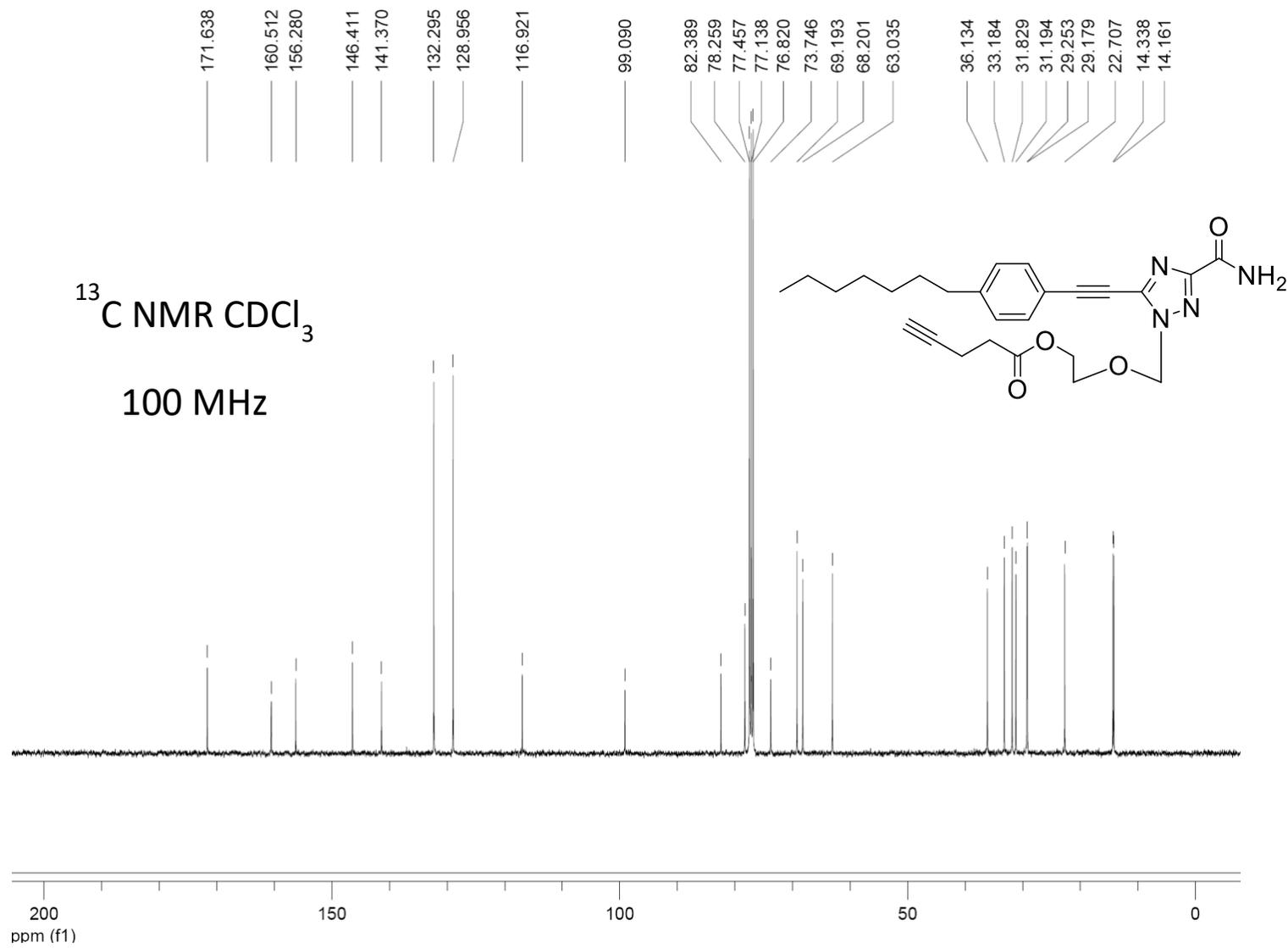
Determination of critical micelle concentration (CMC)

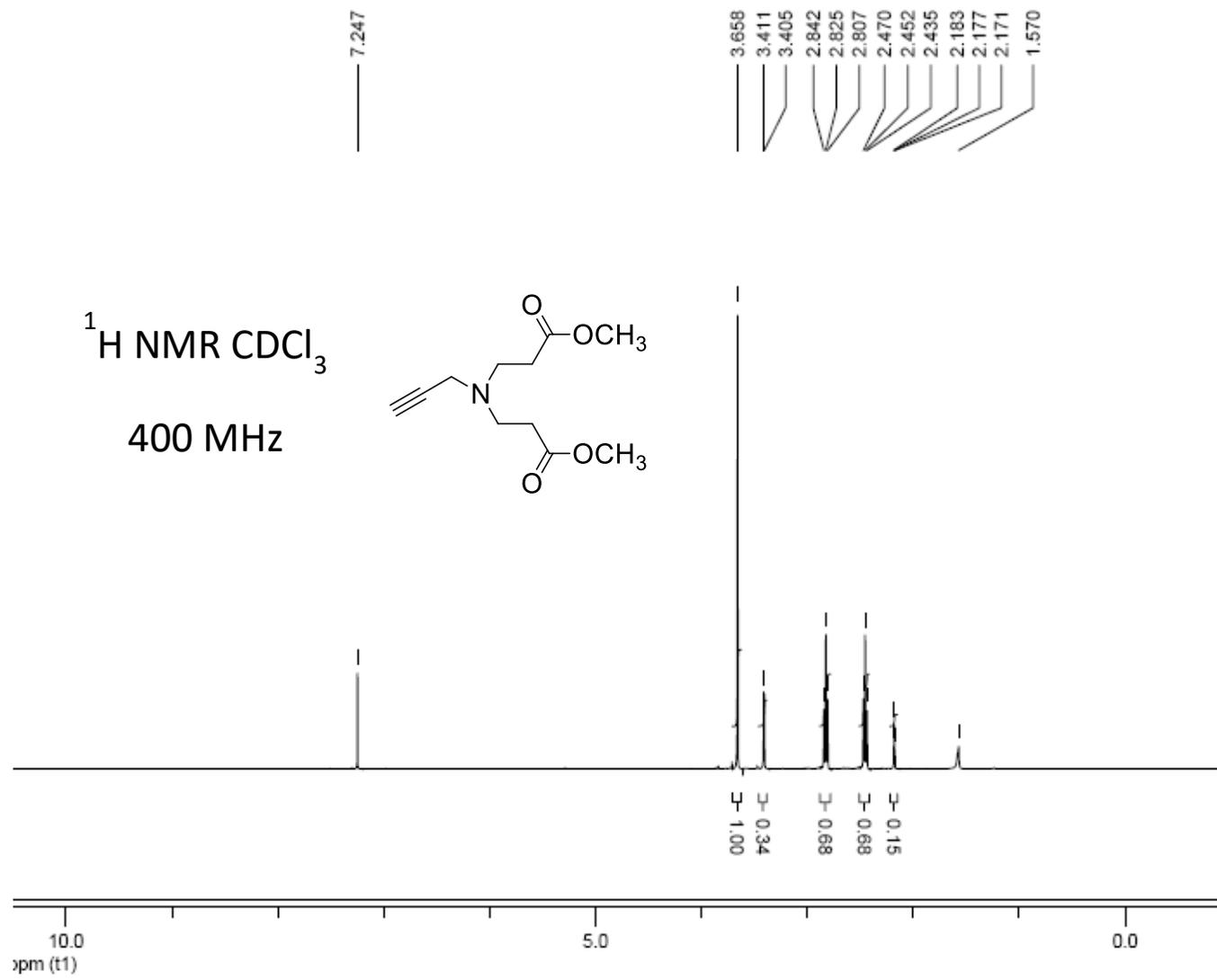
The stock solution of dendrimer conjugate **I** or **II** was prepared in MilliQ water and diluted in MilliQ water to get desired concentration in 1.0 mL assay volume. Then 10 μ L of pyrene solution (10 mg/L in acetone) was added and subsequently sonicated for 30 min to promote the micelle formation. The so-obtained solution was kept at room temperature for 2h. The fluorescence emission at 373 nm and 384 nm was recorded by using an excitation wavelength of 335 nm. Plotting of fluorescence intensity at 373 nm and 384 nm against the compound concentration allowed the determination of CMC value. A sigmoidal best fit analysis to the fluorescence data was applied.

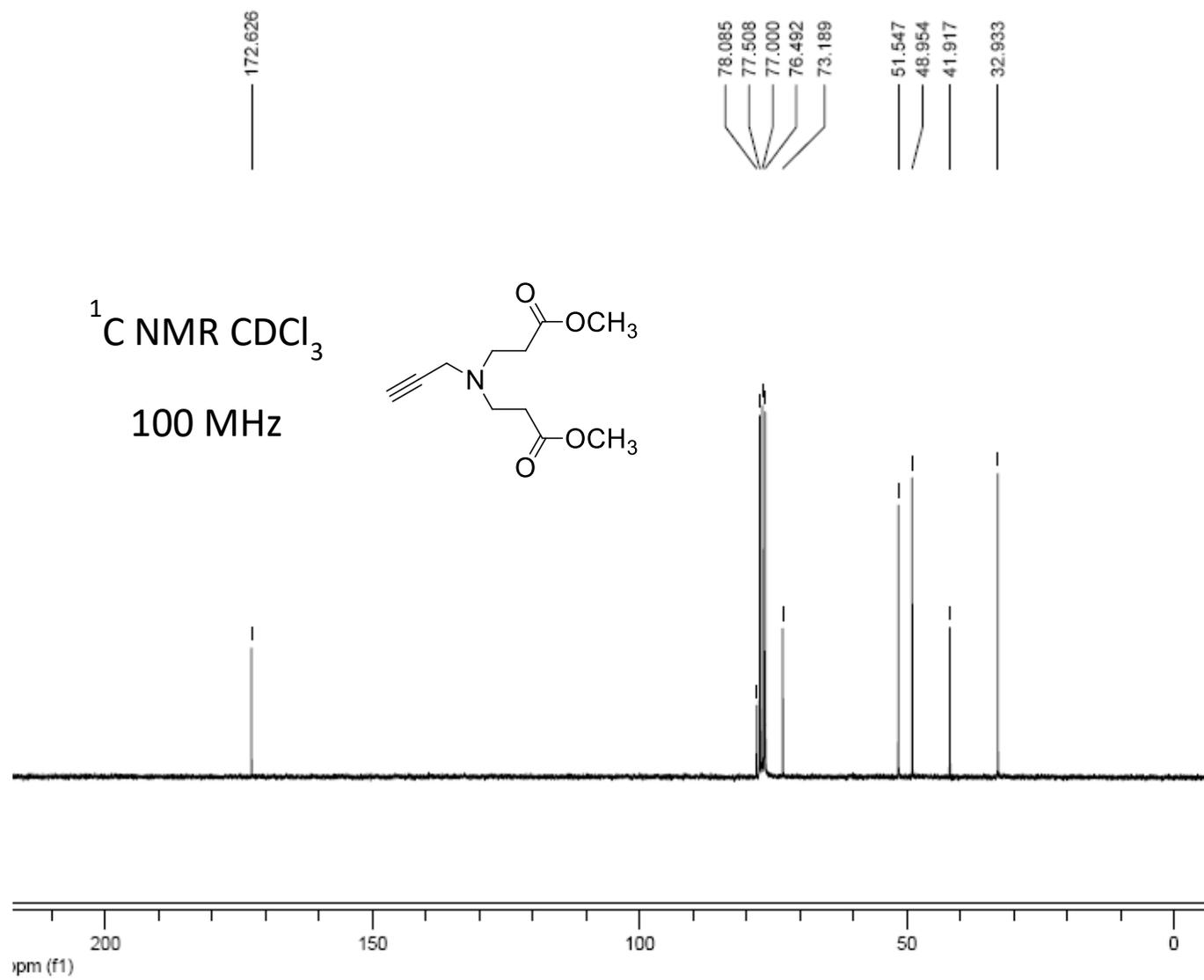
Reference:

1. T. Yu, X. Liu, A. L. Bolcato-Bellemin, Y. Wang, C. Liu, P. Erbacher, F. Qu, P. Rocchi, J. P. Behr and L. Peng, *Angew. Chem. Int. Ed. Engl.*, 2012, **51**, 8478-8484.

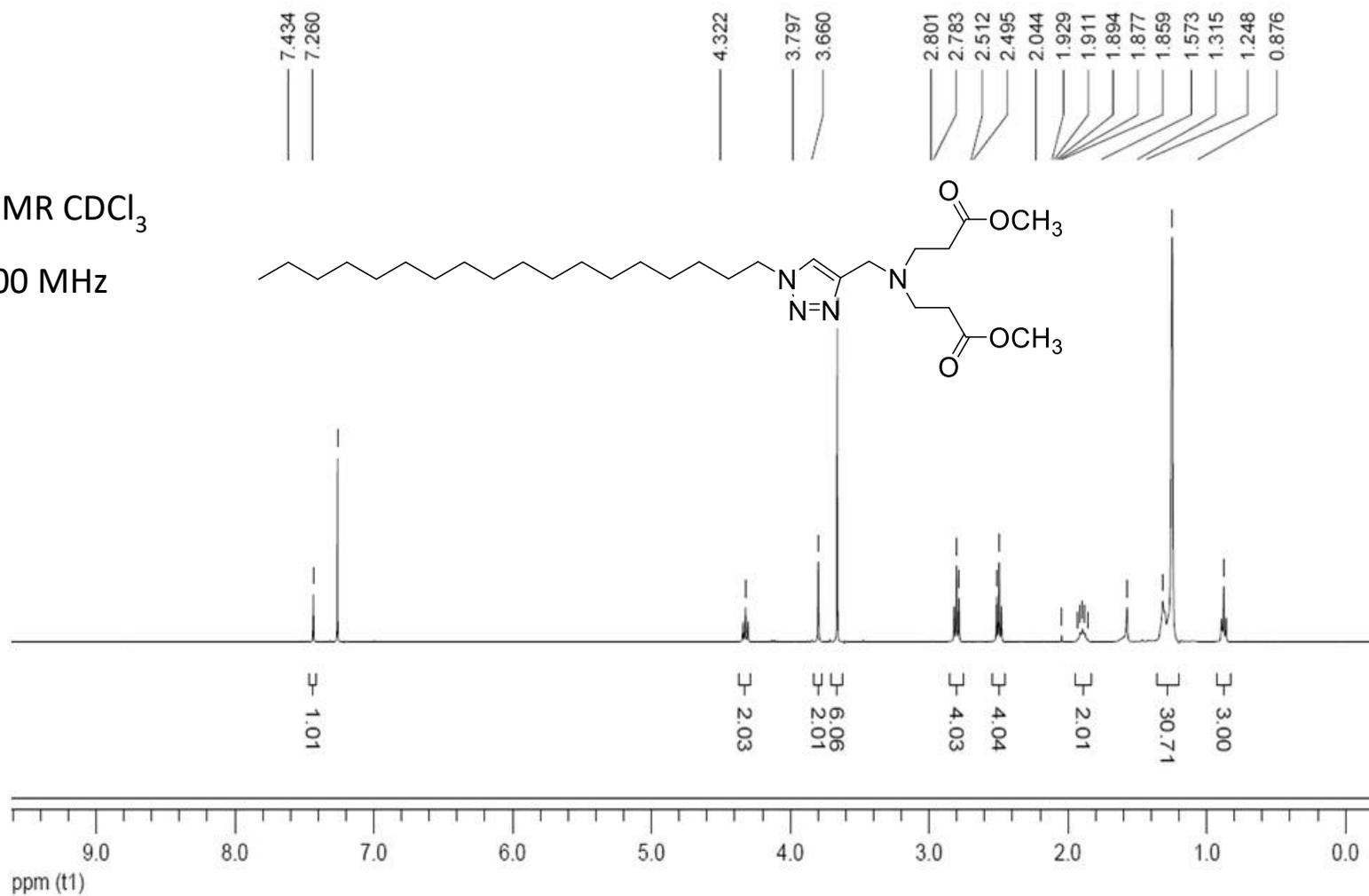


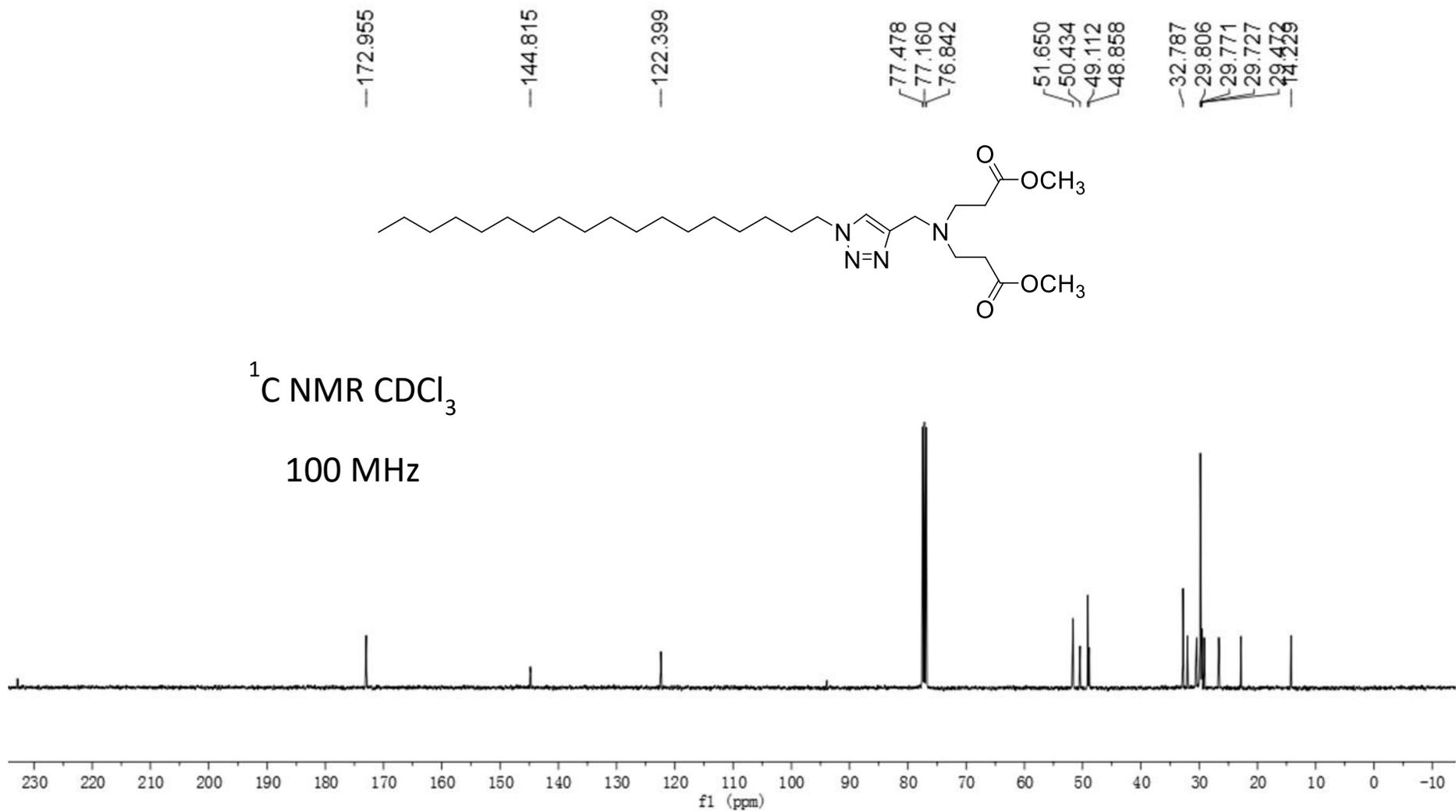




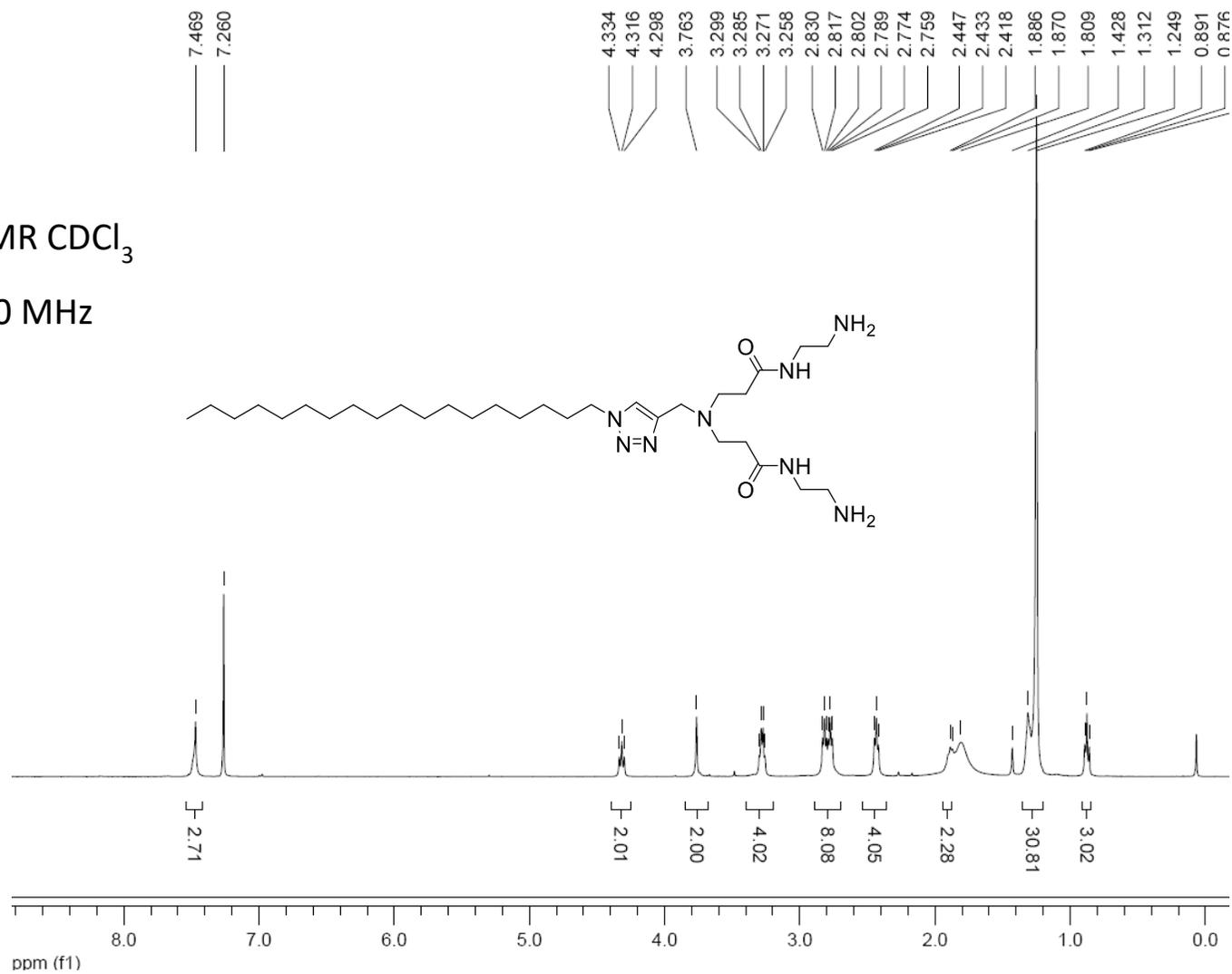


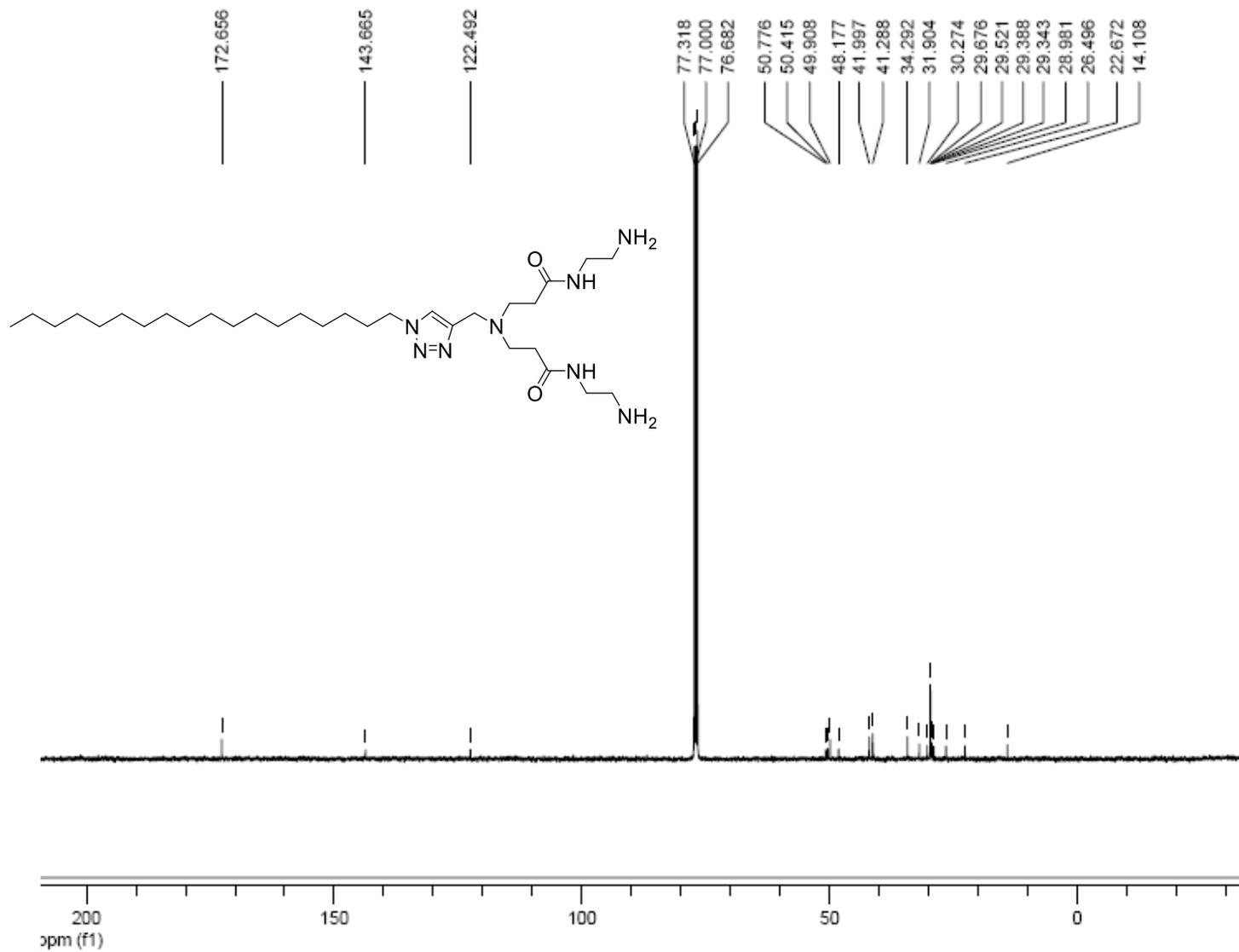
$^1\text{H NMR CDCl}_3$
400 MHz

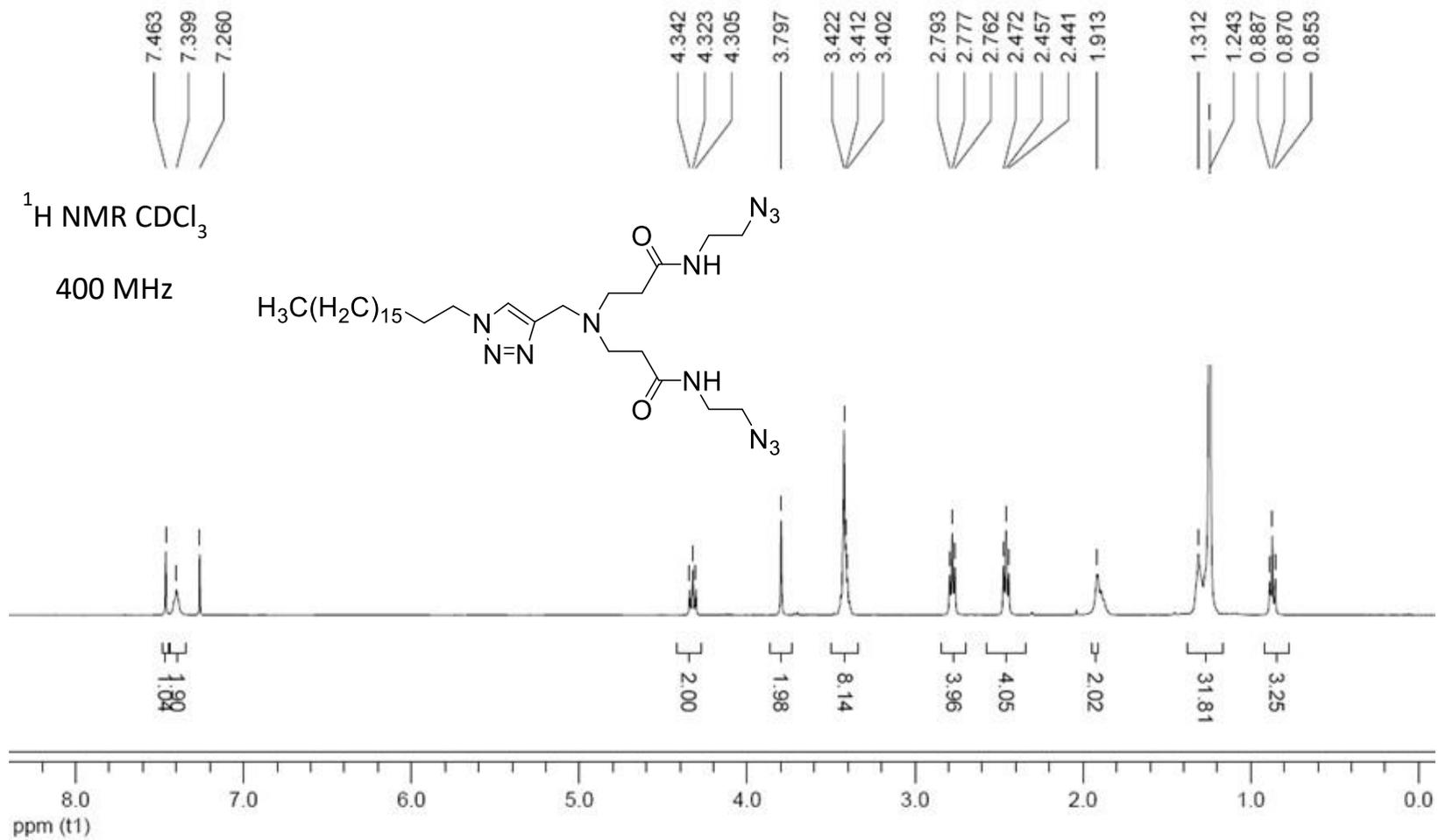




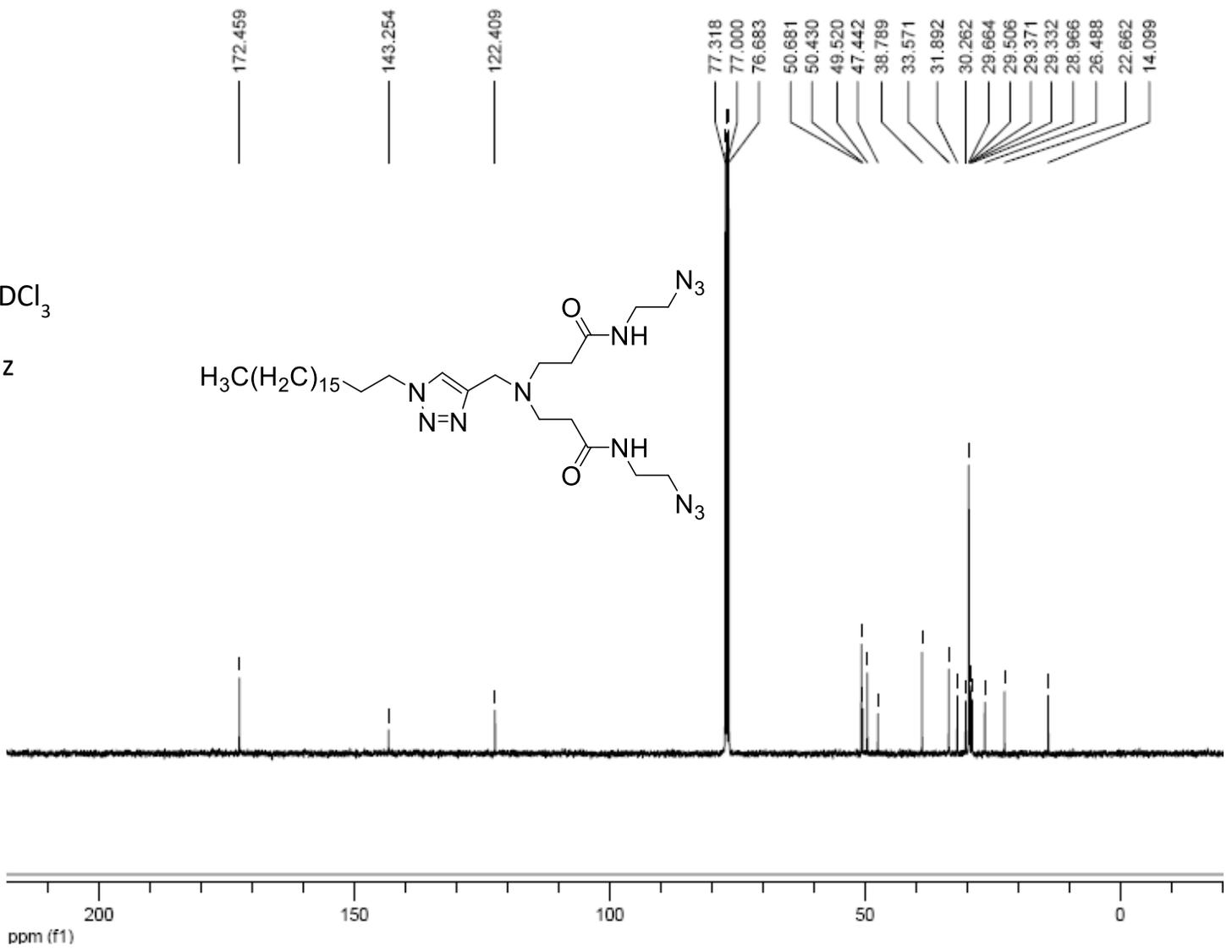
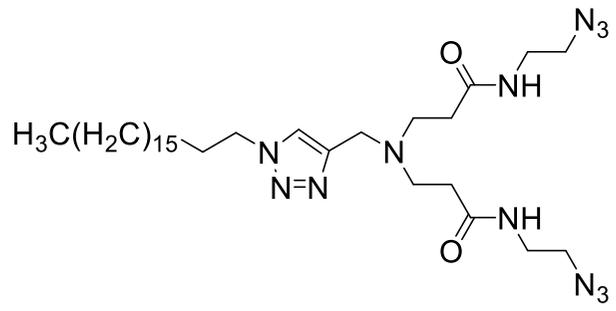
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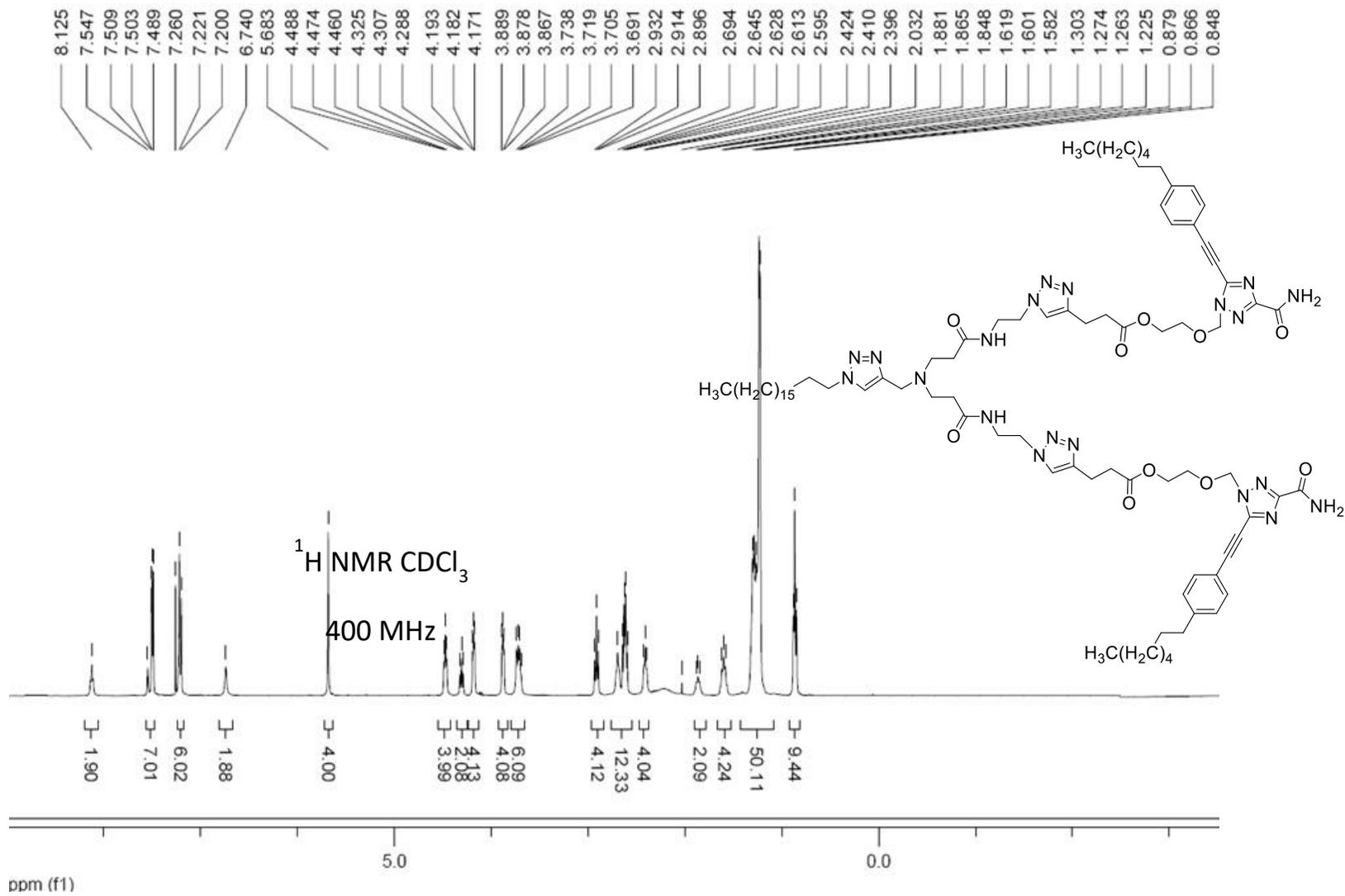


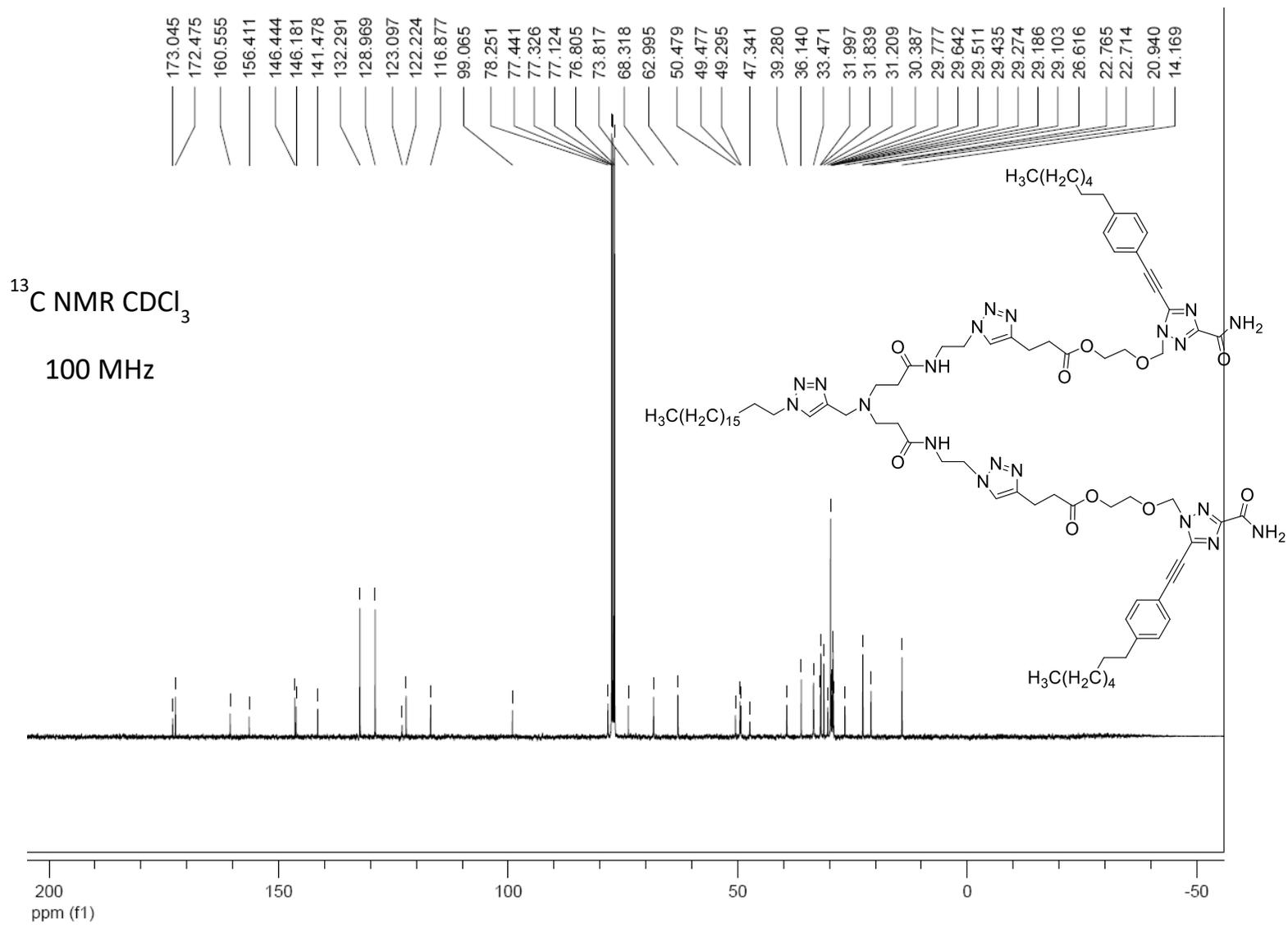


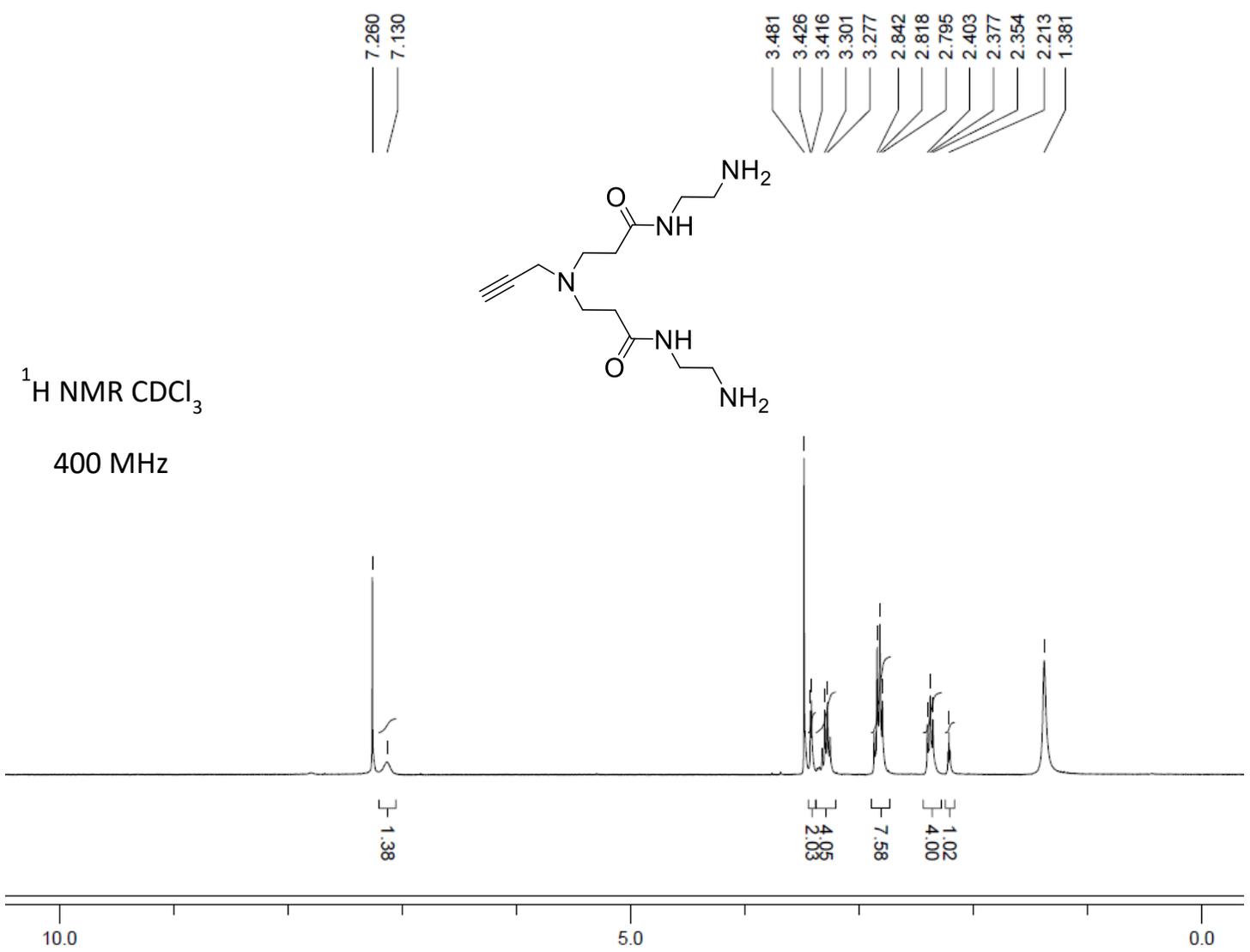


^{13}C NMR CDCl_3
100 MHz

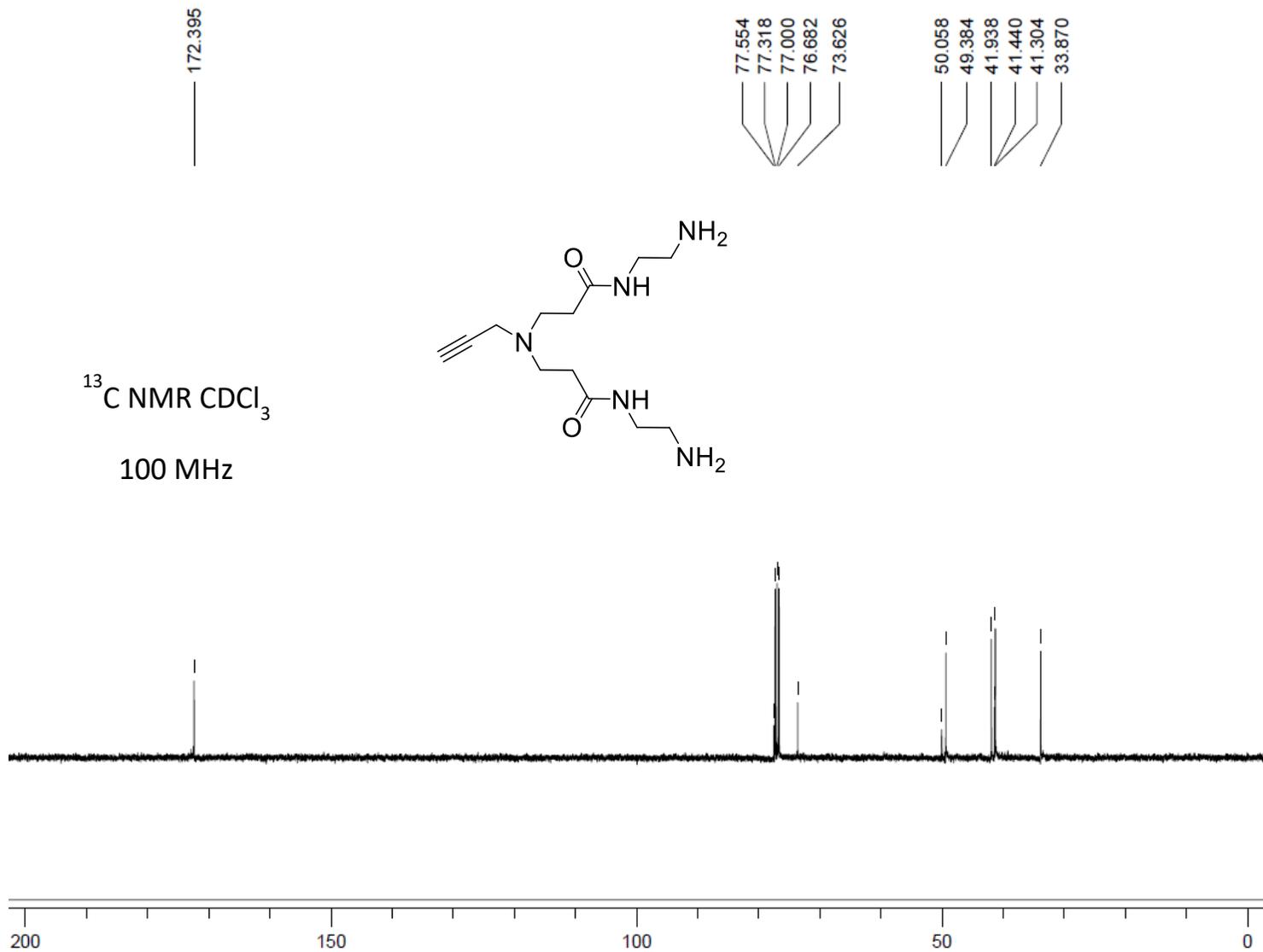
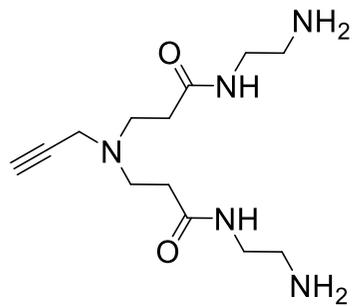


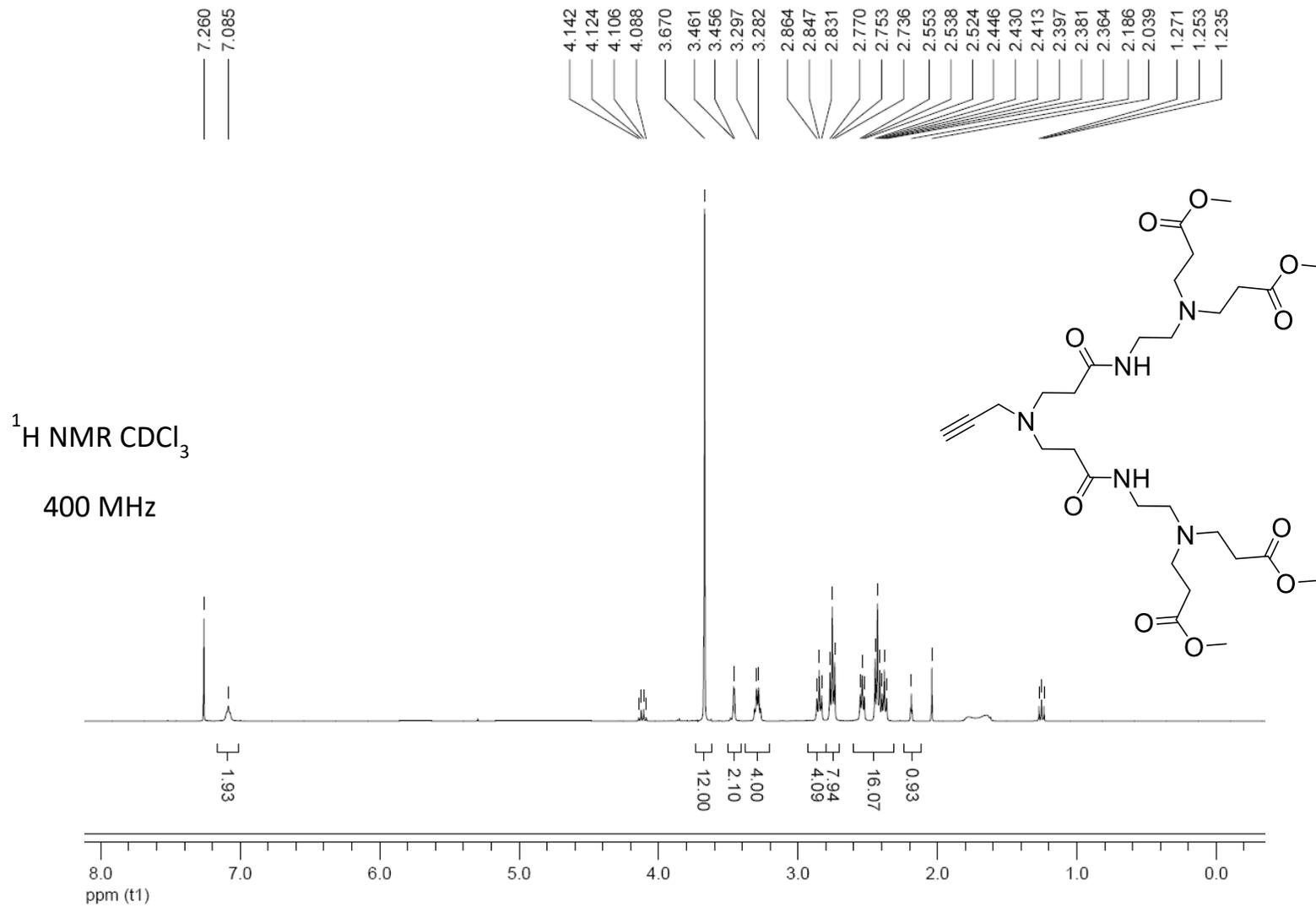


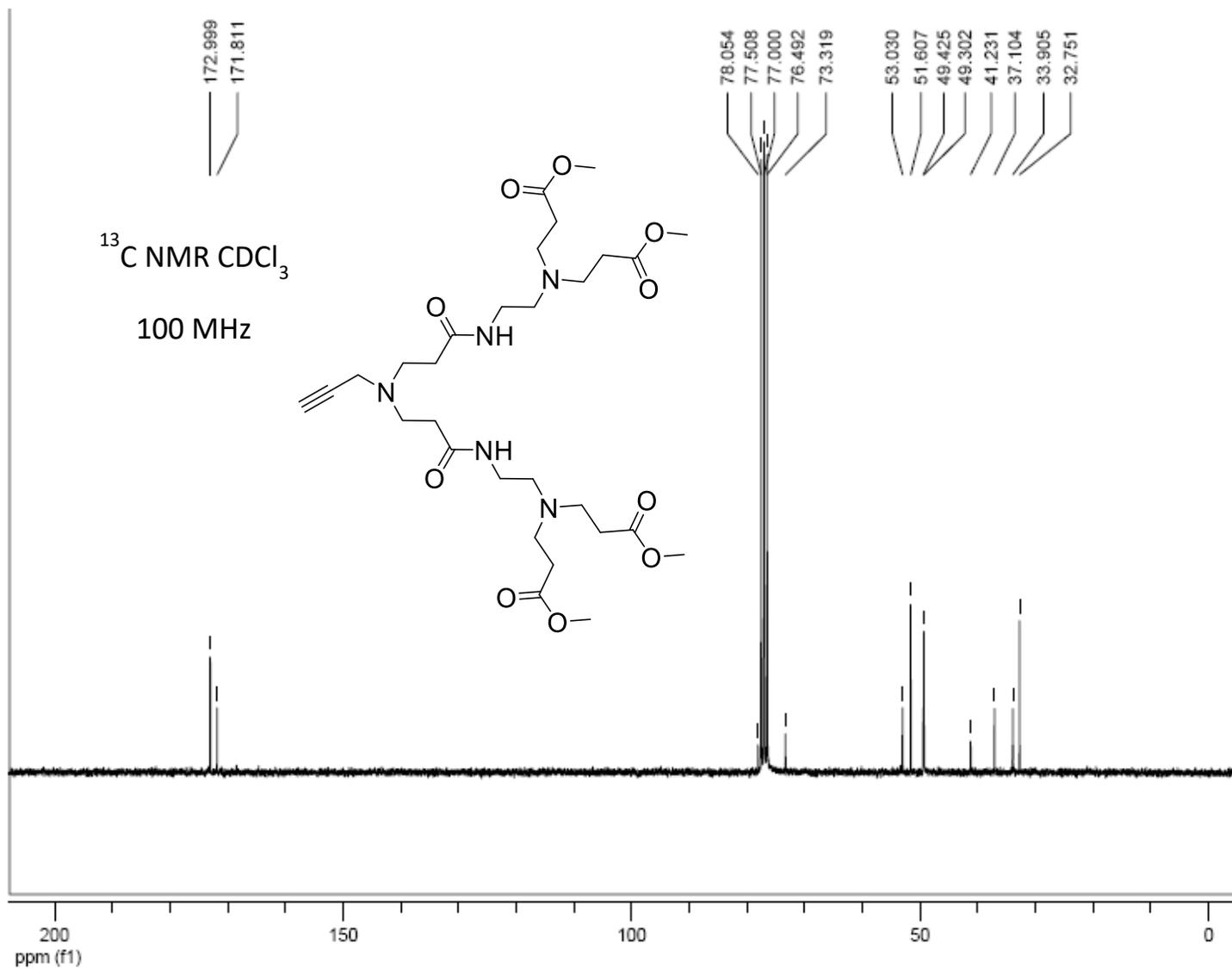


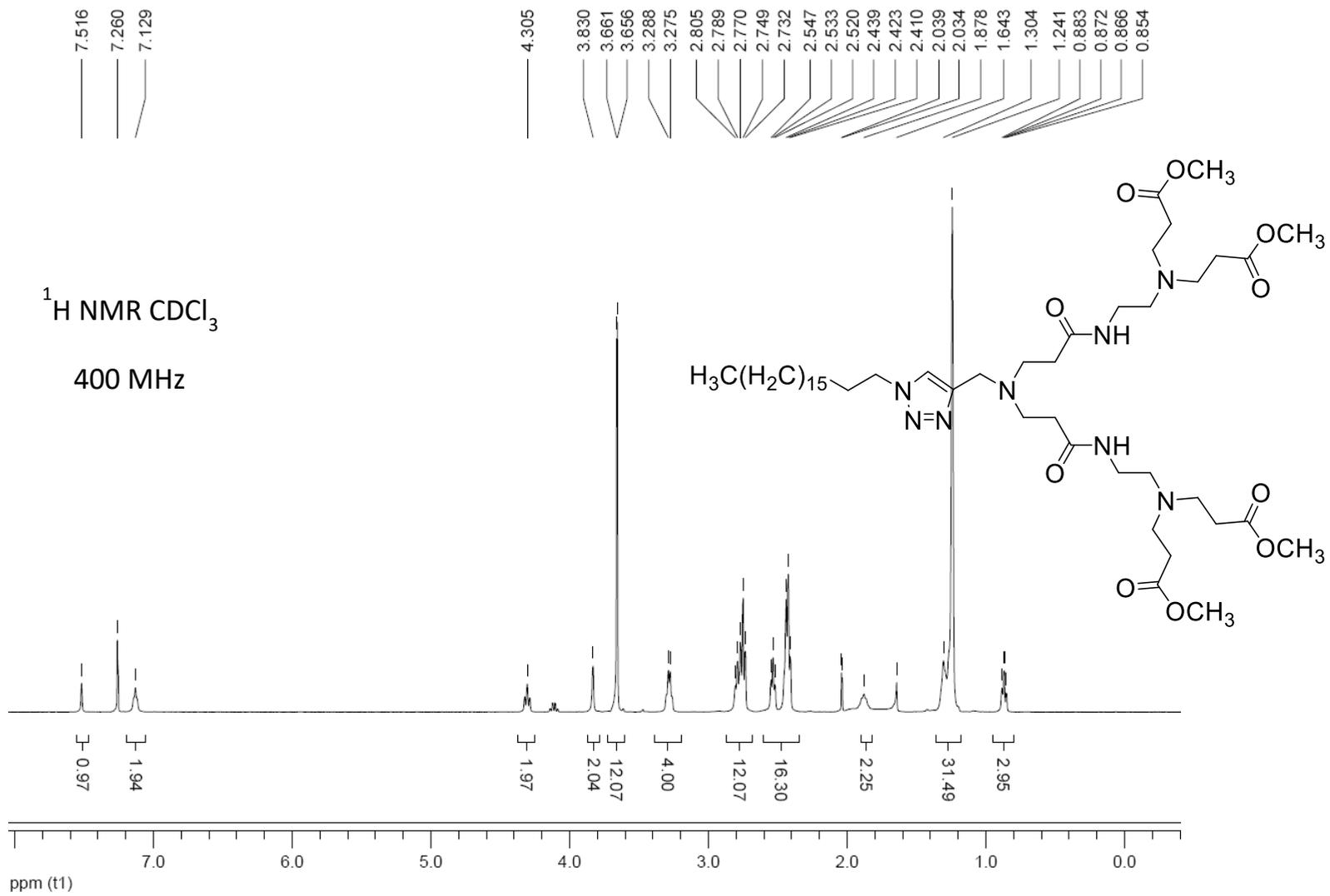


^{13}C NMR CDCl_3
100 MHz

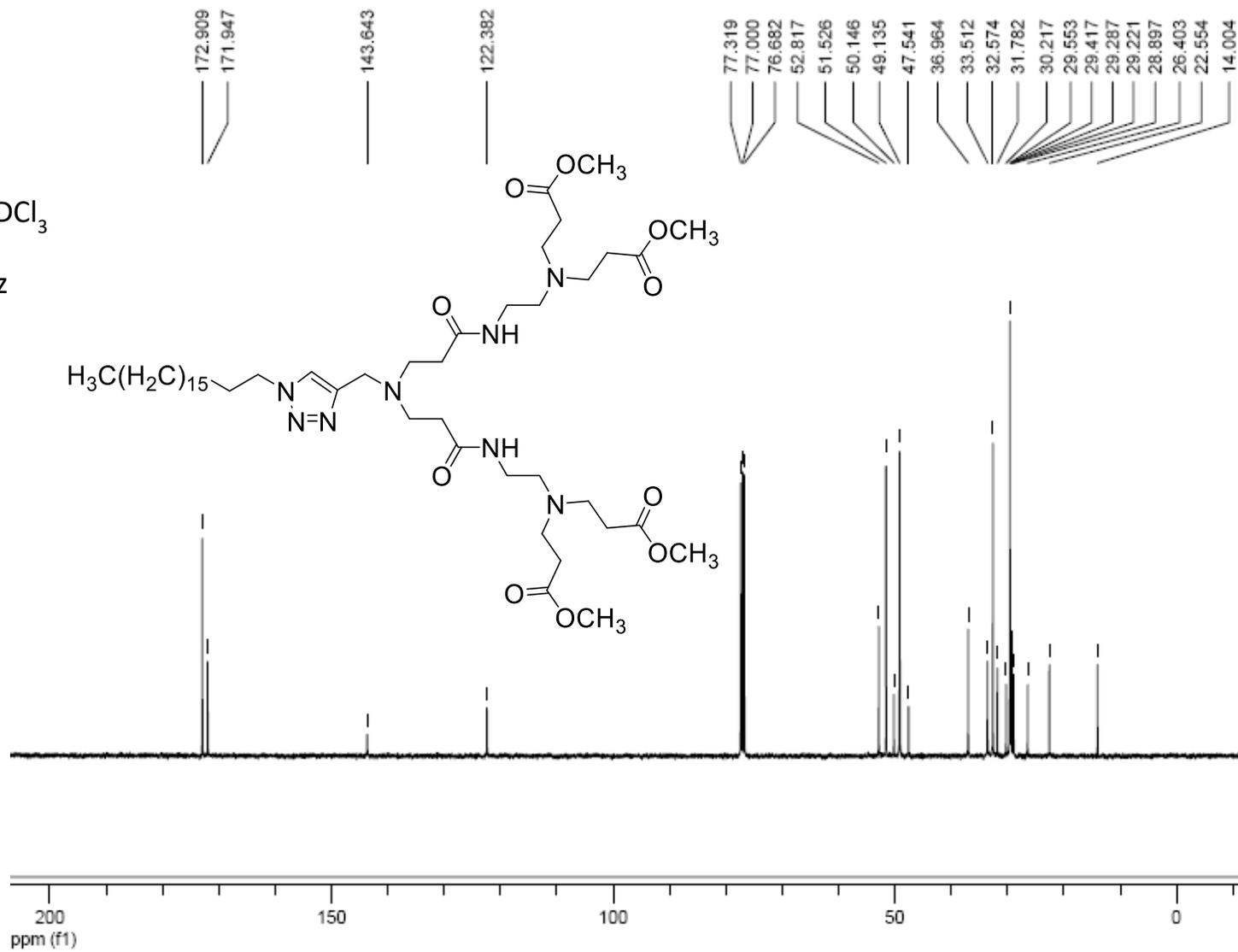




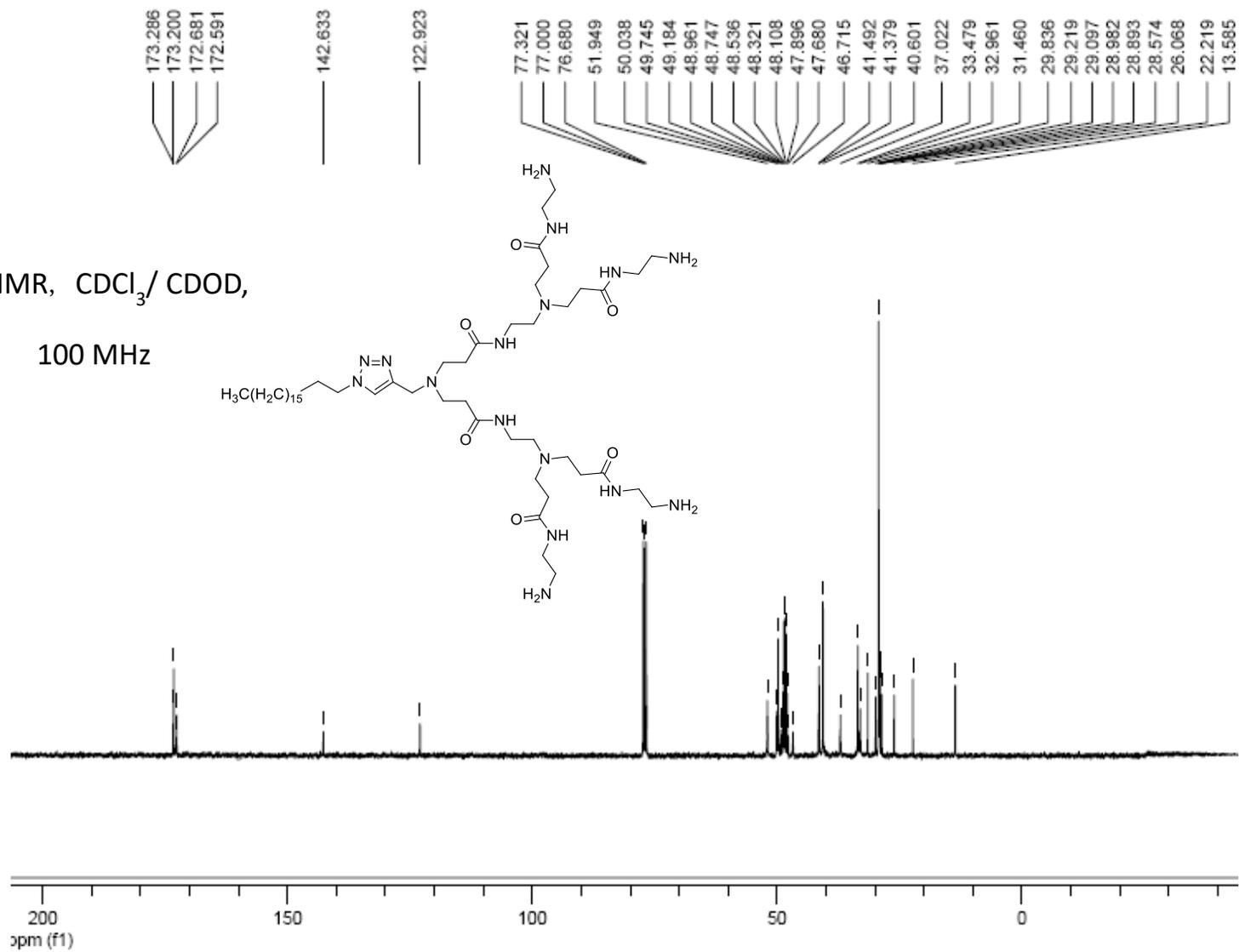


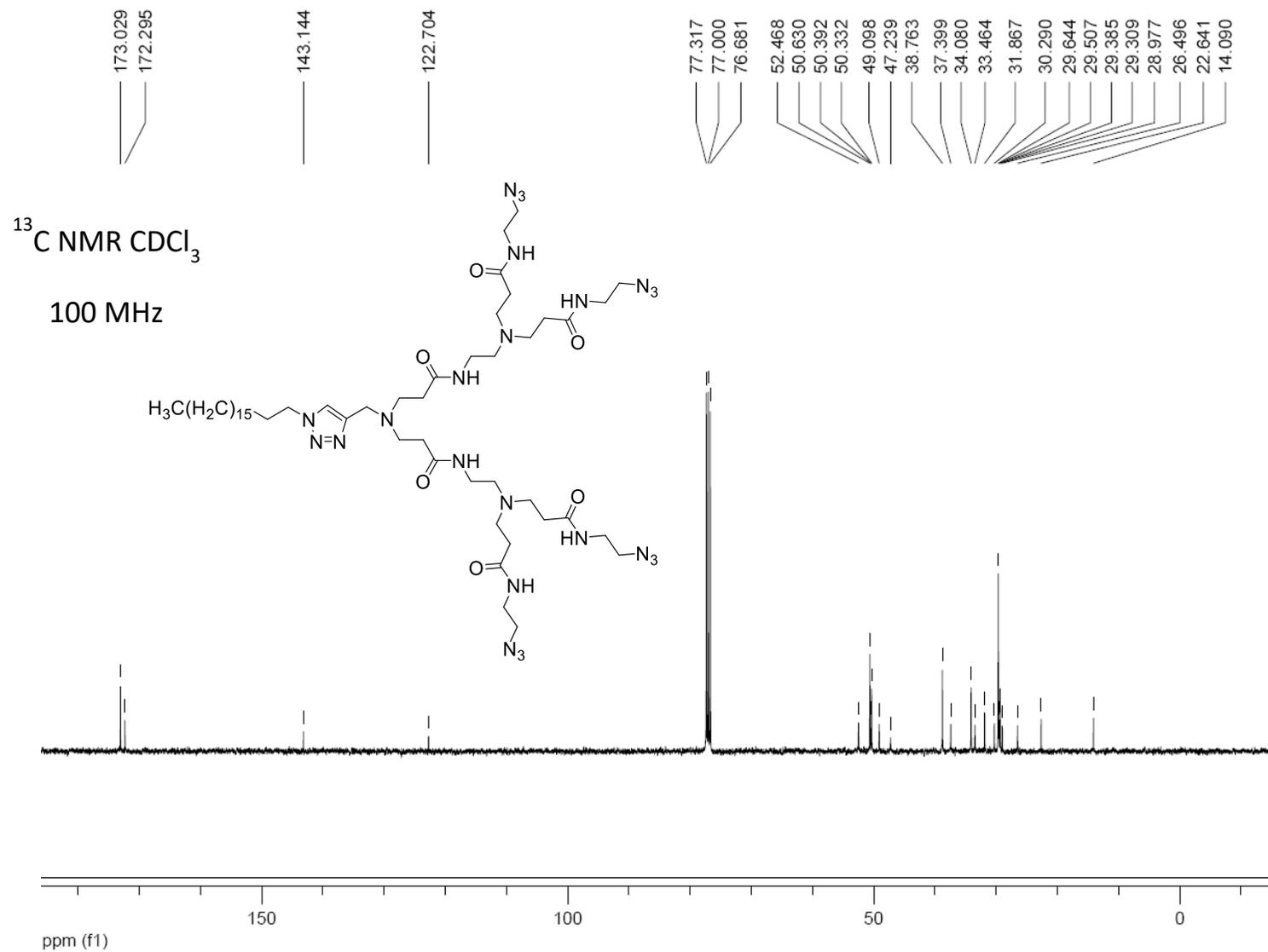


^{13}C NMR CDCl_3
100 MHz

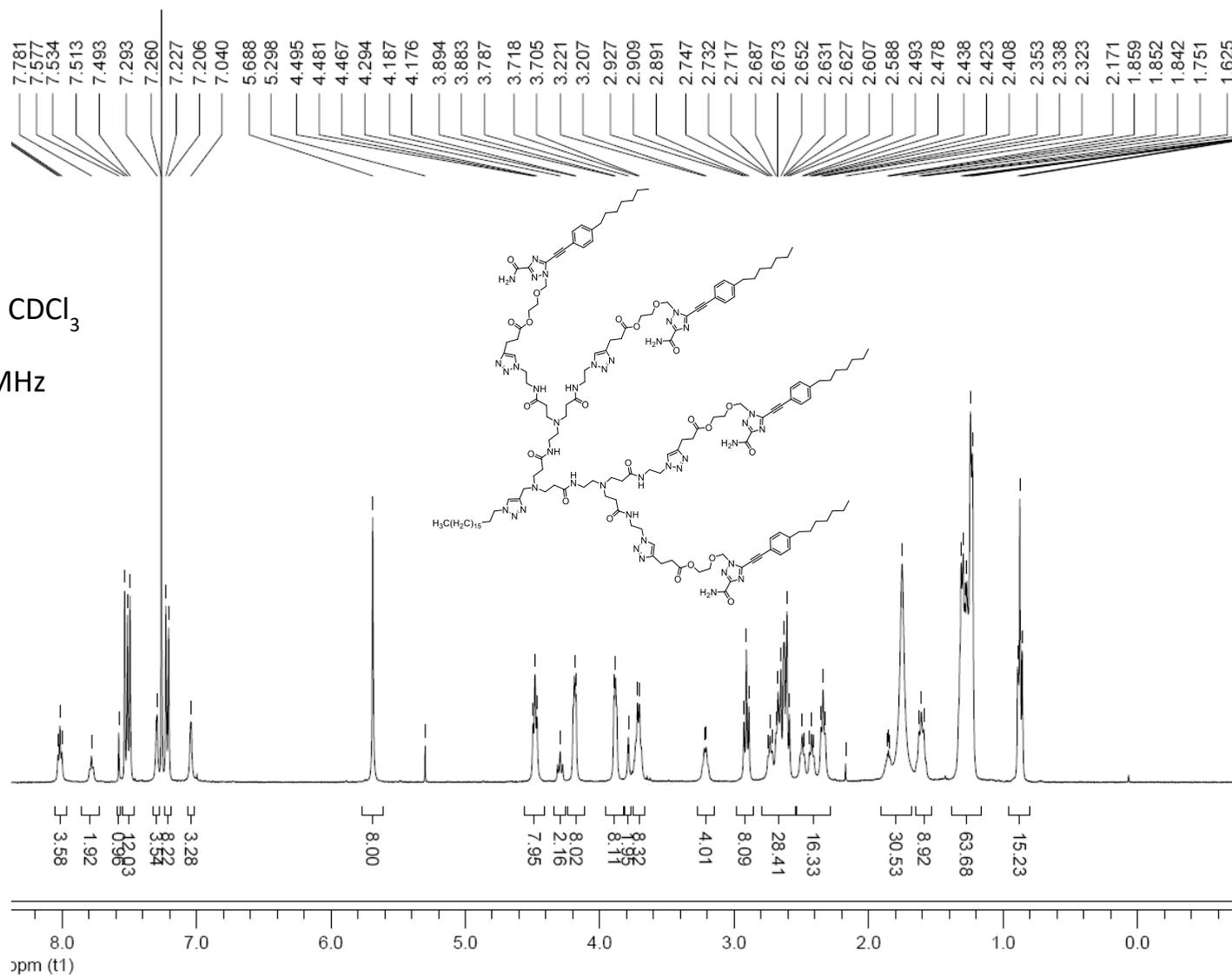


^{13}C NMR, $\text{CDCl}_3/\text{CDOD}$,
100 MHz





$^1\text{H NMR CDCl}_3$
400 MHz



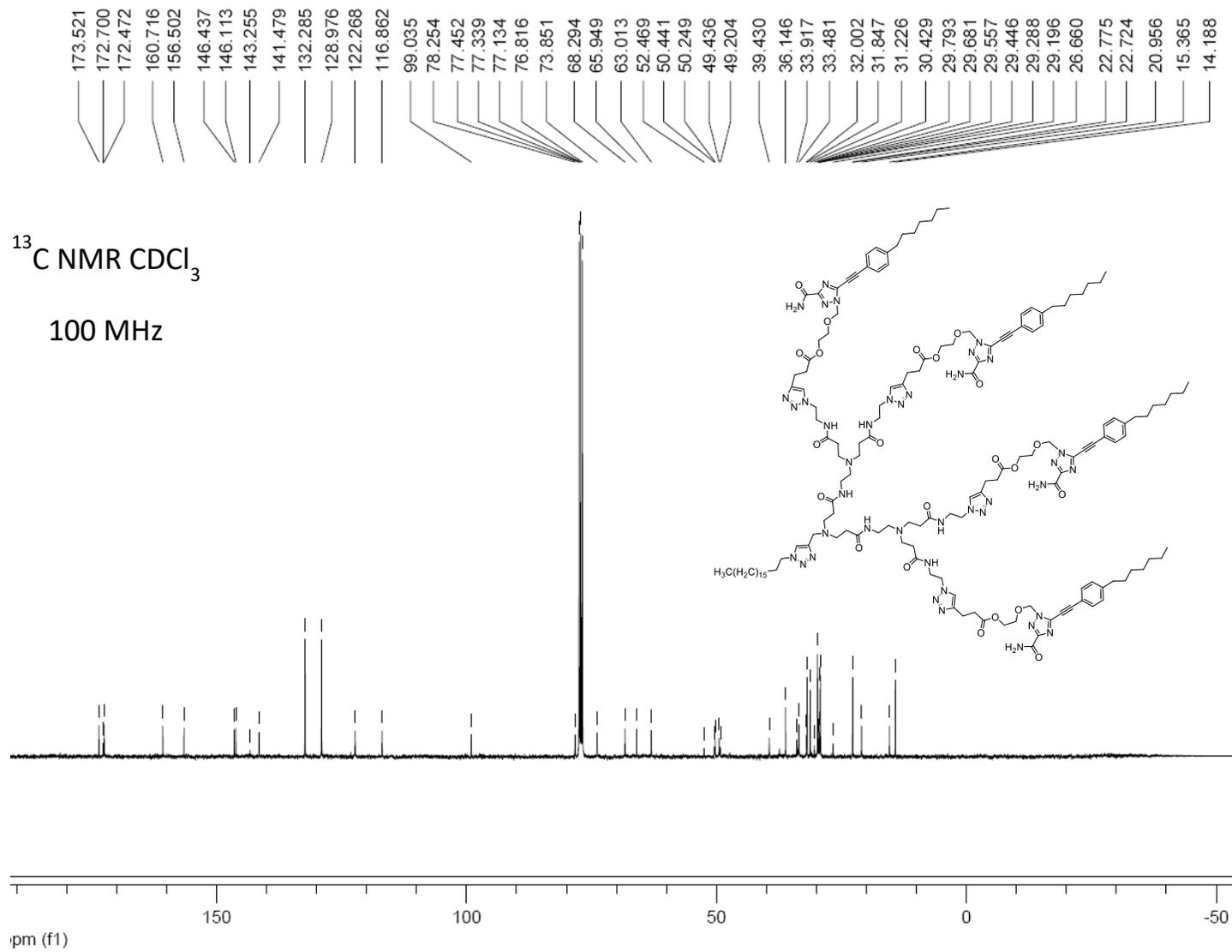
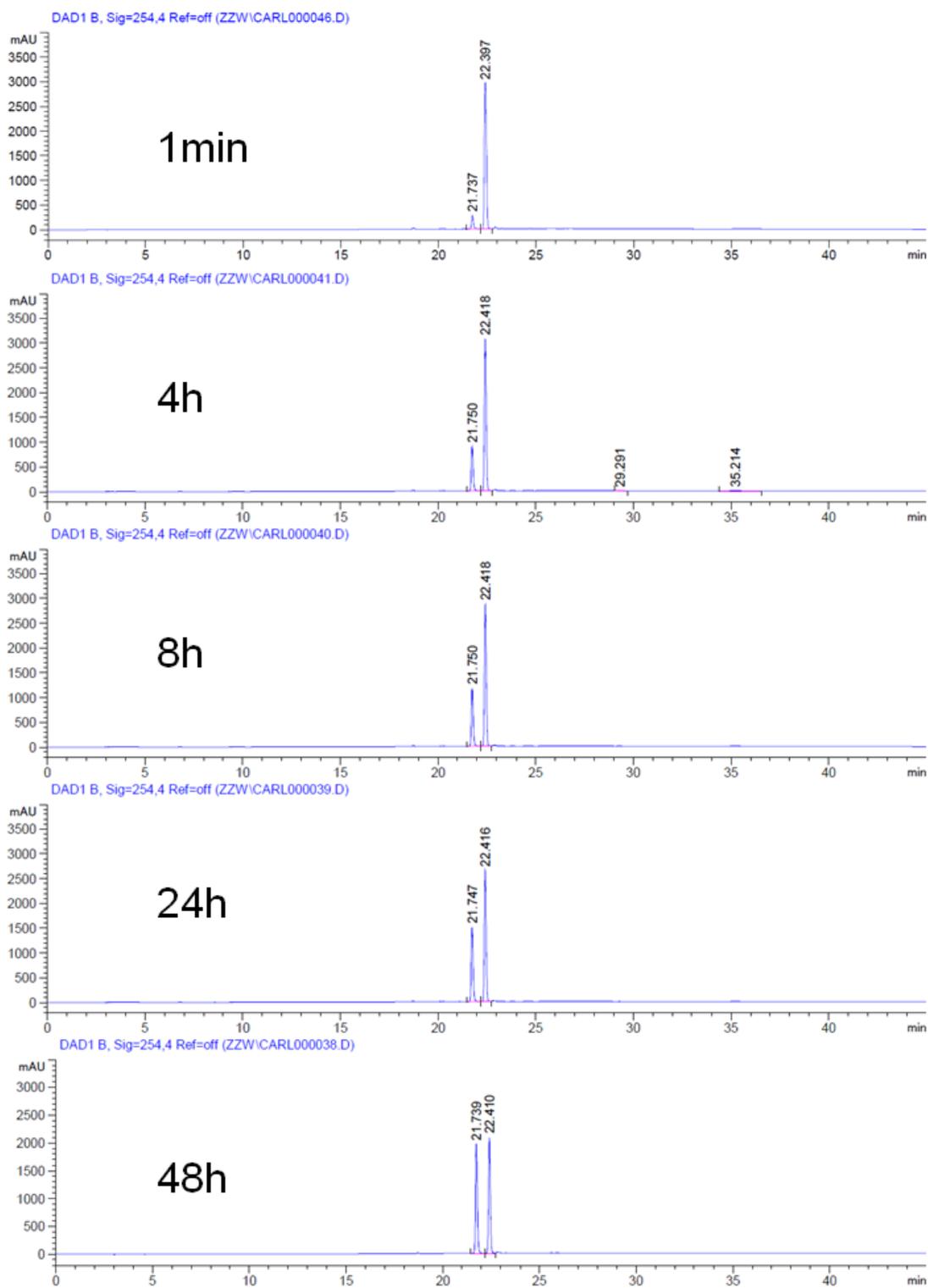
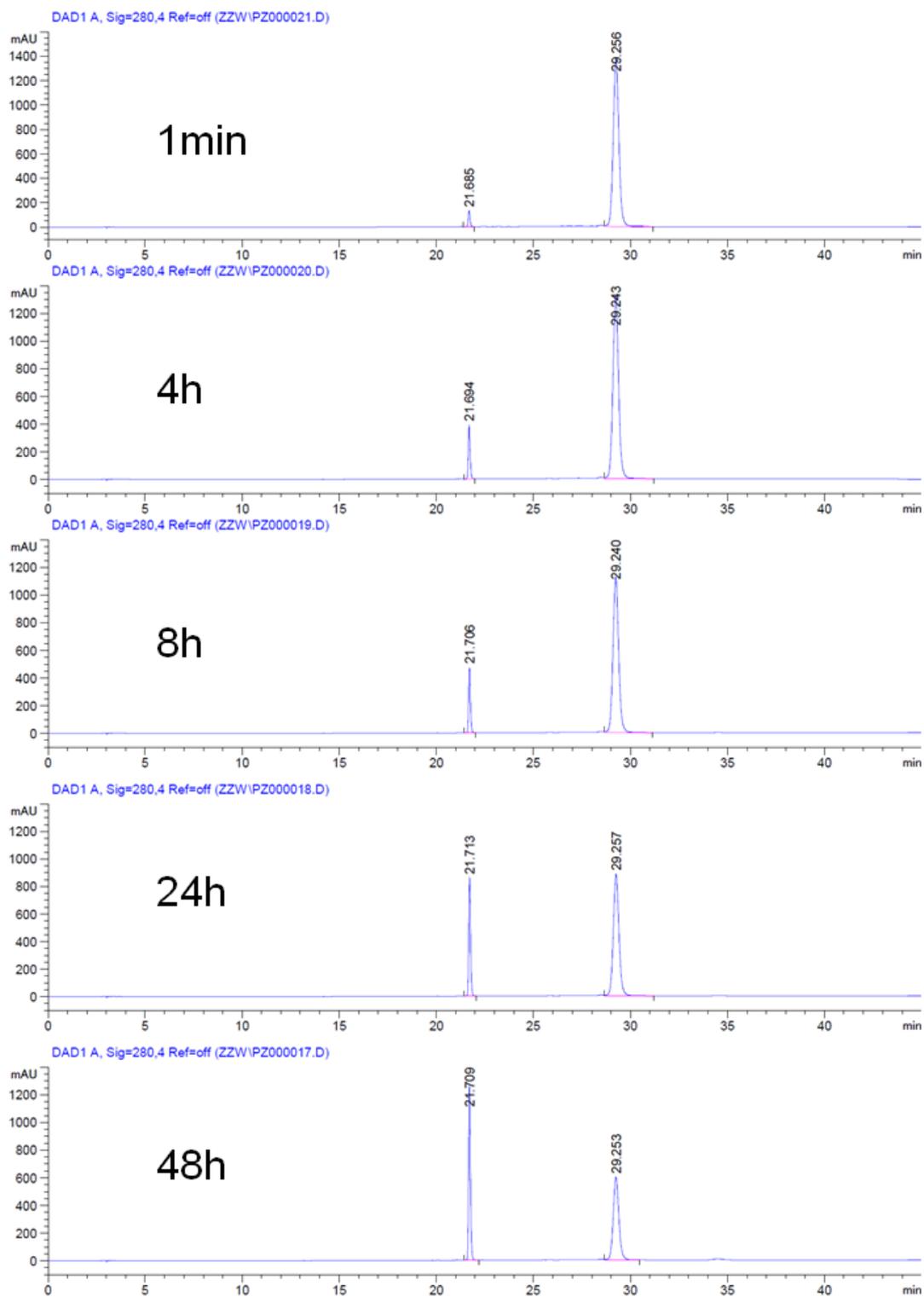


Figure S1: HPLC analysis of the enzymatic release of **1** from **2**, **I** and **II**, respectively.

Compound 2



Compound I



Compound II

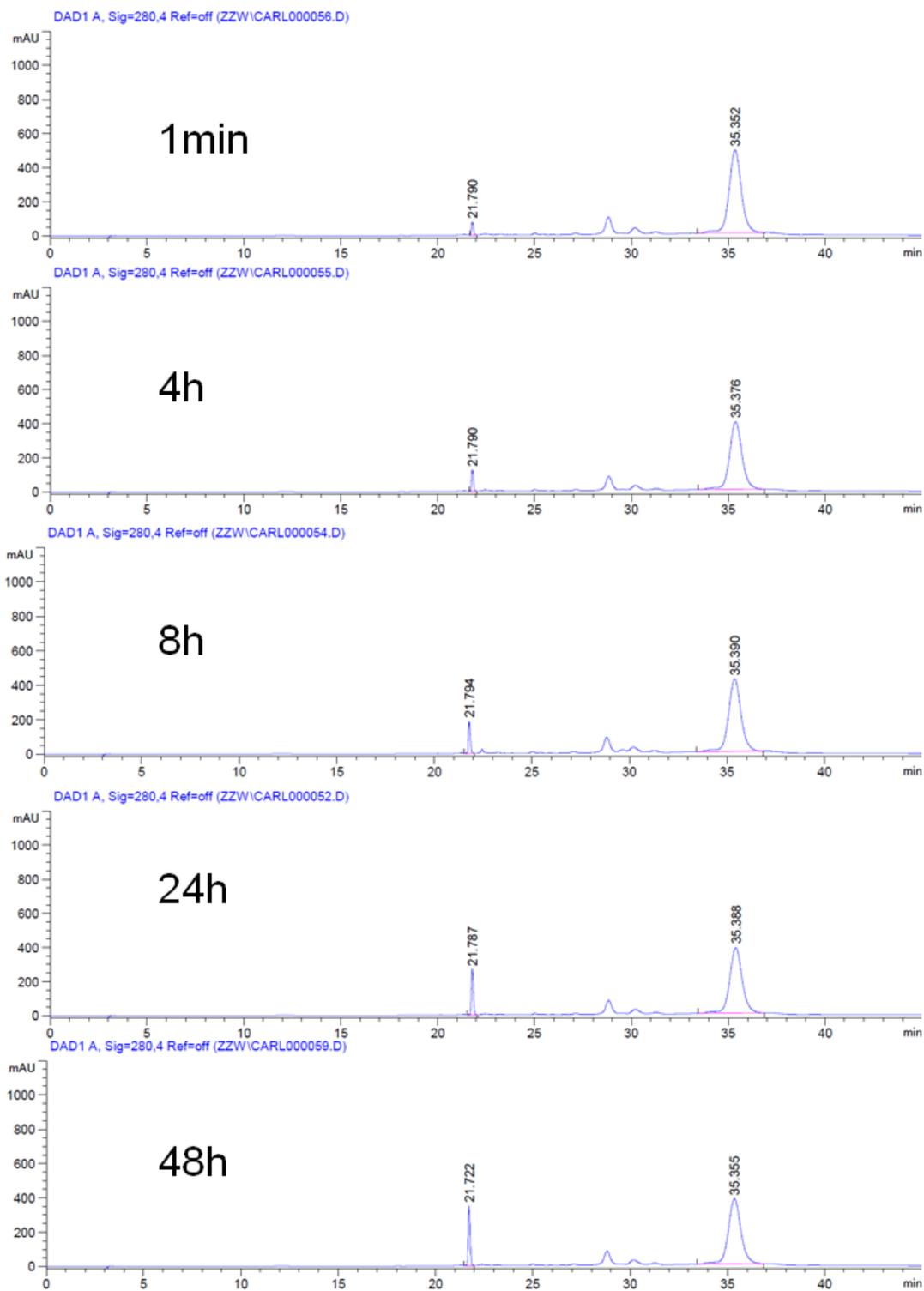


Figure S2: HPLC analysis of the hydrolysis of **I** and **II** at pH = 5.0.

