Supporting Information for

Synthesis and biological evaluation of zinc chelating compounds as metallo-β-lactamase inhibitors

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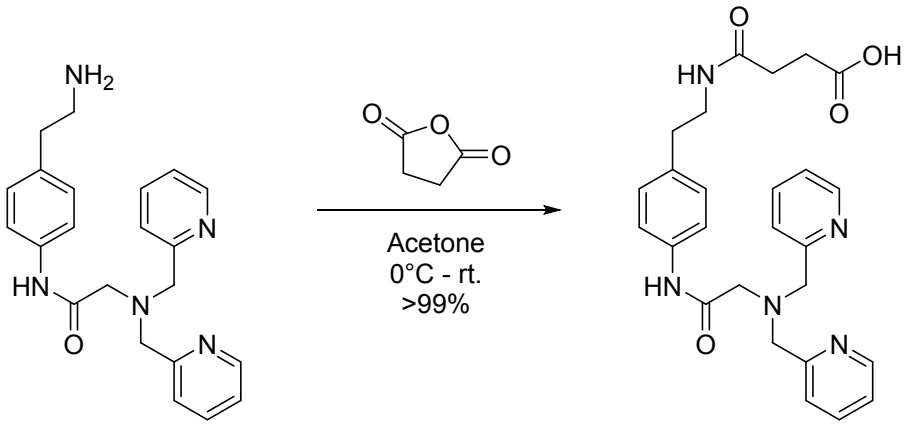
General synthetic information

All reagents and solvents were of analytical grade and were used as received, without further purification. ^{1}H spectra were recorded with Bruker DPX300, AVII400, AVIIIHD400 and AVII600 Fourier transform spectrometers, using an internal deuterium lock, operating at 300 MHz, 400 MHz or 600 MHz resonance frequency for ^{1}H and 75 MHz, 100 MHz and 150 MHz for ^{13}C. All spectra were recorded at 25°C. Chemical shifts are reported in parts per million (ppm) relative to TMS by calibrating either using the residual protons of deuterated solvents, or the ^{13}C resonance from the deuterated solvent itself, with the following shifts: $\delta = 2.50$ ppm for ^{1}H NMR and $\delta = 39.52$ ppm for ^{13}C NMR for DMSO-d_6, $\delta = 7.26$ ppm for ^{1}H NMR and $\delta = 77.16$ ppm for ^{13}C NMR for chloroform-*d*, $\delta = 3.31$ ppm for ^{1}H NMR and $\delta = 49.00$ ppm for ^{13}C NMR for methanol-d_4, $\delta = 5.32$ ppm and 54.00 ppm for ^{13}C NMR for dichloromethane-d_2 and $\delta = 4.79$ ppm for ^{1}H NMR for D_2O. Spectral assignments were in some cases aided by inclusion of ^{1}H-detected, gradient selected 2D experiments (^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HSQC and ^{1}H,^{13}C-HMBC), and carbon multiplicity determined either by APT, DEPT or multiplicity-edited HSQC experiments. Mass spectra were recorded at 70 eV on a Waters Prospec Q or Micromass QTOF 2W spectrometer using ESI or APCI as the method of ionization. High-resolution mass spectra were recorded at 70 eV on a Waters Prospec Q or a Micromass QTOF 2W spectrometer using ESI or APCI as the method of ionization. TLC analyses were carried out using either Merck Silica gel 60 F_{254} plates, Merck Aluminum Oxide (neutral) 60 F_{254} plates, or Merck Silica gel RP-18 60 F_{254} plates, visualized by UV light. Compounds devoid of UV-absorbing chromophores were visualized using ninhydrin stain. The yields reported are of isolated material and are uncorrected for purity.

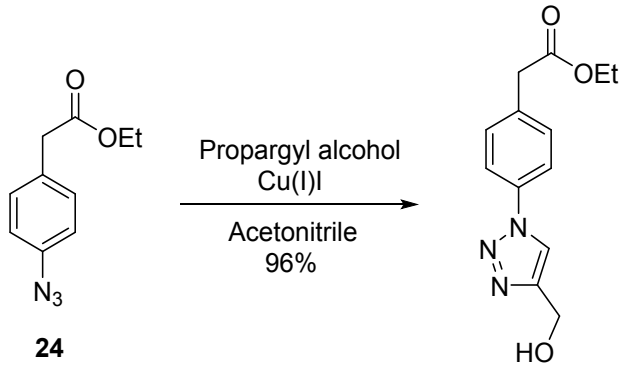
Preparation of 4-((4-(2-(bis(pyridin-2-ylmethyl)amino)acetamido)phenethyl)amino)-4-oxobutanoic acid (1).



5 **1**

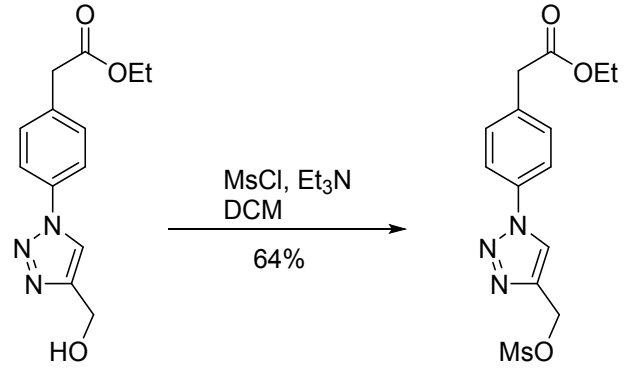
The amine **5** (100 mg, 0.27 mmol), prepared according to literature,[1] was dissolved in 10 mL acetone and cooled to 0°C in an ice-water bath. Succinic anhydride (27 mg, 0.27 mmol) was then added in one portion to the stirring solution. The ice-water bath was removed and the reaction was allowed to stir at room temperature overnight. The crude mixture was then concentrated under reduced pressure to give 126 mg of the title compound as a pale yellow solid (>99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 8.58 (d, J = 4.6 Hz, 2H), 7.94 (s, 1H), 7.76 (t, J = 7.5 Hz, 2H), 7.59 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 7.7 Hz, 2H), 7.32 – 7.21 (m, 2H), 7.16 (d, J = 8.1 Hz, 2H), 3.91 (s, 4H), 3.55 – 3.09 (m, 7H), 2.74 – 2.58 (m, 2H), 2.40 (t, J = 6.9 Hz, 2H), 2.28 (t, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.84, 170.87, 169.02, 158.40, 149.01, 136.81, 136.67, 134.40, 128.89, 123.03, 122.39, 119.01, 59.42, 57.80, 40.32, 34.61, 30.15, 29.36. HR-MS (APCI, pos. mode) *m/z* 476.2292 calculated for $C_{26}H_{30}N_5O_4$ ($[M+H]^+$), found *m/z* 476.2293.

Preparation of 2-(4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)acetic acid (2)



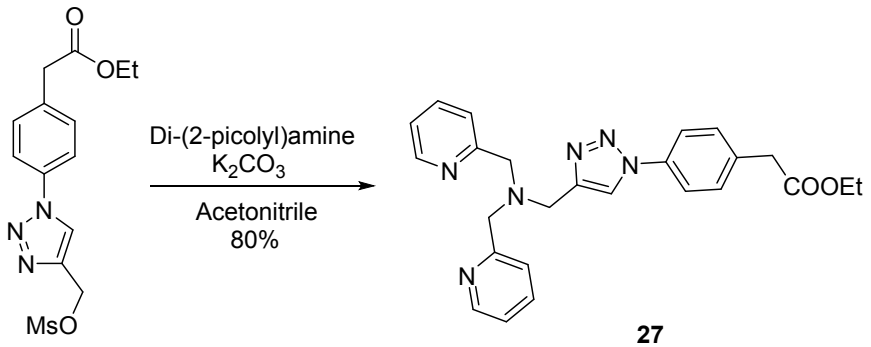
24 **25**

*Ethyl 2-(4-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)acetate (**25**).* To a solution of ethyl 4-azidophenylacetate (**24**, 5.00 g, 24.4 mmol), prepared according to the literature,[2] in 58 mL acetonitrile, propargyl alcohol (2.73 g, 48.7 mmol) and copper(I)iodide (0.928 g, 4.87 mmol) was added. After stirring for 24 h, volatiles were removed under reduced pressure and the crude product purified using column chromatography on silica, using a gradient of ethyl acetate in dichloromethane. Removal of solvent gave the product as a yellow solid (6.14 g, 96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (s, 1H), 7.86 (m, 2H), 7.48 (m, 2H), 5.33 (t, J = 5.6 Hz, 1H), 4.61 (d, J = 5.6 Hz, 2H), 4.11 (q, J = 7.12 Hz, 2H), 3.78 (s, 2H), 1.21 (t, J = 7.1 Hz, 3H). MS (ESI, positive mode) m/z 262.3 $[M+H]^+$.



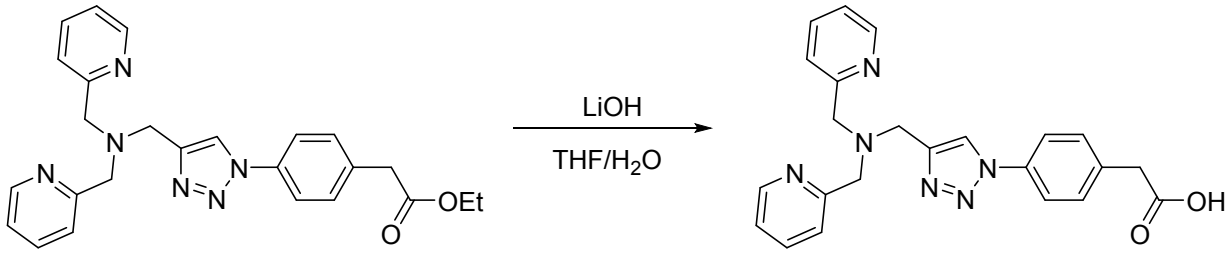
25 **26**

*Ethyl 2-(4-(4-(((methylsulfonyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)acetate (**26**).* Ethyl 2-(4-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)phenyl)acetate (**25**, 6.100 g, 23.35 mmol) was dissolved in 58 mL dichloromethane and kept in an ice bath. Triethylamine (3.544 g, 35.02 mol) was added, followed by methanesulfonyl chloride (3.209 g, 28.02 mmol). The reaction mixture was kept stirring on ice bath for 3 h, after which volatiles were removed under reduced pressure. Purification of the crude product on a silica column, using a gradient of 5%-10% ethyl acetate in dichloromethane, gave, after removal of solvent, the product as a colourless solid (5.11 g, 64%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H), 7.87 (m, 2H), 7.51 (m, 2H), 5.44 (s, 2H), 4.11 (q, J = 7.12 Hz, 2H), 3.79 (s, 2H), 3.29 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H).



26 **27**

*Ethyl 2-(4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)phenyl)acetate (**27**).* Di-(2-picolyl)amine (2.83 g, 14.2 mmol) was dissolved in acetonitrile. Potassium carbonate (3.93 g, 28.4 mmol) was added and the mixture was cooled down in an ice bath. The mesylate **26** prepared in the previous reaction (4.82 g, 14.2 mmol), dissolved in acetonitrile (total volume of acetonitrile in the reaction was 310 mL), was subsequently added dropwise, and after removal of the ice bath, allowed to react at room temperature for 12 hours. Filtration through celite, and with subsequent wash of the celite with dichloromethane, gave, after removal of solvents, a crude product as a dark orange thick oil. Purification on a silica column, using a gradient of methanol in dichloromethane, with additional ammonia added to the mobile phase, gave the product as a dark orange oil. Purification was performed on three combined batches of different sizes, with a combined yield of 6.02 g (80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (s, 1H), 8.50 (m, 2H), 7.87 (m, 2H), 7.78 (td, J = 7.6 Hz, 1.9 Hz, 2H), 7.63 (d, J = 7.9 Hz, 2H), 7.49 (m, 2H), 7.25 (m, 2H), 4.11 (q, J = 7.1 Hz, 2H), 3.86 (s, 2H), 3.82 (s, 4H), 3.78 (s, 2H), 1.20 (t, J = 7.1 Hz, 3H). MS (ESI, positive mode) m/z 443.2 $[M+H]^+$.

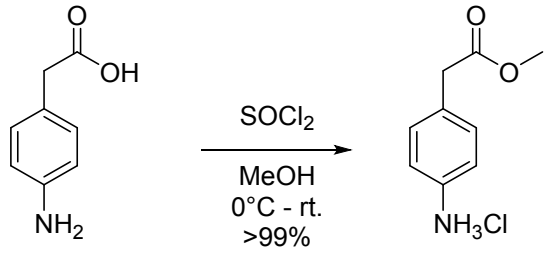


27 **2**

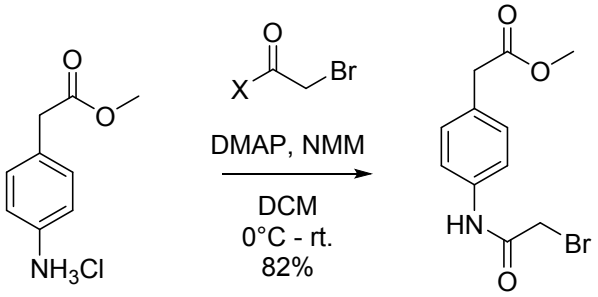
*2-(4-(4-((Bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl) phenyl)acetic acid (**2**).* Ethyl 2-(4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)phenyl)acetate (**27**, 323.1 mg, 0.73 mmol, 1.0 eq.) was dissolved in 10 mL THF and cooled to 0 °C in an ice bath. A solution of LiOH hydrate (61 mg, 1.46 mmol, 2.0 eq.) in 5 mL water was added and the solution stirred at 0 °C until TLC (Alumina, 5% MeOH / CH_2Cl_2) indicated full conversion. The THF was removed under reduced pressure and the residual aqueous solution was adjusted to pH = 6 using HCl (aq.). The solvent was

removed under reduced pressure affording the product in quantitative yield, and used directly in the next step without further purification.

Preparation of 2-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-yl methyl)amino)acetamido)phenyl)acetic acid (3).

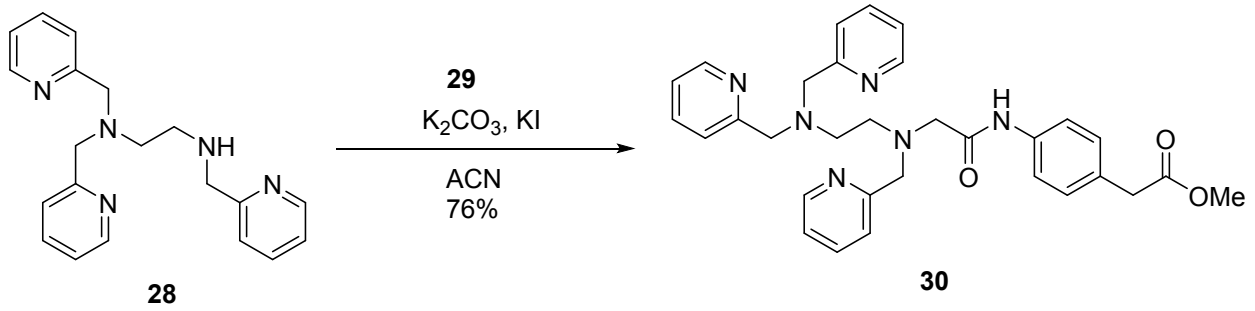


Methyl 2-(4-aminophenyl)acetate hydrochloride. 2-(4-aminophenyl)acetic acid (5.176 g, 34.2 mmol, 1 eq.) was suspended in 100 mL methanol and cooled to 0°C in an ice bath. To this mixture $SOCl_2$ (2.89 mL, 41.07 mmol, 1.2 eq.) was added dropwise over a period of 10 minutes. The mixture was stirred at 0°C for another 30 minutes, 1 h at room temperature and then refluxed for 14 h. The reaction mixture was then concentrated under reduced pressure to afford the title compound as a pale brown solid in quantitative yield that was taken to the next step without further purification. ^{1}H NMR was in accordance with published data.[3]

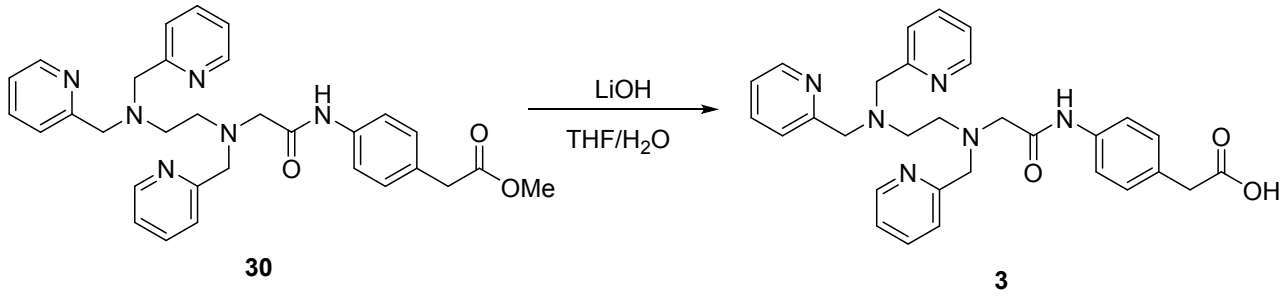


29

*Methyl 2-(4-(2-bromoacetamido)phenyl)acetate (**29**).* Methyl 2-(4-aminophenyl)acetate hydrochloride, prepared as described above (6.89 g, 34.2 mmol, 1 eq.) was suspended in 100 mL DCM and cooled to 0°C in an ice bath. A solution of DMAP (7.51 g, 61.56 mmol, 1.8 eq.) and NMM (3.75 mL, 34.2 mmol, 1 eq.) in 50 mL DCM was added dropwise over 30 minutes via a dropping funnel, followed by dropwise addition of bromoacetyl bromide (1.8 eq.) over 30 minutes at 0°C. The mixture was stirred at 0°C for another 30 minutes and at room temperature for 12 h. The mixture was then washed with 0.1 M HCl (3 x 50 mL) and brine (50 mL) and the organic phase dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The product was isolated via column chromatography on SiO_2 using isocratic elution with a 3:1 mixture of *n*-heptane and EtOAc to afford methyl 2-(4-(2-bromoacetamido)phenyl)acetate (**29**) in 82% yield, as white solids, which could be used directly in the next step. Optional additional purification could be obtained by recrystallization from EtOAc.



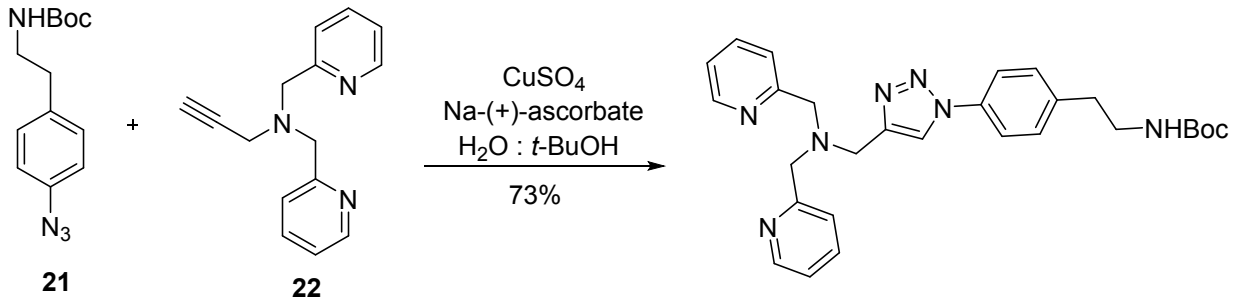
*Methyl 2-(4-(2-((2-(bis(pyridin-2-yl methyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenyl)acetate (**30**).* N^1,N^1,N^2-tris(pyridin-2-ylmethyl)ethane-1,2-diamine (**28**), prepared as described in the literature,[4] was dissolved in 50 mL CH_3CN at room temperature. To this solution was added K_2CO_3 (4 eq.) and KI (0.6 eq.), followed by methyl 2-(4-(2-bromoacetamido)phenyl)acetate (**29**) (1.1 eq.). The mixture was heated to reflux for 16 h until TLC indicated full conversion of the amine. The mixture was then passed through a pad of celite using CH_3CN as eluent and concentrated under reduced pressure. The product was isolated via column chromatography on neutral Al_2O_3 using 1-2% MeOH in DCM as eluent to afford the titled compound in 76% yield as a brown oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 8.52 (dd, J = 4.8, 0.8 Hz, 1H), 8.44 (dd, J = 4.8, 0.9 Hz, 2H), 7.71 (td, J = 7.6, 1.8 Hz, 1H), 7.64 (td, J = 7.6, 1.8 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H), 7.41 (d, J = 7.8 Hz, 2H), 7.33 (d, J = 7.7 Hz, 1H), 7.29 – 7.23 (m, 1H), 7.22 – 7.16 (m, 4H), 3.75 (s, 2H), 3.69 (s, 4H), 3.62 (s, 2H), 3.61 (s, 3H), 3.28 (s, 2H), 2.76 (t, J = 6.4 Hz, 2H), 2.61 (t, J = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.74, 169.46, 158.98, 158.53, 149.03, 148.72, 137.41, 136.66, 136.35, 129.64, 129.17, 123.07, 122.75, 122.39, 122.05, 119.11, 60.16, 59.65, 58.15, 51.74, 51.65, 51.14, 30.67.



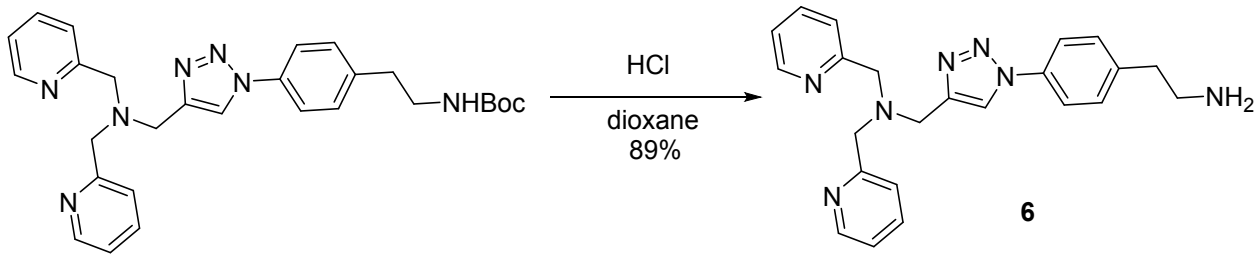
*2-(4-(2-((2-(Bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylethyl)amino)acetamido)phenyl)acetic acid (**3**).* Methyl 2-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenyl)acetate (**30**, 922 mg, 1.7 mmol, 1 eq.) was dissolved in 10 mL THF at 0 °C. To this solution was added LiOH·H_2O (144 mg, 3.4 mmol, 2.0 eq.) in 7 mL dest. H_2O and the solution stirred at 0 °C until TLC indicated full conversion (Al_2O_3, 5% MeOH in DCM). The mixture was then concentrated under reduced pressure to remove the THF, and the residual aq. solution adjusted to pH 7 with 0.5 M HCl. The solvent was removed under reduced pressure to afford the product in

quantitative yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.51 (dd, J = 4.7, 0.7 Hz, 1H), 8.44 (dd, J = 4.8, 0.8 Hz, 2H), 7.71 (td, J = 7.6, 1.8 Hz, 1H), 7.65 (td, J = 7.6, 1.8 Hz, 2H), 7.45 (t, J = 9.3 Hz, 2H), 7.42 (t, J = 9.4 Hz, 2H), 7.33 (d, J = 7.7 Hz, 1H), 7.27 – 7.23 (m, 1H), 7.23 – 7.18 (m, 2H), 7.15 (d, J = 8.4 Hz, 2H), 3.74 (s, 2H), 3.68 (s, 4H), 3.26 (s, 2H), 3.22 (s, 2H), 2.75 (t, J = 6.5 Hz, 2H), 2.60 (t, J = 6.5 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.40, 169.17, 158.98, 158.57, 149.04, 148.76, 136.69, 136.45, 136.00, 134.36, 129.43, 123.09, 122.79, 122.43, 122.14, 118.65, 60.20, 59.65, 58.18, 51.76, 51.13, 44.97.

Perparation of 2-(4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)ethan-1-amine (6).



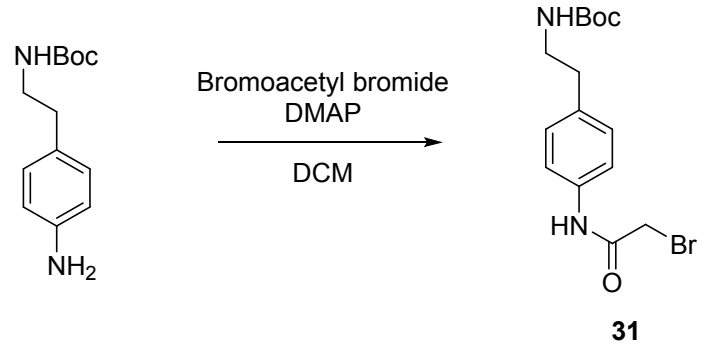
*tert-Butyl (4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)carbamate***.** Sodium-(+)-ascorbate (10.15 g, 51.24 mmol) and $CuSO_4 \cdot 5\ H_2O$ (6.40 g, 25.62 mmol) was mixed rapidly in 60 mL water, and the resulting yellow solution transferred to a flask containing *N*-propargyl-di(2-picolyl)amine (**22**, 6.08 g, 25.62 mmol), prepared as described in the literature,[5] dissolved in 60 mL *tert*-butanol. After rapid stirring, the resulting green solution was transferred to a flask containing *tert*-butyl 4-azidophenethylcarbamate (**21**, 5.60 g, 21.35 mmol), prepared following literature procedures,[6] and allowed to stir for 15 h. The reaction mixture was diluted with ethyl acetate, and washed with 1:1 water/brine. The water phase was then alkalized to pH 9-10 and extracted with ethyl acetate. Combined organic extracts was washed with a 0.025 M EDTA/0.5 M $NaHCO_3$ mixture and dried over sodium sulfate. Filtration and subsequent removal of solvent under reduced pressure gave a crude product as a brown oil which could be purified on neutral alumina, gradient 0-1% methanol in dichloromethane, giving the product as a thick orange oil. Purification was performed on three combined batches of different sizes, with a combined yield of 14.09 g (73%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (s, 1H), 8.51 (m, 2H), 7.83-7.77 (m, 4H), 7.63 (d, J = 7.9 Hz, 2H), 7.41 (m, 2H), 7.27 (m, 2H), 6.92 (br t, NH, 1H), 3.89 (s, 2H), 3.85 (s, 4H), 3.19 (m, 2H), 2.78 (t, J = 7.3 Hz, 2H), 1.37 (s, 9H). MS (ESI, positive mode) m/z 500.5 $[M+H]^+$.



*2-(4-(4-((Bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)phenyl)ethan-1-amine (**6**).* To a solution of *tert*-butyl (4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)phenethyl)carbamate (12.70 g, 25.4 mmol) in 96 mL dioxane, 4 M HCl in dioxane (46 mL) was added slowly. After stirring under Ar overnight, the volatiles were removed under reduced pressure. The crude

product was redissolved in 50 mL saturated $NaHCO_3$ solution (aq.) and extracted with 250 mL dichloromethane. This first extract contained product in a rather low purity. Repeated extractions with 100 mL dichloromethane (>10 repetitions) afforded, after removal of solvent under reduced pressure, pure product as an orange, thick oil (8.98 g, 88.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.50 (m, 2H), 7.82-7.75 (m, 4H), 7.63 (d, J = 7.6 Hz, 2H), 7.42 (m, 2H), 7.25 (m, 2H), 3.86 (s, 2H), 3.82 (s, 4H), 2.83 (m, 2H), 2.73 (m, 2H), 1.74 (br, 2H). MS (ESI, positive mode) m/z 400.4 $[M+H]^+$.

Preparation of *N*-(4-(2-aminoethyl)phenyl)-2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamide (7).



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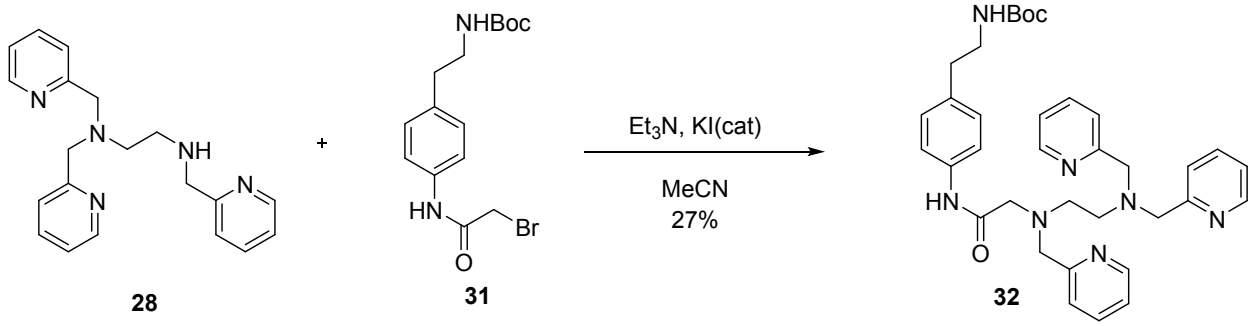
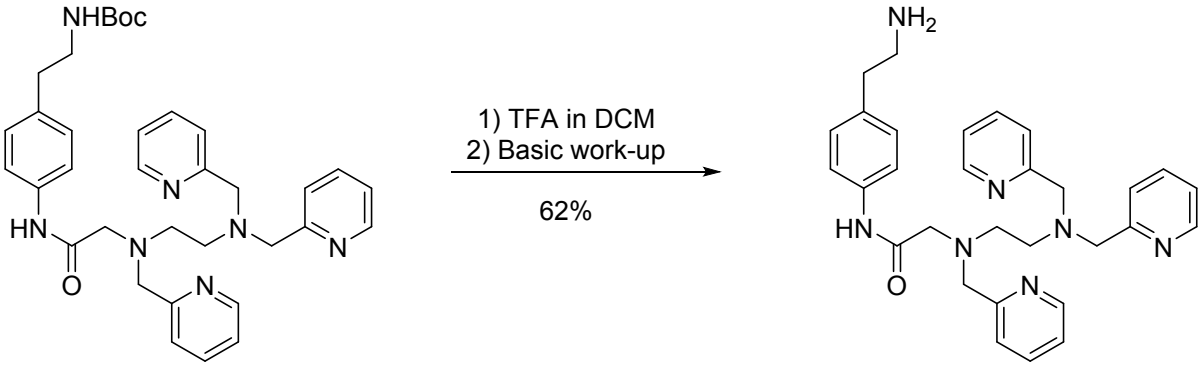
*tert-Butyl (4-(2-bromoacetamido)phenethyl)carbamate (**31**).* *tert*-Butyl (4-aminophenethyl)carbamate (4.5 g, 21.83 mmol, 1.0 eq) was dissolved in DCM (150 mL) and cooled to 0 degrees using an ice bath. DMAP (1.6 eq) was added in one portion to the stirring mixture, followed by bromoacetyl bromide (1.2 eq.), which was added dropwise by the aid of 50 mL DCM. The mixture was stirred for 30 minutes at 0 degrees and then 1.5 hour room temperature before it was concentrated *in vacuo*. The crude material was then purified using column chromatography on SiO_2 with 75-100% EtOAc in heptane as eluent. This gave a colorless solid which was used directly in the next step without further purification.



28 **31** **32**

*tert-Butyl (4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenethyl)carbamate (**32**).* N^1,N^1,N^2-tris(pyridin-2-ylmethyl)ethane-1,2-diamine (**28**, 315 mg, 0.945 mmol) and **31** (387 mg, 1.083 mmol) was dissolved in 300 mL MeCN at room temperature. KI (187 mg, 1.126 mmol) and triethyl amine (1.32 mL, 9.47 mmol) was then added to the stirring mixture. The reaction mixture was heated to reflux and left overnight. The crude mixture was then cooled to room temperature and concentrated under reduced pressure to give a thick oil. The crude product was dissolved in 1M K_2CO_3 (100 mL) and extracted with EtOAc (3x50 mL). The combined organic phases were pooled, dried over K_2CO_3, filtered and concentrated under reduced pressure. The pale green crude product was further purified by column chromatography on a neutral Al_2O_3 column using 0-5% MeOH in DCM as eluent to afford 155 mg (27%) of the titled product. ^{1}H NMR (600 MHz, chloroform-*d*) δ 10.40 (s, 1H), 8.51 – 8.48 (m, 1H), 8.47 – 8.44 (m, 2H), 7.59 (d, *J* =

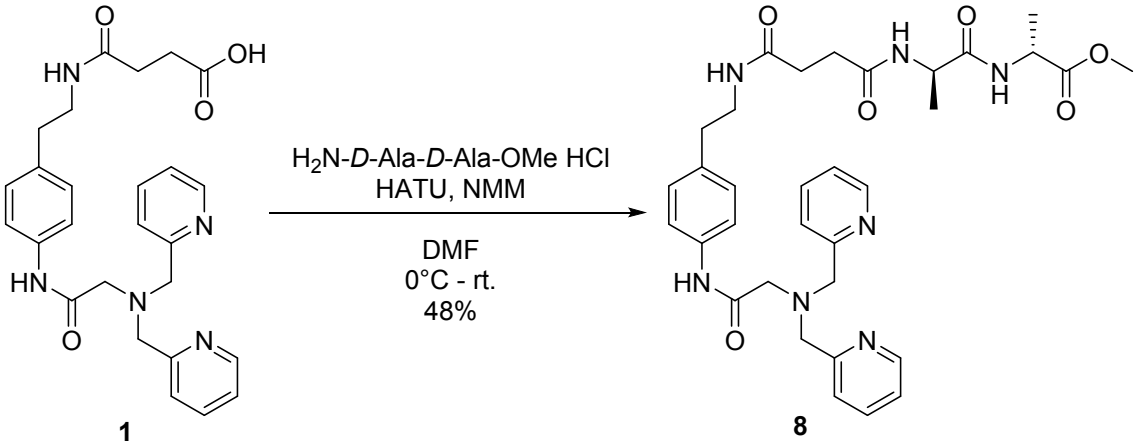
8.4 Hz, 2H), 7.57 – 7.49 (m, 3H), 7.39 (d, *J* = 7.8 Hz, 2H), 7.14 – 7.10 (m, 4H), 7.10 – 7.06 (m, 2H), 3.72 (s, 6H), 3.33 (d, *J* = 6.8 Hz, 2H), 3.27 (s, 2H), 2.81 – 2.71 (m, 4H), 2.68 (t, *J* = 6.4 Hz, 2H), 1.40 (s, 9H). ^{13}C NMR (151 MHz, chloroform-*d*) δ 169.92, 159.14, 158.18, 155.93, 149.55, 149.08, 136.94, 136.59, 136.47, 134.40, 129.17, 123.15, 123.11, 122.56, 122.11, 119.99, 61.01, 60.47, 58.70, 52.09, 51.59, 43.50, 41.90, 35.66, 28.48. HRMS (TOF MS, ESI pos. mode): Calculated mass for $C_{35}H_{43}N_7O_3$ $[M+H]^+$: 610.3505. Found: 610.3511



32 **7**

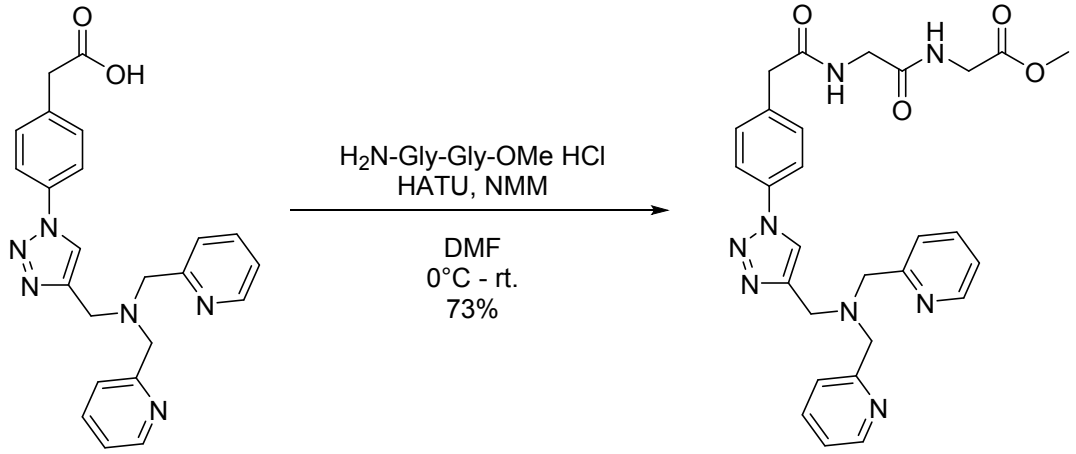
N-(4-(2-Aminoethyl)phenyl)-2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamide (7). The boc protected amine **32** (135 mg, 0.221 mmol) was dissolved in 10 mL DCM and cooled to 0 °C in an ice bath. Trifluoroacetic acid (1 mL, 13,06 mmol) was then dissolved in 10 mL DCM and added dropwise to the stirring mixture. The reaction was then allowed to warm to room temperature and left for 2 hours before it was concentrated under reduced pressure. The crude oil obtained was dissolved in 20 mL DCM and washed with 1M K_2CO_3 (3x20 mL). The organic phase was dried over K_2CO_3, filtered and concentrated under reduced pressure to give 70 mg (62%) of the titled product. ^{1}H NMR (600 MHz, chloroform-*d*) δ 10.39 (s, 1H), 8.54 – 8.51 (m, 1H), 8.50 – 8.46 (m, 2H), 7.63 – 7.59 (m, 2H), 7.59 – 7.52 (m, 3H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.17 – 7.07 (m, 6H), 3.75 – 3.72 (m, 6H), 3.29 (s, 2H), 2.95 (t, *J* = 6.9 Hz, 2H), 2.79 (t, *J* = 6.5 Hz, 2H), 2.74 – 2.67 (m, 4H), 2.16 (s, 2H). ^{13}C NMR (151 MHz, chloroform-*d*) δ 169.94, 159.27, 158.31, 149.66, 149.18, 136.82, 136.66, 136.54, 135.34, 129.29, 123.23, 123.17, 122.62, 122.18, 119.99, 61.09, 60.59), 58.80, 52.20, 51.68, 43.77, 39.68. HRMS (TOF MS, ESI pos. mode): Calculated mass for $C_{30}H_{35}N_7O$ $[M+H]^+$: 510.2981. Found: 510.2987.

Preparation of methyl (4-((4-(2-(bis(pyridin-2-ylmethyl)amino) acetamido)phenethyl)amino)-4-oxobutanoyl)-*D*-alanyl-*D*-alaninate (8).



Compound **1** (0.250 g, 0.526 mmol) was dissolved in DMF (2 mL) and cooled to 0°C in an ice-water bath. *D*-alanyl-*D*-alanine methyl ester hydrochloride (116 mg, 0.551 mmol, 1.05 equiv.) and HATU (211 mg, 0.555 mmol, 1.06 equiv) were then added, before NMM (120 uL, 1.09 mmol, 2.1 equiv.) was added to the stirring mixture. The mixture was left in the ice-water bath for 30 minutes before slowly heating to room temperature. The mixture was then left for 18 hours at room temperature. The mixture was then diluted with 50 mL 0.5 M K_2CO_3 and extracted with 5 x 10 mL EtOAc. The combined organic phases were combined, washed with fresh 0.5 M K_2CO_3 (4 x 25 mL) and dried over K_2CO_3, filtered and concentrated under reduced pressure. This afforded 0.159 mg of the title compound as a pale semisolid (0.252 mmol, 48%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.53 (s, 1H), 8.63 – 8.49 (m, 2H), 8.25 (d, J = 7.1 Hz, 1H), 8.05 (d, J = 7.8 Hz, 1H), 7.92 (t, J = 5.6 Hz, 1H), 7.80 – 7.70 (m, 2H), 7.63 – 7.55 (m, 2H), 7.52 – 7.38 (m, 2H), 7.33 – 7.23 (m, 2H), 7.19 – 7.06 (m, 2H), 4.36 – 4.15 (m, 2H), 3.90 (s, 4H), 3.59 (s, 3H), 3.41 (s, 2H), 3.26 – 3.13 (m, 2H), 2.69 – 2.58 (m, 2H), 2.29 (d, J = 10.0 Hz, 4H), 1.29 (d, J = 7.3 Hz, 3H), 1.19 (d, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.9, 172.3, 171.3, 171.1, 169.0, 158.4, 149.0, 136.8, 136.7, 134.4, 128.9, 123.0, 122.4, 119.0, 59.4, 57.8, 51.8, 47.6, 47.5, 40.20, 38.2, 34.6, 30.6, 17.9, 16.8. HR-MS (ESI, pos. mode) *m*/*z* 654.3011 calculated for $C_{33}H_{41}N_7O_6Na$, found *m*/*z* 654.3026.

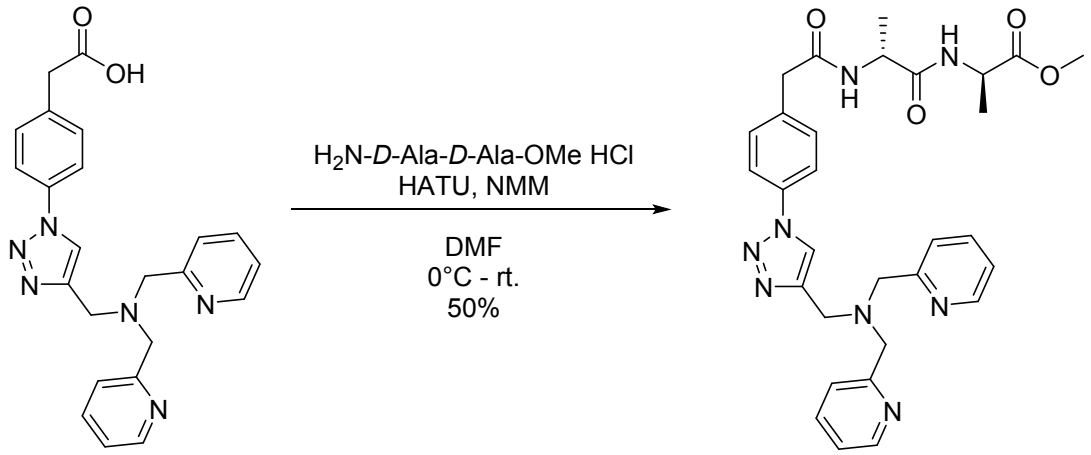
Preparation of methyl (2-(4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)acetyl)glycylglycinate (9).



2 **9**

To a flask containing HATU (154.7 mg, 0.407 mmol) and glycylglycine methyl ester hydrochloride (72.7 mg, 0.398 mmol), 2-(4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)acetic acid (**2**, 0.3175 mmol) dissolved in 3 mL DMF, was added. The mixture was put in an ice bath under nitrogen, to which *N*-methylmorpholine (0.10 mL, 0.91 mmol) was added. After stirring on ice bath for 30 min, the mixture was left at room temperature overnight. Volatile materials were removed under reduced pressure, and the crude product was loaded onto a plug of Bondesil C-18 OH SPE material, and eluted portion-wise with methanol/water mixtures, with percentage of methanol increasing stepwise from 20% to 50%. Fractions containing crude product was collected and solvents evaporated to afford 158.5 mg pure title compound (73.4%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.48 (s, 1H), 8.44 (m, 2H), 7.82-7.78 (m, 4H), 7.70 (d, J = 7.9 Hz, 2H), 7.53 (m, 2H), 7.27 (m, 2H), 3.97 (s, 2H), 3.94 (s, 4H), 3.87 (s, 4H), 3.72 (s, 3H), 3.75 (s, 2H). ^{13}C NMR (100 MHz, methanol-d_4) δ 173.8, 172.0, 171.7, 160.2, 149.5, 146.0, 138.7, 137.8, 137.3, 131.8, 125.0, 123.9, 123.5, 121.6, 60.6, 52.6, 50.0, 43.4, 42.9, 41.8. MS (APCI, positive mode) *m*/*z* 543.2 $[M+H]^+$, HR-MS (APCI, pos. mode) *m*/*z* 543.2463 calculated for $C_{28}H_{31}N_8O_4$, found *m*/*z* 543.2457.

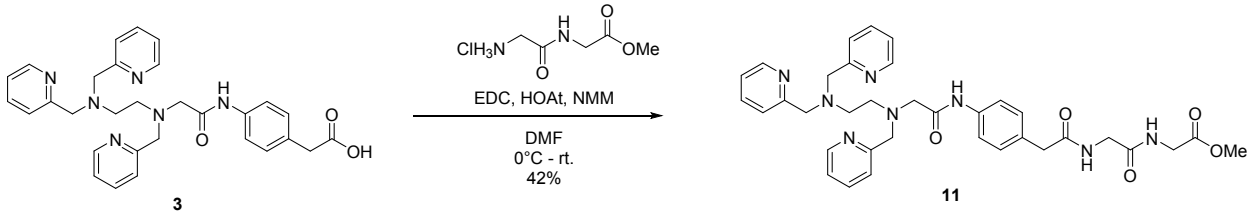
Preparation of methyl (2-(4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)phenyl)acetyl)-D-alanyl-D-alaninate (10).



2 **10**

To a flask containing the acid **2** (0.3175 mmol), HATU (122.9 mg, 0.3232 mmol) and *D*-alanyl-*D*-alanine methyl ester (174.8 mg as a 32.5% mixture with TFA, 0.326 mmol) dissolved in 3 mL DMF, was added. After cooling down in an ice bath under nitrogen, *N*-methylmorpholine (0.15 mL, 1.4 mmol) was added, and the reaction mixture kept in an ice bath for 30 min, and thereafter stirred at room temperature for 4 h. Subsequent removal of volatiles under reduced pressure afforded a crude product which was purified by addition to a plug of Bondesil C-18 OH SPE material, eluted portion-wise with methanol/water mixtures ranging from 40% to 60% methanol content. Pure fractions was pooled and after removal of solvent, 91.4 mg product was obtained (50.4%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.47 (s, 1H), 8.44 (m, 2H), 7.82-7.77 (m, 4H), 7.71 (d, J = 7.9 Hz, 2H), 7.51 (m, 2H), 7.27 (m, 2H), 4.40 (q, J = 7.3 Hz, 1H), 4.38 (q, J = 7.2 Hz, 1H), 3.93 (s, 2H), 3.87 (s, 4H), 3.70 (s, 3H), 3.65 (s, 2H), 1.38 (d, J = 7.2 Hz, 3H), 1.37 (d, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.8, 174.5, 173.0, 160.2, 149.5, 146.0, 138.7, 138.0, 137.2, 131.7, 125.0, 123.9, 123.5, 121.5, 60.6, 52.7, 50.3 (x2), 50.0, 42.7, 18.1, 17.3. MS (APCI, positive mode) m/z 571.3 $[M+H]^+$, HR-MS (APCI, pos. mode) m/z 571.2776 calculated for $C_{30}H_{35}N_8O_4$, found m/z 571.2771.

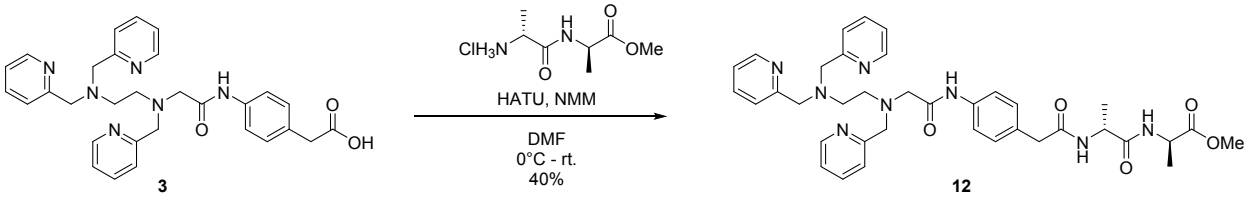
Preparation of methyl 2-(2-(2-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenyl)acetamido)acetamido)acetate (11).



3 11

Glycylglycine methyl ester hydrochloride (37 mg, 0.203 mmol, 1 eq.) was suspended in 3 mL ethanol at room temperature. To this suspension was added *N*-metylmorpholine (49 µL, 2.2 eq.) and the mixture was stirred until all dissolved, followed by addition of 2-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenyl)acetic acid (**3**, 106.7 mg, 0.203 mmol, 1 eq.) and HOAt (5.6 mg, 0.2 eq.). The mixture was cooled to 0 °C in an ice bath, and EDC·HCl (47 mg, 0.244 mmol, 1.2 eq.) was added in one portion. The mixture was stirred at 0 °C for 30 min, then allowed to warm to room temperature and stirred for 16 h. The solvent was removed under reduced pressure, the residue dissolved in a minimum amount of 5% MeOH in CH_2Cl_2 and passed through a plug of neutral alumina using that eluent mixture. The product was isolated via column chromatography on neutral Al_2O_3 using 1% MeOH in CH_2Cl_2 as eluent to afford 54.1 mg (42%) of the product as a yellow oil. ^{1}H NMR (400 MHz, chloroform-*d*) δ 10.49 (s, 1H), 8.48 (d, *J* = 4.2 Hz, 1H), 8.44 (d, *J* = 4.7 Hz, 2H), 7.96 (DMF), 7.63 (d, *J* = 8.4 Hz, 2H), 7.58 – 7.46 (m, 3H), 7.37 (d, *J* = 7.8 Hz, 2H), 7.18 (t, *J* = 10.3 Hz, 2H), 7.15 – 7.01 (m, 5H), 6.65 (s, 1H), 5.25 (s, 0.19H, CH_2Cl_2), 3.91 (dd, *J* = 8.9, 5.5 Hz, 4H), 3.70 (s, 6H), 3.65 (s, 3H), 3.54 (s, 2H), 3.27 (s, 2H), 2.91 (DMF), 2.83 (DMF), 2.76 (t, *J* = 6.2 Hz, 2H), 2.66 (t, *J* = 6.2 Hz, 2H), 2.12 (acetone). ^{13}C NMR (101 MHz, chloroform-*d*) δ 171.93, 170.13, 170.05, 169.32, 159.13, 158.16, 149.54, 149.07, 137.76, 136.62, 136.50, 129.86, 123.15, 123.11, 122.58, 122.13, 120.24, 60.93, 60.45, 58.70, 52.31, 52.04, 51.55, 43.19, 42.82, 41.12. HR-MS (APCI, pos. mode) *m/z* calc. for $C_{35}H_{41}N_8O_5$: 653.3194, found 653.3193 $[M+H]^+$.

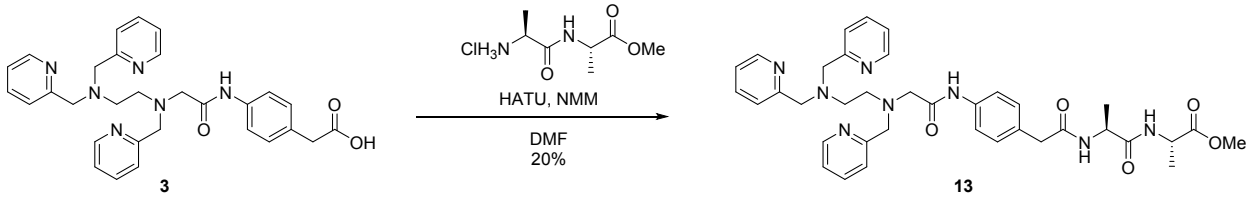
Preparation of (*R*)-methyl 2-((*R*)-2-(2-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenyl)acetamido)propanamido)propanoate (12).



3 **12**

2-(4-(2-((2-(Bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenyl)acetic acid (**3**, 97.1 mg, 0.18 mmol, 1 eq.) was dissolved in 2 mL DMF at 0 °C. To this solution the *D*-Ala-*D*-Ala-OMe·HCl (41 mg, 0.19 mmol, 1.05 eq.) was added, followed by HATU (72 mg, 0.19 mmol, 1.05 eq.) and NMM (41 µl, 2.1 eq.). The mixture was stirred at 0 °C for 30 min, then at room temperature for 16 h. The mixture was diluted with dest. H_2O (20 mL) and 0.5 M K_2CO_3 (20 mL) and the aqueous phase was extracted with EtOAc (5 x 20 mL). The combined organics were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was isolated via column chromatography on neutral Al_2O_3 using 1-5% MeOH in CH_2Cl_2 as eluent to afford the product in 50 mg yield (40%) as a yellow foam. ^{1}H NMR (400 MHz, chloroform-*d*) δ 10.48 (s, 1H), 8.46 (m, 3H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.58 – 7.45 (m, 3H), 7.37 (d, *J* = 7.8 Hz, 2H), 7.18 (d, *J* = 8.4 Hz, 2H), 7.14 – 7.03 (m, 4H), 6.99 (d, *J* = 7.3 Hz, 1H), 6.40 (d, *J* = 7.4 Hz, 1H), 5.25 (s, 0.02H, CH_2Cl_2), 4.55 – 4.39 (m, 2H), 3.70 (s, 6H), 3.67 (s, 3H), 3.50 (s, 2H), 3.27 (s, 2H), 2.76 (t, *J* = 6.3 Hz, 2H), 2.67 (t, *J* = 6.1 Hz, 2H), 1.32 (d, *J* = 7.2 Hz, 3H), 1.29 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 173.13, 171.82, 171.13, 170.11, 159.16, 158.19, 149.60, 149.13, 137.87, 136.66, 136.54, 129.83, 129.80, 123.21, 123.17, 122.62, 122.18, 120.23, 61.01, 60.50, 58.73, 52.49, 52.07, 51.59, 48.84, 48.18, 43.11, 18.28, 18.06. HR-MS (APCI, pos. mode) *m/z* calc. for $C_{37}H_{45}N_8O_5$: 681.3507, found: 681.3501 $[M+H]^+$.

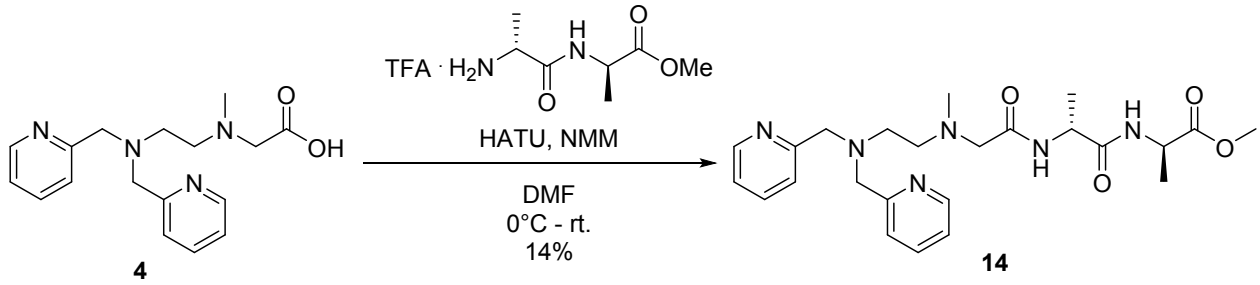
Preparation of (*S*)-methyl 2-((*S*)-2-(2-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenyl)acetamido)propanamido)propanoate (13).



3 13

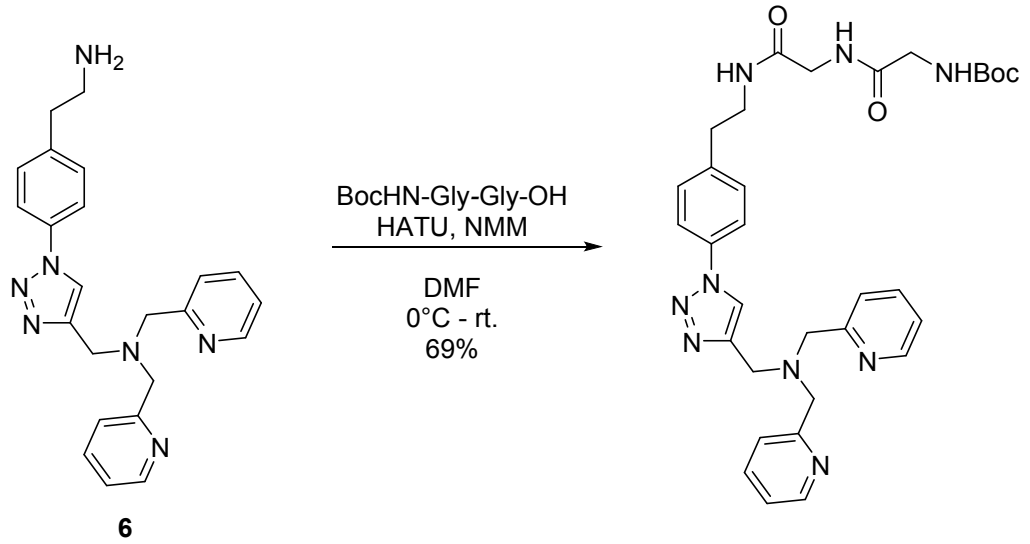
To a solution of *L*-Ala-*L*-Ala-OMe·HCl (0.325 g, 1.54 mmol) in 5 mL DMF was added 2-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenyl)acetic acid (**3**, 0.539 g, 1.02 mmol), HATU (0.586 g, 1.54 mmol) and NMM (0.25 mL, 2.3 mmol). The yellow solution was stirred at room temperature for 3 h. After diluting with dest. H_2O (30 mL) and 0.5 M K_2CO_3 (20 mL), the aqueous phase was extracted with EtOAc (3 x 50 mL). The aqueous phase was diluted further with brine (50 mL) and extracted with EtOAc (2 x 20 mL). The combined organics were dried over K_2CO_3, filtered and concentrated under reduced pressure. The product was isolated via column chromatography on neutral Al_2O_3 using 1-5% MeOH in CH_2Cl_2 as eluent to afford the product in 130.5 mg yield (20%) as a pale yellow semi-solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 10.47 (s, 1H), 8.49 (d, *J* = 4.4 Hz, 1H), 8.46 (ddd, *J* = 4.9, 1.7, 0.9 Hz, 2H), 7.65 (d, *J* = 8.5 Hz, 2H), 7.58-7.51 (m, 3H), 7.38 (d, *J* = 7.8 Hz, 2H), 7.19 (d, *J* = 8.5 Hz, 2H), 7.15 – 7.06 (m, 4H), 6.92 (d, *J* = 7.3 Hz, 1H), 6.33 (d, *J* = 7.5 Hz, 1H), 4.55 – 4.40 (m, 2H), 3.73 (s, 6H), 3.68 (s, 3H), 3.51 (s, 2H), 3.30 (s, 2H), 2.78 (t, *J* = 6.1 Hz, 2H), 2.70 (t, *J* = 6.1 Hz, 2H), 1.32 (d, *J* = 7.2 Hz, 3H), 1.29 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 173.13, 171.87, 171.14, 170.06, 159.05, 158.14, 149.59, 149.13, 137.86, 136.67, 136.56, 129.84, 129.80, 123.23, 123.19, 122.63, 122.21, 120.24, 60.97, 60.44, 58.70, 52.47, 52.03, 51.57, 48.85, 48.18, 43.10, 18.36, 18.02. HR-MS (APCI, neg. mode) *m*/*z* calc. for $C_{37}H_{43}N_8O_5$: 679.3362, found: 679.3342 $[M-H]^-$.

Preparation of methyl *N*-(2-(bis(pyridin-2-ylmethyl)amino)ethyl)-*N*-methylglycyl-*D*-alanyl-*D*-alaninate (14).



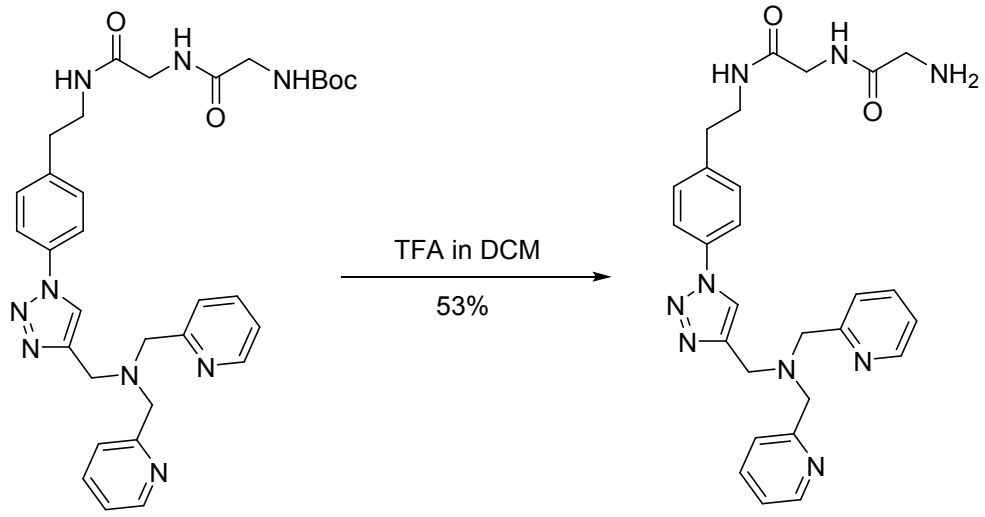
To a flask containing HATU (250.9 mg, 0.6599 mmol) and 2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(methyl)amino) acetic acid (**4**, 205.9 mg, 0.6557 mmol),[7] was added *D*-alanyl-*D*-alanine methyl ester (352 mg as a 32.5% mixture with TFA, 0.657 mmol) dissolved in 5 mL DMF. The mixture was cooled down in an ice bath under nitrogen, and *N*-methylmorpholine (0.35 mL, 3.2 mmol) was added. After stirring in an ice bath for 80 min, the mixture was kept in room temperature overnight, before volatiles were removed under reduced pressure. The product was purified with great difficulty after multiple passages through a plug of Bondesil C-18 OH SPE material, eluted portion-wise with methanol/water mixtures ranging from 20% to 50%. Removal of solvent under reduced pressure of fractions containing pure product afforded product as a pale yellow film (43.8 mg, 14.2%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.53 (br d, J = 4.7 Hz, 2H), 7.63 (dt, J = 1.7 Hz, 7.7 Hz, 2H), 7.25 (dd, J = 4.9 Hz, 7.4 Hz, 2H), 7.18 (d, J = 7.7 Hz, 2H), 4.43 (q, J = 7.1 Hz, 1H), 4.39 (q, J = 7.3 Hz, 1H), 3.97 (s, 4H), 3.93 (br m, 2H), 3.70 (s, 3H), 3.45 (m, 2H), 3.18 (t, J = 5.8 Hz, 2H), 2.93 (s, 3H), 1.41 (d, J = 7.3 Hz, 3H), 1.39 (d, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.47, 174.45, 166.3, 159.3, 149.9, 138.8, 125.0, 124.0, 60.4, 56.8, 55.3, 52.8, 51.0, 50.4, 49.5, 41.6, 18.1, 17.3. MS (APCI, positive mode) *m*/*z* 471.3 $[M+H]^+$, HR-MS (APCI, pos. mode) *m*/*z* 471.2714 calculated for $C_{24}H_{35}N_6O_4$, found *m*/*z* 471.2713.

Preparation of 2-amino-*N*-(2-((4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)phenethyl)amino)-2-oxoethyl)acetamide (16).



6

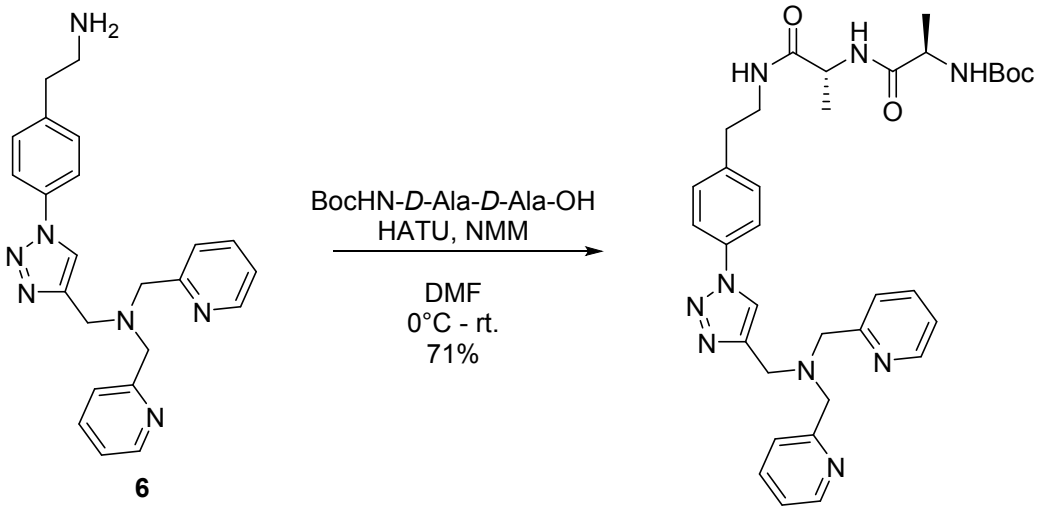
tert-*Butyl (2-((2-((4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)amino)-2-oxoethyl)amino)-2-oxoethyl)carbamate.* To an ice-cooled flask under nitrogen, containing 2-(4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)ethan-1-amine (**6**, 198.1 mg, 0.496 mmol), *N*-boc-glycylglycine (115.4 mg, 0.497 mmol) and HATU (191.4 mg, 0.503 mmol), dissolved in 3 mL DMF, *N*-methylmorpholine (0.06 mL, 0.5 mmol) was added. After stirring for 30 min, the flask was left at room temperature and stirred for 2 days. Product was worked up and purified on Bondesil C18-OH SPE material, as described below for the corresponding *D*-alanyl-*D*-alanine derivative, to afford the title compound as a colourless oil/foam (211.1 mg, 69.3%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.47 (s, 1H), 8.44 (m, 2H), 7.80 (app. dt, J = 1.7 Hz, 7.7 Hz, 2H), 7.76 (m, 2H), 7.71 (d, J = 7.9 Hz, 2H), 7.44 (m, 2H), 7.28 (m, 2H), 3.93 (s, 2H), 3.87 (s, 4H), 3.83 (s, 2H), 3.72 (s, 2H), 3.48 (t, J = 7.2 Hz, 2H), 2.91 (t, J = 7.2 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, methanol-d_4) δ 173.2, 171.7, 160.2, 158.8, 149.5, 146.0, 141.7, 138.7, 136.9, 131.3, 125.0, 123.9, 123.5, 121.6, 80.9, 60.6, 50.0, 45.0, 43.5, 41.8, 36.0, 28.7.



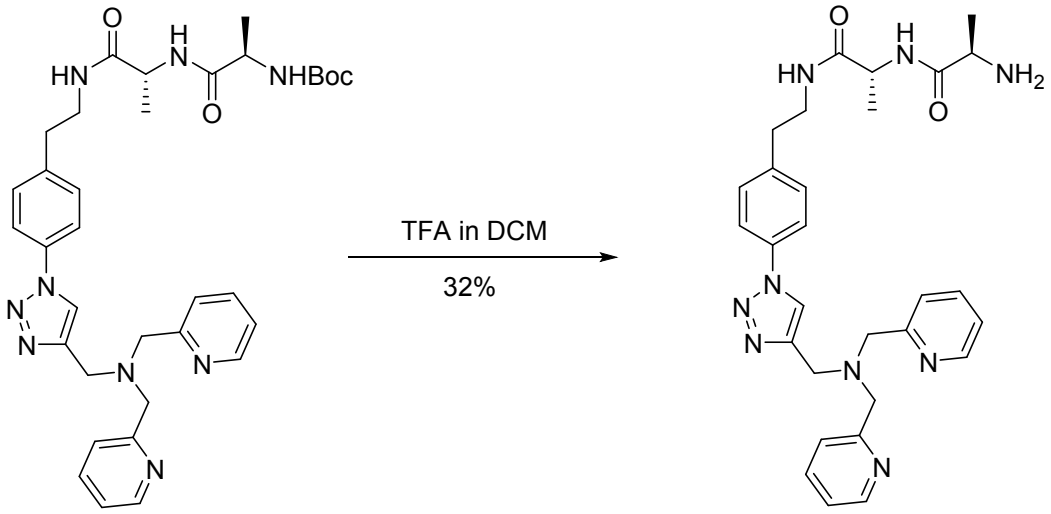
16

*2-Amino-N-(2-((4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)amino)-2-oxoethyl)acetamide (**16**).* The corresponding boc-protected material (203.1 mg, 0.331 mmol), described above, was dissolved in 15 mL dichloromethane, and cooled down in an ice bath under nitrogen. After addition of trifluoroacetic acid (0.6 mL, 8 mmol), the mixture was stirred 30 min in an ice bath, and subsequently 4 h at room temperature. After removal of volatile materials under reduced pressure, the crude product was purified on a plug of strong cationic exchange SPE material, using 1:1 methanol/water and a pH-gradient from pH 3 to pH 10 with formic acid and ammonia as additives. Product eluted at pH 9, and after removal of solvent and drying, the title compound was obtained as a colourless film (90.3 mg, 53.1%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.46 (s, 1H), 8.44 (m, 2H), 7.82-7.74 (m, 4H), 7.70 (d, J = 7.8 Hz, 2H), 7.44 (m, 2H), 7.28 (m, 2H), 3.93 (s, 2H), 3.87 (s, 4H), 3.85 (s, 2H), 3.49 (t, J = 7.2 Hz, 2H), 3.33 (s, 2H), 2.90 (t, J = 7.2 Hz). ^{13}C NMR (100 MHz, methanol-d_4) δ 175.7, 171.7, 160.2, 149.5, 146.0, 141.7, 138.7, 136.9, 131.3, 125.0, 123.9, 123.5, 121.6, 60.6, 49.9, 45.0, 43.3, 41.7, 36.0. MS (APCI, positive mode) m/z 514.3 $[M+H]^+$, HR-MS (APCI, pos. mode) m/z 514.2673 calculated for $C_{27}H_{32}N_9O_2$, found m/z 514.2674.

Preparation of (*R*)-2-amino-*N*-((*R*)-1-((4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)phenethyl)amino)-1-oxopropan-2-yl)propanamide (17)



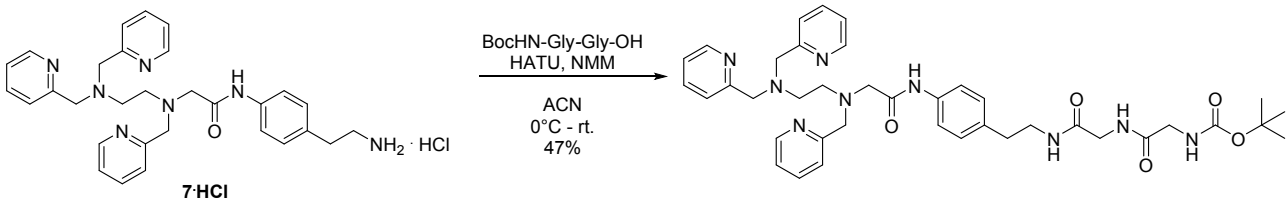
tert-Butyl ((R)-1-(((R)-1-((4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate. To an ice-cooled flask under nitrogen, containing 2-(4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)ethan-1-amine (**6**, 95.4 mg, 0.239 mmol), *N*-boc-*D*-alanyl-*D*-alanioic acid (63.0 mg, 0.242 mmol) and HATU (91.9 mg, 0.242 mmol), dissolved in 2 mL DMF, *N*-methylmorpholine (0.03 mL, 0.3 mmol) was added. After stirring for 30 min, the flask was left at room temperature overnight. Volatiles were removed under reduced pressure and the resulting material loaded onto a plug of Bondesil C-18 OH SPE material. The product was subsequently eluted out using portion-wise additions of methanol/water mixtures, ranging from 30% to 60% methanol. Pure fractions was collected and removal of solvent afforded the title compound as a colourless foam (109.1 mg, 71.1%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.47 (s, 1H), 8.44 (m, 2H), 7.82-7.74 (m, 4H), 7.71 (d, J = 7.9 Hz, 2H), 7.43 (m, 2H), 7.27 (m, 2H), 4.29 (q, J = 7.1 Hz, 1H), 4.01 (q, J = 7.2 Hz, 1H), 3.93 (s, 2H), 3.87 (s, 4H), 3.52 (m, 1H), 3.41 (m, 1H), 2.90 (m, 2H), 1.44 (s, 9H), 1.30 (app. t, J = 7.3 Hz, 6H). ^{13}C NMR (100 MHz, methanol-d_4) δ 175.7, 174.9, 160.2, 158.1, 149.5, 146.0, 141.7, 138.7, 136.9, 131.4, 125.0, 123.9, 123.5, 121.6, 80.8, 60.6, 52.1, 50.4, 50.0, 41.7, 35.9, 28.7, 18.1, 18.0. MS (APCI, positive mode) *m*/*z* 642.3 $[M+H]^+$, HR-MS (APCI, pos. mode) *m*/*z* 642.3511 calculated for $C_{34}H_{44}N_9O_4$, found *m*/*z* 642.3512.



17

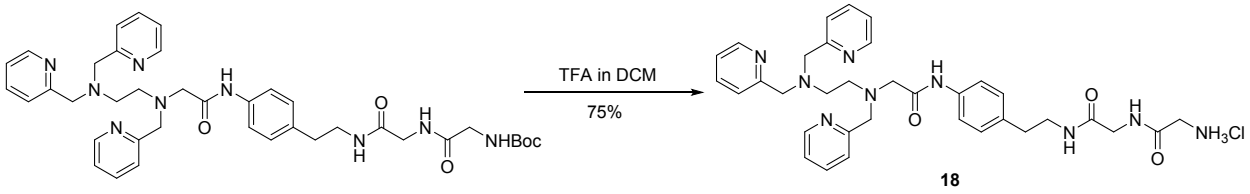
*(R)-2-Amino-N-((R)-1-((4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)amino)-1-oxopropan-2-yl)propanamide (**17**). tert*-Butyl ((*R*)-1-(((*R*)-1-((4-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)phenethyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (108.1 mg, 0.1684 mmol) was dissolved in 5 mL dichloromethane, and cooled down in an ice bath under nitrogen. Trifluoroacetic acid (0.3 mL, 4 mmol) was added and the mixture was stirred 30 min on ice bath, and 150 min in room temperature afterwards. Attempts to isolate product as hydrochloride salt by precipitation from dichloromethane/diethyl ether mixture failed to give the desired purity, and the recovered product was instead purified on a plug of strong cationic exchange SPE material, using 1:1 methanol/water and a pH-gradient from pH 3 to pH 10 with formic acid and ammonia as additives. The product eluted at pH 9-10 and was obtained after removal of solvent under reduced pressure (28.7 mg, 31.5%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.46 (s, 1H), 8.44 (m, 2H), 7.83-7.74 (m, 4H), 7.70 (d, J = 7.8 Hz, 2H), 7.45 (m, 2H), 7.28 (m, 2H), 4.30 (q, J = 7.2 Hz, 1H), 3.94 (s, 2H), 3.87 (s, 4H), 3.69 (q, J = 6.9 Hz, 1H), 3.54-3.42 (m, 2H), 2.90 (m, 2H), 1.38 (d, J = 7.0 Hz, 3H) 1.31 (d, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.8, 174.1, 160.2, 149.5, 145.9, 141.7, 138.8, 136.9, 131.4, 125.0, 123.9, 123.5, 121.6, 60.6, 50.7, 50.4, 49.9, 41.6, 35.9, 19.4, 18.4. MS (APCI, positive mode) m/z 542.3 $[M+H]^+$, HR-MS (APCI, pos. mode) m/z 542.2986 calculated for $C_{29}H_{36}N_9O_2$, found m/z 542.2982.

Preparation of 2-amino-*N*-(2-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenethylamino)-2-oxoethyl)acetamide hydrochloride (18).



7·HCl

tert-Butyl 2-(2-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenethylamino)-2-oxoethylamino)-2-oxoethylcarbamate. *N*-Boc-glycylglycine (108.3 mg, 0.466 mmol, 1.05 eq.) was slurried in 5 mL CH_3CN, cooled to 0 °C in an ice bath. HATU (177 mg, 0.466 mmol, 1.05 eq.), the *N*-(4-(2-aminoethyl)phenyl)-2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamide hydrochloride (**7·HCl**, 242 mg, 0.444 mmol, 1.0 eq.) and NMM (154 µL, 3.0 eq.) were added. The resulting yellow solution was stirred at 0 °C for 30 min and at room temperature for 16 h. The mixture was concentrated under reduced pressure and the residue was dissolved in a minimum amount of 5% MeOH in CH_2Cl_2 and passed through a plug of neutral Al_2O_3 eluting with 5% MeOH in CH_2Cl_2. The solution was concentrated under reduced pressure and the product isolated via column chromatography on neutral Al_2O_3 using 2-5% MeOH in CH_2Cl_2 as eluent to afford 151.6 mg (47%) of the product as a yellow foamy solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.47 (s, 1H), 8.51 (d, J = 4.4 Hz, 1H), 8.47 (d, J = 4.1 Hz, 2H), 7.62 – 7.50 (m, 5H), 7.40 (d, J = 7.8 Hz, 2H), 7.19 – 7.08 (m, 6H), 6.95 (t, J = 5.5 Hz, 1H), 6.45 (s, 1H), 5.52 (s, 1H), 3.86 (d, J = 5.7 Hz, 2H), 3.75 (s, 8H), 3.47 (dd, J = 13.0, 6.8 Hz, 2H), 3.31 (s, 2H), 2.84 – 2.75 (m, 4H), 2.75 – 2.67 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 168.8, 159.1, 158.2, 149.7, 149.2, 137.0, 136.7, 136.6, 134.4, 129.3, 123.3, 123.2, 122.7, 122.3 120.5, 80.6, 61.0, 60.5, 58.8, 52.2, 51.7, 43.3, 40.8, 35.1, 28.5. HR-MS (APCI, pos. mode) *m*/*z* calc. for $C_{39}H_{50}N_9O_5$: 724.3929, found 724.3925 $[M+H]^+$.

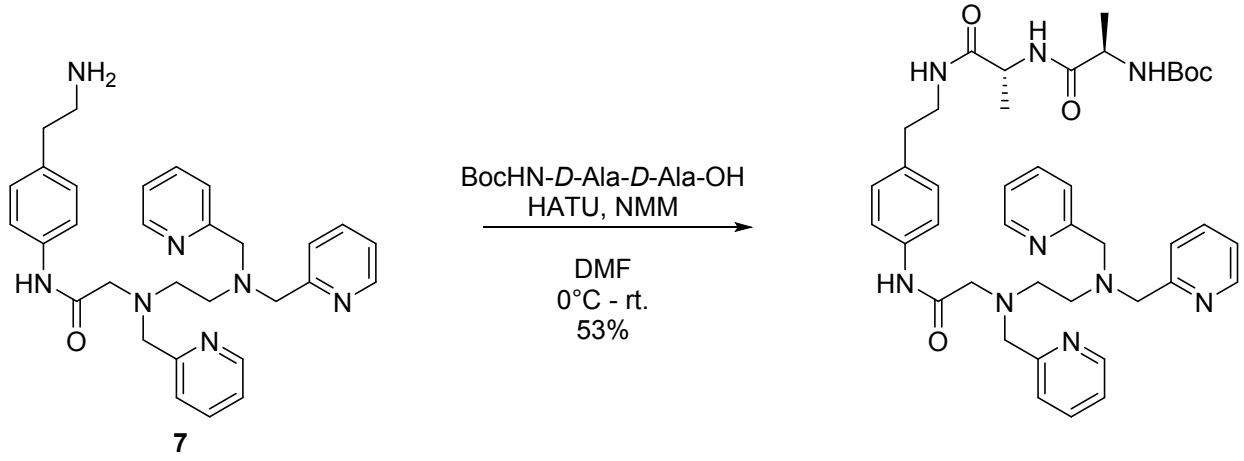


18

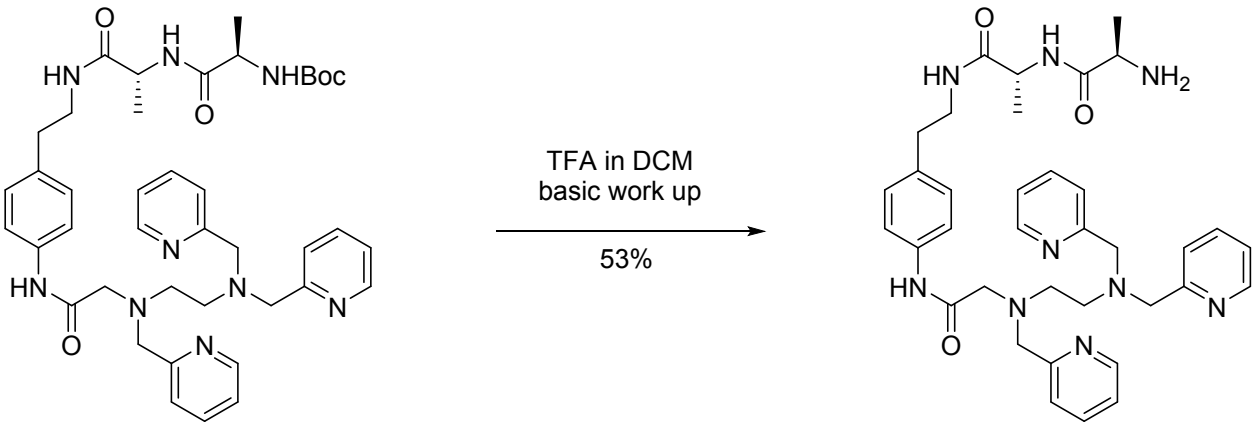
*2-Amino-N-(2-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenethylamino)-2-oxoethyl)acetamide hydrochloride (**18**).* The N-boc protected amine described above (82.9 mg, 0.113 mmol, 1 eq.) was dissolved in 5 mL CH_2Cl_2 cooled to 0 °C in an ice bath. A solution of TFA (433 µL, 5.66 mmol, 50 eq.) in 5 mL CH_2Cl_2 was added dropwise at 0 °C and the mixture allowed to warm to room temperature and stirred for 6 h. The volatiles were

removed under reduced pressure, the residue dissolved in 10 mL CH_2Cl_2 and washed with sat. aq. K_2CO_3 solution (3 x 10 mL). The organic phase was dried over anhydrous K_2CO_3, filtered and concentrated under reduced pressure. The hydrochloric salt was prepared from CH_2Cl_2 (15 mL) solution by dropwise addition of 1 mL of HCl in Et_2O (2.0M) to afford a yellow precipitate and the mixture was left in the fridge for 16 h. The precipitate was filtered off with suction, washed with Et_2O and the sticky solid was dissolved in ethanol and concentrated under reduced pressure to afford 55.9 mg (75%) of product as a yellow solid. ^{1}H NMR (400 MHz, D_2O) δ 8.71 (d, *J* = 5.7 Hz, 3H), 8.47 (m, 3H), 8.07 (d, *J* = 8.0 Hz, 2H), 7.93 (m, 4H), 7.33 (s, 4H), 4.37 (s, 2H), 4.34 (s, 4H), 3.95 (s, 2H), 3.93 (s, 2H), 3.71 (s, 2H), 3.68 (q, ethanol), 3.52 (t, *J* = 7.0 Hz, 2H), 3.21 – 3.12 (m, 2H), 3.09 – 2.99 (m, 2H), 2.88 (t, *J* = 7.0 Hz, 2H), 1.21 (t, ethanol). ^{13}C NMR (101 MHz, D_2O) δ 171.5, 170.4, 168.3, 152.9, 152.7, 147.9, 146.4, 143.3, 142.1, 137.5, 135.1, 130.3, 127.7, 127.3, 127.1, 126.8, 122.2, 58.1 (ethanol), 57.4, 56.2, 52.8, 52.0, 43.0, 41.3, 41.1, 34.8, 17.5 (ethanol). ESI-HRMS *m/z* calc. for $C_{34}H_{42}N_9O_3^+$: 624.3405, found 624.3404.

Preparation of (*R*)-2-amino-N-((*R*)-1-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenethylamino)-1-oxopropan-2-yl)propanamide hydrochloride (19).



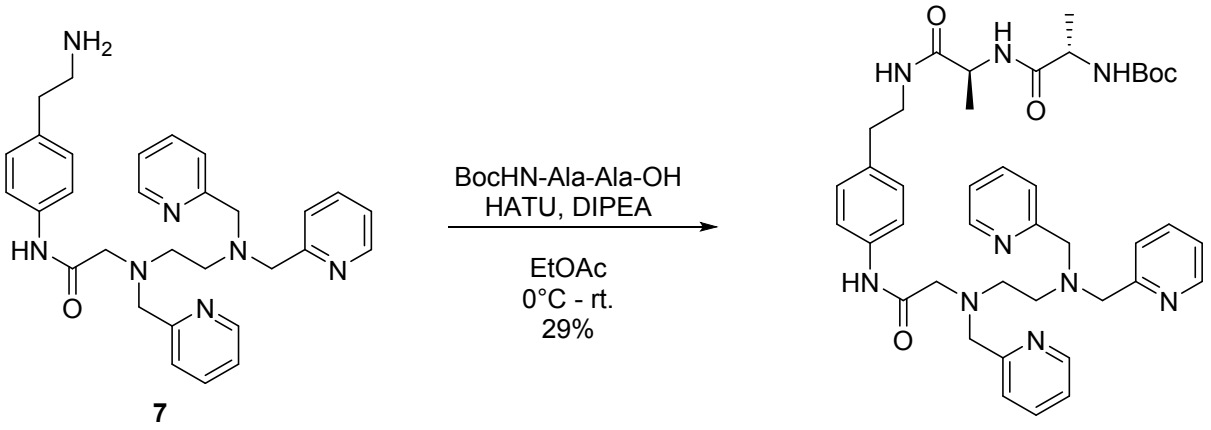
tert-Butyl (R)-1-((R)-1-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenethylamino)-1-oxopropan-2-ylamino)-1-oxopropan-2-ylcarbamate. The amine (**7**, 70 mg, 0.137 mmol) was dissolved in 1 mL DMF and cooled to 0 °C in an ice bath. Boc-D-Ala-D-Ala-OH (37 mg, 0.142 mmol), HATU (88 mg, 0.231 mmol) and NMM (20 µL, 0.181mmol) was then added and the mixture was stirred for 30 minutter in the ice bath and then at room temperature overnight. The mixture was then diluted in 200 mL 1M K_2CO_3 and extracted with 1 x 30 mL and then 4 x 20 mL EtOAc. The combined organic phases were pooled, dried over K_2CO_3, filtered and concentrated under reduced pressure. The crude material was then further purified using column chromatography on a netural Al_2O_3 colunm using 0 - 5 % MeOH in DCM as eluent. This afforded 55 mg (53%) of the title product. ^{1}H NMR (600 MHz, chloroform-*d*) δ 10.43 (s, 1H), 8.56 – 8.42 (m, 3H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.59 – 7.52 (m, 3H), 7.40 (d, *J* = 7.7 Hz, 2H), 7.18 – 7.07 (m, 6H), 6.76 (d, *J* = 7.6 Hz, 1H), 6.53 (s, 1H), 4.39 (p, *J* = 7.1 Hz, 1H), 3.75 (s, 6H), 3.53 – 3.38 (m, 3H), 3.30 (s, 2H), 2.84 – 2.67 (m, 6H), 1.42 (s, 9H) 1.32 (t, *J* = 7.5 Hz, 6H). ^{13}C NMR (151 MHz, chloroform-*d*) δ 172.6, 172.0, 169.9, 159.0, 158.1, 155.9, 149.6, 149.2, 137.0, 136.7, 136.6, 134.3, 129.2, 123.3, 123.2, 122.7, 122.2, 120.1, 80.5, 61.0, 60.4, 58.7, 52.1, 51.6, 50.7, 49.1, 41.0, 35.2, 28.4, 18.3, 18.2. HR-MS: (TOF MS ESI, pos.mode): Calculated mass for $C_{41}H_{54}N_9O_5$ $[M+H]^+$: 752.4242. Found: 752.4263.



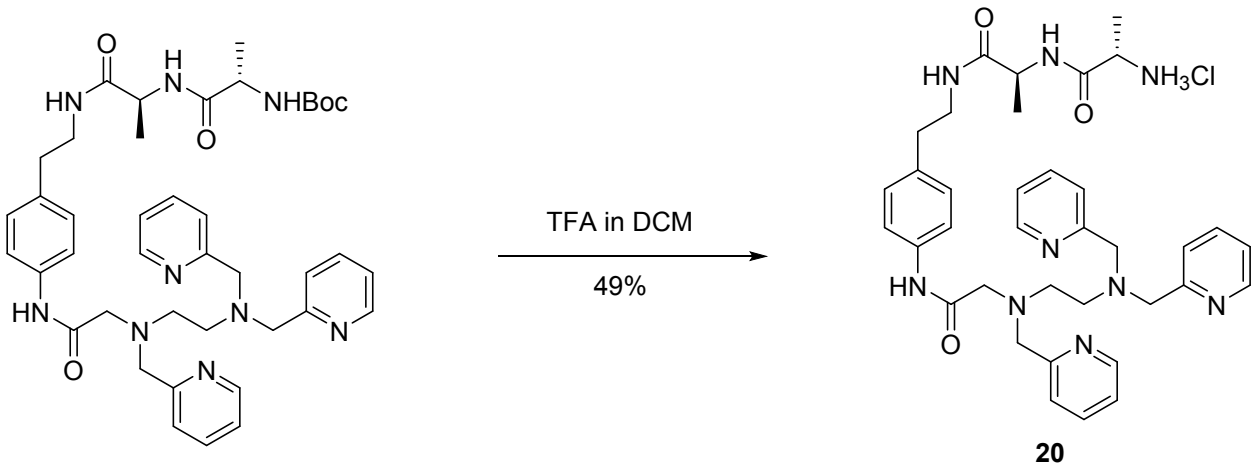
19

*(R)-2-Amino-N-((R)-1-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenethylamino)-1-oxopropan-2-yl)propanamide hydrochloride (**19**).* The N-boc protected amine (64.8 mg, 0.086 mmol, 1 eq.), prepared as described above, was dissolved in 5 mL CH_2Cl_2 and cooled to 0 °C in an ice bath. A solution of TFA (851 µL, 150 eq.) in 1 mL CH_2Cl_2 was added dropwise over 10 min with a dropping funnel at 0 °C. The mixture was warmed to room temperature and stirred for 3 h, then concentrated under reduced pressure. The crude residue was dissolved in 10 mL CH_2Cl_2, washed with sat. aq. K_2CO_3 solution (10 mL), the organic phase dried over anhydrous K_2CO_3, filtered and concentrated under reduced pressure. The hydrochloric salt was prepared from CH_2Cl_2 (5 mL) solution by dropwise addition of HCl in Et_2O (2.0M) to afford a white precipitate and the mixture was left in the fridge for 16 h. The precipitate was filtered off with suction and the sticky solid was dissolved in ethanol and concentrated under reduced pressure to afford 31.6 mg (53%) of product as a beige solid. ^{1}H NMR (400 MHz, D_2O) δ 8.70 (d, J = 5.7 Hz, 3H), 8.46 (q, J = 7.5 Hz, 3H), 8.06 (d, J = 8.0 Hz, 2H), 7.93 (m, 4H), 7.31 (s, 4H), 3.49 – 3.37 (m, 1H), 4.37 (s, 2H), 4.34 (s, 4H), 4.25 (q, J = 7.0 Hz, 1H), 4.10 (q, J = 7.1 Hz, 1H), 3.70 (s, 2H), 3.64 – 3.53 (m, 1H), 3.49 – 3.38 (m, 1H), 3.21 – 3.11 (m, 2H), 3.08 – 2.99 (m, 2H), 2.95 – 2.77 (m, 2H), 1.53 (d, J = 7.1 Hz, 3H), 1.30 (d, J = 7.2 Hz, 3H), 1.20 (ethanol). ^{13}C NMR (101 MHz, D_2O) δ 175.1, 171.1, 170.4, 152.9, 152.7, 147.9, 146.4, 143.3, 142.1, 137.4, 135.1, 130.3, 127.7, 127.4, 127.1, 126.8, 122.2, 63.2 (impurity), 58.14 (ethanol), 58.09, 57.4, 56.2, 52.8, 52.0, 50.6, 49.5, 41.0, 34.7, 17.5 (ethanol), 17.4, 17.1. HR-MS: (TOF MS, ESI pos. mode): Calculated mass for $C_{36}H_{45}N_9O_3$ $[M+H]^+$: 652.3723. Found: 652.3727

Preparation of (*S*)-2-amino-*N*-((*S*)-1-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenethylamino)-1-oxopropan-2-yl)propanamide hydrochloride (20)



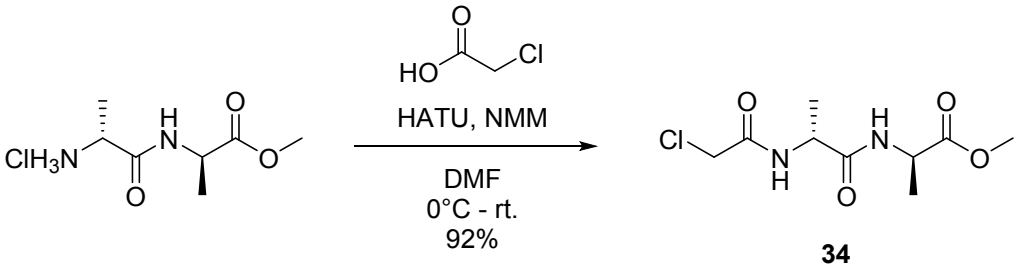
tert-Butyl (S)-1-((S)-1-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenethylamino)-1-oxopropan-2-ylamino)-1-oxopropan-2-ylcarbamate.
Boc-Ala-Ala-OH (276 mg, 0.52 mmol, 1 eq.) and HATU (198 mg, 0.52 mmol, 1 eq.) were suspended in 5 mL EtOAc and cooled to 0 °C in an ice bath. To this mixture was added DIPEA (272 µL, 1.56 mmol, 3 eq.) and after 15 min the *N*-(4-(2-aminoethyl)phenyl)-2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamide hydrochloride (276 mg, 0.52 mmol, 1 eq.). The mixture was stirred at 0 °C for 15 min and at room temperature for 3 h. The mixture was then diluted with 20 mL EtOAc and washed with sat. aq. K_2CO_3 solution (3 x 20 mL). The combined aq. phases were extracted with EtOAc (20 mL) and the combined organic phases dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The product was isolated via column chromatography on neutral Al_2O_3 using 1% MeOH in CH_2Cl_2 as eluent to afford 113.5 mg (29%) of the product as a yellow oil that was taken directly to the deprotection step.



20

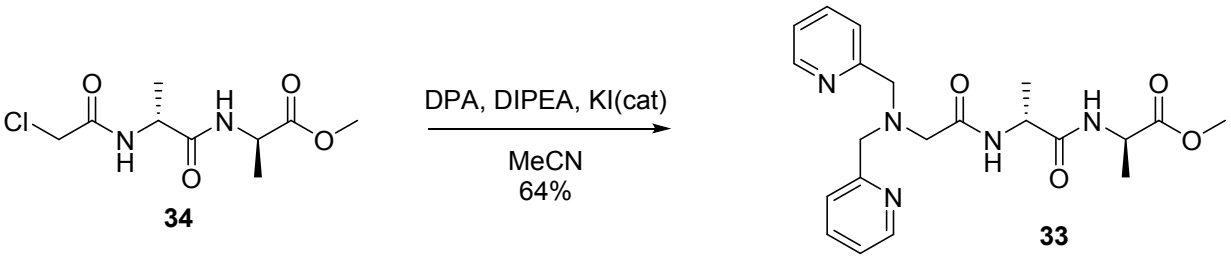
*(S)-2-amino-N-((S)-1-(4-(2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)acetamido)phenethylamino)-1-oxopropan-2-yl)propanamide hydrochloride (**20**).* The N-boc protected amine prepared previously (113.5 mg, 0.151 mmol, 1 eq.) was dissolved in 10 mL CH_2Cl_2 and cooled to 0 °C in an ice bath. A solution of TFA (1.73 mL, 150 eq.) in 5 mL CH_2Cl_2 was added dropwise over 10 min with a dropping funnel at 0 °C. The mixture was then allowed to slowly warm to room temperature and was stirred for 16 h and concentrated under reduced pressure. The crude residue was dissolved in 10 mL CH_2Cl_2, washed with sat. aq. K_2CO_3 solution (10 mL) and the organic phase dried over anhydrous K_2CO_3, filtered and concentrated under reduced pressure. The hydrochloric salt was prepared from CH_2Cl_2 (5 mL) solution by dropwise addition of HCl in Et_2O (2.0M) to afford a white precipitate and the mixture was left in the fridge for 16 h. The precipitate was filtered off with suction and the sticky solid was dissolved in ethanol and concentrated under reduced pressure to afford 50.4 mg (49%) of product as a beige solid. ^{1}H NMR (400 MHz, D_2O) δ 8.67 (d, J = 4.7 Hz, 3H), 8.45 – 8.40 (m, 3H), 8.02 (d, J = 8.0 Hz, 2H), 7.91 – 7.85 (m, 4H), 7.30 (s, 4H), 4.34 (s, 2H), 4.31 (s, 4H), 4.23 (q, J = 7.0 Hz, 1H), 4.08 (q, J = 7.0 Hz, 1H), 3.67 (s, 2H, overlapping with ethanol), 3.56 (dt, J = 14.0, 7.1 Hz, 1H), 3.42 (dt, J = 13.6, 6.8 Hz, 1H), 3.15 (m, 2H), 3.03 (m, 2H), 2.85 (tq, J = 14.2, 7.1 Hz, 2H), 1.52 (d, J = 7.1 Hz, 3H), 1.28 (d, J = 7.2 Hz, 3H), 1.19 (t, ethanol). ^{13}C NMR (101 MHz, D_2O) δ 175.1, 171.1, 170.3, 152.9, 152.8, 147.1, 146.1, 143.5, 142.8, 137.4, 135.1, 130.3, 127.5, 127.2, 126.9, 126.7, 122.2, 58.1 (ethanol), 58.0, 57.4, 56.5, 52.7, 52.0, 50.6, 49.5, 41.1, 34.7, 17.5 (ethanol), 17.4, 17.2. HR-MS (ESI, pos. mode) *m/z* calc. for $C_{36}H_{46}N_9O_3^+$: 652.3718, found 652.3700

Preparation of methyl bis(pyridin-2-ylmethyl)glycyl-*D*-alanyl-*D*-alaninate (33)



34

*Methyl (2-chloroacetyl)-D-alanyl-D-alaninate (**34**)*. Chloromethyl acetic acid (112 mg, 1.19 mmol) was dissolved in DMF (3 mL) and cooled to 0°C in an ice-water bath. *D*-alanyl-*D*-alanine methyl ester hydrochloride (250 mg, 1.19 mmol) and HATU (452 mg, 1.19 mmol) were then added before NMM (288 µL, 2.62 mmol) was added to the stirring mixture. The mixture was left in the ice-water bath for 30 minutes before slowly heating to room temperature. The mixture was then left for 4 hours at room temperature. The mixture was then diluted with 300 mL water and extracted with 5x30 mL EtOAc. The combined organic phases were pooled and washed with 0.5 M $NaHCO_3$ (50 mL), 0.1 M HCl (50 mL) and brine (50 mL) before it was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude material was then purified by flash column chromatography using SiO_2 as stationary phase and 50-100% EtOAc in heptane as eluent. This afforded 274 mg of the title compound as a white powder (92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, *J* = 6.9 Hz, 1H), 8.34 (d, *J* = 7.5 Hz, 1H), 4.43 – 4.18 (m, 2H), 4.09 (s, 2H), 3.62 (s, 3H), 3.30 (s, 0H), 2.50 (s, 8H), 1.28 (d, *J* = 7.3 Hz, 3H), 1.22 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 172.8, 171.7, 165.3, 51.9, 48.0, 47.5, 42.5, 18.3, 16.8. HR-MS (ESI, pos. mode) *m*/*z* 273.0613 calculated for $C_9H_{15}N_2O_4Na$, found *m*/*z* 273.0616.

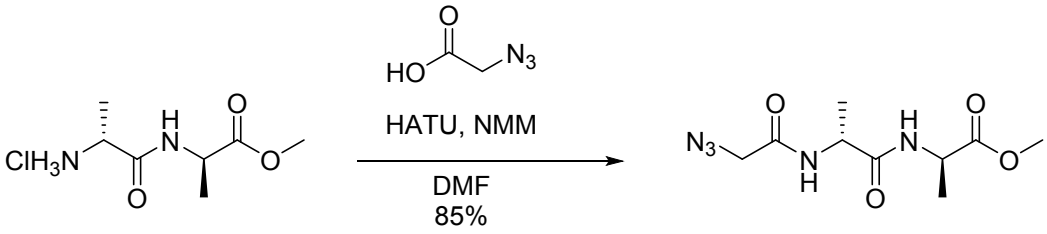


34 **33**

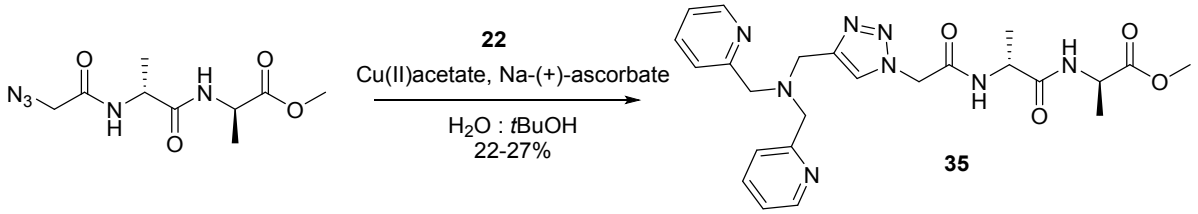
*Methyl bis(pyridin-2-ylmethyl)glycyl-D-alanyl-D-alaninate (**33**).* The α-chloro amide (**34**, 200 mg, 0.80 mmol, 1.0 eq.) and KI (80 mg, 0.48 mmol, 0.6 eq.) were dissolved in 200 mL MeCN and DPA (173 µL, 0.96 mmol, 1.2 eq.) was added to the stirring mixture. DIPEA (1.35 mL, 7.74 mmol, 9.7 eq.) was then added and the mixture was heated to reflux and left for 16 hours. After cooling to room temperature, the mixture was concentrated under reduced pressure and purified using column chromatography. The product was eluted using 0-5 % MeOH in DCM from a neutral alumina column, giving 212 mg (64%). ^{1}H NMR (300 MHz, chloroform-*d*) δ 9.35 (d, *J* = 7.8 Hz, 1H), 8.54 (m, 2H), 7.61 (td, *J* = 7.7, 1.8 Hz, 2H), 7.28-7.22 (m, 2H), 7.20-7.14 (m, 3H), 4.57-4.46 (m, 2H), 3.88 (d, *J* = 3.4 Hz, 4H), 3.67 (s, 3H), 3.38 (s, 2H), 1.46 (d, *J* = 7.1 Hz, 3H), 1.28 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, chloroform-*d*) δ

173.2, 172.3, 171.8, 158.4, 149.4, 136.7, 123.3, 122.6, 77.6, 77.2, 76.7, 60.4, 58.3, 52.5, 49.0, 48.1, 18.3, 17.5. HR-MS (APCI, pos. mode) *m/z* 414.2136 calculated for $C_{21}H_{28}N_5O_4$, found *m/z* 414.2134.

Preparation of methyl (2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-*D*-alanyl-*D*-alaninate (35).



Methyl (2-azidoacetyl)-D-alanyl-D-alaninate. 2-Azidoacetic acid (178 µL, 2,38 mmol) was dissolved in DMF (5 mL) and cooled to 0°C in an ice-water bath. *D*-alanyl-*D*-alanine methyl ester hydrochloride (500 mg, 2,38 mmol) and HATU (904 mg, 2,38 mmol) were then added before NMM (576 µL, 5,24 mmol) was added to the stirring mixture. The mixture was left in the ice-water bath for 30 minutes before slowly heating to room temperature. The mixture was then left for 4 hours at room temperature. The mixture was then diluted with 350 mL water and extracted with 5x50 mL EtOAc. The combined organic phases were pooled and washed with 0.5 M $NaHCO_3$ (50 mL), 0.1 M HCl (50 mL) and brine (50 mL) before it was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude material was then purified by flash column chromatography using SiO_2 as stationary phase and 50-100% EtOAc in heptane as eluent. This afforded 520 mg of the title compound as a white powder (85%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.46 (d, J = 7.1 Hz, 1H), 8.33 (d, J = 7.3 Hz, 1H), 4.35 (p, J = 7.1 Hz, 1H), 4.26 (p, J = 7.3 Hz, 1H), 3.82 (s, 2H), 3.61 (s, 3H), 1.28 (d, J = 7.3 Hz, 3H), 1.21 (d, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.0, 171.9, 167.1, 52.0, 50.4, 47.8, 47.6, 18.4, 16.9.



*Methyl (2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-D-alanyl-D-alaninate (**35**).* Copper acetate (200 mg, 1.0 mmol, 1.0 eq.) in 2.5 mL H_2O and sodium-(+)ascorbate (396 mg, 2.0 mmol, 2.0 eq.) in 2.5 mL H_2O were added simultaneously to a stirring solution of the alkyne **22** (237 mg, 1.0 mmol, 1.0 eq.) in 2.5 mL *t*BuOH. Methyl (2-azidoacetyl)-*D*-alanyl-*D*-alaninate (257 mg, 1.0 mmol, 1.0 eq), prepared as described above, was then added and the solution was stirred at room temperature for 16 hours.

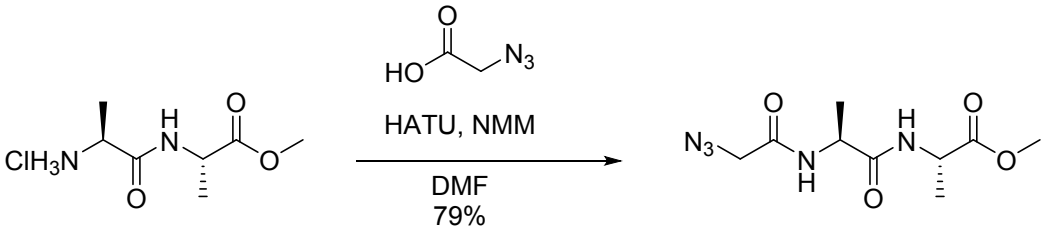
Work-up, alternative I: EDTA (293 mg, 1.0 mmol, 1.0 eq) was added to the stirring solution and left for 60 minutes before the mixture was diluted with 50 mL H_2O and the pH of the mixture was adjusted to

>10 with 1M NaOH. The slurry was then extracted with 2 x 50 mL dichloromethane. The combined organic phases were dried over K_2CO_3 and concentrated under reduced pressure to give a dark red oil. The crude products were purified using column chromatography by eluting a neutral Al_2O_3 column with 0-5% methanol in dichloromethane to give 134 mg of the title compound as a pale orange oil (27%).

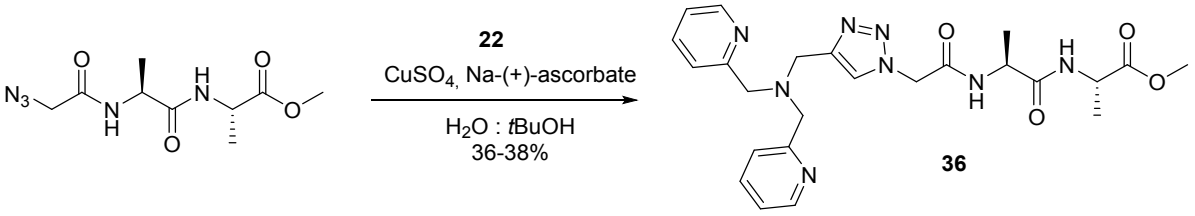
Work-up, alternative II: Chelex 100 (5.0 g) was added and the mixture stirred for 60 min. Filtration, followed by removal of solvent under reduced pressure, gave a crude product which was loaded onto a plug of Bondesil-C18 OH SPE material and purified by running through portions of methanol/water mixture, eluted stepwise from 25% to 50% methanol. To the first two portions, both at 25% methanol content, EDTA was added to the eluent. Fractions containing pure product was collected and the solvent removed under reduced pressure to provide 136 mg of a brown, amorphous solid (22%, starting from 1.24 mmol). Spectral data are reported for material purified in this manner.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, J = 7.6 Hz, 1H), 8.49 (d, J = 4.8 Hz, 2H), 8.44 (d, J = 7.1 Hz, 1H), 8.04 (s, 1H), 7.77 (t, J = 7.6 Hz, 2H), 7.57 (d, J = 7.8 Hz, 2H), 7.27 – 7.23 (m, 2H), 5.13 (s, 2H), 4.36 (p, J = 7.1 Hz, 1H), 4.27 (p, J = 7.2 Hz, 1H), 3.75 (s, 2H), 3.73 (s, 4H), 3.61 (s, 3H), 1.27 (d, J = 7.3 Hz, 3H), 1.24 (d, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.9, 171.8, 165.0, 159.0, 148.8, 143.1, 136.5, 125.3, 122.5, 122.1, 58.7, 51.8, 51.4, 48.0, 47.9, 47.5, 18.3, 16.8. MS (APCI, pos. mode) m/z 495.2 $[M+H]^+$, HR-MS (APCI) m/z 495.2463 calculated for $C_{24}H_{31}N_8O_4$, found m/z 495.2462.

Preparation of methyl (2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-*L*-alanyl-*L*-alaninate (36).



Methyl (2-azidoacetyl)-L-alanyl-L-alaninate. 2-Azidoacetic acid (360 µL, 4.7 mmol) was dissolved in DMF (10 mL) and cooled to 0°C in an ice-water bath. Alanylalanine methyl ester hydrochloride (500 mg, 2.3 mmol) and HATU (1.35 g, 3.6 mmol) were then added before NMM (1.2 mL, 10.9 mmol) was added to the stirring mixture. The mixture was left in the ice-water bath for 30 minutes before slowly heating to room temperature. The mixture was allowed to stir overnight. The mixture was diluted in a mixture of brine (75 mL) and 0.5 M $NaHCO_3$ (aq., 25 mL), and extracted with 4 x 50 mL EtOAc. The combined organic phases were pooled and washed with 0.5 M $NaHCO_3$ (50 mL), 0.1 M HCl (50 mL) and brine (50 mL) before it was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude material was then purified by flash column chromatography using silica as stationary phase and 50-75% EtOAc in heptane as eluent. This afforded 466 mg of the title compound (79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, J = 7.1 Hz, 1H), 8.28 (d, J = 7.7 Hz, 1H), 4.35 (p, J = 7.1 Hz, 1H), 4.27 (p, J = 7.2 Hz, 1H), 3.83 (s, 2H), 3.62 (s, 3H), 1.29 (d, J = 7.3 Hz, 3H), 1.22 (d, J = 7.1 Hz, 3H), ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.9, 171.8, 166.9, 51.9, 50.5, 47.8, 47.5, 18.3, 16.8. MS (ESI, pos. mode) m/z 280.1 $[M+Na]^+$, HR-MS (ESI) m/z 280.1016 calculated for $C_9H_{15}N_5O_4Na$, found m/z 280.1017.



*Methyl (2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-L-alanyl-L-alaninate (**36**).* Copper sulfate (170 mg, 1.05 mmol) in 2.5 mL H_2O was mixed with sodium-(+)-ascorbate (420 mg, 2.1 mmol) and added to a stirring solution of the alkyne **22** (250 mg, 1.05 mmol) mixed with methyl (2-azidoacetyl)-*L*-alanyl-*L*-alaninate (270 mg, 1.05 mmol) in 2.5 mL *t*BuOH. The solution was stirred at room temperature for 18 hours.

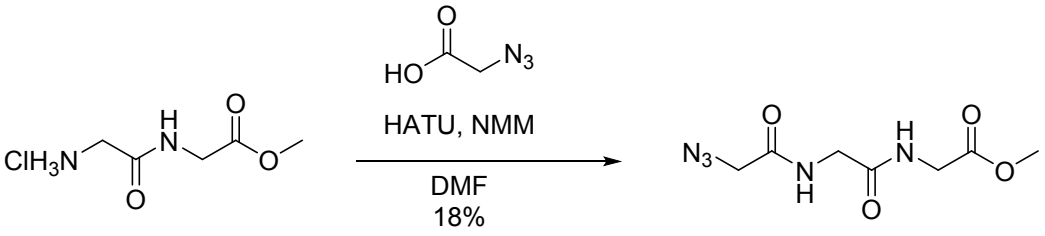
Work-up, alternative I: EDTA was then added to the stirring solution and left for 60 minutes before the mixture was diluted with 10 mL brine, 10 mL 0.5 M $NaHCO_3$ (aq.) and approximately 3 mL 1M NaOH (aq.) to bring pH to 10-11. The product was extracted with 4 x 20 mL 4:1 chloroform/isopropanol and washed with 0.5 M $NaHCO_3$ (15 mL) before drying over Na_2SO_4. Removal of solvent under reduced

pressure gave a crude product which was purified using column chromatography by eluting a neutral Al_2O_3 column with 0-1% methanol in dichloromethane to give 195 mg of the title compound (38%).

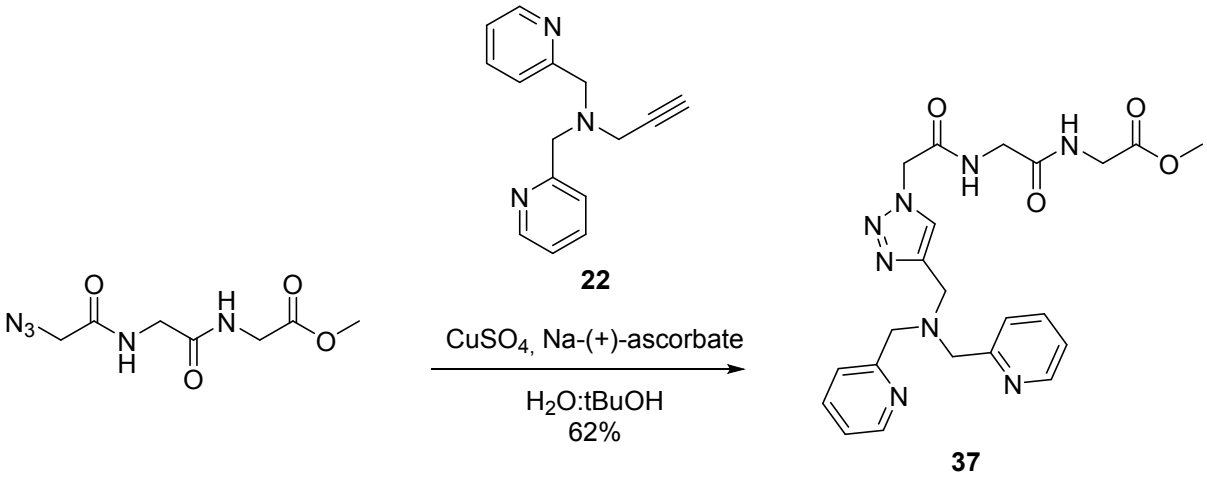
Work-up, alternative II: Chelex 100 (3.0 g) was added and the mixture stirred for 60 min. Filtration and subsequent removal of solvent under reduced pressure gave a crude product which was loaded onto a plug of Bondesil-C18 OH SPE material and purified by running through portions of methanol/water mixture, eluted stepwise from 20% to 60% methanol. To the first two portions, both at 20% methanol content, some EDTA buffered to pH 8 was added to the eluent. Fractions containing pure product was collected and the solvent removed under reduced pressure to provide 186 mg of a brown semisolid (36%). Spectral data are reported for material purified in this manner.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 7.7 Hz, 1H), 8.49 (d, J = 4.8 Hz, 2H), 8.43 (d, J = 7.1 Hz, 1H), 8.04 (s, 1H), 7.77 (t, J = 7.6 Hz, 2H), 7.57 (d, J = 7.8 Hz, 2H), 7.27 – 7.24 (m, 2H), 5.13 (s, 2H), 4.36 (p, J = 7.1 Hz, 1H), 4.27 (p, J = 7.2 Hz, 1H), 3.75 (s, 2H), 3.73 (s, 4H), 3.61 (s, 3H), 1.27 (d, J = 7.3 Hz, 3H), 1.24 (d, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.9, 171.8, 165.0, 159.0, 148.8, 143.1, 136.6, 125.4, 122.5, 122.1, 58.7, 51.9, 51.4, 48.0, 47.9, 47.5, 18.4, 16.8. MS (APCI, pos. mode) m/z 495.2 $[M+H]^+$, HR-MS (APCI) m/z 495.2463 calculated for $C_{24}H_{31}N_8O_4$, found m/z 495.2464.

Preparation of methyl (2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)glycylglycinate (37).



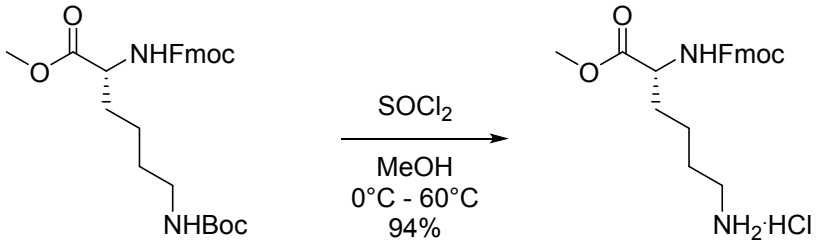
Methyl (2-azidoacetyl)glycylglycinate. H-Gly-Gly-OMe hydrochloric salt (744.2 mg, 4.08 mmol), HATU (1.55 g, 4.08 mmol) and 2-azidoacetic acid (0.33 mL, 4.4 mmol) was stirred in an ice bath with 4 mL dimethylformamide. *N*-Methylmorpholine was added (0.90 mL, 8.2 mmol), and the reaction mixture was kept stirring in an ice bath for 2 h, after which the mixture was concentrated on a rotary evaporator. The residue was partitioned between 50 mL ethyl acetate and 30 mL sat. NaCl (aq.) and 20 mL 0.5 M K_2CO_3 (aq.). The phases were separated and the aqueous phase further extracted with 3 x 50 mL ethyl acetate. Combined organic phases were washed with 50 mL 0.1 M HCl (aq.) followed by 50 mL 0.5 M $NaHCO_3$ (aq.). After drying over $MgSO_4$ (s.), filtration and removal of solvent under reduced pressure, the crude product was dissolved in a mixture of 80 mL ethyl acetate and 50 mL *n*-heptane under gentle heating. Solvent was evaporated under reduced pressure until precipitation was initiated, after which the flask was left to cool. Precipitate was collected on suction, giving a colourless amorphous solid (171.4 mg, 18%). Further material could be recovered by purification of the evaporated filtrate on a silica column, using a gradient of methanol (4-5%) in dichloromethane. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (br m, 2H), 3.88 (s, 2H), 3.86 (d, J = 5.9 Hz, 2H), 3.78 (d, J = 5.8 Hz, 2H), 3.63 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2, 169.1, 167.7, 51.7, 50.7, 41.7, 40.6. MS (ESI, pos. mode) m/z 252.1 $[M+Na]^+$, HR-MS (ESI) m/z 252.0703 calculated for $C_7H_{11}N_5O_4Na$, found m/z 252.0703.



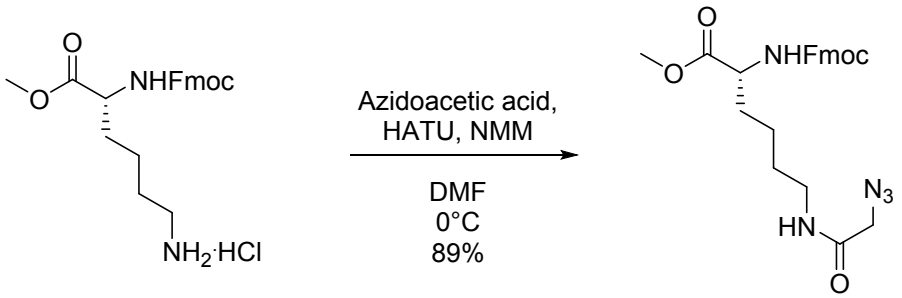
*Methyl (2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)glycylglycinate (**37**)*. A mixture of copper(II)sulfate (41 mg, 0.26 mmol) and sodium-(+)ascorbate (110 mg, 0.557 mmol) in

water (2 mL) was transferred to a flask containing *N*,*N*-bis(pyridin-2-ylmethyl)prop-2-yn-1-amine (**22**, 59 mg, 0.25 mmol) dissolved in *tert*-butanol (2 mL). Under rapid stirring, the methyl (2-azidoacetyl)glycylglycinate (40 mg, 0.17 mmol) from above was added, and the mixture kept under rapid stirring. After 3h reaction time 0.75 g Chelex 100 was added and the mixture stirred for 30 min. Filtration and subsequent evaporation gave a crude product which was loaded onto a plug of Bondesil-C18 OH SPE material and eluted by running through portions of methanol/water mixture, going stepwise from 20% to 50% methanol. To the first two portions, both at 20% methanol content, some EDTA buffered to pH 8 was added to the eluent. Fractions containing product was collected and the solvent removed under reduced pressure to provide a brown oil (49.5 mg, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (br t, 1H), 8.49 (m, 2H), 8.45 (br, 1H), 8.06 (s, 1H), 7.77 (dt, J = 1.9 Hz, 7.6 Hz, 2H), 7.57 (d, J = 7.8 Hz, 2H), 7.25 (m, 2H), 5.17 (s, 2H), 3.86 (d, J = 5.4 Hz, 2H), 3.81 (d, J = 5.2 Hz, 2H), 3.75 (s, 2H), 3.73 (s, 4H), 3.62 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.2, 169.0, 166.0, 159.1, 148.8, 143.2, 136.6, 125.4, 122.6, 122.2, 58.7, 51.8, 51.5, 48.0, 41.9, 40.6. MS (APCI, pos. mode) *m*/*z* 467.2 $[M+H]^+$, HR-MS (APCI) *m*/*z* 467.2150 calculated for $C_{22}H_{27}N_8O_4$, found *m*/*z* 467.2146.

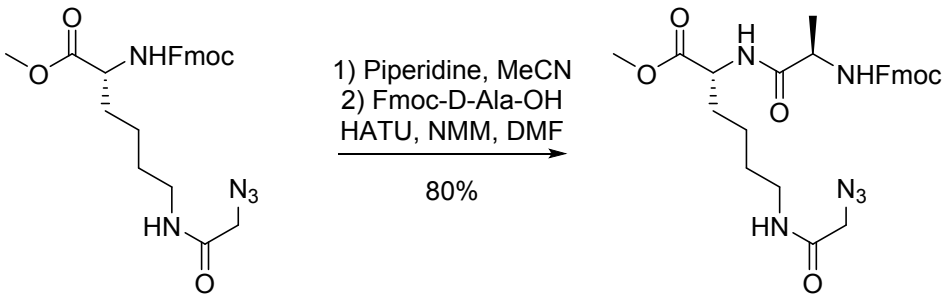
Preparation of methyl N^2-(*D*-alanyl)-N^6-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-*D*-lysinate (38).



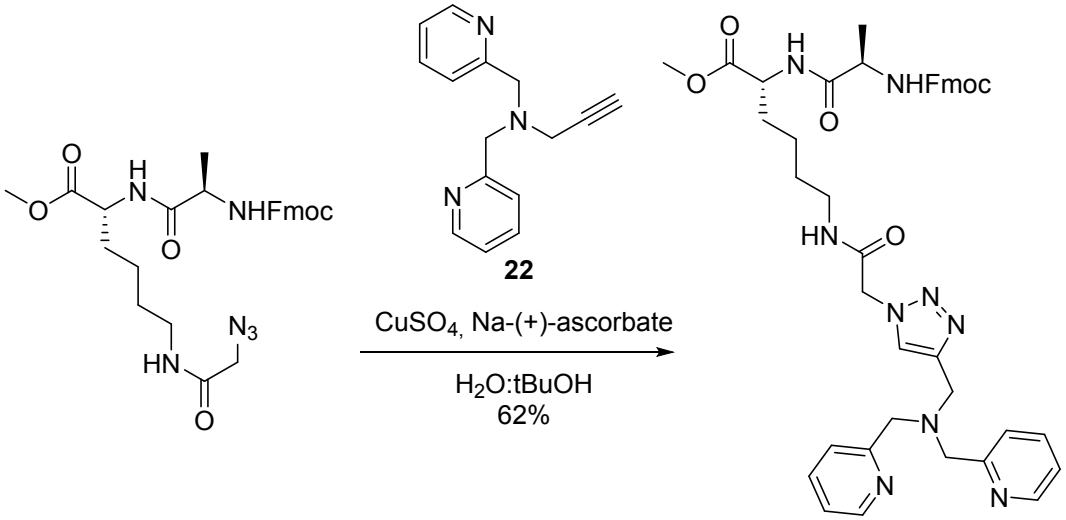
Fmoc-D-Lys(H)-OMe hydrochloride salt. From Fmoc-D-Lys(Boc)-OH (3.47 g, 7.41 mmol) dissolved in 35 mL methanol, thionyl chloride (0.67 mL, 9.2 mmol) was added dropwise and the reaction mixture kept 30 min in an ice bath, followed by heating to 60 °C for 2h. After filtering the reaction mixture, product was precipitated by addition of diethyl ether and isolated as a colourless powder (2.80 g, 94%). ^{1}H NMR data (400 MHz, DMSO-d_6) was in agreement with literature values for corresponding enantiomer.[8]



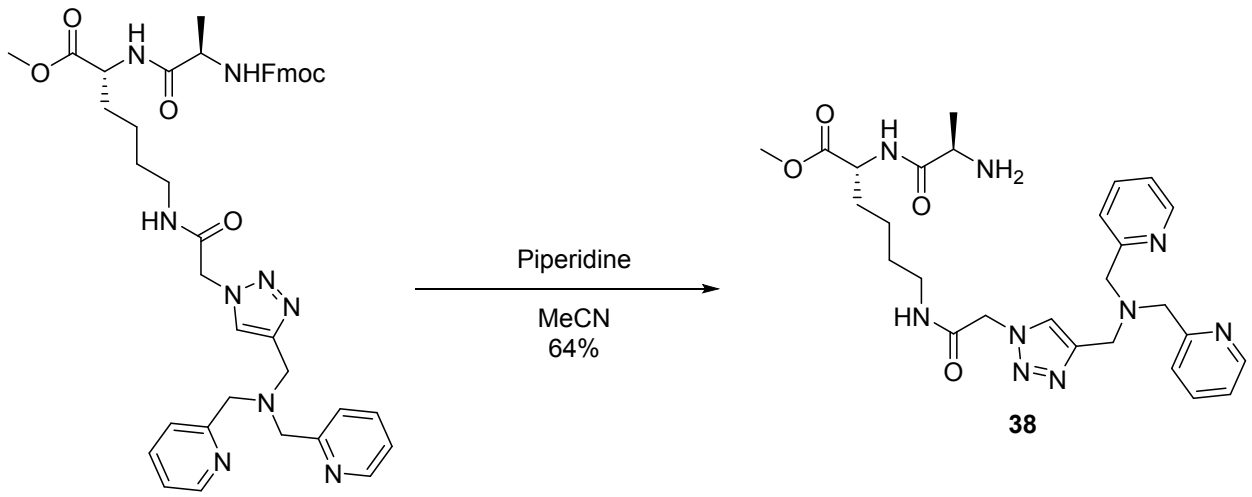
Fmoc-D-Lys(ε-2-azidoacetyl)-OMe. Fmoc-D-Lys(H)-OMe hydrochloride salt (195 mg, 0.484 mmol), 2-azidoacetic acid (0.050 mL, 0.67 mmol) and HATU (185 mg, 0.486 mmol) was stirred in 1.5 mL DMF and cooled in an ice bath. After addition of *N*-methylmorpholine (0.11 mL, 0.997 mmol) the reaction was kept in an ice bath 140 min reaction time, after which the reaction was quenched by addition of 20 mL sat. NaCl (aq.) and 10 mL 0.5 M K_2CO_3. (aq.). The product was extracted with 4 x 20 mL ethyl acetate, washed with 20 mL 0.1 M HCl (aq.) and 20 mL 0.5 M $NaHCO_3$ (aq.). Drying over $MgSO_4$ and subsequent filtration and removal of solvent under reduced pressure afforded a crude product which was further purified on a silica column using a 50%-75% gradient of ethyl acetate in *n*-heptane as eluent. The product was isolated as a clear oil (199.7 mg, 89%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (m, 2H), 7.41 (m, 2H), 7.32 (m, 2H), 6.40 (br, 1H), 5.39 (br, 1H), 4.36-4.45 (m, 3H), 4.23 (t, *J* = 6.9 Hz, 1H), 3.97 (s, 2H), 3.76 (s, 3H), 3.30 (m, 2H), 1.83-1.93 (m, 1H), 1.66-1.75 (m, 1H), 1.52-1.61 (m, 2H) 1.34-1.44 (m, 2H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 173.0, 167.0, 156.2, 143.9, 141.5, 127.9, 127.2, 125.2, 120.2, 67.2, 53.7, 52.8, 52.7, 47.3, 39.2, 32.4, 28.9, 22.5. MS (ESI, pos. mode) *m*/*z* 488.2 $[M+Na]^+$, HR-MS (ESI, pos. mode) *m*/*z* 488.1904 calculated for $C_{24}H_{27}N_5O_5Na$, found *m*/*z* 488.1903.



Methyl N^2*-((((9H-fluoren-9-yl)methoxy)carbonyl)-D-alanyl)-*N^6*-(2-azidoacetyl)-D-lysinate*. To a solution of Fmoc-D-Lys(ε-2-azidoacetyl)-OMe (1.00 mmol) in acetonitrile (50 mL), piperidine (0.5 mL, 5 mmol) was added and stirred overnight. After filtration, volatiles were removed under reduced pressure. The crude product was loaded on a plug of silica, which was eluted portion-wise with pure dichloromethane, 1% methanol in dichloromethane and finally 90:10:1 dichloromethane / methanol / 25% NH_3 (aq.). Fractions containing product was evaporated and the product carried on to react with Fmoc-D-Ala-OH (321 mg, 1.03 mmol) using HATU (411 mg, 1.08 mmol) as coupling reagent in 5 mL DMF. The mixture was cooled on ice bath and *N*-methylmorpholine (0.14 mL, 1.27 mmol) added. After being kept on ice bath for additional 30 min, the reaction was left to stir over-night. The reaction mixture was quenched by addition of 40 mL sat. NaCl (aq.) and 20 mL 0.5 M K_2CO_3. (aq.), then the product was extracted with 4 x 40 mL ethyl acetate, washed with 25 mL 0.1 M HCl (aq.) and 25 mL 0.5 M $NaHCO_3$ (aq.). The organic phase was dried over $MgSO_4$ and after filtration, and removal of solvent under reduced pressure, a crude product was obtained which was further purified on a silica column using 75% ethyl acetate in *n*-heptane as eluent. The product was isolated as a colourless solid (429.1 mg, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, J = 7.5 Hz, 1H), 8.05 (br t, 1H), 7.88 (d, J = 7.5 Hz, 2H), 7.72 (t, J = 6.9 Hz, 2H), 7.46 (d, J = 7.7 Hz, 1H) 7.41 (m, 2H), 7.32 (m, 2H), 4.19-4.26 (m, 4H), 4.10 (m, 1H), 3.77 (s, 2H), 3.61 (s, 3H), 3.06 (m, 2H), 1.69 (m, 1H), 1.61 (m, 1H), 1.39 (m, 2H), 1.30 (m, 2H), 1.22 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 172.5, 167,0, 155.6, 143.8, 140.7, 127.6, 127.1, 125.3, 120.1, 65.6, 51.81, 51.78, 50.8, 49.7, 46.7, 38.2, 30.5, 28.4, 22.6, 18.1. MS (ESI, pos. mode) m/z 559.2 $[M+Na]^+$, HR-MS (ESI, pos. mode) m/z 559.2276 calculated for $C_{27}H_{32}N_6O_6Na$, found m/z 559.2272.

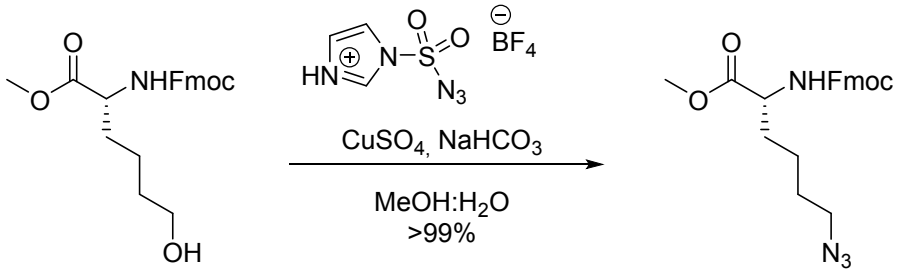


Methyl N^2*-((((9H-fluoren-9-yl)methoxy)carbonyl)-D-alanyl)-*N^6*-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-D-lysinate*. A mixture of copper(II)sulfate (98.9 mg, 0.62 mmol) and sodium-(+)ascorbate (250 mg, 1.26 mmol) in water (7.5 mL) was transferred to a flask containing *N*,*N*-bis(pyridin-2-ylmethyl)prop-2-yn-1-amine (**22**, 151 mg, 0.637 mmol) dissolved in *tert*-butanol (7.5 mL). Under rapid stirring, methyl N^2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)-*D*-alanyl)-N^6-(2-azidoacetyl)-*D*-lysinate (288 mg, 0.538 mmol), prepared above, was added and mixture was kept under rapid stirring for 3 h before dilution with ethyl acetate and transfer to a separatory funnel containing 60 mL water and 40 mL sat. NaCl (aq.). The product was extracted with 4 x 60 mL ethyl acetate, washed with 3 x 70 mL 0.025 M EDTA/0.5 M $NaHCO_3$ (aq.) and sat. NaCl (aq.). After drying over K_2CO_3, filtration and removal of solvent under reduced pressure, a crude product was obtained which was subsequently loaded onto an alumina column, washed with pure ethyl acetate before switching to 1.5% methanol in dichloromethane for elution of product. The product was isolated as a pale oil (260.2 mg, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (m, 2H), 8.28 (br t, 1H), 8.21 (d, J = 7.5 Hz, 1H), 8.03 (s, 1H), 7.87 (d, J = 7.5 Hz, 2H), 7.76 (dt, J = 1.8 Hz, 7.7 Hz, 2H), 7.70 (t, J = 6.8 Hz, 2H), 7.56 (d, J = 7.6 Hz, 2H), 7.50 (d, J = 7.8 Hz, 1H), 7.40 (m, 2H), 7.31 (m, 2H), 7.24 (m, 2H), 5.06 (s, 2H), 4.17-4.25 (m, 4H), 4.09 (m, 1H), 3.74 (s, 2H), 3.72 (s, 4H), 3.60 (s, 3H), 3.07 (m, 2H), 1.69 (m, 1H), 1.61 (m, 1H), 1.40 (m, 2H), 1.32 (m, 2H), 1.22 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 172.6, 165.3, 159.1, 155.7, 148.8, 143.8, 143.1, 140.7, 136.6, 127.7, 127.1, 125.4, 125.3, 122.6, 122.2, 120.1, 65.7, 58.7, 51.88, 51.85, 51.6, 49.7, 48.0, 46.7, 38.6, 30.5, 28.4, 22.7, 18.1. MS (APCI, pos. mode) *m*/*z* 774.4 $[M+H]^+$, HR-MS (APCI, pos. mode) *m*/*z* 774.3722 calculated for $C_{42}H_{48}N_9O_6$, found *m*/*z* 774.3721.

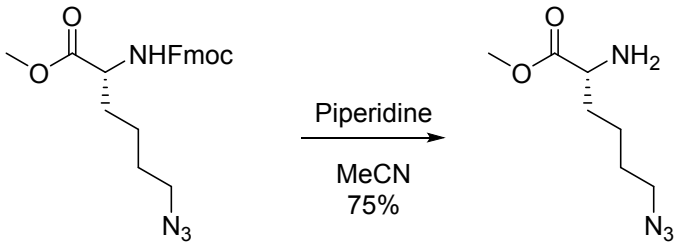


*Methyl N^2-(D-alanyl)-N^6-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-D-lysinate (**38**)*. The available methyl N^2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)-*D*-alanyl)-N^6-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-*D*-lysinate (248 mg, 0.32 mmol) was split in two, and each part dissolved in 10 mL acetonitrile and treated with piperidine (0.08 mL, 0.81 mmol). After 1 h reaction time, the reaction mixtures was loaded onto a plug of alumina, and eluted portion-wise with increasing amounts of methanol (0-10%) in dichloromethane. After combining the reactions, the product was isolated after evaporation of solvent as an off-white foamy solid (113.6 mg, 64%). Some piperidine and dcm was present in the product as a minor impurities. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (m, 2H), 8.36 (br t, 1H), 8.30 (br d, 1H), 8.04 (s, 1H), 7.77 (dt, J = 1.8 Hz, 7.6 Hz, 2H), 7.57 (d, J = 7.8 Hz, 2H), 7.25 (m, 2H), 5.74 (dcm), 5.07 (s, 2H), 4.24 (m, 1H), 3.75 (s, 2H), 3.72 (s, 4H), 3.62 (s, 3H), 3.46 (m, 1H), 3.07 (m, 2H), 2.96 (m, piperidine), 1.69 (m, 1H), 1.62 (m, overlapping with piperidine), 1.54 (m, piperidine), 1.43 (m, 2H), 1.30 (m, 2H), 1.18 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.5, 172.6, 165.4, 159.1, 148.9, 143.2, 136.7, 125.5, 122.7, 122.2, 58.7, 52.0. 51.72, 51.66, 49.5, 48.1, 44.0 (piperidine), 38.6, 30.6, 28.5, 22.7, 22.6 (piperidine), 22.0 (piperidine), 20.4. MS (APCI, pos. mode) m/z 552.3 $[M+H]^+$, HR-MS (APCI, pos. mode) m/z 552.3041 calculated for $C_{27}H_{38}N_9O_4$, found m/z 552.3033.

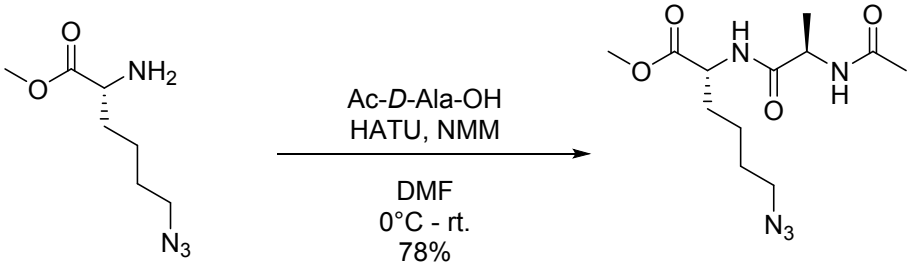
Preparation of methyl (*R*)-2-((*R*)-2-acetamidopropanamido)-6-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)hexanoate (39).



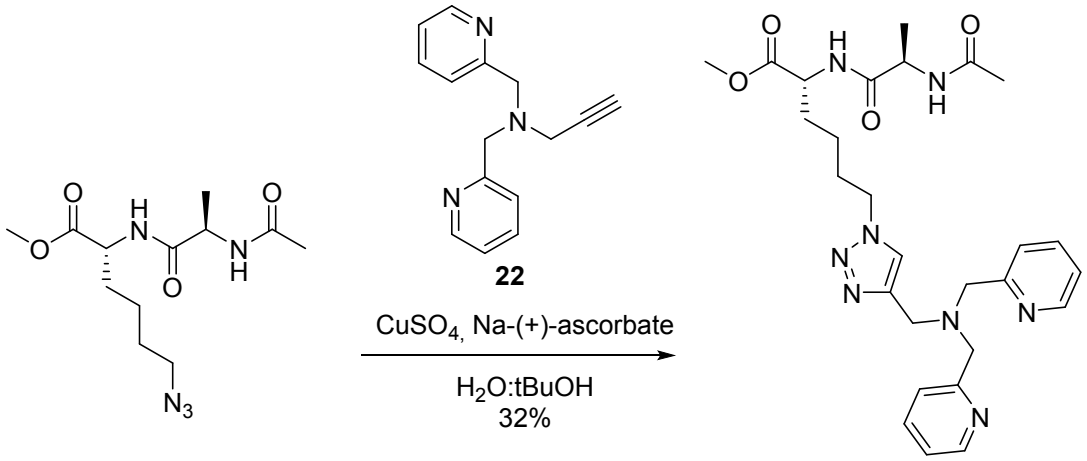
Fmoc-D-Lys(N_3)-OMe. Fmoc-D-Lys(H)-OMe hydrochloride salt (1.0116 g, 2.51 mmol), $NaHCO_3$ (743.1 mg, 8.85 mmol) and $CuSO_4 \cdot 5\ H_2O$ (104.6 mg, 0.419 mmol) was suspended in 4:1 methanol/water (10 mL). 1-(Azidosulfonyl)-1*H*-imidazol-3-ium tetrafluoroborate (795.8 mg, 3.05 mmol) was added, giving evolution of gas. The reaction was stirred for 4.5 h, during which pH was adjusted by small additions of sat. $NaHCO_3$ (aq.). After quenching the reaction by way of addition of 50 mL 0.5 M $NaHCO_3$ (aq.) and $Na_2(SO_4)$ (s.), the product was extracted with 3 x 100 mL ethyl acetate. After washing twice with sat. NaCl (aq.) and drying over Na_2SO_4 (s.), the solution was filtered and the solvent removed under reduced pressure. The product was isolated in quantitative yield as a pale yellow oil and used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (d, J = 7.7 Hz, 2H), 7.60 (br d, 2H), 7.40 (m, 2H), 7.32 (m, 2H), 5.32 (br d, 1H), 4.37-4.43 (m, 3H), 4.23 (t, J = 6.9 Hz, 1H), 3.76 (s, 3H), 3.28 (m, 2H), 1.87 (m, 1H), 1.70 (m, 1H), 1.61 (m, 2H), 1.43 (m, 2H).



H-D-Lys(N_3)-OMe. To a solution of Fmoc-D-Lys(N_3)-OMe (2.51 mmol) in 100 mL acetonitrile, piperidine (5 mL, 50 mmol) was added and the mixture stirred at room temperature for 2 h. After evaporation of solvent, the product was purified by repeated loading onto a silica plug, and elution portion-wise with increasing amounts of methanol (0.5%-10%) in dichloromethane. The product was isolated as an oil (348.9 mg, 75%) and carried along directly to the next step.



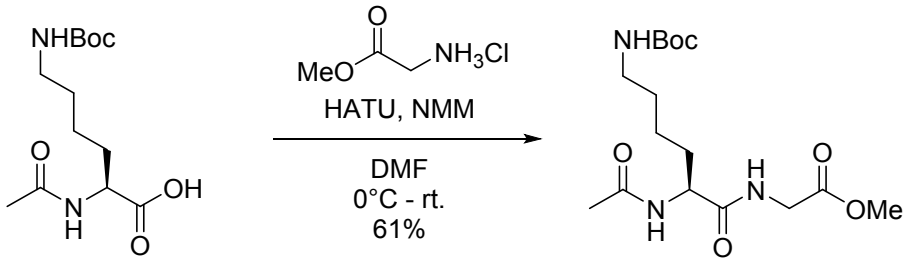
Methyl N^2-(acetyl-D-alanyl)-N^6-diazo-D-lysinate. H-D-Lys(N_3)-OMe (349 mg, 1.88 mmol) and Ac-D-Ala-OH (248 mg, 1.89 mmol) and HATU (723 mg, 1.90 mmol) was stirred in 6 mL DMF and cooled in an ice bath. After addition of *N*-methylmorpholine (0.23 mL, 2.1 mmol), the reaction was kept 30 min on ice bath and thereafter left to stir overnight at room temperature. After quenching the reaction mixture with 40 mL sat. NaCl (aq.) and 20 mL 0.5 M K_2CO_3. (aq.), the product was extracted with 4 x 40 mL ethyl acetate, washed with 20 mL 0.1 M HCl (aq.) and 20 mL 0.5 M $NaHCO_3$ (aq.). The organic phase was dried over $MgSO_4$ and after filtration and removal of solvent under reduced pressure a crude product was obtained which was further purified on a silica column using 8% methanol in dichloromethane as eluent. The product was obtained after removal of solvent under reduced pressure (438.5 mg, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (br d, J = 7.6 Hz, 1H), 7.98 (br d, J = 7.6 Hz, 1H), 4.31 (dq, $J_1 = J_2$ = 7.2 Hz, 1H), 4.22 (m, 1H), 3.61 (s, 3H), 3.31 (t, J = 6.9 Hz, 2H), 1.82 (s, 3H), 1.71 (m, 1H), 1.64 (m, 1H), 1.51 (m, 2H), 1.35 (m, 2H), 1.18 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.4, 168.9, 51.8, 51.7, 50.5, 47.7, 30.3, 27.8, 22.6, 22.4, 18.1. MS (ESI, pos. mode) *m*/*z* 322.1 $[M+Na]^+$, HR-MS (ESI, pos. mode) *m*/*z* 322.1486 calculated for $C_{12}H_{21}N_5O_4Na$, found *m*/*z* 322.1489.



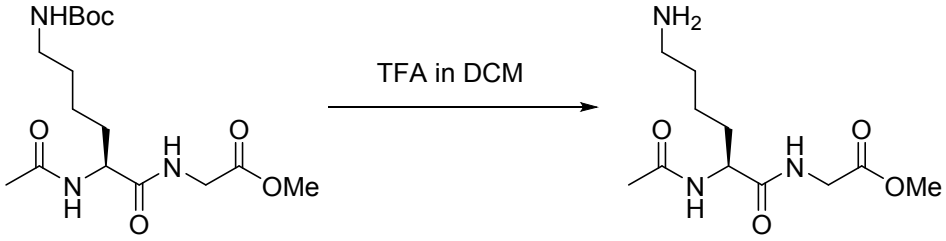
*Methyl (R)-2-((R)-2-acetamidopropanamido)-6-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)hexanoate (**39**)*. A mixture of copper(II)sulfate (266 mg, 1.67 mmol) and sodium-(+)-ascorbate (682 mg, 3.44 mmol) in water (15 mL) was transferred to a flask containing *N*,*N*-bis(pyridin-2-ylmethyl)prop-2-yn-1-amine (**22**, 424 mg, 1.79 mmol) dissolved in *tert*-butanol (15 mL). Under rapid stirring, methyl N^2-(acetyl-*D*-alanyl)-N^6-diazo-*D*-lysinate (427 mg, 1.43 mmol), prepared as described above, was added and the mixture kept under rapid stirring for 5 h before dilution with ethyl acetate, water and sat. NaCl (aq.). Extraction with ethyl acetate was attempted, with only low yields. The product could only be extracted with ethyl acetate after addition of K_2CO_3 and EDTA to the aqueous phase. After removal of solvent under reduced pressure, a crude product was obtained which was subsequently loaded onto an alumina column, and eluted with 1% methanol in dichloromethane, giving a moderately pure product (245.6 mg, 32%). An aliquot was purified on reversed-phase preparative HPLC, using 65:35 water/methanol containing 0.5% trifluoroacetic acid as eluent on a YMC Triart C18 column. After

removal of eluent, the aqueous phase was made basic with sat. K_2CO_3 (aq.) and extracted with ethyl acetate. Subsequent washing with sat. NaCl (aq.) and drying over K_2CO_3, afforded a pure product after removal of solvent under reduced pressure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (m, 2H), 8.20 (br d, J = 7.5 Hz, 1H), 8.05 (s, 1H), 7.97 (br d, J = 7.5 Hz, 1H), 7.76 (td, J = 1.8, 7.6 Hz, 2H), 7.56 (d, J = 7.8 Hz, 2H), 7.24 (m, 2H), 4.26-4.34 (m, 3H), 4.19 (m, 1H), 3.74 (s, 6H), 3.58 (s, 2H), 1.81 (s, 3H), 1.59-1.81 (m, 4H), 1.27 (m, 2H), 1.16 (d, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.4, 168.9, 159.1, 148.8, 143.4, 136.5, 123.6, 122.5, 122.1, 58.9, 51.8, 51.6, 49.0, 48.3, 47.8, 30.1, 29.2, 22.4, 22.3, 18.1. MS (APCI, pos. mode) m/z 537.3 $[M+H]^+$, HR-MS (APCI, pos. mode) m/z 537.2932 calculated for $C_{27}H_{36}N_8O_4$, found m/z 537.2928.

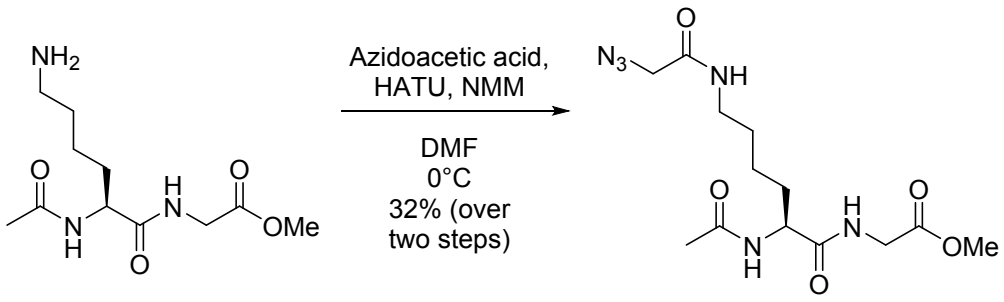
Preparation of methyl N^2-acetyl-N^6-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-*L*-lysylglycinate (40).



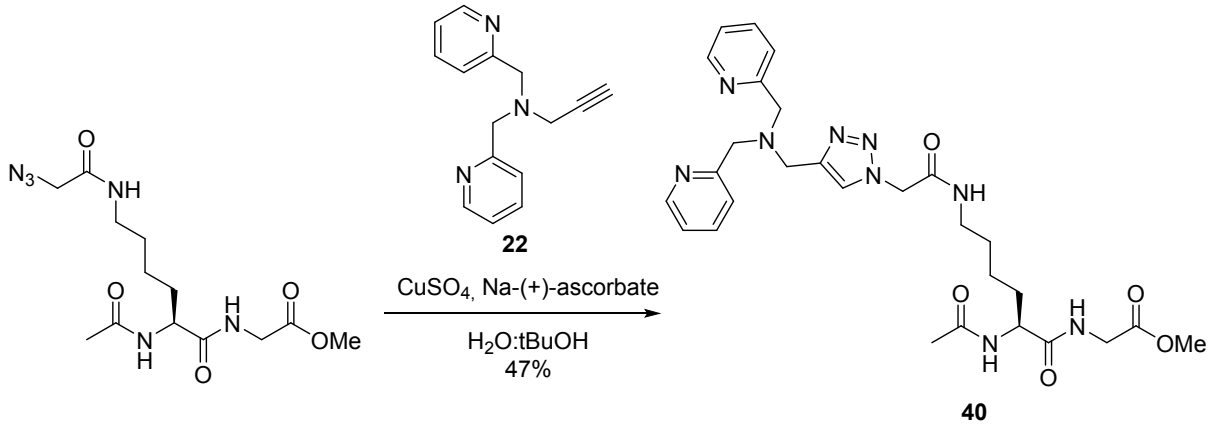
Methyl N^2-acetyl-N^6-(tert-butoxycarbonyl)-L-lysylglycinate. To an ice-cooled flask under nitrogen, containing glycine methyl ester hydrochloride (250.9 mg, 2.00 mmol), (*S*)-2-acetamido-6-((tert-butoxycarbonyl)aminohexanoic acid (593.5 mg, 2.06 mmol) and HATU (772.0 mg, 2.03 mmol), suspended in 5 mL DMF, *N*-methylmorpholine (0.50 mL, 4.5 mmol) was added. The mixture was kept on ice bath for 30 min, and subsequently stirred overnight at room temperature. After removal of volatiles under reduced pressure, the mixture was transferred to a separatory funnel using 15 mL 0.5 M K_2CO_3 (aq.) and 30 mL saturated NaCl (aq.). The product was extracted using 4 x 30 mL ethyl acetate. The combined organic phase was washed with 25 mL 0.1 M HCl (aq.) and 25 mL 0.5 M $NaHCO_3$ (aq.), and then dried over $MgSO_4$. Evaporation of solvent gave a crude product which was recrystallised from a mixture of 30 mL ethyl acetate and 20 mL *n*-heptane to provide the title compound as a colourless solid (435.4 mg, 60.6%). ^{1}H NMR (400 MHz, methanol-d_4) δ 4.34 (dd, J =8.6 Hz, 5.5 Hz, 1H), 3.98 (d, J = 17.5 Hz, 1H), 3.89 (d, J = 17.6 Hz, 1H), 3.72 (s, 3H), 3.04 (app. t, J = 6.7 Hz, 2H), 1.99 (s, 3H), 1.82 (m, 1H), 1.66 (m, 1H), 1.51-1.38 (m, 13H). ^{13}C NMR (100 MHz, methanol-d_4) δ 175.0, 173.4, 171.6, 158.6, 79.9, 54.6, 52.6, 41.8, 41.1, 32.8, 30.6, 28.8, 24.0, 22.4. MS (ESI, positive mode) *m*/*z* 382.2 $[M+Na]^+$, HR-MS (ESI, pos. mode) *m*/*z* 382.1949 calculated for $C_{16}H_{29}N_3O_6Na$, found *m*/*z* 382.1949.



Methyl acetyl-L-lysylglycinate. A solution of methyl N^2-acetyl-N^6-(tert-butoxycarbonyl)-*L*-lysylglycinate (426.3 mg, 1.187 mmol) in 20 mL dichloromethane was kept in an ice bath while trifluoroacetic acid (5 mL, diluted with 5 mL dichloromethane) was added over a period of 1 h. The mixture was stirred on ice bath for additional 100 min, after which the volatile materials were removed three times, with addition of 20 mL toluene ahead of each removal. This gave 608 mg of a crude material, for which full conversion and recovery was assumed, corresponding to 50.6 mass percentage of desired product. Excess mass was assumed to be mainly trifluoroacetic acid and this crude was employed directly in the next step, using additional equivalents of base to correct for this.



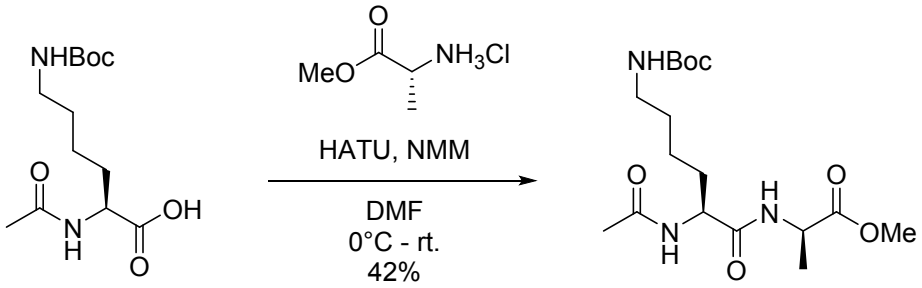
Methyl N^2-acetyl-N^6-(2-azidoacetyl)-L-lysylglycinate. Methyl acetyl-*L*-lysylglycinate (1.187 mmol), available as a 50.6 mass percent mixture with trifluoroacetic acid, as described above, was dissolved in 5 mL DMF. HATU (452.9 mg, 1.191 mmol) was added, and the mixture was subsequently cooled down in an ice bath under nitrogen. After addition of 2-azidoacetic acid (0.13 mL, 1.7 mmol), *N*-methylmorpholine was added (0.45 mL, 4.1 mmol), giving a yellow solution. The mixture was stirred 30 min on the ice bath, and then 4 h in room temperature. After transferal to a separatory funnel, using 15 mL 0.5 M K_2CO_3 (aq.) and 25 mL saturated NaCl (aq.), attempts were made to extract the product with 4 x 25 mL ethyl acetate, albeit with poor recovery. Better results were obtained with 3 x 20 mL 4:1 mixture of chloroform and isopropanol. After drying all extracts over $MgSO_4$ (s) and subsequent filtration, they were combined and, after removal of solvents under reduced pressure, purified on a silica column eluted with 10% methanol in dichloromethane. Fractions containing pure product were combined, and solvents were removed under reduced pressure to give the title compound as a colourless solid (129.5 mg, 31.9%). ^{1}H NMR (400 MHz, methanol-d_4) δ 4.34 (dd, J =8.4 Hz, 5.6 Hz, 1H), 3.99 (d, J = 17.6 Hz, 1H), 3.89 (d, J = 17.5 Hz, 1H), 3.87 (s, 2H), 3.72 (s, 3H), 3.24 (app. t, J = 6.9 Hz, 2H), 1.99 (s, 3H), 1.83 (m, 1H), 1.67 (m, 1H), 1.56 (m, 2H), 1.44 (m, 2H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.9, 173.4, 171.6, 170.1, 54.6, 53.0, 52.6, 41.8, 40.1, 32.7, 30.0, 24.0, 22.4. MS (ESI, positive mode) m/z 365.2 $[M+Na]^+$, HR-MS (ESI, pos. mode) m/z 365.1544 calculated for $C_{13}H_{22}N_6O_5Na$, found m/z 365.1545.



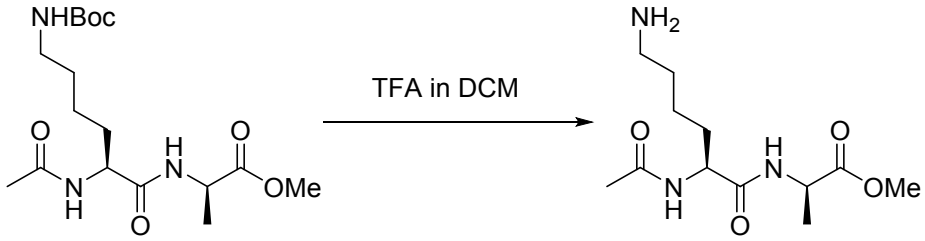
*Methyl N^2-acetyl-N^6-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-L-lysylglycinate (**40**)*. A mixture of copper(II)sulfate (73.5 mg, 0.461 mmol) and sodium-(+)ascorbate (182

mg, 0.919 mmol) in water (3 mL) was transferred to a flask containing *N*,*N*-bis(pyridin-2-ylmethyl)prop-2-yn-1-amine (**22**, 108.8 mg, 0.4591 mmol) dissolved in *tert*-butanol (3 mL). This mixture was then transferred to a flask containing methyl N^2-acetyl-N^6-(2-azidoacetyl)-*L*-lysylglycinate (123.7 mg, 0.3617 mmol). Transfer was aided by rinsing out the flask with additional 2 mL 1:1 *tert*-butanol and water. The reaction mixture was stirred rapidly for 3h, after which Chelex 100 (1.4 g) was added. Stirring was continued for 1 h, during which the reaction mixture went from green to red. Filtration and subsequent evaporation gave a crude product which was loaded onto a plug of Bondesil-C18 OH SPE material and eluted by running through portions of methanol/water mixture, going stepwise from 30% to 60% methanol. To the first two portions, both at 30% methanol concentration, some EDTA buffered to pH 8 was added to the eluent. Fractions containing product was collected and the solvent removed under reduced pressure to provide the title compound as a reddish brown oil (99.0 mg, 47.2%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.44 (m, 2H), 8.03 (s, 1H), 7.80 (m, 2H), 7.67 (d, J = 7.9 Hz, 2H), 7.28 (m, 2H), 5.14 (s, 2H), 4.34 (dd, J =8.4 Hz, 5.7 Hz, 1H), 3.99 (d, J = 17.5 Hz, 1H), 3.90-3.85 (m, 3H), 3.82 (s, 4H), 3.71 (s, 1.6H (loss of signal due to transesterification with the NMR solvent)), 3.25 (app. t, J = 6.8 Hz, 2H), 1.98 (s, 3H), 1.84 (m, 1H), 1.67 (m, 1H), 1.57 (m, 2H), 1.45 (m, 2H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.9, 173.4, 171.7, 167.8, 160.2, 149.5, 145.4, 138.7, 126.9, 124.9, 123.8, 60.3, 54.6, 53.1, 52.6, 49.9, 41.8, 40.3, 32.6, 29.9, 23.9, 22.5. MS (APCI, pos. mode) *m*/*z* 580.3 $[M+H]^+$, HR-MS (APCI) *m*/*z* 580.2990 calculated for $C_{28}H_{38}N_9O_5$, found *m*/*z* 580.2988.

Preparation of methyl N^2-acetyl-N^6-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-*L*-lysyl-*D*-alaninate (41).

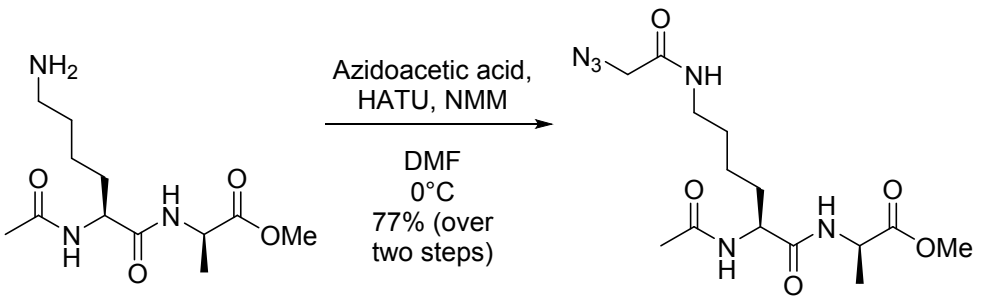


Methyl N^2-acetyl-N^6-(tert-butoxycarbonyl)-L-lysyl-D-alaninate. To an ice-cooled flask under nitrogen, containing *D*-alanine methyl ester hydrochloride (280.1 mg, 2.01 mmol), (*S*)-2-acetamido-6-((tert-butoxycarbonyl)aminohexanoic acid (598.9 mg, 2.08 mmol) and HATU (765.2 mg, 2.01 mmol), suspended in 5 mL DMF, *N*-methylmorpholine (0.50 mL, 4.5 mmol) was added. The mixture was kept on ice bath for 30 min, and subsequently stirred overnight at room temperature. After removal of volatiles under reduced pressure, the mixture was transferred to a separatory funnel using 15 mL 0.5 M K_2CO_3 (aq.) and 30 mL saturated NaCl (aq.). The product was extracted using 4 x 30 mL ethyl acetate. The combined organic phase was washed with 25 mL 0.1 M HCl (aq.) and 25 mL 0.5 M $NaHCO_3$ (aq.), and then dried over $MgSO_4$. Evaporation of solvent gave a crude colorless solid which was attempted recrystallized from a mixture of 20 mL ethyl acetate and 20 mL *n*-heptane. A polymorphous precipitate was formed. Some nice looking crystals were recovered, and the mixture was reheated and redissolved, with the addition of a few more mL of ethyl acetate. The mixture was then slowly cooled down with steady addition of seeding crystals. The desired product was isolated by way of suction filtration as a colourless solid (313.8 mg, 41.8%). ^{1}H NMR (400 MHz, methanol-d_4) δ 4.39 (q, J = 7.3 Hz, 1H), 4. 34 (dd, J =8.3 Hz, 5.9 Hz, 1H), 3.71 (s, 3H), 3.03 (app. t, J = 6.8 Hz, 2H), 1.99 (s, 3H), 1.76 (m, 1H), 1.64 (m, 1H), 1.51-1.35 (m, 16H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.4, 174.1, 173.2, 158.6, 79.9, 54.5, 52.7, 49.5, 41.1, 32.9, 30.6, 28.8, 24.0, 22.5, 17.5. MS (ESI, positive mode) m/z 396.2 $[M+Na]^+$, HR-MS (ESI, pos. mode) m/z 396.2105 calculated for $C_{17}H_{31}N_3O_6Na$, found m/z 396.2105.

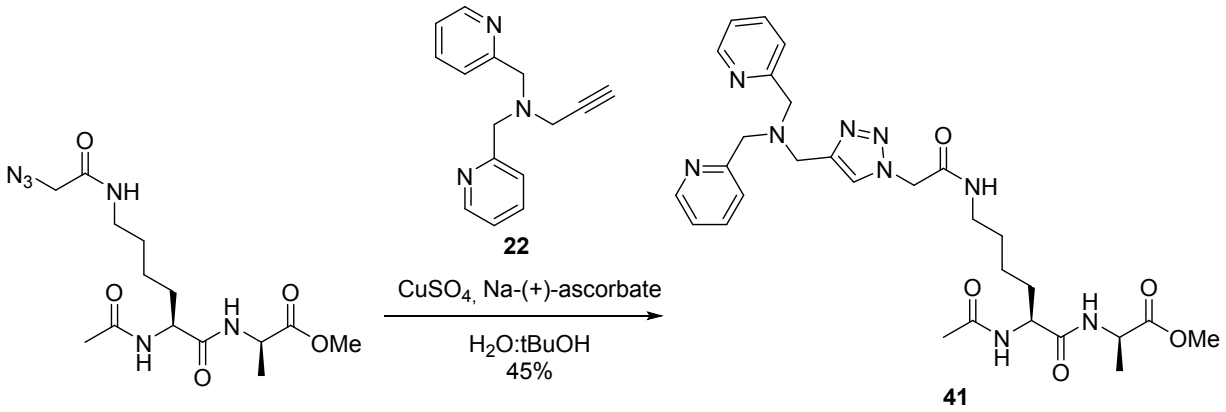


Methyl acetyl-L-lysyl-D-alaninate. A solution of methyl N^2-acetyl-N^6-(tert-butoxycarbonyl)-*L*-lysyl-*D*-alaninate (308.2 mg, 0.826 mmol) in 20 mL dichloromethane was kept in an ice bath while trifluoroacetic acid (5 mL, diluted with 5 mL dichloromethane) was added over a period of 45 min. The mixture was stirred on ice bath for additional 15 min, after which TLC indicated full conversion. The

volatile materials were removed under reduced pressure three times, with addition of 20 mL toluene ahead of each removal. This gave 427 mg of a crude material, for which full conversion and recovery was assumed, corresponding to 52.8 mass percentage of desired product. As for the corresponding glycinate compound, excess mass was assumed to be mainly trifluoroacetic acid and the crude was employed directly in the next step, using additional equivalents of base to correct for this.

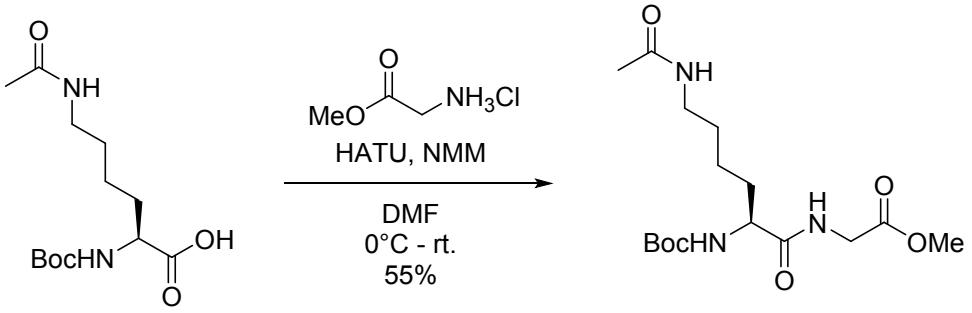


Methyl N^2-acetyl-N^6-(2-azidoacetyl)-L-lysyl-D-alaninate. Methyl acetyl-*L*-lysyl-*D*-alaninate (0.826 mmol), prepared as a mixture with trifluoroacetic acid as described above, was dissolved in 4 mL DMF. HATU (351.0 mg, 0.923 mmol) was added, and the mixture was subsequently cooled down in an ice bath under nitrogen. After addition of 2-azidoacetic acid (0.10 mL, 1.3 mmol), *N*-methylmorpholine was added (0.32 mL, 2.9 mmol). After removal of the ice bath, the mixture was stirred overnight at room temperature. The mixture was transferred to a separatory funnel, using 10 mL 0.5 M K_2CO_3 (aq.) and 20 mL saturated NaCl (aq.), and extracted with 4 x 20 mL 4:1 mixture of chloroform and isopropanol. After drying over $MgSO_4$ (s), filtration, and removal of solvents under reduced pressure, the crude product was purified on a silica column eluted with 7% methanol in dichloromethane. Fractions containing pure product were combined, and solvents removed under reduced pressure to give the title compound as a colourless film which could be scraped to a colourless solid (225.8 mg, 76.7%). ^{1}H NMR (400 MHz, methanol-d_4) δ 4.39 (q, J = 7.3 Hz, 1H), 4.34 (dd, J =8.4 Hz, 5.7 Hz, 1H), 3.87 (s, 2H), 3.71 (s, 3H), 3.22 (app. t, J = 7.0 Hz, 2H), 1.99 (s, 3H), 1.77 (m, 1H), 1.66 (m, 1H), 1.55 (m, 2H), 1.45-1.32 (m, 5H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.4, 174.1, 173.3, 170.1, 54.5, 53.0, 52.7, 49.5, 40.1, 32.8, 30.0, 24.0, 22.5, 17.4. MS (ESI, positive mode) *m*/*z* 379.2 $[M+Na]^+$, HR-MS (ESI, pos. mode) *m*/*z* 379.1700 calculated for $C_{14}H_{24}N_6O_5Na$, found *m*/*z* 379.1701.

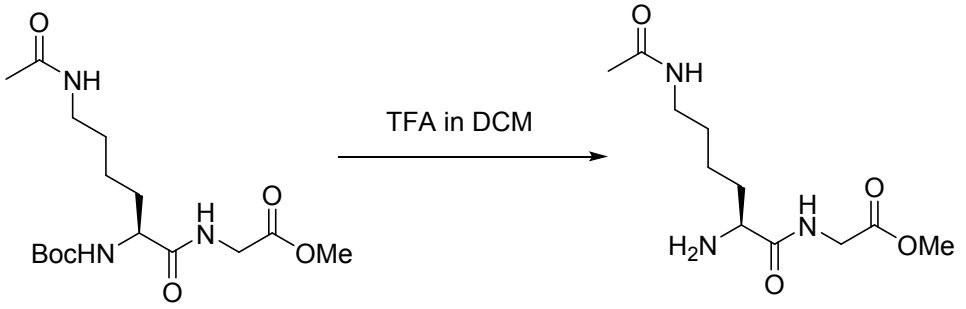


Methyl N^2*-acetyl-*N^6*-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-L-lysyl-D-alaninate* ***(41)***. A rapidly prepared mixture of copper(II)sulfate (127.5 mg, 0.799 mmol) and sodium ascorbate (319.8 mg, 1.615 mmol) in water (5 mL) was transferred to a flask containing *N*,*N*-bis(pyridin-2-ylmethyl)prop-2-yn-1-amine (**22**, 190.3 mg, 0.803 mmol) dissolved in *tert*-butanol (5 mL). This mixture was then transferred to a separate flask containing methyl N^2-acetyl-N^6-(2-azidoacetyl)-*L*-lysyl-*D*-alaninate (218.3 mg, 0.613 mmol), prepared as described above. Transfer was completed by rinsing out the flask with additional 4 mL 1:1 *tert*-butanol and water. The reaction mixture was stirred vigorously for 3h. Chelex 100 (2.5 g) was then added and stirring was continued for 1 h. Filtration and subsequent removal of solvents gave a crude product which was loaded onto a plug of Bondesil-C18 OH SPE material and eluted by running through portions of methanol/water mixture, going stepwise from 30% to 50% methanol. To the first two portions, both at 30% methanol concentration, some EDTA buffered to pH 8 was added to the eluent to aid removal of remaining traces of copper. Fractions containing product was collected, discarding some of the more strongly coloured fractions, and the solvent was removed under reduced pressure to provide the title compound as a brown film which could be scraped to a powder (162.3 mg, 44.6%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.44 (m, 2H), 8.03 (s, 1H), 7.80 (m, 2H), 7.67 (d, J = 7.9 Hz), 7.28 (m, 2H), 5.13 (s, 2H), 4.39 (q, J = 7.2 Hz, 1H), 4.34 (dd, J =8.3 Hz, 5.5 Hz, 1H), 3.86 (s, 2H), 3.82 (s, 4H), 3.70 (s, 3H), 3.23 (app. t, J = 6.9 Hz, 2H), 1.98 (s, 3H), 1.77 (m, 1H), 1.65 (m, 1H), 1.55 (m, 2H), 1.44-1.33 (m, 5H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.4, 174.1, 173.3, 167.8, 160.2, 149.5, 145.4, 138.7, 126.9, 124.9, 123.8, 60.3, 54.5, 53.1, 52.7, 49.9, 49.5, 40.3, 32.8, 29.9, 24.0, 22.5, 17.4. MS (APCI, pos. mode) *m*/*z* 594.3 $[M+H]^+$, HR-MS (APCI) *m*/*z* 594.3147 calculated for $C_{29}H_{40}N_9O_5$, found *m*/*z* 594.3144.

Preparation of methyl N^6-acetyl-N^2-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-*L*-lysylglycinate (42).

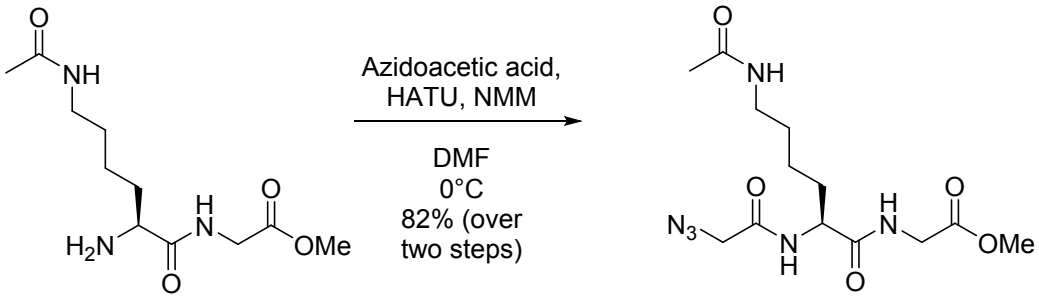


Methyl N^6-acetyl-N^2-(tert-butoxycarbonyl)-L-lysylglycinate. To an ice-cooled flask under nitrogen, containing glycine methyl ester hydrochloride (258.5 mg, 2.06 mmol), (*S*)-6-acetamido-2-((tert-butoxycarbonyl)aminohexanoic acid (593.3 mg, 2.06 mmol) and HATU (759.9 mg, 2.00 mmol), suspended in 5 mL DMF, *N*-methylmorpholine (0.50 mL, 4.5 mmol) was added. The mixture was kept on ice bath for 30 min, and subsequently stirred overnight at room temperature. After removal of volatiles under reduced pressure, the mixture was transferred to a separatory funnel using 15 mL 0.5 M K_2CO_3 (aq.) and 30 mL saturated NaCl (aq.). The product was extracted using 4 x 30 mL ethyl acetate. The combined organic phase was washed with 25 mL 0.1 M HCl (aq.) and 25 mL 0.5 M $NaHCO_3$ (aq.), and then dried over $MgSO_4$. Evaporation of solvent gave a crude product which was purified on a silica column, eluted with 6% methanol in dichloromethane as the eluent. Fractions containing pure product was collected and solvent removed under reduced pressure to give 397.7 mg of the title compound (55.3%). ^{1}H NMR (400 MHz, methanol-d_4) δ 4.06-3.98 (m, 2H), 3.89 (d, J = 17.6 Hz, 1H), 3.72 (s, 3H), 3.17 (app. t, J = 6.8 Hz, 2H), 1.93 (s, 3H), 1.78 (m, 1H), 1.62 (m, 1H), 1.56-1.48 (m, 2H), 1.47-1.40 (m, 11H). ^{13}C NMR (100 MHz, methanol-d_4) δ 175.7, 173.2, 171.6, 157.9, 80.6, 55.9, 52.6, 41.8, 40.2, 33.0, 30.0, 28.7, 24.1, 22.6. MS (ESI, positive mode) m/z 382.2 $[M+Na]^+$, HR-MS (ESI, pos. mode) m/z 382.1949 calculated for $C_{16}H_{29}N_3O_6Na$, found m/z 382.1950.

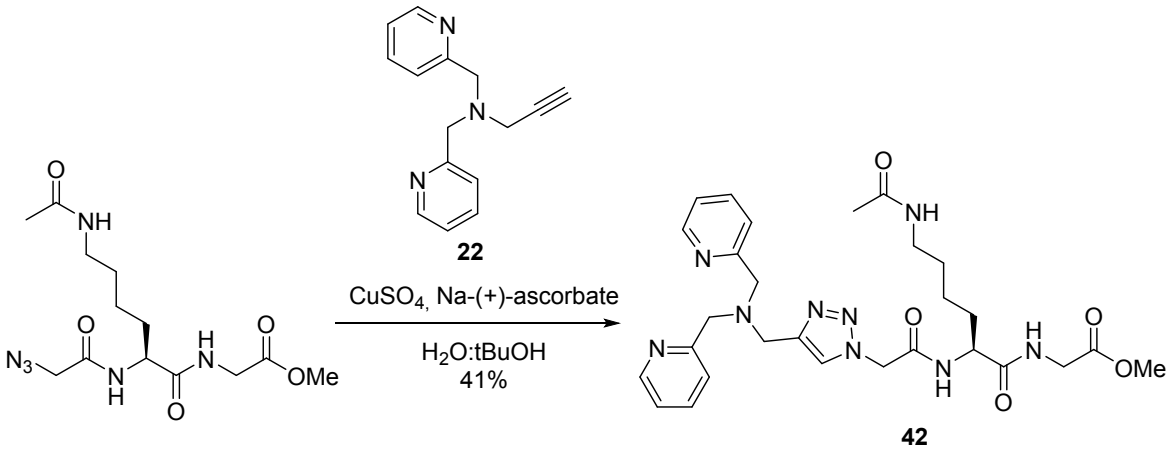


Methyl N^6-acetyl-L-lysylglycinate. To a solution of methyl N^6-acetyl-N^2-(tert-butoxycarbonyl)-*L*-lysylglycinate (391.2 mg, 1.090 mmol) in 20 mL dichloromethane cooled down in an ice bath, trifluoroacetic acid (5 mL, diluted with 5 mL dichloromethane) was added over a period of 1 h. The mixture was kept stirring on the ice bath for additional 3 h. TLC indicated full conversion at this point. The volatile materials were removed under reduced pressure three times, with addition of 20 mL toluene

ahead of each removal. This gave 554 mg of a crude material, for which full conversion and recovery was assumed, corresponding to 51.0 mass percentage of desired product. Excess mass was assumed to be mainly trifluoroacetic acid and the crude was employed directly in the next step, using additional equivalents of base to correct for this.

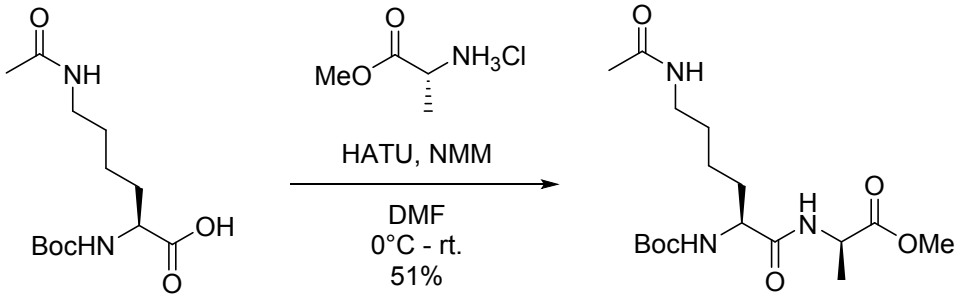


Methyl N^6-acetyl-N^2-(2-azidoacetyl)-L-lysylglycinate. Methyl N^6-acetyl-*L*-lysylglycinate (1.090 mmol), prepared as a mixture with trifluoroacetic acid in the manner as described above, was dissolved in 5 mL DMF. HATU (416.1 mg, 1.094 mmol) was added, and the mixture was cooled down in an ice bath under nitrogen. After addition of 2-azidoacetic acid (0.12 mL, 1.6 mmol), followed by *N*-methylmorpholine (0.40 mL, 3.6 mmol), the mixture was stirred 30 min on the ice bath, giving a yellow solution. Stirring was continued at room temperature for 3.5 h. The mixture was transferred to a separatory funnel, using a mixture of 0.5 M K_2CO_3 (aq.) and saturated NaCl (aq.), and extracted with 4 x 20 mL 4:1 mixture of chloroform and isopropanol. After drying over $MgSO_4$ (s), filtration, and removal of solvents under reduced pressure, the crude product was purified on a silica column eluted with 7% methanol in dichloromethane. Fractions containing pure product were pooled, and solvents removed under reduced pressure to give the title compound as a colourless solid (304.4 mg, 81.6%). ^{1}H NMR (400 MHz, methanol-d_4) δ 4.40 (dd, J =8.4 Hz, 5.5 Hz, 1H), 4.00 (d, J = 17.6 Hz, 1H), 3.93 (d, J = 1.9 Hz, 2H), 3.90 (d, J = 17.6 Hz, 1H), 3.72 (s, 3H), 3.17 (app. t, J = 6.9 Hz, 2H), 1.93 (s, 3H), 1.85 (m, 1H), 1.70 (m, 1H), 1.52 (m, 2H), 1.43 (m, 2H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.5, 173.3, 171.6, 170.2, 54.5, 52.7, 52.6, 41.8, 40.2, 32.8, 29.9, 24.0, 22.6. MS (ESI, positive mode) m/z 365.2 $[M+Na]^+$, HR-MS (ESI, pos. mode) m/z 365.1544 calculated for $C_{13}H_{22}N_6O_5Na$, found m/z 365.1546.

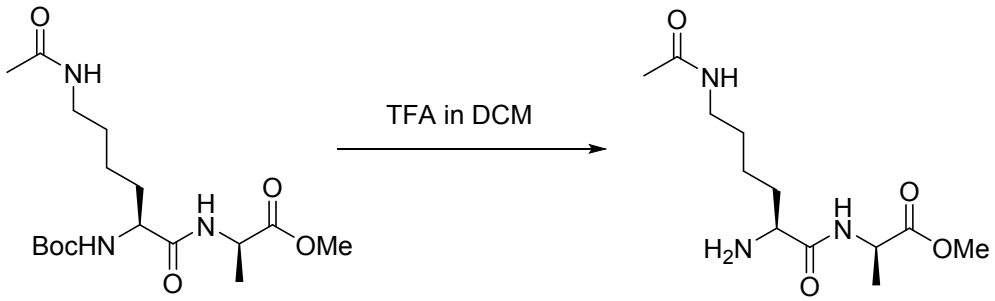


Methyl N^6*-acetyl-*N^2*-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-L-lysylglycinate (**42**)*. A mixture of copper(II)sulfate (185.1 mg, 1.16 mmol) and sodium-(+)ascorbate (458.9 mg, 2.32 mmol) in water (6 mL) was transferred to a flask containing *N*,*N*-bis(pyridin-2-ylmethyl)prop-2-yn-1-amine (**22**, 273.8 mg, 1.155 mmol) dissolved in *tert*-butanol (6 mL). This mixture was then transferred to a flask containing the methyl N^6-acetyl-N^2-(2-azidoacetyl)-*L*-lysylglycinate (297.3 mg, 0.869 mmol) from above. Transfer was completed by rinsing out the flask with additional 4 mL 1:1 *tert*-butanol and water. The reaction mixture was stirred rapidly for 3h, after which Chelex 100 (3.0 g) was added. Stirring was continued for 1 h. Filtration and subsequent evaporation gave a crude product which was loaded onto a plug of Bondesil-C18 OH SPE material and eluted by running through portions of methanol/water mixture, going stepwise from 30% to 50% methanol. To the first two portions, both at 30% methanol concentration, some EDTA buffered to pH 8 was added to the eluent. Fractions containing product was collected and the solvent removed under reduced pressure to provide the title compound as a reddish brown oil (205.3 mg, 40.8%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.43 (m, 2H), 8.03 (s, 1H), 7.80 (app dt, J = 1.7 Hz, 7.7 Hz, 2H), 7.67 (d, J = 7.8 Hz), 7.28 (m, 2H), 5.22 (d, J = 4.2 Hz, 2H), 4.39 (dd, J =8.3 Hz, 5.5 Hz, 1H), 3.99 (d, J = 17.5 Hz, 1H), 3.88 (d, J = 17.8 Hz, 1H), 3.85 (s, 2H), 3.81 (s, 4H), 3.71 (s, 2.2H (probably reduced due to transesterification with the NMR solvent)), 3.17 (app. t, J = 6.7 Hz, 2H), 1.92 (s, 3H), 1.87 (m, 1H), 1.72 (m, 1H), 1.52 (m, 2H), 1.45 (m, 2H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.4, 173.3, 171.6, 167.8, 160.2, 149.5, 145.4, 138.7, 127.0, 124.9, 123.8, 60.4, 54.7, 52.9, 52.6, 49.9, 41.8, 40.2, 32.8, 30.0, 23.9, 22.6. MS (APCI, pos. mode) *m*/*z* 580.3 $[M+H]^+$, HR-MS (APCI) *m*/*z* 580.2990 calculated for $C_{28}H_{38}N_9O_5$, found *m*/*z* 580.2990.

Preparation of methyl N^6-acetyl-N^2-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-*L*-lysyl-*D*-alaninate (43).

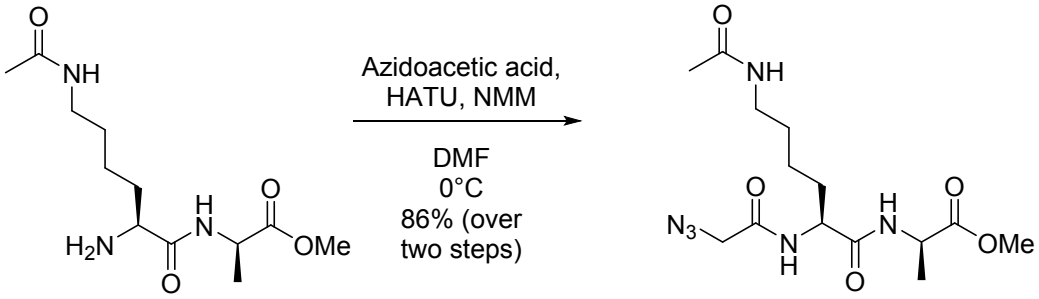


Methyl N^6-acetyl-N^2-(tert-butoxycarbonyl)-L-lysyl-D-alaninate. To an ice-cooled flask under nitrogen, containing *D*-alanine methyl ester hydrochloride (283.3 mg, 2.03 mmol), (*S*)-6-acetamido-2-((tert-butoxycarbonyl)aminohexanoic acid (581.0 mg, 2.01 mmol) and HATU (769.0 mg, 2.02 mmol), suspended in 5 mL DMF, *N*-methylmorpholine (0.50 mL, 4.5 mmol) was added. The mixture was kept on ice bath for 30 min, and subsequently stirred overnight at room temperature. After removal of volatiles under reduced pressure, the mixture was transferred to a separatory funnel using 15 mL 0.5 M K_2CO_3 (aq.) and 30 mL saturated NaCl (aq.). The product was extracted using 4 x 30 mL ethyl acetate. The combined organic phase was washed with 25 mL 0.1 M HCl (aq.) and 25 mL 0.5 M $NaHCO_3$ (aq.), and then dried over $MgSO_4$. Evaporation of solvent gave the crude as a colourless oil. Recrystalisation from ethyl acetate/*n*-heptane mixtures failed, and the product was instead purified on a silica column, gradient eluted with 3-7% methanol in dichloromethane. Fractions containing pure product was combined and solvent evaporated under reduced pressure to provide the title compound as a colourless viscous oil (385.4 mg, 51.3%). ^{1}H NMR (400 MHz, methanol-d_4) δ 4.40 (q, J = 7.3 Hz, 1H), 4. 03 (br t, 1H), 3.71 (s, 3H), 3.16 (app. t, J = 6.9 Hz, 2H), 1.92 (s, 3H), 1.73 (m, 1H), 1.62 (m, 1H), 1.51 (m, 2H), 1.46-1.34 (m, 14H). ^{13}C NMR (100 MHz, methanol-d_4) δ 175.0, 174.4, 173.2, 157.8, 80.7, 55.9, 52.7, 49.5, 40.2, 33.1, 30.0, 28.7, 24.2, 22.6, 17.5. MS (ESI, positive mode) *m*/*z* 396.2 $[M+Na]^+$, HR-MS (ESI, pos. mode) *m*/*z* 396.2105 calculated for $C_{17}H_{31}N_3O_6Na$, found *m*/*z* 396.2106.

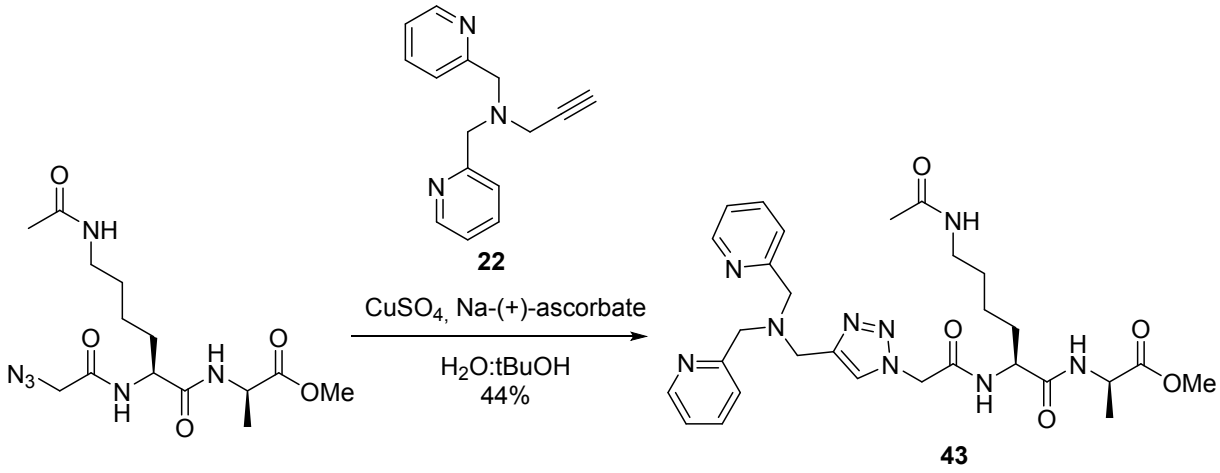


Methyl N^6-acetyl-L-lysyl-D-alaninate. A solution of methyl N^6-acetyl-N^2-(tert-butoxycarbonyl)-*L*-lysyl-*D*-alaninate (376.0 mg, 1.008 mmol) in 20 mL dichloromethane was kept in an ice bath while trifluoroacetic acid (5 mL, diluted with 5 mL dichloromethane) was added over a period of 1 h. The

mixture was stirred on ice bath for additional 1 h, after which TLC indicated full conversion. The volatile materials were removed under reduced pressure three times, with addition of 20 mL toluene ahead of each removal. This gave 525 mg of a crude material, for which full conversion and recovery was assumed, corresponding to 52.4 mass percentage of desired product. Excess mass was assumed to be mainly trifluoroacetic acid and the crude was employed directly in the next step, using additional equivalents of base to correct for this.

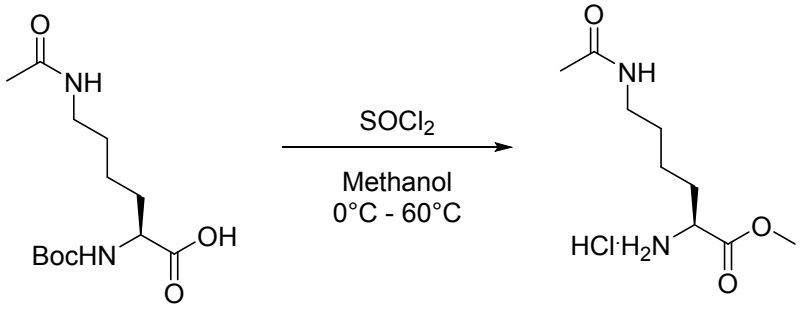


Methyl N^6-acetyl-N^2-(2-azidoacetyl)-L-lysyl-D-alaninate. Methyl N^6-acetyl-*L*-lysyl-*D*-alaninate (1.008 mmol), prepared as a mixture with trifluoroacetic acid as described above, was dissolved in 5 mL DMF. HATU (382.7 mg, 1.006 mmol) was added, and the mixture was subsequently cooled down in an ice bath under nitrogen. After addition of 2-azidoacetic acid (0.11 mL, 1.5 mmol), *N*-methylmorpholine was added (0.37 mL, 3.4 mmol). The mixture was subsequently stirred on ice bath for additional 4 h. The mixture was transferred to a separatory funnel, using 10 mL 0.5 M K_2CO_3 (aq.) and 10 mL saturated NaCl (aq.), and extracted with 4 x 20 mL 4:1 mixture of chloroform and isopropanol. After drying over $MgSO_4$ (s), filtration, and removal of solvents under reduced pressure, the crude product was purified on a silica column eluted with 7% methanol in dichloromethane. Fractions containing pure product were combined, and solvents removed under reduced pressure to give the title compound as a colourless solid (306.8 mg, 85.6%). ^{1}H NMR (400 MHz, methanol-d_4) δ 4.43-4.37 (m, 2H), 3.93 (d, J = 2.5 Hz , 2H), 3.71 (s, 3H), 3.16 (app. t, J = 7.0 Hz, 2H), 1.92 (s, 3H), 1.81 (m, 1H), 1.69 (m, 1H), 1.52 (m, 2H), 1.45-1.33 (m, 5H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.4, 173.7, 173.2, 170.1, 54.4, 52.8 (two exact overlapping signals), 49.6, 40.2, 33.0, 30.0, 24.0, 22.6, 17.4. MS (ESI, positive mode) *m*/*z* 379.2 $[M+Na]^+$, HR-MS (ESI, pos. mode) *m*/*z* 379.1700 calculated for $C_{14}H_{24}N_6O_5Na$, found *m*/*z* 379.1700.

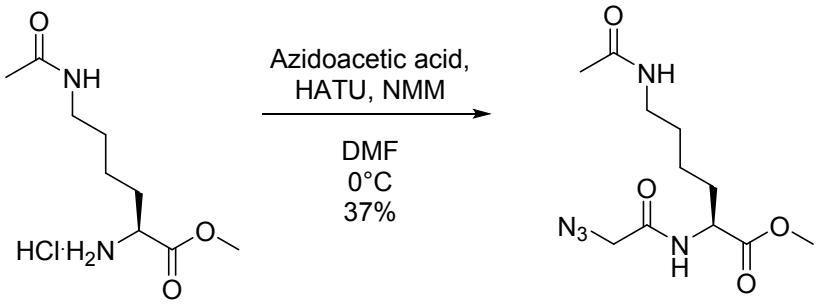


Methyl N^6*-acetyl-*N^2*-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-L-lysyl-D-alaninate (**43**)*. A rapidly prepared mixture of copper(II)sulfate (175.2 mg, 1.098 mmol) and sodium-(+)ascorbate (438.8 mg, 2.216 mmol) in water (6 mL) was transferred to a flask containing *N*,*N*-bis(pyridin-2-ylmethyl)prop-2-yn-1-amine (**22**, 263.6 mg, 1.112 mmol) dissolved in *tert*-butanol (6 mL). This mixture was then transferred to a separate flask containing methyl N^6-acetyl-N^2-(2-azidoacetyl)-*L*-lysyl-*D*-alaninate (300.1 mg, 0.843 mmol). Transfer was completed by rinsing out the flask with additional 4 mL 1:1 *tert*-butanol and water. The reaction mixture was stirred vigorously for 3h. Chelex 100 (3.0 g) was then added and stirring was continued for 1 h. Filtration and subsequent removal of solvents gave a crude product which was loaded onto a plug of Bondesil-C18 OH SPE material and eluted by running through portions of methanol/water mixture, going stepwise from 30% to 50% methanol. To the first two portions, both at 30% methanol concentration, some EDTA buffered to pH 8 was added to the eluent to aid removal of remaining traces of copper. Fractions containing product was collected, discarding some of the more strongly coloured fractions, and the solvent was removed under reduced pressure to provide the title compound as a reddish brown film, which could be scraped to a powder (219.4 mg, 43.8%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.44 (m, 2H), 8.03 (s, 1H), 7.80 (app. dt, J = 1.8 Hz, 7.7 Hz, 2H), 7.67 (d, J = 7.9 Hz, 2H), 7.28 (m, 2H), 5.21 (d, J = 2.8 Hz, 2H), 4.42-4.36 (m, 2H), 3.85 (s, 2H), 3.82 (s, 4H), 3.69 (s, 3H), 3.15 (app. t, J = 7.0 Hz, 2H), 1.92 (s, 3H), 1.81 (m, 1H), 1.70 (m, 1H), 1.51 (m, 2H), 1.44-1.34 (m, 5H). ^{13}C NMR (100 MHz, methanol-d_4) δ 174.4, 173.6, 173.2, 167.7, 160.2, 149.5, 145.4, 138.7, 127.0, 124.9, 123.8, 60.4, 54.6, 53.0, 52.8, 49.9, 49.5, 40.1, 33.0, 30.0, 24.0, 22.6, 17.4. MS (APCI, pos. mode) *m*/*z* 594.3 $[M+H]^+$, HR-MS (APCI, positive mode) *m*/*z* 594.3147 calculated for $C_{29}H_{40}N_9O_5$, found *m*/*z* 594.3146.

Preparation of (*S*)-((6-Acetamido-2-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetamido)hexanamido)methyl)boronic acid (44).



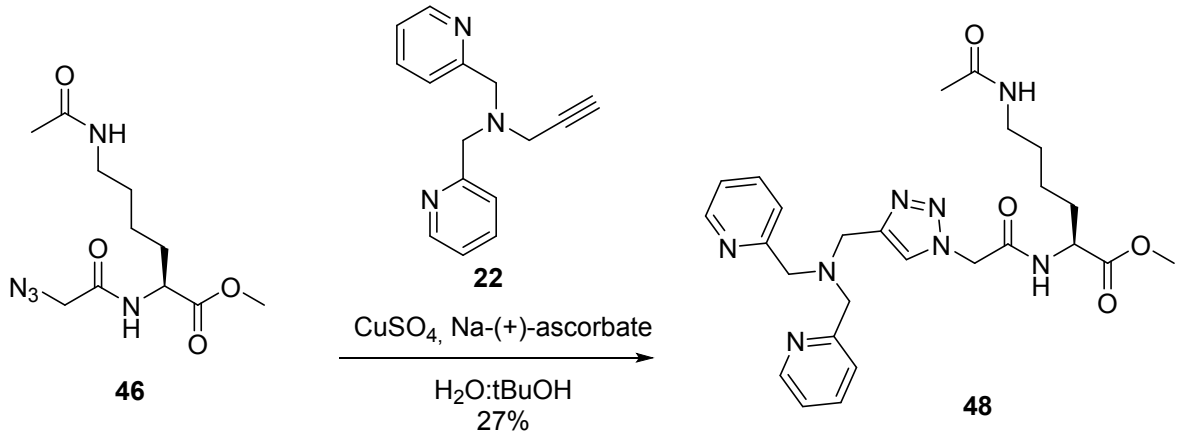
H-D-Lys(Ac)-OMe hydrochloric salt. Boc-Lys(Ac)-OH (1.01 g, 3. 50 mmol) was dissolved in methanol and thionyl chloride (0.32 mL, 4.4 mmol) added dropwise while the reaction mixture was cooled in an ice bath. After 30 min additional cooling, the mixture was heated to 60 °C for 2h. Evaporation of volatiles afforded the product as an oil in quantitative yield. ^{1}H NMR data (400 MHz, DMSO-d_6) was in reasonable agreement with literature values for corresponding trifluoroacetate salt in methanol-d_4.[9]



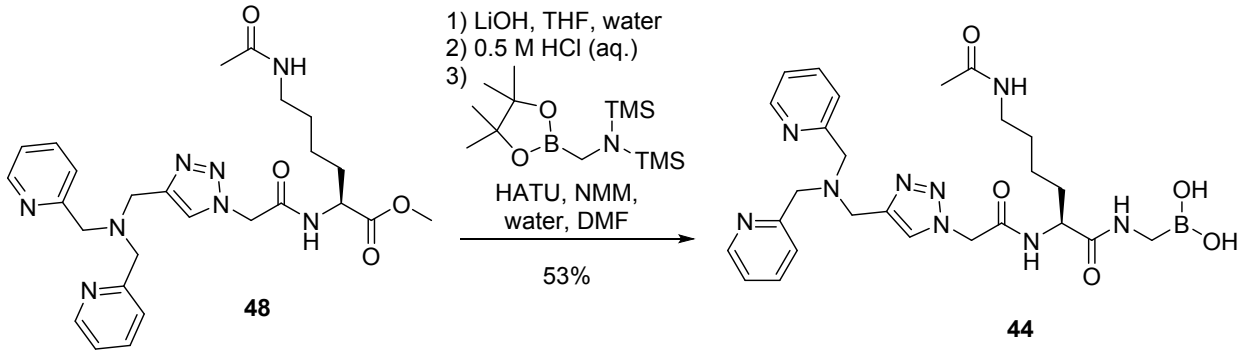
46

*Methyl N^6-acetyl-N^2-(2-azidoacetyl)-L-lysinate (**46**)*. H-D-Lys(Ac)-OMe hydrochloric salt (3.50 mmol), 2-azidoacetic acid (0.3 mL, 4.0 mmol) and HATU (1.35 g, 3.55 mmol) was stirred in 8 mL DMF and cooled in an ice bath. After addition of *N*-methylmorpholine (1.15 mL, 10.4 mmol), the reaction was kept 30 min on ice bath and thereafter left to stir at room temperature. After 4 h reaction time, the reaction was quenched by addition of 40 mL sat. NaCl (aq.) and 20 mL 0.5 M K_2CO_3. (aq.). The product was extracted with 4 x 50 mL ethyl acetate, washed with 35 mL 0.1 M HCl (aq.) and 35 mL 0.5 M $NaHCO_3$ (aq.). Drying over $MgSO_4$ and subsequent filtration and removal of solvent under reduced pressure afforded a crude product which was further purified on a silica column using 5% methanol in dichloromethane as eluent. The product was isolated as a semisolid (370.7 mg, 37%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (br d, J = 7.4 Hz, 1H), 7.78 (br t, 1H), 4.25 (m, 1H), 3.86 (d, J = 2.1 Hz, 2H), 3.64 (s, 3H), 2.99 (q, J = 6.7 Hz, 2H), 1.77 (s, 3H), 1.69 (m, 1H), 1.61 (m, 1H), 1.36 (m, 2H), 1.28 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.2, 169.0, 167.6, 52.0, 51.9, 50.4, 38.2, 30.5, 28.6, 22.7,

22.6. MS (ESI, pos. mode) *m*/*z* 308.1 $[M+Na]^+$, HR-MS (ESI, pos. mode) *m*/*z* 308.1329 calculated for $C_{11}H_{19}N_5O_4Na$, found *m*/*z* 308.1328.



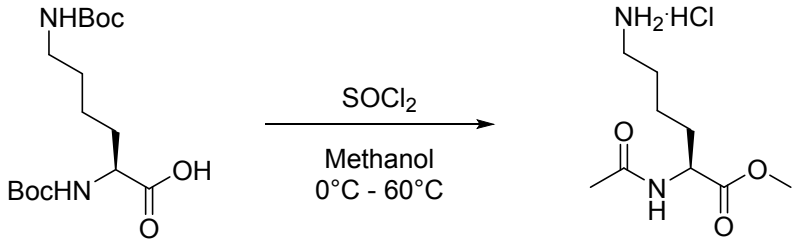
Methyl N^6*-acetyl-*N^2*-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-L-lysinate (**48**)*. A mixture of copper(II)sulfate (247 mg, 1.55 mmol) and sodium-(+)-ascorbate (615 mg, 3.11 mmol) in water (10 mL) was transferred to a flask containing N,N-bis(pyridin-2-ylmethyl)prop-2-yn-1-amine (**22**, 368 mg, 1.55 mmol) dissolved in *tert*-butanol (10 mL). Under rapid stirring, methyl N^6-acetyl-N^2-(2-azidoacetyl)-*L*-lysinate (**46**, 369 mg, 1.29 mmol) was added and the mixture kept under rapid stirring for 4.5 h before dilution with ethyl acetate and transfer to a separatory funnel containing a mixture of 0.5 g EDTA, sat. NaCl (aq.), sat. K_2CO_3 (aq.) to pH 10 and water. The product was extracted using 4 x 50 mL ethyl acetate, washed with 0.5 M $NaHCO_3$ (aq.) containing a pinch of EDTA and sat. NaCl (aq.). After drying over Na_2SO_4, filtration and removal of solvent under reduced pressure, a crude product was obtained which was subsequently loaded onto an alumina column, and eluted with a gradient of methanol (0.5%-1.5%) in dichloromethane. Only moderately pure product was achieved at this stage (180.3 mg, 27%).



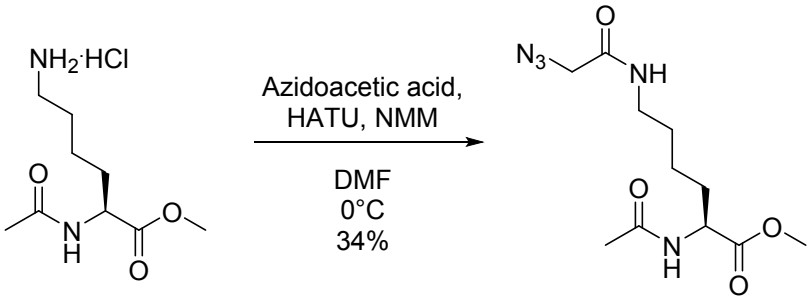
*(S)-((6-Acetamido-2-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetamido)hexanamido)methyl)boronic acid (**44**)*. Methyl N^6-acetyl-N^2-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-*L*-lysinate (**48**, 0.35 mmol) was dissolved in a 1:1 mixture of tetrahydrofuran and water (10 mL). Lithium hydroxide monohydrate was added (60 mg,

1.4 mmol) and the reaction mixture was stirred overnight. After neutralization with 0.5 M HCl, solvents were removed under reduced pressure, and the crude hydrolyzed product carried along and mixed with *bis*-trimethylsilyl-aminomethaneboronate pinacol ester (125mg, 0.415 mmol) and HATU (162 mg, 0.425 mmol) in 2 mL DMF. The mixture was cooled in an ice bath, *N*-methylmorpholine (0.12 mL, 1.1 mmol) added, followed by water (0.05 mL, 2.8 mmol). The mixture was allowed to stir further 30 min on ice bath, before being left at room temperature overnight. For work-up, the solvent was removed under reduced pressure and the crude product loaded onto a plug of Bondesil-C18 OH SPE material. The product was isolated by portion-wise elution using methanol/water-mixtures going stepwise from 10% to 60% methanol. Fractions containing product was pooled and the solvent removed under reduced pressure to afford the product as a "glass-like" pale yellow film (103.9 mg, 53%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45-8.49 (m, 3H), 8.03 (s, 1H), 7.75-7.79 (m, 5H), 7.57 (d, J = 7.8 Hz, 2H), 7.53 (br t, 1H), 7.25 (m, 2H), 5.15 (s, 2H), 4.20 (m, 1H), 3.75 (s, 2H), 3.73 (s, 4H), 2.98 (m, 2H), 2.55 (m, 2H), 1.77 (s, 3H), 1.64 (m, 1H), 1.51 (m, 1H), 1.36 (m, 2H), 1.24 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.0, 169.0, 165.3, 159.0, 148.8, 143.1, 136.6, 125.3, 122.6, 122.1, 58.7, 52.7, 51.5, 48.0, 38.4, 32.0, 28.9, 26.5, 22.7, 22.6. Direct observation of the carbon α to boron (δ_C 26.5) was not possible due to the quadrupolar relaxation exerted on this carbon by the boron atom. Likewise, the resonance was missing in HMBC experiments, probably due to the long delays necessary in the pulse sequence. However, the resonance was observable in HSQC experiments, and reported as such. MS (APCI, negative mode) m/z 520 $[M\text{-}H^{+}\text{-}HBO_2]^{-}$.

Preparation of (*S*)-((2-Acetamido-6-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetamido)hexanamido)methyl)boronic acid (45).

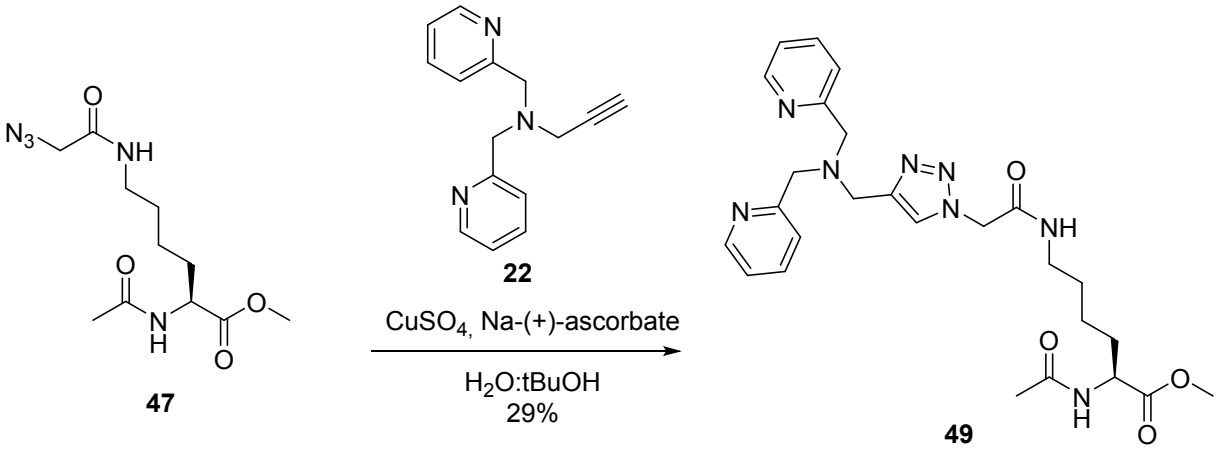


Ac-D-Lys(H)-OMe hydrochloric salt. Ac-D-Lys(Boc)-OH (1.02 g, 3. 53 mmol) was dissolved in methanol and thionyl chloride (0.32 mL, 4.4 mmol) added dropwise while the reaction mixture was cooled in an ice bath. After 30 min additional cooling, the mixture was heated to 60 °C for 2h. Evaporation of volatiles afforded the product as an oil in quantitative yield. ^{1}H NMR data (400 MHz, DMSO-d_6) was in agreement with published values, except for a probably misprinted value of 2.27 ppm for H-6, for which 2.72 ppm was observed.[10]

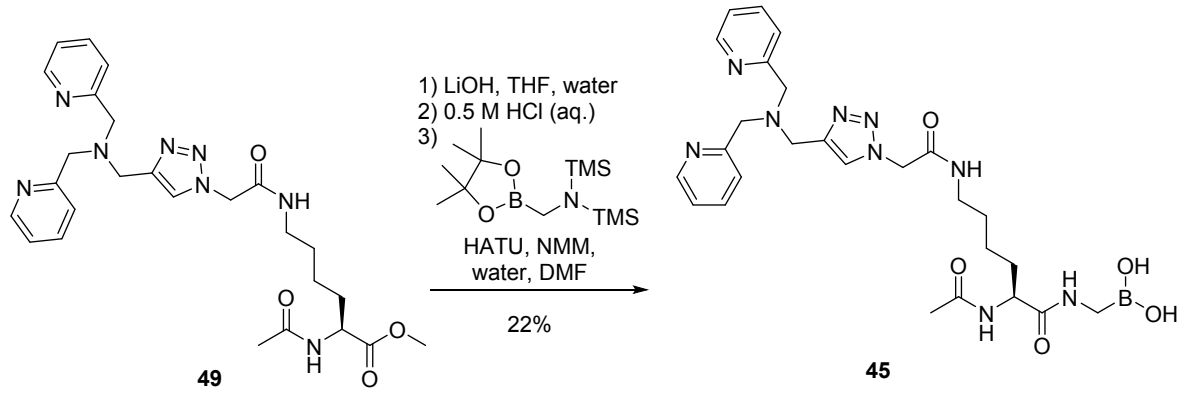


47

*Methyl N^2-acetyl-N^6-(2-azidoacetyl)-L-lysinate (**47**)*. The title compound was prepared from Ac-D-Lys(H)-OMe hydrochloric salt (3.53 mmol) and 2-azidoacetic acid following the exact same procedure as for the synthesis of methyl N^6-acetyl-N^2-(2-azidoacetyl)-*L*-lysinate (**46**). The product was isolated as a colourless semisolid (338.3 mg, 34%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (br d, J = 7.4 Hz, 1H), 8.06 (br t, 1H), 4.18 (m, 1H), 3.78 (s, 2H), 3.61 (s, 3H), 3.06 (q, J = 6.8 Hz, 2H), 1.84 (s, 3H), 1.52-1.69 (m, 2H), 1.40 (m, 2H), 1.28 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 169.5, 167.1, 54.9, 51.9, 51.7, 50.8, 38.4, 30.6, 28.5, 22.7, 22.2. MS (ESI, pos. mode) m/z 308.1 $[M+Na]^+$, HR-MS (ESI, pos. mode) m/z 308.1329 calculated for $C_{11}H_{19}N_5O_4Na$, found m/z 308.1327.



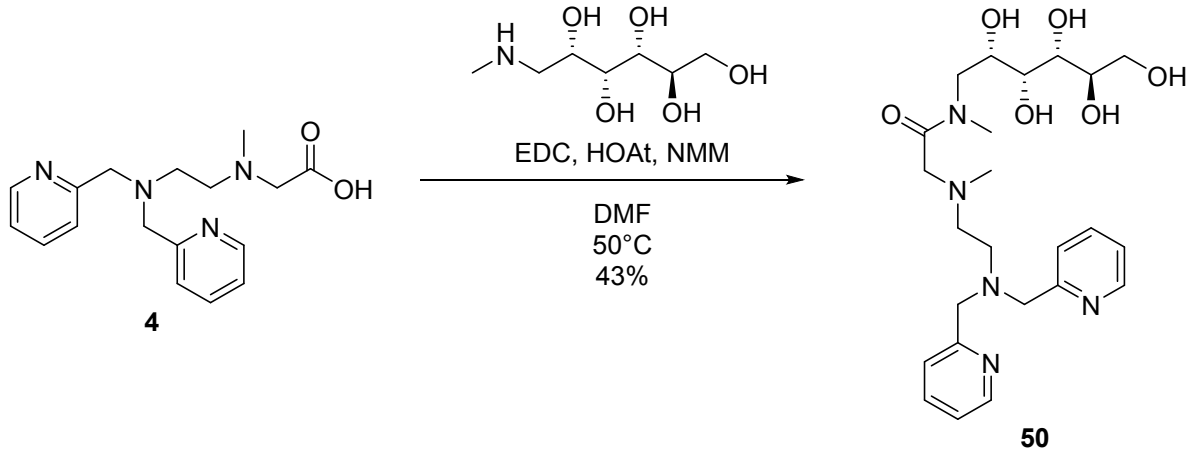
*Methyl N^2-acetyl-N^6-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-L-lysinate (**49**)*. A mixture of copper(II)sulfate (240 mg, 1.50 mmol) and sodium-(+)-ascorbate (597 mg, 3.02 mmol) in water (10 mL) was transferred to a flask containing *N*,*N*-bis(pyridin-2-ylmethyl)prop-2-yn-1-amine (**22**, 357 mg, 1.50 mmol) dissolved in *tert*-butanol (10 mL). Under rapid stirring, methyl N^2-acetyl-N^6-(2-azidoacetyl)-*L*-lysinate (**47**, 338 mg, 1.24 mmol) was added and the mixture kept under rapid stirring for 3 h before dilution with ethyl acetate, water and sat. NaCl (aq.). After dilution, extraction was attempted, resulting in a poor recovery. The product could only be extracted with ethyl acetate after addition of K_2CO_3 and EDTA to the aqueous phase. The extract was dried over K_2CO_3 (s). Filtration and subsequent removal of solvent under reduced pressure gave a crude product, which was purified on an alumina column, using a gradient of methanol (2-3%) in dichloromethane as eluent. Product could not be isolated more than moderately pure at this stage (189.7 mg, 29%).



*(S)-((2-Acetamido-6-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetamido)hexanamido)methyl)boronic acid (**45**)*. Hydrolysis and subsequent coupling to *bis*-trimethylsilyl-aminomethaneboronate pinacol ester was performed from methyl N^2-acetyl-N^6-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-*L*-lysinate (**49**) in a similar manner as for the regiosiomer methyl N^6-acetyl-N^2-(2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-*L*-lysinate (**48**), starting from 0.36 mmol ester. The crude product was loaded

onto a plug of Bondesil-C18 OH SPE material and isolated by portion-wise elution using methanol/water-mixtures going stepwise from 20% to 50% methanol. Fractions containing product was collected and solvent removed under reduced pressure to afford the product as a "glass-like" pale yellow film (44.9 mg, 22%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (m, 2H), 8.29 (br m, 1H), 8.04 (s, 1H), 7.97 (br d, J = 7.8 Hz, 1H), 7.77 (dt, J = 1.7 Hz, 7.7 Hz, 2H), 7.75 (br s, 2H), 7.57 (d, J = 7.8 Hz, 2H), 7.38 (br m, 1H), 7.25 (m, 2H), 5.06 (s, 2H), 4.12 (m, 1H), 3.75 (s, 2H), 3.73 (s, 4H), 3.06 (m, 2H), 2.53 (m, signal partly obscured under solvent peak), 1.83 (s, 3H), 1.60 (m, 1H), 1.35-1.50 (m, 3H), 1.25 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.7, 169.5, 165.3, 159.1, 148.8, 143.1, 136.6, 125.4, 122.6, 122.2, 58.7, 52.7, 51.6, 48.0, 38.7, 31.6, 28.7, 26.3, 22.9, 22.6. See note under compound **44** regarding the carbon α to the boron atom (δ_C 26.3). MS (APCI, negative mode) m/z 520 $[M\text{-}H^{+}\text{-}HBO_2]^{-}$.

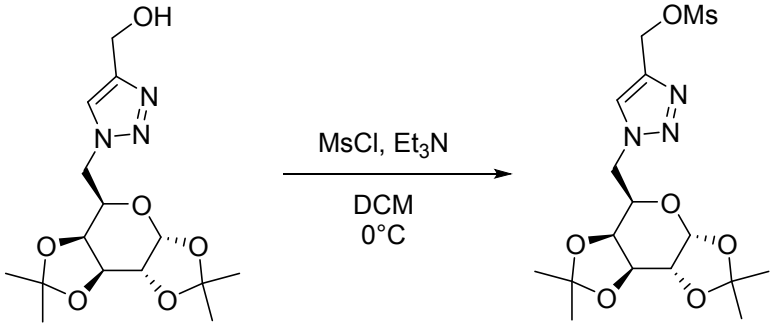
Preparation of 2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(methyl)amino)-*N*-methyl-*N*-((2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl)acetamide (50).



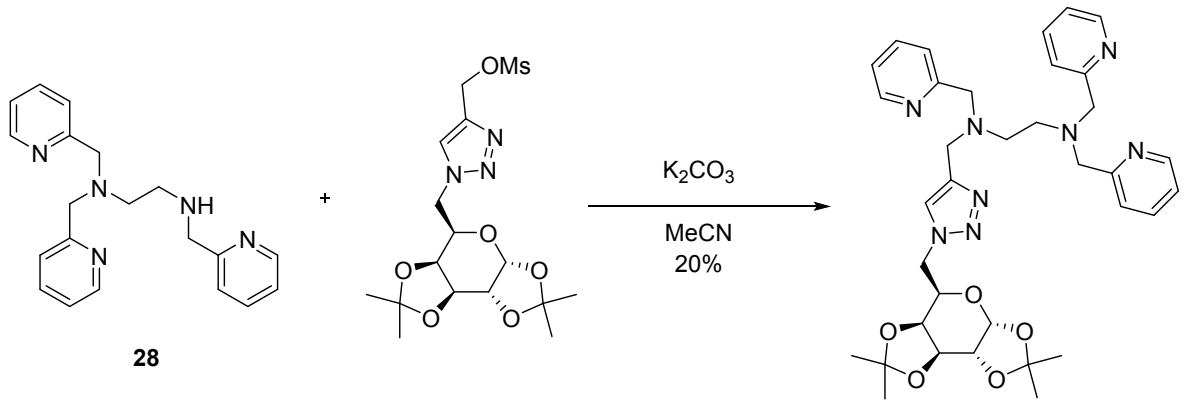
4 **50**

To a flask containing *N*-methyl-*D*-glucamine (219.4 mg, 1.12 mmol), EDC hydrochloride (289.6 mg, 1.51 mmol) and 1-hydroxy-7-azabenzotriazole (203.0 mg, 1.49 mmol), a solution of 2-((2-(bis(pyridin-2-ylmethyl)amino)ethyl)(methyl)amino) acetic acid (**4**, 0.748 mmol) in 5 mL DMF was added, followed by *N*-methylmorpholine (0.20 mL, 1.8 mmol). The mixture was then heated to 50 °C overnight. After removal of solvent under reduced pressure, the crude product was initially purified by loading onto a plug of Bondesil C18-OH SPE material, and eluted portion-wise with pure water followed with increasing amounts of methanol up to 50%. Evaporation of solvent from fractions containing product gave an impure material, which was further purified in aliquots on a plug of strong cationic exchange SPE material, using 1:1 methanol/water and a pH-gradient from pH 3 to pH 10 with formic acid and ammonia as additives. The total amount of product isolated, as a pale yellow oil, was 158.8 mg (43.2%). The NMR spectra had a very complex appearance in the aliphatic region, probably caused by *s-cis*/*trans* isomerism over the amide bond. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (m, 2H), 7.76 (dt, J = 1.8 Hz, 7.7 Hz, 2H), 7.51 (d, J = 7.8 Hz, 2H), 7.24 (m, 2H), 3.75 (m, 4H), 3.60-3.20 (m, 13H), 3.19-3.16 (m, 2H), 3.11 (m, 1H), 2.99 (s, 1.3H), 2.76 (s, 1.7H), 2.57 (m, 3H), 2.10 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 159.4, 159.3, 148.7, 136.5, 122.7, 122.1, 72.4, 71.9, 71.5, 71.4, 71.2, 70.4, 69.8, 69.4, 63.33, 63.27, 60.1, 59.83, 59.79, 59.7, 54.7, 51.8, 51.2, 51.1, 50.9, 48.6, 42.2, 36.3, 33.3. MS (APCI, positive mode) *m*/*z* 492.3 $[M+H]^+$, HR-MS (APCI, pos. mode) *m*/*z* 492.2817 calculated for $C_{24}H_{38}N_5O_6$, found *m*/*z* 492.2814.

Preparation of (3*R*,4*S*,5*R*,6*R*)-6-((4-(((2-(Bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)tetrahydro-2*H*-pyran-2,3,4,5-tetraol (51, mixture of 2*R*/*S* anomers).



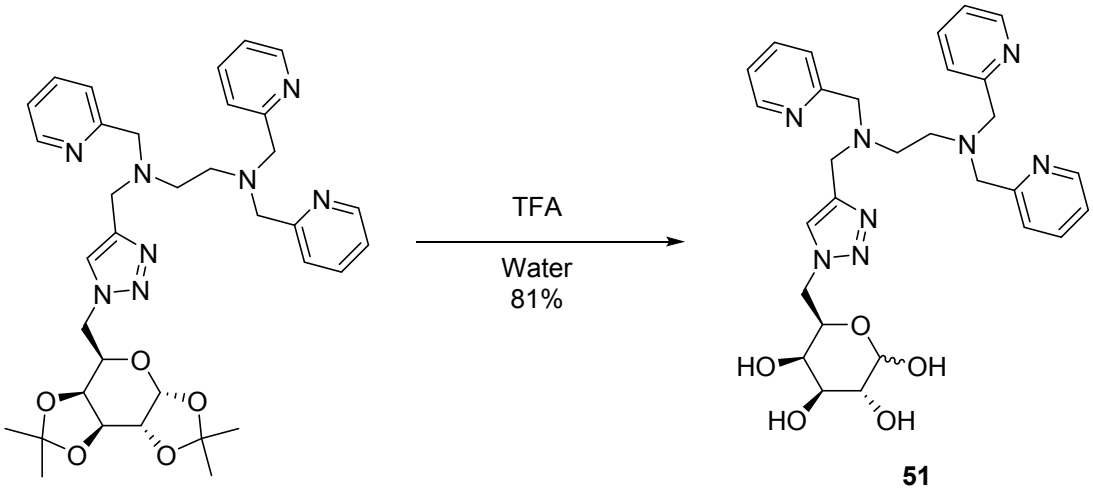
(1-(((3aR,5R,5aS,8aS,8bR)-2,2,7,7-Tetramethyltetrahydro-5H-bis([1,3]dioxolo)[4,5-b:4',5'-d]pyran-5-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl methanesulfonate. 6-Deoxy-6-[4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]-1,2:3,4-bis-O-(1-methylethylidene)-α-*D*-galactopyranose (246.3 mg, 0.722 mmol), available from the Huisgen azide-alkyne cycloaddition of 6-azido-6-deoxy-1,2:3,4-di-O-isopropylidene-α-*D*-galactose with propargyl alcohol,[11] was converted to its corresponding mesylate by dropwise addition of methanesulfonylchloride (0.07 mL, 0.9 mmol) to an ice-cooled solution of the sugar in 5 mL dichloromethane and 0.15 mL (1.1 mmol) triethylamine. After addition, the reaction mixture was allowed to stir at room temperature for 1h, and subsequently added to a plug of silica. The product was eluted with ethyl acetate and solvent was removed under reduced pressure to give the product in quantitative yield. ^{1}H NMR (400 MHz, dichloromethane-d_2) δ 7.89 (s, 1H), 5.48 (d, J = 5.0, 1H), 5.35 (s, 2H), 4.68-4.60 (m, 2H), 4.48 (dd, J = 9.0, 14.3, 1H), 4.34 (dd, J = 2.6, 5.0, 1H), 4.24 (dd, J = 1.9, 7.9, 1H), 4.17 (ddd, J = 1.9, 3.6, 9.0, 1H), 2.94 (s, 3H), 1.48 (s, 3H), 1.37 (s, 3H), 1.36 (s, 3H), 1.28 (s, 3H). ^{13}C NMR (100 MHz, dichloromethane-d_2) δ 131.5, 126.3, 110.5, 109.5, 96.8, 71.7, 71.4, 70.9, 67.7, 63.6, 51.3, 39.3, 26.3, 26.2, 25.2, 24.7.



28

N^1,N^1,N^2-tris(pyridin-2-ylmethyl)-N^2-((1-(((3aR,5R,5aS,8aS,8bR)-2,2,7,7-tetramethyltetrahydro-5H-bis([1,3]dioxolo)[4,5-b:4',5'-d]pyran-5-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)ethane-1,2-diamine. To an ice-cold solution of N^1,N^1,N^2-tris(pyridin-2-ylmethyl)ethane-1,2-diamine (**28**, 241.2 mg, 0.723 mmol) in 10 mL acetonitrile under nitrogen, potassium carbonate (208 mg, 1.50 mmol) was added. A

solution of the mesylate from the previous step (0.722 mmol) in 5 mL acetonitrile was added dropwise. After the addition, the ice bath was removed and the reaction allowed to proceed overnight. The reaction mixture was then filtered through a plug of celite, and after removal of solvent under reduced pressure, purified by way of flash chromatography on an alumina column, using a gradient of 1.0% - 1.5% methanol in dichloromethane as eluent. After removal of solvent from fractions containing the desired product, further purification was achieved by loading onto a plug of Bondesil C18-OH SPE material. Elution in steps from 30% to 70% methanol in water yielded several fractions pure in product, which after evaporation gave 93.8 mg product (19.8%).^{1}H NMR (400 MHz, methanol-d_4) δ 8.42-8.37 (m, 3H), 7.90 (s, 1H), 7.77-7.69 (m, 3H), 7.55 (d, J = 7.8 Hz, 2H), 7.51 (d, J = 7.9 Hz, 1H), 7.28-7.23 (m, 3H), 5.41 (d, J = 4.9 Hz, 1H), 4.67 (dd, J = 2.5 Hz, 7.9 Hz, 1H), 4.61 (dd, J = 3.2 Hz, 14.3 Hz, 1H), 4.46 (dd, J = 9.3 Hz, 14.2Hz, 1H), 4.35 (dd, J = 2.5 Hz, 5.0 Hz, 1H), 4.30 (dd, J = 1.9 Hz, 7.9 Hz, 1H), 4.17 (ddd, J = 1.9 Hz, 3.1 Hz, 9.3 Hz, 1H), 3.75 (s, 6H), 3.68 (s, 2H), 2.72-2.66 (m, 4H), 1.47 (s, 3H), 1.36 (s, 3H), 1.29 (s, 3H), 1.21 (s, 3H). ^{13}C NMR (100 MHz, methanol-d_4) δ 160.68, 160.67, 149.39, 149.37, 145.3, 138.64, 138.60, 126.0, 124.9, 124.8, 123.73, 123.69, 110.9, 110.0, 97.7, 72.6, 72.2, 71.8, 68.8, 61.5, 60.8, 53.4, 52.7, 51.8, 50.2, 26.3,26.2, 25.1, 24.6. MS (APCI, positive mode) m/z 657.4 $[M+H]^+$, HR-MS (APCI, pos. mode) m/z 657.3507 calculated for $C_{35}H_{45}N_8O_5$, found m/z 657.3505.



51

*(3R,4S,5R,6R)-6-((4-(((2-(Bis(pyridin-2-ylmethyl)amino)ethyl)(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrahydro-2H-pyran-2,3,4,5-tetraol (**51**, mixture of 2R/2S anomers)*. From the doubly isopropylidene protected sugar (92.8 mg, 0.141 mmol), the deprotected product was obtained by stirring in a mixture of 15 mL trifluoroacetic acid and 10 mL water for 4 h. Volatiles were removed after repeated additions of 10 mL toluene followed by evaporation on a rotary evaporator. In order to remove traces of unreacted starting material, the crude product was loaded onto a small plug of C18 SPE material and eluted with an 80:20 water/methanol mixture. After another removal of solvent, further purification was achieved by loading onto a plug of strong cationic exchange SPE material, using 1:1 methanol/water and a pH-gradient from pH 3 to pH 10 with formic acid and ammonia as additives. Fractions containing

pure product by TLC was collected and the solvent removed under reduced pressure to afford the title compound as a yellow film, which could be scraped from the flask surface as a powder (65.7 mg, 80.8%). The NMR spectra have a very complex appearance overall due to presence of α and β anomers, and also additional unreported minor peaks, likely from furanose forms. For δ_H between 5 and 3 ppm, only shifts for the main peaks, as observed in the multiplicity edited HSQC spectrum, are reported. Negative peaks are indicated as "methylene". ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51-8.42 (m, 3H), 7.92 (s, 0.5H), 7.91 (s, 0.5H), 7.79-7.66 (m, 3H), 7.46 (d, J = 7.8 Hz, 2H), 7.41 (d, J = 7.9 Hz, 1H), 7.26-7.20 (m, 3H), 6.80-6.55 (m, 0.51H, OH), 6.45-6.17 (m, 0.68H, OH), 6.45-6.17 (m, 0.68H, OH), 4.90, 4.44 (methylene), 4.23, 4.19, 3.84, 3.72, 3.71 (methylene, several), 3.66 (methylene), 3.65, 3.64 (methylene), 3.57, 3.28, 3.27, 2.63 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.38, 159.37, 159.36, 159.35, 148.7, 148.6, 143.3, 143.2, 136.5, 136.4, 124.3, 124.2, 122.6, 122.5, 122.02, 121.95, 97.4, 92.7, 73.0, 72.8, 71.6, 69.4, 68.91, 68.85, 68.78, 68.4, 59.9, 59.5, 51.4, 50.8, 50.69, 50.67, 48.5, 26.3,26.2, 25.1, 24.6. MS (ESI, positive mode) m/z 577.3 $[M+H]^+$, HR-MS (ESI, pos. mode) m/z 577.2881 calculated for $C_{29}H_{37}N_8O_5$, found m/z 577.2879.

Table S1. Intrinsic activity of chelator alone towards different bacteria.

Compound	**MIC (μM)**	
	P. aeruginosa VIM-2	*K. pneumoniae NDM-1*
8	≥1000	≥1000
9	>1000	>1000
10	>1000	>1000
11	>1000	>1000
12	>1000	>1000
13	>1000	>1000
14	>1000	>1000
15	≥1000	≥1000
16	>1000	>1000
17	>1000	>1000
19	>1000	>1000
20	>1000	>1000
27	>1000	>1000
28	1000	≥1000
33	≥1000	≥1000
35	≥1000	≥1000
36	>1000	>1000
37	>1000	>1000
39	>1000	>1000
40	>1000	>1000
41	>1000	>1000
42	>1000	>1000
43	>1000	>1000

44	>1000	>1000
45	>1000	>1000
50	≥1000	≥1000
51	>1000	>1000

Table S2. MIC values of meropenem (MEM) alone and in combination with boronic acid-containing chelators against *E. coli* and *K. pneumoniae* harbouring class A (KPC-2) and class D (OXA-48) β-lactamases together. The boronic acid-containing chelators were tested at a fixed concentration of 125 μM.

Compound	**MIC MEM (mg/L)**	
	E. coli	*K. pneumoniae*
	OXA-48	KPC-2
MEM alone	*0.5 - 2*	*≥64*
44	*2*	*>64*
45	*2*	*>64*

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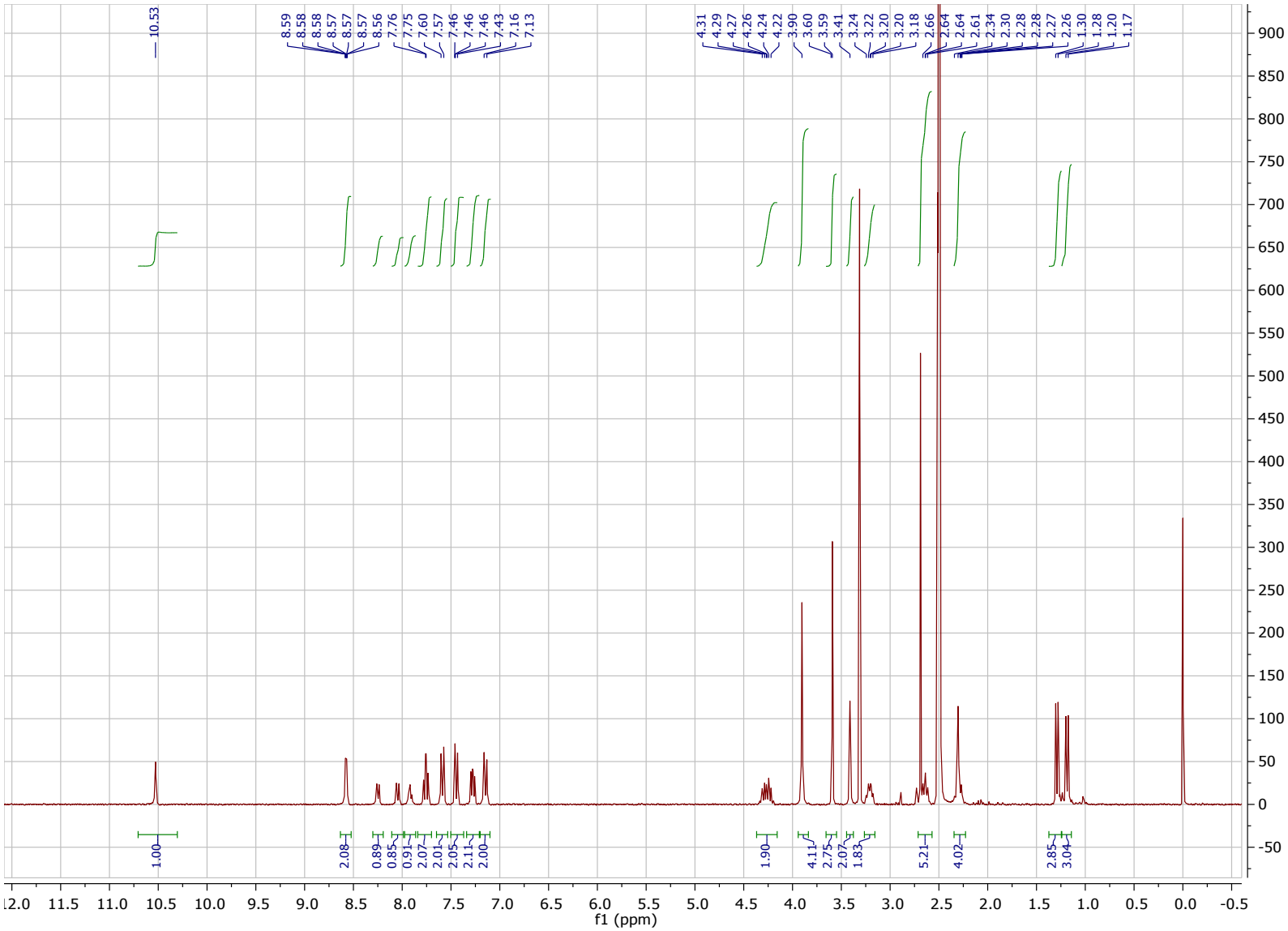
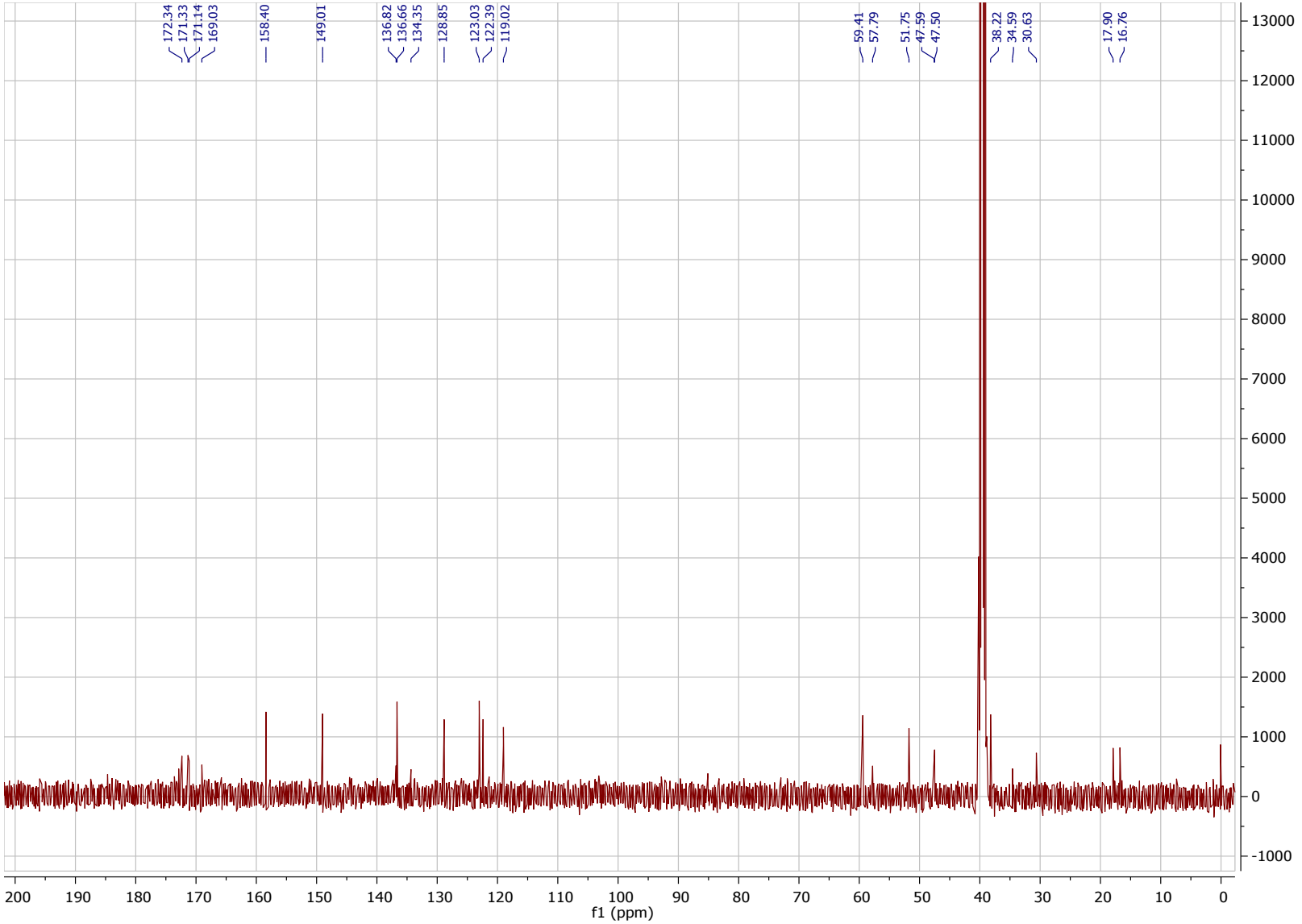
^{1}H NMR and ^{13}C NMR of compound **8**

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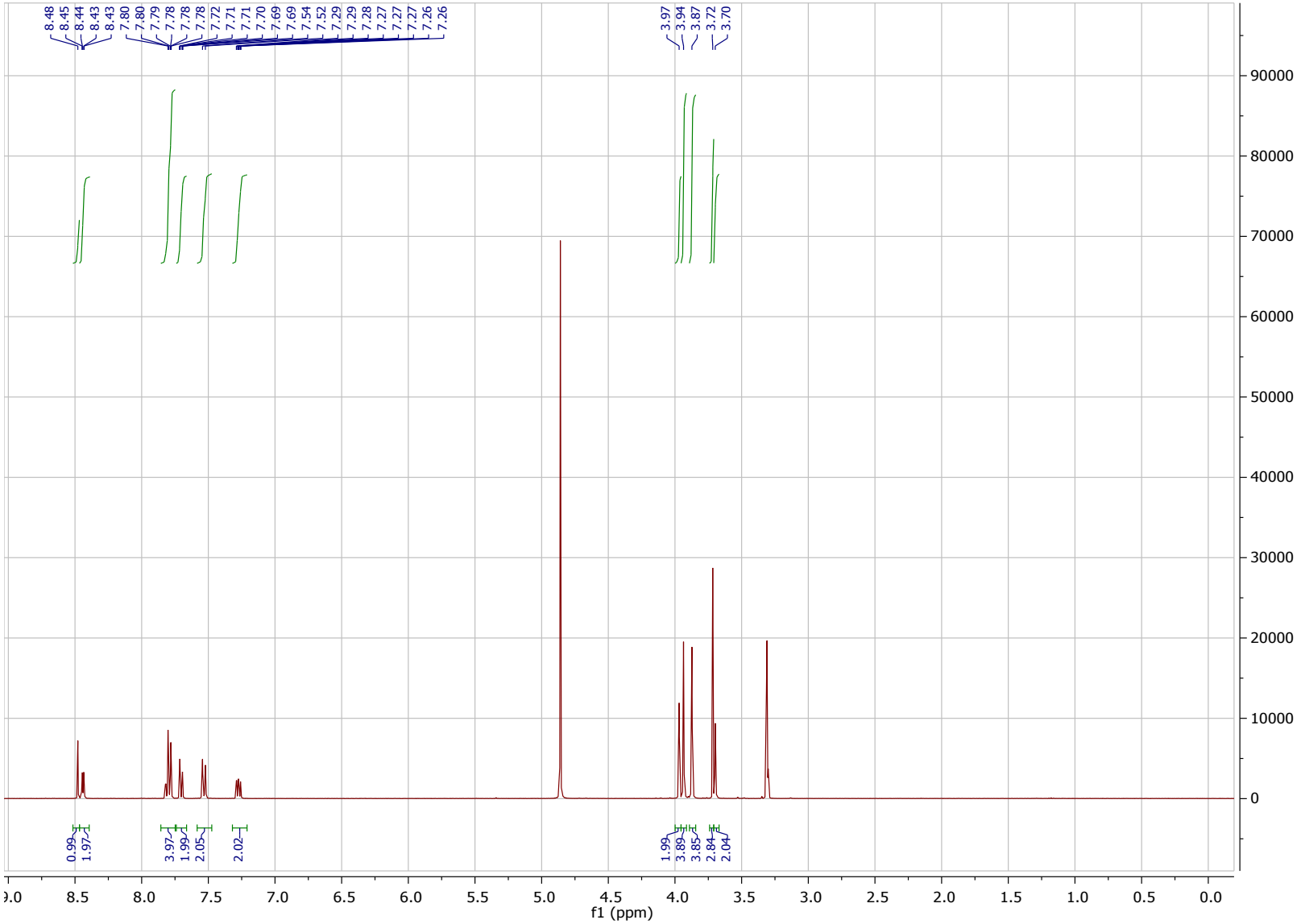
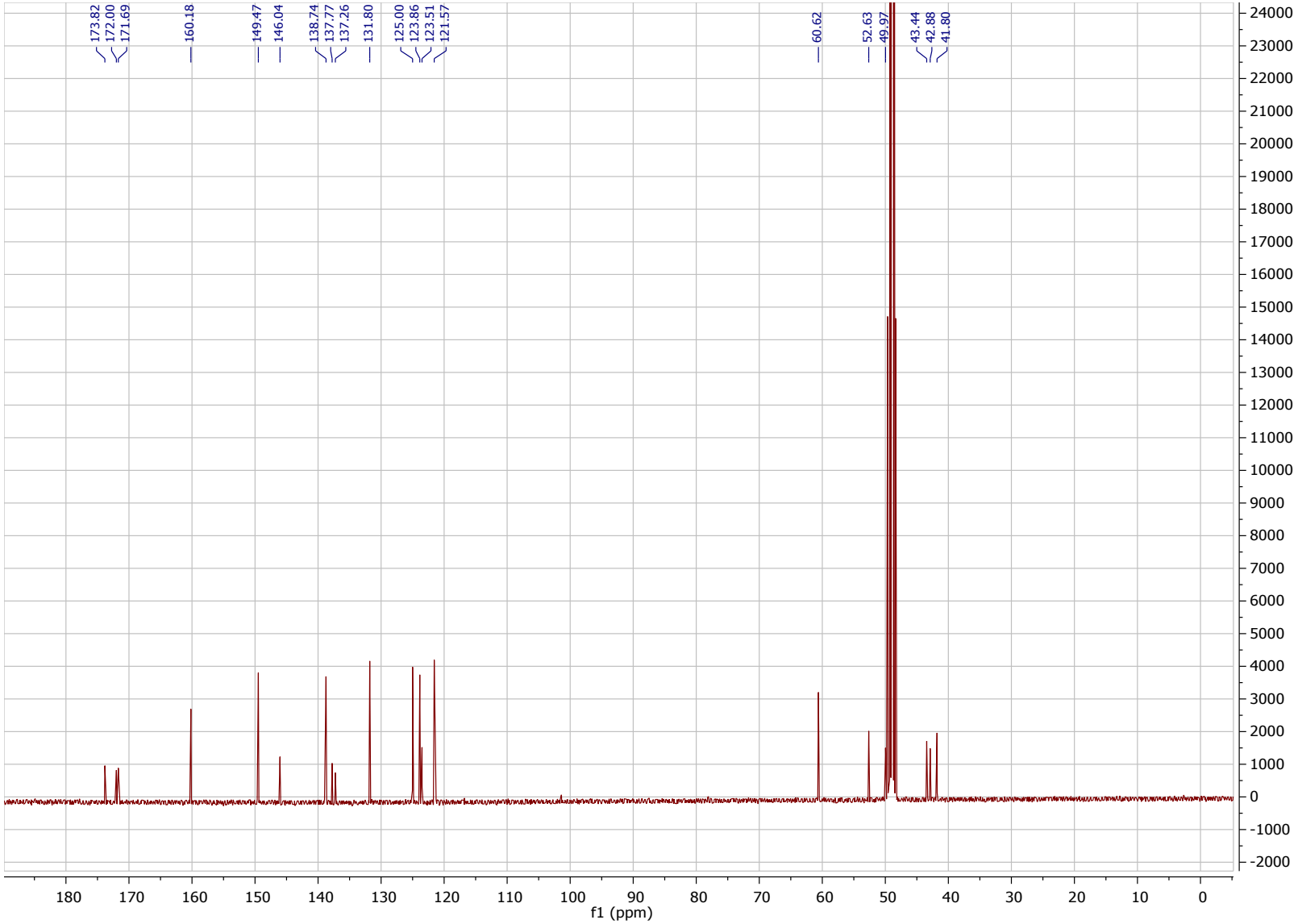
171.33
171.14
169.03
158.40
149.01
136.82
136.66
134.35
128.85
123.03
122.39
119.02
59.41
57.79
51.75
47.59
47.50
38.22
34.59
30.63
17.90
16.76
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **9**

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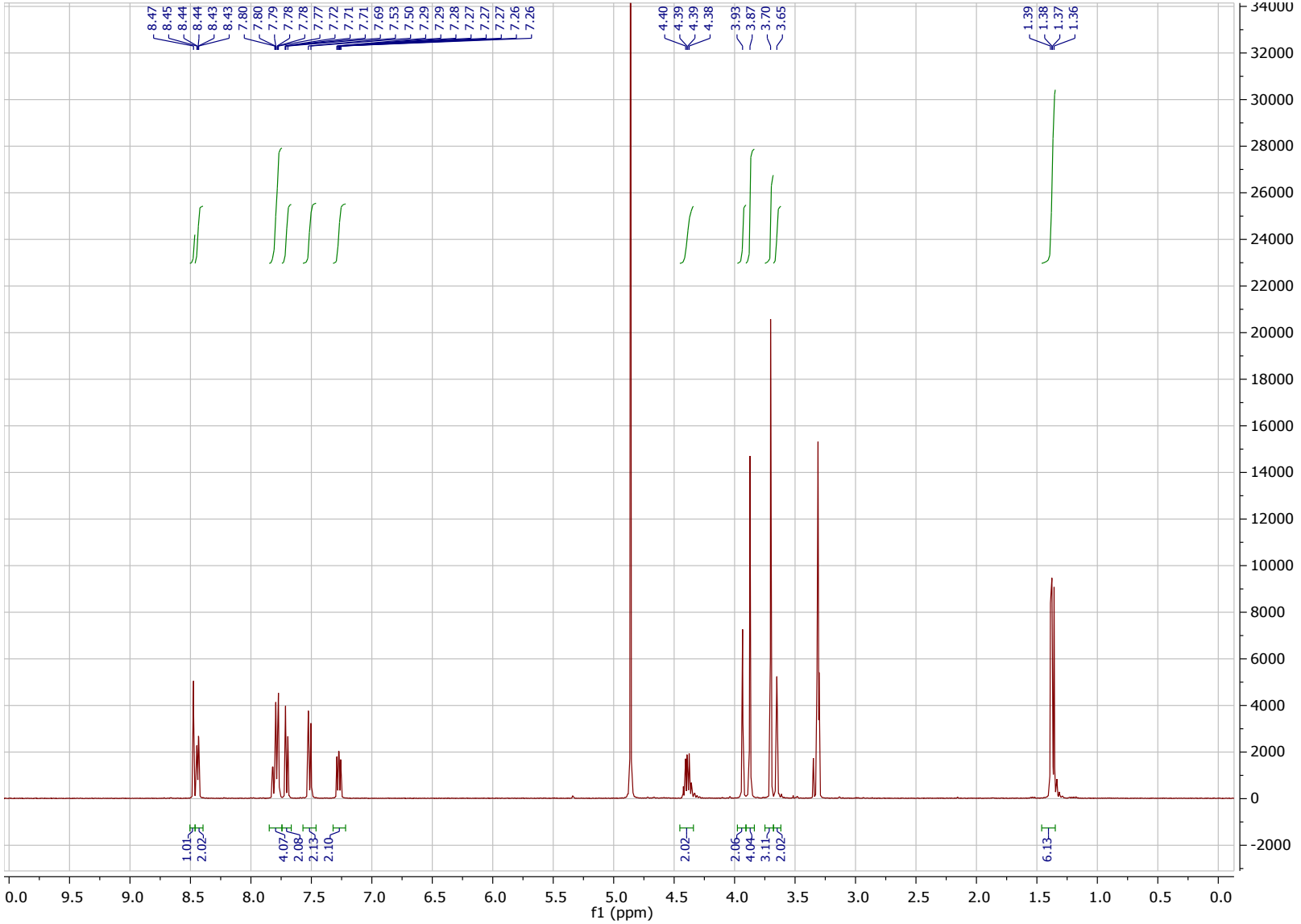
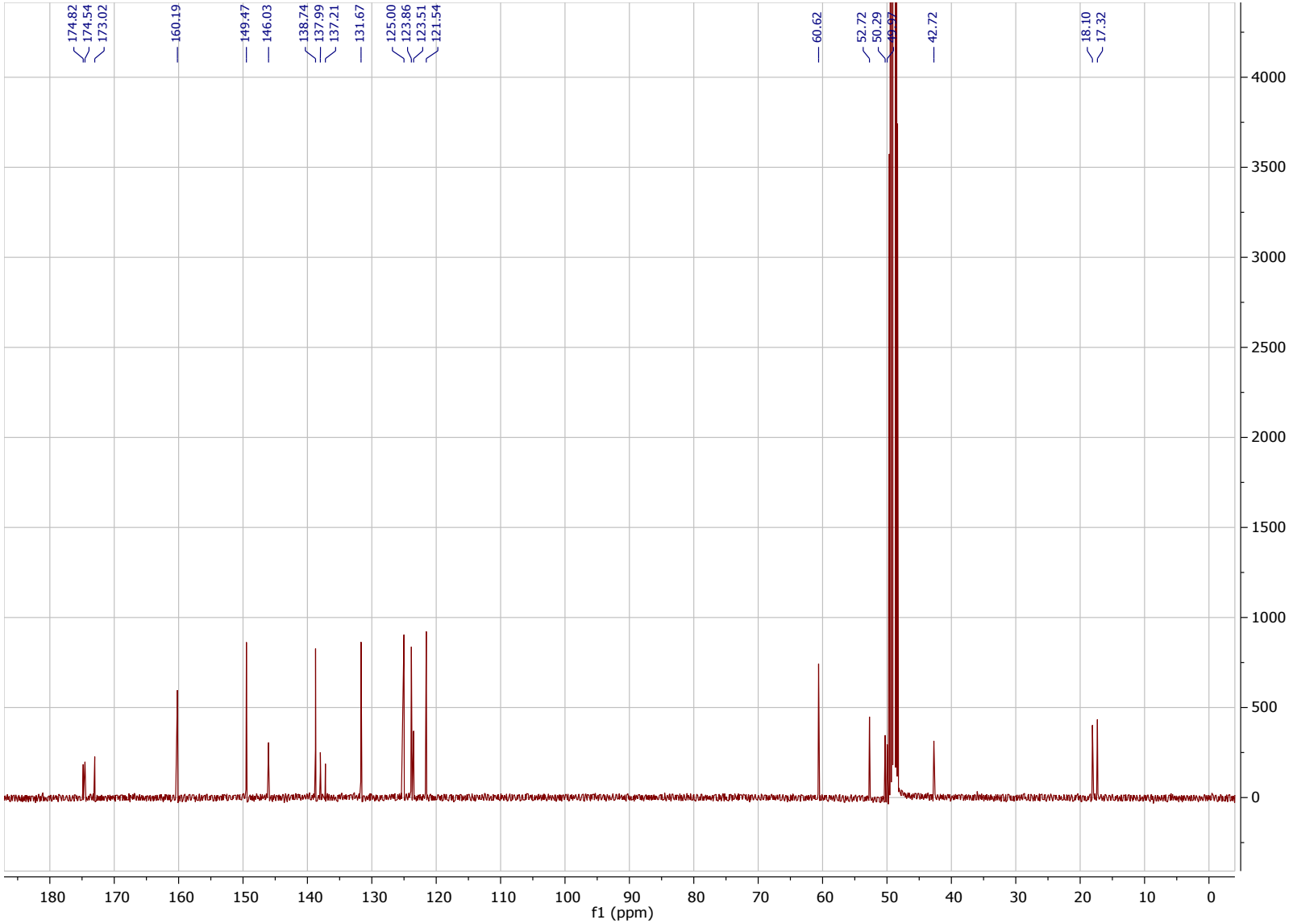
172.00
171.69
160.18
149.47
146.04
138.74
137.77
137.26
131.80
125.00
123.86
123.51
121.57
60.62
52.63
49.97
43.44
42.88
41.80
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **10**

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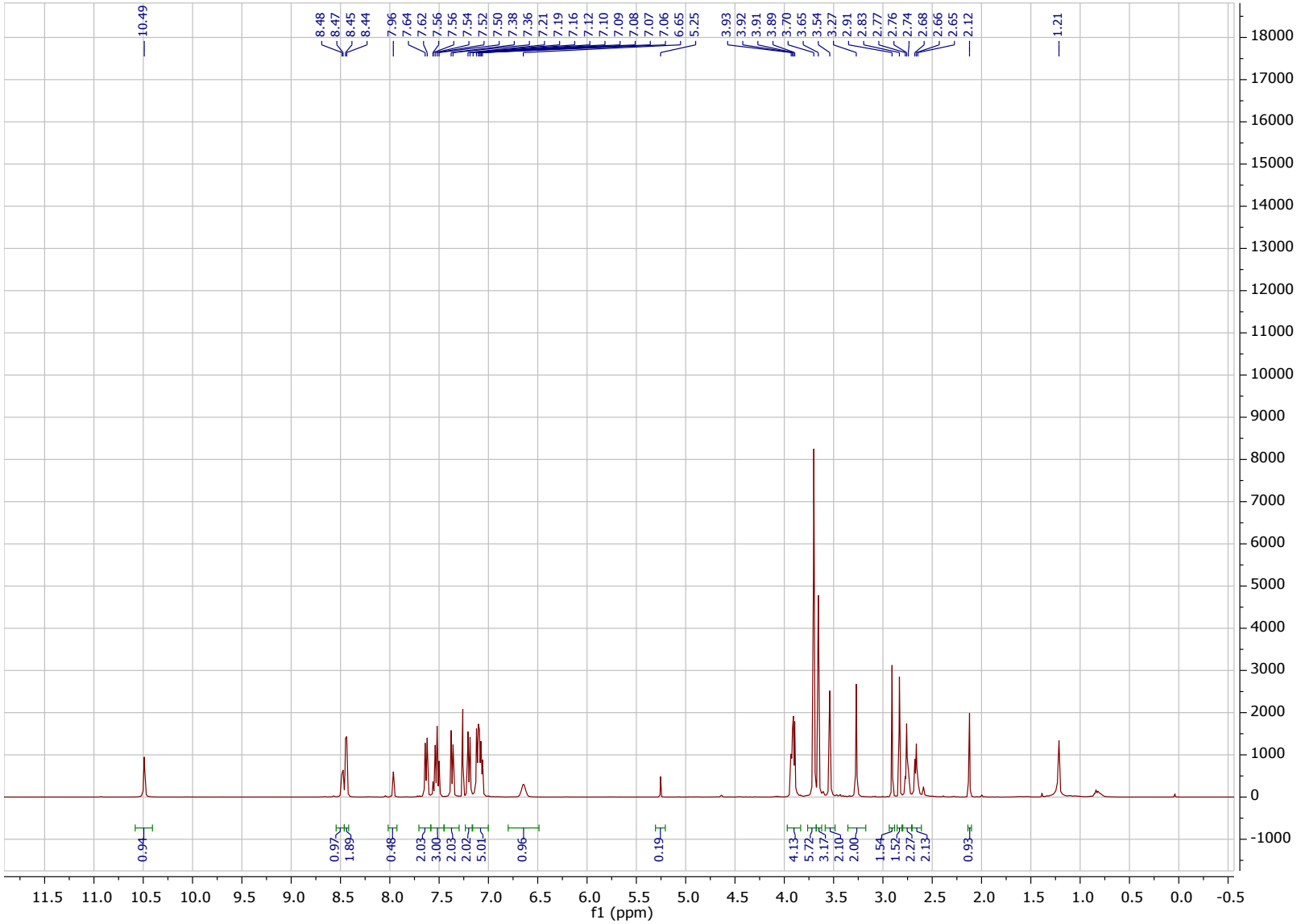
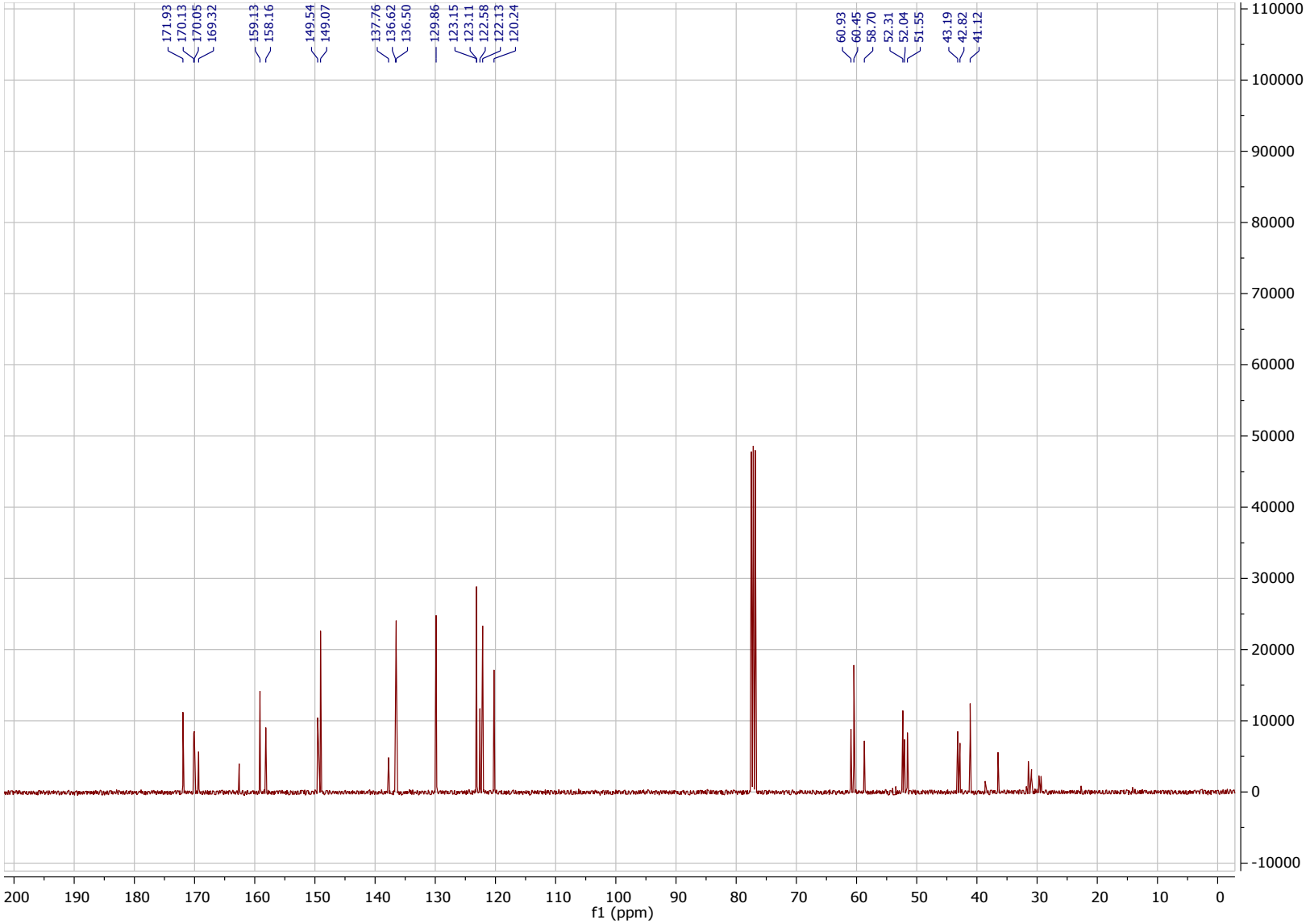
174.54
173.02
160.19
149.47
146.03
138.74
137.99
137.21
131.67
125.00
123.86
123.51
121.54
60.62
52.72
50.29
49.97
42.72
18.10
17.32
4000
3500
3000
2500
2000
1500
1000
500
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **11**

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149.07
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123.11
122.58
122.13
120.24
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60.45
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52.04
51.55
43.19
42.82
41.12
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
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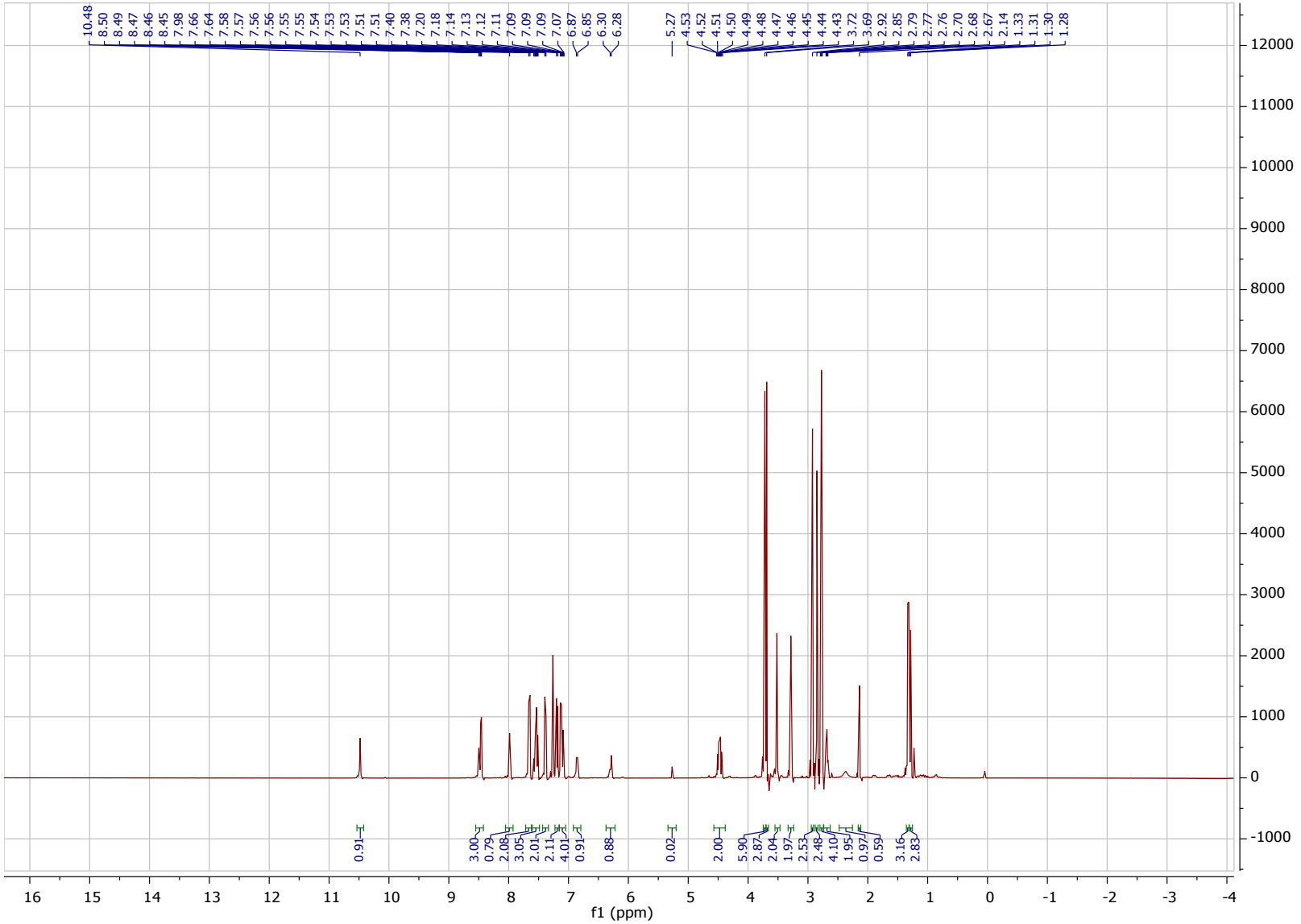
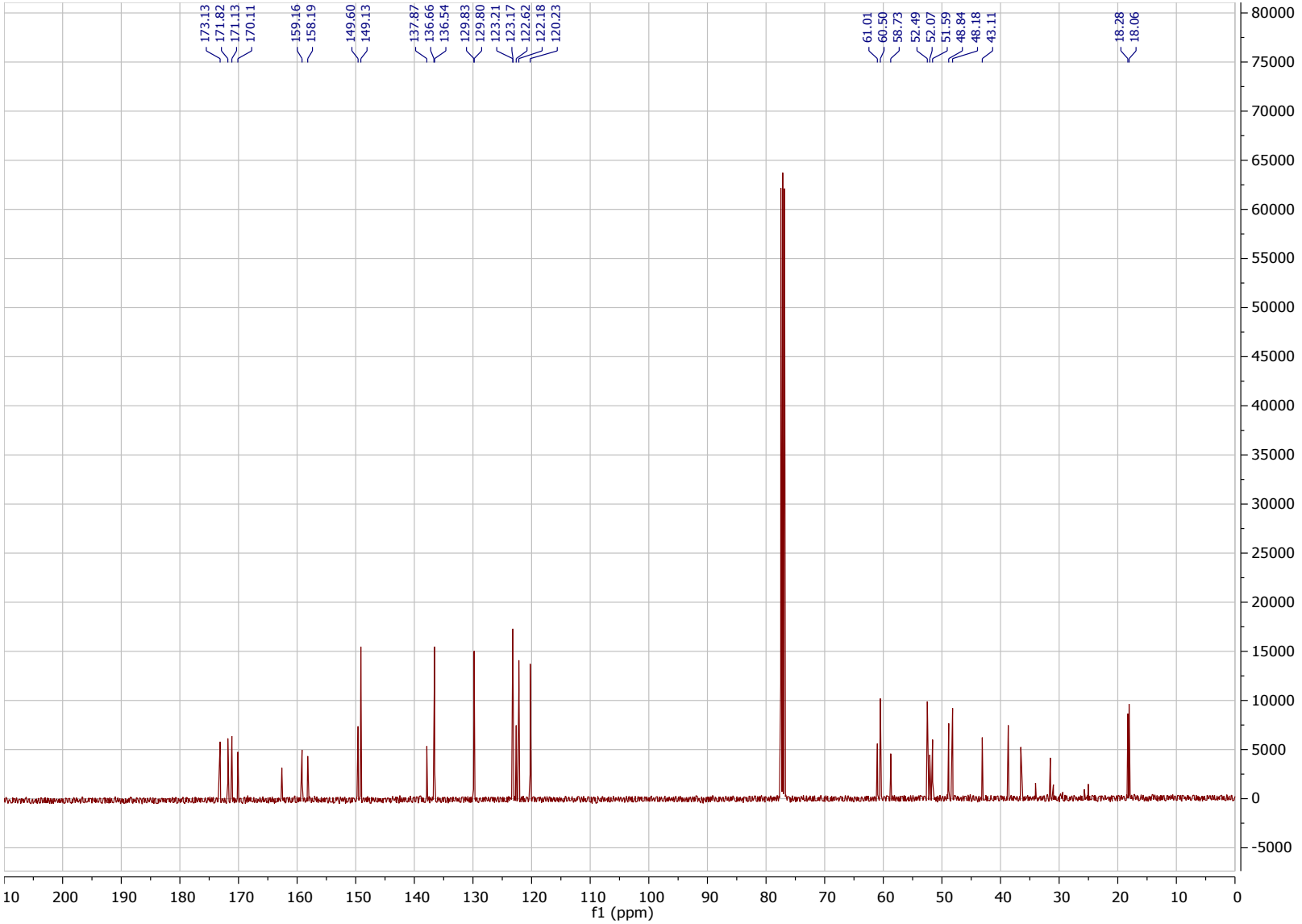
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **12**

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171.82
171.13
170.11
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158.19
149.60
149.13
137.87
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123.17
122.62
122.18
120.23
61.01
60.50
58.73
52.49
52.07
51.59
48.84
48.18
43.11
18.28
18.06
80000
75000
70000
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
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10
200
190
180
170
160
150
140
130
120
110
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90
80
70
60
50
40
30
20
10
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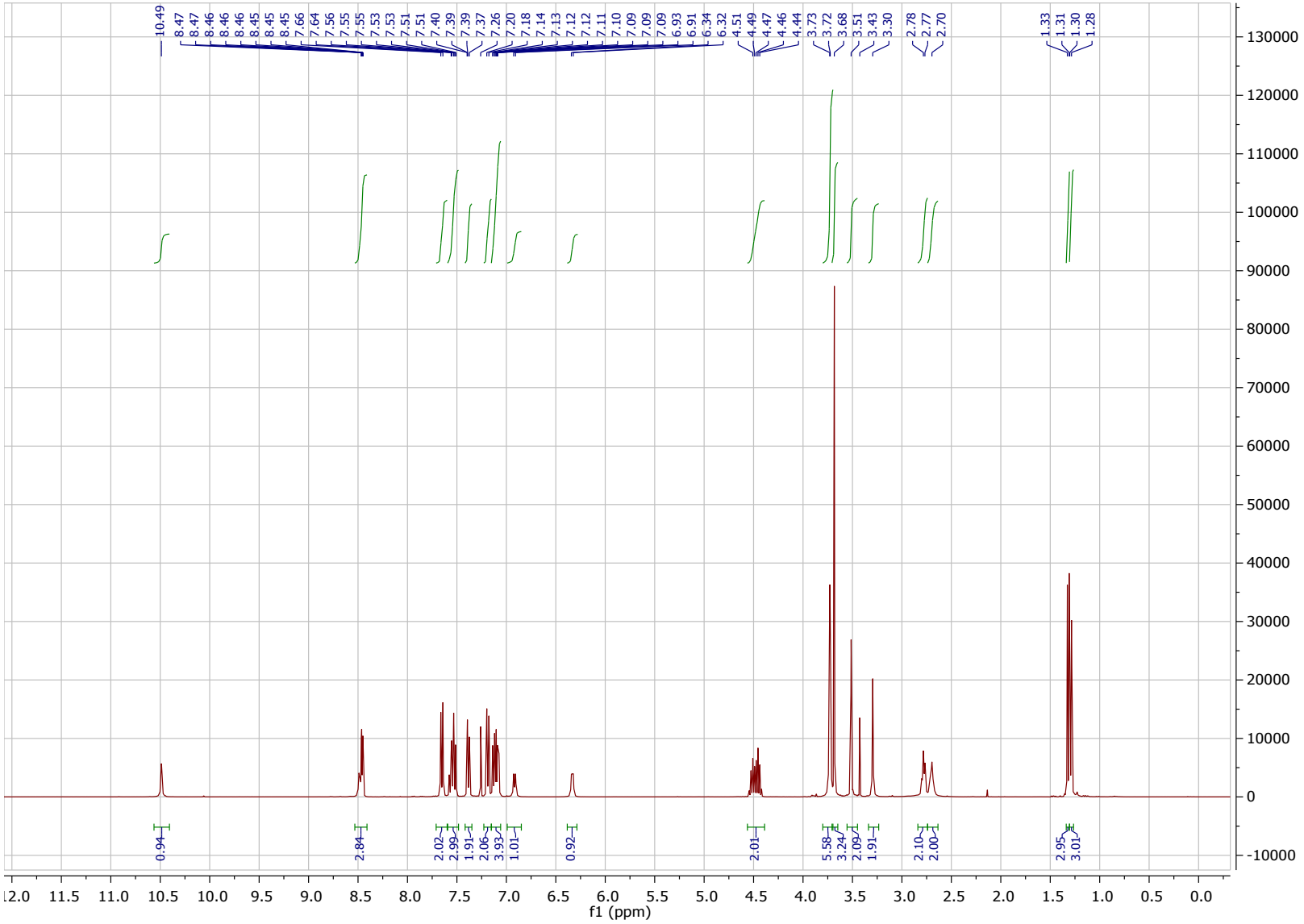
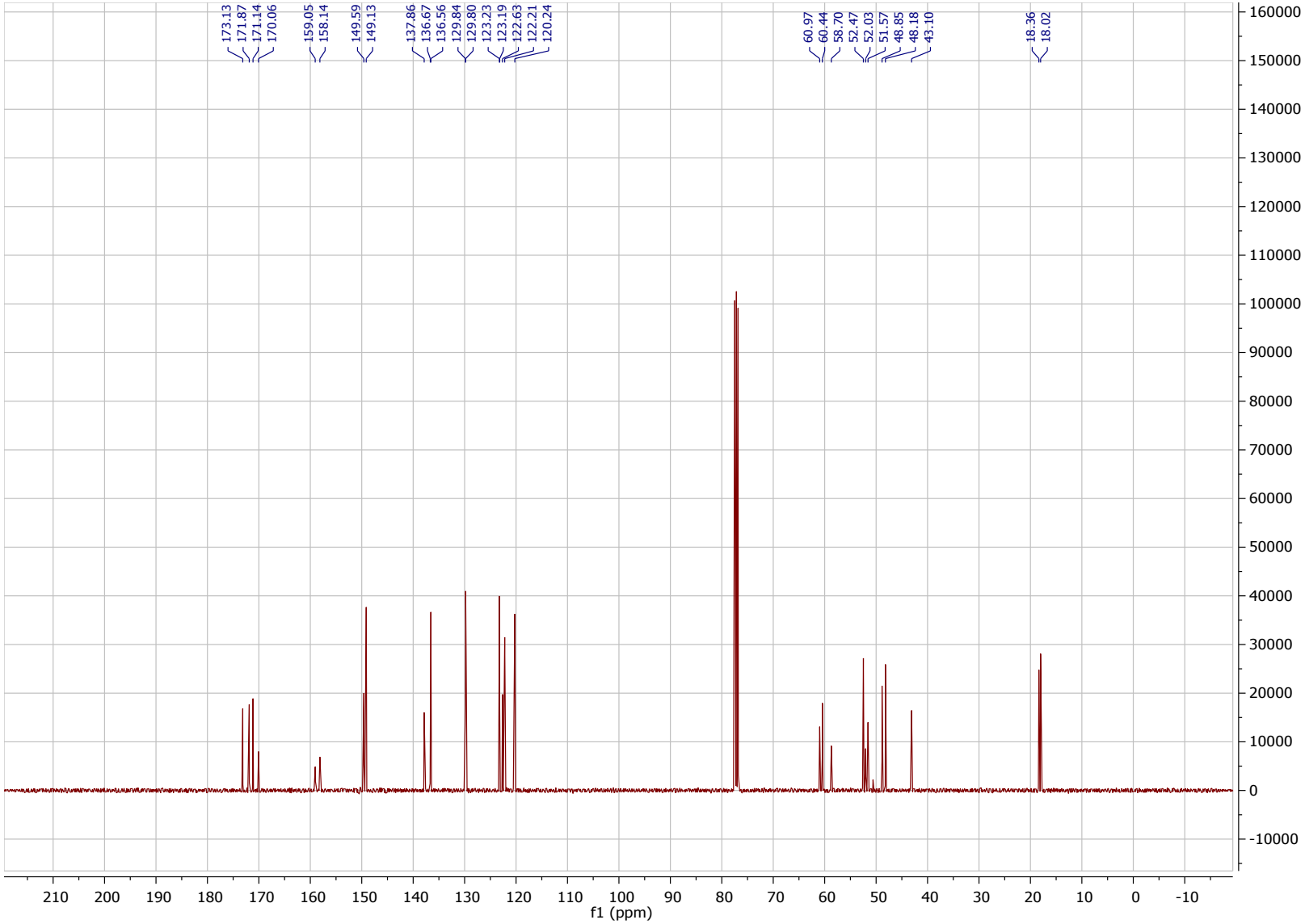
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **13**

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122.63
122.21
120.24
60.97
60.44
58.70
52.47
52.03
51.57
48.85
48.18
43.10
18.36
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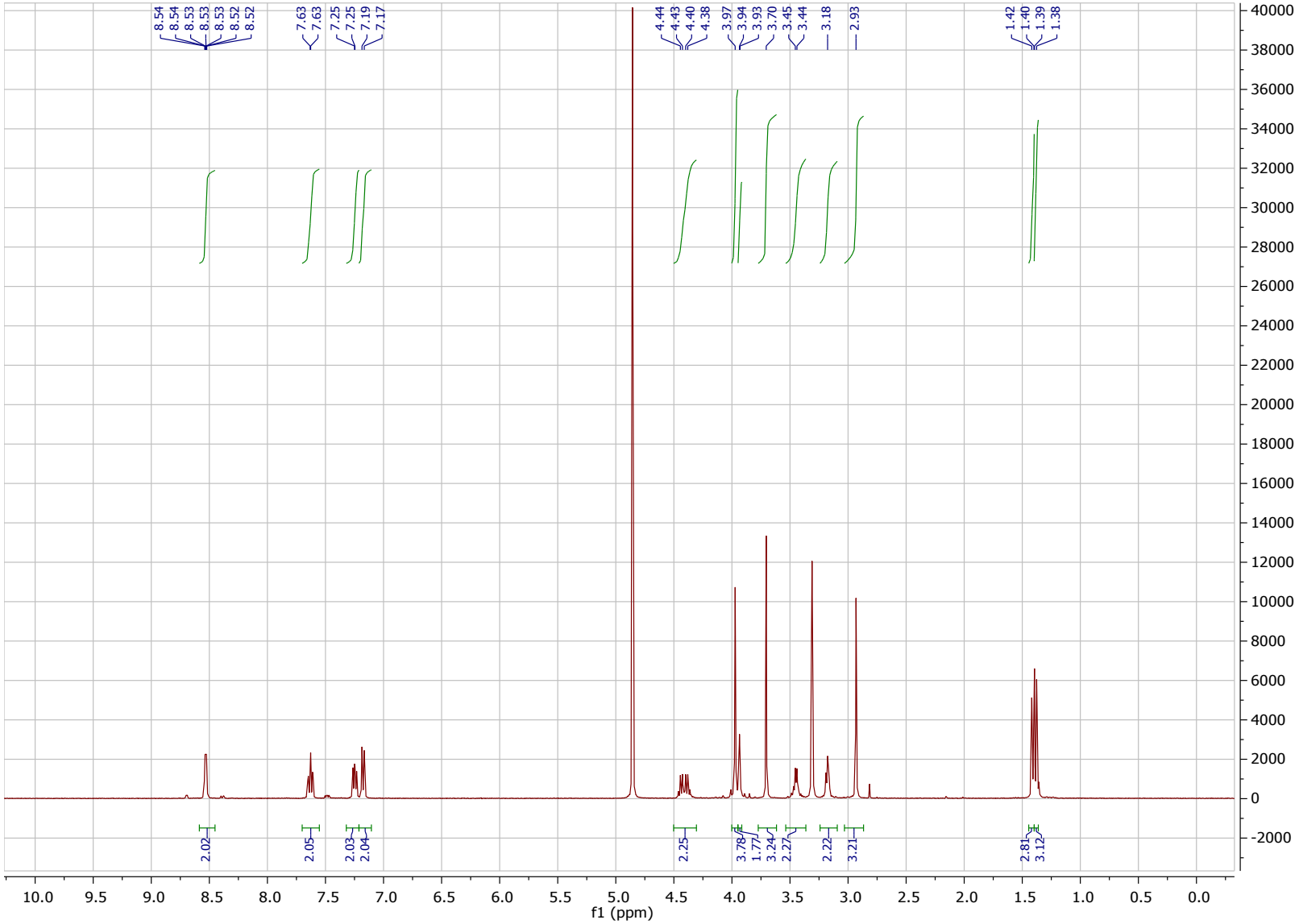
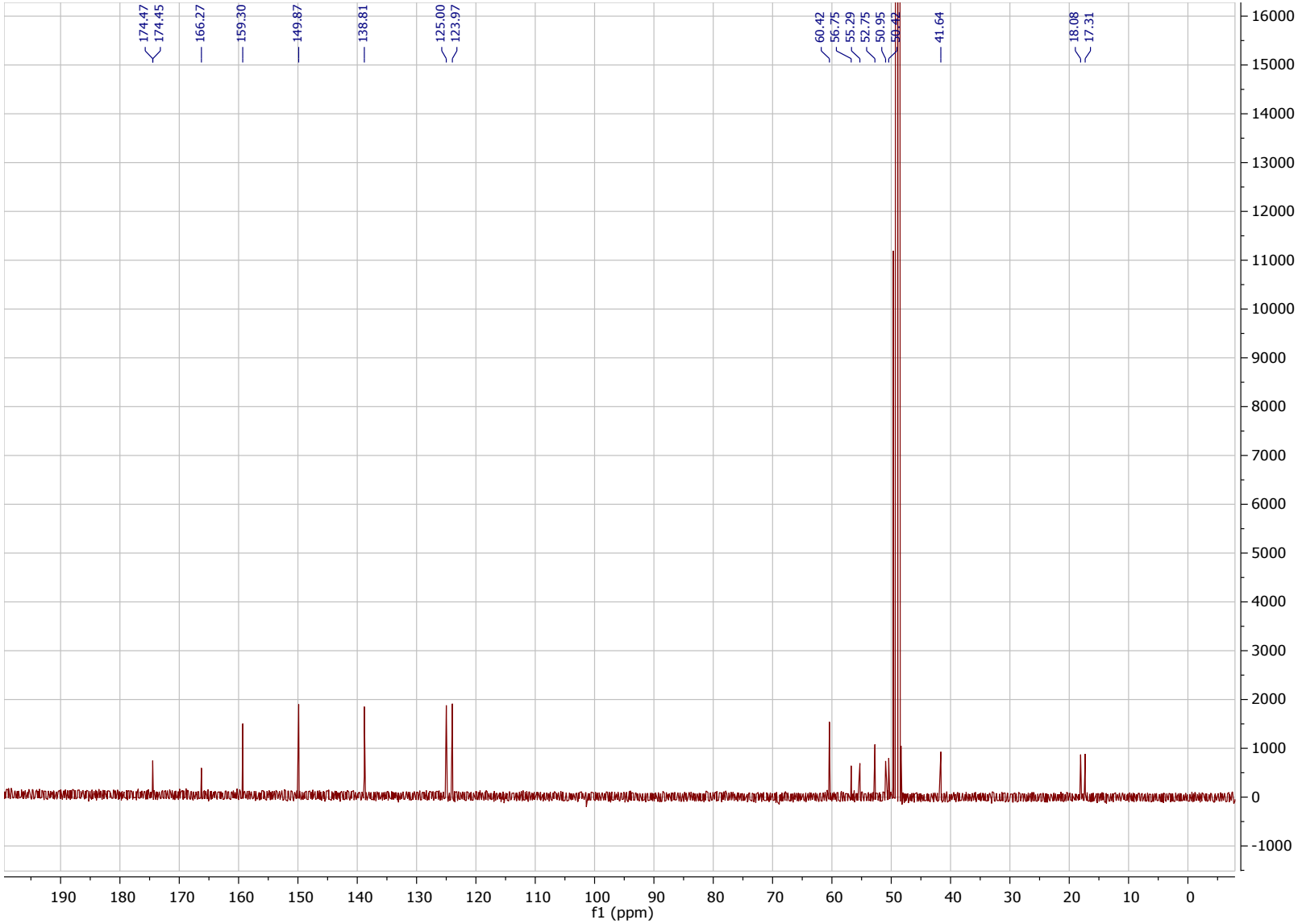
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **14**

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56.75
55.29
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41.64
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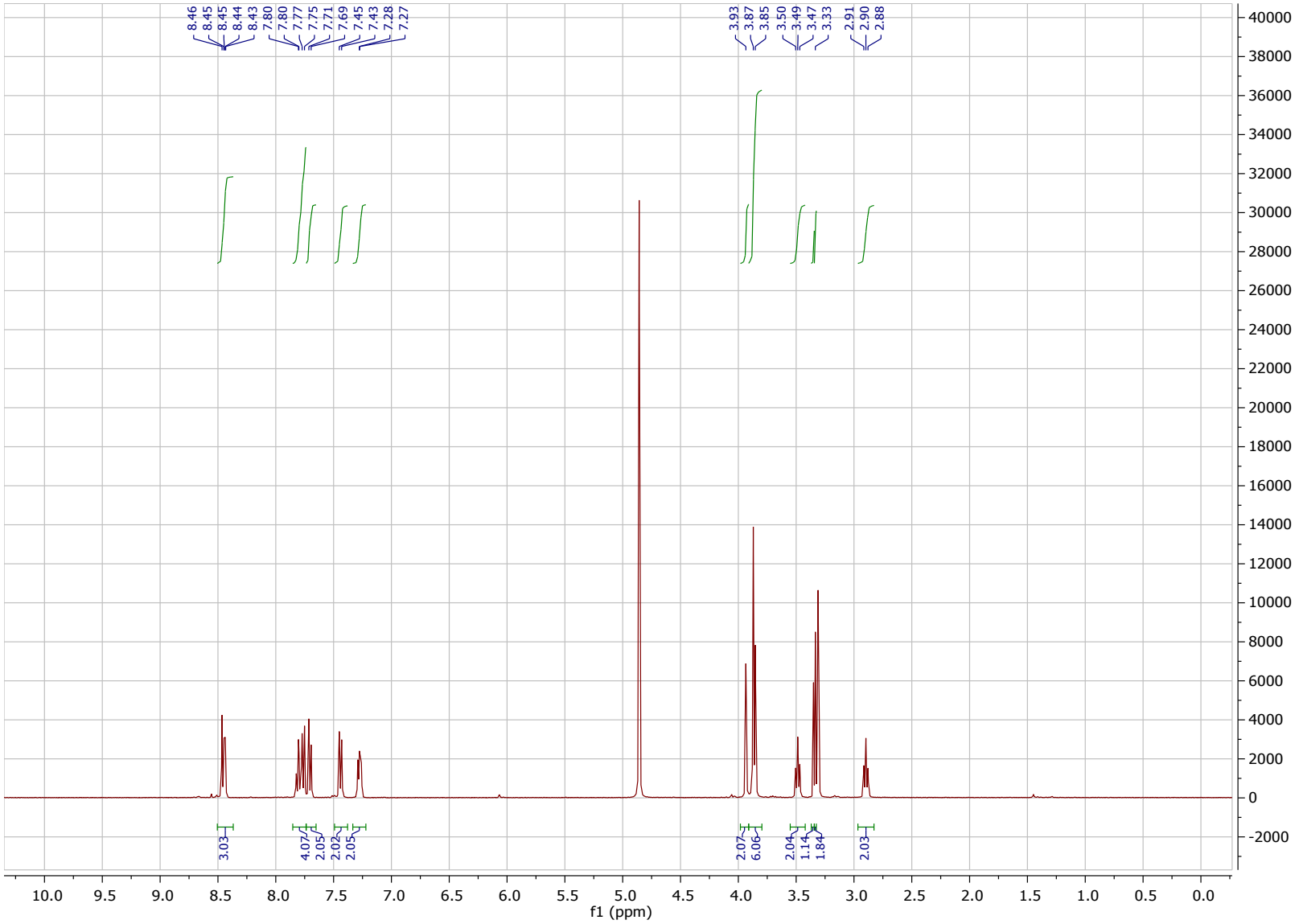
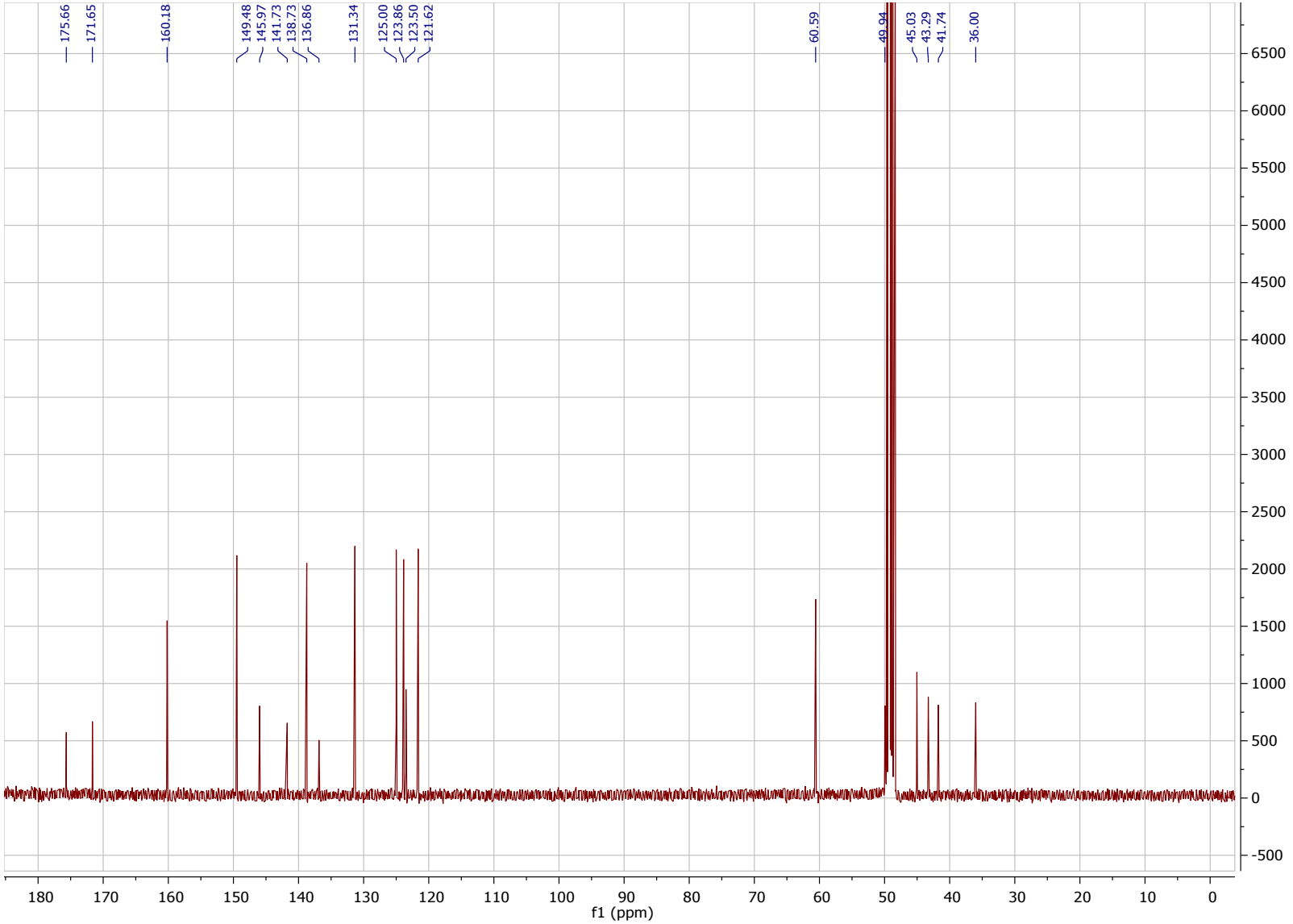
17.31
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **16**

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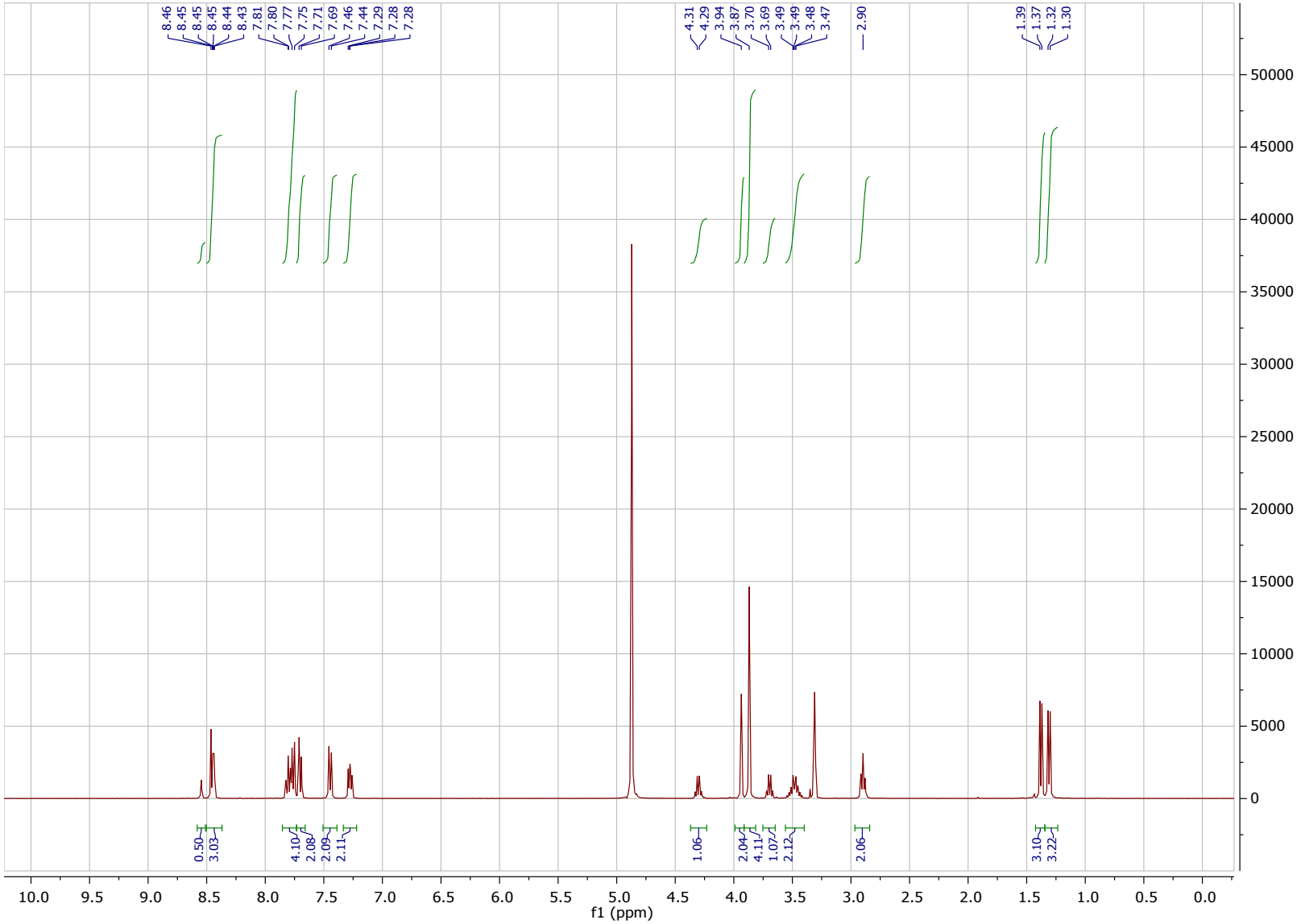
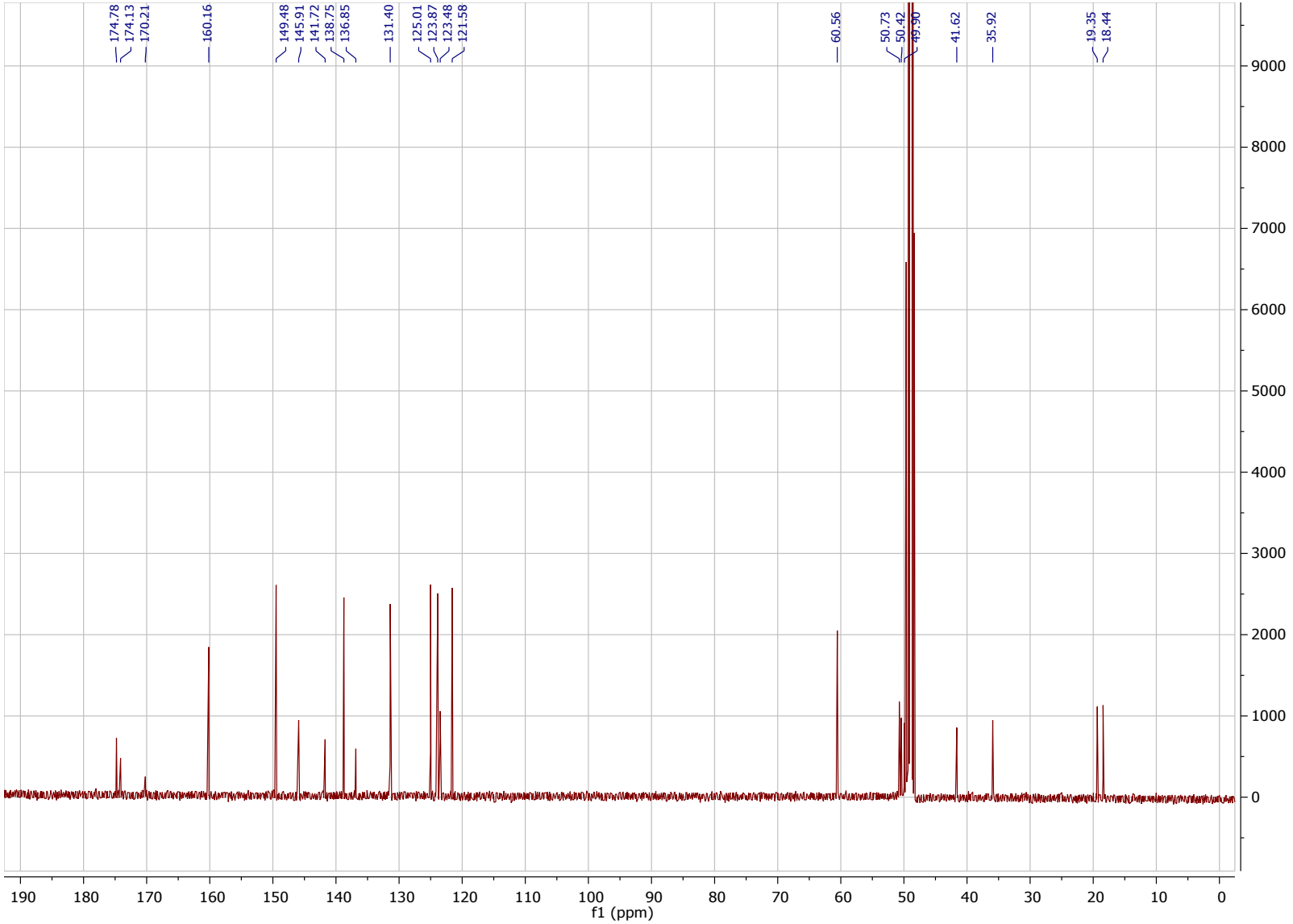
121.62
60.59
49.94
45.03
43.29
41.74
36.00
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **17**

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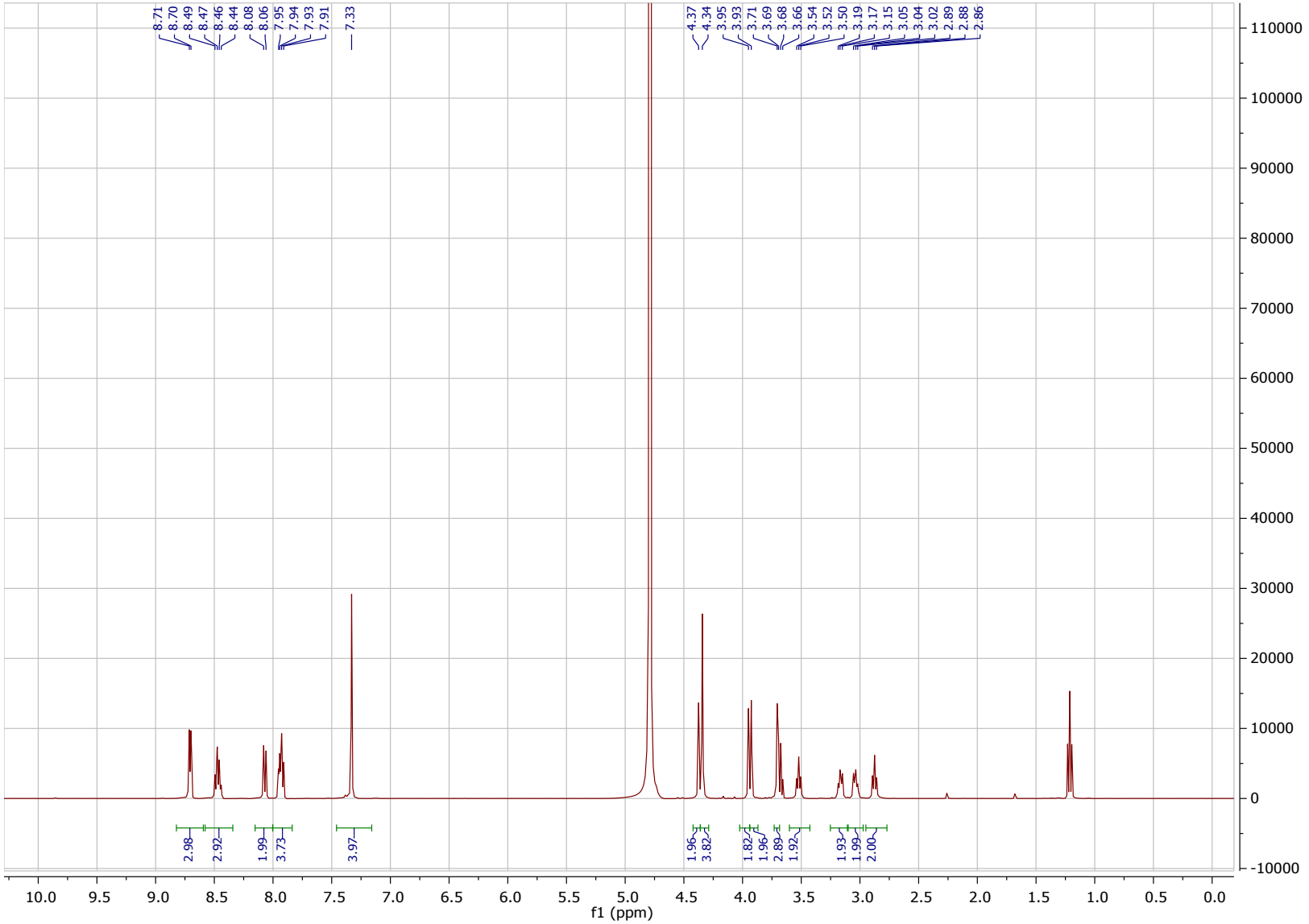
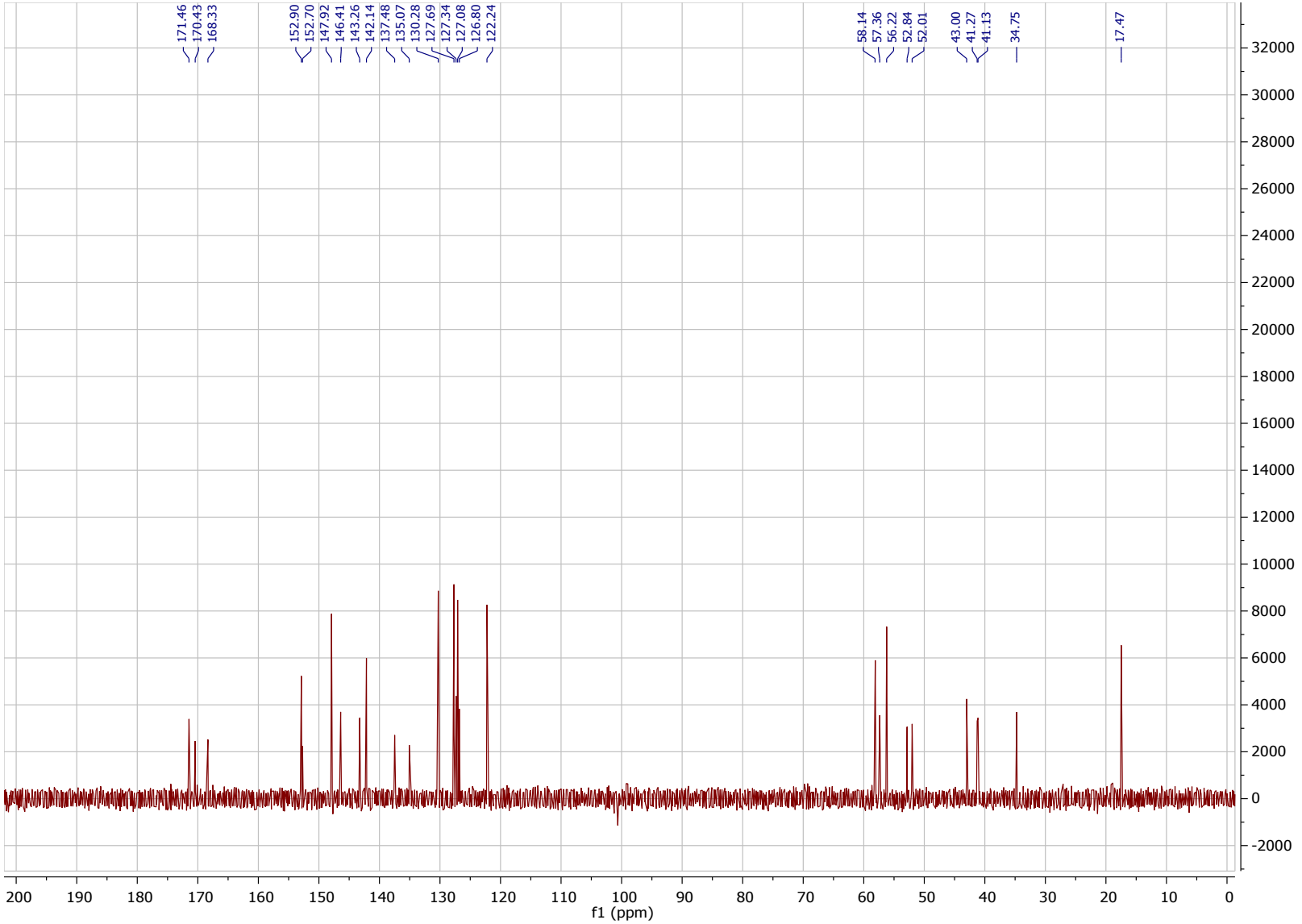
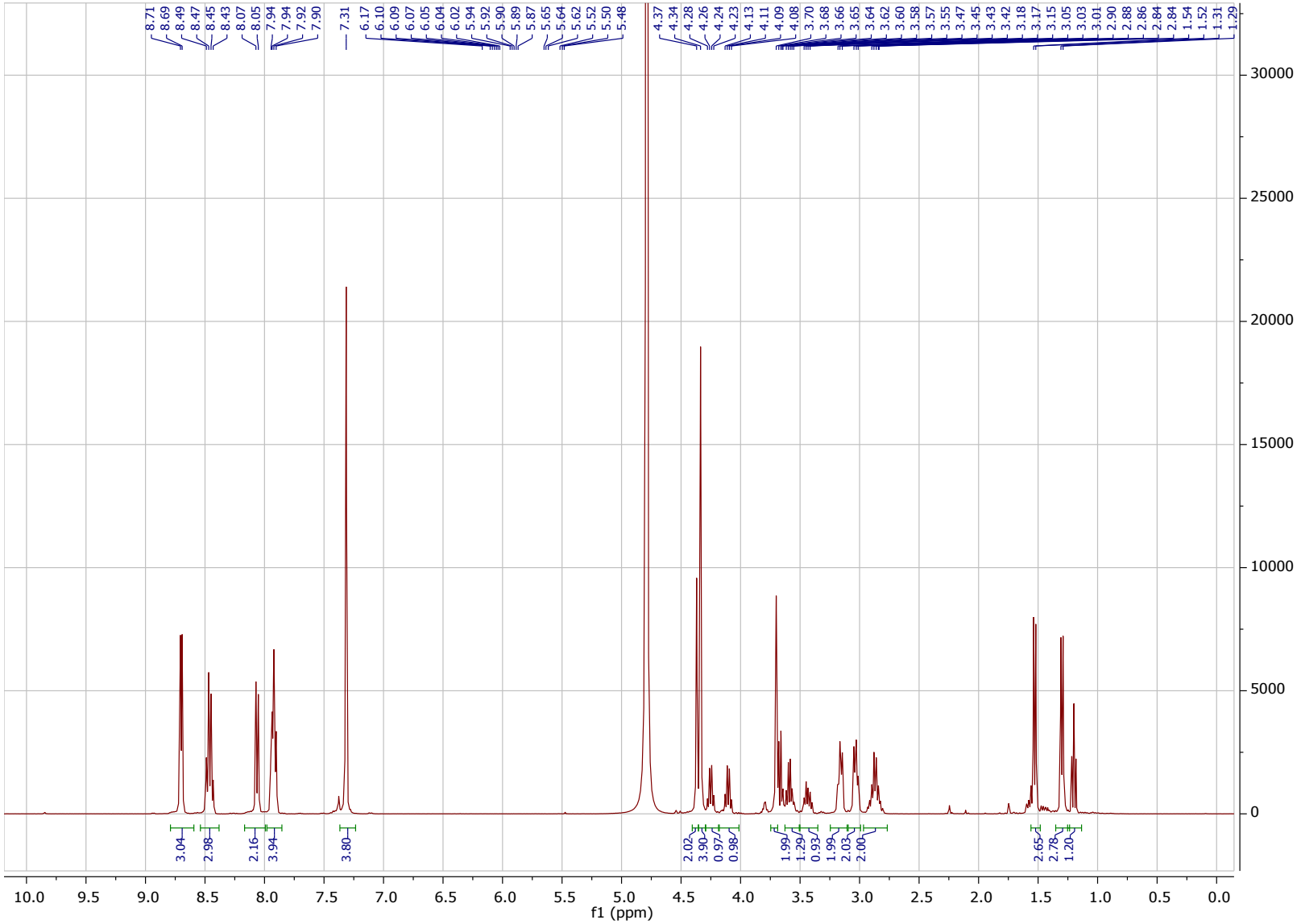
160.16
149.48
145.91
141.72
138.75
136.85
131.40
125.01
123.87
123.48
121.58
60.56
50.73
50.42
49.90
41.62
35.92
19.35
18.44
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

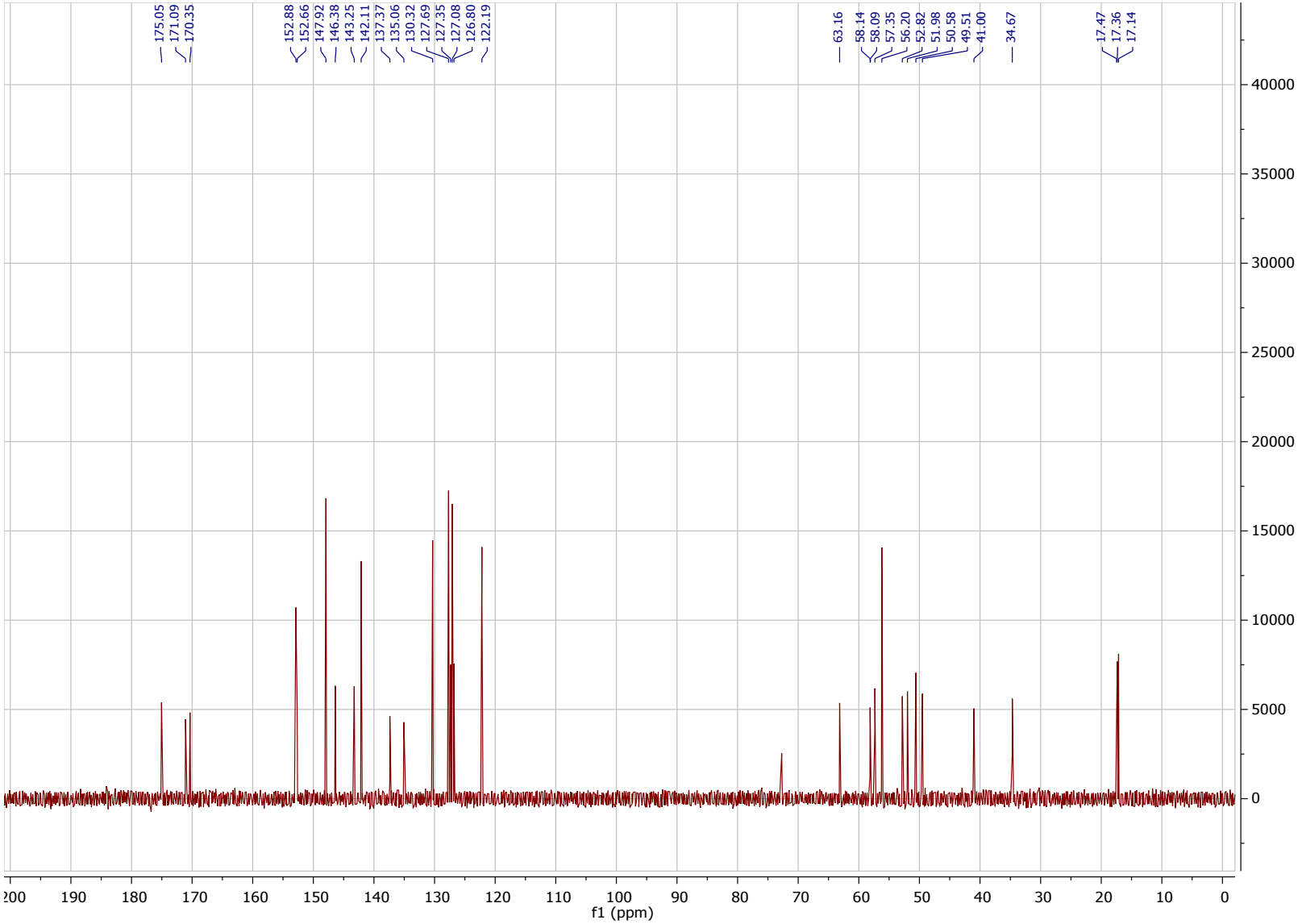
^{1}H NMR and ^{13}C NMR of compound **18**



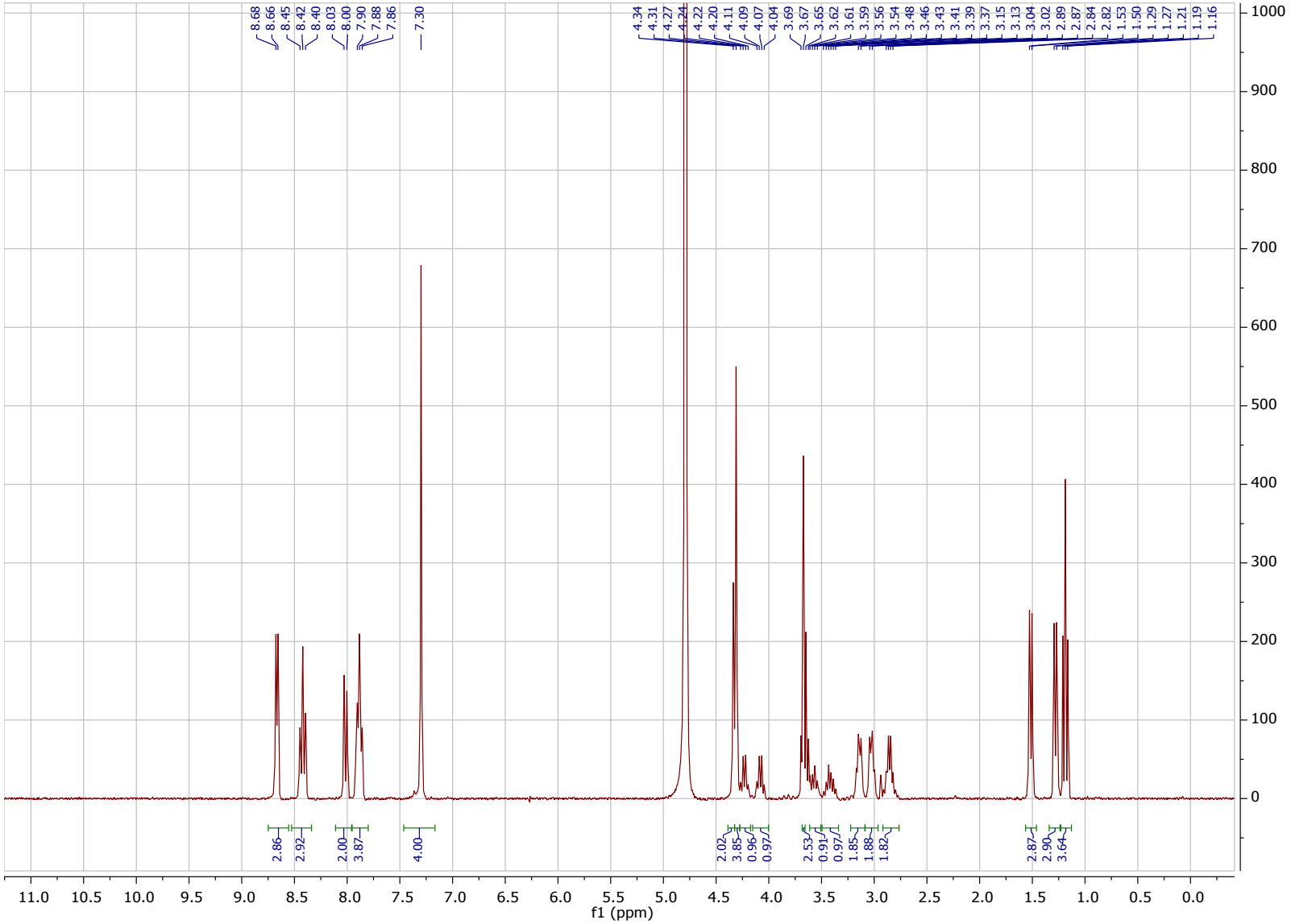

171.46
170.43
168.33
152.90
152.70
147.92
146.41
143.26
142.14
137.48
135.07
130.28
127.69
127.34
127.08
126.80
122.24
58.14
57.36
56.22
52.84
52.01
43.00
41.27
41.13
34.75
17.47
32000
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

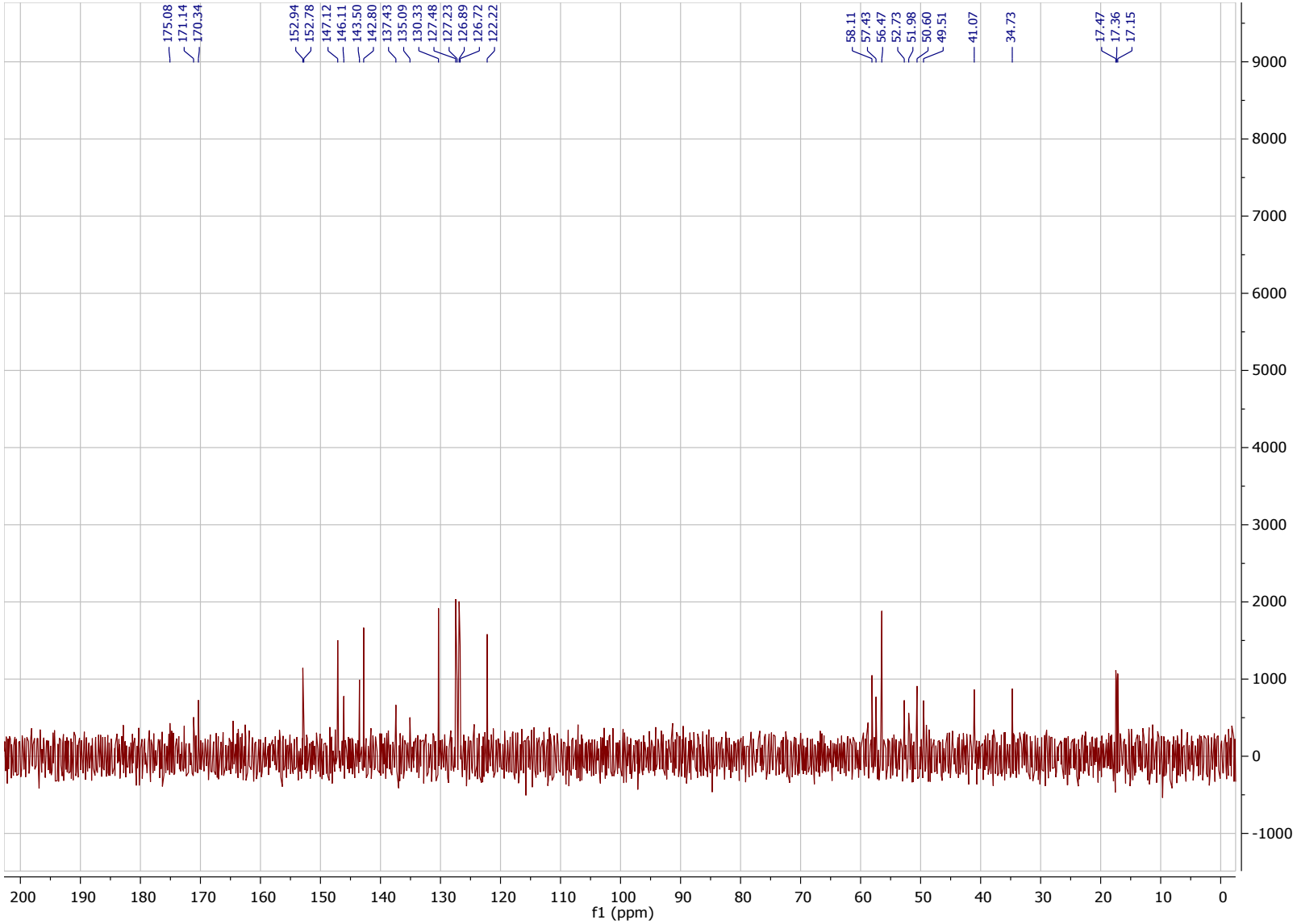
^{1}H NMR and ^{13}C NMR of compound **19**



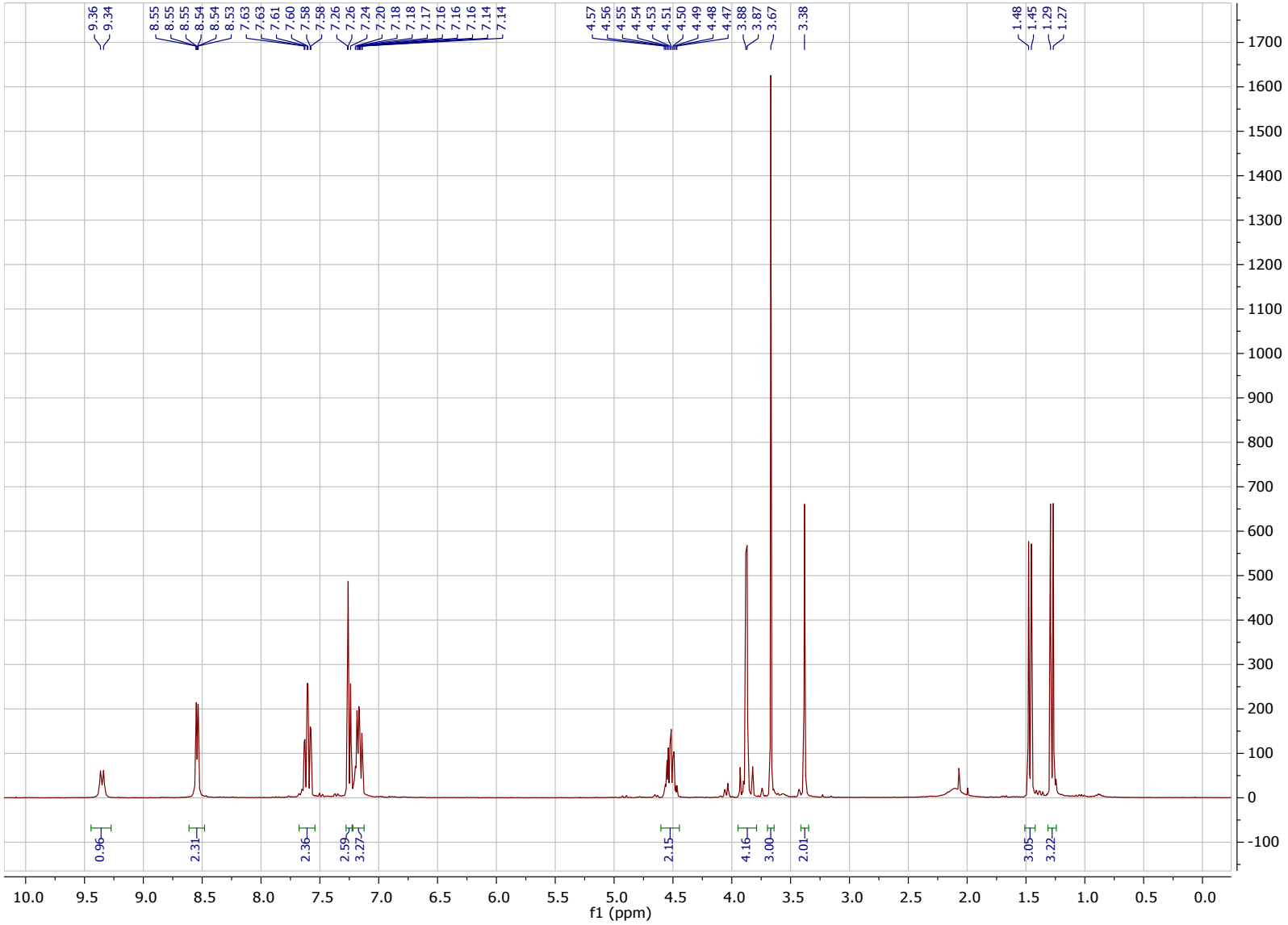

175.05
171.09
170.35
152.88
152.66
147.92
146.38
143.25
142.11
137.37
135.06
130.32
127.69
127.35
127.08
126.80
122.19
63.16
58.14
58.09
57.35
56.20
52.82
51.98
50.58
49.51
41.00
34.67
17.47
17.36
17.14
40000
35000
30000
25000
20000
15000
10000
5000
0
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

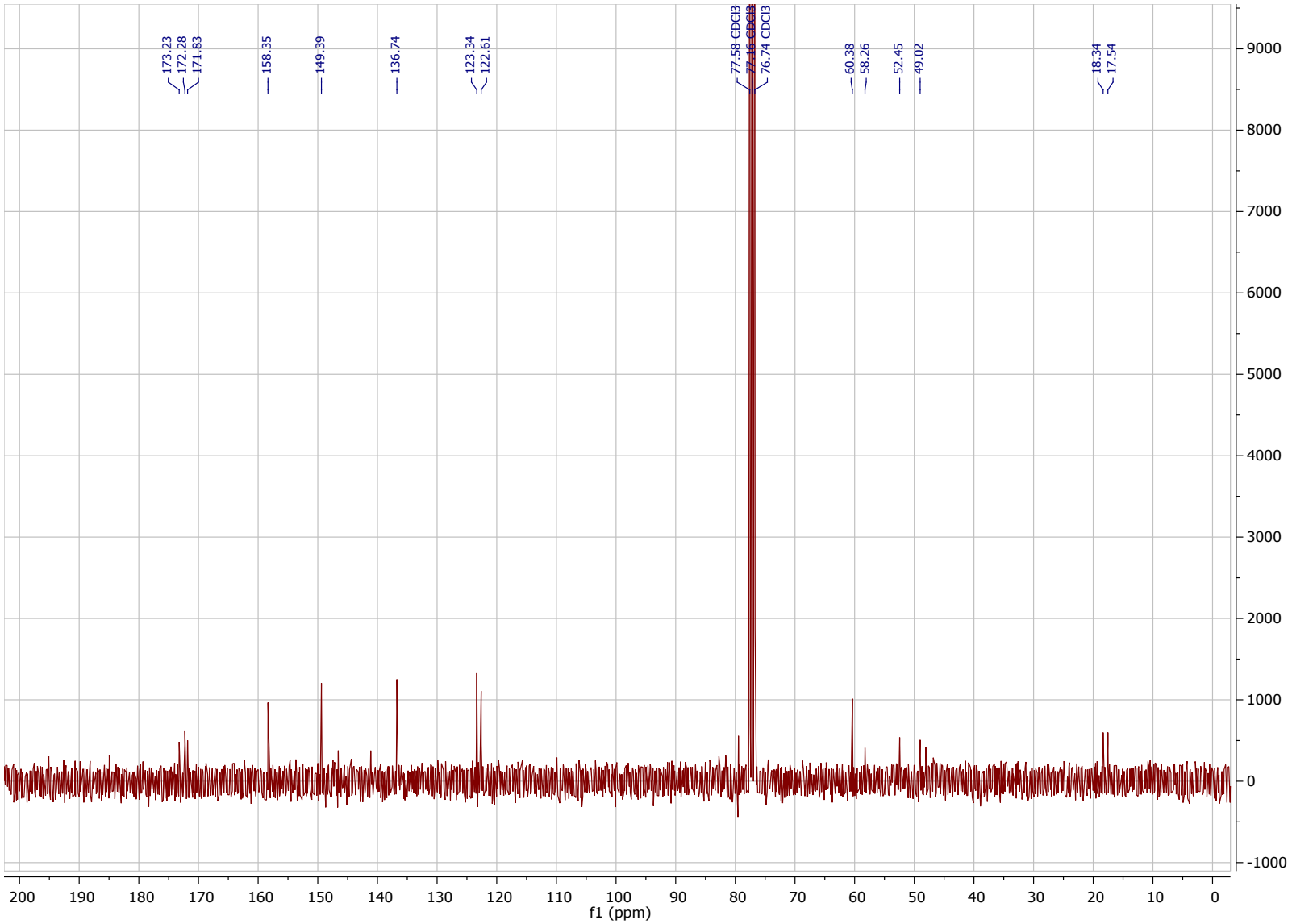
^{1}H NMR and ^{13}C NMR of compound **20**



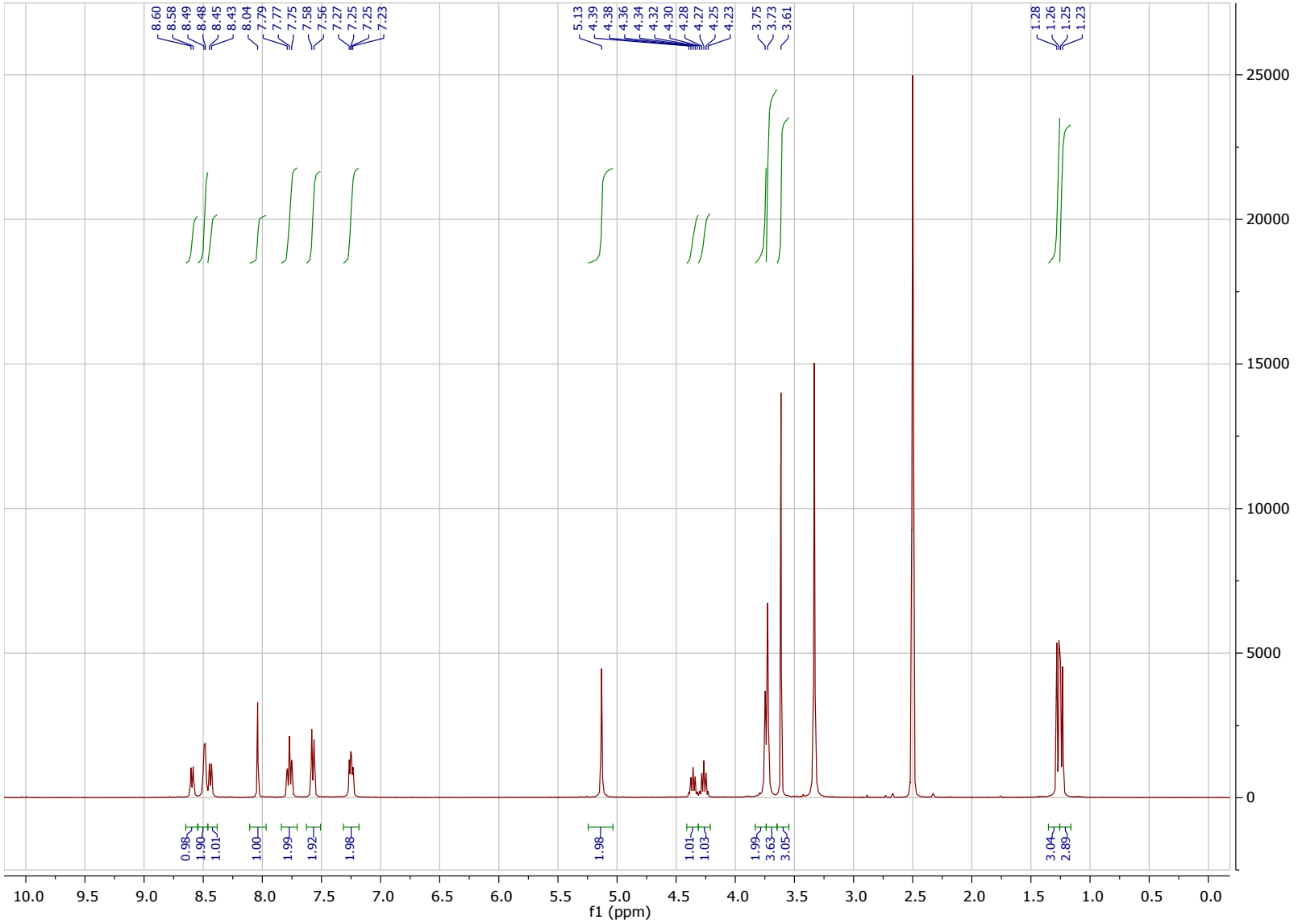

175.08
171.14
170.34
152.94
152.78
147.12
146.11
143.50
142.80
137.43
135.09
130.33
127.48
127.23
126.89
126.72
122.22
58.11
57.43
56.47
52.73
51.98
50.60
49.51
41.07
34.73
17.47
17.36
17.15
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

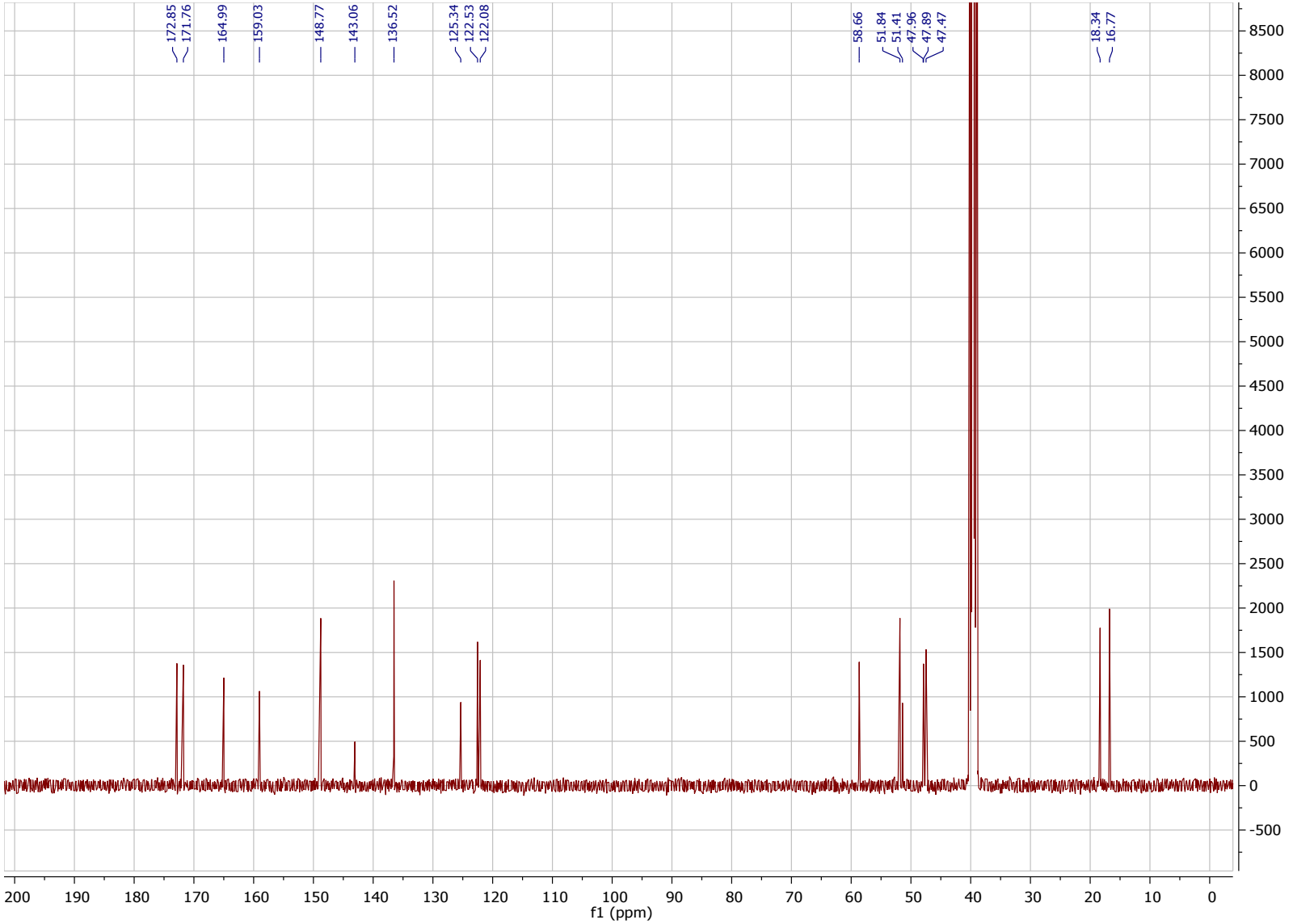
^{1}H NMR and ^{13}C NMR of compound **33**



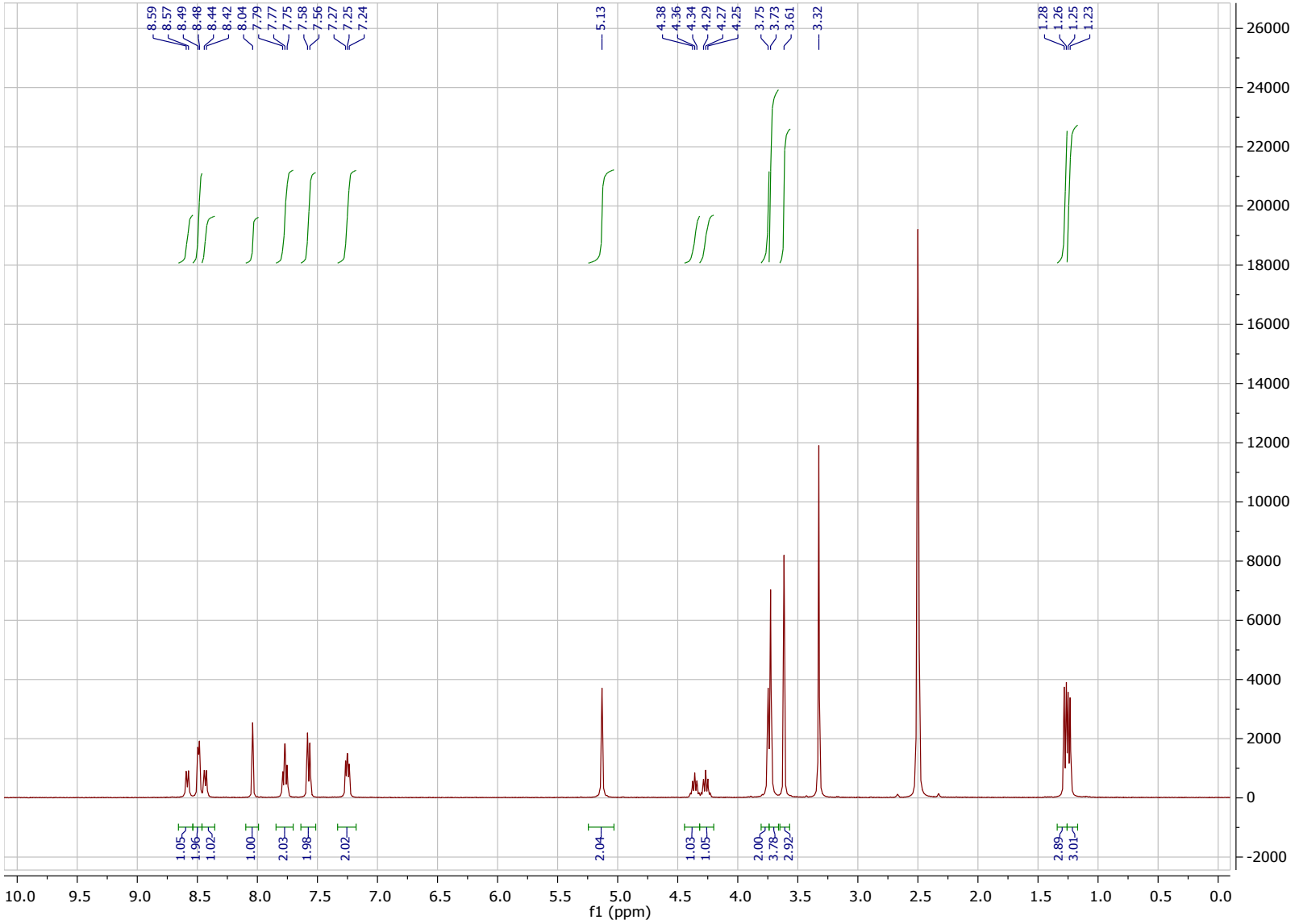

173.23
172.28
171.83
158.35
149.39
136.74
123.34
122.61
77.58 CDCl3
77.16 CDCl3
76.74 CDCl3
60.38
58.26
52.45
49.02
18.34
17.54
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

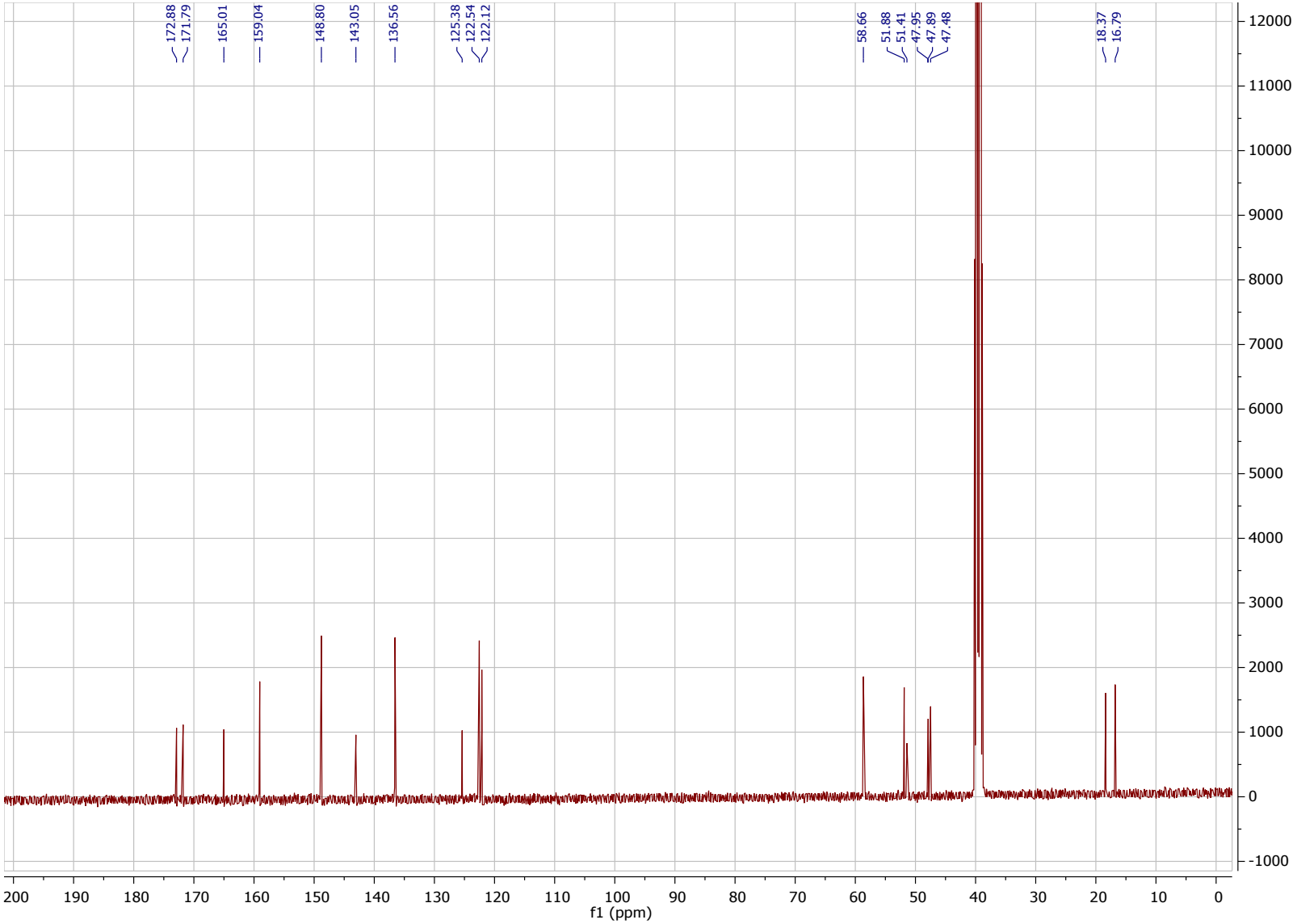
^{1}H NMR and ^{13}C NMR of compound **35**



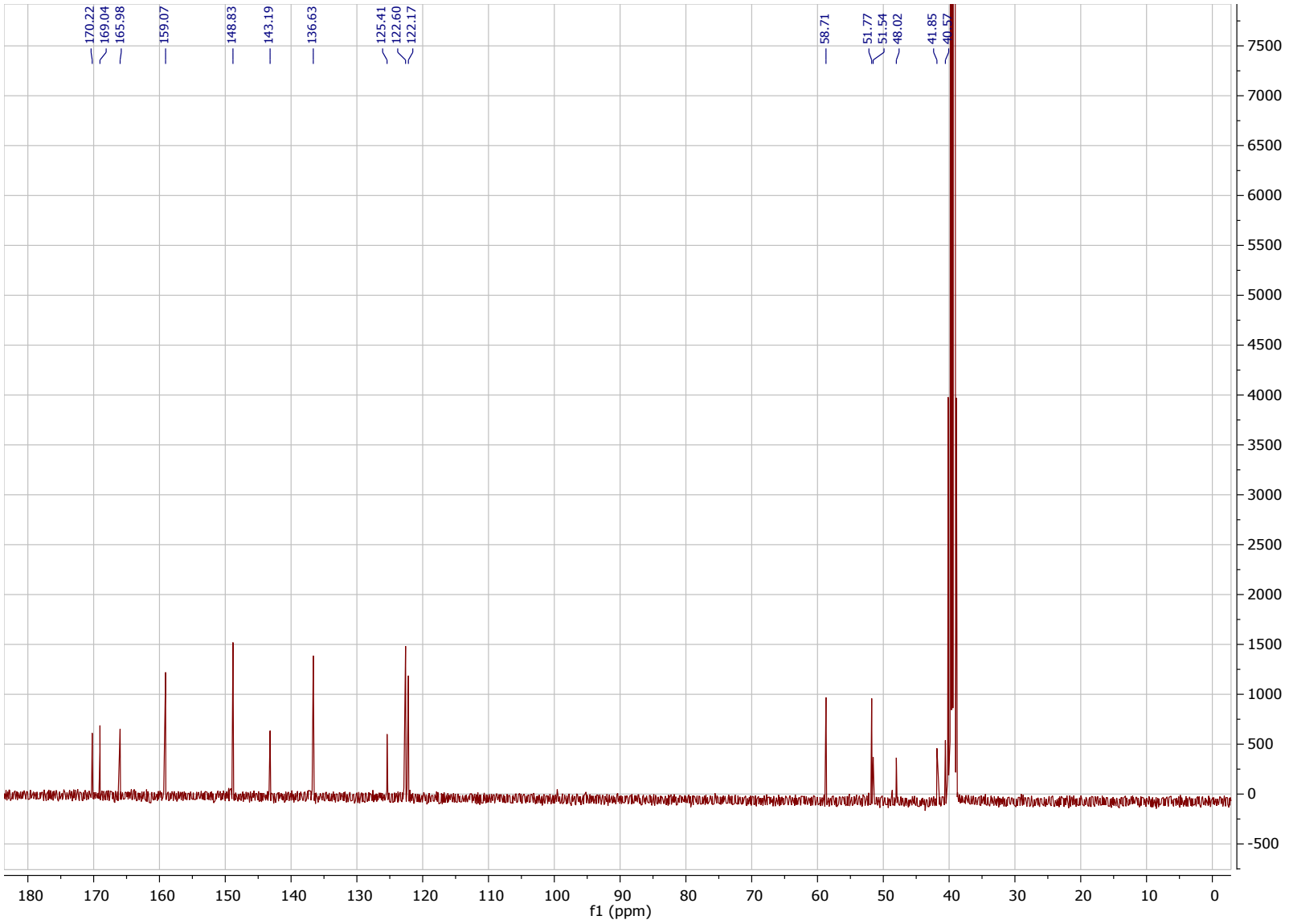

172.85
171.76
164.99
159.03
148.77
143.06
136.52
125.34
122.53
122.08
58.66
51.84
51.41
47.96
47.89
47.47
18.34
16.77
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **36**

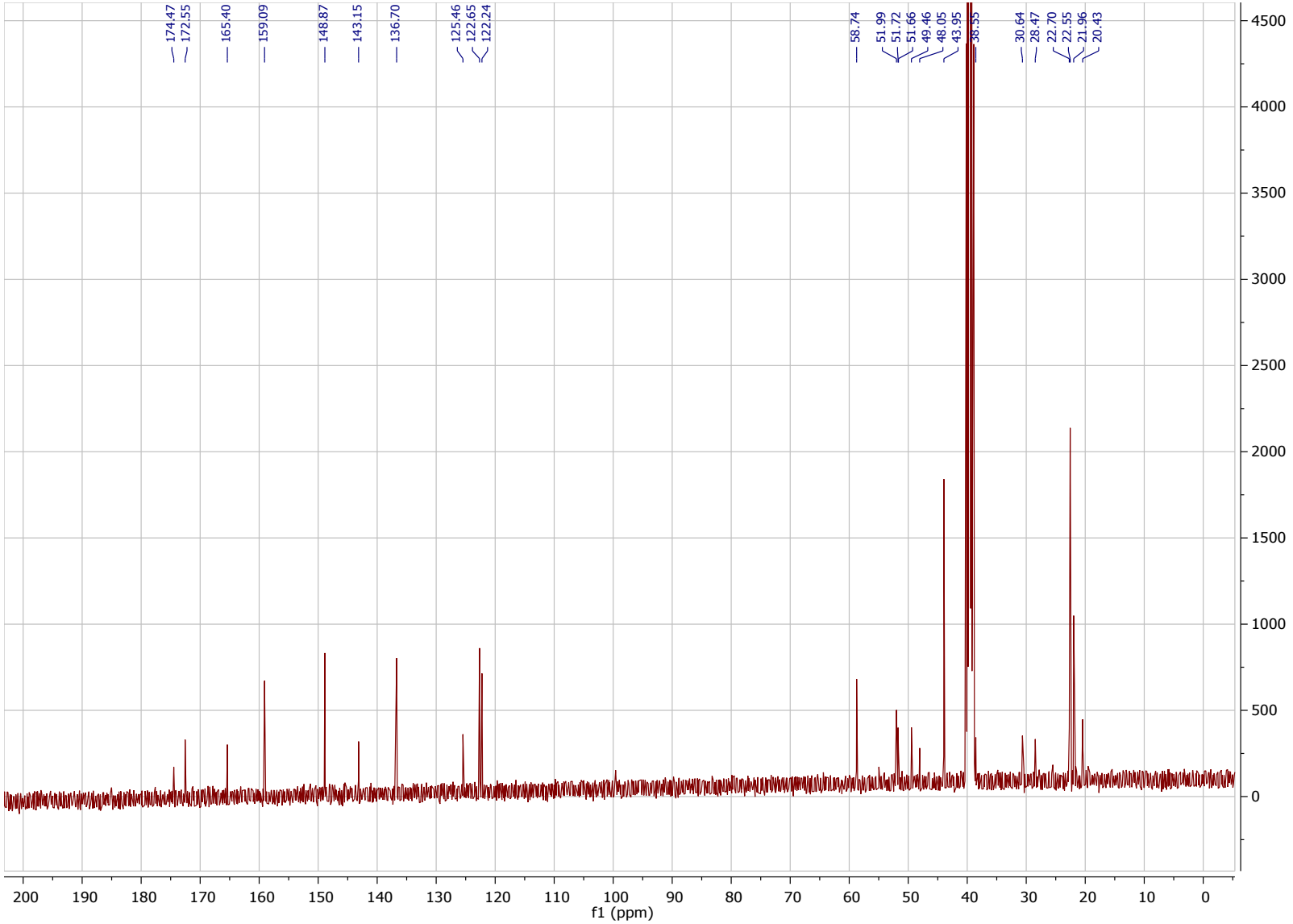



172.88
171.79
165.01
159.04
148.80
143.05
136.56
125.38
122.54
122.12
58.66
51.88
51.41
47.95
47.89
47.48
18.37
16.79
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

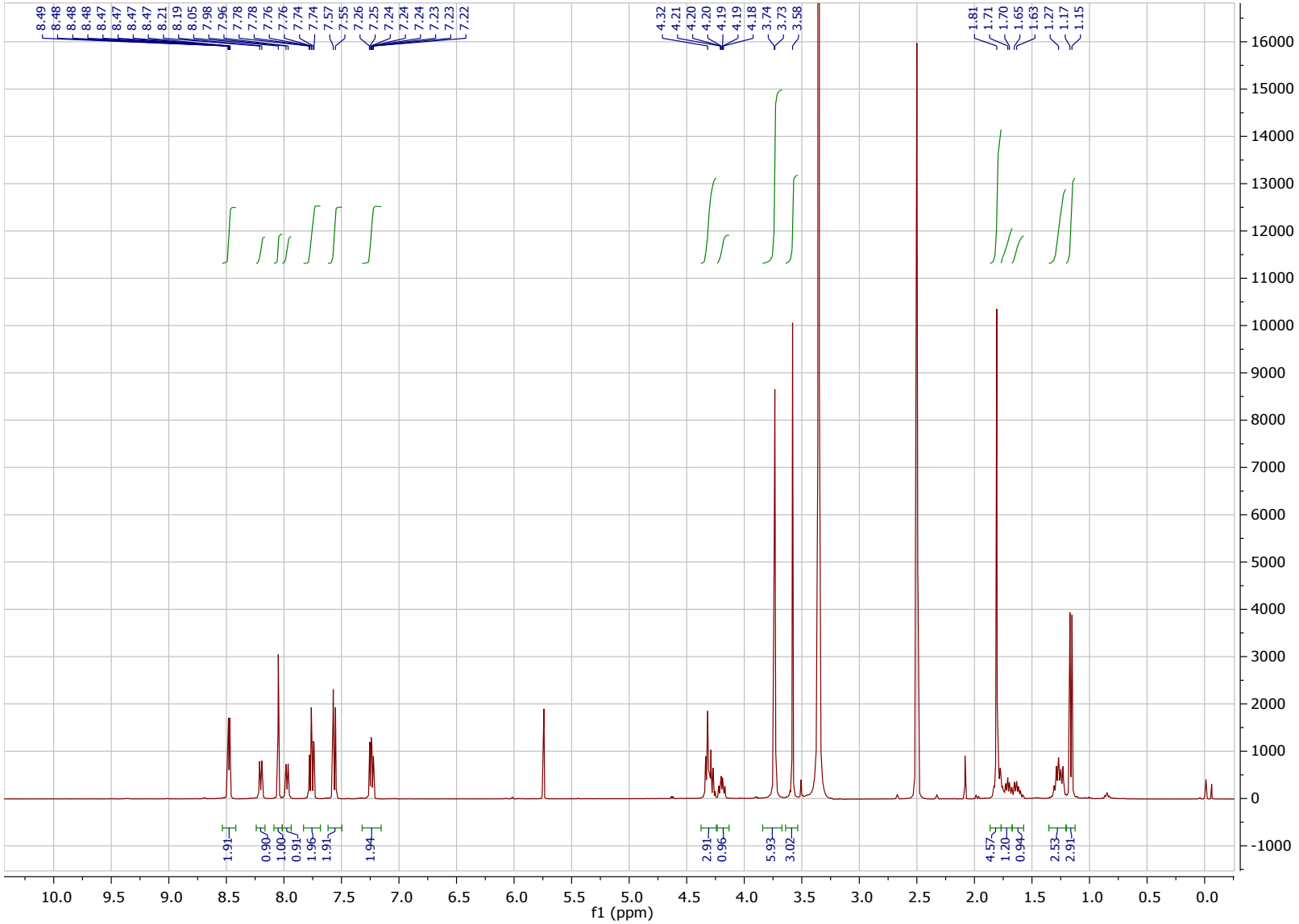
^{1}H NMR and ^{13}C NMR of compound **37**

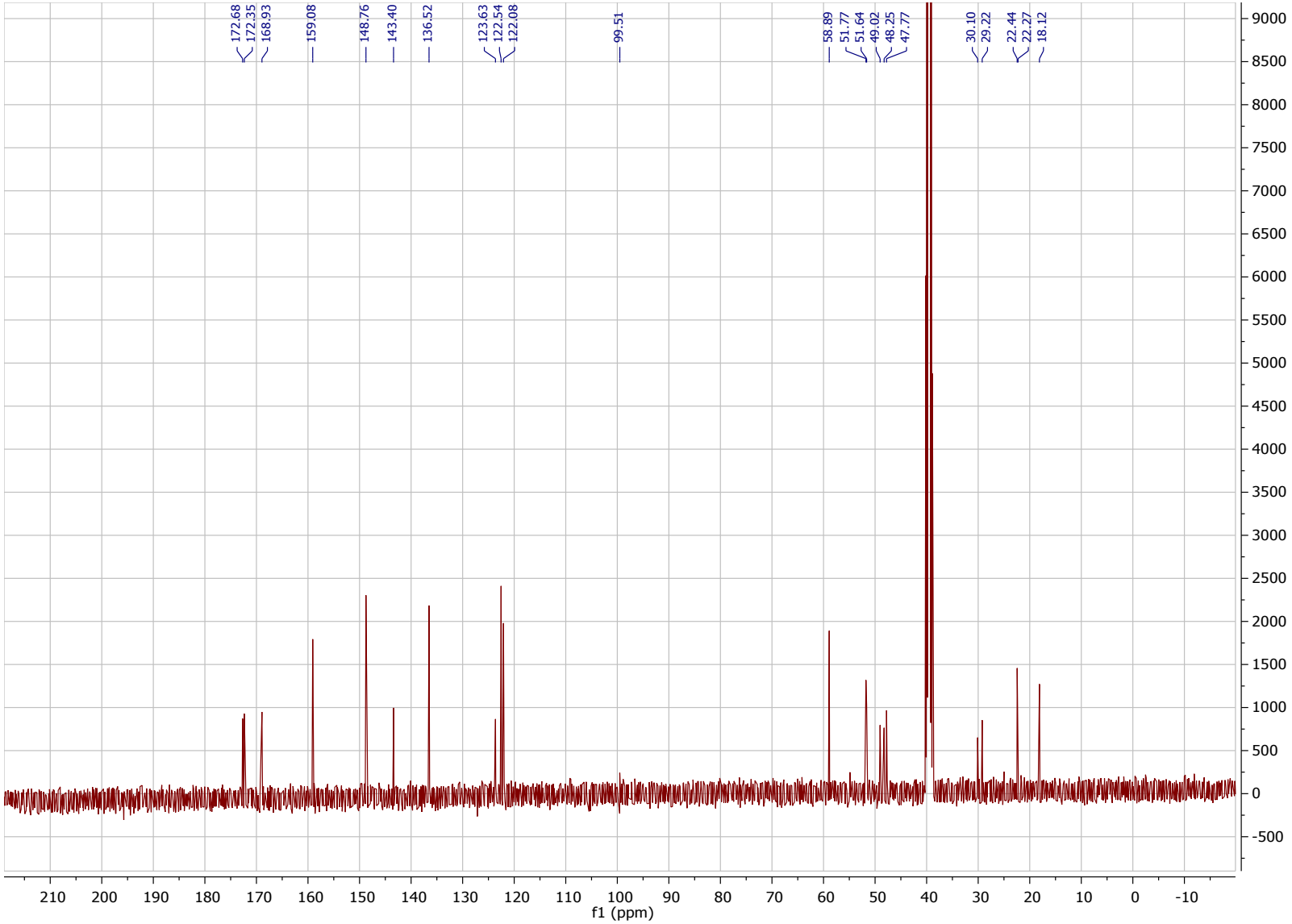

170.22
169.04
165.98
159.07
148.83
143.19
136.63
125.41
122.60
122.17
58.71
51.77
51.54
48.02
41.85
40.57
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **38**

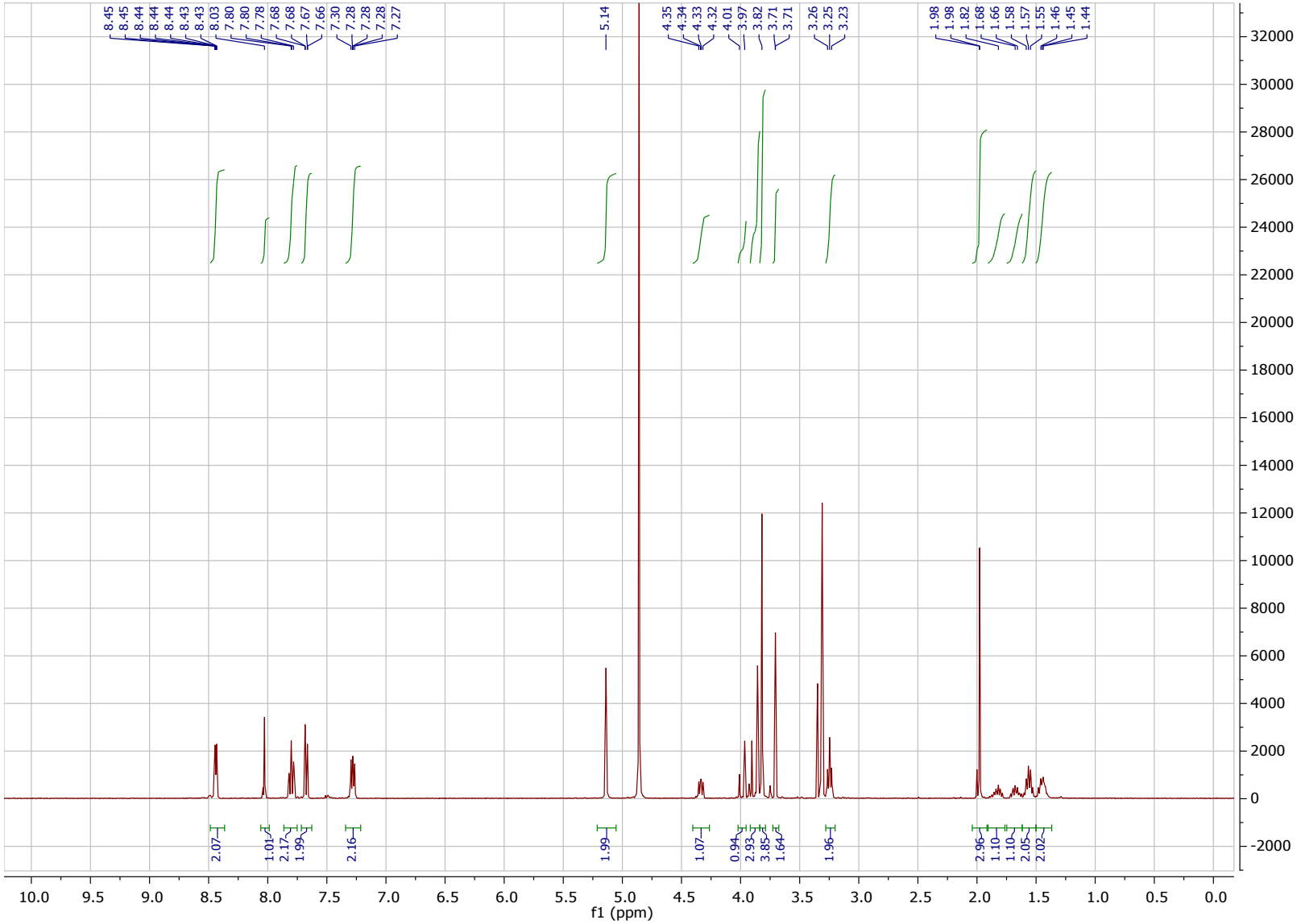

174.47
172.55
165.40
159.09
148.87
143.15
136.70
125.46
122.65
122.24
58.74
51.99
51.72
51.66
49.46
48.05
43.95
38.35
30.64
28.47
22.70
22.55
21.96
20.43
4500
4000
3500
3000
2500
2000
1500
1000
500
0
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

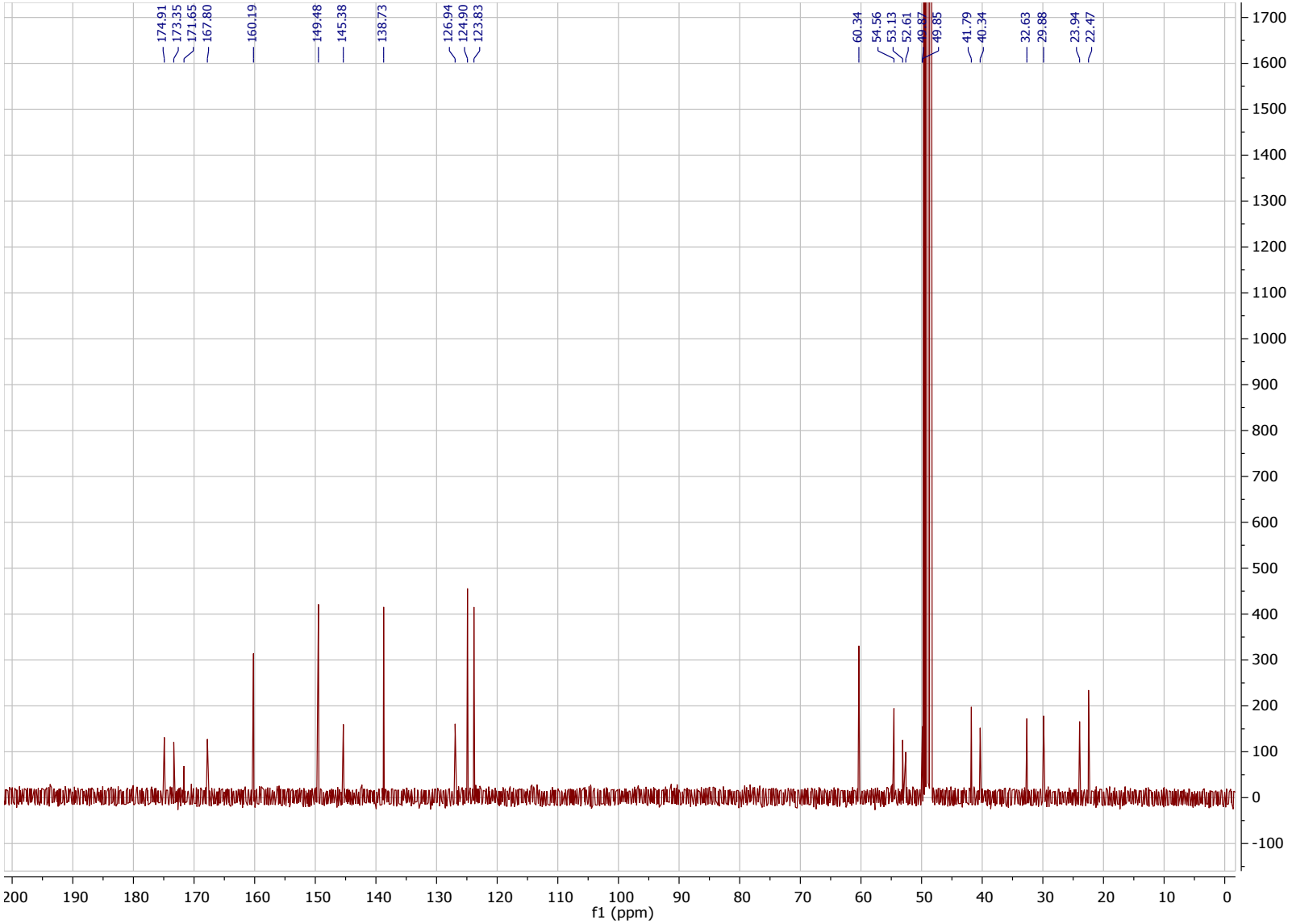
^{1}H NMR and ^{13}C NMR of compound **39**



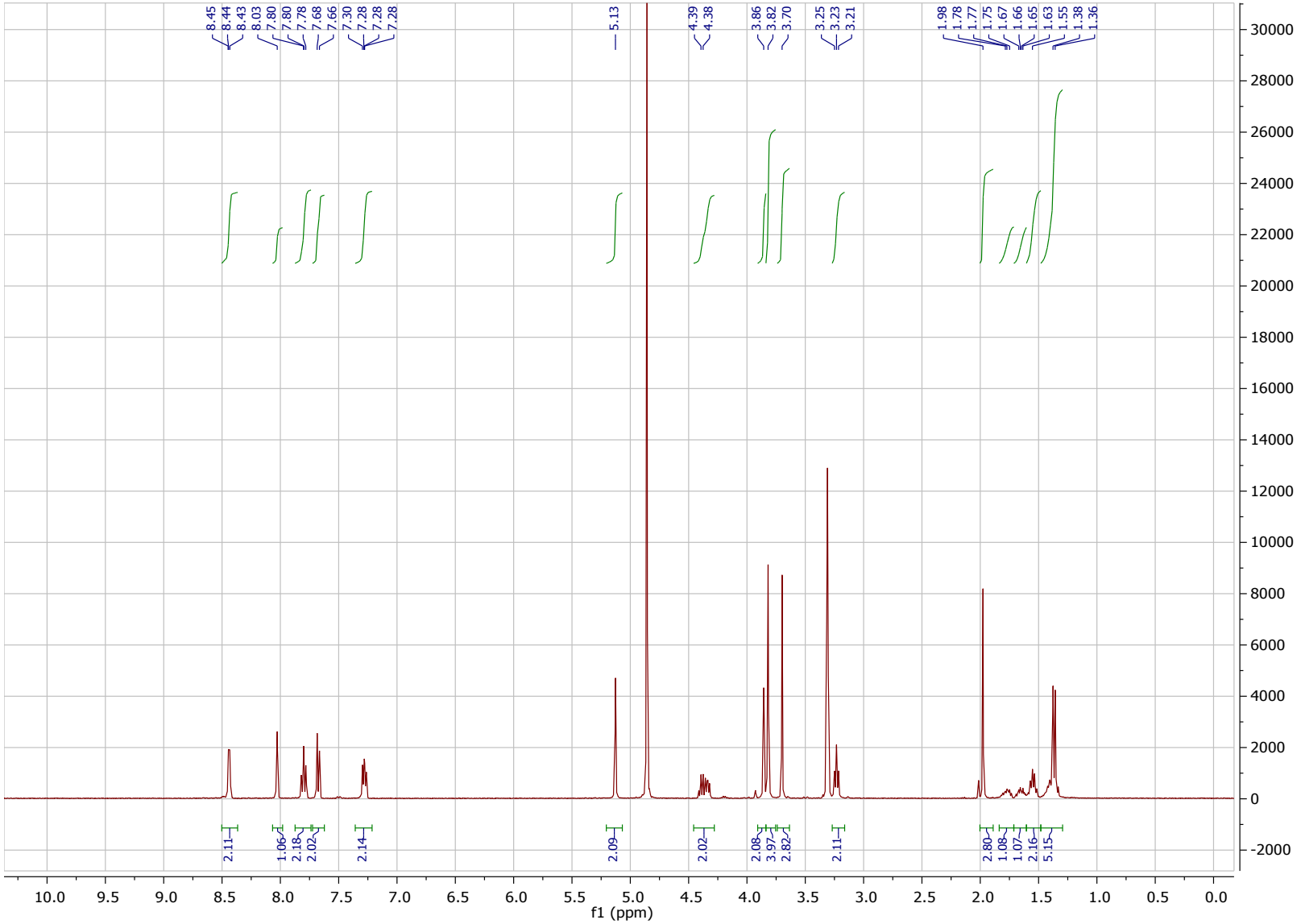

172.68
172.35
168.93
159.08
148.76
143.40
136.52
123.63
122.54
122.08
99.51
58.89
51.77
51.64
49.02
48.25
47.77
30.10
29.22
22.44
22.27
18.12
9000
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

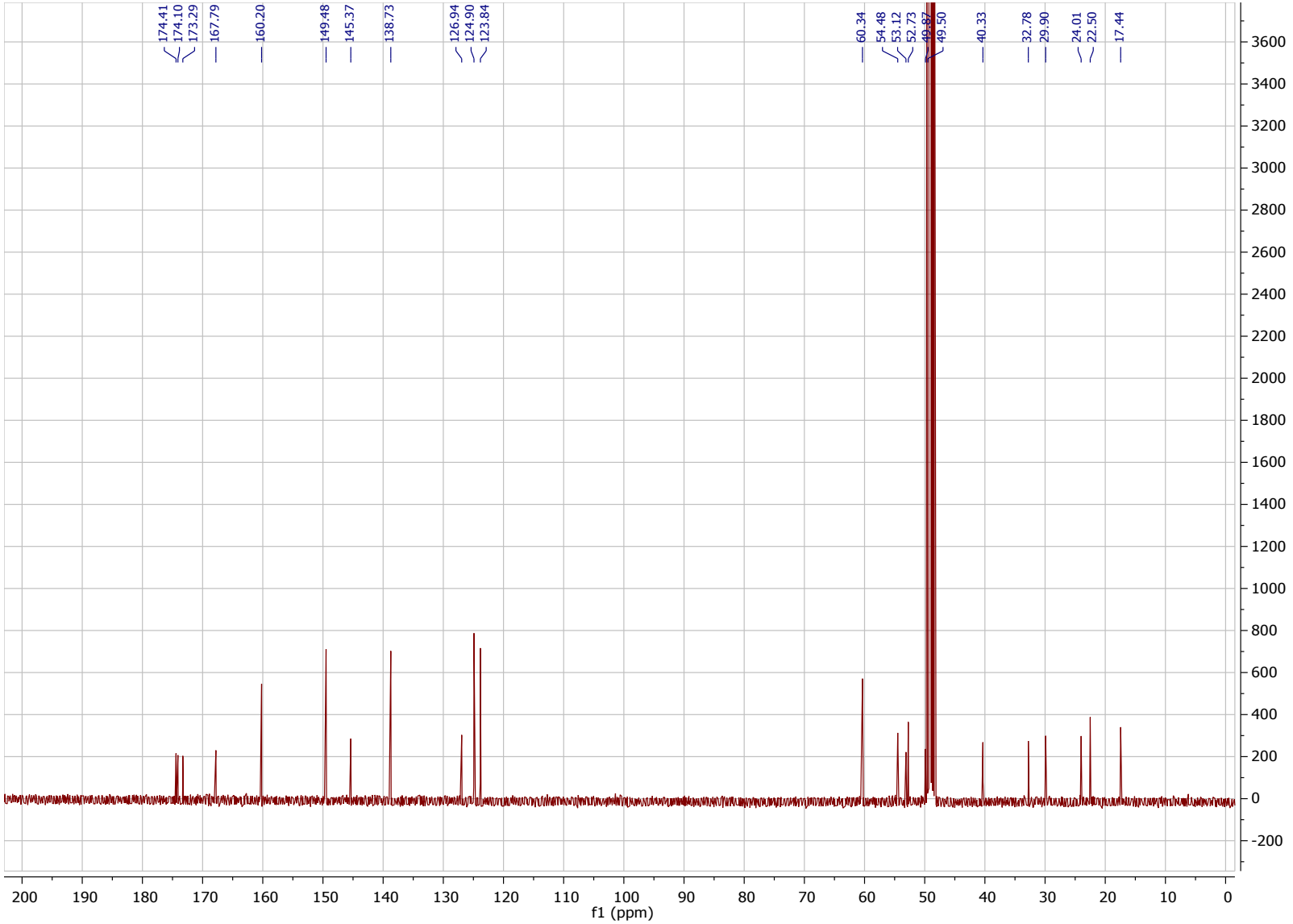
^{1}H NMR and ^{13}C NMR of compound **40**



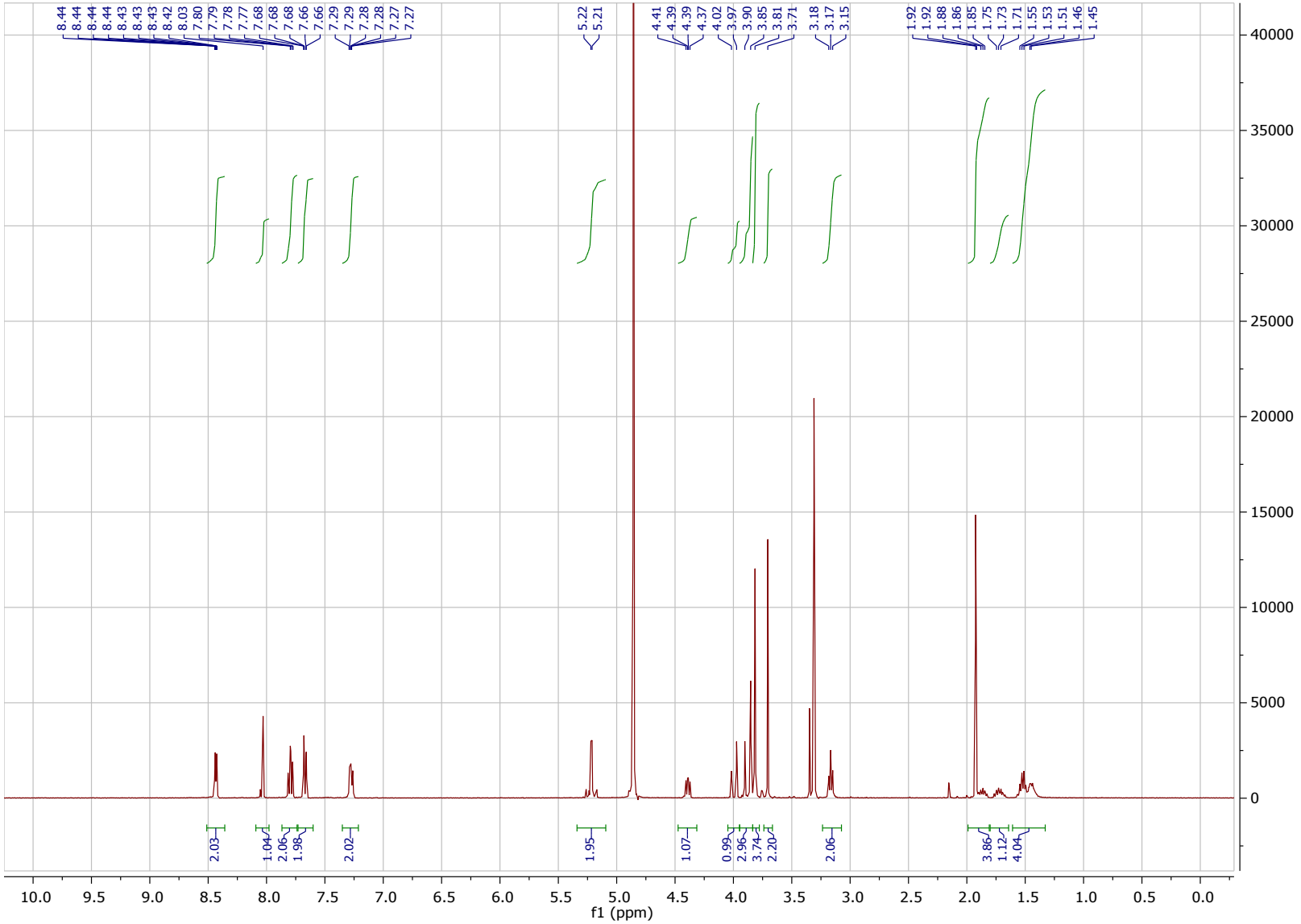

174.91
173.35
171.65
167.80
160.19
149.48
145.38
138.73
126.94
124.90
123.83
60.34
54.56
53.13
52.61
49.85
41.79
40.34
32.63
29.88
23.94
22.47
f1 (ppm)

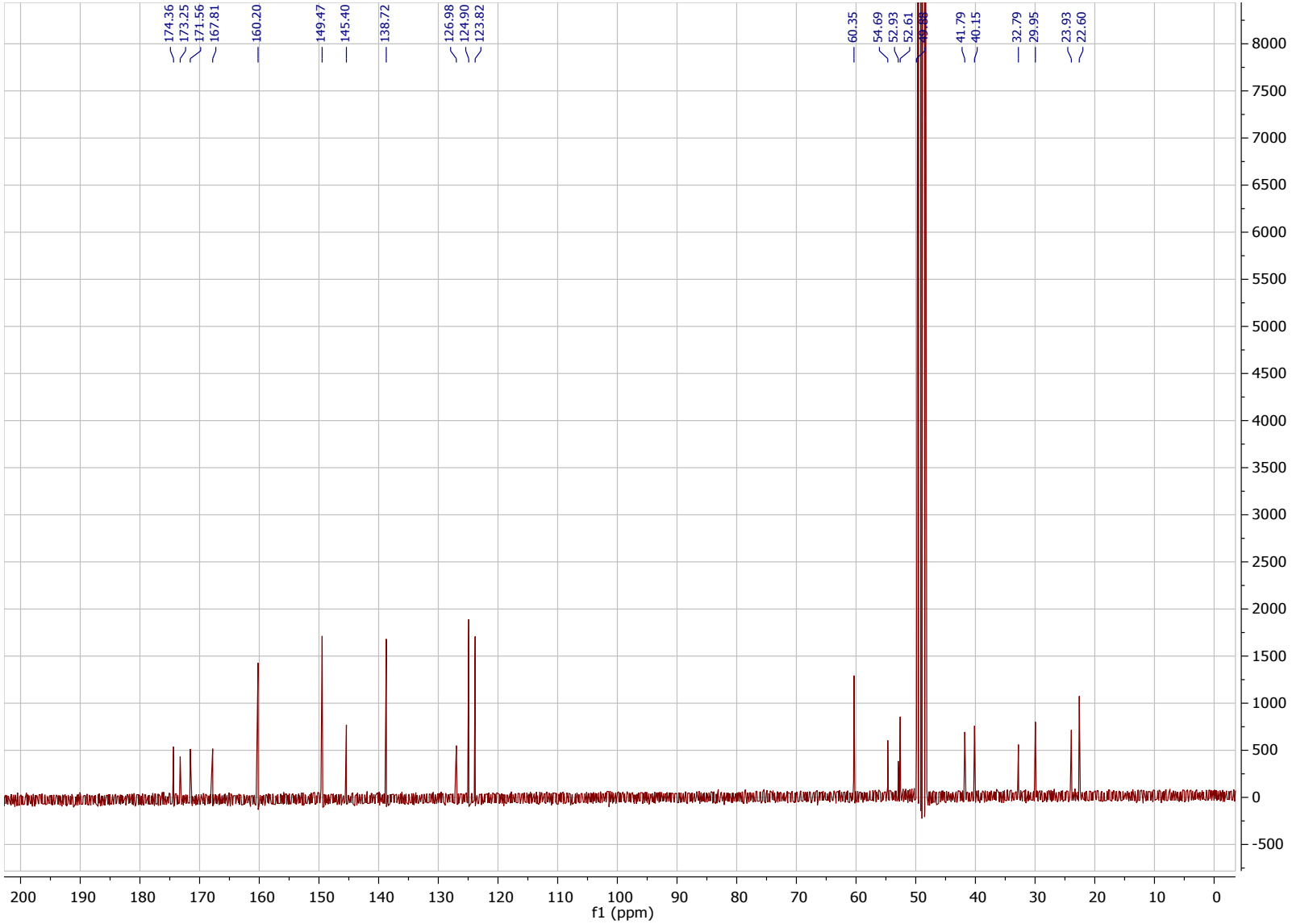
^{1}H NMR and ^{13}C NMR of compound **41**



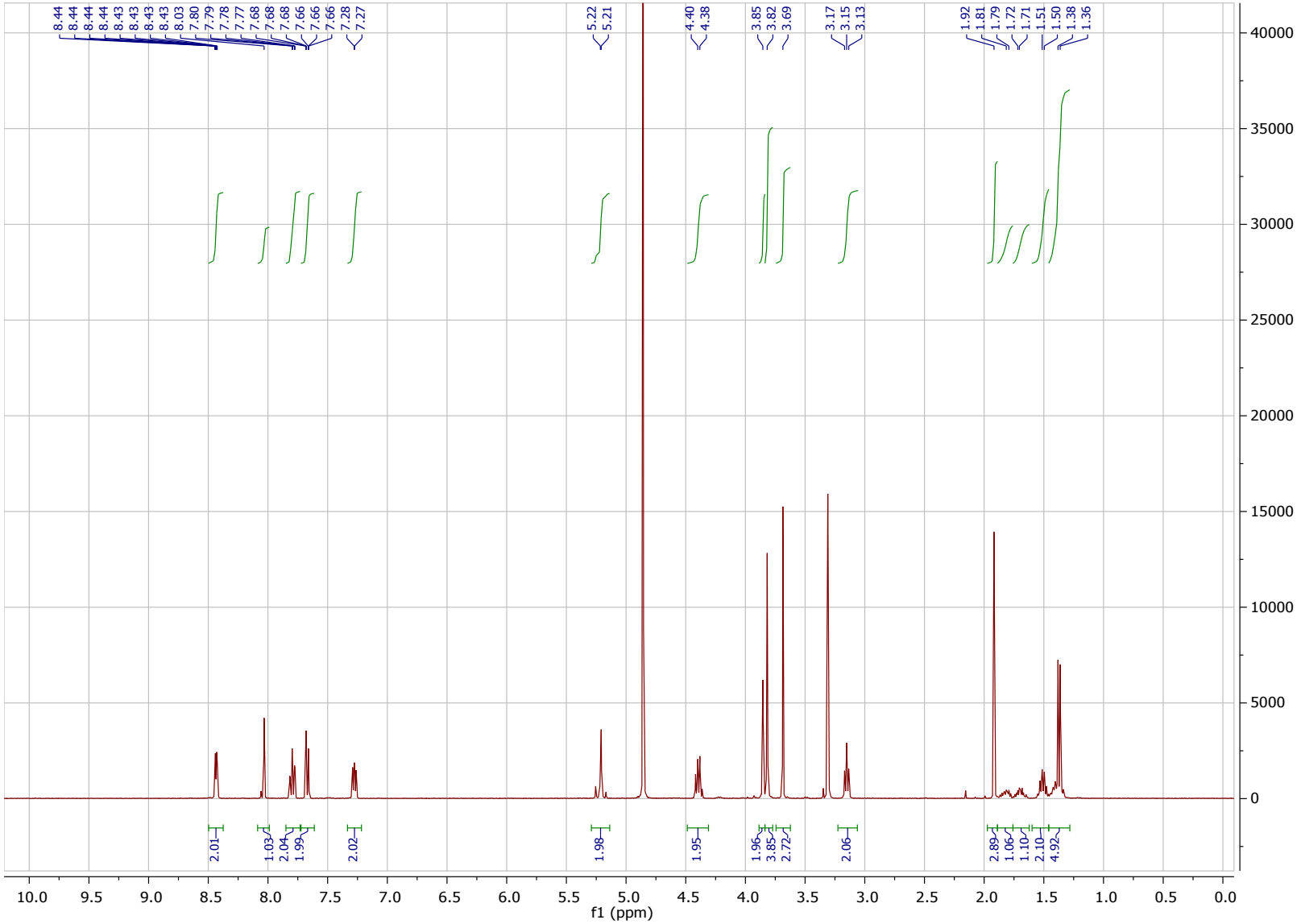

174.41
174.10
173.29
167.79
160.20
149.48
145.37
138.73
126.94
124.90
123.84
60.34
54.48
53.12
52.73
49.50
40.33
32.78
29.90
24.01
22.50
17.44
f1 (ppm)

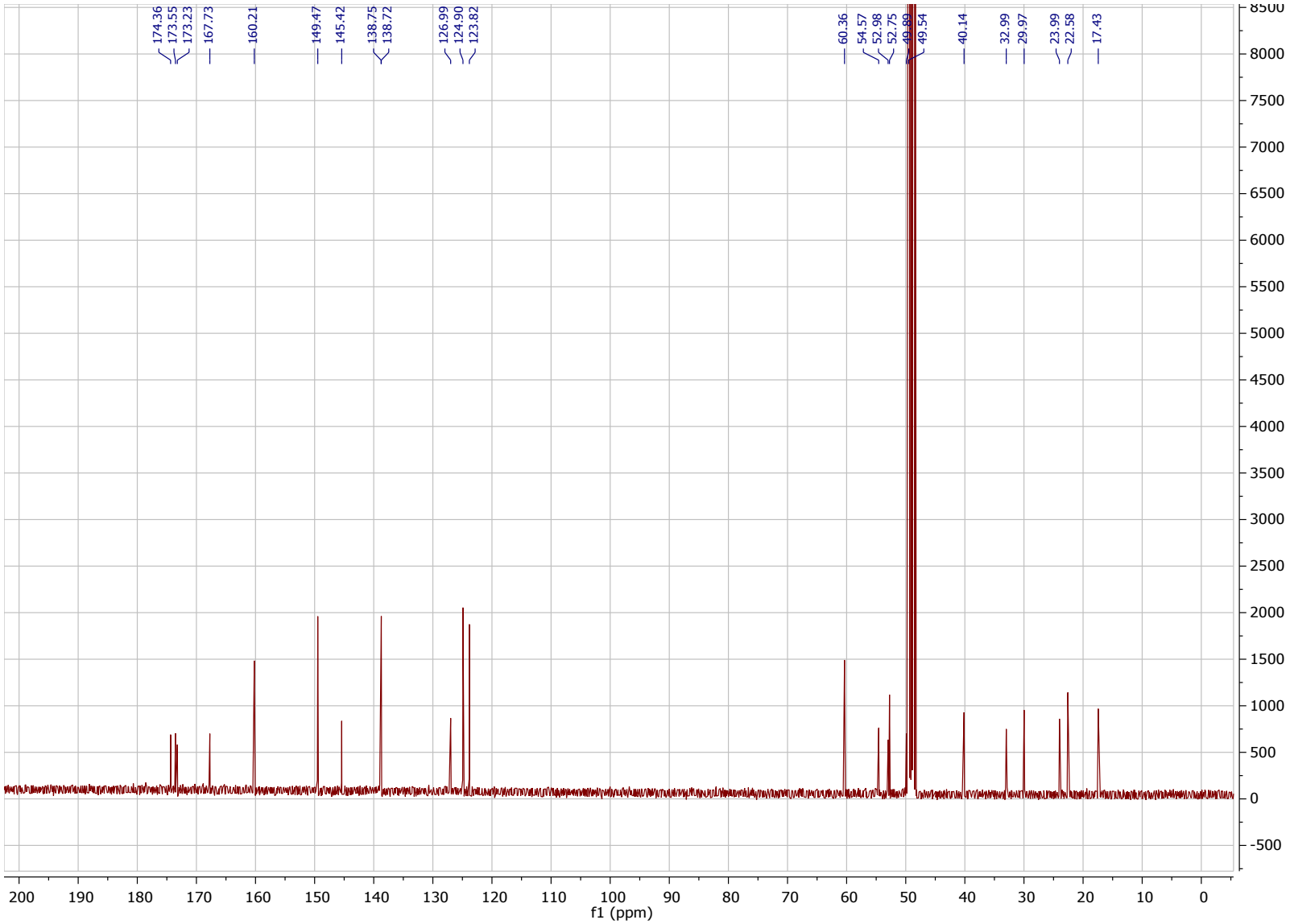
^{1}H NMR and ^{13}C NMR of compound **42**



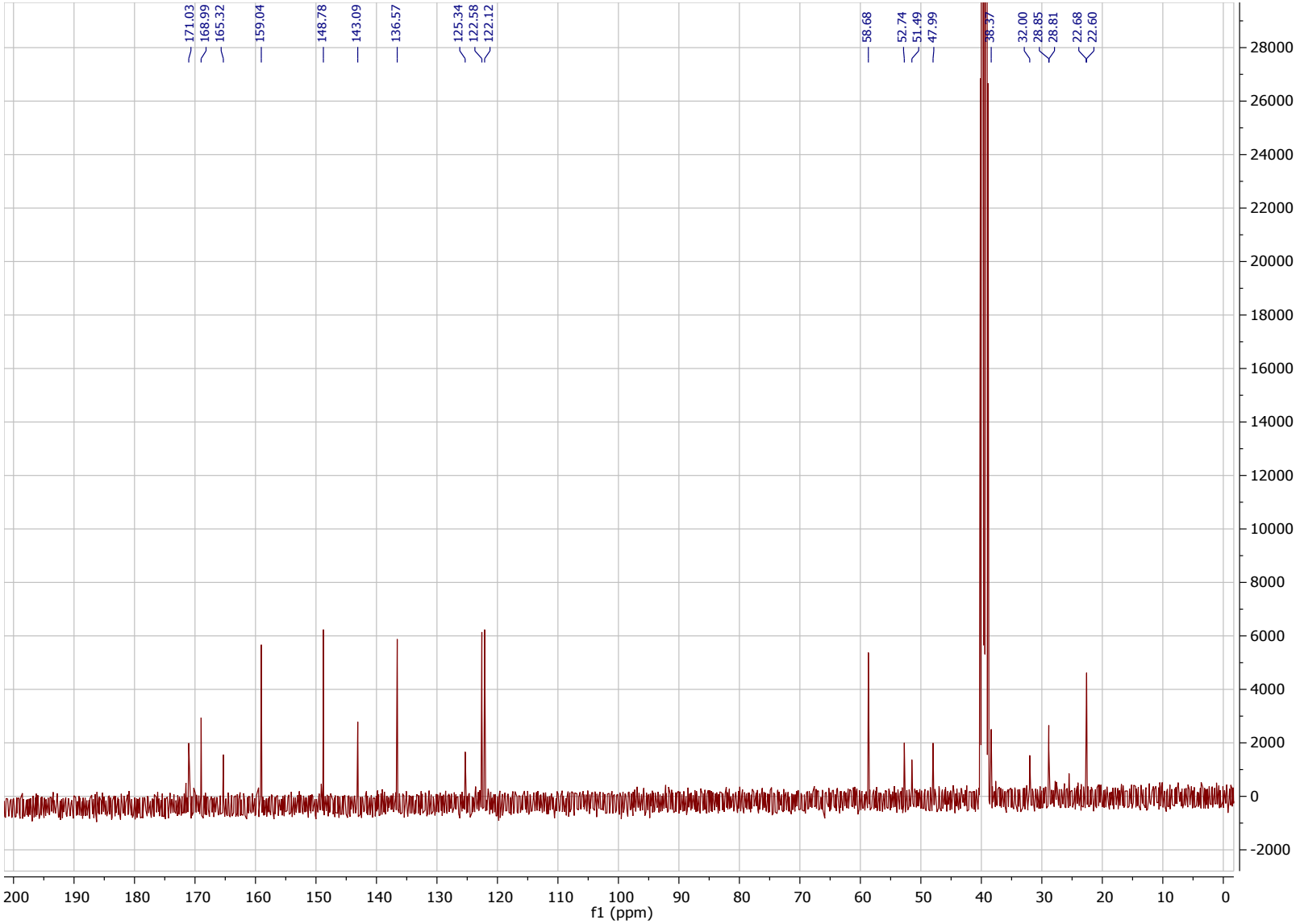

174.36
173.25
171.56
167.81
160.20
149.47
145.40
138.72
126.98
124.90
123.82
60.35
54.69
52.93
52.61
41.79
40.15
32.79
29.95
23.93
22.60
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **43**

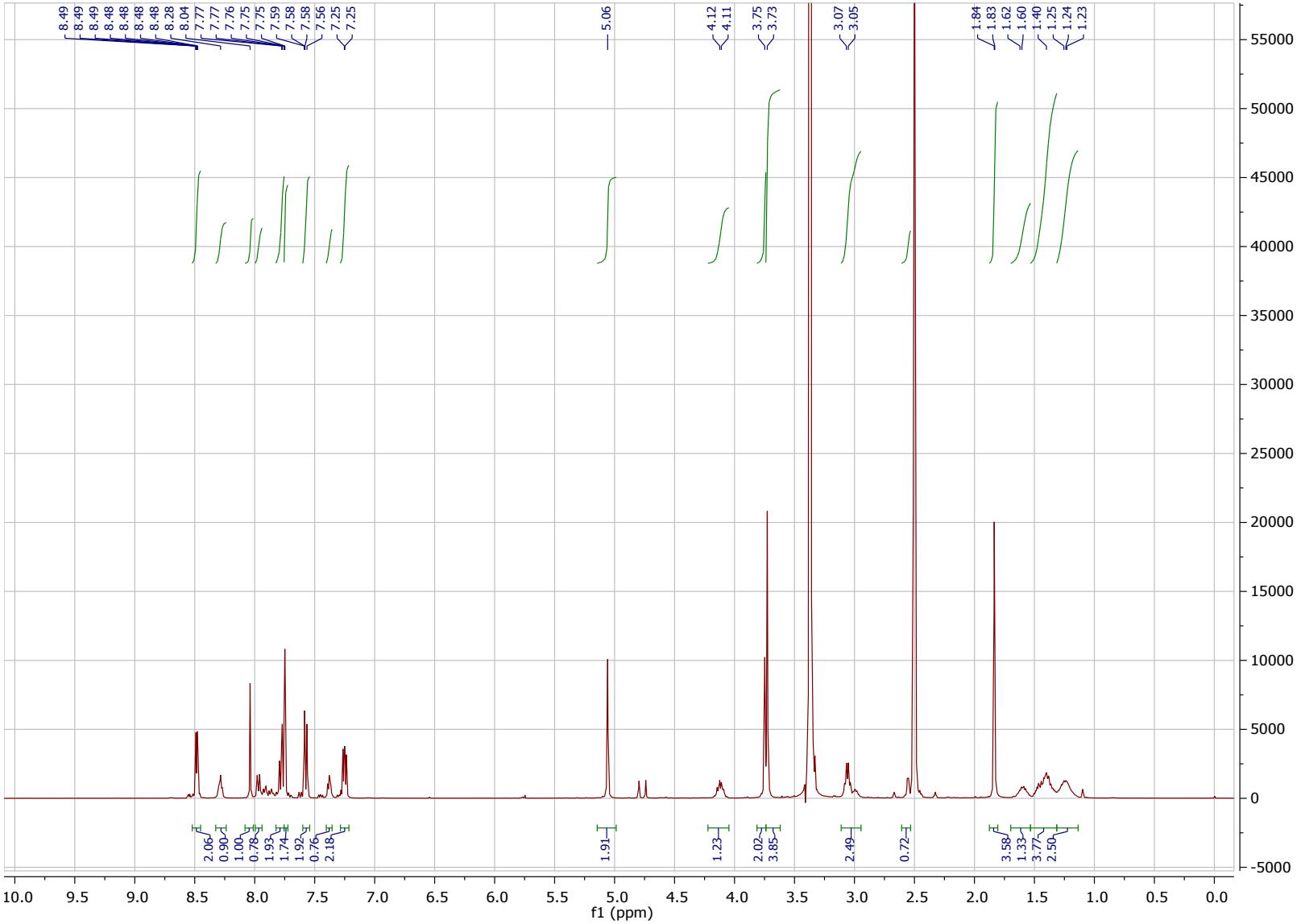


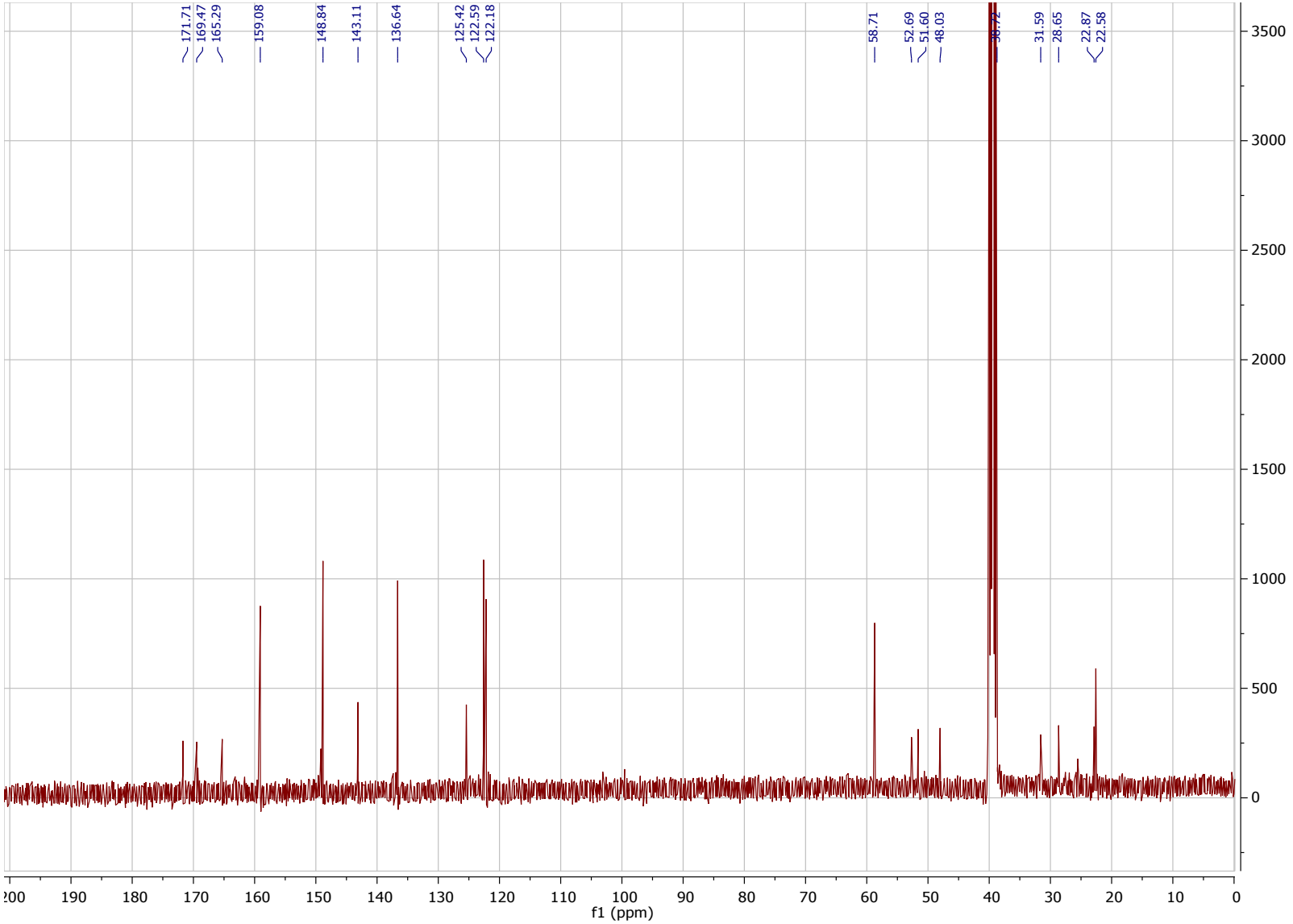

174.36
173.55
173.23
167.73
160.21
149.47
145.42
138.75
138.72
126.99
124.90
123.82
60.36
54.57
52.98
52.75
49.89
49.54
40.14
32.99
29.97
23.99
22.58
17.43
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **44**

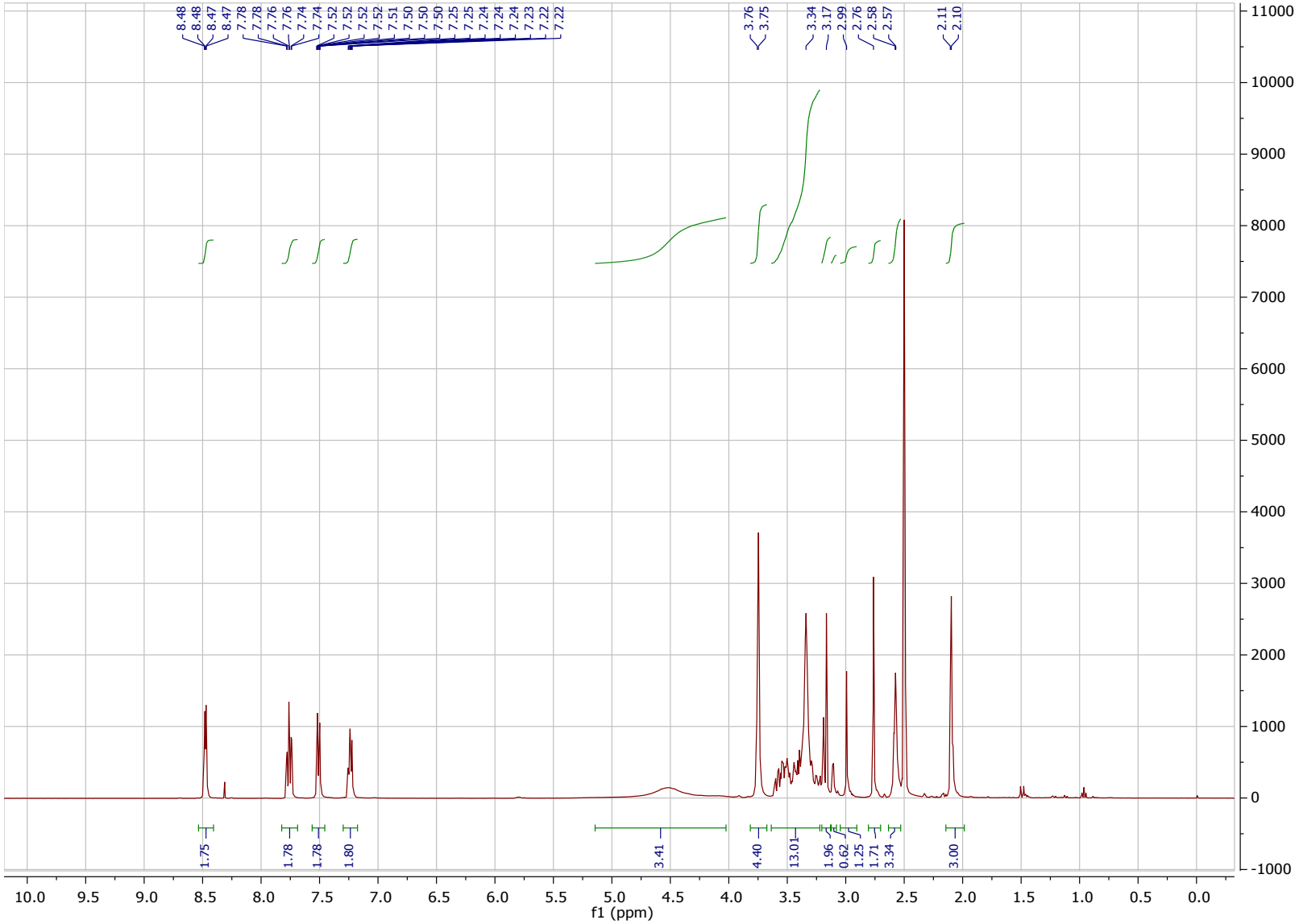

171.03
168.99
165.32
159.04
148.78
143.09
136.57
125.34
122.58
122.12
58.68
52.74
51.49
47.99
38.37
32.00
28.85
28.81
22.68
22.60
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

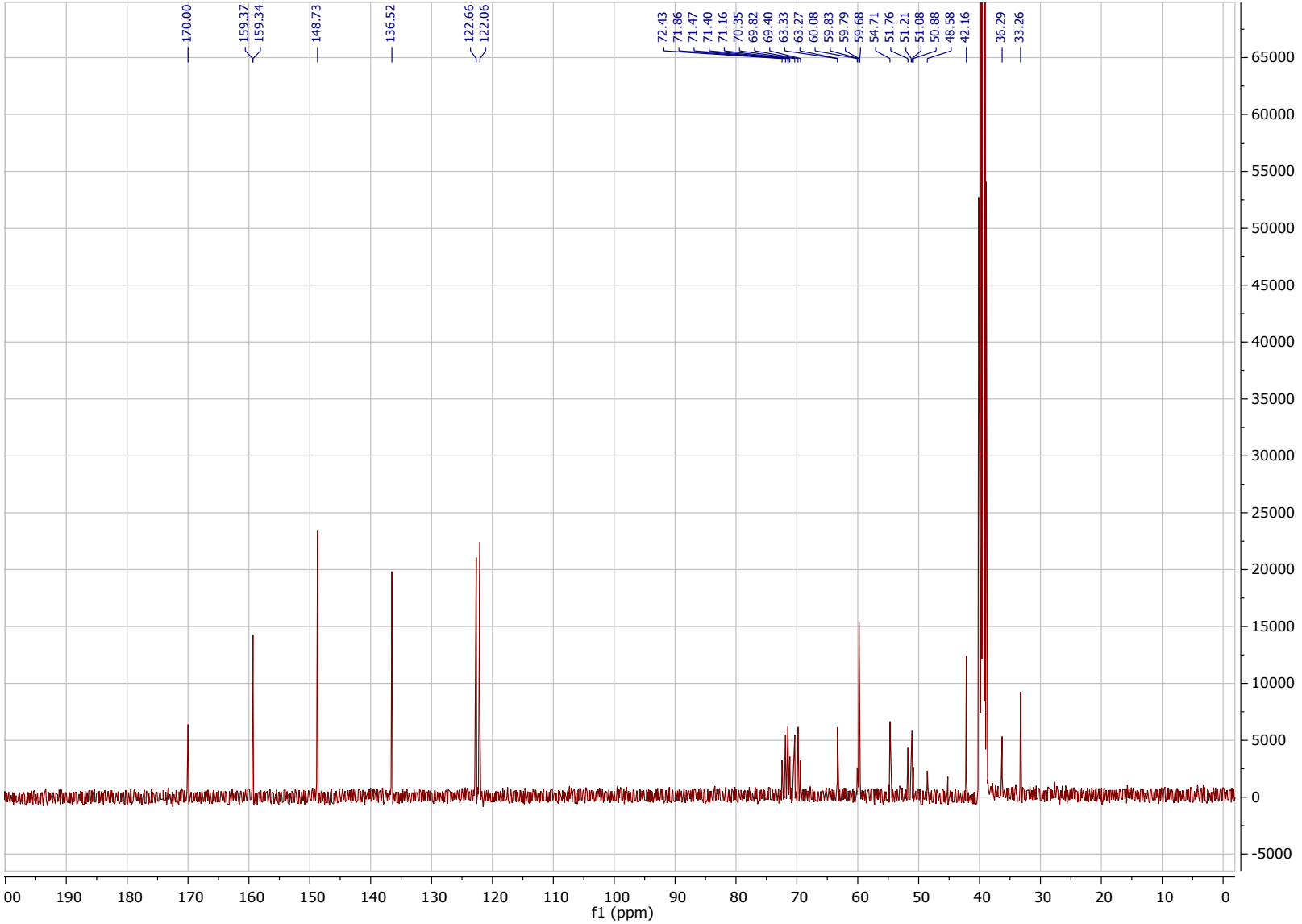
^{1}H NMR and ^{13}C NMR of compound **45**



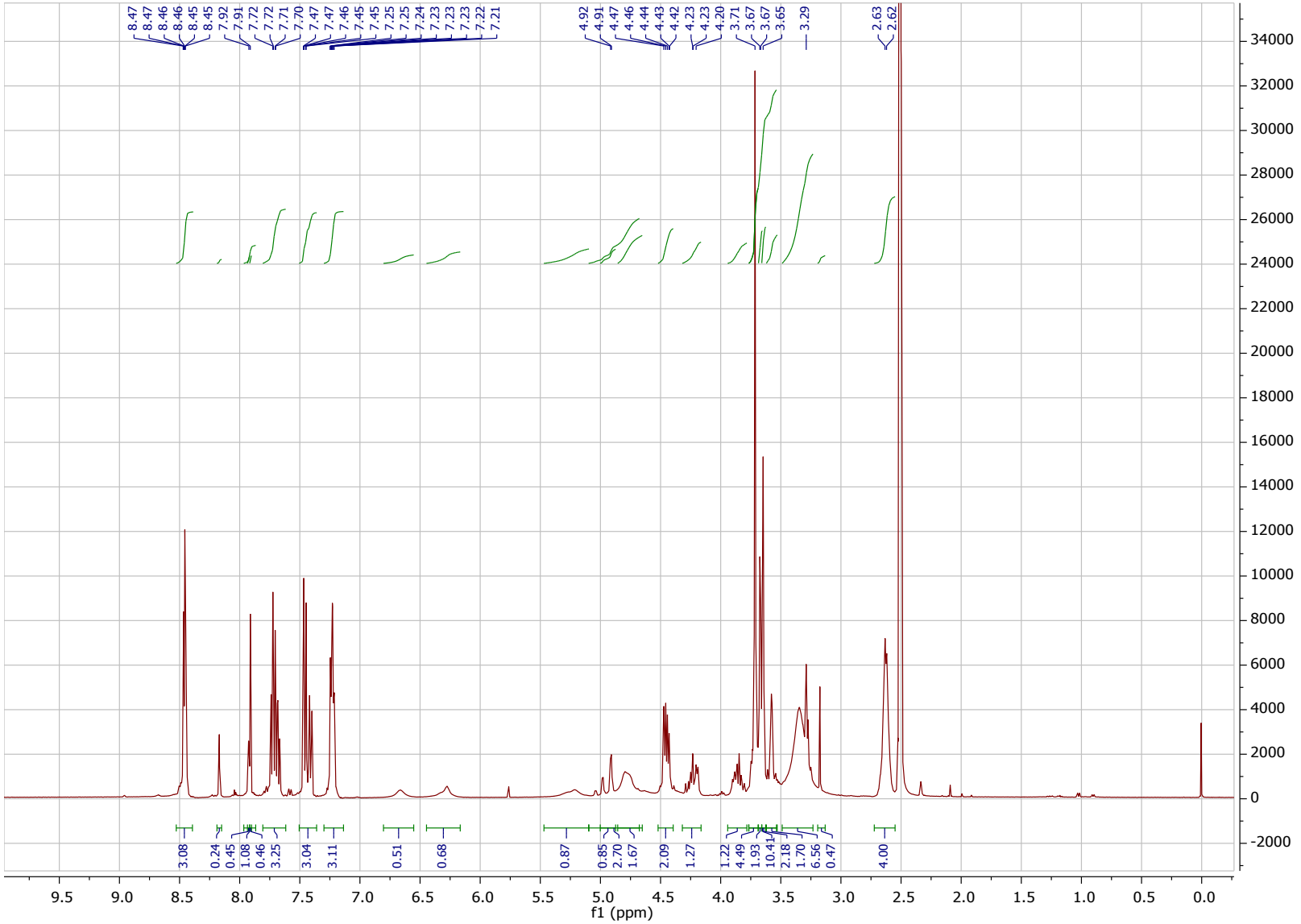

171.71
169.47
165.29
159.08
148.84
143.11
136.64
125.42
122.59
122.18
58.71
52.69
51.60
48.03
39.72
31.59
28.65
22.87
22.58
3500
3000
2500
2000
1500
1000
500
0
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

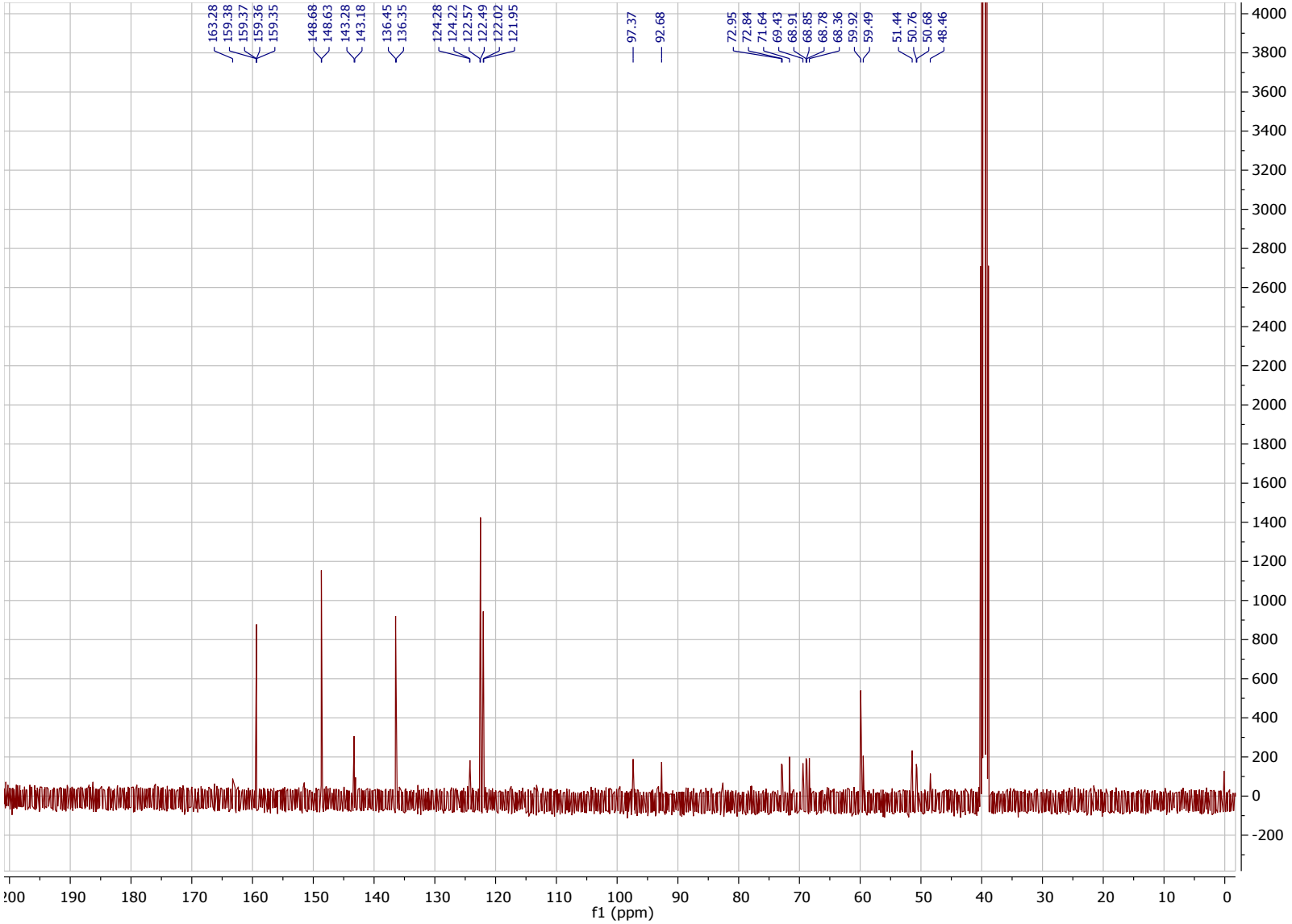
^{1}H NMR and ^{13}C NMR of compound **50**




170.00
159.37
159.34
148.73
136.52
122.66
122.06
72.43
71.86
71.47
71.40
71.16
70.35
69.82
69.40
63.33
63.27
60.08
59.83
59.79
59.68
54.71
51.76
51.21
51.08
50.88
48.58
42.16
36.29
33.26
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR and ^{13}C NMR of compound **51**




163.28
159.38
159.37
159.36
159.35
148.68
148.63
143.28
143.18
136.45
136.35
124.28
124.22
122.57
122.49
122.02
121.95
97.37
92.68
72.95
72.84
71.64
69.43
68.91
68.85
68.78
68.36
59.92
59.49
51.44
50.76
50.68
48.46
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)