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Supporting Information

Synthesis of 1,5-triazole bridged vancomycin CDE-ring bicyclic mimics using RuAAC macrocyclization

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1. Synthetic Procedures

1.1 General Experimental Procedures

Chemicals were used as obtained from commercial sources without further purification unless stated otherwise. Azidoamino acids **16** and **17** were synthesized using literature procedures starting from the corresponding diamino acid residues.[1] The solvents were obtained as peptide synthesis grade and stored over molecular sieves (4 Å) prior to use.

Column chromatography was performed on *Silicycle SiliFlash P60* silica gel (particle size 40-63 μm).

Thin Layer Chromatography was performed on *Merck* precoated silica gel 60F254 glass plates. Compound spots were visualized by UV-quenching, ninhydrin, or Cl_2/TDM.

Optical rotations were measured on a *JASCO* P-1010 Polarimeter using a 10 cm cell with a Na 589 nm filter. The specific concentrations (in g/100 mL) are indicated.

^{1}H-NMR data were acquired on a *Varian* Mercury 300 MHz or a *Varian* Innova 500 MHz spectrometer in $CDCl_3$ or CD_3OD as solvent. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to TMS (0.00 ppm). Coupling constants (*J*) are reported in Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublet (dd), and broad (br). ^{13}C-NMR data were acquired on a *Varian* Mercury 75 MHz or a *Varian* Innova 125 MHz spectrometer in $CDCl_3$ or CD_3OD as solvent. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to the solvent residual signal, $CDCl_3$ (77.0 ppm) or CD_3OD (49.0 ppm).

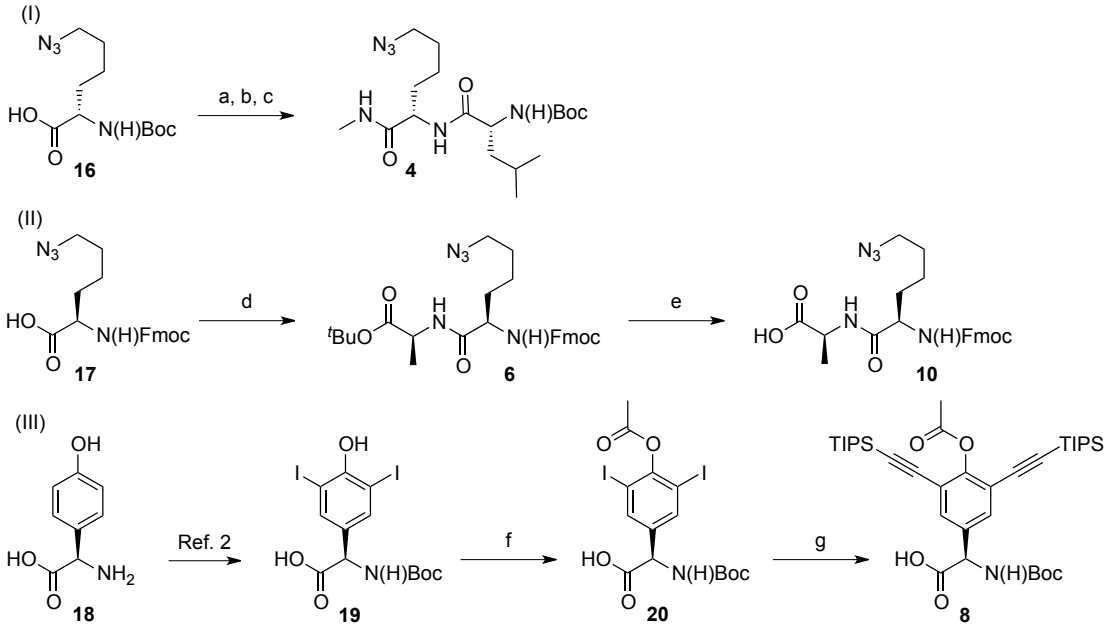
Analytical HPLC was performed on an automated HPLC system (*Shimadzu*) equipped with a UV/Vis detector operating at 220/254 nm and an evaporative light scattering detector using a Dr. Maisch Reprosil-Pur C18-AQ column (pore size: 100Å, particle size: 5 μm; 250×4.6 mm) at a flow rate of 1.0 mL/min (100% buffer A (0.1% TFA in CH_3CN/H_2O 5:95 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 95: 5 v/v) in 60 min).

Preparative HPLC was performed on an automated preparative HPLC system (*Applied Biosystems*) equipped with a UV/Vis detector operating at 214 nm using a Dr. Maisch Reprosil-Pur C18-AQ column (pore size: 100Å, particle size: 10 μm; 250×22 mm) at a flow rate of 2.0 mL/min (100% buffer A (0.1% TFA in CH_3CN/H_2O 95:5 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 5:95 v/v) in 90 min).

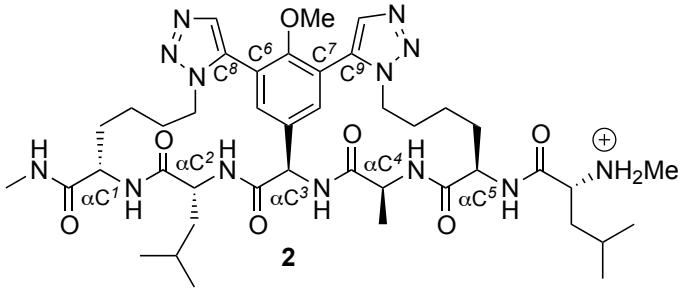
ESI-MS was performed on a *Shimadzu* LCMS-QP8000 electrospray ionization mass spectrometer.

MALDI-TOF MS was performed on a *Shimadzu* Kratos AXIMA-CFR mass spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix and human ACTH (18-39) as the reference.

1.2 Syntheses Schemes



Scheme SI1. Synthesis of, (I) (I) the C-terminal dipeptide: a) $MeNH_2$, BOP, DCM, 20 h; b) TFA/DCM, 1 h; c) Boc-D-Leu-OH, BOP, DIPEA, DCM, 3 h, 82% over three steps; (II) the N-terminal dipeptide: d) H-Ala-O^{t}Bu, BOP, DIPEA, DCM, 3 h, 80%; e) TFA/DCM, 1 h, quant.; and (III) the central amino acid: f) Ac_2O, aq. 1 N NaOH/DCM, 3 h, 88%; g) TIPS-acetylene, $[Pd(PPh_3)_4]$, CuI, DIPEA, THF, 20 h, 91%.



Scheme SI2. The carbon atoms αC^1, αC^2, αC^3, αC^4, αC^5, arom-C^6, arom-C^7 and triazole-C^8 and C^9 have been used as fixed coordinates for superimposition (this Scheme belongs to Figure 1 of the main manuscript).

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1.3 Syntheses and Compound Analyses

Boc-D-Leu-Lys(N_3)-NHMe 4: Boc-Lys(N_3)-OH **16** (2.18 g, 8.0 mmol) was dissolved in DCM (80 mL). To this solution, BOP (3.90 g, 8.8 mmol) was added, and followed by the addition of $MeNH_2$ (2M in THF, 12 mL, 24 mmol) dropwise during 20 min at 0 °C. The mixture was allowed to warm to room temperature and stirred for 20 h. Then, the solvents were removed by evaporation and the residue was redissolved in EtOAc (150 mL). The resulting solution was washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:3 to 1:1, v/v). Boc-Lys(N_3)-NHMe was obtained as white solid (2.10 g, 92%). Subsequently, the obtained amide (1.43 g, 5.0 mmol) was dissolved in DCM (50 mL), and to this solution TFA (30 mL) was added. The reaction mixture was stirred for 1 h, after which the volatiles were removed by evaporation and the residual TFA was removed by coevaporation with DCM (30 mL, twice). After drying for 1 h at high vacuum, the free amine was dissolved in DCM (80 mL). To this solution Boc-D-Leu-OH (1.27 g, 5.5 mmol) and BOP (2.43 g, 5.5 mmol) were added, followed by the addition of D*i*PEA (2.59 mL, 15.0 mmol). The reaction mixture was stirred for 3 h. Then, the solvent was removed and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 3:1 to 1:1, v/v). Boc-D-Leu-Lys(N_3)-NHMe **4** was obtained as viscous oil (1.64 g, 82%); R_f = 0.64 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = -9.0 (c = 1.0 MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.01 (s, 1H), 6.93 (d, *J* = 8.3 Hz, 1H), 5.25 (d, *J* = 5.7 Hz, 1H), 4.41 (q, *J* = 8.3 Hz, 1H), 4.12 – 3.92 (m, 1H), 3.24 (t, *J* = 6.6 Hz, 2H), 2.74 (d, *J* = 4.5 Hz, 3H), 1.91 (s, 1H), 1.75 – 1.52 (m, 5H), 1.52 – 1.30 (m, 12H), 0.91 (t, *J* = 5.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 173.2, 172.0, 156.1, 80.3, 53.8, 53.0, 51.1, 40.8, 31.4, 28.4, 28.3, 26.2, 24.7, 22.9, 22.7, 22.1; MS (ESI) *m/z* calcd for $C_{18}H_{35}N_6O_4$ [M+H]+ 399.27, found 399.45; calcd for $C_{18}H_{34}N_6NaO_4$ [M+Na]+ 421.25, found 421.20.

The synthesis of Fmoc-D-Lys(N_3)-Ala-O^{t}Bu (6) and Fmoc-D-Lys(N_3)-Ala-OH (10): Fmoc-D-Lys(N_3)-OH **17** (680 mg, 1.72 mmol) was dissolved in DCM (30 mL). To this solution H-Ala-O^{t}Bu·HCl (343 mg, 1.89 mmol) and BOP (836 mg, 1.89 mmol) were added, followed by the addition of D*i*PEA (743 µL, 4.30 mmol). The reaction mixture was stirred for 3 h. Then, the solvent was removed and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 4:1, v/v). Fmoc-D-Lys(N_3)-Ala-O^{t}Bu **6** was obtained as a white solid (720 mg, 80%); R_f = 0.76 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = +1.3 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.76 (d, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.40 (dd, *J* = 7.4, 6.9 Hz, 2H), 7.31 (td, *J* = 7.4, 1.2 Hz, 2H), 6.58 (d, *J* = 6.0 Hz, 1H), 5.39 (d, *J* = 8.0 Hz, 1H), 4.44 (dt, *J* = 9.5, 4.8 Hz, 3H), 4.22 (t, *J* = 6.8 Hz, 2H), 3.26 (t, *J* = 6.7 Hz, 2H), 1.98 – 1.78 (m, 1H), 1.76 – 1.51 (m, 3H), 1.48 – 1.40 (m, 12H), 1.37 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz,

$CDCl_3$) δ = 172.1, 170.9, 156.3, 144.0, 144.0, 141.5, 128.0, 127.3, 125.3, 120.2, 82.5, 67.3, 54.8, 51.3, 49.0, 47.4, 32.6, 28.7, 28.2, 22.8, 18.8; MS (ESI) *m/z* calcd for $C_{28}H_{36}N_5O_5$ [M+H]+ 522.27, found 522.00; calcd for $C_{28}H_{35}N_5NaO_5$ [M+Na]+ 544.25, found 544.55.
Fmoc-D-Lys(N_3)-Ala-OH **10** was obtained in quantitative yield by treatment of dipeptide *tert*-butyl ester **6** with TFA and used without further purification.

(*R*)-*N*-α-Boc-(4-hydroxy-3,5-diiodo)phenylglycine 19 was synthesized according to a literature procedure.[2] D-4-Hydroxyphenylglycine **18** (10.5 g, 63.0 mmol) was dissolved in AcOH (90 mL). To this solution ICl (22.5 g, 138.6 mmol) in AcOH (5.0 mL) was added dropwise during 10 min under argon. After stirring for 72 h at room temperature, the reaction mixture was poured into ice water (1000 mL). The precipitated crystals were filtered off, washed with EtOH (100 mL twice) to provide 3,5-diiodo-D-4-hydroxyphenylglycine (22.1 g, 85%) as light brown crystals and was used without further purification in the next step. 3,5-Diiodo-D-4-hydroxy- phenylglycine (4.19 g, 10 mmol) was dissolved in H_2O/dioxane (60 mL, 1:1, v/v). To this solution Boc_2O (2.62 g, 12 mmol) and Et_3N (2.1 mL, 15 mmol) were added. The reaction mixture was stirred for 4 h. The reaction mixture was diluted with EtOAc (50 mL) and the resulting solution was extracted with H_2O (50 mL, twice). The aqueous phase was combined and acidified with $KHSO_4$ to pH 2-3, extracted with EtOAc (100 mL, twice). The organic phase was washed with brine (150 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 3:1, v/v, with 0.1% AcOH). Diiodo compound **19** was obtained as a light yellowish solid (3.54 mg, 68%); R_f = 0.57 (hexane/EtOAc, 1:1, v/v, with 0.1% AcOH); $[\alpha]_D^{20}$ = -94.3 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.02 (s, 1H), 7.75 (s, 2H), 4.97 (d, *J*=4.8 Hz, 1H), 1.29 (s, 9H).

(*R*)-*N*-α-Boc-(4-acetoxy-3,5-diiodo)phenylglycine 20 was synthesized according to a literature procedure.[3] Hydroxy compound **19** (2.60 g, 5.00 mmol) was dissolved in ice-cold aq. 1N NaOH/DCM (60 mL, 1:1, v/v) and to this mixture, Ac_2O (2.36 mL, 25.0 mmol) was added dropwise at 0 °C. Then, the reaction mixture was stirred at room temperature for 3 h. Subsequently, the aqueous solution was acidified with $KHSO_4$ to pH 2-3 and extracted with DCM (30 mL, twice). The organic phases were combined and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:3 to 1:2, v/v, with 0.1% HOAc). Acetyl ester **20** was obtained as a white solid (2.47 g, 88%); R_f = 0.61 (hexane/EtOAc, 1:1, v/v, with 0.1% HOAc); $[\alpha]_D^{20}$ = -72.6 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.08 (s, 1H), 7.86 (s, 2H), 5.02 (d, *J* = 3.7 Hz, 1H), 2.39 (s, 3H), 1.26 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.5, 167.4, 157.0, 151.5, 139.5, 138.3, 90.5, 83.1, 57.3, 28.3, 21.6.

Bis-alkyne 8: Diiodo compound **20** (2.24 g, 4.0 mmol), [$Pd(PPh_3)_4$] (462 mg, 0.4 mmol), and CuI (228 mg, 1.2 mmol) were placed in a flask sealed with a rubber septum. The flask was evacuated and refilled with dry N_2 (repeated for three times). THF (40 mL) (purged with dry N_2 for 1 h prior to use) was added to the flask via a syringe. The resulting solution was degassed again using a freeze-pump-thaw procedure (repeated three times). Then, D*i*PEA (1.39 mL, 8.0 mmol) and TIPS-acetylene (3.60 mL, 16 mmol) were added to the mixture via a

syringe. After stirring the reaction mixture for 20 h at room temperature under N_2, the resulting suspension was filtered through a path of celite and the filtrate was evaporated to dryness. Subsequently the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:4 to 1:2, v/v, with 0.1% AcOH). Bis-alkyne **8** was obtained as a yellowish solid (2.45 g, 91%). R_f = 0.26 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = -51.4 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.99 (s, 1H), 7.51 (s, 2H), 5.04 (s, 1H), 2.28 (s, 3H), 1.26 (s, 9H), 1.11 (s, 42H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.6, 167.4, 156.9, 152.6, 135.9, 132.4, 118.6, 100.6, 96.6, 82.7, 58.2, 27.9, 20.6, 18.6, 11.2; MS (ESI) *m/z* calcd for $C_{37}H_{59}NNaO_6Si_2$ $[M+Na]^+$ 692.38, found 692.25. This compound is abbreviated as: D-Phg(4-OAc-3,5-bis-TIPS-alkyne).

Boc-D-Phg(4-OAc-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe 9:
Boc-D-Leu-Lys(N_3)-NHMe **4** (877 mg, 2.2 mmol) was dissolved in DCM (15 mL), and to this solution, TFA (15 mL) was added. The reaction mixture was stirred for 1 h, after which the volatiles were removed by evaporation and the residual TFA was removed by coevaporation with DCM (20 mL, twice). After drying for 1 h at high vacuum, the free amine was dissolved in DCM (60 mL). To this solution Boc-D-Phg(4-OAc-3,5-bis-TIPS-alkyne) compound **8** (1.34 g, 2.0 mmol) and BOP (973 mg, 2.2 mmol) were added, followed by the addition of D*i*PEA (1.04 mL, 6.0 mmol). This reaction mixture was stirred for 3 h. Then, the solvent was removed by evaporation and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 3:1 to 1:1, v/v). Boc-D-Phg(4-OAc-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **9** was obtained as a diastereoisomeric mixture: *RRS*:*SRS* = 5.4:1 based on analytical HPLC (1.60 g, 84%); MS (ESI) *m/z* calcd for $C_{50}H_{84}N_7O_7Si_2$ $[M+H]^+$ 950.60, found 951.00.

Fmoc-D-Lys(N_3)-Ala-D-Phg(4-OAc-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe 11:
Boc-D-Phg(4-OAc-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **9** (300 mg, 0.316 mmol) was dissolved in 8 mL of DCM, and to this solution TFA (2 mL) was added. The obtained reaction mixture was stirred for 1 h. Then, the volatiles were removed by evaporation and the residual TFA was removed by coevaporation with DCM (10 mL, twice). After drying for 1 h at high vacuum, the free amine was dissolved in DCM (10 mL). To this solution, Fmoc-D-Lys(N_3)-Ala-OH **10** (161 mg, 0.347 mmol), EDCI (66.3 mg, 0.347 mmol), and HOBt (46.9 mg, 0.347 mmol) were added, followed by the addition of D*i*PEA (192 µL, 1.11 mmol), and the reaction mixture was stirred for 4 h. Subsequently the solvent was removed by evaporation and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 1:99 to 2:98, v/v). Fmoc-D-Lys(N_3)-Ala-D-Phg(4-OAc-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **11** was obtained as a white solid (230 mg, 56%); R_f = 0.68 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = +19.7 (c

= 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.76 (d, *J* = 7.4 Hz, 2H), 7.70 (d, *J* = 8.6 Hz, 1H), 7.60 (dd, *J* = 14.2, 7.4 Hz, 2H), 7.45 (s, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.31 (d, *J* = 7.5 Hz, 2H), 7.19 (s, 1H), 7.15 – 7.07 (m, 1H), 6.91 (s, 1H), 6.51 (d, *J* = 16.5 Hz, 1H), 5.20 (d, *J* = 4.4 Hz, 1H), 4.59 – 4.31 (m, 3H), 4.23 (dd, *J* = 13.5, 6.7 Hz, 3H), 4.06 – 3.92 (m, 1H), 3.27 – 3.18 (m, 4H), 2.75 (d, *J* = 3.7 Hz, 3H), 2.31 (s, 3H), 1.95 – 1.65 (m, 6H), 1.60 – 1.52 (m, 4H), 1.47 – 1.34 (m, 8H), 1.11 (s, 42H), 0.92 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 174.0, 173.5, 173.0, 172.7, 169.0, 167.6, 157.8, 153.5, 143.9, 143.6, 141.3, 133.3, 127.7, 127.1, 127.0, 125.2, 125.1, 119.9, 119.6, 100.0, 98.0, 67.1, 59.1, 54.0, 53.6, 52.0, 51.1, 50.6, 47.2, 39.2, 30.4, 29.7, 28.5, 28.4, 26.2, 25.2, 23.3, 23.1, 23.0, 20.7, 20.6, 18.6, 16.3, 11.2, -0.0; MS (ESI) *m/z* calcd for $C_{69}H_{101}N_{12}O_9Si_2$ $[M+H]^+$ 1297.74, found 1297.75.

Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OH-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe 12: Fmoc-D-Lys(N_3)-Ala-D-Phg(4-OAc-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **11** (430 mg, 0.331 mmol) was dissolved in THF (10 mL) and to this solution $(CH_3)_2NH$ (8.28 mL, 2M in THF, 16.6 mmol) was added, followed by the addition of 1-propanethiol (292 µL, 3.31 mmol).[4] The resulting reaction mixture was stirred for 1 h. After removal of the solvent under reduced pressure, the free amine was obtained and after drying for 1 h at high vacuum, the free amine was dissolved in DCM (20 mL), and to this solution Boc-*N*-Me-D-Leu-OH (81 mg, 0.331 mmol), EDCI (69.5 mg, 0.364 mmol), and HOBt (49.2 mg, 0.364 mmol) were added, followed by the addition of D*i*PEA (126 µL, 0.728 mmol). The reaction mixture was stirred for 3 h. Then, the solvent was removed by evaporation and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 1:99 to 2:98, v/v). Hexapeptide, Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OH-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **12** was obtained as a white solid (310 mg, 74%); R_f = 0.55 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = +25.5 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD) δ = 7.43 (s, 2H), 5.14 (s, 1H), 4.65 – 4.48 (m, 1H), 4.40 – 4.11 (m, 3H), 3.29 (d, *J* = 6.4 Hz, 4H), 2.73 (s, 6H), 1.58 – 1.85 (m, 12H), 1.47 (s, 12H), 1.37 (d, *J* = 6.8 Hz, 4H), 1.15 (s, 44H), 1.01 – 0.82 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$) δ = 177.8, 177.1, 177.0, 176.4, 175.4, 162.8, 136.6, 131.3, 115.4, 104.9, 101.0, 84.2, 62.2, 59.6, 58.0, 57.2, 56.5, 54.9, 54.8, 53.0, 43.1, 40.3, 34.8, 33.8, 32.2, 31.8, 31.5, 29.4, 28.6, 28.5, 26.9, 26.7, 26.6, 26.4, 25.0, 24.4, 21.9, 20.3, 15.0; MS (ESI) *m/z* calcd for $C_{64}H_{110}N_{13}O_9Si_2$ $[M+H]^+$ 1260.81, found 1261.20.

Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe 13:

Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OH-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **12** (310 mg, 0.246 mmol) was dissolved in acetone (20 mL). To this solution, MeI (46 µL, 0.738 mmol) and K_2CO_3 (102 mg, 0.738 mmol) were added. The obtained reaction mixture was stirred for 24 h. After the reaction was complete, as judged by ESI-MS, the solvent was removed by evaporation and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, once), saturated $NaHCO_3$ (50 mL, once) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and

removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 2:98, v/v). The protected hexapeptide Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **13** was obtained as a white solid (295 mg, 94%); R_f = 0.55 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = +26.9 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.63 (s, 1H), 7.50 (s, 1H), 7.34 (s, 2H), 6.89 (s, 1H), 6.68 (d, *J* = 17.3 Hz, 1H), 5.19 (s, 1H), 4.53 (s, 1H), 4.42 (s, 1H), 4.26 (s, 1H), 4.01 (s, 1H), 3.99 (s, 3H), 3.26 (d, *J* = 3.1 Hz, 4H), 2.86 – 2.71 (m, 6H), 1.77 (d, *J* = 11.7 Hz, 8H), 1.57 – 1.64 (m, 4H), 1.43 (s, 12H), 1.39 (d, *J* = 6.4 Hz, 4H), 1.12 (s, 44H), 0.93 (s, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 173.5, 173.0, 172.4, 169.3, 163.2, 133.4, 131.0, 119.0, 101.5, 96.8, 80.3, 77.2, 61.1, 58.8, 57.5, 53.5, 51.9, 51.1, 51.1, 50.3, 39.8, 37.0, 30.7, 28.4, 28.3, 26.1, 25.2, 25.0, 23.4, 23.2, 23.0, 21.8, 20.8, 18.6, 16.0, 11.6, 11.3, 11.2, 10.9, -0.0; MS (ESI) *m/z* calcd for $C_{65}H_{112}N_{13}O_9Si_2$ $[M+H]^+$ 1274.82, found 1275.05.

Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-alkyne)-D-Leu-Lys(N_3)-NHMe 14: Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **13** (275 mg, 0.216 mmol) was dissolved in THF/MeOH (20 mL, 19:1, v/v), and to this solution TBAF (0.65 mL, 1M in THF, 0.647 mmol) was added. After stirring for 1 h, another portion of TBAF (0.65 mL, 1M in THF) was added. The obtained reaction mixture was stirred for another 2h. Based on TLC analysis, the reaction was complete. Subsequently the solution was diluted with EtOAc (100 mL) and the resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, once), saturated $NaHCO_3$ (50 mL, once) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 2:98 to 4:96, v/v), and Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-alkyne)-D-Leu-Lys(N_3)-NHMe **14** was obtained as a white solid (190 mg, 91%); R_f = 0.49 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = -15.0 (c = 1.0 MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ = 7.52 (s, 2H), 5.28 (s, 1H), 4.73 – 4.48 (m, 1H), 4.27 (t, *J* = 9.2 Hz, 4H), 3.99 (s, 3H), 3.79 (s, 2H), 3.42 – 3.17 (m, 4H), 2.72 (d, *J* = 4.0 Hz, 6H), 1.96 – 1.51 (m, 10H), 1.51 – 1.22 (m, 20H), 0.93 (dd, *J* = 15.2, 6.1 Hz, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 177.6, 177.1, 177.0, 176.5, 174.9, 166.8, 137.7, 135.7, 120.8, 86.9, 84.1, 82.2, 81.8, 64.3, 61.2, 59.9, 57.6, 57.3, 57.2, 56.7, 54.8, 54.8, 53.0, 43.2, 40.6, 34.7, 34.6, 33.6, 32.1, 31.9, 31.2, 29.1, 28.5, 26.9, 26.7, 26.2, 25.9, 24.4, 21.7, 20.2; MS (ESI) *m/z* calcd for $C_{47}H_{72}N_{13}O_9$ $[M+H]^+$ 962.56, found 963.05; calcd for $C_{47}H_{71}N_{13}NaO_9$ $[M+Na]^+$ 984.54, found 985.30; MALDI-TOF MS *m/z* calcd for $C_{47}H_{71}N_{13}NaO_9$ $[M+Na]^+$ 984.540, found 984.813; calcd for $C_{47}H_{71}KN_{13}NO_9$ $[M+K]^+$ 1000.513, found 1000.788.

***N*-Boc protected bicyclic hexapeptide 15:** Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-alkyne)-D-Leu-Lys(N_3)-NHMe **14** (75 mg, 0.078 mmol) and $[Cp^*RuCl]_4$ (25.2 mg, 0.0234 mmol) were placed in a capped flask. The flask was evacuated and refilled with dry N_2 (repeated three times). Then, THF/MeOH (15.6 mL, 19:1, v/v) was added to the flask via a syringe. The solvents were purged with dry N_2 for 1 h prior to use. The resulting solution was degassed using a free-pump-thaw procedure (repeated three times). Then, the reaction mixture was stirred for 24 h at 50 °C under N_2, after which the solvents were removed under reduced pressure and the residue was absorbed on silica gel and purified by column chromatography (MeOH/DCM, 5:95 to 10:90, v/v). A product fraction was obtained which was further

purified with preparative RP-HPLC. *N*-Boc-protected bicyclic hexapeptide **15** was obtained as a white solid after lyophilization (30 mg, 40%); R_f = 0.25 (DCM/MeOH, 9:1, v/v); ^{1}H NMR (500 MHz, CD_3OD) δ = 8.02 (s, 1H), 7.86 (s, 1H), 7.82 (s, 1H), 7.55 (s, 1H), 5.73 (s, 1H), 4.59 (s, 1H), 4.45 – 4.28 (m, 3H), 4.28 – 4.05 (m, 5H), 3.96 – 3.86 (m, 1H), 3.19 (s, 3H), 2.89 (s, 3H), 2.69 (s, 3H), 2.06 – 1.77 (m, 5H), 1.75 – 1.63 (m, 6H), 1.57 – 1.49 (m, 3H), 1.47 (s, 9H), 1.42 (d, *J* = 7.3 Hz, 3H), 1.29 (s, 3H), 1.23 – 1.04 (m, 2H), 0.96 (d, *J* = 6.5 Hz, 11H), 0.90 (d, *J* = 6.4 Hz, 6H); MS (ESI) *m/z* calcd for $C_{47}H_{72}N_{13}O_9$ $[M+H]^+$ 962.56, found 963.05; calcd for $C_{47}H_{71}N_{13}NaO_9$ $[M+Na]^+$ 984.54, found 984.60; MALDI-TOF MS *m/z* calcd for $C_{47}H_{71}N_{13}NaO_9$ $[M+Na]^+$ 984.540, found 984.740; calcd for $C_{47}H_{71}KN_{13}NO_9$ $[M+K]^+$ 1000.513, found 1000.701.

Bicyclic hexapeptide 2: *N*-Boc-protected bicyclic hexapeptide **15** (20 mg, 0.021 mmol) was treated with TFA (1 mL) in DCM (1 mL) for 1 h, after which the volatiles were removed under reduced pressure. The residue was purified with preparative RP-HPLC. Bicyclic hexapeptide **2** was obtained as a white solid after lyophilization (10.2 mg, 55%); HPLC analysis, R_t = 18.9 min; MS (ESI) *m/z* calcd for $C_{42}H_{64}N_{13}O_7$ $[M+H]^+$ 862.51, found 862.85; MALDI-TOF MS *m/z* calcd for $C_{42}H_{64}N_{13}O_7$ $[M+H]^+$ 862.505, found 862.685; calcd for $C_{42}H_{63}N_{13}NaO_7$ $[M+Na]^+$ 884.487, found 862.685.

1.4 References

1 Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007**, *9*, 3797-3800.
2 Yamada, Y.; Akiba, A.; Arima, S.; Okada, C.; Yoshida, K.; Itou, F.; Kai, T.; Satou, T.; Takeda, K.; Harigaya, Y. *Chem. Pharm. Bull.* **2005**, *53*, 1277-1290.
3 Tran-Huu-Dau, M. E.; Wartchow, R.; Winterfeldt, E.; Wong, Y. S. *Chem. Eur. J.* **2001**, *7*, 2349-2369.
4 Ueki, M.; Nishigaki, N.; Aoki, H.; Tsurusaki, T.; Katoh, T. *Chem. Lett.* **1993**, 721-724.

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2. ITC Experiments

Binding affinity measurement was determined by using microcalorimetry, which was performed on automated MicroCal Auto-iTC$_{200}$ equipment.

ITC (isothermal titration calorimetry) experiment was carried out by injection the ligand solution (10-15 mM) into the cell containing the solution of the synthesized mimics or vancomycin (0.1-0.3 mM) dissolved in a 0.02 M Na-citrate/citric acid buffer (pH 5.1). The typical experiment contains 16 injections in 40 min and the resulting data was analyzed by non-linear fitting in Origin software.

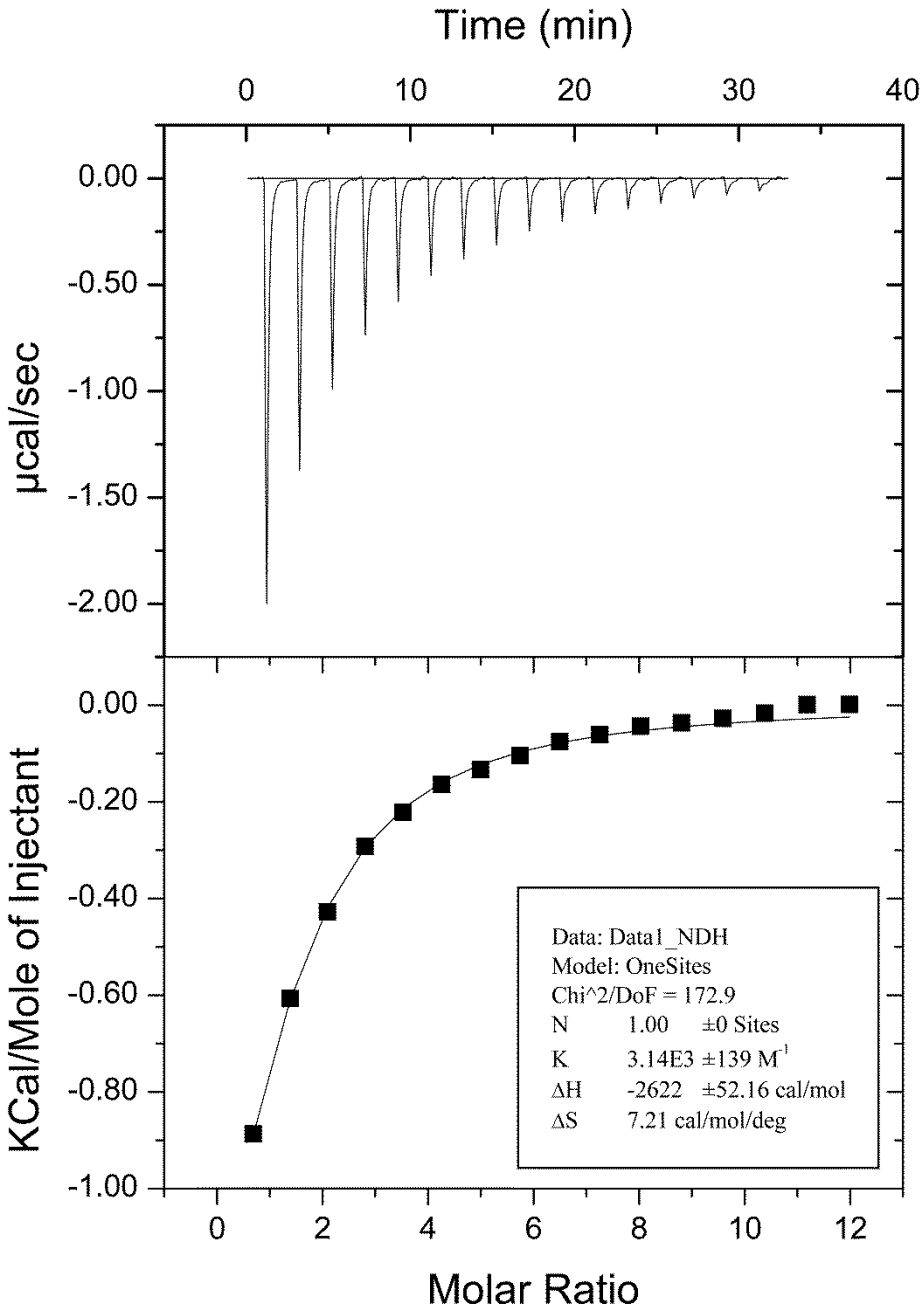

Figure S1. ITC experiment of vancomycin (**1**) + Ac-D-Ala.

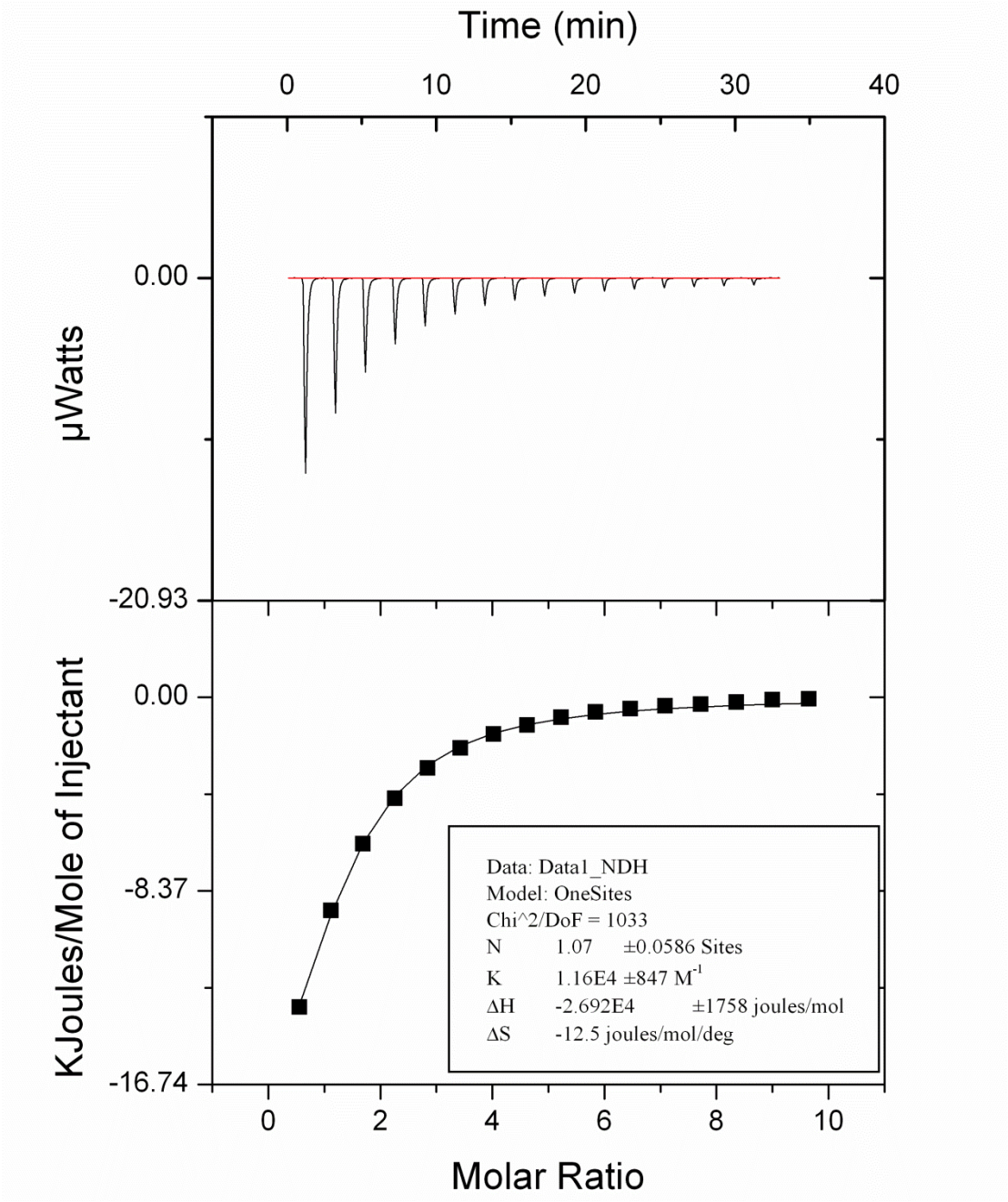

Figure S2. ITC experiment of vancomycin (**1**) + Ac-D-Ala-D-Ala.

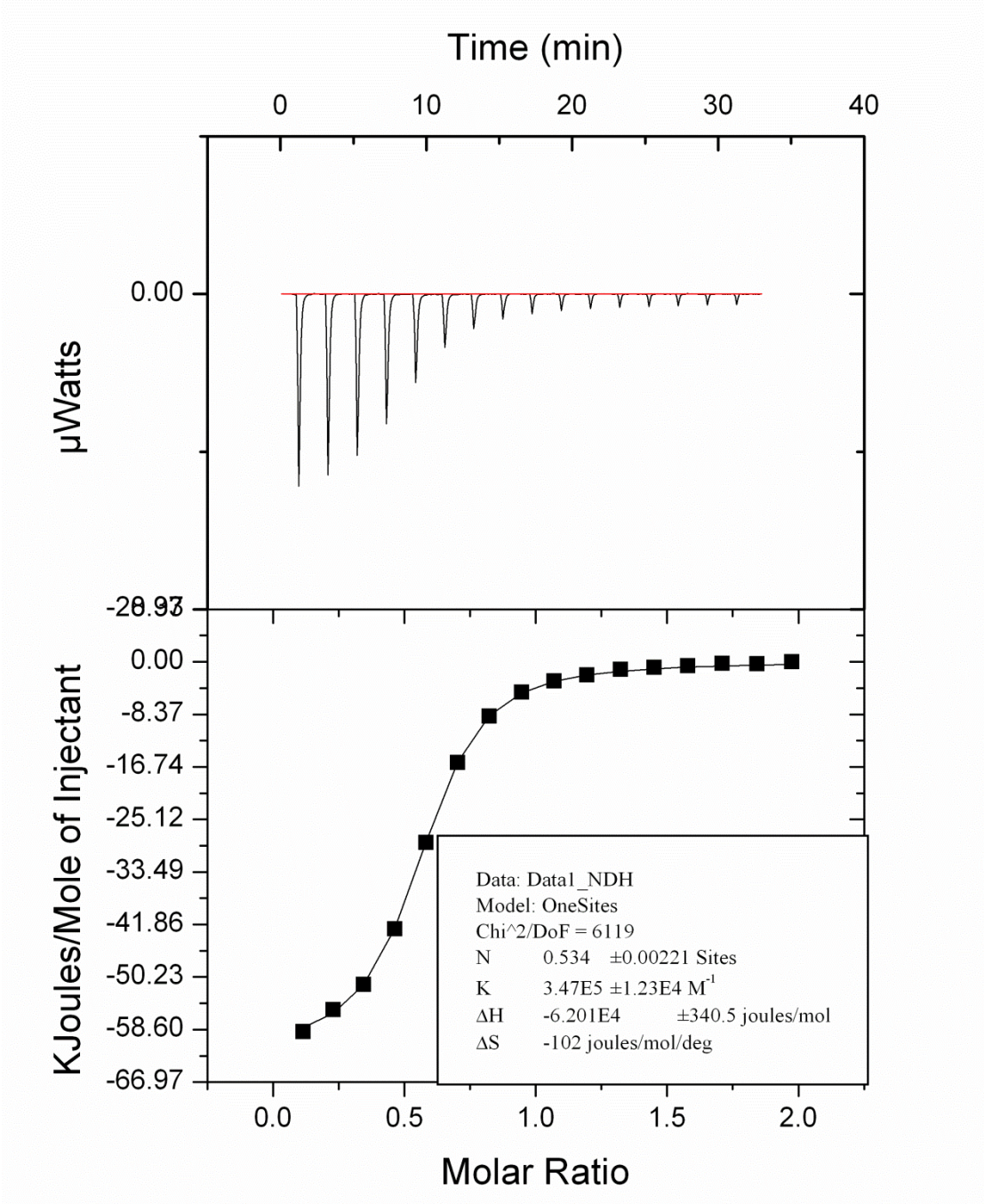

Figure S3. ITC experiment of vancomycin (**1**) + Ac-Lys-D-Ala-D-Ala.

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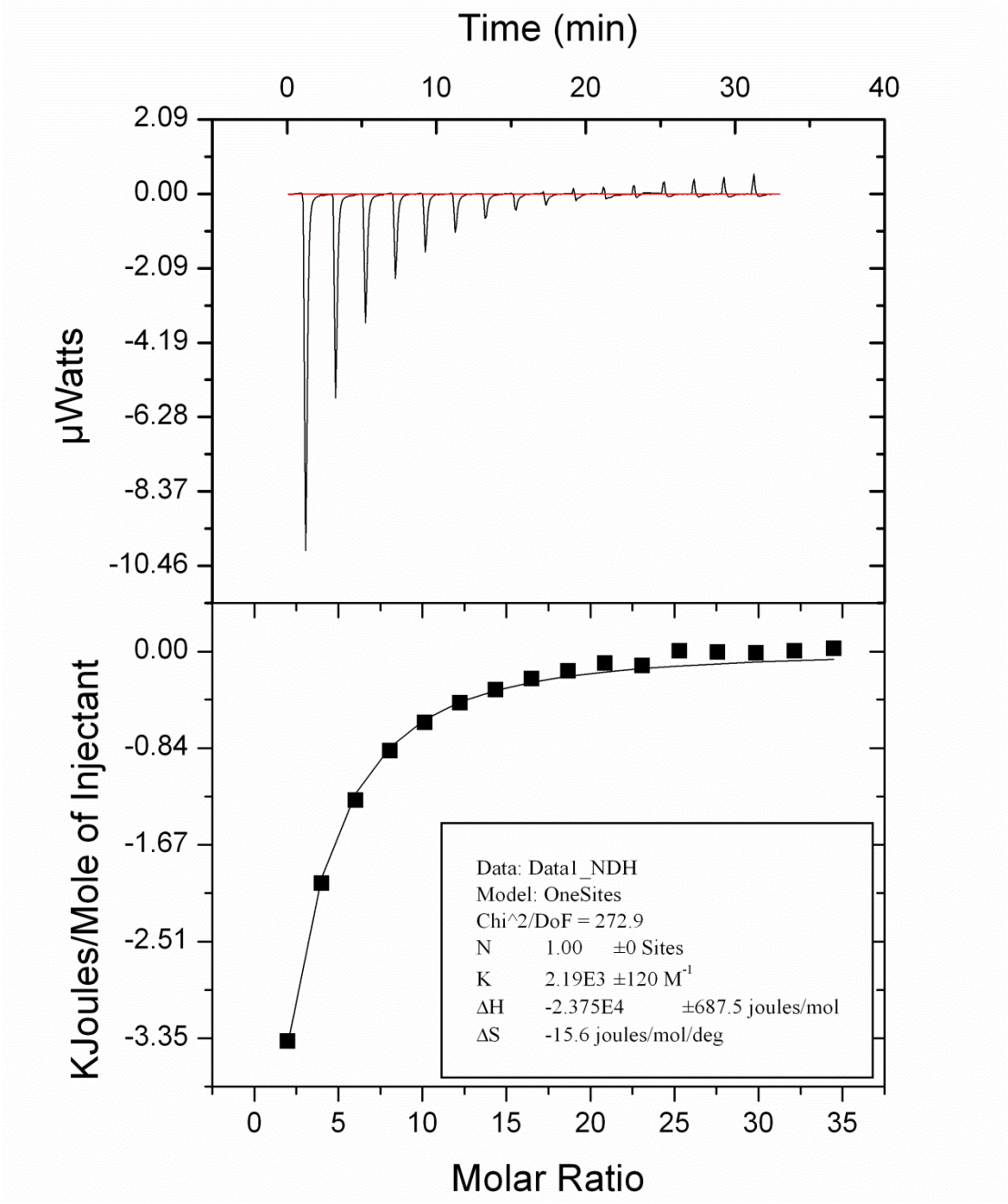

Figure S4. ITC experiment of vancomycin (**1**) + Ac-Lys-D-Ala-D-Lac.

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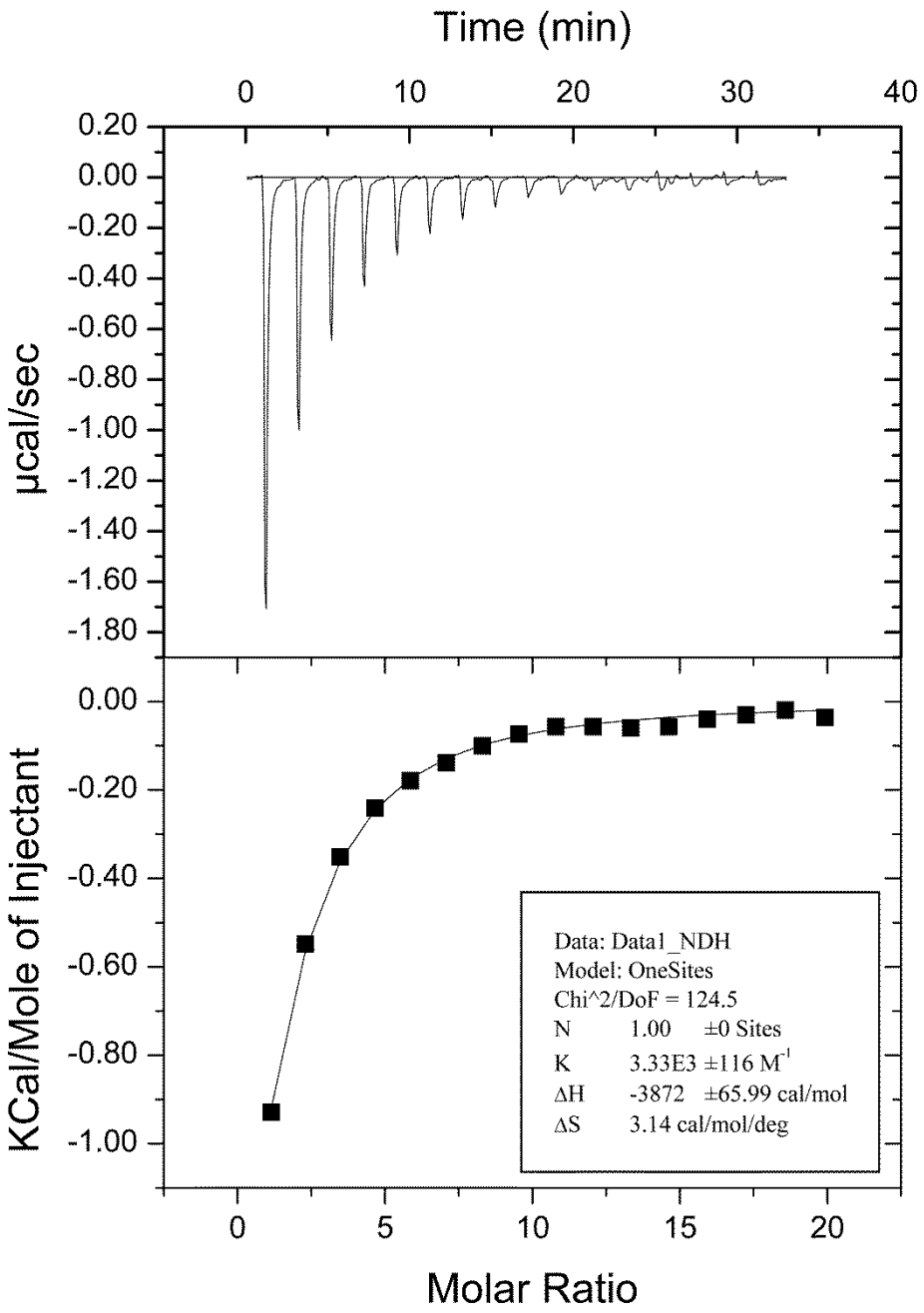

Figure S5. ITC experiment of vancomycin mimic **2** + Ac-D-Ala.

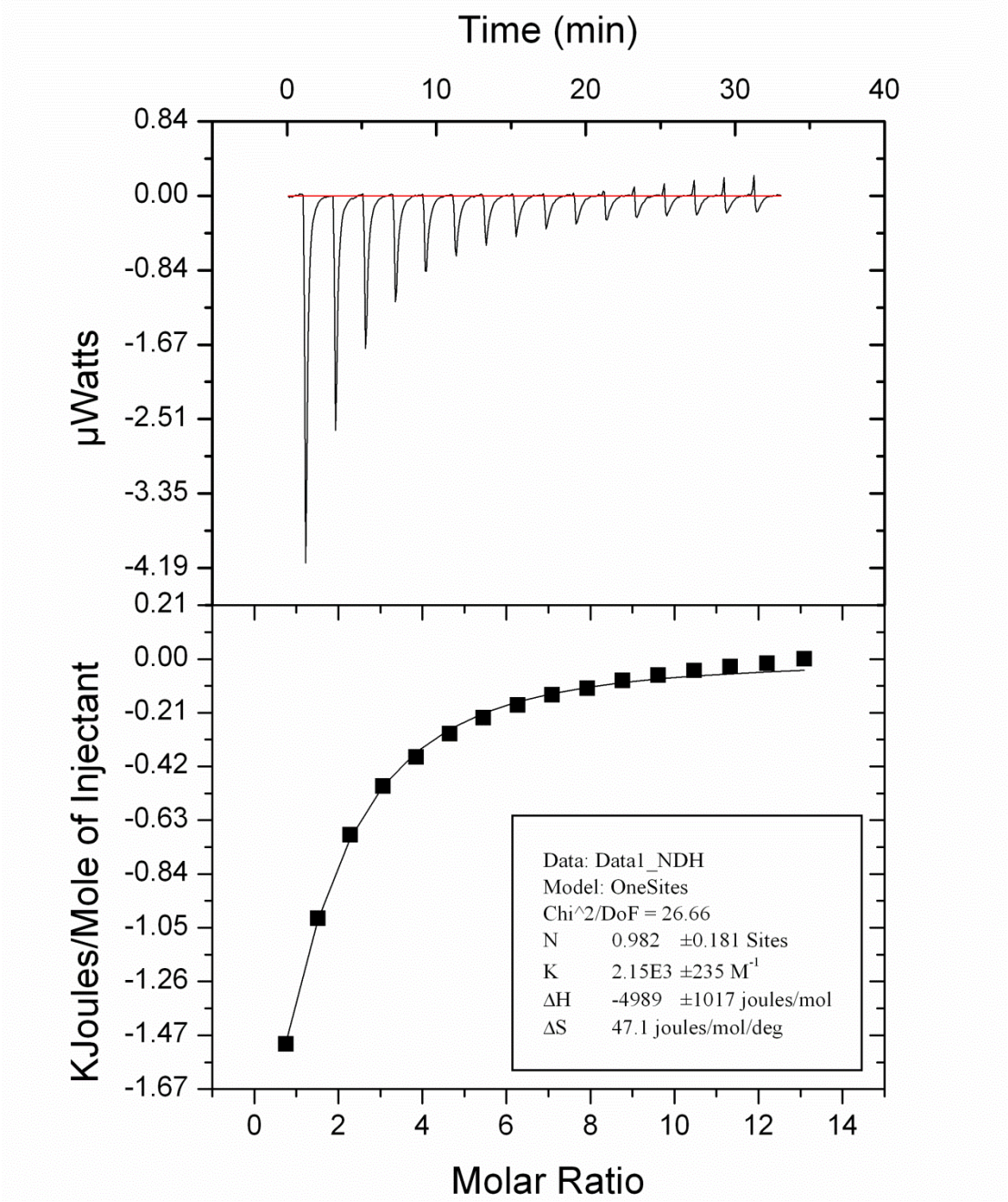

Figure S6. ITC experiment of vancomycin mimic **2** + Ac-D-Ala-D-Ala.

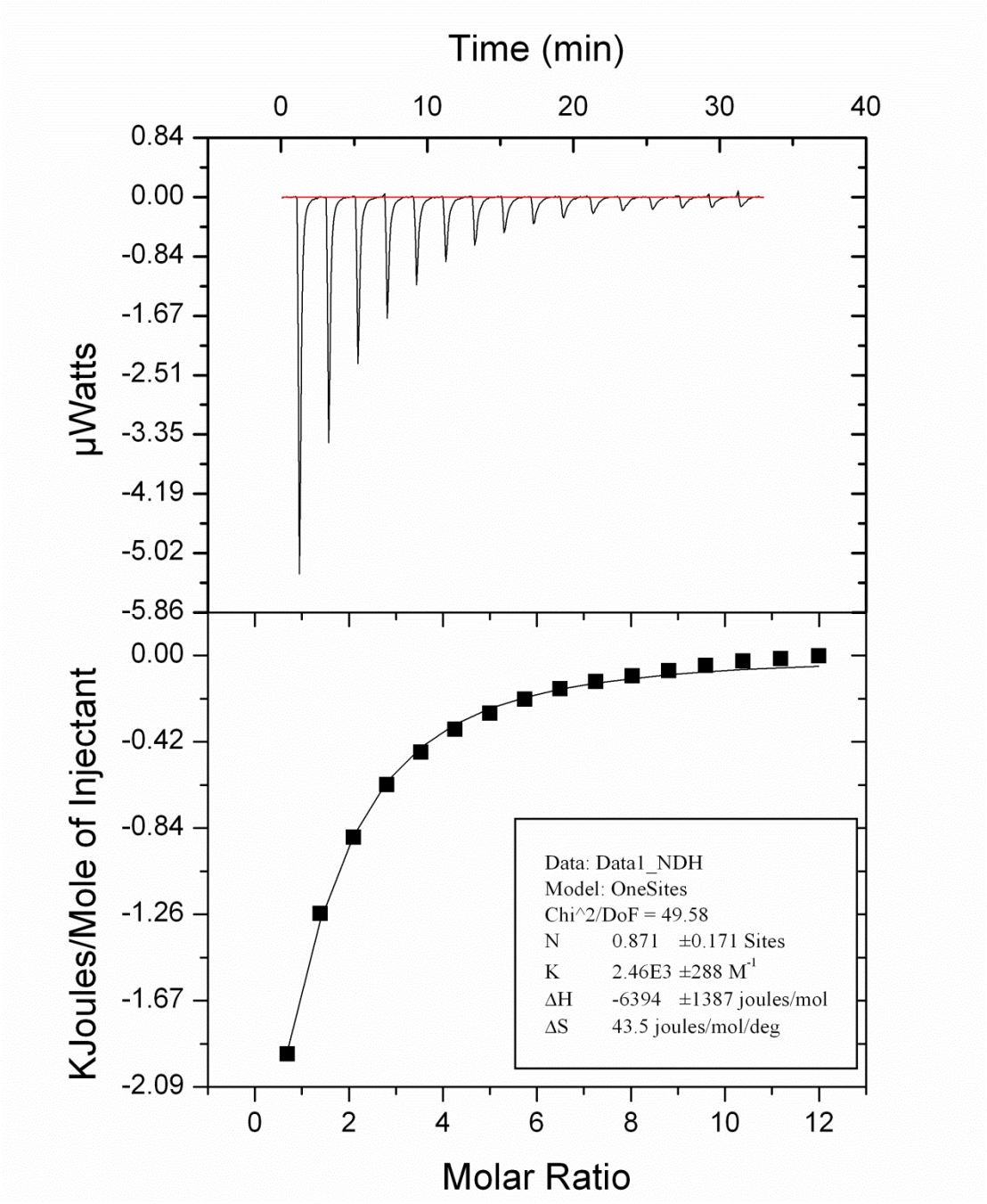

Figure S7. ITC experiment of vancomycin mimic **2** + Ac-Lys-D-Ala-D-Ala.

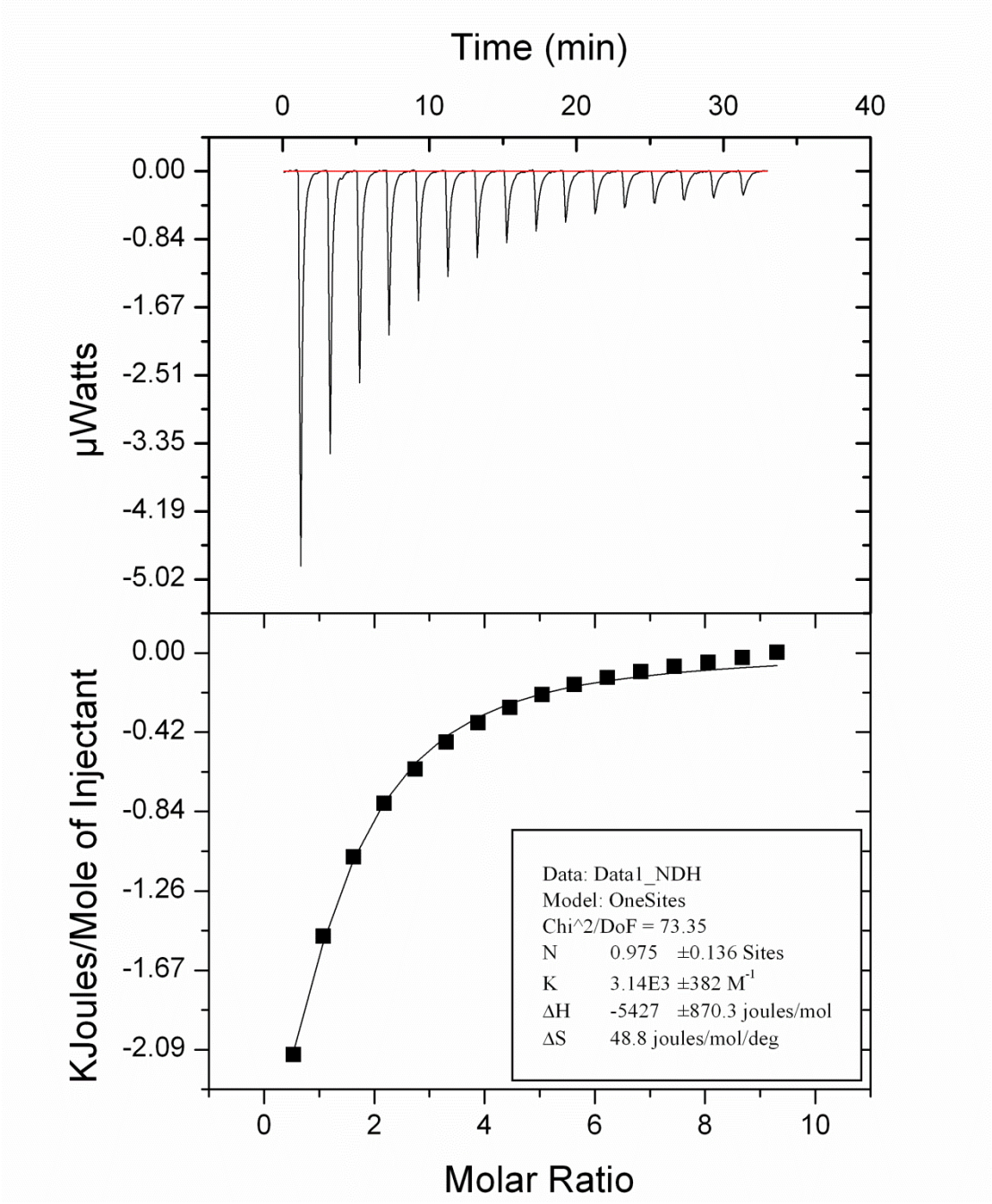

Figure S8. ITC experiment of vancomycin mimic **2** + Ac-Lys-D-Ala-D-Lac.

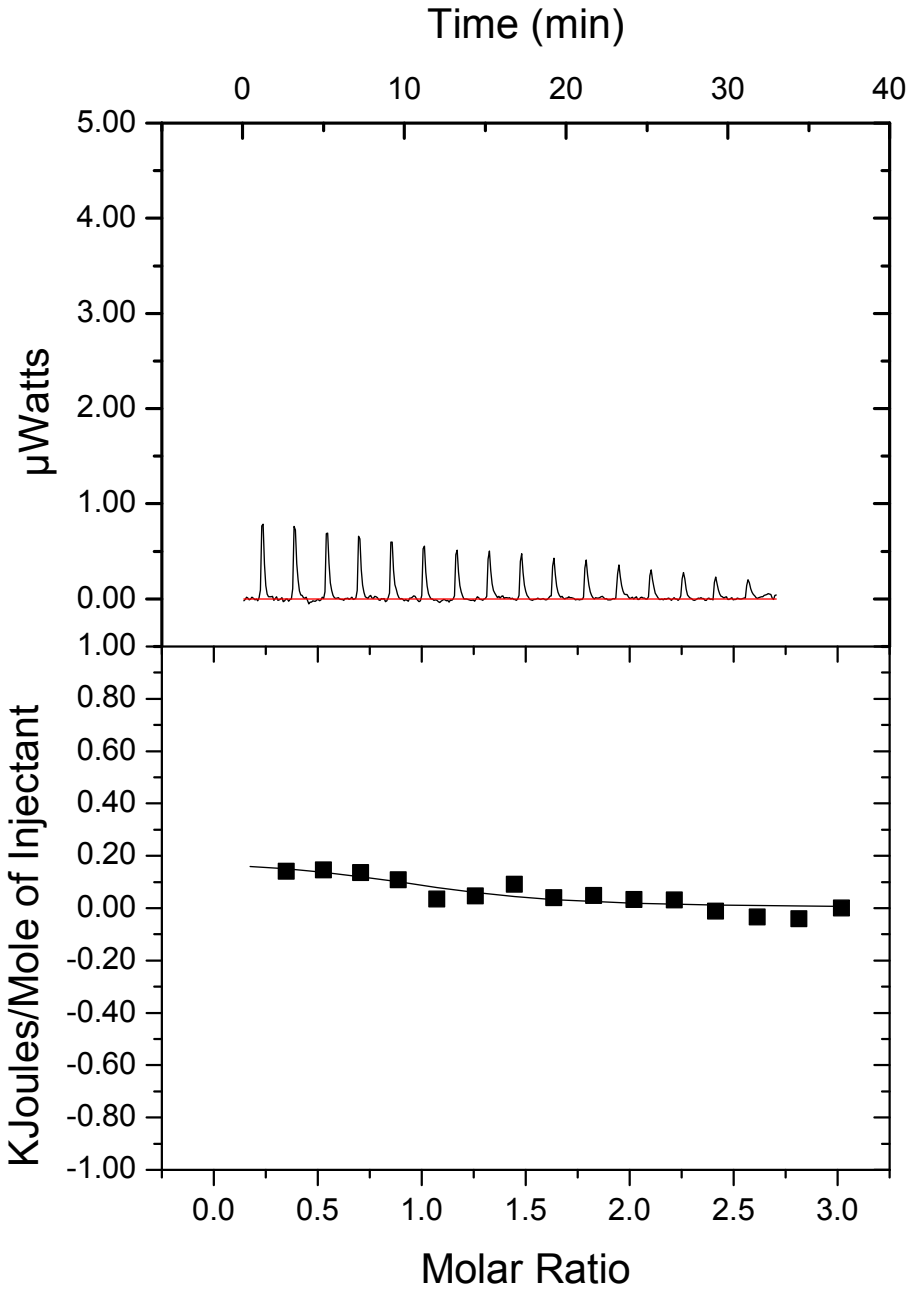

Figure S9. ITC experiment of vancomycin (**1**) + Ac-L-Ala-OH.

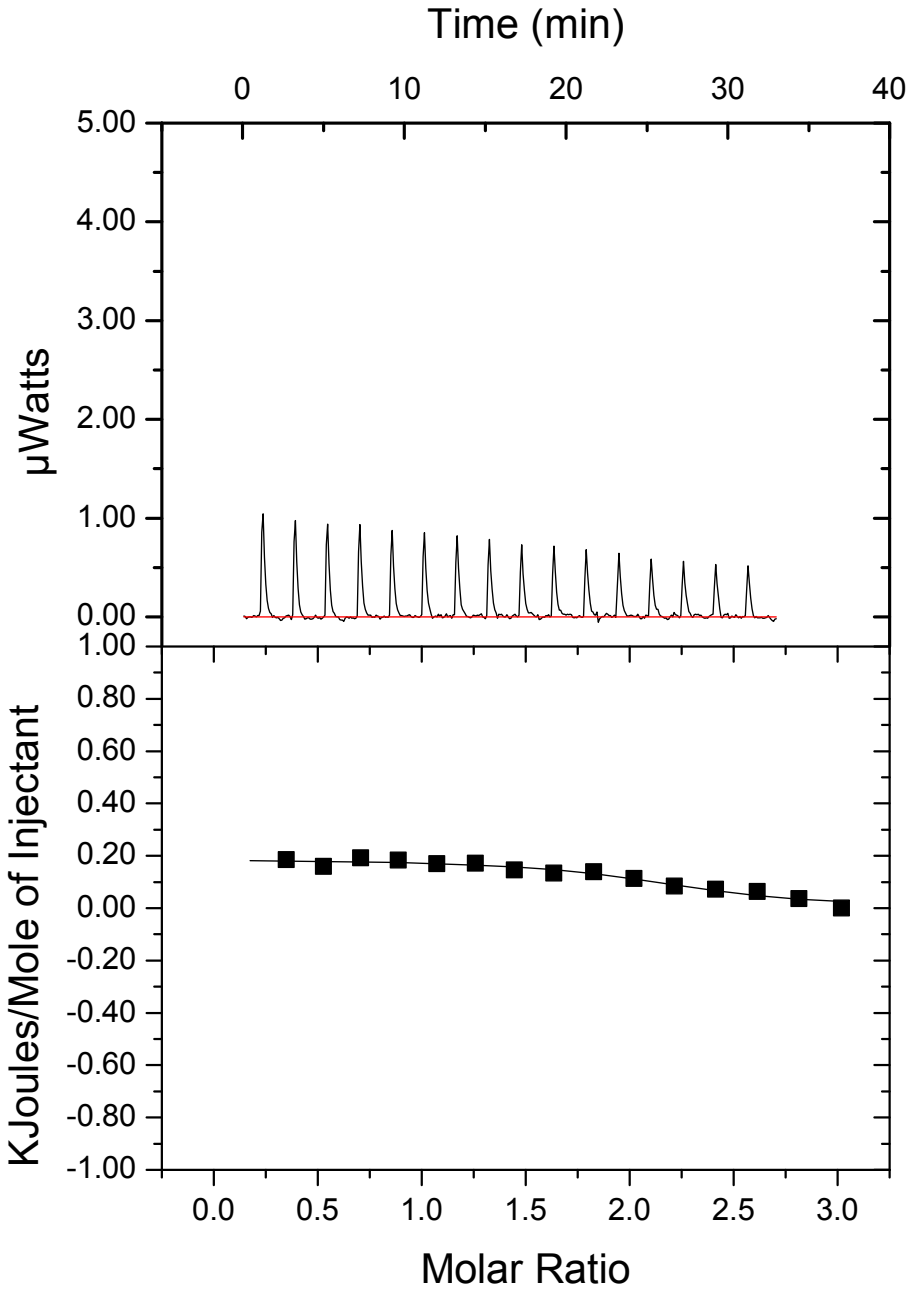

Figure S10. ITC experiment of vancomycin (**1**) + Ac-L-Ala-L-Ala-OH.

3. Copies of the ^{1}H-NMR and ^{13}C-NMR Spectra and the 2D HSQC NMR Spectrum

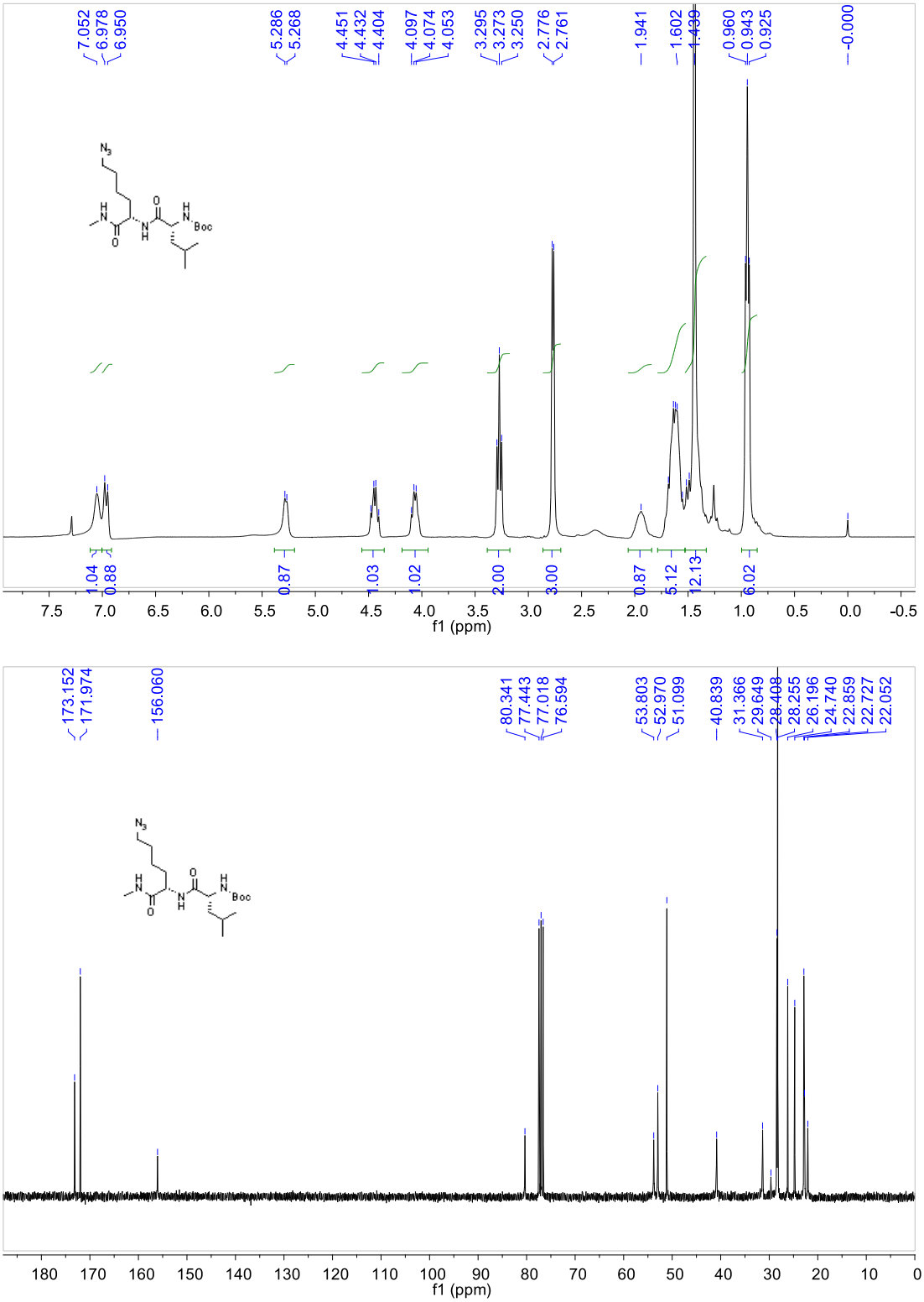

Figure S11 and S12. ^{1}H and ^{13}C NMR spectrum of Boc-D-Leu-Lys(N_3)-NHMe **4**.

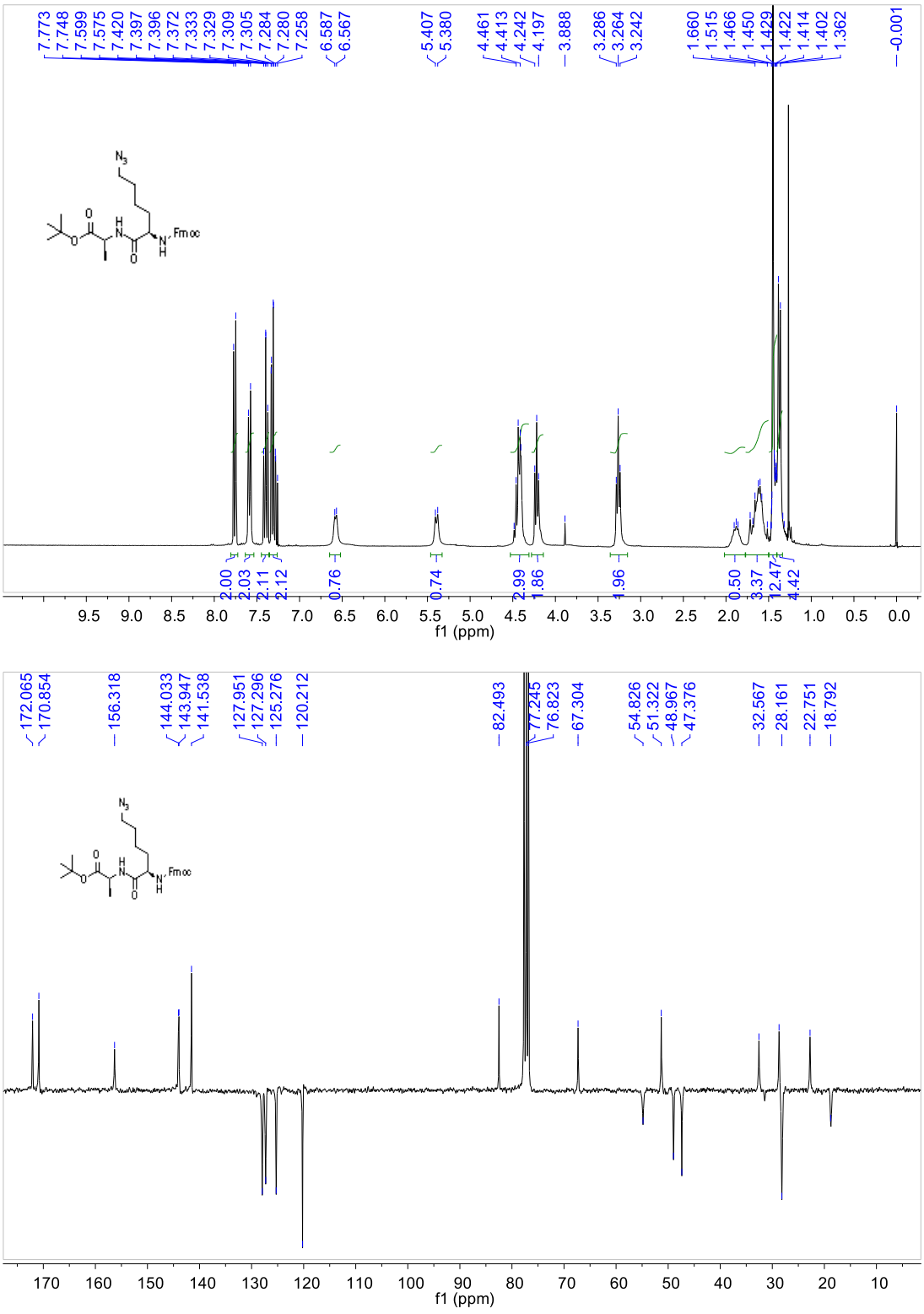

Figure S13 and S14. ^{1}H and ^{13}C NMR spectrum of Fmoc-D-Lys(N_3)-Ala-O^{t}Bu **6**.

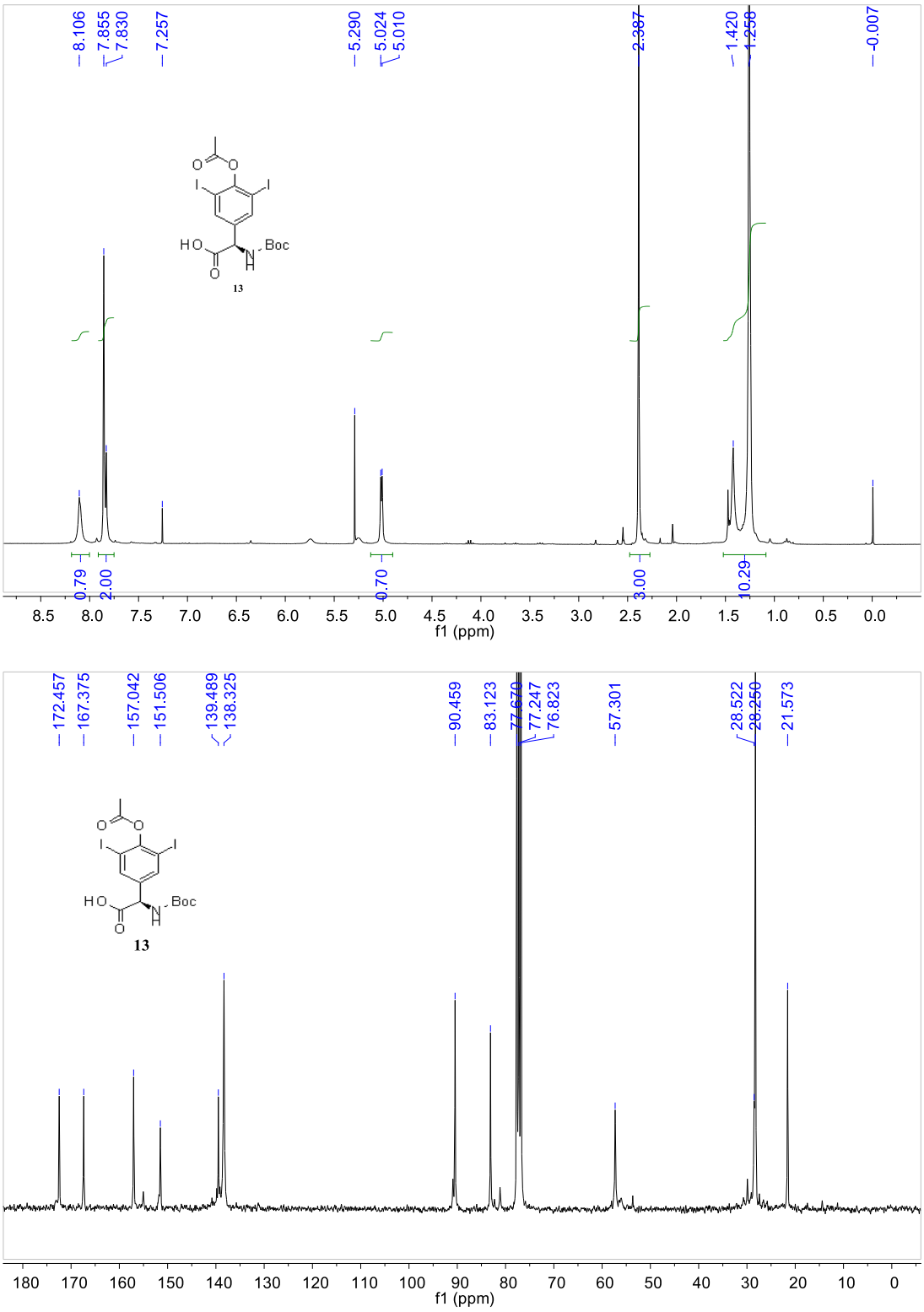

Figure S15 and S16. ^{1}H and ^{13}C NMR spectrum of (*R*)-*N*-α-Boc-(4-acetoxy-3,5-diiodo)phenylglycine **20** (indicated as **13**).

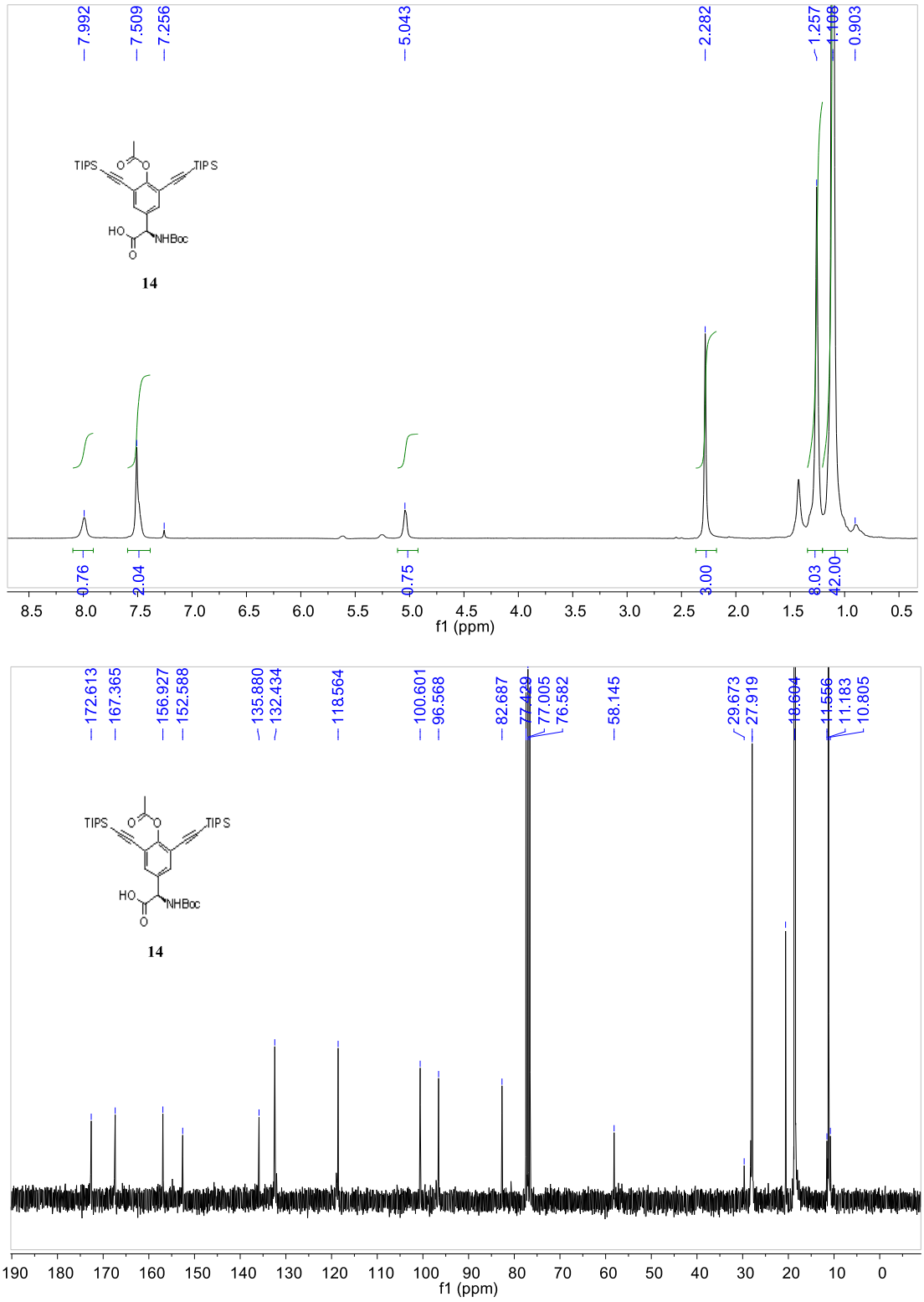

Figure S17 and S18. ^{1}H and ^{13}C NMR spectrum of Boc-D-Phg(4-OAc-3,5-bis-TIPS-alkyne) **8** (indicated as **14**).

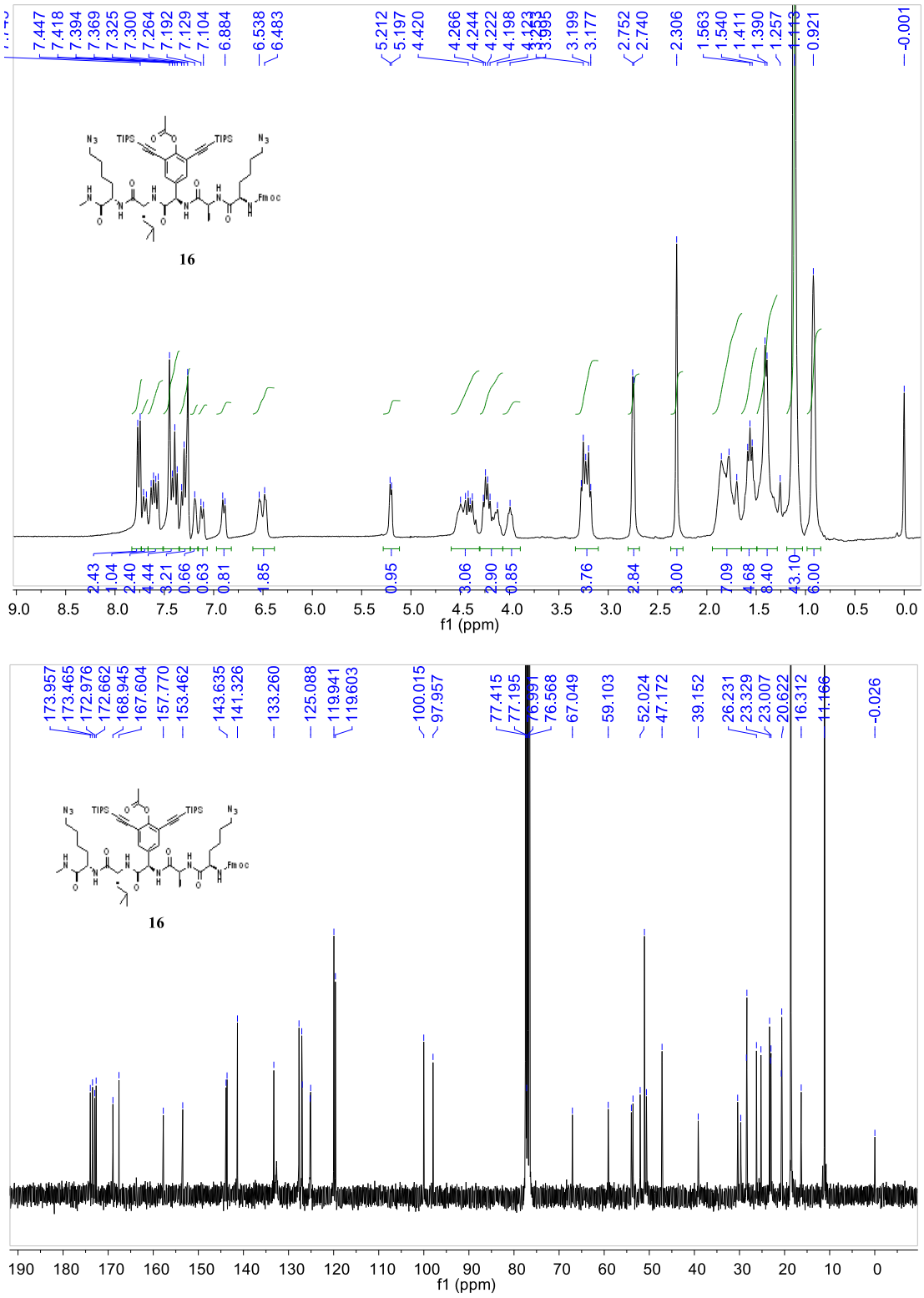

Figure S19 and S20. ^{1}H and ^{13}C NMR spectrum of Fmoc-D-Lys(N_3)-Ala-D-Phg(4-OAc-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **11** (indicated as **16**).

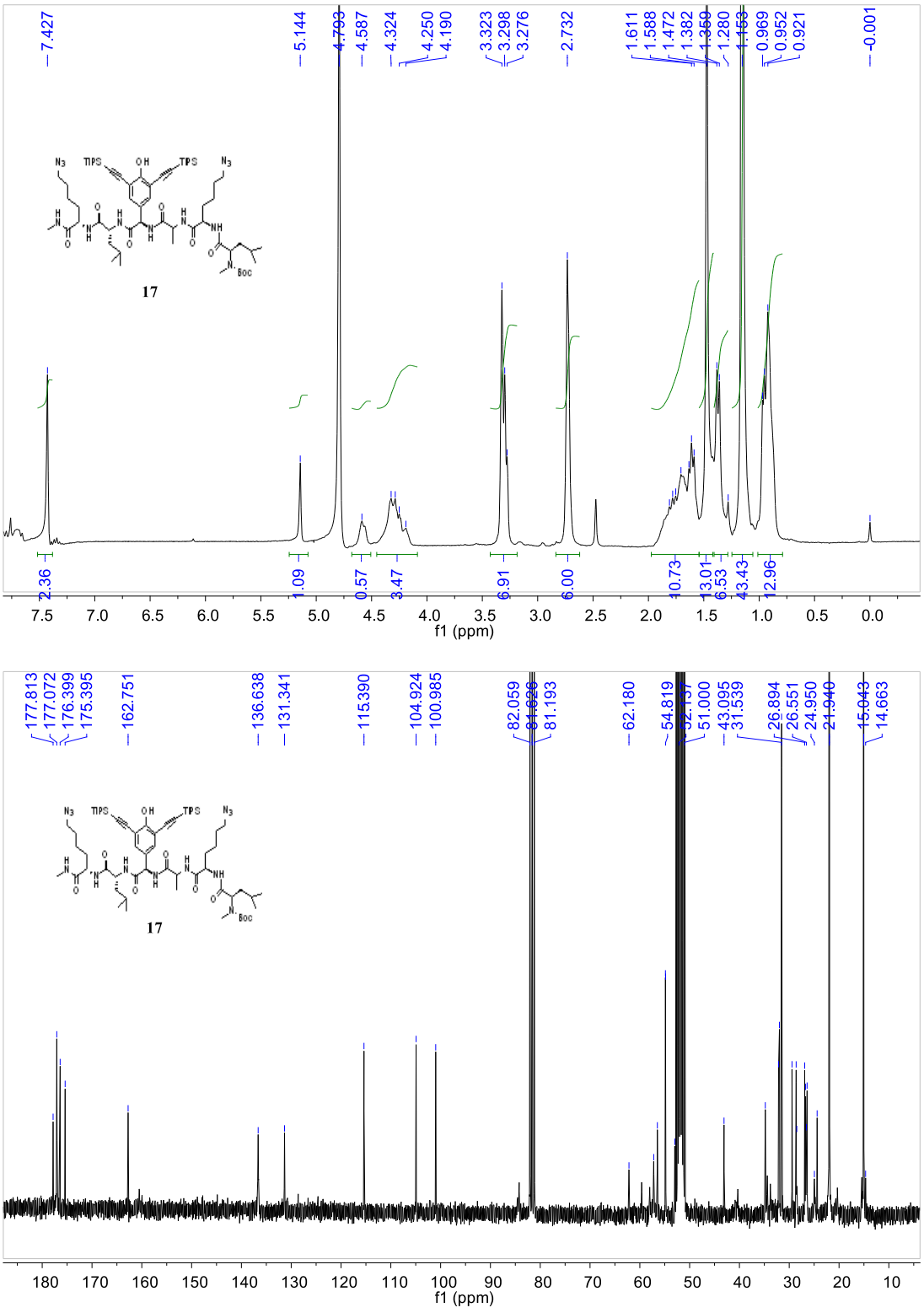

Figure S21 and S22. ^{1}H and ^{13}C NMR spectrum of Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OH-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **12** (indicated as **17**).

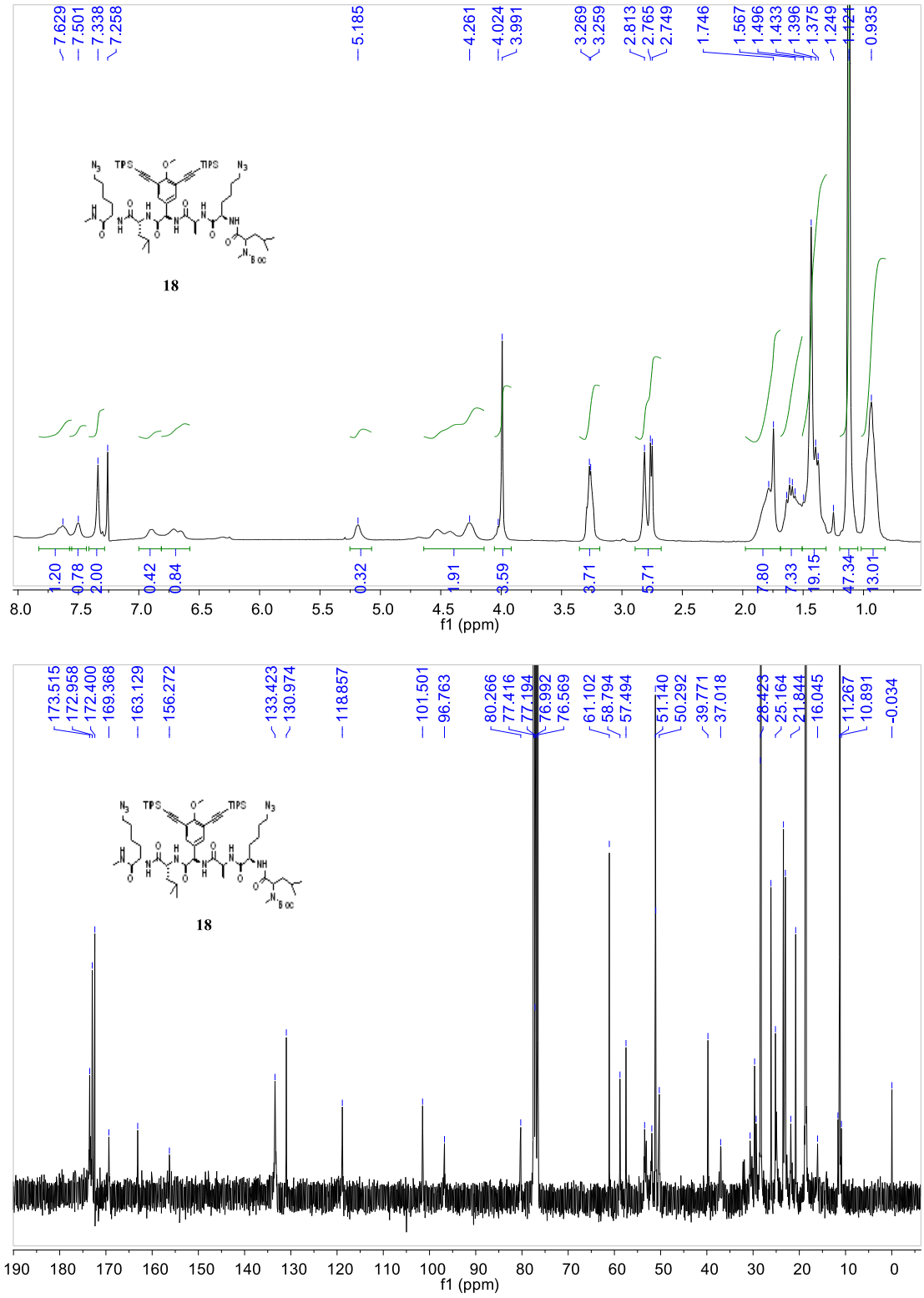

Figure S23 and S24. ^{1}H and ^{13}C NMR spectrum of Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-TIPS-alkyne)-D-Leu-Lys(N_3)-NHMe **13** (indicated as **18**).

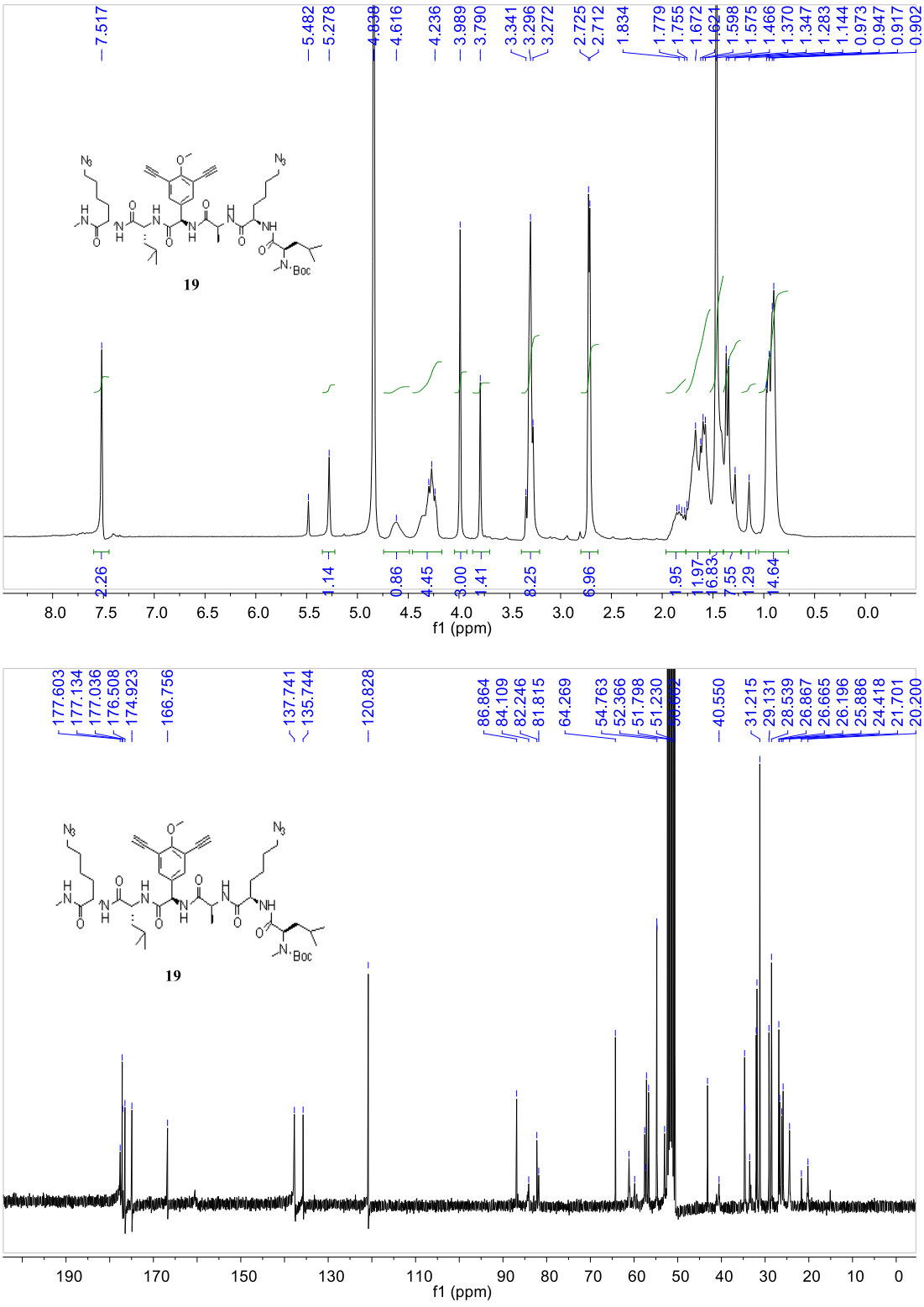

Figure S25 and S26. ^{1}H and ^{13}C NMR spectrum of Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-alkyne)-D-Leu-Lys(N_3)-NHMe **14** (indicated as **19**).

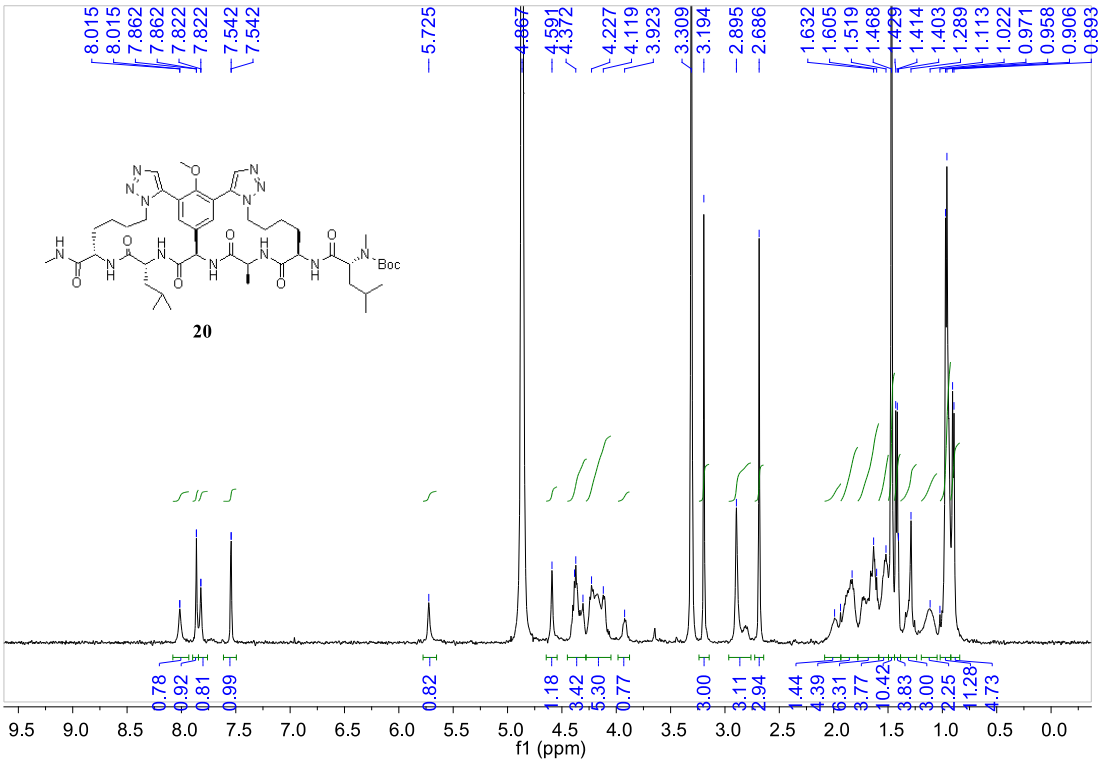

Figure S27. ^{1}H NMR spectrum of *N*-Boc-protected bicyclic hexapeptide **15** (indicated as **20**).

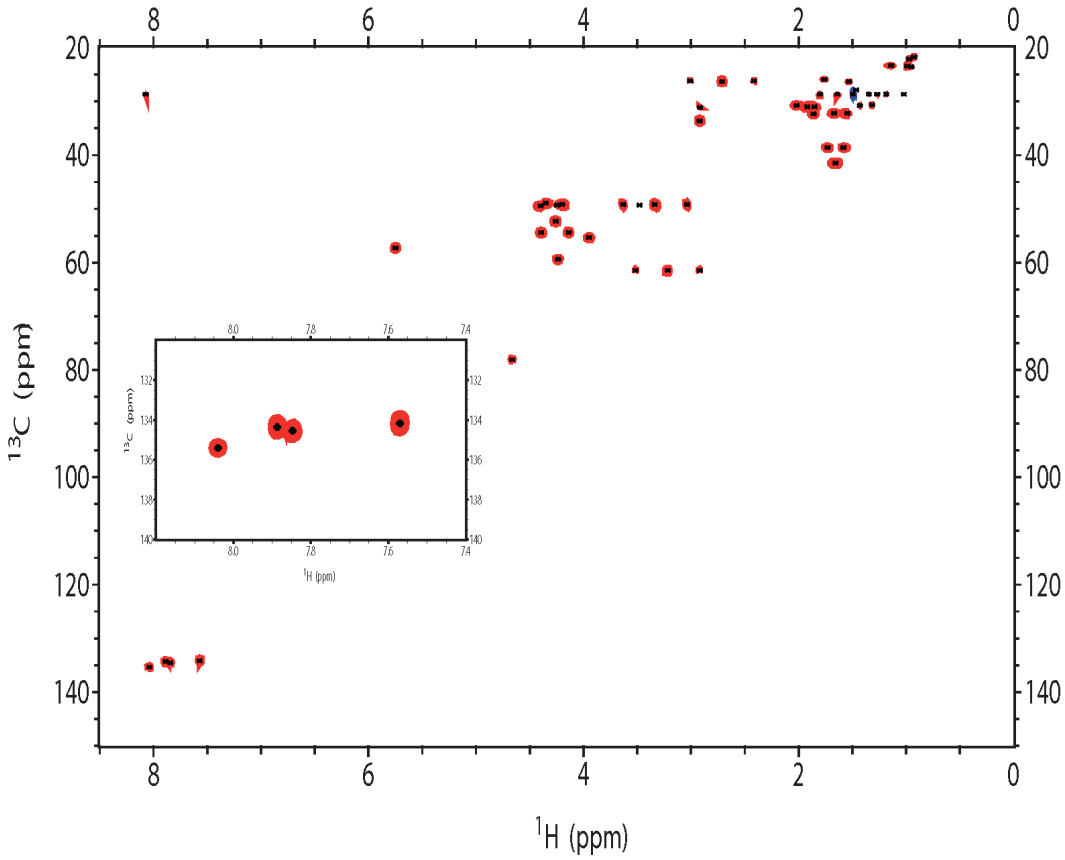

Figure S28. 2D-HSQC spectrum of *N*-Boc-protected bicyclic hexapeptide **15**.

4. Copies of the Analytical HPLC Chromatograms and MALDI-TOF MS Spectra

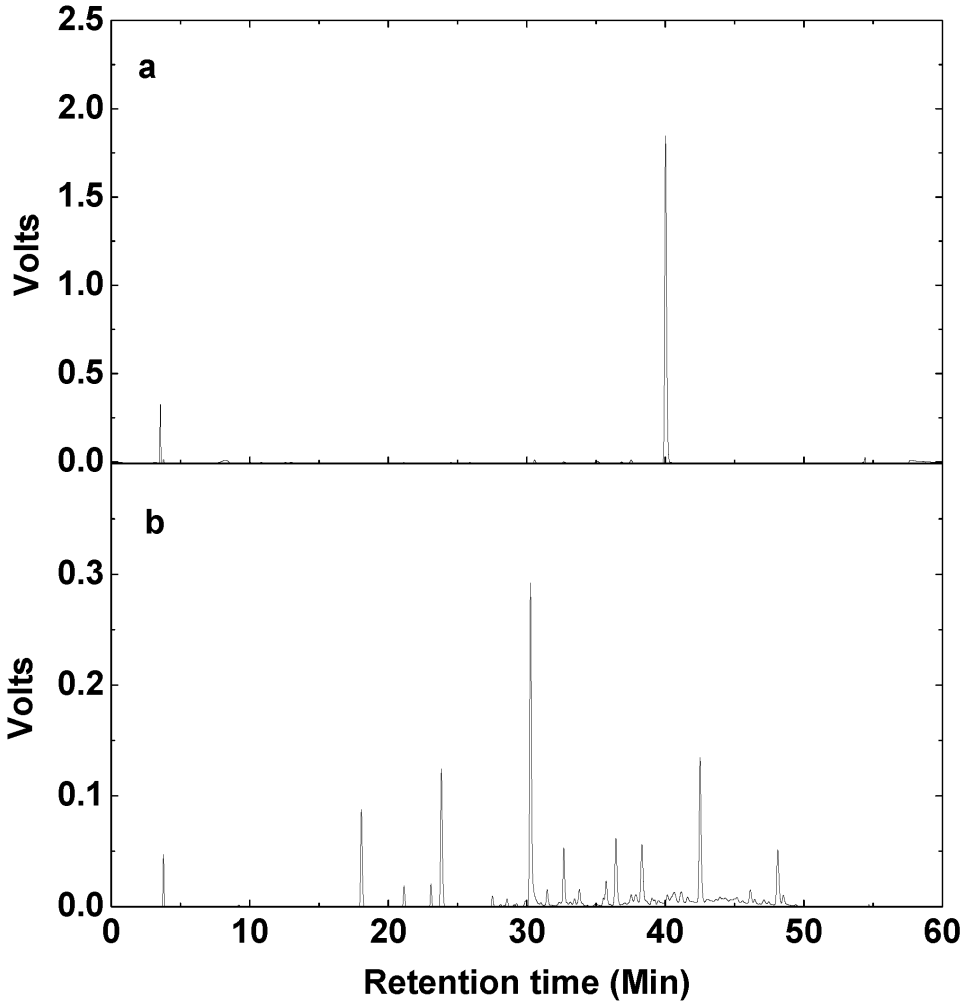

Figure S29. HPLC analysis of (a) the linear hexapeptide **14** (Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-alkyne)-D-Leu-Lys(N_3)-NHMe) and (b) the reaction mixture after RuAAC macrocyclization of linear hexapeptide **14**.

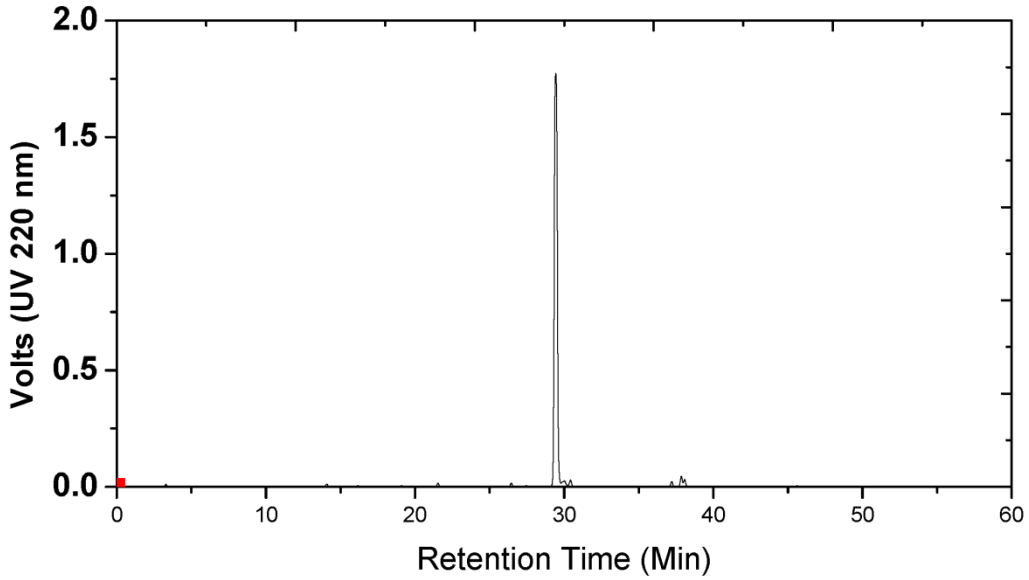

Figure S30. HPLC analysis of *N*-Boc-protected bicyclic hexapeptide **15**.

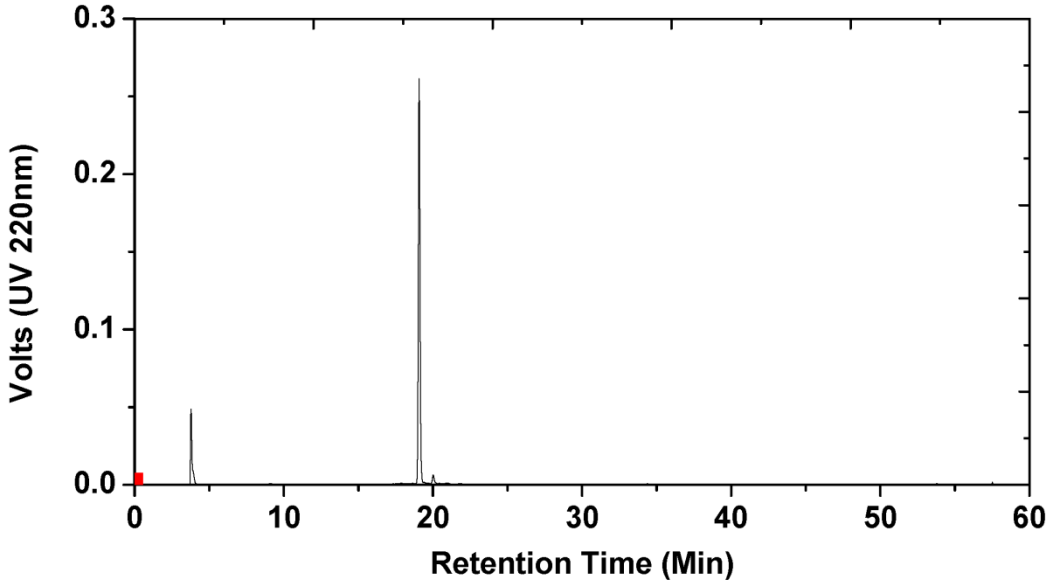

Figure S31. HPLC analysis of bicyclic hexapeptide **2**.

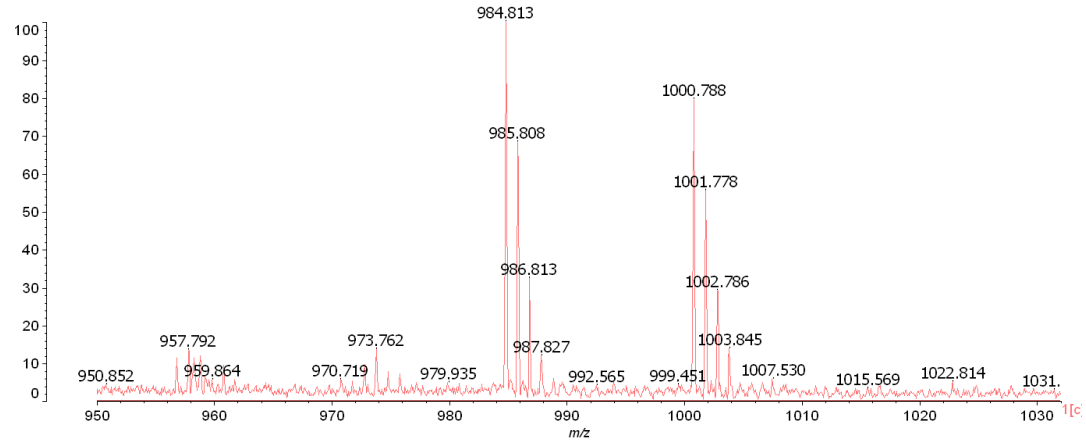

Figure S32. MALDI-TOF MS spectrum of compound **14** (Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-alkyne)-D-Leu-Lys(N_3)-NHMe).

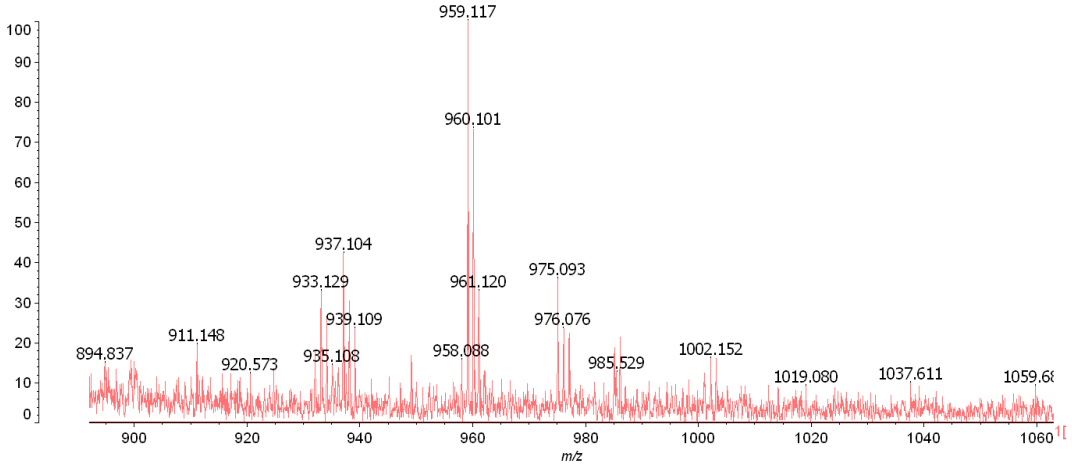

Figure S33. MALDI-TOF MS spectrum of compound **14** after treatment with TCEP.

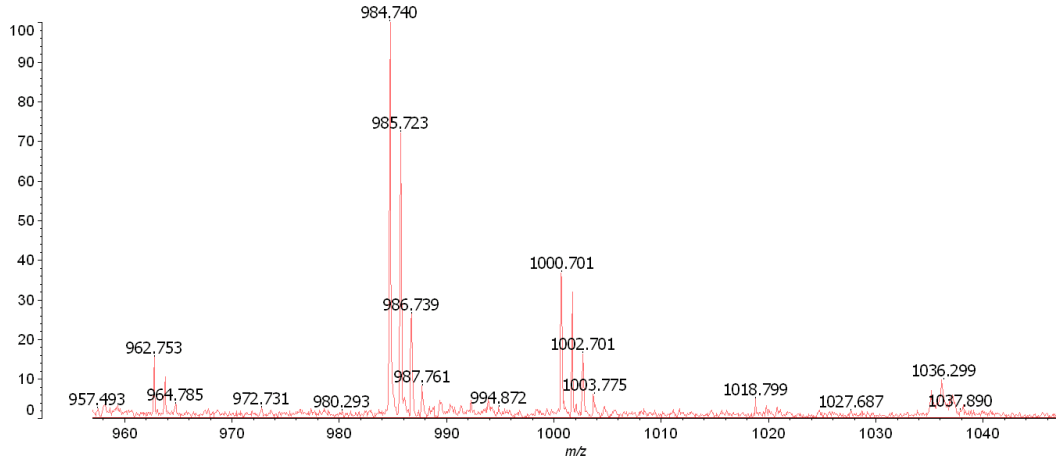

Figure S34. MALDI-TOF MS spectrum of *N*-Boc-protected bicyclic hexapeptide **15**.

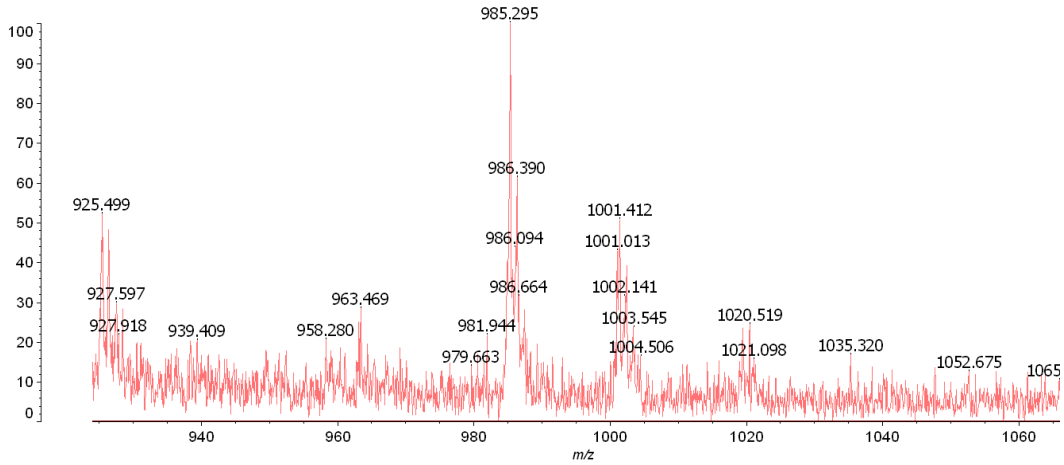

Figure S35. MALDI-TOF MS spectrum of *N*-Boc-protected bicyclic hexapeptide **15** after treatment with TCEP.

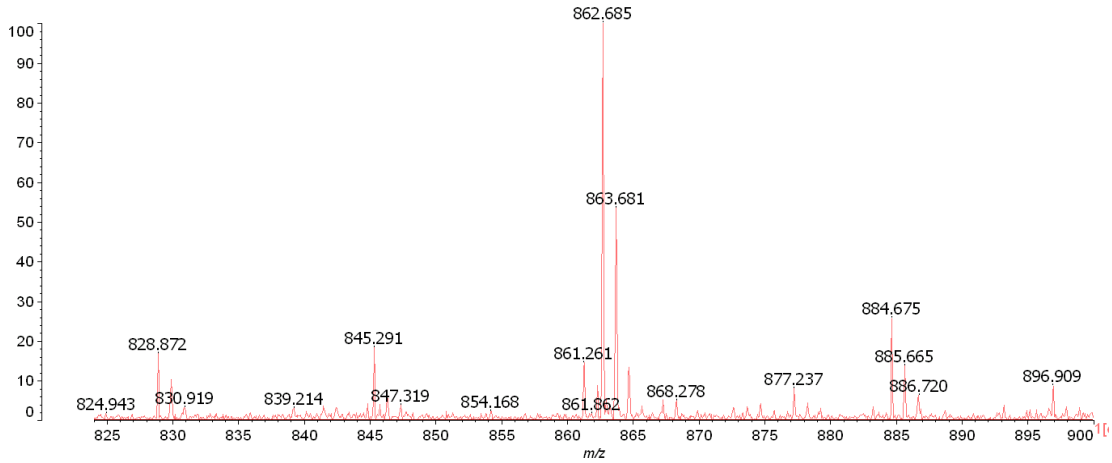

Figure S36. MALDI-TOF MS spectrum of bicyclic hexapeptide **2**.

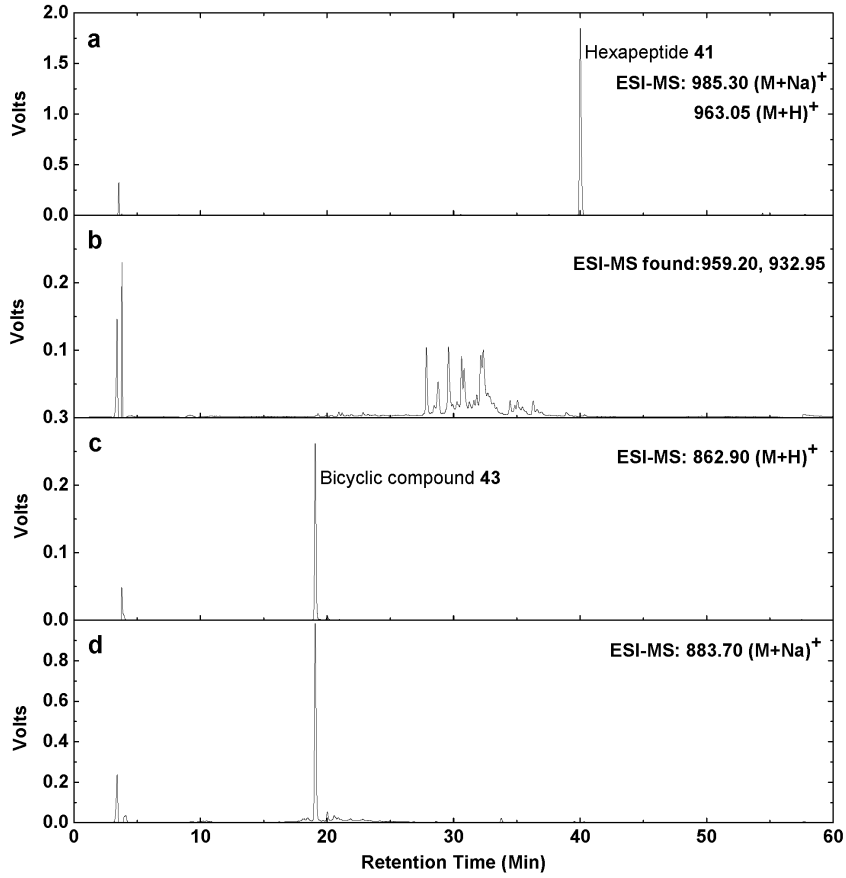

Figure S37. HPLC analysis of the TCEP reduction experiment: (a) linear hexapeptide **14** (indicated as hexapeptide **41**), (b) linear hexapeptide **14** after treatment with TCEP, (c) bicyclic hexapeptide **2** (indicated as bicyclic compound **43**) and (d) bicyclic hexapeptide **2** after treatment with TCEP.

Linear hexapeptide **14**: Boc-*N*-Me-D-Leu-D-Lys(N_3)-Ala-D-Phg(4-OMe-3,5-bis-alkyne)-D-Leu-Lys(N_3)-NHMe.