Supporting Material

Fluorescent analogues of FRH peptide: Cu(II) binding and interactions with ds-DNA/RNA

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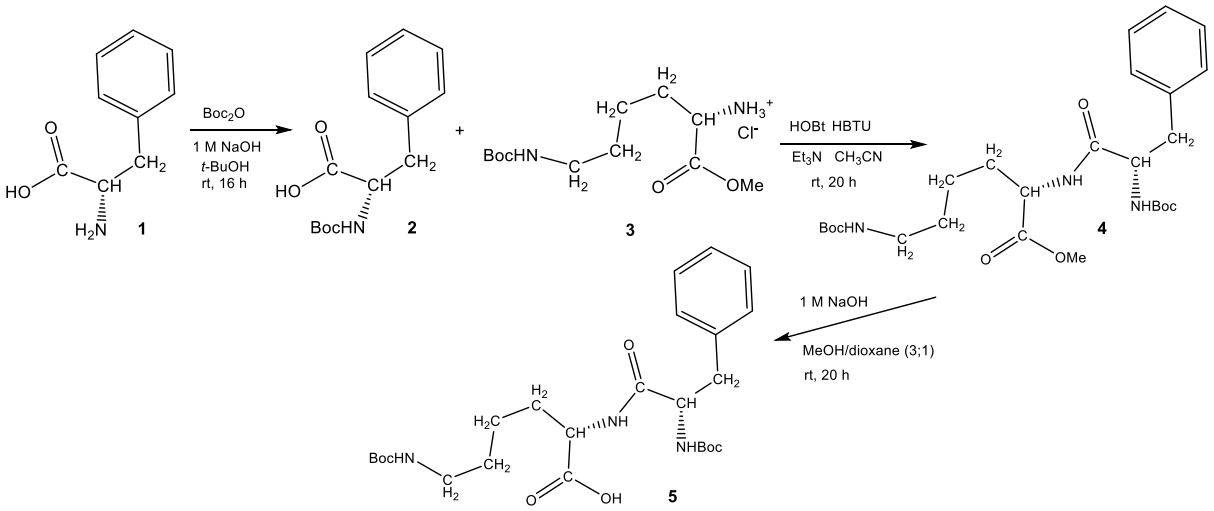
1. Synthesis:

The synthesis of Phe-Lys-Ala(triazole-R)-OMe **A, B** begins with the preparation of the dipeptide Boc-Phe-Lys(Boc)-OH **5** and Ala(triazole-R)-OMe modified amino acids, which in the N1 position of the triazole carry different substituents (R = pivaloyl or 7-hydoxycoumarin-3-yl), followed by standard coupling reaction of both synthons.

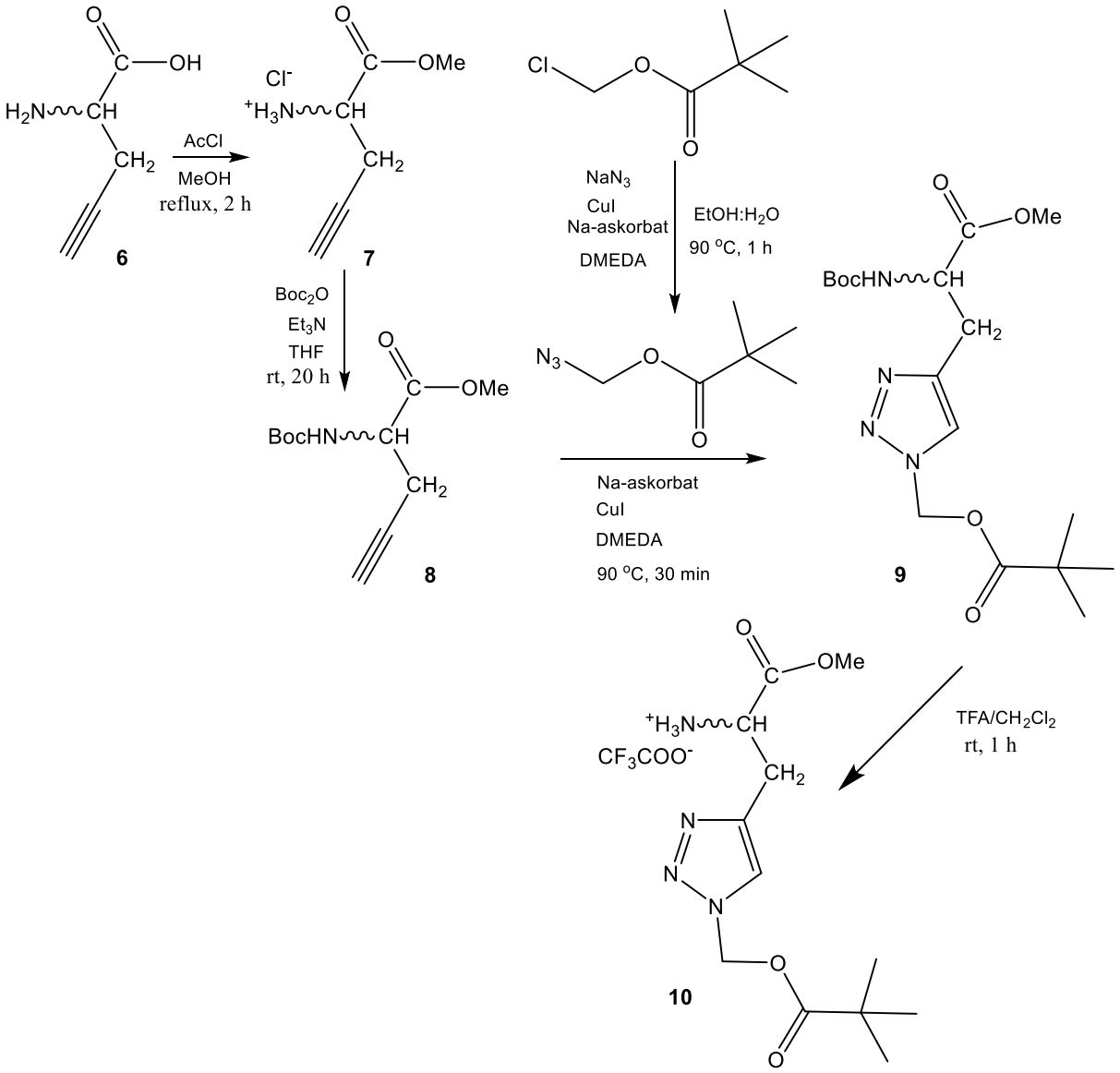
Kirichenko *et al* [1] studied the synthesis of intramolecularly bridged polypeptides and developed a method to prepare the Boc-Phe-Lys(Boc)-OH dipeptide **5**. By this method starting from Fmoc-Lys (Boc)-OH, the carboxyl group was protected with benzyl bromide, and after removal of Fmoc protection, the compound was coupled with Boc-Phe-OH. The desired dipeptide **5** was obtained by cleavage of the benzyl ester group by hydrogenolysis.

In our synthesis, the dipeptide Boc-Phe-Lys(Boc)-OH **5** was synthesized from different starting materials by adopting methods described in the literature (Scheme 1). Starting with *L*-phenylalanine **1**, the amino function was protected with Boc anhydride [2]. giving Boc-Phe-OH **2** in 72 % yield. Protected dipeptide Boc-Phe-Lys(Boc)-OMe **4** was obtained in excellent yield (88 %) by a standard coupling reaction of **2** and commercially available *L*-Lys(Boc)-OMe hydrochloride **3** using HBTU/HOBt coupling reagents and triethylamine in acetonitrile. The ester group in dipeptide **4** was readily hydrolyzed with sodium hydroxide in a mixture of methanol/dioxane (*v*/*v* 3:1) at room temperature giving dipeptide Boc-Phe-Lys(Boc)-OH **5** in 93 % yield.

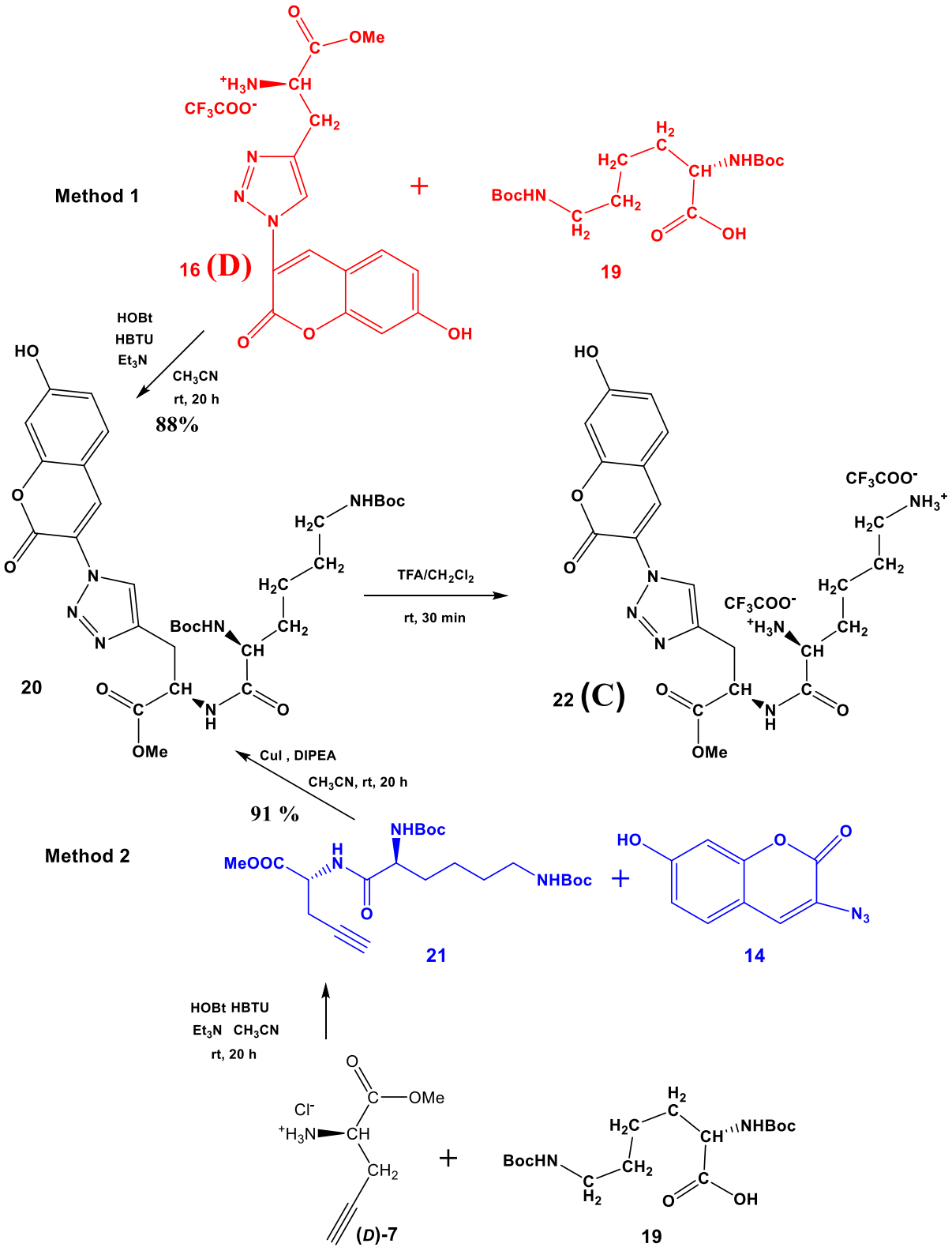


Scheme S1. Synthesis of Boc-Phe-Lys(Boc)-OH dipeptide **5**.

The amino and carboxyl groups of *D,L*-propargylglycine **6** are protected by known methods [3]. reaction of **6** with acetyl chloride in methanol gave propargylglycine methyl ester **7** (97 %), which in the subsequent reaction with Boc-anhydride gave the fully protected compound **8** in 88 % yield.

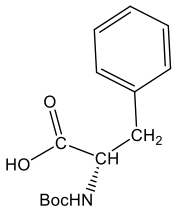


Scheme S2. Synthesis of Ala(triazole-Piv)-OMe **10**.



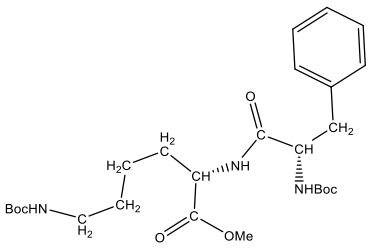
Scheme S3. Two synthetic pathways to Lys-Ala(triazole-coumarin)-OMe **22** (**C**).

Detailed synthetic procedures:



(*tert*-Butoxycarbonyl)-*L*-phenylalanine (2)

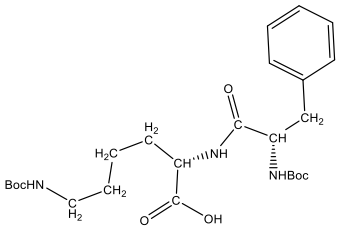
Boc-Phe-OH **2** was prepared with a slight modification of the known procedure [2]. *L*-Phenylalanine **1** (1.006 g, 6.09 mmol) was dissolved in a mixture of *t*-BuOH (4.6 mL) and 1 M NaOH solution (6.7 mL), cooled to 0 °C and di-*tert*-butyl dicarbonate (1.37 g, 6.09 mmol, 97 %) was added dropwise. The reaction was stirred for 15 min at 0 °C, then allowed to warm to room temperature and stirred at room temperature for 16 h. The solvent was partially removed *in vacuo*, and the resulting aqueous residue was washed with ethyl acetate (20 mL). The aqueous solution was acidified to pH 1 with saturated aqueous $KHSO_4$ solution and extracted with ethyl acetate (3x40 mL). The combined organics extracts were washed with water (25 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* to afford the target material Boc-Phe-OH **2** as a white solid (1.17 g, 72 %): R_f = 0.85 (CH_2Cl_2/CH_3OH 3:1); m.p. = 86–88 °C (m.p. 86–88 °C)[2]; ^{1}H NMR (DMSO-d_6) δ/ppm: 12.57 (s, 1H, COOH), 7.30–7.22 (m, 4H, Ar), 7.19 (t, J = 7.1 Hz, 1H, Ar), 7.06 (d, J = 8.4 Hz, 1H, NH), 4.12–4.05 (m, 1H, CH), 3.01 (dd, J = 13.8, 4.5 Hz, 1H, Ha-CH_2), 2.82 (dd, J = 13.8, 10.4 Hz, 1H, Hb-CH_2), 1.32 (s, 9H, $\underline{Me}_3$C-O-); ^{13}C NMR (DMSO-d_6) δ/ppm: 173.6 (HO-$\underline{C}$=O), 155.4 (Me_3C-O-$\underline{C}$=O), 138.0 (Cq, Ar), 129.1 (CH, Ar), 128.1 (CH, Ar), 126.3 (CH, Ar), 78.0 ($Me_3\underline{C}$-O-), 55.1 (CH), 36.4 (CH_2), 28.1 ($\underline{Me}_3$C-O-); (see Supp. Mat. Fig. S32).



Methyl N^6-(*tert*-butoxycarbonyl)-N^2-((*tert*-butoxycarbonyl)-*L*-phenylalanyl)-*L*-lysinate (4)

Boc-Phe-OH **2** (955 mg, 3.6 mmol) and *L*-Lys(Boc)-OMe hydrochloride **3** (1.07 g, 3.6 mmol) were dissolved in dry CH_3CN (33 mL) under argon and HOBt (502 mg, 3.6

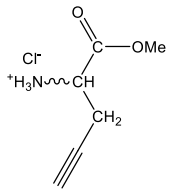
mmol, 97 %), HBTU (1.393 g, 3.6 mmol, 98 %) and dry Et_3N (2.02 mL, 14.4 mmol) were added. The reaction mixture was stirred at room temperature for 20 h. Product **4** (1.6 g, 88 %) was isolated by silica gel chromatography on a funnel (CH_2Cl_2/MeOH 9:1) as a light yellow powder: R_f = 0.86 (CH_2Cl_2/CH_3OH 9:1); m.p. = 91–93 °C; ^{1}H NMR (DMSO-d_6) δ/ppm: 8.22 (d, J = 7.4 Hz, 1H, NH), 7.27 (d, J = 4.2 Hz, 4H, Ar), 7.19 (dd, J = 8.5, 4.3 Hz, 1H, Ar), 6.85 (d, J = 8.7 Hz, 1H, NH), 6.74 (brs, 1H, NH), 4.28–4.16 (m, 2H, 2xCH), 3.62 (s, 3H, OMe), 2.96 (dd, J = 13.8, 3.8 Hz, 1H, Ha-CH_2), 2.89 (dd, J = 12.8, 6.6 Hz, 2H, CH_2), 2.72 (dd, J = 13.8, 10.5 Hz, 1H, Hb-CH_2), 1.74–1.67 (m, 1H, Ha-CH_2), 1.65–1.57 (m, 1H, Hb-CH_2), 1.36 (brs, 11H, Me_3C-O-, CH_2), 1.29 (s, 9H, Me_3C-O-), 1.23 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 172.5 (MeO-<u>C</u>=O), 172.0 (-NH-<u>C</u>=O), 155.6 (Me_3C-O-<u>C</u>=O), 155.2 (Me_3C-O-<u>C</u>=O), 138.1 (Cq, Ar), 129.2 (CH, Ar), 128.0 (CH, Ar), 126.2 (CH, Ar), 78.0 (Me_3<u>C</u>-O-), 77.3 (Me_3<u>C</u>-O-), 55.4 (CH), 51.9 (CH), 51.8 (OCH_3), 40.6 (CH_2 covered with DMSO), 37.3 (CH_2), 30.6 (CH_2), 29.0 (CH_2), 28.2 (<u>Me</u>$_3$C-O-), 28.1 (<u>Me</u>$_3$C-O-), 22.6 (CH_2); (see Supp. Mat. Fig. S33). ESI-MS: m/z calcd. for $C_{26}H_{41}N_3O_7$ $[M+Na]^+$ 530.28, found 530.50.



***N*^{6}-(*tert*-Butoxycarbonyl)-*N*^{2}-((*tert*-butoxycarbonyl)-*L*-phenylalanyl)-*L*-lysine (5)**[1]

To a solution of compound **4** (800 mg, 1.58 mmol) in a 3:1 mixture of methanol/dioxane (8 mL), 1 M NaOH (2.37 mL) was added. The reaction mixture was stirred at room temperature for 20 h. After removing the remaining solvent under reduced pressure, water was added, and pH was adjusted with an aqueous $KHSO_4$ solution (10 %) to 5. The white precipitate which formed was filtered and washed several times with water yielding product **5** (725 mg, 93 %) as a white powder: R_f = 0.25 (CH_2Cl_2/CH_3OH 3:1); m.p. = 106–109 °C; ^{1}H NMR (DMSO-d_6) δ/ppm: 12.58 (brs, 1H, COOH), 8.06 (d, J = 7.8 Hz, 1H, NH), 7.27 (d, J = 4.2 Hz, 4H, Ar), 7.21–7.17 (m, 1H, Ar), 6.87 (d, J = 8.8 Hz, 1H, NH), 6.76 (t, J = 4.9 Hz, 1H, NH), 4.22–4.15 (m, 2H, 2 x CH), 2.97 (dd, J = 13.8, 3.8 Hz, 1H, Ha-CH_2), 2.89 (dd, J = 12.4, 6.4 Hz, 2H, CH_2), 2.77–2.65 (m, 1H, Hb-CH_2), 1.79–1.67 (m, 1H, Ha-CH_2), 1.66–1.54 (m, 1H, Hb-CH_2), 1.36 (brs, 11H, Me_3C-O-, CH_2), 1.29 (s, 9H, Me_3C-O-), 1.23 (brs, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 174.0 (HO-<u>C</u>=O), 171.8 (-NH-<u>C</u>=O), 156.0 (Me_3C-O-<u>C</u>=O), 155.7 (Me_3C-O-<u>C</u>=O),

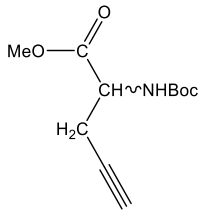
138.8 (Cq, Ar), 129.7 (CH, Ar), 128.4 (CH, Ar), 126.6 (CH, Ar), 78.5 ($Me_3\underline{C}$-O-), 77.8 ($Me_3\underline{C}$-O-), 56.2 (CH), 52.8 (CH), 40.5 (CH_2 covered with DMSO), 37.8 (CH_2), 31.7 (CH_2), 29.7 (CH_2), 28.7 ($\underline{Me}_3$C-O-), 28.6 ($\underline{Me}_3$C-O-), 23.0 (CH_2); (see Supp. Mat. Fig. S34). ESI-MS: *m*/*z* calcd. for $C_{25}H_{39}N_3NaO_7$ $[M+Na]^+$ 516.27, found 516.5.



1-Methoxy-1-oxopent-4-yn-2-aminium chloride (7)

Compound **7** was prepared according to the serine protection procedure [3].

To anhydrous methanol (11.6 mL) cooled with an ice-water bath under argon, acetyl chloride (1.8 mL) was added dropwise. The solution was stirred for a further 5 min. Then solid *D,L*-propargyl glycine **6** (1 g, 8.84 mmol) was added in one portion and the solution was heated to reflux for 2 h. The solvent was removed under reduced pressure to give 1.4 g of crude methyl propargyl glycinate **7** (97 % yield) as a white solid that was used without further purification: ^{1}H NMR (DMSO-d_6) δ/ppm: 8.81 (brs, 3H, NH_3^+), 4.22 (t, J = 5.4 Hz, 1H, CH), 3.76 (s, 3H, OCH_3), 3.14 (t, J = 2.6 Hz, 1H, HC≡C), 2.85 (td, J = 5.7, 2.7 Hz, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 168.4 (MeO-$\underline{C}$=O), 77.3 ($\underline{C}$≡CH), 75.5 (H$\underline{C}$≡C), 53.0 (CH), 50.7 (OCH_3), 20.0 (CH_2); (see Supp. Mat. Fig. S35).

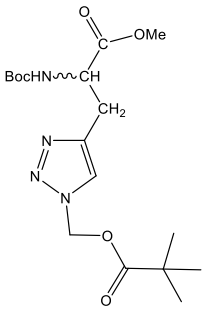


Methyl 2-[(*tert*-butoxycarbonyl)amino]pent-4-ynoate (8)

Compound **8** was prepared according to the serine protection procedure [3].

Suspension of compound **7** (1.4 g, 8.6 mmol) triethylamine (2.57 mL, 18.3 mmol) in dry tetrahydrofuran (27 mL) was cooled with an ice-water bath, and the solution of di-*tert*-butyl dicarbonate (1.9 g, 8.6 mmol, 97 %) in tetrahydrofuran (13 mL) was added dropwise. After 10 min of additional stirring, the ice-water bath was removed, and the suspension was stirred at room temperature for 20 h, then warmed at 50 °C for a further 3 hr. The resulting suspension was filtered, and the filtrate evaporated in a vacuum. The residue was partitioned between diethyl ether (50 mL) and saturated aqueous

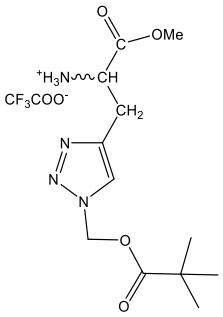
bicarbonate solution (60 mL). The aqueous phase was extracted with three 50 mL portions of diethyl ether. The combined organic phases were dried with anhydrous sodium sulfate and concentrated under reduced pressure to give 1.76 g (88 %) of protected amino acid **8** as a colourless oil: R_f = 0,78 (CH_2Cl_2/EtOAc = 20:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 7.29 (d, J = 8.1 Hz, 1H, NH), 4.14 (dd, J = 14.0, 8.1 Hz, 1H, CH), 3.64 (s, 3H, OCH_3), 2.87 (s, 1H, HC≡C-), 2.63–2.53 (m, 2H, CH_2), 1.38 (s, 9H, Me_3C); ^{13}C NMR (DMSO-d_6) δ/ppm: 171.3 (MeO-<u>C</u>=O), 155.2 (Me_3C-O-<u>C</u>=O), 80.3 (Cq, Me_3<u>C</u>-O-), 78.5 (HC≡<u>C</u>-), 73.0 (H<u>C</u>≡C-), 52.5 (OCH_3 or CH), 52.0 (CH or OCH_3), 28.1 (<u>Me</u>$_3$C-O-), 20.9 (CH_2); (see Supp. Mat. Fig. S36).



(4-(2-((*tert*-Butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-1*H*-1,2,3-triazol-1-yl)methyl pivalate (9)

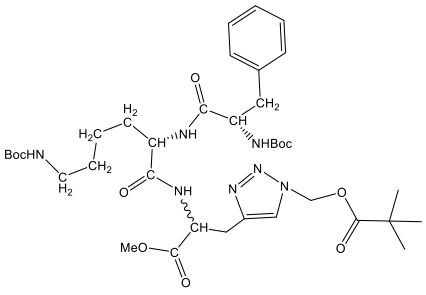
Sodium azide (357.5 mg, 5.5 mmol), CuI (105 mg, 0.55 mmol), sodium ascorbate (60 mg, 0.3 mmol, 99 %) and *N*,*N*-dimethylethylenediamine (DMEDA) (94 µL, 0.83 mmol, 95 %) were added to a solution of chloromethyl pivalate (817 µL, 5.5 mmol, 97 %) in EtOH/H_2O (7:3, *v*/*v*, 7 mL). After the addition of DMEDA, the solution turns blue, then green and finally yellow. After stirring for 30 min, the clear yellow solution changes color to green. The mixture was heated at 90 °C for 1 h, then alkyne **8** (500 mg, 2.2 mmol), sodium ascorbate (60 mg, 0.3 mmol, 99 %), CuI (105 mg, 0.55 mmol) and DMEDA (94 µL, 0.83 mmol, 95 %) were added. The mixture was heated at 90 °C for 30 min and concentrated under reduced pressure. The residue was purified by silica gel chromatography on a funnel (CH_2Cl_2/MeOH 9:1), yielding 754 mg (93 %) of product **9** as a yellow oil: R_f = 0.76 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 7.97 (s, 1H, H-5' triazole), 7.28 (d, J = 8.0 Hz, 1H, NH), 6.28 (s, 2H, N-CH_2-O), 4.24 (td, J = 8.7, 5.6 Hz, 1H, CH), 3.61 (s, 3H, OCH_3), 3.17–2.86 (m, 2H, CH_2), 1.34 (s, 9H, Me_3C-O-), 1.11 (s, 9H, Me_3C-C=O); ^{13}C NMR (DMSO-d_6) δ/ppm: 176.5 Me_3C-<u>C</u>=O), 172.1 (MeO-<u>C</u>=O), 155.3 (Me_3C-O-<u>C</u>=O), 143.3 (Cq, C-4'), 124.2 (CH, C-5'), 78.4 (Cq,

($Me_3\underline{C}$-O-), 69.9 (N-CH_2-O), 53.5 (CH), 51.9 (OCH_3), 38.19 ($Me_3\underline{C}$-C=O), 28.1 ($\underline{Me}_3$C-O-), 27.0 (CH_2), 26.5 ($\underline{Me}_3$C-C=O); (see Supp. Mat. Fig. S37). ESI-MS: *m*/*z* calcd. for $C_{17}H_{28}N_4NaO_6$ $[M+Na]^+$ 407.19, found 407.4.



1-Methoxy-1-oxo-3-(1-(pivaloyloxymethyl)-1*H*-1,2,3-triazol-4-yl)propan-2-aminium 2,2,2-trifluoroacetate (10)

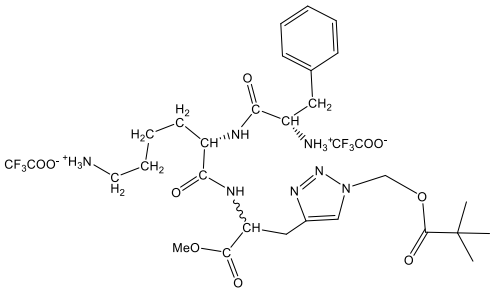
Compound **9** (754 mg, 1.96 mmol) was dissolved in a mixture of TFA/CH_2Cl_2 (*v*/*v* 1:1, 14 mL) and stirred at room temperature for 1 h. After removal of remaining TFA under reduced pressure, product **10** (972 mg, 100 %) was obtained as a yellow/brown foam: R_f = 0.62 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.48 (brs, 3H, NH_3^+), 8.11 (s, 1H, H-5' triazole), 6.30 (s, 2H, N-CH_2-O), 4.41 (brs, 1H, CH), 3.70 (s, 3H, OCH_3), 3.23 (dd, *J* = 5.9, 4.0 Hz, 2H, CH_2), 1.13 (s, 9H, Me_3C-C=O); ^{13}C NMR (DMSO-d_6) δ/ppm: 176.5 (Me_3C-$\underline{C}$=O), 169.0 (MeO-$\underline{C}$=O), 157.8 (CF_3-$\underline{C}$=O), 140.8 (Cq, C-4'), 125.1 (CH, C-5'), 69.9 (N-CH_2-O), 52.9 (CH), 51.6 (OCH_3), 38.2 ($Me_3\underline{C}$-C=O), 27.4 (CH_2), 26.5 ($\underline{Me}_3$C-C=O); (see Supp. Mat. Fig. S38). ESI-MS: *m*/*z* calcd. for $C_{12}H_{21}N_4O_4^+$[M^+] 285.16, found 285.40.



Methyl (6*S*,9*S*)-6-benzyl-9-(4-((*tert*-butoxycarbonyl)amino)butyl)-2,2-dimethyl-4,7,10-trioxo-12-((1-((pivaloyloxy)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)-3-oxa-5,8,11-triazatridecan-13-oate (11)

Dipeptide **5** (174 mg, 0.35 mmol), triazole **10** (139.3 mg, 0.35 mmol), HOBt (48.7 mg, 0.35 mmol, 97 %), and HBTU (135.3 mg, 0.35 mmol 98 %) were dissolved in dry

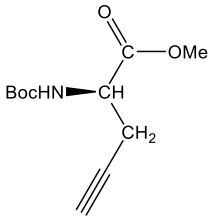
CH_3CN (7 mL), under argon and then dry Et_3N (195 µL, 1.4 mmol) was added. The reaction mixture was stirred at room temperature for 20 h. A product **11** (260.9 mg, 37 %) was obtained as a yellow oil after purifying on preparative TLC plates (CH_2Cl_2/CH_3OH = 9:1): R_f = 0.88 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.47 and 8.40 (2xd, J = 7.5 Hz, 1H, NH), 8.03 and 7.98 (2xs, 1H, H-5′ triazole), 8.01–7.84 (m, 1H, NH), 7.24 (d, J = 4.0 Hz, 4H, Ar), 7.21–7.13 (m, 1H, Ar), 6.92 and 6.88 (2xd, J = 8.5 Hz, 1H, NH), 6.72 (d, J = 5.5 Hz, 1H, NH), 6.28–6.20 (m, 2H, N-CH_2-O), 4.57–4.45 (m, 1H, CH), 4.34–4.21 (m, 1H, CH), 4.22–4.10 (m, 1H, CH), 3.60 and 3.59 (2xs, 3H, OCH_3), 3.19–2.99 (m, 2H, CH_2), 2.99–2.70 (m, 4H, 2xCH_2), 1.70–1.45 (m, 2H, CH_2), 1.36 (brs, 11H, CH_2, Me_3C-O-), 1.28 (brs, 9H, Me_3C-O-), 1.27–1.16 (m, 2H, CH_2), 1.11 and 1.10 (m, 9H, Me_3C=O); ^{13}C NMR (DMSO-d_6) δ/ppm: 176.4 (Me_3C-C=O), 171.7 and 171.5 (MeO-C=O), 171.4 (NH-C=O), 171.3 (NH-C=O), 155.5 (Me_3C-O-C=O), 155.2 (Me_3C-O-C=O), 142.8 (Cq, C-4'), 138.1 (Cq, Ar), 129.2 (CH, Ar), 127.9 (CH, Ar), 126.1 (CH, Ar), 124.3 (CH, C-5'), 78.09 and 78.06 (Me_3C-O-), 77.3 (Me_3C-O-), 69.9 (N-CH_2-O), 55.7 and 55.5 (CH), 52.2 and 52.0 (CH), 51.92 and 51.90 (CH), 51.82 and 51.79 (OCH_3), 38.2 (Me_3C-C=O), 37.3 (CH_2), 32.9 (CH_2), 31.9 (CH_2), 29.2 and 29.1 (CH_2), 28.2 (Me_3C-O-), 28.1 (Me_3C-O-), 27.0 (CH_2), 26.4 (Me_3C-C=O), 22.3 and 22.2 (CH_2); (see Supp. Mat. Fig. S39). ESI-MS: m/z calcd. for $C_{37}H_{58}N_7O_{10}$ $[M+H]^+$ 760.42, found 760.70.



(5*S*)-5-((*S*)-2-ammonio-3-phenylpropanamido)-6-((1-methoxy-1-oxo-3-(1-((pivaloyloxy)methyl)-1*H*-1,2,3-triazol-4-yl)propan-2-yl)amino)-6-oxohexan-1-aminium 2,2,2-trifluoroacetate (12; A)

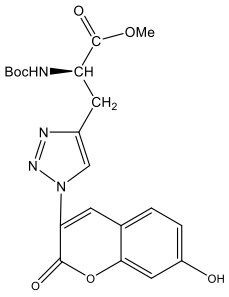
Compound **11** (56 mg, 0.08 mmol) was dissolved in a mixture of TFA/CH_2Cl_2 (*v*/*v* 1:1, 2 mL) and stirred at room temperature for 30 min. After removal of remaining TFA under reduced pressure, product **12** (**A**) (80 mg, 100 %) was obtained as a yellow oil: R_f = 0.3 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.70–8.51 (m, 2H, 2xNH), 8.20 and 8.12 (2xbrs, 3H, NH_3^+), 8.08–7.98 (m, 1H, H-5 triazole), 7.77 (brs, 3H, NH_3^+), 7.38–7.17 (m, 5H, Ar), 6.27–6.23 (m, 2H, N-CH_2-O), 4.64–4.49 (m, 1H, CH), 4.40–4.24

(m, 1H, CH), 4.13–4.07 (m, 1H, CH), 3.62 and 3.58 (2xs, 3H, OCH_3), 3.19–2.96 (m, 4H, 2x CH_2), 2.95–2.85 (m, 1H, Ha-CH_2), 2.78–2.71 (m, 1H, Hb-CH_2), 1.66–1.23 (m, 6H, 3xCH_2), 1.12 and 1.10 (2xs, 9H, Me_3C=O); ^{13}C NMR (DMSO-d_6) δ/ppm: ^{13}C NMR (DMSO-d_6) δ/ppm: 176.4 (Me_3C-C=O), 171.3 and 170.9 (MeO-C=O), 167.8 (NH-C=O), 167.6 (NH-C=O), 158.2, 158.0, and 157.8 (CF_3-C=O), 142.8 and 142.7 (Cq, C-4'),134.9 and 134.7 (Cq, Ar),129.5 (CH, Ar), 128.5 (CH, Ar), 127.1 (CH, Ar), 124.3 (CH, C-5'), 69.9 and 69.8 (N-CH_2-O), 53.2 (CH), 52.2 (CH), 52.0 (CH), 51.94 and 51.86 (OCH_3), 38.7 and 38.6 (CH_2) 38.2 (Me_3C-C=O), 37.3 and 37.0 (CH_2), 31.7 (CH_2).27.3 and 27.0 (CH_2), 26.7 (Me_3C-C=O), 26.5 (CH_2), 21.9 and 21.6 (CH_2); (see Supp. Mat. Fig. S40). HRMS (MALDI-TOF/TOF):*m*/*z* calcd. for $C_{27}H_{41}N_7O_6$ $[M+H]^+$ 560.3197, found 560.3199.



Methyl (*R*)-2-((tert-butoxycarbonyl)amino)pent-4-ynoate (13)

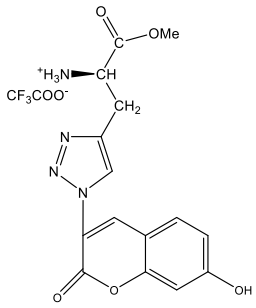
The *R*-enantiomer **13** was synthesized from the purchased *D*-propargylglycine, by the same procedures as racemic compound **8** (shifts in NMR spectra are the same: see Supp. Mat. Fig. S36).



Methyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)propanoate (15)

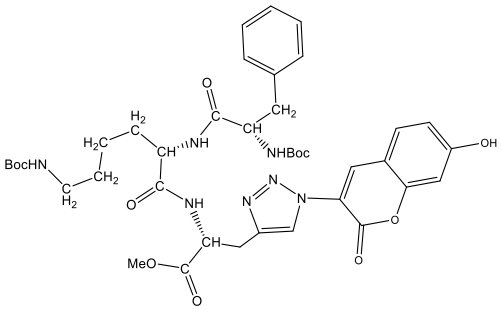
In a mixture of alkyne **13** (740 mg, 3.26 mmol) and 3-azido-7-hydroxycoumarin **14** (662 mg, 3.26 mmol) in water and ethyl alcohol (*v*/*v* = 1:1, 40 mL), sodium ascorbate (390 mg 1.96 mmol) and copper (II) sulfate pentahydrate (50 mg, 0.2 mmol) were added. The yellow mixture was stirred 24 h in the dark (wrapped in Al-foil) at room temperature. The resulting suspension was filtered and the solid was washed with

ethanol/H_2O (*v*/*v* 1:1, 10 mL). Recrystallization from methanol afforded **15** (1.142 g, 81 %) as a light yellow solid: mp 184-186 °C; R_f = 0.74 (CH_2Cl_2/EtOAc = 3:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 10.87 (brs, 1H, OH), 8.59 (s, 1H, H-4''), 8.37 (s, 1H, H-5' triazole), 7.75 (d, J = 8.3 Hz, 1H, H-5''), 7.36 (d, J = 8.0 Hz, 1H, NH), 6.90 (d, J = 8.4 Hz, 1H, H-6''), 6.84 (s, 1H, H-8''), 4.34– 4.26 (m, 1H, CH), 3.64 (s, 3H, OCH_3), 3.23–2.94 (m, 2H, CH_2), 1.35 (s, 9H, Me_3C-C=O); ^{13}C NMR (DMSO-d_6) δ/ppm: 172.1 (CH_3O-<u>C</u>=O), 163.6 (Cq, C-2''), 156.3 (Cq, C-7''), 155.4 (Me_3C-O-<u>C</u>=O), 154.8 (Cq, C-9''), 142.9 (Cq, C-4'), 135.8 (CH, C-4''), 130.8 (CH, C-5''), 123.9 (CH, C-5'), 118.6 (Cq, C-3''), 114.7 (CH, C-6''), 109.8 (Cq, C-10''), 102.2 (CH, C-8''), 78.5 (Cq, (Me_3<u>C</u>-O-C=O), 53.5 (CH), 51.9 (OCH_3), 28.1 (<u>Me</u>$_3$C-O-C=O), 27.0 (CH_2), (see Supp. Mat. Fig. S41); HRMS (MALDI-TOF/TOF): *m*/*z* calcd. for $C_{20}H_{22}N_4O_7$ $[M+Na]^+$ 453.1386, found 453.1395.



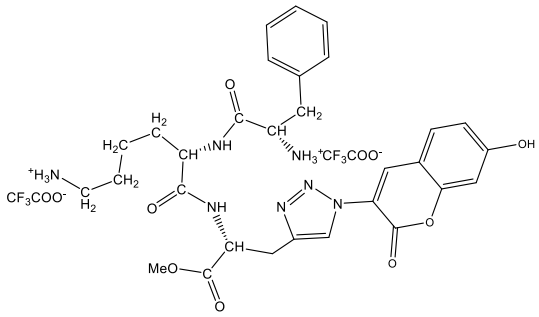
(*R*)-3-(1-(7-Hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)-1-methoxy-1-oxopropan-2-aminium 2,2,2-trifluoroacetate (16; D)

Compound **15** (146 mg, 0.34 mmol) was dissolved in 1:1 mixture of TFA/CH_2Cl_2 (15 mL) and stirred at room temperature for 15 min. After removal of remaining TFA under reduced pressure, product **16 (D)** (150 mg, 100 %) was obtained as a yellow/brown foam: R_f = 0.43 (CH_2Cl_2/EtOAc = 3:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.01 (brs, 1H, OH), 8.59 (s, 1H, H-4''), 8.50 (brs, 3H, NH_3^+), 8.48 (s, 1H, H-5' triazole), 7.78 (d, J = 8.6 Hz, 1H,H-5''), 6.92 (dd, J = 8.5, 2.1 Hz, 1H, H-6''), 6.87 (brd, J = 1.9 Hz, 1H, H-8''), 4.47 (t, J = 5.9 Hz, 1H, CH), 3.75 (s, 3H, OCH_3), 3.32 (d, J = 6.0 Hz, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 169.2 (CH_3O-C=O), 162.6 (Cq, C-2''), 158.30, 158.11, 157.90 and 157.70 (CF_3-<u>C</u>OO^-), 156.2 (Cq, C-7''), 154.6 (Cq, C-9''), 140.6 (Cq, C-4'), 136.0 (CH, C-4''), 131.0 (CH, C-5''), 124.8 (CH, C-5'), 119.2 (Cq, C-3''), 118.27 (<u>C</u>F_3-COO^-), 114.4 (CH, C-6''), 110.3 (Cq, C-10''), 102.2 (CH, C-8''), 52.9 (CH), 51.7 (OCH_3), 26.2 (CH_2); (see Supp. Mat. Fig. S42). HRMS (MALDI-TOF/TOF) *m*/*z* calcd. for $C_{15}H_{15}N_4O_5^+$ $[M^+]$ 331.1042, found 331.1040.



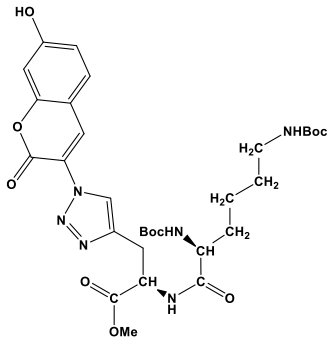
Methyl (6*S*,9*S*,12*R*)-6-benzyl-9-(4-((*tert*-butoxycarbonyl)amino)butyl)-12-((1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate (17; B-boc)

Dipeptide **5** (76.3 mg, 0.155 mmol), triazolo derivative **16** (69 mg, 0.155 mmol), HOBt (21 mg, 0.155 mmol, 97 %), and HBTU (58.8 mg, 0.155 mmol, 98 %) were dissolved in dry CH_3CN (4 mL), under argon and then dry Et_3N (86.4 µL, 0.62 mmol) was added. The reaction mixture was stirred at room temperature for 20 h. A product **17 (B-boc)** (95.2 mg, 76 %) was obtained as a bright yellow crystals, after purifying on preparative TLC plates (CH_2Cl_2/CH_3OH = 9:1): R_f = 0.69 (CH_2Cl_2/CH_3OH 9:1); m.p. = 182-185 °C; ^{1}H NMR (DMSO-d_6) δ/ppm: 10.89 (brs, 1H, OH), 8.53 (s, 1H, H-4''), 8.50 (d, J = 7.7 Hz, 1H,NH), 8.36 (s, 1H, H-5'), 7.84 (d, J = 8.3 Hz, 1H, NH), 7.72 (d, J = 8.7 Hz, 1H, H-5''), 7.27–7.18 (m, 4H, Ar), 7.18–7.11 (m, 1H, Ar), 6.91–6.84 (m, 2H, H-6'' +NH), 6.82 (d, J = 1.8 Hz, 1H, H-8''), 6.68–6.61 (m, 1H, NH), 4.61 (dd, J = 15.2, 7.1 Hz, 1H, CH), 4.29 (dd, J = 13.5, 7.7 Hz, 1H, CH), 4.17–4.10 (m, 1H, CH), 3.65 (s, 3H, OCH_3), 3.22 (dd, J = 14.8, 5.3 Hz, 1H, Ha-CH_2), 3.10 (dd, J = 14.7, 9.1 Hz, 1H, Hb-CH_2), 2.93 (dd, J = 13.8, 3.6 Hz, 1H, Ha-CH_2), 2.80 (dd, J = 13.1, 6.9 Hz, 2H, CH_2), 2.68–2.64 (m, 1H, Hb-CH_2), 1.53 (dt, J = 14.8, 5.5 Hz, 1H, Ha-CH_2), 1.47–1.39 (m, 1H, Hb-CH_2), 1.32 (s, 9H, Me_3C-O), 1.27 (s, 9H, Me_3C-O), 1.27–1.05 (m, 4H, 2xCH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 171.6 (CH_3O-<u>C</u>=O), 171.4 (-NH-<u>C</u>=O), 171.3 (-NH-<u>C</u>=O), 171.3 (Cq, C-2''), 156.1 (Cq, C-7''), 155.4 (Me_3C-O-<u>C</u>=O), 155.2 (Me_3C-O-<u>C</u>=O), 154.6 (Cq, C-9''), 142.6 (Cq, C-4'), 138.1 (Cq, Ar), 135.7 (CH, C-4'') 130.9 (CH, C-5''), 129.1 (CH, Ar), 127.9 (CH, Ar), 126.1 (CH, Ar), 124.0 (CH, C-5'), 119.1 (Cq, C-3''), 114.3 (CH, C-6''), 110.2 (Cq, C-10''), 102.1 (CH, C-8''), 78.1 (Me_3<u>C</u>-O-), 77.2 (Me_3<u>C</u>-O-), 55.7 (CH), 52.1 (CH), 52.0 (CH), 51.8 (OCH_3), 40.1 (CH_2), 37.2 (CH_2), 32.2 (CH_2), 29.1 (CH_2), 28.2 (<u>Me</u>$_3$C-O-), 28.1 (<u>Me</u>$_3$C-O-), 27.2 (CH_2); 22.3 (CH_2); (see Supp. Mat. Fig. S43). HRMS (MALDI-TOF/TOF): m/z calcd. for $C_{40}H_{51}N_7O_{11}$ $[M+Na]^+$: 828.3544, found 828.3530.



(*S*)-5-((*S*)-2-Ammonio-3-phenylpropanamido)-6-(((*R*)-3-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)-1-methoxy-1-oxopropan-2-yl)amino)-6-oxohexan-1-aminium 2,2,2-trifluoroacetate (18; B)

Compound **17** (50 mg, 0.062 mmol) was dissolved in 1:1 mixture of TFA/CH_2Cl_2 (5 mL) and stirred at room temperature for 30 min. After removal of remaining TFA under reduced pressure, product **18** (**B**) (50 mg, 97 %) was obtained as a yellow oil: R_f = 0.50 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.02 (brs, 1H, OH), 8.70 (d, J = 4.0 Hz, 1H, NH), 8.69 (d, J = 4.6 Hz, 1H, NH), 8.53 (s, 1H, H-4''), 8.38 (s, 1H, H-5' triazole), 8.10 (s, 3H, NH_3^+), 7.75–7.73 (m, 4H, NH_3^+, H-5''), 7.29–7.16 (m, 5H, Ar), 6.92 (dd, J = 8.6, 2.3 Hz, 1H, H-6''), 6.85 (d, J = 2.1 Hz, 1H, H-8''), 4.69–4.63 (m, 1H, CH), 4.40–4.37 (m, 1H, CH), 4.09–4.03 (m, 1H, CH), 3.67 (s, 3H, OCH_3), 3.24 (dd, J = 14.9, 5.4 Hz, 1H, Ha-CH_2), 3.13 (dd, J = 14.9, 8.7 Hz, 1H, Hb-CH_2), 3.10–3.03 (m, 1H, Ha-CH_2), 2.88 (dd, J = 14.2, 8.0 Hz, 1H, Hb-CH_2), 2.75–2.65 (m, 2H, CH_2), 1.65–1.56 (m, 1H, Ha-CH_2), 1.52–1.43 (m, 3H, Hb-CH_2, CH_2), 1.31–1.15 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 171.4 (CH_3O-<u>C</u>=O), 170.9 (-NH-<u>C</u>=O),167.7 (-NH-<u>C</u>=O), 162.5 (Cq, C-2''), 158.3, 158.1, 157.9 and 157.7 (CF_3-C=O), 156.2 (Cq, C-7''), 154.6 (Cq, C-9''), 142.6 (Cq, C-4'), 135.8 (Cq, Ar), 134.7 (CH, C-4''), 130.9 (CH, C-5''), 129.5 (CH, Ar), 128.5 (CH, Ar), 127.1 (CH, Ar), 123.9 (CH, C-5'), 119.2 (Cq, C-3''), 114.3 (CH, C-6''), 110.3 (Cq, C-10''), 102.2 (CH, C-8''), 53.2 (CH), 52.2 (CH), 52.1 (CH), 51.9 (OCH_3), 40.1 (CH_2), 36.9 (CH_2), 31.9 (CH_2), 27.3 (CH_2), 26.6 (CH_2), 22.0 (CH_2); (see Supp. Mat. Fig. S44). HRMS (MALDI-TOF/TOF): m/z calcd. for $C_{27}H_{41}N_7O_6$ $[M+Na]^+$ 628.2496, found 628.2468.



Methyl (*R*)-2-((*S*)-2,6-bis((*tert*-butoxycarbonyl)amino)hexanamido)-3-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)propanoate (20)

Method 1

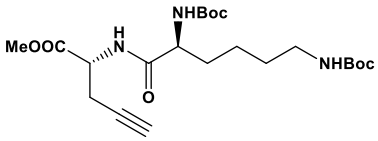
N^2,N^6-Bis(*tert*-butoxycarbonyl)-*L*-lysine **19** (222 mg, 0.64 mmol) and amino acid **16** (**D**) (285 mg, 0.64 mmol) were dissolved in dry CH_3CN (5 mL) under argon and HOBt (89 mg, 6.41 mmol, 97 %), HBTU (248 mg, 6.41 mmol, 98 %) and dry Et_3N (0.357 mL, 2.56 mmol) were added. The reaction mixture was stirred at room temperature for 20 h. Product **20** (370 mg, 88 %) was isolated by preparative chromatography (CH_2Cl_2/MeOH 15:1) as a yellow foam.

Method 2

To a suspension of alkyne **21** (92 mg, 0.2 mmol) in dry acetonitrile (3 mL) was added coumarin azide **14** (49 mg, 0.24 mmol), CuI (76 mg, 0.4 mmol) and dropwise diisopropylethylamine (DIPEA) (0.105 mL, 0.6 mmol). The reaction mixture was stirred at room temperature for 20 h. The solvent was removed in vacuo and the raw product was dissolved in MeOH and filtered through a short Celite column. Product **20** (120 mg, 91 %) was isolated by preparative chromatography (CH_2Cl_2/MeOH 15:1) as a yellow foam.

R_f = 0.45 (CH_2Cl_2/MeOH =15:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.29 (brs, 2H, H-4'' + NH), 8.25 (s, 1H, H-5' triazole), 7.46 (d, J = 8.7 Hz, 1H, NH), 6.80–6.63 (m, 2H, H-5'' + H-6''), 6.54 (d, J = 8.7 Hz, 1H, NH), 6.39 (brs, 1H, H-8''), 4.69–4.49 (m, 1H, CH), 3.97–3.80 (m, 1H, CH), 3.64 (s, 3H, OCH_3), 3.26–3.01 (m, 2H, CH_2), 2.91–2.75 (m, 2H, CH_2), 1.60–1.22 (m, 20H, CH_2 + 2x Me_3C-C=O), 1.29–0.99 (m, 4H, 2x CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 172.3 (MeO-C=O), 171.5 (-NH-C=O), 156.7 (Cq, C-2''), 156.1 (Cq, C-7''), 155.5 (Me_3C-O-C=O), 155.2 (Me_3C-O-C=O), 142.3 (Cq, C-4'), 136.4 (CH, C-4''),

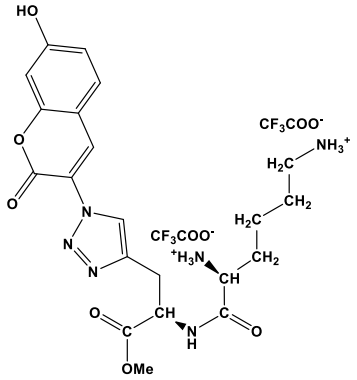
130.4 (CH, C-5''), 123.9 (CH, C-5'), 119.4 (Cq, C-3''), 117.2 (CH, C-6''), 110.1 (Cq, C-10''), 102.5 (CH, C-8), 77.9 ($Me_3\underline{C}$-O-), 77.3 ($Me_3\underline{C}$-O-), 54.0 (CH), 52.0 (CH), 51.8 (OCH_3), 40.4 (CH_2, covered with DMSO), 31.6 (CH_2), 29.1 (CH_2), 28.2 ($\underline{Me}_3$C-O-), 28.1 ($\underline{Me}_3$C-O-), 27.2 (CH_2), 22.6 (CH_2); (see Supp. Mat. Fig. S45). ESI-MS: *m*/*z* calcd. for $C_{31}H_{41}N_6O_{10}^-$ $[M-H]^-$ 657.29, found 657.2.



Methyl (*R*)-2-((*S*)-2,6-bis((*tert*-butoxycarbonyl)amino)hexanamido)pent-4-ynoate (21)

(*R*)-1-methoxy-1-oxopent-4-yn-2-aminium chloride (*D*)-**7** was synthesized from the purchased *D*-propargylglycine by the same procedures as racemic compound **7** (shifts in NMR spectra are the same: see Supp. Mat. Fig. S35).

N^2,N^6-Bis(*tert*-butoxycarbonyl)-*L*-lysine **19** (2.117 g, 6.11 mmol) and (*R*)-1-methoxy-1-oxopent-4-yn-2-aminium chloride (*D*)-**7** (1.06 g, 6.11 mmol) were dissolved in dry CH_3CN (10 mL) under argon and HOBt (851.11 mg, 6.11 mmol, 97 %), HBTU (2.36 mg, 6.11 mmol, 98 %) and dry Et_3N (3.4 mL, 24.44 mmol) were added. The reaction mixture was stirred at room temperature for 20 h. Product **21** (2.11 g, 76 %) was isolated by silica gel chromatography on a funnel (CH_2Cl_2/MeOH 9:1) as a white foam: R_f = 0.85 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.25 (d, J = 7.9 Hz, 1H, NH), 6.80 (d, J = 8.1 Hz, 1H, NH), 6.74 (brs, 1H, NH), 4.46–4.43 (m, 1H, CH), 3.94 (brs, 1H, CH), 3.65 (s, 3H, OCH_3), 2.90–2.84 (m, 3H, HC≡C and CH_2), 2.64–2.54 (m, 2H, CH_2), 1.62–1.44 (m, 2H, CH_2), 1.37 (2xs, 18H, 2x$\underline{Me}_3$C-O-), 1.35–1.29 (m, 2H, CH_2), 1.27–1.18 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 172.3 (MeO-$\underline{C}$=O), 170.6 (-NH-$\underline{C}$=O), 155.5 (Me_3C-O-$\underline{C}$=O), 155.2 (Me_3C-O-$\underline{C}$=O), 79.8 (HC≡$\underline{C}$-), 78.0 ($Me_3\underline{C}$-O-), 77.3 ($Me_3\underline{C}$-O-), 73.2 (H$\underline{C}$≡C-), 54.0 (CH), 52.1 (CH), 50.73 (OCH_3), 40.1 (CH_2, covered with DMSO), 31.7 (CH_2), 29.2 (CH_2), 28.3 ($\underline{Me}_3$C-O-), 28.2 ($\underline{Me}_3$C-O-), 22.7 (CH_2), 21.2 (CH_2); (see Supp. Mat. Fig. S46). ESI-MS: *m*/*z* calcd. for $C_{22}H_{37}N_3O_7$ $[M]^+$ 455.26, found 455.4.



(*S*)-6-(((*R*)-3-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)-1-methoxy-1-oxopropan-2-yl)amino)-6-oxohexane-1,5-diaminium 2,2,2-trifluoroacetate (22 (C))

Compound **20** (200 mg, 0.30 mmol) was dissolved in a 1:1 TFA/CH_2Cl_2 (6 mL) mixture and stirred at room temperature for 20 h. After removal of remaining TFA under reduced pressure, product **22 (C)** (200 mg, 96 %) was isolated by preparative chromatography (CH_2Cl_2/MeOH 9:1) as a yellow foam: R_f = 0.20 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (DMSO-d_6) δ/ppm: ^{1}H NMR (300 MHz, DMSO) δ 8.80 (d, J = 6.2 Hz, 1H, NH), 8.56 (s, 1H, H-4''), 8.38 (s, 1H, H-5' triazole), 8.14-7.29 (brs, 6H, 2xNH_3^+ stretched along the baseline), 7.75 (d, J = 8.6 Hz, 1H, H-5'', covered with NH_3^+), 6.92 (dd, J = 8.5, 2.1 Hz, 1H, H-6''), 6.86 (d, J = 1.9 Hz, 1H, H-8''), 4.80–4.54 (m, 1H, CH), 3.67 (s, 3H, OCH_3), 3.57 (brs, 1H, CH), 3.29–3.12 (m, 2H, CH_2, covered with water), 2.72 (t, J = 7.6 Hz, 2H, CH_2), 1.70–1.38 (m, 4H, 2x CH_2), 1.34–1.16 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 171.33, 171.30 (2x Cq, MeO-C=O and -NH-C=O), 162.7 (Cq, C-2''), 157.8 (CF_3-COO^-), 156.3 (Cq, C-7''), 154.6 (Cq, C-9''), 142.5 (Cq, C-4'), 135.90 (CH, C-4''), 130.9 (CH, C-5''), 124.0 (CH, C-5'), 119.1 (Cq, C-3''), 118.4 and 116.3, (CF_3-COO^-), 114.4 (CH, C-6''), 110.2 (Cq, C-10''), 102.2 (CH, C-8''), 53.1 (CH), 52.1 (CH), 51.7 (OCH_3), 40.1 (CH_2), 38.6 (CH_2), 27.2 (CH_2), 26.7 (CH_2), 21.5 (CH_2); (see Supp. Mat. Fig. S47). HRMS (MALDI-TOF/TOF): m/z calcd. for $C_{21}H_{27}N_6O_6$ $[M+H]^+$: 459,1992, found 459,2002.

2. Spectrophotometric properties:

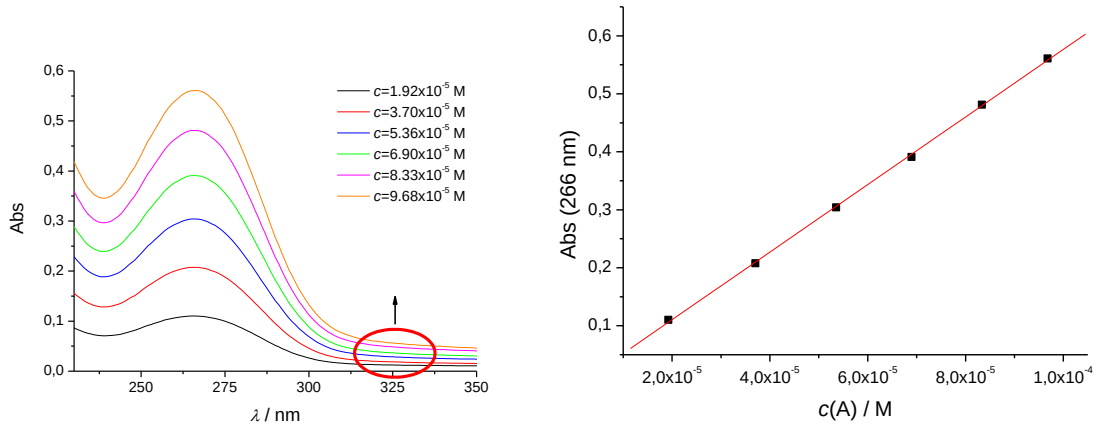


Figure S1. Concentration dependence of **A** UV/Vis spectrum in buffered solution pH 7, *I* = 0.05 M. Note slight increase in baseline at ʎ >325 nm, suggesting colloidisation at higher concentrations, > 4×10^{-5} M.

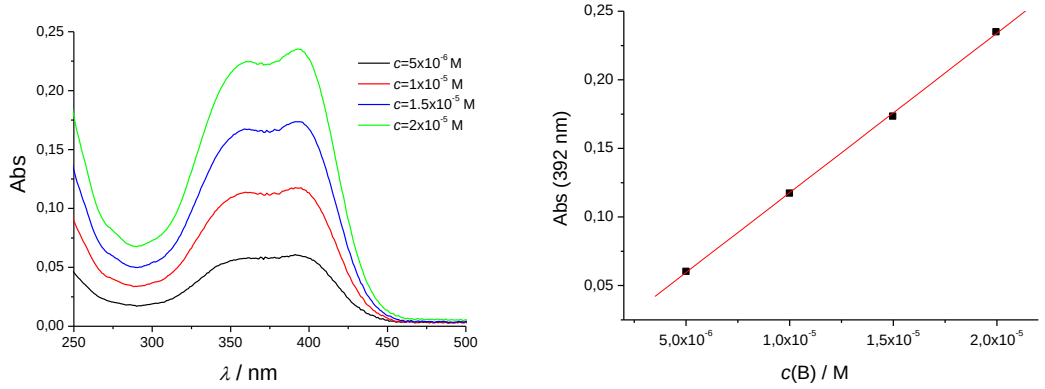


Figure S2. Concentration dependence of **B** UV/Vis spectrum in buffered solution pH 7, *I* = 0.05 M.

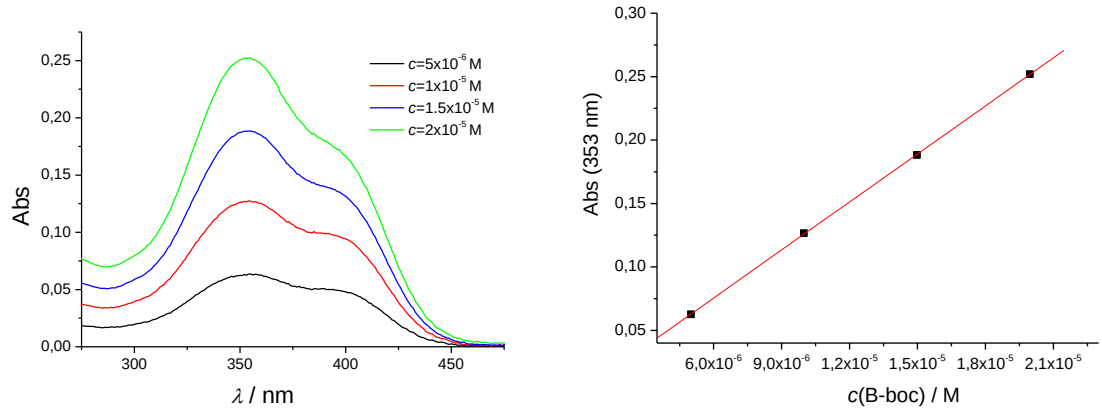


Figure S3. Concentration dependence of **B-boc** UV/Vis spectrum in buffered solution pH 7, *I* = 0.05 M.

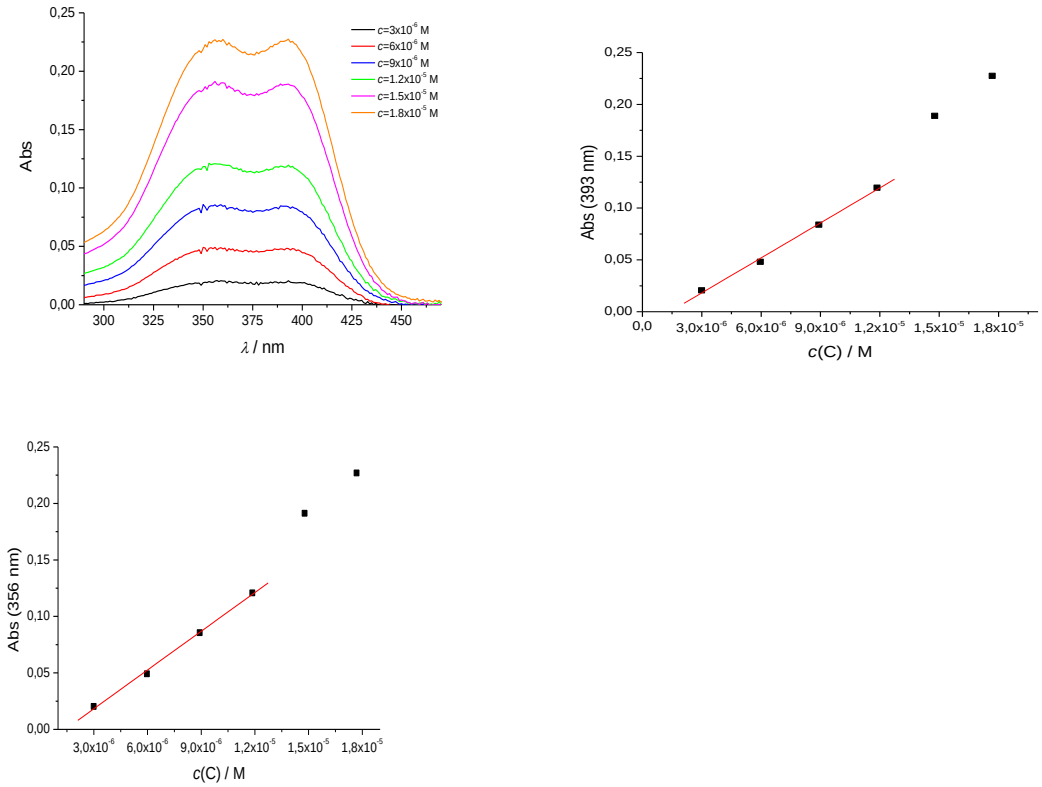


Figure S4. Concentration dependence of **C** UV/Vis spectrum in buffered solution pH 7, *I* = 0.05 M.

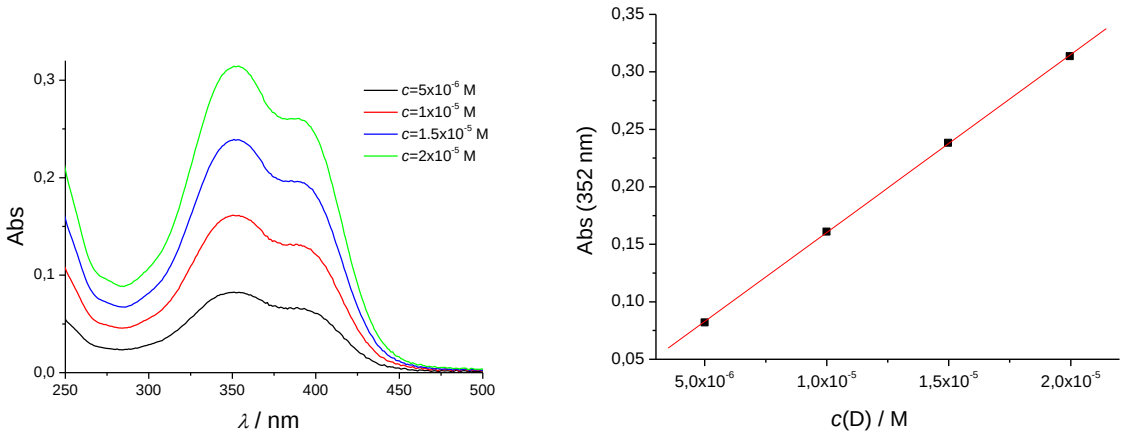


Figure S5. Concentration dependence of **D** UV/vis spectrum in buffered solution pH 7, *I* = 0.05 M.

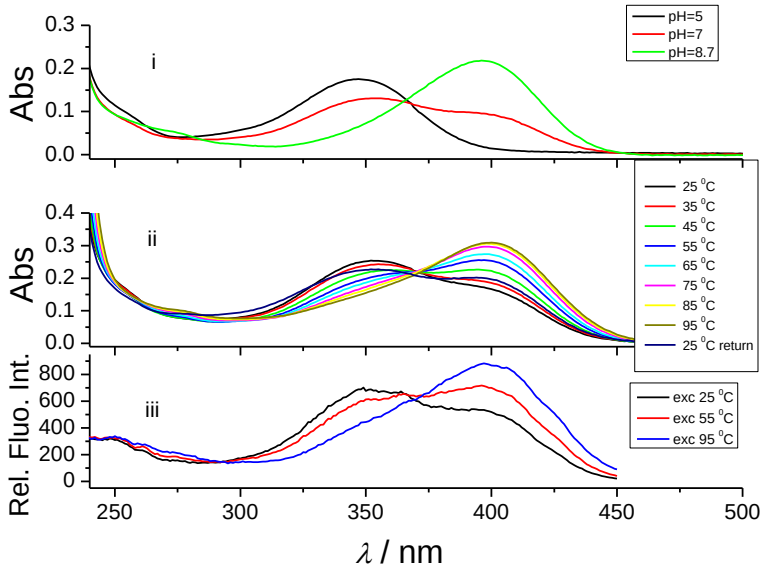


Figure S6. The pH-dependent (i) and temperature-dependent (ii) UV/Vis spectra of compound **B-boc** (c=2×10^{-5} M), and excitation spectrum (iii) at pH 7 (c=2×10^{-6} M; λ_{em}=475 nm).

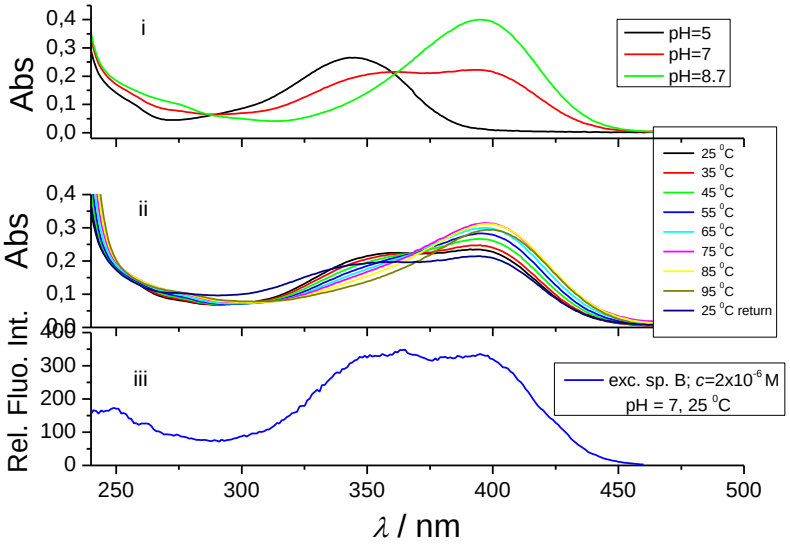


Figure S7. The pH-dependent (i) and temperature-dependent (ii) UV/Vis spectra of compound **B** (c=2×10^{-5} M), and excitation spectrum (iii) at pH 7 (c=2×10^{-6} M; λ_{em}=475 nm).

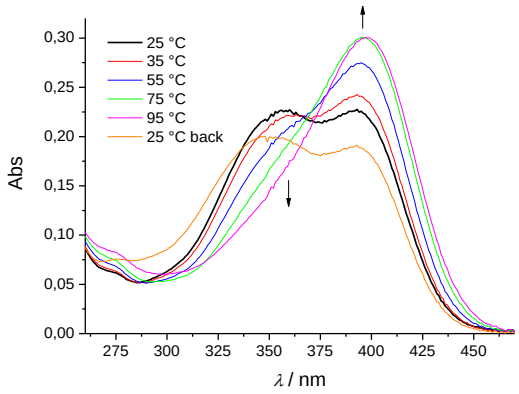


Figure S8. Dependence of UV/Vis spectra on temperature increase of **C** (c=2×10^{-5} M) at pH 7.0, sodium cacodylate buffer, I=50 mM.

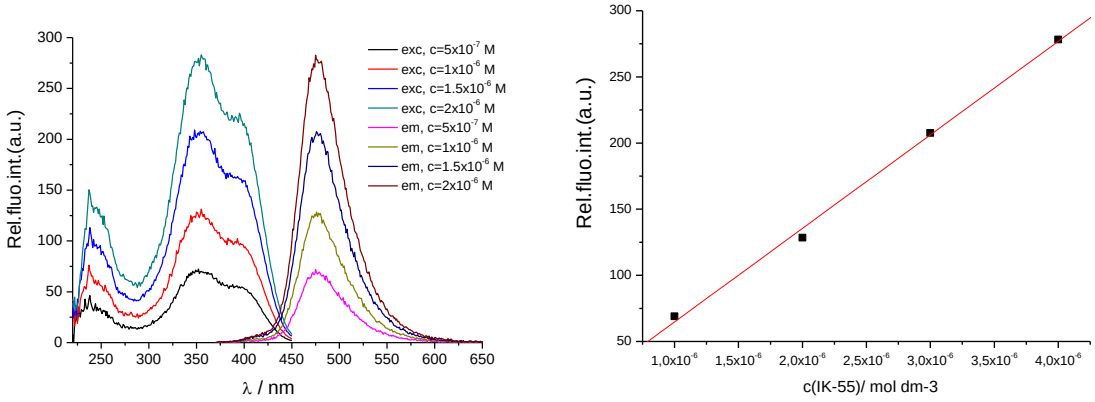


Figure S9. Fluorescence spectra of **B-boc** (λ_{exc}= 353 nm) at micromolar concentrations, in buffer sodium cacodylate (pH 7.0, I = 0.05 M).

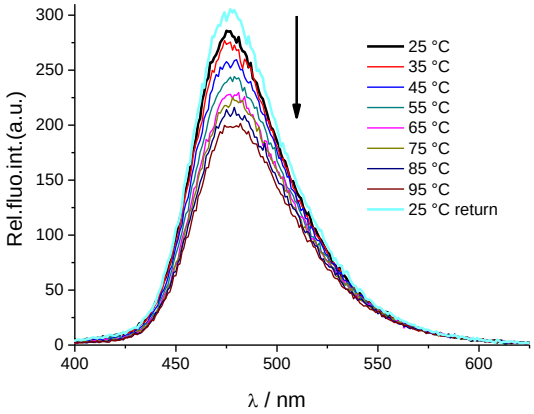


Figure S10. Fluorescence spectra of **B-boc** (λ_{exc}= 353 nm) - temperature dependence with concentration 2×10^{-6} M, in buffer sodium cacodylate (pH 7.0, I = 0.05 M).

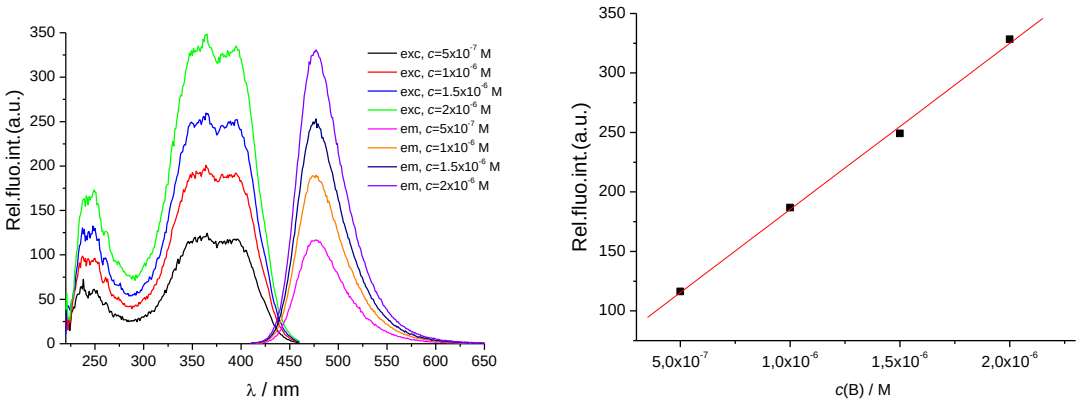


Figure S11. Fluorescence spectra of **B** (λ_{exc}= 392 nm) at micromolar concentrations, in buffer sodium cacodylate (pH 7.0, *I* = 0.05 M). slits 5-2.5

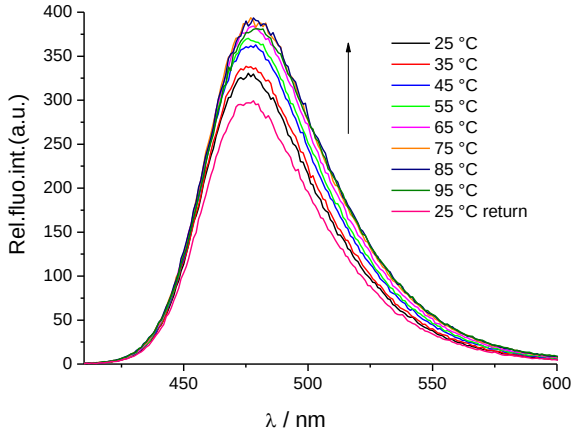
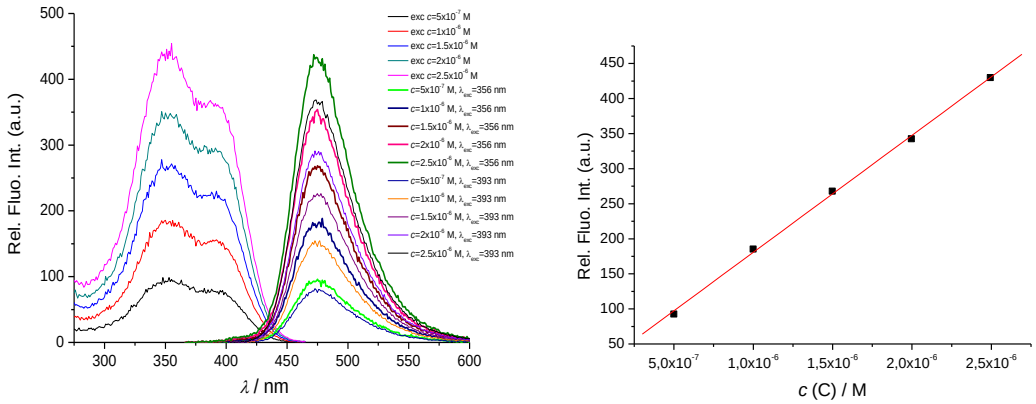


Figure S12. Fluorescence spectra of **B** (λ_{exc}= 392 nm) - temperature dependence with concentration 2×10^{-6} M, in buffer sodium cacodylate (pH 7.0, *I* = 0.05 M).



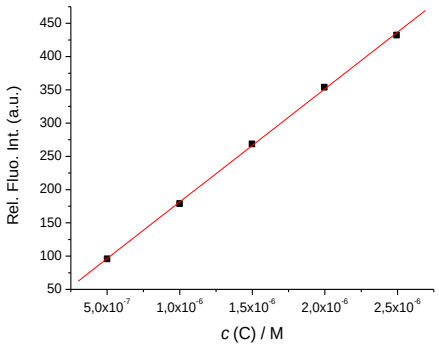


Figure S13. Dependence of fluorescence excitation and emission spectra on concentration increase of **C** on excitation wavelength at 356 nm and emission wavelength at 475 nm. Done at pH 7.0, sodium cacodylate buffer, *I* = 0.05 M.

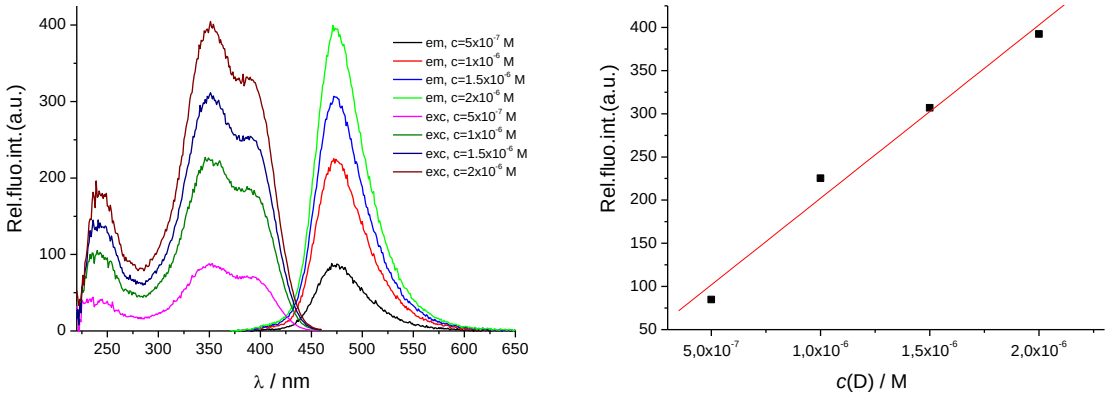


Figure S14. Fluorescence spectra of **D** (λ_{exc}= 353 nm) at micromolar concentrations, in buffer sodium cacodylate (pH 7.0, *I* = 0.05 M).

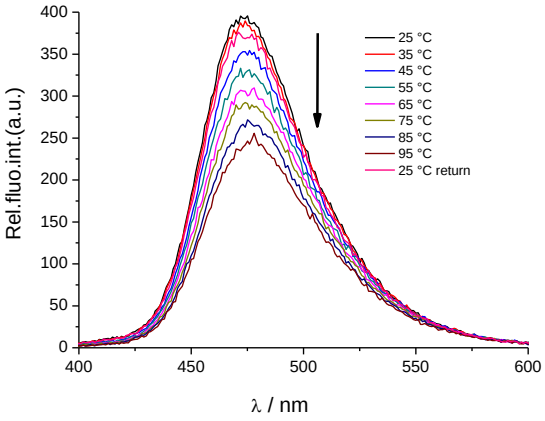


Figure S15. Fluorescence spectra of **D** (2×10^{-6} M, λ_{exc}= 353 nm) - temperature dependence, in buffer sodium cacodylate (pH 7.0, *I* = 0.05 M).

3. **Interactions with $CuCl_2$**

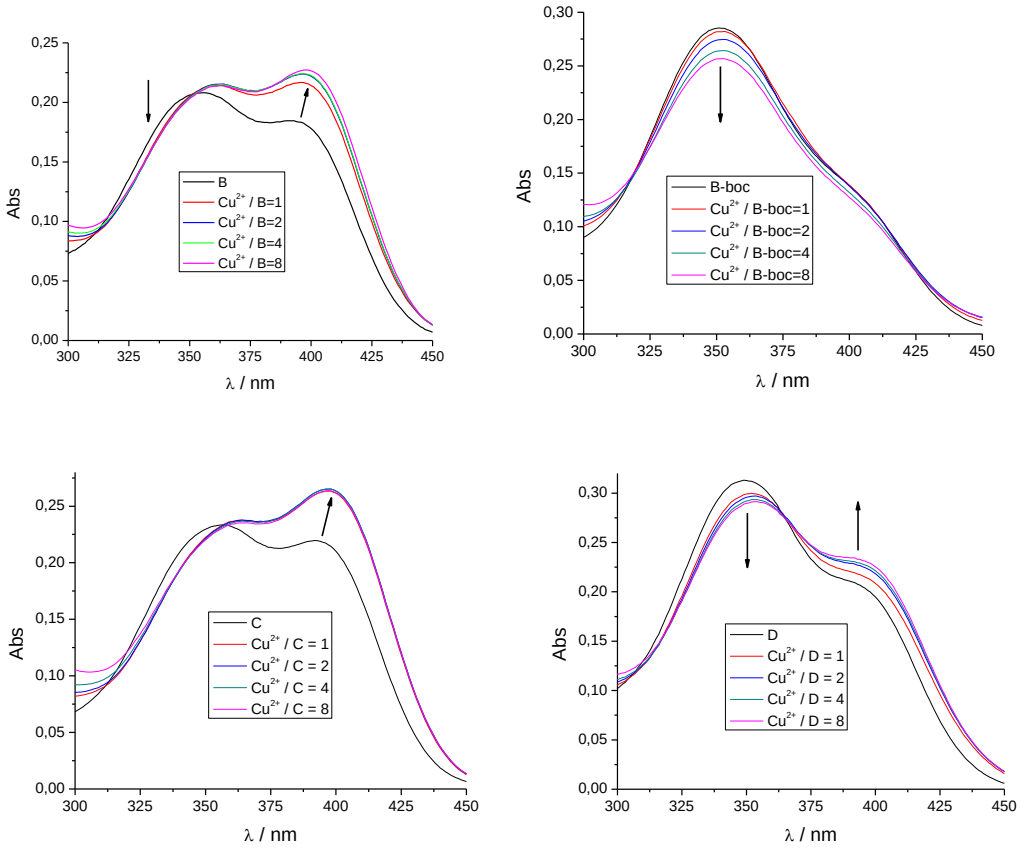


Figure S16. UV/vis titrations of **B, B-boc, C, D** (c = 2×10^{-5} M) with $CuCl_2$**,** for ratios r=[dye]/[Cu^{2+}] = 1-8. Done at pH 7, sodium cacodylate buffer, I = 0.05 M.

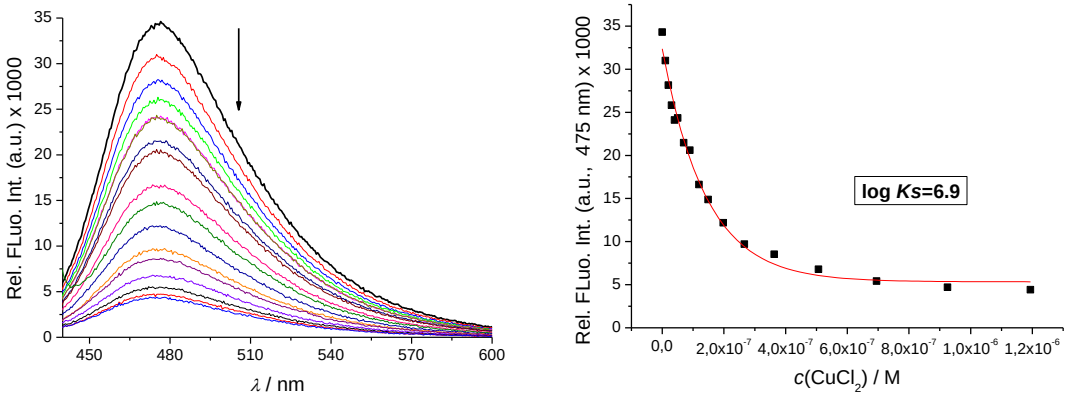


Figure S17. a) Fluorimetric titrations of **C** (c = 1×10^{-8} M; λ_{exc}= 353 nm) with $CuCl_2$; b) dependence of fluorescence at λ_{max}= 475 nm on c($CuCl_2$). Done at pH 7, sodium cacodylate buffer, I = 0.05 M.

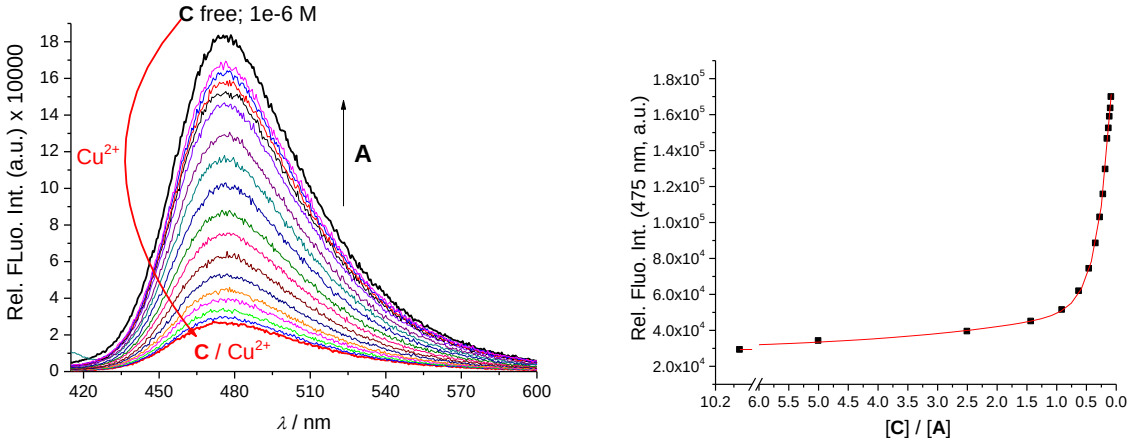


Figure S18. Changes in fluorescence of **C** / Cu^{2+} complex (both, **C** and Cu^{2+} c = 1 × 10^{-6} M, λ_{exc} = 353 nm, λ_{em} = 475 nm) upon addition of **A**. Done at pH 7, sodium cacodylate buffer, I = 0.05 M.

Table S1. The time-correlated single photon counting (TC-SPC) spectrophotometric data of **B, B-boc, C** and **D** upon complexation with Cu^{2+} cation, at pH 7, sodium cacodylate buffer, I = 0.05 M.

	τ / ns (purged)	**χ^2**
B+Cu^{2+} (EPL405)	4.29 (100 %)	1.1755
B-boc+Cu^{2+} (EPLED340)	4.47 (100 %)	1.1399
B-boc+Cu^{2+} (EPL405)	0.18 (1.8 %) 4.44 (98.2 %)	0.9905
C+Cu^{2+} (EPL405)	4.22 (100 %)	1.0102
D+Cu^{2+} (EPL405)	4.21 (100 %)	1.0551
D+Cu^{2+} (EPLED340)	4.20 (100 %)	1.1690

[a] Water solutions were purged by Argon, samples were excited by pulsing diodes at 340 nm and 405 nm, to study neutral an anionic tautomer, respectively. The measurements were performed three times and the average values are reported. The associated errors correspond to the maximum absolute deviation.

CD experiments

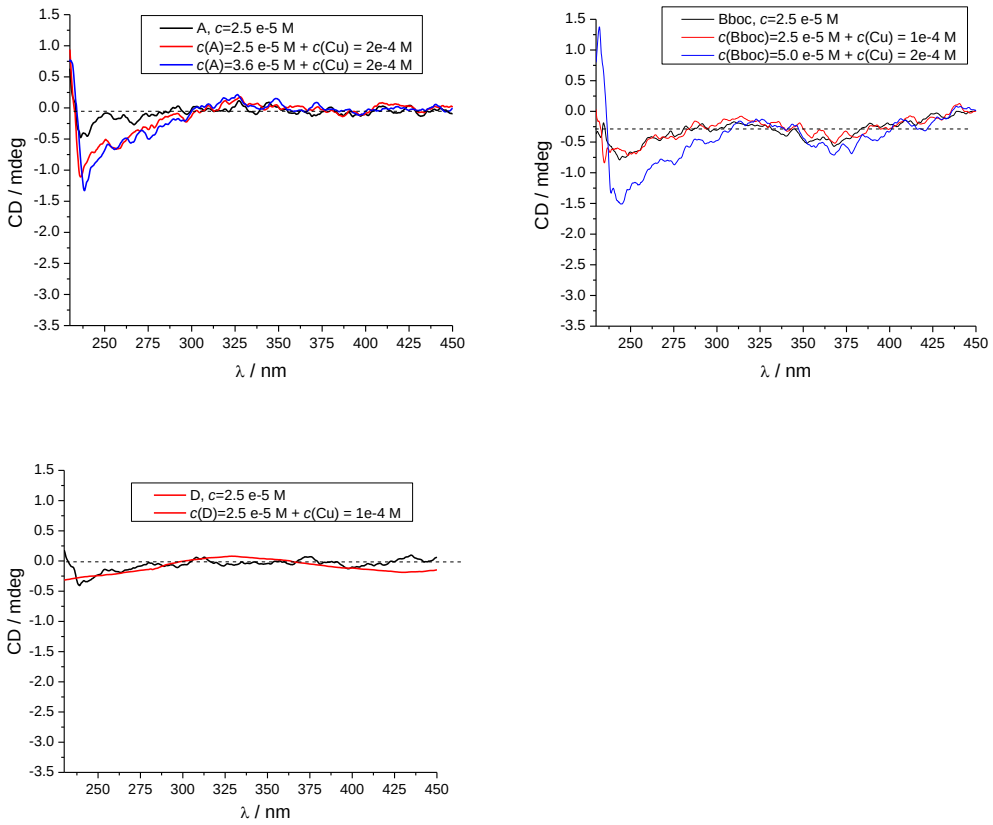


Figure S19. Effect of $CuCl_2$ addition to CD spectra of **A**, **B-boc**, **D** at pH 7.0, sodium cacodylate buffer, *I* = 0.05 M.

4. Interactions with ds-DNA/RNA

Done at the pH 7; sodium cacodylate buffer, *I* = 0.05 M.

Table S2. Groove widths and depths for selected nucleic acid conformations.

Structure type	Groove width [Å]		Groove depth [Å]	
	major	minor	major	minor
[a]**poly rA – poly rU**	**3.8**	10.9	13.5	2.8
[b]**ct-DNA**	11.7	**5.7**	8.5	7.5

[a] A-helical structure (e.g. A-DNA); [b] B- helical structure (e.g. B-DNA)

UV/vis experiments

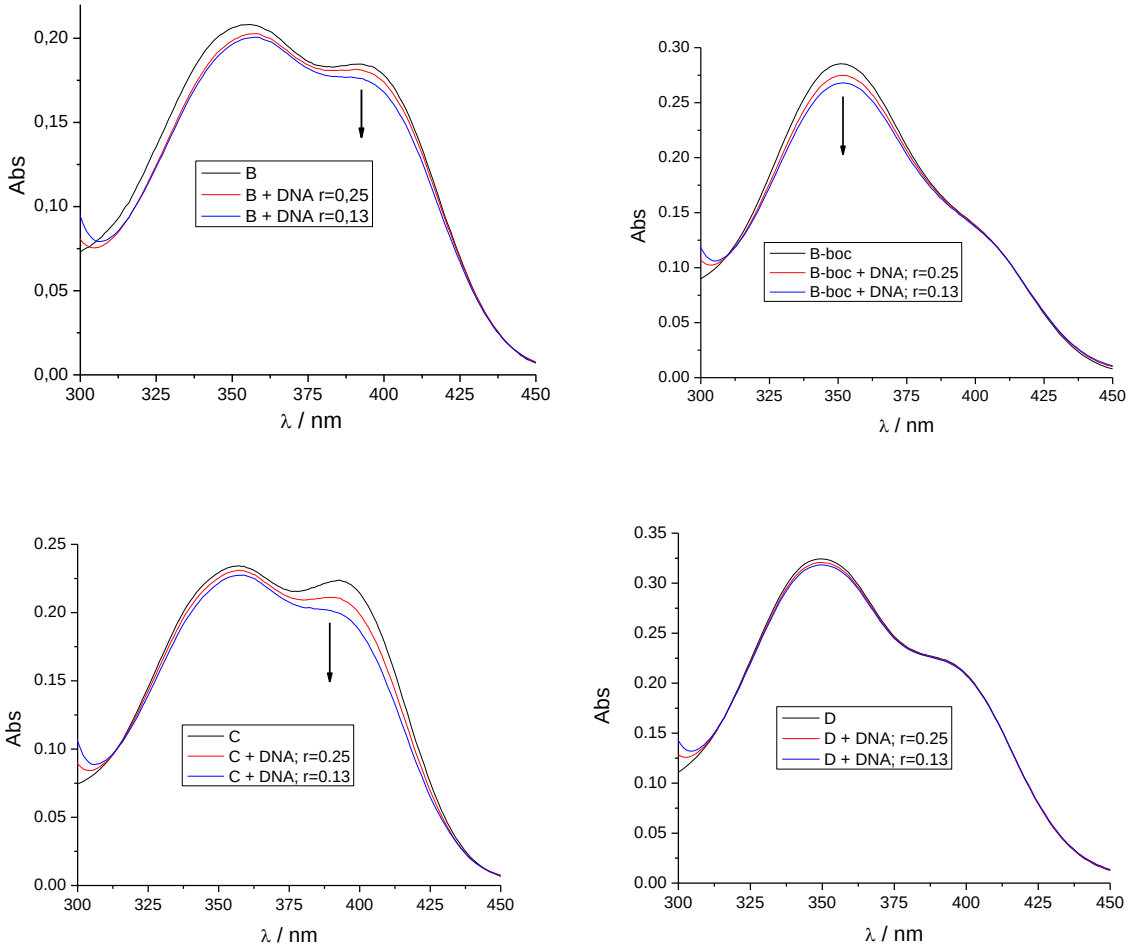


Figure S20. UV/vis titrations of **B, B-boc, C, D** (*c* = 2×10^{-5} M) with ct-DNA**,** for ratios r=[dye]/[DNA]. Done at pH 7, sodium cacodylate buffer, *I* = 0.05 M.

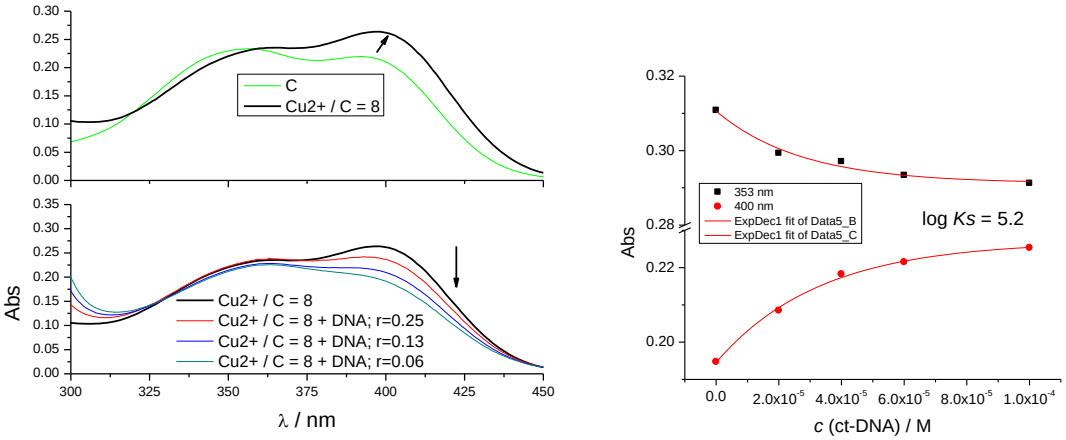


Figure S21. LEFT: up) UV/Vis titration of **C** ($c = 2\times10^{-5}$ M) with $CuCl_2$ (see full titration Figure S17); down) titration of Cu^{2+}/**C** complex with ct-DNA**,** for ratios r=[dye]/[DNA]=0.06-0.25. **RIGHT:** dependence of Abs at λ_{max}= 353 and 400 nm on *c*(ct-DNA), red line denotes non-linear fitting to Scatchard equation for $n_{[bound\ c]/[DNA]}$=0.2. Done at pH 7, sodium cacodylate buffer, *I* = 0.05 M.

Fluorimetric titrations

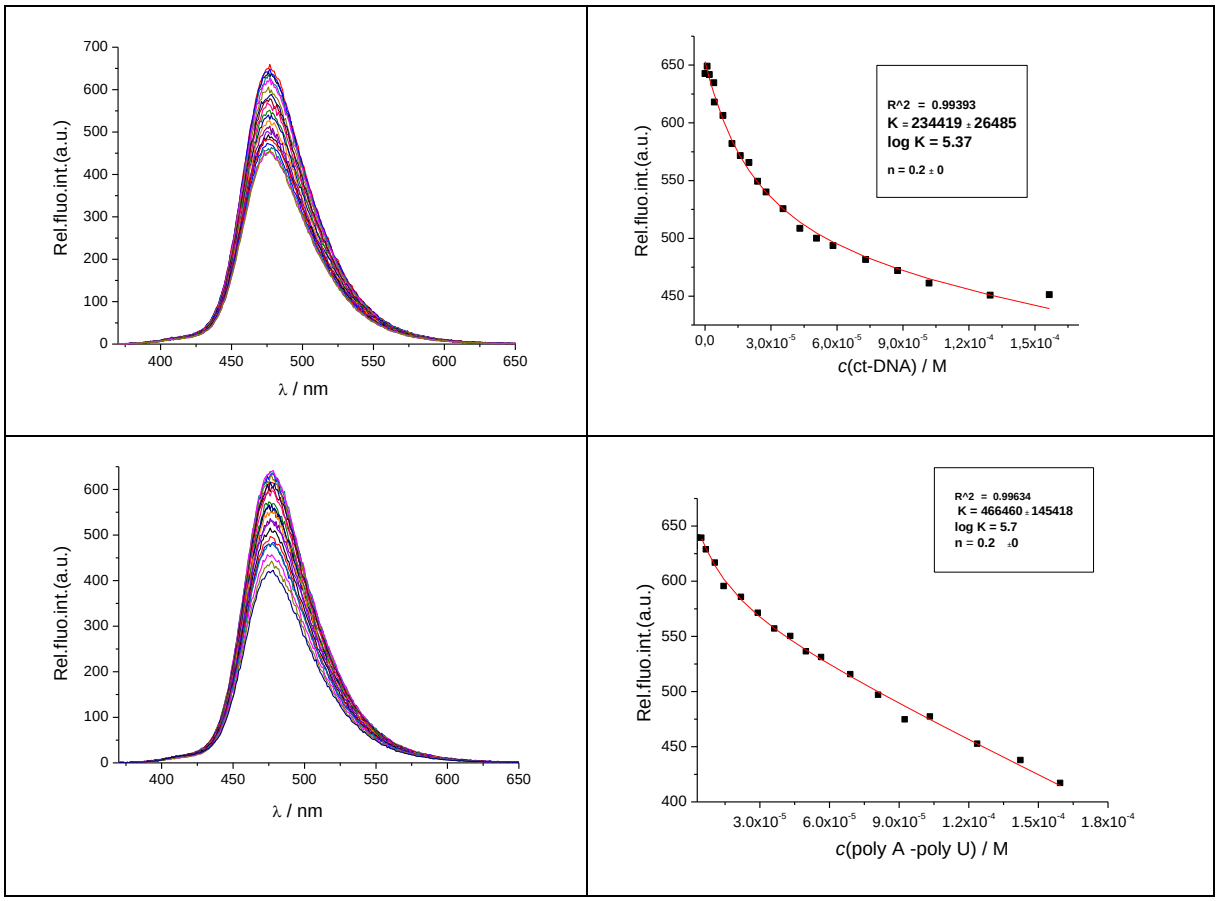


Figure S22. Fluorimetric titrations of **B-boc** (c = 1×10^{-6} M; λ_{exc}= 353 nm) with ds DNA and RNA - **ct-DNA, pApU**. RIGHT: dependence of fluorescence at λ_{max}= 476 nm on c(DNA, RNA). Done at pH 7, sodium cacodylate buffer, I = 0.05 M.

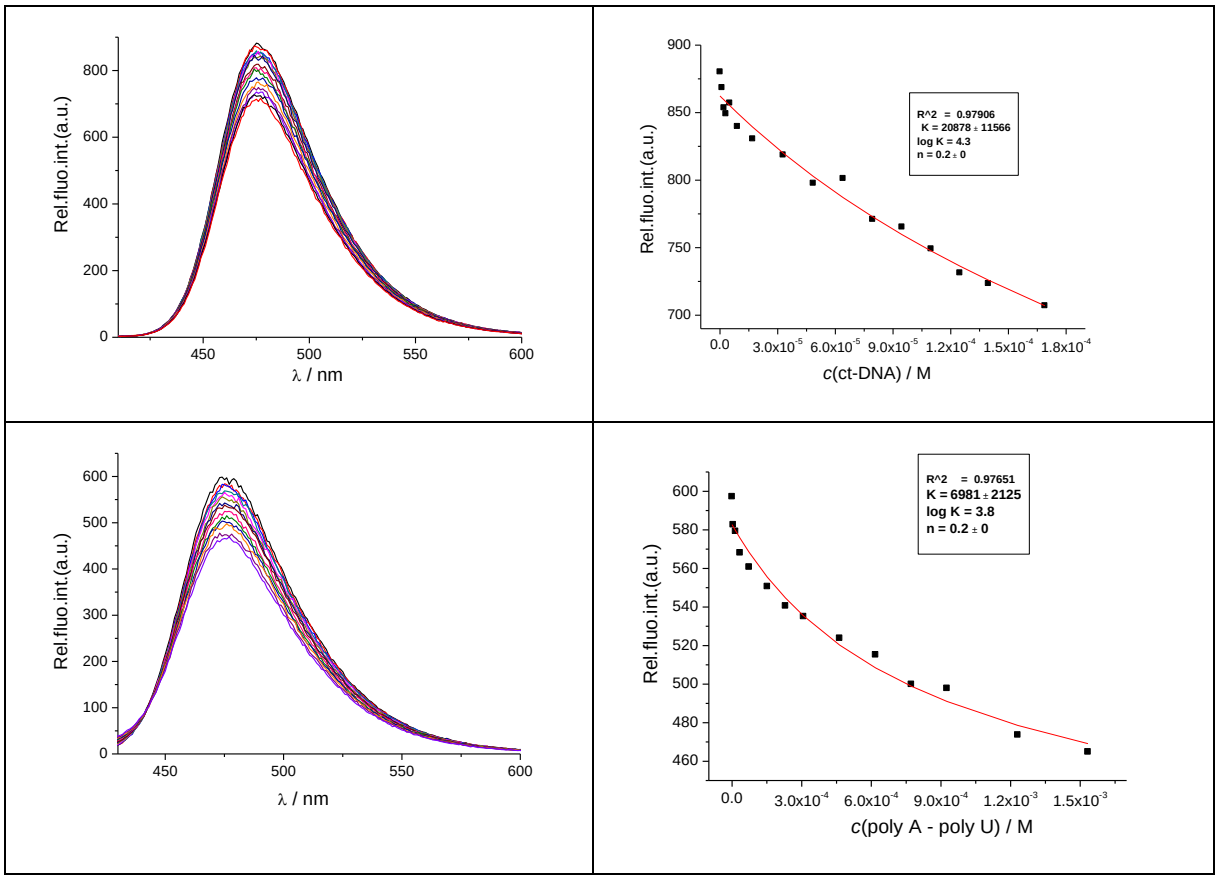


Figure S23. Fluorimetric titration of **B** (c = 1×10^{-6} M; λ_{exc}= 392 nm) with ct-DNA and pApU. RIGHT: dependence of fluorescence at λ_{max}= 476 nm on c(DNA, RNA). Done at pH 7, sodium cacodylate buffer, I = 0.05 M.

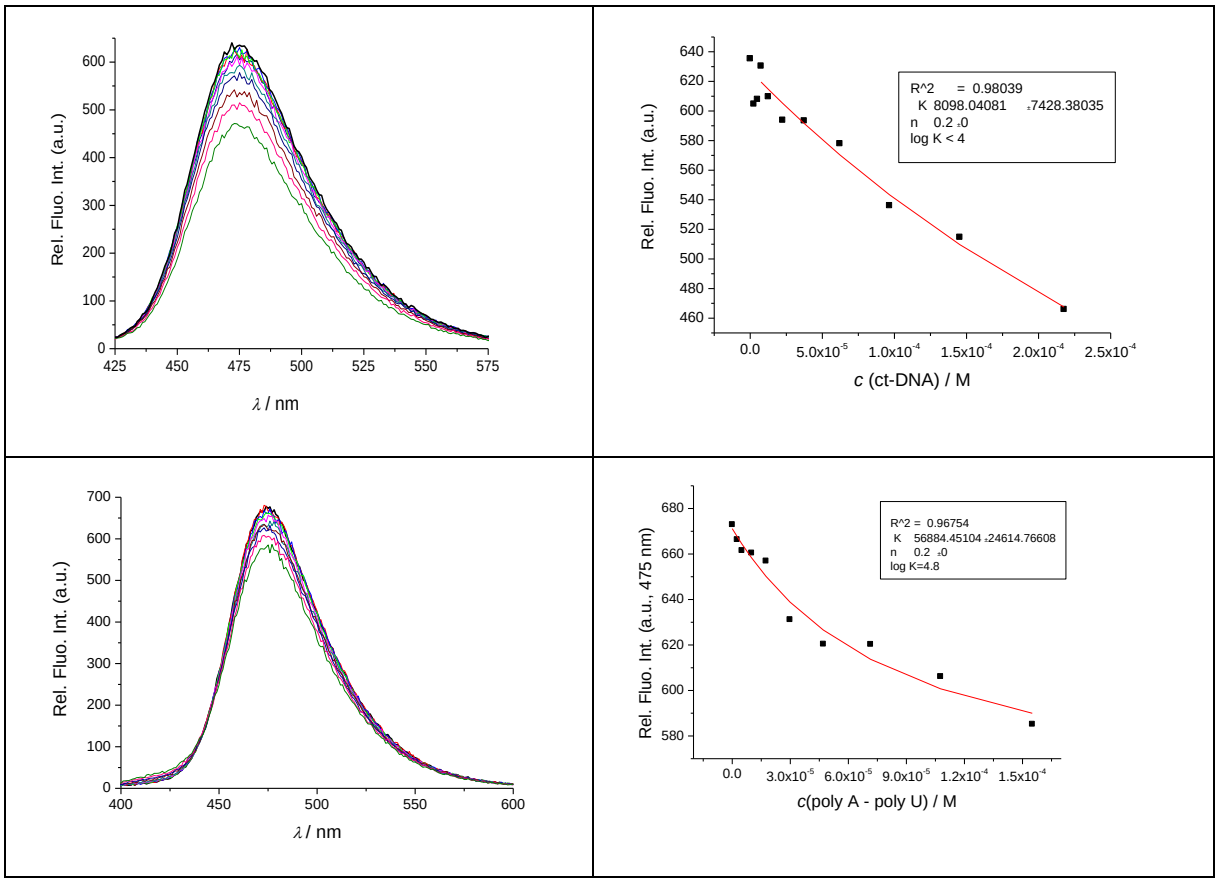


Figure S24. Fluorimetric titration of **C** (c = 1×10^{-6} M; λ_{exc}= 356 nm) with ct-DNA and pApU. RIGHT: dependence of fluorescence at λ_{max}= 475 nm on c(DNA, RNA). Done at pH 7, sodium cacodylate buffer, I = 0.05 M.

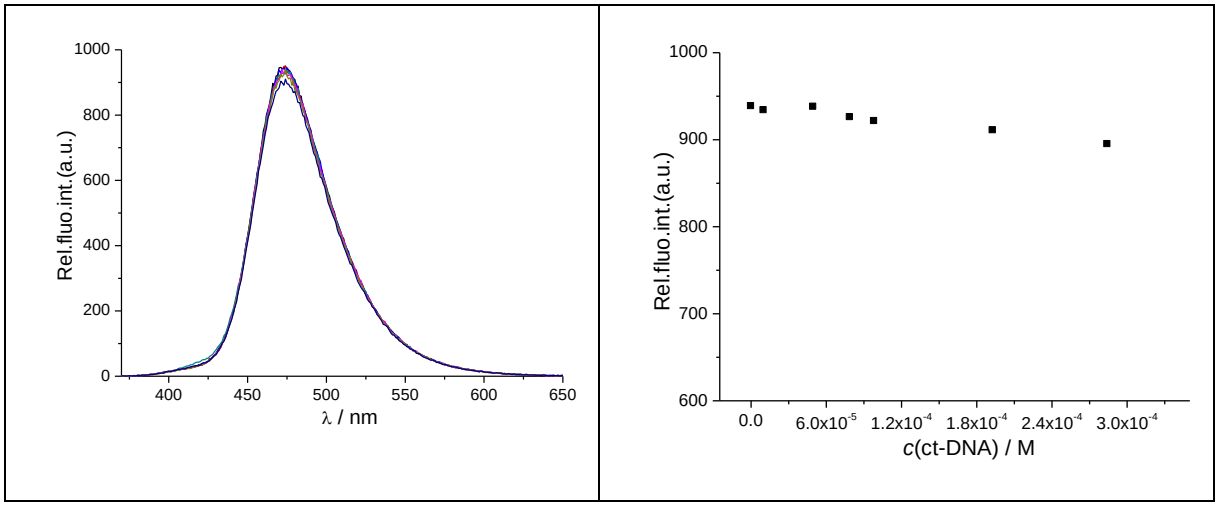


Figure S25. Fluorimetric titration of **D** (c = 1.0 × 10^{-6} M; λ_{exc}= 352 nm) with ct-DNA. RIGHT: dependence of fluorescence at λ_{max}= 476 nm on c(ct-DNA). Done at pH 7.0, sodium cacodylate buffer, I = 0.05 M.

3. Circular dichroism (CD) experiments

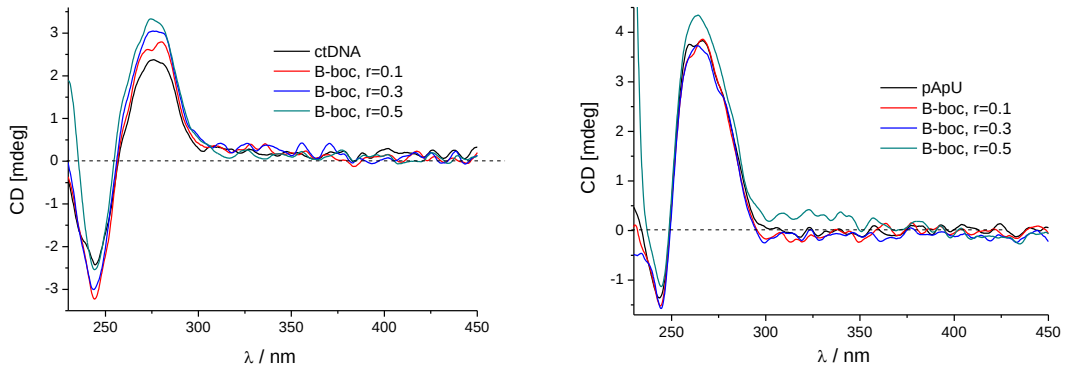


Figure S26. CD titrations of ct-DNA and pApU (c = 2×10^{-5} M) with **B-boc** at molar ratios ***r*** = [compound] / [polynucleotide] (pH 7.0, buffer sodium cacodylate, I = 0.05 M).

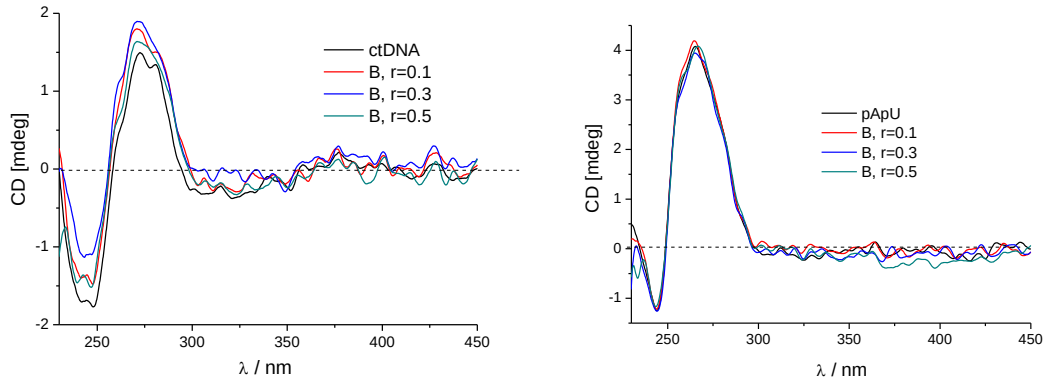
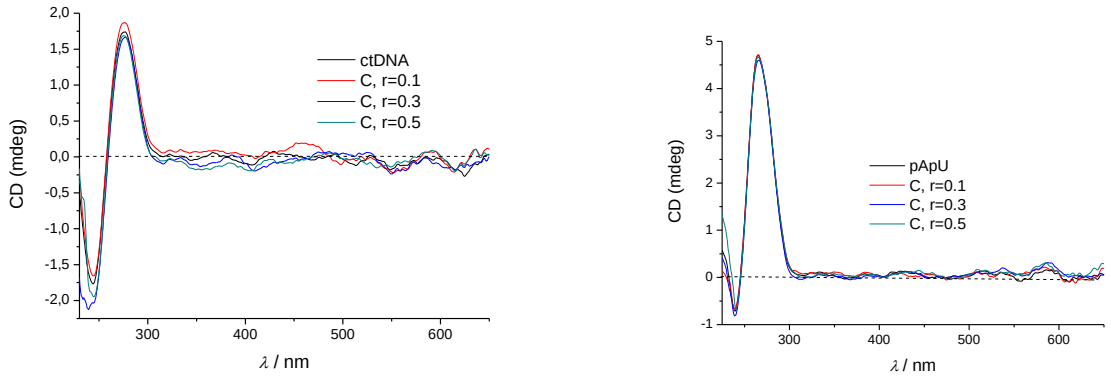


Figure S27. CD titrations of **ctDNA** and **pApU (***c* = 2×10^{-5} M) with **B** at molar ratios ***r*** = [compound] / [polynucleotide] (pH 7.0, buffer sodium cacodylate, I = 0.05 M).



a) b)

Figure S28. CD titration of a) ctDNA, b) pApU (c = 2 × 10^{-5} M) with **C** at molar ratio r = [compound] / [polynucleotide] (pH 7.0, buffer sodium cacodylate, I = 0.05 M).

Thermal denaturation experiments:

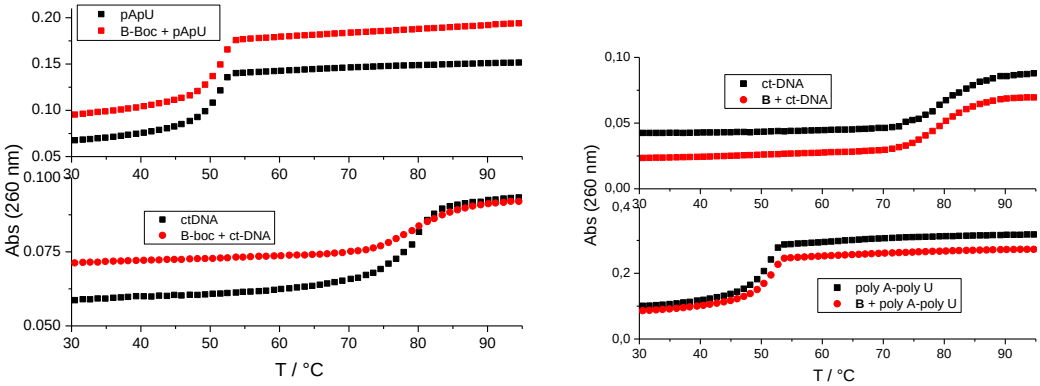


Figure S29. Thermal denaturation curves of ct-DNA (*c*(ct-DNA) = 2.5 × 10^{-5} M, $r_{[(dye)]/[ct\text{-}DNA]}$ = 0.3) and poly A poly U (c(RNA)= 2.5 10^{-5} M, $r_{[(dye)]/[poly\ A\ poly\ U]}$ = 0.3) at pH 7.0 (sodium cacodylate buffer, I = 0.05 M) upon addition of **B-boc** and **B**.

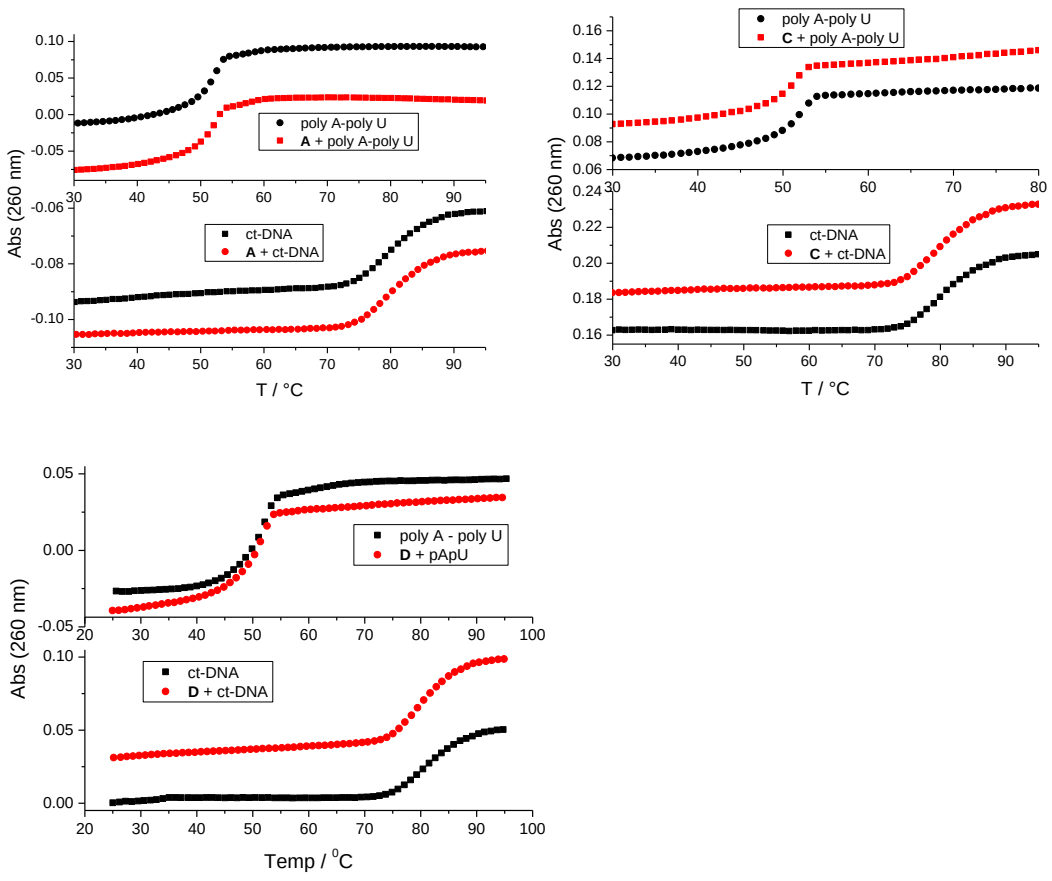


Figure S30. Thermal denaturation curves of ct-DNA (*c*(ct-DNA) = 2.5 × 10^{-5} M, $r_{[(dye)]/[ct\text{-}DNA]}$ = 0.3) and poly A poly U (c(RNA)= 2.5 10^{-5} M, $r_{[(dye)]/[poly\ A\ poly\ U]}$ = 0.3) at pH 7.0 (sodium cacodylate buffer, I = 0.05 M) upon addition of **A**, **C** and **D**.

Addition of A-D complexes with Cu^{2+} cation

$CuCl_2$ was added as 4 equivalents to A-D, to ensure high percentage of complex formed

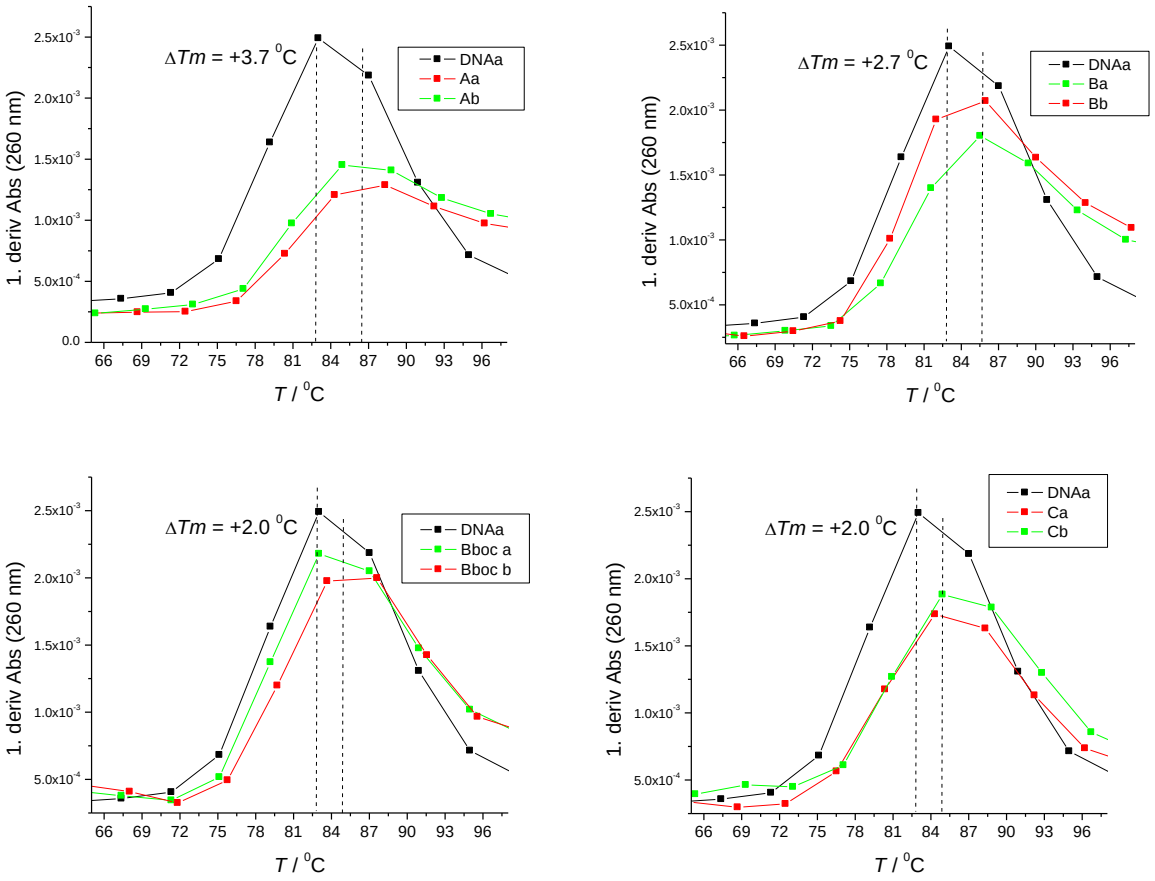


Figure S31. Thermal denaturation curves of ct-DNA (c(ct-DNA) = 2.5 × 10^{-5} M, $r_{[(dye)]/[ct\text{-}DNA]}$ = 0.3) at pH 7.0 (sodium cacodylate buffer, I = 0.05 M) upon addition of complexes **A**, **B, B-boc C** and **D** + 4 equivalents of $CuCl_2$. Referent ct-DNA solutions contained the same amount of $CuCl_2$ and DMSO to as the samples with **A**-**D**.

5. NMR data:

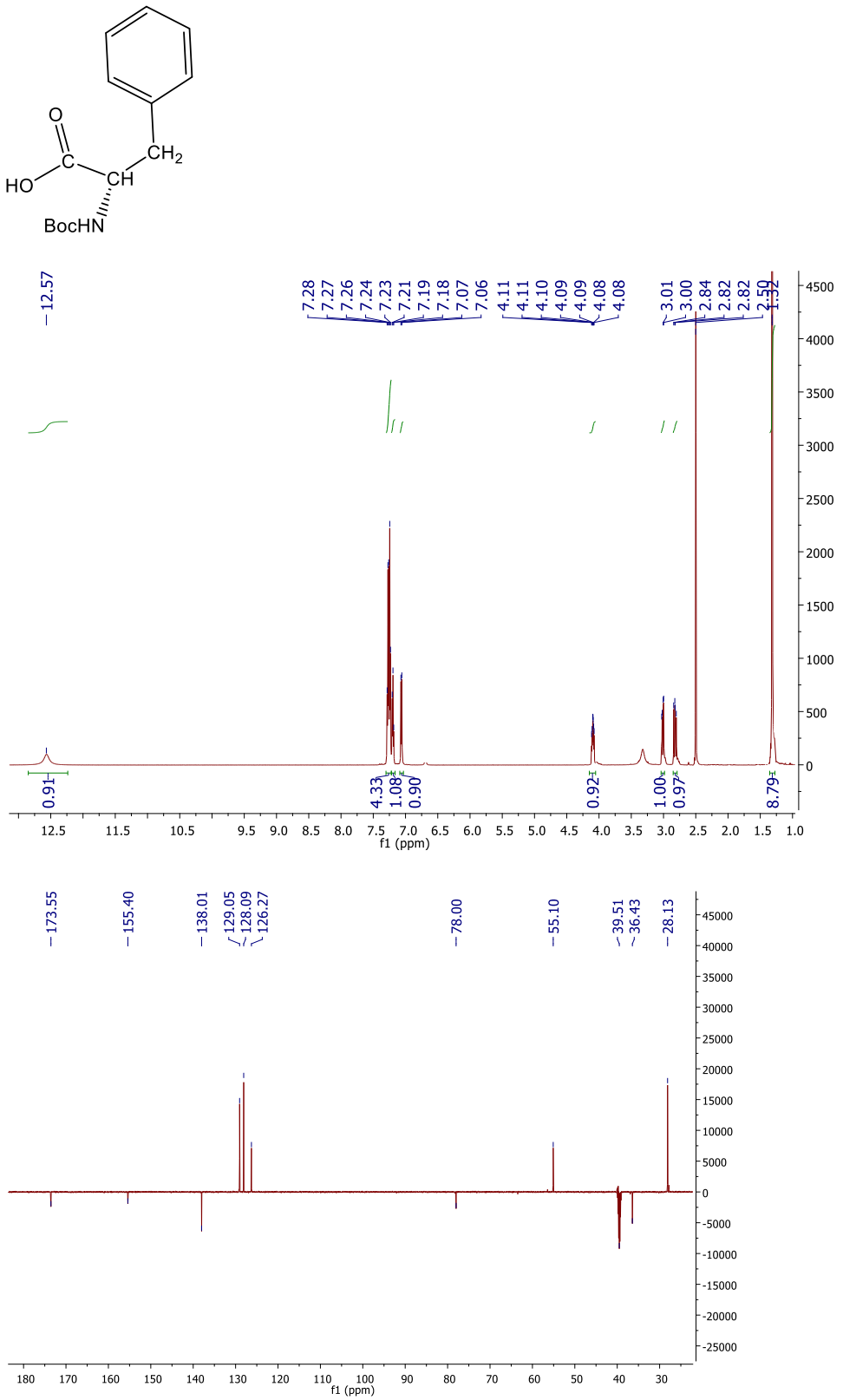


Figure S32. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of compound **2**.

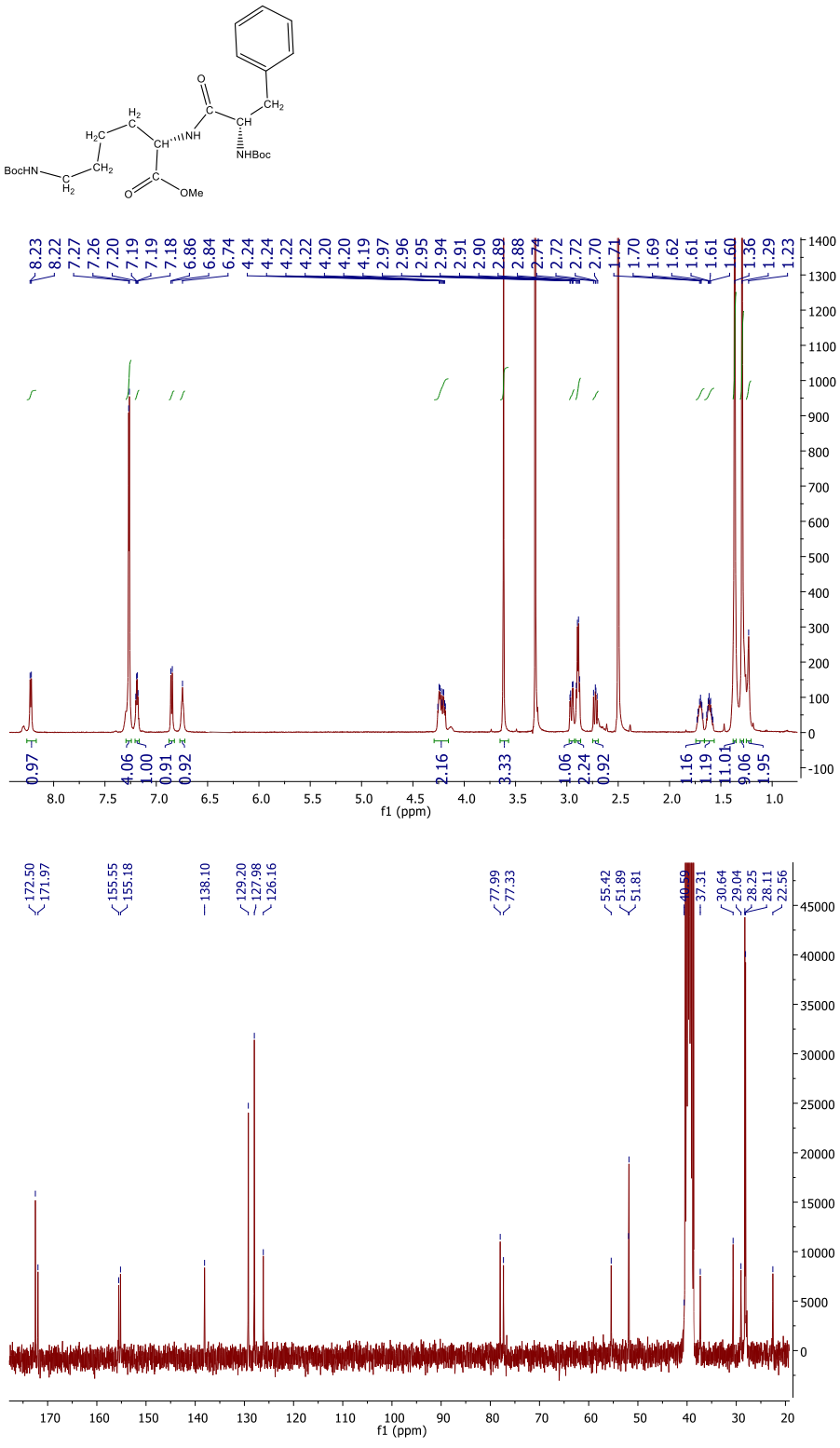


Figure S33. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of compound **4**.

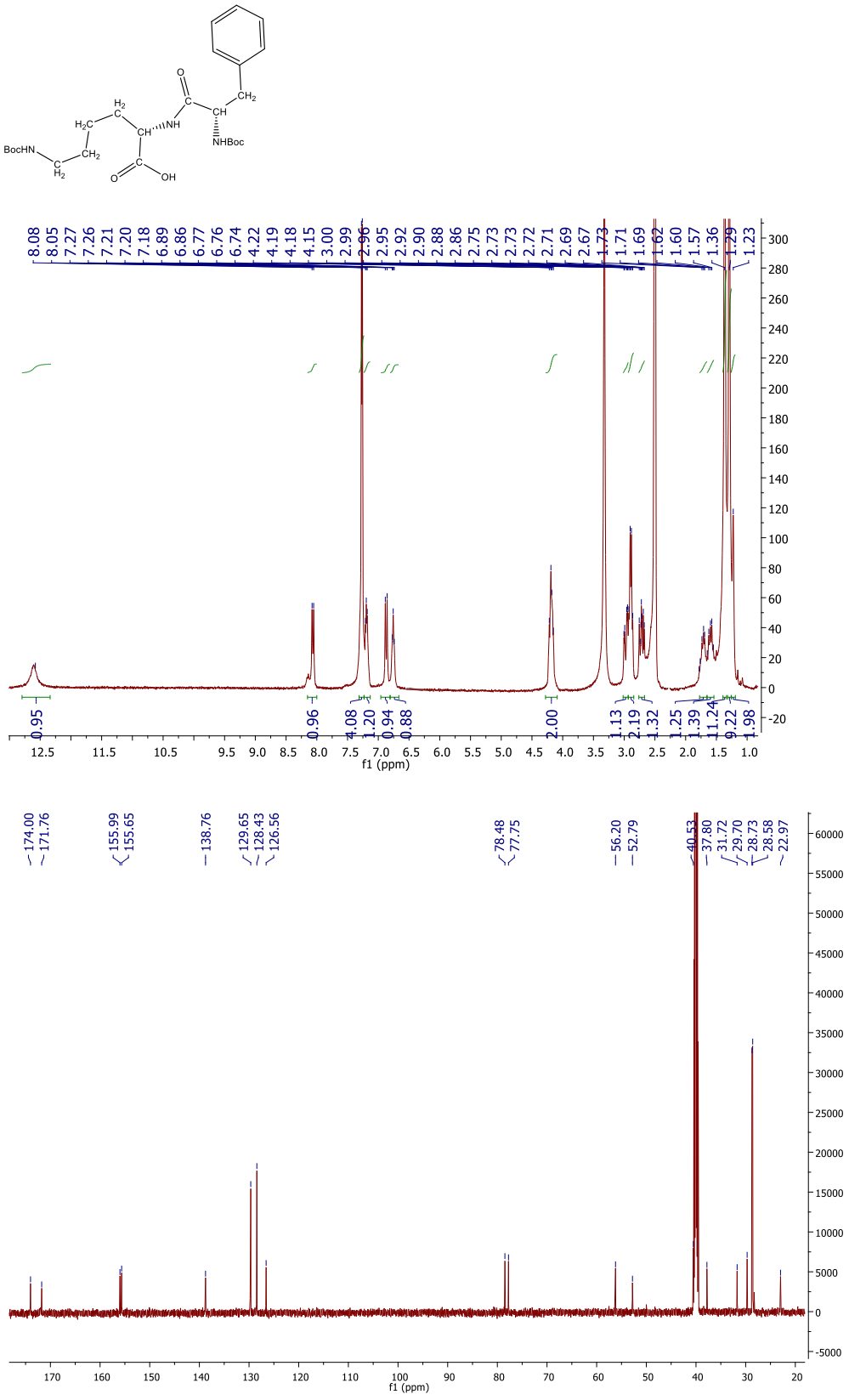


Figure S34. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) spectra of compound **5**.

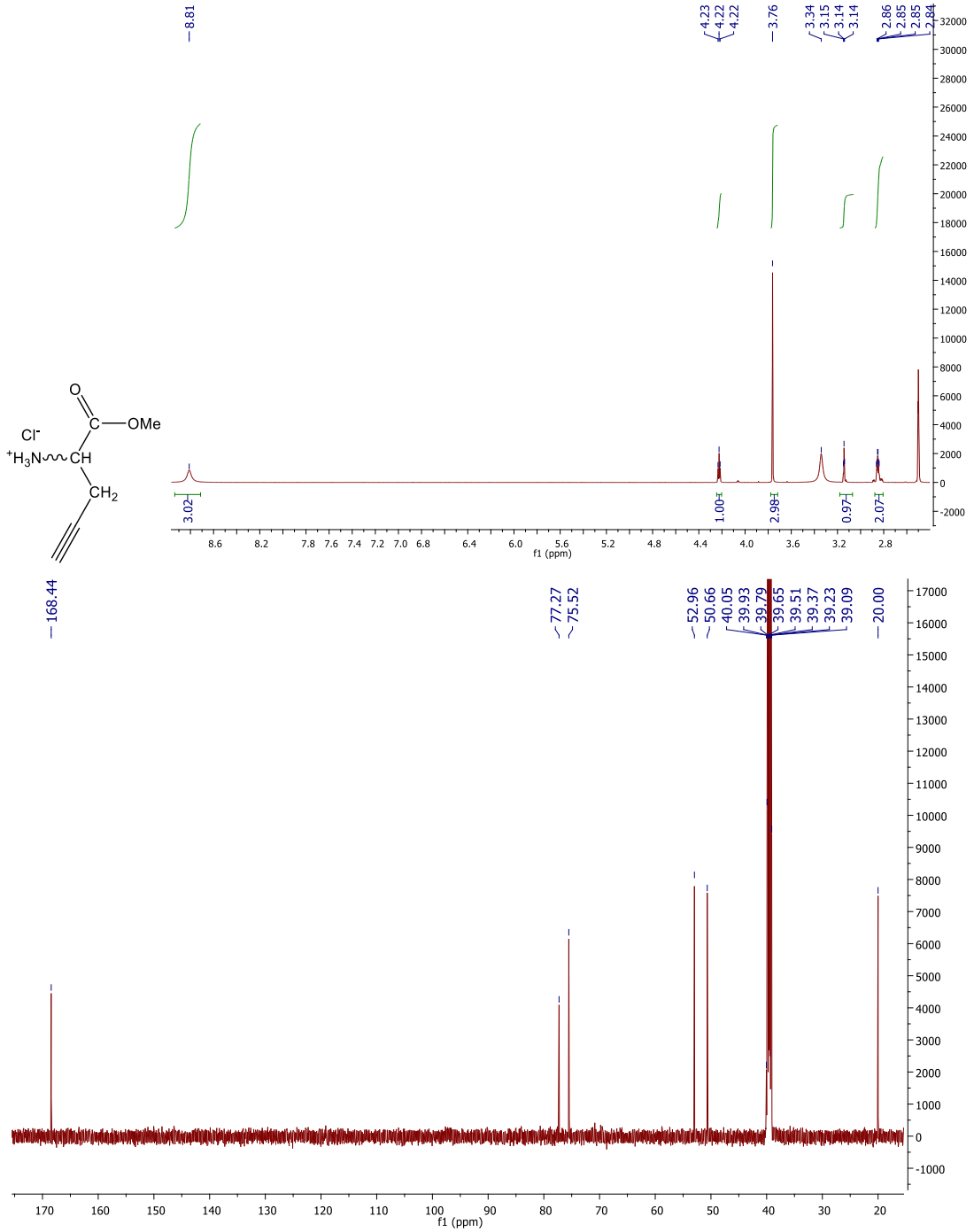


Figure S35. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of compound **7**.

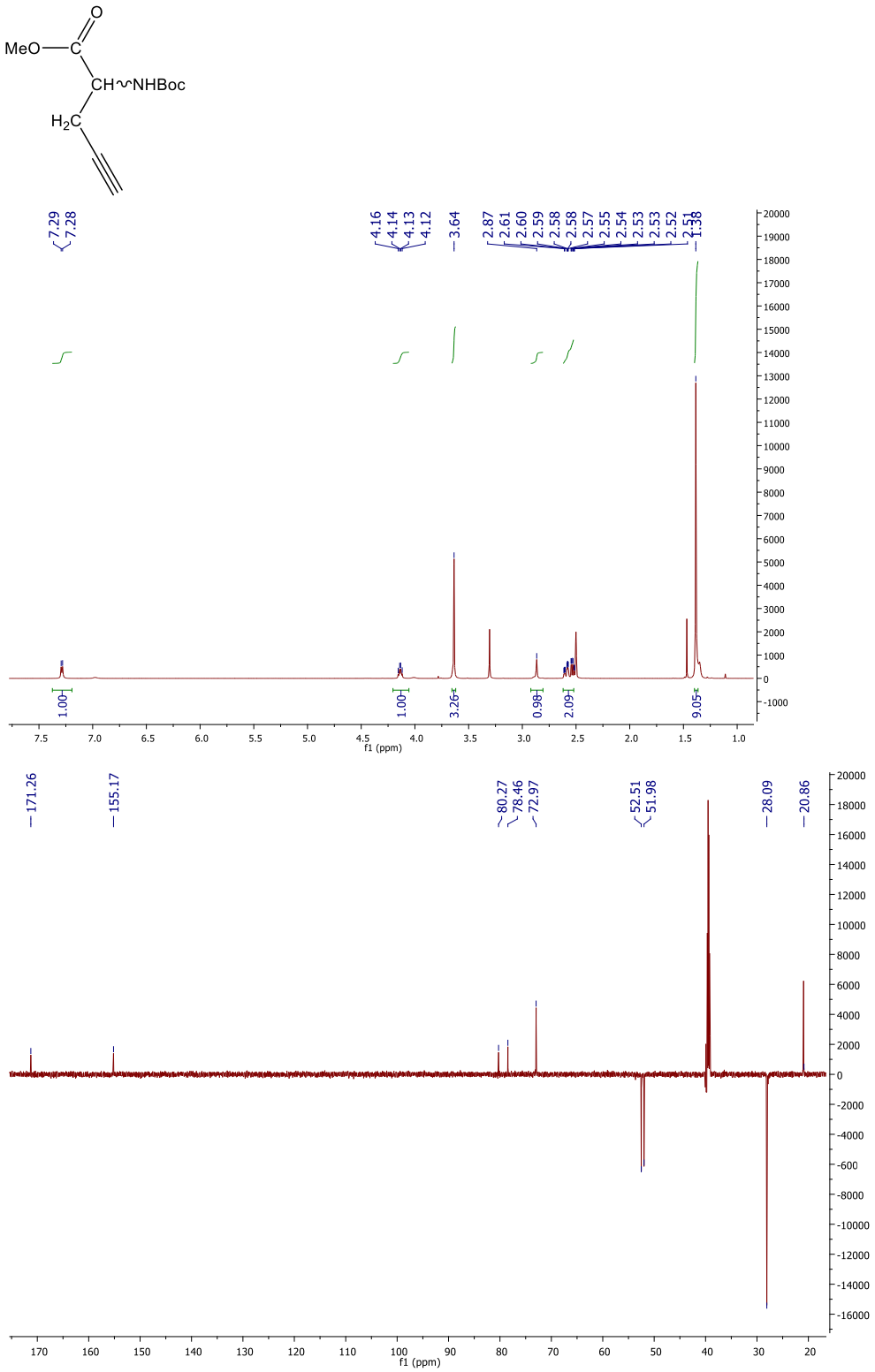


Figure S36. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, APT, DMSO-d_6) spectra of compound **8**.

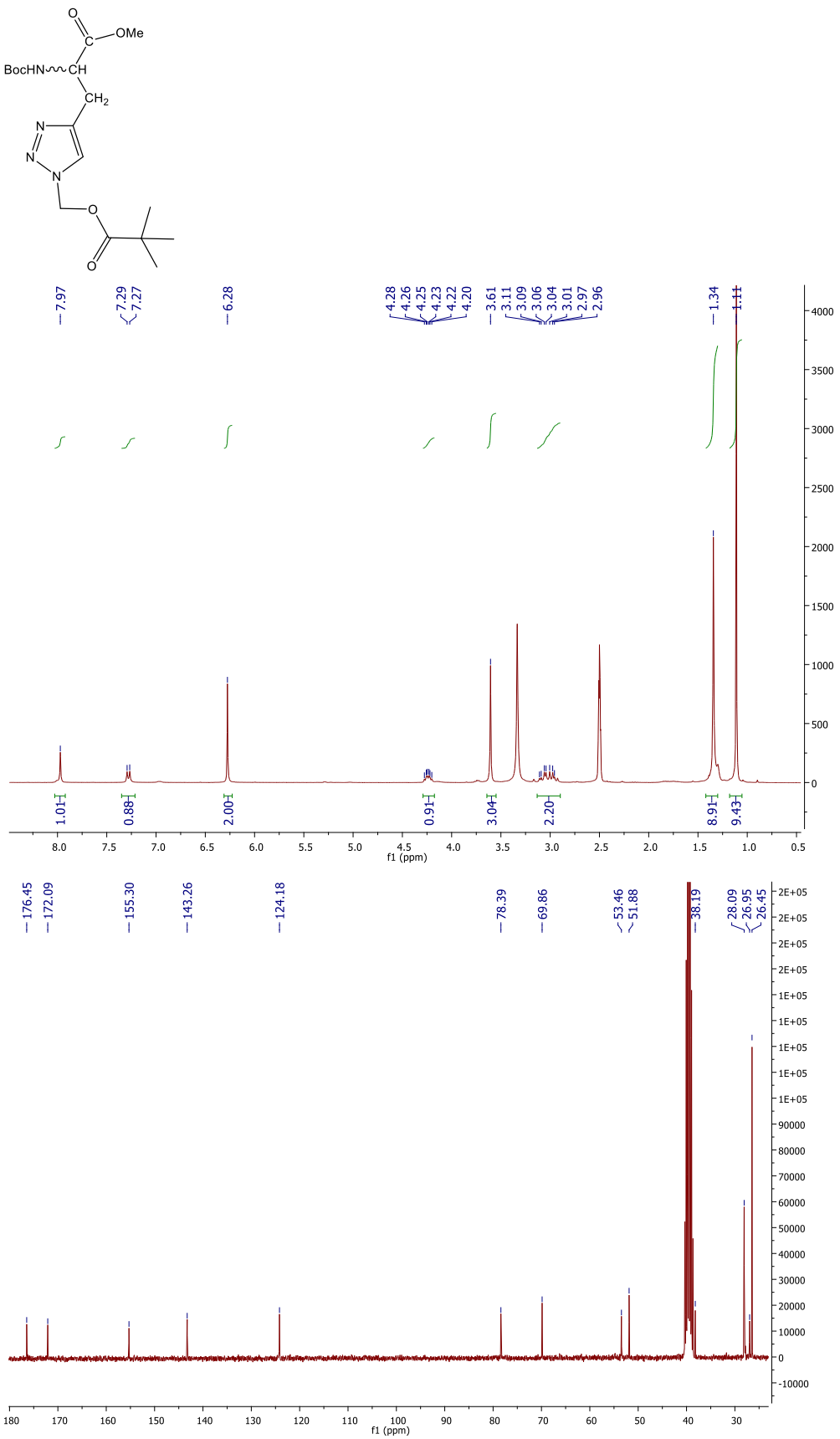


Figure S37. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) spectra of compound **9**.

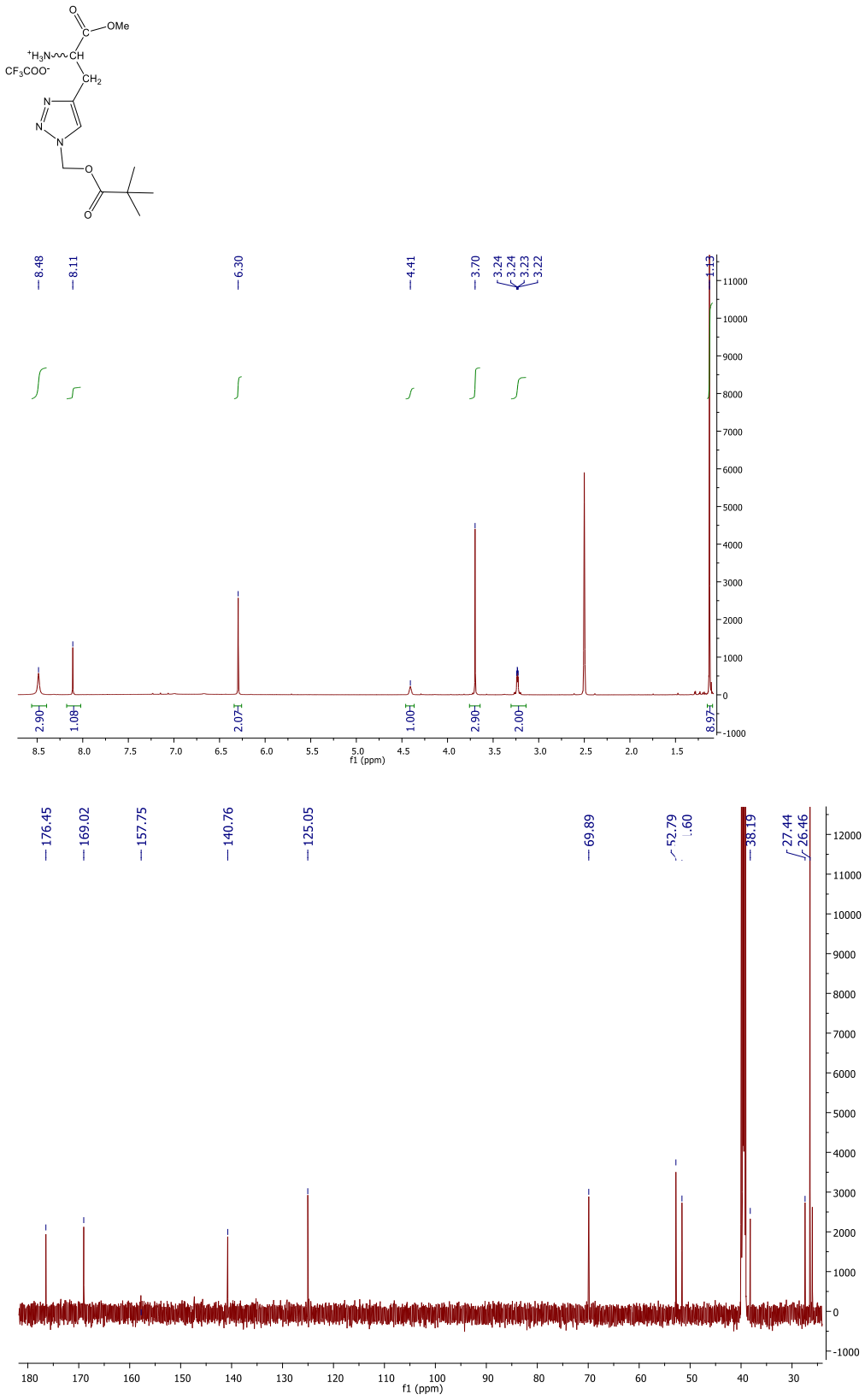
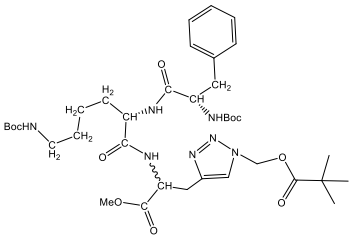
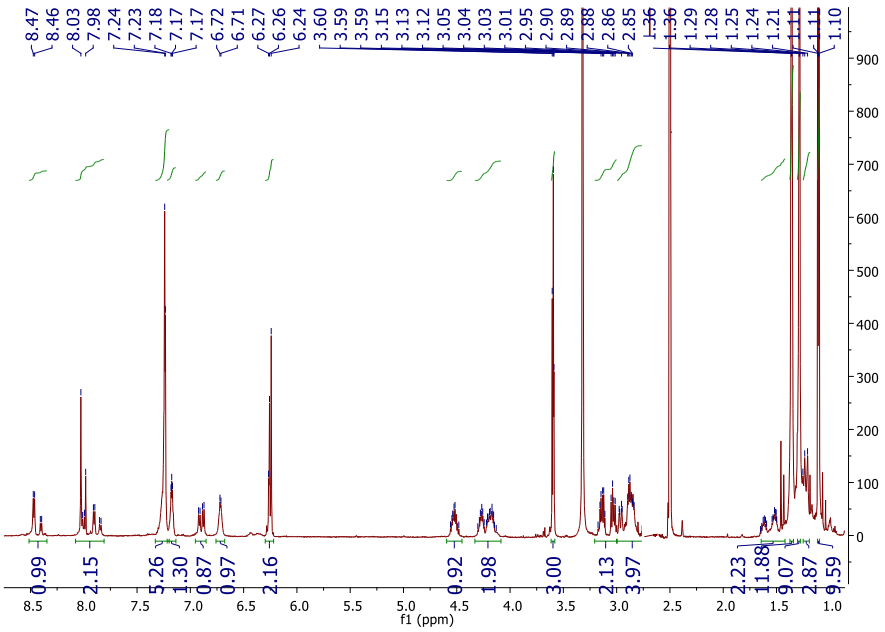
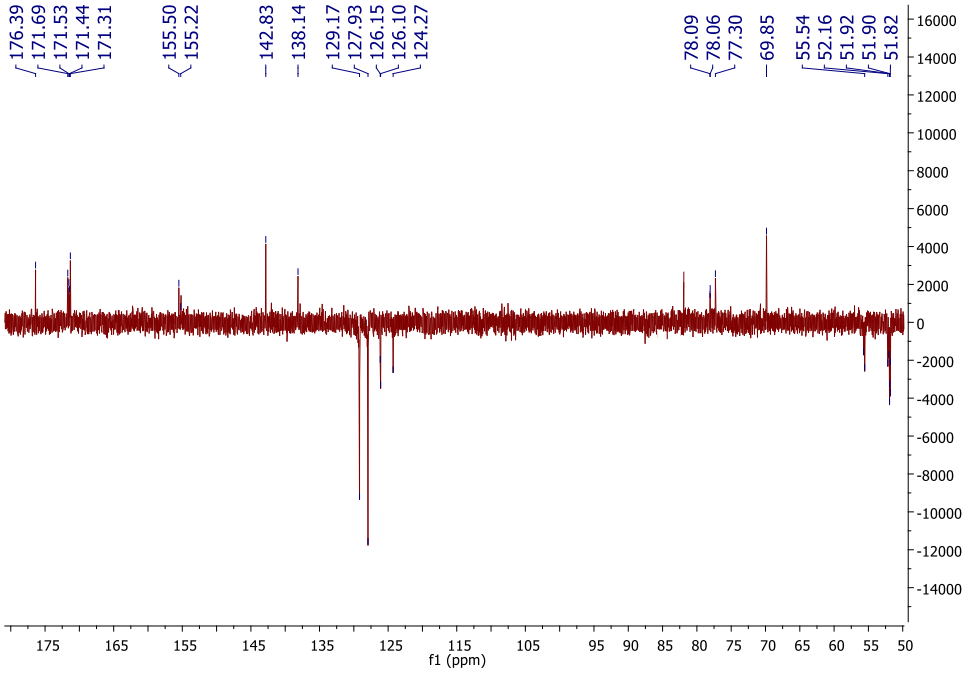


Figure S38. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of compound **10**.


BocHN
NHBoc
MeO
NH
NH
CH
CH
CH
CH2
CH2
CH2
CH2
O
O
O
O
O
O
N
N
N


8.47
8.46
8.03
7.98
7.24
7.23
7.18
7.17
7.17
6.72
6.71
6.27
6.26
6.24
3.60
3.59
3.59
3.15
3.13
3.12
3.05
3.04
3.03
3.01
2.95
2.90
2.89
2.88
2.86
2.85
1.36
1.36
1.29
1.28
1.25
1.24
1.21
1.11
1.11
1.10
0.99
2.15
5.26
1.30
0.87
0.97
2.16
0.92
1.98
3.00
2.13
3.97
2.23
11.88
9.07
2.87
9.59
f1 (ppm)


176.39
171.69
171.53
171.44
171.31
155.50
155.22
142.83
138.14
129.17
127.93
126.15
126.10
124.27
78.09
78.06
77.30
69.85
55.54
52.16
51.92
51.90
51.82
f1 (ppm)

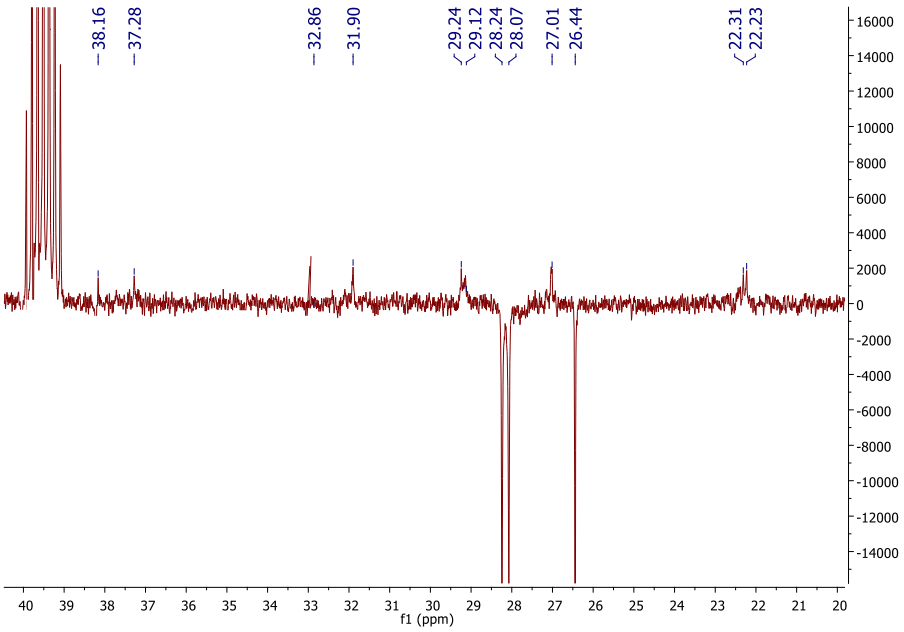


Figure S39. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, APT, DMSO-d_6) spectra of compound **11**.

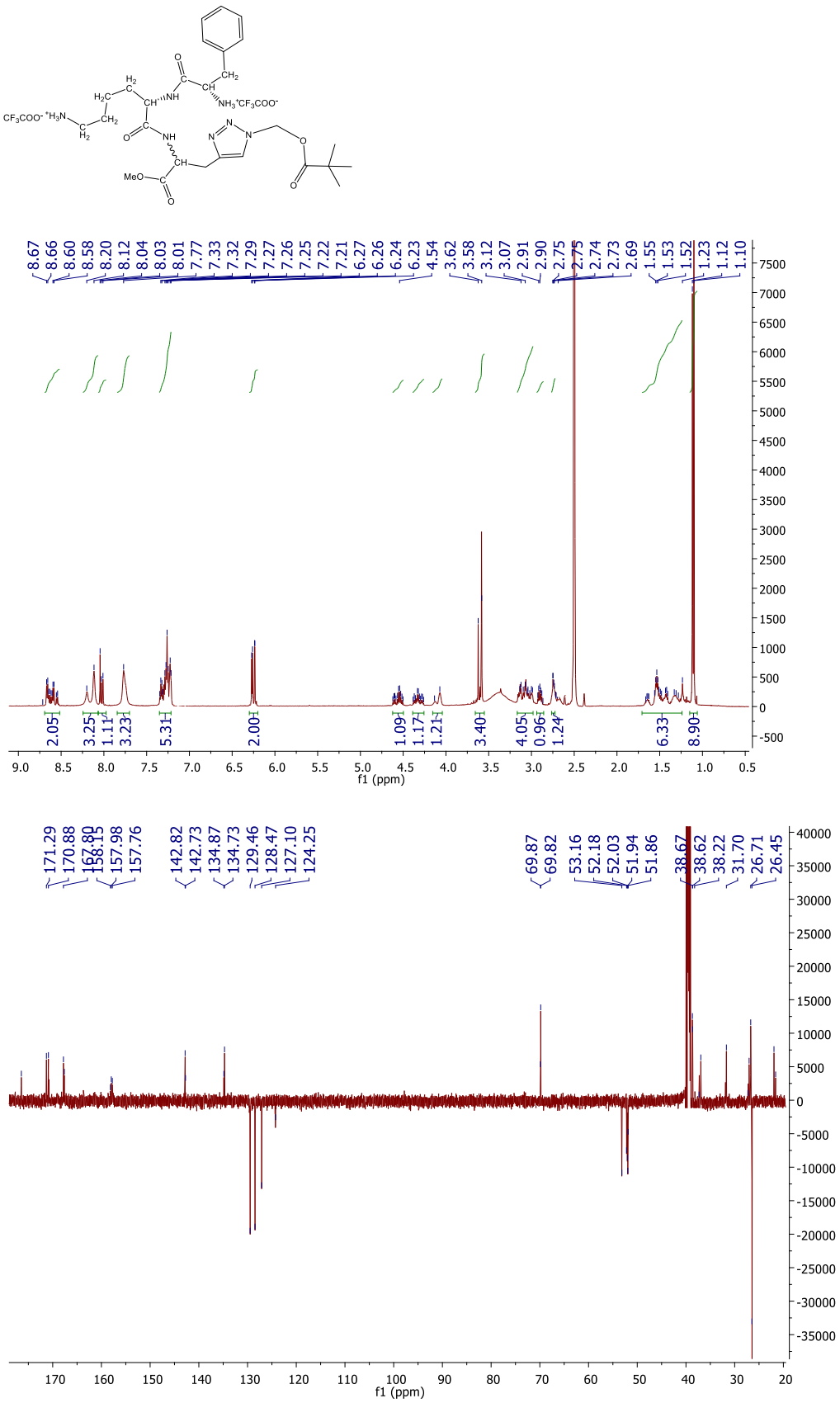
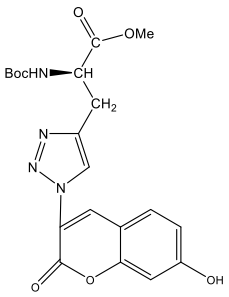
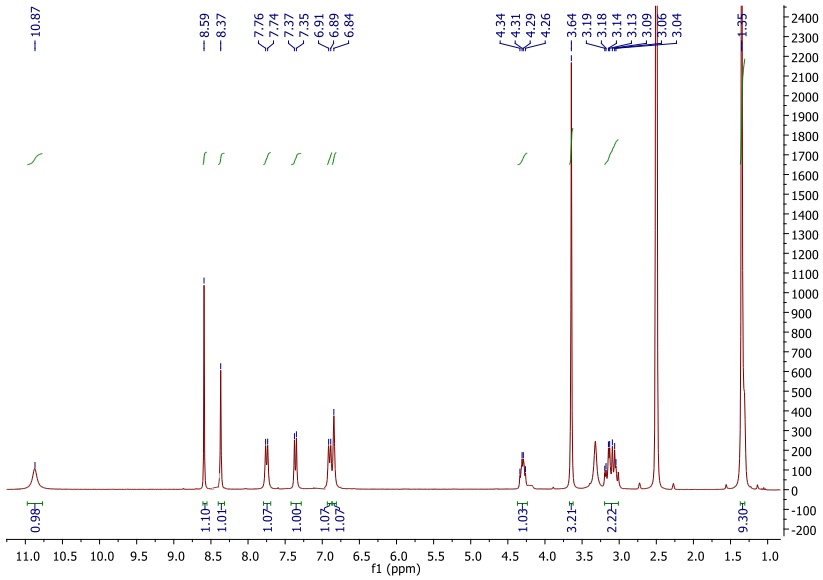
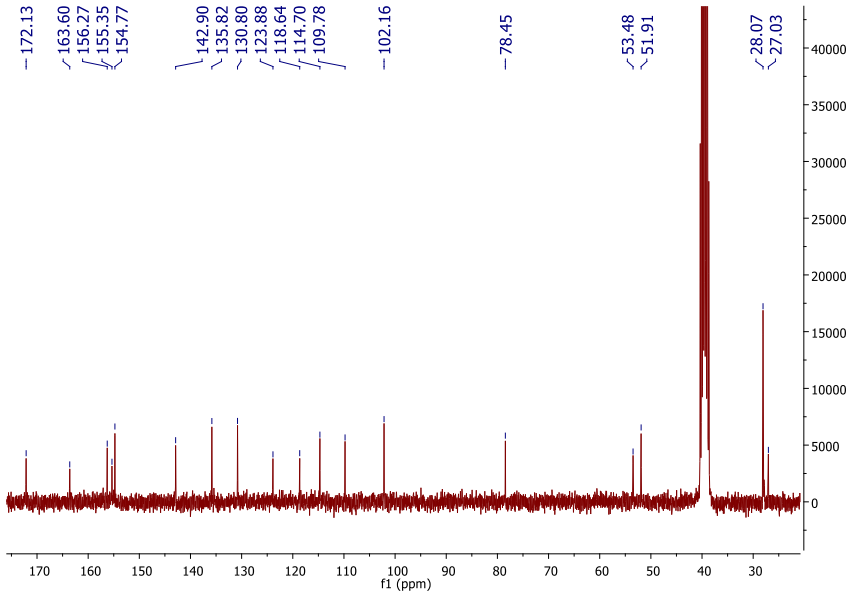


Figure S40. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, APT, DMSO-d_6) spectra of compound (**12; A**).


O
OMe
BocHN
CH
CH2
N
N
N
O
O
OH


10.87
8.59
8.37
7.76
7.74
7.37
7.35
6.91
6.89
6.84
4.34
4.31
4.29
4.26
3.64
3.19
3.18
3.14
3.13
3.09
3.06
3.04
1.35
0.98
1.10
1.01
1.07
1.00
1.07
1.07
1.03
3.21
2.22
9.30
f1 (ppm)


172.13
163.60
156.27
155.35
154.77
142.90
135.82
130.80
123.88
118.64
114.70
109.78
102.16
78.45
53.48
51.91
28.07
27.03
f1 (ppm)

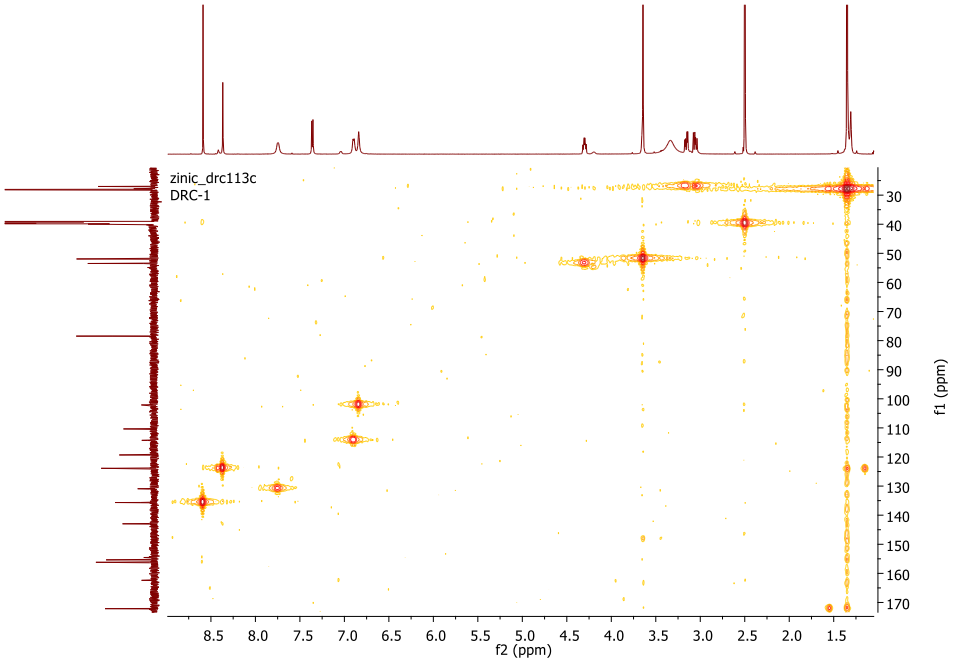


Figure S41. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) and HMQC (151 MHz, DMSO-d_6) spectra of compound **15**.

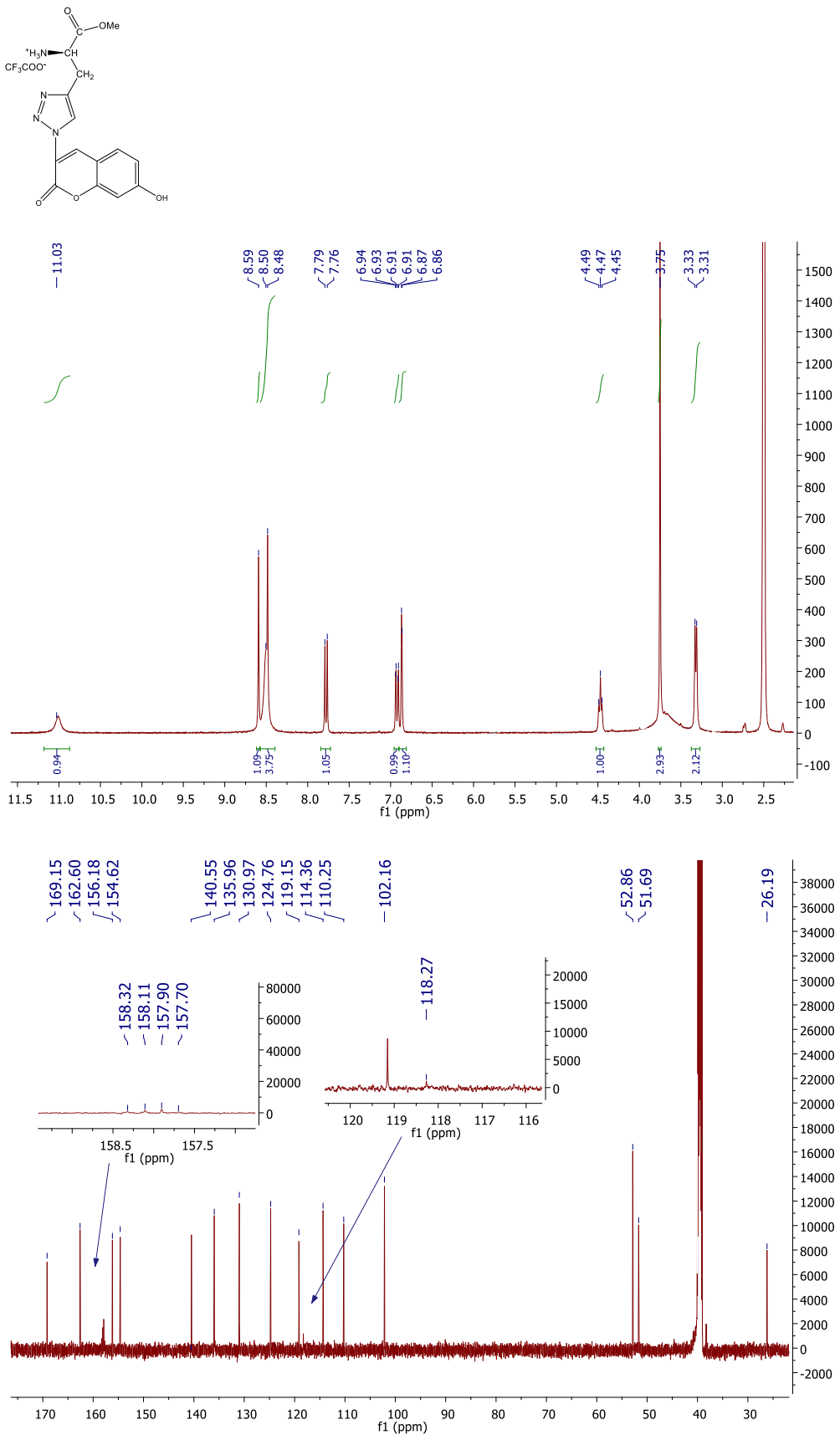
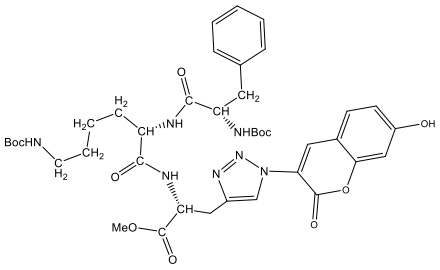
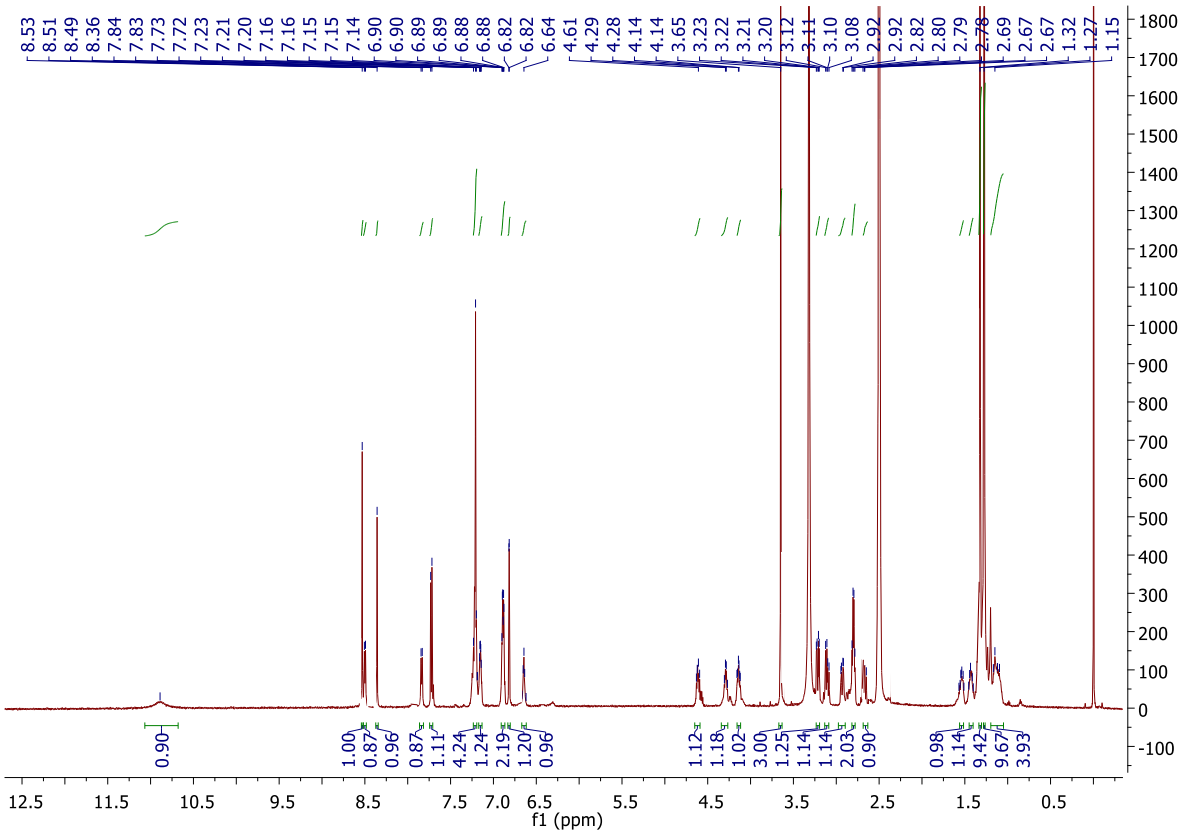


Figure S42. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of compound **16**.


BocHN
NHBoc
NH
MeO
OH
O
N


8.53 8.51 8.49 8.36 7.84 7.83 7.73 7.72 7.23 7.21 7.20 7.16 7.16 7.15 7.15 7.14 6.90 6.90 6.89 6.89 6.88 6.88 6.82 6.82 6.64
4.61 4.29 4.28 4.14 4.14 3.65 3.23 3.22 3.21 3.20 3.12 3.11 3.10 3.08 2.92 2.92 2.82 2.80 2.79 2.78 2.69 2.67 2.67 1.32 1.27 1.15
0.90 1.00 0.87 0.96 0.87 1.11 4.24 1.24 2.19 1.20 0.96 1.12 1.18 1.02 3.00 1.25 1.14 1.14 2.03 0.90 0.98 1.14 9.42 9.67 3.93
f1 (ppm)

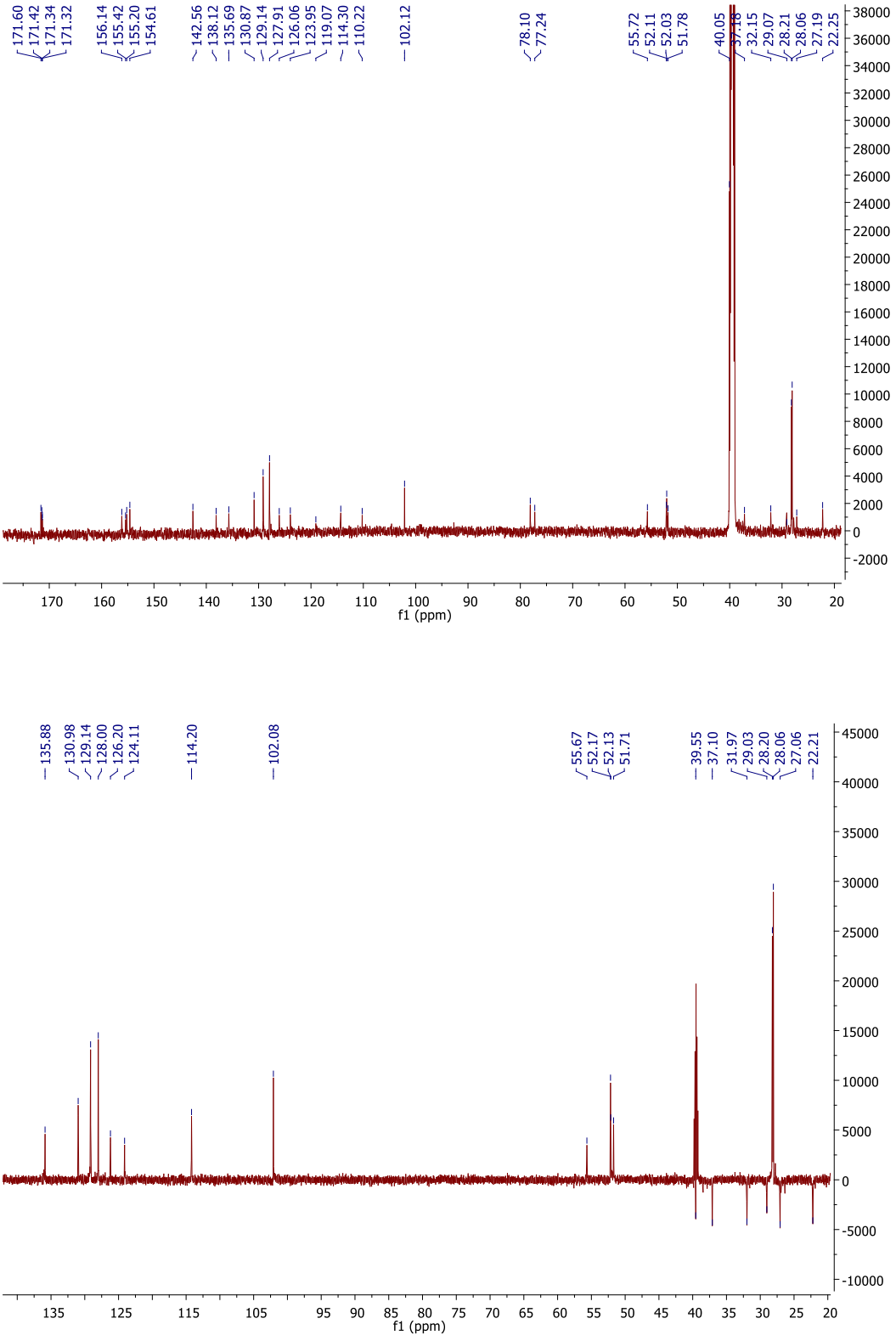


Figure S43. ^{1}H NMR (600 MHz, DMSO-d_6 and DMSO+D_2O), and ^{13}C NMR (151 MHz, DMSO-d_6, DEPT-135) spectra of compound **17 (B-boc)**.

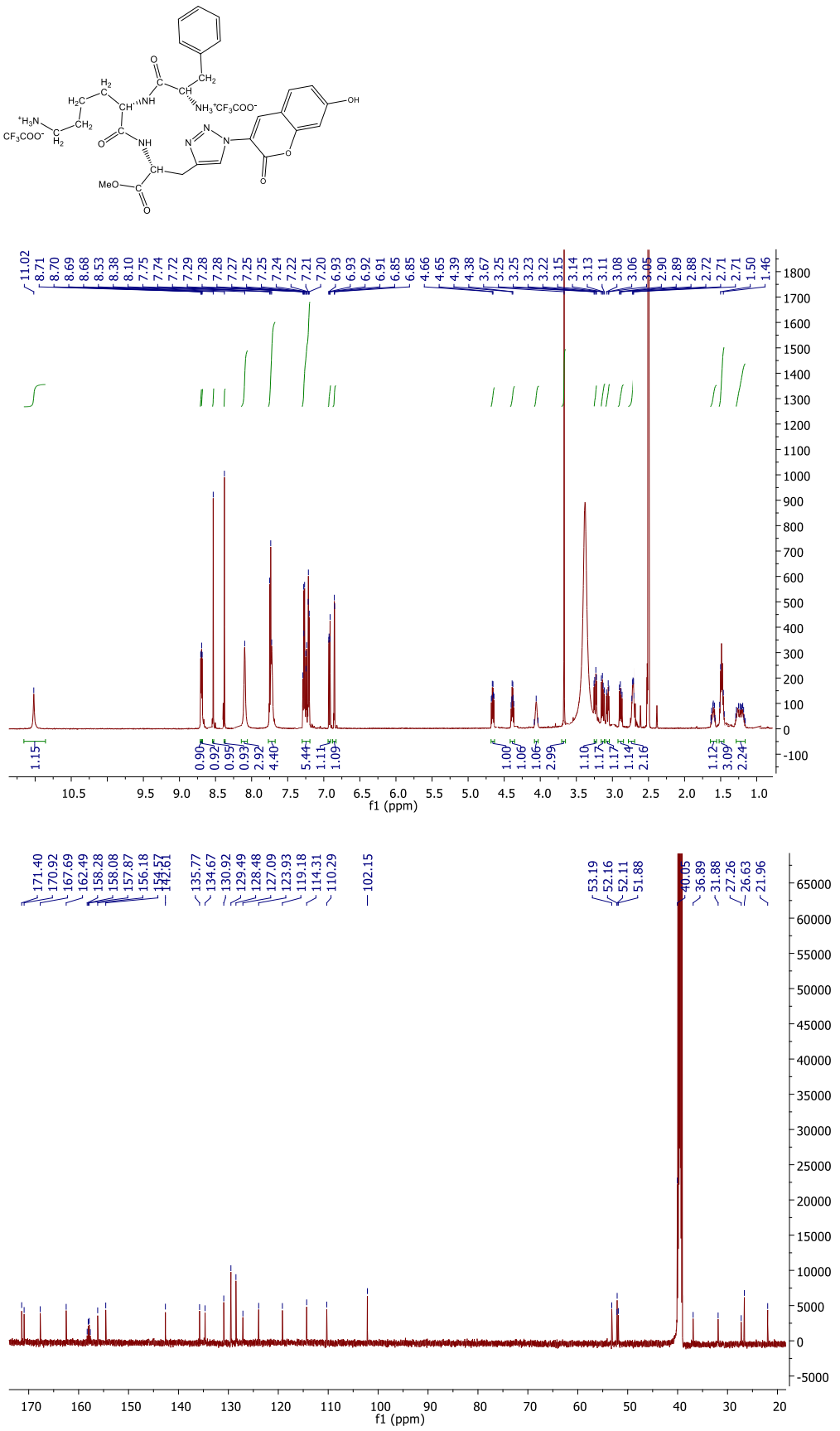
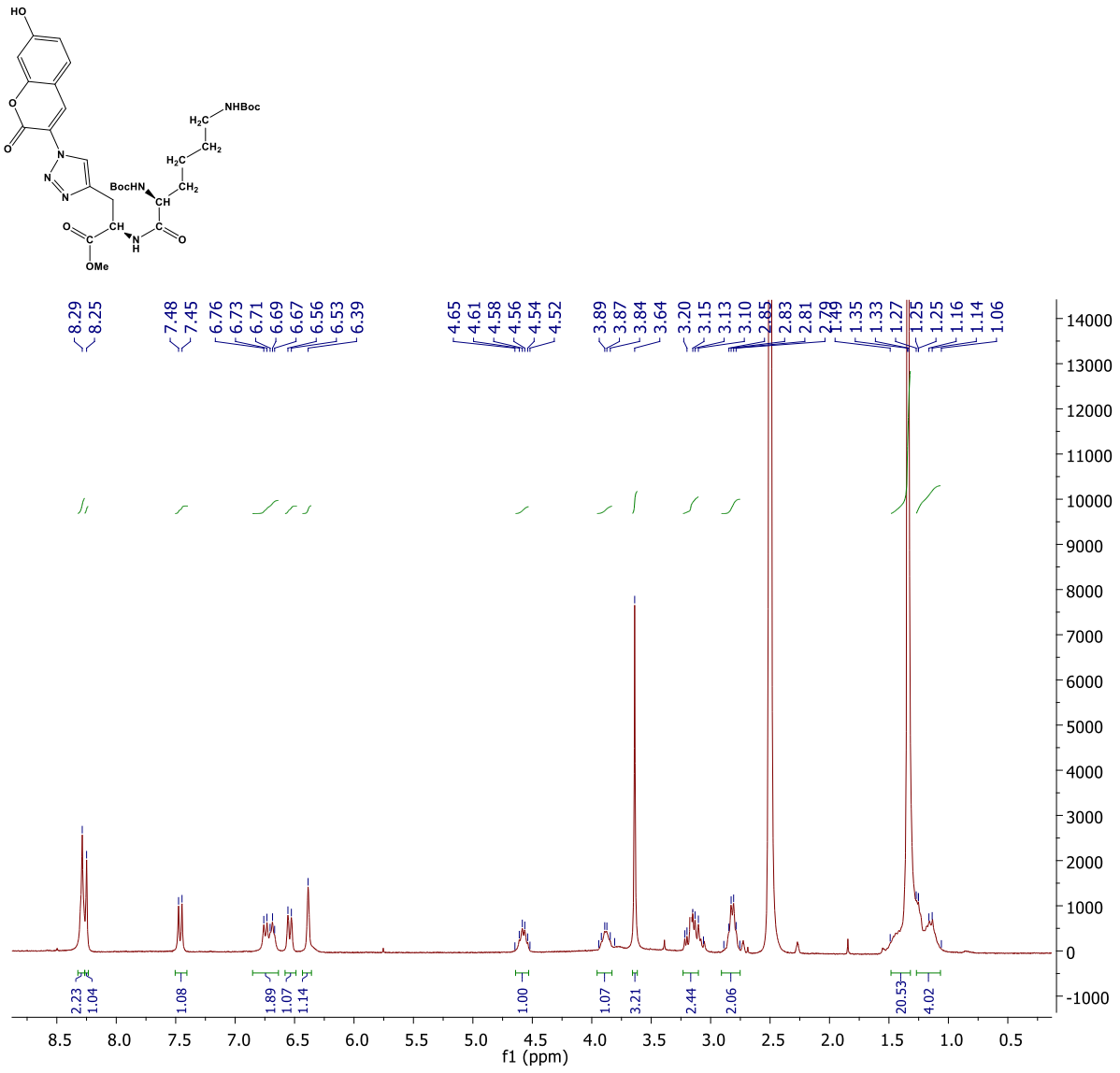


Figure S44. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of compound **18** (**B**).


HO
O
O
N
N
N
BocHN
CH
CH2
H2C
CH2
H2C
NHBoc
O
C
CH
N
H
C
O
OMe
8.29
8.25
7.48
7.45
6.76
6.73
6.71
6.69
6.67
6.56
6.53
6.39
4.65
4.61
4.58
4.56
4.54
4.52
3.89
3.87
3.84
3.64
3.20
3.15
3.13
3.10
2.85
2.83
2.81
1.35
1.33
1.27
1.25
1.25
1.16
1.14
1.06
2.23
1.04
1.08
1.89
1.07
1.14
1.00
1.07
3.21
2.44
2.06
20.53
4.02
f1 (ppm)

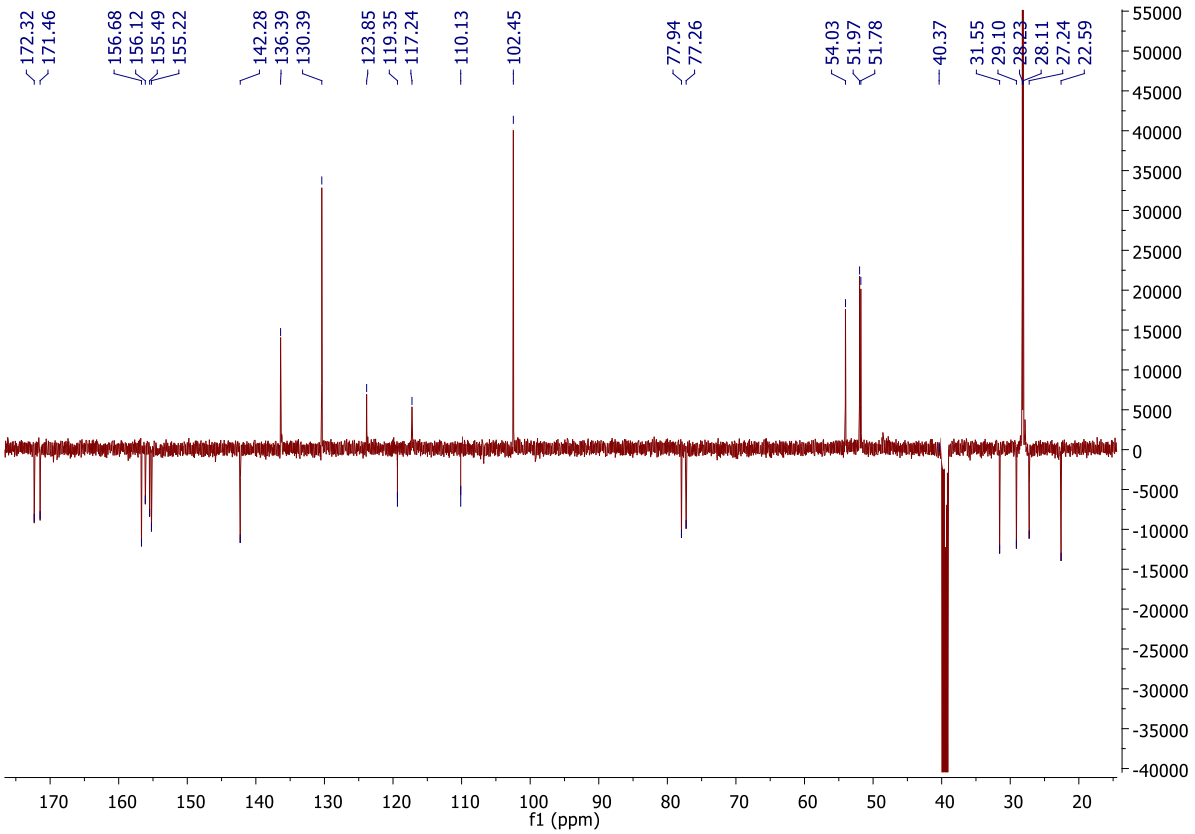
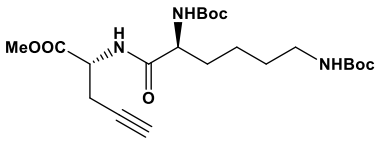
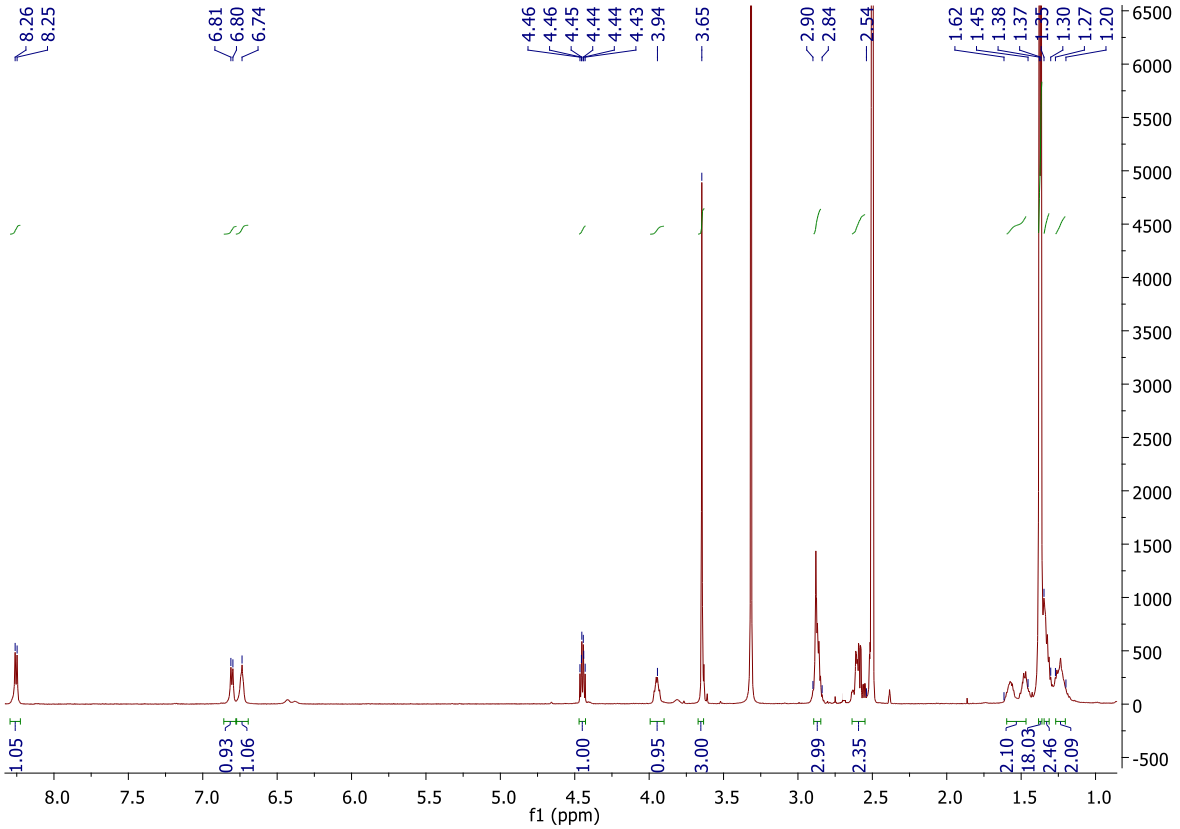


Figure S45. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of compound **20**.


NHBoc
MeOOC
H
N
O
NHBoc


8.26
8.25
6.81
6.80
6.74
4.46
4.46
4.45
4.44
4.44
4.43
3.94
3.65
2.90
2.84
2.54
1.62
1.45
1.38
1.37
1.35
1.30
1.27
1.20
1.05
0.93
1.06
1.00
0.95
3.00
2.99
2.35
2.10
18.03
2.46
2.09
f1 (ppm)

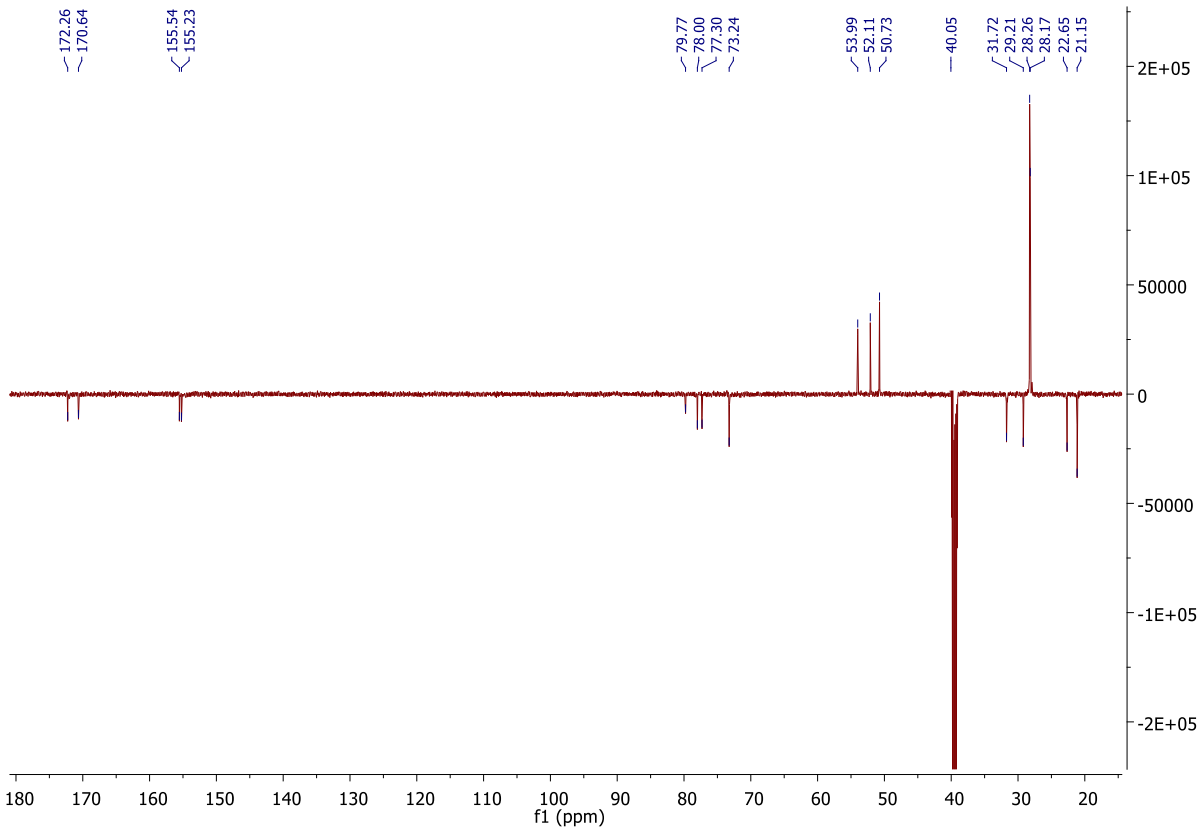
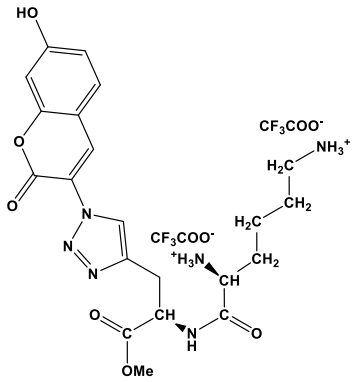
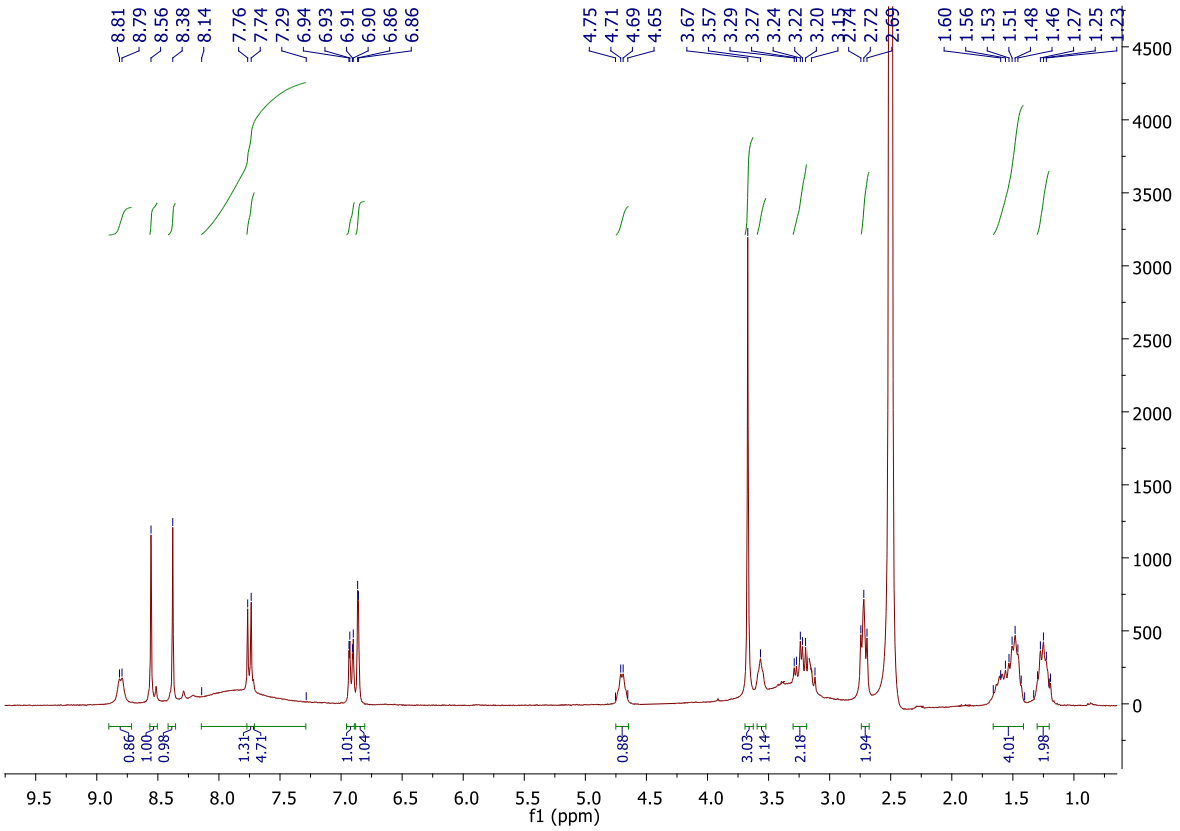


Figure S46. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, APT, DMSO-d_6) spectra of compound **21**.


HO
O
O
N
N
N
CF3COO-
NH3+
H2C
CH2
H2C
CF3COO-
+H3N
CH2
CH
O
C
CH
N
H
C
O
OMe


8.81
8.79
8.56
8.38
8.14
7.76
7.74
7.29
6.94
6.93
6.91
6.90
6.86
6.86
4.75
4.71
4.69
4.65
3.67
3.57
3.29
3.27
3.24
3.22
3.20
3.15
2.74
2.72
2.69
1.60
1.56
1.53
1.51
1.48
1.46
1.27
1.25
1.23
0.86
1.00
0.98
1.31
4.71
1.01
1.04
0.88
3.03
1.14
2.18
1.94
4.01
1.98
f1 (ppm)

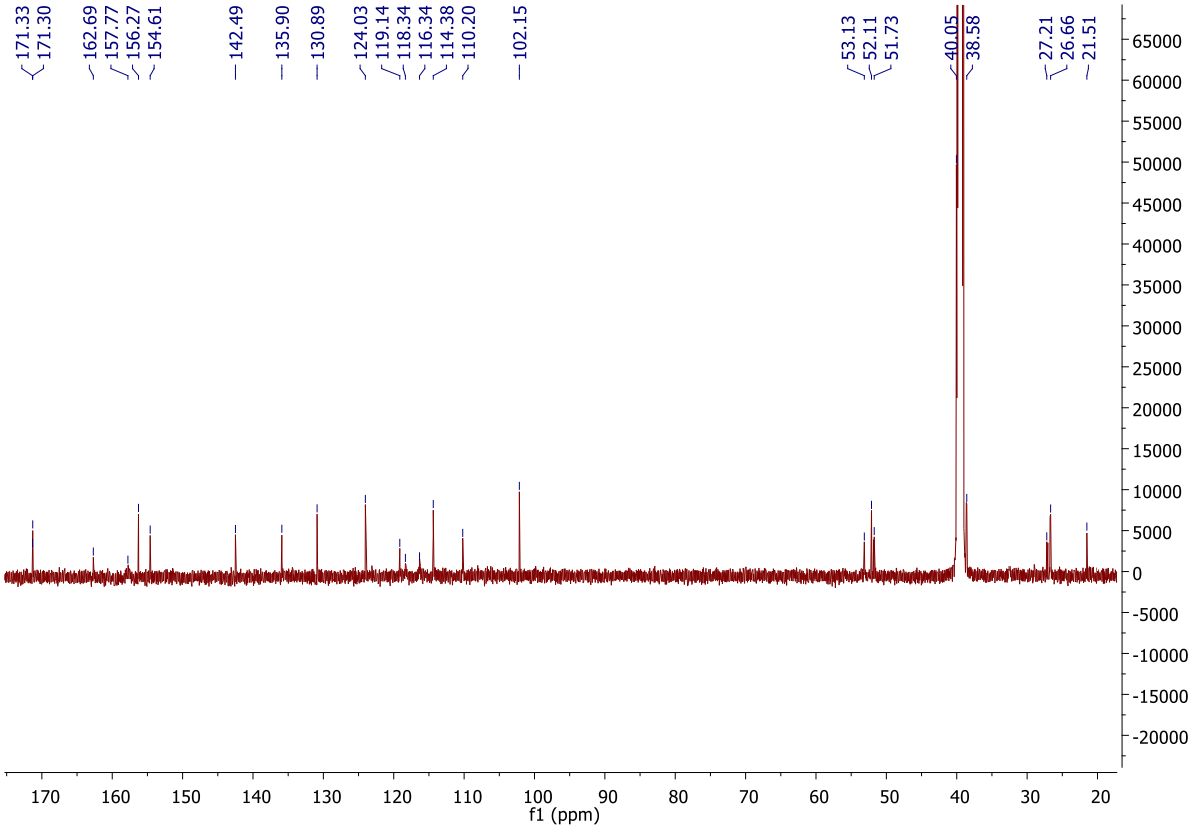


Figure S47. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of compound **22 (C)**.

References:

[1] Kirichenko, K.; Vakulenko, A.; Jeffrey, D. Differentially protected orthogonal lanthionine technology. (PCT/US2010/028620) WO 2010/117652A1, (14 October 2010); https://patents.google.com/patent/WO2010117652A1/fi.

[2] Keller, O.; Keller, W. E.; van Look, G.; Wersin, G. *tert*-Butoxycarbonylation of amino acids and their derivatives: *N-tert*-Butoxycarbonyl-*L*-phenylalanine. *Org. Synth.,* **1985**, *63*, 160. https://doi.org/10.1002/0471264180.os063.19.

[3] Dondoni, A.; Perrone, D. Synthesis of 1,1-dimethylethyl (s)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate by oxidation of the alcohol. *Org. Synth.,* **2000**, *77*, 64. https://doi.org/10.1002/0471264180.os077.07.