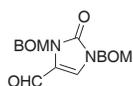


Revisiting the Kinnel–Scheuer hypothesis for the biosynthesis of palau'amine

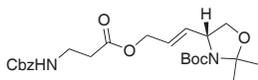
Zhiqiang Ma, Jianming Lu, Xiao Wang and Chuo Chen*

General Experimental Procedures. All reactions were performed in glassware under a positive pressure of argon. Liquids and solvents were transferred via syringe. Organic solutions were concentrated by rotary evaporator at ca. 30 mmHg. Flash column chromatography was performed as described by Still (Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925), employing EMD silica gel 60 (230–400 mesh ASTM). TLC analyses were performed on EMD 250 μm Silica Gel 60 F₂₅₄ plates and visualized by quenching of UV fluorescence ($\lambda_{\text{max}} = 254 \text{ nm}$), or by staining ceric ammonium molybdate. ¹H and ¹³C NMR spectra were recorded on Varian Inova-600, Inova-500, Inova-400. Chemical shifts for ¹H and ¹³C NMR spectra are reported in ppm (δ) relative to the ¹H and ¹³C signals in the solvent (CDCl₃: δ 7.26, 77.16 ppm; DMSO-*d*₆: δ 2.50, 39.51 ppm; CD₃CN: δ 1.94, and 1.32, 118.26 ppm) and the multiplicities are presented as follows: s = singlet, d = doublet, t = triplet, m = multiplet. Mass spectra were acquired on Agilent 6120 Single Quadrupole LC/MS or through the University of Illinois Urbana-Champaign Mass Spectrometer Facility using the indicated ionization method. Preparative HPLC was performed using a Waters Atlantis dC18 OBD 5 μm column with dimension 19×150 mm or Eclipse XDB-C18 5 μm column with dimension 9.4×250 mm.



Aldehyde 11. To a suspension of sodium hydride (60% in mineral oil, 13.20 g, 0.33 mol, 1.1 equiv) and chloromethyl benzyl ether (96.2 mL, 0.63 mol, 2.1 equiv) in 500 mL anhydrous *N,N*-dimethylformamide under argon at 0 °C was added imidazole (20.63 g, 0.3 mol, 1.0 equiv). The reaction was stirred at 23 °C overnight before cooling down to 0 °C. Anhydrous copper(II) chloride (48.40 g, 0.36 mol, 1.2 equiv) and sodium hydride (60% in mineral oil, 26.40 g, 0.63 mol, 2.1 equiv) were added sequentially. Oxygen was then bubbled through at 23 °C and the progress of the reaction was monitored by ¹H NMR. Upon completion, the reaction mixture was once again cooled down to 0 °C and phosphorus oxychloride (83.2 mL, 0.90 mol, 3 equiv) was dropwise added. The reaction mixture was stirred at 23 °C for 2 hours before pouring onto a mixture of sodium carbonate (500 g) and ice (2 kg). The mixture was stirred at 23 °C for about one hour before extracting with ethyl acetate (2 L). The organic layer was washed with brine (500 mL×5), 10% ammonium hydroxide/saturated ammonium chloride (1:1 (v/v), 300 mL each time until the aqueous layer was colorless) and brine (500 mL), and dried over anhydrous sodium sulfate. The organic solution was concentrated to a slurry. Hexanes (300 mL) were added and the mixture was kept at 23 °C overnight. The precipitates were collected, washed with hexanes, and dried under vacuum to give **11** (72.0 g, 68%

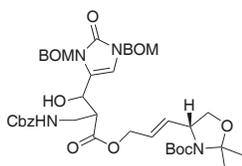
yield). $R_f = 0.37$ (50% ethyl acetate-hexanes); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 9.33 (s, 1H), 7.38 – 7.22 (m, 10H), 7.12 (s, 1H), 5.51 (s, 2H), 5.17 (s, 2H), 4.65 (s, 2H), 4.58 (s, 2H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 177.1, 153.4, 137.6, 136.6, 128.5, 128.2, 128.1, 127.9, 127.64, 127.59, 127.2, 123.4, 72.85, 71.6, 71.4, 71.2.



Ester 14. To a solution of crude alcohol **13** (prepared according to: L. Devel, L.

Hamon, H. Becker, A. Thellend and A. Vidal-Cros, *Carbohydr. Res.*, 2003, **338**,

1591–1601) (1.0 g, 3.9 mmol, 1.0 equiv) and Cbz- β -alanine (0.87 g, 3.9 mmol, 1.0 equiv) in methylene chloride (5 mL) were added *N,N'*-dicyclohexylcarbodiimide (0.85 g, 4.1 mmol, 1.05 equiv) and 4-dimethylaminopyridine (25 mg, 0.20 mmol, 0.05 equiv). After stirring at 23 °C overnight, the reaction mixture was filtered and the solid was washed with methylene chloride. The filtrate was concentrated and the residue was suspended in methylene chloride and hexanes. The solid was filtered off and the solution was concentrated to dryness to give ester **14** as a solid. $R_f = 0.63$ (50% ethyl acetate-hexanes); $^1\text{H NMR}$ (50 °C) (400 MHz, CDCl_3) δ 7.42 – 7.27 (m, 5H), 5.81 – 5.67 (m, 2H), 5.29 – 5.14 (m, 1H), 5.11 (s, 2H), 4.59 (d, $J = 4.7$ Hz, 2H), 4.46 – 4.21 (m, 1H), 4.03 (dd, $J = 6.2, 8.9$ Hz, 1H), 3.73 (d, $J = 8.9$ Hz, 1H), 3.47 (dt, $J = 5.9, 5.9$ Hz, 2H), 2.55 (t, $J = 5.9$ Hz, 2H), 1.51 (s, 3H), 1.48 (s, 3H), 1.45 (s, 9H).

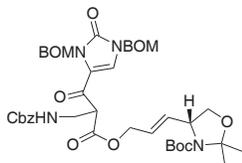


β -Hydroxyester 14a. To a solution of ester **14** (7.59 g, 16.4 mmol, 1.2 equiv) in

tetrahydrofuran (280 mL) at -78 °C was added lithium bis(trimethylsilyl)amide

(1.0 M in tetrahydrofuran, 41 mL, 41 mmol, 3 equiv). After 1 hour, a solution of

aldehyde **11** (4.82 g, 13.7 mmol, 1 equiv) in tetrahydrofuran (140 mL) chilled at -78 °C was introduced via cannula. The reaction was stirred at -78 °C for 2 hours before quenching with saturated ammonium chloride. After removal of the solvent, the mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography to afford **14a** (8.75 g, 79% yield over three steps, as a pair of inseparable diastereomers) as an oil. $R_f = 0.13$ (50% ethyl acetate-hexanes). In addition, aldehyde **11** (676 mg, 14%) was recovered.

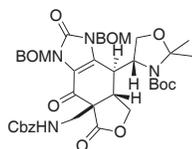


β -Ketoester 15. To a solution of **14a** (8.75 g, 10.7 mmol, 1.0 equiv) in anhydrous

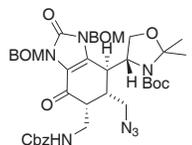
methylene chloride (230 mL) were added Dess–Martin periodinane (8.22 g, 19.3

mmol, 1.8 equiv) and water (0.35 mL). The reaction was stirred at 23 °C for 2

hours. After removal of the solvent, the residue was diluted in ethyl acetate, washed with a solution of sodium thiosulfate (1.0 M in saturated sodium bicarbonate), saturated sodium bicarbonate, and brine, and dried over anhydrous sodium sulfate. Filtration and concentration gave the desired product **15** (8.75 g) and it was used for the next step without purification. $R_f = 0.64$ (50% ethyl acetate-hexanes); $^1\text{H NMR}$ (50 °C) (500 MHz, DMSO- d_6) δ 8.03 (s, 1H), 7.39 – 7.20 (m, 15H), 5.75 – 5.67 (m, 1H), 5.66 – 5.58 (m, 1H), 5.45 (d, $J = 10.3$ Hz, 1H), 5.42 (d, $J = 10.3$ Hz, 1H), 5.18 (d, $J = 10.8$ Hz, 1H), 5.16 (d, $J = 10.8$ Hz, 1H), 5.02 (s, 2H), 4.60 (s, 2H), 4.63 – 4.56 (m, 2H), 4.53 (s, 2H), 4.39 (dd, $J = 6.6, 6.6$ Hz, 1H), 4.28 – 4.22 (m, 1H), 3.95 (dd, $J = 6.6, 8.9$ Hz, 1H), 3.65 – 3.56 (m, 3H), 1.49 (s, 3H), 1.43 (s, 3H), 1.38 (s, 9H); $^{13}\text{C NMR}$ (80 °C) (125 MHz, DMSO- d_6) δ 181.6, 167.1, 155.7, 152.7, 150.8, 137.7, 136.9, 136.6, 133.3, 127.8, 127.8, 127.6, 127.22, 127.17, 127.1, 127.0, 126.84, 126.81, 126.1, 124.4, 120.8, 92.8, 78.7, 72.7, 71.0, 70.3, 69.8, 67.1, 65.2, 64.2, 57.5, 52.9, 39.7, 27.6, 26.1, 23.6; MS(ES) $^+$ calcd for C₄₄H₅₃N₄O₁₁ (M+H) $^+$ 813.4, found 813.5.



Lactone 16. A solution of **15** (8.75 g, 10.7 mmol, 1.0 equiv) in glacial acetic acid (190 mL) was degassed by three freeze-pump-thaw cycles. Manganese(III) acetate dihydrate (8.63 g, 32.2 mmol, 3.0 equiv) was then introduced while the mixture was kept frozen at 0 °C. The reaction vessel was back-filled with argon and warmed to 60 °C. After stirring at 60 °C overnight, acetic acid was removed by vacuum. The residue was dissolved in ethyl acetate and washed with 10% sodium bisulfite solution until the color of the solution became light yellow. The organic phase was then washed with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, concentrated and purified by flash chromatography to afford **16** (3.17 g, 36% yield for two steps) as a solid: $R_f = 0.61$ (50% ethyl acetate-hexanes); $^1\text{H NMR}$ (50 °C) (500 MHz, DMSO- d_6) δ 7.43 – 7.17 (m, 15H), 5.44 – 5.34 (m, 3H), 5.11 – 5.03 (m, 3H), 4.58 – 4.46 (m, 5H), 4.39 – 4.33 (m, 1H), 4.03 (dd, $J = 7.0, 10.1$ Hz, 1H), 3.95 (dd, $J = 6.7, 14.1$ Hz, 1H), 3.65 – 3.56 (m, 3H), 3.51 (dd, $J = 6.0, 14.1$ Hz, 1H), 3.33 (t, $J = 9.0$ Hz, 1H), 1.45 (s, 9H), 1.42 (s, 3H), 1.35 (s, 3H); $^{13}\text{C NMR}$ (50 °C) (125 MHz, DMSO- d_6) δ 174.5, 170.7, 170.5, 156.5, 153.0, 152.4, 137.5, 137.4, 136.7, 136.4, 127.8, 127.6, 127.3, 127.3, 127.1, 127.0, 126.9, 126.7, 118.5, 93.8, 80.5, 71.2, 70.9, 70.3, 69.8, 69.2, 65.5, 63.9, 60.3, 57.7, 44.0, 37.1, 35.1, 27.4, 25.6, 22.5; MS(ES) $^+$ calcd for C₄₄H₅₁N₄O₁₁ (M+H) $^+$ 811.4, found 811.5.

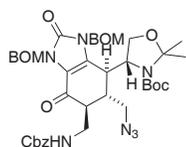


cis-Ketone 16a. To a solution of **16** (2.87 g, 3.53 mmol, 1.0 equiv) in tetrahydrofuran (120 mL) was added aqueous lithium hydroxide (0.34 N, 31.4 mL, 3 equiv). The reaction was stirred at 23 °C for 30 minutes. After removal of tetrahydrofuran, the

mixture was partitioned between ethyl acetate (150 mL) and aqueous hydrogen chloride (0.5 N, 100 mL). The organic layer was washed with saturated sodium bicarbonate and brine, and dried over anhydrous sodium sulfate. After concentration, the crude alcohol was obtained as an oil and directly used for the next step without purification. $R_f = 0.1$ (50% ethyl acetate-hexanes).

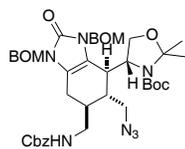
Diethyl azodicarboxylate (923 mg, 5.30 mmol, 1.5 equiv) and diphenylphosphoryl azide (1.46 g, 5.30 mmol, 1.5 equiv) were dropwise added to a solution of the crude alcohol obtained above and triphenylphosphine (1.39 g, 5.30 mmol, 1.5 equiv) in tetrahydrofuran (50 mL) under argon at 0 °C sequentially. The reaction was then stirred at 23 °C overnight. After removal of the solvent, the residue was purified by flash chromatography on a deactivated silica gel column* (10→40% ether-hexanes, then 20% ethyl acetate-hexanes) to give **16a** as a foamy solid (1.21 g, 42% yield for two steps); $R_f = 0.50$ (50% ethyl acetate-hexanes); $^1\text{H NMR}$ (50 °C) (500 MHz, CD_3CN) δ 7.43 – 7.22 (m, 15H), 5.50 (d, $J = 10.4$ Hz, 1H), 5.40 (d, $J = 10.4$ Hz, 1H), 5.32 (d, $J = 11.0$ Hz, 1H), 5.17 (d, $J = 11.0$ Hz, 1H), 5.08 (s, 2H), 4.62 (s, 2H), 4.61 (d, $J = 11.7$ Hz, 1H), 4.57 (d, $J = 11.7$ Hz, 1H), 4.49-4.41 (m, 1H), 4.0-3.90 (m, 1H), 3.80 (d, $J = 10.1$ Hz, 1H), 3.74 – 3.64 (m, 2H), 3.61 (d, $J = 11.4$ Hz, 1H), 3.45 – 3.17 (m, 3H), 3.06 (d, $J = 11.4$ Hz, 1H), 2.80 – 2.73 (m, 1H), 1.59 (s, 3H), 1.48 (s, 3H), 1.39 (s, 9H); $^{13}\text{C NMR}$ (50 °C) (125 MHz, CD_3CN) δ 186.4, 157.6, 155.2, 155.0, 139.9, 139.4, 138.8, 138.6, 129.8 – 128.4 (9 carbons), 118.6, 96.0, 82.2, 72.8, 72.5, 72.2, 71.9, 67.3, 59.2, 51.2, 46.4, 40.9, 39.6, 39.3, 28.8, 28.0, 24.5; MS(ES)^+ calcd for $\text{C}_{43}\text{H}_{52}\text{N}_4\text{O}_{10}$ (M+H) $^+$ 810.4, found 810.6.

*The deactivated silica gel was prepared following the reported procedures (Panne, P.; Fox, J. M. *J. Am. Chem. Soc.* **2007**, *129*, 22–23. In the supporting information). For the workup, the silica gel was washed with methanol, deionized water until neutral, and again with methanol. The deactivated silica gel was air-dried at 23 °C overnight and then dried in a 110 °C oven for 30 minutes.



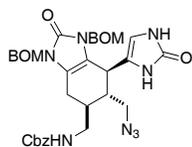
Trans-ketone 16b. To a solution of **16a** (915.6 mg, 1.13 mmol, 1.0 equiv) in tetrahydrofuran (11 mL) at –78 °C was added a solution of lithium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 2.83 mL, 2.83 mmol, 2.5 equiv).

The solution was stirred at –78 °C for 30 minutes and then warmed to 0 °C. After stirring for another 30 minutes, the reaction was quenched with a solution of acetic acid (2 mL) in tetrahydrofuran (6 mL), and diluted with ethyl acetate. The organic layer was washed with water, saturated sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, filtered, and concentrated to afford **16b** (912 mg), which was used directly for the next step without purification: $R_f = 0.60$ (50% ethyl acetate-hexanes).



Azide 17. To a solution of the crude **16b** (912 mg, 1.13 mmol, 1.0 equiv) in tetrahydrofuran (18 mL) at 0 °C was added calcium borohydride bis(tetrahydrofuran) (483.8mg, 2.26 mmol, 2.0 equiv). The reaction was stirred at 23 °C for 30 minutes

before concentrating to dryness. With the residue kept at 0°C, acetic acid (22 mL) was added followed by the addition of sodium cyanoborohydride (224.2 mg, 3.39 mmol, 3 equiv) when the generation of gas had ceased. The reaction was then stirred at 50 °C for 30 minutes before concentrating and re-dissolving in ethyl acetate. The organic phase was washed with aqueous hydrogen chloride (1N), saturated sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, and evaporated to give crude azide **17** (890 mg) as a pale yellow oil, which was used for the next step without purification: $R_f = 0.43$ (70% ethyl acetate-hexanes); $^1\text{H NMR}$ (500 MHz, CD_3CN) δ 7.48 – 7.24 (m, 15H), 5.31 (d, $J = 10.9$ Hz, 1H), 5.19 – 4.95 (m, 5H), 4.59 – 4.29 (m, 4H), 4.06 (s, 1H), 3.89 (dd, $J = 7.2, 9.3$ Hz, 1H), 3.65 (d, $J = 9.3$ Hz, 1H), 3.57 – 3.11 (m, 5H), 2.59 (dd, $J = 5.1, 16.1$ Hz, 1H), 2.21 (dd, $J = 8.6, 16.1$ Hz, 1H), 1.89 – 1.78 (m, 1H), 1.51 (s, 9H), 1.48 (s, 3H), 1.42 (s, 3H); $^{13}\text{C NMR}$ (125 MHz, CD_3CN) δ 157.9, 156.4, 154.7, 139.5, 139.4, 138.7, 129.7, 129.5, 129.4, 129.1, 129.0, 128.9, 128.8, 128.7, 128.7, 120.7, 117.6, 96.0, 81.8, 73.1, 71.514, 71.5, 71.2, 67.2, 66.1, 62.0, 55.0, 44.9, 41.8, 41.3, 38.2, 38.2, 28.9, 26.8, 23.3; MS(ES)^+ calcd for $\text{C}_{43}\text{H}_{54}\text{N}_7\text{O}_8$ ($\text{M}+\text{H}$) $^+$ 796.4, found 796.6.



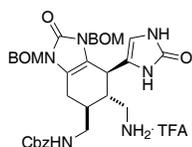
Imidazolinone 18. Trifluoroacetic acid (10% in methylene chloride, 11 mL) was added dropwise to a solution of crude azide **17** (890 mg) in anhydrous methylene chloride (11 mL) cooled at 0 °C under argon. After stirring for 30 minutes, the reaction was

quenched with saturated sodium bicarbonate, and diluted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to afford the crude alcohol (838.2 mg): $R_f = 0.29$ (70% ethyl acetate-hexanes).

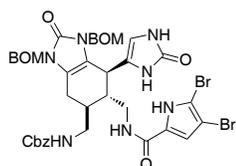
To a solution of the crude alcohol obtained above (838.2 mg) in anhydrous methylene chloride (5 mL) was added Dess–Martin periodinane (718.9 mg) followed by immediate addition of a mixture of water and methylene chloride (30 μL /30 mL). The reaction mixture was stirred at 40 °C for 15 minutes before concentrating and suspending in ethyl acetate. The mixture was washed with a solution of sodium thiosulfate (1.0 M in saturated sodium bicarbonate), saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered, and concentrated to afford the crude aldehyde (810 mg) which was used for the next step without purification: $R_f = 0.68$ (70% ethyl acetate-hexanes).

To a solution of the crude aldehyde (810 mg) in methanol (27 mL) at 0 °C was added a cold solution of aqueous hydrogen chloride (6 N, 27 mL) in methanol (81 mL). The solution was stirred at 40 °C for 2 hours. With the solution cooled in an ice-water bath, the pH of the solution was adjusted to ca. 3.7 by

adding aqueous sodium hydroxide (3 M). The pH value was monitored by a pH meter and meanwhile the temperature of the reaction solution was kept between 10 and 15 °C. Potassium cyanate (3.0 g) and potassium hydrogen phthalate (11.6 g) were then added and the reaction was heated in a sealed reaction vessel at 110 °C for 1.5 hours. After removal of most of the methanol, the mixture was partitioned between ethyl acetate and water. The organic layer was washed with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered and concentrated to afford crude **18**, which was used for the next step without purification.



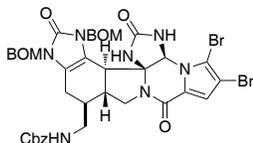
Amine 18a. To a solution of **18** (767 mg) in tetrahydrofuran (11 mL) was added triphenylphosphine (390 mg) and water (1.1 mL). The reaction was stirred at 80 °C for 1 hour before cooling to 23 °C. Upon addition of trifluoroacetic acid (130 μ L), the reaction solution was concentrated to dryness. The residue was then dissolved in a small volume of methylene chloride (0.5 mL). Ether (25 mL) was added to precipitate triphenylphosphine oxide. After filtration, the solution was concentrated and purified by semi-preparative HPLC (Waters Atlantis C-18 column, 19 \times 150 mm, 5 μ m particle S-12 size; eluent A: H₂O with 0.1% TFA, eluent B: MeCN; gradient: T = 0 min: 25% B, T = 50 min: 60% B, 5.0 mL/min) to give **18a** as an oil (61.4 mg, 7–10% yield for 6 steps, retention time: 27.5 min). ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.91 (s, 1H), 9.70 (s, 1H), 7.89 (s, 3H), 7.49 – 7.16 (m, 15H), 6.04 (s, 1H), 5.06 – 4.97 (m, 5H), 4.59 (d, *J* = 10.7 Hz, 1H), 4.52 (d, *J* = 12.1 Hz, 1H), 4.48 (d, *J* = 12.1 Hz, 1H), 4.43 (d, *J* = 12.1 Hz, 1H), 4.39 (d, *J* = 12.1 Hz, 1H), 3.64 (d, *J* = 5.2 Hz, 1H), 3.21 – 2.96 (m, 4H), 2.82 – 2.71 (m, 1H), 2.42 (dd, *J* = 5.2, 16.1 Hz, 1H), 2.16 – 2.10 (m, 1H), 2.07 – 2.02 (m, 1H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 156.6, 154.8, 154.0, 137.9, 137.8, 137.1, 128.6–127.4 (9 carbons), 120.4, 117.5, 112.6, 107.1, 70.3, 70.1, 69.9, 69.7, 65.5, 45.7, 42.8, 39.0, 34.4, 30.8, 20.2; MS(ES)⁺ calcd for C₃₆H₄₀N₆NaO₆ (M+Na)⁺ 675.3, found 675.3.



Pyrrole amide 19. To a solution of **18a** (19.6 mg, 0.0255 mmol, 1.0 equiv) in anhydrous *N,N*-dimethylformamide (1.6 mL) was added 4,5-dibromo-2-(trichloroacetyl)pyrrole (28 mg, 0.0765 mmol, 3 equiv) and triethylamine (0.11 mL, 0.77 mmol, 30 equiv). After stirring at 70 °C for 5 hours, the solvent was removed

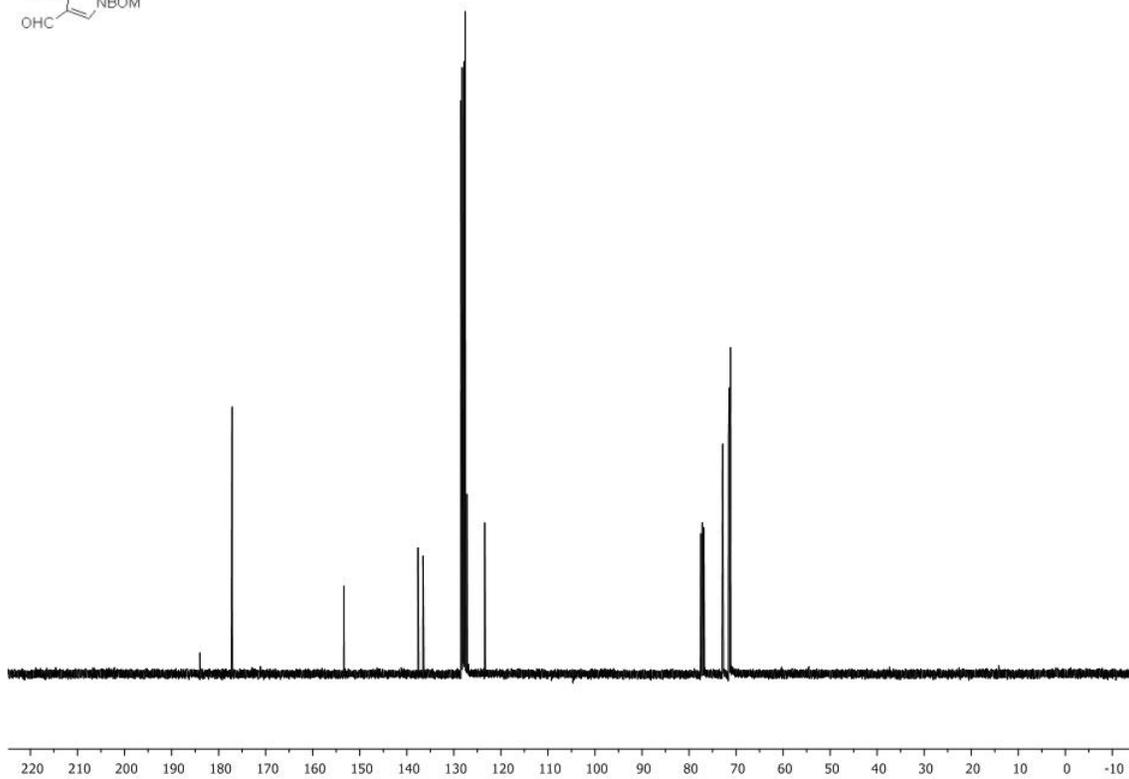
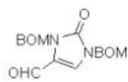
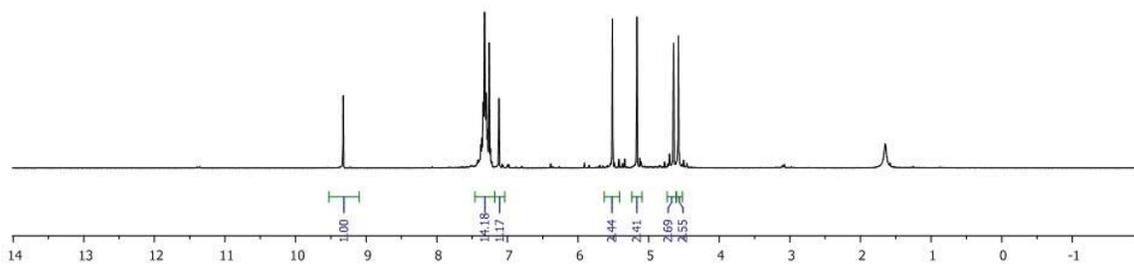
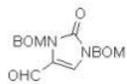
and the residue was purified by semi-preparative HPLC (Waters Atlantis C-18 column, 19 \times 150 mm, 5 μ m particle S-12 size; eluent A: H₂O with 0.1% TFA, eluent B: MeCN; gradient: T = 0 min: 50% B, T = 50 min: 90% B, 5.0 mL/min) to give **19** (7.5 mg, 33% yield, retention time: 24.9 min). ¹H NMR (500 MHz, DMSO-*d*₆) δ 12.78 (s, 1H), 9.88 (s, 1H), 9.64 (s, 1H), 7.86 (s, 1H), 7.44 (t, *J* = 5.5 Hz, 1H), 7.39 – 7.00 (m, 16H), 6.10 (s, 1H), 5.23 – 4.80 (m, 6H), 4.50 (d, *J* = 12.6 Hz, 2H), 4.46 (d, *J* = 12.6 Hz, 2H),

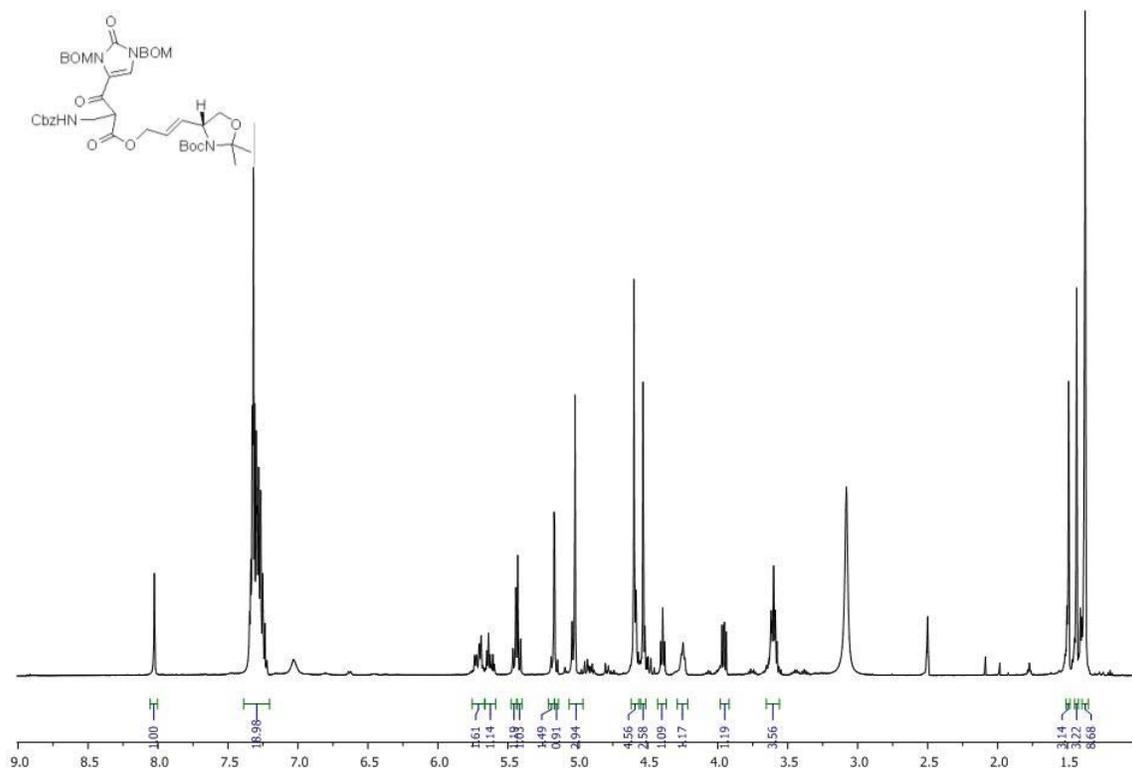
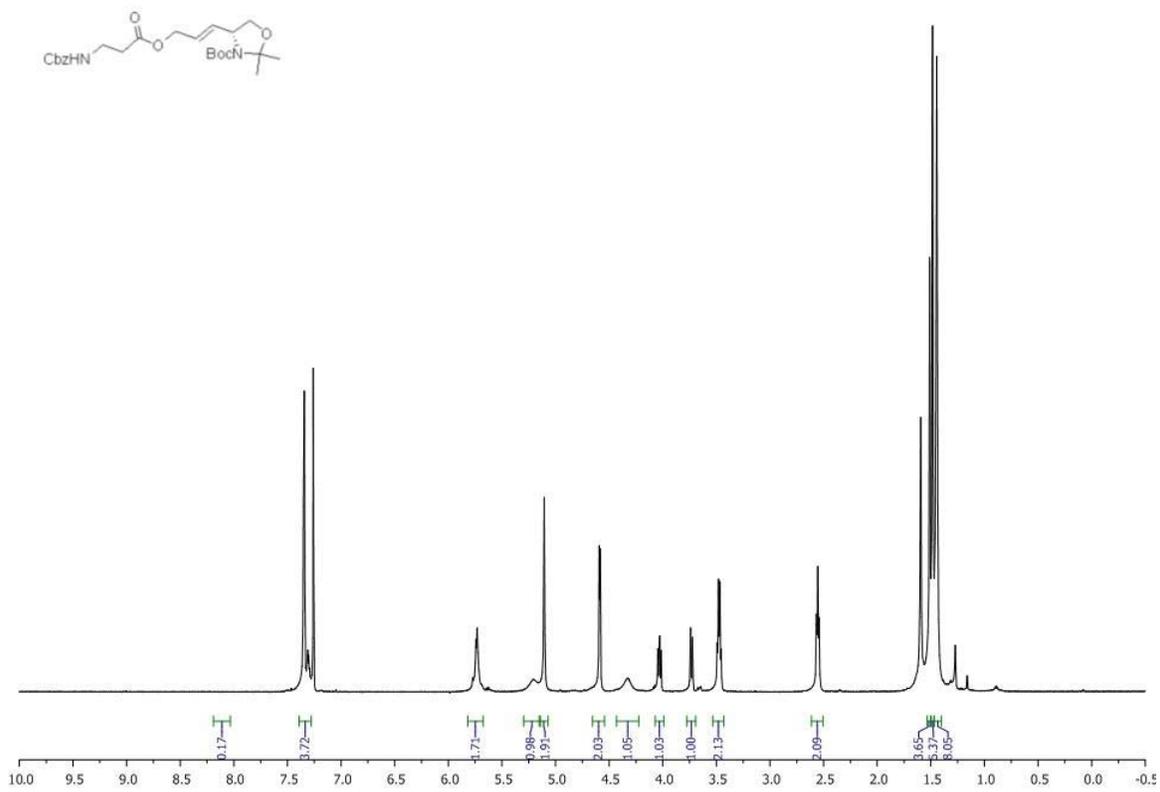
4.33 (d, $J = 12.0$ Hz, 1H), 4.26 (d, $J = 12.0$ Hz, 1H), 3.58 – 3.49 (m, 2H), 3.40 – 3.31 (m, 1H), 3.26 – 3.18 (m, 1H), 3.17 – 3.08 (m, 1H), 2.57 – 2.46 (m, 1H), 2.30 (d, $J = 12.3$ Hz, 1H), 1.91 (s, 2H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 159.5, 156.7, 154.9, 153.9, 137.9, 137.7, 137.1, 128.4, 128.3, 128.1, 127.9, 127.92, 127.86, 127.5, 127.4, 127.3, 121.0, 117.8, 113.5, 113.0, 106.8, 104.8, 97.9, 70.2, 70.0, 69.6, 69.6, 65.5, 43.1, 41.4, 38.1, 35.0, 31.3, 21.4; MS(ES) $^+$ calcd for $\text{C}_{41}\text{H}_{41}\text{Br}_2\text{N}_7\text{NaO}_7$ (M+Na) $^+$ 924.1, found 924.0.

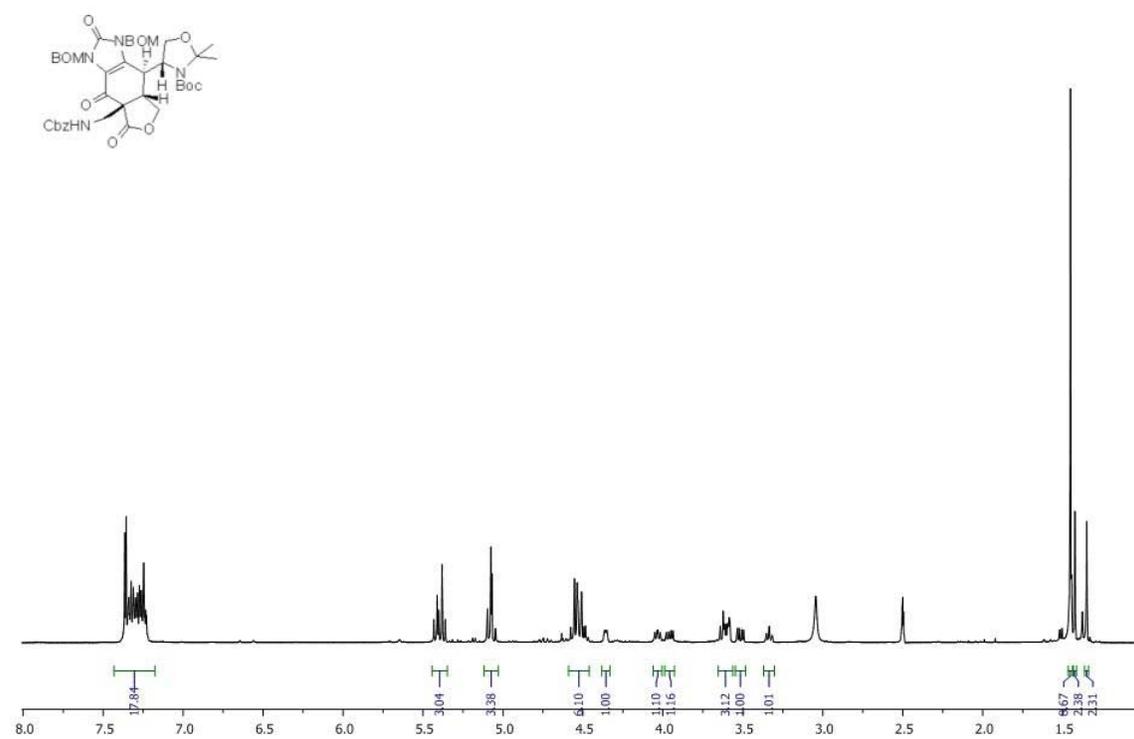
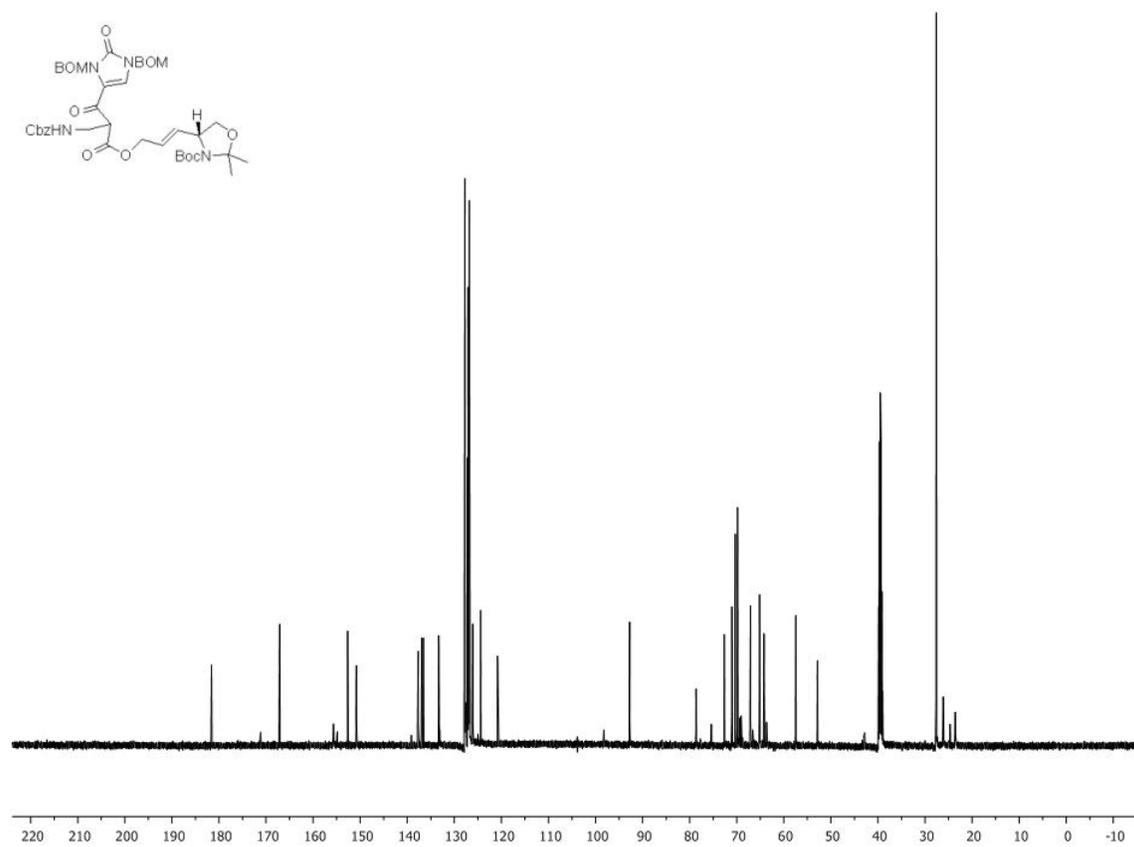


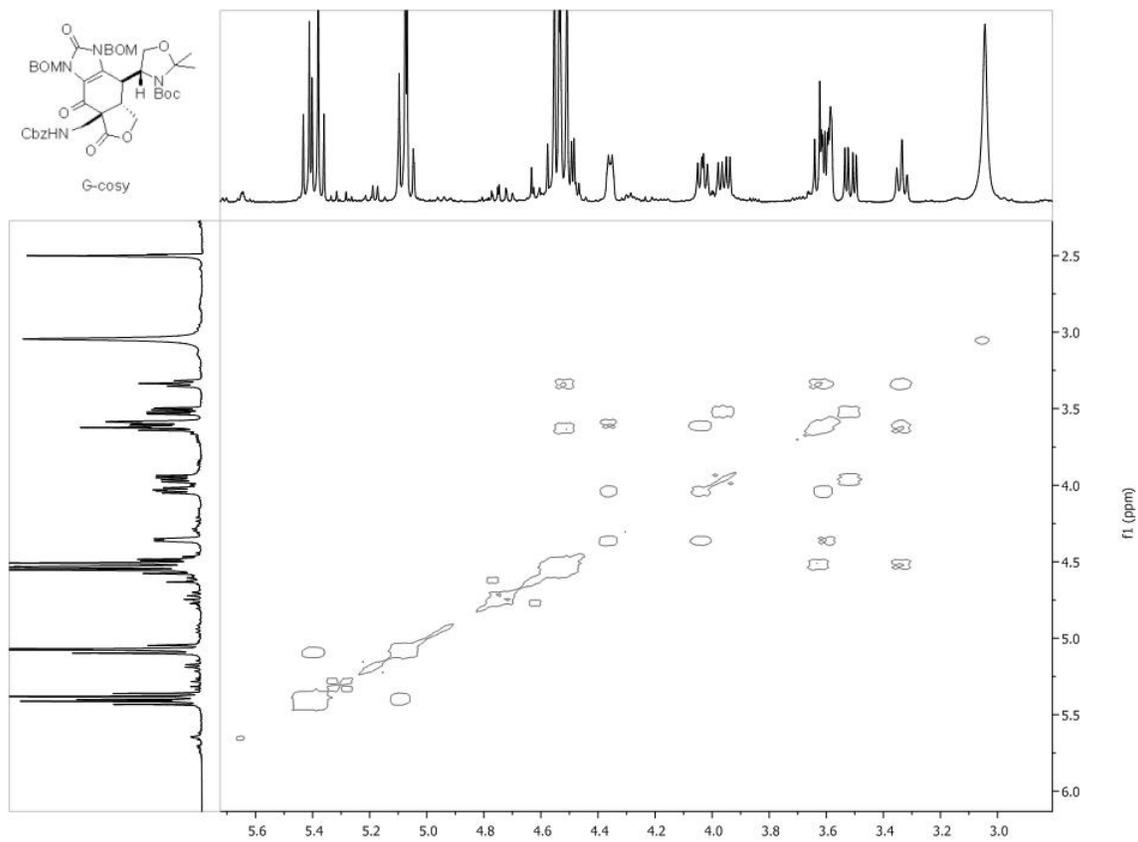
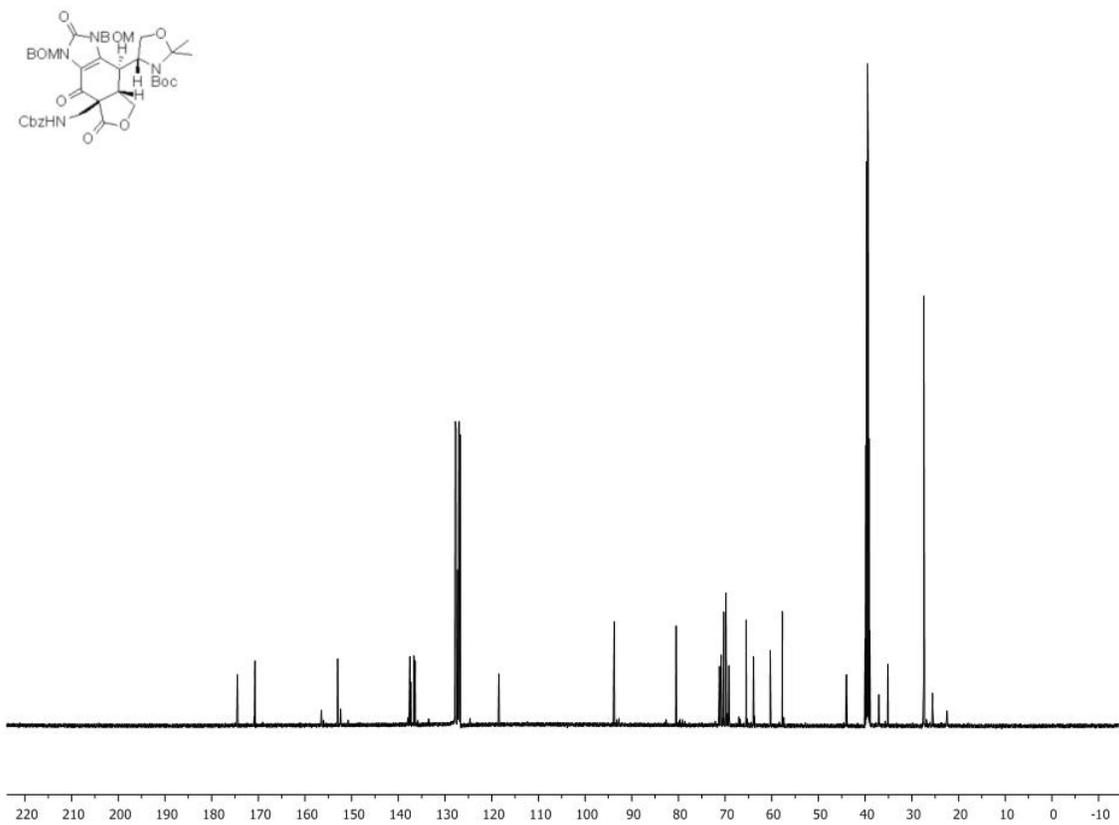
Piperazine 20. Amide **19** (2.5 mg, 2.77 μmol , 1.0 equiv) and anhydrous sodium carbonate (3.0 mg, 28 μmol , 10 equiv) were azeotroped with toluene, and suspended in freshly distilled 2,2,2-trifluoroethanol (0.4 mL). A freshly prepared

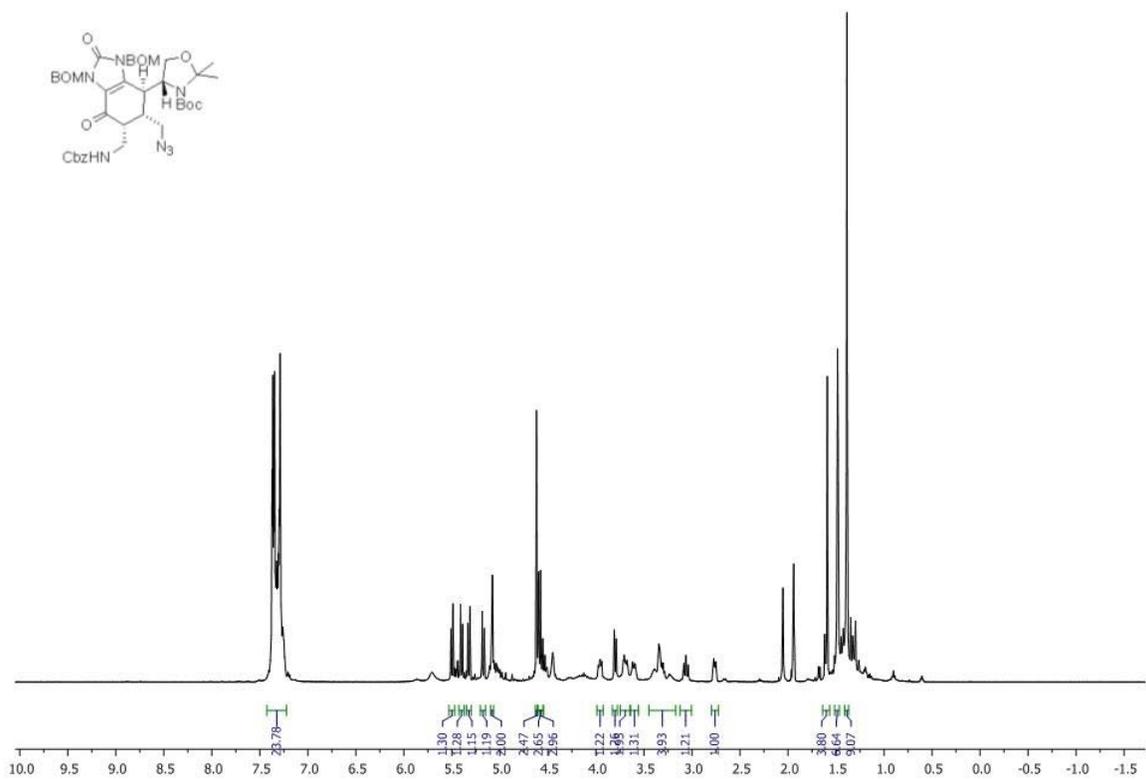
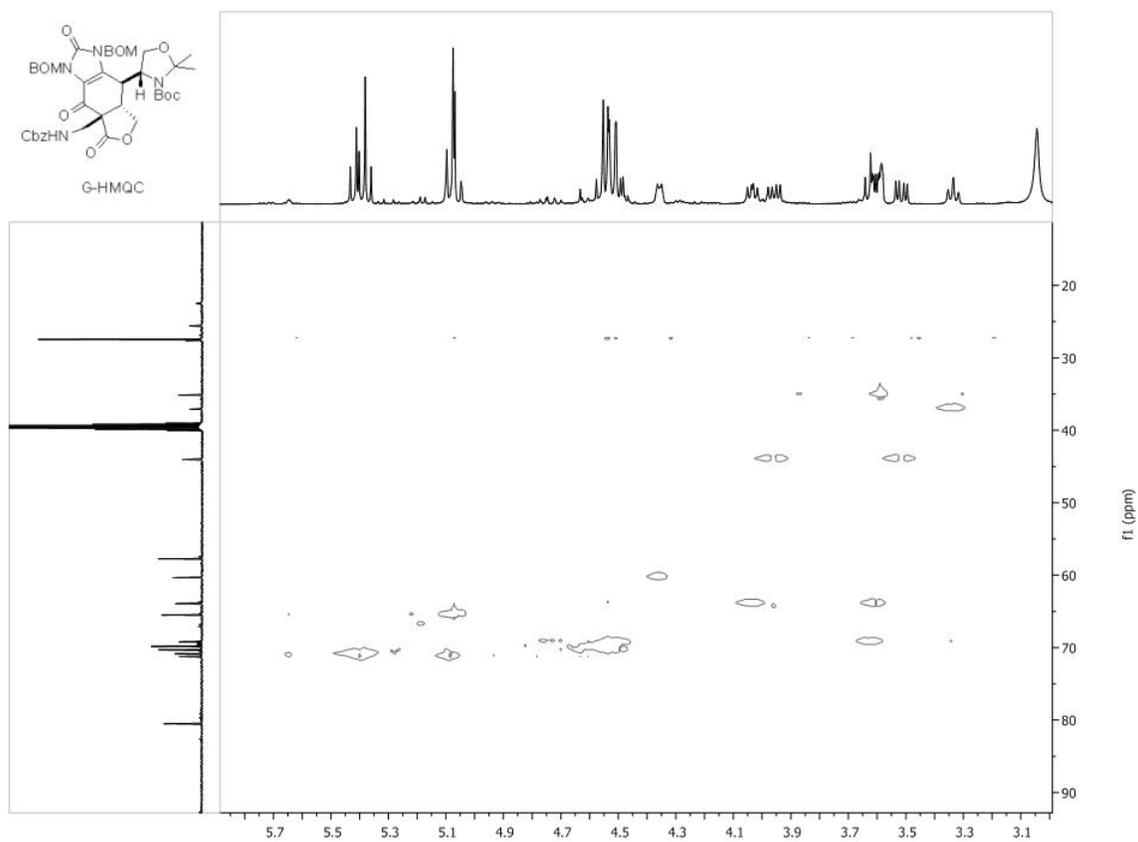
solution of iodosylbenzene (7.1 mg) in 2,2,2-trifluoroethanol (0.45 mL) was then added portionwise at 23 $^{\circ}\text{C}$. Upon consumption of **19** indicated by TLC analysis, the solid was filtered off and the filtrate was concentrated to nearly dryness. The residue was then diluted with dimethyl sulfoxide (0.5 mL) and stirred at 50 $^{\circ}\text{C}$ for 30 minutes. The solvent was subsequently removed by vacuum and the residue was purified by semi-preparative HPLC (Eclipse XDB-C18 5 μm column, 9.4 \times 250 mm; eluent A: H_2O with 0.1% TFA, eluent B: MeCN; gradient: T = 0 min: 35% B, T = 20 min: 70% B, 4.8 mL/min) to give **20** (0.5 mg, 20% yield, retention time: 17.9 min). ^1H NMR (50 $^{\circ}\text{C}$) (600 MHz, DMSO- d_6) δ 8.26 (s, 1H), 8.02 (s, 1H), 7.40 – 7.13 (m, 15H), 6.98 (s, 1H), 6.44 (d, $J = 1.3$ Hz, 1H), 5.40 (d, $J = 11.6$ Hz, 1H), 5.30 (d, $J = 11.6$ Hz, 1H), 5.07 (s, 2H), 5.05 (d, $J = 11.2$ Hz, 1H), 4.96 (d, $J = 11.2$ Hz, 1H), 4.52 (s, 2H), 4.50 (d, $J = 12.2$ Hz, 1H), 4.40 (d, $J = 12.2$ Hz, 1H), 3.89 (dd, $J = 7.7, 10.0$ Hz, 1H), 3.32–3.17 (m, 2H), 3.12 (dd, $J = 10.0, 11.4$ Hz, 1H), 3.08 – 3.02 (m, 1H), 2.60 (dd, $J = 5.0, 16.1$ Hz, 1H), 2.49 – 2.38 (m, 1H), 2.22 (dd, $J = 9.2, 16.1$ Hz, 1H), 1.94 – 1.84 (m, 1H); ^{13}C NMR (50 $^{\circ}\text{C}$) (125 MHz, DMSO- d_6) δ 156.8, 154.2, 153.9, 137.4, 137.1, 137.0, 128.1, 128.0, 127.8, 127.5, 127.5, 127.4, 127.3, 127.3, 127.3, 124.0, 122.5, 114.7, 113.2, 106.0, 101.4, 80.0, 71.9, 70.1, 69.97, 69.76, 66.7, 65.2, 49.8, 44.8, 43.5, 39.9, 35.6, 24.8; (Note: Due to rotamer effect, one carbon can not be found). MS(ES) $^+$ calcd for $\text{C}_{41}\text{H}_{40}\text{Br}_2\text{N}_7\text{O}_7$ (M+H) $^+$ 900.1, found 900.1; HRMS(ES) $^+$ calcd for $\text{C}_{41}\text{H}_{40}\text{Br}_2\text{N}_7\text{O}_7$ (M+H) $^+$ 900.1356, found 900.1346.

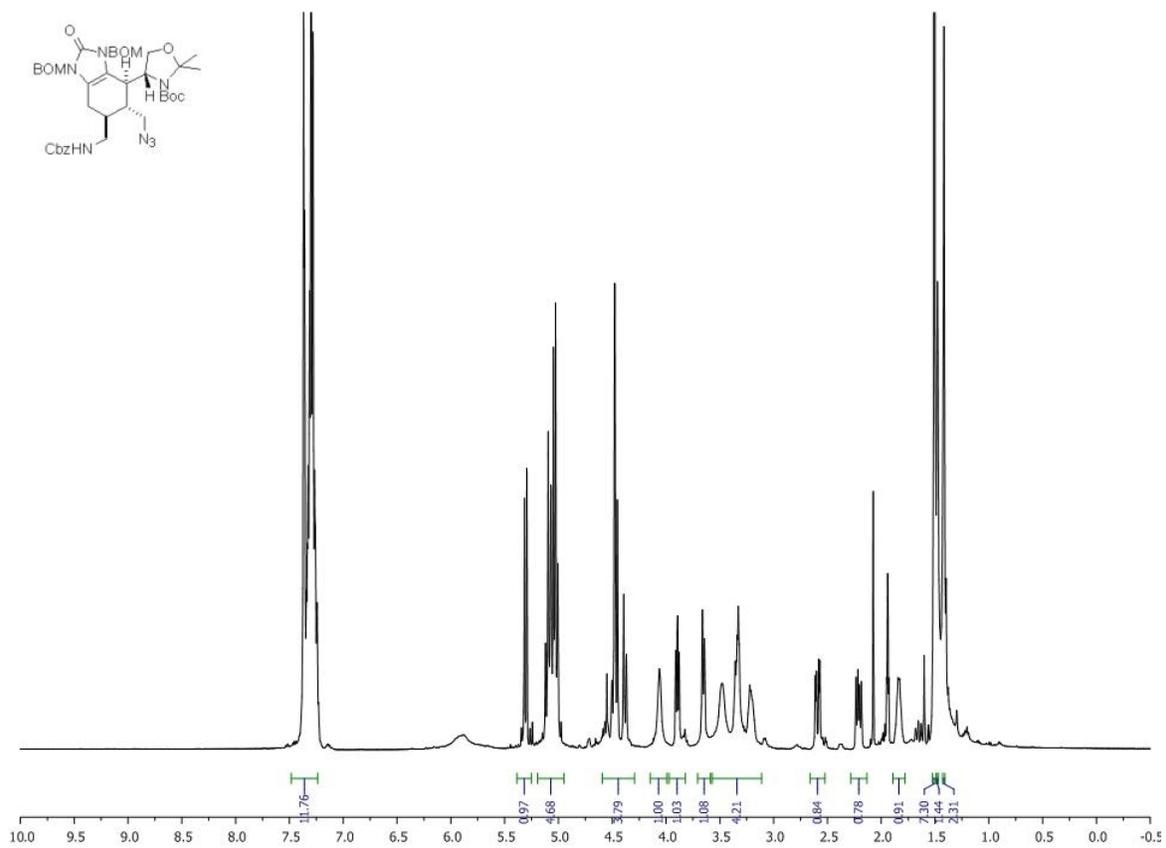
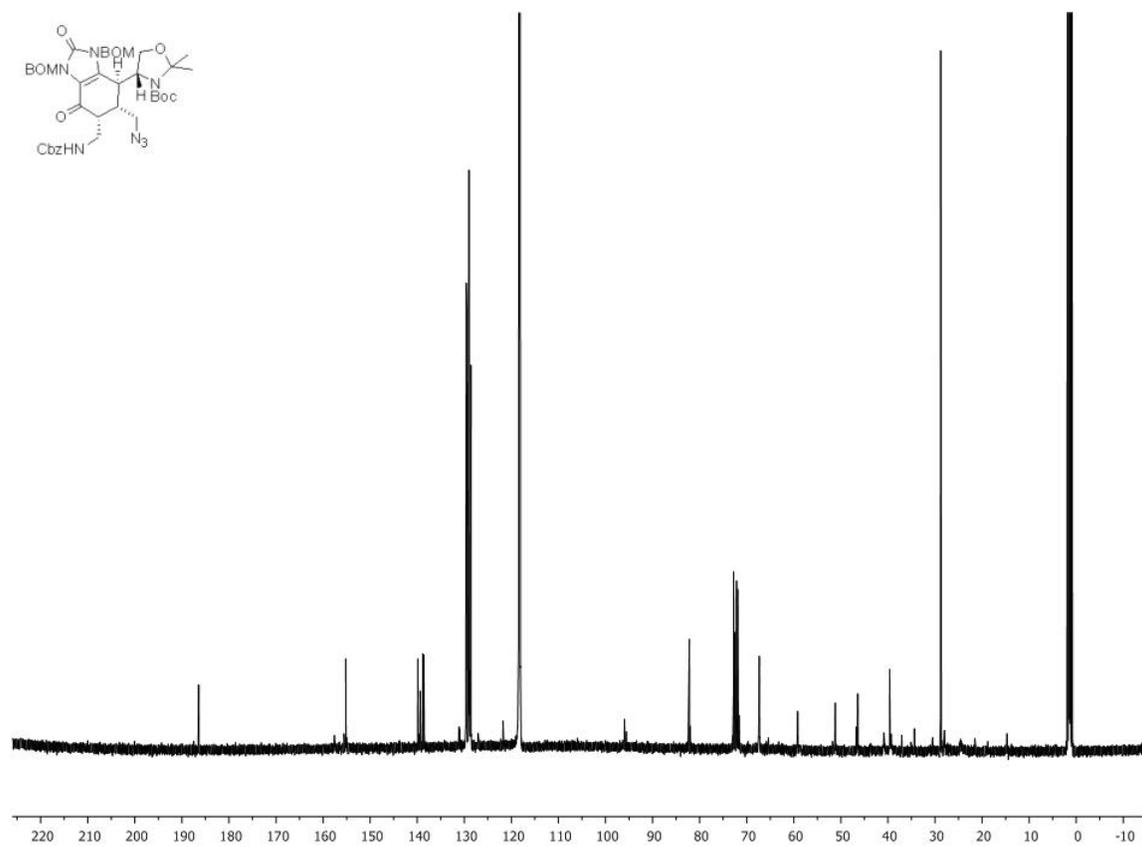


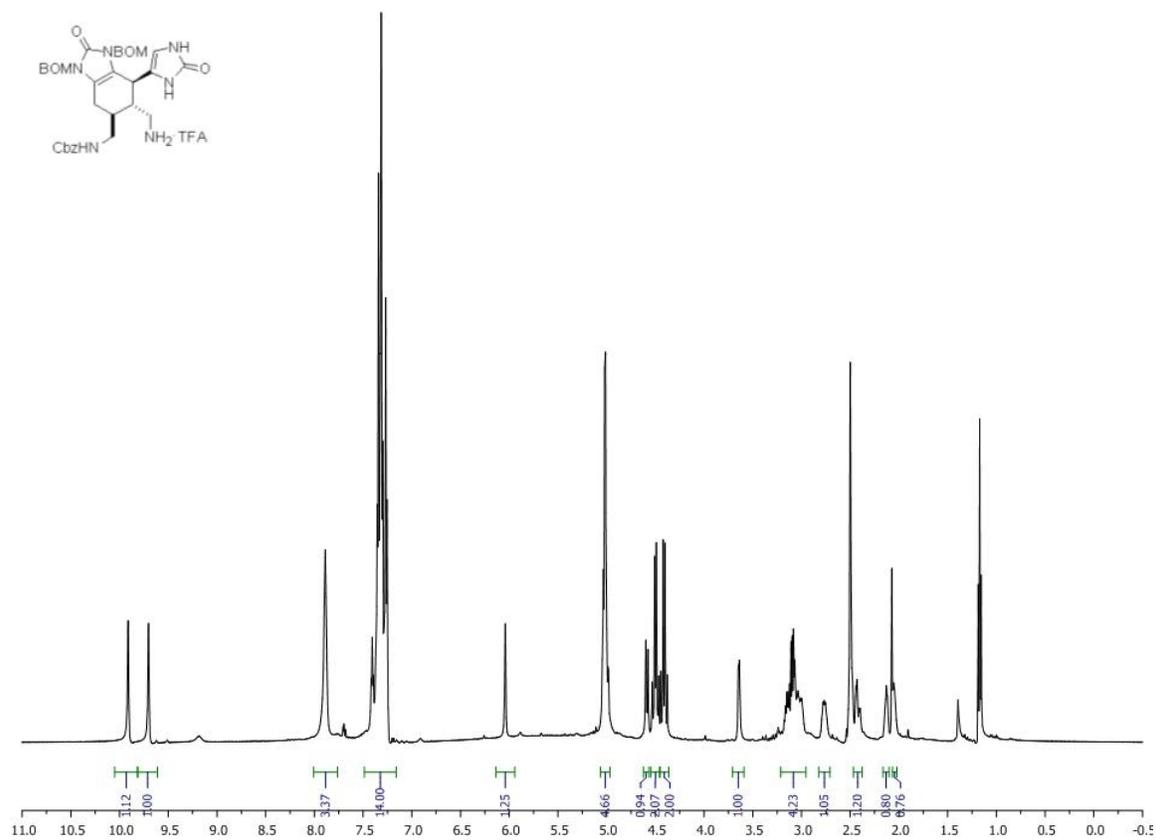
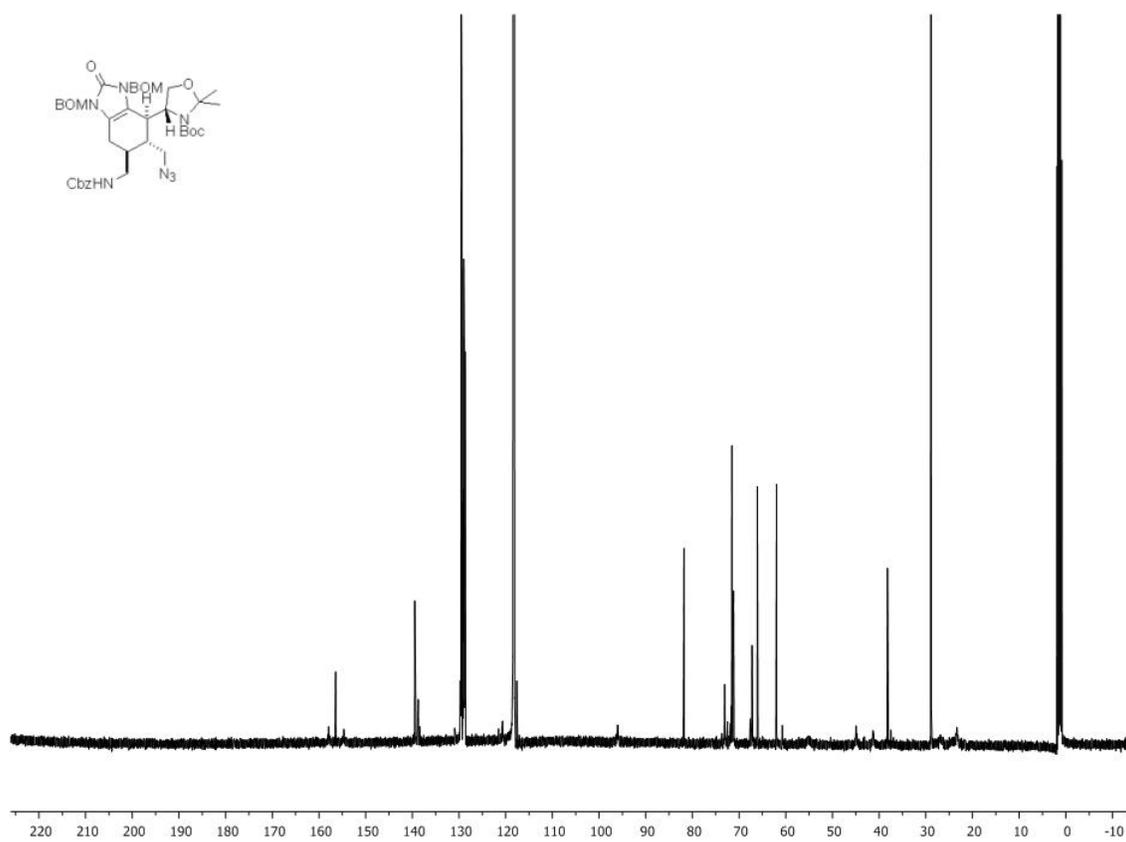


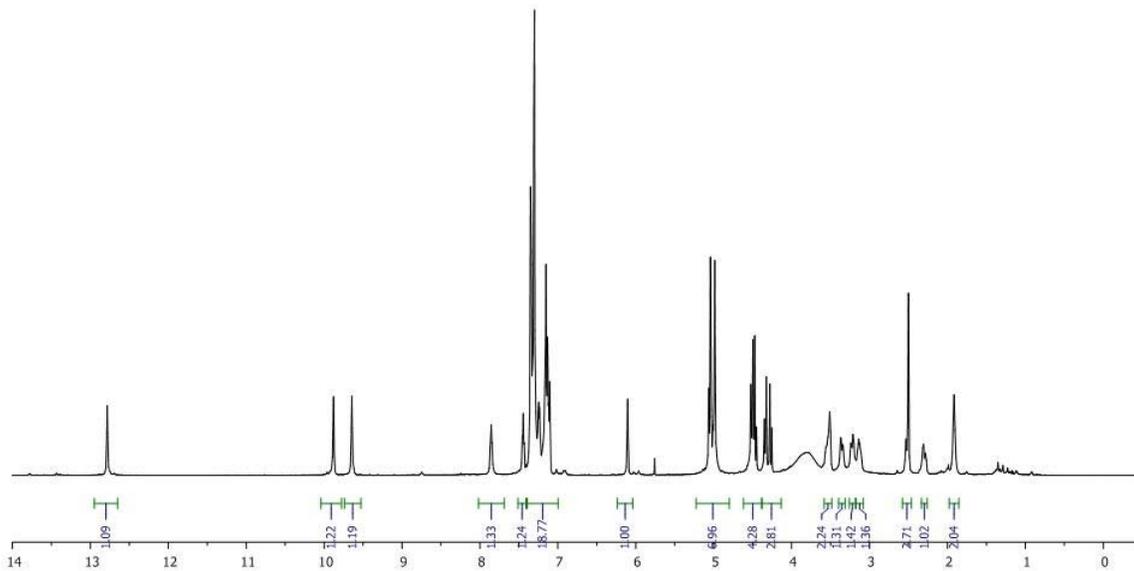
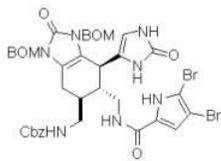
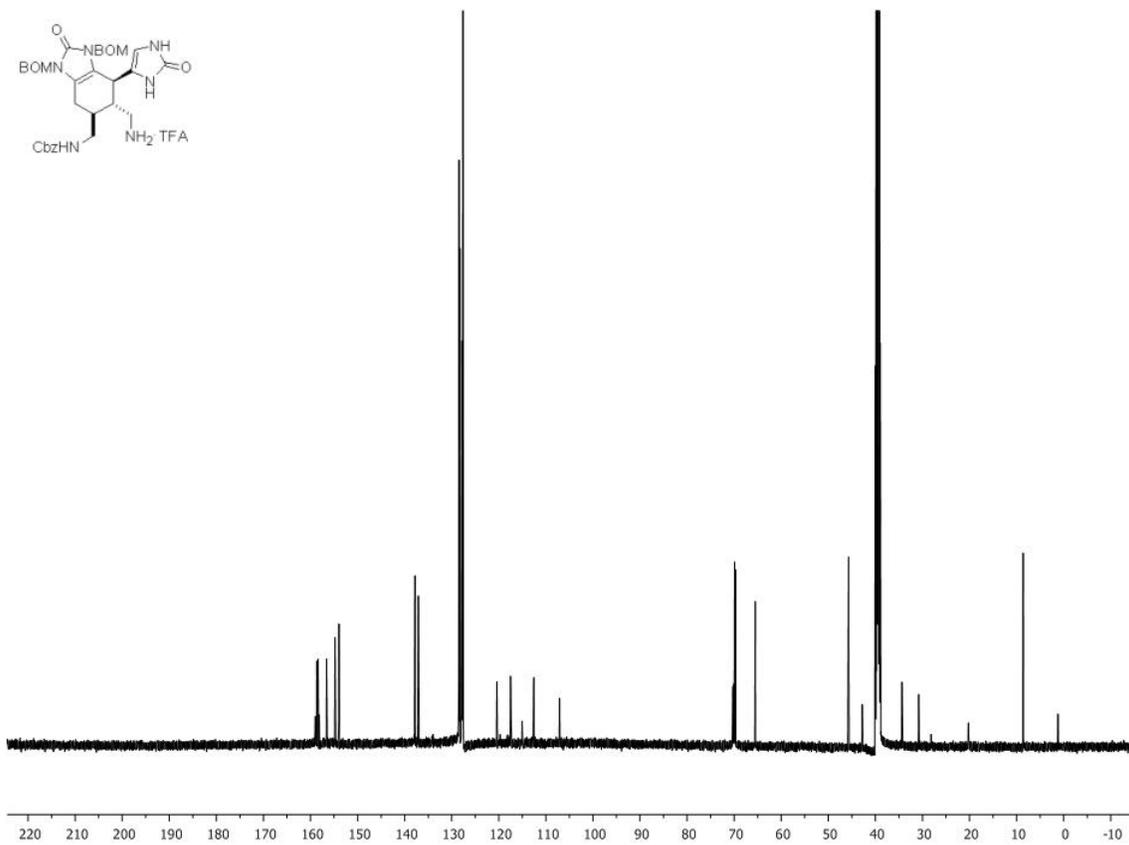
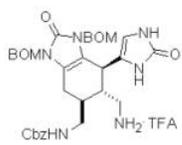


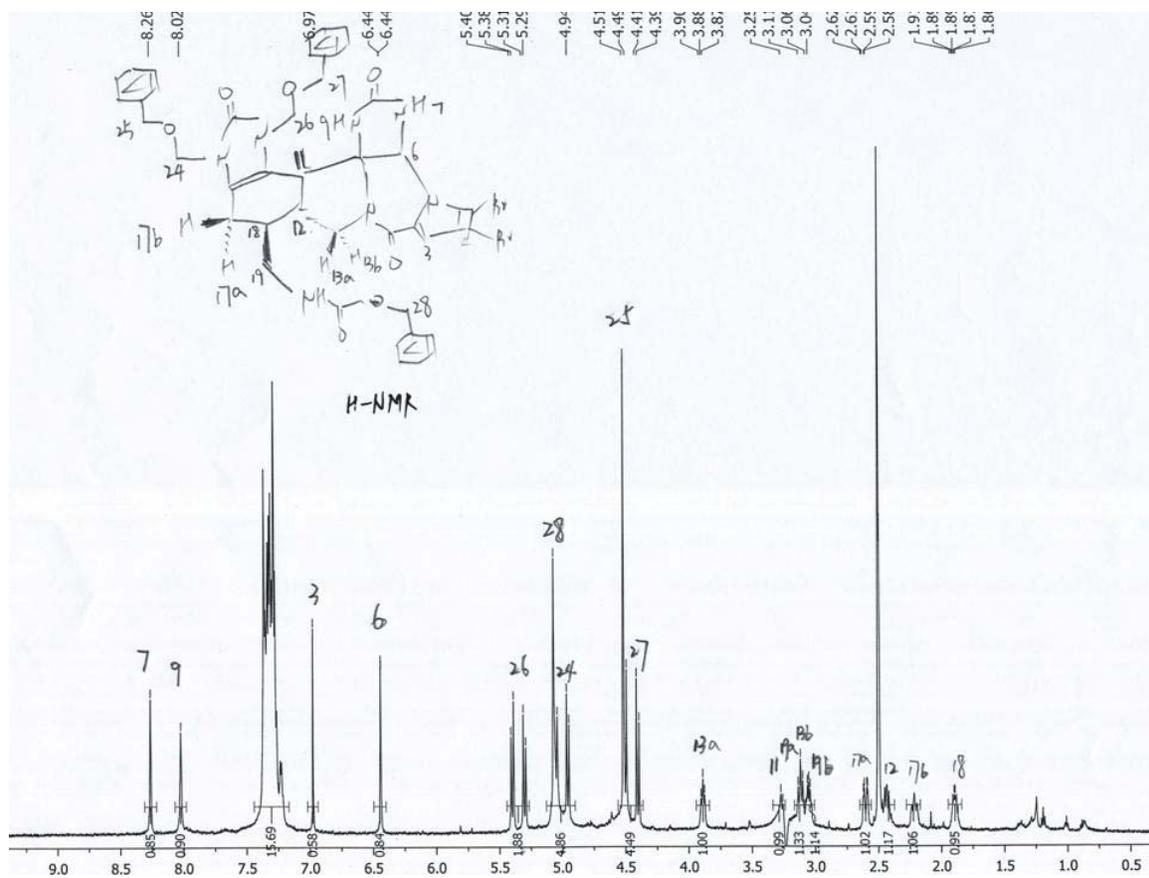
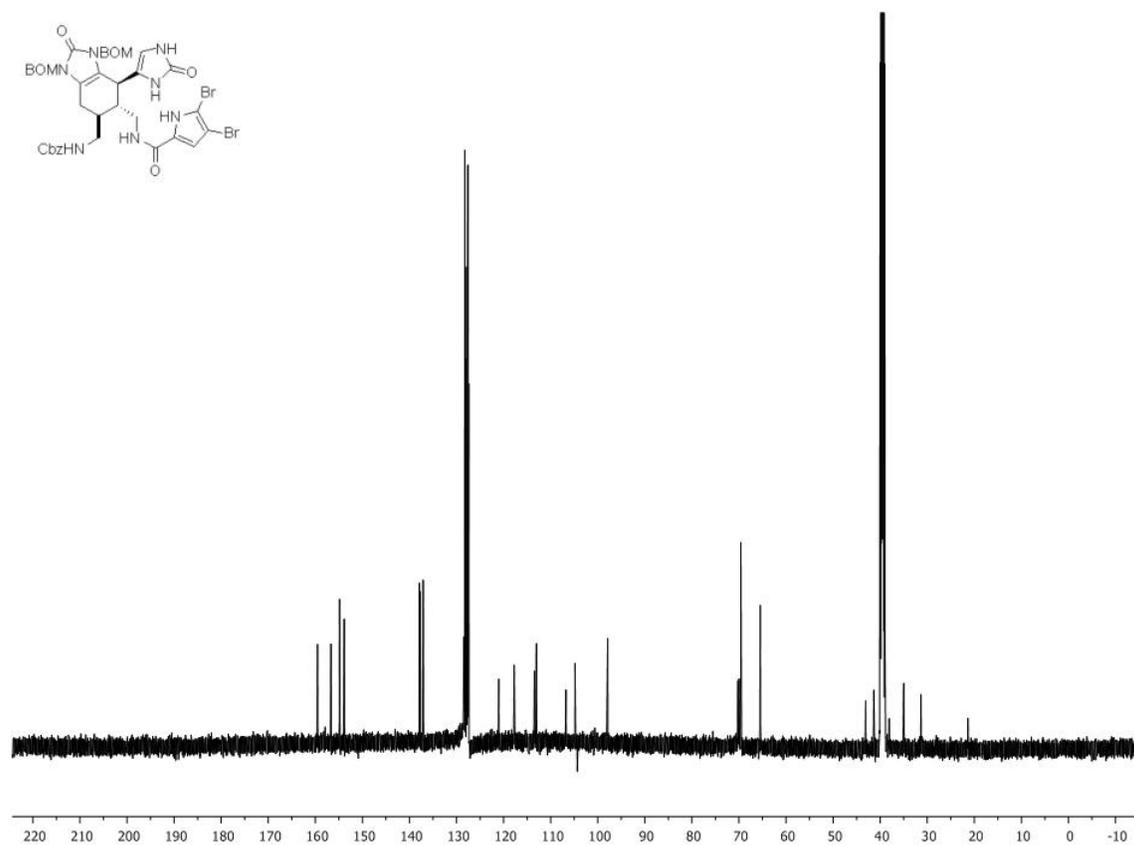


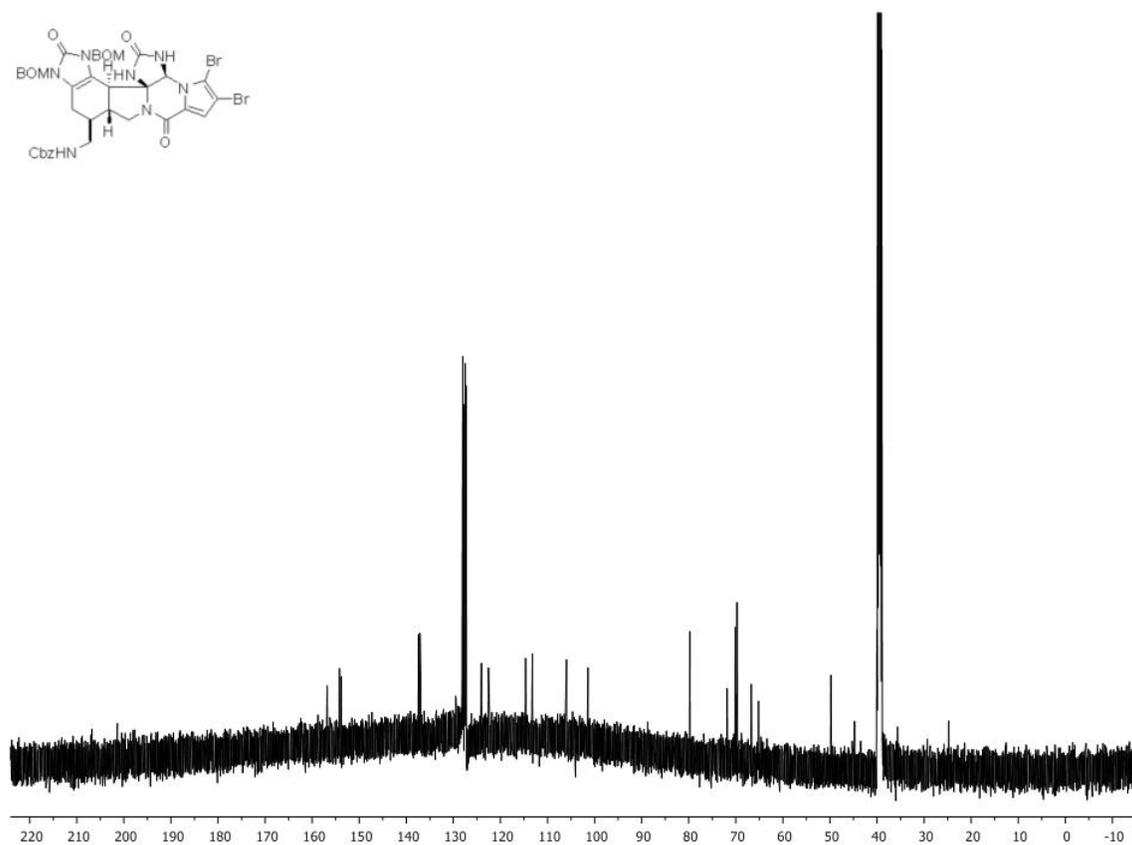
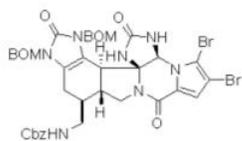












STANDARD PROTON PARAMETERS

Sample Name:
 Data Collected on: sedna-vnmrs600
 Archive directory:
 Sample directory:
 FID file: M3-261-Gcosy50degreeref2.50
 Pulse Sequence: gCOSY
 Solvent: dms0
 Data collected on: Oct 23 2009

Temp. 50.0 C / 323.1 K
 Operator: zhiqiang
 Relax. delay 1.000 sec
 Acq. time 0.150 sec
 Width 5707.8 Hz
 ZD Width 5707.8 Hz
 64 repetitions
 128 increments
 OBSERVE M1, 599.6652760 MHz
 DATA PROCESSING
 Sg, sine bell 0.075 sec
 F1 DATA PROCESSING
 Sg, sine bell 0.022 sec
 FT size 2048 x 2048
 Total time 3 hr, 19 min

