Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Supplemental Information

Synthesis of novel ginkgolide photoaffinity-biotin probes

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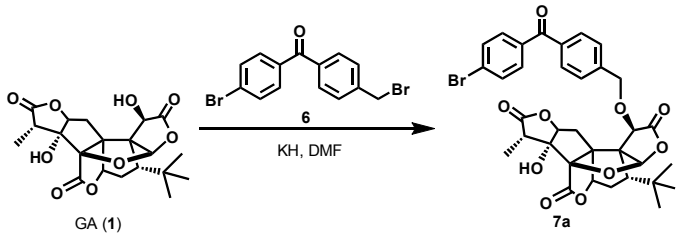
Materials and Methods: All reagents were used as received. Ginkgolides were isolated from Ginkgo biloba extract (*BioGinkgo* 7/27, Pharmanex®. All yields refer to isolated products. Reactions were monitored by TLC (silica gel 60 F254) and spots were visualized by heating and UV (or I_2). Preparatory TLC was performed using silica gel MERCK5715 plates. Column chromatography was performed using silica gel (230-400 mesh). 1H NMR and 13C NMR were recorded on Bruker (300 or 400 MHz) spectrometers. The chemical shifts are reported in ppm (δ) downfield from tetramethylsilane (in $CDCl_3$) or calibrated to solvent residual peak as an internal standard (MeOH-d4, δ 3.31, DMSO-d6, δ 2.51). High-resolution mass spectra (HRMS) were measured on JEOL JMS-HX110/100A HF mass spectrometer under FAB conditions with NBA or glycerol as a matrix.



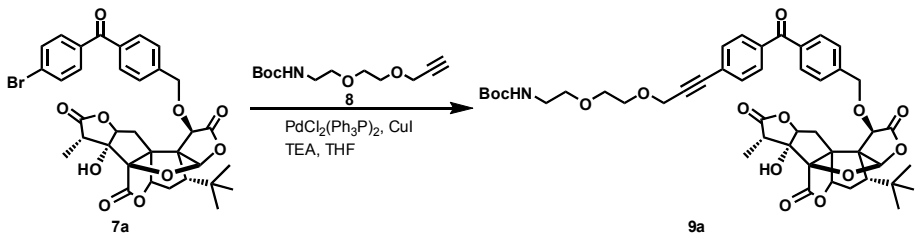
GA (**1**) **7a**

Synthesis of **7a**

GA (**1**) (91.6 mg, 224.5 μmol) was dissolved in DMF (2.5 mL) and KH (37.2 mg, 930 μmol) was added at 0 ºC under nitrogen atmosphere. After stirring for ten minutes, **6** (159.1 mg, 449.0 μmol) was added and stirred for one hour. To this reaction mixture 1 M HCl (20 ml). was added and the mixture extracted with EtOAc (10 ml, 3x). Organic layer was washed with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography (20-50% acetone / hexane) to obtain **7a** (105.5 mg, 69%).

7a. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.79 (2H, d, *J* = 8.2 Hz), 7.69 (2H, d, *J* = 8.7 Hz), 7.65 (2H, d, *J* = 8.7 Hz), 7.42 (2H, d, *J* = 8.2 Hz), 6.00 (1H, s), 5.54 (1H, d, *J* = 11.0 Hz), 4.86 (1H, s), 4.73 (1H, dd, *J* = 7.2, 9.2 Hz), 4.72 (1H, d, *J* = 4.1 Hz), 4.63 (1H, d, *J* = 11.0 Hz), 3.21 (1H, q, *J* = 7.1 Hz), 3.19 (1H, s, -OH), 2.70 (1H, dd, *J* = 7.2, 15.1 Hz), 2.24 (1H, dd, *J*

= 4.8, 13.5 Hz), 2.14 (1H, d, *J* = 9.2, 15.1 Hz), 2.11 (1H, ddd, *J* = 4.1, 13.5, 14.0 Hz), 1.95 (1H, dd, *J* = 4.8, 14.0 Hz), 1.32 (3H, d, *J* = 7.1 Hz), 1.11 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 195.10, 175.84, 171.46, 171.43, 140.77, 137.16, 135.91, 131.71, 131.50, 130.33, 127.80, 127.74, 110.08, 100.32, 86.89, 86.71, 86.52, 75.66, 72.10, 68.71, 67.22, 48.76, 40.57, 37.05, 36.45, 32.23, 29.11, 7.50; HRMS (FAB+):: $[M+H]^+$ *m/z* calcd for $C_{34}H_{34}O_{10}Br$ 681.1335, found 681.1334.

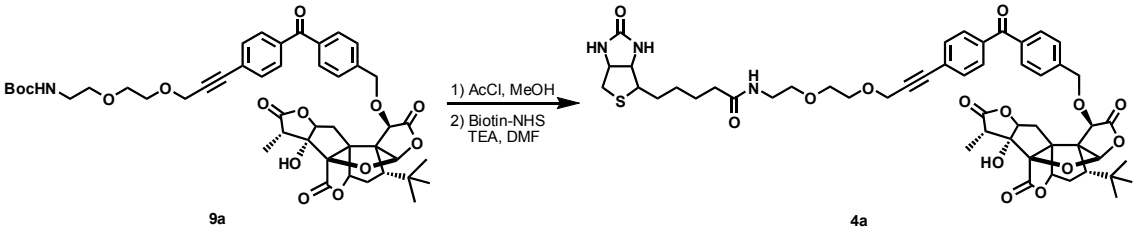


7a 9a

Synthesis of **9a**

Compound **7a** (85.5 mg, 125.5 μmol) and **8** (129.8 mg, 533.5 μmol) were dissolved in THF (3.0 mL) and TEA (1.5 mL) was added under argon atmosphere. To this solution, $PdCl_2(PPh_3)_2$ (5.0 mg, 7.1 μmol) and CuI (2.0 mg, 10.5 μmol) was added and the mixture was refluxed for 21 hours. The reaction mixture was evaporated in vacuum and the residue purified by column chromatography (30-50% acetone / hexane) to obtain **9a** (77.2 mg, 73%).

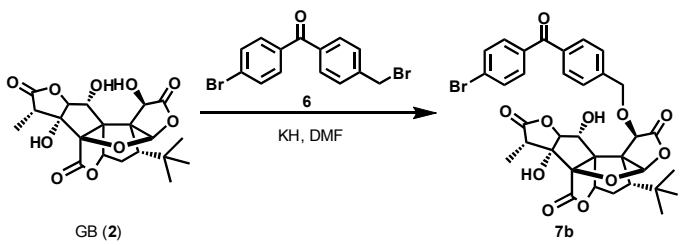
9a. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.80 (2H, d, *J* = 8.1 Hz), 7.76 (2H, d, *J* = 8.5 Hz), 7.56 (2H, d, *J* = 8.5 Hz), 7.42 (2H, d, *J* = 8.1 Hz), 6.00 (1H, s), 5.55 (1H, d, *J* = 10.9 Hz), 5.01 (1H, s, -NH), 4.86 (1H, s), 4.74 (1H, dd, *J* = 7.4, 9.2 Hz), 4.72 (1H, d, *J* = 4.1 Hz), 4.63 (1H, d, *J* = 10.9 Hz), 4.47 (2H, s), 3.79-3.77 (2H, m), 3.70-3.68 (2H, m), 3.57 (2H, t, *J* = 5.2 Hz), 3.34 (2H, dt, *J* = 5.2, 5.2 Hz), 3.32 (1H, s, -OH), 3.21 (1H, q, *J* = 7.0 Hz), 2.70 (1H, dd, *J* = 7.4, 15.1 Hz), 2.24 (1H, dd, *J* = 4.8, 13.6 Hz), 2.15 (1H, dd, *J* = 9.2, 15.1 Hz), 2.11 (1H, ddd, *J* = 4.0, 13.6, 14.0 Hz), 1.96 (1H, dd, *J* = 4.8, 14.0 Hz), 1.44 (9H, s), 1.32 (3H, d, *J* = 7.0 Hz), 1.11 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 195.69, 176.20, 171.84, 171.82, 156.1, 141.09, 137.82, 137.14, 132.08, 130.77, 130.35, 128.17, 127.51, 110.52, 100.80, 88.74, 87.34, 87.14, 86.93, 85.97, 79.69, 76.11, 72.57, 70.75, 70.57, 69.71, 69.18, 67.69, 59.59, 49.26, 41.00, 40.77, 37.54, 36.91, 32.67, 29.56, 28.8, 7.94; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{46}H_{54}O_{14}N$ 844.3544, found 844.3503.



Synthesis of **4a**

Compound **9a** (39.7 mg, 47.0 μmol) was dissolved in MeOH (3.0 mL) and AcCl (300 μL) was added at 0 °C under nitrogen atmosphere. After stirring for three hours at room temperature, solvent was removed under vacuum and the residue was dissolved in DMF (1.0 mL). To this solution, TEA (20 μL) and *N*-hydroxysuccinimide biotin (49.4 mg, 144.7 μmol) was added and stirred for 13 hours at room temperature. After reaction was complete, water (10 ml) was added and extracted with EtOAc (5 ml, 3x). The organic fractions were combined and washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 10/1) to obtain **4a** (34.0 mg, 75%).

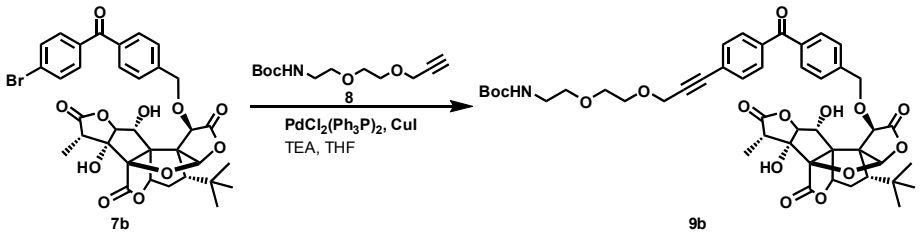
4a. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.79 (2H, d, *J* = 8.2 Hz), 7.77 (2H, d, *J* = 8.4 Hz), 7.55 (2H, d, *J* = 8.4 Hz), 7.42 (2H, d, *J* = 8.2 Hz), 6.72 (1H, t, *J* = 5.5 Hz, -NH), 6.40 (1H, s, -NH), 5.99 (1H, s), 5.70 (1H, s, -NH), 5.55 (1H, d, *J* = 11.0 Hz), 4.86 (1H, s), 4.83 (1H, dd, *J* = 7.8, 7.8 Hz), 4.76 (1H, d, *J* = 3.6 Hz), 4.63 (1H, d, *J* = 11.0 Hz), 4.49 (1H, dd, *J* = 4.8, 7.4 Hz), 4.45 (2H, s), 4.28 (1H, dd, *J* = 4.6, 7.4 Hz), 3.75 (2H, m), 3.69 (2H, m), 3.59 (2H, t, *J* = 5.0 Hz), 3.43 (2H, dt, *J* = 5.5, 5.0 Hz), 3.16 (1H, q, *J* = 7.2 Hz), 3.10 (1H, dt, J = 4.6, 7.2 Hz), 2.87 (1H, dd, *J* = 4.8, 12.8 Hz), 2.72 (1H, dd, *J* = 7.8, 15.2 Hz), 2.71 (1H, d, *J* = 12.8 Hz), 2.23-2.17 (4H, m), 2.11 (1H, ddd, *J* = 3.6, 13.6, 13.6 Hz), 1.95 (1H, dd, *J* = 4.8, 13.6 Hz), 1.75-1.55 (4H, m), 1.39 (2H, t, *J* = 7.4 Hz), 1.31 (3H, d, *J* = 7.2 Hz), 1.10 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 195.52, 176.67, 173.66, 171.74, 171.70, 164.23, 141.14, 137.28, 137.02, 131.79, 130.53, 130.17, 127.73, 126.97, 110.15, 101.18, 88.31, 87.55, 86.82, 86.52, 85.84, 75.96, 72.17, 70.06, 70.00, 69.38, 69.05, 67.36, 61.91, 60.39, 59.29, 55.71, 49.05, 40.94, 40.65, 39.26, 37.38, 36.73, 36.07, 32.40, 29.30, 28.36, 28.17, 25.68, 8.20; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{51}H_{60}O_{14}N_3S$ 970.3796, found 970.3824,;



Synthesis of **7b**

GB **2** (61.1 mg, 144.0 μmol) was dissolved in DMF (1.5 ml) and KH (12.1 mg) was added at 0 ºC under nitrogen atmosphere. After stirring for ten minutes, **6** (101.4 mg, 286.4 μmol) was added and stirred for one hour. To this mixture, 1 M HCl (10ml). was added and extracted with EtOAc (10ml, 3x). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated in vacuum. The residue was purified by column chromatography (25-40% acetone / hexane) to obtain **7b** (70.4 mg, 70%).

7b. ^{1}H NMR (400 MHz, DMSO-d6): δ = 7.79 (2H, d, *J* = 8.5 Hz), 7.72 (2H, d, *J* = 8.3 Hz), 7.66 (2H, d, *J* = 8.5 Hz), 7.56 (2H, d, *J* = 8.3 Hz), 6.48 (1H, s, -OH), 6.18 (1H, d, *J* = Hz), 5.47 (1H, d, *J* = 12.9 Hz), 5.46 (1H, d, *J* = 4.9 Hz, -OH), 5.38 (1H, d, *J* = 4.0 Hz), 5.26 (1H, s), 4.79 (1H, d, *J* = 12.9 Hz), 4.63 (1H, d, *J* = 6.5 Hz), 4.22 (1H, dd, *J* = 4.9, 6.5 Hz), 2.89 (1H, q, *J* = 7.2 Hz), 2.15 (1H, dd, *J* = 4.4, 13.7 Hz), 1.92 (1H, ddd, *J* = 4.0, 13.7, 14.1 Hz), 1.74 (1H, dd, *J* = 4.4, 14.1 Hz), 1.13 (3H, d, *J* = 7.2 Hz), 1.00 (9H, s); ^{13}C NMR (100 MHz, DMSO-d6): δ = 194.52, 176.37, 172.41, 170.30, 142.54, 136.09, 135.74, 131.71, 131.56, 129.78, 126.90, 126.69, 109.55, 99.31, 93.36, 82.85, 78.52, 76.33, 73.79, 71.98, 71.43, 67.43, 48.72, 41.50, 36.68, 31.84, 28.71, 8.16; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{34}H_{34}O_{11}Br$ 697.1284, found 697.1282.

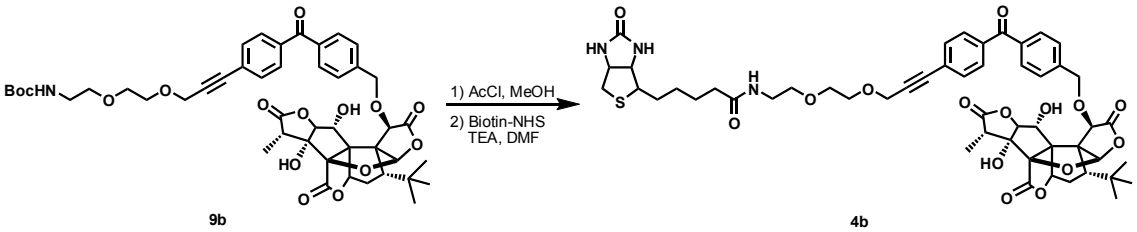


Synthesis of **9b**

7b (60.8 mg, 87.2 μmol) and **8** (89.0 mg, 365.8 μmol) were dissolved in THF (2.0 ml) and TEA (1.0 ml) was added under argon atmosphere. To this solution, $PdCl_2(PPh_3)_2$ (3.2 mg, 4.6 μmol) and CuI (4.8 mg, 25.2 μmol) were added and the mixture was refluxed for 10 hours. The volatiles were removed in vacuum and the residue purified by column chromatography (50-66% ethyl acetate / hexane) to obtain **9b** (54.6 mg, 74%).

9b. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 (2H, d, *J* = 8.2 Hz), 7.74 (2H, d, *J* = 8.4 Hz), 7.56 (2H, d, *J* = 8.4 Hz), 7.49 (2H, d, *J* = 8.2 Hz), 6.02 (1H, s), 5.61 (1H, d, *J* = 9.9 Hz), 5.35 (1H, d, *J* = 3.3 Hz), 5.01 (1H, broad s, -NH), 4.96 (1H, s), 4.69 (1H, d, *J* = 9.9 Hz), 4.57 (1H, d, *J* = 7.9 Hz), 4.47 (2H, s), 4.29 (1H, dd, *J* = 3.4, 7.9 Hz), 3.79-3.76 (2H, m), 3.70-3.67 (2H, m), 3.57 (2H, t, *J* = 5.1 Hz), 3.34 (2H, dt, *J* = 4.8, 5.1 Hz), 3.06 (1H, q, *J* =

7.0 Hz), 2.81 (1H, d, *J* = 3.4 Hz, -OH), 2.32 (1H, broad d, *J* = 13.4 Hz), 1.98 (1H, broad d, *J* = 11.0 Hz), 1.95 (1H, ddd, *J* = 3.3, 11.0, 13.4 Hz), 1.44 (9H, s), 1.31 (3H, d, *J* = 7.0 Hz), 1.16 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 194.97, 175.44, 170.97, 170.82, 155.98, 138 81, 138.28, 136.43, 131.69, 130.88, 129.92, 128.54, 127.21, 110.27, 98.66, 90.58, 88.40, 85.53, 83.46, 79.56, 79.26, 76.24, 74.21, 73.30, 72.47, 70.31, 70.12, 69.26, 67.67, 59.16, 48.94, 41.63, 40.33, 37.11, 32.23, 29.17, 28.37, 7.28; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{46}H_{54}O_{15}N$ 860.3493found 860.3527.

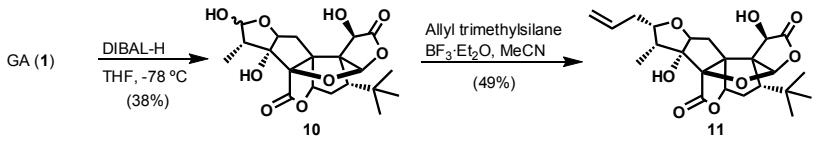


9b 4b

Synthesis of 4b

9b (8.9 mg, 10.3 μmol) was dissolved in MeOH (1.0 ml) and AcCl (100 μl) was added at 0 °C under nitrogen atmosphere. After stirring for three hours at room temperature, the solvent was removed under vacuum and the residue was dissolved in DMF (1.0 ml). To this solution, TEA (20 μl) and *N*-hydroxysuccinimide biotin (12.1 mg, 35.4 μmol) were added and the stirring continued for three hours. Next, water (10ml) was added and extracted with EtOAc (5 ml, 3x). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 10/1) to obtain **4b** (7.5 mg, 74%).

4b. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.80 (2H, d, *J* = 8.3 Hz), 7.77 (2H, d, *J* = 8.5 Hz), 7.60 (2H, d, *J* = 8.5 Hz), 7.59 (2H, d, *J* = 8.3 Hz), 6.15 (1H, s), 5.58 (1H, d, *J* = 11.5 Hz), 5.40 (1H, d, *J* = 4.0 Hz), 5.27 (1H, s), 4.84 (1H, d, *J* = 11.5 Hz), 4.54 (1H, d, *J* = 7.2 Hz), 4.48 (2H, s), 4.47 (1H, dd, *J* = 5.0, 8.0 Hz), 4.30 (1H, d, *J* = 7.2 Hz), 4.28 (1H, dd, *J* = 4.4, 8.0 Hz), 3.77 (2H, m), 3.68 (2H, m), 3.57 (2H, t, *J* = 5.5 Hz), 3.38 (2H, t, *J* = 5.5 Hz), 3.17 (1H, dt, *J* = 4.4, 9.0 Hz), 3.05 (1H, q, *J* = 7.0 Hz), 2.90 (1H, dd, *J* = 5.0, 12.7 Hz), 2.68 (1H, d, *J* = 12.7 Hz), 2.26 (1H, dd, *J* = 4.4, 13.6 Hz), 2.21 (2H, t, *J* = 7.5 Hz), 2.06 (1H, ddd, *J* = 4.0, 13.6, 14.2 Hz), 1.91 (1H, dd, *J* = 4.4, 14.2 Hz), 1.76-1.53 (4H, m), 1.41 (2H, t, *J* = 7.8 Hz), 1.23 (3H, d, *J* = 7.0 Hz), 1.13 (9H, s); ^{13}C NMR (75 MHz, CD_3OD): δ = 197.01, 178.32, 176.16, 173.68, 172.51, 166.09, 142.80, 138.51, 138.32, 132.72, 131.47, 131.13, 129.06, 128.42, 111.84, 100.81, 94.15, 89.54, 86.32, 84.55, 80.47, 77.79, 75.49, 73.83, 73.62, 71.15, 70.65, 70.52, 70.51, 69.24, 63.36, 61.64, 59.79, 56.98, 50.66, 43.29, 41.05, 40.34, 38.27, 36.76, 33.18, 29.74, 29.45, 26.84, 8.23; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{51}H_{60}O_{15}N_3S$ 986.3745, found 986.3780.

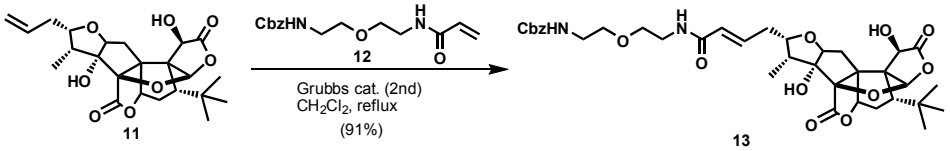


Synthesis of **11**

To a solution of GA **1** (1.00 g, 2.46 mmol) in THF (50 m;), 10 m; of DIBAL-H (1 M solution in hexane) was added dropwise at -78 °C under argon atmosphere. After stirring for two hours at -78 °C, 1 M HCl (10 m;) was added and the resulting solution was extracted with EtOAc (20ml, 3x). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuum. The residue was purified by column chromatography (30-50% acetone/hexane) to obtain **10** as a mixture of epimers (387.6 mg, 38 %) and recover **1** (644.9 mg, 62%).

10 (561.7 mg, 1.37 mmol) and allyltrimethylsilane (1.3 ml, 8.18 mmol) was dissolved in MeCN (10 ml) and $BF_3{\cdot}Et_2O$ (0.5 mL, 4.05 mmol) was added at 0 °C under nitrogen atmosphere. After stirring for 2.5 hours at room temperature, saturated $NaHCO_3$ solution (10ml) was added and the mixture was extracted with EtOAc (20ml, 3x). Organic layer was washed with brine, dried over Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography (30-50% acetone/hexane) to obtain **11** (292.7 mg, 49 %) as a mixture of epimers and recovered **10** (291.4 mg, 51%). Epimeric mixture of 11 was further purified by column chromatography (1-5 % acetone/$CHCl_3$) to obtain 11 (268.1 mg, 44%) and epi-11 (24.2 mg, 5%).

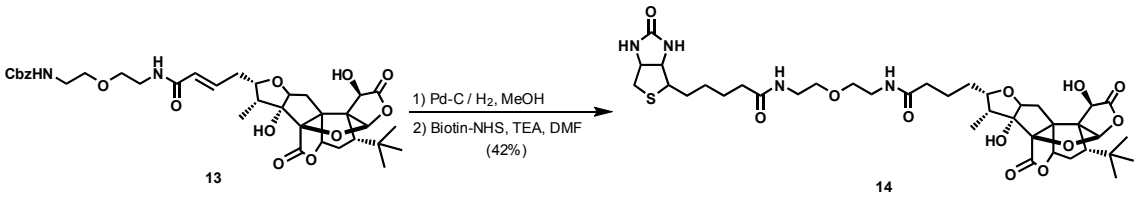
11. ^{1}H NMR (400 MHz, CD_3OD): δ = 5.98 (1H, s), 5.83 (1H, tdd, *J* = 6.8, 10.4, 17.2 Hz), 5.05 (1H, tdd, *J* = 1.8, 1.5, 17.2 Hz), 4.98 (1H, tdd, *J* = 2.0, 1.5, 10.4 Hz), 4.97 (1H, s), 4.75 (1H, d, *J* = 3.6 Hz), 4.49 (1H, dd, *J* = 7.2, 8.0 Hz), 4.34 (1H, ddd, *J* = 3.8, 8.4, 10.0 Hz), 2.90 (1H, dq, *J* = 8.4, 7.2 Hz), 2.45 (1H, d, *J* = 7.2, 15.0 Hz), 2.38 (1H, ddddd, *J* = 1.8, 2.0, 6.8, 8.4, 16.2 Hz), 2.22-2.09 (3H, m), 2.00 (1H, dd, *J* = 8.0, 15.0 Hz), 1.86 (1H, dd, *J* = 5.2, 15.0 Hz), 1.09 (9H, s), 1.05 (3H, d, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ = 176.14, 174.73, 137.25, 116.50, 111.66, 103.19, 91.45, 90.84, 88.12, 85.26, 70.47, 70.31, 68.39, 50.54, 41.71, 38.80, 37.97, 37.31, 33.26, 29.56, 8.43; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{23}H_{31}O_8$ 435.2019, found 435.2053.



Synthesis of **13**

To a solution of **11** (69.9 mg, 0.161 mmol) and **12** (94.3 mg, 0.323 mmol) in dichloromethane (12 ml), Grubbs' second-generation catalyst (7.2 mg, 8.5 μmol) was added and refluxed for 11 hours under nitrogen atmosphere. The reaction mixture was concentrated in vacuo and purified by column chromatography (50-75% ethyl acetate / hexane) to obtain **13** (102.3 mg, 90%).

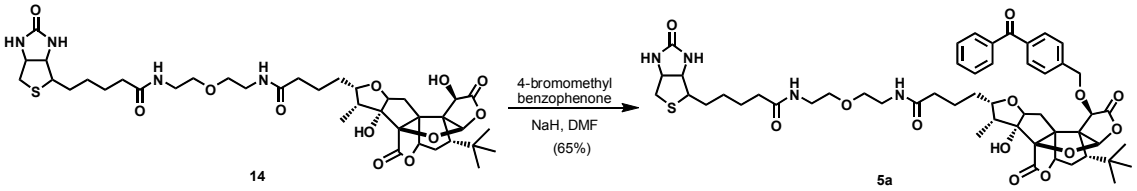
13. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.36-7.27 (5H, m), 6.78 (1H, dt, *J* = 15.5, 6.6 Hz), 6.05 (1H, d, *J* = 15.5 Hz), 5.99 (1H, s), 5.08 (2H, s), 4.99 (1H, s), 4.73 (1H, d, *J* = 3.4 Hz), 4.47 (1H, dd, *J* = 7.0, 7.0 Hz), 4.46-4.40 (1H, m), 3.52 (2H, t, *J* = 5.5 Hz), 3.50 (2H, t, *J* = 5.4 Hz), 3.40 (2H, t, *J* = 5.5 Hz), 3.30 (2H, m), 2.90 (1H, dq, *J* = 7.4, 7.2 Hz), 2.50 (1H, ddd, *J* = 6.6, 9.4, 14.2 Hz), 2.44 (1H, dd, *J* = 7.0, 15.2 Hz), 2.28-20.5 (4H, m), 1.86 (1H, dd, *J* = 5.1, 13.5 Hz), 1.09 (9H, s), 1.04 (3H, d, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ = 176.29, 174.75, 168.81, 158.99, 142.99, 138.39, 129.49, 128.98, 128.76, 125.95, 111.65, 103.44, 91.33, 90.88, 88.32, 84.11, 70.78, 70.56, 70.49, 70.41, 68.46, 67.43, 50.60, 41.76, 41.72, 40.27, 38.99, 37.29, 36.07, 33.28, 29.57, 8.67; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{36}H_{47}O_{12}N_2$ 699.3129, found 699.3146.



Synthesis of **14**

To a solution of **13** (24.1 mg, 34.5 μmol) in methanol (2 ml), 10% Pd/C was added and the mixture was stirred for 9 hours under hydrogen atmosphere. The reaction mixture was filtered through a pad of celite and concentrated in vacuo. The residue was dissolved in DMF (1 ml), TEA (10 μl) and N-hydroxysuccinimide biotin (21.8 mg, 63.9 μmol) were added. After stirring for 3 hours under nitrogen atmosphere, the resulting mixture was dried under vacuum, purified by preparative TLC ($CHCl_3$/ MeOH = 5:1) to obtain **14** (11.6 mg, 42%).

14. ^{1}H NMR (400 MHz, CD_3OD): δ = 5.99 (1H, s), 4.99 (1H, s), 4.76 (1H, d, *J* = 3.5 Hz), 4.50 (1H, dd, *J* = 5.0, 7.8 Hz), 4.46 (1H, dd, *J* = 7.2, 7.2 Hz), 4.31 (1H, dd, *J* = 4.7, 7.8 Hz), 4.33-4.28 (1H, m), 3.53-3.49 (4H, m), 3.37-3.33 (4H, m), 3.21 (1H, dt, *J* = 4.7, 5.7 Hz), 2.93 (1H, dd, *J* = 5.0, 12.7 Hz), 2.86 (1H, dq, *J* = 7.4, 7.2 Hz), 2.70 (1H, d, *J* = 12.7 Hz), 2.46 (1H, dd, *J* = 7.0, 15.2 Hz), 2.30-2.18 (5H, m), 2.15-2.06 (3H, m), 1.86 (1H, dd, *J* = 5.1, 13.7 Hz), 1.78-1.55 (8H, m), 1.44 (2H, t, *J* = 7.6 Hz), 1.10 (9H, s), 1.01 (3H, d, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ = 176.47, 176.31, 176.24, 174.88, 166.11, 111.64, 103.61, 91.23, 91.05, 88.35, 84.96, 70.60, 70.50, 70.48, 70.38, 68.45, 63.37, 61.64, 57.00, 50.64, 41.66, 41.05, 40.30, 40.23, 39.03, 37.32, 36.79, 36.75, 33.29, 32.44, 29.77, 29.58, 29.51, 26.86, 24.26, 8.62; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{38}H_{57}O_{12}N_4S$ 793.3694, found 793.3685.

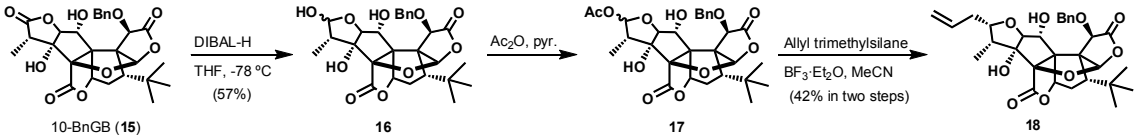


Synthesis of **5a**

To a solution of **14** (9.8 mg, 12.4 μmol) and 4-bromomethylbenzophenone (15.0 mg, 54.5 μmol) in DMF (0.5 mL), NaH (1.4 mg) were added at 0 ºC under nitrogen atmosphere. After stirring for one hour, the reaction mixture was quenched by AcOH (0.1 ml) and the resulting mixture was dried under vacuum. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 10/1) to obtain **5a** (8.0 mg, 65%).

5a. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.81 (2H, d, *J* = 8.2 Hz), 7.80 (2H, d, *J* = 7.8 Hz), 7.61 (1H, t, *J* = 7.4 Hz), 7.50 (2H, dd, *J* = 7.4, 7.8 Hz), 7.41 (2H, d, *J* = 8.2 Hz), 6.78 (1H, t, *J* = 5.4 Hz, -NH), 6.71 (1H, t, *J* = 5.2 Hz, -NH), 6.17 (1H, s, -NH), 5.98 (1H, s), 5.57 (1H, d, *J* = 11.2 Hz), 5.45 (1H, s, -NH), 4.81 (1H, s), 4.69 (1H, d, *J* = 4.0 Hz), 4.61 (1H, d, *J* = 11.2 Hz), 4.53-4.48 (2H, m), 4.36 (1H, dt, *J* = 2.8, 10.6 Hz), 4.30 (1H, dd, *J* = 4.8, 7.2 Hz), 4.15 (1H, s, -OH), 3.55-3.51 (4H, m), 3.46-3.34 (4H, m), 3.13 (1H, dt, *J* = 4.8, 7.2 Hz), 2.91 (1H, dq, *J* = 7.1, 7.1 Hz), 2.89 (1H, dd, *J* = 4.8, 12.8 Hz), 2.71 (1H, d, *J* = 12.8 Hz), 2.42 (1H, dd, *J* = 7.0, 15.0 Hz), 2.29-2.20 (4H, m), 2.17 (1H, dd, *J* = 4.8, 13.8 Hz), 2.11 (1H, dd, *J* = 8.4, 15.0), 2.08 (1H, ddd, *J* = 4.0, 13.8, 13.8 Hz), 1.92 (1H, dd, *J* = 4.8, 13.8 Hz), 1.75-1.59 (8H, m), 1.43 (2H, tt, *J* = 7.5, 7.5 Hz), 1.09 (9H, s), 1.06 (3H, d, *J* = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 196.22, 173.81, 173.66, 172.97, 172.16, 163.79, 140.91, 137.47, 137.35, 132.63, 130.38, 130.03, 128.38, 127.40, 110.12, 101.77, 89.82, 89.81, 86.67, 84.45, 76.05, 71.95, 69.84, 69.57, 69.15, 66.85, 63.72, 61.78, 60.18, 55.46, 48.94, 40.52, 39.12, 38.96,

38.13, 36.47, 36.36, 35.52, 32.24, 31.22, 29.19, 27.93, 27.84, 25.43, 22.98, 7.88; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{52}H_{67}O_{13}N_4S$ 987.4425, found 987.4427.



10-BnGB (**15**) **16** **17** **18**

Synthesis of **18**

To a solution of 10-BnGB **15** (1.66 g, 3.22 mmol) in THF (60 ml), 13 ml of DIBAL-H (solution in hexane) was added dropwise at -78 ºC under argon atmosphere. After stirring for three hours at -78 ºC, 1 M HCl (13 ml) was added and the resulting solution was deluted by H_2O (200 ml) and extracted with EtOAc (60ml, 3x). The organic layer was washed with brine, dried over Na_2SO_4, and the volatiles removed in vacuum. The residue was purified by column chromatography (25-50% acetone/hexane) to obtain **16** (953.9 mg, 57 %) as a mixture of epimers and recover **15** (91.9 mg, 6%).

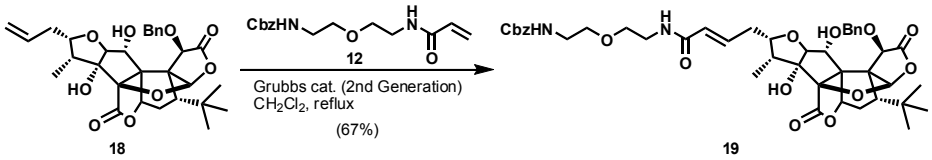
Compound **16** (774.9 mg, 1.50 mmol) was then dissolved in pyridine (15 mL) and acetic anhydride (0.6 mL, 6.35 mmol) was added at 0 ºC under nitrogen atmosphere. After 4 hours, EtOAc (60ml) was added and the reaction mixture was washed with 1M HCl (30ml, 3x), brine and then dried over Na_2SO_4. Solvent was removed in vacuo to obtain **17**, which was directly dissolved in MeCN (10 ml), then allyltrimethylsilane (1.0 ml, 6.29 mmol) was added and the mixture was cooled to 0 ºC. To this solution, $BF_3 \cdot Et_2O$ (0.31 mL, 2.51 mmol) was added under nitrogen atmosphere and the reaction mixture was allowed to warm to room temperature. After stirring for 20 hours, saturated $NaHCO_3$ (30 ml) was added and the mixture was extracted with EtOAc (15 ml, 3x). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography (25-50% acetone/hexane) to obtain **18** (340.5 mg, 42 %) and **16** (329.5 mg, 43%).

16. Mixture of epimers. HRMS (FAB+): $[M+H]^+$ *m/z/* calcd for $C_{27}H_{33}O_{10}$ requires *m/z* 517.2074, found 517.2072.

17. Mixture of epimers. HRMS (FAB+): $[M+Na]^+$ *m/z* calcd for $C_{29}H_{34}O_{11}Na$ 581.1984, found 581.1984.

18. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.42-7.33 (5H, m), 5.98 (1H, s), 5.87 (1H, dddd, *J* = 7.0, 7.0, 10.2, 17.2 Hz), 5.50 (1H, d, *J* = 9.5 Hz), 5.28 (1H, d, *J* = 3.4 Hz), 5.07 (1H, dd, *J* = 1.8, 17.2 Hz), 5.04 (1H, dd, *J* = 1.8, 10.2 Hz), 4.88 (1H, s), 4.58 (1H, d, *J* = 9.5 Hz), 4.45 (1H, ddd, *J* = 3.6, 9.0, 9.8 Hz), 4.34 (1H, d, *J* = 7.6 Hz), 4.20 (1H, dd, *J* = 3.2, 7.6 Hz), 2.82 (1H, dq, *J* = 7.0, 9.0 Hz), 2.76 (1H, d, *J* = 3.2 Hz, -OH), 2.60 (1H, s), 2.40 (1H, ddd, *J* = 7.0,

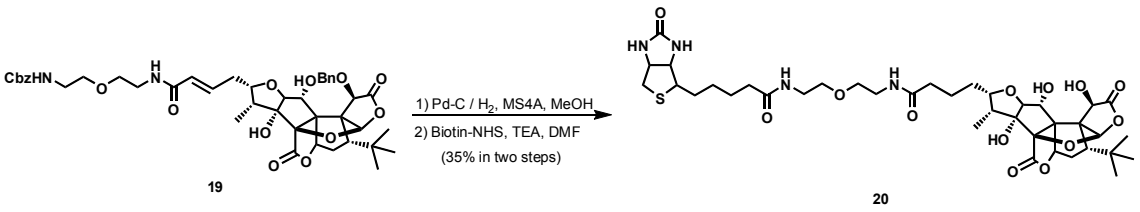
9.8, 14.3 Hz), 2.23 (1H, broad d, *J* = 10.5 Hz), 2.14 (1H, ddd, *J* = 3.6, 7.0, 14.3 Hz), 1.92 (1H, broad d, *J* = 14.0 Hz), 1.87 (1H, ddd, *J* = 3.4, 10.5, 14.0 Hz), 1.13 (9H, s), 1.08 (3H, d, *J* = 7.0 Hz); HRMS (FAB-): $[M-H]^-$ *m/z* calcd for $C_{30}H_{35}O_9$ 539.2281, found 539.2301.



Synthesis of **19**

To a solution of **18** (174.3 mg, 0.323 mmol) and **12** (236.6 mg, 0.809 mmol) in dichloromethane (15 ml), Grubbs' second-generation catalyst (12.5 mg, 14.7 μmol) was added and the mixture refluxed for 16 hours under nitrogen atmosphere. The reaction mixture was concentrated in vacuo and the residue purified by column chromatography (40-60% acetone / hexane) to obtain **19** (174.9 mg, 67%).

19. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40-7.31 (10H, m), 6.77 (1H, ddd, *J* = 7.0, 7.0, 15.5 Hz), 6.03 (1H, t, *J* = 5.0 Hz, -NH), 5.98 (1H, s), 5.84 (1H, d, *J* = 15.5 Hz), 5.50 (1H, d, *J* = 9.5 Hz), 5.26 (1H, d, *J* = 3.8 Hz), 5.10 (2H, s), 4.88 (1H, s), 4.58 (1H, d, *J* = 9.5 Hz), 4.48 (1H, ddd, *J* = 2.4, 8.2, 9.0 Hz), 4.33 (1H, d, *J* = 7.5 Hz), 4.18 (1H, dd, *J* = 3.0, 7.5 Hz), 3.54-3.49 (4H, m), 3.46 (2H, t, *J* = 5.0 Hz), 3.38 (2H, dt, *J* = 5.0, 5.0 Hz), 2.84 (1H, dq, *J* = 7.0, 9.0 Hz), 2.78 (1H, d, *J* = 3.0 Hz, -OH), 2.51 (1H, ddd, *J* = 7.0, 9.0, 14.0 Hz), 2.23 (1H, d, *J* = 9.3 Hz), 2.22-2.17 (1H, m), 1.91 (1H, d, *J* = 14.0 Hz), 1.87 (1H, ddd, *J* = 3.8, 9.3, 14.0 Hz), 1.12 (9H, s), 1.06 (3H, d, *J* = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.28, 171.38, 165.98, 156.57, 140.96, 136.50, 134.73, 129.44, 129.26, 128.77, 128.51, 128.11, 128.09, 125.61, 110.15, 99.11, 93.76, 85.87, 83.20, 79.80, 76.13, 74.66, 73.88, 72.41, 69.86, 69.71, 67.29, 66.70, 48.87, 41.82, 40.80, 39.07, 36.89, 35.21, 32.18, 29.18, 7.55; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{43}H_{53}O_{13}N_2$ 805.3548, found 805.3546.

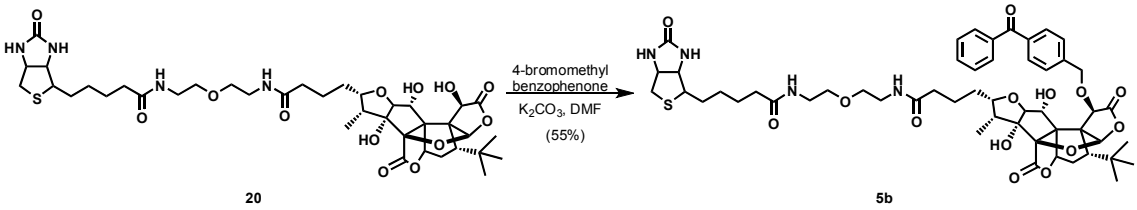


Synthesis of **20**

To a solution of **19** (17.6 mg, 21.9 μmol) in methanol (2 ml), powdered MS4Å and 10% Pd/C was added and stirred for 24 hours under hydrogen atmosphere (balloon). The

reaction mixture was filtered through a pad of celite and concentrated in vacuo. The residue was dissolved in DMF (2 ml) and TEA (10 µl), N-hydroxysuccinimide biotin (15.2 mg, 44.5 µmol) was added. After stirring for 5 hours under nitrogen atmosphere, the resulting mixture was dried under vacuum and purified by preparative TLC ($CHCl_3$/ MeOH = 5:1) to obtain **20** (6.2 mg, 35%).

20. ^{1}H NMR (400 MHz, CD_3OD): δ = 6.03 (1H, s), 5.30 (1H, d, *J* = 4.0 Hz), 5.06 (1H, s), 4.50 (1H, dd, *J* = 5.0, 8.0 Hz), 4.31 (1H, dd, *J* = 4.5, 8.0 Hz), 4.29 (1H, d, *J* = 7.5 Hz), 4.28 (1H, m), 4.10 (1H, d, *J* = 7.5 Hz), 3.53-3.50 (4H, m), 3.37-3.33 (4H, m), 3.21 (1H, dt, *J* = 4.5, 10.2 Hz), 2.93 (1H, dd, *J* = 5.0, 12.8 Hz), 2.77 (1H, dq, *J* = 8.3, 7.0 Hz), 2.70 (1H, d, *J* = 12.8 Hz), 2.26-2.18 (5H, m), 2.04 (1H, ddd, *J* = 4.0, 13.6, 14.1 Hz), 1.86 (1H, dd, *J* = 4.7, 14.1 Hz), 1.82-1.55 (8H, m), 1.44 (2H, t, *J* = 7.6 Hz), 1.10 (9H, s), 1.03 (3H, d, *J* = 7.0 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ = 176.46, 176.31, 175.50, 174.06, 166.12, 111.83, 100.97, 95.65, 87.22, 85.40, 80.90, 75.76, 73.50, 70.86, 70.47, 70.43, 68.71, 63.48, 61.77, 57.00, 50.43, 43.23, 41.04, 40.34, 40.30, 38.13, 36.81, 36.76, 33.32, 32.78, 29.76, 29.55, 29.51, 26.86, 24.16, 8.17; HRMS (FAB+): $[M+H]^+$ *m/z* calcd for $C_{38}H_{57}O_{13}N_4S$ 809.3643, found 809.3634.



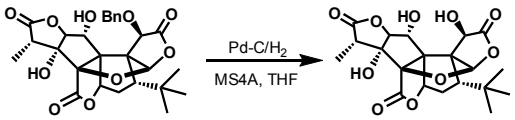
20 **5b**

Synthesis of **5b**

To solution of **20** (5.6 mg, 6.9 µmol) and 4-bromomethylbenzophenone (6.5 mg, 23.6 µmol) in DMF (0.5 ml), K_2CO_3 (3.5 mg, 25.4 µmol) was added at 0 °C under nitrogen atmosphere. After stirring for six hours at room temperature, the reaction mixture was quenched with AcOH (0.2 ml) and the resulting mixture was dried in vacuum. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 10/1) to obtain **5b** (3.8 mg, 55%) as a white solid.

5b. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.82 (2H, d, *J* = 8.0 Hz), 7.77 (2H, d, *J* = 8.0 Hz), 7.66 (1H, t, *J* = 8.0 Hz), 7.59 (2H, d, *J* = 8.0 Hz), 7.54 (2H, t, *J* = 8.0 Hz),6.10 (1H, s), 5.57 (1H, d, *J* = 11.0 Hz), 5.29 (1H, d, *J* = 4.0 Hz), 5.26 (1H, s), 4.85 (1H, d, *J* = 11.0 Hz), 4.47

(1H, dd, J = 5.0, 7.3 Hz), 4.29-4.25 (2H, m), 4.23 (1H, d, J = 7.0 Hz), 4.19 (1H, d, J = 7.0 Hz), 3.55-3.45 (4H, m), 3.35-3.30 (4H, m), 3.19 (1H, dt, J = 4.2, 9.0 Hz), 2.91 (1H, dd, J = 5.0, 12.8 Hz), 2.79 (1H, dq, J = 3.0, 7.0 Hz), 2.68 (1H, d, J = 12.8 Hz), 2.28-2.19 (5H, m), 2.00 (1H, ddd, J = 4.0, 4.0, 13.8), 1.89 (1H, dd, J = 4.0, 14.3 Hz), 1.74-1.53 (8H, m), 1.42 (2H, tt, J = 7.5, 7.5 Hz), 1.14 (9H, s), 1.04 (3H, d, J = 7.0 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ = 197.99, 176.39, 176.24, 173.90, 173.86, 166.10, 142.35, 139.04, 138.69, 133.96, 131.63, 131.03, 129.60, 129.33, 111.81, 101.58, 96.06, 87.20, 85.47, 85.35, 80.59, 77.88, 76.02, 73.95, 73.70, 70.48, 70.44, 68.69, 63.37, 61.64, 57.00, 50.63, 43.19, 41.06, 40.30, 40.25, 38.24, 36.81, 36.76, 33.16, 32.64, 29.75, 29.51, 26.84, 24.14, 8.21; HRMS (FAB+): $[M+H]^+$ m/z calcd for $C_{52}H_{67}O_{14}N_4S$ 1003.4375, found m/z 1003.4391.



10-BnGB (**15**) GB (**2**)

Debenzylation of 10-BnGB (**15**) to GB (**2**)

To a solution of **15** (2.54 g, 4.94 mmol) in THF (100 ml), powdered MS4Å (1.0g) and 10% Pd/C was added and stirred for 48 hours under hydrogen atmosphere (balloon). The reaction mixture was filtered through a pad of celite and concentrated in vacuo. The residue was recrystallized from MeOH to obtain **2** (2.03 g, 97%) whose spectral properties were identical to the authentic sample of GB.