Supplemental Information

Synthesis of novel ginkgolide photoaffinity-biotin probes
Eisuke Kato, Rachel Howitt, Sergei V. Dzyuba and Koji Nakanishi

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 I₂). 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 CDCl₃) 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.

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 °C 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 Na₂SO₄ and evaporated to dryness. The residue was purified by column chromatography (20-50% acetone / hexane) to obtain 7a (105.5 mg, 69%).

7a. 1H NMR (400 MHz, CDCl₃): 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); 13C NMR (75 MHz, CDCl₃): 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 C₃₄H₃₄O₁₀Br 681.1335, found 681.1334.

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, PdCl₂(PPh₃)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. ¹H NMR (400 MHz, CDCl₃): 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); ¹³C NMR (75 MHz, CDCl₃): 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, 70.74, 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 C₄₆H₅₄O₁₄N 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 Na2SO4 and concentrated in vacuo. The residue was purified by preparative TLC (CHCl3/MeOH = 10/1) to obtain 4a (34.0 mg, 75%).

4a. 1H NMR (400 MHz, CDCl3): 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, d, 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);

13C NMR (75 MHz, CDCl3): 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 C51H60O14N3S 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 °C 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 Na2SO4 and evaporated in vacuum. The residue was purified by column chromatography (25-40% acetone / hexane) to obtain 7b (70.4 mg, 70%).

7b. 1H 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); 13C 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 C34H34O11Br 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, PdCl2(PPh3)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. 1H NMR (400 MHz, CDCl3): 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); 13C NMR (75 MHz, CDCl₃): 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, 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 C₄₆H₅₄O₁₅N₈ 860.3493 found 860.3527.

**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 Na₂SO₄ and concentrated in vacuum. The residue was purified by preparative TLC (CHCl₃/MeOH = 10/1) to obtain 4b (7.5 mg, 74%).

**4b.** 1H NMR (400 MHz, CD₃OD): 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); 13C NMR (75 MHz, CD₃OD): 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 C₅₁H₆₀O₁₅N₃S 986.3745, found 986.3780.
Synthesis of 11

To a solution of GA 1 (1.00 g, 2.46 mmol) in THF (50 ml), 10 ml of DIBAL-H (1 M solution in hexane) was added dropwise at -78 ºC under argon atmosphere. After stirring for two hours at -78 ºC, 1 M HCl (10 ml) was added and the resulting solution was extracted with EtOAc (20 ml, 3x). The organic layer was washed with brine, dried over Na₂SO₄, 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 BF₃·Et₂O (0.5 ml, 4.05 mmol) was added at 0 ºC under nitrogen atmosphere. After stirring for 2.5 hours at room temperature, saturated NaHCO₃ solution (10 ml) was added and the mixture was extracted with EtOAc (20 ml, 3x). Organic layer was washed with brine, dried over Na₂SO₄ 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/CHCl₃) to obtain 11 (268.1 mg, 44%) and epi-11 (24.2 mg, 5%).

11. ¹H NMR (400 MHz, CD₃OD): 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); ¹³C NMR (75 MHz, CD₃OD): 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 C₂₃H₃₁O₈ 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. 1H NMR (400 MHz, CD3OD): \( \delta = 7.36-7.27 \) (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, 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); 13C NMR (75 MHz, CD3OD): \( \delta = 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 C36H47O12N2 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 (CHCl3/ MeOH = 5:1) to obtain 14 (11.6 mg, 42%).
14. 1H NMR (400 MHz, CD$_3$OD): $\delta$ = 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);

13C NMR (75 MHz, CD$_3$OD): $\delta$ = 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, 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$_4$S 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. 1H NMR (400 MHz, CD$_3$OD): $\delta$ = 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); 13C NMR (75 MHz, CDCl$_3$): $\delta$ = 196.22, 173.81, 173.66, 172.97, 172.16, 167.39, 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_{4}S 987.4425, found 987.4427.

O

O

O

O

HO

BnO

O

O

O

O

HO

BnO

O

O

O

O

HO

BnO

O

O

O

O

HO

AcO

BnO

O

Ac2O, pyr.

(42% in two steps)

HO

HO

HO

(57%)

Allyl trimethylsilane

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 H2O (200 ml) and extracted with EtOAc (60 ml, 3x). The organic layer was washed with brine, dried over Na2SO4, 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 (60 ml) was added and the reaction mixture was washed with 1M HCl (30 ml, 3x), brine and then dried over Na2SO4. 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, BF3·Et2O (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 NaHCO3 (30 ml) was added and the mixture was extracted with EtOAc (15 ml, 3x). The organic layer was washed with brine, dried over Na2SO4 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.


18. 1H NMR (400 MHz, CDCl3): 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. 1H NMR (400 MHz, CDCl3): 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); 13C NMR (75 MHz, CDCl3): 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 (CHCl₃/MeOH = 5:1) to obtain 20 (6.2 mg, 35%).

20. ¹H NMR (400 MHz, CD₃OD): 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); ¹³C NMR (75 MHz, CD₃OD): 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 C₃₈H₅₇O₁₃N₄S 809.3643, found 809.3634.

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), K₂CO₃ (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 (CHCl₃/MeOH = 10/1) to obtain 5b (3.8 mg, 55%) as a white solid.

5b. ¹H NMR (400 MHz, CD₃OD): 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), 5.40 (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); 13C NMR (75 MHz, CD3OD): 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 C52H67O14N4S 1003.4375, found m/z 1003.4391.

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 (1.0 g) 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.