Supporting Information

Design, Synthesis, and Evaluation of Biotinylated Opioid Derivatives as Novel Probes to Study Opioid Pharmacology

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General:

Unless otherwise mentioned, solvents and reagent were purchased from commercial sources and used as received. ^{1}H NMR spectra were recorded with Me_4Si as internal standard. ^{13}C NMR spectra were recorded on Varian INOVA 400 NMR spectrometers. High-resolution mass data were recorded on a high-resolution mass spectrometer in the ESI or MALDI mode.

Procedure for the substitution reaction of biotin (**5**) with 1,2-bis(2-iodoethoxy) ethane (**6**).

Under N_2 atmosphere, into a 15-mL Schlenk flask containing biotin (**5**) (244 mg, 1.0 mmol) in 5mL DMSO at 25°C, was added NaH (44 mg, 1.1 mmol, 60 % dispersion in mineral oil). The reaction mixture was stirred at this temperature for 10 min. Then 1,2-bis(2-iodoethoxy) ethane (**6**) (555 mg, 1.5 mmol) was added dropwise and the reaction mixture was stirred for another 14 h, followed by adding a half-saturated NH_4Cl-H_2O solution (10 mL) at this temperature. The solution mixture was extracted with EtOAc (25 mL x 3), and the combined organic phase was dried over $MgSO_4$. After the removal of volatile solvents under vacuum, the crude product was further purified by silica gel column chromatography with ethyl acetate/petroleum ether (1: 3) to give product **7** as a white solid, yield 65 % (318 mg). Characterization for compound **7**: $[\alpha]^{25}_D$ = +31.0 (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.81 (s, 1H); 5.23 (s, 1H); 4.52-4.54 (m, 1H); 4.32-4.35 (m, 1H); 4.23-4.28 (m, 2H); 3.78 (t, J = 7.0 Hz, 2H); 3.73 (t, J = 4.5 Hz, 2H); 3.69 (s, 4H); 3.29 (dd, J = 7.0, 6.0 Hz, 2H); 3.16-3.19 (m, 1H); 2.93 (dd, J = 12.5, 5.5 Hz, 1H); 2.76 (d, J = 12.5 Hz, 1H); 2.40 (t, J = 7.0 Hz, 2H); 1.67-1.76 (m, 4H); 1.45-1.50 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.9, 164.0, 72.1, 70.7, 70.4, 69.4, 63.6, 62.1, 60.3, 55.8, 40.8, 34.0, 28.5, 28.4, 24.9, 3.2; HRMS (MALDI) calcd. For $C_{16}H_{28}N_2O_5S$ ($M + H^+$): 487.0758, Found 487.0754.

Preparation of biotinylated (-)-morphine (**2**).

Under N_2 atmosphere, into a 15-mL Schlenk flask containing (-)-morphine (**1**) (29 mg, 0.1 mmol) in 2.0 mL DMSO at 25 ^oC, NaH was added (4.4 mg, 0.11 mmol). The reaction mixture was stirred at this temperature for 10 min. Then compound **7** (49 mg, 0.1 mmol) dissolved in 0.5 mL DMSO was added dropwise and the reaction mixture was stirred for another 12 h. Silica gel column chromatography with methanol/dichloromethane (10 : 1) afforded biotinalyted (-)-morphine (**2**) as an oil, yield 35 % (20 mg). Characterization for compound **2**: $[\alpha]^{25}_D$ = –24.5 (c = 1.25, $CHCl_3$); ^1H NMR (CD_3OD, 400 MHz) δ 6.69 (d, J = 8.2 Hz, 1H); 6.53 (d, J = 8.2 Hz, 1H); 5.62-5.66 (m, 1H); 5.32-5.36 (m, 1H), 4.83 (dd, J = 6.2, 1.2 Hz, 1H); 4.45-4.49 (m, 1H), 4.07-4.30 (m, 7H); 3.78-3.81 (m, 2H), 3.64-3.71 (m, 6H), 3.38-3.41 (m, 1H), 3.16-3.20 (m, 1H), 3.07 (d, J = 19.4 Hz, 1H), 2.91 (dd, J = 12.0, 4.0 Hz, 1H); 2.69 (d, J = 12.0 Hz, 1H), 2.66-2.67 (m, 1H), 2.59-2.63 (m, 1H), 2.44 (s, 3H); 2.39-2.43 (m, 1H), 2.34-2.38 (m, 3H); 2.09 (td, J = 7.5, 4.8 Hz, 1H), 1.80-1.83 (m, 1H), 1.53-1.75 (m, 4H), 1.40-1.48 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.9, 163.6, 147.2, 141.2, 133.6, 131.6, 128.5, 128.1, 119.7, 115.3, 91.7, 70.8, 70.7, 70.1, 69.4, 69.2, 66.7, 63.6, 62.0, 60.2, 59.0, 55.6, 46.6, 43.4, 43.1, 41.0, 40.7, 36.0, 33.9, 28.4, 28.3, 24.9, 20.5. ESI (m/z) 644.2 ($M + H^+$); HRMS (ESI) calc. For $C_{33}H_{46}N_3O_8S^+$ ($M + H^+$): 644.3000, Found 644.2970.

Procedure for AutoDock4 *in silico* docking

The TLR4 pdb file was obtained from RCSB database (ID 2z64) with binding partner MD-2, which was removed as were all ligands via Molegro Molecular Viewer. The modified pdb file was input into AutoDock 4.0, hydrogens added and resaved in pdbqt format. Morphine was obtained using PubChem isomeric SMILES then converted to pdb using a structure file generator (http://cactus.nci.nih.gov/services/translate/). The biotinylated morphine structure was drawn with ChemDraw Ultra 9.0, then converted into pdb format and bond angles optimized with Chem3D Ultra 9.0. In AutoDock, ligand torsion tree roots were generated automatically by choosing 'detect root', and number torsions set at zero for morphine; biotinylated morphine was allowed its natural rotations. Figures shown cover lowest energy results from left portion of receptor only, AutoGrid center set at (-44.725, 4.064, -0.446) with 126 grid points expanding each direction on TLR4, which was kept in its rigid form. The search parameters employed a genetic algorithm set for #GA 100, Max Evals $5x10^6$ and 0.375 Å spacing. All dockings executed with Lamarkian genetic algorithms on Apple desktop computers running OS X 10.4.

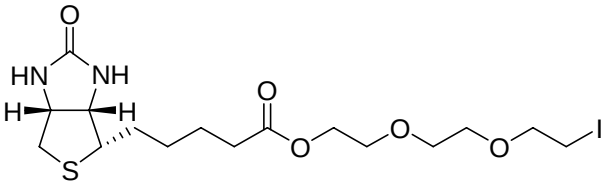
References

(a). Morris, G. M., Goodsell, D. S., Halliday, R.S., Huey, R., Hart, W. E., Belew, R. K. and Olson, A. J. (1998). "Automated Docking using a lamarckian genetic algorithm and an Empirical Binding Free energy function." *J. Comput. Chem.* **19**: 1639-1662.

(b). Molegro Molecular Viewer 1.1, Molegro ApS, Hoegh-Guldbergs Gade 10, Building 1090, DK-8000 Aarhus C, Denmark

(c), ChemOffice Ultra 2000: CambridgeSoft (2001). c/o CHEM Research GmbH, Frankfurt, DM 3,290

^{1}H NMR ($CDCl_3$, 400 MHz)



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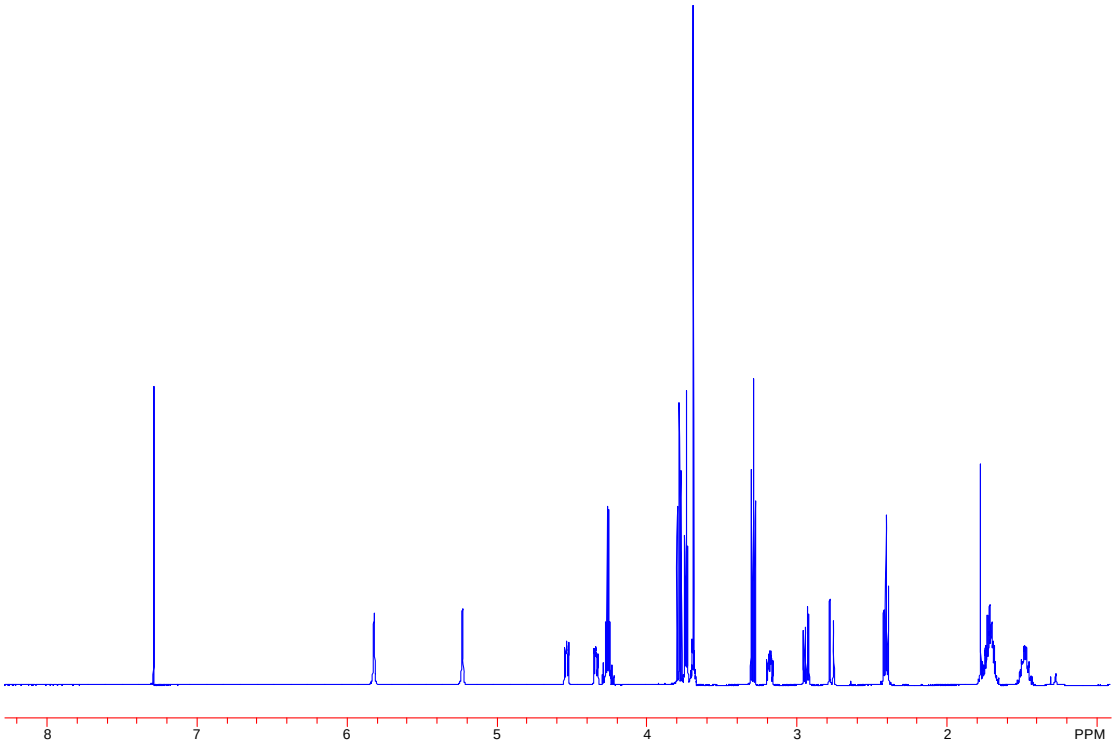

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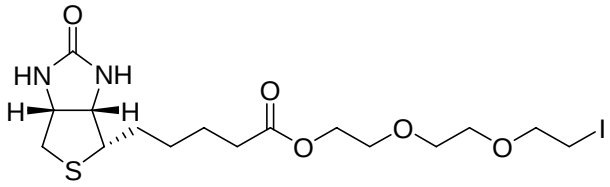

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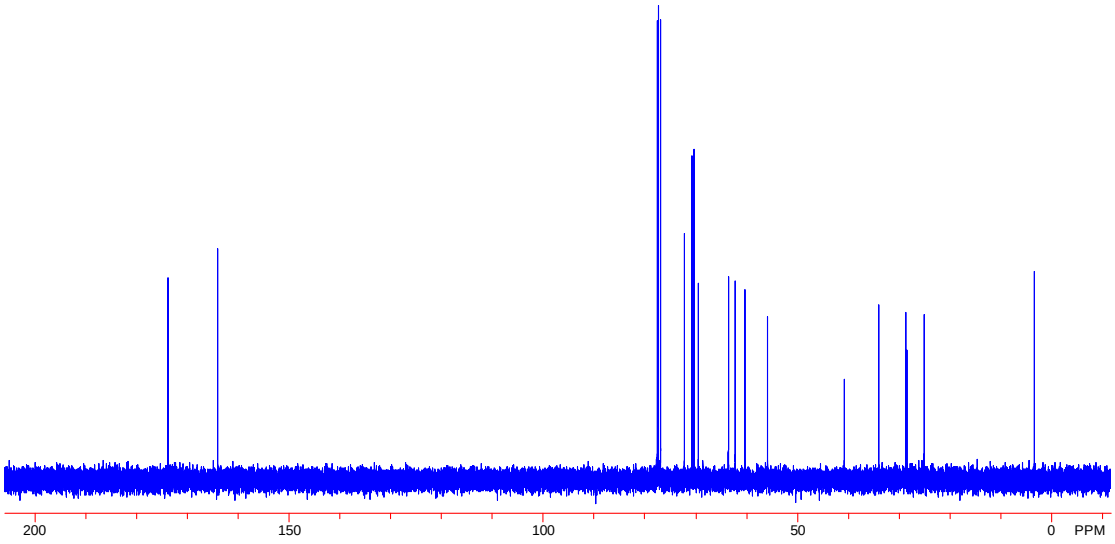

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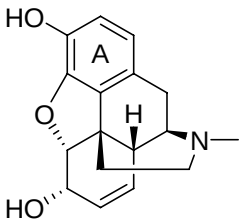
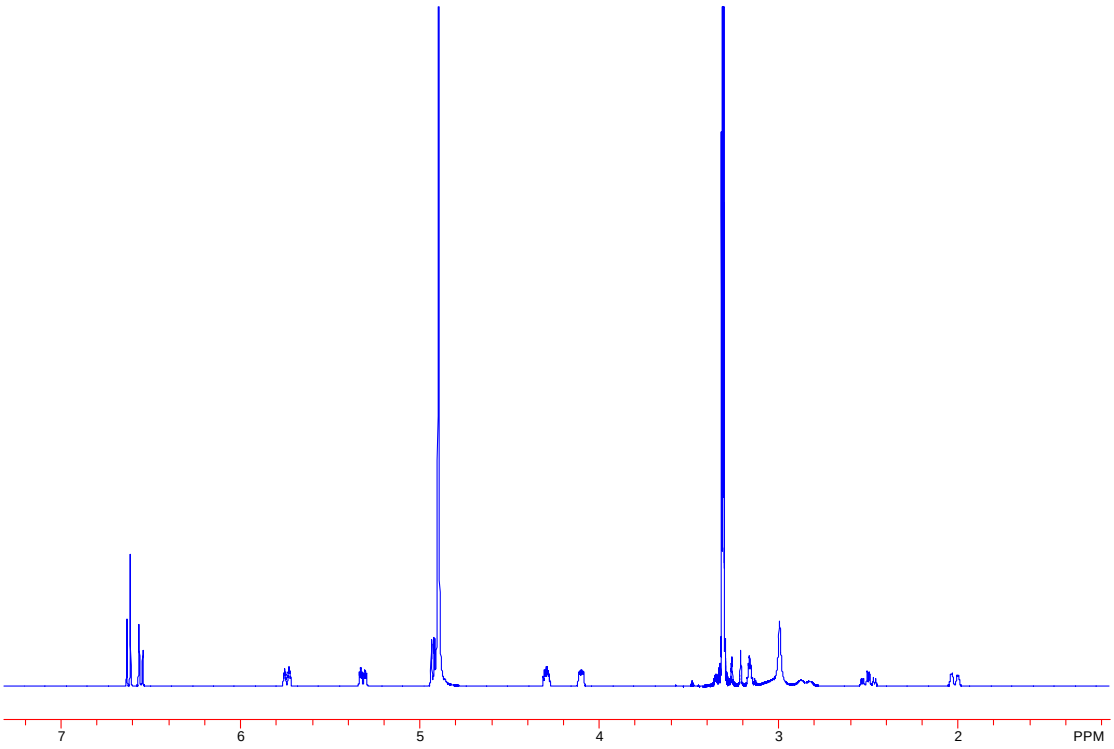


(-)-morphine **1**



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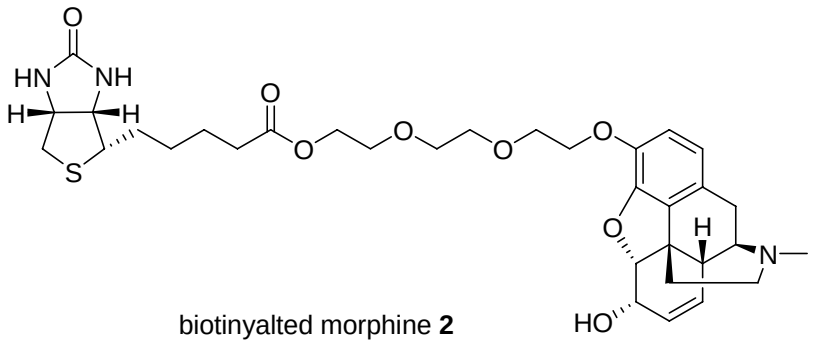
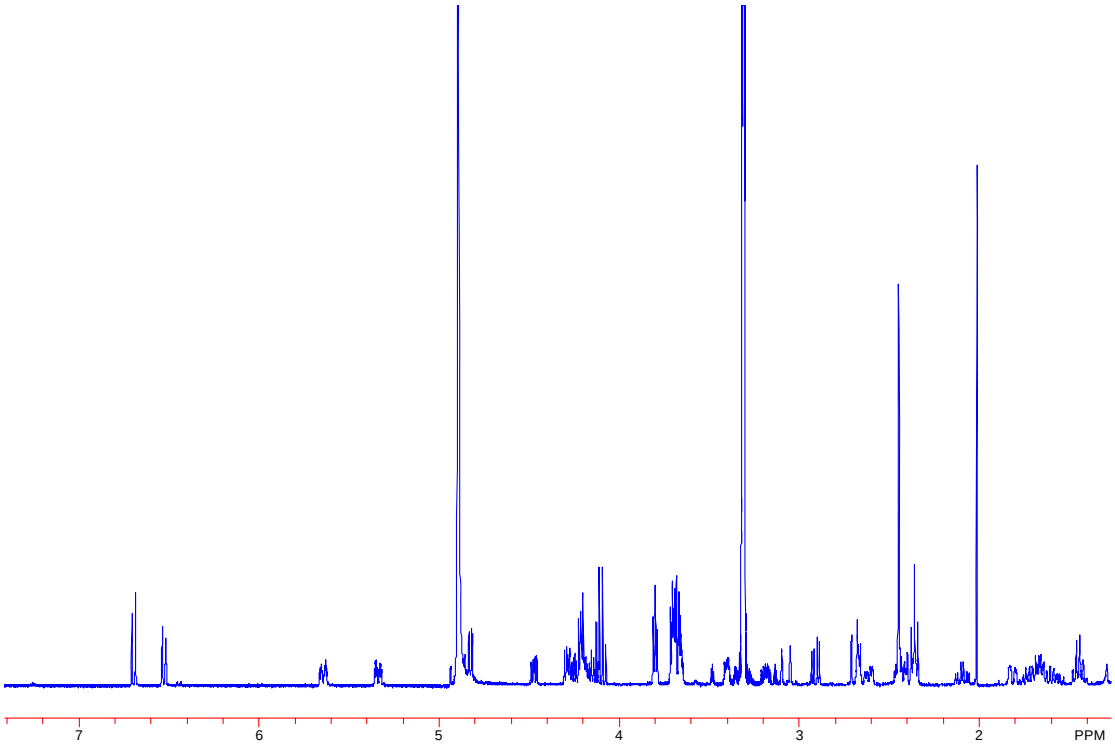
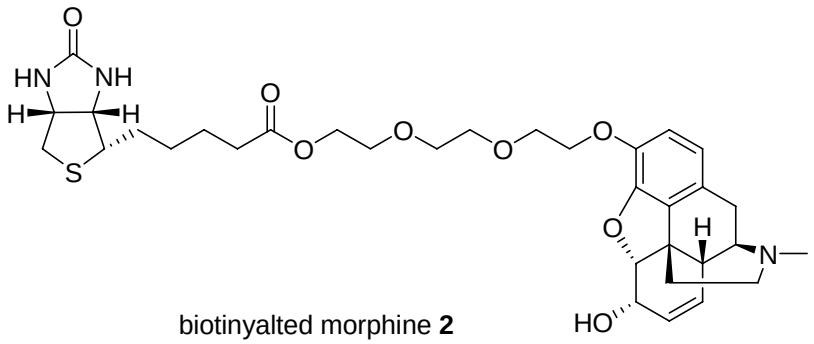


biotinyalted morphine **2**



^{13}C NMR ($CDCl_3$, 100 MHz)



biotinyalted morphine **2**