Supporting Information for

Branched α-D-mannopyranosides; a new class of potent FimH antagonists

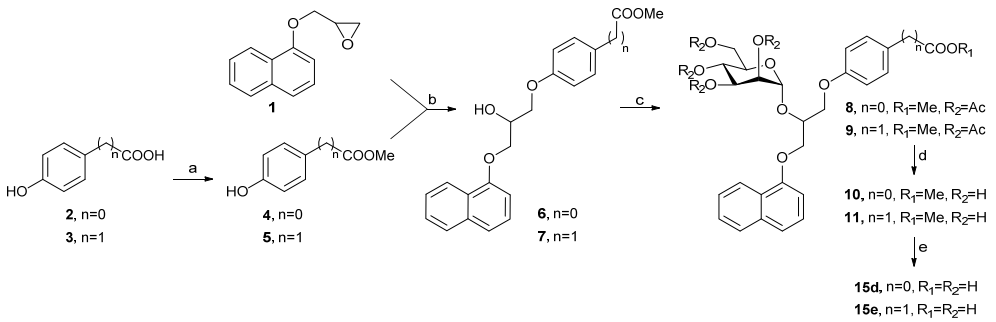
Tihomir Tomašić[a], Said Rabbani[b], Martina Gobec[a], Irena Mlinarič Raščan[a], Črtomir Podlipnik[c], Beat Ernst[b] and Marko Anderluh[a]

[a]University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, SI-1000 Ljubljana, Slovenia.
[b]Institute of Molecular Pharmacy, Pharmacenter, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland.
[c]University of Ljubljana, Faculty of Chemistry and Chemical Technology, Aškerčeva 5, SI-1000 Ljubljana, Slovenia.

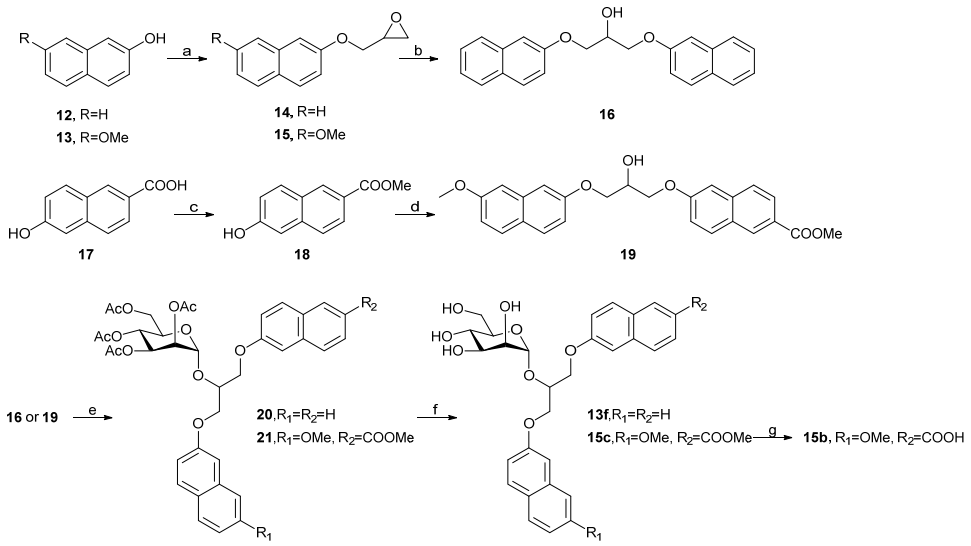
Contents:

1. Chemistry

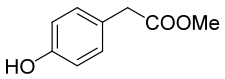
General. Dichloromethane was dried over calcium hydride and *N*,*N*-dimethylformamide over activated molecular sieves. All reagents were used as received from commercial sources without further purification unless otherwise indicated. Analytical TLC was performed on Merck silica gel (60 F 254) plates (0.25 mm) and components visualized with staining reagents or ultraviolet light. Flash column chromatography was carried out on silica gel 60 (particle size 230-400 mesh). Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a Bruker AVANCE III spectrometer in DMSO-d_6, $CDCl_3$ or CD_3OD solution, with TMS as internal standard at 25 °C. Spectra were assigned using gradient COSY, HSQC and DEPT experiments. IR spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR or Nicolet Nexus 470 FT-IR spectrometers. Mass spectra were obtained using a Autospec-Q VGAnalytical mass spectrometer. All reported yields refer to isolated purified products. The synthesis of compounds **12a-g**, **13a-e**, **14a-f** and **15a** is reported in Tomašić *et al*.[1]



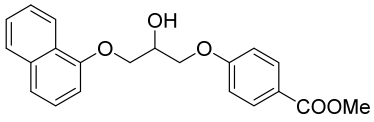
Scheme 1 *Reagents and conditions*: (a) $SOCl_2$, MeOH, reflux, 2 h; (b) (*i*) KOH, MeOH, r.t., 20 min; (*ii*) tetrabutylammonium bromide, toluene/*N*,*N*-dimethylformamide, 90-120 °C, 2-20 h; (c) 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate, TMSOTf, CH_2Cl_2, 0 °C, then r.t., 24 h; (d) NaOMe, MeOH, 1 h, then Amberlite® IR120 H, 15 min; (e) 2 M NaOH, 1,4-dioxane/MeOH, r.t., 24 h.



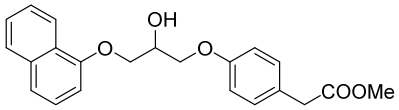
Scheme 2 *Reagents and conditions*: (a) epichlorohydrin, 60 °C, 30 min; (b) (*i*) **12**, KOH, MeOH, r.t., 20 min; (*ii*) tetrabutylammonium bromide, toluene/*N*,*N*-dimethylformamide, reflux, 48 h; (c) $SOCl_2$, MeOH, reflux, 2 h; (d) (*i*) KOH, MeOH, r.t., 20 min; (*ii*) tetrabutylammonium bromide, toluene/*N*,*N*-dimethylformamide, reflux, 48 h; (e) 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate, TMSOTf, CH_2Cl_2, 0 °C, then r.t., 24 h; (f) NaOMe, MeOH, 1 h, then Amberlite® IR120 H, 15 min; (g) 2 M NaOH, 1,4-dioxane/MeOH, r.t., 24 h.



Methyl 2-(4-hydroxyphenyl)acetate (**5**). A solution of 2-(4-hydroxyphenyl)acetic acid (**3**) (4.000 g, 26.3 mmol) in MeOH (10 mL, dried over molecular sieves) was cooled on an ice bath to 0 °C and put under argon. Thionyl chloride (2.10 mL, 28.9 mmol) was then added drop wise. Reaction mixture was stirred at room temperature for 2 h, cooled and the solvent evaporated under reduced pressure. The oily residue was co-evaporated with diethyl ether (2 × 10 mL). The crude product was dissolved in ethyl acetate (20 mL), washed with brine (2 × 20 mL), dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. Yield: 4.336 g (99.2%); yellow liquid; IR (KBr) ν 3400, 3025, 2954, 1718, 1615, 1516, 1439, 1350, 1225, 1170, 1012, 827, 804, 786, 731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.58 (s, 2H, CH_2), 3.73 (s, 3H, CH_3), 6.76 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.12 (d, 2H, *J* = 8.5 Hz, Ar-H) ppm, signal for OH cannot be seen in the spectrum; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.3 (CH_2), 52.3 (CH_3), 115.6, 125.5, 130.5, 155.1 (6 × Ar-C), 173.4 (CO) ppm; MS (ESI+): *m/z* (%) = 167 ($[M+H]^+$), 269 (100%).



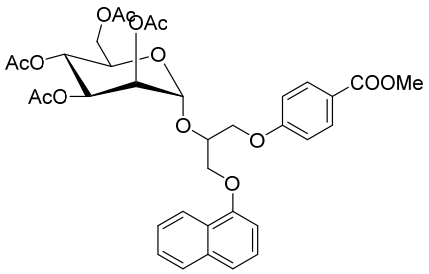
Methyl 4-(2-hydroxy-3-(naphthalen-1-yloxy)propoxy)benzoate (6). To a solution of **4** (1.520 g, 9.99 mmol) in methanol (20 mL) KOH (0.561 g, 9.99 mmol) was added. The reaction mixture was stirred at room temperature until KOH dissolved and then the solvent was evaporated under reduced pressure. The obtained potassium salt of **4** was dissolved in a mixture of toluene (20 mL) and *N*,*N*-dimethylformamide (1 mL) and then **1** (2.000 g, 9.99 mmol) and tetrabutylammonium bromide (0.644 g, 1.99 mmol) were added. Reaction mixture was stirred at 120°C for 20 h. The solvent was evaporated and the oily residue dissolved in ethyl acetate (100 mL). Organic phase was successively washed with water (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. Crude product was purified by flash column chromatography using dichloromethane/MeOH (50:1) as eluent. Yield: 2.450 g (69.6%); yellow oil; IR (KBr) ν 3432, 2947, 1718, 1605, 1579, 1509, 1436, 1399, 1256, 1170, 1103, 1035, 846, 793, 769, 696, 571 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.36 (s, 4H, 2 × CH_2), 3.80 (s, 3H, CH_3), 4.51-4.63 (m, 1H, CH), 5.59 (dd, 1H, J_1 = 4.4 Hz, J_2 = 6.2 Hz, OH), 6.97-7.00 (m, 1H, Ar-H), 7.08-7.12 (m, 1H, Ar-H), 7.38-7.53 (m, 5H, Ar-H), 7.83-7.94 (m, 4H, Ar-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 51.8 (CH_3), 67.3 (CH), 69.3 (CH_2), 69.6 (CH_2), 105.2, 114.5, 120.0, 121.7, 121.9, 124.9, 125.2, 126.2, 126.4, 127.4, 131.2, 134.0, 153.9, 162.4 (16 × Ar-C), 165.8 (CO); HRMS (ESI+): *m/z* for $C_{21}H_{20}O_5$ ([M+H^+]): calcd: 353.1389, found: 353.1388.



Methyl 2-(4-(2-hydroxy-3-(naphthalen-1-yloxy)propoxy)phenyl)acetate (**7**). To a solution of **5** (3.500 g, 21.1 mmol) in methanol (10 mL) KOH (0.591 g, 10.5 mmol) was added. The reaction mixture was stirred at room temperature until KOH dissolved and then the solvent was evaporated under reduced pressure. The obtained potassium salt of **5** was dissolved in a mixture of toluene (10 mL) and *N*,*N*-dimethylformamide (2 mL) and **1** (2.109 g, 10.5 mmol) and tetrabutylammonium bromide (1.358 g, 4.21 mmol) were added. Reaction mixture was stirred at 90 °C for 2 h. The solvent was evaporated and the oily residue dissolved in ethyl acetate (100 mL). Organic phase was successively washed with water (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. Crude

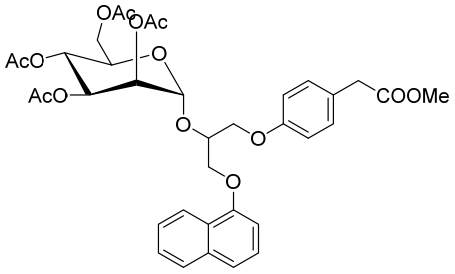
product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 2.937 g (76.1%); yellow oil; IR (KBr) ν 3448, 3052, 2949, 1735, 1580, 1512, 1458, 1438, 1399, 1270, 1240, 1158, 1103, 1043, 895, 824, 793, 772, 734 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.67 (br s, 1H, OH), 3.60 (s, 2H, CH_2COO), 3.71 (s, 3H, CH_3), 4.26 (dd, 1H, J_1 = 5.9 Hz, J_2 = 9.5 Hz, CH_2-H_A), 4.31 (dd, 1H, J_1 = 4.7 Hz, J_2 = 9.5 Hz, CH_2-H_B), 4.37 (d, 2H, J = 5.3 Hz, CH_2), 4.55-4.61 (m, 1H, CH), 6.89 (d, 1H, J = 7.6 Hz, Ar-H), 6.95 (d, 2H, J = 8.7 Hz, Ar-H), 7.24 (d, 2H, J = 8.7 Hz, Ar-H), 7.48 (t, 1H, J = 7.9 Hz, Ar-H), 7.51 (m, 3H, Ar-H), 7.84 (dd, 1H, J_1 = 2.4 Hz, J_2 = 6.9 Hz, Ar-H), 8.26 (dd, 1H, J_1 = 1.3 Hz, J_2 = 8.3 Hz, Ar-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.3 (CH_2), 52.1 (CH_3), 2 × 68.9 (CH_2), 69.1 (CH), 105.0, 114.7, 120.9, 121.7, 125.4, 125.5, 125.8, 126.5, 126.8, 127.6, 130.5, 134.5, 154.1, 157.6 (16 × Ar-C), 172.3 (CO) ppm; HRMS (ESI+): *m/z* for $C_{22}H_{23}O_5$ ($[M+H^+]^+$): calcd 367.1545; found 367.1542.

General procedure A. Glycosylation. To a solution of diarylalcohol (1.2 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (1 mmol) in dry dichloromethane (20 mL) under argon trimethylsilyl trifluoromethanesulfonate (TMSOTf) (1.3 mmol) was added at 0°C. After stirring at 0°C for 30 min the reaction mixture was allowed to warm to room temperature and stirred overnight. Then Et_3N (2.6 mmol) was added, the solvent removed under reduced pressure and crude product purified by flash column chromatography.



Methyl 4-(2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3-(naphthalen-1-yloxy)propoxy)benzoate (8). Prepared from **6** (0.437 g, 1.24 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (0.611 g, 1.24 mmol) according to general procedure A. Crude product was purified by flash column chromatography using ethyl acetate/hexane (1:1) as eluent. Compound **8** was obtained as a mixture of two diastereomers. Yield: 0.329 g (38.9%); yellow oil; IR (KBr): ν 3448, 2954, 1752, 1606, 1581, 1509, 1438, 1371, 1241, 1171, 1137, 1104, 1046, 848, 794, 771, 736, 695, 600, 497 cm^{-1}: ^1H NMR (400 MHz, $CDCl_3$) δ 1.97, 1.98 (2 × s, 3H, $OCOCH_3$), 2.01, 2.02 (2 × s, 3H, $OCOCH_3$), 2.06, 2.08 (2 × s, 3H, $OCOCH_3$), 2.17-2.20 (2 × s, 3H, $OCOCH_3$), 3.85-3.92 (m, 3H, $COOCH_3$), 4.05-

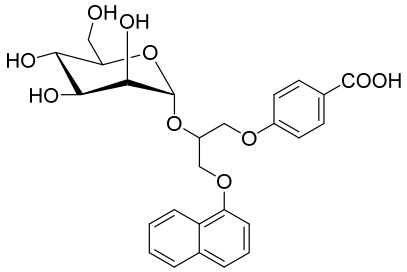
4.11 (m, 1H, mannose-H), 4.31-4.50 (m, 7H, CH_2CHCH_2, 2 × mannose-H), 5.27-5.42 (m, 4H, 4 × mannose-H), 6.87-6.92 (m, 1H, Ar-H), 6.98-7.01 (m, 1H, Ar-H), 7.17-7.21 (m, 1H, Ar-H), 7.38-7.54 (m, 4H, Ar-H), 7.79-7.85 (m, 1H, Ar-H), 8.02-8.06 (m, 2H, Ar-H), 8.17-8.26 (m, 1H, Ar-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 20.4, 20.5, 20.6, 20.7 (4 × $OCO\underline{C}H_3$), 51.8, 59.7, 61.6, 61.9, 65.2, 65.3, 67.4, 67.6, 68.0, 68.1, 68.5, 68.6, 68.7, 68.8, 73.6, 73.9, 96.0, 96.3 ($COO\underline{C}H_3$, $CH_2\underline{C}HCH_2$, 6 × mannose-C), 105.3, 105.5, 114.5, 114.6, 120.2, 120.3, 120.4, 120.5, 121.3, 121.4, 122.2, 124.8, 125.3, 125.4, 126.0, 126.1, 126.4, 126.5, 127.4, 127.5, 131.2, 131.3, 134.0, 134.1, 153.4, 153.5, 162.0, 162.1, 162.9, 165.8 (16 × Ar-C), 169.3, 169.5, 169.6, 169.8, 170.0 (4 × $O\underline{C}OCH_3$, $\underline{C}OOCH_3$) ppm; HRMS (ESI+): *m/z* for $C_{35}H_{38}O_{14}$([M+H$^+$]): calcd: 683.2340, found: 683.2338.



Methyl 2-(4-(2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3-(naphthalen-1-yloxy)propoxy)phenyl)acetate (9). Prepared from **7** (0.500 g, 1.37 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (0.672 g, 1.37 mmol) according to general procedure A. Crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Compound **9** was obtained as a mixture of two diastereomers. Yield: 0.695 g (73.1%); bright yellow oil; IR (KBr) ν 3464, 3055, 2954, 1751, 1582, 1513, 1459, 1438, 1397, 1371, 1240, 1138, 1048, 981, 827, 794, 774, 736 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (2 × s, 3H, $OCOCH_3$), 2.01, 2.02 (2 × s, 3H, $OCOCH_3$), 2.07, 2.08 (2 × s, 3H, $OCOCH_3$), 2.17, 2.19 (2 × s, 3H, $OCOCH_3$), 3.59, 3.60 (2 × s, 2H, CH_2COO), 3.71 (2 × s, 3H, CH_3), 4.03-4.11 (m, 1H, CH_2-H_A), 4.20-4.27 (m, 1H, CH_2-H_B), 4.27-4.35 (m, 2H, CH_2), 4.35-4.42 (m, 2H, mannose-H), 4.42-4.50 (m, 1H, mannose-H), 4.59-4.67 (m, 1H, CH), 5.26-5.38 (m, 3H, mannose-H), 5.39, 5.42 (2 × d, 1H, J = 3.2 Hz, H-1), 6.85-6.92 (2 × dd, 1H, J_1 = 0.8 Hz; J_2 = 7.6 Hz, Ar-H), 6.90-6.95 (2 × d, 2H, J = 8.7 Hz, Ar-H), 7.21-7.27 (2 × d, 2H, J = 8.7 Hz, Ar-H), 7.36-7.44 (2 × t, 1H, Ar-H), 7.45-7.56 (m, 3H, Ar-H), 7.80-7.86 (m, 1H, Ar-H), 8.16-8.20, 8.23-8.27 (2 × m, 1H, Ar-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 20.8, 20.9, 21.0, 21.1 (4 × $OCO\underline{C}H_3$), 40.3 ($\underline{C}H_2COO$), 52.1 ($COO\underline{C}H_3$), 62.2, 62.4, 65.9, 66.0, 67.3, 67.5, 67.7, 67.8, 68.8, 69.0, 69.1, 69.6, 69.7, 74.9, 75.1, 97.4 ($CH_2\underline{C}HCH_2$, 6 ×

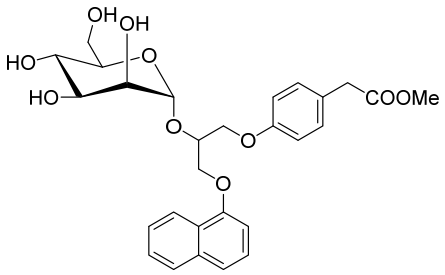
mannose-C), 105.0, 114.5, 114.8, 121.1, 121.7, 125.4,125.5, 125.7, 125.8,126.6, 126.9, 127.6, 130.5, 130.6, 134.5, 134.6, 153.9, 157.5 (16 × Ar-C), 169.7,169.8, 169.9, 170.0, 170.6, 170.8, 171.2, 172.3 (4 × O<u>C</u>OCH$_3$, <u>C</u>OOCH$_3$) ppm; HRMS (ESI+): *m/z* for $C_{36}H_{41}O_{14}$ ([M+H$^+$]$^+$): calcd 697.2496; found 697.2501.

General procedure B. Zemplén deacetylation. The protected mannopyranoside (1 mmol) was dissolved in dry methanol and sodium methanolate solution (30 wt. % in methanol, 0.01 mmol) was added. The reaction mixture was stirred at room temperature for 30 min and then the acidic ion exchange resin Amberlite® IR120 H was added for neutralization. After stirring of mixture for 10 min, the resin was filtered off, washed with methanol and the solvent removed *in vacuo*.

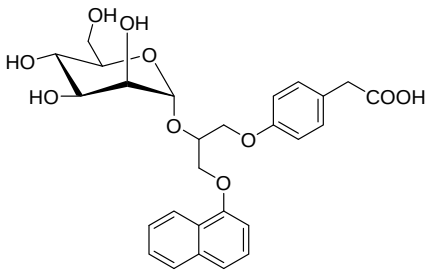


4-(2-(α-D-Mannopyranosyloxy)-3-(naphthalen-1-yloxy)propoxy)benzoate (15d). First compound **10** was prepared from **8** (0,300 g, 0.440 mmol) according to general procedure B. Compound **10** was then without purification used in the next step. Compound **10** was dissolved in a mixture of 1,4-dioxane (5 mL) and methanol (10 mL) and 1 M NaOH (1.32 mL, 1.32 mmol) was added. Reaction mixture was stirred at room temperature for 24 h and concentrated *in vacuo*. Then ethyl acetate (30 mL) and water (10 mL) were added and the mixture acidified with 2 M HCl to pH = 2. White precipitate was filtered off. Water phase was then extracted with ethyl acetate (30 mL). Combined organic phases were dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. The crude product was further purified with Isolera LS flash purification system (Biotage®) using gradient mobile phase (methanol/0.1% trifluoroacetic acid in water). Yield: 0.110 g (37.5%); white amorphous solid; IR (KBr) ν 3422, 3061, 2930, 1685, 1606, 1580, 1509, 1459, 1398, 1240, 1171, 1132, 1103, 1053, 1021, 978, 847, 793, 736 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.62-3.88 (m, 5H, 5 × mannose-H), 3.90 (dt, 1H, J_1 = 1.7 Hz, J_2 =3.3 Hz, mannose-H), 4.40-4.54 (m, 4H,C<u>H$_2$</u>CHC<u>H$_2$</u>), 4.63-4.68 (m, 1H, CH$_2$C<u>H</u>CH$_2$), 5.22, 5.28 (2 × d, *J* = 1.7 Hz, 1H, mannose-H-1), 6.98-7.01 (m, 1H, Ar-H), 7.07-7.11 (m, 2H, Ar-H), 7.38-7.42 (m, 1H, Ar-H), 7.45-7.51 (m, 3H, Ar-H), 7.81-7.83 (m, 1H, Ar-H), 7.98-8.02 (m, 2H, Ar-H), 8.21-8.29 (m, 1H, Ar-H)

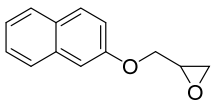
ppm, signals for OH and COOH protons cannot be seen in the spectrum; ^{13}C NMR (100 MHz, CD_3OD) δ 61.3, 61.5, 66.9, 67.0, 67.6, 67.7, 67.8, 67.9, 2 × 70.9, 71.0, 71.1, 73.7, 73.8, 74.1, 74.2 (CH_2CHCH_2, 6 × mannose-C), 100.4, 100.5, 104.7, 104.8, 2 × 114.0, 120.2, 120.3, 121.4, 121.5, 123.0, 124.9, 125.0, 2 × 125.5, 2 × 125.6, 2 × 126.0, 127.1, 127.2, 2 × 131.5, 134.6, 134.7, 154.1, 154.3, 162.7 (16 × Ar-C),168.3 (CO) ppm; HRMS (ESI-): *m/z* for $C_{26}H_{27}O_{10}$ ([M-H]$^-$): calcd 499.1604; found 499.1603.



Methyl 2-(4-(2-(α-D-mannopyranosyloxy)-3-(naphthalen-1-yloxy)propoxy)phenyl) acetate (11). Prepared from **9** (0.600 g, 0.86 mmol) according to general procedure B. Crude product was purified by flash column chromatography using dichloromethane/methanol (9:1) as eluent. Yield: 0.381g (83.7%); yellow viscous oil; IR (KBr) ν 3422, 3054, 2931, 1734, 1581, 1512, 1459, 1438, 1397, 1269, 1239, 1136, 1103, 1055, 1021, 977, 880, 793, 771, 736 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.51 (s, 2H, CH_2COO), 3.64, 3.65 (2 × s, 3H, CH_3), 3.68-3.99 (m, 6H, 6 × mannose-H), 4.08-4.25 (m, 4H, C$\underline{H_2}$CHC$\underline{H_2}$), 4.39-4.48 (m, 1H, $CH_2C\underline{H}CH_2$), 5.19 (2 × s, 1H, mannose-H-1), 6.75 (dd, 1H, J_1 = 1.6 Hz, J_2 =7.6 Hz, Ar-H), 6.78-6.87 (2 × d, 2H, J = 8.7 Hz, Ar-H), 7.10-7.15 (d, 2H, J = 8.7 Hz, Ar-H), 7.29-7.34 (m; 1H, Ar-H), 7.37-7.48 (m, 3H, Ar-H), 7.71-7.77 (m, 1H, Ar-H), 8.12-8.21 (m, 1H, Ar-H) ppm, signals for OH protons cannot be seen in the spectrum; ^{13}C NMR (100 MHz, $CDCl_3$), δ 40.1 (COO$\underline{C}H_2$), 52.0 (CH_3), 61.0, 2 × 66.3, 67.5, 67.8, 71.0, 2 × 71.5, 72.5, 72.6, 74.1, 74.5, 99.7 (CH_2CHCH_2, 6 × mannose-C), 104.8,104.9, 114.5, 114.8, 2 × 120.8, 121.7, 2 × 125.4, 125.5, 125.7, 2 × 126.5, 2 × 127.5, 130.4, 130.5, 134.4, 153.9 154.1, 157.4, 157.6 (16 × Ar-C),172.4 (CO) ppm; HRMS (ESI-): *m/z* for $C_{28}H_{31}O_{10}$ ([M-H]$^-$): calcd 527.1917; found 527.1918.

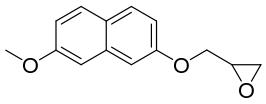


2-(4-(2-(α-D-Mannopyranosyloxy)-3-(naphthalen-1-yloxy)propoxy)phenyl) acetate (15e). Compound **11** (0.270 g, 0.51 mmol) was dissolved in a mixture of 1,4-dioxane (5 mL) and ethanol (20 mL) and 1 M NaOH (1.53 mL, 1.53 mmol) was added. Reaction mixture was stirred at room temperature for 24 h and concentrated *in vacuo*. Then ethyl acetate (40 mL) and water (20 mL) were added and the mixture acidified with 2 M HCl to pH = 2. White precipitate was filtered off. Water phase was then extracted with ethyl acetate (40 mL). Combined organic phases were dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. Yield: 0.209 (79.5%); white amorphous solid; IR (KBr) ν 3422, 3061, 2932, 1708, 1581, 1511, 1458, 1397, 1269, 1240, 1134, 1103, 1054, 1021, 977, 793, 772, 737 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 3.49 (s, 2H, $COOCH_2$), 3.72-3.76 (m, 3H, 3 × mannose-H), 3.85-3.91 (m, 3H, 3 × mannose-H), 4.41 (dd, 2H, J_1 = 3.2 Hz, J_2 = 5.4 Hz, C$\underline{H_2}$CH), 4.45 (dd, 1H, J_1 = 6.2 Hz, J_2 = 10.2 Hz, C$\underline{H_2}$-H_A), 4.54 (dd, 1H, J_1 = 4.1 Hz, J_2 = 10.2 Hz, C$\underline{H_2}$-H_B), 4.61-4.68 (m, 1H, $CH_2C\underline{H}CH_2$), 5.30 (d, 1H, J =1.4 Hz, mannose-H-1), 6.99-7.03 (m, 2H, Ar-H), 7.05 (dd, 1H, J_1 = 0.9 Hz, J_2 = 7.6 Hz, Ar-H), 7.23-7.27 (m, 2H, Ar-H), 7.43 (t, 1H, J= 7.6 Hz, Ar-H), 7.47-7.57 (m, 3H, Ar-H), 7.86-7.88 (m, 1H, Ar-H), 8.27-8.30 (m, 1H, Ar-H) ppm, signals for OH and COOH protons cannot be seen in the spectrum; ^{13}C NMR (100 MHz, DMSO-d_6) δ 61.2, 66.7, 67.3, 67.6, 70.5, 70.8, 73.0, 74.3, 100.1 (CH_2CHCH_2, 6 × mannose-C), 105.4, 114.3, 120.2, 121.4, 124.9, 125.5, 126.2, 126.5, 127.2, 127.5, 130.4, 134.0, 153.8, 157.1 (16 × Ar-C), 173.0 (CO) ppm, signal for $\underline{C}H_2COO$ overlapped with DMSO-d_6; HRMS (ESI-): *m/z* for $C_{27}H_{29}O_{10}$ ($[M-H]^-$): calcd 513.1761; found 513.1757.

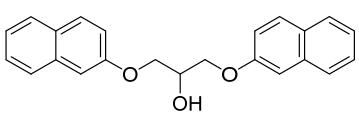


2-((Naphthalen-2-yloxy)methyl)oxirane (14). To a solution of 2-naphthol (**12**) (1.80 g, 12.5 mmol) in methanol (15 mL) KOH (0.701 g, 12.5 mmol) was added. The reaction mixture was stirred at room temperature until KOH dissolved and then the solvent was evaporated under reduced pressure. To the obtained potassium salt of 2-naphthol, epichlorohydrin (9.80 mL, 125 mmol) was added and solution stirred at 60 °C for 30 min. Reaction mixture was cooled and the solvent removed *in vacuo.* To the crude product brine (50 mL) was added and the product extracted with ethyl acetate (2 × 50 mL). Combined organic phases were dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. Crude product was purified by flash column chromatography using ethyl acetate/hexane (1:4) as eluent. Yield: 1.176 g (47.0%); white crystalline solid; mp >300 °C; IR (KBr) ν 3388, 3057, 1684, 1628, 1599, 1558, 1509, 1389, 1354, 1256, 1216, 1181, 1118, 1031, 971, 838, 743, 681 cm^{-1}; ^1H NMR (400 MHz,

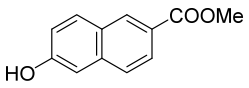
$CDCl_3$) δ 2.84 (dd, 1H, J_1 = 2.7 Hz, J_2 = 4.9 Hz, oxirane CH_2-H_A), 2.98 (dd, 1H, J_1 = 4.2 Hz, J_2 = 4.9 Hz, oxirane CH_2-H_B), 3.44-3.48 (tdd, 1H, J_1 = 2.7 Hz, J_2 = 3.1 Hz, J_3 = 4.1 Hz, J_4 = 5.8 Hz, CH), 4.10 (dd, 1H, J_1 = 5.7 Hz, J_2 = 11.0 Hz, OCH_2-H_A), 4.37 (dd, 1H, J_1 = 3.1 Hz, J_2 = 11.0 Hz, OCH_2-H_B), 7.17 (d, 1H, J = 2.5 Hz, Ar-H), 7.22 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.9 Hz, Ar-H), 7.38 (ddd, 1H, J_1 = 1.2 Hz, J_2 = 6.8 Hz, J_3 = 8.1 Hz, Ar-H), 7.47 (ddd, 1H, J_1 = 1.2 Hz, J_2 = 6.8 Hz, J_3 = 8.2 Hz, Ar-H), 7.74-7.81 (m, 3H, Ar-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.8, 50.1 (CH, CH_2), 68.7 (CH_2), 106.8, 118.8, 123.8, 126.4, 126.8, 127.7, 129.1, 129.5, 134.4, 156.4 (12 × Ar-C) ppm. MS (ESI+): *m/z* (%) = 223.1 ($[M+Na]^+$, 100).



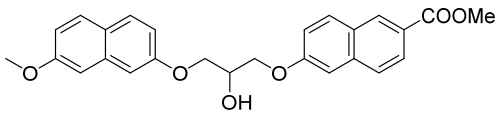
2-(((7-Methoxynaphthalen-2-yl)oxy)methyl)oxirane (15). To a solution of 7-methoxy-2-naphthol (**13**) (1.74 g, 10.0 mmol) in methanol (15 mL) KOH (0.561 g, 10.0 mmol) was added. The reaction mixture was stirred at room temperature until KOH dissolved and then the solvent was evaporated under reduced pressure. To the obtained potassium salt of 7-methoxy-2-naphthol, epichlorohydrin (7.84 mL, 100 mmol) was added and solution stirred at 60 °C for 30 min. Reaction mixture was cooled and the solvent removed *in vacuo.* To the crude product brine (50 mL) was added and the product extracted with ethyl acetate (2 × 50 mL). Combined organic phases were dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. Crude product was purified by flash column chromatography using ethyl acetate/hexane (1:4) as eluent. Yield: 1.508 g (65.6%); white crystalline solid; mp 92-94 °C; IR (ATR) ν 3058, 3005, 2965, 2939, 1627, 1608, 1514, 1464, 1426, 1384, 1348, 1333, 1252, 1219, 1208, 1185, 1170, 1137, 1117, 1081, 1024, 974, 950, 910, 860, 837, 804, 762, 752, 688, 630, 613, 565 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.84 (dd, 1H, J_1 = 4.9 Hz, J_2 = 2.7 Hz, oxirane-CH_2-H_A), 2.97 (dd, 1H, J_1 = 4.9 Hz, J_2 = 4.1 Hz, oxirane-CH_2-H_B), 3.45 (dddd, 1H, J_1 = 5.7 Hz, J_2 = 4.1 Hz, J_3 = 3.2 Hz, J_4 = 2.7 Hz, CH), 3.93 (s, 3H, CH_3), 4.10 (dd, 1H, J_1 = 11.0 Hz, J_2 = 5.7 Hz, OCH_2-H_A), 4.35 (dd, 1H, J_1 = 11.0 Hz, J_2 = 3.2 Hz, OCH_2-H_B), 7.02-7.10 (m, 4H, Ar-H), 7.68 (d, 1H, J = 3.5 Hz, Ar-H), 7.70 (d, 1H, J = 3.7 Hz, Ar-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.8, 50.1 (CH, CH_2), 55.2 (CH_3), 68.7 (CH_2), 105.2, 106.3, 116.1, 116.3, 124.5, 129.1, 129.3, 135.7, 157.0, 158.2 (12 × Ar-C) ppm. HRMS m/z for $C_{14}H_{15}O_3$ ($[M+H]^+$): calcd 231.1021; found 231.1018.



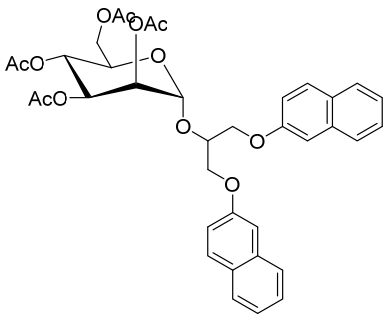
1,3-Bis(naphthalen-2-yloxy)propan-2-ol (16). To a solution of 2-naphthol (0.498 g, 3.45 mmol) in methanol (20 mL) KOH (0.194 g, 3.45 mmol) was added. The reaction mixture was stirred at room temperature until KOH dissolved and then the solvent was evaporated under reduced pressure. The obtained potassium salt of 2-naphthol was dissolved in a mixture of toluene (20 mL) and *N*,*N*-dimethylformamide (5 mL) and **14** (0.536 g, 2.88 mmol) and tetrabutylammonium bromide (0.093 g, 0.29 mmol) were added. Reaction mixture was stirred at 110 °C for 48 h. The solvent was evaporated and the oily residue dissolved in ethyl acetate (50 mL). Organic phase was successively washed with 1 M NaOH (2 × 50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. Crude product was purified by flash column chromatography using ethyl acetate/hexane (1:4) as eluent. Yield: 1.190 g (56.0%); white crystalline solid; mp 97-98 °C; IR (KBr) ν 3448, 3056, 2913, 2877, 1627, 1598, 1508, 1462, 1389, 1352, 1254, 1214, 1178, 1118, 1032, 959, 837, 809, 740, 623 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.33-4.40 (m, 4H, 2 × CH_2), 4.55-4.59 (m, 1H, CH), 7.21-7.24 (m, 4H, Ar-H), 7.36-7.40 (m, 2H, Ar-H), 7.46-7.50 (m, 2H, Ar-H), 7.72-7.82 (m, 6H, Ar-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.8 (2 × CH_2), 77.2 (CH), 106.9, 118.7, 123.9, 126.5, 126.8, 127.7, 129.2, 129.6, 134.4, 156.4 (20 × Ar-C) ppm. MS (ESI+): *m/z* (%) = 345.1 ($[M+H]^+$, 23), 186.2 (100).



Methyl 6-hydroxy-2-naphthoate (18). 6-Hydroxy-2-naphthoic acid (**17**) (5.045 g, 26.8 mmol) was dissolved in MeOH (50 mL), cooled to 0°C and thionyl chloride (4.86 mL, 67.0 mmol) was added drop wise. Reaction mixture was then stirred at reflux for 2 h, cooled and solvent evaporated under reduced pressure. The crude product was dissolved in ethyl acetate (100 mL) and washed successively with saturated aqueous $NaHCO_3$ solution (2 × 50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. Crude product was crystallized from methanol. Yield: 3.264 g (60.2%); white crystalline solid; mp 165-167 °C; IR (ATR) ν 3416, 3003, 2952, 1682, 1628, 1572, 1509, 1483, 1461, 1434, 1351, 1303, 1278, 1242, 1203, 1154, 1129, 1100, 973, 955, 931, 917, 879, 852, 811, 772, 652, 607 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 3H, CH_3), 7.14-7.19 (m, 2H, Ar-H), 7.77 (d, 1H, J = 8.9 Hz, Ar-H), 7.86 (dd, 1H, J_1 = 1.7 Hz, J_2 = 8.6 Hz, Ar-H), 7.98 (d, 1H, J = 8.9 Hz, Ar-H), 8.50 (d, 1H, J = 1.7 Hz, Ar-H), 10.20 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 51.9 (CH_3), 108.7, 119.6, 123.6, 125.1, 126.3, 126.6, 130.5, 131.2, 137.1, 157.8 (10 × Ar-C), 166.5 (CO) ppm.

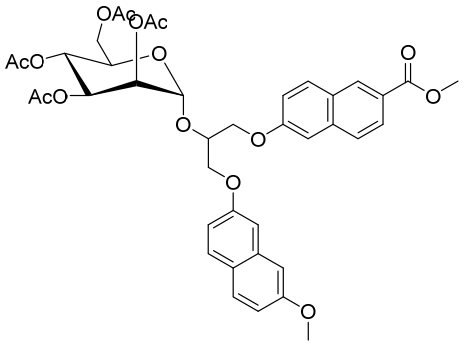


Methyl 6-(2-hydroxy-3-((7-methoxynaphthalen-2-yl)oxy)propoxy)-2-naphthoate (19). To a solution of **18** (0.714 g, 3.53 mmol) in methanol (30 mL) KOH (0.198 g, 3.53 mmol) was added. The reaction mixture was stirred at room temperature until KOH dissolved and then the solvent was evaporated under reduced pressure. The obtained potassium salt of **18** was dissolved in a mixture of toluene (30 mL) and *N*,*N*-dimethylformamide (5 mL) and **15** (0.813 g, 3.53 mmol) and tetrabutylammonium bromide (0.114 g, 0.35 mmol) were added. Reaction mixture was stirred at 110 °C for 48 h. The solvent was evaporated and the oily residue dissolved in ethyl acetate (100 mL). Organic phase was successively washed with 10% citric acid solution (2 × 50 mL), saturated aqueous $NaHCO_3$ solution (2 × 50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. Crude product was purified by flash column chromatography using ethyl acetate/hexane (1:3) as eluent. Yield: 0.771 g (50.5%); white crystalline solid; mp 140 °C; IR (ATR) ν 2952, 1716, 1625, 1603, 1514, 1429, 1388, 1368, 1337, 1291, 1254, 1204, 1158, 1126, 1094, 1028, 962, 951, 916, 850, 822, 792, 768, 753, 698, 660, 641, 631, 613, 582, 558 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 3H, OCH_3), 3.99 (s, 3H, $COOCH_3$), 4.33-4.37 (m, 4H, 2 × CH_2), 4.54-4.60 (m, 1H, CH), 7.03-7.08 (m, 3H, Ar-H), 7.14 (d, 1H, *J*=2.4 Hz, Ar-H), 7.23-7.29 (m, 2H, Ar-H), 7.68 (d, 1H, *J* = 4.8 Hz, Ar-H), 7.70 (d, 1H, *J* = 4.8 Hz, Ar-H), 7.76 (d, 1H, *J* = 8.6 Hz, Ar-H), 7.87 (d, 1H, *J* = 8.9 Hz, Ar-H), 8.06 (dd, 1H, J_1 = 8.6 Hz, J_2 = 1.7 Hz, Ar-H), 8.55 (d, 1H, *J* = 1.2 Hz, Ar-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.0 ($COO\underline{C}H_3$), 55.0 (OCH_3), 67.3, 69.1, 69.5 ($\underline{C}H_2\underline{C}H\underline{C}H_2$), 105.3, 106.2, 106.7, 115.8, 115.9, 119.8, 123.7, 124.5, 125.3, 127.1, 127.4, 128.9, 129.0, 130.3, 131.0, 135.7, 136.8, 156.9, 157.7, 158.5 (20 × Ar-C), 166.4 (CO) ppm. HRMS for $C_{26}H_{25}O_6$ ($[M+H]^+$): calcd 433.1651; found 433.1659.



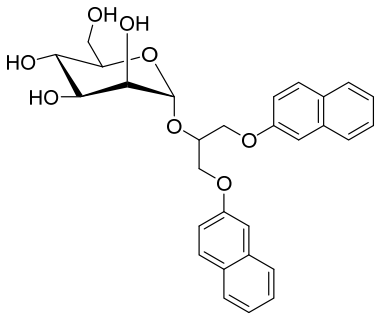
1,3-Bis(naphthalen-2-yloxy)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (20). Prepared from **16** (0.316 g, 0.92 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl

trichloroacetimidate (0.400 g, 0.77 mmol) according to general procedure A. Crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.300 g (58.1%); off-white solid; mp 68-70 °C; $[\alpha]_D$ +26.6 (*c* 0.23, MeOH); IR (KBr) ν 2936, 1742, 1629, 1600, 1510, 1465, 1389, 1213, 1181, 1136, 1039, 979, 836, 811, 747, 623, 598 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 2.02 (s, 3H, $COCH_3$), 2.04 (s, 6H, 2 × $COCH_3$), 2.21 (s, 3H, $COCH_3$), 4.10 (dd, 1H, $J_{5,6}$ = 2.1 Hz, $J_{6,6'}$ = 12.1 Hz, H-6), 4.32-4.42 (m, 6H, H-5, H-6', $C\underline{H_2}CHC\underline{H_2}$), 4.60-4.65 (m, 1H, $CH_2C\underline{H}CH_2$), 5.30 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.33-5.38 (m, 2H, H-2, H-4), 5.43 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 7.16-7.24 (m, 4H, Ar-H), 7.36-7.41 (m, 2H, Ar-H), 7.46-7.50 (m, 2H, Ar-H), 7.76-7.82 (m, 6H, Ar-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 20.7, 20.8, 21.0 (4 × $CO\underline{C}H_3$), 62.4 (C-6), 66.0 (C-4), 67.3, 67.7 (2 × CH_2), 68.9 (C-3), 69.0 (C-2), 69.7 (C-5), 74.9 (CH), 97.4 (C-1), 106.8, 106.9, 118.5, 118.7, 123.9, 124.0, 126.5, 126.6, 126.9, 127.7, 129.2, 129.3, 129.7, 129.8, 134.4, 134.5, 156.2, 156.3 (20 × Ar-C), 169,8, 170.0, 170.1, 170.7 (4 × CO) ppm; HRMS for $C_{37}H_{39}O_{12}$ ($[M+H]^+$): calcd 675.2442; found 675.2432.

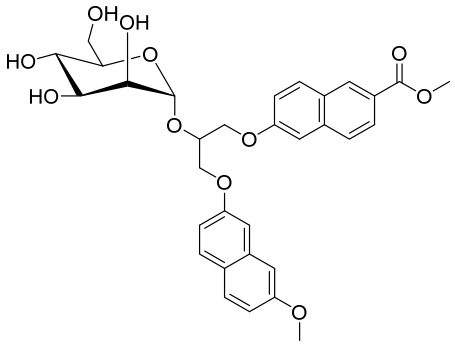


Methyl 6-(2-((2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3-((7-methoxynaphthalen-2-yl)oxy)propoxy)-2-naphthoate (21). Prepared from **19** (0.716 g, 1.66 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (0.816 g, 1.66 mmol) according to general procedure A. Crude product was purified by flash column chromatography using ethyl acetate/hexane (1:3) as eluent. Yield: 0.826 g (65.4%); white solid; mp 61-63 °C; IR (ATR) ν 2952, 2322, 2084, 1989, 1745, 1716, 1627, 1608, 1515, 1457, 1436, 1387, 1368, 1340, 1200, 1164, 1136, 1035, 979, 910, 857, 831, 808, 695, 631, 600, 564, 552 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 2.02-2.04 (m, 9H, 3 × $OCOCH_3$), 2.21 (d, 3H, *J* = 1.2 Hz, $OCOCH_3$), 3.93 (s, 3H, OCH_3), 3.99 (2 × s, 3H, $COOCH_3$), 4.09-4.12 (m, 1H, mannose-H), 4.32-4.43 (m, 6H, 2 × CH_2, 2 × mannose-H), 4.59-4.64 (m, 1H, CH), 5.29 (dd, 1H, J_1 = 5.8 Hz, J_2=1.7 Hz, H-1), 5.33-5.44 (m, 3H, 3 × mannose-H), 7.00-7.09 (m, 3H, Ar-H), 7.14 (dd, 1H, J_1 = 6.2 Hz, J_2 = 2.4 Hz, Ar-H), 7.21-7.28 (m, 2H, Ar-H), 7.67-7.72 (m, 2H, Ar-H), 7.79 (dd, 1H, J_1 = 8.8 Hz,

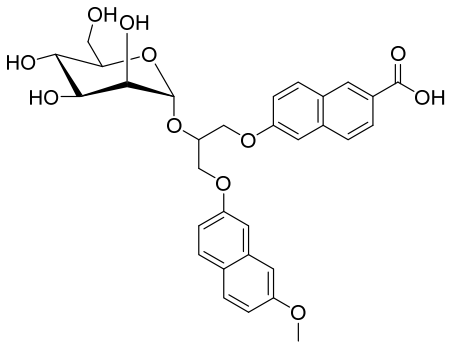
J_2 = 2.1 Hz, Ar-H), 7.90 (dd, 1H, J_1 = 9.1 Hz, J_2 = 5.5 Hz, Ar-H), 8.07 (ddd, 1H, J_1 = 8.6 Hz, J_2 = 3.3 Hz, J_3 = 1.7 Hz, Ar-H), 8.56 (dd, 1H, J_1 = 3.6 Hz, J_2 = 1.5 Hz, Ar-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 20.6, 20.9 (4 × CO<u>C</u>H$_3$), 52.0, 52.1, 55.2 (OCH$_3$, COO<u>C</u>H$_3$), 62.2, 62.3, 65.8, 65.9, 66.9, 67.3, 67.5, 67.8, 68.8, 68.9, 69.6, 69.7, 74.7, 74.9, 97.2, 97.4 (CH_2CHCH_2, 6 × mannose-C), 105.2, 106.3, 106.4, 106.6, 115.7, 115.9, 116.4, 116.5, 119.3, 119.5, 124.4, 124.5, 125.5, 125.6, 126.0, 126.1, 126.9, 128.0, 128.1, 129.1, 129.4, 129.5, 130.8, 131.1, 131.2, 135.6, 135.7, 136.8, 136.9, 156.7, 156.8, 158.0, 158.1, 158.2, 158.3 (20 × Ar-C), 167.2, 167.3, 169.7, 169.9, 170.0, 170.1, 170.6, 170.7 (4 × <u>C</u>OCH$_3$, <u>C</u>OOCH$_3$) ppm; HRMS m/z for $C_{40}H_{43}O_{15}$ ($[M+H]^+$): calcd 763.2602; found 763.2607.



1,3-Bis(naphthalen-2-yloxy)propan-2-yl α-D-mannopyranoside (13f). Prepared from **20** (0.253 g, 0.38 mmol) according to general procedure B. Yield: 0.164 g (86.3%); white crystalline solid; mp 179-180 °C; $[\alpha]_D$ +8.5 (*c* 0.23, MeOH); IR (KBr) ν 3233, 2872, 1628, 1599, 1510, 1456, 1354, 1255, 1215, 1180, 1118, 1033, 974, 833, 808, 742, 682, 623 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), δ 3.69-3.82 (m, 5H, H-3, H-4, H-5, H-6, H-6'), 3.93 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.37-4.50 (m, 4H, C<u>H$_2$</u>CHC<u>H$_2$</u>), 4.60-4.65 (m, 1H, CH_2C<u>H</u>CH_2), 5.25 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 7.20-7.23 (m, 2H, Ar-H), 7.31-7.36 (m, 4H, Ar-H), 7.41-7.45 (m, 2H, Ar-H), 7.76-7.79 (m, 6H, Ar-H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 62.7 (C-6), 68.4 (C-4), 68.9, 69.0 (2 × CH_2), 72.4 (C-2), 72.5 (C-3), 75.0 (C-5), 75.5 (CH), 101.8 (C-1), 107.9, 108.0, 119.7, 119.8, 124.7, 124.8, 127.3, 127.4, 128.0, 128.6, 130.4, 130.5, 130.6, 130.7, 136.1, 158.0, 158.1 (20 × Ar-C) ppm; HRMS for $C_{29}H_{29}O_8$ ($[M-H]^-$): calcd 505.1862; found 505.1856.



Methyl 6-(2-((α-D-mannopyranosyloxy)-3-((7-methoxynaphthalen-2-yl)oxy)propoxy)-2-naphthoate (15c). Prepared from **21** (0.710 g, 0.93 mmol) according to general procedure B. Yield: 0.553 g (100%); white solidified oil; IR (ATR) ν 3392, 2931, 2111, 1710, 1628, 1608, 1515, 1480, 1389, 1340, 1256, 1201, 1160, 1135, 1096, 1020, 977, 913, 855, 829, 678, 630 cm^{-1}; ^1H NMR (400 MHz, CD_3OD), δ 3.67-3.84 (m, 4H, 4 × mannose-H), 3.89-3.92 (m, 5H, OCH_3, 2 × mannose-H), 3.97 (s, 3H, $COOCH_3$), 4.36-4.54 (m, 4H, 2 × CH_2), 4.61-4.64 (m, 1H, CH), 5.24 (t, 1H, J = 1.9 Hz, mannose-H-1), 6.97 (ddd, 1H, J_1 = 8.9 Hz, J_2 = 2.5 Hz, J_3 = 1.2 Hz, Ar-H), 7.05 (ddd, 1H, J_1 = 8.9 Hz, J_2 = 2.5 Hz J_3 = 1.1 Hz, Ar- H), 7.15 (dd, 1H, J_1 = 7.0 Hz, J_2 = 2.5 Hz, Ar- H), 7.25 (d, 1H, J = 2.5 Hz, Ar- H), 7.31 (ddd, 1H, J_1 = 8.9 Hz, J_2 = 4.2 Hz, J_3 = 2.5 Hz, Ar- H), 7.41 (d, 1H, J = 2.1 Hz, Ar- H), 7.65-7.69 (m, 2H, Ar- H), 7.84 (dd, 1H, J_1 = 8.7 Hz, J_2 = 6.4 Hz, Ar- H), 7.93 (dd, 1H, J_1 = 9.1 Hz, J_2 = 2.2 Hz, Ar- H), 8.00 (dd, 1H, J_1 = 9.1 Hz, J_2 = 2.2 Hz, Ar-H), 8.54 (s, 1H, Ar-H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 52.7, 55.7, 62.8, 62.9, 68.4, 68.5, 68.7, 68.8, 69.1, 69.2, 72.3, 72.4, 75.1, 75.3, 75.4 (OCH_3, $COOCH_3$, CH_2CHCH_2, 6 × mannose-C), 101.7, 101.8, 106.3, 106.4, 107.5, 107.6, 107.9, 108.0, 117.0, 117.1, 117.2, 117.3, 120.9, 121.0, 125.9, 126.0, 126.6, 126.7, 128.3, 130.0, 130.2, 130.3, 131.8, 132.1, 132.2, 137.5, 138.8, 158.5, 158.6, 159.7, 160.1, 160.2 (20 × Ar-C), 168.9 (CO) ppm; HRMS m/z for $C_{32}H_{33}O_{11}$ ($[M-H]^-$): calcd 593.2023; found 593.2010.



6-(2-((α-D-Mannopyranosyloxy)-3-((7-methoxynaphthalen-2-yl)oxy)propoxy)-2-naphthoate (15b). Compound **15c** (0.500 g, 0.84 mmol) was dissolved in a mixture of 1,4-dioxane/methanol = 1:1 (10 mL) and 2 M NaOH (1.68 mL, 3.36 mmol) was added. Reaction mixture was stirred at room temperature for 24 h and concentrated *in vacuo*. The obtained solution was acidified to pH = 4 using the acidic ion exchange resin Amberlite® IR120 H. The resin was then filtered off, washed with methanol and the solvent removed *in vacuo*. Yield: 0.453 g (92.9%); white crystalline solid; mp 133-134 °C; IR (ATR) ν 3263, 2935, 1701, 1628, 1607, 1541, 1515, 1463, 1387, 1255, 1208, 1133, 1096, 1053, 1019, 976, 916, 856, 829, 772, 660, 611, 552 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), δ 3.62-3.66 (m, 1H, mannose-H), 3.80-3.84 (m, 3H, 3 × mannose-H), 3.86 (s, 3H, OCH3), 3.95 (d, 1H, *J* =11.1 Hz, mannose-H), 4.01 (s, 1H, mannose-H), 4.33-4.45 (m, 4H, 2 × CH_2), 4.54-4.59 (m, 1H, CH), 5.22 (s, 1H, mannose-H-1), 6.93 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.4 Hz, Ar-H), 6.98 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.4 Hz, Ar-H), 7.06 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.15 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.21 (dd, 1H, J_1 = 9.1 Hz, J_2 = 2.2 Hz, Ar-H), 7.29 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.63 (t, 2H, *J* = 8.8 Hz, Ar-H), 7.69-7.72 (m, 1H, Ar-H), 7.85 (d, 1H, *J* = 9.1 Hz, Ar-H), 8.03 (dd, 1H, J_1 = 8.6 Hz, J_2 = 1.4 Hz, Ar-H), 8.43 (s, 1H, Ar-H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 56.0 (OCH_3), 63.0, 68.5, 68.9, 69.1, 72.4, 72.5, 75.2, 75.3, 101.8 (CH_2CHCH_2, 6 × mannose-C), 106.5, 106.6, 107.7, 108.1, 117.4, 117.5, 120.2, 120.3, 125.9, 127.5, 128.4, 130.3, 130.4, 130.6, 131.9, 137.5, 137.6, 158.7, 159.2, 159.8 (20 × Ar-C), 168.4 (CO) ppm; HRMS m/z for $C_{31}H_{31}O_{11}$ ($[M-H]^-$): calcd 579.1866; found 579.1880.

2. Biological assays

Competitive binding assay

A recombinant protein consisting of the CRD of FimH linked with a thrombin cleavage site to a 6His-tag (FimH-CRD-Th-6His) was expressed in *E. coli* strain HM125 and purified by affinity chromatography.[11] To determine the affinity of the various FimH antagonists, the competitive binding assay described previously was applied.[11] Microtiter plates (F96 MaxiSorp, Nunc) were coated with 100 µL/well of a 10 µg/mL solution of FimH-CRD-Th-6His in 20 mM HEPES, 150 mM NaCl and 1 mM $CaCl_2$, pH 7.4 (assay buffer) overnight at 4 °C. The coating solution was discarded and the wells were blocked with 150 µL/well of 3% BSA in assay buffer for 2 h at 4 °C. After three washing steps with assay buffer (150 µL/well), a four-fold serial dilution of the test compound (50 µL/well) in assay buffer

containing 5% DMSO and streptavidin-peroxidase coupled TM-PAA polymer (50 µL/well of a 0.5 µg/mL solution) were added. On each individual microtiter plate n-heptyl α-D-mannopyranoside[12] was tested in parallel. The plates were incubated for 3 h at 25 °C and 350 rpm and then carefully washed four times with 150 µL/well assay buffer. After the addition of 100 µL/well of the horseradish peroxidase substrate 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), the colorimetric reaction was allowed to develop for 4 min, then stopped by the addition of 2% aqueous oxalic acid before the optical density (OD) was measured at 415 nm on a microplate-reader (Spectramax 190, Molecular Devices, California, USA). The IC_{50} values of the compounds tested in duplicates were calculated with prism software (GraphPad Software, Inc., La Jolla, USA). The IC_{50} defines the molar concentration of the test compound that reduces the maximal specific binding of TM-PAA polymer to FimH-CRD by 50%.

Cell culture

Cell line HepG2 (ATCC / LGC Standards, UK) was cultured in DMEM medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin (all from Sigma-Aldrich) in a humidified chamber at 37 °C and 5% CO_2.

Metabolic activity assay

Cells were seeded into 96-well plates at $1x10^5$ cells/mL (100 µL/well) and treated with 10 µM compound of interest or corresponding vehicle (control cells). The metabolic activity was assessed after 24 h treatment using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA). The absorbance was measured at 492 nm on the automated microplate reader BioTek (BioTek, Winooski, VT, USA). The result is presented as percentage of metabolic activity of control cells stimulated with vehicle (mean ± SD) from three independent experiments, each conducted in triplicates.

3. Molecular modelling

3.1. Computer hardware and software

All the computational work was performed on two workstations. Accelrys Discovery Studio 3.0 (DS)[2] was running on a workstation with Intel core i7 860 CPU processor, 8 GB RAM, two 750 GB hard drives and a Nvidia GT220 GPU graphic card, running Centos 5.5. GOLD

Suite, version 5.2[3] was running on four octal core AMD Opteron CPU processors, 16 GB RAM, two 750 GB hard drives, running 64-bit Scientific Linux 6.0.

3.2. Ligand and protein preparation

The tridimensional models of target compounds (Tables 1 and 2) were built from a standard fragment library in Accelrys Discovery Studio 3.0 (DS).[2] The geometries of the molecules were optimized using CHARMM force field[4] with MMFF94[5] partial atomic charges. The energy was minimized using the Smart Minimizer algorithm in DS until the gradient value was smaller than 0.001 kcal/(mol Å). The optimized structure was further refined with GAMESS interface in ChemBio3D Ultra 13.0 using semiempirical PM3 method, QA optimization algorithm and Gasteiger Hückel charges for all atoms for 100 steps.[6]

Molecular docking calculations were performed using GOLD Suite, version 5.2.[3] Three different crystal structures of FimH-ligand complexes were used for molecular docking studies: 3MCY.pdb as FimH in its closed conformation, 4AUY.pdb as FimH in its half open conformation and 4AV5.pdb as FimH in its open conformation.[7,8] Ligands and water molecules were deleted from the crystal structures of FimH-antagonist complexes, and hydrogen atoms were added to the protein using GOLD. The amino acid residues within a radius of 7 Å around the antagonist were defined as the active site.

3.3. Validation of the docking protocol and ligand docking

In order to validate GOLD 5.2 as a suitable docking program, co-crystallized ligand was re-docked into the FimH-CRD binding site (PDB entries: 3MCY, 4AUY and 4AV5) in 25 independent genetic algorithm (GA) runs. The best ranked GOLD-calculated conformation of co-crystallized antagonists using GOLDscore[9] as a scoring function, had an all heavy atom root-mean square distance (rmsd) values bellow 2 Å compared to the experimentally determined conformation of the ligand in FimH-CRD binding site. These results highlight GOLD in combination with GOLDscore as a suitable tool for binding mode studies of the newly designed ligands.

The target compounds were docked in 25 independent GA runs. The GA parameters were set as suggested by GOLD 5.2. Early termination was allowed if the top 3 solutions were within 1.0 Å of the rmsd value. GOLDscore was used as scoring function. The 10 best ranked docking solutions were inspected visually, and the best ranked GOLD-calculated

conformation was used for analysis and representation. The figures were prepared by Pymol.[10]

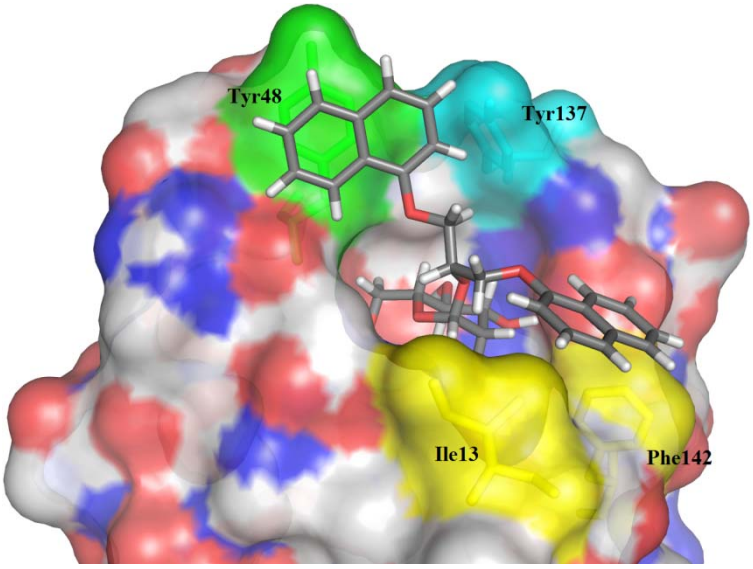
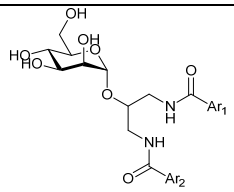


Figure S1. The out-docking mode of compound **13c** (in grey sticks) in the closed conformation of FimH binding site (PDB entry: 3MCY).

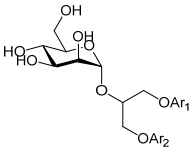
3.4. Calculation of logP values

Calculated logP values (miLogP) for all tested compounds (Table S1) were calculated using Molinspiration Free Web Tools for Cheminformatics Community (http://www.molinspiration.com/cgi-bin/properties).

Table S1. Calculated logP values (miLogP) for **12a-g**, **13a-f**, **14a-f** and **15a-e**.



12



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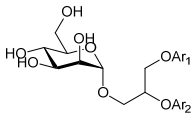

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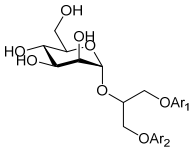
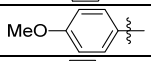
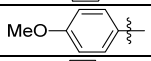
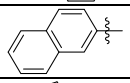
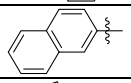
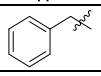
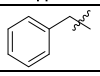
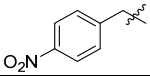
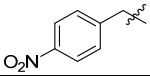
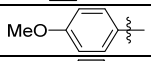
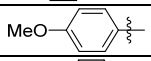
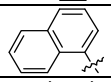
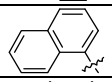
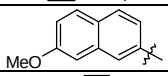
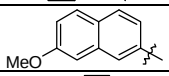
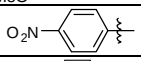
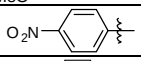
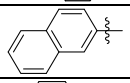
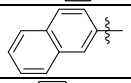
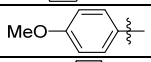
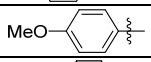
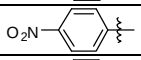
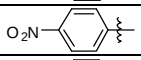
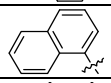
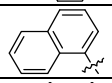
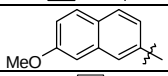
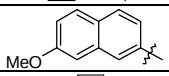


15

Entry	Ar_1	Ar_2	miLogP
12a			-0.40
12b			-0.89
12c			-0.29
12d			1.96
12e			-2.09
12f			-0.22
12g			-0.30
13a			1.32
13b			1.44
13c			3.64
13d			3.76
13e			1.24
13f			3.69
14a			1.32
14b			1.44
14c			1.24
14d			3.64
14e			3.76
14f			3.69
15a		Et-	1.19
15b			3.61 for acid 0.20 for carboxylate
15c			3.87
15d			2.39 for acid -1.02 for carboxylate
15e			1.91 for acid -0.81 for carboxylate

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