First steps towards conformationally selective artificial lectins: the chair-boat discrimination by molecularly imprinted polymers

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Electronic Supplementary Information

Experimental section

General methods

All chemicals were purchased in Aldrich Chemicals Co., Acros Chemicals Co. or ABCR Chemicals Co. and used as purchased, except for AIBN which was recrystallized from methanol and EGDMA which was distilled (bp: 100°C at 7 mbar, 130°C at 18 mbar). THF was distilled over sodium/benzophenone. DCM and acetonitrile were distilled over CaH2. The 2,3,6-tri-O-tert-butyl-dimethylsilyl-α,β-D-galactofuranose was made accordingly to the procedure of Fleet.1 All reactions were monitored by thin-layer chromatography (TLC) carried out on Merck aluminum roll 20 a TOTTOLI-BUCHI Melting Point B-545 apparatus. [α]D are referenced indirectly to TMS via the solvent (or residual solvent) and the detection is generally made using the positive mode. Chemical shifts (δ) are reported in ppm and 35–80 electronic supplementary material (ESI) for Chemical Communications

1-(4-isopropylphenylmethyl)-2,3,6-tri-O-tert-butyl-dimethylsilyl-α,β-D-galactofuranose 4

Magnesium turning (389 mg, 16 mmol) were suspended in anhydrous diethyl ether (8 mL) under argon. p-isobutylbenzyl bromide (2.74 mL, 16 mmol) diluted in diethyl ether (8 mL) was added to the magnesium suspension, to maintain a gentle reflux. This freshly prepared Grignard reagent was slowly added at 0 °C onto a solution of lactone 31 (2.08 g, 4 mmol) in anhydrous diethyl ether (16 mL) under argon. The resulting solution was stirred 4 h at room temperature, and saturated ammonium chloride (30 mL) was added. Dichloromethane (70 mL) was added, and the organic layers were washed by brine (2 x 30 mL), dried over MgSO4, filtrated and concentrated. The residue was purified by flash chromatography (Cyclohexane-AcOEt 98 : 02) to afford 4 (1.73 g, 66%) as a white solid. α/β: 22/78; [α]D 0.50 (Cyclohexane-AcOEt, 9 : 1; m.p.: 75.1 °C; [α]D20 14.7 (c 1.0 in CHCl3); El Analysis for C33H34O2Si: found: C, 62.58, H, 10.17, required: C, 62.33; H, 10.15%; α: 1H NMR (400 MHz, CDCl3): δ 7.24, 7.13 (AB, JAB = 8.0 Hz, 4H, H arom), 4.18 (dd, J3,4 = 2.5 Hz, J2,3 = 1.8 Hz, 1H, H-3), 4.06 (t, J1,2 = 2.5 Hz, 1H, H-4), 3.90 (s, 1H, OH-1), 3.85 (d, J1,2 = 1.6 Hz, 1H, H-2), 3.75-3.57 (m, 3H, H-5, H-6a, H-6b), 3.17, 2.98 (AB, JAB = 13.7 Hz, 2H, H-1'), 2.93 (d, J2H = 6.2 Hz, 1H, OH-5), 2.90-2.80 (m, 1H, CH2Pr), 2.12 (d, JPr = 6.9 Hz, 1H, CH3Pr), 0.93 (s, 9H, SiCMe3), 0.90 (s, 9H, SiCMe3), 0.84 (s, 9H, SiCMe3), 0.12 (s, 3H, SiMe3), 0.07 (s, 3H, SiMe3), 0.06 (s, 3H, SiMe3), 0.03 (s, 3H, SiMe3), -0.09 (s, 3H, SiMe3); 13C NMR (101 MHz, CDCl3): δ 147.0 (C1arom), 133.5 (C4arom), 130.7 (2 CHarom), 130.5 (2 CHarom), 125.9 (2 CHarom), 104.8 (1C), 83.4 (C-4), 80.0 (C-2), 79.7 (C-3), 71.1 (C-5), 63.7 (C-6), 39.7 (C-1'), 33.7 (CH3Pr), 25.9 (Si-C(CH3)2), 25.7 (Si-C(CH3)3), 25.5 (Si-C(CH3)3), 24.1 (CH3Pr), 17.9 (Si-C(CH3)2), 17.8 (Si-C(CH3)3), -4.5 (Si-Me), -4.7 (Si-Me), -4.9 (Si-Me), -5.0 (Si-Me), -5.4 (Si-Me), -5.5 (Si-Me);
β. 1H NMR (400 MHz, CDCl3): δ = 7.29, 7.16 (AB, JAB = 8.0 Hz, 4H, Hα arom), 4.29 (s, 1H, H-4), 4.24 (d, JAs = 1.8 Hz, 1H, OH-1), 4.13 (s, 1H, H-3), 3.75-3.57 (m, 3H, H-5, H-6a, H-6b), 3.20 (d, JAB = 4.8 Hz, 1H, OH-5), 3.15, 2.80 (AB, 1J, JAB = 13.7 Hz, 2H, H-1'), 2.90-2.80 (m, 1H, CH2), 1.23 (d, JAB = 6.9 Hz, 1H, CH2 Pr), 0.94 (s, 9H, SiMe3), 0.87 (s, 9H, SiMe3), 0.86 (s, 9H, SiMe3), 0.18 (s, 3H, SiMe2), 0.15 (s, 3H, SiMe2), 0.13 (s, 3H, SiMe2), 0.09 (s, 3H, SiMe3), 0.04 (s, 3H, SiMe3), 0.02 (s, 3H, SiMe3); 13C NMR (100 MHz, CDCl3): δ 146.8 (C-1α), 133.1 (Cβ), 126.1 (2 Cα), 107.3 (C-1), 85.7 (C-4), 81.7 (C-2), 80.5 (C-3), 71.6 (C-5), 63.7 (C-6), 42.2 (C-1'), 33.7 (CH2 Pr), 25.9 (Si-C(CH3)3), 25.7 (Si-C(CH3)3), 24.0 (CH3 Pr), 18.3 (Si-C(CH3)3), 18.0 (Si-C(CH3)3), 17.7 (Si-C(CH3)3), -4.1 (Si-Me), -4.7 (Si-Me), -5.4 (Si-Me), -5.5 (Si-Me); HRMS (ESI+): m/z: culated for C41H46O5Si2Na [(M+Na)+] = 717.3293, found 717.3283.

(11(1')Z)-1-deoxy-1-(4-isopropylphenyl)methylene-2,3,6-tri-O-tert-butyldimethylsilyl-α,β-D-galactoorosane 1

To a solution of hemiacetal 4 (α/β = 22.78, 300 mg, 0.46 mmol) in anhydrous THF (20.0 mL) at 0°C under argon were added anhydrous pyridine (370 μL, 4.58 mmol) and trifuorooacetic anhydride (32 μL, 2.29 mmol). The mixture was stirred at 0°C during 3 h, then overnight to room temperature. A saturated solution of NaHCO3 (13 mL) was added. The aqueous layer was extracted by dichloromethane (30 mL). The organic layer was then washed by brine (2 x 30 mL), dried over MgSO4, filtrated and concentrated. The residue was dissolved in a solution of methanol/chloroform (1/1 v/v, 20 mL), and catalytic K2CO3 was added. The solution was stirred during 30 min at room temperature, filtered and concentrated. The residue was purifed by flash chromatography (toluene:hexane:DCM = 9:1) to afford 5 (230 mg, 78%) as a colorless oil. Rf = 0.55 (Cyclohexane-EtOAc: 96:4); [α]D 20 +16.4 (c 1.0 in CHCl3); El. Analysis for C43H54O8Si3: found: C, 69.30, H, 10.08; required: C, 69.09, H, 10.12%; 1H NMR (400 MHz, CDCl3): δ 7.61, 7.16 (AB, JAB = 8.0 Hz, 4H, Hα arom), 5.36 (s, 1H, H-1'), 4.51 (t, JAB = 6.4 Hz, 1H, H-4), 4.43 (d, JAB = 2.3 Hz, 1H, H-1), 4.16, (JAB = 2.3 Hz, 1H, H-3), 3.84-3.72 (m, 2H, H-5, H-6a, H-6b), 3.38 (d, JAB = 3.7 Hz, 1H, OH-5), 2.88 (hept, JAB = 6.9 Hz, 1H, CH2Pr), 1.24 (d, JAB = 6.9 Hz, 6H, CH3Pr), 0.93 (s, 9H, SiMe3), 0.92 (s, 9H, SiMe3), 0.88 (s, 9H, SiMe3), 0.20 (s, 3H, SiMe2), 0.18 (s, 3H, SiMe3), 0.14 (s, 3H, SiMe3), 0.13 (s, 3H, SiMe2), 0.12 (s, 3H, SiMe3), 0.11 (s, 3H, SiMe3); 13C NMR (100 MHz, CDCl3): δ 156.4 (C-1'), 146.4 (Cβ), 133.6 (Cα), 128.1 (Cα), 126.3 (Cβ), 101.9 (C-1'), 100.6 (C-2), 79.2 (C-3), 71.2 (C-5), 63.5 (C-6), 34.0 (CH3), 26.0 (Si-(CH3)), 25.9 (Si-(CH3)), 25.8 (Si-(CH3)), 24.1 (CH3 Pr), 18.4 (Si-(CH3)), 18.1 (Si-(CH3)), 18.0 (Si-(CH3)), -4.2 (Si-Me), -4.3 (Si-Me), -4.4 (Si-Me), -4.5 (Si-Me), -5.2 (Si-Me), -5.3 (Si-Me); HRMS (ESI+): m/z: culated for C43H54O8Si3Na [(M+Na)+] = 759.3954, found 759.3954.

1,4-anhydro-1-deoxy-1-hydroxymethyl(4-isopropylphenyl)D-galactopyranose 1

Tetrabutylammonium fluoride trihydrate (287 mg, 0.91 mmol) was added onto a solution of galactoside 7 (198 mg, 0.30 mmol) in anhydrous THF (12 mL) under argon. After stirring overnight at room temperature, the solution was concentrated. The residue was purified by a flash chromatography (AcOEt-EtOH 9:1) followed by second flash chromatography (AcOEt:CH2Cl2, 8:2) to afford 8 (76 mg, 81%) as a colorless oil. Rf = 0.19 (AcOEt-EtOH 9:1); [α]D 20 +56.4 (c 1.0 in MeOH); 1H NMR (400 MHz, MeOD): δ 7.42, 7.19 (AB, JAB = 8.0 Hz, 4H, Hα arom), 4.97 (s, 1H, H-1'), 4.32 (d, JAB = 1.4 Hz, 1H, H-4), 3.80 (ABX X part, JAB = 6.8 Hz, JAX = 6.4 Hz, JAX = 6.4 Hz, 1H, H-5), 3.65 (JAB = 1.1 Hz, 1H, H-3), 3.52 (JAB = 1.1 Hz, 1H, H-2), 3.48, 3.43 (ABX AB part, JAB = 11.2 Hz, JAX = 6.8 Hz, JAX = 6.4 Hz, 2H, H-6), 2.88 (hept, JAB = 6.9 Hz, 1H, CH3Pr), 1.22 (d, JAB = 6.9 Hz, 6H, CH3Pr); 13C NMR (101 MHz, MeOD): δ 148.7 (Cβ), 136.1 (Cα), 127.9 (CH3), 125.7 (CH3), 109.3 (C-1), 83.7 (C-4), 82.6 (C-2), 79.1 (C-3), 76.9 (C-5), 70.6 (C-1'), 62.0 (C-2'), 33.8 (CH3Pr), 23.1 (2 CH3Pr); 1H NMR (400 MHz, acetone-d6): δ 7.42, 7.18 (AB, JAB = 8.2 Hz, 4H, Hα arom), 4.96 (s, 1H, H-1'), 4.35 (s, 1H, H-4), 3.80 (ABX X part, JAX = 6.4 Hz, JAX = 5.6 Hz, 1H, H-5), 3.72 (s, 1H, H-3), 3.67 (s, 1H, H-2), 3.43, 3.35 (ABX AB part, JAB = 11.1 Hz, JAX = 6.4 Hz, JAX = 5.6 Hz, 2H, H-6), 1.22 (d, JAB = 7.1 Hz, 6H, CH3Pr), CH3Pr signal under HDO and D2O signals; 13C NMR (101 MHz, acetone-d6): δ 148.8 (Cβ), 137.7 (Cα), 128.9 (CH3), 126.3 (CH3), 110.0 (C-1), 84.8 (C-4), 84.1 (C-2), 80.4 (C-3), 78.0 (C-5), 71.8 (C-1'), 63.0 (C-6), 35.6 (CH3Pr), 24.4 (CH3Pr), 24.3 (CH3Pr); HRMS (ESI+): m/z: culated for C34H40O5Si [(M+Na)+] = 533.1309.
5-methacryloylaminoboronophthalide (5-methacryloylamino-
benzoboroxole) 7

To a suspension of commercially available 5-amino-
boronophthalide hydrochloride (1.0 g, 5.39 mmol) in dry
 dichloromethane (35 mL) were added at 0 °C disisopropyl-
ethylamine (797 µL, 5.93 mmol) and methacrylic anhydride
(884 µL, 5.93 mmol). The suspension was stirred 16 h at room
 temperature, and then diluted with water (100 mL) and ethyl
acetate (100 mL). The aqueous phase was extracted with ethyl
acetate (2 x 100 mL). The organic layers were combined and
washed with water (100 mL), dried over MgSO₄ and
concentrated. The residue was rinsed with pentane/diethyl ether
(1/1 v/v, 80 mL) to afford 7 (765 mg, 65%) as a yellow powder.

3H NMR (400 MHz, acetone-d₆): δ 9.12 (br s, 1H, NH), 8.18 (s, 1H, OH), 8.13 (s, 1H, H-6), 7.74 (dd, J₆,₇ = 8.2 Hz, J₇,₈ = 2.1 Hz, 1H, H-4), 7.35 (d, J₅,₄ = 8.2 Hz, 1H, H-3), 5.84 (t, J₈,₉ = 0.9 Hz, 1H, H-alcene), 5.47 (dd, J = 1.6 Hz, J₉,₁₀ = 0.9 Hz, 1H, H-alcene), 4.99 (s, 2H, H-1), 2.02 (dd, J₈,₉ = 1.6 Hz, J₉,₁₀ = 0.9 Hz, 3H, H-Me). ¹³C NMR (101 MHz, acetone-d₆): δ 167.3 (C=O), 150.4 (C-4), 142.1 (C-5), 139.1 (C-5), 124.0 (C-4), 122.5 (C-6), 122.1 (C-3), 119.6 (CH₃), 71.0 (C-1), 19.0 (CH₃); ¹¹B NMR (128 MHz, acetone-d₆): δ 31.3; HRMS (ESI-): m/z: calc for C₁₁H₁₁BNO₃·(M-H)⁻ 215.06; found 215.08.

MIP Synthesis

The 1,4-bond locked galactoside 1 (20 mg, 64 µmol) was dissolved with the boronic anchor 7 (56 mg, 256 µmol) in dry acetonitrile (3 mL) with CaH₂ (43 mg, 1.02 mmol) and stirred overnight. The suspension was stirred 16 h at room temperature, and then diluted with water (100 mL) and ethyl acetate (100 mL). The 1,4-bond locked galactoside 1 mixture was sonicated and degassed by three vacuum-argon
cycles. The polymerisation reaction was left for 18 h at 60 °C. The polymerisation reaction was left for 18 h at 60 °C. Once the polymerization completed, the white solid polymer was transferred in an eppendorf and washed three times with a mixture of acetonitrile and water (1/1 v/v, 10 mL), then with pure
acetonitrile (10 mL). For each washing, the solution was shaken in a 500 µL tube, followed by the addition of the cross-linking reagent (286 mg, 80%wt polymer) and AIBN (5 mg). If N,N-
methylenesacrylamide was used, DMSO (3 mL) was added. The mixture was sonicated and degassed by three vacuum-argon
cycles. The polymerisation reaction was left for 18 h at 60 °C. Once the polymerization completed, the white solid polymer was transferred in an eppendorf and washed three times with a mixture of acetonitrile and water (1/1 v/v, 10 mL), then with pure
acetonitrile (10 mL). For each washing, the solution was shaken by centrifugation during 15 min at 500 Hz, followed by centrifugation during 10 min at 3000 rpm and removal of the supernatant. Finally, the resulting powder was dried under vacuum overnight, then crushed in a mortar and passed through a 250 µm sieve.

Template binding

Incubations were made with 10 mg of polymer (theoretical amount of active sites: 1.8 µmol), in 1 mL of template solutions (0.1, 0.5, 1, 3, 5, 10, 15, and 20 equivalents vs theoretical amount of active sites) in acetonitrile, and incubated 24 h at 22 °C. The vials were then centrifuged during 5 min at 10000 rpm, and the resulting supernatants were transferred on vials, for triplicate HPLC analysis. The same template solutions (without polymer) were also analyzed by HPLC, in triplicate, to obtain a calibration curve. All calibration plots displayed r²>0.98.

Table 1 - Imprinting effect of final template –

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<th>Template concentration (mM)</th>
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<th>5.37</th>
<th>8.95</th>
<th>17.9</th>
<th>26.85</th>
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<td>IF (EGDMA-MIP)</td>
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<td>1.41</td>
<td>1.03</td>
<td>3.01</td>
<td>1.83</td>
<td>2.72</td>
<td>2.38</td>
</tr>
<tr>
<td>IF (TETRA-MIP)</td>
<td>2.62</td>
<td>4.95</td>
<td>6.75</td>
<td>4.00</td>
<td>2.10</td>
<td>3.60</td>
<td>1.91</td>
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<tr>
<td>IF (MBAA-MIP)</td>
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<td>1.21</td>
<td>1.20</td>
<td>1.14</td>
<td>1.07</td>
<td>1.12</td>
<td>0.93</td>
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</table>

* Determined by HPLC.

Table 2 - Imprinting effect of the EGDMA MIP with the template as a function of the solvent –

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<th>H₂O/CH₃CN (1/1)</th>
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<tr>
<td>IF (EGDMA-MIP)</td>
<td>2.6</td>
<td>3.1</td>
<td>1.3</td>
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</table>

* Determined by HPLC.

Figure 1 – Cross-linking reagents screened for this study.
Control binding experiments

Incubations with the 4 chair control molecules A, B, C and D
- Incubations were made with 5 mg of polymer (theoretical amount of active sites: 0.9 µmol), in 1 mL of substrate solutions (0.1, 0.5, 1, 3, 5, 10, 15, and 20 equivalents vs theoretical amount of active sites) in 5:95 milli-Q water:acetonitrile, and incubated 24 h at 22°C. The vials were then centrifuged during 5 min at 10000 rpm, and the resulting supernatants were transferred on vials, for triplicate HPLC analysis. The same substrate solutions (without polymer) were also analyzed by HPLC, in triplicate, to obtain a calibration curve. All calibration plots displayed r²>0.98. Importantly, the EGDMA and the TETrA polymers (MIPs and NIPs) were also incubated with the p-nitrophenyl-α- and –β-pyranosides A-D (using 5 mg of polymers in presence of 1 mL of 0.45 to 17.9 mM substrates solution in 95:5 acetonitrile:water solution for solubility reasons).

- The binding experiments with the 4 “chair” molecules A–D were performed either in pure acetonitrile, in water or in a 1/1 mixture of H₂O/CH₃CN. No significant imprinting factors with EGDMA-MIPS could be measured under those conditions.

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References