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ARTICLE TYPE

First steps towards conformationally selective artificial lectins: the chair-boat dicrimation 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 CaH_2. 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 silica gel 60-F254 using UV light and a molybdate-sufuric acid solution as revelator. Merck silica gel (60, particle size 0.040–0.063 mm) was employed for flash column chromatography using technical solvent distilled prior to use as eluting systems. ^{1}H, ^{11}B and ^{13}C NMR spectra were recorded on a JEOL (JNM EX-400). All compounds were characterized by ^{1}H, ^{11}B and ^{13}C NMR as well as by ^{1}H–^{1}H and ^{1}H-^{13}C correlation experiment when necessary. The following abbreviations were used to describe the multiplicities: s= singlet, d= doublet, t= triplet, hept= heptuplet, m= multiplet, br= broad, br s= broad singlet. The numbering of the protons and carbons is analogous to the proton numbers resulting from the name of the compound. Aromatic and *iso*propyl (carbons and protons) are respectively labeled with "arom" and "*i*Pr" subscript, quaternary carbons are indicated with a "q" superscript. Chemical shifts (δ) are reported in ppm and referenced indirectly to TMS via the solvent (or residual solvent) signals. The mass spectra were realized on an Agilent 6120 LC-MS ESI-TOF equipped with a C_{18} Zorbax chromatography column with particles of 3.5 µm (eluent was a mix of water and acetonitrile, with gradient). The ionisation was made at 175 V, and the detection is generally made using the positive mode. Elemental analyses were measured in the Laboratoire d'Analyse CNRS of Vernaison (France). Melting points were measured with a TOTTOLI-BUCHI Melting Point B-545 apparatus. $[\alpha]_D$ are measured on a PERKIN-ELMER 241 polarimeter at room temperature (20 °C), with a sodium lamp (589 nm). HPLC analysis were recorded on a Varian 940LC system, with a Betasil Diol-100 column (250*4.6mm, 5 µm particles) in an isocratic mode with water/acetonitrile 5/95. UV-Detection was set at 256 nm. Polymer shaking was realized on a S50 flask shaker from CAT. Centrifugations were performed on a Universal 320 apparatus from Hettich. Polymers were sieved on a MINOR sieve shaker from Endecotts, with a 250 nm-sieve.

1-(4-isopropyl)phenylmethyl-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-iso*propylbenzyl 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 **3**[1] (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 $MgSO_4$, 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; R_f 0.50 (Cyclohexane-AcOEt, 9 : 1); m.p. : 75.1 °C; $[\alpha]_D^{20}$ -14.7 (*c* 1.0 in $CHCl_3$); El. Analysis for $C_{31}H_{66}O_6Si_3$: found: C, 62.58, H, 10.17, required: C, 62.33; H, 10.15%; **α**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24, 7.13 (AB, J_{AB} = 8.0 Hz, 4H, H_{arom}), 4.18 (dd, $J_{3\text{-}4}$ = 2.5 Hz, $J_{3\text{-}2}$ = 1.8 Hz, 1H, H-3), 4.06 (t, $J_{4\text{-}3}$ = $J_{4\text{-}5}$ = 2.5 Hz, 1H, H-4), 3.90 (s, 1H, OH-1), 3.85 (d, $J_{2\text{-}3}$ = 1.6 Hz, 1H, H-2), 3.75-3.57 (m, 3H, H-5, H-6a, H-6b), 3.17, 2.98 (AB, J_{AB} = 13.7 Hz, 2H, H-1'), 2.93 (d, J_{OH} = 6.2 Hz, 1H, OH-5), 2.90-2.80 (m, 1H, CH_{iPr}), 1.22 (d, J_{iPr} = 6.9 Hz, 1H, $CH_{3\ iPr}$), 0.93 (s, 9H, $SiCMe_3$), 0.90 (s, 9H, $SiCMe_3$), 0.84 (s, 9H, $SiCMe_3$), 0.12 (s, 3H, $SiMe_2$), 0.11 (s, 3H, $SiMe_2$), 0.07 (s, 3H, $SiMe_2$), 0.06 (s, 3H, $SiMe_2$), 0.03 (s, 3H, $SiMe_2$), -0.09 (s, 3H, $SiMe_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 147.0 ($C^q_{p\ arom}$), 133.5 ($C^q_{i\ arom}$), 130.7 (2 CH_{arom}), 130.5 (2 CH_{arom}), 125.9 (2 CH_{arom}), 104.8 (C-1), 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 (CH_{iPr}), 25.9 (Si-C(CH_3)$_3$), 25.7 (Si-C(CH_3)$_3$), 25.5 (Si-C(CH_3)$_3$), 24.1 ($CH_{3\ iPr}$), 17.9 (Si-*C*(CH_3)$_3$), 17.8 (Si-*C*(CH_3)$_3$), -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: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29, 7.16 (AB, J_{AB} = 8.0 Hz, 4H, H_{arom}), 4.29 (s, 1H, H-4), 4.24 (d, J_{OH} = 1.8 Hz, 1H, OH-1), 4.13 (s, 1H, H-3), 3.95 (s, 1H, H-2), 3.75-3.57 (m, 3H, H-5, H-6a, H-6b), 3.20 (d, J_{OH} = 4.8 Hz, 1H, OH-5), 3.15, 2.80 (AB, J_{AB} = 13.7 Hz, 2H, H-1'), 2.90-2.80 (m, 1H, CH_{iPr}), 1.23 (d, J_{iPr} = 6.9 Hz, 1H, $CH_{3\ iPr}$), 0.94 (s, 9H, $SiCMe_3$), 0.87 (s, 9H, $SiCMe_3$), 0.86 (s, 9H, $SiCMe_3$), 0.18 (s, 3H, $SiMe_2$), 0.16 (s, 3H, $SiMe_2$), 0.13 (s, 3H, $SiMe_2$), 0.09 (s, 3H, $SiMe_2$), 0.04 (s, 3H, $SiMe_2$), 0.02 (s, 3H, $SiMe_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 146.8 ($C^q_{p\ arom}$), 133.1 ($C^q_{i\ arom}$), 126.1 (2 CH_{arom}), 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 (CH_{iPr}), 25.9 (Si-C(CH_3)$_3$), 25.7 (Si-C(CH_3)$_3$), 25.6 (Si-C(CH_3)$_3$), 24.0 ($CH_{3\ iPr}$), 18.3 (Si-C(CH_3)$_3$), 18.0 (Si-C(CH_3)$_3$), 17.7 (Si-C(CH_3)$_3$), -4.1 (Si-Me), -4.6 (Si-Me), -4.7 (Si-Me), -5.4 (Si-Me), -5.5 (Si-Me); HRMS (ESI+): *m/z*: calcd for $C_{31}H_{66}O_6Si_3Na$ [(M+Na)$^+$]: 677.4059, found 677.4083.

(1(1')Z)-1-deoxy-1-(4-*iso*propyl)phenylmethylidene-2,3,6-tri-*O*-tert-butyldimethylsilyl-α,β-D-galactofuranose 5

To a solution of hemicetal **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 trifluoroacetic anhydride (323 µl, 2.29 mmol). The mixture was stirred at 0 °C during 3 h, then overnight to room temperature. A saturated solution of $NaHCO_3$ (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 $MgSO_4$, filtrated and concentrated. The residue was dissolved in a solution of methanol/dichloromethane (1/1 v/v, 20 mL), and catalytic K_2CO_3 was added. The solution was stirred during 30 min at room temperature, filtered and concentrated. The residue was purified by flash chromatography (toluene) to afford **5** (230 mg, 78%) as a colorless oil. R_f 0.55 (Cyclohexane-AcOEt, 97 : 03); $[\alpha]_D^{20}$ -16.4 (*c* 1.0 in $CHCl_3$); El. Analysis for $C_{34}H_{64}O_5Si_3$: found: C, 63.90, H, 10.08, required: C, 64.09; H, 10.12%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61, 7.16 (AB, J_{AB} = 8.0 Hz, 4H, H_{arom}), 5.36 (s, 1H, H-1'), 4.51 (t, J_{4-5} = J_{4-3} = 1.8 Hz, 1H, H-4), 4.43 (d, J_{2-3} = 2.3 Hz, 1H, H-2), 4.16 (t, J_{3-4} = J_{3-2} = 2.3 Hz, 1H, H-3), 3.84-3.72 (m, 3H, H-5, H-6a, H-6b), 3.38 (d, J_{OH} = 3.7 Hz, 1H, OH-5), 2.88 (hept, J_{iPr} = 6.9 Hz, 1H, CH_{iPr}), 1.24 (d, J_{iPr} = 6.9 Hz, 6H, $CH_{3\ iPr}$), 0.93 (s, 9H, $SiCMe_3$), 0.92 (s, 9H, $SiCMe_3$), 0.88 (s, 9H, $SiCMe_3$), 0.20 (s, 3H, $SiMe_2$), 0.18 (s, 3H, $SiMe_2$), 0.14 (s, 3H, $SiMe_2$), 0.13 (s, 3H, $SiMe_2$), 0.12 (s, 3H, $SiMe_2$), 0.11 (s, 3H, $SiMe_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.4 (C-1), 146.4 ($C^q_{p\ arom}$), 133.6 ($C^q_{i\ arom}$), 128.1 ($CH_{o\ arom}$), 126.3 ($CH_{m\ arom}$), 101.9 (C-1'), 88.0 (C-4), 79.8 (C-2), 78.0 (C-3), 71.2 (C-5), 63.5 (C-6), 34.0 (CH_{iPr}), 26.0 (Si-C(CH_3)$_3$), 25.9 (Si-C(CH_3)$_3$), 25.8 (Si-C(CH_3)$_3$), 24.1 ($CH_{3\ iPr}$), 18.4 (Si-C(CH_3)$_3$), 18.1 (Si-C(CH_3)$_3$), 18.0 (Si-C(CH_3)$_3$), -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*: calcd for $C_{34}H_{64}O_5Si_3Na$ [(M+Na)$^+$]: 659.3954, found 659.3982.

1,4-anhydro-2,3,6-tri-*O*-tert-butyldimethylsilyl-1-deoxy-1-hydroxymethyl(4-*iso*propyl)phenyl-D-galactopyranose 6

Exoglycal **5** (414 mg, 0.65 mmol) was dissolved in anhydrous dichloromethane (20 mL) under argon and injected in a flame-dried flask under argon containing activated 3Å molecular sieve. After 15 min, D,L-camphorsulfonic acid (15 mg, 65 µmol) and *meta*-chloroperbenzoïc acid (168 mg, 0.97 mmol) were added. The solution was then stirred 3 h at room temperature, and filtered through a pad of Celite. The filtrate was washed by a saturated solution of sodium bicarbonate (20 mL), a saturated solution of sodium thiosulfite (20 mL), a saturated solution of sodium hydrogenocarbonate (20 mL) and brine (20 mL), dried over $MgSO_4$, filtrated and concentrated. The residue was purified by flash chromatography (Cyclohexane-AcOEt 98 : 02) to afford **7** (328 mg, 77%) as a colorless oil. R_f 0.48 (Cyclohexane-AcOEt, 9 : 1); $[\alpha]_D^{20}$ +43.0 (*c* 1.0 in $CHCl_3$); El. Analysis for $C_{34}H_{64}O_6Si_3$: found: C, 62.93, H, 9.81, required: C, 62.52; H, 9.88%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40, 7.17 (AB, J_{AB} = 8.0 Hz, 4H, H_{arom}), 4.93 (s, 1H, H-1'), 4.38 (d, J_{4-3} = 1.4 Hz, 1H, H-4), 3.75-3.65 (m, 3H, H-2, H-3, H-5), 3.53 (dd, J_{6a-6b} = 9.6 Hz, J_{6a-5} = 4.6 Hz, 1H, H-6a), 3.27 (t, J_{6b-5} = J_{6b-6a} = 9.6 Hz, 1H, H-6b), 2.88 (hept, J_{iPr} = 6.9 Hz, 1H, CH_{iPr}), 2.78 (br s, 1H, OH-1'), 1.21 (d, J_{iPr} = 6.9 Hz, 6H, $CH_{3\ iPr}$), 0.88 (s, 9H, $SiCMe_3$), 0.87 (s, 9H, $SiCMe_3$), 0.81 (s, 9H, $SiCMe_3$), 0.04 (s, 3H, $SiMe_2$), 0.03 (s, 3H, $SiMe_2$), 0.02 (s, 6H, $SiMe_2$), 0.01 (s, 3H, $SiMe_2$), -0.02 (s, 3H, $SiMe_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 149.0 ($C^q_{p\ arom}$), 135.0 ($C^q_{i\ arom}$), 128.1 ($CH_{m\ arom}$), 126.1 ($CH_{o\ arom}$), 108.8 (C-1), 84.7 (C-3), 83.4 (C-4), 80.6 (C-2), 76.4 (C-5), 71.4 (C-1'), 62.1 (C-6), 34.0 (CH_{iPr}), 25.9 (2 Si-C(CH_3)$_3$), 25.7 (Si-C(CH_3)$_3$), 24.1 ($CH_{3\ iPr}$), 24.0 ($CH_{3\ iPr}$), 18.2 (Si-C(CH_3)$_3$), 18.0 (Si-C(CH_3)$_3$), 17.9 (Si-C(CH_3)$_3$), -3.9 (Si-Me), -4.2 (Si-Me), -4.4 (Si-Me), -4.8 (Si-Me), -5.3 (Si-Me), -5.4 (Si-Me); HRMS (ESI+): *m/z*: calcd for $C_{34}H_{64}O_6Si_3Na$ [(M+Na)$^+$]: 675.3903, found 675.3874.

1,4-anhydro-1-deoxy-1-hydroxymethyl(4-*iso*propyl)phenyl-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 first flash chromatography (AcOEt-EtOH 9 : 1) followed by second flash chromatography (Acetone-CH_2Cl_2 8 : 2) to afford **1** (76 mg, 81%) as a colorless oil. R_f 0.19 (AcOEt-EtOH 9 : 1); $[\alpha]_D^{20}$ +56.4 (*c* 1.0 in MeOH); ^{1}H NMR (400 MHz, MeOD): δ 7.42, 7.19 (AB, J_{AB} = 8.0 Hz, 4H, H_{arom}), 4.97 (s, 1H, H-1'), 4.32 (d, J_{4-3} = 1.4 Hz, 1H, H-4), 3.80 (ABX X part, J_{AX} = 6.8 Hz, J_{BX} = 6.4 Hz, 1H, H-5), 3.65 (d, J_{3-2} = 1.1 Hz, 1H, H-3), 3.52 (d, J_{3-2} = 1.1 Hz, 1H, H-2), 3.48, 3.43 (ABX AB part, J_{AB} = 11.2 Hz, J_{AX} = 6.8 Hz, J_{BX} = 6.4 Hz, 2H, H-6), 2.88 (hept, J_{iPr} = 6.9 Hz, 1H, CH_{iPr}), 1.22 (d, J_{iPr} = 6.9 Hz, 6H, $CH_{3\ iPr}$); ^{13}C NMR (101 MHz, MeOD): δ 148.7 ($C^q_{p\ arom}$), 136.1 ($C^q_{i\ arom}$), 127.9 ($CH_{o\ arom}$), 125.7 ($CH_{m\ arom}$), 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-6), 33.8 (CH_{iPr}), 23.1 (2 $CH_{3\ iPr}$); ^{1}H NMR (400 MHz, acetone-d6): δ 7.42, 7.18 (AB, J_{AB} = 8.2 Hz, 4H; H_{arom}), 4.96 (s, 1H, H-1'), 4.35 (s, 1H, H-4), 3.80 (ABX X part, J_{AX} = 6.4 Hz, J_{BX} = 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, J_{AB} = 11.1 Hz, J_{AX} = 6.4 Hz, J_{BX} = 5.6 Hz, 2H, H-6), 1.22 (d, J_{iPr} = 7.1 Hz, 6H, $CH_{3\ iPr}$), CH_{iPr} signal under HDO and D2O signals; ^{13}C NMR (101 MHz, acetone-d6): δ 148.8 ($C^q_{p\ arom}$), 137.7 ($C^q_{i\ arom}$), 128.9 ($CH_{o\ arom}$), 126.3 ($CH_{m\ arom}$), 110.0 (C-1), 84.8 (C-4), 84.1 (C-2), 80.4 (C-3), 80.0 (C-5), 71.8 (C-1'), 63.0 (C-6), 35.6 (CH_{iPr}), 24.4 ($CH_{3\ iPr}$), 24.3 ($CH_{3\ iPr}$); HRMS (ESI+): *m/z*: calcd for $C_{16}H_{22}O_6Na$ [(M+Na)$^+$]: 333.1309, found 333.1307.

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 diisopropyl-ethylamine (979 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 $MgSO_4$ 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. R_f 0.57 ($CHCl_3$-MeOH-AcOH 90 : 8 : 2); ^{1}H NMR (400 MHz, acetone-d_6): δ 9.12 (br s, 1H, NH), 8.18 (s, 1H, OH), 8.13 (s, 1H, H-6), 7.74 (dd, J_{4-3} = 8.2 Hz, J_{4-6} = 2.1 Hz, 1H, H-4), 7.35 (d, J_{3-4} = 8.2 Hz, 1H, H-3), 5.84 (t, $J_{alcene-alcene}$ = 0.9 Hz, 1H, H-alcene), 5.47 (dd, J = 1.6 Hz, $J_{alcene-alcene}$ = 0.9 Hz, 1H, H-alcene), 4.99 (s, 2H, H-1), 2.02 (dd, $J_{Me-alcene}$ = 1.6 Hz, $J_{Me-alcene}$ = 0.9 Hz, 3H, H-Me); ^{13}C NMR (101 MHz, acetone-d_6): δ 167.3 (C=O), 150.4 (C-2), 142.1 (-C=), 139.1 (C-5), 124.0 (C-4), 122.5 (C-6), 122.1 (C-3), 119.6 (=CH_2), 71.0 (C-1), 19.0 (CH_3); ^{11}B NMR (128 MHz, acetone-d_6): δ 31.3; HRMS (ESI-): *m/z*: calcd for $C_{11}H_{11}BNO_3$: [(M-H)$^-$]: 216.0832, found 216.0824.

MIP Synthesis

The ^{1,4}*B* locked galactoside **1** (20 mg, 64 µmol) was dissolved with the boronic anchor **7** (56 mg, 256 µmol) in dry acetonitrile (3 mL) with CaH_2 (43 mg, 1.02 mmol) and stirred overnight. The solution was then quickly filtered through Celite and rinsed by 0.5 mL of dry acetonitrile. The filtrate was then injected in a test tube, followed by the addition of the cross-linking reagent (286 mg, 80%wt polymer) and AIBN (5 mg). If *N*,*N*-methylenebisacrylamide 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 with a S50 flask shaker (CAT) 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.

NIP Synthesis

The boronic anchor **7** was injected in a test tube, followed by cross-linking reagent (286 mg, 80%wt polymer) and AIBN (5 mg), degassed by three vacuum-argon cycles, and dissolved in dry acetonitrile (3 mL). If *N*,*N*-methylenebisacrylamide was used, DMSO (3 mL) was added. The mixture was then sonicated. 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 with a S50 flask shaker (CAT) 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.

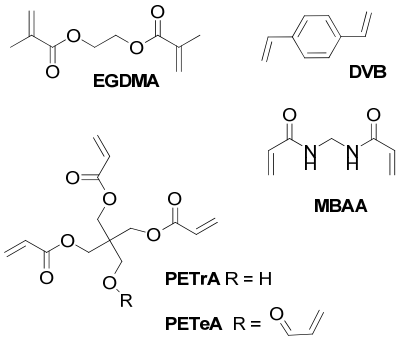

Figure 1 – Cross-linking reagents screened for this study.

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 r^2>0.98.

Table 1 - Imprinting effect of final template –

Template concentration (mM)	0,9	1,79	5,37	8,95	17,9	26,85	35,8
IF (EGDMA-MIP)[a]	1,35	1,41	1,03	3,01	1,83	2,72	2,38
IF (TETrA-MIP) [a]	2,62	4,95	6,75	4,00	2,10	3,60	1,91
IF (MBAA-MIP) [a]	1,67	1,21	1,20	1,14	1,07	1,12	0,93

[a] Determined by HPLC.

Solvent effect on template binding –

The same experiment described above ("*Template binding*") was repeated for the EGDMA-MIP at 3 template concentrations (5, 10 and 20 equivalents equivalents *vs* theoretical amount of active sites) in 1 mL pure CH_3CN, in a 1/1 mixture of H_2O/ CH_3CN and in pure H_2O. The experiment was repeated twice for each experiments and the IF was averaged.

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

solvent	CH_3CN	H_2O/ CH_3CN (1/1)	H_2O
IF (EGDMA-MIP)[a]	2.6	3.1	1.3

[a] Determined by HPLC.

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 $r^2>0.98$. 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 H_2O/ CH_3CN. No significant imprinting factors with EGDMA-MIPS could be measured under those conditions.

Acknowledgements

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