Synthesis, characterization and protein-binding properties of supported dendrons

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Part I. Materials

4-(Bromomethyl)benzaldehyde was synthesized using a literature procedure¹ with slight modifications. Triethyl amine (Et₃N), mesyl chloride (MsCl), 4-bromomethylbenzonitrile, diisobutylaluminiumhydride (DIBAL), ethylene glycol, p-toluenesulfonic acid (PTSA), lithium aluminium hydride (LAH), diisopropyl azodicarboxylate (DIAD), triphenylphosphine (PPh₃), 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald), 3-aminopropyltrimethoxysilane were obtained from Aldrich and used as received. Silica gel 0.04-0.063 mm particle size and 60 Å pore diameter was obtained from Merck; it was dried in vacuo at 110 °C overnight previous to using.

Part II. Synthesis and Characterizations

4-(Bromomethyl)benzaldehyde. 4-(bromromethyl)benzonitrile (25.52 g, 130.2 mmol) was dissolved in dry toluene (400 ml) and cooled to 0°C. 1M DIBAL in toluene (195 ml, 195 mmol) was added dropwise and the reaction mixture was stirred at 0°C for 1 hour. Then, chloroform (200 ml) and 2N HCl (300 ml) were added and the reaction mixture was stirred at room temperature overnight. The organic layer was separated and washed twice with water, dried over magnesium sulfate, filtered, and evaporated to afford the desired product as white flakes weighing 21.45 g (83%).

2-(4-(Bromomethyl)phenyl)-1,3-dioxolane (1). 4-(bromomethyl)benzaldehyde (5.91 g, 29.7 mmol) and p-toluenesulfonic acid monohydrate (0.19 g, 0.99 mmol) were dissolved in toluene (200 ml). Ethylene glycol (4.9 ml, 88 mmol) was added and the reaction mixture was refluxed for 24 hours; water was removed using a Dean-Stark trap. After cooling to room temperature K₂CO₃ (about 14 g) was added and mixture was stirred for 30 min, filtered and evaporated to afford the desired product as a yellow powder weighing 6.32 g (88 %): ¹H-NMR (CDCl₃, 200 MHz) δ 7.46 (d, 2H, J=8 Hz), 7.40 (d, 2H, J=8 Hz), 5.82 (s, 1H), 4.49 (s, 2H), 4.16-3.99 (m, 4H); ¹³C-NMR (CDCl₃, 50MHz) δ 138.6, 138.2, 129.1, 126.9, 103.2, 65.3, 32.9; GC-MS (EI) m/z: M⁺=241.0 (12), M⁺=243.0 (12), 163.2 (100), 119.2 (18), 91.1(58), 73.0 (14), Calc. 242.0.

Methyl 3,5-bis(4-(1,3-dioxolan-2-yl)benzyloxy)benzoate (2). A solution of 3,5dihydroxybenzoic acid methyl ester (1.90 g, 11.3 mmol), 1 (6.32 g, 26.0 mmol), K₂CO₃ (3.59 g, 26.0 mmol) and 18-crown-6 (0.32 g, 1.2 mmol) in acetone (90 ml) was refluxed for 24 hours. Then, acetone was removed under reduced pressure. To the resultant solid was added water and aqueous layer was extracted twice with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, and evaporated to afford the desired product as a white powder weighing 5.40 g (97%): ¹H-NMR (CDCl₃, 200 MHz) δ 7.50 (d, 4H, J=8 Hz), 7.43 (d, 4H, J=8 Hz), 7.27 (d, 2H, J=2Hz), 6.76 (t, 1H, J=2 Hz), 5.83 (s, 2H), 5.08 (s, 4H), 4.17-3.99 (m, 8H), 3.89 (s, 3H); ¹³C-NMR (CDCl₃, 50 MHz) δ 166.7, 159.6, 137.8, 137.5, 132.0, 127.4, 126.8, 108.4, 107.3, 103.4, 69.9, 65.3, 52.3; GC-MS (EI) m/z: M⁺=492.2 (3), 163.2 (75), 119.2 (8), 91.1 (100), 73.0 (18), Calc. 492.2.

(3,5-Bis(4-(1,3-dioxolan-2-yl)benzyloxy)phenyl)methanol (3). To a suspension of lithium aluminum hydride (LAH) (1.25 g, 32.7 mmol) in THF (50 ml) cooled to 0°C was added dropwise a solution of 2 (5.40 g, 11.0 mmol) in THF (90 ml). Then, the solution was allowed to warm to room temperature and stirred for 24 hours. Excess LAH was quenched by slow addition of a semi-saturated solution of sodium sulfate. THF was removed under reduced pressure. The resultant aqueous solution was extracted twice with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated to afford the desired product as a white powder weighing 4.81 g (94%): ¹H-NMR (CDCl₃, 200 MHz) δ 7.49 (d, 4 H, J=8 Hz), 7.42 (d, 4 H, J=8 Hz), 6.59 (d, 2 H, J=2 Hz), 6.51 (t, 1 H, J=2 Hz), 5.82(s, 2 H), 5.05 (s, 4 H), 4.7 (s, 1 H) 4.61 (s, 2 H), 4.17-3.99 (m, 8 H); ¹³C-NMR (CDCl₃, 50 MHz) δ 160.0, 143.4, 137.9, 137.6, 127.4, 126.7, 105.7, 103.5, 101.3, 69.7, 67.9, 65.3; LC-MS (ESI) m/z: 487.1 (M+Na⁺), 503.1 (M+K⁺), Calc.464.1.

3,5-Bis(4-(1,3-dioxolan-2-yl)benzyloxy)benzyl methanesulfonate (4)

To a stirred solution of **3** (0.15 g, 0.31 mmol) in dichloromethane (15 ml), were added triethylamine (0.07 ml, 0.5 mmol) and methanesulfonyl chloride (0.03 ml, 0.4 mmol) at 0 °C. The reaction was stirred for 2 hours. Then, the solution was washed with water and the organic layer was dried under sodium sulphate, filtered and evaporated to afford the

desired product **4** 0.15 g, (89%) as yellowish oil: $\delta_{\rm H}(500 \text{ MHz}; \text{CDCl}_3)$ 7.49 (d, 4 H, J= 8.0), 7.42 (d, 4 H, J= 8.0), 6.60 (m, 3 H), 5.82 (s, 2 H), 5.14 (s, 2 H), 5.06 (s, 4 H), 4.14-4.02 (m, 8 H), 2.81 (s, 3 H); $\delta_{\rm C}(125 \text{ MHz}; \text{CDCl}_3)$ 160.0, 137.7, 137.5, 135.5, 127.3, 126.3, 107.7, 103.4, 103.1, 71.4, 69.8, 65.3, 38.4; m/z LC-MS (APCI): 565.0 (M+Na⁺), Calc. 542.2

Methyl 3.5-bis(3.5-bis(4-(1.3-dioxolan-2-vl)benzyloxy)benzyloxy)benzoate (5). To a mixture of **3** (1.40 g, 3.00 mmol), 3,5-dihydroxy-benzoic acid methyl ester (0.22 g, 1.3 mmol) and triphenylphosphine (PPh₃) (0.80 g, 3.0 mmol) in THF (4 ml) at 0°C was added dropwise a solution of diisopropyl azodicarboxylate (DIAD) (0.6 ml, 3.0mmol) in THF (16 ml). The reaction was warmed to room temperature and stirred for 24 hours. The reaction was stopped by adding water and evaporated to remove THF. The aqueous layer was extracted twice with ethyl acetate, each organic layer was washed with 1 M aqueous KOH and an equal volume of water. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The residue was chromatographed on basic alumina (90/10 to 50/50 petroleum ether /ethyl acetate) followed by a SEC preparative column (SX3 in toluene) to afford the desired product as a white foam weighting 0.56 g (40%): ¹H-NMR (CDCl₃, 200 MHz) δ 7.50 (d, 8H, J=8 Hz), 7.43 (d, 8H, J=8 Hz), 7.28 (d, 2H, J=2 Hz), 6.77 (t, 1H, J=2 Hz), 6.67 (d, 4H, J=2 Hz), 6.55 (t, 2H, J=2 Hz), 5.83 (s, 4H), 5.05 (s, 8H), 4.99 (s, 4H), 4.16-3.99 (m, 16 H), 3.91 (s, 3H); ¹³C-NMR (CDCl₃, 50 MHz) & 166.7, 160.0, 159.6, 138.8, 137.8, 131.9, 127.4, 126.7, 108.4, 107.1, 106.4, 103.4, 101.7, 70.0, 69.7, 65.2, 52.2; MS (MALDI-TOF) m/z: 1083.0 (M+Na⁺), 1099.0 $(M+K^{+})$, Calc. 1060.4.

(3,5-Bis(3,5-bis(4-(1,3-dioxolan-2-yl)benzyloxy)benzyloxy)phenyl)methanol (6). To a suspension of LAH (0.016 g, 0.42 mmol) in THF (5 ml) cooled to 0°C was added dropwise a solution of 5 (0.15 g, 0.14 mmol) in THF (10 ml). Then, the solution was allowed to warm to room temperature and stirred for 24 hours. Excess LAH was quenched by slow addition of semi-saturated solution of sodium sulfate. The THF was removed under reduced pressure. The resultant aqueous solution was extracted twice with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated to afford the desired product as white foam weighed 0.12 g (83%): ¹H-NMR

(CDCl₃, 500 MHz) δ 7.47 (d, 8H, J=8 Hz), 7.41 (d, 8H, J=8 Hz), 6.64 (d, 4H, J=2 Hz), 6.54 (d, 2H, J=2 Hz), 6.53 (t, 2H, J=2 Hz) 6.49 (t, 1H, J=2 Hz), 5.82 (s, 4H), 5.04 (s, 8H), 4.96 (s, 4H), 4.56 (s, 2 H), 4.14-4.02 (m, 16 H); ¹³C-NMR (CDCl₃, 125 MHz) δ 160, 159.9, 143.5, 139.3, 137.8, 137.6, 127.4, 126.7, 106.2, 105.6, 103.5, 101.6, 101.2, 69.8, 69.7, 65.3, 65.1; MS (MALDI-TOF) m/z: 1058.0 (M+Na⁺), Calc. 1032.4.

3,5-Bis(3,5-bis(4-(1,3-dioxolan-2-yl)benzyloxy)benzyloxy)benzyl methanesulfonate (7). To a stirred solution of **6** (1.155 g, 1.12 mmol) in dichloromethane (15 ml) were added triethylamine (0.236 ml, 1.68 mmol) and mathanesulfonyl chloride (0.105 ml, 1.35 mmol) at 0°C. The reaction was stirred for 2 hours. Then, the solution was washed with water and the organic layer was dried under sodium sulfate, filtered and evaporated to afforded the desired product as yellowish oil, weighing 1.152 g (93%): ¹H-NMR (CDCl₃, 500 MHz) δ 7.48 (d, 8H, J=8 Hz), 7.42 (d, 8H, J=8 Hz), 6.64 (d, 4H, J=2 Hz), 6.60-6.59 (m, 3H), 6.54 (t, 2H, J=2 Hz) 5.82 (s, 4H), 5.14 (s, 2H), 5.03 (s, 8H), 4.97 (s, 4H) 4.15-4.06 (m, 16 H), 2.82 (s, 3H); ¹³C-NMR (CDCl₃, 125 MHz) δ 160.0, 138.8, 137.8,137.7, 137.6, 127.4, 126.7, 107.6, 106.3, 103.4, 102.9, 101.6, 71.4, 69.9, 69.7, 65.3, 38.4. MS (MALDI-TOF) m/z: 1114.4 (M+H⁺), Calc.1110.4.

Supplementary Material (ESI) for Journal of Materials Chemistry This journal is (c) The Royal Society of Chemistry 2009



Scheme 1. Synthesis of protected binder molecules. **a**: 3,5-dihydroxybenzoic acid methyl ester, 18-C-6, K_2CO_3 acetone, **b**: LAH, THF, **c**: MsCl, DCM, Et₃N, **d**: 3,5-dihydroxybenzoic acid methyl ester, PPh₃, DIAD, THF.

Part III. Purpald test results





Example of color change by the addition of purpald to **15** (right vial) and **21** (left vial), compounds **14** and **20** showed no color change after the addition of the purpald reagent.



Part. V. References

1. L.Wen, M. Li and J. B. Schlenoff, J. Am. Chem. Soc. 1997, 119, 7726-7733.