Supporting information

Carbohydrate-Functionalized Oligothiophenes for Concanavalin A Recognition

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Materials:
Dichloromethane (Merck) was distilled from CaH₂; N,N-diisopropylethylamine (DIPEA, Sigma Aldrich), copper(I)iodide (Merck), bis(triphenylphosphine) palladium(II)chloride and solvents were purchased from Merck which were distilled prior to use. For purification by column chromatography silica gel 60 (0.040-0.063 mm) from Merck was used. β-D-galactopyranose pentaacetate was purchased from Alfa Aesar. 5,5''α,α-Diiodo[2,2',3',5'']terthiophene 3 and 5,5'''-diiodo-3',5'''-bis(5-iodo-2-thienyl)-2,2':5',2''':4'',2''''-quaterthiophene 4 were prepared according to a literature protocol,1,2 reference compounds 5,5''-bis(trimethylsilyl ethynyl)-2,2':3',2'''-terthiophene 8 and 5,5'''-bis(trimethylsilyl ethynyl)[3',5'''-bis(5-trimethylsilyl ethynyl thien-2-yl)]-2,2':5',2''':4'',2''''-quaterthiophene 9 were also prepared as described.2

Instrumentation:
Nuclear magnetic resonance spectra were recorded on a Bruker AMX 500 (1H NMR: 500 MHz), a Bruker Avance 400 (1H NMR: 400 MHz, 13C NMR: 100 MHz) or a Bruker DPX-400 spectrometer (1H NMR: 400 MHz) at room temperature unless otherwise noted. Chemical shift values (δ) are given in parts per million using residual solvent protons (1H NMR: δH = 7.26 for CDCl₃, δH = 2.49 for DMSO-δ₆; δH = 3.33 for MeOD-δ₆, 13C NMR: δC = 77.0 for CDCl₃, δC = 49.05 for MeOD-δ₆ and 39.43 for DMSO) as internal standard. Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) measurements were carried out on a Bruker Daltonik Reflex III mass spectrometer with the following matrices: 1,2,3-trihydroxyanthracene (dithranol), 2,5-dihydroxybenzoic acid (DHB) and T-2- (3-(4-t-Butylphenyl)-2-methyl-2-propenylidene) malononitrile (DCTB). Elemental analyses were performed on a Elementar Vario EL (University of Ulm) and a Carlo Erba 1104 (University of Stuttgart). Melting points are uncorrected and were determined using a Büchi B-545 apparatus. Absorption spectra were recorded on a Perkin Elmer Lambda 19 spectrometer and fluorescence emission spectra on a Perkin Elmer LS 55 spectrometer in 1 cm cuvettes. All reactions were monitored by TLC (aluminium plates, pre-coated with silica gel, Merck Si60 F254).
Cyclic voltammetry experiments were performed using a computer controlled Metrohm Autolab PGSTAT 10 Potentiostat in a three-electrode single-compartment cell (5 mL). The platinum working electrode consisted of a platinum wire sealed in a soft glass tube with a surface of $A =$
0.785 mm², which was polished down to 0.5 µm with Buehler polishing paste prior to use in order to obtain reproducible surfaces. The counter electrode consisted of a platinum wire and the reference electrode was a Ag/AgCl secondary electrode. A 0.1 M solution of tetrabutylammonium hexafluorophosphate (TBAHFP, Fluka) was used as electrolyte in dichloromethane, distilled over sulfuric acid in an argon atmosphere. In electrochemical characterization concentrations of 10⁻³ mol L⁻¹ of the electroactive species were applied. CV-measurements were performed at 295 K using a scan rate of 100 mV s⁻¹. All potentials were internally referenced to the ferrocene /ferricenium couple (Fc/Fc⁺).
Experimental Section

2-Propynyl-2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannopyranoside, 1.
\(\alpha,\beta\)-D-mannopyranose pentaacetate (3.22 g, 8.24 mmol) and propargyl alcohol (0.77 ml, 13 mmol) were dissolved in 40 mL dry dichloromethane (DCM). The mixture was cooled to 0°C and boron trifluoride etherate (15 mmol, 2.13 g) was added in two portions. After removal of the cooling bath the reaction was stirred for 24 h. Subsequently, the mixture was quenched with saturated NaHCO₃ solution and the organic phase was dried over Na₂SO₄. Small amounts (ca. 7%) of the \(\beta\)-anomere were removed by column chromatography using \(n\)-hexane:ethylacetate as eluent. 2.7 g (84 %) of 1 were obtained as colorless solid.

\(^1\)H-NMR (400 MHz, CDCl₃): \(\delta\) 1.98 (s, 3H); 2.03 (s, 3H); 2.09 (s, 3H); 2.15 (s, 3H), 2.47 (t, 1H, \(J = 2.53\) Hz); 4.01 (m, 1H); 4.11 (dd, 1H, \(J = 12.1\)Hz, 2.5Hz); 4.25-4.30 (m, 3H); 5.02 (d, 1H, 1.96 Hz); 5.25 – 5.36 (3H, m) ppm. \(^1\)C-NMR (101 MHz, CDCl₃): \(\delta\) = 20.6, 20.7, 20.7, 20.8, 54.9, 62.4, 66.1, 68.9, 69.0, 69.4, 75.6, 77.9, 96.3, 169.7, 169.8, 170.6 ppm; gated decoupled \(^1\)C NMR spectroscopy indicated a \(J_{C1-H1}\) value of 174.1 Hz. MS (Cl) \(m/z\) = 387; Elemental analysis (%): found: C: 52.82, H: 5.72, calc. for C\(_{17}\)H\(_{22}\)O\(_{10}\) C: 52.85, H: 5.74, [\(\alpha\)]\(_D\)\(^{23}\) = +57.8 deg, \(c=0.27\) g/dl in DCM.

2-Propynyl-2,3,4,6-tetra-O-acetyl-\(\beta\)-D-galactopyranoside, 2.
3.15 g (3.8 mmol) \(\beta\)-D-galactopyranose pentaacetate and propargyl alcohol (0.27 ml, 4.61 mmol) were dissolved in 30 mL dry dichloromethane. The mixture was cooled to 0°C and boron trifluoride etherate (5.76 mmol, 0.72 ml) was added in two portions. After removal of the cooling bath the reaction was stirred for 2 h. Subsequently, the mixture was quenched with anhydrous K₂CO₃ (480 mg) and after filtration the organic phase was dried over Na₂SO₄. After removal of the solvent small amounts of starting material was separated by column chromatography and 1.0 g (67 %) of 2 was yielded as a colorless solid.

\(^1\)H-NMR (CDCl₃, 400 MHz.) \(\delta\) = 5.76 (d, 1H, 3.3 Hz, H4), 5.20 (dd, 1H, 7.9 Hz, 10.4 Hz, H2) 5.03 (dd, 1 H,10.5 Hz, 2.4 Hz, H-3), 4.72 (d, 1 H, 7.9 Hz,H-1), 4.25 (d, 2 H, J1 ,13 2.4 Hz, H-1), 4.12(m, 2 H, H-6), 3.91 (m, 1 H, H-5), 2.44 (t, 1 H, 2.4 Hz, H-3), 2.12, 2.05, 2.03, 1.96 (4 s, 12H,OCOCH₃) ppm. \(^1\)C-NMR (101 MHz, CDCl₃): \(\delta\) = 20.5, 2x20.6, 20.7, 55.8, 61.1, 66.9, 68.4, 70.7, 70.8, 75.4, 78.1, 98.6, 169.5, 170.1, 170.2, 170.3 ppm.
Elemental analysis (%): found: C: H: 52.91, 5.73, calc. for C\(_{17}\)H\(_{22}\)O\(_{10}\) C: 52.85, H: 5.74.
5,5"α,α-Di(-O-propargyl-2,3,4,6-tetraacetylmannoside)[2,2',3',5"]ter thiophene, 5a.

In a two neck round bottom flask, first 3 (112 mg, 0.224 mmol) in 5 mL THF was degassed completely by bubbling with Ar-flow. Then catalyst PdCl2(PPh3)2 (7.86 mg, 11.2 μmol, 4 mol %) and Cul (1.6 mg, 8.8 μmol, 2 mol%) were added and after 5 min 2-propynyl-α-D- mannopyranoside, 1 (190 mg, 0.492 mmol) and finally degassed diisopropylamine (10 mL) were added. The reaction mixture was stirred for 4-5 hrs. Then the catalytic residues were filtered and the filtrate evaporated in rotary evaporator. The solid obtained was purified by column chromatography (SiO2, gradient elution using DCM → 10% EtOAc/DCM → 30% EtOAc/DCM) to obtain 5a as colorless solid 188 mg (83 %), mp 69-74°C.

1H NMR (CDCl3) δ = 1.98, 2.03, 2.09, 2.15 (24H, COCH3), 4.03 (m, 2H), 4.06 (m, 2H), 4.28 (d, J = 5.04 Hz, 1H), 4.31 (d, J = 4.8 Hz, 1H), 4.49 (s, 4H), 5.06 (m, 2H), 5.2-5.3 (m, 6H), 6.90 (d, J =3.8 Hz, 1H), 6.97 (d, J =3.8 Hz, 1H), 7.10-7.15 (m, 3H), 7.29 (d, J = 5.32 Hz, 1H) ppm.

13C NMR (CDCl3) δ = 20.61, 20.65, 20.70, 20.85 (COCH3), 55.66, 55.72, 60.33, 62.23, 65.88, 68.92, 69.25, 80.11, 80.36, 88.0, 88.44, 96.22, 99.92, 121.86, 123.04, 125.48, 126.58, 127.75, 129.76, 131.23, 131.61, 133.23, 133.28, 136.62, 139.19, 169.65, 169.80, 169.88, 170.60 ppm.

MALDI-TOF mass m/z 1039.3 (M+ + Na), 1055.2 (M+ + K) (cald. for C46H48O20S3: 1016.08).

Elemental Analysis (%): cald. C: 54.32, H 4.76, S 9.46, for C46H48O20S3, found: C: 54.74, H 4.89, S 9.20
5,5'''\alpha,\alpha-Di(-O-propargyl-mannoside)[2,2',3',5''']terthiophene, 5b

5a (80 mg, 0.086 mmol) was dissolved in a mixture of 30 ml methanol (abs.). After adding a catalytic amount sodium methanolate (0.02 ml, 0.3M) the reaction mixture was allowed stirring for 1 hour. Neutralization with ion exchange resin Dowex Marathon C and subsequent filtration provided a clear solution. After removal of the solvent the deprotected 5b was yielded as colorless solid in a 96 % yield (56 mg, 0.08 mmol).

$^1$H-NMR MeOD $d_4$, (400 MHz), δ = 7.49 (d, 5.31 Hz, 1H) 7.20 (d, 5.31 Hz, 1H), 7.18 (d, 3.79 Hz, 1H), 7.15 (d, 3.79 Hz, 1H), 7.02, d, 3.78 Hz, 1H (H1'), 7.00, d, 3.78 Hz, 1H (H1'), 5.01 (d, 1.52Hz , 2H), 4.52, d, 3.28 Hz, 4H, (CH$_2$), 3.88 – 3.82 (m, 4H) 3.60 – 3.76, 6H, (H2'', H3'', H4''), 3.54 – 3.58 (m, 2H H5'') ppm.

$^{13}$C -NMR MeOD $d_4$ (100 MHz), δ =140.16, 137.5, 134.1, 134.0, 133.4, 132.1, 130.6, 129.5, 128.1, 127.2, 125.2, 123.9, 100.2, 100.1, 91.2, 90.6, 80.1, 79.8, 75.2, 75.2, 72.5, 72.0, 68.4, 62.8, 55.7 ppm.

C$_{30}$H$_{32}$O$_{12}$S$_3$· 3H$_2$O Elemental analysis calc. C 49.04, H 5.21, S 13.09, fd. C 49.19, H 5.05, S: 13.53. MS (ESI) calc. for C$_{30}$H$_{32}$O$_{12}$S$_3$ [M + H]$^+$ = 680.8, found 703.2 (M + Na)$^+$.

5,5''''-bis(\alpha,\alpha-Di(-O-propargyl-2,3,4,6-tetraacetylmannoside)-[3',5''-bis(5-\alpha,\alpha-Di(-O-propargyl-2,3,4,6-tetraacetylmannoside thien-2-yl)]-2,2':5',2'':4'',2''''-quaterthiophene, 6a.
In a two neck round bottom flask, first 5,5'''-diiodo-3',5'''-bis(5-iodo-2-thienyl)-2,2':5',2''':4'',2'''', 4, (122 mg, 122 μmol) was dissolved in Ar degassed THF (10 mL). Then catalyst PdCl₂(PPh₃)₂ (8.6 mg, 12.2 μmol, 10 mol %) and CuI (1.2 mg, 6.1 μmol, 5 mol%) were added and after 5 min propargyl mannose (190 mg, 0.492 mmol). Finally Ar degassed diisopropylamine (10 mL) was added. The reaction mixture was stirred for 4-5 hrs at r.t. Then the catalytic residues were filtered and the filtrate evaporated in rotary evaporator. The solid obtained was purified by column chromatography (SiO₂, gradient elution using DCM → 20% EtOAc/DCM → 40% EtOAc/DCM) to obtain 6a as yellowish solid 85 mg (103 mg, 63 %), mp 69-85° C.

¹H NMR (CDCl₃, δ) 1.982, 2.033, 2.095, 2.155, 4.03-4.08 (br, 4H), 4.09, (t, 2H), 4.13, (t, 2H), 4.29 (dd, J = 1.86, 5.02, 2H), 4.31 (dd, J = 1.91, 5.05, 2H), 4.50, 4.51, (s, 8H), 5.05, 5.06, 5.07, 5.07, (s, 4H), 5.25-5.40 (m, 12H), 6.96 (d, J = 3.76 Hz, 2H), 7.00 (d, J = 3.82 Hz, 2H), 7.14 (d, J = 3.83 Hz, 2H), 7.16 (d, J = 3.77 Hz, 2H) 7.18 (s, 2H).¹³C NMR (CDCl₃, δ) 20.60, 20.64, 20.69, 20.75, 55.72, 55.75, 62.31, 66.02, 68.95, 68.96, 69.02, 69.33, 69.35, 80.05, 80.23, 88.31, 88.90, 96.33, 122.53, 123.33, 126.83, 127.18, 127.66, 131.15, 132.06, 133.30, 135.18, 136.07, 138.37, 169.63, 169.76, 169.85, 170.56 ppm.

MALDI-TOF mass: calcd. 2032.1 found 2053.3 (M + Na)⁺, 2069.2 (M + K)⁺


5,5'''-bis(α,α-Di(-O-propargyl-mannoside)-[3',5''-bis(5-α,α-Di(-O-propargylmannoside thien-2-yl)]-2,2':5',2''':4'',2''''-quaterthiophene, 6b.
6a (50 mg, 24.6 μmol) was dissolved in a mixture of 30 ml (v/v) methanol (abs.)/THF mixture. After adding a catalytic amount sodium methanolate (0.02 ml, 0.3M) the reaction mixture was allowed stirring for 1 hour. Neutralization with ion exchange resin Dowex Marathon C and subsequent filtration provided a clear solution. After removal of the solvent the deprotected 6b was yielded as colorless solid in a 89 % yield (30 mg, 22 μmol).

$^1$H-NMR DMSO $d_6$ (400 MHz) $\delta = 7.70, s, 2H$, 7.37, d, 3.79 Hz, 2H, 7.36, d, 3.79 Hz, 2H, 7.30, d, 3.79 Hz, 2H, 7.26, d, 3.79 Hz, 2H, 4.81, d, 1.26 Hz, 4H, 4.48, m (AB-System CH$_2$ propargyl), 8H, (CH$_2$), 3.35 – 3.69, m, 24H (CH$_2$-6 mannoside) ppm.

$^{13}$C-NMR DMSO $d_6$ (100 MHz) $\delta$: 52.7, 54.3, 61.4, 67.2, 70.4, 71.2, 71.3, 74.9, 78.6, 78.9, 91.6, 92.3, 98.9, 99.0, 100.0, 122.67, 123.8, 126.4, 128.9, 129.4, 129.8, 130.3, 132.9, 133.9, 134.2, 135.2, 137.6.

MS (ESI) calc. for C$_{60}$H$_{62}$O$_{24}$S$_6$ [M + H]$^+$ = 1359.2, found 1381.6 (M + Na)$^+$.
5,5′′′′-Bis(α,α-Di(-O-propargyl-2,3,4,6-tetraacetylgalactoside)-[3′,5′′-bis(5-β,β-Di(-O-propargyl-2,3,4,6-tetraacetylgalactoside thien-2-yl)]-2,2′:5′,2′′:4′′,2′′′′-quaterthiophene, 7a

In a two neck round bottom flask, first 4 (49.9 mg, 50 μmol) in 5 mL THF was degassed completely by bubbling with Ar-flow. Then catalyst PdCl₂(PPh₃)₂ (32 mg, 14 μmol, 7 mol %) and CuI (1.6 mg, 5 μmol, 2 mol%) were added and after 5 min 2-propynyl-β-D-galactopyranoside, 2 (85 mg, 0.22 mmol) and finally degassed diisopropylamine (10 mL) were added. The reaction mixture was stirred for 4-5 hrs. Then the catalytic residues were filtered and the filtrate evaporated in rotary evaporator. The solid obtained was purified by column chromatography (SiO₂, gradient elution using DCM → 10% EtOAc/DCM → 30% EtOAc/DCM) to obtain 7a as colorless solid 80 mg (79 %).

¹H-NMR (CDCl₃, 400 MHz) δ: 1.98 (s, 12H, Ac,), 2.04 (s, 12H, Ac), 2.05 (s, 6H, Ac,), 2.06 (s, 6H, Ac), 2.15 (s, 12H, CH₃,), 3.96 (m, 4H, H5), 4.11-4.23 (m, 8H, (CH₂galactose)), 4.62 (2s, CH₂propargyl, 8H), 4.72 (2d, 7.9 Hz, 4H, H1), 5.07 (m, 4H, 3.1 Hz,), 5.23 (m, 4H,), 5.40 (d, 3.3 Hz, 4H), 6.98 (d, 2H, 3.76 Hz,), 7.02 (d, 2H, 3.81 Hz,), 7.13 (d, 2H, 3.81 Hz), 7.16 (d, 2H, 3.76 Hz,), 7.20 (s, 2H) ppm, ¹³C-NMR (CDCl₃, 100.13 MHz) δ: 170.3, 170.2, 170.1, 169.5, 138.3, 136.0, 135.2, 133.0, 132.1, 67.0, 61.2, 57.0, 56.9, 20.8, 20.64, 20.61, 20.5 ppm.

MALDI-TOF mass: calcd. 2032.13 found 2032.0 (M)⁺

Elemental analysis: cald. for C₉₂H₈₀O₄₀S₆ . 8H₂O C: 50.78, H 5.09 fd. C: 50.55, H 5.02

HRMS: calcd. 2030.3646, fd. 2031.4051
5,5''''-Bis(α,α-Di(-O-propargyl-galactoside)-[3',5'''-bis(5-β,β-Di(-O-propargylgalactoside thien-2-yl)]-2,2':5',2'':4'',2'''-quaterthiophene, 7b

7a (50 mg, 24.6 μmol) was dissolved in a mixture of 30 ml (v/v) methanol (abs.)/THF mixture. After adding a catalytic amount sodium methanolate the reaction mixture was allowed stirring for 1 hour. Neutralization with ion exchange resin Dowex Marathon C and subsequent filtration provided a clear solution. After removal of the solvent the 7b was yielded as yellowish solid in a 80.1 % yield (27 mg, 19.9 μmol).

\[ 1^H-NMR \text{ DMSO } \delta = 7.70, s, 2H, 7.36, d, 3.79 \text{ Hz}, 2H, 7.35, d, 3.79 \text{ Hz}, 2H, 7.29, d, 3.79 \text{ Hz}, 2H, 7.26, d, 3.79 \text{ Hz}, 2H, 4.58, m (AB-System CH} \text{propargyl}), 8H, 4.24, d, 6.91 \text{ Hz}, 4H, 3.35 – 3.69, m, 24H (CH} \text{6 galactose) ppm.}\]

\[ 13^C-NMR \text{ (DMSO } \delta = 49.5, 50.2, 56.5, 60.6, 61.3, 67.3.1, 67.9, 68.9, 70.3, 71.2, 71.5, 72.7, 74.1, 74.8, 75.7, 76.1, 77.7, 98.2, 102.2, 123.5, 133.3, 134.1, 134.3, 135.2 (2), 142.8 \text{ ppm.}\]

MS (Maldi tof) calc. for C\text{60H}6\text{2O}2\text{4S}6 \text{ [M + H]}^+ = 1358.2, found 1356.0

Elemental analysis: cald. for C\text{60H}6\text{2O}2\text{4S}6 \cdot 7 \text{ H2O C: 48.51, H 5.16, S 12.95, fd. C: 48.38, H 5.12, S 12.90}
Electrochemical characterization

**Fig. S1** right: CV of 5a and 8 measured in dichloromethane/TBAHFP (0.1M), c = 10^{-3} mol L^{-1} vs. Fc/Fc^+, 295 K, scan rate = 100 mV s^{-1}.

**Fig. S2** left: CV of 6a and 9 measured in dichloromethane/TBAHFP (0.1M), c = 10^{-3} mol L^{-1} vs. Fc/Fc^+, 295 K, scan rate = 100 mV s^{-1}.

**Fig. S3**: CV of 7a measured in dichloromethane/TBAHFP (0.1M), c = 10^{-3} mol L^{-1} vs. Fc/Fc^+, 295 K, scan rate = 100 mV s^{-1}. 
Turbidity analysis

Fig. S4: Turbidity analysis in 0.1 M Hepes Buffer, 0.1 M CaCl₂, MnCl₂, blue curve: change of optical density of 5b upon addition of BSA, black curve: change of optical density of 5b upon addition of Con A, red curve after 25 minutes 1 mg α-methylmannoside was added (measured at 500 nm).

Fig. S5: Turbidity analysis in 0.1 M Hepes Buffer, 0.1 M CaCl₂, MnCl₂, blue curve: change of optical density of 6b upon addition of BSA black curve: change of optical density of 6b upon addition of Con A, red curve after 25 minutes 1 mg α-methylmannoside was added (measured at 500 nm).
Control experiment

Fig. S6: Turbidity analysis in 0.1 M Hepes Buffer, 0.1 M CaCl₂, MnCl₂, black curve: optical density of 7b blue curve: change of optical density of 7b upon addition of Con A, measured at 500 nm

Fig. S7: Fluorescence spectra of 5b, in 0.1 M Hepes Buffer, 0.1 M CaCl₂, MnCl₂, and CaCl₂ [1 mM] (dark line), quenched emission upon addition of Con A (dotted) and 20 min stirring, restored emission after addition of 1 mg α-methylmannoside (red), λ excit = 360 nm.
References