Novel glucose-ferrocenyl derivatives: synthesis and properties

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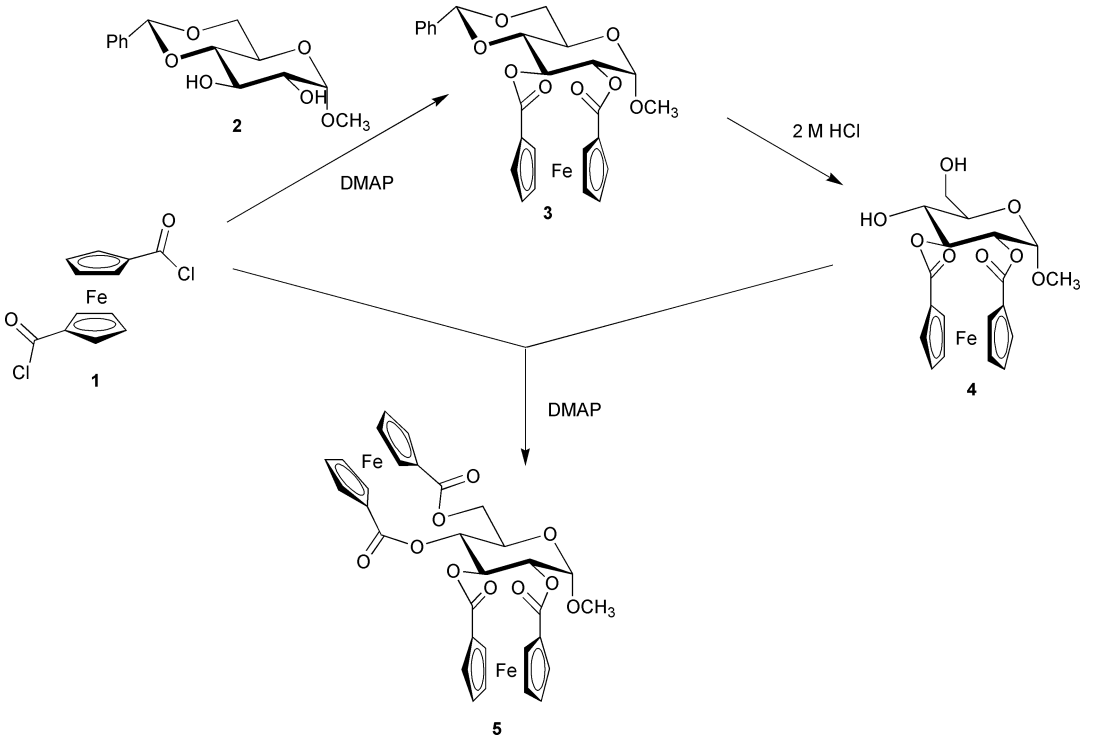
The synthesis and characterization of 4,6-benzyliden-2,3-(ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside, 2,3-(ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside and 4,6 : 2,3-bis(ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside are reported. In addition the cytostatic activity of 2,3-(ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside has been assayed.

Since cisplatin was introduced in 1969 as antitumor agent[1] there has been a growing interest in the use of metal-containing compounds in medicine and biological areas.[2,3] The anticancer activity of several metallocene derivatives has been reported.[4,5] Monosaccharides as basic biomolecules are building blocks for biopolymers like polysaccharides, glycolipids and nucleic acids.[6,7]

A new interesting class of compounds with special chemical, physical and biological properties can be obtained by the combination of metallocenes and biomolecules.[8–12] A number of glucose-ferrocene conjugates and transition metal complexes with carbohydrates are currently known.[13–16] They are extensively investigated as chiral matrices and diagnostic tools in biosciences.[17–19]

Herein we report on the step-by-step synthesis of glucose-ferrocene conjugates with one and two molecules of 1,1′-ferrocenedicarboxylic acid. The antitumor activity of 2,3-(ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside has been screened *in vitro* towards several tumor cell lines. The absolute configuration of this compound has been determined by single crystal X-ray diffraction analysis.

The condensation reaction (Scheme 1) of 1,1′-ferrocenedicarbonyl dichloride, **1**,[20] and 4,6-benzylidene-*O*-methyl-α-D-glucopyranoside, **2**, with *N*,*N*-dimethylaminopyridine (DMAP) gave rise to 4,6-benzylidene-2,3-(ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside, **3**, in 78% yield. Refluxing of **3** in a 1.5 : 1 : 2.5 mixture of 2 M HCl, methanol and THF for 2 h resulted in the loss of the benzylidene group and formation of



Scheme 1 Synthesis of ferrocene-substituted glucopyranosides.

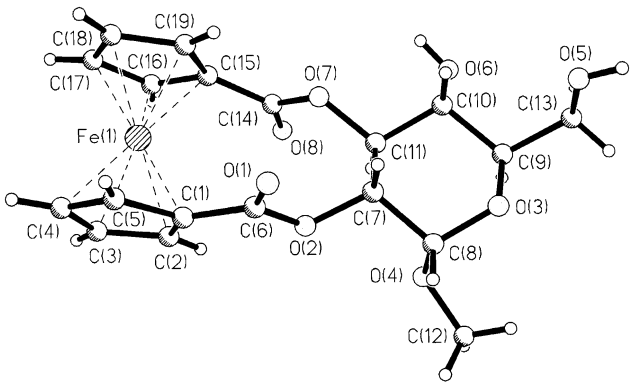


Fig. 1 The perspective view of 2,3-{(*R*,*S*)-(ferrocene-1,1′-dicarbonyl)}-*O*-methyl-α-D-glucopyranoside, **4**. The solvent molecule is omitted for clarity. Selected bond lengths (Å) and angles (°): C(8)–C(7) 1.518(3), C(7)–C(11) 1.518(3), C(11)–C(10) 1.510(3), C(10)–C(9) 1.525(3), C(9)–O(3) 1.454(2), O(3)–C(8) 1.426(2) Å; C(8)–C(7)–C(11) 108.64(15), C(7)–C(11)–C(10) 109.48(15), C(11)–C(10)–C(9) 109.93(15), C(10)–C(9)–O(3) 111.73(14), C(9)–O(3)–C(8) 114.78(14), O(3)–C(8)–C(7) 107.59(14).

2,3-(ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside, **4**, in 80% yield.[21] More prolonged reaction times led to a marked decrease of the yield of **4**. Note that the reaction can also be performed at room temperature. However, much longer reaction times are needed to obtain comparable amounts of the product. It is also worth mentioning that the synthesis of **3** and **4** has also been reported by Itoh *et al.*, but the authors have not provided any analytical data for these compounds.[13] 4,6:2,3-Bis(ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside, **5**, was prepared in 67% yield by a condensation reaction of **4** and **1** with DMAP. Our attempts to perform the synthesis of **5** in one step starting from *O*-methyl-α-D-glucopyranoside resulted in less than 1% yield.

^{1}H and ^{13}C NMR spectra of **3**, **4** and **5** are in agreement with their structures. The most intense peaks in the positive ion ESI mass spectra of **3**, **4** and **5** at m/z 543, 455 and 693, correspondingly, are due to $[M + Na]^+$ ions. The found isotopic distributions are consistent with the known isotopic composition for these ions. In addition, other peaks found can easily be related to species resulting from the fragmentation of the corresponding molecular ions. A strong signal at m/z 414 in the spectrum of **3** presumably arose from the loss of the benzylidene protecting group. A weak signal in the spectrum of **4** at m/z 297 can be attributed to the $[M - Fc]^+$ ion.

The absolute configuration of **4** has been determined by X-ray crystallography. The result of the single crystal X-ray diffraction analysis is shown in Fig. 1. The α-D-glucopyranoside ring adopts a chair conformation. The C(7), C(11), O(3) and C(9) atoms are coplanar within ±0.031 Å. The atoms C(8) and C(10) are on opposite sides of this mean plane at −0.712 and +0.650 Å. The Cp rings assume an arrangement approaching the staggered conformation.

Finally, we tested the cytostatic activity of **4**. Using a concentration range up to 0.5 mM the IC_{50} was determined for the CH1 cells ($IC_{50} = 0.34$ mM). For the other three cell lines only minor cytotoxic effects were observed.

Experimental

The NMR spectra were recorded on a Bruker DPX 400 instrument (Ultrashield™ Magnet) at 400.13 MHz (^{1}H) and 100.63 MHz (^{13}C). Chemical shifts were measured relative to the solvent at 301 K.

The elemental analysis was done by the laboratory for elemental analysis of the Institute of Physical Chemistry, University of Vienna, with a Perkin Elmer 2400 CHN Elemental Analyzer. Mass spectra were measured on a Bruker *esquire*$_{3000}$™ (ESI). The specific rotations were measured on a Perkin Elmer 341.

X-Ray crystallography

The single crystal X-ray diffraction experiments were done on a NONIUS KAPPA instrument with a CCD detector at a temperature of 120 K. $C_{19}H_{22}FeO_9$, formula weight = 450.22, monoclinic, space group $P2_1$ (no. 4), $a = 5.8080(10)$, $b = 10.750(2)$, $c = 14.772(3)$ Å, $\beta = 93.44(3)°$, $U = 920.6(3)$ Å^3, $Z = 2$, $d_{calc.} = 1.624$ g cm^{-3}, $\mu = 8.72$ cm^{-1}, $F(000) = 468$; 4543 unique reflections observed. Refinement on all data and 274 parameters converged at $R_1 = 0.0272$, $wR_2 = 0.0622$ and Flack parameter at −0.001(10).

CCDC reference number 168911. See http://www.rsc.org/suppdata/njc/b2/b200701k/ for crystallographic data in CIF or other electronic format.

Synthesis of 4,6:2,3-Bis(ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside, 5

To a solution of 1,1′-ferrocenyldicarbonyl dichloride, **1** (0.5 g, 1.61 mmol) in CH_2Cl_2 (70 ml) a solution of **4** (0.69 g, 1.61 mmol) and DMAP (0.41 g, 3.38 mmol) in CH_2Cl_2 (100 ml) was slowly added. The reaction mixture was stirred at room temperature for 12 h and washed with cold water and brine. The organic phase was dried over Na_2SO_4 and the solvent was evaporated under vacuum. The crude product was purified by column chromatography (ethyl acetate–hexane 1 : 1). Yield: 0.72 g (67%). $[\alpha]_D^{20} = +261$ (*c* 0.25, CH_2Cl_2); mp: 346–348 °C (decomp.); ^{1}H NMR ($CDCl_3$) δ: 5.77 (tr, 1H, Glc-H3, $^3J = 10$), 5.48 (m, 1H, Glc-H4, $^3J = 9$), 5.39 (dd, 1H, Glc-H2, $^3J = 3.5$), 5.07 (m, 1H, Cp–H), 4.99 (m, 1H, Cp), 4.97–4.96 (m, 2H, Cp), 4.94 (d, 1H, Glc-H1), 4.92 (m, 1H, Cp), 4.85 (m, 1H, Cp), 4.74 (m, 1H, Cp), 4.71 (m, 1H, Cp), 4.69–4.68 (m, 2H, Cp), 4.53–4.47 (m, 4H, Glc-H6e, Glc-H5, Cp), 4.42–4.46 (m, 3H, Glc-H6a, Cp), 4.32 (m, 1H, Cp), 4.27 (m, 1H, Cp), 3.57 (s, 3H, OCH_3); ^{13}C {^{1}H} NMR ($CDCl_3$) δ: 171.57 [C(O)], 171.33 [C(O)], 168.84 [(C(O)], 168.01 [C(O)], 98.40 (Glc-C1), 77.01 (Cp), 75.40 (Cp), 75.37 (Cp), 74.06 (Cp), 73.52 (Cp), 73.45 (Cp), 73.23 (Cp), 73.10 (Cp), 72.53 (Cp), 72.43 (Cp), 72.40 (Cp), 72.28 (Cp), 72.25 (Cp), 72.19 (Cp), 72.14 (Cp), 71.91 (Glc-C4), 71.38 (Cp), 70.55 (Glc-C2), 70.27 (Glc-C3), 66.36 (Glc-C5), 65.38 (Glc-C6), 56.14 (OCH_3); Anal. calcd. for $C_{31}H_{26}Fe_2O_{10}$: C 55.55; H 3.91. Found: C 55.83; H 4.05. MS (ESI$^+$): m/z 693.14 $(M + Na)^+$.

Cytotoxicity

The cytotoxicity of **4** was determined by means of colorimetric microculture assay (MTT assay) using four human tumour cell lines: 41M, CH1 (both ovarian carcinoma), SK-BR-3 (mammary carcinoma), SW480 (colon carcinoma). The compound was dissolved in DMSO and then serially diluted in minimal essential medium (MEM) containing 10% heat-inactivated fetal calf serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 50 U ml^{-1} penicillin and 50 μg ml^{-1} streptomycin (all Gibco) to give a solution containing ⩽0.5% DMSO. Twenty-four hours after seeding adherent microcultures were exposed to this solution for 96 h in a humidified atmosphere containing 5% CO_2 at 37 °C.

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21 4,6-*O*-Benzylidene-2,3-(ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside, **3**. Yield: 2.87 g, (78%). $[\alpha]_D^{20} = +305$ (*c* 0.25, CH_2Cl_2); mp: 244–245 °C; ^{1}H NMR ($CDCl_3$) δ: 7.50 (dd, 2H, Ph–H), 7.37–7.33 (m, 3H, Ph–H), 5.85 (tr, 1H, Glc-H3, $^3J = 10.0$), 5.57 (s, 1H, Ph–CH), 5.36 (dd, 1H, Glc-H2, $^3J = 3.5$), 5.10 (m, 1H, Cp–H), 5.07 (m, 1H, Cp–H), 4.94 (d, 1H, Glc-H1), 4.79 (m, 1H, Cp–H), 4.74 (m, 1H, Cp–H), 4.70 (m, 1H, Cp–H), 4.69 (m, 1H, Cp–H), 4.37 (dd, 1H, Glc-H6e, $^2J = 10.0$, $^3J = 4.5$), 4.34 (m, 1H, Cp–H), 4.31 (m, 1H, Cp–H), 4.05 (m, 1H, Glc-H5, $^3J = 9.5$), 3.90–3.83 (m, 2H, Glc-H4, Glc-H6a), 3.50 (s, 3H, OCH3); ^{13}C {^{1}H} NMR ($CDCl_3$) δ: 171.95 [C(O)], 171.19 [C(O)], 137.26 (Ph), 129.49 (Ph), 128.62 (Ph, 2C), 126.70 (Ph, 2C), 102.00 (CH–Ph), 99.37 (Glc-C1), 79.41 (Glc-C4), 77.43 (Cp), 76.60 (Cp), 75.54 (Cp), 75.31 (Cp), 73.56 (Cp), 73.12 (Cp), 72.44 (Cp), 72.25 (Cp), 72.20 (Cp), 71.46 (Glc-C2), 71.26 (Cp), 69.75 (Glc-C3), 69.34 (Glc-C6), 63.95 (Glc-C5), 55.84 (OCH_3); MS (ESI$^+$): *m*/*z* 542.98 $(M + Na)^+$. 2,3-(Ferrocene-1,1′-dicarbonyl)-*O*-methyl-α-D-glucopyranoside, **4**. Yield: 1.33 g (80%). $[\alpha]_D^{20} = +416$ (*c* 0.25, CH_2Cl_2); mp: 98–99 °C; ^{1}H NMR ($CDCl_3$) δ: 5.59 (tr, 1H, Glc-H3, $^3J = 10$), 5.25 (dd, 1H, Glc-H2, $^3J = 3.5$), 5.07–5.06 (m, 2H, Cp–H), 4.92 (d, 1H, Glc-H1), 4.78 (m, 1H, Cp–H), 4.73–4.71 (m, 2H, Cp–H), 4.67 (m, 1H, Cp–H), 4.34–4.31 (m, 2H, Cp–H), 3.96–3.93 (m, 2H, Glc-H4, Glc-H6e), 3.90 (m, 1H, Glc-H5, $^3J = 5.0$, $^3J = 9.5$), 3.83 (m, 1H, Glc-H6a, $^2J = 7.5$), 3.48 (s, 3H, OCH_3); ^{13}C {^{1}H} NMR ($CDCl_3$) δ: 172.35 [C(O)], 171.81 [C(O)], 91.51 (Glc-C1), 77.01 (Cp), 76.92 (Cp), 75.48 (Cp), 75.45 (Cp), 73.82 (Glc-C4), 73.29 (Cp), 73.26 (Cp), 72.56 (Glc-C5), 72.44 (Cp), 72.34 (Cp), 71.91 (Cp), 71.53 (Cp), 70.73 (Glc-C2), 69.23 (Glc-C3), 62.38 (Glc-C6), 55.76 (OCH_3); MS (ESI$^+$): *m*/*z* 455.04 $(M + Na)^+$.