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SYNTHESIS OF A MACROCYCLIC CONJUGATE OF THE DITERPENOID ISOSTEVIOL AND GLUCURONIC ACID

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A macrocyclic conjugate of the natural diterpenoid isosteviol (16-oxo-ent-beyeran-19-oic acid) and glucuronic acid was synthesized for the first time. The conjugate contained two molecules of dihydroisosteviol β*-D-glucuronoside joined by 1,8-octanedicarboxylate and 1,8-octanedicarbazoyl spacers.*

Keywords: isosteviol, glucuronic acid, glycophanes, macrocycles, macrocyclic glycoterpenoids.

We recently reported the synthesis of the first conjugate of the diterpenoid isosteviol (16-oxo-*ent*-beyeran-19-oic acid) and glucuronic acid [1] in which two molecules of the isosteviol derivative dihydroisosteviol (16-hydroxy-*ent*-beyeran-19-oic acid) functionalized at the carboxyls by *β*-D-glucopyranuronoyl residues were connected by a 1,6-hexanedicarboxylate spacer. In continuation of research on the synthesis of macrocyclic glycoterpenoids [2], herein we present results for the synthesis of a macrocyclic conjugate of isosteviol and glucuronic acid. We used the methodology for synthesizing macrocyclic isosteviol derivatives [3, 4] in which two dihydroisosteviol molecules were first bound through the hydroxyls by reacting them with carboxylic acid chlorides. The resulting diacids (e.g., diacid **4** in Scheme 1) were converted to macrocycles by reacting their carboxylic acid chlorides with diols.

The starting carbohydrate component was D-(+)-glucurono-3,6-lactone (**1**), which was easily converted to methyl-1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronate (**2**) as before [5]. The methyl-1-deoxy-1-bromo-2,3,4-tri-*O*-acetyl-*β*-D-glucopyranuronate **3** that was obtained from it by the literature method [6] was then reacted with diacid **4** in refluxing CH_2Cl_2 in the presence of tetrabutylammonium bromide (TBAB) and potash. Bisglucuronide **5** was obtained in 56% yield. Its anomeric protons appeared in the PMR spectrum as a single doublet at 5.73 ppm with vicinal SSCC 8.02 Hz. This indicated convincingly that the glycoside bonds had the *β*-orientation.

In fact, the anomeric proton of methyl 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronate **2** resonated as a doublet at 5.73 ppm with vicinal SSCC 7.8 Hz [6] whereas the anomeric proton of the corresponding *α*-D-glucopyranuronate was found as a doublet at 6.34 ppm with SSCC 3.7 Hz [5]. Then, bisglucuronoside **5** was reacted at room temperature with hydrazine hydrate as before [7]. The PMR spectrum of the product, which was obtained in 90% yield, lacked the strong singlets corresponding to acetate resonances at 1.96, 1.98, and 1.99 ppm and the ester methyls of the glucopyranuronates at 3.68 ppm, which were observed in the spectrum of **5**. A multiplet that was characteristic of the hydrazide NH proton appeared at 7.69–7.73 ppm. All these data combined with MALDI mass spectral data indicated that dihydrazide **6** had formed. The reaction did not affect the carbohydrate anomeric centers. Therefore, the glycoside bonds in **6** retained the *β*-orientation. Its glucuronate anomeric protons resonated as a single doublet at 3.78 ppm with SSCC 9.63 Hz, which agreed with the literature for various *β*-glycosides of non-acylated glucuronic acid methyl ester (e.g., δ 4.26 ppm and $^3J = 9.4$ Hz [8]) and with previous work [9] in which the PMR spectrum of 1-*O*-*α*-acetyl-D-glucuronic acid was described. The anomeric proton in this compound occupied the *β*-position and still resonated at 5.02 ppm as a doublet with a much smaller constant of 4.2 Hz.

The macrocycle was formed by reacting dihydrazide **6** with sebacic acid dichloride. Bisglucuronoside **6** was thoroughly dehydrated before use. The reaction was carried out for 72 h until the ν(C=O) stretching band at 1800 cm^{-1} that was characteristic of sebacic acid dichloride vibrations disappeared in the IR spectrum of the reaction mixture.

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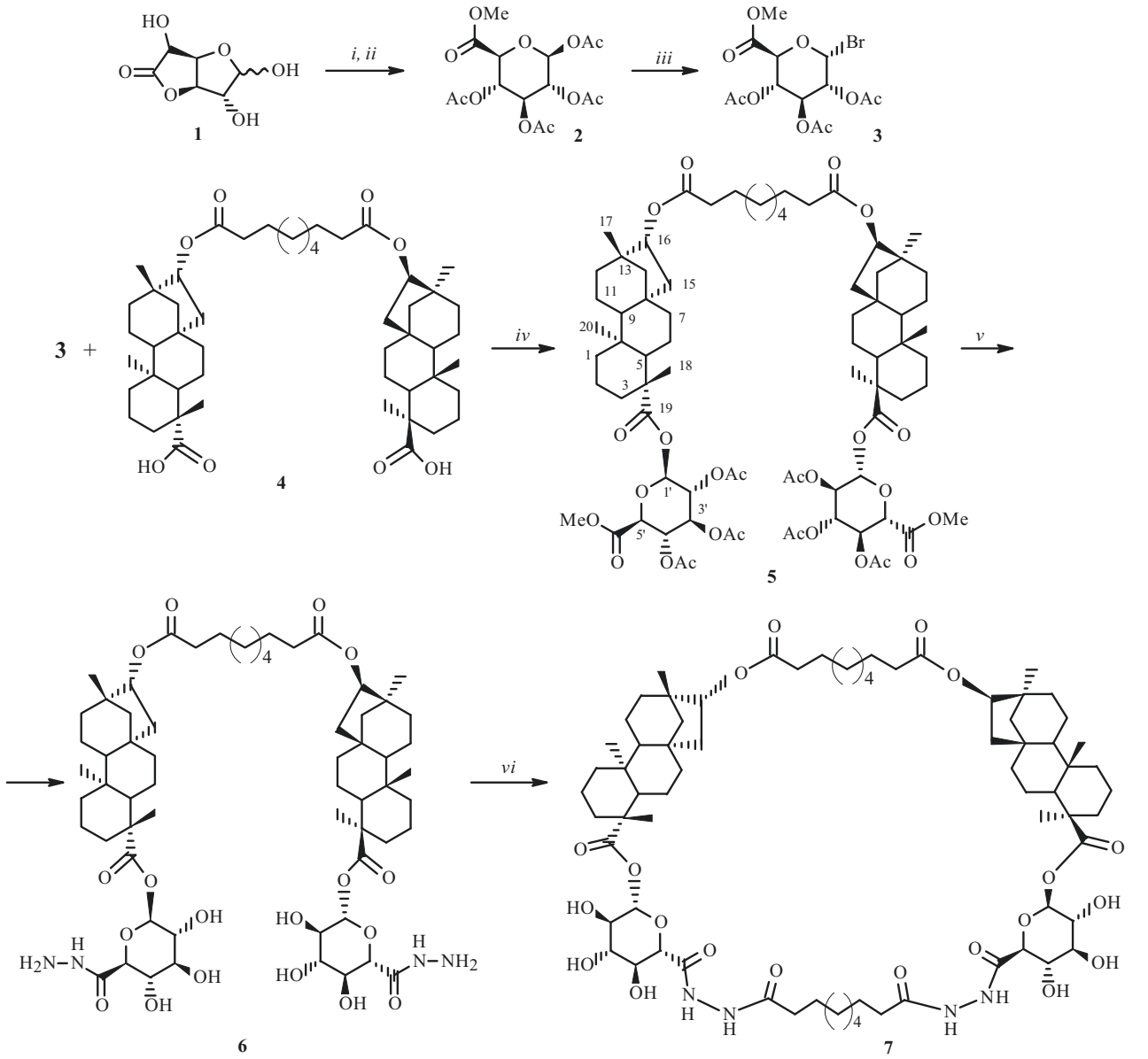

i. MeONa, CH_3OH, room temp., yield 100%; *ii.* Ac_2O, $HClO_4$, 0°C, yield 50%; *iii.* 33% HBr–AcOH, CH_2Cl_2, 0°C, yield 100%; *iv.* TBAB, K_2CO_3, CH_2Cl_2–H_2O, reflux, yield 56%; *v.* MeOH–$CHCl_3$, $NH_2NH_2 \cdot H_2O$, room temp., yield 90%; *vi.* $CHCl_3$–C_5H_5N, $ClOC(CH_2)_8COCl$, 4 Å mol. sieves, room temp., yield 30%

The reaction product that was isolated by column chromatography over silica gel in 30% yield was macrocycle **7** according to MALDI mass spectrometry. The PMR spectrum of the macrocycle contained the same characteristic isosteviol methyl resonances as dihydrazide **6**. Resonances of the 1,8-octanedicarboxylate and 1,8-octanedicarbazoyl spacers overlapped each other in the spectrum. The number of protons was determined from the integrated intensities. The dihydrazide protons resonated as multiplets at 7.31–7.38 and 7.71–7.77 ppm. Like for macrocyclic isosteviol derivatives with the same fragments [3], this was apparently indicative of several hindered conformers of macrocycle **7**. The anomeric protons of its glucuronates were found in the spectrum as a single doublet at 3.88 ppm with SSCC 9.51 Hz. The resonances of the carbohydrate anomeric protons in **5**–**7** as single doublets with SSCC 8–9.6 Hz provided unambiguous evidence that they all were *β*-glucuronosides.

Several macrocyclic glucuronic acid derivatives in which carbohydrate residues were connected by polymethylene [10] or alkenylene [11] spacers were reported. However, macrocycles containing terpenoid fragments in addition to glucuronic acid have not been reported. Thus, it could be concluded that we synthesized for the first time macrocyclic glycoterpenoids as the macrocyclic conjugate of the diterpenoid isosteviol and glucuronic acid.

EXPERIMENTAL

PMR spectra were recorded on Avance-400 and Avance-500 spectrometers (Bruker, Germany). MALDI mass spectra were obtained in an UltraFlex III TOF/TOF time-of-flight mass spectrometer (Bruker Daltonik GmbH, Germany). Measurements were made in the range *m*/*z* 400–3000. The data were processed using the FlexAnalysis 3.0 programs (Bruker Daltonik GmbH, Germany). The matrix consisted of *p*-nitroaniline. Samples were dissolved in $CHCl_3$ or $CHCl_3$–MeOH (1:1) at a concentration of 10^{-3} mg/mL and deposited by the dried drop method. Optical rotation was measured on a PerkinElmer 341 polarimeter (USA). IR spectra were recorded from films on a Vector 22 Fourier spectrometer (Bruker, Germany) in the range 400–4000 cm^{-1}. Melting points were determined on a Boetius apparatus. The course of reactions and purity of products were monitored by TLC on Sorbfil PTSKh-AF-A plate (Krasnodar, Russia) with detection by H_2SO_4 (5%) and heating. Compounds were isolated by flash chromatography over KSK silica gel (0.063–0.125 mm fraction, OOO KhromLab, Russia).

Compound **2** was prepared by the literature method [5], mp 177°C (MeOH), $[\alpha]_D^{20}$ +8.1° (*c* 1.1, $CHCl_3$); lit. mp 176°C [5], mp 177–178°C (EtOH), $[\alpha]_D^{25}$ +7.4° (*c* 2.0, $CHCl_3$) [12]. Compound **3** was prepared by the literature method [6], mp 105–106°C (EtOAc); lit. mp 106–107°C (EtOH) [12]. Diacid **4** was prepared by the literature method [13], mp 108–109°C, $[\alpha]_D^{20}$ –97.7° (*c* 1.1, $CHCl_3$); lit. mp 108–110°C, $[\alpha]_D^{20}$ –99° (*c* 0.17, C_6H_6) [13]. We used commercial D-(+)-glucurono-6,3-lactone (Acros, Belgium).

***Bis*[19-*nor*-4α(2,3,4-tetra-*O*-acetyl-5-methoxycarbonyl-β-D-glucopyranosyloxycarbonyl)-*ent*-beyeran-16-yl]-1,8-octanedicarboxylate (5).** Bromide **3** (0.5 g, 1.26 mmol) and diacid **4** (0.51 g, 0.63 mmol) were dissolved in freshly distilled CH_2Cl_2 (50 mL), treated with K_2CO_3 (0.55 g, 4 mmol) and TBAB (0.4 g, 1.26 mmol), diluted with H_2O (5 mL), stirred for 1 h at room temperature, and refluxed for 16 h. The solution became dark during the reaction. The reaction mixture was cooled, diluted with $CHCl_3$, washed with H_2O, and dried over $MgSO_4$. The solvent was distilled at reduced pressure. Chromatography of the residue over silica gel (petroleum ether–EtOAc eluent, 2:1) afforded a product that was dried *in vacuo* to give a white powder. Yield 0.5 g (56%), mp 114–115°C, $[\alpha]_D^{20}$ –39.9° (*c* 1.1, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1759 (C=O). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.70–1.90 (50H, m, *ent*-beyerane skeleton, spacer fragment $(CH_2)_6$), 0.66 (6H, s, H_3-20), 0.87 (6H, s, H_3-17), 1.16 (6H, s, H_3-18), 1.96 (6H, s, $CH_3C(O)$), 1.98 (6H, s, $CH_3C(O)$), 1.99 (6H, s, $CH_3C(O)$), 2.11 (2H, d, J = 13.08, H_{eq}-3), 2.25 (4H, t, J = 7.43, two "upper" spacer fragments 16-$OC(O)CH_2$), 3.68 (6H, s, H_3-6′), 4.06–4.14 (2H, m, H-5′), 4.66 (2H, dd, J = 10.7, 4.07, H-16), 5.14–5.29 (6H, m, H-2′, 3′, 4′), 5.73 (2H, d, J = 8.02, H-1′). Mass spectrum: *m/z* 1461.8 $[M + Na]^+$, calcd 1461.7 $[M + Na]^+$. $C_{76}H_{110}O_{26}$.

***Bis*[19-*nor*-4α(5-carbazoyl-β-D-glucopyranosyloxycarbonyl)-*ent*-beyeran-16-yl]-1,8-octanedicarboxylate (6).** Bis-derivative **5** (0.5 g, 0.35 mmol) was dissolved in a mixture of MeOH (30 mL) and $CHCl_3$ (10 mL), treated with hydrazine hydrate (0.5 mL), and held at room temperature until a crystalline precipitate formed. The obtained product was rinsed with MeOH. Yield 0.37 g (90%), mp 131–132°C, $[\alpha]_D^{20}$ –41.2° (*c* 0.7, $CHCl_3$–MeOH 1:1). IR spectrum (ν, cm^{-1}): 1373, 1610, 1674 (O=C–NH), 1759 (C=O), 3323 (OH, NH, NH_2). ^{1}H NMR spectrum (400 MHz, $CDCl_3$–CD_3OD 1:1, δ, ppm, J/Hz): 0.80–1.90 (50H, m, *ent*-beyerane skeleton, spacer fragment $(CH_2)_6$), 0.77 (6H, s, H_3-20), 0.89 (6H, s, H_3-17), 1.19 (6H, s, H_3-18), 2.16 (2H, d, J = 13.30, H_{eq}-3), 2.30 (4H, t, J = 7.38, two "upper" spacer fragments 16-$OC(O)CH_2$), 3.31–3.35 (2H, m, H-2′), 3.39–3.46 (4H, m, H-3′, 4′), 3.55–3.61 (2H, m, H-5′), 3.78 (2H, d, J = 9.63, H-1′), 4.67 (2H, dd, J = 10.6, 4.2, H-16), 5.39–5.42 (2H, m, 2 × OH), 7.69–7.73 (2H, m, NH). Mass spectrum: *m/z* 1209.5 $[M + Na]^+$, calcd 1209.7 $[M + Na]^+$. $C_{62}H_{98}N_4O_{18}$.

2,13,16,35-Tetraoxa-19,20,31,32-tetraaza-1,14(16,4α)di(19-*nor*-*ent*-beyerane)-17,34(1,5)di(β-D-glucopyranosyl)cyclohexatriacontaphane-3,12,15,18,21,30,33,36-octaone (7). Dihydrazide **6** (0.3 g, 0.25 mmol) was stored *in vacuo* for 3 h, dissolved in a mixture of anhydrous $CHCl_3$ (50 mL) and freshly distilled Py (5 mL), treated with 4Å molecular sieves, stored for 1 h, treated with sebacic acid chloride (0.06 g, 0.25 mmol), and stirred at room temperature for 72 h. The solvent was removed at reduced pressure. Chromatography of the residue over silica gel (EtOAc–MeOH eluent, 4:1) afforded a white amorphous powder. Yield 0.1 g (30%), mp 176–178°C, $[\alpha]_D^{20}$ –34.9° (*c* 0.55, MeOH). IR spectrum (ν, cm^{-1}): 1372, 1630, 1670 (O=C–NH), 1730 (C=O), 3290 (OH, NH). ^{1}H NMR spectrum (400 MHz, $CDCl_3$–CD_3OD 1:1, δ, ppm, J/Hz): 0.60–1.80 (62H, m, *ent*-beyerane skeleton, "upper" spacer fragment $(CH_2)_6$ and "lower" spacer fragment $(CH_2)_6$), 0.67 (6H, s, H_3-20), 0.79 (6H, s, H_3-17), 1.11 (6H, s, H_3-18), 2.08 (2H, d, J = 12.82, H_{eq}-3), 2.11–2.25 (8H, m, two "upper" spacer fragments 16-$OC(O)CH_2$ and two "lower" spacer fragments $NC(O)CH_2$), 3.38–3.48 (4H, m, H-2′, 3′), 3.51–3.62 (4H, m, H-4′, 5′), 3.88 (2H, d, J = 9.51, H-1′), 4.58 (2H, dd, J = 10.2, 4.7, H-16), 5.38 (2H, d, J = 7.48, 2 × OH), 7.31–7.38 (2H, m, C(O)NH), 7.71–7.77 (2H, m, C(O)NH). Mass spectrum: *m/z* 1375.7 $[M + Na]^+$, calcd 1375.8 $[M + Na]^+$. $C_{72}H_{112}N_4O_{20}$.

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