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Functionalization of the Double Bond in the Glycoside of the *Stevia rebaudiana* Plant Steviolbioside, as a Way to Macrocyclic Glycosides

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Abstract—The C^{16}=C^{17} bond in the glycoside steviolbioside from the plant *Stevia rebaudiana* was oxidized for the first time. Ketone, thiosemicarbazone, and oxime of steviolbioside with a heptaacetylated sophorosyl fragment, as well as binuclear and tetranuclear macrocyclic derivatives of this rebaudioside were synthesized.

Keywords: *Stevia*, glycosides, steviolbioside, macrocyclic compounds

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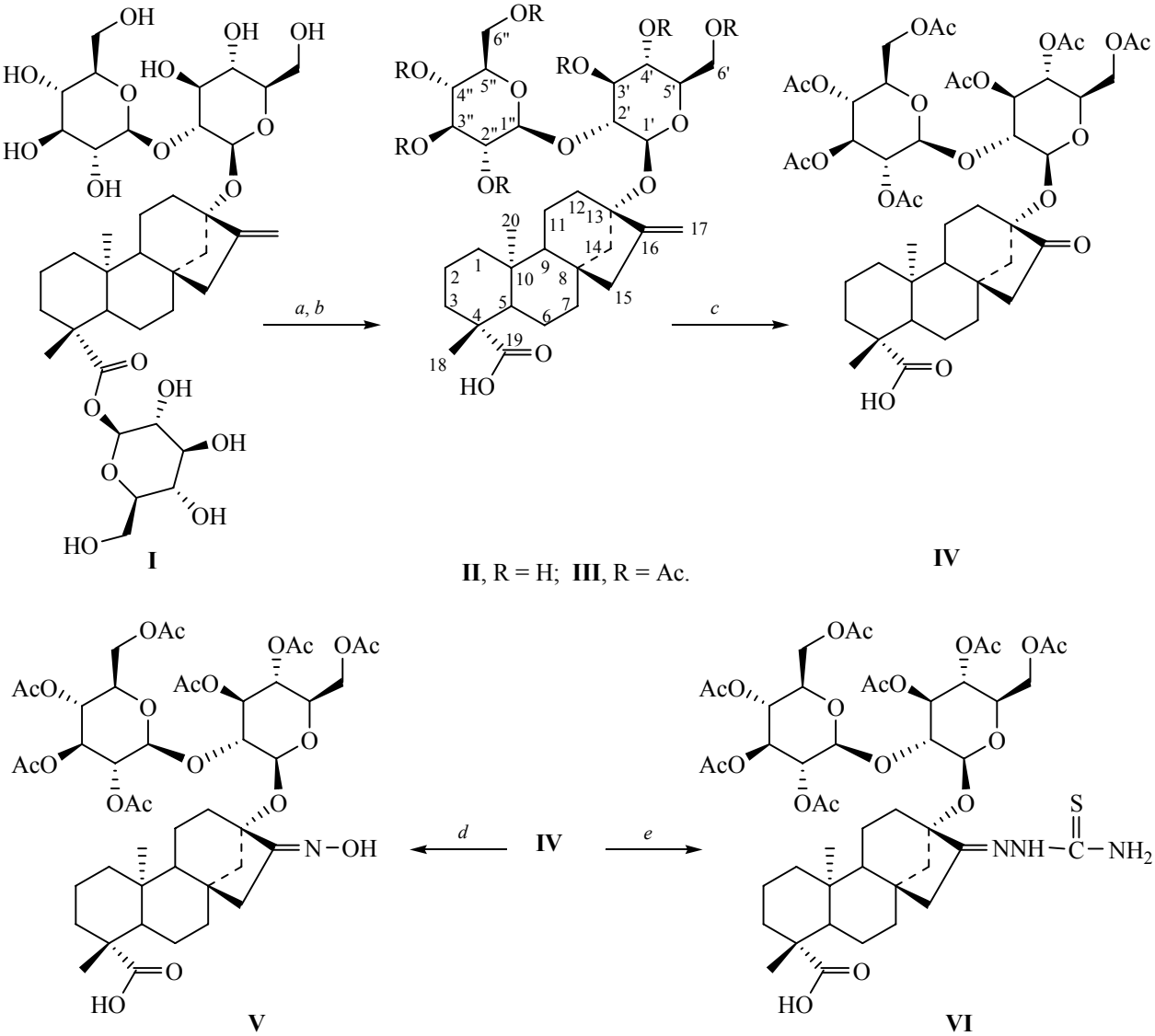
Diterpene glycosides from the plant *Stevia rebaudiana Bertoni* (rebaudiosides) are an attractive scaffold for chemical modification of secondary metabolites of higher plants with the aim to obtain new biologically active compounds. These diterpene glycosides are presently used in Japan, China, and Southeast Asian countries in the production of food sweeteners which are 250–300 times sweeter than sucrose [1]. It is interesting to note that, along with a sweet taste, stevioside **I**, the dominant glycoside of *S. rebaudiana* shows a broad-spectrum biological activity [2, 3], but, to our knowledge, most effort on chemical and enzymatic modifications of both stevioside **I** and glycosides from other plants of the genus *Stevia* was directed to sweetness enhancement and limited to variation of the number of carbohydrate moieties attached to the aglycone [1, 3]. An exception is the *S. rebaudiana* glycoside steviolbioside **II** [4] which was transformed into amides, esters, and hydrazides [5–9]. It will be emphasized that the authors of these works functionalized no other groups in steviolbioside **II** but carboxyl. The C^{16}=C^{17} double bond in **II** have never been functionalized. In the present work we for the first time oxidized this double bond to the oxo group and converted the latter to the thiosemicarbazide and oxime groups, and also synthesized binuclear and tetranuclear derivatives of steviolbioside, in which its molecules are tethered by diester and/or dihydrazonohydrazide linkers.

Steviolbioside **II** was prepared by alkaline hydrolysis of stevioside **I**. The hydroxy groups of the sophorosyl moiety in the latter were protected by acylation with acetic anhydride in pyridine. The C^{16}=C^{17} bond in glycoside **III** was oxidized with 4% aqueous osmium tetroxide [10, 11] to obtain steviolbioside **IV** in 50% yield. The formation of ketone **IV** was evidenced by the fact that the ^{1}H NMR spectrum of the latter no longer contained singlets at 5.10 and 5.76 ppm which are characteristic of the C^{16}=CH_2^{17} protons of steviolbioside **II** (Scheme 1).

Direct evidence for the oxidation of the double bond in the heptaacetylated steviolbioside **III** to the carbonyl group is provided by the fact that the reaction of glycoside **IV** with hydroxylamine hydrochloride forms oxime V, whereas its reaction with thiosemicarbazide gives thiosemicarbazone **VI.**

The ^{1}H NMR spectra of glycosides **I–VI** show characteristic signals of the $C^{20}H_3$ (0.99–1.23 ppm) and $C^{18}H_3$ protons (1.24–1.34 ppm), as well as the $C^{14}H_\alpha$ proton as doublet at 2.70 ppm in stevioside **I** and as doublet of doublets at 2.46–2.56 ppm in glicosides **II**, **IV**. The anomeric protons of the sophorosyl fragment

Scheme 1.



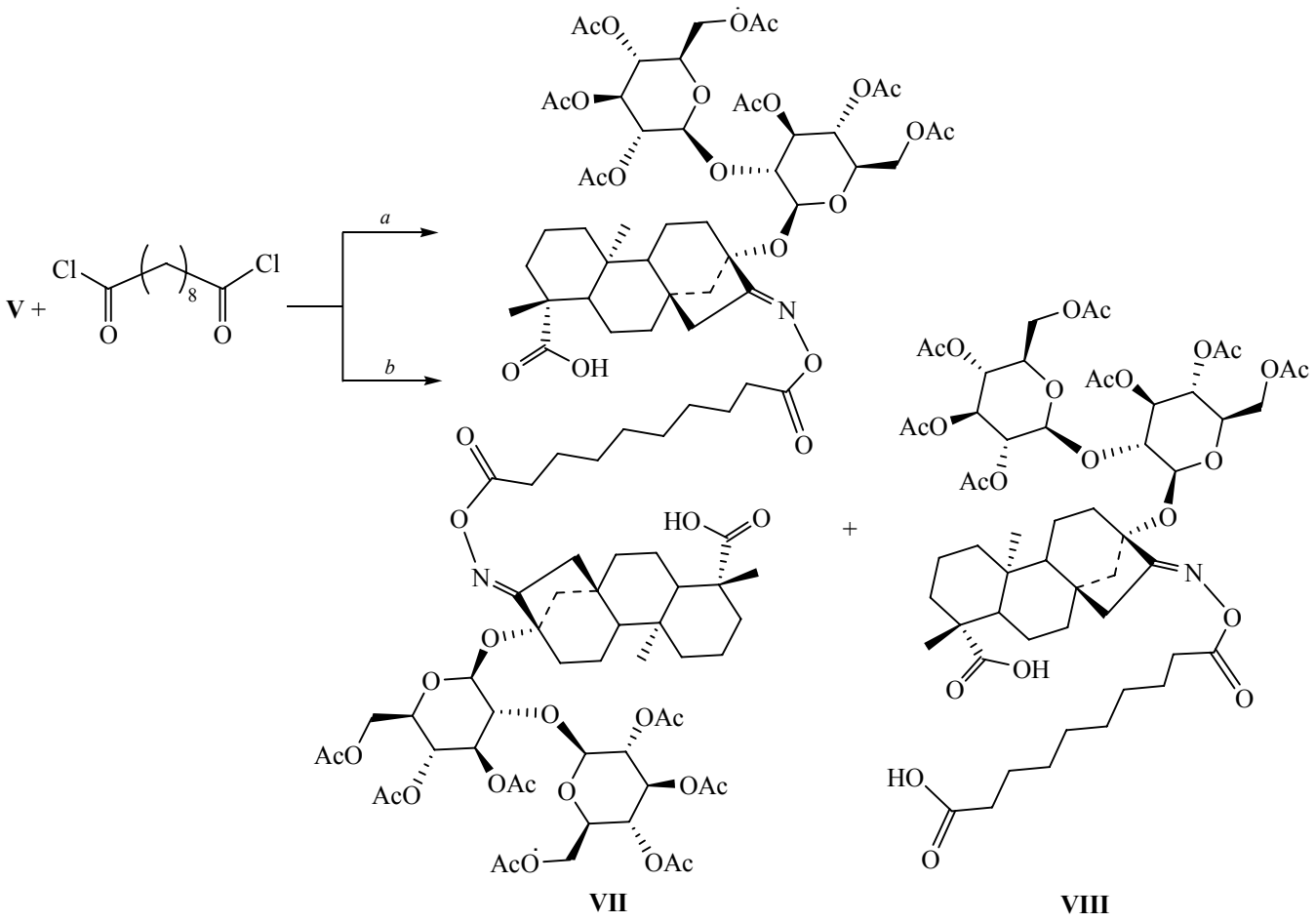
a, 10% KOH; *b*, Ac_2O, Py; *c*, OsO_4 (4%), $NaIO_4$, THF–H_2O, 20°C, 24 h; *d*, $NH_2OH{\cdot}HCl$, AcONa, EtOH, 20°C, 24 h; *e*, $NH_2NHC(S)NH_2$, AcONa, 20% H_2SO_4, EtOH, 20°C, 24 h.

in glycosides **I** and **II** give doublets at 5.13–5.17 and 5.27–5.30 ppm with ^3J 7.8–8 Hz, which implies β orientation of the $C^{1'}$–O and $C^{1''}$–O glycoside bonds in the glucopyranoside fragments in the sophorosyl moiety. The specified chemical shifts and coupling constants agree with published data [7–9, 11–13].

The introduction in the C^{16} position of steviolbioside of reactive ketone or oxime groups opens up a synthetic route to previously unknown macrocyclic derivatives of this glycoside. First we suggested a two-stage procedure involving the coupling of two molecules of glycoside **V** by the oxime groups at the first stage and by the carboxyl groups at the second. At the first stage we made use of a well-known reaction of oximes with carboxylic acid chlorides [14, 15]. Surprisingly, oxime **V** scarcely reacted with sebacoyl dichloride. Binuclear (**VII**) and mononuclear (**VIII**) oxime esters of heptaacetylated steviolbioside **V** were obtained in 1% yield as an unseparable mixture (Scheme 2).

Then we turned to another well-known method of alkylation of oximes, specifically, reaction with dibromoalkanes [16, 17]. An analog of steviolbioside

Scheme 2.



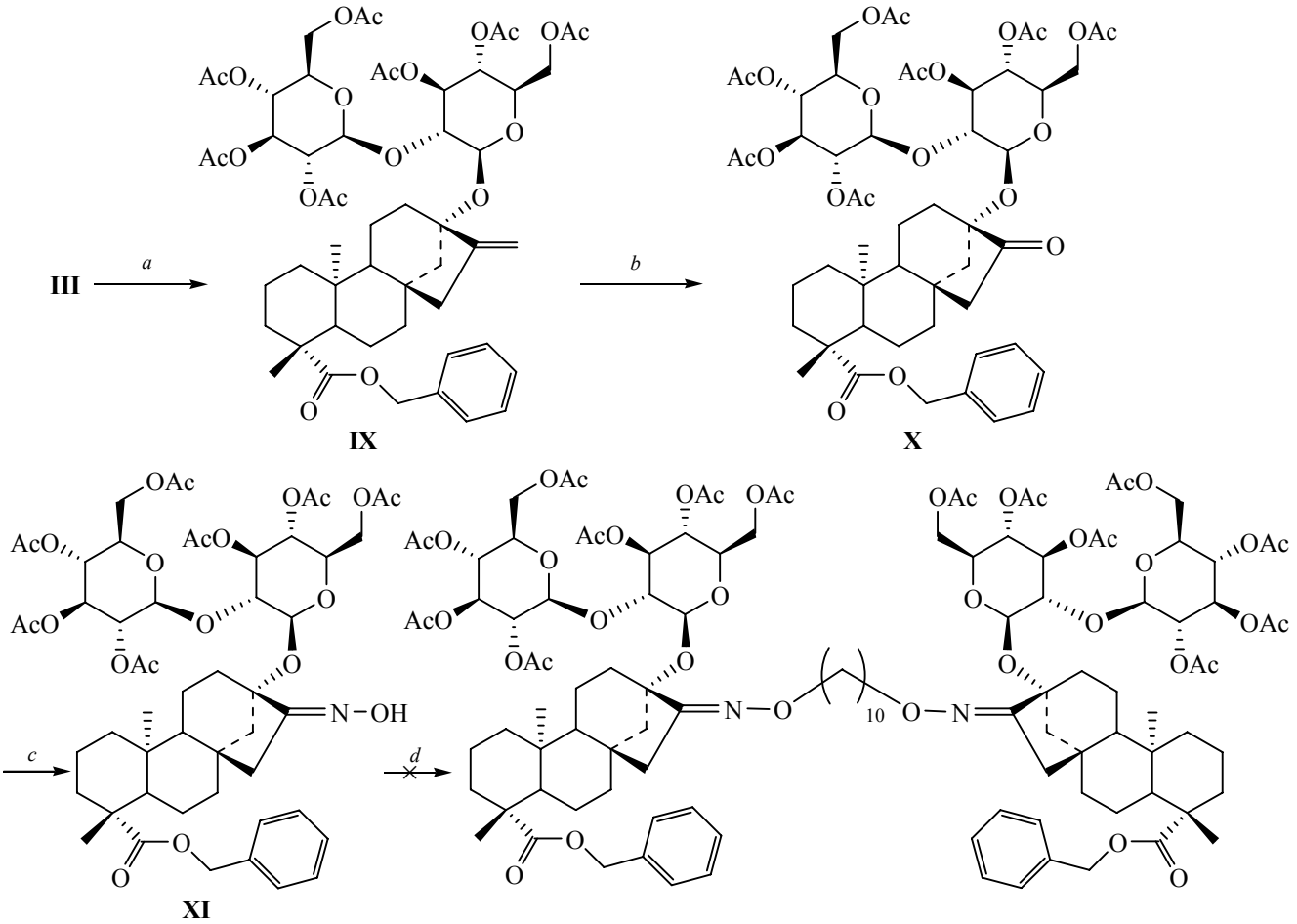
a, Et_3N, CH_2Cl_2, 20°C, 18 h; *b*, DMAP, Py, CH_2Cl_2, 20°C, 18 h.

oxime **V,** oxime **XI** with a protected carboxy group, was used. Unfortunately, the synthesis by a procedure analogous to that described by Akritopoulou-Zanze et al. [17] and involving 30-h refluxing oxime **XI** with 1,10-dibromodecane in a mixture of CH_2Cl_2 and 5% NaOH in the presence of tetrabutylammonium bromide (TBAB) resulted in no alkylation of the oxime group (Scheme 3).

Therefore we decided at the first stage to couple two steviolbioside molecules by the carboxy groups. To this end, heptaacetylated steviolbioside **III** was involved in the reaction with 1,8-dibromooctane in the superbasic KOH–DMSO medium. As a result, binuclear glycoside **XII** was obtained in 90% yield. Then the C^{16}=CH_2^{17} bonds of glycoside **XII** were oxidized with 4% aqueous osmium tetroxide. The resulting ketone **XIII** (yield 45%) was converted to dioxime **XIV** and isolated in 63% yield (Scheme 4).

For macrocyclization of binuclear steviolbioside derivative **XIV** we had to bind two its oxime groups. As the oxime group of glycoside **V** did not react with sebacoyl chloride, and the oxime group of glycoside **XI** did not react with 1,10-di-bromodecane, we made use of another well known [18, 19] reaction of oximes with carboxylic acids. However, the reaction of dioxime **XIV** with sebacic acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), carried out in accordance with the procedure described in [18], did not give macrocycle **XV**. The obtained product was, according to mass spectral data (MALDI), a mixture of the starting dioxime **XIV** and the intermediate product, sebacic acid bis(*N,N*′-

Scheme 3.



a, $BrCH_2Ph$, KOH–DMSO, 40°C, 24 h; *b*, OsO_4 (4%), $NaIO_4$, THF–H_2O, 20°C, 24 h; *c*, $NH_2OH·HCl$, AcONa, EtOH, 20°C, 24 h; *d*, 0.5 mol $Br(CH_2)_{10}Br$, NaOH (5%, 1 equiv), 0.1 mol TBAB, CH_2Cl_2, 40°C, 30 h.

dicyclohexylisoureate) (**XVI**). Note that there are some published precedents where DCC-catalyzed esterification or amidation reactions stopped at the stage of intermediate product formation [20, 21].

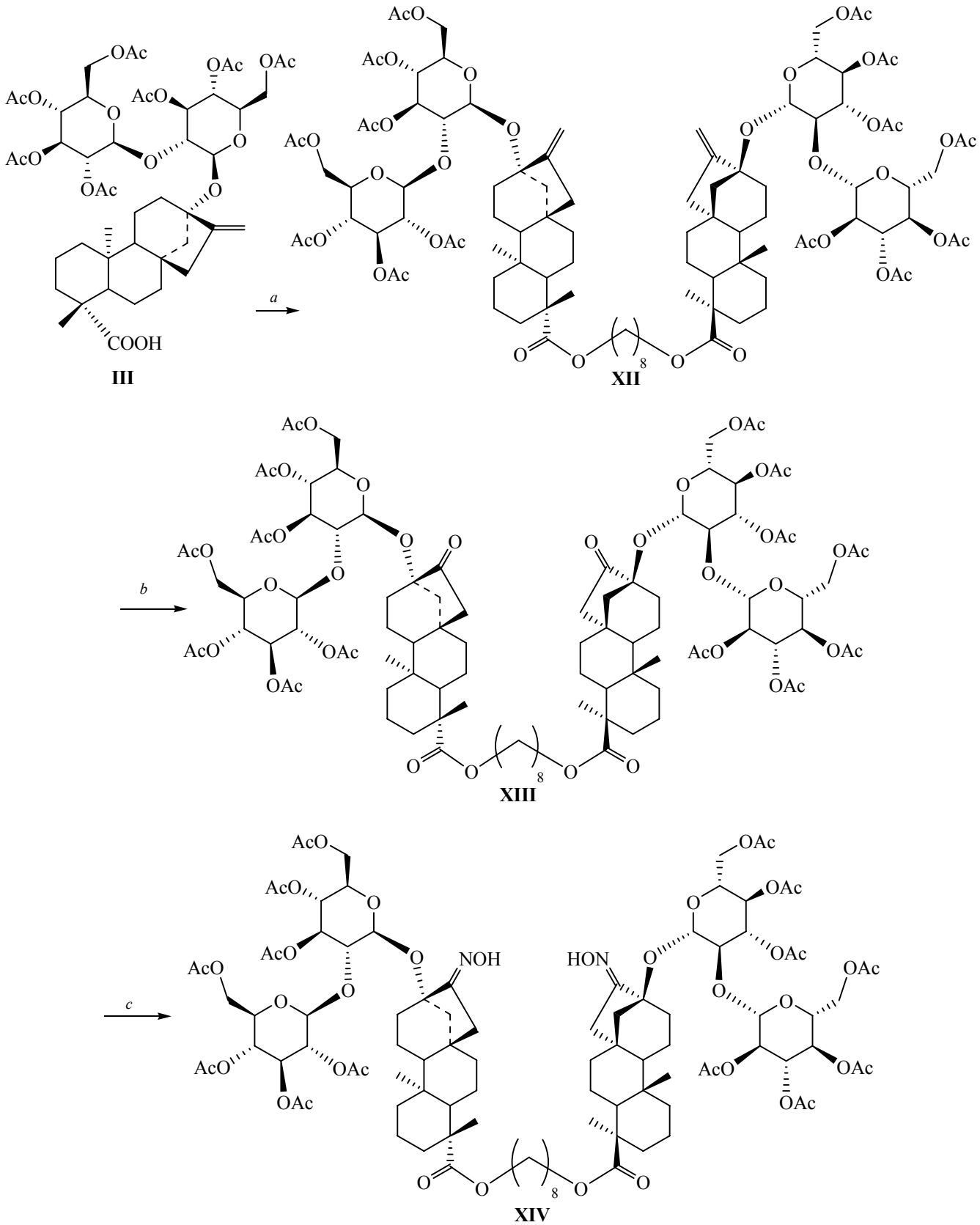
In view of the fact that the macrocyclization involving the oxime groups of glycoside **XIV** proved unsuccessful, we decided to use to this end the oxo groups of its precursor glycoside **XIII**. The latter was reacted with adipic (**XVIIa**), suberic (**XVIIb**), and sebacic dihydrazides (**XVIIc**), respectively (Scheme 5).

The reactions were performed in methanol at room temperature in the presence of trifluoroacetic acid as a catalyst. In all the cases, after removal of unreacted starting compounds, we obtained white powders. By mass spectral data, the powders comprised mixtures of binuclear (**XVIII**) and tetranuclear (**XIX**) macrocycles in which two or four molecules of heptaacetylated steviolbioside **III** are tethered by hydrazonohydrazide and ester linkers. We failed to isolate individual macrocycles, but even the fact of their formation is, to our opinion, very important, because such macrocyclic *O*-glycosides have never been described.

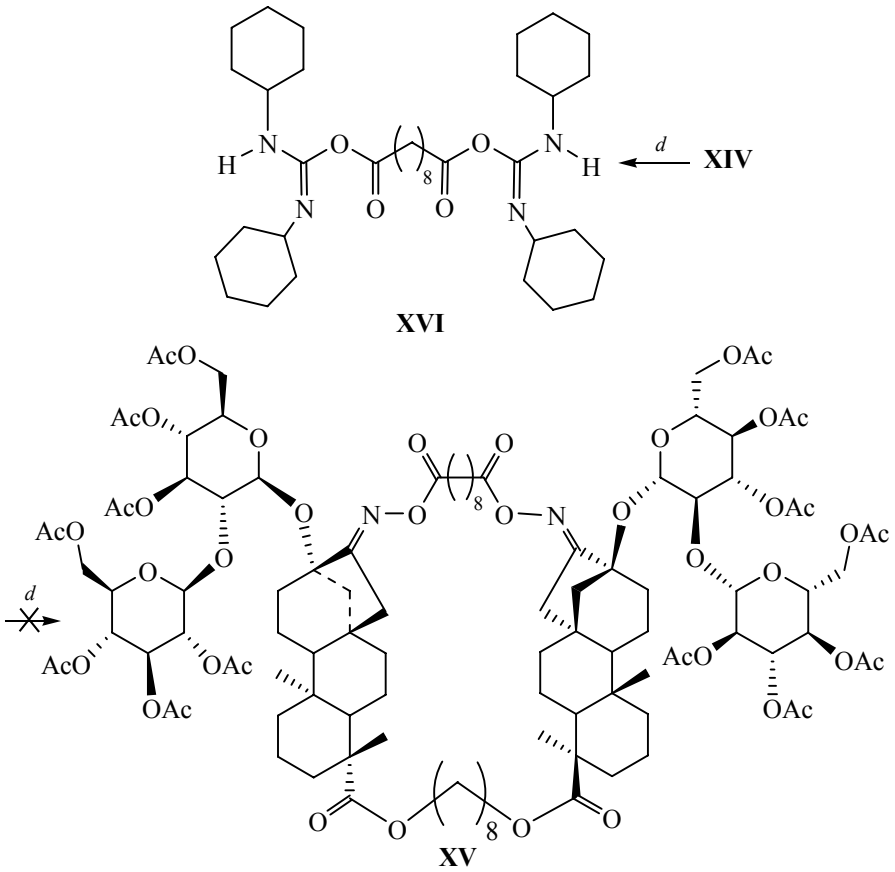
EXPERIMENTAL

The IR spectra were recorded on a Bruker Vector 22 FTIR spectrometer in the range 400–4000 cm^{-1} for thin films. The ^1H NMR spectra were measured on a Bruker Avance-400 spectrometer (400 MHz). The MALDI mass spectra were obtained on a Bruker UltraFlex III TOF/TOF instrument in the linear mode (*m*/*z* 200–6000, matrix 2,5-dihydroxybenzoic acid or *p*-nitroaniline). The melting points were determined on

Scheme 4.



Scheme 4. (Contd.)



a, $Br(CH_2)_8Br$, KOH–DMSO, 50°C, 24 h; *b*, OsO_4 (4%), $NaIO_4$, THF–H_2O, 20°C, 24 h; *c*, $NH_2OH·HCl$, AcONa, EtOH, 20°C, 24 h; *d*, $HOOC(CH_2)_8COOH$, DCC, DMAP, dioxane, 20°C.

a Boetius compact heating table. The reaction completion and product purity were controlled by TLC on Sorbfil plates (Imid, Krasnodar, Russia), development with 5% H_2SO_4 with subsequent heating at 120°C. Individual compounds were isolated by flash chromatography on a KSKG silica column (fraction < 0.063 mm, Khromlab, Lyubertsy, Russia).

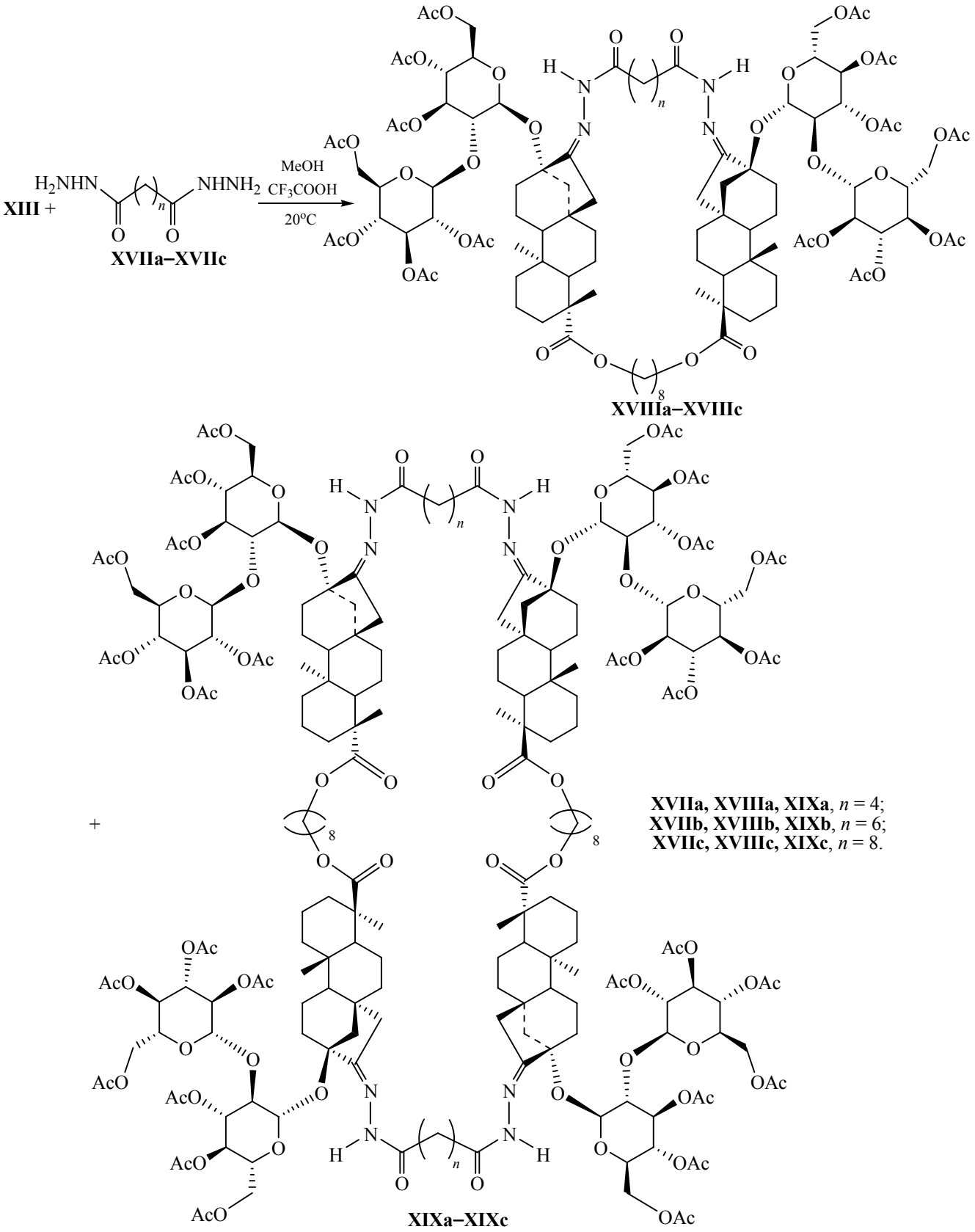
Suberic (**XVIIb**) and sebacic dihydrazides (**XVIIc**) were synthesized by the procedure in [22]. 1,8-Dibromooctane was purchased from Lancaster Synthesis, adipic hydrazide (**XVIIa**) from Alfa Aesar, and 4% aqueous OsO_4, sodium periodate, thiosemicarbazide, and hydroxylamine hydrochloride from Acros Organic.

(19-β-D-Glucosyl-13-*O*-β-D-sophorosyl)-*ent*-kaur-16-ene (I) was isolated from Stevioside sweetener (Travy Baikala, Irkutsk, Russia) by column chromatography (eluent chloroform–methanol, 10 : 0.1). mp 201–203°C (MeOH) (mp 198–202°C from MeOH [4]); $[\alpha]_D^{20}$ –33.7° (*c* = 6.6, H_2O) ($[\alpha]_D^{20}$ –39.3°, *c* = 5.7, H_2O [4]). ^1H NMR spectrum (C_5D_5N), δ, ppm (*J*, Hz): 1.23 s (3H, 20-CH_3), 1.29 s (3H, 18-CH_3), 2.34 d (1H, 3-H_{eq}, *J* 13), 2.70 d (1H, 14-H^{α}, *J* 11.95), 3.59– 4.55 m (18H, sophorosyl), 5.05 s (1H, 17-H_A), 5.13 d (1H, *J* 7.97, H'_{anomer}), 5.27 d (1H, *J* 7.97, H''_{anomer}), 5.68 s (1H, $C^{17}H_B$), 6.08 d (1H, *J* 7.97, H'''_{anomer}).

13-*O*-β-D-Sophorosyl-*ent*-kaur-16-en-19-oic acid (II) was prepared by oxidation of stevioside **I** by the procedure in [4]. mp 190°C (MeOH) (188–192°C from MeOH [4]), $[\alpha]_D^{20}$ –32.5° (*c* = 0.2, MeOH) ($[\alpha]_D^{20}$ –37.4°, *c* = 1.4, dioxane [4]). IR spectrum, ν, cm^{-1}: 3400 (OH), 1691 (C^{19}=O), 1662 (C=C), 1245 (C–O). ^1H NMR spectrum (C_5D_5N), δ, ppm (*J*, Hz): 1.20 s (3H, 20-CH_3), 1.31 s (3H, 18-CH_3), 2.45 d (1H, 3-H_{eq}, *J* 12.8), 2.56 d.d (1H, 14-H^{α}, *J* 12.8, 1.7), 3.69–4.53 m (12H, sophorosyl), 5.10 s (1H, 17-H_A), 5.17 d (1H, *J* 7.8, H'_{anomer}), 5.30 d (1H, *J* 7.8, H''_{anomer}), 5.76 s (1H, $C^{17}H_B$).

13-*O*-(Hepta-*O*-acetyl-β-D-sophorosyl)-*ent*-kaur-16-en-19-oic acid (III) was obtained from

Scheme 5.


XIII +
XVIIa–XVIIc
MeOH
CF3COOH
20°C
XVIIIa–XVIIIc
XVIIa, XVIIIa, XIXa, n = 4;
XVIIb, XVIIIb, XIXb, n = 6;
XVIIc, XVIIIc, XIXc, n = 8.
XIXa–XIXc

steviolbioside **II** by the procedure in [9]. mp 126°C (MeOH) (mp 125°C from MeOH [9]), $[\alpha]_D^{20}$ –39.5° (*c* = 0.2, EtOH) ($[\alpha]_D^{20}$ –28.3°, *c* = 4.26, EtOH [23]). IR spectrum, ν, cm^{-1}: 1754 (C=O), 1664 (C=C), 1230 (C–O). NMR spectrum ^1H (C_5D_5N), δ, ppm: 1.19 s (3H, 20-CH_3), 1.34 s (3H, 18-CH_3), 1.98–2.17 m (21H, 7Ac), 3.97–5.76 (12H, sophorosyl), 5.05 s (1H, 17-H_A), 5.66 s (1H, $C^{17}H_B$).

13-*O*-(Hepta-*O*-acetyl-β-D-sophorosyl)-16-oxo-*ent*-kauran-19-oic acid (IV). To a solution of 1 g (1.01 mmol) of heptaacetylated steviolbioside **III** in a mixture of 7 mL of THF and 5.2 mL of H_2O, 1.5 mL (0.22 mmol) of 4% aqueous OsO_4 was added. The mixture stirred for 15 min at 20°C and, after addition of 2.5 g (11.6 mmol) of $NaIO_4$, stirring was continued for an additional 24 h at the same temperature. The reaction mixture was washed with ethyl acetate (3 × 40 mL), and the organic fractions were combined and dried over anhydrous $MgSO_4$. The solvent was removed, and the residue was purified by silica gel column chromatography [eluent methylene chloride–methanol 10 : (0.1–0.2)]. Yield 0.59 g (59%), white powder, mp 121°C, $[\alpha]_D^{20}$ –26.9° (*c* = 1, $CHCl_3$). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.83–1.95 m (18H, *ent*-kaurane fragment), 1.03 s (3H, 20-CH_3), 1.26 s (3H, 18-CH_3), 1.97 s (3H, CH_3CO), 1.98 s (3H, CH_3CO), 2.01 s (3H, CH_3CO), 2.03 s (3H, CH_3CO), 2.04 s (3H, CH_3CO), 2.06 s [6H, $(CH_3CO)_2$], 2.22 d (1H, H^3, *J* 13.0), 2.46 d.d (H, 14-H^α, *J* 13.5, 2.1), 3.61–5.17 m (13H, sophorosyl), 4.69 d (1H, $H^{1'}$, *J* 7.4). Mass spectrum, *m*/*z*: 961.2 $[M + Na]^+$, 977.2 $[M + K]^+$ (calculated for $C_{45}H_{62}NaO_{21}$: 961.3).

13-*O*-(Hepta-*O*-acetyl-β-D-sophorosyl)-16-hydroxyimino-*ent*-kauran-19-oic acid (V). A solution of 0.3 g (0.3 mmol) of ketone **IV**, 0.11 g (1.5 mmol) of hydroxylamine hydrochloride, and 0.26 g (3.1 mmol) of sodium acetate in a mixture of 30 mL of EtOH and 7 mL of H_2O was stirred for 24 h at 20°C and then diluted with 100 mL of H_2O. The precipitate was filtered off. Yield 0.23 g (75.6%), white powder, mp 147°C, $[\alpha]_D^{20}$ –36.8° (*c* = 1, $CHCl_3$). IR spectrum, ν, cm^{-1}: 3287, 3125 (OH), 1749 [$OC(O)CH_3$], 1690 (COOH), 912 (N–O). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.81–2.31 m (20H, *ent*-kaurane fragment), 0.99 s (3H, 20-CH_3), 1.24 s (3H, 18-CH_3), 1.98 s (3H, CH_3CO), 1.99 s (3H, CH_3CO), 2.02 s (3H, CH_3CO), 2.03 s (3H, CH_3CO), 2.05 s (3H, CH_3CO), 2.06 s (3H, CH_3CO), 2.07 s (3H, CH_3CO), 3.66–5.20 m (12H, sophorosyl), 4.76 d (1H, $H^{1'}$, *J* 7.8), 4.81 d (1H, $H^{1''}$, *J* 7.6). Mass spectrum, *m*/*z*: 954.7 $[M + H]^+$, 976.8 $[M + Na]^+$, 992.7 $[M + K]^+$ (calculated for $C_{45}H_{63}NO_{21}$: 953.4). Found, %: C 56.52; H 6.68; N 1.45. $C_{49}H_{63}NO_{21}$. Calculated, %: C 56.66; H 6.66; N 1.47.

13-*O*-(Hepta-*O*-acetyl-β-D-sophorosyl)-16-thiosemicarbazono-*ent*-kauran-19-oic acid (VI). To a solution of 0.3 g (0.3 mmol) of ketone **IV** and 0.14 g (1.5 mmol) of thiosemicarbazide in 20 mL of EtOH, 3 drops of 20% H_2SO_4 and 0.002 g (0.02 mmol) of sodium acetate. The reaction mixture was stirred for 24 h at 20°C. The solvent was removed by distillation, the residue was washed with hot water (3 × 30 mL) to remove the starting thiosemicarbazide, and was purified by silica gel column chromatography (eluent methylene chloride–methanol, 60 : 1). Yield 0.07 g (22%) white powder, mp 154°C, $[\alpha]_D^{20}$ +8.2° (*c* = 1, $CHCl_3$), $[\alpha]_D^{20}$ +9.8° (*c* = 0.5, $CHCl_3$), $[\alpha]_D^{20}$ +7.0° (*c* = 0.25, $CHCl_3$), $[\alpha]_D^{20}$ +6.8° (*c* = 0.1, $CHCl_3$). IR spectrum, ν, cm^{-1}: 3460 (NH_{ac}), 3320 (NH_c), 3142 (NH), 1752 [$OC(O)CH_3$], 1593 (C=N). NMR spectrum ^1H ($CDCl_3$), δ, ppm (*J*, Hz): 0.84–2.25 m (20H, *ent*-kaurane fragment), 1.03 s (3H, 20-CH_3), 1.24 s (3H, 18-CH_3), 1.98 s (3H, CH_3CO), 1.99 s (3H, CH_3CO), 2.01 s (3H, CH_3CO), 2.04 s (6H, $2CH_3CO$), 2.08 s (3H, CH_3CO), 2.09 s (3H, CH_3CO), 2.36 d.d (1H, 14-H^α, *J* 13.5, 2.1), 3.61–5.15 m (12H, sophorosyl), 4.66 d (1H, $H^{1'}$, *J* 8.0), 4.79 d (1H, $H^{1''}$, *J* 7.5), 6.41 d (1H, NH_2, *J* 3.2), 7.41 d (1H, NH_2, *J* 3.7), 8.53 s (1H, NH). Mass spectrum, *m*/*z*: 1010.3 $[M - H]^+$, 1034.3 $[M + Na]^+$, 1050.2 $[M + K]^+$ (calculated for $C_{46}H_{65}N_3O_{20}S$: 1011.3).

Reaction of oxime V with sebacic chloride. *a.* A solution of 0.0225 g (0.09 mmol) of sebacic chloride in 3 mL of absolute CH_2Cl_2 was added dropwise to a cooled (0°C) solution of 0.14 g (0.15 mmol) of oxime **V** in 7 mL of absolute CH_2Cl_2. The temperature of the reaction mixture was then raised to 20°C, 0.01 mL of Et_3N was added, and stirring was continued for an additional 18 h. The solvent was removed, and the residue was purified by silica gel column chromatography (eluent methylene chloride–ethyl acetate, 1 : 1).

b. 4-Dimethylaminopyridine, 0.006 g (0.049 mmol), and 2 drops of absolute pyridine was added to a solution of 0.14 g (0.15 mmol) of oxime **V** in 7 mL of absolute CH_2Cl_2. A solution of 0.017 g (0.075 mmol) of sebacic chloride in 5 mL of absolute CH_2Cl_2 was added with cooling to 0°C, and the resulting mixture was stirred for 18 h at 20°C. After the reaction had been completed, the mixture was washed with aqueous HCl and water and dried over $MgSO_4$. The solvent was removed, and the residue was purified by silica gel

column chromatography (eluent methylene chloride–ethyl acetate, 1 : 1).

In both cases 0.001 g (1%) of a mixture of compounds **VII** and **VIII** was obtained. Mass spectrum, *m*/*z*: 2097.7 $[M + Na]^+$, 2114.4 $[M + K]^+$ (calculated for $C_{100}H_{140}N_2NaO_4$: 2097.87) (**VII**); 1162.4 $[M + Na]^+$, 1178.0 $[M + K]^+$ (calculated for $C_{55}H_{79}NNaO_{24}$: 1161.5) (**VIII**).

Benzyl-13-*O*-(hepta-*O*-acetyl-β-D-sophorosyl)-*ent*-kaur-16-en-19-oate (IX). Heptaacetylated steviolbioside **III**, 0.7 g (0.7 mmol), was added with stirring at 20°C to a mixture of 0.09 g (1.6 mmol) KOH and 20 mL of DMSO. After 30 min, 0.197 g (1.15 mmol) of benzyl bromide was added. The reaction mixture was stirred for 24 h at 40°C and then diluted with 20 mL of water. The precipitate was filtered off and purified by silica gel column chromatography (eluent petroleum ether–ethyl acetate, 5 : 3). Yield 0.27 g (35%), white powder, mp 95°C, $[\alpha]_D^{20}$ –18.1° (*c* = 1, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 0.79–2.22 m (20H, *ent*-kaurane fragment), 0.87 s (3H, 20-CH_3), 1.18 s (3H, 18-CH_3), 1.99 s (3H, CH_3CO), 2.00 s (3H, CH_3CO), 2.02 s [6H, $(CH_3CO)_2$], 2.03 s (3H, CH_3CO), 2.05 s (3H, CH_3CO), 2.08 s (3H, CH_3CO), 3.63–5.22 m (12H, sophorosyl), 4.05–4.10 m [2H, 19-C(O)OCH_2], 4.59 d (1H, $H^{1'}$, *J* 7.6), 4.68 d (1H, $H^{1''}$, *J* 8.0), 4.80 s (1H, 17-H_A), 5.11 s (1H, 17-H_B), 7.28–7.37 m (5H, Ar). Mass spectrum, *m/z*: 1049.5 $[M + Na]^+$, 1065.4 $[M + K]^+$ (calculated for $C_{53}H_{70}NaO_{20}$: 1049.4).

Benzyl-13-*O*-(hepta-*O*-acetyl-β-D-sophorosyl)-16-oxo-*ent*-kaur-16-en-19-oate (X). To a solution of 0.26 g (0.25 mmol) of benzyl ester **IX** in a mixture of 3.6 mL of THF and 1.75 mL of H_2O, 0.3 mL (0.044 mmol) of 4% aqueous OsO_4 was added dropwise. The mixture was stirred for 15 min at 20°C, 0.6 g (2.8 mmol) of $NaIO_4$ was added, and stirring was continued for 24 h at 20°C. After the reaction had been completed, the mixture was washed with ethyl acetate (3 × 30 mL) and the organic fractions were combined and washed with anhydrous $MgSO_4$. The solvent was removed, and the residue was purified by silica gel column chromatography (eluent petroleum ether–ethyl acetate, 1 : 1). Yield 0.16 g (61.5%), white powder, mp 90°C, $[\alpha]_D^{20}$ –30.2° (*c* = 1, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.81–1.93 m (18H, *ent*-kaurane fragment), 0.87 s (3H, 20-CH_3), 1.21 s (3H, 18-CH_3), 1.96 s (3H, CH_3CO), 1.99 s (3H, CH_3CO), 2.00 s (3H, CH_3CO), 2.04 s [6H, $(CH_3CO)_2$], 2.05 s (3H, CH_3CO), 2.06 s (3H, CH_3CO), 2.24 d (1H, H^3, *J* 13.8), 2.35 d.d (1H, 14-H^α, *J* 14.7, 3.1), 3.59–5.21 m (12H, sophorosyl), 4.04–4.08 m [2H, 19-C(O)OCH_2], 4.74 d (1H, $H^{1'}$, *J* 7.4), 7.30–7.38 m (5H, Ar). Mass spectrum, *m*/*z*: 1051.4 $[M + Na]^+$ (calculated for $C_{52}H_{68}NaO_{21}$: 1051.4).

Benzyl-13-*O*-(hepta-O-acetyl-β-D-sophorosyl)-16-hydroxyimino-*ent*-kauran-19-oate (XI). A mixture of 0.15 g (0.14 mmol) of ketone **X**, 0.07 g (1.02 mmol) of hydroxylamine hydrochloride, 0.128 g (1.4 mmol) of sodium acetate, 15 mL of EtOH, and 3.5 mL of H_2O was stirred for 24 h at 20°C and then diluted with 35 mL of water, and the precipitate was filtered off. Yield 0.07 g (46.6%), white powder, mp 114°C, $[\alpha]_D^{20}$ –33.4° (*c* = 1, CH_3OH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 0.78–2.28 m (20H, *ent*-kaurane fragment), 0.83 s (3H, 20-CH_3), 1.19 s (3H, 18-CH_3), 1.96 s (3H, CH_3CO), 1.99 s (3H, CH_3CO), 2.00 s (3H, CH_3CO), 2.02 s (3H, CH_3CO), 2.03 s (3H, CH_3CO), 2.04 s (3H, CH_3CO), 2.05 s (3H, CH_3CO), 3.61–5.19 m (12H, sophorosyl), 4.12–4.19 m [2H, 19-C(O)OCH_2], 4.76 d (1H, $H^{1'}$, *J* 7.8), 4.81 d (1H, $H^{1''}$, *J* 7.4), 7.29–7.36 m (5H, Ar), 7.85 br s (1H, NOH). Mass spectrum, *m*/*z*: 1066.3 $[M + Na]^+$, 1082.2 $[M + K]^+$ (calculated for $C_{52}H_{69}NNaO_{21}$: 1066.4.

Octane-1,8-diyl bis{13-O-[3',4',6'-tri-*O*-acetyl-β-D-glucopyranosyl-(2→1)-2'',3'',4'',6''-tetra-*O*-acetyl-β-D-glucopyranosyl]-*ent*-kaur-16-en-19-oate} (XII) was synthesized analogously to compound **IX** from 0.18 g (3.2 mmol) of KOH, 0.7 g (0.7 mmol) steviolbioside **III**, and 0.1 g (0.38 mmol) of 1,10-dibromooctane. Yield 1.33 g (90%), white powder, mp 115°C, $[\alpha]_D^{20}$ –31.4° (*c* = 0.7, CH_2Cl_2). IR spectrum, ν, cm^{-1}: 1755 [$OC(O)CH_3$], 1720 [C(O)OC], 1663 ($C{=}CH_2$). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.79–2.19 m [48H, *ent*-kaurane fragments and linker $(CH_2)_4$ fragment], 0.86 s (6H, 20-CH_3, 20'-CH_3), 1.17 s (6H, 18-CH_3, 18'-CH_3), 1.98 s (6H, CH_3CO, C'H_3CO), 1.99 s (6H, CH_3CO, C'H_3CO), 2.00 s (6H, CH_3CO, C'H_3CO), 2.02 s (6H, CH_3CO, C'H_3CO), 2.05 s (6H, CH_3CO, C'H_3CO), 2.06 s (6H, CH_3CO, C'H_3CO), 2.08 s (6H, CH_3CO, C'H_3CO), 3.64–5.19 m [32H, sophorosyl, 19-C(O)$OCH_2C\underline{H}_2$, 19'-C(O)$OCH_2C\underline{H}_2$, 19-C(O)OCH_2, 19'-C(O)OCH_2], 4.61 d (2H, $H^{1'}$, $H^{1''}$, *J* 7.6), 4.69 d (2H, $H^{1'''}$, $H^{1''''}$, *J* 8.2 Hz), 4.80 s (2H, 17-H_A, 17'-H_A), 5.11 s (2H, 17-H_B, 17'-H_B). Mass spectrum, *m*/*z*: 2006.95 $[M + Na]^+$, 2022.96 $[M + K]^+$ (calculated for $C_{100}H_{142}NaO_{40}$: 2006.90).

Octane-1,8-diyl bis{13-O-[3',4',6'-tri-*O*-acetyl-β-D-glucopyranosyl-(2→1)-2'',3'',4'',6''-tetra-*O*-acetyl-β-D-glucopyranosyl]-16-oxo-*ent*-kauran-19-oate}

(XIII) was synthesized from compound **X** from 0.22 g (0.11 mmol) of steviolbioside **XII**, 1 mL (0.15 mmol) of 4% aqueous OsO_4 and 1.74 g (8 mmol) of $NaIO_4$. Yield 0.1 g (45%), white powder, mp 117°C, $[\alpha]_D^{20}$ –31.6° (*c* = 0.6, CH_3OH). IR spectrum, ν, cm^{-1}: 1755 [$OC(O)CH_3$], 1747 (C=O), 1730 [C(O)OC]. ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.82–1.94 m [46H, *ent*-kaurane fragments and linker $(CH_2)_4$ fragment], 0.90 s (6H, 20-CH_3, 20'-CH_3), 1.19 s (6H, 18-CH_3, 18'-CH_3), 1.97 s (6H, CH_3CO, C'H_3CO), 1.98 s (6H, CH_3CO, C'H_3CO), 1.99 s (6H, CH_3CO, C'H_3CO), 2.03 s (6H, CH_3CO, C'H_3CO), 2.04 s (6H, CH_3CO, C'H_3CO), 2.05 s (6H, CH_3CO, C'H_3CO), 2.06 s (6H, CH_3CO, C'H_3CO), 2.20 d (2H, H^3, $H^{3'}$ *J* 12.7), 2.39 d.d (2H, 14-H^{α}, 14'-H^{α}, *J* 13.9, 2.5), 3.60–5.18 m [32H, sophorosyl, 19-C(O)$OCH_2C\underline{H}_2$, 19'-C(O)$OCH_2C\underline{H}_2$, 19-C(O)OCH_2, 19'-C(O)OCH_2], 4.77 d (2H, $H^{1'}$, $H^{1''}$, *J* 7.6), 4.88 d (2H, $H^{1'''}$, $H^{1''''}$, *J* 7.6). Mass spectrum, *m*/*z*: 2010.91 $[M + Na]^+$, 2026.01 $[M + K]^+$ (calculated for $C_{98}H_{138}NaO_{42}$: 2010.86).

Octane-1,8-diyl bis{13-O-[3',4',6'-tri-*O*-acetyl-β-D-glucopyranosyl-(2→1)-2'',3'',4'',6''-tetra-*O*-acetyl-β-D-glucopyranosyl]-16-hydroximino-*ent*-kauran-19-oate} (XIV) was synthesized analogously to compound **XI** from 0.1 g (0.05 mmol) of diketone **XIII**, 0.05 g (0.7 mmol) of hydroxylamine hydrochloride, and 0.136 g (1 mmol) of sodium acetate. Yield 0.063 g (63%), white powder, mp 137°C, $[\alpha]_D^{20}$ –34.5° (*c* = 0.5, $CHCl_3$). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.79–2.28 m [48H, *ent*-kaurane fragments and linker $(CH_2)_4$ fragment], 0.85 s (6H, 20-CH_3, 20'-CH_3), 1.17 s (6H, 18-CH_3, 18'-CH_3), 1.97 s (6H, CH_3CO, C'H_3CO), 1.98 s (6H, CH_3CO, C'H_3CO), 2.00 s (6H, CH_3CO, C'H_3CO), 2.03 s (6H, CH_3CO, C'H_3CO), 2.05 br s (18H, 3CH_3CO, 3C'H_3CO), 3.63–5.18 m [34H, sophorosyl, 19-C(O)$OCH_2C\underline{H}_2$, 19'-C(O)$OCH_2C\underline{H}_2$, 19-C(O)OCH_2, 19'-C(O)OCH_2, $H^{1'''}{}_{anomer}$, $H^{1''''}{}_{anomer}$), 4.77 d (2H, $H^{1'}$, $H^{1''}$, *J* 7.9), 7.81 br.s (2H, NOH, N'OH). Mass spectrum, *m*/*z*: 2018.64 $[M]^+$, 2041.8 $[M + Na]^+$, 2057.83 $[M + K]^+$ (calculated for $C_{98}H_{140}N_2O_{42}$: 2018.9).

Reaction of oxime XIV with sebacic acid. A solution of 0.05 g (0.025 mmol) of oxime **XIV** in 7 mL of absolute dioxane and a solution of 0.0055 g (0.027 mmol) of sebacic acid in 2 mL in absolute dioxane were simultaneously added dropwise to a solution of 0.0076 g (0.037 mmol) of DCC in 2 mL of absolute dioxane, after which 0.002 g (0.02 mmol) of 4-dimethyl-aminopyridine was added. The reaction mixture was stirred for 100 h at 20°C, washed with acidified water, the solvent was removed, and a mixture of compounds **XIV** and **XVI** was obtained as a white powder. Mass spectrum, *m*/*z*: 637.3 $[M + Na]^+$ (calculated for $C_{36}H_{62}N_4NaO_4$: 637.4) (**XVI**); 2041.28 [*M* + Na] (calculated for $C_{98}H_{140}O_{42}N_2Na$: 2041.88) (**XIV**).

Reaction of diketone XIII with adipic (XVIIa), subaric (XVIIb), and sebacic (XVIIc) dihydrazides. Dihydrazide **XVIIa**–**XVIIc** and a few drops of CF_3COOH were added to a solution of 1 mmol of compound **XIII** in absolute methanol. The reaction mixture was stirred for 24 h at 20°C. The solvent was removed, the residue was dissolved in CH_2Cl_2 and washed with water to remove the starting hydrazide. Unreacted diketone **XIII** was removed by reprecepitation from a mixture of petroleum ether and ethyl acetate. A mixture of macrocycles was obtained as a white powder.

Mixture of 1^{13},12^{13}-di[2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-3'',4'',6''-tri-*O*-acetyl-β-D-glucopyranosyl-1''-oxy]-2,3,10,11-tetraaza-14,23-dioxa-1,12(16,4α)-di(19-nor-*ent*-kaurana)cyclotetracosaphane-1^{16}(2),12^{16}(11)-diene-4,9,13,24-tetraone (XVIIIa) and 1^{13},12^{13},25^{13},36^{13}-tetra[2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-3'',4'',6''-tri-*O*-acetyl-β-D-glucopyranosyl-1''-oxy]-2,3,10,11,26,27,-34,35-octaaza-14,23,38,47-tetraoxa-1,12,25,36(16,4α)-tetra(19-nor-*ent*-kaurana)cyclooctacosaphane-1^{16}(2),12^{16}(11),25^{16}(26),36^{16}(37)-tetraen-4,9,13,24,28,33,37,48-octaone (XIXa). Yield 93%. Mass spectrum, *m*/*z*: 2125.89 $[M]^+$, 2148.72 $[M + Na]^+$ (calculated for $C_{104}H_{148}N_4NaO_{42}$: 2148.95) (**XVIIIa**), 4275.69 $[M + Na]^+$ (calculated for $C_{208}H_{296}N_8NaO_{84}$: 4275.91) (**XIXa**).

Mixture 1^{13},14^{13}-di[2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-3'',4'',6''-tri-*O*-acetyl-β-D-glucopyranosyl-1''-oxy]-16,25-dioxa-2,3,12,13-tetraaza-1,14(16,4α)-di(19-nor-*ent*-kaurana)cyclohexacosaphane-1^{16}(2),14^{16}(12)-diene-4,11,15,26-tetraone (XVIIIb) and 1^{13},14^{13},27^{13},40^{13}-tetra[2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl-(1→2)-3'',4'',6''-tri-*O*-acetyl-β-D-glucopyranosyl-1''-oxy]-2,3,12,13,28,29,-38,39-octaaza-16,25,42,51-tetraoxa-1,14,27,40(16,4α)-tetra(19-nor-*ent*-kaurana)cyclodopentacontaphane-1^{16}(2),14^{16}(13),27^{16}(28),40^{16}(39)-tetraen-4,11,15,26,-30,37,41,52-octaone (XIXb). Yield 80%. Mass spectrum, *m*/*z*: 2176.3 $[M + Na]^+$ (calculated for $C_{106}H_{152}N_4NaO_{42}$: 2176.98) (**XVIIIb**), 4307.3 $[M]^+$ (calculated for $C_{212}H_{304}N_8O_{84}$: 4307.98 (**XIXb**).

Mixture of 1^{13},16^{13}-di[2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-3'',4'',6''-tri-*O*-acetyl-β-D-

glucopyranosyl-1''-oxy]-2,3,14,15-tetraaza-18,27-dioxa-1,16(16,4α)-di(19-nor-*ent*-kaurana)cycloocta-cosaphane-1^{16}(2),16^{16}(15)-diene-4,13,17,28-tetraone (XVIIIc) and 1^{13},16^{13},29^{13},44^{13}-tetra[2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-3'',4'',6''-tri-*O*-acetyl-β-D-glucopyranosyl-1''-oxy]-2,3,14,15,30,31,-42,43-octaaza-18,27,46,55-tetraoxa-1,16,29,44(16,4α)-tetra-(19-nor-*ent*-kaurana)-cyclopentacosaphane-1^{16}(2), 16^{16}(15),29^{16}(30),44^{16}(43)-tetraen-4,13,17,28,32,41,45,56-octaone (XIXc). Yield 90%. Mass spectrum, *m*/*z*: 2204.6 $[M + Na]^+$ (calculated for $C_{108}H_{156}N_4NaO_{42}$: 2205.01) (**XVIIIc**), 4364.9 $[M]^+$ (calculated for $C_{216}H_{312}N_8O_{84}$: 4365.05 (**XIXc**).

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