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Macrocyclic Derivatives of Diterpenoid Isosteviol with Hydrazide and Hydrazone Moieties

Bulat F. Garifullin, Olga V. Andreeva, Irina Yu. Strobykina, Vasiliy M. Babaev, and Vladimir E. Kataev[@]

A.E. Arbuzov Institute of Organic and Physical Chemistry of Kazan Scientific Center of Russian Academy of Sciences, 420088 Kazan, Russia
[@]Corresponding author E-mail: kataev@iopc.ru

Novel macrocyclic derivatives of diterpenoid isosteviol (16-oxo-ent-beyeran-19-oic acid) possessing two or four ent-beyerane skeletons bonded with linkers having ester, hydrazide, and hydrazone moieties were synthesized.

Keywords: Macrocycles, macrocyclic diterpenoids, isosteviol, hydrazides, hydrazones.

Макроциклические производные дитерпеноида изостевиола с гидразидными и гидразонными фрагментами

Б. Ф. Гарифуллин, О. В. Андреева, И. Ю. Стробыкина, В. М. Бабаев, В. Е. Катаев[@]

Институт органической и физической химии им. А.Е. Арбузова КазНЦ РАН, 420088 Казань, Россия
[@]E-mail: kataev@iopc.ru

Синтезированы макроциклические производные дитерпеноида изостевиола (16-оксо-энт-бейеран-19-овая кислота) с двумя и четырьмя энт-бейерановыми углеводородными каркасами, соединенными спейсерами, содержащими сложноэфирные, гидразонные и гидразидные фрагменты.

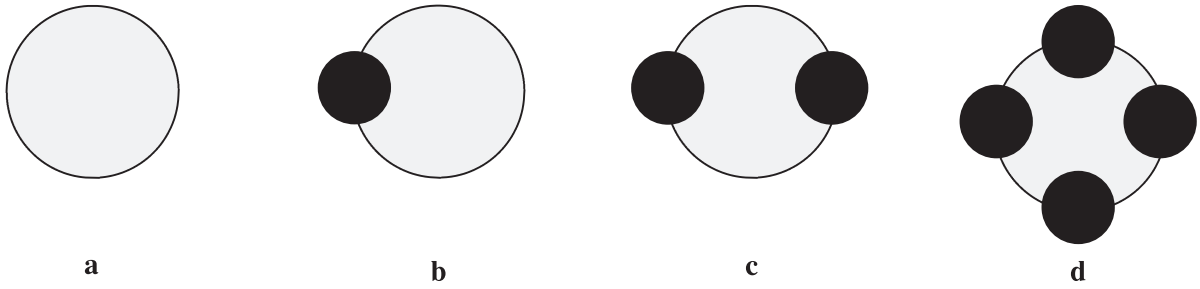
Ключевые слова: Макроциклы, макроциклические дитерпеноиды, изостевиол, гидразиды, гидразоны.

Introduction

Among about 3000 macrocyclic compounds* isolated from various natural sources (macrolactames, macrolides, cyclic peptides, cyclic oligosaccharides *etc*.) about 100 compounds are macrocyclic diterpenoids.[1-3] The most famous are cembranes, jatrophanes, lathyranes; on the contrary, casbanes and dolabellanes are rather rare in nature.[1,3-7] Their carbohydrogen skeleton is 14-, 12, or 11-membered cycle fused with cyclopropane, cyclopentane[1,3,6] or oxirane[7] rings. It should be emphasized that each naturally occurring macrocyclic diterpenoid is a one molecule (Scheme 1a). The literature has provided no examples of naturally occurring macrocyclic diterpenoids that could be formed by macrocyclization of an one diterpenoid molecule (Scheme 1b), or by connecting of several diterpenoid molecules (Scheme 1c,d). Macrocycles having such structures were obtained with the help of synthesis only. Thus macrocycles synthesized on the basis of monoterpenes (myrtenal,[8] 3-carene,[9,10] α-pinene[9,10]), diterpenoids (isosteviol,[11-14] steviol,[15] paclitaxel[16,17]), and bile acids[18,19] have been reported. In these compounds mono- or polycyclic terpene, or steroide skeletons are combined in macrocycle by various linkers.

Noteworthy, macrocyclic derivatives of isosteviol (16-oxo-*ent*-beyeran-19-oic acid, **1**) were previously synthesized not from isosteviol itself. Some of its derivatives were used as starting compounds: isosteviol oxo group was selectively reducted, or the carboxyl group

* A macrocycle is meant to be a compound the molecule of which has a ring formed by more than 10 atoms.



Scheme 1. Schematic representation of macrocyclic terpenoids. (a) "One molecular" macrocyclic terpenoid; (b) mononuclear macrocyclic terpenoid bearing one terpenoid molecule (black circle); (c) binuclear macrocyclic terpenoid constructed from two terpenoid molecules (black circles); (d) tetranuclear macrocyclic terpenoid constructed from four terpenoid molecules (black circles).

was converted into the carbonyl chloride group, or into the hydroxymethyl group.[12,13] The synthetic potential of directly the C^{16}=O group of isosteviol was not realized, though the involving this functionality in reactions with amines should lead to nitrogen containing macrocycles which could exhibit any biological activities. In addition, the course of a macrocyclization reaction of isosteviol derivative bearing the $C^{16}OH$ and the $C^{4}CH_2OH$ groups with chloride-anhydrides of dicarboxylic acids appeared to be dependent on their length. Reactions with acid chlorides, in which the C(O)Cl groups are separated by polymethylene chain having n > 5, led to the formation of mononuclear macrocycles[12] (Scheme 1b), whereas reactions with acid chlorides, in which the C(O)Cl groups are separated by polymethylene chain having n < 5 (*e.g.* adipic and malonic), afforded the mixture of bi- and tetranuclear macrocycles[12] (Scheme 1c,d), each macrocycle existing as several isomers of head-to-head and head-to tail kinds.[12] The reason of the formation of such mixtures seems to be clear: the C(O)Cl groups of acid chlorides reacted with the $C^{16}OH$ and the $C^{4}CH_2OH$ groups of two and more molecules of the reducted isosteviol derivative with equal probability. To avoid the such situation, the macrocyclization of isosteviol and its isomer steviol carried out in several steps at a later time. Two isosteviol molecules were first bonded via the oxo groups into binuclear isosteviol derivative possessing an unfolded structure, and its macrocylization was realized in the last step.[13,15]

Continuing the series of papers concerned with syntheses of macrocyclic isosteviol derivatives, in which isosteviol moieties were connected by various diester linkers,[10-14] herein we report the synthesis of macrocyclic isosteviol derivatives having hydrazide and hydrazone fragments. It was found previously that the combination of two isosteviol molecules with diester linker increased its antituberculosis activity by several times, and the greater is the distance between isosteviol moieties, the greater is the activity.[20] Diacid **10** showed the highest activity (MIC† 12.5 μg/ml) among compounds assayed.[20] The macrocyclization of this acid into a macrocycle of **c** kind (Scheme 1c) resulted in the further increase in bioactivity.[13] We have revealed that some binuclear isosteviol derivatives having an unfolded molecular structure, in which isosteviol moieties are connected by a dihydrazide linker, inhibited the *in vitro* growth of *Mycobacterium tuberculosis* ($H37R_V$) within the range of MIC values from 1.7 to 3.1 μg/ml.[21] Taking into account results aforementioned[13,20] we suppose that a synthesis of macrocycles having several isosteviol moieties connected by dihydrazide and dihydrazone linkers could be an efficient approach to obtain novel isosteviol derivatives exhibiting higher antituberculosis activities.

Experimental

NMR experiments were carried out with Avance-600 (Bruker, Germany) spectrometer in $CDCl_3$ or C_5D_5N at 600.13 MHz at 30 ºC. MALDI mass spectra were measured on DYNAMO MALDI TOF instrument (Thermo BioAnalysis, Santa Fe, New Mexico). Samples were prepared as 0.1 % solutions of compounds in an appropriate solvent. The matrix was *p*-nitroaniline (Acros). IR spectra were recorded with Vector-22 Fourier spectrometer (Bruker, Germany). Melting points of substances were determined on a SMP10 compact heating table (Barloworld Scientific Ltd., South Africa). The completeness of the reaction and the purity of the compounds were monitored by TLC on Sorbfil plates (Sorbfil, Russia). Spots were detected by treatment with the 5% solution of sulfuric acid, followed by heating up to 120 °C. Isolation of individual substances was performed with a dry-column flash chromatography on Silicagel KSK **(**0.063-0.125 mm, Crom-Lab Ltd, Russia).

Isosteviol **1** was obtained by the literature method[22] from the sweetener Sweta (Steivan Biotechnology Corp., Malaysia), m.p. 233-235 °C (231-233 °C[23]). Dihydroisosteviol **9** was prepared by the reaction of isosteviol **1** with $NaBH_4$,[24] m.p. 199-200 °C (m.p. 198-200 °C[24]). Diacid **8** was synthesized by the interaction of isosteviol with adipic acid dihydrazide,[21] m.p. > 300 °C (m.p. > 300 °C[21]). Diacid **10** was obtained from dihydroisosteviol **9**,[13] m.p. 109-110 °C (m.p. 105-110 °C[20]). Acid chloride **12** was synthesized from diacid **10** by a facile rout[13] as an oil that was immediately used in the reactions with adipic acid dihydrazide and hidrazine. Adipic acid dihydrazide was purchased from Alfa Aesar (Massachusetts, USA). All solvents were dried according to standard protocols.

*General procedure for the synthesis of compounds **2** and **3***. A solution of ditosylate (0.5 equiv.) in CH_3CN (10 ml) was added dropwise to a stirred solution of equimolecular amounts of isosteviol **1** and K_2CO_3 in CH_3CN (70 ml) under refluxing for 1 h. Then a reaction mixture was heated under reflux for a further 8 h. A precipitate was filtered off and washed with CH_2Cl_2. The filtrate was evaporated until dry, the precipitate that formed was dissolved in CH_2Cl_2, the solution was washed with water, and extracted. The organic extracts were combined and fractioned by silica gel column chromatography.

† Minimal inhibitory concentration

*Ethane-1,2-diyl-bis(16-oxo-ent-beyeran-19-oat) (**2**)*. 54 %, R_f (hexane - ethyl acetate, 4:1) 0.48, m.p. 185 °C (m.p. 183-185 °C[25]).

*Propane-1,3-diyl-bis(16-oxo-ent-beyeran-19-oat) (**3**)*. 64 %, R_f (hexane - ethyl acetate, 4:1) 0.53, m.p. 155 °C (m.p. 153-155 °C[25]).

*General procedure for the synthesis of compounds **4-7***. A solution of compound **2** (or **3**) in benzene (50 ml) was heated under reflux with a Dean-Stark trap for 1 h. Then a solution was cooled and equimolecular amount of adipic acid dihydrazide and several crystals of TsOH were added. The heterogeneous mixture that formed was refluxed with a Dean-Stark trap for a further 15 h. Then benzene was removed under reduced pressure, a precipitate was dried under vacuum, dissolved in absolute ethanol (40 ml) and refluxed until all the solids went into solution. During further reflux a new precipitate begun to appear. When the formation of the precipitate had finished, a heating was stopped. The precipitate was filtered off from the hot solution, dried firstly in air, and then was dried with $CaCl_2$ in a vacuum-exiccator. The obtained white powder appeared to be the mixture of macrocycles **4** and **6** according to the ^{1}H NMR spectroscopy data.

*The mixture of 14,17-dioxa-2,3,10,11-tetraaza-1,12(16,4α)di(19-nor-ent-beyerana)cyclooctadecaphane-1^{16}(2),12^{16}(11)-diene-4,9,13,18-tetraone (**4**) and 14,17,32,35-tetraoxa-2,3,10,11,20,21,28,29-octaaza-1,12,19,30(16,4α)tetra(19-nor-ent-beyerana)cyclohexatriacontaphane-1^{16}(2),12^{16}(11),19^{16}(20),30^{16}(29)-tetraene-4,9,13,18,22,27,31,36-octaone (**6**)*. Yield 55 %, R_f ($CHCl_3$-CH_3OH, 8:1) 0.50. *m/z* (MALDI TOF) 801.6, [M**4**+H]$^+$; 823.6, [M**4**+Na]$^+$; 839.6, [M**4**+K]$^+$; 1624, [M**6**+Na]$^+$. ^{1}H NMR (C_5D_5N, 303 K) δ_H ppm: 10.21 (2H, s, 2NH), 10.15 (2H, s, 2NH), 10.07 (2H, s, 2NH), 4.33-4.19 (4H, m, C^{19}(O)OCH_2CH_2O(O)C^{19}), 4.19-3.99 (8H, m, 2C$^{19'}$(O)OCH_2CH_2O(O)C$^{19'}$), 3.08-2.93 (2H, m, 2C$^{15}H_\alpha$), 2.88-2.78 (4H, m, 4C$^{15'}H_\alpha$), 2.38-0.53 (138H, mm, six *ent*-beyerane skeletons, three NC(O)$(CH_2)_4$C(O)N linkers), 1.16 (6H, s, 2C$^{18}H_3$), 1.14 (12H, s, 4C$^{18'}H_3$), 1.12 (6H, s, 2C$^{17}H_3$), 1.10 (12H, s, 4C$^{17'}H_3$), 0.72 (12H, s, 4C$^{20'}H_3$), 0.66 (6H, s, 2C$^{20}H_3$).

*The mixture of 14,18-dioxa-2,3,10,11-tetraaza-1,12(16,4α)-di(19-nor-ent-beyerana)cyclononadecaphane-1^{16}(2),12^{16}(11)-diene-4,9,13,19-tetraone (**5**) and 14,18,33,37-tetraoxa-2,3,10,11,21,22,29,30-octaaza-1,12,20,31(16,4α)tetra(19-nor-ent-beyerana)cyclooctatriacontaphane-1^{16}(2),12^{16}(11),20^{16}(21),31^{16}(30)-tetraene-4,9,13,19,23,28,32,38-octaone (**7**)*. Yield 18 %, R_f (CH_2Cl_2-CH_3OH, 40:1) 0.45. *m/z* (MALDI TOF) 815.8, [M**5**+H]$^+$; 853.8, [M**5**+K]$^+$; 1652.6, [M**7**+Na]$^+$. ^{1}H NMR (C_5D_5N, 303 K) δ_H ppm: 10.06 (2H, s, NH), 9.09 (4H, s, NH), 4.36-4.09 (4H, m, 2C^{19}(O)OCH_2), 4.02-3.86 (8H, m, 4C$^{19'}$(O)OCH_2), 3.25-3.14 (4H, m, 4C$^{15'}H_\alpha$), 3.03-2.92 (2H, m, 2C$^{15}H_\alpha$), 2.60-0.51 (144H, mm, six *ent*-beyerane skeletons, three NC(O)$(CH_2)_4$C(O)N linkers, three central CH_2 groups of three C^{19}(O)O$(CH_2)_3$O(O)C^{19} linkers), 1.11 (18H, s, 6C$^{18}H_3$), 1.06 (18H, s, 6C$^{17}H_3$), 0.82 (12H, s, 4C$^{20'}H_3$), 0.72 (6H, s, 2C$^{20}H_3$).

*The reaction of diacid **8** with ethylene glycol ditosylate.* The solution of ethylene glycol ditosylate (0.24 g, 0.65 mmol) in CH_3CN (15 ml) was added dropwise to the refluxing and stirred heterogeneous mixture of diacid **8** (0.5 g, 0.65 mmol) and K_2CO_3 (0.09 g, 0.65 mmol) in CH_3CN (25 ml) for 2 h, and then refluxed for a further 20 h under dry argon. A precipitate was filtered off, washed with water (2×50 ml), dried in air, and refluxed in absolute ethanol (50 ml) until all the solids went into solution. During further reflux a new precipitate begun to appear. When the formation of the precipitate had finished, heating was stopped. The precipitate was filtered off from the hot solution, dried firstly in air, and then was dried with $CaCl_2$ in a vacuum-exiccator. The obtained white powder (0.23 g) appeared to be the mixture of macrocycles **4** and **6** according to the ^{1}H NMR spectroscopy data.

*Bis(19-nor-4α-carbonylchloride-ent-beyeran-16(R)-yl) suberate (**13**)*. To the solution of diacid 11 (0.2 g, 0.26 mmol) in dry CH_2Cl_2 (6 ml) was added thionyl chloride (1 ml), the mixture was refluxed for 6 h under dry argon, and the excess thionyl chloride was then removed under reduced pressure. After addition of CH_2Cl_2, the mixture was stirred, the solvent was removed under reduced pressure, and the precipitate was dried *in vacuo* to give **13** as an oil in quantitative yield (0.21 g). IR (KBr) ν cm^{-1}: 1732, 1796 (C=O).

*2,13-Dioxa-16,17,24,25-tetraaza-1,14(16,4α)di(19-nor-ent-beyerana)cyclohexacosaphane-3,12,15,18,23,26-hexaone (**14**)*. Adipic acid dihydrazide (0.07 g, 0.40 mml) was added to the solution of fresh acid chloride **12** (0.32 g, 0.40 mmol) in pyridine (10 ml), and the mixture was stirred for 8 h at room temperature. Then the flask contents were dissolved in diethyl ether (100 ml), the solution was washed with acidified water (2×50 ml), extracted, and the organic layer was dried with anhydrous Na_2SO_4. After removing diethyl ether under reduced pressure the precipitate was fractioned by silica gel column chromatography. By using ethyl acetate as eluent the remains of adipic acid dihydrazide and diacid 10 were separated, and silica gel was flushed with ethanol. Then the solvent was removed under reduced pressure, a precipitate was recrystallized from methanol, dried *in vacuo*, and the pure product was obtained. Yield 0.26 g (71 %), white powder, m.p. 240 °C (with decomposition). Found: C 71.34, H 10.02, N 5.89. $C_{56}H_{88}N_4O_8$. Calculated: C 71.15, H 9.38, N 5.93. *m/z* (MALDI TOF) 946.4, [M+H]$^+$; 968.4, [M+Na]$^+$; 984.0, [M+K]$^+$. ^{1}H NMR ($CDCl_3$, 303 K) δ_H ppm: 9.50 (2H, s, 2NH), 8.37 (2H, s, 2NH), 4.76-4.70 (2H, m, 2C^{16}H), 2.33-2.21 (4H, m, 2C^{16}OC(O)CH_2), 2.18-2.09 (2H, m, 2C$^3H_{eq}$), 1.22 (6H, s, 2C$^{18}H_3$), 0.93 (6H, s, 2C$^{17}H_3$), 0.75 (6H, s, 2C$^{20}H_3$), 0.8-1.9 (58H, m, two *ent*-beyerane skeletons, the NC(O)$(CH_2)_4$C(O)N linker, the $(CH_2)_6$ fragment of the C^{16}OC(O)$(CH_2)_8$C(O)OC^{16} linker).

*The reaction of acid chloride **13** with adipic acid dihydrozide.* Adipic acid dihydrazide (0.07 g, 0.42 mmol) was added to the solution of fresh acid chloride **13** (0.34 g, 0.42 mmol) in pyridine (30 ml), and the mixture was stirred for 24 h at room temperature. When the reacrion finished pyridine was removed under reduced pressure, a precipitate was dried *in vacuo* and then dissolved in CH_2Cl_2 (50 ml). The solution was washed with acidified water (2×50 ml), and dried with anhydrous Na_2SO_4. After removing the solvent under reduced pressure the precipitate was dried with P_2O_5 in a vacuum-exiccator, and was fractioned by silica gel column chromatography.

*2,11-Dioxa-14,15,22,23-tetraaza-1,12(16,4α)di(19-nor-ent-beyerana)cyclotetracosaphane-3,10,13,16,21,24-hexaone (**15**)*. Yield 0.23 g (59 %), white powder, R_f(CH_2Cl_2-CH_3OH 30:1) 0.36, m.p. 200-205 °C. Found: C 70.42, H 9.95, N 6.16. $C_{54}H_{84}N_4O_8$. Calculated: C 70.71, H 9.23, N 6.11. *m/z* (MALDI TOF) 918, [M+H]$^+$; 939.9, [M+Na]$^+$; 955.9, [M+K]$^+$. ^{1}H NMR ($CDCl_3$, 303 K) δ_H ppm: 9.86 (2H, s, 2NH), 8.50 (2H, s, 2NH), 4.76 (2H, dd, ^1J = 10.3 Hz, ^3J = 2.6 Hz, 2C^{16}H), 2.37-2.17 (4H, m, 2C^{16}OC(O)CH_2), 2.12 (2H, d, ^1J =12.1 Hz, 2C$^3H_{eq}$), 2.06-0.57 (54H, m, two *ent*-beyerane skeletons, the NC(O)$(CH_2)_4$C(O)N linker, the $(CH_2)_4$ fragment of the C^{16}OC(O)$(CH_2)_6$C(O)OC^{16} linker), 1.19 (6H, s, 2C$^{18}H_3$), 0.91 (6H, s, 2C$^{17}H_3$), 0.73 (6H, s, 2C$^{20}H_3$).

*15,24,39,48-Tetraoxa-3,4,11,12,27,28,35,36-octaaza-1,14,25,38(4α,16)tetra(19-nor-ent-beyerana)cyclooctatetracontaphane-2,5,10,13,16,23,26,29,34,37,40,47-dodecaone (**16**)*. Yield 0.03 g (8 %), white powder, R_f= 0.39 (ethyl acetate), m.p. 147-150 °C. Found: C 70.23, H 9.04, N 5.99. $C_{108}H_{168}N_8O_{16}$. Calculated: C 70.71, H 9.23, N 6.11. *m/z* (MALDI TOF) 1834.6, [M+H]$^+$; 1856.6, [M+Na]$^+$; 1872.6, [M+K]$^+$. ^{1}H NMR ($CDCl_3$, 303 K) δ_H ppm: 9.57 (4H, s, 4NH), 8.23 (4H, s, 4NH), 4.71-4.63 (4H, m, 4C^{16}H), 2.39-2.20 (8H, m, 4C^{16}OC(O)CH_2), 2.19-2.13 (4H, m, 4C$^3H_{eq}$), 2.11-0.65 (148H, mm, four *ent*-beyerane skeletons, two NC(O)$(CH_2)_4$C(O)N linkers, two $(CH_2)_4$ fragments of two C^{16}OC(O)$(CH_2)_6$C(O)OC^{16} linkers), 1.24 (12H, s, 4C$^{18}H_3$), 0.91 (12H, s, 4C$^{17}H_3$), 0.74 (12H, s, 4C$^{20}H_3$).

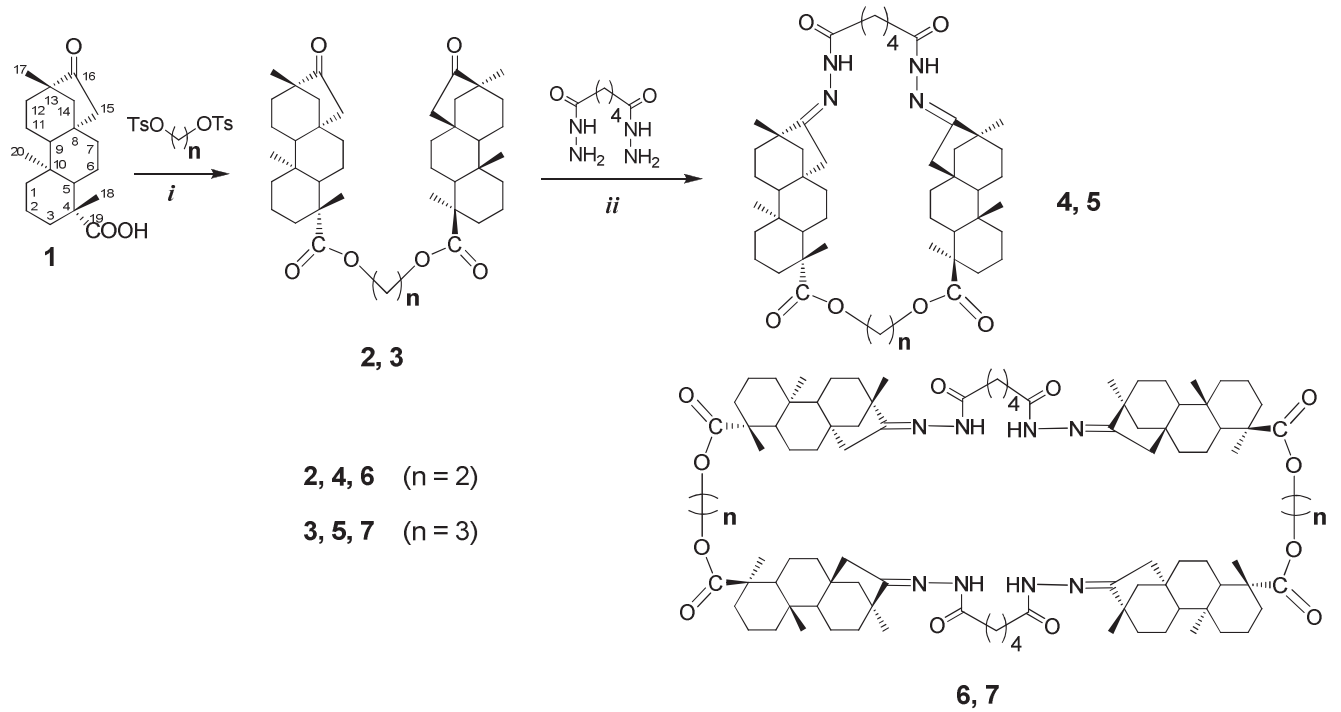
*Bis(19-nor-4α-carbazoyl-ent-beyeran-16(R)-yl)sebacate (**17**)*. Anhydrous hydrazine (0.4 ml, 11 mmol) was added to the

solution of fresh acid chloride **12** (0.19 g, 0.2 mmol) in CH_2Cl_2 (10 ml). The mixture was stirred for 20 h at room temperature, washed with water, and dried with anhydrous Na_2SO_4. Then the solvent and excess thionyl chloride were removed under reduced pressure, the precipitate was dried with P_2O_5 in a vacuum-exiccator to give the pure product as an amorphous powder. Yield 0.15 g (77 %). Found: C 71.05, H 9.37, N 6.64. $C_{50}H_{82}N_4O_6$. Calculated: C 71.90, H 9.90, N 6.71. *m/z* (MALDI TOF) 858.1, $[M+Na]^+$; 873.9, $[M+K]^+$. ^{1}H NMR ($CDCl_3$, 303 K) δ_H ppm: 6.95 (2H, s, C^{19}(O)NH), 4.71 (2H, dd, ^3J_t = 10.5 Hz, ^3J_g =4.4 Hz, $2C^{16}$H), 2.29 (4H, t, J = 7.3 Hz, $2C^{16}OC(O)CH_2$), 2.08 (2H, d, J = 14.1 Hz, $2C^3H_{eq}$), 1.16 (6H, s, $2C^{18}H_3$), 0.91 (6H, s, $2C^{17}H_3$), 0.74 (6H, s, $2C^{20}H_3$), 1.89-0.65 (50H, mm, two *ent*-beyerane skeletons, the $(CH_2)_6$ fragment of the $C^{16}OC(O)(CH_2)_8C(O)OC^{16}$ linker).

*7,18,25,36-Tetraoxa-3,4,21,22-tetraaza-1,6,19,24(4α,16) tetra(19-nor-ent-beyerana)cyclohexatriacontaphane-2,5,8,17, 20,23,26,35-octaone (**18**)*. The solution of dihydrazide **17** (0.15 g, 0.18 mmol) in CH_2Cl_2 (20 ml) was added dropwise to the stirred solution of acid chloride **12** (0.15 g, 0.18 mmol) in CH_2Cl_2 (100 ml) for 1 h under dry argon, then Et_3N (0.05 ml, 0.36 mmol) was added and the mixture was refluxed for a further 18 h. Then the mixture was washed with water, dried with $MgSO_4$, the solvent was removed under reduced pressure, and the precipitate was fractioned by silica gel column chromatography. Yield 0.11 g (40 %), R_f = 0.39 (CH_2Cl_2-CH_3OH, 10:1), white powder, m.p. 134-136 °C. Found: C 74.69, H 9.77, N 3.51 %. $C_{100}H_{156}N_4O_{12}$. Calculated: C 74.77, H 9.79, N 3.49. *m/z* (MALDI TOF) 1628.1, $[M+Na]^+$; 1644.1, $[M+K]^+$. ^{1}H NMR ($CDCl_3$, 303 K) δ_H ppm: 8.71 (4H, s, 4NH), 8.48 (4H, br.s, 4NH), 4.79-4.70 (8H, m, $8C^{16}$H), 2.29 (16H, t, J = 7.21 Hz, $8C^{16}OC(O)CH_2$), 1.22 (12H, s, $4C^{18}H_3$), 1.21 (12H, s, $4C^{18'}H_3$), 0.90 (24H, s, $8C^{17}H_3$), 0.74 (12H, s, $4C^{20}H_3$), 0.72 (12H, s, $4C^{20'}H_3$), 2.12-0.67 (208H, mm, eigth *ent*-beyerane skeletons, four $(CH_2)_6$ fragments of $C^{16}OC(O)(CH_2)_8C(O)OC^{16}$ linkers).

Results and Discussion

We used a two step approach to a synthesis of macrocyclic isosteviol derivatives in this study. The first step consisted in combining two molecules of the starting compound (isosteviol **1** or dihydroisosteviol **9**) into a molecule with unfolded (linear) molecular geometry using one of its functional groups (carboxyl, oxo or hydroxyl). Then this molecule was exposed to macrocyclization using the second (free) functional group. To connect two isosteviol molecules via their carboxyl groups the reaction of isosteviol **1** with ditosylates of diols was applied. The choice of dioxodiesters **2** and **3** (Scheme 2) as the starting compounds for macrocyclization was caused by their unusual molecular geometry. According to the X-ray data these compounds have a tweezer-like structure which is predisposed to macrocyclization because isosteviol moieties are located above each other and their C^{16}=O groups are oriented in the same direction.[25,26] Moreover, the dioxodiester on the basis of isosteviol **1** and diethylene glycol was found to have a tweezer-like structure even in the dilute solution in CCl_4.[27] The compounds **2** and **3** were previously obtained by the reaction of the isosteviol chloride-anhydride with appropriate diols. Using a "tosylate pathway" by the analogy with a synthesis of isosteviol esters[28] enabled us to increase the yields of dioxodiesters **2** and **3**, as well as to avoid the formation of monoalkylated products. The reactions were carried out in CH_3CN under reflux in the presence of K_2CO_3. The macrocyclization of dioxodiesters **2** and **3** was accomplished by the reaction with adipic acid dihydrazide in absolute ethanol under reflux. Surprisingly, in none of the cases the formation of macrocycles was not detected even after 20 hours. We supposed that the reason is the presence of crystalline water in the reagents and subjected them to preliminary heating in benzene in a flask equipped with a Dean-Stark trap for 15 hours. Benzene was then removed under reduced pressure, the reagents were dissolved in boiling ethanol and the reaction was carried out as long as the formation of a precipitate, which is the reaction product,

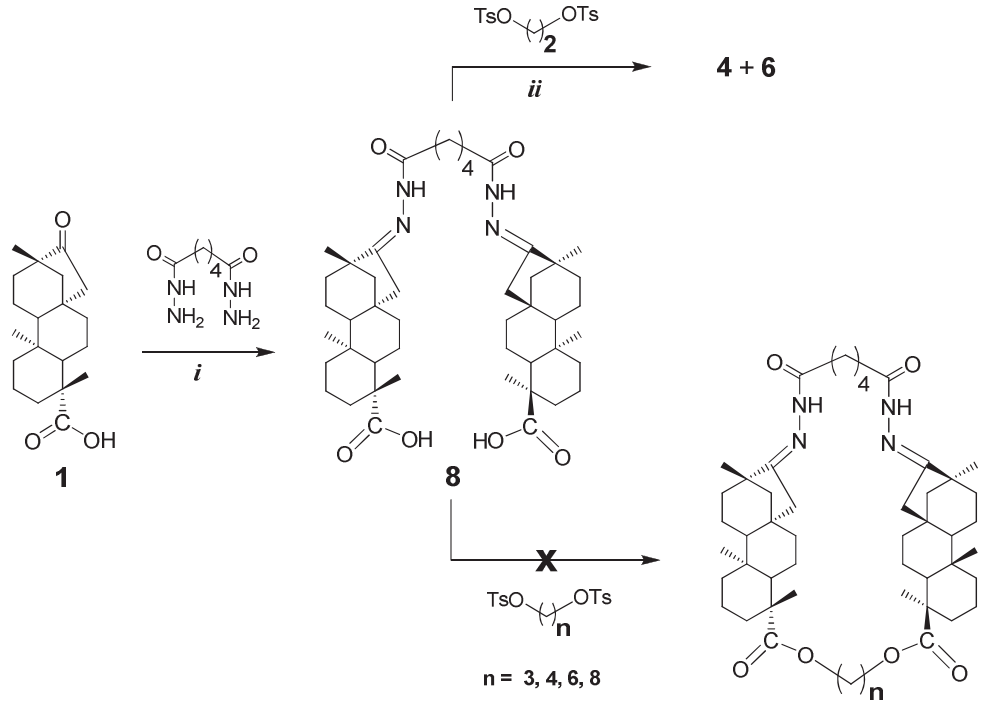


Scheme 2. Reagents and conditions: (i) CH_3CN, K_2CO_3, reflux, yields: **2** 54 %, **3** 64 %; (ii) C_6H_6, reflux, 6 h; C_2H_5OH, reflux, 40 h, yields: (**4** + **6**) 55 %, (**5** + **7**) 18 %.

had finished. The MALDI spectrum of the product of the reaction of dioxodiester **2** with adipic acid dihydrazide was composed of the main peak group involving the peaks at *m/z* 801.6, $[M+H]^+$, *m/z* 823.6, $[M+Na]^+$, and *m/z* 839.6, $[M+K]^+$ corresponding to cationized molecules of binuclear macrocycle **4**, as well as the by-product peak at *m/z* 1624.1, $[M+Na]^+$ corresponding to a trace amount of tetranuclear macrocycle **6**. A similar peak groups were observed in the MALDI spectrum of the product of the reaction of adipic acid dihydrazide with dioxodiester **3**. There were the main peak group involving the peaks at *m/z* 815.8, $[M+H]^+$ and *m/z* 853.8, $[M+K]^+$ corresponding to cationized molecules of binuclear macrocycle **5**, and the peak at *m/z* 1652.6, $[M+Na]^+$ corresponding to a trace amount of cationized molecules of tetranuclear macrocycle **7**. The ^{1}H NMR spectroscopic data also confirmed the formation of the macrocycles mixture. Thus the two sets of signals that are characteristic for isosteviol **1** and its derivatives[11-13,20,21,25,29] were observed in the ^{1}H NMR spectrum of the product of the reaction of dioxodiester **2** with adipic acid dihydrazide. Firstly, these were singlets at δ = 1.16 and 1.14 ppm corresponding to $C^{18}H_3$ groups of different molecules, singlets at δ = 1.12 and 1.10 ppm corresponding to $C^{17}H_3$ groups of different molecules, and singlets at δ = 0.72 and 0.66 ppm corresponding to $C^{20}H_3$ groups of different molecules. Secondly, these were signals in the ranges from 3.08 to 2.93 ppm and from 2.88 to 2.78 ppm corresponding to the $C^{15}H_\alpha$ proton of different molecules. Thirdly, there were signals in the ranges from 4.33 to 4.19 ppm and from 4.19 to 3.99 ppm corresponding to $C^{19}(O)OCH_2CH_2O(O)C^{19}$ linkers of different molecules. The ^{1}H NMR spectrum of the product of the reaction of dioxodiester **3** with adipic acid dihydrazide looked similarly to the one just mentioned. The signals listed above were assigned to binuclear and tetranuclear macrocycles on the basis of integral intensities. The range of hydrazide protons resonances was the distinctive range in the ^{1}H NMR spectra of the reaction products of compounds **2** and **3** with adipic acid dihydrazide. Several signals corresponding to NH protons were observed in the ^{1}H NMR spectra of the mixtures of macrocycles **4**, **6** and **5**, **7**. Thus three singlets at 10.15, 10.21 and 10.07 ppm were observed in the ^{1}H NMR spectrum of the mixtures of macrocycles **4** and **6**, whereas only two singlets at 10.06 и 9.09 ppm were observed in the ^{1}H NMR spectrum of the mixture of macrocycles **5** and **7**. This fact seemed to be the result of a hindered rotation around the NH-C(O) bonds in molecules of macrocycles **4**-**7** that causes the appearance of several hindered conformations with different orientations of the N-H bonds relatively to the C=O and C=N bonds in the hydrazide/hydrazone moieties. Unfortunately, we could not isolated macrocycles **4** and **6** as well as macrocycles **5** and **7** owing to their similar polarity.

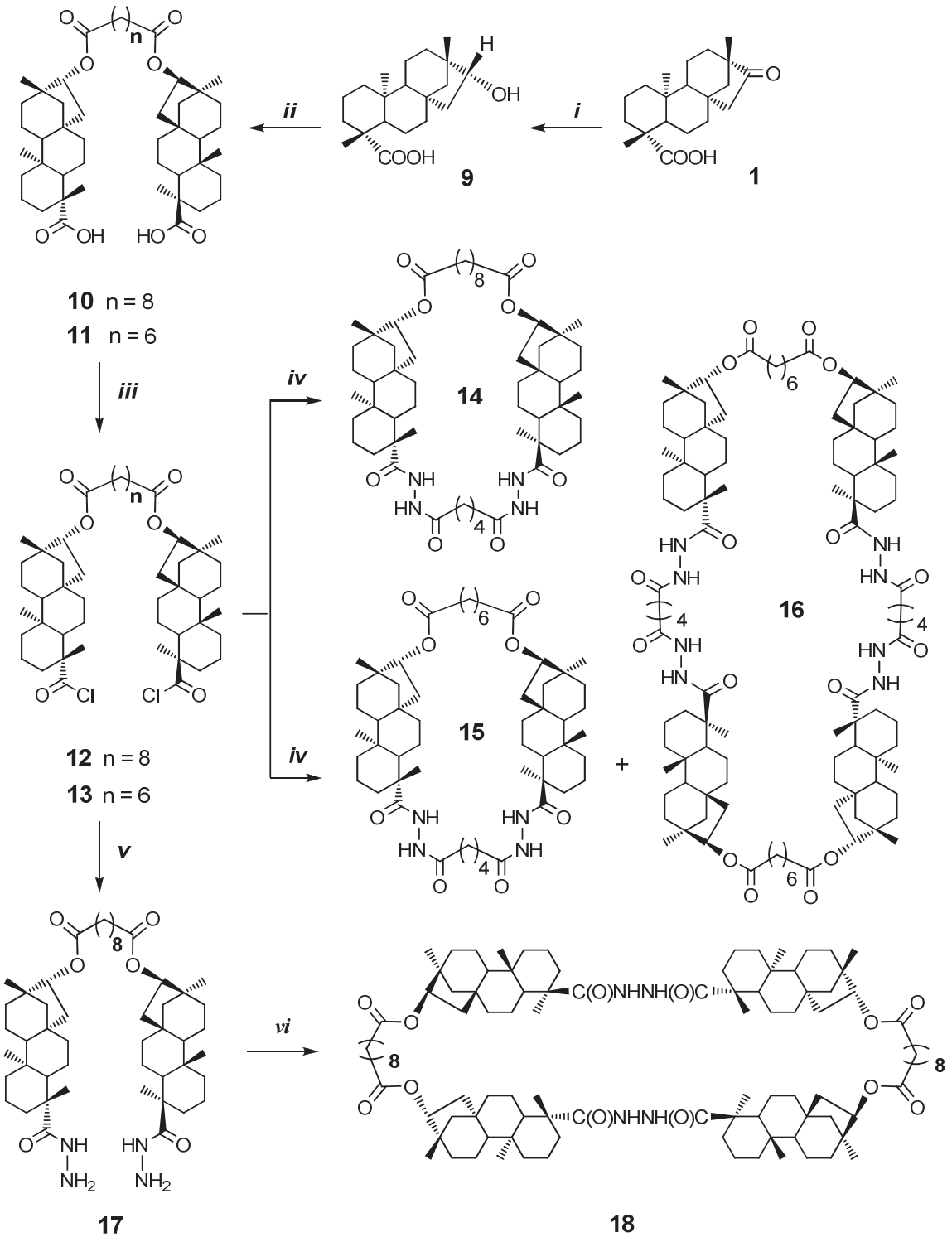
At the next stage of our study we connected two isosteviol molecules via the C^{16}=O groups using its reaction with adipic acid dihydrazide that afforded diacid **8** in 45 % yield (Scheme 3). As a reaction with diol ditosylate showed itself to advantage in coupling isosteviol molecules, we planned to use it for macrocyclization of diacid **8**. Because of a restricted solubility of **8** its reaction with ethylene glycol ditosylate was carried out in boiling CH_3CN for 20 hours. After removing non-reacted starting compounds by boiling ethanol a white powder was obtained which appeared to be the mixture of macrocycles **4** and **6** according to the MS MALDI and NMR spectroscopy data. We did not manage to isolate the macrocycles in individual forms. Moreover we could not involve diacid **8** in the reactions with ditosylate of more lengthy diols.

Having failed in the macrocyclization of diacid **8** we decided to connect two isosteviol molecules via the C^{16}=O groups by a diester linker, instead of a dihydrazide one. For this purpose initially isosteviol oxo group was reducted with $NaBH_4$ using the known method.[24] The reaction proceeded stereospecific to afford C^{16}(*R*)-epimer **9** in accordance with



Scheme 3. Reagents and conditions: (i) CH_3OH, 25 °C, 39 h, yield 61 %; (ii) CH_3CN, K_2CO_3, reflux, 20 h, argon, yield 45 %.

the data previously reported.[24,30-33] Then two molecules of dihydroisosteviol **9** were coupled with a diester linker by the reaction with cebacoyl (or cuberoyl) dichloride (Scheme 4). Diacid **10** was obtained as described previously.[13] Diacid **11** obtained earlier by the reaction of dihydroisosteviol **9** with cuberoyl dichloride in CCl_4 in the presence of $CoCl_2$ in 35 % yield[20] was synthesized now under another condition[13] that increased the yield up to 50 %. To obtain macrocyclic isosteviol derivatives with hidrazide moietis diacids **10** and **11** were converted to their acid chlorides which were further exposed to the macrocyclization by the reaction with adipic acid dihydrazide (Scheme 4). The course of the macrocyclization of acid chlorides **12** and **13** appeared to depend on a correspondence between the distances between carbonyl chloride groups in these compounds which were estimated on the basis of X-ray data[34] and a length of



Scheme 4. Reagents and conditions: (i) $NaBH_4$, CH_3OH, rt, yield 90 %; (ii) $ClOC(CH_2)_nCOCl$, CH_2Cl_2, DMAP, Py; (iii) $SOCl_2$, 40 °C; 2 h, quantitative yield; (iv) $NH_2NHC(O)(CH_2)_4C(O)NHNH_2$, Py, rt, 16 h; (v) NH_2NH_2 (excess), CH_2Cl_2, rt, yield 77 %; (vi) acid chloride **12**, CH_2Cl_2, Et_3N, reflux, yield 40 %.

binucleophile. According to MS MALDI the reaction of acid chloride **12** with adipic acid dihydrazide afforded exclusively binuclear macrocycle **14** (Scheme 4) obtained by column chromatography on silica gel in 71 % yield. Its MALDI spectrum exhibited peaks at *m/z* 946.4, $[M+H]^+$; 968.4, $[M+Na]^+$; 984.0, $[M+K]^+$. The reaction of acid chloride **13** with adipic acid dihydrazide led to the mixture of binuclear macrocycle **15** and tetranuclear macrocycle **16** that were separated by column chromatography on silica gel. Binuclear macrocycle **15** was isolated in 59 % yield, tetranuclear macrocycle **16** was isolated in 8 % yield. Both compounds were characterized by MS MALDI, ^{1}H NMR spectroscopy and elemental analysis data. The reaction was carried out at room temperature in pyridine which perfectly dissolves both reagents, and it was found that the course of the reaction did not depend on the reagent concentrations. Thus the yields of macrocycles **15** and **16** were very similar at the acid chloride – pyridine ratio both 1:70 and 1:900. The ^{1}H NMR spectra of compounds **14-16** showed just a one set of signals characteristic for isosteviol **1** and its derivatives,[11-13,20,21,25,29] namely three singlets corresponding to $C^{18}H_3$, $C^{17}H_3$, $C^{20}H_3$ groups, one multiplet corresponding to $C^{16}H$, and two singlets corresponding to protons of dihydrazide linker.

One can suppose that the reason of good yields of binuclear macrocycles **14** (71 %) and **15** (59 %) is the predisposition of the molecular structures of acid chlorides **12** and **13** to macrocyclization. Probably the distance between carbonyl chloride groups in their molecules is closed to the distance between the terminal amine groups of adipic acid dihydrazide. It follows that if a diamine whose length is smaller than the distance between carbonyl chloride groups of acid chlorides **12** (or **13**) is involved in the reaction with these compounds, it can not cyclize them with the formation of a binuclear macrocycle. Tetranuclear macrocycle should be formed in this case. To prove this hypothesis the reaction of acid chloride **12** with the shortest chain diamine, namely hydrazine, was carried out. For reliability, the macrocyclization was carried out in two steps: initially hydrazine was attached to acid chloride **12**, and then obtained dihydrazide **17** was involved in the reaction with acid chloride **12**. According to MS MALDI data the product of the reaction isolated by column chromatography on silica gel in 40 % yield appeared to be tetranuclear macrocycle **18** (Scheme 4). Its MALDI spectrum showed only peaks corresponding to its cationized molecules, namely *m/z* 1628.1, $[M+Na]^+$, and 1644.1, $[M+K]^+$. The ^{1}H NMR spectrum of the product exhibited characteristic signals corresponding to the $C^{18}H_3$, $C^{17}H_3$, $C^{20}H_3$ groups of *ent*-beyerane skeletons, tryplet at δ = 2.29 ppm corresponding to protons of $C^{16}OC(O)CH_2$ moieties of diester linkers, and multiplet in the range from 4.79 to 4.70 ppm corresponding to $C^{16}H$ protons. All these data confirmed the formulas of macrocycle **18** in Scheme 4. It is noteworthy that two signals at δ = 8.71 and 8.48 ppm were observed in the range corresponding to hydrazide protons of macrocycle **18**, though they seemed to be equivalent. Moreover, in contrast to ^{1}H NMR spectra of macrocycles **14-16** the ^{1}H NMR spectrum of the discussed product showed two signals of $C^{18}H_3$ groups at δ = 1.22 and 1.21 ppm, and two signals of $C^{20}H_3$ groups at δ = 0.74 and 0.72 ppm which were not shifted on heating from 303 K to 403 K. These facts revealed that the product is the mixture of several compounds that have the same molecular mass according to the MS MALDI spectrum and the elemental analysis data, therefore these are diastereomers. Considering the synthetic pathway to macrocycle **18** (Scheme 4) it should be emphasized the following. Individual (-)-isosteviol **1**[23] having four chiral carbons 4(*R*),8(*R*),10(*S*),13(*S*)[35] was used as the starting material. The reduction of its oxo group afforded dihydroisosteviol **9** obtained, like in known papers,[24, 30-33] as the pure 16(*R*)-epimer. Subsequent transformations presented in Scheme 4 could not change the C^{16} carbon configuration, so pure 16(*R*),16'(*R*)-epimers of compounds **12** and **17** were used in the final step of our convergent synthesis. This fact was proved by their ^{1}H NMR spectra which displayed only one set of characteristic signals of $C^{18}H_3$, $C^{17}H_3$, and $C^{20}H_3$ groups. Hence diastereomers of the macrocycle **18** could be formed just in the course of the reaction of acid chloride **12** with dihydrazide **17**. Revealing structures of diastereomers of macrocycle **18** that needs special experiments and computations will be undertaken.

Conclusions

To sum up, unknown before macrocyclic derivatives of diterpenoid isosteviol have been first synthesized. They contain two or four diterpenoid *ent*-beyerane skeletons connected by linkers with ester, hydrazide, or hydrazide/hydrazone moieties. Three approaches were studied to synthesize these macrocycles. The first one consisted in initially coupling of two isosteviol molecules via their carboxyl groups by a reaction with a diol ditosylate, followed by a macrocyclization of obtained diketone (**2** or **3**) that was carried out by a reaction with adipic acid dihydrazide. The second approach consisted in initially coupling of two isosteviol molecules via their carbonyl functionalities by a reaction with adipic acid dihydrazide, followed by a macrocyclization of obtained diacid **8** by its interaction with a diol ditosylate. During the course of the third approach initially selective reduction of isosteviol oxo group with $NaBH_4$ gave dihydroisosteviol **9**. Then two molecules of hydroxyacid **9** were coupled by the reaction with sebacoyl dichloride (or suberoyl dichloride) afforded diacid **10** (or **11**). In the next step diacids obtained were converted to acid chlorides (**12** or **13**) which were macrocyclisized by a reaction with adipic acid dihydrazide in the final step of the convergent synthesis. The first approach led to the mixture of binuclear (compounds **4**, **5**) and tetranuclear (compounds **6**, **7**) macrocycles which we could not separate. Using the second approach we also did not achieve a success. The reaction of diacid **8** with adipic acid dihydrazide also gave the mixture of binuclear macrocycle **4** and tetranuclear macrocycle **6**. Moreover, it appeared to be impossible to involve diacid **8** in the reactions with ditosylates of *n*-propane, *n*-butane, *n*-hexane, and *n*-octane diols because of its restricted solubility. The third approach appeared to be successful. Binuclear macrocycles **14**, **15** and tetranuclear macrocycle **16** were obtained in 71, 59, and 8 % yields, respectively. It was found out that the courses of the reactions of acid chlorides **12** and **13** with adipic acid dihydrazide depend on the distance between isosteviol moieties in molecules of compounds **12** and **13** (sebacoyl and suberoyl linkers, respectively). In the first case binuclear macrocycle

14 was exclusively obtained, whereas in the second one, along with the formation of binuclear macrocycle **15**, tetranuclear macrocycle **16** was formed, albeit in low yield (8 %). We have shown that the shortest linker should be used for the synthesis of tetranuclear macrocycles by a macrocyclization of acid chlorides **12** and **13**. Thus the reaction of acid chloride **12** with dihydrazide **17** afforded tetranuclear macrocycle **18** in a good yield (40 %) as a mixture of diastereomers.

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References

1. Breitmaier E. *Terpenes* Hamburg: Wiley, **2006**. 214 p.
2. Wessjohann L.A., Ruijter E., Garcia-Rivera D., Brandt W. *Mol. Diver.* **2005**, *9*, 171-186.
3. Shi Q.-W., Su X.-H., Kiyota H. *Chem. Rev*. **2008**, *108,* 4295-4327.
4. Devappa R.K., Makkar H.P.S., Becker K. *J. Am. Oil Chem. Soc*. **2011**, *88*, 301-322.
5. Ferreira M.J.U., Duarte N., Lage H., Molnár J. Reversal of Multidrug Resistance by Macrocyclic and Polycyclic Diterpenoids from *Euphorbia* species, Ch. 8. In: *Recent Progress in Medicinal Plants. Vol. 32. Ethnomedicine and Therapeutic Validation* (Kaushik G., Govil J.N., Eds.), Houston: Studium Press Lic., **2012**. 448 p.
6. Duarte N., Jardanhazy A., Molnar J., Hilgeroth A., Ferreira M.-J.U. *Bioorg. Med. Chem*. **2008**, *16*, 9323-9330.
7. Baloch I., Baloch M., Saqib Q. *Planta Med.* **2006**, *72*, 830-834.
8. Alvaro E., de la Torre M.C., Sierra M.A. *Chem. Commun*. **2006**, *9,* 985-987.
9. Larionov S.V., Tkachev A.V., Myachina L.I., Bizyaev S.N., Glinskaya L.A., Klevtsova R.F. *Doklady Chemistry* **2006**, *411*, 206-211.
10. Gorshkov N.B., Agafontsev A.M., Tkachev A.V. *Russ. Chem. Bull., Int. Ed*. **2010**, *59*, 1467-1471.
11. Strobykina I.Yu., Garifullin B.F., Kovylyaeva G.I., Musin R.Z., Gubaidullin A.T., Kataev V.E. *Russ. J. Gen. Chem*. **2007**, *77*, 1066-1068.
12. Garifullin B.F., Strobykina I.Yu., Lodochnikova O.A., Musin R.Z., Gubaidullin A.T., Kataev V.E. *Chem. Nat. Compd*. **2011**, *47*, 422-427.
13. Khaybullin R.N., Strobykina I.Yu., Gubskaya V.P., Fazleeva G.M., Latypov Sh.K., Kataev V.E. *Mendeleev Commun*. **2011**, *21*, 134-136.
14. Korochkina M.G., Nikitashina A.D., Khaibullin R.N., Petrov K.A., Strobykina I.Yu., Zobov V.V., Kataev V.E. *Med. Chem. Commun*. **2012**, *3*, 1449-1454.
15. Khaybullin R.N., Strobykina I.Yu., Dobrynin A.B., Gubaydullin A.T., Chestnova R.V., Babaev V.M., Kataev V.E. *Bioorg. Med. Chem. Lett*. **2012**, *22*, 6909-6913.
16. Ojima I., Lin S., Inoue T., Miller M.L., Borella C.P., Geng X., Walsh J.J. *J. Am. Chem. Soc.* **2000**, *122*, 5343 -5353.
17. Ojima I., Das M. *J. Nat. Prod.* **2009**, *72,* 554-565.
18. Tamminen J., Kolehmainen E. *Molecules* **2001***, 6,* 21-46.
19. Virtanen E., Kolehmainen E. *Eur. J. Org. Chem.* **2004**, 3385-3399.
20. Kataev V.E., Militsina O.I., Strobykina I.Yu., Kovylyaeva G.I., Musin R.Z., Fedorova O.V., Rusinov G.L., Zueva M.N., Mordovskoi G.G., Tolstikov A.G. *Pharm. Chem. J.* **2006**, *40*, 473-475.
21. Garifullin B.F., Strobykina I.Yu., Mordovskoi G.G., Mironov V.F., Kataev V.E. *Chem. Nat. Compd*. **2011**, *47*, 55-58**.**
22. Khaibullin R.N., Strobykina I.Yu., Kataev V.E., Lodochnikova O.A., Gubaidullin A.T., Musin R.Z. *Russ. J. Gen. Chem*. **2009**, *79*, 967-971.
23. Mosettig E., Nes W.R. *J. Org. Chem.* **1955**, *20*, 884-899.
24. Al'fonsov V.A., Bakaleinik G.A., Gubaidullin A.T., Kataev V.E., Kovylyaeva G.I., Konovalov A.I, Litvinov I.A., Strobykina I.Y., Strobykin S.I., Andreeva O.V., Korochkina M.G. *Russ. J. Gen. Chem*. **2000**, *70*, 953-960.
25. Al'fonsov V.A., Andreeva O.V., Bakaleinik G.A., Beskrovnyi D.V., Gubaidullin A.T., Kataev V.E., Kovylyaeva G.I., Konovalov A.I., Korochkina M.G., Latypov Sh.K., Litvinov I.A., Musin R.Z., Strobykina I.Yu. *Russ. J. Gen. Chem*. **2003**, *73*, 1119-1129.
26. Alfonsov V.A., Bakaleynik G.A., Gubaidullin A.T., Kataev V.E., Kovyljaeva G.I, Konovalov A.I., Litvinov I.A., Strobykina I.Yu., Andreeva O.V., Korochkina M.G. *Mendeleev Commun*. **2000**, *5*, 195-196.
27. Kataev V.E., Strobykin S.I., Timosheva A.P., Al'fonsov V.A., Korochkina M.G., Strobykina I.Yu. *Russ. J. Gen. Chem*. **2005**, *75*, 272-275.
28. Wu Y., Yang J.-H., Dai G.-F., Liu C.-J., Tian G.-Q., Ma W.-Y., Tao J.-C. *Bioorg. Med. Chem*. **2009**, *17*, 1464-1473.
29. Korochkina M., Fontanella M., Casnati A., Arduini A., Sansone F., Ungaro R., Latypov Sh., Kataev V., Alfonsov V. *Tetrahedron* **2005**, *61*, 5457-5463.
30. Pariwat P., Homvisasevongsa S., Muanprasat C., Chatsudthipong V. *J. Pharm. Exp. Ther*. **2008**, *324*, 798-805.
31. Wu Y., Dai G.-F., Yang J.-H., Zhang Y.-X., Zhu Y., Tao J.-C. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 1818-1821.
32. An Y.-J., Zhang Y.-X., Wu Y., Liu Z.-M., Pi C., Tao J.-C. *Tetrahedron: Asymmetry* **2010**, *21*, 688-694.
33. Chen J., Sun M., Cai J., Cao M., Zhou W., Ji M. *J. Chem. Crystallogr*. **2011**, *41*, 519-522.
34. Kataev V.E., Militsina O.I., Strobykina I.Yu., Gubaidullin A.T., Zverev V.V., Kataeva O.N., Fedorova O.V., Valova M.S., Rusinov G.L. *J. Incl. Phenom*. **2008**, *60*, 51-58.
35. Alfonsov V.A., Bakaleinic G.A., Gubaidullin A.T., Kataev V.E., Kovylyaeva G.I., Konovalov A.I., Litvinov I.A., Strobykina I.Yu., Strobykin S.I. *Russ. J. Gen. Chem*. **1998**, *68*, 1735-1743.

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