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CONCISE ARTICLE

Unfolded and macrocyclic ammonium derivatives of diterpenoids steviol and isosteviol having choline moieties. Synthesis and inhibitory activities toward acetylcholine- and butyrylcholinesterases

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A series of unfolded and macrocyclic ammonium derivatives of diterpenoids isosteviol **2** and steviol **17** possessing choline moieties have been synthesized and assayed for inhibitory activities toward AchE and BchE. Compounds **5–8**, **13**, **16**, **20**, and **21** showed moderate activity within the range of IC_{50} values 8.0×10^{-4} to 2.2×10^{-6} mol L^{-1}. Isosteviol derivative **16** exhibited the best inhibition selectivity against acetylcholinesterase among the compounds tested. It inhibited AchE of human erythrocytes at $IC_{50} = 80$ μM, whereas inhibition of BchE occurred at $IC_{50} > 1000$ μM.

Introduction

Natural products (NPs) are the most consistently successful source of chemicals leading to the development of many drugs for diverse indications, both historically and currently.[1] Every year many new natural products in themselves, semi-synthetic and NP-derived compounds begin to undergo clinical evaluation or registration in the area of diseases, *i.e.* infectious (bacterial, fungal, parasitic and viral), immunological, cardiovascular, neurological, inflammatory and related diseases as well as oncology.[2] Functionalization of naturally occurring compounds with known pharmacophore moieties[3,4] and synthesis of their hybrid compounds[5] are among the most widely used in medicinal chemistry approaches for obtaining novel therapeutic agents.

Ent-beyeran diterpenoid isosteviol **2** (ref. 6a) (*ent*-16-oxo-beyeran-19-oic acid) is ranked among the most perspective naturally occurring scaffolds for affording novel bioactive compounds. It possesses antihypertension,[6b] hypotension,[6c,d] antihyperglycemic,[6e–g] insulinotropic,[6g] glucanostatic,[6g] anti-proliferation,[6h] cardio-[6h,i] and neuroprotective[6j] effects, prevents the growth of cancer cells[6k,l] and exhibits tuberculostatic activity.[6m] About 100 isosteviol derivatives of various structures have been covered in the literature,[7] many of them being bioactive.[8] For example, lactone derivatives of isosteviol demonstrate anti-inflammatory activity;[8a] functionalization of isosteviol by introducing a double bond at C15,[8b] by interconversions in ring D[8c] and by coupling of two isosteviol molecules with an amide linker[8d] led to novel *ent*-beyeran-type anticancer agents,[8b–d] functionalization of isosteviol with azine, hydrazide, hydrazone moieties provided a new type of antituberculosis agents.[3a] Recently diterpenoid isosteviol **2** was furnished with a choline moiety Me_3N^+–CH_2CH_2–O– and the obtained derivatives exhibited high antimicrobial activity.[3b,c] It is known that alkylammonium compounds represent a considerable class of selective reversible inhibitors of cholinesterase.[9] We considered that it would be interesting to investigate whether ammonium derivatives of diterpenoid isosteviol having acetylcholine-like structure in which an acyl group is replaced with bulky isostevioyl moiety show anti-cholinesterase activity. In our opinion, the approach to design novel inhibitors of AchE (BchE) on the basis of naturally occurring terpenoids functionalized by choline moiety or even only ammonium group seems to be original for the following reasons. Firstly, the literature provides only a few papers concerning semisynthetic terpenoids having choline moieties. These are the derivatives of abietic,[3d] (+)-dehydroabietic,[3e–g] betulinic,[4b] oleanolic,[4a,b] and ursolic[4b] acids, but in none of the cases anti-cholinesterase activity is evaluated. Secondly, no information about macrocycles consisting of terpenoid units connected by linkers having quaternary nitrogens is found in the literature. Many macrocycles consisting of ammonium groups connected by polymethylene,[10] polyoxamethylene[11] (azacoronands) linkers as well as azacyclophanes[12] and azapyrimidinophanes[13] having quaternary nitrogens are known, but in none of the cases anti-cholinesterase activity was evaluated.

In the present work we describe the synthesis of a series of isosteviol derivatives having one or two choline and *ent*-beyeran moieties in particular unfolded compounds **5–8**, macrocyclic compound **12**, ammonium compound **16** possessing a triethylammonium group coupled with isosteviol skeleton by a tri(oxyethylene) linker, and *ent*-kauranes **20** and **21** which are analogous to *ent*-beyeranes **5** and **6**. The inhibitory activities of some synthesized compounds toward AchE and BchE are also discussed.

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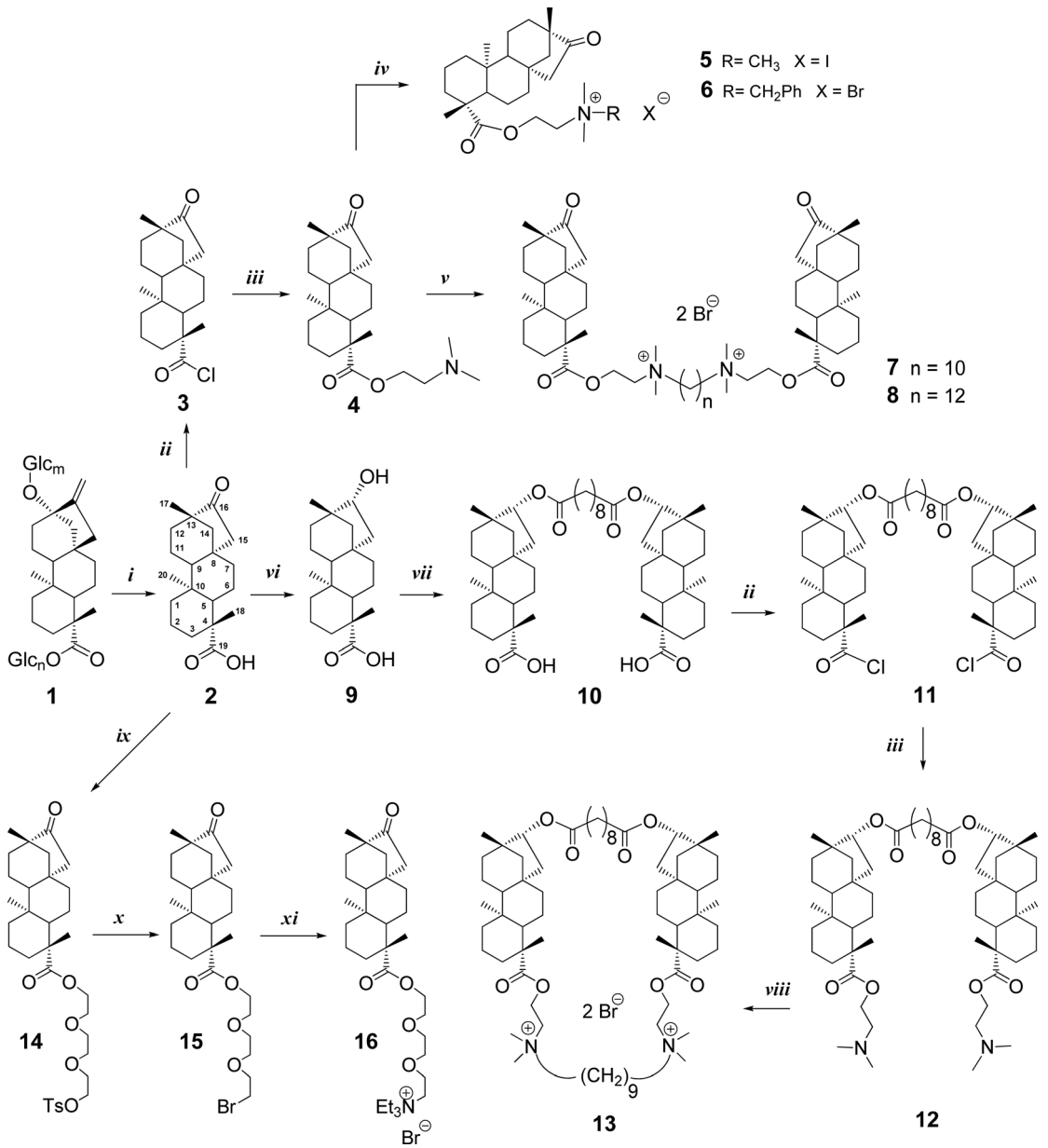
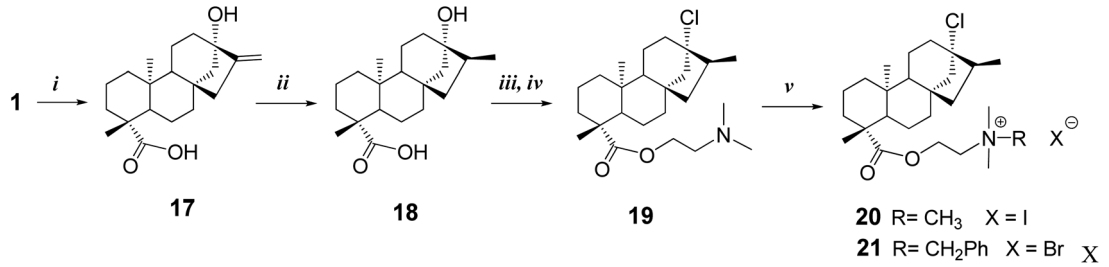
Results and discussion

Isosteviol derivatives **5** and **6** having one choline moiety were synthesized as shown in Scheme 1 according to the procedure previously described.[3b] Isosteviol **2** prepared by acid hydrolysis of sweetener Sweta **1** (ref. 14) as previously described[15] was converted to the isosteviol chloride **3** which was further treated with 2-dimethylaminoethanol to give amine **4**. The reaction of amine **4** with alkyl halides afforded ammonium derivatives **5** and **6** in 80% and 70% yields. Then amine **4** was treated with dibromoalkanes according to the procedure previously described[3c] to give ammonium compounds **7** and **8** in 30% and 45% yields. Macrocycle **13** having two *ent*-beyeran (isosteviol) skeletons and two choline moieties was synthesized from isosteviol **2** as shown in Scheme 1. Initially selective reduction of the isosteviol oxo group with $NaBH_4$ (ref. 16) gave compound **9** in good yield (92%). Treatment of **9** with sebacoyl chloride in CH_2Cl_2 in the presence of Py and DMAP furnished **10** in 58% yield.[17] Then diacid **10** was converted with the excess of thionyl chloride into the corresponding acid dichloride **11** in a quantitative yield. The amination of **11** with *N*,*N*′-dimethylaminoethanol afforded diamine **12** in 22% yield. Then treatment of **12** with 1,9-dibromononane in CH_3CN furnished macrocycle **13** in 6% yield.

The macrocyclization hardly proceeded with low conversion of starting amine **12**. When the reaction is completed the reaction mixture was concentrated under vacuum, the obtained crude material was separated by column chromatography into three fractions: macrocycle **13** in 6% yield, starting amine **12** in roughly



Scheme 1 *Reagents and conditions*: (i) 25% H_2SO_4; (ii) $SOCl_2$, 40 °C; 2 h; (iii) $HOCH_2CH_2N(CH_3)_2$, excess, CH_2Cl_2, reflux, 21 h; (iv) RX, CH_3CN, reflux; (v) $Br(CH_2)_nBr$, CH_3CN, reflux; (vi) $NaBH_4$, CH_3OH, rt; (vii) $ClOC(CH_2)_8COCl$, CH_2Cl_2, DMAP, Py; (viii) $Br(CH_2)_9Br$, CH_3CN, reflux; (ix) $Ts(OCH_2CH_2)_3OTs$, CH_3CN, $CsCO_3$; (x) LiBr, acetone and (xi) Et_3N, CH_3CN.



Scheme 2 *Reagents and conditions*: (i) $NaIO_4$, KOH, H_2O; (ii) $NH_2NH_2 \cdot H_2O$, KOH, H_2O, RANEY® Ni; (iii) $SOCl_2$, 40 °C; 2 h; (iv) $HOCH_2CH_2N(CH_3)_2$, excess, CH_2Cl_2, reflux, 21 h and (iv) RX, CH_3CN, reflux.

80% yield, and a mixture of unidentified products located near the origin of TLC plate (roughly 10% yield).

It should be noted that even ammonium derivatives of isosteviol **5–8** and **12** are restrictedly dissolved in water owing to their lipophilic hydrocarbon skeletons. To enhance the solubility a polymethylene linker bound ammonium group with isosteviol skeleton was replaced by tri(oxyethylene) one. The first water soluble isosteviol derivative **16** was synthesized as shown in Scheme 1. Alkylation of isosteviol **2** with 1,10-ditosyl-1,4,7,10-tetraoxadecane afforded tosylat **14** which was treated with LiBr in acetone to replace the TsO group with bromine. Then the reaction of bromide **15** with Et_3N in CH_3CN gave salt **16** in 57% yield.

To evaluate whether the change of the geometry of diterpenoid skeleton from *ent*-beyeran-type (as in isosteviol **2**) to *ent*-kauran-type (as in its isomer, diterpenoid steviol **17** (ref. 6*a* and 18)) modifies inhibitory activity, *ent*-kauranes **20** and **21** having choline moiety were synthesized from 16(*S*)-dihydrosteviol **18** (ref. 15) as shown in Scheme 2.

The inhibitory activities of compounds **5–8**, **13**, **16**, **20**, and **21** toward human acetyl- (hAchE, EC 3.1.1.7) and butyrylcholinesterase (hBchE, EC 3.1.1.8) were assayed by the spectrophotometric method of Ellman.[19,20] The IC_{50} values and selectivity indices (the IC_{50} BChE/IC_{50} AChE ratios) of compounds synthesized along with the reference selective AchE inhibitor BW284C51 are listed in Table 1.

The analysis of the obtained data showed that all examined compounds exhibited moderate activities within the range of IC_{50} values 10^{-4} to 10^{-6} mol L^{-1}, however some interesting regularities can be revealed. Firstly, the worst inhibition activity against AchE of human erythrocytes was demonstrated by isosteviol derivative **5** having one choline moiety whereas the best inhibition activity was shown by isosteviol derivatives **8** and **13** having two choline moieties. Unfolded compound **8** was formed from two isosteviol molecules coupled by the linker consisting of two choline moieties connected by a dodecamethylene chain. Macrocycle **13** was formed from two isosteviol molecules coupled by two linkers, namely, (1) the similar linker consisting of two choline moieties connected by a nonamethylene chain, and (2) a diester linker on the basis of sebacic acid. So the coupling of two isosteviol derivatives having one choline moiety results in the increase of inhibition activity against AchE of human erythrocytes. Secondly, isosteviol derivatives **7** and **8** differ just in the length of polymethylene linker between choline moieties. Compound **7** having decamethylene linker exhibited a worse inhibition activity than compound **8** having a dodecamethylene linker. So the lengthening of polymethylene linker between choline moieties considerably increases inhibition activity against AchE of human erythrocytes. Thirdly, the change of the geometry of diterpenoid skeleton affects anticholinesterase activity. *Ent*-kauranes (steviol derivatives) **20**, **21** possess 10 times greater activities against AchE of human erythrocytes and BchE, than their *ent*-beyeran analogues (isosteviol derivatives) **5**, **6**. Fourthly, isosteviol derivatives **13** and especially **16** exhibited the highest selectivity toward hAchE among the compounds tested.

Table 1 Inhibitory potency of tested compounds toward cholinesterases[a]

	Compound								
	BW284C51	**5**	**6**	**7**	**8**	**13**	**16**	**20**	**21**
AchE (human erythrocytes; Sigma)	0.03 (ref. 21)	150.0	30.0	88	2.2	5.0	80	34	9.0
BchE (human serum; Sigma)	354.0 (ref. 21)	30.0	6.7	65	10	60	>1000	4.2	1.6
SI[b]	11 800	0.2	0.2	0.7	4.5	12	>12.5	0.1	0.2

[a] Inhibitory concentrations (IC_{50}) in μM. [b] Selectivity indices (the IC_{50} BChE/IC_{50} AChE ratio).

Conclusions

A series of unfolded and macrocyclic ammonium derivatives of diterpenoids isosteviol **2** and steviol **17** possessing choline moieties have been synthesized and assayed for inhibitory activities toward AchE and BchE. Compounds **5–8**, **13**, **16**, **20** and **21** showed moderate activity within the range of IC_{50} values 8.0×10^{-4} to 2.2×10^{-6} mol L^{-1}. Isosteviol derivative **16** exhibited the best inhibition selectivity against acetylcholinesterase among the compounds tested. It inhibited AchE of human erythrocytes at $IC_{50} = 80$ μM, whereas inhibition of BchE occurred at $IC_{50} > 1000$ μM.

Experimental

General

All reagents used were of analytical grade. Solvents were dried if necessary by standard methods. Column chromatography was performed on silica gel (particle size 63–200, mm) using light

petroleum–EtOAc as eluent. NMR experiments were carried out with a Bruker AVANCE-600 spectrometer (14.1 T) equipped with a pulsed gradient unit capable of producing magnetic field pulse gradients in the *z*-direction of 56 G cm^{-1}. All spectra were acquired in a 5 mm inverse probehead working at 600.13 MHz in ^1H experiments. Chemical shifts are reported on the δ (ppm) scale and are relative to the residual ^1H signal of $CDCl_3$.

Dichloride 11. Diacid **10** (0.9 g, 1.1 mmol) was dissolved in a large excess $SOCl_2$ and heated for 2 h at 40 °C. The reaction mixture was concentrated under vacuum to afford **11** as viscous oil. Yield: 0.94 g (100%). IR spectrum (ν/cm^{-1}): 1732, 1795 (C=O). ^1H NMR ($CDCl_3$, 600 MHz), δ (ppm), *J* (Hz): 0.79–2.00 (m, 50H, *ent*-beyeran skeleton and –$(CH_2)_6$–); 0.82 (s, 6H, $C^{20'}H_3$, $C^{20}H_3$); 0.91 (s, 6H, $C^{17'}H_3$, $C^{17}H_3$); 1.32 (s, 6H, $C^{18'}H_3$, $C^{18}H_3$); 2.32 (m, 6H, $C^{3'}H_{eq}$, C^3H_{eq} and $C^{16'}$,C^{16}–OC(O)C$H_2$$CH_2$); 4.75 (dd, 2H, *J* = 10.5, 4.3, $C^{16'}H$, $C^{16}H$).

Diamine 12. The solution of dichloride **11** in CH_2Cl_2 (50 mL) was added dropwise to a stirred solution of *N*,*N*-dimethylethanolamine (5 mL) in CH_2Cl_2 (50 mL) under argon. The mixture was refluxed for 21 h under argon. After that the reaction mixture was washed with saturated $NaHCO_3$ aqueous solution, the organic layer was dried with $CaCl_2$ and concentrated under vacuum. The residue was purified by column chromatography on silica (chloroform–methanol = 6 : 1, v/v) to give diamine **12** (0.26 g, 22%) as viscous oil. ^1H NMR ($CDCl_3$, 600 MHz), δ (ppm): 0.69–2.11 (m, 50H, *ent*-beyeran skeleton and –$(CH_2)_6$–), 0.69 (s, 6H, $C^{20'}H_3$, $C^{20}H_3$), 0.88 (s, 6H, $C^{17'}H_3$, $C^{17}H_3$), 1.14 (s, 6H, $C^{18'}H_3$, $C^{18}H_3$), 2.14 (d, 2H, $C^{3'}H_{eq}$, C^3H_{eq}, *J* = 13.3 Hz), 2.25 (s, 12H, –N(CH_3)$_2$), 2.28 (t, 2H, $C^{16'}$,C^{16}–OC(O)C$H_2$$CH_2$, *J* = 7.5 Hz), 2.53 (m, 4H, –C(O)OCH_2CH_2N(CH_3)$_2$), 4.10 (m, 4H, C(O)OCH_2–), 4.70 (dd, 2H, $C^{16'}H$, $C^{16}H$, *J* = 10.3, 4.6 Hz). MALDI-TOF MS, *m*/*z*: 950 $[M + H]^+$; *m*/*z* (calc.): 948.72 ($C_{58}H_{96}N_2O_8$).

Macrocycle 13. The solution of 1,9-dibromononane (0,10 mL, 0.49 mmol) in CH_3CN (15 mL) was added to a stirred solution of **12** (0.26 g, 0.27 mmol) in CH_3CN (100 mL) and the reaction mixture was refluxed for 50 h under argon. After that the reaction mixture was concentrated under vacuum, and the residue was purified by column chromatography on silica (chloroform–methanol = 2 : 1, v/v) to give macrocycle **13** (0.02 g, 6.0%), white powder, mp 194–198 °C. ^1H NMR ($CDCl_3$, 600 MHz), δ (ppm): 0.66–2.10 (m, 64H, *ent*-beyeran skeleton, –$(CH_2)_6$– and –$(CH_2)_7$–), 0.66 (s, 6H, $C^{20'}H_3$, $C^{20}H_3$), 0.90 (s, 6H, $C^{17'}H_3$, $C^{17}H_3$), 1.18 (s, 6H, $C^{18'}H_3$, $C^{18}H_3$), 2.12 (d, 2H, $C^{3'}H_{eq}$, C^3H_{eq}, *J* = 13.3 Hz), 2.32 (m, 2H, $C^{16'}$,C^{16}–OC(O)C$H_2$$CH_2$), 3.41 (s, 12H, –N($CH_3$)$_2$), 3.89 (m, 4H, –NC$H_2$($CH_2$)$_7CH_2$N–), 4.07 (m, 4H, –C(O)OCH_2CH_2N(CH_3)$_2$–), 4.45 and 4.63 (m, 4H, C(O)OCH_2–), 4.68 (dd, 2H, $C^{16'}H$, $C^{16}H$, *J* = 10.3, 4.6 Hz). MALDI-TOF MS, *m*/*z*: 1060 $[M - CH_3]^+$, 1154 $[M - Br]^+$. 1228 [M-DHB + H]$^+$. Elemental anal. C 65.43; H 8.97; Br 13.09; N 2.35, calcd for $C_{67}H_{114}Br_2N_2O_8$, C 65.14; H 9.30; Br 12.94; N 2.27%.

Tosylate 14. α,ω-Ditosyl triethylene glycol (10.8 g, 23.5 mmol) was added to a stirred solution of isosteviol **2** (5.0 g, 15.7 mmol) in CH_3CN (250 mL). Then the reaction mixture was heated up to 70 °C and K_2CO_3 (3.3 g, 23.8 mmol) was added. After heating for 6 h at 70 °C the precipitate was filtered and the mixture was concentrated under vacuum. The crude product was purified by column chromatography on silica (petroleum ether 40–70%–ethyl acetate = 2 : 1, v/v) to give tosylate **14** (5.0 g, 53%) as viscous oil. ^1H NMR ($CDCl_3$, 600 MHz), δ (ppm): 0.62–2.14 (m, 18H, *ent*-beyeran skeleton), 0.70 (s, 3H, $C^{20}H_3$), 0.96 (s, 3H, $C^{17}H_3$), 1.18 (s, 3H, $C^{18}H_3$), 2.17 (d, 1H, C^3H_{eq}, *J* = 13.2 Hz), 2.62 (dd, 1H, $C^{15}H_a$, *J* = 3.4, 19.1 Hz), 3.56 (brs, 4H, OCH_2-CH_2O), 3.64 (t, 2H, C(O)OCH_2CH_2O–, *J* = 4.7 Hz), 3.68 (t, 2H, –OCH_2CH_2OTs, *J* = 4.7 Hz), 4.15 (m, 4H, C(O)OCH_2– and –CH_2OTs). MALDI-TOF MS, *m*/*z*: 627 $[M + Na]^+$, 643 $[M + K]^+$. Elemental anal. C 65.56; H 8.24; S 5.16, calcd for $C_{33}H_{48}O_8S$, C 65.54; H 8.00; S 5.30%.

Bromide 15. Lithium bromide (0.79 g, 9.1 mmol) was added to a stirred solution of tosylate **14** (5.0 g, 8.3 mmol) in acetone (25 mL). The reaction mixture was refluxed for 16 h. After that the precipitate was filtered and the mixture was concentrated under vacuum. The crude product was purified by column chromatography on silica (petroleum ether–ethyl acetate = 2 : 1, v/v) to give bromide **15** (3.7 g, 88%) as viscous oil. ^1H NMR ($CDCl_3$, 600 MHz), δ (ppm): 0.71–2.15 (m, 18H, *ent*-beyeran skeleton), 0.71 (s, 3H, $C^{20}H_3$), 0.97 (s, 3H, $C^{17}H_3$), 1.19 (s, 3H, $C^{18}H_3$), 2.19 (d, 1H, C^3H_{eq}, *J* = 13.5 Hz), 2.62 (dd, 1H, $C^{15}H_a$, *J* = 3.7, 18.6 Hz), 3.45 (t, 2H, –CH_2Br, *J* = 6.3 Hz), 3.64 (m, 4H, OCH_2CH_2O), 3.69 (m, 2H, –OC$H_2$$CH_2$Br), 3.80 (t, 2H, C(O)OC$H_2CH_2$O–, *J* = 6.2 Hz), 4.18 (m, 2H, C(O)OCH_2–). MALDI-TOF MS, *m*/*z*: 537 $[M + Na]^+$, 552 $[M + K]^+$. Elemental anal. C 60.27; H 8.09; Br 15.45, calcd for $C_{26}H_{41}BrO_5$, C 60.81; H 8.05; Br 15.56%.

Compound 16. Triethylamine (9.0 mL, 66.2 mmol) was added to a stirred solution of bromide **15** (3.4 g, 6.6 mmol) in CH_3CN (80 mL). The reaction mixture was refluxed for 17 h, concentrated under vacuum, and the crude product was purified by column chromatography on silica (chloroform/methanol) to give compound **16** (2.3 g, 57%) as a white powder, mp 90–93 °C. ^1H NMR ($CDCl_3$, 600 MHz), δ (ppm): 0.67–2.02 (m, 18H, *ent*-beyeran skeleton), 0.67 (s, 3H, $C^{20}H_3$), 0.94 (s, 3H, $C^{17}H_3$), 1.16 (s, 3H, $C^{18}H_3$), 1.37 (t, 9H, N($CH_2C H_3$)$_3$, *J* = 7.2), 2.14 (d, 1H, C^3H_{eq}, *J* = 13.2 Hz), 2.56 (dd, 1H, $C^{15}H_a$, *J* = 3.6, 18.4 Hz), 3.54 (m, 8H, –CH_2N(CH_2CH_3)$_3$), 3.64 (m, 4H, OCH_2CH_2O), 3.75 (m, 2H, OCH_2), 3.95 (m, 2H, C(O)OCH_2CH_2O), 4.13 (m, 2H, C(O)OCH_2–). MALDI-TOF MS, *m*/*z*: 534 $[M - Br]^+$. Elemental anal. C 62.43; H 9.30; N 2.37; Br 13.19, calcd for $C_{32}H_{56}Br\,NO_5$, C 62.53; H 9.18; Br 13.01; N 2.28%.

General procedure for synthesis of compounds 20 and 21

Amine 19. 13-Dihydrosteviol **18** (0.5 g, 1.6 mmol) was dissolved in a large excess $SOCl_2$ and heated for 3 h at 40 °C. The reaction mixture was concentrated under vacuum affording appropriate dichloride of hydroxyacid **18** as viscous oil. A solution of crude product (0.4 g, 1 mmol) in CCl_4 (30 mL) was added dropwise to a stirred solution of *N*,*N*-dimethylethanoloamine (2 mL, 20 mmol) in CH_2Cl_2 (30 mL) under argon and the mixture was refluxed for 24 h under argon. After that the reaction mixture was washed with saturated $NaHCO_3$ aqueous solution, the organic layer was dried with $CaCl_2$ and concentrated under vacuum. The residue was purified by column

chromatography on silica (chloroform–methanol = 9 : 1, v/v) to give amine **19** (0.25 g, 54%) as viscous oil. ^{1}H NMR ($CDCl_3$, 600 MHz), δ (ppm): 0.72–2.24 (m, 20H, *ent*-kaurane skeleton), 0.83 (s, 3H, $C^{20}H_3$), 1.03 (s, 3H, $C^{17}H_3$), 1.13 (s, 3H, $C^{18}H_3$), 2.25 (s, 6H, –N(CH_3)$_2$), 2.33 (m, 1H), 2.54 (m, 2H, –C(O)OCH$_2$CH_2N(CH$_3$)$_2$), 4.10 (m, 2H, C(O)OCH_2–). MALDI-TOF MS, *m*/*z*: 410 $[M]^+$.

Alkyl halide (0.6 mmol) was added to a stirred solution of amine **19** (0.6 mmol) in CH_3CN (5 mL). After heating for 10 h at 80 °C, the mixture was concentrated under vacuum and the crude product was recrystallized from hexane.

Compound 20. Yield 55%; mp 226–228 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ (ppm): 0.78–2.32 (m, 21H, *ent*-kaurane skeleton), 0.82 (s, 3H, $C^{20}H_3$), 1.06 (s, 3H, $C^{17}H_3$), 1.21 (s, 3H, $C^{18}H_3$), 3.57 (s, 9H, –N(CH_3)$_3$), 4.11 (m, 2H, –C(O)OCH$_2$CH_2N(CH$_3$)$_3$), 4.55 (m, 2H, C(O)OCH_2–). MALDI-TOF MS, *m*/*z*: 424 $[M - I]^+$. Elemental anal. C 54.12; H 7.40; Cl 7.19; I 22.48; N 2.79; calcd for $C_{25}H_{43}ClINO_2$; C 54.40; H 7.85; Cl 6.72; I 22.99; N 2.54%.

Compound 21. Yield 34%; mp 190–192 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ (ppm): 0.75–2.15 (m, 21H, *ent*-kaurane skeleton), 0.75 (s, 3H, $C^{20}H_3$), 1.06 (s, 3H, $C^{17}H_3$), 1.16 (s, 3H, $C^{18}H_3$), 3.40 (s, 6H, –N(CH_3)$_2$), 4.06 (m, 2H, –C(O)OCH$_2$CH_2N(CH$_3$)$_3$), 4.57 (m, 2H, C(O)OCH_2–), 5.20 (s, 2H, –CH_2Ph), 7.47–7.70 (m, 5H, Ph). MALDI-TOF MS, *m*/*z*: 500 $[M - Br]^+$. Elemental anal. C 63.99; H 8.83; Br 14.05; Cl 7.07; N 2.06; calcd for $C_{31}H_{47}BrClNO_2$, C 64.08; H 8.15; Br 13.75; Cl 6.1; N 2.41%.

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