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Synthesis and biological activity of chiral tetrahydrofuranyl amino acids as building moieties of pamamycin analogues

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A new synthetic route to chiral tetrahydrofuranyl amino acids is described starting with D-glucose. The 17-step and a 20-step procedures, respectively, furnished compounds of the general formulas **1** and **2** mimicking the dimethylamino carboxylic acid part of pamamycin (**3**). In comparison with the absolute stereochemistry of **3** the acyclic compounds **1a–c** and **2a–c** are enantiomers. Despite of the presence of a dimethylamino function they showed neither antibacterial activity nor protonophoric effects on an artificial bilayer membrane suggesting that the macrodiolide ring of **3** forms an indispensable prerequisite for the known interaction of this antibiotic with biological systems.

1. Introduction

Pamamycin-607 (**3**) from *Streptomyces alboniger* and its higher homologues as products of some other Streptomyces strains are characterized by the presence of a 16-membered macrodiolide ring which is substituted by a side chain with a dimethylamino function [1–3]. Both the ring and the side chain contain tetrahydrofuranyl groups as nucleophilic centers. The relative stereochemistry of pamamycin was established by NOE enhancements and H-H coupling [3]. Its absolute stereochemistry was determined by conversion of the lower part of pamamycin-635 to its (*S*)- and (*R*)-MTPA derivatives and application of the advanced Mosher's method [4]. In addition to strong activity against some Gram-positive bacteria and fungi the pamamycins display a series of other interesting biological effects which rendered them useful candidates for chemical modifications, characterization of structure-activity relationships and lead structure development. Thus, induction of aerial mycelium formation in *Streptomyces* cultures [5], inhibition of myosine light-chain kinase [6], post-mortem anti-autolytic effects on chicken embryo blood vessels [7] and protonophoric activities of pamamycins [8] (used either as pamamycin-607 (**3**) or as mixture of several homologues) in artificial membranes have been reported. Especially, the latter proton-translocating activity of **3** using an artificial lipid bilayer membrane model intended us to synthesize analogues of the upper part of the pamamycin molecule containing the dimethylamino group as the suggested proton-binding region, carboxylic and tetrahydrofuranyl functions. The aim was to compare the protonophoric and antibacterial activities of the synthetic dimethylamino acids mimicking parts of the pamamycin constitution with those of the entire pamamycin. In particular we wanted to better understand the role of the macrodiolide ring and its distance from the dimethylamino group in the chelation of protons and their transport through artificial bilayer membranes. In order to simplify the synthetic problems with pamamycin analogues as 'seco'-pamamycins we omitted the second tetrahydrofuranyl ring which should play a minor role in proton chelation. Moreover, we decided to synthesize enantiomeric structures in comparison with the naturally occurring pamamycins [3] because proton chelation involving the macrodiolide ring and the dimethylamino group should not require a special absolute configuration.

Here we report a new synthetic route to chiral tetrahydrofuranyl amino acids such as **1a–c** and **2a–c** as enantiomeric analogues of the dimethylamino moiety of pamamycin-607 (Scheme 1) and comparison of their antibacterial and protonophoric activities with those of **3**.

2. Investigations, results and discussion

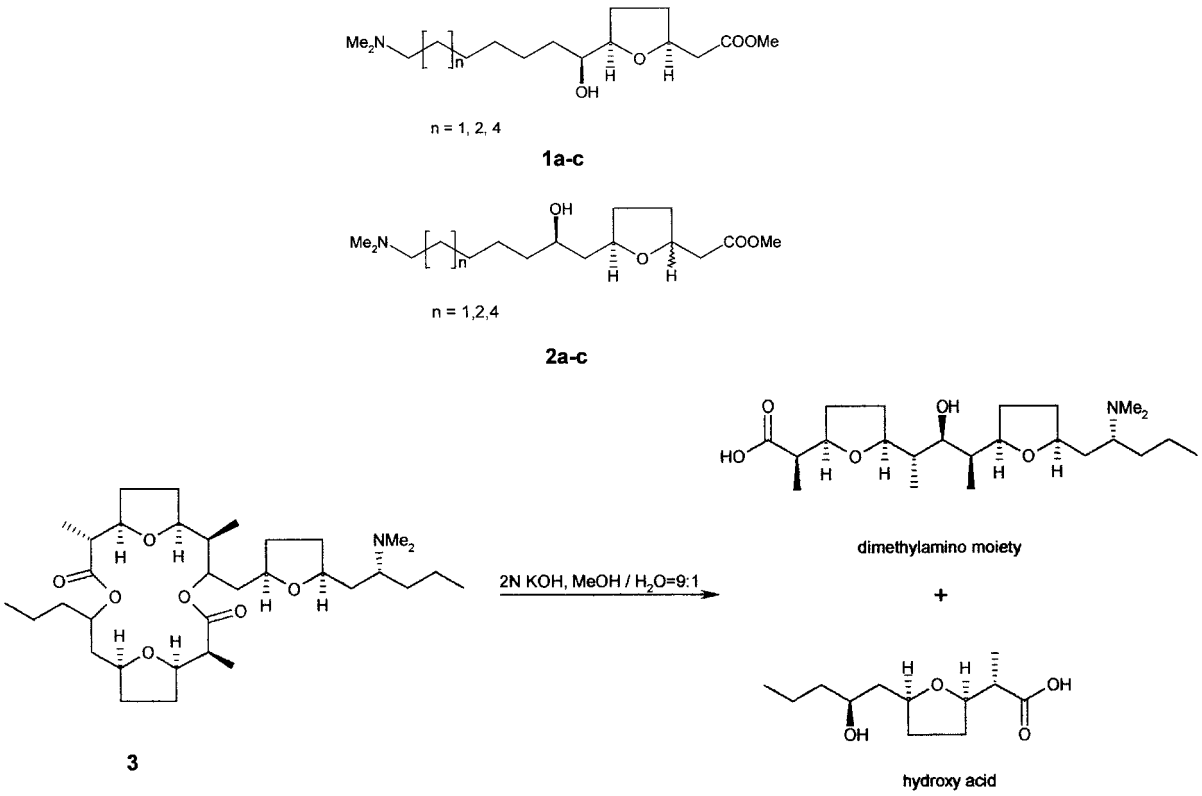
The synthetic routes towards chiral tetrahydrofuranyl amino acids such as **1a–c** and **2a–c** as 'seco'-pamamycin analogues are depicted in Schemes 2 and 3.

For the synthesis of **1a–c** we started from D-glucose. Therefrom we obtained the highly functionalized *cis*-2,5-disubstituted tetrahydrofuran **5** via several subsequent steps as it has been described in the literature [9–11]. Subsequently, the aldehyde **5** was protected as ethylene acetal in the presence of *p*-toluenesulfonic acid as catalyst, and the iodine substituent was replaced by the nitrile function with lithium cyanide under reflux in THF [16]. Obviously, under these alkaline conditions isomerisation at C-2 of the tetrahydrofuran ring occurred. Thus, in the ^{13}C NMR spectrum of **7** twice the theoretical number of signals was visible. For the isomerization the mechanism shown in Scheme 4 was suggested.

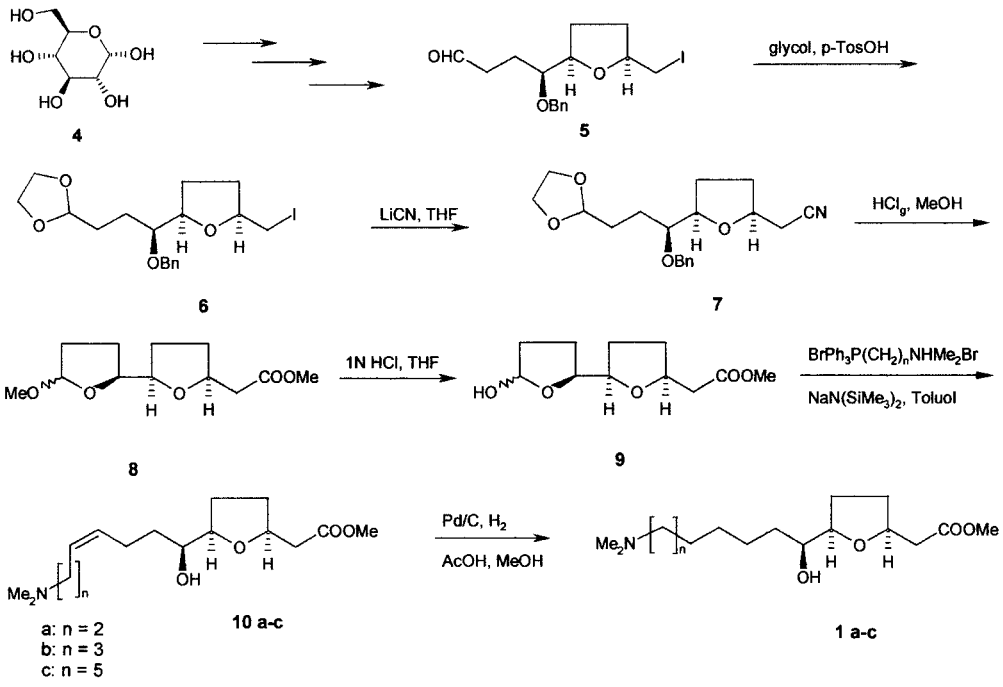
7 was separated readily from the minor component **7a** by column chromatography on silica gel. The relative stereochemistry of **7** was settled on the basis of the ^{1}H,^{1}H NMR and NOESY connectivities. Hydrolysis of **7** was furnished with methanol saturated by hydrochloric acid. The resulting bis-tetrahydrofuranyl ester **9** was subjected to Wittig reaction with 3-(dimethylamino)-propyl-, 4-(dimethylamino)-butyl- and 6-(dimethylamino)-hexyl-triphenylphosphoniumbromide-hydrobromide. As could be expected, the use of sodium bistrimethylsilylamide as base the Wittig reaction furnished the (*Z*)-alkenes in a large excess [12]. In the case of the methylesters **10a–c** the (*E*)-isomer was observed in 10% amount only.

After hydrogenation over Pd/C dimethylamino-alkyl-tetrahydrofuranyl acetic acid methyl esters **1a–c** were afforded as enantiomeric analogues of the upper part of the pamamycin molecule (dimethylamino carboxylic acid moiety, 'seco'-pamamycins). They are distinguishable by different distances between the carboxyl function at C-1, the tetrahydrofuranyl ring and the hydroxyl group, at the one side, and the dimethylamino group at the end of the aliphatic side chain, at the other.

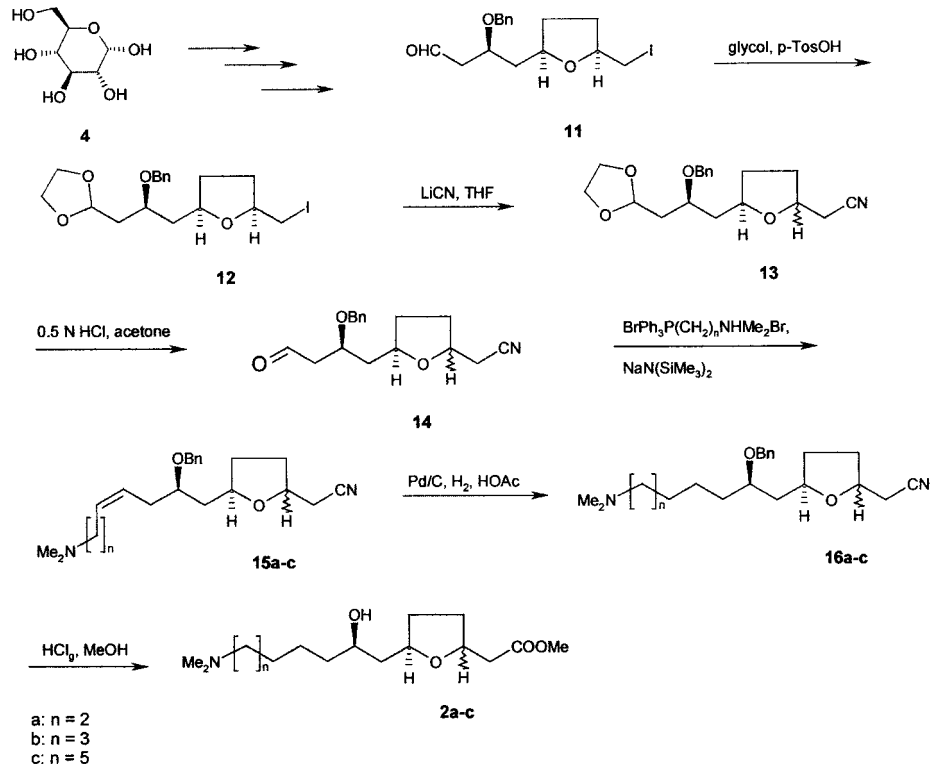
Scheme 1



Scheme 2



Scheme 3



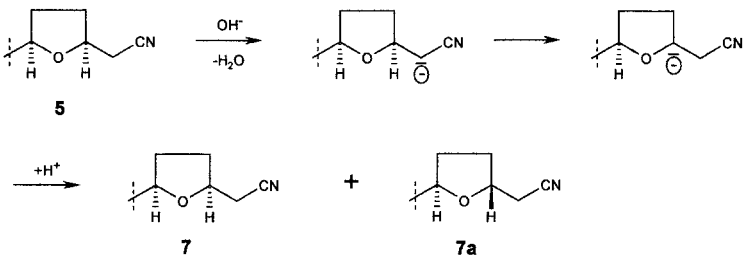
The synthesis of seco-pamamycin-type analogues harbouring a hydroxyl group at C-2 of the alkyl chain (**2a–c**) was carried out in a similar manner. As was described above D-glucose **4** was the starting material, too. A series of known reaction steps [9, 13–15, 11] furnished the *cis*-2,5-disubstituted tetrahydrofuran **11**. Protection of the aldehyde group and Kolbe's nitrile synthesis led to the acetonitrile **13**. In this case isomerisation at C-2 of the THF ring occurred again. However, separation of these two isomers (*cis*- and *trans*-THF compounds) occurring in approximate 60 : 40 ratio was not successful. Consequently, the subsequent reactions furnished racemates at C-2. In the case of the nitrile **13** treatment with HCl-saturated methanol afforded the α,β-unsaturated aldehyde **13a** in addition to **14** (Scheme 5).

Hence the aldehyde was deprotected with 0.5 N hydrochloric acid in an aqueous solution of acetone to yield **14**. Thereafter, the Wittig reaction was performed with the dimethylaminoalkylphosphonium salts as mentioned above. The acetonitriles **15a–c** displayed only the (*Z*)-configuration as was shown by ^{1}H NMR spectroscopy. After hydrogenation of the resulting alkenes **15a–c** and hydrolysis of the nitrile function with HCl-saturated methanol the compounds **2a–c** were obtained.

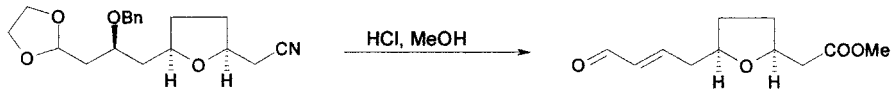
The above synthetic procedure thus enabled the formation of chiral and racemic tetrahydrofuranyl amino acids with variable distance between the carboxylic and amino groups. They appear as enantiomeric analogues of the dimethylamino building moiety of pamamycin-607 (**3**). The physicochemical properties of the new compounds **1a**, **1b**, **1c**, **2a**, **2b** and **2c** are shown in the Experimental Part.

The antibacterial activities of compounds **1a–c** and **2a–c** were compared with **3** using the agar dilution assay. In contrast to **3** a concentration of 100 μg **1a–c** and **2a–c**/ml displayed no inhibitory effects on a series of Gram-positive bacteria and fungi. Shown in the Fig. is the effect of pamamycin and compounds **1a–c** and **2a–c** on the conductance of an artificial model membrane constituted of soybean lecithine [8]. Below pH 6.5 the presence of **3** caused a strong passive transport of protons through the bilayer membrane as was reported previously [8]. Other cations such as sodium and potassium ions were translocated across the membrane at pH < 6.5 with a more than two hundred-fold lower rate than the protons. In full accord with the protonophoric properties of **3** the conductivity of the membrane declined by a factor of more than 200 if the pH was increased to 8.5. Under alkaline condi-

Scheme 4



Scheme 5



13 **13a**

tions the proton conductivity became comparable to the conductivity for sodium and potassium ions. In comparison to **3** the tetrahydrofuranyl amino acid esters **1a–c** and **2a–c** displayed only 0.1% of proton conductivity of pamamycin at pH 6.0 (Fig.).

3. Discussion

A new synthetic pathway was developed for the synthesis of enantiomeric analogues of the dimethylamino moiety of the macrodiolide antibiotic pamamycins with variable distance between the carboxylic and dimethylamino group. The latter has been suggested as the proton binding region of the pamamycin molecule [8] and reason for its activity as proton carrier. However, compounds **1a–c** and **2a–c** displayed only a very weak proton translocating activity in an artificial membrane model suggesting that the entire macrodiolide ring of **3** forms an additional, essential prerequisite for the protonophoric activity of pamamycin. Thus the data support the contention that drastic increase of proton permeability of an artificial membrane in presence of pamamycin is due to the formation of a lipophilic, cage-like proton chelate as a membrane-permeable carrier whereby the protonated dimethylamino group is arranged above the plain of the macrodiolide ring. Apparently the latter is acting as a nucleophilic ligand binding hydronium ions which were bound to the dimethylamino group. Moreover, the missing antibacterial effect of **1a–c** and **2a–c** suggests that carrier-mediated passive proton translocation across the membrane forms a constitutive part of mode of antibacterial action of pamamycin **3**.

4. Experimental

4.1. Materials

THF, diethylether, methanol and benzene were dried by distillation from Na/benzophenone. Dichloromethane and chloroform were dried over calciumchloride and distilled. All other materials and solvents were of analytical grade. CC was performed on silica gel (60–230 mesh; Merck). TLC was performed on silica gel F_{254} (Merck). Soybean lecithine was purchased from Sigma. Pamamycin (as a mixture of pamamycins-607 and −621) was isolated from cultures of *Streptomyces* sp. [8].

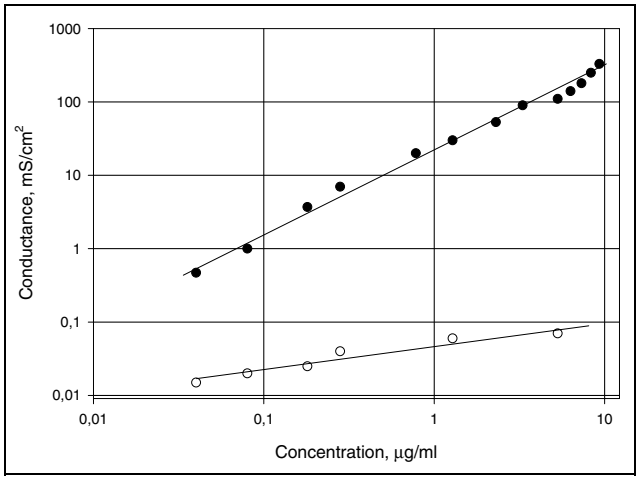


Fig.: Conductivity of an artificial membrane versus concentration of pamamycin (3; –●–) and 1a–c resp. 2a–c (-o-)

4.2. Instruments

HREI-MS investigations were carried on a double-focussing sector field instrument AMD-402 (AMD Intectra, Harpstedt, Germany), HRESI-MS on a Finnigan MAT 9582 instrument (Finnigan, Bremen, Germany) and ESI-MS on a triple-quadrupole instrument Quattro (VG Biotech, Altrincham, England). 1D and 2D NMR measurements (^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC) used Bruker Avance DPX 300 (300 MHz) and Bruker Avance DRX 500 (500 MHz) spectrometers.

4.3. Synthesis of ethylene acetals **6** *and* **12**

To a solution of the aldehyde **5** or **11** in benzene 1,2 equivalents ethylene glycol and a trace *p*-toluenesulfonic acid were added. The solution was stirred and heated under reflux for 90 min. After cooling the solution was washed with 10% potassium hydroxide solution and water. The organic layer was dried over potassium carbonate and the solvent was removed under reduced pressure. The product was obtained as weak yellow oil. A further purification was not necessary.

4.3.1. 2-[(3S)-(Benzyloxy)-3-[(2S,5R)-5-(iodomethyl)tetrahydrofuran-2-yl]-propyl}-1,3-dioxolane (**6**)

Attempt: **5**: 27.0 g (69.6 mmol). Yield: 28.8 g (95.8%). TLC: hexane/ethyl acetate (2 : 1): $R_f = 0.59$. MS (ESI): m/z (rel. Int.): 438 ($[M + H]^+$, 23), 460 ($[M + Na]^+$, 100). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 1.63–1.78 (m, 4 H), 1.84–2.05 (m, 4 H), 3.16 (dd, J = 10.0/6.9, 1 H), 3.23 (dd, J = 10.0/5.2, 1 H), 3.59 (m, 1 H), 3.83 (m, 2 H), 3.95 (m, 2 H), 4.0 (m, 2 H), 4.71/4.61 (AB, J = 11.5, 2 H), 4.80 (t, J = 4.8, 1 H), 7.30 (m, 5 H). ^{13}C NMR (500 MHz, $CDCl_3$, ppm): 10.01, 25.84, 25.99, 29.72, 31.38, 64.83, 64.86, 73.07, 78.88, 79.60, 82.96, 104.45, 127.44, 127.74, 128.26, 138.83.
$C_{18}H_{25}IO_4$ (437.0)

4.3.2. 2-[(2S)-(Benzyloxy)-3-[(2S,5R)-5-(iodomethyl)tetrahydrofuran-2-yl]-propyl}-1,3-dioxolane (**12**)

Attempt: **11**: 15.5 g (40 mmol). Yield: 16.5 g (95.5%). TLC: hexane/ethyl acetate (2 : 1): $R_f = 0.47$. MS (ESI): m/z (rel. Int.): 438 ($[M + H]^+$, 37), 460 ($[M + Na]^+$, 100). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 1.58 (m, 1 H), 1.73 (m, 2 H), 1.90 (m, 2 H), 2.02 (m, 3 H), 3.16 (dd, J = 9.9/6.9, 1 H), 3.25 (dd, J = 9.9/4.5 Hz, 1 H), 3.77 (m, 1 H), 3.84 (m, 2 H), 3.93 (m, 1 H), 3.96 (m, 2 H), 4.07 (m, 1 H), 4.51/4.55 (AB, J = 11.5, 2 H), 5.02 (dd, J = 5.8/4.3, 1 H), 7.30 (m, 5 H). ^{13}C NMR (500 MHz, $CDCl_3$, ppm): 11.11, 31.34, 31.46, 38.66, 40.71, 64.64, 64.80, 70.92, 73.53, 77.47, 78.15, 102.30, 127.46, 127.78, 128.26, 138.67.
$C_{18}H_{25}IO_4$ (437.0)

4.4. Synthesis of the tetrahydrofuranyl acetonitriles **7** *and* **13**

To a solution of the ethylene acetal protected 5-iodomethyl-tetrahydrofuranyl-butanal in dry THF three equivalents of lithium cyanide were added under an atmosphere of argon. The suspension was stirred and heated under reflux for 48 h. After cooling the solvent was evaporated to dryness. The residue was suspended in diethylether and washed successively with brine and water. The aqueous layer was extracted twice with ether and the combined organic extracts were dried over sodium sulfate, filtered and the solvent was removed on a rotary evaporator. The crude product was purified by chromatography on silica gel with 2 : 1 hexane/ethyl acetate as the eluent to obtain **7** and **13** as colourless oil.

4.4.1. 2-[(3S)-(Benzyloxy)-3-[(2R,5S)-5-(3-(1,3-dioxolan-2-yl)propyl]tetrahydrofuran-2-yl}-acetonitrile **7**

Attempt: **6**: 20.2 g (46.76 mmol). Yield: 6.8 g (44%). TLC: hexane/ethyl acetate (2 : 1): $R_f = 0.23$. MS (ESI): m/z (rel. Int.) 332 ($[M + H]^+$, 12), 354 ($[M + Na]^+$, 100). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.6–2.15 (m, 9 H), 2.53 (d, J = 5.8, 2 H), 3.62 (dd, J = 10.0, 5.5, 1 H), 3.86 (m, 2 H), 3.97 (m, 2 H), 4.15 (m, 1 H), 4.62/4.68 (AB, J = 11.5, 2 H), 4.87 (t,

J = 4.3, 1 H), 7.3 (m, 5 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 24.02, 25.67, 25.79, 29.59, 30.63, 64.86, 73.0, 74.32, 79.5, 82.56, 104.38, 117.46, 127.5, 127.73, 128.29, 138.69.
$C_{19}H_{25}NO_4$ (331.0)

4.4.2. 2-{(2S)-(Benzyloxy)-3-[(2R,5)-5-[3-(1,3-dioxolan-2-yl)-propyl]tetrahydrofuran-2-yl}-acetonitrile (**13**)

Attempt: **12**: 8.64 g (20.0 mmol). Yield: 6.45 g (97.7%; mixture of *cis*- and *trans*-THF). TLC: hexane/ethyl acetate (2 : 1): $R_f = 0.16$. MS (ESI): m/z (rel. Int.) 332 ($[M + H]^+$, 15), 354 ($[M + Na]^+$, 100), 370 ($[M + K]^+$, 8). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.6–2.15 (m, 8 H), 2.55 (dd, J = 5.6/3.8, 2 H), 3.75 (m, 1 H), 3.84 (m, 2 H), 3.97 (m, 2 H), 4.1 (m, 1 H), 4.22 (m, 1 H), 4.51/4.56 (AB, J = 12.2, 2 H), 5.02 (t, J = 4.8, 1 H), 7.32 (m, 5 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 24.50, 30.49, 31.19, 38.63, 40.29, 64.66, 64.81, 70.94, 73.45, 73.80, 77.31, 102.27, 117.43, 127.49, 127.80, 128.28, 138.65.
$C_{19}H_{25}NO_4$ (331.0)

4.5. Procedure for the hydrolysis of the nitriles to the methyl esters **8** *and* **2a–c**

7 respectively **16a–c** were diluted in dry methanol. The solution was saturated with gaseous hydrochloric acid at room temperature. Afterwards it was heated to reflux for 2 h.
Half of the methanol was evaporated, the concentrated solution was diluted with water and let stirred overnight. Then water was added and the aqueous solution was extracted three times with methylene chloride. The combined organic extracts were washed with water, dried over sodium sulfate, filtered and the solvent was removed in vaccuum.

4.5.1. Methyl[(2S,2′S,5R)-5′-methoxyoctahydro-2,2′-bisfuran-5-yl]acetate (**8**)

The crude product was purified by chromatography on silica gel with 2 : 1 hexane/ethyl acetate as the eluent to obtain **8** as colourless oil. The signals in the NMR spectra were assigned to the two isomers with (*R*)- resp. (*S*)-configuration at C-5′ of **8**.
Attempt: **7**: 3.0 g (9.06 mmol). Yield: 1.72 g (77.8%). TLC: hexane/ethyl acetate (2 : 1): $R_f = 0.37$. MS (HREI): m/z = 244.1279 ($[M-CH_2]^+$, calcd. 244.1295 for $C_{11}H_{18}O_5$). (*R*)-isomer: ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.5–2.1 (m, 8 H), 2.43 (dd, J = 6.6/2.7, 1 H), 2.54 (dd, J = 6.6/1.0, 1 H), 3.25 (s, 3 H), 3.60 (s, 3 H), 3.78 (m, 1 H), 3.91 (m, 1 H), 4.20 (m, 1 H), 4.94 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 25.15, 27.64, 30.62, 31.67, 40.47, 51.32, 54.36, 75.58, 80.75, 82.15, 105.12, 171.34.
(*S*)-isomer: ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.5–2.1 (m, 8 H), 2.37 (dd, J = 6.5 / 2.5, 1 H), 2.59 (dd, J = 6.5/1.0, 1 H), 3.23 (s, 3 H), 3.60 (s, 3 H), 3.78 (m, 1 H), 3.91 (m, 1 H), 4.20 (m, 1 H), 4.87 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 26.85, 27.47, 30.91, 32.61, 40.58, 51.32, 54.29, 75.61, 80.20, 82.32, 105.06, 171.43.
$C_{12}H_{20}O_5$ (244.0)

4.5.2. Methyl[(2R,5)-5-[(2R)-7-(dimethylamino)-2-hydroxyheptyl]tetrahydrofuran-2-yl}acetate (**2a**)

Attempt: **16a**: 1.0 g (2.79 mmol). Yield: 483 mg (57.5%). TLC ($CHCl_3$/MeOH = 5 : 1): $R_f = 0.27$. MS (HREI): m/z 301.2235 (M^+, calcd. 301.2253 for $C_{16}H_{31}NO_4$). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.40 (m, 6 H), 1.67 (m, 5 H), 2.12 (m, 2 H), 2.23 (m, 2 H), 2.49 (s, 6 H), 2.64 (m, 2 H), 2.73 (m, 2 H), 3.81 (s, 3 H), 3.92 (m, 1 H), 4.13 (m, 1 H), 4.46 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 25.14, 27.15, 27.22, 30.47, 31.72, 37.33, 40.86, 42.94, 44.56, 51.60, 59.14, 71.37, 76.00, 79.72, 171.34.
$C_{16}H_{31}NO_4$ (301.0)

4.5.3. Methyl{(2R,5)-5-[(2R)-8-(dimethylamino)-2-hydroxyoctyl]tetrahydrofuran-2-yl}acetate (**2b**)

Attempt: **16b**: 0.5 g (1.344 mmol). Yield: 280 mg (62.7%). TLC ($CHCl_3$/MeOH = 5 : 1): $R_f = 0.30$. MS (HREI): m/z 315.2424 (M^+, calcd. 315.2410 for $C_{17}H_{33}NO_4$). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.20–1.40 (m, 7 H), 1.40–1.67 (m, 8 H), 2.04 (m, 2 H), 2.23 (s, 6 H), 2.29 (m, 2 H), 2.50 (m, 2 H), 3.63 (s, 3 H), 3.72 (m, 1 H), 4.08 (m, 1 H), 4.29 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 25.27, 26.77, 27.25, 29.43, 31.07, 31.70, 37.39, 40.84, 42.90, 44.67, 51.57, 59.27, 71.46, 75.97, 80.28, 171.57.
$C_{17}H_{33}NO_4$ (315.0)

4.5.4. Methyl{(2R,5)-5-[(2R)-10-(dimethylamino)-2-hydroxydecyl]tetrahydrofuran-2-yl}acetate (**2c**)

Attempt: **16c**: 1.0 g (2.5 mmol). Yield: 425 mg (49.6%). TLC ($CHCl_3$/MeOH = 5 : 1): $R_f = 0.34$. MS (HREI): m/z 343.2732 (M^+, calcd. 343.2723 for $C_{19}H_{37}NO_4$). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.26–1.40 (m, 8 H), 1.43–1.60 (m, 7 H), 2.06 (m, 3 H), 2.22 (s, 6 H), 2.27 (m, 3 H), 2.40 (m, 2 H), 2.56 (m, 2 H), 3.65 (s, 3 H), 3.76 (m, 1 H), 4.04 (m, 1 H), 4.24 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 25.06, 26.72, 27.26, 27.41, 29.51, 30.51, 31.78, 32.59, 37.54, 40.44, 42.94, 45.03, 51.64, 59.59, 71.66, 76.05, 79.83, 171.45.
$C_{19}H_{37}NO_4$ (343.0)

4.6. Methyl[(2S,2′S,5R)-5′-hydroxyoctahydro-2,2′-bifuran-5-yl]acetate (**9**)

To a solution of **8** (1.72 g, 7.05 mmol) in THF (100 ml) was added 1 N aqueous hydrochloric acid (10 ml). The solution was stirred at room temperature for 20 h. Neutralization was done with solid $NaHCO_3$. The mixture was diluted with water (100 ml) and extracted with trichloromethane three times. The combined organic extracts were washed with water, dried over sodium sulfate, filtered and the solvent was removed under reduced pressure. Compound **9** was obtained as colourless oil. Isomers with (*R*)- and (*S*)-configuration at C-5′ are available in a ratio of 1 : 1.5.
Attempt: **8**: 1.7 g (7 mmol). Yield: 1.18 g (73%). TLC (ethyl acetate): $R_f = 0.60$. MS (ESI): m/z (rel. Int.) 253 ($[M + Na]^+$, 100), 213 ($[M - OH]^+$, 28).
(*R*)-isomer: ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.5–2.1 (m, 8 H), 2.45 (m, 1 H), 3.60 (s, 3 H), 3.78 (m, 1 H), 3.91 (m, 1 H), 4.20 (m, 1 H), 5.35 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 23.84, 26.96, 30.84, 34.20, 40.35, 51.56, 75.70, 79.93, 81.27, 98.50, 171.34.
(*S*)-isomer: ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.5–2.1 (m, 8 H), 2.40 (m, 1 H), 2.59 (m, 1 H), 3.62 (s, 3 H), 3.78 (m, 1 H), 3.91 (m, 1 H), 4.20 (m, 1 H), 5.48 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 25.25, 27.34, 30.63, 32.53, 40.44, 51.44, 75.58, 80.78, 81.35, 98.60, 171.51.
$C_{11}H_{18}O_5$ (230.0)

4.7. Olefination of the aldehydes: Synthesis of **10a–c** *and* **15a–c**

To a stirred solution of sodium hexamethyldisilazide (8 eq.) in dry toluene under nitrogen at 23 °C dimethylaminoalkyltriphenylphosphoniumbromide hydrobromide (4 eq.) was added. The solution was stirred for 45 min at room temperature and afterwards heated to reflux for 1 h. The red coloured suspension was cooled to −40 °C. To this a solution of the aldehyde respectively the semi acetal (1 eq.) in toluene was slowly added. The mixture was stirred further at this temperature for 1 h and, later, it was stirred overnight at room temperature. The reaction was then quenched with water. The organic layer was separated and the aqueous layer was extracted twice with diethylether. The combined organic layers were washed with water, dried over sodium sulfate, filtered and the solvent was evaporated. The residue was purified by chromatography on silica gel.

4.7.1. Methyl{(2R,5S)-5-[(1S,4Z)-7-(dimethylamino)-1-hydroxyhept-4-enyl]tetrahydrofuran-2-yl}acetate (**10a**)

Colourless oil. Attempt: **9**: 0.5 g (2.174 mmol). Yield: 267 mg (40.8%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.14$. MS (ESI): m/z (rel. Int.) 300 ($[M + H]^+$, 88), 322 ($[M + Na]^+$, 98). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.43 (m, 4 H), 1.75 (m, 4 H), 1.85 (m, 1 H), 2.07 (m, 1 H), 2.23 (m, 1 H), 2.30 (m, 3 H), 2.39 (m, 1 H), 2,53 (m, 1 H), 2.72 (br, OH), 3.06 (m, 1 H), 3.25 (m, 1 H), 3.67 (s, 3 H), 3.73 (m, 1 H), 5.35 (m, 1 H), 5.45 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 22.80, 25.48, 29.22, 30.44, 31.97, 40.96, 45.22, 51.59, 59.88, 70.06, 76.13, 81.61, 128.60, 130.80, 170.23.
$C_{16}H_{29}NO_4$ (299.0)

4.7.2. Methyl{(2R,5S)-5-[(1S,4Z)-8-(dimethylamino)-1-hydroxyoct-4-enyl]tetrahydrofuran-2-yl}acetate (**10b**)

Colourless oil. Attempt: **9**: 0.5 g (2.174 mmol). Yield: 243 mg (35.7%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.21$. MS (ESI): m/z (rel. Int.) 314 ($[M + H]^+$, 100), 336 ($[M + Na]^+$, 12). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.34–1.70 (m, 6 H), 1.70–2.10 (m, 7 H), 2.18 (s, 6 H), 2.25 (m, 2 H), 2.51 (m, 2 H), 3.21 (m, 1 H), 3.67 (s, 3 H), 3.72 (m, 1 H), 3.82 (m, 1 H), 5.34 (m, 2 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 23.58, 23.82, 24.72, 27.44, 31.12, 32.12, 32.40, 40.48, 45.38, 51.64, 59.02, 70.35, 75.26, 83.17, 129.50, 130.04, 171.80.
$C_{17}H_{31}NO_4$ (313.0)

4.7.3. Methyl{(2R,5S)-5-[(1S,4Z)-10-(dimethylamino)-1-hydroxydec-4-enyl]tetra-hydrofuran-2-yl}acetate (**10c**)

Colourless oil. Attempt: **9**: 0.5 g (2.174 mmol). Yield: 316 mg (42.7%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.23$. MS (ESI): m/z (rel. Int.) 342 ($[M + H]^+$, 70), 364 ($[M + Na]^+$, 85). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.20–1.50 (m, 8 H), 1.57–2.00 (m, 7 H), 2.04 (m, 2 H), 2.19 (s, 6 H), 2.21 (m, 2 H), 2.50 (m, 2 H), 3.68 (s, 3 H), 3.75 (m, 1 H), 3.84 (m, 1 H), 4.13 (m, 1 H), 5.34 (m, 2 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 23.68, 26.85, 26.96, 27.35, 27.45, 29.43, 31.11, 32.63, 40.48, 45.38, 51.69, 59.82, 70.68, 75.28, 83.14, 129.28, 130.67, 171.39.
$C_{19}H_{35}NO_4$ (341.0)

4.7.4. {(2R,5)-5-[(2R,4Z)-(Benzyloxy)-7-(dimethylamino)-hept-4-enyl]tetrahydrofuran-2-yl}acetonitrile (**15a**)

Colourless oil. Attempt: **14**: 1.5 g (5.23 mmol). Yield: 1.10 g (59%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.27$. MS (ESI): m/z (rel. Int.) 357 ($[M + H]^+$, 93). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.5–1.8 (m, 4 H), 1.88–2.1 (m, 6 H), 2.22 (s, 6 H), 2.35 (m, 2 H), 2.53 (m, 2 H), 3.54 (m, 1 H), 4.01 (m, 1 H), 4.20 (m, 1 H), 4.45/4.58 (AB, J = 11.7, 2 H), 5.48 (m, 2 H), 7.35 (m, 5 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 24.50, 25.97, 30.52, 31.00, 31.61, 39.79, 45.36, 59.41, 70.75, 73.75, 76.21, 77.66, 117.50, 126.32, 127.48, 127.76, 128.29, 132.12, 138.72.
$C_{22}H_{32}N_2O_2$ (356.0)

4.7.5. {(2R,5)-5-[(2R,4Z)-(Benzyloxy)-8-(dimethylamino)-oct-4-enyl]tetrahydrofuran-2-yl}acetonitrile (**15b**)

Colourless oil. Attempt: **14**: 1.5 g (5.23 mmol). Yield: 1.29 g (66.6%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.27$. MS (ESI): m/z (rel. Int.) 371 ($[M + H]^+$, 100). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.5–1.8 (m, 5 H), 1.86–2.10 (m, 3 H), 2.18 (m, 2 H); 2.22 (s, 6 H), 2.28 (m, 2 H), 2.35 (m, 2 H), 2.53 (m, 2 H), 3.52 (m, 1 H), 4.04 (m, 1 H), 4.19 (m, 1 H), 4.45/4.57 (AB, J = 11.6, 2 H), 5.46 (m, 2 H), 7.32 (m, 5 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 24.45, 25.25, 27.40, 27.52, 30.48, 31.44, 39.74, 45.33, 59.25, 70.67, 73.70, 76.19, 77.22, 117.32, 125.50, 127.44, 127.71, 128.24, 132.36, 138.70.
$C_{23}H_{34}N_2O_2$ (370.0)

4.7.6. {(2R,5)-5-[(2R,4Z)-(Benzyloxy)-10-(dimethylamino)-dec-4-enyl]tetrahydrofuran-2-yl}acetonitrile (**15c**)

Colourless oil. Attempt: **14**: 1.5 g (5.23 mmol). Yield: 1.16 g (55.8%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.43$. MS (ESI): m/z (rel. Int.) 399 ($[M+H]^+$, 100). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 1.25–1.50 (m, 6 H), 1.58–1.80 (m, 3 H), 1.85–2.10 (m, 5 H), 2.23 (s, 6 H), 2.26 (m, 2 H), 2.35 (m, 2 H), 2.53 (m, 2 H), 3.53 (m, 1 H), 4.05 (m, 1 H), 4.20 (m, 1 H), 4.45/4.59 (AB, J = 11.7, 2 H), 5.45 (m, 2 H), 7.32 (m, 5 H). ^{13}C NMR (500 MHz, $CDCl_3$, ppm): 24.48, 27.13, 27.39, 27.46, 29.50, 30.50, 31.47, 39.76, 45.33, 59.74, 70.67, 73.70, 76.24, 77.69, 117.42, 125.02, 127.44, 127.73, 128.25, 132.01, 138.72.
$C_{25}H_{38}N_2O_2$ (398.0)

4.8. Hydrogenation of the olefins to 1a–c *and* 16a–c

To a solution of the olefin in methanol acetic acid (2 eq.) was added. Hydrogenation was carried out over 10% Pd/C in catalytic amounts for 3 h. The mixture was filtered over Celite and the solvent was evaporated to dryness. The residue was distributed between trichloromethane and water. Sodium hydrogen carbonate was added for neutralisation. The layers were separated and the aqueous layer extracted two times with trichloromethane. The combined organic layers were washed with water, dried over sodium sulfate, filtered and the solvent was removed. The products were obtained as colourless oils.

4.8.1. Methyl{(2R,5S)-5-[(1S)-7-(dimethylamino)-1-hydroxyheptyl]tetrahydrofuran-2-yl}acetate (**1a**)

Attempt: **10a**: 1.0 g (3.34 mmol). Yield: 0.99 g (98.5%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.17$. MS (HREI): m/z 301.2235 (M^+, calcd. 301.2253 for $C_{16}H_{31}NO_4$). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.26 (m, 6 H), 1.43 (m, 4 H), 1.74 (m, 3 H), 2.03 (m, 1 H), 2.19 (s, 6 H), 2.2–2.45 (m, 4 H), 2.50 (m, 1 H), 3.23 (m, 1 H), 3.65 (m, 3 H), 3.7 (m, 1 H), 4.41 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 25.18, 27.38, 29.23, 29.57, 30.40, 30.94, 31.95, 40.01, 45.22, 51.53, 59.70, 73.96, 79.27, 82.38, 170.44.
$C_{16}H_{31}NO_4$ (301.0)

4.8.2. Methyl{(2R,5S)-5-[(1S)-8-(dimethylamino)-1-hydroxyoctyl]tetrahydrofuran-2-yl}acetate (**1b**)

Attempt: **10b**: 1.0 g (3.2 mmol). Yield: 0.98 g (97.2%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.19$. MS (HREI): m/z 315.2424 (M^+, calcd. 315.2410 for $C_{17}H_{33}NO_4$). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.20–1.38 (m, 9 H), 1.40–1.52 (m, 3 H), 1.65 (m, 1 H), 1.75 (m, 1 H), 1.92 (m, 2 H), 2.05 (m, 1 H), 2.20 (s, 6 H), 2.23 (m, 2 H), 2.51 (m, 2 H), 2.7 (br, OH), 3.68 (s, 3 H), 3.78 (m, 1 H), 3.86 (m, 1 H), 4.30 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 23.32, 25.96, 27.40, 27.59, 29.46, 29.57, 31.14, 32.49, 40.49, 45.37, 51.73, 59.83, 71.13, 75.26, 83.13, 171.83.
$C_{17}H_{33}NO_4$ (315.0)

4.8.3. Methyl{(2R,5S)-5-[(1S)-10-(dimethylamino)-1-hydroxydecyl]tetrahydrofuran-2-yl}acetate (**1c**)

Attempt: **10c**: 1.5 g (4.4 mmol). Yield: 1.34 g (89%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.25$. MS (HREI): m/z 343.2733 (M^+, calcd. 343.2723 for $C_{19}H_{37}NO_4$). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.20–1.45 (m, 13 H), 1.60–2.10 (m, 5 H), 2.20 (s, 6 H), 2.22 (m, 2 H), 2.51 (m, 4 H), 2.63 (br, OH), 3.68 (s, 3 H), 3.85 (m, 1 H), 4.15 (m, 1 H), 4.35 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 23.69, 25.08, 26.98, 27.41, 27.51, 29.26, 29.45, 31.11, 32.06, 32.63, 40.61, 45.39, 51.69, 59.87, 71.11, 75.31, 83.11, 171.40.
$C_{19}H_{37}NO_4$ (343.0)

4.8.4. {(2R,5)-5-[(2R)-Benzyloxy-7-(dimethylamino)-heptyl]tetrahydrofuran-2-yl}acetonitrile (**16a**)

Attempt: **15a**: 1.0 g (2.81 mmol). Yield: 1.0 g (99.4%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.37$. MS (HREI): m/z 357.2530 ($[M - H]^-$, calcd. 357.2542 for $C_{22}H_{33}N_2O_2$). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 1.27 (m, 4 H), 1.43 (m, 5 H), 1.56 (m, 4 H), 1.72 (m, 1 H), 2.18 (s, 6 H), 2.21 (m, 2 H), 2.52 (m, 2 H), 3.46 (m, 1 H), 4.02 (m, 1 H), 4.18 (m, 1 H), 4.45/4.49 (AB, J = 11.6, 2 H), 7.30 (m, 5 H). ^{13}C NMR (500 MHz, $CDCl_3$, ppm): 24.50, 25.10, 27.61, 29.46, 30.59, 31.50, 33.75, 39.80, 45.39, 59.75, 70.56, 73.56, 76.36, 77.65, 117.43, 127.42, 127.77, 128.26, 138.84.
$C_{22}H_{34}N_2O_2$ (358.0)

4.8.5.{(2R,5)-5-[(2R)-Benzyloxy-8-(dimethylamino)-octyl]tetrahydrofuran-2-yl}acetonitrile (**16b**)

Attempt: **15b**: 1.2 g (3.24 mmol). Yield: 1.19 g (98.7%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.40$. MS (HREI): m/z 371.2674, ($[M - H]^-$, calcd. 371.2699 for $C_{23}H_{35}N_2O_2$). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 1.24–1.40 (m, 8 H), 1.40–1.50 (m, 2 H), 1.52–1.70 (m, 3 H), 1.74 (m, 1 H), 1.97 (m, 1 H), 2.18 (m, 1 H), 2.20 (s, 6 H), 2.22 (m, 2 H), 2.54 (m, 2 H), 3.48 (m, 1 H), 3.99 (m, 1 H), 4.20 (m, 1 H), 4.46/4.51 (AB, J = 11.6, 2 H), 7.27 (m, 5 H). ^{13}C NMR (500 MHz, $CDCl_3$, ppm): 24.51, 25.13, 27.44, 27.67, 29.46, 30.51, 31.52, 33.78, 39.82, 45.44, 59.86, 70.55, 73.55, 76.40, 77.68, 117.43, 127.41, 127.76, 128.25, 138.86.
$C_{23}H_{36}N_2O_2$ (372.0)

4.8.6. {(2R,5)-5-[(2R)-Benzyloxy-10-(dimethylamino)-decyl]tetrahydrofuran-2-yl}acetonitrile (**16c**)

Attempt: **15c**: 1.1 g (2.76 mmol). Yield: 1.08 g (97.7%). TLC ($CHCl_3$/MeOH = 9 : 1): $R_f = 0.32$. MS (HREI): m/z 399.3006 ($[M - H]^-$, calcd. 399.3012 for $C_{25}H_{39}N_2O_2$). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 1.26 (m, 6 H), 1.52 (m, 4 H), 1.55 (m, 2 H), 1.62 (m, 2 H), 1.74 (m, 2 H), 1.95 (m, 2 H), 2.06 (m, 2 H), 2.19 (s, 6 H), 2.20 (m, 2 H), 2.52 (m, 2 H), 3.47 (m, 1 H), 4.02 (m, 1 H), 4.19 (m, 1 H), 4.45/4.50 (AB, J = 11.6, 2 H), 7.27 (m, 5 H). ^{13}C NMR (500 MHz, $CDCl_3$, ppm): 24.52, 25.14, 27.47, 27.72, 29.53, 30.52, 31.13, 31.51, 32.23, 33.81, 39.83, 45.46, 59.92, 70.55, 73.70, 76.44, 77.30, 117.43, 127.41, 127.77, 128.26, 138.88.
$C_{25}H_{40}N_2O_2$ (400.0)

4.9. {(2R,5)-5-[(2S)-Benzyloxy-4-oxobutyl]tetrahydrofuran-2-yl}acetonitrile (**14**)

To a stirred solution of **13** (6.5 g, 19.6 mmol) in acetone (150 ml) and water (25 ml) 0.5 N hydrochloric acid (100 ml) was slowly added. The mixture was stirred further overnight. Neutralisation was done with solid sodium hydrogencarbonate. Subsequently the acetone was evaporated in vacuo. The aqueous mixture was extracted three times with trichloromethane. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and the solvent was evaporated to obtain **14** as a weakly yellow oil.
Yield: 5.52 g (98.2%). TLC (hexane/ethylacetate = 2 : 1): $R_f = 0.36$. MS (ESI): m/z (rel. Int.) 288 ($[M + H]^+$, 38), 310 ($[M + Na]^+$, 12). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.50–2.22 (m, 6 H), 2.50–2.75 (m, 4 H), 3.92–4.20 (m, 3 H), 4.53 (m, 2 H), 7.33 (m, 5 H), 9.78 (dd, J = 2.3, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$, ppm): 24.46, 30.29, 31.31, 39.63, 48.10, 71.06, 71.87, 74.00, 76.66, 117.35, 126.91, 127.78, 128.37, 138.05, 201.49.
$C_{17}H_{21}NO_3$ (287.0)

4.10. Measurement of membrane activity of 1a–c, 2a–c *and* 3 *in an artificial membrane model*

Planar bilayer lipid membranes (BLM) were prepared from soybean phosphatidylcholine (Sigma, P5638) 20 mg/ml n-heptane) [8]. The measuring glass cell (25 ml of total volume) was equipped with a teflon cylinder (1 cm diameter) which contained a hole of 0.5 mm diameter to harbour the black lipid membrane (BLM). Formation of the BLM was controlled by the use of a binocular microscope. Both the measuring cell (10 ml outside (cis)-volume) and the inner side of teflon cylinder (trans-volume; 1 ml) were filled with a solution of sodium and potassium chloride ranging from 100 to 1000 mM, and pH 4–9 depending on the type of experiment. Membrane current was measured by the voltage-clamp method [8]. The current measuring device consisted of an operational amplifier model Keithly 301 (USA). Amplitude current noise of the amplifier was less than 10^{-13} in the frequency

range 0.1–20 . Output of the operational amplifier was connected to the pen chart X-Y plotter (Endim – 622, Germany). The experimental set up allowed to register currents <1 pA with time resolution of about 25 msec.
Compounds **1a–c, 2a–c** and **3** (1–10 μl of 0.5 mg/ml in DMSO stock solution) were added into 10 ml constantly stirred volume of electrolyte, at the cis side o BLM. To avoid the noise caused by the BLM vibration the magnetic stirrer was switched off during recording of the single ion channel currents. In general case, the membrane current started to increase within about 1 min after addition of the samples and reached a relatively stable level after approximately 3–4 min whereby it changed less then about 10%/min. That values of the membrane currents were used for plotting the conductance versus concentration dependence.

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