Bioorganic & Medicinal Chemistry 19 (2011) 7720–7727

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Anti-cancer activity of 5-*O*-alkyl 1,4-imino-1,4-dideoxyribitols

Claudia Bello [a,†], Giovanna Dal Bello [b,†], Michele Cea [c,†], Aimable Nahimana [d], Dominique Aubry [d], Anna Garuti [c], Giulia Motta [c], Eva Moran [c], Floriana Fruscione [e], Paolo Pronzato [b], Francesco Grossi [b], Franco Patrone [c], Alberto Ballestrero [c], Marc Dupuis [a], Bernard Sordat [a], Kaspar Zimmermann [f], Jacqueline Loretan [f], Markus Wartmann [f], Michel A. Duchosal [d], Alessio Nencioni [c], Pierre Vogel [a,*]

[a] *Laboratory of Glycochemistry and Asymmetric Synthesis, Swiss Federal Institute of Technology (EPFL), Batochime, CH-1015 Lausanne, Switzerland*
[b] *National Cancer Research Institute, 16132 Genoa, Italy*
[c] *Department of Internal Medicine, University of Genoa, Italy*
[d] *Service and Central Laboratory of Hematology, University Hospital (CHUV), Lausanne, Switzerland*
[e] *Department of Experimental Medicine, University of Genoa, Italy*
[f] *Novartis, Institutes for Biomedical Research, CH-4002 Basel, Switzerland*

ARTICLE INFO

Article history:
Received 26 April 2011
Revised 21 July 2011
Accepted 23 July 2011
Available online 30 July 2011

Keywords:
Breast cancer
Iminoalditols
JURKAT cells
Oleyl derivative

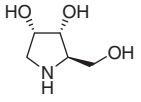
ABSTRACT

New derivatives of 1,4-dideoxy-1,4-imino-D-ribitol have been prepared and evaluated for their cytotoxicity on solid and haematological malignancies. 1,4-Dideoxy-5-*O*-[(9*Z*)-octadec-9-en-1-yl]-1,4-imino-D-ribitol (**13**, IC_{50} ~2 μM) and its C_{18}-analogues (IC_{50} <10 μM) are cytotoxic toward SKBR3 (breast cancer) cells. **13** also inhibits (IC_{50} ~8 μM) growth of JURKAT cells.

1. Introduction

1,4-Dideoxy-1,4-imino-D-ribitol **1** is a natural product with important biological activities. It was first isolated from the root bark of Mulberry tree (*Morus Alba* L.).[1] This compound has been studied in some details because it is a mimic of furanosides and, consequently, might interact with carbohydrate-processing enzymes.[2] In addition to the glycosidase inhibitory activity, 1,4-dideoxy-1,4-imino-D-ribitol strongly inhibits the activities of eukaryotic DNA polymerases with almost no activity on prokaryotic DNA polymerases, nor on DNA metabolic enzymes.[3] These properties suggested a potential clinical application of **1** as anti-HIV agent.[4] This compound has been proposed to be also an anti-neoplastic and anti-proliferative agent. Unfortunately, the in vitro studies on cancer cell lines did not show any cytotoxic activity, probably because of the high polarity of **1** that hampers the compound to cross cell membranes.

Numerous 2-methyleneamino derivatives of **1** were synthesized in order to increase lypophilicity and cell membrane permeability. This has led to the discovery of compounds with promising anti-cancer activities most probably related to their α-mannosidase inhibitory activity.[5,6]



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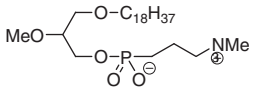

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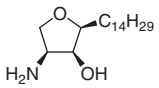

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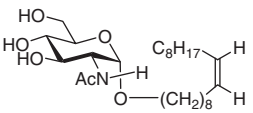

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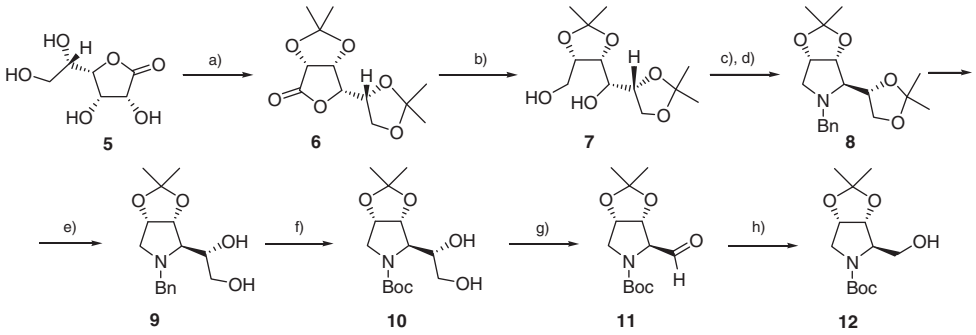
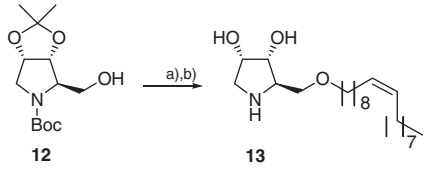
Derivatization of iminosugars with aliphatic side chains has been extensively studied in order to improve their biological activity, lipophilicity and bioavailability.[7] For instance N-alkylation of 1-deoxynojirimycin has led to compounds of therapeutic interest, like *N*-butyl-1-deoxynojirimycin (Zavesca) that has been approved as a drug for the treatment of Gaucher's disease,[8] or like *N*-nonyl-1-deoxynojirimycin, that inhibits hepatitis B virus in cell based as-

* Corresponding author.
E-mail address: pierre.vogel@epfl.ch (P. Vogel).
† These authors equally contributed to this work.

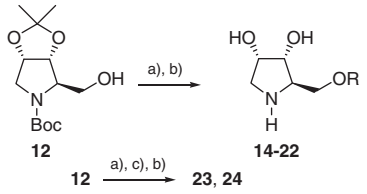
0968-0896/$ - see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2011.07.053



Scheme 1. Synthesis of 1,4-dideoxy-1,4-imino-D-ribitol 12. (a) Acetone/DMP (5:1), *p*-TSAcat, rt, 99% yield; (b) (1) Red-Al (2 equiv), THF/toluene, 0 °C; (2) Rochelle's salt (sat aq. solution), rt, 62% yield; (c) MsCl (3 equiv), NEt_3 (3.5 equiv), CH_2Cl_2, 0 °C; (d) Benzylamine, 65 °C, 2d, 77% yield (two steps); (e) AcOH, 55 °C, 50% conversion; (f). $Pd(OH)_2$-C_{cat}, H_2, Boc_2O (3 equiv), MeOH, rt, 73% yield; (g) $NaIO_4$ (1.5 equiv), MeOH/H_2O (4:1), 0 °C, 1 h, 90% yield; (h) $NaBH_4$ (1.5 equiv), MeOH, 0 °C, 72% yield.



Scheme 2. Synthesis of oleyl pyrrolidine **13**. Reagents and conditions: (a) RBr (1.5 equiv), NaH (1.5 equiv), DMF, 60 °C, 40% yield; (b) TFA/H_2O (4:1), 0 °C, 43% yield.



Scheme 3. Synthesis of pyrrolidine derivatives **14**–**24** analogues of oleyl derivative **13**. Reagents and conditions: (a) RBr or ROMs, Bu_4NI, 50% aq NaOH; (b) TFA/H_2O (4:1), 0 °C; (c) $Pd(OH)_2$/charcoal, H_2/MeCN. Total yields obtained for **14**–**24** were 84%(**14**: R = pent-5-en-1-yl), 38% (**15**: R = *n*-C_7H_{15}), 26% (**16**: R = *n*-$C_{10}H_{21}$), 28% (**17**: R = *n*-$C_{14}H_{29}$), 57% (**18**: R = palmitoleyl = *cis*-Δ^9 $C_{16}H_{31}$), 32% (**19**: R = linoleyl = *cis,cis*-Δ^9,Δ^{12} $C_{18}H_{33}$), 44% (**20**: R = linolenyl = *cis,cis,cis*-Δ^9,Δ^{12},Δ^{15} $C_{18}H_{31}$), 44% (**21**: R = benzyl), 40% (**22**: R = *p*-phenylbenzyl), 82% (**23**: R = pentyl), 75% (**24**: R = octadecyl).

says.[9] α-1-*C*-Alkyl-1-deoxynojirimycin derivatives have shown inhibitory activity toward intestinal isomaltase and their potency was highly dependent on the alkyl chain length.[10] *N*-Alkylated-D-fagosamine derivatives and *N*-alkyl-(2*R*,3*S*,4*R*,5*S*)-2-(hydroxymethyl)-5-methylpyrrolidine-3,4-diol derivatives with long alkyl chains exhibited enhanced cytotoxic activities on a panel of cancer cell lines compared to their non alkylated progenitors.[11]

We hypothesized that attaching another natural compound having lipophilic character to the hydroxymethylene lateral chain of the dihydroxypyrrolidine core could lead to new pyrrolidine derivatives (e.g., **13**) with potent anticancer activity. Our attention was attracted by oleyl alcohol that exhibits weak in vitro cytotoxic activity toward cancer cell lines.[12] The oleyl moiety would make the new compound more lipophilic, allowing cell penetration, and could also be directly involved in its cytotoxic activity.

In addition, 1,4-dideoxy-1,4-imino-D-ribitol imitates the polar moieties of anti-tumor compounds such as Edelfosine (**2**),[13] Jaspine B (**3**)[14] or oleyl 2-acetamido-2-deoxy-α-D-glucopyranosides (e.g., **4**),[15] while the oleyl group would have the same role than the less polar side-chain of the latter cytotoxic agents. This might lead to novel anti-cancer agents.

2. Results and discussion

2.1. Synthesis of pyrrolidine-fatty alcohol conjugates

N-tert-Butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-1,4-imino-D-ribitol **12** was prepared by reduction of the aldehyde **11**,[16] synthesized in turn from D-gulonic acid γ-lactone **5** following a modified procedure (Scheme 1) based on Fleet's synthesis.[17] The modifications allowed us to perform the synthesis faster and in better overall yield, thanks to faster work-up and purification of the intermediates.

The targeted compound **13** was obtained through S_N2 displacement of oleyl bromide with alcohol **12** followed by deprotection of both the diol and amine groups by treatment with trifluoroacetic acid and water (Scheme 2).[18]

In order to study the role of the aliphatic side-chain in the mechanism of action of this compound and to out-line a structure–activity relationship, we prepared a class of derivatives (**14**–**24**, Scheme 3) containing the same pyrrolidine moiety but with side chains being either fatty alkyl groups of different lengths (**14**, **15**, **16**, **17**, **18**, **23**, **24**) and with various degrees of unsaturation (**19**, **20**), or containing aromatic groups like benzyl (**21**) or *p*-phenylbenzyl (**22**) groups that are found in many drugs.[19–21]

These compounds were obtained via S_N2 displacements by **12** of the methanesulfonates or bromides derived from the corresponding fatty alcohols, without solvent, in 50% aqueous solution of NaOH as a base, and in the presence of tetrabutylammonium iodide as a phase transfer catalyst.

2.2. Biological results

Compound **13** and its analogues were evaluated for their ability to inhibit SKBR3 (HER2+ breast cancer) cell growth, using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) assay, which determines the number of metabolically active cells, after 72 h exposure. SKBR3 cells were selected for these experiments based on preliminary data indicating that this cell line is highly susceptible to the activity of **13** and related compounds. Namely the IC_{50} of these drugs is up to 10-times lower in these cells as compared to other cancer cell lines tested (including breast, lung, prostate cancer, and glioblastoma cells).

Oleyl pyrrolidine **13** killed almost all SKBR3 cells already at a concentration of 6.25 μM. An IC_{50} ~2 μM can be estimated from Figure 1. The results obtained with analogues **14**–**24** (Fig. 1) allowed us to establish a structure–activity relationship as a function of the lateral chain length. We observed that the cytotoxic activity starts with a chain length of more than seven carbon atoms, as the heptyl derivative **15** was not active while the decyl **16** already

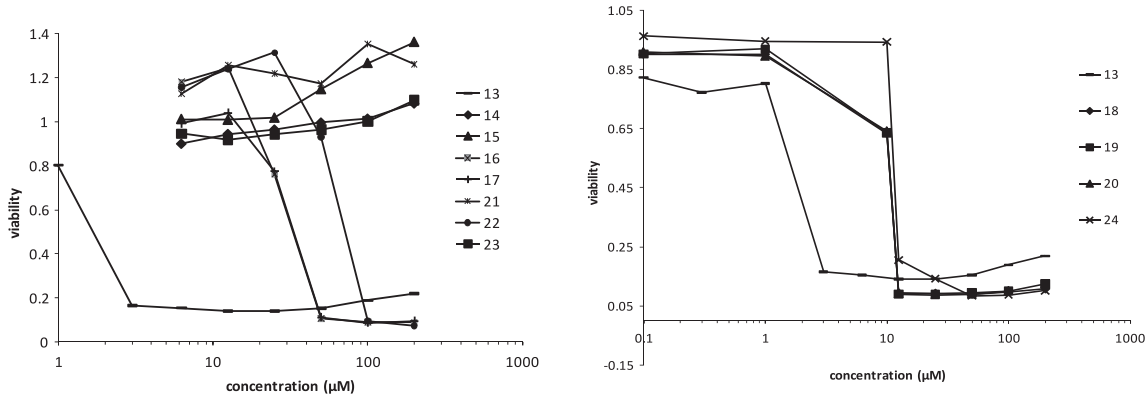


Figure 1. Viability assays of compounds **13**–**23** (left) and **18**–**24** (right, **13** is used as reference) on SKBR3 breast cancer cell lines after 72 h exposure. Results are presented as means of duplicate wells.

showed some activity, although much weaker than that of **13** (Fig. 1).

With **18** that has a chain length of sixteen carbon atoms the cytotoxic activity was almost the same as that of the oleyl derivative **13** (Fig. 1). These results indicate that the anti-cancer activity of the new compounds increase with the chain length (best activities with minimum 14 carbon atoms), in accordance with the hypothesis that a higher lypophilicity would help the transport through the cytoplasmic membrane or an incrustation of the compound in the membrane.

The comparison between the results obtained with the oleyl compound **13** and that with compounds bearing linoleyl **19**, linolenyl **20** and octadecyl **24** pointed out that in the absence of unsaturation or in the presence of two or three double bonds the cytotoxicity was slightly reduced (Fig. 1).This suggests that the double bond could play a role, even if not determinant, in the cancer cell growth inhibitory activity of these compounds.

The presence of an aromatic moiety instead of the aliphatic side chain lowered the cytotoxic activity: the benzyl derivative **21** did not kill cancer cells even at high concentrations, while the *p*-phenylbenzyl analogue **22** exhibited a moderate effect (Fig. 1) that is in the same range of that displayed by the α-mannosidase inhibitors we described in a previous work.[6]

Contrary to **13** and its analogues **14**–**21** and **23**–**24** that do not inhibit α-mannosidase from jack beans (results not shown), **22** shows a moderate α-mannosidase inhibitory activity. It is thus possible that the weak cytotoxic activity of **22** arises from its α-mannosidase inhibitory activity (as thought to be the case with other dihydroxypyrrolidine derivatives we reported recently[6]). This is not the case for **13**.

In order to compare the effect of the drugs on solid and hematological malignancies, the cytotoxicity of compounds **13** and **22** was also evaluated toward JURKAT cells (a well-established model of acute T-cell lymphoblastic leukemia) (Fig. 2). Apoptotic cells were quantified by staining with annexin-V and 7-ADD and flow cytometric analysis. Cell viability was monitored using Trypan blue assay.

The compounds have a similar effect on both solid and hematological malignancies: indeed, SKBR3 cells and JURKAT cells were sensitive to both compounds, the most active being **13** (IC_{50} ~8 μM, estimated from Fig. 2), while **22** killed all the cells only at a concentration of 100 μM.

Compound **13** induced time-dependent cell death in JURKAT cells, as shown in the time course analysis of cell death induced by 10 μM of **13** on JURKAT cells (Fig. 3).

In a preliminary assay compound **13** was evaluated on normal hematopoietic progenitor cells (HPC) and the results were compared to those obtained toward JURKAT cells (Fig. 3, material and methods ESI). This comparison disclosed that compound **13** induces cell death in normal cells to a lower extent than in cancer cells, or, at least, that the cell death is induced more slowly in normal cells than in cancer cells. In fact, after 24 h of exposure to 10 μM of **13**, the percentage of cell death in JURKAT cells was 100 (Fig. 3A, fourth bar), 60% were in the late apoptotic phase (black part of the histogram) and 40% in the early apoptotic phase (white part of the histogram), whereas under the same conditions the percentage of cell death was 60 in normal cells (Fig. 3B, second bar), 20% of $CD34^{+}CD38^{low}$ cells were in the early apoptotic phase and 40% in the late apoptotic phase.

Further studies will be performed in order to confirm these encouraging observations and to have a better insight into the selectivity of compound **13** and its analogues.

The mechanism of action of our compounds has not been established yet. As α-mannosidase from Jack bean was not inhibited by **13** it is possible that human α-mannosidases, key enzymes of the N-glycosidation pathway in cells,[22] might not be the targets of **13** (and its *O*-alkyl derivatives).

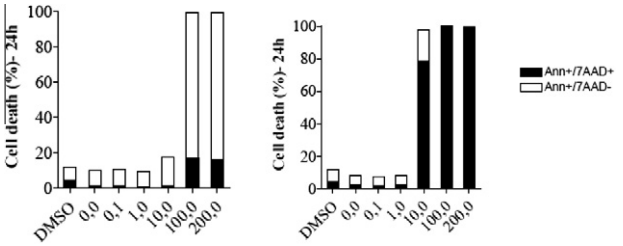


Figure 2. Sensitivity of JURKAT cells toward compounds **22** (left) and **13** (right). The percentage of early apoptotic cells (annexin V+7AAD−) are shown as white columns and that of late apoptotic cells (annexin V+7AAD+) are shown as solid black columns.

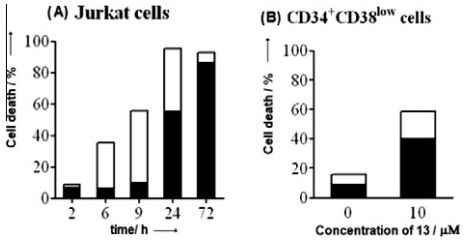


Figure 3. (A) 10 μM **13** induces cell death in JURKAT cells in a time dependent manner and (B, measured after 24 h) in a less extent in normal hematopoietic progenitor cells (HPC).

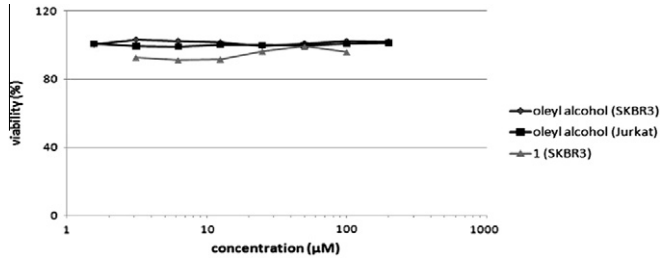


Figure 4. Viability assays of oleyl alcohol on SKBR3 and on JURKAT cells and of 1,4-dideoxy-1,4-imino-D-ribitol **1** on SKBR3 cancer cells. Results are means of triplicata wells.

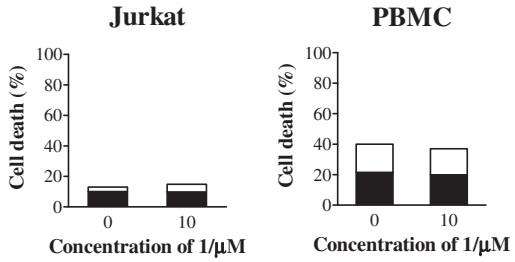


Figure 5. Sensitivity of JURKAT cells and peripheral blood mononuclear cells toward compounds 1,4-dideoxy-1,4-imino-D-ribitol **1**. The percentage of early apoptotic cells (annexin V+7AAD−) are shown as white columns and that of late apoptotic cells (annexin V+7AAD+) are shown as solid black columns.

Preliminary assays showed that compound **13** is an effective cell cycle modulator: the treatment of SKBR3 cells with **13** results in inhibition of cell growth accompanied by enhanced accumulation of cells in the G_0/G_1 phase of the cell cycle and reduction in S phase. Higher concentrations of **13** led to cell demise with DNA fragmentation and appearance of hypodyploid cell nuclei. The cytotoxic activity of the drug was detected after 6–12 h of incubating and was initially characterized by extensive intracellular vacuolization.

Compounds **13**, **17**–**20** and **22** have been tested on 15 Ba/F_3 cells models whose proliferation depends on the engineered expression of specific tyrosine kinases.[23] None of the compounds reproducibly achieved half-maximal suppression of cell proliferation (resazurin sodium salt reducing ability, determined after 48 h of potential drug incubation, 9 point IC_{50} curves determined in three independent experiments at concentrations below 10 μM). Thus **13** and analogues can be considered as devoid of relevant inhibitory activity against the respective kinases as assessed in the Ba/F3 model. However, as the Ba/F3 model represents a cellular system, one can deduce that compound **13** (as well as **17**–**20**, **22**) does not show unspecific cytotoxity at concentrations lower than 10 μM.

Interestingly, we found that neither oleyl alcohol nor 1,4-dideoxy-1,4-imino-D-ribitol alone exhibited significant cytotoxic activity in our cellular models (Figs. 4 and 5). Thus the activity of **13** and its analogues appears to rely on both 1,4-dideoxy-1,4-imino-D-ribitol and amphiphilic tail, although, again, the reason for this requirement remains to be determined.

With respect to the mechanism of action of **13** and its analogues an important feature has to be taken into account: as they are amphiphilic, they could have detergent activity,[24] causing cell death through membrane disruption.[19b–f,25] Although not all soluble amphiphiles have comparable detergent proprieties, we cannot exclude at this stage that our compounds can have this activity. Further studies have to be done in order to determine their detergent power, as it has been done, for example, for Edelfosine.[13] More exhaustive studies, about the mechanism of action of these new series of compounds are now in course and, together with the analysis of their toxicity/selectivity, will be discussed in a forthcoming report.

3. Conclusions

In summary, we have prepared a library of new 5-*O*-alkyl and 5-*O*-aryl 1,4-imino-1,4-dideoxy-D-ribitols. These compounds are active as inhibitors of cancer cell growth; their activity depends of the length of the alkyl side chain. The oleyl derivative **13** and its C-18 analogues **19**, **20** and **24** exhibited the most potent anti-cancer activity, with **13** being the most efficient in killing tumor cells. These results indicate an active role of the side chain and of the number and position of the insaturations in determining the biological properties of this class of compounds. Studies on the mechanism of action are now in course, in order to establish if they act via the same pathway than Edelfosine **2**, Jaspine B **3** or glycosides of type **4**.

4. Experimental

4.1. General methods

All commercially available reagents (Fluka, Aldrich, Acros) were used without further purification. Technical solvents were used for extraction without any purification. For reactions requiring anh. conditions, dry solvents were bought (Fluka) or filtered prior to use (Innovative Technology). In absence of any particular notification, experiments were carried out under argon atmosphere. Reactions were monitored by thin layer chromatography (Merck silica gel $60F_{254}$ plates). Revelation was carried out by UV light (254 nm) and $KMnO_4$ [MnO_4 (3 g), K_2CO_3 (20 g, AcOH (0.25 ml) and H_2O (300 ml)] or Pancaldi [$(NH_4)_6Mo_4$ (21 g), $Ce(SO_4)_2$ (1 g), H_2SO_4 (31 ml) and H_2O (470 ml)] reagents. Purifications were performed by flash chromatography (Fluka silica gel 60, 230–400 mesh, 0.04–0.063 mm). ^{1}H NMR spectra were recorded on Bruker ARX-400 and DPX-400 spectrometers at 400 MHz, on Bruker Avance-500 at 500 MHz and on Bruker AvanceII-800 at 800 MHz. The signals of residual solvents were used as reference (MeOH-d_4: 3.32 ppm, $CDCl_3$: 7.27 ppm). Coupling constants are given in Hz. When necessary, the ^{1}H-signal assignments were confirmed by COSY-45 or by analogy with spectra of similar compounds. ^{13}C NMR spectra were recorded on Bruker ARX-400 and DPX-400 spectrometers at 101 MHz, on Bruker Avance-500 at 125 MHz and on Bruker Avance II-800 at 200 MHz. The signals of residual solvents were used as reference (MeOH-d_4: 49 ppm, $CDCl_3$: 77 ppm). When necessary, the ^{13}C-signals assignments were confirmed by HSQC spectra. *IR spectra* were recorded on Perkin Elmer Paragon 1000 FT-IR spectrometer or on Perkin Elmer Spectrum One FT-IR spectrometer. *UV spectra* were recorded on a Perkin Elmer Lambda 10 UV/VIS spectrometer. *Optical rotations* were recorded at 25 °C on a Jasco P-1020 polarimeter. *Mass spectra:* GC-IE/CI/MS experiments were perfomed by Francisco Sepulveda with a 1200L instrument (Varian) coupled to a GC CP-3800 (Varian). ESI-TOF-MS experiments were performed on a Q-Tof Ultima mass spectrometer (Waters) fitted with a standard Z-spray ion source and operated in the positive ionization mode. MALDI-TOF-MS experiments were performed with an AXIMA CFR-Plus instrument (Shimadzu) operated in the positive reflectron ionization mode.

4.2. General procedure 1: S_N2 reactions

Tetrabutylammonium iodide (TBI: 1–1.4 equiv) was added to R-X (5.2 equiv or more) and the mixture was stirred at 25 °C for 20 min. *N-tert*-Butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopro pylidene-1,4-

imino-D-ribitol **12** then NaOH (0.11–0.3 ml of a 50% aqueous solution, 4.5–5 equiv) were added and the solution was stirred for 5 h at 25 °C (reaction monitored by TLC, petroleum ether/ethyl acetate 1:4). Ethyl acetate (5 ml) and water (5 ml) were added and the phases were separated. The aqueous phase was extracted with ethyl acetate (3 × 5 ml), the collected organic layers were washed with brine (5 ml) and dried ($MgSO_4$). Solvent evaporation in vacuo and flash chromatography gave the pure product.

4.3. General procedure 2: deprotection of Boc and acetonides

A 0.3–0.5 M solution of the protected pyrrolidine-fatty alcohol conjugate in cold trifluoroacetic acid was stirred for 10 min at 0 °C. Water (0.05 ml) was added and the solution was stirred at 0 °C for 10 min, then at room temperature until consumption of the starting material (monitored by TLC, pertroleum ether/ ethyl acetate 5:1 and CH_2Cl_2/MeOH 9:1 with 1% NH_4OH 25% aq). The solvent was evaporated in vacuo and the crude was neutralized with 25% aqueous solution of NH_4OH (0.035 ml). Solvent evaporation and flash chromatography gave the pure products.

4.3.1. 1,4-Dideoxy-5-*O*-[(9*Z*)-octadec-9-en-1-yl]-1,4-imino-D-ribitol 13

N-Benzyl-1,4-dideoxy-2,3-*O*-isopropylidene-1,4-imino-D-allitol **9**[26] (1.005 g, 3.42 mmol) was dissolved in dry methanol (35 ml), Boc_2O (2.378 g, 12.8 mmol, 2 equiv) was added and then $Pd(OH)_2$-C as catalyst under Argon atmosphere. The mixture was stirred under H_2 atmosphere for 3 h. The catalyst was filtered off on a Celite pad and the product was purified by flash chromatography (diethyl ether/petroleum ether 4: 1 to diethyl ether 100%) giving N-*tert*-butyloxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-1,4-imino-D-allitol **10**[26] (0.474 g, 1.56 mmol, 46% yield) as white solid.

$NaIO_4$ (0.53 g, 2.47 mmol, 2.8 equiv) was added to a solution of **10** (0.261 g, 0.86 mmol) in methanol/water (6.7 ml, 4:1) at 0 °C and the solution was stirred at 0 °C for 1 h. The solution was poured into water and ethyl acetate was added. The two phases were separated and the aqueous phase was extracted twice with ethyl acetate. The combined organic phases were washed with brine, dried with $MgSO_4$ and the solvent was evaporated in vacuo giving pure **11**[17] (0.209 g, 0.77 mmol, 90% yield) that was used without any further purification.

$NaBH_4$ (0.031 g, 0.8 mmol, 1.6 equiv) was added portionwise to a solution of aldehyde **11** (0.145 g, 0.5 mmol) in methanol (1.5 ml) at 0 °C. The solution was stirred for 1 h at 0 °C, cold water was added (0.5 ml) and then ethyl acetate (1 ml). The two phases were separated, the aqueous phase was extracted twice with ethyl acetate (2 × 0.5 ml) and the collected organic layers were washed with brine, dried with $MgSO_4$ and the solvent was evaporated in vacuo to afford *N*-*tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-1,4-imino-D-ribitol **12**[17] (0.099 g, 0.36 mmol, 72% yield).

12 (0.099 g, 0.36 mmol) was dissolved in DMF (2 ml), NaH (0.022 g (60% in oil), 1.5 equiv) and then oleyl bromide (0.149 g, 0.45 mmol, 1.25 equiv) was added and the mixture was stirred at room temperature for 1 h, then at 60 °C for 18 h. Methanol was added, then water (2 ml) and the solution was extracted with diethyl ether (3 × 2 ml). The combined organic phases were washed successively with water and brine, dried ($MgSO_4$) and the solvent was evaporated in vacuo. After flash chromatography (petroleum ether/AcOEt 7:1) *N*-*tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-O-[(9*Z*)-octadec-9-en-1-yl]-1,4-imino-D-ribitol was obtained (0.075 g, 0.14 mmol, 40% yield) as a colourless oil.

Deprotection of Boc and acetonide protecting groups (TFA/H_2O, 4:1 vol: vol, 0 °C for 3 h) and purification by flash chromatography (CH_3CN/NH_4OH 12: 1) gave **13** (0.021 g, 0.06 mmol, 43% yield) as a white foam.

$[\alpha]_D^{25}$ +13; $[\alpha]_{577}^{25}$ +4; $[\alpha]_{435}^{25}$ +16; $[\alpha]_{405}^{25}$ +24 (*c* = 0.076, MeOH). IR (neat): 3346, 2920, 2851, 1664, 1203, 1185, 1138, 1096, 850, 798, 723. ^{1}H NMR (800 MHz, MeOH-d_4, ol = oleyl): δ 5.35 (m, 2H, H–C(9) and H–C(10) ol) 4.27 (m, 1H, H–C(4)) 4.12 (m, 1H, H–C(3)) 3.77 (dd, ^2J = 10.5, ^3J = 2.3, 1H, H*H*–C(6)) 3.66 (dd, ^2J = 10.5, ^3J = 6.4, 1H, *H*HC-(6)) 3.62 (m, 1H, H–C(2)) 3.59 (m, 1H, *H*HC(1) ol) 3.53 (m, 1H, H*H*C(1) ol) 3.27 (dd, ^2J = 14.6, ^3J = 10.1, 1H, H*H*C(5)) 3.23 (dd, ^2J = 14.6, ^3J = 7.4, 1H, *H*HC(5)) 2.06 (m, 4H, H_2-C(8) and H_2-C(11) ol) 1.64 (m, 2H, H_2C(2) ol) 1.35 (m, 22 H, C(2)-$(CH_2)_5$-C(8) and C(11)-$(CH_2)_6$-C(18) ol) 0.93 (t, ^3J = 7.1, 3H, CH_3 ol). ^{13}C NMR (200 MHz, MeOH-d_4, ol = oleyl): δ: 130.9 (C(9) ol) 130.8 (C(10) ol) 73.5 (C(3)) 72.8 (C(1) ol) 71.2 (C(4)) 68.6 (C(2)) 62.3 (C(6)) 51.2 (C(5)) 33.1 (CH_2 ol) 30.3 (CH_2 ol) 30.8 (CH_2 ol) 30.6 (CH_2 ol) 30.6 (CH_2 ol) 30.6 (CH_2 ol) 30.4 (CH_2 ol) 30.3 (CH_2 ol) 28.1 (CH_2 ol) 28.1 (CH_2 ol) 27.2 (CH_2 ol) 23.7 (CH_2 ol) 14.4 C(18) ol). HR-MALDI-TOF-MS: calcld for $C_{23}H_{45}NO_3$: 384.3477, found 384.3473 ($[M+H]^+$).

4.3.2. 1,4-Dideoxy-5-O-(pent-5-en-1-yl)-1,4-imino-D-ribitol 14

General procedure 1. TBI: 0.137 g, 0.37 mmol, R–X: 1-bromo-5-pentene (0.5 ml, 0.412 g, 1.41 mmol, 5.2 equiv), **12**: 0.101 g, 0.37 mmol, NaOH: 0.15 ml. Pure *N*-*tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-*O*-(pent-5-en-1-yl)-1,4-imino-D-ribitol was obtained (0.124 g, 0.35 mmol, 96% yield) as a colourless oil.

Deprotection performed following the general procedure 2: protected **14**: 0.072 g, 0.21 mmol, TFA: 0.45 ml. Pure **14** (0.037 g, 0.18 mmol, 88% yield) was obtained as a white foam after flash column chromatography on silica gel (CH_2Cl_2/MeOH 87:13, 1% NH_3 aq).

$[\alpha]_D^{25}$ +35; $[\alpha]_{577}^{25}$ +29; $[\alpha]_{435}^{25}$ +56; $[\alpha]_{405}^{25}$ +68 (*c* = 0.11, MeOH). IR (neat): 3488, 3269, 2940, 2913, 2865, 2506, 1770, 1316, 1191, 1079, 873, 576. ^{1}H NMR (400 MHz, MeOH-d_4, pent = pentenyl): δ 5.84 (ddt, ^3J = 10.9, ^3J = 6.7, ^3J = 6.7, H–C(4) pent) 5.03 (ddd, ^2J = 17.1, ^3J = 3.5, ^4J = 1.7, 1H, H_{trans}-C(5) pent) 4.96 (bd, ^3J = 11.2, 1H, H_{cis}-C(5) pent) 4.05 (m, 1H, H–C(4)) 3.81 (dd, ^2J = 7.1, ^3J = 5.3, 1H, H–C(3)) 3.59 (dd, ^2J = 9.8, ^3J = 3.7, *H*H–C(6)) 3.48–3.52 (m, 3H, H_2-C(1) pent and H*H*–C(6)) 3.18 (dd, ^2J = 12.0, ^3J = 5.4, 1H, *H*H–C(5)) 3.09 (m, 1H, H–C(2)) 2.81 (dd, ^2J = 12.0, ^3J = 3.9, 1H, H*H*–C(5)) 2.13 (m, 2H, H_2 -C(3) pent) 1.68 (m, 2H, H_2-C(2) pent). ^{13}C NMR (101 MHz, MeOH-d_4, pent = pentenyl): δ 139.5 (d, ^1J = 192, C(4) pent) 115.2 (t, ^1J = 154, C(5) pent) 74.6 (d, ^1J = 144, C(4)) 72.5 (d, ^1J = 154, C(3)) 71.8 (t, ^1J = 144, H_2C-C(2)) 71.7 (t, ^1J = 144, C(1) pent) 63.3 (d, ^1J = 138, C(2)) 52.4 (t, ^1J = 139, C(5)) 31.41 (t, ^1J = 145, C(2) pent) 30.0 (t, ^1J = 125, C(3) pent). HR-ESI-TOF-MS: calcd for $C_{10}H_{19}NO_3$: 202.1438, found: 202.1434 ($[M+H]^+$).

4.3.3. 1,4-Dideoxy-5-*O*-heptyl-1,4-imino-D-ribitol 15

General procedure 1. TBI: 0.164 g, 0.44 mmol, 1 equiv, R-X: heptyl methanesulfonate (0.5 ml, excess), **12**: 0.10 g, 0.37 mmol, NaOH: 0.15 ml. Pure *N*-*tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-*O*-heptyl-1,4-imino-D-ribitol was obtained (0.070 g, 0.19 mmol, 51% yield) as a colourless oil after flash column chromatography on silica gel (petroleum ether/ethyl acetate 100:0 → 95:5 → 90:10). Deprotection performed following the general procedure 2: protected **15**: 0.070 g, 0.19 mmol, TFA: 0.45 ml. Pure **15** (0.033 g, 0.14 mmol, 75% yield) was obtained as white foam after flash column chromatography on silica gel CH_2Cl_2/MeOH 93:7, 1% NH_3 aq).

$[\alpha]_D^{25}$ +40; $[\alpha]_{577}^{25}$ +43; $[\alpha]_{435}^{25}$ +74; $[\alpha]_{405}^{25}$ +84 (*c* = 0.0605, MeOH) IR (pure): 3484, 3267, 2917, 2870, 1081, 873, 658. ^{1}H NMR (400 MHz, MeOH-d_4, hep = heptyl): δ 4.04 (m, 1H, H–C(4)) 3.79 (dd, ^3J = 5.4, ^3J = 6.9, 1H, H–C(3)) 3.59 (dd, ^3J = 3.6, ^2J = 9.8, 1H, *H*H–C(6)) 3.46–3.51 (m, 3H, H_2-C(1) hep and H*H*–C(6)) 3.15 (dd, ^3J = 5.4, ^2J = 12.0, 1H, *H*H–C(5)) 3.08 (m, 1H, H–C(2)) 2.80 (dd, ^3J = 4.0,

^2J = 12.0, 1H, H*H*–C(5)) 1.59 (m, 2H, H_2-C(2) hep) 1.32–1.39 (m, 8H, C(2)-$(CH_2)_4$-C(7) hep) 0.91 (t, ^3J = 6.8, 3H, H_3-C(7) hep). ^{13}C NMR (101 MHz, MeOH-d_4): δ 74.7 (d, ^1J = 147, C(3)) 72.6 (t, ^1J = 139, C(6)) 72.5 (d, ^1J = 149, C(4)) 71.8 (t, ^1J = 142, C(1) hep) 63.3 (d, ^1J = 140, C(2)) 52.4 (t, ^1J = 140, C(5)) 33.0 (t, ^1J = 124, C(2) hep) 30.7 (t, ^1J = 123, C(3) hep) 30.3 (t, ^1J = 124, C(4) hep) 29.2 (t, ^1J = 126, C(5) hep) 23.7 (t, ^1J = 124, C(6)hep) 14.4 (q, ^1J = 124, C(7) hep). HR-ESI-MS: calcd for $C_{12}H_{25}NO_3$: 232.1907, found 232.1914 ([M+H]$^+$).

4.3.4. 1,4-Dideoxy-5-*O*-decyl-1,4-imino-D-ribitol 16

General procedure 1. TBI: 0.163 g, 0.44 mmol, 1.2 equiv, R–X: decyl bromide (0.5 ml, excess), **12**: 0.098 g, 0.36 mmol; NaOH: 0.2 ml. Pure *N-tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-*O*-decyl-1,4-imino-D-ribitol was obtained (0.050 g, 0.12 mmol, 34% yield) as a colourless oil (flash column chromatography: petroleum ether/ ethyl acetate 9:1).

Deprotection performed following the general procedure 2: protected **16**: 0.050 g, 0.12 mmol, TFA: 0.5 ml. Pure **16** (0.0252 g, 0.09 mmol, 77% yield) was obtained as a white foam after flash column chromatography on silica gel (CH_2Cl_2/MeOH 9:1, 1% NH_3 aq).

$[\alpha]_D^{25}$ +31; $[\alpha]_{577}^{25}$ +23; $[\alpha]_{435}^{25}$ +57; $[\alpha]_{405}^{25}$ +77 (*c* = 0.0575, MeOH). IR (pure): 3511, 3268, 2917, 2850, 1078, 982, 874. ^{1}H NMR (400 MHz, $CDCl_3$, dec = decyl): δ 4.12 (m, 1H, H–C(4)) 3.86 (m, 1H, H–C(3)) 3.54 (m, 2H, H_2-C-C(2)) 3.44 (t, ^3J = 6.7, 2H, H_2-C(1) dec) 3.28 (m, 2H, 2× H–O) 3.22 (dd, ^2J = 12.0, ^3J = 5.1, 1H, *H*H–C(5)) 3.11 (m, 1H, H–C(2)) 2.90 (dd, ^2J = 12.0, ^3J = 3.1, 1H, H*H*–C(5)) 1.55 (m, 2H, H_2-C(2) dec) 1.26 (br s 14H, C(2)-$(CH_2)_7$-C(10) dec) 0.88 (t, ^3J = 6.7, 3H, H_2-C(10) dec). ^{13}C NMR (101 MHz, $CDCl_3$): δ 74.4 (d, ^1J = 143, C(3)) 71.6 (t, ^1J = 139, C(1) dec) 71.5 (d, ^1J = 149, C(4)) 71.2 (t, ^1J = 139, H_2C-C(2)) 62.2 (d, ^1J = 139, C(2)) 52.0 (t, ^1J = 139, C(5)) 31.9 (t, ^1J = 122, C(2) dec) 29.6 (t, ^1J = 124, C(4) and C(3) dec) 29.6 (t, ^1J = 125, C(5) dec) 29.5 (t, ^1J = 125, C(6) dec) 29.3 (t, ^1J = 124, C(7) dec) 26.1 (t, ^1J = 124, C(8) dec) 22.7 (t, ^1J = 124, C(9) dec) 14.1 (q, ^1J = 124, C(10) dec). HR-ESI-TOF-MS: calcd for $C_{15}H_{31}NO_3$: 274.2382, found 274.2381 ([M+H]$^+$).

4.3.5. 1,4-Dideoxy-5-*O*-tetradecyl-1,4-imino-D-ribitol 17

General procedure 1. TBI: 0.100 g, 0.27 mmol, 1 equiv, R-X: tetradecyl methanesulfonate (0.412 g, 1.41 mmol, 5.2 equiv), CH_3CN (0.5 ml) **12**: 0.073 g, 0.27 mmol, NaOH: 0.25 ml. Pure *N-tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-*O*-tetradecyl-1,4-imino-D-ribitol was obtained (0.046 g, 0.1 mmol, 37% yield) as a colourless oil (flash column chromatography: PE/ AcOEt 93:7). Deprotection performed following the general procedure 2: protected **17**: 0.046 g, 0.1 mmol, TFA: 0.4 ml. Pure **17** (0.021 g, 0.063 mmol, 63% yield) was obtained as a white foam after flash column chromatography on silica gel (CH_2Cl_2/MeOH 9:1, 1% NH_3 aq). $[\alpha]_D^{25}$ +27; $[\alpha]_{577}^{25}$ +25; $[\alpha]_{435}^{25}$ +50; $[\alpha]_{405}^{25}$ +65 (*c* = 0.052, MeOH). IR (neat): 3417, 3280, 3239, 2917, 2849, 1470, 1376, 1341, 1125, 1094, 832, 718. ^{1}H NMR (400 MHz, $CDCl_3$, td = tetradecyl): δ 4.12 (m, 1H, H–C(4)) 3.86 (m, 1H, H–C(3)) 3.49–3.56 (m, 2H, H_2C-C(2)) 3.44 (t, ^3J = 6.7, 2H, H–C(1) td) 3.22 (dd, ^2J = 12.0, ^3J = 5.1, 2H, H*H*C(5) and H–O) 3.10–3.14 (m, 2H, H–C(2) and H–O) 2.91 (dd, ^2J = 12.0, ^3J = 5.1, 1H, *H*HC(5)) 1.55 (m, 2H, H_2C(2) td) 1.26 (m, 22H, $(CH_2)_{11}$ td) 0.88 (t, ^3J = 6.5, 3H, H_3C(14) td). ^{13}C NMR (100 MHz, $CDCl_3$, td = tetradecyl): δ 74.4 (d, ^1J = 145, C(3)) 71.7 (t, ^1J = 141, C(1) td) 71.6 (d, ^1J = 150, C(4)) 71.3 (t, ^1J = 141, H_2C-C(2)) 62.2 (d, ^1J = 137, C(2)) 52.0 (t, ^1J = 138, C(5)) 31.9 (t, ^1J = 124, CH_2 td) 29.7 (t, ^1J = 124, $(CH_2)_2$ td) 29.6 (t, ^1J = 124, $(CH_2)_2$ td) 29.6 (t, ^1J = 124, CH_2 td) 29.6 (t, ^1J = 124, CH_2 td) 29.6 (t, ^1J = 124, CH_2 td) 29.3 (t, ^1J = 124, CH_2 td) 26.1 (t, ^1J = 125, CH_2 td) 22.7 (t, ^1J = 127, CH_2 td) 14.1 (q, ^1J = 124, C(14) td). HR-ESI-MS: calcd for $C_{19}H_{39}NO_3$: 330.3003, found: 330.2991 ([M+H]$^+$).

4.3.6. 1,4-Dideoxy-5-*O*-[(*9Z*)-hexadec-9-en-1-yl]-1,4-imino-D-ribitol 18

General procedure 1. TBI: 0.132 g, 0.36 mmol, 1.3 equiv, R-X: palmitoleyl methanesulfonate (0.5 ml excess), **12**: 0.074 g, 0.27 mmol, NaOH: 0.11 ml. Pure *N-tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-O-[(9*Z*)-hexadec-9-en-1-yl]-1,4-imino-D-ribitol was obtained (0.090 g, 0.18 mmol, 67% yield) as a colourless oil (flash column chromatography: PE/AcOEt 7:1). Deprotection performed following the general procedure 2: protected **18**: 0.090 g, 0.18 mmol TFA: 0.34 ml. Pure **18** (0.054 g, 0.15 mmol, 84% yield) was obtained as a white foam after flash column chromatography on silica gel (CH_2Cl_2/MeOH 9:1, 1% NH_3 aq). $[\alpha]_D^{25}$ +21; $[\alpha]_{577}^{25}$ +50; $[\alpha]_{435}^{25}$ +46; $[\alpha]_{405}^{25}$ +48 (*c* = 0.1160, $CHCl_3$). IR (pure): 3313, 3257, 2913, 2848, 1080, 1034, 874. ^{1}H NMR (400 MHz, $CDCl_3$, pal = palmitoleyl): δ 5.44 (m, 2H, H–C(9) and H–C(10) pal) 4.14 (m, 1H, H–C(4)) 3.89 (m, 1H, H–C(3)) 3.69 (br s 3H, 2 xH–O and H-N) 3.57 (dd, ^2J = 9.7, ^3J = 4.5, 1H, *H*HC(6)) 3.53 (dd, ^2J = 9.7, ^3J = 5.2, 1H, H*H*C(6)) 3.44 (t, ^3J = 6.7, 2H, H_2C(1) pal) 3.24 (dd, ^2J = 12.0, ^3J = 5.1, 1H, *H*HC(5)) 3.17 (m, 1H, H–C(2)) 2.96 (dd, ^2J = 9.7, ^3J = 3.0, 1H, H*H*C(5)) 2.00 (m, 4H, H_2C(8) and H_2C(11) pal) 1.55 (m, 2H, H_2C(2) pal) 1.28 (br s, 18H, C(2)-$(CH_2)_5$-C(8) and C(11)-$(CH_2)_4$-C(16) pal) 0.88 (t, ^3J = 6.8, 3H, H_3C(16) pal). ^{13}C NMR (101 MHz, $CDCl_3$, pal = palmitoleyl): δ 129.9 (d, ^1J = 153, C(9) pal) 129.8 (d, ^1J = 153, C(10) pal) 74.1 (d, ^1J = 146, C(3)) 71.7 (t, ^1J = 139, C(1) pal) 71.4 (d, ^1J = 150, C(4)) 70.8 (t, ^1J = 142, C(6)) 62.1 (d, ^1J = 138, C(2)) 51.8 (t, ^1J = 138, C(5)) 31.7 (t, ^1J = 127, H_2C pal) 29.7 (t, ^1J = 125, H_2C pal) 29.7 (t, ^1J = 124, H_2C pal) 29.5 (t, ^1J = 124, H_2C pal) 29.5 (t, ^1J = 124, H_2C pal) 29.4 (t, ^1J = 124, H_2C pal) 29.3 (t, ^1J = 124, H_2C pal) 28.9 (t, ^1J = 124, H_2C pal) 27.2 (t, ^1J = 124, H_2C pal) 27.2 (t, ^1J = 124, H_2C pal) 26.1 (t, ^1J = 125, H_2C pal) 22.6 (t, ^1J = 124, H_2C pal) 14.1 (q, ^1J = 124, C(16) pal). HR-ESI-TOF-MS: calcd for $C_{21}H_{41}NO_3$: 356.3159, found 356.2715 ([M+H]$^+$).

4.3.7. 1,4-Dideoxy-5-*O*-[(*9Z,12Z*)-octadeca-9,12-dien-1-yl]-1,4-imino-D-ribitol 19

General procedure 1. TBI: 0.153 g, 0.41 mmol, 1.2 equiv, R-X: linoleyl methanesulfonate (0.5 ml, excess), **12**: 0.090 g, 0.33 mmol, NaOH: 0.12 ml. Pure *N-tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-O-[(9*Z,12Z*)-octadeca-9,12-dien-1-yl]-1,4-imino-D-ribitol was obtained (0.086 g, 0.17 mmol, 52% yield) as a colourless oil (flash column chromatography: PE/AcOEt 7:1). Deprotection performed following the *general procedure* 2: protected **19**: 0.090 g, 0.18 mmol TFA: 0.34 ml. Pure **19** (0.039 g, 0.10 mmol, 61% yield) was obtained as a white foam after flash column chromatography on silica gel (CH_2Cl_2/MeOH 9:1, 1% NH_3 aq). $[\alpha]_D^{25}$ +35 $[\alpha]_{577}^{25}$ $[\alpha]_{435}^{25}[\alpha]_D^{25}$ (*c* = 0.1460, MeOH) IR (pure): 3293, 3202, 2918, 2850, 1094, 1079, 981, 827, 720. ^{1}H NMR (400 MHz, $CDCl_3$, lin = linoleyl): δ 5.39–5.30 (m, 4H, H–C(9), H–C(10), H–C(12) and H–C(13) lin) 4.28 (m, 3H, 2× H–O and H-N) 4.18 (m, 1H, H–C(2)) 3.96 (m, 1H, H–C(3)) 3.61 (dd, ^2J = 9.7, ^3J = 4.1, 1H, *H*HC(5)) 3.55 (dd, ^2J = 9.7, ^3J = 5.1, 1H, H*H*C(5)) 3.45 (m, 2H, H_2C(1) lin) 3.29–3.25 (m, 2H, H–C(4) and *H*HC(1)) 3.08 (bd, dd, ^2J = 10.2, 1H, H*H*C(1)) 2.77 (m, 2H, H_2C(11) lin) 2.05 (m, 4H, H_2C(8) and H_2C(14) lin) 1.54 ((m, 2H, H_2C(2) lin) 1.30 (m, 16H, C(2)-$(CH_2)_5$-C(8) and C(14)-$(CH_2)_3$-C(18) lin) 0.89 (t, ^3J = 6.8, 3H, H_3C(18) lin). ^{13}C NMR (101 MHz, $CDCl_3$, lin = linoleyl): δ 130.2 (d, ^1J = 161, C(9) lin) 130.1 (d, ^1J = 161, C(10) lin) 128.0 (d, ^1J = 149, C(12) lin) 127.9 (d, ^1J = 149, C(13) lin) 74.0 (d, ^1J = 143, C(3)) 71.7 (t, ^1J = 142, C(1) lin) 71.2 (d, ^1J = 149, C(2)) 70.2 (t, ^1J = 143, C(5)) 62.0 (d, ^1J = 126, C(4)) 51.5 (t, ^1J = 146, C(1)) 31.5 (t, H_2C lin) 29.7 (t, H_2C lin) 29.5 (t, ^1J = 125, H_2C lin) 29.5 (t, ^1J = 125, H_2C lin) 29.5 (t, ^1J = 125, H_2C lin) 29.3 (t, ^1J = 120, H_2C lin) 29.3 t, ^1J = 120, H_2C lin) 27.2 (t, H_2C lin) 27.2 t, H_2C lin) 26.1 (t, ^1J = 124, H_2C lin) 25.6 (t, ^1J = 125, H_2C lin) 22.5 (t, ^1J = 117, H_2C lin) 14.0 (q, ^1J = 123, C(18) lin). HR-ESI-TOF-MS: calcd for $C_{23}H_{43}NO_3$: 382.3316, found 382.3544 ([M+H]$^+$).

4.3.8. 1,4-Dideoxy-5-*O*-[(*9Z,12Z,15Z*)-octadec-9,12,15-trien-1-yl]-1,4-imino-D-ribitol 20

General procedure 1. TBI: 0.136 g, 0.37 mmol, 1.2 equiv, R-X: linolenyl methanesulfonate (0.5 ml, excess), **12**: 0.082 g, 0.30 mmol, NaOH: 0.12 ml. Pure *N-tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-O-[(9*Z,12Z,15Z*)-octadeca-9,12,15-trien-1-yl]-1,4-imino-D-ribitol was obtained (0.103 g, 0.2 mmol, 67% yield) as a colourless oil (flash column chromatography: PE/AcOEt 7:1). Deprotection performed following the *general procedure* 2: protected **20**: 0.103 g, 0.2 mmol TFA: 0.34 ml. Pure **20** (0.048 g, 0.13 mmol, 65% yield) was obtained as a white foam after flash column chromatography on silica gel (CH_2Cl_2/MeOH 9:1, 1% NH_3 aq). $[\alpha]_D^{25}$ +30; $[\alpha]_{577}^{25}$ +54; $[\alpha]_{435}^{25}$ +73; $[\alpha]_{405}^{25}$ +84 (*c* = 0.103, MeOH). IR (neat): 3502, 3277, 3007, 2919, 2851, 1081, 981, 872, 721. ^{1}H NMR (400 MHz, $CDCl_3$, lin = linolenyl): δ 5.42–5.28 (m, 6H, H–C(9), H–C(10) H–C(12), H–C(13), H–C(15) and H–C(16) lin) 4.14 (m, 1H, H–C(2)) 3.89 (m, 1H, H–C(3)) 3.73 (br s 3H, 2 xH–O and H-N) 3.57 (dd, ^2J = 9.7, ^3J = 4.3, 1H, *H*HC(5)) 3.52 (dd, ^2J = 9.7, ^3J = 5.3, 1H, H*H*C(5)) 3.45 (t, ^3J = 13.2, 2H, H_2C(1) lin) 3.23 (dd, ^2J = 12.0, ^3J = 4.9, 1H, *H*HC(1)) 3.18 (m, 1H, H–C(4)) 2.97 (bd, ^2J = 9.7, 1H, H*H*C(1)) 2.80 (m, 4H, H_2C(11) and H_2C(14) lin) 2.11–2.02 (m, 4H, H_2C(8) and H_2C(17) lin) 1.55 (m, 2H, H_2C(2) lin) 1.28 (br s 10H, C(2)-$(CH_2)_5$-C(8) lin) 0.97 (t, ^3J = 7.5, 3H, H_3C(18) lin). ^{13}C NMR (101 MHz, $CDCl_3$, lin = linolenyl): δ 131.9 (d, ^1J = 152, C(9) lin) 130.3 (d, ^1J = 152, C(10) lin) 128.2 (d, ^1J = 154, C(12) lin) 128.2 (d, ^1J = 154, C(13) lin) 127.7 (d, ^1J = 154, C(15) lin) 127.1 (d, ^1J = 155, C(16) lin) 74.1 (d, ^1J = 140, C(3)) 71.6 (t, ^1J = 139, C(1) lin) 71.4 (d, ^1J = 144, C(2)) 70.7 (t, ^1J = 134, C(5)) 62.1 (d, ^1J = 138, C(4)) 51.7 (t, ^1J = 140, C(1)) 29.6 (t, ^1J = 123, H_2C lin) 29.5 (t, ^1J = 123, H_2C lin) 29.5 (t, ^1J = 123, H_2C lin) 29.4 (t, ^1J = 123, H_2C lin) 29.2 (t, ^1J = 123, H_2C lin) 27.2 (t, ^1J = 124, H_2C lin) 26.1 (t, ^1J = 118, H_2C lin) 25.6 (t, ^1J = 117, H_2C lin) 25.5 (t, ^1J = 117, H_2C lin) 20.5 (t, ^1J = 121, H_2C lin) 14.2 (q, ^1J = 129, C(18) lin). HR-ESI-TOF-MS: calcd for $C_{23}H_{41}NO_3$: 380.3159, found 380.2331 ([M+H]$^+$).

4.3.9. 1,4-Dideoxy-5-*O*-benzyl-1,4-imino-D-ribitol 21

General procedure 1. TBI: 0.124 g, 0.37 mmol, 1.4 equiv, R-X: benzyl bromide (0.5 ml, excess), **12**: 0.074 g, 0.27 mmol, NaOH: 0.17 ml. Pure *N-tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-O-benzyl-1,4-imino-D-ribitol was obtained (0.066 g, 0.18 mmol, 68% yield) as a colourless oil after flash column chromatography on silica gel (petroleum ether/ ethyl acetate 9:1). Deprotection performed following the *general procedure* 2: protected **21**: 0.037 g, 0.10 mmol, TFA: 0.4 ml. Pure **21** (0.014 g, 0.065 mmol, 65% yield) was obtained as a white foam after flash column chromatography on silica gel CH_2Cl_2/MeOH 9:1, 1% NH_3 aq). $[\alpha]_D^{25}$ +57; $[\alpha]_{577}^{25}$ +52; $[\alpha]_{435}^{25}$ +94; $[\alpha]_{405}^{25}$ +109 (*c* = 0.0635, MeOH) IR (neat): 3465, 3279, 2948, 2919, 2900, 2871, 2564, 1088, 1020, 728. ^{1}H NMR (400 MHz, MeOH-d_4, bn = benzyl): δ 7.38–7.28 (m, 5H, H–C bn) 4.56 (m, 2H, H_2-C bn) 4.05 (m, 1H, H–C(2)) 3.84 (dd, ^3J = 7.0, ^3J = 5.4, 1H, H–C(3)) 3.66 (dd, ^2J = 9.7, ^3J = 3.6, 1H, *H*H–C(5)) 3.56 (dd, ^2J = 9.7, ^3J = 5.5, 1H, HH–C(5)) 3.20–3.12 (m, 2H, H–C(4) and *H*H–C(1)) 2.81 (dd, ^2J = 12.0, ^3J = 3.7, 1H, H*H*C(1)). ^{13}C NMR (101 MHz, MeOH-d_4, bn = benzyl): δ 139.6 (s, C bn) 129.4 (d, ^1J = 159, C bn) 128.9 (d, ^1J = 158, C bn) 128.7 (d, ^1J = 158, C bn) 74.6 (d, ^1J = 143, C(2)) 74.3 (t, ^1J = 141, H_2C bn) 72.5 (d, ^1J = 148, C(3)) 71.3 (t, ^1J = 139, H_2C(5)) 63.3 (d, ^1J = 138, C(4)) 52.3 (t, ^1J = 139, C(1)). HR-ESI-TOF-MS: calcd for $C_{12}H_{17}NO_3$: 224.1287, found 224.1280 ([M+H]$^+$).

4.3.10. 1,4-Dideoxy-5-*O*-(*p*-phenylbenzyl)-1,4-imino-D-ribitol 22

General procedure 1. TBI: 0.126 g, 0.34 mmol, 1.3 equiv, R-X: *p*-phenylbenzyl bromide (0.5 ml, excess), CH_3CN (0.4 ml), **12**: 0.070 g, 0.26 mmol, NaOH: 0.35 ml. Pure *N-tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-*O*-(*p*-phenylbenzyl)-1,4-imino-D-ribitol was obtained (0.080 g, 0.18 mmol, 71% yield) as a colourless oil after flash column chromatography on silica gel (petroleum ether/ ethyl acetate 5:1). Deprotection performed following the *general procedure* 2: protected **22**: 0.080 g, 0.18 mmol, TFA: 0.5 ml. Pure **22** (0.029 g, 0.1 mmol, 56% yield) was obtained as a white foam after flash column chromatography on silica gel CH_2Cl_2/MeOH 9:1, 1% NH_3 aq). $[\alpha]_D^{25}$ +31; $[\alpha]_{577}^{25}$ +12; $[\alpha]_{435}^{25}$ +27; $[\alpha]_{405}^{25}$ +33 (*c* = 0.0515, MeOH). IR (pure): 3437, 3309, 2920, 2881, 2693, 1488, 1452, 1092. ^{1}H NMR (400 MHz, $CDCl_3$, bPh = biphenyl): δ 7.58–7.30 (m, 9H, H–C bPh) 4.57 (m, 2H, H_2C bPh) 4.12 (m, 1H, H–C(2)) 3.91 (m, 1H, H–C(3)) 3.63 (m, 2H, H_2-C(5)) 3.25–3.18 (m, 4H, H–C(4), *H*H–C(1) and 2× H–O)) 2.92 (bd, ^2J =10.0, 1H, HH–C(1)). ^{13}C NMR (101 MHz, $CDCl_3$, bPh = biphenyl): δ 140.7 (s, C(4) bPh) 140.7 (s, C(1') bPh) 136.9 (s, C(1) bPh) 128.8 (d, ^1J = 160, CH bPh) 128.2 (d, ^1J = 162, CH bPh) 127.3 (d, ^1J = 162, C(4') bPh) 127.2 (d, ^1J = 162, CH bPh) 127.1 (d, ^1J = 162, CH bPh) 74.3 (d, ^1J = 148, C(2)) 73.1 (t, ^1J = 141, H_2C-bPh) 71.5 (d, ^1J = 152, C(3)) 70.9 (t, ^1J = 143, C(5)) 62.3 (d, ^1J = 137, C(4)) 52.0 (t, ^1J = 138, C(1)). HR-ESI-TOF-MS: calcd for $C_{18}H_{21}NO_3$: 300.1600 found 300.1600 ([M+H]$^+$).

4.3.11. 1,4-Dideoxy-5-*O*-pentyl-1,4-imino-D-ribitol 23

N-tert-Butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-O-(pent-5en-1-yl)-1,4-imino-D-ribitol (0.051 g, 0.15 mmol) (see preparation of **14**) was dissolved in methanol (1.5 ml) and the solution was degassed with argon. Palladium hydroxide on activated charcoal (0.08 g, 10% mol) was added and the solution was stirred under hydrogen atmosphere at 25 °C for 1 h (reaction monitored by ^{1}H NMR). The suspension was filtered on a Celite pad (eluent: ethyl acetate) in order to eliminate the catalyst, and the solvent was evaporated in vacuo, giving *N-tert*-butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-*O*-pentyl-1,4-imino-D-ribitol quantitatively. This product was dissolved in cold trifluoroacetic acid (0.5 ml) and the solution was stirred at 0 °C for 10 min. Water (0.07 ml) was added and the solution was stirred at 0 °C for 30 min, then at 25 °C for 1 h (reaction monitored by TLC, petroleum ether/ethyl acetate 3:1 and CH_2Cl_2/CH_3OH 9:1 (1% aqueous ammonia)). The solvent was evaporated in vacuo and the product was dissolved in CH_2Cl_2 and neutralized with ammonia. After solvent evaporation in vacuo, the product was purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH 88.5:11.5 (1% aqueous ammonia)). Pure **23** was obtained (0.024 g, 0.13 mmol, 82% yield) as white foam.

$[\alpha]_D^{25}$ +40; $[\alpha]_{577}^{25}$ +43; $[\alpha]_{435}^{25}$ +75; $[\alpha]_{405}^{25}$ +90 (*c* = 0.067, MeOH). IR (pure): 3503, 3269, 2931, 2870, 2812, 1080, 990, 873, 658. ^{1}H NMR (400 MHz, MeOH-d_4, pen = pentyl): δ 4.05 (m, 1H, H–C(2)) 3.80 (dd, ^3J = 7.0, ^3J = 5.4, 1H, H–C(3)) 3.59 (dd, ^2J = 9.8, ^3J = 3.6, 1H, *H*H–C(5)) 3.46–3.52 (m, 3H, H_2-C(1)pen and H*H*–C(5)) 3.17 (dd, ^2J = 12.0, ^3J = 5.4, 1H, *H*H-C(1)) 3.09 (m, 1H, H-C(4)) 2.81 (dd, ^2J = 12.0, ^3J = 3.9, 1H, H*H*-C(1)) 1.59 (m, 2H, H_2-C(2) pen) 1.30–1.38 (m, 4H, H_2-C(3) and H_2-C(4) pen) 0.93 (t, ^3J = 7.0, 3H, H_3-C(5) pen). ^{13}C NMR (101 MHz, MeOH-d_4, pen = pentyl): δ 74.6 (d, ^1J = 144, C(2)) 72.5 (d, ^1J = 148, C(3)) 72.5 (t, ^1J = 148, C(5)) 71.7 (t, ^1J = 149, C(1)pen) 63.3 (d, ^1J = 139, C(4)) 52.4 (t, ^1J = 138, C(1)) 30.4 (t, ^1J = 123, C(2)pen) 29.5 (t, ^1J = 125, C(3) pen) 23.6 (t, ^1J = 126, C(14) pen) 14.4 (q, ^1J = 124, C(5) pen). HR-ESI-MS: calcd for $C_{10}H_{21}NO_3$: 204.1594, found 204.1601 ([M+H]$^+$).

4.3.12. 1,4-Dideoxy-5-*O*-octadecyl-1,4-imino-D-ribitol 24

N-tert-Butoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-5-O-[(9*Z*)-octadec-9-en-1-yl]-1,4-imino-D-ribitol (0.043 g, 0.08 mmol) (see preparation of **13**) was dissolved in methanol (1 ml) and the solution was degassed with argon. Palladium hydroxide on activated charcoal (0.007 g, 12% mol) was added and the solution was stirred under hydrogen at 25 °C for 1 h (reaction monitored

by ^{1}H NMR). The suspension was filtered on a Celite pad (eluent: ethyl acetate) in order to eliminate the catalyst, and the solvent was evaporated in vacuo, obtaining *N-tert*-butoxycarbonyl-1, 4-dideoxy-2,3-*O*-isopropylidene-5-*O*-octadecyl-1,4-imino-D-ribitol quantitatively. This product was dissolved in cold trifluoroacetic acid (0.32 ml) and the solution was stirred at 0 °C for 10 min. Water (0.08 ml) was added and the solution was stirred at 0 °C for 30 min, then at 25 °C for 1 h (reaction monitored by TLC, petroleum ether/ethyl acetate 7:1 and CH_2Cl_2/CH_3OH 9:1 (1% aqueous ammonia)). The solvent was evaporated in vacuo and the product was dissolved in CH_2Cl_2 and neutralized with ammonia. After solvent evaporation in vacuo, the product was purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH 9:1 (1% aqueous ammonia)). Pure 1,4-dideoxy-5-*O*-octadecyl-1,4-imino-D-ribitol **24** (0.022 mg, 0.06 mmol, 75% yield) was obtained as white foam.

$[\alpha]_D^{25}$ +20; $[\alpha]_{577}^{25}$ +56; $[\alpha]_{435}^{25}$ +70; $[\alpha]_{405}^{25}$ +80 (c = 0.087, MeOH). IR (pure): 3294, 2917, 2848, 1470, 1118, 1103, 717. ^{1}H NMR (400 MHz, Me OH-d_4, od = octadecyl): δ 4.06 (m, 1H, H-C(2)) 3.84 (dd, ^2J = 7.2, ^3J = 5.0, 1H, H–C(3)) 3.56 (dd, ^2J = 10.1, ^3J = 3.5, 1H, H*H*–C(5)) 3.52–3.41 (m, 3H, *H*HC(1) od, H–C(4) and *H*H–C(5)) 3.20–3.14 (m, 2H, H*H*C(1) and H*H*C(1) od) 2.88 (dd, ^2J = 12.1, ^3J = 3.4, 1H, *H*HC(1)) 1.55 (m, 2H, H_2C(2) od) 1.26 (br s 30 H, C(2)-$(CH_2)_{15}$-C(18) od) 0.87 (t, ^3J = 6.7, 3H, H_3C(18) od). ^{13}C NMR (101 MHz, MeOH-d_4, od = octadecyl): δ 74.3 (C(3)) 72.6 (C(1) od) 72.2 (C(2)) 70.9 (H_2C(5)) 63.1 (C(4)) 52.1 (C(1)) 33.1 (CH_2 od) 30.8 ((CH_2)$_{10}$ od) 30.7 (CH_2 od) 30.6 (CH_2 od) 30.5 (CH_2 od) 27.3 (CH_2 od) 23.7 (CH_2 od) 14.4 (C(14) od). HR-ESI-TOF-MS: calcd for $C_{23}H_{47}NO_3$: 386.3629, found: 386.3492 ([M+H]$^+$).

Acknowledgments

This work has been generously supported by the Associazione Italiana per la ricerca sul cancro (AIRC) by the Swiss National Science Foundation and the European FP7 – HEALTH-2010-2-4-1-6 (PANACREAS) programme. CB was supported by an EPFL Innogrant; AN was supported by FBM Interdisciplinary Research Grant and Fondation Dr H Dubois-Ferrière Dinu Lipattic

A. Supplementary data

Supplementary data (viability assays experimental protocols) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.07.053.

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