Synthesis and cancer growth inhibitory activities of 2-fatty-alkylated pyrrolidine-3,4-diol derivatives

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Dedicated to Prof. Pierre Vogel on the occasion of his 70th anniversary

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Abstract
A series of new amphiphilic pyrrolidines containing dodecyl and oleyl apolar side chains were prepared and evaluated for their ability to inhibit the growth of pancreatic ductal adenocarcinoma cells in vitro. The new compounds are shown to exhibit anticancer activity at concentrations in the μM range.

Keywords: Amphiphilic compounds, pyrrolidines, iminoalditols, pancreatic cancer

Introduction
In recent years, several compounds with amphiphilic structure have been proposed as a new type of potential anticancer agents due to the lipophilic nature of their lipid chains. Series of such compounds isolated from natural sources have indeed shown promising anticancer activities. For instance, Ircinamine B, isolated from marine sponge Dactylia,1 and Melophlins Q2 isolated from Palauan marine sponge are active against mouse leukemia cell lines in the μM range. Alkylglycerols3 from shark liver also show anticancer activity in vivo in the μM range with no toxicity on human umbilical vein endothelial cells, the best compounds bearing unsaturated 16 and 18 carbon alkyl chains. Motuporamine C from a marine Sponge,4 interferes with the migration of human breast carcinoma and glioma cells in culture with low toxicity in vitro. Sphingosine 4-sulfate5 from the sponge Spirastrella abata shows cytotoxic effects on several human tumor cell lines. The glycosyl ceramide, Turbostatin 1,2,3,4,6 from Asian marine mollusk Turbo stenogyrus, is active against leukemia cells in the low μM range.
Notably, synthetic amphiphilic compounds have also shown interesting anticancer properties. As shown by Gajate and Mollinedo, edelfosine (Figure 1), a lipid analogue of lysophosphatidyl-choline induces apoptosis in several tumor cell lines through its lipid ether moiety. Once edelfosine is inside the cell, it induces selective apoptosis in cancer cells through the activation of the Fas death receptor located on the surface of cells without the participation of the corresponding Fas ligand.

Figure 1

Other compounds such as Jaspines, having also amphiphilic character by combining a polar head (amino and hydroxy moieties) with aliphatic long chains, have shown good anticancer activities as well. Among the different diastereoisomeric Jaspines evaluated against human alveolar cell lines, Jaspine B (Figure 1) was the most active, showing cytotoxicity at nanomolar concentrations in in vitro assays.

Due to their amphiphilic nature, glycolipids and their analogues also show therapeutic potential. Numerous glycolipid derivatives have been proposed as antibacterial/antiviral agents, cell adhesion mediators, and oral drug carriers. In the past few years, glycolipids have also been proposed as new potential anticancer agents due to the lipophilic nature of their aglycones. A variety of synthetic glycolipids have been prepared and their anticancer properties evaluated. Thus, oleyl N-acetyl-α- and β-D-glucosaminides and their thioglycosyl analogues 1a and 1b (Figure 2) exhibited antimitotic activity on rat glioma (C6) and human lung carcinoma (A549) cell cultures in the μM range. Triazologlycolipids 2 showed cytotoxicity against a panel of cancer cell lines in the μM range.

Figure 2

Derivatization of iminosugars with aliphatic side chains has been extensively studied in order to improve their biological activity, lipophilicity and bioavailability. For instance N-nonyl-1-
deoxynojirimycin inhibits hepatitis B virus in cell based assays.\textsuperscript{20} \(\alpha\)-1-C-Alkyl-1-deoxynojirimycin derivatives have shown inhibitory activity toward intestinal isomaltase and their potency was highly dependent on the alkyl chain length.\textsuperscript{21} \(N\)-alkylated-D-fagomine derivatives \textsuperscript{3} and \(N\)-alkylated hydroxylated pyrrolidine \textsuperscript{4} (Figure 3) bearing a long alkyl chain, not only presented an improved inhibitory selectivity towards \(\alpha\)-D-glucosidase and \(\alpha\)-L-fucosidase, respectively, but also exhibited enhanced cytotoxic activities on a panel of cancer cell lines compared to their non-alkylated progenitors.\textsuperscript{22} Other \(N\)-alkylated pyrrolidine derivatives such as \textsuperscript{5}\textsuperscript{23} and \textsuperscript{6}\textsuperscript{24,25} presented interesting inhibition against \(N\)-acetylglucosaminidases and \(\alpha\)-mannosidases, respectively.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{5-O-Alkyl 1,4-dideoxy-1,4-imino-D-ribitols bearing different long alkyl chains 7-12 (Figure 3) were evaluated as enzyme inhibitors and for their toxicity on solid and hematological malignancies showing activities in the \(\mu\)M range.\textsuperscript{26,27} Even though these compounds are not inhibitors of \(\alpha\)-mannosidases, they show promising cytotoxicity on solid and hematological malignancies, their activity depending on the length of the alkyl side chain. The oleyl derivative 9 and its C-18 analogues, linoleyl 10, linolenyl 11 and octadecyl 12, exhibited the most potent anti-cancer activity, with oleyl-conjugate 9 being the most efficient in killing tumor cells. These results indicated that the side chain and the number and position of the unsaturations play a role in determining the biological properties of this class of compounds. The anticancer activities increased with the length of the alkyl chain, in accordance with the reported data for other iminosugars\textsuperscript{22} and with the hypothesis that a higher lipophilicity would facilitate compound transport through the cell membrane.

Compound 9 was chosen as representative of the new class of compounds because of its interesting anticancer activities against breast, lung, prostate cancer, and glioblastoma cells in the \(\mu\)M range (IC\textsubscript{50} between 2 and 21 \(\mu\)M).\textsuperscript{26,27}
Results and Discussion

The promising results shown by compound 9 and derivatives, prompted us to explore further structural variations in order to improve their anticancer properties, in particular in pancreatic cancer cell lines. Thus, different series of compounds bearing amphiphilic character were prepared for structure-activity relationship studies by combining polyhydroxylated pyrrolidines with 2-fatty-alkyl moieties.

We report herein the synthesis of a series of this type of compounds starting from different sugars as source of chirality and the results of their evaluation in terms of toxicity in pancreatic cancer cell lines. The structural variations of the new compounds are shown in Figure 4. The diversity at the new structures will allow us to examine the role of different configurations at the stereogenic centers, the type of linker (X), the distance between the side chain and the pyrrolidine skeleton and the role of different functionalities at the nitrogen atom on their anticancer activity.

Thus, compound 19 was prepared from D-ribose as outlined in Scheme 1. Starting from the known D-ribose derivative 13,28 reaction with ethyl triphenylphosphoranylidene acetate in dichloromethane at reflux afforded a mixture of alkenes 14 and 15 in 86% yield and a ratio E:Z 1:3.6. The stereoselectivity in the Wittig reaction was in accordance with that described in the literature for other ribo-lactols.29 Mesylation of the free OH group in both alkenes followed by treatment with ammonia in ethanol led to pyrrolidine 16 as unique product (94% yield), through tandem conjugate addition-internal SN2 displacement. Reductive amination with butanal, ester hydrolysis and amide coupling with oleyl amine in the presence of PyBOP and DIPEA afforded compound 18 in 30% yield (two last steps). Deprotection with TBAF led to pyrrolidine 19 in quantitative yield. Attempts to deprotect 19 with cold TFA aq. were unsuccessful. The preparation of other acetonide protected pyrrolidines with the same configuration have been reported.30
The effect of a triazole linker between the pyrrolidine skeleton and the side chain was also studied. These compounds were prepared by click chemistry upon reaction of the corresponding pyrrolidine azides with tetradec-1-yne.

**Scheme 1.** Synthesis of 19.

Differently configurated pyrrolidine azides were obtained from D-mannose, D-gulono-γ-lactone and D-arabinose (Figure 5).

**Figure 5.** Retrosynthesis for triazole-pyrrolidine compounds.

Differently configured pyrrolidine azides were obtained from D-mannose, D-gulono-γ-lactone and D-arabinose (Figure 5).

The preparation of the azido-pyrrolidines is outlined in Scheme 2. Starting from known aldehyde 20,31 reduction and tosylation gave derivative 21, which after displacement with
sodium azide in DMF furnished azido derivative 22. Similarly, derivative 25 was obtained by azido displacement of the corresponding tosyl derivative 24 via azido displacement of the corresponding tosyl derivative 24, obtained through oxidation, reduction and tosylation of known diol 23. Derivative 28 was prepared from pyrrolidine ester 26 by Cbz protection and reduction with LiAlH₄ followed by mesylation and azido displacement.

Scheme 2. Synthesis of azido-pyrrolidines.

The preparation of these azido derivatives requires benzyloxycarbonyl as N protecting group. The use of other carbamates as protecting groups for the preparation of the azido pyrrolidines gave unexpected results. Thus, tosylation of Boc-protected alcohol 29 did not lead to the corresponding tosyl derivative as expected. Instead, we mostly obtained cyclic carbamate 30 (Scheme 3) as confirmed by its spectral data. The ¹H- and ¹³C NMR spectra of 30 did not show the typical signals for the tosyl and tert-butyl groups. However, a signal at 152.9 ppm was observed in the ¹³C NMR spectra, corresponding to the carbonyl group of the carbamate.

Scheme 3. Participation of the N-protecting group.
The formation of cyclic carbamate 30 depends on the protecting group used in the introduction of the azide group at C-1' of the pyrrolidine. Boc protecting group as well as Fmoc exhibit a hydrogen atom at the β position with respect to the oxygen atom, which is not the case in the Cbz group. Under mild basic conditions, the H at β position is released with the concomitant displacement of the leaving group, promoting the formation of the cyclic carbamate, as outlined in Scheme 4.

**Scheme 4.** Participation of carbamates on leaving group departure.

Compounds bearing a triazole moiety were prepared by copper-catalyzed dipolar cycloaddition of pyrrolidine azides (22, 25 and 28) and tetradec-1-yne in the presence of CuI and DIPEA. Subsequent deprotection reactions led to derivatives 31, 32 and 33 in good overall yields, and their structures were confirmed by NMR and HRMS spectra (Scheme 5).

**Scheme 5.** Preparation of triazole derivatives.
Biological evaluation. The cytotoxic activity of the new amphiphilic derivatives has been evaluated on the pancreatic cancer cell lines MiaPaCa2 and PK9. The results are summarized in Table 1. They all exhibited antiproliferative activity in the μM range.

Although undoubtedly much research needs to be done in this area, the results shown here indicate that the amphiphilic compounds combining unsaturated alkyl chains and iminoalditols of defined configurations might be of interest for the definition of new anticancer agents to be used in the treatment of pancreatic cancer.

Table 1. IC_{50} of analogues of 9 toward pancreatic cancer cell lines. IC_{50} (μM)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>19</th>
<th>31</th>
<th>32</th>
<th>33</th>
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<tbody>
<tr>
<td>MiaPaCa2</td>
<td>3.6</td>
<td>21</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td>PK9</td>
<td>17</td>
<td>19</td>
<td>45</td>
<td>16</td>
</tr>
</tbody>
</table>

Experimental Section

General. Optical rotations were measured in a 1.0 cm or 1.0 dm tube with a Perkin–Elmer 241MC spectropolarimeter. \(^1\)H and \(^13\)C NMR spectra were obtained for solutions in CDCl\(_3\), [d\(_6\)]DMSO and CD\(_3\)OD; \(J\) values are given in Hz and \(\delta\) in ppm. All the assignments were confirmed by two-dimensional NMR experiments (COSY and HSQC). The LSIMS mass spectra were obtained using glycerol or 3-nitrobenzyl alcohol as the matrix. NMR and Mass spectra were registered in CITIUS (University of Seville). TLC was performed over silica gel HF\(_{254}\) (Merck), with detection by UV light charring with ninhydrin or with Pancaldi reagent [(NH\(_4\))\(_6\)MoO\(_4\), Ce(SO\(_4\))\(_2\), H\(_2\)SO\(_4\), H\(_2\)O]. Silica gel 60 (Merck, 63-200 μm) was used for preparative chromatography.

\((E)-\) and \((Z)-\)Ethyl 7-O-tert-butyldiphenylsilyl-2,3-dideoxy-4,5-O-isopropylidene-D-ribohept-2-enoate (14 and 15). Ethoxycarbonyltriphenylmethyleneephosphorane (7 g, 20.1 mmol) was added to a solution of 13^28 (3.75 g, 8.76 mmol) in dry CH\(_2\)Cl\(_2\) (80 mL) and the mixture was heated at reflux for 6 h. After evaporation of the solvent, the residue was purified by column chromatography (ethyl acetate/petroleum ether 1:10) to afford 14 and 15 as oils (3.74 g, 86%, 14:15 = 1:3.6). Data for 14: [\(\alpha\)]\(_D\)^29 +9.2 (c 1.24, CH\(_2\)Cl\(_2\)); IR (ν cm\(^{-1}\)) 3462, 2931, 2857, 1718, 1657, 1428, 1110, 1059, 822, 740, 701, 614. \(^1\)H NMR (300 MHz, CDCl\(_3\), \(\delta\) ppm, \(J\) Hz) \(\delta\) 7.69-7.64 (m, 4H, H-arom.), 7.46-7.36 (m, 6H, H-arom.), 7.12 (dd, 1H, \(J_{3,2} 15.6, J_{3,4} 4.9, H-3\)), 6.14 (dd, 1H, \(J_{2,4} 1.6, H-2\)), 4.86 (m, 1H, H-4), 4.25-4.18 (m, 3H, H-5, C\(_2\)H\(_2\)CH\(_3\)), 3.84 (dd, 1H, \(J_{2,4} 1.6, H-2\)), 4.86 (m, 1H, H-4), 4.25-4.18 (m, 3H, H-5, C\(_2\)H\(_2\)CH\(_3\)), 3.84 (dd, 1H, \(J_{7a,7b} 10.4, J_{7a,5} 3.3, H-7a\)), 3.76 (dd, 1H, \(J_{7b,6} 5.5, H-7b\)), 3.63 (m, 1H, H-6), 2.58 (d, 1H, \(J_{OH,6} 5.7, OH\)), 1.41, 1.35 (2s, 3H each, C(CH\(_3\))\(_2\)). \(^13\)C NMR (75.4 MHz, CDCl\(_3\), \(\delta\) ppm) \(\delta\) 166.2 (COOC\(_2\)), 143.8 (C-3), 135.6, 132.8, 129.8, 127.7 (C-arom.), 122.3 (C-2), 109.4 (C(CH\(_3\))\(_2\)), 77.4 (C-5), 76.8 (C-4), 69.8 (C-6), 65.2 (C-7), 60.4
(CH₂CH₃), 27.6 (C(CH₃)₂), 26.8 (C(CH₃)₂), 25.3 (C(CH₃)₂), 19.3 (C(CH₃)₃), 14.2 (CH₂CH₃). CIMS m/z 499 [1%, (M+H)+]. HRCIMS m/z found 499.2512, calc. for C₂₈H₃₉O₆Si: 499.2516.

Data for 15: [α]D₂⁰ +76.2 (c 1.05, CH₂Cl₂); IR (ν cm⁻¹) 2931, 2857, 1716, 1698, 1427, 1380, 1190, 1055, 869, 822, 701, 615. ¹H NMR (300 MHz, CDCl₃, δ ppm, J Hz) 7.68-7.66 (m, 4H, H-arom.), 7.45-7.35 (m, 6H, H-arom.), 6.23 (dd, 1H, J₃,₂ 11.7, J₅,₄ 8.4, H-3), 5.94 (dd, 1H, J₂,₄ 1.1, H-2), 5.74 (m, 1H, H-4), 4.38 (dd, 1H, J₅,₄ 8.1, J₅,₆ 6.3, H-5), 4.19 (q, 2H, J₃,₅ 6.9, CH₂CH₃), 3.84-3.75 (m, 2H, H-7a, H-7b), 3.66 (m, 1H, H-6), 2.80 (d, 1H, J₆,₇ 4.5, OH), 1.38, 1.35 (2s, 3H each, C(CH₃)₂), 1.30 (t, 3H, CH₂CH₃), 1.06 (s, 9H, C(CH₃)₃). ¹³C NMR (75.4 MHz, CDCl₃, δ ppm) 166.1 (C OOEt), 144.6 (C-3), 135.6, 133.2, 129.7, 127.7 (C-arom.), 122.2 (C-2), 109.1 (C(CH₃)₂), 73.9 (C-4), 70.3 (C-6), 65.1 (C-7), 60.6 (CH₂CH₃), 27.8 (C(CH₃)₂), 26.8 (C(CH₃)₂), 25.4 (C(CH₃)₂), 19.3 (C(CH₃)₃), 14.2 (CH₂CH₃). CIMS m/z 499 [1%, (M+H)+]. HRCIMS m/z found 499.2498, calc. for C₂₈H₃₉O₆Si: 499.2516.

Ethyl 7-O-tert-butyldiphenylsilyl-2,3,6-trideoxy-3,6-imino-4,5-O-isopropylidene-L-galacto-heptanoate ((2S,3R,4S,5R)-2-tert-butyldiphenylsilyloxyethyl-5-ethoxycarbonylmethyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol) (16). A solution of 14 and 15 (2.22 g, 4.45 mmol) in dry CH₂Cl₂ (10 mL) was added dropwise to a stirred solution of MsCl (1.2 mL, 16.0 mmol) in dry pyridine (4 mL) cooled to 0 ºC. After stirring at r.t. overnight, the mixture was cooled to 0 ºC, H₂O (5 mL) was added and the reaction stirred for 15 min at r.t. The solvent was then evaporated, the crude diluted with dichloromethane and washed with H₂O and brine. The organic phase was dried, filtered and concentrated. The residue was dissolved in absolute EtOH (45 mL), cooled to 0 ºC and saturated with NH₃. After 4 days at r.t., the solvent was evaporated and the residue was treated with NH₄OH (25%, 30 mL) and extracted with CH₂Cl₂ (5x30 mL). The organic phase was washed with satd. aq. sol. of NaHCO₃ (30 mL) and H₂O until neutral pH, dried (Na₂SO₄), filtered and concentrated. The residue was purified by column chromatography (AcOEt:petroleum ether (1:6), Et₃N (1%)) to give pure 16 as an oil (2.09 g, 94%). 

(2S,3R,4S,5R)-N-Butyl-2-tert-butyldiphenylsilyloxyethyl-5-ethoxycarbonylmethyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol (17). Butanal (0.3 mL, 3.38 mmol) and NaBH(OAc)₃ (752 mg, 3.55 mmol) were added to a solution of 16 (840 mg, 1.69 mmol) in 1,2-dichloroethane (10 mL). The reaction mixture was stirred overnight at r.t. Then, aq. sat. sol. of NaHCO₃ was added.
and the mixture extracted with CH$_2$Cl$_2$. The organic phases were dried (Na$_2$SO$_4$) and evaporated in vacuo. Purification of the residue by column chromatography (AcOEt:petroleum ether 1:10) afforded 17 as an oil (825 mg, 88%). [$\alpha$]$^D$_{29}$$^0$ +1.9 (c 1.15, CH$_2$Cl$_2$); IR (v cm$^{-1}$) 2931, 2857, 1732, 1110, 1087, 823, 738, 701, 613. $^1$H NMR (300 MHz, CDC$_3$, $\delta$ ppm, J Hz) $\delta$ 7.73-7.67 (m, 4H, H-arom.), 7.42-7.35 (m, 6H, H-arom.), 4.62-4.60 (m, 2H, H-4, H-3), 4.15 (qd, 2H, 3 $^3$J$_{H,H}$ 7.1, J 1.4, OCH$_2$CH$_3$), 3.98 (dd, 1H, J$_{1''a,1'b}$ 10.2, J$_{1'a,2}$ 6.8, H-1'a), 3.75 (dd, 1H, J$_{1'b,2}$ 5.4, H-1'b), 2.85 (m, 1H, H-5), 2.72-2.63 (m, 2H, H-2, H-1''a), 2.61-2.40 (m, 3H, H-1''b, CH$_2$N), 1.39, 1.29 (2s, 3H each, C(CH$_3$)$_2$), 1.25 (t, 3H, OCH$_2$CH$_3$), 1.24-1.09 (m, 4H, C(H$_2$C$_2$)), 1.05 (s, 9H, C(CH$_3$)$_3$), 0.83 (t, 3H, $^3$J$_{H,H}$ 7.2, CH$_3$). $^{13}$C NMR (75.4 MHz, CDCl$_3$, $\delta$ ppm) $\delta$ 172.4 (C=OEt), 135.7, 135.6, 133.9, 133.6, 129.5, 127.6, 127.5 (C-arom.), 110.8 (C(CH$_3$)$_2$), 79.4, 79.1 (C-4, C-3), 67.7 (C-2), 62.7, 62.5 (C-1', C-5), 60.3 (OCH$_2$CH$_3$), 49.5 (C-1'''), 33.2 (CH$_2$-N), 27.1 (CH$_2$), 26.8 (C(CH$_3$)$_3$), 26.0, 25.7 (C(CH$_3$)$_3$), 20.7 (CH$_3$), 19.2 (C(CH$_3$)$_3$), 14.2, 14.0 (OCH$_2$CH$_3$, CH$_2$CH$_3$). CIMS m/z 554 [15%, (M+H)$^+$]. HRCIMS m/z found 554.3283, calc. for C$_{32}$H$_{48}$NO$_5$Si: 554.3302.

(2S,3R,4S,5R)-N-Butyl-2-tert-butyldiphenylsilyloxyethyl-3,4-O-isopropylidene-5-oleylcarbamoylmethyl pyrrolidine-3,4-diol (18). A solution of 17 (119 mg, 0.23 mmol) in EtOH:1M NaOH (1:1, 3 mL) was stirred for 2 h at r.t. Then, the mixture was neutralized with IRA-120 (H$^+$) resin, filtered and concentrated. The residue thus obtained was dissolved in DMF (2 mL) and oleyl amine (67 mg, 0.25 mmol), DIPEA (118 $\mu$L, 0.69 mmol) and PyBOP (130 mg, 0.25 mmol) were added. After stirring at r.t. overnight, the mixture was evaporated to dryness and the residue was dissolved in CH$_2$Cl$_2$ and washed with 1M HCl, sat. aq. soln. of NaHCO$_3$ and sat. aq. soln. of NaCl. The dried organic phase (MgSO$_4$) was purified by chromatography over silica gel (AcOEt:petroleum ether 1:3) to give 18 as an oil (53 mg, 30%). [$\alpha$]$^D$_{29}$$^0$ -1.7 (c 1.05, CH$_2$Cl$_2$); IR (v cm$^{-1}$) 2921, 2852, 1643, 1110, 1092, 823, 738, 701, 614. $^1$H NMR (300 MHz, CDCl$_3$, $\delta$ ppm, J Hz) $\delta$ 7.73-7.67 (m, 4H, H-arom.), 7.42-7.35 (m, 6H, H-arom.), 6.56 (br t, 1H, NH), 5.38-5.32 (m, 2H, CH$_2$=CH), 4.62 (dd, 1H, J 6.5, J 4.8, H-3), 4.50 (br dd, 1H, H-4), 3.76 (dd, 1H, J$_{1'a,2}$ 5.0, H-1'b), 3.20 (q, 2H, J$_{H,H}$ 6.4, CH$_2$-NH), 2.83 (m, 1H, H-5), 2.70 (m, 1H, H-2), 2.63-2.35 (m, 4H, H-1''a, H-1''b, CH$_2$N), 2.02-1.98 (m, 4H, CH$_2$-CH=CH), 1.39 (s, 3H, C(CH$_3$)$_2$), 1.48-1.09 (m, 31H, 14C(H$_2$)), 1.05 (s, 9H, C(CH$_3$)$_3$), 0.88 (t, 3H, J$_{H,H}$ 6.3, CH$_3$), 0.83 (t, 3H, J$_{H,H}$ 7.2, CH$_3$). $^{13}$C NMR (75.4 MHz, CDCl$_3$, $\delta$ ppm) $\delta$ 171.3 (C=O), 135.7, 135.5, 133.7, 133.5 (C-arom.), 129.9, 129.7 (CH=CH), 129.6, 129.5, 127.6, 127.5 (C-arom.), 110.7 (C(CH$_3$)$_3$), 79.2 (C-4), 79.0 (C-3), 67.6 (C-2), 63.4 (C-5), 62.5 (C-1'), 49.4 (C-1''), 35.2 (CH$_2$-N), 32.6 (CH$_2$), 31.9 (CH$_2$), 29.7-29.2 (CH$_2$), 27.2, 27.1 (CH$_2$), 26.7 (C(CH$_3$)$_3$), 25.7, 25.0 (C(CH$_3$)$_3$), 22.6 (CH$_2$), 20.6 (CH$_2$), 19.1 (C(CH$_3$)$_3$), 14.1, 14.0 (2CH$_3$). CIMS m/z 775 [100%, (M+H)$^+$]. HRCIMS m/z found 775.5804, calc. for C$_{48}$H$_{79}$N$_2$O$_4$Si: 775.5809.

(2S,3R,4S,5R)-N-Butyl-2-hydroxymethyl-3,4-O-isopropylidene-5-oleylcarbamoylmethyl pyrrolidine-3,4-diol (19). TBAF (1 M in THF, 0.25 mL) was added to a solution of 18 (59.4 mg, 0.071 mmol) in THF (1 mL). After stirring at r.t. for 5 h, the solvent was evaporated and the residue purified by chromatography column over silica gel (ether/acetone 50:1) to afford 19 as
an oil (38 mg, quant.). $[\alpha]_{D}^{28}$ -10.6 (c 1.04, CH$_2$Cl$_2$); IR (ν cm$^{-1}$) 3306 (OH, NH), 2924, 2855, 1648 (C=O), 1458, 1206, 1043, 713. $^1$H NMR (300 MHz, CDCl$_3$, δ ppm, J Hz) δ 6.54 (br t, 1H, NH), 5.39-5.32 (m, 2H, CH$_2$=CCH$_2$); IR (ν/cm$^{-1}$) 3306 (OH, NH), 2924, 2855, 1648 (C=O), 1458, 1206, 1043, 713.

$^1$H NMR (300 MHz, CDCl$_3$, δ ppm, J Hz) 6.54 (br t, 1H, N), 5.39-5.32 (m, 2H, C$_{13}$H$_{12}$), 4.69 (dd, 1H, J 5.1, J 6.3, H-3 or H-4), 4.58 (dd, 1H, J 5.1, J 6.0, H-3 or H-4), 3.94 (dd, 1H, $^2$J$_{1'a,1'b}$ 11.7, $^2$J$_{1'a,2}$ 2.5, H-1'a), 3.82 (dd, 1H, J$^1$ 5.8, H-1'b), 3.25-3.18 (m, 2H, C$_3$H$_2$-NH), 3.02 (br s, 1H, H-5), 2.68-2.45 (m, 5H, H-2, H-1''a, H-1''b, C$_3$H$_2$-N), 2.07-1.91 (m, 4H, C$_3$H$_2$-CH=CH-C$_3$H$_2$), 1.49 (s, 3H, C(C$_3$H$_3$)$_2$), 1.36-1.22 (m, 36H, 14C$_3$H$_2$), 0.94-0.83 (m, 6H, 2 C$_3$H$_3$). 13C NMR (75.4 MHz, CDCl$_3$, δ ppm) 171.0 (C=O), 130.0, 129.7 (C$_6$H$_5$), 111.2 (C$_6$H$_5$), 80.3, 79.4 (C-3, C-4), 65.7 (C-2), 62.7 (C-5), 59.9 (C-1'), 48.8 (C-1''), 39.4 (C$_3$H$_2$-NH), 31.9 (C$_3$H$_2$-N), 29.7-20.7 (16 C$_3$H$_2$, C(C$_3$H$_3$)$_2$), 14.1, 13.9 (2 C$_3$H$_3$).

CIMS m/z 537 [100%, (M+H)$_+^\dagger$]. HRCIMS m/z found 537.4637, calc. for C$_{32}$H$_{61}$N$_2$O$_4$: 537.4631.

$^{(2S,3S,4R,5S)}$-N-Benzylxycarbonyl-2-tosyloxymethyl-3,4-O-isopropylidene-5-methylpyrrolidine-3,4-diol (21).

NaBH$_4$ (0.43 g, 4.75 mmol) was added to a solution of 20 (735 mg, 2.30 mmol) in MeOH (11 mL), and the mixture stirred at r.t for 20 min. Then, AcOH (0.1 mL) was added and the mixture subsequently diluted with AcOEt and washed with sat. aq. soln. of NaHCO$_3$. The organic phases were dried, filtered and concentrated. The residue was purified by chromatography column over silica gel (AcOEt:petroleum ether 1:2) to afford the corresponding alcohol (0.63 g, 85%).

To a 0 ºC solution of the alcohol (383 mg, 1.19 mmol) in dry pyridine (10 mL) was slowly added TsCl (0.69 g, 3.56 mmol). After stirring at r.t for 4.5 h, the mixture was cooled to 0 ºC, water was slowly added (0.5 mL), and the mixture was allowed to warm to r.t. Solvent was then removed, and the residue was diluted with AcOEt, washed with HCl (1N), sat. aq. soln. of NaHCO$_3$ and brine, dried, filtered, and concentrated. Purification by chromatography column (AcOEt:cyclohexane 1:4) afforded 21 as an oil (414 mg, 73%). $[\alpha]_{D}^{28}$ + 18.2 (c 0.51, CH$_2$Cl$_2$). IR ν$_{max}$ 2986, 2938, 1698 (C=O), 1356 (S=O), 1175, 814 cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-d$_6$, 363 K, δ ppm) 7.78-7.73 (m, 2H, H-arom. of Ts), 7.47-7.44 (m, 2H, H-arom. of Ts), 7.39-7.28 (m, 5H, H-arom.), 5.09 (d, 1H, $^2$J$_{H,H}$ 12.7, C$_3$H$_2$ of Cbz), 5.04 (d, 1H, CH$_2$ of Cbz), 4.60 (dd, 1H, J$^3,4$ 5.7, J$^3,2$ 1.5, H-3), 4.38 (dd, 1H, J$^1,4$ 1.4, H-4), 4.19 (dd, 1H, $^2$J$_{1'a,1'b}$ 9.8, $^2$J$_{1'a,2}$ 3.7, H-1'a), 4.09 (dd, 1H, J$^1,b,2$ 5.9, H-1'b), 4.05 (m, 1H, H-2), 3.98 (qd, 1H, J$_5$Me 6.9, H-5), 2.42 (s, 3H, Me of Ts), 1.33, 1.25 (2s, 3H each, C(C$_3$H$_3$)$_2$), 1.13 (d, 3H, Me). 13C NMR (75.4 MHz, DMSO-d$_6$, 363 K, δ ppm) δ 153.3 (C=O of Cbz), 144.6, 136.2, 132.1, 129.6, 127.8, 127.3, 127.0, 126.7, 125.1 (C-arom.), 110.8 (C(CH$_3$)$_2$), 84.2 (C-4), 80.5 (C-3), 68.4 (C-1'), 65.9 (CH$_2$ of Cbz), 62.8 (C-2), 59.6 (C-5), 26.6, 24.6 (C(CH$_3$)$_2$), 20.5 (Me of Ts), 18.2 (Me). CIMS m/z 476 [1%, (M+H)$_+^\dagger$], 340 [10%, (M-Cbz+H)$_+^\dagger$]. HRCIMS m/z found 476.1736, calc. for C$_{24}$H$_{30}$NO$_7$S [M+H]$^+$: 476.1743.

$^{(2S,3S,4R,5S)}$-N-Benzylxycarbonyl-2-azidomethyl-3,4-O-isopropylidene-5-methylpyrrolidine-3,4-diol (22). NaN$_3$ (137 mg, 2.18 mmol) was added to a solution of 21 (414 mg, 0.87 mmol) in DMF (7.5 mL). After heating at 70 ºC for 3 h, the solvent was evaporated and the residue diluted with CH$_2$Cl$_2$ and washed with water and brine. The organic phase was dried, filtered, and concentrated. Purification by chromatography column (AcOEt:cyclohexane 1:6) afforded 22 as an oil (276 mg, 92%). $[\alpha]_{D}^{28}$ + 60.4 (c 0.55, CH$_2$Cl$_2$). IR ν$_{max}$ 2986, 2938, 2103.
(N₃), 1693 (C=O), 1403, 1210, 1026, 697 cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆, 363 K, δ ppm) δ 7.39-7.28 (m, 5H, H-arom.), 5.14 (d, 1H, ²J_H,H 12.5, CH₂ of Cbz), 5.08 (d, 1H, ²J_H,H 6.4, H-5), 3.66 (dd, 1H, ²J₁,a₁;b 12.8, J₁,a₂ 6.0, H-1’a), 3.53 (dd, 1H, ²J₁,b₂ 3.5, H-1’b), 1.40, 1.30 (2s, 3H each, C(CH₃)₂), 1.29 (d, 3H, Me). ¹³C NMR (75.4 MHz, DMSO-d₆, 363 K, /g303 ppm) /g303 153.6 (C=O of Cbz), 136.2, 127.8, 127.4, 127.2 (C-arom.), 110.4 (C(CH₃)₂), 80.7 (C-3), 79.4 (C-4), 65.8 (CH₂ of Cbz), 61.8 (C-2), 56.6 (C-5), 50.5 (C-1’), 25.4, 24.4 (C(CH₃)₂), 14.6 (Me).

CIMS m/z 347 [2%, (M+H)+], 290 [26%, (M-CH₂N₃)+]. HRCIMS m/z found 347.1727, calcd. for C₁₇H₂₃N₄O₄ [M+H]+: 347.1719.

(2R,3R,4S)-N-Benzoylcarbonyl-2-azidomethyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol (25). A solution of NaIO₄ (3.0 g, 14.0 mmol) in H₂O (40 mL) was added dropwise to a 0 ºC solution of diol 23 (2.36 g, 7.0 mmol) in THF (35 mL). After 45 min., THF was evaporated and the mixture extracted with CH₂Cl₂. The organic phases were washed with water, sat. aq. soln. of NaHCO₃ and brine, dried, filtered and concentrated. The aldehyde obtained was then dissolved in MeOH (40 mL), cooled to 0 ºC and NaBH₄ (260 mg, 6.8 mmol) was added. After 30 min., the mixture was diluted with CH₂Cl₂ and washed with water and brine. The organic phase was dried, filtered and concentrated. The crude alcohol so-obtained was then dissolved in dry pyridine (25 mL), TsCl (4.34 g, 22.8 mmol) was added and the mixture stirred at 20 ºC for 12 h. Water was added dropwise under stirring and the mixture evaporated to dryness. The residue was dissolved in CH₂Cl₂ and washed with water and brine. The organic phase was dried, filtered and concentrated. The tosylate ester 24 so-obtained was dissolved in DMF (30 mL) and NaN₃ (0.93 g, 14.2 mmol) was added. After heating to 50 ºC for 2.5 h, the solvent was evaporated and the residue diluted with CH₂Cl₂ and washed with water and brine. The organic phase was dried, filtered, and concentrated. Purification by chromatography column (AcOEt:cyclohexane 1:1) afforded 25 (1.68 g, 72 %, 4 steps). [α]D²⁷ = -46.0 (c 0.87, CH₂Cl₂). IR ν_max 2996, 2938, 2103 (N₃), 1698 (C=O), 1350, 1043, 769 cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆, 363 K) δ 7.33-7.29 (m, 5H, H-arom.), 5.12 (s, 2H, CH₂ of Cbz), 4.77 (m, 1H, H-4), 4.57 (dd, 1H, J₂,₃ 6.0, J₁,₂ 0.6, H-3), 4.07 (br. t, 1H, J₂,a₁;b 5.7, H-2), 3.74 (dd, 1H, J₁,a₂ 1.2, J₁,b₂ 12.9, H-5a), 3.56-3.44 (m, 3H, H-5b, H-1’a, H-1’b), 1.32, 1.26 (2s, 3H each, C(CH₃)₂), 1.30 (2s, 3H each, C(CH₃)₂). ¹³C NMR (75.4 MHz, DMSO-d₆, 363 K) δ 153.7 (C=O), 136.4, 127.8, 127.3, 126.8 (C-arom.), 110.6 (C(CH₃)₂), 81.7 (C-3), 78.2 (C-4), 65.9 (CH₂ of Cbz), 62.6 (C-2), 51.4 (C-5), 50.3 (C-1’), 26.3 (C(CH₃)₂), 24.4 (C(CH₃)₂). HRCIMS: calculated for C₁₆H₂₃N₄O₄ [M+H]+: 333.1563, found 333.1570 [M+H]+.
sat. aq. soln. of Na₂SO₄ (20 mL) was added dropwise and the mixture extracted with AcOEt. The dried organic phase was evaporated and the obtained residue was purified by column chromatography over silica gel (toluene:acetone, 5:1) to afford the corresponding hydroxyethyl pyrrolidine 27 (171 mg, 70%). To a solution of this compound (221 mg, 0.69 mmol) in anh. CH₂Cl₂ (4 mL), anh. pyridine (3 mL) and MeSOCl (160 μL, 2.06 mmol) were added. After 1.5 h, the reaction was quenched with water (4 mL) and evaporated. The residue was diluted with CH₂Cl₂, washed with water and brine, dried, filtered and concentrated. The crude product was then dissolved in anh. DMF (5 mL) and NaNO₃ (135 mg, 2.06 mmol) was then added. The mixture was heated at 70 °C for 3 h and then evaporated. The residue was dissolved in CH₂Cl₂, washed with water and brine, dried, filtered and concentrated. The residue was purified by column chromatography (AcOEt:toluene:acetone 1:4) to give pure 28 as an oil (144.8 mg, 61%, 2 steps).

27 D/g302+80.1 (c 0.96, CH₂Cl₂). IR /g542max 2918, 2849, 1726 (C=O), 1201, 1027, 738 cm⁻¹. 1H NMR (300 MHz, DMSO-d₆, 363 K, δ ppm, J Hz) 7.54 (s, 1H, H-5''), 7.41-7.32 (m, 5H, H-arom.), 4.52 (dd, 1H, J₁''H,1'b 3.6, H-1''b), 4.34 (t, 1H, J₁'H,4 6.0, H-4), 4.27 (m, 1H, H-2), 3.38 (q, 1H, J₅Me 6.0, H-5'), 2.60 (t, 2H, J₇H,₇H 7.5, -CH₂(CH₂)₁₀CH₃), 1.58 (q, 2H, J₇H,₇H 6.3, -CH₂CH₂(CH₂)₉CH₃), 1.37-1.23 (m, 27H, -CH₂CH₂(C₆H₄)₉CH₃, -C(CH₃)₂, Me), 0.87 (t, 3H, J₇H,₇H 7.2, -(CH₂)₁₁C₆H₃). 13C NMR (75.4 MHz, DMSO-d₆, 363 K, δ ppm) δ 153.6 (C=O), 146.8 (C-4''), 136.2, 127.8, 127.4, 127.3 (C-arom.), 122.0 (C-5''), 110.3 (+C(CH₃)₂), 80.2 (C-4), 79.4 (C-3), 65.8 (CH₂ of Cbz), 62.4 (C-2), 56.3 (C-5), 48.4 (C-1'), 30.7, 28.4, 28.3, 28.2, 28.1, 27.9, 25.3, 24.4, 21.4, 14.4 (11 -CH₂, -C(CH₃)₂, Me), 13.2 (-C(CH₂)₁₁CH₃). LSIMS m/z found 563 [52 %, (M+Na)⁺]. HRRLSIMS m/z found 563.3564, calc. for C₃₁H₄₈N₄O₄Na: 563.3573. Deprotection of this compound (49.5 mg, 0.092 mmol) was carried out in HCl (1 M):THF 1:1 (5 mL) at r.t overnight. Solvent was then evaporated and the residue was purified by chromatography column over silica gel (CH₂Cl₂:MeOH, 20:1) affording the corresponding 3,4-O-unprotected derivative (47.6 mg, 95%). To a solution of this derivative (43.6 mg, 0.087 mmol) in MeOH (2 mL), Pd/C
(10%) and HCl (5 M, 70 µL) were added. The mixture was hydrogenated at 1 atm for 3 h and then diluted with MeOH, filtered through Celite, and evaporated, to afford 31 as an amorphous solid (33.2 mg, 95%). \([\delta^1^H]\) -16.5 (c 0.95, MeOH). IR \(\nu_{max}\) 3374 (OH, NH), 2920, 2851, 1636, 1120 cm\(^{-1}\). \(\text{H NMR (300 MHz, MeOD, } \delta \text{ ppm)} \delta 8.38 (s, 1H, H-5''), 7.51-7.31 (m, 2H, H-1'a', H-1'b), 4.29-4.07 (m, 3H, H-2, H-3, H-4), 3.88 (m, 1H, H-5), 2.84 (br s, 2H, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{10}\text{CH}_3\)), 1.74 (br s, 2H, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{10}\text{CH}_3\)), 1.41-1.29 (m, 23H, CH2(C2H5)10CH3, Me), 0.89 (t, 3H, J_HH 6.6, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{10}\text{CH}_3\)). \(^{13}\text{C NMR (75.4 MHz, MeOD, } \delta \text{ ppm)} \delta 148.5 (C-4''), 126.5 (C-5''), 75.4, 72.6, 61.2 (C-2, C-3, C-4), 59.2 (C-5), 51.8 (C-1'), 33.0, 30.7, 30.6, 30.4, 30.3, 30.1, 30.0, 25.5, 23.6 (11 CH2), 14.4 (\(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{10}\text{CH}_3\)), 12.1 (Me). LSIMS m/z found 367.3056, calc. for C20H39N4O2: 367.3073.

\((2R,3R,4S)-2-\{(4''-\text{dodecyl})-1H-1,2,3-triazole-1-yl\}-\text{methyl}\)-pyrrolidine-3,4-diol hydrochloride (32). To a solution of 25 (75 mg, 0.23 mmol) in toluene (2 mL) were added tetradec-1-yne (72 µL, 0.30 mmol), DIPEA (85.1 µL, 0.83 mmol) and CuI (13 mg, 0.07 mmol). The mixture was stirred at r.t. for 20 h and then, a sat. aq. soln. of NaHCO3 was added and extracted with AcOEt. The organic phases were dried, filtered and concentrated. Purification by chromatography column (AcOEt:cyclohexane 1:2) afforded the corresponding triazole derivative (95 mg, 80%). \([\delta^1^H] +22.9 (c 0.96, \text{MeOH})). IR \(\nu_{max}\) 3382, 3240 (OH, NH), 2921, 2851, 1638, 1136 cm\(^{-1}\). \(\text{H NMR (300 MHz, DMSO-}d_6, 363 K, \delta \text{ ppm)} \delta 7.66 (s, 1H, H-5''), 7.37-7.28 (m, 5H, H-arom.), 5.11 (d, 1H, J_HH 12.9, C=CH of Cbz), 5.01 (d, 1H, C=CH of Cbz), 4.72 (d, 1H, J_3,4 5.7, H-3), 4.59 (dd, 1H, J_1'a,1'b 14.1, J_1'a',2 6.6, H-1'a), 4.45 (dd, 1H, J_1'b,2 5.7, H-1'b), 4.35 (t, 1H, J_2,5 6.0, H-2), 3.67 (d, 1H, J_5a,5b 12.8, H-5a), 3.10 (dd, 1H, H-5b), 2.60 (t, 2H, J_HH 7.4, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{10}\text{CH}_3\)), 1.59 (q, 2H, J_HH 7.3, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{10}\text{CH}_3\)), 1.30-1.25 (m, 24H, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{9}\text{CH}_3\)), 0.87 (t, 3H, J_HH 6.6, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{9}\text{CH}_3\)). \(^{13}\text{C NMR (75.4 MHz, DMSO-}d_6, 363 K, \delta \text{ ppm)} \delta 153.7 (C=O), 146.8 (C-4''), 122.8 (C-5''), 73.0 (C-3), 68.3 (C=CH of Cbz), 63.0 (C-2), 51.0 (C-5), 48.3 (C-1'), 30.7, 28.4, 28.2, 28.0, 27.9, 26.2, 24.4, 24.3, 21.4 (10 CH2, C-CH2), 13.2 (\(-\text{CH}_2\text{(CH}_2\text{)}_{2}\)). HRLSIMS calculated for C30H46N4O4Na: 549.3417, found 549.3409 [M+Na]+. Deprotection of this compound (80.5 mg, 0.149 mmol) as indicated for the preparation of 31 afforded 32 as an amorphous solid (44.4 mg, 92%). \([\delta^1^H] +22.9 (c 0.96, \text{MeOH})). IR \(\nu_{max}\) 3380, 3240 (OH, NH), 2921, 2851, 1638, 1136 cm\(^{-1}\). \(\text{H NMR (300 MHz, DMSO-}d_6, \delta \text{ ppm)} \delta 10.03 (br s, 1H, NH), 9.80 (br s, 1H, NH), 8.13 (s, 1H, H-5''), 4.97 (dd, 1H, J_1'a,1'b 14.3, H-1'a), 4.74 (m, 1H, H-1'b), 4.11 (m, 1H, H-4), 3.91 (m, 1H, H-3), 3.67 (m, 1H, H-2), 3.38 (m, 1H, H-5a), 3.01 (m, 1H, H-5b), 2.59 (br t, 2H, J_HH 6.99, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{10}\text{CH}_3\)), 1.57 (m, 2H, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{10}\text{CH}_3\)), 1.23 (m, 18H, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{10}\text{CH}_3\)), 0.84 (m, 3H, \(-\text{CH}_2\text{CH}_2\text{(CH}_2\text{)}_{10}\text{CH}_3\)). \(^{13}\text{C NMR (75.4 MHz, DMSO-}d_6, 363 K, \delta \text{ ppm)} \delta 153.7 (C=O), 146.8 (C-4''), 136.4, 127.8, 127.2, 126.7 (C-arom.), 121.9 (C-5''), 110.6 (C(CH3)2), 81.6 (C-3), 78.1 (C-4), 65.8 (C=CH of Cbz), 63.0 (C-2), 51.0 (C-5), 48.3 (C-1'), 30.7, 28.4, 28.2, 28.0, 27.9, 26.2, 24.4, 24.3, 21.4 (10 CH2, C-CH2), 13.2 (\(-\text{CH}_2\text{(CH}_2\text{)}_{2}\)). HRLSIMS calculated for C30H46N4O4Na: 549.3417, found 549.3409 [M+Na]+.
(2R,3S,4R)-2-[(4”-Dodecyl)-1H-1,2,3-triazole-1-yl)ethyl]-pyrrolidine-3,4-diol hydrochloride (33). Tetradec-1-yn (47 µL, 0.19 mmol), DIPEA (110 µL, 0.64 mmol) and Cul (11 mg, 0.06 mmol) were added to a solution of 28 (60 mg, 0.173 mmol) in toluene (1.5 mL). The mixture was stirred at r.t. for 24 h and then, a sat. aq. soln. of NaHCO₃ was added and extracted with AcOEt. The organic phases were dried, filtered and concentrated. Purification by chromatography column (AcOEt:cyclohexane 1:2) afforded the triazole derivative (76 mg, 81%). Deprotection of this compound (60 mg, 0.111 mmol) as indicated for the preparation of 31 afforded 33 as an amorphous solid (81%, 2 steps). [α]D²⁰⁻8.3 (c 0.73, MeOH); IR (v cm⁻¹) 3362 (OH), 2923, 2852, 1636, 1126, 1048. ¹H-NMR (300 MHz, MeOD, δ ppm) δ 8.34 (s, 1H, H-5”), 4.72 (m, 2H), 4.43 (m, 1H), 4.19 (m, 1H), 3.64 (m, 1H), 3.15 (m, 1H), 2.84 (m, 2H), 2.63-2.46 (m, 2H, -CH₂(CH₂)₆CH₃), 1.75 (br s, 2H, -CH₂CH₂(CH₂)₃CH₃), 1.34 (m, 18H, -CH₂CH₂(CH₂)₉CH₃), 0.89 (t, 3H, J H,H 5.9, -(CH₂)₁₁C₃). ¹³C-NMR (75.4 MHz, MeOD, δ ppm) 146.6 (C-4”), 128.7 (C-5”), 72.0, 71.6, 60.7, 51.4, 32.9, 30.6, 30.5, 30.3, 30.2, 29.6, 28.3, 25.1, 23.6, 14.3 (-(CH₂)₁₁CH₃). CIMS m/z 367 [100%, (M+H)⁺]. HRCIMS m/z found 367.3071, calc. for C₂₀H₃₉N₄O₂: 367.3073.

Cell lines and culture conditions. MiaPaCa2 and PK9 pancreatic ductal adenocarcinoma cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, U.S.A.). Cells were cultured in RPMI (Gibco, Paisley, United Kingdom) supplemented with 10% heat inactivated fetal calf serum (FCS; Amimed, BioConcept, Allschwil, Switzerland) and 1% penicillin/streptomycin at 37°C (BioConcept) in a humidified atmosphere of 95% air and 5% CO₂.

Colorimetric cell growth assays. 5 x 10³ MiaPaCa2 or PK9 cells/well were plated in 96-well plates and allowed to adhere overnight. Thereafter, cells were incubated with or without compounds in a final volume of 200 µL medium for 72h. Subsequently, cell viability was measured with CellTiter96 Aqueous1 (Promega) according to the manufacturer’s instructions. Means of triplicate wells were analyzed. IC₅₀s were estimated using GraphPad Software (GraphPad, La Jolla, CA, USA).

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References


   [http://dx.doi.org/10.1016/j.carres.2010.01.015](http://dx.doi.org/10.1016/j.carres.2010.01.015)
   PMID:20170905

   [http://dx.doi.org/10.1016/S0005-2736(98)00058-3](http://dx.doi.org/10.1016/S0005-2736(98)00058-3)

   [http://dx.doi.org/10.1016/j.bmc.2007.07.048](http://dx.doi.org/10.1016/j.bmc.2007.07.048)
   PMID:17851079

   [http://dx.doi.org/10.1021/jm200961q](http://dx.doi.org/10.1021/jm200961q)
   PMID:21866909

   [http://dx.doi.org/10.4137/CMO.S7685](http://dx.doi.org/10.4137/CMO.S7685)
   PMID:22084619 PMCID:PMC3201112


   [http://dx.doi.org/10.1016/j.ijpharm.2005.11.017](http://dx.doi.org/10.1016/j.ijpharm.2005.11.017)
   PMID:16377108

   [http://dx.doi.org/10.1016/S0014-5793(98)00525-0](http://dx.doi.org/10.1016/S0014-5793(98)00525-0)

   PMID:15546715

   [http://dx.doi.org/10.1007/s10719-009-9276-3](http://dx.doi.org/10.1007/s10719-009-9276-3)
   PMID:20041292
