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 Q[2] isolated from Palauan marine sponge are active against mouse leukemia cell lines in the μM range. Alkylglycerols[3] 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-sulfate[5] 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,[7-9] 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.[10]

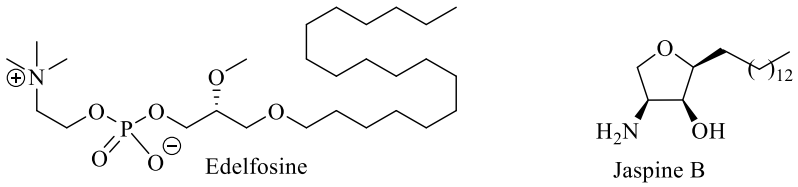


Figure 1

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.[11]

Due to their amphiphilic nature, glycolipids and their analogues also show therapeutic potential. Numerous glycolipid derivatives have been proposed as antibacterial/antiviral agents,[12,13] cell adhesion mediators,[14] and oral drug carriers.[15] 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.[16,17] Triazologlycolipids **2** showed cytotoxicity against a panel of cancer cell lines in the μM range.[18]

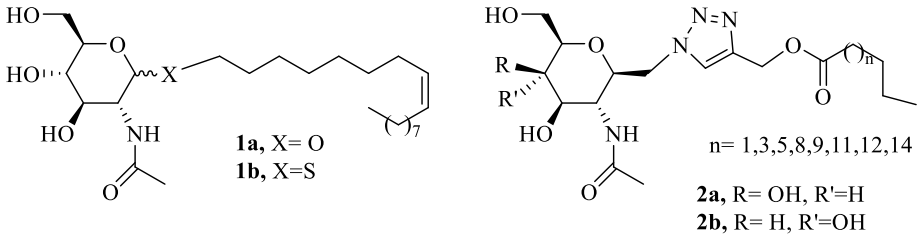


Figure 2

Derivatization of iminosugars with aliphatic side chains has been extensively studied in order to improve their biological activity, lipophilicity and bioavailability.[19] For instance *N*-nonyl-1-

deoxynojirimycin inhibits hepatitis B virus in cell based assays.[20] 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.[21] *N*-alkylated-D-fagomine derivatives **3** and *N*-alkylated hydroxylated pyrrolidine **4** (Figure 3) bearing a long alkyl chain, not only presented an improved inhibitory selectivity towards α-D-glucosidase and α-L-fucosidase, respectively, but also exhibited enhanced cytotoxic activities on a panel of cancer cell lines compared to their non-alkylated progenitors.[22] Other *N*-alkylated pyrrolidine derivatives such as **5**[23] and **6**[24,25] presented interesting inhibition against *N*-acetylglucosaminidases and α-mannosidases, respectively.

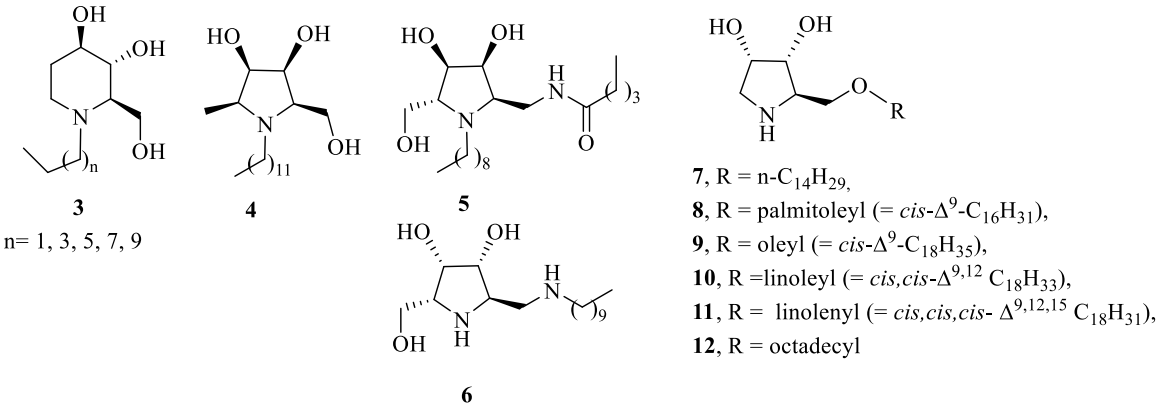


Figure 3

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 μM range.[26,27] Even though these compounds are not inhibitors of α-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[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 μM range (IC_{50} between 2 and 21 μM).[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.

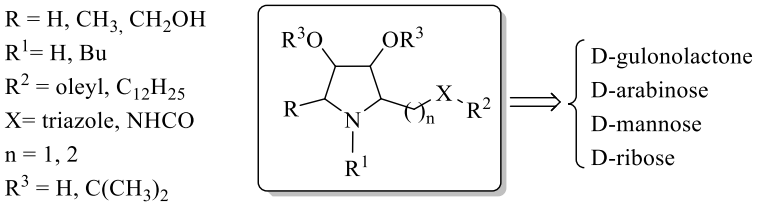
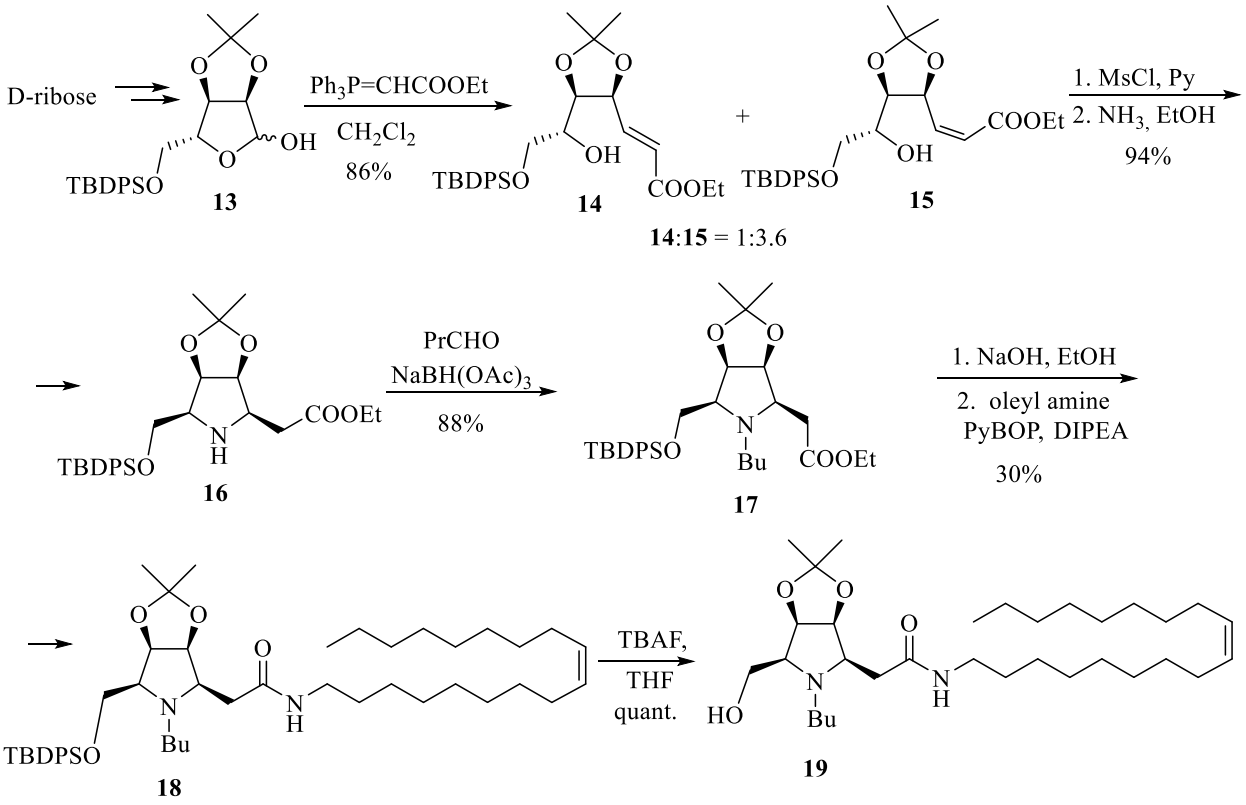


Figure 4. Structural variations for new amphiphilic pyrrolidine-3,4-diol derivatives.

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 S_N2 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]



Scheme 1. Synthesis of **19.**

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.

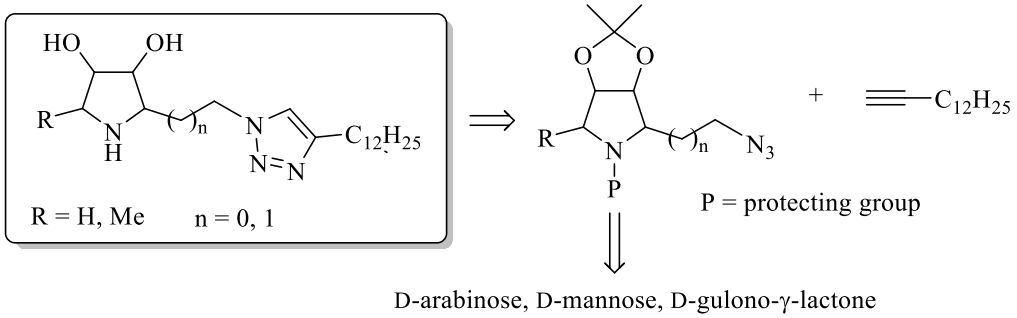
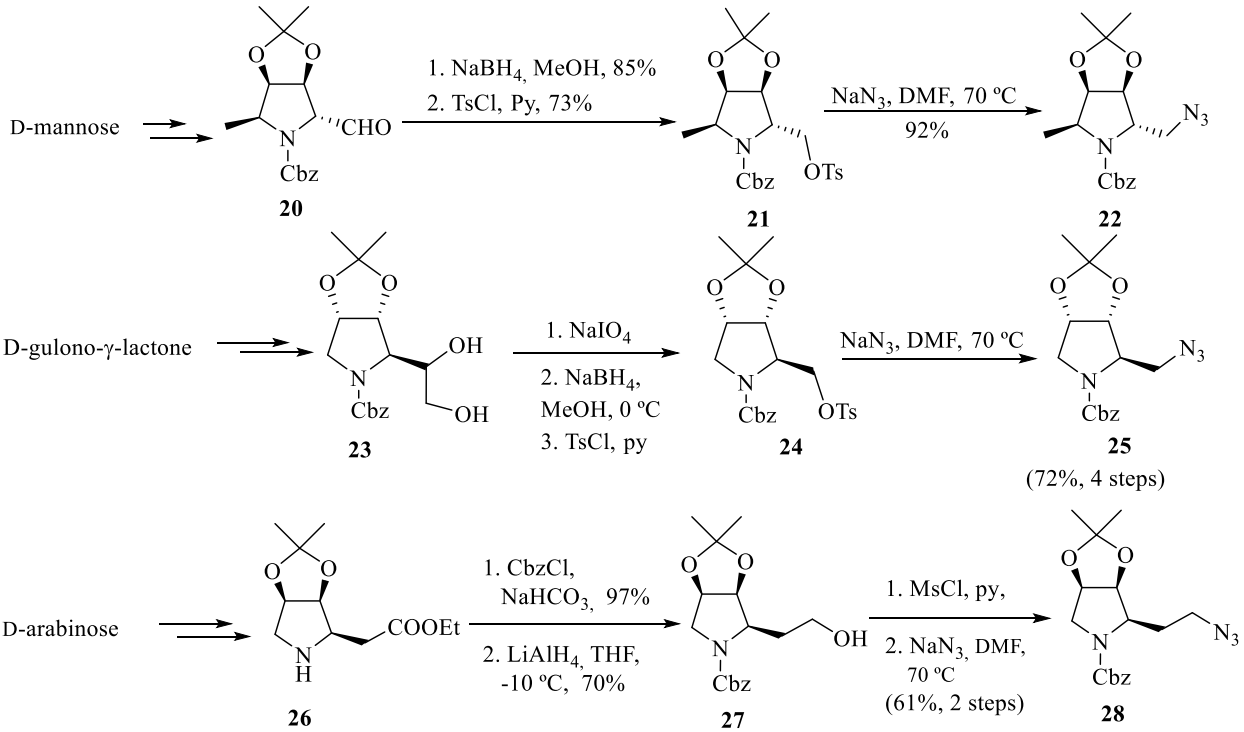


Figure 5. Retrosynthesis for triazole-pyrrolidine compounds.

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

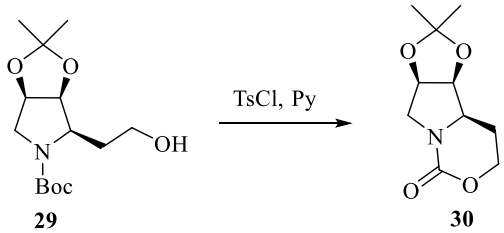
The preparation of the azido-pyrrolidines is outlined in Scheme 2. Starting from known aldehyde **20**,[31] reduction and tosylation gave derivative **21**, which after displacement with

sodium azide in DMF furnished azido derivative **22**. Similarly, derivative **25** was obtained by azido displacement of the corresponding tosyl derivative **24**[32], obtained through oxidation, reduction and tosylation of known diol **23**.[33] Derivative **28** was prepared from pyrrolidine ester **26**,[34] by Cbz protection and reduction with $LiAlH_4$ followed by mesylation and azido displacement.



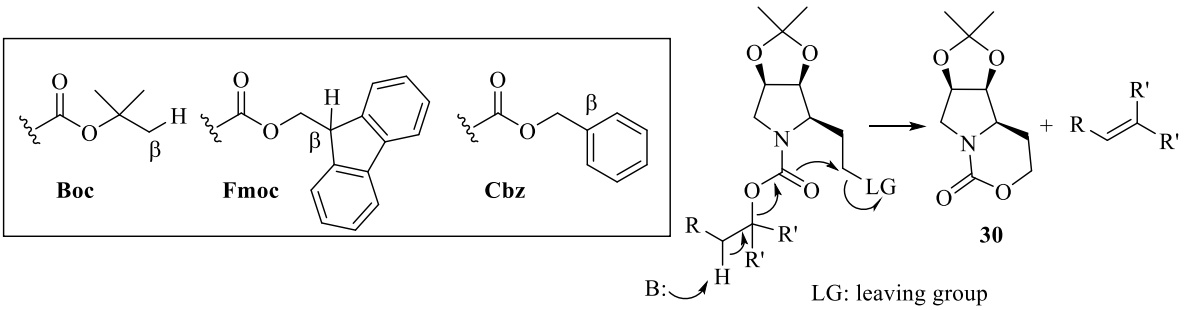
Scheme 2. Synthesis of azido-pyrrolidines.

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 ^{1}H- and ^{13}C 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 ^{13}C NMR spectra, corresponding to the carbonyl group of the carbamate.



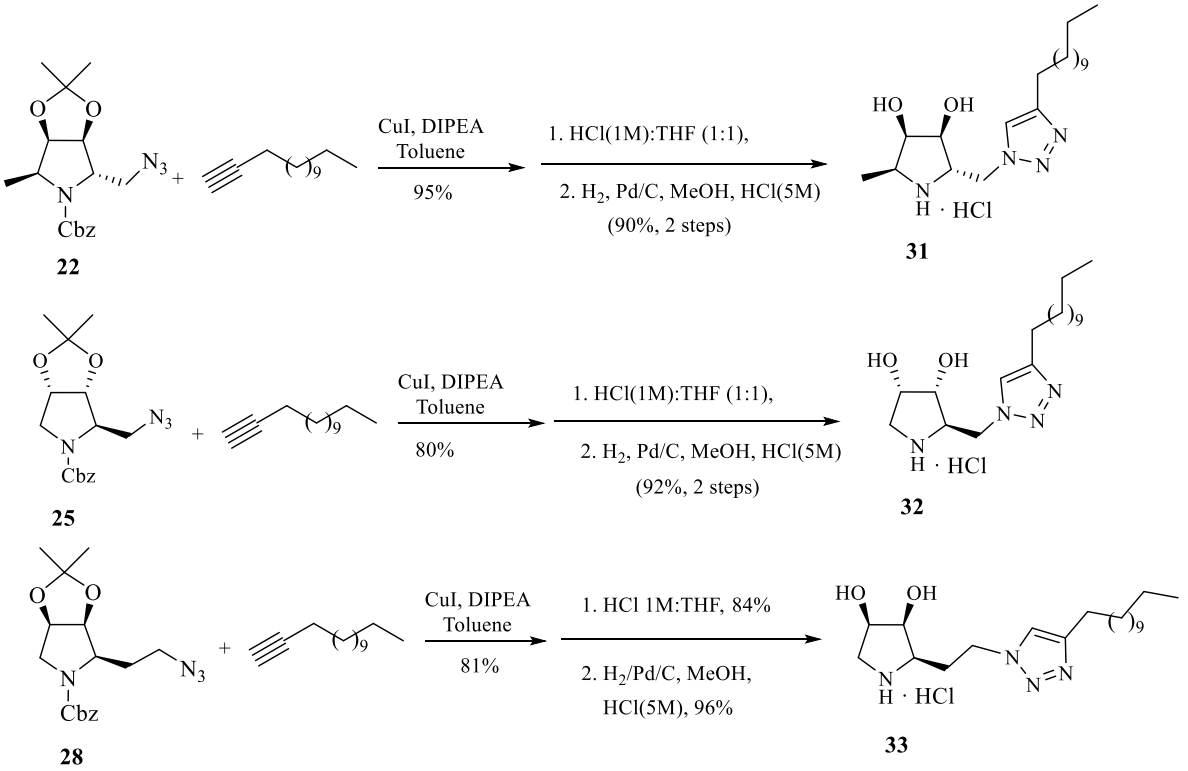
Scheme 3. Participation of the *N*-protecting group.

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[35-37] 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)

	Compounds	**19**	**31**	**32**	**33**
Cell line	MiaPaCa2	3.6	21	35	14
	PK9	17	19	45	16

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_3OD; *J* values are given in Hz and *δ* in ppm. All the assignments were confirmed by two-dimensional NMR experiments (COSY and HSQC). The LSI 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 [$(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O]. 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-*ribo*hept-2-enoate (14 and 15). Ethoxycarbonyltriphenylmethylenephosphorane (7 g, 20.1 mmol) was added to a solution of **13**[28] (3.75 g, 8.76 mmol) in dry CH_2Cl_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_2Cl_2); IR (ν cm^{-1}) 3462, 2931, 2857, 1718, 1657, 1428 , 1110, 1059, 822, 740, 701, 614. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 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, CH_2CH$_3$), 3.84 (dd, 1H, $J_{7a,7b}$ 10.4, $J_{7a,6}$ 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, O*H*), 1.41, 1.35 (2s, 3H each, C(CH_3)$_2$), 1.30 (t, 3H, $J_{H,H}$ 7.2, CH_2CH_3), 1.07 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 166.2 (*C*OOEt), 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

(*C*H_2CH$_3$), 27.6 (C(*C*H$_3$)$_2$), 26.8 (C(*CH*$_3$)$_3$), 25.3 (C(*C*H$_3$)$_2$), 19.3 (*C*(CH$_3$)$_3$), 14.2 (CH$_2$*C*H$_3$). CIMS *m/z* 499 [1%, (M+H)$^+$]. HRCIMS *m/z* found 499.2512, calc. for $C_{28}H_{39}O_6Si$: 499.2516.

Data for **15**: $[\alpha]_D^{29}$ +76.2 (*c* 1.05, CH_2Cl_2); IR (ν cm^{-1}) 2931, 2857, 1716, 1698, 1427, 1380, 1190, 1055, 869, 822, 701, 615. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 7.68-7.66 (m, 4H, H-arom.), 7.45-7.35 (m, 6H, H-arom.), 6.23 (dd, 1H, $J_{3,2}$ 11.7, $J_{3,4}$ 8.4, H-3), 5.94 (dd, 1H, $J_{2,4}$ 1.1, H-2), 5.74 (m, 1H, H-4), 4.38 (dd, 1H, $J_{5,4}$ 8.1, $J_{5,6}$ 6.3, H-5), 4.19 (q, 2H, $J_{H,H}$ 6.9, C*H*$_2$CH$_3$), 3.84-3.75 (m, 2H, H-7a, H-7b), 3.66 (m, 1H, H-6), 2.80 (d, 1H, $J_{OH,6}$ 4.5, O*H*), 1.38, 1.35 (2s, 3H each, C(C*H*$_3$)$_2$), 1.30 (t, 3H, CH$_2$C*H*$_3$), 1.06 (s, 9H, C(C*H*$_3$)$_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 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*(CH$_3$)$_2$), 78.1 (C-5), 73.9 (C-4), 70.3 (C-6), 65.1 (C-7), 60.6 (*C*H$_2$CH$_3$), 27.8 (C(*C*H$_3$)$_2$), 26.8 (C(*CH*$_3$)$_3$), 25.4 (C(*C*H$_3$)$_2$), 19.3 (*C*(CH$_3$)$_3$), 14.2 (CH$_2$*C*H$_3$). CIMS *m/z* 499 [1%, (M+H)$^+$]. HRCIMS *m/z* found 499.2498, calc. for $C_{28}H_{39}O_6Si$: 499.2516.

Ethyl 7-*O*-*tert*-butyldiphenylsilyl-2,3,6-trideoxy-3,6-imino-4,5-*O*-isopropylidene-L-*galacto*-heptanoate ((2*S*,3*R*,4*S*,5*R*)-2-*tert*-butyldiphenylsilyloxymethyl-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 CH_2Cl_2 (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, H_2O (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 H_2O 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 NH_3. After 4 days at r.t., the solvent was evaporated and the residue was treated with NH_4OH (25%, 30 mL) and extracted with CH_2Cl_2 (5x30 mL). The organic phase was washed with satd. aq. sol. of $NaHCO_3$ (30 mL) and H_2O until neutral pH, dried (Na_2SO_4), filtered and concentrated. The residue was purified by column chromatography (AcOEt:petroleum ether (1:6), Et_3N (1%)) to give pure **16** as an oil (2.09 g, 94%). $[\alpha]_D^{26}$ +12.3 (*c* 1.25, CH_2Cl_2); IR (ν cm^{-1}) 2930, 2857, 1731, 1108, 1091, 822, 739, 701, 615. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 7.72-7.68 (m, 4H, H-arom.), 7.41-7.34 (m, 6H, H-arom.), 4.63 (dd, 1H, $J_{5,4}$ 5.7, $J_{5,6}$ 4.1, H-5), 4.60 (dd, 1H, $J_{4,3}$ 4.2, H-4), 4.15 (m, 2H, C*H*$_2$CH$_3$), 3.90 (dd, 1H, $J_{7a,7b}$ 10.4, $J_{7a,6}$ 7.1, H-7a), 3.83 (dd, 1H, $J_{7b,6}$ 6.7, H-7b), 3.17 (ddd, 1H, $J_{3,2b}$ 7.6, $J_{3,2a}$ 6.4, H-3), 3.02 (m, 1H, H-6), 2.66 (dd, 1H, $^2J_{2a,2b}$ 17.0, H-2a), 2.59 (dd, 1H, H-2b), 1.81 (br s, 1H, NH), 1.39, 1.29 (2s, 3H each, C(C*H*$_3$)$_2$), 1.26 (t, 3H, CH$_2$C*H*$_3$), 1.06 (s, 9H, C(C*H*$_3$)$_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 172.2 (*C*OOEt), 135.7, 135.6, 133.7, 133.6, 129.5, 127.6, 127.5 (C-arom.), 111.0 (*C*(CH$_3$)$_2$), 81.3 (C-4), 80.6 (C-5), 63.6 (C-6), 62.5 (C-7), 60.4 (*C*H$_2$CH$_3$), 58.1 (C-3), 34.1 (C-2), 26.8 (C(*CH*$_3$)$_3$), 25.7, 24.5 (C(*C*H$_3$)$_2$), 19.2 (*C*(CH$_3$)$_3$), 14.2 (CH$_2$*C*H$_3$). CIMS *m/z* 497 [1%, (M)$^+$], 440 [50%, (M-^{t}Bu)$^+$]. HRCIMS *m/z* found 497.2591, calc. for $C_{28}H_{39}NO_5Si$: 497.2598.

(2*S*,3*R*,4*S*,5*R*)-*N*-Butyl-2-*tert*-butyldiphenylsilyloxymethyl-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 $NaHCO_3$ was added

and the mixture extracted with CH_2Cl_2. The organic phases were dried (Na_2SO_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%). $[\alpha]_D^{29}$ +1.9 (*c* 1.15, CH_2Cl_2); IR (ν cm^{-1}) 2931, 2857, 1732, 1110, 1087, 823, 738, 701, 613. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 7.73-7.68 (m, 4H, H-arom.), 7.42-7.34 (m, 6H, H-arom.), 4.64-4.62 (m, 2H, H-4, H-3), 4.15 (qd, 2H, $^3J_{H,H}$ 7.1, *J* 1.4, OC*H*$_2$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, C*H*$_2$-N), 1.39, 1.29 (2s, 3H each, C(C*H*$_3$)$_2$), 1.25 (t, 3H, OCH$_2$C*H*$_3$), 1.24-1.09 (m, 4H, C*H*$_2$C*H*$_2$), 1.05 (s, 9H, C(C*H*$_3$)$_3$), 0.83 (t, 3H, $^3J_{H,H}$ 7.2, C*H*$_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 172.4 (*C*OOEt), 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 (O*C*H$_2$CH$_3$), 49.5 (C-1''), 33.2 (*C*H$_2$-N), 27.1 (*C*H$_2$), 26.8 (C(*C*H$_3$)$_3$), 26.0, 25.7 (C(*C*H$_3$)$_2$), 20.7 (*C*H$_2$), 19.2 (*C*(CH$_3$)$_3$), 14.2, 14.0 (OCH$_2$*C*H$_3$, CH$_2$*C*H$_3$). CIMS *m/z* 554 [15%, $(M+H)^+$]. HRCIMS *m/z* found 554.3283, calc. for $C_{32}H_{48}NO_5Si$: 554.3302.

(2*S*,3*R*,4*S*,5*R*)-*N*-Butyl-2-*tert*-butyldiphenylsilyloxymethyl-3,4-*O*-isopropylidene-5-oleyl-carbamoylmethyl 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 μL, 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_2Cl_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%). $[\alpha]_D^{29}$ -1.7 (*c* 1.05, CH_2Cl_2); IR (ν cm^{-1}) 3299, 2921, 2852, 1643, 1110, 1092, 823, 738, 701, 614. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 7.73-7.67 (m, 4H, H-arom.), 7.42-7.35 (m, 6H, H-arom.), 6.56 (br t, 1H, N*H*), 5.38-5.32 (m, 2H, C*H*=C*H*), 4.62 (dd, 1H, *J* 6.5, *J* 4.8, H-3), 4.50 (br dd, 1H, H-4), 3.97 (dd, 1H, $^2J_{1'a,1'b}$ 10.0, $J_{1'a,2}$ 7.0, H-1'a), 3.76 (dd, 1H, $J_{1'b,2}$ 5.0, H-1'b), 3.20 (q, 2H, $J_{H,H}$ 6.4, C*H*$_2$-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, C*H*$_2$-N), 2.02-1.98 (m, 4H, C*H*$_2$-CH=CH-C*H*$_2$), 1.39 (s, 3H, C(C*H*$_3$)$_2$), 1.48-1.09 (m, 31H, 14C*H*$_2$, C(C*H*$_3$)$_2$), 1.05 (s, 9H, C(C*H*$_3$)$_3$), 0.88 (t, 3H, $J_{H,H}$ 6.3, C*H*$_3$), 0.83 (t, 3H, $J_{H,H}$ 7.2, C*H*$_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 171.3 (*C*=O), 135.7, 135.5, 133.7, 133.5 (C-arom.), 129.9, 129.7 (*C*H=*C*H), 129.6, 129.5, 127.6, 127.5 (C-arom.), 110.7 (*C*(CH$_3$)$_2$), 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 (*C*H$_2$-N), 32.6 (*C*H$_2$), 31.9 (*C*H$_2$), 29.7-29.2 (*C*H$_2$), 27.2, 27.1 (*C*H$_2$), 26.7 (C(*C*H$_3$)$_3$), 25.7, 25.0 (C(*C*H$_3$)$_2$), 22.6 (*C*H$_2$), 20.6 (*C*H$_2$), 19.1 (*C*(CH$_3$)$_3$), 14.1, 14.0 (2*C*H$_3$). CIMS *m/z* 775 [100%, $(M+H)^+$]. HRCIMS *m/z* found 775.5804, calc. for $C_{48}H_{79}N_2O_4Si$: 775.5809.

(2*S*,3*R*,4*S*,5*R*)-*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_2Cl_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, N*H*), 5.39-5.32 (m, 2H, C*H*=C*H*), 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, $^2J_{1'a,1'b}$ 11.7, $J_{1'a,2}$ 2.5, H-1'a), 3.82 (dd, 1H, $J_{1'b,2}$ 5.8, H-1'b), 3.25-3.18 (m, 2H, CH_2-NH), 3.02 (br s, 1H, H-5), 2.68-2.45 (m, 5H, H-2, H-1''a, H-1''b, CH_2-N), 2.07-1.91 (m, 4H, CH_2-CH=CH-CH_2), 1.49 (s, 3H, C(CH_3)$_2$), 1.36-1.22 (m, 36H, 14CH_2, C(CH_3)$_2$), 0.94-0.83 (m, 6H, 2 CH_3). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 171.0 (*C*=O), 130.0, 129.7 (*C*H=*C*H), 111.2 (*C*(CH$_3$)$_2$), 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*H$_2$-NH), 31.9 (*C*H$_2$-N), 29.7-20.7 (16*C*H$_2$, C(*C*H$_3$)$_2$), 14.1, 13.9 (2*C*H$_3$). CIMS *m/z* 537 [100%, $(M+H)^+$]. HRCIMS *m/z* found 537.4637, calc. for $C_{32}H_{61}N_2O_4$: 537.4631.

(2*S*,3*S*,4*R*,5*S*)-*N*-Benzyloxycarbonyl-2-tosyloxymethyl-3,4-*O*-isopropylidene-5-methyl-pyrrolidine-3,4- diol (21). $NaBH_4$ (0.43 g, 4.75 mmol) was added to a solution of **20**[31] (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_2Cl_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) δ 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, $^2J_{H,H}$ 12.7, CH_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, $J_{4,5}$ 1.4, H-4), 4.19 (dd, 1H, $^2J_{1'a,1'b}$ 9.8, $J_{1'a,2}$ 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(CH_3)$_2$), 1.13 (d, 3H, Me). ^{13}C 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*(*C*H$_3$)$_2$), 84.2 (C-4), 80.5 (C-3), 68.4 (C-1'), 65.9 (*C*H$_2$ of Cbz), 62.8 (C-2), 59.6 (C-5), 26.6, 24.6 (C(*C*H$_3$)$_2$), 20.5 (Me of Ts), 18.2 (Me). CIMS *m/z* 476 [1%, $(M+H)^+$], 340 [10%, $(M-Cbz+H)^+$]. HRCIMS *m/z* found 476.1736, calcd. for $C_{24}H_{30}NO_7S$ $[M+H]^+$: 476.1743.

(2*S*,3*S*,4*R*,5*S*)-*N*-Benzyloxycarbonyl-2-azidomethyl-3,4-*O*-isopropylidene-5-methyl-pyrrolidine-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_2Cl_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_2Cl_2). IR ν_{max} 2986, 2938, 2103

(N_3), 1693 (C=O), 1403, 1210, 1026, 697 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 363 K, δ ppm) δ 7.39-7.28 (m, 5H, H-arom.), 5.14 (d, 1H, $^2J_{H,H}$ 12.5, CH_2 of Cbz), 5.08 (d, 1H, CH_2 of Cbz), 4.68 (t, 1H, $J_{4,3}$= $J_{4,5}$ 6.2, H-4), 4.56 (dd, 1H, $J_{3,2}$ 1.0, H-3), 3.99 (m, 1H, H-2), 3.90 (q, 1H, $J_{5,Me}$ 6.4, H-5), 3.66 (dd, 1H, $^2J_{1'a,1'b}$ 12.8, $J_{1'a,2}$ 6.0, H-1'a), 3.53 (dd, 1H, $J_{1'b,2}$ 3.5, H-1'b), 1.40, 1.30 (2s, 3H each, C(CH_3)$_2$,) 1.29 (d, 3H, Me). ^{13}C NMR (75.4 MHz, DMSO-d_6, 363 K, δ ppm) δ 153.6 (C=O of Cbz), 136.2, 127.8, 127.4, 127.2 (C-arom.), 110.4 (*C*(CH_3)$_2$), 80.7 (C-3), 79.4 (C-4), 65.8 (*C*H_2 of Cbz), 61.8 (C-2), 56.6 (C-5), 50.5 (C-1'), 25.4 , 24.4 (C(*C*H_3)$_2$), 14.6 (Me). CIMS *m/z* 347 [2%, $(M+H)^+$], 290 [26%, $(M-CH_2N_3)^+$]. HRCIMS *m/z* found 347.1727, calcd. for $C_{17}H_{23}N_4O_4$ $[M+H]^+$: 347.1719.

(2*R*,3*R*,4*S*)-*N*-Benzyloxycarbonyl-2-azidomethyl-3,4-*O*-isopropylidene-pyrrolidine-3,4-diol (25). A solution of $NaIO_4$ (3.0 g, 14.0 mmol) in H_2O (40 mL) was added dropwise to a 0 ºC solution of diol **23**[33] (2.36 g, 7.0 mmol) in THF (35 mL). After 45 min., THF was evaporated and the mixture extracted with CH_2Cl_2. The organic phases were washed with water, sat. aq. soln. of $NaHCO_3$ and brine, dried, filtered and concentrated. The aldehyde obtained was then dissolved in MeOH (40 mL), cooled to 0 ºC and $NaBH_4$ (260 mg, 6.8 mmol) was added. After 30 min., the mixture was diluted with CH_2Cl_2, 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 CH_2Cl_2 and washed with water and brine. The organic phase was dried, filtered and concentrated. The tosylate ester **24**[32] so-obtained was dissolved in DMF (30 mL) and NaN_3 (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 CH_2Cl_2 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). $[\alpha]_D^{27}$ = -46.0 (*c* 0.87, CH_2Cl_2). IR ν_{max} 2996, 2938, 2103 (N_3), 1698 (C=O), 1350, 1043, 769 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 363 K) δ 7.33-7.29 (m, 5H, H-arom.), 5.12 (s, 2H, CH_2 of Cbz), 4.77 (m, 1H, H-4), 4.57 (dd, 1H, $J_{3,4}$ 6.0, $J_{3,2}$ 0.6, H-3), 4.07 (br. t, 1H, $J_{2,1'a}$ 5.7, H-2), 3.74 (dd, 1H, $J_{5a,4}$ 1.2, $^2J_{5a,5b}$ 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(CH_3)$_2$). ^{13}C NMR (75.4 MHz, DMSO-d_6, 363 K) δ 153.7 (C=O), 136.4, 127.8, 127.3, 126.8 (C-arom.), 110.6 (*C*(CH_3)$_2$), 81.7 (C-3), 78.2 (C-4), 65.9 (*C*H_2 of Cbz), 62.6 (C-2), 51.4 (C-5), 50.3 (C-1'), 26.3 (C(*C*H_3)$_2$), 24.4 (C(*C*H_3)$_2$). HRCIMS: calculated for $C_{16}H_{21}N_4O_4$: 333.1563, found 333.1570 $[M+H]^+$.

(2*R*,3*S*,4*R*)-*N*-Benzyloxycarbonyl-2-azidoethyl-3,4-*O*-isopropylidene-pyrrolidine-3,4-diol (28). $NaHCO_3$ (155 mg, 1.84 mmol) and CbzCl (310 μL, 2.02 mmol) were added to a solution of **26** [34] (421.7 mg, 1.84 mmol) in EtOH:H_2O (1:1, 10 mL), and the mixture stirred at r.t for 3 h. Then, it was poured into a sat. aq. soln. of $NaHCO_3$ and extracted with AcOEt. The dried organic phase was evaporated and the obtained residue was purified by column chromatography over silica gel (AcOEt:cyclohexane, 1:3) affording the corresponding *N*-protected pyrrolidine (594.9 mg, 97%). Then, to a cooled (-10 ºC) suspension of $LiAlH_4$ (35 mg, 0.91 mmol) in anh. THF (3 mL), the protected pyrrolidine (274 mg, 0.756 mmol) in THF (5 mL) was added. After 10 min.,

sat. aq. soln. of Na_2SO_4 (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. CH_2Cl_2 (4 mL), anh. pyridine (3 mL) and MsCl (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 CH_2Cl_2, washed with water and brine, dried, filtered and concentrated. The crude product was then dissolved in anh. DMF (5 mL) and NaN_3 (135 mg, 2.06 mmol) was then added. The mixture was heated at 70 ºC for 3 h and then evaporated. The residue was dissolved in CH_2Cl_2, washed with water and brine, dried, filtered and concentrated. The resulting residue was purified by column chromatography (AcOEt:cyclohexane 1:4) to give pure **28** as an oil (144.8 mg, 61%, 2 steps). $[\alpha]_D^{28}$ -53.6 (*c* 1.04, CH_2Cl_2); IR (ν cm^{-1}) 2981, 2937, 2093 (N_3), 1700 (C=O), 1410, 1206, 1084, 747. ^{1}H-NMR (300 MHz, DMSO-*d*$_6$, 363 K, δ ppm, *J* Hz) 7.37-7.30 (m, 5H, H-arom.), 5.01 (s, 2H, C*H*$_2$ of Cbz), 4.79-4.72 (m, 2H, H-4, H-3), 3.96-3.81 (m, 1H, H-2), 3.79-3.73 (m, 1H, H-5a), 3.41-3.28 (m, 3H, H-2', H-5b), 2.16-2.05 (m, 1H, H-1'a), 2.01-1.92 (m, 1H, H-1'b), 1.44, 1.30 (2s, 3H each, -C(C*H*$_3$)$_2$). ^{13}C-NMR (75.4 MHz, DMSO-*d*$_6$, 363 K, δ ppm) 154.2 (C=O), 136.5, 128.0, 127.4, 127.1 (C-arom.), 111.5 (-*C*(CH$_3$)$_2$), 79.2, 77.0 (C-3, C-4), 65.9 (*C*H$_2$ of Cbz), 57.2 (C-2), 50.4 (C-5), 48.0 (C-2'), 28.0 (C-1'), 25.9 (-C(*C*H$_3$)$_2$), 24.6 (-C(*C*H$_3$)$_2$). CIMS *m/z* 347 [11%, (M+H)$^+$]. HRCIMS *m/z* found 347.1712, calc. for $C_{17}H_{23}N_4O_4$: 347.1719.

(2*S*,3*S*,4*R*,5*S*)-2-[(4''-Dodecyl)-1H-1,2,3-triazole-1-yl)methyl)]-5-methylpyrrolidine-3,4-diol hydrochloride (31). Tetradec-1-yne (43 µL, 0.21 mmol), DIPEA (41 µL, 0.42 mmol) and CuI (7 mg, 0.03 mmol) were added to a solution of **22** (39.6 mg, 0.114 mmol) in toluene (1 mL). The mixture was stirred at r.t. for 20 h and then, a sat. aq. soln. of $NaHCO_3$ was added and extracted with AcOEt. The organic phases were dried, filtered and concentrated. Purification by chromatography column (Et_2O:cyclohexane 2:1) afforded the triazole derivative (58.3 mg, 95%). $[\alpha]_D^{27}$ +80.1 (*c* 0.96, CH_2Cl_2). IR ν_{max} 2918, 2849, 1726 (C=O), 1201, 1027, 738 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d*$_6$, 363 K, δ ppm) δ 7.54 (s, 1H, H-5''), 7.41-7.32 (m, 5H, H-arom.), 5.13 (d, 1H, $^2J_{H,H}$ 12.3, C*H*$_2$ of Cbz), 5.07 (d, 1H, C*H*$_2$ of Cbz), 4.74 (d, 1H, $J_{3,4}$ 6.0, H-3), 4.62 (dd, 1H, $J_{1'a,2}$ 6.3, $^2J_{1'a,1,b}$ 13.8, H-1'a), 4.52 (dd, 1H, $J_{1'b,2}$ 3.6, H-1'b), 4.34 (t, 1H, $J_{4,5}$ 6.0, H-4), 4.27 (m, 1H, H-2), 3.38 (q, 1H, $J_{5,Me}$ 6.0, H-5), 2.60 (t, 2H, $J_{H,H}$ 7.5, -C*H*$_2$(CH$_2$)$_{10}$CH$_3$), 1.58 (q, 2H, $J_{H,H}$ 6.3, -CH$_2$C*H*$_2$(CH$_2$)$_9$CH$_3$), 1.37-1.23 (m, 27H, -CH$_2$CH$_2$(C*H*$_2$)$_9$CH$_3$, -C(C*H*$_3$)$_2$, Me), 0.87 (t, 3H, $J_{H,H}$ 7.2, -(CH$_2$)$_{11}$C*H*$_3$). ^{13}C NMR (75.4 MHz, DMSO-*d*$_6$, 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*(CH$_3$)$_2$), 80.2 (C-4), 79.4 (C-3), 65.8 (*C*H$_2$ 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 -*C*H$_2$, -C(*C*H$_3$)$_2$, Me), 13.2 (-(CH$_2$)$_{11}$*C*H$_3$). LSIMS *m/z* 563 [52 %, (M+Na)$^+$]. HRLSIMS *m/z* found 563.3564, calc. for $C_{31}H_{48}N_4O_4Na$: 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 (CH_2Cl_2: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%). $[\alpha]_D^{27}$ -16.5 (*c* 0.95, MeOH). IR ν_{max} 3374 (OH, NH), 2920, 2851, 1636, 1120 cm^{-1}. ^1H NMR (300 MHz, MeOD, δ ppm) δ 8.38 (s, 1H, H-5''), 5.10-4.90 (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, -CH_2(CH_2)$_{11}CH_3$), 1.74 (br s, 2H, -CH_2CH_2(CH_2)$_9CH_3$), 1.41-1.29 (m, 23H, CH_2(CH_2)$_{10}CH_3$, Me), 0.89 (t, 3H, $J_{H,H}$ 6.6, -(CH_2)$_{11}$CH_3). ^{13}C NMR (75.4 MHz, MeOD, δ ppm) δ 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 CH_2), 14.4 (-(CH_2)$_{11}$CH_3), 12.1 (Me). LSIMS *m/z* 367 [62 %, $(M+H)^+$]. HRLSIMS *m/z* found 367.3056, calc. for $C_{20}H_{39}N_4O_2$: 367.3073.

(2*R*,3*R*,4*S*)-2-[(4''-dodecyl)-1H-1,2,3-triazole-1-yl)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 $NaHCO_3$ 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%). $[\alpha]_D^{27}$ -58.6 (*c* 0.81, CH_2Cl_2). IR ν_{max} 2917, 2848, 1692 (C=O), 1425, 1053, 700 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 363 K, δ ppm) δ 7.66 (s, 1H, H-5''), 7.37-7.28 (m, 5H, H-arom.), 5.11 (d, 1H, $^2J_{H,H}$ 12.9, CH_2 of Cbz), 5.01 (d, 1H, CH_2 of Cbz), 4.72 (d, 1H, $J_{3,4}$ 5.7, H-3), 4.59 (dd, 1H, $J_{4,5}$ 10.8, H-4), 4.54 (dd, 1H, $^2J_{1'a,1'b}$ 14.1, $J_{1a',2}$ 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, $^2J_{5a,5b}$ 12.8, H-5a), 3.10 (dd, 1H, H-5b), 2.60 (t, 2H, $J_{H,H}$ 7.4, -CH_2(CH_2)$_{10}CH_3$), 1.59 (q, 2H, $J_{H,H}$ 7.3, -CH_2CH_2(CH_2)$_9CH_3$), 1.30-1.25 (m, 24H, -CH_2CH_2(CH_2)$_9CH_3$, -C(CH_3)$_2$), 0.87 (t, 3H, $^2J_{H,H}$ 6.6, -(CH_2)$_{11}$CH_3). ^{13}C NMR (75.4 MHz, DMSO-d_6, 363 K, δ ppm) δ 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*(CH_3)$_2$), 81.6 (C-3), 78.1 (C-4), 65.8 (*C*H_2 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 CH_2, -C(*C*H_3)$_2$), 13.2 (-(CH_2)$_{11}$*C*H_3). HRLSIMS calculated for $C_{30}H_{46}N_4O_4Na$: 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%). $[\alpha]_D^{27}$ +22.9 (*c* 0.96, MeOH). IR ν_{max} 3382, 3240 (OH, NH), 2921, 2851, 1638, 1136 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.03 (br s, 1H, NH), 9.80 (br s, 1H, NH), 8.13 (s, 1H, H-5''), 4.97 (dd, 1H, $^2J_{1'a,1'b}$ 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, $^2J_{H,H}$ 6.99, -CH_2(CH_2)$_{10}CH_3$), 1.57 (m, 2H, -CH_2CH_2(CH_2)$_9CH_3$), 1.23 (m, 18H, -CH_2CH_2(CH_2)$_9CH_3$), 0.84 (m, 3H, -(CH_2)$_{11}$CH_3. ^{13}C NMR (75.4 MHz, DMSO-d_6, 363 K, δ ppm) δ 146.8 (C-4''), 122.8 (C-5''), 73.0 (C-3), 68.3 (C-4), 59.6 (C-2), 49.3 (C-5), 48.2 (C-1'), 31.2, 29.0, 28.9, 28.8, 28.7, 28.6, 28.5, 24.9, 22.0 (11 CH_2), 13.9 (-(CH_2)$_{11}$*C*H_3). LSIMS *m/z* 353 [80%, $(M+H)^+$], 375 [100%, $(M+Na)^+$]. HRLSIMS calculated for $C_{19}H_{37}N_4O_2$: 353.2917, found 353.2929 $[M+H]^+$.

(2*R*,3*S*,4*R*)-2-[(4''-Dodecyl)-1H-1,2,3-triazole-1-yl)ethyl)-pyrrolidine-3,4-diol hydrochloride (33). Tetradec-1-yne (47 μL, 0.19 mmol), DIPEA (110 μL, 0.64 mmol) and CuI (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 $NaHCO_3$ 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). $[\alpha]_D^{26}$ -8.3 (*c* 0.73, MeOH); IR (ν cm^{-1}) 3362 (OH), 2923, 2852, 1636, 1126, 1048. ^{1}H-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), 3.44 (m, 1H), 3.15 (m, 1H), 2.84 (m, 2H), 2.63-2.46 (m, 2H, -C*H*$_2$(CH$_2$)$_{11}$CH$_3$), 1.75 (br s, 2H, -CH$_2$C*H*$_2$(CH$_2$)$_9$CH$_3$), 1.34 (m, 18H, -CH$_2$CH$_2$(C*H*$_2$)$_9$CH$_3$), 0.89 (t, 3H, $J_{H,H}$ 5.9, -(CH$_2$)$_{11}$C*H*$_3$). ^{13}C-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, 30.6, 30.5, 30.3, 30.2, 29.6, 28.3, 25.1, 23.6, 14.3 (-(CH$_2$)$_{11}$*C*H$_3$). CIMS *m/z* 367 [100%, $(M+H)^+$]. HRCIMS *m/z* found 367.3071, calc. for $C_{20}H_{39}N_4O_2$: 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% CO_2.

Colorimetric cell growth assays. 5 x 10^3 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. IC_{50}s were estimated using GraphPad Sofware (GraphPad, La Jolla, CA, USA).

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