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Single- and double-chained truncated jaspine B analogues: asymmetric synthesis, biological evaluation and theoretical study of an unexpected 5-*endo-dig* process

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ABSTRACT

An optimized synthesis of jaspine B analogues bearing an *n*-octyl or a *p*-fluorophenethyl lipophilic appendage was developed. Key to the approach was the use of acetylenic nucleophiles for the stereocontrolled introduction of the side chain and the implementation of a novel cyclization procedure to build the tetrahydrofuran ring. Three *N*-substituted amine or amide derivatives were also accessed. The biological activity of these four jaspine B analogues was shown to strongly depend on the nature of both the *N*-substituent and the aliphatic moiety connected to the tetrahydrofuran ring. Gratifyingly, the truncated jaspine B derivative proved to be a pro-apoptotic inhibitor of the conversion of ceramide into sphingomyelin. Finally, the efficient formation of a fused bis-furan derivative according to a 5-*endo-dig* process was observed under saponification conditions. On the basis of a theoretical study, a mechanistic pathway was delineated highlighting the Lewis acidity of the K^+ ion as the driving force for this transformation in a strongly alkaline medium.

1. Introduction

Jaspine B (**1**) is an analogue of sphingolipid isolated in 2003 by Debitus from the marine sponge *Jaspis* sp. (Fig 1).[1] The same natural product **1**, named pachastrissamine, was also identified from extracts of the sponge *Pachatrissa* sp.[2] Both studies reported a potent cytotoxicity for compound **1**, with IC_{50} values in the submicromolar range. Synthetic jaspine B was found to act through a dihydroceramide-mediated autophagy in A549 lung cancer cells.[3] We evidenced that the natural compound triggered apoptosis of melanoma cells by blocking the conversion of ceramide into sphingomyelin.[4] The amphiphilic structure of jaspine B embeds a phytosphingosine-like C_{18} skeleton. Central to its structure is the all-*cis* trisubstituted tetrahydrofuran polar head, bearing an apolar myristyl residue. Four distinct non-natural stereoisomers of jaspine B have been disclosed to date, indicating a variable influence of the stereochemistry on cytotoxicity, evaluated on A549 and MCF7 breast cancer cells.[3,5] Assessing the contribution of the lipophilic portion of the molecule, we developed a series of novel cytotoxic sphingomyelin synthase (SMS) inhibitors.[6] We also found that non-amphiphilic analogues retained a substantial potency. In particular, we described the short chain jaspine B analogue **3** displaying cytotoxicity towards melanoma cells comparable to that of the parent natural compound.[7]

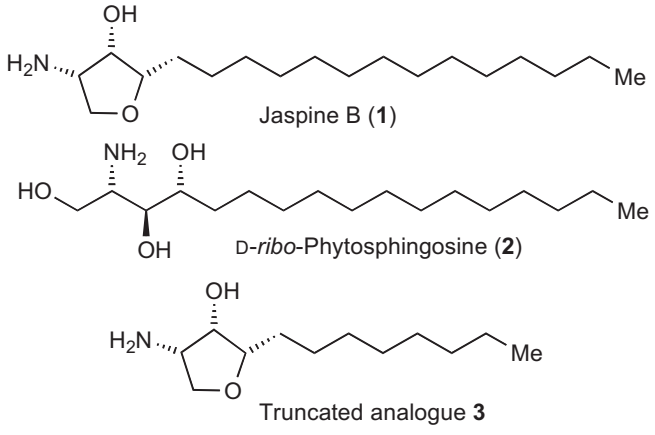

Fig. 1. Structure of the natural jaspine B (**1**), the natural phytosphingosine (**2**) and the synthetic truncated analogue **3**.

Along these lines, we wish to report here the optimized synthesis and the full characterization of a series of single- and double-

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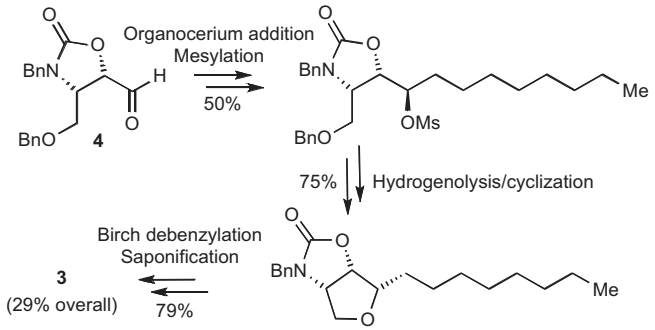
chained truncated jaspine B analogues, as well as a study of the mode of action of the previously described cytotoxic derivative **3**.

2. Synthetic studies

2.1. Synthetic approach

Our previous synthesis[7] of **3** starting from the aldehyde **4** relied on two key steps: (1) the diastereoselective addition of an organocerium reagent in order to introduce the lipophilic chain and (2) the construction of the tetrahydrofuran core by means of a benzylic ether hydrogenolysis-induced intramolecular cyclization of a mesylate intermediate (Scheme 1).[8,9,10–18] Despite of being straightforward, this route suffered from several weak points, such as (1) the moderate efficiency of the aliphatic organometallic addition onto the aldehyde; (2) the use of an hydrogen-promoted cyclization process and (3) the requirement of harsh Birch reaction conditions in order to achieve the *N*-deprotection.

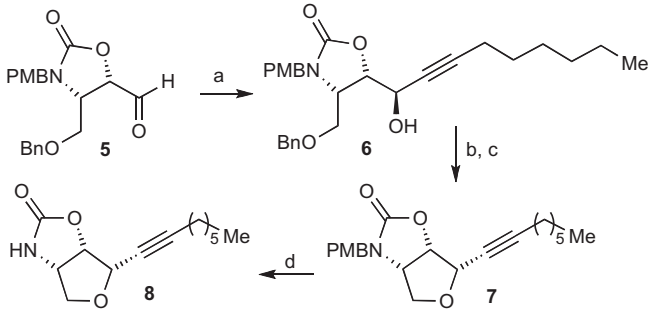


Scheme 1. Previous synthetic route to **3**.[7]

In light of our recent synthetic development, we sought of optimizing this reaction sequence. In particular, our plan was to start from the second-generation *N*-*p*-methoxybenzyl-protected pivotal aldehyde **5** and to take advantage of the new convenient amine-promoted cyclization protocol.[6]

2.2. Preparation of truncated aliphatic analogue 3

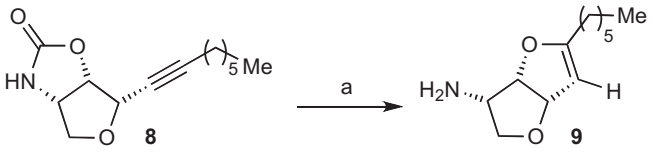
The synthetic sequence leading to **3** started from aldehyde **5**. In order to improve the efficiency of the introduction of the lipophilic chain, we switched from an aliphatic to an acetylenic residue. This was also expected to favor the cyclization process onto the activated propargylic center (vide infra). Thus, treatment of aldehyde **5** with the preformed 1-octyne-derived organocerium reagent led to the secondary aminoalcohol **6**, isolated in 87% yield as a single diastereoisomer (Scheme 2).



Scheme 2. En route to **3**. Reagents and conditions: (a) (i) $CeCl_3$, THF, rt, 16 h; (ii) *n*-BuLi, 1-octyne, THF, 0 °C, 1 h; (iii) aldehyde **5**, THF, −78 °C, 7 h, 87% yield, 100% de; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 1 h, 82%; (c) $BnNH_2$, Et_3N, DMSO, 80 °C, 4 h, 84%; (d) CAN, $MeCN/H_2O$ (9:1), rt, 4 h, 77%.

With the acetylenic intermediate **6** in hand, we implemented the new cyclization protocol. The required mesylate was initially isolated in 82% yield after chromatography. Due to its propensity to undergo spontaneous cyclization, the latter could however not be properly characterized, mass spectrometry and NMR analyses being always 'contaminated' with the corresponding tetrahydrofuran. We thus found more convenient to engage the crude mesylation product in the cyclization step. After smooth heating in DMSO with Et_3N and $BnNH_2$, the expected cyclized product **7** was isolated in 70% yield overall from the alcohol **6** (84% yield based on the chromatographically purified mesylate intermediate). Even though the exact role of both amines is not elucidated, previous studies demonstrated that the process was more efficient in their presence.[6]

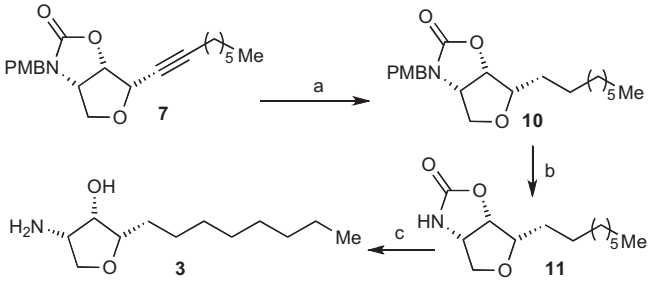
In order to facilitate the oxazolidinone saponification, the nitrogen atom in **7** was first deprotected. Oxidative cleavage of the *p*-methoxybenzyl group proceeded uneventfully to give the expected intermediate **8** in 77% yield. At this stage we explored the possibility of preparing the unsaturated analogue of **3**. Saponification of the acetylenic intermediate **8** led, however, to the highly efficient formation of an unexpected product. Although the mass spectrometry analysis displayed an $[M+H^+]$ ion at *m*/*z* 212 that could account for the expected product, the NMR analysis did not match with the desired structure. In particular, the appearance of a strongly unshielded quaternary ^{13}C at 164.9 ppm and the persistence of three ^1H signals above 4 ppm were puzzling. Extensive 2D NMR analysis led us to propose the bicyclic structure **9** (Scheme 3).



Scheme 3. The unexpected 5-*endo*-*dig* cyclization. Reagents and conditions: (a) KOH, $EtOH/H_2O$ (8:2), 85 °C, 7 h, 92%.

The high efficiency of this unexpected transformation prompted us to explore its mechanism by means of a theoretical study (vide infra).

In order to ensure access to **3**, saturation of the side chain in **7** was thus performed affording **10** (Scheme 4). Smooth cleavage of the *N*-*p*-methoxybenzyl group to give **11** was followed by a final saponification delivering the expected aminoalcohol **3** in high yield.



Scheme 4. The oxazolidinone hydrolysis leading to **3**. Reagents and conditions: (a) $Pd(OH)_2$, MeOH/EtOAc (1:1), 1 bar H_2, rt, 24 h, 98%; (b) CAN, $MeCN/H_2O$ (9:1), rt, 4 h, 78%; (c) KOH, $EtOH/H_2O$ (8:2), 85 °C, 7 h, quant.

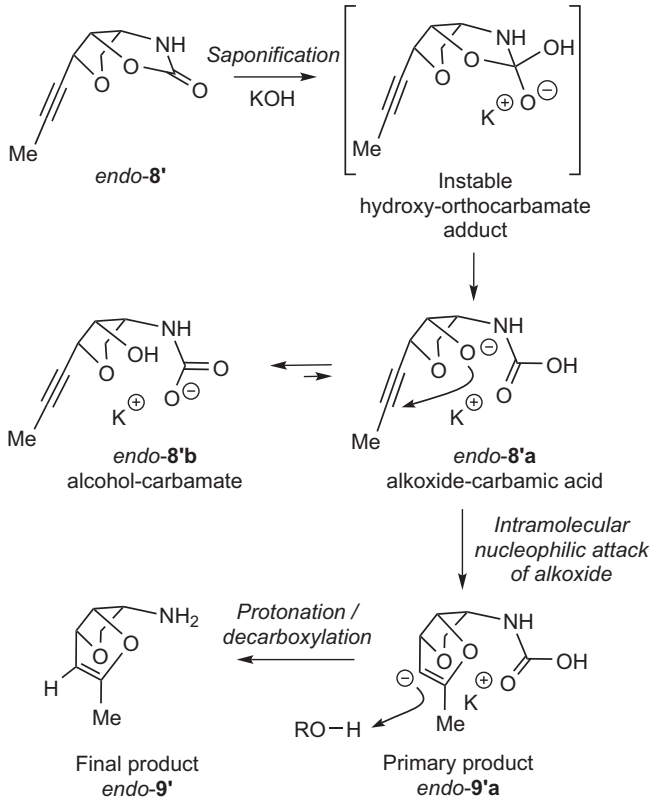
The optimized five-step synthetic sequence thus led to **3** in 46% overall yield from the pivotal aldehyde **5**.

2.3. *N*-Functionalized aliphatic analogues 12, 13, and 14

We then explored the N-functionalization of the truncated jaspine B analogue **3**. Indeed, the *N*-substituent of sphingolipids, by influencing the interaction of these lipids with membranes, largely

orientates their cellular outcome.[19–23] For instance, *N*-acylation of the somewhat water-soluble sphingosine with a fatty acid leads to ceramide, that is, confined to lipid bilayers, the synthetic *N*-acetyl analogue (known as 'C2–ceramide') being on the contrary cell permeable. The overall biological effect of sphingolipids is therefore finely modulated by their *N*-functionalization. For instance, whereas sphingosine is a substrate of sphingosine kinases,[24] the *N*,*N*-dimethyl sphingosine is a potent competitive inhibitor of the isoform 1 of the kinase.[25] Despite early reports of naturally occurring anhydrocerebrins, identified as 1,4-anhydrophytosphingosines *N*-acylated with cerebronic (2-hydroxytetracosanoic) acid,[26] the *N*-functionalization of the jaspine B has merely never been studied to date. The C_{12} skeleton of **3**, in minimizing the amphiphilic character of the molecule, appeared appropriate for the introduction of a second truncated lipophilic moiety.

Introduction of an *N*-alkyl group was envisioned by mean of a reductive amination reaction. Thus, treatment of **3** with an excess of formaldehyde in the presence $NaBH_3CN$ gave the *N*,*N*-dimethyl derivative **12** (Scheme 5). The *N*-hexyl analogue **13**, comprising the same overall length of lipophilic chain as the parent jaspine B (C_{14}), was also obtained from the reaction with *n*-hexanal.



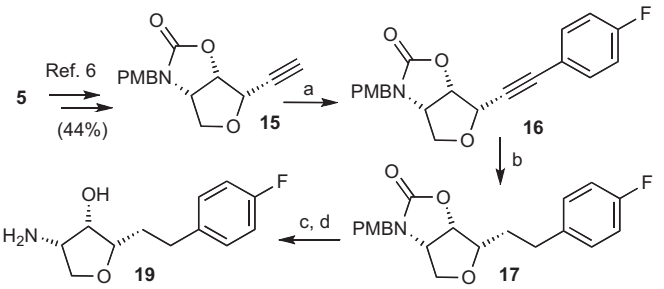
Scheme 5. *N*-Functionalization of **3**. Reagents and conditions: (a) aq HCHO, $NaBH_3CN$, AcOH, $MeCN/H_2O$ (3:1), rt, 24 h, 43%; (b) *n*-hexanal, $NaBH_3CN$, MeOH, rt, 20 h, 75%; (c) *p*-nitrophenyl caprilate, THF, rt, 48 h, 63%.

In order to assess the influence of the basic nitrogen atom, we also prepared an amide derivative. The *N*-octanoyl derivative **14** was obtained reacting **3** with *p*-nitrophenyl caprilate (Scheme 5).

2.4. Preparation of truncated aromatic analogue 19

We previously reported several aromatic ring-containing chain-modified jaspine B analogues displaying potent cytotoxic effects and SMS inhibitory activities.[6] In the context of the present study, we envisioned the replacement of the *n*-octyl chain in **3** by a short aromatic ring-ended lipophilic appendage, such as a *p*-fluorophenethyl radical, embedding the same total number of carbon atom.

The targeted compound was prepared from the acetylenic building block **15**, obtained in four steps and 44% overall yield from aldehyde **5** (Scheme 6).[6] Sonogashira coupling with 4-fluoroiodobenzene proceeded efficiently to afford the arylation product **16** in 70% yield. After catalytic hydrogenation, the structure of the saturated intermediate **17** was confirmed by X-ray diffraction analysis of a crystalline sample (Fig. 2).



Scheme 6. Synthesis of the aromatic analogue **17**. Reagents and conditions: (a) IC_6H_4F, $PdCl_2(PPh_3)_2$, CuI, $(i\text{-}Pr)_2NH$, THF, rt, 4 h, 70%; (b) $Pd(OH)_2$, MeOH/EtOAc (1:1), 1 bar H_2, rt, 24 h, 96%; (c) CAN, $MeCN/H_2O$ (9:1), rt, 4 h, 93%; (d) KOH, $EtOH/H_2O$ (8:2), 85 °C, 7 h, 96%.

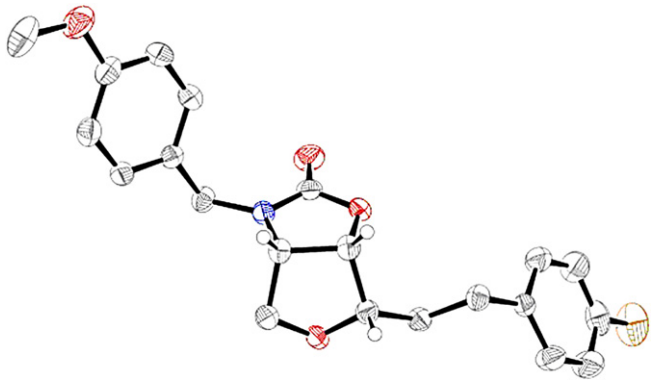


Fig. 2. Molecular view of the oxazolidinone **17** in the solid state (thermal ellipsoids at 50% probability); hydrogen are omitted for clarity excepted on asymmetric carbons.[27]

Importantly, this structure further confirmed the all-*cis* arrangement of the three substituents present on the tetrahydrofuran ring. Finally, a high yielding two-step deprotection sequence delivered the expected jaspine B analogue **19**.

3. Biological studies

3.1. Cell viability assays

The lethal effect of **3** and its *N*-functionalized derivatives **12**, **13** and **14** were evaluated on tumor cell growth and compared to that obtained with the natural jaspine B. As illustrated in Fig. 3, the single-chained truncated compound **3** exhibited a dose-dependent cytotoxic activity, leading to a reduction of 50 % in cell viability of B16 melanoma cells after 24 h exposure to 2.5 μM. Derivative **3** was somewhat less effective than the natural molecule bearing an aliphatic C_{14} skeleton but displayed a higher or similar cytotoxicity than its double-chained analogues **13** and **14**. The presence of an alkyl chain in the *N*-hexyl compound **13** led to a decrease of cytotoxicity compared to the *N*-octanoyl analogue **14**. Conversely, the dimethylation of the amine in **3** (compound **12**) leads to a complete loss of cytotoxicity, even at concentrations above 10 μM. The

replacement of the *n*-octyl chain in **3** by a short aromatic ring-ended chain (compound **19**) also abolished all cytotoxic effect at the concentrations used. Altogether, these results suggest that, while the length of the aliphatic chain of jaspine B has a moderate effect on its cytotoxicity, the nature of the *N*-substituent and the aliphatic portion of the molecule can strongly impact its biological activity.

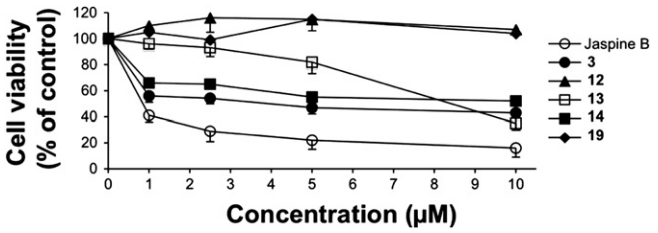


Fig. 3. Effect of natural jaspine B, single- and double-chained truncated analogues on B16 melanoma cell viability.

3.2. Study of the biological effects of the truncated analogue 3

The ability of the single-chained aliphatic truncated jaspine B analogue **3** to induce apoptotic cell death and to alter sphingolipid metabolism was evaluated in B16 melanoma cells. Indeed, we previously observed that the natural anhydrophytosphingosine was able to induce apoptosis of melanoma cells by acting on the conversion of pro-apoptotic ceramide into sphingomyelin (SM), through the inhibition of SMS1 activity.[4] Here, we reported that executioner caspase activity, a marker of apoptosis, measured by the cleavage of the fluorogenic tetrapeptide substrate DEVD-AMC, increased in melanoma cells within 6 h, peaking at 16 h after treatment with compound **3** (Fig 4A). Activation of caspase-3, i.e., the appearance of cleaved forms, was further demonstrated by Western blot analysis (Fig. 4B).

SM. As illustrated in Fig. 4D, the truncated analogue **3** elicited a decrease in SMS activity. All these effects were comparable to that observed with the natural jaspine B.

4. Theoretical studies

Saponification of the acetylenic intermediate **8** led to **9** via the formation of the dihydrofuran ring that would result from an intramolecular attack of the transient potassium alkoxide onto the triple bond, according to a 5-*endo*-*dig* process (Scheme 3).[28] Such a pathway, believed to be favored by proper orbital overlaps, is in agreement with the Baldwin's rules. However, its high efficiency in the absence of any apparent acidic activation of the acetylenic moiety seemed surprising. This serendipitous observation prompted us to seek for mechanistic insights with the help of a theoretical study of this cyclization process.

Thorough examination of the literature indicated that formation of a dihydrofuran via the intramolecular cyclization of a homopropargylic alcohol under alkaline conditions had been only sporadically reported. After the pioneering study by Holand and Epsztein,[29] similar transformations were either used as synthetic steps[30,31] or observed as side reactions.[32] Recently, an example related to ours, yet relying on the promoting effect of a fluorine atom at the propargylic position, was described.[33] It was noteworthy to us that all these examples concerned homopropargylic alcohol substrates bearing an oxy group at the α-,[30] β-,[29,33] or γ-position[31,32] with respect to the attacked triple bond.

As in the above examples, the feasibility of the 5-*endo*-*dig* conversion of **8** to **9** could be attributed to a specific role of the alkaline metal cation in electrophilic activation of the alkynyl substituent towards the homopropargylic alcoholic terminus. The K^+ ion would thus play the role of the 'a priori required acid in the overall strongly basic medium'. This hypothesis was examined through DFT calculations on a model compound **8′** of **8** where the fatty alkyl substituent of the triple bond was restricted to a methyl

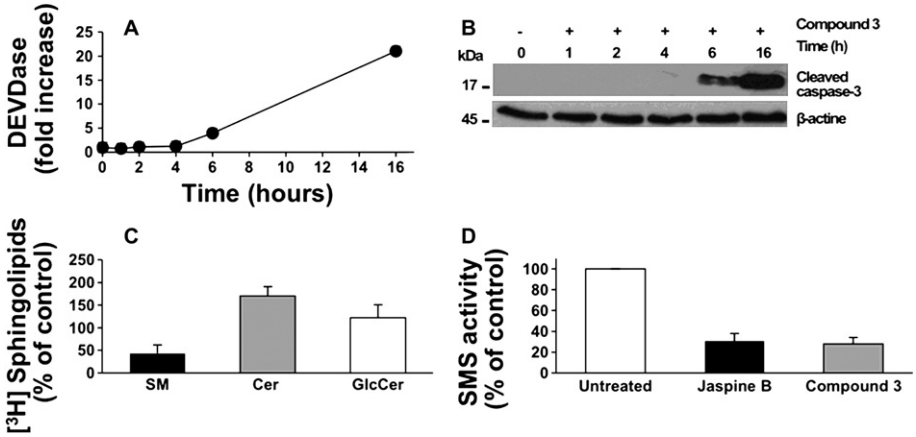


Fig. 4. Effect of the single-chained truncated compound **3** on caspase activation and sphingolipid metabolism in B16 melanoma cells.

After labelling B16 cells with radioactive sphingosine, we observed that the sphingolipid pattern was perturbed in cells treated by analogue **3** as compared to untreated cells. Interestingly, the ceramide content has strongly increased whereas SM level has decreased by 60 % (Fig. 4C). In sharp contrast, no variation was observed for the cellular content of glucosylceramide (GlcCer). As inhibition of SMS activity can account for the changes observed in the levels of SM and ceramide, we measured the in situ SMS activity by incubating intact living cells with a fluorescent analogue of ceramide (C_6–NBD–ceramide), that is, converted into fluorescent

group. Most of the calculations were performed taking into account a water medium within SCRF calculations (see computational details) as a model of the experimental ethanol/water solvent mixture.

The oxygen atom of the starting all-*cis* tetrahydrofuran ring may be pointing either *towards* or *away* from the oxazolidinone ring (Fig. 5). At the B3PW91/6-31G** level of calculation, the respective *endo* and *exo* conformations of **8′** are isoenergetic. Both conformers were thus considered as possible reactants in the various reaction pathways investigated.

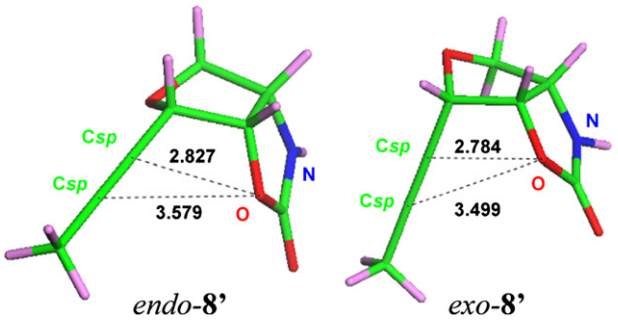
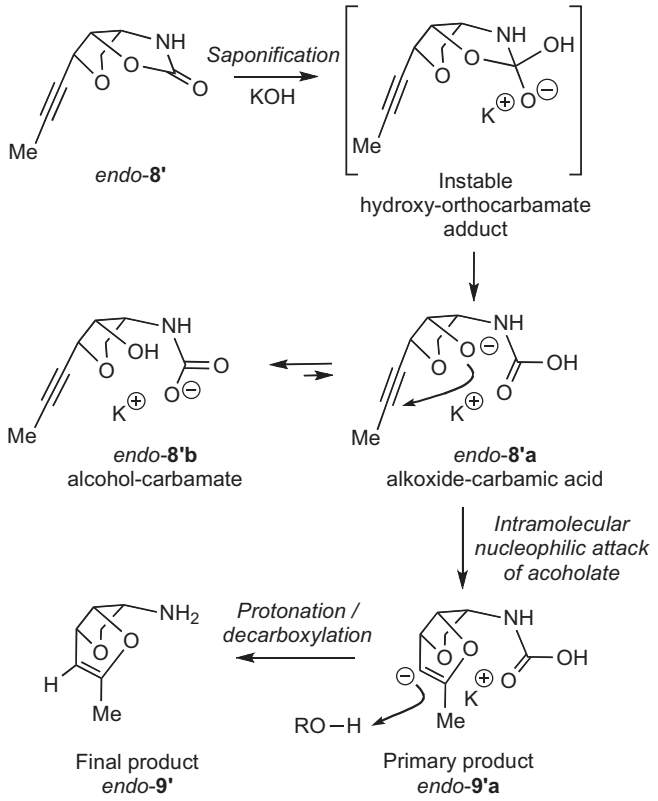


Fig. 5. Isoenergetic *endo* and *exo* conformations of **8′**. Bond distances are given in Å. B3PW91/6-31G** level of calculation.

Since the presence of oxy groups in the vicinity of the reacting site seemed to be required from the literature (vide supra), the computational studies focused on a cyclization step directly ensuing from the initial saponification of the oxazolidinone **8′**, thus yielding a primary carbamic acid **9′** (Scheme 7). Although unlikely in view of the present study, the participation of amino alkoxide intermediates could not be formally excluded. Subsequent protonation and decarboxylation of **9′** was expected to yield the final fused bis-furan derivative **9**.



Scheme 7. Structure of the saponification product of *endo*-**8′** and possible tautomeric forms resulting from the initial hydrolysis of the oxazolidinone ring anticipated from SCRF calculations performed in aqueous medium (PCM, ε=78.3553) at the B3PW91/6-31G** level.

The primary 'hydroxy-orthocarbamate' adduct (Scheme 7) expected from the saponification of *endo*-**8′**, could not be isolated from SCRF calculations as a minimum on the PES. Two tautomers **8′a**,**b** resulting from the opening of oxazolidinone ring were obtained instead. The alkoxide–carbamic acid intermediate *endo*-**8′a** was calculated to be less stable by 19.1 kcal/mol than its alcohol/carbamate tautomer *endo*-**8′b** (Table S1, Scheme 7). Starting from the *exo*-**8′** conformer, a similar situation was found, albeit at slightly higher energies (Table S1, Fig. 6)

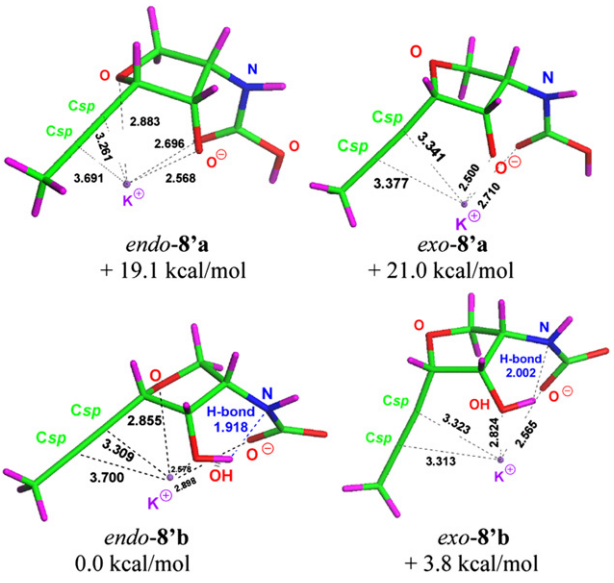


Fig. 6. Calculated structures and Gibbs energies of *endo* and *exo* conformations of the intermediates **8′a** and **8′b** in aqueous medium. Bond distances are given in Å. SCRF calculations (PCM, ε=78.3553) performed at the B3PW91/6-31G** level.

In the calculated structures of **8′a** and **8′b**, as drawn in Scheme 7, the $K^{+}\cdots O^{-}$ distances ranging from 2.6 to 2.7 Å (Fig. 6) were indicative of strong K–O bonds with partial ionic character. Coordination of the potassium cation to other oxygen atoms was indicated by still short $K^{+}\cdots O$ distances lying in the 2.8–2.9 Å range. Electrophilic activation of the C≡C bond by the potassium cation was revealed by rather short K···C*sp* distances ranging from 3.3 to 3.7 Å, which are shorter than a non-bonding $K^{+}\cdots C$ contact distance (3.73 Å) and much shorter than the sum of van der Waals radii of the corresponding neutral atoms (4.45 Å).

In *endo*-**8′a** and *endo*-**8′b**, coordination of the tetrahydrofuran oxygen atom to K^{+} was likely to be responsible for a stabilization with respect to the *exo*-isomers, wherein this oxygen atom is located far away from the potassium centre (e.g., by 3.8 kcal/mol for *endo*-**8′b** vs *exo*-**8′b**). Whereas the triple bond was symmetrically 'η^2-coordinated' in the *exo* isomer, the additional O→K bond of the *endo* isomer shifted the potassium cation closer to the internal C*sp* atom, thus repelling it away from the external C*sp* atom. This desymmetrization would make the distal C*sp* atom regioselectively more electrophilic and thus more prone to undergo nucleophilic attack by the homopropargylic oxygen atom.

Starting from either the *endo* or *exo* conformations of **8′a** and **8′b**, four reaction pathways were investigated. All the processes were found endothermic and the corresponding Gibbs free energy profiles are shown in Fig. 7. The highest energy barriers (ca. 35–40 kcal/mol) were obtained for the most stable *exo* and *endo* tautomers **8′b**, and the energy barrier was lower for the *endo* isomer in agreement with the structural analysis of the reactants (Table 1). In contrast, the lowest energy barriers obtained from the less stable tautomers **8′a** were almost identical for both the *endo* and *exo* conformers (ca. 20 kcal/mol).

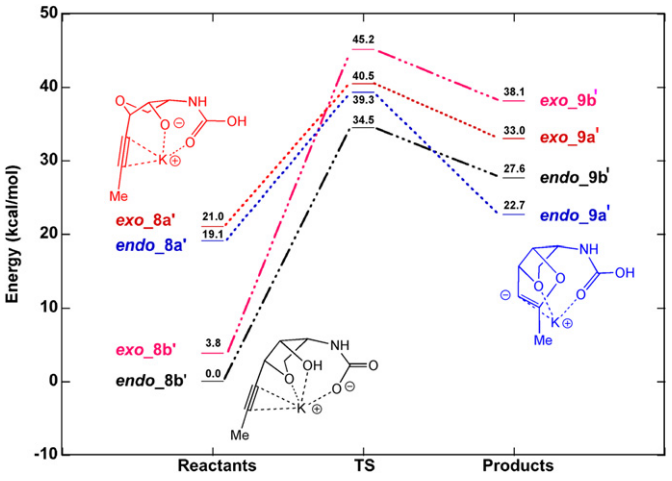


Fig. 7. Computed Gibbs energy profiles of the determining steps of the KOH-induced 5-*endo-dig* cyclization of **8′** in aqueous medium. SCRF calculations (PCM, ε=78.3553) performed at the B3PW91/6-31G** level.

Table 1
Gibbs energies (ΔG) and Gibbs energy barriers ($\Delta G^{\#}$) (in kcal/mol at 298.15 K) of the reaction pathways shown in Fig. 4. SCRF calculations (PCM, ε=78.3553) performed at the B3PW91/6-31G** level

Reactant	$\Delta G^{\#}$	ΔG
endo-**8′a**	20.19	3.57
exo-**8′a**	19.45	11.96
endo-**8′b**	34.49	27.63
exo-**8′b**	41.32	34.29

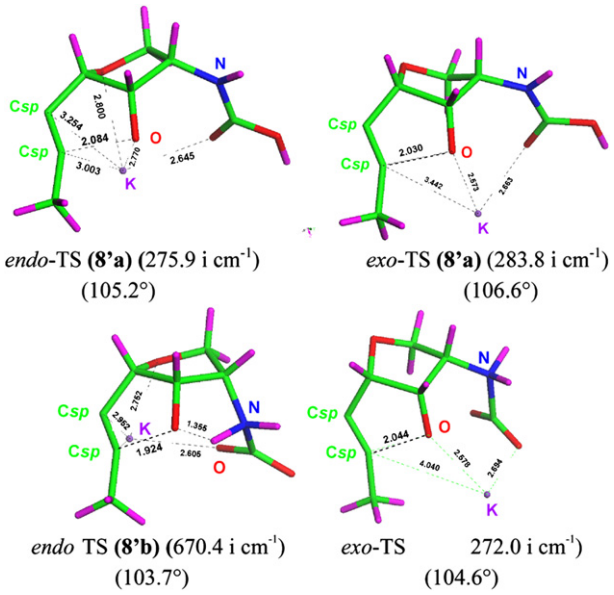


Fig. 8. Calculated structures of *endo* and *exo* transition states in aqueous medium. *CH*$_3$–*Csp*–O angles are given in parentheses. Bond distances are given in Å. SCRF calculations (PCM, ε=78.3553) performed at the B3PW91/6-31G** level.

According to the Curtin–Hammett principle, the kinetically favored cyclization pathway would thus proceed through the **8′a** tautomer and involve the most nucleophilic homopropargylic oxygen atom, namely an alkoxide. Although slightly less stable than the *endo*-**8′a** conformer by 1.9 kcal/mol (Table S1), the most effective reacting conformer was *exo*-**8′a** (albeit by only a 0.74 kcal/mol difference in Gibbs energy barrier). The corresponding absolute Gibbs energy barrier of 19.5 kcal/mol is compatible with the experimental conditions (vide supra).

The calculated structures of the transition states are shown in Fig. 8, whereas those of the primary cyclization products **9′a**,**b** are given in Supplementary data (Fig. S1).

In agreement with the Hammond postulate, the four endothermic processes involved late transition states (Fig. 8), whose structures were indeed very close to those of the corresponding primary products (Fig. S1). The C*H*$_3$–C*sp*–O angles deviated by ca. 15° from the ideal 120° value of the Baldwin rules,[28] but the normal mode related to the imaginary frequency involved mainly a shortening of the O–C*sp* bond, as expected. Starting from the *endo*-**8′b** reactant, the reaction coordinate indeed combined a shortening of the O–C*sp* bond and a proton transfer from the reacting hydroxyl to the nitrogen atom of the carbamate moiety.

Finally, calculations indicated that, in the absence of a potassium cation, the bicyclic structure of **8′** is retained upon addition of OH^- to the carbamate carbonyl group. The resulting anion is however unlikely to occur because of its high calculated energy as compared to those of *endo*-**8′a** and *endo*-**8′b**. These results showed that the potassium cation would play the determining role in the 5-*endo-dig* cyclization of homopropargylic alcohol derivatives under basic conditions, in enhancing the electrophilic character of the C≡C bond. The Lewis acidity of the K^+ ion could therefore be considered as the driving force of the process in a strongly alkaline medium.

5. Conclusion

An optimized preparation of the C_{12} jaspine B analogue **3** was accomplished relying on a new cyclization process to build the tetrahydrofuran core. Structural variations of the jaspine B were also explored through the synthesis of four other single or double-chained analogues. Biological evaluations of the prepared analogues showed that, even though varying the nature of the *N*-substituent or the lipophilic chain could not enhance the cytotoxicity, the truncated analogue of reference, **3**, displayed a characterized pro-apoptotic behavior towards B16 melanoma cells. Finally, an unexpected, yet highly efficient, 5-*endo-dig* cyclization was observed in the course of the saponification of an oxazolidinone intermediate. On the basis of theoretical calculations, a mechanistic scenario was elaborated that emphasized the key role of the potassium cation in enhancing the electrophilicity of the acetylenic partner.

6. Experimental section

6.1. General details

The following solvents and reagents were dried prior to use: CH_2Cl_2, MeOH, DMF (from calcium hydride), 1,2-dimethoxy-ethane, Et_2O, petroleum ether, THF, toluene (freshly distilled from sodium/benzophenone), Et_3N (from calcium hydride, stored over KOH pellets). Analytical thin layer chromatography (TLC) was performed using Merck silica gel $60F_{254}$ precoated plates. Chromatograms were observed under UV light and/or were visualised by heating plates that were dipped in 10% phosphomolybdic acid in EtOH or Dragendorff reagent. Column chromatographies were carried out with SDS 35–70 μm flash silica gel. NMR spectroscopic data were obtained with Bruker Advance 300. Chemical shifts are quoted in parts per million (ppm) relative to residual solvent peak. *J* values are given in hertz. For matter of homogeneity, sphingolipid numbering is used for NMR assignment throughout the experimental section. Infrared (IR) spectra were recorded on a Perkin–Elmer FT-IR 1725X spectrometer. Mass spectrometry (MS) data were obtained on a ThermoQuest TSQ 7000 spectrometer. High-

resolution mass spectra (HRMS) were performed on a Thermo-Finnigan MAT 95 XL spectrometer. Optical rotations were measured on a Perkin–Elmer model 241 spectrometer. Crystallographic data were collected at low temperature (180 K) on a Bruker Kappa Apex II diffractometer using a graphite-monochromated Mo Kα radiation (λ=0.71073 Å) and equipped with an Oxford Cryosystems Cryostream cooler device. The structure was solved by direct methods[34] and all non hydrogen atoms were refined anisotropically using the least-squares method on F^2.[35] Natural jaspine B was purified from crude extracts of the sponge *Jaspis* sp. kindly provided by Dr C. Debitus (UMR 152 IRD, Toulouse, France). DMEM, trypsin–EDTA and fetal calf serum (FCS) were from Invitrogen (Cergy-Pontoise, France). 3-(4,5-Dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide (MTT) was supplied from Euromedex (Mundolsheim, France). C_6–NBD–ceramide was purchased from Interchim (Montluçon, France). Murine B16 melanoma cell line was purchased from American Type Culture Collection (LGC, Molsheim, France).

6.2. General chemical procedures

6.2.1. General procedure A: oxidative cleavage of the p-methoxybenzyl group. To a solution of *N*-PMB intermediate (1.0 equiv) in CH_3CN/H_2O (9:1) (0.05 M) was added CAN (6.0 equiv). The mixture was stirred at room temperature for 2 h, then diluted with water and extracted three times with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and with brine, dried over $MgSO_4$, and concentrated in vacuo.

6.2.2. General procedure B: saponification of the carbamate. KOH (10 equiv) was added to a solution of oxazolidinone (1.0 equiv) in $EtOH/H_2O$ (8:2) (0.05 M). The mixture was heated at 85 °C for 7–10 h (TLC monitoring) before being cooled to room temperature and diluted with EtOAc and brine. The mixture was extracted three times with EtOAc and the combined extracts were concentrated in vacuo.

6.2.3. General procedure C: hydrogenation of the triple bonds. A solution of unsaturated compound (1.0 equiv) in EtOAc/MeOH (1:1) (0.1 M) containing $Pd(OH)_2$ (20 wt %) was stirred under H_2 at atmosphere pressure (balloon) overnight. The reaction mixture was then filtered over Celite. The precipitate was rinsed with CH_2Cl_2 and the filtrate was concentrated in vacuo.

6.3. Specific chemical procedures and characterization data

*6.3.1. (4S,5S)-4-(Benzyloxymethyl)-5-((R)-1-hydroxynon-2-ynyl)-3-(4-methoxybenzyl) oxazolidin-2-one (**6**).* A suspension of anhydrous $CeCl_3$ (0.86 g, 3.52 mmol) in anhydrous THF (10 mL) under nitrogen atmosphere was stirred overnight at room temperature. The suspension of $CeCl_3$ was cooled to −78 °C and a freshly prepared solution of octynyl lithium generated by addition of *n*-BuLi (2.65 mL of a 1.6 M commercial solution in hexanes, 4.20 mmol) to 1-octyne (620 μL, 4.20 mmol) in anhydrous THF (10 mL) at −20 °C was added dropwise. The reaction mixture was stirred at −78 °C for 1 h. Then aldehyde **5** (250 mg, 0.70 mmol) in solution in anhydrous THF (2 mL) was added and the mixture stirred at −78 °C for 6 h. The reaction was quenched by addition of a saturated aqueous solution of NH_4Cl and the mixture was directly filtered over Celite eluted by EtOAc. The aqueous layer was extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude material was purified by column chromatography on SiO_2 eluted with PE/EtOAc (70:30 to 60:40) to give **6** (285 mg, 87%) as a colorless oil. $[\alpha]_D^{20}$ +27.8 (*c* 0.8, $CHCl_3$); IR (film) ν_{OH} 3402, $\nu_{C=O}$ 1752 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42–7.22 (5H, m, OCH_2Ph), 7.09 (2H, d, ^3J 8.6 Hz, NCH_2Ph), 6.81 (2H, d, ^3J 8.6 Hz, NCH_2Ph), 4.70 (1H, d, $^2J_{gem}$ 15.0 Hz, NCH_2Ph), 4.70–4.65 (1H, m, H_4), 4.48 (2H, ABq, $^2J_{gem}$ 11.8, $\Delta\delta$ 40.4 Hz, OCH_2Ph), 4.47 (1H, dd, ^3J 8.1, 5.4 Hz, H_3), 3.94 (1H, d, $^2J_{gem}$ 15.0 Hz, NCH_2Ph), 3.88–3.64 (6H, m, OCH_3, 2×H_1, H_2), 2.17 (2H, td, $^3J_{H7-H8}$ 7.1, $^5J_{H7-H4}$ 1.9 Hz, H_7), 1.54–1.41 (2H, m, 2×H_8), 1.40–1.18 (6H, m, 2×H_9, 2×H_{10}, 2×H_{11}), 0.87 (3H, t, ^3J 7.0 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.3 (Cq, Ph), 157.6 (C=O), 136.6 (Cq, Ph), 129.5, 128.7, 128.4, 128.3 (CH, Ph), 127.9 (Cq, Ph), 114.2 (CH, Ph), 88.1 (C_5), 77.7 (C_3), 77.2 (C_6), 73.7 (OCH_2Ph), 65.2 (C_1), 61.2 (C_4), 55.8 (C_2), 55.3 (OCH_3), 45.9 (NCH_2Ph), 31.4, 28.7, 28.4, 22.6, 18.8 (5×CH_2, C_7 to C_{11}), 14.1 (CH_3); MS (DCI/CH_4) *m*/*z* 466 $[M+H]^+$, 100%; 494 $[M+C_2H_5]^+$, 56%; HRMS (CI) *m*/*z* $C_{28}H_{36}NO_5$ requires 466.2593; found 466.2601.

*6.3.2. (3aS,6S,6aS)-3-(4-Methoxybenzyl)-6-(oct-1-ynyl)tetrahydrofuro[3,4-d]oxazol-2(3H)-one (**7**).* To solution of alcohol **6** (210 mg, 0.45 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 °C under nitrogen atmosphere was added Et_3N (156 μL, 1.12 mmol). After 15 min of stirring at the same temperature mesyl chloride (190 μL, 1.12 mmol) was added and the solution was stirred at 0 °C for 30 min then at room temperature for 1 h. The reaction was quenched by addition of water and the mixture was extracted three times with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude material was purified by column chromatography on SiO_2 eluted with PE/EtOAc (70:30 to 60:40) to give the expected mesylate (200 mg, 82%) as a colorless oil. However, this intermediate gave rise to spontaneous cyclization and was thus routinely used without purification for the next step.

To a solution of the chromatographically purified mesylate (195 mg, 0.36 mmol) in anhydrous DMSO (6 mL) at room temperature and under nitrogen atmosphere were added Et_3N (150 μL, 1.08 mmol) and $BnNH_2$ (59.0 μL, 0.54 mmol). The solution was heated at 80 °C with stirring for 4 h. The mixture was then diluted with CH_2Cl_2 and the reaction quenched by addition of water. The mixture was extracted three times with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude material was purified by column chromatography on SiO_2 eluted with PE/EtOAc (60:40) to give **7** (108 mg, 84%) as a white solid. This sequence was run without purification of the intermediate mesylate with equivalent efficiency (i.e., 70% overall yield). $[\alpha]_D^{20}$ +105.2 (*c* 1.2, $CHCl_3$); IR (film) $\nu_{C=O}$ 1740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.18 (2H, d, ^3J 8.6 Hz, NCH_2Ph), 6.86 (2H, d, ^3J 8.6 Hz, NCH_2Ph), 4.80 (1H, dd, $^3J_{H3-H2}$ 7.6, $^3J_{H3-H4}$ 4.2 Hz, H_3), 4.65 (1H, d, $^2J_{gem}$ 15.0 Hz, NCH_2Ph), 4.34–4.27 (1H, m, H_4), 4.11 (1H, d, $^2J_{gem}$ 15.0 Hz, NCH_2Ph), 4.07 (1H, dd, $^3J_{H2-H3}$ 7.9, $^3J_{H2-H1'}$ 4.0 Hz, H_2), 3.91 (1H, d, $^2J_{gem}$ 10.6 Hz, H_1), 3.85 (3H, s, OCH_3), 3.36 (1H, dd, $^2J_{gem}$ 10.6, $^3J_{H1'-H2}$ 4.0 Hz, $H_{1'}$), 2.24 (2H, td, $^3J_{H7-H8}$ 7.1, $^5J_{H7-H4}$ 1.8 Hz, H_7), 1.60–1.46 (2H, m, H_8), 1.44–1.18 (6H, m, 3×CH_2, H_9 to H_{11}), 0.87 (3H, t, ^3J 6.7 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.4(Cq, Ph), 157.0 (C=O), 129.5 (CH, Ph), 127.1 (Cq, Ph), 114.2 (CH, Ph), 90.5 (C_5 or C_6), 77.1 (C_3), 73.6 (C_4), 71.3 (C_6 or C_5), 69.3 (C_1), 59.4 (C_2), 55.2 (OCH_3), 46.2 (NCH_2Ph), 31.2, 28.4, 28.2, 22.4, 18.8 (C_7 to C_{11}), 13.9 (Me); MS (ESI) *m*/*z* 380 $[M+Na]^+$, 100%; HRMS (CI) *m*/*z* $C_{21}H_{27}NO_4Na$ requires 380.1838; found 380.1858.

*6.3.3. (3aS,6S,6aS)-6-(Oct-1-ynyl)tetrahydrofuro[3,4-d]oxazol-2(3H)-one (**8**).* Prepared from *N*-PMB derivative **7** (70.0 mg, 0.19 mmol) according to the general procedure A. The crude material was purified by column chromatography on SiO_2 eluted with CH_2Cl_2/EtOAc (60:40 to 50:50) to give **8** (30.0 mg, 65%) as a white solid. $[\alpha]_D^{20}$ +153.4 (*c* 1.4, $CHCl_3$); IR (film) $\nu_{C=O}$ 1727 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.80 (1H, ls, NH), 4.97 (1H, dd, $^3J_{H3-H2}$ 7.6, $^3J_{H3-H4}$ 4.0 Hz, H_3), 4.39 (1H, dd, $^3J_{H2-H3}$ 7.6, $^3J_{H2-H1}$ 4.2 Hz, H_2), 4.30 (1H, dt, $^3J_{H4-H3}$ 4.0, $^5J_{H4-H7}$ 1.9 Hz, H_4), 3.98 (1H, d, $^2J_{gem}$ 10.5 Hz, H_1), 3.57 (1H, dd, $^2J_{gem}$ 10.5, $^3J_{H1'-H2}$ 4.2 Hz, $H_{1'}$), 2.24 (2H, dt, $^3J_{H7-H8}$ 7.1, $^5J_{H7-H4}$ 1.9 Hz, H_7), 1.58–1.46 (2H, m, H_8), 1.44–1.16 (6H,

m, H_9 to H_{11}), 0.86 (3H, t, ^3J 6.8 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.2 (C=O), 90.3 (C_5 or C_6), 80.3 (C_3), 73.4 (C_4), 73.2 (C_1), 71.4 (C_6 or C_5), 56.7 (C_2), 31.2, 28.4, 28.2, 22.4, 18.7 (C_7 to C_{11}), 13.9 (Me); MS (DCI/NH_3) m/z 260 $[M+Na]^+$, 100%; HRMS (CI) m/z $C_{13}H_{19}NO_3Na$ requires 260.1263; found 260.1270.

*6.3.4. (3S,3aS,6aS)-5-Hexyl-2,3,3a,6a-tetrahydro-furo[3,2-b]furan-3-amine (**9**).* Prepared from oxazolidinone **8** (17.2 mg, 0.07 mmol) according to the general procedure B. The crude material was purified by column chromatography on SiO_2 eluted with EtOAc/MeOH/NH_4OH (94.2:5:0.8) to give **9** (14.0 mg, 91%) as a colorless oil. $[\alpha]_D^{20}$ −4.2 (*c* 1.3, $CHCl_3$); IR (film) ν_{NH} 3430 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.29 (1H, dd, $^3J_{H4-H3}$ 6.2, $^3J_{H4-H5}$ 2.6 Hz, H_4), 4.69 (1H, d, $^3J_{H5-H4}$ 2.6 Hz, H_5), 4.64 (1H, pseudot, $^3J_{H3-H4} \approx {}^3J_{H3-H2} \approx 5.9$ Hz, H_3), 3.86 (1H, dd, $^2J_{H1-H1'}$ 8.6, $^3J_{H1-H2}$ 6.7 Hz, H_1), 3.45 (1H, ddd, $^3J_{H2-H1'}$ 10.4, $^3J_{H2-H1}$ 6.7, $^3J_{H2-H3}$ 5.6 Hz, H_2), 3.02 (1H, dd, $^3J_{H1'-H2}$ 10.4, $^2J_{H1'-H1}$ 8.6 Hz, $H_{1'}$), 2.17 (2H, t, $^3J_{H7-H8}$ 7.6 Hz, 2×H_7), 1.60–1.44 (4H, m, 2×H_8, NH_2), 1.40–1.20 (6H, m, 3×CH_2), 0.86 (3H, t, ^3J 6.8 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.9 (C_6), 95.5 (C_5), 85.7 (C_4), 83.7 (C_3), 68.4 (C_1), 56.1 (C_2), 31.5 (C_7), 28.9, 27.9, 26.7, 22.6 (C_8 to C_{11}), 14.1 (C_{12}); SM (DCI/NH_3) m/z 212 $[M+H]^+$, 60%; 234 $[M+Na]^+$, 65%; HRMS (CI) m/z $C_{12}H_{22}NO_2$ requires 212.1651; found 212.1653.

*6.3.5. (3aS,6S,6aS)-3-(4-Methoxybenzyl)-6-octyl-tetrahydrofuro[3,4-d]oxazol-2(3H)-one (**10**).* Prepared from alkyne **7** (80.0 mg, 0.22 mmol) according to the general procedure C. The crude material was purified by column chromatography on SiO_2 eluting with CH_2Cl_2/EtOAc (95:5) to give **10** (79.0 mg, 98%) as a white solid. $[\alpha]_D^{20}$ +70.8 (*c* 1.0, $CHCl_3$); IR (film) $\nu_{C=O}$ 1737 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (2H, d, ^3J 8.6 Hz, NCH_2*Ph*), 6.86 (2H, d, ^3J 8.6 Hz, NCH_2*Ph*), 4.74 (1H, dd, $^3J_{H3-H2}$ 7.6, $^3J_{H3-H4}$ 3.8 Hz, H_3), 4.69 (1H, d, $^2J_{gem}$ 15.0 Hz, NCH_2Ph), 4.07 (1H, d, $^2J_{gem}$ 15.0 Hz, NCH_2Ph), 4.06 (1H, dd, $^3J_{H2-H3}$ 7.6, $^3J_{H2-H1'}$ 4.0 Hz, H_2), 3.92 (1H, d, $^2J_{gem}$ 10.6 Hz, H_1), 3.78 (3H, s, OCH_3), 3.48 (1H, td, $^3J_{H4-H5}$ 6.8, $^3J_{H4-H3}$ 3.8 Hz, H_4), 3.29 (1H, dd, $^2J_{gem}$ 10.6, $^3J_{H1'-H2}$ 4.0 Hz, $H_{1'}$), 1.82–1.68 (2H, m, 2×H_5), 1.50–1.16 (12H, m, 6×CH_2), 0.86 (3H, t, ^3J 6.7 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.4(Cq, Ph), 157.5 (C=O), 129.5 (CH, Ph), 127.3 (Cq, Ph), 114.2 (CH, Ph), 83.4 (C_4), 77.7 (C_3), 69.2 (C_1), 59.8 (C_2), 55.2 (OCH_3), 46.1 (NCH_2Ph), 31.7, 29.5, 29.3, 29.1, 27.9, 25.9, 22.5 (C_5 to C_{11}), 14.0 (Me); MS (DCI/CH_4) m/z 362 $[M+H]^+$, 100%; 390 $[M+C_2H_5]^+$, 18%; HRMS (CI) m/z $C_{21}H_{32}NO_4$ requires 362.2331; found 362.2329.

*6.3.6. (3aS,6S,6aS)-6-Octyltetrahydrofuro[3,4-d]oxazol-2(3H)-one(**11**).* Prepared from *N*-PMB derivative **10** (55.8 mg, 0.15 mmol) according to the general A. The crude material was purified by column chromatography on SiO_2 eluted with CH_2Cl_2/EtOAc (70:30 to 50:50) to give **11** (28.8 mg, 77%) as a white solid. $[\alpha]_D^{20}$ +77.7 (*c* 0.6, $CHCl_3$); IR (film) $\nu_{C=O}$ 1740 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.95 (1H, br s, NH), 4.88 (1H, dd, $^3J_{H3-H4}$ 3.6, $^3J_{H3-H2}$ 7.5 Hz, H_3), 4.30 (1H, dd, $^3J_{H2-H3}$ 7.5, $^3J_{H2-H1}$ 3.9 Hz, H_2), 3.66 (2H, AB of an ABX, $^2J_{gem}$ 10.5, $^3J_{H1-H2}$ 3.9, $^3J_{H1'-H2}$ 0.0 Hz, $\Delta\delta$ 129 Hz, 2×H_1), 1.75–1.65 (2H, m, 2×H_5), 1.40–1.15 (12H, m, 6×CH_2), 0.81 (3H, t, ^3J 7.0 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.0 (C=O), 83.2 (C_4), 81.0 (C_3), 73.3 (C_1), 57.2 (C_2), 31.8, 29.6, 29.4, 29.2, 28.1, 26.0, 22.6 (C_5 to C_{11}), 14.1 (Me); MS (DCI/NH_3) m/z 259 $[M+NH_4]^+$, 100%; HRMS (CI) m/z $C_{13}H_{24}NO_3$ requires 242.1756; found 242.1757.

*6.3.7. (2S,3S,4S)-4-Amino-2-(octyl)tetrahydrofuran-3-ol (**3**).* Prepared from oxazolidinone **11** (48.0 mg, 0.22 mmol) according to the general procedure B. The crude material was purified by column chromatography on SiO_2 eluted with EtOAc/MeOH/NH_4OH (69.2:30:0.8) to give **3** (45.5 mg, quant.) as a white solid. $[\alpha]_D^{20}$ +20.0 (*c* 0.5, $CHCl_3$); IR (neat) $\nu_{OH,\ NH}$ 3340 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.93 (1H, dd, $^2J_{H1-H1'}$ 8.6, $^3J_{H1-H2}$ 7.5 Hz, H_1), 3.86 (1H, dd, $^3J_{H3-H2}$ 5.0, $^3J_{H3-H4}$ 3.6 Hz, H_3), 3.73 (1H, ddd, $^3J_{H4-H5}$ 7.5, $^3J_{H4-H5'}$ 6.3, $^3J_{H4-H3}$ 3.4 Hz, H_4), 3.66 (1H, pseudot, $^3J_{H2-H1}$ 7.0, $^3J_{H2-H3}$ 5.2 Hz, H_2), 3.51 (1H, dd, $^2J_{H1'-H1}$ 8.6, $^3J_{H1'-H2}$ 6.8 Hz, $H_{1'}$), 1.90–1.60 (5H, m, 2×H_5, NH_2, OH), 1.50–1.20 (12H, m, 6×CH_2), 0.90 (3H, t, ^3J 7.0 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 83.2 (C_4), 72.3 (C_1), 71.7 (C_3), 54.3 (C_2), 31.9 (C_5), 29.8, 29.7, 29.5, 29.3, 26.3, 22.7 (C_6 to C_{11}), 14.1 (C_{12}); SM (DCI/NH_3) m/z 216 $[M+H]^+$, 62%; 233 $[M+NH_4]^+$, 100%; HRMS (CI) m/z $C_{12}H_{26}NO_2$ requires 216.1964; found 216.1965.

*6.3.8. (2S,3S,4S)-4-(Dimethylamino)-2-octyltetra-hydrofuran-3-ol (**12**).* To a solution of amine **3** (22.0 mg, 0.10 mmol) in MeCN/H_2O (3:1) (2 mL) at room temperature were added formaldehyde (30.0 μL of a 37% aqueous solution, 0.40 mmol), sodium cyanoborohydride $NaBH_3CN$ (19.6 mg, 0.30 mmol), and acetic acid (12.0 μL, 0.20 mmol). The mixture was stirred for 24 h after which it was diluted with water. The mixture was extracted three times with CH_2Cl_2 and the combined extracts were washed with brine and dried over $mgSO_4$. The solvent was evaporated in vacuo and the crude material was purified by column chromatography on SiO_2 eluted with EtOAc/CH_2Cl_2/NH_4OH (49.2:50:0.8) to give **12** (10.7 mg, 43%) as colorless oil. $[\alpha]_D^{20}$ +36.9 (*c* 0.2, $CHCl_3$); IR (film) ν_{OH} 3430 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.99 (1H, dd, $^3J_{H3-H2}$ 4.0, $^3J_{H3-H4}$ 2.8 Hz, H_3), 3.92–3.76 (3H, m, $H_{1'}$, H_1, H_4), 2.99 (1H, br s, OH), 2.82 (1H, ddd, $^3J_{H2-H1'}$ 9.2, $^3J_{H2-H1}$ 7.7, $^3J_{H2-H3}$ 4.1 Hz, H_2), 2.31 (6H, br s, 2×NCH_3), 1.76–1.62 (2H, m, 2×H_5), 1.40–1.20 (12H, m, 6×CH_2), 0.87 (3H, t, ^3J 6.7 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 83.4 (C_4), 70.4 (C_3), 70.2 (C_2), 66.6 (C_1), 44.2 (2×NCH_3), 31.9 (C_5), 29.8, 29.5, 29.3, 29.2, 26.2, 22.6 (C_6 to C_{11}), 14.1 (C_{12}); MS (DCI/CH_4) m/z 244 $[M+H]^+$, 100%; HRMS (CI) m/z $C_{14}H_{30}NO_2$ requires 244.2277; found 244.2256.

*6.3.9. (2S,3S,4S)-4-(Hexylamino)-2-octyltetra-hydrofuran-3-ol (**13**).* To a solution of amine **3** (20.0 mg, 0.09 mmol) in distilled MeOH (1.5 mL) at room temperature and under nitrogen atmosphere were added sodium cyanoborohydride $NaBH_3CN$ (11.6 mg, 0.18 mmol), acetic acid (5.30 μL, 0.09 mmol), and hexanal (11.0 μL, 0.09 mmol). The mixture was stirred for 20 h after which it was diluted with water. The mixture was extracted three times with diethyl ether and the combined extracts were washed with brine and dried over $mgSO_4$. The solvent was evaporated in vacuo and the crude material was purified by column chromatography on SiO_2 eluted with EtOAc/MeOH/NH_4OH (84.2:15:0.8) to give **13** (20.7 mg, 75%) as a white solid. $[\alpha]_D^{20}$ +22.1 (*c* 1.0, $CHCl_3$); IR (film) ν_{OH} 3100, ν_{NH} 3275 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.96–3.88 (2H, m, H_1, H_3), 3.69 (1H, td, $^3J_{H4-H5}$ 6.9, $^3J_{H4-H3}$ 3.0 Hz, H_4), 3.51 (1H, dd, $^2J_{H1'-H1}$ 8.5, $^3J_{H1'-H2}$ 7.2 Hz, $H_{1'}$), 3.35 (1H, td, $^3J_{H2-H1'}$ 7.2, $^3J_{H2-H3}$ 4.9 Hz, H_2), 2.70–2.50 (2H, m, 2×H_5), 1.78–1.58 (2H, m, 2×H_6), 1.54–1.18 (20H, m, 10×CH_2), 0.92–0.80 (6H, m, 2×Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 83.5 (C_4), 70.6 (C_1), 69.6 (C_3), 61.9 (C_2), 48.8 (NCH_2), 31.9, 31.7, 30.2, 29.8, 29.5, 29.3, 29.2, 26.8, 26.3, 22.6, 22.5 (11×CH_2), 14.1, 14.0 (2×CH_3); MS (DCI/CH_4) m/z 300 $[M+H]^+$, 100%; HRMS (CI) m/z $C_{18}H_{38}NO_2$ requires 300.2903; found 300.2892.

*6.3.10. N-((3S,4S,5S)-4-Hydroxy-5-octyltetra-hydrofuran-3-yl)octanamide (**14**).* To a solution of amine **3** (13.7 mg, 0.06 mmol) in anhydrous THF (1.5 mL) at room temperature and under nitrogen atmosphere was added 4-nitrophenyl caprylate (31.0 μL, 0.12 mmol). The mixture was stirred for 2 days after which solvent was removed in vacuo. The crude material was purified by column chromatography on SiO_2 eluted with PE/EtOAc (70:30) to give **14** (13.8 mg, 63%) as a white solid. $[\alpha]_D^{20}$ +5.3 (*c* 0.9, $CHCl_3$); IR (film) $\nu_{OH,\ NH}$ 3326, $\nu_{NHC=O}$ 1641 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.13(1H, d, ^3J 7.6 Hz, NH), 4.57 (1H, pseudoqd, $^3J_{H2-H1'}$ 7.6, ^3J 5.0 Hz, H_2), 4.14–4.04 (2H, m, H_1, H_3), 3.76 (1H, td, $^3J_{H4-H5}$ 6.9, $^3J_{H4-H3}$

2.8 Hz, H_4), 3.57 (1H, dd, $^2J_{H1'-H1}$ 8.8, $^3J_{H1'-H2}$ 7.3 Hz, $H_{1'}$), 2.20 (2H, pseudot, $^3J \approx 7.6$ Hz, 2×H_5), 1.74–1.54 (4H, m, 2×CH_2), 1.38–1.18 (20H, m, 10×CH_2), 0.94–0.80 (6H, m, 2×Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5 (C=O), 82.1 (C_4), 72.0 (C_3), 70.6 (C_1), 52.8 (C_2), 36.6, 31.8, 31.6, 29.7, 29.4, 29.3, 29.2, 28.9, 28.8, 26.0, 25.6, 22.6, 22.5 (13×CH_2), 14.1, 14.0 (2×CH_3); MS (DCI/CH_4) *m*/*z* 342 $[M+H]^+$, 15%; 364 $[M+Na]^+$, 100%; HRMS (CI) *m*/*z* $C_{20}H_{40}NO_3$ requires 342.3008; found 342.2990.

*6.3.11. (3aS,6S,6aS)-6-((4-Fluorophenyl)ethynyl)-3-(4-methoxybenzyl)-tetrahydrofuro[3,4-d]oxazol-2(3H)-one (**16**).* To a solution of alkyne **15** (83.0 mg, 0.30 mmol) and iodofluorobenzene (81.0 mg, 0.36 mmol) in degassed THF (5 mL) under argon in the dark were added $Pd(PPh_3)_2Cl_2$ (8.00 mg), CuI (2.30 mg), and diisopropylamine (106 μL, 0.75 mmol). The reaction mixture was stirred for 4 h at room temperature and quenched with saturated NH_4Cl solution. The mixture was extracted at three times with EtOAc and the combined extracts were washed with brine, dried over $mgSO_4$, and concentrated in vacuum. The crude material was purified by column chromatography on SiO_2 eluted with CH_2Cl_2/EtOAc (95:5) to give **16** (78.0 mg, 70%) as colorless oil. $[\alpha]_D^{20}$ +151.7 (*c* 0.9, $CHCl_3$); IR (film) $\nu_{C=O}$ 1737 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (2H, dd, ^3J 8.8, ^4J 5.4 Hz, *Ph*F), 7.20 (2H, d, ^3J 8.6 Hz, NCH_2Ph), 7.00 (2H, d, ^3J 8.8 Hz, *Ph*F), 6.87 (2H, d, ^3J 8.6 Hz, NCH_2Ph), 4.91 (1H, dd, $^3J_{H3-H2}$ 7.8, $^3J_{H3-H4}$ 4.4 Hz, H_3), 4.66 (1H, d, $^2J_{gem}$ 15.0 Hz, NCH_2Ph), 4.56 (1H, d, $^3J_{H4-H3}$ 4.4 Hz, H_4), 4.17 (1H, d, $^2J_{gem}$ 15.0 Hz, NCH_2Ph), 4.14 (1H, dd, $^3J_{H2-H3}$ 7.7, $^3J_{H2-H1'}$ 4.3 Hz, H_2), 3.97 (1H, d, $^2J_{gem}$ 10.6 Hz, H_1), 3.79 (3H, s, OCH_3), 3.46 (1H, dd, $^2J_{gem}$ 10.6, $^3J_{H1'-H2}$ 4.3 Hz, $H_{1'}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.8(d, Cq, *Ph*F, ^1J 250.3 Hz), 159.5(Cq, Ph), 157.0 (C=O), 134.0 (d, CH, *Ph*F, ^3J 8.5 Hz), 129.5 (CH, Ph), 127.0 (Cq, Ph), 117.9 (d, Cq, *Ph*F, ^4J 3.4 Hz), 115.5 (d, CH, *Ph*F, ^2J 21.1 Hz), 114.3 (CH, Ph), 88.0 (C_5), 80.1 (C_6), 77.1 (C_3), 73.7 (C_4), 69.7 (C_1), 59.5 (C_2), 55.2 (OCH_3), 46.4 (NCH_2Ph); MS (DCI/CH_4) *m*/*z* 368 $[M+H]^+$, 66%; HRMS (CI) *m*/*z* $C_{21}H_{19}NO_4F$ requires 368.1298; found 368.1310.

*6.3.12. (3aS,6S,6aS)-6-(4-Fluorophenethyl)-3-(4-methoxybenzyl)tetrahydrofuro[3,4-d]oxazol-2(3H)-one (**17**).* Prepared from alkyne **16** (62.3 mg, 0.17 mmol) according to the general procedure C to give **17** (60.1 mg, 95%) as a white solid. $[\alpha]_D^{20}$ +65.4 (*c* 1.3, $CHCl_3$); IR (film) $\nu_{C=O}$ 1738 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24–7.10 (4H, m containing a d, ^3J 8.6 Hz, NCH_2Ph, *Ph*F), 6.95 (2H, t, ^3J 8.7 Hz, *Ph*F), 6.87 (2H, d, ^3J 8.6 Hz, NCH_2Ph), 4.72 (1H, dd, $^3J_{H3-H2}$ 7.6, $^3J_{H3-H4}$ 3.9 Hz, H_3), 4.69 (1H, d, $^2J_{gem}$ 15.0 Hz, NCH_2Ph), 4.09 (1H, d, $^2J_{gem}$ 15.0 Hz, NCH_2Ph), 4.06 (1H, dd, $^3J_{H2-H3}$ 7.6, $^3J_{H2-H1'}$ 4.0 Hz, H_2), 3.94 (1H, d, $^2J_{gem}$ 10.6 Hz, H_1), 3.79 (3H, s, OCH_3), 4.45 (1H, ddd, $^3J_{H4-H5}$ 7.6, $^3J_{H4-H5'}$ 6.0, $^3J_{H4-H3}$ 3.9 Hz, H_4), 3.28 (1H, dd, $^2J_{gem}$ 10.6, $^3J_{H1'-H2}$ 4.0 Hz, $H_{1'}$), 2.84–2.62 (2H, m, 2×H_5), 2.20–1.94 (2H, m, 2×H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.3(d, Cq, *Ph*F, ^1J 244 Hz), 159.4(Cq, Ph), 157.3 (C=O), 136.7 (d, Cq, *Ph*F, ^4J 3.2 Hz), 129.7 (d, CH, *Ph*F, ^3J 7.8 Hz), 129.5 (CH, Ph), 127.2 (Cq, Ph), 115.1 (d, CH, *Ph*F, ^2J 21.1 Hz), 114.2 (CH, Ph), 82.0 (C_4), 77.5 (C_3), 69.2 (C_1), 59.9 (C_2), 55.2 (OCH_3), 46.1 (NCH_2Ph), 31.1 (C_6), 29.5 (C_5); MS (DCI/CH_4) *m*/*z* 372 $[M+H]^+$, 100%; $[M-CH_3OPh]^+$, 28%; $[M+C_2H_5]^+$, 15%; HRMS (CI) *m*/*z* $C_{21}H_{23}NO_4F$ requires 372.1611; found 372.1606. Selected crystallographic data for *x*: $C_{21}H_{22}FNO_4$, *M*=371.4, triclinic, space group *P*1, *a*=4.6676(4) Å, *b*=17.2070(13) Å, *c*=17.7086(13) Å, α=75.915(4)°, β=87.483(4)°, γ=82.706(4)°, *V*=1368.23(19) Å^3, *Z*=3, crystal size 0.60×0.38×0.10 mm^3, 29,473 reflections collected (8223 independent, R_{int}=0.0297), 733 parameters, *R*1 [$I>2\sigma(I)$]=0.0421, *wR*2 [all data]=0.1134, largest diff. peak and hole: 0.362 and −0.195 e Å^{-3}.

*6.3.13. (3aS,6S,6aS)-6-(4-Fluorophenethyl)tetra-hydrofuro[3,4-d]oxazol-2(3H)-one (**18**).* Prepared from *N*-PMB derivative **17** (56.0 mg, 0.15 mmol) according to the general A. The crude material was purified by column chromatography on SiO_2 eluted with CH_2Cl_2/EtOAc (60:40 to 50:50) to give **18** (35.2 mg, 93%) as a white solid. $[\alpha]_D^{20}$ +94.0 (*c* 1.4, $CHCl_3$); IR (film) ν_{NH} 3272, $\nu_{C=O}$ 1709 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (2H, dd, ^3J 8.5, ^4J 5.5 Hz, *Ph*F), 6.95 (2H, t, ^3J 8.7 Hz, *Ph*F), 6.82 (1H, br s, N*H*), 4.89 (1H, dd, $^3J_{H3-H2}$ 7.5, $^3J_{H3-H4}$ 3.7 Hz, H_3), 4.36 (1H, dd, $^3J_{H2-H3}$ 7.5, $^3J_{H2-H1'}$ 3.9 Hz, H_2), 3.95 (1H, d, $^2J_{gem}$ 10.4 Hz, H_1), 3.54–3.4 (2H, m, H_4, $H_{1'}$), 2.88–2.64 (2H, m, 2×H_5), 2.20–1.90 (2H, m, 2×H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.3(d, Cq, *Ph*F, ^1J 244 Hz), 159.4 (C=O), 136.7(d, Cq, *Ph*F, ^4J 3.2 Hz), 129.7 (d, CH, *Ph*F, ^3J 7.8 Hz), 115.1 (d, CH, *Ph*F, ^2J 21.1 Hz), 81.8 (C_4), 80.8 (C_3), 73.3 (C_1), 57.2 (C_2), 31.2 (C_6), 29.7 (C_5); MS (DCI/CH_4) *m*/*z* 252 $[M+H]^+$, 100%; HRMS (CI) *m*/*z* $C_{13}H_{15}NO_3F$ requires 252.1036; found 252.1028.

*6.3.14. (2S,3S,4S)-4-Amino-2-(4-fluorophenethyl)-tetrahydrofuran-3-ol (**19**).* Prepared from oxazolidinone **18** (30.0 mg, 0.12 mmol) according to the general procedure B. The crude material was purified by column chromatography on SiO_2 eluted with EtOAc/$MeOH/NH_4OH$ (84.2:15:0.8) to give **19** (25.8 mg, 96%) as a white solid. $[\alpha]_D^{20}$ +22.0 (*c* 1.1, $CHCl_3$); IR (film) $\nu_{OH,\ NH}$ 2937 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 7.21 (2H, dd, ^3J 8.2 Hz, ^4J 5.7 Hz, *Ph*F), 6.97 (2H, t, ^3J 8.8 Hz, *Ph*F), 3.98–3.76 (3H, m, H_1, H_3, H_4), 3.55–3.38 (2H, m, $H_{1'}$, H_2), 2.78–2.56 (2H, m, H_5), 1.90 (2H, dd, $^2J_{gem}$ 14.8, $^3J_{H6-H5}$ 7.8 Hz, H_6); ^{13}C NMR (75 MHz, CD_3OD) δ 162.7(d, Cq, *Ph*F, ^1J 242 Hz), 139.3(d, Cq, *Ph*F, ^4J 3.2 Hz), 131.0 (d, CH, *Ph*F, ^3J 7.8 Hz), 115.9 (d, CH, *Ph*F, ^2J 21.3 Hz), 83.3 (C_4), 73.3 (C_3), 72.2 (C_1), 56.1 (C_2), 32.8 (C_6), 32.4 (C_5); MS (DCI/CH_4) *m*/*z* 226 $[M+H]^+$, 25%; HRMS (CI) *m*/*z* $C_{12}H_{17}NO_2F$ requires 226.1243; found 226.1250.

6.4. Biological evaluations

Murine B16 melanoma cells were grown in a humidified 5% CO_2 atmosphere at 37 °C in DMEM medium containing Glutamax (2 mM), and heat-inactivated FCS (10%). All compounds were added to the cells as ethanolic solution. Control cells were treated with the same concentration of solvent (which did not exceed 0.5%).

6.4.1. Cell viability. After treatment with the indicated concentrations of natural Jaspine B or synthetic truncated analogues for 24 h in the absence of FCS, viability of murine B16 melanoma cells was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[36]

6.4.2. Caspase activation. After incubation with 5 μM of compound **3** for the indicated times in the absence of FCS, cells were sedimented and washed with PBS. Cell pellets were homogenized in 10 mM HEPES (pH 7.4), 42 mM KCl, 5 mM $MgCl_2$, 0.5% CHAPS, 1 mM dithiothreitol, 1 mM PMSF, and 2 μg/ml leupeptin. Reaction mixtures contained 100 μl of cell lysates and 100 μl of 40 μM Ac-Asp-Glu-Val-Asp-aminomethylcoumarin (Ac-DEVD-AMC). After 30 min incubation at room temperature, the amount of the released fluorescent product aminomethylcoumarin was determined at 351 and 430 nm for the excitation and emission wavelengths, respectively. Protein concentrations were determined according to the Bradford method.

Then, equal amounts of proteins were separated in a 15% SDS/polyacrylamide gel and transferred to a nitrocellulose membrane and blotted with monoclonal anti-caspase-3 or anti-β-actin (used as a control for protein loading) antibodies. Proteins were detected using an ECL detection system.

6.4.3. Sphingolipid turnover. Sphingolipids were labeled by incubating B16 cells for 6 h with 0.33 μCi/mL [3-^{3}H]-sphingosine in the presence or absence of 5 μM of compound **3**. Then, cells were washed twice with PBS, harvested and centrifuged. Lipids were extracted and separated by TLC using chloroform/methanol/water (100:42:6, by vol). Radiolabeled sphingolipids were detected using a Berthold radiochromatoscanner, identified using lipid standards and then scraped before counting radioactivity by liquid scintillation.

6.4.4. SMS activity. B16 cells were incubated for 6 h with 5 μM of Jaspine B or compound **3** in the absence of FCS. Then, 5 μM C_6–NBD–ceramide was added to the medium as an ethanolic solution. After incubation for 2 hours at 37 °C, cellular lipids were extracted with chloroform/methanol (2:1, v/v).[37] After centrifugation (1000×*g*, 10 min), the lower phases were concentrated under nitrogen and resolved by TLC developed in chloroform/methanol/30% ammonia/water (70:30:3:2, by vol). Fluorescent lipids were eluted from the silica and the specific activity of SMS was determined by quantifying the fluorescence of SM at 470 and 530 nm for the excitation and emission wavelengths, respectively.

6.5. Computational details

Geometry optimizations were carried out without any symmetry restriction at the B3PW91/6-31G** level of calculation using Gaussian 09.[38] Vibrational analysis was performed at the same level of calculation in order to check the nature of the stationary points found on the ground state potential energy surface (PES), and to extract the enthalpic and entropic corrections required for the calculation of Gibbs energies at 298.15 K. From these optimized structures, the PES was explored along the reaction coordinate (O–C*sp* distance) at the same calculation level. The intrinsic reaction coordinate was followed using the IRC technique implemented in Gaussian 09 for all located transition states. Solvent effects were included within self-consistent reaction field (SCRF) calculations using the polarizable continuum model (PCM, water, ε=78.3553).[38]

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Supplementary data

Supplementary data contains copies of the ^{13}C NMR spectra for compounds **3**, **6**–**14**, **16**–**19** as well as Fig. S1 and Table S1. Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2011.04.027.

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