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Synthesis of Cyclopropene Analogues of Ceramide and Their Effect on Dihydroceramide Desaturase

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The synthesis of several analogues of the *N*-[(1*R*,2*S*)-2-hydroxy-1-hydroxymethyl-2-(2-tridecyl-1-cyclopropenyl)ethyl]octanamide (GT11), the first reported inhibitor of dihydroceramide desaturase, as well as their effects on this enzyme, are described. Modifications of the parent structure include variations on the cyclopropene ring, the *N*-acyl chain length, the configuration of the stereocenters, and the hydroxyl group at C1. The key intermediates for the synthesis are the products resulting from the addition of suitable organolithium compounds to either Garner's aldehyde or its enantiomer. The final products are obtained by TMSTf-induced cleavage of the protecting groups and *N*-acylation, both under specific conditions. An alternative method for *N*-Boc deprotection is also reported that allows us to obtain the cyclopropene analogue of sphingosine **12a**, which can be transformed into GT11 upon acylation. The procedure consists of the conversion of the Garner aldehyde addition products into the bicyclic dihydrooxazolo[3,4,0]oxazol-3-ones **19** by transesterification in basic medium of the *tert*-butyl group with the hydroxyl function at C3. Mild cleavage of the *N*,*O*-isopropylidene cyclic acetal present in **19** affords the oxazolidin-2-one **20**, which gives **12a** upon saponification. Furthermore, compound **20** is also the key intermediate in the synthesis of the terminal deoxy, methoxy, and fluoro derivatives **9**, **10**, and **11**, respectively. Determination of dihydroceramide desaturase activity in vitro showed that GT11 was a competitive inhibitor (K_i = 6 μM) and that its analogues with *N*-hexanoyl (**6**) and *N*-decanoyl (**7**) moieties inhibited the enzyme with similar potencies (IC_{50} = 13 and 31 μM, respectively). No decrease in dihydroceramide desaturase activity was observed with any of the other compounds tested.

Introduction

Over the past decade, sphingolipids have emerged as a new class of modulators of various cell functions. Ceramide, which is the central molecule in the biosynthesis of sphingolipids and glycosphingolipids, is involved in the regulation of different cellular events, including cell senescence, differentiation, and apoptosis.[1] Intracellular generation of ceramide can occur by different metabolic routes. In the de novo pathway,[2] L-serine is condensed with palmitoyl-CoA to give 3-ketosphinganine, which affords dihydroceramide upon reduction to sphinganine and subsequent *N*-acylation. Ceramide is finally generated by introduction of a (*E*)-4 double bond into dihydroceramide under the catalysis of dihydroceramide desaturase.[3] Taking into consideration that dihydroceramide does not mimic the effects of ceramide,[3d,e] dihydroceramide desaturase is an attractive target for therapeutic intervention in diseases that result from alterations in the de novo biosynthesis of ceramide.[4]

Dihydroceramide desaturase belongs to the family of membrane-bound desaturases and hydroxylases.[3,5] In a previous paper,[6] we reported on the design and synthesis of the cyclopropene ceramide GT11 (Chart 1), as well as its activity as the first inhibitor of dihydroceramide desaturase. Recent work in our laboratories has shown that GT11 is metabolized at both the amido linkage and the hydroxyl group at C1 (Triola, G. Unpublished results). In light of published reports,[7] we envisaged that modifications of either the hydroxyl group at C1 or the

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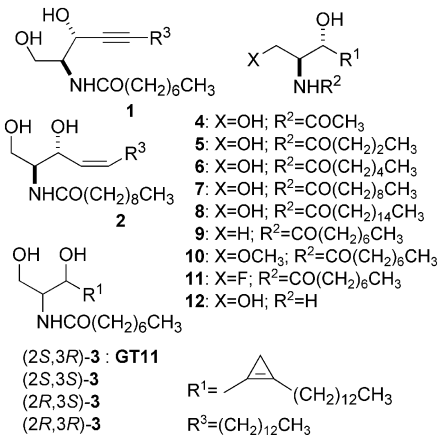
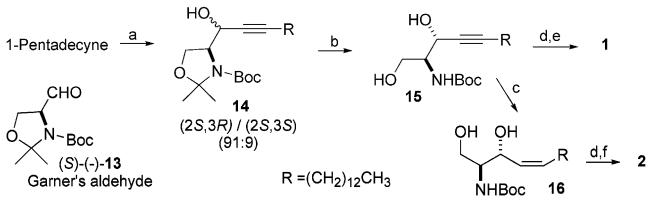
CHART 1. Sphingolipid Analogues Synthesized as Potential Dihydroceramide Desaturase Inhibitors



SCHEME 1[a]



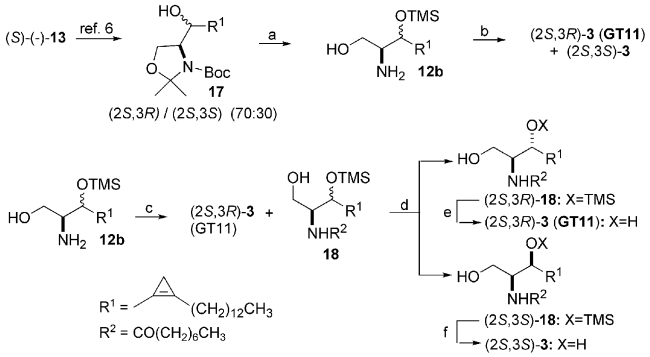
[a] Reagents and conditions: (a) *n*-BuLi, hexane/THF, −23 °C, 1 h, then (*S*)-**13**, 35 °C 2 h; (b) column chromatography then cat. TsOH, MeOH, 25 °C, 2 h; (c) H_2/Lindlar, AcOEt 25 °C, 4 h; (d) 1 N HCl/dioxane 100 °C, 30 min; (e) THF/50% NaOAc, 0 °C 10 min, then *n*-octanoyl chloride, 25 °C, 5 h; (f) THF/50% NaOAc 0 °C, 10 min, then *n*-decanoyl chloride, 25 °C, 5 h.

amide acyl moiety might yield GT11 analogues of higher metabolic stability and thereby greater inhibitory potency. Furthermore, the overall biochemical information gathered with different GT11 analogues should enable us to obtain structure−activity relationships of interest for the future design of new inhibitor molecules. In this paper, we describe the synthesis of some analogues of **GT11** (Chart 1) and their effect on dihydroceramide desaturase activity.

Results and Discussion

Synthesis. Acetylenic and Olefinic Analogues. The synthesis of acetylene **1** and olefin **2** was carried out as reported by Herold[8] (Scheme 1). Briefly, addition of 1-pentadecynyllithium to Garner's aldehyde afforded **14** ((2*S*,3*R*)/(2*S*,3*S*)[9] (91:9), HPLC), which gave the *N*-protected aminodiol **15** after column chromatography purification and mild acid hydrolysis. Partial hydrogenation of **15** furnished **16**, which was converted into the (*Z*)-olefin analogue **2** upon hydrolysis of the *tert*-butoxycarbonyl group and final *N*-acylation. On the other hand, strong acid treatment of **15** followed by Schotten−Baumann acylation with *n*-octanoyl chloride gave the final acetylenic analogue **1**.

SCHEME 2[a]



[a] Reagents and conditions: (a) TMSTf/2,6-lutidine, CH_2Cl_2, 0 °C, 30 min; (b) *n*-octanoyl chloride/pyridine, 25 °C, 24 h; (c) THF/50% NaOAc, 0 °C, 10 min, then *n*-octanoyl chloride, 25 °C, 5 h; (d) column chromatography; (e) pyridine·Cl, $CHCl_3$, 25 °C, 24 h; (f) TBAF, THF, 25 °C, 3 h.

Synthesis of (2*S*,3*R*)-3 (GT11) and Its Epimer (2*S*,3*S*)-3. In a previous paper,[6] we reported the preparation of GT11 (compound (2*S*,3*R*)-**3**,[9] Chart 1) by addition of 2-tridecyl-1-cyclopropenyllithium to Garner's aldehyde, which afforded **17** as a diastereomeric mixture (2*S*,3*R*)/(2*S*,3*S*) (70:30) in THF as solvent (Scheme 2). In agreement with the literature,[8] the use of HMPA as cosolvent improved the (2*S*,3*R*)/(2*S*,3*S*)-**17** ratio to (90:10),[6] and the (2*S*,3*S*) epimer was the major compound ((2*S*,3*R*)/(2*S*,3*S*)-**17** (9:91)) when the coupling was carried out in the presence of $ZnBr_2$. However, since both epimers were sought for biochemical investigation, the separation of diastereomeric mixtures (2*S*,3*R*)/(2*S*,3*S*)-**17** (70:30) (or products thereof) was considered convenient from a preparative point of view. Although column chromatography of (2*S*,3*R*)/(2*S*,3*S*)-**17** furnished the individual (2*S*,3*R*) enantiomer, pure (2*S*,3*S*)-**17** could not be isolated. The hydrolysis of the *N*-Boc group was carried out successfully under neutral conditions by reaction of **17** (epimeric mixture) with TMSTf in the presence of 2,6-lutidine as acid scavenger. Although partial decomposition of the cyclopropene ring occurred after quenching the reaction with MeOH,[10] the expected products with a trimethylsilyloxy group at C3 ((2*S*,3*R*)/(2*S*,3*S*)-**12b**) were obtained by replacing MeOH with phosphate buffer (pH 8). Since column chromatography separation of (2*S*,3*R*)- and (2*S*,3*S*)-**12b** was unsuccessful and it originated a considerable loss of material, the synthesis was continued with the (2*S*,3*R*)/(2*S*,3*S*)-**12b** epimeric mixture. Therefore, the direct acylation of crude (2*S*,3*R*)/(2*S*,3*S*)-**12b** was thus attempted. Using *n*-octanoyl chloride in chloroform and pyridine as base,[11] both (2*S*,3*R*)- and (2*S*,3*S*)-**12b** were concomitantly desilylated and *N*-acylated to give an inseparable mixture of (2*S*,3*R*)-**3** (GT11) and its epimer (2*S*,3*S*)-**3**. However, when the diastereomeric mixture (2*S*,3*R*)/(2*S*,3*S*)-**12b** was *N*-acylated in THF/50% NaOAc[12] and ice-cold 0.1 M NH_4OH was used in the workup, a

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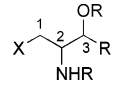
(9) To designate the absolute configuration of the stereogenic centers present in the compounds described throughout this paper, we use the numbering of the sphingolipid chain.



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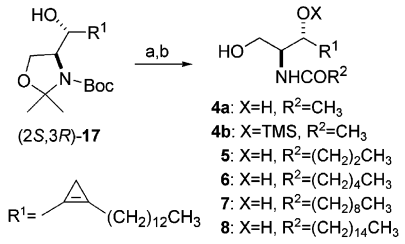
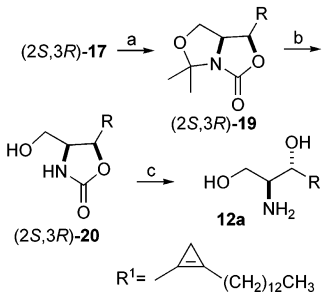
SCHEME 3[a]



[a] Reagents and conditions: (a) TMSTf/2,6-lutidine, CH_2Cl_2, 0 °C, 30 min; (b) THF/50% NaOAc, 0 °C, 10 min, then *n*-acyl chloride, 25 °C, 5 h.

SCHEME 4[a]



[a] Reagents and conditions: (a) NaH, THF, 0 °C, then 25 °C, 18 h; (b) cat. TsOH, MeOH, 25 °C, 6 h; (c) 2 N NaOH/EtOH, 80 °C, 3 h.

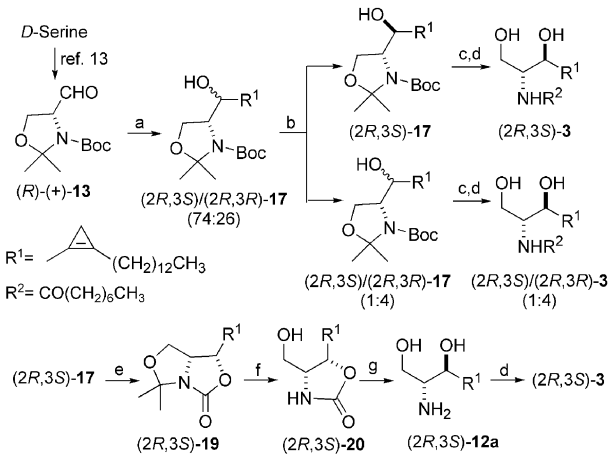
mixture of (2*S*,3*R*)/(2*S*,3*S*)-**3** and their *O*-TMS derivatives was obtained. This mixture was subjected to columnchromatography, which allowed us to separate and isolate the pure silylated derivatives (2*S*,3*R*)-**18** and (2*S*,3*S*)-**18**. Whereas (2*S*,3*R*)-**18** was transformed almost quantitatively into (2*S*,3*R*)-**3** by treatment with pyridinium hydrochloride in chloroform, (2*S*,3*S*)-**18** remained unaltered under these conditions. However, the trimethylsilyl group of (2*S*,3*S*)-**18** could be removed, although in low yields (20−25%), with TBAF in THF, thus affording pure (2*S*,3*S*)-**3**.

Synthesis of GT11 Analogues with Different Acyl Chain Lengths. The preparation of the analogues **4**−**8** was accomplished by treatment of (2*S*,3*R*)-**17**, obtained by column chromatography separation of a 70:30 diastereomeric mixture of (2*S*,3*R*)/(2*S*,3*S*)-**17**, with TMSTf and 2,6-lutidine, followed by Schotten−Baumann reaction of the deprotection crude with the suitable acylating agent in each case (Scheme 3).

Synthesis of the Cyclopropene Long-Chain Base (2*S*,3*R*)-12a. The isolation and purification of the amine (2*S*,3*R*)-**12a** from the crude obtained from the reaction of (2*S*,3*R*)-**17** with TMSTf/2,6-lutidine was not possible. All the attempts resulted in poor yields of impure material. As a consequence, a stepwise procedure was undertaken (Scheme 4). Thus, (2*S*,3*R*)-**17** was transformed into bicyclic (2*S*,3*R*)-**19** with NaH in THF. The cyclization crude was treated with mild acid to afford (2*S*,3*R*)-**20**, which after basic hydrolysis of the carbamate function gave (2*S*,3*R*)-**12a**, a cyclopropene analogue of sphingosine.

It is worth mentioning that, since *N*-acylation of (2*S*,3*R*)-**12a** furnishes GT11, this is a new approach for the synthesis of this compound. Unfortunately, this procedure is not appropriate to prepare the (2*S*,3*S*)-**3** epimer, since cyclization of (2*S*,3*S*)-**17** to (2*S*,3*S*)-**19** (Scheme 4) takes place very slowly and the starting material decomposes upon long reaction times.

SCHEME 5[a]



[a] Reagents and conditions: (a) 2-tridecyl-1-cyclopropenyllithium, THF, −23 °C, 2 h; (b) column chormatography; (c) TMSTf/2,6-lutidine, CH_2Cl_2, 0 °C, 30 min; (d) THF/50% NaOAc, 0 °C, 10 min, then *n*-octanoyl chloride, 25 °C, 5 h; (e) NaH, THF, 0 °C, then 25 °C, 18 h; (f) cat. TsOH, MeOH, 25 °C, 6 h; (g) 2 N NaOH/EtOH, 80 °C, 3 h.

Synthesis of (2*R*,3*S*)/(2*R*,3*R*)-3. The synthesis of these compounds was accomplished as outlined in Scheme 5. Following a reported methodology,[13] the (*R*)-(+)-enantiomer of Garner aldehyde (*R*)-**13** was obtained from D-serine with a 93% ee (Mosher ester analysis after reduction with $NaBH_4$ in MeOH). Coupling of the aldehyde with 2-tridecyl-1-cyclopropenyllithium[14] afforded a diastereomeric mixture of (2*R*,3*S*)-**17**/(2*R*,3*R*)-**17** in a 74:26 ratio (Scheme 5). Column chromatography separation furnished pure (2*R*,3*S*)-**17** and a mixture of (2*R*,3*S*)-**17**/(2*R*,3*R*)-**17** (1:4). Treatment of (2*R*,3*S*)-**17** with TMSTf/2,6-lutidine and further Schotten−Baumann acylation gave pure (2*R*,3*S*)-**3**. Alternatively, (2*R*,3*S*)-**17** was cyclized to (2*R*,3*S*)-**19**,[15] which was converted into the carbamate (2*R*,3*S*)-**20**. Basic hydrolysis of (2*R*,3*S*)-**20** gave the aminodiol (2*R*,3*S*)-**12a**, which yielded (2*R*,3*S*)-**3** upon *N*-acylation. On the other hand, deprotection (TMSTf/2,6-lutidine) and acylation (*n*-octanoyl chloride/pyridine) of the (2*R*,3*S*)-**17**/(2*R*,3*R*)-**17** (1:4) mixture afforded (2*R*,3*S*)-**3**/(2*R*,3*R*)-**3** in the same ratio. The preliminary biochemical experiments were performed with this mixture of epimers.

GT11 Analogues Modified at C1. Compounds **9**, **10**, and **11** (Chart 1) were obtained, respectively, by hydrolysis and *N*-acylation of carbamates **22**, **23**, and **24**, which were in turn prepared from the common precursor (2*S*,3*R*)-**20** (Scheme 6). Mesylation of (2*S*,3*R*)-**20** and reduction of the resulting sulfonate **21** with $NaBH_4$ in dimethyl sulfoxide[16] afforded **22**. On the other hand, tri-

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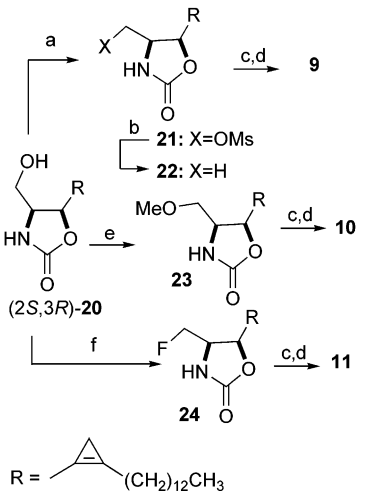
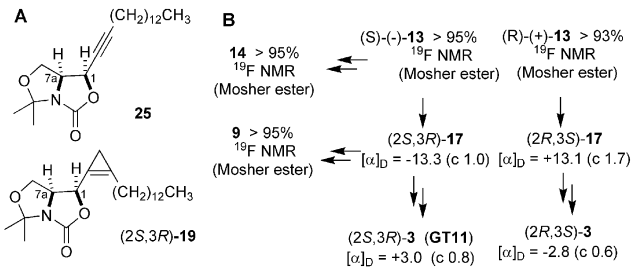
(16) Hutchins, R. O.; Hoke, D.; Keogh, J.; Koharshi, D. *Tetrahedron Lett.* **1969**, *10*, 3459−3498.

SCHEME 6[a]



[a] Reagents and conditions: (a) MsCl, NEt_3/cat. DMAP CH_2Cl_2, 0 °C; (b) $NaBH_4$, DMSO, 40 °C, 7 h; (c) 2 N NaOH/EtOH, 80 °C, 3 h; (d) THF/50%NaOAc, 0 °C, 10 min, then *n*-octanoyl chloride, 25 °C, 5 h; (e) $Me_3O{\cdot}BF_4$/2,6-di-*tert*-butylpyridine, CH_2Cl_2, 25 °C, 24 h; (f) DAST, CH_2Cl_2 −78 °C 1 h, −78 → +25 °C, 9 h, then 25 °C, 12 h.

CHART 2. Stereochemical Analysis



methyloxonium tetrafluoroborate methylation of (2*S*,3*R*)-**20** gave **23**. Other methylation procedures applied to (2*S*,3*R*)-**20**, such as the treatment with base followed by MeI alkylation, afforded the *N*-methyl derivative as the main product. Attempts to obtain the fluorinated cyclopropene ceramide precursor **24** by treatment of sulfonate **21** with either TBAF[17] or KF[18] gave complex mixtures of products. However, the direct reaction of (2*S*,3*R*)-**20** with DAST yielded **24** in good yields.

Stereochemical Analysis. The relative stereochemistry of the products formed upon addition of the organolithium reagents to L- and D-serine-derived aldehydes was determined by NMR studies on the bicyclic derivatives **19** and **25** (Chart 2A). Among other data, the NOE values of 15−19% observed between H1 and H7a were of diagnostic value and indicate a relative cis stereochemistry in the bicycles, which implies a 2*S*,3*R* configuration in their precursors **17** (see Scheme 4) and **14** (see Scheme 1), respectively. Further confirmation of these assignments was inferred from the acetylenic adduct **14**, which is in full agreement with the stereochemistry described for this compound.[8]

The absolute stereochemistry of C3 in **14** (Chart 2B, see Scheme 1) was determined by ^{19}F NMR of the corresponding Mosher ester (>95%). Since no racemization occurs in the following reactions,[8] a similar enantiomeric purity was assumed for the final acetylenic and olefinic analogues **1** and **2**, respectively.

Mosher ester preparation from the final cyclopropene ceramide analogues was not possible, since a complete transformation was never achieved and complex mixtures of products were always obtained. Furthermore, attempted conversion of the cyclopropene alcohols (2*S*,3*R*)-**17** and (2*R*,3*S*)-**17** to the corresponding Mosher esters was also unsuccessful. Therefore, the enantiomeric purity of the ceramide analogues had to be assessed indirectly. First, as stated above, the starting Garner aldehyde (*S*)-**13** was of ee > 95%, as determined after reduction of an aliquot into the corresponding alcohol, which was converted to the Mosher ester and analyzed by ^{19}F NMR. Similarly, the enantiomeric aldehyde (*R*)-**13** was found to have an ee > 93%. On the other hand, ^{19}F NMR analysis of its Mosher ester evidenced that **9** (see Chart 1) was >95% enantiomerically pure, identical to the ee displayed by its synthetic precusor (*S*)-**13**. This result confirmed the stereochemical integrity of C2 in the starting (*S*)-**13** throughout the synthesis of **9**. As a consequence, an ee > 95% can also be inferred for (2*S*,3*R*)-**17**, an intermediate along the synthetic pathway transforming (*S*)-**13** to **9** (see Scheme 6). Finally, the optical rotation values found for (2*S*,3*R*)-**17** and (2*R*,3*S*)-**17**, on one side, and (2*S*,3*R*)-**3** and (2*R*,3*S*)-**3** (see Chart 2), on the other, are in agreement with similar enantiomeric purities of both the L- and D-series. Since no racemization is expected to occur during the transformation of the **17** into the final products, these were deemed to possess enantiomeric purities similar to those of the starting aldehydes.

Dihydroceramide Desaturase Activity. The effect of the compounds synthesized on dihydroceramide desaturase activity was screened following the previously reported procedure,[6] using equimolar concentrations (50 μM) of substrate (*N*-octanoylsphinganine) and each test compound. As summarized in Figure 1, removal, methylation, or replacement by fluorine of the primary hydroxyl group of GT11 afforded inactive compounds. These results demonstrate that the presence of a free hydroxyl function at C1 is crucial for inhibition and suggest that the C1-OH group is probably required for the inhibitor interaction with the enzyme active site.

Besides the C1-OH function, the cyclopropene ring is also essential for dihydroceramide desaturase inhibition, since its replacement by a (*Z*)-double bond or a triple bond yielded inactive molecules, such as **1** and **2**.

Among the several analogues of GT11 differing in the *N*-acyl chain length, only the *N*-hexanoyl- (**6**) and *N*-decanoylcyclopropene (**7**) ceramides retained inhibitory activity, with IC_{50} values of 31 and 13 μM, respectively, at a fixed substrate concentration of 50 μM (Figure 2). Kinetic studies using different concentrations of substrate and inhibitor evidenced that GT11 was a competitive inhibitor with a K_i of 6 μM (Figure 3). This type of inhibition may account for the lack of activity of **4**, **5**, and **8**, which probably bear two different *N*-acyl chains to efficiently compete with *N*-octanoylsphinganine used as the desaturase substrate. In contrast, the inhibitory

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(18) Badone, D.; Jommi, R.; Pagliarin, P.; Tavecchia, P. *Synthesis* **1987**, 920−921.

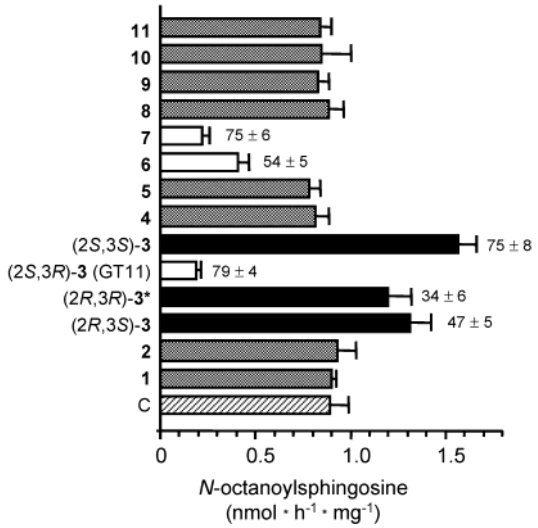

FIGURE 1. Effect of compounds **1**−**11** on dihydroceramide desaturase. The biochemical experiments were performed as described in the Experimental Section. Data correspond to the mean ± standard deviation of three to five replicates. The amounts of *N*-octanoylsphingosine are significantly different (unpaired, two-tail *t* test, $p \leq 0.025$) from controls (C, bottom bar) with compounds GT11, **6** and **7** (lower amounts, white bars), and the stereoisomers of GT11 (higher amounts, black bars). The percentages of increase or decrease with respect to the control value are indicated as mean ± standard deviation next to each bar. Compound **12a** could not be tested due to its insolubility in the assay buffer. *Contains 20% of (2*R*,3*S*)-**3**.

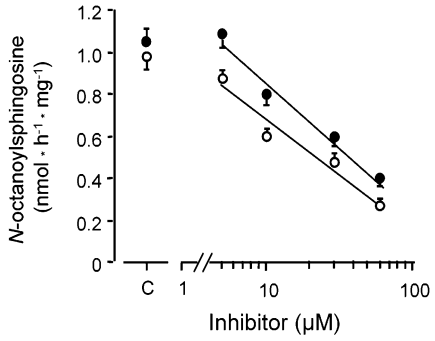

FIGURE 2. Concentration dependence of dihydroceramide desaturase inhibition by the *N*-hexanoyl and *N*-decanoyl derivatives of GT11. Inhibitory effect was investigated in rat liver microsomes, which were incubated in the absence (controls, C) or presence of different amounts of either the *N*-hexanoyl (filled circles) or the *N*-decanoyl (empty circles) analogues of GT11 with a constant concentration (50 μM) of substrate (*N*-octanoylsphinganine). Data correspond to mean ± standard deviation, $n = 4-5$.

activity is maintained in **6** and **7**, with *N*-acyl chains more similar to that of the desaturase substrate.

None of the stereoisomers of GT11 inhibited the conversion of *N*-octanoylsphinganine into *N*-octanoylsphingosine. Furthermore, the amounts of *N*-octanoylsphingosine contained in extracts after incubation with either (2*S*,3*S*)-, (2*R*,3*S*)-, or (2*R*,3*R*)-**3** were higher than those present in controls. These results seemed to indicate an activation of dihydroceramide desaturase. However, since inhibition of the neutral ceramidase by *N*-acylsphingosines structurally similar to (2*S*,3*S*)-, (2*R*,3*S*)-, and (2*R*,3*R*)-**3** has been reported[19] and neutral

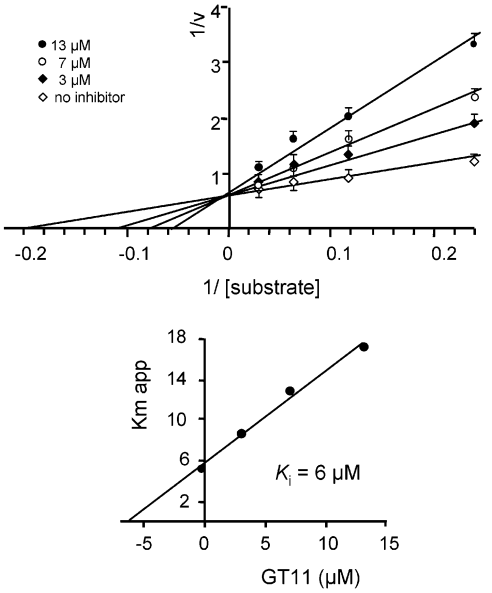

FIGURE 3. Type of inhibition of of dihydroceramide desaturase by GT11. Inhibitory effect was investigated in rat liver microsomes, which were incubated with different amounts of GT11 at different concentrations of substrate (*N*-octanoylsphinganine). The experiments were performed as described in the Experimental Section. Data correspond to mean ± standard deviation, $n = 4$.

ceramidase activity occurs in the desaturase-containing microsomal preparation, we suggest that the rise in the amounts of *N*-octanoylsphingosine observed with the above three compounds is not due to an increased desaturase activity, but to inhibition of the ceramidase-catalyzed hydrolysis of the *N*-octanoylsphingosine formed in the desaturation reaction. Current experiments both in vitro and in cultured neurons support this hypothesis.

In summary, these overall results indicate that the presence of a cyclopropene ring in place of the ceramide double bond and the natural 2*S*,3*R* stereochemistry are required for inhibition of dihydroceramide desaturase. In addition, the presence of a free hydroxyl group at C1 and a similar *N*-acyl chain length in both substrate and cyclopropene inhibitor seem to be essential for in vitro inhibitory activity. Further work along this line is currently under way in our laboratories.

Experimental Section

Dihydroceramide Desaturase Activity. Enzymatic activities were determined using rat liver microsomes in phosphate buffer (0.1 M, pH 7.4), with (2*S*,3*R*)-*N*-octanoylsphingosine as substrate.[6] The indicated concentrations of substrate and inhibitor were solubilized in phosphate buffer/ethanol 9:1 (v/v, 100 μL) containing 15 nmol of BSA, combined with the microsome suspension (0.6 mg of protein) and NADH (30 μL, 1 μM in phosphate buffer), and made up to a final volume of 300 μL with phosphate buffer. The suspension was incubated at 37 °C for 30 min, and the reactions were stopped by addition of $CHCl_3$ (0.5 mL) containing (2*S*,3*R*)-*N*-hexanoylsphingosine (1 nmol) as an internal standard for quantification. The lipids were extracted with $CHCl_3$, the combined organic layers were evaporated under a stream of nitrogen, and the residue was derivatized with BSTFA. After derivatization, $CHCl_3$ was

(19) Usta, J.; El Bawab, S.; Roddy, P.; Szulc, Z. M.; Hannun, Y. A.; Bielawska, A. *Biochemistry* **2001**, *40*, 9657−9668.

added and the samples were analyzed by gas chromatography coupled to electron-impact (70 eV) mass spectrometry using a Fisons gas chromatograph (8000 series) coupled to a Fisons MD-800 mass-selective detector. The system was equipped with a nonpolar Hewlett-Packard HP-1 capillary column (30 m × 0.20 mm i.d.), which was programmed from 100 to 340 °C at 7 °C per min.

Mosher Ester Preparation. Oxalyl chloride (3.6 equiv) was added to a mixture of (*S*)-(−)−α-methoxy-α-trifluoromethylphenylacetic acid (1.2 equiv) and DMF (4.8 equiv) in dry hexane (30 mM Mosher acid) under argon at room temperature. After 2 h, the formed precipitate was filtered off, and solvent was evaporated to dryness at reduced pressure. This residue was dissolved in dry CH_2Cl_2 (10 mL/mmol of Mosher acid) and added to a mixture of the alcohol (1 equiv), dry Et_3N (5 equiv), and DMAP (catalytic amount). After 2 h of stirring at room temperature, the solvent was removed under vacuum, and $CDCl_3$ containing a 0.5% of $CFCl_3$ was added for ^{19}F NMR recording.

***N*-Boc Deprotection on *tert*-Butyl 2,2-Dimethyloxazolidine-3-carboxylates with TMSTf and Further *N*-Acylation. General Procedure.** To the starting *tert*-butyl 2,2-dimethyloxazolidine-3-carboxylates (0.5 mmol) dissolved in anhydrous CH_2Cl_2 (5 mL) was added under argon at 25 °C 0.4 mL (3.5 mmol) of freshly distilled 2,6-lutidine. After being stirred for 10 min, the solution was cooled to 0 °C, and 0.27 mL (1.5 mmol) of TMSTf was added dropwise. Stirring was mantained at 0 °C for 30 min, and the reaction mixture was quenched with 0.1 M phosphate buffer at pH 8.0. The product was extracted with CH_2Cl_2, and the organic layer was washed sequentially with phosphate buffer and a saturated solution of $NaHCO_3$ and dried ($MgSO_4$). Solvent was evaporated, and the resulting residue was directly submitted to *N*-acylation with the corresponding acid chloride in $CHCl_3$/pyridine (method A) or THF/50% aqueous NaOAc (method B).

***N*-Acylation. General Method A.** The residue obtained from TMSTf deprotection of 0.5 mmol of starting *tert*-butyl 2,2-dimethyloxazolidine-3-carboxylate was reacted with *n*-octanoyl chloride (0.7 mmol) and anhydrous pyridine (7 mmol) in dry chloroform (10 mL) at 25 °C until the consumption of starting material. After this time, the solvent was evaporated, and the resulting residue was extracted with Et_2O. The organic layers were washed with brine and dried, the solvent was removed under vacuum, and the resulting crude was purified by column chromatography using chloroform/methanol mixtures.

***N*-Acylation. General Method B.** The residue obtained from TMSTf deprotection of 0.5 mmol of starting *tert*-butyl 2,2-dimethyloxazolidyne-3-carboxylate was dissolved in THF (5 mL) and added at 0 °C to a 50% aqueous solution of NaOAc (5 mL). After the mixture was stirred for 10 min, the acid chloride or the anhydride (0.7 mmol) was added dropwise, and stirring was continued at 25 °C for 5 h. After this time, $CHCl_3$ (10 mL) was added, the organic layer was washed with brine, and the solvent was evaporated. The resulting residue was dissolved in Et_2O (15 mL) and washed sequentially with ice-cold 0.1 N NH_4OH and brine. The organic layer was dried, and the solvent was removed to furnish a crude that was purified by column chromatography.

Amide 1. *tert*-Butyl (1*S*,2*R*)-*N*-(2-hydroxy-1-hydroxymethyl-3-heptadecynyl)carbamate **15** was prepared from 1-pentadecyne and Garner's aldehyde as reported by Herold.[9] A solution of 0.04 g (0.085 mmol) of this compound in dioxane (2.5 mL) and 1 N HCl (0.4 mL) was heated to reflux for 30 min and cooled to room temperature, 2 N NaOH (1 mL) was added, and the mixture was extracted with ether (3 × 20 mL). The extracts were combined, washed with saturated NaCl, dried, and evaporated yielding the crude alkynylaminodiol intermediate that was directly acylated with octanoyl chloride according to method B. The crude product was purified by chromatography (MeOH/CH_2Cl_2 95/5) to give 0.022 g (0.052 mmol) of **1**. Mp: 77 °C. IR: 3288, 2923, 2214, 1646, 1540. ^1H NMR: δ 0.88 (t, J = 6.5, 6H), 1.20−1.40 (m, 28H), 1.51 (t, J = 7.5 Hz 2H), 1.64 (t, J = 7.0, 2H), 2.22 (dt, J = 7.5 and 2.0, 2H), 2.25 (t, J = 7.5, 2H), 3.76 (AB system, J = 4.0, 11.5, 1H), 4.04 (m, 1H), 4.12 (AB system, J = 4.0, 11.5, 1H) 4.60 (s, 1H), 6.34 (d, J = 7.0, 1H). ^{13}C NMR: 14.0, 14.1, 22.6, 22.7, 25.7, 28.5, 28.9, 29.0, 29.1, 29.2, 29.4, 29.5, 29.6, 31.7, 31.9, 36.8, 54.7, 62.7, 64.6, 88.3, 93.9, 174.32. $[\alpha]_D$: −11.7 (*c* 1.35, $CHCl_3$). ESI-MS: *m*/*z* 424 $[M + 1]^+$, 446 $[M + Na]^+$, 869 $[2M + Na]^+$. HRMS: calcd for $C_{26}H_{49}NO_3$ 423.3712, found 423.3709.

Amide 2. According to Herold, [9] hydrogenation of *N*-Boc-alkynylaminodiol **15** with Lindlar catalyst followed by recrystallization from hexane gave *tert*-butyl (3*Z*,1*S*, 2*R*)-*N*-(2-hydroxy-1-hydroxymethyl-3-heptadecenyl)carbamate **16**. A solution of 0.008 g (0.02 mmol) of this compound in in dioxane (1 mL) and 1 N HCl (0.1 mL) was heated to reflux for 30 min and cooled to room temperature. NaOH (2 N, 0.2 mL) was added and the mixture extracted with ether (3 × 5 mL). The extracts were combined, washed with saturated NaCl, dried, and evaporated yielding the crude alkenylaminodiol intermediate that was directly acylated with decanoyl chloride according to method B. The crude product was purified by chromatography (MeOH/CH_2Cl_2 97/3) to give 0.005 g (0.011 mmol) of **2**. Mp: 69−70 °C. IR: 3292, 1646, 1550, 1464, 1374, 724. ^1H NMR: 0.87 (t, J = 6.9, 3H), 1.2−1.4 (m, 20 H),1.58 (m, 2H), 1.65 (t, J = 6.9, 2H) 2.11 (m, 2H), 2.34 (t, J = 7.5, 2H,), 2.58 (bs, 1H) 2.71 (bs, 1H), 3.7 (m, 1H) 3.86 (m, 1H), 3.98 (dd, 1H, J = 3.6 J = 11.7, H1), 4.68 (m, 1H,) 5.49 (m,1H), 5.59 (m, 1H), 6.22 (d, J = 7.2, 1H).^{13}C NMR: 14.1, 22.7, 25.7, 27.9, 29.3, 29.3, 29.4, 29.6, 29.7, 31.9, 36.8, 54.7, 62.6, 69.8, 128.4, 134.8, 173.9. $[\alpha]_D$: −7 (*c* 0.3, $CHCl_3$). ESI-MS: *m*/*z* 436 $[M - 18 + 1]^+$, 476 $[M + Na]^+$. HRMS: calcd for $C_{28}H_{55}NO_3$ 453.4182, found 453.4188.

Alcohol (2*S*,3*R*)-17. Following a reported procedure,[7] to a solution of 1,1,2-tribromo-2-tridecylcyclopropane[14] (3.60 g, 7.52 mmol) in THF (40 mL) was added, at −78 °C under argon, a 1.3 M solution of *n*-BuLi in hexane (12.8 mL, 16.4 mmol). The reaction mixture was warmed to room temperature over 45 min and cooled again to −23 °C. A solution of *tert*-butyl (*S*)-(−)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate (*S*)-**13** (1.43 g, 6.26 mmol) in 14 mL of THF was added at −23 °C and the mixture stirred for 2 h at this temperature. The reaction was quenched with aqueous saturated NH_4Cl and extracted with ether. Evaporation of the solvent and flash chromatography (hexane/AcOEt 8:1) of the reaction crude (which contained a 3:1 mixture of (2*S*,3*R*)-**17** and (2*S*,3*S*)-**17**) resulted in partial separation of pure diastereomers that were obtained in 88% combined yield. A 0.48 g (1.06 mmmol) amount of pure (HPLC) (2*S*,3*R*)-**17** was obtained in addition to several fractions containing mixtures of (2*S*,3*R*)-**17** and (2*S*,3*S*)-**17** that after combination and evaporation gave 2.01 g (4.44 mmmol).

When dry HMPA (approximately0.08 mL of HMPA/mL of THF) was added at −78 °C to the organolithium solution prior to the addition of the aldehyde (*S*)-**13**, the diastereoselectivity obtained was 90:10 (2*S*,3*R*)-**17**/(2*S*,3*S*)-**17**.

(2*S*,3*R*)-17. Oil. IR: 3400, 2852, 1703. ^1H NMR: 0.89 (t, J = 6.6, 3H), 0.93 (d, AB system, J = 8.8, 1H), 0.99 (d, AB system, J = 8.8, 1H), 1.20−1.40 (m, 20H), 1.48 (m, 14H), 1,59 (m, 2H), 2.43 (t, J = 7.0, 2H), 2.43 (t, J = 6.0, 2H), 4.8−5.0 (m, 4H). ^{13}C NMR: 8.4, 14.0, 22.6, 25.8, 25.9, 27.3, 28.3, 29.3, 29.4, 29.5, 29.6, 29.7, 31.8, 61.8, 64.1, 69.0, 80.5, 94.6, 107.7, 115.1, 154.0. $[\alpha]_D$: −13.3 (*c* 1.03, $CHCl_3$). Anal. Calcd for $C_{27}H_{49}NO_4$: C, 71.80; H, 10.93; N, 3.10; Found: C, 71,77; H, 10,79; N, 3,01.

Amides (2*S*,3*R*)-18 and (2*S*,3*S*)-18. The general procedure for *N*-Boc deprotection with TMSTf followed by acylation under method B was applied to 0.20 g (0.46 mmol) of a 3:1 mixture of (2*S*,3*R*)-**17**/(2*S*,3*S*)-**17**. Flash chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 97:3) afforded (2*S*,3*R*)-**18** (39 mg, 0.08 mmol) and (2*S*,3S)-**18** (9 mg, 0.02 mmol) in addition to a mixture of the corresponding *O*-desilylated compounds (2*S*,3*R*)-**3 (**GT11**)** and (2*S*,3*S*)-**3** (87 mg, 0.18 mmol).

(2*S*,3*R*)-18. Oil. ^1H NMR: 0.11 (s, 9H), 0.86 (t, J = 6.9, 6H), 0.98 (d, AB system, J = 8.3, 1H), 1.01 (d, AB system, J = 8.3,

1H), 1.20−1.40 (m, 28H), 1.56 (t, J = 7.0, 2H), 1.65 (t, J = 7.6, 2H), 2.23 (t, J = 7.5, 2H), 2.43 (t, J = 7.6, 2H), 3.28 (dd, J = 10.4, 1.6, 1H), 3.57 (ddd, J = 3.3, 11.8, 14.8, 1H), 3.98 (dt, J = 1.8, 11.8, 1H), 4.05 (m, 1H), 4.95 (s, 1H), 6.37 (d, J = 7.8, 1H). ^{13}C NMR: −0.3, 8.7, 14.0, 14.0, 22.5, 22.6, 25.6, 25.8, 27.1, 29.2, 29.9, 29.3, 29.4, 29.5, 29.7, 30.0, 31.8, 36.7, 53.5, 64.1, 68.8, 107.8, 115.8, 174.2. HRMS: calcd for $C_{30}H_{59}NO_3Si$ 509.4264, found 509.4266.

(2*S*,3*S*)-18. Oil. ^{1}H NMR: 0.11 (s, 9H), 0.86 (t, J = 7.0, 6H), 0.94 (s, 2H), 1.40−1.20 (m, 28H), 1.60 (m, 4H), 2.10 (t, J = 7.0, 2H), 2.40 (t, J = 7.0, 2H), 3.69 (m, 2H), 4.16 (m, 1H), 4.88 (s, 1H), 6.02 (d, J = 4.8, 1H). ^{13}C NMR: 0.9, 8.6, 14.0, 14.1, 22.5, 22.6, 25.6, 25.7, 27.0, 28.9, 29.2, 29.3, 29.4, 29.5, 29.6, 29.6, 31.8, 36.7, 52.5, 63.3, 71.8, 108.3, 114.8, 174.0. HRMS: calcd for $C_{30}H_{59}NO_3Si$ 509.4264, found 509.4255.

Amide (2*S*,3*R*)-3 (GT11). From (2*S*,3*R*)-17. The general procedure for *N*-Boc deprotection and acylation under method A was applied to 0.12 g (0.27 mmol) of pure (2*S*,3*R*)-**17** to give (2*S*,3*R*)-**3** (0.11 g, 0.25 mmol, 93%).

From (2*S*,3*R*)-18. A solution of (2*S*,3*R*)-**18** (6.2 mg, 12.2 μmol) and pyridinium chloride (2.15 mg, 18.7 μmol) in chloroform (2 mL) was stirred at 25 °C for 24 h. The solvent was evaporated, and the residue was extracted with Et_2O. The organic layers were washed with brine and dried and the solvent evaporated to yield 4.8 mg (11 μmol, 95%) of pure (2*S*,3*R*)-**17**. Mp: 69 °C. IR: 1550, 1644, 3010, 3287. ^{1}H NMR: 0.87 (t, J = 6.0, 6H), 1.01 (s, 2H), 1.25 (s, 28H), 1.60 (m, 4H), 2.24 (t, J = 7.0, 2H), 2.45 (dt, J = 7.5, 1.0, 2H), 3.02 (bs, 1H), 3.37 (bs, 1H), 3.71 (dd, J = 11.5, 3.0, 1H), 3.87 (dd, J = 11.0, 4.0, 1H), 4.18 (m, 1H), 4.85 (s, 1H), 6.37 (bs, 1 H). ^{13}C NMR: 8.4, 14.0, 14.1, 22.6, 22.7, 25.7, 25.8, 27.3, 29.00, 29.2, 29.3, 29.4, 29.5, 29.6, 29.6, 29.6, 29.6, 29.7, 31.6, 31.9, 36.7, 53.9, 63.1, 70.7, 107.6, 115.7, 174.00. [α]$_D$: +3.0 (*c* 0.8, $CHCl_3$). HRMS: calcd for $C_{27}H_{51}NO_3$ 437.3869, found 437.3870. Anal. Calcd for $C_{27}H_{51}NO_3$: C, 74.09; H, 11.74; N, 3.20. Found: C, 74.27; H, 11.69; N, 3.01.

Amide (2*S*,3*S*)-3. A solution of TBAF (16.2 mg, 0.051 mmol) and (2*S*,3*S*)-**18** (26 mg, 0.0051 mmol) in dry THF (2 mL) was stirred for 3 h at 25 °C under argon. After this time, the solvent was removed under vacuum, and the resulting crude was dissolved in AcOEt. The organic solution was washed sequentially with Na_2CO_3 and brine and dried. Solvent removal afforded a crude oil that was purified by column chromatography. Elution with (CH_2Cl_2/MeOH 97:3) furnished 7 mg (0.016 mmol, 31%) of (2*S*,3*S*)-**3** as a thick oil. IR: 3290, 1650, 1545. ^{1}H NMR: 0.87 (t, J = 6.0, 6H), 0.98 (s, 1H), 1.20−1.40 (s, 28H), 1.60 (m, 4H), 2.20 (t, J = 7.0, 2H), 2.44 (t, J = 7.5, 2H), 2.93 (bs, 1H), 3.84 (dd, J = 2.5, 2.0, 1H), 4.18 (m, 1H), 4.93 (d, J = 3.0, 1H), 6.15 (d, 1H, J = 7.5 Hz); ^{13}C NMR: 8.4, 14.0, 14.0, 22.5, 22.6, 25.7, 27.3, 28.9, 29.1, 29.3, 29.3, 29.4, 29.5, 29.6, 29.6, 31.6, 31.8, 36.7, 53.9, 63.9, 69.0, 107.8, 115.9, 174.0. [α]$_D$: −7.1 (*c* 0.51, $CHCl_3$). HRMS: calcd for $C_{27}H_{51}NO_3$ 437.3869, found 437.3867.

Amide 4a. The general procedures of hydrolysis with TMSTf and *N*−acylation (method B) was applied to 0.25 g (0.56 mmol) of (2*S*, 3*R*)-**17** to give the *O*-silyl derivative (2*S*, 3*R*)-**4b** (10 mg, 0.023 mmol, 4%) and (2*S*, 3*R*)-**4a** (93 mg, 0.26 mmol, 46%).

(2*S*,3*R*)-4b. Oil. ^{1}H NMR: 0.12 (s, 9H), 0.88 (t, J = 6.5, 3H), 0.96 (AB system, J = 8.5, 1H), 1.05 (dd, AB system, J = 8.5, 0.5, 1H), 1.40−1.20 (m, 20 H), 1.56 (t, J = 6.5, 2H), 2.05 (s, 2H), 2.44 (dt, J = 7.0, 1.0, 2H), 3.55 (dd, J = 3.5, 11.5, 1H), 3.97 (dd, J = 2.5, 11.5, 1H), 4.06 (m, 1H), 4.97 (d, J = 1.0, 1H), 6.37 (d, J = 7.0 Hz, 1H). ^{13}C NMR: 0.3, 8.7, 14.1, 22.7, 23.4, 25.7, 27.2, 29.3, 29.4, 29.5, 29.6, 31.9, 52.5, 63.2, 71.8, 107.8, 115.2, 170.0.

(2*S*,3*R*)-4a. Mp: 66 °C. IR: 1544, 1660, 3295, 3098. ^{1}H NMR: 0.87 (t, J = 6.5, 3H), 1.00 (s, 2H), 1.2−1.4 (s, 20H), 1.56 (7, J = 7.0, 2H), 2.04 (s, 3H), 2.45 (dt, J = 7.0, 1. 0, 2H), 3.51 (bs, 2H), 3.72 (dd, J = 11.0, 1H), 3.86 (dd, J = 11.0, 4.0, 1H), 4.14 (m, 1H), 4.81 (s, 1H), 6.65 (d, J = 8.0, 1H). ^{13}C NMR: 8.3, 14.0, 22.6, 23.2, 25.7, 27.2, 29.3, 29.3, 29.4, 29.5, 29.6, 31.8, 53.7, 63.7, 70.2, 107.5, 115.3, 171.0. [α]$_D$: +2.45 (*c* 1.1, $CHCl_3$). ESI-MS: *m*/*z* 354 [M + 1]$^+$, 376 [M + Na]$^+$, 729 [2M + Na]$^+$. HRMS: calcd for $C_{21}H_{39}NO_3$ 353.2930, found 353.2926.

Amide 5. The general procedures of hydrolysis with TMSTf and *N*-acylation (method B) applied to 0.20 g (0.46 mmol) of (2*S*,3*R*)-**17** afforded (2*S*,3*R*)-**5** (25 mg, 0.068 mmol, 15%). Mp: 72 °C. IR: 1549, 1636, 3284, 3085. ^{1}H NMR: 0.88 (t, J = 6.5, 3H), 0.97 (t, J = 7.0, 3H), 1.02 (s, 2H), 1.2−1.4 (s, 20H), 1.57 (t, J = 7. 5, 2H), 1.70 (m, J = 7. 5, 2H), 2.23 (t, J = 7.0, 2H), 2.46 (dt, J = 7.0, 1.0, 2H), 3.74 (dd, J = 11.5, 3. 5, 1H), 3.90 (dd, J = 11.5, 4.0, 1H), 4.18 (m, 1H), 4.84 (s, 1H), 6.39 (d, J = 7.5, 1H). ^{13}C NMR: 8.2, 13.6, 14.1, 19.1, 22.6, 25.8, 27.2, 29.3, 29.4, 29.5, 29.6, 31.8, 38.6, 53.6, 63.1, 70.5, 107.5, 115.4, 173.7. [α]$_D$: −1.03 (*c* 0.93, $CHCl_3$). ESI-MS: *m*/*z* 382 [M + 1]$^+$, 404 [M + Na]$^+$, 785 [2M + Na]$^+$. HRMS: calcd for $C_{23}H_{43}NO_3$ 381.3243, found 381,3251.

Amide 6. The general procedures of hydrolysis with TMSTf and further *N*-acylation under method B were applied to 0.20 g (0.46 mmol) of (2*S*,3*R*)-**17** to give (2*S*,3*R*)-**6** (105 mg, 0.30 mmol, 54%). Mp: 63 °C. IR: 3289, 3010, 1636, 1550. ^{1}H NMR: 0.88 (q, 6H, J = 6.5 Hz), 1.01 (s, 1H), 1.2−1.4 (s, 26H), 1.58 (t, J = 7. 0, 2H), 1.65 (t, J = 7.5, 2H), 2.24 (t, J = 7.5, 2H), 2.45 (dt, J = 7.5, 1.5, 2H), 3.72 (dd, J = 11.0, 3.5, 1H), 3.87 (dd, J = 11.5, 3.5, 1H), 4.16 (m, 1H), 4.83 (s, 1H), 6.37 (d, J = 7.0, 1H). ^{13}C NMR: 8.4, 13.9, 14.1, 22.4, 22.7, 25.4, 25.8, 27.3, 29.3, 29.4, 29.6, 29.6, 31.4, 31.9, 36.7, 53.7, 63.2, 70.6, 107.5, 115.4, 174.0. [α]$_D$: +1.58 (*c* 1.08, $CHCl_3$). ESI-MS: *m*/*z* 410 [M + 1]$^+$, 432 [M + Na]$^+$, 841 [2M + Na]$^+$. HRMS: calcd for $C_{25}H_{47}NO_3$ 409.3556, found 409.3555.

Amide 7. The general procedures of hydrolysis with TMSTf and *N*-acylation (method B) to 0.20 g (0.46 mmol) of (2*S*,3*R*)-**17** afforded (2*S*,3*R*)-**7** (104 mg, 0.22 mmol, 48%). Mp: 73 °C; IR: 3294, 3105, 1649, 1542. ^{1}H NMR: 0.88 (t, J = 6.5, 6H), 1.01 (s, 2H), 1.2−1.4 (s, 32H), 1.59 (m, 4H), 2.24 (t, J = 7.0, 2H), 2.46 (t, J = 7.0, 2H), 3.74 (dd, J = 11.5, 3.5, 1H), 3.89 (dd, J = 11.5, 4.0, 1H), 4.17 (m, 1H), 4.84 (s, 1H), 6.3 (d, J = 7.5, 1H). ^{13}C NMR: 8.3, 14.0, 22.6, 25.7, 25.8, 27.2, 29.2, 29.3, 29.4, 29.6, 31.8, 31.9, 36.7, 53.7, 63.1, 70.5, 107.5, 115.4, 174.0. [α]$_D$: +0.81 (*c* 0.68, $CHCl_3$). ESI-MS: *m*/*z* 466 [M + 1]$^+$, 488 [M + Na]$^+$, 953 [2M + Na]$^+$. HRMS: calcd for $C_{29}H_{55}NO_3$ 465.4182, found 465.4180.

Amide 8. The general procedures of hydrolysis with TMSTf and *N*-acylation (method B) applied to 0.19 g (0.43 mmol) of (2*S*,3*R*)-**17** afforded (2*S*,3*R*)-**8** (0.2 g, 0.34 mmol, 80%) as a cerous solid of very poor solubility in organic and aqueous solvents. Mp: 156−157 °C dec. IR (KBr): 1558, 1645, 3309. ^{1}H NMR: 0.88 (t, J = 6.5, 6H), 1.02 (s, 2H), 1.2−1.4 (s, 32H), 1.64 (m, 4H), 2.24 (t, J = 7.0, 2H), 2.46 (dt, J = 7.0, 1.5, 2H), 2.66 (bs, 1H), 3.02 (bs, 1H), 3.75 (d, J = 9.5, 1H), 3.92 (d, J = 10.0, 1H), 4.17 (m, 1H), 4.85 (s, 1H), 6.33 (d, J = 7.5, 1H). ^{13}C NMR: 8.3, 14.1, 22.7, 25.7, 25.8, 27.3, 29.3, 29.5, 29.6, 31.9, 36.7, 53.7, 63.3, 70.7, 107.5, 115.5, 173.9. ESI-MS: *m*/*z* 550 [M + 1]$^+$, 572 [M + Na]$^+$, 1121 [2M + Na]$^+$. Anal. Calcd for $C_{35}H_{67}NO_3$: C, 76.44; H, 12.28; N, 2.55. Found: C, 76,81; H, 12,29; N, 2,73.

Alcohol (2*R*,3*S*)-17. The enantiomer of Garner aldehyde (*R*)-(+)-**13** with 93% ee was obtained from D-serine following the reported procedures.[13] Addition of tridecylcyclopropenyllithium[14] to 0.50 g (2.17 mmol) of aldehyde (*R*)-**13** as previously described[7] gave a diastereomeric mixture (2*R*,3*S*)-**17**/(2*R*,3*R*)-**17** (74:26 by HPLC). Purification by column chromatography (hexane/EtOAc 8:1) afforded 66 mg (0.15 mmol, 7%) of pure (2*R*,3*S*)-**17** and several fractions with mixtures of (2*R*,3*S*)-**17**/(2*R*,3*R*)-**17** in different proportions (530 mg, 1.17 mmol, 54%).

(2*R*,3*S*)-17. Oil. IR: 3400, 2852, 1703. ^{1}H NMR: 0.88 (t, J = 6.5, 3H), 0.93 (AB system, J = 8.5, 1H), 0.96 (AB system, J

= 10.5, 1H), 1.2−1.4 (m, 20H), 1.48 (m, 15 H), 1.56 (m, 2H), 2.43 (t, J = 7.0, 2H), 4.8−5.0 (m, 4H). ^{13}C NMR: 8.4, 14.1, 22.6, 25.9, 26.0, 27.3, 28.3, 29.3, 29.46 29.4, 29.5, 29.6, 31.9, 61.9, 64.8, 69.0, 81.0, 94.6, 107.8, 115.1, 153.8. $[\alpha]_D$: +11.6 (*c* 1.7, $CHCl_3$). Anal. Calcd for $C_{27}H_{49}NO_4$: C, 71.80; H, 10.93; N, 3.10. Found: C, 71,62; H, 11,16; N, 2,86.

Amide (2*R*,3*S*)-3. The general procedures of hydrolysis with TMSTf and further *N*-acylation under method B was applied to 50 mg (0.12 mmol) of pure (2*R*,3*S*)-**17** to obtain (2*R*,3*S*)-**3** (18 mg, 0.04 mol, 32%). Mp: 66−67 °C. IR: 3294, 1650, 1545. ^{1}H NMR: 0.88 (t, J = 6.5, 6H), 1.02 (s, 1H), 1.2−1.4 (s, 28H), 1.60 (m, 4H), 2.25 (t, J = 7.0, 2H), 2.46 (dt, J = 7.5, 1.5, 2H), 3.72 (dd, J = 11.0, 3.5, 1H), 3.88 (dd, J = 11.5, 3.5, 1H), 4.18 (m, 1H), 4.85 (s, 1H), 6.36 (d, J = 7.0, 1H). ^{13}C NMR: 8.3, 14.0, 14.1, 22.6, 22.7, 25.7, 25.8, 27.3, 29.0, 29.2, 29.3, 29.4, 29.5, 29.6, 31.6, 31.9, 36.8, 53.7, 63.2, 70.7, 107.5, 115.5, 173.9. $[\alpha]_D$: −2.8 (*c* 0. 58, $CHCl_3$). HRMS: calcd for $C_{27}H_{51}NO_3$ 437.3869, found 437.3853.

Dihydrooxazolo[3,4,0]oxazol-3-one (2*S*,3*R*)-19. To 355 mg (0.78 mmol) of (2*S*, 3*R*)-**17** dissolved in anhydrous THF (10 mL) was added, at 0 °C under argon, 63 mg (1.57 mmol) of NaH. After the mixture was stirred at 25 °C for 18 h, saturated aqueous $NaHCO_3$ was added at 0 °C and the product was extracted with Et_2O. The combined organic layers were washed with brine and dried. Solvent removal afforded (2*S*,3*R*)-**19** (250 mg, 0.66 mmol, 85%), which was submitted to the following reaction without purification. ^{1}H NMR: 0.90 (t, J = 6.3, 3H), 1.05 (s, 2H), 1.20−1.40 (m, 20H), 1.44 (s, 3H) 1.54 (t, J = 7.0, 2H), 1.72 (s, 3H), 2.47 (dt, J = 1.5, 8.3, 2H), 3.56 (t, J = 8.5, 1H), 3.94 (dd, J = 8.5, 6.5, 1H), 4.55 (ddd, J = 6.4, 8.0, 8.5, 1H), 5.55 (d, J = 8.5, 1H); ^{13}C NMR (50 MHz): 9.3, 14.1, 22.6, 23.3, 25.8, 27.0, 27.8, 29.2, 29.3, 29.5, 29.6, 31.9, 61.4, 64.9, 71.5, 94.8, 103.1, 118.9, 158.5.

Oxazolidin-2-one (2*S*,3*R*)-20. A solution of crude (2*S*,3*R*)-**19** (250 mg, 0.66 mmol) and TsOH (12 mg, 0.06 mmol) in MeOH (10 mL) was stirred at 25 °C for 6 h. After this time, the solvent was removed under vacuum and the resulting residue was dissolved in EtOAc. The organic solution was washed sequentially with a saturated solution of $NaHCO_3$ and brine and dried. Removal of solvent gave a crude oil that was purified by column chromatography ($CHCl_3$/MeOH (97:3)) to furnish 150 mg (0.44 mmol, 67%) of (2*S*,3*R*)-**20**. Mp: 50 °C. IR: 3315, 1726, 2858. ^{1}H NMR: 0.88 (t, J = 6.5, 3H), 1.08 (d, AB system, J = 8.0, 21, 1H), 1.15 (d, AB system, J = 8.0, 21, 1H), 1.2−1.4 (m, 20H), 1.58 (t, J = 7.0, 2H), 2.49 (dt, 2H, J = 7.0, 1.0 Hz), 3.09 (bs, 1H), 3.63 (m, 2H), 4.12 (m, 1H), 5.64 (d, J = 8.5, 1H), 6.59 (bs, 1H). ^{13}C NMR: 9.7, 14.1, 22.7, 25.9, 26.9, 29.3, 29.3, 29.4, 29.6, 29.6, 29.7, 31.9, 57.5, 62.4, 74.3, 102.6, 119.6, 159.7. $[\alpha]_D$: −29.9 (*c* 0.88, $CHCl_3$). ESI-MS: *m*/*z* 338 $[M + 1]^+$, 360 $[M + Na]^+$, 697 $[2M + Na]^+$. HRMS: calcd for $C_{20}H_{35}NO_3$ 337.2617, found 337.2611.

Aminodiol (2*S*,3*R*)-12a. A mixture of (2*S*,3*R*)-**20** (75 mg, 0.222 mmol), 2 N NaOH (12 mL), and EtOH (12 mL) was heated at 80 °C for 3 h. The mixture was cooled to 25 °C, the solvent was removed under vacuum, and the product was extracted with Et_2O. The organic layer was washed with brine and dried, and the solvent was evaporated giving 63 mg (0.202 mmol, 91%) of (2*S*,3*R*)-**12a**. Oil. IR: 3306, 2853. ^{1}H NMR: 0.87 (t, J = 6.5, 3H), 0.98 (d, AB system, J = 8.4, 1H), 1.06 (d, AB system, J = 8.5, 1H), 1.20−1.40 (m, 20 H), 1.60 (t, J = 7.0, 2H), 1.99 (bs, 4H), 2.51 (dt, J = 7.5, 1.2, 2H), 3.65 (m, 3H), 4.63 (d, J = 4.0 Hz); ^{13}C NMR: 8.1, 14.2, 22.6, 26.0, 27.3, 29.4, 29.4, 29.6, 29.6, 31.9, 56.1, 64.3, 69.9, 107.8, 115.2. $[\alpha]_D$: −4.5 (*c* 1.0, $CHCl_3$). ESI-MS: *m*/*z* 312 $[M + 1]^+$, 623 $[2M + 1]^+$. HRMS: calcd for $C_{19}H_{37}NO_2$ 311.2824, found 311.2822.

Oxazolidin-2-one (2*R*,3*S*)-20. The same procedure used to transform (2*S*,3*R*)-**17** into (2*S*,3*R*)-**19** was applied to 250 mg (0.55 mmol) of (2*R*,3*S*)-**17** to obtain 200 mg (0.53 mmol, 96%) of the intermediate (1*S*,7a*R*)-5,5-dimethyl-1-(2-tridecyl-1-cyclopropenyl)dihydrooxazolo[3,4-*c*]oxazol-3-one (2*R*,3*S*)-**19**, with spectral data identical to its enantiomer (2*S*,3*R*)-**19**. Crude (2*R*,3*S*)-**19** (200 mg, 0.53 mmol) and TsOH (12 mg, 0.06 mmol) in MeOH (20 mL) were stirred at 25 °C for 6 h. After this time, the solvent was removed under vacuum, and the resulting residue was dissolved in EtOAc. The organic solution was washed sequentially with a saturated solution of $NaHCO_3$ and brine and dried. Removal of solvent gave a crude oil that was purified by column chromatography ($CHCl_3$/MeOH (97:3)) to furnish 62 mg (0.2 mmol, 38%) of (2*R*,3*S*)-**20** after column chromatography (CH_2Cl_2/MeOH 97:3). Mp: 48 °C. IR: 3289, 1741, 2852. ^{1}H NMR: 0.88 (t, J = 6.5, 3H), 1.07 (d 1H, AB system, J = 8.4, 21.5 Hz), 1.14 (d 1H, AB system, J = 8.0, 21.0 Hz), 1.20−1.40 (m, 20H), 1.58 (t, J = 7.0, 2H), 2.49 (dt, J = 7.0, 1.0, 2H), 2.69 (bs, 1H), 3.64 (m, 2H), 4.12 (m, 1H), 5.65 (d, J = 8.0, 1H), 6.29 (bs, 1H). ^{13}C NMR: 9.7, 14.1, 22.7, 25.9, 26.9, 29.3, 29.3, 29.3, 29.6, 29.6, 29.7, 31.9, 57.4, 62.5, 74.3, 102.6, 119.6, 159.4. $[\alpha]_D$: +25.81 (*c* 1.12, $CHCl_3$). HRMS: calcd for $C_{20}H_{35}NO_3$ 337.2617, found 337.2616.

Aminodiol (2*R*,3*S*)-12a. The same procedure used for the preparation of (2*S*,3*R*)-**12a** was applied to (2*R*,3*S*)-**20** (13 mg, 0.038 mmol) to obtain 11 mg (0.035 mmol, 93%) of (2*R*,3*S*)-1**2a**. Oil. IR: 3306, 2853. ^{1}H NMR: 0.88 (t, J = 6.5, 3H), 0.98 (d, AB system, J = 8.5, 1H), 1.05 (d, AB system, J = 8.5, 1H), 1.20−1.40 (m, 20 H), 1.58 (t, J = 7.0, 2H), 1.98 (bs, 4H), 2.47 (dt, J = 7.5, 1.0, 2H), 3.65 (m, 3H), 4.64 (d, J = 4.0 Hz); ^{13}C NMR: 8.0, 14.1, 22.7, 25.9, 27.3, 29.3, 29.4, 29.6, 29.6, 31.9, 56.1, 64.2, 69.7, 107.8, 115.1. $[\alpha]_D$: +3.0 (*c* 0.87, $CHCl_3$). HRMS: calcd for $C_{19}H_{37}NO_2$, 311.2824, found 311.2825.

Amide (2*R*,3*S*)-3. *N*-Acylation of (2*R*,3*S*)-**12a** (6.0 mg, 0.019 mmol) using method B gave 6.5 mg (0.015 mmol, 79%) of (2*R*,3*S*)-**3**.

Amides (2*R*,3*S*)-3/(2*R*,3*R*)-3. The general procedures of hydrolysis with TMSTf and further *N*-acylation under method B were applied to 181 mg (0.40 mmol) of a (1:4) mixture of (2*R*,3*S*)-**17**/(2*R*, 3*R*)-**17** to obtain a (1:4) mixture of (2*R*,3*S*)-**3**/(2*R*,3*R*)-**3** (136 mg, 0.31 mmol, 78%).

Oxazolidin-2-one (2*S*,3*R*)-23. A solution of (2*S*,3*R*)-**20** (90 mg, 0.278 mmol) and 2,6-di-*tert*-butylpyridine (0.92 mL, 4.12 mmol) in 10 mL of anhydrous CH_2Cl_2 was added onto 411 mg (2.78 mmol) of trymethyloxonium tetrafluoborate. After 24 h of stirring at 25 °C, brine was added, and the product was extracted with EtOAc, washed with brine, and dried. Solvent removal furnished an oil that was purified by column chromatography (CH_2Cl_2/MeOH 97:3) to give (2*S*,3*R*)-**23** (43 mg, 0.126 mmol, 45%) as a yellowish oil. ^{1}H NMR: 0.87 (t, J = 6.5, 3H), 1.04 (d, J = 8.0, 1H), 1.11 (d, AB system, J = 8.0, 0.5, 1H), 1.2−1.4 (m, 20H), 1.58 (t, J = 7.0, 2H), 2.48 (t, J = 7.0, 2H), 3.32 (s, 3H), 3.31 (m, 2H), 4.16 (m, 1H), 5.58 (d, J = 8, 1H); ^{13}C NMR: 8.7, 14.1, 22.7, 25.9, 26.9, 29.3, 29.3, 29.4, 29.6, 29.6, 31.9, 55.5, 59.3, 72.2, 73.8, 102.8, 119.3, 158.5. $[\alpha]_D$: −20.38 (*c* 1.04, $CHCl_3$). HRMS: calcd for $C_{21}H_{37}NO_3$ 351.2773, found 351.2777.

Amide 10. A mixture of (2*S*,3*R*)-**23** (22 mg, 0.06 mmol), 2 N NaOH (2 mL), and EtOH (2 mL) was heated at 80 °C for 3 h. The mixture was cooled to 25 °C, and the product was extracted with Et_2O. The organic layer was washed with brine and dried, and the solvent was evaporated. The resulting residue was dissolved in THF (3 mL) and added at 0 °C to a 50% aqueous solution of NaOAc (3 mL). After the mixture was stirred for 10 min, *n*-octanoyl chloride (17 μL, 0.07 mmol) was added dropwise, and stirring was continued at 25 °C for 5 h. After this time, $CHCl_3$ (10 mL) was added, the organic layer was washed with brine, and the solvent was evaporated. The resulting residue was dissolved in Et_2O (15 mL), and the organic solution was washed sequentially with 0.1 N NH_4OH and brine. The organic layer was dried, and the solvent was removed to furnish 14 mg (0.03 mmol, 50%) of **10** as a white solid after column chromatography (CH_2Cl_2/MeOH 95:5). Mp: 55 °C. IR: 3297, 2930, 2852, 1654. ^{1}H NMR: 0.87 (t, J = 6.5, 6H), 0.99 (d, AB system, J = 8.5, 1H), 1.03 (d, AB system, J = 8.5, 1H), 1.40−1.20 (m, 20H), 1.59 (m, 4H), 2.22 (t, J = 7.5, 2H), 2.45 (t, J = 7.0, 1.0, 2H), 3.32 (s, 3H), 3.54 (dd, J = 2.5, 9.5, 1H), 3.62 (dd, J = 3.5, 9.5, 1H), 4.27 (m, 1H), 4.69 (s, 1H), 6.28 (d, J = 8.5, 1H); ^{13}C NMR 8.5, 14.0, 14.1, 22.6, 22.7, 25.7,

25.9, 27.3, 29.0, 29.2, 29.3, 29.4, 29.4, 29.5, 29.6, 29.7, 31.7, 31.9, 36.8, 51.7, 59.2, 70.7, 73.3, 108.1, 114.7, 173.3. $[\alpha]_D$: +2.26 (*c* 0.53, $CHCl_3$). ESI-MS: *m*/*z* 452 $[M + 1]^+$, 474 $[M + Na]^+$, 927 $[2M + Na]^+$. HRMS: calcd for $C_{28}H_{53}NO_3$ 451.4025, found 451.4030.

Mesylate 21. To a solution of (2*R*,3*S*)-**20** (123 mg, 0.38 mmol), NEt_3 (106 μL, 0.76 mmol), and a catalytic amount of DMAP in anhydrous CH_2Cl_2 (10 mL) was added under stirring at 0 °C 59 μL (0.76 mmol) of freshly distilled MsCl. After the cooling bath was removed, the reaction was completed (TLC) after 10 min at 25 °C. The reaction mixture was poured onto 1 N HCl and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried. Solvent removal afforded a solid that was purified by chromatography (CH_2Cl_2/MeOH 97:3) to give 133 mg (0.32 mmol, 84%) of **21**, which was used without purification. Mp: 83−84 °C. IR: 3128, 2923, 1726, 1350, 1170. ^{1}H NMR: 0.86 (t, *J* = 6.5, 3H), 1.10 (d, AB system, *J* = 8.0, 1H), 1.17 (d, AB system, *J* = 8.0, 1H), 1.2−1.4 (m, 20H), 1.57 (t, *J* = 6.5, 2H), 2.49 (dt, *J* = 7.5, 2H), 3.07 (s, 3H), 4.12 (dd, *J* = 8.5, 10.5, 1H), 4.24 (dd, *J* = 3.5, 10.0, 1H) 4.36 (c, *J* = 3.9, 8.5, 8.0, 1H), 5.68 (d, *J* = 8.0, 1H), 6.77 (bs, 1H). ^{13}C NMR: 9.8, 14.1, 22.6, 22.6, 25.9, 26.9, 29.3, 29.3, 29.3, 29.5, 29.6, 29.6, 31.9, 37.6, 54.7, 67.7, 73.5, 101.6, 120.9, 158.6. $[\alpha]_D$: −26.15 (*c* 0.91, $CHCl_3$). ESI-MS: *m*/*z* 416 $[M + 1]^+$, 438 $[M + Na]^+$, 853 $[2M + Na]^+$. HRMS: calcd for $C_{21}H_{37}NO_5S$ 415.2392, found 415.2401.

Oxazolidinone 22. A mixture of **21** (45 mg, 0.1 mmol), $NaBH_4$ (41 mg, 1.1 mmol), and dry DMSO (4 mL) was stirred at 40 °C for 7 h. Water (20 mL) was added, and the product was extracted with EtOAc. The organic layer was washed with brine, dried, and evaporated to give 35 mg of a yellow oil. Purification by column chromatography (hexane/EtOAc (2:1)) afforded 23 mg (0.07 mmol, 66%) of **22**. ^{1}H NMR: 0.87 (t, *J* = 6.3, 6H), 1.08 (d, AB system, *J* = 8.5, 1H), 1.13 (d, AB system, *J* = 8.0, 1H), 1.12 (d, *J* = 6.5, 3H), 1.40−1.20 (m, 20H), 1.58 (m, 4H), 2.45 (t, *J* = 7.0, 0.5, 2H), 4.15 (m, 1H), 5.56 (d, *J* = 7.5, 1H), 5.66 (bs). ^{13}C NMR: 9.6, 14.1, 17.2 22.7, 25.9, 27.0, 29.3, 29.3, 29.6, 29.6, 29.7, 31.9, 51.8, 76.5, 103.6, 118.7, 159.0. $[\alpha]_D$: −3.77 (*c* 0.99, $CHCl_3$). HRMS: calcd for $C_{20}H_{35}NO_2$ 321.2668, found 321.2671.

Amide 9. The same procedure used to obtain **10** from **23** was applied to **22** (28 mg, 0.087 mmol) to give **9** (13 mg, 0.031 mmol, 35%) as a white solid after column chromatography purification (CH_2Cl_2/MeOH 96:4). Mp: 56−57 °C. IR: 3304, 2926, 1636. ^{1}H NMR: 0.87 (t, *J* = 6.5, 6H), 0.98 (s, 2H), 1.12 (d, *J* = 6.5, 3H), 1.40−1.20 (m, 20H), 1.59 (m, 4H), 2.17 (t, *J* = 7.0, 2H), 2.44 (t, *J* = 7.0, 2H), 4.31 (dq, *J* = 2.5, 7.0, 1H), 4.66 (s, 1H), 5.67 (d, *J* = 7.5, 1H). ^{13}C NMR: 8.3, 14.0, 14.1, 15.7, 22.6, 22.7, 25.7, 25.9, 27.3, 29.0, 29.2, 29.3, 29.4, 29.4, 29.6, 29.6, 31.7, 31.9, 36.8, 49.7, 71.5, 107.7, 115.1, 173.6. $[\alpha]_D$: −19.15 (*c* 0.38, $CHCl_3$). ESI-MS: *m*/*z* 422 $[M + 1]^+$, 444 $[M + Na]^+$, 865 $[2M + Na]^+$. HRMS: calcd for $C_{27}H_{51}NO_2$ 421.3920, found 421.3920.

Fluorooxazolidinone 24. A solution of **20** (86 mg, 0.26 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated, at −78 °C under argon, with DAST (68 μL, 0.52 mmol). The mixture was allowed to warm to 25 °C during 9 h and stirred at this temperature for 12 h. The solution was cooled to 0 °C, and a few drops of MeOH were added. The product was extracted with Et_2O, and the organic layer was washed sequentially with saturated aqueous $NaHCO_3$ and brine and dried. Solvent elimination and column chromatography of the residue (hexane/EtOAc 2:1) gave 70 mg (0.21 mmol, 82%) of **24**. Mp: 33 °C. ^{1}H NMR: 0.87 (t, *J* = 6.5, 6H), 1.08 (d, AB system, *J* = 7.5, 1H), 1.14 (d, AB system, *J* = 8.0 Hz), 1.2−1.4 (m, 20 H), 1.58 (m, 4H), 2.49 (dt, *J* = 7.0 and 1.0, 2H), 4.28 (m, 2H), 4.48 (m, 1H) 5.67 (d, *J* = 7.5, 1H), 6.1 (bs, 1H).^{13}C NMR: 9.7, 14.1, 22.7, 25.9, 26.9, 29.3, 29.3, 29.6, 29.6, 31.9, 55.4 (d, *J* = 20.5), 69.8, 73.4 (d, *J* = 65), 82.4 (d, *J* = 170), 102.0, 120.3, 158.5. ^{19}F NMR: −35.47 (m). $[\alpha]_D$: −20.62 (*c* 016, $CHCl_3$). HRMS: calcd for $C_{20}H_{34}NO_2F$ 339.2574, found 339.2577.

Amide 11. The same procedure used to obtain **10** from **23** was applied to **24** (70 mg, 0.21 mmol) to obtain **11** (19 mg, 0.044 mmol, 20%) as a white solid after column chromatography (CH_2Cl_2/MeOH 99:1). Mp: 74−75 °C. IR: 3292, 3075, 2922, 1649. ^{1}H NMR: 0.88 (t, *J* = 6.0, 6H), 1.02 (s, 2H), 1.2−1.4 (m, 20H), 1.62 (m, 4H), 2.23 (t, *J* = 7.0, 2H), 2.46 (dt, *J* = 7.0, 2.0, 2H), 4.30 (dm, *J* = 28.0, 1H), 4.54 (AB system, *J* = 3.0, 10.0, 47.5, 1H), 4.64 (AB system, *J* = 4.0, 10.0, 47.0, 1H) 4.78 (bs, 1H), 6.07 (bs, *J* = 8.5 Hz). ^{13}C NMR: 8.3, 14.0, 14.1, 22.6, 22.7, 25.6, 25.8, 27.3, 28.8, 29.2, 29.4, 29.4, 29.6, 29.6, 31.6, 31.9, 36.7, 52.8 (d, *J* = 17.4), 68.8, 83.2 (d, *J* = 168.5), 107.1, 115.9, 173.5. ^{19}F NMR: −40.20 (dt, *J* = 28, 47). $[\alpha]_D$: −5.34 (*c* 0.78, $CHCl_3$). ESI-MS: *m*/*z* 440 $[M + 1]^+$, 462 $[M + Na]^+$, 903 $[2M + Na]^+$. HRMS: calcd for $C_{27}H_{50}NO_2F$ 439.3826, found 439.3830.

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Supporting Information Available: General experimental details and copies of ^{13}C and/or ^{1}H NMR spectra for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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