A mechanism-based sphingosine-1-phosphate lyase inhibitor

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Abstract

The synthesis of a series of vinylated analogs of sphingosine-1-phosphate, together with their unambiguous configurational assignment by VCD methods is reported. Among them, compound **RBM10-8** can irreversibly inhibit human sphingosine-1-phosphate lyase (hS1PL), while behaving also as an enzyme substrate. These findings, together with the postulated mechanism for S1PL activity, reinforce the role of **RBM10-8** as a new mechanism-based hS1PL inhibitor.

INTRODUCTION

Sphingosine-1-phosphate (S1P) is a sphingolipid (SL) that acts as a signaling molecule regulating diverse cellular processes such as cell proliferation and differentiation, angiogenesis, immune function, inflammation and development, among others.¹ S1P homeostasis is tightly regulated by its synthesis and degradation. S1P is synthesized by the phosphorylation of the long-chain base sphingosine (So) by cytosolic sphingosine kinase-1 (SoK1) or nuclear and mitochondria-associated sphingosine kinase-2 (SoK2), whereas sphingosine-1-phosphate lyase (S1PL) catalyzes the irreversible degradation of S1P in the final step of the sphingolipid catabolic pathway. This latter enzyme is ubiquitously expressed and is located in the endoplasmic reticulum (ER).² It is a 63.5 KDa³ pyridoxal 5'-phosphate (PLP)-dependent lyase that cleaves the C2-C3 bond of the S1P substrate to give *trans*-2-hexadecenal and phosphoethanolamine (PEA) (Scheme 1)

Scheme 1. Irreversible degradation of S1P and analogs **RBM10** catalyzed by S1PL and PLP as cofactor.

S1PL modulates neuronal autophagy by PEA production,⁴ whereas *trans*-2-hexadecenal indirectly induces the transcription of inflammatory cytokines.⁵ It also forms DNA⁶ and protein adducts with glutathione⁷ and Bax,⁵ whose activation is related with the induction of apoptosis. Although a number of selective S1PL inhibitors to control S1P and *trans*-2-hexadecenal levels have been reported,⁸ activity-based irreversible inhibitors are not known. In this paper, we wish

to report on a series of stereodefined S1P analogs (**RBM10-5** to **RBM10-8**, Scheme 2) arising from the introduction of a C3-vinyl moiety on the sphingoid chain in the search of putative S1PL mechanism-based covalent inhibitors (Scheme 1)^{9,10}. The choice of the vinyl group was dictated by mechanistic considerations, based on the accepted S1PL catalytic cycle,¹¹ and on the expected reactivity of the resulting α,β -unsaturated ketone **10** as a Michael acceptor to irreversibly bind S1PL. Although the natural stereochemistry was expected to be selectively used by S1PL to yield the α,β -unsaturated ketone, the inherent conformational flexibility of SLs made us consider the four possible stereoisomers of **RBM10** as potential mechanism-based inhibitors.

RESULTS AND DISCUSSION

Synthesis and configurational assignments

The synthesis of the natural (2*S*) or the non-natural (2*R*) series was carried out from *L*- or *D*-Garner's aldehyde,¹² respectively, as shown in Scheme 2.

Scheme 2. Synthesis of (2*S*)-3-vinylS1P analogs: Conditions: a: Magnesium dodecyl bromide, THF (**1**, 92%); b: Pyridinium dichromate, DCM (**2**, 74%); c: vinylmagnesium bromide, FeCl₂, MTBE-THF (**3a**, 52% and **3b**, 23%); d: Acetyl chloride, MeOH (**RBM10-1**, 84% from **3a**, **RBM10-2**, 80% from **3b**); e: pTsOH, MeOH (**4a**, 97% from **3a** and **4b**, 95 % from **3b**); f:

N-methylimidazole, dimethyl chlorophosphate (**5a**, 74% from **4a** and **5b**, 78% from **4b**); g: trimethyl silyl bromide (TMSBr), MeOH-H₂O (**RBM10-5**, 80% from **5a**, **RBM10-6**, 85% from **5b**). The same sequence led to the enantiomeric 2*R* series from *D*-Garners's aldehyde in comparable yields (see the Supporting Information)

Addition of a vinyl organometallic to ketone (*S*)-**2** was explored using different additives. In all cases,¹³ the major stereoisomer was assigned as **3a** (Scheme 2), whose formation can be interpreted according to the a Cram-chelation model.¹⁴ Considering that we were interested in both diastereomers, the use of FeCl₂ (1 equiv/mol) at -78 °C in MBTE was preferred, since it afforded the higher **3b/3a** ratio, which, nonetheless, was never superior to a modest 3:7. Luckily, both diastereomers could be separated by flash chromatography and their relative configurations were unambiguously assigned as described below (See also the Supporting Information). The same protocol starting from *D*-serine led to the corresponding enantiomers *ent*-**3a** and *ent*-**3b**. Aminodiols **RBM10-1** to **RBM10-4** were obtained in a single step from the corresponding precursors **3**, whereas the corresponding amino phosphates **RBM10-5** to **RBM10-8** required the multistep sequence of acetonide removal, phosphorylation and final deprotection shown in Scheme 2.

Configurational assignments of vinyl derivatives **3a** and **3b** were initially attempted by *nOe* experiments from the bicyclic derivatives **6a** and **6b**, obtained by intramolecular NaH-promoted cyclization, similarly as reported by us for a related system.¹⁵ The weak *nOe* effect (around 2%) measured between the H_a and H_b protons in **6a** (Scheme 3) led us infer a tentative (2*S*,3*S*) configuration for its precursor **3a**. However, the absence of any measurable *nOe* effect in **6b** did not allow the unambiguous configurational assignment for the precursor **3b**. At this stage, vibrational circular dichroism (VCD)¹⁶ was considered as a suitable alternative method that helped us confirm the above predictions.

Scheme 3. Cyclization of **3a** and **3b** and observed *nOe* effects in the corresponding cyclic **6a** and **6b** derivatives

Vibrational circular dichroism (VCD) is a powerful technique commonly used for the stereochemical analysis of complex natural products. It is based on the measure of the differential absorption of left versus right circularly polarized infrared (IR) radiation by molecular vibrational transitions, having the advantages of both CD and IR features.¹⁷ In combination with density functional theory (DFT) calculations, VCD has become one of the most reliable methods to determine the chirality of a variety of small molecules.¹⁸ Examples of the application of this technique to the stereochemical analysis of glycerolipids¹⁹ and sphingosine²⁰ have been reported.

As expected, the experimental IR profiles obtained for both diastereomers show high similarity (Figure 1). Nonetheless, the disparities observed between their VCD spectra (predicted correctly by quantum chemical calculations) allowed us to confirm the absolute configuration of the differential stereocenter (C₃) in both species. These differences occur in the region around 1300 cm⁻¹, where, in agreement with their calculated values, vibrations due to the deformation of the vinyl group at C₃ are expected. In this region, the negative signal observed at 1294 cm⁻¹ in the VCD spectrum of **6b** finds no counterpart in the spectrum of **6a** (Figure 1), an observation that can be justified by theoretical calculations. Thus, while normal modes 105 to 101 (their scaled values range from 1305 to 1289 cm⁻¹) present a +/+/-/-/- pattern in the (2*S*,3*R*)

diastereomer, with dominating negative contributions (Table S3), in the (2*S*,3*S*) diastereomer the same modes present a -/-/+/- pattern with calculated intensities that cause negative and positive contributions to cancel (Table S2). Remarkably, normal modes 105 to 101 are robust, according to the definition of Nicu *et al.*²¹ as it can be seen from the values of the calculated angle between the electric and magnetic dipole transition moments (highlighted in Tables S2 and S3). This implies that, according to Nicu's definition of robustness, the signs of the bands in the region would be reliable regardless of the calculation method used,²¹ which reinforces our conclusion.

Figure 1. Experimental (colored line) vs theoretical (black line) IR and VCD spectra in the 1400-1000 cm^{-1} region for **6a** (left) and **6b** (right).

Also, in this region, the negative band at 1310 cm^{-1} in the VCD spectrum of **6a**, that can be attributed to normal mode 109 (-) according to its calculated value, is absent in the spectrum of the (2*S*,3*R*) diastereomer, for which the calculated intensity of the 109 normal mode (-) is almost

negligible (Table S3). Moreover, the strong negative feature observed at *ca.* 1335 cm⁻¹ in the VCD spectra of both diastereomers is attributable in both cases to vibrational motions that involve C₂, that has the same configuration in both species (modes 112 and 113, -/-, Tables S2 and S3). It is worth mentioning that, except for the vibrational band observed in the VCD spectra at *ca.* 1350 cm⁻¹, the remaining features observed in the above commented region of the VCD spectra have no counterpart in the experimental IR spectra. This can be explained based on the negligible IR intensities that are calculated for the normal modes in this area (Tables S2 and S3) and represent a clarifying example of the exquisite structural sensitivity of the VCD technique as compared with IR spectroscopy. Finally, a merely visual inspection shows that the overall agreement between experimental and theoretical IR and VCD profiles is reasonably good in the 1400-1000 cm⁻¹ range (Figure 1). Nonetheless, to avoid a biased comparison, the match between the profiles is expressed in terms of the neighborhood similarity (NS) and enantiomeric similarity indices (ESI) according to the methodology reported elsewhere.^{22,23} The values obtained for both species, as reported in Figure 1, suggest that their absolute configurations have been assigned correctly with a high confidence level (the so-called CompareVOA index).

Inhibition of recombinant hS1PL by compounds RBM10

Compounds **RBM10-1** to **RBM10-8** were first tested as inhibitors of recombinant hS1PL. Only the amino phosphate **RBM10-8** behaved as hS1PL inhibitor (at 100 μM), using **RBM148**²⁴ as fluorogenic substrate (Fig S6). Slightly higher potencies were observed when S1P was used as substrate and hS1PL inhibition was measured by quantitation (UPLC-TOF-MS) of the resulting 2-hexedecenal, after derivatization with isoniazid²⁵ (Figure 2A). As expected, the non-phosphorylated precursor alcohols **RBM10-1** to **RBM10-4** were inactive in both enzyme assays. Importantly, the adduct **11**, which results from reaction of isoniazid with

1-pentadecen-3-one (**10**), was also detected in the chromatograms. Since ketone **10** is the expected reaction product from the metabolization of amino phosphates **RBM10** by hS1PL, this finding unveiled amino phosphates **RBM10** as hS1PL substrates, particularly **RBM10-8**, with an unnatural *2R,3S* configuration (Scheme 4 and Figure 2B). Examples where sphingolipid analogs with non-natural configurations are metabolized by the target enzymes have been preceded.^{26–28} In addition, previous results from our group on differently configured 2-vinyl S1P analogs, proved the ability of the hS1PL active site to discriminate between the different stereoisomers of the amino diol phosphate framework.²⁹

Figure 2. Inhibition of recombinant hS1PL by amino diols and amino phosphates **RBM10**. Inhibitor **12**³⁰ (see structure in Supplementary Material) is used as reference. A) Compounds (**RBM10**, 100 μ M; **12**, 200 nM) were incubated with hS1PL in the presence of 10 μ M S1P. The amount of *trans*-2-hexadecenal was quantified after chemical derivatization with isoniazid. B) Quantitative analysis of adduct **11**, arising from reaction of isoniazid with **10**, the putative reaction product from hS1PL catalyzed lysis of compounds **RBM10**. Data (mean $\pm$ SD) were obtained from two experiments with triplicates.

Inhibition of hS1PL by **RBM10-8**, together with its ability to act also as hS1PL substrate, prompted us to consider **RBM10-8** as a putative hS1PL mechanism-based inhibitor. Under this scenario, the enzymatically generated 1-pentadecen-3-one (**10**) would be able to react as a Michael-type acceptor with a nearby nucleophile close to the enzyme active site (Scheme 4). In support of this assumption, **RBM10-9** (the saturated analog of **RBM10-8**) was totally devoid of inhibitory activity, even though formation of the corresponding isoniazid adduct with ethyl ketone **13** was detected (results not shown). This result sustains the essential requirement of the vinyl moiety as a Michael acceptor for inhibition. Interestingly, exogenous vinyl ketone **10** was inactive as inhibitor. (Figure S6).

Scheme 4. Proposed mechanism for the mechanism-based inhibition of hS1PL by **RBM10-8**. For the synthesis of **RBM10-9**, see the Experimental Section.

To confirm this assumption, a kinetic experiment³¹ with **RBM10-8**, using recombinant hS1PL, **RBM10-9** as negative control, and inhibitor **12**³⁰ as a positive control for reversible inhibition, was performed. Incubation with the above compounds at the indicated concentrations (Figure 3), followed by 1/100 dilutions, led to an apparent reversible inhibition for **12** (at 200 nM) and **RBM10-9** (at 5 mM), as evidenced by the progressive increase of the fluorescence at longer incubation times. However, incubation with **RBM10-8** at 5 mM (50 μ M at final dilution, non-inhibitory concentration in the enzyme assay), under the same conditions, prevented the recovery of the enzyme activity, even at long incubation times (140 min) (Figure 3).

Figure 3. Irreversibility assay on hS1PL. hS1PL activity kinetics of 140 min using **RBM148** as fluorogenic substrate in the presence of **RBM10-8** and **RBM10-9**. Compound **12** was used as a control of reversible inhibition. Data (mean $\pm$ SD), expressed in fluorescence arbitrary units, were obtained from two experiments with triplicates.

The maximal rate of inactivation (k_{inact}) and the concentration at 50% k_{inact} (K_I) for **RBM10-8** by varying seven different inhibitor concentrations were also calculated, as shown in Figure 4.³² These findings are in agreement with a mechanism-based inhibition for **RBM10-8**.

Figure 4. (A): hS1PL (0.5 μL of a 300 $\mu\text{g/mL}$ hS1PL solution) was preincubated at 37 $^{\circ}\text{C}$ with six concentrations of **RBM10-8** at a final volume of 1 μL ($[I] = 0.08\text{--}5 \text{ mM}$). At each time point, the enzymatic reaction started by adding 94 μL buffer and **RBM148** (5 μL from a 2.5 mM DMSO solution, final concentration 125 μM) and it was stopped after 1h. (B) Inactivation plot, logarithm of the % remaining activity versus preincubation time. (C) Nonlinear regression analysis of the negative slopes against inhibitor concentration gives rise to k_{inact} and K_I values, as indicated.

Covalent adducts between ketone 10 and purified hS1PL

In order to gain insight into the mechanism-based irreversible inhibition of hS1PL by the putative covalent bonding of the resulting vinyl ketone **10**, hS1PL was incubated with **RBM10-8** and subsequently treated with the endoproteinase Lys-C and trypsin, as described in

the Experimental Section. The ESI-spectra of the peptides resulting from the treatment of hS1PL with **RBM10-8** were compared to those from a control experiment in order to assess the presence of new peptides arising from the incorporation of the putative vinyl ketone **10** ($m/z=+224.2140$). As shown in Figure 5, two peptides with mass increases matching those expected after reaction with vinyl ketone **10** were identified. In this way, peptide A ($m/z = 1467.6963 \pm 0.05$) and peptide B ($m/z = 1436.2698 \pm 0.05$) can be assigned as the corresponding adducts with fragments 279-303 and 132-155, respectively. Interestingly, these fragments are surrounding the enzyme active site in the hS1PL dimer (see Figure S7).

Figure 5. Sequence of hS1PL (PDB 4Q6R) and UPLC-MS-TOF peaks of tryptic hS1PL after incubation with **RBM10-8** (up) compared to control (down). (A) and (B) are peaks matching the expected mass for the yellow and green hS1PL sequences (fragments 279-303 and 132-155, respectively) covalently bonded to vinyl ketone **10**.

In agreement with our mechanistic hypothesis, which requires the presence of nucleophilic residues as Michael donors for reaction with vinyl ketone **10**, the use of a general electrophilic reagent should diminish or abolish the enzyme activity. Among the different reagents currently used for the covalent modification of nucleophilic residues, phenylmethylsulfonyl fluoride (PMSF) is a well-recognized inhibitor of some serine proteases (although not all serine residues are equally reactive),³³ whereas diethylpyrocarbonate (DEPC) is an unspecific electrophile that reacts with histidine, lysine, cysteine and tyrosine residues.³⁴ In our case, the hS1PL activity was completely abolished by DEPC, although it was not significantly affected by PMSF, a fact that can be probably related to the above referred inherent selectivity of this reagent (Figure 6).

Figure 6. hS1PL activities after treatment with **PMSF** (1 mM) and **DEPC** (0.1%) using **RBM148** as fluorogenic substrate. Inhibitor **12**³⁰ (500 nM) was used as reference. Enzyme activity is expressed as % relative to control. Data (mean $\pm$ SD) were obtained from one experiment with triplicates.

CONCLUSIONS

Starting from *L*- or *D*-Garner's aldehyde, a series of configurationally defined vinylated analogs of S1P is reported. Their absolute configuration has been unambiguously confirmed by VCD

methods through the bicyclic derivatives **6a** and **6b**. Compound **RBM10-8** behaves as a mechanism-based hS1PL inhibitor, as evidenced by the irreversible alkylation of nucleophilic residues close to the enzyme active site by the electrophilic vinyl ketone **10** arising from the metabolism of **RBM10-8** by hS1PL, in agreement with the accepted mechanism for this enzyme.¹¹ These findings paves the way to the design of a suitably modified analog of **RBM10-8** for the *in vivo* imaging of this interesting enzyme. Current work along this line is currently underway in our laboratory and will be reported in due course.

EXPERIMENTAL SECTION

Synthesis

Unless otherwise stated, reactions were carried out under argon atmosphere. Dry solvents were obtained by passing through an activated alumina column on a Solvent Purification System (SPS). Methanol and ethanol were dried over CaH₂ and distilled prior to use. Commercially available reagents and solvents were used with no further purification. All reactions were monitored by TLC analysis using ALUGRAM[®] SIL G/UV₂₅₄ precoated aluminum sheets (Machery–Nagel). UV light was used as the visualizing agent and a 5% (w/v) ethanolic solution of phosphomolybdic acid as the developing agent. Usual workup stands for extraction of the reaction mixture with the appropriate solvent, followed by washings with brine, drying over Na₂SO₄, filtration and evaporation of the organic phase to dryness. Flash column chromatography was carried out with the indicated solvents using flash–grade silica gel (37–70 μm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. NMR spectra were recorded at room temperature on a Varian Mercury 400 instrument. The chemical shifts (δ) are reported in ppm relative to the solvent signal and coupling constants (*J*) are reported in Hertz (Hz). Deuterated solvents were used as internal standards (CDCl₃: δ = 7.26 ppm for ¹H, δ = 77.16 ppm for ¹³C; CD₃OD: δ = 3.31 ppm for ¹H,

δ = 49.00 ppm for ^{13}C . Chemical shift (δ) values are reported in ppm. ^{31}P chemical shifts are relative to an aqueous 85 % H_3PO_4 solution as external reference (0 ppm). Signal characterization is described using the next abbreviations: s (singlet), d (doublet), t (triplet), q and m (multiplet). Coupling constant values are presented in Hertz (Hz). High Resolution Mass Spectrometry analyses were carried out on an Acquity UPLC system coupled to a LCT Premier orthogonal accelerated time-of-flight mass spectrometer (Waters) using electrospray ionization (ESI) technique. Optical rotations were measured at room temperature on a Perkin Elmer 341 polarimeter.

***tert*-Butyl (4*S*,1'*RS*)-4-(1-hydroxytridecyl)-2,2-dimethyloxazolidine-3-carboxylate (1).**

A solution of magnesium dodecyl bromide (40 mL of a 0.8 M solution in THF, 32 mmol) was added dropwise to a solution of L-Garner's aldehyde ¹² (4.6 g, 20 mmol) in 10 mL THF. After stirring for 12h at rt, the resulting yellow solution was poured over crushed ice and extracted with DCM (3x 20 mL). Usual workup afforded an oil, which after flash chromatography on hexanes/EtOAc (from 0 to 15%) afforded alcohol **1** (6.96 g, 92% yield) as a mixture of diastereomers.

^1H NMR (CDCl_3 , 400 MHz): δ 4.25-3.60 (broad, 5H), 1.55 (s, 3H), 1.47 (s, 12H), 1.41-1.19 (broad, 22H), 0.89 (t, 3H). $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, Chloroform-*d*) δ 94.1, 81.2 (broad), 74.0 and 72.7 (rotamers), 64.8 and 62.4 (rotamers), 31.9, (29.6-29.3), 28.3, 26.4, 26.1, 22.6, 14.0. HRMS (TOF): m/z calc. for $\text{C}_{23}\text{H}_{45}\text{NNaO}_4^+$ $[\text{M}+\text{Na}]^+$ 422.3241; Found: 422.3246.

Starting from D-Garner's aldehyde, a mixture of diastereomers **1**, of identical composition to that described above, was obtained in comparable yields.

***tert*-Butyl (*S*)-2,2-dimethyl-4-tridecanoyloxazolidine-3-carboxylate (*S*)-2 and (*R*)-2**

A solution of pyridinium dichromate (12.63 g, 24.62 mmol) in DCM (125 mL) was added dropwise over a solution of **1** (6.9 g, 17.4 mmol) in DCM (25 mL). The resulting brown solution was covered with aluminum foil and the reaction mixture was kept under stirring at room temperature. After 48h, an excess NaSO₃ (4.5 g, 36 mmol) was added portionwise, which made the solution turn from brown to green. This solution was washed with water (3x50 mL) and the organic phase was dried and evaporated to dryness to give an oil, which after chromatography on hexanes/EtOAc (95:5) afforded (*S*)-**2** (5.1 g, 74% yield) as a yellow oil.

$[\alpha]_{\text{D}}^{25}$ -38.1 (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, Chloroform-*d*) δ 4.42 (dd, *J* = 7.3, 2.5 Hz) and 4.30 (dd, *J* = 7.6, 2.7 Hz), rotamers 1H, 4.12 (dt, *J* = 12.5, 8.9 Hz), 3.91 (dd, *J* = 9.3, 2.7 Hz) and 3.87 (dd, *J* = 9.4, 2.8 Hz), rotamers 2H, 2.54 – 2.39 (m, 2H), 1.69 (s), 1.64 (s) and 1.51 (s), rotamers 6H, 1.56 (t, 2H), 1.30-1.22 (broad, 18H), 0.86 (t, *J* = 6.8 Hz, 5H). ¹³C{¹H} (101 MHz, Chloroform-*d*) δ 208.6 and 208.2 (rotamers), 152.4 and 151.5 (rotamers), 95.2 and 94.5 (rotamers), 80.9 and 80.5 (rotamers), 65.8 and 65.4 (rotamers), 65.3 and 65.2 (rotamers), 39.1 and 38.5 (rotamers), 29.7-29.2, 28.4 and 28.3, and 23.7 (rotamers), 26.3, 23.2 and 22.8 (rotamers), 22.7, 14.2. HRMS (TOF): *m/z* calc. for C₂₃H₄₃NNaO₄⁺ [M+Na]⁺ 420.3084; found 420.3096.

Starting from D-Garner's aldehyde, ketone (*R*)-**2** was obtained in comparable yields. $[\alpha]_{\text{D}}^{25}$ +41.5 (*c* 1.0, CHCl₃); HRMS (TOF): *m/z* calc. for C₂₃H₄₃NNaO₄⁺ [M+Na]⁺ 420.3084; found 420.3088.

***tert*-Butyl (S)-4-((S)-3-hydroxypentadec-1-en-3-yl)-2,2-dimethyloxazolidine-3-carboxylate (3a), *tert*-butyl (S)-4-((R)-3-hydroxypentadec-1-en-3-yl)-2,2-dimethyloxazolidine-3-carboxylate (3b), *ent*-3a and *ent*-3b.**

A solution of vinylmagnesium bromide (1.0 M in THF, 1.7 mL) was added dropwise over a solution of ketone (*S*)-**2** (660 mg, 1.66 mmol) in MTBE (10 mL) at -78°C containing FeCl₂

(215 mg, 1.7 mmol). The reaction was stirred for 24h, while the temperature was allowed to rise to rt over that time. The reaction mixture was next quenched with few drops of water and concentrated to remove most of the MTBA. The residue was taken up in DCM-H₂O (1:1, 15 mL) and worked up as usual to afford a yellow oil, which was purified by flash chromatography on hexanes/EtOAc (from 9:1 to 7:3) to afford 375 mg (52% yield) of **3a** and 162 mg (23% yield) of **3b**.

Analytical data for **3a** (2*S*,3*S*): Yellow oil. $[\alpha]_{\text{D}}^{25}$ -3.9 (*c* 1.0, CHCl₃). TLC (Hexane: Et₂O, 9:1); *R*_f 0.28. ¹H NMR (400 MHz, Chloroform-*d*) δ 5.79 (dd, *J* = 16.4, 10.8 Hz, 1H), 5.46 (d, *J* = 16.8 Hz, 1H), 5.19 (d, *J* = 10.5 Hz, 1H), 4.84 (s, 1H), 4.01-3.88 (broad, 3H), 1.59 (s, 3H), 1.49 (broad, 12H), 1.45 – 1.38 (m, 2H), 1.24 (s, 20H), 0.87 (t, *J* = 6.6 Hz, 3H). ¹³C{¹H} (101 MHz, Chloroform-*d*) δ 155.6, 141.3, 115.7, 94.5, 81.4, 77.6, 66.2, 64.6, 35.4, 30.3, 29.6, 29.6, 29.3, 28.3, 26.2, 24.2, 23.1, 22.7, 14.1. HRMS (TOF): *m/z* calc. for C₂₅H₄₇NNaO₄⁺ [M+Na]⁺ 448.3397; found 448.3404.

Analytical data for **3b** (2*S*,3*R*): Yellow oil. $[\alpha]_{\text{D}}^{25}$ -5.4 (*c* 1.0, CHCl₃). TLC (Hexane: Et₂O, 9:1); *R*_f 0.45. ¹H NMR (400 MHz, Chloroform-*d*) δ 5.77 (dd, *J* = 16.9, 10.6 Hz, 1H), 5.47 (s, 1H), 5.39 (dd, *J* = 17.0, 2.1 Hz, 1H), 5.15 (d, *J* = 10.6 Hz, 1H), 4.07 (s, 1H), 3.97 (dd, *J* = 16.7, 10.4 Hz, 2H), 1.54-1.39 (broad, 15H), 1.31-1.18 (broad, 22H), 0.86 (t, *J* = 6.9 Hz, 3H). ¹³C{¹H} (101 MHz, Chloroform-*d*) δ 155.6, 140.5, 114.4, 94.5, 81.4, 77.8, 65.6, 64.5, 39.0, 31.9, 30.2, 29.61, 29.59, 29.57, 29.3, 28.2, 25.9, 24.5, 22.9, 22.6, 14.1. HRMS (ESI): *m/z* calc. for C₂₅H₄₇NNaO₄⁺ [M+Na]⁺ 448.3397; found 448.3411.

Starting from ketone (*R*)-**2**, a mixture of alcohols *ent*-**3a** and *ent*-**3b** was obtained in comparable yields.

ent-**3a**: $[\alpha]_{\text{D}}^{25}$ +4.7 (*c* 1.0, CHCl₃). HRMS (ESI): *m/z* calc. for C₂₅H₄₇NNaO₄⁺ [M+Na]⁺ 448.3397; found 448.3392.

ent-3b: $[\alpha]_{\text{D}}^{25} +5.2$ (c 1.0, CHCl_3). HRMS (ESI): m/z calc. for $\text{C}_{25}\text{H}_{47}\text{NNaO}_4^+$ $[\text{M}+\text{Na}]^+$ 448.3397; found 448.3404

***tert*-Butyl (1,3-dihydroxy-3-vinylheptadecan-2-yl)carbamate (4a, 4b, ent-4a and ent-4b).**

A solution of *p*-TsOH (35 mg, 0.21 mmol) in MeOH (5 mL) was added dropwise to a solution of **3** (200 mg, 0.47 mmol) in MeOH (35 mL) and the reaction was kept under stirring at rt. After 24 h, the reaction was neutralized with aq sat NaHCO_3 and the mixture was concentrated *in vacuo*. The residue was diluted with water, extracted with DCM (2 x 20 mL) and worked-up as usual. The resulting oil was chromatographed on DCM/MeOH (from 0 to 1% MeOH) to afford the corresponding carbamate **4**.

4a (2*S*,3*S*) was obtained in 97% yield (175.8 mg) as a white powder from **3a**. $[\alpha]_{\text{D}}^{25} -3.3$ (c 1.0, CHCl_3). ^1H NMR (400 MHz, Chloroform-*d*) δ 5.75 (dd, $J = 17.3, 10.8$ Hz, 1H), 5.25 (dd, $J = 17.4, 1.4$ Hz, 1H), 5.16 (d, $J = 10.9$ Hz, 1H), 3.95 (d, $J = 11.5$ Hz, 1H), 3.78 (dt, $J = 10.9, 4.2$ Hz, 1H), 3.57 (dt, $J = 8.4, 3.7$ Hz, 1H), 1.70 – 1.50 (m, 2H), 1.39 (s, 9H), 1.23 (broad, 20H), 0.85 (t, $J = 6.7$ Hz, 3H). $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, Chloroform-*d*) δ 156.2, 140.6, 114.3, 79.5, 78.6, 62.9, 56.1, 38.7, 31.9, 30.0, 29.62, 29.60, 29.56, 29.51, 29.3, 28.3, 23.4, 22.7, 14.1. HRMS (TOF): m/z calc. for $\text{C}_{22}\text{H}_{43}\text{NNaO}_4^+$ $[\text{M}+\text{Na}]^+$ 408.3084; found 408.3071

4b (2*S*,3*R*) was obtained in 95% yield (172.2 mg) as an oil from **3b**. $[\alpha]_{\text{D}}^{25} -4.6$ (c 1.0, CHCl_3). ^1H NMR (400 MHz, Chloroform-*d*) δ 5.82 (dd, $J = 17.2, 10.8$ Hz, 1H), 5.36 (d, $J = 17.0$ Hz, 2H), 5.22 (dd, $J = 10.8, 1.3$ Hz, 1H), 3.89 (d, $J = 11.0$ Hz, 1H), 3.71 – 3.64 (m, 1H), 3.56 (d, $J = 9.2$ Hz, 1H), 1.63 – 1.52 (m, 2H), 1.43 (s, 9H), 1.21 (s, 21H), 0.85 (t, $J = 6.8$ Hz, 3H). $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, Chloroform-*d*) δ 156.4, 141.0, 114.2, 79.5, 79.2, 63.7, 55.7, 38.1, 31.9, 30.0, 29.59, 29.58, 29.47, 29.28, 28.3, 22.9, 22.6, 14.0. HRMS (TOF): m/z calc. for $\text{C}_{22}\text{H}_{43}\text{NNaO}_4^+$ $[\text{M}+\text{Na}]^+$ 408.3084; found 408.3092

ent-**4a** (2*R*,3*R*) was obtained in 92% yield (166.7 mg) as an oil from *ent*-**3a**. $[\alpha]_{\text{D}}^{25}$: +2.6 (*c* 1.0, CHCl₃). Spectroscopic data were identical to those of **4a** (see above). HRMS (TOF): *m/z* calc. for C₂₂H₄₃NNaO₄⁺ [M+Na]⁺ 408.3084; found 408.3098.

ent-**4b** (2*R*,3*S*) was obtained in 91% yield (164.9 mg) as an oil from *ent*-**3b**. $[\alpha]_{\text{D}}^{25}$: +5.6 (*c* 1.0, CHCl₃). Spectroscopic data were identical to those of **4b** (see above). HRMS (TOF): *m/z* calc. for C₂₂H₄₃NNaO₄⁺ [M+Na]⁺ 408.3084; found 408.3091.

tert-Butyl N-[1-[(dimethoxyphosphoryl)oxy]-3-hydroxy-3-vinylpentadecan-2-yl]carbamate (5a, 5b, *ent*-5a and *ent*-5b)

A solution of the starting diol **4** (0.5 mmol) in DCM (15 mL) at 0 °C was treated successively with *N*-methylimidazole (1.5 equiv/mol) and dimethyl chlorophosphate (1.2 equiv/mol). The reaction mixture was stirred at rt for 1 h, cooled down to 0 °C and quenched by the dropwise addition of saturated aqueous NH₄Cl (10 mL). The resulting mixture was extracted with DCM (3 x 15 mL) and the combined organic layers were combined and worked-up as usual to afford a crude mixture, which was purified by flash chromatography on DCM/MeOH (0 to 5%).

5a (2*S*,3*S*) was obtained in 74% yield (182.6 mg) as a white powder from diol **4a**; TLC (DCM/MeOH, 9:1): R_f: 0.1. $[\alpha]_{\text{D}}^{25}$ -4.7 (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, Chloroform-*d*) δ 5.73 (dd, *J* = 17.2, 10.8 Hz, 1H), 5.31 – 5.23 (m, 1H), 5.20 (d, *J* = 9.4 Hz, 1H), 5.13 (d, *J* = 11.0 Hz, 1H), 4.28 (m, 1H), 4.15 (m, 1H), 3.83 – 3.75 (m, 1H), 3.72 (dd, *J* = 11.1, 2.2 Hz, 6H), 1.54 (m, 2H), 1.37 (s, 9H), 1.20 (s, 20H), 0.83 (t, *J* = 6.8 Hz, 3H). ¹³C{¹H} (101 MHz, Chloroform-*d*) δ 156.1, 140.2, 114.4, 79.4, 77.1, 66.88, 66.83, 56.8, 56.7, 54.42, 54.40, 54.36, 54.34, 38.2, 31.9, 30.0, 29.60, 29.58, 29.55, 29.50, 29.29, 28.3, 23.3, 22.6, 14.1. ³¹P NMR (162 MHz, CDCl₃) δ 1.3. HRMS (TOF): *m/z* calc. for C₂₄H₄₈NNaO₇P⁺ [M+Na]⁺ 516.3061; found 516.3037.

5b (2*S*,3*R*) was obtained in 78% yield (192.5 mg) as a white powder from diol **4b**; TLC (DCM/MeOH, 9:1): R_f : 0.1. $[\alpha]_D^{25} +1.0$ (c 1.0, CHCl₃). ^1H NMR (400 MHz, Chloroform-*d*) δ 5.79 (dd, $J = 17.3, 10.8$ Hz, 1H), 5.37 – 5.19 (m, 3H), 4.22 – 4.08 (m, 2H), 3.78 (dd, $J = 10.5, 6.8$ Hz, 1H), 3.72 (d, $J = 11.1$ Hz, 6H), 1.63 – 1.46 (m, 2H), 1.42 (s, 9H), 1.21 (s, 20H), 0.85 (t, $J = 6.8$ Hz, 3H). $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, Chloroform-*d*) δ 156.3, 140.4, 114.5, 79.0, 77.1, 67.62, 67.57, 56.38, 56.32, 54.27, 54.24, 54.21, 54.18, 38.4, 31.8, 30.0, 29.52, 29.51, 29.42, 29.21, 28.3, 22.9, 22.6, 14.0. ^{31}P NMR (162 MHz, CDCl₃) δ 1.3. HRMS (TOF): m/z calc. for C₂₄H₄₈NNaO₇P⁺ [M+Na]⁺ 516.3061; found 516.3072.

ent-**5a** (2*R*,3*R*) was obtained in 70% yield (172.8 mg) as a white solid from *ent*-**4a**. $[\alpha]_D^{25} +4.0$ (c 1.0, CHCl₃). Spectroscopic data were identical to those of **5a** (see above). HRMS (TOF): m/z calc. for C₂₄H₄₈NNaO₇P⁺ [M+Na]⁺ 516.3061; found 516.3078.

ent-**5b** (2*R*,3*S*) was obtained in 72% yield (177.7 mg) as a white solid from *ent*-**4b**. $[\alpha]_D^{25} -1.0$ (c 1.0, CHCl₃). Spectroscopic data were identical to those of **5b** (see above). HRMS (TOF): m/z calc. for C₂₄H₄₈NNaO₇P⁺ [M+Na]⁺ 516.3061; found 516.3080.

Cyclization of **3a** and **3b** into oxazolo[3,4-*c*]oxazol-3-ones **6a** and **6b**

A solution of **3a** or **3b** (50 mg, 0.12 mmol) in THF (2 mL) was added dropwise over a suspension of NaH (17 mg of a 60% dispersion in mineral oil, equivalent to 10 mg, 0.42 mmol) in THF (1 mL). After stirring for 16h at rt, the reaction was quenched with few drops of water and concentrated *in vacuo*. The residue was taken up in water and extracted with DCM (3 x 5 mL). After usual workup, the resulting yellow oil was flash chromatographed on hexanes/EtOAc (5%) to afford the corresponding cyclic carbamate **6**.

6a: 29.5 mg (70% yield); $[\alpha]_D^{25} -9.8$ (c 1.0, CHCl₃). ^1H NMR (400 MHz, Chloroform-*d*) δ 5.82 (dd, $J = 17.2, 10.9$ Hz, 1H), 5.41 (d, $J = 17.2$ Hz, 1H), 5.25 (d, $J = 10.9$ Hz, 1H), 4.05 (dd, $J = 8.4, 6.2$ Hz, 1H), 3.93 (dd, $J = 8.6, 6.2$ Hz, 1H), 3.75 (t, $J = 8.5$ Hz, 1H), 1.76 – 1.66 (s, 3H +

m, 2H), 1.58 – 1.49 (m, 2H), 1.39 (s, 3H), 1.22 (broad, 18H), 0.85 (t, $J = 6.8$ Hz, 3H). $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, Chloroform- d) δ 156.3, 138.5, 115.3, 94.6, 82.3, 66.6, 64.0, 35.1, 32.0, 29.84, 29.74, 29.73, 29.70, 29.60, 29.50, 29.45, 28.0, 23.7, 23.5, 22.8, 14.2. HRMS (ESI): m/z calc. for $\text{C}_{21}\text{H}_{38}\text{NO}_3^+ [\text{M}+\text{H}]^+$ 352.2846; found 352.2844

6b: 31.2 mg (74% yield); $[\alpha]_{\text{D}}^{25}$ -84.6 (c 1.0, CHCl_3). ^1H NMR (400 MHz, Chloroform- d) δ 5.64 – 5.56 (m, 1H), 5.45 (dd, $J = 17.1, 1.1$ Hz, 1H), 5.30 (dd, $J = 10.8, 1.0$ Hz, 1H), 4.04 (dd, $J = 8.8, 6.1$ Hz, 1H), 3.86 (dd, $J = 8.7, 6.1$ Hz, 1H), 3.58 (t, $J = 8.7$ Hz, 1H), 1.88 – 1.78 (m, 1H), 1.76 – 1.67 (m, 1H), 1.64 (s, 3H), 1.43 (s, 3H), 1.23 (s, 20H), 0.86 (t, $J = 6.8$ Hz, 3H). $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, Chloroform- d) δ 156.0, 134.6, 116.7, 94.3, 82.6, 66.6, 65.3, 40.5, 31.9, 29.59, 29.56, 29.48, 29.38, 29.30, 27.9, 23.3, 23.0, 22.7, 14.1. HRMS (ESI): m/z calc. for $\text{C}_{21}\text{H}_{38}\text{NO}_3^+ [\text{M}+\text{H}]^+$ 352.2846; found 352.2852.

Using the same protocol, cyclization of *ent*-**3a** and *ent*-**3b** afforded cyclic carbamates *ent*-**6a** and *ent*-**6b**, respectively, whose NMR spectra were identical to those of their corresponding enantiomers.

ent-**6a**: 30.8 mg (73% yield); $[\alpha]_{\text{D}}^{25}$ +10.2 (c 1.0, CHCl_3). HRMS (ESI): m/z calc. for $\text{C}_{21}\text{H}_{38}\text{NO}_3^+ [\text{M}+\text{H}]^+$ 352.2846; found 352.2851.

ent-**6b**: 31.6 mg (75% yield); $[\alpha]_{\text{D}}^{25}$ +86.4 (c 1.0, CHCl_3). HRMS (ESI): m/z calc. for $\text{C}_{21}\text{H}_{38}\text{NO}_3^+ [\text{M}+\text{H}]^+$ 352.2846; found 352.2839.

Synthesis of RBM10-9 from *ent*-**3b**

tert-butyl (*R*)-4-[(*R*)-3-hydroxypentadecan-3-yl]-2,2-dimethyloxazolidine-3-carboxylate (**7**)

A solution of *ent*-**3b** (300 mg, 0.70 mmol) in EtOH (4 mL) was added onto a suspension of 10% Pd/C (10 mg) in EtOH (1 mL). The system was purged with H_2 and kept under stirring at rt for 12h. Filtration over a plug of Celite and evaporation to dryness afforded a crude, which

was purified by passing it through a short path of silica and elution with hexanes/EtOAc 9:1 to afford chromatographically pure **7** (298 mg, 99% yield). $[\alpha]_{\text{D}}^{25} +17.3$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, Chloroform-*d*) δ 4.14 (s, 1H), 4.02 – 3.92 (m, 1H), 3.82 (s, 1H), 1.66 – 1.53 (broad, 6H), 1.48 (s, 9H), 1.35 – 1.11 (m, 24H), 0.96 – 0.80 (m, 6H). ¹³C{¹H} (101 MHz, Chloroform-*d*) δ 155.8, 94.5, 81.5, 75.9, 64.8, 64.6, 39.0, 37.0, 36.6, 31.9, 30.5, 29.65, 29.63, 29.62, 29.60, 29.59, 29.31, 28.9, 28.3, 26.0, 24.1, 23.1, 22.7, 16.3, 15.0, 14.8, 14.1, 7.4. HRMS (ESI): *m/z* calc. for C₂₅H₅₀NO₄⁺ [M+H]⁺ 428.3734; found 428.3741.

***tert*-butyl N-[(2*R*,3*R*)-3-ethyl-1,3-dihydroxypentadecan-2-yl]carbamate (**8**)**

Following the same protocol described above for the synthesis of *ent*-**4b**, compound **7** (200 mg, 0.47 mmol) afforded 160.3 mg (88% yield) of carbamate **8**. $[\alpha]_{\text{D}}^{25} -17.8$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, Chloroform-*d*) δ 4.02 – 3.91 (m, 1H), 3.76 (d, *J* = 10.4 Hz, 1H), 3.55 (d, *J* = 9.0 Hz, 1H), 1.67 (dq, *J* = 14.8, 7.4 Hz, 1H), 1.54 (dq, *J* = 14.7, 7.3, 6.7 Hz, 1H), 1.43 (s, 9H), 1.22 (s, 22H), 0.87 (2 x t, 6H). ¹³C{¹H} (101 MHz, Chloroform-*d*) δ 156.2, 79.4, 78.0, 63.5, 54.6, 34.8, 31.9, 30.3, 30.1, 29.62, 29.60, 29.46, 29.31, 28.7, 28.4, 23.1, 22.7, 14.1, 7.9. HRMS (ESI): *m/z* calc. for C₂₂H₄₆NO₄⁺ [M+H]⁺ 388.3421; found 388.3434.

***tert*-butyl N-[(2*R*,3*R*)-1-[(dimethoxyphosphoryl)oxy]-3-ethyl-3-hydroxypentadecan-2-yl]carbamate (**9**)**

Following the same protocol described above for the synthesis of *ent*-**5b**, compound **8** (175 mg, 0.45 mmol) afforded 174.5 mg (78% yield) of phosphate **9**. $[\alpha]_{\text{D}}^{25} -15.4$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, Chloroform-*d*) δ 5.25 (d, *J* = 9.4 Hz, 1H), 4.28 – 4.14 (m, 2H), 3.75 (d, *J* = 1.0 Hz, 3H), 3.73 (d, *J* = 1.0 Hz, 3H), 3.67 (broad, 1H), 1.61 (dq, *J* = 13.3, 6.7, 5.8 Hz, 1H), 1.41 (s, 9H + broad 1H), 1.22 (s, 22H), 0.86 (2 x t, *J* = 10.6, 6H). ¹³C{¹H} (101 MHz, Chloroform-*d*) δ 156.1, 79.2, 76.1, 67.4, 67.3, 55.09, 55.03, 54.41, 54.35, 35.0, 31.8, 30.1, 29.58, 29.57, 29.44, 29.27, 28.29, 23.1, 22.6, 14.6, 14.3, 7.8. ³¹P NMR (162 MHz, CDCl₃) δ 1.32. HRMS (ESI): *m/z* calc. for C₂₄H₅₁NO₇P⁺ [M+H]⁺ 496.3398; found 496.3409.

Adduct 11 from reaction of isoniazid with pentadec-1-en-3-one (10).

To a stirred solution of isonicotinic acid hydrazide (Isoniazid) (500 mg, 3.65 mmol) in a 3:1 mixture of CH₃CN and H₂O (24 mL) was added dropwise a solution of vinyl ketone **10**³⁵ (80 mg, 0.36 mmol) in CH₃CN (4 mL) at rt. After stirring for 3 h, the volatiles were removed *in vacuo* and the residue was diluted with water (25 mL) and extracted with EtOAc (3 x 10 mL). After usual workup, purification of the crude by column chromatography on silica gel (from 0 to 5 % MeOH in MTBE) afforded adduct **11** (67 mg, 51 %) as a colourless solid.

¹H NMR (400 MHz, CDCl₃) δ 8.74 (d, *J* = 4.6 Hz, 2H), 7.58 (d, *J* = 5.9 Hz, 2H), 7.24 (br s, 1H), 3.16 (t, *J* = 6.2 Hz, 2H), 2.72 (t, *J* = 6.2 Hz, 2H), 2.36 (t, *J* = 7.5 Hz, 2H), 1.50 – 1.41 (m, 2H), 1.33 – 1.14 (m, 18H), 0.88 (t, *J* = 6.9 Hz, 3H). ¹³C{¹H} (101 MHz, CDCl₃) δ 210.8, 164.5, 150.8, 140.1, 121.0, 52.2, 43.4, 40.5, 32.1, 29.8, 29.7, 29.6, 29.5, 29.3, 23.8, 22.8, 14.3. HRMS calcd. for C₂₁H₃₆N₃O₂ ([M + H]⁺): 362.2802, found: 362.2790.

2-amino-3-vinylpentadecane-1,3-diol (RBM10-1, RBM10-2, RBM10-3, and RBM10-4)

Acetyl chloride (900 μL, 6% volume) was added to a solution of the corresponding starting **3** (340 mg, 0.80 mmol) in MeOH (15 mL). The reaction was completed in 1 h, as monitored by TLC (hexane/AcOEt 1:1). Methanol was removed *in vacuo* and the resulting residue was purified by flash chromatography (CH₂Cl₂/MeOH/NH₃ 9:0.9:0.1) to give of the corresponding diol.

RBM10-1 (2*S*,3*S*), was obtained in 84% yield (191.8 mg) from **3a**. [α]_D²⁵ +2.1 (*c* 1.0, MeOH). ¹H NMR (400 MHz, Methanol-*d*₄) δ 5.82 (dd, *J* = 17.2, 10.9 Hz, 1H), 5.30 (dd, *J* = 40.2, 14.0 Hz, 2H), 3.89 – 3.78 (m, 1H), 3.62 – 3.50 (m, 1H), 2.90 (d, *J* = 4.1 Hz, 1H), 1.63 – 1.46 (m, 2H), 1.27 (s, 20H), 0.88 (t, *J* = 6.4 Hz, 3H). ¹³C{¹H} (101 MHz, Methanol-*d*₄) δ 140.2, 114.4, 75.6, 60.5, 58.6, 36.8, 31.7, 29.80, 29.38, 29.35, 29.32, 29.30, 29.06, 22.9, 22.3, 13.1. HRMS (ESI): *m/z* calc. for C₁₇H₃₆NO₂⁺ [M+H]⁺ 286.2741; found 286.2734.

RBM10-2 (2*S*,3*R*), was obtained in 80% yield (182.7 mg) from **3b**. $[\alpha]_{\text{D}}^{25}$ -20.8 (*c* 1.0, MeOH). ^1H NMR (400 MHz, Methanol-*d*₄) δ 5.79 (dd, *J* = 17.3, 10.9 Hz, 1H), 5.28 (dd, *J* = 17.3, 1.9 Hz, 1H), 5.16 (dd, *J* = 10.9, 1.9 Hz, 1H), 3.67 (dd, *J* = 11.0, 3.4 Hz, 1H), 3.42 (dd, *J* = 11.0, 7.5 Hz, 1H), 2.70 (dd, *J* = 7.4, 3.4 Hz, 1H), 1.62 – 1.51 (m, 2H), 1.27 (s, 20H), 0.90 (t, *J* = 6.1 Hz, 3H). $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, Methanol-*d*₄) δ 140.4, 113.5, 76.2, 62.3, 59.0, 37.7, 31.7, 29.83, 29.37, 29.35, 29.33, 29.05, 22.8, 22.3, 13.0. HRMS (ESI): *m/z* calc. for C₁₇H₃₆NO₂⁺ [M+H]⁺ 286.2741; found 286.2739.

RBM10-3 (2*R*,3*R*), was obtained in 88% yield (200.9 mg) from *ent*-**3a**. $[\alpha]_{\text{D}}^{25}$ -2.8 (*c* 1.0, MeOH). Spectroscopic data were identical to those indicated for **RBM10-1**. HRMS (ESI): *m/z* calc. for C₁₇H₃₆NO₂⁺ [M+H]⁺ 286.2741; found 286.2738.

RBM10-4 (2*R*,3*S*), was obtained in 79% yield (180.4 mg) from *ent*-**3b**. $[\alpha]_{\text{D}}^{25}$ +24.1 (*c* 1.0, MeOH). Spectroscopic data were identical to those indicated for **RBM10-2**. HRMS (ESI): *m/z* calc. for C₁₇H₃₆NO₂⁺ [M+H]⁺ 286.2741; found 286.2740.

***tert*-Butyl N-(3-hydroxy-1-(phosphonoxy)-3-vinylpentadecan-2-yl)carbamate (RBM10-5, RBM10-6, RBM10-7, and RBM10-8)**

To an ice cooled solution of the starting dimethyl phosphate **5** (0.2 mmol) in dry CH₃CN (8 mL), was added dropwise TMSBr (5 equiv/mol). After stirring for 3 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was then taken up in 10 mL MeOH/H₂O (95:5) and stirred for an additional hour at rt. Evaporation of the solvent afforded a crude mixture, which was dissolved in methanol and loaded on an Amberlite[®] XAD4 column (10 g), which had been washed thoroughly with acetone and then equilibrated with water. Elution with a linear gradient from 0 to 50 % CH₃CN in H₂O provided the expected compounds.

Compound **RBM10-5** (2*S*,3*S*) was obtained from **5a** in 80% yield (58.5 mg) as a white powder. Spectroscopic data were identical to those of **RBM10-7** (see below). HRMS (TOF): m/z calc. for $C_{17}H_{37}NO_5P^+$ $[M+H]^+$ 366.2404; found 366.2420.

Compound **RBM10-6** (2*S*,3*R*) was obtained from **5b** in 85% yield (62.1 mg) as a white powder. Spectroscopic data were identical to those of **RBM10-8** (see below). HRMS (TOF): m/z calc. for $C_{17}H_{37}NO_5P^+$ $[M+H]^+$ 366.2404; found 366.2418.

Compound **RBM10-7** (2*R*,3*R*) was obtained in 83% yield (60.7 mg) from *ent*-**5a**, as a white powder. 1H NMR (400 MHz, Methanol- d_4) δ 5.80 (dd, $J = 17.2, 10.7$ Hz, 1H), 5.48 (dd, $J = 17.2, 1.5$ Hz, 1H), 5.35 (dd, $J = 10.7, 1.5$ Hz, 1H), 4.21 (ddd, $J = 11.2, 7.4, 3.4$ Hz, 1H), 3.99 (ddd, $J = 11.7, 9.3, 7.9$ Hz, 1H), 3.30 (m, 1H), 1.58 (t, $J = 7.7$ Hz, 2H), 1.27 (s, 20H), 0.88 (t, $J = 6.9$ Hz, 3H). $^{13}C\{^1H\}$ (101 MHz, Methanol- d_4) δ 139.1, 116.1, 73.7, 61.6, 36.3, 31.6, 29.6, 29.33, 29.30, 29.18, 29.0, 22.9, 22.3, 13.0. HRMS (TOF): m/z calc. for $C_{17}H_{37}NO_5P^+$ $[M+H]^+$ 366.2404; found 366.2422. ^{31}P NMR (162 MHz, $CDCl_3$) δ 1.22.

Compound **RBM10-8** (2*R*,3*S*) was obtained in 80% yield (58.5 mg) from *ent*-**5b**, white powder. 1H NMR (400 MHz, Methanol- d_4) δ 5.79 (dd, $J = 17.2, 10.8$ Hz, 1H), 5.47 (dd, $J = 17.2, 1.6$ Hz, 1H), 5.34 (dd, $J = 10.8, 1.6$ Hz, 1H), 4.12 (ddd, $J = 11.8, 6.9, 3.4$ Hz, 1H), 3.86 (ddd, $J = 11.7, 9.3, 7.7$ Hz, 1H), 3.35 (dd, $J = 9.3, 3.4$ Hz, 1H), 1.67 – 1.54 (m, 2H), 1.28 (d, $J = 14.0$ Hz, 20H), 0.92 – 0.84 (m, 3H). $^{13}C\{^1H\}$ (101 MHz, Methanol- d_4) δ 137.5, 116.3, 73.5, 61.98, 61.94, 59.19, 59.13, 37.69, 31.64, 29.6, 29.4, 29.33, 29.32, 29.28, 29.0, 22.37, 22.30, 13.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 0.98. HRMS (TOF): m/z calc. for $C_{17}H_{37}NO_5P^+$ $[M+H]^+$ 366.2404; found 366.2412.

(2*R*,3*R*)-2-amino-3-ethyl-3-hydroxypentadecyl dihydrogen phosphate (RBM10-9)

Following the method described above for the synthesis of **RBM10-8**, compound **9** (75 mg, 0.15 mmol) afforded amino phosphate **RBM10-9** (44.5 mg, 80% yield). 1H NMR (400 MHz,

Methanol-*d*₄) δ 4.14 (m, 1H), 4.01 – 3.89 (m, 1H), 3.39 (dd, *J* = 9.3, 3.2 Hz, 1H), 1.55 (m, 2H), 1.32 (broad, 22H), 0.92 (2 x t, 6H). ¹³C{¹H} (101 MHz, Methanol-*d*₄) δ 73.7, 63.2, 63.1, 59.23, 59.17, 36.4, 33.1, 31.2, 30.78, 30.77, 30.75, 30.71, 30.5, 28.5, 23.9, 23.7, 14.4, 7.8. ³¹P NMR (162 MHz, CDCl₃) δ 1.15. HRMS (ESI): *m/z* calc. for C₁₇H₃₉NO₅P⁺ [M+H]⁺ 368.2560; found 368.2572.

In vitro S1PL inhibition using a fluorogenic substrate. In vitro S1PL inhibition assays were performed as previously described.²⁴ In brief, to each of the 96-well plate were added, for a final volume of 100 μ L: 45 μ L of a solution of the tested inhibitors or vehicles in the reaction buffer (0.1 M HEPES, 0.1 mM PLP, 0.1 mM EDTA, 0.05% Triton X-100, 0.01% Pluronic F127, pH=7.4), 5 μ L of 2.5 mM **RBM148** and 50 μ L of 1.6 μ g/mL purified hS1PL in the reaction buffer. In experiments with PMSF or DEPC, these additives were added to the reaction buffer to a final concentration of 1 mM or 0.1%, respectively. Inhibitors were tested at 100 and 200 μ M, final concentration, except compound **12** that was assayed at 200 nM. After 1 h of incubation at 37°C, the reaction was stopped by adding 50 μ L of MeOH and 100 μ L of 0.2 M Gly-NaOH buffer. The 96-well plate was further incubated in the dark at 37°C for 20 min and the amount of umbelliferone formed was determined by spectrofluorometry (Spectramax M5 Microplate Reader) at λ_{ex} 355 and λ_{em} 460nm.

In vitro S1PL inhibition using S1P as substrate. In vitro S1PL inhibition using S1P assays were performed as described.²⁵ Briefly, to each microcentrifuge tube were added, for a final volume of 100 μ L: 45 μ L of a solution of the tested inhibitors or vehicle in the reaction buffer (0.1 M HEPES, 0.1 mM PLP, 0.1 mM EDTA, 0.05% Triton X-100, 0.01% Pluronic F127, pH=7.4), 5 μ L of 0.2 mM S1P and 50 μ L of 1.6 μ g/mL purified hS1PL in the reaction buffer. Compounds were tested at 100 μ M. After 1h of incubation at 37°C, the reaction was stopped by adding 5 μ L of 0.148 mM pentadecanal (as internal standard) in ACN and 95 μ L of 6.5 mM

isoniazid in ACN / 0.3M H₂SO₄ (1:1). Samples were analyzed using a Waters Acquity UPLC system connected to a Waters LCT Premier Orthogonal Accelerated Time of Flight Mass Spectrometer (Waters, Millford, MA), operated in positive electrospray ionization mode. Exact masses ([M+H]⁺) of both (E)-N0-[(E)-hexadec-2-en-1-ylidene] isonicotinohydrazide (2EC16-ISO) and (E)-N'-(1-pentadecylidene)isonicotinohydrazide (C15-ISO) (m/z: 358.2858 and 346.2858, respectively) were extracted from TIC chromatograms and the area of the resulting peaks was calculated using MassLynx.

Identification of covalent adducts between hS1PL and ketone 10

Purified hS1PL (25 µg) was mixed with a 0.1 M HEPES, 0.1 mM PLP, 0.1 mM EDTA, 0.05% Triton X-100, 0.01% Pluronic F127 buffer at pH=7.4 and incubated for 1h at 37°C with 200 µM **RBM10-8** in a final volume of 100 µL. The protein was precipitated with cold acetone at 4°C. After centrifuging at 2000 g for 10 min, the protein pellet was dissolved in the digestion buffer (15 µL of 50 mM (NH₄)₂CO₃, 30 µL of H₂O, 2 µL of 500 mM DTT). After denaturation at 95°C for 10 min, the samples were incubated with 1 µg of Lys-C at 37°C and then were further digested overnight with 1 µg trypsin at 37°C.⁴¹ The resulting peptides were dissolved in CH₃CN:H₂O (1:1) to a final volume of 150 µL. Comparison of ESI-spectra from incubation with **RBM10-8** and a control experiment showed two new adducts: adduct A, rt = 2.05 min, (m/z): 1467.6963±0.05) from fragment 279-303, and adduct B, rt = 3.27 min (m/z): 1436.2698±0.05, from fragment 132-155. These adducts identified with the help of the software “Protein Prospector”, from the University of California at San Francisco (<http://prospector.ucsf.edu>).

Irreversibility assay. A preincubation between hS1PL and the tested compounds was carried out by adding to each of the 96-well plate, for a final volume of 1 µL: 0.5 µL of a solution of the tested compounds or vehicle in the reaction buffer (0.1 M HEPES, 0.1 mM PLP, 0.1 mM

EDTA, 0.05% Triton X-100, 0.01% Pluronic F127, pH=7.4), and 0.5 μ L 160 μ g/mL purified hS1PL in the reaction buffer. Compounds were tested at: 5 mM / 1 mM for RBM10-7 and RBM10-8, and 200 nM / for 12.³⁰ After 30 min preincubation at 37°C, a 1/100 dilution was performed³¹ by adding to each well 94 μ L of the reaction buffer and 5 μ L of 2.5 mM RBM148.²⁴ Final concentrations after 1/100 dilution were: 0.8 hS1PL μ g/mL hS1PL, 125 μ M RBM148, 50 μ M / 10 μ M RBM10-7 and RBM10-8, 2 nM for 12. Incubations were performed in the spectrofluorometer (Biotek Synergy 2 Multi-Mode Microplate Reader) and a 140 min kinetic curve was constructed by measuring the fluorescence at λ_{ex} 355 nm and λ_{em} 460 nm every 10 min.

Determination of k_i/K_i

In 96-well plates 0.5 μ L of hS1PL 300 μ g/mL was preincubated with different concentrations of **RBM10-8** in DMSO at a final volume of 1 μ L. The reaction mixture was preincubated at room temperature for different times (from 1 to 60 min). At each time point, the enzymatic reaction started by adding 94 μ L of HEPES buffer pH 7.4, containing 0.1 mM EDTA, 0.05 % Triton X-100, 0.01 % Pluronic F127 (Biotium) and 100 μ M pyridoxal 5'-phosphate, and 5 μ L of a 2.5 mM DMSO solution **RBM7-148** as fluorogenic hS1PL substrate (final concentration: 125 μ M). The mixture was incubated at 37 °C for 1 h and the enzymatic reaction was stopped by the addition of 50 μ L of MeOH. Next, 100 μ L of a 100 mM glycine-NaOH buffer pH 10.6 were added to the resulting solution and the mixture was incubated for 20 additional min at 37 °C. Fluorescence was measured on a Synergy 2 (BioTek) microplate reader ($\lambda_{\text{ex/em}}$ = 355/460 nm). In all cases, the fluorescence of a blank solution, without enzyme, was subtracted from the total fluorescence. To determine the inactivation constants, the inactivation plot (logarithm of the % remaining activity versus preincubation time) was represented. Nonlinear regression analysis of the negative slopes against inhibitor concentration gives rise to k_{inact} and K_i values.

UPLC-MS-TOF conditions. Samples were analyzed using ACQUITY UPLC system (Waters) connected to OA-TOF MS LCT Premier (Waters) operated in positive and negative electrospray ionization mode (ESI+ and ESI-). Accuracy and reproducibility was maintained using LockSpray (Waters), an independent reference. An analytical column 100 mm x 2.1 mm i.d., 1.7 μ m C18 ACQUITY UPLC BEH (Waters) was used. The two mobile phases were acetonitrile (phase A) and water (phase B), both phases with 0.2% formic acid (v/v). The linear gradient was programmed at: 0 min: 90% B; 5 min: 100% A; 6,5 min: 100% A; 7,2 min: 90% B; 8 min: 90% B. Flow rate was 0.3 mL/min. Column was maintained at 30°C. Quantification was performed using ion chromatogram extracted from each compound using windows of 50 mDa. The lineal dynamic interval was determined injecting standards. Positive identification of compounds was based on the exact mass measurement with an error <5ppm and its retention time compared to authentic standards.

Statistical analysis. All experiments were replicated at least two times and data sets were expressed as means $\pm$ standard deviations (SD). Statistical significance was determined by the unpaired student's t-test, one-way ANOVA verifying previously homoscedasticity, normality and independence of datasets (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Tukey's multiple comparison test was employed to analyse difference between groups in case of positive signification in ANOVA ($p < 0.05$). Statistical analysis was performed using the software GraphPad Prism Version 7 (<http://www.graphpad.com/scientific-software/prism/>).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI. Computational details and conformational search results for **6a** and **6b** species,

vibrational circular dichroism experiments, Figures S1 to S7, Tables S1 to S3 and copies of NMR spectra of synthesized compounds.

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Notes The authors declare no competing financial interest

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Supporting Information for

A mechanism-based sphingosine-1-phosphate lyase inhibitor

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Structure of inhibitor 12

The hS1PL inhibitor **12** was a donation from Novartis (see ref ¹)

12

Computational details and conformational search results for 6a and 6b species.

A scheme of the workflow in the conformational search and the definition of the theoretical model is shown in Figure S1. A Montecarlo conformational search has been performed over the structures of **6a** and **6b** with the MMFF force field as implemented in Spartan08 program package.² The selected nonredundant structures were subsequently optimized and confirmed as minima in the potential energy surfaces (PES) of the systems by computation of their harmonic vibrational frequencies with the B3LYP^{3,4} and M062X⁵ functionals in conjunction with the 6-31+G* basis set.^{6,7} Those conformers lying in an energy threshold of 3 kcal mol⁻¹ were further optimized to consider the effect of the solvent in their structures by applying integral equation formalism (IEF) version of the continuum PCM solvation model⁸⁻¹² using the dielectric constant value corresponding to CCl₄. In these calculations, the basis set used was the so-called cc-pVTZ.¹³ The final set of minima, those representing 98% of the Boltzmann population of the total conformational sample composition at the PCM/M062X/cc-pVTZ, were re-optimized at PCM/B3LYP/cc-pVTZ at which level the harmonic vibrational IR and VCD spectra were calculated. No imaginary vibrational frequencies were calculated for any of the structures in the final set of conformers. Their calculated Gibbs free energies (H) along with the relative values of this magnitude referred to the global minima (kcal mol⁻¹) and the subsequently calculated Boltzmann populations are reported in Table S1). The cartesian coordinates matrices of the 17 structures (9 for 6a and 8 for 6b) used for the theoretical estimation of the vibrational spectra are also reported as part of the Supplementary Material. DFT calculations were performed according to the methods related above as implemented in the Gaussian09 program package.¹⁴ Geometry optimization criteria were SCF=tight and Int=Ultrafine.

The results of the exploration of the potential energy surfaces of **6a** and **6b** point out that the global minima for both species in CCl₄ solution (PCM/M062x/cc-pVTZ) are characterized by

the linear (all trans) arrangement of the side alkyl chain and the syn-periplanar orientation of the vinyl group towards the endocyclic $C_3 - O$ bond ($\phi_{C=C-C_3O} \approx 0^\circ$), suggesting the possible incidence of an intramolecular hydrogen bond ($d_{O...H} = 2,45\text{\AA}$). Calculated Boltzmann's populations for these structures are 35,8% and 47,4% for **6a** and **6b**, respectively (Table S1, Figure S2). For both diastereomers, the sets of local minima include both structures with folded alkyl chains and syn-periplanar vinyl arrangements and with linear alkyl chains and anti-periplanar arrangements of the vinyl moiety.

It is well known that, although the linear arrangement of alkyl chains is more energetically favorable, in long enough chains intramolecular dispersion forces may cause attractive intramolecular interactions that promote the occurring of folded conformations.^{15,16} It is also known that B3LYP hybrid functional does not account for long range dispersion interactions,¹⁷ which may justify the observed B3LYP's overestimation of the contribution of structures with linear side chains to the total composition of the conformational mixture (Table S1). In contrast, dispersion corrected Thrular's M062x functional⁵ allows a finer description of the conformational landscape of both systems. Therefore, we have used Boltzmann's populations calculated with the M062x functional to balance the individual contributions of each conformer to the overall spectral IR and VCD profiles calculated for **6a** and **6b** with the B3LYP method, that is well-known to have proven efficiency in the estimation of the VCD signals intensity and signs.

Following this methodology, theoretical IR and VCD spectra were built using GaussView6.¹⁸ FWHMs of the bands were set to 8 cm^{-1} . Calculated harmonic vibrational wavenumbers were scaled by 0.977 (**6a**) and 0.980 (**6b**) to maximize the similarity between experimental and theoretical profiles according to the statistical validation of the absolute configuration performed by CompareVOA.^{19,20} The use of this scale factor is justified considering the recommended value of 0.965.²¹ The correctness of the approximation proposed can be inferred from the theoretically supported interpretation of the IR and VCD spectra of both diastereomers in the $1400\text{-}1000\text{ cm}^{-1}$ region (see Figure 1 in the manuscript). The complete lists of vibrational modes calculated in this region for the two main conformers of **6a** and **6b** are also reported as Supporting Information in Tables S2 and S3.

Vibrational Circular Dichroism

The experimental IR and VCD spectra of **6a** and **6b** and their respective enantiomers are reported in Figures S3-S5. For the recording of the spectra, fixed pathlength cuvettes (100 mm) equipped with BaF₂ windows were used. Concentrations were optimized to maximize IR absorbance considering the limitations derived by the availability of samples. VCD spectra were recorded using a Bruker PMA50 optical bench coupled to a Vertex 70 FT-IR spectrometer. In the PMA50, the infrared radiation is focused by a BaF₂ lens, passing an optical filter (3800-600 cm⁻¹ range) and a ZnSe photo-elastic modulator (PEM 50 kHz). The light beam is finally collected by a D313/QMTC detector with non-dichroic BaF₂ windows. A previous calibration of the PEM at a fixed wavenumber is required before recording a VCD spectrum. Calibration at 1250 cm⁻¹ allowed us to obtain a VCD signal over the whole infrared region of interest, 1400-1000 cm⁻¹. Every VCD spectrum was the result of averaging a minimum of 50000 scans (18h acquisition time) at a spectral resolution 4 cm⁻¹. After data acquisition, individual baselines of the VCD spectra were corrected according to the standard methodology.²² VCD profiles were also smoothed using a 7 points Savitzky-Golay algorithm.

Figure S1. Schematic view of the workflow in the conformational search and definition of the theoretical models

Table S1. Absolute values of G (H), ΔG (kcal mol⁻¹) and Boltzmann populations (%) for the set of conformers of **6a and **6b**.***

Conf.	G (H)	ΔG (kcal mol ⁻¹)	Boltzmann Pop.(%)	Conformer	G (H)	ΔG (kcal mol ⁻¹)	Boltzmann Pop. (%)
PCM / M062X / cc-pVTZ					PCM / B3LYP / cc-pVTZ		
		6a (2S, 3S)					
6a-1	-1102.374133	0.00	35.8	6a-1	-1102.872516	0.00	91.6
6a-2	-1102.373455	0.43	17.5	6a-9	-1102.869127	2.13	2.5
6a-3	-1102.373301	0.52	14.8	6a-3	-1102.868970	2.23	2.1
6a-4	-1102.373124	0.63	12.3	6a-2	-1102.868900	2.27	2.0
6a-5	-1102.372363	1.11	5.5	6a-4	-1102.868220	2.70	1.0
6a-6	-1102.371998	1.34	3.7	6a-10	-1102.867975	2.85	0.7
6a-7	-1102.371909	1.40	3.4				
6a-8	-1102.371795	1.47	3.0				
6a-9	-1102.371766	1.49	2.9				
		6b (2S, 3R)					
6b-1	-1102.375857	0.00	47.4	6b-1	-1102.874236	0.00	90.1
6b-2	-1102.375171	0.43	22.9	6b-2	-1102.871553	1.68	5.3
6b-3	-1102.374537	0.83	11.7	6b-4	-1102.871446	1.75	4.7
6b-4	-1102.374289	0.98	9.0				
6b-5	-1102.373207	1.66	2.9				
6b-6	-1102.372930	1.84	2.1				
6b-7	-1102.372858	1.88	2.0				
6b-8	-1102.372856	1.88	2.0				

*No imaginary frequencies were calculated for any of the structures, confirming them as real minima of the PES

Table S2. PCM/B3LYP/cc-pVTZ calculated harmonic wavenumbers in the region of interest for the main conformer of **6a** (6a-1).

Normal mode	ω (cm^{-1})	ω_{scaled} (cm^{-1})*	IR intensity	Rotor strength	I-M angle	Description**
122	1422	1392	17	2	89	$\delta_{\text{CH}_2(\text{ring})}$
121	1413	1383	2	0	90	$\delta_{\text{CH}_2(\text{chain})}$
120	1412	1382	27	22	80	$\delta_{\text{CH}_2(\text{ring})} + \delta_{\text{HC}_2-\text{ring}}$
119	1407	1377	25	-11	94	$\delta_{\text{CH}_2(\text{ring})}$
118	1405	1376	8	2	82	$\delta_{\text{CH}_2(\text{chain})}$
117	1402	1373	1	-2	98	$\delta_{\text{CH}_2(\text{chain})}$
116	1400	1371	5	7	81	$\delta_{\text{CH}_2(\text{chain})}$
115	1398	1369	2	-4	95	$\delta_{\text{CH}_2(\text{chain})}$
114	1391	1362	5	-1	93	$\delta_{\text{CH}_2(\text{chain})}$
113	1374	1346	37	-15	95	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}}$
112	1367	1338	77	-41	96	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}}$
111	1352	1324	4	2	86	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}} + \delta_{\text{CH}_2(\text{ring})}$
110	1351	1323	10	2	60	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}} + \delta_{\text{CH}_2(\text{ring})}$
109	1343	1315	11	-22	104	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}} + \delta_{\text{CH}_2(\text{ring})}$
108	1341	1312	0	3	43	$\delta_{\text{CH}_2(\text{chain})}$
107	1338	1310	3	-9	106	$\delta_{\text{CH}_2(\text{chain})}$
106	1333	1305	0	2	61	$\delta_{\text{CH}_2(\text{chain})}$
105	1332	1304	5	-15	112	$\delta_{\text{CH}_2(\text{chain})}$
104	1327	1299	0	-18	121	$\delta_{\text{HC}-\text{vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
103	1327	1299	1	29	22	$\delta_{\text{HC}-\text{vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
102	1321	1294	7	20	56	$\delta_{\text{HC}-\text{vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
101	1317	1289	1	-8	129	$\delta_{\text{CH}_2(\text{chain})}$
100	1296	1269	7	2	87	$\delta_{\text{CH}_2(\text{chain})}$
99	1293	1266	1	1	79	$\delta_{\text{CH}_2(\text{chain})}$
98	1272	1245	52	-17	92	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{CH}_2(\text{ring})}$
97	1265	1238	142	-25	92	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{CH}_2(\text{ring})}$
96	1264	1237	1	-1	93	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}} + \delta_{\text{CH}_2(\text{ring})}$
95	1261	1235	64	23	86	$\delta_{\text{CH}_2(\text{ring})} + \delta_{\text{HC}_2-\text{ring}}$
94	1246	1219	173	-62	94	$\delta_{\text{CH}_2(\text{ring})} + \delta_{\text{HC}_2-\text{ring}}$
93	1240	1214	7	-1	90	$\delta_{\text{CH}_2(\text{chain})}$
92	1233	1208	73	-43	160	$\delta_{\text{CH}_2(\text{ring})} + \delta_{\text{HC}_2-\text{ring}}$
91	1231	1205	4	-2	93	$\delta_{\text{CH}_2(\text{chain})}$
90	1216	1190	5	-7	105	$\delta_{\text{CH}_2(\text{chain})}$
89	1204	1179	5	66	31	$\delta_{\text{CH}_2(\text{chain})}$
88	1190	1165	56	16	84	$\delta_{\text{CH}_2(\text{ring})} + \delta_{\text{HC}_2-\text{ring}}$
87	1159	1135	39	-41	100	$\nu_{\text{C}_3-\text{C}(\text{chain})} + \delta_{\text{HC}-\text{vinyl}} + \delta_{\text{CH}_2(\text{ring})}$
86	1150	1126	38	-4	96	$\nu_{\text{C}_2-\text{N}} + \delta_{\text{CH}_2(\text{ring})}$
85	1146	1122	68	-52	100	$\nu_{\text{C}_2-\text{N}} + \delta_{\text{CH}_2(\text{ring})}$
84	1133	1109	14	13	76	$\nu_{\text{C}_2-\text{C}_3} + \delta_{\text{CH}_2(\text{ring})}$
83	1102	1079	25	25	75	$\delta_{\text{CH}_2(\text{chain})}$
82	1092	1069	16	-16	106	$\delta_{\text{CH}_2(\text{chain})}$
81	1068	1045	26	5	88	$\delta_{\text{CH}_2(\text{chain})}$
80	1066	1044	10	-7	97	$\delta_{\text{CH}_2(\text{chain})}$
79	1065	1043	6	-17	142	$\delta_{\text{CH}_2(\text{chain})}$
78	1063	1041	8	8	85	$\delta_{\text{CH}_2(\text{chain})}$
77	1058	1036	35	40	55	$\delta_{\text{CH}_2(\text{chain})}$
76	1056	1034	96	5	68	$\delta_{\text{HC}-\text{vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
75	1054	1032	18	-10	94	$\delta_{\text{HC}-\text{vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
74	1042	1020	1	-2	102	$\delta_{\text{HC}-\text{vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
73	1040	1018	6	-88	147	$\delta_{\text{HC}-\text{vinyl}} + \delta_{\text{CH}_2(\text{chain})}$

*Wavenumbers scaled by 0.980 to maximize the similarity between experimental and theoretical spectra according to the statistical validation of the absolute configuration performed by CompareVOA.

**Symbols: $\delta_{\text{CH}_2(\text{ring})}$: deformation of the HC-X angles in methyl and methylene groups linked to/in the fused ring; $\delta_{\text{CH}_2(\text{chain})}$: deformation of the HC-X angles in methyl and methylene groups in the alkyl side-chain; $\delta_{\text{HC}_2-\text{ring}}$: deformation of the HC₂-X angles; $\delta_{\text{HC}-\text{vinyl}}$: deformation of the HC-X angles in the vinyl moiety.

Table S3. PCM/B3LYP/cc-pVTZ calculated harmonic wavenumbers in the region of interest for the main conformer of **6b** (6b-1).

Normal mode	ω (cm^{-1})	ω_{scaled} (cm^{-1})*	IR intensity	Rotor strength	I-M angle	Description**
122	1420	1391	13	8	85	$\delta_{\text{CH}_2(\text{ring})}$
121	1413	1385	10	-11	105	$\delta_{\text{CH}_3(\text{chain})}$
120	1413	1385	29	11	85	$\delta_{\text{CH}_2(\text{ring})} + \delta_{\text{HC}_2-\text{ring}}$
119	1405	1377	28	-12	93	$\delta_{\text{CH}_2(\text{ring})}$
118	1403	1375	3	1	88	$\delta_{\text{CH}_2(\text{chain})}$
117	1402	1374	4	0	92	$\delta_{\text{CH}_2(\text{chain})}$
116	1399	1371	3	1	89	$\delta_{\text{CH}_2(\text{chain})}$
115	1398	1370	1	-1	103	$\delta_{\text{CH}_2(\text{chain})}$
114	1390	1362	1	-4	111	$\delta_{\text{CH}_2(\text{chain})}$
113	1373	1346	52	-34	102	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}}$
112	1372	1344	79	-63	101	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}}$
111	1355	1328	12	28	75	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}} + \delta_{\text{CH}_2(\text{ring})}$
110	1349	1322	5	-7	102	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}} + \delta_{\text{CH}_2(\text{ring})}$
109	1344	1317	7	-2	94	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{HC}_2-\text{ring}} + \delta_{\text{CH}_2(\text{ring})}$
108	1341	1314	0	0	76	$\delta_{\text{CH}_2(\text{chain})}$
107	1339	1312	1	1	87	$\delta_{\text{CH}_2(\text{chain})}$
106	1334	1307	0	1	82	$\delta_{\text{CH}_2(\text{chain})}$
105	1332	1305	3	1	85	$\delta_{\text{CH}_2(\text{chain})}$
104	1327	1301	1	33	53	$\delta_{\text{HC-vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
103	1327	1300	6	-53	155	$\delta_{\text{HC-vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
102	1321	1295	4	-9	121	$\delta_{\text{HC-vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
101	1317	1291	3	-7	127	$\delta_{\text{CH}_2(\text{chain})}$
100	1297	1272	8	3	86	$\delta_{\text{CH}_2(\text{chain})}$
99	1292	1267	9	-6	102	$\delta_{\text{CH}_2(\text{chain})}$
98	1280	1254	159	-40	92	$\nu_{\text{N-C}} + \delta_{\text{HC}_2-\text{ring}}$
97	1270	1245	3	7	82	$\delta_{\text{CH}_2(\text{chain})}$
96	1265	1240	163	-24	93	$\delta_{\text{CH}_2(\text{chain})} + \delta_{\text{CH}_2(\text{ring})}$
95	1263	1238	34	9	86	$\delta_{\text{CH}_2(\text{chain})}$
94	1255	1229	103	-87	107	$\delta_{\text{CH}_2(\text{ring})} + \delta_{\text{HC}_2-\text{ring}}$
93	1243	1218	17	-22	107	$\delta_{\text{CH}_2(\text{chain})}$
92	1233	1209	16	-19	98	$\delta_{\text{CH}_2(\text{ring})} + \delta_{\text{HC}_2-\text{ring}}$
91	1231	1207	1	0	78	$\delta_{\text{CH}_2(\text{chain})}$
90	1220	1195	2	-5	103	$\delta_{\text{CH}_2(\text{chain})}$
89	1207	1183	2	-8	99	$\delta_{\text{CH}_2(\text{chain})}$
88	1191	1167	70	80	79	$\delta_{\text{CH}_2(\text{ring})} + \delta_{\text{HC}_2-\text{ring}}$
87	1169	1145	20	16	84	$\nu_{\text{N-C}} + \delta_{\text{HC}_2-\text{ring}}$
86	1157	1134	11	2	87	$\nu_{\text{C}_2-\text{C}_3} + \delta_{\text{HC-vinyl}}$
85	1141	1118	23	37	55	$\delta_{\text{HC-vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
84	1129	1106	69	7	87	$\nu_{\text{C}_2-\text{C}_3} + \delta_{\text{HC-vinyl}}$
83	1108	1086	11	30	59	$\delta_{\text{CH}_2(\text{chain})}$
82	1090	1068	30	14	84	$\delta_{\text{CH}_2(\text{chain})}$
81	1073	1051	25	-44	106	$\delta_{\text{CH}_2(\text{chain})}$
80	1067	1045	1	5	54	$\delta_{\text{CH}_2(\text{chain})}$
79	1065	1044	16	-34	114	$\delta_{\text{CH}_2(\text{chain})}$
78	1064	1043	48	96	69	$\delta_{\text{CH}_2(\text{chain})}$
77	1062	1040	105	-4	90	$\delta_{\text{CH}_2(\text{chain})}$
76	1057	1036	1	-8	126	$\delta_{\text{HC-vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
75	1051	1030	10	7	69	$\delta_{\text{HC-vinyl}} + \delta_{\text{CH}_2(\text{chain})}$
74	1047	1026	23	-75	108	$\delta_{\text{HC-vinyl}} + \delta_{\text{CH}_2(\text{chain})} + \nu_{\text{CO}}$
73	1041	1020	11	1	89	$\delta_{\text{HC-vinyl}} + \delta_{\text{CH}_2(\text{chain})}$

*Wavenumbers scaled by 0.980 to maximize the similarity between experimental and theoretical spectra according to the statistical validation of the absolute configuration performed by CompareVOA.

**Symbols: $\delta_{\text{CH}_2(\text{ring})}$: deformation of the HC-X angles in methyl and methylene groups linked to/in the fused ring; $\delta_{\text{CH}_2(\text{chain})}$: deformation of the HC-X angles in methyl and methylene groups in the alkyl side-chain; $\delta_{\text{HC}_2-\text{ring}}$: deformation of the HC₂-X angles; $\delta_{\text{HC-vinyl}}$: deformation of the HC-X angles in the vinyl moiety.

Figure S2. PCM/M062X/cc-pVTZ Boltzmann populations of the main conformers of **6a** and **6b** and molecular structure of the global minima.

Figure S3. IR spectra of **6a** and **6b** and their respective enantiomers

Figure S4. VCD spectra of **6a** and its enantiomer.

Figure S5. VCD spectra of **6b** and its enantiomer.

Figure S6. hS1PL activities of compounds **RBM10** (100 and 200 μ M) and vinyl ketone **10** using **RBM148** as fluorogenic substrate. Inhibitor **12**¹ (200 nM) was used as reference. Enzyme activity is expressed as % relative to control. Data (mean $\pm$ SD) were obtained from two experiments with triplicates.

Figure S7. Backbone of hSPL dimer (PDB 4Q6R, chain A in gray and chain B in salmon) showing inhibitor **13** (red) and highlighted fragments 132-155 (green) and 279-303 (yellow).

Cartesian Coordinates matrices of the optimized (PCM/M062X/cc-pVTZ) structures of the set of conformers of 6a and 6b.

6a-1

C	4.60746900	-1.60752200	-1.00370500
H	3.98991000	-2.06324100	-0.22212100
H	4.34031700	-2.02600900	-1.97102000
C	6.49302900	-0.89311800	0.17244400
C	4.54894400	-0.08695900	-0.93204800
H	4.94643100	0.34412100	-1.85127300
C	7.77720900	-0.32922900	-0.40158400
H	8.48507600	-1.13789100	-0.57745100
H	8.21323900	0.38369700	0.29640200
H	7.57430700	0.17414800	-1.34550900
C	6.71566000	-1.57953800	1.50604300
H	7.11042400	-0.89105700	2.24742300
H	7.42796100	-2.38587300	1.34019500
H	5.78656600	-2.00226100	1.88529200
O	5.98212500	-1.85388800	-0.77099200
N	5.44284700	0.14843000	0.19523300
C	4.69324000	0.46056300	1.31174600
C	3.26770800	0.63902300	-0.48530000
O	5.05739100	0.55721300	2.44875100
O	3.40711500	0.68970200	0.94193600
C	3.27036700	2.03934900	-1.04409700
H	3.13424100	2.08947800	-2.12038900
C	3.43131300	3.14180700	-0.33349100
H	3.43508600	4.11289600	-0.80831200
H	3.55810000	3.10661400	0.74044800
C	1.98248800	-0.10060100	-0.83949800
H	1.95733600	-0.22772900	-1.92607800
H	2.02500300	-1.09876300	-0.40036200
C	0.71732500	0.60531200	-0.36710800
H	0.80027800	0.80827800	0.70325700
H	0.62920600	1.57556000	-0.86117700
C	-0.53486800	-0.21913800	-0.63781000
H	-0.59949500	-0.44320500	-1.70748600
H	-0.45243500	-1.18312500	-0.12644800
C	-1.81317900	0.47882400	-0.19171700
H	-1.89988500	1.43958600	-0.70849300
H	-1.74400100	0.71015700	0.87552200
C	-3.06707800	-0.34702800	-0.44883500
H	-3.13296600	-0.58265500	-1.51576200
H	-2.98117400	-1.30593600	0.07179000
C	-4.34688900	0.35180200	-0.00807700
H	-4.27980900	0.58985300	1.05805900
H	-4.43413600	1.30966500	-0.53046800
C	-5.60063000	-0.47564100	-0.26119200
H	-5.51379700	-1.43262800	0.26290000
H	-5.66653500	-0.71554100	-1.32714100
C	-6.88106800	0.22359100	0.17713900
H	-6.81461900	0.46469300	1.24269200
H	-6.96864700	1.18001100	-0.34787800
C	-8.13455000	-0.60489800	-0.07404400
H	-8.04711500	-1.56093900	0.45173600
H	-8.20052200	-0.84680400	-1.13954100
C	-9.41521100	0.09419500	0.36315100
H	-9.35009200	0.33674700	1.42863500
H	-9.50400700	1.05020000	-0.16273900
C	-10.66916600	-0.73443800	0.11291400
H	-10.57870300	-1.68839900	0.63899400
H	-10.73238100	-0.97575500	-0.95140800
C	-11.94069700	-0.02159900	0.55518800
H	-12.82754200	-0.62679900	0.36950400

H	-12.06080400	0.92236000	0.02131900
H	-11.90629100	0.20476500	1.62198000

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C	-3.93926400	-0.31380800	-1.86127400
H	-4.05529300	0.76972900	-1.75012500
H	-3.49162100	-0.53651800	-2.82675500
C	-5.55546900	-1.15628900	-0.40395200
C	-3.20565300	-0.91175300	-0.66767900
H	-2.87509900	-1.92361800	-0.90348700
C	-5.98931200	-2.59892400	-0.23966300
H	-6.82336800	-2.80829100	-0.90784700
H	-6.30006000	-2.77727900	0.78853500
H	-5.16464300	-3.26657700	-0.48404700
C	-6.66059500	-0.18201600	-0.04496600
H	-7.50192400	-0.38918700	-0.70396600
H	-6.33709600	0.84640000	-0.19872100
H	-6.97084800	-0.29552000	0.98958500
O	-5.19277400	-0.96760300	-1.78474800
N	-4.28352800	-0.91975300	0.31391300
C	-4.08788900	0.11081700	1.21151400
C	-2.10628500	-0.12388900	0.06645300
O	-4.83882900	0.51851200	2.05094200
O	-2.84463700	0.62888400	1.03956100
C	-1.18713900	-1.09218400	0.76750500
H	-0.56650300	-1.68119500	0.09866600
C	-1.11304300	-1.25905700	2.07636600
H	-0.43801400	-1.98566700	2.50631200
H	-1.71915500	-0.67264600	2.75414500
C	-1.32971100	0.83517000	-0.82901400
H	-2.03798100	1.51087500	-1.31116200
H	-0.85705500	0.24586800	-1.62106600
C	-0.27656900	1.65024300	-0.08850800
H	0.45358000	0.97469000	0.36153700
H	-0.75249000	2.18723500	0.73627100
C	0.42660000	2.63980800	-1.01042800
H	-0.32383300	3.25569500	-1.51265500
H	0.94580200	2.08969800	-1.80044600
C	1.40858400	3.55842100	-0.28378000
H	0.84813600	4.20603600	0.39516700
H	1.88446500	4.21466000	-1.01703800
C	2.49501700	2.83109000	0.50807400
H	3.16027800	3.57505700	0.95371400
H	2.04693400	2.28871000	1.34524300
C	3.32613100	1.86388300	-0.32705200
H	3.71436200	2.38586300	-1.20822200
H	2.69108500	1.05686200	-0.70527400
C	4.48463400	1.25361500	0.45195900
H	5.13554900	2.05359600	0.81800300
H	4.09390400	0.74708700	1.34029400
C	5.30873000	0.26901400	-0.36760600
H	4.65782200	-0.53213800	-0.73201600
H	5.69769100	0.77527700	-1.25672200
C	6.46727500	-0.34039300	0.41166900
H	6.07812200	-0.84623500	1.30092000
H	7.11863600	0.46023200	0.77592200
C	7.29098400	-1.32543700	-0.40750200
H	6.64005800	-2.12593800	-0.77357600
H	7.68244400	-0.81975500	-1.29599500
C	8.44824600	-1.93727900	0.37233500
H	9.09687800	-1.13665300	0.73729600
H	8.05530000	-2.44192000	1.25884000
C	9.26208200	-2.91941900	-0.46043500
H	8.63542000	-3.74003000	-0.81317400
H	10.08451500	-3.34789300	0.11158300
H	9.68450300	-2.42626900	-1.33727200

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C	4.69842400	1.54475300	-0.46361400
H	4.06669200	1.41859200	-1.34958500
H	4.68409200	2.58563000	-0.14977900
C	6.18036900	-0.25185900	-0.62122300
C	4.32165700	0.54807700	0.62505400
H	4.76981900	0.84606600	1.57324900
C	7.39903600	-0.51760500	0.23948900
H	8.27573800	-0.06051700	-0.21702200
H	7.56059800	-1.59045800	0.33154700
H	7.25484400	-0.09212400	1.23133500
C	6.31647700	-0.85231300	-2.00694200
H	6.43427200	-1.93112000	-1.96388700
H	7.19600400	-0.40652800	-2.46822000
H	5.44391800	-0.62307500	-2.61677800
O	6.03729200	1.17635600	-0.74009100
N	4.95095000	-0.65552800	0.09535600
C	3.98011100	-1.47533700	-0.44435600
C	2.86409600	0.09297100	0.81588300
O	4.12458000	-2.46184500	-1.10816400
O	2.75306100	-1.01740300	-0.08630400
C	2.67521900	-0.38600700	2.23312100
H	2.70830900	0.40313000	2.97848800
C	2.49445700	-1.64460900	2.59308500
H	2.38027000	-1.91345300	3.63383700
H	2.44996500	-2.43956500	1.86046800
C	1.83196100	1.15079300	0.44160500
H	2.00243600	1.44962100	-0.59407900
H	2.01292400	2.03197400	1.06486200
C	0.38996100	0.68475500	0.60177000
H	0.25409200	-0.24986200	0.05247600
H	0.18885700	0.45958900	1.65164300
C	-0.60792100	1.72275700	0.10422700
H	-0.44756800	2.66678400	0.63486800
H	-0.41974500	1.92948600	-0.95391900
C	-2.05668000	1.28551000	0.28033100
H	-2.25429300	1.10350600	1.34196300
H	-2.20113600	0.32912300	-0.22825900
C	-3.05141100	2.31326300	-0.24849800
H	-2.81639400	3.28760000	0.18861500
H	-2.91474400	2.41973900	-1.32926900
C	-4.51061400	1.97354300	0.04419700
H	-4.65834600	1.92042500	1.12751900
H	-5.14574600	2.78882400	-0.31269400
C	-4.98576500	0.66978700	-0.58813000
H	-4.43459100	-0.17366600	-0.16394600
H	-4.75439400	0.68157300	-1.65852400
C	-6.47835500	0.42977300	-0.39570300
H	-6.70545200	0.45928900	0.67325500
H	-7.04022300	1.25142200	-0.85258100
C	-6.94757700	-0.89326500	-0.99148900
H	-6.45369800	-1.71720700	-0.46674100
H	-6.61378500	-0.94788500	-2.03122900
C	-8.45937800	-1.09807600	-0.94087200
H	-8.95202200	-0.30078500	-1.50683900
H	-8.71161000	-2.03399300	-1.44694700
C	-9.03716800	-1.13536400	0.47029900
H	-8.88006100	-0.17234400	0.95990000
H	-8.48995800	-1.87570700	1.06105800
C	-10.52417100	-1.46734300	0.47550800
H	-11.08592700	-0.73071500	-0.10120800
H	-10.93072300	-1.48156900	1.48635900
H	-10.70488700	-2.44519000	0.02659500

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C	2.64417800	-0.82729300	-1.83716900
H	1.57545900	-0.73945400	-1.61284300

H	2.85443100	-0.36925500	-2.80055100
C	3.29075300	-2.66670300	-0.55458600
C	3.49150800	-0.30177500	-0.68554600
H	4.52040100	-0.15865200	-1.01657300
C	4.62877700	-3.37796400	-0.57082200
H	4.60160000	-4.19148900	-1.29426700
H	4.84469500	-3.78301400	0.41654700
H	5.41765700	-2.68195600	-0.85142900
C	2.15909300	-3.58662000	-0.13917700
H	2.31210700	-3.97910700	0.86185700
H	2.13022700	-4.40765300	-0.85339200
H	1.20404100	-3.06392000	-0.16406900
O	3.03976300	-2.18568800	-1.88868200
N	3.38253200	-1.42244700	0.24055700
C	2.50469600	-1.09083300	1.25312000
C	3.01510100	0.87913800	0.17866800
O	2.04082900	-1.80197200	2.09796200
O	2.22905800	0.23759000	1.19398000
C	4.21131800	1.54550200	0.80961400
H	4.83971800	2.08796900	0.10939200
C	4.52202300	1.49427200	2.09303200
H	5.40733000	1.98671600	2.47012900
H	3.89958300	0.96555500	2.80275700
C	2.14148500	1.88067800	-0.56893200
H	1.30133100	1.33694800	-1.00190000
H	2.72536500	2.28714000	-1.40106900
C	1.62662300	3.01211300	0.31342300
H	2.47211300	3.56933600	0.71956900
H	1.10478600	2.58104100	1.16982200
C	0.70511900	3.97724400	-0.43244900
H	0.36495500	4.73998200	0.27203300
H	1.28039500	4.50068900	-1.20086700
C	-0.51696200	3.32564400	-1.08111100
H	-0.20756000	2.69696700	-1.92111600
H	-1.14219000	4.11104400	-1.51326700
C	-1.35918800	2.49508200	-0.11920900
H	-1.61714000	3.10331600	0.75410300
H	-0.77386400	1.65208400	0.26101000
C	-2.63357800	1.96138700	-0.76127200
H	-2.37110700	1.36542900	-1.64157100
H	-3.23328600	2.80007100	-1.12853400
C	-3.47573500	1.11529300	0.18497500
H	-2.87447500	0.27918600	0.55518100
H	-3.74214700	1.71193200	1.06295600
C	-4.74503700	0.57599300	-0.46202300
H	-4.47750800	-0.02149500	-1.33933200
H	-5.34560000	1.41242100	-0.83340500
C	-5.58962000	-0.26926400	0.48293200
H	-4.98813600	-1.10375200	0.85671400
H	-5.86019900	0.32876500	1.35878200
C	-6.85582100	-0.81249000	-0.16637300
H	-6.58566300	-1.41157100	-1.04191100
H	-7.45781700	0.02160500	-0.54107600
C	-7.70208200	-1.65761100	0.77770500
H	-7.09922400	-2.48942300	1.15124500
H	-7.97160000	-1.05777600	1.65090400
C	-8.96328600	-2.19403700	0.11276000
H	-9.59040400	-1.37578200	-0.24466400
H	-8.71235200	-2.81725700	-0.74698000
H	-9.55702100	-2.79549200	0.80048200

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C	-4.48120800	1.11520800	-1.18797800
H	-4.33504600	1.81788500	-0.36044700
H	-4.26941700	1.61232200	-2.13148100
C	-5.96138900	-0.45210500	-0.29125600
C	-3.69864500	-0.17151500	-0.95851000

H	-3.64304100	-0.74397100	-1.88478100
C	-6.65223900	-1.58372300	-1.02552900
H	-7.61481400	-1.24089100	-1.40210200
H	-6.80973800	-2.42248000	-0.34932700
H	-6.04137200	-1.91207000	-1.86494900
C	-6.75880800	0.02132300	0.90828200
H	-6.90077800	-0.77617400	1.63183600
H	-7.72668600	0.35805300	0.54096600
H	-6.26037200	0.85357200	1.40278300
O	-5.81465300	0.64015600	-1.21894500
N	-4.56345600	-0.82272700	0.01785600
C	-3.99641800	-0.71004100	1.27175600
C	-2.34343800	-0.15720900	-0.22981000
O	-4.48298700	-0.97138900	2.33455400
O	-2.71995300	-0.26318600	1.15078100
C	-1.55127600	-1.37529000	-0.63291700
H	-1.19165200	-1.35418700	-1.65740500
C	-1.29912400	-2.41145500	0.14761200
H	-0.73496900	-3.25782600	-0.21828900
H	-1.64331800	-2.44175400	1.17294900
C	-1.54326100	1.12387200	-0.43655200
H	-1.36332500	1.23757500	-1.51027600
H	-2.15600200	1.97038500	-0.12232600
C	-0.21986900	1.14770300	0.31856600
H	-0.40856300	0.96319600	1.37953300
H	0.41049900	0.32758600	-0.03111700
C	0.50945400	2.47517000	0.14789600
H	0.74206700	2.62510100	-0.91008600
H	-0.16207100	3.29074700	0.42819700
C	1.78399100	2.58178000	0.98524800
H	1.50790700	2.59976100	2.04258000
H	2.26430300	3.54075800	0.77419200
C	2.79874100	1.45980200	0.76088200
H	3.66777600	1.65628500	1.39118000
H	2.38445500	0.50715000	1.10436200
C	3.24259400	1.31529000	-0.69118900
H	2.36952200	1.11939500	-1.31868700
H	3.66107200	2.26725400	-1.03541800
C	4.26307600	0.20158700	-0.91705300
H	4.45270000	0.10489300	-1.98954300
H	3.83237000	-0.75187700	-0.59441700
C	5.59497400	0.41389400	-0.20508300
H	5.98196400	1.40816400	-0.45234000
H	5.44839600	0.40097900	0.87775700
C	6.63411900	-0.63792700	-0.57372000
H	6.80426600	-0.61492500	-1.65472500
H	6.23627500	-1.63250100	-0.34802100
C	7.96193200	-0.45187400	0.14880200
H	8.35701100	0.54546100	-0.06963300
H	7.79406200	-0.48380200	1.23001400
C	9.00378900	-1.49786900	-0.22821400
H	9.17239900	-1.46231900	-1.30770300
H	8.60622800	-2.49304100	-0.01236000
C	10.32415600	-1.30115800	0.50554900
H	11.05813300	-2.05564300	0.22399100
H	10.17935100	-1.36052200	1.58533800
H	10.74844400	-0.32088000	0.28295400

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C	1.38628500	-1.33854600	-1.45135000
H	0.54623400	-0.99320100	-0.83850100
H	1.15209000	-1.20009800	-2.50400200
C	2.45418000	-2.86044100	-0.04190300
C	2.69377500	-0.69846000	-1.00220000
H	3.45994900	-0.84642100	-1.76370900
C	3.59437300	-3.79852400	-0.38264200
H	3.19394700	-4.75329700	-0.72035700

H	4.21529800	-3.96100600	0.49693600
H	4.20486800	-3.37085400	-1.17630300
C	1.58952100	-3.39581700	1.08293800
H	2.16070600	-3.52519700	1.99749300
H	1.19470900	-4.35710300	0.75884000
H	0.75702800	-2.72374700	1.28664700
O	1.64697200	-2.71161100	-1.22493400
N	2.96815200	-1.49224400	0.18922500
C	2.67899800	-0.74272200	1.31168200
C	2.72796000	0.73784300	-0.44989500
O	2.62442200	-1.10313300	2.45269400
O	2.47587100	0.55043500	0.95085100
C	4.10812000	1.31240400	-0.64308000
H	4.36084800	1.52661100	-1.67727900
C	4.97834000	1.54298500	0.32435100
H	5.95799200	1.94442400	0.10616500
H	4.73579400	1.34323600	1.35968900
C	1.65100200	1.64718100	-1.03116300
H	1.77764100	1.67553700	-2.11822400
H	0.68315900	1.18546200	-0.83294100
C	1.67816000	3.06086900	-0.46120800
H	1.65200300	3.00170000	0.62855600
H	2.62487500	3.53827600	-0.71831300
C	0.52564900	3.92913800	-0.96693900
H	0.60507300	4.91233000	-0.49701200
H	0.64092900	4.08892400	-2.04236400
C	-0.86702100	3.35985500	-0.69058000
H	-1.03232000	2.47384400	-1.30759400
H	-1.61573300	4.09006200	-1.01013700
C	-1.10132300	3.02029600	0.77864000
H	-0.43543900	2.20669400	1.08343100
H	-0.82283900	3.88553200	1.38731400
C	-2.53983200	2.61959100	1.09404800
H	-2.64285200	2.48059600	2.17330200
H	-3.21457100	3.43737300	0.82183400
C	-2.99209500	1.34272000	0.39275900
H	-2.26540400	0.54706900	0.59236400
H	-2.99826400	1.49175900	-0.69031600
C	-4.37552400	0.88333400	0.83584800
H	-4.37306400	0.72418700	1.91849900
H	-5.09855700	1.68316800	0.64601500
C	-4.84098500	-0.39010600	0.14069700
H	-4.12621100	-1.19520800	0.34239400
H	-4.82751100	-0.22956100	-0.94054700
C	-6.23176500	-0.83040800	0.58442400
H	-6.24997300	-0.88818600	1.67603200
H	-6.95714800	-0.05971100	0.30433600
C	-6.67474700	-2.17204500	0.00511200
H	-7.64500100	-2.43802700	0.42901700
H	-5.97468300	-2.94832500	0.32557400
C	-6.77475300	-2.17395700	-1.51665800
H	-7.18301200	-3.11505400	-1.88397600
H	-5.79939400	-2.03429500	-1.98230900
H	-7.42626300	-1.36845400	-1.86129500

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C	-3.57286100	-1.73150500	-0.00531000
H	-3.15296400	-1.60489300	-1.00905100
H	-3.05462400	-2.53713500	0.50902900
C	-5.72108300	-0.84468400	-0.22359400
C	-3.59705400	-0.40878100	0.74983200
H	-3.76866100	-0.58970900	1.81122300
C	-6.84215800	-0.88643600	0.79526700
H	-7.44971500	-1.77580700	0.63452200
H	-7.46796300	-0.00122700	0.69343600
H	-6.42961600	-0.91767500	1.80244700
C	-6.24266000	-0.76818200	-1.64534200

H	-6.83535200	0.12794300	-1.80382500
H	-6.85982000	-1.64927600	-1.81222200
H	-5.42185400	-0.77171600	-2.36098200
O	-4.95057900	-2.05166400	-0.06966600
N	-4.75403200	0.21935500	0.12416000
C	-4.32255100	1.18633400	-0.76158500
C	-2.49946200	0.64835000	0.53535100
O	-4.96447600	1.79358400	-1.57028900
O	-2.99548500	1.40634200	-0.57777500
C	-2.41649200	1.53393200	1.75287400
H	-2.01271600	1.04386100	2.63391100
C	-2.79735700	2.79844300	1.80105100
H	-2.71901200	3.36736300	2.71683800
H	-3.19224500	3.30165000	0.92842700
C	-1.13713300	0.06275500	0.18063900
H	-0.84512400	-0.60809800	0.99294400
H	-1.24417500	-0.54719300	-0.71883400
C	-0.06627700	1.12444800	-0.05350100
H	-0.47039900	1.88308400	-0.72608900
H	0.15885100	1.63035700	0.88833100
C	1.21934000	0.55119700	-0.64304700
H	1.00201500	0.09679400	-1.61464200
H	1.91427000	1.37188800	-0.83670700
C	1.90737500	-0.47677400	0.24866100
H	1.26244200	-1.35010000	0.38086200
H	2.05522400	-0.04844300	1.24603900
C	3.25014900	-0.93722000	-0.30539400
H	3.10338900	-1.36708200	-1.30204500
H	3.89504000	-0.06397700	-0.44119200
C	3.94137500	-1.95941200	0.58979100
H	4.15771200	-1.49876800	1.55777800
H	3.24711200	-2.77901300	0.79344400
C	5.22308000	-2.53901800	-0.00866700
H	4.96558100	-3.12044300	-0.89794300
H	5.66133400	-3.24106600	0.70566300
C	6.27393300	-1.49476500	-0.38474900
H	7.15873400	-2.00682600	-0.77393500
H	5.89723900	-0.87706100	-1.20284600
C	6.69209600	-0.60728600	0.78427900
H	6.92601900	-1.24324700	1.64329500
H	5.85034100	0.02225300	1.08889600
C	7.88954900	0.28794800	0.47722500
H	8.16573900	0.84109800	1.37913600
H	8.75297500	-0.33449900	0.22122900
C	7.64259500	1.28479600	-0.65081900
H	6.72666300	1.84447200	-0.43834700
H	7.46683900	0.74896100	-1.58560500
C	8.80705100	2.25014900	-0.83330100
H	8.62604800	2.95072500	-1.64792800
H	9.72685200	1.70714400	-1.05653300
H	8.97764400	2.82834700	0.07605200

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C	-4.33025000	-0.91052700	-1.37318000
H	-3.56565400	-0.40360400	-1.97179500
H	-4.40426800	-1.95103700	-1.67985000
C	-5.69969000	0.84915000	-0.68337700
C	-4.08746300	-0.70350300	0.11647300
H	-4.68684800	-1.40930900	0.69203300
C	-7.02615200	0.75516700	0.04340500
H	-7.83851100	0.72004100	-0.68099900
H	-7.15750700	1.62307900	0.68766700
H	-7.05538200	-0.14782000	0.65114200
C	-5.59461800	2.10848900	-1.52146800
H	-5.67416800	3.00186700	-0.90905600
H	-6.40898500	2.08623900	-2.24351000
H	-4.64777100	2.14000300	-2.05850100

O	-5.59575700	-0.29959700	-1.54565200
N	-4.58179700	0.65978800	0.26667100
C	-3.50928000	1.52244500	0.37354100
C	-2.66064000	-0.59369900	0.68243700
O	-3.51237100	2.71993500	0.34610100
O	-2.37039000	0.80578100	0.55483100
C	-2.67502900	-0.97622000	2.14084300
H	-2.85276000	-2.03202100	2.32274000
C	-2.50435600	-0.13876800	3.14875200
H	-2.54213700	-0.48612300	4.17168400
H	-2.31710600	0.91365800	2.98142000
C	-1.62314900	-1.39594500	-0.09511000
H	-1.64428600	-1.07180400	-1.13699300
H	-1.92956300	-2.44638200	-0.07839600
C	-0.20620400	-1.25693500	0.44824900
H	-0.16078900	-1.64994100	1.46649700
H	0.04878500	-0.19652200	0.51312700
C	0.81589300	-1.97826000	-0.42113700
H	0.54190500	-3.03471100	-0.50629900
H	0.78266900	-1.56964800	-1.43598100
C	2.23668300	-1.86755300	0.11727000
H	2.27913200	-2.29627100	1.12408800
H	2.49239800	-0.80945600	0.22429400
C	3.26124500	-2.56181600	-0.77243800
H	3.27356700	-2.07490500	-1.75125900
H	2.93612800	-3.59011700	-0.95184900
C	4.67162900	-2.59008000	-0.18330400
H	5.34473300	-3.06846900	-0.89977500
H	4.66736800	-3.22399100	0.70743500
C	5.24103100	-1.22094400	0.19097900
H	4.65830900	-0.78540200	1.00829400
H	6.24971700	-1.36639000	0.58186400
C	5.28029300	-0.23455600	-0.97179400
H	4.26765500	-0.08671500	-1.35456300
H	5.85923400	-0.67047500	-1.79321900
C	5.86643600	1.12767500	-0.60628700
H	5.76230400	1.80081600	-1.46153400
H	5.27592500	1.56807000	0.20361400
C	7.33367900	1.09127800	-0.19243600
H	7.91356300	0.57035200	-0.96200900
H	7.45192300	0.51170100	0.72667400
C	7.92077900	2.48086000	0.02516900
H	7.82295700	3.05966600	-0.89690600
H	7.32879600	3.00471000	0.78036100
C	9.38173600	2.43950900	0.45446000
H	9.49502400	1.89024400	1.39038900
H	9.99219100	1.93838700	-0.29840800
H	9.78671900	3.44019800	0.60235300

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C	4.92345200	0.07552100	-1.81539600
H	3.96046200	-0.34471800	-2.11025800
H	5.27708800	0.74684000	-2.59255800
C	5.72565100	-1.54782300	-0.36602800
C	4.87712100	0.71734200	-0.42900800
H	5.65756700	1.47688100	-0.34802600
C	7.08635600	-1.96968200	0.14292200
H	7.50688600	-2.73052400	-0.51292900
H	6.98688000	-2.37834500	1.14757600
H	7.75124600	-1.10886700	0.16839200
C	4.74855100	-2.71116200	-0.43125300
H	4.56360700	-3.10691200	0.56515200
H	5.17140600	-3.49429800	-1.05789500
H	3.79941800	-2.39103500	-0.86162200
O	5.89249700	-0.95167800	-1.64955000
N	5.18052100	-0.42182800	0.41233400
C	4.37655600	-0.47744600	1.50778000

C	3.55594500	1.24398900	0.20309300
O	4.34123800	-1.31992000	2.36335600
O	3.59236100	0.63525900	1.52346900
C	3.63637100	2.73232000	0.38602300
H	4.32077000	3.04048500	1.16961200
C	3.01438400	3.63807200	-0.34956300
H	3.17735400	4.69354300	-0.18277200
H	2.32349800	3.36766000	-1.13787400
C	2.29671000	0.74282100	-0.49479900
H	2.39245000	-0.34058000	-0.61438200
H	2.25501800	1.16277600	-1.50363000
C	0.99977800	1.03046800	0.25448000
H	0.86512500	2.10650800	0.37580300
H	1.06949700	0.60977700	1.25909400
C	-0.21004400	0.44500900	-0.46391200
H	-0.27047900	0.85957200	-1.47556500
H	-0.07561700	-0.63473100	-0.58210000
C	-1.52016400	0.71031100	0.26649300
H	-1.65636100	1.78973000	0.38486500
H	-1.45934500	0.29640700	1.27747200
C	-2.73162900	0.12434500	-0.44727300
H	-2.59478800	-0.95526400	-0.56419400
H	-2.78989000	0.53706400	-1.45947200
C	-4.04383400	0.38893700	0.27983400
H	-4.18086600	1.46847600	0.39745500
H	-3.98606500	-0.02419100	1.29162900
C	-5.25450600	-0.19728800	-0.43518400
H	-5.31103900	0.21513500	-1.44755500
H	-5.11738300	-1.27692100	-0.55194100
C	-6.56794800	0.06739100	0.28967600
H	-6.70510400	1.14699000	0.40678300
H	-6.51189300	-0.34535700	1.30180600
C	-7.77796600	-0.51879300	-0.42655500
H	-7.83308500	-0.10676400	-1.43915900
H	-7.64104200	-1.59853900	-0.54276600
C	-9.09206800	-0.25345100	0.29644100
H	-9.23011000	0.82626500	0.41286200
H	-9.03824300	-0.66539800	1.30921500
C	-10.30236900	-0.83965700	-0.41995900
H	-10.35379500	-0.42787500	-1.43129900
H	-10.16311400	-1.91785600	-0.53431700
C	-11.60829100	-0.56458800	0.31451700
H	-11.58474200	-0.99073100	1.31866700
H	-11.77680000	0.50867800	0.41543500
H	-12.46326100	-0.99017000	-0.21001900

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C	5.41477600	-1.67137800	0.75379500
H	5.42639500	-1.18572000	1.73178800
H	5.26076400	-2.73978800	0.87679500
C	6.66644500	-0.17338200	-0.48138200
C	4.41266800	-1.00815000	-0.19124600
H	4.08995900	-1.71664300	-0.95755600
C	7.48719700	-0.20944600	-1.75115200
H	8.52059300	-0.46037300	-1.51640300
H	7.45652900	0.76819300	-2.23019700
H	7.08037400	-0.95618200	-2.42980500
C	7.19562800	0.83984200	0.52219500
H	7.17049200	1.84171200	0.09942700
H	8.22223700	0.58059400	0.77485800
H	6.59127100	0.83079600	1.42996800
O	6.65119200	-1.48112800	0.08058400
N	5.23303600	0.04041200	-0.76724700
C	4.58468500	1.23790000	-0.78815200
C	3.18971800	-0.20586200	0.33651000
O	5.00805000	2.30720000	-1.13453100
O	3.30395800	1.04656600	-0.37137100

C	3.27017000	0.05609600	1.81564000
H	3.08342600	-0.81243700	2.44069500
C	3.54679100	1.22761100	2.36464900
H	3.59231900	1.34038700	3.43898200
H	3.72190200	2.10687500	1.75850000
C	1.87331400	-0.86563900	-0.05084000
H	1.85162200	-1.86119900	0.40404500
H	1.88278700	-1.01067000	-1.13385100
C	0.63657300	-0.07534800	0.35418500
H	0.60238600	0.02632900	1.44172400
H	0.71221300	0.93697500	-0.04948900
C	-0.65013100	-0.72897200	-0.13415600
H	-0.71372100	-1.74789500	0.26125400
H	-0.61861200	-0.82382200	-1.22385600
C	-1.89853800	0.04583900	0.26752600
H	-1.92658500	0.14675400	1.35692600
H	-1.83603400	1.06234200	-0.13260100
C	-3.18905000	-0.60683500	-0.21097700
H	-3.25159700	-1.62262600	0.19209800
H	-3.15989600	-0.71082400	-1.30012600
C	-4.43742400	0.17013400	0.18712700
H	-4.46256200	0.28051800	1.27581500
H	-4.37791400	1.18361000	-0.22168700
C	-5.72932900	-0.48700400	-0.28162600
H	-5.70364800	-0.59980400	-1.37003900
H	-5.78939700	-1.49966700	0.12954400
C	-6.97746400	0.29149700	0.11426400
H	-6.91966300	1.30260000	-0.30079200
H	-7.00045300	0.40848800	1.20232100
C	-8.27000200	-0.36915700	-0.34787300
H	-8.24680200	-0.48745900	-1.43579200
H	-8.32801900	-1.37982000	0.06849300
C	-9.51800600	0.40973900	0.04685400
H	-9.46240100	1.41977700	-0.37154600
H	-9.54075300	0.53067500	1.13463500
C	-10.81138800	-0.25272200	-0.41137100
H	-10.86513800	-1.26085700	0.00789600
H	-10.78681900	-0.37301900	-1.49761000
C	-12.04952100	0.53840900	-0.00929600
H	-12.96525000	0.05135400	-0.34316500
H	-12.10372600	0.64763300	1.07497000
H	-12.02577800	1.54018700	-0.44098800

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C	2.72004700	-2.07328600	-0.50862200
H	2.53420900	-2.28012400	0.54747000
H	1.92350500	-2.49998800	-1.11179000
C	5.01637900	-1.81883000	-0.49524400
C	2.95151100	-0.58269700	-0.75623800
H	2.70693400	-0.32896400	-1.79027100
C	6.13883400	-1.91917700	-1.50387100
H	6.52633300	-2.93669200	-1.52617900
H	6.94011300	-1.23748500	-1.22214000
H	5.76966400	-1.65242900	-2.49194300
C	5.46333700	-2.19958200	0.90791200
H	6.25616800	-1.53678200	1.24734300
H	5.83127500	-3.22396300	0.89276000
H	4.62948900	-2.13022200	1.60728700
O	3.94707500	-2.65281000	-0.92881000
N	4.37979200	-0.48613800	-0.52250300
C	4.69346700	0.57447900	0.27220700
C	2.39382700	0.50685100	0.20259100
O	5.76943400	0.88800600	0.70408900
O	3.56375600	1.29750200	0.49914000
C	1.85442700	-0.07418600	1.48112400
H	0.90737800	-0.59646800	1.37675300
C	2.44457800	0.01125100	2.66208100

H	1.99790100	-0.43538700	3.53955800
H	3.38068000	0.53978200	2.78728300
C	1.36617900	1.39288700	-0.48793900
H	0.54080200	0.74876600	-0.80702800
H	1.82621400	1.79335300	-1.39539200
C	0.85469900	2.53375000	0.38271600
H	0.31212800	2.12649100	1.23819800
H	1.71064300	3.07296000	0.79158700
C	-0.03755400	3.50816600	-0.38520000
H	0.56261000	4.01806100	-1.14301000
H	-0.38567600	4.27979100	0.30556100
C	-1.24847600	2.86288400	-1.05891300
H	-0.91964100	2.20021100	-1.86493600
H	-1.84068000	3.64575100	-1.53928200
C	-2.14506800	2.08186000	-0.10454000
H	-1.59590700	1.23269700	0.31491500
H	-2.41316700	2.71917500	0.74459800
C	-3.41343100	1.56707500	-0.77348700
H	-3.98096300	2.41411000	-1.17089400
H	-3.14162600	0.95047400	-1.63641000
C	-4.30377200	0.75883900	0.16165300
H	-3.73857900	-0.09276200	0.55369300
H	-4.57075600	1.37300400	1.02733400
C	-5.57495200	0.25315000	-0.50848300
H	-5.30764800	-0.35662800	-1.37731300
H	-6.14230600	1.10505800	-0.89603500
C	-6.46362900	-0.56113000	0.42311600
H	-5.89672700	-1.41396500	0.80974900
H	-6.73042200	0.04812700	1.29238300
C	-7.73500800	-1.06479900	-0.24765100
H	-7.46907100	-1.67281700	-1.11827900
H	-8.30361500	-0.21237400	-0.63279400
C	-8.62368500	-1.88171900	0.68216100
H	-8.05442400	-2.73266700	1.06534700
H	-8.88802900	-1.27313600	1.55088300
C	-9.89044300	-2.37706000	-0.00373400
H	-9.64576400	-3.00776800	-0.85978700
H	-10.51439700	-2.95920500	0.67372700
H	-10.48492200	-1.53894600	-0.37061900

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C	-5.51146100	-0.09573900	-1.27484900
H	-6.25725000	0.18172100	-0.52221100
H	-5.75800500	0.37688600	-2.22196800
C	-4.68102300	-2.10299800	-0.43263000
C	-4.09863200	0.18374400	-0.77994600
H	-3.40540000	0.16978100	-1.62266900
C	-3.76196600	-3.10990800	-1.09319200
H	-4.35337900	-3.85833700	-1.61865600
H	-3.14992200	-3.60154100	-0.33854000
H	-3.11262200	-2.60583600	-1.80744000
C	-5.59641800	-2.74617100	0.59208600
H	-5.03136100	-3.22471200	1.38633300
H	-6.19681900	-3.48970900	0.07092800
H	-6.26107400	-2.00613400	1.03659900
O	-5.47671900	-1.49848300	-1.46498400
N	-3.89948200	-0.96117100	0.09495600
C	-3.91352300	-0.55482000	1.41188200
C	-3.80116000	1.38102200	0.14794400
O	-3.92178100	-1.23755500	2.39691700
O	-3.88402100	0.79913100	1.46744600
C	-4.85835500	2.44905300	0.06592100
H	-5.79440300	2.18772300	0.54695900
C	-4.73238300	3.62154600	-0.53369600
H	-5.55870900	4.31819400	-0.55800500
H	-3.81515700	3.93376600	-1.01448500
C	-2.38535800	1.91062200	-0.06046900

H	-2.23454600	2.74130500	0.63219800
H	-2.32408800	2.31926900	-1.07237100
C	-1.28025900	0.87816400	0.13416100
H	-1.34389600	0.45871000	1.14118600
H	-1.40856200	0.04350900	-0.56019900
C	0.10266200	1.48669500	-0.06818700
H	0.23849700	2.31705500	0.63124000
H	0.16729600	1.91670800	-1.07273900
C	1.22881300	0.47870800	0.12105500
H	1.09036800	-0.35360500	-0.57600000
H	1.16527500	0.05054900	1.12590700
C	2.61196800	1.08294000	-0.08447900
H	2.75075400	1.91423400	0.61376900
H	2.67288500	1.51333600	-1.08911500
C	3.74047800	0.07654400	0.10048300
H	3.59926200	-0.75677500	-0.59496900
H	3.68244900	-0.35108800	1.10621100
C	5.12308000	0.67990200	-0.11161700
H	5.17967400	1.10857100	-1.11721400
H	5.26476800	1.51269600	0.58436000
C	6.25267100	-0.32582900	0.07043700
H	6.10926800	-1.15997000	-0.62360800
H	6.19834900	-0.75253800	1.07685500
C	7.63465100	0.27722400	-0.14679600
H	7.68796300	0.70487300	-1.15300400
H	7.77857000	1.11080900	0.54783200
C	8.76481200	-0.72802100	0.03268600
H	8.62103400	-1.56261500	-0.66097500
H	8.71371900	-1.15522900	1.03925900
C	10.14694900	-0.12547600	-0.18716300
H	10.19539800	0.30144900	-1.19235500
H	10.28917100	0.70691200	0.50700700
C	11.26572800	-1.14336600	-0.00612000
H	11.15403200	-1.97018600	-0.70924000
H	12.24667800	-0.69710500	-0.16687700
H	11.24868300	-1.56224200	1.00121100

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C	-5.91153100	1.15834400	-0.47537700
H	-6.06043700	1.44420400	0.57215200
H	-6.08190500	2.02099600	-1.11411600
C	-6.27339100	-1.14774000	-0.45950500
C	-4.55883100	0.48886800	-0.67004200
H	-4.29799700	0.46935900	-1.72895900
C	-6.37540400	-2.07655000	-1.65260700
H	-7.41539200	-2.15776300	-1.96525100
H	-6.00315400	-3.06443300	-1.38577700
H	-5.78738700	-1.68453100	-2.48101300
C	-7.05701000	-1.66086500	0.73325300
H	-6.69507600	-2.63079300	1.06135200
H	-8.09771300	-1.74395000	0.42491100
H	-6.99187800	-0.96545700	1.56888300
O	-6.80484900	0.12844600	-0.85690300
N	-4.85332400	-0.84607200	-0.16644000
C	-4.27561200	-1.00530700	1.07784000
C	-3.34378700	0.90969000	0.17905700
O	-4.43326000	-1.89945000	1.85913300
O	-3.42556400	0.02388900	1.31587700
C	-3.46233700	2.32155900	0.68548300
H	-4.06468500	2.42844100	1.58078700
C	-2.93690800	3.38951600	0.10828200
H	-3.10663500	4.37704400	0.51376900
H	-2.32013100	3.32371000	-0.77900500
C	-2.05124400	0.60474400	-0.57422000
H	-2.00137100	1.25725000	-1.44976000
H	-2.13428000	-0.41690900	-0.95715000
C	-0.78223600	0.73577900	0.25743100

H	-0.68370200	1.75881100	0.62644500
H	-0.86154400	0.09220700	1.13572800
C	0.46203000	0.36167400	-0.53913000
H	0.53237600	0.99886800	-1.42669900
H	0.36385900	-0.66490600	-0.90533000
C	1.74562000	0.48569600	0.27151100
H	1.84592300	1.51307500	0.63511200
H	1.67335500	-0.14822800	1.16041900
C	2.99152200	0.10660400	-0.51870900
H	3.06030200	0.73757900	-1.41056100
H	2.89198400	-0.92227100	-0.87847000
C	4.27699100	0.23516000	0.28849800
H	4.37718200	1.26438400	0.64725000
H	4.20792900	-0.39455000	1.18096300
C	5.52252900	-0.14598000	-0.50142800
H	5.42326800	-1.17614000	-0.85771800
H	5.58958000	0.48184800	-1.39558400
C	6.80909300	-0.01377100	0.30345500
H	6.74232500	-0.64132600	1.19769000
H	6.90841400	1.01648000	0.65952900
C	8.05426700	-0.39505200	-0.48704400
H	7.95562200	-1.42587500	-0.84168500
H	8.11977000	0.23143400	-1.38225400
C	9.34144000	-0.26049000	0.31608500
H	9.27748300	-0.88730800	1.21125500
H	9.44082800	0.77017400	0.67140700
C	10.58708900	-0.64077400	-0.47475800
H	10.64876300	-0.01418400	-1.36839900
H	10.48633800	-1.67031200	-0.82814600
C	11.86542200	-0.49985200	0.34163600
H	12.74632100	-0.77448700	-0.23787800
H	11.99534100	0.52839600	0.68270200
H	11.83269500	-1.13892300	1.22536800

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C	-2.68872000	-0.69466600	-1.83542800
H	-3.73054600	-0.42665300	-2.02410800
H	-2.06116900	-0.30839700	-2.63413000
C	-2.95879200	-2.57539900	-0.52105000
C	-2.24294900	-0.26354300	-0.43879400
H	-1.15914400	-0.12081800	-0.41237400
C	-2.13835400	-3.79346900	-0.16003200
H	-2.33732100	-4.59680900	-0.86782100
H	-2.40599200	-4.12521700	0.84221500
H	-1.07970500	-3.54385900	-0.18633700
C	-4.45246800	-2.86308800	-0.52275700
H	-4.77800200	-3.17830300	0.46629100
H	-4.65772000	-3.65616900	-1.23962500
H	-5.01372000	-1.97178600	-0.80497500
O	-2.53080900	-2.10607600	-1.79504600
N	-2.62865000	-1.42364200	0.34134000
C	-3.24934400	-1.07160200	1.50067500
C	-2.94809500	0.88606100	0.33428100
O	-3.74194600	-1.78826700	2.32926000
O	-3.20283100	0.28328300	1.62087000
C	-4.26249900	1.27913100	-0.28454800
H	-4.19165100	1.79672800	-1.23467900
C	-5.44974900	1.02919300	0.24469000
H	-6.35518000	1.34287800	-0.25617900
H	-5.55120200	0.51721600	1.19247600
C	-2.02557000	2.07788000	0.55370600
H	-1.15718800	1.70609100	1.10207500
H	-2.53357700	2.79602900	1.20091200
C	-1.58336900	2.75540100	-0.73912600
H	-1.12357600	2.01816100	-1.40464800
H	-2.45251000	3.14840200	-1.26849200
C	-0.60629500	3.90846100	-0.50298000

H	-1.12126100	4.69907400	0.04810200
H	-0.33434900	4.33214100	-1.47269200
C	0.67096800	3.52679700	0.24449100
H	0.43902900	3.27003300	1.28149400
H	1.31900600	4.40537600	0.29251600
C	1.44229000	2.37844800	-0.39604400
H	0.84944100	1.45906200	-0.35094600
H	1.59553400	2.59130700	-1.45946900
C	2.78900000	2.12529000	0.26969300
H	3.39897700	3.03150200	0.20507500
H	2.63375900	1.93622200	1.33655000
C	3.55258600	0.95838300	-0.34317200
H	2.94605400	0.05022300	-0.26901000
H	3.69838500	1.14301100	-1.41220800
C	4.90503400	0.71457100	0.31410200
H	4.75959700	0.53354000	1.38365100
H	5.51282200	1.62154000	0.23708100
C	5.66710000	-0.45521900	-0.29542400
H	5.06050000	-1.36276900	-0.21539000
H	5.80951300	-0.27571200	-1.36577700
C	7.02146000	-0.69623400	0.35846500
H	6.88027600	-0.87456300	1.42923100
H	7.62951100	0.21043500	0.27733900
C	7.78340800	-1.86746900	-0.24922500
H	7.17530800	-2.77200600	-0.16589800
H	7.92208300	-1.68827100	-1.31866000
C	9.13594900	-2.09401400	0.41397400
H	9.01527700	-2.29914600	1.47876100
H	9.66726100	-2.93473000	-0.03111800
H	9.76717000	-1.20926400	0.31775100

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C	-3.80232900	1.37841400	0.73187100
H	-3.68869000	0.93481400	1.72344700
H	-3.37355800	2.37702500	0.72658600
C	-5.63625500	0.21243100	-0.05203500
C	-3.24733100	0.46290300	-0.35864800
H	-2.96092400	1.04778400	-1.23641800
C	-6.70908100	0.42378700	-1.09684700
H	-7.56548000	0.92770300	-0.65120100
H	-7.02633300	-0.54120900	-1.48966700
H	-6.31568900	1.03158200	-1.90898000
C	-6.12893800	-0.61524700	1.12520500
H	-6.44115200	-1.60199000	0.79021200
H	-6.97454600	-0.10593400	1.58407500
H	-5.33827800	-0.73476700	1.86671200
O	-5.17495500	1.48730300	0.38253900
N	-4.40904900	-0.35823800	-0.64209400
C	-4.08867100	-1.67871700	-0.72840400
C	-2.16430800	-0.61202400	-0.06083400
O	-4.82465200	-2.61487100	-0.88550400
O	-2.73615400	-1.80181200	-0.64364700
C	-1.96651800	-0.84017700	1.41358100
H	-1.48776800	-0.02693400	1.94811000
C	-2.33841600	-1.93108200	2.06435400
H	-2.16345200	-2.02778200	3.12692400
H	-2.81569900	-2.75823200	1.55581400
C	-0.85568500	-0.32396500	-0.78464600
H	-1.08326300	-0.25858000	-1.85151700
H	-0.19156200	-1.17954100	-0.64776400
C	-0.16397700	0.95472400	-0.32521400
H	-0.86882100	1.79414200	-0.34743700
H	0.16011500	0.84724500	0.71150900
C	1.03899700	1.29391100	-1.19983800
H	0.71380700	1.35494500	-2.24110500
H	1.75490200	0.46816800	-1.15306100
C	1.72560300	2.60336200	-0.81647200

H	2.55953800	2.77628300	-1.49968600
H	1.02743200	3.42957200	-0.97422500
C	2.23129800	2.65665400	0.62746900
H	1.38083400	2.69489600	1.31218800
H	2.77532800	3.59389500	0.76923900
C	3.13978100	1.49315000	1.02210800
H	3.45425400	1.62840100	2.06016000
H	2.57810400	0.55387300	0.99790600
C	4.37944800	1.35410300	0.14545200
H	4.08556100	1.13190900	-0.88454000
H	4.90810200	2.31263800	0.11273000
C	5.33024200	0.26776400	0.63253700
H	4.79404200	-0.68541000	0.68138200
H	5.64463800	0.49446300	1.65604300
C	6.56199900	0.10732900	-0.24919300
H	6.24710600	-0.12572100	-1.27123200
H	7.09466100	1.06203700	-0.30333000
C	7.51611600	-0.97382700	0.24166400
H	6.98361400	-1.92856600	0.29859300
H	7.83428300	-0.74014800	1.26269100
C	8.74631700	-1.13727600	-0.64233000
H	9.27604300	-0.18272000	-0.69891300
H	8.42651900	-1.37170200	-1.66101100
C	9.69081500	-2.22069900	-0.13767400
H	10.56438700	-2.32517000	-0.78043200
H	10.04108500	-1.99037000	0.86966900
H	9.18565900	-3.18699200	-0.09856000

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C	-0.84762700	-2.16053000	1.76903900
H	-1.38224600	-3.03640300	1.39272000
H	-0.09294400	-2.47098800	2.48730500
C	-2.43569800	-0.50961300	1.43165700
C	-0.30489900	-1.31024900	0.62366900
H	0.51278800	-0.68072400	0.98418400
C	-2.65539700	0.88738700	1.96836300
H	-3.29435300	0.85082200	2.84963900
H	-3.13097900	1.50056800	1.20281900
H	-1.69721200	1.33112800	2.23363900
C	-3.73602300	-1.20242400	1.05834100
H	-4.26363700	-0.64180500	0.29066200
H	-4.35857000	-1.27190800	1.94852200
H	-3.53964800	-2.20724100	0.68265000
O	-1.73376000	-1.26221900	2.41968600
N	-1.47064400	-0.50815700	0.31343100
C	-1.77090900	-0.52716400	-1.01475800
C	0.05216000	-1.92346800	-0.76118100
O	-2.70625300	-0.01786200	-1.57165200
O	-0.79394700	-1.19795200	-1.67604000
C	-0.26743000	-3.38956100	-0.84743800
H	0.33244600	-4.02657500	-0.20550400
C	-1.19958000	-3.91610600	-1.62494900
H	-1.37096300	-4.98346000	-1.63884400
H	-1.80748400	-3.29815400	-2.27189500
C	1.49485200	-1.61628400	-1.14735400
H	1.59377700	-0.52964100	-1.11050600
H	1.64521600	-1.91688200	-2.18622400
C	2.53928600	-2.27504600	-0.25208200
H	2.28011500	-2.12357200	0.80019000
H	2.53523400	-3.35383600	-0.41535800
C	3.95432000	-1.74880800	-0.51073900
H	4.25311400	-2.02869100	-1.52385100
H	4.64205500	-2.25848800	0.16823600
C	4.11669300	-0.23720000	-0.34295000
H	5.16131500	0.02611500	-0.52883200
H	3.53997800	0.28210600	-1.11104000
C	3.72017300	0.27386300	1.03814300

H	4.30952100	-0.25505000	1.79257900
H	2.67645900	0.02325000	1.24407200
C	3.91412300	1.77996500	1.21296600
H	3.67193000	2.04888900	2.24448800
H	4.97068500	2.02097800	1.06928300
C	3.07132200	2.64390000	0.27266400
H	3.27734600	3.69549000	0.48787400
H	3.37928500	2.48438900	-0.76415000
C	1.56953800	2.40623300	0.39152700
H	1.26613500	2.51778300	1.43875200
H	1.32828400	1.37501900	0.11447800
C	0.73952100	3.33861500	-0.48234300
H	0.98291700	4.37833000	-0.24253600
H	1.01937100	3.19109500	-1.53027500
C	-0.75849300	3.11604200	-0.32292600
H	-1.04548500	3.32656400	0.71291200
H	-0.99004400	2.05908000	-0.49057700
C	-1.61057400	3.96080000	-1.26054100
H	-1.32643800	3.74331000	-2.29323600
H	-1.39311600	5.01960800	-1.09514400
C	-3.09923600	3.69603000	-1.07329900
H	-3.70508600	4.29260700	-1.75493100
H	-3.32211400	2.64276400	-1.25303000
H	-3.40820300	3.93660000	-0.05390500

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C	3.73295200	-1.67462200	-1.24608800
H	3.46568900	-2.45445400	-0.52316400
H	3.34962700	-1.94755100	-2.22593400
C	5.59438100	-0.63695500	-0.29019500
C	3.28311600	-0.30865200	-0.74964600
H	3.27422100	0.41191300	-1.56870600
C	6.48394100	0.41303500	-0.92533900
H	7.31559000	-0.07173200	-1.43453700
H	6.87337700	1.08150200	-0.15904300
H	5.91614100	0.99331700	-1.65100500
C	6.33308000	-1.45809000	0.74893300
H	6.70870200	-0.83591400	1.55593200
H	7.16609900	-1.94413300	0.24398500
H	5.68395300	-2.22270100	1.17297700
O	5.13566900	-1.50924100	-1.33785700
N	4.34485200	-0.01543200	0.20496200
C	3.86523800	-0.17648900	1.49008900
C	2.01400500	-0.18247800	0.11024300
O	4.48166100	-0.17604300	2.51711100
O	2.51707900	-0.31205300	1.45431800
C	1.01180900	-1.28399400	-0.12666400
H	0.81849100	-1.92687900	0.72414900
C	0.39451100	-1.48002500	-1.28061500
H	-0.31515100	-2.28538000	-1.40654200
H	0.57548200	-0.84669600	-2.14227300
C	1.39560400	1.20650400	-0.04668800
H	1.03348600	1.28112100	-1.07505000
H	2.19069300	1.95036400	0.06812800
C	0.26926500	1.48624000	0.93967800
H	-0.49928700	0.71631200	0.83357100
H	0.65759500	1.39950700	1.95481600
C	-0.35544500	2.86800400	0.75204500
H	0.39463700	3.63360400	0.96554700
H	-1.14214400	2.99763800	1.49766500
C	-0.93011200	3.11576000	-0.64585700
H	-0.11352800	3.22631100	-1.36324800
H	-1.45695900	4.07326000	-0.64105300
C	-1.88195800	2.02609500	-1.13774900
H	-2.23248400	2.28838300	-2.13939400
H	-1.34205100	1.07897200	-1.24431200
C	-3.09019400	1.80637300	-0.23464700

H	-2.76093000	1.49978600	0.76228400
H	-3.62105400	2.75511300	-0.10358800
C	-4.05106800	0.75722200	-0.77995900
H	-4.38778800	1.05720400	-1.77728700
H	-3.51382100	-0.18826200	-0.90927900
C	-5.26336300	0.52929600	0.11365200
H	-5.79878200	1.47503000	0.24370600
H	-4.92638600	0.22893300	1.11070000
C	-6.22311400	-0.52117400	-0.43066900
H	-6.55845000	-0.22199000	-1.42874900
H	-5.68847600	-1.46778200	-0.55839300
C	-7.43705200	-0.74564300	0.46143000
H	-7.97284800	0.20043000	0.58874600
H	-7.10270900	-1.04404500	1.46015500
C	-8.39732600	-1.79698100	-0.08111700
H	-8.72964300	-1.49765200	-1.07859900
H	-7.86080500	-2.74112400	-0.20674100
C	-9.60577800	-2.00837900	0.82188800
H	-10.16896200	-1.08113300	0.93806800
H	-10.28172100	-2.76292700	0.42066800
H	-9.29398500	-2.33190900	1.81614400

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Copies of NMR spectra

Copies of the NMR spectra of all the synthesized compounds can be found in the following pages.

ent-3a (2R,3R)

ent-3b (2R,3S)

—156.2

—140.6

—114.3

79.5

78.6

77.3 cdd13

77.0 cdd13

76.7 cdd13

—62.9

—56.1

38.7

31.9

30.0

29.6

29.6

29.6

29.5

29.3

28.3

23.4

22.6

—14.1

70 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

ent-4a (2R,3R)

ent-5a (R,R)

ent-5b (2R,3S)

Chemical shift values (ppm) are listed above the spectrum:

- 156.0
- 134.5
- 116.7
- 94.3
- 82.6
- 66.6
- 65.3
- 40.5
- 31.9
- 29.6
- 29.6
- 29.5
- 29.4
- 29.3
- 27.9
- 23.3
- 22.9
- 22.6
- 14.1

ent-6b

11

8.75
8.74

7.59
7.57

7.26 cdd13
7.24

3.18
3.16
3.14

2.73
2.72
2.70

2.38
2.36
2.34

1.49
1.47
1.45

1.44
1.29
1.25

1.19
0.89
0.88

0.86

RBM10-1 (2S,3S)

RBM10-1 (2S,3S)

—140.2

—114.4

—75.6

—60.5

—58.6

36.8

31.7

29.8

29.4

29.3

29.3

29.3

29.1

22.9

22.3

—13.1

RBM10-2 (2*S*,3*R*)

RBM10-2 (*2S,3R*)

RBM10-3 (2*R*,3*R*)

RBM10-5 (2S,3S)

RBM10-6 (2S,3R)

RBM10-7 (2*R*,3*R*)

RBM10-7 (2*R*,3*R*)

RBM10-8 (2R,3S)

RBM10-8 (*2R,3S*)

RBM10-8 (2R,3S)

RBM10-9 (*2R,3R*)

RBM10-9 (2R,3R)