UNIVERSITAT ROVIRA I VIRGILI

Cross-metathesis optimization as a key step for the synthesis of sphingosine analogues

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12th June 2017

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Chemistry degree's final project

Contents

1. Introduction

1.1. Sphingolipids, sphingosine and new targets for cancer treatment

Traditionally lipids have been considered structural and energy storage molecules. Despite this fact, in the past decades, the discovery that they are involved in signalling and regulatory processes has come to the limelight.[1]

Among all the bioactive lipids, special attention has been paid to sphingolipids. Sphingolipids are a type of lipids, derived from sphingosine (Figure 1.1), which main roles are the regulation of the actin cytoskeleton, endocytosis, the cell cycle and apoptosis.

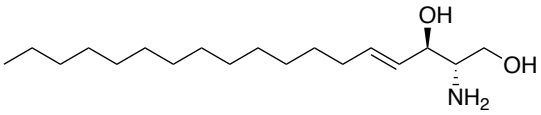


Figure 1.1. Sphingosine structure**.**

Even more interest was devoted to the metabolism of ceramide (Cer) and sphingosine-1-phosphate (S1P). The formation of S1P is partially controlled by the enzyme sphingosine kinase (SphK). Specifically, there are two isoforms of this enzyme (SphK1 and SphK2) that are able to transform sphingosine to sphingosine-1-phosphate. Despite this fact, SphK2 is more related with signalling pathways associated with cancer cell proliferation, metastasis processes and multi drug resistance.[2]

Cancer cells fate has proven to be influenced by the dynamic balance between ceramide, sphingosine and sphingosine-1phosphate. The Cer-to-Sph-to-S1P balance involves deacylation of ceramide by ceramidases to provide sphingosine, which is further phosphorylated by the sphingosine kinase. Higher intracellular concentrations of Cer favour the apoptotic process while the accumulation of S1P promotes cancer cell survival (Figure 1.2).[2]

Regarding all the information above, the inhibition of SphK1 activity has become a novel approach for cancer treatment.

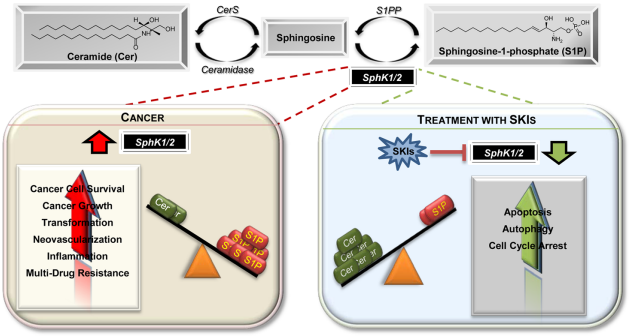


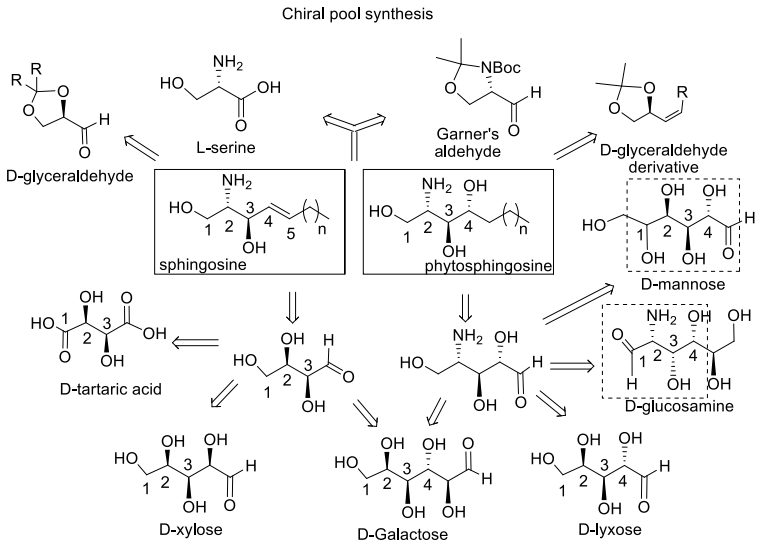
Figure 1.2. Cer/S1P rheostat and effects on cancer cells with and without SphK1 inhibition.

During the past decade, special attention has been paid to find possible sphingosine kinase inhibitors (SKI), being the most important sphingolipid analogues, natural products and nonlipidic small molecules. Consequently, new synthetic approaches to obtain these molecules are of great interest.

1.2 Synthetic methodology targeting sphingosine

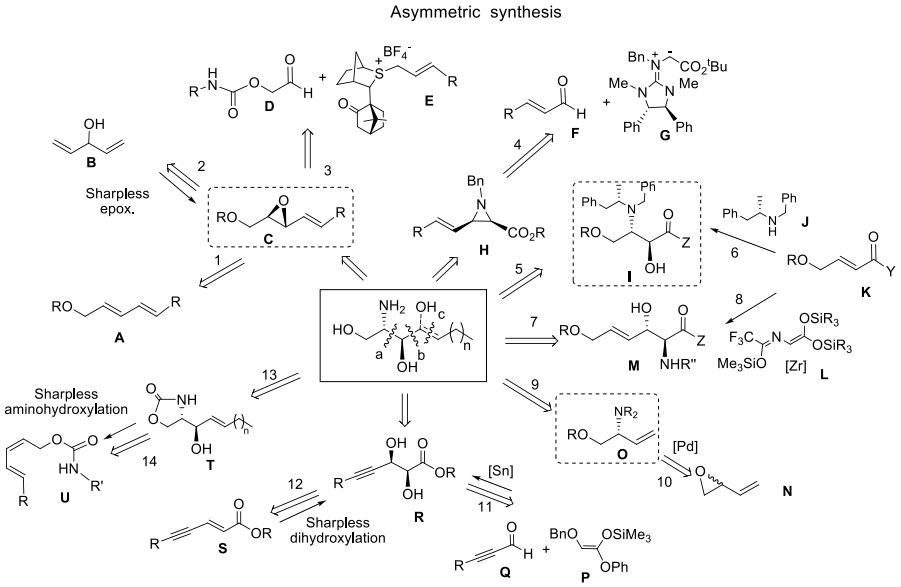
Owing the relevant biological role of sphingolipids, developing methods for the synthesis of sphingosine has attracted the interest of researches. Particularly, two synthetic strategies have been followed in order to prepare sphingosine and other derivatives.

The first procedure is the chiral pool synthesis. L-Serine, and specially Gardner's aldehyde, are considered important precursors because the hydroxyl-amino groups match those of sphingosine. In the other hand, sugars as D-galactose and D-xylose among others represent a family of compounds which can be easily modified to achieve sphingosine structure with the introduction of the amino function by inversion of configuration at C-5 of the sugar as key strategic step. Scheme 1.1. shows a summary of procedures based on the use of the chiral pool.[3]



Scheme 1.1. Overview of the most important chiral precursors of sphingosine and phytosphingosine.

Asymmetric synthesis has also been an attractive methodology targeting sphingosine and its derivatives. Among all the reactions, asymmetric epoxidation, dihydroxylation and aminohydroxilation represent the most common strategies. Moreover, asymmetric aziridination has emerged as an important functionalization because the nucleophilic ring opening of the nitrogen-cycle with a large variety of nucleophiles offers the desired trans-configuration of different functional groups. Scheme 1.2 shows a summary of the most relevant procedures used in the synthesis of sphingoides bases.[3]



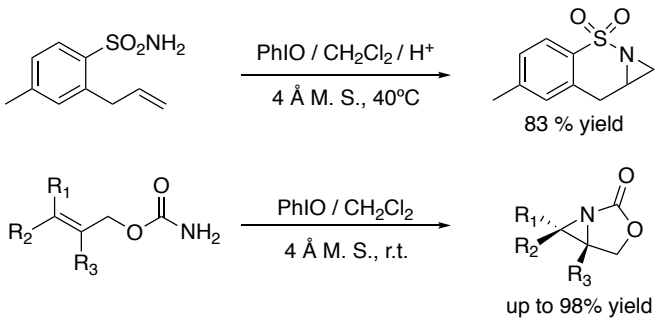
Scheme 1.2. Overview of the most important asymmetric synthesis targeting sphingosine and phytosphingosine. Scheme extracted from [3].

Despite the large quantity of methods available, new direct, flexible and efficient procedures are currently object of development because the growing importance sphingolipids on medical and pharmaceutical areas.

1.3 Hypervalent iodine aziridination

Hypervalent iodine compounds have demonstrated to be useful reagents for a large number of synthetic transformations as selective oxidations, rearrangements and aminations. Compounds of iodine possess reactivity similar to that of transition metals but with the advantage of being environmental sustainable.[4]

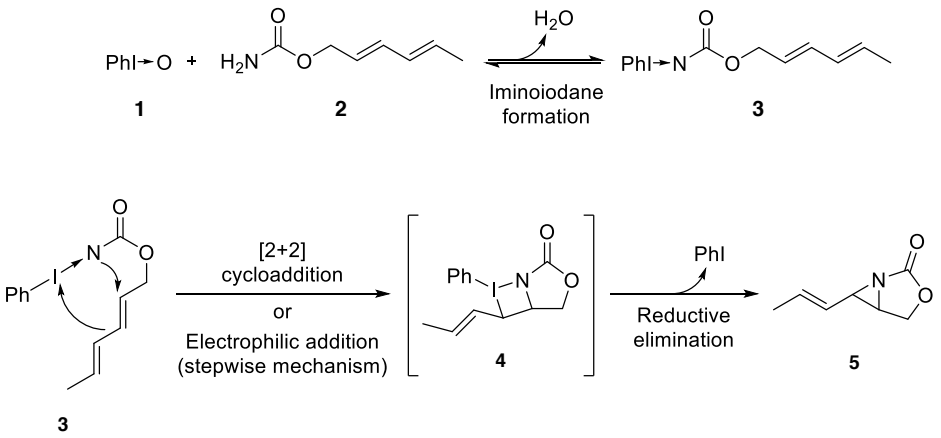
In amination reactions, hypervalent iodine compounds have revealed promising results for the metal-free intramolecular aziridnation of carbamate and sulphonamide systems regardless conformational restrains (Scheme 1.3).[5,6]



Scheme 1.3. Metal-free intramolecular aziridination of sulphonamides and allylic carbamates.

The use of this type of reagents tackles the formation of aziridines through an addition of a nitrene to an olefin.[7] Nitrenes are molecular fragments that present a monovalent nitrogen atom with a sextet of electrons, which makes them electrophilic. One of the major limitations of working with nitrenes is that they are highly unstable since they possess a nitrogen atom prone to react in order to attain the octet of electrons. Within this problematic, hypervalent iodine reagents have proved to be successful in the stabilization of nitrenes as iminoiodanes.[8]

Taking into account the mechanistic insights for intramolecular aziridination reported in the literature previously mentioned, in the research group a plausible mechanism for the aziridination of dyenyl carbamates was proposed (Scheme 1.4).



Scheme 1.4. Proposed mechanism for the intramolecular aziridination of 5 with PhIO.

The mechanism can be explained first with the reaction of iodosobenzene **1** (PhIO) and carbamate **2** to form iminoiodane **3**. Secondly, nucleophilic attack of the π-density of the double bond into the electrophilic iodine atom followed by nitrogen attack to the positively charged carbon atom of the former double bond, in a stepwise manner, would render intermediate **4**. Finally, a reductive elimination would yield the desired vinyl aziridine intermediate **5**.

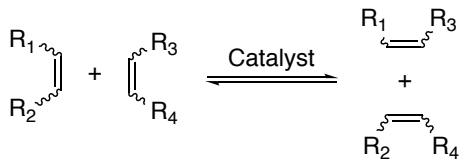
1.4 Cross metathesis

Olefins are one of the most important functional groups in organic synthesis not only because its prevalence in all kinds of natural products, but also because they serve as versatile intermediates in the preparation of other functional groups.

Consequently, synthetic methodologies to achieve olefination are very important to organic chemists. In this area, apart from the elimination processes, traditionally olefination strategies allowing carbon-carbon double bonds formation relied on the functionalization of a carbonyl compound with a carbon nucleophile (Wittig, Horner-Wadsworth-Emmons or Julia olefinations). These reactions often require mandatory protection of functional groups prior to the olefination, thus lengthening the synthesis with additional protection and deprotection steps. Moreover, they generate stoichiometric formation of byproducts.[9]

In the other hand, new modern methodologies involving organometallic reagents have been developed, being the palladium cross coupling reactions one of the most important (Heck, Stille, Suzuki-Miyaura and Shonogashira couplings).[10] In this case, previous activation of the substrates is generally necessary.

In this scenario, cross metathesis emerges as an alternative approach to olefination. This reaction is defined as the metal-catalysed redistribution of carbon-carbon double bonds (Scheme 1.5) and has the main advantages using exclusively olefins as starting materials, the easy of removal of the by-products formed (mainly ethylene) and a vast functional group tolerance.[11]



Scheme 1.5. General representation of olefin cross metathesis**.**

Despite its main advantages in comparison to the other olefinations, some problems are still encountered. The incompatibly with basic functional groups (nitriles, azides and amines), the selectivity of the reaction and the difficulty of some tri or tetrasubstituted olefins to undergo cross metathesis are the main drawbacks.[12]

1.4.1 Catalysts and mechanism

The most widely used catalysts for olefin cross metathesis are ruthenium carbene complexes ($L_2X_2Ru=CHR$) (Figure 1.3), although molybdenum and tungsten complexes have also been used with success.

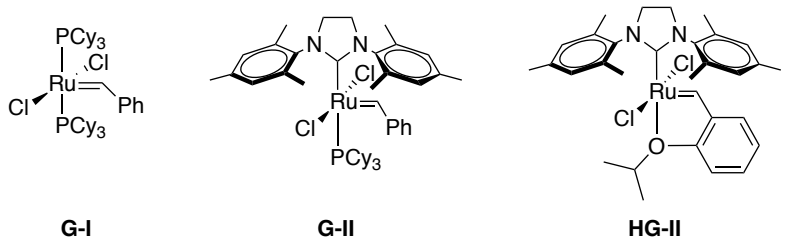
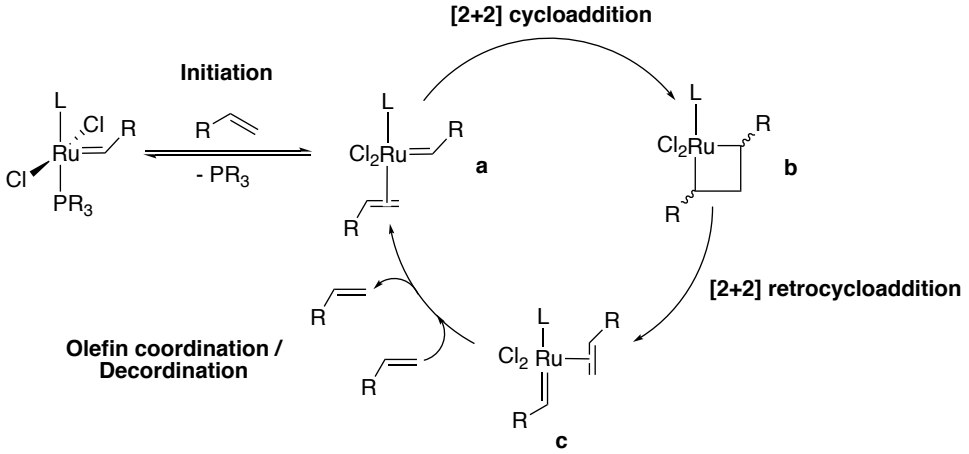


Figure 1.3. Most common cross metathesis Ru catalysts: **GI:** Grubbs catalyst 1st generation, **G-II:** Grubbs catalyst 2nd generation and **HG-II:** Hoveyda-Grubbs 2nd generation catalyst.

From the first generation (G-I) to the most modern ones (HG-II) significant catalytic activity has been improved with the incorporation of electron donating groups (N-heterocyclic carbenes in substitution of tricyclohexylphosphine) and the use of hemilabile ligands (2-isopropoxyphenylmethylene instead of tricyclohexylphosphine) to facilitate the dissociation of the complex and the incorporation of an olefin, which is the rate-limitign step.[13]

The cross metathesis mechanism was originally proposed by Hérison and Chauvin in 1971 and it has been fully validated experimentally by Grubbs and coworkers (Scheme 1.6).[14,15]



Scheme 1.6. Catalytic cycle of the cross metathesis reaction.

The reaction starts with the dissociative coordination of an olefin to the metal complex (initiation). This step is considered the rate-limiting step and justifies the improvements on the catalysts mentioned above. The active specie **a** undergoes a [2+2]-cycloaddition between the coordinated olefin and the metal alkylidene to form a metallocyclobutane intermediate **b**. This intermediate reacts by a [2+2]-retrocycloaddition to form **c**. Finally, the last intermediate releases the cross

metathesis product and incorporates another olefin to the metal coordination sphere closing, in this manner, the catalytic cycle.[16]

It is interesting to emphasize that one of the most important steps, in the catalytic cycle, is a thermic [2+2]-cycloaddition which is, theoretically, forbidden by the Woodward-Hoffman rules. However, this specific transformation in cross metathesis is allowed because of the symmetry of the interaction of a *d*-orbital of the metal centre (HOMO) with the π-antibonding orbital of the olefin (LUMO) (Figure 1.4).[17]

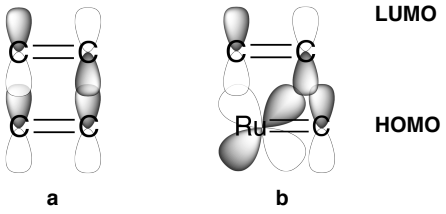
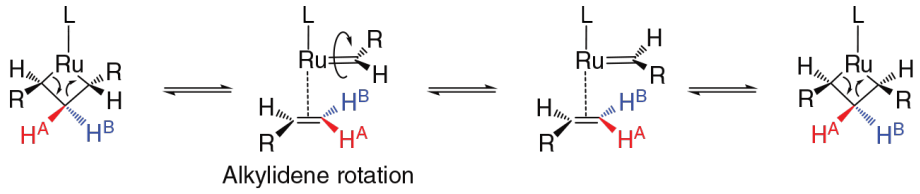


Figure 1.4. [2+2] interactions. **a**: symmetry forbidden interaction; **b**: symmetry allowed interaction.

1.4.2 E:Z selectivity

Cross metathesis is thermodynamically controlled which means that the reaction will continue, as long as the catalyst is active, and eventually equilibrium will be reached. Thus, E-olefins are expected to be the major product since they are the most thermodynamically stable ones.

Although the E-olefins are expected to be the major product, the stereochemistry of the initially formed product is determined by the interactions of the olefin substituents and the permanent ligands of the metal centre in the metallocyclobutane intermediate. In the ring formed, 1,2-and 1,3-interactions play an important role being the first the most destabilising ones (Scheme 1.7).[18]



Scheme 1.7. Intermediate equilibration through 1,3 intractions.

1.4.3 Cross-product selectivity

Cross metathesis reaction first appeared not to be attractive for synthetic organic chemists due to the lack of predictability in product selectivity. The metathesis mechanism allows a product distribution regarding the relative energies of the olefins formed once the equilibrium has been reached. Despite this fact, the activation barriers for electronically deactivated and sterically

hindered alkenes are high and thus diminish its reactivity. The consequence is that if a product is kinetically trapped in the cycle will not undergo secondary metathesis.[19]

In 2007 Grubbs and coworkers published a general model to predict the formation of the major product of a cross metathesis reaction and, thus, overcome the selectivity issue.[20]

The model is based on the classification of olefins in four different families according to its reactivity. This reactivity is measured as the ability of the molecules to undergo homodimerization relative to other olefins. Comparing their propensity to homodimerize, and the subsequent reactivity of the homodimers, olefins fall into different classes (Table 1.1). It must be said that the classification depends on the type of catalyst because its activity affects the homodimerization ability of the substrates.

Table 1.1. Olefin categories for selective cross metathesis with Grubbs 2nd generation catalyst.

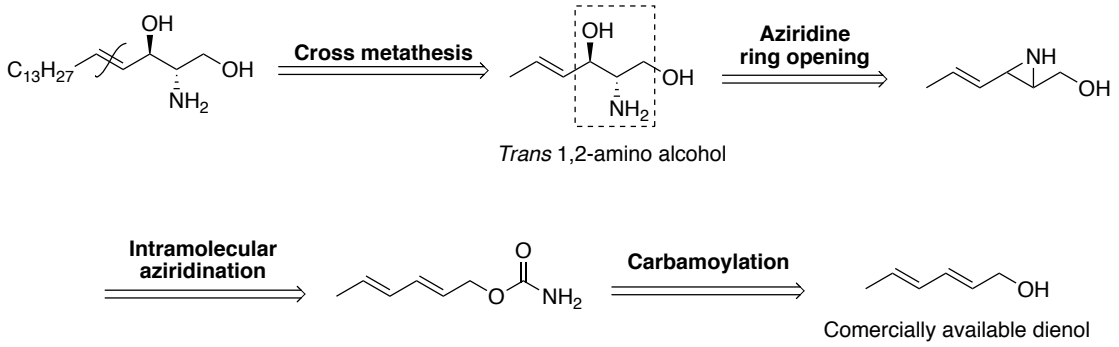
Olefin type	Homodimerization ability	Homodimer activity	Examples
Type I	Rapid homodimerization	Homodimers consumable	Terminal olefins, 1º allylic alcohols, allyl halides, styrenes, protected allyl amines
Type II	Slow homodimerization	Homodimers sparingly consumable	Acrylates, vinyl ketones, unprotected 3º allylic alcohols, 2º allylic alcohols.
Type II	No homodimerization	-	1,1- disubstituted olefins, non-bulky trisub. olefins, protected 3º allylic alcohols.
Type IV	Olefins inert to C.M. but do not deactivate the catalyst	-	Vinyl nitro olefins

Having this classification in hand, olefins of the same class are expected to give near-statistical mixture of all the possible products and, thus, the selectivity is controlled with the excess of equivalents of one olefin in relation to the other. Furthermore, selective cross metathesis can be achieved with olefins of different type.[21]

2. Aims and objectives

One of the main research lines in the group, where I have carried out my final chemistry degree project (T.F.G.), is focused on the development of new synthetic methodologies targeting sphingosine analogues as possible sphingosine kinase inhibitors (SKI). The molecules synthetized are further submitted to biological tests to find whether they are suitable drugs for cancer treatment.

Among this research line, it has been studied a synthesis of sphingosine analogues based on the intramolecular aziridination of dienyl carmabates followed by regioselective opening and cross-metathesis reaction (Scheme 2.1).



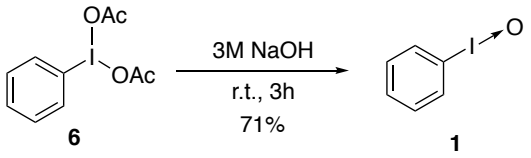
Scheme 2.1. Retrosynthetic strategy for the synthesis of sphingosine developed in the research group.

However, preliminary experiments in the cross metathesis step afforded low yields. Regarding this problematic, the main objective of the present project is to reproduce the initial steps of the synthesis and optimize the cross metathesis reaction with the aim of developing an optimal procedure for the incorporation of the side chain of the molecule.

3. Results and discussion

3.1 Synthesis of starting materials

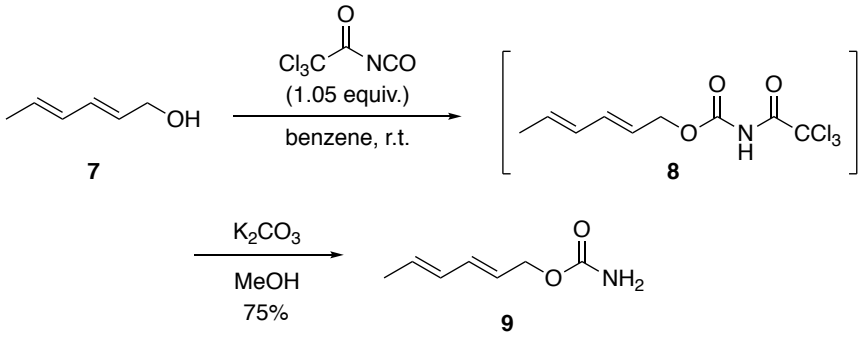
Synthesis of PhIO. Hypervalent iodine reagent, PhIO **1** (iodosobenzene), was readily synthetized in 71% yield from commercially available iodosobenzene diacetate **6** upon treatment with aqueous NaOH solution (Scheme 3.1).



Scheme 3.1. Preparation of iodosobenzene **1** from commercially available iodosobenzene diacetate **6.**

3.2 Synthesis of dienyl carbamate

Dienyl carbamate **9** was obtained in 75% yield upon treatment of dienol **7** with trichloroacetyl isocyanate (TAI), to afford intermediate **8**, and subsequent methanolysis with sodium carbonate in methanol (Scheme 3.2).

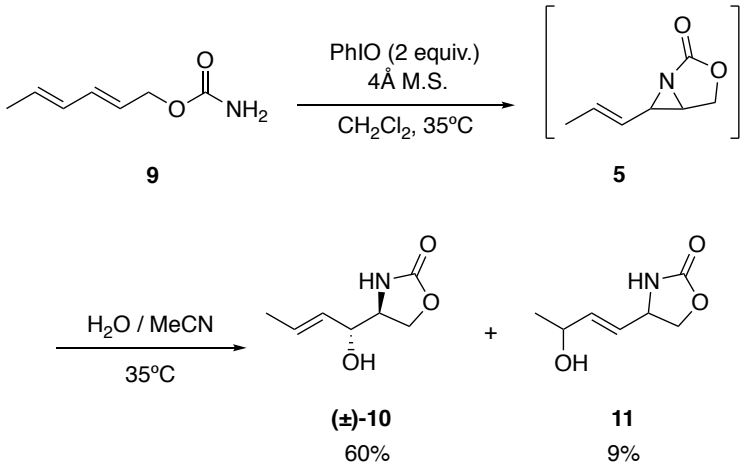


Scheme 3.2. Preparation of carbamate **9** from commercially available dienol **7**.

3.3 *One pot* intramolecular aziridination and regioselective ring opening

Oxazolidinone **(±)-10** was obtained in a 60% yield by treatment of carbamate **9** with iodosobenzene (PhIO) in the presence of 4Å molecular sieves followed by nucleophilic ring opening of the aziridine intermediate **5** with water.

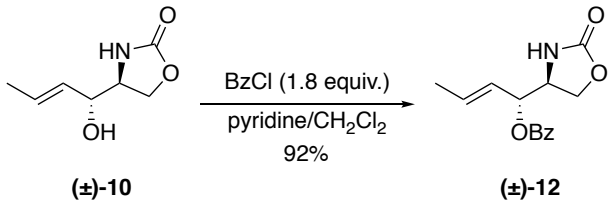
The reaction takes place by initial aziridination of the double bond neighbouring to the carbamate group to generate the bicyclic aziridine **5**, and concomitant aziridine opening by attack of water. This opening reation produces compounds **(±)-10** (major) and **11** (minor) as a result of S_N2 and S_N2', respectively.



Scheme 3.3. Tandem aziridination ring opening of carbamate **9**.

3.4 Alcohol protection

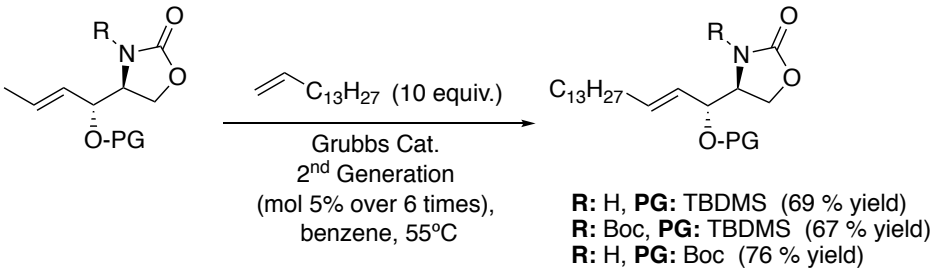
Next, the free hydroxyl group in **(±)-10** was protected by reaction with benzoyl chloride in a mixture of pyridine and dichloromethane to afford compound **(±)-12** in 92% yield.



Scheme 3.4. Benzoylation reaction of alcohol **(±)-10**.

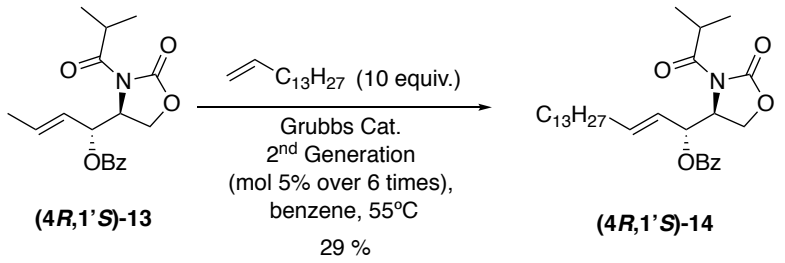
3.5 Cross metathesis: reaction optimisation.

Regarding our retrosynthetic analysis (Scheme 2.1), chain elongation was tackled via a cross metathesis reaction. According to a reported work by Katsumara and coworkers, similar substrates to oxazolidinone **(±)-12** underwent cross metathesis with 1-pentadecene with good yields.[22]



Scheme 3.5. Reported cross metathesis by Katsumara and coworkers.

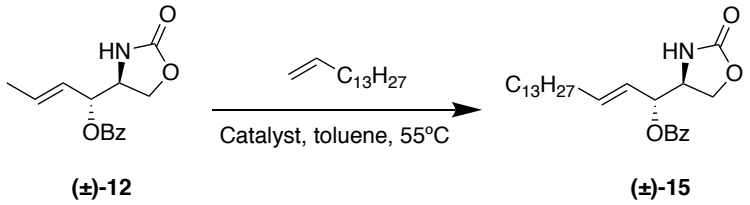
Previous work within the group involved the study of the cross metathesis of (**4*R*,1'*S*)-13** for the preparation of **(4*R*,1'*S*)-14** under reported Katsumara conditions. However, optimisation of reaction conditions needed to be done in order to improve final yields.



Scheme 3.6. Cross metathesis of compound under the reported conditions.[22]

Optimisation of the reaction conditions was carried out by an initial screening of cross metathesis catalysts, different addition methods and reaction times, applying the general conditions reported by Katsumara and coworkers, but changing the solvent (toluene instead of benzene because safety concerns). Yields were calculated by isolation of **(±)-15** using column chromatography

Table 3.1. Cross metathesis of **(±)-12**. Catalyst, addition mode and reaction time screening.



Entry	Catalyst (mol %)	Method[b]	Time (h)	Yield (%)	E:Z (%)
1	**G-I** (5)	A	24	No reaction	-
2	**G-I** (5)	B	24	No reaction	-
3	**G-II** (5)	A	24	46	68:32
4	**G-II** (5)	B	24	44	69:31
5	**HG-II** (5)	A	24	41	83:17
6	**HG-II** (5)	B	24	51	83:17
7	**G-II** (5)	A	72	60	83:17
8	**HG-II** (5)	A	72	46	85:15

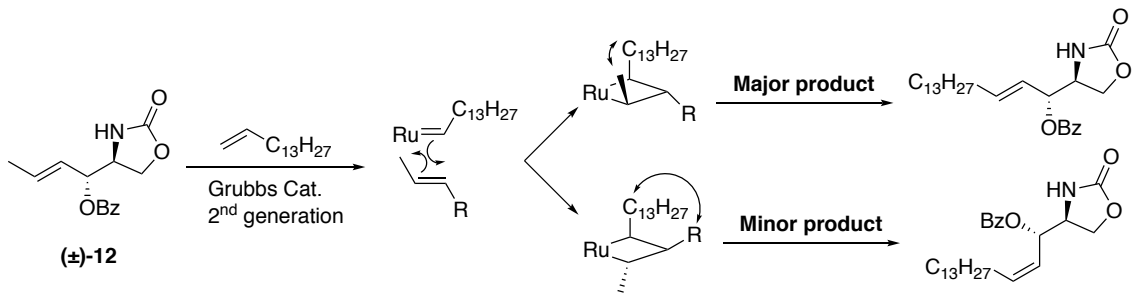
[a] **General conditions:** Oxazolidinone **(±)-12** (1 equiv.), 1-pentadecene (10 equiv.), PhMe (0.05M), 55ºC.
[b] **Method A**: catalyst was added in a single batch; **Method B**: catalyst was added over 5 times in intervals of 1 hour.

Exposure of the starting material oxazolidinone **(±)-12** to 1st generation Grubbs catalyst did not show any conversion presumably because its low catalytic activity (Table 3.1, entries 1 and 2). This is in agreement with the reported results in the literature with similar subsrates.[22,23]

The use of 2nd generation Grubbs catalyst and 2nd generation Hoveyda-Grubbs catalyst resulted in moderate yields with similar values (Table 3.1, entries 3 to 6). Interestingly, no significant difference was found between the single batch and the fractional addition.

In order to decide which of the previous catalyst was the most adequate for the present cross-metathesis reaction, two more experiments were performed with methodology A under longer reaction time (Table 3.1, entries 7 and 8). Interestingly, 2nd generation Grubbs catalyst proved to afford the best results.

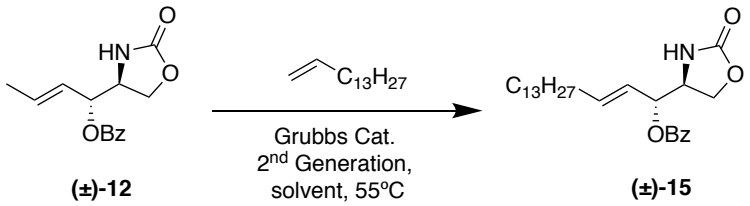
Regarding the *E:Z* selectivity, it does not seem to be affected by the catalyst addition methodology (Table 3.1, entries 3 and 4 or 5 and 6); so, for practical reasons, method A was selected for future experiments. However, it is interesting to stress that 2nd generation Hoveyda-Grubbs catalyst does provide a better *E:Z* selectivity than 2nd generation Grubbs catalyst in 24h reactions (Table 3.1, entries 3 and 5). On the other hand, when the reaction is stirred for 72 hours the selectivity is similar for both catalysts (Table 3.1, entries 7 and 8), and higher than in the previous case (Table 3.1, entries 3 and 5). This observation is consistent with the fact that cross metathesis reaction is ruled by thermodynamics, longer reaction times will allow products equilibration rendering the more stable product as it was expected (Scheme 3.7). Taking into account this information, 72 hours is chosen as optimal reaction time.



Scheme 3.7. 1,3 and 1,2 interactions on the cyclobutallocene intermediate of the model substrate.

Once the type of catalyst, the addition mode and the reaction time had been optimised, a solvent screening was performed.

Table 3.2. Cross metathesis of **(±)-12**. Solvent screening.



Entry	Solvent (M)	Temperature (ºC)	Yield (%)	E:Z
1	Perfluorotoluene (0.05)	55	48	84:16
2	Dichloromethane (0.05)	55	71	85:15
3	Toluene (0.05)	100	Complex mixture	-

[a] **General conditions:** Oxazolidinone **(±)-12** (1 equiv.), 1-pentadecene (10 equiv.), Grubbs 2nd generation catalyst (mol 5%, Method A: catalyst was added in a single batch), reaction time: 72h.

Fluorinated aromatic solvents have demonstrated to be an alternative medium for promoting challenging olefin metathesis reactions.[24,25] It is believed that fluorinated aromatic hydrocarbons (FAH) interact electronically with the *N*-mesityl groups at the cross metathesis catalysts. These π-π stacking[26] interactions favour an enhancement on the catalyst activity.

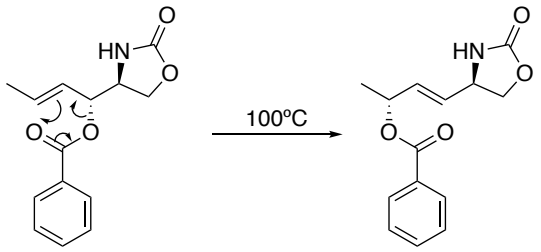
Bearing in mind the mentioned effects of FAH, perfluorotoluene was tested as an alternative solvent (Table 3.2, entry 1). Disappointingly, any improvement in final yield was found comparing with initial experiments in toluene (table 3.2 entry 7). These results could be justified due to poor solubility of oxazolidinone **(±)-12** in this reaction media.

On the other hand, dichloromethane is also a common solvent for cross metathesis reactions.[27] To our delight, when the reaction was carried out in this solvent yields rised up to 71%, being the highest yield achieved among all the tests (Table 3.2, entry 2).

Moreover, *E/Z* selectivity remained unaltered independently of the solvent of choice, supporting the hypothesis of the importance of products equilibration through longer reaction times (Table 3.2, entries 1 and 2).

Finally, a preliminary attempt of using higher temperature for the cross metathesis reaction proved to be unsuccessful, yielding a complex mixture of products. ^{1}H NMR of the reaction crude showed new aromatic signals along with olefinic protons and deshielded methyl signals. A Claisen [3,3]-

sigmatropic rearrangement was proposed as a plausible explanation for byproduct formation (Scheme 3.8).



Scheme 3.8. Plausible reaction mechanism to the formation of byrpoducts in Table 3.2, entry 3.

After former optimisation work, extensive analysis of the different column chromatography fractions NMR spectra enlightened the presence of an unidentified compound. Further purification allowed us to confirm the formation of compound **(±)-16** under reaction conditions (See ^{1}H NMR spectrum in Figure 3.1; selected spectroscopic data in Table 3.3).

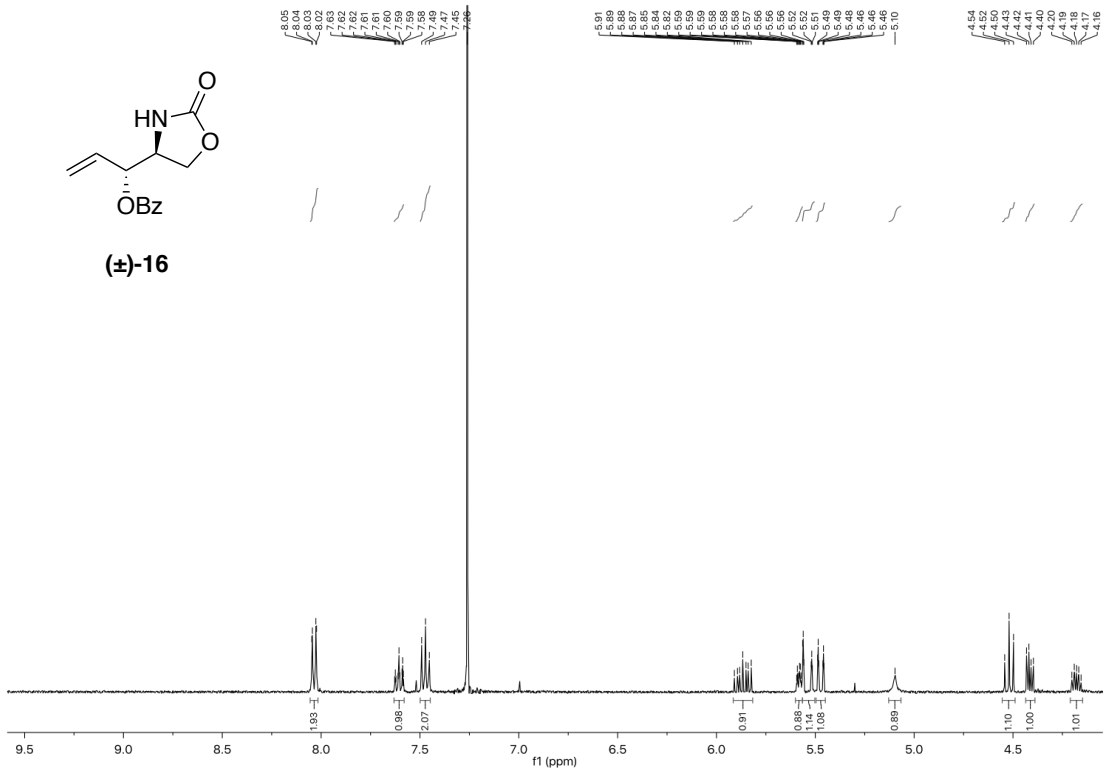


Figure 3.1. ^{1}H NMR spectrum of the minor compound **(±)-16** isolated in the cross metathesis reaction.

The ^{1}H-NMR spectrum showed a very similar signal profile as the starting material except for the double bond region and the lack of the methyl signal at 0.86 ppm. A more detailed study at the vinylic region revealed the signals listed in Table 3.3.

Table 3.3. Spectral NMR data from the vinylic region of the unidentified compound.

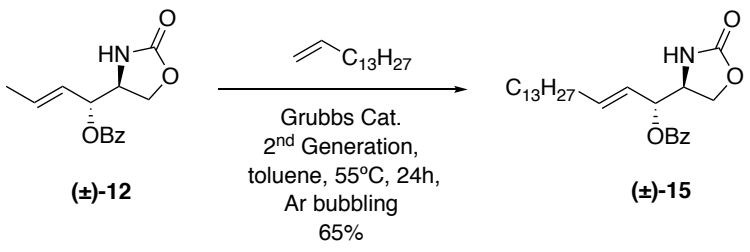
Entry	δ (ppm)	Integration	Multiplicity	Coupling constants (Hz)
1	5.47	1	dt	1.2, 17.2
2	5.54	1	dt	1.2, 10.6
3	5.56	1	ddt	1.2, 4.7, 5.8
4	5.87	1	ddd	6.0, 10.6, 17.1

[a] ^{1}H-NMR data (400 MHz, $CDCl_3$).

One of the most characteristic signals encountered in the ^{1}H NMR of the minor product is the one at 5.87 ppm (Table 3.3, entry 4). The multiplicity exhibited by this signal and the coupling constant values (6.0 Hz, 10.6 Hz and 17.1 Hz, the last two characteristics coupling constants of a double bond proton *cis* and *trans* coupled), together with the two signals at approximately 5 ppm corresponding to double bond protons, are indicative of the presence of a terminal double bond. Moreover, the rest of the signals do match with those of the starting material (Table 3.3, entries 1 to 3). In addition, the proposed structure for oxazolidinone **(±)-16** was confirmed by mass spectrometry (ESI-TOF).

Finally, it was found that oxazolidinone **(±)-16** was present in all the purified fractions of cross metathesis, ranging from 15% to 25 % yield. Regarding the fact that all reactions were performed in schlenk sealed tubes and the homocoupling of 1-pentadecene releases ethylene, our first hypothesis was that the starting material oxazolidinone **(±)-12** could react with the ethylene present in the media to form **(±)-16**.

So as to verify our prediction, a reaction was carried out under optimized reaction conditions in a round bottom flask with argon stream and fitted with an opened reflux condenser (Table 3.1, entry 4). This setting was chosen to ensure complete elimination of ethylene from the reaction media.



Scheme 3.9. Cross coupling reaction under ethylene removal conditions.

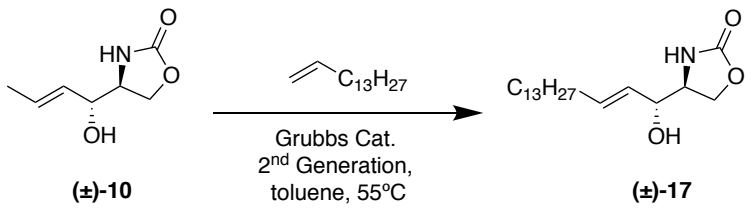
Unexpectedly, after flash column chromatography, former intermediate **(±)-16** was also detected within the starting material in 13% yield. It is important to point out that both reaction yield and

E/Z relationship were higher than the the corresponding to the closed system (Table 3.1, entry 4) and also exhibited a better *E:Z* relationship. Therefore, the first hypothesis appears to be invalid.

A deeper insight into the cross-metathesis mechanism showed that the formation of product **(±)-16** can only be explained by the reaction of the starting material oxazolidinone **(±)-12**, or the desired product protected sphingosine **(±)-15**, with a $Ru{=}CH_2$ complex. Taking into account this information, a new hypothesis was proposed: the by-product **(±)-16** could be formed as result of the large excess of 1-pentadecene (10 equiv.). Having such excess of side chain in the reaction media can be unproductive since the catalytic cycle will be dominated by the reaction of 1-pentadecene to form its homocoupling product. This saturation of the catalytic cycle leads to the formation of important amounts of $Ru{=}CH_2$ intermediates that can react with the starting material, or the product, to form the by-product **(±)-16**. Regarding Grubbs model of selectivity, this information is supported by the fact that 1-pentadecene is a Type I olefin and the oxazolidinone **(±)-12** is, presumably, a type III (since homodimerization has not been observed), so the catalytic cycle will be dominated by the most reactive species.

Performing the reaction with a lesser excess of 1-pentadecene could reduce the amount of homocoupling formed avoiding the saturation with $Ru{=}CH_2$ species. Unfortunately, due to time constrains further exploration on this matter is still pending.

Owing the fact that Katsumara and coworkers tried to perform cross metathesis with substrates having unprotected hydroxyl groups, we decided to carry out a control experiment with oxazolidinone **(±)-10** in order to explore the reaction scope.[22]

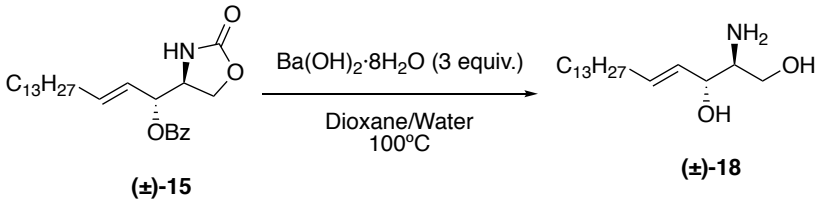


(±)-10 **(±)-17**

Scheme 3.10. Cross coupling reaction with a free hydroxyl grop. **General conditions:** Oxazolidinone **(±)-10** (1 equiv.), 1-pentadecene (10 equiv.), Grubbs 2nd generation catalyst (mol 5%, Method A: catalyst was added in a single batch), reaction time: 72h.

Disappointingly, only a 20% yield was obtained. This result may be explained thorough the formation of ruthenium inactive chelates with Lewis-basic sites at the substrate, in this case, the alcohol moiety. A possible solution, the use of Lewis-acids, such as $Ti(O^iPr)_4$, may decrease the amount of Ru-substrate chelates and, thus, increase the amount of desired product.[23]

3.6 Final deprotection



Scheme 3.11. Deprotection of **(±)-15** to yield racemic sphingosine **(±)-18**.

Oxazolidinone **(±)-15** was heated to 100ºC in a mixture of dioxane and water in presence of $Ba(OH)_2 \cdot 8H_2O$ so as to perform the basic hydrolysis of the oxazilinone and benzoate moieties. Unfortunately, final isolation of racemic sphingosine after deprotection could not be achieved due to purification difficulties.

4. Conclusions and future work

We have optimized the cross metathesis key step for the proposed synthesis of sphingosine. The new conditions enable to couple the side chain to the protected polar head of the sphingosine in a 71% yield which is considered good for the type of substrate.

Further research is still needed in order to fully understand how the intermediate **(±)-16** is formed and, thus, find a way of improving the reaction yield.

Future work will involve the application of the optimised cross metathesis conditions for the introduction of different side chains to render a variety of sphingosine analogues as potential SK1 inhibitors.

5. Experimental section

5.1 General methods

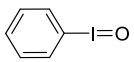
All chemicals used were reagent grade and used as supplied unless otherwise specified. HPLC grade dichloromethane, 1,2-dichloroethane, benzene and chloroform were purified using standard procedures.[28]

^{1}H and ^{13}C NMR spectra were recorded on a Varian® Mercury VX 400 (400 MHz and 100.6 MHz respectively) or Varian 400-MR spectrometer in $CDCl_3$ as solvent, with chemical shifts (δ) referenced to internal standards $CDCl_3$ (7.26 ppm ^{1}H, 77.16 ppm ^{13}C) or Me_4Si as an internal reference (0.00 ppm). 2D correlation spectra (gCOSY, gHSQC, gHMBC) were visualized using VNMR program (Varian®). ESI MS were run on an Agilent® 1100 Series LC/MSD instrument. IR spectra were recorded on a JASCO FT/IR-600 plus Fourier Transform Infrared Spectrometer ATR Specac Golden Gate.

Reactions were monitored by TLC carried out on 0.25 mm E. Merck® silica gel 60 F_{254} glass or aluminium plates. Developed TLC plates were visualized under a short-wave UV lamp (254 nm) and by heating plates that were dipped in a solution of p-anisaldehyde in ethanol/H_2SO_4/AcOH (90:3:1). Flash column chromatography (FCC) was performed using flash silica gel (32–63 μm) and using a solvent polarity correlated with TLC mobility.

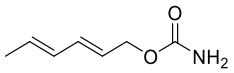
5.2 Compound characterisation

Iodosobenzene (PhIO) (1).



A sodium hydroxide solution (3*N*, 20 mL) was added over a 10-minutes period to finely ground recrystallised (5*M* AcOH) iodosobenzene diacetate (4.0 g, 12.4 mmol) in a 100 mL beaker under vigorous stirring. The reaction mixture was left at room temperature until completion (c.a. 45 minutes); then, H_2O (15 mL) was added and the crude, solid iodosobenzene, was filtered on a Büchner funnel. The wet solid was returned to the beaker, triturated with H_2O (25 mL), collected again on a Büchner funnel, washed with water and dried overnight under vacuum. Final purification was effected by triturating the dried solid with chloroform (10 mL) in a beaker, separating it by filtration and drying under vacuum to afford 1.9 g (71% yield) of iodosobenzene 11 as a yellow powder.[29]

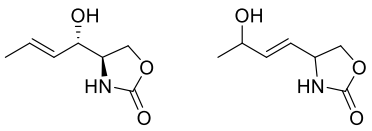
(2*E*,4*E*)-Hexa-2,4-dien-1-yl carbamate (2).



The title compound was synthesized following the general carbamoylation procedure starting from commercially available (2*E*,4*E*)-hexa-2,4-dien-1-ol (1.65 mL, 15.0 mmol) and TAI (1.8 mL, 15.75 mmol). The crude was purified by column chromatography (AcOEt/hexanes from 30:70 to 50:50). Compound 11 was recrystallised by slow diffusion of pentane into a solution of the carbamate in THF to afford 1.6 g (75% yield) of pure carbamate 2 as a white powder.

R_f = 0.20 (30:70 AcOEt/Hexanes). Mp = 93-95 ºC. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 6.24 (dd, *J* = 15.2, 10.5 Hz, 1H, H-3), 6.04 (ddd, *J* = 14.2, 10.5, 1.3 Hz, 1H, H-4), 5.74 (dq, *J* = 14.2, 6.7 Hz, 1H, H-5), 5.62 (dt, *J* = 15.2, 6.6 Hz, 1H, H-2), 4.85 (brs, 2H, NH_2), 4.55 (d, *J* = 6.6 Hz, 2H, H-1), 1.75 (dd, *J* = 6.7, 1.3 Hz, 3H, H-6).**^{13}C NMR** (100 MHz, $CDCl_3$): δ = 157.0 (C=O), 134.7 (C-3), 131.3 (C-5), 130.5 (C-4), 124.1 (C-2), 65.7 (C-1), 18.3 (C-6). **ESI-TOF** $[M+Na]^+$ calc for $C_7H_{11}NNaO_2$: 164.0682, found: 164.0682. **FT-IR (ATR)** ν in cm^{-1}: 3426, 3296, 3213, 2911, 2851, 1652, 1616, 1429, 1344, 1314, 1108, 1054, 990.

***u*-4-[(*E*)-1-Hydroxybut-2-en-1-yl]oxazolidin-2-one ((±)-10) and 4-[(*E*)-3-Hydroxybut-1-en-1-yl]oxazolidin-2-one (11).**



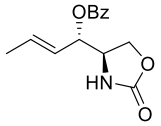
The title compounds were synthesized following PhIO mediated aziridination/ring-opening procedure starting from carbamate 11 (70.6 mg, 0.5 mmol), PhIO (220.0 mg, 1 mmol) and using a mixture of 50 drops of H_2O in MeCN (7.5 mL) as nucleophile to afford a mixture of compounds (±)-10 and 11. The crude solution was filtered over celite, washed with MeOH and concentrated under reduced pressure. NMR-yield: 81% (ratio (±)-10:11 = 86:14). The crude was purified by column chromatography (80:20 AcOEt/hexanes to AcOEt) to afford 564.6 mg (60% yield) of oxazolidinone (±)-10 as a white solid and regioisomer 11 as a colourless oil.

Compound (±)-10: R_f = 0.26 (AcOEt). Mp = 110-115 ºC. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 6.03 (brs, 1H, NH/OH), 5.85 (dq, *J* = 15.3, 6.5 Hz, 1H, H-3'), 5.40 (ddq, *J* = 15.3, 7.0, 1.6 Hz, 1H, H-2'), 4.40 (t, *J* = 8.8 Hz, 1H, $H\text{-}5_a$), 4.33 (dd, *J* = 8.9, 5.0 Hz, 1H, $H\text{-}5_b$), 4.13-4.09 (m, 1H, H-1'), 3.88-3.83 (m, 1H, H-4), 2.88 (brs, 1H, NH/OH), 1.73 (dd, *J* = 6.5, 1.6 Hz, 3H, H-4'). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 160.5 (C=O), 131.4 (C-3'), 128.0 (C-2'), 73.3 (C-1'), 66.5 (C-5), 56.4 (C-

4), 18.1 (C-4'). **ESI-TOF** $[M+Na]^+$ calc for $C_7H_{11}NNaO_3$: 180.0631, found: 180.0627. **FT-IR (ATR)** ν in cm^{-1}: 3389, 2918, 1781, 1716, 1478, 1418, 1233, 1089, 1020, 966.

Compound 11: $\mathbf{R_f}$ = 0.13 (AcOEt). **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 5.83 (ddd, *J* = 15.4, 5.5, 1.2 Hz, 1H, H-2'), 5.68 (ddd, *J* = 15.4, 7.6, 2.1 Hz, 1H, H-1'), 5.11 (brs, 1H, NH/OH), 4.54 (td, *J* = 8.5, 2.1 Hz, 1H, H-5_a), 4.43-4.33 (m, 2H, H-4, H-3'), 4.07 (ddd, *J* = 8.5, 6.7, 4.0 Hz, 1H, H-5_b), 1.29 (d, *J* = 6.5 Hz, 3H, H-4'). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 159.3 (C=O), 138.9 (C-2'), 127.0 (C-1'), 70.2 (C-5), 67.7 (C-3'), 54.5 (C-4), 23.5 (C-4'). **ESI-TOF** $[M+Na]^+$ calc for $C_7H_{11}NNaO_3$: 180.0631, found: 180.0628. **FT-IR (ATR)** ν in cm^{-1}: 3296, 2967, 2921, 2852, 1728, 1403, 1237, 1063, 1022, 971.

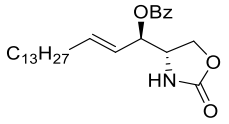
***u*-4-[(*E*)-1-Benzoyloxybut-2-en-1-yl]-oxazolidin-2-one ((±)-12)**



To a solution of alcohol (±)-10 (564.6 mg, 3.6 mmol) in anhydrous pyridine (10.6 ml) and dichloromethane (31.8 ml) was added benzoyl chloride (0.75 ml) at 0ºC. The reaction mixture was then allowed to warm to room temperature, stirred for 3 hours and poured into a saturated aqueous $CuSO_4$ solution. The organic layer was washed several times with the $CuSO_4$ solution. Finally, the combined organic phases were washed with brine and dried over Na_2SO_4. Purification was effected by column chromatography (AcOEt/hexanes from 3:7 to 5:5) to afford 863.5 mg (92% yield) of benzoylated compound (±)-12 as a colourless syrup.

$\mathbf{R_f}$ = 0.20 (50:50 AcOEt/Hexanes). **^{1}H NMR** (400 MHz, $CDCl_3$, δ in ppm): 8.02 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.56 (tt, *J* = 7.5, 1.4 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 6.37 (brs, 1H), 6.02-5.91 (m, 1H), 5.51-5.43 (m, 2H), 4.47 (t, *J* = 8.9 Hz, 1H), 4.34 (dd, *J* = 9.0, 4.8 Hz, 1H), 4.11-4.07 (m, 1H), 1.74 (dd, *J* = 6.6, 1.1 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$, δ in ppm): 165.6 (C=O), 160.1 (C=O), 134.1 (C3'), 133.5 (Ar), 129.8 (Ar), 128.6 (Ar), 128.5 (Ar), 123.5 (C2'), 75.4 (C1'), 66.4 (C5), 54.9 (C4), 18.1 (C4'). **ESI-TOF** $[M+Na]^+$ calc for $C_{14}H_{15}NNaO_4$: 284.0893, found: 284. 0845. **FT-IR (ATR)** ν in cm^{-1}: 3292, 2922, 2853, 1754, 1720, 1267, 1109, 712.

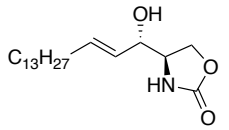
***u*-4-[(*E*)-1-Benzoyloxyhexadeca-2-en-1-yl]-oxazolidin-2-one ((±)-15).**



To a solution of benzoate (±)-12 (28.5 mg, 0.1 mmol) in dry dichlorometane (2.2 mL), liquid 1-pentadecene (0.29 mL, 1.1 mmol) was added. Grubbs catalyst 2nd Generation (4.6 mg, 5 mol%) was then added and the reaction mixture was stirred overnight at 55ºC. Finally the crude mixture was concentrated under reduced pressure and purified by column chromatography (AcOEt/hexanes 4:6 to 7:3) to afford 33.4 mg (71% yield) of oxazolidinone (±)-15 as a white oil.

$\mathbf{R_f}$ = 0.42 (50:50 AcOEt/Hexanes). **^{1}H NMR** (400 MHz, $CDCl_3$, δ in ppm): 8.03 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.54 (tt, *J* = 7.4, 1.4 Hz, 1H), 7.42 (t, *J* = 7.7 Hz, 2H), 6.64 (brs, 1H), 5.93 (dt, *J* = 13.5, 6.8 Hz, 1H), 5.49-5.40 (m, 2H), 4.45 (t, *J* = 8.9 Hz, 1H), 4.33 (dd, *J* = 9.0, 4.5 Hz, 1H), 4.08 (dt, *J* = 8.7, 4.5 Hz, 1H), 2.04 (q, *J* = 6.8 Hz, 2H), 1.36-1.33 (m, 2H), 1.26-1.22 (m, 20H), 0.86 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$, δ in ppm): 165.5 (C=O), 160.0 (C=O), 139.2 (C3'), 133.4 (Ar), 129.8 (Ar), 128.6 (Ar), 122.0 (C2'), 75.3 (C1'), 66.2 (C5), 54.9 (C4), 32.5 (C4'), 32.0, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 28.8, 22.8, (C5'-C15'), 14.2 (C16'). **ESI-TOF** $[M+Na]^+$ calc for $C_{26}H_{39}NNaO_4$: 452.2771, found: 452.2769. **FT-IR (ATR)** ν in cm^{-1}: 2921, 2851, 1755, 1720, 1601, 1451, 1407, 1314, 1264, 1177, 1109, 1069, 1025, 970, 936, 765, 709, 666, 616.

u-4-[(E)-1-hydroxyhexadec-2-en-1-yl]-oxazolidin-2-one((±)-17).



To a solution of alcohol (±)-10 (69.6 mg, 0.44 mmol) in dry toluene (8.9 mL), liquid 1-pentadecene (1.18 mL, 4.4 mmol) was added. Grubbs catalyst 2nd Generation (18.8 mg, 5 mol%) was then added and the reaction mixture was stirred 72h at 55ºC. Finally the crude mixture was concentrated under reduced pressure and purified by column chromatography (DCM/MeOH/NH_4OH 96:4:1) to afford 28.7 mg (20 % yield) of oxazolidinone (±)-17 as a white oil.

$\mathbf{R_f}$ = 0.24 (AcOEt). **Mp** = 76-79 ºC. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 5.93 (brs, 1H, NH), 5.84 (m, 1H, H-3'), 5.37 (ddt, *J* = 15.4, 6.9, 1.4 Hz, 1H, H-2'), 4.40 (t, *J* = 8.8 Hz, 1H, H-5_a), 4.33 (dd, *J* = 8.8, 5.1 Hz, 1H, H-5_b), 4.12 (m, 1H, H-1'), 3.85 (m, 1H, H-4), 2.76 (d, *J* = 3.5 Hz, 1H, OH),

2.04 (q, *J* = 6.9 Hz, 2H, H-4'), 1.40-1.20 (m, 22H, H5'-H15'), 0.87 (t, *J* = 6.9 Hz, 3H, H-16'). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 160.3 (C=O), 136.8 (C3'), 126.59 (C2'), 73.4 (C4), 66.5 (C5), 56.3 (C1'), 32.5 (C4'), 32.1, 29.84, 29.8, 29.8, 29.8, 29.6, 29.5, 29.4, 29.1, 22.8 (C5'-C15'), 14.3 (C16'). **ESI-TOF** $[M+Na]^+$ calc for $C_{19}H_{35}NNaO_3$: 325.2617, found: 325.2628. **FT-IR (ATR)** ν in cm^{-1}: 3385, 2917, 1849, 2358, 1777, 1699, 1474, 1435, 1233, 1092, 1027, 977, 722.

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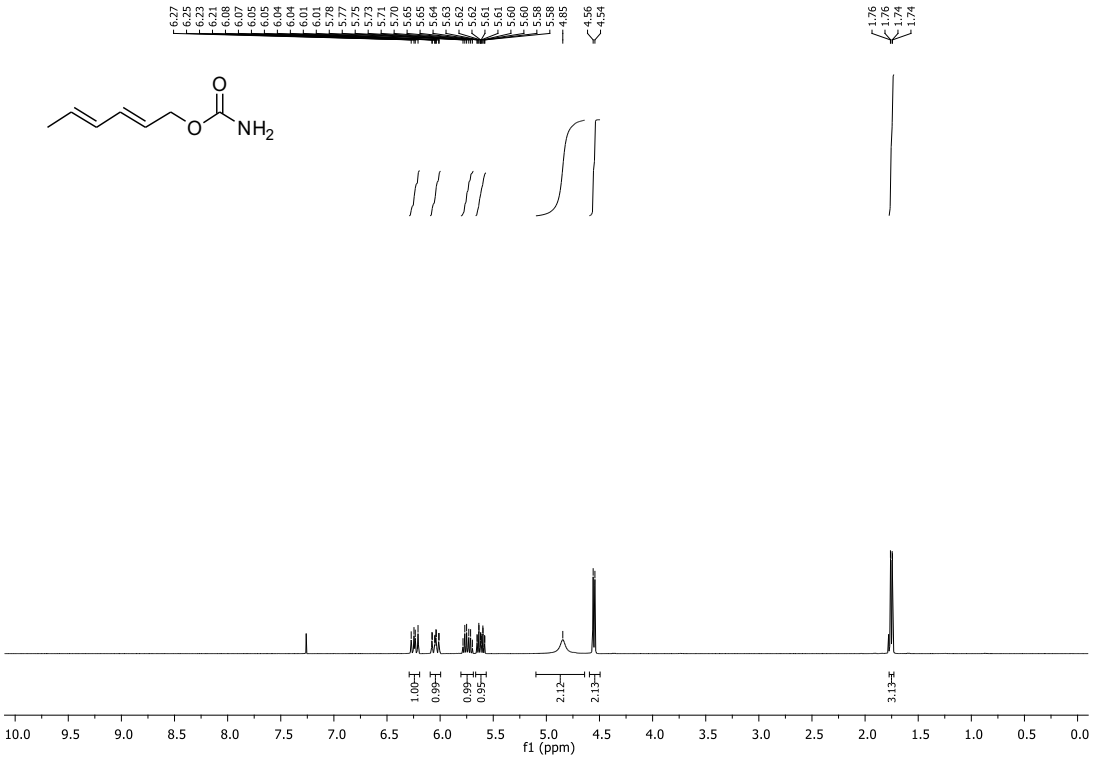
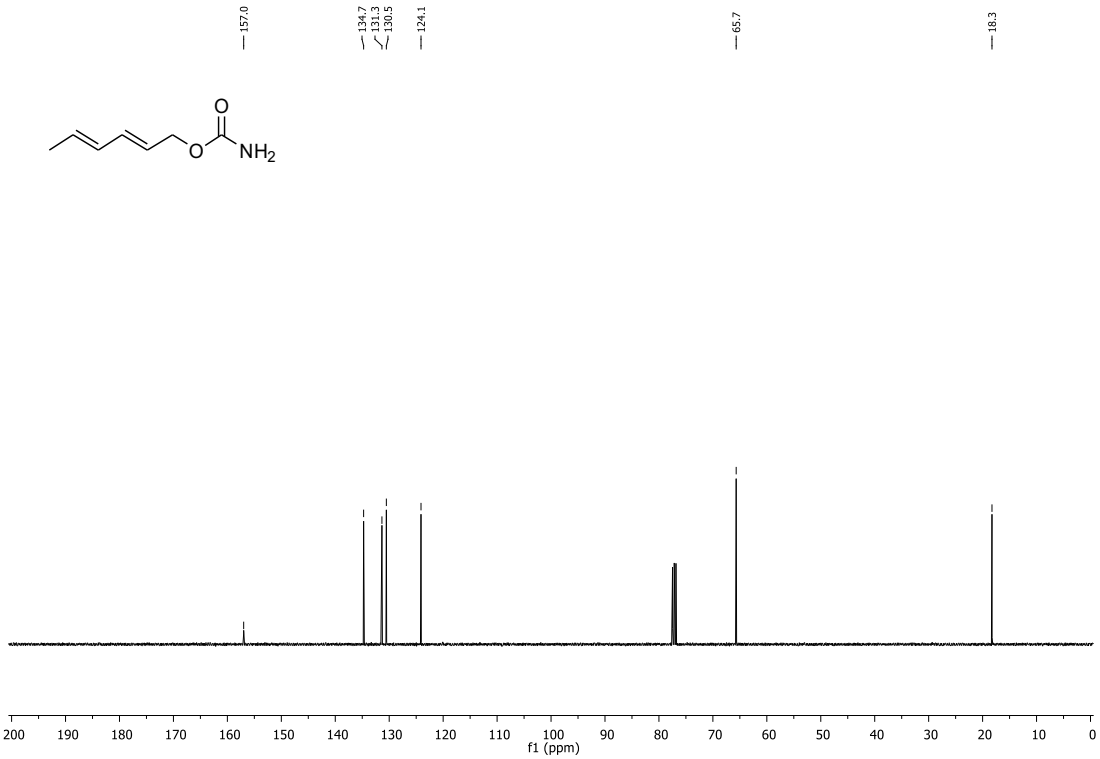
Annex

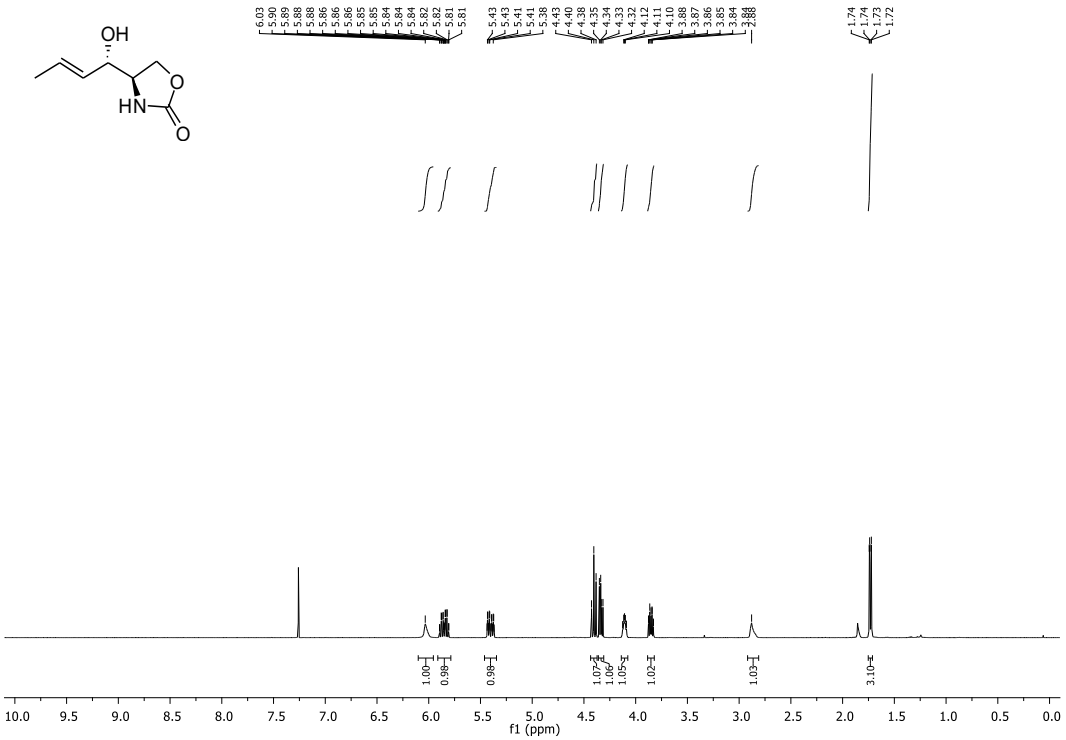
^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm) of (2*E*,4*E*)-hexa-2,4-dien-1-yl carbamate (2).



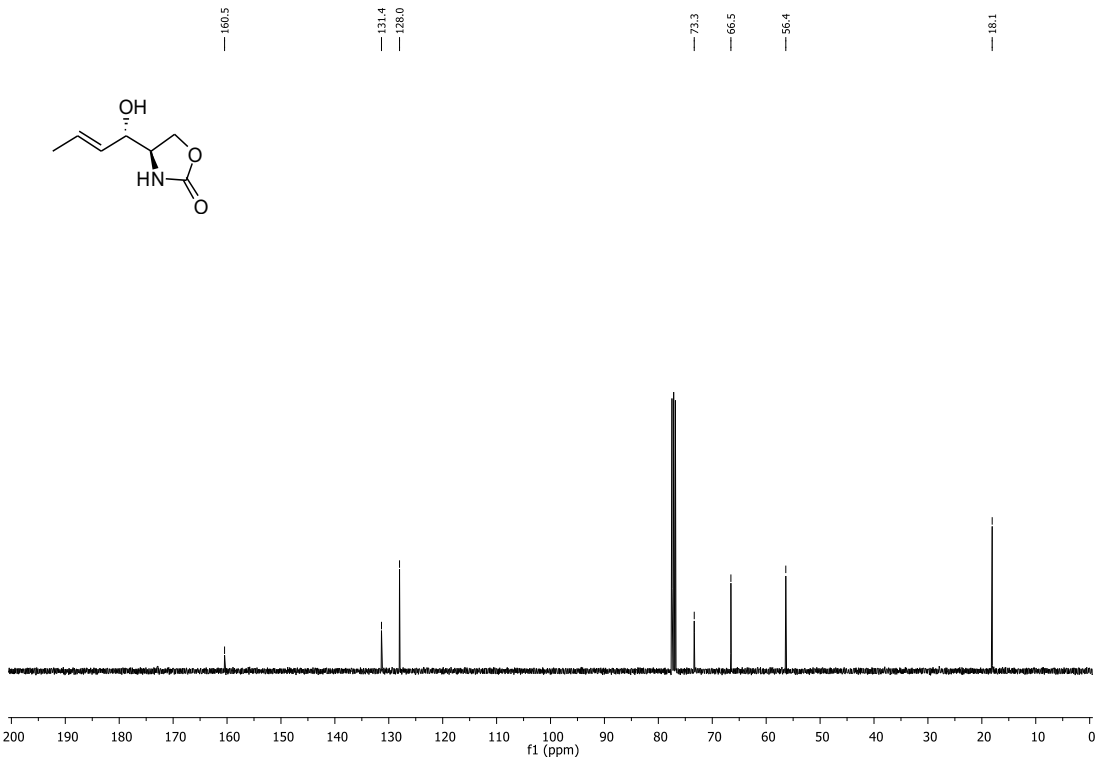
^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm) of (2*E*,4*E*)-hexa-2,4-dien-1-yl carbamate (2).



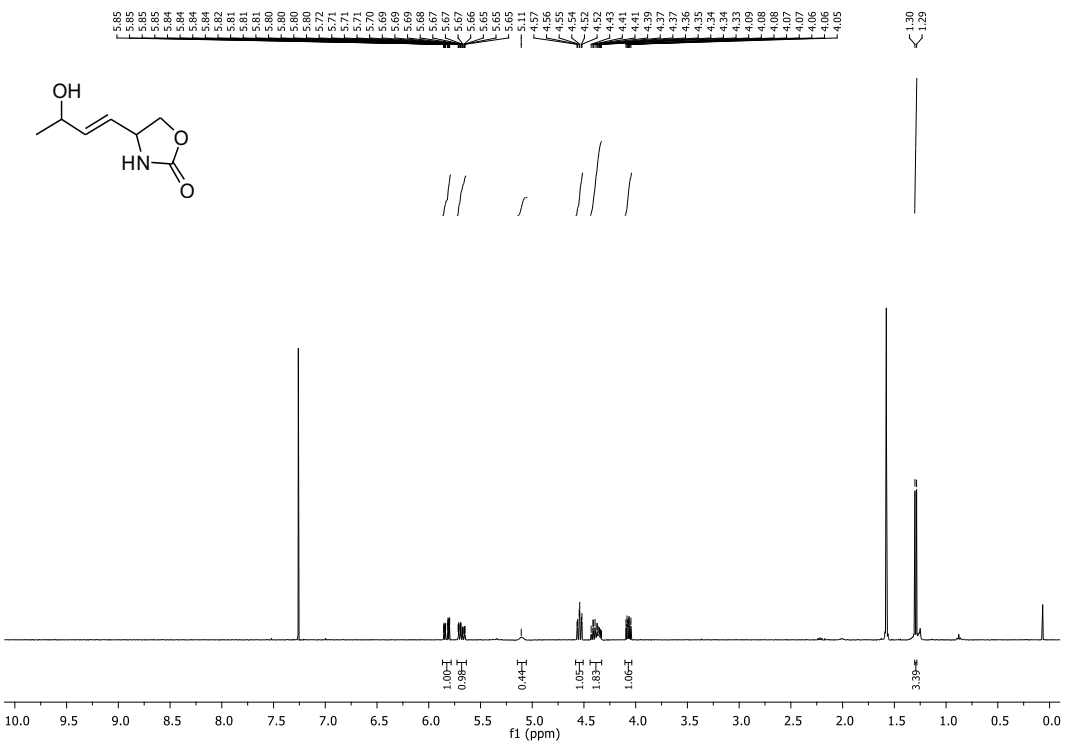
^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm) of *u*-4-[(*E*)-1-hydroxybut-2-en-1-yl]oxazolidin-2-one ((±)-10).



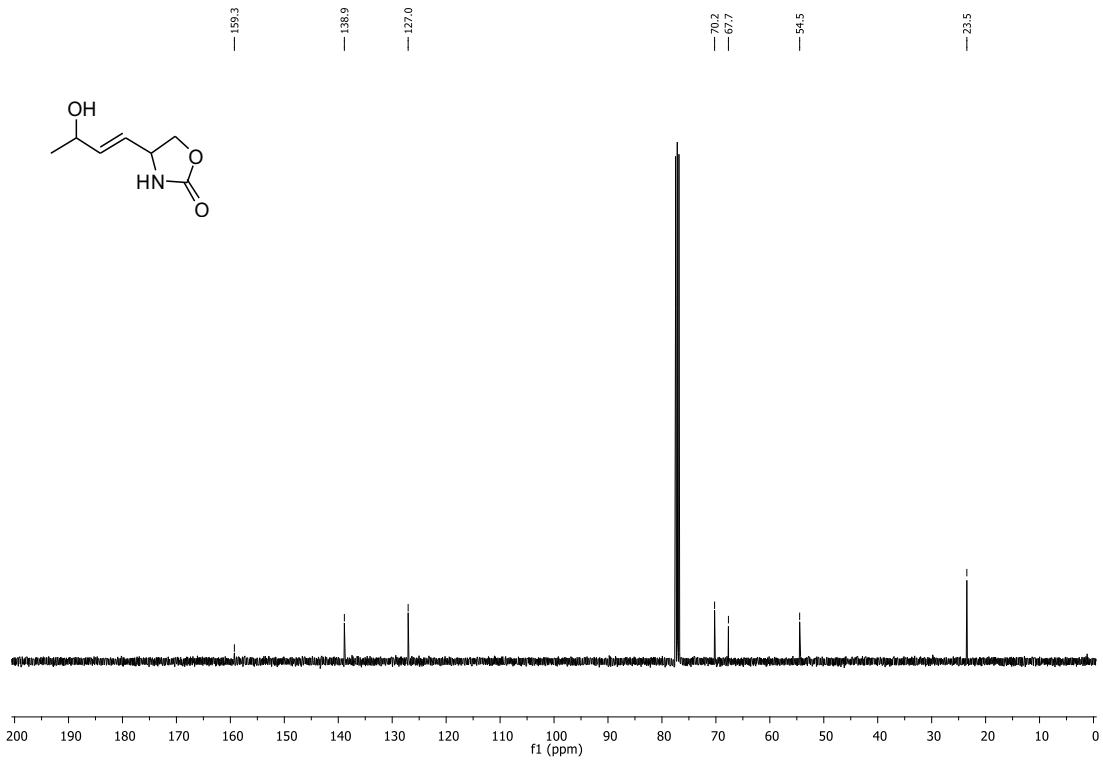
^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm) of *u*-4-[(*E*)-1-hydroxybut-2-en-1-yl]oxazolidin-2-one ((±)-10).



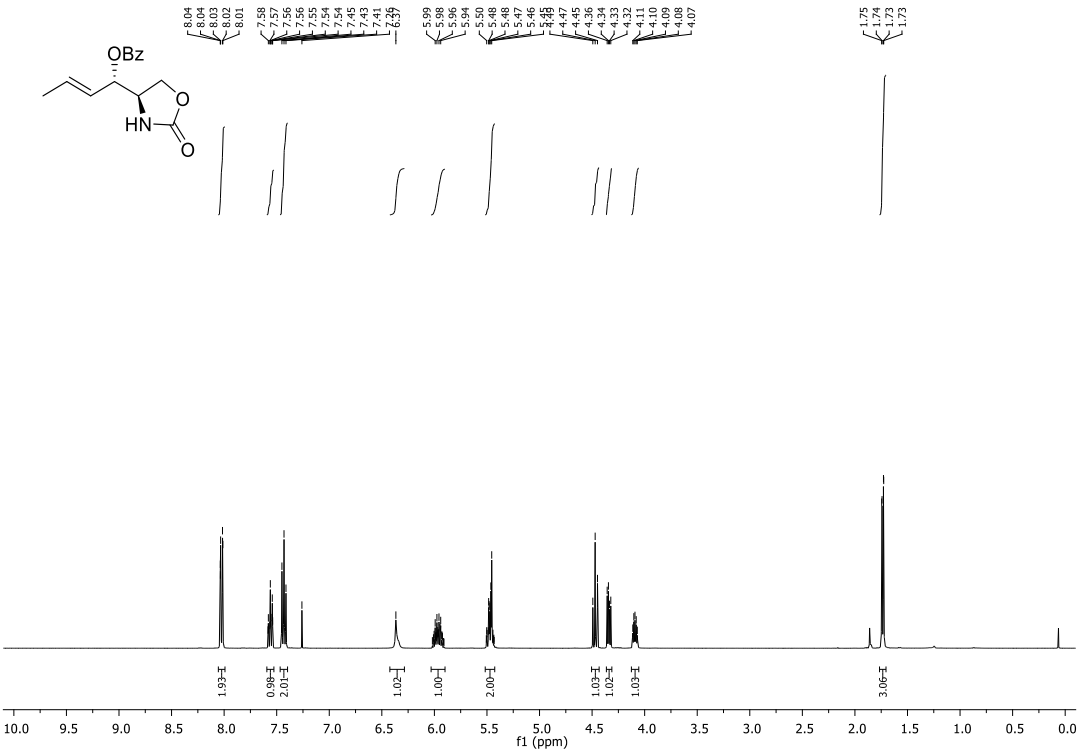
^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm) of 4-[(*E*)-3-hydroxybut-1-en-1-yl]oxazolidin-2-one (11).



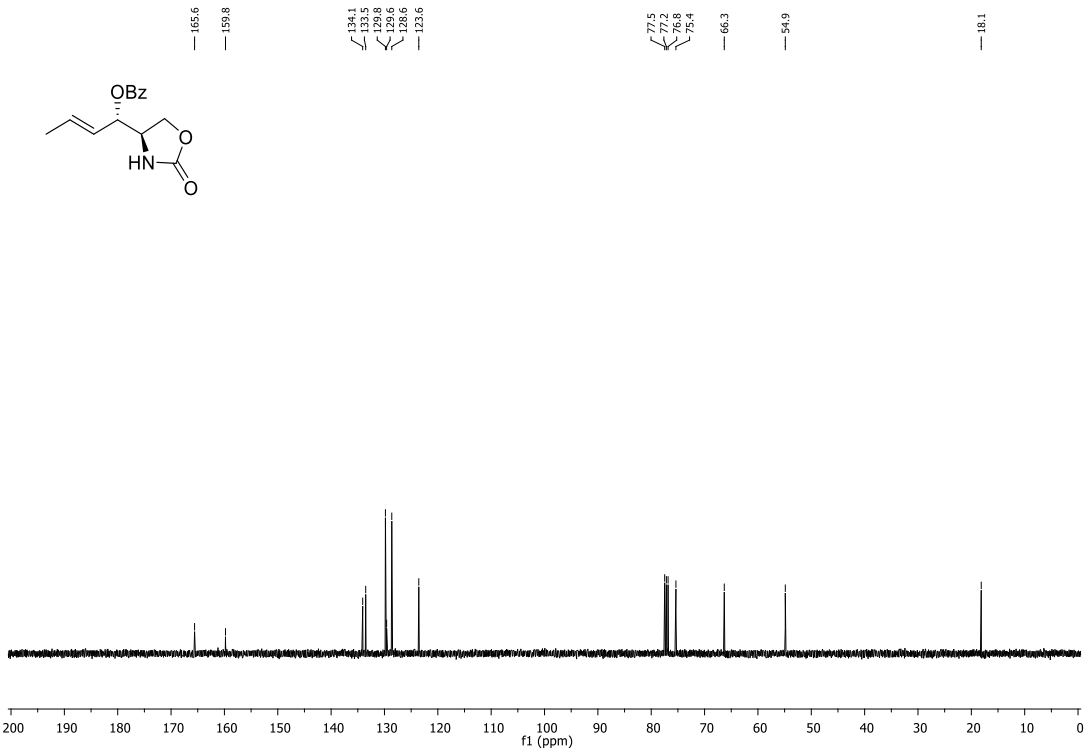
^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm) of 4-[(*E*)-3-hydroxybut-1-en-1-yl]oxazolidin-2-one (11).



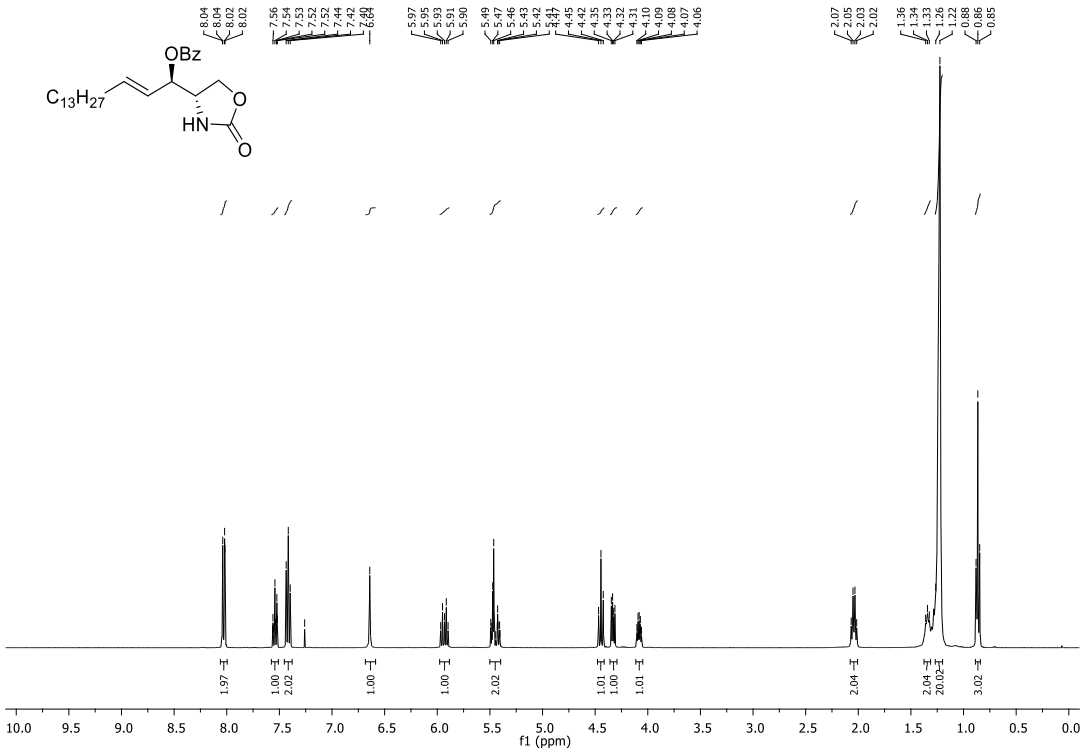
^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm) of *u*-4-[(*E*)-1-benzoyloxybut-2-en-1-yl]-oxazolidin-2-one ((±)-12).



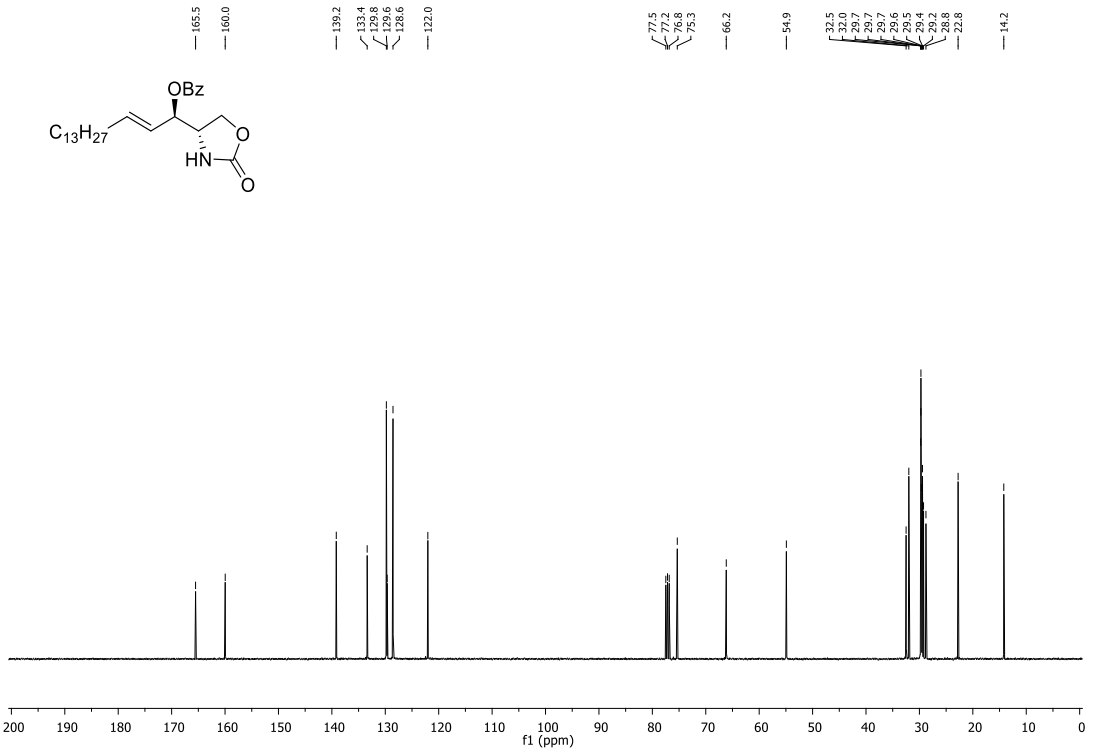
^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm) of *u*-4-[(*E*)-1-benzoyloxybut-2-en-1-yl]-oxazolidin-2-one ((±)-12).



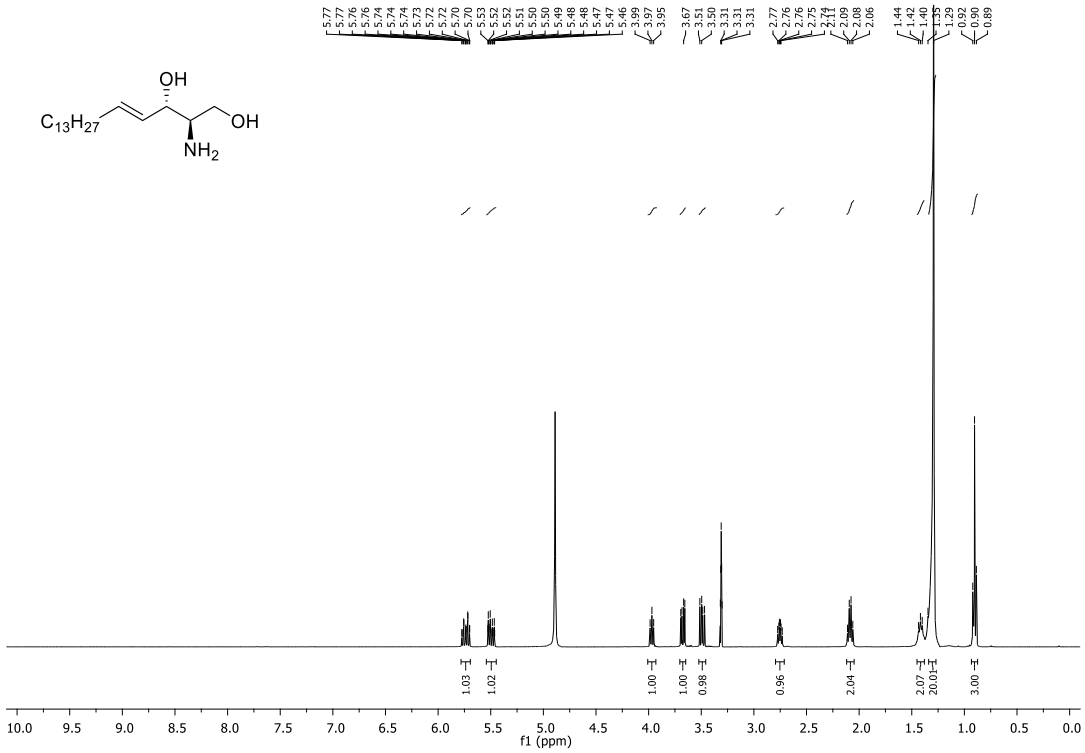
^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm) of *u*-4-[(*E*)-1-benzoyloxyhexadeca-2-en-1-yl]-oxazolidin-2-one ((±)-15).



^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm) of *u*-4-[(*E*)-1-benzoyloxyhexadeca-2-en-1-yl]-oxazolidin-2-one ((±)-15).



^{1}H NMR (CD_3OD, 400 MHz, δ in ppm) of (2*R*,3*S*)-2-aminooctadec-4-ene-1,3-diol ((*R*,*S*)-17).



^{13}C NMR (CD_3OD, 100 MHz, δ in ppm) of (2*R*,3*S*)-2-aminooctadec-4-ene-1,3-diol ((*R*,*S*)-17).