Cross Metathesis Route in Sphingomyelin Synthesis

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Cross metathesis reaction of short chain Boc sphingosine using Grubbs' 2nd generation catalyst proceeded in stereoselective manner to afford Boc sphingosine in good yield. An efficient synthesis of sphingomyelin was achieved from the obtained Boc sphingosine using our own phosphorylation reagent.

Sphingolipids are well known as an important class of second metabolites found in almost every cell membrane and cytoplasm. They are regarded as the lipid secondary messenger in a cell, and are now accepted to play an important role in signal transduction and in molecular recognition processes in the cell membrane.[1] Representative sphingolipids include sphingomyelin, ceramide, sphingosine, and sphingosine 1-phosphate. Among them, sphingomyelin has been paid particular attention because of its important role in maintaining the microdomain structure in cell membranes which is the so-called raft.[2]

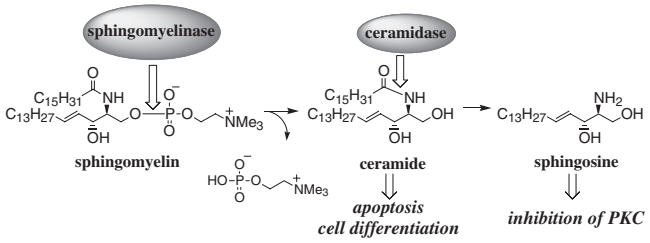


Figure 1. Sphingolipids metabolism.

During the course of our study on sphingolipid synthesis, we have already achieved the syntheses of not only various sphingolipids, such as natural sphingomyelin, ceramide, sphingosine, sphingosine 1-phosphate, and their short chain analogues,[3] but also fluorescence[4] and photo affinity labeled sphingolipids.[5] These labeled sphingolipids have been accepted as important tool molecules for the elucidation of the sphingolipid recognition processes. However, further convenient and divergent methods for the synthesis of these various kinds of sphingolipids derivatives including the derivatives in which fluorescence and photoaffinity groups are introduced into the sphingosine backbone skeleton, have been desired.

From this aspect, the cross metathesis reaction using a ruthenium catalyst[6] is very attractive for providing these various kinds of sphingolipids from a common intermediate. Quite recently, two groups independently reported the syntheses of sphingosine and ceramide by utilizing the olefin cross metathesis method.[7]

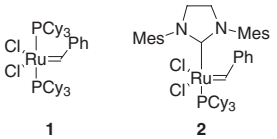
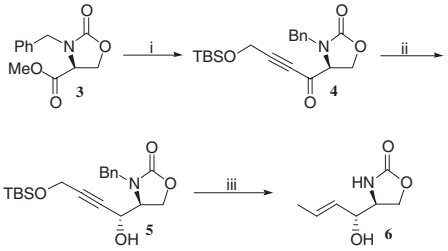


Figure 2. Grubbs' catalysts.

We have also independently investigated this method for the synthesis of the sphingolipid derivatives. In this paper, we disclose our own results of the olefin metathesis for the synthesis of sphingomyelin as a representative example of sphingolipids.

The substrates were synthesized from oxazolidinone methyl ester **3** as a chiral building block.[8] The monoalkynylation of the ester **3** with the *tert*-butyldimethylsilyl ether of propalgyl alcohol under −100 °C followed by stereoselective reduction of the resulting ketone **4** with diisobutylaluminum 2,6-di-*tert*-butyl-4-methylphenoxide[9] provided the corresponding alcohol **5** in 75% yield. The subsequent Birch reduction gave disubstituted olefin **6** in good yield. Other related compounds **8**–**13**, which were derived from compound **6** by protecting group manipulation, were used as the substrates of the cross metathesis reaction. Another segment, 1-pentadecene **7**, was synthesized by the Swern oxidation of commercially available 1-tetradecanol followed by the Wittig olefination.

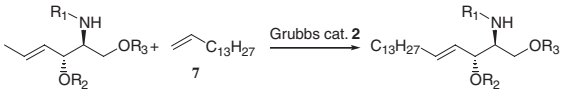


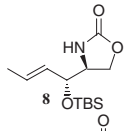
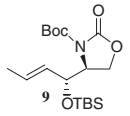
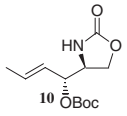
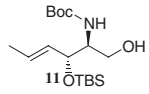
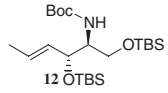
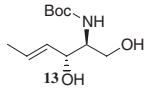
Scheme 1. Reagents and conditions: i) $TBSOCH_2CCH$, *n*-BuLi, THF, −100 °C, 82%, ii) Diisobutylaluminum 2,6-di-*tert*-butyl-4-methylphenoxide, toluene, 0 °C, 92%, iii) Li, NH_3, THF, −78 °C, 90%.

Substrate **8** was used to investigate the reaction conditions and 10 equivalents of olefin **7** were generally used. Although the desired cross metathesis reaction did not proceed using the first generation catalyst **1**,[10] the 2nd generation catalyst **2**[11] effectively catalyzed the reaction to produce the desired compounds. After several trials, we found that the reaction was completed by the addition of 5 mol % catalyst over 6 times. As a result, the substrate **8** stereoselectively provided the desired coupling compound of the *E* form as the sole product in 69% yield after flash column chromatography.

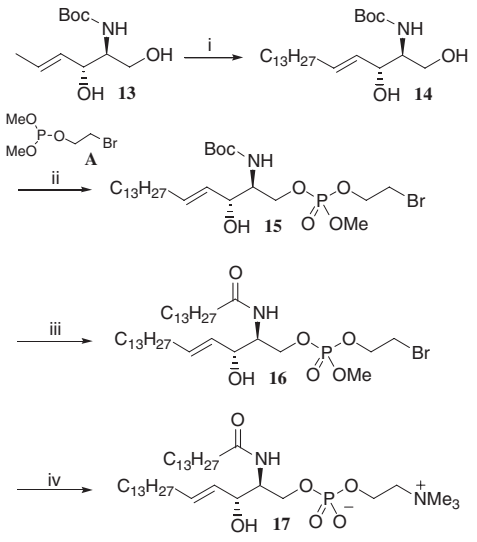
The reactions with other substrates were examined and these results are summarized in Table 1. For the *N*-Boc compound **9** (Entry 2), almost the same result was obtained. On the other hand, the reaction of *O*-Boc compound **10** (Entry 3) showed no stereoselectivity in 76% yield. For the *O*-protected opened chain compounds **11** and **12** (Entries 4 and 5), the coupling products were obtained in low yield or with no stereoselectivity. Fortunately, the desired *N*-Boc sphingosine possessing an *E* olefin was obtained from alcohol **13** in 62% yield as the sole product by the use of an 8 mol % catalyst (Entry 6). The use of a smaller amount of the olefin **7** resulted in a low yield of the desired prod-

Table 1. Cross metathesis reactions using Ru cat. **2**[a]



Entry	Substrate	Yield/%	Selectivity	Catalyst/equiv.
1	 **8**	69	*E* only	0.05 X 6
2	 **9**	67	*E* only	0.05 X 6
3	 **10**	76	*E* : *Z*=18 : 1	0.05 X 6
4	 **11**	18	N. D.	0.05 X 6
5	 **12**	N. D.	mixture of *E*&*Z*	0.05 X 6
6		62	*E* only	0.05 + 0.03
7	**13**	30[b]	*E* only	0.05 + 0.03

[a]All reactions were conducted in benzene at 55 °C.
[b]2 equivalents of **7** were used.



Scheme 2. Reagents and conditions: i) see Table 1. ii) CBr_4, **A**, pyr., 0 °C, 89%. iii) a) TFA, CH_2Cl_2, 0 °C. b) $C_{13}H_{27}COCl$, K_2CO_3, THF, H_2O, 85% for 2 steps. iv) NMe_3, MeOH, 46%.

uct (Entry 7).

Furthermore, natural sphingomyelin was conveniently synthesized from the obtained *N*-Boc sphingosine **14**. Phosphorylation of the compound **14** was successfully realized by a treatment with compound **A**,[12,13] which was developed in our laboratory, and carbon tetrabromide in pyridine to give compound **15** in 89% yield. It is noteworthy that the phosphorylation selectively proceeded at the primary hydroxy group without protection of the secondary hydroxy group. The obtained bromide showed a normal polarity and was easily purified by column chromatography. Removal of the *tert*-butoxycarbonyl group of **15** by treatment with trifluoroacetic acid followed by the introduction of an acyl group onto the resulting primary amine produced amide **16**. Finally, the obtained bromide **16** was treated with trimethylamine in methanol in an autoclave at 60 °C to afford natural sphingomyelin **17** in 46% yield. This is a new route for the synthesis of sphingomyelin.

In conclusion, the cross metathesis method is a very convenient way to produce sphingolipid derivatives and the further development of this method for the synthesis of other sphingolipid derivatives such as fluorescence and photoaffinity labeled compounds is now in progress.

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References and Notes

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