Concise Total Synthesis of (-)-D-*erythro*-Sphingosine and Sphingosine-1-Phosphate

By

Hao Yang
B.E., East China University of Science and Technology, 1999
M.E., East China University of Science and Technology, 2002

Advisor: Lanny S. Liebeskind, Ph.D.

A Thesis submitted to the Faculty of the Graduate School of Emory University in partial fulfillment of the requirements for the degree of Master of Science

Department of Chemistry
Graduate School of Arts and Sciences

2007

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Abstract

A short and efficient synthesis of high enantiopurity (-)-D-*erythro*-sphingosine has been achieved in 71% yield over 6 steps from *N*-Boc-L-serine. The key steps are high yield, racemization-free, palladium-catalyzed, copper(I)-mediated coupling of the thiophenyl ester of *N*-Boc-*O*-TBS L-serine with *E*-1-pentadecenyl boronic acid and the highly diastereoselective reduction of the resulting peptidyl ketone with $LiAl(OtBu)_3$. Using this concise route (-)-D-*erythro*-sphingosine can be prepared on large scale and in high enantio- and diastereopurity (ee >99%, de up to 99%).

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Acknowledgments

Of my graduate education at Emory University, I would like to acknowledge many people who were both directly and indirectly involved in my research projects. I would first like to thank my advisor, Dr. Lanny S. Liebeskind, for his mentorship, encouragement and support throughout these years. I would also like to thank my committee members, Dr. Conticello and Dr. Padwa, for their helpful comments during my annual research reports and the preparation of this thesis. I would like to thank all the organic faculty professors, Dr. Blakey, Dr. Liotta, Dr. Mcdonald, Dr. Menger and Dr. Mohler for their invaluable teaching for my education.

I would also like to thank all the present and past members of the Liebeskind group. I really enjoyed working with so many nice people in this group. I want to thank all the staff from the chemistry department. Especially, Dr. Shaoxiong Wu and Dr. Strobel, Fred provided fantastic instrumental support for identification and characterization of my research results.

My family is always supportive throughout my life. I am truly blessed by their unconditional love from my dad, Mr. Yunchang Yang and my mother, Mrs. Baohuan Liu. Finally, to my loving wife Chunyuan, it is so hard to express my appreciation for what you have done for me. You are truly my love, backbone, and soul mate.

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List of Abbreviations

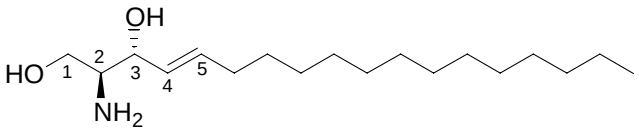
app	apparent
Ar	aryl
Bn	benzyl
Boc	*t*-butoxycarbonyl
bp	boiling point
br	broad
Bu	butyl
ºC	degrees Celsius
calcd	calculated
Cbz	benzyloxycarbonyl
CuTC	copper(I) thiophene-2-carboxylate
cm^{-1}	wavenumber unit
δ	chemical shilft (in ppm for NMR)
d	doublet
dba	dibenzylideneacetone
DCC	1,3-dicyclohexylcarbodiimide
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMAP	*N*,*N*-dimethylaminopyridine
DMSO	dimethylsulfoxide
equiv.	equivalent
Et	ethyl
EtOAc	ethyl acetate
Fmoc	9-fluorenylmethoxycarbonyl

g	gram(s)
Hex	hexane
HOBt	1-hydroxybenzotriazole
HPLC	high pressure liquid chromatography
hrs	hour(s)
HRMS	high-resolution mass spectrometry
Hz	hertz
IR	infrared spectroscopy
J	coupling constant
L	liter
M	molar
Me	methyl
MeCN	acetonitrile
mg	milligram
MHz	megahertz
ml	milliliter
mmol	millimole
mol %	mole percent
mol	mole
Mp	melting point
N	normal
N_3	azide
NMM	*N*-methyl morpholine
OAc	acetate
Ph	phenyl
PMB	*p*-methoxybenzyl

ppm	parts per million
py	pyridine
q	quartet
s	singlet
Ser	serine
t	triplet
TBAF	tetrabutylammonium fluoride
TBS	*t*-butyldimethylsilyl
TFP	tri(2-furyl)phosphine
THF	tetrahydrofuran
TLC	thin layer chromatography
Tol	toluene
Trityl	triphenylmethyl
UV	ultraviolet
w	weak

1. Introduction

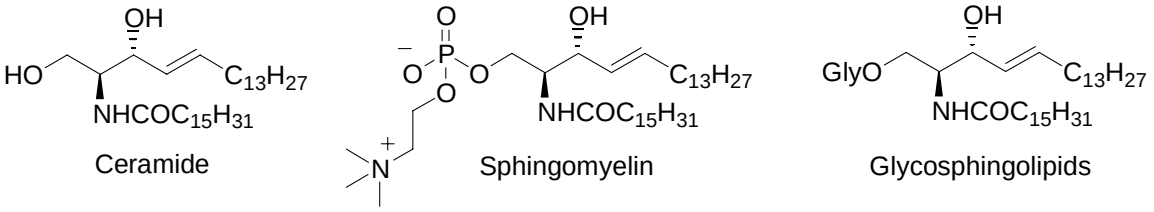
Figure 2. (-)-D-*erythro*-Sphingosine



1

Naturally occurring sphingolipids are important lipids structurally derived from (-)-D-*erythro*-sphingosine (**1**, Figure 1). They consist of three classes of lipids: ceramides, sphingomyelins and glycosphingolipids (Figure 2).[1] Sphingolipids can be isolated from cell membranes and neural tissues. Their biological activities have been associated with the control of cell growth, maturity, survival, and death. Interestingly, sphingolipids have shown promising efficacy for suppressing the growth of cancer cells.[2] In addition to the purpose of curing diseases, the related *N*-acylsphingosines (ceramides) are already widely used in the cosmetic industry as active ingredients to improve skin cell cohesion.[3]

Figure 2. Typical sphingolipids

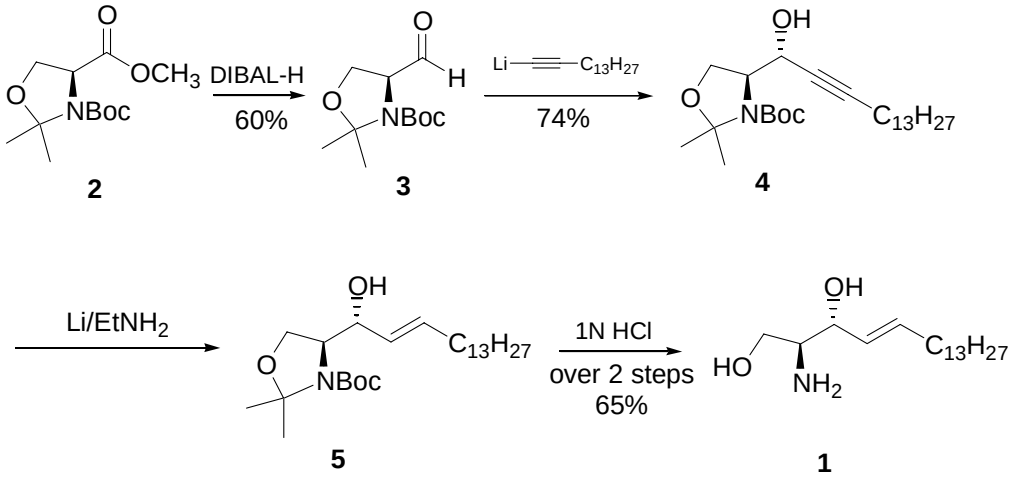


The versatile biological activities of sphingolipids make it a valuable pursuit for chemical synthesis. Since the structure elucidation of D-*erythro*-sphingosine by Jenny and Grob,[4] more than 50 syntheses of sphingosine have been disclosed.[5] Of these, the most economical approaches utilize serine as the starting material.[6] The benefit of this strategy originates from the presence of the hydroxyl group and amino group as well as the stereocenter inherent from the serine. However, the easy racemization of the serine under

both acid and base conditions limits its application to the synthesis of high enantiopure (-)-D-*erythro*-sphingosine (ee>99%).

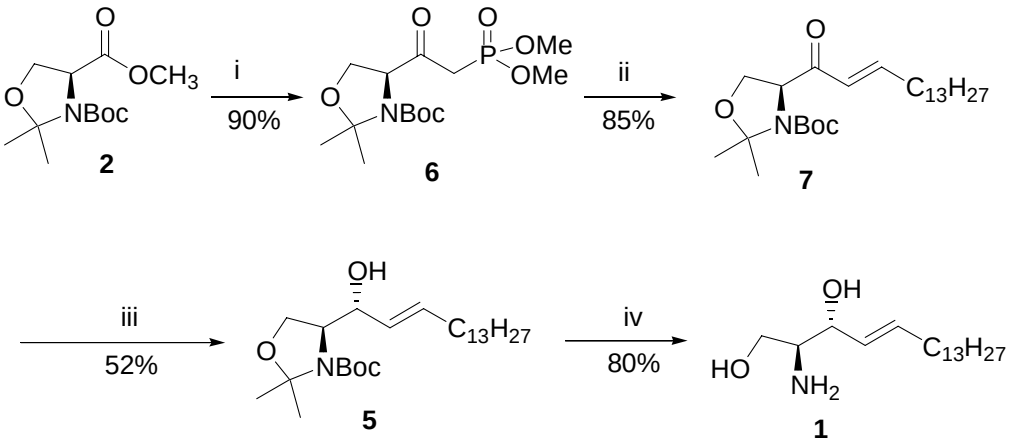
Garner *et al.* established a short and efficient synthesis of sphingosine by a stereocontrolled addition of alkynyl lithium to the key oxazolidine aldehyde **3** followed by reduction with Benkeser's reduction conditions using lithium in ethylamine and deprotection.[7] However, it was difficult to obtain the key oxazolidine aldehyde **3** with very high enantiopurity (only 95-98%).[8]

Scheme 1. Garner's sphingosine synthesis



A more practical strategy reported by Koskinen[9] took advantage of Horner-Wadsworth-Emmons reaction to prepare an enone **7** from a high enantiopure L-serine-derived ketophosphonate **6**. Although initial attempts involved racemization of the enone **7** product, K_2CO_3/MeCN was found to be a good base/solvent system to overcome the racemization problem (Scheme 2).

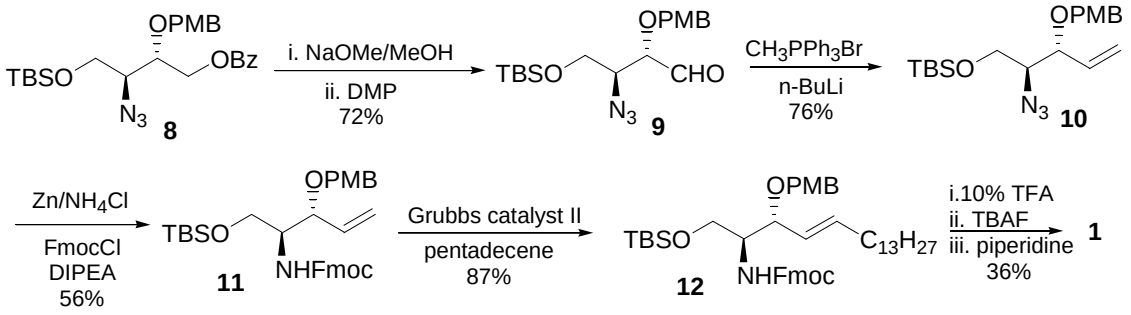
Scheme 2. Synthesis of sphingosine *via* Horner-Wadsworth-Emmons reaction



Conditions: (i) *n*-BuLi/Dimethylmethylphosphophosphonate/THF (ii) $C_{13}H_{27}CHO/K_2CO_3$/ MeCN (iii) L-Selectride/THF (iv) 1N HCl

As an important olefination method, cross metathesis was demonstrated by Somfai[10] and Basu[11] (Scheme 3) as an effective strategy to synthesize sphingosine because of its mildness and high selectivity which yielded only *trans* alkene. However, an excessive of pentadecene was incorporated to avoid *homo* metathesis of the substrates.

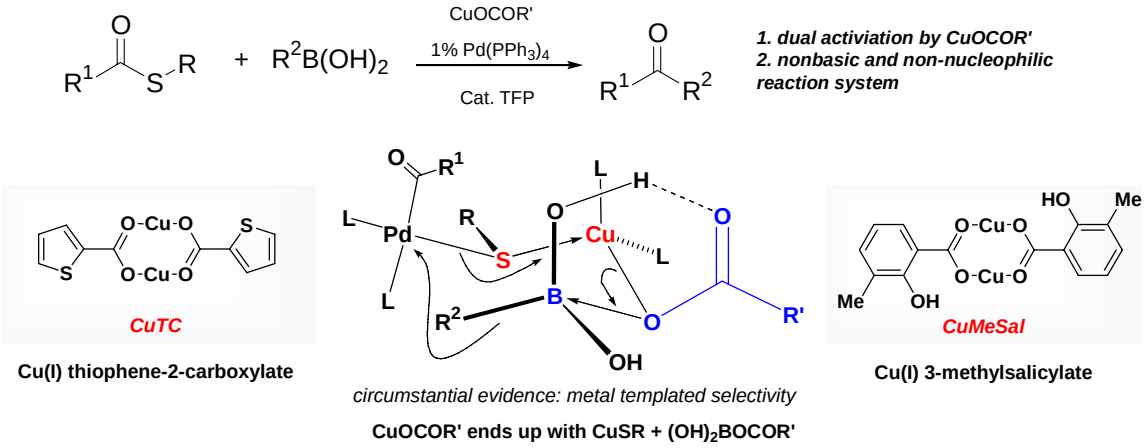
Scheme 3. **Cross-metathesis strategy**



Liebeskind *et al.* invented an extremely mild ketone synthesis by cross coupling of thiol esters and boronic acids.[12] This new reaction utilizes catalytic palladium(0) to activate thiol esters by a initial oxidative addition of carbonyl sulfur bond. The resulting R^1COPdL_2SR complex possesses a strong Pd-S bond which allows only strong

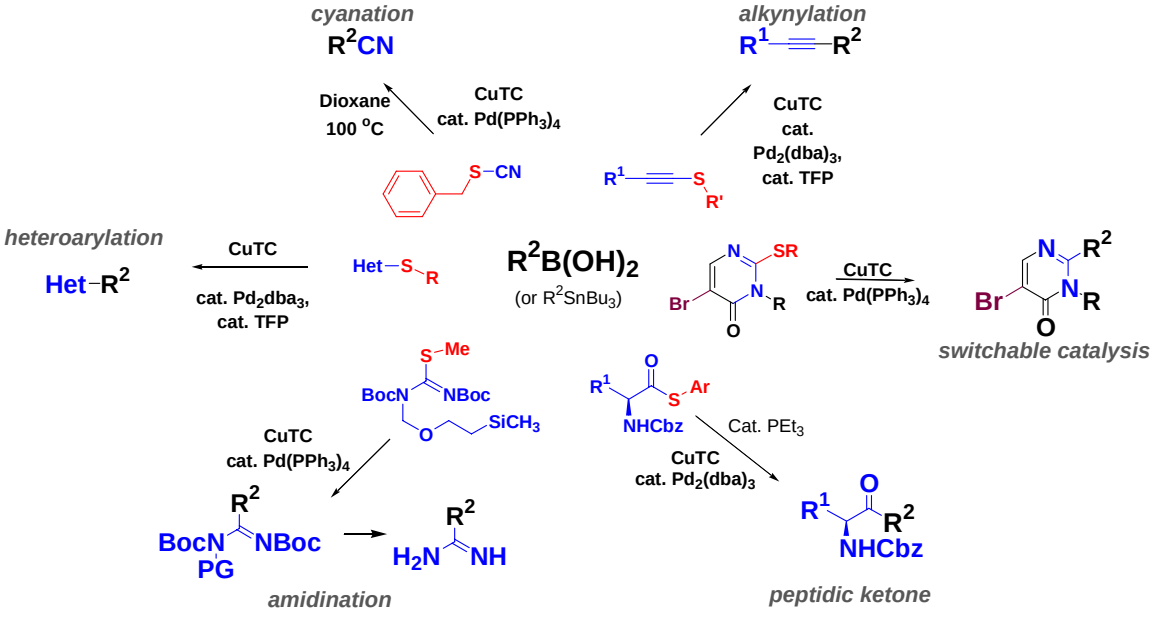
nucleophilic reagents such as organo lithium, magnesium or zinc to perform a transmetalation and reductive elimination which leads to ketone product. [13] The introduction of a Cu(I) carboxylate in the Liebeskind-Srogl reaction provides a dual activation of thiol esters and boronic acids by a "soft-soft" (S-Cu) and "hard-hard" (O-B) interactions, which is depicted in the Figure 3 as a proposed transition state of this reaction. With this dual activation by Cu(I) carboxylate, thiol esters efficiently couple with either boronic acids or organostannanes to produce ketones.

Figure 3. Liebeskind-Srogl cross coupling



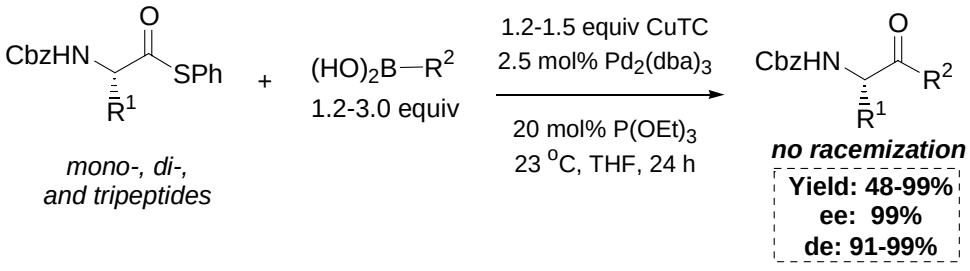
Based on the same principle, the Liebeskind group developed a series of thiol organic-based cross couplings to synthesize alkynes, [14] nitriles, [15] substituted heterocycles, [16] functionalized amidines [17] as well as substituted pyrimidines by an interesting switchable catalysis (Scheme 4). [18]

Scheme 4. Liebeskind thio-organic cross coupling

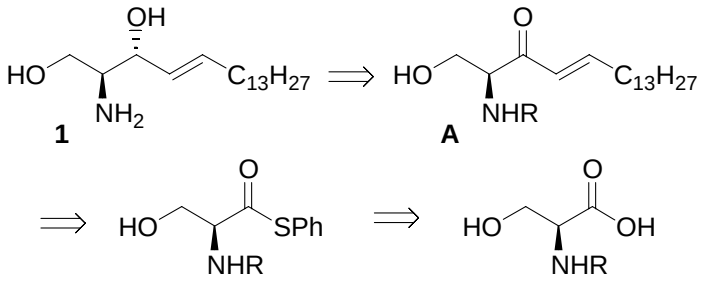


Recently, the Liebeskind laboratory extended this principle by developing a high enantiopurity peptidic ketone synthesis (Scheme 5).[19] The successful development of this methodology was based on the screening of various supporting ligands. Addition of triethyl phosphite ($P(OEt)_3$) as a supporting ligand was able to suppress a decarbonylation side reaction. As a very mild approach, this reaction is carried out at room temperature in the absence of base. This neutral and efficient methodology was capable of making various highly enantiopurity α-amino ketone and peptidic ketone without racemization.

Scheme 5. Synthesis of high enantiopurity peptidic ketone



Scheme 6. Retrosynthetic analysis



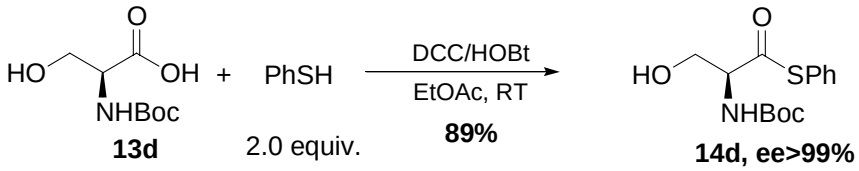
The retrosynthetic analysis of the (-)-D-erythro-sphingosine is rendered in Scheme 6. The key to generating high enantiopurity sphingosine from L-serine is the efficient construction of enone **A** without racemization which can be realized by cross coupling of serine thiol ester and *trans*-1-pentadecenyl boronic acid.

2. Results and Discussion

2.1 Synthesis of L-Serine Derived Thiol Ester

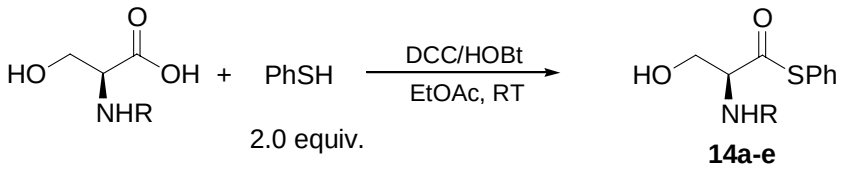
During the course of our study on peptidic ketone synthesis, a serious racemization was found in the preparation of peptidic thiol ester when DCC was used as the dehydration reagent. Fortunately, this problem was solved by adding HOBt as racemization suppressing reagent as well as by using excessive thiophenol. Following the same protocol, in the presence of 1.05 equivalent of DCC and 1.0 equivalent of HOBt, *N*-Boc-L-Serine coupled with thiophenol (2.0 equiv.) to produce *N*-Boc-L-Ser-SPh in high yield (89%) without racemization (ee>99%).

Scheme 7. Synthesis of thiol ester with high enantiopurity



With the development of standard conditions to prepare the L-serine derived thiophenyl ester, a typical selection of protected serines was converted to the corresponding thiophenyl esters from good to excellent yields (Table 1).

Table 1. Synthesis of various *N*-protected L-serine thiophenyl esters

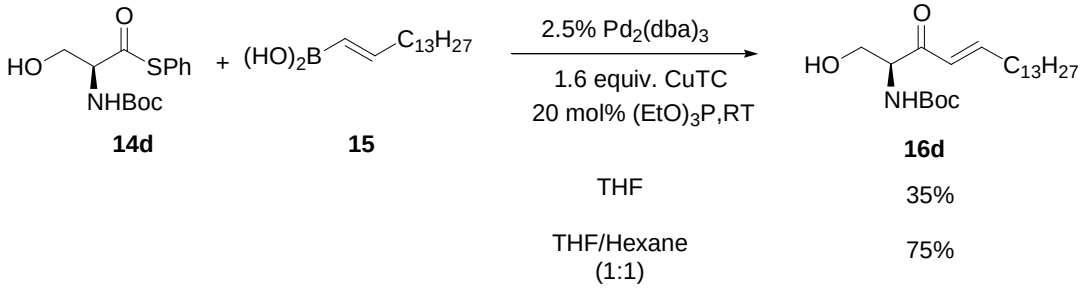


14a-e

entry	No	R^1	Yield (%)[a]
1	**14a**	$COC_{15}C_{31}$	65
2	**14b**	Cbz	47
3	**14c**	Trityl	67
4	**14d**	Boc	89
5	**14e**	Fmoc	77

An initial attempt of cross coupling with *N*-Boc-L-Ser-SPh and *trans*-1-pentadecenyl boronic acids gave a low yield of ketone **16d** (35%) (Scheme 8). Although replacement of THF by THF/hexanes (1:1) as the solvent system greatly improved the yield (75%), the extension of this reaction into a larger scale (0.5 gram) failed to give satisfactory yield (<20%).

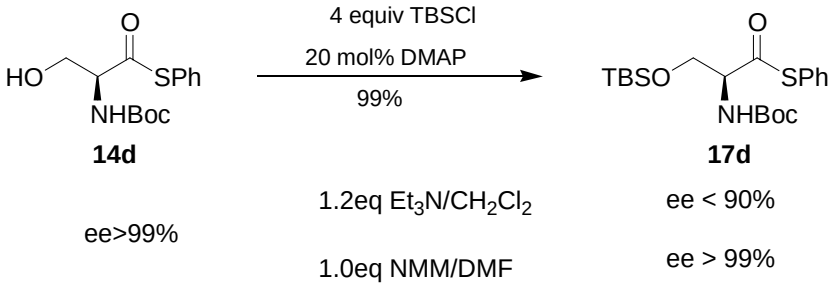
Scheme 8. The solvent effect of cross coupling



This observation suggested that an appropriate 1-OH protecting group was necessary to obtain a good cross coupling. An *O*-silylation of the **14d** was carried out

using TBSCl/TEA in CH_2Cl_2. Unfortunately, a significant racemization occurred during the silylation process (>5%). Upon switching to DMF as the solvent, the racemization dropped to only 2%. Attempts to use DIPEA or K_2CO_3 as the base exacerbated the racemization. Finally, employing *N*-methyl morpholine (NMM) as the base in the DMF furnished the requisite silylated product **17d** in excellent yield less than 30 minutes without racemization (ee > 99%, Scheme 9).

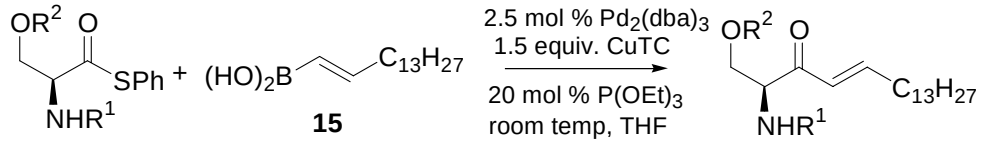
Scheme 9. Racemization of *O*-silylation



2.2 Cross Coupling Reactions

An investigation was conducted to evaluate the cross coupling efficiency of the various *N*-protected serine derived thiophenyl ester with *trans*-1-pentadecenyl boronic acid **15**. Table 2 summarizes the overall results of cross coupling. From a typical selection of amino protecting groups, this cross-coupling showed very good reactivity regardless of the protecting groups, except for the hindered trityl group. Of the protected thiol esters studied, mono *N*-Boc-*O*-TBS L-serine thiophenyl ester **17d** gave the highest yield of ketone **18d** in less than 6 hr at room temperature (94%). In addition to the yield, the enantiopurity of the ketones was of concern. To our delight, no racemization was detected from the analysis of the products on a chiral HPLC by comparing with their racemic mixtures.

Table 2. The cross-coupling.



		R^2 = TBDMS	R^2 = H
entry	R^1	ketone no./yield (%)[a]	
1	$COC_{15}C_{31}$	**18a**, 60	**16a**, 37
2	Cbz	**18b**, 78	**16b**, 40
3	Trityl	**18c**, 0[b]	**16c**, 0[b]
4	Boc	**18d**, 94[d]	**16d**, 75[c]
5	Fmoc	**18e**, 73	**16e**, 32

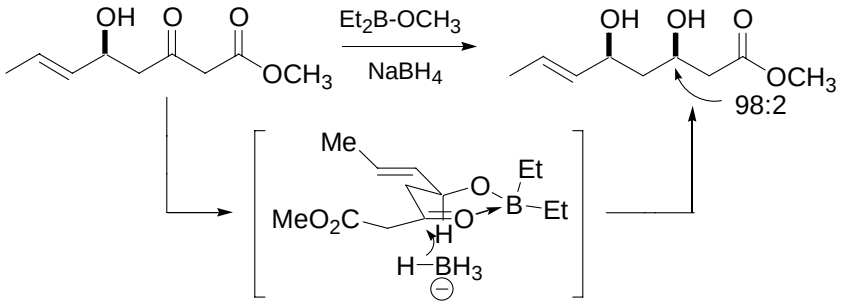
[a] Isolated yield. [b] Starting material was recovered. [c] THF/ hexanes (1:1) used as solvent (30% yield using pure THF as the solvent). [d] ee > 99%.

2.3 Asymmetric Reduction

Having obtained high enantiopurity ketone, further work was focused on the asymmetric reduction of the enone to the corresponding *anti* featured amino alcohol. We noticed that enone **16d** bears a potential hydroxyl group *beta* adjacent to the ketone, which can be utilized to induce a diastereoselective reduction.

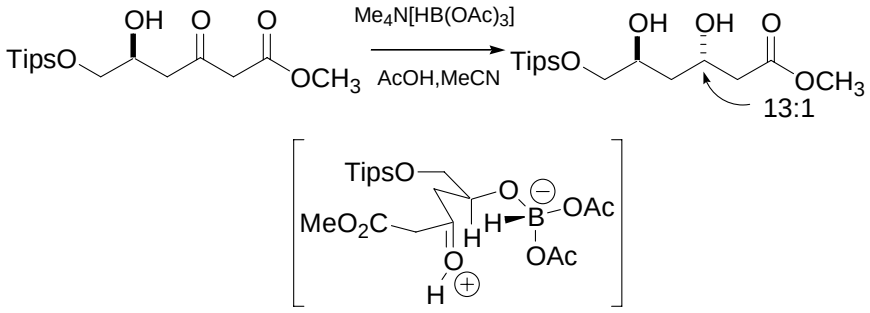
In fact, 1,3-chelation-control is a common strategy to direct a stereoselective reduction of ketone which possesses a *beta*-hydroxyl group. By using a dialkylboron chelating agent, Shapiro realized a *syn*-selective reduction of ketone to generate a 1,3-*syn* diol product[20] (Scheme 10).

Scheme 10. Diastereoselective chelation-controlled reduction of β-hydroxyketone



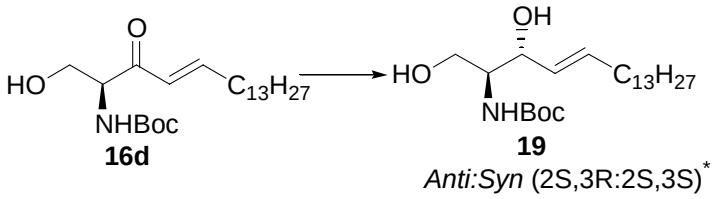
On the contrary, Evans *et al.* employed triacetoxyborohydride to obtain an *anti*-selective reduction from an acyclic *beta*-hydroxyketone substrate[21] (Scheme 11).

Scheme 11. Hydroxyl-directed ketone reduction gives the *anti*-diol



Inspired by 1,3-chelation controlled reduction, we expected to get either *anti*- or *syn*- protected amino alcohol by the employment of the above reduction conditions. However, both methods led to an *anti*-favored selectivity (Scheme 12).

Scheme 12. Diastereoselective reduction



Anti:Syn (2S,3R:2S,3S)*

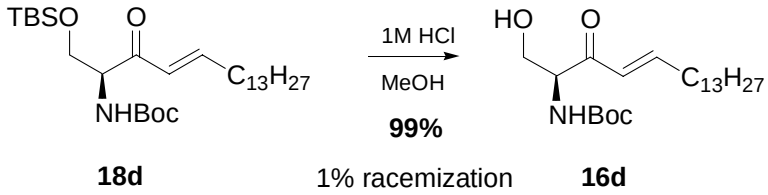
$Me_4N[B(OAc)_3H]$	7:1
$Et_2BOMe/NaBH_4$	13:1

*determined by ^{1}H-NMR

Although $Et_2BOMe/NaBH_4$ gave a decent *anti* selectivity (13:1), HPLC analysis of the products showed four distinct isomers. Further investigation revealed that enone **16d** contained 1% of the other enantiomer. It turned out the racemization resulted during the desilylation process. In order to eliminate the racemization problem, a TBAF-mediated desilylation was carried out. Unfortunately, it gave a quite messy mixture of products. Other efforts were tried to improve the outcome such as changing the solvent,

lowering the temperature, and increasing the concentration of HCl. Unfortunately, more than 1% chirality loss was inevitable.

Scheme 13. Racemization during desilylation

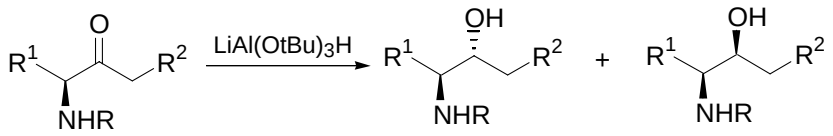


18d 1% racemization **16d**

These disappointing results led us to seek a new diastereoselective reduction method based on substrate **18d** rather than the desilylated alcohol **16d** (Scheme 13).

Hoffman *et al.* developed a highly diastereoselective syntheses of both *syn*- and *anti*-1,2-amino alcohols from the reduction of *N*-protected α-amino ketones,[22] depending on the nature of the amino protective groups, as shown in Scheme 14.

Scheme 14. *syn*- and *anti*- Selective reduction of *N*-protected α-amino ketone

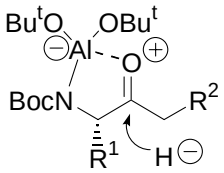


R=carbamate anti selectivity > 19:1

R=trityl syn selectivity > 4:1

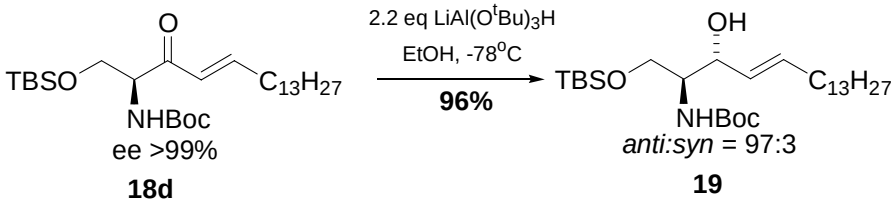
The transition state of the resulting *anti* selectivity was suggested by the author in Figure 4, which was depicted as a five-membered ring chelation controlled stereo-selective addition. The first equivalent of the $LiAl(OtBu)_3H$ deprotonates the carbamate NH, a subsequent chelation will form a five member ring so that the second equivalent of $LiAl(OtBu)_3H$ prefer to addition from the less hindered face. The overall effect led to an *anti* amino alcohol.

Figure 4. Proposed transition state



Following Hoffman's approach, the cross coupling product **18d** was reduced by $LiAl(OtBu)_3H$ in ethanol at -78 ºC to give alcohol **19** (Scheme 15). To our delight, an excellent *anti* diastereoselectivity (97:3) was obtained as well as an outstanding yield (96%).

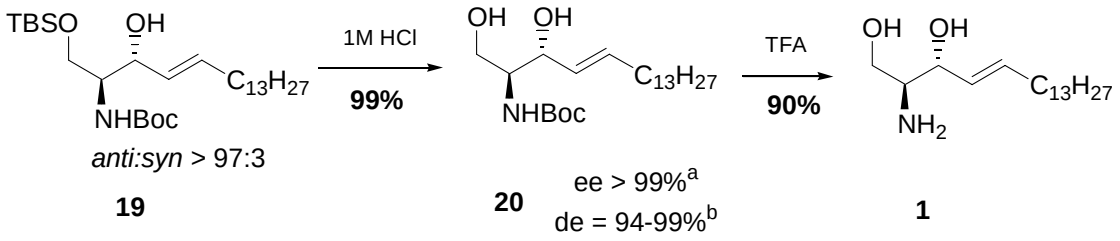
Scheme 15. ***anti*- Selective reduction**



2.4 Completion of Total Synthesis of (-)-D-*erythro*-Sphingosine

For completion of the total synthesis of (-)-D-*erythro*-sphingosine, the subsequent desilylation quantitatively afforded diol **20** with a 94% de. Both HPLC and LC-MS showed high enantiopurity for each diastereomer (ee > 99%). To further improve the diastereomeric purity, diol **20** was recrystallized in an isopropyl ether/hexane (1:1) solvent system giving pure **20** with high optical purity (de>99% ee>99%). The final deprotection of *N*-Boc afforded the (-)-D-*erythro*-sphingosine **1** in 90% yield. Spectroscopic data and optical rotatory value were identical to the reported values.[23]

Scheme 16. **Completion of total synthesis of (-)-D-*erythro*-sphingosine**



anti:syn > 97:3

19

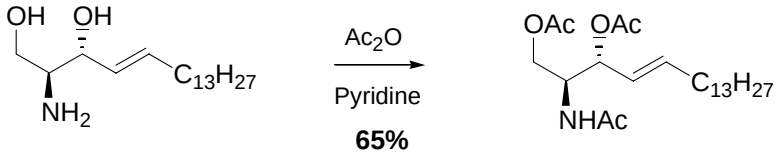
20 ee > 99%[a] de = 94-99%[b]

1

a. Determined by Chiral HPLC, AS-RH. b. Determined by ^{1}H NMR and chiral HPLC, OD-RH.

As a supplementary method to identify the synthesized (-)-D-*erythro*-sphingosine, its corresponding triacetate derivative **21** was also synthesized (Scheme 17). All the spectroscopic data matched with the reported literature values.[24]

Scheme 17. Synthesis of sphingosine triacetate derivative



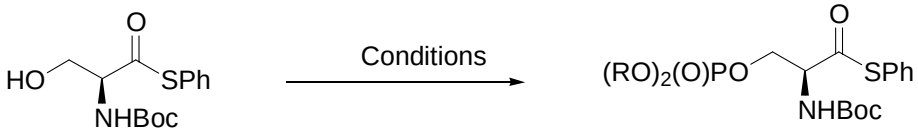
1 **21**

2.5 Synthesis Sphingosine Derivatives

As discussed in the introduction section, the entire sphingolipid family derives from the core structure of (-)-D-*erythro*-sphingosine **1**. The benefit of our synthesis relies on the very mild peptidyl ketone synthesis without racemization. The subsequent challenging question is "Can this ketone synthesis be carried out on a more complex substrate?". In order to demonstrate the versatility of our strategy for other sphingolipids syntheses, a cross coupling study was carried out on the more complex substrates such as *O*-phosphorylated serine derived thiol ester and *O*-glycosyl functionalized serine thiol ester.

Synthesis of these thiophenyl esters was not straightforward. Attempts to phosphorylate *N*-Boc-Ser-SPh using $P(OMe)_3$ and CBr_4 failed to generate the corresponding dimethyl phosphate ester. Surprisingly, the more active dimethyl chlorophosphate was not able to convert thiol ester **14d** to the phosphorylated product, even though many conditions were tried (employing various bases such as K_2CO_3, pyridine, Et_3N and DMAP). Finally, the phosphorylation was realized by utilizing diphenylchlorophosphate as the phosphorylation reagent (Table 3).

Table 3. **Phosphorylation of *N*-Boc L-serine thiophenyl ester**

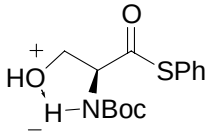


14d

Entry	R	Conditions	Yield (%)
1	Me	$P(OMe)_3/CBr_4$/pyridine	0
2	Et	$P(O)(OEt)_2Cl/Et_3N$/THF	0
3	Ph	$P(O)(OPh)_2Cl/Et_3N$/THF	30
4	Ph	Cat.DMAP/$P(O)(OPh)_2Cl/Et_3N$/THF	82

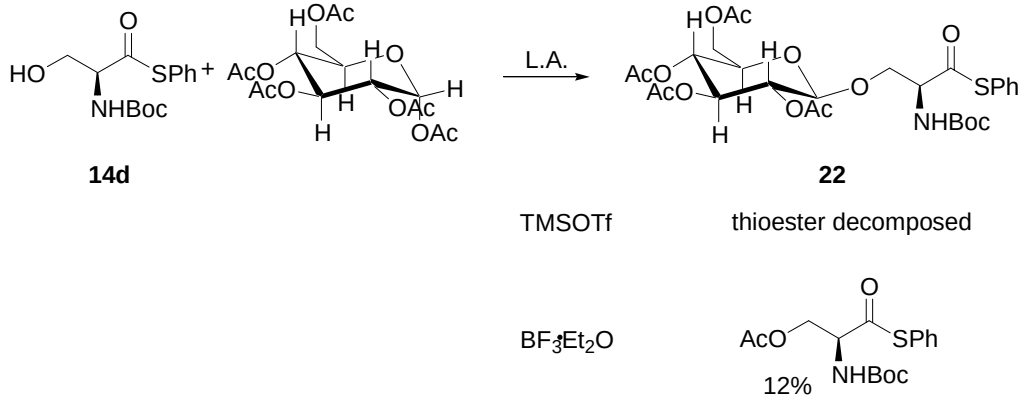
The difficulty of the above *O*-phosphorylation might be rationalized by an intramolecular hydrogen bonding interaction of 1-O(H)····HNBoc within the structure of serine thiophenyl ester,[25] which decreases the nucleophility of the 1-OH (Figure 5). A similar difficulty was also encountered in the *O*-glycosylation of the serine thiol ester.

Figure 5. Intramolecular hydrogen bonding interaction



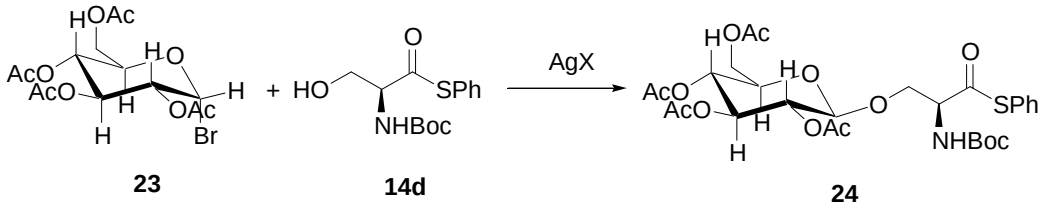
Activation of the typical glucose *penta*- acetate by TMSOTf did not generate any trace of *tetra*-acetate glucosyl thiophenyl ester **22**. To our surprise, an undesired *O*-acetylated thiol ester **22** was obtained when $BF_3{\cdot}Et_2O$ was used as the Lewis acid (Scheme 18).

Scheme 18. Glycosylation with glucose *penta*-acetate



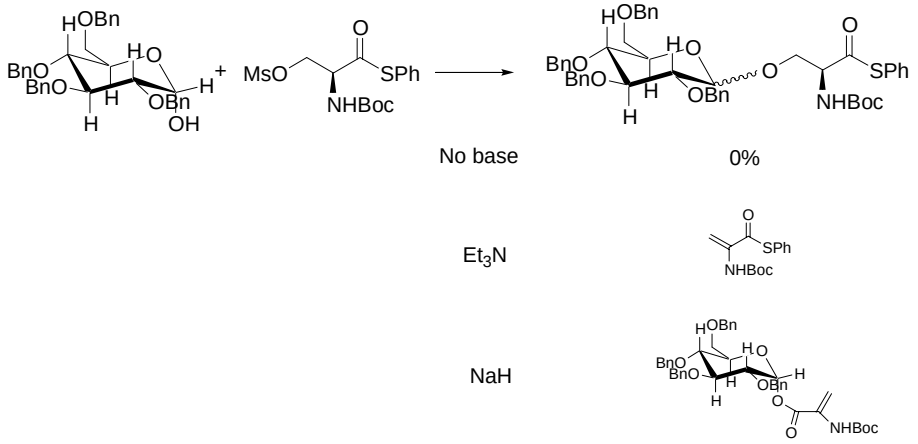
To overcome the low nucleophility of the hydroxyl group on thiophenyl serine, we tried to use a more active glycosyl donor like glucosyl bromide. Unfortunately, no matter what kind of silver salts were used (Ag_2CO_3, $AgPF_6$, AgOAc and AgOTf), no glycosylation product was obtained with this Koenig-Knorr glycosylation reaction[26] (Scheme 19).

Scheme 19. Koenig-Knorr glycosylation



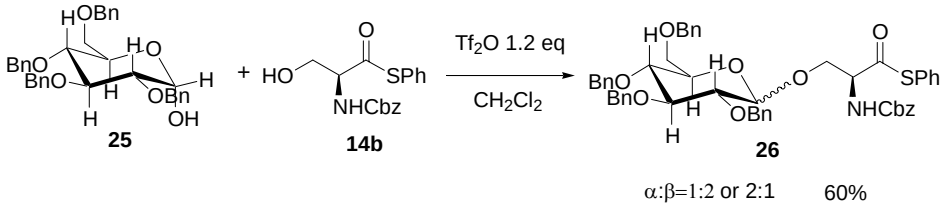
Attempts to use the glycodonor as a nucleophile rather than an electrophile gave either elimination product or a vinyl glycosylated ester (Scheme 20).

Scheme 20. Using glycodonor as nucleophile



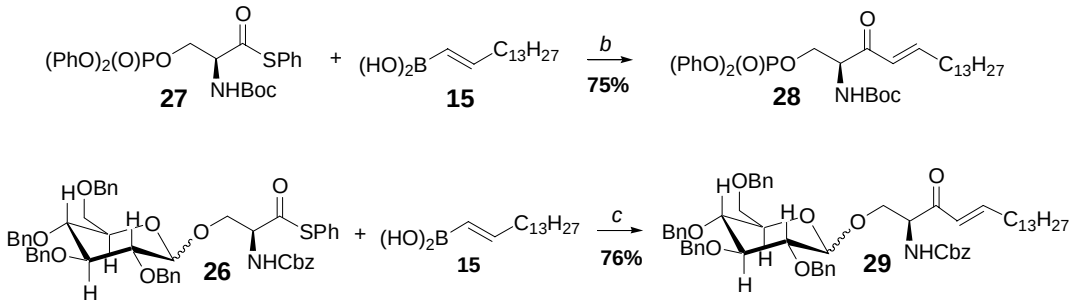
Finally, using the Ramage method,[27] *tetra*-benzyl glucose **25** coupled with **14b** to form the desired glycosylated thiol ester **26** in 60% yield by the Tf_2O mediated dehydration at room temperature (Scheme 21).

Scheme 21. **Tf_2O mediated glycosylation**



The subsequent cross coupling of the **26** and **27** with *trans*-1-pentadecenyl boronic acid **15** with our optimized cross-coupling conditions worked very well. Using a mixed solvent system of THF and hexanes (1:1) both produced a higher yield of ketone rather than using pure THF. More importantly, phosphate and glycoside were well tolerated under our cross coupling (Scheme 22).

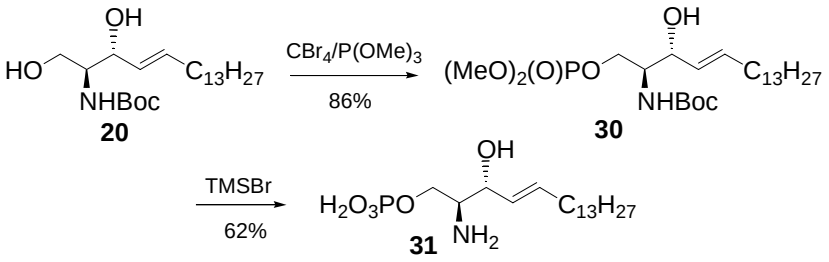
Scheme 22. ***O*-Functionalized derivatives**



a. All starting material stereoprofiles were conserved in the products.
b. 2.5 mol % $Pd_2(dba)_3$, 20 mol % $P(OEt)_3$, 1.6 equiv CuTC, room temp, THF/hexanes 1:1.
c. 2.5 mol % $Pd_2(dba)_3$, 20 mol % $P(OEt)_3$, 2.0 equiv CuTC, room temp, THF/hexanes 1:1.

With the synthesis of high enantiopurity (-)-D-*erythro*-sphingosine was synthesized, it is convenient to transform sphingosine **1** into other useful sphingolipids such as sphingosine-1-phosphate (S1P). Following the reported method,[28] selective phosphorylation of the 1-OH group of **20** using $P(OMe)_3/CBr_4$/pyridine followed by deprotection of the resulting phosphate ester using a TMSBr-mediated cleavage gives sphingosine-1-phosphate (S1P) **31** in 62% yield.

Scheme 23. Synthesis of sphingosine-1-phosphate



3. Conclusions

In summary, a concise total synthesis (6 steps, 71% overall yield from *N*-Boc-L-serine) of high enantiopurity (-)-D-*erythro*-sphingosine and sphingosine-1-phosphate was established by employing a key peptidic thiol ester and boronic acid cross coupling for the critical bond-forming step. Problems during the protection and deprotection steps were completely solved. This method not only provides a rapid, mild and efficient synthesis of sphingosine, but it also provides a powerful toolbox for rapid construction of sphingosine-related lipid family. Our future work will demonstrate the mildness and versatility of this method toward synthesizing various amino acid derived sphingosine analogues.

4. References

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23) see experimental section

24) see experimental section

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5. Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded on Varian Inova 600 MHz and 400 MHz spectrometers or a Mercury 300 MHz spectrometer in deuteriochloroform ($CDCl_3$) with the solvent residual peak as internal reference unless otherwise stated ($CDCl_3$: ^{1}H = 7.26 ppm, ^{13}C = 77.23 ppm). Data are reported in the following order: chemical shifts are given (δ); multiplicities are indicated as br (broad), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), app (apparent); coupling constants, *J*, are reported (Hz); integration is provided. Infrared spectra were recorded on a Nicolet 510 FT-IT or ASI ReactIR 1000 spectrometer. Peaks are reported (cm^{-1}) with the following relative intensities: vs (very strong), s (strong), m (medium), w (weak), and br (broad). Optical rotation values were measured at 20 °C on a Perkin Elmer Model 341 polarimeter with chloroform ($CHCl_3$) as solvent. Uncalibrated melting points were taken on a *Thomas-Hoover* melting point apparatus in open capillary tubes.

Analytical thin-layer chromatography (TLC) was performed using Merck silica gel glass plates with F-254 indicator. Visualization was accomplished by UV light, or with solutions of $K_2CO_3/KMnO_4$ in water, phosphomolybdic acid in ethanol, or *p*-anisaldehyde in ethanol. Solvents for reactions and chromatography were reagent grade and used as received. Flash column chromatography was performed by the method of Still[1] with 32-63 μm silica gel 60 (Woelm). HPLC analyses were carried out using an Agilent 1100 system with a quaternary pump. Separations were achieved on a Zorbax Eclipse XDB C8 4.6 x 150 mm column or DAICEL chiral AD, AS, OD reversed phase column. Solvents used as reaction media were purchased in > 99% purity purged for

[1] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

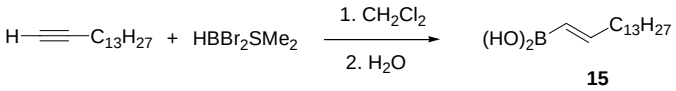
several minutes with argon then dried and stored over 4Å molecular sieves (water content below 10 ppm). All reactions requiring an inert atmosphere were carried out under dry argon in oven-dried glassware. "Brine" refers to a saturated aqueous solution of NaCl. Unless otherwise specified, solutions of NH_4Cl and $NaHCO_3$ refer to saturated aqueous solutions.

Starting Materials. *N*-Fmoc-L-serine and 1-pentadecyne were purchased from Fluka. *N*-Palmitoyl-L-serine was purchased from Timtec. All other protected serines were purchased from Sigma-Aldrich. Also purchased from Sigma-Aldrich were *N*,*N*'-dicyclohexylcarbodiimide (DCC), thiophenol, triethylamine, *tert*-butyldimethylsilyl chloride, 4-(dimethylamino)-pyridine, 4-methylmorpholine, lithium tri-*tert*-butoxylaluminohydride, dibromoborane-methyl sulfide complex, palmitic acid, 2,3,4,6-*tetra*-*O*-benzyl-D-glucopyranose, diphenylchlorophosphate, trimethyl phosphate, carbon tetrabromide, bromotrimethylsilane and solvents.

Tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$), 1-hydroxybenzotriazole (HOBt), and triethylphosphite ($P(OEt)_3$) were purchased from Acros. Triethylphosphite was purified by distillation at 1 atm (157 °C).[2] Cu(I) thiophenecarboxylate (CuTC) was prepared using a previously reported procedure.[3]

Experimental Procedures

E-Pentadecene Boronic Acid. [15]



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[2] Taira, K.; Gorenstein, D. G. *Tetrahedron* **1984**, *40*, 3215-3222.

[3] Liebeskind, L. S.; Srogl, J. *J. Am. Chem. Soc.* **2000**, *122*, 11260-11261.

To the solution of 1-pentadecyne (4.16 g, 20 mmol) in methylene chloride (20 mL) was added dibromoborane dimethyl sulfide (4.66 g, 20 mmol) at 0 ºC. The reaction mixture was stirred at room temperature for 6 hr until all of the pentadecyne was consumed. Diethyl ether (20 mL) and cold water were then added into the reaction mixture followed by stirring for 1 hr. The reaction mixture was washed with $NaHCO_3$ and then extracted into ethyl acetate (40 mL x 2). The combined organic layers were concentrated to give the crude product that was further purified by flash column chromatography (silica gel, hexane/ethyl acetate = 2:1) to afford *E*-pentadecene boronic acid **15** as a white powder. Yield: 3.91 g (77%). Mp = 76-78 ºC. TLC (R_f = 0.5, silica gel, hexane/ethyl acetate = 2:1). ^{1}H NMR (600MHz, $CDCl_3$) δ 6.95 (dt, *J* = 6.6, 18.0 Hz, 1H), 5.51 (d, *J* = 18.0 Hz, 1H), 2.19 (q, *J* = 6.6 Hz, 2H), 1.43 (m, 2H), 1.24 (m, 20H), 0.87 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.1, 153.4, 35.9, 35.8, 32.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 28.6, 28.4, 22.9, 14.3. IR (neat, cm^{-1}) 3350 (w), 2918 (s), 2853 (m), 1640 (w), 1363 (m), 1046 (m).

General Procedures.

Procedure A *(Cross Coupling of Protected Serine Thiophenyl Esters and E-Pentadecene Boronic Acid,* ***15****)*

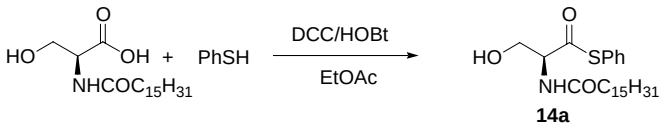
To a mixture of the thiol ester (1.0 equiv), boronic acid **15** (1.7 equiv), $Pd_2(dba)_3$ (2.5 mol %), and CuTC (1.7 equiv) in an argon flushed flask at room temperature was added $P(OEt)_3$ (20 mol %) and THF (to bring the thiol ester to 0.1 M). The reaction mixture was stirred at room temperature until TLC analysis indicated completion of the reaction (4-10 hr). The reaction mixture was then concentrated under vacuum to remove most of the THF. The resulting material was then diluted with ethyl acetate and washed with 2

wt % aqueous ammonium hydroxide, 0.1 M HCl and finally $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the resulting crude product was purified by flash chromatography to give the ketone products.

Procedure B *(O-Silylation of N-Protected Serine Thiophenyl Esters)*

To a solution of *N*-protected-L-Ser-SPh (1.0 equiv), TBSCl (4.0 equiv) and DMAP (0.2 equiv) in DMF (to bring the thiol ester to 0.2 M) was added *N*-methylmorpholine (1.0 equiv.) at 0 ºC. The resulting solution was stirred for 30 min at room temperature. The reaction mixture was then diluted with ethyl acetate and washed with 0.1 M HCl, $NaHCO_3$ and then brine. The organic layer was concentrated *in vacuo* to give the *O*-TBS protected serine thiophenyl ester.

2-(*S*)-Hexadecanoylamino-3-hydroxythiopropionic acid *S*-phenyl. [**14a**]

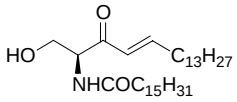


14a

Thiophenol (220 mg, 2 mmol) was added to a solution of *N*-palmitoyl-L-serine (343 mg, 1 mmol) and HOBt (203 mg, 1.5 mmol) in dry ethyl acetate (10 mL) at 0 ˚C followed by the addition of 1,3-dicyclohexylcarbodiimide (DCC) (206 mg, 1 mmol) slowly in small portions. The mixture was stirred for 16 hr at room temperature and the reaction progress was monitored by HPLC analysis. At the end of the reaction 0.5 mL of 50 % acetic acid in ethyl acetate was added. The reaction mixture was filtered through a short plug of Celite™ and washed with 1M HCl and $NaHCO_3$ followed by concentration *in vacuo*. The crude product was further purified by flash chromatography (silica gel, ethyl acetate/hexane = 4:1) to give *N*-palmitoyl-L-serine thiophenyl ester **14a** as white solid. Yield: 285 mg (65%). Mp = 103-106 ºC. TLC (R_f = 0.40, silica gel, ethyl acetate/hexanes

= 1:4). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.41 (m, 5H), 6.56 (d, *J* = 8.4 Hz, 1H), 4.91 (m, 1H), 4.17 (dd, *J* = 11.6, 3.6 Hz, 1H), 3.83 (dd, *J* = 11.2, 3.6 Hz, 1H). 2.40 (br, 1H), 2.32 (t, *J* = 3.6 Hz, 2H), 1.70 (m, 2H), 1.24 (m, 24H), 0.87 (t, *J* = 2.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.4, 173.9, 134.8, 129.9, 129.5, 126.8, 63.4, 60.6, 36.8, 32.1, 29.9, 29.7, 29.5, 29.5, 25.7, 22.9, 14.3. IR (neat, cm^{-1}) 3489 (br), 3293 (m), 2922 (s), 2853 (m), 1679 (m), 1656 (s), 1532 (m), 1046 (m). HRMS (FAB) Calcd for $C_{25}H_{42}NO_3S$ ($[M+H]^+$): 436.2867. Found: 436.2879.

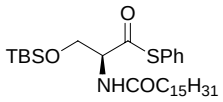
1-Hydroxy-2-(*S*)-hexadecanoylamino-octadec-4-en-3-one. [**16a**]



Following the general procedure **A**, *N*-palmitoyl-L-Ser-SPh (30 mg, 0.06 mmol) was allowed to react with *E*-pentadecene boronic acid (31 mg, 0.11 mmol) for 10 hr at room temperature. The product was purified by flash chromatography (silica gel, hexane/ethyl acetate = 3:2) to give the above enone **16a** as a colorless oil. Yield: 13 mg (37%). TLC (R_f = 0.4, silica gel, hexane/ethyl acetate = 3:2). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.10 (dt, *J* = 15.6, 7.2 Hz, 1H), 6.74 (d, *J* = 6.0 Hz, 1H), 6.26 (d, *J* = 16.0 Hz, 1H), 4.90 (m, 1H), 3.96 (dd, *J* = 11.6, 2.8 Hz, 1H), 3.81 (dd, *J* = 11.6, 4.8 Hz, 1H), 2.27 (m, 2H), 1.64 (m, 2H), 1.47 (m, 2H), 1.26 (m, 44H), 0.88 (t, *J* = 6.8 Hz, 3H).[4] ^{13}C NMR (100 MHz, $CDCl_3$) 195.4, 174.6, 151.9, 126.5, 65.1, 59.8, 36.7, 33.0, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 28.1, 25.8, 22.9, 14.3. IR (neat, cm^{-1}) 3350 (br), 2918 (s), 2853 (s), 1633 (s), 1467 (m). HRMS (FAB) Calcd for $C_{34}H_{66}NO_3$ ($[M+H]^+$): 536.5037. Found: 536.5032.

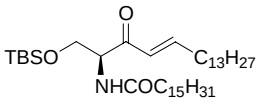
[4] Goldstein, A. S.; Gelb, M. H.; Yager, P. *Chem. Phys. Lipids.* **2001**, *109*, 1-14.

3-(*tert*-Butyldimethylsilanyloxy)-2-(*S*)-hexadecanoylaminothiopropionic acid *S*-phenyl ester. [**17a**]



Following the general silylation procedure **B**, *N*-palmitoyl-L-Ser-SPh (56 mg, 0.13 mmol) was allowed to react with TBSCl (77 mg, 0.51 mmol) for 30 min to afford *N*-palmitoyl-O-TBS-Ser-SPh **17a** as a colorless oil. Yield: 58 mg (83%). TLC (R_f = 0.3, silica gel, ethyl acetate/hexane = 1:10). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.39 (m, 5H), 6.41 (d, *J* = 8.4 Hz, 1H), 4.84 (m, 1H), 4.20 (dd, *J* = 10.0, 2.0 Hz, 1H), 3.77 (dd, *J* = 10.4, 4.0 Hz, 1H), 2.34 (m, 2H), 1.71 (m, 2H), 1.29 (m, 24H), 0.91 (s, 9H), 0.87 (t, *J* = 2.8 Hz, 3H), 0.06 (d, *J* = 2.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.3, 173.2, 134.8, 129.7, 129.4, 127.4, 63.7, 60.3, 36.9, 32.1, 29.9, 29.8, 29.8, 29.7, 29.5, 29.5, 26.0, 25.6, 22.9, 18.4, 14.3, -5.3. IR (neat, cm^{-1}) 3285 (m), 2926 (s), 2856 (s), 1702 (m), 1656 (m), 1116 (m), 837 (m). HRMS (FAB) Calcd for $C_{31}H_{56}NO_3SSi$ ($[M+H]^+$): 550.3744. Found: 550.3739.

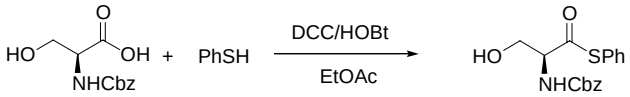
1-(*tert*-Butyl-dimethyl-silanyloxy)-2-(*S*)-hexadecanoylamino-octadec-4-en-3-one. [**18a**]



Following general procedure **A**, *N*-palmitoyl-*O*-TBS-L-Ser-SPh (130 mg, 0.24 mmol) was allowed to react with *E*-pentadecene boronic acid (104 mg, 0.40 mmol) for 10 hr at room temperature. The product was purified by flash chromatography (silica gel, hexane/ethyl acetate = 4:1) to give the above enone **18a** as a white powder. Yield: 94 mg (60%). Mp = 45-46 ºC. TLC (R_f = 0.6, silica gel, hexane/ethyl acetate = 20:3). ^{1}H NMR

(400MHz, $CDCl_3$) δ 6.99 (dt, *J* = 15.6, 6.8 Hz, 1H), 6.50 (d, *J* = 7.2 Hz, 1H), 6.25 (d, *J* = 15.6 Hz, 1H), 4.86 (m, 1H), 4.00 (dd, *J* = 10.4, 3.2 Hz, 1H), 3.83 (dd, *J* = 10.0, 4.0 Hz, 1H), 2.23 (m, 4H), 1.63 (m, 2H), 1.45 (m, 2H), 1.25 (m, 44H), 0.89 (m, 6H), 0.83 (s, 9H), -0.01 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.2, 173.0, 149.9, 127.0, 63.6, 58.3, 36.9, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 29.4, 28.2, 25.9, 22.9, 18.3, 14.3, -5.3. IR (neat, cm^{-1}) 3312 (w), 2926 (s), 2856 (s), 1656 (m), 1467 (m), 1112 (m), 837 (m). HRMS (FAB) Calcd for $C_{40}H_{80}NO_3Si$ ($[M+H]^+$): 650.5902. Found: 650.5875.

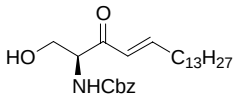
2-(*S*)-Benzyloxycarbonylamino-3-hydroxy-thiopropionic acid *S*-phenyl ester. [**14b**]



Thiophenol (606 mg, 5.5 mmol) was added to a solution of *N*-Cbz-L-serine (1196 mg, 5 mmol) and HOBt (1013 mg, 7.5 mmol) in dry ethyl acetate (15 mL) at 0 ºC, followed by the addition of 1,3-dicyclohexylcarbodiimide (DCC) (1135 mg, 5.5 mmol) slowly in small portions. The mixture was stirred for 16 hr at room temperature and the reaction progress was monitored by HPLC analyses. At the end of the reaction 1 mL of 50 % acetic acid in ethyl acetate was added. The reaction mixture was filtered through a short plug of Celite™ and washed by 1M HCl and then $NaHCO_3$ followed by concentration *in vacuo*. The crude product was triturated with hexanes to remove excess thiophenol. The resulting solid was dissolved in chloroform and crystallization was induced by addition of hexanes. After filtration and drying under vacuum, the *N*-Cbz-L-serine-SPh **14b** was obtained as a white solid. Yield: 780 mg (47%). Mp = 108-110 ºC. TLC (R_f = 0.40, silica gel, ethyl acetate/hexane = 1:1). ^1H NMR (400MHz, $CDCl_3$) δ 7.43-7.31 (m, 10H), 5.87 (d, *J* = 8.8 Hz, 1H), 5.20 (s, 2H), 4.64 (m, 1H), 4.17 (dd, *J* = 11.2, 2.8 Hz, 1H), 3.86 (dd,

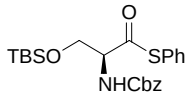
J = 11.6, 4.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.9, 156.3, 136.1, 134.8, 129.9, 129.5, 128.8, 128.5, 128.4, 126.9, 67.7, 63.2, 62.4. IR (neat, cm^{-1}) 3377 (br), 3065 (w), 2937 (w), 1698 (s), 1521 (s), 1254 (s), 1058 (s), 694 (m). HRMS (FAB) Calcd for $C_{17}H_{18}NO_4S$ ($[M+H]^+$): 332.0951. Found: 332.0944.

1-Hydroxy-2-(*S*)-benzyloxycarbonylamino-octadec-4-en-3-one. [**16b**]



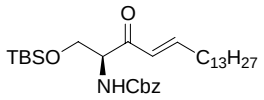
Following general procedure **A**, *N*-Cbz-L-Ser-SPh (30 mg, 0.06 mmol) was allowed to react with *E*-pentadecene boronic acid (31 mg, 0.11 mmol) for 10 hr at room temperature. The product was purified by flash chromatography (silica gel, hexane/ethyl acetate 2:1) to give the above enone **16b** as a white solid. Yield: 13 mg (40%). TLC (R_f = 0.5, silica gel, hexane/ethyl acetate = 2:1). Mp = 50-51 ºC. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.35 (m, 5H), 7.07 (dt, *J* = 15.6, 7.2 Hz, 1H),6.27 (d, *J* = 16.0 Hz, 1H), 6.00 (d, *J* = 6.0 Hz, 1H), 5.12 (s, 2H), 4.67 (m, 1H), 3.97 (dd, *J* = 12.0, 3.2 Hz, 1H), 3.89 (dd, *J* = 11.6, 4.4 Hz, 1H), 2.24 (q, *J* = 14.0, 7.2 Hz, 2H), 1.46 (m, 2H), 1.25 (m, 20H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.5, 156.8, 151.6, 136.2, 128.7, 128.4, 128.3, 126.3, 67.4, 64.1, 60.5, 33.0, 32.1, 29.8, 29.7, 29.5, 29.4, 28.1, 22.9, 14.3. IR (neat, cm^{-1}) 3381 (br), 2918 (s), 2853 (s), 1671 (s), 1532 (m), 1251 (m), 1058 (m). HRMS (FAB) Calcd for $C_{26}H_{42}NO_4$($[M+H]^+$): 432.3108. Found: 432.3097.

2-(*S*)-Benzyloxycarbonylamino-3-(*tert*-butyl-dimethyl-silanyloxy)-thiopropionic acid *S*-phenyl ester. [**17b**]



Following the general silylation procedure **B**, *N*-Cbz-L-Ser-SPh (340 mg, 1.0 mmol) was allowed to react with TBSCl (615 mg, 4.0 mmol) for 30 min to afford *N*-Cbz-*O*-TBS-Ser-SPh **17b** as a white powder. Yield: 378 mg (85%). Mp = 50-51 ºC. TLC (R_f = 0.3, silica gel, ethyl acetate/hexane = 1:20). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.42 (m, 5H), 5.82 (d, *J* = 8.8 Hz, 1H), 5.23 (s, 2H), 4.58 (dt, *J* = 8.8, 2.8 Hz, 1H), 4.22 (dd, *J* = 10.0, 3.2 Hz, 1H), 3.84 (dd, *J* = 3.6, 10.0 Hz, 1H), 0.92 (s, 9H), 0.07 (d, *J* = 2.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.9, 156.2, 136.3, 134.8, 129.7, 129.4, 128.8, 128.6, 128.5, 127.6, 67.6, 63.7, 62.5, 26.0, 18.5, -5.2, -5.3. IR (neat, cm^{-1}) 3443 (w), 2953 (m), 1702 (s), 1498 (s), 1212 (s), 1108 (s), 837 (m), 702 (m). HRMS (FAB) Calcd for $C_{23}H_{32}NO_4SSi$ ($[M+H]^+$): 446.1815. Found: 446.1832.

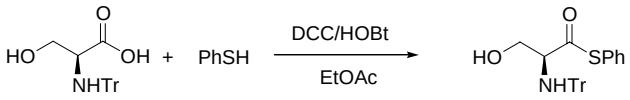
1-(*tert*-Butyldimethylsilanyloxy)-2-(*S*)-benzyloxycarbonylamino-octadec-4-en-3-one. [**18b**]



Following general procedure **A**, *N*-Cbz-*O*-TBS-L-Ser-SPh (71 mg, 0.16 mmol) was allowed to react with *E*-pentadecyne boronic acid (73 mg, 0.28 mmol) for 10 hr at room temperature. The product was purified by flash chromatography (silica gel, hexane/ethyl acetate 5:1) to give the above enone **18b** as a colorless oil. Yield: 74 mg (86%). TLC (R_f = 0.6, silica gel, hexane/ethyl acetate = 5:1). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.36-7.28 (m, 5H), 6.98 (dt, *J* = 15.6, 6.8 Hz, 1H), 6.28 (d, *J* = 15.6 Hz, 1H),5.82 (d, *J* = 7.6 Hz, 1H), 5.11 (s, 2H), 4.60 (m, 1H), 4.00 (dd, *J* = 3.2, 10.0 Hz, 1H), 3.85 (dd, *J* = 4.8, 10.4 Hz, 1H), 2.22 (q, *J* = 7.2, 14.0 Hz, 2H), 1.45 (m, 2H), 1.26 (m, 20H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.83 (s, 9H), -0.01 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.0, 156.0, 149.8, 136.6,

128.7, 128.3, 128.2, 126.8, 67.0, 63.7, 60.1, 32.8, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 28.2, 25.9, 22.9, 18.3, 14.3, -5.3, -5.4. IR (neat, cm^{-1}) 3428 (w), 2926 (s), 2856 (s), 1725 (s), 1698 (s), 1498 (s), 1112 (s), 837 (m), 689 (m). HRMS (FAB) Calcd for $C_{32}H_{56}NO_4Si$ ($[M+H]^+$): 546.3973. Found: 546.3967.

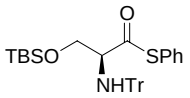
2-(*S*)-Tritylamino-3-Hydroxythiopropionic acid *S*-phenyl ester. [**14c**]



Thiophenol (220 mg, 2.0 mmol) was added to a solution of *N*-trityl-L-serine (697 mg, 2.0 mmol) in dry ethyl acetate (10 mL) at 0 ºC, followed by the addition of 1,3-dicyclohexyl-carbodiimide (DCC) (414 mg, 2.0 mmol) slowly in small portions. The mixture was stirred for 16 hr at room temperature and the reaction progress was monitored by HPLC analysis. At the end of the reaction 1 mL of 50 % acetic acid in ethyl acetate was added. The reaction mixture was filtered through a short plug of Celite™ and washed with 1M HCl then $NaHCO_3$. After concentration under vacuum the crude product was triturated with hexanes to remove excess thiophenol and then dissolved in MeOH. Crystallization was induced by the addition of water. After filtration and drying under vacuum, *N*-trityl-L-serine thiol phenyl ester **14c** was obtained as a white solid. Yield: 590 mg (67%). Mp = 167-169 ºC. TLC (R_f = 0.5, silica gel, ethyl acetate/hexanes = 4:1). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.61–7.12 (m, 20H), 3.68-3.64 (m, 1H), 3.55 (m, 1H), 3.18 (d, *J* = 8.8 Hz, 1H), 2.76 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.3, 146.1, 134.7, 129.4, 129.0, 128.8, 128.4, 127.0, 71.8, 64.8, 64.1. IR (neat, cm^{-1}) 3443 (br), 3323 (m), 3061 (m), 2937 (m), 1695 (s), 1447 (m), 1065 (m), 748 (m).

Attempted cross-coupling of this thiol ester returned only starting material.

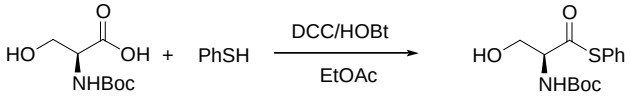
3-(*tert*-Butyl-dimethyl-silanyloxy)-2-(*S*)-tritylamino-thiopropionic acid *S*-phenyl ester. [**17c**]



Following the general silylation procedure **B**, *N*-trityl-L-Ser-SPh (100 mg, 0.23 mmol) was allowed to react with TBSCl (136 mg, 0.92 mmol) for 30 min to afford *N*-trityl-*O*-TBS-L-Ser-SPh **17c** as a colorless oil. Yield: 102 mg (82%). TLC (R_f = 0.8, silica gel, ethyl acetate/hexane = 1:2). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.67-7.22 (m, 20H), 3.58 (dd, *J* = 2.8, 10.0 Hz, 1H), 3.54 (s, 1H), 3.24 (d, *J* = 7.6 Hz, 1H), 2.17 (dd, *J* = 9.6, 3.6 Hz, 1H), 0.85 (s, 9H), -0.09 (s, 3H), -0.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.0, 146.6, 134.7, 130.2, 129.2, 129.1, 128.9, 128.4, 126.8, 71.9, 64.8, 63.9, 25.9, 18.4, -5.4, -5.4. IR (neat, cm^{-1}) 2930 (m), 1702 (s), 1467 (m), 1252 (m), 1092 (s), 837 (m), 706 (m).

Attempted cross-coupling of this thiol ester returned only starting material.

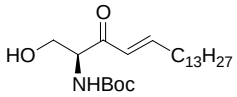
2-(*S*)-*tert*-Butoxycarbonylamino-3-hydroxy-thiopropionic acid *S*-phenyl ester, [**14d**]



Thiophenol (4.4 g, 40 mmol) was added to a solution of *N*-Boc-L-serine (4.1 g, 20 mmol) and HOBt (2.7 g, 20 mmol) in dry ethyl acetate (200 mL) at 0 ºC, followed by the addition of 1,3-dicyclohexylcarbodiimide (DCC) (4.3 g, 21 mmol) slowly in small portions. The mixture was stirred for 16 hr at room temperature and the reaction progress was monitored by HPLC analyses. At the end of the reaction 2 mL of 50 % acetic acid in ethyl acetate was added. The reaction mixture was filtered through a short plug of Celite™ and concentrated *in vacuo*. The crude product was triturated with hexanes and

the solid filtered to remove excess thiophenol. The resulting solid was dissolved in chloroform and crystallization was induced by the addition of hexanes. After filtration and drying at under vacuum, *N*-Boc-L-serine-SPh **14d** was obtained as a white solid. Yield: 5.3 g (89%). Mp = 133-135 ºC. TLC (R_f = 0.5, silica gel, ethyl acetate/hexane = 1:1). HPLC Chiral OD-RH, λ= 210 nm, Method: Flow: 1.0 mL/min; T= 30 °C; Isogradient: 40 % H_2O in CH_3CN for 17.0 min, L-isomer t_R = 9.2 min, D-isomer t_R = 10.4 min, ee > 99%. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.41 (m, 5H), 5.67 (d, *J* = 8.8 Hz, 1H), 4.55 (m, 1H), 4.13 (dd, *J* = 10.8, 2.8 Hz, 1H), 3.83 (dd, *J* = 11.2, 4.0 Hz, 1H), 1.50 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.6, 155.7, 134.8, 129.8, 129.5, 127.3, 80.9, 63.3, 62.1, 28.6. IR (neat, cm^{-1}) 3389 (br, m), 2980 (m), 1695 (s), 1505 (m), 1166 (m), 1058 (m), 748 (m). HRMS (FAB) Calcd for $C_{14}H_{20}NO_4S$ ($[M+H]^+$): 298.1113. Found: 298.1110. $[\alpha]^{20}_D$ = -85.2 (c = 0.99, $CHCl_3$).

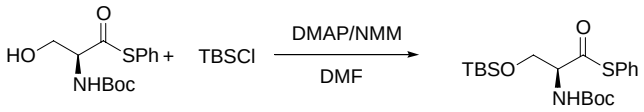
1-Hydroxy-2-(*S*)-*tert*-butoxycarbonylamino-octadec-4-en-3-one. [**16d**]



Following general procedure **A**, *N*-Boc-L-Ser-SPh (60 mg, 0.20 mmol) was allowed to react with *E*-pentadecene boronic acid (81 mg, 0.32 mmol) using THF/hexane (2 mL, 1:1) for 10 hr at room temperature. The product was purified by flash chromatography (silica gel, hexane/ethyl acetate = 1:1) to give the above enone **16d** as a colorless oil. Yield: 60 mg (75%). TLC (R_f = 0.45, silica gel, hexane/ethyl acetate = 1:1). ^{1}H NMR (600MHz, $CDCl_3$) δ 7.06 (dt, *J* = 15.6, 7.2 Hz, 1H), 6.27 (d, *J* = 16.0 Hz, 1H), 5.73 (d, *J* = 4.8 Hz, 1H), 4.62 (s, 1H), 3.93 (dd, *J* = 11.4, 2.4 Hz, 1H), 3.85 (dd, *J* = 12.0, 4.2 Hz, 1H), 2.84 (br s, 1H), 2.24 (app q, *J* = 7.2 Hz, 2H), 1.45 (m, 11H), 1.25 (m, 20H), 0.87 (t, *J* = 7.2 Hz,

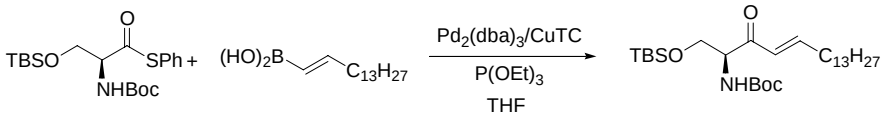
3H). ^{13}C NMR (100 MHz, $CDCl_3$) 196.0, 156.4, 151.2, 126.6, 80.5, 64.5, 60.1, 32.9, 32.1, 29.8, 29.7, 29.5, 29.4, 28.5, 28.1, 22.8, 14.3. IR (neat, cm^{-1}) 3385 (br), 2926 (s), 2856 (m), 1691 (m), 1170 (m). HRMS (FAB) Calcd for $C_{23}H_{44}NO_4$ ($[M+H]^+$): 398.3270. Found: 398.3278.

2-(*S*)-*tert*-Butoxycarbonylamino-3-(*tert*-butyldimethylsilanyloxy)-thiopropionic acid *S*-phenyl ester. [**17d**]



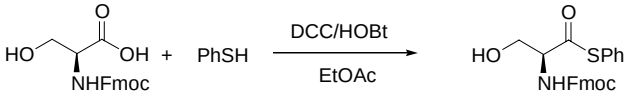
Following the general silylation procedure **B**, *N*-Boc-L-Ser-SPh (2.37 g, 8.0 mmol) was allowed to react with TBSCl (4.80 g, 32.0 mmol) for 30 min to afford *O*-TBS-*N*-Boc-L-serine thiophenyl ester **17d** as a colorless oil. Yield: 3.29 g (99%). TLC (R_f = 0.5, silica gel, ethyl acetate/hexane = 1:6). HPLC Chiral OD-RH, λ= 210 nm, Method: Flow: 1.0 mL/min; T= 30 °C; Isogradient: 50 % H_2O in CH_3CN for 17.0 min, L-isomer t_R = 12.7 min, D-isomer t_R = 13.2 min, ee > 99%. ^{1}H NMR (600MHz, $CDCl_3$) δ 7.39 (m, 5H), 5.52 (d, *J* = 9.0 Hz, 1H), 4.47 (m, 1H), 4.17 (dd, *J* = 10.2, 2.4 Hz, 1H), 3.79 (dd, *J* = 10.2, 3.6 Hz, 1H), 1.52 (s, 9H), 0.91 (s, 9H), 0.06 (d, *J* = 7.2 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.3, 155.4, 134.7, 129.4, 129.3, 127.8, 80.6, 63.6, 61.9, 28.5, 25.9, 18.4, -5.4. IR (neat, cm^{-1}) 2934 (m), 2860 (w), 1706 (s), 1490 (m), 1166 (m), 837 (m). HRMS (FAB) Calcd for $C_{20}H_{33}NO_4SSiNa$ ($[M+Na]^+$): 434.1792. Found: 434.1792. $[\alpha]^{20}_D$ = -79.5 (c = 1.97, $CHCl_3$).

1-(*tert*-Butyldimethylsilanyloxy)-2-(*S*)-*tert*-Butoxycarbonylamino-octadec-4-en-3-one. [**18d**]



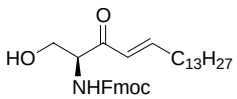
Following the general procedure **A**, *N*-Boc-*O*-TBS-L-Ser-SPh (2.30 g, 5.61 mmol) was allowed to react with *E*-pentadecyne boronic acid (2.41 g, 9.53 mmol) for 10 hr at room temperature. The crude product was further purified *via* flash chromatography (silica gel, hexane/ethyl acetate 20:3) to give enone **18d** as a colorless oil. Yield: 2.69 g (94%). TLC (R_f = 0.6, silica gel, hexane/ethyl acetate 20:3). HPLC Chiral OD-RH, λ = 210 nm, Method: Flow: 1.0 mL/min; T= 30 °C; Isogradient: 50 % H_2O in CH_3CN for 20.0 min, L-isomer t_R = 15.8 min, D-isomer t_R = 14.9 min, ee > 99%. ^{1}H NMR (400MHz, $CDCl_3$) δ 6.96 (dt, *J* = 16.0, 7.2 Hz, 1H), 6.26 (d, *J* = 16.0 Hz, 1H), 5.53 (d, *J* = 7.2 Hz, 1H), 4.53 (m, 1H), 3.96 (dd, *J* = 10.0, 3.2 Hz, 1H), 3.82 (dd, *J* = 10.0, 4.4 Hz, 1H), 2.21 (m, 2H), 1.44 (s, 11H), 1.25 (m, 20H), 0.87 (t, *J* = 6.8Hz, 3H), 0.83 (s, 9H), 0.00 (d, *J* = 2.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) 196.70, 155.56, 149.48, 127.10, 79.82, 63.88, 59.69, 32.85, 32.13, 29.86, 29.73, 29.61, 29.57, 29.42, 28.57, 28.21, 25.95, 22.90, 18.41, 14.33, -5.36. IR (neat, cm^{-1}) 2926 (s), 2856 (m), 1725 (s), 1698 (s), 1502 (s), 1254 (s), 1112 (s), 833 (s). HRMS (FAB) Calcd for $C_{29}H_{58}NO_4Si$ $[M+Li]^+$): 512.4129. Found: 512.4139. [α] $^{20}_D$ = +37.7 (c = 1.12, $CHCl_3$)

2-(*S*)-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxythiopropionic acid *S*-phenyl ester. [**14e**]



Thiophenol (330 mg, 3.0 mmol) was added to a solution of *N*-Fmoc-L-serine (654 mg, 2.0 mmol) in dry ethyl acetate (20 mL) at 0 ºC, followed by the addition of 1,3-dicyclohexylcarbodiimide (DCC) (454 mg, 2.2 mmol) slowly in small portions. The mixture was stirred for 16 hr at room temperature and the reaction progress was monitored by HPLC analysis. At the end of the reaction 1 mL of 50 % acetic acid in ethyl acetate was added. The reaction mixture was filtered through a short plug of Celite™ and washed with 1M HCl and then $NaHCO_3$. After concentration under vacuum the crude solid was triturated with hexanes to remove excess thiophenol and then dissolved in MeOH. Crystallization was induced by the addition of water. After filtration and drying under vacuum, *N*-Fmoc-L-serine thiol phenyl ester **14e** was obtained as a white solid. Yield: 648 mg (77%). Mp = 69-72 ºC. [lit: 129-130 ºC].[5] TLC (R_f = 0.55, silica gel, ethyl acetate/hexanes = 1:1). ^{1}H NMR (400MHz, $CDCl_3$) showing 2 rotamers δ 7.78-7.27 (m, 13.8 H), 6.53 (d, *J* = 9.2 Hz, 0.2H), 5.98 (d, *J* = 8.8 Hz, 0.8H), 5.85 (d, *J* = 8.0 Hz, 0.2 H), 4.63-4.36 (m, 3H), 4.29-4.05 (m, 2H), 3.88-3.82 (m, 1H), 2.35 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.0, 156.3, 143.8, 141.5, 134.8, 130.1, 129.9, 129.6, 129.5, 128.0, 127.3, 127.0, 125.3, 125.3, 120.2, 67.5, 63.1, 62.5, 47.4, 47.3. IR (neat, cm^{-1}) 3389 (br), 3065 (w), 2937 (w), 1695 (s), 1517 (m), 1251 (s), 1058 (s), 690 (m). HRMS (FAB) Calcd for $C_{24}H_{22}NO_4S$ ($[M+H]^+$): 420.1264. Found: 420.1265.

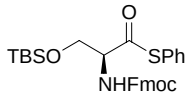
1-Hydroxy-2-(*S*)-(9*H*-fluoren-9-ylmethoxycarbonylamino)-octadec-4-en-3-one. [**16e**]



[5] Ishii, A.; Hojo, H.; Nakahara, Y.; Ito, Y.; Nakahara, Y. *Biosci. Biotech. Biochem.* **2002**, *66*, 225-232.

Following the general procedure **A**, *N*-Fmoc-L-Ser-SPh (130 mg, 0.31 mmol) was allowed to react with *E*-pentadecene boronic acid (127 mg, 0.50 mmol) for 10 hr at room temperature. The product was purified by flash chromatography (silica gel, hexane/ethyl acetate 2:1) to give the above enone **16e** as a white solid. Yield: 52 mg (32%). Mp = 63-66 ºC. TLC (R_f = 0.4, silica gel, hexane/ethyl acetate = 3:1). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.32 (d, *J* = 7.6 Hz, 2H), 7.09 (dt, *J* = 15.6, 7.2 Hz, 1H), 6.28 (d, *J* = 15.6 Hz, 1H), 5.99 (d, *J* = 6.0 Hz, 1H), 4.68 (m, 1H), 4.42 (d, *J* = 7.2 Hz, 2H), 4.23 (t, *J* = 7.2 Hz, 1H), 3.94 (m, 2H), 2.25 (m, 2H), 1.46 (m, 2H), 1.26 (m, 20H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) 195.5, 156.8, 151.7, 143.9, 141.5, 127.9, 127.3, 126.3, 125.2, 120.2, 67.4, 64.1, 60.4, 47.3, 33.0, 32.1, 29.8, 29.7, 29.6, 29.4, 28.1, 22.9, 14.3. IR (neat, cm^{-1}) 3405 (br), 2926 (s), 2853 (s), 1695 (s), 1513 (m), 1058 (m), 741 (m). HRMS (FAB) Calcd for $C_{33}H_{46}NO_4$ ($[M+H]^+$): 520.3421. Found: 520.3405.

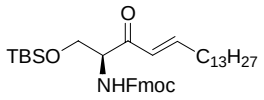
3-(*tert*-Butyl-dimethyl-silanyloxy)-2-(*S*)-(9*H*-fluoren-9-ylmethoxycarbonylamino)-thiopropionic acid *S*-phenyl ester. [**17e**]



Following the general silylation procedure **B**, *N*-Fmoc-L-Ser-SPh (118 mg, 0.28 mmol) was allowed to react with TBSCl (169 mg, 1.13 mmol) for 30 min to afford *N*-Fmoc-*O*-TBS-L-Ser-SPh **17e** as colorless oil. Yield: 52 mg (36%). TLC (R_f = 0.5, silica gel, ethyl acetate/hexane = 1:4). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.6 Hz, 2H), 7.67 (t, *J* = 8.4 Hz, 2H), 7.43-7.37 (m, 7H), 7.33 (t, *J* = 7.2 Hz, 2H), 5.80 (d, *J* = 8.8 Hz, 1H), 4.57 (m, 2H), 4.40 (t, *J* = 8.0 Hz, 1H), 4.34 (t, *J* = 7.2 Hz, 1H), 4.21 (dd, *J* = 10.0, 2.4 Hz, 1H),

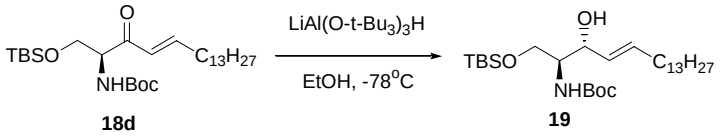
3.83 (dd, *J* = 3.6, 10.0 Hz, 1H), 0.94 (s, 9H), 0.09 (d, *J* = 5.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.8, 156.1, 144.0, 143.8, 141.5, 134.8, 129.7, 129.4, 128.0, 127.5, 127.3, 125.4, 125.3, 120.3, 67.7, 63.7, 62.4, 47.4, 26.0, 18.5, -5.3. IR (neat, cm^{-1}) 2953 (m), 1702 (s), 1498 (m), 1252 (m), 1108 (s), 837 (m), 706 (m). HRMS (FAB) Calcd for $C_{30}H_{36}NO_4SSi$ ($[M+H]^+$): 534.2128. Found: 534.2124.

1-(*tert*-Butyldimethylsilanyloxy)-2-(*S*)-(9*H*-fluoren-9-ylmethoxycarbonylamino)-octadec-4-en-3-one. [**18e**]



Following the general procedure **A**, *N*-Fmoc-*O*-TBS-L-Ser-SPh (35 mg, 0.06 mmol) was allowed to react with *E*-pentadecene boronic acid (30 mg, 0.11 mmol) for 10 hr at room temperature. The product was purified by flash chromatography (silica gel, hexane/ethyl acetate = 5:1) to give the above enone **18e** as a colorless oil. Yield: 30 mg (73%). TLC (R_f = 0.5, silica gel, hexane/ethyl acetate = 5:1). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.62 (t, *J* = 6.4 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), 7.01 (dt, *J* = 15.6, 6.8 Hz, 1H), 6.30 (d, *J* = 15.6 Hz, 1H), 5.88 (d, *J* = 7.6 Hz, 1H), 4.64 (m, 1H), 4.37 (d, *J* = 7.2 Hz, 2H), 4.24 (t, *J* = 6.8 Hz, 1H), 4.02 (dd, *J* = 10.0, 3.6 Hz, 1H), 3.88 (dd, *J* = 10.0, 4.4 Hz, 1H), 2.24 (m, 2H), 1.45 (m, 2H), 1.26 (m, 20H), 0.88 (t, *J* = 6.4 Hz, 3H), 0.86 (s, 9H), -0.02 (d, *J* = 2.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) 196.1, 156.0, 150.0, 144.1, 144.0, 141.5, 127.9, 127.2, 126.9, 125.4, 120.1, 67.2, 63.7, 60.0, 47.3, 32.9, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 28.2, 25.9, 22.9, 18.4, 14.3, -5.3. IR (neat, cm^{-1}) 3428 (w), 2926 (s), 2856 (s), 1725 (s), 1695 (s), 1502 (s), 1251 (s), 837 (m). HRMS (FAB) Calcd for $C_{39}H_{60}NO_4Si$ ($[M+H]^+$): 634.4286. Found: 634.4279.

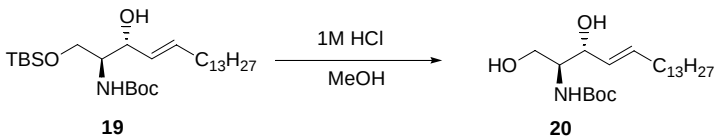
1-(*tert*-Butyl-dimethyl-silanyloxy)-2-(*S*)-*tert*-butyloxycarbonylamino-octadec-4-en-3-(*R*)-ol. [**19**]



18d **19**

To a solution of $LiAlH(O\text{-}t\text{-}Bu)_3$ (1.14 g, 4.4 mmol) in ethanol (20 mL) at -78 ºC was added dropwise a solution of **18d** (1.02 g, 2.0 mmol) in EtOH (10 mL). After 2 hr the reaction was quenched with HCl (0.1M). The reaction mixture was then diluted with 100 mL ethyl acetate. The organic layer was washed with $NaHCO_3$, brine and then dried over $MgSO_4$. The concentrated crude product was purified by a short flash column (silica gel, hexane/ethyl acetate = 10:1) to give the above alcohol **19** as a white waxy material. Yield: 988 mg (96%). TLC (R_f = 0.60, silica gel, hexane/ethyl acetate = 10:1). ^{1}H NMR (400MHz, $CDCl_3$) δ 5.75 (dt, *J* = 15.2, 6.8 Hz, 1H), 5.51 (dd, *J* = 15.2, 6.0 Hz, 1H), 5.24 (d, *J* = 8.0 Hz, 1H), 4.19 (t, *J* = 4.8 Hz, 1H), 3.94 (dd, *J* = 10.0, 3.0 Hz, 1H), 3.82 (d, *J* = 7.6 Hz, 1H), 3.56 (m, 1H), 2.05 (app q, *J* = 6.8 Hz, 2H), 1.45 (s, 9H), 1.37 (m, 2H), 1.33 (m, 20H), 0.88 (m, 12H), 0.07 (d, *J* = 1.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 133.3, 129.6, 79.7, 74.9, 63.7, 54.6, 32.5, 32.1, 29.9, 29.7, 29.6, 29.4, 28.6, 26.0, 22.9, 18.3, 14.3, -5.4, -5.4. IR (neat, cm^{-1}) 3451 (br), 2926 (s), 1718 (m), 1502 (m), 1254 (m), 1173 (m), 837 (m). HRMS (FAB) Calcd for $C_{29}H_{60}NO_4Si$ ($[M+H]^+$): 514.4286. Found: 514.4282. $[\alpha]^{20}_D$ = +11.0 (c = 0.91, $CHCl_3$). [Lit: $[\alpha]^{23.8}_D$ = +11.9 (c = 1.02, $CHCl_3$)].[7]

***N*-Boc-sphingosine.** [**20**]



19 **20**

To a solution of **19** (422 mg, 0.82 mmol) in methanol (5 mL) was added 1M HCl (5 mL) at 0 ºC. The reaction mixture was then stirred at room temperature for another 30 min. The solution was diluted with ethyl acetate and the organic layer was washed with $NaHCO_3$, brine, and dried over $MgSO_4$. Concentration under vacuum gave the above diol **20** as a white powder. Yield: 325 mg (99%). Mp = 63–64 ºC. [lit: 64-66 ºC].[6] TLC (R_f = 0.3, silica gel, hexane/ethyl acetate 1:1). HPLC Chiral AS-RH, λ= 210 nm, Method: Flow: 0.65 mL/min; T= 30 °C; Isogradient: 50 % H_2O in CH_3CN for 20.0 min, 2*S*, 3*R*-isomer t_R = 25.9 min, 2*R*, 3*S*-isomer (enantiomer) t_R = 23.1 min, ee > 99%. de > 94% (by NMR). After recrystalliztion from isopropyl ether (243 mg from 325 mg). HPLC Chiral OD-RH, λ= 210 nm, Method: Flow: 1.0 mL/min; T= 30 °C; Isogradient: 50 % H_2O in CH_3CN for 20.0 min, 2*S*, 3*R*-isomer t_R = 29.2 min, 2*S*, 3*S*-isomer (diastereomer) t_R = 31.4 min, de > 99% (by NMR). ^{1}H NMR (600 MHz $CDCl_3$) δ 5.77 (dt, *J* = 15.6, 7.8 Hz, 1H), 5.26 (dd, *J* = 15.6, 6.6 Hz, 1H), 5.32 (d, *J* = 7.2 Hz, 1H), 4.30 (s, 1H), 3.92 (dd, *J* = 11.4, 3.6 Hz, 1H), 3.69 (dd, *J* = 11.4, 3.0 Hz, 1H), 3.59 (s, 1H), 2.85 (br, 2H), 2.21 (dd, *J* = 14.4, 7.2 Hz, 2H), 1.44 (s, 9H), 1.35 (m, 2H), 1.25 (m, 20H), 0.87 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 134.4, 129.1, 80.0, 75.2, 62.9, 55.5, 32.5, 32.1, 29.9, 29.8, 29.7, 29.6, 29.4, 29.3, 28.6, 22.9, 14.3. IR (neat, cm^{-1}) 3347 (br), 2926 (s), 1718 (m), 1502 (m), 1254 (m), 1173 (m), 837 (m). HRMS (FAB) Calcd for $C_{23}H_{46}NO_4$ ($[M+H]^+$): 400.3421. Found: 400.3420. $[\alpha]^{20}_D$ = -1.5 (c = 1.12, $CHCl_3$) [Lit: -2.3 c = 0.88 $CHCl_3$].[7]

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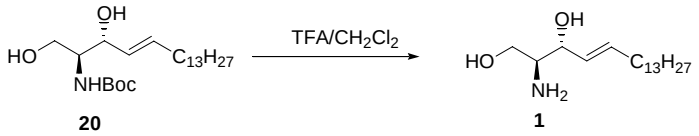
[6] Merino, P.; Jimenez, P.; Tejero, T. *J. Org. Chem*. **2006**, *71*, 4685-4688.

[7] Yamamoto, T.; Hasegawa, H.; Hakogi, T.; Katsumura, S. *Org. Lett.* **2006**, *8*, 5569-5572.

Sphingosine. [**1**]



20 **1**

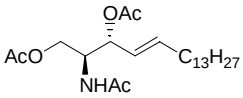
To a solution of **20** (200 mg, 0.5 mmol) in methylene chloride (5 mL) was added TFA (5 mL) at 0 ºC. The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under vacuum and the crude material was dissolved in methanol followed by evaporation under vaccum. This process was repeated three times to remove excess TFA. The crude TFA salt was then dissolved in 0.5 mL methanol and mixed with 20 mL 1M NaOH. This mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic layers were concentrated *in vacuo* to afford the crude product. Further purification was accomplished by flash chromatography (silica gel, $CHCl_3/CH_3OH/NH_4OH$ = 135:25:4) to give **1** as a white solid. Yield: 132 mg (90%). Mp = 72-73 ºC. [Lit: 75-80 ºC[8], 73-75ºC.][9] TLC (R_f = 0.3, silica gel, $CHCl_3/CH_3OH/ NH_4OH$ = 135:25:4) ^{1}H NMR (400 MHz $CDCl_3$) δ 5.73 (dt, *J* = 15.2, 7.4 Hz, 1H), 5.45 (dd, *J* = 15.2, 6.8 Hz, 1H), 4.0 (s, 1H), 4.30 (s, 1H), 3.65 (m, 2H), 2.83 (s, 1H), 2.68 (br s, 4H), 2.04 (q, *J* = 7.2 Hz, 2H), 1.37 (m, 2H), 1.25 (m, 20H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.8, 129.4, 75.3, 63.9, 56.3, 32.6, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 22.9, 14.3. IR (neat, cm^{-1}) 3366 (br), 2918 (s), 2853 (m), 1467 (m), 1046 (m), 968 (m). HRMS (FAB) Calcd for $C_{18}H_{38}NO_2$ ($[M+H]^+$): 300.2897. Found: 300.2895. [α]

[8] Duclos R. I. *Chem. Phys. Lipids*. **2001**, *111*, 111-138.

[9] Olofsson, B.; Somfai, P. *J. Org. Chem.* **2003**, *68*, 2514-2517.

$^{20}_{D}$ = -1.4 (c = 1.5, $CHCl_3$). [Lit: -1.4 (c = 0.42, $CHCl_3$)[10], -1.2 (c = 1.74, $CHCl_3$),[11] -2.9 (c = 1.0, $CHCl_3$)[12]].

***N, O, O* – Triacetyl- D-*erythro*-sphingosine** [**21**]



Following the reported method, [14] D-*erythro*-sphingosine triacetate was synthesized from D-*erythro*-sphingosine as a white solids. Mp = 102-103 ºC. [Lit: 101-102 ºC][14]. TLC (R_f = 0.4, silica gel, EtOAc/hexanes = 1:1) ^{1}H NMR (400 MHz $CDCl_3$) δ 5.76 (dt, *J* = 15.6, 7.4 Hz, 1H), 5.65 (d, *J* = 8.8 Hz, 1H), 5.36 (dd, *J* = 15.2, 7.2 Hz, 1H), 5.26 (m, 1H), 4.40 (m, 1H), 4.28 (dd, *J* = 11.2, 6.0 Hz, 1H), 4.01 (dd, *J* = 12.0, 4.0 Hz, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (m, 2H), 1.96 (s, 3H), 1.34-1.23 (m, 22H), 0.85 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 170.2, 169.9, 137.7, 124.2, 74.0, 62.8, 50.8, 32.5, 32.1, 29.9, 29.8, 29.6, 29.5, 29.3, 29.1, 23.6, 22.9, 21.3, 21.0, 14.4. IR (neat, cm^{-1}) 3289 (m), 2922 (s), 2853 (m), 1737 (s), 1656 (m), 1552 (m), 1231 (s). HRMS (FAB) Calcd for $C_{24}H_{44}NO_5$ ($[M+H]^+$): 426.3214. Found: 426.3212. $[\alpha]^{20}_{D}$ = -13.3 (c = 0.95, $CHCl_3$). [Lit: -13.2 (c = 1.0, $CHCl_3$)[10], -13.0 (c = 1.6, $CHCl_3$),[13] -13.2 (c = 1.04, $CHCl_3$)[14]].

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[10] Van den Berg, R. J. B. H. N.; Korevaar, C. G. N.; Overkleeft, H. S.; Van der Marel, G. A.; Van Boom, J. H. *J. Org. Chem.* **2004**, *69*, 5699-5704.

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[12] Lu, X.; Bittman, R. *Tetrahedron Lett.* **2005**, *46*, 1873-1876

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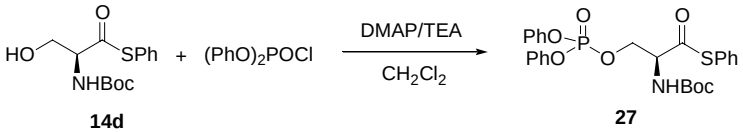
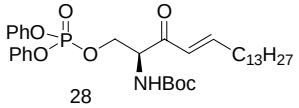
2-(*S*)-*tert*-Butoxycarbonylamino-3-(diphenoxyphosphoryloxy)-thiopropionic acid *S*-phenyl ester. [**27**]



14d **27**

To a solution of *N*-Boc-L-Ser-SPh (60 mg, 0.2 mmol), diphenylchlorophosphate (108 mg, 0.4 mmol) and DMAP (2.4 mg, 0.02 mmol) in CH_2Cl_2 (2 mL) was added triethylamine (24 mg, 0.24 mmol). The reaction mixture was stirred in room temperature for 3 hr. The reaction mixture was then diluted with ethyl acetate (10 mL) and washed with HCl (1M) and $NaHCO_3$. The crude product was further purified by flash chromatography (silica gel, hexane / ethyl acetate = 2:1) give the desired product **27** as a colorless oil. Yield: 54 mg (51%). TLC (R_f = 0.5, silica gel, hexane/ethyl acetate = 2:1). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.44-7.31 (m, 9H), 7.22-7.19 (m, 6H), 5.63 (d, J = 9.2 Hz, 1H), 4.76 (m, 2H), 4.46 (m, 1H), 1.49 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.4, 155.2, 150.5 (d, $J_{c\text{-}p}$ = 7.4 Hz), 134.8, 130.1 (d, $J_{c\text{-}p}$ = 2.2 Hz), 129.8, 129.5, 127.0, 125.9 (d, $J_{c\text{-}p}$ = 4.5 Hz), 120.3 (t, $J_{c\text{-}p}$ = 4.5 Hz), 81.2, 68.6 (d, $J_{c\text{-}p}$ = 5.9 Hz), 60.2 (d, $J_{c\text{-}p}$ = 7.4 Hz), 28.5. ^{31}P NMR (161.89 MHz, $CDCl_3$) δ -11.11. IR (neat, cm^{-1}) 1706 (s), 1490 (s), 1189 (s), 957 (s), 690 (m). HRMS (FAB) Calcd for $C_{26}H_{29}NO_7PS$ ($[M+H]^+$): 530.1397. Found: 530.1394.

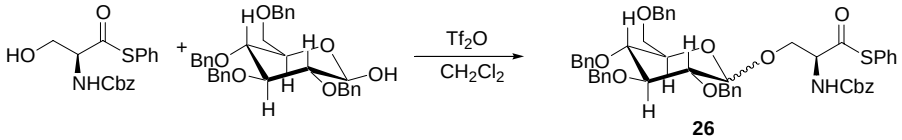
Phosphoric acid 2-(*S*)-*tert*-butoxycarbonylamino-3-oxo-octadec-4-enyl ester diphenyl ester. [**28**]



28

Following the general procedure **A** described above, *N*-Boc-Ser-SPh diphenyl phosphate **27** (37.5 mg, 0.07 mmol) was allowed to react with *E*-pentadecene boronic acid(36 mg, 0.13 mmol) in THF/hexane (2 mL, 1:1) for 4 hr at room temperature. The product was purified by flash chromatography (silica gel, hexane/ethyl acetate = 2:1) to give the above enone **28** as a colorless oil. Yield: 33 mg (76%). TLC (R_f = 0.5, silica gel, hexane/ethyl acetate = 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.30 (m, 4H), 7.20-7.14 (m, 6H), 6.98 (dt, *J* = 15.6, 6.8 Hz, 1H), 6.21 (d, *J* = 15.6 Hz, 1H), 5.57 (d, *J* = 7.2 Hz, 1H), 4.73 (m, 1H), 4.55 (m, 2H), 2.18 (m, 2H), 1.42 (s, 11H), 1.25 (s, 20H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) 194.1, 155.3, 151.4, 150.5, 130.0 (d, $J_{c\text{-}p}$ = 4.4 Hz), 125.9, 125.7, 120.3 (d, $J_{c\text{-}p}$ = 5.1 Hz), 80.4, 68.3 (d, $J_{c\text{-}p}$ = 6.0 Hz), 57.8 (d, $J_{c\text{-}p}$ = 8.2 Hz), 32.9, 32.1, 29.8, 29.7, 29.6, 29.4, 28.4, 28.0, 22.9, 14.3. ^{31}P NMR (161.89 MHz, $CDCl_3$) δ -11.18. IR (neat, cm^{-1}) 2926 (s), 2853 (s), 1698 (s), 1529 (s), 1193 (s), 961 (s), 687 (m). HRMS (FAB) Calcd for $C_{35}H_{53}NO_7P$ ($[M+H]^+$): 630.3554. Found: 630.3551.

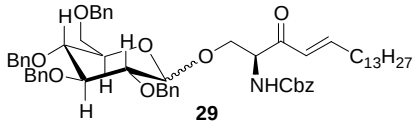
2-(*S*)-Benzyloxycarbonylamino-3-(3,4,5-*tris*-benzyloxy-6-benzyloxymethyl-tetrahydro-D-pyran-2-yloxy)-thiopropionic acid *S*-phenyl ester. [**26**]



To a solution of *N*-Cbz-L-serine (66 mg, 0.2 mmol) and 2,3,4,6-*tetra*-*O*-benzyl-D-glucopyranose (108 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) was added Tf_2O (56 mg, 33 μL) dropwise at room temperature. After stirring for 1 hr, the reaction mixture was washed with $NaHCO_3$ and brine. The organic solution was concentrated *in vacuo*. The crude product was further purified by flash chromatography (silica gel, hexane/ethyl acetate = 2:1) to give the above thiol ester **26** as a colorless oil. Yield: 80 mg (47%). TLC (R_f =

0.40, silica gel, hexane/ethyl acetate = 2:1). ^{1}H NMR (400MHz, $CDCl_3$) δ showing two diastereomers 7.39-7.10 (m, 30H), 6.08 (d, *J* = 9.2 Hz, 0.6 H), 5.99 (d, *J* = 8.4 Hz, 0.3H), 5.16 (m, 2H), 4.95-4.33 (m, 10H), 4.16 (dd, *J* = 11.2, 3.6 Hz, 1H), 3.95-3.42 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ showing two diastereomers 198.1, 197.7, 156.3, 156.2, 138.8, 138.7, 138.3, 138.2, 138.0, 136.2, 134.9, 134.8, 129.7, 129.4, 128.8, 128.7, 128.6, 128.5, 128.2, 128.1, 128.1, 127.9, 127.8, 127.8, 127.2, 103.8, 98.7, 84.7, 82.0, 81.9, 80.0, 75.9, 75.8, 75.4, 75.3, 75.1, 73.7, 73.1, 71.2, 69.5, 69.2, 68.8, 68.4, 67.7, 67.6, 65.6, 61.3, 61.1. IR (neat, cm^{-1}) 3088 (m), 3065 (m), 3034 (m), 2922 (m), 2868 (m), 1725 (s), 1702 (s), 1498 (m), 1251 (m), 1069 (s), 698 (m). HRMS (FAB) Calcd for $C_{51}H_{52}NO_9S$ ($[M+H]^+$): 854.3357. Found: 854.3354.

2-(*S*)-Benzyloxycarbonylamino-1-(3,4,5-*tris*-benzyloxy-6-benzyloxymethyl-tetrahydro-D-pyran-2-yloxy)-octadec-4-en-3-one. [**29**]

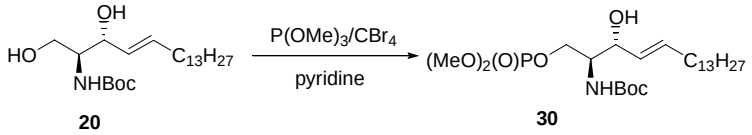


29

Following the general procedure **A** described above, thiol ester **26** (33 mg, 0.04 mmol) was allowed to react with *E*-pentadecene boronic acid (18 mg, 0.07 mmol) in THF/hexane (2 mL, 1:1) for 4 hr at room temperature. The product was further purified *via* flash chromatography (silica gel, hexane/ethyl acetate = 2:1) to give the above enone **29** as a colorless oil. Yield: 26 mg (75%). TLC (R_f = 0.65, silica gel, hexane/ethyl acetate = 2:1). ^{1}H NMR (400MHz, $CDCl_3$) δ showing two diastereomers 7.34-7.19 (m, 23H), 7.13-7.08 (m, 2H), 7.00 (m, 1H), 6.30 (d, *J* = 15.6 Hz, 0.6H), 6.28 (d, *J* = 15.6 Hz, 0.4H), 6.00 (d, *J* = 7.6 Hz, 0.6H), 5.91 (d, *J* = 7.2 Hz, 0.4H), 5.09 (m, 2H), 4.89 (m, 1H), 4.82-4.26 (m, 9H), 3.97 (dd, *J* = 3.6, 10.8 Hz, 0.6H), 3.89 (dd, *J* = 10.4, 4.4 Hz, 0.4H), 3.80 (m,

1H), 3.68-3.49 (m, 5H), 3.36 (m, 1H), 2.14 (m, 2H), 1.38-1.21 (m, 22H), 0.87 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.6, 156.1, 150.6, 138.9, 138.4, 138.1, 137.4, 136.5, 128.7, 128.6, 128.6, 128.3, 128.1, 127.9, 127.8, 126.5, 104.3, 98.5, 84.6, 82.0, 80.1, 75.9, 75.0, 74.7, 73.7, 73.2, 70.9, 69.2, 68.4, 67.2, 58.6, 32.9, 32.1, 29.8, 29.7, 29.6, 29.5, 28.1, 22.9, 14.3. IR (neat, cm^{-1}) 3034 (w), 2926 (s), 2856 (s), 1725 (m), 1698 (m), 1069 (s), 698 (m). HRMS (FAB) Calcd for $C_{60}H_{76}NO_9$ ($[M+H]^+$): 954.5514. Found: 954.5534.

Dimethyl-2-(*S*)-(*tert*-butyloxycarbonylamino)-3-(*R*)-hydroxy-4-pentenylphosphate. [**30**]



20 **30**

To a mixture of **20** (104 mg, 0.26 mmol) and carbon tetrabromide (122 mg, 0.36 mmol) was added pyridine (1 mL) at 0 ºC. $P(OMe)_3$ (45 mg, 43 μL) was then added dropwise slowly. The reaction mixture was stirred at 0 ºC for 30 min followed by slow warming to room temperature. After 3 hr the reaction mixture was diluted with ethyl acetate and washed with 1M HCl, $NaHCO_3$ and dried over $MgSO_4$. The organic layer was concentrated in vacuum and purified by flash chromatography (silica gel, ethyl acetate/hexane = 2:1) to give the above alcohol **30** as a colorless oil. Yield: 110 mg (86%). TLC (R_f = 0.25, silica gel, hexane/ethyl acetate = 1:1) ^{1}H NMR (400 MHz $CDCl_3$) δ 5.74 (dt, *J* = 14.8, 6.8 Hz, 1H), 5.48 (dd, *J* = 15.2, 7.2 Hz, 1H), 5.05 (d, *J* = 9.2 Hz, 1H), 4.31 (m, 1H), 4.12 (m, 2H), 3.77 (dd, *J* = 10.8, 2.0 Hz, 7H), 2.75 (br s, 1H), 2.02 (app q, *J* = 6.4 Hz, 2H), 1.42 (s, 9H), 1.35 (m, 2H), 1.25 (m, 20H), 0.86 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) 155.8, 135.0, 128.6, 79.9, 72.6, 66.9 (d, $J_{c\text{-}p}$ = 5.2 Hz), 55.0, 54.8 (d, $J_{c\text{-}p}$ = 6.0 Hz), 32.5, 32.1, 29.8, 29.8, 29.7, 29.5, 29.4, 29.3, 28.5, 22.9, 14.3. ^{31}P

NMR (161.89 MHz, $CDCl_3$) δ 2.87. IR (neat, cm^{-1}) 3362 (br), 2926 (s), 2856 (m), 1714 (s), 1529 (m), 1251 (s), 1038 (s). HRMS (FAB) Calcd for $C_{25}H_{51}NO_7P$ ($[M+H]^+$): 508.3397. Found: 508.3394. $[\alpha]^{20}_D$ = +4.7 (c = 1.1, $CHCl_3$). [Lit: +4.3 (c = 1.0, $CHCl_3$)].[15]

Sphingosine-1-phosphate. [**31**]

With protection from light a solution of **30** (110 mg, 0.22 mmol) in acetonitrile (4 mL) was treated with bromotrimethylsilane (118 mg, 100 μL) at room temperature. After stirring for 2 hr, the reaction mixture was diluted with methylene chloride and concentrated *in vacuo*. The crude material was added to hot acetic acid followed by the addition of ice water. A white precipitate formed. The solution was centrifuged and the solvent was removed. The resulting precipitate was washed with water followed by centrifuging to form a white pellet. Following the same procedure (washing/centrifuging), the white pellet was worked up using acetone/water sequence for two times to give pure sphingosine-1-phosphate **31** (53 mg, 62%) as white powder. Mp > 200 ºC. TLC (R_f = 0.55, silica gel, *n*-BuOH/H_2O/AcOH = 5:1:1) ^{1}H NMR (600 MHz CD_3OD) δ 5.84 (dt, *J* = 14.4, 7.2 Hz, 1H), 5.45 (dd, *J* = 15.0, 6.6 Hz, 1H), 4.24 (t, *J* = 5.4 Hz, 1H), 4.06 (m, 1H), 3.96 (m, 1H), 3.32 (m, 1H), 2.07 (app q, *J* = 7.2 Hz, 2H), 1.40 (m, 2H), 1.26 (m, 20H), 0.87 (t, *J* = 6.6 Hz, 3H). ^{1}H NMR (600 MHz CD_3COOD) δ 5.98 (dt, *J* = 15.6, 6.6 Hz, 1H), 5.61 (dd, *J* = 15.6, 6.6 Hz, 1H), 4.55 (m, 1H), 4.32 (m, 2H), 3.75 (m, 1H), 2.15 (m,

[15] Szulc, Z. M.; Hannun, Y. A.; Bielawska, A. *Tetrahedron Lett.* **2000**, *41*, 7821-7824

2H), 1.54-1.36 (m, 22H), 0.95 (t, J = 6.6 Hz, 3H). ^{13}C NMR (125 MHz, CD_3COOD) δ 137.5, 126.9, 70.7, 62.7, 57.2, 33.5, 33.1, 32.8, 32.1, 30.6, 30.5, 30.5, 30.3, 29.7, 23.5, 14.4. IR (neat, cm^{-1}) 3435 (br), 2916 (s), 2848 (m), 1029 (s), 925 (s). HRMS (FAB) Calcd for $C_{18}H_{39}NO_5P$ ($[M+H]^+$): 380.2560. Found: 380.2556. $[\alpha]^{20}_D$ = -3.1 (c = 0.28, CH_3COOH). [Lit: -1.22 (c = 0.4, CH_3COOH)][16].

[16] Lim, H-S.; Oh, Y-S.; Suh, P-G.; Chung, S-K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 237-240.