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Mapping the Pathway toward Thiophosphinic Pseudopeptides. Synthesis of Suitably Protected PG-Phe-Ψ[P(S)(OX)CH_2]-Gly-OY Analogues as Thiophosphinyl Dipeptide Isosters (TDI), a Comparative Study for Selective Deprotection and Further Elongation

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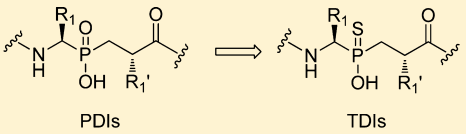
Supporting Information

ABSTRACT: In the present study, we describe in detail the first synthesis of a new class of phosphorus compounds, thiophosphinyl pseudopeptides. We prepared several fully protected thiophosphinate pseudodipeptides of the general formula PG-Phe-Ψ[P(S)(OX)CH_2]-Gly-OY starting from the corresponding phosphinate pseudodipeptide using Lawesson's reagent. Selective deprotection, further elongation, and stability of these compounds were studied, and the results are disclosed. These compounds can be used as transition-state-mimicking inhibitors for several zinc metalloproteases.



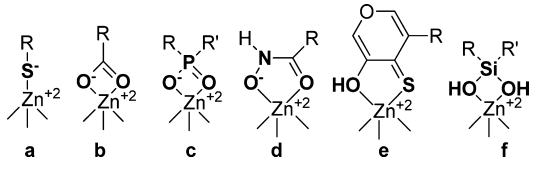
INTRODUCTION

The backbone modification of bioactive peptides with replacement of the scissile peptide bond in enzymatic hydrolysis is a well-established strategy for developing protease inhibitors.[1,2] In particular, for zinc metalloproteases, which contain a zinc atom in their active site, several successful modifications have been reported over the past years.[3,4]

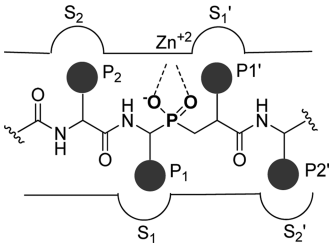
The presence of a zinc ion in the active site of these proteases and the functional role of this atom in substrate hydrolysis led to the suggestion that potent synthetic inhibitors could be developed by grafting to short peptide sequences chemical groups able to interact potently with the zinc ion. The choice of a particular zinc-binding group may critically determine the inhibitor selectivity profile. In fact, the contribution of the zinc-binding group to overall inhibitor affinity depends on its chemical structure and differs for each zinc-binding group (Scheme 1).

An important property of the zinc-binding group, depending on its chemistry, is the ability to use both primed and nonprimed subsites when developing inhibitors (Scheme 2). With the exception of phosphinic and silanediol groups, such inhibitor structures cannot be prepared when other zinc-binding groups are used. Exploiting both the primed and nonprimed subsites of the enzyme active site can be critical to the control of both inhibitor potency and selectivity.

Scheme 1. Six Zinc-Binding Groups Shown in Bold [(a) Thiolate, (b) Carboxylate, (c) Phosphinate, (d) Hydroxamate, (e) Hydroxythiopyrone, and (f) Diolsilane)] in Complex with Zinc Ion



Scheme 2. Representation of the Subsites of a Zinc Protease and a Phosphinic Peptide Sequence



Phosphinic pseudopeptides are among the best candidates when addressing the challenge to potent and selectively inhibit zinc proteases.[5−7] Phosphinyl dipeptide isosters (PDIs) (Scheme 3) are the building blocks for the synthesis of phosphinic pseudopeptides, and their chemistry is well documented.[8−10] PDIs, presented as NH_2XaaΨ[P(O)(OH)-CH_2]XaaOH, mimic the transition state for tetrahedral geometry of a scissile peptide bond during enzymatic hydrolysis.

In contrast to phosphinyl dipeptide isosters, there is no scientific report concerning thiophosphinyl dipeptide isosters (TDIs). A thorough overview of the literature[11] revealed the absence of any synthetic method providing this class of

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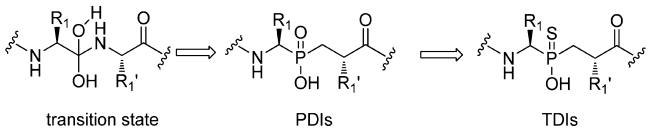
Scheme 3. Similarity between the Structure of a Peptidic Sequence in the so-Called Tetrahedral Intermediate, a Phosphinic Dipeptide Isoster (PDI), and a Thiophosphinic Dipeptide Isoster (TDI)



compounds. The only relevant approaches dealt with the synthesis of Cbz-GlyΨ[P(S)(OH)]CH_3[12] and Cbz-GlyΨ[P(S)(OH)]CH_2OH[13] as a potent inhibitors of bacterial ureases.

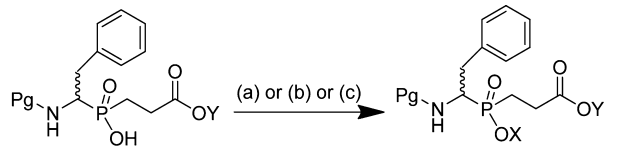
Herein, we present the first synthesis of fully protected thiophosphinate pseudodipeptides of the general formula PG-Phe-Ψ[P(S)(OX)CH_2]-Gly-OY, a systematic deprotection study, as well as further elongation and stability behavior.

RESULTS AND DISCUSSION

Phosphinic pseudodipeptides (Scheme 4) are typically defined as dipeptide analogues that replace the amide bond with the phosphinate moiety. The most traditional approach to obtain a pseudodipeptide skeleton involves the phospha-Michael addition of a suitably *N*-protected *α*-amino-*H*-phosphinic acid to an acrylate. The *H*-phosphinic acid requires activation to the more nucleophilic tervalent ester form that is typically achieved in the presence of a silylation agent (Scheme 4).[14]

The phosphinic pseudodipeptide scaffold Phe-Ψ[P(O)(OH)CH_2]-Gly was used as a template in this study. PG-Phe-Ψ[P(O)(OH)CH_2]-Gly-OY **1**, **2**, **3**, and **4** were prepared using the well-described procedures[15,16] based on the Michael addition of a disilyl phosphonite to a Michael acceptor. Special citation is needed concerning the synthesis of Cbz-Phe-Ψ[P(O)(OH)CH_2]-Gly-OAllyl **5**. This compound was prepared using TMSCl as silylating agent, allyl acrylate as Michael acceptor, and imidazole as base to avoid the corresponding rearrangement product.[17] Two protecting groups, methyl (for **6**, **8**, **10**, **11**) and adamantyl (for **7** and **9**) covering the alkaline and the acidic removal, respectively, were used for the esterification of the hydroxyphosphinyl group. Introduction of the methyl group was achieved by warming **1**, **4**, and **5** (Scheme 5) with trimethylphosphite $P(OCH_3)_3$ at 90 °C for 10 h, providing the corresponding esters in excellent yield.[18] Exceptionally, carbodiimide-mediated esterification of **2** with methanol furnished **8**. Introduction of the adamantyl group, particularly useful in the phosphinopeptide chemistry, was feasible by mixing **1** and **3** with 1-adamantyl bromide in refluxing chloroform followed by portion-wise addition of silver(I) oxide.[16] Because of the two chiral centers, P and C atoms, in all cases **6**–**11**, mixtures of diastereomers were obtained, the ratio being around 70/30, as judged by ^{31}P NMR.

Scheme 5. Synthesis of Protected Phosphinates[a]



1: Pg=Cbz, Y=Et
2: Pg=Fmoc, Y=*t*-Bu
3: Pg=Boc, Y=Et
4: Pg=Cbz, Y=*t*-Bu
5: Pg=Cbz, Y=Allyl

6: Pg=Cbz, X=Me, Y=Et
7: Pg=Cbz, X=Ad, Y=Et
8: Pg=Fmoc, X=Me, Y=*t*-Bu
9: Pg=Boc, X=Ad, Y=Et
10: Pg=Cbz, X=Me, Y=*t*-Bu
11: Pg=Cbz, X=Me, Y=Allyl

[a]Reagents and conditions: (a) $P(OCH_3)_3$, 90 °C, 10 h, 93% for **6**, 91% for **10**, 93% for **11**; (b) 1-Ad-Br, Ag_2O, $CHCl_3$, reflux, 3 h, 82% for **7**, 81% for **9**; (c) EDC·HCl, MeOH, CH_2Cl_2, rt, 1 h, 91% for **8**.

Then, we focused on establishing a synthetic methodology to obtain PG-Phe-Ψ[P(S)(OX)CH_2]-Gly-OY. Starting point seemed to be the corresponding phosphinate, and two thionating agents, P_4S_{10}[19] and Lawesson's reagent[20] (LR), were the most promising to be tested. These two thionating agents have been extensively used to transform mainly carbonyl to thiocarbonyl compounds as well as for the synthesis of a wide range of heterocyclic compounds having a sulfur atom. Generally it is claimed that LR has advantages over P_4S_{10} in terms of requirements for excess P_4S_{10} and longer reaction time. This was confirmed in our case, since in initial experiments using Cbz-Phe-Ψ[P(O)(OMe)CH_2]-Gly-OEt and P_4S_{10} in warm toluene, no thionation product was observed. By contrary, using LR in warm toluene the desired product Cbz-Phe-Ψ[P(S)(OMe)CH_2]-Gly-OEt appeared both in mass spectrometry and phosphorus NMR.

Because Lawesson's reagent is also the reagent of choice for transformations of functional groups such as urethanes and esters,[21] we should carefully examine the reactivity of Lawesson's reagent toward the carbonyl group present in our substrate. Indeed, by careful control of the temperature and by adding 0.5 equiv of LR, we selectively thionated the phosphorus atom without affecting the carbamate or the ester function. Therefore, after detailed experimentation, we found that the optimal reaction conditions were heating at 95 °C, for 2 h in dry toluene using 0.5 equiv of Lawesson's reagent.

Under these conditions, compound **12** was obtained in 89% yield after column purification (Table 1). It can be noted the less polar character of this compound allowed the use of CH_2Cl_2/EtOAc as eluent compared to CH_2Cl_2/MeOH needed for its oxygen counterpart. Of special interest, the increase of the ^{31}P NMR chemical shift, 54.44 and 55.44 ppm for the two diasteroisomers of **6** compared to 104.87, 106.09 ppm for **12**, is very diagnostic. The absence of any overthionation product was confirmed by means of mass spectrometry and ^{13}C NMR (C═O appears around 155 ppm for C═ONH, whereas C═S for

Scheme 4. General Structure of a Phosphinic Pseudodipeptide Analogue and a Common Synthetic Strategy Leading to It

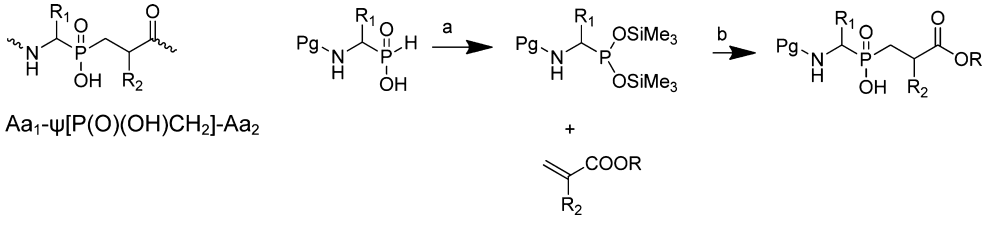


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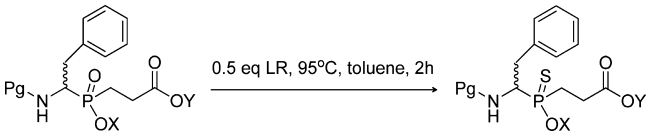
Table 1. Synthesis of Thiophosphinates from the Corresponding Phosphinates Using Lawesson's Reagent



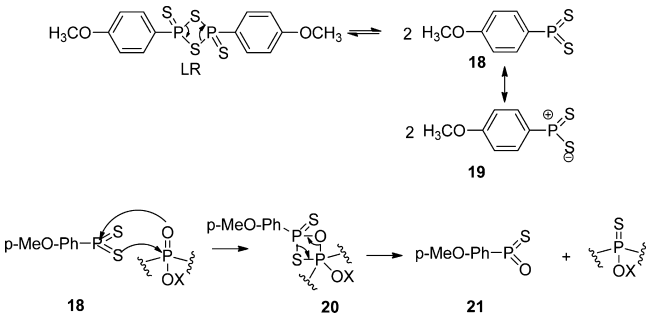
product	Pg	X	Y	yield (%)
12	Cbz	Me	Et	89
13	Cbz	Ad	Et	84
14	Fmoc	Me	*t*-Bu	82
15	Boc	Ad	Et	80
16	Cbz	Me	*t*-Bu	89
17	Cbz	Me	allyl	92

C═SNH is usually observed around 195 ppm).[22] The presence of the bulky adamantyl hydroxyphosphinyl protecting group did not affect the reaction progress, and thus thionation of **7** proceeded smoothly under the previously described conditions, providing **13** in 84% yield. The Fmoc- and Boc-protected thiophosphinic dipeptide analogues **14** and **15** were easily and selectively prepared without affecting either the N^{α}-carbamate carbonyl or the ester carbonyl. The presence of the allyl ester in **11** was equally well tolerated to give **17**, adding an extra aspect of orthogonality in this type of molecules.

Apart from the downfield shift of about 50 ppm observed in ^{31}P NMR, a similar trend was recorded in ^{1}H NMR with the PC*H*$_2$ and C*H*(Ph)P protons appearing in higher chemical shift, about 0.15−0.20 ppm for the thiophosphinates compared to their phosphinates counterparts. In ^{13}C spectra, the two carbons directly bonded to phosphorus, PCH$_2$ and CH(Ph)P, were 3−5 ppm downfield shifted. This increase in chemical shift can be attributed to the electron density, which is located more on the sulfur bound to phosphorus, compared to oxygen.

The mechanism of thionation can be rationalized as follows (Scheme 6). LR is in equilibrium with the highly reactive

Scheme 6. Possible Mechanism for the Thionation of a Phosphinate



dithiophosphine ylide (**18**, **19**). Both mesomeric structures **18** and **19** can react with a P═O-containing compound to form thiaoxaphosphetane **20**, which decomposes in a Wittig-analogous reaction to the corresponding thiophosphinate. The whole process may be summarized as *cis-syn* addition−ellimination. Thermodynamically, **21** is more stable than **18**, given the fact that the P═O bond is much stronger than the P═S bond (bond energy is 120 kcal/mol for P═O in $POCl_3$ vs 76 kcal/mol for P═S in $PSCl_3$), and this must be the driving force behind this mechanism.[23,24]

With these thiophosphinates in hand, their reactivity was examined using common deprotection conditions. Therefore, thiophosphinates **12**, **13**, **14**, **15**, **16**, and **17** were subjected to common acidic and basic deprotection conditions (Table 2). The results of the reactions were analyzed by ^{31}P NMR and mass spectrometry. Phosphorus NMR was the most valuable tool during this study since, during the deprotection course, three main types of compounds (Scheme 7) appeared to a smaller or a lesser extent. Compounds of the general formula **I**, whose thiophosphinate is protected and the carboxy group is still protected or not, appear in a range from 90 to 106 ppm. An upfield shift toward 67−75 ppm occurs when the thiophosphinyl functionality is deprotected (**II**), with the carboxyl group again protected or not. With TFA treatment, S→O exchange was observed in substrates **13** and **16** (entries f, h, j, k, Table 2). The presence of water did not seem to be the crucial factor. This exchange has been again observed under TMSBr-mediated deprotection of a methyl thiophosphinate in an analogue substrate in one of our previous work.[12] It was not observed under alkaline hydrolysis conditions, which is again confirmed in this work. A further upfield shift, reaching the area of 53−57 ppm, is indicative of this S→O exchange, characteristic of phosphinates **III**. Since hydrogenolytic removal of the N^{α}-Cbz group is not possible due to the poisonous effect of sulfur to Pd-catalyst, **12** was mixed with 33% HBr/AcOH for 1 h. To our delight, the Cbz group was completely removed while no S→O exchange was observed after workup (entry l, Table 2). Of special interest, Fmoc-thiophosphinate **24** is a suitable block for future Fmoc-solid phase thiophosphinic peptide synthesis (entry g, Table 2).

From the saponification experiment for **12** (entry a, Table 2), it was concluded that the thiophosphinyl methyl ester is 85% stable during the removal of the carboxyl ethyl ester, which is a great difference from the corresponding phosphinyl methyl ester, which was quantitatively removed under the same conditions in 40 min.[25]

Removal of the thiophosphinyl protecting group, taking into consideration the S→O exchange under acidic conditions, is feasible under basic conditions. Therefore a volatile amine, aqueous NH_3 in methanol, was first checked using **12** and **16** as substrates. Due to both its high basicity and nucleophilicity, demethylation occurred smoothly, and at the same time the carboxyl ethyl ester was transformed to the corresponding amide **26**, in the case of **12** (entry m, Table 2). In the case of **16**, however, no product was detected during the same period of time. This can be attributed to the insolubility of the starting material in the reaction mixture. The use of *n*-butanol instead of methanol increased the solubility of the starting materials but also increased the reaction time. Reaction of **16** with aqueous dimethylamine instead of aqueous NH_3 in methanol gave a demethylated **27** in 8 days (entry p, Table 2). In order to obtain the demethylated thiophosphinyl derivative with free carboxyl group instead of amide, we thought to use aqueous NH_3 in methanol for the saponified derivative **22**. Surprisingly, no reaction took place even after 10 days (entry q, Table 2). Compounds **26** and **27** were loaded on silica gel column chromatography but they were completely decomposed during the elution with a mixture CH_2Cl_2/MeOH−acetic acid, most probably due to the acidic nature of the eluent. The same was observed when HPLC characterization was attempted. Due to the presence of TFA in the solvent system, thiophosphinate **26** was transformed to its oxygen analogue. Therefore we decided to isolate **26** and **27** in the form of their salt with the

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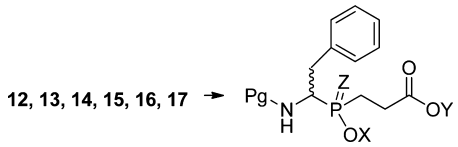
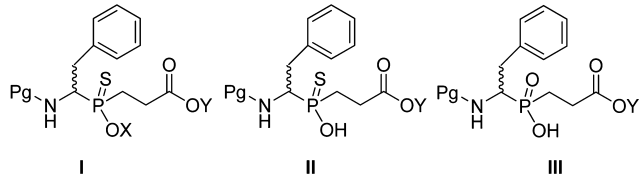
Table 2. Reactivity of 12−17 under Acidic/Basic Conditions



entry	reactant	conditions[a]	product(s) ratio[b]	
a	**12**	A	X = Me, Y = H, Z = S, **22**, 85%	X = Na, Y = Na, Z = S, 15%
b	**13**	A	X = Ad, Y = H, Z = S, **23**, 100%	
c	**16**	A	X = Me, Y = *t*-Bu, Z = S, 10%	X = Na, Y = *t*-Bu, Z = S + X = Na, Y = Na, Z = S, 90%
d	**17**	A	X = Me, Y = allyl, Z = S, 30%	X = Na, Y = allyl, Z = S + X = Na, Y = Na, Z = S, 70%
e	**12**	B	X = Me, Y = Et, Z = S, **12**, 100%	
f	**13**	B	X = Ad, Y = Et, Z = S, 46%, X = H, Y = Et, Z = S, 48%	X = H, Y = Et, Z = O, 6%
g	**14**	C	X = Me, Y = H, Z = S, **24**, 100%	
h	**16**	B	X = Me, Y = *t*-Bu, Z = S + X = Me, Y = H, Z = S, 90%	X = Me, Y = H, Z = O + X = H, Y = H, Z = O, 10%
i	**17**	B	X = Me, Y = allyl, Z = S, **17**, 100%	
j	**16**	D	X = Me, Y = *t*-Bu, Z = S + X = Me, Y = H, Z = S, 95%	X = Me, Y = H, Z = O + X = H, Y = H, Z = O, 5%
k	**16**	E	X = Me, Y = H, Z = S, **22**, 80%	X = Me, Y = H, Z = O + X = H, Y = H, Z = O, 20%
l	**12**	F	Pg = HBr, X = Me, Y = Et, Z = S, **25**, 100%	
m	**12**	G	Y = NH_2, X = NH_4, Z = S, **26**, 100%	
n	**16**	G	**16**, 100%	
o	**12**	H	Y = NH_2, X = NH_4, Z = S, **26**, 100%	
p	**16**	I	Y = O^{t}Bu, X = dimethylamine, Z = S, **27**, 100%	
q	**22**	G	**22**, 100%	

[a](A) 0.35 N NaOH/MeOH,1 h; (B) TFA/CH_2Cl_2/H_2O, 50/49.5/0.5, 1 h; (C) TFA/CH_2Cl_2/TIPS, 50/49.5/0.5, 30 min; (D) TFA/CH_2Cl_2, 50/50, 30 min; (E) TFA/CH_2Cl_2, 90/10, 30 min; (F) 33% HBr/AcOH, 1 h; (G) 25% NH_4OH/MeOH, 50/50, 5 days; (H) 25% NH_4OH/*n*-BuOH, 50/50, 19 days; (I) 40% aq dimethylamine/MeOH, 50/50, 8 days. [b]Determined by ^{31}P NMR.

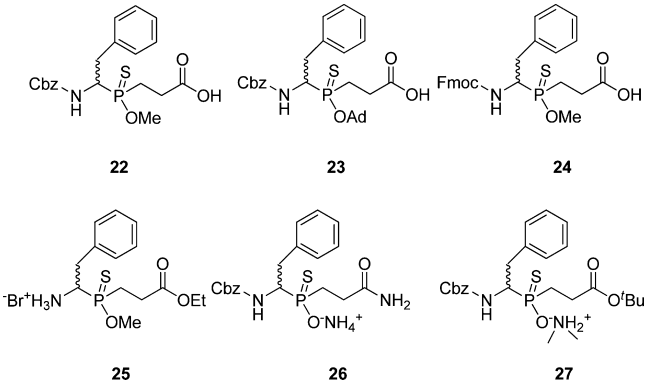
Scheme 7. Products of the Deprotection Study and ^{31}P NMR Area in Which They Appear



corresponding amine after trituration with $CHCl_3$ and filtration. In this form, these compounds are stable for months, as judged by ^{31}P NMR stability control.

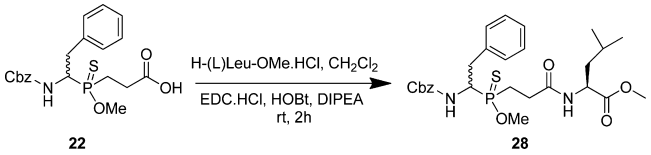
Thiophosphinates that were selectively deprotected and characterized are summarized in Scheme 8.

Scheme 8. Thiophosphinates Selectively Deprotected



Reading carefully the results of the deprotection study, we were able to proceed in C-terminal and N-terminal elongation. For the C-elongation, compound **22**, derived either from entry a or from entry k (Table 2), was coupled with H-(L)Leu-OMe·HCl using standard EDC·HCl procedure, providing **28** in high yield after column chromatography (Scheme 9).

Scheme 9. C-Elongation of Thiophosphinate 22



For the N-elongation hydrobromic salt **25**, Table 2, entry l was neutralized with DIPEA and coupled with Cbz-L-Phe-OH to give pseudotripeptide **29** in good yield (Scheme 10).

■ CONCLUSION

We have devised the first procedure that enables access to orthogonally protected thiophosphinyl dipeptide isoster (TDI) PG-Phe-Ψ[P(S)(OX)CH_2]-Gly-OY. The synthetic strategy is based on the logical selection of protecting groups as well as on the careful reaction conditions between the corresponding phosphinyl dipeptide isoster (PDI) and Lawesson's reagent. In addition, each functionality's selective deprotection was successfully studied, giving the possibility for further C-and N-elongation. Derived thiophosphinyl pseudopeptides are stable for months if isolated in the form of their salt.

Taking into consideration the increasing interest for zinc binding groups with unusual structural features due to their improved biological properties, the described synthesis is of particular practical value.

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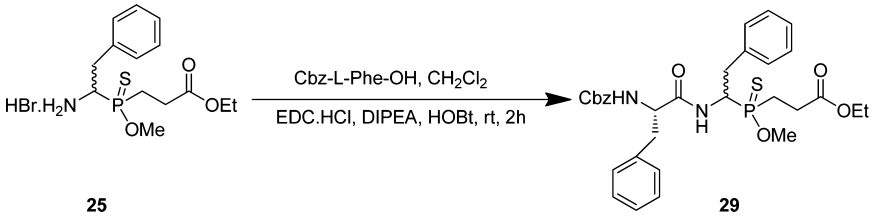
Scheme 10. N-Elongation of Thiophosphinate 25



25 29

EXPERIMENTAL SECTION

General. Dry methylene chloride (CH_2Cl_2) was obtained by distillation of commercially available predried solvent from NaH. Toluene was dried and kept over 4 Å molecular sieves. Reagents were purchased at the highest commercial quality and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel plates (silica gel 60F254), and components were visualized by the following methods: UV light absorbance, and/or charring after spraying with a solution of NH_4HSO_4, and/or an aqueous solution of cerium molybdate/H_2SO_4 ("Blue Stain"), and heating. Purification of compounds by column chromatography was carried out on silica gel (70−230 mesh) and the indicated solvents. ^1H, ^{13}C, and ^{31}P NMR spectra were recorded on a 200 MHz spectrometer. ^1H and ^{13}C spectra are referenced according to the residual peak of the solvent based on literature data. ^{31}P NMR chemical shifts are reported in ppm downfield from 85% H_3PO_4 (external standard). ^{13}C and ^{31}P NMR spectra are fully proton decoupled. The presence of asymmetric centers and rotamers in these compounds complicates the interpretation of the spectra. Numbers I and II were used to describe the ^{13}C resonances corresponding to the different diastereoisomers. ESI mass spectral analyses were performed on a mass spectrometer, using direct sample injection. Negative or positive ion ESI spectra were acquired by adjusting the needle and cone voltages accordingly. HRMS spectra were registered using a Orbitrap mass spectrometer in the m/z range of 100−700.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)methyloxyphosphinyl) Propanoic Acid Ethyl Ester (6).[25] Trimethylphosphite (1 mL) was added to **1** (650 mg, 1.55 mmol), and the reaction mixture was warmed to 90 °C for 10 h. The crude product was taken up by Et_2O and extracted with NaOH 0.1 N twice, with H_2O, dried over Na_2SO_4, and purified by column chromatography using CH_2Cl_2/MeOH 9.8−0.2 as eluent. Phosphinate **6** was obtained as a sticky gum. Yield 624 mg (93%). R_f = 0.58 (ethyl acetate). ^1H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼75:25, only signals of the major isomer are given: δ 1.24 (t, J = 7.2 Hz, 3H), 1.99−2.20 (m, 2H), 2.40−2.62 (m, 2H), 2.70−3.00 (m, 1H), 3.09−3.34 (m, 1H), 3.61(s, 3H/2), 3.68 (s, 3H/2), 4.14 (q, J = 7.2 Hz, 2H), 4.28−4.48 (m, 1H), 5.00 (s, 2H), 6.87 (d, J = 10.1 Hz, 1H), 7.10−7.36 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.8, 20.6 (d, $^1J_{PC}$ = 90.9 Hz), 21.3 (d, $^1J_{PC}$ = 90.9 Hz), 26.3, 33.7, 50.6 (d, $^1J_{PC}$ = 106.3 Hz), 51.4, 60.8, 66.4, 126.4, 126.5, 127.3, 127.5, 128.1, 128.2, 128.8, 136.1, 136.2, 136.4, 156.1 (d, $^3J_{PC}$ = 4.9 Hz), 171.8 (d, $^3J_{PC}$ = 15.1 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 54.44, 55.44; ESMS m/z calcd for $C_{22}H_{29}NO_6P$ $(M + H)^+$ 434.2, found 434.1.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)adamantyloxyphosphinyl) Propanoic Acid Ethyl Ester (7).[25] To a refluxing solution of compound **1** (360 mg, 0.86 mmol) and 1-adamantyl bromide (0.22 g, 1.03 mmol) in $CHCl_3$ (8 mL) was added silver(I) oxide (0.24 g, 1.03 mmol) portion wise over 1 h. After the solution was refluxed for 2 h, the solvent was removed in vacuo, and the residue was treated with Et_2O (10 mL). The resulting mixture was filtered through Celite, and the filtrate was evaporated. The residue was purified by column chromatography using CH_2Cl_2/MeOH 9.8−0.2 as eluent to give 0.39 g of **7** as white solid (82%). R_f = 0.79 (ethyl acetate). ^1H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼65:45, only signals of the major isomer are given: δ 1.23 (t, J = 7.0 Hz, 3H), 1.52−1.66 (m, 6H), 2.00−2.19 (m, 11H), 2.20−2.42 (m, 2H), 2.77−3.00 (m, 1H), 3.13−3.36 (m, 1H), 4.11 (q, J = 7.0 Hz, 2H), 4.06−4.32 (m, 1H), 4.95 (s, 2H), 6.41 (d, J = 10.3 Hz, 1H), 7.09−7.36 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0, 23.3 (d, $^1J_{PC}$ = 90.0 Hz), 27.0, 31.0, 34.3, 35.5, 44.4, 50.8 (d, $^1J_{PC}$ = 108.3 Hz), 60.6, 66.3, 82.9, 126.3, 126.4, 127.3, 127.5, 127.7, 127.8, 128.1, 128.2, 129.0, 136.1, 136.4, 136.7, 137.0, 137.2, 156.2 (d, $^3J_{PC}$ = 6.0 Hz), 172.2 (d, $^3J_{PC}$ = 17.1 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 48.51, 48.76; ESMS m/z calcd for $C_{31}H_{41}NO_6P$ $(M + H)^+$ 554.3, found 554.2.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-(9-Fluorenylmethoxycarbonyl)-amino)-2′-phenylethyl)methyloxyphosphinyl) Propanoic Acid *tert*-Butyl Ester (8). To a solution of **2** (470 mg, 0.88 mmol) and MeOH (0.5 mL) in CH_2Cl_2 (5 mL) at room temperature was added EDC·HCl (203 mg, 1.06 mmol). After stirring for 1 h, the mixture was diluted with CH_2Cl_2 and washed successively with 5% $NaHCO_3$, H_2O, dried over Na_2SO_4, and evaporated to give the crude product, which was further purified by column chromatography using CH_2Cl_2/MeOH 9.8−0.2 as eluent to give 0.44 g of **8** as white solid (91%). R_f = 0.75 (CH_2Cl_2/MeOH 9.5−0.5). ^1H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼70:30, only signals of the major isomer are given: δ 1.42 (s, 9H), 2.00−2.22 (m, 2H), 2.40−2.67 (m, 2H), 2.80−3.05 (m, 1H), 3.10−3.36 (m, 1H), 3.71 (s, 3H/2), 3.77 (s, 3H/2), 3.97−4.10 (m, 2H), 4.14−4.44 (m, 2H), 6.55 (d, J = 9.6 Hz, 1H), 7.10−7.73 (m, 13H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.9 (d, $^1J_{PC}$ = 88.6 Hz), 21.9 (d, $^1J_{PC}$ = 90.4 Hz), 25.5, 28.2, 34.0, 47.3, 50.5 (d, $^1J_{PC}$ = 106.4 Hz), 52.3, 67.4, 81.3, 120.1, 125.2, 125.3, 125.4, 127.0, 127.2, 127.9, 128.7, 129.4, 129.5, 136.9, 137.1, 141.4, 143.9, 144.1, 156.5 (d, $^3J_{PC}$ = 5.7 Hz), 171.7 (d, $^3J_{PC}$ = 14.3 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 54.32, 55.56; ESMS (m/z) calcd for $C_{31}H_{36}NO_6P$ $(M + H)^+$ 550.2, found 550.3. HRMS (ESI-orbitrap) m/z calcd for $C_{31}H_{36}NO_6PNa$ $[M + Na]^+$ 572.2178, found 572.2163.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-tert-Butoxycarbonyl)-amino)-2′-phenylethyl)adamantyloxyphosphinyl) Propanoic Acid Ethyl Ester (9). Following the same procedure as described for compound **7**, 0.59 g of compound **9** was obtained as gummy solid in 91% yield starting from **3**. R_f = 0.82 (ethyl acetate). ^1H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼72:33, only signals of the major isomer are given: δ 1.20−l.30 (s+t, J = 7.2 Hz, 12H), 1.46−1.75 (m, 6H), 2.00−2.22 (m, 11H), 2.40−2.90 (m, 3H), 3.10−3.35 (m, 1H), 4.01−4.24 (m+q, J = 7.2 Hz, 3H), 4.68 (d, J = 10.6 Hz, lH), 7.21−7.31 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 24.0 (d, $^1J_{PC}$ = 89.0 Hz), 24.2 (d, $^1J_{PC}$ = 89.0 Hz), 28.3, 31.4, 34.2, 35.6, 44.6, 50.0 (d, $^1J_{PC}$ = 111.3 Hz), 60.7, 79.6, 83.9 (d, $^2J_{PC}$= 10.1 Hz), 126.6, 126.7, 128.5, 129.4, 136.9, 137.0, 155.4 (d, $^3J_{PC}$ = 7.1 Hz), 175.6 (d, $^3J_{PC}$ = 17.4 Hz); ^{31}P NMR (81 MHz, $CDCl_3$) 49.65, 49.97; ESMS m/z calcd for $C_{28}H_{42}NO_6P$ $(M + H)^+$ 520.3, found 520.4. HRMS (ESI-orbitrap) m/z calcd for $C_{28}H_{42}NO_6PNa$ $[M + Na]^+$ 542.2647, found 542.2658.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)methyloxyphosphinyl) Propanoic Acid *tert*-Butyl Ester (10). Following the same procedure as described for compound **6**, 1.12 g of compound **10** was obtained as gummy solid in 91% yield starting from **4**. R_f = 0.70 (CH_2Cl_2/MeOH 9.5−0.5). ^1H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼68:32, only signals of the major isomer are given: δ 1.38 (s, 9H), 1.90−2.18 (m, 2H), 2.37−2.60 (m, 2H), 2.65−2.98 (m, 1H), 3.06−3.30 (m, 1H), 3.60(s, 3H/2), 3.65 (s, 3H/2), 4.18−4.40 (m, 1H), 4.94 (s, 2H), 6.61 (d, $^3J_{HH}$ = 10.0 Hz, 1H), 7.10−7.40 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.5 (d, $^1J_{PC}$ = 91.5 Hz), 21.4 (d, $^1J_{PC}$ = 91.5 Hz), 28.2, 34.1, 34.7 (d, $^2J_{PC}$ = 4.0 Hz), 50.6 (d, $^1J_{PC}$ = 105.5 Hz), 52.3, 67.0, 81.3, 126.9, 127.0, 127.8, 128.0, 128.1, 128.2, 128.6, 129.4, 136.6, 136.7, 137.2, 156.5 (d, $^3J_{PC}$ =

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5.3 Hz), 171.8 (d, $^3J_{PC}$ = 14.7 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 54.63, 55.87; ESMS m/z calcd for $C_{24}H_{32}NO_6P$ (M + H)$^+$ 462.2, found 462.2. HRMS (ESI-orbitrap) m/z calcd for $C_{24}H_{32}NO_6PNa$ [M + Na]$^+$ 484.1865, found 484.1872.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)methyloxyphosphinyl) Propanoic Acid Allyl Ester (11). Following the same procedure as described for compound **6**, 0.30 g of compound **11** were obtained as gummy solid in 93% yield starting from **5**. R_f = 0.73 (CH_2Cl_2/MeOH 9.5−0.5). ^{1}H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼62:38, only signals of the major isomer are given: δ 1.90−2.20 (m, 2H), 2.48−2.70 (m, 2H), 2.73−2.97 (m, 1H), 3.07−3.30 (m, 1H), 3.62(s, 3H/2), 3.68 (s, 3H/2), 4.19−4.38 (m, 1H), 4.45−4.60 (m, 2H), 5.00 (s, 2H), 5.16−5.30 (m, 2H), 5.72−5.97 (m,1H), 6.37 (d, $^3J_{HH}$ = 9.7 Hz, 1H), 7.05−7.38 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.7 (d, $^1J_{PC}$ = 90.7 Hz), 21.2 (d, $^1J_{PC}$ = 90.7 Hz), 26.7, 34.1, 50.5 (d, $^1J_{PC}$ = 116.8 Hz), 52.2, 65.9, 67.0, 118.7, 126.9, 127.1, 127.8, 128.1, 128.2, 128.8, 129.4, 136.4, 136.6, 136.8, 137.0, 132.0, 156.5 (d, $^3J_{PC}$ = 5.1 Hz), 172.3 (d, $^3J_{PC}$ = 14.0 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 54.18, 55.13; ESMS m/z calcd for $C_{23}H_{28}NO_6P$ (M + H)$^+$ 446.2, found 446.1. HRMS (ESI-orbitrap) m/z calcd for $C_{23}H_{28}NO_6PNa$ [M + Na]$^+$ 468.1552, found 468.1547.

General Procedure for the Synthesis of Thiophosphinates (A). To a stirred solution of phosphinate (1 mmol) in dry toluene (5 mL) under argon atmosphere was added Lawesson's reagent (0.5 mmol), and the reaction mixture was heated at 95 °C for 2 h. The solvent was removed in vacuo, and the residue was purified with column chromatography using CH_2Cl_2/EtOAc 9.5−0.5 as eluent.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)methyloxythiophosphinyl) Propanoic Acid Ethyl Ester (12). Following the general procedure (A), compound **12** was obtained from phosphinate **6** as solid in 89% yield. R_f = 0.40 (CH_2Cl_2/EtOAc 9.5−0.5). ^{1}H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼75:25, only signals of the major isomer are given: δ 1.27 (t, J = 7.2 Hz, 3H), 2.17−2.36 (m, 2H), 2.57−2.70 (m, 2H), 2.76−3.00 (m, 1H), 3.09−3.31 (m, 1H), 3.62 (s, 3H/2), 3.69 (s, 3H/2), 4.14 (q, J = 7.2 Hz, 2H), 4.40−4.61 (m, 1H), 4.98 (s, 2H), 5.05 (d, J = 11.0 Hz, 1H), 7.18−7.30 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.8, 27.0 (d, $^1J_{PC}$ = 70.0 Hz), 27.7, 36.0, 53.6 (d, $^1J_{PC}$ = 75.8 Hz), 51.5, 61.0, 66.7, 126.2, 126.4, 127.1, 127.4, 127.5, 128.2, 128.3, 128.8, 136.1, 136.3, 136.4, 156.0 (d, $^3J_{PC}$ = 5.0 Hz), 171.8 (d, $^3J_{PC}$ = 15.4 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 104.87, 106.09; ESMS m/z calcd for $C_{22}H_{28}NO_5PS$ (M + H)$^+$ 450.1, found 450.0. HRMS (ESI-orbitrap) m/z calcd for $C_{22}H_{28}NO_5PSNa$ [M + Na]$^+$ 472.1323, found 472.1313.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)adamantyloxythiophosphinyl) Propanoic Acid Ethyl Ester (13). Following the general procedure (A), compound **13** was obtained from phosphinate **7** as solid in 84% yield. R_f = 0.65 (CH_2Cl_2/EtOAc 9.5−0.5). ^{1}H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼65:45, only signals of the major isomer are given: δ 1.25 (t, J = 7.0 Hz, 3H), 1.54−1.64 (m, 6H), 2.05−2.20 (m, 11H), 2.20−2.40 (m, 2H), 2.50−2.70 (m, 2H), 2.72−3.00 (m, 1H), 3.11−3.35 (m, 1H), 3.38−3.55 (m, 1H) 4.12 (q, J = 7.0 Hz, 2H), 4.06−4.40 (m, 1H), 4.95 (s+d, 3H), 7.10−7.40 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.4, 28.4, 29.3 (d, $^1J_{PC}$ = 81.3 Hz), 31.0, 34.8, 36.3, 44.9, 52.8 (d, $^1J_{PC}$ = 90.2 Hz), 60.7, 67.0, 84.9, 126.9, 127.9, 127.8, 128.1, 129.2, 129.4, 136.3, 136.7, 137.0, 137.2, 156.6 (d, $^3J_{PC}$ = 5.7 Hz), 172.4 (d, $^3J_{PC}$ = 15.2 Hz); ^{31}P NMR (81 MHz, $CDCl_3$) δ 91.90, 92.15; ESMS m/z calcd for $C_{31}H_{40}NO_5PS$ (M + H)$^+$ 570.2 found 570.4. HRMS (ESI-orbitrap) m/z calcd for $C_{31}H_{40}NO_5PSNa$ [M + Na]$^+$ 592.2263, found 592.2249.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-(9-Fluorenylmethoxycarbonyl)-amino)-2′-phenylethyl)methyloxythiophosphinyl) Propanoic Acid *tert*-Butyl Ester (14). Following the general procedure (A), compound **14** was obtained from phosphinate **8** as solid in 82% yield. R_f = 0.40 (CH_2Cl_2).^{1}H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼70:30, only signals of the major isomer are given: δ 1.42 (s, 9H), 2.18−2.38 (m, 2H), 2.43−2.68 (m, 2H), 2.79−3.00 (m, 1H), 3.11−3.30 (m, 1H), 3.64(s, 3H/2), 3.71 (s, 3H/2), 3.98−4.13 (m, 2H), 4.14−4.40 (m, 2H), 5.10 (d, J = 10.5 Hz, 1H), 7.10−7.78 (m, 13H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.2 (d, $^1J_{PC}$ = 76.6 Hz), 27.3 (d, $^1J_{PC}$ = 76.6 Hz), 28.2,, 28.7 (d, $^2J_{PC}$ = 17.0 Hz), 34.9 (d, $^2J_{PC}$ = 17.3 Hz), 47.2, 50.5 (d, $^1J_{PC}$ = 84.3 Hz), 52.8, 67.5, 81.3, 120.2, 125.2, 125.3, 127.1, 127.3, 127.9, 128.7, 129.4, 129.5, 136.5, 136.7, 141.4, 143.7, 144.0, 155.9 (d, $^3J_{PC}$ = 5.4 Hz,), 171.4 (d, $^3J_{PC}$ = 15.4 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 106.52; ESMS m/z calcd for $C_{31}H_{36}NO_5PS$ (M + Na)$^+$ 588.2, found 588.3. HRMS (ESI-orbitrap) m/z calcd for $C_{31}H_{36}NO_5PSNa$ [M + Na]$^+$ 588.1950, found 588.1946

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-*tert*-Butoxycarbonyl)-amino)-2′-phenylethyl)adamantyloxythiophosphinyl) Propanoic Acid Ethyl Ester (15). Following the general procedure (A), compound **15** was obtained from phosphinate **9** as solid in 80% yield. R_f = 0.77 (CH_2Cl_2/EtOAc 9.5−0.5). ^{1}H NMR (200 MHz, $CDCI_3$) mixture of two diastereoisomers ∼72:33, only signals of the major isomer are given: δ 1.20-l.38 (s+t, J = 7.2 Hz, 12H), 1.61 (bs, 6H), 2.05−2.22 (m, 9H), 2.25−2.40 (m, 2H, PCH_2), 2.50−2.98 (m, 3H), 3.13−3.30 (m, 1H), 4.01−4.24 (m+q, J = 7.2 Hz, 3H) 4.70 (d, J = 11.0 Hz, lH,), 7.21−7.31 (m, 5H); ^{13}C NMR (50 MHz, $CDCI_3$) δ 14.4, 28.3, 29.4 (d, $^1J_{PC}$ = 82.6 Hz), 31.5, 34.8 (d, J = 5.9 Hz), 36.0, 44.4 (d, J = 4.2 Hz), 52.0 (d, $^1J_{PC}$ = 92.1 Hz), 61.0, 80.2, 85.3 (d, $^2J_{PC}$= 10.8 Hz), 126.7, 126.8, 128.5, 129.4, 137.1, 137.4, 155.1 (d, $^3J_{PC}$ = 6.0 Hz), 175.1 (d, $^3J_{PC}$ = 7.1 Hz); ^{31}P NMR (81 MHz, $CDCl_3$) δ 91.21, 92.47; ESMS m/z calcd for $C_{28}H_{42}NO_5PS$ (M + H)$^+$ 536.3, found 536.4. HRMS (ESI-orbitrap) m/z calcd for $C_{28}H_{42}NO_5PSNa$ [M + Na]$^+$ 558.2419, found 588.2409.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)methyloxythiophosphinyl) Propanoic Acid *tert*-Butyl Ester (16). Following the general procedure (A), compound **16** was obtained from phosphinate **10** as solid in 89% yield. R_f = 0.72 (CH_2Cl_2/EtOAc 9.5−0.5). ^{1}H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼68:32, only signals of the major isomer are given: δ 1.43 (s, 9H), 2.06−2.38 (m, 2H), 2.42−2.62 (m, 2H), 2.67−2.97 (m, 1H), 3.08−3.29 (m, 1H), 3.63(s, 3H/2), 3.68 (s, 3H/2), 4.22−4.60 (m, 1H), 4.97 (s, 2H), 7.05−7.38 (m, 11H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.4 (d, $^1J_{PC}$ = 69.5 Hz), 27.2 (d, $^1J_{PC}$ = 69.5 Hz), 28.3, 34.9 (d, $^2J_{PC}$ = 7.1 Hz), 35.4 (d, $^2J_{PC}$ = 5.0 Hz), 53.0 (d, $^1J_{PC}$ = 83.9 Hz), 52.4, 67.3, 81.4, 127.0, 127.1, 128.0, 128.2, 128.3, 128.4, 129.4, 136.3, 136.4, 137.2, 155.8 (d, $^3J_{PC}$ = 5.6 Hz), 171.5 (d, $^3J_{PC}$ = 13.8 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 105.1, 106.4; ESMS m/z calcd for $C_{24}H_{32}NO_5PS$ (M + H)$^+$ 477.2, found 422.2 (M − ^{t}Bu). HRMS (ESI-orbitrap) m/z calcd for $C_{24}H_{32}NO_5PSNa$ [M + Na]$^+$ 500.1637, found 500.1633.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)methyloxythiophosphinyl) Propanoic Acid Allyl Ester (17). Following the general procedure (A), compound **17** was obtained from phosphinate **11** as solid in 92% yield. R_f = 0.78 (CH_2Cl_2/EtOAc 9.5−0.5). ^{1}H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼62:38, only signals of the major isomer are given: δ 2.10−2.40 (m, 2H), 2.47−2.68 (m, 2H), 2.71−2.98 (m, 1H), 3.05−3.27 (m, 1H), 3.61(s, 3H/2), 3.68 (s, 3H/2), 4.37−4.47 (m, 1H), 4.50−4.65 (m, 2H), 4.96 (s, 2H), 5.10−5.30 (m, 2H), 5.78−6.00 (m, 1H), 6.97 (d, $^3J_{HH}$ = 9.7 Hz, 1H), 7.10−7.40 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.0 (d, $^1J_{PC}$ = 70.2 Hz), 27.4 (d, $^1J_{PC}$ = 70.2 Hz), 27.6, 34.1 ($^2J_{PC}$ = 5.8 Hz), 53.4 (d, $^1J_{PC}$ = 85.0 Hz), 52.8, 65.9, 67.3, 118.8, 127.0, 127.1, 127.8, 128.1, 128.2, 128.8, 129.4, 136.3, 136.6, 136.8, 132.1, 156.0 (d, $^3J_{PC}$ = 6.0 Hz), 172.2 (d, $^3J_{PC}$ = 15.2 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 104.8, 105.9; ESMS m/z calcd for $C_{23}H_{28}NO_5PS$ (M + H)$^+$ 462.1, found 462.2. HRMS (ESI-orbitrap) m/z calcd for $C_{23}H_{28}NO_5PSNa$ [M + Na]$^+$ 484.1323, found 484.1310.

General Procedure for the Saponification Experiments (B). To a stirred solution of thiophosphinate (25 mg) in MeOH (1 mL) was added a 4 N aqueous solution of NaOH (0.1 mL). After 1 h at room temperature, the reaction mixture was concentrated in vacuo, and the conversion ratios appearing in Table 2 were determined by ^{31}P NMR using D_2O as solvent. The products were acidified and purified, when possible, using appropriate eluent systems and characterized.

General Procedure for the Acid-Catalyzed Experiments (C). Method (i). The thiophosphinate (25 mg) was dissolved in the appropriate solution of TFA/CH_2Cl_2/H_2O, and the resulting mixture was stirred at room temperature. After the indicated reaction time, the mixture was concentrated to dryness. The determination of the

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conversion percentages reported in Table 2 was based on ^{31}P NMR spectra of the crude reaction product in $CDCl_3$.

Method (ii). Thiophosphinate **12** (30 mg) was stirred for 1 h in 33% HBr/AcOH (0.5 mL) in a flask fitted with a $CaCl_2$ drying tube. Toluene was added, and the volatiles were removed in vacuo. The procedure was repeated twice, and the residue was analyzed by ^{31}P NMR and mass spectrometry.

Method (iii). Thiophosphinate **14** (50 mg, 0.09 mmol) was treated with TFA/CH_2Cl_2/TIPS 50−49.5−0.5, at room temperature for 30 min followed by concentration to dryness. CH_2Cl_2 was added and concentrated again. The crude reaction mixture was purified by column chromatography using $CHCl_3$/MeOH 9.5−0.5 as eluent to give **24** as a glassy solid in 98% yield (44 mg).

General Procedure for the Base-Catalyzed Experiments (D). The thiophosphinate (25 mg) was stirred for the time and the conditions mentioned in Table 2 until total consumption of the starting material as judged by TLC. Then the volatile materials were removed in vacuo, and the residue was treated with $CHCl_3$. The solid was filtered and stored in the form of the salt.

General Procedure for the Coupling Experiments (E). EDC·HCl (1.2 equiv) and *N*,*N*-diisopropylethylamine (3 equiv) were added to a solution of amine (1 equiv), acid (1 equiv), and HOBt (1.2 equiv) in CH_2Cl_2 (5 mL/1 mmol) and stirred for 18 h at room temperature. The solvent was removed in vacuo, the residue was partitioned between EtOAc/H_2O, and the organic phase was washed with 5% $NaHCO_3$, 0. 5N HCl, and H_2O, and dried over Na_2SO_4. The organic solvent was removed, and the residue was purified by column chromatography.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)methyloxyphosphinyl) Propanoic Acid (22). Following the general procedure (B), compound **22** was obtained as foamy solid in 85% yield after column chromatography using $CHCl_3$/MeOH 9−1 as eluent. R_f = 0.39 ($CHCl_3$/MeOH 9−1). ^1H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers only signals of the major isomer are given: δ 2.17−2.36 (m, 2H), 2.57−3.00 (m, 3H), 3.09−3.25 (m, 1H), 3.62 (s, 3H/2), 3.69 (s, 3H/2), 4.40−4.61 (m, 1H), 4.98 (s, 2H), 5.25 (d, J = 5.5 Hz, 1H), 7.00−7.40 (m, 10H), 9.25 (bs, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.0 (d, $^1J_{PC}$ = 71.0 Hz), 27.9, 34.9, 52.5, 53.7 (d, $^1J_{PC}$ = 73.8 Hz), 66.7, 126.3, 127.4, 128.4, 128.8, 128.9, 136.0, 136.4, 156.2, 177.4. ^{31}P NMR (81 MHz, $CDCl_3$) δ 104.87, 106.09; ESMS m/z calcd for $C_{20}H_{24}NO_5PS$ $(M + H)^+$ 422.1, found 422.1. HRMS (ESI-orbitrap) m/z calcd for $C_{20}H_{24}NO_5PSNa$ $[M + Na]^+$ for 444.1010, found 444.1006.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)adamantyloxythiophosphinyl) Propanoic Acid (23). Following the general procedure (B), compound **23** was obtained as foamy solid quantitatively after column chromatography using CH_2Cl_2/isopropyl alcohol 9−1 as eluent. R_f = 0.62 (CH_2Cl_2/isopropyl alcohol 9−1). ^1H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers ∼65:45, only signals of the major isomer are given: δ 1.54−1.64 (m, 6H), 2.05−2.20 (m, 11H), 2.22−2.42 (m, 2H), 2.55−3.00 (m, 3H), 3.11−3.35 (m, 1H), 3.40−3.52 (m, 1H), 4.40−4.55 (m, 1H), 4.91−5.17 (m, 3H), 7.15−7.30 (m, 10H), 8.05 (bs, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.4, 29.2 (d, $^1J_{PC}$ = 72.3 Hz), 28.5, 34.6, 35.9, 44.5, 53.2 (d, $^1J_{PC}$ = 85.2 Hz), 67.3, 85.7, 126.9, 128.0, 128.3, 128.4, 128.7, 129.5, 136.3, 136.3, 136.8, 137.2, 156.1, 177.4; ^{31}P NMR (81 MHz, $CDCl_3$) δ 91.73, 91.51, 90.49, 89.24; ESMS m/z calcd for $C_{29}H_{36}NO_5PS$ $(M - H)^-$ 540.2 found 540.1. HRMS (ESI-orbitrap) m/z calcd for $C_{29}H_{36}NO_5PSNa$ $[M + Na]^+$ 564.1950, found 564.1947.

(*R*,*R*,*S*,*S*)-3-((1′-(*N*-(9-Fluorenylmethoxycarbonyl)-amino)-2′-phenylethyl)methyloxyphosphinyl) Propanoic Acid (24). Following the general procedure (C), method (iii), compound **24** was obtained. R_f = 0.30 ($CHCl_3$/MeOH 9.5−0.5). ^1H NMR (200 MHz, $CDCl_3$) mixture of two diastereoisomers only signals of the major isomer are given: δ 2.15−2.45 (m, 2H), 2.55−2.85 (m, 3H), 3.10−3.27 (m, 1H), 3.63 (s, 3H/2), 3.68 (s, 3H/2), 4.00−4.51 (m, 4H), 5.07 (d, J = 5.1 Hz, 1H), 7.00−7.91 (m, 14H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.4 (d, $^1J_{PC}$ = 78.5 Hz), 27.8, 34.8, 47.1, 52.6 (d, $^1J_{PC}$ = 79.8 Hz), 54.1, 66.6, 120.2, 125.2, 127.3, 128.0, 128.8, 129.4, 136.3, 141.5, 143.9, 156.0, 177.5 (d, $^3J_{PC}$ = 8.5 Hz). ^{31}P NMR (81 MHz, $CDCl_3$) δ 104.54, 106.00; ESMS m/z calcd for $C_{27}H_{27}NO_5PS$ $(M - H)^+$ 508.1, found 508.1. HRMS (ESI-orbitrap) m/z calcd for $C_{27}H_{28}NO_5PSNa$ $[M + Na]^+$ 532,1323, found 532,1329.

(*R*,*S*)-3-((1′-(*N*-Benzyloxycarbonylamino)-2′-phenylethyl)-hydroxythiophosphinyl) Propanamide Ammonium Salt (26). Following the general procedure (D), compound **26** was obtained as solid in 76% yield. R_f = 0.29 ($CHCl_3$/MeOH/AcOH 7−0.5−0.5). ^1H NMR (DMSO-d_6) δ 1.62−1.85 (bq, 2H), 2.00−2.25 (bq, 2H), 2.58−2.80 (m, 1H), 3.00−3.20 (m, 1H), 3.70−3.85 (m, 1H), 4.86 (s, 2H), 6.41 (d, J = 6.4 Hz, 1H), 6.68 (bs, 1H), 7.13−7.23 (m, 11H); ^{13}C NMR (DMSO-d_6) δ 29.7, 31.6 (d, $^1J_{PC}$ = 68.9 Hz), 34.7, 55.8 (d, ^1JPC = 77.1 Hz), 65.5, 126.3, 127.0, 127.8, 128.3, 128.5, 129.5, 129.8, 138.0, 140.7, 156.2 (d, $^3J_{PC}$ = 5.3 Hz), 175.4 (d, $^3J_{PC}$ = 15.4 Hz); ^{31}P NMR (DMSO-d_6) δ 71.21; ESMS m/z calcd for $C_{19}H_{23}N_2O_4PS$ $(M - H - NH_3)^-$ 405.1, found 405.1. HRMS (ESI-orbitrap) m/z calcd for $C_{19}H_{22}N_2O_4PS$ $[M - H - NH_3]^-$ 405.1038, found 405.1033.

(*R*,*S*)-3-((1′-(*N*-Benzyloxycarbonyl)-amino)-2′-phenylethyl)-hydroxy thiophosphinyl) Propanoic Acid *tert*-Butyl Ester Dimethylammonium Salt (27). Following the general procedure (D), compound **27** was obtained as solid in 70% yield. R_f = 0.48 ($CHCl_3$/MeOH/AcOH 7−0.5−0.5). ^1H NMR (200 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.90−2.10 (m, 2H), 2.51−2.70 (m, 8H), 2.75−3.10 (m, 2H), 4.00−4.20 (m, 1H), 4.91 (s, 2H), 5.28 (d, J = 5.3 Hz 1H), 7.05−7.38 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 30.0 (d, $^1J_{PC}$ = 64.3 Hz), 28.3, 29.7, 30.0 (d, $^1J_{PC}$ = 64.3 Hz), 35.2, 54.1 (d, $^1J_{PC}$ = 86.1 Hz), 67.8, 81.1, 126.6, 127.9, 128.7, 129.7, 136.8, 138.3, 156.2 (d, $^3J_{PC}$ = 4.6 Hz), 173.4 (d, $^3J_{PC}$ = 7.8 Hz);^{31}P NMR (81 MHz, $CDCl_3$) δ 77.45; ESMS m/z calcd for $C_{23}H_{29}NO_5PS$ $(M - H -$ dimethylamine$)^-$ 462.1, found 462.2. HRMS (ESI-orbitrap) m/z calcd for $C_{23}H_{29}NO_5PS$ $[M - H -$ dimethylamine$]^-$ 462.1510, found 462.1524.

Cbz-(R,S)Phe-ψ[P(S)(OMe)]Gly-(S)Leu-OMe (28). Following the general procedure (E), using H-(L)Leu-OMe.HCl as amine and **22** as acid, compound **28** was obtained as solid in 70% yield after column chromatography using $CHCl_3$/EtOAc 4−1 as eluent. R_f = 0.72 ($CHCl_3$/ethyl acetate 1−1). ^1H NMR (200 MHz, $CDCl_3$): mixture of four diastereoisomers, δ 0.92 (s, 3H), 1.11(s, 3H), 1.23 (s, 1H), 1.40−1.70 (m, 4H), 2.20−2. 90 (m, 3H), 3.08−3.32 (m, 1H), 3.64 (s, 3H/2), 3.70 (s, 3H/2), 4.40−4.58 (m, 2H), 4.98 (bs, 2H), 5.40 (bd, 1H), 6.35 (bd, 1H), 7.10−7.30 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.1, 23.4, 25.0, 27.3 (set of 3d, $^1J_{PC}$ = 68.7 Hz), 29.4, 34.8, 41.6, 51.2, 51.6, 52.3, 53.3 (d, $^1J_{PC}$ = 71.7 Hz), 67.3, 126.7, 127.7, 128.0, 128.4, 128.3, 129.5, 135.9, 136.7, 156.5, 171.1, 176.3. ^{31}P NMR (81 MHz, $CDCl_3$) δ 106.3, 106.0, 105.7, 105.4; ESMS m/z calcd for $C_{27}H_{37}N_2O_6PS$ $(M + H)^+$ 549.2, found 549.4. HRMS (ESI-orbitrap) m/z calcd for $C_{27}H_{37}N_2O_6PSNa$ $[M + Na]^+$ 571.2008, found 571.2005.

Cbz-(S)Phe-(R,S)Phe-ψ[P(S)(OMe)]Gly-OEt (29). Following the general procedure (E), using **25** as amine and Cbz-(L)Phe-OH as acid, compound **29** was obtained as solid in 61% yield after column chromatography using $CHCl_3$/EtOAc 4−1 as eluent. R_f = 0.59 ($CHCl_3$/EtOAc 4−1). ^1H NMR (200 MHz, $CDCl_3$): mixture of four diastereoisomers, δ 1.10−1.40 (m, 2H), 1.80−2.90 (m, 4H), 3.08−3.32 (m, 1H), 3.30−3.50 (m, 1H), 3.64 (d, 3H/2), 3.70 (d, 3H/2), 4.00−4.20 (m, 2H), 4.20−4.40 (bd, 1H), 5.00 (bs, 2H), 5.30 (bd, 1H), 6.40 (bd, 1H), 7.10−7.40 (m, 15H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.4, 26.5 (d, $^1J_{PC}$ = 74.7 Hz), 27.7, 34.5, 37.7, 52.8, 52.9 (3d, $^1J_{PC}$ = 72.0 Hz), 61.3, 67.5, 127.1, 128.0, 128.2, 128.4, 128.8, 129.5, 136.2, 136.6, 156.1, 171.0, 172.7. ^{31}P NMR (81 MHz, $CDCl_3$) δ 106.2, 106.1, 105.0, 104.7; ESMS m/z calcd for $C_{31}H_{37}N_2O_6PS$ $(M + H)^+$ 597.2, found 597.2. HRMS (ESI-orbitrap) m/z calcd for $C_{31}H_{38}N_2O_6PS$ $[M + H]^+$ 597.2188, found 597.2192.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of ^1H, ^{13}C, and ^{31}P NMR spectra of **6**, **8**−**12**, **14**−**17**, **22**−**24**, and **26**−**29**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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