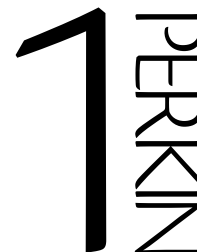

Synthesis and chemical reactivity of thiophenoxyphenylalanine bioisosteres, suitable synthons for the design of HIV protease inhibitors

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Phenylalanine in which the methylene group is replaced by a sulfur atom could be a useful bioisostere for the design of HIV-protease inhibitors. Due to the chemical instability of hemiaminal intermediates, these bioisosteres have to be prepared from α-hydroxyglycine following specific synthetic routes. In this paper, we report the synthesis of sulfenylated phenylalanine bioisosteres **2** and **3**, which represent two original building-blocks for peptide solid phase synthesis.

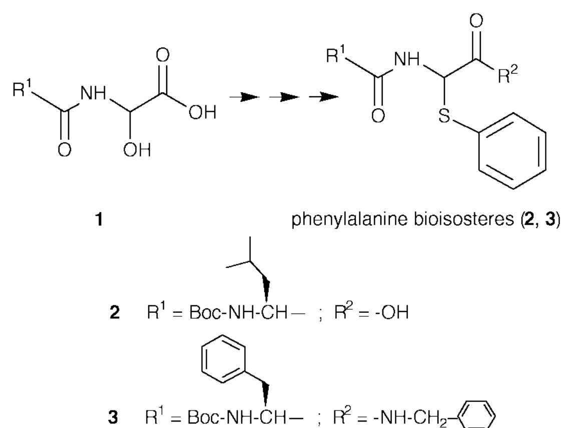
Introduction

HIV aspartylprotease represents an alternative target for a therapeutic intervention. One of the interests of our research is to find a new series of compounds which can inhibit the activity of the HIV protease.

Several synthetic peptides, for example Ile-Arg-Lys-Ile-Leu-Phe-Leu-Asp-Gly-Ile, were found to be substrates of the recombinant HIV protease,[1–3] and were cleaved specifically between the Leu and Phe residues. Based on this observation, we have attempted the synthesis of new modified thiophenoxy-peptides containing a thiophenoxyphenylalanine bioisostere moiety.

We have previously reported through molecular model studies[4,5] that the bioisosteric replacement of a methylene group by a sulfur atom in a benzyl group of various enzymatic substrates does not induce drastic changes in the conformational energies.

The synthesis and the anti-HIV properties of the two original key-synthons **2** and **3** which are sulfenylated phenylalanine bioisosteres (Scheme 1) are described in this paper. The chemical reactivity of these intermediates led us also to report their chemical stabilities under various conditions.

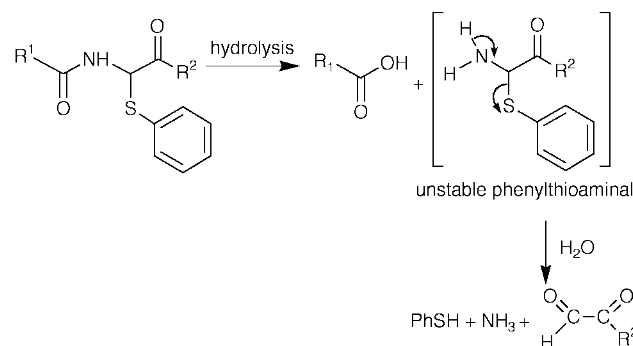


Scheme 1 Structure of phenylalanine bioisosteres **2** and **3**.

Results and discussion

α-Hydroxyglycine derivatives **1** (Scheme 1), which are often used as key synthons in the design of various protease inhibitors,[6] have been extensively studied.[7,8] We therefore think that α-hydroxyglycine could be a suitable synthon for the synthesis of the various phenylalanine bioisosteres **2** and **3**.

N-Carbonyl-α-thiophenoxyglycine derivatives are known to be chemically stable; in contrast, when the amide function is hydrolyzed (chemically or enzymatically), the resulting unstable phenylthioaminal intermediate decomposed directly into thiophenol and aldehyde products[9] as shown in Scheme 2. It is



Scheme 2 Hydrolysis of *N*-carbonyl α-thiophenoxyglycine derivatives.

foreseeable that liberation of thiophenol and glyoxylic acid derivatives may cause a dysfunction of the cellular mechanism which will induce the death of the infected cell. One could take advantage of this specific reactivity of *N*-carbonyl-α-thiophenoxyglycine compounds for the design of novel HIV-protease inhibitors. Therefore the syntheses of bioisosteres **2** and **3** are of major interest since they could be used as phenylalanine peptidomimetic synthons suitable for modified peptide solid phase synthesis.

Usually, the most straightforward synthesis of *N*-(1-hydroxyalkyl)amides involves the addition of primary or secondary amides to aldehydes or ketones. This reaction is an equilibrium process which usually disfavours the adduct except for special cases. Indeed, reactive aldehydes like formaldehyde, chloral

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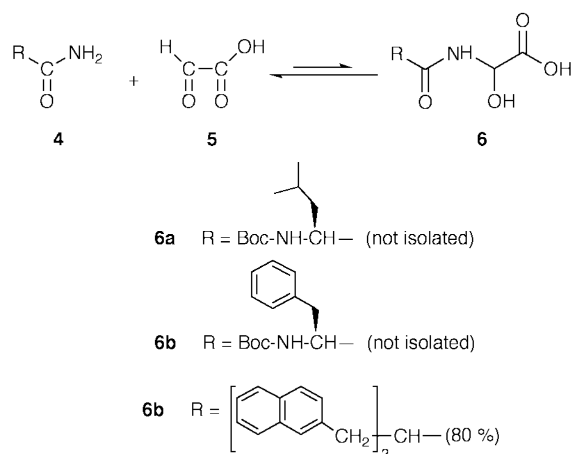
(trichloroacetaldehyde), glyoxylic acid and its derivatives react with amides to give fairly stable *N*-(1-hydroxyalkyl)amides[7,8] (Scheme 3).

In fact, we found that when glyoxylic acid **5** was condensed with amide substrates **4** (BocLeuNH$_2$ or BocPheNH$_2$), isolation of the corresponding carboxylic hemiaminals **6a** and **6b** was not possible. Only highly hydrophobic amides like [bis(1-naphthyl)methyl]acetamide led to pure hemiaminal **6c** in 80% yield[6] (Scheme 3).

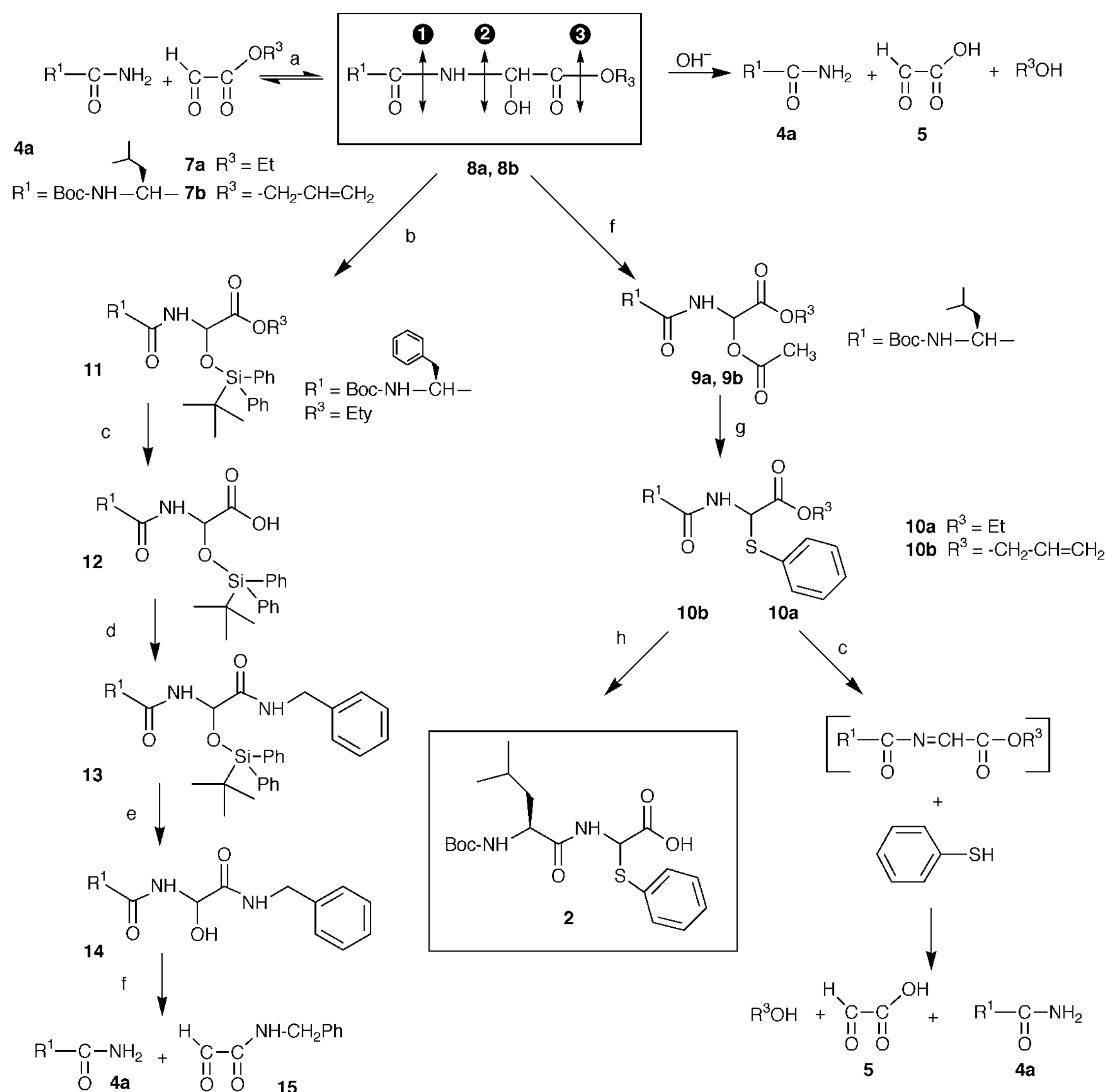


Scheme 3 Synthesis of *N*-(1-hydroxyalkyl)amides.

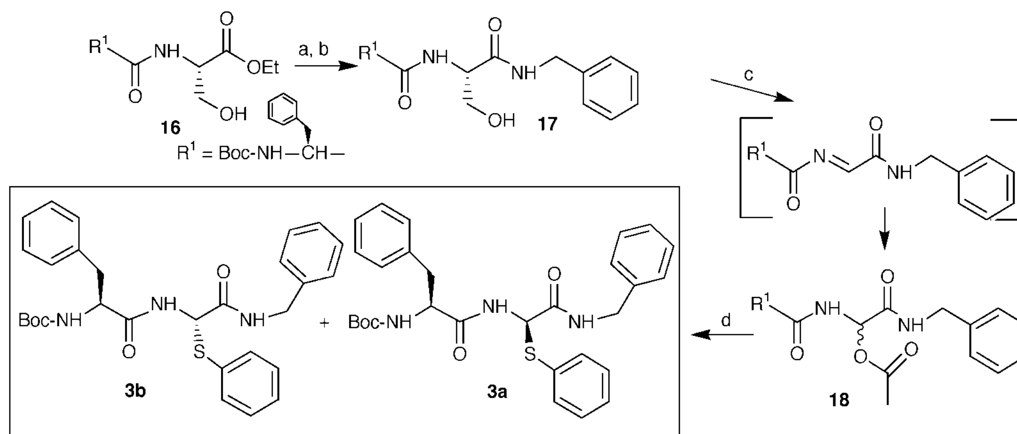
In contrast, when various esters of glyoxylic acid (**7a**: R^3 = ethyl; **7b**: R^3 = allyl) were condensed with BocLeuNH$_2$ **4a**, the corresponding hemiaminal esters **8a** and **8b** were isolated in relatively good yields,[10] 50 and 60% respectively, (Scheme 4).

Our goal was to use intermediates **8a** and **8b** for the synthesis of α-sulfenylated carboxylic acid **2** (R^1 = Boc(L)Leu-; R^2 = -OH) or amide **3** (R^1 = Boc(L)Phe-; R^2 = -NH-CH$_2$Ph) (Scheme 1) and we have investigated several routes. The most direct route attempted with esters **8a** (R^3 = Et) and **8b** (R^3 = allyl) involved initial *O*-acylation of the hydroxy group using acetic anhydride, followed by the substitution of the *O*-acetoxy group of compounds **9a** and **9b** by thiophenol, leading to the corresponding α-thiophenoxy esters **10a** and **10b** (Scheme 4). Unfortunately, classical hydrolysis of the ester function (1 M sodium hydroxide solution)[11] led to the decomposition products: thiophenol, glyoxylic acid **5**, and the starting amide **4a**, according to the mechanism shown in Scheme 4. This result prompted us to study the hydrolysis stability of hemiaminal esters **8a** and **8b**.

Three different hydrolysis sites of cleavage 1, 2 and 3 of intermediates **8a** and **8b** are indicated on Scheme 4. The sites of cleavage depend on the following structural parameters: the presence or not of a protecting group on the free hydroxy function, and the nature of the terminal carbonyl function (ester or amide). Direct basic hydrolysis of esters **8a** and **8b** gave



Scheme 4 Synthesis of phenylalanine bioisostere **2**. a, Et$_3$N, CH$_2$Cl$_2$, rt, 72 h, 50–60%; b, t-Bu(Ph)$_2$SiCl, imidazole, DMF, 62%; c, 1 M NaOH, CH$_3$OH, quantitative; d, PhCH$_2$NH$_2$, BOP, Et$_3$N, CH$_2$Cl$_2$, 50%; e, (nBu)$_4$N$^+$F$^-$, THF, 66%; f, Ac$_2$O, Pyr, CH$_2$Cl$_2$; g, PhSH, (iPr)$_2$NEt, DMF, rt, 72 h, 58%; h, potassium 2-ethylhexanoate, Pd(PPh$_3$)$_4$, PPh$_3$, EtOAc, rt, 16 h, 70%.



Scheme 5 Synthesis of diastereoisomers **3a** and **3b**. a, 1 M NaOH, CH_3OH, quantitative; b, $PhCH_2NH_2$, BOP, Et_3N, CH_2Cl_2, 96%; c, $Pb(OAc)_4$, molecular sieves 4 Å, EtOAc reflux, 1 h, quantitative; d, PhSLi, THF, 0 °C, 1 h, 61%.

the initial amide **4a**, glyoxylic acid **5**, and the corresponding alcohol (type 2 cleavage). In contrast, basic hydrolysis[11] of protected ester **11** (OH protected by a *tert*-butyldiphenylsilyl group[12]) led to the expected corresponding acid **12** (type 3 cleavage).

O-Silyl deprotection of *N*-benzylamide **13** with tetrabutylammonium fluoride,[12] and treatment of the resulting hydroxy-derivative **14** with acetic anhydride did not lead to the expected *O*-acetoxy analogue, but to the initial amide **4a** and glyoxylic amide **15** (type 2 cleavage).

These results convinced us to use alternative routes to access the desired synthons **2** and **3**.

Hydrogenation of α-thiophenoxy allyl ester **10b** using tetrakis(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$] in the presence of PPh_3[13] led to the desired α-thiophenoxy acid **2** in acceptable yield (65%) (Scheme 4). α-Thiophenoxyamide bioisosteres **3a** and **3b** were obtained using the following synthetic route (Scheme 5). Boc-protected (L)-phenylalanyl-(L)-serine ethyl ester **16** after saponification[11] was condensed with benzylamine in the presence of BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate) reagent (96% yield).[14] The resulting amide **17** was refluxed with lead tetraacetate[15] in ethyl acetate and led quantitatively to the corresponding 1 : 1 ratio of α-acetoxybenzylamide diastereoisomers **18**. Racemisation occurred during the nucleophilic addition of acetic acid onto the two diastereofaces of the imine intermediate. The asymmetric carbon of the phenylalanine residue appears too far removed to affect the diastereoselectivity during this process. Nucleophilic substitution of the *O*-acetoxy group by lithium thiophenoxide led to the corresponding diastereoisomers **3a** and **3b** in acceptable yield (61%). The ratio of diastereoisomers **3a** and **3b** was calculated from the integration of the different ^1H NMR signals: two broad doublet peaks for the N*H* benzylamide protons at 6.81 and 6.94 ppm and for the N*H* protons (4.91 and 5.05 ppm); two doublet peaks for the C*H*-SPh protons at 5.69 and 5.72 ppm; and two singlet peaks for the *N*-Boc protons at 1.24 and 1.27 ppm.

In conclusion, versatile sulfenylated phenylalanine bioisosteres **2** and **3** can be used as original building blocks in the solid phase synthesis for the design of various protease inhibitors. Indeed compound **2** was found to be stable enough for solid phase synthesis of various HIV inhibitor peptides.[10] The fusogenic effect of HIV-1 on MT_4 cell line (*syncitia* formation) was determined for the peptides shown in Table 1 as described by Rey and co-workers.[16,17] EC_{50} values (concentration required to inhibit 50% *syncitia* formation) ranging from 5 to 10 μM were found for these peptides (Table 1). The mixture of diastereoisomers **3a** and **3b** showed potent anti-HIV activity (EC_{50} = 5 μM). However, the separation of the two isomers has not yet been achieved and it can only be suggested that one isomer should be more active than the other, as already reported in specific cases of antiprotease inhibitors.[18,19] Investigations into the determination of the enzymatic mechanism of action of such HIV inhibitors are currently in progress.

Table 1 Anti-HIV activities of synthetic [-Leu-Phe-(S)-] peptides and mixture of compounds **3a** and **3b**

Compounds	EC_{50}[a] /μM	TI[b] (CC_{50}[c]/ EC_{50}) /μM
Ile-Arg-Lys-Ile-Leu-Phe-(S)[d]-Leu-Asp-Gly-NH_2	5	50
Ile-Arg-Lys-Ile-Leu-Phe-(S)[d]-Leu-Asp-Gly-Ile-OH	10	10
Boc-Phe-(S)[d]-NH-CH_2Ph (mixture of **3a** and **3b**)	5	10

[a] EC_{50}: concentration required to inhibit 50% *syncitia* formation. [b] TI: therapeutic index. [c] CC_{50}: concentration required to cause death of 50% of uninfected MT_4 cells. [d] Phe-(S) represents thiophenoxyphenylalanine bioisostere residue: –NH–CH(SPh)–CO–.

Several analogs of compound **3**, with various functions (OH, COOH, NH_2) at the *para* position of the benzamide group could be prepared using a similar route to that described in Scheme 5.

Experimental

General

Nuclear magnetic resonance spectra were recorded with a Bruker AC-250 (^1H, ^{13}C NMR); chemical shifts are expressed as δ units (parts per million) downfield from TMS. Infrared spectra were obtained using a Perkin-Elmer 1600 FT-IR spectrophotometer, values are expressed in cm^{-1}. Fast atom bombardment mass spectral analysis was performed by Dr Astier (Laboratoire de Mesures Physiques-RMN, USTL, Montpellier, France) on a JEOL DX-100 using a caesium ion source and glycerol–thioglycerol (1 : 1) (GT) or *m*-nitrobenzyl alcohol (NOBA) as matrix. Mass calibration was performed using caesium iodide. Microanalyses were within 0.4% of the theoretical values. Microanalyses were performed by the Service Central d'Analyses, CNRS, Vernaison-Lyon, France. Preparative flash column chromatography was carried out on silica gel (230–240 mesh, G60 Merck). All reagents were of commercial quality (Aldrich Company) from freshly opened containers.

Anti-HIV evaluation assay

The CEM cell line and the T Leukaemia virus type one (HTLV-1) CD4-positive T cell line were cultured in RPMI–10% FCS (fetal calf serum) and refed twice a week. The laboratory-adapted strain HIV^{LAV} clade B stock was prepared from the supernatant of the infected CEM cell line and aliquots were

kept frozen at −80 °C until use. Anti-HIV activity was monitored as the efficiency of drug compounds to inhibit *syncytia* formation after HIV infection of MT_4 as already described. Briefly, 3×10^5 MT_4 cells were initially pre-incubated with 100 µL of various concentrations of drug compounds dissolved in phosphate buffer saline solution for 1 h at 37 °C. Then 100 µL of an appropriate virus dilution was added to the mixture and incubated at 37 °C for 1 h. After three washes, the cells were resuspended in the culture medium in the presence or absence of drug compounds. Cultures were then grown for 7 days at 37 °C, under 5% CO_2 atmosphere and refed at day 3 post-infection with culture medium supplemented (or not) with drug compounds. Each culture well was duplicated. The appearance of *syncytia* was followed each day with an inverted optical microscope. Typically, the virus dilution used in the assay (multiplicity of infection of 0.1 $TCID_{50}$/CELL) allowed *syncytia* formation at day 5 post-infection. The inhibitory concentration of drug compounds was expressed as the concentration that caused 50% inhibition of *syncytia* formation (EC_{50}) without direct toxicity for the cells. Cytotoxicity concentration (CC_{50}) of the drug compounds was monitored by the growth of non-infected cells using the trypan blue exclusion assay and corresponded to the concentration required to cause 50% of cell death.

N-*tert*-Butyloxycarbonyl-L-leucyl-DL-α-hydroxyglycine allyl ester (8b)

To a solution of allyl glyoxalate (2.0 g; 16.0 mmol) in dry dichloromethane (40 cm^3) *N-tert*-butyloxycarbonyl-L-leucine (2.8 g; 12.0 mmol) and triethylamine (4.0 cm^3; 29.0 mmol) were added. The solution was stirred at 40 °C for 72 hours and then dichloromethane was removed. The crude residue was purified by flash column chromatography on silica gel (solvent: dichloromethane–methanol 98:2) to give compound **8b** (1.81 g, 44%).

HMRS calcd for $C_{16}H_{28}N_2O_6$: 344.4111. Found: 344.4120. ν_{max} (film/cm^{-1}) 3310, 1790, 1653, 1596. δ_H (250 MHz; $CDCl_3$) 8.10 (br s, 1H, N*H*), 6.00–5.80 (m, 2H, C*H*=CH_2 + NH-C*H*-OH), 5.40–5.20 (m, 2H, C*H*$_2$=CH), 4.60 (m, 3H, NH-C*H*-CH_2 + O-C*H*$_2$-CH), 4.20 (br s, 1H, O*H*), 1.80–1.50 (m, 3H, C*H*$_2$-C*H*(CH_3)$_2$), 1.40 (s, 9H, (C*H*$_3$)$_3$CO), 0.80 (2d, 6H, (C*H*$_3$)$_2$CH). δ_C (62.8 MHz; $CDCl_3$) 175.4 (*C*O), 154.3 (*C*O), 135.5 (*C*H=CH_2), 110.3 (CH=*C*H$_2$), 80.1 (*C*H-OH), 70.3 (*C*H$_2$-CH=CH_2), 39.6 (*C*H$_2$CH(CH_3)$_2$), 28.5 ((*C*H$_3$)$_3$CO), 20.3 ((*C*H$_3$)$_2$CH).

N-*tert*-Butyloxycarbonyl-L-leucyl-DL-α-acetoxyglycine allyl ester (9b)

Compound **8b** (1.70 g; 5.10 mmol) was dissolved in dry pyridine (10 cm^3) and cooled to 0 °C, before acetic anhydride (20 cm^3; 0.21 mol) was added. The mixture was stirred at room temperature for 72 hours. After evaporation of solvent, ethyl acetate was added. The organic layer was washed with 5% sodium hydrogen carbonate solution and brine, dried on $MgSO_4$ and evaporated to afford compound **9b** (2.0 g, quantitative) which was used without further purification.

HMRS calcd for $C_{18}H_{30}N_2O_7$: 386.4490. Found: 386.4485. ν_{max} (film/cm^{-1}) 1788, 1774, 1654, 1588. δ_H (250 MHz; $CDCl_3$) 6.35 (m, 1H, C*H*=CH_2), 5.80 (m, 1H, CH=C*H*$_2$), 5.20–5.00 (m, 1H, C*H*=CH_2), 4.60 (m, 2H, C*H*-$OCOCH_3$ + O-C*H*$_2$-CH), 4.20–4.00 (m, 1H, NH-C*H*-CH_2), 2.5 (s, 3H, C*H*$_3$CO), 1.80–1.30 (m, 3H, C*H*$_2$-C*H*(CH_3)$_2$), 1.20 (s, 9H, (C*H*$_3$)$_3$CO), 0.80 (2d, 6H, (C*H*$_3$)$_2$CH).

N-*tert*-Butyloxycarbonyl-L-leucyl-DL-α-thiophenoxyglycine allyl ester (10b)

To a solution of compound **9b** (1.91 g; 5.10 mmol) dissolved in dry *N,N*-dimethylformamide (10 cm^3), thiophenol (0.67 g; 6.10 mmol) and *N,N*-diisopropylethylamine (0.79 g; 6.10 mmol) were added. The solution was stirred for 72 hours and then the solvent was evaporated. The crude residue was dissolved in water and the aqueous phase was extracted twice with ethyl acetate. The organic layer was dried on $MgSO_4$ and evaporated. The crude residue was purified by flash column chromatography on silica gel (solvent: toluene–methanol 9:1) to give compound **10b** (1.28 g, 58%).

HMRS calcd for $C_{22}H_{32}N_2O_5S$: 436.5747. Found: 436.5741. ν_{max} (film/cm^{-1}) 1788, 1655, 1592, 750, 698. δ_H (250 MHz; $CDCl_3$) 7.40–7.30 (m, 5H, C*H*$_{arom}$), 6.30 (m, 1H, C*H*=CH_2), 5.80 (m, 1H, CH=C*H*$_2$), 5.20–5.05 (m, 1H, C*H*=CH_2), 4.60–4.50 (m, 2H, C*H*-SPh + O-C*H*$_2$-CH), 4.20 (m, 1H, NH-C*H*-CH_2), 1.60–1.40 (m, 3H, C*H*$_2$-C*H*(CH_3)$_2$), 1.40 (s, 9H, (C*H*$_3$)$_3$CO), 0.90 (2d, 6H, (C*H*$_3$)$_2$CH). δ_C (62.8 MHz; $CDCl_3$) 172.3 (*C*O), 154.2 (*C*O), 136.5 (*C*H=CH_2), 135.4 ($C^{quat.}_{arom}$), 130.4, 129.5, 128.6 (*C*H$_{arom}$), 112.8 (CH=*C*H$_2$), 71.6 (*C*H$_2$-CH=CH_2), 68.4 (*C*H-SPh), 40.2 (*C*H$_2$CH(CH_3)$_2$), 28.4 ((*C*H$_3$)$_3$CO), 20.3 ((*C*H$_3$)$_2$CH).

N-*tert*-Butyloxycarbonyl-L-leucyl-DL-α-thiophenoxyglycine (2)

To a solution of compound **10b** (1.07 g; 2.45 mmol) in dry ethyl acetate (10 cm^3) a 0.5 M potassium 2-ethylhexanoate solution in ethyl acetate (0.63 cm^3; 0.3 mmol) was added. The solution was stirred at room temperature for 6 hours, then $Pd(PPh_3)_4$ (113.0 mg; 0.09 mmol) and PPh_3 (25.0 mg; 0.04 mmol) were successively added. The mixture was stirred for 16 hours at room temperature. The solvent was removed and the desired compound **2** (0.67 g, 70%) was obtained after purification by flash column chromatography on silica gel (solvent: dichloromethane–methanol 85:15).

HMRS calcd for $C_{19}H_{28}N_2O_5S$: 396.5094. Found: 396.4088 (Found: C, 57.7; H, 7.4; N, 7.2. Requires C, 57.5; H, 7.1; N, 7.0%). ν_{max} (film/cm^{-1}) 2312, 1718, 1654, 752, 702, 658. δ_H (250 MHz; $CDCl_3$) 8.00 (br s, 1H, N*H*), 7.40–7.10 (m, 5H, C*H*$_{arom}$), 4.50–4.20 (m, 2H, C*H*-SPh + NH-C*H*-CH_2), 4.10 (br s, 1H, NH), 1.60–1.40 (m, 3H, C*H*$_2$-C*H*(CH_3)$_2$), 1.40 (s, 9H, (C*H*$_3$)$_3$-CO), 0.90 (2d, 6H, (C*H*$_3$)$_2$CH). δ_C (62.8 MHz; $CDCl_3$) 180.1 (*C*OOH), 156.3 (*C*O), 136.0 ($C^{quat.}_{arom}$), 130.4, 129.5, 128.6 (*C*H$_{arom}$), 70.6 ($C^{quat.}_{Boc}$), 68.5 (*C*H-SPh), 55.0 (*C*H-CH_2(CH_3)$_2$), 40.3 (*C*H$_2$CH(CH_3)$_2$), 28.5 ((*C*H$_3$)$_3$CO), 20.4 ((*C*H$_3$)$_2$CH). FABMS *m*/*z* 395 $(MH)^-$.

N-*tert*-Butyloxycarbonyl-L-phenylalanyl-DL-α-(*tert*-butyldiphenylsilyloxy)glycine ethyl ester (11)

To a solution of compound **8a** (200 mg; 0.55 mmol) in dry *N,N*-dimethylformamide (15 cm^3), imidazole (260 mg; 3.82 mmol) and *tert*-butyldiphenylsilyl chloride (1.05 g; 3.82 mmol) were added. The mixture was stirred overnight, then *N,N*-dimethylformamide was removed and the crude residue was dissolved in ethyl acetate. The organic layer was washed twice with 5% citric acid solution, water, dried on $MgSO_4$ and evaporated. The crude residue was purified by flash column chromatography on silica gel (solvent: *n*-hexane–ethyl acetate 4:1) to afford **11** as a pale yellow oil (0.20 g, 62%).

HMRS calcd for $C_{34}H_{44}N_2O_6Si$: 604.8255. Found: 604.8310. ν_{max} (film/cm^{-1}) 1701, 1592, 909, 737, 668. δ_H (250 MHz; $CDCl_3$) 7.56–7.50 (m, 5H, C*H*$_{arom}$-Si), 7.28–7.19 (m, 5H, C*H*$_{arom}$-CH_2), 7.19–6.99 (m, 5H, C*H*$_{arom}$-Si), 5.56 (2d, 1H, C*H*-OSi, $J = 8.42$ Hz), 4.73 (br s, 1H, N*H*), 4.55 (br s, 1H, N*H*), 4.12 (m, 1H, C*H*-CH_2Ph), 3.97–3.76 (2q, 2H, O-C*H*$_2$-CH_3, $J = 7.41$ Hz), 2.92–2.69 (m, 2H, Ph-C*H*$_2$-CH), 1.20 (2s, 9H (C*H*$_3$)$_3$C-O), 0.95 (2t, 3H, O-CH_2-C*H*$_3$, $J = 7.20$ Hz), 0.90 (2s, 9H (C*H*$_3$)$_3$C-Si). δ_C (62.8 MHz; $CDCl_3$) 170.9 (*C*O), 168.7 (*C*O), 158.2 (*C*O), 136.7, 136.2, 136.1, 135.5, 135.4, 135.3, 132.9, 132.7, 132.3, 130.6, 130.3, 129.6, 128.9, 127.8, 127.1 (*C*H$_{arom}$), 73.1 (NH-*C*H-OSi), 61.9 (O-*C*H$_2$-CH_3), 38.1 (*C*H$_2$-Ph), 28.2 ((*C*H$_3$)$_3$C-O), 27.0 ((*C*H$_3$)$_3$C-Si), 14.3 (O-CH_2-*C*H$_3$).

N-tert-Butyloxycarbonyl-L-phenylalanyl-DL-α-(*tert*-butyl-diphenylsilyloxy)glycine (12)

To a solution of compound **11** (120 mg; 0.20 mmol) in methanol (5 cm^3) 1 M sodium hydroxide solution (0.5 cm^3; 0.45 mmol) was slowly added. The mixture was stirred for one hour then 1 M hydrochloric acid solution (0.20 cm^3) was added before methanol was evaporated. The residue was cooled to 0 °C before slow addition of 1 M hydrochloric acid solution (0.3 cm^3). The mixture was extracted three times with ethyl acetate. The organic layer was dried and evaporated to give **12** (0.09 g, 85%) which was used without further purification.

HMRS calcd for $C_{32}H_{40}N_2O_6Si$: 576.7714. Found: 576.7742. ν_{max} (film/cm^{-1}) 2358, 1698, 1662, 913, 737, 650. δ_H (250 MHz; $CDCl_3$) 7.50–7.47 (m, 5H, CH_{arom}-Si), 7.16–7.07 (m, 5H, CH_{arom}-CH_2), 7.03–6.89 (m, 5H, CH_{arom}-Si), 5.52 (2d, 1H, C*H*-OSi, J = 9.42 Hz), 4.93 (br s, 1H, N*H*), 4.59 (br s, 1H, N*H*), 4.07 (m, 1H, C*H*-CH_2Ph), 2.79–2.47 (m, 2H, Ph-CH_2-CH), 1.16 (2s, 9H, $(CH_3)_3$C-O), 0.86 (2s, 9H, $(CH_3)_3$C-Si).

N-Benzyl-[*N-tert*-butyloxycarbonyl-L-phenylalanyl-DL-α-(*tert*-butyldiphenylsilyloxy)]glycinamide (13)

Compound **12** (770 mg; 1.33 mmol) was dissolved in dry dichloromethane (10 cm^3) then benzylamine (0.15 cm^3; 1.33 mmol), BOP reagent (710 mg; 1.60 mmol) and triethylamine (0.46 cm^3; 3.32 mmol) were added. The mixture was stirred overnight before the solvent was evaporated. The crude residue was dissolved in ethyl acetate and the organic layer was successively washed with 5% sodium hydrogen carbonate solution, brine, dried on $MgSO_4$ and evaporated. The crude residue was purified by flash column chromatography on silica gel (solvent: *n*-hexane–ethyl acetate 7:3) to give **13** (0.39 g, 45%) as a yellow oil.

HMRS calcd for $C_{39}H_{47}N_3O_5Si$: 665.9125. Found: 665.9128. ν_{max} (film/cm^{-1}) 2359, 1683, 1558, 973, 737, 650. δ_H (250 MHz; $CDCl_3$) 7.42–6.92 (m, 20H, CH_{arom}), 6.76 (d, 1H, N*H*, J = 8.09 Hz), 6.65 (d, 1H, N*H*, J = 8.43 Hz), 6.37 + 6.29 (2br m, 2H, 2N*H*), 5.34 (2s, 1H, C*H*-OSi, J = 8.47 Hz), 4.54 (br m, 1H, N*H*), 4.54 + 4.45 (2br s, 2H, N*H*), 4.20–4.08 (m, 2H, Ph-CH_2-NH), 4.08–3.90 (m, 1H, C*H*-CH_2Ph), 2.84–2.40 (m, 2H, Ph-CH_2-CH), 1.12 (2s, 9H, $(CH_3)_3$C-O), 0.80 (2s, 9H, $(CH_3)_3$C-Si). δ_C (62.8 MHz; $CDCl_3$) 171.3, 170.2 (*C*O), 158.4 (*C*O), 136.7, 136.2, 136.1, 135.5, 135.4, 135.3, 132.9, 132.7, 132.3, 130.6, 130.3, 129.6, 128.9, 127.8, 127.1 (CH_{arom}), 73.5 (NH-*C*H-OSi), 47.5 (NH-*C*H_2-Ph), 38.2 (CH-*C*H_2-Ph), 28.3 ($(CH_3)_3$C-O), 27.1 ($(CH_3)_3$C-Si).

N-Benzyl-(*N-tert*-butyloxycarbonyl-L-phenylalanyl-DL-α-hydroxy)glycinamide (14)

To a solution of compound **17** (400 mg; 0.60 mmol) in dry tetrahydrofuran (5 cm^3) tetrabutylammonium fluoride (0.60 cm^3; 0.62 mmol) was added. The mixture was stirred for 30 minutes, then tetrahydrofuran was evaporated and the crude residue was dissolved in dichloromethane. The organic layer was washed twice with water, dried on $MgSO_4$ and evaporated. Compound **14** (0.17 g, 66%), mp 135–137 °C was obtained after purification by flash column chromatography on silica gel (solvent: *n*-hexane–ethyl acetate 2:3) as a white solid.

HMRS calcd for $C_{23}H_{29}N_3O_5$: 427.5046. Found: 427.5120. ν_{max} (film/cm^{-1}) 2360, 1694, 1592, 909, 737, 668. δ_H (250 MHz; $CDCl_3$) 7.25–7.12 (m, 10H, CH_{arom}), 6.07 (br s, 1H, N*H*), 5.86 (br m, 2H, N*H* + O*H*), 5.16 (br m, 1H, N*H*), 5.00 (2d, 1H, C*H*-OH, J = 8.08 Hz), 4.31 (m, 2H, Ph-CH_2-NH), 4.06 (m, 1H, C*H*-CH_2Ph), 2.96 (m, 2H, Ph-CH_2-CH), 1.31 (s, 9H, $(CH_3)_3$-C-O). δ_C (62.8 MHz; $CDCl_3$) 136.8, 135.2, 129.5, 129.1, 128.9, 128.7, 128.3, 127.2 (*C*H_{arom}), 78.6 (NH-*C*H-OH), 47.4 (NH-*C*H_2-Ph), 38.7 (CH-*C*H_2-Ph), 28.5 ($(CH_3)_3$C-O). FABMS *m*/*z* 428 $(MH)^+$.

N-tert-Butyloxycarbonyl-L-phenylalanyl-L-serine ethyl ester (16)

To a solution of *N-tert*-butyloxycarbonyl-L-phenylalanine (2.34 g; 8.84 mmol) in dry dichloromethane (70 cm^3) L-serine ethyl ester hydrochloride (1.50 g; 8.84 mmol), BOP reagent (4.69 g; 10.60 mmol) and triethylamine (3.10 cm^3; 22.0 mmol) were added. The mixture was stirred overnight at room temperature. The solvent was removed and the crude residue was dissolved in ethyl acetate. After four washes with 5% sodium hydrogen carbonate solution, brine, 5% citric acid solution and brine, successively, the organic layer was dried on $MgSO_4$ and evaporated. Compound **16** (3.09 g, 92%), mp 113–115 °C was purified by flash column chromatography on silica gel (solvent: *n*-hexane–ethyl acetate 2:3) as a white solid.

HMRS calcd for $C_{19}H_{28}N_2O_6$: 380.4448. Found: 380.4398. ν_{max} (film/cm^{-1}) 3598, 1702, 1658, 908, 731, 658. δ_H (250 MHz; $CDCl_3$) 7.40 (br d, 1H, N*H*, J = 7.21 Hz), 7.19–7.14 (m, 5H, CH_{arom}), 5.57 (d, 1H, N*H*, J = 7.73 Hz), 4.55 (m, 1H, C*H*-CH_2OH), 4.45 (m, 1H, C*H*-CH_2Ph), 4.15–4.00 (q, 2H, O-CH_2-CH_3, J = 10.65 Hz), 3.91 (d, 2H, CH_2-OH, J = 5.70 Hz), 3.81 (br s, 1H, O*H*), 3.10–2.75 (m, 2H, Ph-CH_2-CH), 1.26 (s, 9H, $(CH_3)_3$C-O), 1.13 (t, 3H, O-CH_2-CH_3, J = 7.13 Hz). δ_C (62.8 MHz; $CDCl_3$) 172.8 (*C*O), 170.6 (*C*O), 156.2 (*C*O), 137.0 ($C^{quat.}_{arom}$), 129.6, 128.6, 126.9 (CH_{arom}), 80.4 ($C^{quat.}_{t-Bu}$), 62.7 (*C*H_2-OH), 62.0 (O-*C*H_2-CH_3), 56.0 (Ph-CH_2-*C*H), 50.5 (*C*H-CH_2-OH), 38.7 (CH-*C*H_2-Ph), 28.4 ($(CH_3)_3$C-O), 14.2 (*C*H_3-CH_2). FABMS *m*/*z* 381 $(MH)^+$.

N-Benzyl-(*N-tert*-butyloxycarbonyl-L-phenylalanyl)-L-serinamide (17)

Compound **16** (1.50 g; 3.94 mmol) was dissolved in methanol (10 cm^3), then 1 M sodium hydroxide solution (8.70 cm^3; 8.67 mmol) was added slowly. After 3 hours, 1 M hydrochloric acid solution (4 cm^3) was added and methanol was removed, then 1 M hydrochloric acid solution (4.7 cm^3) was added slowly at 0 °C. The aqueous layer was extracted twice with ethyl acetate, and the organic layer was dried on $MgSO_4$. The crude carboxylic acid intermediate, obtained in a quantitative yield (3.05 g; 8.67 mmol), was dissolved in dry dichloromethane (30 cm^3) then benzylamine (0.94 cm^3; 8.67 mmol), BOP reagent (4.60 g; 10.40 mmol) and triethylamine (3.01 cm^3; 21.67 mmol) were added. After one night's stirring at room temperature, the solvent was removed and the residue dissolved in ethyl acetate. The organic layer was washed successively with 5% sodium hydrogen carbonate solution, 5% citric acid solution and brine, and then dried and evaporated. The crude residue was purified by flash column chromatography on silica gel (solvent: ethyl acetate) to afford **17** (1.66 g, 96%), mp 150–154 °C as a white solid.

HMRS calcd for $C_{24}H_{31}N_3O_5$: 441.5317. Found: 441.5220. ν_{max} (film/cm^{-1}) 3573, 1705, 1662, 1562, 910, 736, 660. δ_H (250 MHz; $CDCl_3$) 7.40 (br m, 1H, N*H*), 7.20–6.95 (m, 10H, CH_{arom}), 6.82 (d, 1H, N*H*, J = 7.78 Hz), 5.00 (br m, 1H, N*H*, J = 7.21 Hz), 4.39 (m, 1H, C*H*-CH_2OH), 4.31–4.25 (m, 3H, C*H*-CH_2Ph + NH-CH_2Ph), 4.16–3.97 (m, 2H, CH_2-OH), 2.97 (m, 2H, Ph-CH_2-CH), 1.26 (s, 9H, $(CH_3)_3$C-O). δ_C (62.8 MHz; $CDCl_3$) 172.8, 170.5 (*C*O), 137.1 ($C^{quat.}_{arom}$), 129.6, 128.6, 128.4, 127.3, 127.2, 127.0 (CH_{arom}), 80.3 ($C^{quat.}_{t-Bu}$), 62.6 (*C*H_2-OH), 56.0 (Ph-CH_2-*C*H), 43.2 (Ph-*C*H_2-NH), 38.5 (CH-*C*H_2-Ph), 28.3 ($(CH_3)_3$C-O). FABMS *m*/*z* 442$(MH)^+$.

N-Benzyl-(*N-tert*-butyloxycarbonyl-L-phenylalanyl-DL-α-acetoxy)glycinamide (18)

Compound **17** (750 mg; 1.51 mmol) was dissolved in dry ethyl acetate (30 cm^3) then lead tetraacetate (2.00 g; 4.53 mmol) and molecular sieves 4 Å (3 g) were added. The mixture was refluxed under nitrogen for 2 hours. The precipitate was filtrated on Celite and the organic layer was stirred with 10% citric acid solution during 20 minutes. The organic layer was washed with

water and dried on $MgSO_4$. The crude product **18** (0.67 g, 96%) was used in the next step without purification.

ν_{max} (film/cm^{-1}) 3405, 2245, 1690, 1497, 908, 731, 668. δ_H (250 MHz; $CDCl_3$) 7.79 (d, 1H, N*H*, *J* = 8.53 Hz), 7.68 (d, 1H, N*H*, *J* = 8.72 Hz), 7.20–6.93 (m, 10H, CH_{arom}), 6.45 (2d, 1H, NH-C*H*-OCOCH$_3$, *J* = 8.88 Hz), 5.20 (br m, 1H, N*H*), 4.50–4.40 (m, 1H, C*H*-CH$_2$Ph), 4.31 (m, 2H, NH-CH_2Ph), 3.08–2.93 (m, 2H, Ph-CH_2-CH), 1.95 (2s, 3H, CH_3-CO), 1.26 (2s, 9H, (CH_3)$_3$C-O). δ_C (62.8 MHz; $CDCl_3$) 173.1, 172.1 (*C*O), 165.6 (*C*O), 137.5, 137.0 ($C^{quat.}{}_{arom}$), 129.6, 129.5, 129.3, 128.9, 128.8, 127.8, 127.3, 127.2 (CH_{arom}), 73.3 (NH-*C*H-OCOCH$_3$), 44.0 (Ph-*C*H$_2$-NH), 28.4 ((*C*H$_3$)$_3$C-O), 21.0 (*C*H$_3$-CO). FABMS *m*/*z* 470 (MH)$^+$.

N-Benzyl-(*N-tert*-butyloxycarbonyl-L-phenylalanyl-DL-α-thiophenoxy)glycinamide (3a) and (3b)

Compound **18** (2.71 g; 5.77 mmol) dissolved in dry tetrahydrofuran (20 cm^3) was cooled to 0 °C. A solution of 1.0 M lithium thiophenoxide in tetrahydrofuran (6.92 cm^3; 6.92 mmol) was added slowly. After one hour, the solvent was removed, the residue was dissolved in dichloromethane and the organic layer was washed with water, and then dried on $MgSO_4$ and evaporated. The crude residue was purified by flash column chromatography on silica gel (solvent: toluene–ethyl acetate 3:7) to afford a mixture of **3a** and **3b** (1.82 g, 61%), mp 70–72 °C as yellow crystals.

Compound **3a**: HMRS calcd for $C_{29}H_{33}N_3O_4S$: 519.6680. Found: 519.6695 (Found: C, 67.1; H, 6.6; N, 8.2. Requires C, 67.0; H, 6.4; N, 8.0%). ν_{max} (film/cm^{-1}) 3418, 2253, 1667, 1495, 908, 735, 660. δ_H (250 MHz; $CDCl_3$) 1.24 (s, 9H, (CH_3)$_3$CO), 2.76–2.99 (m, 2H, PhCH_2-CH), 4.02–4.38 (m, 3H, C*H*-CH$_2$Ph + NH-CH_2Ph), 4.91 (br d, 1H, N*H*, *J* = 7.45 Hz), 5.68 (d, 1H, NH-C*H*-SPh, *J* = 8.21 Hz), 6.81 (br d, 1H, N*H*), 7.00–7.28 (m, 15H, CH_{arom}). δ_C (62.8 MHz; $CDCl_3$) 171.1, 170.9, 167.0 (*C*O), 137.5, 136.5, 134.6 ($C^{quat.}{}_{arom}$), 130.4, 129.4, 129.0, 128.9, 128.6, 128.5, 127.8, 127.5, 126.8 (CH_{arom}), 57.7 (Ph-CH$_2$-*C*H), 44.0 (Ph-*C*H$_2$-NH), 38.3 (Ph-*C*H$_2$-CH), 28.2 ((*C*H$_3$)$_3$CO). FABMS *m*/*z* 520 (MH)$^+$.

Compound **3b**: HMRS calcd for $C_{29}H_{33}N_3O_4S$: 519.6680. Found: 519.6695 (Found: C, 67.1; H, 6.6; N, 8.2. Requires C, 67.0; H, 6.4; N, 8.0%). ν_{max} (film/cm^{-1}) 3418, 2253, 1667, 1495, 908, 735, 660. δ_H (250 MHz; $CDCl_3$) 1.27 (s, 9H, (CH_3)$_3$CO), 2.76–2.99 (m, 2H, PhCH_2-CH), 4.02–4.38 (m, 3H, C*H*-CH$_2$Ph + NH-CH_2Ph), 5.05 (br d, 1H, N*H*, *J* = 9.66 Hz), 5.72 (d, 1H, NH-C*H*-SPh, *J* = 8.25 Hz), 6.94 (br d, 1H, N*H*), 7.00–7.28 (m, 15H, CH_{arom}). δ_C (62.8 MHz; $CDCl_3$) 171.1, 170.9, 167.0 (*C*O), 137.5, 136.5, 134.6 ($C^{quat.}{}_{arom}$), 130.4, 129.4, 129.0, 128.9, 128.6, 128.5, 127.8, 127.5, 126.8 (CH_{arom}), 57.7 (Ph-CH$_2$-*C*H), 44.0 (Ph-*C*H$_2$-NH), 38.3 (Ph-*C*H$_2$-CH), 28.2 ((*C*H$_3$)$_3$CO). FABMS *m*/*z* 520 (MH)$^+$.

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