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Parallel synthesis of new phenylalanino-2-(phenylthio)pyrrolidin-3-one scaffold-based analogues

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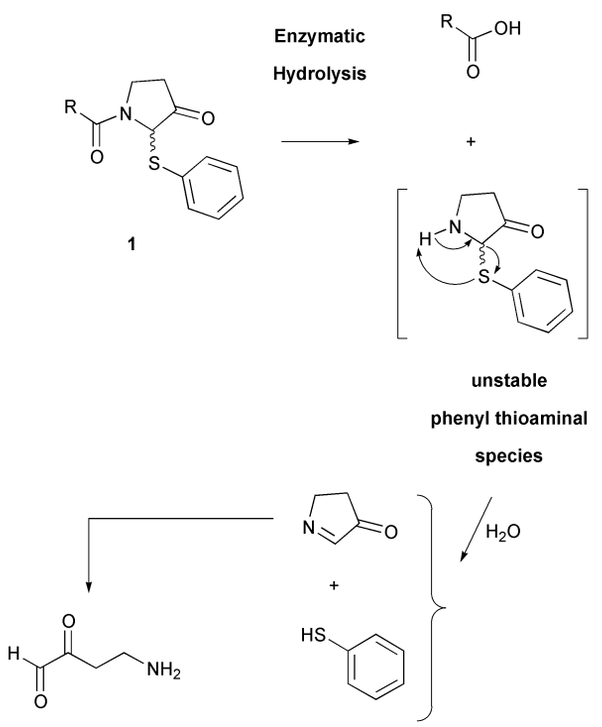
Based on the specific PhePro proteolytic cleavage of the HIV-protease, we synthesized short pseudopeptides incorporating a 2-(phenylthio)pyrrolidin-3-one ring. We previously described various analogues based on this scaffold. We report herein a parallel methodology with improved efficiency applied to the synthesis of new derivatives. For this purpose, we selected an intermediate, *i.e.* **8**, which can be used as a versatile synthon after simple removal of the *N*-Boc protecting group of the starting material as the penultimate reaction step. This new route improved the synthesis of these molecules in terms of numbers of reaction and in terms of both reagent and reaction time, and allowed the preparation of a small library focused on the phenylalanino-2-(phenylthio)pyrrolidin-3-one scaffold. A phenylthio group at the 2-position confers on the resulting derivative a high reactivity, which could be unmasked upon hydrolysis, and which led to the release of toxophoric thiophenol as detected by Ellman's reagent. Biological activity of these compounds was analysed towards various representative proteolytic enzymes. Pure diastereoisomers **7**(***R***) and **7**(***S***) displayed potent inhibitory activities against HIV-protease.

Introduction

Viral proteases are crucial in the replication cycle of many viruses, including retroviruses such as Human Immunodeficiency Virus (HIV); herpes viruses; picorna viruses such as rhinovirus; and flaviviruses such as Hepatitis C Virus (HCV). Viral proteases have therefore been favoured as targets for antiviral agents.[1] Proteases cleave newly expressed precursor polyproteins into smaller, mature viral proteins. For example, in HIV replication, HIV protease is an aspartate protease which cleaves *gag* and *pol* precursor proteins[2] whereas herpes virus proteases that have Ser-His-His as the catalytic triad[3] or HCV proteases encoded by the non-structural NS3 domain[4] are serine proteases. Rhinovirus 3C protease is a cysteine protease involved in the proteolytic cleavage of the viral precursor polyprotein to both capsid and functional proteins required for RNA replication.[5–7] Chiral 2-substituted pyrrolidin-3-ols or pyrrolidin-3-ones have been widely used in the design of pseudopeptides as aspartate,[8–10] serine,[11] and cysteine[12,13] protease inhibitors or non-peptidic neuroamidase inhibitors.[14]

We have recently reported new HIV-aspartyl protease inhibitors, which incorporate a 2-(phenylthio)pyrrolidin-3-one moiety as proline replacement.[10] Introduction of a phenylthio group at position 2 of a pyrrolidinone ring could be of interest since hydrolysis of the amido bond leads to an unstable phenylthioaminal intermediate which undergoes a chemical rearrangement allowing the release of toxophoric thiophenol as shown in Scheme 1. Such a mechanism has already been reported by Kingsbury *et al.*[15] during the enzymatic hydrolysis of Ala-(*S*)-(phenylthio)glycine. Acylation of the intracyclic amine provides stabilization of the resulting molecule by delocalization of the nitrogen electrons into the amide bond. If compounds such as **1** (Scheme 1), where R represents various amino acid residues, are recognized as substrates by intracellular peptidases, hydrolysis of the peptide bond should result in the release of thiophenol. These observations prompted us to investigate the synthesis of a focused library of new derivatives containing a 2-(phenylthio)pyrrolidin-3-one moiety, to test their activities (substrate or inhibitor) on representative families of proteases: aspartate protease (HIV-aspartyl protease), serine protease (α-chymotrypsin and trypsin) and cysteine protease (caspase 3); and to confirm the ability of these compounds to release the toxophoric thiophenol upon hydrolysis.



Scheme 1 Structure of *N*-substituted 2-(phenylthio)pyrrolidin-3-ones **1** and their mode of breakdown after peptidase cleavage.[15]

Results and discussion

Since 2-(phenylthio)pyrrolidin-3-one is an unstable species which cannot be isolated (Scheme 1), the synthesis of the target

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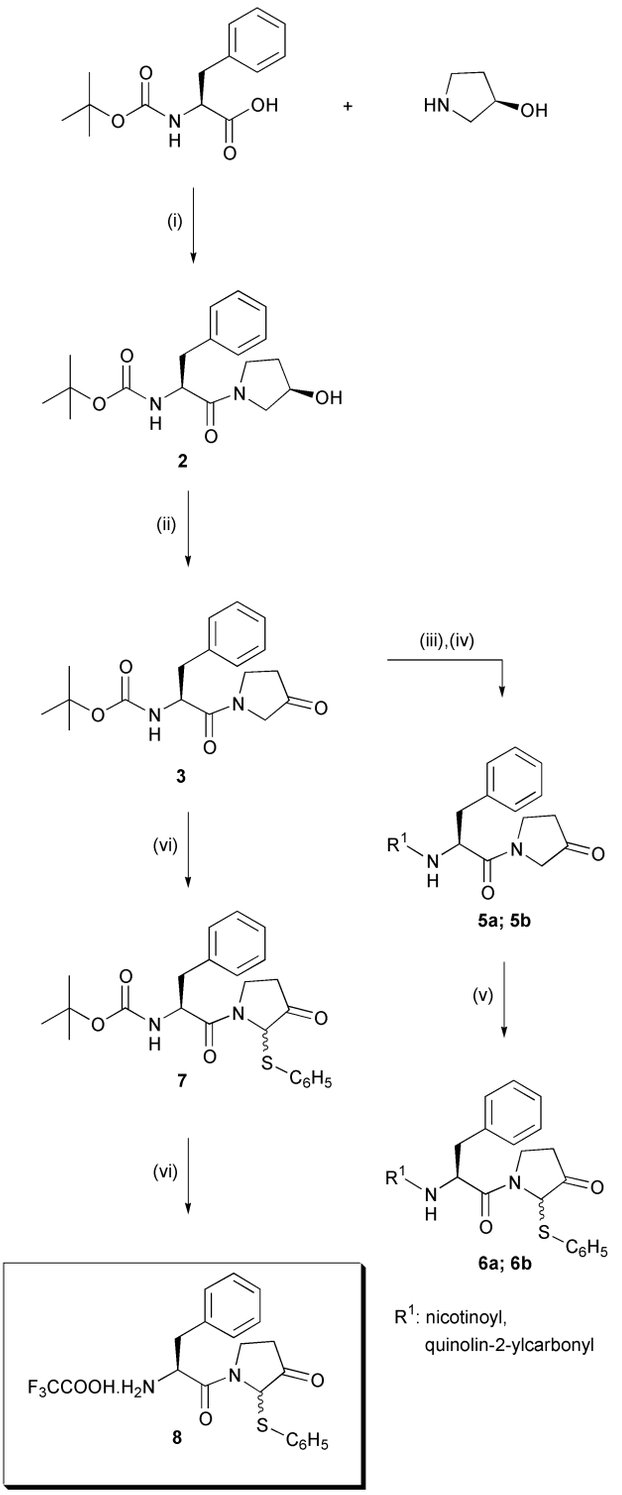
compounds through direct coupling between L-amino acid residues with 2-(phenylthio)pyrrolidin-3-one cannot be considered. Therefore the synthesis of the new derivatives required a specific synthetic route as summarized in Scheme 2. Compound **2** was obtained through a standard coupling reaction between Boc-L-PheOH and (*R*)-pyrrolidin-3-ol using (benzotriazol-1-yloxy) tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as coupling agent.[16] Direct oxidation of this compound under Swern's conditions[17] led to the corresponding pyrrolidin-3-one derivative **3**. As far as sulfenylation at the 2-position of the pyrrolidinone ring was the most crucial step, we were able to use two different chemical pathways to perform the synthesis of the target molecules. On the one hand, intermediate **3** was first deprotected and then coupled to various moieties. Resulting compounds could be submitted to a sulfenylation reaction in order to obtain the desired derivatives. We used and described previously this methodology to perform the synthesis of such nicotinoyl and quinolin-2-carbonyl analogues **6a** and **6b**, respectively (Scheme 2, Route 1).[10] On the other hand, **3** could be first sulfenylated. The resulting *N*-Boc 2-phenylthio derivative **7** was then deprotected to lead to the versatile TFA salt synthon **8**. This intermediate was coupled to various reagents to give the desired derivatives (Scheme 2, Route 2).



Scheme 2 Synthesis of 2-(phenylthio)pyrrolidin-3-one analogues **6a**–**b** and synthon **8**. *Reagents and conditions*: (i) BOP, Et_3N, CH_2Cl_2, RT (91%); (ii) TFAA, DMSO, DIEA, CH_2Cl_2, −60 °C (80%); Route 1: (iii) TFA, CH_2Cl_2 (10 : 90), RT; (iv) **4a** or **4b**, DMF, HOBt, DCC, Et_3N (**5a**: 70%, **5b**: 38%); (v) (a) *sec*-BuLi, toluene, −78 °C; (b) $PhSO_2SPh$, toluene, −78 °C (**6a**: 10%; **6b**: 22%); Route 2: (vi) (a) *sec*-BuLi, THF, −78 °C; (b) $PhSO_2SPh$, THF, −78 °C (39%); (vii) TFA–CH_2Cl_2 (10 : 90), RT.

As mentioned above and for different reasons, the most crucial step of these synthetic pathways was the sulfenylation of the pyrrolidin-3-one ring. Indeed, stereoselectivity and regioselectivity were both important parameters involved during this reaction. Besides, it was the synthetic sequence's yield-limiting step.

Various attempts were made to optimize all these parameters and, of course, the yield of the reaction. Compound **3**, easily obtained in a two-step reaction, was used to optimize the limiting sulfenylation reaction.

The sulfenylation step was performed at −78 °C in toluene or tetrahydrofuran (THF) using one equivalent of various bases *versus* various stoichiometric amounts of substrate **3** and sulfenylating agent; this latter could be phenyl disulfide or *S*-phenyl benzenethiosulfonate.[18] An α-keto carbanion in equilibrium with its two possible corresponding enolates was *in-situ* base-generated before an electrophilic addition of the sulfenylating agent.[19] Two α-keto positions were available on the pyrrolidin-3-one ring during this α-sulfenylation. For this reason, it was necessary to control the regioselectivity of the monosubstitution reaction which must preferentially occur at the 2-position of the ring and not at the 4-position.

The different bases used during this study were *sec*-butyllithium, lithium bis(trimethylsilyl)amide (LiHMDS) and lithium diisopropylamide (LDA). As shown in Table 1, the best results were obtained using the following stoichiometric conditions: base : sulfenylating agent : substrate **3** (1 : 1.5 : 1.5). As expected, we observed the formation of 2- and 4-mono- and polysubstituted pyrrolidin-3-one species, which were fully characterized. The desired monosulfenylated derivative **7** was the main reaction product. The main by-product was the 4,4-disulfenylated analogue **7a** and the minor one was the 2,4,4-trisulfenylated derivative **7b** (Scheme 3). We never observed the presence of any analogues disubstituted at the 2-position or monosubstituted at the 4-position of the pyrrolidin-3-one ring. The presence of 4,4-disubstituted derivatives and the absence of 4-monosubstituted analogues can be explained by the acidic character of the geminal proton[20] of the *in-situ*-generated 4-monosubstituted intermediate. This proton was then easily substituted by a second molecule of the electrophilic agent.

At the 2-position of the pyrrolidin-3-one ring, the second proton was less available for a second base abstraction. Indeed, the steric hindrance due to the presence on this carbon of three bulky groups, *i.e.* a trisubstituted nitrogen atom, a carbonyl group and a phenylthio substituent, reduced drastically the availability of the last proton towards the base. Even if a strong base such as *sec*-butyllithium could extract this proton, the steric hindrance was too great for electrophilic attack by another bulky phenylthio group to succeed.

The ratio between the sulfenylated isolated compounds was dependent of the various reaction parameters mentioned above. The 2,4,4-trisubstituted analogue **7b** was always present in the

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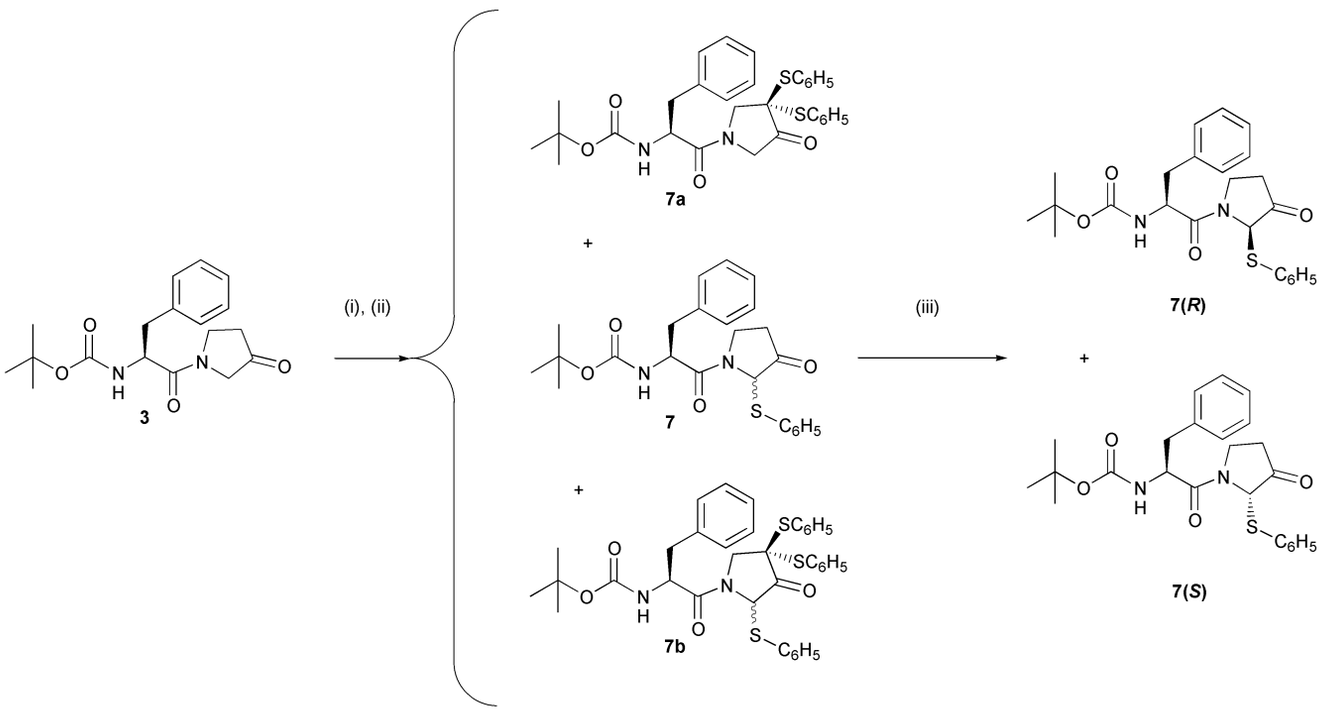
Table 1 Sulfenylation study of **3** (Scheme 3)

Base (1.0 equiv.)	**3** (mol equiv.)	$PhSO_2SPh$ (mol equiv.)	Molar ratio **7** : **7a**	Molar ratio **7**(***R***) : **7**(***S***)	Yield of **7** (%)
sec-BuLi[a]	1.5	1.5	5.0 : 1.0	1 : 1	39
sec-BuLi[a]	1.0	2.0	1.8 : 1.0	1 : 1	13
sec-BuLi[b]	1.0	2.0	1.2 : 1.0	1 : 1	17
sec-BuLi[a]	1.5	2.0	4.7 : 1.0	1 : 1	25
sec-BuLi[a]	0.5	2.0	0.8 : 1.0	1 : 1	10
LiHMDS[a]	1.0	2.0	1.0 : 1.0	1 : 1	12
LDA[a]	1.0	2.0	2.3 : 1.0	1 : 1	15
LDA[b]	1.0	2.0	1.5 : 1.0	1 : 1	8

Reaction time to generate the enolate of substrate **3**:[a] 1 hour; [b] 2 hours.



Scheme 3 Synthesis and separation of the diastereoisomeric mixture **7**. *Reagents and conditions*: (i) *sec*-BuLi, THF, −78 °C; (ii) $PhSO_2SPh$, THF, −78 °C; (iii) HPLC, C18 Waters Spherisorb column, eluent CH_3CN–H_2O (60 : 40) [**7**(***R***) : **7**(***S***) 1 : 1].

crude reaction mixture but only in a small amount, less than 5%. As mentioned above, the two major compounds were the 2-monosulfenylated and 4,4-disulfenylated derivatives, respectively **7** and **7a**.

The best molar ratio between **7** and **7a** was observed under the following conditions: 1 equivalent of *sec*-butyllithium, 1.5 equivalent of *S*-phenyl benzenethiosulfonate and 1.5 equivalent of pyrrolidin-3-one derivative **3** in toluene or THF at −78 °C (Table 1). Under these experimental conditions, the yield in compound **7** was 39%. Despite the real improvement in the yield of this step, this reaction remained the yield-limiting step of this synthetic route. We observed the same phenomenon when the sulfenylation reaction was the final step involved in the synthesis of the nicotinoyl (**6a**) and quinolin-2-ylcarbonyl (**6b**) analogues with yields of 10 and 22%, respectively.[10] It was obvious that it was easier to prepare a large amount of the *N*-Boc-protected synthon **3** *via* a two-step reaction than to prepare each *N*-substituted phenylalanine analogue before the final reaction which should be the sulfenylation reaction. In this case, it was required to obtain a large amount of the penultimate intermediate. For this reason, we performed the synthetic sequence according to the procedure involving the sulfenylation of the *N*-Boc-protected synthon **3** (Scheme 2, Route 2).

The 2-monosubstituted pyrrolidin-3-one derivative **7** was isolated as a mixture of the two corresponding diastereoisomers **7**(***R***) and **7**(***S***). Indeed, we did not observe any isomerization of the phenylalanine residue, which conserved its L-configuration. The ratio of the two diastereoisomers was determined by analytical HPLC. In each case, we observed the same ratio, *i.e.* **7**(***R***) : **7**(***S***) 1 : 1 (Scheme 3).

During this sulfenylation reaction, we were able to control the regioselectivity of the electrophilic addition. We always obtained the desired monosulfenylated derivative **7** as a mixture of its two diastereoisomers. The following steps were performed on this mixture of diastereoisomers.

Synthesis of a small focused library from synthon 8 (Scheme 4). The removal of the *N*-Boc protecting group led to the corresponding TFA salt **8** which represents a versatile and very useful synthon in a parallel synthetic procedure.

In order to prepare different analogues, we investigated the parallel coupling reaction of this synthon **8** with several functionalized reagents selected from the following families of reagents: sulfonyl chlorides leading to the corresponding sulfonamides; chloroformates to give carbamates; carbamoyl chlorides and isocyanates to give ureas; amino acids and peptides to give amides.

As representative sulfonyl chlorides, we selected 4-nitrobenzenesulfonyl chloride **9a** and 4-methoxybenzenesulfonyl chloride **9b** for the synthesis of the corresponding sulfonamides **10a** and **10b**. 4-Nitrophenyl chloroformate **9c** was used to prepare the carbamate analogue **10c**. Morpholine-4-carbonyl chloride **9d** and isocyanates **9e** and **9f** gave the corresponding ureas **10d**, **10e** and **10f**.

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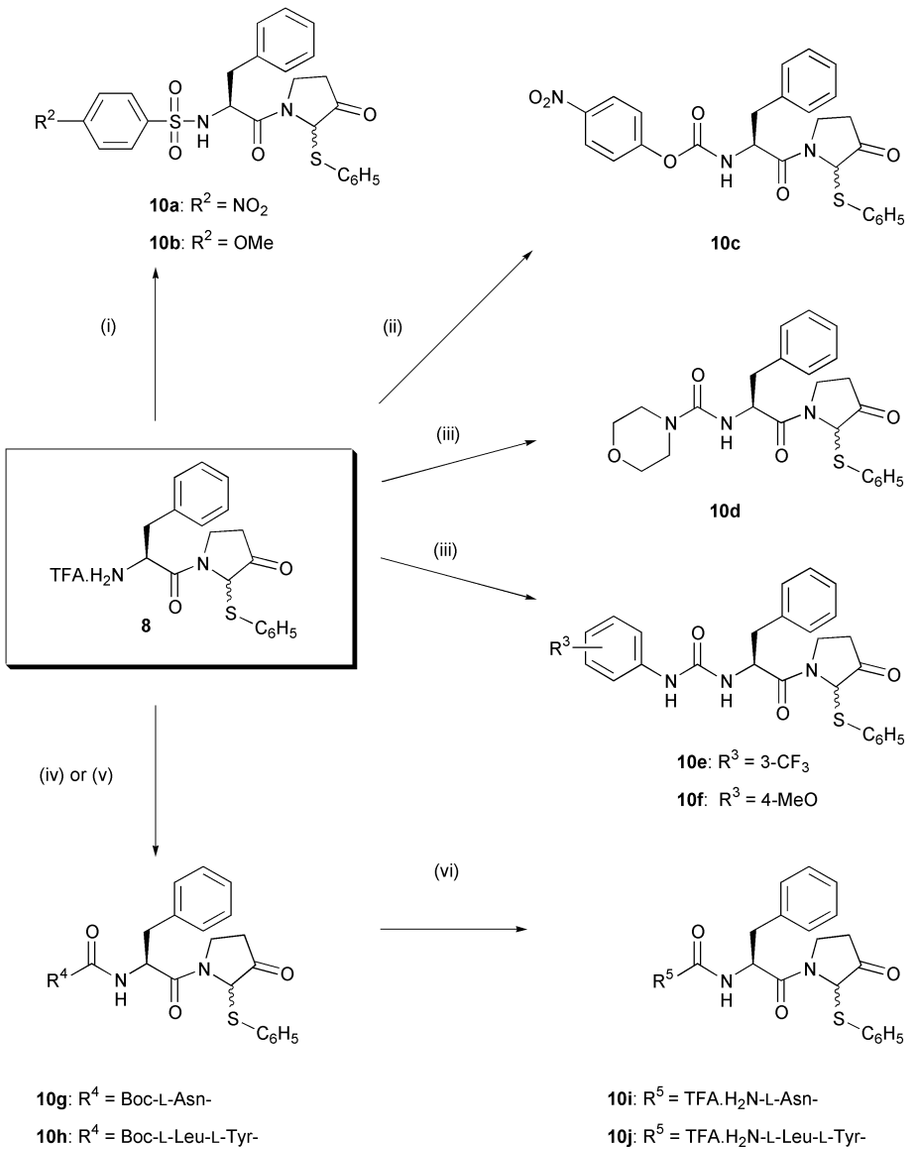

Scheme 4 Synthesis of 2-(phenylthio)pyrrolidin-3-one analogues **10a**–**j**. *Reagents and conditions*: (i) **9a** or **9b**, Et_3N, CH_3CN (**9a**) or CH_2Cl_2 (**9b**), RT [from **7** : **10a**, 42% ($R^2 = NO_2$); **10b**, 53% (R^2 = OMe)]; (ii) **9c**, Et_3N, CH_2Cl_2, RT (from **7** : **10c**, 34%); (iii) **9d**, **9e** or **9f**, Et_3N, CH_2Cl_2, RT [from **7** : **10d**, 54%; **10e**, 71% ($R^3 = 3\text{-}CF_3$); **10f**, 67% (R^3 = 4-MeO)]; (iv) **9g**, EDC, DIEA, HOBt, DMF, RT [from **7** : **10g**, 55% (R^4 = Boc-L-Asn–)]; (v) BOP, DIEA, **9h**, CH_2Cl_2, RT [from **7** : **10h**, 64% (R^4 = Boc-L-Leu-L-Tyr–)]; (vi) TFA, CH_2Cl_2 (10 : 90), RT [**10i**, quantitative (R^5 = TFA·H_2N-L-Asn–); **10j**, 93% (R^5 = TFA·H_2N-L-Leu-L-Tyr–)].

Boc-L-AsnOH **9g** and dipeptide Boc-L-Leu-L-TyrOH **9h** led, respectively, to derivatives **10g** and **10h**. It should be pointed out that activation of the carboxy group of asparagine derivatives is known to be complicated by the formation of cyanoalanine analogues.[21,22] 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC)–HOBt was proven to been an efficient coupling system for the activation of the carboxylic function of Boc-L-AsnOH.[22] Application of this procedure led to a moderate yield (55%) for the two-step synthesis of the *N*-Boc-L-asparagyl analogue **10g**. In the case of the dipeptide **9h**, BOP reagent was successfully used to give the corresponding pyrrolidinone derivative **10h** in moderate yield (64%). Products **10g** and **10h** were deprotected and the corresponding TFA salts **10i** and **10j**, respectively, were isolated in good yields.

The parallel synthesis of this small library using the versatile TFA salt synthon **8** as intermediate involved only a small amount of the *N*-Boc-protected 2-sulfenylated pyrrolidin-3-one starting material **7**, in contrast to the other synthetic sequence (Scheme 2, Route 1) which required a larger amount of the penultimate compound to perform the ultimate sulfenylation reaction with a very low yield.

As demonstrated, the purpose of this study was to perform the synthesis of a small library of 2-(phenylthio)pyrrolidin-3-one analogues thanks to the versatility of **8**. This procedure could be extended to a larger library.

Diastereoisomeric separation. As mentioned previously, the *N*-Boc-protected 2-sulfenylated pyrrolidin-3-one **7** and the final compounds **10a**–**j** were obtained as diastereoisomeric mixtures. As we observed no isomerization of the C_1 stereogenic center when *sec*-butyllithium was used as base in the sulfenylation step of **3**, the derivatives were mixtures of only two diastereoisomers in which asymmetric carbon C_1 of the phenylalanine residue was in its L-configuration. Separation of the diastereoisomeric mixtures could be achieved by a semipreparative method using a reversed-phase C18 Waters Spherisorb column and CH_3CN–H_2O (60 : 40) as eluent. This procedure, when applied to diastereoisomeric mixture **7**, led to both pure stereoisomers **7**(***R***) and **7**(***S***), which were fully characterized. It should be underlined that *R* and *S* assignments of the asymmetric carbon C_2 had not then been determined.

Thiophenol release evidenced by chemical hydrolysis. The concept of the release of the toxophoric thiophenol (Scheme 1) according to Kingsbury *et al.*[15] was validated upon chemical hydrolysis. Indeed, when the mixture **7** was submitted to basic

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hydrolysis, *i.e.* 0.1 M NaOH in H_2O–THF 1 : 1 (v : v), the release of thiophenol was identified by using Ellman's reagent 5,5′-dithiobis(2-nitrobenzoic acid).[23] Thiophenol release was spectrophotometrically monitored (λ_{max} = 412 nm), thereby confirming the initial hypothesis summarized in Scheme 1.

Protease-inactivation studies. The activities of these new 2-(phenylthio)pyrrolidin-3-one analogues were evaluated on representative classes of proteases including: aspartyl protease (HIV-protease), serine protease (α-chymotrypsin and trypsin) and cysteine protease (caspase 3). Enzymatic-activity evaluation was first performed directly on diastereoisomeric mixtures; only pure diastereoisomers which derived from mixtures showing substantial inhibitory properties were separately assayed. We found that none of the different mixtures **10a**–**j** showed any significant antiprotease activity on serine and cysteine proteases. Only pure diastereoisomers **7**(***R***) and **7**(***S***), which mixture was active on HIV-aspartyl protease (IC_{50} = 0.1 μM, the concentration required to inhibit 50% of the activity of the enzyme), were tested separately. *R* and *S* assignment of the asymmetric carbon C_2 had not then been determined but one of the two diastereoisomers was found to be more potent than its homologue: IC_{50}-values of 0.008 μM and 1 μM.[10]

In summary, we report herein the parallel synthesis of a small focused library based on the phenylalanine-2-(phenylthio)-pyrrolidin-3-one scaffold. The versatile synthon **8** was coupled to different reagents as various as sulfonyl chlorides; chloroformates; carbamoyl chlorides and isocyanates; amino acids and peptides. The synthetic results were promising and allowed the preparation of a larger library. Our interest in the phenylthio group at the 2-position of the pyrrolidinone ring was based on the fact that hydrolysis (chemically or enzymatically) of the exocyclic amide bond of these compounds led to the release of toxophoric thiophenol as spectrophotometrically demonstrated by using Ellman's reagent. The small parallel library was biologically tested towards various representative proteolytic enzymes, *i.e.* HIV-aspartyl protease, α-chymotrypsin and trypsin as serine protease, and caspase 3 as cysteine protease. Only diastereoisomeric mixture **7** and both purified diastereoisomers **7**(***R***) and **7**(***S***) diplayed an inhibitory activity *versus* HIV-aspartyl protease. No crucial activity was pointed out towards the serine and cysteine proteases.

Experimental

Unless otherwise noticed, starting materials and reagents were obtained from commercial suppliers and were used without purification. All the protected amino acids and peptide-coupling reagents were purchased from Bachem, Neosystem and Aldrich. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl immediately prior to use. Methylene dichloride (CH_2Cl_2) was distilled over P_2O_5 just prior to use. Dimethylformamide (DMF), toluene and dimethylsulfoxide (DMSO) were of anhydrous quality from commercial suppliers (Aldrich). Nuclear magnetic resonance spectra were recorded at 250 MHz for ^1H and 62.9 MHz for ^{13}C on a Brüker AC-250 spectrometer and at 300 MHz for ^1H on a Brüker Avance 300. Chemical shifts are expressed as δ-units (ppm) downfield from TMS (tetramethylsilane). Fast-atom bombardment (FAB^+) mass spectral analyses were obtained 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) or *m*-nitrobenzyl alcohol (NOBA) as matrix. Mass calibration was performed using caesium iodide. IR spectra were recorded on a Perkin-Elmer FTIR 1605 spectrophotometer. Analytical HPLC was performed on a Waters 600E instrument with a Waters 991 detector using a Waters Spherisorb S5 ODS2 column (4.6 × 150 mm; 5 μM) and a two-mobile phase system (0.1 % formic acid in water/0.1 % formic acid in acetonitrile: 40 : 60; flow rate 1 mL min^{-1}). Semi-preparative HPLC was performed by using a reversed-phase C18 Waters Spherisorb column and CH_3CN–H_2O (60 : 40) as eluent. Microanalyses were carried out by Service Central d'Analyses du CNRS (Venaison, France) and were within 0.4% of the theoretical values. UV spectra for chemical analysis and biological assays were obtained with an Uvikon 930 spectrophotometer (Kontron Instruments). Analytical thin-layer chromatography (TLC) was performed using silica gel plates 0.2 mm thick ($60F_{254}$ Merck). Preparative flash-column chromatography was carried out on silica gel (230–240 mesh, G60 Merck).

(*R*)-*N*-[*N*-(*tert*-Butoxycarbonyl)-L-phenylalanyl]pyrrolidin-3-ol **2**[10,16]

Boc-L-PheOH (4.44 g, 1.0 equiv., 16.75 mmol) was dissolved in 100 mL of anhydrous CH_2Cl_2. 1.2 Equiv. of BOP (8.89 g, 20.10 mmol) was then added. The resulting solution was cooled to 0 °C and 1.0 equiv. of Et_3N (2.3 mL, 16.75 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred for 30 min. The solution was then cooled to 0 °C and a solution of (*R*)-pyrrolidin-3-ol (1.46 g, 1.0 equiv., 16.75 mmol) and Et_3N (3.5 mL, 1.5 equiv., 25.13 mmol) in 20 mL of anhydrous CH_2Cl_2 was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred for 2 hours. The reaction was monitored by TLC (EtOAc–hexane 9 : 1). The solution was concentrated under reduced pressure. The residue was diluted in ethyl acetate (120 mL) and washed successively with H_2O (50 mL), 5% aq. $NaHCO_3$ (50 mL), H_2O (50 mL), 5% aq. citric acid (2 × 50 mL) and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography (EtOAc–hexane 9 : 1) to give **2** as a white hygroscopic powder (5.07 g, 91%), R_f 0.19 (EtOAc–hexane 9 : 1); mp 56–58 °C; ^1H NMR (250 MHz; $CDCl_3$) δ 1.21 (s, 9 H, CH_3 Boc), 1.40–1.80 (m, 2 H, –$NCH_2CH_2CH(OH)$–), 2.30–2.80 (m, 3 H, –$NCH_2CH_2CH(OH)$– + CH^aH^b β Phe), 3.21–3.37 (m, 3 H, –$NCH_2CH(OH)$– + CH^aH^b β Phe), 3.90 (br s, 1 H, O*H*), 4.05–4.20 (m, 1 H, –C*H*OH), 4.25–4.50 (m, 1 H, C*H* α Phe), 5.15–5.32 (m, 1 H, N*H*), 7.01–7.07 (m, 5 H, C_6H_5); ^{13}C NMR (62.9 MHz; $CDCl_3$) δ_C 27.1, 35.1, 39.4, 40.4, 50.8, 52.5, 73.1, 79.9, 126.8, 128.4, 129.5, 135.9, 155.2, 171.1; ν_{max} (KBr)/cm^{-1} 3335, 2952, 1750, 1680; MS (FAB > 0, GT) *m*/*z* 357 $(M + Na)^+$. Calc. for $C_{18}H_{26}N_2O_4$: 334.41 g mol^{-1}. Found: C, 64.28; H, 8.02; N, 8.52. $C_{18}H_{26}N_2O_4$ requires C, 64.65; H, 7.84; N, 8.38%.

N-[*N*-(*tert*-Butoxycarbonyl)-L-phenylalanyl]pyrrolidin-3-one **3**[10,17]

Anhydrous DMSO (2.54 mL, 2.0 equiv., 35.88 mmol) was dissolved in 32 mL of anhydrous CH_2Cl_2. The solution was cooled to −60 °C and 1.5 equiv. of trifluoroacetic anhydride (TFAA, 3.80 mL, 26.91 mmol) was added dropwise. The reaction mixture was stirred at −60 °C for 15 min and a solution of 1.0 equiv. of alcohol **2** (6.00 g, 17.94 mmol) in 32 mL of anhydrous CH_2Cl_2 was then added. The solution was stirred at −60 °C for 1 hour. The reaction was monitored by TLC (EtOAc–hexane 9 : 1). Diisopropylethylamine (DIEA) (13.75 mL, 4.4 equiv., 78.94 mmol) was then added dropwise to the reaction mixture, which was then stirred for 15 min at −60 °C. The solution was allowed to warm to room temperature and 100 mL of H_2O was added. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc–hexane 4 : 6) to give the desired ketone **3** as a pale yellow hygroscopic solid (4.70 g, 80%), R_f 0.15 (EtOAc–hexane 4 : 6); mp 106–108 °C; ^1H NMR (300 MHz; $CDCl_3$) δ 1.25 (br s, 9 H, CH_3 Boc), 2.17–2.28 (m, 2 H, –$NCH_2CH_2C(O)$–), 2.71–2.88 (m, 4 H, CH_2 β Phe + –$NCH_2CH_2C(O)$–), 3.43–3.71 (m, 2 H, –$NCH_2C(O)$–), 4.22–4.50 (m, 1 H, C*H* α Phe), 5.15 (br t,

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1 H, N*H*), 6.95–7.15 (m, 5 H, C_6H_5); ^{13}C NMR (62.9 MHz; $CDCl_3$) δ_C 28.4, 36.1, 39.5, 42.3, 52.0, 53.9, 79.5, 126.9, 128.4, 129.3, 136.4, 155.1, 170.9, 209.1; ν_{max} (KBr)/cm^{-1} 2980, 1762, 1760; MS (FAB > 0, GT) *m*/*z* 333 (M + H)$^+$. Calc. for $C_{18}H_{24}N_2O_4$: 332.39 g mol^{-1}. Found: C, 64.90; H, 7.14; N, 8.72. $C_{18}H_{24}N_2O_4$ requires C, 65.04; H, 7.28; N, 8.43%.

N-(*N*-Nicotinoyl-L-phenylalanyl)pyrrolidin-3-one 5a [10]

The previous ketone **3** (560 mg, 1.0 equiv., 1.68 mmol) was dissolved in 5 mL of CH_2Cl_2. 10.0 Equiv. of TFA (1.29 mL, 16.80 mmol) were added dropwise. The reaction mixture was stirred for 2 hours at room temperature. The reaction was monitored by TLC (EtOAc–hexane 4 : 6). The solvent and excess of TFA were removed under reduced pressure. The desired TFA salt was used immediately without any further purification for the following step. The TFA salt was diluted in 15 mL of anhydrous DMF with 1.1 equiv. of nicotinic acid **4a** (228 mg, 1.85 mmol) and 2.0 equiv. of HOBt (454 mg, 3.36 mmol). DCC (519 mg, 1.5 equiv., 2.52 mmol) was added as a solid followed by Et_3N (700 µL, 3.0 equiv., 5.04 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC (CH_2Cl_2–MeOH 9 : 1). The solution was diluted with 10 mL of diethyl ether and filtered to remove the solid 1,3-dicyclohexylurea (DCU). The solid was washed with diethyl ether (3 × 10 mL) and the filtrate was concentrated under reduced pressure. The crude material was purified by flash chromatography (CH_2Cl_2–MeOH 9 : 1) to give the desired compound **5a** (389 mg, 70%) as a colourless oil, R_f 0.36 (CH_2Cl_2–MeOH 9 : 1); ^{1}H NMR (250 MHz; CD_3OD) δ 2.15–2.27 (m, 2 H, –NCH$_2$C*H*$_2$C(O)–), 2.70–3.05 (m, 4 H, C*H*$_2$ β Phe + –NC*H*$_2$CH$_2$C(O)–), 3.45–3.75 (m, 2 H, –NC*H*$_2$C(O)–), 4.70–4.85 (m, 1 H, C*H* α Phe), 6.95–7.15 (m, 5 H, C_6H_5), 7.40–7.75 (m, 4H, C_5H_4N); ^{13}C NMR (62.9 MHz; CD_3OD) δ_C 36.2, 39.6, 42.5, 52.1, 53.9, 123.1, 126.9, 127.4, 128.4, 136.1, 136.4, 151.7, 158.4, 171.0, 173.1, 210.2; ν_{max} (KBr)/cm^{-1} 2979, 1763, 1762, 1759, 1640; MS (FAB > 0, GT) *m*/*z* 338 (M + H)$^+$. Calc. for $C_{19}H_{19}N_3O_3$: 337.37 g mol^{-1}. Found: C, 67.89; H, 5.32; N, 12.75. $C_{19}H_{19}N_3O_3$ requires C, 67.54; H, 5.68; N, 12.46%.

N-[*N*-(Quinolin-2-ylcarbonyl)-L-phenylalanyl]pyrrolidin-3-one 5b [10]

The title compound was prepared according to the two-step procedure described above from the protected ketone **3** (294 mg, 1.0 equiv., 0.75 mmol) which was deprotected with TFA before the coupling reaction with 2-quinaldic acid **4b** (144 mg, 1.1 equiv., 0.83 mmol). The crude material was purified by flash chromatography (CH_2Cl_2–MeOH 9 : 1) to give the desired compound **5b** (110 mg, 38%) as a colourless oil, R_f 0.71 (CH_2Cl_2–MeOH 9 : 1); ^{1}H NMR (250 MHz; CD_3OD) δ 2.31–2.53 (m, 2 H, –NCH$_2$C*H*$_2$C(O)–), 3.00–3.90 (m, 6 H, C*H*$_2$ β Phe + –NC*H*$_2$CH$_2$C(O)– + –NC*H*$_2$C(O)–), 4.80–4.88 (m, 1 H, C*H* α Phe), 7.05–7.20 (m, 5 H, C_6H_5), 7.40–8.15 (m, 6H, C_9H_6N); ^{13}C NMR (62.9 MHz; CD_3OD) δ_C 37.0, 39.4, 42.3, 52.2, 53.8, 126.9, 128.3, 128.4, 129.2, 129.3, 129.7, 129.7, 130.2, 133.1, 136.3, 138.2, 149.3, 171.0, 172.2, 210.0; ν_{max} (KBr)/cm^{-1} 2981, 1762, 1760, 1757, 1680, 1641; MS (FAB > 0, GT) *m*/*z* 388 (M + H)$^+$. Calc. for $C_{23}H_{21}N_3O_3$: 387.43 g mol^{-1}. Found: C, 70.15; H, 5.74; N, 11.10. $C_{23}H_{21}N_3O_3$ requires C, 71.30; H, 5.46; N, 10.85%.

N-(*N*-Nicotinoyl-L-phenylalanyl)-2-(phenylthio)pyrrolidin-3-one 6a [10]

The *N*-substituted ketone **5a** (300 mg, 1.5 equiv., 0.89 mmol) was dissolved in 5 mL of anhydrous toluene and the solution was cooled to −78 °C. 1.0 Equiv. of *sec*-butyllithium [1.3 M in cyclohexane (460 µL)] was then added dropwise. The reaction mixture was stirred at −78 °C for 1 hour before addition of a toluene solution of 1.5 equiv. of *S*-phenyl benzenethiosulfonate (223 mg, 0.89 mmol). The reaction was monitored by TLC (toluene–MeOH 9 : 1). After 30 min at −78 °C, the solution was allowed to warm to room temperature and the reaction was quenched by addition of 5 mL of saturated aq. NH_4Cl. The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography to give the desired compound **6a** as a pale yellow oil (40 mg, 10%), R_f 0.20 (toluene–MeOH 9 : 1); ^{1}H NMR (250 MHz; CD_3OD) δ 2.15–2.25 (m, 2 H, –NCH$_2$C*H*$_2$C(O)–), 2.70–3.00 (m, 4 H, C*H*$_2$ β Phe + –NC*H*$_2$CH$_2$C(O)–), 4.75–4.85 (m, 1 H, C*H* α Phe), 5.13–5.30 (m, 1 H, C*H*SPh), 7.00–7.15 (m, 10 H, C_6H_5), 7.40–8.75 (m, 4H, C_5H_4N); ^{13}C NMR (62.9 MHz; CD_3OD) δ_C 36.1, 39.2, 42.6, 52.1, 70.4, 123.1, 126.9, 127.3, 128.3, 129.3, 129.8, 130.1, 134.0, 134.4, 136.0, 136.4, 151.7, 158.4, 171.0, 174.0, 210.2; ν_{max} (KBr)/cm^{-1} 2980, 1763, 1760, 1680, 1640; MS (FAB > 0, GT) *m*/*z* 446 (M + H)$^+$. Calc. for $C_{25}H_{23}N_3O_3S$: 445.53 g mol^{-1}. Found: C, 67.03; H, 4.88; N, 9.36. $C_{25}H_{23}N_3O_3S$ requires C, 67.39; H, 5.20; N, 9.43%.

N-[*N*-(Quinolin-2-ylcarbonyl)-L-phenylalanyl]-2-(phenylthio)-pyrrolidin-3-one 6b [10]

The title compound **6b** was obtained according to the procedure described above from the corresponding ketone **5b** (90 mg, 1.5 equiv., 0.23 mmol) and after purification by flash chromatography (EtOAc–hexane 2 : 3) as a pale yellow oil (25 mg, 22%), R_f 0.29 (EtOAc–hexane 2 : 3); ^{1}H NMR (250 MHz; CD_3OD) δ 2.30–2.45 (m, 2 H, –NCH$_2$C*H*$_2$C(O)–), 2.75–3.50 (m, 4 H, C*H*$_2$ β Phe + –NC*H*$_2$CH$_2$C(O)–), 4.80–4.95 (m, 1 H, C*H* α Phe), 5.15–5.25 (m, 1 H, C*H*SPh), 7.00–7.15 (m, 10 H, C_6H_5), 7.40–8.15 (m, 6H, C_9H_6N); ^{13}C NMR (62.9 MHz; CD_3OD) δ_C 37.0, 39.4, 42.4, 52.1, 72.4, 126.9, 128.3, 128.7, 129.3, 129.6, 129.8, 130.1, 130.2, 133.2, 134.0, 136.4, 138.3, 149.3, 170.9, 172.0, 209.1; ν_{max} (KBr)/cm^{-1} 2980, 1760, 1758, 1680, 1640; MS (FAB > 0, GT) *m*/*z* 496 (M + H)$^+$. Calc. for $C_{29}H_{25}N_3O_3S$: 495.59 g mol^{-1}. Found: C, 70.07; H, 4.81; N, 8.26. $C_{29}H_{25}N_3O_3S$ requires C, 70.28; H, 5.08; N, 8.48%.

N-[*N*-(*tert*-Butoxycarbonyl)-L-phenylalanyl]-2-(phenylthio)-pyrrolidin-3-one 7 [10]

The ketone **3** (1.00 g, 1.5 equiv., 2.99 mmol) was dissolved in 12.5 mL of freshly distilled THF. The solution was cooled to −78 °C. *sec*-BuLi [1.3 M in cyclohexane (1.53 mL, 1.0 equiv., 1.99 mmol)] was then added dropwise. The reaction mixture was stirred for 1 hour at −78 °C. A solution of 1.5 equiv. of *S*-phenyl benzenethiosulfonate (0.75 g, 2.99 mmol) in 3 mL of freshly distilled THF was then added dropwise. The resulting mixture was stirred for 1 hour at −78 °C. The reaction was monitored by TLC (EtOAc–hexane 3 : 7). The reaction mixture was then allowed to warm to room temperature and 5 mL of saturated aq. NH_4Cl was added. The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography to give the desired compound **7** as a pale yellow solid (514 mg, 39%), R_f 0.20 (EtOAc–hexane 3 : 7); mp 56–58 °C; ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.45 (br s, 9 H, C*H*$_3$ Boc), 1.75–2.45 (m, 2 H, –NCH$_2$C*H*$_2$C(O)–), 2.70–3.05 (m, 4 H, C*H*$_2$ β Phe + –NC*H*$_2$CH$_2$C(O)–), 4.40–4.90 (m, 1 H, C*H* α Phe), 5.00–5.30 (m, 1 H, C*H*SPh), 5.50 (br s, 1 H, N*H*), 6.90–7.40 (m, 10 H, Ar*H*); ^{13}C NMR (62.9 MHz; $CDCl_3$) δ_C 28.4, 39.7, 39.9, 42.7, 53.2, 65.6, 78.0, 126.0–138.0, 155.0, 170.0, 206.1; ν_{max} (KBr)/cm^{-1} 2980, 1760, 1757, 1680; MS (FAB > 0, GT) *m*/*z* 441 (M + H)$^+$. Calc. for $C_{24}H_{28}N_2O_4S$: 440.54 g mol^{-1}. Found: C, 65.38; H, 6.65; N, 6.27. $C_{24}H_{28}N_2O_4S$ requires C, 65.43; H, 6.41; N, 6.36%.

The pure diastereoisomers were separated by semi-preparative HPLC using a reversed-phase C18 Waters Spherisorb column and CH_3CN–H_2O (60 : 40) as eluent.

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First stereoisomer: t_R: 11.9 min; ^{1}H NMR (250 MHz; $CDCl_3$) δ 1.50 (br s, 9 H, CH_3 Boc), 1.75–2.45 (m, 2 H, –NCH$_2$-CH_2C(O)–), 2.72–3.05 (m, 4 H, CH_2 β Phe + –NCH_2CH$_2$-C(O)–), 4.85–5.00 (m, 1 H, CH α Phe), 5.25–5.45 (m, 1 H, CHSPh), 5.65 (br s, 1 H, NH), 7.08–7.50 (m, 10 H, ArH); MS (FAB > 0, GT) m/z 441 (M + H)$^+$.

Second stereoisomer: t_R: 12.6 min; ^{1}H NMR (250 MHz; $CDCl_3$) δ 1.41 (br s, 9 H, CH_3 Boc), 1.75–2.45 (m, 2 H, –NCH$_2$-CH_2C(O)–), 2.69–3.07 (m, 4 H, CH_2 β Phe + –NCH_2CH$_2$-C(O)–), 4.40–4.75 (m, 1 H, CH α Phe), 5.10–5.30 (m, 1 H, CHSPh), 5.55 (br s, 1 H, NH), 7.05–7.50 (m, 10 H, ArH); MS (FAB > 0, GT) m/z 441 (M + H)$^+$.

N-[L-Phenylalanyl-2-(phenylthio)pyrrolidinone trifluoroacetic acid salt 8

General procedure. The *N*-Boc-protected ketone **7** (1.0 equiv.) was dissolved at 0 °C in 2 mL of a 10% solution of TFA in CH_2Cl_2. The reaction mixture was allowed to warm to room temperature and was stirred for 2 hours. The reaction was monitored by TLC (EtOAc–hexane 3 : 7 and EtOAc). The solvent was removed under reduced pressure and the excess of TFA was co-evaporated successively with CH_2Cl_2 and toluene to give the desired TFA salt **8** as a black velvet solid which was used immediately without any further purification for the following step, R_f 0.13 (EtOAc); MS (FAB > 0, GT) m/z 341 (M + H)$^+$. Calc. for $C_{19}H_{20}N_2O_2S$: 340.43 g mol^{-1}.

N-[*N*-(4-Nitrophenylsulfonyl)-L-phenylalanyl]-2-(phenylthio)-pyrrolidin-3-one 10a

The TFA salt **8** (obtained from 90 mg, 0.20 mmol of **7**) was dissolved in 1 mL of anhydrous acetonitrile and the resulting solution was cooled to 0 °C. 4-Nitrobenzenesulfonyl chloride **9a** (58 mg, 1.3 equiv., 0.27 mmol) was then added as a solid followed by K_2CO_3 (34 mg, 1.2 equiv., 0.25 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC (EtOAc and EtOAc–hexane 3 : 7). The solvent was removed under reduced pressure. The residue was dissolved in 10 mL of EtOAc and washed with H_2O (2 × 5 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc–hexane 3 : 7) to give the desired compound **10a** (45 mg, 42%) as a yellow solid, R_f 0.23 (EtOAc–hexane 3 : 7); mp 140–142 °C; ^{1}H NMR (300 MHz; $CDCl_3$) δ 2.00–2.50 (m, 2 H, –NCH$_2$CH_2C(O)–), 2.80–3.30 (m, 4 H, CH_2 β Phe + –NCH_2CH$_2$C(O)–), 4.90–5.10 (m, 1 H, CH α Phe), 5.25–5.40 (m, 1 H, CHSPh), 5.60 (br s, 1 H, NH), 7.10–7.55 (m, 14 H, ArH); ^{13}C NMR (62.9 MHz; $CDCl_3$) δ_C 39.5, 39.6, 42.0, 52.5, 71.0, 123.9, 124.9, 125.7, 126.4, 126.6, 127.9, 128.4, 128.7, 135.6, 140.2, 145.4, 151.6, 173.9, 207.1; ν_{max} (KBr)/cm^{-1} 1766, 1761, 1758, 1325, 1160; MS (FAB > 0, GT) m/z 526 (M + H)$^+$. Calc. for $C_{25}H_{23}N_3O_6S_2$: 525.57 g mol^{-1}. Found: C, 56.95; H, 4.18; N, 8.12. $C_{25}H_{23}N_3O_6S_2$ requires C, 57.13; H, 4.41; N, 7.99%.

N-[*N*-(4-Methoxyphenylsulfonyl)-L-phenylalanyl]-2-(phenylthio)pyrrolidin-3-one 10b

The TFA salt **8** (obtained from 100 mg, 0.23 mmol of **7**) was dissolved in 1 mL of anhydrous CH_2Cl_2 and the resulting solution was cooled to 0 °C. 4-Methoxybenzenesulfonyl chloride **9b** (61 mg, 1.3 equiv., 0.29 mmol) was then added as a solid followed by Et_3N (73 µL, 2.3 equiv., 0.52 mmol). The reaction mixture was stirred for 2 hours at room temperature. The reaction was monitored by TLC (EtOAc and EtOAc–hexane 1 : 1). The solvent was removed under reduced pressure. The residue was dissolved in 10 mL of EtOAc and successively washed with H_2O (5 mL), 5% aq. citric acid (5 mL), and H_2O (5 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc–hexane 1 : 1) to give the desired compound **10b** (61 mg, 53%) as a yellow solid, R_f 0.37 (EtOAc–hexane 1 : 1); mp 112–114 °C; ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.60–2.20 (m, 2 H, –NCH$_2$CH_2C(O)–), 2.60–2.90 (m, 4 H, CH_2 β Phe + –NCH_2CH$_2$C(O)–), 3.60 (br s, 3 H, OCH_3), 4.25–4.55 (m, 1 H, CH α Phe), 4.90–5.10 (m, 1 H, CHSPh), 5.50 (br s, 1 H, NH), 6.55–7.70 (m, 14 H, ArH); ^{13}C NMR (62.9 MHz; $CDCl_3$) δ_C 38.7, 39.7, 42.5, 53.1, 56.0, 71.8, 114.4, 125.0, 125.1, 126.5, 126.6, 127.5, 127.8, 128.0, 131.6, 134.6, 139.3, 165.2, 171.0, 209.8; ν_{max} (KBr)/cm^{-1} 1765, 1760, 1757, 1155; MS (FAB > 0, GT) m/z 511 (M + H)$^+$. Calc. for $C_{26}H_{26}N_2O_5S_2$: 510.61 g mol^{-1}. Found: C, 60.95; H, 5.02; N, 5.86. $C_{26}H_{26}N_2O_5S_2$ requires C, 61.16; H, 5.13; N, 5.49%.

N-[*N*-(4-Nitrophenyloxycarbonyl)-L-phenylalanyl]-2-(phenylthio)pyrrolidin-3-one 10c

The title compound **10c** was prepared according to a similar procedure as described as previously, starting from 90 mg (0.20 mmol) of *N*-Boc-protected **7**. The resulting TFA salt **8** was treated with 4-nitrophenyl chloroformate **9c** (49 mg, 1.2 equiv., 0.25 mmol) and Et_3N (65 µL, 2.3 equiv., 0.47 mmol). The residue of the reaction after work-up was purified by flash chromatography (EtOAc–hexane 3 : 7) to give the desired compound **10c** (35 mg, 34%) as an yellow oil, R_f 0.22 (EtOAc–hexane 3 : 7); ^{1}H NMR (300 MHz; $CDCl_3$) δ 2.00–2.50 (m, 2 H, –NCH$_2$CH_2C(O)–), 2.65–3.10 (m, 4 H, –NCH_2CH$_2$C(O)– + CH_2 β Phe), 4.70–4.95 (m, 1 H, CH α Phe), 5.05–5.40 (m, 1 H, CHSPh), 6.45 (br s, 1 H, NH), 6.70 (d, 2 H, ArH, ^3J = 9.0 Hz), 6.90–7.40 (m, 10 H, C$_6H_5$), 7.95 (d, 2 H, ArH, ^3J = 9.0 Hz); ^{13}C NMR (62.9 MHz; $CDCl_3$) δ_C 37.8, 41.4, 42.0, 56.4, 70.2, 122.0, 124.3, 124.8, 126.6, 127.5, 127.9, 128.8, 128.9, 135.6, 141.2, 145.2, 157.5, 158.5, 171.0, 209.3; ν_{max} (KBr)/cm^{-1} 2975, 1763, 1757, 1753, 1342; MS (FAB > 0, GT) m/z 506 (M + H)$^+$. Calc. for $C_{26}H_{23}N_3O_6S$: 505.52 g mol^{-1}. Found: C, 61.50; H, 4.22; N, 8.12. $C_{26}H_{23}N_3O_6S$ requires C, 61.77; H, 4.59; N, 8.31%.

N-[*N*-(4-Morpholinylcarbonyl)-L-phenylalanyl]-2-(phenylthio)-pyrrolidin-3-one 10d

The title compound **10d** was prepared according to a similar procedure as described as previously, starting from 52 mg (0.12 mmol) of *N*-Boc-protected **7**. The resulting TFA salt **8** was treated with morpholine-*N*-carbonyl chloride **9d** (17 µL, 1.2 equiv., 0.15 mmol) and Et_3N (38 µL, 2.3 equiv., 0.27 mmol). The residue of the reaction after work-up was purified by flash chromatography (EtOAc) to give the desired compound **10d** (40 mg, 54%) as a brown solid, R_f 0.33 (EtOAc); mp 86–88 °C; ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.80–2.50 (m, 2 H, –NCH$_2$-CH_2C(O)–), 2.60–3.55 (m, 12 H, 4 × CH_2 morpholine + CH_2 β Phe + –NCH_2CH$_2$C(O)–), 4.65–5.00 (m, 1 H, CH α Phe), 5.05–5.35 (m, 1 H, CHSPh), 5.72 (br s, 1 H, NH), 6.95–7.40 (m, 10 H, ArH); ^{13}C NMR (62.9 MHz; $CDCl_3$) δ_C 36.3, 39.1, 42.4, 52.5, 58.1, 70.2, 70.8, 125.0, 125.9, 126.6, 127.6, 128.4, 128.7, 137.1, 138.6, 164.2, 172.1, 208.0; ν_{max} (KBr)/cm^{-1} 2981, 1760, 1756, 1754; MS (FAB > 0, GT) m/z 454 (M + H)$^+$. Calc. for $C_{24}H_{27}N_3O_4S$: 453.54 g mol^{-1}. Found: C, 63.18; H, 6.25; N, 9.43. $C_{24}H_{27}N_3O_4S$ requires C, 63.55; H, 6.00; N, 9.26%.

N-[*N*-(α,α,α-Trifluoro-*m*-tolylcarbamoyl)-L-phenylalanyl]-2-(phenylthio)pyrrolidin-3-one 10e

The title compound **10e** was prepared according to a similar procedure as described as previously, starting from 70 mg (0.16 mmol) of *N*-Boc-protected **7**. The resulting TFA salt **8** was treated with α,α,α-trifluoro-*m*-tolyl isocyanate **9e** (26 µL, 1.2 equiv., 0.19 mmol) and Et_3N (66 µL, 3.0 equiv., 0.43 mmol). The residue of the reaction after work-up was purified by flash chromatography (EtOAc–hexane 3 : 7) to give the desired compound **10e** (60 mg, 71%) as a brown oil, R_f 0.10 (EtOAc–hexane 3 : 7); ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.80–2.50 (m, 2 H,

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–NCH$_2$C*H$_2$*C(O)–), 2.75–3.20 (m, 4 H, –NC*H$_2$*CH$_2$C(O)– + C*H$_2$* β Phe), 4.80–5.20 (m, 4 H, C*H* α Phe + C*H*SPh + 2 N*H*), 6.80–7.80 (m, 14 H, Ar*H*); ^{13}C NMR (62.9 MHz; CDCl$_3$) δ_C 37.4, 39.2, 41.4, 57.0, 79.7, 117.2, 119.3, 120.9, 123.7, 124.9, 125.7, 126.8, 127.9, 128.4, 128.7, 129.0, 131.2, 136.5, 138.5, 140.1, 158.0, 171.0, 209.5; ν_{max} (KBr)/cm^{-1} 2975, 1770, 1765, 1680; MS (FAB > 0, GT) *m*/*z* 528 (M + H)$^+$. Calc. for $C_{27}H_{24}F_3N_3O_3S$: 527.55 g mol^{-1}. Found: C, 61.12; H, 4.42; N, 11.05. $C_{27}H_{24}F_3N_3O_3S$ requires C, 61.47; H, 4.59; N, 10.80%.

N-[*N*-(4-Methoxyphenylcarbamoyl)-L-phenylalanyl]-2-(phenylthio)pyrrolidin-3-one 10f

The title compound **10f** was prepared according to a similar procedure as described previously, starting from 85 mg (0.19 mmol) of *N*-Boc-protected **7**. The resulting TFA salt **8** was treated with 4-methoxyphenyl isocyanate **9f** (30 µL, 1.2 equiv., 0.23 mmol) and Et$_3$N (81 µL, 3.0 equiv., 0.59 mmol). The residue of the reaction after work-up was purified by flash chromatography (EtOAc–hexane 3 : 7 then 1 : 1) to give the desired compound **10f** (65 mg, 67%) as a clear brown solid, R_f 0.28 (EtOAc–hexane 1 : 1); mp 90–92 °C; ^{1}H NMR (300 MHz; CDCl$_3$) δ 1.90–2.40 (m, 2 H, –NCH$_2$C*H$_2$*C(O)–), 2.75–3.10 (m, 4 H, –NC*H$_2$*CH$_2$C(O)–) + C*H$_2$* β Phe), 3.56 (s, 3 H, OC*H$_3$*), 4.25–5.15 (m, 2 H, C*H* α Phe + C*H*SPh), 6.50–7.35 (m, 14 H, Ar*H*); ^{13}C NMR (62.9 MHz; CDCl$_3$) δ_C 37.4, 39.2, 42.3, 58.0, 72.9, 114.3, 121.4, 124.9, 125.4, 125.7, 127.1, 128.7, 128.8, 130.5, 134.3, 138.7, 157.6, 161.0, 171.2, 174.7, 210.2; ν_{max} (KBr)/cm^{-1} 1762, 1750, 1640; MS (FAB > 0, GT) *m*/*z* 490 (M + H)$^+$. Calc. for $C_{27}H_{27}N_3O_4S$: 489.57 g mol^{-1}. Found: C, 65.95; H, 5.72; N, 8.21. $C_{27}H_{27}N_3O_4S$ requires C, 66.24; H, 5.56; N, 8.58%.

N-[*N*-(*tert*-Butoxycarbonyl)-L-asparagyl-L-phenylalanyl]-2-(phenylthio)pyrrolidin-3-one 10g

Boc-L-AsnOH **9g** (142 mg, 3.0 equiv., 0.16 mmol) was dissolved in 2 mL of anhydrous DMF. HOBt (55 mg, 2.0 equiv., 0.41 mmol) was then added and the reaction mixture was cooled to 0 °C. EDC (78 mg, 2.0 equiv., 0.41 mmol) was added as a solid. The TFA salt **8** (1.0 equiv., 0.20 mmol) obtained from 90 mg (0.20 mmol) of **7** dissolved in 2 mL of anhydrous DMF was then added to the reaction mixture. After a few minutes, 3.0 equiv. of DIEA (107 µL, 0.61 mmol) were added and the reaction mixture was stirred at 0 °C for 1 hour, then overnight at room temperature. The reaction was monitored by TLC (EtOAc). The solvent was removed under reduced pressure and the residue was dissolved in 10 mL of EtOAc. The organic layer was successively washed with H$_2$O (2 × 5 mL), 5% aq. NaHCO$_3$ (2 × 5 mL), and brine (5 mL), dried over anhydrous MgSO$_4$, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (EtOAc) to give the desired compound **10g** as a yellow solid (85 mg, 55%), R_f 0.22 (EtOAc); mp 116–118 °C; ^{1}H NMR (250 MHz; CDCl$_3$) δ 1.37–1.42 (2 br s, 9 H, C*H$_3$* Boc), 1.90–2.40 (m, 2 H, –NCH$_2$C*H$_2$*C(O)–), 2.60–3.10 (m, 6 H, –NC*H$_2$*CH$_2$C(O)– + C*H$_2$* β Phe + C*H$_2$* β Asn), 4.30–4.50 (m, 1 H, C*H* α Asn), 4.70–5.30 (m, 2 H, C*H* α Phe + C*H*SPh), 7.00–7.60 (m, 10 H, Ar*H*); ^{13}C NMR (62.9 MHz; CDCl$_3$) δ_C 28.7, 37.9, 38.2, 38.8, 41.4, 52.5, 54.9, 70.6, 79.7, 124.9, 125.7, 126.6, 127.9, 128.4, 128.7, 135.6, 140.2, 157.5, 173.9, 175.4, 177.2, 207.1; ν_{max} (KBr)/cm^{-1} 1762, 1757, 1750, 1680; MS (FAB > 0, GT) *m*/*z* 555 (M + H)$^+$. Calc. for $C_{28}H_{34}N_4O_6S$: 554.64 g mol^{-1}. Found: C, 60.86; H, 6.35; N, 9.98. $C_{28}H_{34}N_4O_6S$ requires C, 60.63; H, 6.18; N, 10.10%.

N-[*N*-(*tert*-Butoxycarbonyl)-L-leucyl]-L-tyrosine 9h

Boc-L-Tyr-OBzl (1.00 g, 1.0 equiv., 2.70 mmol) was dissolved in 15 mL of CH$_2$Cl$_2$. At 0 °C, 10.0 equiv. of TFA (2.1 mL, 27.00 mmol) were added dropwise and the solution was stirred at room temperature for 2 hours. The reaction was monitored by TLC (EtOAc–hexane 1 : 1). The solvent and the excess of TFA were removed under reduced pressure and co-evaporated successively with CH$_2$Cl$_2$ and toluene to give quantitatively the desired TFA salt (1.04 g) as an oil, ^{1}H NMR (250 MHz; CD$_3$OD) δ 3.10 (d, 2 H, C*H$_2$* β Tyr), 4.27 (t, 2 H, C*H* α Tyr), 5.23 (AB, 2 H, C*H$_2$*C$_6$H$_5$, J_{gem} = 12.0 Hz), 6.71 (d, 2 H, Ar*H*, ^3J = 8.5 Hz), 6.97 (d, 2 H, Ar*H*, ^3J = 8.5 Hz), 7.32–7.43 (m, 5 H, C$_6$*H$_5$*).

Boc-L-LeuOH (0.67 g, 1.0 equiv., 2.70 mmol) was dissolved in 20 mL of anhydrous CH$_2$Cl$_2$. 1.0 Equiv. of BOP (1.19 g, 2.70 mmol) was added. The resulting solution was cooled to 0 °C and 1.0 equiv. of DIEA (0.47 mmol, 2.70 mmol) was added dropwise. The solution was allowed to warm up to room temperature and was stirred for 30 min before being cooled to 0 °C. A solution of 1.0 equiv. of the previous TFA salt (1.04 g, 2.70 mmol) and 3.0 equiv. of DIEA (1.41 mL, 8.10 mmol) in 5 mL of anhydrous CH$_2$Cl$_2$ was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred for 2 hours. The reaction was monitored by TLC (EtOAc–hexane 2 : 3). The solvent was removed under reduced pressure. The residue was diluted in 30 mL of EtOAc, washed successively with H$_2$O (20 mL), 1 M HCl (20 mL), brine (20 mL), and 5% aq. NaHCO$_3$ (20 mL), dried over anhydrous MgSO$_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc–hexane 2 : 3) to give the desired diprotected dipeptide (1.14 g, 87%), R_f 0.20 (EtOAc–hexane 3 : 7); ^{1}H NMR (300 MHz; CDCl$_3$) δ 0.69 (d, 3 H, C*H$_3$* δ Leu), 0.70 (d, 3 H, C*H$_3$* δ Leu), 1.20–1.32 (m, 11 H, C*H$_3$* Boc + C*H$_2$* β Leu), 1.35–1.46 (m, 1 H, C*H* γ Leu), 2.81–2.88 (m, 2 H, C*H$_2$* β Tyr), 3.90 (br s, 1 H, C*H* α Leu), 4.63–4.78 (m, 2 H, C*H* α Tyr + O*H*), 4.95 (AB, 2 H, C*H$_2$*C$_6$H$_5$, J_{gem} = 12.1 Hz), 5.65 (br s, 1 H, N*H*), 6.39–6.52 (m, 3 H, N*H* + Ar*H*, ^3J = 8.5 Hz), 6.65 (d, 2 H, Ar*H*, ^3J = 8.5 Hz), 7.10–7.25 (m, 5 H, C$_6$*H$_5$*); MS (FAB > 0, GT) *m*/*z* 485 (M + H)$^+$. Calc. for $C_{27}H_{36}N_2O_6$: 484.58 g mol^{-1}.

1.0 Equiv. of this *N*-Boc-protected dipeptide (1.02 g, 2.10 mmol) was dissolved in 20 mL of MeOH. A suspension of 100 mg of 10% Pd/C in 1 mL of MeOH was then added and the resulting mixture was stirred for 3 hours at room temperature under H$_2$ at atmospheric pressure. The reaction was monitored by TLC (EtOAc–hexane 3 : 7 and EtOAc). The suspension was filtered on Celite and the solvent was removed under reduced pressure to give the corresponding acid **9h** as a white solid (0.82 g, quantitative), R_f 0.15 (EtOAc); ^{1}H NMR (300 MHz; DMSO-d$_6$) δ 0.62 (d, 3 H, C*H$_3$* δ Leu), 0.65 (d, 3 H, C*H$_3$* δ Leu), 1.12–1.22 (m, 11 H, C*H$_3$* Boc + C*H$_2$* β Leu), 1.27–1.40 (m, 1 H, C*H* γ Leu), 2.59 (dd, 1 H, C*H^a*H^b β Tyr, J_{gem} = 13.9 Hz, ^3J = 8.2 Hz), 2.70 (dd, 1 H, CH^a*H^b* β Tyr, J_{gem} = 13.9 Hz, ^3J = 5.2 Hz), 3.70–3.80 (m, 1 H, C*H* α Leu), 4.10–4.22 (m, 1 H, C*H* α Tyr), 6.43 (d, 2 H, Ar*H*, ^3J = 8.4 Hz), 6.66 (d, 1 H, N*H*, ^3J = 8.7 Hz), 6.77 (d, 2 H, Ar*H*, ^3J = 8.4 Hz), 7.57 (d, 1 H, N*H*, ^3J = 7.7 Hz), 8.98 (s, 1 H, O*H*), 12.53 (br s, 1 H, COO*H*); MS (FAB > 0, GT) *m*/*z* 395 (M + H)$^+$. Calc. for $C_{20}H_{30}N_2O_6$: 394.46 g mol^{-1}.

N-{*N*-[*N*-(*tert*-Butoxycarbonyl)-L-leucyl-L-tyrosyl]-L-phenylalanyl}-2-(phenylthio)pyrrolidin-3-one 10h

Boc-L-Leu-L-TyrOH **9h** (90 mg, 1.0 equiv., 0.23 mmol) was dissolved in 1 mL of anhydrous CH$_2$Cl$_2$. The solution was cooled to 0 °C. BOP (100 mg, 1.0 equiv., 0.23 mmol) was then added followed by 1.0 equiv. of DIEA (40 µL, 0.23 mmol). The reaction mixture was allowed to warm to room temperature and was stirred for 30 min. The solution was once again cooled to 0 °C. A solution of the TFA salt **8** (obtained from 100 mg, 0.23 mmol of **7**) in 1 mL of anhydrous CH$_2$Cl$_2$ was then added followed by 2.0 equiv. of DIEA (40 µL, 0.45 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC (EtOAc and EtOAc–hexane 1 : 1). The organic layer was diluted with 10 mL of CH$_2$Cl$_2$ and successively washed with H$_2$O (5 mL), 5% aq. citric acid (2 × 5 mL), H$_2$O (5 mL), 5% aq. NaHCO$_3$ (2 × 5 mL), and brine (5 mL), dried over anhydrous MgSO$_4$, filtered, and

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concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc–hexane 1 : 1 then 2 : 1) to give the desired compound **10h** (103 mg, 64%) as a white solid, R_f 0.33 (EtOAc–hexane 2 : 1); mp 130–132 °C; ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.70–0.90 (m, 6 H, 2 × C*H*$_3$ δ Leu), 1.35 (br s, 11 H, C*H*$_3$ Boc + C*H*$_2$ β Leu), 1.50 (br s, 1 H, C*H* γ Leu), 1.80–2.40 (m, 2 H, –NCH$_2$C*H*$_2$C(O)–), 2.65–3.15 (m, 6 H, C*H*$_2$ β Tyr + C*H*$_2$ β Phe + –NC*H*$_2$CH$_2$C(O)–), 3.60–3.80 (m, 1 H, C*H* α Leu), 3.92–4.15 (m, 1 H, C*H* α Tyr), 4.60–5.40 (m, 3 H, C*H* α Phe + C*H*SPh + O*H* Tyr), 6.50–7.50 (m, 14 H, Ar*H*); ^{13}C NMR (62.9 MHz; $CDCl_3$) δ_C 21.6, 22.4, 28.4, 37.6, 37.9, 39.8, 41.1, 41.4, 54.3, 54.9, 56.7, 72.3, 77.1, 115.6, 124.9, 125.7, 126.6, 127.9, 128.4, 128.7, 129.3, 132.8, 135.6, 140.2, 154.5, 157.5, 173.9, 174.7, 175.4, 209.5; ν_{max} (KBr)/cm^{-1} 1762, 1759, 1755, 1750, 1680; MS (FAB > 0, GT) *m*/*z* 717 (M + H)$^+$. Calc. for $C_{39}H_{48}N_4O_7S$: 716.90 g mol^{-1}. Found: C, 64.98; H, 6.45; N, 7.61. $C_{39}H_{48}N_4O_7S$ requires C, 65.34; H, 6.75; N, 7.82%.

N-(*N*-L-Asparagyl-L-phenylalanyl)-2-(phenylthio)pyrrolidin-3-one trifluoroacetic acid salt 10i

The *N*-Boc-protected derivative **10g** (30 mg, 0.05 mmol) was dissolved at 0 °C in 1 mL of a solution of TFA in CH_2Cl_2 (v/v 1 : 9). The reaction mixture was stirred at room temperature for 2 hours. The reaction was monitored by TLC (EtOAc). The solvents were evaporated off under reduced pressure and the excess of TFA was co-evaporated with CH_2Cl_2. The desired TFA salt was isolated as a brown solid (31 mg, quantitative), ^{1}H NMR (250 MHz; CD_3OD) δ 1.80–2.40 (br s, 2 H, –NCH$_2$C*H*$_2$C(O)–), 2.70–3.20 (m, 6 H, –NC*H*$_2$CH$_2$C(O)– + C*H*$_2$ β Phe + C*H*$_2$ β Asn), 4.00–4.20 (m, 1 H, C*H* α Asn), 4.90–5.30 (m, 2 H, C*H* α Phe + C*H*SPh), 7.05–7.55 (m, 10 H, Ar*H*); ^{13}C NMR (62.9 MHz; CD_3OD) δ_C 37.5, 39.7, 41.0, 41.2, 53.9, 57.5, 68.3, 123.9, 125.4, 125.7, 126.6, 128.2, 128.6, 132.3, 138.5, 174.3, 175.4, 177.2, 208.0; MS (FAB > 0, GT) *m*/*z* 455 (M + H)$^+$. Calc. for $C_{23}H_{26}N_4O_4S$: 454.53 g mol^{-1}. Calc. for $C_{23}H_{26}N_4O_4S{\cdot}CF_3COOH$: 568.56 g mol^{-1}.

N-{*N*-[*N*-(L-Leucyl-L-tyrosyl)-L-phenylalanyl]-2-(phenylthio)-pyrrolidin-3-one trifluoroacetic acid salt 10j

The title TFA salt **10j** was prepared according to a similar procedure as described as previously, from the *N*-Boc-protected derivative **10h** (42 mg, 0.06 mmol). After evaporation and co-evaporation of the solvent and excess of TFA, the salt was isolated as a black solid (40 mg, 93%), ^{1}H NMR (300 MHz; DMSO-d$_6$) δ 0.54–0.74 (m, 6 H, 2 × C*H*$_3$ δ Leu), 0.95–1.05 (m, 3 H, C*H*$_2$ β Leu + C*H* γ Leu), 2.00–2.50 (m, 2 H, –NCH$_2$C*H*$_2$C(O)–), 2.70–3.10 (m, 6 H, C*H*$_2$ β Tyr + C*H*$_2$ β Phe + –NC*H*$_2$CH$_2$C(O)–), 3.40–3.80 (m, 2 H, C*H* α Tyr + C*H* α Leu), 4.30–4.70 (m, 2 H, C*H* α Phe + C*H*SPh), 6.25–7.50 (m, 14 H, Ar*H*); ^{13}C NMR (62.9 MHz; DMSO-*d*$_6$) δ_C 22.5, 23.9, 37.9, 38.3, 38.4, 42.4, 43.9, 54.8, 55.7, 57.7, 69.3, 116.5, 123.3, 126.6, 127.5, 127.9, 128.4, 128.6, 129.1, 131.2, 136.5, 139.3, 155.4, 173.9, 174.7, 174.8, 206.3; MS (FAB > 0, GT) *m*/*z* 617 (M + H)$^+$. Calc. for $C_{34}H_{40}N_4O_5S$: 616.75 g mol^{-1}. Calc. for $C_{34}H_{40}N_4O_5S{\cdot}CF_3COOH$: *m*/*z* 730.78 g mol^{-1}.

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