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Use of BOP-Cl in the Presence of Boc-Amino Monothioacids for the Thioacylation of Iminoacid Residues

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Abstract—BOP-Cl was found to be an efficient coupling reagent for the introduction of thiopeptide bonds on imino acid residues (Pro, Sar). Boc-amino monothioacids were coupled at moderate temperature (0 °C–RT) with fair yields and with retained optical purity. The mechanism of the coupling reaction is discussed. Copyright © 1996 Elsevier Science Ltd

Introduction

One approach to the stabilization of amide linkages against enzymatic degradation is the replacement of the peptide bond with a thioamide linkage.[1] This modification does not change to a great extent the geometry of the starting carboxamide bond.[2] However, biological studies have shown that the behavior of the resulting thiopeptides is unpredictable.[3] This may be explained by the fact that the presence of a thioamide bond will influence the formation of the secondary structure of the polypeptide backbone. This change may be due to a different pattern in forming hydrogen bonds.[4] Thus special interest might arise from the incorporation of thioamide bonds in connection with structure–function studies of biologically active peptides.

The synthesis of endothiopeptides has received increasing attention. The use of the Lawesson reagent or similar reagents for the conversion of carbonyl compounds into thiocarbonyl analogues has led to important developments.[5] Thiopeptide linkage can be formed by stepwise elongation from the N-terminus or the C-terminus of thiodipeptides. Those methods, however, are limited by regioselectivity and racemization.[6] An alternative strategy involves thioacylation, but known reagents such as thiobenzimidazolone derivatives[4,7] seem less readily available than the classical amino acid methyl dithioesters. Dithioesters proved to be efficient reagents for the synthesis of endothiopeptides whenever used in the presence of DMAP and CF_3CO_2Cs and thiopeptide analogues are obtained without racemization.[8] However, the usual method for dithioester synthesis is not suitable for asparagine and glutamine derivatives which need two additional steps for their preparation.[9] Hoeg-Jensen et al.[10] have recently shown that N-protected-amino monothioacids, which are potentially available from all amino acids, can be activated by use of the phosphorus containing PyBOP, to generate in situ new thioacylating reagents for the monothionation of peptides retaining optical purity.

Preliminary studies in our laboratory were performed to evaluate Hoeg-Jensen's method for the formation of thioamide bonds on sterically hindered amino acids. We report here on the reactivity of several phosphorus-based coupling reagents for the thioamide linkage formation between Boc-amino monothioacid and iminoacid residues (Pro, Sar). A preliminary study of the mechanism for thioamide formation is discussed, using a model system consisting of Boc-Leu-SH and piperidine.

Results and Discussion

Several phosphorus reagents such as DPPA,[11] t-PNC,[12] BOP,[13] BOP-Cl,[14] BrOP,[15] FDPP,[16] and PyBOP[17] have been tested in a model designed to evaluate the thioamide formation by coupling Boc-Leu-SH to piperidine. The products (the thioamide and the undesired oxoamide derivatives) were measured by HPLC by comparison with authentic samples (Table 1). Our results are well correlated with those obtained by Hoeg-Jensen et al.[10a] The ratio of the S/O-selectivity depends on the nature of the phosphorus functionality and the kind of leaving group involved. The leaving groups which have high charge density (low polarizability) such as Cl^- (BOP-Cl) or OBt^- (PyBOP) have promoted the formation of the thioamide. Lowering of the charge density (delocalized charge) diminished the yields of thioamide as observed clearly with N_3^- (DPPA; case A), or Br^- (BrOP; case K), or $PfpO^-$ (FDPP; case L). The maximum ratio for S/O selectivity was even higher with BOP-Cl (3:1) than for PyBOP (1.08:1). Nevertheless, the yield of the overall amide plus thioamide formation obtained with BOP-Cl, was slightly decreased (75%) as compared to that obtained with PyBOP (95%). The nonpolar solvents such as THF gave the best results (3:1) while the lowest ratios for S/O selectivity were obtained in the more polar solvents CH_3CN or DMF (0.30:1, 0.33:1). In some cases preactivation conditions, in which the N-protected amino acid was allowed to react with BOP-Cl before adding the amino-component, were

Table 1. Reaction of Boc-Leu-SH with piperidine in the presence of various phosphorus coupling reagents

No	Coupling reagents	Solvent	Activation at 0 °C	Yield[g] (%)	Thio/oxo	Conditions
A[a]	DPPA (1.2 eq)	DMF	1 h	80	0	10 h, RT
B[b]	t-PNC (0.5 eq)	AcOEt	1 h	50	0.66	5 h, RT
C	t-PNC (1.5 eq)	AcOEt	1 h	80	0.43	5 h, RT
D	t-PNC (0.5 eq)	AcOEt	without	40	0.01	5 h, RT
E	t-PNC (0.5 eq)	DCM	1 h	45	0.40	5 h, RT
F	BOP (1.0 eq)	DCM	without	70	0.02	5 h, RT
G	BOP-Cl[f] (1.5 eq)	THF	1 h	75	3.00	6 h, RT
H	BOP-Cl[f] (1.5 eq)	THF	without	42	0.66	6 h, RT
I	BOP-Cl[f] (1.5 eq)	CH_3CN	1 h	55	0.30	6 h, RT
J	BOP-Cl[f] (1.5 eq)	DMF	1 h	60	0.33	6 h, RT
K[c]	BrOP (1.0 eq)	DCM	without	81	0.10	4 h, RT
L[d]	FDPP (1.2 eq)	DMF	without	81	0.03	4 h, RT
M[e]	PyBOP (1.0 eq)	DCM	without	95	1.08	4 h, RT
N	PyBOP (1.0 eq)	DCM	1 h	70	0.05	4 h, RT
O	PyBOP (1.0 eq)	THF	1 h	65	0.43	4 h, RT

[a]Conditions as in ref. 11.
[b]Conditions as in ref. 12.
[c]Conditions as in ref. 15.
[d]Conditions as in ref. 16.
[e]Conditions as in ref. 17.
[f]BOP-Cl (Fluka) was recrystallized[25] from CH_3CN.
[g]Yield calculated by HPLC from the starting Boc-amino monothioacid.

used to limit the reaction of the amino-component with BOP-Cl and hence increase overall yields and the S/O-selectivity (75%; 3:1 in the G case or 42%; 0.66:1 in the H case). Conversely, the preactivation with PyBOP did not increase the yield of thioamides. Thus a different mechanism may be involved for the thioamidation via PyBOP.

In Table 2 are reported the results obtained with Boc-amino monothioacids reacted with PyBOP and several sterically hindered amino acids labeled with the paranitroanilide group (pNA) which allows an easy monitoring of the reactions by HPLC. In these experiments, the ratio of thioamide formation is decreased (1.5–0.1) when compared to the ratio reported by Hoeg-Jensen et al.[10b] for the coupling of primary amines, using PyBOP.

In Table 3 are reported the results obtained with the same amino reacting molecules using BOP-Cl as an activating reagent. Imino acid derivatives led to the highest yields of thiopeptide (Pro: 47–51%, Sar: 38–46%). Interestingly enough, primary amino derivatives such as H-Val-pNa, led to poor yields of thioamide (11–18%). Steric hindrance in the amino-components may play a larger role than the nucleophilicity in S/O selectivity. These results confirm the exceptional properties of BOP-Cl in terms of increased efficiency for creating a peptide bond with *N*-alkyl-amino acids (imino acid).[18]

Using PyBOP or BOP-Cl, racemization was evaluated by HPLC of the reaction mixtures in comparison with authentic reference samples. In both experiments (Tables 2 and 3), products of high optical purity were obtained (racemization less than 0.30–1.00%).

BOP-Cl is as a good reagent to obtain thioamide formation preferentially with substituted or secondary, sterically hindered amino groups, whereas PyBOP remains the reagent of choice for thioamide formation on primary amino groups, in the absence of steric hindrance.

Table 2. Boc-AA_1-SH + H-AA_2-pNA $\xrightarrow[4-6\ h,\ RT]{+PyBOP}$ Boc-$AA_1\psi$(CSNH)AA_2-pNA

Boc-AA-SH	Amino acid (or amine)	Product	Yield (%) (HPLC)	Thio/oxo (HPLC)	*D*% (thio) (HPLC)
Boc-D-Leu-SH	H-Val-pNA	Boc-D-Leuψ (CSNH) Val-pNA	81	0.34	97.4
Boc-L-Leu-SH	H-Val-pNA	Boc-L-Leuψ (CSNH) Val-pNA	80	0.33	3.00
Boc-L-Leu-SH	piperidine	Boc-L-Leu-thiopiperidide	95	1.08	
Boc-L-Leu-SH	H-Pro-pNA	Boc-L-Leuψ (CSNH) Pro-pNA	68	0.15	
Boc-L-Leu-SH	H-Sar-pNA	Boc-L-Leuψ (CSNH) Sar-pNA	70	0.16	
Boc-D-Val-SH	H-Val-pNA	Boc-D-Valψ (CSNH) Val-pNA	78	0.01	99.6
Boc-L-Val-SH	H-Val-pNA	Boc-L-Valψ (CSNH) Val-pNA	79	0.02	0.50
Boc-L-Val-SH	piperidine	Boc-L-Val-thiopiperidide	72	1.50	
Boc-L-Val-SH	H-Pro-pNA	Boc-L-Valψ (CSNH) Pro-pNA	86	0.03	
Boc-L-Val-SH	H-Sar-pNA	Boc-L-Valψ (CSNH) Sar-pNA	71	0.11	

Table 3. Boc-AA_1-SH + BOP-Cl $\xrightarrow[\text{(ii) H-}AA_2\text{-pNA}]{\text{(i) DIEA, 0 °C, 1 h}}$ Boc-$AA_1\psi$(CSNH)AA_2-pNA
4–10 h, RT

Boc-AA-SH	Amino acid (or amine)	Product	Yield (%) (HPLC)	Thio/oxo (HPLC)	*D*% (thio) (HPLC)	Yield (% isolated thio)
Boc-D-Leu-SH	H-Val-pNA	Boc-D-Leuψ (CSNH) Val-pNA	71	0.38	99.2	18
Boc-L-Leu-SH	H-Val-pNA	Boc-L-Leuψ (CSNH) Val-pNA	70	0.37	1.00	17
Boc-L-Leu-SH	piperidine	Boc-L-Leu-thiopiperidide	75	3.00		55
Boc-L-Leu-SH	H-Pro-pNA	Boc-L-Leuψ (CSNH) Pro-pNA	91	1.13		47
Boc-L-Leu-SH	H-Sar-pNA	Boc-L-Leuψ (CSNH) Sar-pNA	80	1.38		46
Boc-D-Val-SH	H-Val-pNA	Boc-D-Valψ (CSNH) Val-pNA	80	0.18	99.7	12
Boc-L-Val-SH	H-Val-pNA	Boc-L-Valψ (CSNH) Val-pNA	78	0.19	0.50	11
Boc-L-Val-SH	piperidine	Boc-L-Val-thiopiperidide	75	6.70		63
Boc-L-Val-SH	H-Pro-pNA	Boc-L-Valψ (CSNH) Pro-pNA	97	1.17		51
Boc-L-Val-SH	H-Sar-pNA	Boc-L-Valψ (CSNH) Sar-pNA	78	1.04		38

^{13}C NMR experiments were performed to further investigate the nature of the reaction intermediates. After 1 h, the reaction of Boc-Leu-SH with BOP-Cl in THF-d^8 at 0 °C led to the formation of a unique intermediate signal (222.59 ppm) concomitant with the disappearance of the thiocarboxylate signal (202.95 ppm). After the piperidine was added and allowed to react for 2 h at room temperature the signal attributed to the intermediate disappeared slowly whereas the thioamide signal appeared at 204.67 ppm. We could not observe the amide signal (170.25 ppm) under these conditions (Fig. 1).

The reaction pathway could be tentatively depicted as in Scheme 1. The intermediate **I** might be transformed to the thioamide whereas the amide formation could involve another intermediate **II** issued from **I** after contact with the amino attacking moiety. The intermediate **II** might be formed slowly and then rapidly disappear towards the amide formation and hence was not observed in ^{13}C NMR experiments. Thus, these observations suggest that the orientation of the S/O selectivity from a unique intermediate is highly dependent on the nature of the amino attacking moiety.

It is noteworthy that Hoeg-Jensen et al.[10a] have postulated the formation of two different intermediates leading respectively to the formation of an oxo- or a thioamide bond, when reacting a *N*-protected monothioacid to PyBOP. The reaction mechanism involved in the activation of Boc-amino monothioacids by BOP-Cl is probably different from the mechanism of the reaction via PyBOP which does not need any preactivation step.

From these preliminary results, we propose BOP-Cl as a reagent for the activation of thiocarbamate protected amino acids and their subsequent coupling to *N*-alkyl-amino acids (imino acid) giving high yields of the thiopeptides with negligible levels of racemization.

Experimental

Reagents, solvents, and Boc-amino acids used for the reactions were purchased from Fluka, Novabiochem or Neosystem. Progress of the reactions and purity of the products were determined by analytical TLC on precoated Merck silica gel F_{250} plates, visualized by UV, iodine vapor, ninhydrine or by a spray of *o*-toluidine after chlorination.[19] Values on TLC refer to the following solvent systems (v:v): (A) CH_2Cl_2:AcOH (15:1); (B) CH_2Cl_2:ether (3:1); (C) heptane:ether (1:1); (D) CH_2Cl_2:MeOH (9:1). Products were purified on silica gel columns 60, 230–400 mesh ASTM (Merck). Organic solvents were removed on a rotary evaporator under the vacuum of a water pump with bath temperature at 30 °C or lower. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. UV spectra were recorded on a Perkin–Elmer Lambda 5 UV–Vis spectrophotometer. Melting points were taken on a Leitz microscope apparatus and are corrected. Mass spectra were determined at the Service de Spectrométrie de Masse de l'Institut de Chimie des Substances Naturelles (ICSN) on an AEI MS50 spectrometer or an MS80 RT Kratos spectrometer. ^1H, ^{13}C NMR spectra, ^1H–^1H COSY, were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker AM400 or AM300 spectrometer. Evaluations of the yields or of the racemization were performed on a Waters HPLC 600E, with a reversed-phase Alltima C-18, 5 μm column (4.6 mm i.d. × 250 mm) by isocratic elution (I): 10% A, 90% B; (II): 40% A, 60% B with the solvent system: A: 95% water, 5% CH_3CN, 0.1% TFA; B: 100% CH_3CN, 0.1% TFA. The flow rate was 1 mL/min and the detection wavelength was 190, 230 nm (amino acids), 265 nm (thioamide bonds) or 309 nm (-pNA group).

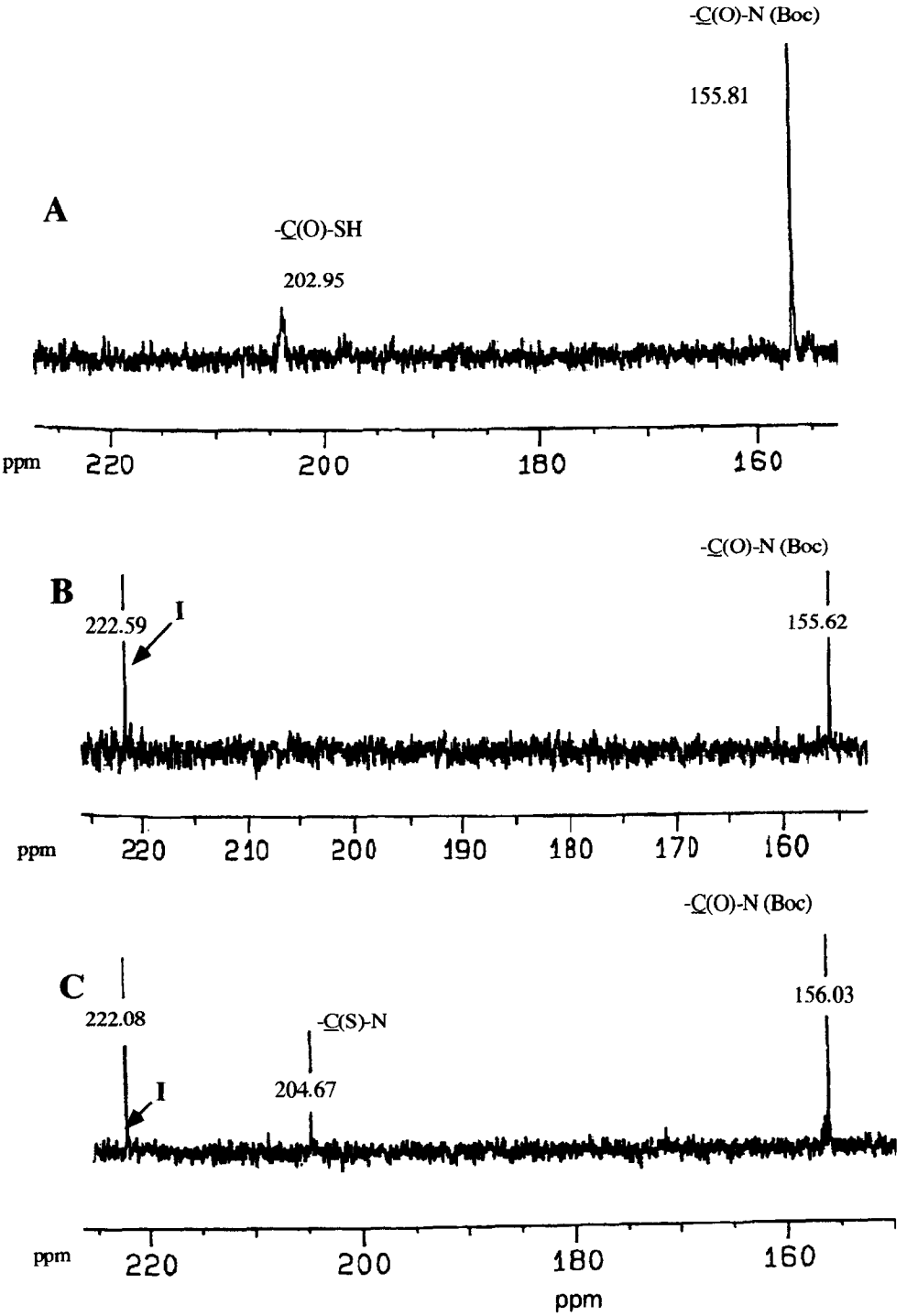
Boc-amino monothioacids

The amino monothioacids, Boc-AA-SH, were obtained directly from the Boc-AA-OH which were activated by IBCF and reacted with H_2S. High yields and pure products were obtained (87–92% with no racemization detected). This method, which is very simple and efficient, is as an improvement of the usual method using activated esters of amino acid.[20]

IBCF (1 eq) was added to a stirred solution of Boc-amino acids (1 eq) and NMM (1 eq), cooled at

−20 °C in THF. The mixture was allowed to react 15 min, then H_2S and NMM (4 eq) were added and allowed to react for 1–3 h at 0–5 °C. The reaction was monitored by TLC. After completion, the solution was acidified with 1 N HCl to pH 3 and concentrated in vacuo, the residue was extracted into ethyl acetate, washed with H_2O, then dried over $MgSO_4$ and evaporated in vacuo to dryness. The product was further dried over KOH for 1 day.

The stereospecificity of the reactions were evaluated by HPLC by coupling with H-Val-pNA[20b] and comparison with authentic samples ($k'_{\text{H-L-Leu-Val-pNA}}$: 13.12, $k'_{\text{H-D-Leu-Val-pNA}}$: 14.7, $k'_{\text{H-L-Val-Val-pNA}}$: 7.9, $k'_{\text{H-D-Val-Val-pNA}}$: 8.3, HPLC system: Waters 600 E + 486; column RP SFCC C18 Nucléosil 5 µm (4.6 mm × 250 mm); isocratic elution 75% A, 25% B (A: 80% sodium acetate 0.02 M at pH 4, 20% MeOH; B: MeOH); flow rate 0.8 mL/min; UV: 309 nm).



A: Boc-Leu-SH; B: Boc-Leu-SH + BOP-Cl + DIEA, 1 h, 0°C; C: reaction mixture, RT + piperidine

Figure 1.

Boc-Leu-SH (1). Required the same procedure using 7.48 g (30 mmol) of Boc-Leu-OH, H_2O, to give a white powder 6.6 g (90%); R_f(A): 0.65 $[\alpha]_D^{20}$ (*c* 1.18, AcOEt): −66.1; UV (EtOAc) λ_{max} (ε): 251 (3.6×10^3), *k'*(I): 0.69, FABMS, *m/z*: 270 $(M+23)^+$, 248 $(M+H)^+$, 148 $(M+H\text{-Boc})^+$; ^{1}H NMR ($CDCl_3$): 4.92 (b, 1H, COSH), 4.28 (b, 1H, CH^γ), 1.75–1.60, 1.45 (m, 3H, CH_2^β, CH^γ), 1.35 (s, 9H, Boc), 0.95–0.85 (m, 6H, CH_3^δ); ^{13}C NMR ($CDCl_3$): 203.78 (COSH), 156.72 (C=O, Boc), 79.67 (C^q, Boc), 61.35 (C^γ), 41.51 (C^β), 28.85 (CH_3, Boc), 25.10 (C^γ), 23.65, 21.58 (C^δ).

Boc-D-Leu-SH (2). Using 2.7 g (10 mmol) of Boc-D-Leu-OH, H_2O, to give a white powder 2.1 g (87%); $[\alpha]_D^{20}$ (*c* 1.30, AcOEt): +63.8; FABMS, *m/z*: 270 $(M+23)^+$, 248 $(M+H)^+$, 148 $(M+H\text{-Boc})^+$.

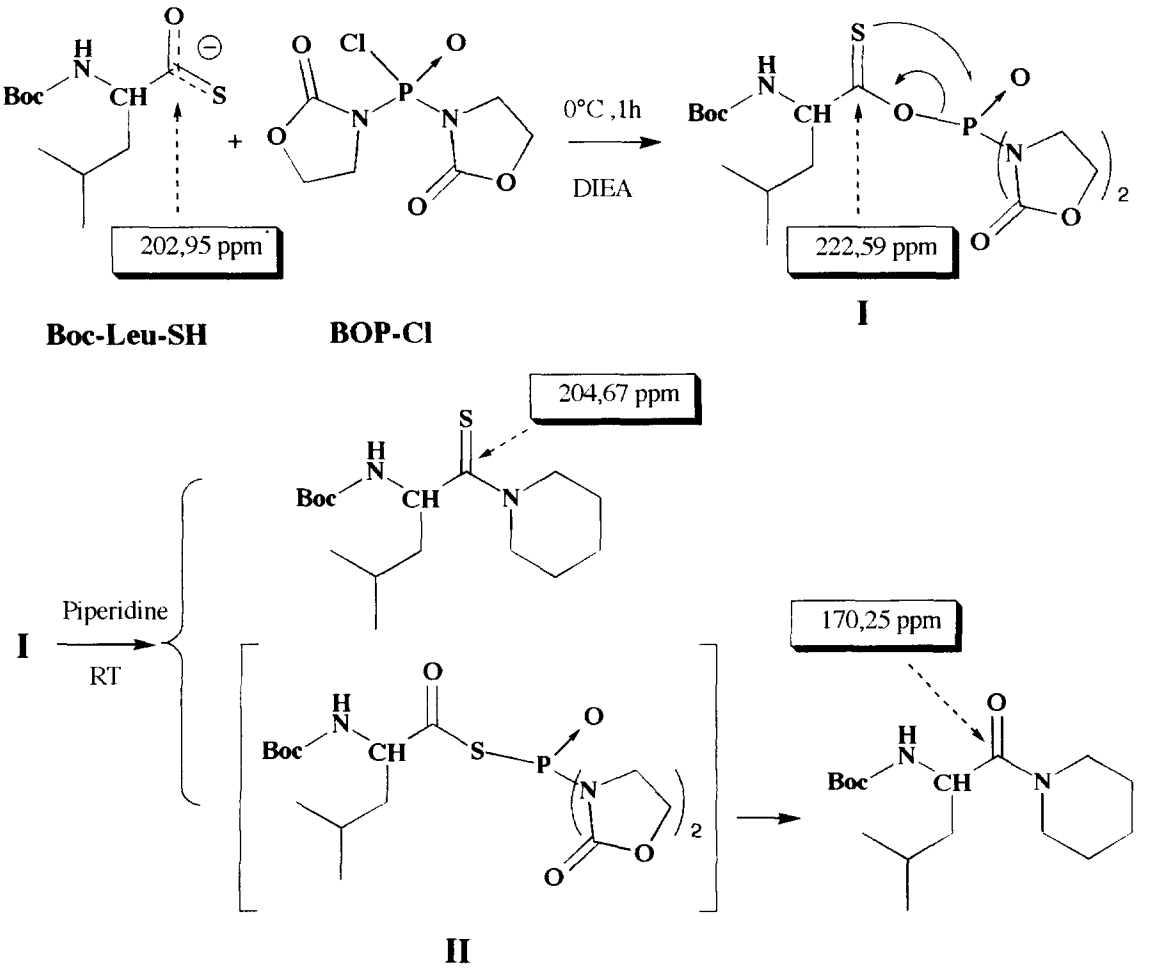
Boc-Val-SH (3). Using 6.5 g (30 mmol) of Boc-Val-OH, to give a white powder 6.4 g (92%); R_f (A): 0.66; $[\alpha]_D^{20}$ (*c* 1.22, AcOEt): −41.6, UV (AcOEt); λ_{max} (ε): 253 (2.7×10^3); *k'*(I): 0.57; FABMS, *m/z*: 257 $(M+H+Na)^+$, 234 $(M+H)^+$; ^{1}H NMR ($CDCl_3$): 4.90 (b, 1H, COSH), 4.30 (b, 1H, CH^γ), 2.30 (m, 1H, CH^β), 1.25 (s, 9H, Boc), 1.05, 0.95 (m, 6H, CH_3^γ); ^{13}C NMR ($CDCl_3$): 204.25 (COSH), 156.35 (C=O, Boc), 80.17 (C^q, Boc), 63.75 (C^γ), 29.15 (C^β), 19.05, 17.95 (C^γ).

Boc-D-Val-SH (4). Using 2.1 g (10 mmol) of Boc-D-Val-OH, to give a white powder 2.1 g (90%); $[\alpha]_D^{20}$ (*c* 1.02, AcOEt): +40.1; FABMS, *m/z*: 257 $(M+H+Na)^+$, 234 $(M+H)^+$.

Boc-Gly-SH (5). Using 5.25 g (30 mmol) of Boc-Gly-OH, to give a white powder 5.20 g (91%); R_f(A): 0.53; UV (AcOEt) λ_{max} (ε): 252 (2.8×10^3); *k'*(I): 0.61; FABMS, *m/z*: 215 $(M+H+23)^+$, 192 $(M+H)^+$; ^{1}H NMR ($CDCl_3$): 4.91 (b, 1H, COSH), 4.28 (b, 2H, CH^γ), 1.15 (s, 9H, Boc); ^{13}C NMR ($CDCl_3$): 202.85 (COSH), 156.65 (C=O, Boc), 79.95 (C^q, Boc), 59.85 (C^γ), 28.45 (CH_3, Boc).

Amino acid-4-nitroanilides

H-Sar-pNA (6). Compound **6** was obtained according to Schoutkowski et al.[21] by reacting 4-nitrophenylisocyanate with Boc-Sar-OH (1.9 g, 10 mmol). The crude product was purified on a silica gel column with the solvent system: heptane:EtOAc (9:1). The product, treated by TFA (10 eq, 40% in CH_2Cl_2), gave a pale yellow solid 1.3 g (65%); mp 244–246 °C, Lit.[21] mp. 245 °C; *k'*(II): 0.39; UV (acetic acid), λ_{max} (ε): 304 (2.3×10^4); FABMS, *m/z*: 210 $(M+H)^+$, 154 $(M+H\text{-}56)^+$; ^{1}H NMR (DMSO-d_6): 9.2 (s, 1H, CONH, pNA), 8.4 (d, 2H, aromatic, pNA), 8.01 (d, 2H,



Scheme 1. Attribution of ^{13}C NMR signals to possible reaction intermediates.

aromatic, pNA), 4.20 (s, 2H, CH^{γ}_2), 2.73 (s, 3H, N-CH_3); ^{13}C NMR (DMSO-d_6): 165.31 (C=O), 144.40, 142.96, 125.29, 119.36 (aromatic, pNA), 50.06 (C^{γ}), 32.95 (N-CH_3).

H-Val-pNA and H-Pro-pNA were purchased from Bachem.

Boc thiodipeptide-4-nitroanilides or Boc-amino thioacid-piperidides

BOP-Cl (1.5 eq) was added a stirred soln of Boc-amino thioacids (1 eq) in THF and two equivalents of DIEA. The mixture was allowed to react 1 h at 0 °C. Piperidine or amino acid-4-nitroanilide (1.2 eq) was added and allowed to react for 4–10 h at room temperature (the reaction was monitored by TLC and HPLC) under nitrogen. After completion, the solution was diluted with EtOAc, filtered, washed with 3% HCl, H_2O, then dried over $MgSO_4$ and evapd in vacuo. The crude product was purified by silica gel column chromatography with a mixture of CH_2Cl_2:ether (9:1) as eluent.

Boc-Leu-thiopiperidide (7). Gave a colorless oil; yield 55%; R_f(B): 0.87; R_f(C): 0.45; $[\alpha]_D^{20}$: +31.3 (*c* 1.01, AcOEt); UV (AcOEt), λ_{max} (ε) : 280 (1.78×10^4); *k'*(I): 1.57; FABMS, *m/z*: 315 $(M+H)^+$, 215 $(M+H\text{-Boc})^+$; ^1H NMR ($CDCl_3$): 5.60 (b, 1H, NH), 4.85 (m, 1H, CH^{α}), 4.15, 3.70 (m, 4H, α-CH_2(piperidine)), 1.70 (m, 1H, CH^{γ}), 1.65, 1.55 (m, 6H, β, γ-CH_2(piperidine)), 1.45 (m, 2H, CH^{β}_2), 1.35 (s, 9H, CH_3, (Boc)), 0.95, 0.85 (m, 6H, CH^{δ}_3); ^{13}C NMR($CDCl_3$): 203.45 (C=S), 155.43 (C=O, Boc), 79.36 (C^{q}, Boc), 52.85 (C^{α}, Leu), 52.13, 50.65 (C^{α}, piperidine), 46.16 (C^{β}, Leu), 28.37 (CH_3, (Boc)), 24.29 (C^{γ}, Leu), 26.82, 25.30, 24.26 (C^{β}, C^{γ}, piperidine), 21.90, 19.10 (C^{δ}, Leu).

Boc-L-Leuψ(CSNH)Val-pNA (8). Gave a pale yellow solid; mp 80–82 °C; yield 17%; R_f(B): 0.68; $[\alpha]_D^{20}$: −157.3 (*c* 1.02, AcOEt); UV (AcOEt) λ_{max} (ε): 312 (3×10^4), 275 (2.6×10^4), *k'*(II) : 11.16; FABMS, *m/z*: 467 $(M+H)^+$, 367 $(M+H\text{-Boc})^+$; ^1H NMR ($CDCl_3$), 8.95 (b, 1H, NH, pNA), 8.10, 7.75 (m, 4H, aromatic, pNA), 5.65 (b, 1H, NH, Val), 5.15 (b, 1H, NH, Leu), 4.85 (m, 1H, CH^{α}, Val), 4.38 (m, 1H, CH^{α}, Leu), 2.49 (m, 1H, CH^{β}, Val), 1.80, 1.50 (m, 2H, CH^{β}, Leu), 1.60 (m, 1H, CH^{γ}, Leu), 1.27 (s, 9H, CH_3, Boc), 0.95, 0.90 (m, 6H, CH^{γ}_3, Val), 0.85, 0.80 (m, 6H, CH^{δ}_3, Leu); ^{13}C NMR ($CDCl_3$): 205.77 (C=S, Leu), 168.51 (C=O, Val), 156.47 (C=O, Boc), 143.83, 143.63, 124.91, 119.72 (aromatic, pNA), 81.78 (C^{q}, Boc), 64.50 (C^{α}, Val), 61.97 (C^{α}, Leu), 43.58 (C^{β}, Leu), 29.83 (C^{β}, Val), 28.31 (CH_3, Boc), 25.28 (C^{γ}, Leu), 23.18, 21.50 (C^{δ}, Leu), 19.58, 18.05 (C^{γ}, Val).

Boc-D-Leuψ(CSNH)Val-pNA (9). Gave a pale yellow solid; mp 73–76 °C; yield 18%; $[\alpha]_D^{20}$ −75.5 (*c* 1.06, AcOEt); *k'*(II): 11.58; FABMS, *m/z*: 467 $(M+H)^+$, 367 $(M+H\text{-Boc})^+$.

Boc-Leuψ(CSNH)Pro-pNA (10). Gave a pale yellow solid; mp 89–91 °C; yield 47%; R_f(B): 0.64, $[\alpha]_D^{20}$ −126.2 (*c* 1, AcOEt); UV (AcOEt) λ_{max} (ε): 313 (2.7×10^4), 282 (2.9×10^4); *k'*(II): 8.31; FABMS, *m/z*: 465 $(M+H)^+$, 365 $(M+H\text{-Boc})^+$; ^1H NMR ($CDCl_3$): 8.95 (b, 1H, NH, pNA), 8.08, 7.75 (m, 4H, aromatic, pNA), 5.35 (m, 1H, CH^{α}, pro), 5.22 (b, NH, Leu), 4.50 (m, 1H, CH^{α}, Leu), 4.20, 3.65 (m, 2H, CH^{δ}_2, Pro), 2.45, 2.15 (m, 4H, CH^{β}_2, CH^{γ}_2, Pro), 1.80, 1.50 (m, 2H, CH^{β}_2, Leu), 1.60 (m, 1H, CH^{γ}, Leu), 1.35 (s, 9H, Boc), 0.95, 0.85 (m, 6H, CH^{δ}_3, Leu); ^{13}C NMR ($CDCl_3$): 206.72 (C=S, Leu), 168.34 (C=O, Pro), 154.80 (C=O, Boc), 143.74, 143.10, 124.72, 120.27 (aromatic, pNA), 80.58 (C^{q}, Boc), 67.63 (C^{α}, Leu), 56.48 (C^{α}, Pro), 50.78 (C^{δ}, Pro), 43.28 (C^{β}, Leu), 28.63 (C^{β}, Pro), 28.43 (CH_3, Boc), 24.90 (C^{γ}, Leu), 24.47 (C^{γ}, Pro), 23.59, 21.86 (C^{δ}, Leu).

Boc-Leuψ(CSNH)Sar-pNA (11). Gave a pale yellow solid; mp 171–172 °C; yield: 46%; R_f(B): 0,56, $[\alpha]_D^{20}$: +3.5 (*c* 1.2, AcOEt); UV(AcOEt); λ_{max} (ε): 311 (1.9×10^4), 283 (2×10^4); *k'*(II): 7.80; FABMS, *m/z*: 439 $(M+H)^+$, 339 $(M+H\text{-Boc})^+$; ^1H NMR ($CDCl_3$): 9 01 (b, 1H, NH, pNA), 8.10, 7.75 (m, 4H, aromatic, pNA), 5.35 (b, 1H, NH, Leu), 5.20 (s, 2H, CH^{α}_2, Sar), 4.75 (m, 1H, CH^{α}, Leu), 3.50 (s, 3H, N-CH_3, Sar), 1.75, 1.40 (m, 2H, CH^{β}_2, Leu), 1.55 (m, 1H, CH^{γ}, Leu), 1.35 (s, 9H, CH_3, Boc), 0.95, 0.90 (m, 6H, CH^{δ}_3, Leu); ^{13}C NMR ($CDCl_3$): 205.25 (C=S, Leu), 166.24 (C=O, Sar), 157.07 (C=O, Boc), 144.48, 144.20, 125.42, 120.53 (aromatic, pNA), 81.43 (C^{q}, Boc), 60.84 (C^{α}, Sar), 55.20 (C^{α}, Leu), 44.90 (C^{β}, Leu), 41.31 (N-CH_3, Sar), 29.03 (CH_3, Boc), 25.49 (C^{γ}, Leu), 24.08, 22.40 (C^{δ}, Leu).

Boc-Val-thiopiperidide (12). Gave a colorless oil; yield 63%; R_f(B) : 0.87, R_f(C): 0.60, $[\alpha]_D^{20}$ +26.8 (*c* 1.01, AcOEt); UV(AcOEt), λ_{max} (ε): 281 (1.16×10^4); *k'*(I): 1.30; FABMS, *m/z*: 301 $(M+H)^+$, 201 $(M+H\text{-Boc})^+$; ^1H NMR ($CDCl_3$): 5.65 (b, 1H, NH, Val), 4.62 (m, 1H, CH^{α}, Val), 4.30, 3.90 (m, 4H, CH^{α}_2, piperidine), 2.00–1.90 (m, 1H, CH^{β}, Val), 1.70, 1.50 (m, 6H, CH^{β}_2, CH^{γ}_2, piperidine), 1.40 (s, 9H, CH_3, Boc), 1.00, 0.90 (m, 6H, CH^{γ}_3, Val); ^{13}C NMR ($CDCl_3$): 202.50 (C=S, Val), 155.37 (C=O, Boc), 79.16 (C^{q}, Boc), 59.01 (C^{α}, Val), 51.91, 51.13 (C^{α}, piperidine), 34.20 (C^{β}, Val), 28.15 (CH_3, Boc), 26.74, 25.46, 24.11 (C^{β}, C^{γ}, piperidine), 19.68, 17.67 (C^{γ}, Val).

Boc-Valψ(CSNH)Val-pNA (13). Gave a pale yellow solid; mp 90–92 °C; yield: 11%, R_f(B): 0.59; $[\alpha]_D^{20}$ −87.5 (*c* 1.02, AcOEt); UV(AcOEt), λ_{max} (ε): 313 (3.1×10^4), 276 (2.6×10^4); *k'*(II): 7.95; FABMS, *m/z*: 453 (M+H), 353 $(M+H\text{-Boc})^+$; ^1H NMR ($CDCl_3$): 9.01 (b, 1H, NH, pNA), 8.45 (b, 1H, NH, Val-pNA), 8.10, 7.82 (m, 4H, aromatic, pNA), 5.15 (b, 1H, NH, Val), 4.85 (m, 1H, CH^{α}, Val-pNA), 4.31 (m, 1H, CH^{α}, Val), 2.05 (m, 2H, CH^{β}, Val-pNA, Val), 1.35 (s, 9H, CH_3, Boc), 1.00–0.80 (m, 12H, CH^{γ}_3, Val-pNA, Val); ^{13}C NMR ($CDCl_3$): 203.88 (C=S, Val), 167.06 (C=O, Val-pNA), 156.18 (C=O, Boc), 81.07 (C^{q}, Boc), 143.18, 143.05, 124.25, 119.43 (aromatic, pNA), 68.07 (C^{α}, Val), 64.29 (C^{α}, Val-pNA), 31.92 (C^{β}, Val), 29.08

(C^{β}, Val-pNA), 27.61 (CH_3, Boc), 19.29, 17.56 (C^{γ}, Val), 19.06, 17.15 (C^{γ}, Val-pNA).

Boc-D-Valψ(CSNH)Val-pNA (14). Gave a pale yellow solid, yield: 12%; $[\alpha]_D^{20}$ −45.6 (*c* 1.05, AcOEt); *k'*(II): 8.25; FABMS, *m/z*: 453 (M+H), 353 (M+H-Boc)$^+$.

Boc-Valψ(CSNH)Pro-pNA (15). Gave a pale yellow amorphous solid; yield: 51%; R_f (B): 0.65; $[\alpha]_D^{20}$ −97.2 (*c* 1.3, AcOEt); UV (AcOEt), λ_{max} (ε): 316 (1.8×10^4), 285 (1.85×10^4); *k'*(II): 6.11; FABMS, *m/z*: 451 (M+H)$^+$, 351 (M+H-Boc)$^+$; ^{1}H NMR ($CDCl_3$): 9.20 (b, 1H, NH, pNA), 8.10, 7.70 (m, 4H, aromatic, pNA), 5.35 (m, 1H, CH^{α}, Pro), 5.30 (b, 1H, NH, Val), 4.48 (m, 1H, CH^{α}, Val), 4.12, 3.85 (m, 2H, CH_2^{δ}, Pro), 2.40, 2.20 (m, 2H, CH_2^{β}, Pro), 2.18 (m, 2H, CH_2^{γ}, Pro), 2.01 (m, 1H, CH^{β}, Val), 1.30 (s, 9H, CH_3, Boc), 1.00–0.90 (m, 6H, CH_3^{γ}, Val).; ^{13}C NMR ($CDCl_3$): 205.15 (C=S, Val), 168.01 (C=O, Pro), 155.80 (C=O, Boc), 143.60, 143.24, 124.58, 119.87 (aromatic, pNA), 79.50 (C^q, Boc), 66.51 (C^{α}, Val) 62.81 (C^{α}, Pro), 51.53 (C^{δ}, Pro), 32.02 (C^{β}, Val), 27.99 (CH_3, Boc), 28.62 (C^{β}, Pro), 24.35 (C^{γ}, Pro), 18.93, 18.06 (C^{γ}, Val).

Boc-Valψ(CSNH)Sar-pNA (16). Gave a pale yellow solid; mp 160–168 °C; yield: 38%, R_f(B): 0.51; $[\alpha]_D^{20}$ −4.1 (*c* 1.03, AcOEt); UV(AcOEt), λ_{max} (ε): 310 (2.06×10^4), 287 (2.1×10^4); *k'*(II): 5.82; FABMS, *m/z*: 425 (M+H)$^+$, 324 (M-Boc)$^+$; ^{1}H NMR ($CDCl_3$): 9.01 (b, 1H, NH, pNA), 8.02, 7.80 (m, 4H, aromatic, pNA), 5.28 (b, 1H, NH, Val), 5.18 (s, 2H, CH_2^{α}, Sar), 4.50 (m, 1H, CH^{α}, Val), 3.45 (s, 3H, N-CH_3, Sar), 1.95 (m, 1H, CH^{β}, Val), 1.30 (s, 9H, CH_3, Boc), 1.00, 0.85 (m, 6H, CH_3, Val); ^{13}C NMR ($CDCl_3$): 208.01(C=S, Val), 165.17 (C=O, Sar), 155.60 (C=O, Boc), 143.50, 143.15, 124.46, 119.62 (aromatic, pNA), 80.06 (C^q, Boc), 61.20 (C^{α}, Val), 60.19 (C^{α}, Sar), 40.74 (N-CH_3, Sar), 33.85 (C^{β}, Val), 28.09 (CH_3, Boc), 18.86, 18.45 (C^{γ}, Val).

Boc-dipeptide-4-nitroanilides or Boc amino acids piperidides

Boc-dipeptide-4-nitroanilides were obtained according to Coste et al.[22] using PyBOP as a coupling reagent. DIEA (2 eq) was added to a mixture (cooled to 0 °C) of Boc-amino-acid (1 eq), amino acid-4-nitroanilide (1.1 eq) and PyBOP (1 eq) in CH_2Cl_2 (1 mL/mmol), and stirred for 1 min cold and 1–4 h at room temperature. After completion, the residue was extracted into EtOAc, washed with 2 N HCl, 5% $NaHCO_3$, H_2O, then dried over $MgSO_4$. The crude peptide was purified on column chromatography with the solvent system AcOEt:n-heptane (2:8).

Boc-amino acid piperidides were obtained following Anderson et al.[23], from Boc-amino acid (1 eq), NMM (1 eq), IBCF (1 eq), and piperidine (1.2 eq) in THF for 15 min at −15 °C, 1 h at 0 °C then 4 h at room temperature. The product was purified by flash chromatography.

Boc-Leu-piperidide (17). Gave a colorless solid; mp 48–50 °C, Lit.[24] oil; yield 90%; R_f(B): 0.69, R_f(D): 0.88; *k'*(I): 1.02; FABMS, *m/z*: 299 (M+H)$^+$, 198 (M–Boc)$^+$; ^{1}H NMR ($CDCl_3$) : 5.28 (b, 1H, NH), 4.50 (m, 1H, CH^{α}), 3.45, 3.35 (m, 4H, α-CH_2(piperidine)), 1.65 (m, 1H, CH^{γ}), 1.59, 1.48 (m, 6H, β, γ-CH_2(piperidine)), 1.40 (m, 2H, CH_2^{β}), 1.35 (s, 9H, CH_3(Boc)), 0.95, 0.85 (m, 6H, CH_3^{δ}); ^{13}C NMR($CDCl_3$): 170.58 (C=O), 155.09 (C=O, Boc), 78.77 (C^q, Boc), 47.95 (C^{α}, Leu), 45.95, 44.21 (C^{α}, piperidine), 42.65 (C^{β}, Leu), 27.86 (CH_3, Boc), 24.11 (C^{γ}, Leu), 25.88, 25.01, 23.99 (C^{β}, C^{γ}, piperidine) 22,91, 21.41 (C^{δ}, Leu).

Boc-Leu-Val-pNA (18). Gave a pale yellow amorphous solid; yield 82%; R_f(B): 0,57; *k'*(II): 4.96; FABMS, *m/z*: 451 (M+H)$^+$, 351 (M+H-Boc)$^+$; ^{1}H NMR ($CDCl_3$), 8.93 (b, 1H, NH, pNA), 8.09, 7.65 (m, 4H, aromatic, pNA), 7.25 (b, 1H, NH, Val), 5.13 (b, 1H, NH, Leu), 4.55 (m, 1H, CH^{α}, Val), 4.28 (m, 1H, CH^{α}, Leu), 2.40 (m, 1H, CH^{β}, Val), 1.75, 1.45 (m, 2H, CH_2^{β}, Leu), 1,58 (m, 1H, CH^{γ}, Leu), 1.30 (s, 9H, CH_3, Boc), 0.95, 0.9 (m, 6H, CH_3^{γ}, Val), 0.85, 0.8 (m, 6H, CH_3^{δ}, Leu); ^{13}C NMR ($CDCl_3$): 170.20 (C=O, Leu), 167.60 (C=O, Val), 155.48 (C=O, Boc), 143.60, 143.42, 124.70, 119.25 (aromatic, pNA), 80.25 (C^q, Boc), 63.40 (C^{α}, Val), 57.20 (C^{α}, Leu), 42.70 (C^{β}, Leu), 29.35 (C^{β}, Val), 28.25 (CH_3, Boc), 25.20 (C^{γ}, Leu), 23.10, 21.40 (C^{δ}, Leu), 19.40, 17.90 (C^{γ}, Val).

Boc-Leu-Pro-pNA (19). Gave a pale yellow solid; yield 79%; R_f(B): 0.54; *k'*(II): 4.01; FABMS, *m/z*: 449 (M+H)$^+$, 349 (M+H-Boc)$^+$; ^{1} H NMR ($CDCl_3$): 9.01 (b, 1H, NH, pNA), 8.15, 7.80 (m, 4H, aromatic, pNA), 5.25 (m, 1H, CH^{α}, pro), 5.17 (b, NH, Leu), 4.27 (m, 1H, CH^{α}, Leu), 4.15, 3.20 (m, 2H, CH_2^{δ}, Pro), 2.40, 2.10 (m, 4H, CH_2^{β}, CH_2^{γ}, Pro), 1.72, 1.40 (m, 2H, CH_2^{β}, Leu), 1.55 (m, 1H, CH^{γ}, Leu), 1.30 (s, 9H, Boc), 0.85, 0.8 (m, 6H, CH_3^{δ}, Leu); ^{13}C NMR ($CDCl_3$): 170.65 (C=O, Leu), 167.85 (C=O, Pro), 155.35 (C=O, Boc), 143.75, 143.52, 124.71, 118.25 (aromatic, pNA), 79.85 (C^q, Boc), 58.75 (C^{α}, Leu), 54.25 (C^{α}, Pro), 49.50 (C^{δ}, Pro), 41.95 (C^{β}, Leu), 28.60 (C^{β}, Pro), 28.15 (CH_3, Boc), 24.80 (C^{γ}, Leu), 23.45 (C^{γ}, Pro), 23.35, 22.15 (C^{δ}, Leu).

Boc-Leu-Sar-pNA (20). Gave a pale yellow amorphous solid; yield 75%; R_f(B): 0.45; *k'*(II): 3.45; FABMS, *m/z*: 423 (M+H)$^+$, 323 (M+H-Boc)$^+$; ^{1}H NMR ($CDCl_3$): 9.00 (b, 1H, NH, pNA), 8.15, 7.70 (m, 4H, aromatic, pNA), 5.20 (b, 1H, NH, Leu), 4.85 (s, 2H, CH_2^{α}, Sar), 4.35 (m, 1H, CH^{α}, Leu), 3.15 (s, 3H, N-CH_3, Sar), 1.80, 1.45 (m, 2H, CH_2^{β}, Leu), 1.60 (m, 1H, CH^{γ}, Leu), 1.30 (s, 9H, CH_3, Boc), 0.95, 0.85 (m, 6H, CH_3^{δ}, Leu); ^{13}C NMR ($CDCl_3$): 171.15 (C=O), Leu), 167.25 (C=O, Sar), 156.15 (C=O, Boc), 144.25, 144.10, 124.85, 121.32 (aromatic, pNA), 80.25 (C^q, Boc), 58.25 (C^{α}, Sar), 53.15 (C^{α}, Leu), 43.15 (C^{β}, Leu), 38.75 (N-CH_3, Sar), 28.35 (CH_3, Boc), 25.35 (C^{γ}, Leu), 23.82, 21.95 (C^{δ}, Leu).

Boc-Val-piperidide (21). Gave a colorless oil; yield 90%; R_f(B): 0.69; R_f(D): 0.75; *k'*(I): 0.83; FABMS, *m/z*: 285 (M+H)$^+$; ^{1}H NMR ($CDCl_3$): 5.35 (b, 1H,

NH, Val), 4.40 (m, 1H, CH^{α}, Val), 3.45, 3.40 (m, 4H, CH_2^{α}, piperidine), 1.85 (m, 1H, CH^{β}, Val), 1.55, 1.40 (m, 6H, CH_2^{β}, CH_2^{γ}, piperidine), 1.30 (s, 9H, CH_3, Boc), 0.9, 0.80 (m, 6H, CH_3^{γ}, Val); ^{13}C NMR ($CDCl_3$) : 171.05 (C=O, Val), 156.64 (C=O, Boc), 79.95 (C^{q}, Boc), 55.39 (C^{α}, Val), 47.57, 43.89 (C^{α}, piperidine), 32.05 (C^{β}, Val), 29.12 (CH_3, Boc), 27.62, 26.42, 25.21 (C^{β}, C^{γ}, piperidine), 20.43, 17.76 (C^{γ}, Val).

Boc-Val-Val-pNA (22). Gave a pale yellow oil; yield: 79%; R_f(B): 0.48; k'(II): 3.47; FABMS, *m/z*: 437 $(M+H)^+$, 337 $(M+H-Boc)^+$; ^1H NMR ($CDCl_3$): 8.95 (b, 1H, NH, pNA), 8.15, 7.76 (m, 4H, aromatic, pNA), 7.21 (b, 1H, NH, Val-pNA), 5.13 (b, 1H, NH, Val), 4.80 (m, 1H, CH^{α}, Val-pNA), 4.15 (m, 1H, CH^{α}, Val), 1.95 (m, 2H, CH^{β}, Val-pNA,Val), 1.30 (s, 9H, CH_3, Boc), 0.95, 0.85 (m, 12H, CH_3^{γ}, Val-pNA, Val); ^{13}C NMR ($CDCl_3$): 170.25 (C=O, Val), 155.85 (C=O, Boc), 168.15 (C=O, Val-pNA), 79.87 (C^{q}, Boc), 143.20, 143.10, 124.35, 119.40 (aromatic, pNA), 65.25 (C^{α}, Val), 64.01 (C^{α}, Val-pNA), 31.85 (C^{β}, Val), 29.07 (C^{β}, Val-pNA), 28.01 (CH_3, Boc), 19.45, 17.35 (C^{γ}, Val), 19.35, 17.25 (C^{γ}, Val-pNA).

Boc-Val-Pro-pNA (23). Gave a pale yellow solid; yield: 76%; R_f(B): 0.55; k' (II): 2.95; FABMS, m/z: 435 $(M+H)^+$, 334 $(M-Boc)^+$; ^1H NMR ($CDCl_3$): 9.01 (b, 1H, NH, pNA), 8.15, 7.78 (m, 4H, aromatic, pNA), 5.28 (m, 1H, CH^{α}, Pro), 5.25 (b, 1H, NH, Val), 4.20 (m, 1H, CH^{α}, Val), 4.15, 3.25 (m, 2H, CH_2^{δ}, Pro), 2.35, 2.2 (m, 2H, CH_2^{β}, Pro), 2.18 (m, 2H, CH_2^{γ}, Pro), 2.00 (m, 1H, CH^{β}, Val), 1.35 (s, 9H, CH_3, Boc), 0.95, 0.9 (m, 6H, CH_3^{γ}, Val).; ^{13}C NMR ($CDCl_3$): 170.95 (C=O, Val), 167.95 (C=O, Pro), 156.05 (C=O, Boc), 143.75, 143.20, 123.95, 119.25 (aromatic, pNA), 80.25 (C^{q}, Boc), 63.25 (C^{α}, Val), 61.75 (C^{α}, Pro), 50.87 (C^{δ}, Pro), 31.75 (C^{β}, Val), 28.03 (CH_3, Boc), 27.95 (C^{β}, Pro), 24.25 (C^{γ}, Pro), 19.01, 18.01 (C^{γ}, Val).

Boc-Val-Sar-pNA (24). Gave a pale yellow oil; yield: 74%; R_f(B): 0.40; k' (II): 2.51; FABMS, *m/z*: 409 $(M+H)^+$, 309 $(M+H-Boc)^+$; ^1H NMR ($CDCl_3$): 9.00 (b, 1H, NH, pNA), 8.01, 7.75 (m, 4H, aromatic, pNA), 5.18 (b, 1H, NH, Val), 4.9 (s, 2H, CH_2^{α}, Sar), 4.23 (m, 1H, CH^{α}, Val), 3.40 (s, 3H, N-CH_3, Sar), 1.95 (m, 1H, CH^{β}, Val), 1.35 (s, 9H, CH_3, Boc), 0.95, 0.85 (m, 6H, CH_3^{γ}, Val); ^{13}C NMR ($CDCl_3$): 170.23 (C=O, Val), 166.27 (C=O, Sar), 155.92 (C=O, Boc), 143.45, 143.15, 124.35, 119.45 (aromatic, pNA), 79.82 (C^{q}, Boc), 58.57 (C^{α}, Val), 58.17 (C^{α}, Sar), 39.25 (N-CH_3, Sar), 32.98 (C^{β}, Val), 28.01 (CH_3, Boc), 19.02, 18.35 (C^{γ}, Val).

Monitoring of the thioamides formation by ^{13}C NMR

The ^{13}C NMR spectra were recorded in THF-d_8 on a Bruker AM 400 with a magnetic field of 9.395 T, operating at 100.614 MHz, NS: 4000, RD: 1 s, at 263 K and 300 K.

BOP-Cl (76.3 mg, 0.3 mmol) was added to a stirred solution of Boc-Leu SH (49.4 mg, 0.2 mmol) and DIEA (68.5 µL, 0.4 mmol in THF-d^8 (0.5 mL). After reaction for 1 h at 0 °C under argon, the solution was centrifuged and monitored using ^{13}C NMR in a 5-mm NMR tube at 263 K (see spectra **B**). Then piperidine (23.7 µL, 0.24 mmol) was added and allowed to react for 2 h at room temperature. The solution was monitored using ^{13}C NMR at 300 K (see spectra **C**).

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