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N-Urethane-Protected Amino Alkyl Isothiocyanates: Synthesis, Isolation, Characterization, and Application to the Synthesis of Thioureidopeptides

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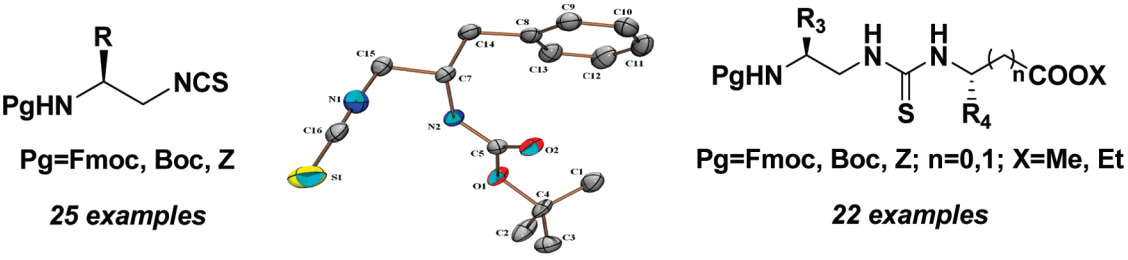

N-Boc-Phe-ψ[CH_2NCS]

Synthetically useful *N*-Fmoc amino-alkyl isothiocyanates have been described, starting from protected amino acids. These compounds have been synthesized in excellent yields by thiocarbonylation of the monoprotected 1,2-diamines with CS_2/TEA/*p*-TsCl, isolated as stable solids, and completely characterized. The procedure has been extended to the synthesis of amino alkyl isothiocyanates from Boc- and Z-protected amino acids as well. The utility of these isothiocyanates for peptidomimetics synthesis has been demonstrated by employing them in the preparation of a series of dithioureidopeptide esters. Boc-Gly-OH and Boc-Phe-OH derived isothiocyanates **9a** and **9c** have been obtained as single crystals and their structures solved through X-ray diffraction. They belong to the orthorhombic crystal system, and have a single molecule in the asymmetric unit ($Z' = 1$). **9a** crystallizes in the centrosymmetric space group *Pbca*, while **9c** crystallizes in the noncentrosymmetric space group $P2_12_12_1$.

Introduction

The synthesis of backbone modified peptides and their screening is an integral part of the present day drug development processes owing to the dramatic improvement in the pharmacokinetic properties of the bioactive peptides caused by the replacement of one or more peptide bonds with unnatural linkages.[1,2] Thus, several classes of peptidomimetics that contain non-native bonds such as ureas, carbamates, oligosulfonamides, peptoids, hydrazino peptides, aminoxy peptides, and heterocycles have been synthesized and employed for therapeutic applications.[3,4]

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Among these compounds, oligoureas and ureidopeptides have been extensively studied as inhibitors and antagonists of various enzymes and receptors as well as important structural motifs in de novo design.[5,6] Isoelectronic replacement of the oxygen atom of the uredio bond results in another biologically, medicinally, and structurally relevant linkage, the thioureido bond. The importance of the thioureido group in conferring the required potency to bioactive molecules can be seen in its insertion into many medicinally valuable compounds. Many of the substituted thioureas are active as anti-HIV,[7] antiviral,[8] antimicrobial,[9] antituberculor,[10] antitumor,[11] antihypertensive,[12] and anticarcinogenic agents.[13] The high acidity of the −NH−CS−NH− protons, in correlation with strong hydrogen bonding property has been exploited in the design of self-assembling macromolecules and stabilization of secondary structures.[14] Thiourea moieties

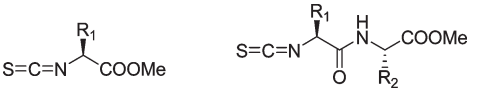

FIGURE 1. Amino/peptide acid ester derived isothiocyanates well known in the literature.[21,22]

embedded into macromolecules like pseudo-oligosaccharides provide anchoring points for hydrogen bonding recognition of complementary functional groups with specific orientation.[15] They are also used as catalysts for asymmetric organic synthesis.[16−19] RGD peptides and lysine derivatives functionalized with thioureido moieties have been used to facilitate cell adhesion, and as transfecting agents.[20]

Substituted thioureas (both symmetrical and unsymmetrical, Figure 1) are largely prepared by two main routes: (i) coupling of primary/secondary amines in the presence of CS_2,[23] thiophosgene,[24] or its equivalent,[25] triphenylphosphine thiocyanogen,[26] thiourea, or H_2S on substituted guanidines[27] [several thiocarbonylating agents such as 1-(methyldithiocarbonyl)imidazole,[28] 1,1′-thiocarbonyldiimidazole (TDI),[29] di-2-pyridyl thionocarbonate (DPT),[30] thiocarbonyl(bis-benzotriazole),[31] 1,3-diphenyl thiourea,[32] and molybdenum xanthate complexes[33] have also been developed] and (ii) straightforward reaction of amines with isothiocyanates, which is widely used.[34]

Isothiocyanates are versatile synthetic intermediates whose strong electrophilicity enables them to readily take part in nucleophilic addition and cycloaddition reactions. Apart from being precursors for thioureas, they are also starting materials for a broad spectrum of compounds such as thiohydantoins, sulfur heterocycles, viz., mercaptoimidazoles, thioquinazolines, thiopyrimidones, thioamidazolones, and benzothiazines.[25,35]

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Glycosyl isothiocyanates are well known and highly useful building blocks in carbohydrate chemistry.[36] Their stereospecific synthesis, stability and characterization aspects, and synthetic applications are widely reported.[37] They have been employed in the preparation of thiourea-linked glyco-oligomers that mimic the branching patterns of oligosaccharides and multivalent glycosides, glycosyl heterocycles, and glyco-conjugates such as *N*-nucleosides.[38]

In peptidomimetic chemistry, while the bioisosteric replacement of the −C═O/−C−O−C− bond with the −C═S/−C−S−C− bond can be profoundly found in the case of the synthesis of thiopeptides/thioamides and thiazoles with new properties compared to parent peptides/amides or oxazoles,[3a,39] similar modification in the case of the other hetero bonds, −NH−CO−NH− (ureido), −NCO (isocyano), and −NH−CO−O−R (carbamate), is less commonly reported. Again, when diversely substituted peptidyl ureas, e.g., *N*,*N*; *N*,*N*′, linked oligoureas, α-peptidyl, *β*-peptidyl ureas etc.,[40,41] have been reported, the known types of peptidyl thioureas are restricted to two examples, viz., α-amino acid esters linked through thioureido linkages[42] and Boc-NH-$(CH_2)_2$-NHCSNH-R synthesized starting from ethylene diamine.[42,43] Similarly, there are few reports on the synthesis of isothiocyanates derived from amino acids/peptides, and all of these describe only α-isothiocyanato alkyl esters, SCN−CHR−COOY, obtained by converting the α-amino group of amino acid esters into the isothiocyano group under different thiocarbonylation conditions. Halpern et al. synthesized them by reacting α-amino acid esters with CS_2 and subsequent decomposition of the dithiocarbamate intermediate with chloroformate ester.[44] Kunze et al. prepared α-isothiocyanato alkyl esters employing thiophosgene in water.[21] The products were purified by vacuum distillation and used for the preparation of thiocarbamoylphosphines. Nowick et al. prepared peptide ester isothiocyanates, SCN−CHR−CONH−CHR−COOY by treating amino free peptidyl ester with thiophosgene under biphasic conditions.[22] Boas et al. generated isothiocyanates by treating the resin bound Phe and Tyr with CS_2 in the presence of HBTU and PyBOP and coupled with amino acid esters to obtain thioureas.[42]

In this context, we envisaged the preparation of a hitherto unreported class of *N*-urethane-protected amino alkyl isothiocyanates. Owing to the vast diversity of synthetic applications of isothiocyanates, these novel compounds could find utility as valuable intermediates in the synthesis of several new classes of peptidomimetics including N-protected thioureidopeptide esters, amino acid analogues, and peptide conjugates. Further, our interest in the design and synthesis of Fmoc-amino acid derived novel monomeric building blocks that possess a reactive functional group inserted in place of the carboxyl moiety of amino acids and useful in preparing backbone modified peptides with N as well as C terminus[41,45,46] led us to focus attention on the synthesis of yet another new class of N^{β}-Fmoc amino alkyl isothiocyanates. Accordingly, we herein report the first synthesis and isolation of *N*-Fmoc-*β*-amino alkyl isothiocyanates and demonstrate their utility in the preparation of dithioureidopeptides which, to the best of our knowledge, are hitherto unreported. The protocol has been extended to obtain Boc and Z amino acid derived isothiocyanates as well.

Results and Discussion

Synthesis of Fmoc-N^{β}-amino Alkyl Isothiocyanates Fmoc-Xaa-ψ[CH_2NCS] 2. The initial part of the study involved the synthesis of title isothiocyanates **2** employing Fmoc chemistry. The synthesis was pursued with two different routes.

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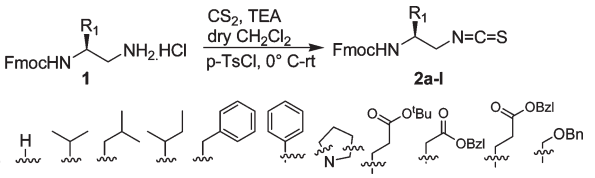
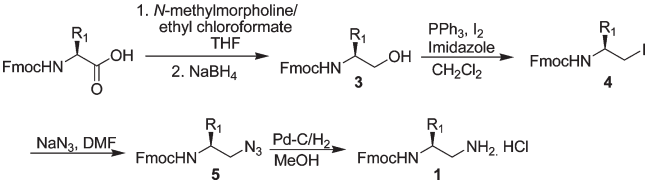
SCHEME 1



SCHEME 2



The direct thionation of known isocyanates (Fmoc-Aaa-ψ[CH_2NCO] into isothiocyanates with P_2S_5[47] or Lawesson reagent)[48] and reaction of *N*-Fmoc-β-amino alkyl iodides with isothiocyanate ions.[49] But both these routes turned out to be unsuccessful with the former resulting in very low yields of the desired isothiocyanates and the later yielding a larger product fraction as thiocyanate due to the ambident nature of isothiocyanate ion.[50] In view of these results, studies on thiocarbonylation of amines **1** were undertaken (Scheme 1). Fmoc-amino acids were initially reduced to the corresponding β-amino alcohols via treatment of the mixed anhydride of the acid with $NaBH_4$.[51] The alcohols were then converted into the alkyl iodides **4** under Mitsunobu conditions, which after isolation and purification through flash chromatography were treated with NaN_3 in DMF at room temperature for 3−4 h to obtain the azides **5** as white solids.[52] On subjecting the azides to catalytic hydrogenation with Pd/C, amines **1** were obtained quantitatively which were isolated as HCl salts by the addition of $CHCl_3$ to the reaction mixture according to Liskamp et al.[4b] (Scheme 2). In the case of Fmoc-Asp/Glu(Bzl)-OH and Fmoc-Ser(Bzl)-OH, the desired amines were prepared by treating the corresponding alkyl azides **5** with PPh_3 in THF.[53]

In the next step, conversion of the amine into isothiocyanate was undertaken. For this, the amine **1** was reacted with thiocarbonylating agents thiophosgene, DPT, and TDI. With thiophosgene, though the yield of the desired isothiocyanate was satisfactory, handling problems, difficulty in removal of the excess of the reagent, and the toxicity discouraged further usage. With both DPT and TDI the reaction was slow and also the yields were unsatisfactory. Consequently, generation of the dithiocarbamic salt by reaction of the amine with CS_2 followed by decomposition into the isothiocyanate in the presence of TsCl,[54] DCC,[55] and H_2O_2[56] was pursued. Alternatively, reduction of the alkyl azide with PPh_3 in the presence of CS_2 was also carried out.[57] The results of these studies are summarized in Table 1. The reactions involving DCC and H_2O_2 were sluggish and less yielding, harsh and involved tedious workup with difficult product isolation. Finally, the reaction of amine with CS_2/TsCl/TEA was found to be attractive in terms of furnishing good yield, involving milder reaction conditions, less expense, and commercial availability of the reagents.

TABLE 1. Comparative Study of the Reagents for the Synthesis of 2f

entry	reagent	reaction time (h)	yield (%)
1	thiophosgene	0.5	65
2	dipyridyl thionocarbonate (DPT)	1.0	52
3	1,1′-thiocarbonyldiimidazole (TDI)	1.0	56
4	CS_2/TEA/tosyl chloride	2.0	93
5	CS_2/DCC	3.5	54
6	CS_2/TEA/H_2O_2	1.5	63
7	CS_2/PPh_3 (for direct conversion of azide **5** into isothiocyanate)	1.1	56

In a typical experiment, a solution of Fmoc-Ala-ψ[$CH_2NH_2\cdot$HCl] **1b** in THF was treated with an equimolar quantity of CS_2 in the presence of TEA at 0 °C for 20 min, and the in situ generated dithiocarbamic salt was decomposed with *p*-TsCl for about 30 min at the same temperature (Scheme 1). Upon completion of the reaction (from TLC analysis) isothiocyanate **2b** was isolated by a simple workup and purified through column chromatography as analytically pure solid in 92% yield (as evident by HPLC). The same procedure was successfully extended to prepare a series of novel isothiocyanates **2a**−***l*** from Fmoc amino acids including those derived from side chain functionalized, viz., aspartic acid, glutamic acid, and serine which were fully characterized (Table 2). The ^{13}C NMR of these compounds contains a characteristic signal at around δ 132.0 ppm corresponding to isothiocyanate carbon, and the IR spectrum exhibits a sharp and intense peak at around 2090 cm^{-1} characteristic of the isothiocyanato group. All the isothiocyanates showed exceptional stability; they were stable toward column chromatography and long time storage (for few months) at room temperature with no noticeable degradation as analyzed by HPLC.

Synthesis of *N*-Boc/Z-Amino Alkyl Isothiocyanates Boc/Z-Xaa-ψ[CH_2NCS] 8 and 9. To obtain differentially N-protected isothiocyanate derivatives of amino acids to be useful in diverse conditions of peptide and peptidomimetic synthesis, and also to demonstrate the generality of the present protocol to yield the isothiocyanates containing the other commonly employed urethane protections Boc and Z, we extended the synthesis to prepare *N*-Boc- and Z-protected isothiocyanates as well. The required alkyl diamines, Pg-NH-Xaa-ψ[CH_2NH_2] **7** where Pg = Boc/Z, were synthesized through a different route that involved the reduction of *N*-Boc/Z-protected α-amino nitriles with $LiAlH_4$, due to the tolerance of Boc and Z groups toward $LiAlH_4$, unlike the Fmoc group. The resulting mono Boc/Z-protected alkyl

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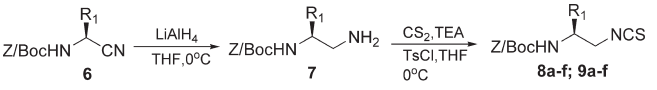
TABLE 2. List of *N*-Fmoc-Aaa-ψ[CH_2NCS]s Prepared

entry	R_1	yield (%)	mp (°C)	$[\alpha]^{25}_D$ (*c* 1, $CHCl_3$)	HRMS ($M + Na^+$) calcd	HRMS ($M + Na^+$) obsd
2a	H	90	171		347.0830	347.0830
2b	CH_3	92	183	−67.0	361.0987	361.0974
2c	$CH(CH_3)_2$	90	164	−71.0	389.1300	389.1314
2d	$CH_2CH(CH_3)_2$	88	168	−87.0	403.1456	403.1470
2e	$CH(CH_3)C_2H_5$	90	154	−71.0	403.1456	403.1453
2f	$CH_2C_6H_5$	93	187	−17.0	437.1300	437.1274
2g	C_6H_5	85	148	−16.0	423.1143	423.1140
2h	C_6H_5 (D-amino acid)	82	145	+15.0	423.1143	423.1142
2i	-$(CH_2)_3$- (Proline)	87	Oil	−52.0	387.1143	387.1140
2j	$(CH_2)_2COO^tBu$	87	123	−44.0	475.1667	475.1689
2k	$CH_2COOBzl$	91	175	−36.0	495.1354	495.1352
2*l*	$(CH_2)_2COOBzl$	88	186	−23.0	509.1511	509.1502
2m	CH_2OBzl	84	142	−17.0	467.1405	467.1402

SCHEME 3



Amino acids: 8a:Ala; **8b**:Val; **8c**:Leu; **8d**:Ile; **8e**:Phe; **8f**:Phg; **9g**:Pro
9a:Gly; **9b**:Val; **9c**:Phe; **9d**:Phg; **9e**:Met; **9f**:Cys(Bzl)

diamines were then converted to isothiocyanates by following the same procedure used for the preparation of Fmoc-protected isothiocyanates (Scheme 3). In these studies also, the desired isothiocyanates Boc/Z-Xaa-ψ[CH_2NCS] **8** and **9** were obtained in 80−89% yields. All of these compounds (Table 3) were isolated as stable solids after column purification, completely characterized, and found to be shelf-stable like their Fmoc counterparts.

Synthesis of Thioureidodipeptides. Dithioureidopeptides were synthesized by coupling the isothiocyanates **2** with amino acid esters. Compounds **2a**−**d** and **2f** were reacted with amino acid methyl/ethyl esters (obtained by deprotonation of the HCl salt of the amino ester with activated Zn)[58] in the presence of DIEA to obtain the thioureidodipeptides **10a**−**g** in 65−74% yields after column purification, which were adequately characterized (Scheme 4, Table 4). The ^{13}C NMR of thioureas **10** showed a signal at around δ 181.00 corresponding to thiocarbonyl carbon. No significant difference was observed with respect to reaction time or yields upon changing the base from DIEA to TEA or pyridine or *N*-methylmorpholine (NMM). These isothiocyanates take a longer time (4 h) to couple with amino acid esters compared to the related class of isocyanates which readily couple with the amines within 30 min.[6a]

Isothiocyanates derived from Boc and Z amino acids were also employed in the synthesis of dithioureidopeptides. Reaction of **8** or **9** with amino acid esters in the presence of DIEA readily yielded the corresponding Boc- and Z-protected dithioureidopeptides **11** and **12** in 67−74% yield (Figure 2, Table 4). Again, all the thioureas thus obtained were well characterized.

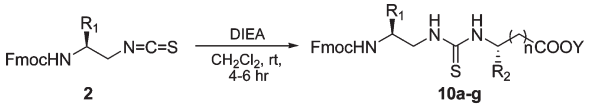
Racemization Studies. The optical purity of the isothiocyanates as well as that of the thioureidopeptides was evaluated by ^1H NMR studies of the model dithioureidopeptides prepared via the present protocol starting from Fmoc, Boc, as well as Z amino acids. For this, three sets of epimeric dithioureidopeptides **13a**,**b** (Fmoc protected), **14a**,**b** (Boc protected), and **15a**,**b** (Z protected) were synthesized by coupling Fmoc/Boc-Phe-ψ[CH_2NCS] and Z-Phg-ψ[CH_2NCS] with (*R*)-1-phenylethylamine and (*S*)-1-phenylethylamine separately (Figure 3). In each set, ^1H NMR of the particular epimer contained a single distinct methyl group doublet. Observed δ values for the $-CH_3$ group of the compounds are as follows: **13a** 1.40, 1.38 and **13b** 1.49, 1.47; **14a** 1.42, 1.41 and **14b** 1.44, 1.43; and **15a** 1.28, 1.27 and **15b** 1.31, 1.32 ppm. Further, the samples **13c**, **14c**, and **15c** prepared by coupling the isothiocyanates with racemic 1-phenylethaneamine showed methyl group resonances at δ values of 1.37, 1.40, 1.47, 1.48; 1.45, 1.44, 1.41; and 1.13, 1.15, 1.17, respectively, indicating the presence of two isomers with well-separated methyl group doublets. Also, the HPLC profile of the two epimers, **13a** and **13b**, had peaks at R_t values of 17.8 and 19.2 min, respectively. Similarly, HPLC of the crude samples of compound **10b** and its epimer Fmoc-Phe-ψ[$CH_2NHCSNH$]-D-Ala-OMe had major peaks at R_t values of 20.13 and 21.17 min, respectively, and the equimolar mixture of these epimers that was prepared by coupling racemic alanine methyl ester with Fmoc-Phe-ψ[CH_2NCS] showed two well-separated peaks corresponding to the thioureas at R_t 20.14 and 21.21 min. Thus, from the above studies it was evident that the samples analyzed were optically pure and the synthesis of isothiocyanates as well as their coupling with amino acid esters takes place with retention of configurations at the chiral center of the isothiocyanate as well as that of the newly coupled amino acid ester residue.

TABLE 3. List of Z/Boc-Aaa-ψ[CH_2NCS]s Synthesized

entry	isothiocyanates	yield (%)	mp (°C)	$[\alpha]^{25}_D$ (*c* 1, $CHCl_3$)	HRMS ($M + Na^+$) calcd	HRMS ($M + Na^+$) obsd
8a	Z-Ala-ψ[CH_2NCS]	88	112	−95.0	273.0	272.9[a]
8b	Z-Val-ψ[CH_2NCS]	85	119	−103.0	301.0987	301.0980
8c	Z-Leu-ψ[CH_2NCS]	86	108	−110.0	315.1	315.0[a]
8d	Z-Phe-ψ[CH_2NCS]	88	128	−23.0	349.0	349.0[a]
8e	Z-Phg -ψ[CH_2NCS]	85	132	+11.8	335.0830	335.0837
8f	Z-Pro-ψ[CH_2NCS]	80	gum	−88.0	299.0830	299.0822
9a	Boc-Gly-ψ[CH_2NCS]	89	65		225.0674	225.0680
9b	Boc-Val-ψ[CH_2NCS]	82	78	−45.0	267.1143	267.1140
9c	Boc-Phe-ψ[CH_2NCS]	87	107	−40.0	315.1	315.0[a]
9d	Boc-D-Phg-ψ[CH_2NCS]	85	75	−30.0	301.0	300.9[a]
9e	Boc-Met-ψ[CH_2NCS]	80	68	−46.7	299.0864	299.0867
9f	Boc-Cys(Bzl)-ψ[CH_2NCS]	82	gum	−39.0	361.1020	361.1029

[a]ESI-MS of the isothiocyanates.

SCHEME 4. Synthesis of Thioureidodipeptides



Compound	R_1	R_2	Y	n
10a	H	$CH_2C_6H_5$	CH_3	0
10b	$CH_2C_6H_5$	CH_3	CH_3	0
10c	$CH_2CH(CH_3)_2$	H	CH_3	1
10d	$CH_2C_6H_5$	H	CH_3	1
10e	CH_3	H	CH_3	1
10f	CH_3	H	C_2H_5	1
10g	C_6H_5	H	C_2H_5	1

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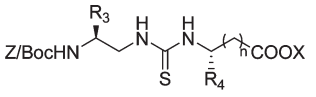
TABLE 4. List of Dithioureido Peptides

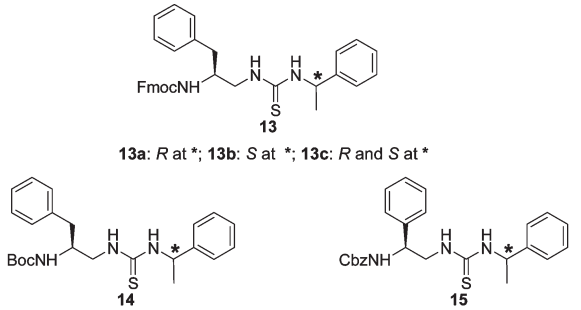
S1 no.	dithioureido peptides	yield[a] (%)
10a	Fmoc-Gly-ψ[CH_2-NH-CS-NH]-Phe-OMe	73
10b	Fmoc-Phe-ψ[CH_2-NH-CS-NH]-Ala-OMe	71
10c	Fmoc-Leu-ψ[CH_2-NH-CS-NH]-β-Ala-OMe	74
10d	Fmoc-Phe-ψ[CH_2-NH-CS-NH]-β-Ala-OMe	65
10e	Fmoc-Ala-ψ[CH_2-NH-CS-NH]-β-Ala-OMe	67
10f	Fmoc-Ala-ψ[CH_2-NH-CS-NH]-β-Ala-OEt	69
10g	Fmoc-D-Phg-ψ[CH_2-NH-CS-NH]-β-Ala-OEt	73
11a	Z-Phe-ψ[CH_2-NH-CS-NH]-Leu-OMe	72
11b	Z-Cys(Bzl)-ψ[CH_2-NH-CS-NH]-Ala-OMe	70
11c	Z-Phe-ψ[CH_2-NH-CS-NH]-β-Ala-OEt	67
11d	Z-Phg-ψ[CH_2−NH−CS-NH]-β-Ala-OMe	71
12a	Boc-Gly-ψ[CH_2-NH-CS-NH]-Phe-OMe	73
12b	Boc-Ala-ψ[CH_2-NH-CS-NH]-Val-OEt	74
12c	Boc-Ala-ψ[CH_2-NH-CS-NH]-β-Ala-OMe	70
12d	Boc-Leu-ψ[CH_2-NH-CS-NH]-β-Ala-OMe	70
12e	Boc-Phe-ψ[CH_2-NH-CS-NH]-Phe-OMe	73

[a]Isolated yield after column purification.



11b, 12a, 12c : n=0; **11a, 11c-d, 12b. 12d** : n=1

FIGURE 2. Thiourea-linked dipeptidomimetics.



13a: *R* at *; **13b**: *S* at *; **13c**: *R* and *S* at *

14a: *R* at *; **14b**: *S* at *; **14c**: *R* and *S* at * **15a**: *R* at *; **15b**: *S* at *; **15c**: *R* and *S* at *

FIGURE 3. Epimeric dithioureidopeptides synthesized for racemization studies.

Crystal Structures of Boc-Gly-ψ[CH_2-NCS] 9a and Boc-Phe-ψ[CH_2-NCS] 9c. X-ray diffraction of single crystals of amino acid derivatives generates valuable information on the structural properties which would influence the reactivity and chemical behavior of these compounds during peptide coupling and further in peptidomimetic synthesis. Nevertheless, only a small fraction of the vast number of reported amino acid derivatives have been crystallized, and generally these compounds are the ones with conformationally restricted $C^{\alpha,\alpha}$ disubstituted glycine residue that show higher crystallinity.[59] In the present study, we have obtained single crystals of two isothiocyanates **9a** and **9c** containing proteinogenic amino acids, Gly and Phe, respectively. To the best of our knowledge, this is the first report on the crystal structure analysis of amino acid derivatives containing an isothiocyanato group.

The crystal structures indicate the stability of this class of isothiocyanates compared to their isocyanate counterparts which are not stable and hence have not been isolated.[4a,40f] Hence, the present crystal structure studies would also be helpful in evaluating the structural parameters of N-blocked amines functionalized at the β position. The compounds **9a** and **9c** belong to the orthorhombic crystal system, and have a single molecule in the asymmetric unit ($Z' = 1$) (Figure 4). However, while **9a** crystallizes in the centrosymmetric space group *Pbca*, **9c** crystallizes in the noncentrosymmetric space group $P2_12_12_1$. Selected bond lengths and torsion angles, hydrogen bonding geometries, and parts of the crystal structures are given in the Supporting Information.

(59) For a review on X-ray diffraction analysis of N-protected amino acid halides, esters, anhydrides, UNCAs, azides, and amides see : Toniolo, C.; Crisma, M.; Formaggio, F. *Biopolymers Peptide Science* **1996**, *40*, 627−651 and references cited therein.

Conclusion

In summary, we describe the synthesis of N^{β}-Fmoc/Boc/Z-amino isothiocyanates by the reaction of *N*-Fmoc/Boc/Z-amino acid derived alkyl amines with CS_2 in the presence of TEA and *p*-TsCl. The isothiocyanates were isolated as stable solids and characterized through NMR, IR, and mass spectrometry. These isothiocyanates **2**, **8**, and **9** were conveniently used as building blocks for the synthesis of thioureido-linked dipeptidomimetics **10**, **11**, and **12**. Crystal structures of isothiocyanates **9a** and **9c** that are synthesized from Boc-Gly-OH and Boc-Phe-OH, respectively, have been solved, which constitutes the first report on the crystal structure of amino acid derivatives containing an isothiocyanate moiety. Application of the present isothiocyanates in the synthesis of eoligothioureidopeptides, peptide heterocycles, and thiocarbamate-linked peptides is being investigated.

Experimental Section

General Procedure for the Synthesis of *N*-Fmoc Amino Alkyl Isothiocyanates 2. To a chilled solution of *N*-Fmoc-amino alkyl amine **3** (1.3 mmol) in dry THF was added CS_2 (1.3 mmol) followed by TEA (3.5 mmol). The ice bath was removed and stirring was continued for another 10 min. The reaction mixture was again chilled; *p*-toluene sulfonyl chloride (1.5 mmol) was added and the solution was stirred for another 25 min or until the completion of reaction as judged by TLC. An excess of CH_2Cl_2 (20 mL) was added and the organic layer was washed twice with citric acid solution (10%, 15 mL), followed by water (15 mL) and brine (10 mL). It was then dried over anhydrous sodium sulfate and concentrated under vacuum. The resulting crude product was subjected to column chromatography (silica gel 100−200 mesh, 20% ethyl acetate in hexane) to afford the title compound as a white solid. Batch reactions up to 50 mmol were safely carried out and the products were isolated in excellent yield.

***N*-Fmoc-Thioureido Dipeptidyl Esters 10.** A solution of amino acid ester (1.2 mmol, obtained by neutralizing the hydrochloride salt by treatment with zinc dust) in dry CH_2Cl_2 was added to a stirred solution of *N*-Fmoc-amino alkyl isothiocyanate **2** (1 mmol) followed by DIEA (1.5 mmol) at 0 °C. The solution was then allowed to warm to room temperature and stirred for 3 h. After completion of the reaction, the solution was diluted with 10% citric acid (8 mL) and the layers were allowed to separate. The organic phase was washed with 10% Na_2CO_3 (10 mL), water (2 × 10 mL), and brine (10 mL) and dried over anhydrous sodium sulfate. After the removal of the solvent under vacuum, the crude was purified through column chromatography (silica gel 100−200 mesh, 30−40% ethyl acetate in hexane) to afford the thioureido dipeptidyl thiourea esters as off-white to white semisolids.

Spectral Characterization Data of Representative Compounds 2b and 10c. (*S*)-(9*H*-Fluoren-9-yl)methyl 1-isothiocyanatopropan-2-ylcarbamate (*N*-Fmoc-Ala-ψ[CH_2NCS]) (2b): yield 92% of white solid; mp 183 °C; R_f (10% EtOAc:hexane) 0.32;

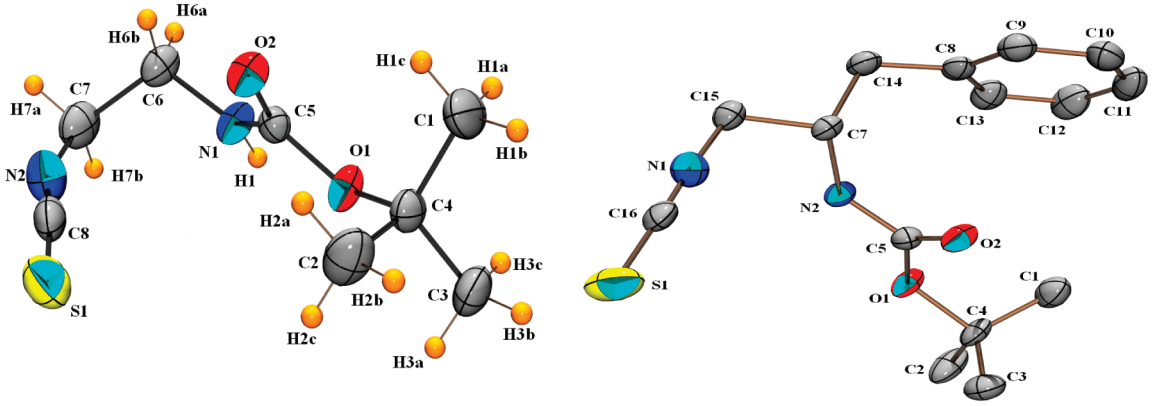


FIGURE 4. The molecular structure of Boc-Gly-ψ[CH_2-NCS] **9a** (left) and Boc-Phe-ψ[CH_2-NCS] **9c** (right) showing the atom-labeling scheme. Displacement ellipsoids are drawn at the 50% probability level. H atoms are shown as small spheres of arbitrary radii.

$[\alpha]^{25}_D$ −67.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.26 (d, J = 6.4 Hz, 3H), 3.94 (br, 1H), 4.21 (d, J = 6.4 Hz, 2H), 4.35−4.49 (m, 3H), 4.83 (d, J = 6.0 Hz, 1H), 7.30−7.42 (m, 4H), 7.58 (d, J = 7.2 Hz, 2H), 7.76 (d, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 18.4, 47.3, 47.7, 50.5, 67.3, 120.5, 125.6, 127.6, 128.3, 133.0, 141.8, 144.2, 155.9; HRMS calcd for $C_{19}H_{18}N_2O_2S$ *m*/*z* 361.0987 (M^+ + Na), found 361.0974.

(*S*)-Methyl-3-(3-(2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-4-methylpentyl)thio ureido)propanoate (*N*-Fmoc-Leu-ψ[CH_2-NHCSNH]-β-Ala-OMe) (10c): yield 74%; R_f (30% EtOAc:hexane) 0.32; $[\alpha]^{23}_D$ −12.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.92 (d, J = 6.8 Hz, 6H), 1.37 (m, 2H), 1.66 (m, 1H), 2.58 (br, 2H), 3.58 (d, 3H), 3.65−3.80 (m, 4H), 4.02 (br, 1H), 4.19 (t, J = 6.8 Hz, 1H), 4.35 (m, 2H), 5.21 (br, 1H), 6.96 (br, 2H), 7.22−7.76 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 22.4, 23.5, 25.3, 34.0, 42.3, 44.4, 47.6, 50.2, 52.3, 53.9, 67.4, 120.5, 125.6, 127.6, 128.2, 141.7, 144.2, 157.8, 173.5, 183.2; HRMS calcd for $C_{26}H_{33}N_3O_4S$ *m*/*z* 506.2089 (M^+ + Na), found 506.2112.

Test for Racemization. To a solution of isothiocyanate **2f** (300 mg, 0.72 mmol) in dry CH_2Cl_2 (5.0 mL) at 0 °C was added (*R*)-1-phenylethylamine (95 mg, 0.79 mmol) followed by DIEA (0.12 mL, 1.5 mmol) and the reaction mixture was stirred for 2.5 h, then it was diluted with CH_2Cl_2 (10 mL), washed with 5% citric acid (10 mL), water (2 × 10 mL), and brine (10 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to afford the product as a single diastereomer of the thiourea. **(*S*)-(9*H*-fluoren-9-yl)methyl (*S*)-3-phenyl-1-(3-((*R*)-1-phenylethyl)thioureido)propan-2-ylcarbamate (Fmoc-Phe-ψ-[CH_2NHCSNH]-(*R*)-1-phenethylamine) (13a):** yield 72%; R_t (HPLC) 17.8 min; ^{1}H NMR ($CDCl_3$) δ 1.39 (d, J = 6.0 Hz, 3H), 2.75 (m, 2H), 3.51 (m, 1H), 3.80 (m, 1H), 4.17 (t, J = 6.9 Hz, 1H), 4.31 (t, J = 7.2 Hz, 2H), 5.23 (m, 1H), 6.06 (br, 2H), 7.08 (d, J = 6.9 Hz, 2H), 7.26−7.43 (m, 14H), 7.53 (t, J = 7.2 Hz, 2H), 7.76 (d, J = 7.2 Hz, 2H). When the experiment was repeated with **2f** and (*S*)-1-phenylethylamine, the other diastereomer **(*S*)-(9*H*-fluoren-9-yl)methyl (*S*)-3-phenyl-1-(3-((*S*)-1-phenylethyl)thioureido)propan-2-ylcarbamate (Fmoc-Phe-ψ[CH_2NHCSNH]-(*S*)-1-phenethylamine) (13b)** was obtained in 74% yield: R_t (HPLC) 19.2 min; ^{1}H NMR ($CDCl_3$) δ 1.48 (d, J = 6.6 Hz, 3H), 2.73 (m, 2H), 3.53 (m, 1H), 3.85 (m, 1H), 4.14 (t, J = 6.9 Hz, 1H), 4.35 (t, J = 7.2 Hz, 2H), 5.29 (m, 1H), 6.04 (br, 2H), 7.07 (d, J = 6.9 Hz, 2H), 7.24−7.44 (m, 14H), 7.51 (t, J = 7.5 Hz, 2H), 7.77 (d, J = 7.2 Hz, 2H). Finally, **2f** (0.3 g, 0.72 mmol) was treated with racemic-(1)-phenylethylamine (95 mg, 0.79 mmol) and DIEA (0.12 mL, 1.5 mmol). After the usual workup, the product **13c** isolated was found to be a 1:1 mixture of both diastereomers (**Fmoc-Phe-ψ[CH_2NHCSNH]-(*R* and *S*)-(+)-phenylethylamine**): R_t (HPLC) 17.8 and 19.2 min.

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Supporting Information Available: General experimental procedure for N-protected amino alkyl amines and the product characterization data of all the compounds discussed along with copies of ^{1}H NMR, ^{13}C NMR, and mass spectra for **4d**−**g**, **4i**, **4k**−**m**, **5d**−**e**, **5g**, **5k**−**m**, **1a**−**c**, **1e**, **1g**, **1k**, **1m**, **2a**−**m**, **10a**−**g**, 13a−**c**, **8a**−**f**, **11a**−**d**, **15a**,**b**, **9a**−**f**, **12a**−**e**, **14a**,**b**, and CIFs of compounds **9a** and **9c**. This material is available free of charge via the Internet at http://pubs.acs.org.