Chapter 3. Simple approach for the synthesis of new class of dithiocarbamate-linked peptidomimetics

This chapter describes an efficient protocol for the synthesis of a new series of dithiocarbamate-linked peptidomimetics. The dithiocarbamic acid intermediate generated in situ by a reaction of an amino acid ester and carbon disulfide in the presence of triethylamine was treated with N-protected amino alkyl iodide to afford the title compounds in good to moderate yields. The protocol has also been extended to synthesize dithiocarbamate-linked tripeptidomimetics, N,N'-orthogonally protected dipeptidomimetics as well.

3.1. INTRODUCTION

Several classes of peptidomimetics have been tailored by replacing the native amide bond with various other tethers and are biologically scrutinized. Such non-amidic insertions would bring out shelf stability, alter the secondary structures and induce rigidity and thus would suffice the basic needs for a drug candidate.\textsuperscript{1} The importance of unnatural linkages in the peptide backbone and fruitful results obtained from biological screening of many of those class of molecules prompts one to occupy this area of research.\textsuperscript{2} With the background of our group’s success in designing novel peptidomimetics through incorporation of biologically active and valuable linkages into peptide backbone as well as developing simpler routes for the existing ones,\textsuperscript{3-5} we turned our attention towards another useful and biologically important functionality namely dithiocarbamate.\textsuperscript{6}

Reports in the literature demonstrate that the dithiocarbamate containing molecules show antibacterial, anthelmintic, anti-cancer, fungicidal, herbicidal, algicidal and growth depressant properties.\textsuperscript{7-9} They are extensively used as pharmaceuticals, as radical precursors and more recently as starting materials for ionic liquids.\textsuperscript{10} A limited number of this class of molecules have found application in photopolymerisation and vulcanization outlets.\textsuperscript{11} The utility of dithiocarbamate group as linkers in solid phase organic synthesis is also well documented.\textsuperscript{12} The dithiocarbamate functionality chelates heavy metals that make them versatile ligands and are applicable as NO scavengers and antidotes in nickel and copper poisoning.\textsuperscript{13} Furthermore, the functionalized dithiocarbamates such as benzamide-based thiolcarbamates have been developed as HIV-I NCp7 inhibitors.\textsuperscript{14} Cao and co-workers synthesized 4(3H)-quinazoline derivatives bearing dithiocarbamate side
chains and screened for antitumor activity.\textsuperscript{9b} It was found that certain class of dithiocarbamates such as (4-methanesulfinyl-butyl)dithiocarbamic acid methyl ester \textbf{A} (Figure 3.1) and 5-oxohexyl dithiocarbamic acid methyl ester \textbf{B} are potent phase II enzyme inducers. Subtle attention has also been thrown on anticancer activity of RWJ-025856 \textbf{C} that has been found to possess attenuating effects on tumor necrosis factor $\alpha$ induced apoptosis in murine fibrosarcoma WEHI 164 cells.\textsuperscript{15} 4-Substituted-piperazine-1-carbodithioic acid 3-cyano-3,3-diphenyl-propyl esters (\textbf{D} and \textbf{E}) have also been tested for anticancer activity (Figure 3.1).\textsuperscript{7} Further, the dithiocarbamate derivatives of itols and carbohydrates were synthesized and screened for antifungal activity.\textsuperscript{16-18}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3_1.png}
\caption{Selected biologically active compounds bearing dithiocarbamate unit.}
\end{figure}

Literature survey made over the protocols for the synthesis of dithiocarbamates directed us to a handful of reports.\textsuperscript{19} Villa \textit{et al.}, reported the synthesis and antifungal activity of bis(dithiocarbamate) derivatives of glycerol by a reaction of dithiocarbamic acid salt with 1,3-dichloro-1,3-didoxglycerol.\textsuperscript{16} The
precursor dithiocarbamic acid salts were prepared by the reaction of secondary amines with CS$_2$ (Scheme 3.1).

**SCHEME 3.1.**

![Scheme 3.1](image)

Meng-Shen Cai and co-workers synthesized dithiocarbamate derivatives via a simple one-pot protocol involving reaction between an amine and an alkyl halide in the presence of CS$_2$/anhydrous potassium phosphate (K$_3$PO$_4$). The reaction was carried out in acetone and a moderate yield of the product was obtained.$^{20}$ A three-component reaction between an amine, a substituted halide and CS$_2$ was carried out employing cesium carbonate (Cs$_2$CO$_3$) and tetrabutylammonium iodide (TBAI) by Jung et al. The study involved the reaction of several primary and secondary amines with alkyl halides in the presence of CS$_2$ to afford respective class of dithiocarbamates in moderate yield (Scheme 3.2).$^{21}$ The same condition was explored to access thiocarbonates also by replacing the amine component with substituted alcohols.

**SCHEME 3.2.**

![Scheme 3.2](image)
Guo’s group described a reaction of electrophilic alkenes with alkyl/aryl amines in the presence of CS$_2$ and K$_3$PO$_4$ to synthesize corresponding class of dithiocarbamates. The yields were moderate and a range of amines, both primary and secondary were selected to conjugate with a variety of alkenes (Scheme 3.3).$^{22}$

**SCHEME 3.3.**

\[
R'\text{NH} + CS_2 + XCH=CH-EWG \xrightarrow{K_3PO_4 / MeOH \text{ rt}} R'\text{N=S-X}\text{EWG}
\]

Similar conditions were followed by Cao et al., who synthesized a library of 4(3H)-quinazoline derivatives with dithiocarbamate side chains.$^{9b}$ The reaction was carried out in DMF for 2 h and the isolated compounds were tested for antitumor activity (Scheme 3.4).

**SCHEME 3.4.**

Later, Saidi and co-workers reported a one-pot solvent-free synthesis of dithiocarbamates starting from alkyl amines and alkyl/aryl halides in the absence of any catalysts. Simple mixing of amine component with halide compound in the presence of equimolar quantity of CS$_2$ afforded the dithiocarbamate product at rt in about 5 h in affordable yield (Scheme 3.5).$^{23}$
Saidi’s group observed that the synthesis of dithiocarbamates can be accelerated if the reaction is carried out in water. They reported a one-pot Michael type reaction of several primary and secondary amines with α,β-unsaturated compounds in the presence of CS₂ at rt (Scheme 3.6). However the reaction took 6-18 h for completion.

Another one-pot synthesis of dithiocarbamates starting from amines and alcohols was developed by Chaturvedi and Ray employing Mitsunobu’s reagent. They envisaged that the unstable dithiocarbamic acid generated by the reaction of CS₂ with an amine can be reacted with triphenylphosphine (PPh₃) and diethyl azodicarboxylate (DEAD) to form stable Mitsunobu zwitterionic species that underwent S-alkylation with an alcohol to afford corresponding dithiocarbamate.

Epoxide opening with an amine in the presence of CS₂ was also shown to yield corresponding dithiocarbamate in good yield. Saidi demonstrated an eco-friendly efficient protocol that involved one-pot mixing of an amine, epoxide and CS₂ in the absence of any catalyst under solvent-free condition. The reaction was complete in 2-8 h, with yields exceeding 85% (Scheme 3.7).
Recently, Ranu and co-workers\textsuperscript{28} reported that ionic liquids such as 1-methyl-3-pentylimidazolium bromide ([pmIm]Br) accelerate the formation of dithiocarbamates. A three component reaction was designed wherein a mixture of an amine, CS\textsubscript{2} and [pmIm]Br was treated with an electrophilic center such as an epoxide or an alkene or a methylene halide. A battery of compounds was synthesized in a very short duration (Scheme 3.8).

In spite of vast reports on the biological and synthetic applications of dithiocarbamate containing compounds, the peptidomimetics bearing this functionality are yet to be addressed. In correlation with other non-amide tethers such as ureas, carbamates \textit{etc}., incorporation of dithiocarbamate linkage into peptide backbone may result in interesting class of molecules. Thus we envisaged a simple synthesis of dithiocarbamate-linked peptidomimetics through the reaction of CS\textsubscript{2} with suitably chosen amino acid derived amine and halide components in the presence of TEA.
3.2. PRESENT WORK

Reaction between carbon disulfide and a N-nucleophile involves the addition of CS$_2$ to NH bonds.$^{6a}$ This gives rise to dithiocarbamate salt that can be transformed into various intermediates and products. Recently, Sureshbabu et al., reported the preparation of N-protected amino alkyl isothiocyanates.$^{29}$ During that study, N-protected amino alkyl amine was treated with CS$_2$ in the presence of TEA. The *in situ* generated dithiocarbamic acid salt was decomposed employing $p$-toluenesulfonyl chloride ($p$-TsCl) to afford corresponding isothiocyanate. The dithiocarbamic acid salt forms an addition product with $p$-Ts group, which subsequently undergoes decomposition to generate the isothiocyanate functionality. Thus it was envisaged that the intermediate dithiocarbamic acid salt reacts with electron deficient carbon centers such as alkyl halides to afford respective class of dithiocarbamate compounds. This chemistry was explored to prepare new class of peptidomimetics and the results are discussed here.

The first target of the present study was a dipeptidomimetic of the type 3.3. This was accomplished by a reaction of an amino acid ester with N-protected amino alkyl iodide in the presence of CS$_2$ and TEA. The iodo intermediates used in the present studies were prepared by following reported protocols.$^{29,30}$ Briefly, an N-protected amino acid was reduced to corresponding aminol and was subjected to Mitsunobu reaction (PPh$_3$, Im, I$_2$) to afford corresponding N-protected amino alkyl iodide 3.1. The iodo compound was obtained in good yield after a column chromatography (Scheme 3.9).
Then, in the next step, the preparation of compounds 3.3 was undertaken. In a typical reaction, a chilled solution of HCl-H-Phe-OMe in dry THF was treated with TEA and CS₂. To the in situ generated dithiocarbamic acid salt, a solution of N-Fmoc-Val-ψ[CH₂I] 3.1 in THF was added at the same temperature. The reaction was complete in about an hour as adjudged by TLC. A simple work-up followed by column chromatography yielded pure N-protected dithiocarbamate-linked dipeptidomimetic 3.3a in 72% yield (Scheme 3.10).

The generality of this reaction was demonstrated with a series of N-Fmoc/Z-amino alkyl iodides 3.1 and amino acid esters 3.2 to afford corresponding dipeptidomimetics 3.3b-g in good to moderate yields (Table 3.1). The products were found to be analytically pure.
To confirm the optical purity of the synthesized dithiocarbamates, a model study was carried out as follows: two epimeric dithiocarbamate compounds were prepared by coupling N-Fmoc-Phe-[CH2I] with (R)- and (S)-1-phenylethylamines separately to obtain diastereomeric compounds (S, R)3.3h and (S, S)3.3h respectively (Scheme 3.11). In the 1H NMR spectra of these samples, the methyl group appeared as distinct doublets at δ values 1.35, 1.37 (for (S, R)3.3h) and 1.38, 1.40 (for (S, S)3.3h) indicating the absence of racemization during the course of the reaction. Also, the HPLC profiles of these two diastereomers had exclusive major peak at R_t values 16.4 and 17.1 respectively, while the equimolar mixture of these epimers prepared by
the reaction of racemic phenylethylamine with Fmoc-Phe-\(\gamma\)[CH\(_2\)I] had two well separated peaks at R\(_t\) 16.5 and 17.1 min. This also confirmed that the samples were pure.

**SCHEME 3.11.**

In the subsequent study, chain elongation of dipeptidomimetics 3.3 on N-terminus was undertaken to obtain tripeptidomimetics containing two dithiocarbamate linkages in the peptidomimetic backbone. Starting with 3.3a, the amine was deprotected using 50% DEA in DCM. The solvent and excess DEA were removed under vacuum and the resulting amino-free dipeptidomimetic was isolated by trituration with ether. This, without further purification was treated with CS\(_2\), TEA and then N-Fmoc-Ala-\(\gamma\)[CH\(_2\)I] to afford N-Fmoc-protected tripeptidomimetic 3.4a possessing two dithiocarbamate groups (Scheme 3.12). Employing this protocol, four more dithiocarbamate-linked tripeptidomimetics 3.4b-e were prepared, isolated and were adequately characterized (Table 3.2).
Finally, the protocol was explored to describe two other types of dithiocarbamate-linked peptidomimetics starting from N-protected amino acid derived vicinal diamines 3.5. The amine precursors were prepared using known protocol.\textsuperscript{29,31} Separate procedures were followed based on the nature of N-protecting groups. To mention particularly, the \textit{N}-Fmoc amino alkyl amines were prepared by reducing the corresponding alkyl azides under catalytic hydrogenation, while \textit{N}-Boc and \textit{N}-Z amino alkyl amines were obtained through LiAlH\textsubscript{4} reduction of their nitriles (Scheme 3.13).
In the next stage, N-protected vicinal diamine 3.5 was treated with CS$_2$ in the presence of TEA to get corresponding dithiocarbamic acid intermediate which without isolation was reacted with either N-protected amino alkyl iodide 3.1 or bromo acetic acid ester 3.6 to afford dithiocarbamate-linked $N,N'$-orthogonally protected dipeptidomimetics 3.7a-c and simple dipeptidomimetics 3.8a-c respectively (Scheme 3.14, Table 3.3). All the compounds were isolated after a simple work-up as gummy solids and fully characterized using mass and NMR spectroscopic techniques.
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TABLE 3.3. Dithiocarbamate-linked dipeptidomimetics 3.7 and 3.8

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Dithiocarbamates</th>
<th>([\alpha]^{25}_D) c 1, CHCl_3</th>
<th>Yield (%)</th>
<th>HR-MS* Obsd (Calcd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.7a</td>
<td>BocNH-(\text{Ph})N(\text{H})N(\text{S})(\text{H})N(\text{Fmoc})</td>
<td>-46.8</td>
<td>72</td>
<td>670.2757 (670.2749)</td>
</tr>
<tr>
<td>3.7b</td>
<td>ZN(\text{H})N(\text{H})N(\text{S})(\text{H})N(\text{Fmoc})</td>
<td>-19.3</td>
<td>68</td>
<td>614.2119 (614.2123)</td>
</tr>
<tr>
<td>3.7c</td>
<td>Fmoc(\text{N})N(\text{H})N(\text{S})(\text{H})N(\text{HNZ})</td>
<td>-12.7</td>
<td>65</td>
<td>614.2133 (614.2123)</td>
</tr>
<tr>
<td>3.8a</td>
<td>BocNH-(\text{Ph})N(\text{H})N(\text{S})(\text{COOMe})</td>
<td>+29.7</td>
<td>62</td>
<td>421.1230 (421.1232)</td>
</tr>
<tr>
<td>3.8b</td>
<td>Fmoc(\text{N})N(\text{H})N(\text{S})(\text{COOEt})</td>
<td>+33.5</td>
<td>67</td>
<td>481.1220 (481.1232)</td>
</tr>
<tr>
<td>3.8c</td>
<td>ZN(\text{H})N(\text{H})N(\text{S})(\text{COOMe})</td>
<td>-8.2</td>
<td>70</td>
<td>421.1229 (421.1232)</td>
</tr>
</tbody>
</table>

*\([M+Na]^+\)  

In summary, a simple and mild protocol for the synthesis of a new class of dithiocarbamate-linked peptidomimetics starting from amino acid ester, CS_2, TEA and N-protected amino alkyl iodides is reported. The reaction is fast, high yielding and is devoid of the use of toxic reagents. The protocol is racemization free and all the synthesized compounds were isolated and well characterized. These dithiocarbamate linked dipeptidomimetics were subjected to chain extension on N-termini to afford tripeptidomimetics bearing two dithiocarbamate linkages. Also, the protocol was extended to prepare two more class of dipeptidomimetics starting from N-protected amino alkyl amines. With the increasing progress on the synthesis of new variety of peptidomimetics, the present work seems to get considerable attention.
3.3. EXPERIMENTAL

**Benzyloxy carbonyl amino acids (Z-amino acids)**

To a vigorously stirred solution of amino acid (10 mmol) in 4N NaOH (2.5 mL) and acetone (2.5 mL) at 0 °C, benzyloxy carbonyl chloride (1.8 mL, 10 mmol) was added in ten small portions during 2 h period maintaining the pH at 9-10 using 4N NaOH. Stirring was continued at 0 °C and at room temperature overnight. The reaction mixture was diluted with water (10 mL) and extracted with ether (3 x 50 mL). The aqueous phase was acidified with 6N HCl and the compound separated was extracted with EtOAc (3 x 20 mL). The organic extract was washed with water and saturated brine solution, dried over anhydrous Na$_2$SO$_4$ and the solvent removed *in vacuo*.

**tert-Butyloxy carbonyl amino acids (Boc-amino acids)**

A mixture of the amino acid (10 mmol), Boc-ON (11 mmol), Et$_3$N (13 mmol), dioxane (7 mL) and water (7 mL) was stirred at room temperature till it becomes homogeneous. Water (7 mL) was added to the reaction mixture which was then extracted with ether (3 x 50 mL). The aqueous phase was acidified with 10% KHSO$_4$ or citric acid solution and the Boc-amino acid was extracted into EtOAc (4 x 20 mL). The combined organic layer was washed with water (3 x 20 mL), dried over anhydrous Na$_2$SO$_4$ and evaporated *in vacuo*.
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General experimental for the preparation of N\textsuperscript{\textalpha}-protected aminols\textsuperscript{29}

![Chemical Reaction 1](image1)

To a stirred solution of N\textsuperscript{\textalpha}-protected amino acid (1.5 mmol) in dry THF at -10\degree C was added NMM (1.8 mmol) and ECF (1.8 mmol). After 10 min, the inorganics were filtered off and the filtrate was treated with moist NaBH\textsubscript{4} (2.0 mmol) at sub-zero temperature for 10-15 min. Excess water was added and the product was either collected by filtration or extracted with EtOAc.

General experimental for the synthesis of N\textsuperscript{\textalpha}-protected amino alkyl iodides 3.1\textsuperscript{29}

![Chemical Reaction 2](image2)

A solution of N\textsuperscript{\textalpha}-protected aminol (1 mmol) in dry DCM (10 mL) was added to a stirred mixture of PPh\textsubscript{3} (3 mmol), imidazole (5 mmol) and iodine (3 mmol) at rt and the reaction mixture was stirred for 6 h. It was concentrated and column chromatographed to afford the iodo derivative as white solid in excellent yield and purity.\textsuperscript{29}
3.3.1. Synthesis of dithiocarbamate-linked dipeptidomimetic 3.3

To a chilled solution of hydrochloride salt of an amino acid ester (3 mmol) and TEA (8 mmol) in dry THF (15 mL) was added CS₂ (6 mmol). After 10 min, a solution of N-protected amino alkyl iodide 3.1 (N²-Pg-CHR-[CH₂I], 4 mmol) in THF was added slowly and the reaction mixture was allowed to warm to rt gradually and stirred for 2 h. After completion of the reaction (TLC), it was evaporated under vacuum and the crude was partitioned between EtOAc (15 mL) and water. The organic layer was washed with citric acid solution (10%, 10 mL), water and brine. After drying over Na₂SO₄, the organic phase was concentrated and the crude was purified through column chromatography using EtOAc in hexane (10-15%) as eluent.

3.3.1.1. Fmoc-Val-[CH₂S-CS]-Phe-OMe 3.3a

Yield 72%; colorless gum.

^1^H NMR (300 MHz, CDCl₃) δ 0.93 (d, J = 7.1 Hz, 6H), 1.87 (m, 1H), 3.03 (d, J = 4.5 Hz, 2H), 3.52 (d, J = 4.3 Hz, 2H), 3.75 (s, 3H), 4.33-4.45 (m, 2H), 4.13 (t, J = 5.3 Hz, 1H), 4.23 (d, J = 6.2 Hz, 2H), 4.87 (d, J = 5.5 Hz, 1H), 5.22 (d, J = 5.7 Hz, 1H), 7.11-7.93 (m, 13H).

^1^C NMR (100 MHz, CDCl₃) δ 18.4, 19.3, 29.6, 38.1, 47.3, 52.3, 54.8, 55.8, 65.2, 66.6, 126.3, 126.6, 127.4, 128.3, 129.3, 129.4, 129.8, 136.4, 141.2, 143.5, 152.6, 165.2, 188.4.

IR ν max = 1738, 1689, 1120 cm⁻¹.
3.3.1.2. Fmoc-Ala-[CH$_2$S-CS]-Leu-OMe 3.3b

Yield 74%; gum.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.94 (d, $J = 6.3$ Hz, 6H), 1.30 (d, $J = 5.8$ Hz, 3H), 1.72-1.74 (m, 3H), 2.04 (m, 2H), 3.73 (s, 3H), 3.77 (m, 1H), 3.80 (m, 1H), 4.20 (t, $J = 4.8$ Hz, 1H), 4.39 (d, $J = 6.7$ Hz, 2H), 5.15 (br, 1H), 5.16 (br, 1H), 7.29-7.76 (m, 8H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 18.2, 19.9, 20.3, 23.4, 28.1, 40.1, 43.3, 47.5, 50.7, 52.5, 65.2, 126.6, 127.2, 127.9, 128.1, 141.2, 143.7, 154.6, 173.0, 195.3.

IR $\nu_{\text{max}}$ = 1741, 1696, 1119 cm$^{-1}$.

3.3.1.3. Fmoc-Pro-[CH$_2$S-CS]-Met-OMe 3.3c

Yield 65%; brownish gum.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.71 (m, 2H), 1.92 (m, 2H), 2.03 (s, 3H), 2.55 (m, 4H), 2.83 (d, $J = 5.5$ Hz, 2H), 3.72 (t, $J = 7.1$ Hz, 2H), 3.76 (s, 3H), 4.13 (t, $J = 4.8$ Hz, 1H), 4.23 (d, $J = 6.2$ Hz, 2H), 4.42 (m, 2H), 5.29 (br, 1H), 7.31-7.77 (m, 8H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 16.3, 21.4, 26.4, 29.2, 30.7, 33.7, 46.3, 47.5, 49.8, 50.9, 55.6, 65.4, 127.2, 127.7, 128.4, 128.7, 140.0, 141.3, 155.7, 172.4, 194.2.

IR $\nu_{\text{max}}$ = 1747, 1712, 1125 cm$^{-1}$.

3.3.1.4. Fmoc-Phg-$\psi$[CH$_2$S-CS]-Cys(Bzl)-OMe 3.3d

Yield 68%; pale liquid.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 3.46 (m, 1H), 3.75 (s, 2H), 3.80 (d, $J = 5.5$ Hz, 2H), 3.81 (m, 1H), 3.94 (s, 3H), 4.11 (t, $J = 4.6$ Hz, 1H), 4.25 (d, $J = 6.1$ Hz, 2H), 4.28 (d, $J = 3.2$ Hz, 2H), 6.62 (br, 2H), 6.81-7.66 (m, 18 H).
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$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 29.4, 36.1, 37.2, 39.3, 47.6, 51.4, 62.1, 62.1, 126.2, 126.3, 126.8, 127.3, 127.4, 128.3, 128.4, 128.8, 129.2, 129.4, 139.2, 140.3, 141.5, 142.7, 154.3, 173.4, 195.1.

IR $\nu_{\text{max}}$ = 1742, 1690, 1185 cm$^{-1}$.

3.3.1.5. Z-Phe-$\psi$(CH$_2$S-CS)-Gly-OEt 3.3e

Yield 70%; gummy solid.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.27 (t, $J$ = 2.7 Hz, 3H), 2.79 (d, $J$ = 6.5 Hz, 2H), 3.19 (d, $J$ = 5.2 Hz, 2H), 3.41 (m, 1H), 3.53 (s, 2H), 3.82 (m, 2H), 5.1 (s, 2H), 6.32-6.51 (br, 2H), 6.82-7.13 (m, 10H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.6, 37.2, 38.3, 42.1, 55.6, 62.9, 63.1, 126.1, 127.1, 127.6, 128.2, 128.9, 129.0, 140.8, 141.2, 155.6, 169.5, 201.3.

IR $\nu_{\text{max}}$ = 1737, 1696, 1165 cm$^{-1}$.

3.3.1.6. Z-Phe-$\psi$(CH$_2$S-CS)-Aib-OMe 3.3f

Yield 69%; gum.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.29 (s, 6H), 2.36 (d, $J$ = 7.1 Hz, 2H), 3.61 (s, 3H), 3.71 (d, $J$ = 6.6 Hz, 2H), 3.82 (m, 1H), 5.11 (s, 2H), 5.62 (br, 2H), 7.11-7.62 (m, 10H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 22.3, 39.6, 43.1, 51.3, 52.2, 60.1, 66.2, 127.1, 127.4, 127.6, 128.3, 128.8, 129.1, 140.1, 140.7, 154.3, 172.4, 192.3.

IR $\nu_{\text{max}}$ = 1738, 1693, 1179 cm$^{-1}$.

3.3.1.7. Fmoc-Leu-$\psi$(CH$_2$S-CS)-Phe-Ala-OMe 3.3g

Yield 62%; low melting solid.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.97 (d, $J$ = 5.8 Hz, 6H), 1.22-1.45 (m, 5H), 1.65 (m, 1H), 2.82
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(d, J = 6.1 Hz, 2H), 3.75 (s, 3H), 3.87-4.07 (m, 3H), 4.17-4.28 (m, 3H), 4.45 (m, 2H),
4.87-5.00 (brm, 1H), 5.21-5.45 (brm, 1H), 7.11-7.87 (m, 13H).

13C NMR (100 MHz, CDCl3) δ 17.1, 22.9, 29.3, 30.2, 36.5, 38.1, 41.2, 41.8, 45.6,
52.3, 55.3, 63.1, 126.0, 126.6, 126.7, 127.3, 127.9, 128.4, 128.8, 139.6, 141.2, 143.3,
153.2, 170.5, 172.1, 191.3.

IR νmax = 1738, 1712, 1160 cm⁻¹.

3.3.1.8.  (9H-fluoren-9-yl)methyl  (S)-3-phenyl-1-((R)-1-phenylethyl)
carbamothioylthio) propan-2-ylcarbamate (S, R)3.3h

Yield 77%; gum.

1H NMR (300 MHz, CDCl3) δ 1.36 (d, J = 6.0 Hz, 3H),
2.65-2.78 (m, 4H), 3.78 (m, 1H), 4.07 (m, 1H), 4.24-4.34 (m, 3H),
6.65 (br, 1H),
7.12-7.78 (m, 18H), 8.12 (m, 1H).

13C NMR (100 MHz, CDCl3) δ 19.3, 37.6, 42.1, 46.5, 53.3, 54.2, 65.3, 126.1, 126.5,
126.8, 127.5, 127.9, 128.3, 128.6, 128.8, 129.1, 138.3, 141.3, 142.1, 143.1, 154.9,
201.7.

HR-MS Calcd for C33H32N2NaO2S2 575.1803, found 575.1818 [M+Na]⁺

3.3.1.8.  (9H-fluoren-9-yl)methyl  (S)-3-phenyl-1-((S)-1-phenylethyl)
carbamothioylthio) propan-2-ylcarbamate (S, S)3.3h

Yield 80%; gum.

1H NMR (300 MHz, CDCl3) δ 1.39 (d, J = 6.0 Hz, 3H),
2.60 (d, J = 5.5 Hz, 2H), 2.78 (m, 2H), 3.78 (m, 1H), 4.10 (m, 1H), 4.17 (t, J = 3.8 Hz,
1H), 4.34 (d, J = 4.9 Hz, 2H), 6.54-6.78 (br, 2H), 7.12-7.67 (m, 18H), 8.23 (m, 1H).
3.3.2. Synthesis of dithiocarbamate-linked tripeptidomimetics 3.4

*N*-Fmoc-protected dithiocarbamate linked dipeptidomimetic 3.3 (0.2 g) was stirred in a mixture of dry DCM/DEA (1:1, 8 mL) for 1 h. After the complete removal of Fmoc group (TLC), the reaction mixture was concentrated and the residue was triturated with ether to remove the non-polar impurities. In the subsequent step, the free amino dipeptidomimetic was treated with CS₂, TEA and an N-protected amino alkyl iodide 3.1 as described previously to obtain tripeptidomimetic 3.4 containing two dithiocarbamate moieties. It was isolated pure by column chromatography.

**3.3.2.1 Fmoc-Ala-\textsuperscript{γ}[[CH\textsubscript{2}S-CS]-Val-\textsuperscript{γ}[[CH\textsubscript{2}S-CS]-Phe-OMe 3.4a**

Yield 58%; gummy solid.

\[^{1}\text{H} \text{NMR} (300 \text{ MHz, CDCl}_3) \delta \ 0.96 (d, J = 5.7 \text{ Hz, } 6\text{H}), 1.27 (d, J = 7.1 \text{ Hz, } 3\text{H}), 1.82 (m, 1H), 2.87 (d, J = 5.6 \text{ Hz, } 2\text{H}), 3.55 (s, 3H), 3.62 (m, 1H), 3.82-3.94 (m, 6H), 4.17 (t, J = 3.3 \text{ Hz, } 1\text{H}), 4.32 (d, J = 6.6 \text{ Hz, } 2\text{H}), 5.82 (br, 2H), 6.11 (br, 1H), 7.32-7.89 (m, 13H).**

\[^{13}\text{C} \text{NMR} (100 \text{ MHz, CDCl}_3) \delta \ 16.8, 18.3, 29.3, 33.1, 35.1, 38.2, 45.3, 45.6, 53.7, 54.3, 58.2, 65.2, 126.9, 127.3, 127.5, 128.0, 128.5, 128.7, 129.0, 139.2, 142.1, 144.3, 154.1, 170.8, 192.6, 195.1.**

HR-MS Calcd for C\textsubscript{35}H\textsubscript{41}N\textsubscript{3}NaO\textsubscript{4}S\textsubscript{7} 718.1878, found: 718.1870 [M+Na]⁺.

IR \text{ v}_{\text{max}} = 1752, 1712, 1075, 1110 \text{ cm}⁻¹.
3.3.2.2. Fmoc-Phe-\textsuperscript{$\varphi$}[CH\textsubscript{2}S-CS]-Ala-\textsuperscript{$\varphi$}[CH\textsubscript{2}S-CS]-Leu-OMe \textbf{3.4b}

Yield 61%; gummy solid.

\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta\) 0.95 (d, \(J = 5.2\) Hz, 6H), 1.65 (m, 1H), 1.28 (d, \(J = 5.8\) Hz, 3H), 1.87 (m, 2H), 2.48 (d, \(J = 5.7\) Hz, 2H), 2.51-2.67 (m, 4H), 3.58 (s, 3H), 3.71-3.92 (m, 3H), 4.17-4.32 (m, 3H), 5.92 (br, 1H), 6.11 (br, 2H), 7.25-7.79 (m, 13H).

\textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 17.1, 17.4, 31.2, 32.5, 35.4, 40.7, 44.1, 45.6, 48.6, 49.8, 52.1, 65.8, 68.8, 126.6, 127.3, 127.5, 128.0, 128.3, 128.6, 129.1, 139.6, 140.7, 142.3, 160.2, 173.4, 192.5, 193.2.

ESI-MS Calcd for C\textsubscript{36}H\textsubscript{36}N\textsubscript{3}NaO\textsubscript{4}S\textsubscript{4} 732.2, found: 732.65 [M+Na]\textsuperscript{+}.

IR \(\nu_{\text{max}}\) = 1752, 1708, 1085, 1101 cm\textsuperscript{-1}.

3.3.2.3. Fmoc-Val-\textsuperscript{$\varphi$}[CH\textsubscript{2}S-CS]-Gly-\textsuperscript{$\varphi$}[CH\textsubscript{2}S-CS]-Met-OMe \textbf{3.4c}

Yield 55%.

\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta\) 1.00 (d, \(J = 4.2\) Hz, 6H), 2.05 (s, 3H), 2.58-3.64 (m, 4H), 2.81 (m, 1H), 3.77 (s, 3H), 4.13-4.27 (m, 3H), 4.52-4.62 (m, 2H), 4.71-5.00 (m, 6H), 6.3 (br, 2H), 6.31 (d, \(J = 5.2\) Hz, 1H), 7.32-7.87 (m, 8H).

\textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 15.5, 17.8, 29.4, 29.8, 32.3, 36.2, 39.7, 42.4, 43.2, 53.6, 55.3, 58.3, 65.4, 126.9, 127.6, 128.2, 128.8, 141.1, 142.2, 156.3, 167.8, 199.7, 203.1.

HR-MS Calcd for C\textsubscript{30}H\textsubscript{39}N\textsubscript{3}NaO\textsubscript{4}S\textsubscript{4} 688.1442, found: 688.1434 [M+Na]\textsuperscript{+}.

IR \(\nu_{\text{max}}\) = 1742, 1710, 1072 cm\textsuperscript{-1}.
3.3.2.4. Fmoc-Leu-$\psi$(CH$_2$S-CS)-Pro-$\psi$(CH$_2$S-CS)-Met-OMe 3.4d

Yield 60%; gummy solid.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.00 (d, $J$ = 7.2 Hz, 6H), 1.28 (m, 2H), 1.58 (m, 1H), 1.62-1.67 (m, 4H), 2.19 (s, 3H), 2.62-2.66 (m, 4H), 2.82 (m, 2H), 3.41 (m, 4H), 3.81 (s, 3H), 4.07 (t, $J$ = 3.3 Hz, 1H), 4.21 (d, $J$ = 6.5 Hz, 2H), 4.33 (m, 2H), 5.10 (m, 1H), 6.82 (br, 2H), 7.11-7.32 (m, 8H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.7, 19.2, 21.6, 21.6, 28.7, 28.8, 31.3, 33.2, 37.1, 37.2, 43.6, 45.3, 49.2, 53.4, 54.6, 55.6, 65.5, 126.3, 128.5, 128.6, 129.1, 140.7, 142.1, 153.6, 170.3, 198.3, 203.5.

HR-MS Caled for C$_{34}$H$_{45}$N$_3$NaO$_4$S$_7$ 742.1911, found 742.1926 [M+Na$^+$].

IR $\nu_{\text{max}}$ = 1734, 1709, 1062 cm$^{-1}$.

3.3.2.5. Fmoc-Ile-$\psi$(CH$_2$S-CS)-Ala-$\psi$(CH$_2$S-CS)-Leu-OMe 3.4e

Yield 53%, gum.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.96-1.06 (m, 12H), 1.10 (m, 1H), 1.25 (m, 2H), 1.65 (d, $J$ = 7.1 Hz, 3H), 1.69 (m, 2H), 1.90 (m, 1H), 3.23-3.33 (m, 3H), 3.74 (s, 3H), 3.81-4.03 (m, 4H), 4.17-4.29 (m, 3H), 6.82 (br, 1H), 6.91 (br, 2H), 7.13-7.52 (m, 8H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 11.3, 14.3, 19.3, 22.9, 25.1, 27.6, 30.2, 32.6, 36.3, 41.8, 44.8, 50.3, 53.7, 54.0, 55.6, 65.3, 67.8, 126.8, 127.6, 127.7, 128.5, 139.8, 140.3, 153.5, 172.3, 197.4, 199.8.

HR-MS Caled for C$_{33}$H$_{45}$N$_3$NaO$_4$S$_4$ 698.2191, found 698.2181 [M+Na$^+$].
General experimental for the preparation of N⁶-Fmoc amino alkyl amines 3.5²⁹

\[
\text{Fmoc-NH} \xrightarrow{\text{NaN₃, DMF}} \text{Fmoc-NH} \xrightarrow{\text{Pd/C, H₂, MeOH}} \text{FmocNH} \xrightarrow{\text{3.5 NH₃}} \text{NH₂}
\]

To a solution of N⁶-Fmoc-amino alkyl iodide 3.1 (1 mmol) in DMF, sodium azide (2 mmol) was added and the reaction mixture was stirred for 3 h at rt. After the completion of reaction (TLC), the azido product was extracted into EtOAc. The resulting N⁶-Fmoc-amino alkyl azide (1 mmol) was subjected to catalytic hydrogenation using Pd/C-H₂ to afford corresponding amine 3.5 in good yield.²⁹

General experimental for the preparation of N⁶-Z/Boc-amino alkyl amines 3.5²⁹

\[
\text{PgNH} \xrightarrow{\text{COOH}} \xrightarrow{\text{1. NMM ECF, 2. aq. NH₃}} \text{PgNH} \xrightarrow{\text{CONH₂, POCl₃/Py, dry DCM}} \text{PgNH} \xrightarrow{\text{CN \xrightarrow{\text{LiAlH₄}}} \text{PgNH} \xrightarrow{\text{3.5 NH₃}} \text{NH₂}}
\]

N⁶-Z/Boc-amino acids were first converted to corresponding amides and then dehydrated to their nitriles using POCl₃/Py. In the next step, an ice-cold solution of N⁶-Z/Boc-amino alkyl nitrile (1 mmol) in dry THF (8 mL) was treated with LiAlH₄ (1.5 mmol) for 2 h. After the completion of reaction, excess reagent was quenched with aq. NH₄Cl and the amino product 3.5 was isolated through a simple work-up.²⁹

3.3.3. Synthesis of N,N'-orthogonally protected dithiocarbamate linked dipeptidomimetic 3.7

\[
\text{CS₂, TEA, THF, 0 °C-rt} \xrightarrow{\text{3.5, 3.1 PG₁-NH}} \text{NHPG₂}
\]
To a chilled solution of N-protected amino alkyl amine 3.5 (N°-Pg-CHR-\(\psi\)[CH\(_2\)NH\(_2\)], 1 mmol) and TEA (2.5 mmol) in dry THF (8 mL) was added CS\(_2\) (2 mmol). After 10 min, a solution of N-protected amino alkyl iodide 3.1 (N°-Pg-CHR-\(\psi\)[CH\(_2\)I], 1.1 mmol) in THF was added slowly and the reaction mixture was allowed to warm to rt gradually and stirred for 2 h. It was worked up as described in section 3.3.1 and column chromatographed.

### 3.3.3.1. Boc-Phe-\(\psi\)[CH\(_2\)NH-CS-S-CH\(_2\)CH(i-Bu)-NH-Fmoc] 3.7a

Yield 72%.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 0.93 (d, \(J = 5.2\) Hz, 6H), 1.25 (s, 9H), 1.77 (m, 3H), 2.48-2.53 (m, 4H), 2.61 (d, \(J = 4.9\) Hz, 2H), 3.48 (m, 1H), 3.51 (m, 1H), 4.06 (t, \(J = 4.2\) Hz, 1H), 4.31 (d, \(J = 7.7\) Hz, 2H), 6.47 (br, 2H), 7.11-7.82 (m, 13H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 19.7, 23.1, 27.3, 38.7, 39.8, 41.5, 46.7, 47.7, 48.9, 51.3, 65.2, 81.2, 127.3, 127.5, 127.6, 128.2, 128.4, 128.9, 129.1, 141.6, 142.2, 143.4, 155.7, 156.3, 194.2.

IR \(v_{\text{max}}\) = 1712, 1696, 1059 cm\(^{-1}\).

### 3.3.3.2. Z-Ala-\(\psi\)[CH\(_2\)NH-CS-S-CH\(_2\)CH(i-Pr)-NH-Fmoc] 3.7b

Yield 68%, gum.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 0.87 (d, \(J = 7.1\) Hz, 6H), 1.21 (d, \(J = 6.2\) Hz, 3H), 1.62 (m, 1H), 3.63-3.71 (m, 2H), 3.85 (dd, \(J = 4.1\) Hz, 2H), 4.01 (d, \(J = 5.5\) Hz, 2H), 4.22-4.42 (m, 3H), 5.00 (s, 2H), 6.31-6.42 (br, 2H), 6.71 (br, 1H), 7.19-7.55 (m, 13H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 16.9, 18.3, 27.2, 35.2, 38.1, 42.1, 48.7, 51.4, 66.3, 68.3, 126.5, 127.2, 127.5, 127.8, 128.1, 128.8, 129.0, 139.3, 142.0, 143.6, 151.4, 154.4, 194.6.
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IR $\nu_{\text{max}} = 1710, 1696, 1052$ cm$^{-1}$.

3.3.3.3. Fmoc-Ile-$\psi[\text{CH}_2\text{NH-CS-SC}_2\text{H}_4\text{-NH-Z}]$ 3.7c

Yield 65%, colorless gum.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.93-1.10 (m, 6H), 1.15 (m, 1H), 1.25 (m, 2H), 3.37-3.52 (m, 4H), 3.41 (m, 1H), 3.74 (m, 1H), 4.11 (t, $J$ = 4.5 Hz, 1H), 4.36 (d, $J$ = 6.5 Hz, 2H), 5.11 (s, 2H), 6.30-6.36 (br, 2H), 6.62 (br, 1H), 7.22-7.67 (m, 13H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 11.3, 14.3, 25.1, 32.3, 37.2, 40.3, 43.1, 43.7, 53.1, 62.3, 66.6, 126.3, 127.2, 127.4, 127.7, 128.3, 128.6, 129.0, 138.9, 142.1, 144.0, 153.2, 155.4, 198.3.

IR $\nu_{\text{max}} = 1716, 1701, 1042$ cm$^{-1}$.

3.3.4. Synthesis of dithiocarbamate linked dipeptidomimetic 3.8

To a chilled solution of N-protected amino alkyl amine 3.5 (N$^\alpha$-Pg-CHR-$\psi[\text{CH}_2\text{NH}_2]$, 1 mmol) and TEA (2.5 mmol) in dry THF (8 mL) was added CS$_2$ (2 mmol). After 10 min., a solution of bromo acetic acid ester (1.1 mmol) in THF was added slowly and the reaction mixture was allowed to warm to rt gradually and stirred for 2 h. It was worked up as described in section 3.3.1 and column chromatographed.

3.3.4.1. Boc-Phe-$\psi[\text{CH}_2\text{NH-CS-SCH}_2\text{COOMe}]$ 3.8a

Yield 62%; gum.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.31 (s, 9H), 2.62 (m,
2H), 2.85 (d, $J = 6.2$ Hz, 2H), 3.42 (s, 2H), 3.56 (m, 1H), 3.81 (s, 3H), 6.52-6.62 (br, 2H), 6.91-7.11 (m, 5H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 29.1, 35.6, 39.3, 44.9, 51.6, 52.3, 80.1, 127.2, 127.8, 128.1, 139.6, 154.3, 167.2, 195.6.

IR $v_{\text{max}}$ = 1740, 1689, 1154 cm$^{-1}$.

### 3.3.4.2. Fmoc-Ala-$\psi$(CH$_2$NH-CS-SCH$_2$COEt) 3.8b

Yield 67%; gum.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.21 (d, $J = 6.5$ Hz, 3H), 1.31 (t, $J = 4.5$ Hz, 3H), 3.65 (s, 2H), 3.72-3.81 (m, 3H), 3.86 (m, 2H), 4.11 (t, $J = 4.3$ Hz, 1H), 4.23 (d, $J = 5.5$ Hz, 2H), 6.82 (br, 2H), 7.22-7.46 (m, 8H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 13.6, 17.9, 36.4, 37.4, 47.2, 49.3, 63.1, 65.3, 126.7, 127.6, 127.9, 128.8, 139.9, 140.6, 153.2, 167.5, 196.4.

### 3.3.4.3. Z-Leu-$\psi$(CH$_2$NH-CS-SCH$_2$COOMe) 3.8c

Yield 70%; gum.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.01 (d, $J = 5.8$ Hz, 6H), 1.62 (m, 2H), 1.77 (m, 1H), 2.61 (m, 2H), 3.41 (m, 1H), 3.42 (s, 3H), 3.65 (s, 2H), 5.07 (s, 2H), 6.37 (br, 2H), 6.92-7.34 (m, 5H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 19.6, 22.8, 37.2, 41.7, 43.4, 51.2, 53.1, 63.4, 127.3, 127.7, 128.6, 140.8, 154.6, 166.8, 191.5.

IR $v_{\text{max}}$ = 1734, 1689, 1147 cm$^{-1}$. 
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$^1$H NMR of Fmoc-Val-$\psi$(CH$_2$S-CS)-Phe-OMe 3.3a

$^{13}$C NMR of Fmoc-Val-$\psi$(CH$_2$S-CS)-Phe-OMe 3.3a
HR-MS of Fmoc-Val-$\psi$(CH$_2$S-CS)-Phe-OMe 3.3a

HR-MS of Fmoc-Phg-$\psi$(CH$_2$S-CS)-Cys(Bzl)-OMe 3.3d
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\[ \text{H NMR of Fmoc-Leu-\(\psi\)(CH}_2\text{S-CS})\text{-Phe-Ala-OMe 3.3g} \]

\[ \text{Chromatogram of Fmoc-Ala-\(\psi\)(CH}_2\text{S-CS})\text{Val-\(\psi\)(CH}_2\text{S-CS})\text{-Phe-OMe 3.4a} \]

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HR-MS of Fmoc-Leu-$\psi$(CH$_2$S-CS)-Pro-$\psi$(CH$_2$S-CS)-MetOMe 3.4d

HR-MS of Z-Ala-$\psi$(CH$_2$NH-CS-SCH$_2$CH(i-Pr)-NHFmoc] 3.7b
3.4. REFERENCES


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