CHAPTER 3

Synthesis of Thioureido Linked Peptidomimetics, Neoglycoconjugates and Glycosylated Amino Acids Employing Bis(benzotriazolyl)methanethione

3.1. Introduction

Peptidomimetics and glycomics are known to exhibit improved therapeutical and biological properties. Their ability to sustain enzymatic hydrolysis due to the presence of unnatural linkages provides them longer shelf life while the better solubility in the biological system helps to deliver the desired drug action effectively. Among various backbone modifications explored so far, several classes of pseudopeptides and neoglycoconjugates have been developed by inserting urea, sulfonamide, phosphoramid, carbamate functionalities and also several heterocycles (tetrazole, triazole, oxadiazole, thiazole) as peptide bond surrogates. Among them, the ureidopeptides and oligoureas have gained special interest due to their significance in medicinal chemistry. Similarly, thioureido (Figure 3.1) linkage has also received greater attention because of its interesting biological as well as structural aspects.

![Figure 3.1](image_url)

The ability to provide hydrogen bond donor and acceptor points makes it an efficient anion receptor and serves as building block in the construction of supramolecular structures. A number of thioureido derivatives have been reported to exhibit marked anti-arrhythmic, anti-viral, anti-cancer activities. Their utility as non-nucleoside inhibitors of HIV-1, HIV-2 RT, antagonists for the human NK1 tachykinin receptor, influenza virus and potential anti-TB
therapeutic agents\textsuperscript{22} is also documented (Figure 3.2).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{molecules.png}
\caption{Thiourea substituted biologically potent molecules}
\end{figure}

The -NHCSNH- moiety has been widely employed to bridge carbohydrates and related glycoconjugates,\textsuperscript{23,24} which are used in tailoring oligonucleoside analogues. Thiourea linkage is the key structural unit in many organocatalysts being explored in the asymmetric synthesis in a wide variety of reactions (Figure 3.3).\textsuperscript{25} Further, the thiourea-linked glycooligomers have been employed as phosphate binders in water,\textsuperscript{26} and in designing the mimics of natural oligosaccharides.\textsuperscript{27}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{catalysts.png}
\caption{Thiourea based organocatalysts}
\end{figure}

Several strategies are available for the synthesis of symmetrical and unsymmetrical thioureas. The reaction of two equivalents of a primary or secondary amine with thiophosgene is the earliest method of preparing symmetrical thioureas.\textsuperscript{28} The unpleasant nature of thiophosgene could be avoided by using thiophosgene equivalents such as 1,1'-thiocarbonyldiimidazole.\textsuperscript{29,30} Reaction of isothiocyanates with primary or secondary amines is another method available for
preparing unsymmetrical thioureas.\textsuperscript{31-33} But, the protocol suffers from the side reactions like urethane formation (in alcoholic medium where the reaction is often carried out) or exchange between the amine and isothiocyanates.\textsuperscript{34} Many isothiocyanates are tedious to prepare and display poor long term stability. Hence, numerous alternative methods have been developed for the synthesis of both symmetrical and unsymmetrical thioureas avoiding the use of isothiocyanates. Some of the protocols developed for the preparation of \(N,N'\)-disubstituted symmetrical thioureas are depicted in the scheme 3.1.

![Scheme 3.1: Literature methods for the preparation of symmetrical thioureas](image)

**SCHEME 3.1. Literature methods for the preparation of symmetrical thioureas**

Maddani \textit{et al.},\textsuperscript{41} reported the synthesis of substituted thioureas by the reaction of primary amines with molybdenum dialkyl dithiocarbamate. To a solution of primary alkyl or aryl amines in toluene, 0.5 equiv. of molybdenum xanthate was added and the resulting reaction mixture was refluxed for about 4 h to obtain the corresponding thioureas in moderate yields (\textbf{Scheme 3.2}).
Mohanta *et al.*,\(^4^2\) used 1-(methylthiocarbonyl)imidazole and its \(N\)-methyl quaternary salts for the preparation of unsymmetrical thioureas. The equimolar mixture of primary amines and 1-(methylthiocarbonyl)imidazole was refluxed in ethanol for about 6 h to afford the corresponding thiocarbamate intermediate. The reaction was continued further by adding one more equivalent of an amine and refluxed for about 6 h to yield the desired thioureas. In this reaction, the imidazole reagent acts as thiocarbonyl transfer agent *via* the formation of methylthiocarbonyl intermediate (Scheme 3.3).

Sugimoto\(^4^3\) employed 2-halo-3-alkyl-4-phenylthiazolium salt for the activation of dithiocarbamate salt, which facilitates the formation of thioureas by nucleophilic displacement with amines. Initially, secondary amine was converted into the corresponding dithiocarbamate salt in the presence of \(\text{CS}_2\) and a base in an appropriate solvent such as THF or MeCN. The activated dithiocarbamate (after the addition of onium salt) undergoes nucleophilic substitution with amines leading to the corresponding unsymmetrical thioureas, but the yields were moderate (Scheme 3.4).
SCHEME 3.4.

Ramadas et al.,\textsuperscript{44} demonstrated the synthesis of $N,N'$-disubstituted unsymmetrical thioureas from symmetrical thioureas. The formation of unsymmetrical thioureas was unexpected as the reagent diphenylthiourea was originally meant to obtain $N,N'$-substituted guanidines. But the reagent underwent successive nucleophilic substitutions in the presence of triethylamine (TEA) with two different amines to form unsymmetrical thioureas (Scheme 3.5).

SCHEME 3.5.

Xian et al.,\textsuperscript{45} obtained $N$-mono- or di-alkyl substituted thioureas readily by the reaction of $N$-nitroso-1,3-dimethylthiourea (DMNT) and $N$-nitroso-trimethylthiourea (TMNT) from a series of alkyl amines. Because of the high bond energies of the N-NO bond, the reaction proceeds \textit{via} direct nucleophilic substitution with amines (Scheme 3.6).

SCHEME 3.6.

Shusheng et al.,\textsuperscript{46} reported the synthesis of novel glycosyl thiourea derivatives, which were found to possess potential antitumor activity. The method involves the preparation of per-$O$-acetylated glycosyl isothiocyanates from the corresponding sugar amines, and coupling with heterocyclic hydrazide to obtain thiourea-linked pseudonucleosides (Scheme 3.7).
There are very few reports regarding the preparation of thioureidopeptides. Nowick et al.,\textsuperscript{47} reported the synthesis of thioureidopeptides by the reaction of peptide ester isothiocyanates with \textit{N}-ethylaniline. Isothiocyanates in turn were prepared by the addition of a solution of thiophosgene in toluene to a solution of a hydrochloride salt of peptide ester in an ice-cooled mixture of DCM and saturated aqueous NaHCO\textsubscript{3} solution (Scheme 3.8).

Boas et al.,\textsuperscript{48} carried out the solid phase synthesis of thioureas using peptide coupling agents. In this approach isothiocyanates anchored on solid support were coupled with different amines. Initially, Fmoc-amino acid was loaded on to the resin followed by the removal of Fmoc group. The resulting resin bound free amine was treated with CS\textsubscript{2}, \textit{N,N}-diisopropylethylamine (DIEA) and a coupling agent \textit{[O-(benzotriazole-1-yl)-\textit{N},\textit{N},\textit{N}',\textit{N}'-tetramethyluronium hexafluorophosphate (HBTU)] or \textit{O-(7-azabenzotriazol-1-yl)-\textit{N},\textit{N},\textit{N}',\textit{N}'-tetramethyluronium hexafluorophosphate (HATU)]} to obtain the corresponding isothiocyanate intermediate, which was then reacted with an amine to obtain the thioureas. Finally, treatment of thioureidyl resin with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) gave the thioureas (Scheme 3.9).
Katritzky and co-workers have demonstrated the applications of bis(benzotriazolyl)methanethione (Bt-CS-Bt), as a thiocarbonyl transfer reagent for the synthesis of alkyl and arylthiocarbonyl benzotriazoles. The intermediate 1-(alkyl/arylthiocarbamoyl)benzotriazole formed by the replacement of one Bt-group from Bt-CS-Bt acts as masked isothiocyanate equivalent which on treating with an amine affords the substituted thiourea (Scheme 3.10).\(^4^9\)

Generally, benzotriazole acts as a good synthetic auxiliary which offers many advantages. First, it can be easily removed at the end of the synthetic sequence and also can be recovered and reused. Second, it is easy to introduce readily at the beginning of the sequence. Third, it is stable during various synthetic operations and sometimes exerts an activating influence on other part of the molecule (Scheme 3.11). Further, it is an inexpensive, stable solid, soluble in ethanol, benzene, toluene, chloroform, DMF, and sparingly soluble in water.
As a leaving group

\[
\begin{align*}
\text{X} & \text{R} & \text{Bt} \\
\text{R'} & \text{MgX} & \text{X} & \text{R'} \\
\text{R} & \text{Bt} & \text{R'} & \text{Bt} \\
\end{align*}
\]

As a proton activator

\[
\begin{align*}
\text{R} & \text{Bt} & \text{BuLi} & \text{Li} & \text{R'} \\
\text{Br} & \text{R'} & \text{Bt} & \text{Bt} \\
\end{align*}
\]

As a cation stabilizer

\[
\begin{align*}
\text{Y} & \text{Bt} & \text{ArH} & \text{Ar} & \text{Bt} \\
\text{R} & \text{Bt} & \text{R'} & \text{Bt} \\
\end{align*}
\]

As an anion precursor

\[
\begin{align*}
\text{R} & \text{Bt} & \text{2e}^- & \text{CH}_2 & \text{Bt} \\
\text{R'} & \text{C} & \text{Q} & \text{R'} & \text{Bt} \\
\end{align*}
\]

As a radical precursor

\[
\begin{align*}
\text{R} & \text{Bt} & \text{e}^- & \text{CH}_2 & \text{Bt} & \text{trapped} \\
\text{R} & \text{Bt} & \text{Bt} \\
\end{align*}
\]

SCHEME 3.11.

In continuation of our interest in the synthesis of ureidopeptidomimetics, a facile and convenient protocol for the synthesis of thioureidopeptides, thiourea tethered glycosylated amino acids and neoglycoconjugates was developed. Many of these findings are described in this part of the thesis. The protocol employs a step-wise replacement of benzotriazoly1 unit of bis(benzotriazoly1)methanethione 3 with a suitably protected amino acid or carbohydrate derived amine.

3.2. Present Work

Bis(benzotriazoly1)methanethione (Bt-CS-Bt) 3 was prepared by the reaction of trimethylsilyl benzotriazole with thiophosgene. The required trimethylsilyl derivative 2 was synthesized from benzotriazole 1 and hexamethyldisilazane (HMDS) according to the Birkofer procedure (Scheme 3.12).50 We then initiated our studies on the synthesis of thioureidopeptides
possessing both N and C terminals. A peptidomimetic with N and C termini, similar to the natural peptides, will enable further chain extensions/modifications. For such molecular

![Scheme 3.12. Preparation of bis(benzotriazolyl) methanethione](image)

**SCHEME 3.12. Preparation of bis(benzotriazolyl)methanethione**

assembly, one of the contributing amine has to be derived from an N-protected amino acid through suitable carboxy modification, and the amino acid ester is an obvious choice as another substrate. Initially for the synthesis of required monoprotected diamine \(8\), Fmoc-\(\alpha\)-amino acid \(4\) was converted to the corresponding alcohol \(5\) by sodium boro hydride (NaBH\(_4\))\(^{51}\) reduction of its mixed anhydride prepared using \(N\)-methylmorpholine (NMM) and ethyl chloroformate (ECF). The hydroxy group was transformed to azide \(7\) via the iodo intermediate \(6\) under Mitsunobu conditions employing triphenylphosphine (PPh\(_3\)), imidazole and iodine (I\(_2\)).\(^{52}\) On subjecting the \(N\)-Fmoc-\(\alpha\)-amino alkyl azide \(7\) to catalytic hydrogenation using Pd/C, the corresponding amine \(8\) was obtained in good yield and purity. Several amines were isolated as HCl salts by the addition of CHCl\(_3\) to the reaction mixture (Scheme 3.13).\(^{53}\)

![Scheme 3.13.](image)

**SCHEME 3.13.**

In the case of tert-butyloxy carbonyl (Boc) and benzyloxy carbonyl (Z) compounds, monoprotected Z/Boc-vicinal diamines \(15\) and \(16\) were prepared by reducing the \(N\)-Z/Boc-protected \(\alpha\)-amino nitriles \(13\) and \(14\) using lithium aluminium hydride (LiAlH\(_4\)) (Scheme 3.14).\(^{54}\)
The nitriles in turn have been prepared by the dehydration of corresponding Z/Boc-amino acid amides employing trifluoroacetic anhydride (TFAA) and pyridine.\textsuperscript{55}

\begin{center}
\begin{align*}
\text{Z/BocH}_2\text{N}&-\text{OH} \xrightarrow{\text{(Boc)}_2\text{O, pyridine, NH}_4\text{HCO}_3} \text{Z/BocH}_2\text{N} \xrightarrow{\text{rt, 5 h}} \text{Z/BocH}_2\text{N} \\
&\text{NH}_2 \xrightarrow{\text{TFAA, pyridine}} \text{Z/BocH}_2\text{N} \xrightarrow{\text{DCM, 0 °C, 3 h}} \text{Z/BocH}_2\text{N} \\
\text{LiAlH}_4 &\xrightarrow{\text{THF, 0 °C}} \text{Z/BocH}_2\text{N} \xrightarrow{\text{NH}_2} \text{Z/BocH}_2\text{N} \\
&\text{NH}_2 \\
\end{align*}
\end{center}

**SCHEME 3.14.**

In the following step towards thioureidopeptides 21, we undertook tandem nucleophilic replacement of the Bt groups of 3 with the amines in hand. Thus, a reaction of monoprotected Fmoc/Boc/Z-amino alkyl amine 8, 15 and 16 with Bt-CS-Bt at room temperature yielded the corresponding monosubstituted thiocarbohydronbenzotriazoles 17 in yields greater than 80\% (Scheme 3.15, Table 3.1).

**SCHEME 3.15. Preparation of monosubstituted thiocarbohydronbenzotriazoles**

Employing a similar approach, an amino acid ester 18 was reacted with 3 to afford monosubstituted thiocarbohydronbenzotriazoles 19. The isolated monosubstituted thiocarbohydronbenzotriazoles 17 and 19 were further utilized for the synthesis of unsymmetrical thioureas. It seemed possible because replacement of the first Bt moiety by an amine nucleophile takes place readily at room temperature whereas the second Bt group gets replaced by another nucleophile only in the presence of a base. All derivatives 17 and 19 were isolated as crystalline
solids and were adequately characterized. They were found to be stable and could be stored at ambient temperature for long periods without any decomposition.

**TABLE 3.1. Monosubstituted thiocarboxylbenzotriazoles**

<table>
<thead>
<tr>
<th>entry</th>
<th>monosubstituted thiocarboxylbenzotriazoles</th>
<th>mp (°C)</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17a</td>
<td><img src="image" alt="Structure 17a" /></td>
<td>112-114</td>
<td>89</td>
</tr>
<tr>
<td>17b</td>
<td><img src="image" alt="Structure 17b" /></td>
<td>72-74</td>
<td>92</td>
</tr>
<tr>
<td>17c</td>
<td><img src="image" alt="Structure 17c" /></td>
<td>110-112</td>
<td>87</td>
</tr>
<tr>
<td>17d</td>
<td><img src="image" alt="Structure 17d" /></td>
<td>98-100</td>
<td>90</td>
</tr>
<tr>
<td>17e</td>
<td><img src="image" alt="Structure 17e" /></td>
<td>108-110</td>
<td>82</td>
</tr>
<tr>
<td>17f</td>
<td><img src="image" alt="Structure 17f" /></td>
<td>126-128</td>
<td>91</td>
</tr>
<tr>
<td>19a</td>
<td><img src="image" alt="Structure 19a" /></td>
<td>87-89</td>
<td>89</td>
</tr>
<tr>
<td>19b</td>
<td><img src="image" alt="Structure 19b" /></td>
<td>78-80</td>
<td>89</td>
</tr>
</tbody>
</table>

In the next step, isothiocyanate equivalents 17a-f and 19a-b were further substituted. Thus, a reaction of 17a with β-glycine methyl ester 20 in the presence of DIEA afforded the desired thioureidopeptide 21a (Scheme 3.16, Table 3.2). The N-Fmoc thioureidopeptides 21a-c
were obtained as low melting solids, whereas those of Boc and Z compounds 21d-f were gummy products which were characterized using IR, NMR, and mass spectrometry.

![Reaction Scheme](image)

**SCHEME 3.16.**

In a similar approach, the reaction of valyl methyl ester with 19a and alanyl methyl ester with 19b in the presence of DIEA afforded unsymmetrical and symmetrical thioureidopeptides 21g and 21h respectively. Further, a reaction of 2,3,4,6-tetra-O-acetyl-β-D-glucosyl-1-amine 22 with Boc-Phe-ψ[CH₂NHCSBt] 17f yielded thiourea linked glycosylated amino acid 21i in good yield and purity (Table 3.2).

The absence of racemization during the course of the reaction sequence was verified through ¹H NMR analysis of the unsymmetrical thioureas prepared by the reaction of Fmoc-Phe-ψ[CH₂NHCSBt] with optically pure R and S-1-phenylethylamine. The methyl protons of the phenylethylamine moiety in Fmoc-Phe-ψ[CH₂NHCSNH]-R-(+)-1-phenylethylamine and Fmoc-Phe-ψ[CH₂NHCSNH]-S-(−)-1-phenylethylamine were observed as distinct doublets at δ 1.30, 1.32 and δ 1.29, 1.31, respectively. For Fmoc-Phe-ψ[CH₂NHCSNH]-R,S-(±)-1-phenylethylamine, the corresponding methyl resonances were observed as two doublets at δ 1.32, 1.30 and δ 1.31, 1.29. This clearly indicates the absence of an epimeric mixture (absence of two –CH₃ doublets when optically pure phenylethylamine was coupled) during the reaction.
TABLE 3.2. Characterization data for thiourea linked peptidomimetics

<table>
<thead>
<tr>
<th>entry</th>
<th>X</th>
<th>Y</th>
<th>mass (calcd/ obsd)</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21a</td>
<td>FmocHN&lt;br&gt;(\text{N}^\text{f})</td>
<td>(\text{R}^\text{X})</td>
<td>506.2089/506.2079&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87</td>
</tr>
<tr>
<td>21b</td>
<td>FmocHN&lt;br&gt;(\text{N}^\text{f})</td>
<td>(\text{R}^\text{X})</td>
<td>520.2246/520.2241&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92</td>
</tr>
<tr>
<td>21c</td>
<td>FmocHN&lt;br&gt;(\text{N}^\text{f})</td>
<td>(\text{R}^\text{X})</td>
<td>606.2614/606.2618&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85</td>
</tr>
<tr>
<td>21d</td>
<td>ZHN&lt;br&gt;(\text{N}^\text{f})</td>
<td>(\text{R}^\text{X})</td>
<td>418.1776/418.1781&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90</td>
</tr>
<tr>
<td>21e</td>
<td>ZHN&lt;br&gt;(\text{N}^\text{f})</td>
<td>(\text{R}^\text{X})</td>
<td>554.2123/554.2119&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86</td>
</tr>
<tr>
<td>21f</td>
<td>BocHN&lt;br&gt;(\text{N}^\text{f})</td>
<td>(\text{R}^\text{X})</td>
<td>418.1776/418.1782&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85</td>
</tr>
<tr>
<td>21g</td>
<td>O&lt;br&gt;(\text{N}^\text{f})</td>
<td>(\text{R}^\text{X})</td>
<td>375.1354/375.1351&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83</td>
</tr>
<tr>
<td>21h</td>
<td>O&lt;br&gt;(\text{N}^\text{f})</td>
<td>(\text{R}^\text{X})</td>
<td>271.1/271.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87</td>
</tr>
<tr>
<td>21i</td>
<td>BocHN&lt;br&gt;(\text{N}^\text{f})</td>
<td>(\text{R}^\text{X})</td>
<td>910.2986/910.2965&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79</td>
</tr>
</tbody>
</table>

<sup>a</sup>HRMS [M+Na]<sup>-</sup>  <sup>b</sup>ESI-MS [M+Na]

Sugar isothiocyanates constitute an important class of intermediates but the synthetic difficulty involved in the preparation of glycosyl isothiocyanate is a major concern in assembling a diverse array of thiourea tethered carbohydrate derivatives.<sup>56</sup> Hence, a sugar isothiocyanate equivalent 23 was prepared by replacing one Bt group of 3 with an amino sugar 22. O-Protected
sugar-1-amines 22 were prepared from glucose, galactose and lactose through established protocols using acetyl or benzoyl group for hydroxy protection.\textsuperscript{57} The masked sugar isothiocyanates 23a-d from both monosaccharide and disaccharides were isolated in good yield after a simple work-up and were characterized by IR, NMR, and mass spectrometry (Scheme 3.17, Table 3.3).

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme_3.17}
\end{center}

\textbf{SHHEME 3.17. Preparation of masked sugar isothiocyanates}

\begin{table}[h]
\centering
\caption{Characteristic data of masked sugar isothiocyanates}
\begin{tabular}{|c|c|c|c|}
\hline
entry & $O$-protected glycosylamino thiocarbonyl benzotriazoles & mp (°C) & yield (%) \\
\hline
23a & \begin{center}\includegraphics[width=0.3\textwidth]{table_3.3_23a}\end{center} & 116-118 & 79 \\
23b & \begin{center}\includegraphics[width=0.3\textwidth]{table_3.3_23b}\end{center} & 82-84 & 67 \\
23c & \begin{center}\includegraphics[width=0.3\textwidth]{table_3.3_23c}\end{center} & 72-74 & 75 \\
23d & \begin{center}\includegraphics[width=0.3\textwidth]{table_3.3_23d}\end{center} & 84-86 & 72 \\
\hline
\end{tabular}
\end{table}
Further, the reaction of the compounds 23 with amino sugars in the presence of DIEA yielded symmetrical and unsymmetrical thioureido linked neoglycoconjugates 24a-c which were isolated and recrystallized using DCM and n-hexane before being fully characterized (Scheme 3.18, Table 3.4).

**SCHEME 3.18. Synthesis of pseudo glycoconjugates**

In addition to the above, a new class of glycosyl amino acids bearing thiourea segment as a linker has been synthesized by the addition of O-protected glycosylamino thiocarbonyl benzotriazoles 23 to an amine functionalized amino acid 26. It is known that the molecules bearing β-amino-L-alanine unit have acquired profound synthetic and biological interest and are present in several natural products of therapeutical significance.\textsuperscript{58} \(N^\alpha\)-Z-β-Amino-L-alanine methyl ester 26 was synthesized through Hofmann rearrangement of Z-Asn 25 using iodosobenzene diacetate (PIDA) as reported by Zhang et al (Scheme 3.19).\textsuperscript{59}

**SCHEME 3.19.**

The resulting free γ-amine was reacted with various O-protected glycosylamino thiocarbonyl benzotriazoles 23 to afford the corresponding thiourea tethered glycosylated amino acid conjugates 27a-d as solids in 85-90% yields (Scheme 3.20, Table 3.4).
### TABLE 3.4. Characteristic data of neoglycoconjugates and glycosylated amino acids

<table>
<thead>
<tr>
<th>entry</th>
<th>pseudo-glycoconjugates / neoglycosylated amino acids</th>
<th>mp (°C)</th>
<th>mass$^a$ (calcd/ obsd)</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24a</td>
<td><img src="image" alt="24a structure" /></td>
<td>113</td>
<td>759.1895/759.1891</td>
<td>81</td>
</tr>
<tr>
<td>24b</td>
<td><img src="image" alt="24b structure" /></td>
<td>115</td>
<td>1007.2521/1007.2518</td>
<td>89</td>
</tr>
<tr>
<td>24c</td>
<td><img src="image" alt="24c structure" /></td>
<td>116</td>
<td>1047.2740/1047.2735</td>
<td>80</td>
</tr>
<tr>
<td>27a</td>
<td><img src="image" alt="27a structure" /></td>
<td>68</td>
<td>664.1788/664.1785</td>
<td>88</td>
</tr>
<tr>
<td>27b</td>
<td><img src="image" alt="27b structure" /></td>
<td>96</td>
<td>912.2414/912.2409</td>
<td>79</td>
</tr>
<tr>
<td>27c</td>
<td><img src="image" alt="27c structure" /></td>
<td>90</td>
<td>952.2633/952.2638</td>
<td>81</td>
</tr>
<tr>
<td>27d</td>
<td><img src="image" alt="27d structure" /></td>
<td>94</td>
<td>1386.3729/1386.3719</td>
<td>76</td>
</tr>
</tbody>
</table>

$^a$ HRMS
In summary, the synthesis of thioureido linked peptidomimetics, neoglycosylated amino acids and pseudoglycoconjugates by employing Bt-CS-Bt as thiocarbonyl transfer reagent is described. The step wise replacement of each Bt group of the reagent 3 with appropriate amines has resulted in thiourea linked products. The method obviates the use of hazardous reagents. The protocol is simple and efficient giving near quantitative yields in all the steps.

### 3.3. Experimental Section

#### 3.3.1. Fmoc-Amino Acids 4: General Procedure.\(^{60}\)

See section 2.4.1 for details. The yields and physical constants of Fmoc-amino acids prepared are listed in the Table 3.5.

#### 3.3.2. Z-Amino Acids 9: General Procedure.\(^ {61}\)

See section 2.4.4 for detail. The yields and physical constants of all the Z-amino acids prepared are listed in the Table 3.5.

#### 3.3.3. Boc-Amino Acid 10: General Procedure.\(^ {62}\)

See section 1.4.2 for detail. The yield and physical constants of Boc-Phe are listed in the Table 3.5.

### Table 3.5. List of the \(N^\alpha\)-protected amino acids

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>compound</th>
<th>yield (%)</th>
<th>mp (°C)</th>
<th>[\alpha](^{\text{D}}) (c=1, DMF)</th>
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<td>144-46</td>
<td>-15.4</td>
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<tr>
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<td>152-54</td>
<td>-25.1</td>
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<tr>
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<td>147-49</td>
<td>-14.8</td>
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<tr>
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<td>Boc-Phe-OH</td>
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<td>78-80</td>
<td>-4.6</td>
</tr>
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</table>
3.3.4. Methyl esters of amino acids: General Procedure.\textsuperscript{63}

See section 1.4.3. for details.

3.3.4.1. Deprotonation of hydrochloride salts of amino acid methyl esters.\textsuperscript{64}

To a suspension of amino acid methyl ester hydrochloride salt (1 mmol) in DCM or THF (10 mL) was added activated zinc dust (100 mg) in one portion. The mixture was stirred for about 5 min at rt. After the completion of the reaction, the mixture was filtered, evaporated \textit{in vacuo} and precipitated using dry ether to obtain free methyl esters as crystalline solids.

3.3.5. Per-\textit{O}-Benzyol Glycopyranose: General Procedure.\textsuperscript{65}

To a solution of pyridine (12.6 mL) in DCM (10 mL) at -10 °C (using ice-salt bath), a solution of benzoyl chloride (10.4 mL, 90 mmol) in DCM (10 mL) was added dropwise, followed by the addition of dry β-\textit{D}-glycopyranose (28 mmol) with vigorous stirring below 10 °C. The resulting pink-coloured solution was allowed to stand at 0 °C for 24 h. The reaction mixture was diluted with DCM (40 mL) and washed with 5% H\textsubscript{2}SO\textsubscript{4} (2 x 20 mL), water (2 x 20 mL), saturated NaHCO\textsubscript{3} (2 x 20 mL) and dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}. Finally, the solvent was evaporated \textit{in vacuo} and recrystallized using acetone-water to afford the title compound.

3.3.6. Per-\textit{O}-Acetyl Glycopyranose: General Procedure.\textsuperscript{65}

Anhydrous sodium acetate (1.4 g, 17 mmol) and β-\textit{D}-glycopyranose (10 mmol) were ground together and the powdered mixture was taken in a 200 mL flask. Acetic anhydride (9 mL, 95 mmol) was added and the reaction mixture was irradiated with microwaves for 30 sec to 1 min. The clear solution obtained was poured into a beaker containing crushed ice and allowed to stand for an hour. The resulting residue was filtered and recrystallized using ethanol.

3.3.7. Per-\textit{O}-Acetyl/Benzyol Glycopyranosyl Bromide: General Procedure.\textsuperscript{65}

To a solution of per-\textit{O}-acetyl/benzyol glycopyranose (10 mmol) in dry DCM (10 mL) was added 33% of HBr in acetic acid (0.7 mL, 11 mmol) and kept for stirring at room
temperature for about 2 h. Reaction mixture was poured into water. The aqueous layer was extracted with DCM (3 x 20 mL) and the combined organic extracts were washed successively with 5% NaHCO₃ (3 x 25 mL) and brine (2 x 30 mL). The resulting crude product was used directly in the next step.

3.3.8. Per-O-Acetyl/Benzoyl Glycopyranosyl Azide: General Procedure.⁶⁵

To a solution of per-O-acetyl/benzoyl glycopyranosyl bromide (10 mmol) in DMF was added NaN₃ (0.9 g, 15 mmol) and sonicated for 10 min. After the complete consumption of starting material (TLC analysis), the reaction mixture was quenched with water (20 mL) and extracted with DCM (3 x 20 mL). The combined organic layer was washed with brine (2 x 30 mL) and dried over anhydrous Na₂SO₄. The solvent was evaporated under vacuum to furnish the crude product which was used as such in the next step.

3.3.9. Per-O-Acetyl/Benzoyl Glycopyranosyl Amine: General Procedure.⁶⁵

To a solution of per-O-acetyl/benzoyl glycopyranosyl azide (10 mmol) in MeOH (25 mL) was added Pd/C (0.1 g, 10% wt.) and stirred under hydrogen atmosphere. After the completion of the reaction (TLC), the reaction mixture was filtered through celite bed and washed with methanol (2 x 10 mL). The filtrate was concentrated under reduced pressure to afford the product.

3.3.10. Bis(benzotriazolyl)methanethione 3.⁶⁶

To a stirring solution of 1-trimethylsilylbenzotriazole 2 (23.6 g, 169 mmol) in CCl₄ (25 mL) at 0 °C was added dropwise a solution of thiophosgene (6.5 mL, 85 mmol) in CCl₄ (25 mL) over 90 min. When two-thirds of the thiophosgene solution has been added, yellow crystals of bis(benzotriazolyl)methanethione 3 began to precipitate. To ensure the completion of the reaction, stirring was continued at room temperature for another 8 h. After cooling the reaction
mixture in an ice-water bath, the crystals were collected, giving 13.10 g of analytically pure \( \text{3, mp 171-173 °C (lit. 176-78 °C).} \)

### 3.3.11. N-Fmoc-\( \beta \)-Amino Alcohols 5: General Procedure.

To a solution of \( N^\alpha \)-Fmoc amino acid 4 (1.0 mmol) in THF at -15 °C (ice-salt bath) under nitrogen atmosphere was added NMM (0.11 mL, 1.0 mmol) and ECF (0.09 mL, 1.0 mmol) successively. After 20 min, the reaction mixture was filtered. The filtrate was cooled to -15 °C, and a solution of NaBH₄ (0.06 g, 1.5 mmol) in H₂O (0.5 mL) was added in one portion, followed by the addition of water (25 mL). After precipitation of the product, the suspension was filtered. The residue was washed with water (2 x 5 mL) and hexane (2 x 5 mL), and dried in a vacuum desiccator to obtain alcohol 5 as a white solid.

#### 3.3.11.1. Fmoc-Ile-\( \psi \)(CH₂OH) (5a): mp 116-18 °C; \( ^1 \)H NMR (CDCl₃, 300 MHz) \( \delta \) 0.96 (m, 6H), 1.40 (m, 2H), 1.79 (m, 1H), 2.21 (br, 1H), 3.54 (m, 2H), 3.67 (m, 1H), 4.35 (t, \( J = 6.5 \) Hz, 1H), 4.76 (d, \( J = 6.7 \) Hz, 2H), 7.25 (m, 8H); \( ^{13} \)C NMR (CDCl₃, 100 MHz) \( \delta \) 11.5, 15.6, 24.8, 36.2, 47.5, 53.4, 63.2, 67.0, 120.0, 126.2, 126.6, 127.3, 127.5, 128.4, 129.0, 140.1, 142.6, 155.9; ESI-MS caled for C₂₁H₂₅NO₃ \( m/z \) 362.2 [M+Na]⁺, found 362.2.

#### 3.3.11.2. Fmoc-Leu-\( \psi \)(CH₂OH) (5b): mp 110-12 °C; \( ^1 \)H NMR (CDCl₃, 300 MHz) \( \delta \) 1.01 (d, \( J = 5.7 \) Hz, 6H), 1.38 (m, 2H), 1.77 (m, 1H), 3.55 - 3.62 (m, 2H), 3.92 (m, 1H), 4.20 (t, \( J = 6.7 \) Hz, 1H), 4.67 (d, \( J = 6.9 \) Hz, 2H), 7.27 - 7.80 (m, 8H); \( ^{13} \)C NMR (CDCl₃, 100 MHz) \( \delta \) 22.1, 22.9, 23.4, 42.1, 47.0, 50.5, 64.8, 66.9, 119.8, 120.1, 124.9, 125.2, 126.9, 127.1, 139.9, 142.3, 156.0; ESI-MS caled for C₂₁H₂₅NO₃ \( m/z \) 362.2 [M+Na]⁺, found 362.3.
3.3.11.3. Fmoc-Asp(O’Bu)-ψ[CH$_2$OH] (5c): mp 85-87 °C; $^1$H NMR (CDCl$_3$, 300 MHz) δ 1.39 (s, 9H), 2.40 (m, 2H), 2.77 (br, 1H), 3.70 (m, 2H), 4.12 (m, 1H), 4.35 (t, $J$ = 6.1 Hz, 1H), 4.67 (d, $J$ = 6.3 Hz, 2H), 6.12 (br, 1H), 7.25 – 7.82 (m, 8H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 28.0, 37.5, 47.4, 49.2, 64.5, 67.0, 81.8, 119.9, 125.3, 125.9, 126.2, 126.5, 127.1, 128.3, 139.9, 141.8, 155.9, 171.8; ESI-MS calcd for C$_{23}$H$_{27}$NO$_5$ m/z 420.1787 [M+Na]$^+$, found 362.2.

3.3.12. N-Fmoc-β-Iodoamines 6: General Procedure.$^{52}$

To N-Fmoc-β-amino alcohol 5 (1.0 mmol) in dry DCM at 0 °C was added pre-mixed solution of PPh$_3$ (0.79 g, 3.0 mmol), iodine (0.76 g, 3.0 mmol), and imidazole (0.34 g, 5.0 mmol) in dry DCM (1.2 mL). The reaction mixture was stirred at room temperature for 3 h. After the completion of the reaction, the solvent was evaporated under reduced pressure and the corresponding iodide 6 was purified by silica gel column chromatography (10% EtOAc in n-hexane).

3.3.12.1. Fmoc-Ile-ψ[CH$_3$I] (6a): mp 115-117 °C; $^1$H NMR (CDCl$_3$, 300 MHz) δ 0.95 (m, 6H), 1.31 (m, 2H), 1.79 (m, 1H), 3.20 (m, 2H), 3.92 (m, 1H), 4.27 (t, $J$ = 6.1 Hz, 1H), 4.65 (d, $J$ = 6.2 Hz, 2H), 6.12 (br, 1H), 7.25 – 7.70 (m, 8H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 10.1, 11.9, 13.5, 22.4, 38.1, 46.5, 52.8, 66.3, 120.4, 210.8, 126.0, 126.1, 127.4, 128.5, 128.9, 140.1, 143.1; ESI-MS calcd for C$_{23}$H$_{34}$INO$_2$ m/z 472.1 [M+Na]$^+$, found 472.1

3.3.12.2. Fmoc-Leu-ψ[CH$_3$I] (6b): mp 110-112 °C; $^1$H NMR (CDCl$_3$, 300 MHz) δ 0.98 (d, $J$ = 6.2 Hz, 6H), 1.30 (m, 2H), 1.72 (m, 1H), 3.25 – 3.31 (m, 2H), 3.89 (m, 1H), 4.35 (t, $J$ = 5.8 Hz, 1H), 4.70 (d, $J$ = 6.0 Hz, 1H), 7.29 – 7.81 (m, 8H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 11.9, 21.8, 22.5, 22.6, 39.4, 45.8, 51.2, 66.9, 119.4, 120.1, 126.3,
126.7, 127.4, 127.9, 128.1, 140.1, 143.1, 160.0; ESI-MS calcd for C_{21}H_{24}INO_{2} m/z 472.1 [M+Na]^+; found 472.1

3.3.12.3. Fmoc-Asp(OtBu)-\(\text{\textgreek{psi}}\)\{CH_{2}I\} (6c): mp 142-144 °C; \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 1.36 (s, 9H), 2.25 (m, 2H), 3.29 – 3.32 (m, 2H), 4.19 (m, 1H), 4.38 (t, \(J = 6.4\) Hz, 1H), 4.62 (d, \(J = 6.6\) Hz, 2H), 6.45 (br, 1H), 7.25 – 7.79 (m, 8H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 11.7, 28.9, 42.1, 49.8, 67.0, 82.4, 119.4, 119.8, 126.4, 126.8, 128.3, 128.7, 129.0, 139.8, 141.1, 156.0, 172.8; ESI-MS calcd for C_{23}H_{26}INO_{4} m/z 530.1 [M+Na]^+; found 530.2.

3.3.13. N-Fmoc-\(\text{\textgreek{beta}}\)-Amino Alkyl Azides 7: General Procedure.\(^53\)

To the solution of N-Fmoc-\(\text{\textgreek{beta}}\)-iodoamines 6 (0.13 g, 1.0 mmol) in DMF (5 mL), NaN\(_3\) (2.0 mmol) was added and stirred at room temperature for about 4 h. After the solvent removal and purification using silica gel column chromatography (10% EtOAc in n-hexane), the azide 7 was obtained as a white solid in high yield.

3.3.13.1. Fmoc-Ile-\(\text{\textgreek{psi}}\)\{CH_{2}N\(_3\)\} (7a): mp 110-12 °C; \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 0.98 (m, 6H), 1.22 (m, 2H), 1.79 (m, 1H), 3.52 (m, 2H), 3.79 (m, 1H), 4.39 (t, \(J = 6.8\) Hz, 1H), 4.70 (d, \(J = 5.8\) Hz, 2H), 7.27 – 7.83 (m, 8H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 11.6, 15.5, 25.2, 37.4, 47.1, 53.4, 55.2, 67.1, 120.1, 120.7, 125.2, 125.7, 126.8, 127.0, 128.1, 141.3, 142.8, 155.6; ESI-MS calcd for C_{21}H_{24}N_{4}O_{2} m/z 387.2 [M+Na]^+; found 387.2.

3.3.13.2. Fmoc-Leu-\(\text{\textgreek{psi}}\)\{CH_{2}N\(_3\)\} (7b): mp 85-87 °C; \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 0.95 (d, \(J = 6\) Hz, 1H), 1.30 (m, 2H), 1.75 (m, 1H), 3.55 (m, 2H), 3.95 (m, 1H), 4.40 (t, \(J = 6.1\) Hz, 1H), 4.75 (d, \(J = 6.5\) Hz, 2H), 7.26 – 7.79 (m, 8H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 21.5, 23.0, 23.3, 39.4, 47.1, 47.9, 55.4, 67.8, 119.4, 119.9, 125.0, 125.6, 127.0, 128.9, 140.1, 143.2, 155.9; ESI-MS calcd for C_{21}H_{24}N_{4}O_{2} m/z 387.2 [M+Na]^+; found 387.2.
3.3.13.3. Fmoc-Asp(O'Bu)-ψ[CH$_2$N$_3$] (7c): mp 90-92 °C; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 1.46 (s, 9H), 2.54 (m, 2H), 3.51 (m, 2H), 4.16 (m, 1H), 4.40 (t, $J = 6.6$ Hz, 1H), 4.70 (d, $J = 6.8$ Hz, 2H), 6.23 (br, 1H), 7.25 – 7.80 (m, 8H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 28.0, 35.6, 47.0, 49.1, 52.3, 66.9, 82.0, 120.1, 125.1, 125.4, 126.2, 127.8, 128.4, 128.9, 140.1, 142.5, 155.6, 171.0; ESI-MS calec for C$_{23}$H$_{26}$N$_4$O$_4$ m/z 445.2 [M+Na]$^+$, found 445.2.

3.3.14. N-Fmoc-Amino Alkyl Amine hydrochloride salts 8: General Procedure.$^{53}$

To the solution of N-Fmoc amino alkyl azide 7 (1.0 mmol) in THF (10 mL) and CHCl$_3$ (0.2 mL) catalytic amount of 10% palladium on carbon (20 mg) was added, and stirred at room temperature under hydrogen atmosphere (balloon pressure) until the completion of the reaction (TLC). The reaction mixture was filtered over celite, and the solvent was evaporated to afford the Fmoc-amino alkyl amine as hydrochloride salt.

3.3.14.1 Fmoc-Ile-ψ[CH$_2$NH$_2$] (8a): $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 0.79 (m, 6H), 1.00 (m, 2H), 2.93 (m, 1H), 3.10 (m, 2H), 3.82 (br, 1H), 4.13 – 4.31 (m, 3H), 6.12 (d, $J = 9.2$ Hz, 1H), 7.18 – 7.34 (m, 4H), 7.52 – 7.69 (m, 4H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 11.5, 15.6, 26.1, 37.6, 47.4, 54.1, 57.5, 68.1, 120.0, 126.0, 128.6, 130.8, 141.9, 144.8, 157.2; HRMS calcd for C$_{21}$H$_{26}$N$_2$O$_2$ m/z 339.2072 [M+H]$^+$, found 339.2069.

3.3.14.2 Fmoc-Leu-ψ[CH$_2$NH$_2$] (8b): $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 0.90 (d, $J = 6.7$ Hz, 6H), 1.25 (m, 2H), 1.89 (m, 1H), 3.00 (m, 2H), 3.82 (m, 1H), 4.43 (t, $J = 6.6$ Hz, 1H), 4.72 (d, $J = 6.7$ Hz, 2H), 7.25 – 7.80 (m, 8H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 22.1, 23.3, 23.7, 39.5, 45.1, 47.9, 66.5, 120.3, 126.5, 126.7, 128.6, 130.8, 141.0, 144.5, 156.3; ESI-MS calcd for C$_{21}$H$_{26}$N$_2$O$_2$ m/z 339.2 [M+H]$^+$, found 339.2.

3.3.14.3 Fmoc-Asp(O'Bu)-ψ[CH$_2$NH$_2$] (8c): $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 1.38 (s, 9H), 2.45 (m, 2H), 3.10 (m, 2H), 4.35 – 4.42 (m, 2H), 4.75 (d, $J = 6.6$ Hz, 2H), 6.12 (br,
1H); 7.25 – 7.82 (m, 8H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 28.9, 35.7, 44.9, 47.5, 53.4, 66.7, 81.9, 119.8, 120.1, 126.5, 127.7, 140.3, 143.1, 156.8, 172.5; HRMS calcd for C$_{23}$H$_{28}$N$_2$O$_4$ $m/z$ 396.2049 [M+H]$^+$, found 396.2057.

3.3.15. Boc/Z-Amino Acid Amides 11 and 12: General Procedure.$^{54}$

To a solution of N-Boc/Z-amino acid 9 or 10 (1.0 mmol) in THF at -15 °C (ice-salt bath), NMM (0.11 mL, 1.0 mmol), and ECF (0.09 mL, 1.0 mmol) were added and stirred at the same temperature for about 20 min. A solution of 30% aq. ammonia (2.0 mL) was added and stirring was continued for another 5 h. Solvent was evaporated and the residue was diluted with water, filtered and dried to afford the corresponding amide 11 and 12 as a solid. Alternatively, the crude compound was diluted with EtOAc (15 mL) and the organic layer was washed with water, brine and dried over anhydrous Na$_2$SO$_4$. Evaporation of the organic layer yielded the corresponding amide in good yield.

3.3.15.1. Z-Leu-NH$_2$ (11a): $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 0.95 (d, $J$ = 6.7 Hz, 6H), 1.53 (m, 2H), 1.75 (m, 1H), 4.20 (m, 1H), 5.40 (s, 2H), 6.12 (br, 1H), 7.18 – 7.35 (m, 5H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 21.0, 22.1, 22.4, 38.1, 52.2, 66.7, 126.1, 128.6, 129.0, 140.3, 156.2, 176.8; HRMS calcd for C$_{14}$H$_{20}$N$_2$O$_3$ $m/z$ 287.1372 [M+Na]$^+$, found 287.1366.

3.3.15.2. Z-Cys(Bzl)-NH$_2$ (11b): $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 2.75 (m, 2H), 3.80 (s, 2H), 4.54 (m, 1H), 5.25 (s, 2H), 6.37 (br, 1H), 7.15 – 7.36 (m, 10H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 31.7, 39.2, 54.4, 66.7, 126.2, 127.7, 127.8, 128.0, 128.4, 128.9, 136.0, 141.1, 156.1, 177.5; HRMS calcd for C$_{18}$H$_{20}$N$_2$O$_3$S $m/z$ 367.1092 [M+Na]$^+$, found 367.1102.
3.3.15.3. Boc-Phe-NH₂ (12a): \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 1.38 (s, 9H), 2.91 (m, 2H), 4.10 (m, 1H), 6.20 (br, 1H), 7.18 – 7.33 (m, 5H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 28.8, 35.6, 55.9, 78.9, 127.1, 128.2, 128.9, 139.7, 156.0, 177; ESI-MS calcd for C\(_{14}\)H\(_{20}\)N\(_2\)O\(_3\) m/z 287.1 [M+Na]\(^+\), found 287.1.

3.3.16 N-Boc/Z-α-Amino Nitriles 13 and 14: General Procedure.\(^{55}\)

A solution of N-Boc/Z-amino acid derived amide 11 or 12 (1.0 mmol) in dry DCM was cooled to -0 °C to which pyridine (0.2 mL, 2.5 mmol) and TFAA (0.21 mL, 1.5 mmol) were added and the reaction mixture was stirred for about 3 h until the completion of the reaction (TLC analysis). Then the reaction mixture was diluted with DCM (5 mL), and the organic phase was washed successively with 10% citric acid solution (2 x 5 mL), 10% NaHCO\(_3\) solution (2 x 5 mL), water (2 x 5 mL) and brine (2 x 5 mL). It was dried over anhydrous Na\(_2\)SO\(_4\) and evaporated under reduced pressure to afford the corresponding nitriles 13 and 14.

3.3.16.1. Z-Leu-ψ[CN] (13a): \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 0.98 (d, \(J = 6.7\) Hz, 6H), 1.67 (m, 2H), 1.87 (m, 1H), 4.20 (m, 1H), 5.40 (s, 2H), 7.15 – 7.35 (m, 5H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 21.0, 22.2, 22.6, 39.1, 41.7, 66.7, 120.1, 126.4, 127.5, 128.9, 129.0, 141.2, 156.0; HRMS calcd for C\(_{14}\)H\(_{18}\)N\(_2\)O\(_2\) m/z 269.1266 [M+Na]\(^+\), found 269.1275.

3.3.16.2. Z-Cys(Bzl)-ψ[CN] (13b): \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 3.12 (m, 2H), 3.57 (s, 2H), 4.70 (m, 1H), 5.25 (s, 2H), 7.17 – 7.37 (m, 5H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 32.7, 37.8, 44.5, 66.9, 116.4, 126.3, 127.0, 127.4, 128.1, 128.3, 128.9, 135.8, 142.2, 156.0; ESI-MS calcd for C\(_{18}\)H\(_{18}\)N\(_2\)O\(_2\)S m/z 349.1 [M+Na]\(^+\), found 367.1102.

3.3.16.3. Boc-Phe-ψ[CN] (14a): \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 1.40 (s, 9H), 2.98 – 3.05 (m, 2H), 4.70 (m, 1H), 6.20 (br, 1H), 7.15 – 7.32 (m, 5H); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 28.6, 39.5, 42.0, 79.0, 118.4, 126.2, 127.6, 127.8, 140.1, 155.9; ESI-MS
calcd for C₁₄H₁₈N₂O₂ m/z 269.1 [M+Na]^⁺, found 269.2.

3.3.17. N-Boc/Z-Amino Alkyl Amines 15 and 16: General Procedure.⁵⁴

To a solution of N°-Boc/Z-amino nitrile 13 or 14 (1.0 mmol) in dry THF at -15 °C (ice-salt bath), LiAlH₄ (0.01 g, 0.25 mmol) was added slowly in portions. The progress of the reaction was monitored through TLC. After the completion of the reaction, it was quenched with 10% aq. ammonium chloride solution and filtered through celite bed. The celite bed was washed with EtOAc (2 x 10 mL), the filtrate was dried over anhydrous Na₂SO₄ and concentrated in vacuo to afford the N-Boc/Z-amino alkyl amine 15 and 16.

3.3.17.1. Z-Leu-ψ[CH₂NH₂] (15a): ¹H NMR (CDCl₃, 300 MHz) δ 1.01 (d, J = 5.9 Hz, 6H), 1.60 (m, 2H), 1.87 (m, 1H), 2.55 (m, 2H), 3.95 (m, 1H), 5.40 (s, 2H), 7.25 – 7.45 (m, 5H); ¹³C NMR (CDCl₃, 100 MHz) δ 21.0, 22.3, 22.7, 41.2, 44.8, 67.0, 127.1, 127.8, 129.2, 156.2; ESI-MS calcd for C₁₄H₂₂N₂O₂ m/z 250.2 [M+Na]^⁺, found 250.2.

3.3.17.2. Z-Cys(Bzl)-ψ[CH₂NH₂] (15b): ¹H NMR (CDCl₃, 300 MHz) δ 2.55 – 2.70 (m, 4H), 3.56 (s, 2H), 4.20 (m, 1H), 5.27 (s, 2H), 6.26 (br, 1H), 7.10 (m, 5H); ¹³C NMR (CDCl₃, 100 MHz) δ 33.4, 38.9, 42.8, 53.7, 67.0, 126.5, 126.9, 127.0, 127.9, 128.1, 128.5, 136.8, 140.1, 156.0; ESI-MS calcd for C₁₈H₂₃N₂O₂S m/z 353.1 [M+Na]^⁺, found 353.1.

3.3.17.3. Boc-Phe-ψ[CH₂NH₂] (16a): ¹H NMR (CDCl₃, 300 MHz) δ 1.40 (s, 9H), 2.62 – 2.75 (m, 4H), 4.15 (m, 1H), 6.12 (br, 1H), 7.15 – 7.32 (m, 5H); ¹³C NMR (CDCl₃, 100 MHz) δ 28.9, 39.6, 42.0, 54.1, 79.0, 126.5, 127.1, 127.8, 139.4, 155.8; ESI-MS calcd for C₁₄H₂₂N₂O₂ m/z 273.2 [M+Na]^⁺, found 273.1.
3.3.18. Preparation of 17, 19 and 23: General Procedure.

To a solution of N-Fmoc/Boc/Z-amino alkyl amine 8, 15 and 16 (1.0 mmol), amino acid ester 18 or O-protected-β-glycosyl amine 22 (1.0 mmol) in DCM (10 mL), Bt-CS-Bt 3 (0.28 g, 1.0 mmol) was added at room temperature and the reaction mixture was stirred overnight. After the completion of reaction (TLC), it was diluted with DCM. The organic layer was washed with 10% NaHCO₃ (2 x 10 mL), brine (2 x 10 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure to afford the 17, 19 and 23, which were recrystallized from DCM/n-hexane.

3.3.18.1. Fmoc-Ile-ψ(CH₂NHCSBt) (17a): ¹H NMR (CDCl₃, 400 MHz) δ 0.85 – 1.05 (m, 6H), 1.23 (m, 2H), 1.67 (m, 1H), 3.45 (m, 1H), 3.85 – 4.00 (m, 2H), 4.29 – 4.38 (m, 1H), 5.10 (br, 1H), 7.15 – 7.72 (m, 10H), 8.02 (d, 1H), 8.85 (d, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 11.6, 15.3, 25.5, 37.5, 41.2, 54.9, 66.8, 116.0, 119.8, 120.2, 124.9, 125.3, 126.9, 127.6, 130.0, 132.4, 141.2, 143.7, 144.3, 147.0, 156.9, 175.2; HRMS calcd for C₂₈H₂₉N₅O₂S m/z 522.1940 [M+Na]⁺, found 522.1949.

3.3.18.2. Fmoc-Leu-ψ(CH₂NHCSBt) (17b): ¹H NMR (CDCl₃, 400 MHz) δ 0.95 (d, J = 5.6 Hz, 6H), 1.35 (m, 2H), 2.05 (m, 1H), 3.45 (m, 1H), 3.50 (m, 1H), 4.05 – 4.20 (m, 3H), 5.65 (br, 1H), 7.20 – 7.65 (m, 10H), 8.12 (d, 1H), 8.78 (d, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 21.9, 22.9, 24.8, 41.9, 47.2, 49.1, 50.3, 66.7, 115.9, 119.9, 120.2, 124.9, 125.6, 126.9, 127.6, 130.1, 132.4, 141.3, 143.7, 147.0, 156.8, 175.2; HRMS calcd for C₂₈H₂₉N₅O₂S m/z 522.1940 [M+Na]⁺, found 522.1944.

3.3.18.3. Fmoc-Asp(OBu)-ψ(CH₂NHCSBt) (17c): ¹H NMR (CDCl₃, 400 MHz) δ 1.39 (s, 9H), 2.30 – 2.62 (m, 2H), 2.70 – 2.93 (m, 2H), 4.05 (m, 1H), 4.49 (t, J = 5.2 Hz, 1H), 4.87 (d, J = 5.2 Hz, 2H), 5.96 (br, 1H), 7.26 – 7.89 (m, 10H), 8.10 (d,
1H), 8.80 (d, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 29.0, 36.5, 46.3, 47.1, 49.2, 66.7, 80.5, 118.1, 119.3, 126.5, 126.7, 128.8, 128.9, 131.0, 139.5, 141.0, 143.2, 145.5, 155.2, 171.9; HRMS calcd for C$_{30}$H$_{31}$N$_2$O$_4$S $m/z$ 580.1994 [M+Na]$^+$, found 580.1201.

3.3.18.4. **Z-Leu-$\psi$(CH$_2$NHCSBt) (17d):** $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 0.95 (d, $J = 5.6$ Hz, 6H), 1.31 (m, 2H), 1.79 (m, 1H), 2.35 – 2.86 (m, 2H), 4.03 (m, 1H), 5.35 (s, 2H), 6.12 (br, 1H), 7.09 – 7.36 (br, 9H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 22.2, 23.6, 40.6, 46.3, 50.6, 67.2, 119.0, 126.2, 127.9, 128.1, 128.8, 129.2, 129.7, 131.1, 136.7, 156.8, 172.2; HRMS calcd for C$_{21}$H$_{23}$N$_5$O$_2$S $m/z$ 434.1627 [M+Na]$^+$, found 434.1633.

3.3.18.5. **Z-Cys(Bzl)-$\psi$(CH$_2$NHCSBt) (17e):** $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 2.50 – 2.65 (m, 2H), 3.30 (m, 1H), 3.42 (m, 1H), 3.67 (s, 2H), 4.17 (m, 1H), 5.41 (s, 2H), 6.05 (br, 1H), 7.10 – 7.50 (m, 14H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 31.1, 39.5, 46.3, 51.4, 65.5, 117.9, 118.5, 126.4, 127.5, 127.9, 128.8, 129.7, 133.6, 139.1, 141.1, 142.8, 155.1, 171.9; HRMS calcd for C$_{23}$H$_{25}$N$_5$O$_2$S$_2$ $m/z$ 514.1347 [M+Na]$^+$, found 514.1355.

3.3.18.6. **Boc-Phe-$\psi$(CH$_2$NHCSBt) (17f):** $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.37 (s, 9H), 2.28 – 2.96 (m, 2H), 3.05 – 3.16 (m, 1H), 4.67 (m, 1H), 5.62 (br, 1H), 7.12 – 7.86 (br, 9H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 29.1, 34.4, 43.2, 51.6, 79.5, 118.1, 121.3, 125.2, 127.8, 135.1, 139.3, 145.5, 152.1, 155.8; ESI-MS calcd for C$_{21}$H$_{23}$N$_5$O$_2$S $m/z$ 434.1627 [M+Na]$^+$, found 434.1619.

3.3.18.7. **MeO-Phe-NHCSBt (19a):** $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 3.05 – 3.10 (m, 2H), 3.67 (s, 3H), 4.05 (m, 1H), 7.15 – 7.55 (m, 9H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 33.4, 55.6, 58.7, 116.2, 118.3, 120.4, 127.3, 128.7, 128.9, 139.0, 142.1, 171.0, 172.3; ESI-MS calcd for C$_{17}$H$_{16}$N$_4$O$_2$S $m/z$ 363.1 [M+Na]$^+$, found 363.1.
3.3.18.8. MeO-Ala-NHCSBt (19b): $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.05 (d, $J = 5.8$ Hz, 3H), 3.57 (s, 3H), 4.15 (m, 1H), 5.98 (br, 1H), 7.20 – 7.45 (m, 4H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 15.9, 50.1, 56.5, 170.9, 172.5, 118.3, 127.4, 127.9, 134.5, 134.9, 171.3, 172.1; ESI-MS calcd for C$_{11}$H$_{12}$N$_4$O$_2$S $m/z$ 287.1 [M+Na]$^+$, found 287.1.

3.3.18.9. N-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1H-benzo[1,2,3]triazole-1-carbothioamide (23a): $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 2.02 – 2.08 (m, 12H), 4.20 (dd, $J = 12.0$ Hz, 1H), 4.25 (dd, $J = 12.1$ Hz, 1H), 4.45 (m, 1H), 4.78 (m, 2H), 5.31 (m, 1H), 5.64 (m, 1H), 6.03 (br, 1H), 7.53 – 7.80 (m, 4H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.5, 21.1, 21.5, 63.1, 69.3, 71.2, 75.1, 77.6, 85.3, 116.3, 121.3, 125.8, 127.4, 135.0, 146.2, 171.1, 171.2, 171.8, 171.9, 173.5; HRMS calcd for C$_{21}$H$_{24}$N$_4$O$_6$S $m/z$ 531.1162 [M+Na]$^+$, found 531.1170.

3.3.18.10. N-(2,2',3,3',4',6,6'-Hepta-O-acetyl-β-D-lactosyl)-1H-benzo[1,2,3]triazole-1-carbothioamide (23b): $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 2.04 (m, 18H), 2.10 (s, 3H), 4.02 – 4.15 (m, 4H), 4.32 (m, 1H), 4.76 – 4.92 (m, 3H), 5.32 (m, 2H), 5.67 (m, 2H), 6.09 (br, 1H), 7.35 – 7.56 (m, 4H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.3, 20.5, 20.7, 21.4, 58.1, 61.6, 61.8, 61.9, 63.5, 67.1, 67.9, 71.6, 72.3, 73.4, 74.3, 84.9, 168.1, 168.3, 168.9, 169.2, 169.7, 169.9, 170.4; HRMS calcd for C$_{33}$H$_{40}$N$_4$O$_{17}$S $m/z$ 819.2007 [M+Na]$^+$, found 819.2007.

3.3.18.11. N-(2,3,4,6-Tetra-O-benzoyl-β-D-galactopyranosyl)-1H-benzo[1,2,3]triazole-1-carbothioamide (23c): $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 4.02 – 4.34 (m, 2H), 4.67 (m, 1H), 5.09 (m, 1H), 5.69 (m, 1H), 5.90 (m, 1H), 7.20 – 7.55 (m, 20H), 7.95 (d, 1H), 8.13 (d, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 66.3, 69.5, 73.5, 79.4, 83.5,
127.5, 128.8, 129.5, 130.1, 130.3, 131.5, 131.6, 133.4, 133.9, 134.5, 136.2, 165.0, 166.4, 168.2, 169.1, 173.5; HRMS calcd for C_{41}H_{22}N_{4}O_{8}S \text{ m/z } 779.1788 \text{ [M+Na]}^{+}, \text{ found } 779.1792.

3.3.18.12. \textit{N-(2',2',3',3',4',6,6'-Hepta-\textit{O}-benzoyl-\textit{\beta}-\textit{D}-lactosyl)-1H-benzo[1,2,3]triazole-1-carbothioamide (23d): }\textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) \text{ ð } 4.42 \text{ – } 4.65 (m, 4H), 4.70 \text{ – } 4.92 (m, 3H), 5.17 (m, 1H), 5.41 \text{ – } 5.55 (m, 3H), 5.68 (m, 1H), 5.85 (m, 1H), 5.97 (m, 1H), 7.20 \text{ – } 7.89 (m, 30H), 7.95 – 8.00 (m, 4H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 100 MHz) \text{ ð } 59.2, 62.1, 63.2, 67.8, 69.9, 70.4, 70.9, 71.9, 72.3, 73.2, 75.4, 83.5, 119.0, 119.3, 119.6, 120.1, 120.4, 120.8, 121.1, 121.5, 127.2, 127.4, 128.0, 128.1, 128.4, 128.8, 129.0, 129.5, 130.4, 130.5, 131.4, 132.4, 133.9, 134.4, 135.7, 169.5, 169.9, 170.1, 170.5, 171.0, 171.4, 171.7, 171.9; HRMS calcd for C_{68}H_{54}N_{4}O_{17}S \text{ m/z } 1253.3102 \text{ [M+Na]}^{+}, \text{ found } 1253.3110.

3.3.19. \textit{N\textsuperscript{\alpha-}Z-\textit{\beta}-Amino-\textit{L}-Alanine Methyl Ester 26}. \textsuperscript{58}

A solution containing \textit{N\textsuperscript{\alpha-}Z}-Asn 25 (5.0 g, 18.8 mmol), EtOAc (24 mL), acetonitrile (24 mL), water (12 mL), PIDA (7.26 g, 22.5 mmol) was cooled to 10 °C and stirred for 30 min. The temperature was allowed to reach 20 °C, and the reaction was stirred until completion (4 h). The resulting reaction mixture was cooled to 5 °C, and the product was filtered, washed with EtOAc (10 mL), and dried under vacuum at 50 °C to give \textit{N\textsuperscript{\alpha-}Z-\beta}-amino-\textit{L}-alanine (3.9 g, 87% yield). To the suspension of acid in MeOH at 0 °C, SOCl\textsubscript{2} (1.3 mL, 18.8 mmol) was added drop wise and allowed to stir at room temperature for 3 h. The solvent was removed \textit{in vacuo} to yield the title compound 26 in 89% yield. \textsuperscript{1}H NMR (DMSO-\textit{d}_{6}, 400 MHz) \text{ ð } 3.01 \text{ – } 3.19 (m, 1H), 3.67 (s, 3H), 4.20 (m, 1H), 5.29 (s, 2H), 7.25 \text{ – } 7.67 (m, 5H); \textsuperscript{13}C NMR (DMSO-\textit{d}_{6}, 400 MHz) \text{ ð } 39.5, 50.2, 55.7, 64.9, 128.2, 128.3, 128.8, 135.5, 155.3, 171.2; HRMS calcd for C_{12}H_{17}N_{2}O_{4} \text{ m/z } 311.0775 \text{ [M+Na]}^{+}, \text{ found } 311.0782.
3.3.20. Preparation of 21a-i, 24a-c and 27a-d: General Procedure.

To a stirring solution of 17, 19 or 23 (1.0 mmol) in DCM (10 mL) was added amino-free amino acid ester (1.0 mmol), O-protected-β-glycosyl amine 22 (1.0 mmol) or Nα-Z-β-amino-L-alanine methyl ester 26 (0.25 g, 1.0 mmol) followed by the addition of DIEA (0.17 mL, 1.0 mmol) at room temperature. It was stirred for about 5-6 h until completion (TLC analysis). The solvent was removed in vacuo and the residue was taken into EtOAc (10 mL). The organic layer was washed with 10% citric acid solution (2 x 10 mL), 10% NaHCO₃ solution (2 x 10 mL) solution, brine (2 x 10 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and recrystallized from DCM and n-hexane to afford the title compounds.

3.3.20.1. Fmoc-Ile-ψ(CH₂NHCSNH)-β-Gly-OMe (21a): ¹H NMR (CDCl₃, 400 MHz) δ 0.83 – 0.96 (m, 6H), 1.25 (m, 2H), 2.00 (m, 2H), 2.35 (t, J = 8.0 Hz, 2H), 3.37 (t, J = 8.0 Hz, 2H), 3.56 (s, 3H), 3.67 (m, 2H), 3.81 (m, 1H), 4.18 (m, 1H), 4.37 (m, 2H), 5.35 (br, 1H), 6.84 (br, 1H), 7.31 – 7.76 (m, 8H); ¹³C NMR (CDCl₃, 100 MHz) δ 12.1, 15.9, 25.8, 30.9, 33.9, 37.8, 40.1, 47.6, 52.3, 56.4, 67.5, 125.6, 125.7, 127.6, 128.2, 141.7, 144.2, 158.0, 173.5, 183.1; HRMS calcd for C₂₊H₁₃N₃O₄S m/z 506.2089 [M+Na]⁺, found 506.2079.

3.3.20.2. Fmoc-Leu-ψ(CH₂NHCSNH)-β-Gly-OMe (21b): ¹H NMR (CDCl₃, 400 MHz) δ 0.95 (d, J = 6.7 Hz, 6H), 1.07 (d, J = 5.8 Hz, 3H), 1.37 (m, 2H), 2.01 (m, 1H), 2.30 – 2.45 (m, 2H), 3.35 – 3.47 (m, 2H), 3.67 (s, 3H), 3.97 (m, 1H), 4.05 (m, 1H), 4.35 (t, J = 5.3 Hz, 1H), 4.67 (d, J = 5.3 Hz, 2H), 7.25 – 7.65 (m, 8H); ¹³C NMR (CDCl₃, 100 MHz) δ 18.5, 20.5, 22.1, 22.3, 35.4, 41.7, 43.2, 47.7, 50.1, 52.0, 53.4, 65.2, 126.0, 126.3, 128.1, 137.5, 140.1, 155.4, 171.9, 182.3; HRMS calcd for C₂₇H₃₅N₅O₄S m/z 520.2246 [M+Na]⁺, found 520.2241.
3.3.20.3. Fmoc-Asp(O’Bu)-ψ(CH$_2$NHCSNH)-β-Val-OMe (21c): $^1$H NMR (CDCl$_3$, 400 MHz) δ 0.95 (d, $J = 5.8$ Hz, 6H), 1.45 (s, 9H), 2.17 (m, 1H), 2.25 – 2.55 (m, 4H), 3.67 (s, 3H), 3.50 – 3.72 (m, 2H), 4.05 (m, 1H), 4.45 – 4.52 (m, 2H), 4.75 (d, $J = 5.6$ Hz, 2H), 7.38 – 7.75 (m, 8H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 18.9, 19.0, 28.0, 30.2, 35.9, 42.3, 49.1, 51.7, 52.0, 53.5, 56.4, 69.1, 84.0, 126.1, 126.4, 127.5, 128.9, 139.7, 143.2, 155.1, 171.9, 172.5, 182.6; HRMS calcld for C$_{31}$H$_{41}$N$_3$O$_6$S $m/z$ 606.2614 [M+Na]$^+$, found 606.2618.

3.3.20.4. Z-Leu-ψ(CH$_2$NHCSNH)-β-Gly-OMe (21d): $^1$H NMR (CDCl$_3$, 400 MHz) δ 0.89 (d, $J = 6.7$ Hz, 6H), 1.33 (m, 2H), 1.69 (m, 1H), 2.59 (m, 2H), 3.45 – 3.59 (m, 4H), 3.64 (s, 3H), 3.81 (m, 1H), 5.04 (s, 2H), 5.36 (br, 1H), 6.98 (br, 1H), 7.26 – 7.35 (m, 5H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 22.4, 23.5, 25.3, 34.0, 40.3, 42.3, 50.1, 52.4, 67.4, 128.4, 128.6, 129.0, 136.7, 157.8, 173.5, 182.5; HRMS calcld for C$_{19}$H$_{26}$N$_3$O$_4$S $m/z$ 418.1776 [M+Na]$^+$, found 418.1781.

3.3.20.5. Z-Cys(Bzl)-ψ(CH$_2$NHCSNH)-β-Leu-OMe (21e): $^1$H NMR (CDCl$_3$, 400 MHz) δ 0.98 (d, $J = 6.8$ Hz, 6H), 1.23 (m, 2H), 1.76 (m, 1H), 2.40 (m, 2H), 2.53 – 2.67 (m, 2H), 3.35 (m, 1H), 3.47 (m, 1H), 3.77 (m, 3H), 3.89 – 3.99 (m, 4H), 5.04 (s, 2H), 5.04 (s, 2H), 5.30 (br, 1H), 7.23 – 7.33 (m, 10H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 21.9, 22.6, 23.0, 25.2, 34.1, 36.9, 44.5, 44.6, 49.6, 55.4, 55.6, 67.3, 127.7, 128.4, 128.6, 128.9, 129.6, 136.9, 137.1, 138.1, 156.7, 175.1, 183.8; HRMS calcld for C$_{27}$H$_{37}$N$_3$O$_4$S$_2$ $m/z$ 554.2123 [M+Na]$^+$, found 554.2119.

3.3.20.6. Boc-Phe-ψ(CH$_2$NHCSNH)-β-Gly-OMe (21f): $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.39 (s, 9H), 2.35 – 2.62 (m, 4H), 3.67 (s, 3H), 3.69 – 3.96 (m, 4H), 4.35 (m, 1H), 7.23 – 7.69 (m, 5H); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 28.0, 33.5, 39.4, 42.2,
50.6, 51.3, 55.2, 79.1, 126.5, 127.2, 128.0, 132.6, 155.1, 171.6, 182.9; HRMS calced for C_{15}H_{29}N_{3}O_{4}S m/z 418.1776 [M+Na]^+, found 418.1782.

3.3.20.7. MeO-Phe-NHC-SNH-Val-OMe (21g): $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 0.95 (d, $J = 7.6$ Hz, 6H), 2.23 (m, 1H), 3.12 (m, 2H), 3.34 (m, 1H), 3.67 (s, 3H), 3.76 (s, 3H), 3.83 (m, 1H), 6.80 (br, 1H), 7.10 – 7.32 (m, 5H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 18.4, 18.5, 30.5, 37.1, 52.2, 52.6, 60.1, 64.2, 126.8, 127.5, 129.4, 136.3, 168.8, 173.1, 183.4; HRMS calced for C$_{17}$H$_{24}$N$_{2}$O$_{4}$S m/z 375.1354 [M+Na]$^+$, found 375.1351.

3.3.20.8. MeO-Ala-NHC-SNH-Ala-OMe (21h): $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.49 (d, $J = 6.7$ Hz, 3H), 1.65 (d, $J = 6.7$ Hz, 3H), 3.75 (s, 3H), 3.76 (s, 3H), 4.20 (m, 1H), 5.33 (m, 1H), 8.42 (br, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 14.2, 16.6, 50.6, 50.7, 52.6, 54.7, 172.1, 172.2, 182.5, 182.7; ESI-MS calced for C$_{6}$H$_{16}$N$_{2}$O$_{4}$S m/z 271.1 [M+Na]$^+$, found 271.2.

3.3.20.9. $N,N'$-Bis(2,3,4,6-tetra-$O$-acetyl-$\beta$-$D$-glucopyranosyl)-thiourea (24a): $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 2.05 – 2.25 (m, 18H), 2.40 (m, 3H), 2.45 (s, 3H), 4.01 – 4.35 (m, 4H), 4.50 (m, 2H), 4.79 – 4.92 (m, 2H), 5.25 (m, 2H), 5.47 (m, 1H), 5.52 (m, 1H), 5.87 – 5.92 (m, 2H), 6.75 (br, H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.1, 20.5, 20.7, 23.1, 25.2, 58.9, 59.1, 62.0, 62.2, 65.1, 67.5, 70.1, 72.4, 73.5, 77.1, 80.8, 81.0, 168.1, 168.5, 169.0, 169.1, 169.3, 170.2, 170.4, 170.9, 182.7; HRMS calced for C$_{29}$H$_{40}$N$_{2}$O$_{18}$S m/z 759.1895 [M+Na]$^+$, found 759.1890.

3.3.20.10. $N$-(2,3,4,6-tetra-$O$-acetyl-$\beta$-$D$-glucopyranosyl)-$N'$-(2,3,4,6-tetra-$O$-benzoyl-$\beta$-$D$-galactopyranosyl)thiourea (24b): $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 2.02 (s, 3H), 2.05 (s, 3H), 2.07 – 2.10 (m, 6H),
4.20 – 4.54 (m, 4H), 4.69 (m, 1H), 4.80 (m, 1H), 4.85 – 5.00 (m, 3H), 5.25 (m, 2H), 5.45 (m, 1H), 5.89 (m, 1H), 5.95 (m, 1H), 6.75 (br, 1H), 7.25 – 7.99 (m, 20H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.3, 20.9, 21.0, 21.9, 22.1, 24.7, 55.4, 59.2, 60.5, 63.1, 65.4, 66.7, 69.5, 72.1, 75.9, 79.0, 80.2, 80.9, 166.0, 166.5, 166.9, 169.5, 169.7, 170.0, 170.6, 182.9; HRMS calcd for C$_{49}$H$_{48}$N$_2$O$_{18}$S $m/z$ 1007.2521 [M+Na]$^+$, found 1007.2535.

3.3.20.11. $N$-(2,2',3,3',4',6,6'-hepta-$O$-acetyl-$\beta$-$\delta$-lactosyl)-$N'$-[(2,3,4,6-tetra-$O$-acetyl-$\beta$-$\delta$-glucopyranosyl)thiourea (24c): $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 2.02 – 2.08 (m, 27H), 2.20 (s, 3H), 2.23 (s, 3H), 3.97 – 4.20 (m, 6H), 4.45 – 4.52 (m, 3H), 4.76 – 4.90 (m, 3H), 5.02 – 5.05 (m, 2H), 5.25 (m, 1H), 5.55 – 5.67 (m, 2H), 5.70 – 5.85 (m, 2H), 6.25 (br, 1H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.1, 20.4, 20.5, 20.9, 21.4, 21.9, 22.2, 55.1, 56.9, 60.5, 61.9, 68.5, 70.2, 70.9, 75.9, 77.5, 77.9, 80.1, 83.5, 83.9, 89.5, 90.1, 95.2, 95.8, 169.1, 169.3, 169.4, 169.7, 169.8, 170.0, 170.5, 170.9, 171.3, 171.9, 182.7; HRMS calcd for C$_{41}$H$_{56}$N$_2$O$_{28}$S $m/z$ 1047.2740 [M+Na]$^+$, found 1047.2751.

3.3.20.12. Methyl (S)-2-[(benzyloxy carbonyl)amino]-3-(2,3,4,6-tetra-$O$-acetyl-$\beta$-$\delta$-glucopyranosyl-thioureido)propionate (27a): $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 2.02 – 2.05 (m, 12H), 3.78 (s, 3H), 3.82 (m, 1H), 4.11 (dd, $J = 11.2$ Hz, 1H), 4.29 (dd, $J = 10.0$ Hz, 1H), 4.31 (m, 3H), 4.52 (m, 1H), 4.96 – 5.00 (m, 4H), 5.09 (s, 2H), 5.29 (m, 2H), 6.03 (br, 1H), 6.95 (br, 1H), 7.27 – 7.36 (m, 5H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.5, 21.1, 21.5, 47.1, 53.7, 54.2, 62.1, 67.9, 68.6, 71.2, 73.3, 73.7, 85.1, 128.3, 128.9, 129.2, 136.6, 157.5, 170.1, 170.4, 170.9, 171.3, 171.7, 183.2; HRMS calcd for C$_{27}$H$_{35}$N$_2$O$_{13}$S $m/z$ 664.1788 [M+Na]$^+$, found 664.1785.
3.3.20.13. Methyl (S)-2-[(benzyloxy carbonyl)amino]-3-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-thioureido)propionate (27b): $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 2.05 (br, 1H), 3.64 (s, 3H), 3.93 (m, 1H), 4.18 (m, 1H), 4.41 (m, 2H), 4.51 (m, 2H), 5.06 (m, 3H), 5.64 (s, 2H), 5.96 (br, 1H), 7.14 – 8.01 (m, 25H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 53.6, 53.8, 62.7, 68.0, 69.7, 69.9, 71.9, 72.3, 77.8, 83.3, 128.2, 128.8, 128.9, 129.0, 129.2, 129.4, 130.4, 130.5, 131.1, 133.6, 133.9, 134.2, 134.4, 136.1, 157.2, 165.9, 166.5, 168.2, 168.9. 171.2, 183.2; HRMS calcd for C$_{47}$H$_{63}$N$_5$O$_{15}$S $m/z$ 912.2414 [M+Na]$^+$, found 912.2409.

3.3.20.14. Methyl (S)-2-[(benzyloxy carbonyl)amino]-3-(2,2',3,3',4',6,6'-hepta-O-acetyl-β-D-lactosyl-thioureido)propionate (27c): $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 1.97 (s, 3H), 2.03 – 2.07 (m, 15H), 2.16 (s, 3H), 3.76 (s, 3H), 3.86 (m, 3H), 4.05 – 4.10 (m, 3H), 4.25 (m, 1H), 4.44 (m, 1H), 4.46 – 4.48 (m, 2H), 4.92 – 4.96 (m, 2H), 5.08 (s, 2H), 5.31 (m, 2H), 6.07 (br, 1H), 7.26 – 7.35 (m, 5H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 20.3, 20.4, 20.6, 22.2, 24.1, 50.6, 50.7, 52.7, 55.6, 58.1, 61.5, 61.6, 61.9, 63.5, 67.7, 68.2, 71.6, 72.3, 73.4, 74.3, 85.5, 128.1, 128.3, 129.1, 136.5, 150.1, 168.6, 168.9, 169.1, 169.6, 169.7, 169.8, 169.9, 170.1; HRMS calcd for C$_{59}$H$_{65}$N$_5$O$_{27}$S $m/z$ 952.2633 [M+Na]$^+$, found 952.2644.

3.3.20.15. Methyl (S)-2-[(benzyloxy carbonyl)amino]-3-(2,2',3,3',4',6,6'-hepta-O-benzoyl-β-D-lactosyl-thioureido)propionate (27d): $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 2.06 (br, 1H), 3.64 (s, 3H), 3.87 (m, 1H), 4.42 – 4.49 (m, 3H), 4.62 – 4.66 (m, 5H), 4.82 (m, 1H), 5.00 – 5.04 (m, 3H), 5.17 (m, 1H), 5.41 (m, 3H), 5.61 (m, 1H), 5.70 (m, 1H), 5.93 – 5.98 (m, 2H), 7.20 – 8.08 (m, 40H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 50.1, 53.5, 56.1, 63.2, 67.8, 68.5, 69.9, 70.5, 71.5, 71.9, 72.1, 73.0, 73.1, 75.2, 82.6.
102.1, 119.0, 119.1, 119.6, 120.1, 120.4, 120.7, 121.1, 121.4, 127.7, 127.8, 127.9, 128.2, 128.3, 128.4, 128.8, 129.1, 129.5, 130.3, 130.4, 130.5, 131.1, 131.3, 132.0, 132.3, 133.9, 134.4, 136.5, 137.1, 137.2, 157.6, 170.1, 170.2, 170.4, 170.9, 171.0, 171.3, 171.7, 171.9, 183.1; HRMS calc'd for C_{74}H_{65}N_{21}O_{21}S m/z 1386.3729 [M+Na]^+, found 1386.3729.
3.4. Spectra

3.4.1. HRMS spectrum of Fmoc-Leu-ψ[CH₂NHCSBt] 17b

3.4.2. HRMS spectrum of Boc-Phe-ψ[CH₂NHCSBt] 17f
3.4.3. ESI-MS spectrum of OMe-Phe-NHCSBt 19a

3.4.4. HRMS spectrum of Boc-Phe-ψ[CH₂NHCSNII]-β-Gly-OMe 21a
3.4.5. $^1$H NMR spectrum of Fmoc-Ile-$\psi$[CH$_2$NHCSN]t-$\beta$-Gly-OMe 17a

3.4.6. $^1$H NMR spectrum of Fmoc-Ile-$\psi$[CH$_2$NHCSN]t-$\beta$-Gly-OMe 21a
3.4.7. $^1$H NMR spectrum of Z-Leu-$\psi$(CH$_2$NHCSNH)$-\beta$-Gly-OMe 21d

3.4.8. $^1$H NMR spectrum of Boc-Phe-$\psi$(CH$_2$NHCSNH)$-\beta$-Gly-OMe 21f
3.4.9. $^1$H NMR spectrum of Methyl (S)-2-[(benzyloxy carbonyl) amino]-3-(2,3,4,6-tetra-\textit{O}-acetyl-\textit{\textbeta-}D-glucopyranosyl-thioureido)propionate 27a

3.4.10. $^1$H NMR spectrum of Methyl (S)-2-[(benzyloxy carbonyl) amino]-3-(2,3,4,6-tetra-\textit{O}-benzoyl-\textit{\textbeta-}D-galactopyranosyl-thioureido)propionate 27b
3.4.11. $^1$H NMR spectrum of Methyl (S)-2-[(benzyloxy carbonyl) amino]-3-(2,2',3,3',4,6,6'-hepta-O-acetyl-β-D-lactosyl-thioureido)propionate 27c

3.4.12. $^1$H NMR spectrum of MeO-Ala-NHCSNH-Ala-OMe 21h
3.4.14. $^{13}$C NMR spectrum of Fmoc-Leu-$\psi$/[CH$_2$NHCS]t 17b

3.4.15. $^1$H NMR spectrum of Z-Leu-$\psi$/[CH$_2$NHCSNH]-β-Gly-OMe 21b
3.5. References


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