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A New Photochemical Method For The Preparation Of Amino Acid-Î±-Phenylthioesters And Peptide-Î±-Phenylthioesters

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A NEW PHOTOCHEMICAL METHOD FOR THE PREPARATION OF AMINO ACID-α-PHENYLTHIOESTERS AND PEPTIDE-α-PHENYLTHIOESTERS

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DEDICATIONS

Dad and Mom, Mike and Scott
HOGENAUER

Your love and support guides my every decision
Thank you….

A NEW PHOTOCHEMICAL METHOD FOR THE PREPARATION OF AMINO ACID-α-PHENYLTHIOESTERS AND PEPTIDE-α-THIOESTERS

by

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ABSTRACT

We describe the development of a novel resin with a photoreactive *N*-acylnitroindoline linker, that allows for the synthesis of amino acid- (in solution) or peptide-α-thioesters using standard Fmoc/t-Bu standard solid phase peptide synthesis (SPPS). Recent advances have shown that the yields of this thioesterification reaction have dramatically increased due to a simple change in the order of addition of reagents. Upon illumination with UV-light, in the presence of *N*-hydroxybenzotriazole (HOBt), the amino acid-α- or peptide-α-OBt ester is generated by direct photo-release from the nitroindoline linker with minimal epimerization, followed by reaction with thiophenol in the dark to produce amino acid-α-phenylthioesters or peptide-α-phenylthioesters.

Nine amino acid-α-phenylthioesters of the twenty proteinogenic amino acids were prepared in high yield and high purity (**75-83**). Eight photoreactive tripeptides were prepared on Rink Amide resin following Fmoc-based SPPS strategies and cleaved to give their corresponding photoreactive tripeptide amides (**67-74**). Seven tripepide-α-phenylthioesters were prepared in high yield and high purity (**84-90**). Three tripeptide-α-phenylthioesters were prepared in good yield by direct photo-release from the solid support (**86, 87, 90**).

Five photoreactive glycopeptides based on sequences found in human erythropoietin (**98 and 99**) and the GP-120 HIV-1 envelope protein (**100**, **101**, and **102**) were synthesized and cleaved from Rink Amide resin for future conversion to their corresponding thioesters.

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CHAPTER I

Introduction

1.1 Brief History

The Chemical Society publication *Annual Reports on the Progress of Chemistry* for 1963 informed readers of all the highly significant advances in all the major fields of pure chemistry during that year[1]. Fortunately, the section on peptide chemistry drew attention to a paper by R. B. Merrifield which had just been published in the *Journal of the American Chemical Society*[2]:

A novel approach to peptide synthesis has been the use of a chloromethylated polystyrene polymer as an insoluble but porous solid phase on which the coupling reactions are carried out. Attachment to the polymer constitutes protection of the carboxyl group (as a modified benzyl ester), and the peptide is lengthened …… by successive carbodiimide couplings. The method has been applied to the synthesis of a tetrapeptide, but incomplete reactions lead to the accumulation of by-products. Further development of this interesting method is awaited[2].

A second paper from Merrifield appeared in the same journal that described the synthesis of a nona-peptide which causes blood vessels to dilate: bradykinin[3]. This article was added proof of the usefulness of this new technique.

Merrifield's work constitutes the beginning of solid phase peptide synthesis (SPPS), and has paved the way for a vast number of peptides to be synthesized according to what is now known as the Merrifield method (**Figure 1**)[1].

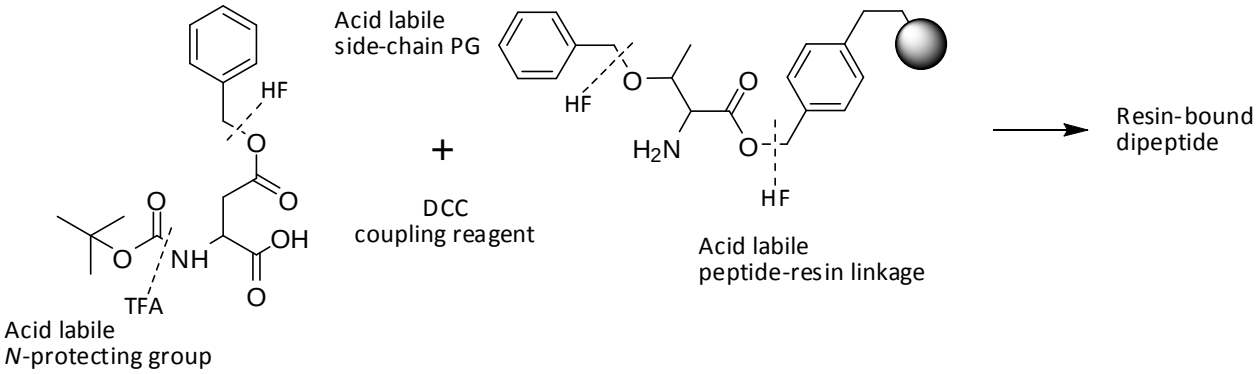
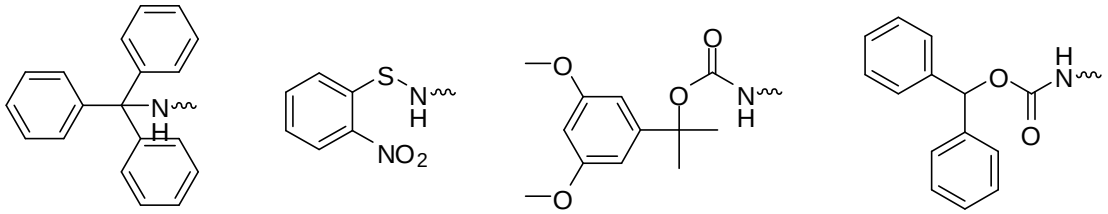


Figure 1. The Merrifield method for SPPS[1]

The Merrifield method of SPPS excited the chemical community because of its ease of use. With the growing peptide bound to a solid support, by-products could easily be removed by simple filtration, a technique that circumvents the need for purification after the installation of each amino acid. However exciting it was seen to be, this method is not without its limitations. Merrifield's technique made use of protecting groups based predominantly on benzyl and *t*-butyl derivatives[3]. Both of these protecting groups are labile to acidic conditions and complete selectivity cannot be achieved. The loss of some small portion of the side-chain protecting group and peptide-resin linkage must also occur at every cycle. This leads to undesired side products as some unprotected side-chain functionalities will compete during each coupling reaction. Alternatively potential yield reduction will occur due to cleavage of some portion of the peptide from the resin. Further, the final, global deprotection of the permanent protecting groups with concomitant cleavage from the resin requires liquid hydrogen fluoride. Liquid hydrogen fluoride is a particularly unpleasant and hazardous reagent requiring special equipment for its safe handling[3].

Due to the harsh conditions employed in the Merrifield method, the search began for a more reactive protecting group strategy that would require less acidic conditions to realize the peptide of

interest. N-terminal protecting groups such as *N*-trityl (**1**), *o*-nitophenylsulphenyl (**2**), α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl (**3**) and biphenlyisopropoxycarbonyl (Bpoc, **4**) were among those explored (**Figure 2**) [4,5].



1 **2** **3** **4**

Figure 2. Acid labile N-terminal protecting groups[4,5]

The obvious next step was to search for base labile protecting groups for the N-terminus. A strategy such as this would create an orthogonal protecting group that would ultimately eliminate the need for the liquid hydrogen fluoride condition upon completion of the desired resin bound peptide. Caprino's 9-fluorenylmethoxycarbonyl group (**Fmoc**, **5**), introduced in 1972 captured this idea perfectly (**Figure 3**) [6].

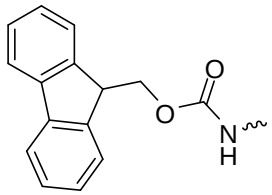


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Figure 3. *N*-Fmoc protecting group[6]

Fluorenylmethoxycarbonyl derivatives are cleaved by organic bases with the initial formation of the non-volatile and reactive dibenzofulvene[6]. However, SPPS reduces the chance of product contamination by simple filtration and washing steps (**Figure 4**).

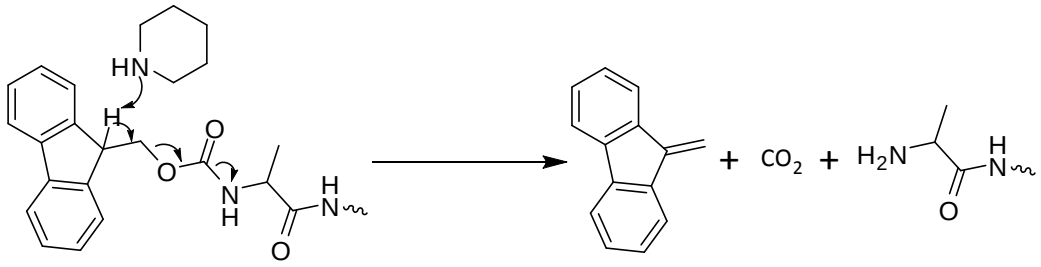


Figure 4. Cleavage of the *N*-Fmoc protecting group by an organic base[6]

Fmoc-based SPPS offered an efficient alternative to the Merrifield method. With the addition of more improved, commercially available, activated esters such as *N*-hydroxysuccinimidyl- (**6**), pentafluorophenyl- (**7**) and the more popular *N*-hydroxybenzotriazolyl esters (**8**) of *N*-Fmoc amino acids, Fmoc-based SPPS quickly became the dominant method for the solid phase synthesis of peptides (**Figure 5**) [1,7].

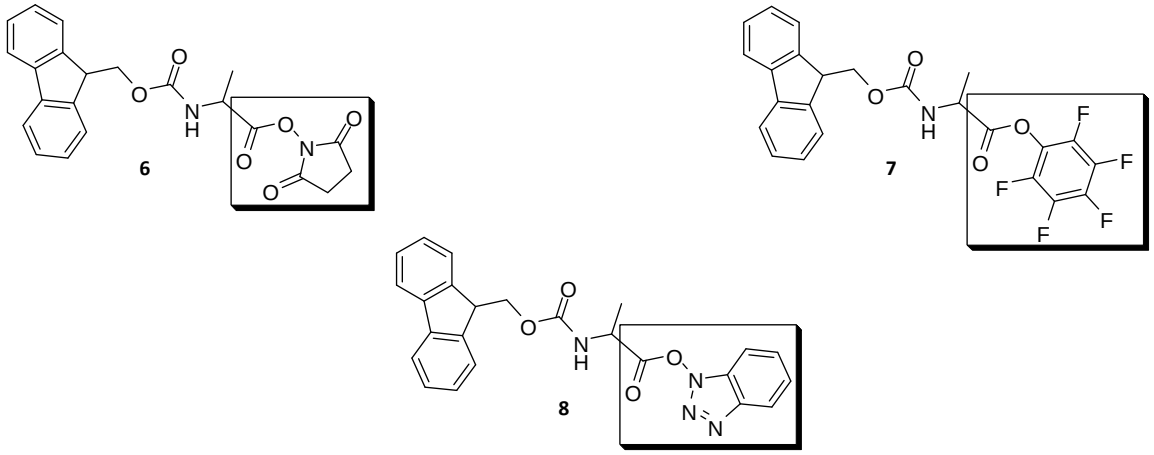


Figure 5. Commercially available *N*-Fmoc amino acid activated esters[1,7]

Additionally, fluorene derivatives have strong ultraviolet absorption in an accessible part of the spectrum. The take-up of fluorene derivatives from solution onto the solid phase and vice versa could now be followed spectrometrically. This property created new advancements in automation. Inclusion of a UV cell within the flowing reagent system of continuous flow automated synthesizers provided a complete and immediate record of the acylation and deprotection steps of the synthesis as they were occurring by measuring the ultraviolet absorption of the fluorene derivatives during these steps.

A more detailed investigation of each coupling step was also undertaken by the use of spectrometric analysis. Peptide chemists often talk about 'difficult sequences' during the synthesis of peptides. Incomplete solvation, interchain hydrogen bonding, chain aggregation and the folding of the resin bound peptide create incomplete coupling of the incoming amino acid[8]. An evolved understanding of these phenomenon based on the spectrometric analysis using *N*-Fmoc amino acids allowed for solutions to overcome these problems. It has been established that the introduction of tertiary amide bonds at every fifth or sixth residue interrupts interchain hydrogen bonding and ultimately reduces chain aggregation. Appropriate alkyl or aryl *N*-substituents, such as *N*-hydroxymethoxybenzyl (Hmb) or *N*-Dimethoxybenzyl (Dmb) (**Fig. 6**) provide the requisite sterics with the simultaneous removal of a hydrogen-bond donor, effectively eliminating the problems associated with difficult sequences[8].

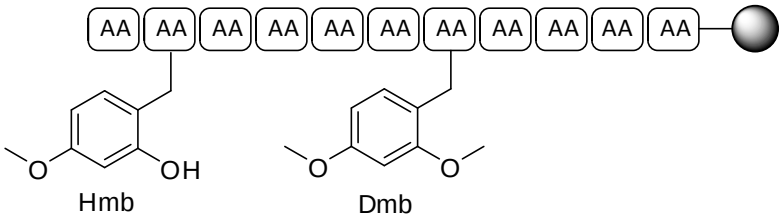


Figure 6. *N*-Hmb-substituted amino acid[8]

Fmoc-based SPPS is now firmly established alongside the Merrifield (Boc) method. It has provided for the synthetic community the ability to monitor in real time the coupling of each incoming amino acid to the growing peptide chain. It has encouraged the construction of continuous-flow synthesizers and advanced automatic peptide synthesizers. The routine synthesis of natural peptides and modified peptides based on unnatural amino acids has been established and continually improved over the years since the introduction of the Merrifield method for SPPS. The remainder of this dissertation will focus on Fmoc-based SPPS.

1.2 Synthesis of Peptides

The general scheme for Fmoc-based solid phase peptide synthesis can be described as the sequential addition of α-amino and side-chain protected amino acid residues to an insoluble polymeric support (**Figure 7**).

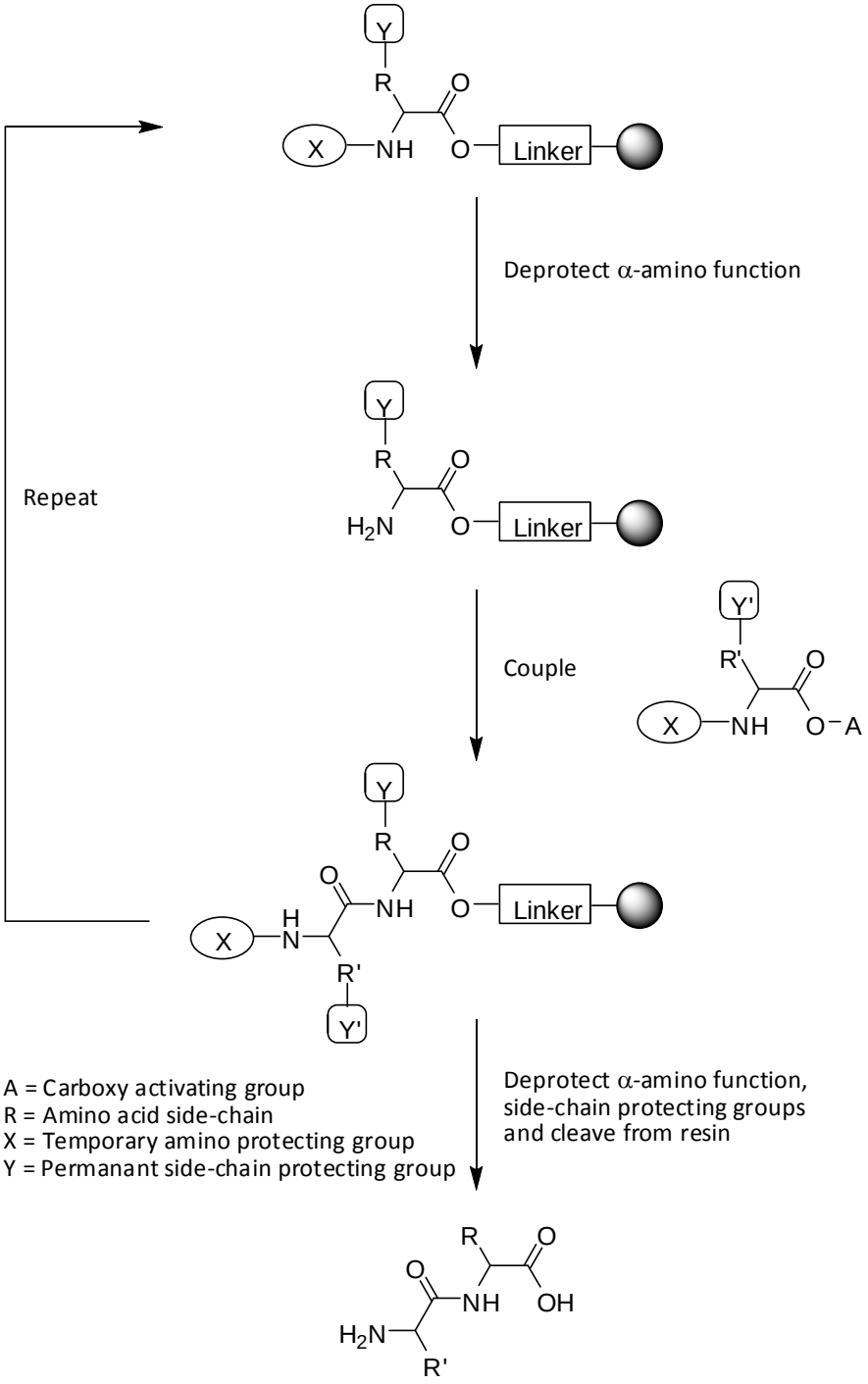


Figure 7. Principle of solid phase peptide synthesis

The base-labile Fmoc-group is used for *N*-α-protection and the side chain functionality is generally, but not exclusively, protected by the acid-labile ^{t}butyl-group. In the first step, an amino acid is attached to

a suitable insoluble resin equipped with the desired linker that produces the C-terminal functionality of interest at the end of the synthesis. Upon successful attachment of the first amino acid to the resin bound linker, the N-terminus is deprotected, reacted with a C-terminal activated amino acid then analyzed spectrometrically or by colorometric tests. The process is then repeated for each additional amino acid until the desired peptide length has been reached. The resin bound peptide is then cleaved from the resin, with simultaneous cleavage of the side-chain protecting groups, using an organic acid solution.

There are a number of commercially available resins for SPPS. The choice of resin depends on a number of factors: swelling properties, loading capacity, and the desired functionality at the C-terminus of the cleaved peptide[9]. The resin of choice must have suitable swelling properties in the solvent to be used throughout the synthesis. Poor swelling will result in the incomplete loading of the first amino acid, and will ultimately reduce the overall yield of the synthesized peptide.

The loading capacity of the resin is defined as the amount, in moles, of the amino acid that can be coupled to the resin. The loading capacity of the resin can determine the success of the desired synthesis. If the loading capacity of the resin is too high, steric crowding may inhibit the successful coupling of each amino acid. Conversely, if the loading capacity is low, a large amount of resin must be used to obtain the desired amount of peptide. The choice of loading capacity depends on the nature of the peptide to be synthesized. Commercially available resins utilize a variety of loading capacities that allow for a variety of syntheses to be accomplished.

A number of C-terminal peptide functionalities can be installed with the proper choice of resin. For the production of C-terminal peptide acids the Wang resin can be used (**Figure 8**)[9].

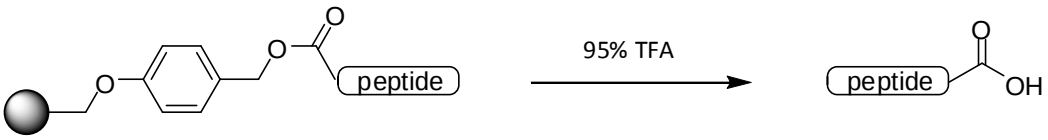


Figure 8. Resin for the production of peptide acids[9]

C-terminal peptide amides can be obtained with the use of Rink Amide resin (**Figure 9**)[7].

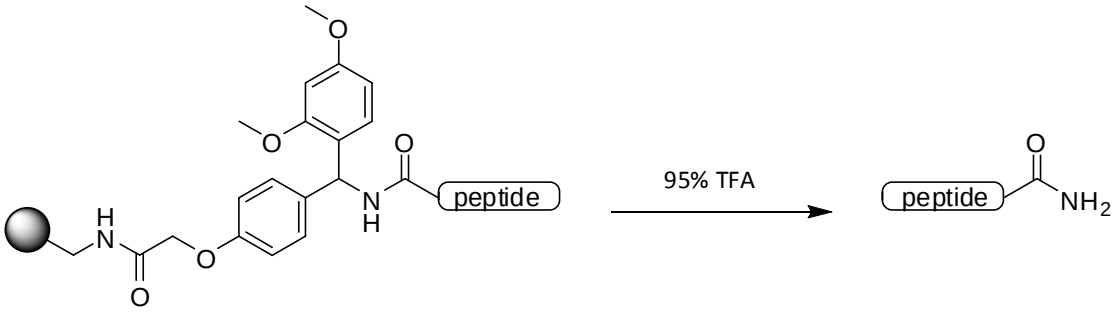


Figure 9. Resin for the production of peptide amides[7]

For C-terminal peptide esters, the HMBA-AM resin can be used (**Figure 10**)[7].

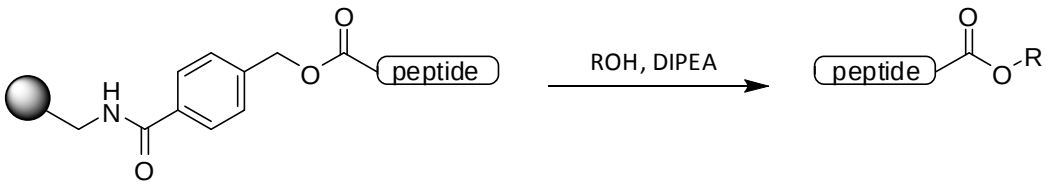


Figure 10. Resin for the production of peptide alcohols[7]

A final example is the production of C-terminal peptide thioesters using the 4-Sulfamylbutyryl AM resin (**Figure 11**)[7].

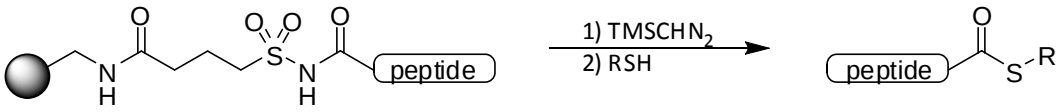


Figure 11. Resin for the production of peptide thioesters[7]

The above examples illustrate that with the proper choice of resin, any number of C-terminal peptide functionalities could be realized.

After the choice of resin has been reached, SPPS can begin. Efficient peptide bond formation of incoming amino acids requires chemical activation of the C-terminal carboxylic acid of the *N*-α-protected amino acid. The choice of reaction or activating group must be chosen carefully to achieve high coupling efficiency and to avoid unwanted side reactions. There are essentially four major types of coupling methods employed by peptide chemists to achieve step-wise addition of amino acids: carbodiimides, preformed symmetrical anhydrides, activated esters and coupling reagents[1,9]. Each has its own particular advantage in the hands of individual chemists.

A commonly used carbodiimide is dicyclohexylcarbodiimide (DCC, **9**), but other carbodiimides such as diisopropylcarbodiimide (DIPCDI, **10**), *t*-butylmethylcarbodiimide (MCDI, **11**) and *t*-butylethylcarbodiimide (ECDI, **12**) have also found uses in SPPS (**Figure 12**) [9,10].

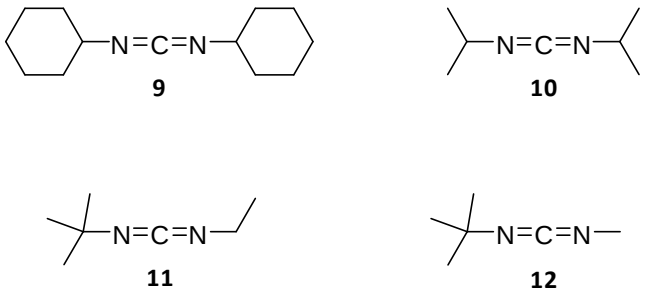


Figure 12. Carbodiimide coupling reagents[9,10]

Carbodiimides have found much popularity as *in situ* activation reagents for peptide synthesis. DCC was first described in 1955 and remains a popular choice for the apolar nature of polystyrene resins[11]. Reactions are carried out in dichloromethane (DCM) for 12 h. The advantage of performing coupling reaction in DCM is that it is an excellent solvent for the swelling of the solid support. A disadvantage of DCC is the dehydration to nitriles of the unprotected side-chains of asparagine and glutamine during activation[12]. In addition, the principle drawback of using DCC is the formation of the DCM-insoluble dicyclohexylurea (DCU) as a by-product during coupling reactions which cannot be removed from the resin by simple filtration. Conversely, DIPCDI, MCDI and ECDI have been found to give rise to urea derivatives that are more DCM-soluble[13,14,15].

Performed symmetrical anhydrides (PSA) have been used by many research groups, mainly in Boc-based SPPS[16,17]. PSA's are generated by reaction of two equivalents of *N*-α-protected amino acid in the presence of DCC using DCM as solvent. The DCU that forms is filtered off and then couplings proceed in dimethylformamide (DMF). The most obvious drawback of PSA's is the waste involved in their preparation: one full equivalent is lost. It is also recommended that PSA's be made fresh prior to use[18].

Extensive studies have been performed on the use of Fmoc amino acid activated esters. Of the many active esters investigated, pentafluorophenyl esters (OPfp) have found the most application in Fmoc-based SPPS. They are efficient acylating agents and their use is generally regarded to be free from side-reactions[1,9]. Further, most of the proteinogenic amino acids are commercially available as their *N*-Fmoc protected OPfp ester derivatives (**Figure 13**)[9].

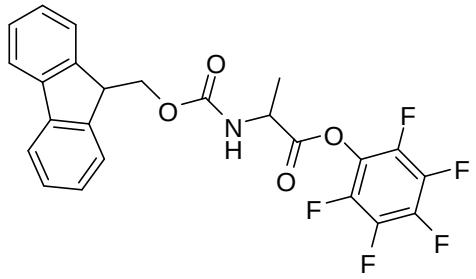


Figure 13. Pentafluorophenol derived activated ester[9]

Probably the most widely used method for the coupling of amino acids in SPPS is the use of the aminium and phosphonium-based coupling reagents. Among the more popular reagents are PyBOP[20], TBTU[21] and HBTU[22]. These coupling reagents smoothly convert Fmoc-amino acids, in the presence of a tertiary base, into their corresponding OBt esters (**Figure 14**)[22].

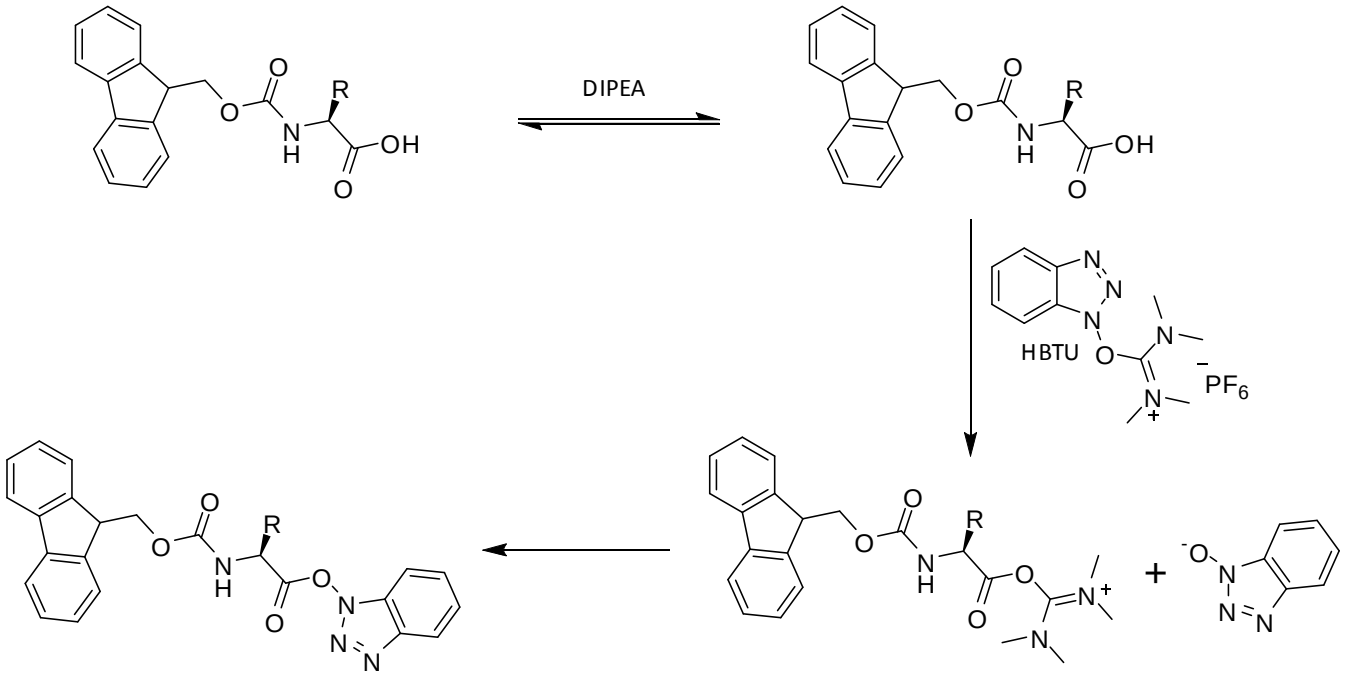


Figure 14. Preparation of OBt-esters using HBTU[22]

The reactivity of the analogous aminium and phosphonium derivatives are essentially equivalent. However, the aminium-based reagents have been observed to cap the resin-bound amino groups through formation of N-terminally guanidinated peptides (**Figure 15**) [1].

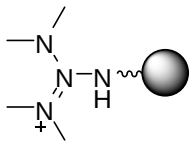


Figure 15. Capping of resin bound amino groups[1]

The methods for coupling amino acids during SPPS involve the *in situ*- or pre-activation of the carboxyl function of the incoming protected amino acids. In order to drive the acylation reaction to completion a large excess of activated reagent is used, usually 2-10 times the resin functionality. Each of the methods has found success in SPPS[1,9].

1.3 Side Reactions and Size Limitations of SPPS

SPPS is not without its limitations. Of the twenty proteinogenic amino acids all but glycine have a chiral center of L-configuration at their α-carbon atoms. The chirality of peptides and proteins give rise to their biological activity, so maintaining the integrity of these centers is of paramount importance in peptide synthesis. Epimerization, with subsequent loss of chiral integrity, is among the most important side reaction to be suppressed when synthesizing peptides: both solution phase and solid phase. Epimerization is a process that occurs at the C-terminal amino acid, upon activation of its carboxyl group, through an intramolecular cyclization reaction to from a planar intermediate known as an azlactone, followed by a deprotonation step to give a resonance-stabilized anion (**Figure 16**) [23].

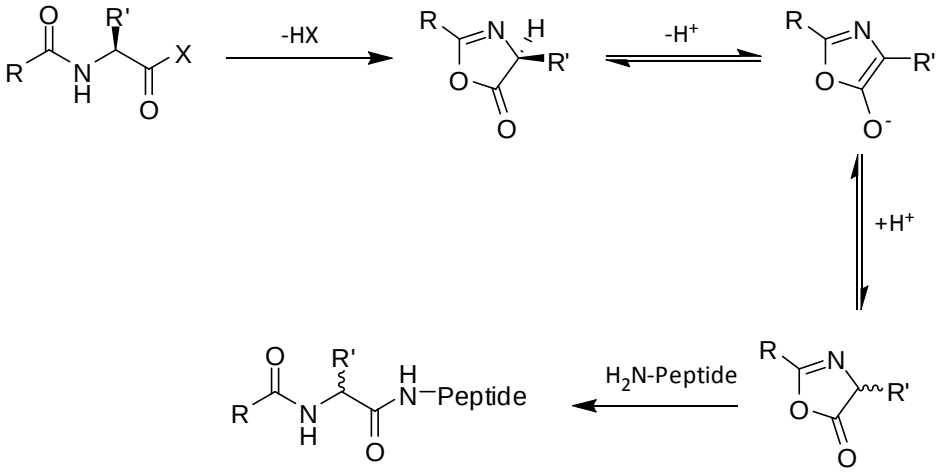


Figure 16. Epimerization via azlactone formation[23]

Due to its acidity (pKa ~ 8.9)[23] the azlactone is prone to deprotonation by excess base present in the reaction solution. Fortunately, the addition of HOBt (pKa ~ 4.6)[9] to most coupling reactions will suppress this deprotonation and help to retain the chiral integrity of the synthesized peptide.

A particular side reaction can occur at the dipeptide level, after removal of the N-terminal protecting group, resulting in the loss of overall yield of the desired peptide: diketopiperizine formation (**Figure 17**)[24].

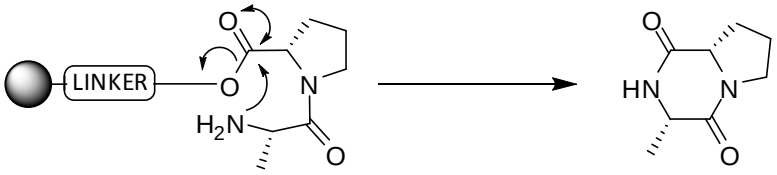


Figure 17. Diketopiperizine formation[24]

Peptides containing proline or *N*-alkylated amino acids in the C-terminal dipeptide sequence present special problems because of the ease with which these dipeptides cyclize[25]. This problem can be minimized, if not eliminated, by the use of the bulky 2-chlorotrityl resin, by introduction of a

preformed dipeptide to the first amino acid attached to the resin, or by avoiding a C-terminal ester linkage[26].

Aspartimide formation is the side-reaction most likely to be encountered in the routine SPPS of peptides containing aspartic acid or asparagine residues. The reaction involves the attack of the C-terminal nitrogen of the amide bond of aspartic acid or asparagine, on the side-chain ester or amide group respectively, resulting in the formation of a succinimide. This intermediate can suffer a number of fates: it can undergo ring opening with piperidine during Fmoc-removal, leading to the formation of the corresponding α- (**13**) or β-piperidine derived amides (**14**), or it can survive cleavage from the resin, to later hydrolyze in solution, producing the corresponding α- (**15**) or β-aspartyl peptides (**16**) (**Figure 18**) [27,28,29].

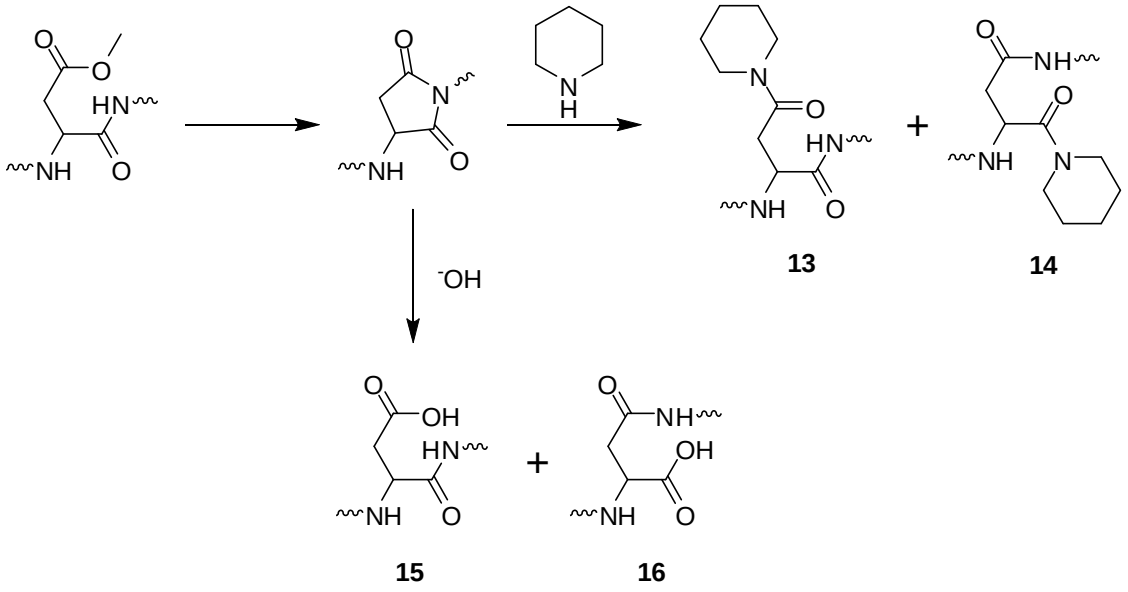


Figure 18. Aspartimide formation[27,28,29]

This reaction is highly sequence specific, occurring frequently in peptides containing the Asp(OtBu)-X motif. Where X = Asn(Trt), Gly, Ser or Thr. The only solution to this problem that is completely

effective is the temporary protection of the vicinal nitrogen. The amino acid preceding the problematic Asp or Asn residue can be protected as its *N*-Hmb amino acid prior to coupling. The Hmb-protecting group is removed simultaneously with cleavage of the peptide from the resin[30,31].

With the improvements in linker, protecting group, and cleavage strategies made over the last decades, the cause of failure in Fmoc-based SPPS is now most likely aggregation[32]. Growing peptide chains built up on the solid support can form secondary structures or aggregates either with other peptide chains or with the polymer support. Inter- and intra-chain hydrogen bonding and hydrophobic interaction are thought to be the cause of this phenomenon. Insertion of proline or other secondary amino acids at every fifth or sixth residue can disrupt peptide aggregation. Additionally, incorporation of secondary amino acids has the added benefit of improved solubility properties[33].

With the careful execution of the standard methods developed for SPPS, it is not unreasonable to expect good to moderately pure peptides of up to 60 amino acids[1]. However, as the chain lengths increases, the separation of the desired peptide from the accumulated by-products becomes increasingly difficult. Incomplete Fmoc-removal gives rise to truncated sequences. Hyrodgen-bonding leads to chain aggregation, reducing the solubility of the peptide, and chain folding hinders reactants from reaching the reactive sites on the peptide. For these reasons, peptide chemists have developed convergent methods for the synthesis of large peptides and small proteins: chemoselective ligations.

1.4 Peptide Ligation Methods

One of the most powerful ligation methods for the productions of large peptides or small proteins is a technique called Native Chemical Ligation (NCL) developed by Kent (**Figure 19**) [34].

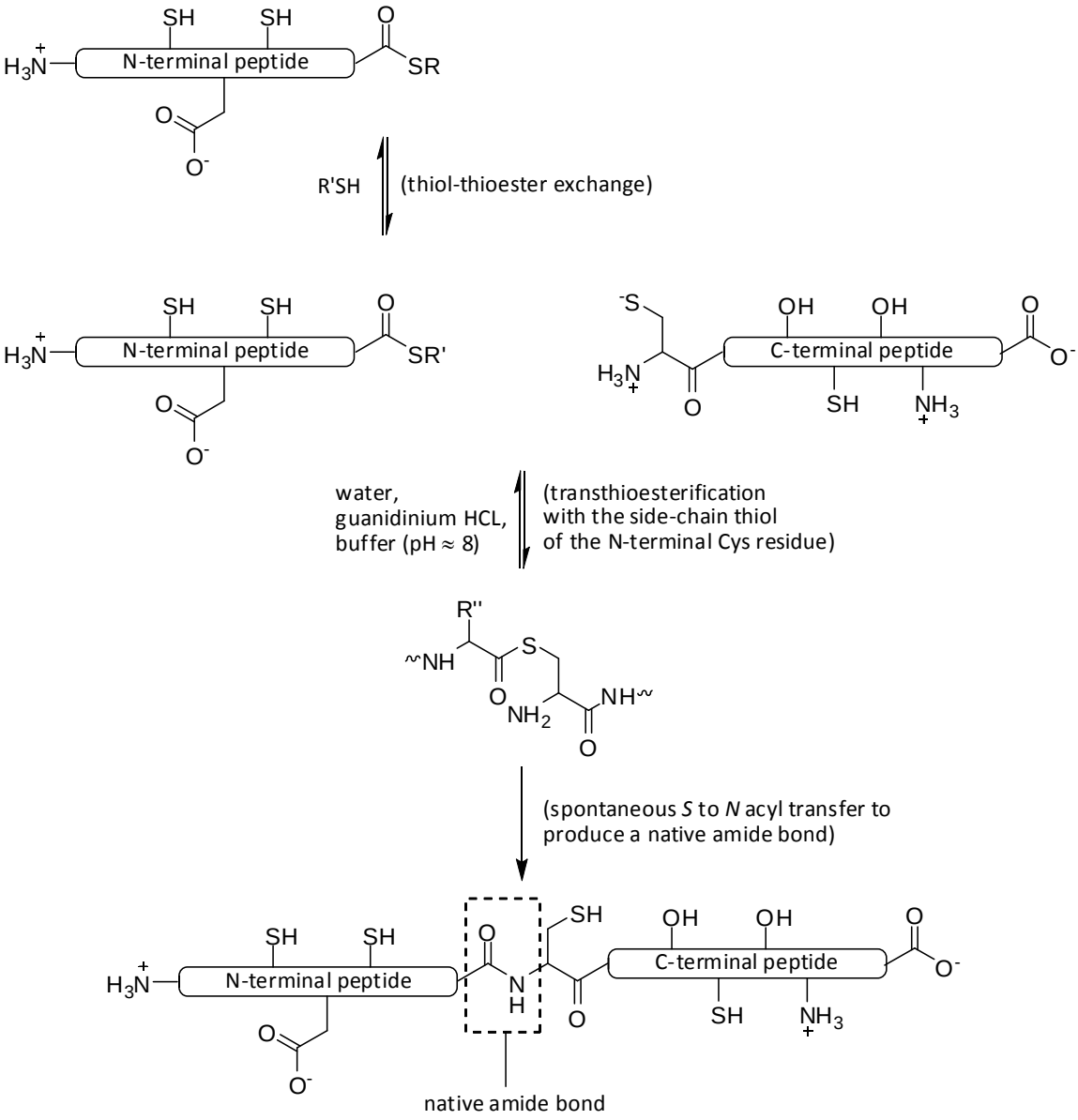


Figure 19. General scheme of Native Chemical Ligation[34]

In this technique, an unprotected synthetic peptide bearing a C-terminal α-thioester is reacted in a chemoselective manner with an unprotected peptide containing an N-terminal cysteine residue in an aqueous environment under denaturing conditions at pH 8. Though powerful, native chemical ligation has a serious limitation. The method has an absolute reliance on the formation of a peptide bond to a cysteine residue. Naturally occurring proteins have a low abundance of cysteine residues; comprising

only 1.7% of all residues[35]. Natural Xaa-Cys bonds may not repeat in a protein within few enough residues for peptide segments to be constructed by SPPS.

Methods have been designed that circumvent the need for a cysteine residue at the ligation site. One such strategy is the use of removable auxiliaries. These auxiliaries act as cysteine surrogates to mediate the ligation of peptide fragments. Auxiliaries are appended to the N-terminus of a peptide. A second peptide is activated as a C-terminal thioester. After a capture step, *S* to *N* acyl transfer produces a native amide bond (**Figure 20**)[36-39].

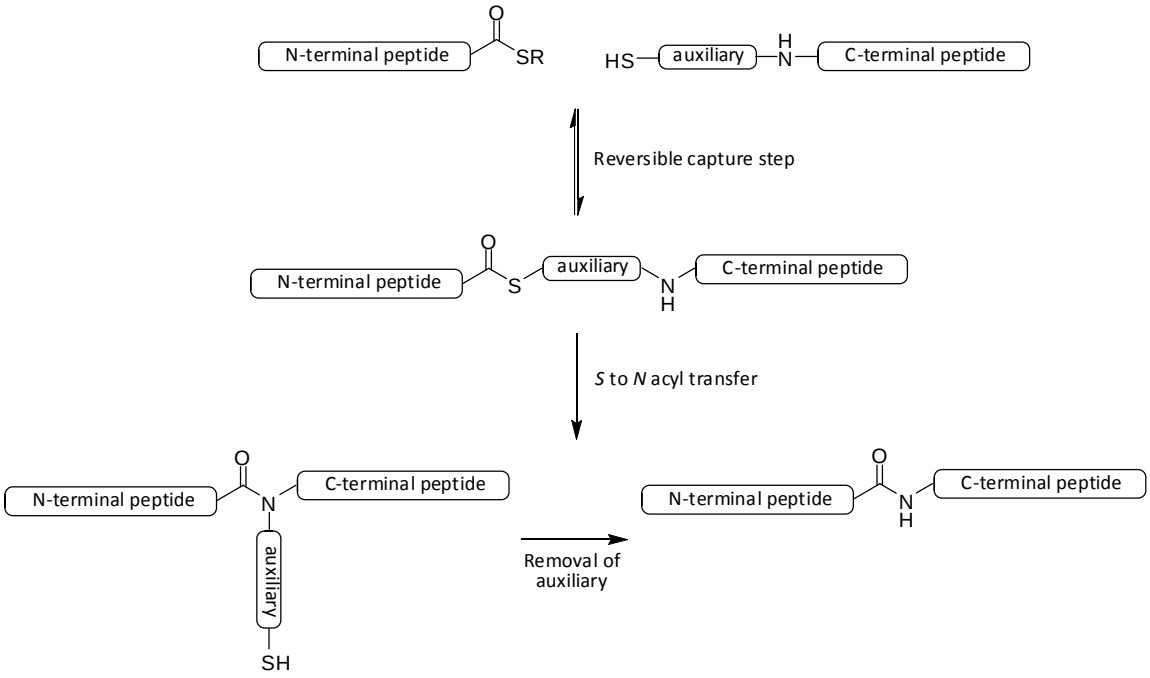


Figure 20. Auxiliary-mediated peptide ligation[36-39]

Three classes of auxiliaries have been designed to accomplish the successful ligation of peptide fragments: *N*-ethanthiol and *N*-oxyethanethiol (**class 1**)[36], *N*-2-mercaptobenzylamine based auxiliaries (**class 2**)[37] and an *N*-(1-phenyl-2-mercaptoethyl) based auxiliaries (**class 3**)[38,39] (**Figure 21**).

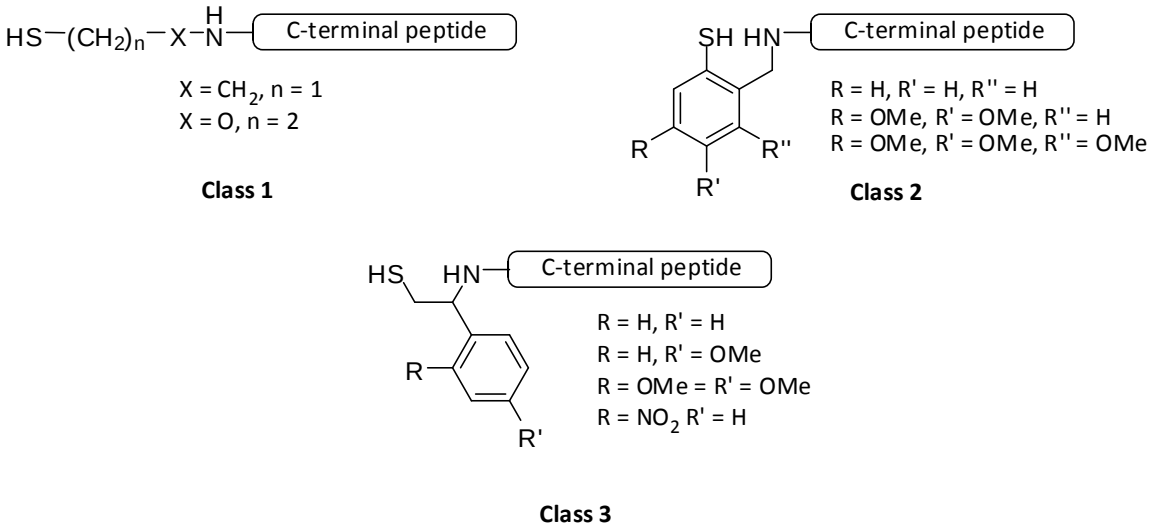


Figure 21. Classes of removable auxiliaries[36-39]

To date, however, removable auxiliaries have been successful only for glycine or alanine N-terminal residues.

The removable auxiliary strategy is modeled after NCL, where the peptide fragments are first captured in a thiol-exchange reaction followed by *S* to *N* acyl transfer to produce the native peptide bond. An orthogonal approach to NCL is a method termed the Staudinger ligation. In the capture step, a peptide that has a C-terminal phosphinothioester reacts with a peptide that has an N-terminal azide to form an iminophosphorane (**Figure 22**)[40].

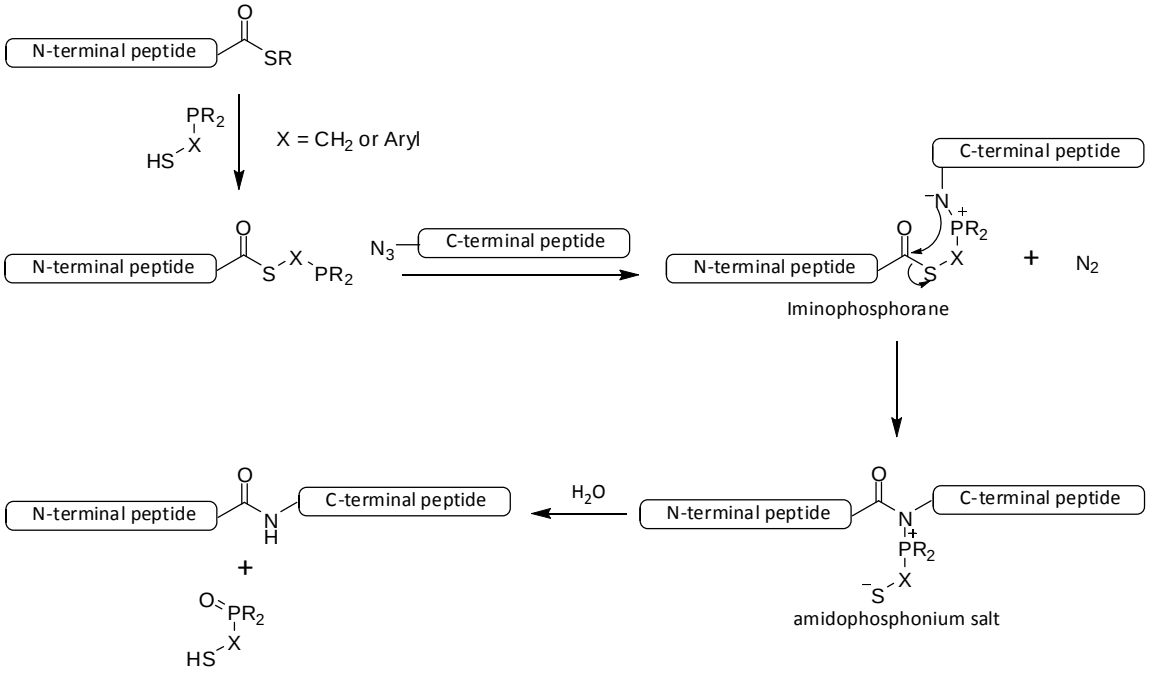
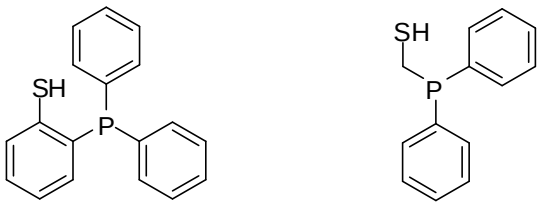


Figure 22. Staudinger ligation mediated by phosphinothiol[40]

Two phosphinothiol derivatives have been used in the successful ligation of peptide fragments (**Figure 23**)[41].



17 **18**

Figure 23. Phosphinothiols used in the Staudinger ligation[41]

The aryl derivative (**17**) was a first generation phosphinothiol which reacts through a six-membered ring transition state and resulted in low yields and long reaction times. The alkyl derivative (**18**)

reduces the ring size in the transition state to the more favorable five-membered ring. This phosphinothiol derivative was used in the assembly of RNase A[41].

Native Chemical Ligation requires an N-terminal cysteine residue for successful ligation with a C-terminal thioester. Removable auxiliaries circumvent the need for an N-terminal cysteine residue by using a cysteine surrogate in reaction with C-terminal thioesters. The Staudinger ligation presented an orthogonal approach to both of these reactions by utilizing an N-terminal azide. However, in each case the preparation of a C-terminal thioester is imperative.

Glycopeptides profoundly affect protein folding, stability, immunogenicity, and biological activity. To understand the relationship between glycan structure and glycopeptide function, and to facilitate the development of glycopeptide pharmaceuticals, homogeneous glycopeptides bearing well-defined glycan structures must be obtained. Glycopeptides can be readily prepared through standard Fmoc-based SPPS. The extension to small or medium sized glycoproteins can be obtained by combining SPPS with peptide ligation techniques.

1.5 Preparation of C-terminal Peptide-α-Thioesters

Thioesters can be prepared routinely by the Merrifield method for SPPS however, this technique is not compatible with the preparation of glycopeptides[42,43,44]. After the introduction of NCL, chemists sought ways to combine the preparation of the requisite C-terminal thioesters with the glycopeptide compatible Fmoc-based SPPS technique. The problem with this strategy is that thioesters are labile to aminolysis during the Fmoc deprotection step.

Fukati *et al.* performed the first peptide thioester synthesis using Fmoc-based SPPS. After peptide elongation and cleavage to give the free acid, the desired C-terminal thioester was installed

using DCC in the presence of HOBt[45]. Aimoto *et al.* prepared a 25-mer peptide thioester on a Fmoc-Gly-SC(CH_3)$_2$$CH_2$CONH-SAL-β-Ala-MBHA resin using an Fmoc-deblocking solution consisting of 1-methylpyrrolidine, hexamethyleneimine and HOBt in a one to one mixture of NMP and DMSO[46]. In a similar fashion, Clippingdale *et al.* reported the synthesis of a 10-mer peptide thioester using the non-nucleophilic base 1,8-diaza-bicyclo[5.4.0]undec-7-ene (DBU) in combination with HOBt in DMF for the deprotection of the *N*-Fmoc protecting group during SPPS[47]. Epimerization, undesired cleavage of the peptide from the solid support at the mono and dipeptide level and aspartimide formation, respectively, are the major limitations of these developed methods.

Alsina *et al.* reported the introduction of the α-thioester group at the end of the synthesis of the target peptide. Key to their approach was the use of the backbone amide linker (BAL) which was employed to generate medium sized peptide thioesters using an Fmoc-based strategy (**Figure 24**)[48].

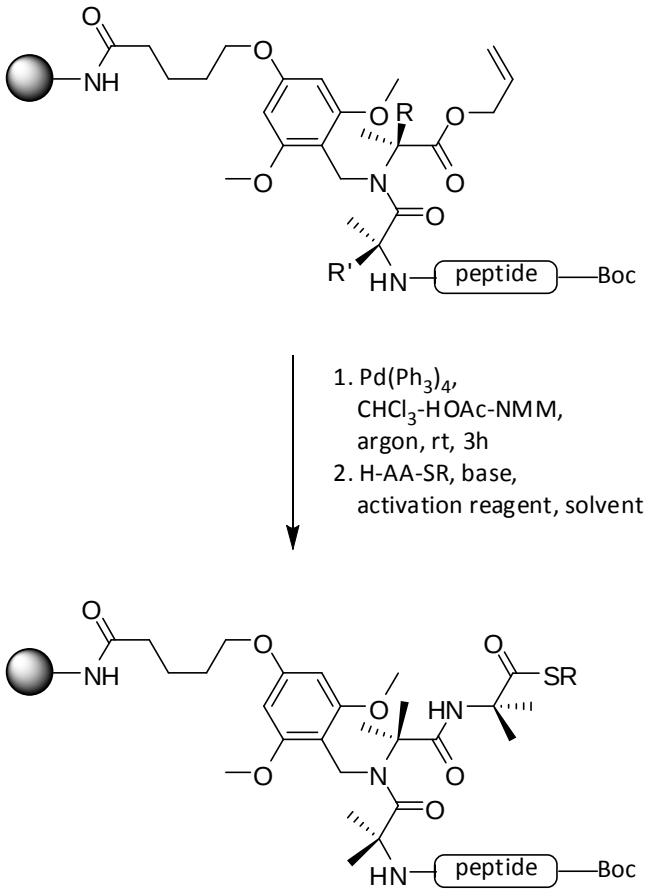


Figure 24. Fmoc-based method for the preparation of C-terminal thioesters[48]

This strategy relies on anchoring the growing peptide through the backbone nitrogen instead of the C-terminal carboxyl group. Once the synthesis is completed, the carboxyl protecting group is selectively deprotected and then thioesterified with a preformed amino acid thioester residue[49]. The main limitations of this approach are epimerization of the C-terminal residue and diketopiperizine (DKP) formation.

Brask *et al.* overcame the DKP formation by anchoring an amino trithioortho ester derived from glycine to the BAL support. Trithioortho esters are resistant to nucleophilic attack and therefore remain intact at the dipeptide level. Trifluoroacetic acid cleavage from the BAL support realizes the C-terminal glycylthioester (**Figure 25**)[50].

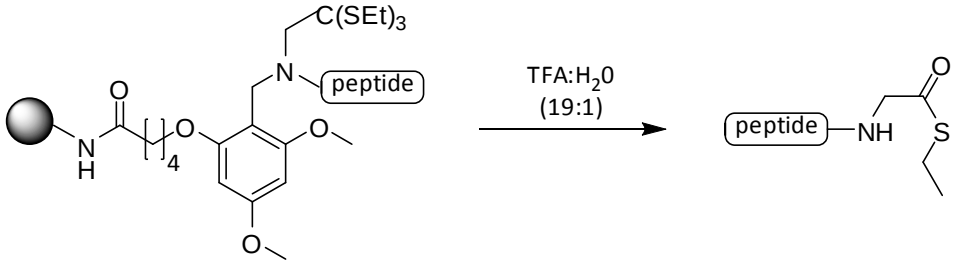


Figure 25. C-terminal thioesters by masking as triorthothioesters[50]

An alternative Fmoc-compatible approach for the production of peptide α-thioesters was first reported by Swinnen *et al.* This synthetic scheme involves the assembly of the target peptide in either a PAM or Wang resin using standard Fmoc-based SPPS and then cleavage of the protected peptide resin with an excess of Me_2AlCl and EtSH in DCM (**Figure 26**)[51].

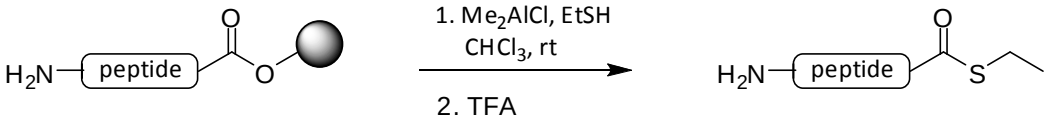


Figure 26. Direct cleavage of thioesters from Pam or Wang resins[51]

This approach was used to generate different peptide α-thioesters in different yields, ranging from 20-60%. However, some problems arising from epimerization of C-terminal residues other than glycine and undesired reactions, especially formation of side chain thioesters and aspartimide rearrangement, were also observed.

Ingenito *et al.*[52] and Shin *et al.*[53] independently reported in 1999 the Fmoc-based SPPS of C-terminal thioesters using Kenner's acyl-sulfonamide safety catch linker modified by Backes *et al.*[54] (**Figure 27**)[52,53].

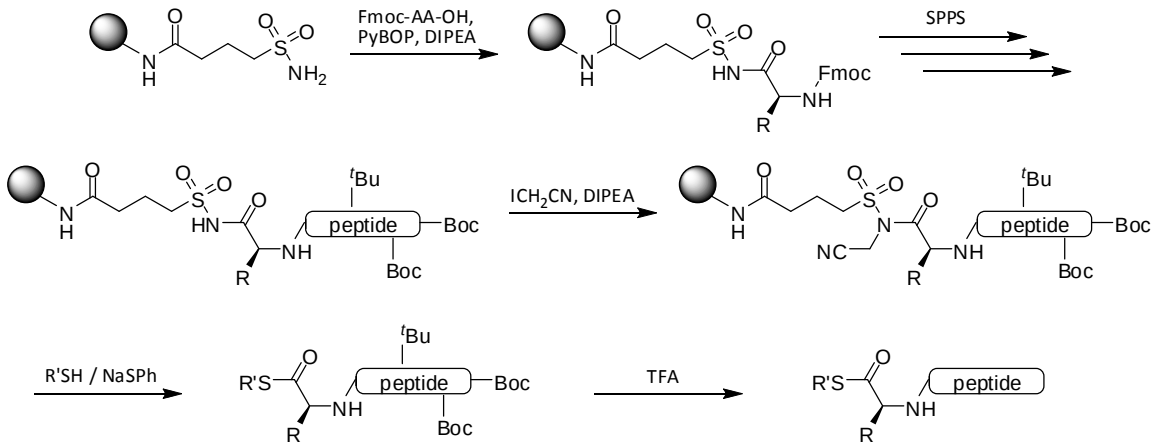
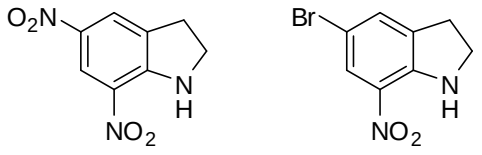


Figure 27. C-terminal thioesters using an alkanesulfonamide "safety catch" linker[52,53]

This acyl-sulfonamide linker is completely stable to basic or strongly nucleophilic conditions and can be activated by treatment with either trimethylsilyl-diazomethane or iodoacetonitrile to provide an *N*-alkyl acylsulfonamide, which is susceptible to nucleophilic attack. Since its introduction in 1997, this approach has been used by several groups for the synthesis of different peptide thioesters involved in the production of different glycoproteins[55], phosphoproteins[56,57], Se-containing proteins[58] and cyclic proteins[59] by NCL.

1.6 Photochemistry of Nitroindoline Derivatives

In 1975 Amit *el al*. introduced to the chemical community protecting groups for carboxylic acids based on a 7-nitroindoline motif that utilizes photochemistry for their removal (**Figure 28**)[60].



19 **20**

Figure 28. 7-Nitroindoline derivatives[60]

Irradiation of amides**,** derived from 5,7-dinitroindoline (**19**) and 5-bromo-7-nitroindoline (**20**)**,** in the presence of water (approx. 1% in an aprotic organic solvent), furnished the free carboxylic acids and the starting indolines in nearly quantitative yields.

Six years later Pass *et al*. utilized the unique photochemical properties of **20** in their development of a method for the racemization-free coupling of peptide segments in combination with Fmoc-based solution phase peptide synthesis (**Figure 29**)[61].

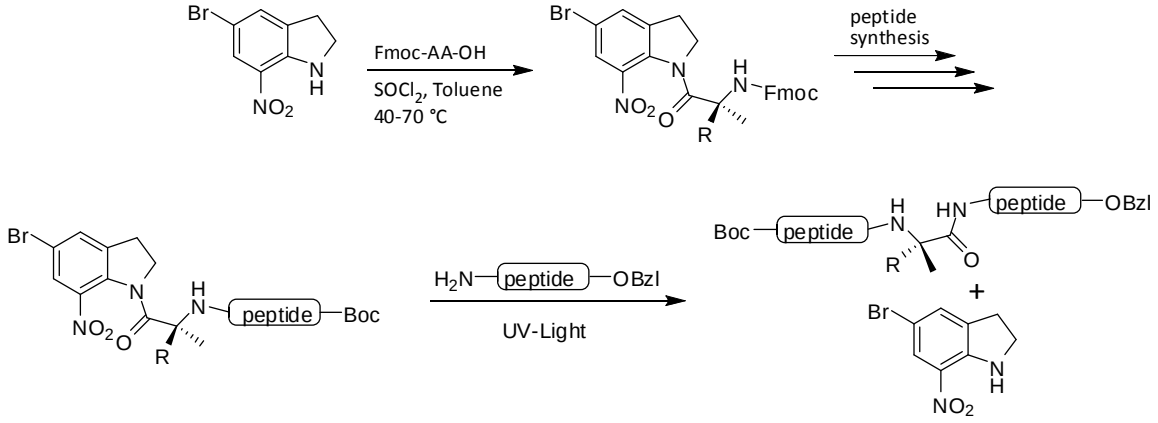


Figure 29. Photochemical coupling of peptide segments[61]

Papageorgiou *et al*. investigated the use of *N*-acyl-7-nitroindolines for the rapid release of glutamic acid in aqueous media (**Figure 30**)[62].

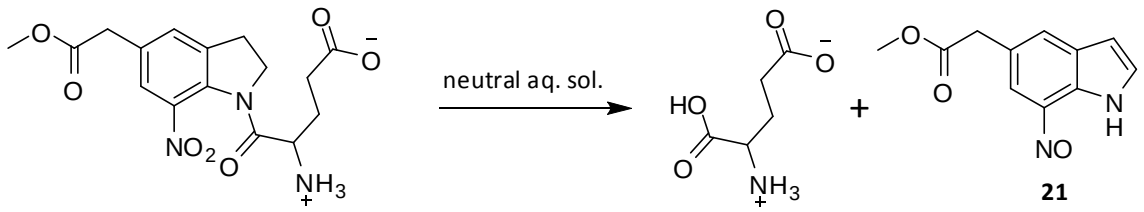


Figure 30. Photorelease of L-glutamate[62]

During their investigation, the Corrie group witnessed the formation of a product that had not been reported in literature. L-glutamate was efficiently released but the by-product was not the 7-nitroindoline derivative that they expected, instead they observed the formation of the 7-nitrosoindole derivative (**21**).

Formation of different products implies a different mechanism. Different photoreactivity of nitroaryl compounds in aqueous or organic solvents has been explained by formation of a highly polarized π,π^* triplet state in water and an n,π^* state in organic solvents[63]. An investigation into the mechanism of the photorelease of carboxylic acids from 1-acyl-7-nitroindolines was performed by Papageorgiou *et al*. The data supports a mechanism that involves reaction of a photo-generated nitronic anhydride (**21**), obtained via photochemical transfer of the acetyl group from the amide nitrogen to one of the oxygen atoms of the nitro group. Two competing pathways from reaction of **21** produce two different products. In solutions of higher water content, the predominant reaction pathway of **21** results in an intramolecular redox reaction of the aromatic ring system, releasing the carboxylic acid and 7-nitrosoindole (**22**). In solutions of low water content, the major pathway for hydrolysis of **21** is by nucleophilic attack of water as the nucleophile, releasing the carboxylic acid and 7-nitroindoline (**23**) (**Figure 31**)[64].

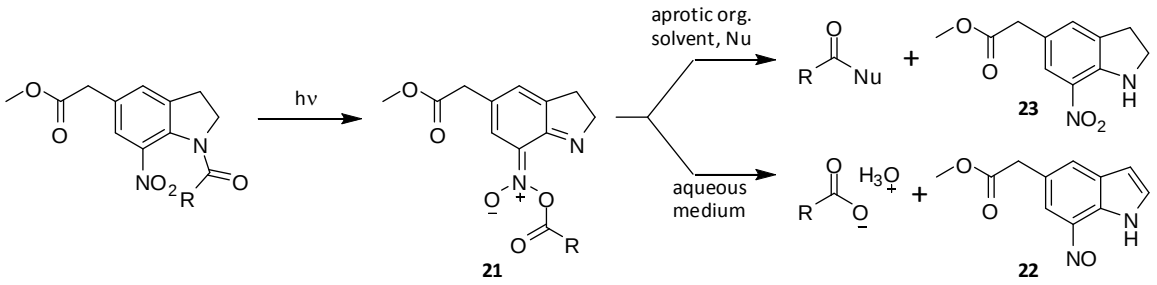


Figure 31. Proposed mechanism of photorelease[64]

Michael *et al*. extended the photochemistry of 7-nitroindoline derivatives to the glycosylation of *N*-protected asparagine residues (**Figure 32**)[65].

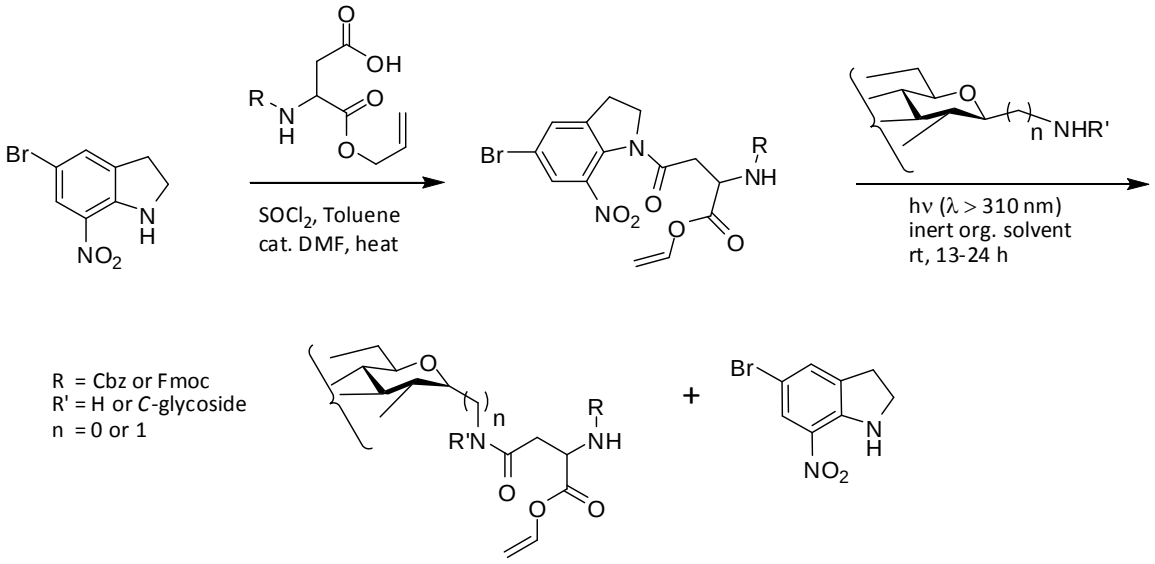


Figure 32. Glycosylation of photoreactive amino acids[65]

The *N*-aspartyl-5-bromo-7-nitroindolines are latent nitronic anhydrides, which become activated upon irradiation with near-UV light. In inert organic solvents (THF or TMU) amino saccharides, including relatively weakly nucleophilic glycosylamines and partially unprotected 1-*C*-aminomethyl-β-D-glucopyranosides, become acylated.

Kaneshiro and Michael utilized this chemistry to develop a method for the convergent synthesis of glycopeptides (**Figure33**)[66].

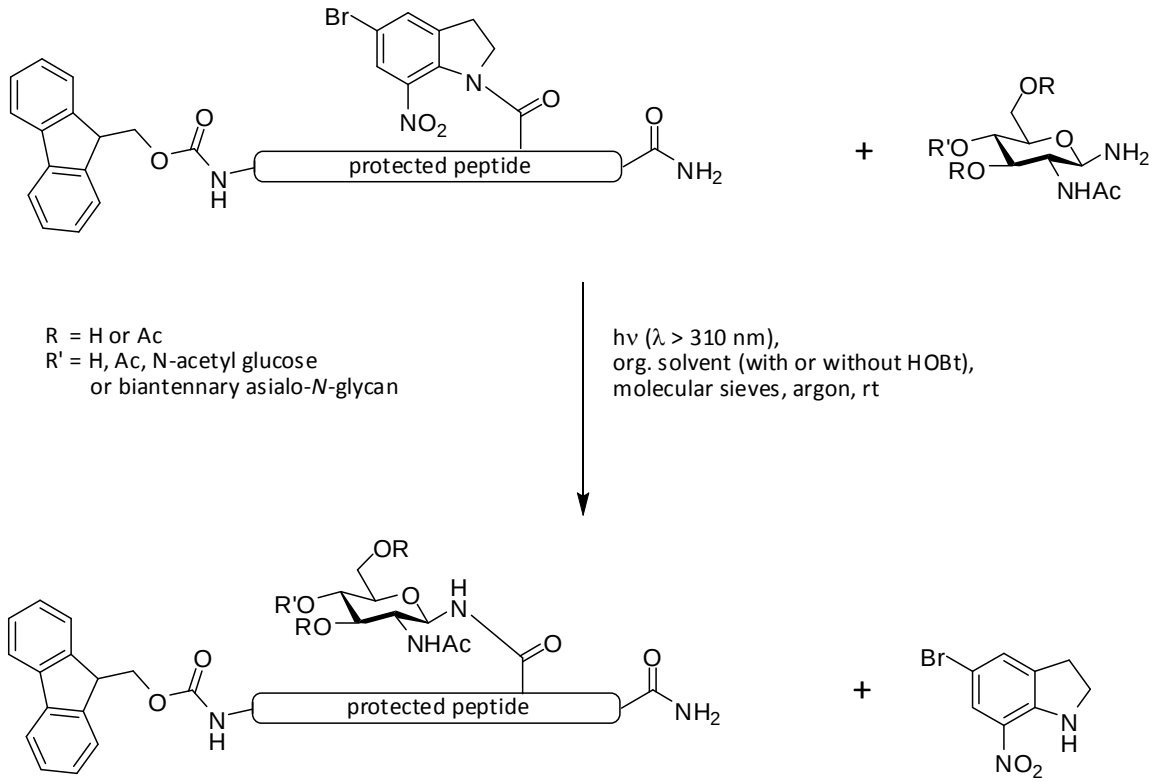


Figure 33. Convergent photochemical glycopeptide synthesis[66]

The photocoupling reaction is conducted under neutral conditions and offers a solution to the long-standing "aspartimide problem". This method provides a shorter synthetic route to *N*-glycopeptides by UV-light activation of the same group that serves as a protecting group for the aspartic acid side chain during peptide synthesis.

The following summarizes the most important milestones toward the development of Fmoc-compatible peptide-α-thioester synthesis over the last decades. Merrifield *et al*. introduced the community to a novel method for the synthesis of peptides (SPPS). This method opened the door for

the synthesis of moderately sized peptides, however, access to glycopeptides was not available. Caprino *et al*. introduced the Fmoc-group which provided an orthogonal protecting group strategy that both expanded the utility of SPPS and gave access to the biologically important phospho- and glycopeptides. Kent *et al*. expanded SPPS further with the technique of NCL, allowing chemist to synthesize large glycopeptides and small proteins. This method required chemists to develop high yielding methods that produce optically pure C-terminal peptide thioesters. The chemical community devised a number of ways to achieve this goal. Each of the developed methods has found utility in Fmoc-based SPPS, however, each suffer from undesired side-reactions, low yield or are amino acid dependent. This dissertation offers a solution to the problems associated with C-terminal peptide-α-thioester syntheses, by describing a more general, high yielding method that produces optically pure products. Our method takes advantage of the photochemical acylation of thiols under neutral conditions, minimizing the notorious problems of thioester cleavage and epimerization.

CHAPTER II

Results and Discussion

2.1 Hypothesis

Protected amino acid-α-thioesters and protected peptide-α-thioesters can be synthesized, photochemically, in high yield and purity, in the absence of protic solvents, with negligible epimerization at the C-terminal amino acid, using a developed 7-nitroindoline-5-carboxylic acid linker in combination with Fmoc-based solid phase peptide synthesis (SPPS). Peptide-α-thioesters can be cleaved from the solid support allowing for analysis and photochemical thioesterification in solution, alternatively, the peptide-α-thioesters can be prepared be direct photorelease from the resin.

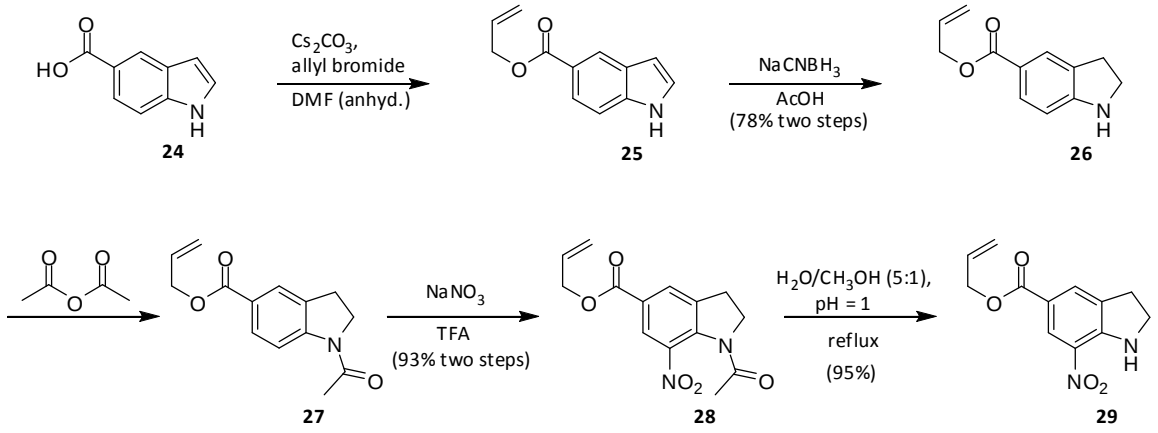
2.2 Goals and Objectives

In order to prove the hypothesis correct a number of goals would have to be reached. Our first goal was to design a synthetic scheme based on literature protocols that allows for a high yielding route to the desired photoreactive linker that is compatible with SPPS. After successful completion of the synthesis, our next goal was to prepare three photoreactive tripeptides using Fmoc-based SPPS strategies. The prepared tripeptides were then to be utilized in our efforts to prove the hypothesis correct. In order to accomplish this task, a search for the optimal conditions for a high yielding reaction that produces high purity peptide-α-thioesters would begin. With success at this stage, a communication was going to be prepared for publication. The next goal was to expand the scope of this chemistry to include a variety of C-terminal amino acids, creating a general method for the preparation of protected amino acid-α-phenythioesters. We would then build on this success with the

expansion of the peptide thioester method to a general method that includes C-terminal amino acids representative of those found in naturally occurring proteins. The final goal was to extend the developed methodology to the production of glycopeptide-α-thioesters. After accomplishing these goals, our developed methods, in combination with NCL, would be applicable to the construction of natural and designer proteins.

2.3 Synthesis of the 7-Nitroindoline Linker for SPPS

The synthesis of the desired 7-nitroindoline linker began with the commercially available indole-5-carbxylic acid (**24**) and was modeled after a similar synthesis by Nicolau *et al*[67] (**Scheme 1**)[68].



Scheme 1. Synthesis of nitroindoline linker SPPS[68]

This synthesis required a careful protecting group strategy for success. The carboxy function had to be protected in such a way as to survive the conditions of the entire synthesis. To this end, indole-5-carboxylic acid (**24**) was converted to its allyl ester using cesium carbonate and allyl bromide in dimethylformamide (DMF) to give allyl indole-5-carboxylate (**25**) which did not require purification.

Converting the free acid to its allyl ester in the first step fulfills a number of requirements: increased solubility in a number of organic solvents, ease of purification and complete orthogonality to both the *N*-acetyl protecting group on the indoline nitrogen and the *N*-Fmoc protecting group on the amino acids. Furthermore, a free carboxylic acid in the developed linker would result in unwanted products during the coupling of amino acids.

The indole (**25**) was then reduced using sodium cyanoborohydride in acetic acid to give allyl indoline-5-carboxylate (**26**) which was purified prior to acetylation. The indole starting material (**25**) and the indoline product (**26**) have very close R_f values in a number of solvent systems, however, they differ in their activity toward long-wave UV light (λ = 365 nm): **26** fluoresces whereas **25** does not. This observation allowed for the proper analysis of the reaction by TLC and to determine the end of the reaction. The purified allyl indoline-5-carboxylate (**26**) was then acetylated, quantitatively, using acetic anhydride to give allyl *N*-acetylindoline-5-carboxylate (**27**). The *N*-acetyl protecting group and the allyl ester are orthogonal which allowed for the selective removal of one in the presence of the other. Site directed nitration of (**27**) using sodium nitrate in trifluoroacetic acid (TFA) resulted in allyl 7-nitro-*N*-acetylindoline-5-carboxylate (**28**). Finally, removal of the *N*-acetyl protecting group in refluxing methanolic aqueous acid at pH 1 resulted in the desired product, allyl 7-nitroindoline-5-carboxylate (**29**) in 69 % overall yield. Alternatively, the *N*-acetyl protecting group could be removed by photolysis in 1% aqueous acetonitrile over 24h.

The successful construction of allyl 7-nitroindoline-5-carboxylate (**29**) provided the basis compound for the construction of the desired photoreactive amino acids and photoreactive peptides to be utilized throughout the method development.

2.4 Synthesis of our Initial Three Photoreactive Tripeptides

Early on we envisioned that the small non-sterically demanding amino acids glycine (Gly) and alanine (Ala) would allow us to accomplish our first goal with the least problems. Glycine is the single proteinogenic amino acid that is achiral and without a formal side-chain. These two properties eliminate the worry of epimerization and steric hindrance that is associated with the chemistry of C-terminal amino acids. Alanine contains the simple side-chain methyl group and is a chiral amino acid. Preparation of photoreacitve peptides from both the L- (**31**) and D- (**32**) stereoisomers of alanine would allow us to study the extent of epimerization that the C-terminal amino acid may undergo during thioesterification (**Figure 34**).

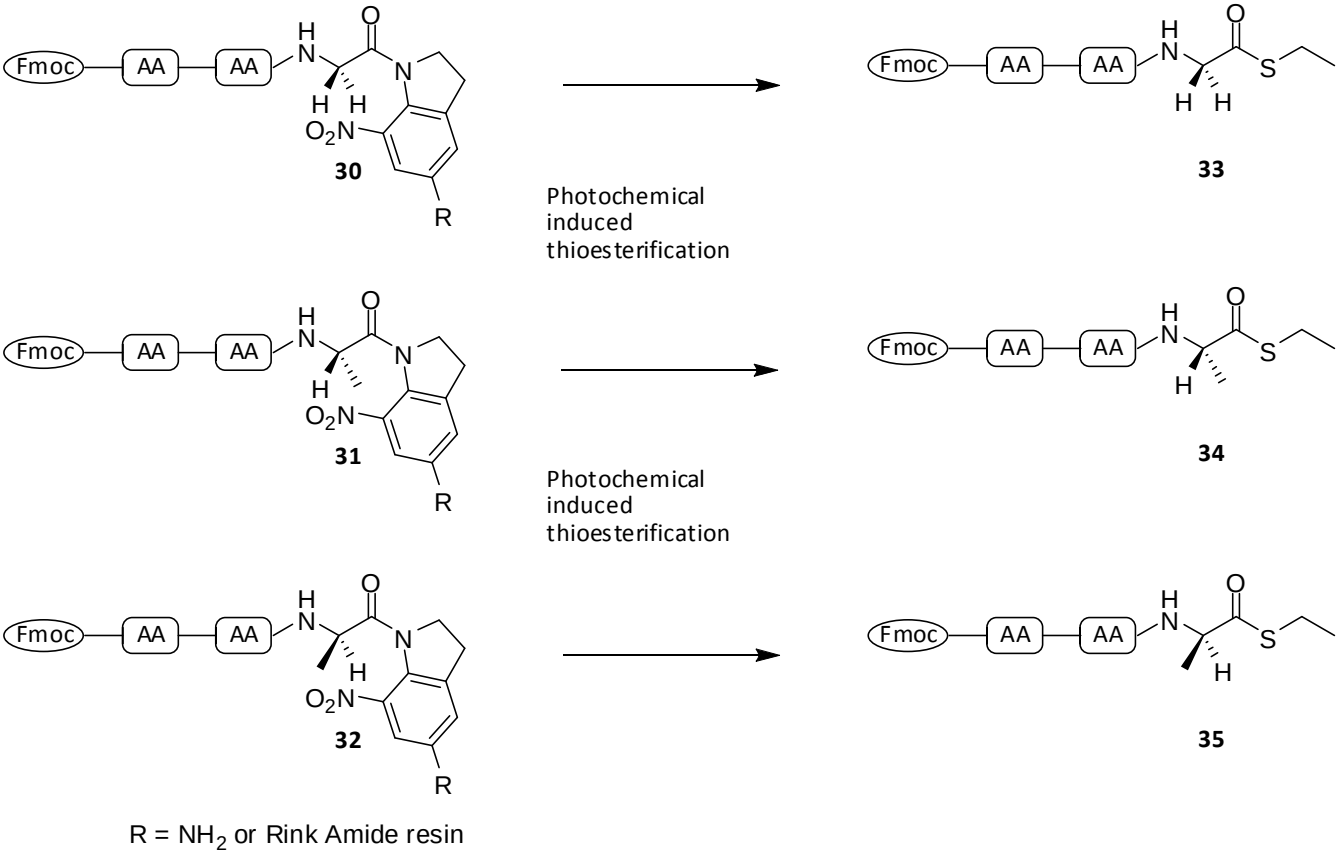
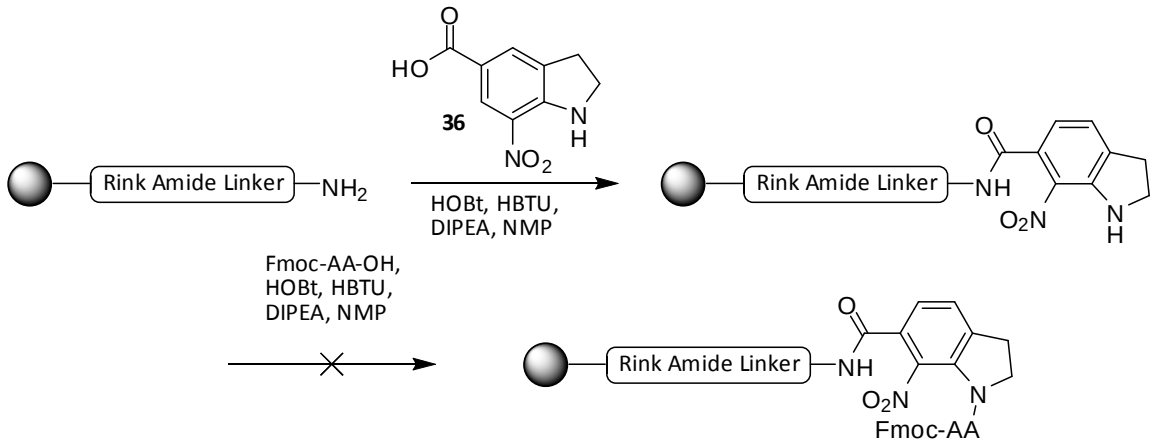


Figure 34. General scheme for C-terminal thioesterification

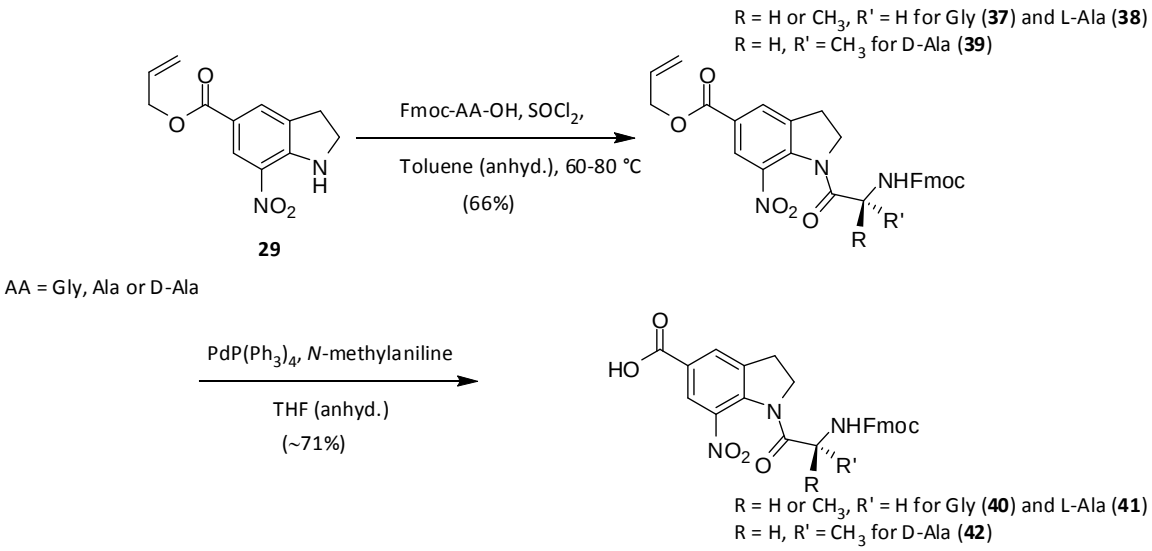
The original synthetic scheme called for the attachment of the photoreactive linker (**29**), after deallylation to give the free carboxylic acid (**36**), to Rink Amide resin followed by coupling of the C-terminal amino acid under standard coupling conditions (HOBt, HBTU, DIPEA, in NMP). Unfortunately, this scheme failed in our hands (**Scheme 2**).



Scheme 2. Original method for attachment of linker to Rink Amide resin

It is believed that due to the weak nucleophilicity of the nitrogen in (**36**) a more reactive acyl-donor is required to accomplish the coupling. A more reactive acyl donor is the amino acid chloride which can be prepared *in situ* from an amino acid and thionyl chloride. The high reactivity of acid chlorides outweighs the low nucleophilicity of the nitroindoline nitrogen and results in a smooth coupling. However, the loss of stability of the resin bound linker during the highly acidic amino acid chloride coupling reaction prevents its use as an acyl donor in the original scheme.

To successfully accomplish a convenient synthesis of photoreactive tripeptides through Fmoc-based SPPS a revised approach was taken (**Scheme 3**) [68].

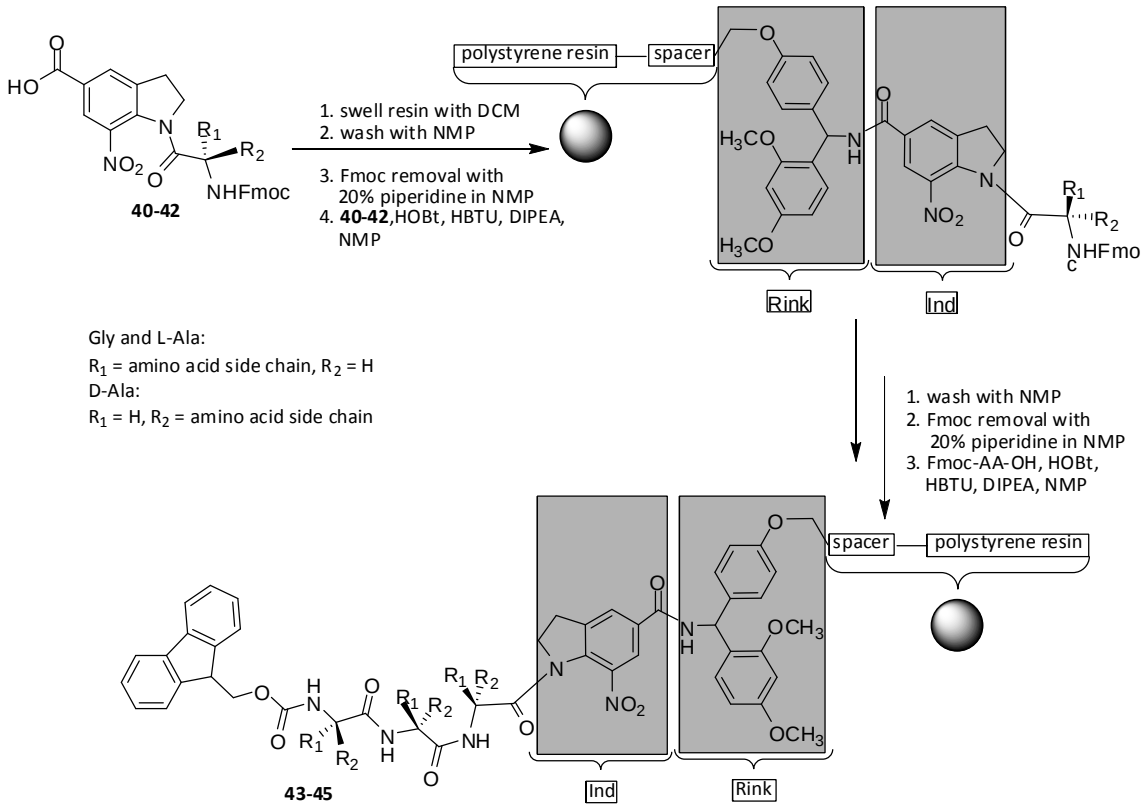


Scheme 3. Preparation of photoreactive amino acids[68]

The photoreactive nitroindoline derivative (**29**) was coupled to an *N*-Fmoc-protected amino acid chloride, generated *in situ* from thionyl chloride in anhydrous toluene, to give the amino acyl derived nitroindoline linker (**37-39**). With the amino acid successfully coupled to the photoreactive linker, deallylation and attachment to the solid support could be readily accomplished. Deallylation of **37-39** in the presence of Pd(0) and *N*-methylaniline in anhydrous THF gave *N*-aminoacyl-7-nitroindoline-5-carboxylic acids (**40-42**).

With the desired amino acid derived linkers (**40-42**) in hand, the Fmoc-based solid phase synthesis of the photoreactive tripeptides began with the proper choice of solid support. It was envisioned that attaching the prepared *N*-amino acyl-7-nitroindoline linker to Rink Amide resin would generate a solid support with two orthogonal linkers suitable for SPPS, providing chemical flexibility for cleaving the peptides from the solid support.

The Fmoc group of commercially available Rink Amide resin was removed with 20% piperidine and *N*-amino acyl-7-nitroindoline-5-carboxylic acids (**40-42**) were coupled, followed by the remaining two amino acids, using standard Fmoc-based SPPS techniques to give the resin bound photoreactive tripeptides (**43-45**) (**Scheme 4**) [68].



Scheme 4. SPPS of photoreactive tripeptides[68]

With the successful construction of our photoreactive tripeptide starting materials, the search for the optimal conditions for a high yielding reaction that produces high purity peptide-α-thioesters could begin.

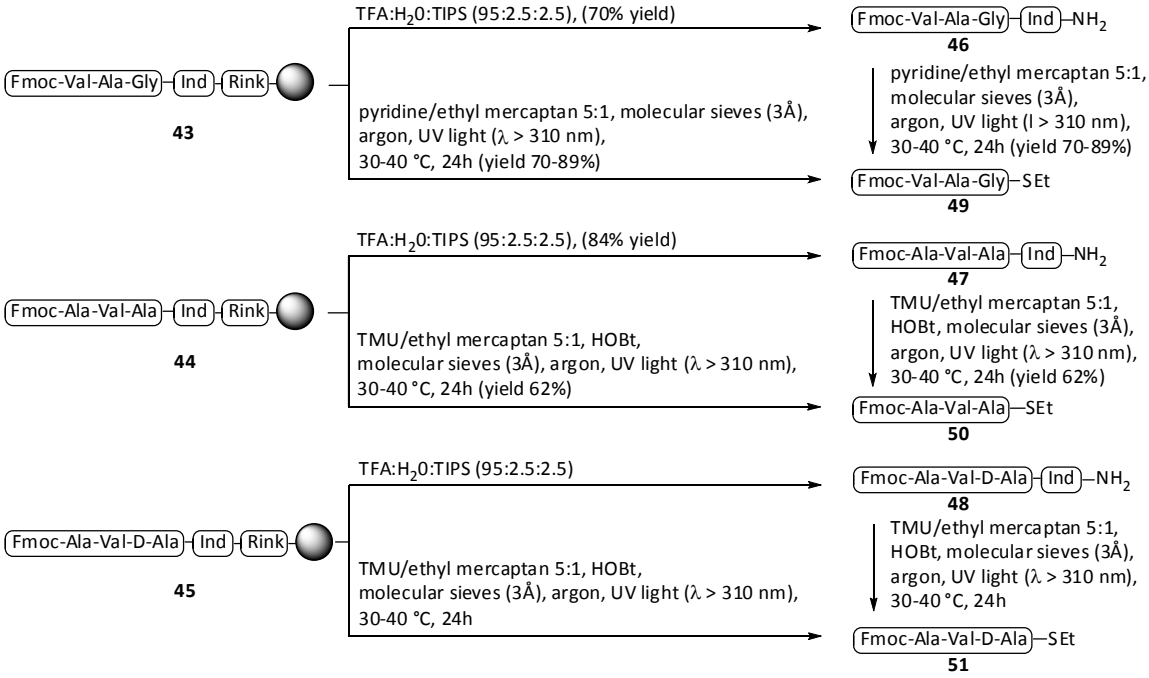
2.5 Initial Method Development

Drawing upon the knowledge gained in this lab from the photoinduced glycosylation of amino acids[65] and the convergent approach for the photoinduced glycosylation of peptides[66], we believed that the photochemical preparation of peptide-α-thioesters could be readily accomplished.

Upon illumination of the photoreactive peptide amides in solution, or directly from the beads, a nitronic anhydride intermediate is produced[64], which, in the presence of ethylmercaptan and inert aprotic solvents, can lead to nucleophilic displacement of the peptide from the nitronic anhydride intermediate, resulting in the desired C-terminal peptide-α-thioester and a nitroindoline amide. However, in the presence of a nucleophile and protic solvents, such as water, a redox mechanism predominates over nucleophilic displacement[64], producing the C-terminal peptide acids and a nitrosoindole (**Figure 31**). As a result, our initial idea was to run the photochemical reactions under dry conditions using an anhydrous aprotic solvent, molecular sieves, HOBt and ethylmercaptan as the nucleophile to produce C-terminal peptide-α-ethylthioesters.

Attempts to generate ethylthioesters in solution or directly from the beads with UV-light (λ > 310 nm), in the presence of ethylmercaptan/DCM (1:6), produced low yields[67,68]. Similar results were observed by others when alcohol nucleophiles were used in their attempts to photoacylate alcohols to produce esters[67]. For the C-terminal glycine tripeptide (**43 or 46**) it was found that the use of pyridine as the solvent led to the desired thioester (**49**) in good yield[68] (**Scheme 5**). A small amount of the peptide acid is usually also present. It originates mostly from base catalyzed hydrolysis of thioester **49** upon prolonged exposure to pyridine under anhydrous conditions. Unfortunately, pyridine can only be used for the non-chiral glycine residue, as it results in significant amounts of unwanted epimerization of all the chiral C-terminal amino acid residues.

In order to investigate the potential occurrence of epimerization, two tripeptides, **47** and **48**, which differ only in their C-terminal configuration, were prepared. Photothioesterification in pyridine gave 86% yield, but resulted in partial epimerization (**50** : **51** ~ 11 : 89). We found that epimerization could be largely suppressed by conducting the photothioesterification in a non-basic solvent in the presence of HOBt. Although substantial amounts of the undesired peptide acids formed when DMSO was used as a solvent, these reaction mixtures proved useful for studying epimerization. HPLC analysis showed that L-configured **47** reacts to the L-configured peptide acid, and the L-configured thioester **50**. Likewise, D-configured **48** reacts to the D-configured peptide acid and the D-configured thioester **51**. The epimerization levels are less than 5%. *N*,*N*,*N*',*N*'-tetramethyl urea (TMU) proved to be a better solvent than DMSO as photoreactive tripeptides (**44** or **47, 45 or 48)** were converted to their ethylthioesters (**50** or **51**) in moderate yield with less than 4% epimerization[68] (**Scheme 5**) [68].



Scheme 5. Thioesterification of photoreactive tripeptides[68]

The results of the thioesterifications using photoreactive tripeptides **43-45** or **46-48** proved that the hypothesis was correct. The developed nitroindoline linker in combination with Rink Amide resin, ethylmercaptan and UV light were successful in photoreleasing three C-terminal (Gly, L-Ala or D-Ala) peptide-α-thioesters. Further, neutral reaction conditions kept epimerization to a minimum as determined by HPLC[68]. With these results we realized the first three of our goals which produced a communication published in the *Journal of Organic and Biomolecular Chemistry*[68].

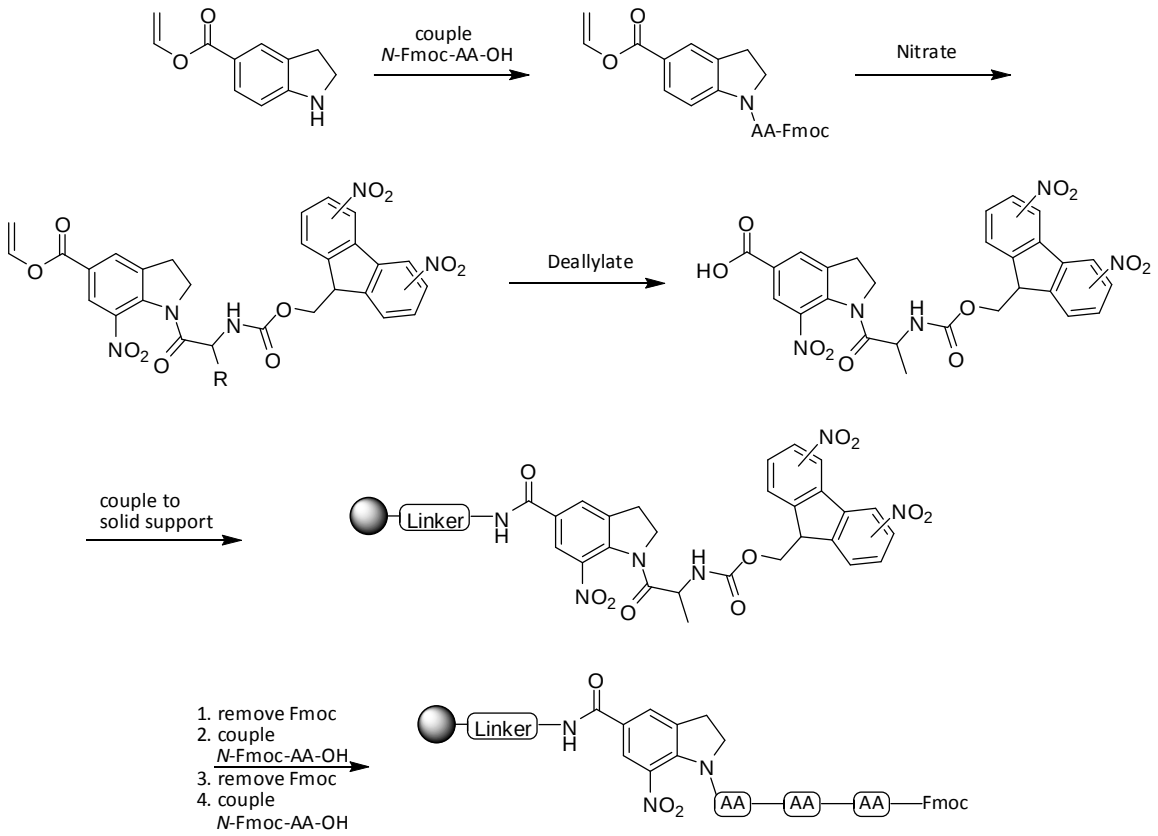
2.6 Initial Efforts to Produce a General Method

For the development of a general method in organic synthesis, the choice of compounds studied must be such that the method tolerates a variety of functional group reactivity. To this end nine amino acids, representative of the R-group functionality present in the 20 proteinogenic amino acids were chosen. Coupling of these amino acids to the developed nitroindoline linker (**29**) was accomplished successfully following Patchornik's acylation procedure with thionyl chloride[60] (**Scheme 3**) (**Table 1**).

Table 1. Protected photoreactive amino acids

Allyl *N*-Fmoc-aminoacyl-7-nitroindoline-5-carboxylate (Scheme 3)	**Isolated % yield**
Fmoc-Gly-Ind-OAll (**49**)	95
Fmoc-Ala-Ind-OAll (**50**)	91
Fmoc-Ile-Ind-OAll (**51**)	88
Fmoc-Phe-Ind-OAll (**52**)	86
Fmoc-Pro-Ind-OAll (**53**)	86
Fmoc-Lys(Tfa)-Ind-OAll (**54**)	86
Fmoc-Glu(OBn)-Ind-OAll (**55**)	86
Fmoc-Thr(OBn)-Ind-OAll (**56**)	84
Fmoc-Cys(^{t}buThio)-Ind-OAll (**57**)	68
***N*-Fmoc-aminoacyl-7-nitroindoline-5-carboxylic acid (Scheme 3)**	**Isolated % yield**
Fmoc-Gly-Ind-OH (**58**)	95
Fmoc-Ala-Ind-OH (**59**)	91
Fmoc-Ile-Ind-OH (**60**)	95
Fmoc-Phe-Ind-OH (**61**)	93
Fmoc-Pro-Ind-OH (**62**)	97
Fmoc-Lys(Tfa)-Ind-OH (**63**)	95
Fmoc-Glu(OBn)-Ind-OH (**64**)	94
Fmoc-Thr(OBn)-Ind-OH (**65**)	96
Fmoc-Cys(^{t}buThio)-Ind-OH (**66**)	ND

However before our success was realized, we investigated two alternative methods. The yields for the preparation of photoreactive amino acids (**37-39**) were only moderate (**Scheme 3**). If our method was going to prove applicable to the community, our yields needed increasing at this step. We knew that the nucleophilicity of the indoline nitrogen substituted with an *o*-nitro group was low. We also observed that the acylation of indoline **26** is quantitative. We reasoned that attachment of the desired *N*-Fmoc-protected amino acid to indoline (**26**) prior to nitration would result in similar yields (**Scheme 6**).

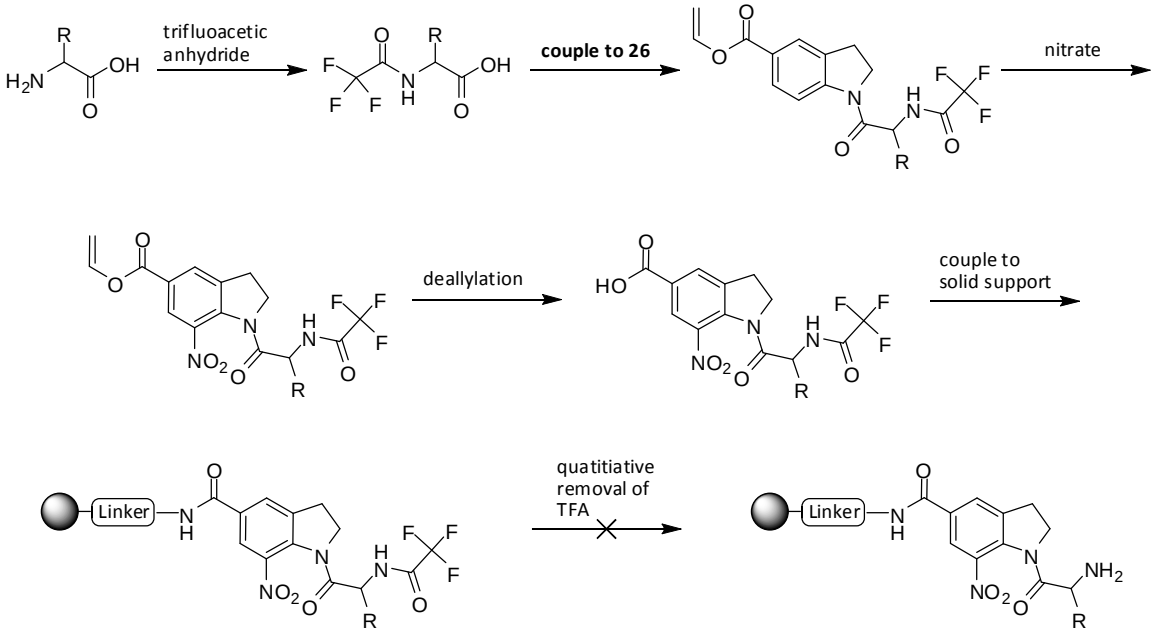


Scheme 6. Alternative method for the production of photoreactive tripeptides

Nitration at this point would not only nitrate the 7-position of the indoline ring but also the aromatic rings on the *N*-Fmoc-protecting group. This was not seen as a problem as the nitrated *N*-Fmoc-protected amino acid after attachment to the resin would then be removed and the continuation of the synthesis would proceed by standard SPPS techniques, realizing the desired photoreacitve tripeptide. Unfortunately this reaction scheme did not prove fruitful, as the nitration reaction did not result in an acceptable yield of the nitrated product(s).

A second alternative method for the preparation of photoreactive tripeptides was designed due to the failure of the previous scheme. We remained confident that the acylation of **26** was the best path to high yields. Instead of using *N*-Fmoc-protected amino acids we decided to replace the Fmoc group with the trifluoroacetyl (Tfa) group which could be removed by aminolysis. Coupling an *N*-Tfa-

protected amino acid to **26** would relieve the problems of nitration that the Fmoc group presented (**Scheme 7**).



Scheme 7. Second alternative for the production of photoreactive tripeptides

A number of reactions were performed, in solution, to find the best conditions for the quantitative removal of the trifluoroacetyl group from TFA-Val-OH for future solid phase syntheses. Along with the work performed by Dr. Cai, the following are other reaction conditions attempted: 1 M aq. piperidine, 10% aq. piperidine, 20% diethylamine in NMP, 1 M ammonia in THF and 0.5 M ammonia in THF:NMP (1:1). The most promising cleavage condition, as determined by TLC analysis, was 0.5 M ammonia in THF:NMP (1:1). Preparation of the *N*-Tfa-aminoacyl-nitroindoline derivative was accomplished and attached to the solid support. Unfortunately, attempts to quantitatively remove the *N*-Tfa protecting group of the resin bound amino acid failed. This result would limit its use in SPPS and therefore the idea was discarded.

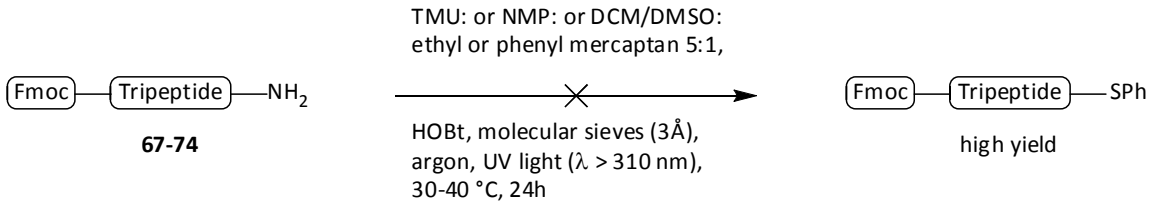
These failures lead to a reevaluation of our original coupling scheme. Fortunately it was found that the purity of the thionyl chloride reagent was of paramount importance. The use of fresh, pure thionly chloride under the conditions of **Scheme 3** resulted in the improved yields obtained shown in **Table 1**. Having successfully prepared the requisite photoreactive amino acids (**58-65**), SPPS of eight photoreactive tripeptides (**67-74**) based on sequences found in human erythropoietin (h-EPO) was accomplished using Fmoc-based techniques (**Scheme 4**) (**Table 2**).

Table 2. Protected photoreactive tripeptides

***N*-Fmoc-Tripeptide-Ind-NH_2 (Scheme 4)**	**Isolated % Yield**
Fmoc-Val-Ala-**Gly**-Ind-NH_2 (**67**)	86
Fmoc-Ala-Val-**Ala**-Ind-NH_2 (**68**)	89
Fmoc-Leu-Gly-**Ile**-Ind-NH_2 (**69**)	86
Fmoc-Lys(TFA)-Leu-**Phe**-Ind-NH_2 (**70**)	82
Fmoc-Phe-Ala-**Pro**-Ind-NH_2 (**71**)	90
Fmoc-Glu(OBn)-Ala-**Lys(TFA)**-Ind-NH_2 (**72**)	88
Fmoc-Val-Leu-**Glu(OBn)**-Ind-NH_2 (**73**)	88
Fmoc-Thr(OBn)-Ile-**Thr(OBn)**-Ind-NH_2 (**74**)	84

Drawing on our initial success with the preparation of peptide-α-ethylthioesters (**49-51**), the photoreactive tripeptides (**67-74**) were subjected to the previously developed conditions (**Scheme 4**). In addition to ethanethiol we also employed thiophenol as the acyl acceptor. The ability to isolate phenyl thioesters would have great implications for native chemical ligation as the phenyl thioester is known to be the more reactive thioester[69]. Generally, in literature procedures, a less reactive thioester is installed at the C-termini of peptides. NCL is then run with an excess of thiophenol to produce, through thiol exchange, the more reactive phenyl thioester, which is the reacting species that undergoes transthioesterification and ultimately leads to the ligated product[69].

Attempts to produce both the more stable ethyl thioesters and the more reactive phenyl thioesters in high yields failed (**Scheme 8**).



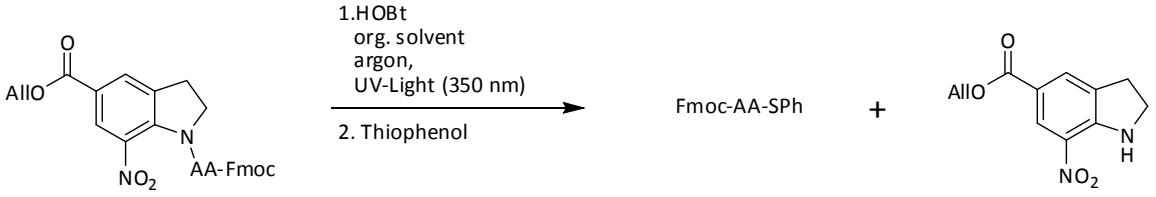
Scheme 8. Low yielding preparations of tripeptide thioesters

The yields of the more stable ethyl thioester were moderate as unacceptable amounts of the peptide free acids were obtained. Additionally, we were unable to produce any phenyl thioesters as thiophenol undergoes photodegredation in the presence of UV-light. The negative results led us to explore new options.

2.7 Amino Acid Phenyl Thioesters

Semi-emperical calculations of the potential energy surface, performed by the Dirk group (University of Texas, El Paso), on the photochemistry of an acylated nitroindoline determined that upon illumination with UV-light (λ = 350 nm) a nitronic anhydride intermediate forms from a suprafacial [1-5] sigmatropic shift involving the amide carbonyl carbon and one of the oxygens of the *o*-nitro group. If the nitronic anhydride intermediate becomes protonated either at the anhydride oxygen or the carbonyl oxygen, then the undesired pathway to the free acid predominates over nucleophilic displacement (**Figure 31**). The pKa of thiols range between 8 and 11, this level of acidity proved to be high enough to cause the unwanted protonation.

To circumvent this problem a new strategy was devised based on the data from the computational analysis. It was believed that the protonation issue could be solved by first generating, *in situ*, Fmoc-AA(PG)-OBt esters photochemically, followed by acylation of a thiol, in the dark, to produce the desired Fmoc-AA(PG)-thioesters (**Scheme 9**).



Scheme 9. Revised approach to the preparation of thioesters

A test irradiation of a mixture of Fmoc-Phe-Ind-OAll (**52**) and HOBt in NMP, followed by the addition of thiophenol, resulted in, for the first time, the production of the desired phenythioester. At this point it was decided to optimize the reaction at the amino acid level instead of using the precious photoreactive tripeptides.

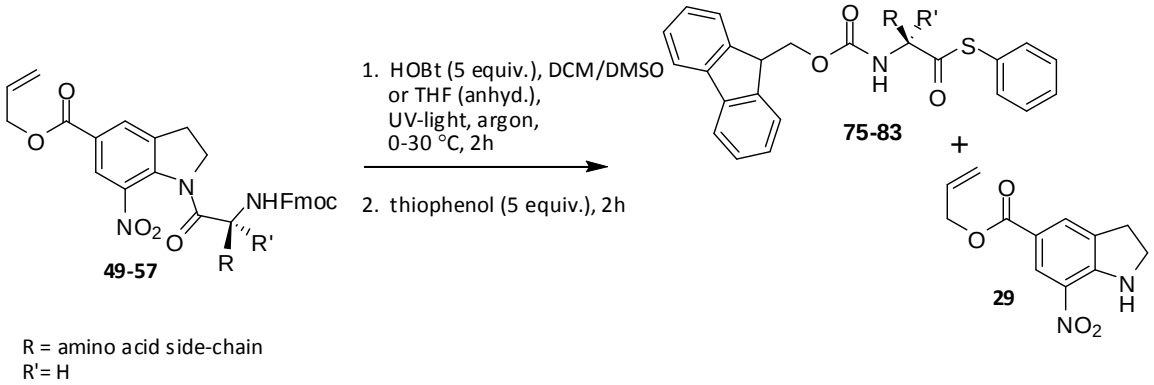
This reaction requires that the starting materials are soluble in an aprotic organic solvent. The starting photoreactive amino acids (**49-57**) are soluble in a number of aprotic organic solvents. HOBt was found to dissolve readily in DMSO, NMP or TMU, however, because of their high boiling points removal of large volumes of these solvents requires vacuum distillation. We believed that solvent mixtures containing a volatile aprotic organic solvent and just enough NMP, DMSO or TMU to dissolve the HOBt would relieve the difficulties in product isolation. Test reactions using acetonitrile/DMSO, acetonitrile/NMP, DCM/DMSO and DCM/NMP were conducted. Analysis by TLC showed that in each of the solvent systems, the starting material was completely consumed and the ratio of thioester to nitroindoline products implied high yields. We decided to use the DCM/DMSO solvent system as each

of these solvents was readily available in our lab as anhydrous solvents. Water can act as a nucleophile, so its absence throughout the reaction has to be beneficial. Later we found that THF could dissolve both the starting material and HOBt and its use gave similar results to the DCM/DMSO mixture.

HOBt has served as an auxiliary nucleophile in peptide coupling reactions for many decades, and it has shown its usefulness to produce thioesteters in test thioesterifications. Other nucleophiles were investigated to determine the optimal reagent for our method. *N*-hydoxysuccinimde, pentafluorophenol and HOAt were each used in test reactions and evaluated by TLC. It was found that in each reaction the product thioester was observed. In the case of *N*-hydroxysuccinimide and pentafluorophenol a smaller amounts of products were observed when compared to those reactions run using HOBt. HOAt gave results similar to HOBt but as it is a more expensive reagent, we decide to run all subsequent reactions with HOBt. We attempted one additional reaction where we irradiated a photoreactive amino acid in DCM/DMSO, without an auxiliary nucleophile, followed by addition of thiophenol. TLC analysis showed the complete consumption of starting material, however only a small amount of thioester was observed.

With the solvent system and the auxiliary nucleophile determined, we investigated the stoichiometry of the reagents. Our initial test reaction utilized a large excess of HOBt (20+ equiv.) and thiophenol (20+ equiv.). A reduction in the excess of reagents used would facilitate purification, save on cost, reduce waste and make the method more attractive to the chemical community. Test reactions were run using HOBt in two, five, ten and fifteen fold excess. It was determined by TLC analysis that the starting material was consumed in two hours when using a five-fold excess, where two-fold excess required longer reaction time. Thiophenol was used in five fold excess.

With the optimal conditions determined, a high yielding method was developed that produced high purity *N*-Fmoc protected amino acid thioesters (**Scheme 10**) (**Table 3**).



Scheme 10. General preparation of *N*-Fmoc-amino acid-phenylthioesters

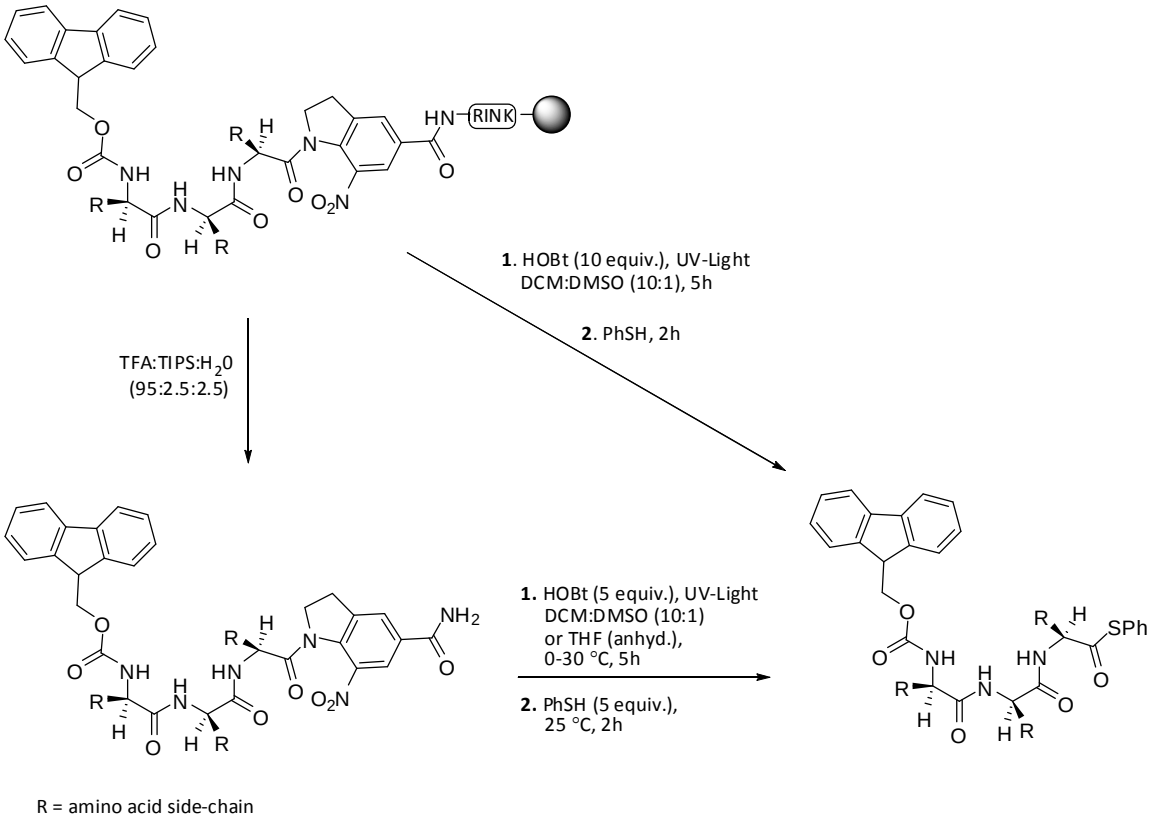
Table 3. *N*-Fmoc-amino acid-phenylthioesters

***N*-Fmoc-amino acid-phenylthioesters (Scheme 9)**	**Solvent(s)**	**Isolated % Yield**
Fmoc-Gly-SPh (**75**)	HOBt	93
Fmoc-Ala-SPh (**76**)	HOBt	90
Fmoc-Ile-SPh (**77**)	DCM/DMSO	89
Fmoc-Phe-SPh (**78**)	DCM/DMSO	92
Fmoc-Pro-SPh (**79**)	HOBt	91
Fmoc-Lys(Tfa)-SPh (**80**)	DCM/DMSO	84
Fmoc-Glu(OBn)-SPh (**81**)	DCM/DMSO	90
Fmoc-Thr(Obn)-SPh (**82**)	DCM/DMSO	89
Fmoc-Cys(^{t}buThio)-SPh (**83**)	HOBt	90

The results from the thioesterifications of **49-57** to give the Fmoc-amino acid-phenylthioesters (**75-83**) gave us great confidence that the extension of this method to the preparation of *N*-Fmoc-tripeptide-phenylthioesters could be readily accomplished.

2.8 Extension to Peptide Thioesters

The photoreactive tripeptides (**67-74**) could now be converted to their corresponding thioesters under similar reaction conditions developed at the amino acid level (**Scheme 11**).



Scheme 11. Preparation of *N*-Fmoc-tripeptide-phenylthioesters

It was envisioned that attaching the prepared *N*-amino acyl-7-nitroindoline linker to Rink Amide resin would generate a solid support with two orthogonal linkers suitable for SPPS, providing chemical flexibility for cleaving the peptides from the solid support. In order to perform quality control the resin bound photoreactive tripeptides were cleaved using a trifluoroacetic acid (TFA), triisopropylsilane

(TIPS), water solutuion (95:2.5:2.5) to give photoreactive tripeptide amides (**67-74**). Cleaving of these photoreactive peptide amides with TFA provided for proper spectroscopic analysis and yields. In addition, starting from pure starting material of known weight, we could get accurate yields for the thioesterification reactions. Tripeptides (**67-74**) and HOBt were dissolved in either DCM/DMSO or THF (anhyd.) under an argon atmosphere, then irradiated in a Rayonet photoreactor (λ = 350nm) for 5 h (0 to 30°C). Upon complete consumption of the starting material, the lights were turned off, and then thiophenol was added to produce the desired *N*-Fmoc-tripepetide-phenylthioesters (**84-90**) in high yield (**Table 4**). Alternatively, tripeptide thioesters could be produced by direct photorelease from the resin. The resin bound derivatives of photoreactive tripeptides (**69**, **70**, **74**) containing the C-terminal Ile, Phe, or Thr(OBn) residues, respectively, were suspended in a solution of THF (anhyd.) (**70**) or NMP (**69, 74**) and HOBt (5 eq for **70**, 10 eq for **69**, **74**), then irradiated (λ = 350nm) for 5 h. Addition of thiophenol produced the corresponding *N*-Fmoc-tripeptide-phenylthioesters in moderate to good yield (**Table 4**). The reduced yields from the solution phase reactions can be rationalized. If the preparation of the resin bound photoreactive peptide starting material was not accomplished in high yield then the corresponding photorelease of the peptide-α-thioester would be obtained in a lower yield. Alternatively, if the preparation of the resin bound photoreactive peptide was peformed in high yield then it is possible that due to the porous nature of the solid support itself, longer irradiation may be required in order to convert, completely, the starting material into the peptide-Obt ester intermediate.

Table 4. *N*-Fmoc-tripeptide-phenylthioesters

***N*-Fmoc-tripeptide-phenylthioesters (Scheme 10)**	**Solvent**	**Isolated Yield**
Fmoc-Val-Ala-**Gly**-SPh (**84**)	THF	86
Fmoc-Ala-Val-**Ala**-SPh (**85**)	THF	84
Fmoc-Leu-Gly-**Ile**-SPh (**86**)	DCM/DMSO or NMP*	79/68*
Fmoc-Lys(TFA)-Leu-**Phe**-SPh (**87**)	DCM/DMSO or THF*	84/78*
Fmoc-Phe-Ala-**Pro**-SPh (**88**)	THF	88
Fmoc-Glu(Obn)-Ala-**Lys(TFA)**-SPh (**89**)	DCM/DMSO	82
Fmoc-Thr(Obn)-Ile-**Thr(Obn)**-SPh (**90**)	DCM/DMSO or NMP*	82/66*

* = direct photorelease of peptide-α-thioester from solid support

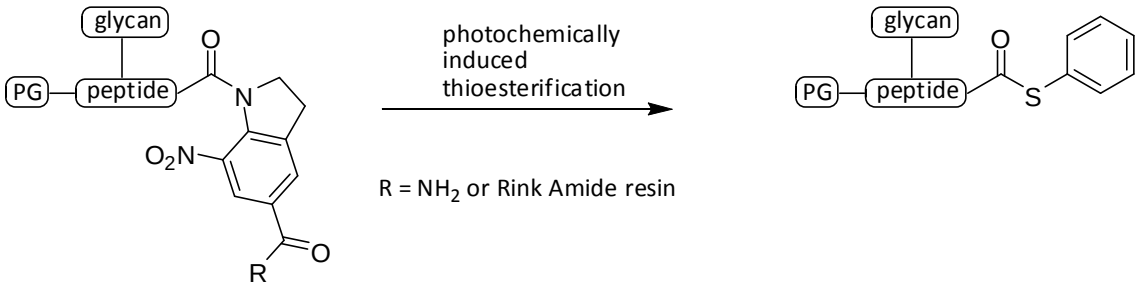
The successful preparation of C-terminal peptide-α-thioesters (**84-90**) proved our hypothesis correct. Additionally, the original method was expanded to include eight different C-terminal amino acids of varying side-chain functionality, creating a general, high yielding method for the thioesterification of *N*-Fmoc-protected photoreactive tripeptides. Further, this method allows for two orthogonal cleavage pathways giving the user a flexibility not seen in other methods.

Attemps were made to convert *N*-Boc-protected photoreactive peptides into their corresponding peptide-α-phenylthioesters. The conversion of *N*-Boc-protected-Epo 23-27-Ind-NH_2 was accomplished in 32% yield, representing the most productive of the *N*-Boc-protected compounds that underwent thioesterification. Similar yields were reported by the Bochet group in their attempts to photo-acylate amines with Boc-, Fmoc- or CBz- derived 5,7-dinitroindolines[70].

2.9 Ground Work for the Preparation of Glycopetide Thioesters

Peptide thioesters are of great interest to the peptide community for ligation and cylization reactions, additionally, they have application to the glycopeptide community. Glycosylation of peptides/proteins via post translational modifications is, in many cases, imperative for their biological acitivity. Protein folding, cellular communication/recognition and increased solubility of glycopeptides

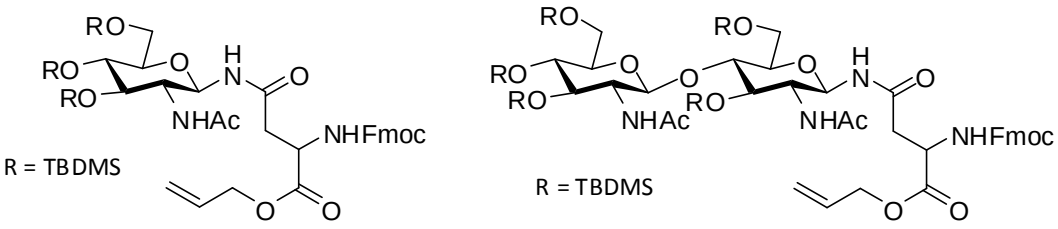
in an aqueous environment are just a few examples of the effect of glycosylation on protein/peptide scaffolds[71]. The preparation of glycopeptides and small glycoproteins is of great interest to this lab. The method we developed for the synthesis of peptide-α-phenylthioesters can be extended to the synthesis of glycopeptide-α-phenythioesters (**Scheme 12**).



Scheme 12. General preparation of glycopeptide phenylthioesters

N-Glycopeptides can be generated by a stepwise approach where the glycan is attached first to the side chain carboxyl of aspartic acid, producing a building block for SPPS. Alternatively, glycosylation can be achieved by a convergent approach where the glycan is attached, after peptide synthesis, as a post translational mimetic. Both schemes are valid in glycopeptide synthesis, the choice of which to use is generally determined by the size and complexity of the glycan to be attached. Simple glycans like chitobiosylamine or *N*-acetylglucosamine are relatively easy to make and can be used in the building block approach as the large excess used in SPPS will not be overly expensive. As the glycan moiety becomes more and more complex, its synthesis becomes costly and the material becomes precious. The convergent approach requires only stoichiometric amounts of glycan for coupling.

In order to extend the peptide thioester method to glycopeptides, it was necessary to prepare the glycosylasparagine building blocks (**91** and **92**) for SPPS of glycopeptides (**Fig 35**).

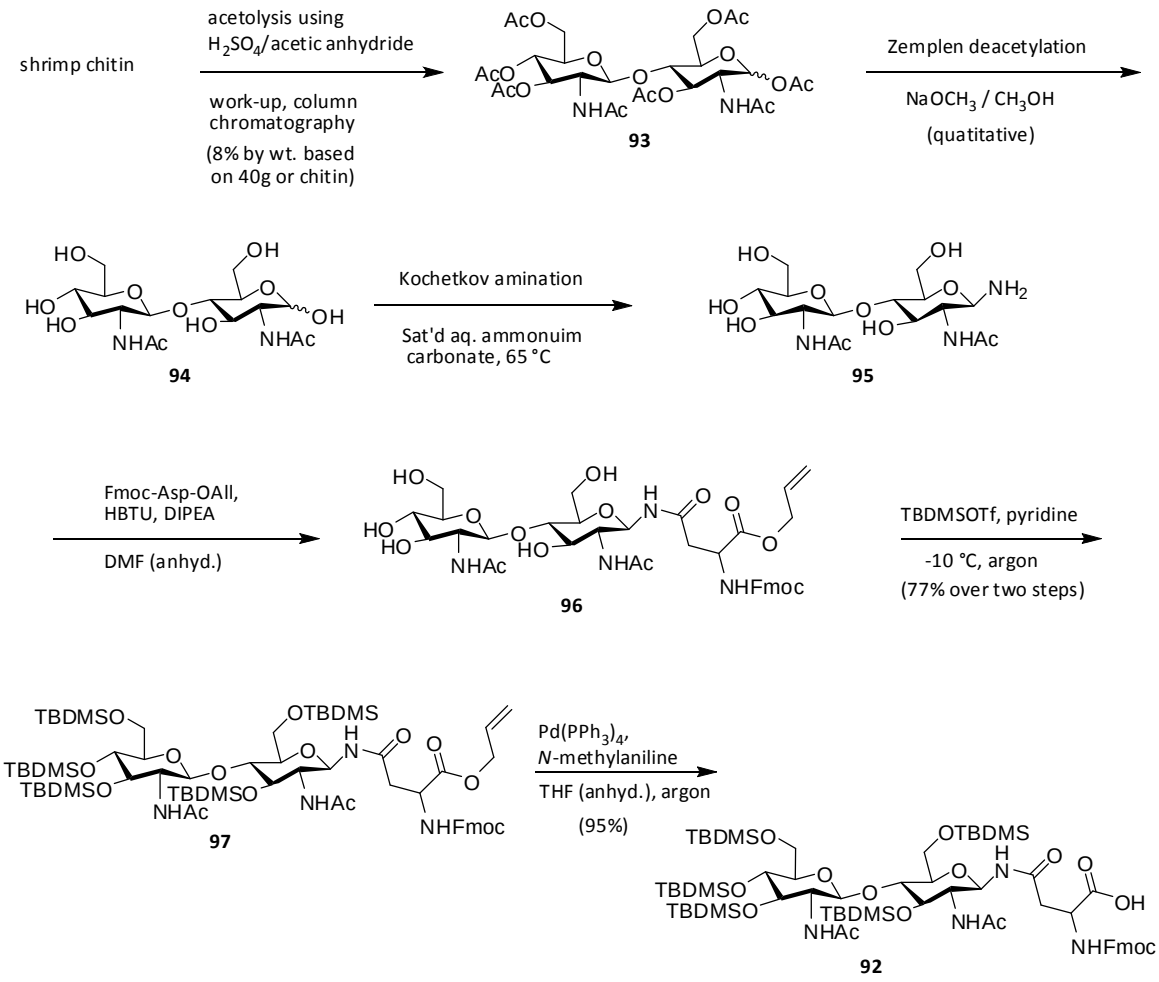


Fmoc-Asn[GlcNAc(TBDMS)$_3$]-OH
91

Fmoc-Asn[Chito(TBDMS)$_5$]-OH
92

Figure 35. Glycosylasparagine building blocks

We chose two simple glycans, *N*-acetylglucosamine and its dimer chitobiosylamine, as model glycans for our glycopeptide thioester synthesis. *N*-acetylglucosamine is cheap enough to buy direct, however chitobiosylamine is not. The chitobiosyl containing building block was prepared starting from the cheap shrimp chitin (**Scheme 13**).



Scheme 13. Synthesis of glycosyl-asparagine building blocks for SPPS

Chitin is the world's second most abundant biopolymer which consists of repeating *N*-acetylglucose subunits. Acetolysis of chitin results in the monomer, dimer, trimer and larger polymer chains of peracetylated-*N*-acetylglucosamine. The dimer, chitobiose (**93**), was isolated by repeated column chromatography and recrystallization. With the desired glycan in hand, manipulation to the building block could begin. Zemplen deacetylation of **93** followed by Kochetkov amination of **94** resulted in the fully deprotected chitobiosylamine (**95**). Attachment of **95** to the protected aspartic acid residue (Fmoc-Asp(OH)-OAll) was accomplished under standard coupling conditions. After hydroxyl protection as silyl ethers (**96**), the fully protected glycosyl amino acid (**97**) was obtained. Deallylation using Pd(0)

and *N*-methylaniline resulted in the desired glycosylasparagine building block (**92**). The synthesis of the glycosyl amino acid (**91**) derivative followed a similiar procedure.

Preparation of glycosylasparagine building blocks (**91, 92**) enabled this lab to synthesize five glycopeptides of biological relevance: small glycopeptide fragments from both human erythropoietin (**98** and **99**) and the GP-120 HIV-1 envelope protein (**100**, **101**, and **102**). Fmoc-Asn[GlcNAc(TBDMS)$_3$]-Ile-Thr-Thr-Gly-Ind-NH_2 (**98**), Fmoc-Asn[chitobiose(TBDMS)$_5$]-Ile-Thr-Thr-Gly-Ind- NH_2 (**99**), Fmoc-Asn[chitobiose (Ac)$_5$]-Ser-Thr-Ala-Thr-Leu-Ind- NH_2 (**100**), Fmoc-Gly-Ala-Asp-Asn-Asn[GlcNAc(Ac)$_3$]-Ile-Thr-Leu-Pro-Ind- NH_2 (**101**), Fmoc-Gly-Ala-Asp-Asn-Asn[chitobiose(TBDMS)$_5$]-Ile-Thr-Leu-Pro-Ind- NH_2 (**102)**.

CHAPTER III

Conclusions

This dissertation offers a solution to the problems associated with C-terminal peptide-α-thioester syntheses, by describing a more general, high yielding method that produces optically pure amino acid- and peptide-α-phenylthioesters. Our method takes advantage of the photochemical acylation of thiols under neutral conditions, minimizing the notorious problems of thioester cleavage and epimerization. Further, this method offers the possibility to cleave the peptide from the solid support in two different ways, which has been described by only one other group[72]. By attaching our nitroindoline derived linker to Rink Amide resin, our peptides can be cleaved either with TFA or with UV-light[68]. Specifically, the direct photorelease of the peptide-α-thioesters from the solid support may give one the ability to release highly acid sensitive peptides in high yields. Moreover, a photoreactive peptide amide can be cleaved from the solid support with TFA at the Rink Amide linker allowing for spectroscopic analysis and an accurate determination of yield of both the desired peptide, after SPPS, and the corresponding peptide-α-thioesters after reaction with a thiol. By conducting the peptide thioester synthesis in a two-reaction-sequence, i.e. photochemical formation of a peptide activated ester followed by acylation of a thiol in the dark, allows us to utilize photosensitive thiols.

We believe that the method developed in this dissertation can be extended to the photochemical synthesis of glycopeptide-α-thioesters since the carbohydrate moieties should not interfere with the photochemistry at the C-terminus of a peptide. The ability to synthesize glycopeptides of all sizes has great implication for the glycobiology community. Although glycoproteins can be isolated from biomaterials, their separation into single glycoforms of sufficient quantities is very difficult. For medicinal and pharmaceutical purposes the generation of homogeneous glycoproteins

with a tailored glycosylation pattern is of high interest. Furthermore, the biological role of individual glycoforms is of great interest to glycobiologists. The successful extension of our method to the synthesis of glycopeptide-α-thioesters fragments for use in NCL will most likely provide easier access to these highly desired glycopeptides/proteins.

CHAPTER IV

Experimental

All solvents and reagents used in the reactions were purchased from commercial sources (Aldrich, Fischer, etc.) and used without further purification unless otherwise noted. ^{1}H NMR spectra were recorded on Bruker 300 MHz and JEOL 600 MHz NMR spectrometers. ^{13}C NMR spectra were recorded on Bruker 300 MHz (at 75 MHz) and JEOL 600 MHz NMR (at 125 MHz) spectrometers. Optical rotations were measured on an ATAGO AP-300 Automatic Polarimeter. All reactions were monitored by TLC on Silica Gel 60 F_{254} (EMD Chemicals Inc., affiliate of Merck). Spots were detected under UV light and/or by charring with 2% H_2SO_4 in EtOH or 5% ninhydrin in EtOH. Column chromatography was perform on Silica Gel 60 230-400 mesh (Natland International Corp.); the ratio between silica gel and crude product ranged from 50:1 to 200:1 (w/w).

3.1 Synthesis of the Nitroindoline Linker

Allylation of Indole-5-Carboxylic Acid (25): Indole-5-carboxylic acid (93.07 mmol) and cesium carbonate (48.52 mmol) were added to anhydrous DMF (100 ml) and the solution stirred at room temperature. Allyl bromide (930.2 mmol) was added drop-wise over 30 min and the solution stirred for 2.5 h. The DMF was removed by vacuum distillation and the resulting solid was dissolved in ethyl acetate, washed with water and brine then dried over magnesium sulfate. After filtration and concentration a solid crude product 20.18 g (quantitative) was obtained and used without further purification as determined by ^{1}H NMR. $\boldsymbol{R_f}$ 0.52 (1:1 EtOAc:Hex); **^{1}H NMR** (300 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.73-8.58 (br s, 1H, N**H**), 8.46 (s, 1H, Ar**H**), 7.93 (d, *J* = 8.4 Hz, 1H, Ar**H**), 7.40 (d, 1H, Ar**H**), 7.26 (d, *J* = 5.5

Hz, 1H, NC**H**=CH), 6.64 (d, *J* = 4.7 Hz, 1H, N-CH=C**H**), 6.12-6.03 (m, 1H, CH_2=C**H**), 5.43 (dd, *J* = 1.4 Hz, 17.2 Hz, 1H, C**H**$_2$=CH), 5.28 (dd, *J* = 1.4 Hz, 10.3 Hz, 1H, C**H**$_2$=CH), 4.86 (d, *J* = 5.5 Hz, 2H, C**H**$_2$CH=CH_2); **^{13}C NMR** (75 MHz, 23.4 °C, $CDCl_3$, δ_C) 167.65, 138.60, 132.76, 127.58, 125.76, 123.96, 123.50, 121.88, 117.95, 110.93, 104.06, 65.38.

Reduction of Allyl Indole-5-Carboxylate (26): Allyl indole-5-carboxylate (201.22 mg, 1 mmol) and acetic acid (2 mL) were stirred at room temperature for 15 min then the solution was placed in ac ice-water bath. Sodium cyanoborohydride (157.14 mg, 2.5 mmol) was added and the solution was allowed to stir at 10 °C. The reaction was quenched after 5 h by addition of 50% sodium hydroxide solution. The solution was trassfered to a separatory funnel and extracted with ethyl acetate (3 x 5 mL). The combined organic solution was washed by brine and dried over magnesium sulfate. After filtration and concentration the solid crude product was purified by column chromatography (hex: EtOAc 2:1) to give the desired product (159 mg, 78%, two steps). ***R*$_f$** 0.52 (1:1 EtOAc:Hex); **^{1}H NMR** (300 MHz, 23.4 °C, $CDCl_3$, δ_H) 7.82-7.79 (m, 2H, Ar**H**), 6.65-6.62 (m, 1H, Ar**H**), 6.07-5.96 (m, 1H, CH_2=C**H**), 5.42-5.24 (m, 2H, C**H**$_2$=CH), 4.78-4.76 (m, 2H, C**H**$_2$-O), 3.68 (t, *J* = 8.9 Hz, 2H, NC**H**$_2$$CH_2$), 3.09 (t, *J* = 8.9 Hz, 2H, NCH_2C**H**$_2$); **^{13}C NMR** (75 MHz, 23.4 °C, $CDCl_3$, δ_C) 166.67, 156.10, 132.94, 130.91, 128.77, 126.28, 119.58, 117.68, 107.49, 64.97, 47.37, 28.94.

Acetylation of Allyl Indoline-5-carboxylate (27): Allyl indoline-5-carboxylate (11.55 mmol) was dissolved in acetic anhydride (14 mL) and the solution stirred at room temperature for 50 min. Ice-cold water was added to hydrolyze the remaining acetic anhydride then the aqueous solution was extracted with ethyl acetate (3 x 20 mL). The combined ethyl acetate extracts were washed with saturated sodium bicarbonate solution, water and brine then dried over magnesium sulfate. After filtration and

concentration a solid crude product 2.747 g (quantitative) was obtained and used without further purification as determined by ^{1}H NMR. R_f 0.43 (1:1 EtOAc:Hex); **^{1}H NMR** (300 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.19 (d, *J* = 8.9 Hz, 1H, Ar**H**), 7.90, (d, *J* = 6.9 Hz, 1H, Ar**H**), 7.82 (s, 1H, Ar**H**), 6.10-5.93 (m, 1H, CH_2=C**H**), 5.38-5.36 (m, 1H, C**H**$_2$=CH), 5.26-5.24 (m, 1H, C**H**$_2$=CH), 4.78-4.76 (m, 2H, C**H**$_2$-O), 4.06 (t, *J* = 8.2 Hz, 2H, NC**H**$_2$), 3.18 (t, *J* = 8.9 Hz, 2H, NCH_2C**H**$_2$), 2.20 (s, 3H, C**H**$_3$); **^{13}C NMR** (75 MHz, 23.4 °C, $CDCl_3$, δ_C) 169.32, 166.01, 146.98, 132.46, 131.41, 130.30, 126.03, 125.20, 118.20, 116.20, 65.47, 49.23, 27.59, 24.38.

Nitration of Allyl *N*-Acetylindoline-5-carboxylate (28): Allyl *N*-acetylindoline-5-carboxylate (11.19 mmol) was dissolved in TFA (25 ml) at 0 °C. Sodium nitrate (14.56 mmol) was added slowly over 5 min. The solution warmed to room temperature and stirred for 8 h. The TFA was removed by evaporation and the resulting solid was dissolved in ethyl acetate, washed with water and brine then dried over magnesium sulfate. After filtration and concentration a solid crude product was obtained and purified by column chromatography to give 3.3567 g (93%, two steps). R_f 0.35 (1:1 EtOAc:Hex); **^{1}H NMR** (300 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.21 (s, 1H, Ar**H**), 7.94 (s, 1H, Ar**H**), 6.09-5.98 (m, 1H, CH_2=C**H**), 5.43 (dd, *J* = 4.1 Hz, 19.0 Hz, 1H, C**H**$_2$=CH), 5.29 (dd, *J* = 1.4 Hz, 10.2 Hz, 1H C**H**$_2$=CH), 4.80 (d, *J* = 6.9 Hz, 2H, C**H**$_2$CH=CH_2), 4.11 (t, *J* = 8.2 Hz, 2H, NC**H**$_2$), 3.22 (t, *J* = 8.2 Hz, 2H, NCH_2C**H**$_2$), 2.24 (s, 3H, C**H**$_3$); **^{13}C NMR** (75 MHz, 23.4 °C, $CDCl_3$, δ_C) 168.81, 164.17, 140.06, 138.09, 137.24, 131.84, 129.19, 126.39, 124.84, 118.96, 66.20, 50.45, 28.56, 23.46.

De-Acetylation of Allyl 7-Nitro-*N*-Acetylindoline-5-Carboxylate (29): Allyl 7-nitro-*N*-acetylindoline-5-carboxylate (6.89 mmol) was added to a solution of methanol (10 ml) and aqueous HCl (pH 1, 200 ml). The solution was brought to reflux and stirred for 5 h with periodic additions of methanol to maintain the solubility of the starting material in the solution. The methanol was removed by evaporation then

the aqueous solution was extracted with ethyl acetate. The combined ethyl acetate extracts were washed with brine then dried over magnesium sulfate. After filtration and concentration a solid crude product was obtained and purified by column chromatography to give 1.6591 g (97 %) of the desired product. R_f 0.54 (1:1 EtOAc:Hex); **^{1}H NMR** (300 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.59 (s, 1H, Ar**H**), 7.82 (s, 1H, Ar**H**), 7.10 (br s, 1H, N**H**), 6.10-5.95 (m, 1H, CH_2=C**H**), 5.39 (dd, *J* = 1.4 Hz, *J* = 17.1 Hz, 1H, C**H**$_2$=CH), 5.29 (dd, *J* = 1.4 Hz, *J* = 10.3 Hz, 1H, C**H**$_2$=CH), 4.81 (d, *J* = 5.5 Hz, 2H, C**H**$_2$CH=CH_2), 3.95 (t, *J* = 8.2 Hz, 2H, NC**H**$_2$), 3.22 (t, J = 8.9 Hz, 2H, NCH_2C**H**$_2$); **^{13}C NMR** (75 MHz, 23.4 °C, $CDCl_3$, δ_C) 165.11, 151.52, 133.98, 132.32, 129.41, 128.22, 126.52, 118.58, 118.49, 65.68, 47.19, 27.75.

3.2 Synthesis of Photoreactive Amino Acids

General procedure for the coupling of Fmoc-AA-OH to Allyl 7-Nitro-1*H*-Indoline-5-Carboxylate (49-57): Allyl 7-nitro-1-*H*-indoline-5-carboxylate (0.2452 mmol, 1.0 equiv.) and Fmoc-Pro-OH (0.4907 mmol, 2.0 equiv.) were dissolved in anhydrous toluene (10 mL) and the solution stirred at 60 °C under an argon atmosphere. Thionyl chloride (0.7356 mmol, 3.0 equiv.) was added and the solution stirred for 24 h. The solution was diluted with ethyl acetate then washed with water and saturated sodium chloride solution then dried over magnesium sulfate. After filtration and evaporation a crude solid was obtained and purified by flash chromatography to give 0.1197 g (86%) of the desired product.

Fmoc-Pro-Ind-NH_2 (53): R_f 0.38 (1:1 EtOAc:Hex); **$[\alpha]^{24.6}$** = -216.88 (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.37 (s, 1H, Ar**H**), 8.07 (s, 1H, Ar**H**), 7.75-7.73 (m, 2H, Ar**H**), 7.58-7.53 (m, 2H, Ar**H**), 7.39-7.36 (m, 2H, Ar**H**), 7.30-7.27 (m, 2H, Ar**H**), 6.03-5.99 (m, 1H, CH_2=C**H**)**,** 5.41-5.38 (m, 1H, C**H**=CH), 5.31-5.29 (m, 1H, C**H'**=CH), 4.96-4.92 (m, 1H, NC**H**), 4.81 (d, *J* = 5.5 Hz, 2H, C**H$_2$**CH=CH_2), 4.70-4.68 (m,

1H, α-C**H**), 4.36-4.33 (m, 1H, Fmoc-C**H**), 4.29-4.23 (m, 3H, NC**H'**, Fmoc-C**H_2**), 3.78-3.75 (m, 1H, δ-C**H**), 3.58-3.54 (m, 1H, δ-C**H'**), 3.49-3.45 (m, 1H, NCH_2C**H**), 3.20-3.15 (m, 1H, NCH_2C**H'**), 2.32-2.20 (m, 3H, γ-C**H**, β-C**H_2**), 2.02-1.99 (m, 1H, γ-C**H'**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 172.22, 164.17, 155.17, 143.99, 143.69, 141.26, 140.05, 138.81, 137.40, 131.72, 129.39, 127.68, 127.02, 125.18, 125.04, 119.94, 118.98, 67.65, 66.17, 58.87, 50.19, 47.10, 46.83, 29.47, 28.96, 28.59.

Fmoc-Gly-Ind-OAll (49): R_f 0.40 (1:1 EtOAc:Hex); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.35 (s, 1H, Ind-Ar**H**), 8.09 (s, 1H, Ind-Ar**H**), 7.74 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.58 (d, 2H, Fmoc-Ar**H**), 7.38 (dd, *J* = *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.30 (dd, 2H, Fmoc-Ar**H**), 6.04-5.99 (m, 1H, CH_2=C**H**), 5.74 (dd, *J* = *J* = 4.86 Hz, 1H, N**H**), 5.43-5.38 (m, 1H, C**H'**=CH), 5.33-5.30 (m, 1H, C**H'**=CH), 4.84-4.82 (m, 2H, C**H_2**CH=CH_2), 4.37 (d, *J* = 6.9 Hz, 2H, α-C**H_2**), 4.29 (t, 2H, NC**H_2**), 4.22-4.19 (m, 3H, Fmoc-C**H**, Fmoc-C**H_2**), 3.30 (t, 2H, NCH_2C**H_2**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 167.38, 164.04, 156.41, 143.80, 141.35, 140.20, 137.65, 136.70, 131.71, 129.48, 127.82, 127.34, 127.19, 125.22, 125.11, 120.07, 119.22, 67.46, 66.37, 49.14, 47.12, 44.44, 28.82.

Fmoc-Ala-Ind-OAll (50): R_f 0.48 (1:1 EtOAc:Hex); **$[\alpha]^{24.6}$** = -313.65° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.33 (s, 1H, Ind-Ar**H**), 8.03 (s, 1H, Ind-Ar**H**), 7.70 (d, *J* = 6.9 Hz, 2H, Fmoc-Ar**H**), 7.55-7.51 (m, 2H, Fmoc-Ar**H**), 7.35-7.32 (m, 2H, Fmoc-Ar**H**), 7.26-7.22 (m, 2H, Fmoc-Ar**H**), 6.04-5.98 (m, 2H, CH_2=C**H,** N**H**), 5.41-5.38 (m, 1H, C**H**=CH), 5.32-5.30 (m, 1H, C**H'**=CH), 4.81 (d, *J* = 6.2 Hz, 2H, C**H_2**CH=CH_2), 4.73 (p, 1H, α-C**H**), 4.58-4.53 (m, 1H, NC**H_2**), 4.31 (dd, *J* = *J* = 7.6 Hz, 2H, Fmoc-C**H_2**), 4.21-4.09 (m, 2H, NC**H'**, Fmoc-C**H**), 3.33-3.27 (m, 1H, NCH_2C**H**), 3.15-3.10 (m, 1H, NCH_2C**H'**), 1.47 (d, *J* = 6.9 Hz, 2H, β-C**H_3**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 172.17, 164.13, 156.10, 143.84, 143.73, 141.30,

138.34, 131.81, 129.49, 127.81, 127.15, 127.05, 125.23, 125.18, 124.95, 120.05, 119.11, 67.25, 66.34, 50.14, 49.43, 47.10, 28.94, 18.13.

Fmoc-Ile-Ind-OAll (51): R_f 0.56 (1:1 EtOAc:Hex); $[\alpha]^{24.6}$ = -270.27° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.34 (s, 1H, Ind-Ar**H**), 8.05 (s, 1H, Ind-Ar**H**), 7.72-7.70 (m, 2H, Fmoc-Ar**H**), 7.55-7.51 (m, 2H, Fmoc-Ar**H**), 7.37-7.32 (m, 2H, Fmoc-Ar**H**), 7.28-7.22 (m, 2H, Fmoc-Ar**H**), 6.03-5.98 (m, 1H, CH_2=C**H**), 5.58 (d, *J* = 8.94 Hz, 1H, N**H**), 5.41-5.38 (m, 1H, C**H**=CH), 5.32-5.30 (m, 1H, C**H'**=CH), 4.81-4.80 (m, 2H, C**H**$_2$CH=CH_2), 4.74-7.70 (m, 1H, NC**H**), 4.56-4.52 (m, 1H, α-C**H**), 4.36-4.22 (m, 3H, Fmoc-C**H$_2$**, NC**H'**), 4.14 (dd, *J* = *J* = 7.6 Hz, 1H, Fmoc-C**H**), 3.38-3.31 (m, 1H, NCH_2C**H**), 3.18-3.12 (m, 1H, NCH_2C**H'**), 1.93-1.88 (m, 1H, β-C**H**), 1.69-1.64 (m, 1H, γ-C**H**), 1.26-1.18 (m, 1H, γ-C**H'**), 1.09 (d, *J* = 6.8 Hz, 3H, γ-C**H$_3$**), 0.97 (dd, *J* = *J* = 7.6 Hz, 3H, δ-C**H$_3$**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 171.58, 163.90, 156.50, 143.55, 141.09, 140.39, 137.25, 131.58, 129.19, 127.59, 126.92, 124.96, 124.70, 119.84, 118.90, 67.13, 66.11, 60.28, 57.74, 50.26, 46.93, 38.05, 28.61, 24.35, 20.94, 15.19, 14.09, 11.08.

Fmoc-Phe-Ind-OAll (52): R_f 0.54 (1:1 EtOAc:Hex); $[\alpha]^{24.6}$ = -90.09° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.37 (s, 1H, Ind-Ar**H**), 8.03 (s, 1H, Ind-Ar**H**), 7.74 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.55-7.52 (m, 2H, Fmoc-Ar**H**), 7.39-7.23 (m, 9H, 4 x Fmoc-Ar**H,** 5 x Phenyl**H**), 6.05-5.98 (m, 1H, CH_2=C**H**), 5.64 (d, *J* = 8.3 Hz, 1H, N**H**), 5.41-5.38 (m, 1H, C**H**=CH), 5.32-5.30 (m, 1H, C**H'**=CH), 4.91-4.87 (m, 1H, α-C**H**), 4.82 (d, *J* = 5.5 Hz, 2H, C**H$_2$**CH=CH_2), 4.33-4.29 (m, 3H, Fmoc-C**H$_2$**, NC**H**), 4.17 (t, 2H, Fmoc-C**H**), 3.48-3.43 (m, 1H, NC**H'**), 3.19-3.09 (m, 3H, NCH_2C**H**, β-C**H$_2$**), 2.90-2.85 (m, 1H, NCH_2C**H'**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 170.74, 164.09, 155.97, 143.78, 141.34, 140.65, 137.91, 137.42, 135.96, 131.72, 129.67, 129.45, 128.89, 127.81, 127.37, 127.16, 125.21, 125.03, 120.07, 119.15, 67.42, 66.34, 55.15, 50.07, 47.10, 39.64, 30.96, 28.74.

Fmoc-Lys(Tfa)-Ind-OAll (54): R_f 0.46 (1:1 EtOAc:Hex); **[α]$^{24.6}$** = -170.17° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.36 (s, 1H, Ind-Ar**H**), 8.08 (s, 1H, Ind-Ar**H**), 7.72 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.54-7.50 (m, 2H, Fmoc-Ar**H**), 7.37-7.34 (m, 2H, Fmoc-Ar**H**), 7.27-7.22 (m, 2H, Fmoc-Ar**H**), 6.69 (t, 1H, N**H**COCF3), 6.05-5.98 (m, 1H, CH_2=C**H**), 5.78 (d, *J* = 8.3 Hz, 1H, N**H**), 5.42-5.38 (m, 1H, C**H**=CH), 5.33-5.30 (m, 1H, C**H'**=CH), 4.82 (d, *J* = 5.5 Hz, 2H, C**H$_2$**CH=CH_2), 4.72-4.68 (m, 1H, α-C**H**), 4.66-4.62 (m, 1H, NC**H**), 4.34-4.32 (m, 2H, Fmoc-C**H$_2$**), 4.28-4.23 (m, 2H, NC**H'**), 4.14-4.11 (m, 1H, Fmoc-C**H**), 3.47-3.34 (m, 3H, 2 x γ-C**H**, NCH_2**CH**), 3.22-3.17 (m, 1H, NCH_2**CH'**), 1.95-1.89 (m, 1H, β-C**H**), 1.72-1.63 (m, 2H, δ-C**H$_2$**), 1.55-1.52 (m, 2H, γ-C**H$_2$**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 171.61, 164.05, 156.41, 143.71, 141.34, 140.37, 137.98, 137.40, 130.70, 129.64, 127.81, 127.42, 127.13, 125.11, 124.96, 128.07, 119.21, 67.33, 66.40, 53.11, 47.10, 39.49, 32.25, 28.96, 28.37, 21.75, 14.28.

Fmoc-Glu(OBn)-Ind-OAll (55): R_f 0.46 (1:1 EtOAc:Hex); **[α]$^{24.6}$** = -230.23° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.37 (s, 1H, Ind-Ar**H**), 8.09 (s, 1H, Ind-Ar**H**), 7.74-7.72 (m, 2H, Fmoc-Ar**H**), 7.56-7.53 (m, 2H, Fmoc-Ar**H**), 7.38-7.24 (m, 9H, 4 x Fmoc-Ar**H**, 5 x Phenyl**H**), 6.05-5.98 (m, 1H, CH_2=C**H**), 5.60 (d, *J* = 8.3 Hz, 1H, N**H**), 5.42-5.39 (m, 1H, C**H**=CH), 5.33-5.30 (m, 1H, C**H'**=CH), 5.19-5.11 (m, 2H, Benzyl-C**H$_2$**), 4.86-4.82 (m, 3H, α-C**H**, C**H$_2$**CH=CH_2), 4.55-4.51 (m, 1H, NC**H**), 4.35-4.33 (m, 2H, Fmoc-C**H$_2$**), 4.26-4.15 (m, 2H, NC**H'**, Fmoc-C**H**), 3.35-3.29 (m, 1H, NCH_2C**H**), 3.16-3.11 (m, 1H, NCH_2C**H'**), 2.63-2.53 (m, 2H, γ-C**H$_2$**), 2.26-2.22 (m, 1H, β-C**H**), 1.95-1.92 (m, 1H, β-C**H'**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 172.81, 171.48, 164.11, 156.63, 143.86, 143.71, 141.30, 140.24, 138.12, 137.12, 137.66, 135.92, 131.84, 129.51, 128.69, 128.45, 128.40, 127.80, 127.16, 125.22, 125.19, 124.90, 120.04, 119.11, 67.33, 66.68, 66.34, 52.60, 50.30, 47.12, 29.48, 28.88, 27.72.

Fmoc-Thr(OBn)-Ind-OAll (56): R_f 0.46 (1:1 EtOAc:Hex); **[α]$^{24.6}$** = -166.83° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.43 (s, 1H, Ind-Ar**H**), 8.04 (s, 1H, Ind-Ar**H**), 7.72-7.70 (m, 2H, Fmoc-Ar**H**), 7.57-7.54 (m, 2H, Fmoc-Ar**H**), 7.39-7.24 (m, 9H, 4 x Fmoc-Ar**H**, 5 x Phenyl**H**), 6.05-5.98 (m, 1H, CH_2=C**H**), 5.87 (d, *J* = 7.56 Hz, 1H, N**H**), 5.42-5.39 (m, 1H, C**H**=CH), 5.32-5.30 (m, 1H, C**H'**=CH), 4.82 (d, *J* = 5.46, 2H, C**H$_2$**CH=CH_2), 4.77-4.74 (m, 1H, α-C**H**), 4.67 (d, *J* = 11.7 Hz, 1H, Benzyl-C**H**), 4.59-4.53 (m, 2H, Benzyl-C**H'**, NC**H**), 4.38-4.32 (m, 2H, Fmoc-C**H$_2$**), 4.25-4.21 (m, 1H, NC**H'**), 4.16 (t, 1H, Fmoc-C**H**), 3.99-3.96 (m, 1H, β-C**H**), 3.26-3.21 (m, 1H, NCH_2C**H**), 3.04-2.99 (m, 1H, NCH_2C**H'**), 1.33 (d, *J* = 6.18 Hz, 3H, γ-C**H$_3$**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 169.50, 164.13, 156.41, 143.82, 143.71, 141.34, 140.69, 138.00, 137.97, 137.61, 131.80, 129.43, 128.57, 128.17, 128.00, 127.83, 127.18, 125.23, 124.91, 120.07, 119.12, 71.46, 67.37, 66.34, 57.65, 50.83, 47.16, 28.84, 15.94.

Fmoc-Cys(S-^{t}BuThio)-Ind-OAll (57): R_f 0.49 (1:1 EtOAc:Hex); **[α]$^{24.6}$** = -100.10° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, $CDCl_3$, δ_H) 8.33 (s, 1H, Ind-Ar**H**), 8.05 (s, 1H, Ind-Ar**H**), 7.69 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.54, (dd, 2H, Fmoc-Ar**H**), 7.35-7.31 (m, 2H, Fmoc-Ar**H**), 7.26-7.21 (m, 2H, Fmoc-Ar**H**), 6.04-5.97 (m, 2H, N**H**, CH_2=C**H**), 5.40-5.37 (m, 1H, C**H**=CH), 5.30-5.29 (m, 1H, C**H'**=CH), 5.04-5.00 (m, 1H, α-C**H**), 4.80 (d, *J* = 5.5 Hz, 2H, C**H$_2$**CH=CH_2), 4.63-4.60 (m, 1H, NC**H**), 4.39-4.33 (m, 3H, NC**H'**, Fmoc-C**H$_2$**), 4.15 (dd, *J* = *J* = 6.8 Hz, 1H, Fmoc-C**H**), 3.32-3.26 (m, 1H, NCH_2C**H**), 3.22-3.17 (m, 1H, NCH_2C**H'**), 3.15-3.12 (m, 1H, β-C**H**), 2.99-2.96 (m, 1H, β-C**H'**), 1.35 (s, 9H, 3 x C**H$_3$**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 170.32, 164.13, 156.25, 143.72, 141.30, 140.25, 138.05, 137.82, 131.75, 129.58, 127.79, 127.25, 127.14, 125.24, 124.95, 120.02, 119.11, 67.48, 66.34, 53.16, 50.17, 50.53, 48.63, 47.09, 42.25, 30.01, 28.96, 25.39.

General Procedure for the Deallylation of Allyl 7-Nitro-*N*-(Fmoc-aminoacyl)-Indoline-5-carboxylate (58-66): Fmoc-Glu(OBn)-Ind-OAll (0.4364 mmol, 1.0 equiv.), tetrakistriphenylphosphine palladium (0)

(0.0537 mmol, 0.12 equiv.) and *N*-methylaniline (4.3571 mmol, 10 equiv.) were dissolved in anhydrous THF (5 mL). The solution stirred for 25 min at room temperature. The THF was removed by evaporation and the resulting solid purified by flash chromatography to give 0.2665 g (94%) of the desired product. The loss of the allyl group peaks in the ^{1}H NMR and one spot on TLC confirmed that Fmoc-Glu(OBn)-Ind-OH was obtained and was used as a building block for future solid phase peptide synthesis.

3.3 Synthesis of Photoreacitve Tripeptides

General Procedure for the Synthesis of Protected Tripeptides by SPPS (67-74): Fmoc-protected Rink Amide AM resin (0.1753 g, 0.1297 mmol, 1.00 equiv.) was placed in a 10 mL peptide synthesis reaction vessel. The resin was swelled for 30 min. in dichloromethane then washed with *N*-methylpyrolidinone (5 x 1 min). The Fmoc-protecting group was removed with a solution of 20 % piperidine in NMP (3 x 2 min) followed by another washing step with NMP (5 x 1 min). Fmoc-Lys(Tfa)-Ind-OH (0.2125 g, 0.3246 mmol, 2.50 equiv), HOBt (0.0438 g, 0.3246 mmol, 2.50 equiv), HBTU (0.1219 g, 0.3213 mmol, 2.48 equiv) and DIPEA (0.113 mL, 0.6470 mmol, 4.99 equiv) were dissolved in NMP (1.3 mL) then poured into the peptide synthesis reaction vessel and allowed to shake for 3 h. The reaction solution was drained and the beads washed with NMP (5 x 1 min). Following a negative bromophenol blue test of the beads, the N-terminal Fmoc-protecting group was cleaved using a solution of 20% piperidine in NMP (3 x 2 min). The beads were washed with NMP (5 x 1 min), then Fmoc-Ala-OH (0.2292 g, 0.6486 mmol, 5.00 equiv), HOBt (0.0876 g, 0.6483 mmol, 5.00 equiv), HBTU (0.2436 g, 0.6421 mmol, 4.95 equiv) and DIPEA (0.227 mL, 1.297 mmol, 10.00 equiv) were dissolved in NMP then poured into the peptide synthesis vessel and allowed to shake for 3 h. The reaction solution was drained and the beads

washed with NMP (5 x 1 min). Following a negative bromophenol blue test of the beads, the N-terminal Fmoc-protecting group was cleaved using a solution of 20 % piperidine in NMP (3 x 2 min). The beads were washed with NMP (5 x 1 min), then Fmoc-Glu(OBn)-OH (0.3011 g, 0.6485 mmol, 5.00 equiv), HOBt (0.0876 g, 0.6483 mmol, 5.00 equiv), HBTU (0.2436 g, 0.6421 mmol, 4.95 equiv) and DIPEA (0.226 mL, 1.297 mmol, 10.00 equiv) were dissolved in NMP then poured into the peptide synthesis vessel and allowed to shake for 3 h. The reaction solution was drained and the beads washed with NMP (5 x 1 min). Following a negative bromophenol blue test, the bead were dried-down by sequential washing with DCM (5 x 1 min), MeOH (5 x 1 min) and hexane (5 x 1 min) then dried on full pump vacuum for 17 h.

The Fmoc-protected tripeptide was cleaved from the resin by shaking in a solution of TFA:H_2O:TIPS (95:2.5:2.5) for 1 h. The filtrate was evaporated to dryness under reduced pressure then the peptide was cryatallized by swirling in cold diethyl ether. Decanting off the ether followed by drying on full pump vacuum gave a solid crude product. Purification by column chromatography resulted in the title compound.

Fmoc-Glu(OBn)-Ala-Lys(Tfa)-Ind-NH_2 (72): R_f 0.43 (9:1 $CHCl_3$-MeOH); $[\alpha]_{24.5}$ = -170.17° (*c* = 1.0, CH_3OH); ^{1}**H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 9.36 (t, 1H, N**H**$COCF_3$), 8.30 (d, *J* = 6.9 Hz, 1H, N**H** [Lys]), 8.14 (s, 1H, Ind-Ar**H**), 8.03 (s, 1H, Ind-Ar**H**), 7.97 (d, *J* = 6.9 Hz, 1H, N**H** [Ala]), 7.84 (d, *J* = 7.56 Hz, 2H, Fmoc-Ar**H**), 7.67 (dd, *J* = 7.6 Hz, *J* = 5.5 Hz, 2H, Fmoc-Ar**H**), 7.51 (d, *J* = 3.4 Hz, 1H, N**H** [Glu]), 7.37 – 7.26 (m, 9H, 4 x Fmoc-Ar**H**, 5 x Phenyl**H** [Glu]), 5.04 (s, 2H, Benzyl-C$\mathbf{H}_2$), 4.61 – 4.54 (m, 2H, α-C**H** [Lys], Fmoc-C**H**), 4.28 – 4.16 (m, 5H, Fmoc-C$\mathbf{H}_2$, α-C**H** [Ala], NC$\mathbf{H}_2$), 4.02 – 3.98 (m, 1H, α-C**H** [Glu]), 3.24 – 3.12 (m, 4H, ε-C$\mathbf{H}_2$ [Lys], $NCH_2C\mathbf{H}_2$), 2.39 – 2.35 (m, 2H, γ-C$\mathbf{H}_2$ [Glu]), 1.93 – 1.87 (m, 1H, β-C**H** [Glu]), 1.79 – 1.67 (m, 2H, β-C**H'** [Glu], β-C**H** [Lys]), 1.60 – 1.54 (m, 1H, β-C**H'** [Lys]), 1.49 – 1.44 (m, 2H, δ-C$\mathbf{H}_2$ [Lys]),

1.38 – 1.31 (m, 2H, γ-C**H**$_2$ [Lys]), 1.13 (d, *J* = 6.8 Hz, 3H, β-C**H**$_3$ [Ala]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 173.11, 172.80, 171.79, 171.27, 166.06, 156.37, 144.43, 144.12, 141.24, 139.78, 138.51, 136.82, 136.79, 131.20, 128.94, 128.63, 128.50, 128.42, 128.15, 127.57, 125.81, 122.28, 120.62, 66.21, 65.98, 54.11, 51.76, 50.39, 48.35. 47.20, 31.01, 30.68, 28.90, 28.55, 27.71, 22.57, 18.60, 14.35, 11.31; **MS** (ESI) Calc. for $[C_{47}H_{48}F_3N_7O_{11}]Na^+$: 966.3. Found: 966.8.

Fmoc-Val-Ala-Gly-Ind-NH$_2$ (67): ***R*$_f$** 0.38 (9:1 $CHCl_3$-MeOH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 8.20 (dd, *J* = *J* = 5.5 Hz, 1H, N**H** [Gly]), 8.12-8.11 (m, 2H, Ind-Ar**H**, CON**H**), 8.04-8.03 (m, 2H, Ind-Ar**H**, N**H** [Ala]), 7.85 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.70 (dd, *J* = 7.6 Hz, *J* = 12.4 Hz, 2H, Fmoc-Ar**H**), 7.52 (s, br, 1H, CON**H'**), 7.40-7.36 (m, 2H, N**H** [Val], Fmoc-Ar**H**), 7.28 (dd, 2H, Fmoc-Ar**H**), 4.39-4.34 (m, 1H, α-C**H** [Ala]), 4.28-4.23 (m, 3H, NC**H$_2$**, Fmoc-C**H**), 4.20-4.09 (m, 4H, Fmoc-C**H$_2$**, α-C**H$_2$** [Gly]), 3.85 (dd, *J* = 6.9 Hz, *J* = 8.9 Hz, 1H, α-C**H** [Val]), 3.22 (dd, *J* = *J* = 8.3 Hz, 2H, NCH_2CH_2), 1.97-1.92 (m, 1H, β-C**H** [Val]), 1.19 (d, *J* = 7.6 Hz, 3H, β-C**H$_3$** [Ala]), 0.83 (d, *J* = 6.9 Hz, 3H, γ-C**H$_3$** [Val]), 0.80 (d, 3H, γ-C**H$_3$'** [Val]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 172.74, 170.79, 167.76, 165.53, 156.08, 143.89, 140.67, 139.35, 137.78, 136.04, 130.63, 127.94, 127.61, 127.03, 125.35, 121.74, 120.06, 65.64, 59.95, 48.96, 47.87, 46.66, 42.17, 30.35, 28.34, 19.23, 18.38, 18.14.

Fmoc-Ala-Val-Ala-Ind-NH$_2$ (68): ***R*$_f$** 0.45 (9:1 $CHCl_3$-MeOH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 8.40 (d, *J* = 6.2 Hz, 1H, N**H** [Ala]), 8.14 (s, 1H, Ind-Ar**H**), 8.03 (s, 1H, Ind-Ar**H**), 7.85 (d, *J* = 7.5 Hz, 2H, Fmoc-Ar**H**), 7.69-7.63 (m, 3H, 2 x Fmoc-Ar**H**, N**H** [Val]), 7.52 (s, 1H, N**H** [Ala]), 7.37 (dd, 2H, Fmoc-Ar**H**), 7.29 (dd, 2H, Fmoc-Ar**H**), 4.67-4.63 (m, 1H, α-C**H** [Ala]), 4.58-4.54 (m, 1H, NC**H**), 4.26-4.16 (m, 5H, NC**H'**, Fmoc-C**H**, Fmoc-C**H$_2$**, α-C**H** [Val]), 4.11-4.06 (m, 1H, α-C**H** [Ala]), 3.26-3.14 (m, 2H, NCH_2CH_2), 1.91-1.86 (m, 1H, β-C**H** [Val]), 1.25 (d, *J* = 6.9 Hz, 3H, β-C**H**$_3$ [Ala]), 1.16 (d, *J* = 8.1 Hz, 3H, β-C**H**$_3$ [Ala]), 0.78 (d, *J* =

6.9 Hz, 3H, γ-C**H**$_3$ [Val]), 0.74 (d, *J* = 6.5 Hz, 3H, γ-C**H**$_3$ [Val]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 172.86, 172.32, 171.44, 166.09, 156.17, 144.28, 141.23, 139.74, 138.50, 137.11, 131.15, 128.65, 128.15, 127.60, 125.76, 122.33, 123.63, 66.12, 57.39, 50.59, 50.43, 47.76, 47.19, 31.47, 28.96, 19.68, 18.66, 18.45, 17.01.

Fmoc-Leu-Gly-Ile-Ind-NH$_2$ (69): R_f: 0.41 (9:1 $CHCl_3$-MeOH); **$[\alpha]_{24.0}$** = -200.20° (*c* = 1.0, CH_3OH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 8.13-8.10 (m, 3H, Ind-CON**H$_2$**, N**H** [Gly]) 8.04-8.02 (m, 2H, N**H** [Ile], Ind-Ar**H**) 7.85 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.68 (dd, 2H, Fmoc-Ar**H**), 7.52-7.49 (m, 2H, Ind-Ar**H**, N**H** [Leu]), 7.37 (dd, 2H, Fmoc-Ar**H**), 7.30-7.28 (m, 2H, Fmoc-Ar**H**), 4.69-4.66 (m, 1H, NC**H**), 4.54 (dd, *J* = *J* = 6.2 Hz, 1H, α-C**H** [Ile]), 4.32-4.28 (m, 1H, Fmoc-C**H**), 4.24-4.16 (m, 3H, NC**H'**, Fmoc-C**H$_2$**), 4.00-3.96 (m, 1H, α-C**H** [Leu]), 3.75-3.65 (m, 2H, α-C**H$_2$** [Gly]), 3.28-3.25 (m, 1H, NCH_2C**H**), 3.18-3.13 (m, 1H, NCH_2C**H'**), 1.81-1.77 (m, 1H, β-C**H** [Ile]), 1.58-1.36 (m, 4H, γ-C**H** [Leu], γ-C**H** [Ile], β-C**H** [Leu], β-C**H'** [Leu]), 1.14-1.06 (m, 1H, γ-C**H'** [Ile], 0.90 (d, *J* = 6.8 Hz, 3H, γ-C**H$_3$** [Ile]), 0.83 (d, *J* = 6.9 Hz, 3H, δ-C**H$_3$** [Ile]), 0.81-0.79 (m, 6H, 2 x δ-C**H$_3$** [Leu]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 173.20, 171.31, 169.67, 166.05, 156.53, 144.45, 144.26, 141.25, 140.27, 138.56, 136.45, 131.39, 128.58, 128.13, 127.58, 125.81, 122.25, 120.60, 66.06, 55.98, 53.62, 50.64, 47.27, 36.81, 28.87, 24.62, 23.56, 21.93, 15.21, 11.42; **MS** (ESI) Calc. for $[C_{38}H_{44}N_6O_8]Na^+$: 735.3. Found: 735.7.

Fmoc-Lys(Tfa)-Leu-Phe-Ind-NH$_2$ (70): R_f 0.46 (9:1 $CHCl_3$-MeOH); **$[\alpha]_{24.5}$** = –176.84° (*c* = 1.0, CH_3OH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 9.35 (s, 1H, N**H**$COCF_3$), 8.42 (d, *J* = 7.6 Hz, 1H, N**H** [Phe]), 8.13 (s, 1H, Ind-Ar**H**), 8.11 (s, 1H, Ind-CON**H**), 8.01 (s, 1H, Ind-Ar**H**), 7.85 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.76 (d, *J* = 8.9 Hz, 1H, N**H** [Lys]), 7.68-7.65 (m, 2H, Fmoc-Ar**H**), 7.53 (s, 1H, N**H**$COCF_3$), 7.43 (d, *J* = 8.2 Hz, 1H, N**H** [Leu]), 7.28-7.26 (m, 4H, 4 x Phenyl**H**), 7.23 (dd, 2H, Fmoc-Ar**H**), 7.16 (dd, *J* = *J* = 6.8 Hz, 1H,

Phenyl**H**), 4.89-4.85 (m, 1H, α-C**H** [Phe]), 4.41-4.36 (m, 1H, NC**H**), 4.31-4.27 (m, 1H, α-C**H** [Lys]), 4.22-4.16 (m, 3H, Fmoc-C**H$_2$**, Fmoc-C**H**), 3.98-3.90 (m, 2H, NC**H'**, α-C**H** [Leu]), 3.99-2.99 (m, 5H, NCH$_2$C**H**, γ-C**H$_2$** [Lys], NCH$_2$C**H'**, β-C**H** [Phe]), 2.90-2.85 (m, 1H, β-C**H'** [Phe]), 1.56-1.15 (m, 9H, β-C**H$_2$** [Leu], δ-C**H$_2$** [Lys], γ-C**H$_2$** [Lys], β-C**H$_2$** [lys], γ-C**H** [Leu], 0.78 (d, *J* = 6.9 Hz, 3H, δ-C**H$_3$** [Leu]), 0.75 (d, *J* = 6.2 Hz, 3H, γ-C**H$_3$** [Leu]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, **δ_C**) 172.69, 173.13, 170.97, 166.05, 156.48, 144.46, 144.25, 121.24, 139.99, 138.45, 137.49, 136.59, 131.35, 129.84, 128.73, 128.56, 128.15, 127.58, 127.08, 125.83, 125.78, 122.34, 120.62, 66.11, 55.01, 53.27, 51.14, 50.35, 47.20, 41.43, 40.59, 37.14, 31.89, 28.85, 28.44, 24.56, 23.33, 22.04; **MS** (ESI) Calc for [$C_{47}H_{50}F_3N_7O_9$]Na^+: 936.4. Found: 937.4.

Fmoc-Phe-Ala-Pro-Ind-NH$_2$ (71): ***R*$_f$** 0.38 (9:1 $CHCl_3$-MeOH); **[α]$_{24.5}$** –185.36° (*c* = 1.0, CH_3OH); **^{1}H NMR**: (600 MHz, 23.4 °C, DMSO-$_{d6}$, **δ_H**) 8.19 (d, *J* = 7.6 Hz, 1H, N**H** [Ala]), 8.16 (s, 1H, Ind-Ar**H**), 8.05 (s, 1H, Ind-Ar**H**), 7.84 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.60-7.52 (m, 4H, 2 x Fmoc-Ar**H**, N**H** [Phe], Ind-CON**H**), 7.38-7.33 (m, 2H, Fmoc-Ar**H**), 7.28-7.20 (m, 6H, 2 x Fmoc-Ar**H**, 4 x Phenyl**H**), 7.15-7.13 (m, 1H, Phenyl**H**), 4.76-4.71 (m, 2H, α-C**H** [Pro], NC**H**), 4.54-4.49 (m, 1H, α-C**H** [Ala]), 4.26-4.21 (m, 2H, α-C**H** [Phe], NC**H'**), 4.13-4.06 (m, 3H, Fmoc-C**H**, Fmoc-C**H$_2$**), 3.67-3.63 (m, 1H, δ-C**H** [Pro]), 3.55-3.51 (m, 1H, δ-C**H'** [Pro]), 3.32-3.24 (m, 1H, NCH$_2$C**H**), 3.21-3.19 (m, 1H, NCH$_2$C**H'**), 2.97-2.94 (m, 1H, β-C**H** [Phe]), 2.73-2.69 (m, 1H, β-C**H'** [Phe]), 2.17-2.12 (m, 1H, β-C**H** [Pro]), 2.04-1.98 (m, 1H, γ-C**H** [Pro]), 1.93-1.82 (m, 2H, γ-C**H'** [pro], β-C**H'** [Pro]), 1.13 (d, *J* = 6.9 Hz, 3H, β-C**H$_3$** [Ala]). **^{13}C NMR**: (150 MHz, 23.4 °C, DMSO-$_{d6}$, **δ_C**) 171.84, 171.49, 170.93, 166.08, 156.29, 144.23, 141.18, 139.68, 138.64, 138.39, 137.64, 131.09, 129.77, 128.73, 128.54, 128.14, 127.58, 125.84, 125.78, 122.39, 120.60, 66.15, 58.74, 56.44, 50.47, 47.23, 47.09, 46.65, 37.97, 28.94, 28.68, 25.36, 17.38.

Fmoc-Val-Leu-Glu(OBn)-Ind-NH_2 (73): R_f 0.48 (9:1 $CHCl_3$-MeOH); **$[\alpha]_{24.5}$** –198.74° (*c* = 1.0, CH_3OH); **^{1}H NMR**: (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 8.34 (d, *J* = 7.6 Hz, 1H, N**H** [Glu]), 8.14-8.12 (m, 2H, Ind-CON**H_2**), 8.03 (s, 1H, Ind-Ar**H**), 7.88-7.85 (m, 3H, N**H** [Leu], Fmoc-Ar**H**), 7.71-7.68 (m, 2H, Fmoc-Ar**H**), 7.53 (s, 1H, Ind-Ar**H**), 7.39-7.36 (m, 3H, N**H** [Val], Fmoc-Ar**H**), 7.32-7.26 (m, 7H, 5 x Phenyl**H**, 2 x Fmoc-Ar**H**), 5.08 (d, *J* = 12.4 Hz, 1H, Benzyl**H**), 5.04 (d, 1H, Benzyl**H'**), 4.71-4.67 (m, 1H, α-C**H** [Glu]), 4.48-4.46 (m, 1H, NC**H**), 4.34-4.30 (m, 1H, α-C**H** [Leu]), 4.25-4.13 (m, 4H, Fmoc-C**H**, Fmoc-C**H_2**, NC**H'**), 3.83-3.81 (m, 1H, α-C**H** [Val]), 3.24-3.11 (m, 2H, NCH_2**CH_2**), 2.51-2.43 (m, 2H, γ-C**H_2** [Glu]), 2.01-1.79 (m, 3H, 2 x β-C**H_2** [Glu], β-C**H** [Val]), 1.57-1.50 (m, 1H, γ-C**H** [Leu]), 1.40-1.30 (m, 2H, 2 x β-C**H** [Leu]), 0.79-0.75 (m, 12H, 2 x δ-C**H_3** [Leu], 2 x γ-C**H_3** [Val]); **^{13}C NMR**: (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 172.89, 172.70, 171.47, 171.29, 166.04, 159.59, 144.40, 144.24, 141.23, 139.90, 138.60, 136.78, 136.65, 131.33, 128.95, 128.55, 128.50, 128.16, 127.58, 125.86, 122.22, 120.62, 66.11, 60.67, 51.09, 50.94, 50.46, 47.22, 41.15, 30.85, 29.76, 28.93, 26.71, 24.55, 23.56, 21.98, 19.72, 18.72; **MS** (ESI) Calc. for $[C_{47}H_{52}N_6O_{10}]Na^+$: 883.4. Found: 883.5.

Fmoc-Thr(OBn)-Ile-Thr(OBn)-Ind-NH_2 (74): R_f 0.51 (9:1 $CHCl_3$-MeOH); **$[\alpha]_{24.5}$** = –136.80° (*c* = 1.0, CH_3OH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 8.34 (d, *J* = 6.84 Hz, 1H, N**H** (Thr)), 8.13-8.12 (m, 2H, Ind-ArH, CON**H** (Ind)), 8.03 (s, 1H, Ind-Ar**H**), 7.85 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.82 (d, *J* = 8.9 Hz, 1H, N**H** [Ile]), 7.72-7.68 (m, 2H, Fmoc-Ar**H**), 7.54 (s, 1H, Ind-CON**H**), 7.42 (d, *J* = 8.9 Hz, 1H, N**H** [Thr]), 7.38-7.36 (m, 2H, Fmoc-Ar**H**), 7.35-7.18 (m, 10H, Phenyl**H**), 4.78 (dd, *J* = *J* = 6.8 Hz, 1H, α-C**H** [Thr]), 4.65-4.61 (m, 1H, NC**H**), 4.52 (s, 2H, Benzyl-C**H_2** [Thr]), 4.46-4.36 (m, 3H, Benzyl-C**H_2** [Thr], α-C**H** [Ile]), 4.28-4.13 (m, 5H, Fmoc-C**H**, Fmoc-C**H_2**, α-C**H** [Thr], NC**H'**), 3.88-3.84 (m, 1H, β-C**H** [Thr]), 3.81-3.77 (m, 1H, β-C**H** [Thr]), 3.20-3.14 (m, 1H, NCH_2C**H**), 3.11-3.05 (m, 1H, NCH_2C**H'**), 1.66-1.60 (m, 1H, β-C**H** [Ile]), 1.38-1.32 (m, 1H, γ-C**H** [Ile]), 1.16 (d, *J* = 6.2 Hz, 3H, γ-C**H_3** [Thr]), 0.99-0.97 (m, 1H, γ-C**H'** [Ile]), 0.72 (d, *J* = 6.2 Hz,

3H, γ-C**H$_3$** [Thr]), 0.66 (t, 3H, δ-C**H$_3$** [Ile]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, **δ_C**) 171.83, 170.06, 169.34, 156.70, 144.43, 144.24, 141.23, 140.29, 139.24, 139.01, 138.51, 136.42, 131.50, 128.66, 128.17, 127.91, 127.88, 127.69, 127.59, 125.90, 122.31, 120.61, 75.57, 75.10, 70.09, 70.74, 66.34, 59.73, 57.02, 56.26, 50.26, 47.22, 37.43, 28.84, 24.61, 16.86, 16.27, 15.69, 11.45; **MS** (ESI) Calc. for $[C_{52}H_{56}N_6O_{10}]Na^+$: 947.4. Found: 947.2.

3.4 Preparation of Amino Acid-α-Phenylthioesters

General Procedure for the Thioesterification of Fmoc-AA-Ind-OAll (42-49): In a microcetrifuge tube were placed Fmoc-Phe-Ind-OAll (0.0102 g, 0.01651 mmol, 1.00 equiv) and HOBt (0.0346 g, 0.2561 mmol, 15.51 equiv). The combined solids were dissolved in DCM (1 mL) and d_6-DMSO (0.1 mL) then injected into a pre-dried, argon flushed NMR tube attached with a septum. The NMR tube was placed in a photoreactor and illuminated with light (350nm) for 2 h. PhSH (0.05 mL, large molar excess) was injected and the tube shaken by hand for 5 min. After sitting in the dark for 2 h, the contents of the NMR tube were transferred to a round bottom flask and evaporated to dryness. The resulting solid was then purified by column chromatograpy (silica gel) to give 0.0073 g (92%) of Fmoc-Phe-SPh.

Fmoc-Phe-SPh (78): ***R*$_f$** 0.71 (1:1 EtOAc:Hex); **[α]$_{24.5}$** = -60.06° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, **δ_H**) 7.76 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.55 (dd, *J* = 17.9, 1H, Fmoc-Ar**H**), 7.43-7.27, (m, 12 H, 5 x S-Phenyl**H**, 5 x Phenyl**H**, 2 x Fmoc-Ar**H**), 7.18 (d, 2H, Fmoc-Ar**H**), 5.21 (d, *J* = 8.9 Hz, 1H, N**H**), 4.86-4.82 (m, 1H, α-C**H**), 4.46-4.40 (m, 2H, Fmoc-C**H$_2$**), 4.22 (dd, *J* = *J* = 6.8 Hz, 1H, Fmoc-C**H**), 3.22-3.12 (m, 2H, β-C**H$_2$**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, **δ_C**) 198.75, 155.67, 143.80, 143.72, 141.42, 135.43,

134.71, 129.73, 129.53, 129.39, 128.90, 127.84, 127.45, 127.17, 125.15, 120.09, 67.32, 61.41, 47.24, 38.47.

Fmoc-Gly-SPh (75): R_f 0.54 (1:1 EtOAc:Hex); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 7.76 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.60 (d, *J* = 8.2 Hz, 2H, Fmoc-Ar**H**), 7.43-7.38 (m, 7H, 5 x S-Phenyl**H**, 2 x Fmoc-Ar**H**), 7.30 (dd, 2H, Fmoc-Ar**H**), 5.35 (dd, *J* = *J* = 6.8 Hz, 1H, N**H**), 4.46 (d, *J* = 7.6 Hz, 2H, Fmoc-C$\mathbf{H_2}$), 4.26-4.23 (m, 3H, Fmoc-C**H**, α-C$\mathbf{H_2}$); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 146.91, 143.88, 141.29, 135.02, 129.84, 129.46, 127.84, 127.18, 125.13, 120.10, 67.41, 50.56, 46.98, 29.82.

Fmoc-Ala-SPh (76): R_f 0.63 (1:1 EtOAc:Hex); $[\alpha]_{24.5}$ = -33.37° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 7.76 (d, *J* = 7.6 Hz, 2H, Ar**H**), 7.62 (dd, 2H, Ar**H**), 7.42-7.37 (m, 7H, 5 x S-Phenyl**H**, 2 x Fmoc-Ar**H**), 7.31 (dd, 2H, Fmoc-Ar**H**), 5.27 (d, *J* = 8.3 Hz, 1H, N**H**), 4.62-4.57 (m, 1H, α-C**H**), 4.55-4.52 (m, 1H, Fmoc-C**H**), 4.41-4.38 (m, 1H, Fmoc-C**H'**), 4.25 (dd, *J* = *J* = 6.8 Hz, 1H, Fmoc-C**H**), 1.48 (d, *J* = 6.9 Hz, 3H, β-C$\mathbf{H_3}$); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 199.53, 155.62, 143.77, 141.42, 141.42, 134.77, 129.67, 129.36, 127.84, 127.19, 125.19, 125.12, 120.11, 67.25, 56.72, 47.28, 19.01.

Fmoc-Ile-SPh (77): R_f 0.66 (1:1 EtOAc:Hex); $[\alpha]_{24.5}$ = -56.72° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 7.77 (d, 2H, *J* = 7.6 Hz, Fmoc-Ar**H**), 7.63 (dd, 2H, Fmoc-Ar**H**), 7.42-7.38 (m, 7H, 5 x S-Phenyl**H**, 2 x Fmoc-Ar**H**), 7.31 (dd, 2H, Fmoc-Ar**H**), 5.29 (d, *J* = 9.6 Hz, 1H, N**H**), 4.57-4.53 (m, 1H, Fmoc-C**H**), 4.52-4.49 (m, 1H, α-C**H**), 4.46-4.43 (m, 1H, Fmoc-C**H'**), 4.26 (dd, *J* = *J* = 6.8 Hz, 1H, Fmoc-C**H**), 2.10-2.05 (m, 1H, β-C**H**), 1.53-1.47 (m, 1H, γ-C**H**), 1.21-1.13 (m, 1H, γ-C**H'**), 1.01 (d, *J* = 6.8 Hz, 3H, γ-C$\mathbf{H_3}$), 0.94 (dd, *J* = *J* = 6.9 Hz, 3H, δ-C$\mathbf{H_3}$); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 198.92, 156.22, 143.91,

143.77, 141.45, 134.70, 129.64, 129.36, 127.84, 127.20, 125.14, 120.11, 67.24, 65.49, 47.36, 37.27, 24.50, 16.00, 11.78.

Fmoc-Lys(Tfa)-SPh (80): R_f 0.54 (1:1 EtOAc:Hex); $[\alpha]_{24.5}$ = -36.70° (*c* = 1.0, CH_3OH); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 7.76 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.61 (d, *J* = 6.9 Hz, 2H, Fmoc-Ar**H**), 7.42-7.37 (m, 7H, 5 x S-Phenyl**H**, 2 x Fmoc-Ar**H**), 7.31 (dd, *J* = *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 6.46 (s, br, 1H, N**H**$COCF_3$), 5.34 (d, *J* = 8.9, 1H, N**H**), 4.56-4.52 (m, 2H, Fmoc-C**H**, α-C**H**), 4.43-4.40 (m, 1H, Fmoc-C**H'**), 4.24 (dd, *J* = *J* = 6.9 Hz, 1H, Fmoc-C**H**), 3.39-3.35 (m, 2H, ε-C$\mathbf{H_2}$), 2.00-1.94 (m, 1H, β-C**H**), 1.78-1.56 (m, 3H, β-C**H'**, δ-C$\mathbf{H_2}$), 1.47-1.43 (m, 2H, γ-C$\mathbf{H_2}$); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 207.07, 198.89, 156.11, 143.79, 141.45, 134.71, 129.81, 129.42, 127.86, 127.19, 129.70, 125.11, 120.12, 67.29, 60.37, 47.28, 39.53, 32.42, 31.01, 28.27, 22.34.

Fmoc-Glu(OBn)-SPh (81): R_f 0.64 (1:1 EtOAc:Hex); $[\alpha]_{24.5}$ = -23.36° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 7.75 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.62 (dd, 2H, Fmoc-Ar**H**), 7.41-7.29 (m, 14 H, 5 x S-Phenyl**H**, 5 x Phenyl**H**, 4 x Fmoc-Ar**H**), 5.57 (d, *J* = 8.8 Hz, 1H, N**H**), 5.13 (d, *J* = 2.0 Hz, 2H, Benzyl-C$\mathbf{H_2}$), 4.61-4.57 (m, 1H, α-C**H**), 4.40-4.37 (m, 2H, Fmoc-C$\mathbf{H_2}$), 4.24 (dd, *J* = *J* = 8.8 Hz, 1H, Fmoc-C**H**), 2.57-2.45 (m, 2H, γ-C$\mathbf{H_2}$), 2.35-2.29 (m, 1H, β-C**H**), 2.08-2.02 (m, 1H, β-C**H'**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 198.64, 172.79, 155.95, 143.69, 141.43, 135.67, 134.73, 129.75, 129.39, 128.71, 128.48, 128.42, 127.86, 127.20, 125.20, 125.16, 120.10, 67.34, 66.38, 60.46, 47.28, 30.40, 27.64.

Fmoc-Thr(OBn)-SPh (82): R_f 0.63 (1:1 EtOAc:Hex); $[\alpha]_{24.5}$ = -60.06° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 7.76 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.66 (d, *J* = 8.3 Hz, 2H, Fmoc-Ar**H**), 7.43-7.29 (m, 14H, 5 x S-Phenyl**H**, 5 x Benzyl**H**, 4 x Fmoc-Ar**H**), 5.76 (d, *J* = 9.6 Hz, 1H, N**H**), 4.62-4.56 (m, 2H, Fmoc-

C**H$_2$**), 4.48-4.42 (m, 3H, α-C**H**, Benzyl-C**H$_2$**), 4.35-4.28 (m, 2H, β-C**H**, Fmoc-C**H**), 1.26 (d, *J* = 6.0 Hz, 3H, γ-C**H$_3$**); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 199.40, 156.63, 143.90, 143.72, 141.45, 134.79, 129.60, 129.35, 128.51, 127.99, 127.88, 127.84, 127.20, 125.24, 125.19, 120.11, 74.77, 71.83, 67.47, 65.69, 47.35, 16.79; **MS** (ESI) Calc. for $[C_{32}H_{29}NO_6S]H^+$: 524.18. Found: 524.19.

Fmoc-Cys(*t*bu-Thio)-SPh (83): R_f 0.62 (1:1 EtOAc:Hex); **$[\alpha]_{24.5}$** = -48.84° (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (600 MHz, 23.4 °C, $CDCl_3$, δ_H) 7.76 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.65 (d, *J* = 6.9 Hz, 2H, Fmoc-Ar**H**), 7.41-7.37 (m, 9H, 5 x S-Phenyl**H**, 4 x Fmoc-Ar**H**) 5.74 (d, *J* = 8.9 Hz, 1H, N**H**), 4.87-4.85 (m, 1H, α-C**H**), 4.53-4.50 (m, 1H, Fmoc-C**H**), 4.45-4.43 (m, 1H, Fmoc-C**H'**), 4.29 (dd, *J* = *J* = 8.9 Hz, 1H, Fmoc-C**H**), 3.20 (d, *J* = 6.2 Hz, 2H, β-C**H$_2$**), 1.34 (s, 9H, S-C(C**H$_3$**)$_3$); **^{13}C NMR** (150 MHz, 23.4 °C, $CDCl_3$, δ_C) 198.08, 155.78, 143.81, 143.77, 141.42, 134.71, 129.73, 129.38, 127.59, 127.21, 125.28, 120.10, 67.59, 60.48, 48.71, 47.24, 42.44, 29.92.

3.5 Preparation of Tripeptide-α-Phenylthioesters

General Procedure for the Thioesterification of Fmoc-Tripeptide-Ind-NH$_2$

In a microcetrifuge tube were placed Fmoc-Leu-Gly-Ile-Ind-NH_2 (0.0126 g, 0.0177 mmol, 1.00 equiv) and HOBt (0.0361 g, 0.2672 mmol, 15.11 equiv). The combined solids were dissolved in DCM (1 mL) and d_6-DMSO (0.1 mL) then injected into a pre-dried, argon flushed NMR tube attached with a septum. The NMR tube was placed in a photoreactor and illuminated with light (350nm) for 5 h. PhSH (0.1 mL, large molar excess) was injected and the tube shaken by hand for 5 min. After sitting in the dark overnight, the contents of the NMR tube were transferred to a round bottom flask and evaporated to

dryness. The resulting solid was then purified by column chromatograpy (silica gel) to give 0.0086 g (79%) of Fmoc-Leu-Gly-Ile-SPh.

Fmoc-Val-Ala-Gly-SPh (84): R_f 0.61 (9:1 $CHCl_3$-MeOH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 7.76-7.74, (m, 2H, Fmoc-Ar**H**), 7.76-7.54 (m, 2H, Fmoc-Ar**H**), 7.40-7.34 (m, 7H, 2 x Fmoc-Ar**H**, 5 x S-Phenyl**H**), 7.31-7.27 (m, 2H, Fmoc-Ar**H**), 6.96-6.94 (m, 1H, N**H** [Gly]), 6.46 (d, *J* = 6.9 Hz, 1H, N**H** [Ala]), 5.33 (d, *J* = 6.9 Hz, 1H, N**H** [Val]), 4.60-4.55 (m, 1H, α-C**H** [Ala]), 4.45 (dd, *J* = 6.8 Hz, *J* = 10.3 Hz, 1H, Fmoc-C**H**), 4.36 (dd, 1H, Fmoc-C**H'**), 4.26 (d, *J* = 5.2 Hz, 2H, α-C**H$_2$** [Gly]), 4.19 (dd, *J* = *J* = 6.8 Hz, 1H, Fmoc-C**H**), 4.01-3.97 (m, 1H, α-C**H** [Val]), 2.17-2.12 (m, 1H, β-C**H** [Val]), 1.42 (d, *J* = 6.90 Hz, 3H, β-C**H$_3$** [Ala]), 0.96 (d, *J* = 6.60 Hz, 3H, γ-C**H$_3$** [Val]), 0.92 (d, *J* = 6.18 Hz, 3H, γ-C**H$_3$'** [Val]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 195.09, 172.19, 171.33, 156.69, 143.79, 141.40, 134.78, 129.83, 129.42, 127.86, 127.17, 126.27, 125.04, 120.10, 67.24, 60.74, 49.00, 48.88, 47.23, 30.92, 29.79, 19.34, 17.91.

Fmoc-Ala-Val-Ala-SPh (85): R_f 0.65 (9:1 $CHCl_3$-MeOH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 8.72 (d, *J* = 6.84 Hz, 1H, N**H** [Ala$_1$]), 7.86 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.70-7.67 (m, 3H, Fmoc-Ar**H**, N**H** [Val]), 7.54 (d, *J* = 7.6 Hz, 1H, N**H** [Ala$_2$]), 7.42-7.28 (m, 9H, 5 x S-Phenyl**H**, 4 x Fmoc-Ar**H**), 4.48-4.44 (m, 1H, α-C**H** [Ala$_1$]), 4.32-4.32 (dd, *J* = 5.9 Hz, *J* = 8.6 Hz, 1H, α-C**H** [Val]), 4.24 (d, *J* = 6.9 Hz, 2H, Fmoc-C**H$_2$**), 4.18 (dd, *J* = *J* = 6.9 Hz, 1H, Fmoc-C**H**), 4.15-4.10 (m, 1H, α-C**H** [Ala$_2$]), 2.12-2.06 (m, 1H, β-C**H** [Val]), 1.31 (d, *J* = 6.8 Hz, 3H, β-C**H$_3$** [Ala$_1$]), 1.19 (d, *J* = 6.9 Hz, 3H, β-C**H$_3$** [Ala$_2$]), 0.88 (d, *J* = 6.2 Hz, 3H, γ-C**H$_3$** [Val]), 0.86 (d, *J* = 6.9 Hz, 3H, γ-C**H$_3$'** [Val]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 199.76, 172.99, 171.81, 156.19, 144.40, 144.34, 141.17, 134.95, 129.89, 128.13, 127.75, 127.55, 125.78, 120.62, 66.14, 57.45, 55.46, 50.53, 47.12, 31.39, 19.99, 18.71, 18.12, 17.65.

Fmoc-Leu-Gly-Ile-SPh (86): R_f 0.69 (9:1 $CHCl_3$-MeOH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 8.27 (d, *J* = 8.2 Hz, 1H, N**H** [Ile]), 8.23 (dd, *J* = *J* = 6.2 Hz, 1H, N**H** [Gly]), 7.85 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.69-7.67 (m, 2H, Fmoc-Ar**H**), 7.55 (d, *J* = 7.6 Hz, 1H, N**H** [Leu]), 7.42-7.37 (m, 5H, 3 x S-Phenyl**H**, 2 x Fmoc-Ar**H**), 7.31-7.27 (m, 4H, 2 x S-Phenyl**H**, 2 x Fmoc-Ar**H**), 4.39 (dd, *J* = 6.5 Hz, *J* = 8.2 Hz, α-C**H** [Ile]), 4.29-4.27 (m, 1H, Fmoc-C**H**), 4.21-4.17 (m, 2H, Fmoc-C**H$_2$**), 4.04-4.01 (m, 1H, α-C**H** [Leu]), 3.86 (dd, *J* = 5.5 Hz, *J* = 16.5 Hz, 1H, α-C**H** [Gly]), 3.76 (dd, 1H, α-C**H'** [Gly]), 1.92-1.89 (m, 1H, β-C**H** [Ile]), 1.63-1.57 (m, 1H, γ-C**H** [Ile]), 1.51-1.38 (m, 3H, β-C**H$_2$** [Leu], γ-C**H'** [Ile]), 1.22-1.14 (m, 1H, γ-C**H** [Leu]), 0.86-0.77 (m, 12H, 2 x δ-C**H$_3$** [Leu], δ-C**H$_3$** [Ile], γ-C**H$_3$'** [Ile]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 198.85, 173.32, 170.20, 165.57, 144.42, 141.25, 134.99, 129.90, 128.16, 127.81, 127.58, 125.84, 120.64, 66.11, 63.83, 53.61, 47.23, 42.46, 41.12, 36.92, 24.88, 24.70, 23.61, 21.93, 15.99, 11.69; **MS** (ESI) Calc. for $[C_{35}H_{41}N_3O_5S]Na^+$: 638.27. Found: 638.07. Calc for $[C_{35}H_{41}N_3O_5S]K^+$: 654.37. Found: 654.04.

Fmoc-Lys(Tfa)-Leu-Phe-SPh (87): R_f 0.70 (9:1 $CHCl_3$-MeOH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 9.34 (t, 1H, N**H**COCF3), 8.71 (d, *J* = 7.6 Hz, 1H, N**H** [Phe]), 7.69-7.66 (m, 3H, N**H** [Leu], 2 x Fmoc-Ar**H**), 7.46-7.37 (m, 6H, N**H** [Lys], 5 x S-Phenyl**H**), 7.30-7.27 (m, 2H, Fmoc-Ar**H**), 7.24-7.16 (m, 5H, Phenyl**H**), 4.66-4.62 (m, 1H, α-C**H** [Phe]), 4.38-4.34 (m, 1H, α-C**H** [Leu]), 4.25-4.16 (m, 3H, Fmoc-C**H**, Fmoc-C**H$_2$**), 3.98-3.94 (m, 1H, α-C**H** [Lys]), 3.13-3.05 (m, 3H, ε-C**H$_2$** [Lys], β- C**H** [Phe]), 2.96 (dd, *J* = 9.7 Hz, *J* = 13.7 Hz, 1H, β-C**H** [Phe]), 1.59-1.35 (m, 7H, δ-C**H$_2$** [Lys], γ-C**H** [Leu], γ-C**H$_2$** [Lys], β-C**H$_2$** [Leu]), 1.26-1.22 (m, 2H, β-C**H$_2$** [Lys]), 0.82 (d, *J* = 6.8 Hz, 3H, δ-C**H$_3$** [Leu]), 0.78 (d, *J* = 6.2 Hz, 3H, δ-C**H$_2$** [Leu]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 198.64, 172.99, 172.20, 156.47, 144.43, 144.26, 141.25, 137.22, 135.01, 129.98, 129.88, 129.59, 128.76, 128.16, 127.98, 127.57, 126.96, 125.81, 120.63, 66.11, 60.83, 54.96, 51.27, 47.17, 41.23, 40.61, 36.99, 32.06, 28.51, 24.61, 23.46, 22.22.

Fmoc-Phe-Ala-Pro-SPh (88): R_f 0.62 (9:1 $CHCl_3$-MeOH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 8.13 (d, *J* = 6.9 Hz, 1H, N**H** [Ala]), 7.84 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.60 (d, *J* = 6.9 Hz, 1H, N**H** [Phe]), 7.58 (d, *J* = 8.3 Hz, 2H, Fmoc-Ar**H**), 7.42-7.21 (m, 14 H, 4 x Fmoc-Ar**H**, 5 x Phenyl**H**, 5 x S-Phenyl**H**), 4.60-4.56 (m, 2H, α-C**H** [Ala], α-C**H** [Pro]), 4.28-4.23 (m, 1H, α-C**H** [Phe]), 4.13-4.07 (m, 3H, Fmoc-C**H**, Fmoc-C**H$_2$**), 3.74-3.70 (m, 1H, δ-C**H** [Pro]), 3.66-3.62 (m, 1H, δ-C**H'** [Pro]), 2.99 (dd, *J* = 2.7 Hz, *J* = 12.4 Hz, 1H, β-C**H** [Phe]), 2.71 (dd, 1H, β-C**H'** (Phe)), 2.22-2.19 (m, 1H, β-C**H** [Pro]), 2.04-2.00 (m, 1H, β-C**H'** [Pro]), 1.97-1.91 (m, 2H, γ-C**H$_2$** [Pro]), 1.30 (d, *J* = 6.8 Hz, 3H, β-C**H$_3$** [Ala]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 198.80, 171.94, 171.70, 156.25, 144.26, 141.27, 135.06, 130.00, 129.89, 129.75, 128.56, 128.14, 127.59, 127.45, 126.75, 125.88, 125.28, 120.60, 66.20, 56.43, 47.42, 47.07, 46.91, 38.00, 29.86, 25.13, 17.18.

Fmoc-Glu(OBn)-Ala-Lys(Tfa)-SPh (89): R_f 0.67 (9:1 $CHCl_3$-MeOH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 9.36 (t, 1H, N**H**COCF_3), 8.62 (d, *J* = 7.6 Hz, 1H, N**H** [Lys]), 8.09 (d, *J* = 6.9 Hz, 1H, N**H** [Ala]), 7.84 (d, *J* = 7.6 Hz, 2H, Fmoc-Ar**H**), 7.68 (dd, 2H, Fmoc-Ar**H**), 7.51 (d, *J* = 8.2 Hz, 1H, N**H** [Glu]), 7.42 - 7.26 (m, 14H, 4 x Fmoc-Ar**H**, 5 x phenyl**H** [Glu], 5 x S-Phenyl**H**), 5.05 (s, 2H, Benzyl-C**H$_2$**) 4.38 – 4.32 (m, 2H, α-C**H** [Lys], α-C**H** [Ala]), 4.28 – 4.16 (m, 3H, Fmoc-C**H**, Fmoc-C**H$_2$**) 4.06 – 4.02 (m, 1H, α-C**H** [Glu]), 3.13 (ddd, 2H, *J* = 6.2 Hz, ε-C**H$_2$** [Lys]), 2.41 – 2.38 (m, 2H, γ-C**H$_2$** [Glu]), 1.96 – 1.90 (m, 1H, β-C**H** [Glu]), 1.82 – 1.74 (m, 2H, β-C**H'** [Glu], β-C**H'** [Lys]), 1.66 – 1.59 (m, 2H, β-C**H'** [Lys]), 1.48 – 1.40 (m, 2H, δ-C**H$_2$** [Lys]), 1.37 – 1.26 (m, 5H, β-C**H$_3$** [Ala], γ-C**H$_2$** [Lys]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 199.68, 173.30, 172.81, 171.44, 156.54, 144.44, 144.25 141.25, 136.74, 135.08, 129.93 129.85, 128.94, 128.49, 128.42, 128.16, 127.58, 125.82, 120.64, 66.18, 65.97, 59.65, 54.05, 48.56, 47.20, 39.42, 31.04, 30.68, 28.25, 27.77, 22.91, 18.18, 14.35, 11.36; **MS** (ESI) Calc. for $[C_{44}H_{45}F_3N_4O_8S]Na^+$: 869.3. Found: 868.9.

Fmoc-Thr(OBn)-Ile-Thr(OBn)-SPh (90): R_f 0.72 (9:1 $CHCl_3$-MeOH); **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6}$, δ_H) 7.86 (m 3H, N**H** [Thr], Fmoc-Ar**H**), 7.72-7.68 (m,3H, N**H** [Thr], Fmoc-Ar**H**), 7.42 (d, *J* = 8.9 Hz, 1H, N**H** [Ile]), 7.37 (dd, *J* = *J* = 6.8 Hz, 2H, Fmoc-Ar**H**), 7.28-7.18 (m, 17H, 2 x Fmoc-Ar**H**, 10 x Phenyl**H**, 5 x S-Phenyl**H**), 4.46-4.38 (m, 6H, 2 x Benzyl-C**H$_2$**, Fmoc-C**H**, α-C**H** [Thr]), 4.25-4.17 (m, 4H, Fmoc-C**H$_2$**, α-C**H** [Thr], α-C**H** [Thr]), 4.04-4.03 (m, 1H, β-C**H** [Thr]), 3.87-3.83 (m, 1H, β-C**H** [Thr]), 1.74-1.70 (m, 1H, β-C**H** [Ile]), 1.44-1.39 (m, 1H, γ-C**H** [Ile]), 1.06-0.99 (m, 7H, 2 x γ-C**H$_3$** [Thr], γ-C**H** [Ile]), 0.81 (d, *J* = 6.8 Hz, 3H, γ-C**H$_3$** [Ile]), 0.72 (dd, *J* = *J* = 7.6 Hz, 3H, δ-C**H$_3$** [Ile]); **^{13}C NMR** (150 MHz, 23.4 °C, DMSO-$_{d6}$, δ_C) 206.98, 170.08, 156.65, 144.43, 144.25, 141.23, 139.64, 139.27, 129.45, 128.50, 128.48, 128.15, 127.99, 127.94, 127.81, 127.69, 127.58, 121.99, 120.61, 75.70, 70.96, 70.79, 66.35, 59.72, 57.49, 47.20, 37.34, 31.21, 24.64, 17.01, 15.88, 11.68.

3.6 Synthesis of Photoreactive Glycopeptides

Preparation of Chitobiose Octaacetate from Shrimp Chitin Acidolysis: Shrimp chitin (40 g) was added to a cooled solution of acetic anhydride (200 mL) and H_2SO_4 conc. (26 mL) in a 500 mL E-flask. The solution was allowed to warm to room temperature and was swirled occasionally, by hand, for two days. The flask was then placed in a temperature controlled oil bath set to 50 °C for 7 h. The flask was removed from the oil bath and to it was added sodium acetate (150 g in 1 L H_20) and the solution was allowed to sit at room temperature for 2 h. The solid was removed by filtration and the solution was neutralized to pH 7 using solid sodium bicarbonate. The neutralized solution was extracted with chloroform. The combined chloroform extracts were washed with water and brine then dried over magnesium sulfate. After filtration and concentration a solid crude product was obtained and purified

by flash chromatography to give 3.097 g (8% by weight based on 40 g chitin) of chitobioseoctaacetate **(94)**. R_f 0.60 (9:1 $CHCl_3$-MeOH); **^{1}H NMR** (300 MHz, 23.4 °C, $CDCl_3$, δ_H) 8.01 (d, *J* = 8.9 Hz, 1H, N**H**Ac), 7.95 (d, *J* = 8.9 Hz, 1H, N**H'**Ac), 5.82 (d, *J* = 4.3 Hz, 1H, **H**-β), 5.13-3.81 (m, 12 H, 2 x C**H$_2$**, 8 x C**H**, **H'**-α), 2.16 (s, 3H, C**H$_3$**), 2.06 (s, 3H, C**H$_3$**), 2.01 (s, 3H, C**H$_3$**), 1.99 (s, 3H, C**H$_3$**), 1.96 (s, 3H, C**H$_3$**), 1.90 (s, 3H, C**H$_3$**), 1.78 (s, 3H, C**H$_3$**), 1.74 (s, 3H, C**H$_3$**).

β-Amination of Chitobiose: Initial attempts to convert chitobioseoctaacetate to its β-amine directly using the Hackenberger method failed. As a result the strategy was changed to utilize the more promising Kochetkov method which required the use of chitobiose as starting material.

De-acetylation of chitobioseoctaacetate under Zemplen conditions (95): In a 500 ml flask were placed chitobioseoctaacetate (1.72 g), dry methanol, and a sliver of sodium metal. The reaction was allowed to stir until TLC analysis showed complete consumption of the starting material and the convergence of all spots produced into one base line spot. The solution was neutralized to pH 7 using the ion exchange resin amberlyst 15. After filtration and evaporation, crude chitobiose was recovered in quantitative yield and used without purification. TLC analysis showed one spot.

Kochetkov β-amination of chitobiose (96): The crude product from the Zemplen de-acetylation reaction was added to a saturated ammonium carbonate solution and allowed to stir at 45 °C for 2 d. The β-amine of chitobiose was isolated by repeated lyophilization. The crude product was used without purification. TLC analysis showed one spot.

Preparation of Fmoc-Asn[chitobiose(TBDMS)$_5$]-OAll (97): Fmoc-Asp-OAll (1.00 mmol), HOBt (1.12 mmol) and HBTU (1.12 mmol) were dissolved in anhydrous DMF (15 ml). DIPEA (2.2 mmol) was added and the solution stirred under an argon atmosphere for 15 min. Chitobiosylamine (1.14 mmol) was added and the solution stirred for 20 h. TLC indicated the full consumption of the Fmoc-asp-oall starting material. The DMF was removed by evaporation and the resulting solid was stirred in ethyl acetate for 8 h. The undissolved solid was isolated by filtration then dried on full pump vacuum to give 0.7119 g (89%) of the desired product which analyzed as one spot on TLC. A portion of the crude product (**96**) (56 mg) was then dissolved in anhydrous pyridine (3 mL) under an argon atmosphere. Excess tetrabutyldimethylsilylchloride, TBDMS-Cl, (0.5 mL) was added dropwise at 0 °C and the solution was stirred for 24 h. The pyridine was removed by evaporation and the resulting solid was purified by flash chromatography to give 80.3 mg (87%) of the desired product. **^{1}H NMR** (300 MHz, 23.4 °C, $CDCl_3$, δ_H) 7.75 (d, *J* = 8.4 Hz, 2H, Fmoc-Ar**H**), 7.62 (d, *J* = 6.9 Hz, 2H, Fmoc-Ar**H**), 7.40 (dd, *J* = *J* = 8.2 Hz, 2H, Fmoc-Ar**H**), 7.31 (dd, *J* = *J* = 6.9 Hz, 2H, Fmoc-A**rH**), 6.84 (d, *J* = 8.2 Hz, 1H, N**H**), 6.59 (d, *J* = 8.2 Hz, 1H, N**H**), 6.13 (d, *J* = 8.9 Hz, 1H, N**H**), 5.94-5.83 (m, 1H, CH_2=C**H**), 5.74 (d, *J* = 10.2 Hz, 1H, N**H**), 5.31 (d, *J* = 17.8 Hz, 1H, C**H**$_2$=CH), 5.23 (d, *J* = 8.9 Hz, 1H, C**H**$_2$=CH), 4.95 (d, *J* = 14.3 Hz, 2H, C**H**$_2$-CH=CH_2), 4.66- 3.66 (m, 18 H, 4 x C**H**$_2$, 8 x C**H**, **H**-α, **H**'-α), 3.00 (d, *J* = 6.9 Hz, 1H Fmoc-C**H**), 2.67 (d, *J* = 8.4 Hz, 1H, α-C**H**), 0.93-0.87 (m, 45H, 5 x (C**H$_3$**)$_3$), 0.18-0.025 (m, 30H, 5 x (C**H$_3$**)$_3$).

Deallyation of Fmoc-Asn[chitobiose(TBDMS)$_5$]-OAll (92): Fmoc-Asn[chitobiose(TBDMS)$_5$]-OAll (0.330 mmol), tetrakistriphenylphosphine palladium (0) (0.0334 mmol) and *N*-methylaniline (3.309 mmol) were dissolved in anhydrous THF (5 mL) and stirred for 1 h. The THF was removed by evaporation and the crude product mixture was purified by flash chromatography to give the desired product (95%). The loss of the allyl group peaks in a crude ^{1}H NMR and one spot on TLC confirmed that Fmoc-

Asn[chitobiose(TBDMS)$_5$]-OH was obtained and was used as a building block for future solid phase peptide synthesis.

β-Amination of *N*-Acetylglucose: Initial attempts to convert *N*-acetylglucosamine to its β-amine directly using the Hackenberger method failed. As a result the strategy was changed to utilize the more promising Kochetkov method.

Kochetkov β-amination of *N*-Acetylglucose: *N*-acetylglucose (1.0869 g) was added to a saturated ammonium carbonate solution and allowed to stir at 45 °C for 2 d. The β-amine of *N*-acetylglucose was isolated by repeated lyophilization. The crude product (1.3937 g) was used without purification.

Preparation of Fmoc-Asn[GlcNAc(TBDMS)$_3$]-OAll: Fmoc-Asp-OAll (0.998 mmol), HOBt (1.11 mmol) and HBTU (1.11 mmol) were dissolved in anhydrous DMF (15 mL). DIPEA (2.2 mmol) was added and the solution stirred under an argon atmosphere for 15 min. *N*-acetylglucosamine (1.12 mmol) was added and the solution stirred for 20 h. TLC indicated the full consumption of the Fmoc-asp-oall starting material. The DMF was removed by evaporation and the resulting solid stirred in ethyl acetate for 8 h. The undissolved solid was isolated by filtration then dried on full pump vacuum to give 0.5335 g (89%) of the desired product which analyzed as one spot on TLC. A portion of the crude product (0.4517 g) was then dissolved in anhydrous pyridine (10 mL) under an argon atmosphere. Excess tetrabutyldimethylsilylchloride, TBDMS-Cl, (2.8 mL) was added dropwise at 0 °C and the solution stirred for 24 h. The pyridine was removed by evaporation and the resulting solid was purified by flash chromatography to give 0.6104 g (85%) of the desired product.

Deallyation of Fmoc-Asn[GlcNAc(TBDMS)$_3$]-OAll: Fmoc-Asn[GlcNAc(TBDMS)$_3$]-OAll (0.7797 mmol), tetrakistriphenylphosphine palladium (0) (0.0881 mmol) and *N*-methylaniline (7.813 mmol) were dissolved in anhydrous THF (5 mL) and stirred for 1 h. The THF was removed by evaporation and the crude product mixture was purified by flash chromatography to give the desired product (99%). The loss of the allyl group peaks in a crude ^{1}H NMR and one spot on TLC confirmed that Fmoc-Asn[GlcNAc(TBDMS)$_3$]-OH was obtained and was used as a building block for future solid phase peptide synthesis.

SPPS of Fmoc-Asn[GlcNAc(OH)$_3$]-Ile-Thr-Thr-Gly-Ind-NH$_2$ (98): The synthesis of glycopeptide partial sequence Asn 24 – Gly 28 of human erythropoietin was accomplished by solid phase peptide synthesis using standard Fmoc/t-Bu chemistry. Coupling of Fmoc-Gly-Ind-OH (3.00 equiv.) to Rink Amide AM resin (0.74 mmol/g loading capacity) was achieved using HOBt (3.00 equiv.), HBTU (2.97 equiv.) and DIPEA (6 equiv.) following Fmoc group removal using a solution of 20% piperidine in NMP. The subsequent three amino acids were coupled using amino acid (5 equiv.), HOBt (5 equiv.), HBTU (4.95 equiv.) and DIPEA (10 equiv.) following Fmoc group removal. Fmoc-Asn[GlcNAc(TBDMS)$_3$]-OH (4.82 equiv) was coupled using HOBt (4.82 equiv.), HBTU (4.77 equiv.) and DIPEA (9.62 equiv.) following Fmoc group removal. Upon completion the desired glycopeptide was cleaved from the resin with a solution of TFA/H_2O/TIPS (95/2.5/2.5 v/v/v). HPLC showed a total of six peaks, isolation and analysis showed three of the peaks to be product with varying numbers of silyl groups still attached. **MS** (ESI) Calc. for $[C_{52}H_{66}N_{10}O_{18}]Na^+$: 1141.45. Found: 1141.67.

SPPS of Fmoc-Asn[chitobiose(OH)$_5$]-Ile-Thr-Thr-Gly-Ind-NH$_2$ (99): The synthesis of glycopeptide partial sequence Asn 24 – Gly 28 of human erythropoietin protein was accomplished by solid phase peptide synthesis using standard Fmoc/t-Bu chemistry. Coupling of Fmoc-Gly-Ind-OH (3.00 equiv.) to

Rink Amide AM resin (0.74 mmol/g loading capacity) was achieved using HOBt (3.00 equiv.), HBTU (2.97 equiv.) and DIPEA (6 equiv.) following Fmoc group removal using a solution of 20% piperidine in NMP. The subsequent three amino acids were coupled using amino acid (5 equiv.), HOBt (5 equiv.), HBTU (4.95 equiv.) and DIPEA (10 equiv.) following Fmoc group removal. Fmoc-Asn[chitobiose(TBDMS)$_5$]-OH (4.72 equiv.) was coupled using HOBt (4.72 equiv.), HBTU (4.67 equiv.) and DIPEA (9.44 equiv.) following Fmoc group removal. Upon completion the desired glycopeptide was cleaved from the resin with a solution of TFA/H_2O/TIPS (90/5/5 v/v/v). The cleavage cocktail was altered to accomplish complete removal of the silyl protecting groups during cleavage from the resin. HPLC analysis showed two major peaks. It is believed that the two peaks are different conformers (cis/trans isomerization of the prolyl peptide bond) of the desired glycopeptide. This is supported by reinjection of each isolated peak and observation of the two peaks with the same retention times as the original injection. **MS** (TOF) Calc. for $[C_{60}H_{79}N_{11}O_{23}]H^+$: 1322.54. Found: 1322.71.

SPPS of Fmoc-Asn[chitobiose(Ac)$_5$]-Ser-Thr-Ala-Thr-Leu-Ind-NH$_2$ (100): The synthesis of this glycopeptide was accomplished using starting material previously prepared in this lab. Fmoc-Leu-Ind-OH (5 equiv.), HOBt (5 equiv.), HBTU (4.95 equiv.) and DIPEA (10 equiv.) were used to accomplish attachment to Rink Amide AM Resin (0.63 mmol/g loading capacity). Each subsequent amino acid including the Fmoc-Asn[chitobiose(Ac)$_5$]-OH building block were successfully coupled using 5 equivalents each of the amino acid and HOBt, 4.95 equivalents of HBTU and 10 equivalents of DIPEA following Fmoc removal with a solution of 20% piperidine in NMP. Each coupling reaction was considered complete upon observation of a negative test when reacted with a solution of 0.5% bromophenol blue in NMP. Upon completion the desired glycopeptide was cleaved from the resin with a solution of TFA/H_2O/TIPS (95/2.5/2.5 v/v/v).

SPPS of Fmoc-Gly-Ala-Asp-Asn-Asn[GlcNAc(Ac)$_3$]-Ile-Thr-Leu-Pro-Ind-NH$_2$ (101): The synthesis of the glycopeptide partial sequence Gly 411 – Pro 419 of gp120 HIV envelope protein was accomplished by solid phase peptide synthesis using standard Fmoc/t-Bu chemistry. Coupling of Fmoc-Pro-Ind-OH (4 equiv) to Rink Amide AM resin (0.63 mmol/g loading capacity) was achieved using HOBt (4.03 equiv), HBTU (3.99 equiv) and DIPEA (8.06 equiv). Each subsequent amino acid coupling was performed with 5 equivalents each of the amino acid and HOBt, 4.95 equivalents of HBTU, and 10 equivalents of DIPEA following Fmoc removal with a solution of 20 % piperidine in NMP. Each coupling reaction was considered complete upon observation of a negative test when reacted with solution of 0.5% bromophenol blue in NMP. Upon completion the desired glycopeptide was cleaved from the resin with a solution of TFA/H_2O/TIPS (95/2.5/2.5 v/v/v).

SPPS of Fmoc-Gly-Ala-Asp-Asn-Asn[chitobiose(TBDMS)$_5$]-Ile-Thr-Leu-Pro-Ind-NH$_2$ (102): The synthesis of the glycopeptide partial sequence Gly 411 – Pro 419 of gp120 HIV envelope protein was accomplished by solid phase peptide synthesis using standard Fmoc/t-Bu chemistry. Coupling of Fmoc-Pro-Ind-OH (4 equiv) to Rink Amide AM resin (0.63 mmol/g loading capacity) was achieved using HOBt (4.03 equiv.), HBTU (3.99 equiv.) and DIPEA (8.06 equiv.). Each subsequent amino acid coupling was performed with 5 equivalents each of the amino acid and HOBt, 4.95 equivalents of HBTU, and 10 equivalents of DIPEA following Fmoc removal with a solution of 20 % piperidine in NMP. Each coupling reaction was considered complete upon observation of a negative test when reacted with a solution of 0.5% bromophenol blue in NMP. Upon completion the desired glycopeptide was cleaved from the resin with a solution of TFA/H_2O/TIPS (95/2.5/2.5 v/v/v).

3.6 Synthesis of *N*-Tfa-Val-OH and Attempts Towards its Quatitative Removal

Trifluoroacetylation of L-valine: The trifluoroacetylation of L-valine was attempted a number of times under varying conditions. Varying concentrations of trifluoroacetic anhydride were attempted in each solvent: dry pyridine, dry dichloromenthane, or dry THF. The reaction was also attempted using trifluoroacetic anhydride in dry dichloromethane with addition of triethylamine. Finally, the reaction was attempted using trifluorocaetic anhydride as the solvent with no other additions. In each reaction the yields were not as sufficient as that obtained from the following procedure.

L-valine (0.9390 mmol) was dissolved in TFA (1 mL). Trifluoroacetic anhydride (2.1568 mmol) was added slowly over 1 min and the solution was allow to stir under argon for 24 h. Cold water was added to hydrolyze the remaining trifluoroacetic anhydride and then the solvent was removed by evaporation to give 0.1475 g (68%) of crude Tfa-Val-OH. **^{1}H NMR** (600 MHz, 23.4 °C, DMSO-$_{d6,}$ δ_{H}) 9.6 (d, *J* = 3.4 Hz, 1H, N**H**), 4.12 (t, *J* = 6.9 Hz, 1H, α-C**H**), 2.15 (m, 1H, β-C**H**), 0.90 (d, *J* = 7.2 Hz, 6H, 2 x γ-C**H$_3$**).

Deprotection of TFA-Val-OH: A number of reactions were performed, in solution, to find the best conditions for the quantitative removal of the trifluoroacetyl group from Tfa-Val-OH for future solid phase syntheses. Along with the work performed by Dr. Zhefeng Cai, the following are other reaction conditions attempted:

1. 1 M aq. Piperidine: by-products observed.
2. 10% aq. Piperidine: by-products observed.
3. 20% diethylamine in NMP: by-products observed.
4. 1 M ammonia in THF: by-products observed

5. 0.5 M ammonia in THF/NMP (1:1): by-products observed on TLC. This Looked to be the most promising condition, however, an attempt at the quantitative removal of the *N*-Tfa group in a solid phase reaction failed.

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73.

LIST OF ABBREVIATIONS

λ	wavelength
AA/aa	amino acid
Ac	acetyl
AcOH	acetic acid
Ala	alanine
Ar	aryl
Asn	asparagine
Asp	aspartic acid
aq	aqueous
BAL	backbone amide linker
Boc	tert-butyloxycarbonyl
Bn	benzyl
Bpoc	biphenyloxycarbonyl
cat	catalytic
Cbz	benzyloxycarbonyl
Cys	cysteine
DBU	1,8-diazabicycloundec-7-ene
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DCU	dicyclohexyl urea
DIPCDI	diisopropylcarbodiimide

DIPEA	diisopropylethylamine
DKP	diketopiperizine
Dmb	dimethoxybenzyl
DMF	dimethylformamide
DMSO	dimethylsulfoxide
$DMSO_{d\text{-}6}$	deuterated dimethylsulfoxide
ECD	tert-butylethylcarbodiimide
ESI	electrospray ionization
EtOAc	ethyl acetate
EtSH	ethane thiol
Equiv	equivalents
Fmoc	fluorenylmethyloxycarbonyl
g	gram
GlcNAc	*N*-acetylglucosamine
Gly	glycine
Glu	glutamic acid
Hex	hexanes
Leu	leucine
Lys	lysine
h	hour
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetrametylaminiumhexafluorophosphate
HCl	hydrochloric acid
h-EPO	Human erythropoietin

Hmb	hydroxymethoxybenzyl
HMBA-AM	4-hydroxymethylbenzoic acid AM resin
HOAc	acetic acid
HOAt	9-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
ICH_2CN	iodoacetonitrile
Ile	isolucine
Ind	indoline
M	molar
MCD	tert-butylmethylcarbodiimide
MBHA	methylbenzhydrylamine
Me	methyl
mg	milligram
$MgSO_4$	magnesium sulfate
mL	milliliter
min	minute(s)
mmol	millimole
MS	mass spectrometry
N_2	nitrogen gas
$NaCNBH_3$	sodium cyanoborohydride
$NaNO_3$	sodium nitrate
$NaOCH_3$	sodium methoxide

NaSPh	sodium thiophenylate
NCL	native chemical ligation
NMM	*N*-methylmorpholine
NMP	*N*-methylpyrollidinone
NMR	nuclear magnetic resonance
Nu	nucleophile
OAll	allyl ester
OBt	oxybenzotriazole ester
$^{-}$OH	hydroxide ion
OPfp	pentafluorophenyl ester
org.	organic
OtBu	tert-butyl ester
PAM	phenylacetamidomethyl
Ph	phenyl
pH	negative log of the hydronium ion concentration
Phe	phenylalanine
PhSH	thiophenol
pKa	negative logarithm of the acidity constant
Pro	proline
PSA	preformed symmetrical anhydrides
PyBOP	benzotriazole-1-yl-oxy-tris-pyrrolidino-phophonium hexafluorophosphate
RNase A	Ribonuclease A
rt	room temperature

SAL	sugar assisted ligation
Sat'd	saturated
Ser	serine
Sol.	solution
SPPS	solid phase peptide synthesis
TBDMS	tert-butyldimethylsilyl
TBDMSCl	tert-butyldimethylsilyl chloride
TBDMSOTf	tert-butyldimethylsilyl triflate
TBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
t-Bu	tert-butyl
Tfa	trifluoroacetyl
TFA	trifluoroacetic acid
THF	tetrahydofuran
Thr	threonine
TIPS	triisopropylsilane
TLC	thin layer chromatography
TMS	trimethylsilyl
$TMSCHN_2$	trimethylsilyldiazomethane
TMU	*N*,*N*,*N*',*N*'-tetramethyl urea
TOF	time of flight
Trt	trityl
UV	ultra violet
Val	valine

Xaa	generic amino acid

SUPPLEMENTAL INFORMATION

Allyl indole-5-carboxylate (25): ^{1}H NMR p.101

Allyl indole-5-carboxylate (25): ^{13}C NMR p.102

Allyl indoline-5-carboxylate (26): ^{1}H NMR p.103

Allyl indoline-5-carboxylate (26): ^{13}C NMR p.104

Allyl 1-acetylindoline-5-carboxylate (27): ^{1}H NMR p.105

Allyl 1-acetylindoline-5-carboxylate (27): ^{13}C NMR p.106

Allyl 7-nitro-1-acetylindoline-5-carboxylate (28): ^{1}H NMR p.107

Allyl 7-nitro-1-acetylindoline-5-carboxylate (28): ^{13}C NMR p.108

Allyl 7-nitroindoline-5-carboxylate (29): ^{1}H NMR p.109

Allyl 7-nitroindoline-5-carboxylate (29): ^{13}C NMR p.110

Fmoc-Lys(Tfa)-Leu-Phe-Ind-OAll (70): ^{1}H NMR p.111

Fmoc-Lys(Tfa)-Leu-Phe-Ind-OAll (70): ^{13}C NMR p.112

Fmoc-Lys(Tfa)-Leu-Phe-Ind-OAll (70): COSY p.113

Fmoc-Lys(Tfa)-Leu-Phe-Ind-OAll (70): MS p.114

Fmoc-Glu(OBn)-Ala-Lys(Tfa)-Ind-OAll (72): ^{1}H NMR p.115

Fmoc-Glu(OBn)-Ala-Lys(Tfa)-Ind-OAll (72): ^{13}C NMR p.116

Fmoc-Glu(OBn)-Ala-Lys(Tfa)-Ind-OAll (72): COSY p.117

Fmoc-Glu(OBn)-Ala-Lys(Tfa)-Ind-OAll (72): MS p.118

Fmoc-Glu(OBn)-SPh (81): ^{1}H NMR p.119

Fmoc-Glu(OBn)-SPh (81): ^{13}C NMR p.120

Fmoc-Glu(OBn)-SPh (81): COSY p.121

Fmoc-Thr(OBn)SPh (82): ^{1}H NMR p.122

Fmoc-Thr(OBn)SPh (82): ^{13}C NMR p.123

Fmoc-Thr(OBn)SPh (82): COSY p.124

Fmoc-Thr(OBn)-SPh (82): MS p.125

Fmoc-Lys(Tfa)-Leu-Phe-SPh (87): ^{1}H NMR p.126

Fmoc-Lys(Tfa)-Leu-Phe-SPh (87): ^{13}C NMR p.127

Fmoc-Lys(Tfa)-Leu-Phe-SPh (87): COSY p.128

Fmoc-Lys(Tfa)-Leu-Phe-SPh (87): MS p.129

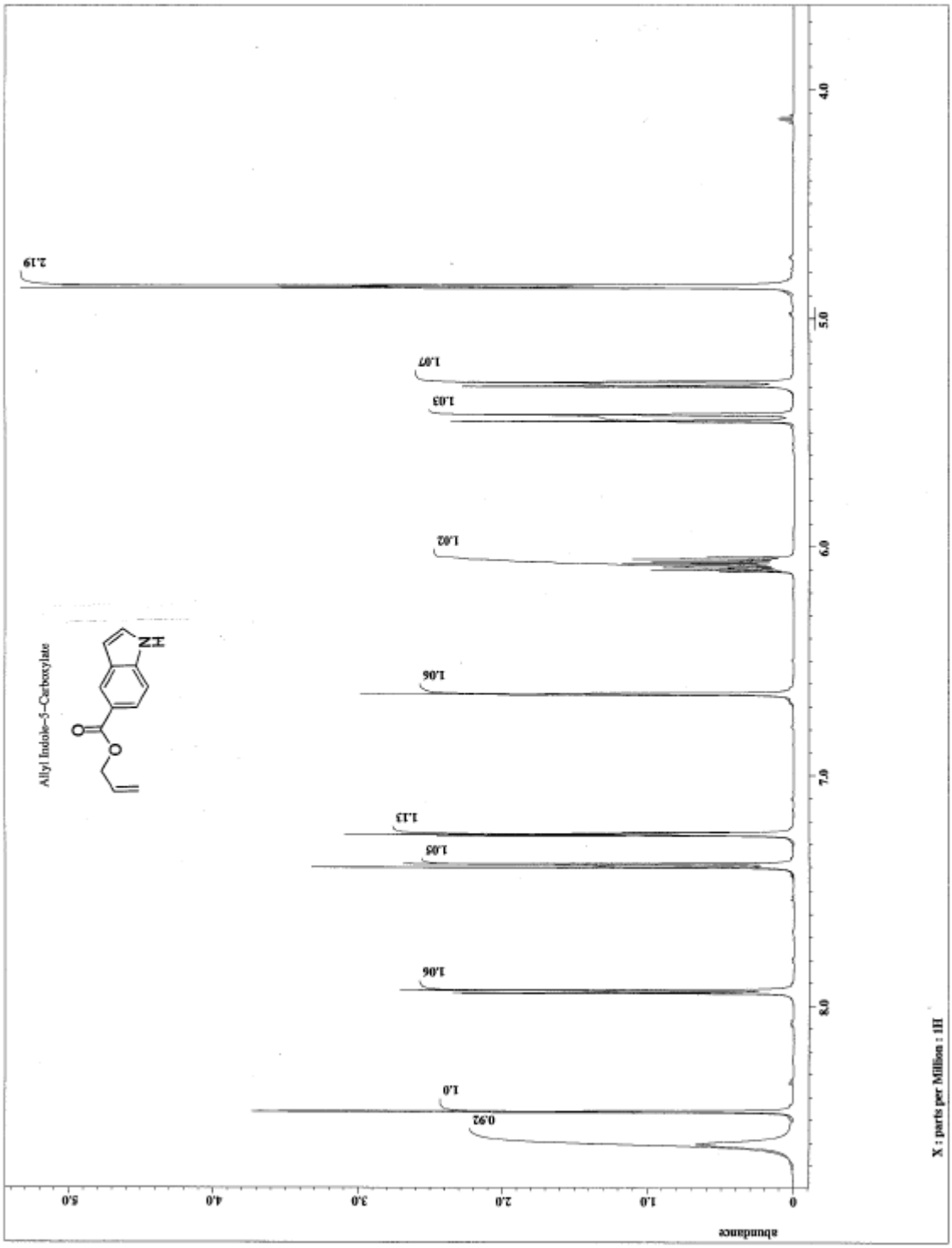
Fmoc-Glu(OBn)-Ala-Lys(Tfa)-SPh (89): ^{1}H NMR p.130

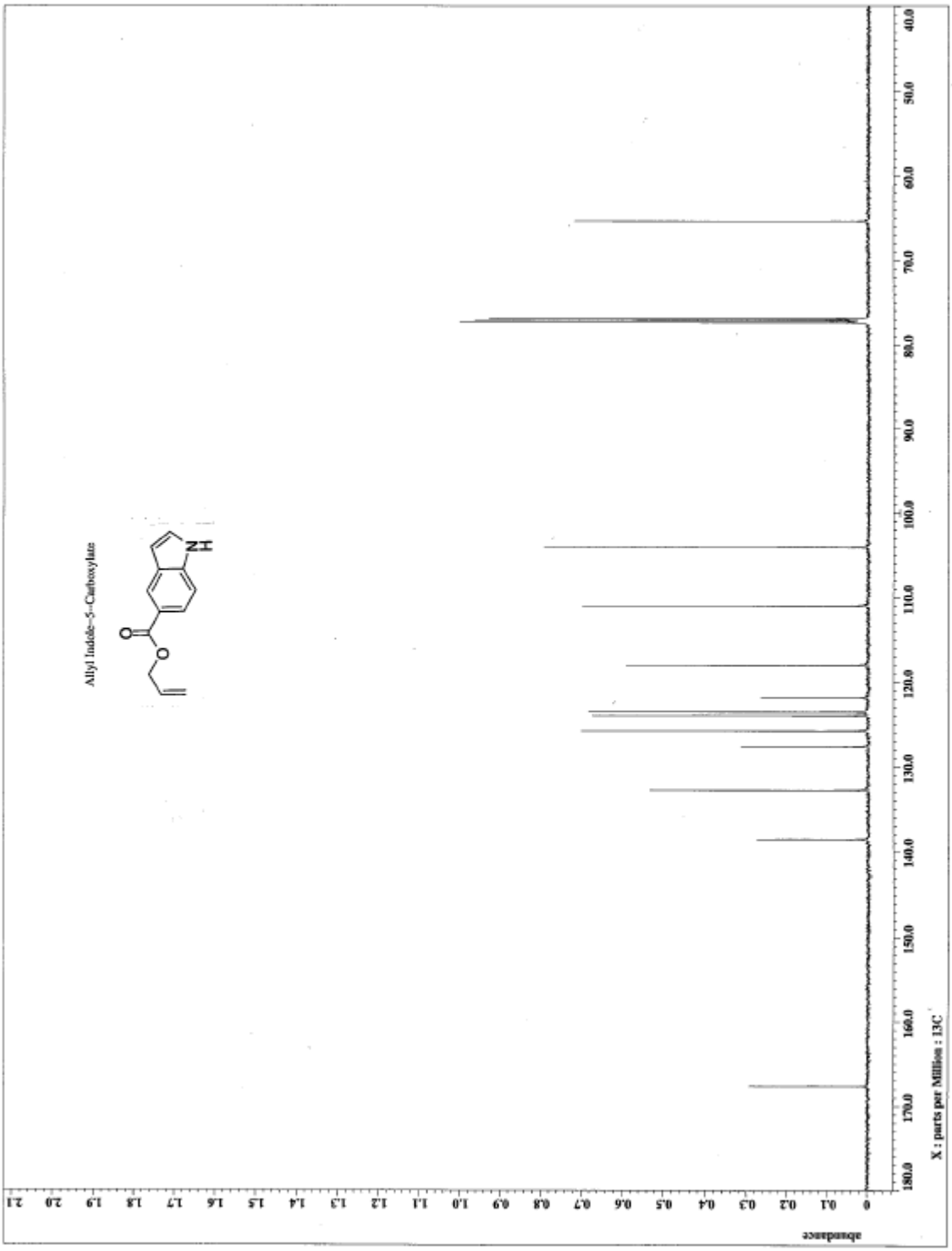
Fmoc-Glu(OBn)-Ala-Lys(Tfa)-SPh (89): ^{13}C NMR p.131

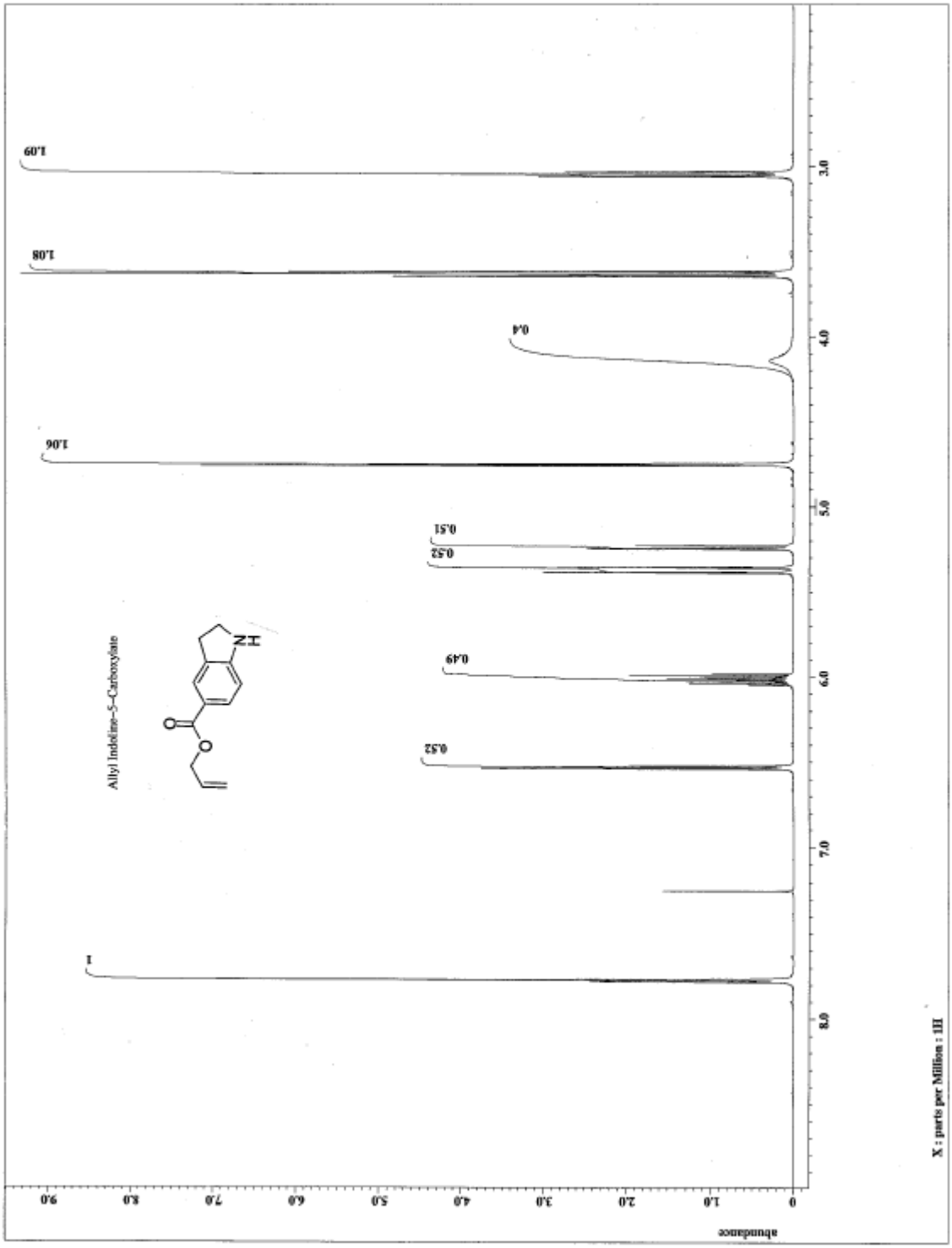
Fmoc-Glu(OBn)-Ala-Lys(Tfa)-SPh (89): COSY p.132

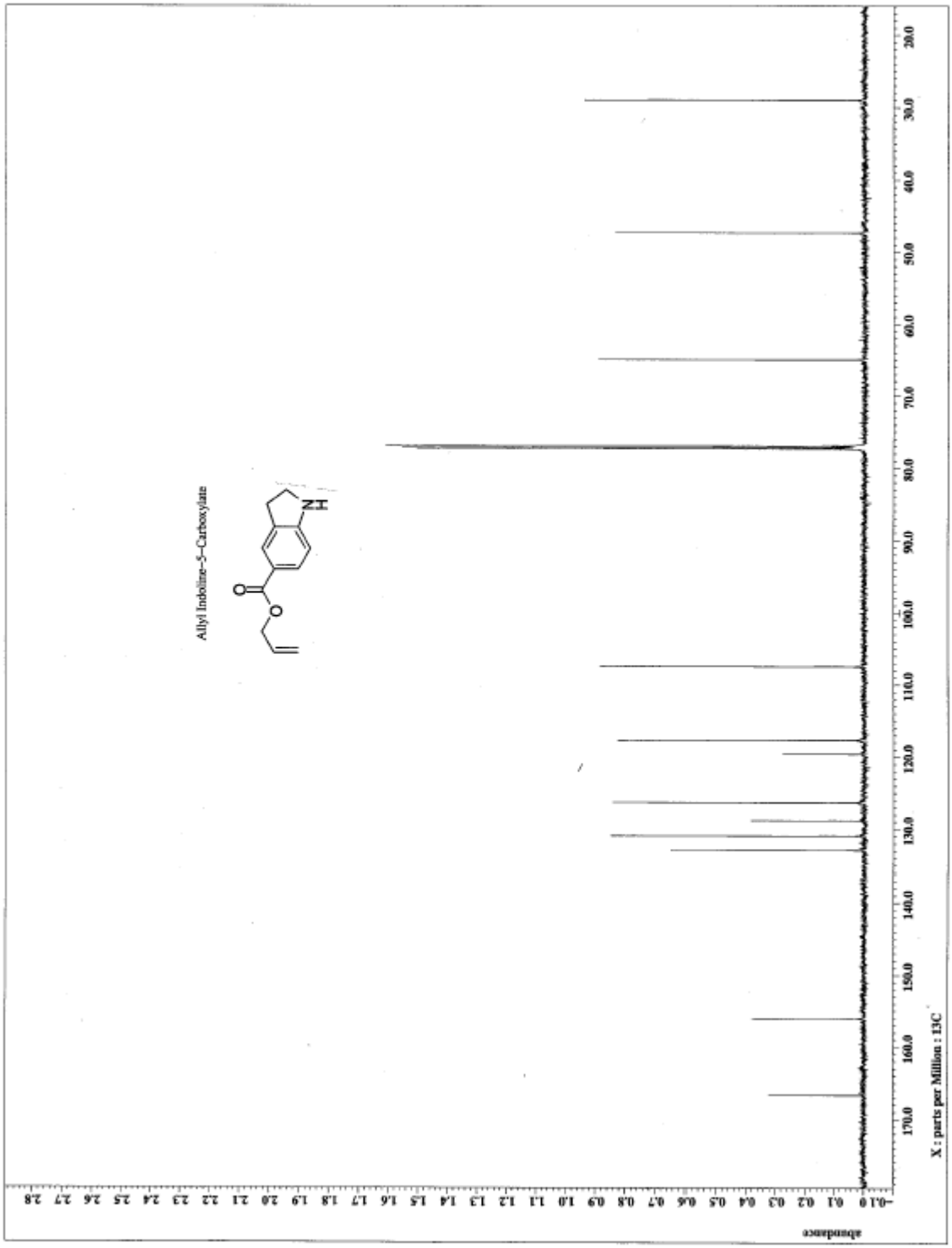
Fmoc-Asn[GlcNAc(OH)$_3$]-Ile-Thr-Thr-Gly-Ind-NH$_2$ (98): MS p.133

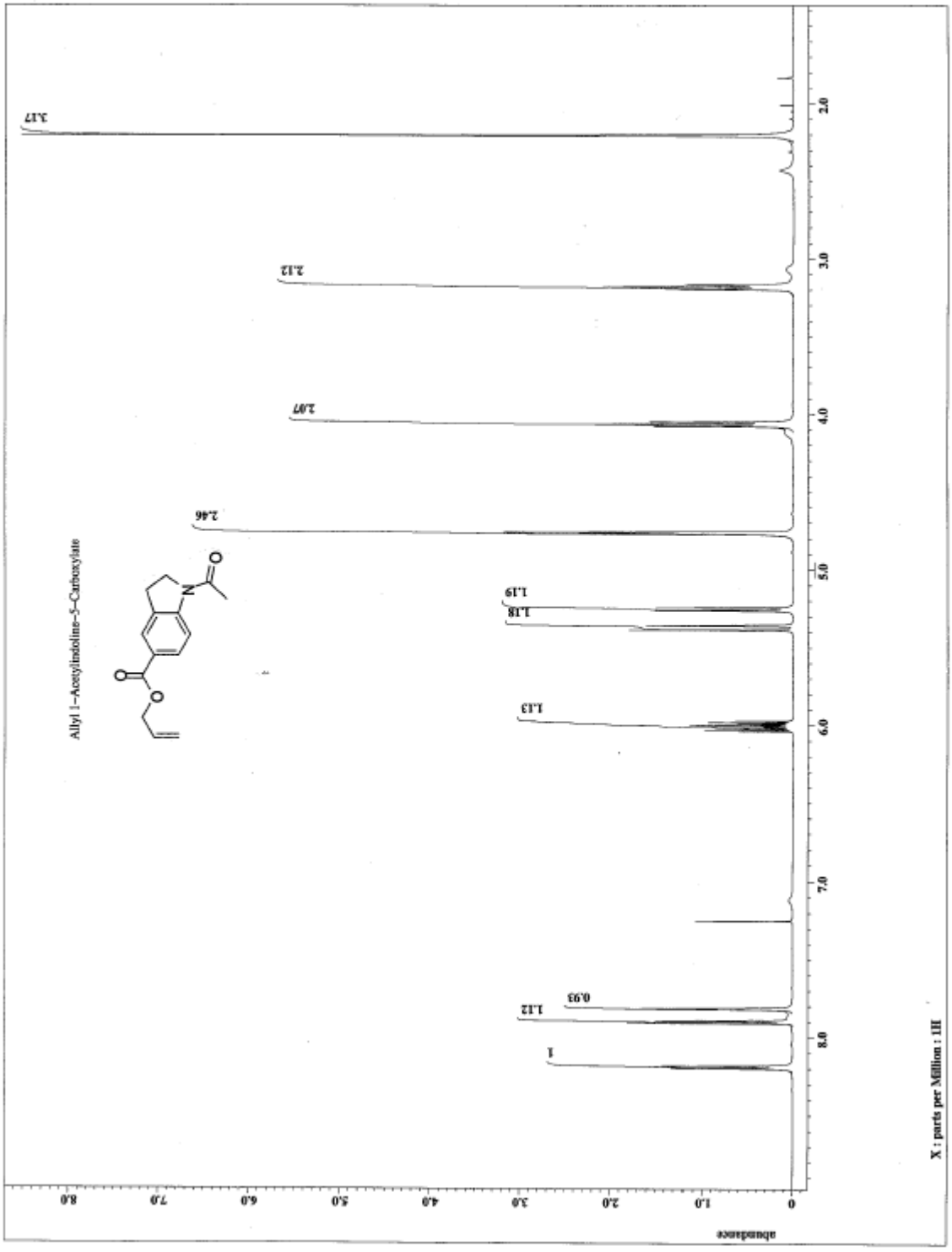
Fmoc-Asn[chitobiose(OH)$_5$]-Ile-Thr-Thr-Gly-Ind-NH$_2$ (99): MS p.134

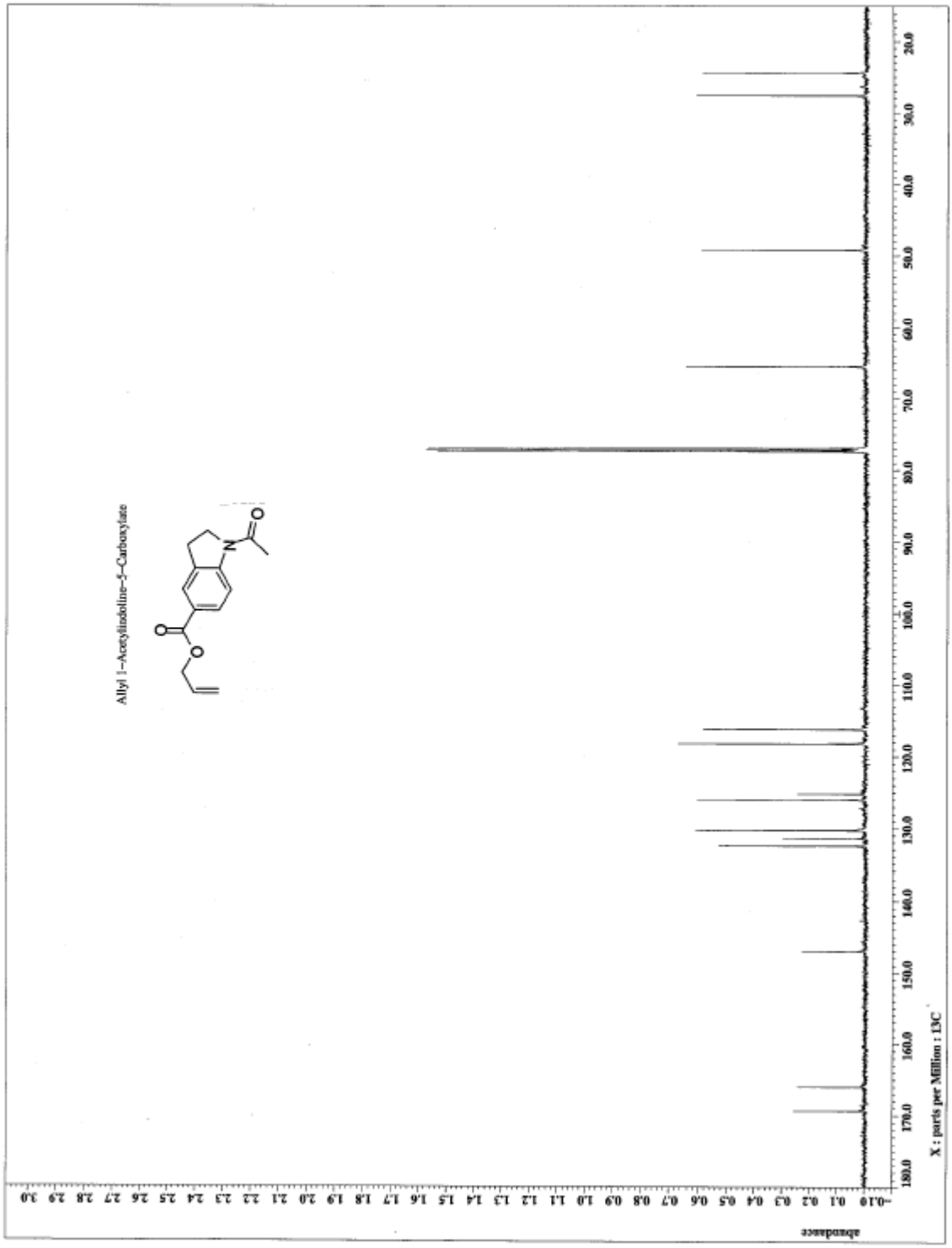

Allyl Indole-3-Carboxylate
2.19
1.07
1.03
1.02
1.06
1.13
1.05
1.06
1.0
0.92
X : parts per Million : 1H
abundance

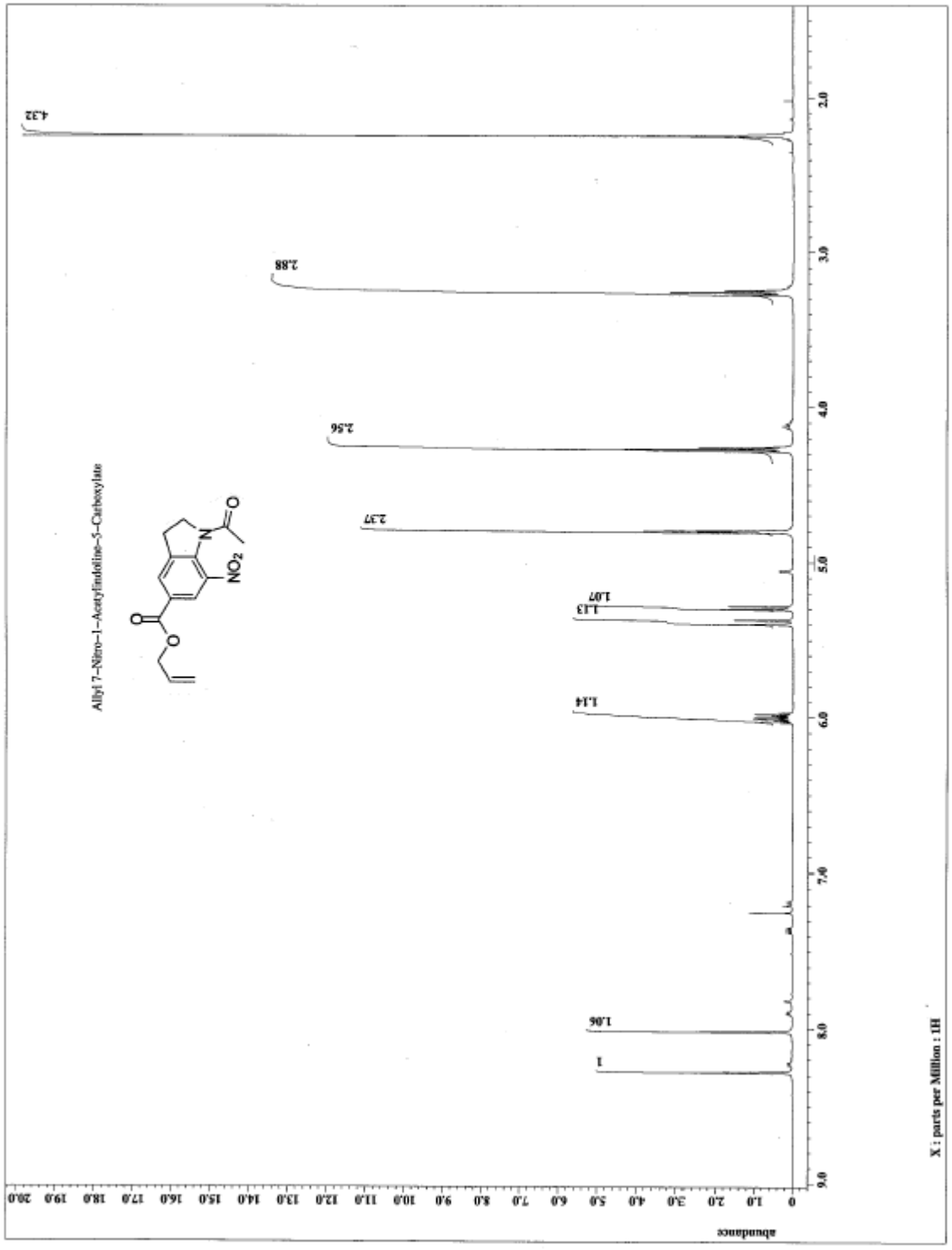

Allyl Indole-5-Carboxylate
NH
O
O
abundance
X : parts per Million : 13C

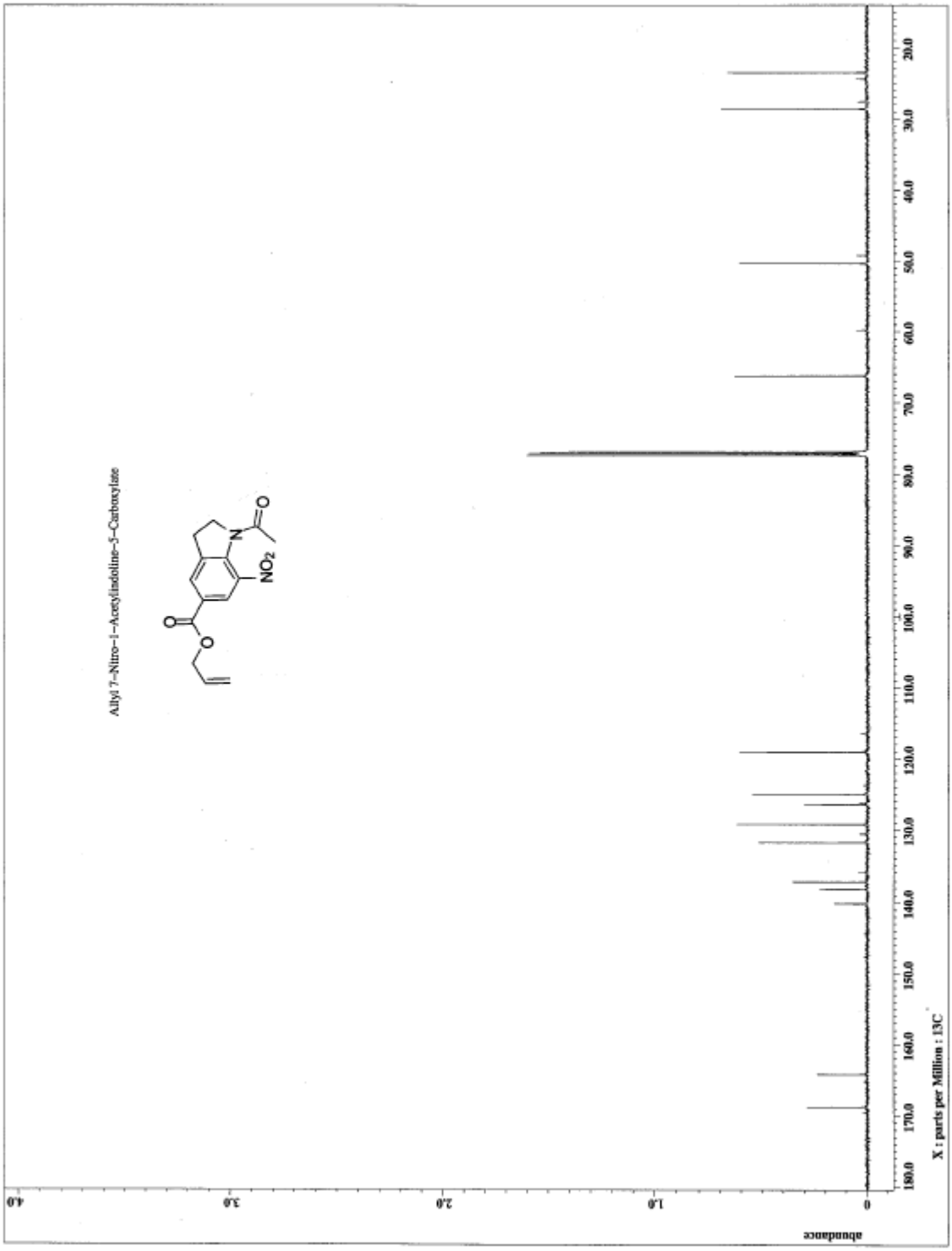

Allyl Indoline-5-Carboxylate
1
1.06
0.4
1.08
1.09
0.52
0.49
0.52
0.51
X : parts per Million : 1H
abundance

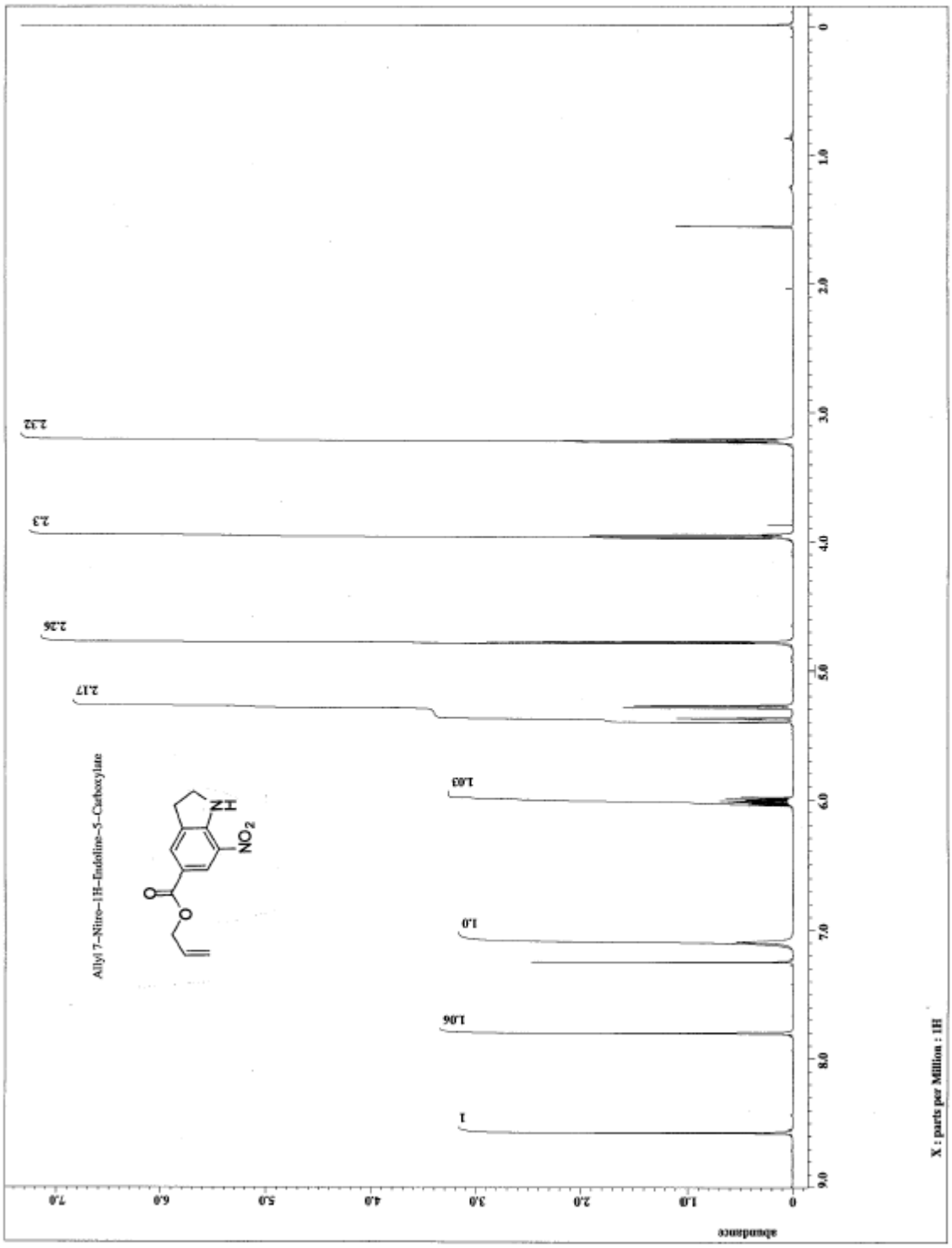

Allyl Indoline--5--Carboxylate
NH
O
O
abundance
X : parts per Million : 13C

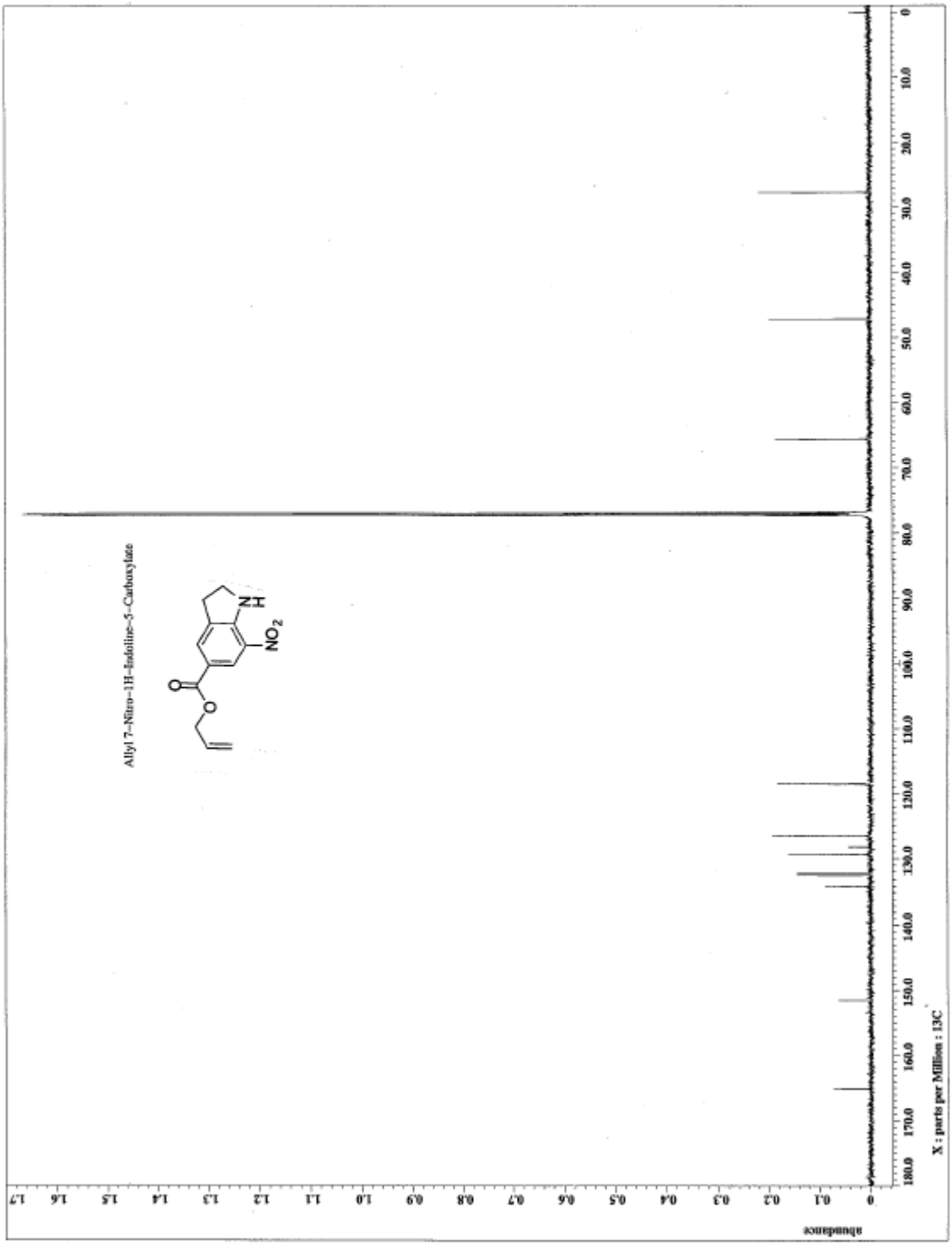

Allyl 1-Acetylindoline-5-Carboxylate
3.17
2.12
2.07
2.46
1.19
1.18
1.13
0.93
1.12
1
X : parts per Million : 1H
abundance


Allyl 1-Acetylindoline-5-Carboxylate
X : parts per Million : 13C

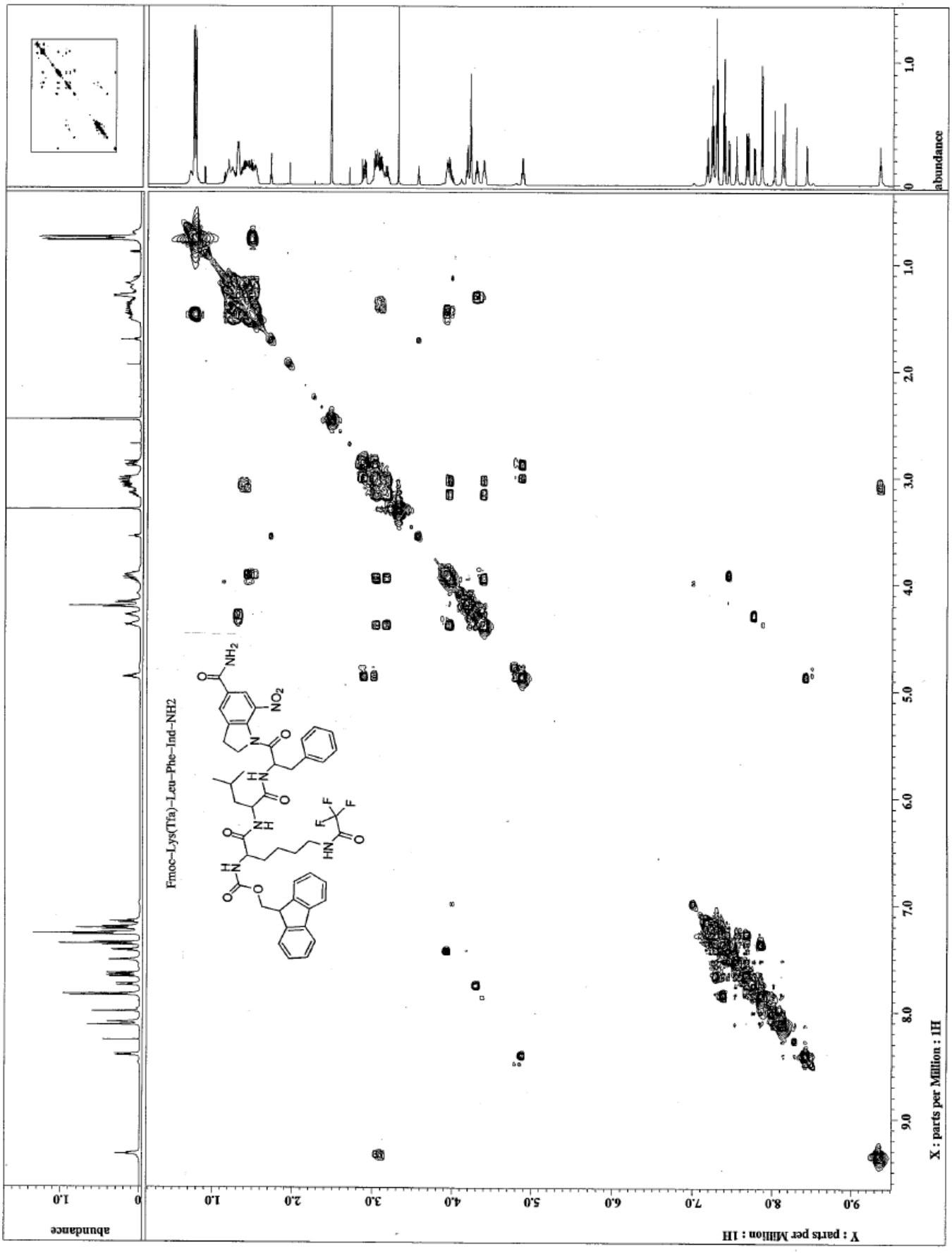

Allyl 7-Nitro-1-AcetylIndoline-5-Carboxylate
4.32
2.88
2.56
2.37
1.07
1.13
1.14
1.06
1
X : parts per Million : 1H
abundance

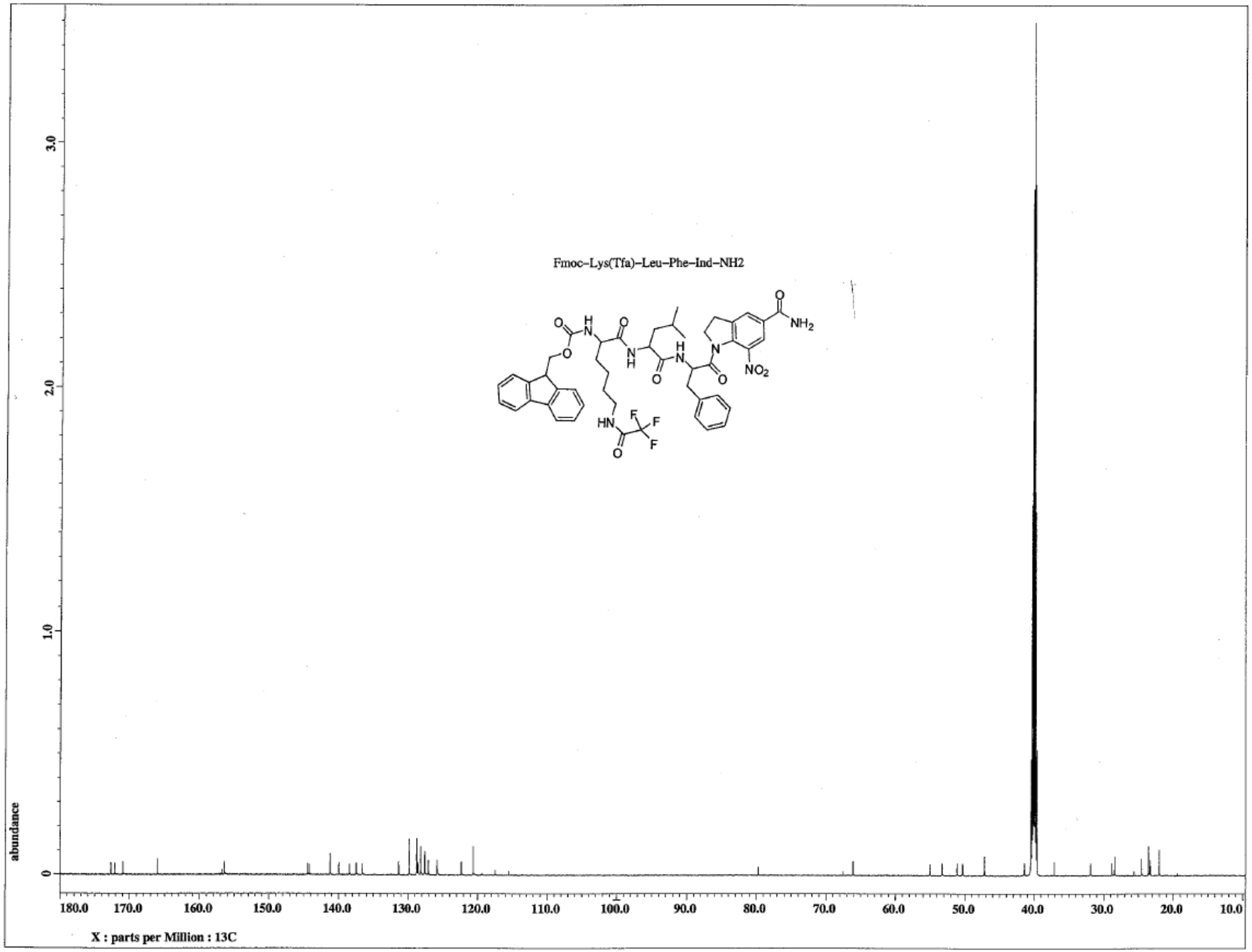

Allyl 7-Nitro-1-Acetylindoline-5-Carboxylate
O
N
NO2
O
O
abundance
0
1.0
2.0
3.0
4.0
X : parts per Million : 13C
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0


Allyl 7-Nitro-1H-Indoline-5-Carboxylate
NH
NO2
O
O
2.32
2.3
2.36
2.17
1.03
1.0
1.06
1
abundance
X : parts per Million : 1H


Allyl 7-Nitro-1H-Indoline-5-Carboxylate
NH
NO2
O
O
abundance
X : parts per Million : 13C

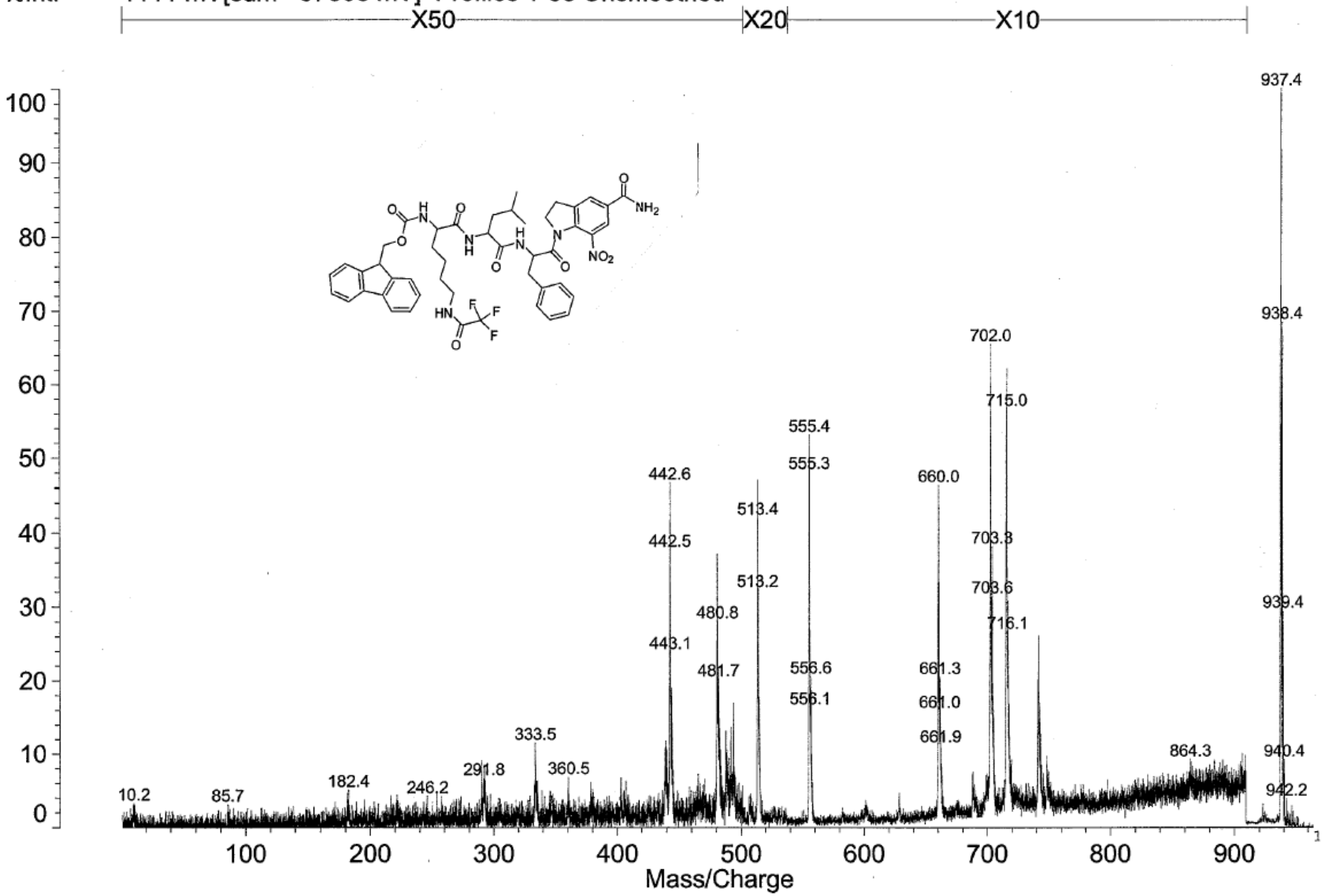
Fmoc-Lys(Tfa)-Leu-Phe-Ind-NH2
abundance
X : parts per Million : 1H
11.99
8.93
6.06
5.88
2.46
2.19
2.21
2.08
2.05
1.93
1.09
1.06
1.00
0.98
0.97
0.96
0.95
0.93

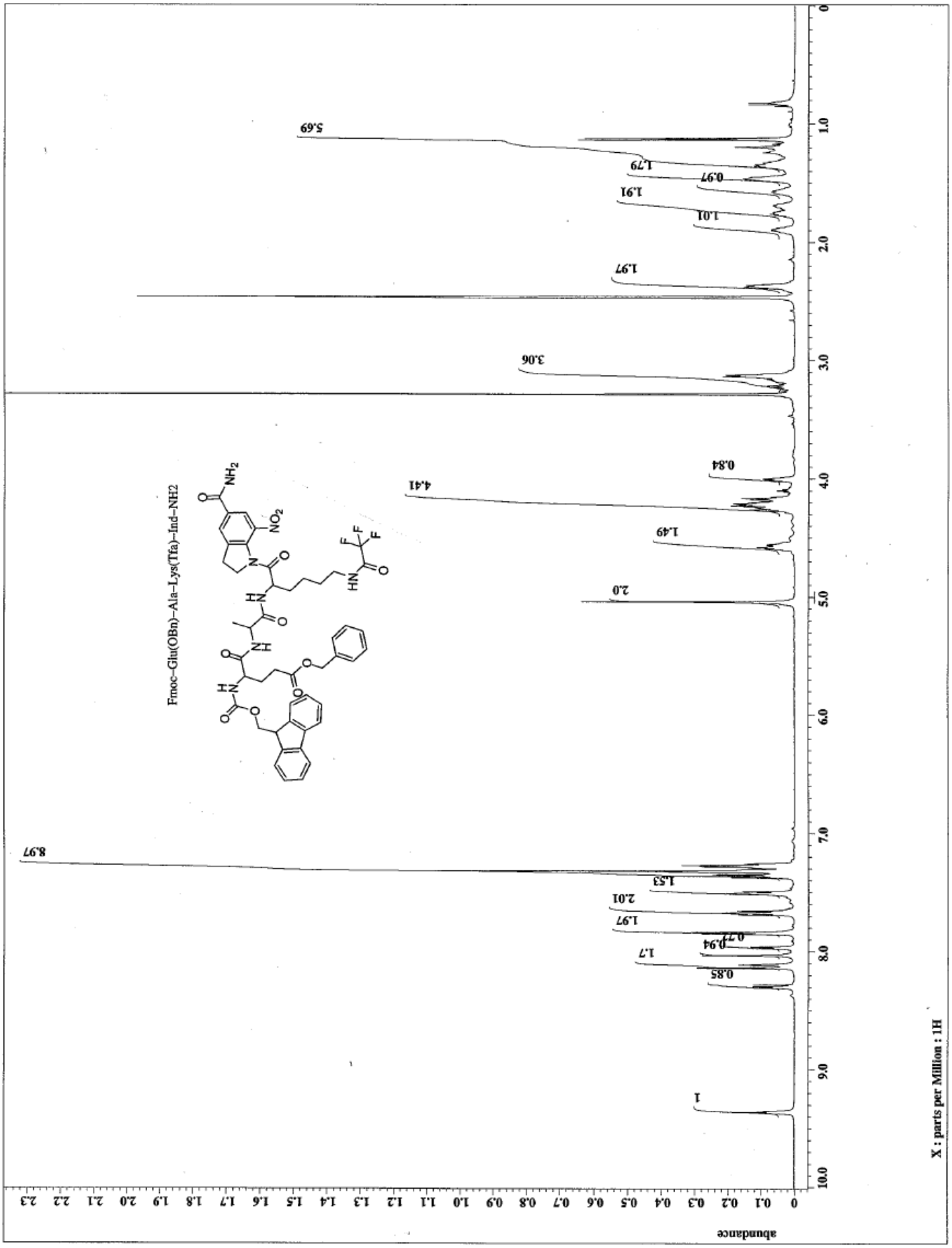

Fmoc–Lys(Tfa)–Leu–Phe–Ind–NH2
X : parts per Million : 1H
Y : parts per Million : 1H
abundance
abundance

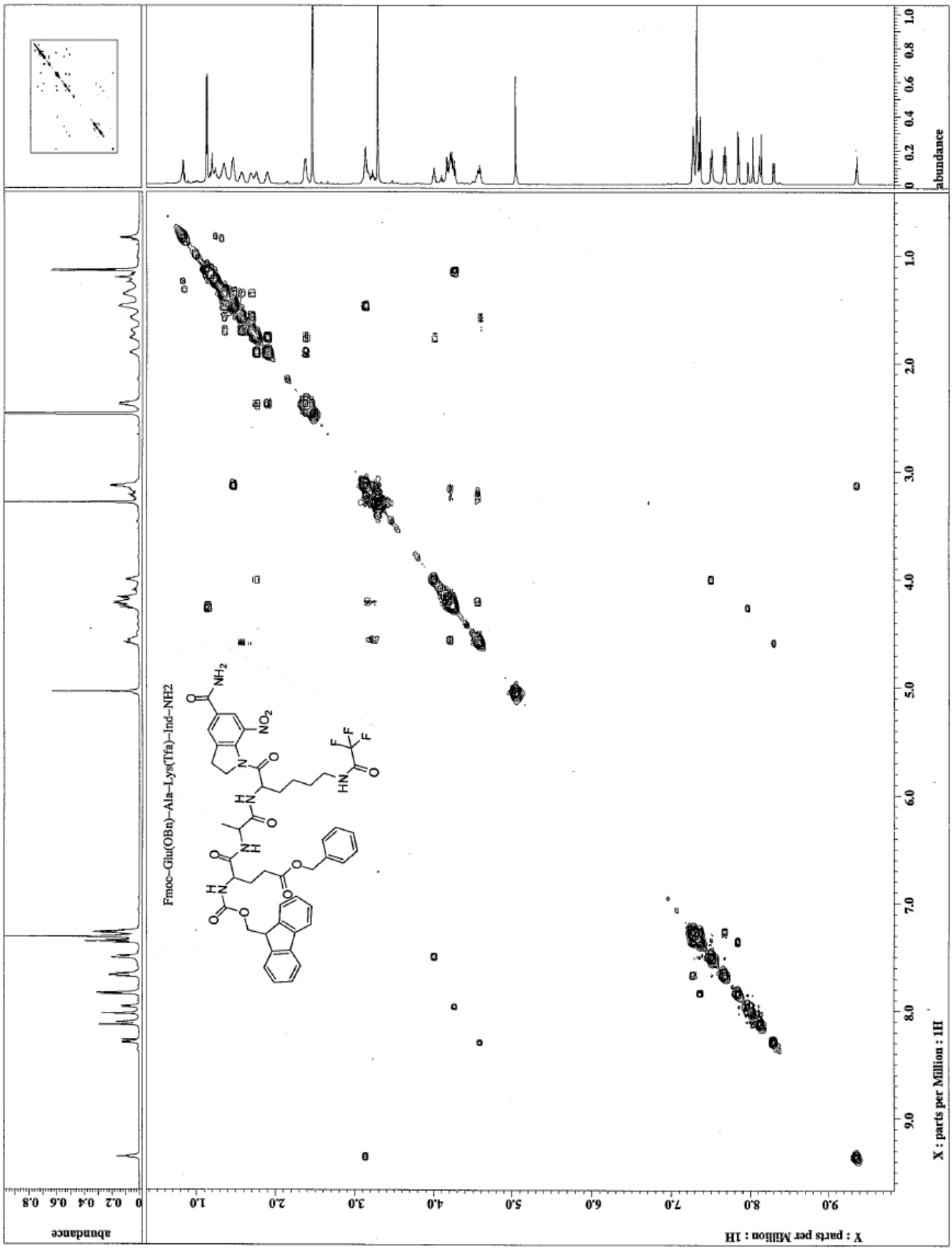

Fmoc-Lys(Tfa)-Leu-Phe-Ind-NH2
abundance
3.0
2.0
1.0
0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
X : parts per Million : 13C

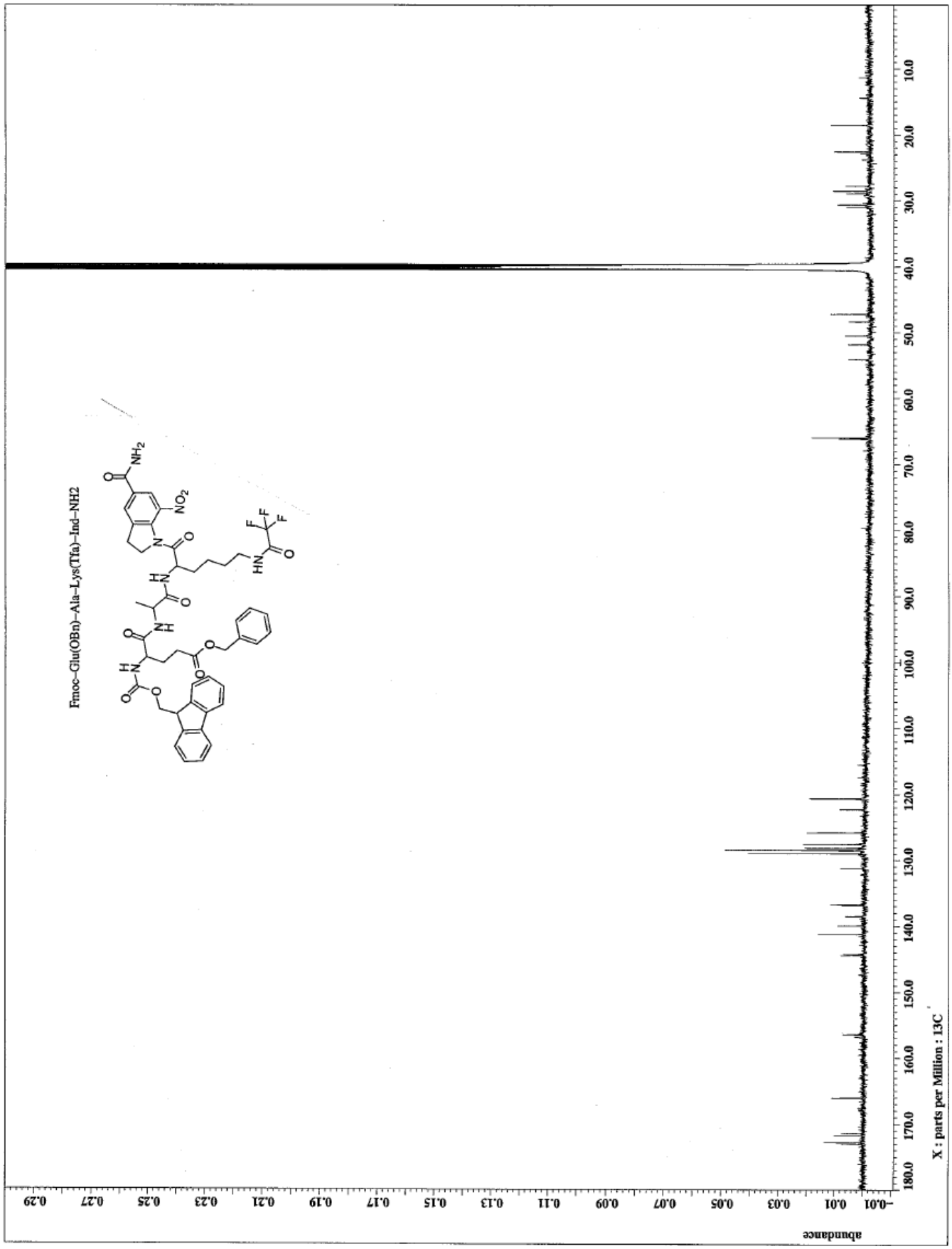
ZfC(II)_p4(CHCA(937PSD)

Data: ZfC(II)_p45_CHCA(937PSD)0001.H16 7 Oct 2008 17:18 Cal: Refl_Franz011607 7 Oct 2008 17:02 (PSD of 937.43
Kratos PC Axima CFR V2.3.4: Mode Reflectron, Power: 111, Gate: 922.34-952.58, P.Ext. @ 940 (bin 102)
%Int. 1111 mV[sum= 97808 mV] Profiles 1-88 Unsmoothed



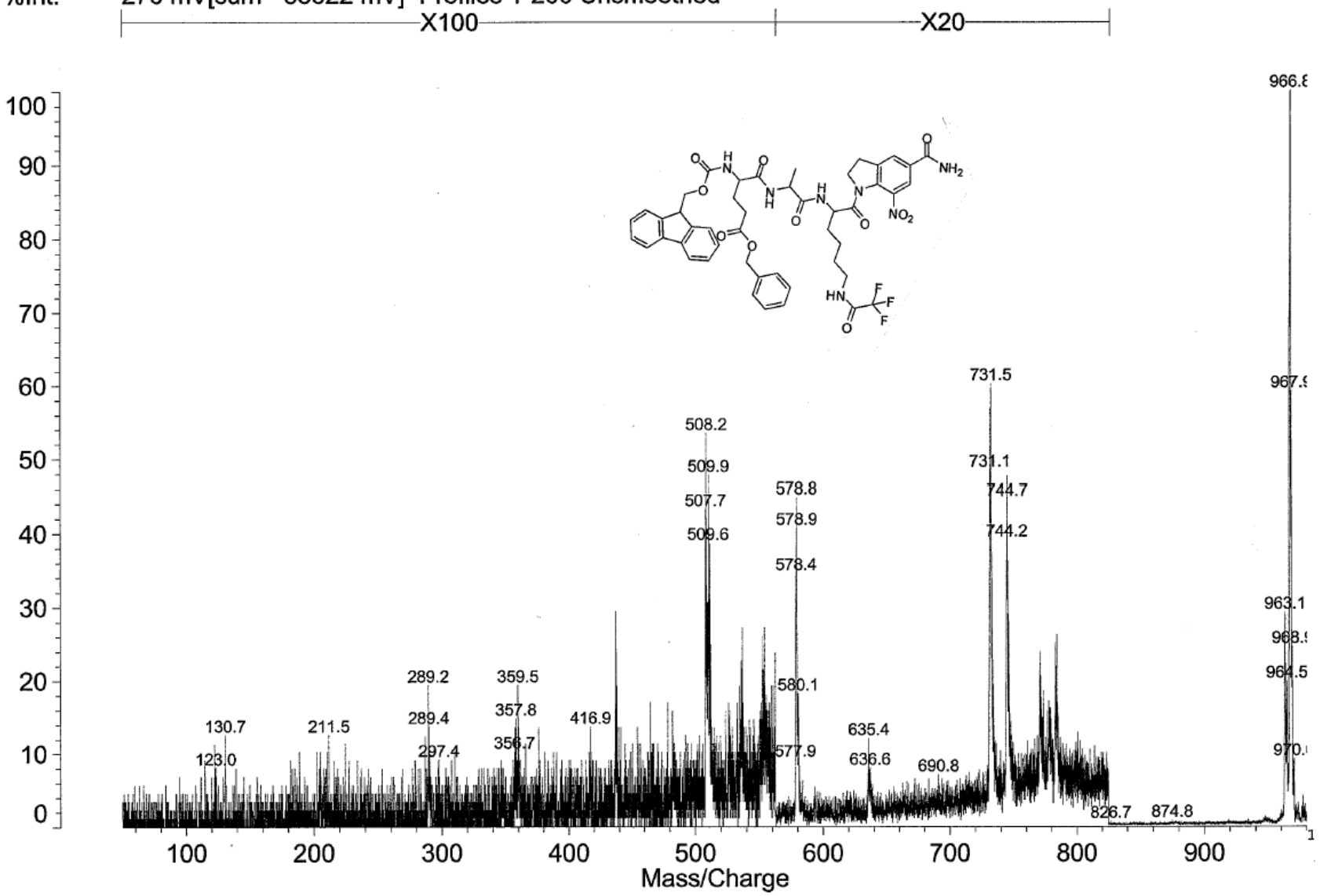

Fmoc–Glu(OBn)–Ala–Lys(Tfa)–Ind–NH2
X : parts per Million : 1H
abundance
8.97
5.69
4.41
3.06
2.0
1.97
1.91
1.79
1.53
1.49
1.01
0.97
0.94
0.85
0.84
1

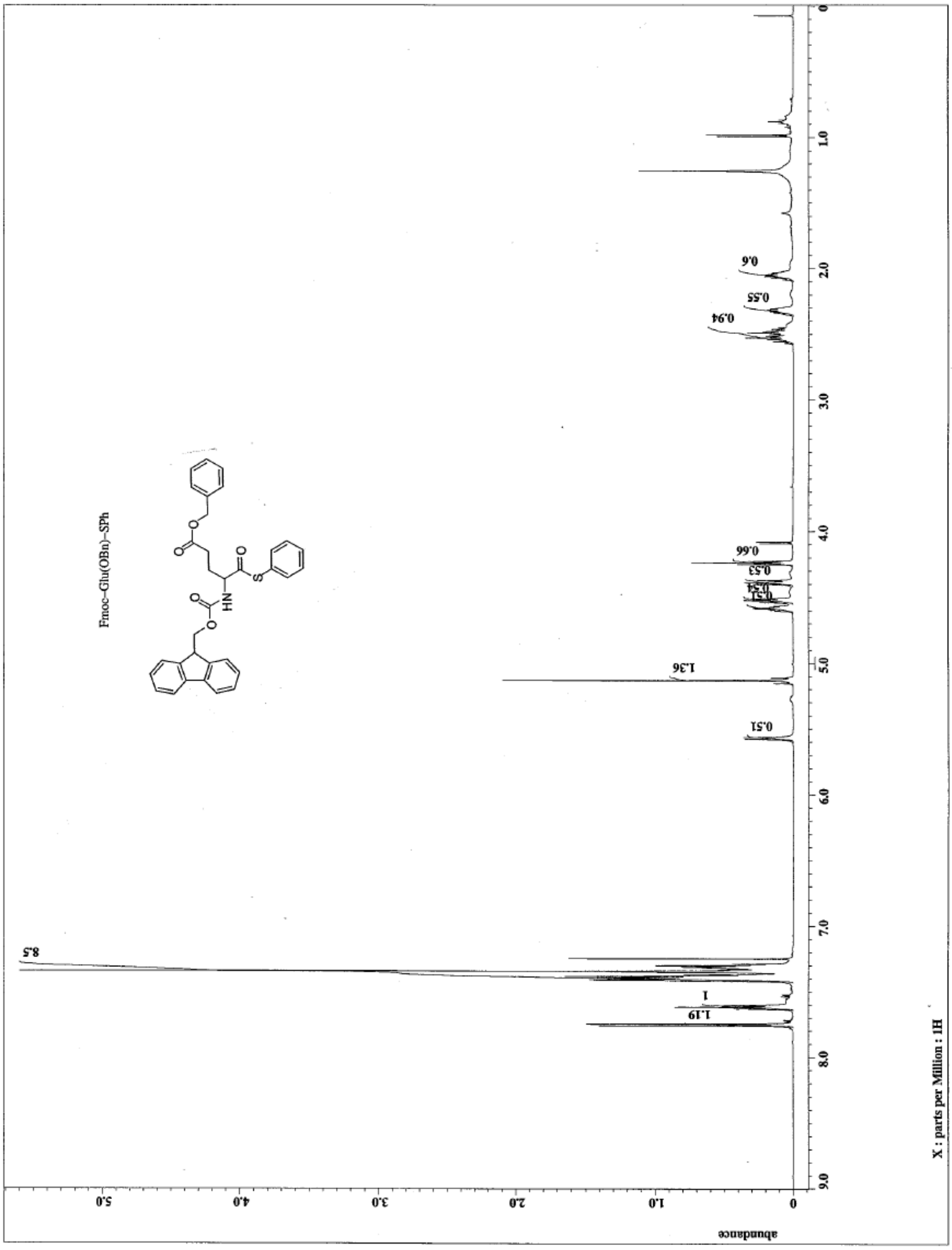

Fmoc–Glu(OBn)–Ala–Lys(Tfa)–Ind–NH2
X : parts per Million : 1H
abundance

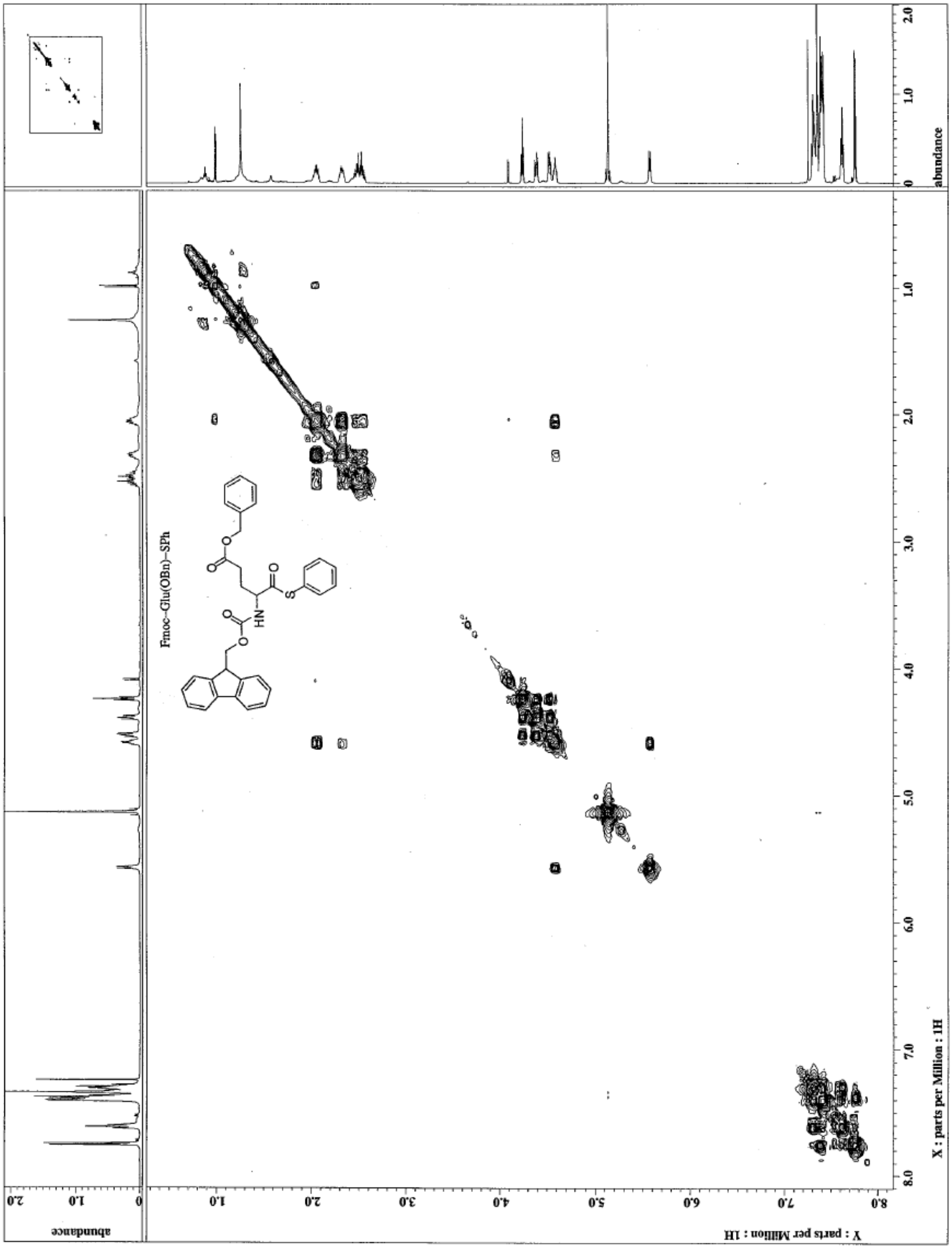

Fmoc–Glu(OBn)–Ala–Lys(Tfa)–Ind–NH2
abundance
X : parts per Million : 13C

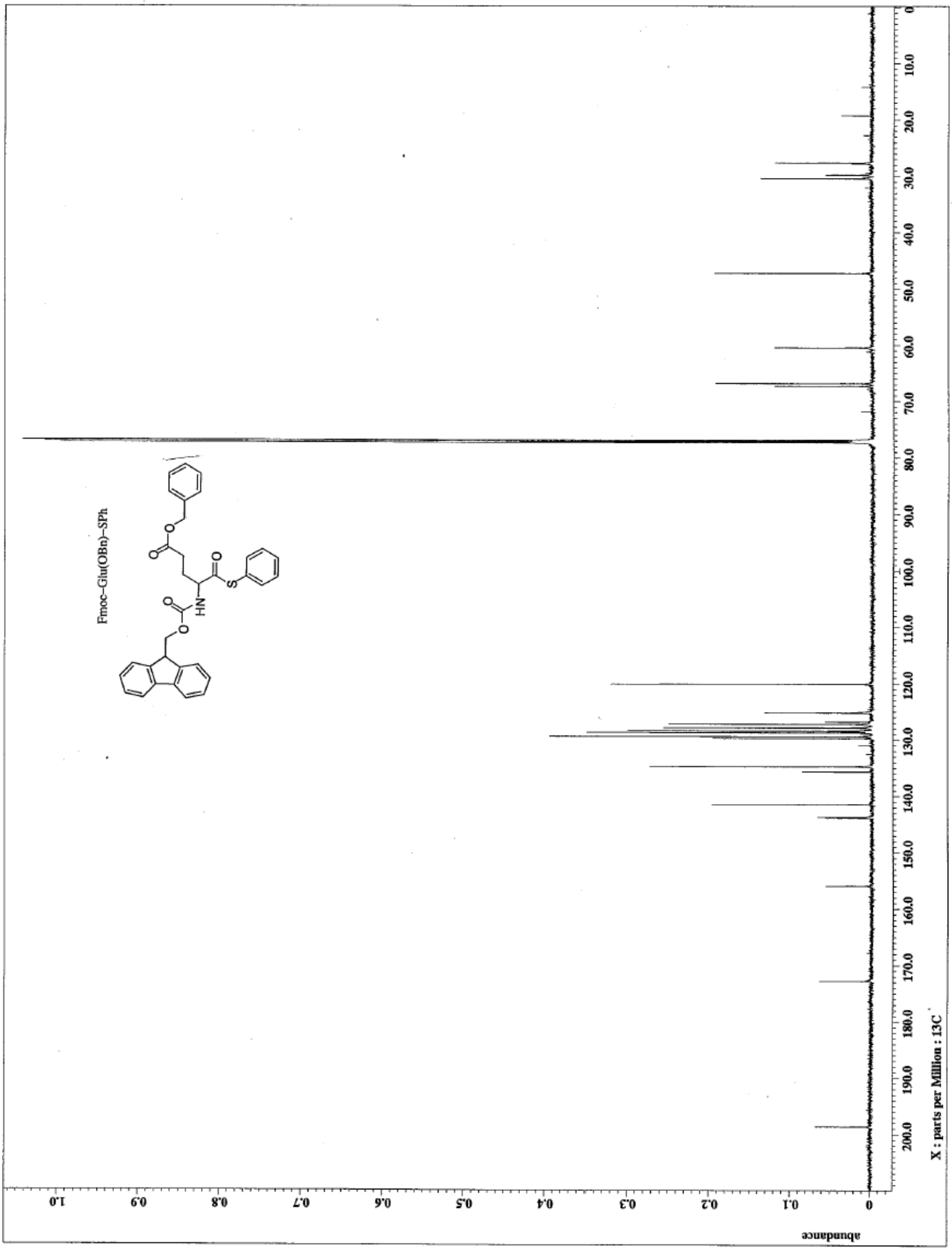
2FC(III)_p5_28min_af012809_CHCA(966PSD)

Data: 2FC(III)_p5_28min_af012809_CHCA(966PSD)0001.N23 28 Jan 2009 14:31 Cal: Refl_Franz011607 28 Jan 2009 14
Kratos PC Axima CFR V2.3.4: Mode Reflectron, Power: 85, Gate: 950.95-982.69, P.Ext. @ 960 (bin 103)
%Int. 270 mV[sum= 53922 mV] Profiles 1-200 Unsmoothed

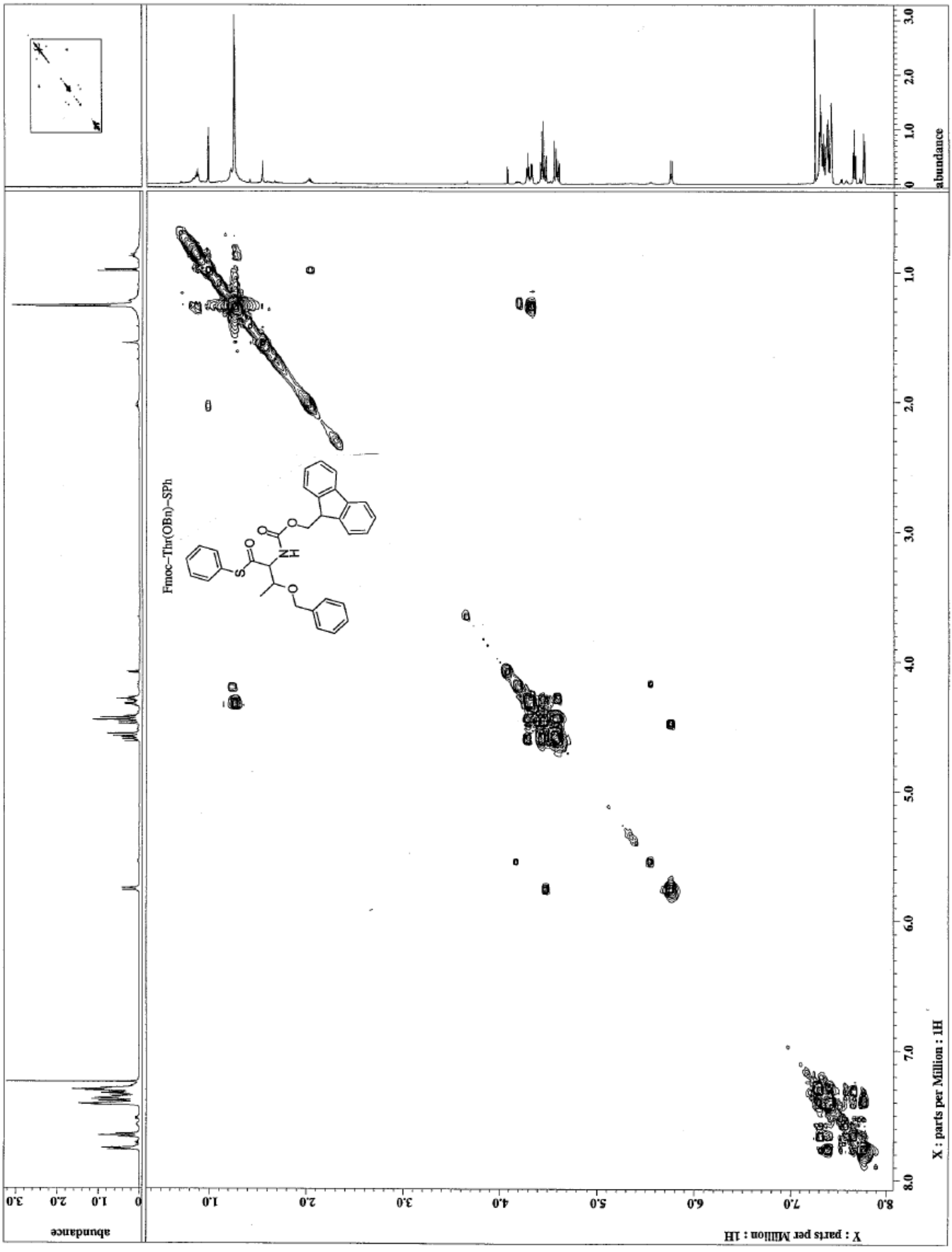


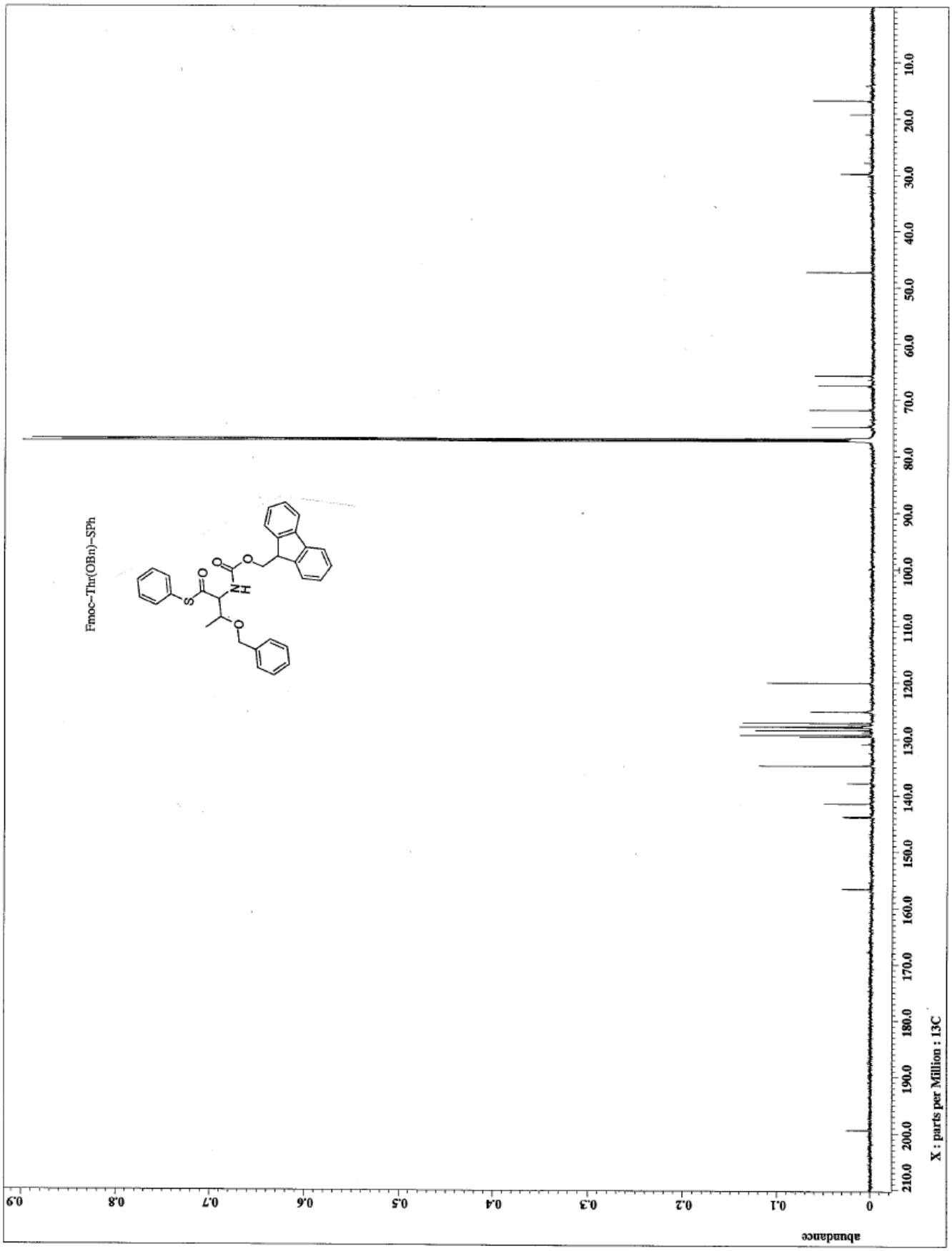

Fmoc–Glu(OBn)–SPh
NH
8.5
1.19
1
0.51
1.36
0.51
0.54
0.53
0.66
0.94
0.55
0.6
abundance
X : parts per Million : 1H


Fmoc–Glu(OBn)–SPh
HN
abundance
X : parts per Million : 1H
Y : parts per Million : 1H


Fmoc–Glu(OBn)–SPh
abundance
X : parts per Million : 13C

Fmoc-Thr(OBn)-SPh
7.98
1.07
1
0.51
1.08
1.57
1.13
2.71
abundance
X : parts per Million : 1H


Fmoc–Thr(OBn)–SPh
X : parts per Million : 1H
Y : parts per Million : 1H
abundance
abundance


Fmoc-Thr(OBn)-SPh
abundance
X : parts per Million : 13C

6/24/2009 9:19:09 AM

File: franz_062209
Sample:

Date Run: 6/22/2009 (6:19:09)

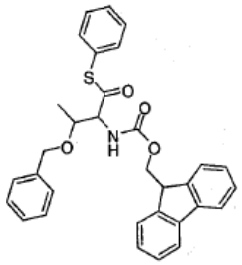
Instrument: AccuTOF

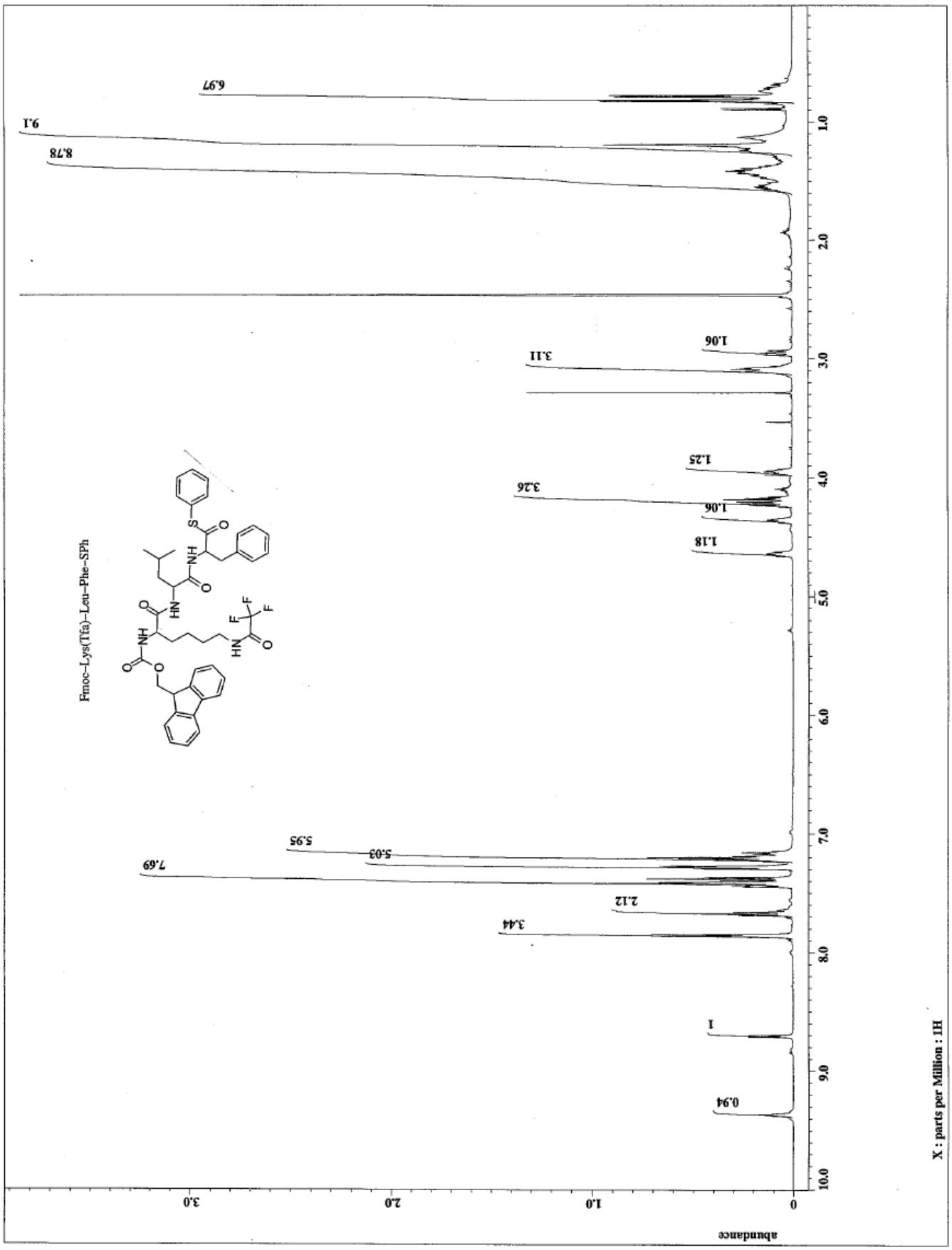
Ionization mode: ESI+

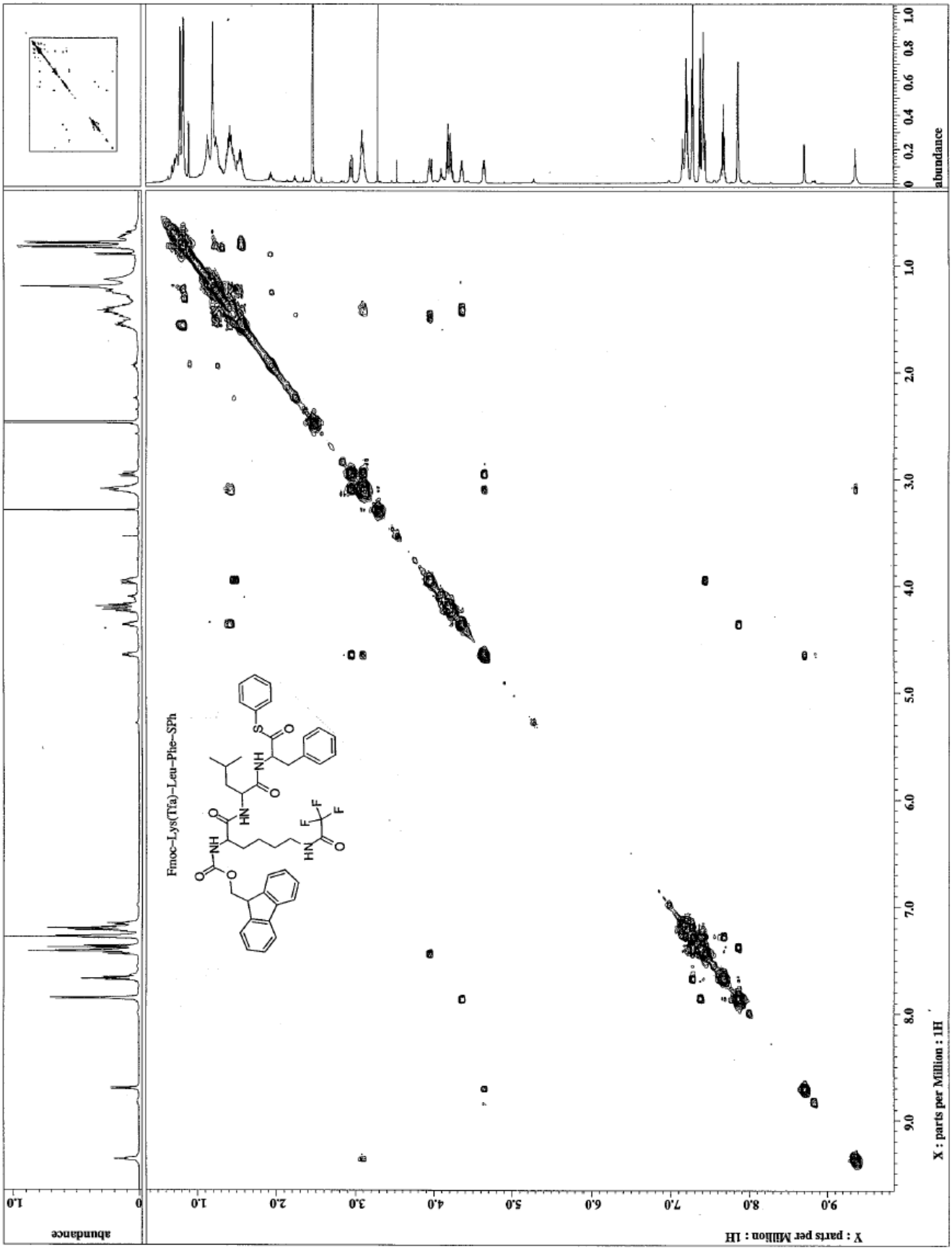
Scan: 492-495 (409-418)
TH(VI)p93 DART 220C (NH4OH added to sample gap)
TIC: 701811 (Max Inten : 145860)

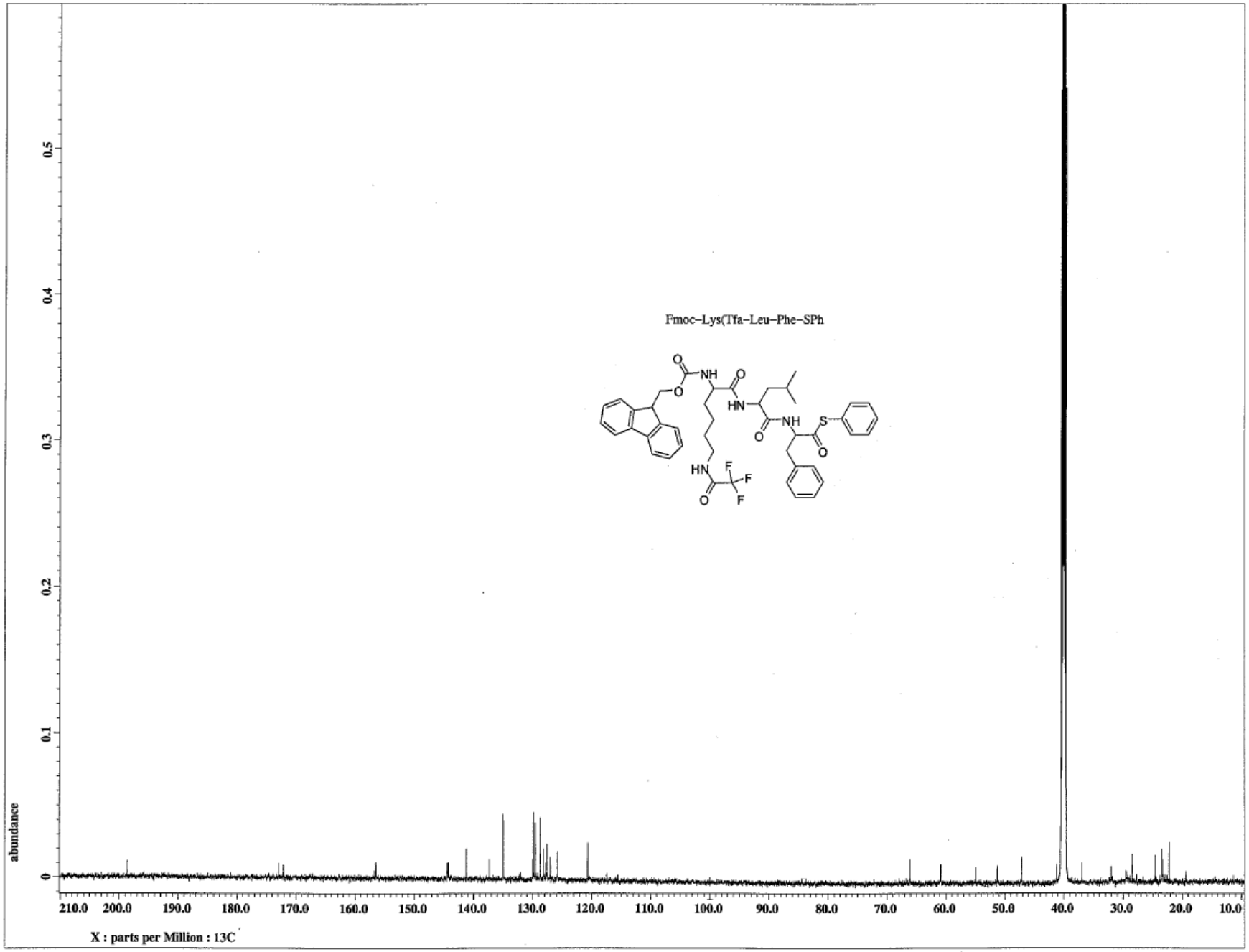
R.T.: 8.23

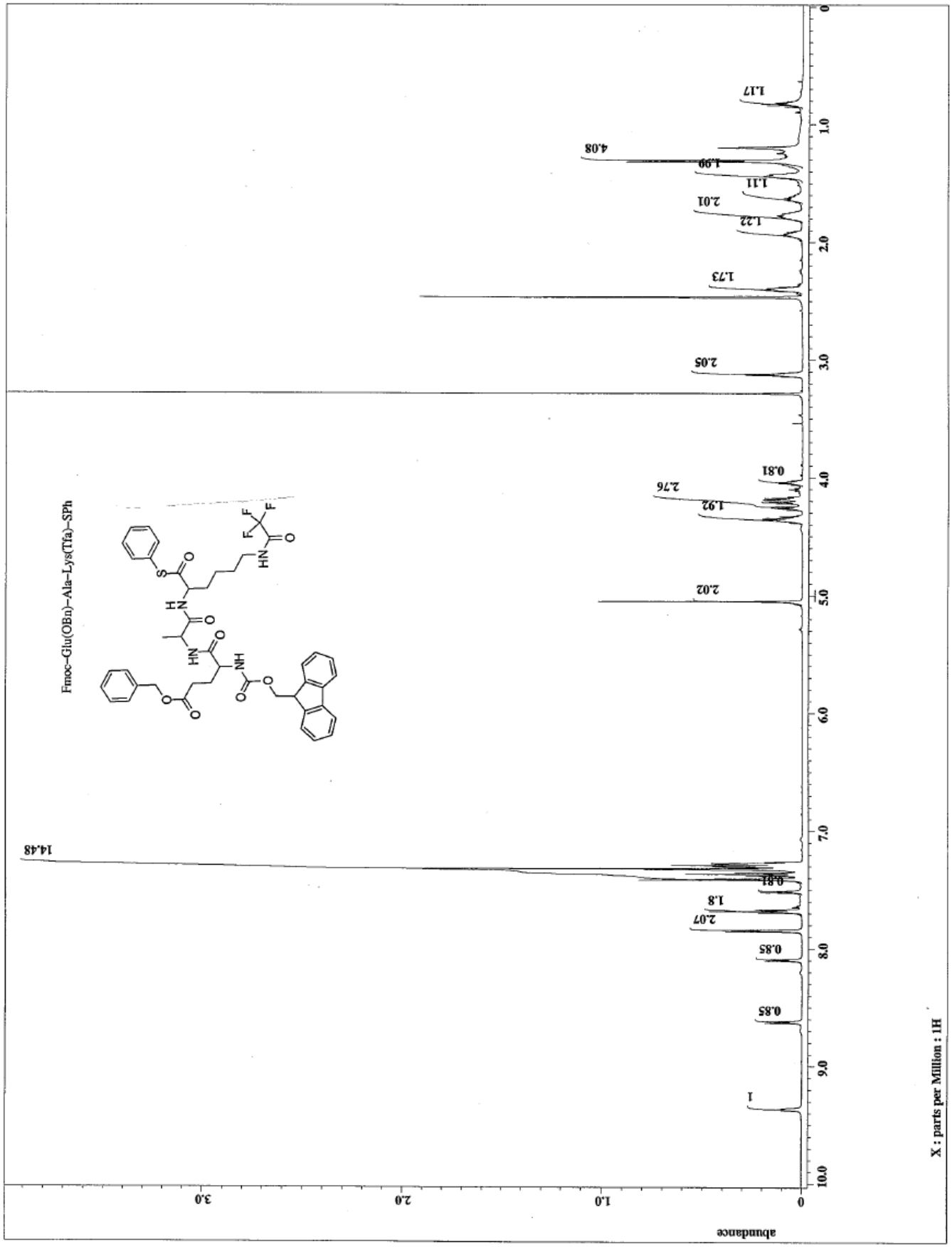
Base: m/z 524; 16.3%FS

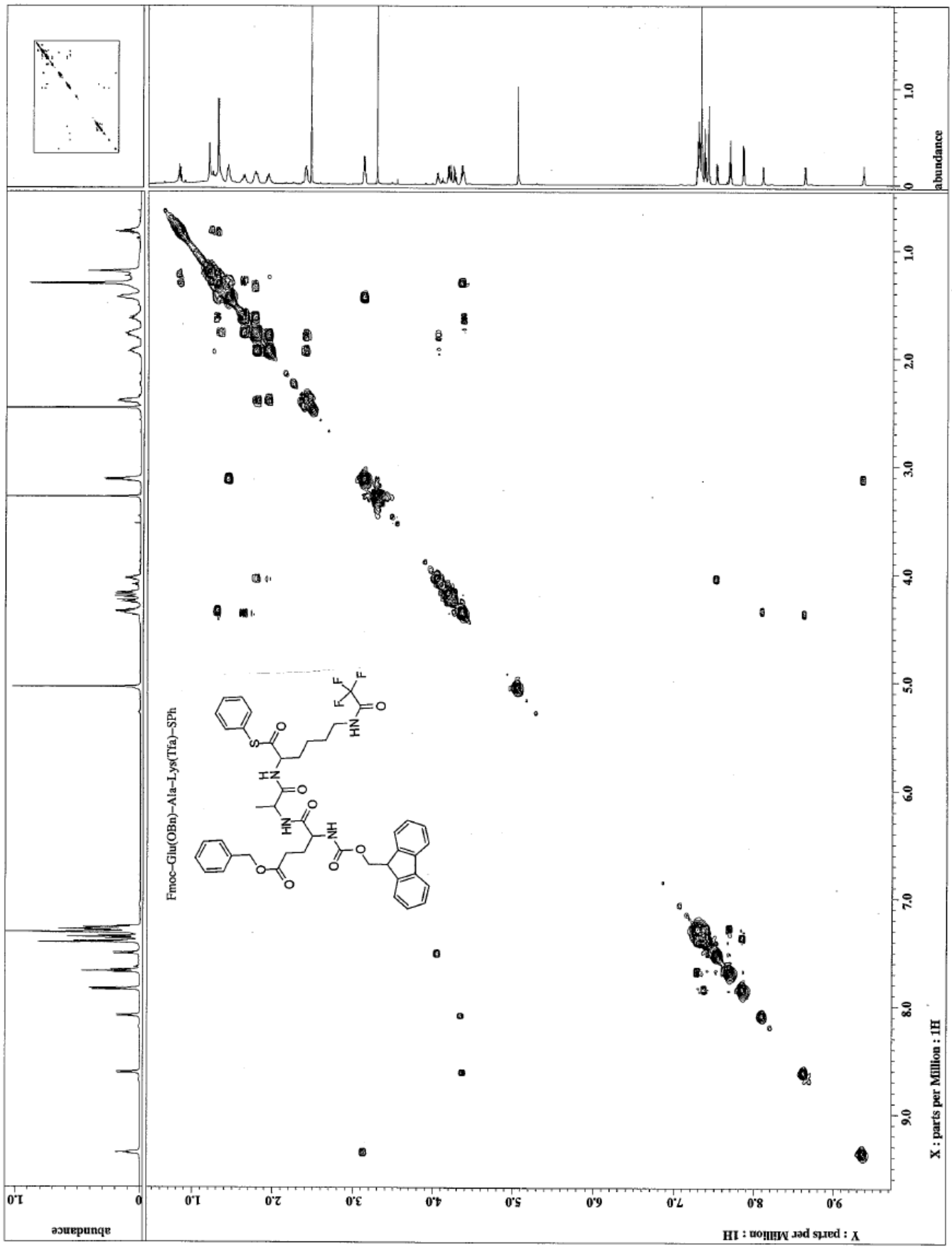


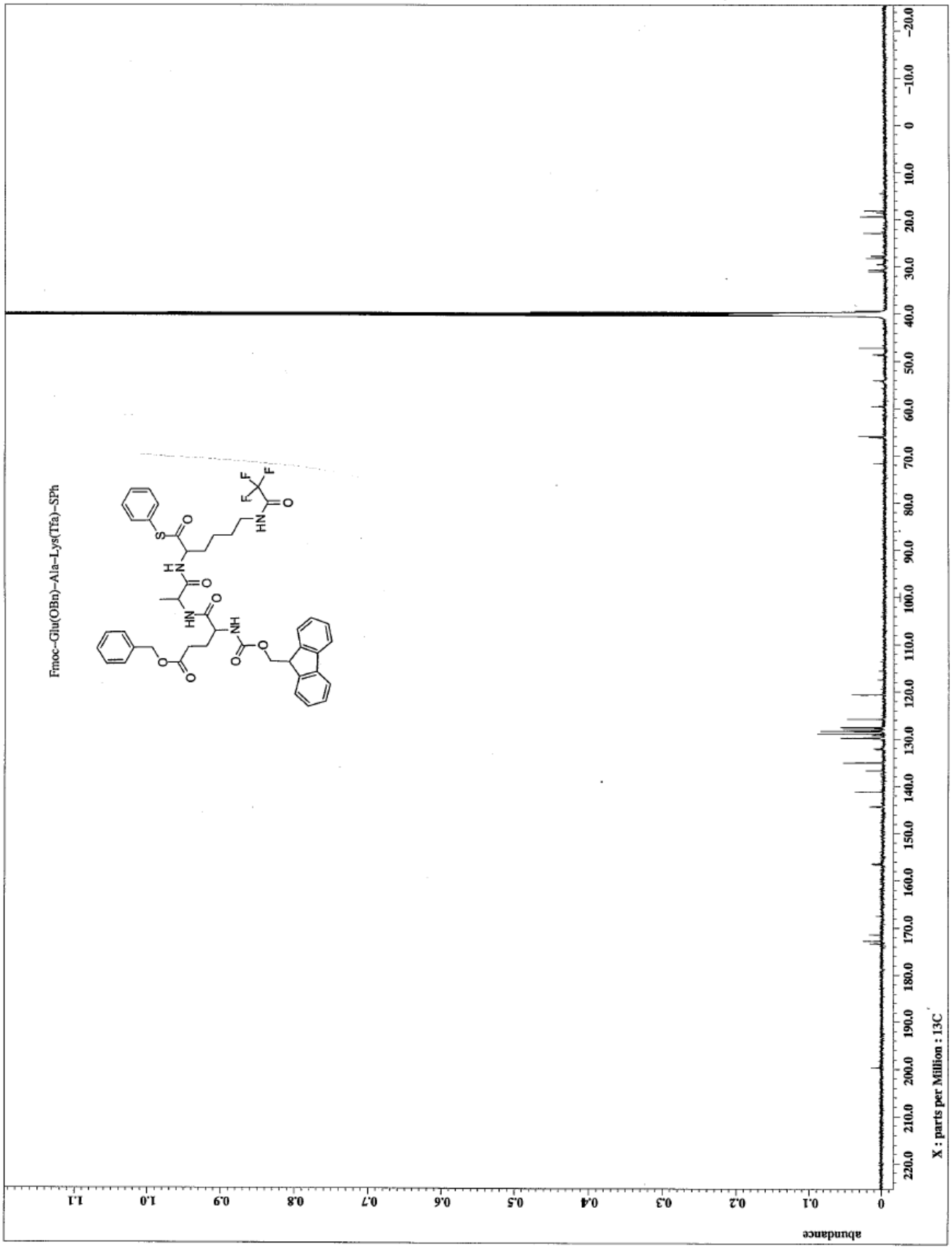

Fmoc–Lys(Tfa)–Leu–Phe–SPh
X : parts per Million : 1H
abundance
0.94
1
3.44
2.12
7.69
5.03
5.95
1.18
1.06
3.26
1.25
3.11
1.06
8.78
9.1
6.97


Fmoc-Lys(Tfa)-Leu-Phe-SPh
X : parts per Million : 1H
abundance


Fmoc-Lys(Tfa-Leu-Phe-SPh
abundance
0
0.1
0.2
0.3
0.4
0.5
210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0
X : parts per Million : 13C

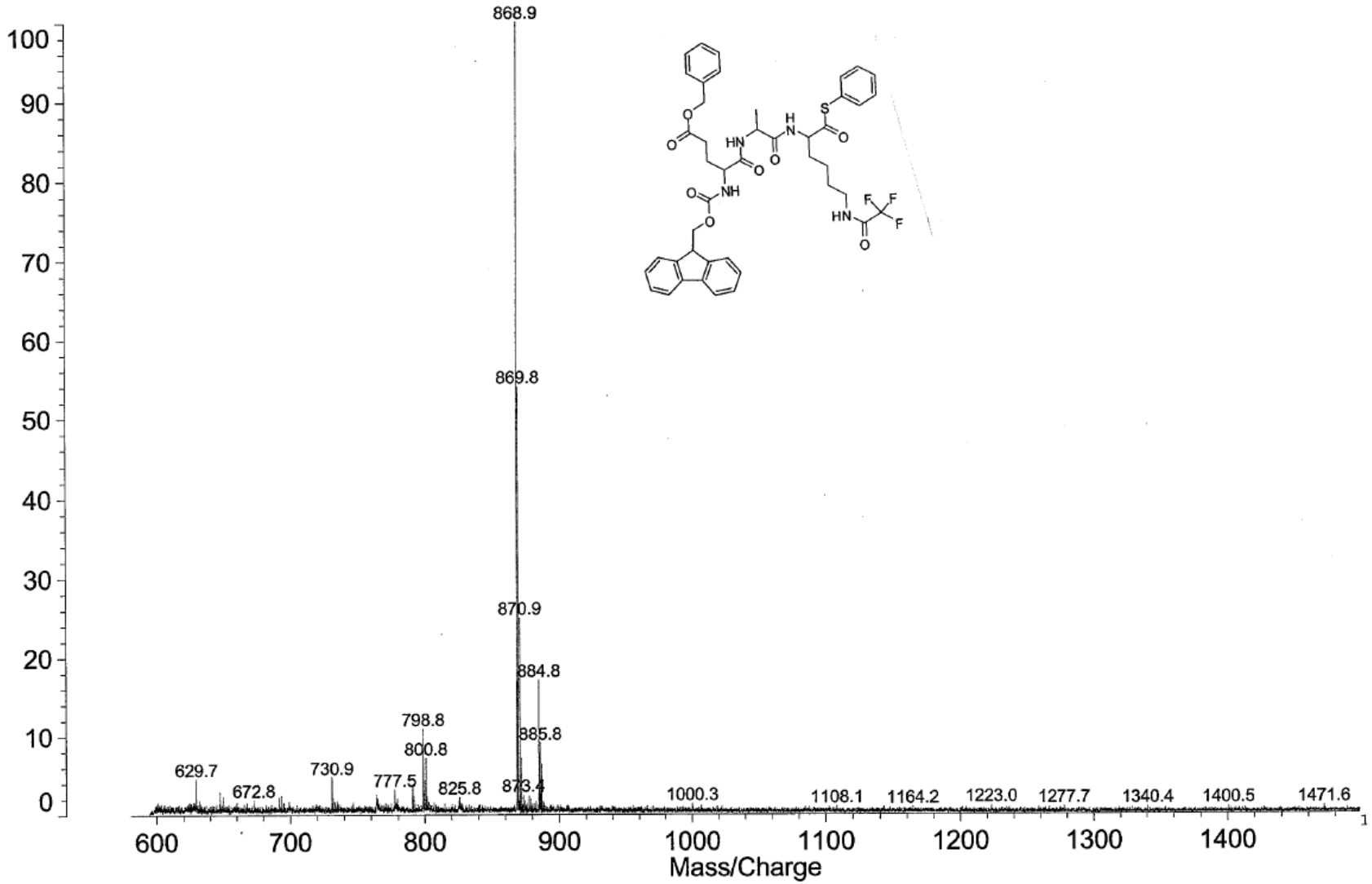

Fmoc-Glu(OBn)-Ala-Lys(Tfa)-SPh
X : parts per Million : 1H
abundance
14.48
2.07
1.8
0.85
0.85
1
2.02
2.76
1.92
0.81
2.05
1.73
2.01
1.22
1.11
1.99
4.08
1.17


Fmoc–Glu(OBn)–Ala–Lys(Tfa)–SPh
X : parts per Million : 1H
Y : parts per Million : 1H
abundance
abundance


Fmoc-Glu(OBn)-Ala-Lys(Tfa)-SPh
abundance
X : parts per Million : 13C

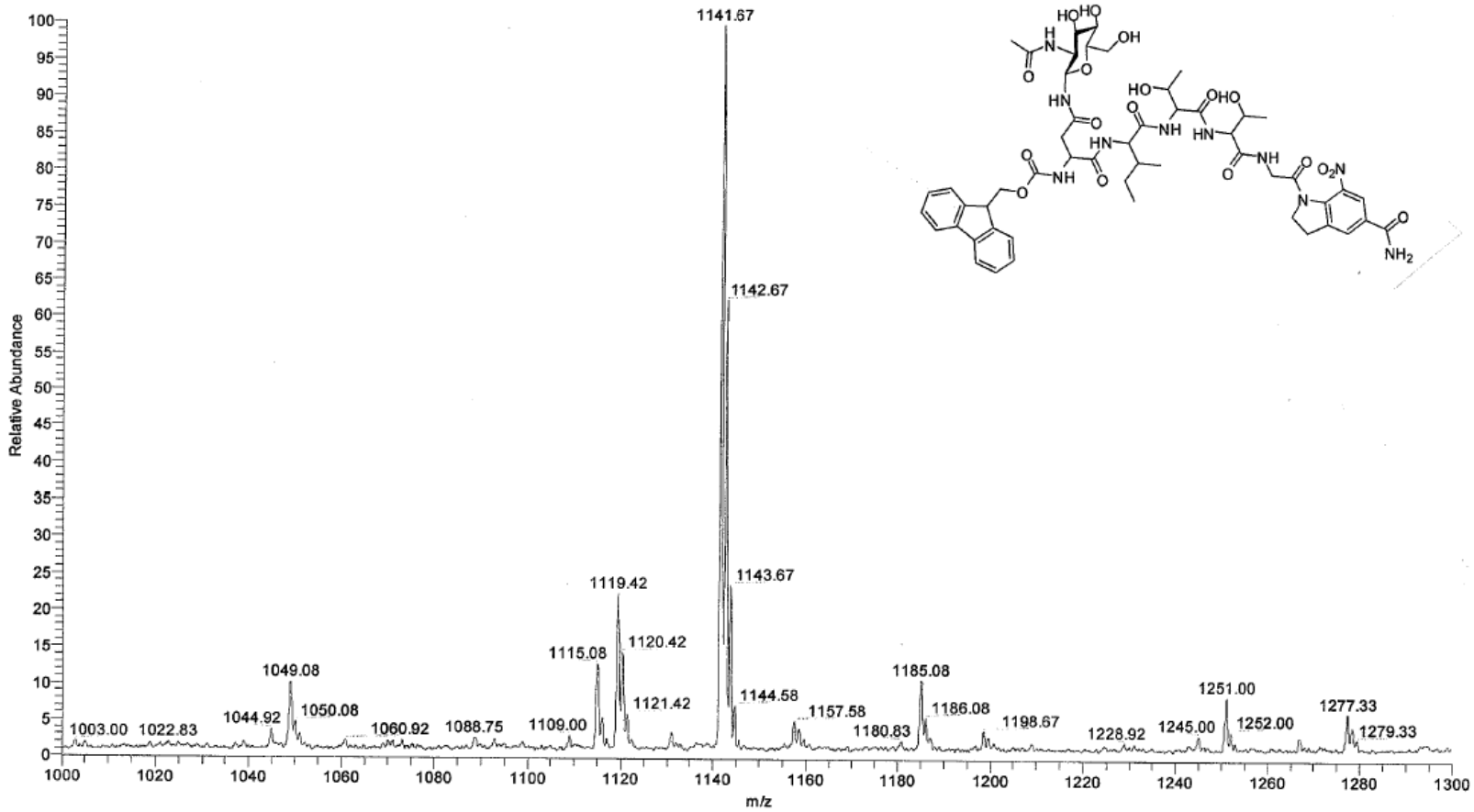
TH(VI)p31_Fmoc-Glu(OBn)-Ala-Lys(tfa)-SPh_af030609_CHCA

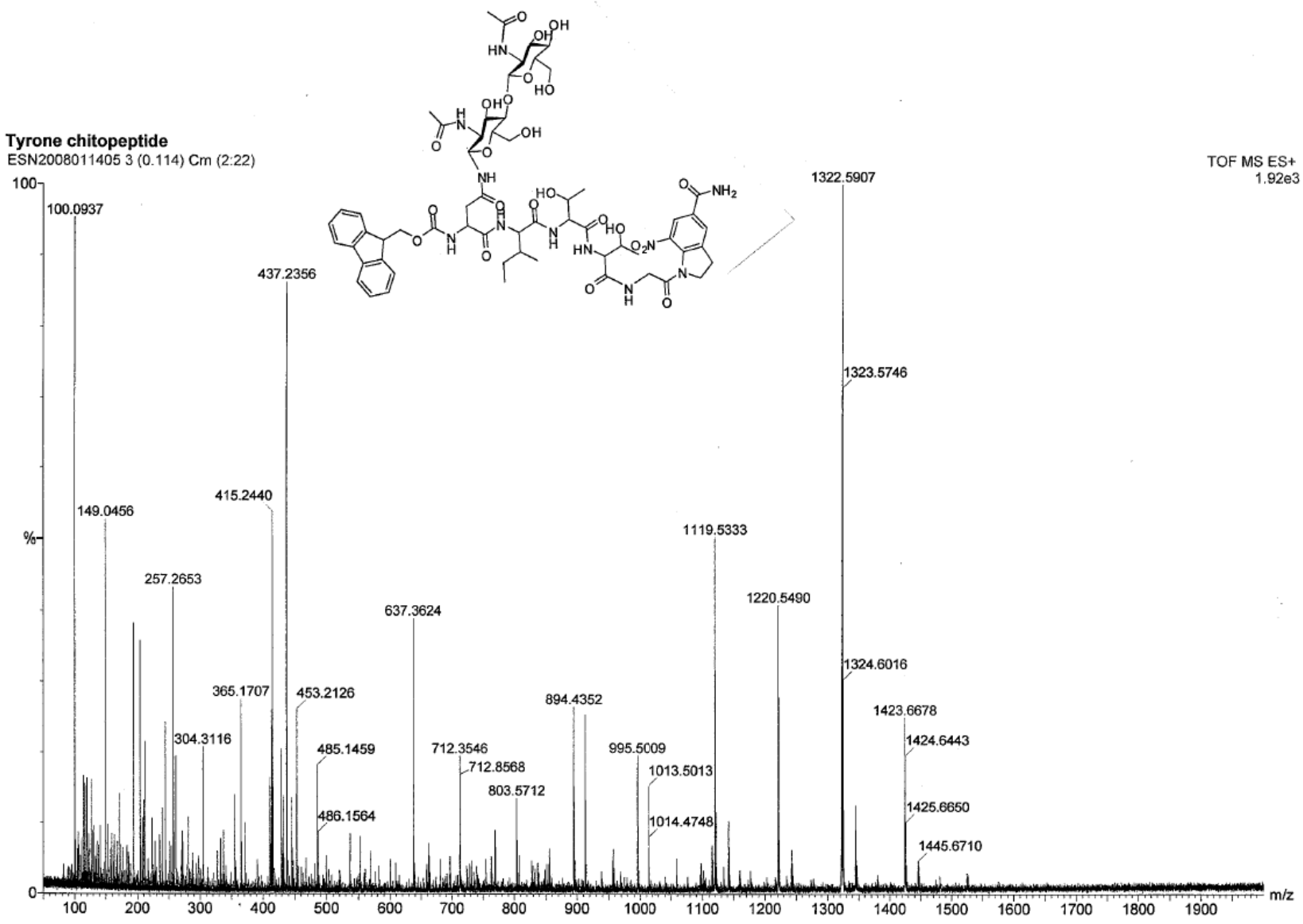
Data: TH(VI)p31_Fmoc-Glu(OBn)-Ala-Lys(tfa)-SPh_af030609_CHCA0001.C4 6 Mar 2009 12:37 Cal: Refl_Franz011607 6
Kratos PC Axima CFR V2.3.4: Mode Reflectron, Power: 60, P.Ext. @ 1000 (bin 105)
%Int. 543 mV[sum= 27169 mV] Profiles 1-50 Unsmoothed



C:\Xcalibur\Data\ica2007112106 11/21/2007 3:58:53 PM 38(Tyrone),1/2dil,mz1000-1300
infusion 500nl/min 50%ACN/0.05%FA

ica2007112106 #1-171 RT: 0.00-1.08 AV: 171 NL: 2.07E5
T: ITMS + p NSI Full ms [1000.00-1300.00]




Tyrone chitopeptide
ESN2008011405 3 (0.114) Cm (2:22)
TOF MS ES+
1.92e3
100
%
0
100.0937
149.0456
257.2653
304.3116
365.1707
415.2440
437.2356
453.2126
485.1459
486.1564
637.3624
712.3546
712.8568
803.5712
894.4352
995.5009
1013.5013
1014.4748
1119.5333
1220.5490
1322.5907
1323.5746
1324.6016
1423.6678
1424.6443
1425.6650
1445.6710
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
m/z

VITA

I was born in Seattle, WA in the fall of 1974 to parents Edward and Susan Hogenauer. I moved to Walnut, CA in the summer of 1981. I was educated in the public school systems and graduated high school in the summer of 1993. I attended Bellevue Community College (Bellevue, WA) for a couple of years, then Fullerton Junior College (Fullerton, CA) for a couple more years as I decided what path I was going to take. I decided to pursue a degree in chemistry after I took an organic chemistry course which I fell in love with. I graduated from California State University, Fullerton in the summer of 2002. I entered the Masters program, at the same school, in the fall of 2002 and graduated two years later in the summer of 2004. Having obtained the Masters of Science degree under the tutelage of Dr. Gene Hiegel, I was encouraged to continue on for the Ph.D. I entered the University of Hawaii, Manoa chemistry program in the fall of 2004. I worked briefly with Dr. Marcus Tius before finding a home in the laboratory of Dr. Katja Michael. In the summer of 2006, I was offered a place in Dr. Michael's laboratory at the University of Texas, El Paso upon her move from Hawaii. I accepted and four years later, in the summer of 2010, I graduated with my Ph.D. in Chemsitry.

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This dissertation was typed by Tyrone Justin Hogenauer