Synthesis of Fmoc-3-(N-ethyl-3-carbazolyl)-L-alanine and Its Incorporation into a Cyclic Peptide

By

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Abstract

Ghadiri reported the first synthetic peptide nanotubue in 1993, which has triggered extensive studies on peptide-based nanotubes and their potential application in molecular wires, catalysts and novel drug delivery vehicles. Our concerns focus on chromophore-modified cyclic peptides, which open a new way to design and synthesize novel nanoscale electronic or photonic devices, and are expected to provide the highly efficient electron and energy transfer that such devices require.

This research concerned the design and synthesis of chiral α-amino acids with specific chromophores, including N-ethyl-3-carbazolylalanine and 9-anthrylalanine, and an 8-mer linear peptide (H-Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe-OH) and its corresponding cyclic peptide *cyclo*(Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe) that incorporate the N-ethyl-3-carbazolylalanine. This thesis describes the relevant background, synthetic strategies, experiments and results in detail.

The carbazole derivatives were found to be very labile to strong acid, which might have caused self-condensation. In order to avoid the formation of acid-derived side-products, the Wittig-Horner reaction was used successfully in preparation of N-protected-3-(N'-ethyl-3-carbazolyl)-DL-alanine methyl ester. Dual enzymatic hydrolyses were developed to produce the chiral amino acids with high enantiomeric excess. ChiroCLEC-BL was used to selectively hydrolyze the N-acetyl-L-amino acid methyl ester, while amanoacylase was adopted to remove the acetyl group from the resulting N-acetyl-L-amino acid.

Two model peptides were synthesized, a 4-mer peptide (H-Car-D-Ala-Bpa-D-Ala-OH) via the Boc-strategy, and an 8-mer peptide (H-D-Ala-Npa-D-MeAla-Ala-D-Ala-Bpa-D-Ala-Ala-OH) by the Fmoc-strategy. Eventually, the target linear peptide was synthesized via the Fmoc-strategy and then cyclized in solution.

Acknowledgements

I would like to thank my advisor Prof. Stephen Weininger for his extensive knowledge and valuable advice given to me. His sharp insight made me stay on the right track and approach the final target. I would express many thanks to Prof. Grant McGimpsey for this interesting project he chose and his patient instruction, especially in intramolecular electron and energy transfer.

When I began with solid-phase peptide synthesis and cyclization, I got many benefits from the previous work of Chris Cooper, John Benco, and Dr. Hubert Nienaber. Many helpful discussions with them, plus Zhonghong Tao's experimental data, helped me avoid many frustrating trials. I couldn't forget David Ferguson and Man Phewluangdee. When I just came to the lab, they kindly shared their previous experience in amino acid synthesis with me.

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Chapter 1 Introduction

1. 1 Research goal

One fundamental goal of our laboratory is to design and synthesize bichromophore-containing cyclic peptides, and to explore their self-assembly into peptide-based nanotubes. If successful, it would open a new way to self-assemble novel nanoscale electronic or photonic devices. It is anticipated that such structures could be applied in a high-resolution display. The building units would transmit information via highly efficient intramolecular energy transfer between the chromophores.

This research concerned the design and synthesis of chiral α-amino acids with specific chromophores, incorporating them into a bichromophoric linear 8-mer peptide, and cyclizing the linear peptide. The cyclic peptides will be used to investigate photoinduced intramolecular energy transfer between the chromophores in order to further develop novel molecular devices in the near future.

1. 2 Peptide based nanotubes

With the fast-increasing demand for high-speed electronic devices, e.g. molecular computers, molecular nanotechnology featuring the miniaturization of physical components of devices has made great progress in designing and controlling organic molecules and carbon nanotubes in the past decade.[1]

Chemists obtained great inspiration from the way that biological systems create life and self-assemble the supramolecular structures into the living units, and began to explore artificial approaches to intelligent functional supramolecular materials.[2] The artificial peptide-based nanotube was a successful landmark.[3]

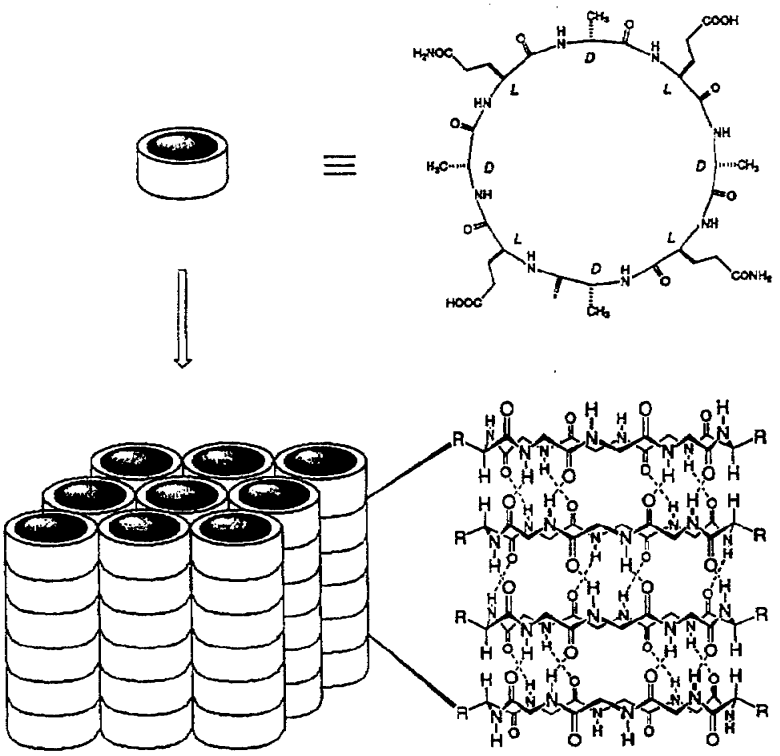


Figure 1-1 A cyclic peptide, *cyclo*[-(D-Ala-Glu-D-Ala-Gln)$_2$-] and the nanotubes formed by its self-assembly.[1]

In nature, many peptide and protein-based microtubules build their sub-cellular structure by self-assembly techniques. In 1993, the Ghadiri group reported the first synthetic peptide nanotube (Figure1-1). The cyclic peptide *cyclo*[-(D-Ala-Glu-D-Ala-Gln)$_2$-] with an even number of alternating D- or L-amino acids, which favors a low-

energy flat conformation, was used as the molecular building block of the nanotubes. All the side chains of the amino acids are allowed to point outwards. The amide backbone forms hydrogen bonds in a direction perpendicular to the plane of the cyclic peptide. When two cyclic peptides stack upon one another, a hydrogen-bonding network is formed, which looks like a contiguous, anti-parallel β sheet, commonly found in natural proteins. As this hydrogen-bonded lattice propagates in a direction perpendicular to the plane of the cyclic peptide, a highly ordered parallel array of nanotubular structure with fixable internal diameter and modifiable exterior surface is formed by self-assembly. Hundreds of these nanotubes are tightly packed to form crystalline fibers.[1]

This nanotube used a pH triggered self-assembly mechanism. The protonation of two glutamic acid residues was designed as the switch. At high or neutral pH, the cyclic peptide has a negative charge and is water-soluble, and the repulsive charges on the cyclic peptides resist the amide backbone hydrogen bonding. When the pH is lowered, the glutamic acids are protonated and the solubility decreases. In the meantime, the repulsive charges are removed, which induces amide backbone hydrogen bonding and self-assembly into tubular microcrystals with a diameter of 7.5 Å. When the number of amino acid residues making up of the cyclic peptide is increased from eight to twelve, another large-size microcrystalline nanotube (*cyclo*[-(D-Ala-Glu-D-Ala-Gln)$_3$-]) is formed with a 13 Å diameter, which displays greater stability in a broad range of pHs and solvents.[4]

Another group of nanotubes designed by the Ghadiri group adopted a highly hydrophobic outer surface and hydrophilic inner pore, such as *cyclo*[-(Phe-D-^{Me}NAla-Hag-D-^{Me}NAla)$_2$-] (Hag represents homoallylglycine)[5] and *cyclo*[-(Trp-D-Leu)$_3$-Gln-D-

Leu].[6] Such nanotubes are easily inserted into a lipid bilayer. Some ions were found to be able to pass through the open ends of the nanotubes, which would allow new applications as highly efficient ion channels. Nanotubes with slightly larger pores have been shown to transport small molecules, such as glucose.[7]

The theoretical and applied studies on peptide-based nanotubes have attracted the attention of many research labs.[8,9] Based on the experimental results, Lewis calculated the energetics, electronic structure and vibrational spectra of *cyclo*[-(D-Ala-Glu-D-Ala-Gln)$_{m=1-4}$].[10] Such research could provide the theoretical guide for future work on computer-aided molecular design of peptide nanotube-based molecular devices. By adjusting the number and choice of the amino acid residues, the cyclic peptide nanotubes can be designed with desirable surface characteristics and pore sizes. They can be easily synthesized by combining self-assembly with standard solid phase peptide synthesis.

The potential applications of peptide-based nanotubes cover nanowires, optical and electronic devices, catalytic media, therapeutic agents, transmembrane channels, and novel drug delivery systems.[1]

1. 3 Why did we consider cyclic peptides as investigative candidates?

Research on photoinduced intramolecular energy and charge transfer in polychromophoric helical peptides composed of various numbers of unnatural amino acid residues carrying the chromophores on the peptide backbone has been undertaken.[11,12,13] The research has been concerned mainly with varying the distance between chromophores, devising less complicated means of incorporating various chromophores

into the peptide, and forming the secondary helical structure to limit the change of conformation.

Our latest task with chromophore-modified cyclic peptides is made easier by their tendency to self-assemble into peptide nanotubes. At the same time, photochemical research on the chromophore-modified cyclic peptide nanotube is an unexplored field as yet. There are a couple of factors that led us into this research.

- The cyclic peptide adopts a stable conformation that facilitates unidirectional, highly efficient energy transfer in molecular devices.
- Automatic solid-phase peptide synthesis can be utilized to simplify synthesis and purification.
- Flexible adjustments of the distance between the chromophores can be made by changing their attachment positions and the internal diameter of the cycle peptide.
- The property of the exterior surface can be controlled by changing the chromophores.
- Favorable self-assembly creates nanoscale devices. The intermolecular hydrogen bonding and ring stacking interactions of peptide subunits in the β conformation would be energetically favored under appropriate conditions, and would thus produce open-ended, hollow, tubular ensembles.

1. 4 Photoinduced intramolecular energy transfer between chromophores[14]

There are two important mechanisms that theoretically describe the photoinduced intramolecular energy transfers, the Förster mechanism and the Dexter mechanism (Figure 1-2).

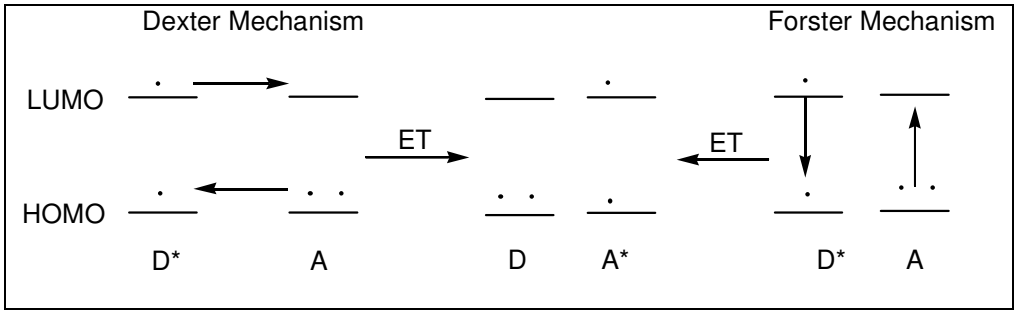


Figure 1-2 Photoinduced energy transfer pathways. D represents the donor; A represents the acceptor; the horizontal line means a molecular orbital; a dot stands for an electron, and * indicates an excited state.

The Dexter mechanism is based on electron exchange. It occurs when one electron moves from the LUMO of the donor to a LUMO of the acceptor, while at the same time, another electron is exchanged from HOMO of the acceptor to one of the donor. As a result, the excited-state donor returns to the ground state while the acceptor is excited. A through-bond pathway is effective over a range of 10-15 Å. A through-space path is also available if there is good overlap of the acceptor and donor orbitals.

The Förster mechanism is based on an electrodynamic interaction between the donor and acceptor. The oscillating donor dipole in the excited state induces oscillation of the acceptor dipole. This through-space mechanism is effective over many tens of Å. Singlet-

singlet energy transfer (SSET) is viable via this mechanism, while triplet-triplet energy transfer (TTET) is forbidden here.

There are three chromophores - anthracene, benzophenone and N-ethylcarbazole - that we considered for incorporation into cyclic peptides, based on preliminary photophysical investigations.[15,16] The α-alanine skeleton would be used as a spacer, with the chromophore attached to C-3. An energy diagram illustrating the predicted pathways of intramolecular energy transfer is shown in Figure 1-3. Carbazole has the highest first excited singlet energy level (S_1) and first triplet energy level (T_1), while anthrance has the lowest S_1 and T_1. Carbazole will always act as a donor, while anthracene will always be an acceptor. Both singlet–singlet energy transfer (SSET) and triplet-triplet energy transfer (TTET) will be expected to occur. Benzophenone can act as the donor to anthrance or the acceptor from carbazole. Interestingly, benzophenone and carbazole have the same T_1 energy. Thus highly efficient reversible TTET would be expected.

We planned to attach two of these chromophores to the 8-mer linear and cyclic peptide scaffolds, and then observe the energy transfer efficiency. In the first instance, two chromophores would be separated by three other amino acid residues.

1. 5 Synthesis of optically active α-amino acid

The design and synthesis of novel, unnatural, optically active α-amino acids have generated interest due to their potential in improvement of pharmacological and chemical applications and their flexibility in incorporation into synthetic peptides.[17,18] With the different chromophores attached to the β-carbon of the amino acids, these derivatives

would provide specific photophysical and photochemical properties.

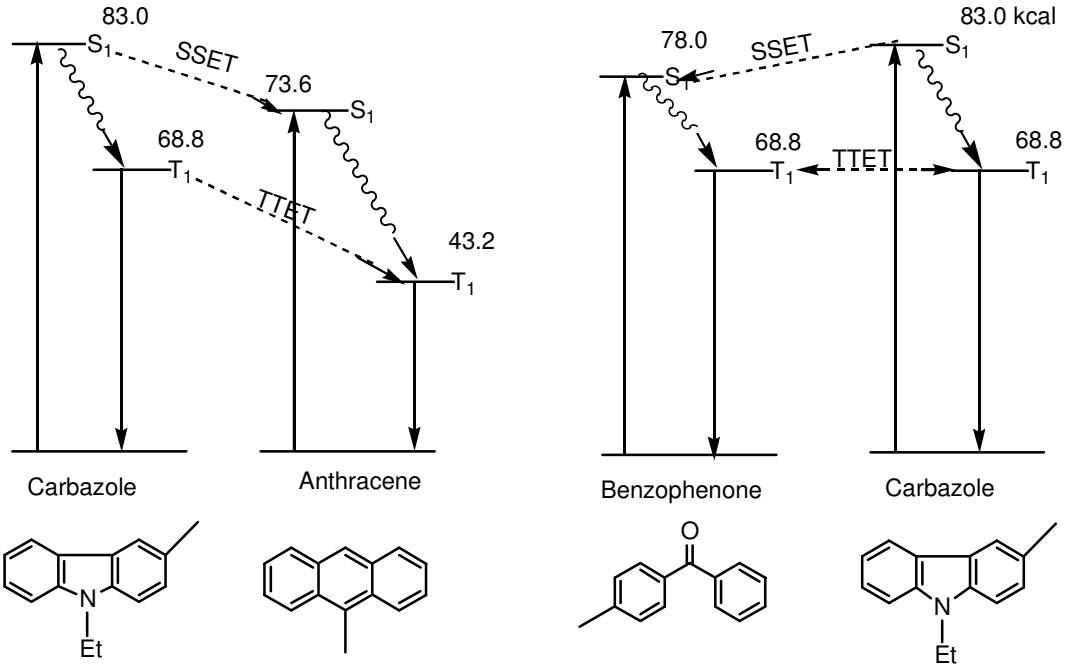


Figure 1-3 Energy diagram for intramolecular energy transfer among the proposed chromophores (broken dashed arrows show possible pathways of energy transfer).

1. 5. 1 Building the α-amino acid skeleton

There are several methods that have been used to build the amino acid skeleton.

- **Sorensen method**[19]

The Sorensen method starts with alkylation of N-acetylaminomalonic ester by an alkyl halide, using a strong base as deprotonating reagent. The diester product is hydrolyzed and decarboxylated to give the N-protected α-amino acid (Figure 1-4). Sisido made 3-(9-anthryl)-DL-alanine via this way,[20] while Morrison prepared 3-(2-fluorenyl)-DL-alanine

following the same procedure.[21]

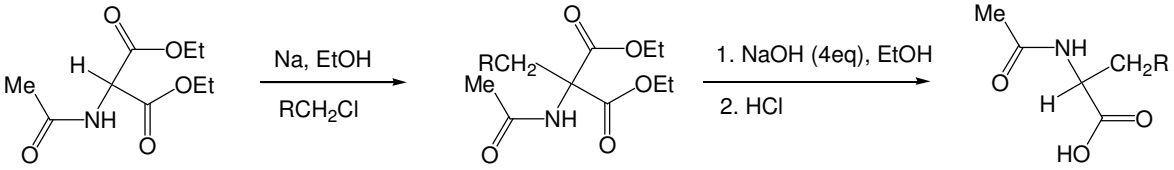


Figure 1-4 General pathway for the Sorensen method to make the α-amino acid derivatives.

- **Erlenmeyer's azlactone method**[19,22]

Erlenmeyer's azlactone synthesis is based on the Perkin reaction. First, an acylglycine is converted into an azlactone by the dehydration of its enol tautomer with Ac_2O. The azlactone, whose methylene group is activated, undergoes Perkin condensation with the aldehyde. This product is then reduced and hydrolyzed to give an α-amino acid (Figure 1-5). Taku reported the preparation of 3-(N-ethyl-3-carbazolyl)-DL-alanine by this method.[23]

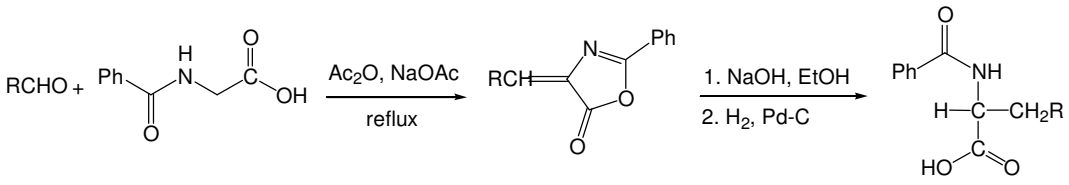


Figure 1-5 General pathway for Erlenmeyer's azlactone synthesis of the α-amino acid derivatives.

- **Wittig-Horner synthesis**[24]

The Wittig-Horner reaction occurs via condensation between phosphonate anions and aldehydes, and has been extensively used to synthesize dehydroamino acids, with a

preference for forming the Z-isomer (Figure 1-6). The reaction is carried out under mild conditions, especially suitable for sensitive chromophores, such as our substrates, the carbazolyl derivatives. Their syntheses will be discussed later.

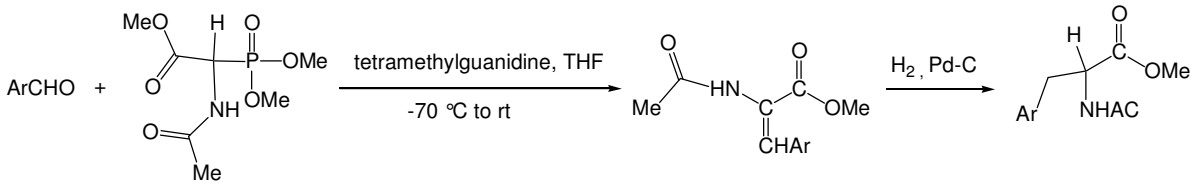


Figure 1-6 General pathway for Wittig-Horner reaction used to build the α-amino acid derivatives (Z may be Me, O-*t*-Bu or Cbz groups).

- **Strecker synthesis**[25]

The Strecker synthesis, a traditional approach to making unprotected racemic amino

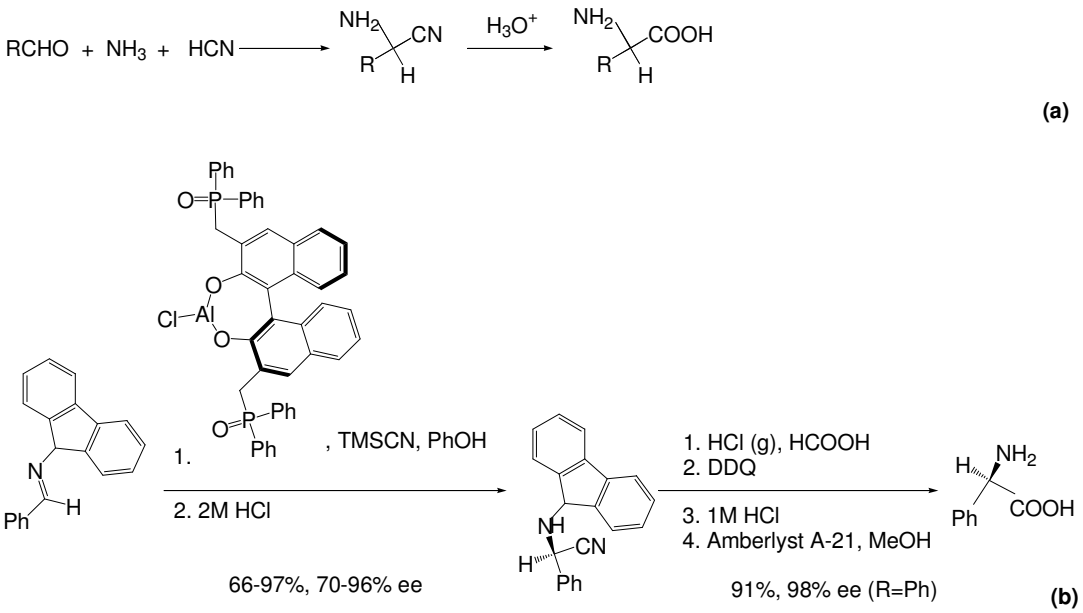


Figure 1-7 **(a)** General pathway for the traditional Strecker synthesis of α-amino acid derivatives; **(b)** asymmetric Strecker-type synthesis of D-phenylalanine.

acids, involves treatment of aldehydes with hydrogen cyanide and ammonia. The intermediate α-aminonitrile is hydrolyzed with H_3O^+ to provide an α-amino acid (Figure 1-7a). Recently, attention has turned to the catalytic asymmetric Strecker-type synthesis, which provides a direct method for asymmetric synthesis of α-amino acid derivatives (Figure 1-7b).

- **Alkylation of glycinate imines under phase transfer conditions**[26]

In 1989, O' Donnell reported the catalytic enantioselective alkylation of the benzophenone imine of a glycine alkyl ester by pseudoenantiomeric phase transfer catalysis, to give enantiomerically enriched α-amino acids (Figure 1-8). Further recrystallization or enzymatic resolution was required to obtain a highly optically pure isomer.

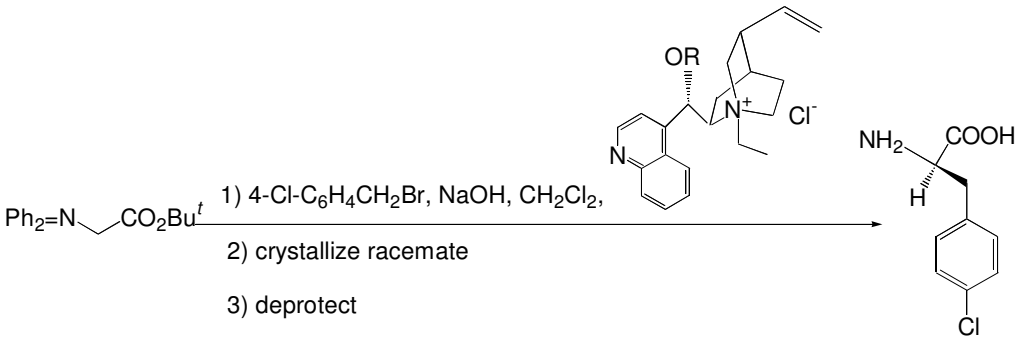


Figure 1-8 Catalytic enantioselective alkylation of glycinate imine under phase transfer catalysis.

- **Asymmetric hydrogenation**[27]

Asymmetric hydrogenation of dehydroamino acids using transition metal complexes with chiral ligands (e.g., BICPO) has provided an extremely useful approach to making a

variety of optically active amino acids, e.g., N-acetyl-3-(2-naphthyl)-(R or S)-alanine methyl ester (Figure 1-9).

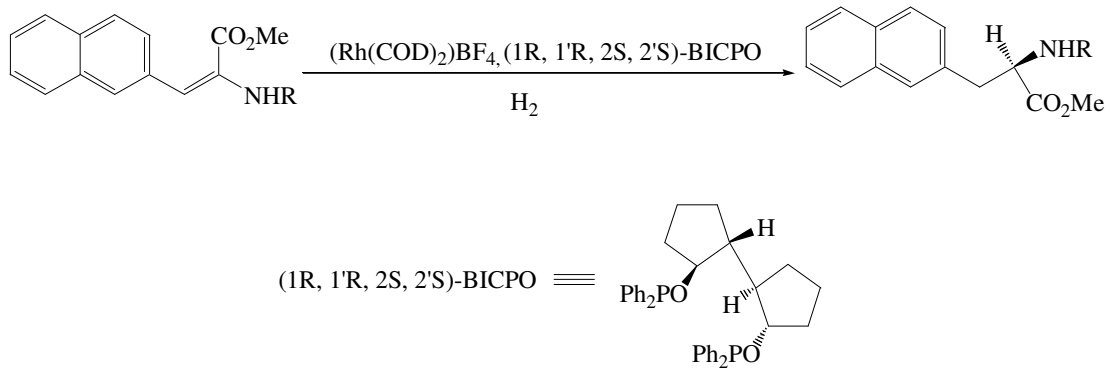


Figure 1-9 Rh-catalyzed asymmetric hydrogenation of an acetamidocinnamic ester.

However, for bulky substrates such as these containing 9-anthryl and N-ethylcarbazolyl side groups, there is no effective catalyst reported that can produce high enantiomeric excesses. The expensive chiral ligands also decreased our desire for attempting to find the optimal chiral ligand for our substrates.

Asymmetric syntheses can build chiral amino acids directly with the aid of various chiral auxiliary reagents. The attainable enantiometric excess is in the range of 70-98%, but is heavily dependent on the stereochemical characteristics of the substrate and chiral reagents. In order to secure high enantiometric excess, enzymatic resolution seemed to us the most promising.

1. 5. 2 Enzymatic resolution

Enantiomers have identical physical and chemical properties, and so can't be resolved by conventional methods, such as crystallization and standard chromatography. Traditionally, the enantiomers have been separated by converting them into

diastereomeric salts. The salts can be isolated by crystallization or chromatography and then decomposed into the pure enantiomers.

Our interest focused on enzymatic resolution. One isomer of the racemate is preferentially transformed into its hydrolytic product under enzymatic catalysis. The major approaches include stereospecific cleavage of N-acyl groups by acylases, and stereospcific ester hydrolysis by proteases.

- **Ester hydrolysis catalyzed by esterases**[17]

A practical resolution of racemic amino acid esters has been obtained using protease VIII (Figure 1-10). The L-methyl (or benzyl) ester can be enantiospecifically hydrolyzed in pH 8 buffer. It was noted that the benzyl ester is hydrolyzed three times faster than the corresponding methyl ester. It is important to monitor the reaction and stop it when it reaches 50% conversion. Organic co-solvents such as DMF, dioxane and CH_3CN (up to 30% v/v) do not significantly lower the enzyme activity, while enhancing the solubility of the substrates. The major advantages of this method are enzyme stability and ease of product isolation. The resultant enantiomeric excess and hydrolytic yield depend heavily on the structure of the substrate and the hydrolytic conditions.

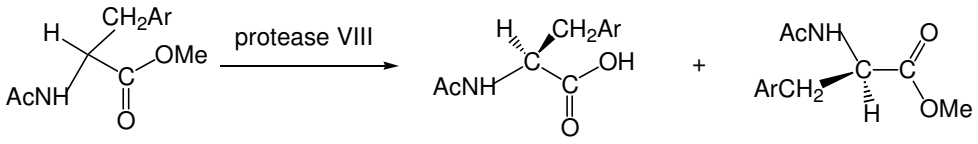


Figure 1-10 General pathway for enantiospecific ester hydrolysis by protease VIII.

The protease subtilisin is broadly applied in stereospecific ester hydrolyses. Its disadvantages are its low stability in water and water-miscible organics, and low activity in neat organic solvents, which limit its application.

Subtilisin ChiroCLEC-BL is a crosslinked, crystalline subtilisin.[28] Crosslinking confers high stability and activity at elevated temperature and in organic solvents on the enzyme, which exhibited much better activity for N-acetyl-3-(2-fluorenyl)-L-alanine methyl ester than the protease subtilisin did, as recently found in our lab.

- **Deacylation by acylases**[17]

Mori and Iwasawa reported the acylase-catalyzed deacetylation of N-acetyl-DL-amino acid derivatives, and used it to generate the enantiomers of *threo*-2-amino-3-methylhexanoic acid. The reaction was carried out at pH 6.7 at 37 °C for four days in the presence of $CoCl_2$. The enzyme selectively deacylated the L-isomer and provided both enantiomers in high optical purity.[11]

There are mainly two types of the acylases used for deacylation, one extracted from hog kidney, which has shown poor activity for substrates with bulky aromatic groups, and the other from *aspergillus melleus* with good activity for aromatic substrates.[29] Sisido reported that an acylase (type unknown) failed to deacylate N-acetyl-3-(9-anthryl)-L-alanine,[20] while Matsubara found that acylase from *aspergillus melleus* succeeded in removing the acetyl group from N-acetyl-3-(2-anthraquinonyl)alanine.[30] The reason for this difference wasn't stated, but the type of acylases that made this difference is reasonably assumed.

There are several commercial acylases obtained from *aspergills melleus* with different enzymatic activities, distributed by Aldrich, Fluka and TCI. Amanoacylase from Aldrich has the highest activity at 30,000 μ/g, while another acylase from Fluka has 500 μ/g; no activity data was available for the TCI product. Compared with Fluka's acylase, Aldrich's amanoacylase is more efficient, which means that a lower catalyst/substrate ratio and shorter reaction time are required for the hydrolysis (Figure 1-11).

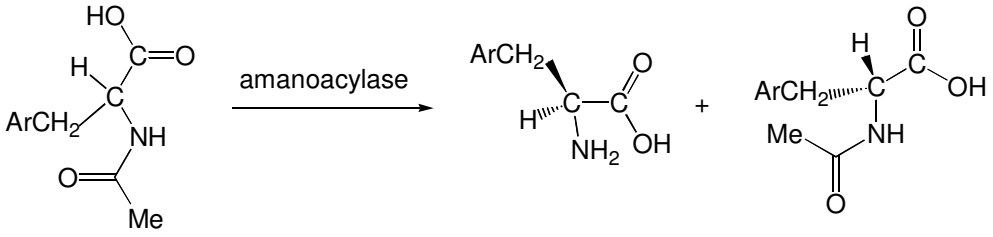


Figure 1-11 General pathway for deacylation by amanoacylase.

1. 5. 3 Protection and deprotection of α-amino acid derivatives[31]

Protection of amino or carboxyl groups are very important processes for peptide synthesis, in order to block functional groups that may participate in unwanted peptide bond formation. The nucleophilic reactivity of the α-amino group can be suppressed by pulling its electron density into a protective group or by shielding it sterically. Good protection and deprotection steps are those that can be done under mild, racemization-free conditions. The protection should be easy to achieve and maintain until deprotection is required. The protective groups should be removable under mild conditions that have no adverse effect on other substitutents or the chiral center. Boc and Fmoc protection of amino groups are two common transformations used in solid-phase peptide synthesis.

- **Boc protection and deprotection**

The Boc group (t-BuOCO-) is applied extensively in peptide synthesis for amine protection, and is introduced with t-butoxycarbonyl azide or di-t-butyldicarbonate. A certain amount of side product formation, attributed to dipeptide or tripeptide formation, has been observed.[32] To prevent these side reactions, the carboxyl group should be first converted to a trimethylsilyl ester with trimethylsilyl chloride. The trimethylsilyl ester is easily hydrolyzed back to the carboxylic acid after Boc protection. The Boc group is inert to basic hydrolysis and catalytic hydrogenation. It can be easily removed by TFA/CH_2Cl_2 or HCl/dioxane.

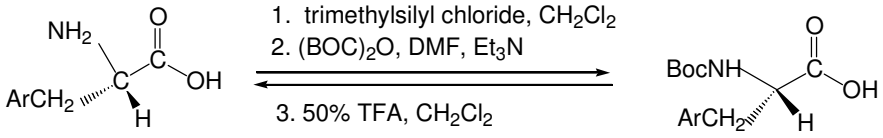


Figure 1-12 General pathway for Boc protection and deprotection of the amino groups on α-amino acids.

- **Fmoc protection and deprotection**

The Fmoc group may be introduced by 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) or 9-fluorenylmethyloxycarbonyl-N-hydroxysuccinimide (Fmoc-OSu).[31] For the amino acids with less hindered side chains, such as glycine or alanine, Fmoc-Cl can give small but detectable amounts of dipeptides and even tripeptides as side products. Fmoc-OSu is the reagent of choice for eliminating this oligomer formation. The Fmoc group has excellent acid stability. It can be quickly cleaved by 25% piperidine in DMF to afford the free amine and dibenzofulvene (Figure 1-13).

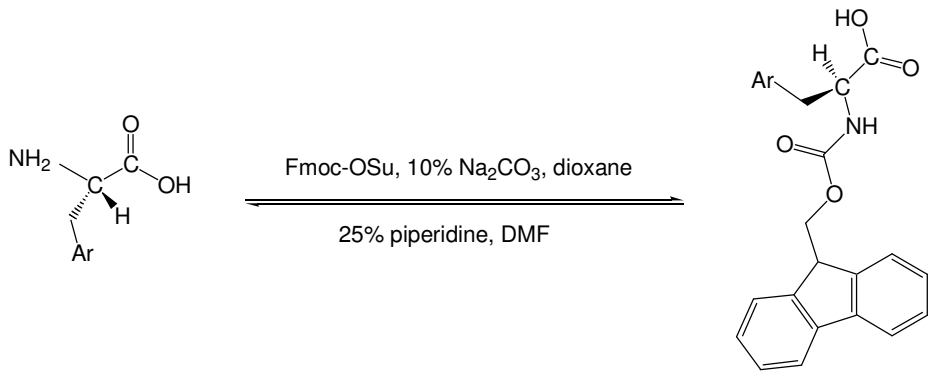


Figure 1-13 General pathways for Fmoc protection and deprotection of α-amino groups on the amino acids.

1. 6 Solid-phase peptide synthesis[33,34]

Peptide synthesis involves the formation of amide linkages (referred to as peptide bonds), which are formed by the reaction between the amino group of one amino acid and the carboxyl group of another amino acid (Figure 1-14). The formation of the peptide bond requires two fundamental operations: (1) activation of the carboxyl group, and (2) N-protection of the activated amino acid species, C-protection of the other one, and protection of any reactive functional groups on the side chain that may lead to side reaction during coupling.

Peptide synthesis, whether in solution or on a resin, is a repetitive multi-step process, consisting of protection, activation, coupling and selective deprotection. The traditional solution-phase peptide synthesis requires equimolar amounts of reagents and complicated purification at each stage to remove the impurities. Such processes are time consuming and costly. Solid-phase peptide synthesis avoids these problems.

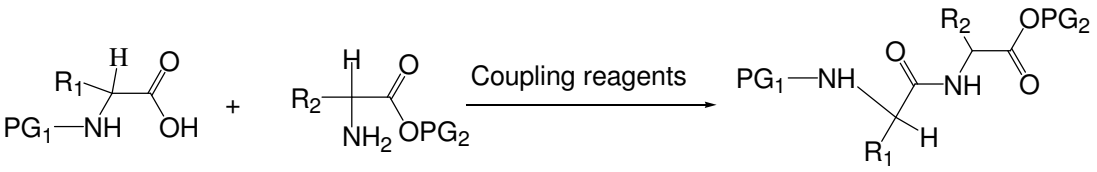


Figure 1-14 The formation of the peptide bond. R represents the alkyl or aromatic groups, and PG indicates the protective group.

The concept of solid-phase peptide synthesis (SPPS) was conceived by Merrifield in 1959. Merrifield stated that, "One day, I had an idea about how the goal of a more efficient synthesis might be achieved. The plan was to assemble a peptide chain in a stepwise manner while it was attached at one end to a solid support. With the growing chain covalently anchored to an insoluble matrix at all stages of the synthesis, the peptide would also be completely insoluble and, furthermore, would be in a suitable physical form to permit rapid filtration and washing after completion of each of the synthetic reactions. The intermediate peptides in the synthesis would thus be purified by a very sample, rapid procedure rather than by the usual tedious crystallization methods. When a multistep process, such as the preparation of a long polypeptide or protein, is contemplated, the saving in time, effort and materials could be very large. The fact that all of the steps just described are heterogeneous reactions between a soluble reagent in the liquid phase and a growing peptide chain in the insoluble solid phase led to the introduction of the name – solid phase peptide synthesis."[34] With the developments in automation technology and parallel synthesis, the automatic solid-phase peptide synthesis was implemented, and further simplified peptide synthesis.

After the Boc-strategy peptide synthesis originally used by Merrifield, the Fmoc-strategy was proposed by Sheppard in 1975. Although these methods adopt different protection groups for the amino groups, they use similar strategies: a resin support, excess coupling reagents, and building the peptide in a C→N terminal direction. The resin supports are swollen at the start in solvents and expand to many times their original size. Thus, the reactions don't occur on the surface of a rigid particle, but within the solvated gel that the resin forms. Swelling permits easy access to the growing peptide chain. Reagents are used in excess, which drives the reactions to completion in the minimum time, resulting in faster synthesis of peptides and reducing side reactions or racemization. Proceeding in a C→N terminal direction allows use of racemization-limiting amine protection for the activated species.

1. 6. 1 Activation and coupling[35,36]

Effective coupling requires chemical activation of the carboxyl group of an N-protected α-amino acid. There are currently four major types of activating techniques, including use of acid halides, preformed symmetrical anhydrides, active esters and *in situ* activating reagents. Active esters and *in situ* reagents are the most commonly used coupling techniques for solid-phase synthesis.

A pentafluorophenyl amino acid ester (Opfp) is a pre-activated amino acid, and can be stored and placed in the synthesizer during coupling. By contrast, an OBt ester formed from DCC and HOBt, BOP, PyBOP, TBTU or HATU *in situ* (Figure 1-15) reacts so fast that it can't be isolated.

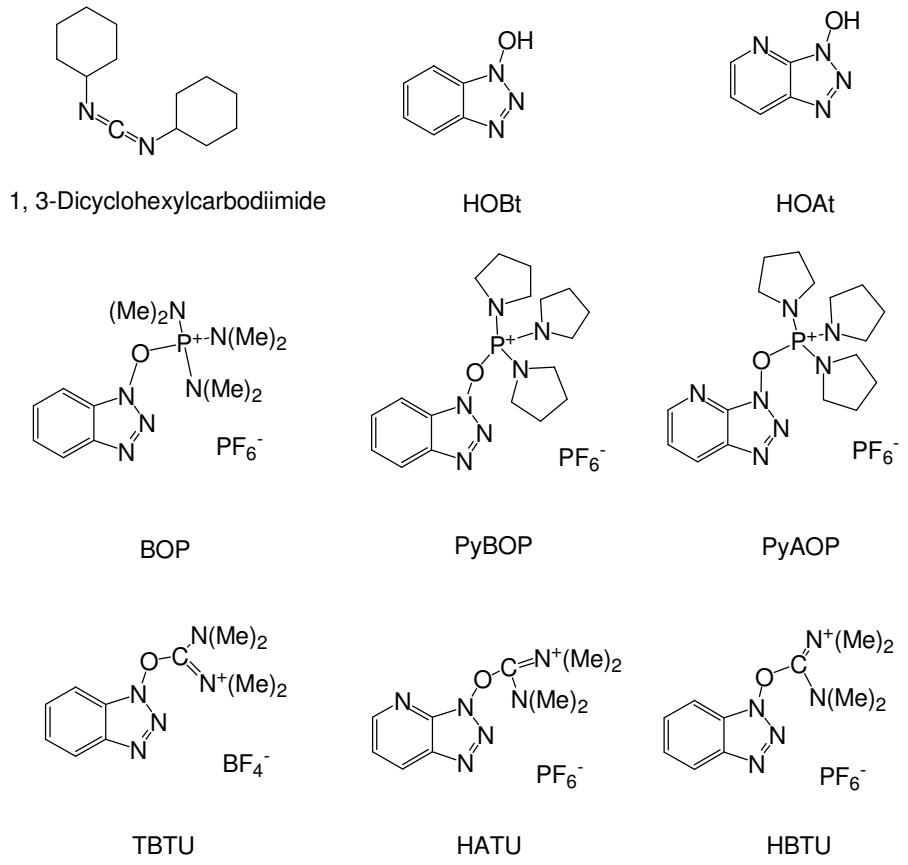


Figure 1-15 Some common coupling reagents.

In situ reagents are mixed with the amino acid derivative in the synthesizer, just prior to coupling. *In situ* reagents include DCC, BOP, PyBOP, HATU, TBTU, and PyAOP. Figure 1-16 describes the mechanism of peptide bond formation through DCC activation. HOBt and HOAt are used as the coupling additive here. The use of additives can inhibit side reactions and reduce racemization.

More recently, HOAt has been found to be a more efficient additive that shortens coupling time and reduces the loss of chirality. The uronium and phosphonium salts are the most common coupling reagents, which in the presence of a tertiary base can smoothly convert protected amino acids to a variety of activated species. The most

common coupling reagents – BOP, PyBOP, HBTU and TBTU – generate HOBt esters. HATU is the uronium salt of HOAt, and has shown to be superior to other *in situ* reagents; it is used for the synthesis of difficult peptides (e. g., hydrophobic peptides, peptides containing hindered amino acids), and for assembly of peptide libraries. PyAOP, the phosphonium salt of HOAT, provides enhanced coupling activity and can be used as a direct substitute for BOP or PyBOP. It is beneficial in synthesis of cyclic peptides and for the addition of the first amino acid onto a hydroxyl resin.

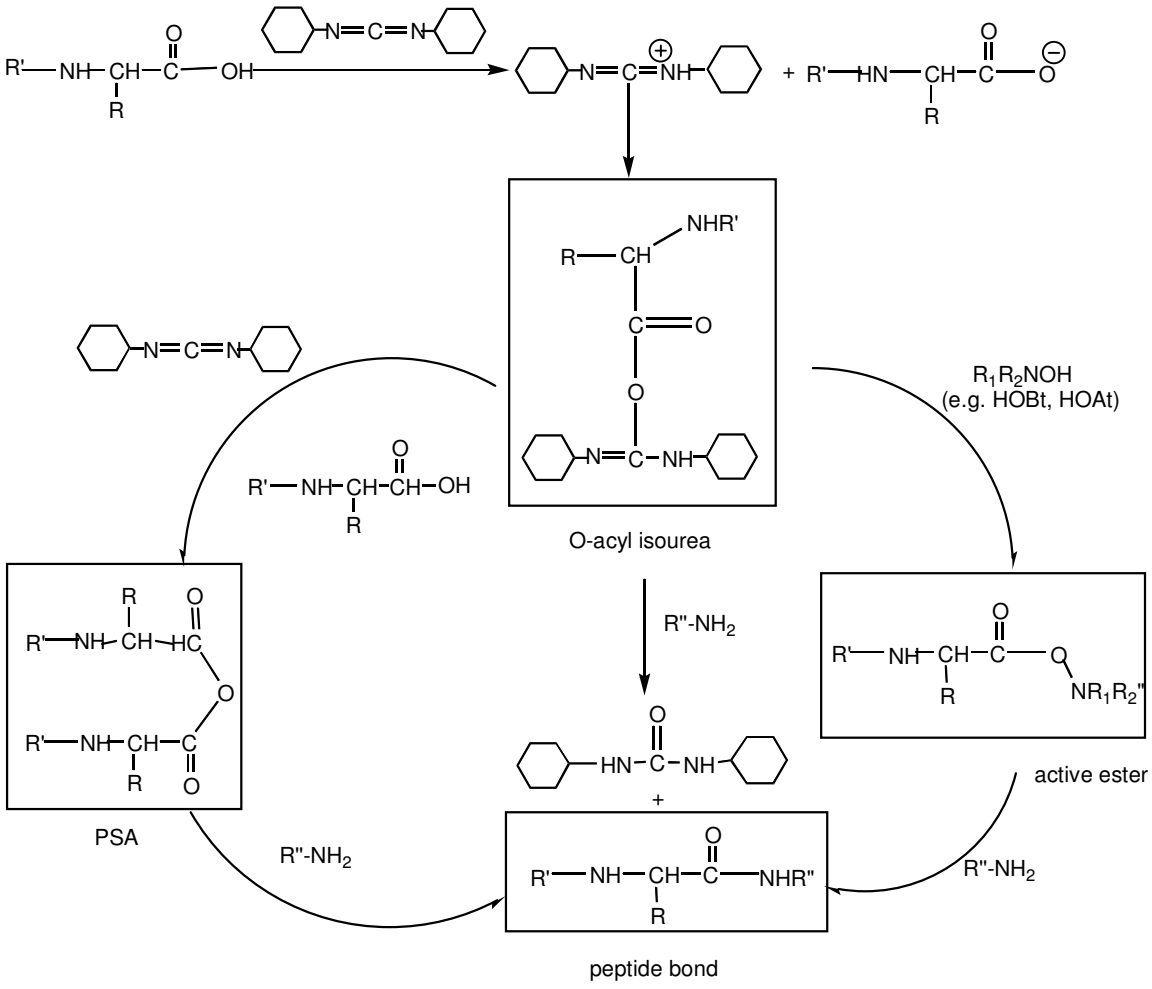


Figure 1-16 The mechanism of activation and coupling through DCC.

1. 6. 2 Merrifield synthesis (Boc-strategy solid-phase peptide synthesis)[37]

This methodology is characterized by use of *tert*-butyl based amino group protection (Figure 1-17). The first-loaded N-Boc amino acid is covalently attached to the resin, e.g., PAM resin. The use of PAM resin takes advantage of the fact that the presence of the electron-withdrawing 4-(aminoacyloxymethyl)phenylacetamidomethyl group increases the stability of the peptide ester in 50% TFA/CH_2Cl_2 during deprotection.

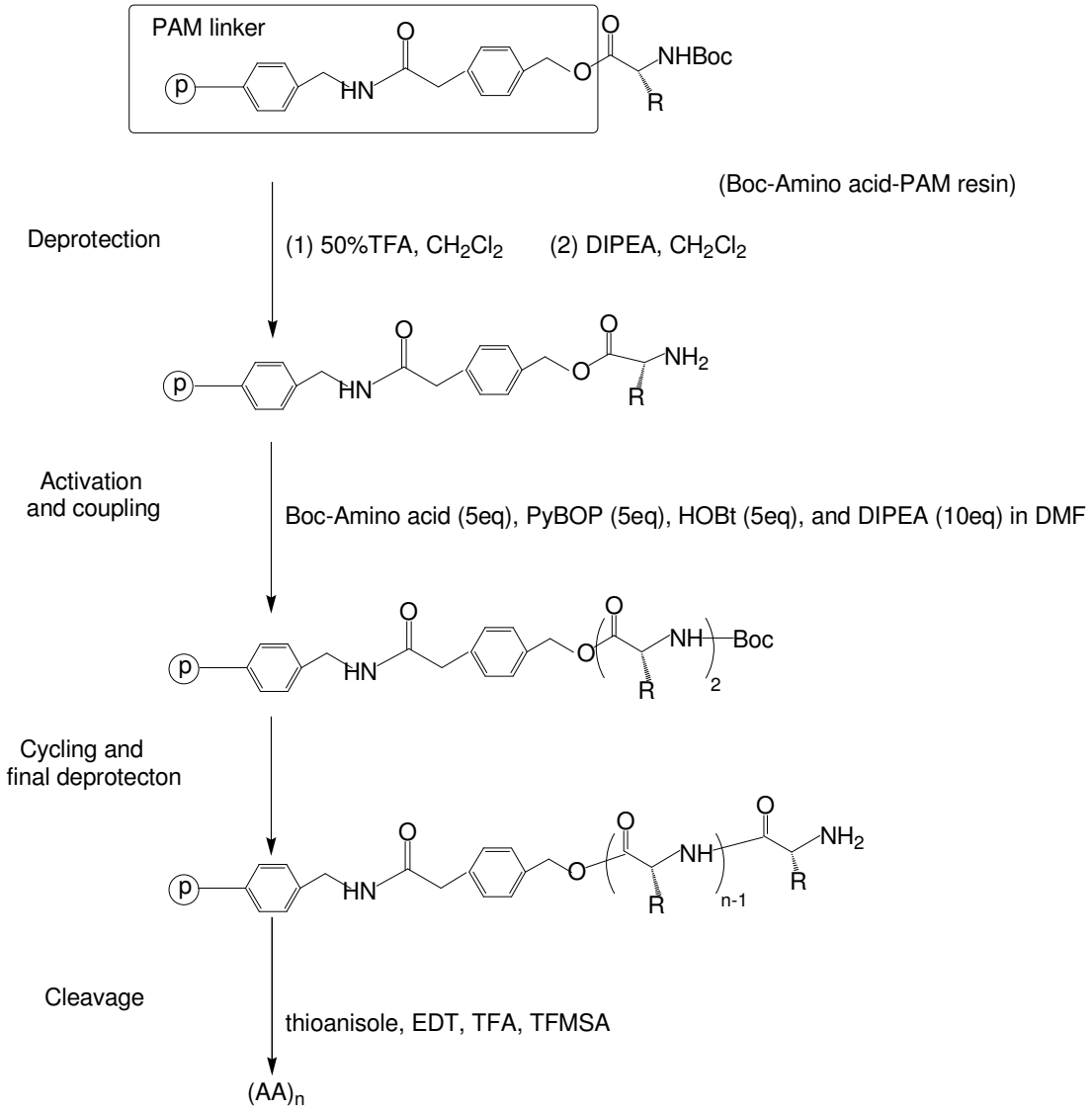


Figure 1-17 General pathway for BOC-strategy solid-phase peptide synthesis.

Boc-deprotection is followed by neutralization of the resulting ammonium salt with a hindered tertiary base, such as diisopropylethylamine (DIPEA) or triethylamine (TEA). The next N-Boc amino acid is activated and coupled with the resin-bound amino acid to yield an N-Boc dipeptide. The Boc-group is removed again, and the peptide chain continues to extend until completion of desired sequence. Before final cleavage of the target peptide from the resin, all side-chain protecting groups are removed. The anchoring ester bond between peptide and resin is broken by strong acids, usually HF or trifluoromethanesulphonic acid (TFMSA). Such procedures may require special apparatus (for HF) or scavenger reagents (for TFMSA) because the highly acidic conditions catalyze several possible rearrangements. The crude peptide is released and further purified by HPLC.

1. 6. 3 Fmoc-strategy solid-phase peptide synthesis[37]

The Fmoc-strategy arises from the adoption of the base labile Fmoc protecting group for α-amino group protection (Figure 1-18). Wang resin, one of the most common resins, consists of polystyrene beads onto which the acid-labile *p*-hydroxybenzyl alcohol linker is attached. As a result of the electron donating *para* oxygen atom stabilizing the resultant carbocation, cleavage of the peptide from the resin occurs under rather mild acid conditions, typically TFA in the presence of scavengers.

1. 6. 4 Comparison of Boc- and Fmoc-strategies

In the Boc-strategy, repetitive TFA acidolysis to remove Boc protecting groups may lead to acid-catalyzed side reactions. Cleavage of the peptide from the resin requires the

use of strong acids, which may lead to more side products, reduce the purity of the desired peptide and complicate the purification. The advantage of the Fmoc-strategy is that the growing peptide is subjected to mild bases such as piperidine during deprotection; TFA is required only for the final cleavage. In addition, the progress of each coupling and deprotection step can be monitored by measurement of the absorbance of the cleaved Fmoc group at 300-320 nm.

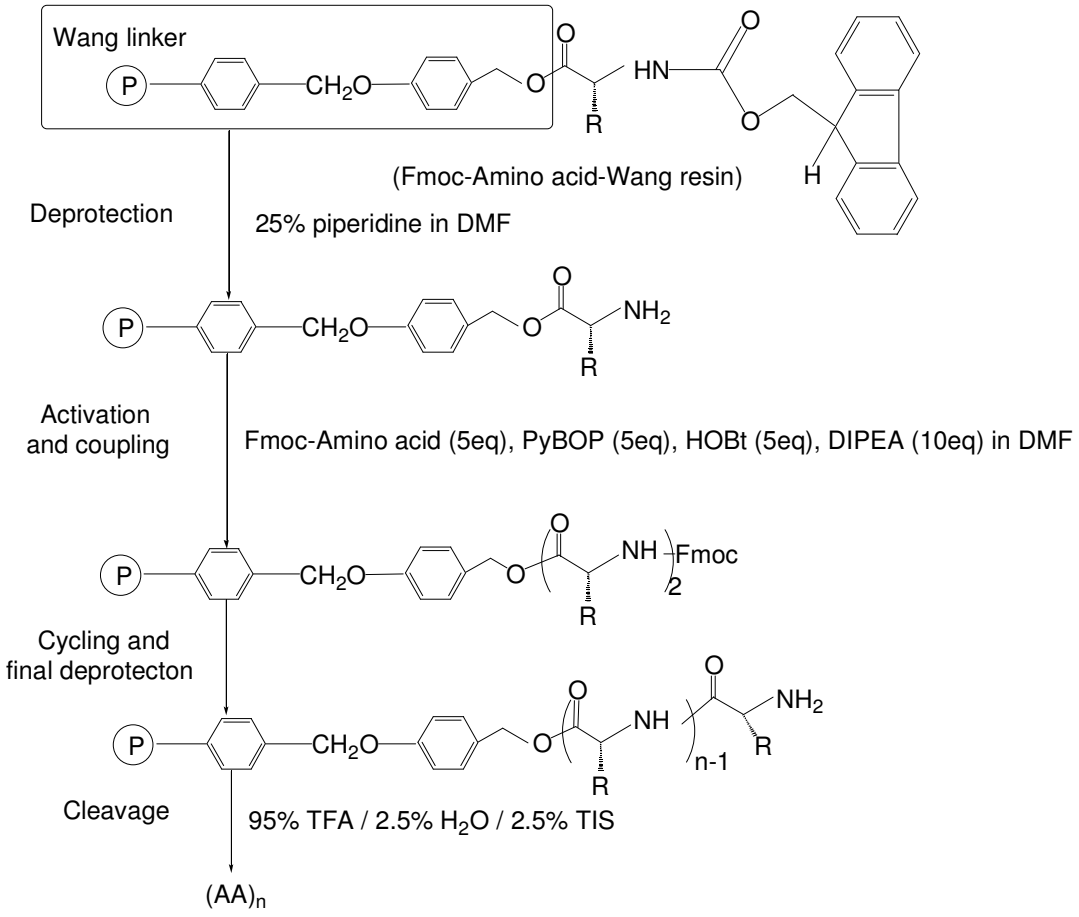


Figure 1-18 General pathway for Fmoc-strategy solid-phase peptide synthesis.

1. 6. 5 Cyclic peptides and peptide cyclization[38]

There are two types of cyclic peptides: homodetic and heterodetic. In homodetic cyclic peptides, the amino acid residues are connected only by the usual amide linkage between amino and carboxylic acid groups, whereas the presence of other linkages such as lactone, ether, thioether, or the disulfide bridge make a peptide heterodetic.

- **Cyclization in solution**

In general, cyclic peptides are synthetically challenging.[39] Cyclic peptides are more difficult to synthesize than linear oligomers, since the desirable intramolecular cyclization may be replaced by the competitive linear peptide chain propagation or intermolecular cyclization, unless a particularly stable ring is formed or specific molecular auxiliaries are employed.

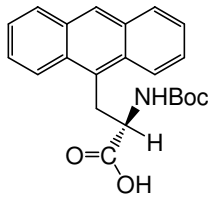
The protected peptide must be cleaved from the solid support before cyclization in solution is attempted. The cyclization conditions are similar to those used in coupling of the linear peptide. The only difference is that very dilute concentrations of the linear peptide (usually, 0.5-1 mM) and coupling reagents (0.5-3 mM) are used in order to minimize the formation of the cyclodimer and oligomers. Linear dimerization, cyclodimerization and cyclooligomerizations may still occur even under high dilution as side reactions. Generally, it is necessary to isolate the desired peptide from excess reagents and side products by HPLC to obtain the product with good purity.[5,40]

- **Cyclization on a resin**[8,41]

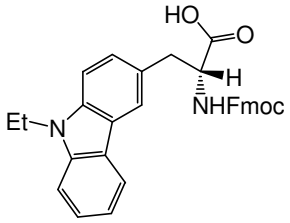
On-resin cyclization requires that two reactive groups, an amino and a carboxylic acid group, not be directly attached to the resin and that another functional group on the side chain is used to make the covalent link to the resin. This set of characteristics makes it possible to cyclize the linear peptide on the solid resin, where pseudodilution is achieved because the solid support provides a large distance between the different peptide molecules. Thus intramolecular is preferred over intermolecular cyclization.

Chapter 2 Synthetic Targets and Procedures

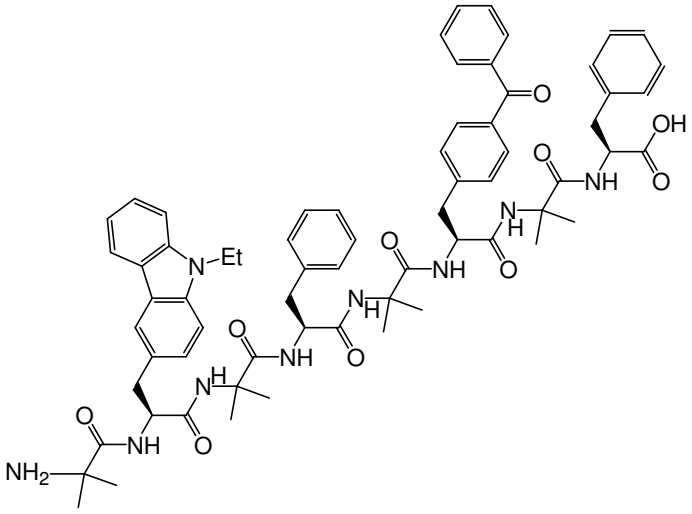
The chromophore-modified chiral amino acids that we designed and synthesized are N-Boc-3-(9-anthryl)-L-alanine (Boc-An-Ala, **7**) and N-Fmoc-3-(N-ethyl-3-carbazolyl)-L-alanine (Fmoc-Car-Ala, **25**). Fmoc-Car-Ala was to be incorporated into an 8-mer bichromophoric linear peptide with benzophone as the second chromophore (H-Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe-OH, **28**). This linear peptide was then cyclized to the corresponding cyclic peptide, *cyclo*(Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe, **29**) (Figure 2-1).



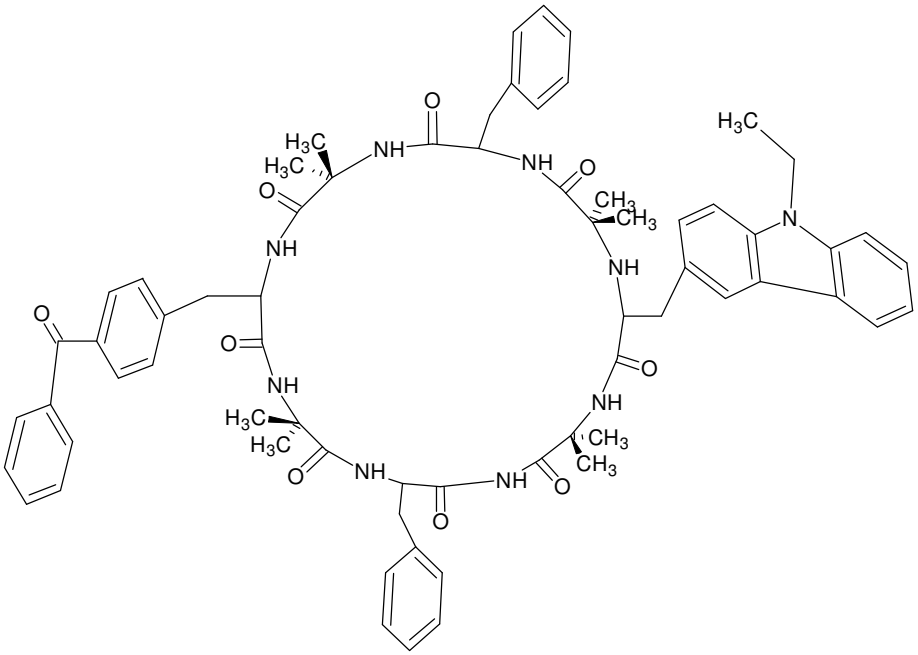
Boc-An-Ala, **7**



Fmoc-Car-Ala, **25**



H-Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe-OH, **28**



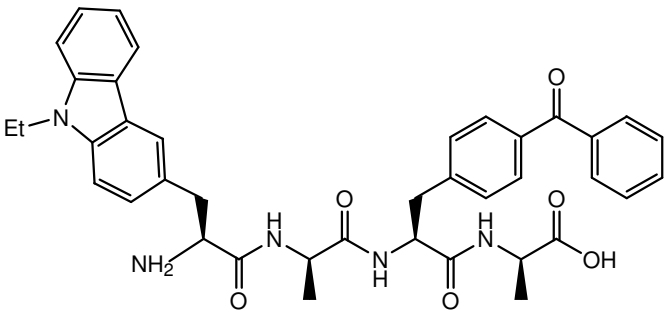
Cyclo(Phe-Aib-Car-Aib-Phe-Aib-Bpa-Aib), **29**

Figure 2-1 Our intermediates and target products.

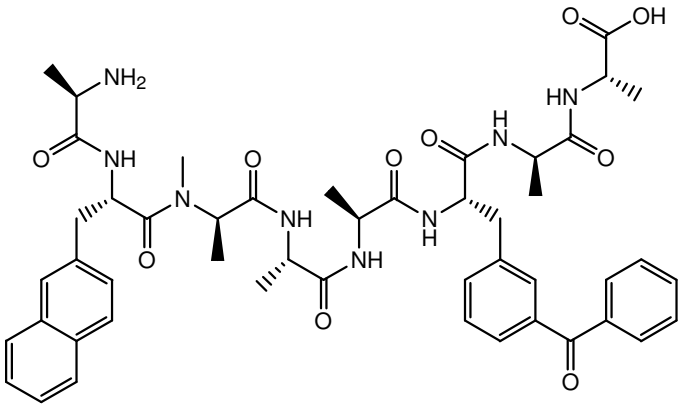
We decided to resolve the D,L-amino acids by enzymatic hydrolysis to secure a high enantiomeric excess. The Sorensen method, Erlenmeyer's azlactone method and the Wittig-Horner reaction were each tried in our attempts to build the amino acid skeletons.

For peptide synthesis, suitable protection of amino or carboxyl group is necessary. We attempted two most common amino group transformations, Boc or Fmoc protection, depending on the strategy for peptide synthesis that we chose, and methyl esterification for C-protection.

Solid-phase peptide synthesis was carried out. Two model peptides were initially synthesized, one by Boc-strategy, and the other by Fmoc-strategy, to seek the optimal conditions before we started with the target peptide (Figure 2-2).



H-Car-D-Ala-Bpa-D-Ala-OH, **26**



H-D-Ala-Nap-D-MeAla-Ala-D-Ala-Bpa-D-Ala-Ala-OH, **27**

Figure 2-2 Two model linear peptides.

2. 1 Synthesis of N-Boc-9-anthryl-L-alanine

Sisido had reported the synthesis of N-Ac-3-(9-anthryl)-DL-alanine by the Sorensen method (Figure 2-3).[20,42,43] First, sodium ethoxide deprotonated the active hydrogen from diethyl acetamidomalonate. The carbanion formed attacked 9-chloromethylanthracene to give an alkylated diester, which was hydrolyzed and decarboxylated.

Erlenmeyer's azlactone route is not applicable for this compound, because reduction of the exocyclic double bond in the intermediate also leads to reduction at the anthracene 9, 10-positions (Figure 2-4).[22]

We chose Sisido's pathway for synthesis of N-Ac-3-(9-anthryl)-DL-alanine (Scheme 3-1), except for small adjustments made following David Ferguson's procedure for synthesis of 3-(2-fluorenyl)-DL-alanine.[44] NaH was used as the base instead of sodium due to ease of handling of the former.

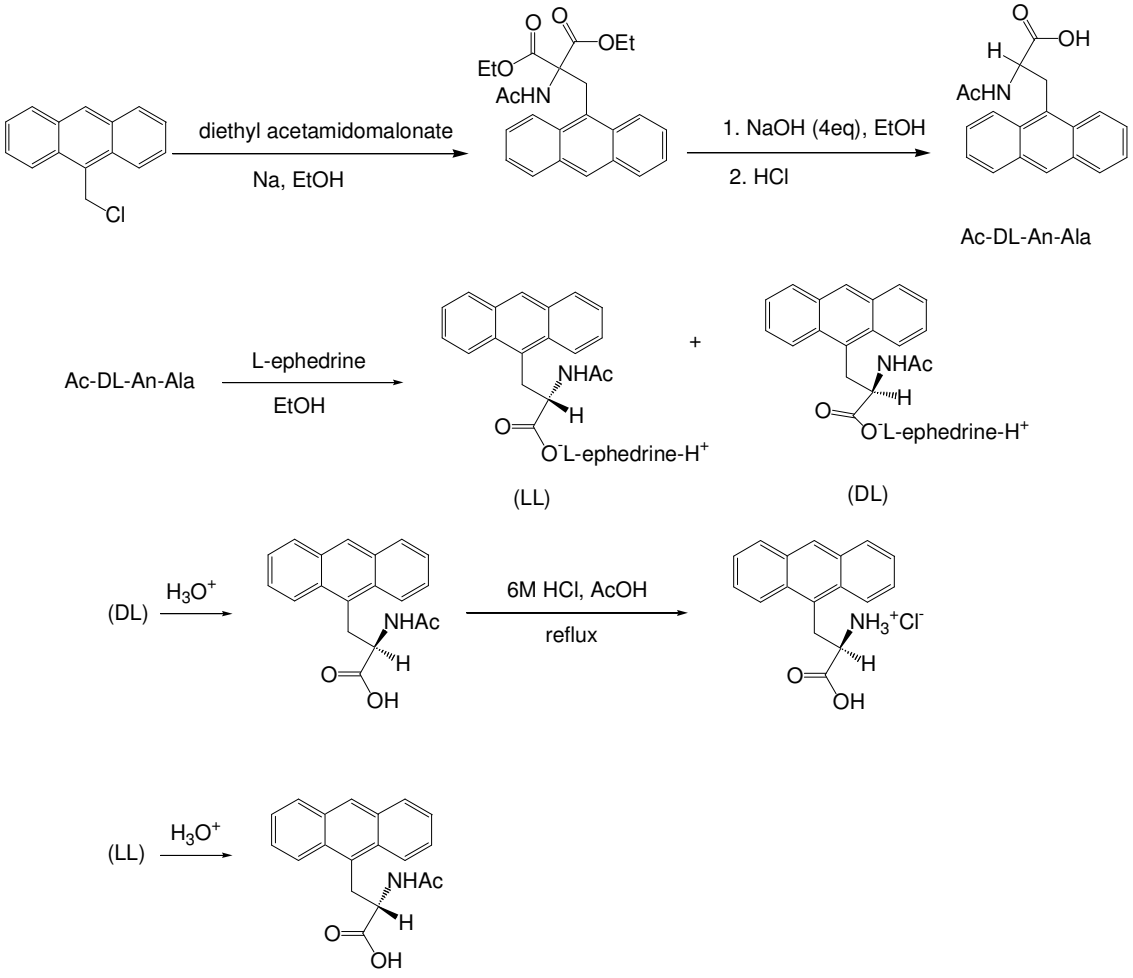


Figure 2-3 The pathway for synthesis of Ac-L-An-Ala and D-An-Ala via Sorensen method by Sisido.[20]

According to Sisido's report, acylase failed to remove the acetyl group from N-Ac-3-(9-anthryl)-DL-alanine.[20] Nestor also reported that enzymatic hydrolysis didn't work for N-Ac-3-(9-anthryl)-DL-alanine methyl ester and the derivative of 3-(9,10-dihydro-9-anthryl)-DL-alanine.[22] The major reason is the bulkiness and hydrophobicity of the 9-anthryl group. Sisido used a chiral auxiliary, L-ephedrin, that can form salts with N-Ac-DL-amino acids. The diastereoisomeric products were separated by stepwise precipitation, and then decomposed into the two enantiomers, whose methyl ester derivatives were identified by NMR with the aid of a chiral shift reagent.[20,42] The same experiment was repeated in our lab, but good resolution couldn't be achieved because the compound didn't dissolve well in hot EtOH as reported, or in other solvents, such as acetone, CH_2Cl_2, $CHCl_3$, EtOAc, and MeCN.

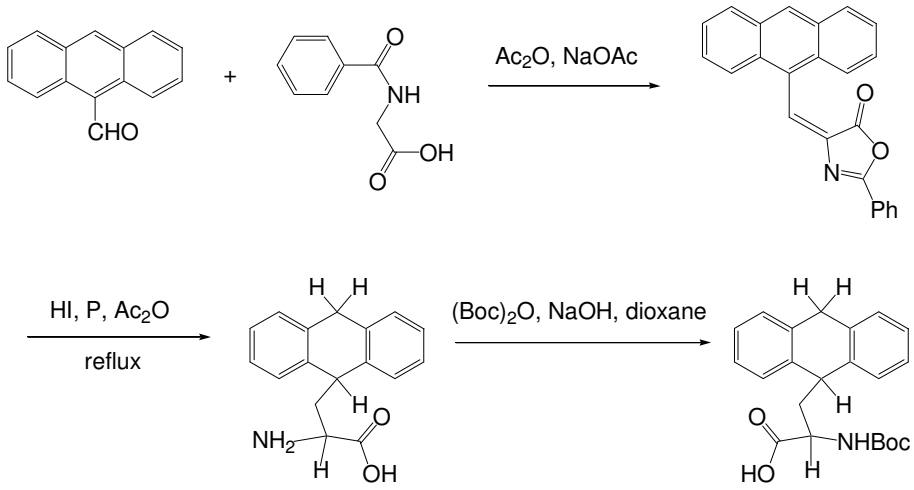


Figure 2-4 The pathway for synthesis of N-Boc-3-(9,10-dihydro-9-anthryl)-DL-alanine via the Erlenmeyer azlactone method by Nestor.[22]

Before enzyme-catalyzed ester hydrolysis, methyl esterification is an indispensable step. BF_3•Et_2O was chosen as the catalyst for this transformation instead of conc. HCl, to avoid the introduction of any water that might affect the yield. In addition, two other methods for methyl esterification were attempted in order to seek higher yields (Figure 2-5). Thionyl chloride[18] and trimethylsilyldiazomethane[45] were used as the reactive reagents; the reaction involving the latter proceeds in a shorter time at room temperature.

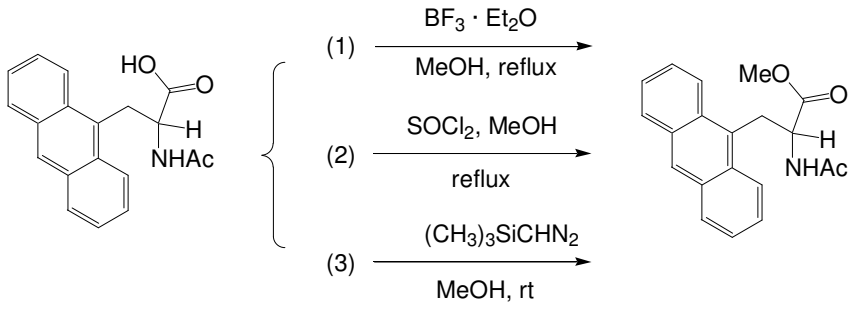


Figure 2-5 Different methods for methyl esterification of N-Ac-3-(9-anthryl)-DL-alanine.

Subtilisin ChiroCLEC-BL and acylase from *aspergillus melleus* are two powerful enzymes. The former efficiently removed the methyl group from N-Ac-3-(2-fluorenyl)-DL-alanine methyl ester (Figure 2-6(a)),[44] while the latter removed the acetyl group from N-acetyl-3-(2-anthraquinonyl)-DL-alanine (Figure 2-6(b)).[30] In our substrate, C-9 of the anthryl group is attached to C-3 of the alanine, which may be more sterically hindered than having C-2 of the fluorenyl group attached to C-3. Moreover, the solubility of the 9-anthryl derivative is much poorer than that of the 2-fluorenyl one in acetone or other organic solvents. As for the two anthryl derivatives, one is substituted at C-9, the other at C-2. The steric hindrance is probably greater for the first case. Since the stereochemical interaction between enzyme and substrate will determine the hydrolytic efficiency, higher

steric hindrance and poor solubility of the substrates may make the resolution slower and selectivity poorer.

We adopted both these enzymes to sequentially remove the two protective groups from our substrates. The dual enzymatic resolutions simplify the purification because the D-isomer has been removed before deacylation, securing high enantiometric excess and avoiding the acid-catalyzed side reactions during normal deacylation that take place in conc. HCl/AcOH under lengthy reflux.

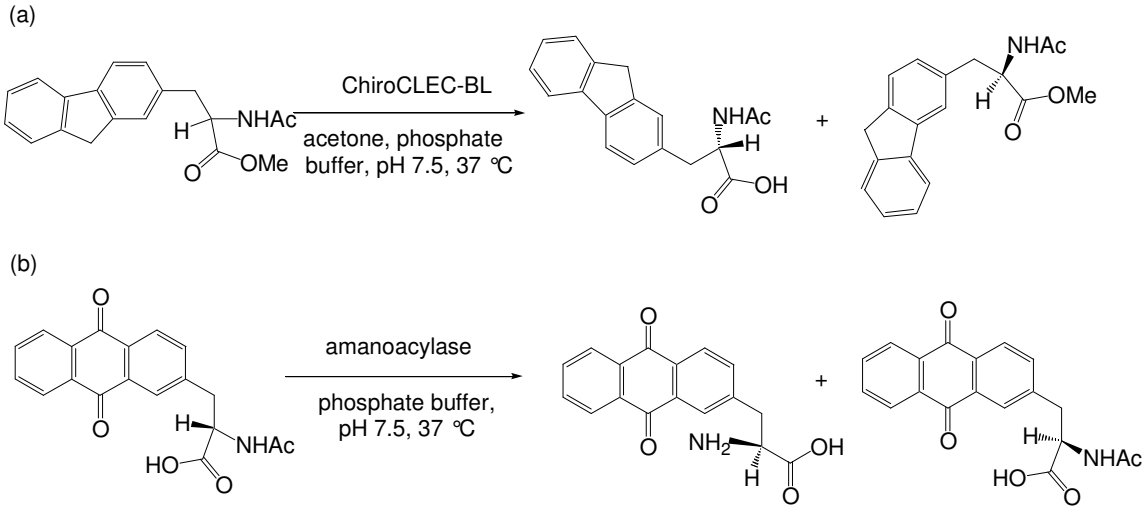


Figure 2-6 **(a)** Ester hydrolysis catalyzed by ChiroCLEC-BL;

(b) deacylation catalyzed by *aspergillus* acylase.

The normal conditions for Boc protection of the free amino group, like that in L-proline, is shown in Figure 2-7. When these conditions are applied to sterically hindered amino acids, only 40-60% yields have been obtained.[18] For Boc protection of 3-(9-anthryl)-L-alanine, DMF was chosen as the solvent to enhance solubility of the starting

material.

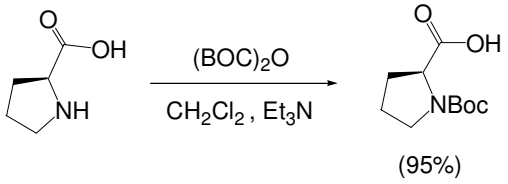


Figure 2-7 Boc-protection of L-proline.

An alternative synthetic scheme was designed to directly make the N-Boc amino acid derivatives,[46,47,48] where N-Boc-amidomalonate replaced its acetyl derivative to give an N-Boc diester (Figure 2-8a). ChiroCLEC-BL can be used to remove the methyl ester

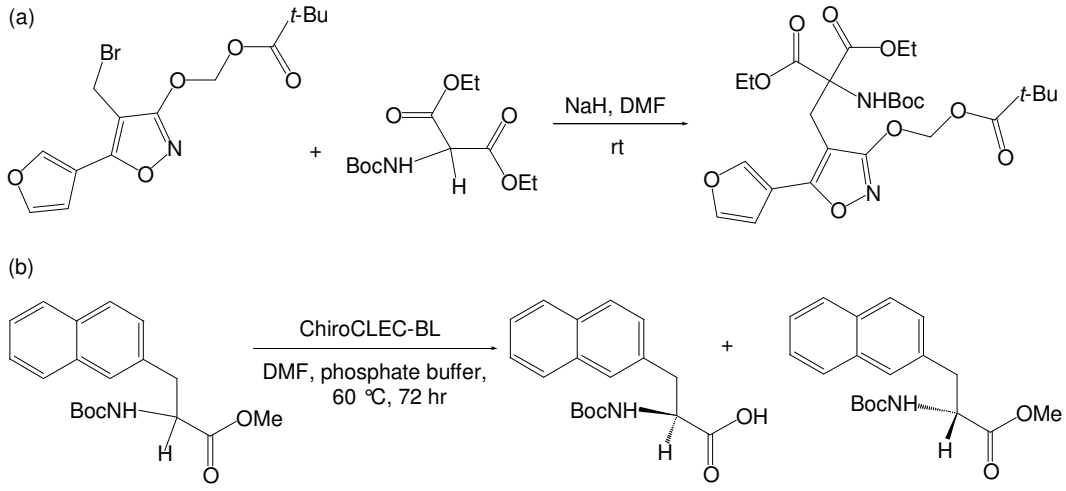


Figure 2-8 **(a)** Modified Sorensen method to directly generate an N-Boc diester;

(b) ester hydrolysis of N-Boc-3-(2-naphthyl)-L-alanine methyl ester catalyzed by ChiroCLEC-BL.

from N-Boc derivatives, according to Wang's report that ChiroCLEC-BL hydrolyzed N-Boc-3-(2-naphthyl)-L-alanine methyl ester (Figure 2-8b).[28] We

attempted to synthesize N-Boc-3-(9-anthryl)-DL-alanine methyl ester first, followed by ester hydrolysis via the catalysis of ChiroCLEC-BL (Scheme 3-2).

2. 2 Synthesis of 3-(N-ethyl-3-carbazolyl)-L-alanine and its derivatives

In my initial attempt to obtain 3-(N-ethyl-3-carbazolyl)-DL-alanine via the Sorensen method, the desired intermediate, N-ethyl-3-chloromethylcarbazole, could not be obtained from $SOCl_2$ and the corresponding alcohol (Figure 2-9). Instead, an unexpected blue side product was observed.

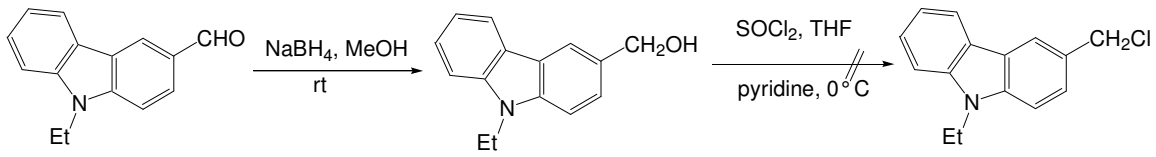


Figure 2-9 Unsuccessful pathway to N-ethyl-3-chloromethylcarbazole.

Taku reported the preparation of 3-(N-ethyl-3-carbazolyl)-DL-alanine by the azlactone route (Figure 2-10).[23] We repeated the synthesis (Scheme 3-3), but a major problem was met during removal of the benzoyl group by refluxing with conc. HCl/AcOH. It will be discussed in detail later.

An alternative approach to removing the benzoyl group was attempted, where the oxazolone is treated with red P, Ac_2O and aq. HI. The mixture was heated to 140 °C (Figure 2-11). The reactions, involving reduction, ring-opening, and deacylation took place simultaneously in one pot.

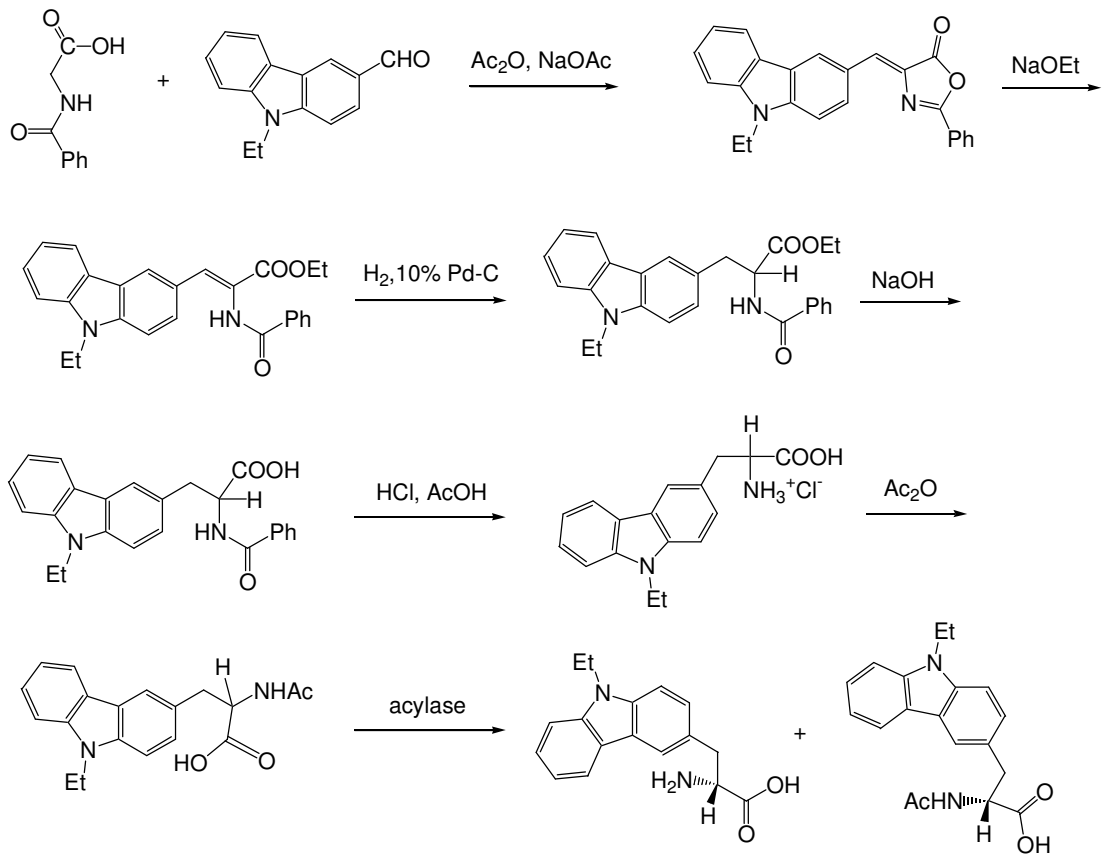


Figure 2-10 Erlenmeyer azlactone route to produce 3-(N-ethyl-3-carbazolyl)-DL-alanine by Taku.[23]

The Wittig-Horner reaction provides mild conditions to make N-protected dehydroamino acid methyl esters,[49,50] and was our next choice for synthesis of 3-(N-ethyl-3-carbazolyl)-DL-alanine (Scheme 3-4).

Figure 2-11 Reduction, hydrolysis and debenzoylation of 4-(3-(N-ethylcarbazolylidene)-2-phenyloxazol-5-one.

The Wittig-Horner reaction starts with an aromatic aldehyde and an N-protected phosphonoglycinate. N-Cbz-phosphonoglycinate is the only commercial derivative, which can be easily converted to Boc and Ac derivatives (Figure 2-12).[51] The Cbz group is removed first by catalytic hydrogenation, followed by Boc-protection. A one-step conversion has also been applied to transform the Cbz to the acetyl derivative. In the Wittig-Horner reaction of aldehydes with phosphonates, tetramethylguanidine is a common base used to create the carbanion, which attacks the carbonyl group and couples the two components together (Figure 2-13).[52]

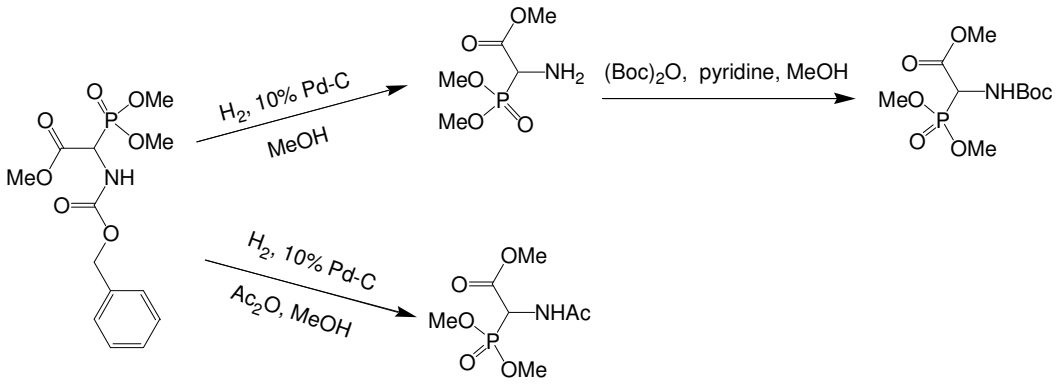


Figure 2-12 The conversion of an N-Cbz-phosphonoglycinate to its corresponding Boc or Ac derivatives.[51]

The hydrogenated product carrying two protecting groups, the N-protective group (N-Boc or N-Ac) and the methyl ester, would be ready for selective hydrolysis by the two kinds of the enzymes to provide the desired L-isomer.

For Fmoc protection, Fmoc-OSu was chosen as the Fmoc reagent to avoid side products, which may form when Fmoc-Cl is used. Many factors, such as solubility of the substrate, possible racemization, and deprotection under basic conditions had to be

considered in choosing the best conditions for protecting our substrate. Protection and deprotection seemed to be competitive reactions under basic conditions. In order to avoid deprotection of the product, fast reaction is necessary. Strong base may cause both deprotection and racemization of the chiral amino acids, so the pH is generally kept between 9 and 11 (Figure 1-13).

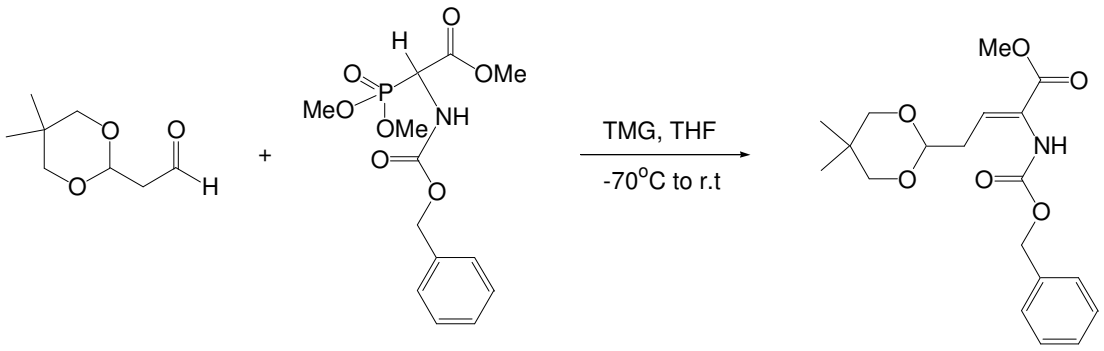


Figure 2-13 Synthesis of methyl 2-Cbz-5,5-(2,2-dimethylpropane-1,3-diyldioxy)pent-2-enoate by the Wittig-Horner reaction with a phosphonoglycinate.[52]

2. 3 Syntheses of polypeptides[35,36]

The major task of this research was to incorporate the chromophore-modified amino acids into the peptide skeletons. Before building the target linear peptide **28** and cyclic peptide **29**, two model peptides were synthesized by Boc-strategy SPPS and Fmoc-strategy SPPS, respectively, in order to compare the feasibility of two synthetic methods.

- **4-Mer model peptide obtained by Boc-strategy SPPS (26)**

For the 4-mer model peptide synthesis via the Boc-strategy, there are a number of common conditions that apply to all steps of the same procedure. As we have discussed

above, PAM resin was chosen for Boc-strategy syntheses.

Swelling: N_2 bubbling in 10 mL of CH_2Cl_2 with 0.2-1 g of resin.

Deprotection: use of 50% TFA/CH_2Cl_2 for 30 min, twice. The end point is signaled by a positive ninhydrin test.

Washing after deprotection: washing with CH_2Cl_2, neutralizing with 20% DIPEA in CH_2Cl_2, and washing with DMF, MeOH, and CH_2Cl_2.

Activation and coupling: adding 5 eq N-Boc amino acid, 5 eq PyBOP, 5 eq HOBT, and 10 eq DIPEA dissolved in DMF. The coupling reagents are poured onto the resin with N_2 bubbling for the required time period. A negative dye test signals the completion of coupling. If the dye test is positive, one repetition of the coupling step may be required.

Washing after coupling: washing with DMF, MeOH, EtOH and CH_2Cl_2.

Final washing before cleavage: washing with DMF, CH_2Cl_2, MeOH, and EtOAc and drying the resin in vacuum.

Cleavage: mixing the dry resin with thiolanisole, ethanedithiol, TFA and TFMSA and stirring for one hour.

- **8-Mer model peptide by Fmoc-strategy SPPS (27)**

For the 8-mer model peptide synthesis via the Fmoc-strategy, some additional details need be mentioned. DMF was used instead of CH_2Cl_2, and no neutralization required.

Resin choice: Wang resin was chosen.

Swelling: N_2 bubbling in 10 mL of CH_2Cl_2 with 0.2-1 g of resin.

Deprotection: use of 25% piperidine in DMF for 30 min, twice. The Kaiser test is used to indicate the presence of primary amines, the chloranil test for secondary amines, and the TNBS test for highly hindered primary amines.

Washing: washing with DMF, CH_2Cl_2, and DMF. No neutralization is required.

Activation and coupling: same as above, but for difficult sequences, extension of the reaction time, or more powerful coupling reagents are used (HOAt and PyBOP or HOAt and HATU).

Cleavage: use of TFA/H_2O (95:5) for two hours. Scavenging reagents may be required, depending on the amino acids present.

- **Linear target peptide (28)**

Based on the results of the syntheses of two model peptides, an improved synthetic strategy was designed to achieve the target peptide.

- **Cyclic target peptide (29)**

Cyclization was carried out according to Ghadiri's procedure for an 8-mer cyclic peptide, *cyclo*(-L-Phe-D-*N*-MeAla-)$_4$.[5] The cyclization was done in DMF (1 mM substrate) at 0 °C in the presence of 3 eq HATU, 3 eq HOAt, and 10 eq DIPEA to give a 70% yield of the cyclic peptide after further purification by RP-HPLC. HOAT and HATU were used in place of HOBT and PyBOP, which speeded up the cyclization.

Chapter 3 Results and Discussion

This chapter describes the syntheses and resolutions of two unnatural amino acids, and their use in several linear and cyclic peptides.

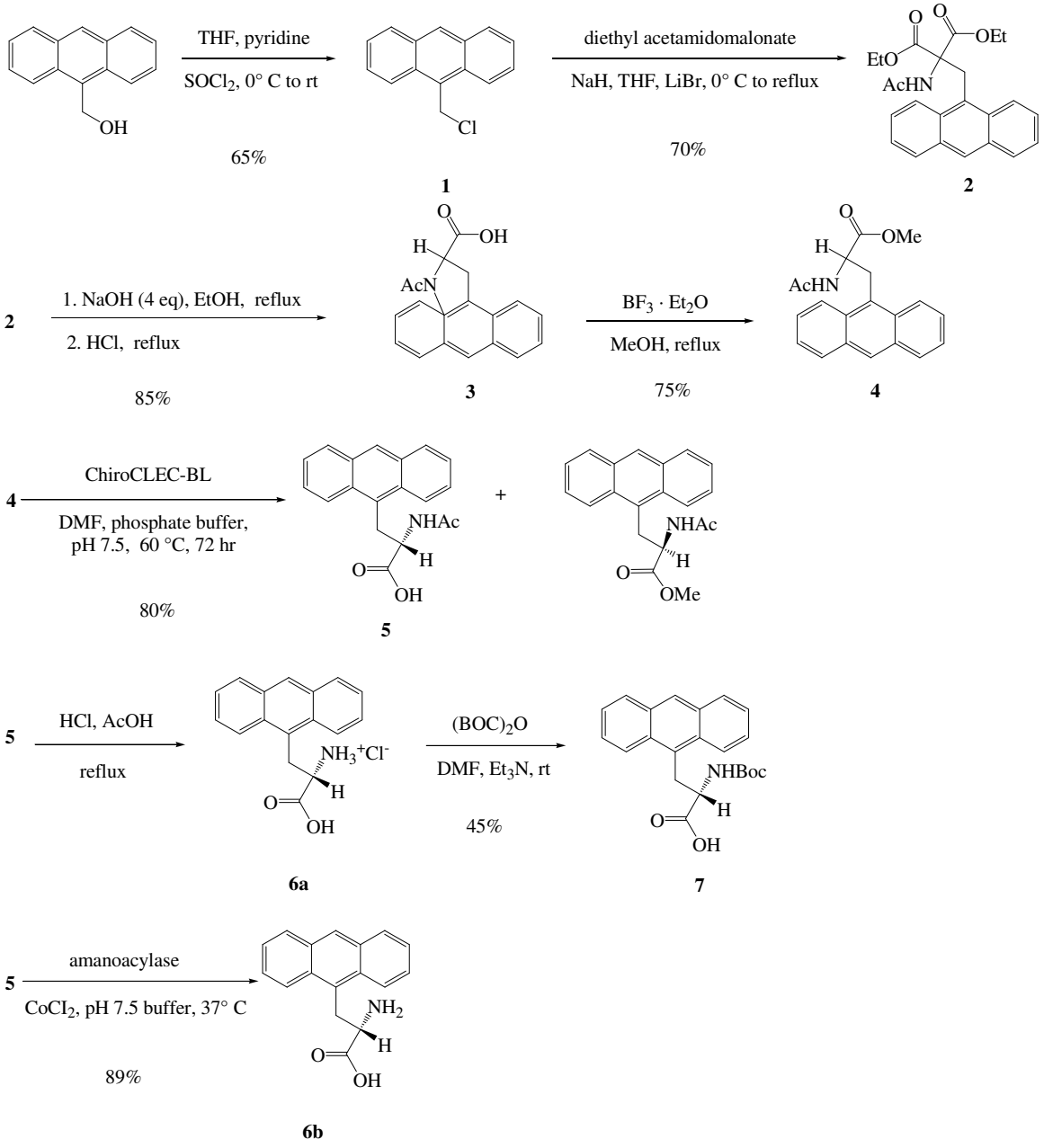
3. 1 Syntheses of Boc-3-(9-anthryl)-L-alanine

The Sorensen method was used to synthesize N-Ac-3-(9-anthryl)-L-alanine (**5**, Scheme 3-1). Many strategies were considered in order to increase the yield of alkylation of diethyl acetamidomalonate by 9-chloromethyl-anthracene. According to the SN_2 mechanism, extended reaction time, high concentrations of the starting materials, and elevated temperature increase the yield to some degree. The leaving activity of chloride is lower than bromide. Therefore, addition of a small amount of LiBr as catalyst transformed the chloride to a bromide intermediate, and sped up the conversion. The yield was increased to 70% from 39% as a result.

Enzymatic hydrolysis is the key step in this synthesis. Dual enzymatic resolutions were designed to remove the methyl group of **4** by ChiroCLEC-BL and the acetyl group of **5** by amanoacylase.

ChiroCLEC-BL failed to remove the methyl group from N-Ac-9-anthryl-L-alanine methyl ester (**4**) under routine conditions in acetone-phosphate buffer at 37 °C for four days with shaking, due to the bulkiness of the 9-anthryl group and poor solubility of this

Scheme 3-1



substrate. The temperatures and solvents were varied to seek the optimal hydrolytic conditions (Table 3-1). Finally, we achieved a good resolution in DMF at 60 °C for three

days. The conversion was 40% (yield 80%), which was calculated from NMR-7. There were two peaks assigned to the CH_3 groups of the acetyl groups, one belonging to **4** located at δ 1.69, the other belonging to **5** at δ 1.67. The ratio of the integrals was 6:4 (**4/5**, Figure 3-1).

Table 3-1 Experiments aimed at optimizing the enzymatic resolution of N-Ac-3-(9-anthryl)-DL-alanine methyl ester (**4**).

Experiment No.	Enzyme Types	Solvent	Temperature (°C)	Time (days)	Yield by TLC (%)
1	Protease	Acetone	37	2 4	~ 10% No obvious change on TLC
2	ChiroCLEC-BL	Acetone	37	2 4	~ 10% No obvious change on TLC
3	ChiroCLEC-BL	DMF	60	2 3 5	~ 60% ~ 80% No obvious change on TLC
4	ChiroCLEC-BL	Acetone DMF	60	2 4 5	~ 20% ~ 60% No obvious change on TLC
5	ChiroCLEC-BL	THF DMF	37 60 60	1 2 5 6 8	No obvious reaction ~10% ~ 60% ~ 70% ~ 70%

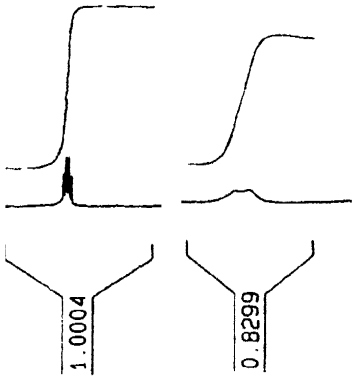
There are peaks due to unknown impurities appearing on NMR-9 (N-Ac-3-(9-anthryl)-L- alanine, **5**) and on NMR-6 (N-Ac-3-(9-anthryl)-DL-alanine methyl ester, **4**) after resolution. It was assumed that a small part of ChiroCLEC-BL might have dissolved in DMF after three days of enzymatic hydrolysis at 60 °C, or that it wasn't completely removed by centrifugation. The impurity wasn't observed on TLC, but it did contaminate the NMR spectrum.

The enantiomeric excess is commonly determined from NMR spectra with the aid of a chiral shift reagent. Tris[3-(trifluoromethylhydroxymethylene)-d-camphorato]europium (III) was used as the chiral reagent to resolve the signals from N-Ac-3-(9-anthryl)-DL-alanine methyl ester (**4**). A quartet assigned to CH showed up at δ 4.99-5.04 without the chiral reagent (NMR-6), while two broadened and low-intensity peaks overlapped each other at δ 5.89-6.12 after addition of the chiral reagent (NMR-8). There is no clear border between two peaks and so it is impossible to integrate the individual peaks to calculate the relative amount of each (Figure 3-2).

Figure 3-1 ^{1}H NMR CH_3 peaks of the acetyl groups assigned to compounds **4** (left) and **5** (right), respectively.

Next, deacylation was done in two ways: by heating **4** to reflux in strong acid and by using a powerful enzyme, amanoacylase. Hydrolysis under the first conditions did not

give a clean product — an unknown side product was observed by TLC, and black spots (which may come from decomposition of the impurity) were seen with the naked eye. Deacylation by amanoacylase was clean and efficient; 89% yield was observed by TLC after two days. The mild reaction conditions (pH 7.5 buffer, 37 °C) avoided racemization or acid-catalyzed side-reactions.



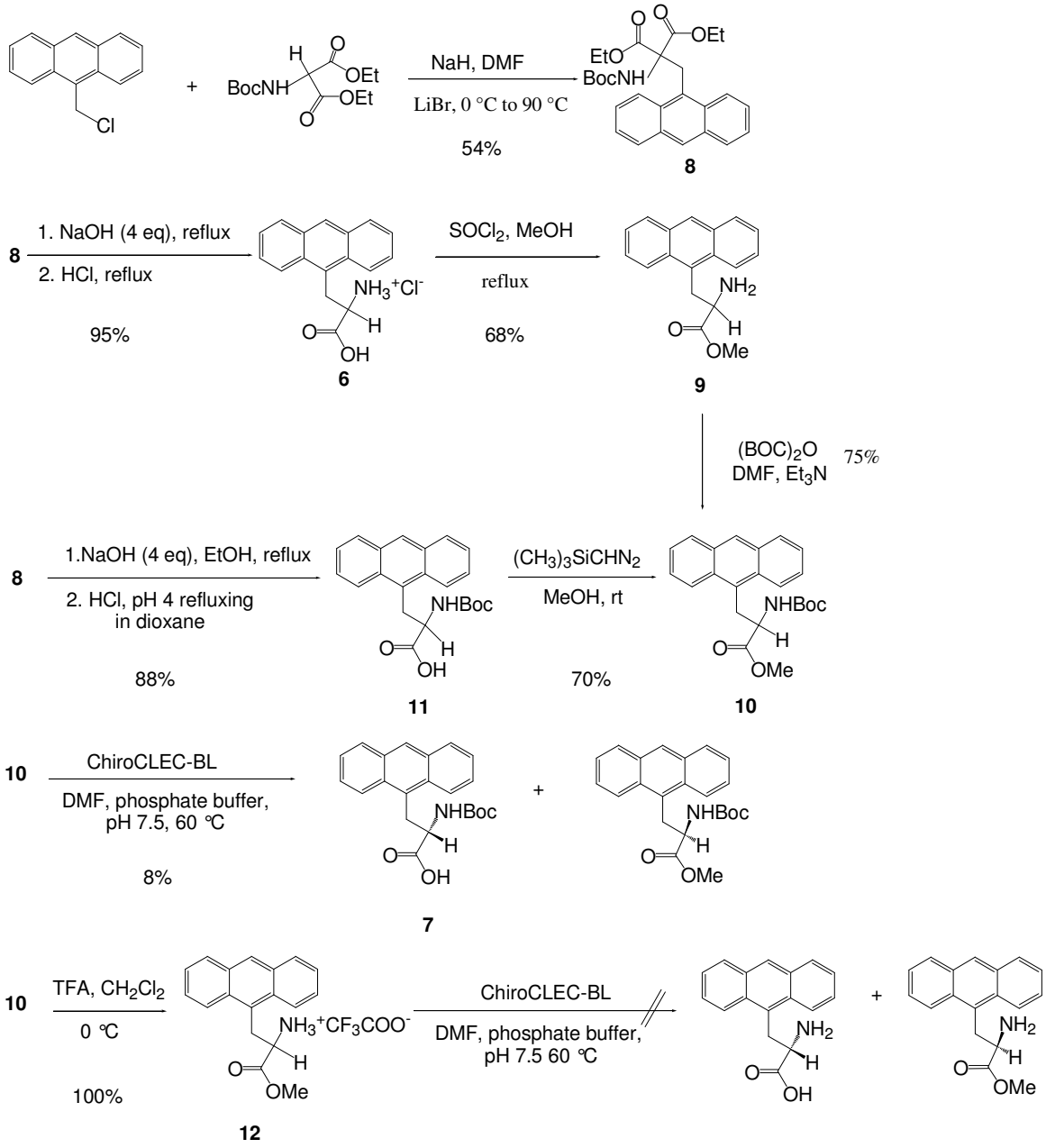
(a) (b)

Figure 3-2 The CH peak in the ^{1}H NMR spectrum of N-Ac-3-(9-anthryl)-DL-alanine methyl ester **(a)** without the chiral shift reagent; **(b)** with the chiral shift reagent. (sample/chiral shift reagent 5:3 mole ratio).

The overall yield for the process shown in Scheme 3-1 was 9.5%.

Scheme 3-2 represents an alternative route for synthesizing N-Boc-3-(9-anthryl)-L-alanine (7). When one carboxylic group was removed from the dicarboxylic acid by heating to reflux in a weakly acidic media overnight, the Boc group was also removed. The free amino group had to be Boc-protected again after methyl esterification.

Scheme 3-2



In order to protect the Boc group during decarboxylation, the pH was adjusted to 7 and the solution was heated to reflux in dioxane for five hours. The Boc group survived this

time. The following methyl esterification with trimethylsilyl-diazomethane gave a 70 % yield.

There is no big difference in yield for three methyl esterfication conditions that we have adopted. The advantage of using trimethylsilyldiazomethane was that the reaction proceeded rapidly at rt under acid-free conditions.The Boc protection step gave higher yields with the C-protected amino acid derivative (75%) than with the free amino acid (45%).

The enzyme-catalyzed hydrolysis of the Boc-protected esters became more difficult due to greater steric hindrance and poorer solubility. Different solvents (DMF, THF, dioxane), temperatures (37-60 °C), enzyme concentrations and time periods (up to five days) were tried and failed to bring about good resolution. After comparing the sizes of the spots assigned to N-Boc-3-(9-anthryl)-L-alanine (**7**) and N-Boc-3-(9-anthryl)-DL-alanine methyl ester (**10**) on TLC, it was estimated that about 20% of yield was achieved under one set of conditions (DMF, 60 °C, three days), but after further work-up, only 8% of the desired product was obtained. It is assumed that either the size of the spots on TLC didn't reflect the content of each component for this system, or that the applied work-up conditions were faulty.

This N-Boc-amino acid methyl ester didn't seem to be a good substrate for ChiroCLEC-BL. It may be due to its hydrophobicity or to the bulky Boc group that may affect complexation between the enzyme and its substrate. Another kind of enzyme, papain, was reported to be used for hydrolysis of the N-Boc-amino acid methyl ester, but the enantiomeric excess (80%) wasn't really satisfactory.[24] The same problem was met in enzymatic hydrolysis of N-Boc-3-(N'-ethyl-3-carbazolyl)-DL-alanine methyl ester (**21a**)

later. The enzymatic hydrolysis of 3-(N-ethyl-3-carbazolyl)-DL-alanine methyl ester also failed, and multiple side-products were displayed on TLC. This failure may be attributable to the weak bonding between this enzyme and its substrate.

3. 2 Attempted syntheses of 3-(N-ethyl-3-carbazolyl)-DL-alanine

The first attempt at synthesis of 3-(N-ethyl-3-carbazolyl)-DL-alanine by the Sorensen method failed in the second step, where N-ethyl-3-hydroxymethylcarbazole (**13**) was expected to be converted to the chloride by $SOCl_2$. The solution turned blue when it was warmed to rt after addition of $SOCl_2$. Figure 3-3 suggests the potential side reactions under these conditions.

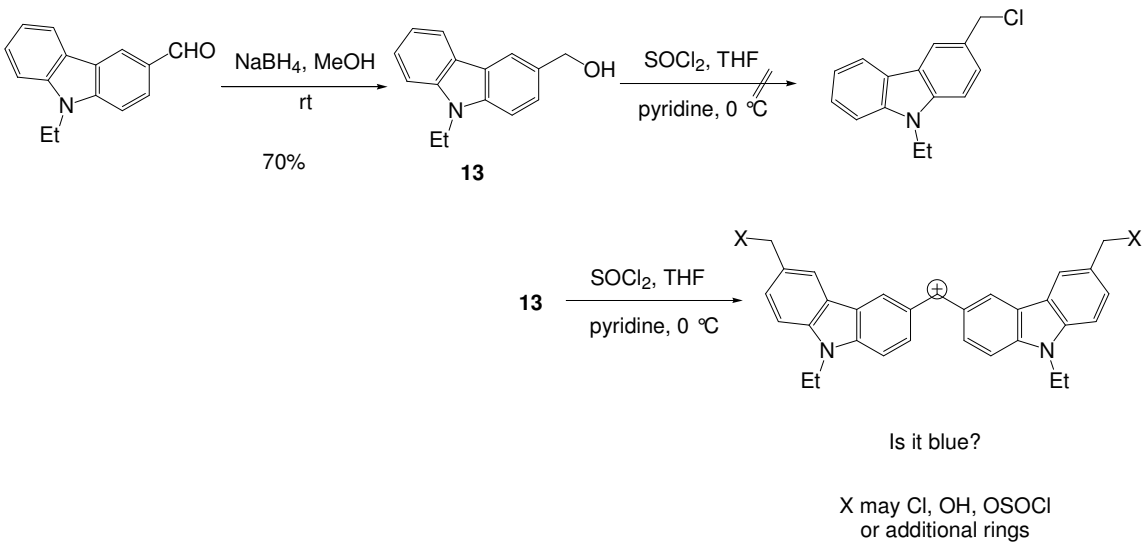


Figure 3-3 Potential side reactions in the transformation from a carbazolylmethanol to the chloride derivative.

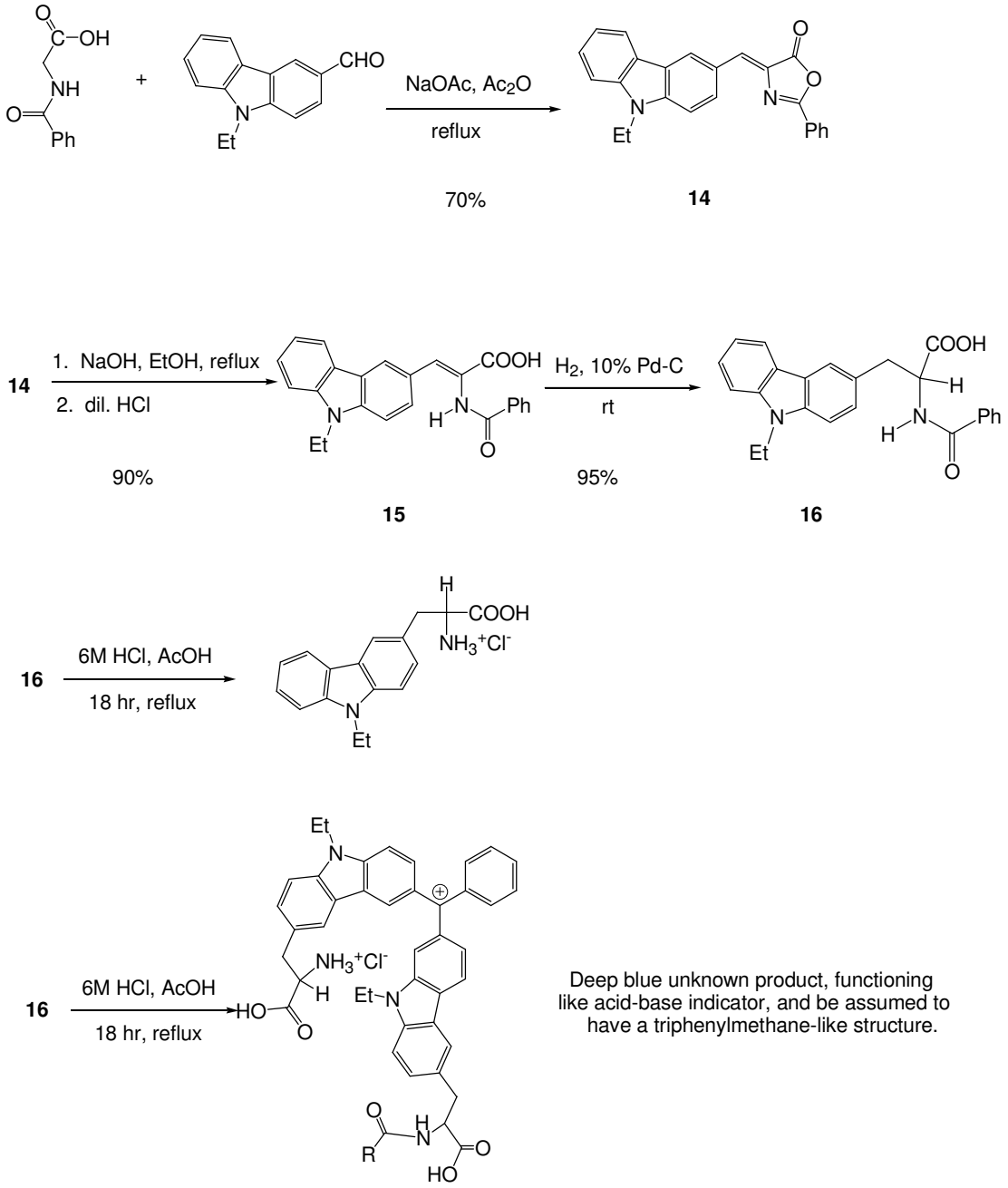
The azlactone route was used to synthesize 3-(N-ethyl-3-carbazolyl)alanine (Scheme 3-3). The oxazolone was prepared by condensation between carbazolecarboxaldehyde and benzoylglycine.

The ring was opened in basic solution and the double bond was reduced by catalytic hydrogenation. The following step, heating in 6M HCl/AcOH, was required to remove the benzoyl group. The deep blue byproduct was observed after four hours of reflux. The blue disappeared when the solution was made basic, and the blue came back when the acid was added again. It functioned as a reversible acid-base indicator such as those with the blue product showed similarity to that of the starting material, although there was a slight change in the integration of the aromatic protons. In order to speed up the large conjugated structures (e.g., as triphenylmethane dyes). The ^{1}H NMR spectrum of deacylation, hippuric acid was replaced with N-acetyl-glycine and 4-nitrobenzoylglycine, which were condensed with the aldehyde. During deacylation, the blue byproducts were again present. HBr and HI were used in place of HCl to speed up the reaction, and the reaction temperature was reduced to 80 °C, but the blue product appeared again after five hours of refluxing.

Two small reactions were set up, where carbazole and 9-ethyl-3-carbazole-carboxaldehyde, respectively, were refluxed in HCl/AcOH solution. After a couple of hours, the deep blue color was observed in both cases, which may be the best evidence that the reaction involved the carbazole ring. But its potential structure was unknown until a compound called carbazole blue was located in a 1920 journal article.[52] Carbazole blue was first obtained by Suida from the fusing of carbazole with oxalic acid in 1879. Copisarow made carbazole violet when 9-ethylcarbazole was fused with oxalic

acid. Copisarow thought that carbazole condenses in this case, not with oxalic acid itself, but with its decomposition product, formic acid. Figure 3-4 describes the potential reactions and the deduced structure of the products.

Scheme 3-3



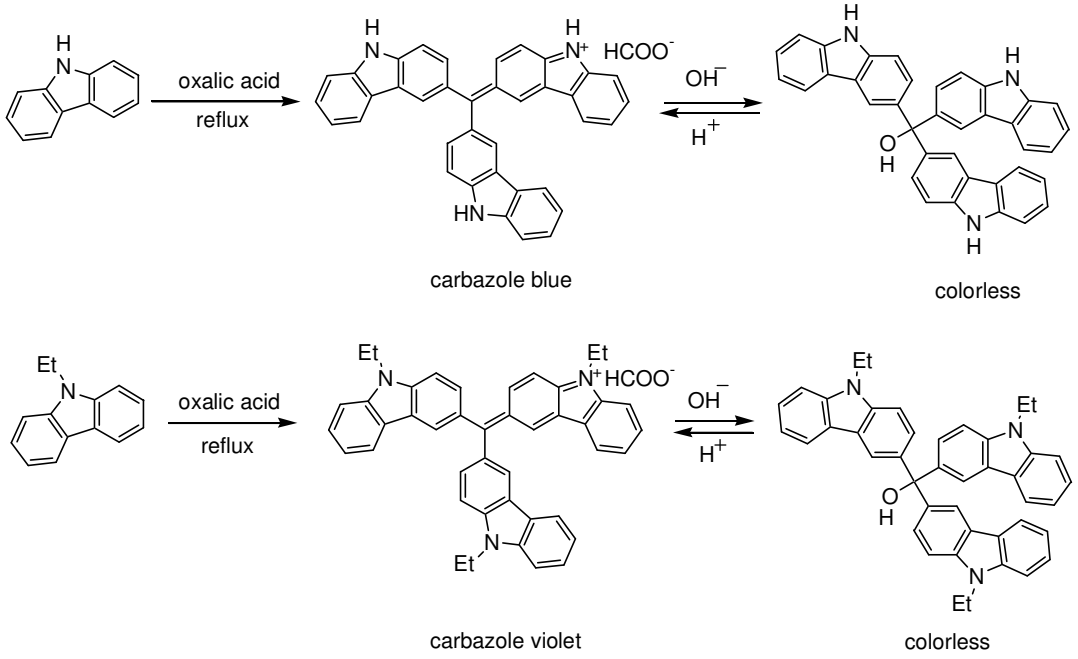


Figure 3-4 Formation and assumed structures of carbazole blue and carbazole violet.[56]

The proposed reaction in our case is described in Figure 3-5. The two carbazole rings were assumed to condense with benzoic acid instead of formic acid.

In order to securely remove the benzoyl group, an alternative method was adopted, where the oxazolone was treated with red phosphorus, Ac_2O and aqueous HI. A series of reactions, involving reduction, ring-opening, and deacylation took place during seven hours of refluxing. The blue product wasn't observed this time. A positive ninhydrin test indicated that the benzoyl group had been removed, but there were several TLC spots showing positive ninhydrin tests. Their similar polarities made them hard to separate.

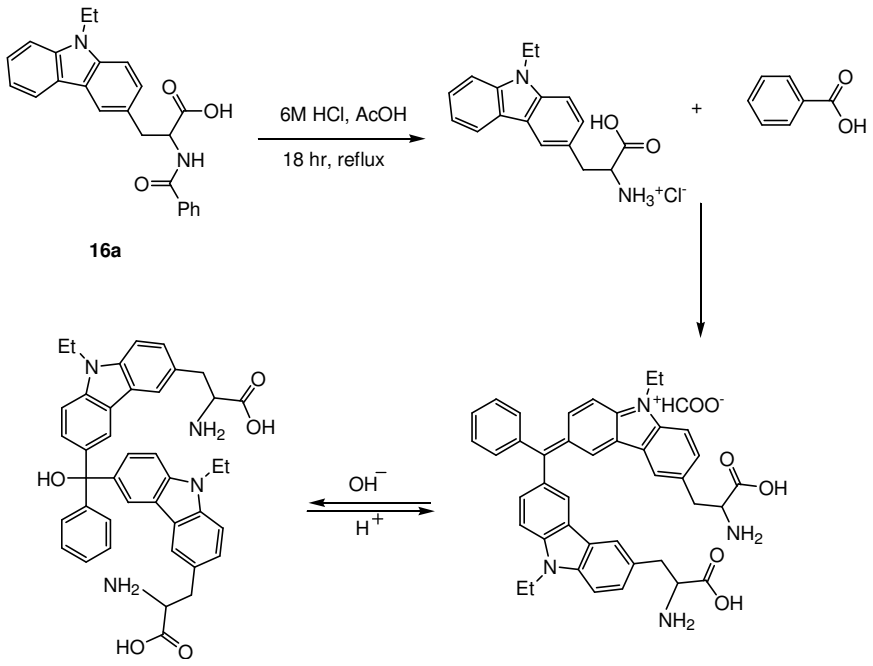
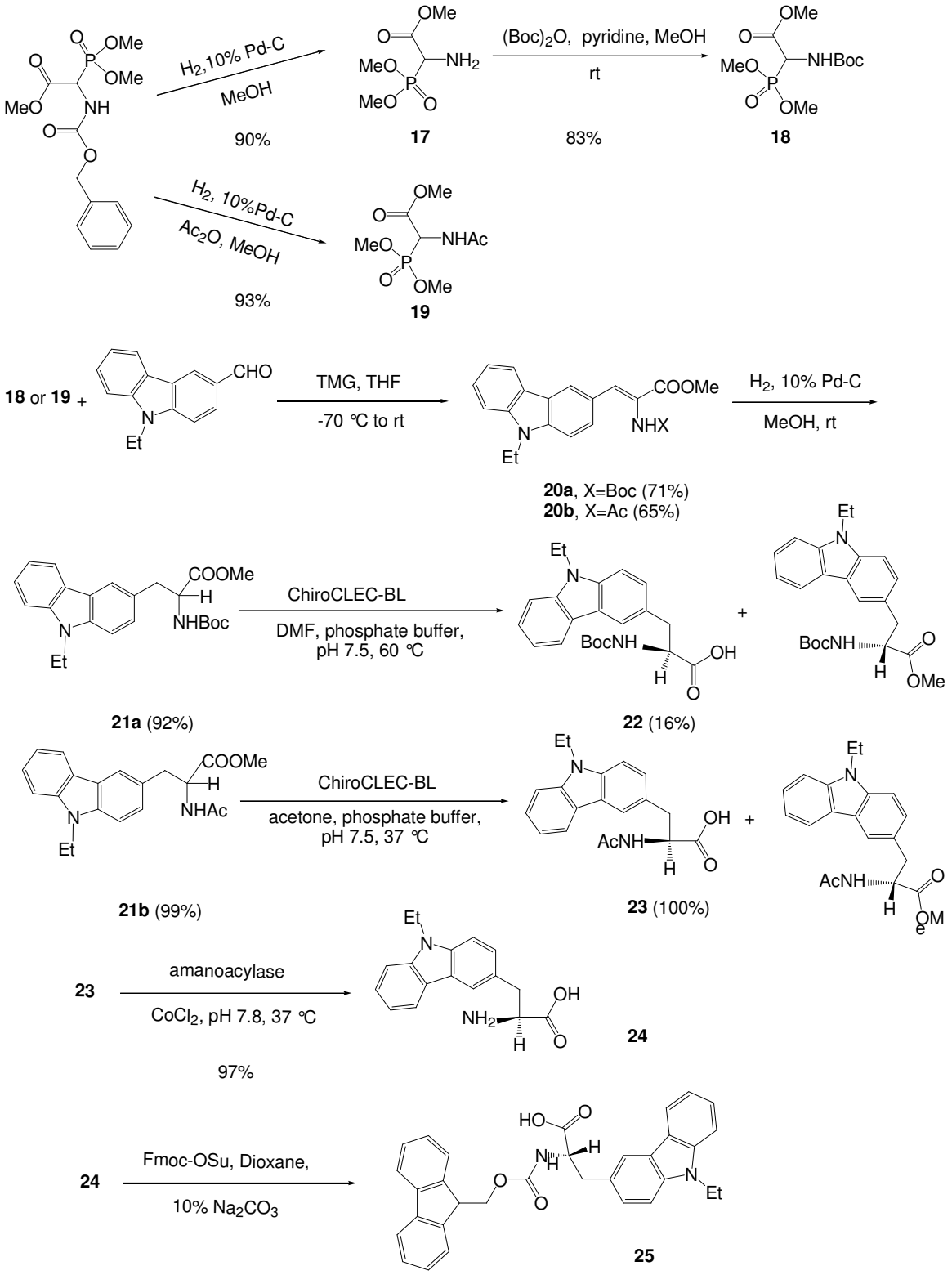


Figure 3-5 The assumed side reaction during debenzoylation of compound **16a.**

3. 3 Syntheses of N-Fmoc-3-(N'-ethyl-3-carbazolyl)-L-alanine and its derivatives via Wittig-Horner reactions followed by two-step enantiomeric resolutions

The liability of 3-(N-ethylcarbazoyl)-DL-alanine to acid blocked its synthesis by the Sorensen or azlactone routes. Thus, the Wittig-Horner route was a good alternative to create the amino acid backbone. All reactions took place under mild conditions at rt. The yield for each step was fairly good (Scheme 3-4).

Scheme 3-4


H2,10% Pd-C
MeOH
90%
17
(Boc)2O, pyridine, MeOH
rt
83%
18
H2, 10%Pd-C
Ac2O, MeOH
93%
19
18 or 19 +
TMG, THF
-70 ℃ to rt
H2, 10% Pd-C
MeOH, rt
20a, X=Boc (71%)
20b, X=Ac (65%)
ChiroCLEC-BL
DMF, phosphate buffer,
pH 7.5, 60 ℃
21a (92%)
22 (16%)
ChiroCLEC-BL
acetone, phosphate buffer,
pH 7.5, 37 ℃
21b (99%)
23 (100%)
amanoacylase
CoCl2, pH 7.8, 37 ℃
97%
24
Fmoc-OSu, Dioxane,
10% Na2CO3
40%
25

Thus theWittig-Horner reaction was adopted to create this amino acid. CBZ-protected phosphonoglycinate was converted to its N-Boc derivative (**18**) by hydrogenolysis and N-Boc protection, and to N-Ac derivative (**19**) by one-pot reaction involving CBZ-deprotection and acylation. The following Wittig-Horner reaction proceeded under mild conditions, where TMG was used as base. The reaction was stirred at -70 °C for one hour, and was continued at rt for 18 hours. On the ^{1}H NMR spectrum of **20b** (Figure 3-6(a)), there were two singlet peaks located at δ 2.15 and 1.85, which were assigned to the acetyl groups of N-acetyl-3-(N'-ethyl-3-carbazolyl)-DL-acrylic acid methyl ester (**20b**), belonging to E and Z isomers, respectively. After hydrogenation, these two CH_3 peaks merged into one on the ^{1}H NMR spectrum of **21b** (Figure 3-6(b)).

ChiroCLEC-BL was chosen to selectively hydrolyze the methyl ester, but it didn't work well for the N-Boc-amino acid methyl ester (**21a**). Changing solvents (DMF, THF, and dioxane), temperatures (37-60 °C), amount of enzyme and time periods (up to five days) failed to produce good resolutions. After comparing the sizes of spots assigned to N-Boc-3-(N'-ethyl-3-carbazolyl)-L-alanine (**22**) and N-Boc-3-(N'-ethyl-3-carbazolyl)-DL-alanine methyl ester (**21a**) on TLC, it was estimated that about 40% of yield was achieved under favorable conditions (DMF, 60 °C, 72 hr). Eventually, only 16% of the desired product was isolated.

N-Ac-3-(N'-ethyl-3-carbazolyl)-DL-alanine methyl ester (**21b**) was synthesized by the same pathway, as shown in Scheme 3-4. Its L-isomer (**23**) was found to be a good substrate for ChiroCLEC-BL. The hydrolysis was complete in acetone-phosphate buffer at 37 °C after 24 hr (yield 100%). This hydrolytic product is also a good substrate for amanoacylase. The deacylation was complete in pH 7.5 buffer at 37 °C after 24 hr (yield

100%). Dry reverse-phase flash column chromatography was used to purify the highly polar 3-(N-ethyl-3-carbazolyl)-L-alanine (**24**). The difference between this technique and the conventional wet reverse-phase flash column chromatography is that the former relies on suction rather than a slight positive pressure to force the organic mobile phase through

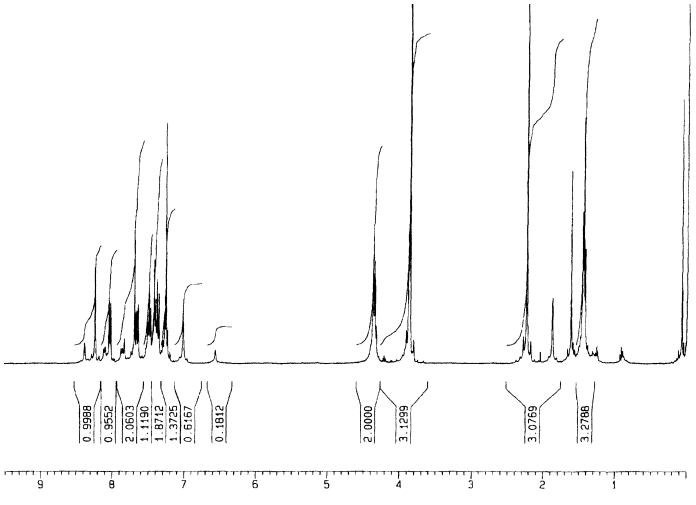


Figure 3-6 ^{1}H NMR spectra for **20b** (a) and **21b** (b).

a silica-gel column, which enables rapid and efficient mobile phase flow and gives the possibility of separating polar compounds on a preparative scale.[18] It can also avoid the tailings and loss of the free amino acid that occurs when a normal-phase silica gel column is used.

The chiral reverse-phase TLC was used to attempt to resolve the D,L mixture of the free amino acids and estimate the enantiomeric excess. Separations of DL-methionine and DL-proline were observed on the chiral TLC plates. However, only one spot with a long tail was observed for DL-alanine, and only one spot was seen for DL-aspartic acid and DL-threonine. There was no separation observed for the 3-(N-ethyl-3-carbazolyl)-L-alanine (**24**), but that fact can't guarantee that only one isomer exists in the product.

Fmoc protection was carried out in 5% Na_2CO_3/dioxane at rt for four hours (yield 40%). After purification by normal-phase column chromatography, a light yellow solid was obtained. Two close spots appeared on TLC plate. Final recrystallization removed the impurity and the product was obtained purely as a white solid.

The N-acetyl-3-(N'-ethyl-3-carbazolyl)alanine derivatives have low-melting points and good solubility in most organic solvents. They do not form good crystals, but instead a foam-like solid after drying under the vacuum. The solvent may be trapped inside the foam, and difficult to completely remove. That may be the cause of the impurity peaks showing up on the NMR spectra.

The overall yield for synthesizing **25** by Scheme 3-4 was 23%.

3. 4 Solid-phase peptide synthesis

In comparing the Boc-strategy and Fmoc-strategy solid-phase peptide syntheses, it is evident that better purity and yield would be achieved with the Fmoc-strategy SPPS. The latter is much easier to operate, especially for peptides with sensitive groups. In the Boc-strategy, strong acids, e.g., TFMSA, are applied to cleave the desired peptides from the resin. This process is very susceptible to salt and scavenger association. The precipitated peptides have to be neutralized and the salts removed either by ion exchange or by Sephadex column chromatography before further purification by HPLC.[36]

The above analysis was confirmed by our preliminary attempt at syntheses of two model peptides, 4-mer (**26**) and 8-mer (**27**) linear peptides. Eventually, the Fmoc-strategy was adopted to make the desired 8-mer linear peptide, H-Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe-OH (**28**). Subsequent cyclization in solution afforded the target cyclic peptide, c*yclo*(Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe) (**29**).

The first model peptide, H-Car-D-Ala-Bpa-D-Ala-OH (**26**), was synthesized by the Boc-strategy. The reaction was carried out on Pam resin. After the peptide was cleaved from the resin, purification became a big problem. First, this peptide didn't precipitate in cold Et_2O, even though TFA had been almost completely removed. The residue was heated to 80 °C to remove the solvents as much as possible. A light yellow precipitate was obtained and analyzed by ES-MS. Its ES-MS spectrum was very complicated and many impurity peaks showed up (MS-3). The molecular ion peak couldn't be located. A major peak at *m/z* 791.4, which was assigned to the TFA salt of the desired peptide (calcd. 790.8), was observed.

An 8-mer peptide, H-D-Ala-Npa-D-MeAla-Ala-D-Ala-Bpa-D-Ala-Ala-OH (**27**), was synthesized by the Fmoc strategy. The reaction was carried out on the Wang-resin. Before the final deprotection, 70 mg of resin-bound peptide, H-D-FmocAla-Npa-D-MeAla-Ala-D-Ala-Bpa-D-Ala-Ala-OH, was set aside. The Fmoc group was removed from the remaining 200 mg of the resin by 25% piperidine. The two peptide-bound resin samples were cleaved under mild conditions (TFA/H_2O 95:5). They both gave precipitates in cold Et_2O. The ES-MS spectrum of the Fmoc-deprotected peptide seemed pretty clean (MS-4). The molecular ion peak $[M + 1]^+$ was identified at *m/z* 907.2 (calcd. 907.0). For the Fmoc protected peptide, no molecular ion peak was observed (calcd *m/z* 1115.1 $[M + 1]^+$, (MS-7)). Its absence may be due to poor solubility of the N-Fmoc peptide in TFA, and so the cleaved peptide may stay with the resin during workup.

Syntheses of the 8-mer target peptide (**28**) were carried out twice by the Fmoc-strategy. The first time, after Fmoc-Aib was attached to the resin and then its Fmoc group removed, the ninhydrin test gave a negative result. In the following attachment of Fmoc-Bpa to the resin, another negative ninhydrin test was observed after deprotection. It wasn't clear which step had failed to attach an amino acid onto the resin-bound peptide. After the total synthesis had been completed, the ES-MS gave the major peak at *m/z* 917.6 (MS-8), which belonged to H-Aib-Car-Aib-Phe-Aib-Aib-Phe-OH, Therefore, Fmoc-Bpa failed to attach to the resin-bound peptide. The second synthesis was successful. TNBS and chloranil tests were adopted to monitor the deprotection after the Fmoc-Aib attachment. Only one peak appeared on MS-5, which corresponded to the molecular ion peak, $[M + 1]^+$, found at *m/z* 1168.8 (calcd. 1168.6).

Two cyclization attempts were made for synthesis of *cyclo*(Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe, **29**). The first gave a 40% yield. There were three major peaks in its MS spectrum (MS-6), at *m/z* 1150.8 (belonging to $[M + 1]^+$), at *m/z* 1172.8 (belonging to $[M + Na]^+$), and at *m/z* 1354.8 (presumably an impurity). The second run gave a 70% yield. There were the same three peaks but with different ratios (MS-9).

There are two possible reasons for the existence of the $[M + Na]^+$ peak in the ES-MS spectrum. This polar molecule may trap Na^+ present in the mass spectrometer during ionization. Another reason was related to the work-up method. In the first cyclization, $NaHCO_3$ was first used to remove the linear peptide and water-soluble impurities, and then $KHSO_4$ was added to remove the remaining linear peptide and water-soluble impurities. For the second cyclization, $KHSO_4$ was used first, and $NaHCO_3$ followed. Only a $[M + Na]^+$ but no $[M + K]^+$ peak was observed on MS-6 and MS-9. It may indicate that the size of the hollow crevice of the cyclic peptide fits the diameter of sodium cation well, but not that of potassium ion.

Chapter 4 Experimental

4. 1 Materials

ChiroCLEC-BL subtilisin was purchased from Altus Biologics, amanoacylase from Aldrich, and (±)-Z-α-phosphonoglycine trimethyl ester from Fluka. Boc-D-Ala-PAM resin, Fmoc-Phe-Wang resin, various commercial amino acids and coupling reagents including 1-hydroxybenzo- triazole (HOBT) and benzotriazol-1-yloxytrispyrrolidino-phosphonium hexafluorophosphate (PyBOP) were purchased from NovaBioChem and Advanced ChemTech, and 1-hydroxy-9-azabenzotriazole (HOAT) and O-(7-azabenzotriazol-1-yl)-N,N,N',N' -tetramethyluronium-hexafluoro phosphate (HATU) from Aldrich. The other reagents were purchased from Aldrich. All dry solvents were purchased from Aldrich and used without further purification. The other solvents were purchased from Aldrich or VWR.

4. 2 Analyses and peptide syntheses

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance 400 (400 MHz) NMR spectrometer. Unless otherwise noted, spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS) at 0.00 ppm. Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded on the same spectrometer at 100 MHz. Melting points were determined on a Thomas-Hoover Capillary melting point apparatus and are uncorrected. Analytal thin layer chromatography was performed using precoated silica gel plates

(Whatman, KF6F, silica gel 60A, 250 μm; KCF18, silica gel 60A, 200 μm; Sorbtech, CHIRALPLATE 25, RP silica gel coated with Cu^{2+} and a chiral ligand on glass) with a 254 nm fluorescence indicator. The plates were visualized under a UV lamp, with a PMA stain (5 wt % phosphomolybdic acid in EtOH), ninhydrin spray (2 wt % ninhydrin in 1-butanol) or I_2. Wet normal-phase flash column chromatography was performed on Mallinckrodt Baker 40 μm silica gel under a positive air pressure. Dry reverse-phase flash column chromatography was performed on octadecyl-functionalized silica gel under negative pressure. Manual peptide syntheses were carried out in a QPE-3 peptide synthesis vessel (supplied by Quark Glass). ES-MS spectra were provided by Synpep Corporation.

4. 3 Dye tests[35,36]

The following tests were used to monitor completion of the coupling reaction in solid-phase peptide synthesis.

Kaiser test (for primary amines)

- Dissolve5 g of ninhydrin in 100 mL of EtOH
- Dissolve 80 g of phenol in 20 mL of EtOH
- Add 2 mL of 1 mmol aqueous KCN to 98 mL of pyridine
- Sample a few resin beads and wash several times with EtOH
- Transfer to a small glass tube and add 2 drops of each of the above solutions

- Mix well and heat to 120 °C for 4-6 min. A positive test is indicated by formation of blue colored beads.

TNBS test (for hindered primary amines)

- Make a 10% DIPEA solution in DMF
- Make a 1% 2,4,6-trinitrobenzenesulfonic acid (TNBS) solution in DMF
- Sample a few resin beads and wash several times with EtOH
- Suspend the beads in DMF and add 2 drops of each of the solutions above and leave for 5 min
- Wash the beads with DMF to remove the red solution. A positive test is indicated by formation of red colored beads.

Chloranil test (for secondary amines)

- To 1-5 mg of resin, add one drop of 2% acetaldehyde in DMF followed by one drop of 2% *p*-chloranil in DMF
- Allow to stand at rt for 5 min. Blue colored beads indicate the presence of secondary amines.

4. 4 Dry flash column chromatography[18]

The practical approach is fully described in reference 18.

4. 5 Syntheses of chromophore-modified chiral amino acids

The following sections described the details of the experiments.

4. 5. 1 Synthesis of N-Boc-3-(9-anthryl)-L-alanine (Schemes 3-1 and 3-2)

9-Chloromethylanthracene (1). To a stirred solution of 9-anthracenemethanol (2.08 g, 10 mmol) in 20 mL of dry THF under N_2, was added dry pyridine (1.2 mL, 14 mmol), giving a yellow solution. After the solution was cooled to 0 °C, $SOCl_2$ (1 mL, 13.4 mmol) was added dropwise to the flask. The mixture was allowed to return to rt, and heated to reflux for four hours. The solvent was removed on a rotary evaporator. The crude product was washed with MeOH in order to remove the residual 9-anthracenemethanol, and recrystallized from CH_2Cl_2 twice to afford the yellow, long needle-like solid **1** (1.48g, 65%): mp 137-139 °C (lit.[53] 138-140 °C); ^{1}H NMR δ 5.61 (s, 2H, CH_2), 7.51 (t, 2H, *J* = 4.1 Hz, ArH), 7.60 (t, 2H, *J* = 5.1 Hz, ArH), 8.02 (d, 2H, *J* = 8.5 Hz, ArH), 8.30 (d, 2H, *J* = 8.9 Hz, ArH), 8.48 (s, 1H, ArH); ^{13}C NMR (not available).

Diethyl 9-anthrylmethylacetamidomalonate (2). Diethyl acetamidomalonate (1.42 g, 6.5 mmol), NaH (0.24 g, 10 mmol) and LiBr (0.43 g, 5 mmol) were placed into a dry, N_2-purged three-neck flask immersed in an ice-bath. Dry THF (10 mL) was dropped in slowly with stirring, followed by dry EtOH (0.19 mL). After one hour, **1** (1.47 g, 6.5 mmol) in dry THF (10 mL) was added and the ice-bath was removed. The mixture was heated to reflux for 18 hr. The progress of the reaction was followed by reverse-phase TLC (EtOH/H_2O 4:1, R_f 0.7). The solvent was removed on a rotary evaporator. The crude product was dissolved in hot EtOH, and insoluble impurities were removed by filtration.

The EtOH was removed on a rotary evaporator. No further purification was done. The product was dried in vacuum to give **2** as a yellow solid (1.85 g, 70%): mp (not available); ^{1}H NMR (DMSO-d_6) δ 1.11 (t, 6H, *J* = 6.9 Hz, CH_3), 1.70 (s, 3H, CH_3), 3.55 (s, 1H, NH), 3.98 (m, 4H, CH_2), 4.57 (s, 2H, CH_2), 7.50 (m, 4H, ArH), 8.07 (m, 4H, ArH), 8.54 (s, 1H, ArH); ^{13}C NMR (not available).

N-Acetyl-3-(9-anthryl)-DL-alanine (3). Compound **2** (1.84 g, 4.6 mmol) was mixed with NaOH (0.74 g, 18.4 mmol) in 20% aq. EtOH solution (75 mL) with stirring. After the solution was heated to reflux for four hours, the EtOH was removed on a rotary evaporator. Then HCl (3 M, 6.1 mL) was added. The suspension was heated to reflux overnight. The resultant precipitate was isolated by filtration and dissolved in NaOH solution (0.2 M, 100 mL). The solution was extracted with EtOAc (3 × 80 mL). The organic phase was discarded. The aqueous phase was adjusted to pH 4 with HCl (3 M), and then extracted with EtOAc (5 × 250 mL). The combined organic phase was washed with brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The product **3** was a light yellow solid (1.18 g, 85%): mp 265-7 °C (lit.[20] 269-70 °C); ^{1}H NMR (DMSO-d_6) δ 1.50 (s, 3H, CH_3), 3.76 (m, 1H, CH_2), 3.90 (m, 1H, CH_2), 4.44 (m, 1H, CH), 7.38 (m, 4H, ArH), 7.91 (d, 2H, *J* = 8.2 Hz, ArH), 8.24 (d, 2H, *J* = 8.7 Hz, ArH), 8.28 (d, 1H, *J* = 8.2 Hz, NH), 8.36 (s, 1H, ArH), 12.8 (s, 0.6H, OH); ^{13}C NMR (not available).

N-Acetyl-3-(9-anthryl)-DL-alanine methyl ester (4). To a solution of **3** (1.15 g, 3.7 mmol) in dry MeOH (35 mL) was added dropwise BF_3 Et_2O (1.15 g, 8 mmol) under N_2. The solution was heated to reflux for two hours. The solvent was removed on a rotary evaporator. The crude product was dissolved in EtOAc. The organic phase was washed

with 5% of Na_2CO_3, saturated NH_4Cl and brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The crude product was purified by normal-phase column chromatography (hexane/EtOAc 1:1) to afford **4** as a yellow solid (0.9 g, 75%): mp 205-7 °C (lit.[20] 208-9 °C); ^{1}H NMR (DMSO-d_6) δ 1.68 (s, 3H, CH_3), 3.25 (s, 3H, CH_3), 3.92 (m, 1H, CH_2), 4.01 (m, 1H, CH_2), 4.55 (m, 1H, *J* = 7.5 Hz, CH), 7.49 (m, 4H, ArH), 8.02 (d, 2H, *J* = 8.1 Hz, ArH), 8.21 (d, 2H, *J* = 8.7 Hz, ArH), 8.48 (s, 1H, ArH), 8.56 (d, 1H, *J* = 7.7 Hz, NH); ^{13}C NMR δ 23.5 (CH_3), 30.1 (CH_2), 52.7 (OCH_3), 53.9 (CH), 124.3 (CH), 125.3 (CH), 126.6 (CH), 127.6 (CH), 128.5 (C), 129.7 (CH), 131.0 (C), 131.8 (C), 170.2 (C=O), 173.0 (C=O).

N-Acetyl-3-(9-anthryl)-L-alanine (5). Compound **4** (880 mg, 2.7 mmol) was mixed with DMF (40 mL) and phosphate buffer (0.5 M, pH 7.5, 25 mL). ChiroCLEC-BL (40 mg) was added with stirring under N_2. The mixture was heated to 60 °C for 72 hr. The progress of the reaction was followed by reverse-phase TLC. It was observed that the size of a new spot (EtOH/H_2O 4:1, R_f 0.90) assigned to **5** became larger with time. The enzyme was removed by centrifugation and the solvents were removed on a rotary evaporator. The residue was brought to pH 4, and extracted with EtOAc (3 × 200 mL). The organic phase was washed with brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The ^{1}H NMR spectrum of the crude product showed that there were two methyl signals, assigned to the acetyl CH_3 groups, one belonging to **4** at δ 1.69, the other belonging to **5** at δ 1.67 (NMR-8). The ratio of the integrals was 6:4, which meant that 40% of **4** had been converted into **5** (yield 80%). Aq. Na_2CO_3 (5%) was added to the mixture, and undissolved **4** extracted into EtOAc (3 × 200 mL). The organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed on a

rotary evaporator to yield **4** (520 mg). The aqueous phase was adjusted to pH 4, and extracted with EtOAc (5 × 200 mL). The organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed on a rotary evaporator to give **5** as a light yellow solid (340 mg, 40%): mp (not available); ^{1}H NMR (DMSO-d_6) δ 1.66 (s, 3H, CH_3), 3.92 (m, 1H, CH_2), 4.06 (m, 1H, CH_2), 4.59 (m, 1H, CH), 7.57 (m, 4H, ArH), 8.10 (d, 2H, *J* = 8.2 Hz, ArH), 8.42 (d, 2H, *J* = 8.8 Hz, ArH), 8.52 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ 22.7 (CH_3), 30.1 (CH_2), 54.2 (CH), 124.8 (CH), 125.3 (CH), 126.2 (CH), 126.7 (CH), 129.3 (C), 130.3 (CH), 130.9 (C), 131.4 (C), 169.4 (C=O), 173.6 (C=O).

9-Anthryl-L-alanine hydrochloride (6a) from acid-catalyzed hydrolysis of 5. Compound **5** (180 mg, 0.59 mmol) was suspended in conc. HCl (6 mL) and AcOH (2 mL). The mixture was heated to reflux overnight. The solvent was removed on a rotary evaporator. The product **6a** was dried in vacuum to give a brownish yellow solid (174 mg, positive ninhydrin test). The other analytical data are not available.

9-Anthryl-L-alanine hydrochloride (6b) from enzyme-catalyzed hydrolysis of 5. Compound **5** (150 mg, 0.49 mmol) was dissolved in KOH (1 M), and then HCl (2 M) and a small amount of H_2O were added to adjust the pH to 7.8. The final concentration of the substrate was kept close to 0.05M. $CoCl_2$ (2 mg, 0.05 M) and amanoacylase (5 mg) were added. The mixture was set on a shaker at 200 rpm at 37 °C for 48 hr. A yellow precipitate was observed. A positive ninhydrin test was shown on normal-phase TLC ($CHCl_3$/MeOH/ AcOH 9:1:0.1). The precipitate was isolated by filtration and washed with H_2O. It was dissolved in EtOH and a small amount of HCl solution (1M) was added to dissolve the desired product completely. The insoluble residue was separated. The filtrate was heated to 50 °C with a small amount of charcoal in order to denature the

amanoacylase. The charcoal was removed by passing the solution through a short column filled with Celite. The solvent was evaporated to afford the product **6b** as a light yellow solid (116 mg, 89%, positive ninhydrin test). The other analytical data are not available.

N-t-Butyloxycarbonyl-3-(9-anthryl)-L-alanine (7). To a solution of **6a** (100 mg, 0.38 mmol) in dry DMF (2 mL) were added Et_3N (0.8 mL) and $(Boc)_2O$ (82 mg, 0.38 mmol). The mixture was stirred at rt overnight. The solvent was removed on a rotary evaporator. The residue was dissolved in EtOAc. The solution was washed with 5% aq. citric acid and brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator to give **7** as a yellow solid (60 mg, 45%): mp 186-189 °C; ^{1}H NMR (DMSO-d_6) δ 0.94 (s, 9H, CH_3), 3.87 (m, 2H, CH_2), 4.07 (t, 1H, CH), 7.37 (m, 4H, ArH), 7.89 (d, 2H, *J* = 8.1 Hz, ArH), 8.24 (d, 2H, *J* = 7.9 Hz, ArH), 8.26 (s, 1H, ArH); ^{13}C NMR (not available).

Diethyl 9-anthrylmethyl-N-(t-butyloxycarbonyl)aminomalonate (8). A three-neck flask purged with N_2 was charged with NaH (0.2 g, 8.3 mmol) and LiBr (0.37g, 4.2 mmol) in an ice-bath. Dry DMF (10 mL) was added with stirring, and then diethyl N-(t-butyloxycarbonyl)-aminomalonate (1.7 g, 6.1 mmol) was slowly added. Dry EtOH (0.24 mL) was added dropwise. After one hour, a solution of 9-chloromethylanthracene (1.2 g, 5.3 mmol) in dry THF (10 mL) was added, and the ice-bath was removed. The solution was heated to 90 °C for 18 hr. The solvent was removed on a rotary evaporator. The residue was dissolved in EtOAc, and washed with aq. citric acid (5%) and brine. The solvent was dried over Na_2SO_4, and then removed on a rotary evaporator. The product **8** was obtained as a yellow solid (1.32 g, 54%): mp (not available); ^{1}H NMR δ 1.32 (t, 6H, *J* = 7.0 Hz, CH_3), 1.49 (s, 9H, CH_3), 4.03-4.18 (m, 4H, CH_2), 4.73 (s, 2H, CH_2), 5.47 (s, 1H, NH), 7.48 (m, 4H, ArH), 8.01 (d, 2H, *J* = 5.3 Hz, ArH), 8.30 (d, 2H, *J* = 4.1, ArH),

8.40 (s, 1H, ArH); ^{13}C NMR δ 14.2 (CH_3), 28.7 (CH_3), 30.6 (CH_2), 62.8 (CH_2), 67.8 (C), 125.1 (CH), 125.2 (CH), 125.8 (CH), 127.9 (CH), 128.1 (C), 129.4 (CH), 131.8 (C), 132.0 (C), 154.7 (C=O), 168.4 (C=O).

3-(9-Anthryl)-DL-alanine hydrochloride (6) from hydrolysis, decarboxylation and Boc deprotection of 8. Compound **8** (1.3 g, 2.8 mmol) was mixed with NaOH (0.45 g, 11.2 mmol) in 20% aq. EtOH solution (50 mL). The mixture was heated to reflux for four hours. The EtOH was removed on a rotary evaporator and then HCl (3 M, 3.7 mL) was added. The mixture was heated to reflux overnight, and a yellow precipitate was formed. The precipitate was collected to give **6** as a light yellow solid (0.7 g, 95%, positive ninhydrin test). The other analytical data are not available.

3-(9-Anthryl)-DL-alanine methyl ester (9). To a solution of **6** (0.69 g, 2.6 mmol) in dry MeOH (20 mL) under N_2, cooled in an ice-bath, $SOCl_2$ (0.29 mL, 3.9 mmol) was added dropwise. The mixture was heated to reflux overnight. The solvent was removed on a rotary evaporator. The residue was dissolved in EtOAc (250 mL). The organic phase was washed with 5% aq. Na_2CO_3 and brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The crude product was purified by normal-phase column chromatography (hexane/EtOAc 1:2.5). Compound **9** (0.5 g, 68%), was obtained as an oily yellow solid: mp 180•Cdec; ^{1}H NMR δ 1.62 (s, 2H, NH_2), 3.63 (s, 3H, CH_3), 3.94 (m, 1H, CH_2), 4.10 (m, 1H, CH_2), 4.17 (m, 1H, CH), 7.50-7.57 (m, 4H, ArH), 8.05 (d, 2H, *J* = 8.3 Hz, ArH), 8.35 (d, 2H, *J* = 8.9 Hz, ArH), 8.43 (s, 1H, ArH); ^{13}C NMR (not available).

N-t-Butyloxycarbonyl-3-(9-anthryl)-DL-alanine methyl ester (10) from Boc-protection of 9. To a stirred solution of **9** (0.48 g, 1.7 mmol) in dry DMF (5 mL) were

added Et_3N (2 mL) and $(BOC)_2O$ (0.6 g, 2.7 mmol). The mixture was stirred at rt overnight. The solvent was removed on a rotary evaporator. The residue was dissolved in EtOAc (200 mL). The organic phase was washed with 5% aq. citric acid and brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The crude product was further purified by normal-phase column chromatography (hexane/EtOAc 3:1). Compound **10** was obtained as a yellow solid (0.49 g, 75%): mp 190-193 °C; ^{1}H NMR δ 1.29 (s, 9H, CH_3), 3.28 (s, 3H, CH_3), 3.99 (m, 1H, CH_2), 4.04 (m, 1H, CH_2), 4.67 (t, 1H, CH), 5.22 (s, 1H, NH), 7.48 (m, 4H, ArH), 7.95 (d, 2H, *J* = 8.3 Hz, ArH), 8.24 (d, 2H, *J* = 7.1 Hz, ArH), 8.33 (s, 1H, ArH); ^{13}C NMR (not available).

N-t-Butyloxycarbonyl-3-(9-anthryl)-DL-alanine (11). Compound **8** (1.2 g, 2.6 mmol) was mixed with NaOH (0.42 g, 10.4 mmol) in 20% aq. EtOH solution (46 ml) with stirring. The mixture was heated to reflux for four hours. The EtOH was removed on a rotary evaporator, then HCl (1 M) was added slowly and the pH adjusted to 4. The formed precipitate was collected by filtration, and then dissolved in dioxane. The dioxane solution was heated to reflux for five hours. The solvent was removed on a rotary evaporator. The residue was dissolved in EtOAc, washed with brine, and dried over Na_2SO_4. The crude product was purified by normal-phase column chromatography ($CHCl_3$/MeOH 9:1) to give **11** as a light yellow solid (0.82 g, 88%): mp (not available); ^{1}H NMR (DMSO-d_6) δ 0.94 (s, 9H, CH_3), 3.87 (m, 2H, CH_2), 4.07 (m, 1H, CH), 7.37 (m, 4H, ArH), 7.89 (d, 2H, *J* = 8.1 Hz, ArH), 8.26 (d, 2H, *J* = 7.9 Hz, ArH), 8.30 (s, 1H, ArH); ^{13}C NMR (not available).

N-t-Butyloxycarbonyl-3-(9-anthryl)-DL-alanine methyl ester (10) from methyl esterification of 11. To a solution of **11** (0.8 g, 2.2 mmol) in dry MeOH (20 mL), was

added dropwise trimethylsilyldiazomethane (2.2 mL, 2 M in hexane, 4.4 mmol) with stirring at rt under N_2 for five hours. The solvent was removed on a rotary evaporator. The residue was dissolved in EtOAc (50 mL), washed with 5% aq. citric acid and brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The crude product was purified by normal-phase column chromatography (hexane/EtOAc 3:1) to give **10** as a yellow solid (0.55 g, 70%): mp 191-193 ^oC; ^1H NMR δ 1.40 (s, 9H, CH_3), 3.33 (s, 3H, CH_3), 4.04 (m, 1H, CH_2), 4.07 (m, 1H, CH_2), 4.78 (t, 1H, J = 7.1 Hz, CH), 5.30 (s, 1H, NH), 7.50 (m, 2H, ArH), 7.58 (m, 2H, ArH), 7.95 (d, 2H, J = 8.3 Hz, ArH), 8.33 (d, 2H, J = 8.6 Hz, ArH), 8.43 (s, 1H, ArH); ^{13}C NMR (not available).

N-t-Butyloxycarbonyl-3-(9-anthryl)-L-alanine (7) from enzyme-catalyzed hydrolysis of 10. Compound **10** (500 mg, 1.3 mmol) was suspended in DMF (30 mL) and phosphate buffer (0.5 M, pH 7.5, 15 mL). ChiroCLEC-BL (40 mg) was added with stirring at 60 °C for 72 hr. The progress of the reaction was followed by reverse-phase TLC. A new spot with R_f 0.9 (reverse-phase TLC, EtOH/H_2O 4:1) increased with time. The enzyme was removed by centrifugation and the solvents were removed on a rotary evaporator. The residue was dissolved in EtOAc (200 mL). The organic phase was washed with 5% aq. $NaHCO_3$ (3 × 50 mL), washed with brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator to recover 10 (460 mg). The aqueous phase was brought to pH 4, and extracted with EtOAc (5 × 200 mL). The organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed on a rotary evaporator to give **7** as a light yellow solid (20 mg, 8%). The analytical data are not available.

Attempted enzyme-catalyzed hydrolysis of 3-(9-anthryl)-DL-alanine methyl ester trifluoroacetate (12). Compound **10** (100 mg, 0.26 mmol) was mixed with TFA (5 mL)

and CH_2Cl_2 (5 mL) with stirring for 30 min in an ice-bath. The solvents were completely removed on a rotary evaporator to give the intermediate **12**. Compound **12** was dissolved in DMF (7 mL) and phosphate buffer (0.5 M, pH 7.5, 3 mL). ChiroCLEC-BL (10 mg) was added and the mixture stirred at 60 °C for 72 hr. The progress of the reaction was followed by normal-phase TLC ($CHCl_3$/MeOH/AcOH 5:1:0.1, visualized by UV lamp and ninhydrin spray), which showed that the conversion was less than 20%, and several side products had been formed. No further purification was carried out.

4. 5. 2 Synthesis of N-ethylcarbazolyl-L-alanine and its derivatives (Schemes 3-3 and 3-4)

N-Ethyl-3-hydroxymethyl carbazole (13).[54] N-Ethyl-3-carbazolecarboxaldehyde (2.24 g, 10 mmol) was dissolved in MeOH containing 2% NaOH (50 mL). $NaBH_4$ (0.21 g, 5.5 mmol) was added, and the solution was kept at rt overnight. The reaction mixture was diluted with H_2O and extracted with benzene (3 × 100 mL). The organic phase was dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The crude product was recrystallized twice from EtOH at -10 °C, giving white, felt-like needles **13** (1.57 g, 70%): mp 75-76 °C (lit.[54] 75-76 °C); ^{1}H NMR (DMSO-d_6 + D_2O) δ 1.28 (t, 3H, J = 7.1 Hz, CH_3), 4.42 (m, 2H, CH_2), 4.63 (s, 2H, CH_2), 5.15 (s, 0.7H, OH), 7.18 (t, 1H, J = 7.4 Hz, ArH), 7.42 (t, 1H, J = 7.4 Hz, ArH), 7.44 (d, 1H, J = 7.6 Hz, ArH), 7.55 (d, 2H, J = 8.2 Hz, ArH), 8.00 (s, 1H, ArH), 8.10 (d, 1H, J = 7.7 Hz, ArH); ^{13}C NMR (not available).

Attempted synthesis of N-ethyl-3-chloromethylcarbazole. To a solution of **13** (1.55 g, 6.9 mmol) in dry THF (20 mL), was added dry pyridine (1.2 mL, 14 mmol) with stirring

under N_2. After the mixture was cooled to 0 °C, $SOCl_2$ (1 mL, 13.4 mmol) was added dropwise. The mixture was allowed to return to rt and the solution turned blue slowly. No further work-up was undertaken.

4-(3-(N-Ethylcarbazolylidene))-2-phenyloxazol-5-one (14). A stirred mixture of 9-ethyl-3-carbazolecarboxaldehyde (1.12 g, 5 mmol), powdered, dry hippuric acid (0.9 g, 5 mmol), and powdered, freshly fused NaOAc (1.2 g, 15 mmol) in Ac_2O (10 mL) was heated to reflux for four hours. A deep yellow precipitate was observed and then hot EtOH (15 mL) was added into the flask. During the addition, the round-bottom flask was slightly cooled. After the reaction mixture was allowed to stand overnight, the crystalline product was collected by filtration and washed with ice-cold EtOH twice and boiling water twice to give **14** as a bright yellow solid (1.28 g, 70%): mp (not available); ^{1}H NMR δ 1.45 (t, 3H, J = 7.2 Hz, CH_3), 4.37 (q, 2H, J = 7.2 Hz, CH_2), 7.32 (t, 1H, ArH), 7.47 (m, 3H, ArH, =CH), 7.55 (m, 4H, ArH), 8.17 (m, 3H, ArH), 8.39 (d, 1H, ArH), 8.93 (s, 1H, ArH); ^{13}C NMR (not available).

N-Benzoyl-3-(N'-ethyl-3-carbazolyl)acrylic acid (15). Compound **14** (1.2g, 3.3 mmol) was suspended in EtOH (30 mL). The suspension was heated until the solid dissolved. NaOH (0.5 M, 20 mL) was added and the mixture was heaed to reflux for two hours. The solution turned light yellow. H_2O (30 mL) was added and the EtOH was removed on a rotary evaporator. When HCl (6 M, 2 mL) was slowly added to the residue, a light yellow precipitate was formed. The mixture was kept at an ice-bath for 30 min. The precipitate was collected by filtration, washed with H_2O, and dried in vacuum to give **15** as a light yellow solid (1.16 g, 90%): mp (not available); ^{1}H NMR (DMSO-d_6) δ 1.33 (t, 3H, J = 7.4 Hz, CH_3), 4.44 (q, 2H, J = 6.6 Hz, CH_2), 7.23 (t, 1H, J = 7.3 Hz, ArH), 7.49 (t, 1H, J

= 7.4 Hz, ArH), 7.62 (m, 5H, ArH), 7.85 (d, 2H, J = 7.3 Hz, ArH), 7.64 (s, 1H, =CH), 7.98 (d, 1H, J = 7.6 Hz, ArH), 8.07 (d, 1H, J = 7.2 Hz, ArH), 8.50 (s, 1H, NH), 12.66 (s, 1H, OH); ^{13}C NMR (not available).

N-Benzoyl-3-(N'-ethyl-3-carbazolyl)alanine (16). To a solution of 15 (1.0 g, 2.6 mmol) in THF (30 mL) was added 10% Pd-C catalyst (100 mg). The mixture was shaken on a Parr shaker (30-40 psi) for 48 hr. The catalyst was removed by filtration through a pad of Celite and the solvent was removed on a rotary evaporator. The residue was dissolved in hot NaOH (0.2 M, 15 mL). When HCl (3 M, 1 mL) was slowly dropped into the solution, a white precipitate was formed. The precipitate was collected by filtration and dissolved in EtOAc (200 mL). The organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed on a rotary evaporator to give **16** as a white solid (0.95 g, 95%): mp (not available); ^{1}H NMR (DMSO-d_6) δ 1.26 (t, 3H, J = 7.0 Hz, CH_3), 3.21 (m, 1H, CH_2), 3.36 (m, 1H, CH_2), 4.38 (m, 2H, CH_2), 4.69 (m, 1H, CH), 7.15 (t, 1H, J = 7.6 Hz, ArH), 7.42 (m, 3H, ArH), 7.47 (d, 2H, J = 7.7 Hz, ArH), 7.52 (d, 1H, J = 8.2 Hz, ArH), 7.77 (d, 2H, J = 7.1 Hz, ArH), 8.04 (d, 2H, J = 8.3 Hz, ArH), 8.76 (d, 1H, J = 8.1 Hz, ArH), 12.7 (s, 1H, OH); ^{13}C NMR (not available).

Attempted acid-catalyzed hydrolysis of 16. Compound **16** (400 mg, 1.0 mmol) was suspended in a mixture of HCl (6 M, 15 mL) and AcOH (7 mL). The mixture was heated to reflux. The solution turned blue after three hours. The blue color disappeared when the solution was made basic with NaOH (0.2 M), and came back again when the pH was adjusted to 4 with HCl (1 M). Then the mixture was extracted with EtOAc (2 × 50 mL). The organic solutions were combined and the solvent was removed on a rotary evaporator. The NMR spectrum of the blue residue was similar to that of the starting

material, except that the relative integration of the aromatic protons was slightly reduced. The other analytical data are not available.

Attempted one-pot synthesis of 3-(N-ethyl-3-carbazolyl)-DL-alanine from 14. To a solution of **14** (1.0 g, 2.7 mmol) in Ac_2O (5 mL) was added red phosphorus (0.6 g, 19 mmol) and HI (47%, 4 mL, dropwise). The mixture was heated to 140-150 °C for six hours. The progress of the reaction was followed by normal-phase TLC ($CHCl_3$/MeOH/ AcOH 5:1:0.1). Several new spots with positive ninhydrin tests were observed on TLC. The desired product couldn't be clearly isolated from the other side products due to their similar R_f values. The other analytical data are not available.

Methyl 2-amino-2-(dimethoxyphosphonyl)acetate (17).[51] A solution of (±)-Z-α-phosphono-glycine trimethyl ester (1.5 g, 4.5 mmol) in MeOH (25 mL) was mixed with 10% Pd-C (0.1 g) with stirring under a H_2-filled balloon for 24 hr. The catalyst was removed by filtration through a short column filled with Celite. The filtrate was concentrated to give **17** as a transparent solid (0.8 g, 90%). The analytical data are not available.

Methyl 2-(t-butoxycarbonylamino)-2-(dimethoxyphosphonyl)acetate (18).[51] To a stirred solution of 17 (0.8 g, 4.1 mmol) and di-t-butyldicarbonate (1.2 g, 5.5 mmol) in CH_2Cl_2 (20 mL) was added pyridine (0.3 mL, 4.1 mmol) at rt. The mixture was stirred for 48 hr. The solvent was removed on a rotary evaporator, and the residue dissolved in EtOAc (50 mL). The solution was washed with 5% aq. citric acid, saturated Na_2CO_3 and brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The residue was purified by normal-phase column chromatography (hexane/EtOAc 1:5) to give a semi-transparent solid (1.0 g, 83%): mp 44-46 °C (lit.[51] 44-46 °C); ^{1}H NMR δ 1.50

(s, 9H, CH_3), 3.81 (d, 6H, J = 4.0, CH_3), 3.85 (s, 3H, CH_3), 4.86-4.94 (m, 1H, CH), 5.37 (d, 1H, NH); ^{13}C NMR δ 28.6 (CH_3), 51.3 (OCH_3), 52.7 (OCH_3), 53.7 (OCH_3), 54.6 (CH), 81.4 (C), 167.9 (C=O).

Methyl 2-acetamino-2-(dimethoxyphosphonyl)acetate (19).[50] A solution of (±)-Z-α-phosphonoglycine trimethyl ester (4.9 g, 14.8 mmol) in MeOH (75 mL) was mixed with 10% Pd-C (0.5 g) and Ac_2O (4 mL) with stirring for 24 hr under a H_2 atmosphere maintained with a balloon. The catalyst was removed by filtration through a short column filled with Celite. The solvent was removed on a rotary evaporator and hexane was added to aid in the crystallization. The mixture was stored in a refrigerator overnight. The product was collected by filtration, giving **19** as a semi-transparent solid (3.3 g, 93%): mp 85-87 °C (lit.[51] 85-87 °C); ^{1}H NMR δ 1.98 (s, 3H, CH_3), 3.83 (d, 6H, J = 2.8, CH_3), 3,85 (s, 3H, CH_3), 5.07-5.17 (m, 1H, CH), 6.30 (d, 1H, NH); ^{13}C NMR δ 23.3 (CH_3), 49.6 (OCH_3), 51.1 (OCH_3), 53.8 (OCH_3), 54.7 (CH), 167.6 (C=O), 170.0 (C=O).

N-t-Butoxycarbonyl-3-(N'-ethyl-3-carbazolyl)-DL-acryl acid methyl ester (20a). To a stirred solution of 18 (0.8 g, 2.7 mmol) in dry THF (10 mL) was added 1,1,3,3-tetramethyl-guanidine (0.35 mL, 2.8 mmol) dropwise at -70 °C. N-Ethyl-3-carbazole-carboxaldehyde (0.6 g, 2.7 mmol) in dry THF (7 mL) was added slowly. The mixture was kept at -70 °C for one hour and then warmed to rt and stirred overnight. The progress of the reaction was followed by normal-phase TLC (hexane/EtOAc 1:3) and two adjacent new spots with R_f 0.5 were observed on TLC. Two peaks at δ 1.42 and 0.95 were observed by ^{1}H NMR and assigned to CH_3 peaks belonging to the E and Z isomers, respectively. The solvent was removed on a rotary evaporator. The residue was dissolved in EtOAc (200 mL) and the solution was washed with 5% aq. citric acid, H_2O and brine,

and dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The crude product was purified by normal-phase column chromatography (hexane/EtOAc 1:1), giving **20a** as a white solid (E,Z mixture, 0.76 g, 71%): mp (not available); ^{1}H NMR δ 1.39-1.57 (m, 12H, 4CH_3), 3.87 (s, 3H, CH_3), 4.38 (q, 2H, CH_2), 7.25 (t, 1H, J = 7.2 Hz, ArH), 7.37 (d, 1H, J = 6.4 Hz, ArH), 7.41 (d, 1H, J = 8.1 Hz, ArH), 7.47 (t, 1H, J = 7.1 Hz, ArH), 7,57 (s, 1H, =CH), 7.75 (m, 1H, ArH), 8.07 (d, 1H, J = 7.8 Hz, ArH), 8.34 (s, 1H, NH); ^{13}C NMR (not available).

N-Acetyl-3-(N'-ethyl-3-carbazolyl)-DL-acrylic acid methyl ester (20b). To a stirred solution of 19 (3.2 g, 13.3 mmol) in dry THF (40 mL), cooled to -70 °C, was added tetramethylguanidine (1.5 g, 13.3 mmol) dropwise. N-Ethyl-3-carbazolecarboxaldehyde (2.7 g, 12 mmol) in dry THF (32 mL) was added slowly. The mixture was kept at -70 °C for one hour. The mixture was warmed to rt and stirred overnight. The progress of the reaction was followed by normal phase TLC. Two adjacent new spots were observed on the TLC plate. The solvent was removed on a rotary evaporator. The residue was dissolved in EtOAc (50 mL). The organic phase was washed with 5% aq. citric acid and brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The crude product was purified by normal-phase column chromatography (hexane/EtOAc 1:3), giving **20b** as a white solid (E, Z mixture, 2.6 g, 65%). The peaks at δ 2.15 and 1.85 observed by NMR, were assigned to CH_3 groups belonging to the E and Z isomers, respectively. Mp (not available); ^{1}H NMR δ 1.38 (t, 3H, J = 7.2 Hz, CH_3), 1.85 (s, 0.6H, CH_3), 2.15 (s, 2.4H, CH_3), 3.87 (s, 3H, CH_3), 4.36 (m, 2H, CH_2), 6.57 (s, 0.2H, NH), 7.02 (s, 0.8H, NH), 7.31 (t, 1H, J = 6.8 Hz, ArH), 7.43 (d, 1H, J = 8.1 Hz, ArH), 7.47 (t, 1H, J = 7.5 Hz, ArH), 7.51 (d, 1H, J = 7.7 Hz, ArH), 7.65 (s, 0.8H, =CH), 7.69 (d, 0.8H, J = 8.7

Hz, ArH), 7.84 (s, 0.2H, =CH), 7.85 (d, 0.2H, ArH), 8.03 (d, 0.8H, J = 7.7 Hz, ArH), 8.05 (d, 0.2H, ArH), 8.24 (s, 0.8H, ArH), 8.39 (s, 0.2H, ArH); ^{13}C NMR δ 14.3 (CH_3), 24.0 (CH_3), 38.1 (CH_2), 53.0 (OCH_3), 109.0 (=CH), 109.2 (=C), 119.9 (CH), 120.8 (CH), 121.7 (CH), 123.2 (C), 123.4 (C), 124.7 (C), 126.6 (C), 127.0 (CH), 128.2 (CH), 135.4 (CH), 140.8 (CH), 141.0 (C), 166.6 (C=O), 169.5 (C=O).

N-t-Butoxycarbonyl-3-(N'-ethyl-3-carbazolyl)-DL-alanine methyl ester (21a). A solution of **20a** (0.75 g, 1.9 mmol) in MeOH (15 mL) was mixed with 10% Pd-C (50 mg) with stirring under a H_2-filled balloon at rt for 24 hr. The catalyst was removed by filtration through a column filled with Celite. The filtrate was evaporated to dryness and the residue was purified by normal-phase column chromatography (hexane/EtOAc 3:1) to give **21a** as a white solid (0.69 g, 92%): mp 155-156 °C; ^{1}H NMR δ 1.53 (s, 9H, CH_3), 1.70 (t, 3H, J = 8.3 Hz, CH_3), 3.40 (m, 2H, CH_2), 3.88 (s, 3H, OCH_3), 4.48 (q, 2H, J = 7.2 Hz, CH_2), 4.76 (q, 1H, J = 6.1 Hz, CH), 5.15 (d, 1H, NH), 7.32 (t, 2H, J = 7.7 Hz, ArH), 7.41 (d, 1H, J = 7.5 Hz, ArH), 7.45 (d, 1H, J = 8.3 Hz, ArH), 7.50 (d, 1H, J = 7.8 Hz, ArH), 7.96 (s, 1H, ArH), 8.19 (d, 1H, J = 7.7 Hz, ArH); ^{13}C NMR (not available).

N-Acetyl-3-(N'-ethyl-3-carbazolyl)-DL-alanine methyl ester (21b). A solution of **20b** (2.55 g, 7.5 mmol) in MeOH (40 mL) was mixed with 10% Pd-C (0.25 g) with stirring under a H_2-filled balloon at rt for 48 hr. The catalyst was filtered through a column filled with Celite. The filtrate was evaporated to dryness and the residue purified by normal-phase column chromatography (hexane/EtOAc 1:3) to give **21b** as a white solid (2.55 g, 99%): mp 80 °C; ^{1}H NMR δ 1.45 (t, 3H, *J* = 7.2 Hz, CH_3), 2.06 (s, 3H, CH_3), 3.33 (m, 2H, CH_2), 3.77 (s, 3H, CH_3), 4.38 (q, 2H, *J* = 7.2 Hz, CH_2), 4.97 (q, 1H, *J* = 5.8 Hz, CH), 5.98 (d, 1H, NH), 7.20 (t, 1H, *J* = 6.7 Hz, ArH), 7.27 (d, 1H, *J* = 5.8 Hz, ArH), 7.35 (d,

1H, *J* = 8.3 Hz, ArH), 7.42 (d, 1H, *J* = 8.2 Hz, ArH), 7.48 (t, 1H, *J* = 6.0 Hz, ArH), 7.84 (s, 1H, ArH), 8.07 (d, 1H, *J* = 7.7 Hz, ArH); ^{13}C NMR δ 14.3 (CH_3), 23.6 (CH_3), 38.0 (CH_2), 38.4 (CH_2), 52.7 (CH), 54.2 (OCH_3), 108.5 (CH), 108.9 (CH), 116.8 (CH), 121.2 (C), 122.6 (C), 123.7 (C), 124.2 (C), 125.9 (CH), 126.7 (CH), 138.8 (CH), 139.9 (CH), 142.3 (C), 170.2 (C=O), 172.9 (C=O).

N-t-Butoxycarbonyl-3-(N'-ethyl-3-carbazolyl)-L-alanine (22). Compound **21a** (160 mg, 0.42 mmol) was suspended in a solution of DMF (10 mL) and phosphate buffer (0.5 M, pH 7.7, 8 mL). ChiroCLEC-BL (16 mg) was added. The mixture was stirred at 60 °C for 72 hr. The progress of the reaction was followed by reverse-phase TLC. A new spot with R_f 0.9 (EtOH/H_2O 4:1) became bigger with time. The enzyme was removed by centrifugation and the solvents were removed on a rotary evaporator. The base-soluble product was dissolved in 5% aq. Na_2CO_3, and the unhydrolytic starting material was extracted with EtOAc. The organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed on a rotary evaporator to give 21a (140 mg). The aqueous phase was brought to pH 4 and extracted with EtOAc (5 × 200 mL). The organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed on a rotary evaporator to give **22** as a white solid (10 mg, 16%). Better conversion couldn't be achieved by varying the temperature or the solvents. The analytical data are not available.

N-Acetyl-3-(N'-ethyl-3-carbazolyl)-L-alanine (23). Compound 21b (2.50 g, 7.4 mmol) was mixed with acetone (40 mL) and phosphate buffer (0.25 M, pH 7.5, 60 mL). ChiroCLEC-BL (80 mg) was added. The mixture was agitated on an orbital shaker at 200 rpm at 37 °C for 24 hr. The progress of the reaction was followed by reverse-phase TLC (EtOH/H_2O 4:1, R_f 0.9). The enzyme was removed by centrifugation and the solvents

were removed on a rotary evaporator. Then the hydrolyzed product was dissolved in 5% aq. Na_2CO_3, and unhydrolyzed starting material was extracted with EtOAc. The organic phase was dried over Na_2SO_4. The solvent was removed on a rotary evaporator to give **21b** (120 mg, enriched in D-isomer). The aqueous phase was brought to pH 4 and extracted with EtOAc (5 × 200 mL). The organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed on a rotary evaporator to give **23** as an off-white foam (110 mg, 100%): mp 75 °C; ^{1}H NMR δ 1.40 (t, 3H, *J* = 7.1 Hz, CH_3), 2.05 (s, 3H, CH_3), 3.35 (m, 2H, CH_2), 4.32 (m, 2H, CH_2), 4.95 (m, 1H, CH), 6.18 (d, 1H, *J* = 7.1 Hz, NH), 7.20 (t, 1H, *J* = 7.3 Hz, ArH), 7.26 (d, 1H, *J* = 5.7 Hz, ArH), 7.32 (d, 1H, *J* = 8.3 Hz, ArH), 7.39 (d, 1H, *J* = 8.1 Hz, ArH), 7.41 (t, 1H, *J* = 7.2 Hz, ArH), 7.88 (s, 1H, ArH), 8.06 (d, 1H, *J* = 7.7 Hz, ArH) 8.65 (bs, 1H, OH); ^{13}C NMR δ 14.2 (CH_3), 23.4 (CH_3), 37.7 (CH_2), 38.0 (CH_2), 54.3 (CH), 108.9 (CH), 109.1 (CH), 119.2 (CH), 120.7 (CH), 121.5 (CH), 122.9 (C), 123.5 (C), 126.1 (C), 126.2 (CH), 127.1 (CH), 139.6 (C), 140.5 (C), 171.4 (C=O), 175.9 (C=O).

3-(N-Ethyl-3-carbazolyl)-L-alanine (24). Compound **23** (1.20 g, 3.7 mmol) was dissolved in NaOH (20 mL, 1 M). Then HCl (2 M) and H_2O were added in order to adjust the pH to 8 and the final concentration of the substrate to 0.05-0.1 M. Amanoacylase (30 mg) and $CoCl_2$ (10 mg) were added. The mixture was placed on a shaker at 37 °C for 24 hr. A light pink precipitate formed. The progress of the reaction was followed by normal-phase TLC ($CHCl_3$/MeOH/AcOH 5:1:0.1). The spot assigned to **23** (R_f 0.6) almost disappeared on the TLC plate and a new spot assigned to **24** (R_f 0.8) gave a positive ninhydrin test. The solid was collected by centrifugation and dissolved again in EtOH (250 mL) containing HCl (1 M, 5 mL). An insoluble solid was removed by filtration. The

filtrate was heated to 50 °C with a small amount of charcoal in order to denature the amanoacalyse and the charcoal was removed by filtration thought a short column filled with Celite. The solution was evaporated to dryness. A dry reverse-phase flash column chromatography (MeCN/H_2O/MeOH 2:1:0.5) was carried out and gave **24** as a white solid (1.02 g, 97%): mp (not available); ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3H, *J* = 6.9 Hz, CH_3), 3.01 (m, 1H, CH_2), 3.04 (m, 1H, CH_2), 3.06-3.33 (m, 4H, OH, NH_2, CH), 4.41 (m, 2H, CH_2), 7.15 (t, 1H, *J* = 7.4 Hz, ArH), 7.37 (d, 1H, *J* = 8.3 Hz, ArH), 7.41 (t, 1H, *J* = 7.8 Hz, ArH), 7.45 (d, 1H, *J* = 7.4 Hz, ArH), 7.57 (d, 1H, *J* = 8.1 Hz, ArH), 7.60 (s, 1H, ArH), 8.06 (d, 1H, *J* = 7.7 Hz, ArH), 12.8 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ 13.6(CH_3), 36.8(CH_2), 38.7(CH_3), 56.0(CH), 108.8, 108.9, 118.4, 120.1, 120.8, 121.9, 122.1, 125.4, 127.2, 127.5, 138.4, 139.6, 169.5(C=O); ES-MS calcd for $C_{17}H_{19}N_2O_2$ [M + 1]$^+$ 283.1, found 283.2.

N-Fmoc-3-(N'-ethyl-3-carbazolyl)-L-alanine (25). Aq. Na_2CO_3 (10%, 10 mL) was added to **24** (1.0 g, 3.5 mmol), and a small amount of $NaHCO_3$ and H_2O were added to dissolve the substrate and adjust the pH to 10-11. Then dioxane (10 mL) was added and the solution was cooled to 0 °C. Fmoc-OSu (1.1 g, 3.2 mmol) was dissolved in dioxane (5 mL) and added dropwise. The mixture was stirred at 0 °C for 30 min, and at rt for an additional four hours. A white precipitate formed during the reaction. The progress of the reaction was followed by normal-phase TLC ($CHCl_3$/MeOH/AcOH 9:1:0.1). Et_2O (30 mL) was added to extract impurities. The aqueous solution was brought to pH 3 and extracted with EtOAc. The organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed on a rotary evaporator and the residue was purified by normal-phase column chromatography (CH_2Cl_2/MeOH 11:1). The material isolated by

chromatography showed two spots close to each other on TLC plate. Further purification was done by recrystallization from $CHCl_3$ to give **25** as a pure white powder (0.65 g, 40%): mp 158-160 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 (t, 3H, *J* = 7.0 Hz, CH_3), 3.05 (m, 1H, CH_2), 3.65 (m, 1H, CH_2), 4.14 (m, 3H, CH, CH_2), 4.17 (m, 1H, CH), 4.41 (m, 2H, CH_2), 7.15 (m, 2H, ArH), 7.17 (t, 1H, *J* = 7.4 Hz, ArH), 7.37 (m, 4H, ArH), 7.50 (m, 1H, ArH), 7.57 (m, 2H, ArH), 7.84 (d, 2H, *J* = 7.4 Hz, ArH), 8.06 (s, 1H, ArH), 8.07 (d, 1H, *J* = 3.7 Hz, ArH), 12.8 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ13.6(CH_3), 36.5(CH_2), 36.8(CH_2), 46.4 (CH_2,), 56.2(CH), 65.5(CH), 108.6, 108.9, 118.4, 119.9, 120.1, 120.5, 121.8, 121.9, 125.2, 125.5, 126.9, 127.5, 128.1, 138.3, 139.6, 140.5, 143.5, 143.6, 155.9(C=O), 173.5(C=O); ES-MS calcd for $C_{32}H_{29}N_2O_4$ $[M + 1]^+$ 505.2, found 505.4.

4. 6 Solid-phase peptide synthesis

4. 6. 1 4-Mer model peptide by Boc-strategy solid-phase peptide synthesis (26, H-Car-D-Ala-Bpa-D-Ala-OH)

- Swelling: Boc-D-Ala-PAM resin (0.2 g, 0.16 mmol) was swollen in CH_2Cl_2 (10 mL) for one hour with N_2 bubbling, and the CH_2Cl_2 drained.
- Deprotection: The resin was treated twice with 50% TFA/CH_2Cl_2 (10 mL) with N_2 bubbling for 30 min. A positive ninhydrin test (blue beads and blue solution) indicated the presence of a primary amine.

- Washing: The resin was washed with CH_2Cl_2, 20% DIPEA/CH_2Cl_2, DMF, MeOH, and CH_2Cl_2 (5 × 10 mL each), the solvents drained and the resin dried in vacuum.
- Activation and coupling: The coupling reagents (listed in Table 3.1) were mixed in a round flask and stirred for 5 min, and then added to the resin with N_2 bubbling for the indicated period. The negative ninhydrin test signaled complete coupling.

Table 4-1 The quantities of coupling reagents and reaction times for synthesis of 4-mer model peptide (**26**).

Step	Coupling reagents	Reaction time (hr)
1	Boc-4-benzoyl-L-phenylalanine (296 mg, 0.8 mmol), PyBOP (416 mg, 0.8 mmol), HOBT (122 mg, 0.8 mmol), and DIPEA (0.28 mL) dissolved in DMF (5 mL)	16
2	Boc-D-alanine (151 mg, 0.8 mmol), PyBOP (416 mg, 0.8 mmol), HOBT (122 mg, 0.8 mmol), and DIPEA (0.28 mL) dissolved in DMF (5 mL)	4
3	N-Boc-3-(N'-ethyl-3-carbazolyl)-L-alanine (191 mg, 0.5 mmol), PyBOP (260 mg, 0.5 mmol), HOBT (77 mg, 0.5 mmol), and DIPEA (0.18 mL) dissolved in DMF (3.2 mL)	18

- Washing after coupling: The solvents were removed and the resin was washed with DMF, MeOH, EtOH and CH_2Cl_2 (5 × 10 mL each), the solvent drained, and the resin dried in vacuum.
- Final washing before cleavage: The resin was washed with DMF, CH_2Cl_2, MeOH, and Et_2O (5 × 10 mL each), the solvents drained, and the resin dried in vacuum. It weighed 0.22 g.
- Cleavage: The dry resin was placed into a round-bottom flask. Thiolanisole (0.5 mL) and ethanedithiol (0.25 mL) were added with stirring for 2 min. After the solution was cooled to 0 °C, TFA (5 mL) was dropped in. After 10 min, TFMSA

(0.5 mL) was added dropwise. Then the ice-bath was removed and the mixture was stirred at rt for 1 hour. After that, the resin was removed on a sintered funnel under reduced pressure. The resin was washed with TFA twice. Then cold Et_2O (100 mL) was added into the filtrate, and the mixture was stored in the refrigerator overnight. No precipitate was observed. The TFA was removed under vacuum. The residue was poured into cold Et_2O (100 mL) and the mixture was stored in the refrigerator overnight. Again, no precipitate was observed. The solvents were evaporated under vacuum (< 80 °C). Finally, a yellow solid-like residue with an oily liquid on the surface was obtained. Cold Et_2O was used to wash away the oily impurity. After filtration and drying, this light yellow solid weighed 0.55 g and showed a positive Kaiser test. It was washed with cold Et_2O again. It weighed 0.45 g (> 100% yield). ES-MS calcd for $C_{41}H_{43}N_5O_8$, 790.8 $[M + 1]^+$ as a TFA salt, found 791.4.

4. 6. 2 8-Mer model peptide by Fmoc-strategy solid-phase peptide synthesis (27, H-D-Ala-Nap-D-MeAla-Ala-D-Ala-Bpa-D-Ala-Ala-OH)

- Swelling: Fmoc-Ala-Wang resin (0.3 g, 0.18 mmol) was swollen in DMF (10 mL) for 1 hour with N_2 bubbling, and the CH_2Cl_2 drained.
- Deprotection: The resin was treated with 25% piperidine/DMF (10 mL) with N_2 bubbling for 30 min, twice. A positive Kaiser test indicated the presence of a primary amine and a positive chloranil test indicated a secondary amine (deep blue beads).

- Washing: The resin was washed with DMF, CH_2Cl_2, and DMF (5 × 10 mL each), the solvents drained, and dried in vacuum.
- Activation and coupling: The coupling reagents (listed in Table 4.2) were mixed in a round flask and stirred for 5 min, and then added to the resin with N_2 bubbling for the indicated period. The negative Kaiser or Chloranil test signaled complete coupling.

Table 4-2 The quantities of coupling reagents and reaction times for synthesis of 8-mer model peptide (**27**).

Step	Coupling reagents	Reaction time (hr)
1, 3, 7	Fmoc-D-alanine (280 mg, 0.9 mmol), PyBOP (468 mg, 0.9 mmol), HOBT (137 mg, 0.9 mmol), and DIPEA (0.31 mL) dissolved in DMF (6 mL)	4
2	Fmoc-4-benzoyl-L-phenylalanine (265 mg, 0.54 mmol), PyBOP (281 mg, 0.54 mmol), HOBT (137 mg, 0.54 mmol), and DIPEA (0.19 mL) dissolved in DMF (3.6 mL)	16
4	Fmoc-alanine (280 mg, 0.9 mmol), PyBOP (468 mg, 0.9 mmol), HOBT (137 mg, 0.9 mmol), and DIPEA (0.31 mL) dissolved in DMF (6 mL)	4
5	N-Fmoc-N'-methyl-D-alanine (293 mg, 0.9 mmol), PyBOP (468 mg, 0.9 mmol), HOBT (137 mg, 0.9 mmol), and DIPEA (0.31 mL) dissolved in DMF (6 mL)	4
6	Fmoc-L-naphthylalanine (236 mg, 0.54 mmol), PyBOP (281 mg, 0.54 mmol), HOBT (137 mg, 0.54 mmol), and DIPEA (0.19 mL) dissolved in DMF (6 mL)	16

- Washing after coupling: The resin was washed with DMF, CH_2Cl_2, MeOH, and DMF (5 × 10 mL each), the solvents drained, and the resin dried in vacuum.
- Washing before cleavage: The resin was washed with DMF, CH_2Cl_2, MeOH, and Et_2O (5 × 10 mL each), the solvent drained and the resin dried in vacuum. Fmoc-8-mer peptide-bound resin (270 mg) was obtained. A part of the resin (70 mg) was taken out before deprotection.

- Deprotection: The remaining resin (200 mg) was treated with 25% piperidine/ DMF (10 mL) for 30 min.
- Cleavage: Fmoc-protected and Fmoc-deprotected peptide-bound resins were cleaved according to the same procedure. The Fmoc-protected resin was treated with TFA (1.75 mL) and H_2O (0.10 mL). The suspension was stirred in an ice-bath for 30 min and at rt for an additional two hours. The Fmoc-deprotected resin was treated with TFA (4.75 mL) and H_2O (0.25 mL). The suspension was stirred in an ice-bath for 30 min and at rt for an additional two hours. The resin was separated on a sintered funnel and then washed twice with a small amount of TFA. The filtrate was evaporated to dryness (< 50 °C). Cold Et_2O was added into the residue, and the mixture was stored in a refrigerator overnight. The solvent was removed by centrifugation to give a white precipitate, which was dried in vacuum (20 mg for Fmoc-protected peptide and 90 mg for Fmoc-deprotected peptide).
- For the N-deprotected-peptide, ES-MS calcd for $C_{48}H_{59}N_8O_{10}$ $[M + 1]^+$ 907.4, found 907.2. For the N-Fmoc protected-peptide, no molecular ion peak or any fragment peak was identified (calcd for $C_{63}H_{69}N_8O_{12}$ $[M + 1]^+$ 1129.50).

4. 6. 3 8-Mer bichromophoric peptide by Fmoc-strategy solid-phase peptide synthesis (28, H-Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe-OH)

- Swelling: Fmoc-Phe-Wang resin (0.35 g, 0.224 mmol) was swollen in DMF (5 mL) for 1 hour, and drained the solvents.
- Deprotection: The resin was treated with 25% piperidine in DMF (5 mL) with N_2 bubbling for 30 min, twice. A positive Kaiser test indicated the presence of a

primary amines and positive TBNS (orange beads) or chloranil test indicated a primary amines with a highly-hindered Aib unit, especially.

- Washing: The resin was washed with DMF, CH_2Cl_2, and DMF (5 × 10 mL each), drained the solvents and the resin dried in vacuum.
- Activation and coupling: The coupling reagents (listed in Table 4.3) were mixed in a round flask and stirred for 5 min, and then added to the resin with N_2 bubbling for the indicated period. Negative Kaiser, TBNS or chloranil tests signaled complete coupling.

Table 4-3 The quantities of coupling reagents and reaction times for synthesis of 8-Mer bichromophoric peptide (**28**).

Step	Coupling reagents	Reaction time (hr)
1, 3, 5, 7	Fmoc-α-aminoisobutyric acid (364 mg, 1.12 mmol), PyBOP (582 mg, 1.12 mmol), HOBT (170 mg, 1.12 mmol), and DIPEA (0.4 mL) dissolved in DMF (7.5 mL)	6
2	Fmoc-4-benzoyl-L-phenylalanine (329 mg, 0.67 mmol), PyBOP (348 mg, 0.67mmol), HOBT (102 mg, 0.67 mmol), and DIPEA (0.25 mL) dissolved in DMF (4.5 mL)	16
4	Fmoc-D-phenylalanine (434mg, 1.12 mmol), PyBOP (582 mg, 1.12 mmol), HOBT (170 mg, 1.12 mmol), and DIPEA (0.4 mL) dissolved in DMF (7.5 mL)	12
6	N-Fmoc-3-(N'-ethyl-3-carbazolyl)-L-alanine (283 mg, 0.56 mmol), PyBOP (291 mg, 0.56 mmol), HOAT (85 mg, 0.56 mmol), and DIPEA (0.2 mL) dissolved in DMF (4 mL)	18

- Washing: The resin was washed with DMF, CH_2Cl_2, MeOH and DMF (5 × 10 mL each), drained the solvents and the resin dried in vacuum.
- Final washing: The resin was washed with DMF, CH_2Cl_2, MeOH, and Et_2O (5 × 10 mL each), drained the solvents and the resin dried in vacuum.

- Cleavage: The dry resin (0.3 g) was treated with a solution of TFA/H_2O/ triisopropylsilane (95: 2.5: 2.5, 8 mL). The suspension was stirred at an ice-bath for 30 min and at rt for an additional three hours. The resin was separated on a sintered funnel, and washed with a small amount of TFA, twice. The filtrate was evaporated to dryness (< 50 ^oC). The cold Et_2O was added into the residue, and a white precipitate formed. The mixture was stored in a refrigerator overnight. The solvent was removed by centrifugation and the white solid was dried in vacuum (150 mg, 60%). ES-MS calcd for $C_{67}H_{78}N_9O_{10}$ $[M + 1]^+$ 1168.6, found 1168.8.

4. 6. 4 Cyclization in solution (29, c*yclo*(Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe))

The linear peptide **28** (100 mg, 0.086 mmol) was dissolved in DMF (15 ml). A solution including HATU (43 mg, 0.11 mmol) and HOAt (16 mg, 0.11 mmol) in DMF (2 mL) was added with stirring under N_2. The mixture was cooled to 0 °C. DIPEA (0.15 mL) was added dropwise. The color of the solution changed from colorless to yellow. The progress of the reaction was followed by normal-phase TLC ($CHCl_3$/MeOH 10:1). After being stirred overnight, the reaction was quenched with a small amount of H_2O. Aq. Na_2CO_3 (5%, 60 mL) was added, and then EtOAc was used to extract the desired product (3 × 100 mL). The combined organic phase was washed with brine, $KHSO_4$ (3 × 20 mL) and brine. The solvent was dried over Na_2SO_4 and removed on a rotary evaporator to give an oily residue. It was dissolved in $CHCl_3$, and then the solvent was removed. Cold Et_2O was added slowly and the mixture was stored in the refrigerator overnight for crystallization. After removal of the solvent by centrifugation, a semitransparent solid,

cyclo(Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe, was obtained (70 mg, 70%). ES-MS calcd for $C_{67}H_{76}N_9O_9$ $[M + 1]^+$ 1150.6, found 1150.8, 1172.8 $[M + Na]^+$.

Chapter 5 Summary

1. The Sorensen method was adopted to synthesize N-Ac (or Boc)-3-(9-anthryl)-DL-alanine methyl ester.
2. Erlenmeyer's azlactone method was found to be unsuitable for the synthesis of 3-(N-ethyl-3-carbazolyl)-DL-alanine due to sensitivity of the carbazole ring to strong acids and electrophilic reagents. N-Ac (or N-Boc)-3-(N'-ethyl-3-carbazolyl)-DL-alanine methyl ester was successfully obtained by a Wittig-Horner reaction under mild conditions.
3. Dual enzymatic hydrolysis was adopted to obtain amino acids with high enantiomeric purity.

    - ChiroCLEC-BL was used to selectively remove the methyl group from L-amino acid methyl esters. N-acetyl-3-(N'-ethyl-3-carbazolyl)-L-alanine was completely hydrolyzed in acetone-phosphate buffer at 37 °C after 24 hr; these conditions didn't work for N-acetyl-3-(9-anthryl)-L-alanine methyl ester and their Boc derivatives. A 40% of conversion for this resolution was obtained in DMF-phosphate buffer at 60 °C for 72 hr only for N-Ac-3-(9-anthryl)-L-alanine methyl ester. But this enzyme doesn't work well for their Boc derivatives.

- Amanoacylase was used to remove the acetyl groups. Complete deacylation was achieved after 24 hr for N-acetyl-3-(N'-ethyl-3-carbazolyl)-L-alanine and 48 hr for N-acetyl-3-(9-anthryl)-L-alanine, both at 37 °C.

4. The 8-mer linear peptide (H-Aib-Car-Aib-Phe-Bpa-Aib-Phe-OH) was effectively synthesized via Fmoc-strategy solid-phase peptide synthesis, and its corresponding cyclic peptide was obtained in solution. ES-MS confirmed the molecular weights of all target compounds.

Chapter 6 Future Work

Possible future work could include the following investigations.

1. The photophysics of the target linear peptide and its corresponding cyclic peptide. Absorption, fluorescence, and phosphorescence spectroscopy, and laser flash photolysis could be studied to determine the pathway of the intramolecular energy transfer and its efficiency.
2. X-ray analysis of the target peptides could be used to determine their conformations.
3. IR analyses of the target peptides can be used to study their hydrogen-bonding interactions.
4. Based on the above experimental results, computational chemistry studies could be used to seek good calculational models and design novel peptide-based nanotubes with desirable properties.
5. Another set of linear and cyclic peptides incorporating a different chromophore, H-D-Ala-Car-D-Ala-Phe-D-Ala-Bpa-D-Ala and *cyclo*(D-Ala-Car-D-Ala-Phe-D-Ala-Bpa-D-Ala), where Ala is to be replaced by Aib, would be synthesized. This cyclic compound would be expected to self-assemble into nanotubes.
6. Another new bichromophoric peptide pair, including anthracene and benzophenone, would be synthesized to make a contrasting investigation in search of the high-performance molecular wire.
7. Changing the attachment position of the bichromophores can be used to study the influence of distance between the chromophores on intramolecular energy transfer.

Chapter 7 References

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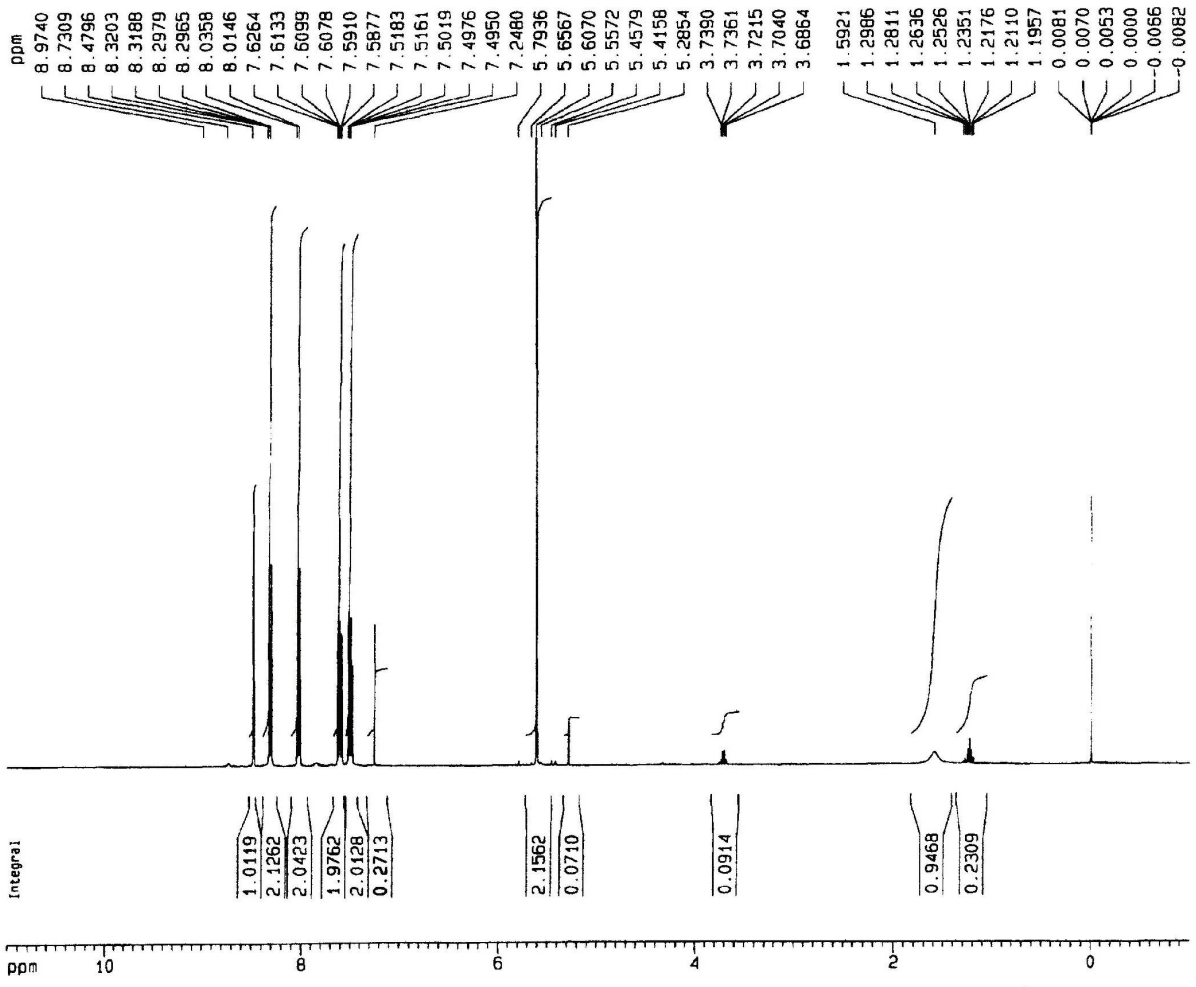
9-Chloromethylanthracene (NMR-1)

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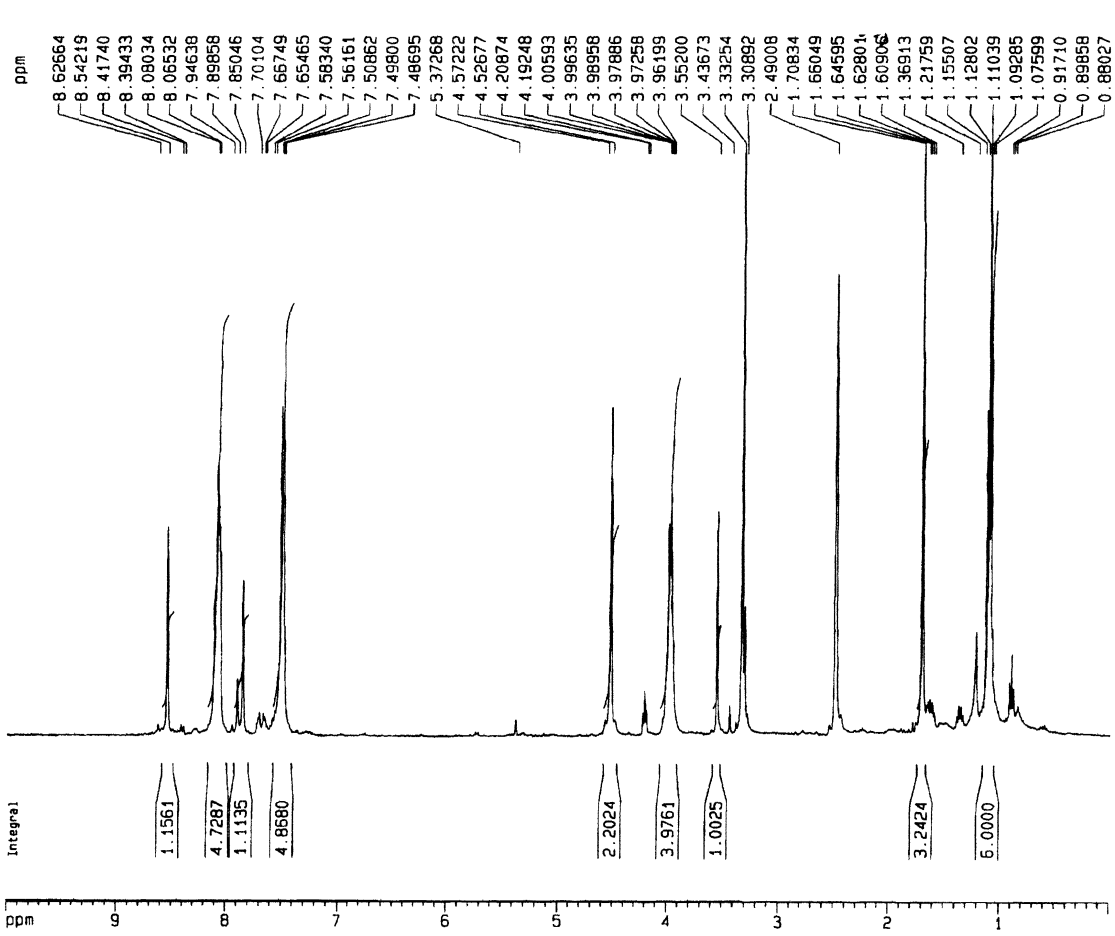

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1D NMR plot parameters

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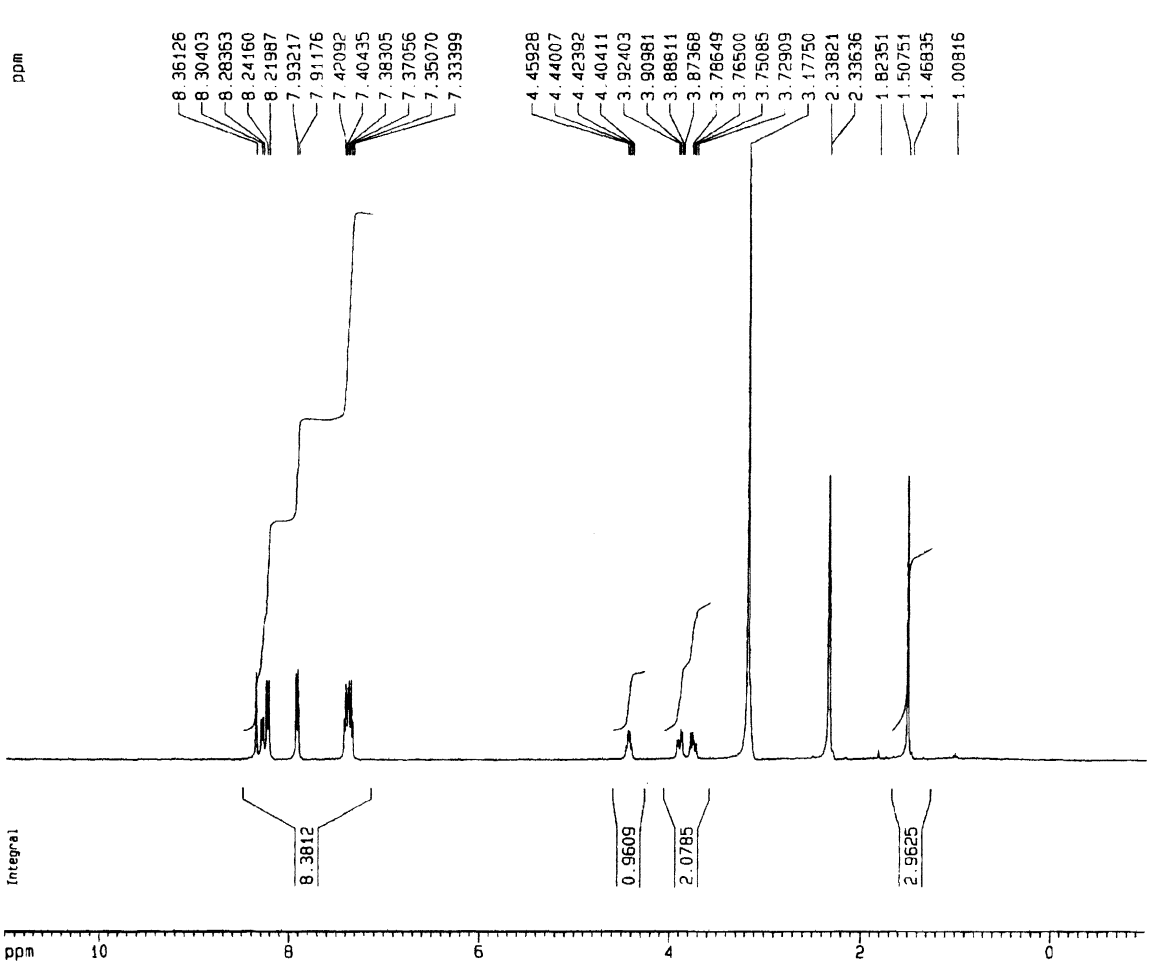
N-Acetyl-3-(9-anthryl)-DL-alanine (NMR-3)

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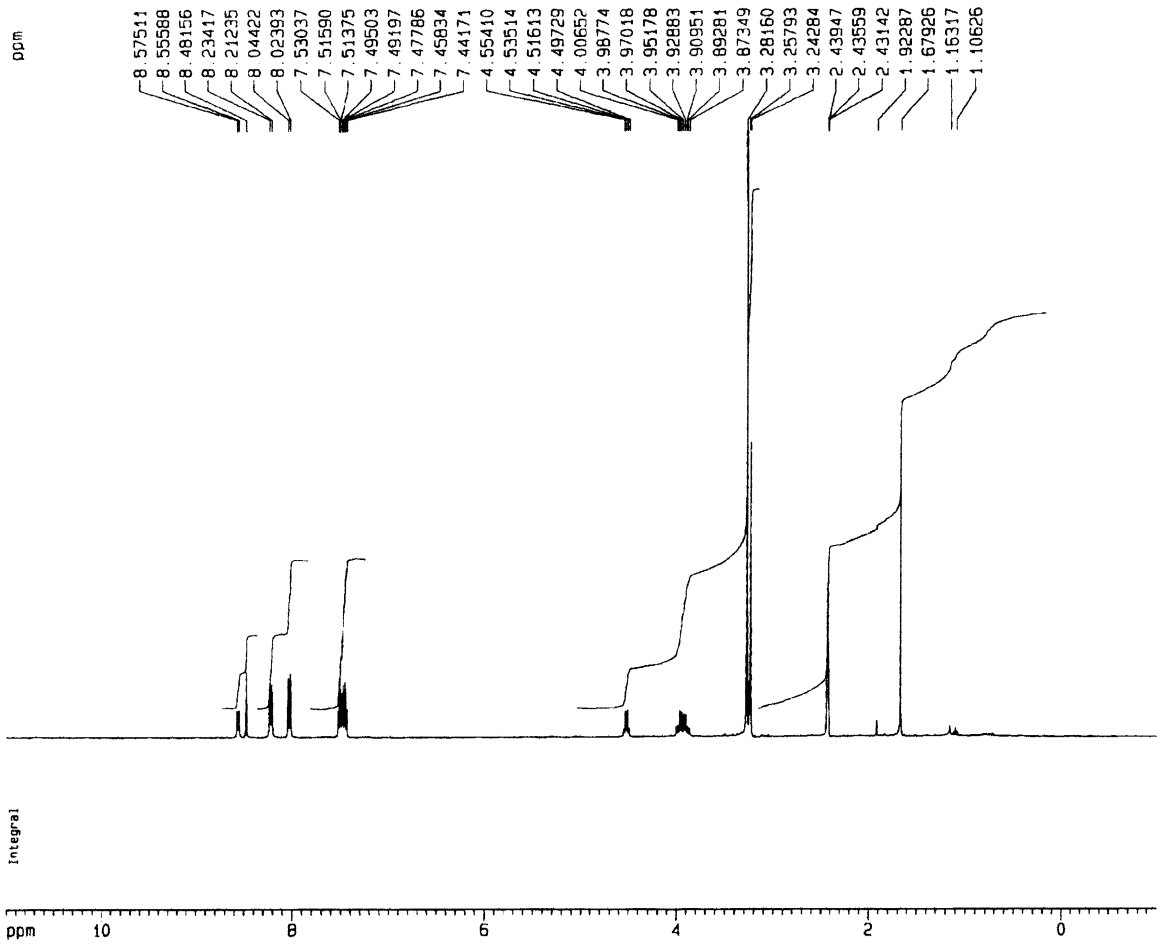

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1D NMR plot parameters

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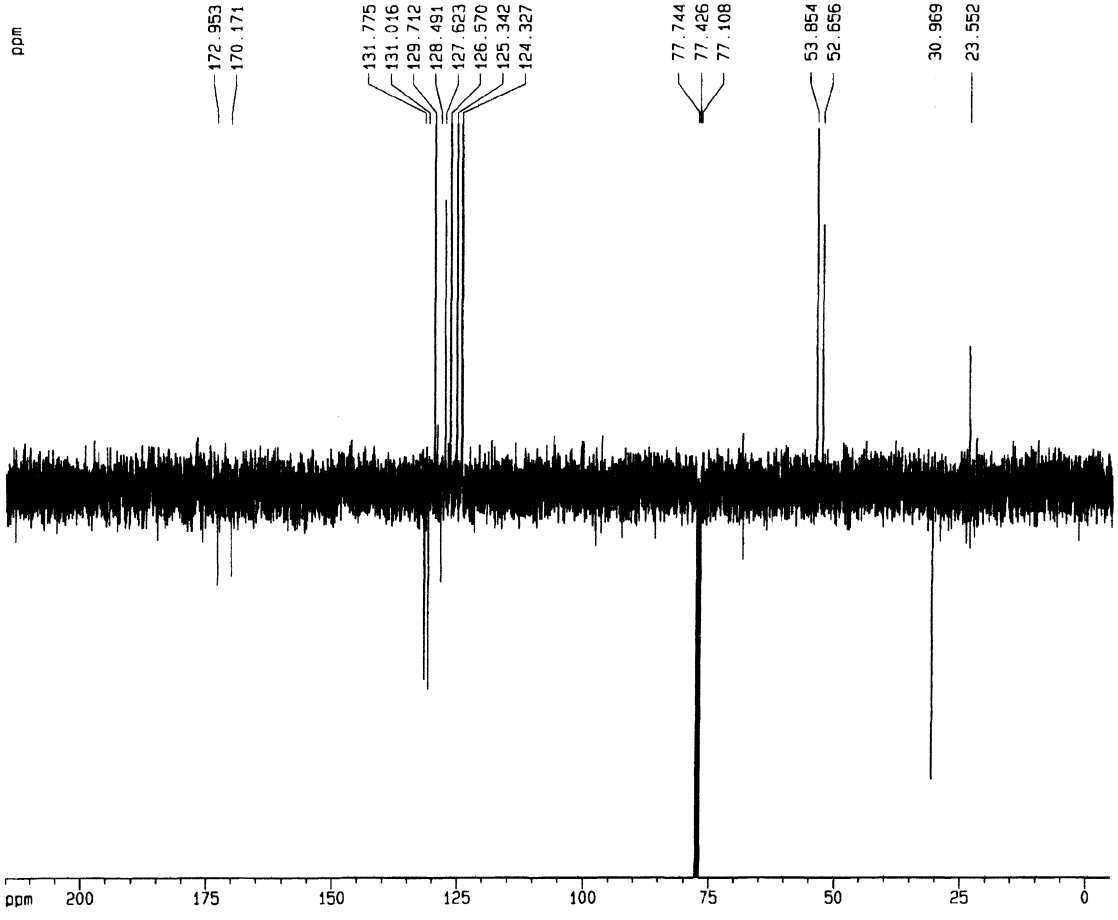
N-Acetyl-3-(9-anthryl)-DL-alanine methyl ester (NMR-5)

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p2                         21.40 usec
PL1                        -1.00 dB
SFO1                100.6237959 MHz

========== CHANNEL f2 ==========
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NUC2                         1H
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1D NMR plot parameters
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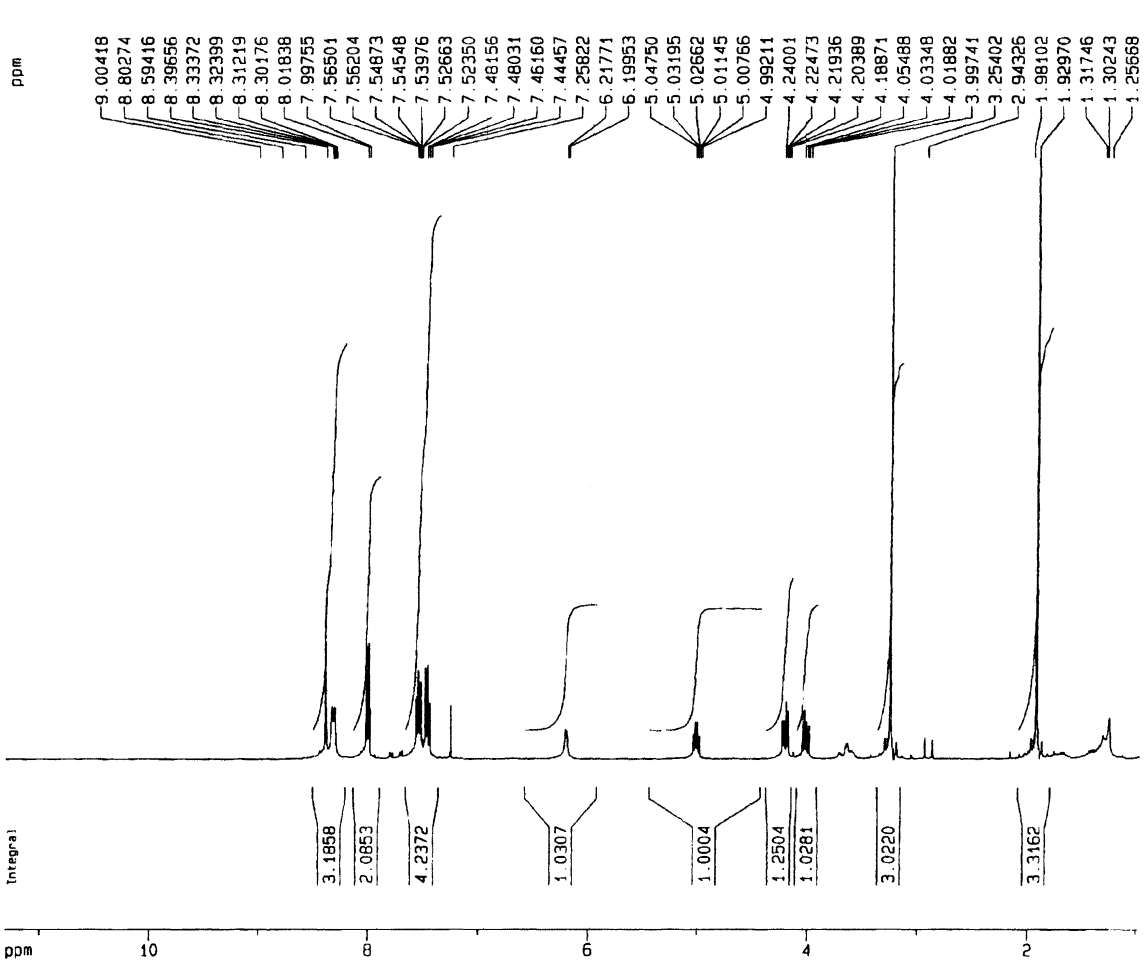

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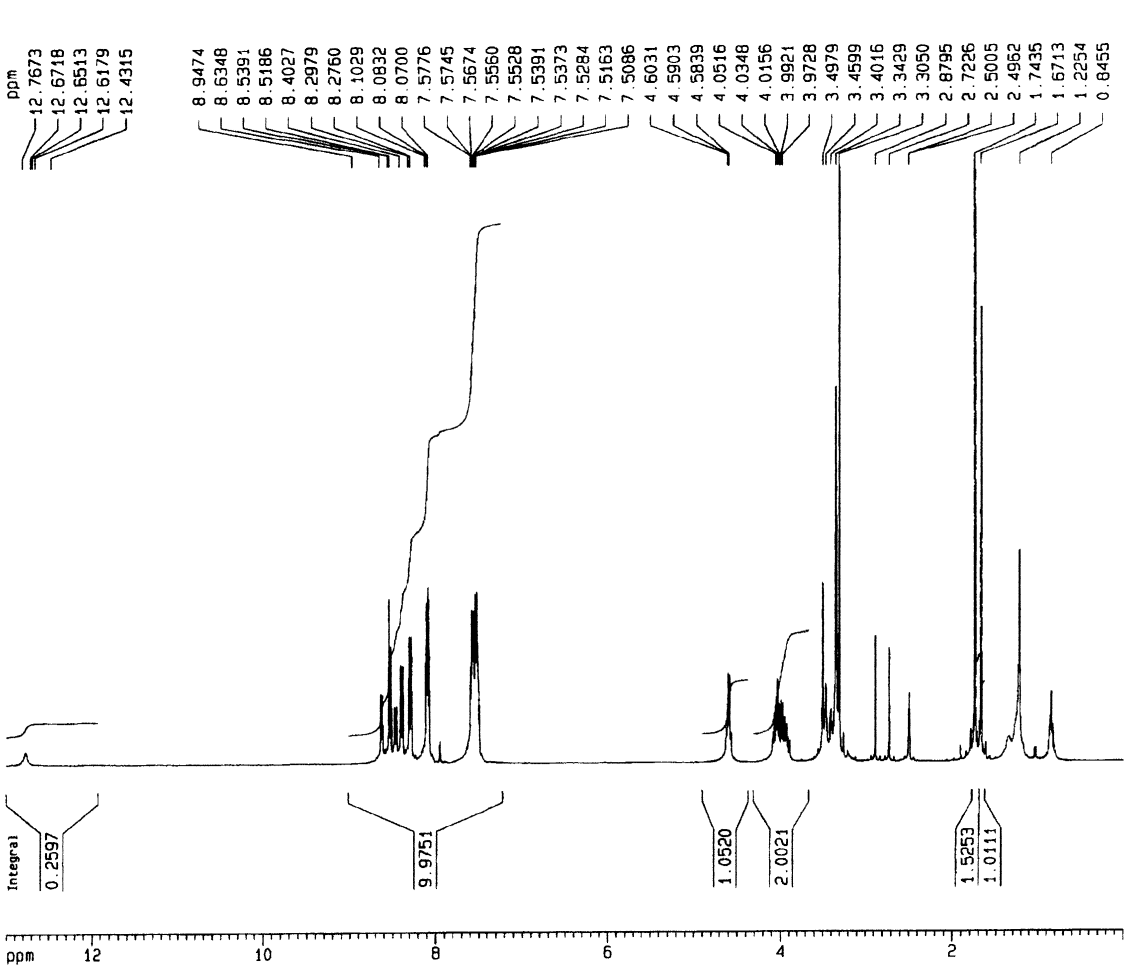

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========== CHANNEL f1 ========

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1D NMR plot parameters

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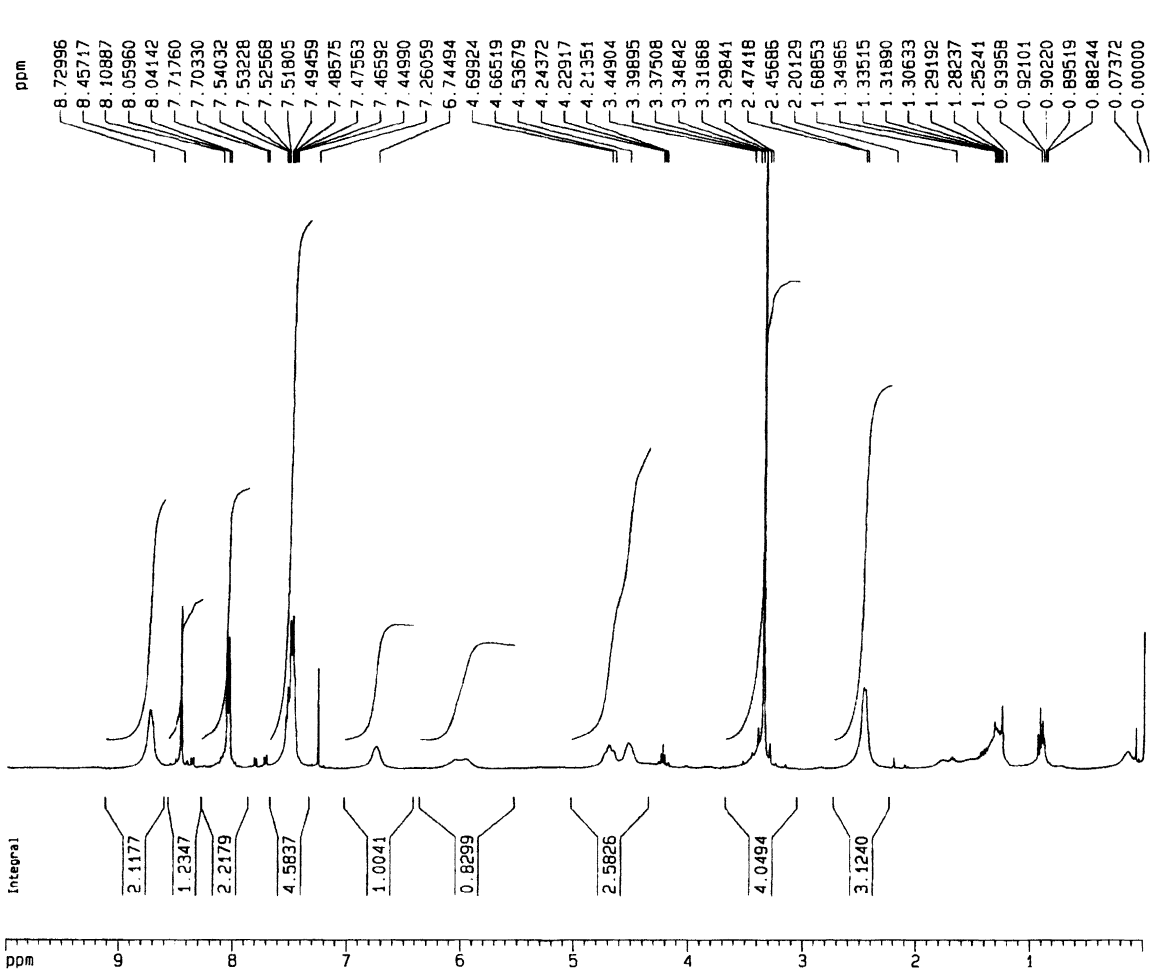
N-Acetyl-3-(9-anthryl)-DL-alanine methyl ester (with chiral reagent, NMR-8)



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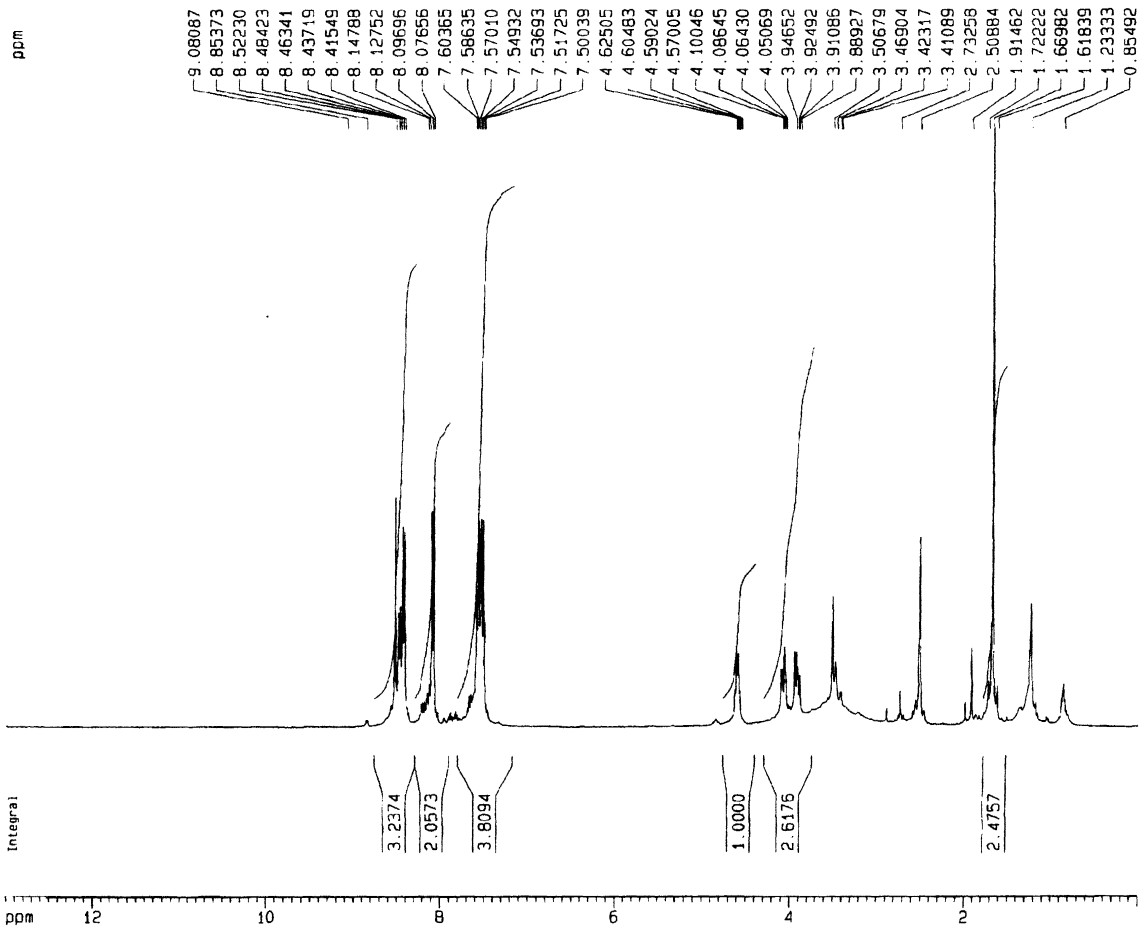
1D NMR plot parameters

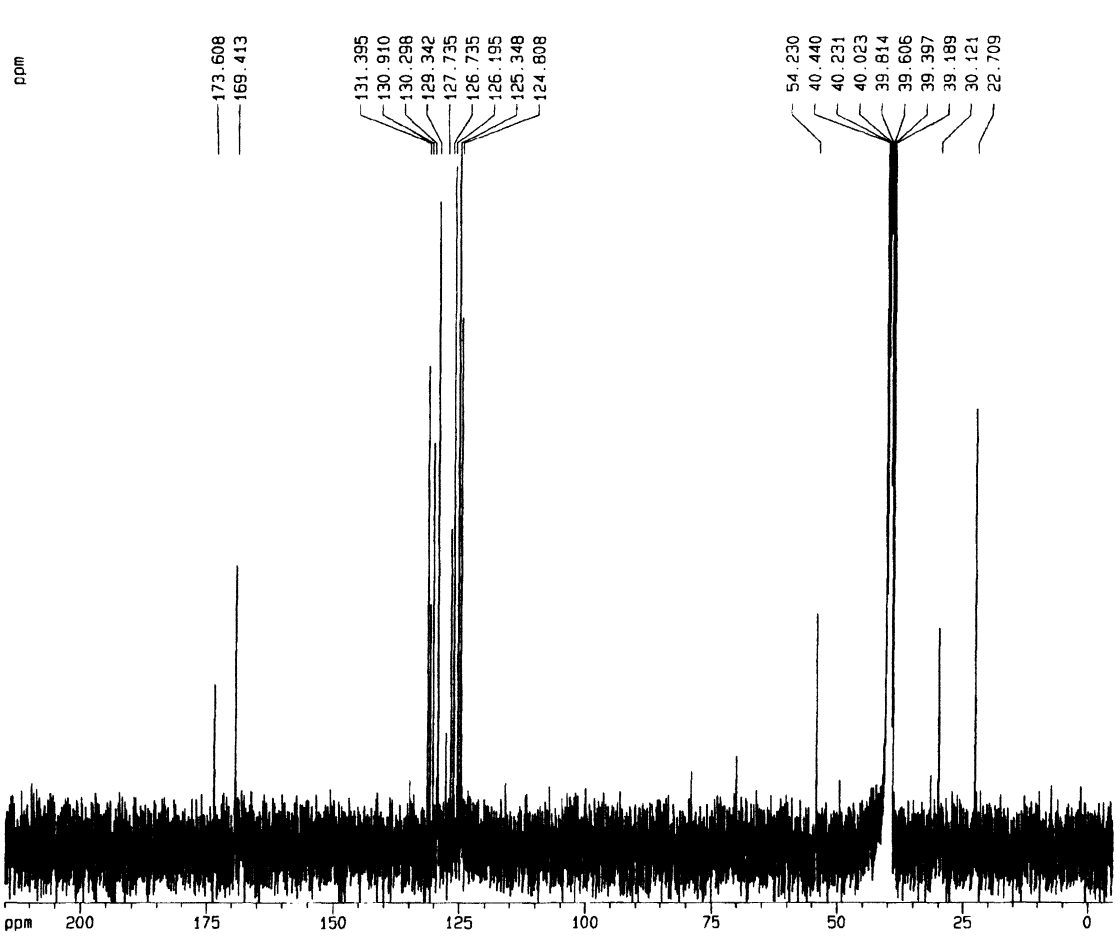
CX	20.00 cm
F1P	10.000 ppm
F1	4001.30 Hz
F2P	0.000 ppm
F2	0.00 Hz
PPMCM	0.50000 ppm/cm
HZCM	200.06500 Hz/cm

N-Acetyl-3-(9-anthryl)-L-alanine (NMR-9)



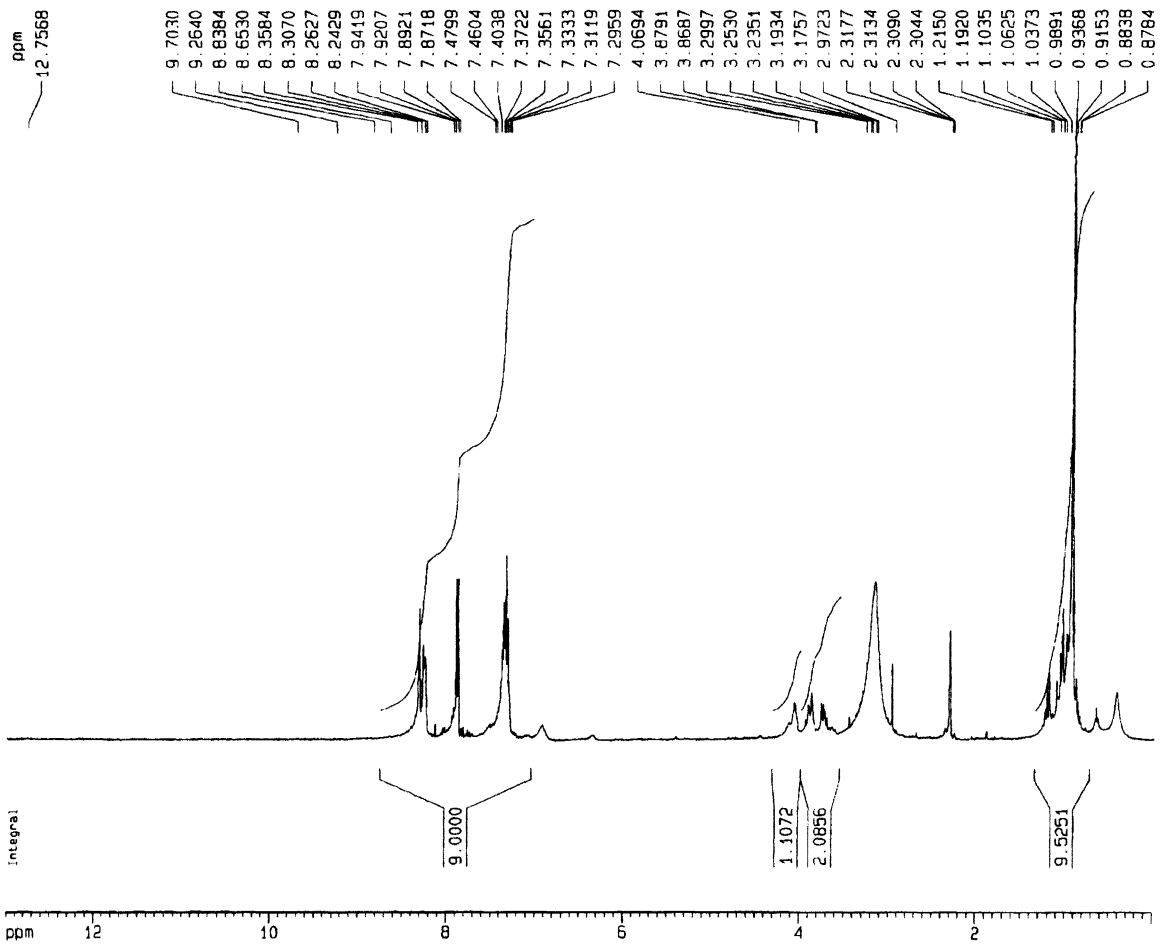

```
Current Data Parameters
NAME                 p00051701
EXPNO                        2
PROCNO                       1

F2 - Acquisition Parameters
Date_               20000517
Time                    16.56
INSTRUM                 spect
PROBHD     5 mm Multinu
PULPROG                 zg30
TD                      32768
SOLVENT                 DMSO
NS                         16
DS                          2
SWH                 8278.146 Hz
FIDRES              0.252629 Hz
AQ                  1.9792372 sec
RG                        256
DW                     60.400 usec
DE                      6.00 usec
TE                     300.0 K
D1                  1.00000000 sec

========== CHANNEL f1 ========
NUC1                       1H
P1                       9.00 usec
PL1                      0.00 dB
SFO1                400.1324710 MHz

F2 - Processing parameters
SI                      32768
SF                  400.1300000 MHz
WDW                        EM
SSB                         0
LB                       0.30 Hz
GB                          0
PC                       1.00

1D NMR plot parameters
CX                      20.00 cm
F1P                    13.000 ppm
F1                    5201.69 Hz
F2P                     0.000 ppm
F2                      0.00 Hz
PPMCM                0.65000 ppm/cm
HZCM                260.08450 Hz/cm
```

N-Acetyl-3-(9-anthryl)-DL-alanine (NMR-10)



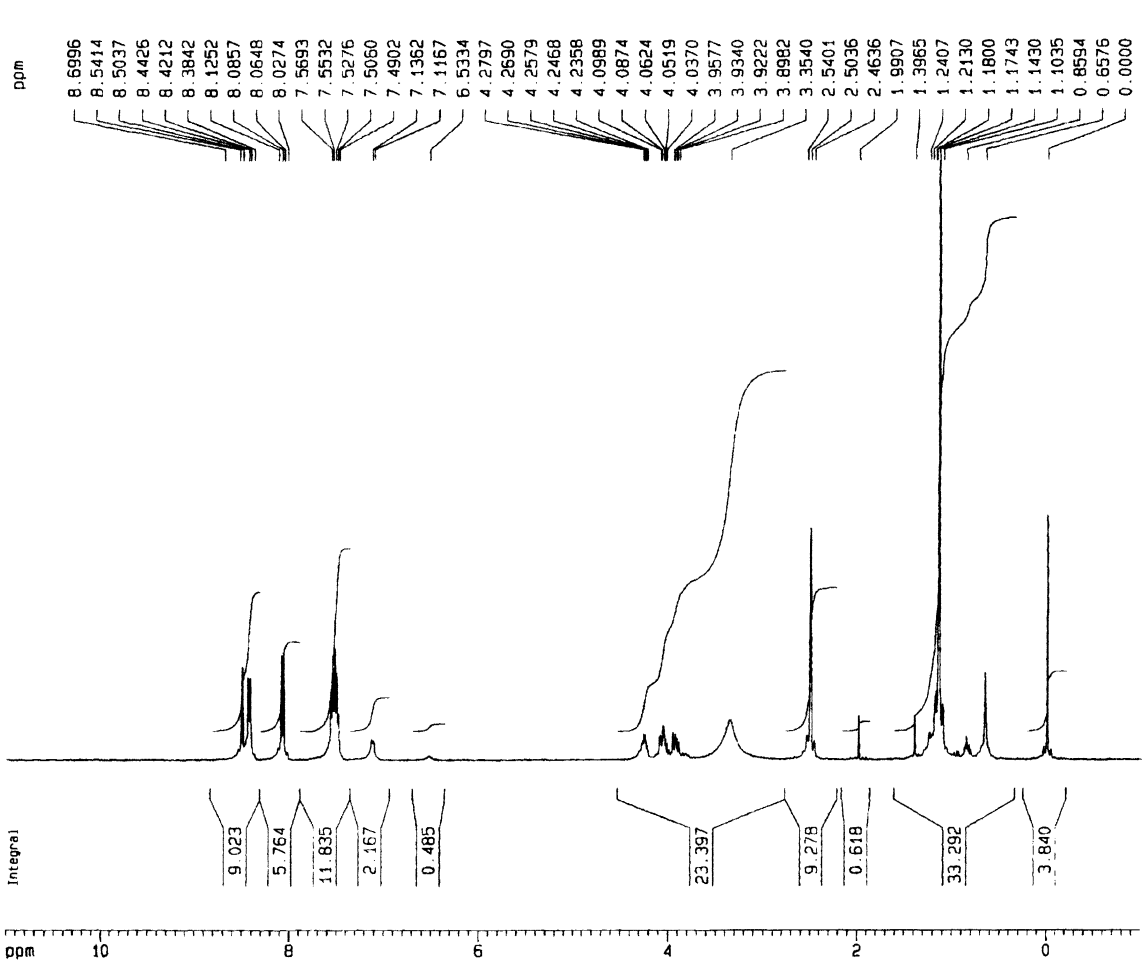

```
Current Data Parameters
NAME            p00051702
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date_            20000517
Time                17.25
INSTRUM             spect
PROBHD     5 mm Multinu
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                   512
DS                     4
SWH           25125.629 Hz
FIDRES        0.383387 Hz
AQ            1.3042164 sec
RG                  8192
DW               19.900 usec
DE                6.00 usec
TE               300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

========== CHANNEL f1 ==========
NUC1                13C
P1                10.70 usec
PL1               -1.00 dB
SFO1         100.6237959 MHz

========== CHANNEL f2 ==========
CPDPRG2         waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                0.00 dB
PL12              20.00 dB
PL13              20.00 dB
SFO2         400.1316005 MHz

F2 - Processing parameters
SI                 32768
SF           100.6127793 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40

1D NMR plot parameters
CX                20.00 cm
F1P              215.000 ppm
F1             21631.75 Hz
F2P               -5.000 ppm
F2              -503.06 Hz
PPMCM          11.00000 ppm/cm
HZCM         1106.74060 Hz/cm
```

N-t-Butyloxycarbonyl-3-(9-anthryl)-L-alanine (NMR-11)



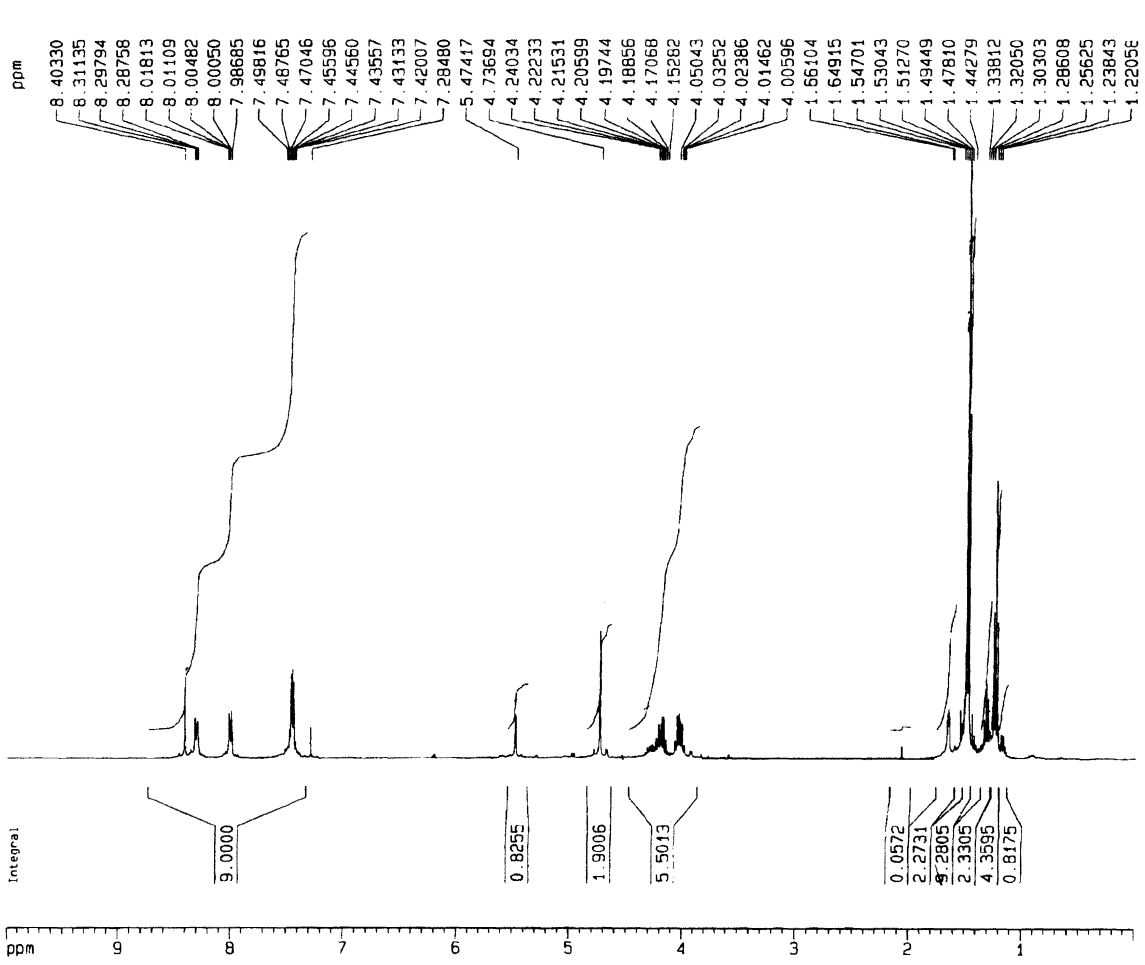

```
Current Data Parameters
NAME          p000709002
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_          20000710
Time              16.17
INSTRUM           spect
PROBHD     5 mm Multinu
PULPROG           zg30
TD               32768
SOLVENT           DMSO
NS                   16
DS                    2
SWH           8278.146 Hz
FIDRES        0.252629 Hz
AQ            1.9792372 sec
RG                  512
DW               60.400 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec

========== CHANNEL f1 ========
NUC1                 1H
P1                9.00 usec
PL1               0.00 dB
SFO1        400.1324710 MHz

F2 - Processing parameters
SI               32768
SF          400.1300795 MHz
WDW                 EM
SSB                  0
LB                0.30 Hz
GB                   0
PC                1.00

1D NMR plot parameters
CX               20.00 cm
F1P             13.000 ppm
F1             5201.69 Hz
F2P              0.000 ppm
F2               0.00 Hz
PPMCM         0.65000 ppm/cm
HZCM          260.08453 Hz/cm
```

N-t-Butyloxycarbonyl-3-(9-anthryl)-L-alanine (NMR-12)



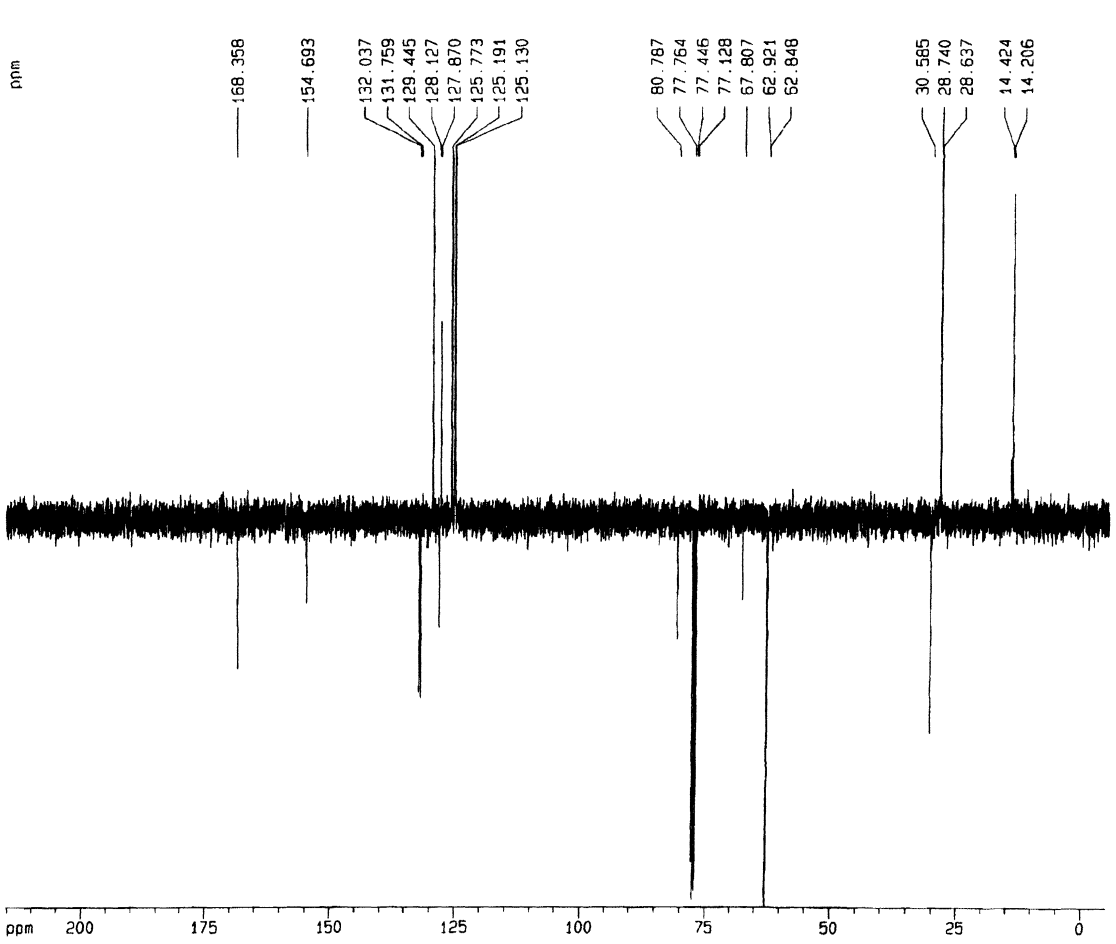

```
Current Data Parameters
NAME      Apr15-2-2001-p
EXPNO               10
PROCNO               1

F2 - Acquisition Parameters
Date_          20010415
Time              22.41
INSTRUM           spect
PROBHD     5 mm Multinu
PULPROG           zg30
TD               32768
SOLVENT           DMSO
NS                   16
DS                    2
SWH           8278.146 Hz
FIDRES        0.252629 Hz
AQ            1.9792372 sec
RG                574.7
DW               60.400 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec

========== CHANNEL f1 ========
NUC1                 1H
P1                9.00 usec
PL1               0.00 dB
SF01        400.1324710 MHz

F2 - Processing parameters
SI               32768
SF          400.1300022 MHz
WDW                 EM
SSB                  0
LB                0.30 Hz
GB                   0
PC                1.00

1D NMR plot parameters
CX               20.00 cm
F1P             11.000 ppm
F1             4401.43 Hz
F2P             -1.000 ppm
F2              -400.13 Hz
PPMCM          0.60000 ppm/cm
HZCM          240.07800 Hz/cm
```

Diethyl 9-anthrylmethyl-N-(t-butyloxycarbonyl)aminomalonate (NMR-13)



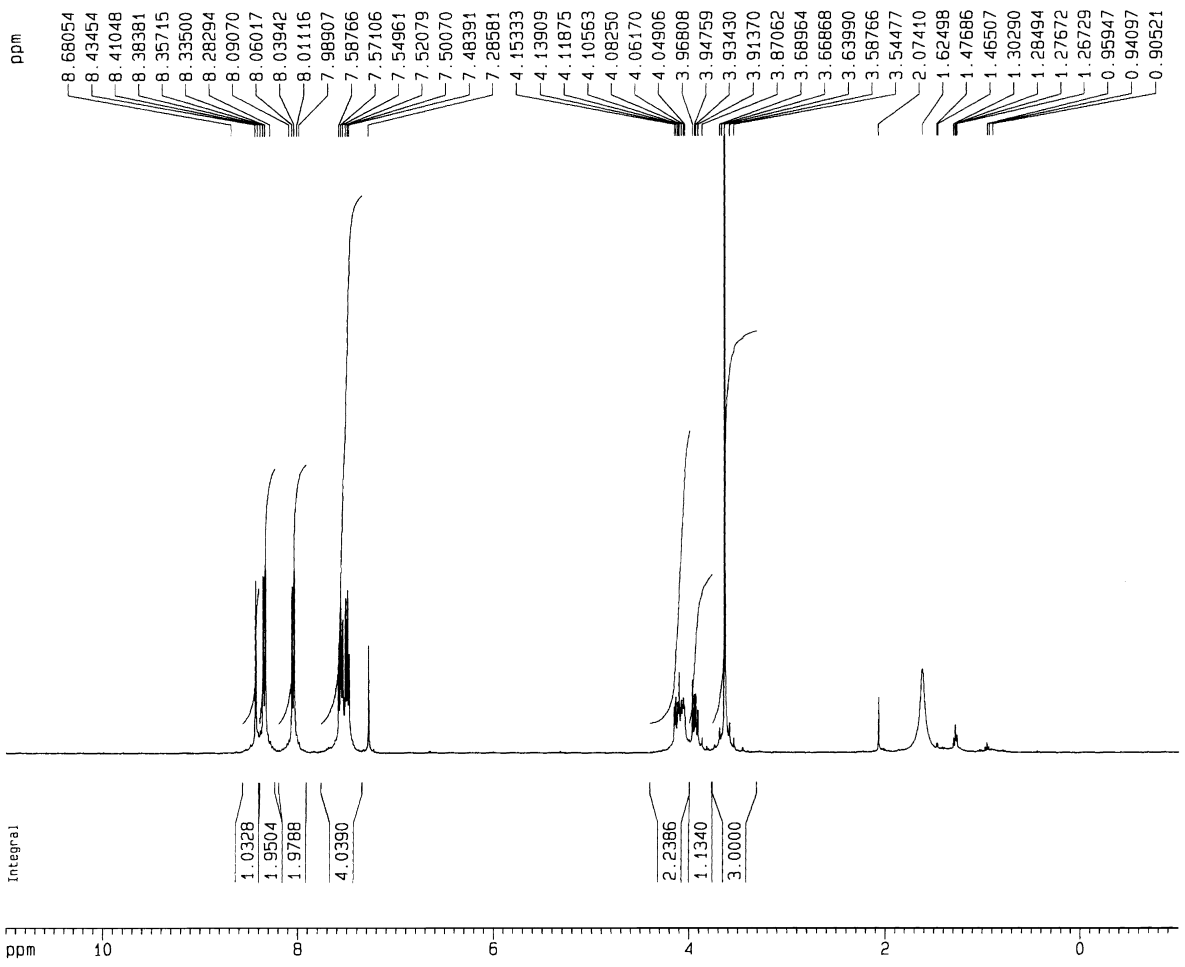

```
Current Data Parameters
NAME                  p00052302
EXPNO                        10
PROCNO                        1

F2 - Acquisition Parameters
Date_                 20000523
Time                     22.43
INSTRUM                  spect
PROBHD     5 mm Multinu
PULPROG                  zg30
TD                      32768
SOLVENT                  CDCl3
NS                          16
DS                           2
SWH                 8278.146 Hz
FIDRES              0.252629 Hz
AQ                  1.9792372 sec
RG                       161.3
DW                    60.400 usec
DE                     6.00 usec
TE                     300.0 K
D1                  1.00000000 sec

========== CHANNEL f1 ========
NUC1                         1H
P1                     9.00 usec
PL1                     0.00 dB
SFO1               400.1324710 MHz

F2 - Processing parameters
SI                          32768
SF                 400.1300000 MHz
WDW                            EM
SSB                             0
LB                           0.30 Hz
GB                              0
PC                           1.00

1D NMR plot parameters
CX                          20.00 cm
F1P                        10.000 ppm
F1                       4001.30 Hz
F2P                         0.000 ppm
F2                          0.00 Hz
PPMCM                    0.50000 ppm/cm
HZCM                  200.06500 Hz/cm
```

Diethyl 9-anthrylmethyl-N-(t-butyloxycarbonyl)aminomalonate (NMR-14)



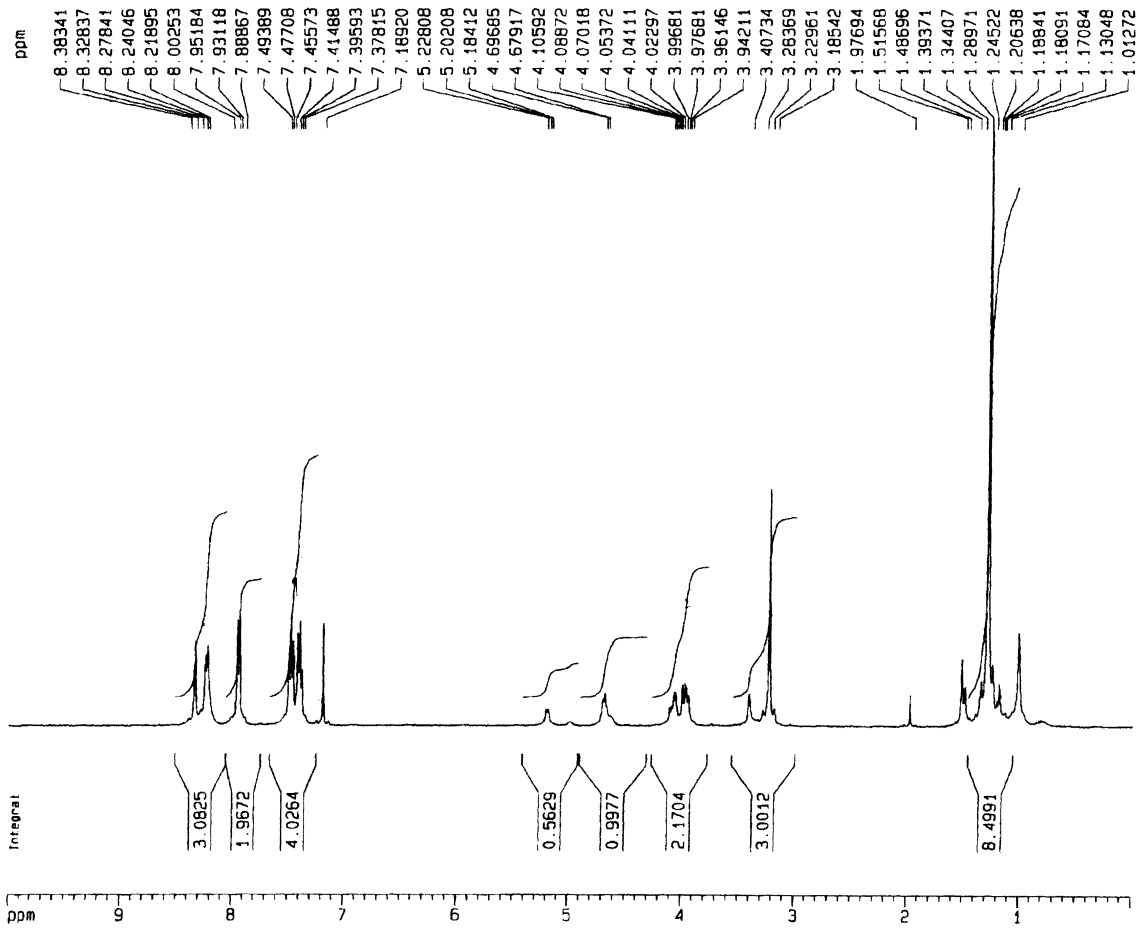

```
Current Data Parameters
NAME            p00052302
EXPNO                  11
PROCNO                  1

F2 - Acquisition Parameters
Date_            20000523
Time                22.58
INSTRUM             spect
PROBHD     5 mm Multinu
PULPROG             jmod
TD                 65536
SOLVENT             CDCl3
NS                    256
DS                      4
SWH           25125.629 Hz
FIDRES        0.383387 Hz
AQ            1.3042164 sec
RG                   4096
DW               19.900 usec
DE                6.00 usec
TE                300.0 K
CNST2        145.0000000
CNST11         1.0000000
D1             2.00000000 sec
d13            0.00000300 sec
d20            0.00689655 sec
DELTA     6366.18261719 sec

========== CHANNEL f1 ==========
NUC1                  13C
P1                 10.70 usec
p2                 21.40 usec
PL1                -1.00 dB
SFO1         100.6237959 MHz

========== CHANNEL f2 ==========
CPDPRG2          waltz16
NUC2                  1H
PCPD2              80.00 usec
PL2                0.00 dB
PL12              20.00 dB
SFO2         400.1316005 MHz

F2 - Processing parameters
SI                  32768
SF           100.6127290 MHz
WDW                    EM
SSB                     0
LB                 1.00 Hz
GB                      0
PC                 1.40

1D NMR plot parameters
CX                20.00 cm
F1P              215.000 ppm
F1             21631.74 Hz
F2P               -5.000 ppm
F2              -503.06 Hz
PPMCM           11.00000 ppm/cm
HZCM          1106.73999 Hz/cm
```

3-(9-Anthryl)-DL-alanine methyl ester (NMR-15)



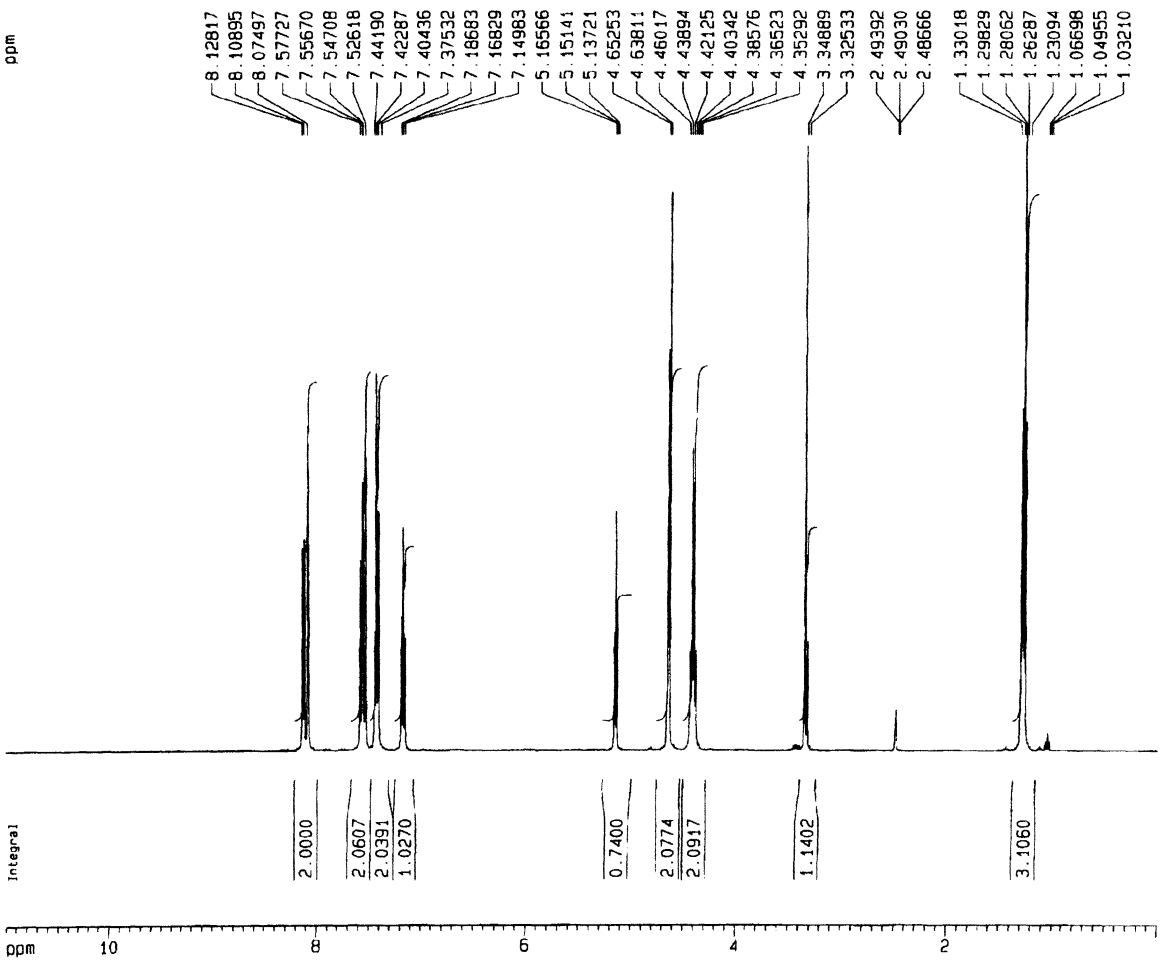

```
Current Data Parameters
NAME            p00060601
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date_            20000606
Time                20.16
INSTRUM             spect
PROBHD      5 mm Multinu
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8278.146 Hz
FIDRES          0.252629 Hz
AQ             1.9792372 sec
RG                 406.4
DW                60.400 usec
DE                 6.00 usec
TE                300.0 K
D1             1.00000000 sec

============ CHANNEL f1 ========
NUC1                   1H
P1                  9.00 usec
PL1                 0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00

1D NMR plot parameters
CX                 20.00 cm
F1P               11.000 ppm
F1              4401.43 Hz
F2P               -1.000 ppm
F2              -400.13 Hz
PPMCM           0.60000 ppm/cm
HZCM          240.07800 Hz/cm
```

N-t-Butyloxycarbonyl-3-(9-anthryl)-DL-alanine methyl ester (NMR-16)



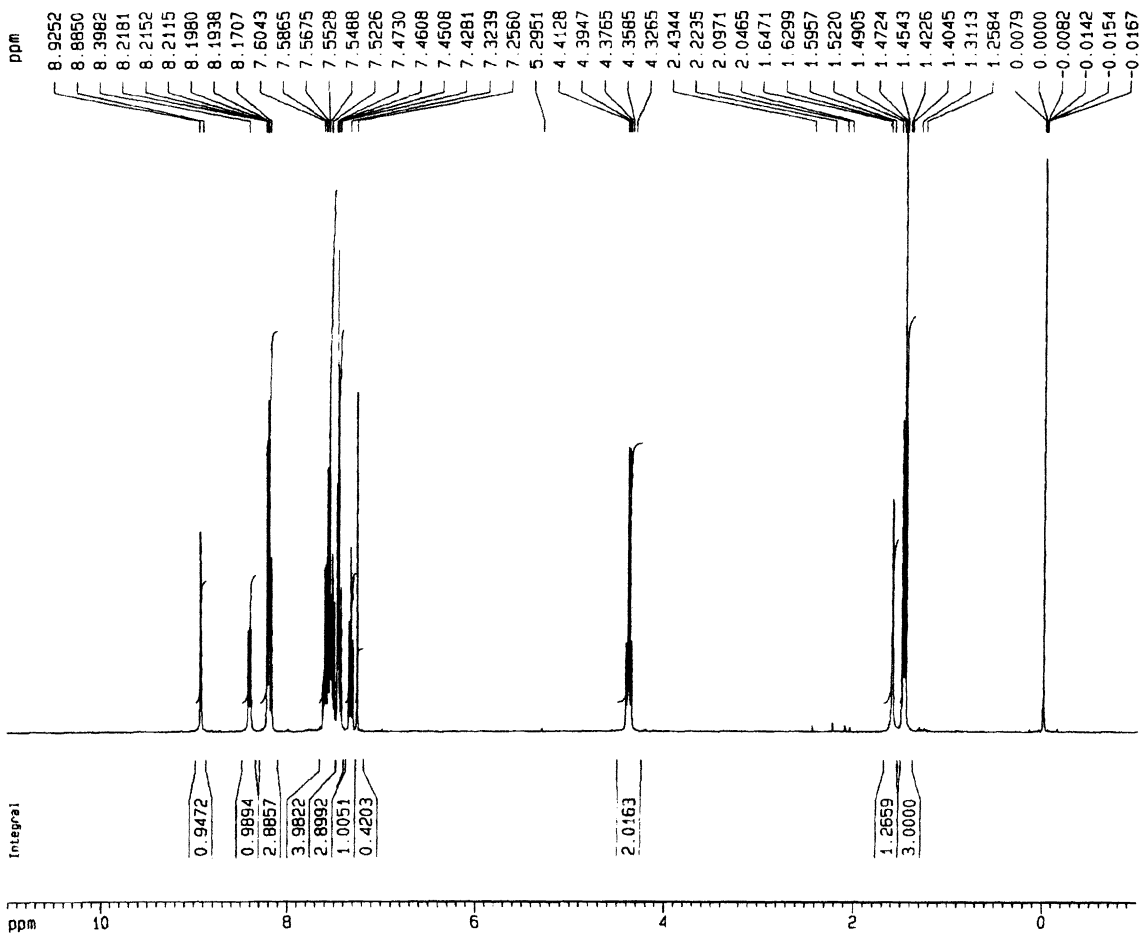

```
Current Data Parameters
NAME                 p00060902
EXPNO                       10
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20000609
Time                     16.49
INSTRUM                  spect
PROBHD     5 mm Multinu
PULPROG                  zg30
TD                      32768
SOLVENT                  CDCl3
NS                          16
DS                           2
SWH                 8278.146 Hz
FIDRES              0.252629 Hz
AQ                  1.9792372 sec
RG                         362
DW                    60.400 usec
DE                     6.00 usec
TE                     300.0 K
D1                  1.00000000 sec

========== CHANNEL f1 ========
NUC1                         1H
P1                     9.00 usec
PL1                    0.00 dB
SFO1               400.1324710 MHz

F2 - Processing parameters
SI                          32768
SF                 400.1300382 MHz
WDW                         EM
SSB                          0
LB                     0.30 Hz
GB                           0
PC                        1.00

1D NMR plot parameters
CX                    20.00 cm
F1P                  10.000 ppm
F1                  4001.30 Hz
F2P                   0.000 ppm
F2                     0.00 Hz
PPMCM                0.50000 ppm/cm
HZCM               200.06502 Hz/cm
```

N-Ethyl-3-hydroxymethyl carbazole (NMR-17)



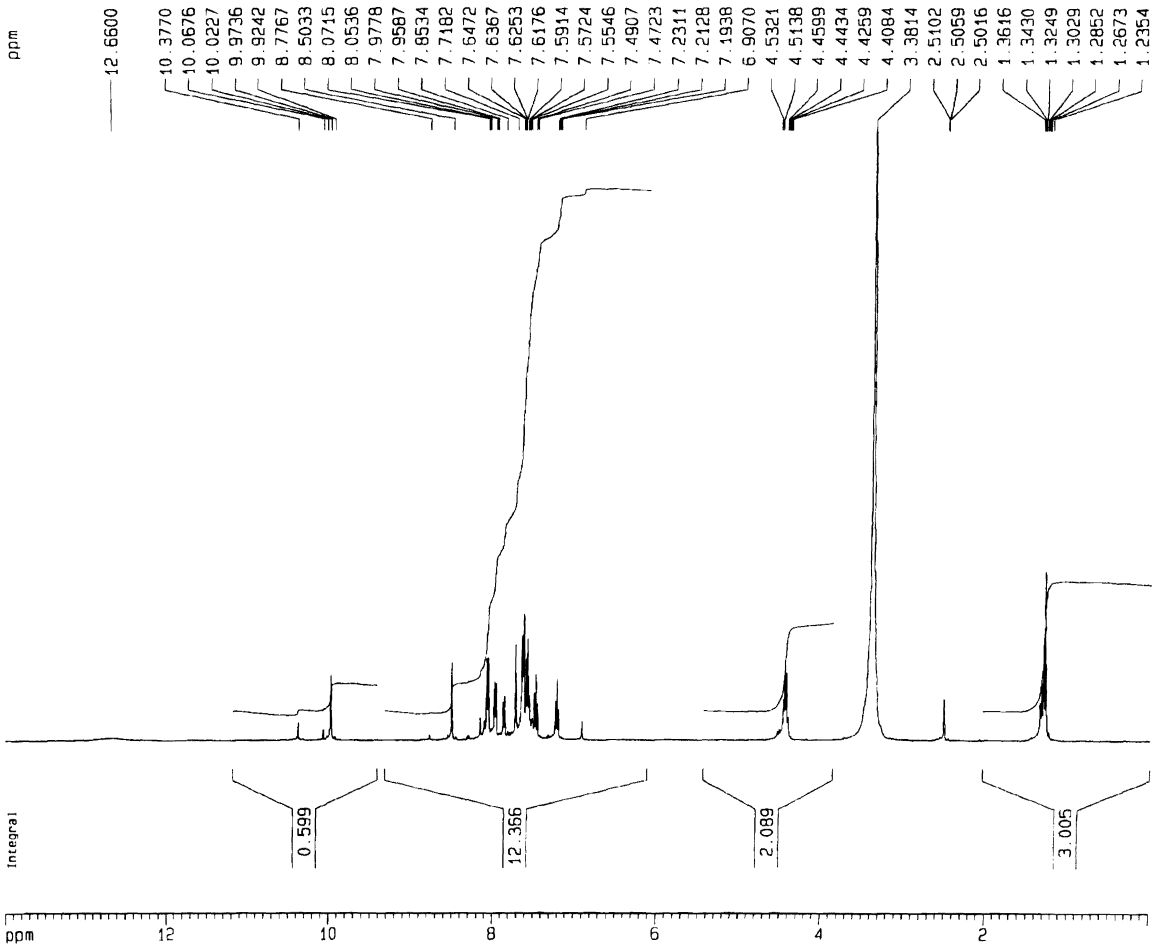

```
Current Data Parameters
NAME                  pB01301
EXPNO                       21
PROCNO                       1

F2 - Acquisition Parameters
Date_                   990813
Time                     21.44
INSTRUM                  spect
PROBHD     5 mm BBO BB-1
PULPROG                  zg30
TD                      32768
SOLVENT                  DMSO
NS                          16
DS                           2
SWH                 8223.685 Hz
FIDRES              0.250967 Hz
AQ                  1.9923444 sec
RG                         256
DW                    60.800 usec
DE                     6.00 usec
TE                     300.0 K
D1                  1.00000000 sec
P1                     8.70 usec
SFO1              400.1324710 MHz
NUC1                       1H
PL1                    0.00 dB

F2 - Processing parameters
SI                       16384
SF                400.1300070 MHz
WDW                        EM
SSB                         0
LB                     0.50 Hz
GB                          0
PC                     1.00

1D NMR plot parameters
CX                    20.00 cm
F1P                  11.000 ppm
F1                  4401.43 Hz
F2P                   0.000 ppm
F2                    0.00 Hz
PPMCM              0.55000 ppm/cm
HZCM             220.07150 Hz/cm
```

4-(3-(N-Ethylcarbazolylidene))-2-phenyloxazol-5-one (NMR-18)



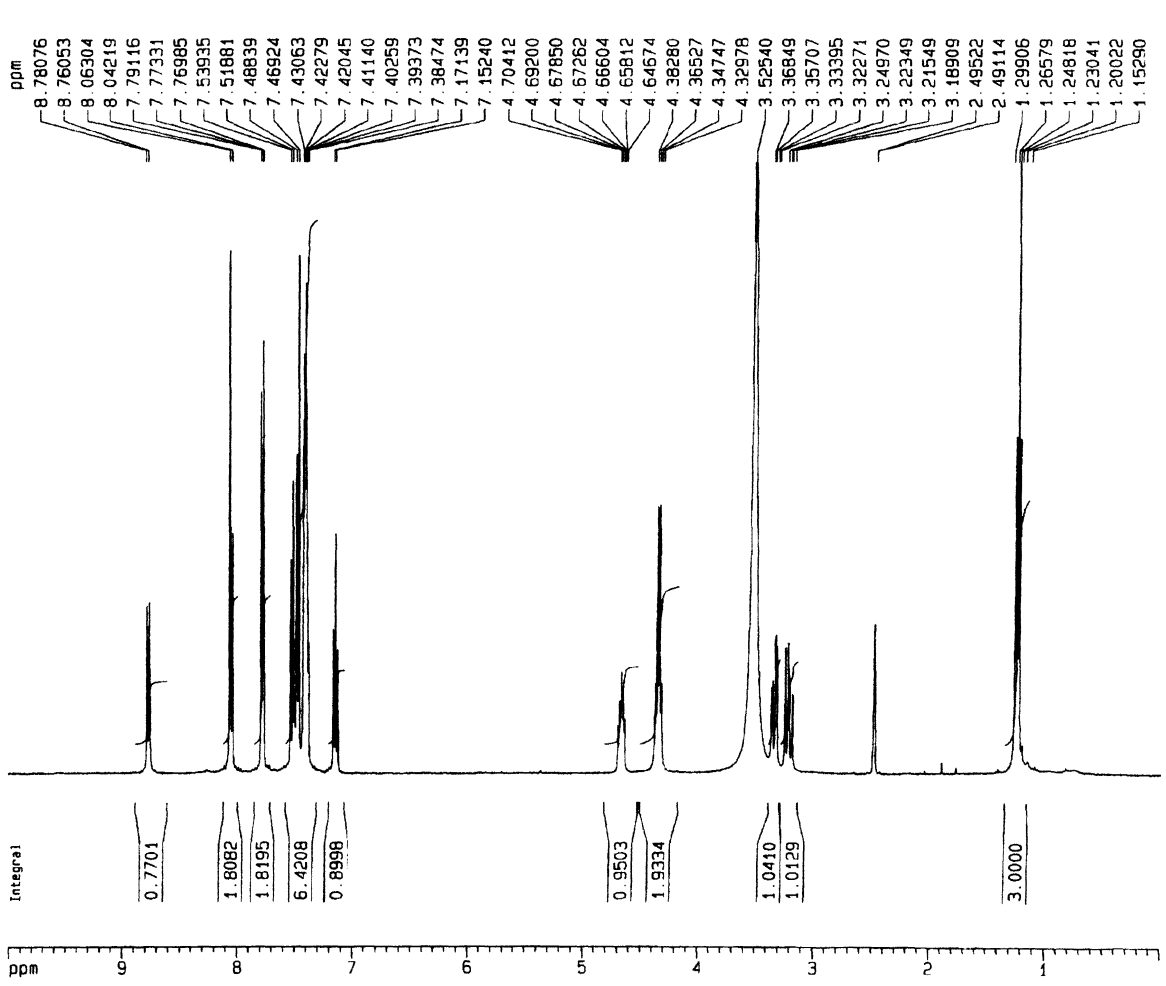

```
Current Data Parameters
NAME                  p902501
EXPNO                      10
PROCNO                      1

F2 - Acquisition Parameters
Date_                  990925
Time                    11.34
INSTRUM                 spect
PROBHD     5 mm BBO BB-1
PULPROG                 zg30
TD                     32768
SOLVENT                 CDCl3
NS                         16
DS                          2
SWH                 8278.146 Hz
FIDRES              0.252629 Hz
AQ                1.9792372 sec
RG                        256
DW                   60.400 usec
DE                    6.00 usec
TE                    300.0 K
D1                1.00000000 sec

========== CHANNEL f1 ========
NUC1                       1H
P1                      8.70 usec
PL1                     0.00 dB
SFO1              400.1324710 MHz

F2 - Processing parameters
SI                     32768
SF                400.1300113 MHz
WDW                        EM
SSB                         0
LB                      0.30 Hz
GB                          0
PC                       1.00

1D NMR plot parameters
CX                     20.00 cm
F1P                   11.000 ppm
F1                   4401.43 Hz
F2P                  -1.000 ppm
F2                   -400.13 Hz
PPMCM                0.60000 ppm/cm
HZCM               240.07800 Hz/cm
```

N-Benzoyl-3-(N'-ethyl-3-carbazolyl)acrylic acid (NMR-19)



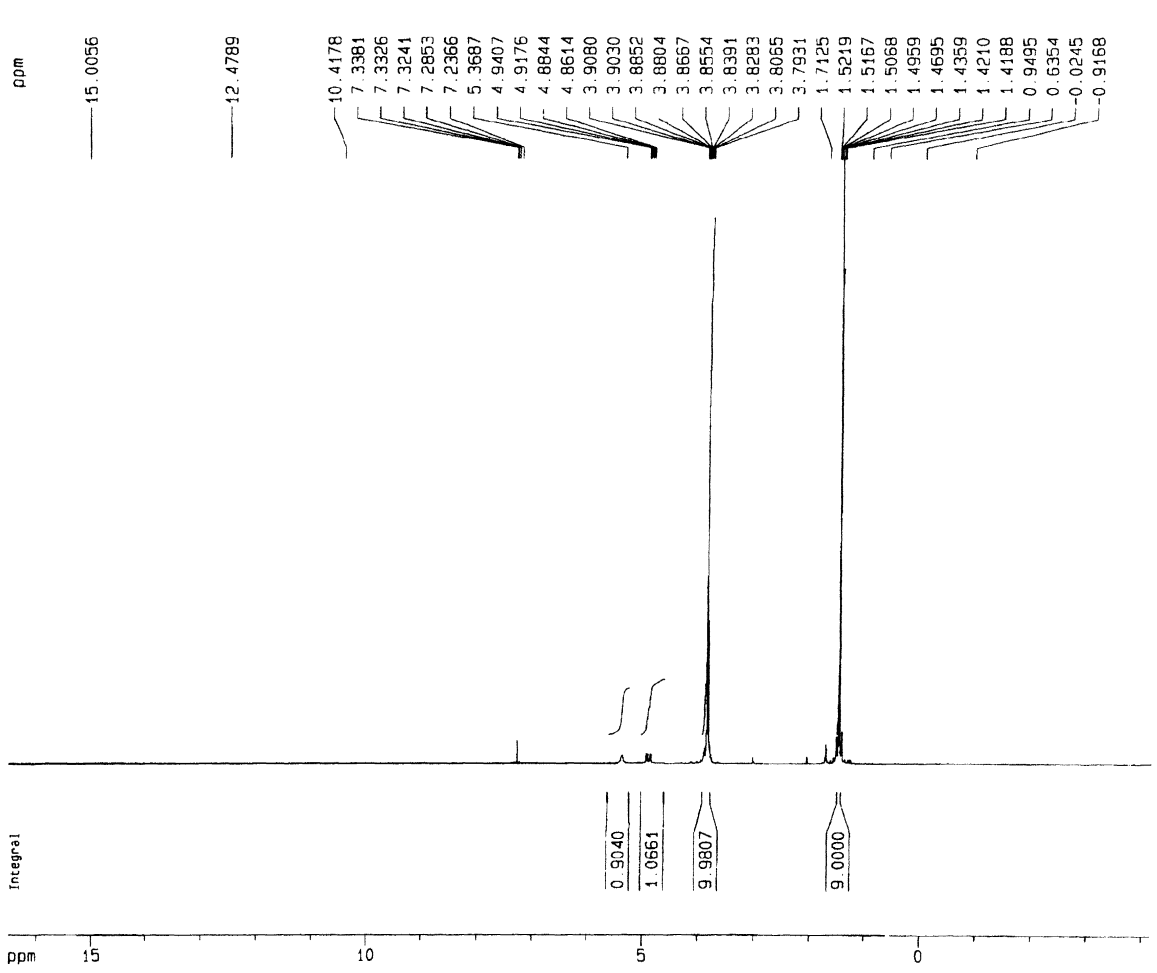

```
Current Data Parameters
NAME          Oct27-1999
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_             991027
Time                0.17
INSTRUM            spect
PROBHD     5 mm Multinu
PULPROG            zg30
TD                32768
SOLVENT            DMSO
NS                    16
DS                     2
SWH           8278.146 Hz
FIDRES        0.252629 Hz
AQ            1.9792372 sec
RG                287.4
DW               60.400 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec

========== CHANNEL f1 ========
NUC1                  1H
P1                 8.70 usec
PL1                0.00 dB
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00

1D NMR plot parameters
CX                20.00 cm
F1P              14.000 ppm
F1              5601.82 Hz
F2P               0.000 ppm
F2                0.00 Hz
PPMCM          0.70000 ppm/cm
HZCM          280.09100 Hz/cm
```

N-Benzoyl-3-(N'-ethyl-3-carbazolyl)alanine (NMR-20)



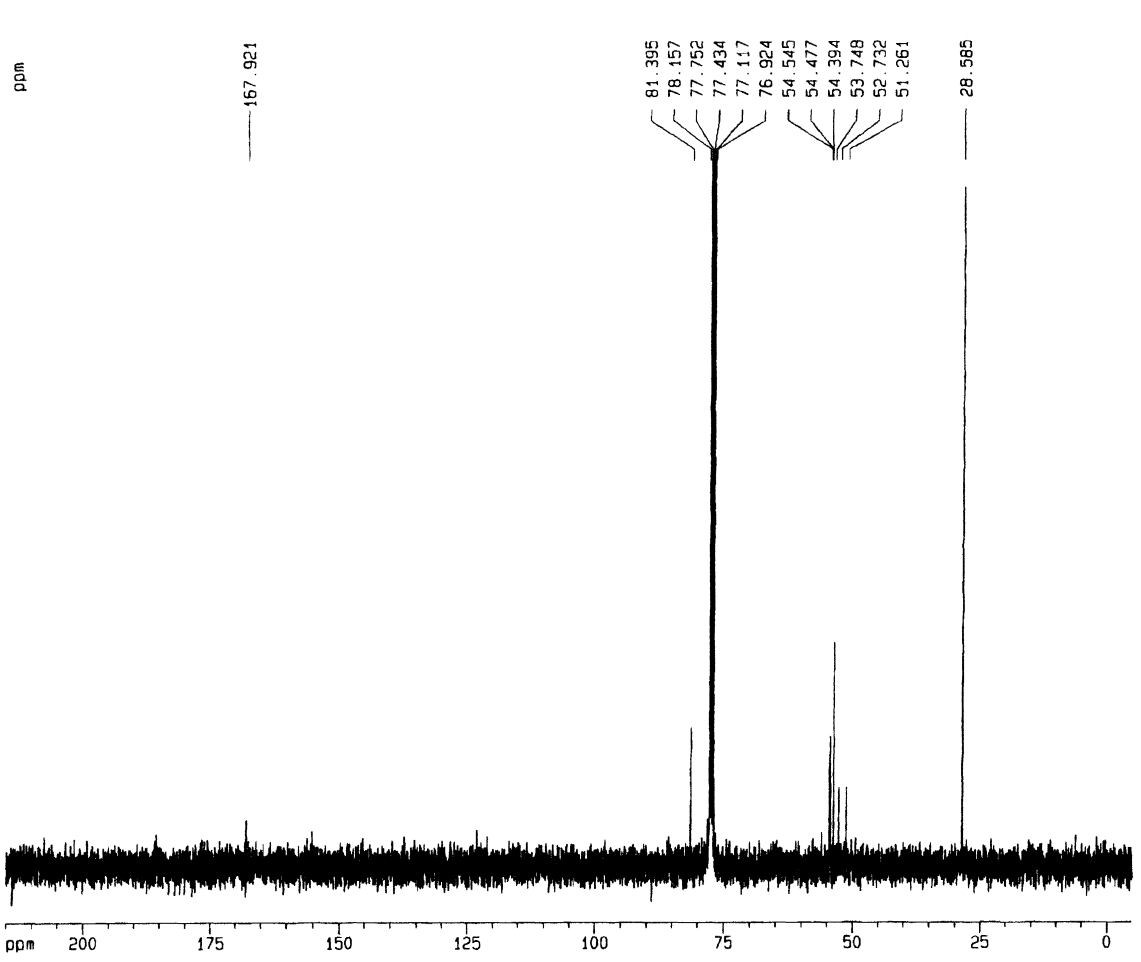

```
Current Data Parameters
NAME                  p101702
EXPNO                      10
PROCNO                      1

F2 - Acquisition Parameters
Date_                  991017
Time                    14.12
INSTRUM                 spect
PROBHD     5 mm BBO BB-1
PULPROG                 zg30
TD                     32768
SOLVENT                 DMSO
NS                         16
DS                          2
SWH                8278.146 Hz
FIDRES             0.252629 Hz
AQ                 1.9792372 sec
RG                        362
DW                   60.400 usec
DE                    6.00 usec
TE                   300.0 K
D1                 1.00000000 sec

============ CHANNEL f1 ========
NUC1                       1H
P1                    8.70 usec
PL1                    0.00 dB
SFO1           400.1324710 MHz

F2 - Processing parameters
SI                         32768
SF                400.1300068 MHz
WDW                        EM
SSB                         0
LB                     0.30 Hz
GB                          0
PC                      1.00

1D NMR plot parameters
CX                     20.00 cm
F1P                   10.000 ppm
F1                   4001.30 Hz
F2P                   0.000 ppm
F2                     0.00 Hz
PPMCM                0.50000 ppm/cm
HZCM               200.06500 Hz/cm
```

Methyl 2-(t-butoxycarbonylamino)-2-(dimethoxyphosphonyl)acetate (NMR-21)



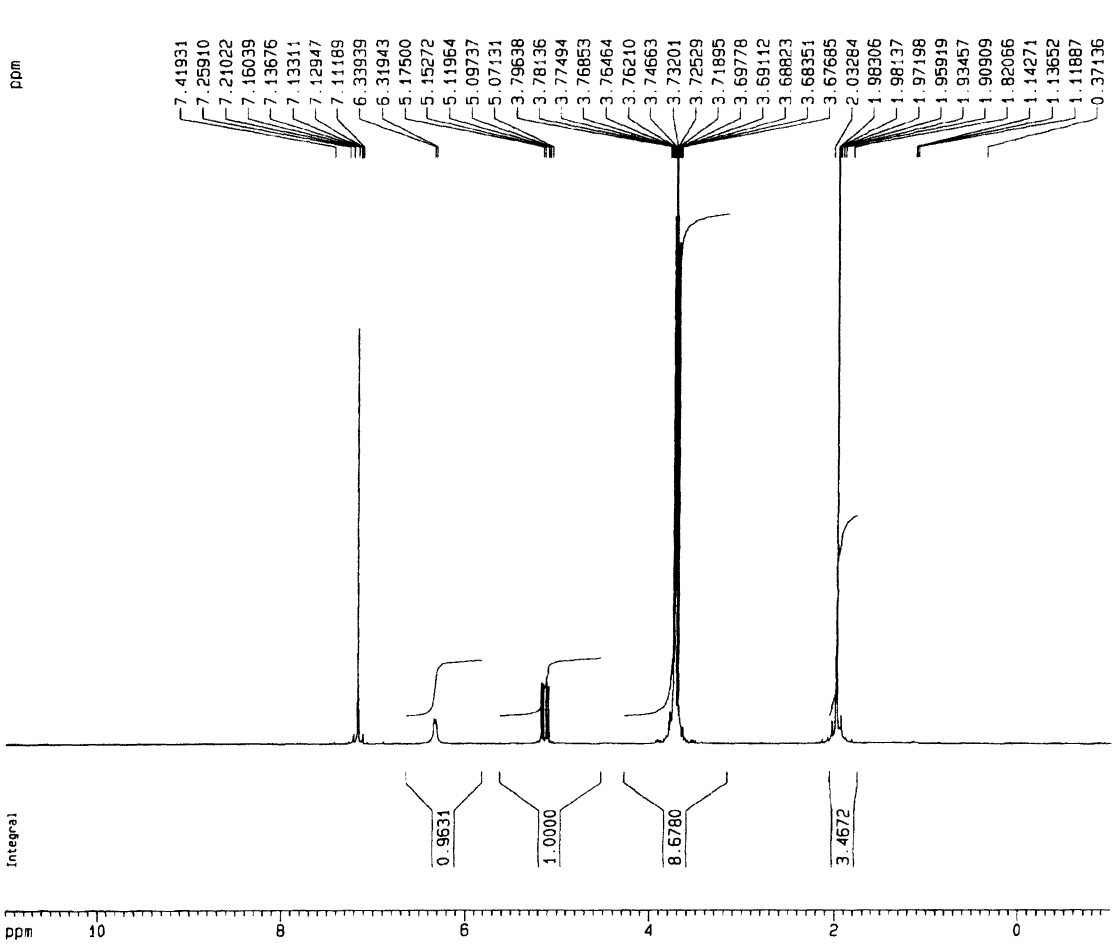

```
Current Data Parameters
NAME                  p00060801
EXPNO                        20
PROCNO                        1

F2 - Acquisition Parameters
Date_                 20000608
Time                     19.59
INSTRUM                  spect
PROBHD       5 mm Multinu
PULPROG                   zg30
TD                       32768
SOLVENT                  CDCl3
NS                          16
DS                           2
SWH                 8278.146 Hz
FIDRES              0.252629 Hz
AQ                  1.9792372 sec
RG                       322.5
DW                      60.400 usec
DE                       6.00 usec
TE                      300.0 K
D1                  1.00000000 sec

========== CHANNEL f1 ========
NUC1                         1H
P1                       9.00 usec
PL1                      0.00 dB
SFO1               400.1324710 MHz

F2 - Processing parameters
SI                       32768
SF                 400.1300000 MHz
WDW                         EM
SSB                          0
LB                       0.30 Hz
GB                           0
PC                       1.00

1D NMR plot parameters
CX                      20.00 cm
F1P                     16.520 ppm
F1                    6610.01 Hz
F2P                     -4.169 ppm
F2                    -1668.13 Hz
PPMCM                  1.03443 ppm/cm
HZCM                 413.90729 Hz/cm
```

Methyl 2-(t-butoxycarbonylamino)-2-(dimethoxyphosphonyl)acetate (NMR-22)



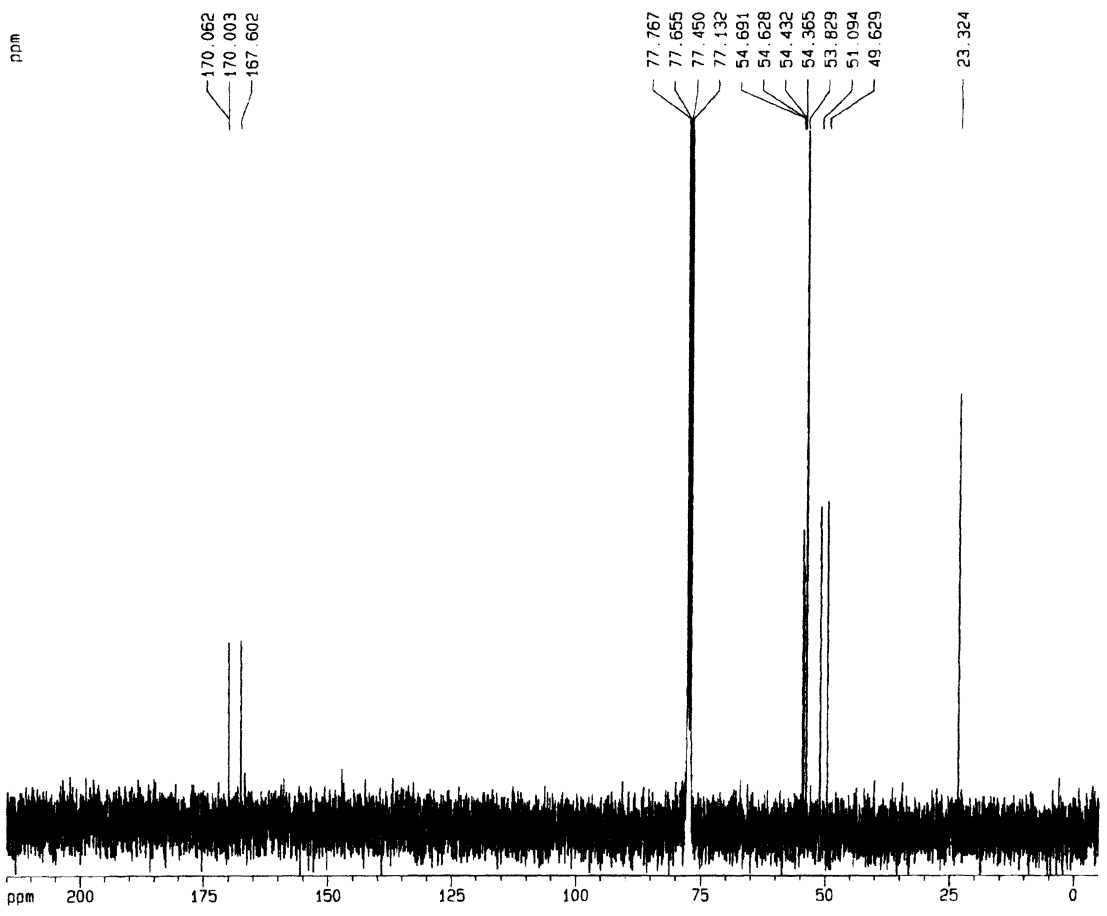

```
Current Data Parameters
NAME            p00060802
EXPNO                  11
PROCNO                  1

F2 - Acquisition Parameters
Date_           20000608
Time                20.43
INSTRUM             spect
PROBHD     5 mm Multinu
PULPROG          zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   512
DS                     4
SWH           25125.629 Hz
FIDRES        0.383387 Hz
AQ            1.3042164 sec
RG                 13004
DW               19.900 usec
DE                6.00 usec
TE                300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

========== CHANNEL f1 ==========
NUC1                13C
P1                10.70 usec
PL1               -1.00 dB
SFO1         100.6237959 MHz

========== CHANNEL f2 ==========
CPDPRG2         waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                0.00 dB
PL12              20.00 dB
PL13              20.00 dB
SFO2         400.1316005 MHz

F2 - Processing parameters
SI                 32768
SF           100.6127290 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40

1D NMR plot parameters
CX                20.00 cm
F1P              215.000 ppm
F1               21631.74 Hz
F2P               -5.000 ppm
F2               -503.06 Hz
PPMCM          11.00000 ppm/cm
HZCM         1106.73999 Hz/cm
```

Methyl 2-acetamino-2-(dimethoxyphosphonyl)acetate (NMR-23)



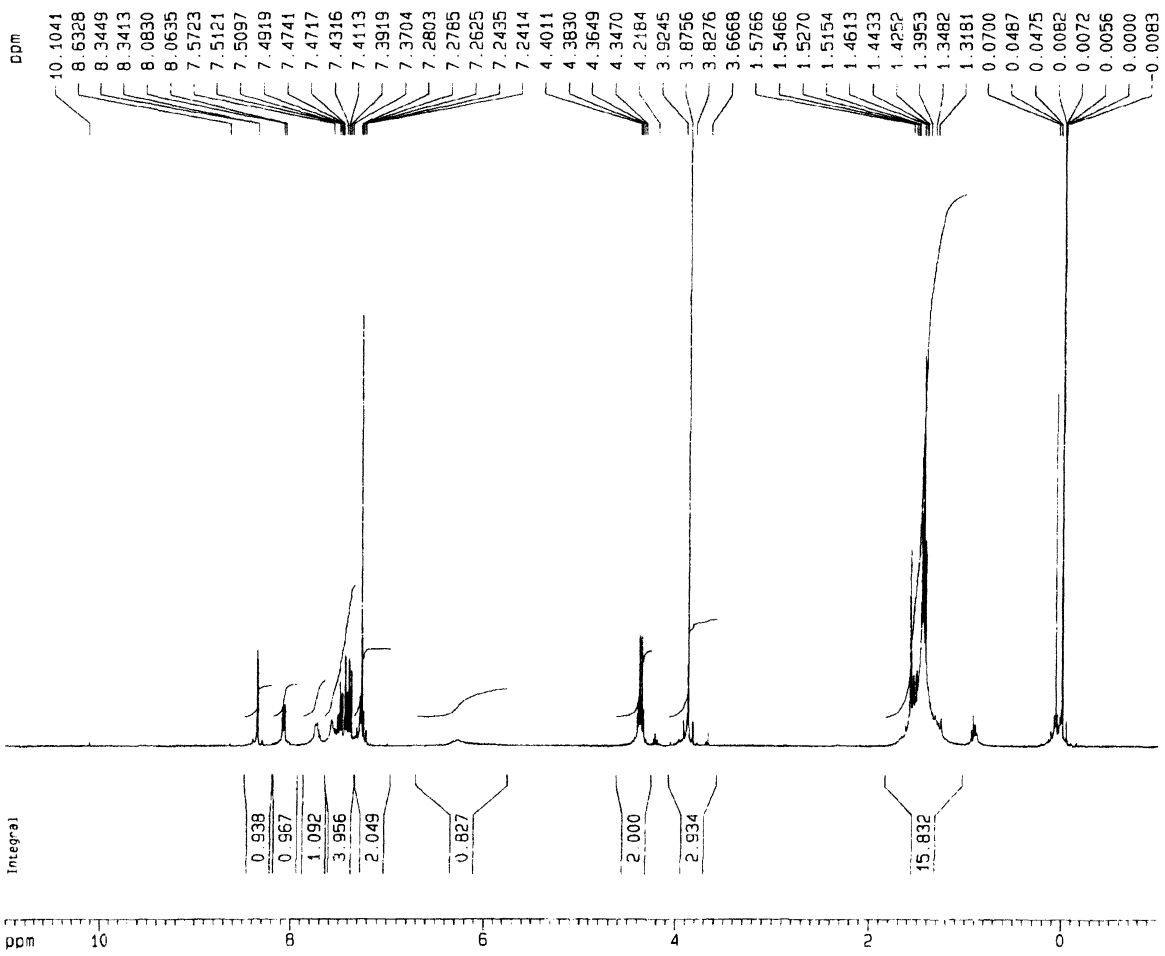

```
Current Data Parameters
NAME      Feb20-2001-pan
EXPNO                 12
PROCNO                 1

F2 - Acquisition Parameters
Date_           20010220
Time               15.24
INSTRUM            spect
PROBHD     5 mm Multinu
PULPROG            zg30
TD                32768
SOLVENT           CDCl3
NS                   16
DS                    2
SWH           8278.146 Hz
FIDRES        0.252629 Hz
AQ            1.9792372 sec
RG                406.4
DW               60.400 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec

========== CHANNEL f1 ========
NUC1                  1H
P1                 9.00 usec
PL1                0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                32768
SF          400.1300495 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00

1D NMR plot parameters
CX                20.00 cm
F1P               11.000 ppm
F1              4401.43 Hz
F2P               -1.000 ppm
F2              -400.13 Hz
PPMCM          0.60000 ppm/cm
HZCM          240.07802 Hz/cm
```

Methyl 2-acetamino-2-(dimethoxyphosphonyl)acetate (NMR-24)



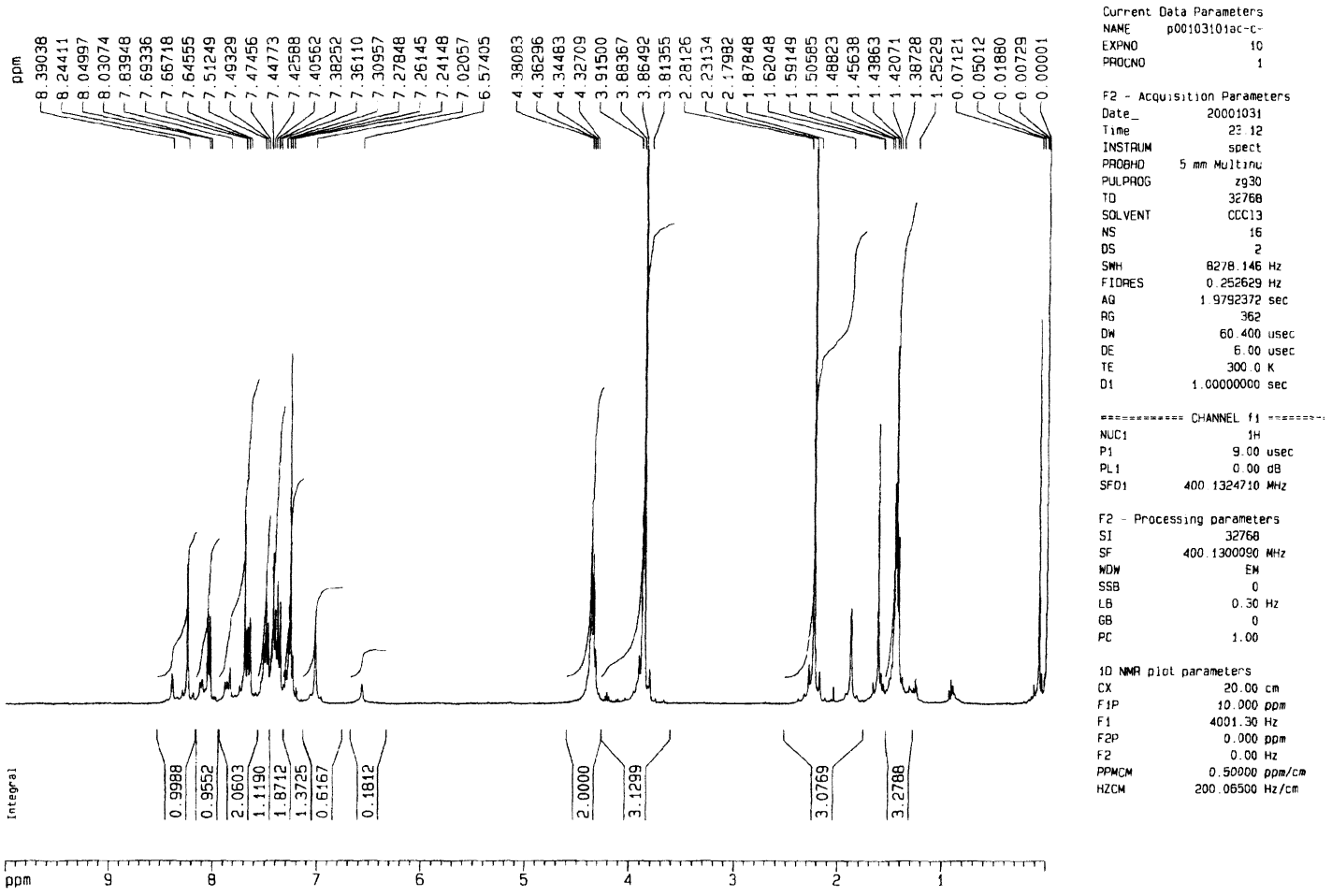

```
Current Data Parameters
NAME      Nov20-2000-p-2
EXPNO               21
PROCNO               1

F2 - Acquisition Parameters
Date_          20001120
Time               6.16
INSTRUM           spect
PROBHD     5 mm Multinu
PULPROG         zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    4
SWH           25125.629 Hz
FIDRES        0.383387 Hz
AQ            1.3042164 sec
RG                 4096
DW               19.900 usec
DE                6.00 usec
TE               300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

********** CHANNEL f1 **********
NUC1                13C
P1               10.70 usec
PL1              -1.00 dB
SFO1         100.6237959 MHz

********** CHANNEL f2 **********
CPDPRG2         waltz16
NUC2                1H
PCPD2            80.00 usec
PL2               0.00 dB
PL12             20.00 dB
PL13             20.00 dB
SFO2         400.1316005 MHz

F2 - Processing parameters
SI                 32768
SF           100.6127290 MHz
WDW                   EM
SSB                    0
LB                1.00 Hz
GB                     0
PC                1.40

1D NMR plot parameters
CX               20.00 cm
F1P             215.000 ppm
F1             21631.74 Hz
F2P             -5.000 ppm
F2              -503.06 Hz
PPMCM          11.00000 ppm/cm
HZCM         1106.73999 Hz/cm
```

N-t-Butoxycarbonyl-3-(N'-ethyl-3-carbazolyl)-DL-acryl acid methyl ester (NMR-25)




```
Current Data Parameters
NAME      p001101B-C-d-M
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_          20001101
Time              20.53
INSTRUM           spect
PROBHD     5 mm Multinu
PULPROG           zg30
TD               32768
SOLVENT           CDCl3
NS                   16
DS                    2
SWH           8278.146 Hz
FIDRES        0.252629 Hz
AQ            1.9792372 sec
RG                574.7
DW               60.400 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec

========== CHANNEL f1 ========
NUC1                  1H
P1                 9.00 usec
PL1                0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                   32768
SF          400.1300085 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00

1D NMR plot parameters
CX                20.00 cm
F1P               11.000 ppm
F1                4401.43 Hz
F2P               -1.000 ppm
F2                -400.13 Hz
PPMCM             0.60000 ppm/cm
HZCM          240.07800 Hz/cm
```

N-Acetyl-3-(N'-ethyl-3-carbazolyl)-DL-acrylic acid methyl ester (NMR-26)



Current Data Parameters
NAME p00103101ac-c-
EXPNO 10
PROCNO 1

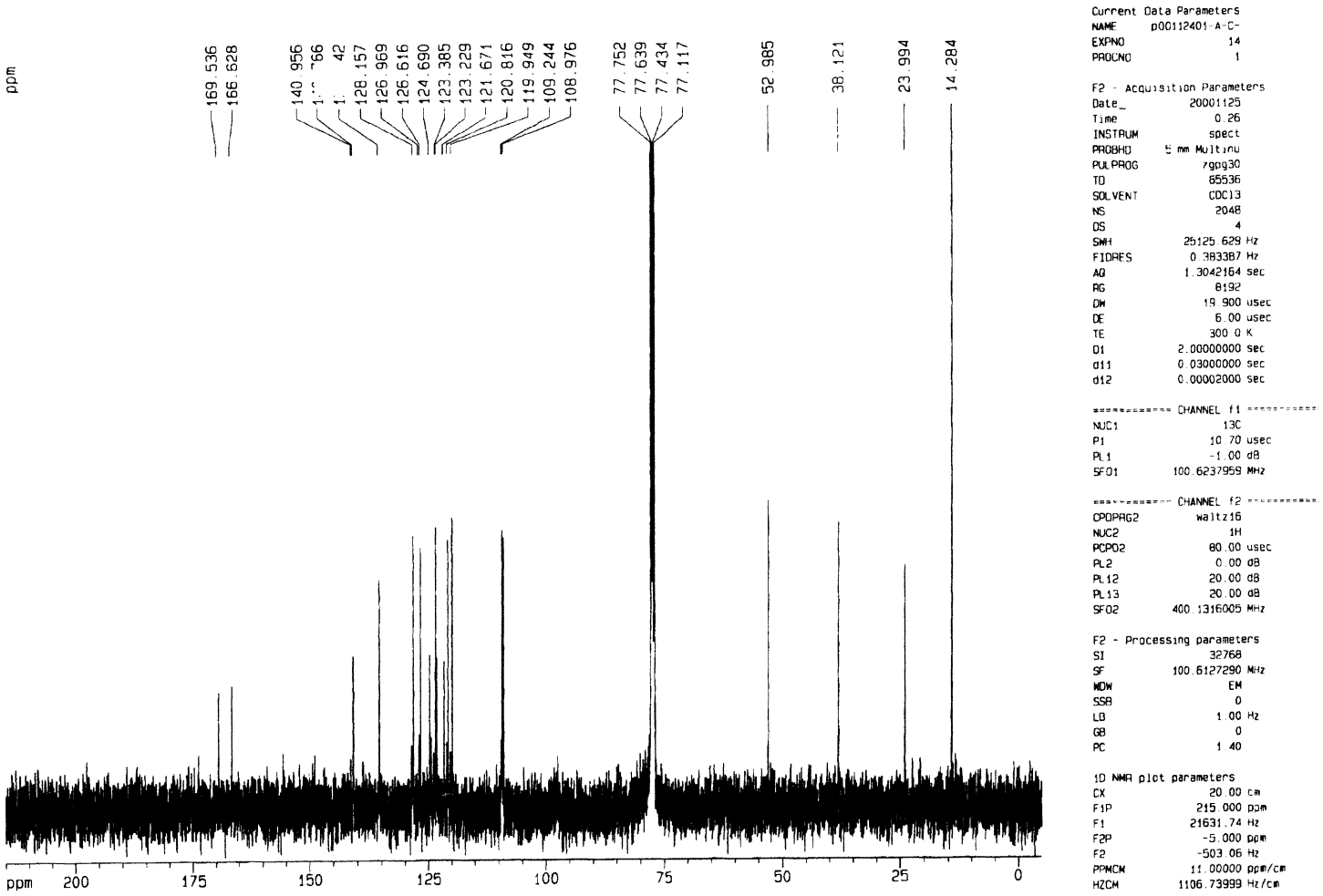
F2 - Acquisition Parameters
Date_ 20001031
Time 23 12
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.252629 Hz
AQ 1.9792372 sec
RG 362
DW 60.400 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec

========== CHANNEL f1 ========
NUC1 1H
P1 9.00 usec
PL1 0.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300090 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
F1P 10.000 ppm
F1 4001.30 Hz
F2P 0.000 ppm
F2 0.00 Hz
PPMCM 0.50000 ppm/cm
HZCM 200.06500 Hz/cm

N-Acetyl-3-(N'-ethyl-3-carbazolyl)-DL-acrylic acid methyl ester (NMR-27)



Current Data Parameters
NAME p00112401-A-C-
EXPNO 14
PROCNO 1

F2 - Acquisition Parameters
Date_ 20001125
Time 0.26
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 2048
DS 4
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 8192
DW 19.900 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
d12 0.00002000 sec

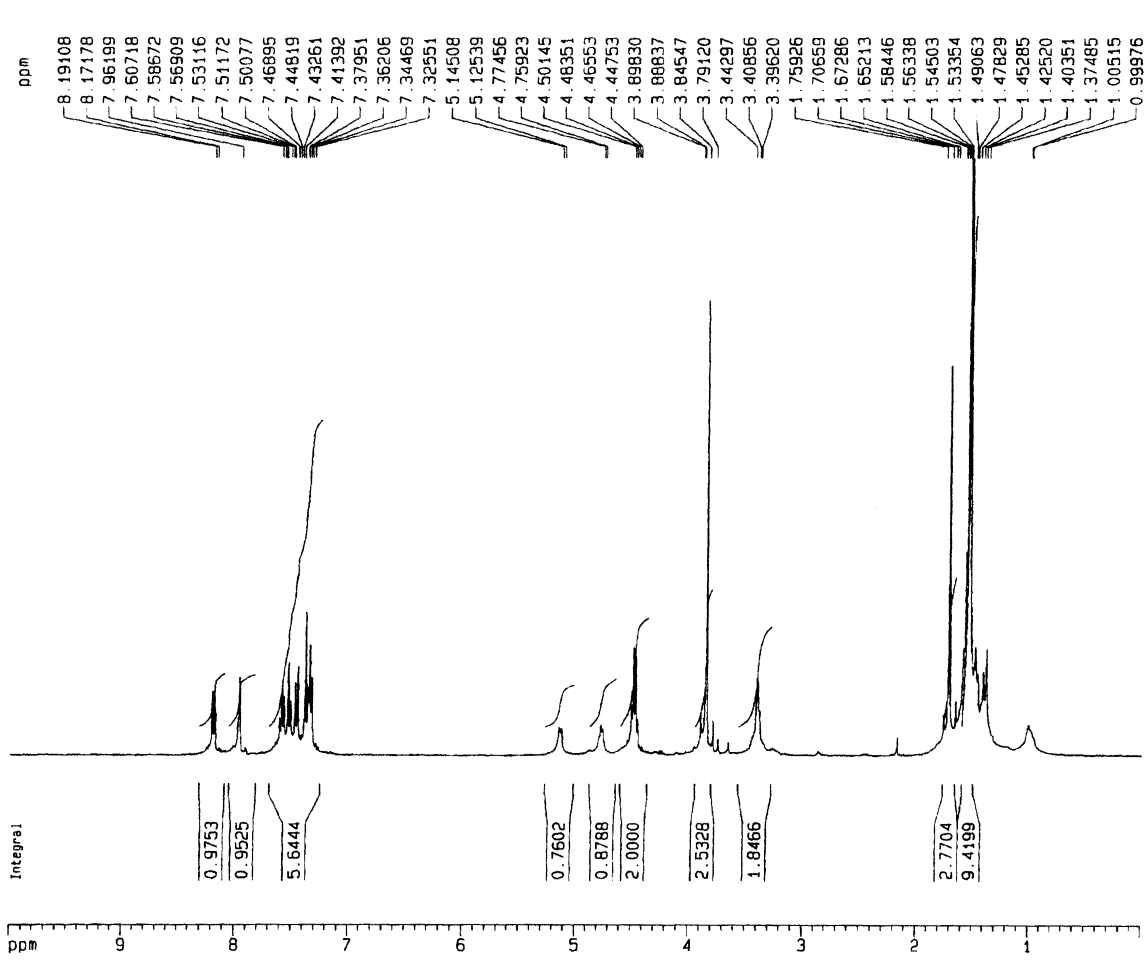
========== CHANNEL f1 ==========
NUC1 13C
P1 10.70 usec
PL1 -1.00 dB
SFO1 100.6237959 MHz

========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 20.00 dB
PL13 20.00 dB
SFO2 400.1316005 MHz

F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

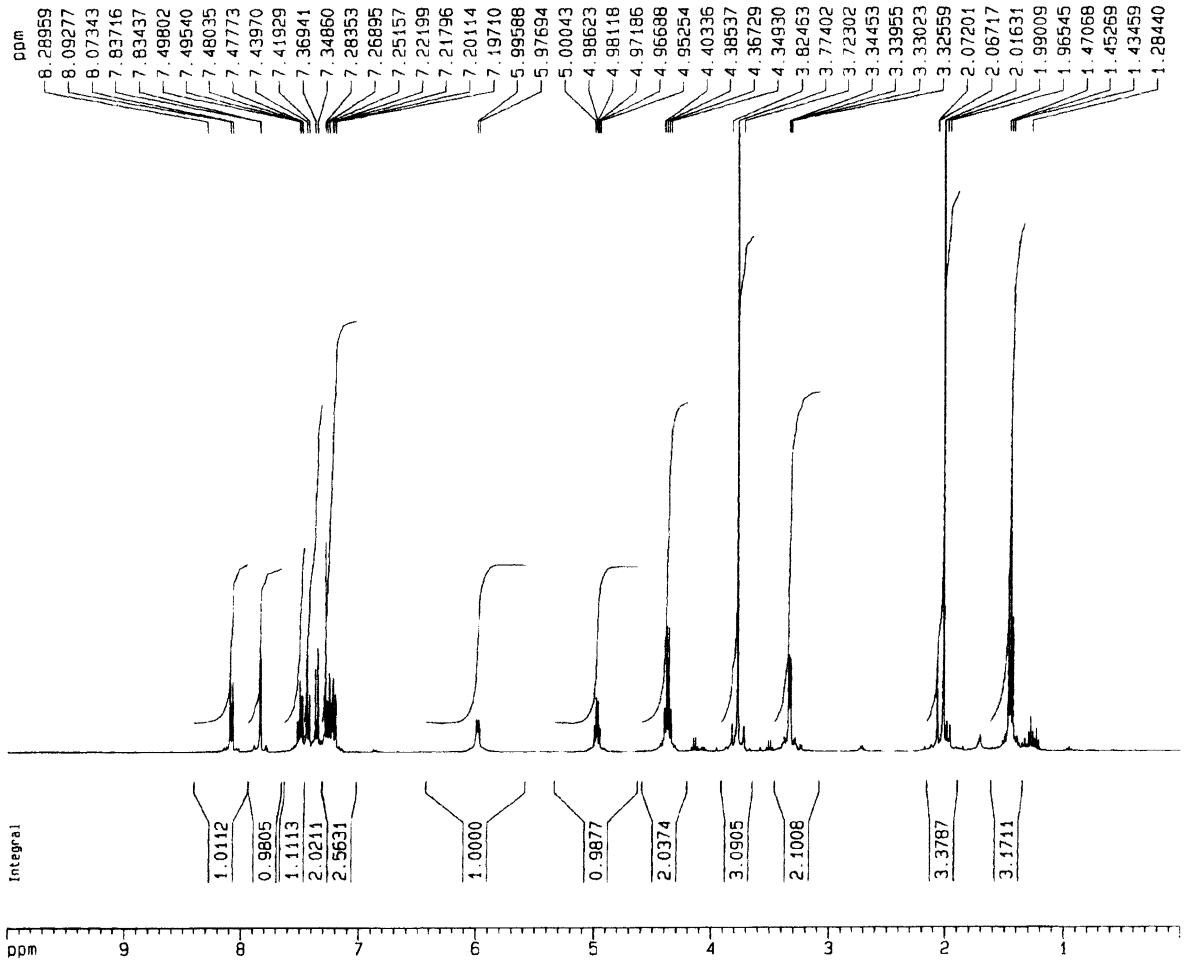
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.74 Hz
F2P -5.000 ppm
F2 -503.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.73999 Hz/cm

N-t-Butoxycarbonyl-3-(N'-ethyl-3-carbazolyl)-DL-alanine methyl ester (NMR-28)



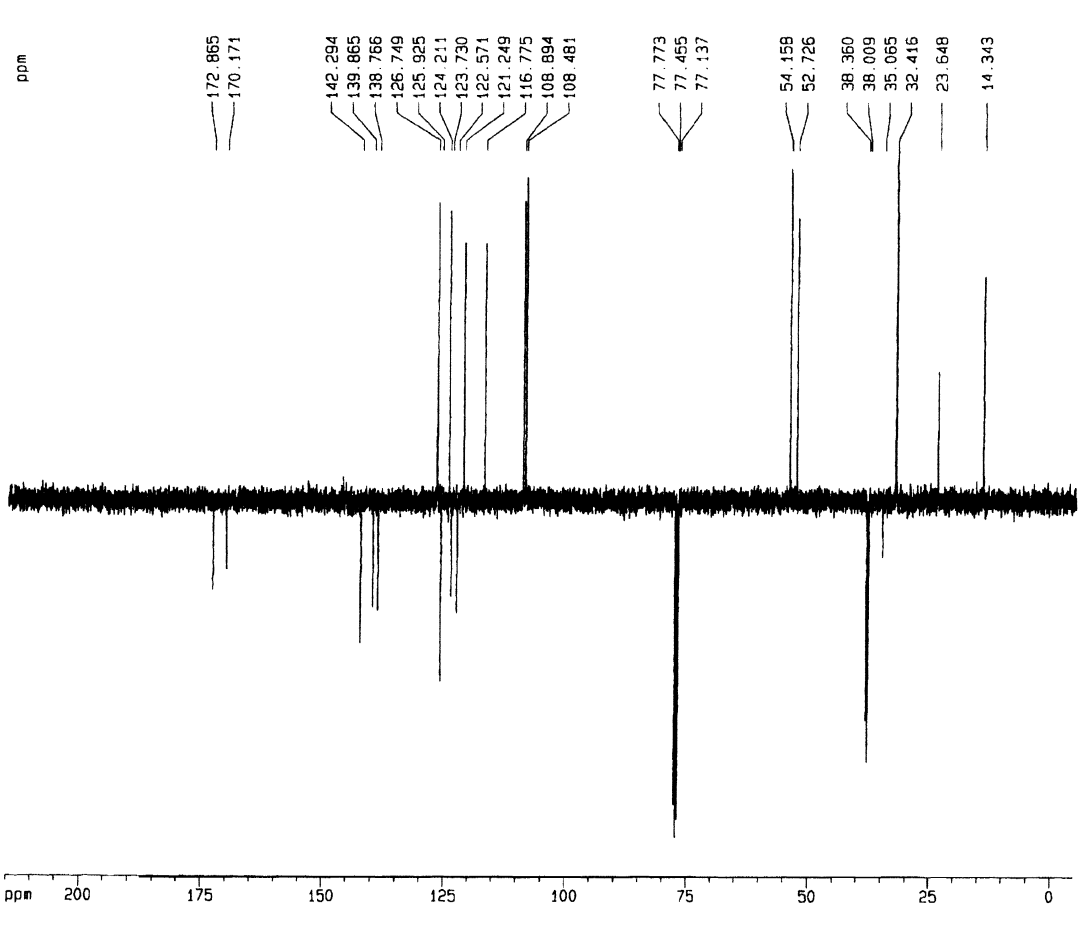

```
Current Data Parameters
NAME          p000709001
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_          20000710
Time              16.10
INSTRUM           spect
PROBHD     5 mm Multinu
PULPROG           zg30
TD               32768
SOLVENT          CDCl3
NS                   16
DS                    2
SWH           8278.146 Hz
FIDRES        0.252629 Hz
AQ            1.9792372 sec
RG                456.1
DW               60.400 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec

========== CHANNEL f1 ========
NUC1                  1H
P1                 9.00 usec
PL1                0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI               32768
SF          400.1299613 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00

1D NMR plot parameters
CX                20.00 cm
F1P               10.000 ppm
F1              4001.30 Hz
F2P               0.000 ppm
F2                0.00 Hz
PPMCM          0.50000 ppm/cm
HZCM          200.06499 Hz/cm
```

N-Acetyl-3-(N'-ethyl-3-carbazolyl)-DL-alanine methyl ester (NMR-29)



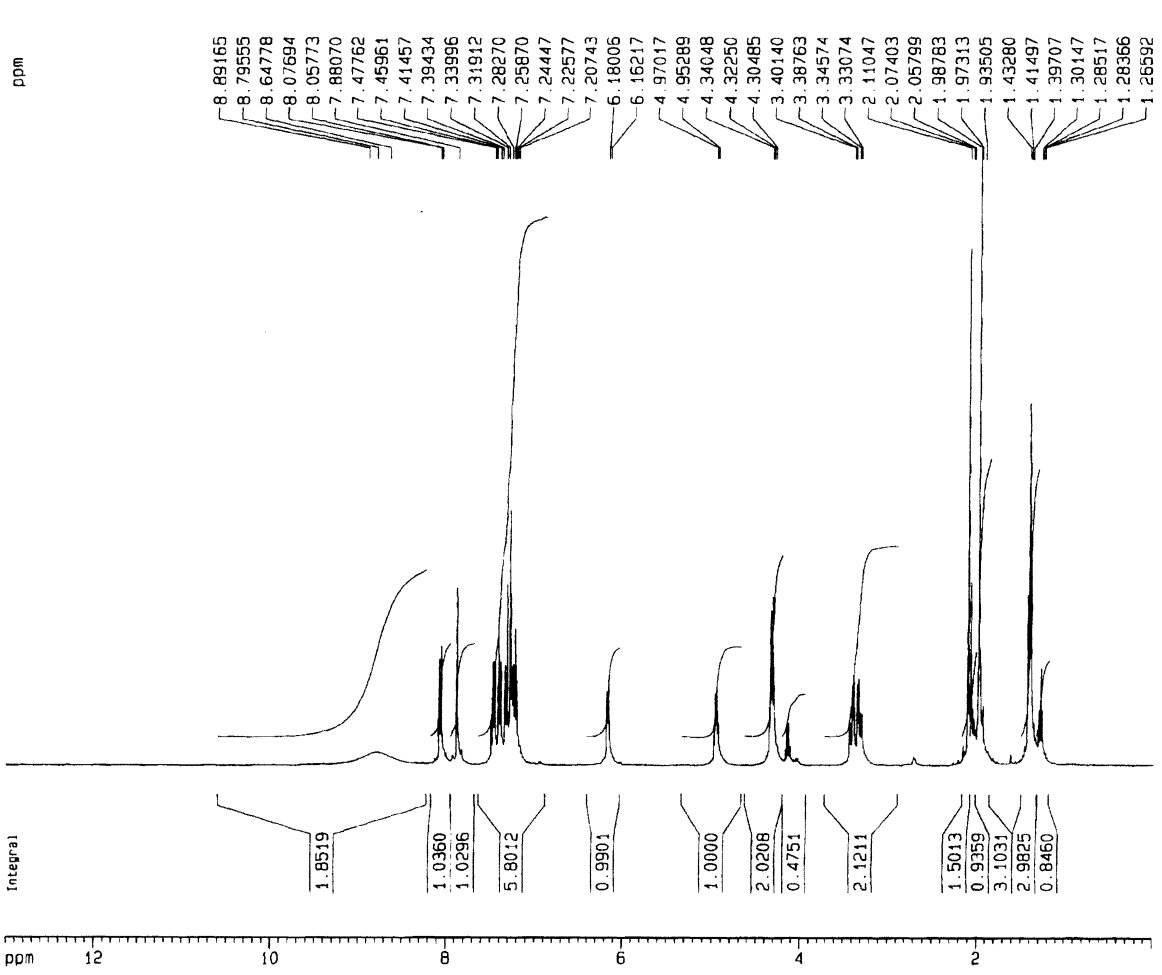

```
Current Data Parameters
NAME      p00112601-A-C-
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_          20001126
Time              21.23
INSTRUM           spect
PROBHD     5 mm Multinu
PULPROG           zg30
TD               32768
SOLVENT          CDCl3
NS                  16
DS                   2
SWH           8278.146 Hz
FIDRES        0.252629 Hz
AQ            1.9792372 sec
RG                 181
DW              60.400 usec
DE               6.00 usec
TE              300.0 K
D1           1.00000000 sec

========== CHANNEL f1 ========
NUC1                1H
P1               9.00 usec
PL1              0.00 dB
SFO1        400.1324710 MHz

F2 - Processing parameters
SI               32768
SF          400.1300000 MHz
WDW                 EM
SSB                  0
LB               0.30 Hz
GB                   0
PC                1.00

1D NMR plot parameters
CX              20.00 cm
F1P             10.000 ppm
F1            4001.30 Hz
F2P              0.000 ppm
F2               0.00 Hz
PPMCM         0.50000 ppm/cm
HZCM        200.06500 Hz/cm
```

N-Acetyl-3-(N'-ethyl-3-carbazolyl)-DL-alanine methyl ester (NMR-30)



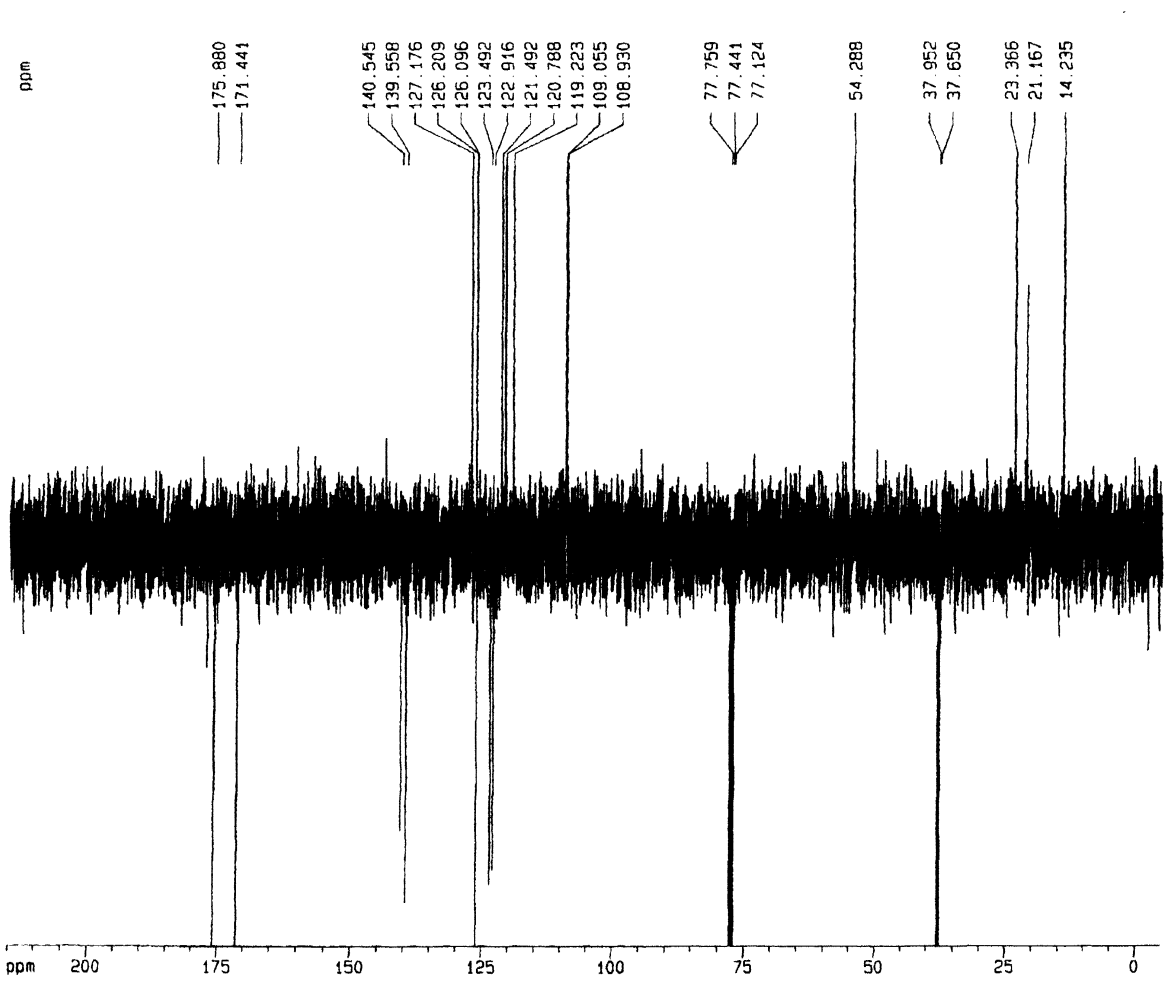

```
Current Data Parameters
NAME        p00102402-c-a
EXPNO               10
PROCNO               1

F2 - Acquisition Parameters
Date_          20001024
Time              15.26
INSTRUM           spect
PROBHD     5 mm Multinu
PULPROG           jmod
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    4
SWH           25125.629 Hz
FIDRES        0.383387 Hz
AQ            1.3042164 sec
RG                 4096
DW               19.900 usec
DE                6.00 usec
TE               300.0 K
CNST2        145.0000000
CNST11         1.0000000
D1             2.00000000 sec
d13            0.00000300 sec
d20            0.00689655 sec
DELTA       6366.18261719 sec

========== CHANNEL f1 ==========
NUC1               13C
P1               10.70 usec
p2               21.40 usec
PL1              -1.00 dB
SF01         100.6237959 MHz

========== CHANNEL f2 ==========
CPDPRG2          waltz16
NUC2               1H
PCPD2            80.00 usec
PL2               0.00 dB
PL12             20.00 dB
SF02         400.1316005 MHz

F2 - Processing parameters
SI                32768
SF           100.6127290 MHz
WDW                EM
SSB                0
LB              1.00 Hz
GB                 0
PC              1.40

1D NMR plot parameters
CX              20.00 cm
F1P            215.000 ppm
F1           21631.74 Hz
F2P             -5.000 ppm
F2           -503.06 Hz
PPMCM         11.00000 ppm/cm
HZCM        1106.73999 Hz/cm
```

N-t-Butoxycarbonyl-3-(N'-ethyl-3-carbazolyl)-L-alanine (NMR-31)



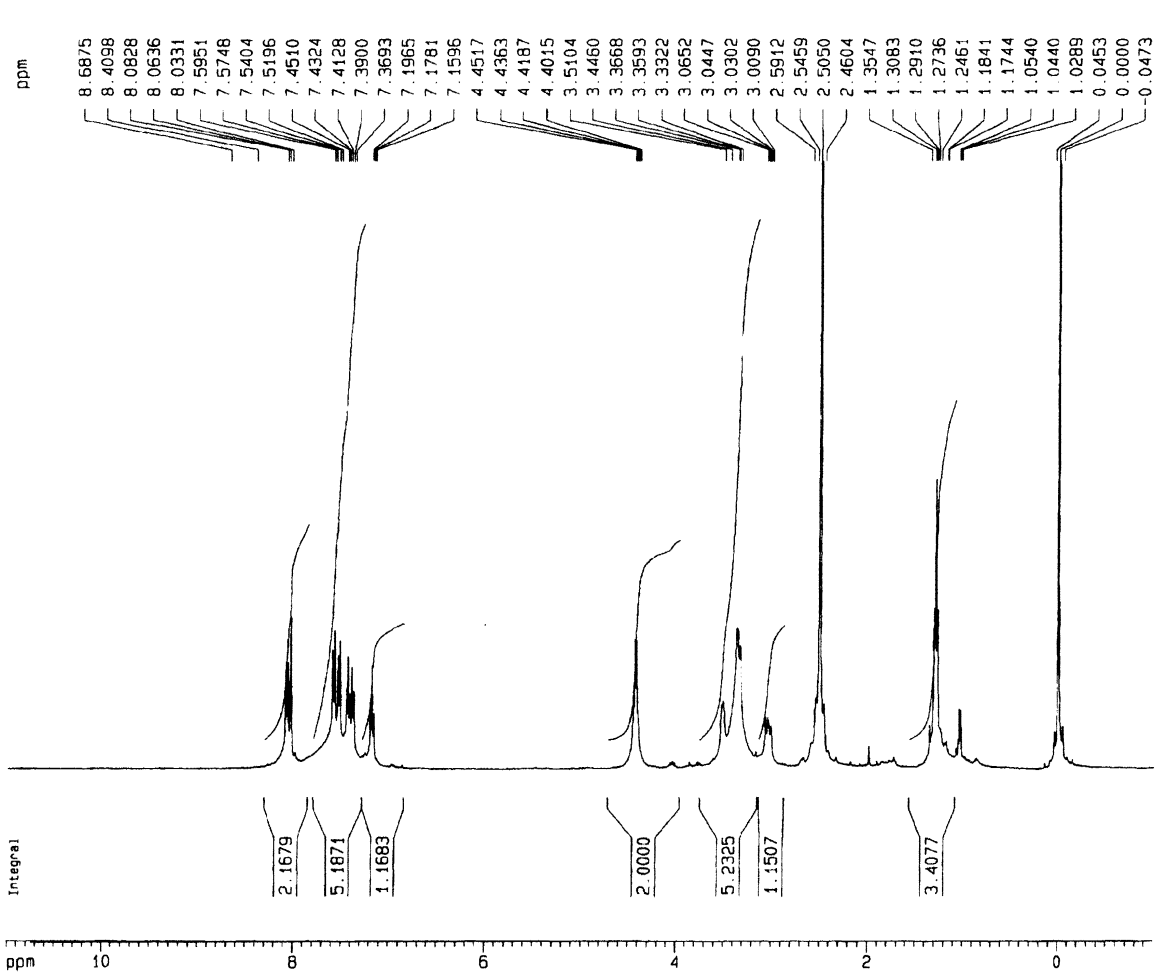

```
Current Data Parameters
NAME      p00110401-ac-c
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20001104
Time               22.04
INSTRUM            spect
PROBHD     5 mm Multinu
PULPROG            zg30
TD                32768
SOLVENT           CDCl3
NS                    16
DS                     2
SWH           8278.146 Hz
FIDRES        0.252629 Hz
AQ            1.9792372 sec
RG                203.2
DW               60.400 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec

========== CHANNEL f1 ========
NUC1                  1H
P1                 9.00 usec
PL1                0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300000 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                 1.00

1D NMR plot parameters
CX                20.00 cm
F1P               13.000 ppm
F1                5201.69 Hz
F2P                0.000 ppm
F2                 0.00 Hz
PPMCM          0.65000 ppm/cm
HZCM          260.08450 Hz/cm
```

N-t-Butoxycarbonyl-3-(N'-ethyl-3-carbazolyl)-L-alanine (NMR-32)



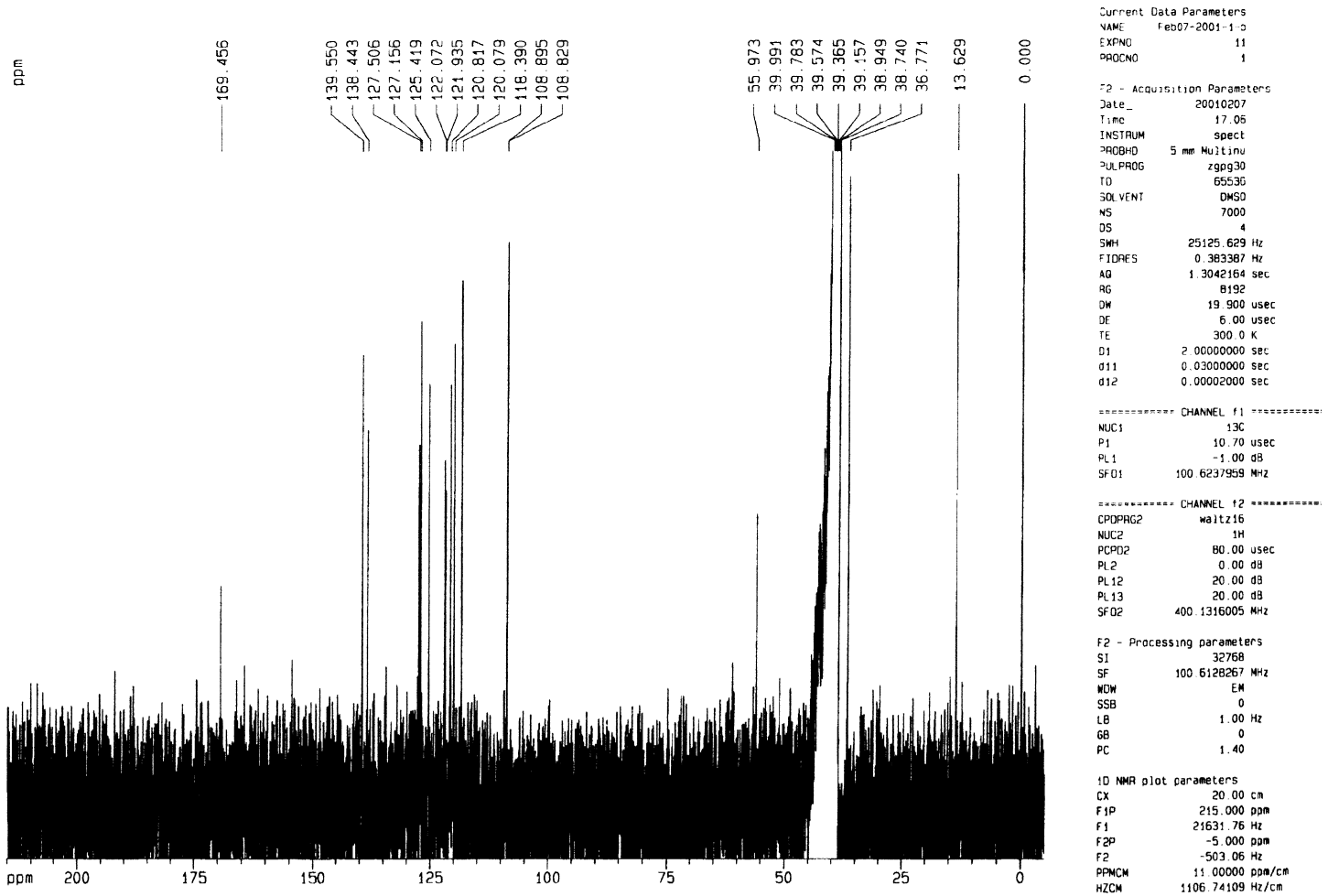

```
Current Data Parameters
NAME        p00110401-ac-c
EXPNO                   11
PROCNO                   1

F2 - Acquisition Parameters
Date_             20001104
Time                 22.19
INSTRUM              spect
PROBHD     5 mm Multinu
PULPROG              jmod
TD                  65536
SOLVENT             CDCl3
NS                    256
DS                      4
SWH              25125.629 Hz
FIDRES           0.383387 Hz
AQ               1.3042164 sec
RG                    4096
DW                19.900 usec
DE                 6.00 usec
TE                300.0 K
CNST2          145.0000000
CNST11           1.0000000
D1               2.00000000 sec
d13              0.00000300 sec
d20              0.00689655 sec
DELTA         6366.18261719 sec

========== CHANNEL f1 ==========
NUC1                    13C
P1                   10.70 usec
p2                   21.40 usec
PL1                  -1.00 dB
SFO1            100.6237959 MHz

========== CHANNEL f2 ==========
CPDPRG2             waltz16
NUC2                    1H
PCPD2                80.00 usec
PL2                   0.00 dB
PL12                 20.00 dB
SFO2            400.1316005 MHz

F2 - Processing parameters
SI                      32768
SF              100.6127290 MHz
WDW                        EM
SSB                         0
LB                   1.00 Hz
GB                          0
PC                       1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                215.000 ppm
F1                 21631.74 Hz
F2P                 -5.000 ppm
F2                -503.08 Hz
PPMCM             11.00000 ppm/cm
HZCM           1106.73999 Hz/cm
```

3-(N-Ethyl-3-carbazolyl)-L-alanine (NMR-33)




```
Current Data Parameters
NAME      Feb07-2001-1-p
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20010207
Time                1.52
INSTRUM            spect
PROBHD     5 mm Multinu
PULPROG            zg30
TD                65536
SOLVENT            DMSO
NS                  128
DS                    0
SWH          8278.146 Hz
FIDRES       0.126314 Hz
AQ           3.9584243 sec
RG               645.1
DW              60.400 usec
DE               6.00 usec
TE              300.0 K
D1           2.00000000 sec

========== CHANNEL f1 ========
NUC1                  1H
P1                 9.00 usec
PL1                0.00 dB
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300005 MHz
WDW                   EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00

1D NMR plot parameters
CX                20.00 cm
F1P              11.000 ppm
F1             4401.43 Hz
F2P              -1.000 ppm
F2             -400.13 Hz
PPMCM          0.60000 ppm/cm
HZCM         240.07800 Hz/cm
```

3-(N-Ethyl-3-carbazolyl)-L-alanine (NMR-34)



Current Data Parameters
NAME Feb07-2001-1:5
EXPNO 11
PROCNO 1

F2 - Acquisition Parameters
Date_ 20010207
Time 17.05
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 7000
DS 4
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 8192
DW 19.900 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
d12 0.00002000 sec

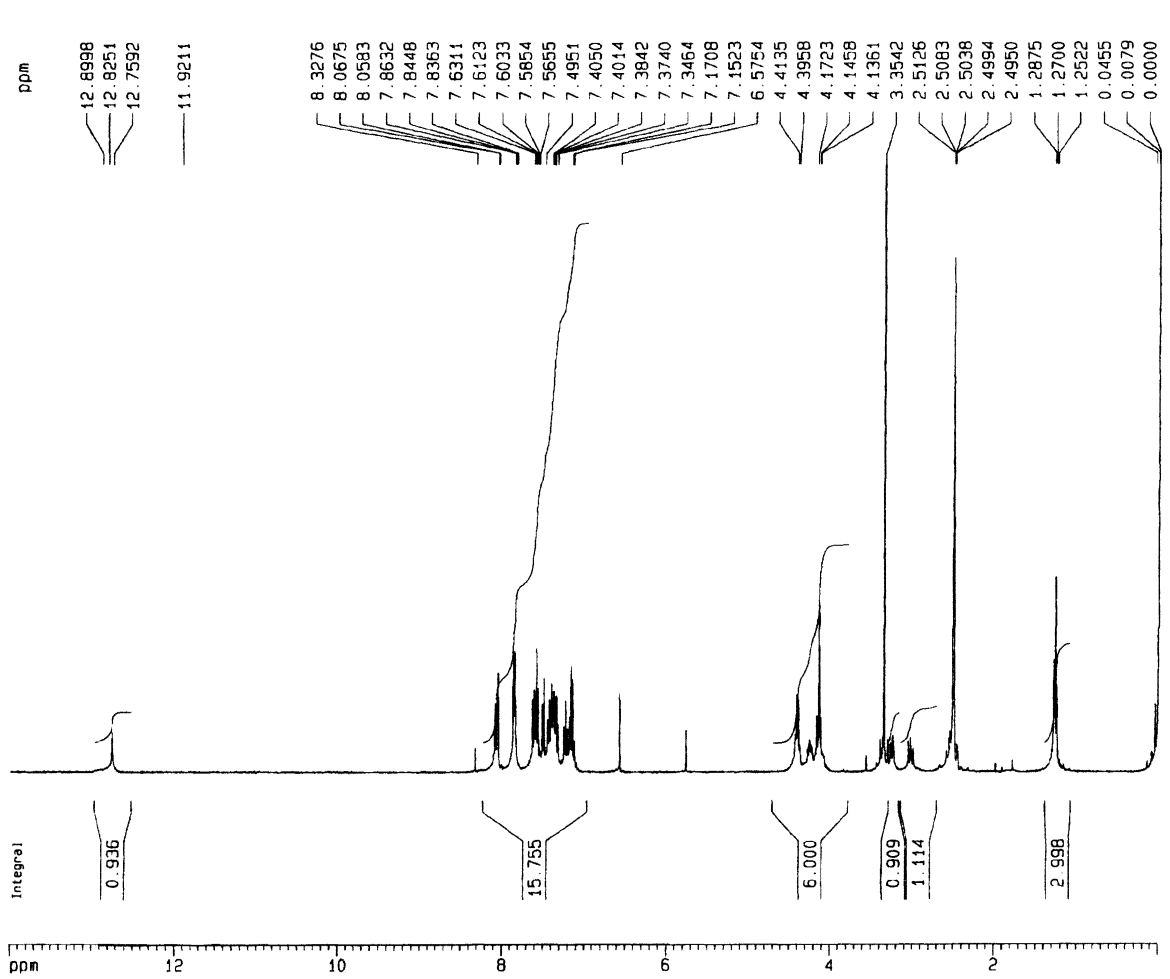
========== CHANNEL f1 ==========
NUC1 13C
P1 10.70 usec
PL1 -1.00 dB
SFO1 100.6237959 MHz

========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 20.00 dB
PL13 20.00 dB
SFO2 400.1316005 MHz

F2 - Processing parameters
SI 32768
SF 100.6128267 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

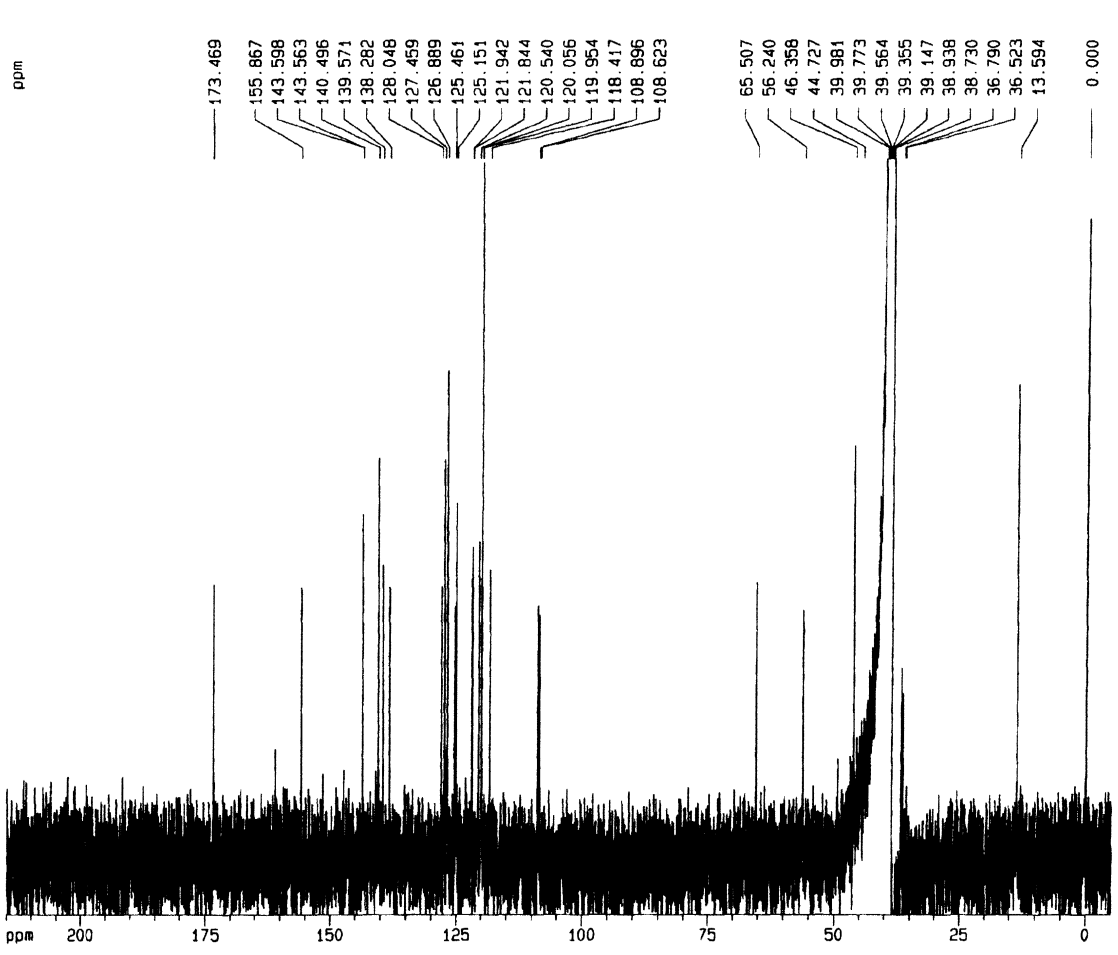
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.76 Hz
F2P -5.000 ppm
F2 -503.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.74109 Hz/cm

N-Fmoc-3-(N'-ethyl-3-carbazolyl)-L-alanine (NMR-35)



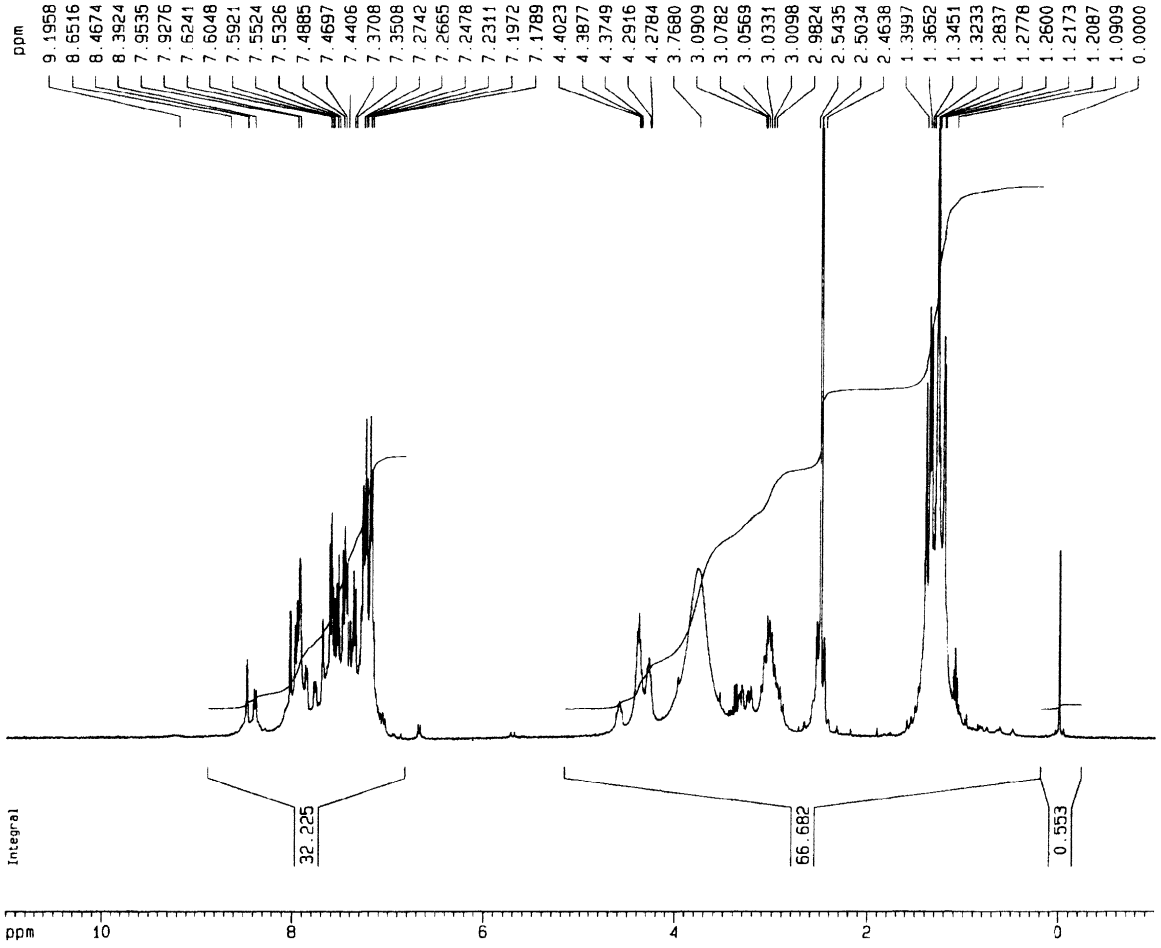

```
Current Data Parameters
NAME          Mar10-2001
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_          20010310
Time              19.36
INSTRUM           spect
PROBHD     5 mm Multinu
PULPROG           zg30
TD               32768
SOLVENT           DMSO
NS                   16
DS                    2
SWH           8278.146 Hz
FIDRES        0.252629 Hz
AQ            1.9792372 sec
RG                  512
DW             60.400 usec
DE              6.00 usec
TE              300.0 K
D1           1.00000000 sec

========== CHANNEL f1 ========
NUC1                1H
P1               9.00 usec
PL1              0.00 dB
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300016 MHz
WDW                   EM
SSB                    0
LB                0.30 Hz
GB                     0
PC                  1.00

1D NMR plot parameters
CX                20.00 cm
F1P              14.000 ppm
F1              5601.82 Hz
F2P               0.000 ppm
F2                0.00 Hz
PPMCM          0.70000 ppm/cm
HZCM          280.09100 Hz/cm
```

N-Fmoc-3-(N'-ethyl-3-carbazolyl)-L-alanine (NMR-36)




```
Current Data Parameters
NAME      Apr09-2001-pan
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20010409
Time               14.47
INSTRUM            spect
PROBHD     5 mm Multinu
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                14000
DS                    4
SWH           25125.629 Hz
FIDRES        0.383387 Hz
AQ            1.3042154 sec
RG                16384
DW               19.900 usec
DE                6.00 usec
TE               300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

============ CHANNEL f1 ============
NUC1                13C
P1                10.70 usec
PL1               -1.00 dB
SFO1         100.6237959 MHz

============ CHANNEL f2 ============
CPDPRG2         waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                0.00 dB
PL12              20.00 dB
PL13              20.00 dB
SFO2         400.1316005 MHz

F2 - Processing parameters
SI                32768
SF           100.6128268 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40

1D NMR plot parameters
CX                20.00 cm
F1P              215.000 ppm
F1             21631.76 Hz
F2P               -5.000 ppm
F2              -503.06 Hz
PPMCM          11.00000 ppm/cm
HZCM         1106.74109 Hz/cm
```

H-Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe-OH (NMR-37)




```
Current Data Parameters
NAME      Apr15-2001-1-p
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20010416
Time                1.09
INSTRUM             spect
PROBHD     5 mm Multinu
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                   128
DS                     0
SWH           8278.146 Hz
FIDRES        0.126314 Hz
AQ            3.9584243 sec
RG                574.7
DW               60.400 usec
DE                6.00 usec
TE               300.0 K
D1            2.00000000 sec

============ CHANNEL f1 ========
NUC1                  1H
P1                 9.00 usec
PL1                0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300022 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00

1D NMR plot parameters
CX                 20.00 cm
F1P               11.000 ppm
F1              4401.43 Hz
F2P               -1.000 ppm
F2              -400.13 Hz
PPMCM           0.60000 ppm/cm
HZCM          240.07800 Hz/cm
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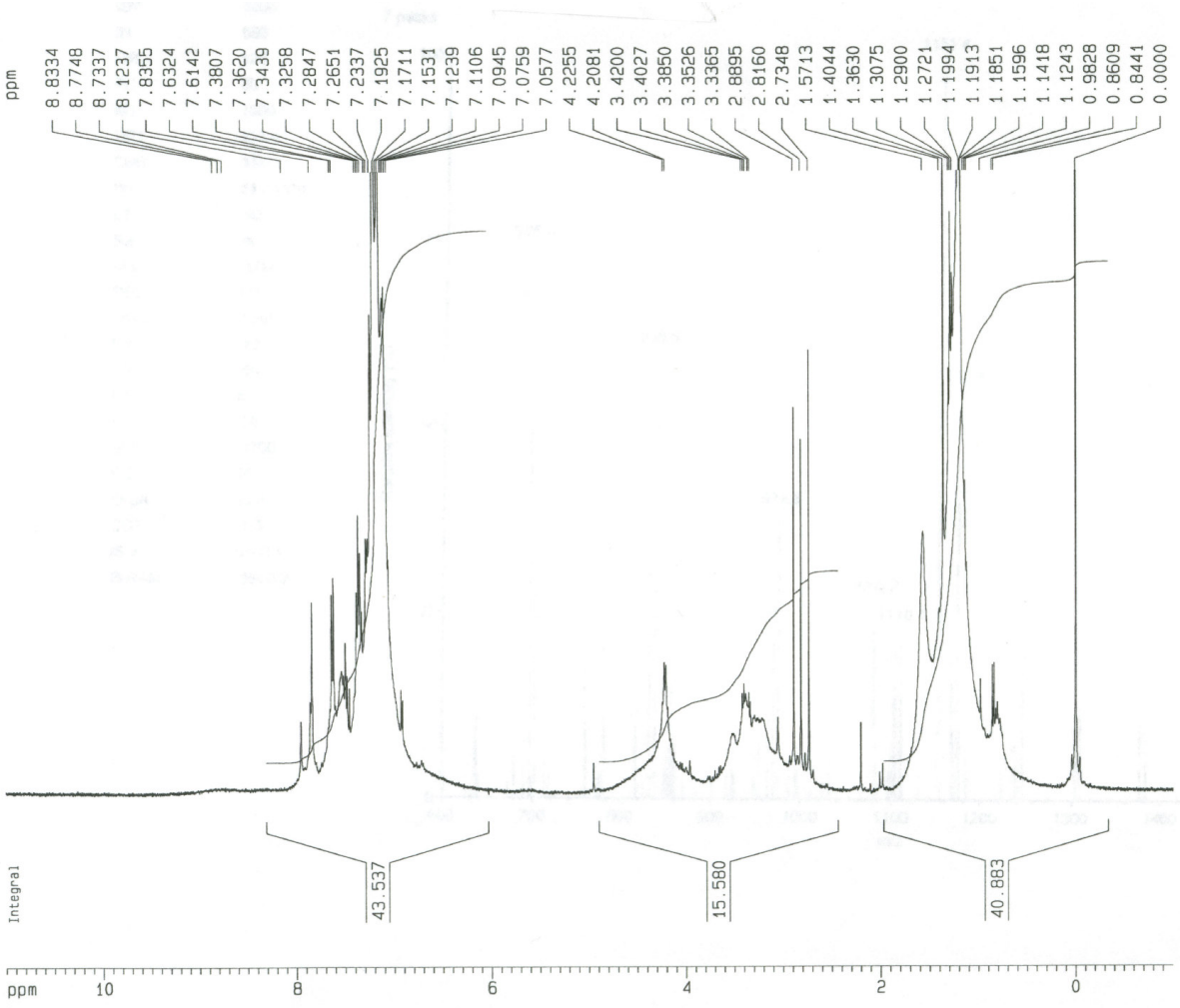
c*yclo*(Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe) (NMR-38)

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Current Data Parameters
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EXPNO                 10
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F2 - Acquisition Parameters
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============ CHANNEL f1 ========
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F2 - Processing parameters
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PC                  1.00

1D NMR plot parameters
CX                20.00 cm
F1P               11.000 ppm
F1              4401.43 Hz
F2P               -1.000 ppm
F2              -400.13 Hz
PPMCM          0.60000 ppm/cm
HZCM          240.07802 Hz/cm
```
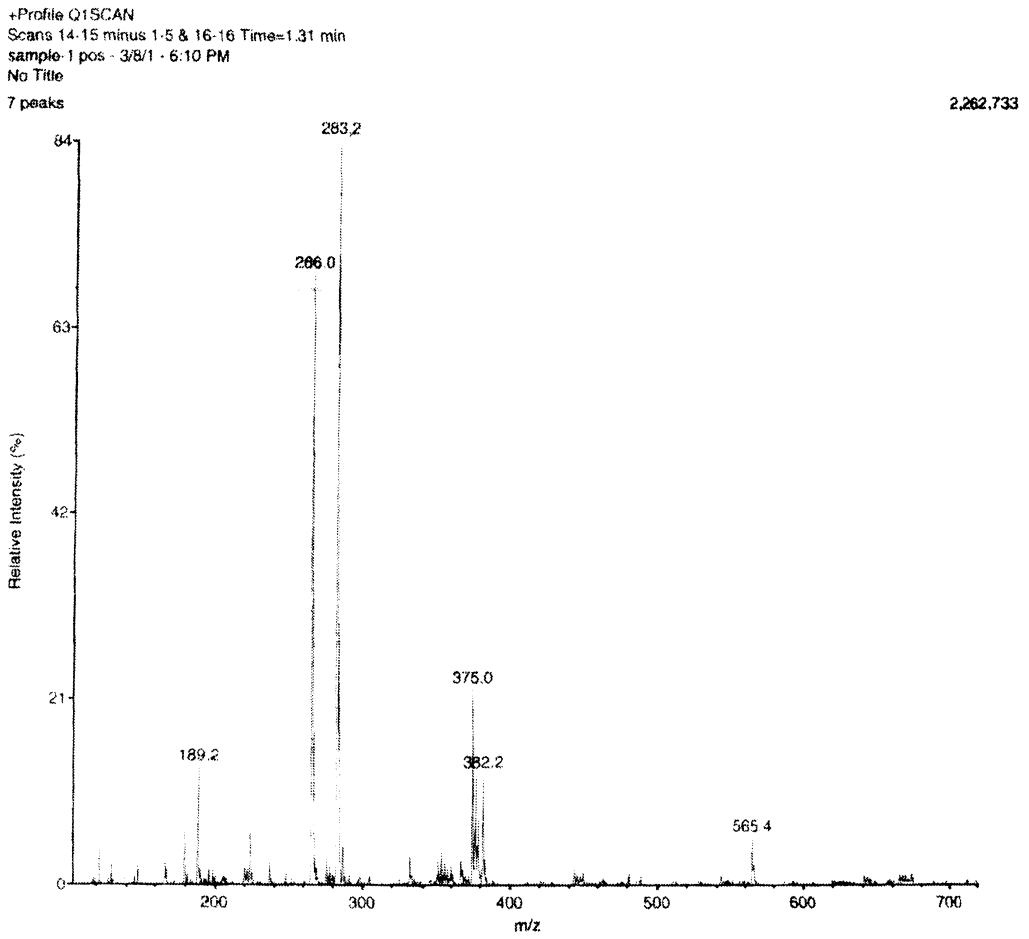
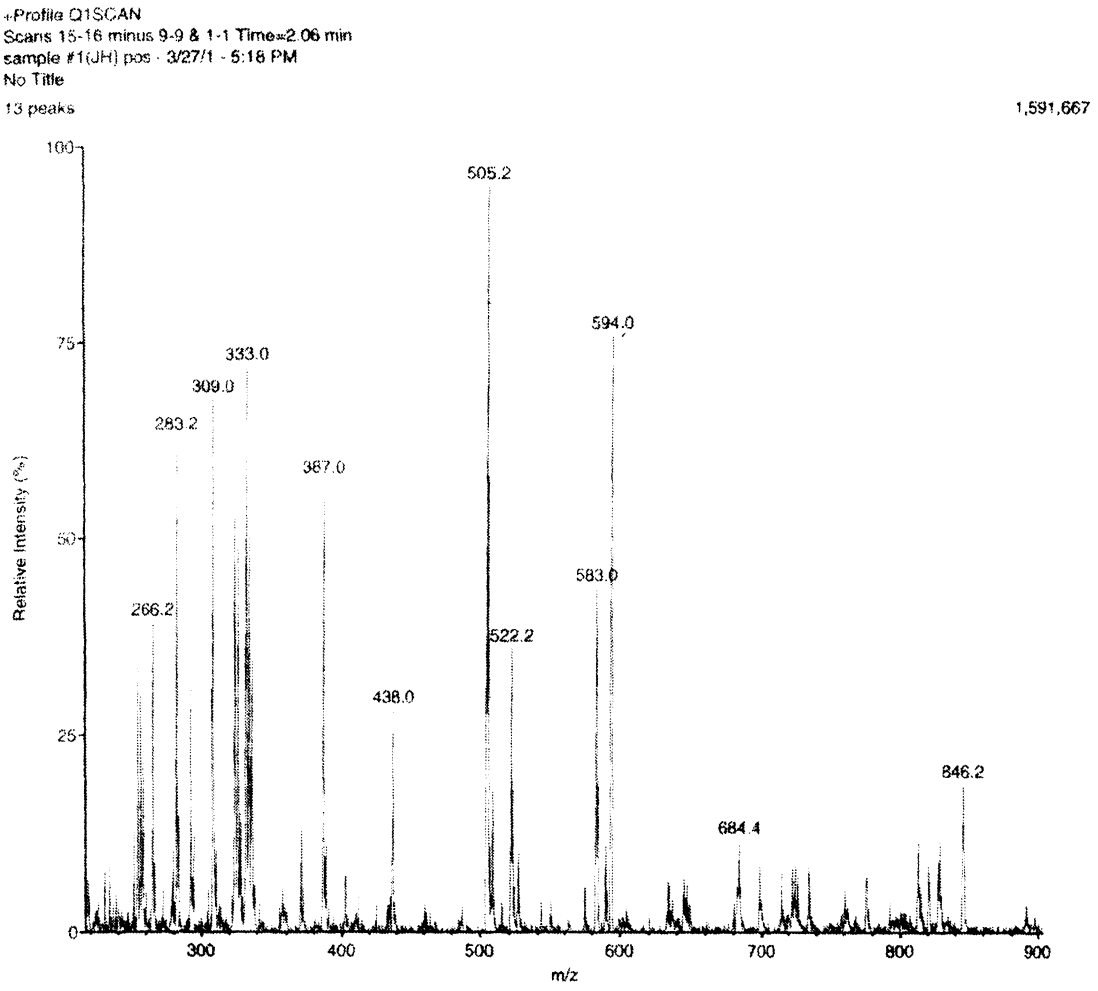

3-(N-Ethyl-3-carbazolyl)-L-alanine (MS-1)

DI	50
ISV	5000
IN	650
OR	60
R0	30
M1	1000
RE1	122
DM1	0.060
R1	26.00000
L7	-40
R2	-60
M3	1000
RE3	118
DM3	0.060
RX	-10
R3	-50
L9	50
FP	-50
MU	-4700
CC	10
DI µA	1.6
CGT	0
IS v	5119.7
B-RAM	-10,004.9



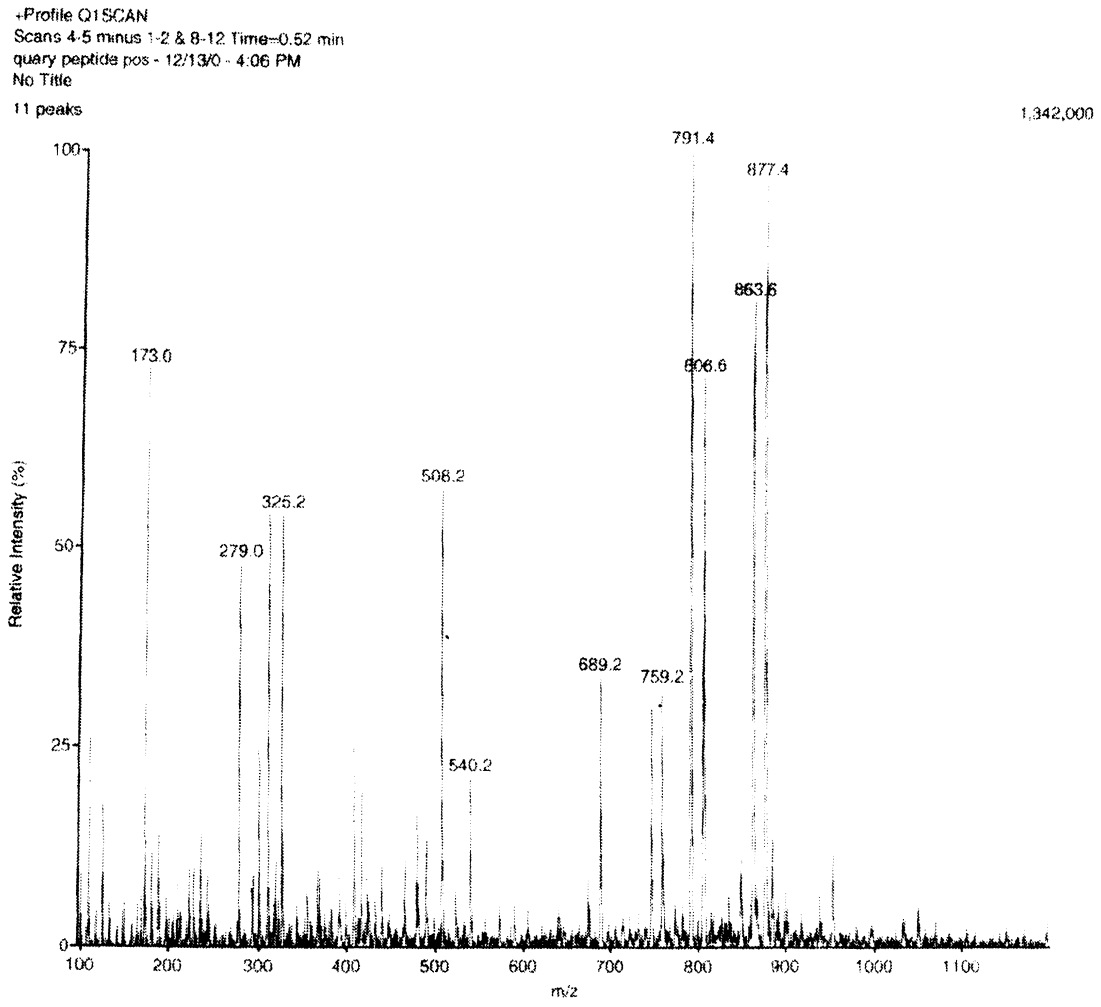
N-Fmoc-3-(N'-ethyl-3-carbazolyl)-L-alanine (MS-2)

DI	50
ISV	5000
IN	650
OR	60
R0	30
M1	1000
RE1	122.4
DM1	0.060
R1	26.00000
L7	-40
R2	-60
M3	1000
RE3	118
DM3	0.060
RX	-10
R3	-50
L9	50
FP	-50
MU	-4700
CC	10
DI µA	3.2
CGT	0
IS v	5085.5
B-RAM	-10,004.9



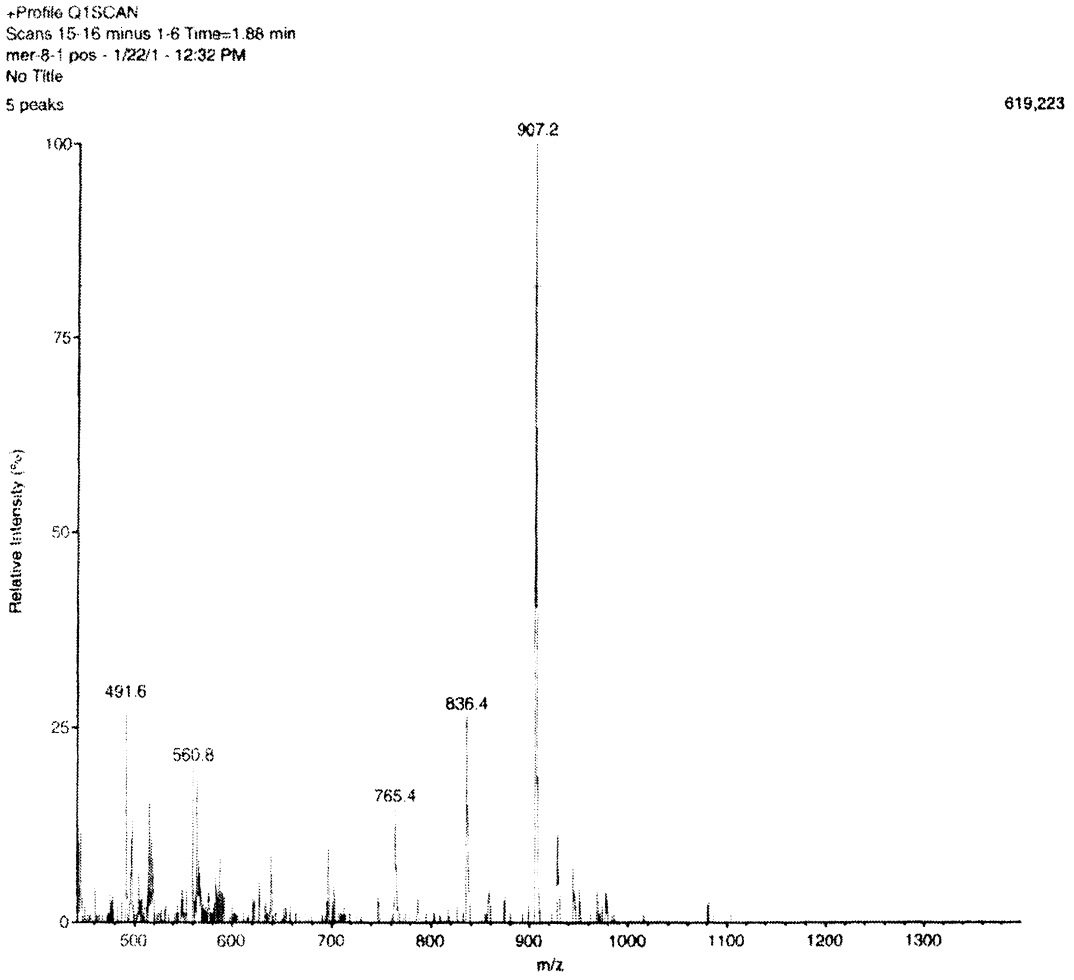
H-Car-D-Ala-Bpa-D-Ala-OH (MS-3)

DI	50
ISV	5000
IN	650
OR	120
R0	30
M1	1000
RE1	122.7
DM1	0.040
R1	26.5
L7	-50
R2	-50
M3	1000
RE3	118
DM3	0.060
RX	-10
R3	-50
L9	50
FP	-50
MU	-4200
CC	1
Di µA	3.9
CGT	-1.3
IS v	5090.4
B-RAM	-10,004.9



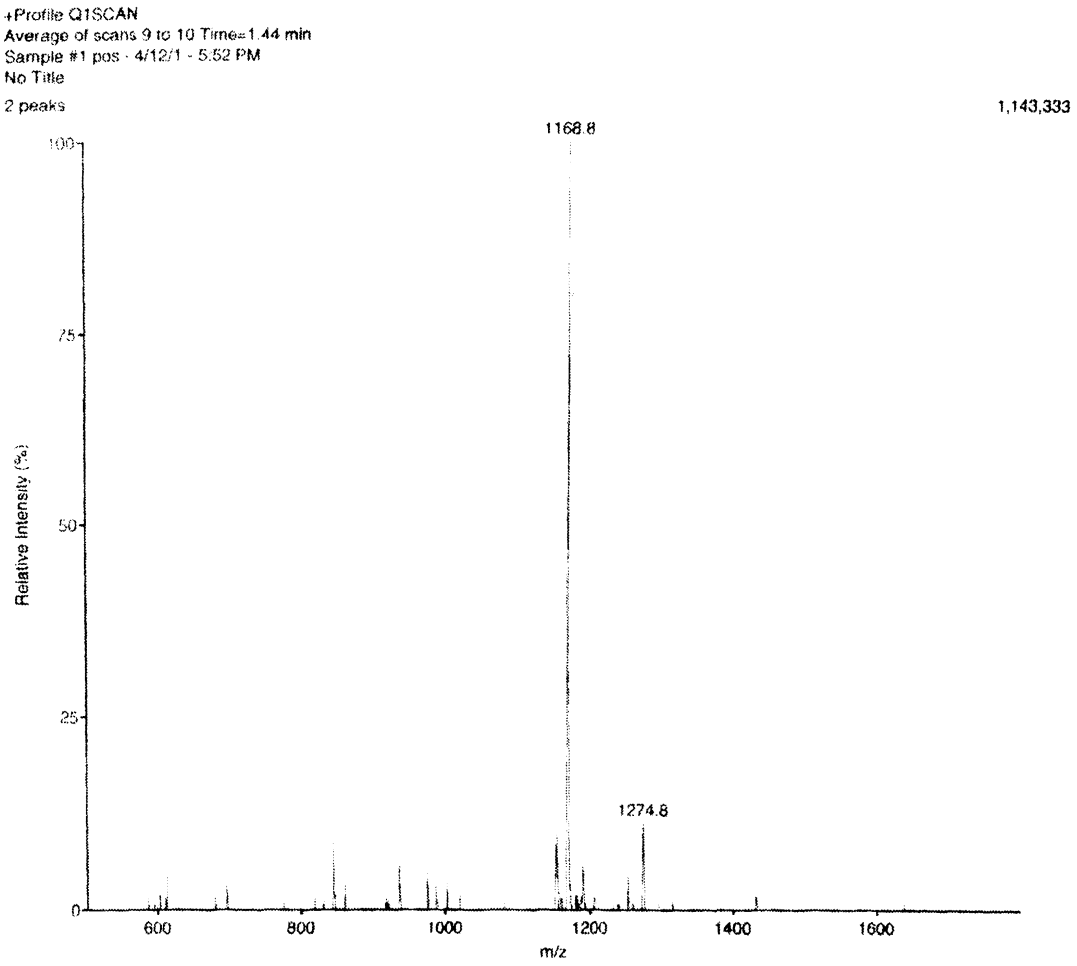
H-D-Ala-Nap-D-MeAla-Ala-D-Ala-Bpa-D-Ala-Ala-OH (MS-4)

DI	50
ISV	5000
IN	650
OR	100
R0	30
M1	1000
RE1	122.4
DM1	0.050
R1	26.00000
L7	-40
R2	-60
M3	1000
RE3	118
DM3	0.060
RX	-10
R3	-50
L9	50
FP	-50
MU	-4700
CC	10
DI µA	14.3
CGT	-1.3
IS v	5100.1
B-RAM	-10,004.9



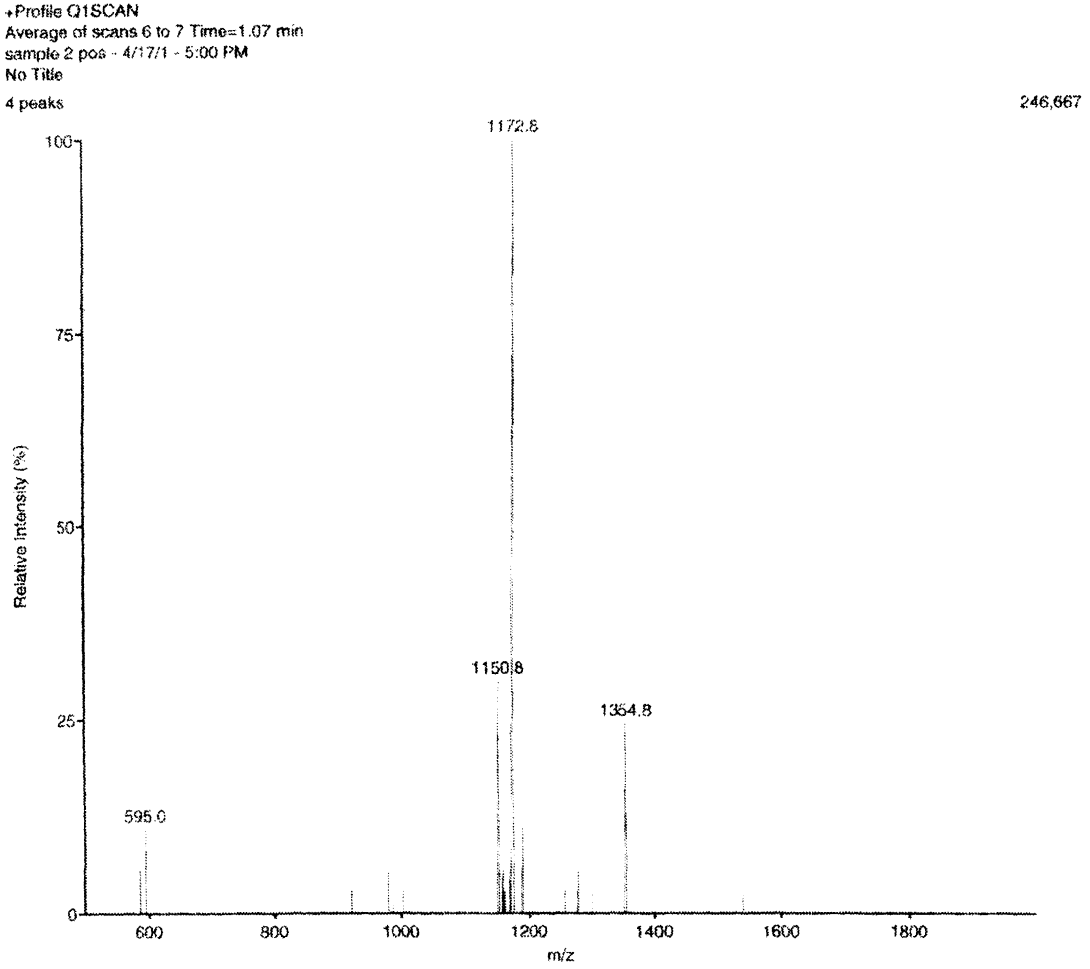
H-Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe-OH (MS-5)

DI	50
ISV	5000
IN	650
OR	80
R0	30
M1	1000
RE1	121.8
DM1	0.060
R1	26.00000
L7	-40
R2	-60
M3	1000
RE3	118
DM3	0.060
RX	-10
R3	-50
L9	50
FP	-50
MU	-4700
CC	10
DI µA	20.3
CGT	1.3
IS v	5124.6
B-RAM	-10,004.9



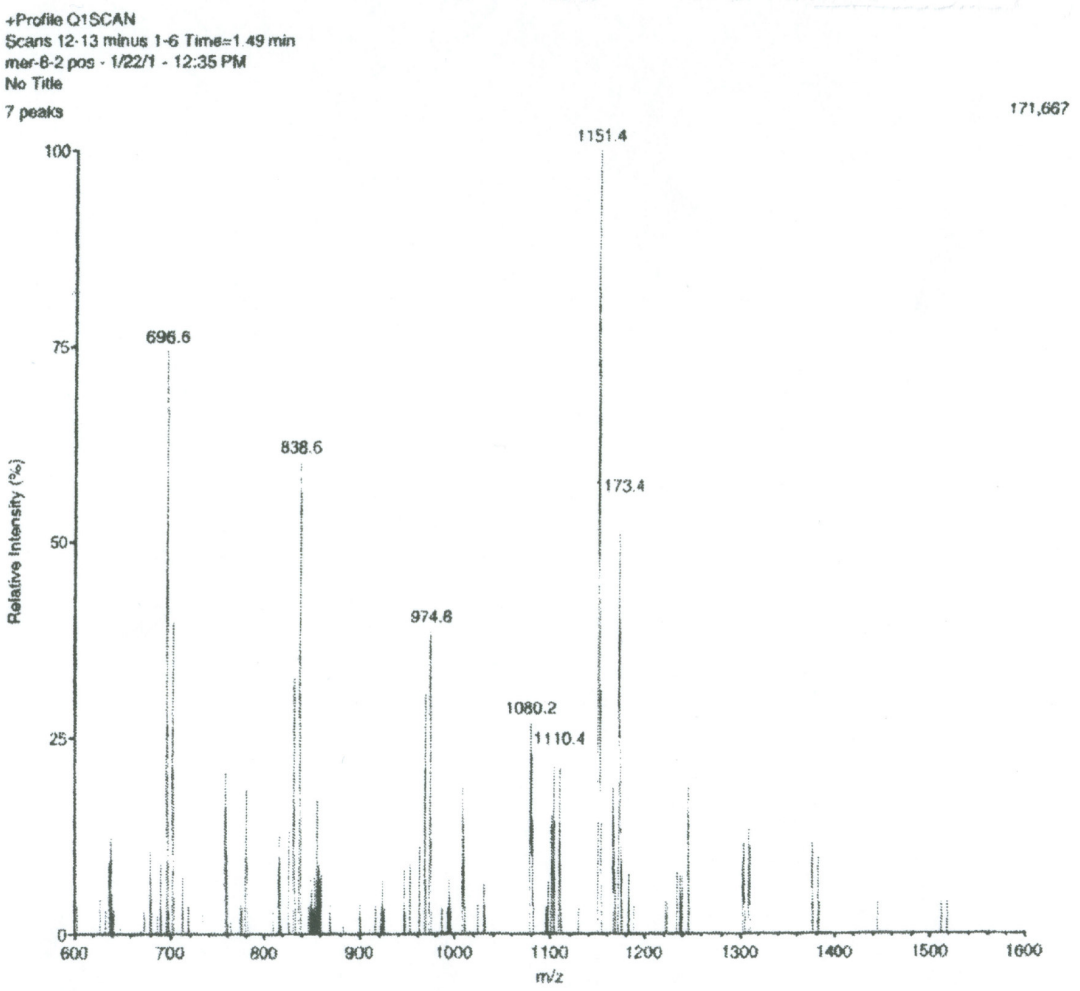
cyclo(Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe) (MS-6)

DI	50
ISV	5000
IN	650
OR	120
R0	30
M1	1000
RE1	121.9
DM1	0.060
R1	26.00000
L7	-40
R2	-60
M3	1000
RE3	118
DM3	0.060
RX	-10
R3	-50
L9	50
FP	-50
MU	-4700
CC	10
DI µA	1.7
CGT	-1.3
IS v	5129.5
B-RAM	-10,004.9



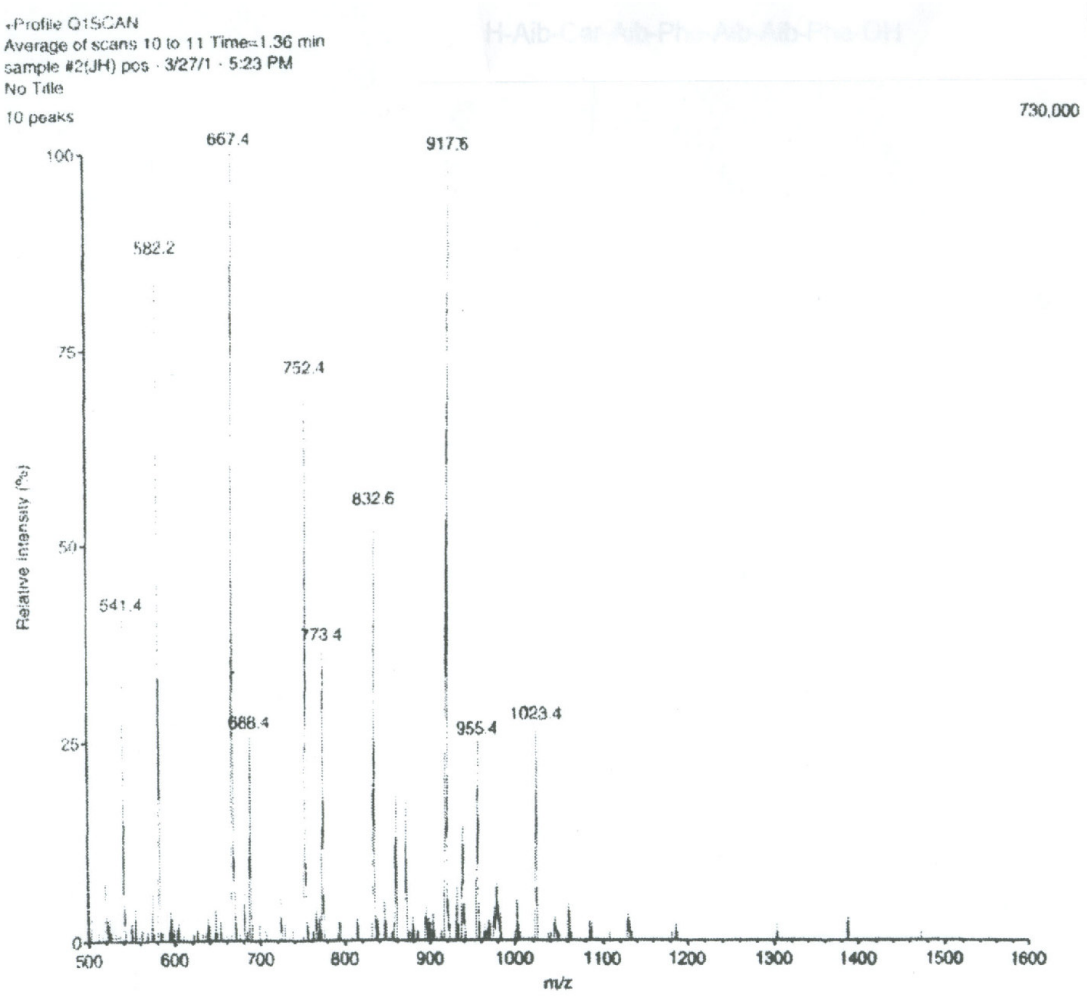
H-D-Ala-Nap-D-MeAla-Ala-D-Ala-Bpa-D-Ala-Ala-OH (MS-7)

DI	50
ISV	5000
IN	650
OR	120
R0	30
M1	1000
RE1	122.4
DM1	0.050
R1	26.00000
L7	-40
R2	-60
M3	1000
RE3	118
DM3	0.060
RX	-10
R3	-50
L9	50
FP	-50
MU	-4700
CC	10
Di µA	23.2
CGT	-1.3
IS v	5100.1
B-RAM	-9902.3



H-Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe-OH (MS-8)

DI	50
ISV	5000
IN	650
OR	120
R0	30
M1	1000
RE1	122.4
DM1	0.060
R1	26.00000
L7	-40
R2	-60
M3	1000
RE3	118
DM3	0.060
RX	-10
R3	-50
L9	50
FP	-50
MU	-4700
CC	10
DI µA	5.007
CGT	-1.3
IS v	5114.8
B-RAM	-10,004.9



c*yclo*(Aib-Car-Aib-Phe-Aib-Bpa-Aib-Phe) (MS-9)

DI	50
ISV	5000
IN	650
OR	90
R0	30
M1	1000
RE1	121.7
DM1	0.040
R1	26.00000
L7	-40
R2	-60
M3	1000
RE3	118
DM3	0.060
RX	-10
R3	-50
L9	50
FP	-50
MU	-4700
CC	10
DI µA	1.7
CGT	-1.3
IS v	5080.6
B-RAM	-10,004.9