Development Toward the Synthesis of New Pharmacophores

An Major Qualifying Project
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by
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Abstract

The concerted [3+2] ylide-alkene photocyclization proceeds through trans-fused ylide intermediates. This reaction has been demonstrated to produce multicyclic heterocycles with up to six chiral centers in excellent yields from simple starting materials. This reaction can be used to develop novel compounds toward the discovery of new pharmacophores. Developments toward an asymmetric variant of this reaction have been undertaken.

Acknowledgments

I would like to thank Professor Dittami for his resources and time, without whom this project would not have been possible. I would also like to thank Victor Kiryak for his time and knowledge, and for answering all of those questions that come up in a new lab space. I would also like to extend my gratitude to Professor McGimpsey and Professor Lambert as well as the other members of their research group for the use of materials and for their time.

Background

Amino acids are the monomeric species which make up proteins and enzymes in living things. The 20 natural amino acids (AAs) are primarily responsible for the structure and function of biological entities. Proteins, made up of polymerized strings of the 20 natural AAs, are responsible in the body for an overwhelming variety of enzymatic and structural functions. AAs are also involved in innumerable biochemical interactions, including metabolism and catabolism, regulation of pathways, and immunostimulation and suppression[1]. Because of their close tie to biochemical effects, use of unnatural amino acids to prepare synthetic enzymes as well as other peptide drugs has grown[2]. As developments in proteomics and genomics gain knowledge into the structure and activity relationships of enzymes and other peptides, there is a drive to create compounds with novel structural properties, in the hopes that affecting these enzymes will impart pharmacological properties. Incorporating unnatural AAs in peptide analogues has been used to alter conformational flexibility, enzymatic stability, and pharmacodynamics

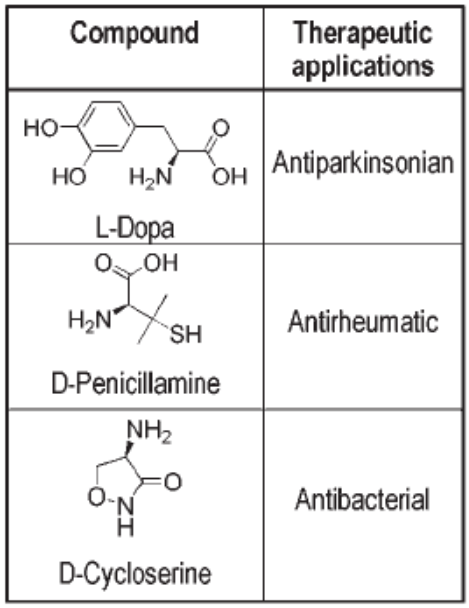

Figure 1 - Pharmacologically Active Amino Acid Derivatives[4]

[1] Voet, D., Voet, J. G., & Pratt, C. W. (2006). *Fundamentals of biochemistry: life at the molecular level*. New York: Wiley.

[2] A. Hasuoka, Y. Nishikimi, Y. Nakayama, K. Kamiyama, M. Nakao, et al; J Antibiot **55** 322-336 (2002); T.R. Ryder, L.-Y. Hu, M.F. Rafferty, S.M. Lotarski, D.M. Rock, et al.; Drug Des. Discovery **16** 317-322 (2000); A.Y. Jeng, P. Savage, M.E. Beil, W. Bruseo, D. Hoyer, et al; Clin. Sci. 103-Suppl **48**, 98-101S (2002); A.G. Beck-Sickinger; Methods Mol. Biol. (Totowa, N. J.) **73** 61-73 (1997); F.A. Davis, B.-C. Chen; Chem. Soc. Rev. **27** 13-18 (1998)

and bioavailablity[3,4]. Incorporation of AAs into proteins has also proven to be a valuable tool to aid in the study of structure-function activities in biochemistry[5].

There are a number of drugs on the market today which incorporate unnatural AA components. L-DOPA, shown in Figure 1, used for the treatment of Parkinson's disease, is a derivative of tyrosine, and a direct metabolic precursor to dopamine. Low levels of dopamine are characteristic of Parkinson's disease, causing violent, uncontrollable shaking[6]. D-Penicillamine is a metabolite of penicillin, a derivative of the cysteine, and has no antibiotic properties. However, it acts as an immunosuppressant and is thus utilized as a treatment for rheumatoid arthritis[7]. D-Cycloserine is an antibiotic which is typically used as a second-line drug for mycobacterium tuberculosis, if one or more first line drugs cannot be used[8]. Unnatural amino acids are also incorporated as components to many drugs on the market, including amoxicillin and ampicillin.

Researchers have also been successful at using the natural mechanisms of the cell's ribosome in order to allow for the use of unnatural amino acids in peptide chains[9,5]. This advancement will allow for the design of synthetic enzymes made up of synthetic amino acids which are assembled by the preexisting biological components of bacteria (or other organisms). This process allows for the encoding of a synthetic enzyme made up of synthetic amino acids into ribonucleic acid sequences (RNAs), a great advance for biotechnology.

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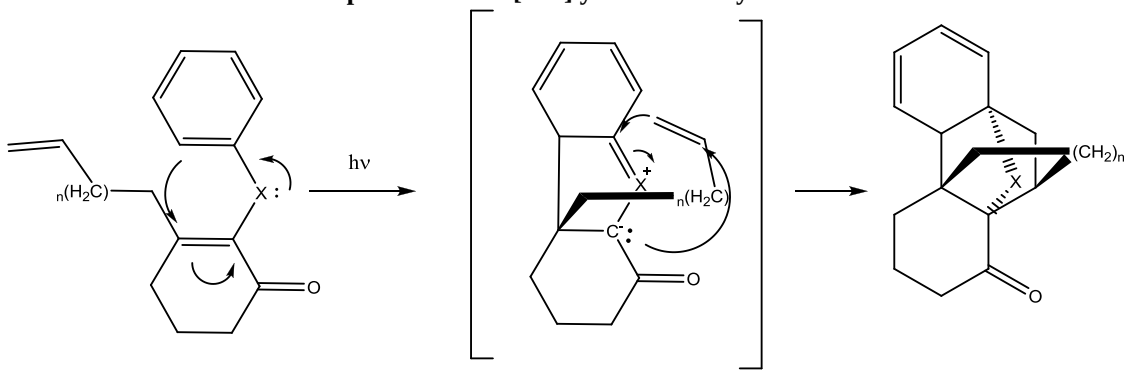
[3] M. Goodman, H. Shao; Pure Appl. Chem. **68** 1303-1308 (1996); G. Lubec, G. A. Rosenthal; Amino Acids: Chemistry, Biology, and Medicine; B. V., Leiden, Neth., 1990;
[4] J. Ma; Chimica Oggi. 65-68 (2003);
[5] Monahan, S., Lester, H., Dougherty, D.; Chemistry & Biology, **10** 573–580 (2003)
[6] F. Hefti, E. Melamed; *Trends in Neurosciences* **3**, 10 229-231 (1980)
[7] Walshe JM ; *Mov. Disord.* **18** (8): 853–9 (2003)
[8] G.B.M. Clarke, A.J.O'Hea; *Br Med J* **1** 636-638 (1961)
[9] Feng Tian, Meng-Lin Tsao, and Peter G. Schultz; J. AM. CHEM. SOC. **126** 15962-15963 (2004)

There is a clear drive to synthesize unnatural amino acids in the pursuit of compounds which exhibit useful biological activity. The synthesis of amino acids presents specific difficulties to organic synthesis. Amino acids include mutually reactive functional groups, and use of functional groups is required to mask the functionality of part of the molecule. Protection steps can often lead to racemization, which leads to complications in purification steps. AAs typically exist in a zwitterionic form at neutral pH. Amino acids are quite soluble in water but insoluble in many common organic solvents. Amino acids are chiral molecules and usually only a single enantiomer is of interest, so separation of enantiomers is required for symmetric syntheses. This problem creates a need for synthetic reactions which can exhibit stereospecificity to maximize yield efficiency. The development of stereospecific reactions to form complex unnatural amino acids is clearly of scientific merit.

Synthesis of chirally specific compounds requires the utilization of reactions which exhibit a high degree of stereospecificity. Stereospecific reactions are those which yield different stereoisomers depending on the stereochemistry of the reactant. For example, the standard S_N2 reaction which involves the nucleophilic displacement of a leaving group from carbon is stereospecific, resulting in the inversion of stereochemistry. Photochemical reactions proceed through predictable transition states and often exhibit a high degree of stereospecificity. A huge benefit to photochemical reactions is that the consumption of stoichiometric quantities of reagents are not required to transform the substrate, only the substrate itself. Photochemistry has been used to generate many complex compounds in relatively few steps, from simple starting materials, and could be employed with great utility in the synthesis of novel amino acids.

Scheme 1 – Mechanism of the photoinitiated [3+2] ylide-alkene cycloaddition

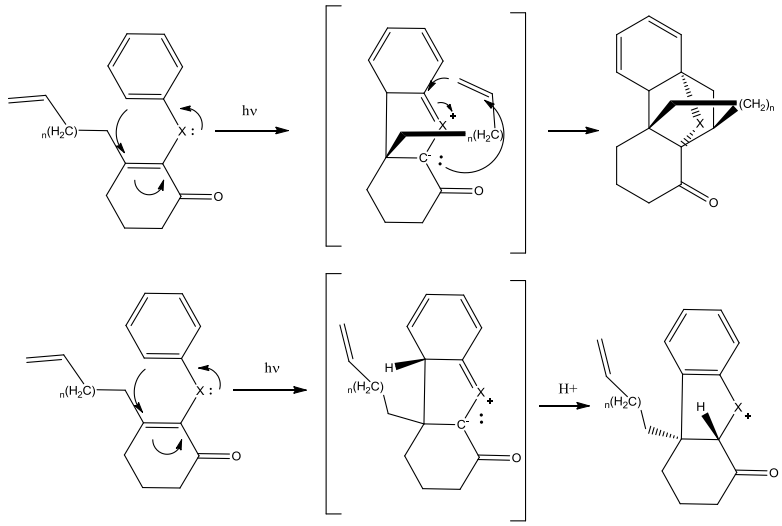


The photoinitiated intramolecular ylide-alkene cycloaddition has been used to generate multicyclic heterocycles with up to 6 chiral centers by exploiting the use of an intramolecular [3+2] cycloaddition to the intermediate ylide. This reaction, shown in Scheme 1, has been demonstrated by many researchers, and much is known about its mechanism and use[10,11,12,13,14].

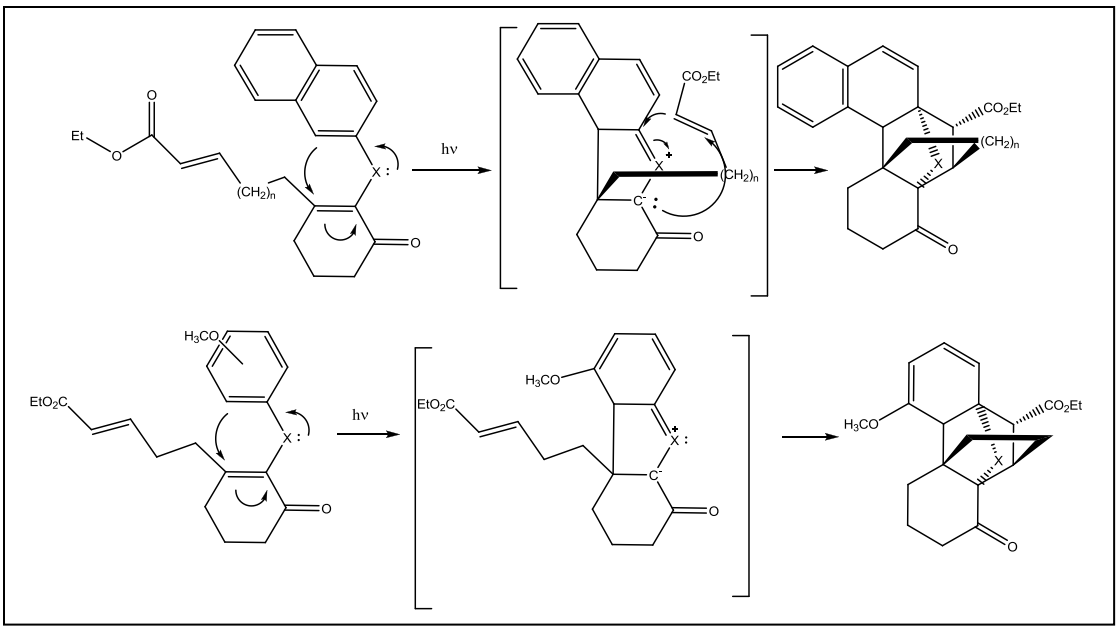
This reaction has been explored as a means to generate complex heterocycles from simple starting materials. This reaction is reported to proceed through a trans-fused ylide intermediate, shown in Scheme 1. This reaction can be directed based on reaction conditions to undergo a hydrogen shift rearrangement or to undergo an intramolecular addition reaction to give multicyclic addition products[14], as shown in Scheme 2.

[10] Dittami, J.P., Ramanathan, H., et al.; *Tetrahedron Lett.*, **30** 795 (1989)
[11] Dittami, J.P., Nie X-Y, et al.; *Syn. Commun.*, **20** (4) 541 (1990)
[12] Dittami, J.P., Nie X-Y, et al.; *Tetrahedron Lett.*, 3821 (1990)
[13] Dittami, J.P., Nie X-Y, et al.; *J. Org. Chem.*, **56** 5572 (1991)
[14] Dittami, J.P., Nie X-Y, et al.; *J. Org. Chem.*, **57** 1151 (1992)

Scheme 2 –Reaction Pathways



Several structural features have been shown to facilitate the [3+2] cycloaddition reaction over the hydrogen shift reaction product. Compounds which contain an oxygen for the heteroatom (X=O) as opposed to a sulfur, or an electron donating group in the aromatic ring show a greater tendency to undergo the [3+2] cycloaddition. Use of a naphthalene ring or other multicyclic heterocycle enhances the yield of [3+2] product[13,14]. Additionally, the incorporation of an electron withdrawing group conjugated to the alkene in the side chain helps promote formation of the [3+2] adducts[14].



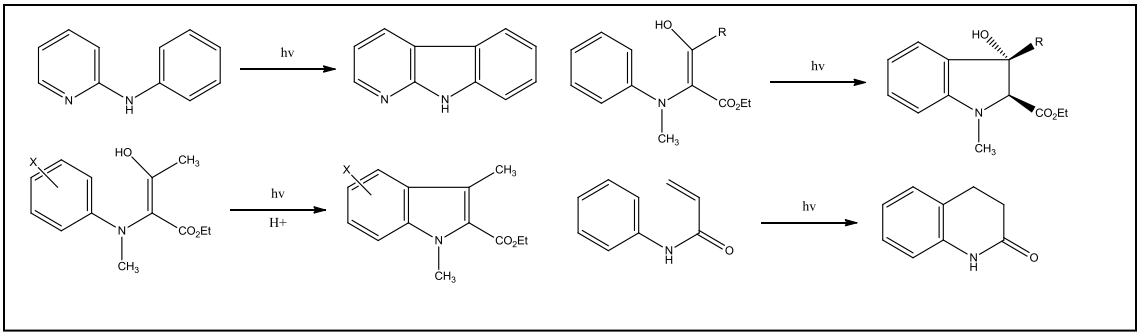
The rationalization behind the relative reactivity between each compound is straightforward, and can be easily expressed in terms of the stability of the ylide intermediate. The use of a naphthalene ring, multicyclic heteroaromatic, or an aromatic ring with an electron donating substituent such as a methoxy group in the starting material play a large role in the formation and stability of the ylide reaction intermediate[15]. The ylide formed in the intermediate still retains a degree of aromatic character if a multicyclic aromatic is used. Compounds which contain an electron donating group in the aromatic ring stabilize the intermediate ylide by donating electron density to the ylide intermediate, stabilizing the ylide and prolonging its lifetime.

Stabilization of the ylide through resonance benefits the reactivity of the intermediate species toward formation of the [3+2] cycloaddition adducts. According to Frontier Molecular Orbital (FMO) Theory, the cycloaddition involves the Highest Occupied Molecular Orbital (HOMO) of the dipole (ylide) which is in an excited state. The Lowest Unoccupied Molecular Orbital (LUMO) of the dipolarophile (alkene in the

[15] Schultz, A.G. *Acc. Chem. Res.* **16** 210 (1983)

side chain) is at a higher energy than the dipole HOMO, and this energy difference is required to undergo the [3+2] cycloaddition. The incorporation of an electron withdrawing group, such as the ethyl ester group, in conjugation to the alkene lowers the energy of the LUMO of the side chain, and thus lowers the energy required for the reaction between the ylide and the alkene. Lowering this energy increases the rate of reaction and this is evidenced by the reaction yields[16,17,18].

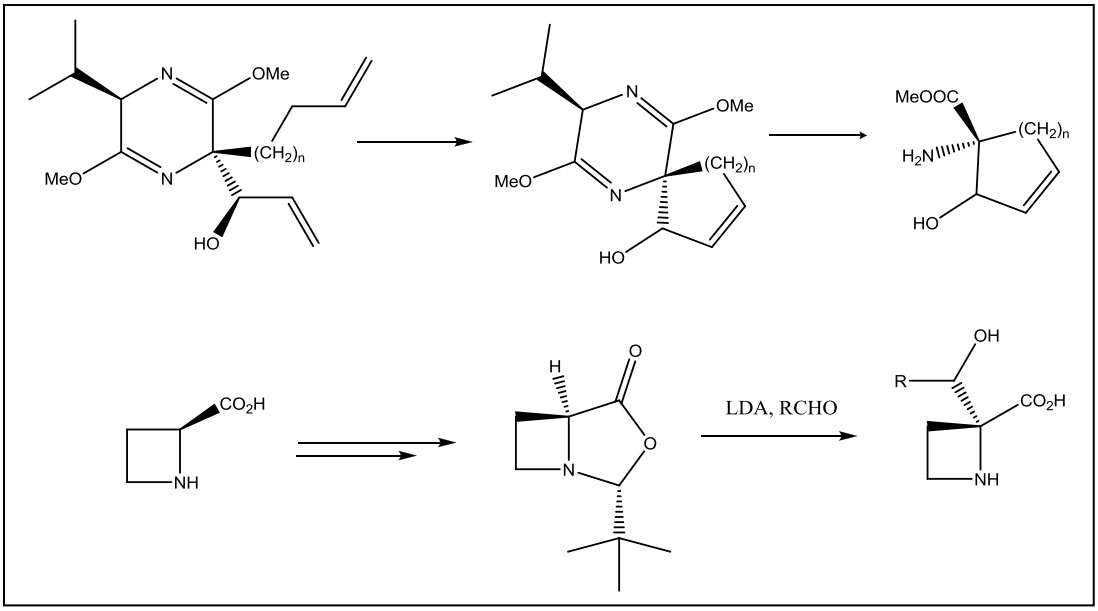
There are many examples in the research to support the use of a nitrogen atom to be incorporated in the reaction, which is a requirement for the development of the intramolecular [3+2] ylide-alkene cycloaddition toward the synthesis of amino acid derivatives[19],[20],[21].



A problem with the synthetic utility of the [3+2] ylide-alkene cycloaddition reaction has to do with its potential to create enantiomers. In developing the utility of the concerted reaction for the synthesis of amino acid derivatives, efforts toward an asymmetric variant of the reaction were taken. In order to impose asymmetry on the reaction sequence, there needs to be some method to restrict or lock the conformation in

[16] Houk, K. N., Sims, J., et al.; *J. Am. Chem. Soc.* **95** 7287 (1973)
[17] Curran, D.P., Ed., *Advances in Cycloaddition* I (1988)
[18] Huisgen, R. Fulka, C. et al.; *Bull. Soc. Chim. Beig.* **93** (1984)
[19] Schultz, A.G. Hagmann, W.K.; *Chem. Commun.* 726 (1976)
[20] Hagmann, W.K.; Ph.D. Thesis, Cornell University
[21] Schultz, A.G. Hagmann, W.K.; *J. Org. Chem.* **43** 4231 (1978)

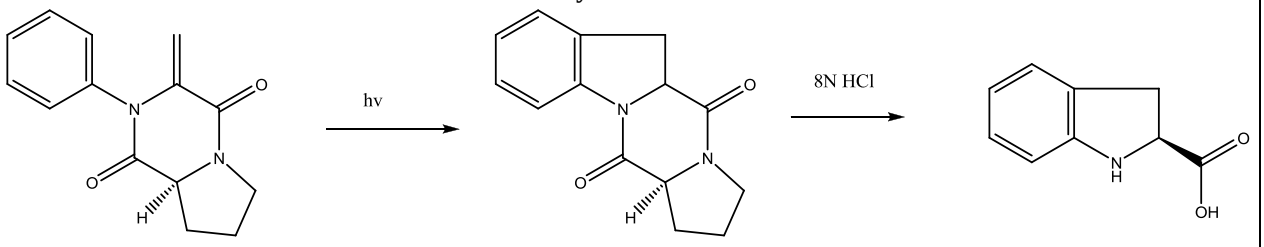
an asymmetric way. A common method to control the stereochemical configuration of a compound during synthesis is to impose a bulky chiral auxiliary group to achieve stereospecificity through steric interactions. In many cases, a ring is used to constrain the conformation. For example, in the synthesis of unnatural AAs shown below, a chiral ring component was used to maintain asymmetry[22,23,24].



There is precedent to achieve the six electron heterocyclization using S-proline to retain chiral configuration during the cyclization reaction. The S-proline can be removed after the cyclization to yield adducts of 98% optical purity[25].

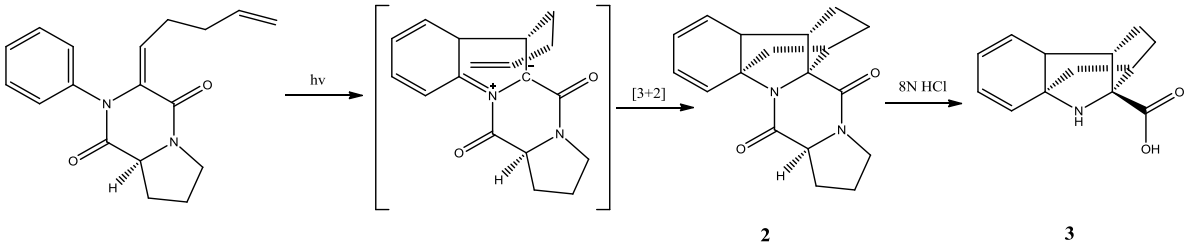
[22] Hammer, K., Undheim, K. *Tetrahedron* **53** 16 5925-5936 (1997)
[23] Park, K. Kurth, M. *Tetrahedron* **58** 8629–8659 (2002)
[24] Xiao, D. et al.; *Tetrahedron: Asymmetry*, **8** 18 3043-3046 (1997)
[25] Castle, R.; *Lectures in Heterocyclic Chemistry* **9** (1987)

Scheme 3 – Conformational Restriction of Photoarylation Reaction[25]



This reaction is reported to proceed via a ylide intermediate, in the same fashion as the photoinitiated six electron cyclization. Approaching the idea of asymmetry in terms of the concerted cyclization and intramolecular [3+2] addition outlined above would allow for the production of complex heterocycles of high optical purity. Incorporation of a pendant alkene side chain into the compound above should allow for this reaction to proceed. The model compound to test in the concerted six electron heterocyclization and ylide-alkene cycloaddition is shown in below. This compound can illustrate the potential feasibility of this reaction to achieve high chiral purity from simple starting materials.

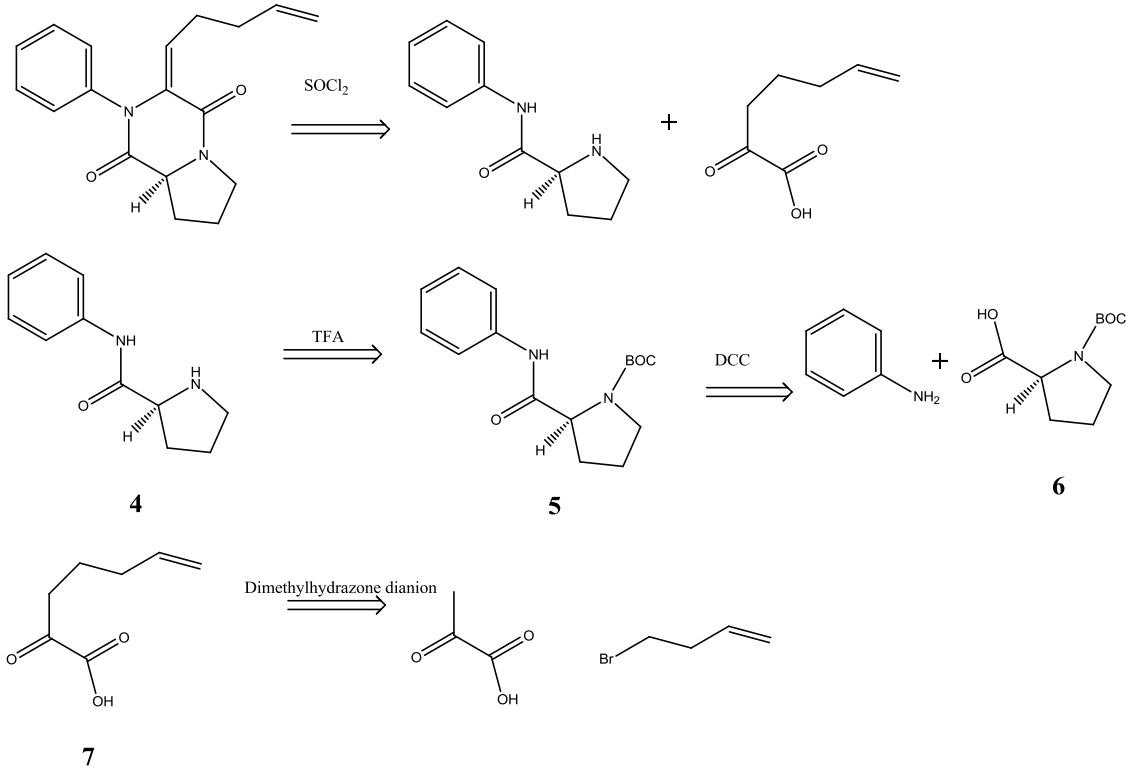
Scheme 4 – Asymmetric Variant of the Photoinitiated [3+2] ylide-alkene cycloaddition



Since compound **1** is not commercially available, a synthesis was devised. Retrosynthetic analysis shows the compound could be synthesized from a coupling of a pyruvic acid derivative with proline-aniline amide. The pyruvic acid butane derivative could be made from the pyruvic acid and 4-bromo-butene, by using the dianion of the

dimethyl hydrazone of pyruvic acid[26]. Pyruvic acid and 4-bromobutene are commercially available. The proline-aniline amide product could be made through an amide condensation of aniline and s-proline, both commercially available.

Scheme 5 - Retrosynthetic Analysis



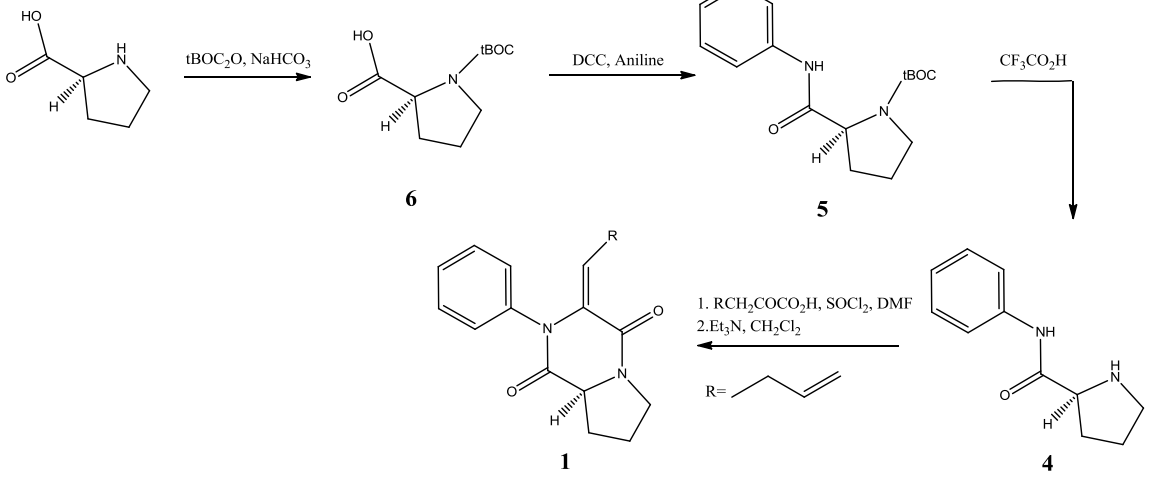
4 **5** **6** **7**

There is ample precedent for these conversions in literature. The coupling of the proline-aniline amide **4** with pyruvic acid has been shown[27], requiring treatment with thionyl chloride followed by treatment with triethylamine. The reaction yields the α,β-unsaturated carbonyl compound. The proline-aniline amide **4** can be obtained by the dicyclohexylcarbodiimide coupling of t-BOC protected proline **6** with aniline[28]. The t-

[26] Tapia, I. Alcazar, V. *The Chemical Society of Japan*, 697-700 (1990)
[27] Roloff, A. et al.; *Pure Appl. Chem.*, **58.** 1267 (1986)
[28] Han, S-Y.; Kim, Y-A.; *Tetrahedron*, **60**,11, 2447-2467 (2004)

BOC protected proline group can be synthesized by the reaction of tBOC anhydride with s-proline[29].

Scheme 6 - Synthesis Plan for Compound 1



In summary, there is ample precedent to support the synthesis plan of **1**. Compound **1** should be easily obtained from s-proline, aniline, and the pyruvic acid derivative, which are all structurally simple compounds. Compound **1** is expected to yield **2** upon irradiation with ultraviolet light in a high degree of enantiomeric purity. Compound **2** should then yield the synthetic amino acid **3** in an enantiomerically pure form.

[29] Gleason, J.L, Boeckman, R.K., *Org. Syn. Coll. Vol.* **10,** 12 (2004)

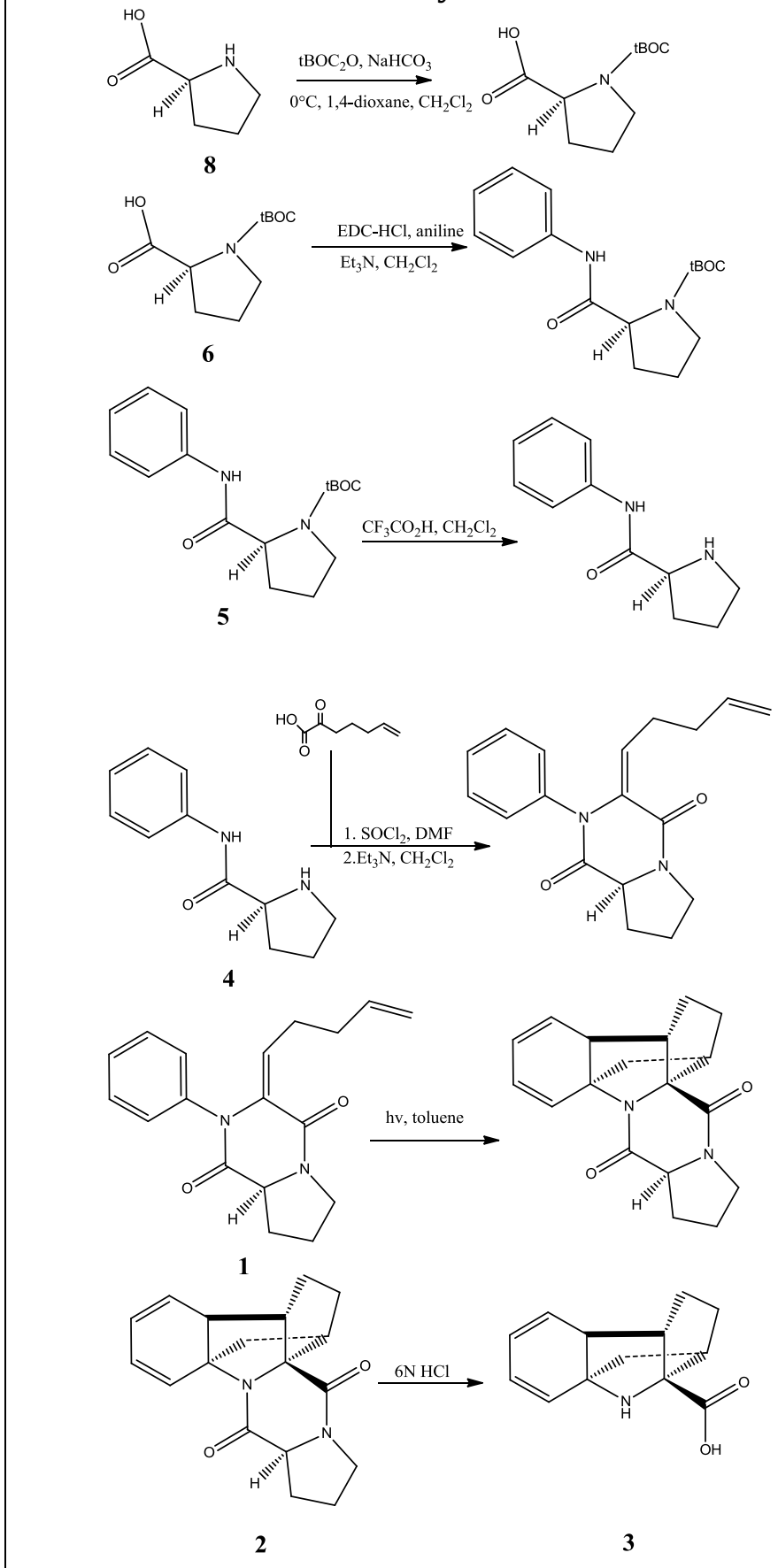
Results and Discussion

The overall goal of this project is to develop the methodology of the photoinitiated ylide-alkene [3+2] cycloaddition toward an asymmetric variant of the reaction. The synthesis plan, outlined in Scheme 7, should show the efficacy of this method to synthesize complex heterocycles of high optical purity. The use of chiral ring auxiliaries to control the stereochemistry of substrates is a commonly employed method in synthesis. The model compound **1** was selected at the outset of the project which would contain an S-proline ring component in order to direct the stereochemistry of the ylide-alkene cycloaddition.

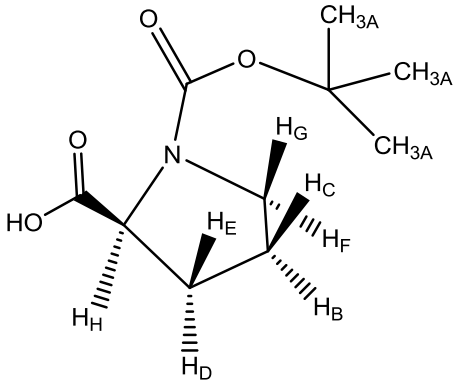
The product of the reaction sequence is a synthetic amino acid derivative of proline, which may exhibit many uses either as a potentially biologically active compound or as an artificial amino acid for use in artificial protein synthesis.
The first goal of the project was to synthesize **1**. This compound incorporates a phenyl vinyl amine as well as an alkene side chain, and contains a conformational restriction imposed by the chirality of the ring system. Upon irradiation with ultraviolet light, **1** is expected to yield **2** which, upon treatment with acid, should yield the amino acid derivative **3**[30]. The first step was to synthesize protected S-proline 6 so that it could be coupled to aniline with DCC. The choice of the t-butoxycarbonyl (tBOC) group was made because of its stability to basic conditions, and its facile cleavage in trifluoroacetic acid (TFA)[31]. tBOC anhydride ($tBOC_2O$) is a reagent commonly employed to introduce the tBOC group to primary as well as secondary amines.

[30] Castle, R.; *Lectures in Heterocyclic Chemistry* **9** (1987)
[31] Gleason, J.L, Boeckman, R.K., *Org. Syn. Coll. Vol.* **10,** 12 (2004)

Scheme 7 – Synthesis Plan



Treatment of S-proline with aqueous bicarbonate and tBOC anhydride in 1,4-dioxane yielded tBOC-S-proline (**6)** in ~60% isolated yield. ^{1}HNMR analysis in $CDCl_3$ showed what appeared to be poor resolution, with broadened and indistinct multiplets. To improve resolution, several recrystallizations from 1:3 EtOAc-Hexane were done. The spectra did not seem to show any improvement. There was a doublet observed at δ=1.45-1.51ppm, J=30.25Hz which integrated to 9H corresponding to the t-butyl protons on the tBOC protecting group. This was strange, as a singlet was expected for this signal.



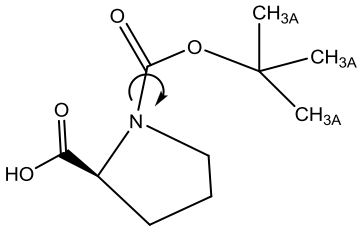
Due to the highly asymmetric nature of the molecule, it was hypothesized that virtual coupling was the cause of the poor resolution. Virtual coupling is known to occur in chiral ring structures, leading to solvent-dependent coupling interactions that can make ^{1}HNMR spectra difficult to interpret[32]. These problems often occur with chiral ring systems such as glycosides, where prochiral hydrogens appear as broadened signals due to virtual coupling interactions[33]. It was hypothesized that protons B-G, as shown in the figure, interact through virtual coupling and thus cause complicated multiplicities, causing overlap of many of the signals and the apparently low resolution of the spectra. It

[32] Robins, M. MacCoss, M.; *J. Am. Chem. Soc.*, **99**, 14, 4660–4666 (1977)
[33] Pauli, G.; *J. Nat. Prod.* **63**, 834-838 (2000)

was thought that protons B, C, and D overlap in the region of δ=1.8-2.2ppm, proton E in the region δ=2.2-2.4, proton F and G at δ=3.3-3.6ppm, and proton H at δ= 4.2-4.4ppm. These observations agree with literature results[34].

Amide bond rotation has been known to cause splitting of signals[35]. Since amide bonds rotate freely, and when the amide adopts a planar conformation and allows for resonance interaction between the lone pair of electrons on the amide nitrogen and the carbonyl group, there is the possibility for signals to appear as distinct in spectra. If the resonance stabilization can slow the rotation of the amide so it is slower than an NMR transition, splitting will occur in the protons affected by the rotation. In tBOC-S-proline, the 9 t-butyl hydrogens appear as a doublet at δ=1.455ppm and 1.519ppm, with J=30.25Hz, a large coupling constant consistent with amide bond rotation interactions[36].

Amide rotation



Since the virtual coupling effects and the amide rotation effects are solvent dependent[37,38], deuterated methanol was employed to test for these effects. When the compound was analyzed in d-methanol, a sharpening of the multiplets and a change in the coupling constants for the t-butyl protons was observed. As shown in Figure 2, the signals observed in the spectrum taken with d-methanol as the solvent were more crisp and distinct, evidence of complicated virtual coupling interactions as hypothesized. The

[34] Fu, Y-Q., Li. Z-C.; *Tetrahedron: Asymmetry*, **17**, 3351-3357 (2006)
[35] Pavia, D. L. (2009). *Introduction to spectroscopy*. Belmont, CA: Brooks/Cole, Cengage Learning.
[36] Quintanilla-Licea**,** R.; *Molecules*, **7**, 662-673 *(*2002)
[37] Pauli, G.; *J. Nat. Prod.* **63**, 834-838 (2000)
[38] Musher, J.I., Corey, E.J., *Tetrahedron*, **18,** 6, 791 (1962)

coupling constant for the doublet observed for the t-butyl protons was 18.85Hz, as opposed to 30.25Hz in $CDCl_3$. This is evidence of the amide rotation effect.

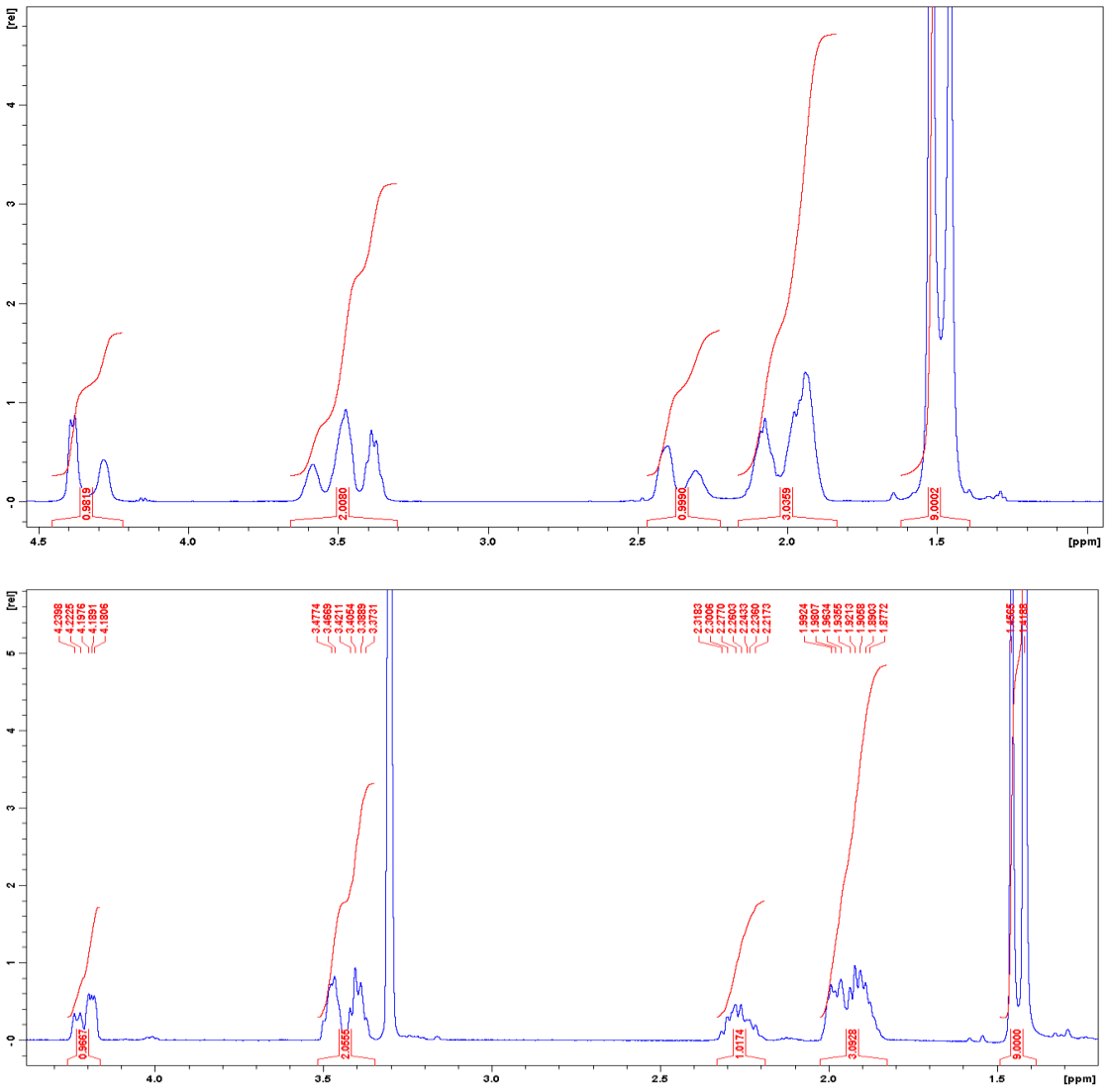
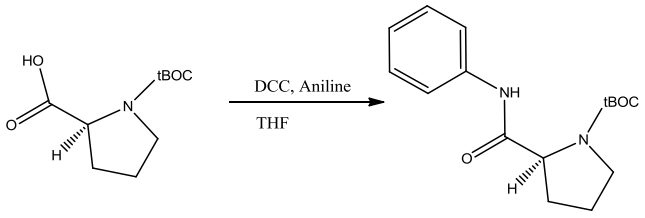


Figure 2 - HNMR spectra of nBOC-proline in CDCl3 (top) and d-methanol (bottom)

Once the concerns with the NMR spectra were clarified, the next step in the synthesis was undertaken. This reaction, the coupling of tBOC-S-proline with aniline to form compound **5**, was attempted using dicyclohexylcarbodiimide (DCC) in THF.



6 **5**

This reaction gave a considerably low yield (~16%) and it was hypothesized that the age of the DCC reagent combined with the wetness of the THF contributed to the loss of product. In order to compensate for the possible wetness of solvents, a more stable reagent was chosen. 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC-HCl) was available on-hand, and is known to be stable in an aqueous environment and used in the coupling of amines to carboxylic acids, and was selected for use in the synthesis of **5**[39]. Treatment of aniline with triethylamine in dichloromethane yielded ~80% of compound **5**.

The ^{1}HNMR spectra of compound **5** in $CDCl_3$ contained many aspects which affected the interpretation of the tBOC-S-proline (**6**). For example, the broadening and interference of multiplets was observed for the compound, appearing to be low resolution. It was hypothesized that the signals would show a difference in d-methanol, just like **6**, and indeed, these effects were observed. The spectra show a solvent dependent change in the multiplicity pattern, indicating virtual coupling is present. There is also a singlet observed for the t-butyl protons in the $CDCl_3$ spectrum whereas a doublet of doublets was observed for the d-methanol spectrum. This can be rationalized in terms of the amide bond rotation frequency which is also solvent dependent.

[39] Sathapornvagana, S., Vilaiva, T.; *Tetrahedron*, **63**, 10253-10259 (2007)

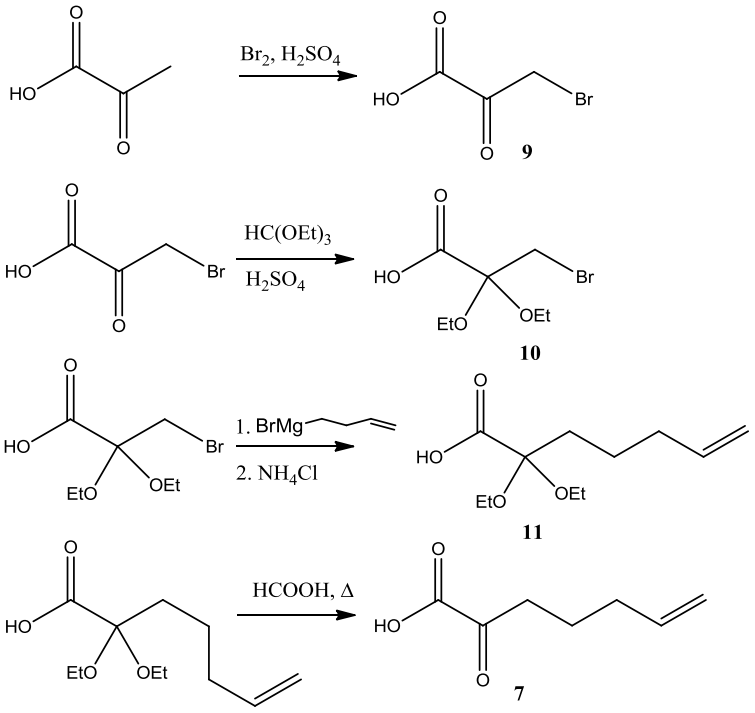
Following the coupling reaction, it was necessary that the tBOC protecting group be removed. The standard procedure for this transformation is treatment with a 1:1 mixture of trifluoroacetic acid and methylene chloride[39]. The product of this reaction, compound **4**, showed a much more defined and crisp NMR spectrum than the tBOC-protected compound.

The last step in synthesizing the target compound **1** was to couple compound **4** with the pyruvic acid derivative **7**. Unfortunately, compound **7** is not readily available, so a synthesis was devised. The most direct route to this compound is by allowing the dimethylhydrazone of pyruvic acid with 4-bromobutene[40]. Unfortunately, dimethylhydrazine was not readily available, and the synthesis of pyruvic acid derivative **7** was not achieved. Upon the synthesis of **7**, the synthesis of **1** should be easily carried out, so an alternate synthesis was devised.

Due to the structure of pyruvic acid, the regiospecific addition of the alkene side chain was a difficulty which warrants concern. Pyruvic acid has two electrophilic carbonyl groups, both susceptible to attack from a nucleophilic reagent. There are also two acidic protons which can lend themselves to interactions with reagents. The synthesis plan must take these problems into consideration. The synthesis plan is outlined below. The synthesis of 3-bromopyruvic acid can be achieved by treatment of pyruvic acid with elemental bromine and H_2SO_4[41]. In order to achieve the selective alkylation at the brominated site of compound **9**, the alpha-ketone can be protected as a diethyl acetal by treatment with triethylorthoformate and H_2SO_4[42]. Acetal **10** should react with the 4-bromobutene Grignard reagent to yield compound **11**, which can be subsequently

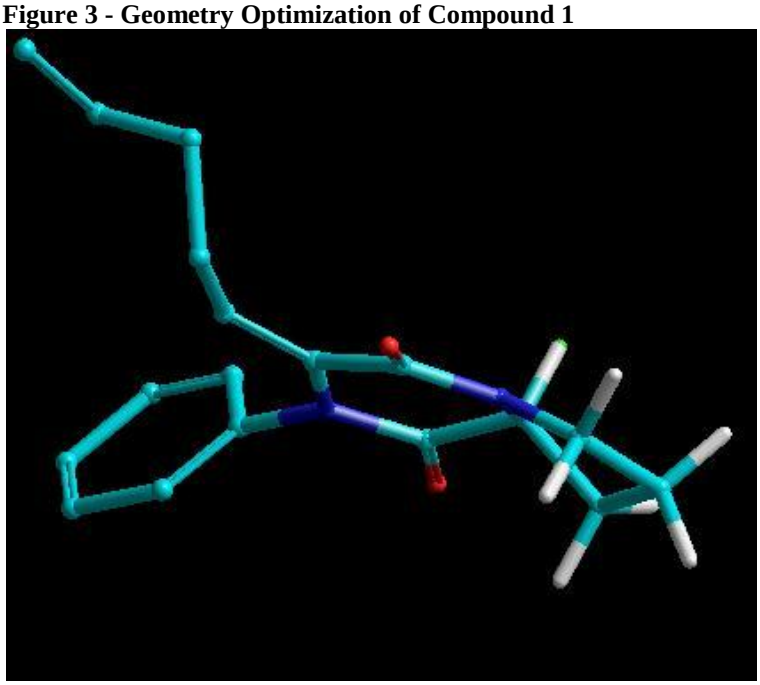
[40] Tapia, I. Alcazar, V. *The Chemical Society of Japan*, 697-700 (1990)
[41] Springson**,** Chargraff. *J. Biol. Chem.*, 164, 424 (1946).
[42] Yan, Y-L., Cohen, S. *Org. Lett.* 9, 13, 2517 (2007)

deprotected by treatment in hot formic acid to yield the required pyruvic acid alkene **7**[42]. Upon the completion of this leg of the convergent synthesis, the target compound **1** should be ready at hand.



The photoreaction of **1** is expected to yield **2** in a high degree of enantiomeric purity. This stereospecificity can be rationalized in terms of the conformational restriction imposed by the proline ring system, which due to its chiral nature, favors a specific stereoisomer depending on the chirality of the proline ring. The ring imposes a U shape into the overall molecule, where the alkene side chain can more easily access the outer edge of the U than the inner, thus resulting in the predicted specificity. The lowest energy conformation was predicted using Hyperchem software, utilizing the Polak-Ribiere (conjugate gradient) geometry optimization (ab initio, 6-31G* basis set). The result of this model is shown below.

Figure 3 - Geometry Optimization of Compound 1

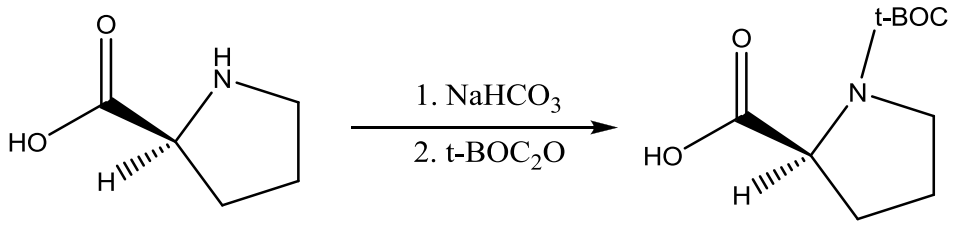


In summary, although the synthesis of **1** was not achieved, it should be readily synthesized from proline-aniline amide **4** and pyruvic acid derivative **7**. Due to the geometric restrictions imposed upon compound **1** by the chiral ring auxiliary, irradiation with ultraviolet light is expected to yield **2** in a high degree of enantiomeric purity. Compound **2** can then be transformed by treatment with acid to amino acid derivative **3**.

Experimental Section

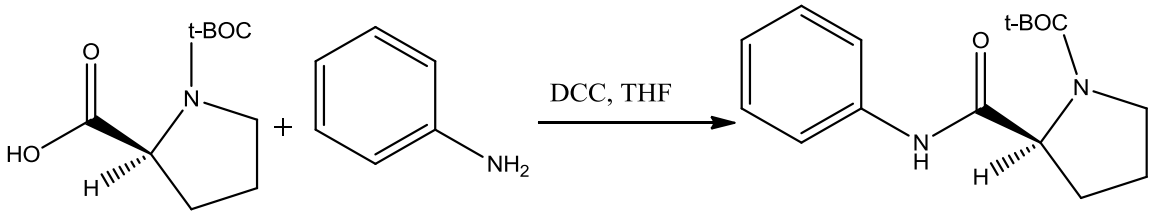
General Methods. Analytical thin-layer chromatography (TLC) was performed on precoated glass-backed silica plates (0.25 mm thickness with a 254 nm fluorescent indicator). Visualization was performed using a UV lamp (254 nm) and by staining with a p-anisaldehyde solution. Infrared spectra (IR) were recorded on a Bruker Vertex 70 Infrared Spectrometer with a 4 cm^{-1} resolution, scanning from 4000 to 650 cm-1 over 16 scans. ^{1}H NMR spectra were recorded on a Bruker Avance III (500 MHz) NMR Spectrometer. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) at 0.00. Solvents and reagents were used as purchased from Sigma-Aldrich or VWR.

(S)-1-(tert-butoxycarbonyl)pyrrolidine-2-carboxylic acid (6) TSV-I-038



A 200mL round bottom flask was charged with 1.1008g solid s-proline and 4.2114g $NaHCO_3$. 50mL water was added and stirred with a magnetic stirbar. The solution was chilled to 0ºC in an ice-bath. A solution of 2.3310g (1.1 equivalents) in 30mL 1,4-dioxane was added by the pipette-full Throughout addition, solution became more and more cloudy. After about 15 minutes, a white precipitate appeared in the mixture. TLC analysis after 100 minutes was taken, the white precipitate was made soluble in MeOH with a few drops of 1M NaOH. Analysis showed consumption of starting materials. The flask was removed from ice and allowed to warm to room temperature. Aqueous solution was extracted twice with 25mL EtOAc. The extract was backextracted twice with 10mL 0.1M NaOH. Aqueous layers recombined. The solution was acidified to pH 1 with 2M HCl. Extracted aqueous solution with 30mL EtOAc. The organic extracts were combined and washed with 20mL water, which was back extracted with EtOAc. The organic layers were combined and dried over $MgSO_4$. The solvent was stripped via rotovap, yielding 1.5486g of clear oil which crystallized upon standing to clear white, needle shaped crystals (74% yield). Product purified by several recrystallizations from 1:3 EtOAc:Hexane. ^{1}HNMR ($CDCl_3$ 500MHz) δ 1.45-1.55 (d, 9H, J=30.25Hz), 1.91-2.10 (m, 3H), 2.29-2.40 (m, 1H), 3.37-3.58 (m, 2H), 4.28-4.40 (m, 1H), 7.29 (s, 1H exchanged). IR 3070, 3020, 2400, 1750, 1640, 1450, 1200, 900cm^{-1}

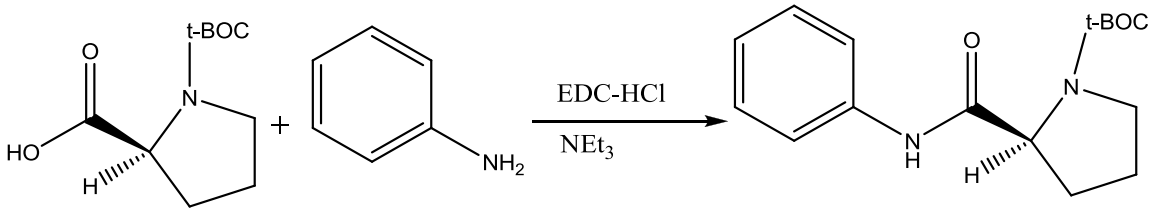
(S)-tert-butyl 2-(phenylcarbamoyl)pyrrolidine-1-carboxylate (5) TSV-I-039a



6 **5**

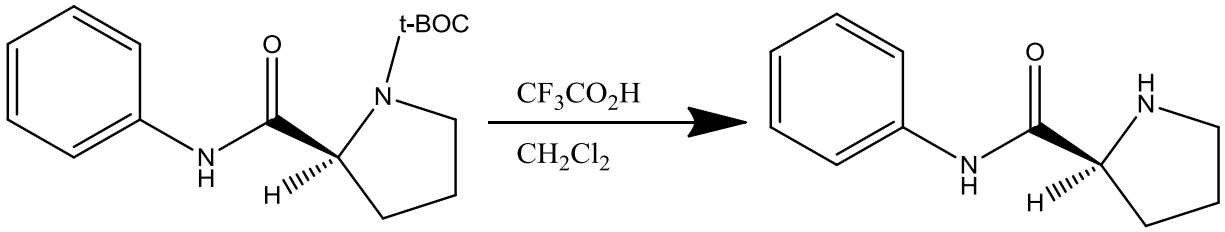
515.8mg Aniline was added to a round bottom flask. A solution of 1.126g dicyclohexylcarbodiimide (DCC) in 30mL THF was added to the flask. 626.2mg t-BOC s-proline was added in small spoonfuls. The color became more yellow, the reaction mixture was heterogeneous. Reaction mixture was left stirring overnight. The mixture had become opaque and milky overnight, TLC (Silica, mobile: 5%MeOH in DCM) showed product spot at Rf=0.7. Solvent was stripped from reaction mixture via rotovap, and the residue was dissolved in dichloromethane (DCM). Added 10mL AcOH to neutralize reactants, at which point mixture became clear and transparent instantly. Basified to about pH 10 at which point a white precipitate appeared which stayed with the organic layer. The white urea precipitate was filtered and washed with DCM. DCM solution dried in $MgSO_4$, and then stripped of solvent via rotovap yielding 245.3mg of a yellow solid (24% yield). ^{1}HNMR ($CDCl_3$ 500MHz) δ 1.52 (s, 9H), 1.92-2.20 (m, 3H), 2.31-2.45 (m, 1H), 3.37-3.65 (m, 2H), 4.22-4.56 (m, 1H), 7.05-7.65 (m, 5H)

(S)-tert-butyl 2-(phenylcarbamoyl)pyrrolidine-1-carboxylate (5) TSV-I-039b-d



150mg aniline was combined with 10mL DCM and 150mg triethylamine. 313mg tBoc-S-Proline was added after several minutes with magnetic stirring. 307mg 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC-HCl) was added. The reaction mixture was left stirring overnight. TLC (silica, mobile: 1:! Hexane:EtOAc) showed a spot overlapping aniline spot at Rf=0.45. Reaction mixture was washed with water and then dried with $MgSO_4$. The solvent was stripped, yielding 350mg of yellow oil which crystallized upon standing (80% yield). The solid was purified by recrystallization from 2% v/v EtOH in EtOAc. ^{1}HNMR ($CDCl_3$ 500MHz) δ 1.52 (s, 9H), 1.92-2.20 (m, 3H), 2.31-2.45 (m, 1H), 3.37-3.65 (m, 2H), 4.22-4.56 (m, 1H), 7.05-7.65 (m, 5H). IR 3350, 2990, 2420, 1640, 1600, 1580, 1560, 1430, 1210, 1090, 900cm^{-1}

(S)-N-phenylpyrrolidine-2-carboxamide (4) TSV-I-040

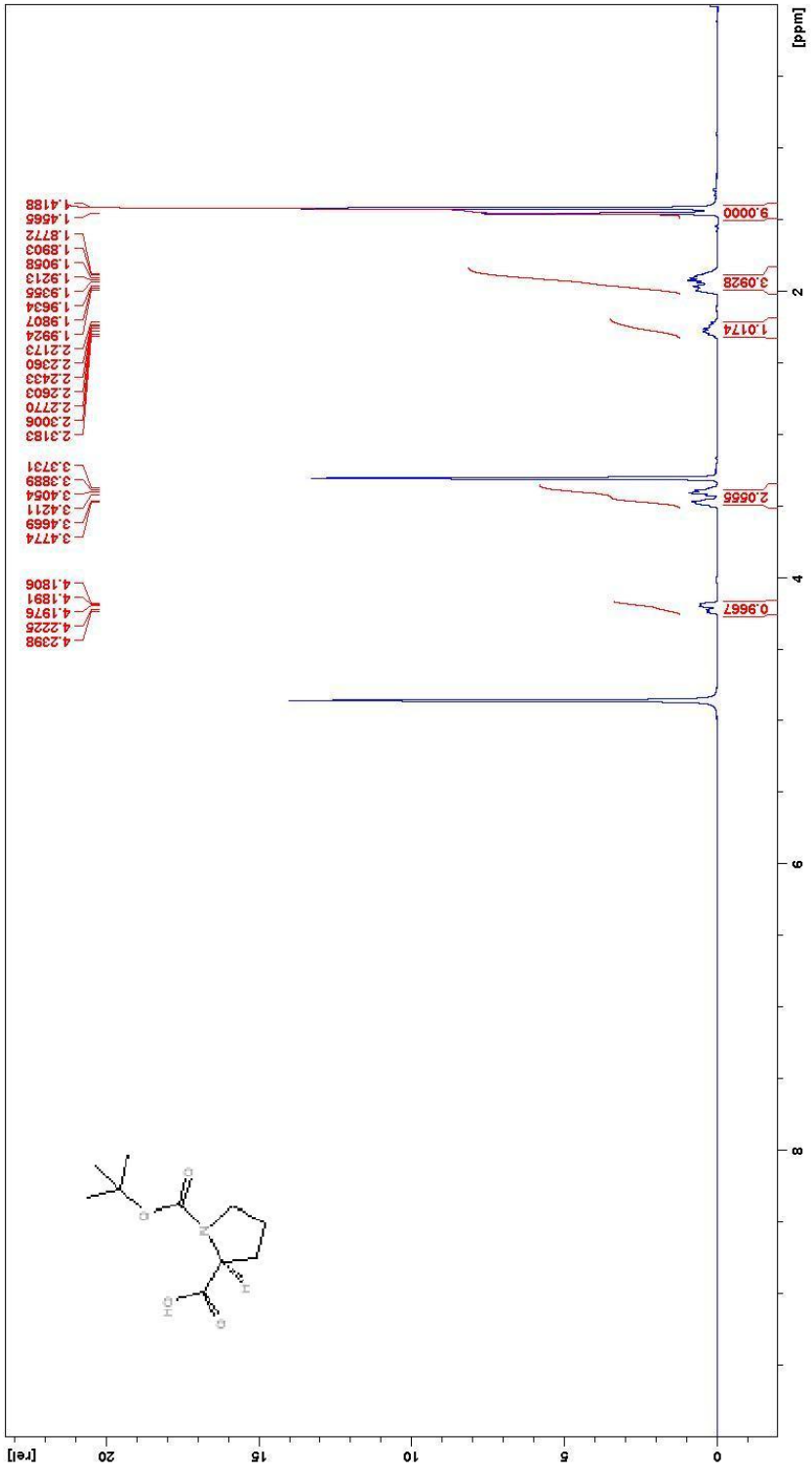


5 **4**

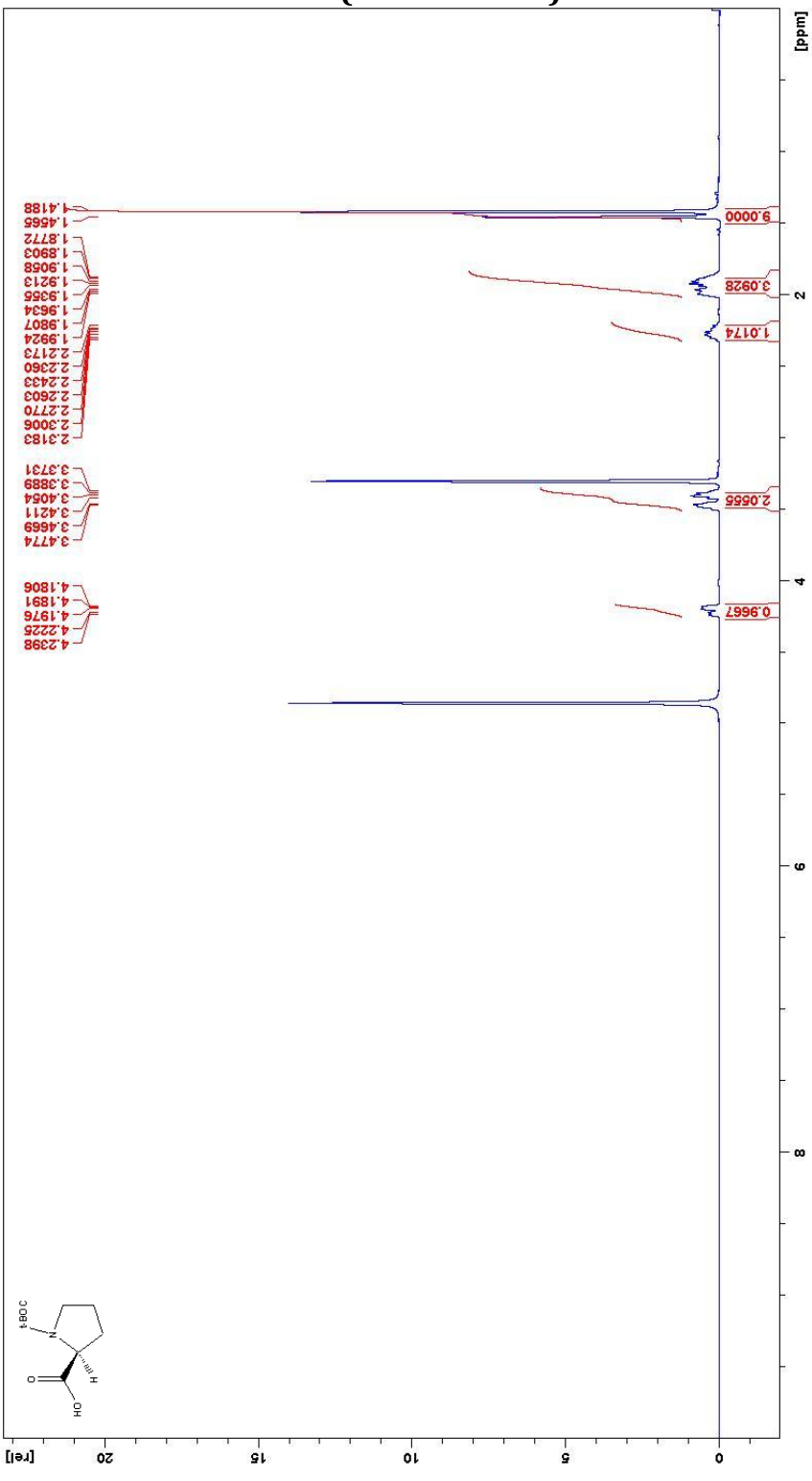
To a solution of 100mg proline-aniline amide (**5**) in 10mL dichloromethane was added 10mL of a 1:1 solution of trifluoroacetic acid in dichloromethane. The mixture was stirred in an ice-bath for 2 hours. The product was washed with 10% NaOH solution and stripped of solvent yielding **4** as the trifluoroacetic acid salt in nearly quantitative yield. ^{1}HNMR (CD_3OD, 500MHz) δ 1.04 (m, 1H), 1.25 (m, 1H), 1.51 (m, 1H), 1.92-2.10 (m, 3H), 3.44 (m, 1H), 7.05-7.65 (m, 5H)

Appendix: Spectral Data

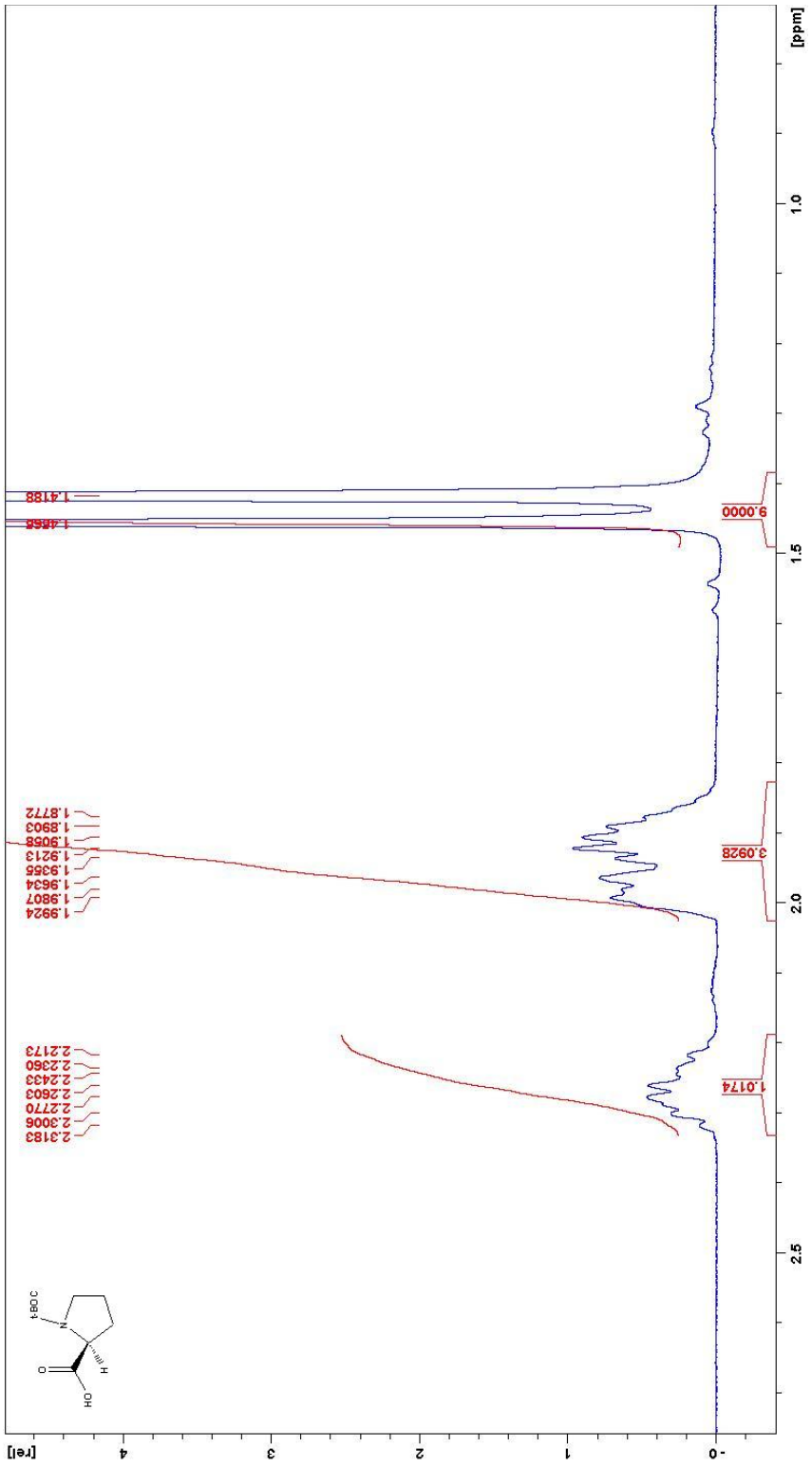
n-tBOC-S-proline HNMR in d-chloroform (TSV-I-038d)



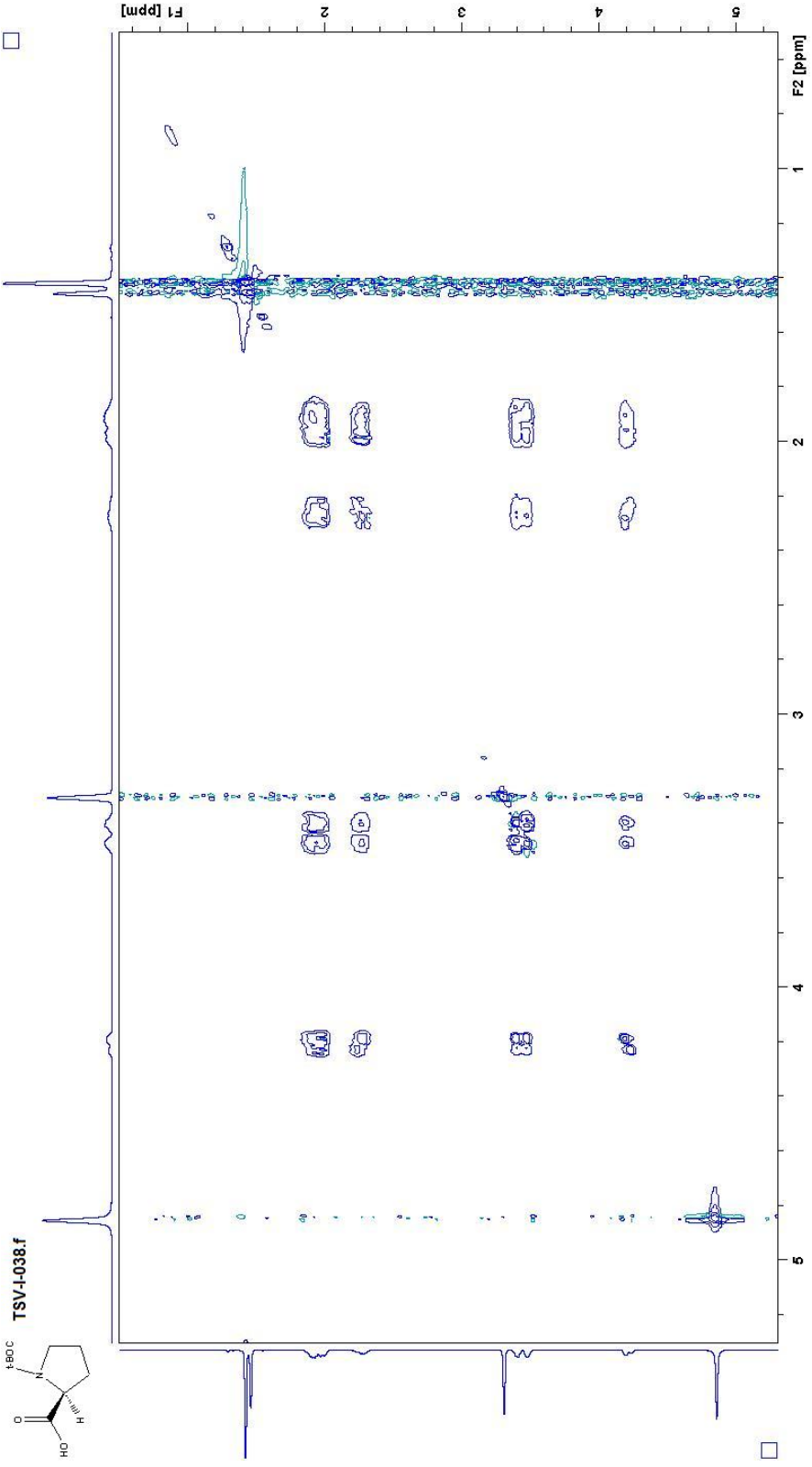
n-tBOC-S-proline HNMR in d-methanol (TSV-I-038e.a)



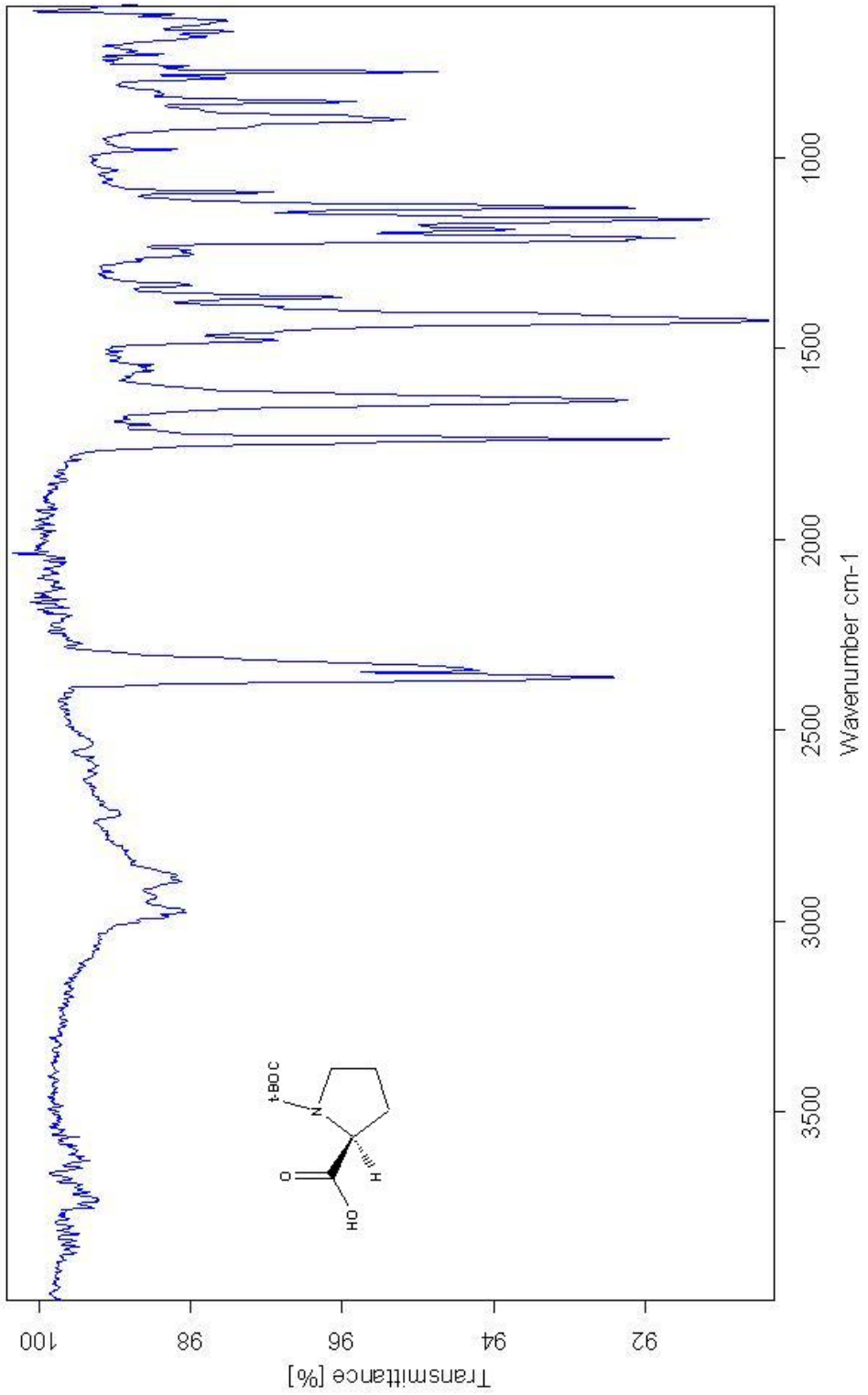
n-tBOC-S-proline HNMR in d-methanol multiplet expansion (TSV-I-038e.a)



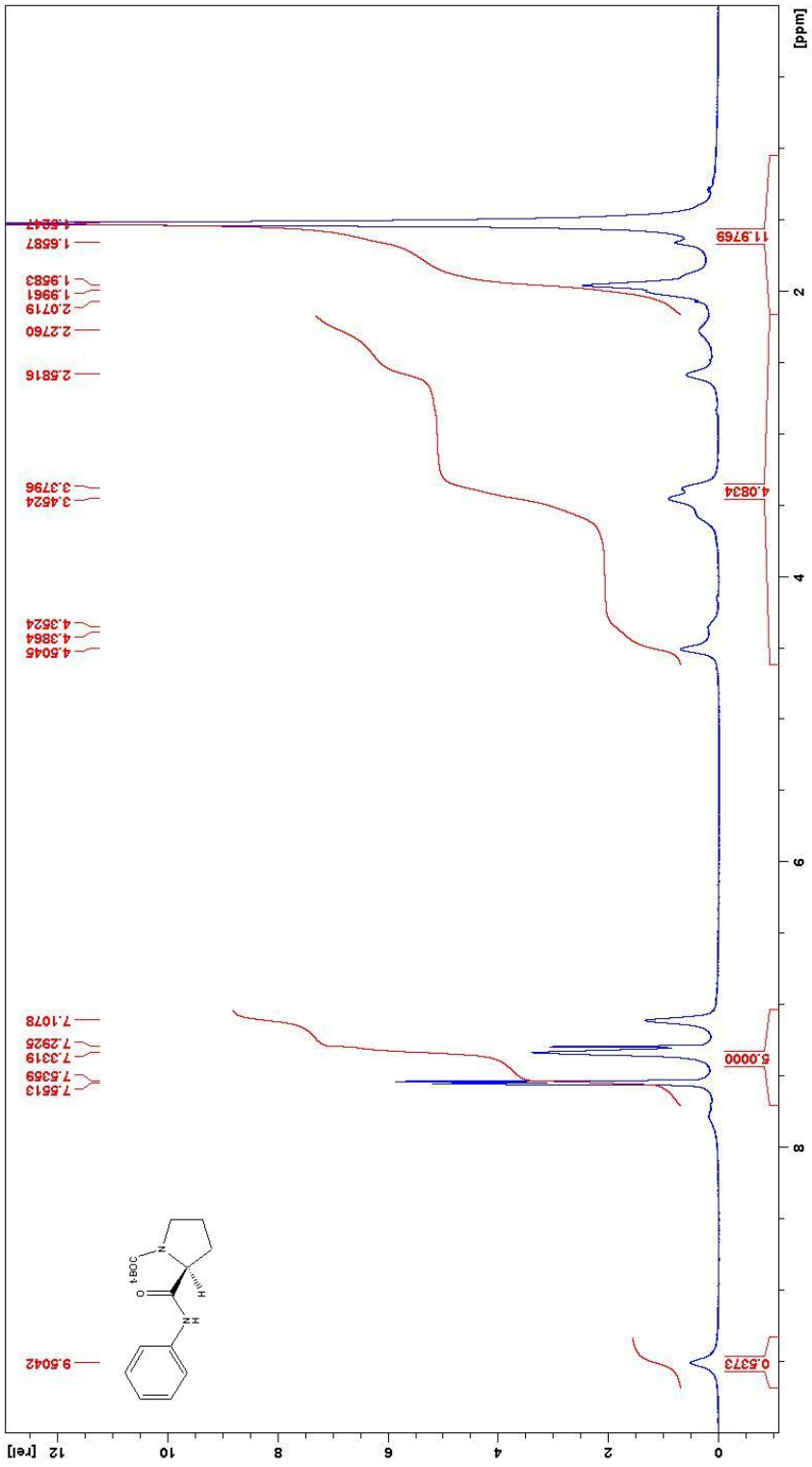
n-tBOC-S-proline, TOCSY in d-methanol (TSV-I-038f)



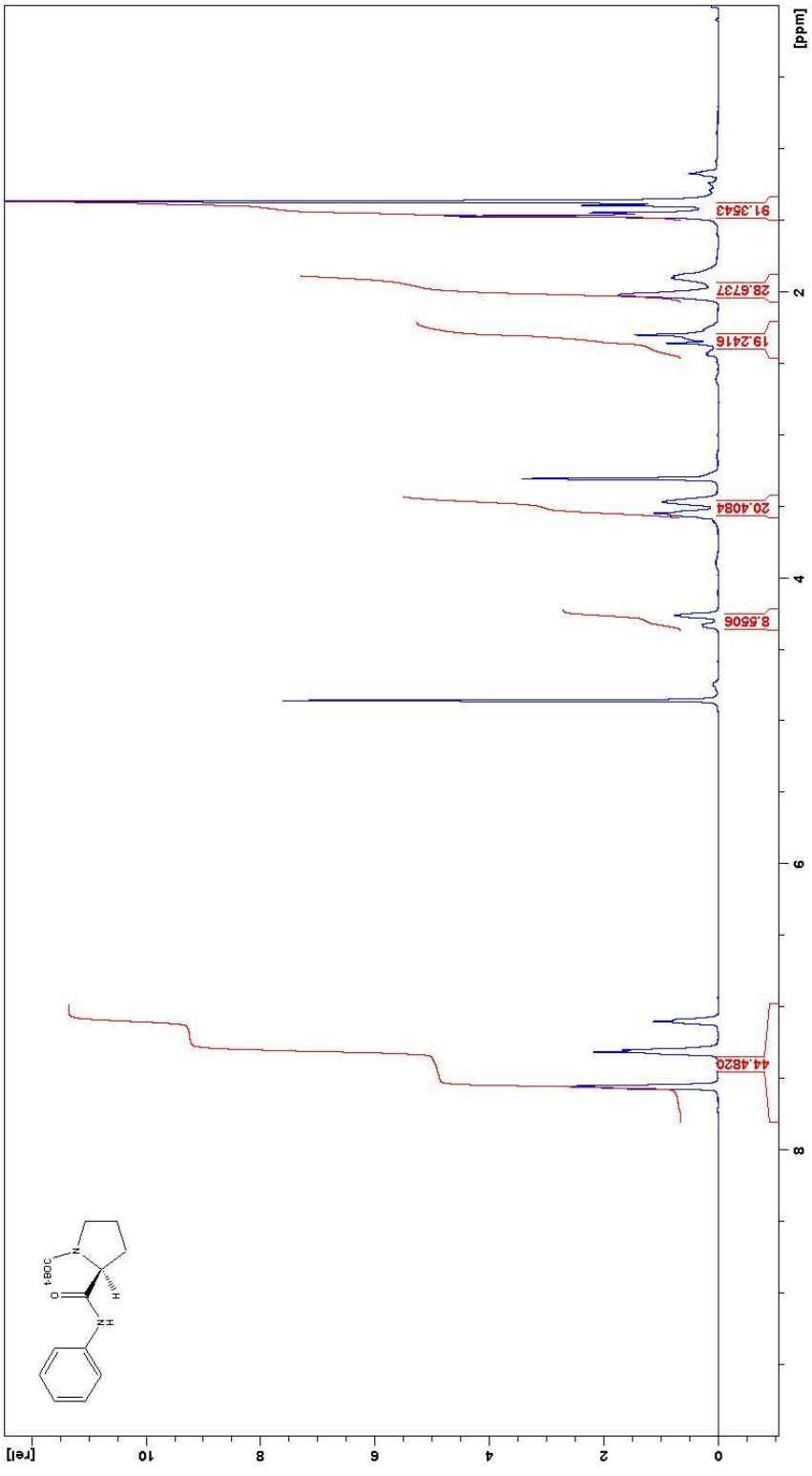
n-tBOC-S-proline, IR spectrum (neat)



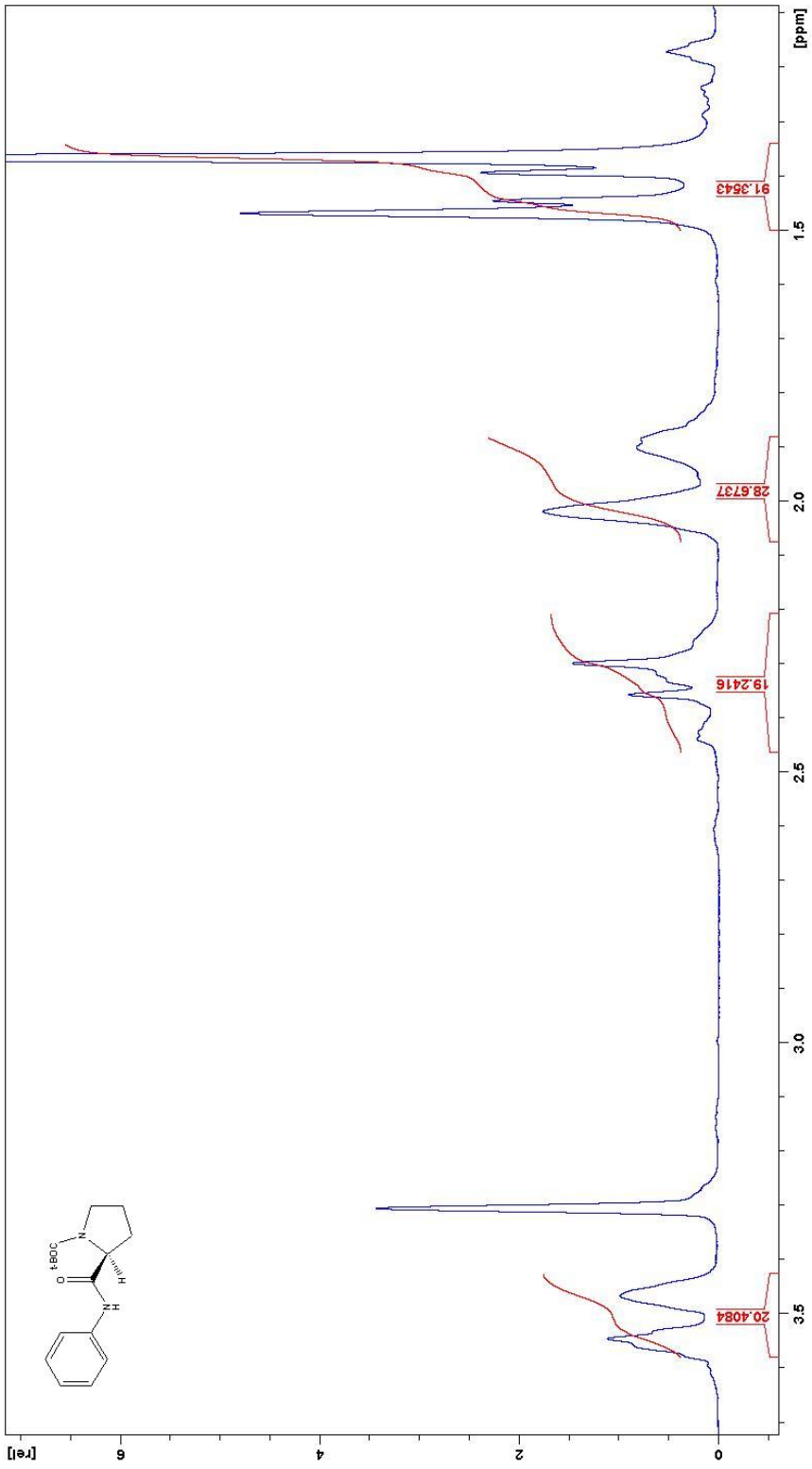
(S)-tert-butyl 2-(phenylcarbamoyl)pyrrolidine-1-carboxylate, HNMR spectrum in d-chloroform (TSV-I-039b)



(S)-tert-butyl 2-(phenylcarbamoyl)pyrrolidine-1-carboxylate, HNMR spectrum in d-methanol (TSV-I-039d)



(S)-tert-butyl 2-(phenylcarbamoyl)pyrrolidine-1-carboxylate, d-methanol expansions (TSV-I-039d)



(S)-tert-butyl 2-(phenylcarbamoyl)pyrrolidine-1-carboxylate, IR (neat)