THE REDUCTIVE AMINATION CHEMISTRY OF AZIRIDINE ALDEHYDES

by

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A thesis submitted in conformity with the requirements

for the degree of Master of Science

Department of Chemistry

University of Toronto

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Abstract: Amino aldehydes without *N*-protecting groups are subject to self-condensation, since the amine is able to react with an aldehyde to form an imine/enamine system. As a result, the aldehyde functional group is prematurely destroyed. In order to make it behave as an aldehyde, protection is unavoidable. Even if the unprotected amine and aldehyde groups can co-exist under special conditions, the aldehyde or amine function is unable to react independently. Our recent interest in amphoteric molecules has led to the discovery of stable compounds that contain unprotected amine and aldehyde functionalities. The so-called kinetic amphoterism has been coined in order to describe the co-existence of a secondary amine and aldehyde in such molecules. Amphoteric aziridine aldehydes exhibit different properties from normal aminoaldehydes and have great potential for the construction of peptidomimetic conjugates.

Acknowledgements

During the past two years, my advisor Prof. Andrei K. Yudin influenced my research greatly. His solid knowledge of chemistry and brilliant ideas has inspired my research. I learned lots of important lab skills from my labmates Mr. Ryan Hili and Dr. Sivaraj Baktharaman. The helpful discussions with them have benefited me a lot. Also, the other group members helped me a lot in the daily work, and Prof. Ronald Kluger spent precious time in reading and correcting the thesis. Thank you.

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Introduction

1.1 Properties of aziridines

The aziridine ring is recognized as a valuable building block for the construction of nitrogen-containing molecules.[1] The synthetic applications of aziridines usually take advantage of their ring-strain (26.8 kcal/mol).[2] The strain energy is caused principally by the deformation of ideal tetrahedral bond angles among the atoms of the ring. The bond angles of aziridines deviate away from the regular tetrahedral angle of 109° in order to fit their 60° triangular structures. X-ray crystallography indicates that the average bond length of C-C bond in aziridines is 1.480 Å and that of C-N bond is 1.472 Å. The value for C-C bond is between the standard value of Csp^2-Csp^2 (1.33 Å) and Csp^3-Csp^3 (1.54 Å), whereas the value for C-N bond is close to the standard value of Csp^3-Nsp^3 (1.469 Å). The release of strain provides the driving force to the ring-opening reactions.[3]

The pK_{aH} values of the aziridine and selected other amine species are shown in Figure 1.1.[4] Ohwada explained this difference by the fact that aziridines have larger pyramidalization angles[5] and, accordingly show smaller local electron-donating abilities at the nitrogen center as compared with piperidines.[6] Therefore, aziridines have weaker

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[1] *Aziridines and epoxides in organic synthesis*; Yudin, A. K., Ed. John Wiley & Sons, 2006; Mitsunobu, O, *Comprehensive organic synthesis*; Trost, B. M., Fleming, I., Winterfeldt, E. Eds. Pergamon: Oxford, 1990; Vol 7, 65-101; Kemp J. E. G. *Comprehensive organic synthesis*; Trost, B. M., Fleming, I., Winterfeldt, E. Eds. Pergamon: Oxford, 1990; Vol 7, 469-512; Tanner, D. *Angew, Chem. Int. Ed.*, **1994**, *33*, 599-619; McCoull, W., Davis, F. A. *Synthesis* **2000**, (10), 1347-1365; Sweeney J. B. *Chem. Sco. Rev.* **2002**, *31*, 247-258; Hu, X. E. *Tetrahedron* 2004, *60*, 2701-2743; Watson I. D. G. Yu, L., Yudin, A. K. *Acc. Chem. Res.* **2006**, *39*, 194-206.

[2] Dudev, T.; Lim, C. *J. Am. Chem. Soc.* **1998,** *120,* 4450-4458.

[3] Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen A. G.; Taylor R. *J. Chem. Soc., Perkin Trans.* 2, **1987**, S1 - S19.

[4] *Organic Chemistry*, Clayden, J.; Geeves, N.; Warren S.; Wothers; P. 1st. Ed, Oxford University Press, Oxford, 2000; 1126.

[5] For pyramidalization angle see: Vanzquez S.; Camps P. *Tetrahedron Lett.* **2005**, *21*, 5147-5208; Haddon R. *Pure&Appl. Chem.* **1999,** *71,* 289-294;

[6] Ohwada T.; Hirao H.; Ogawa A. *J. Org. Chem.* **2004,** *69*, 7486-7494.

basicity and nucleophilicity than common secondary amines.

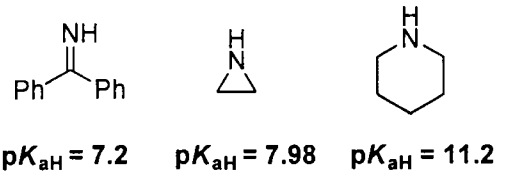

Figure 1.1 pK_{aH} values of selected nitrogen-containing molecules

Due to the ring strain, aziridines also exhibit better stability towards oxidation than secondary amines. The oxidation peak potentials of aziridines and regular secondary amines are shown in Figure 1.2.[7] A significant increase in oxidative stability makes aziridines useful in many synthetic transformations. Regular secondary amines, on the other hand, are vulnerable under certain oxidation conditions. Therefore, protection or deprotection steps are necessary for the N-H moiety during these processes. However, aziridines can be employed in the protection group-free transformations,[8] which increases the synthetic efficiency of these operations.

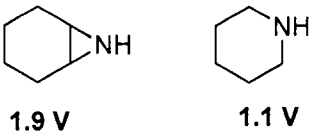

Figure 1.2 Oxidation peak potentials of aziridine and piperidine

1.2 Protease inhibitors

Proteases are enzymes that selectively catalyze hydrolysis of peptide bonds. Due to their control over protein synthesis, turnover, and function, they regulate physiological processes such as digestion, fertilization, growth, differentiation, cell signaling,

[7] Tung Siu, Doctoral Thesis, University of Toronto, Toronto, ON, 2002.
[8] Chen, G.; Sasaki, M.; Li, X.; and Yudin, A. K., *J. Org. Chem.* **2006**, *71*, 6067-6073.

immunological defense, wound healing and apoptosis.[9] Since proteases play an important role in disease propagation, protease inhibitors continue to attract a lot of attention in therapeutic applications.[10] Replacement of a cleavable amide bond within a specific peptide sequence by a suitable mimetic is a general method for the development of such inhibitors.[11] The so-called reduced amide bond isosteres are well-known constituents of peptidomimetics. These molecules contain an aminomethylene functional group in place of the selected amide linkage (Figure 1.3A). This structural fragment is is isosteric with the tetrahedral transition state formed during amide hydrolysis. This replacement enables the inhibitor to bind to the protease target without being cleaved by hydrolysis. It always leads to a binding that is tighter than that of a peptide prototype (Figure 1.3B).[12]

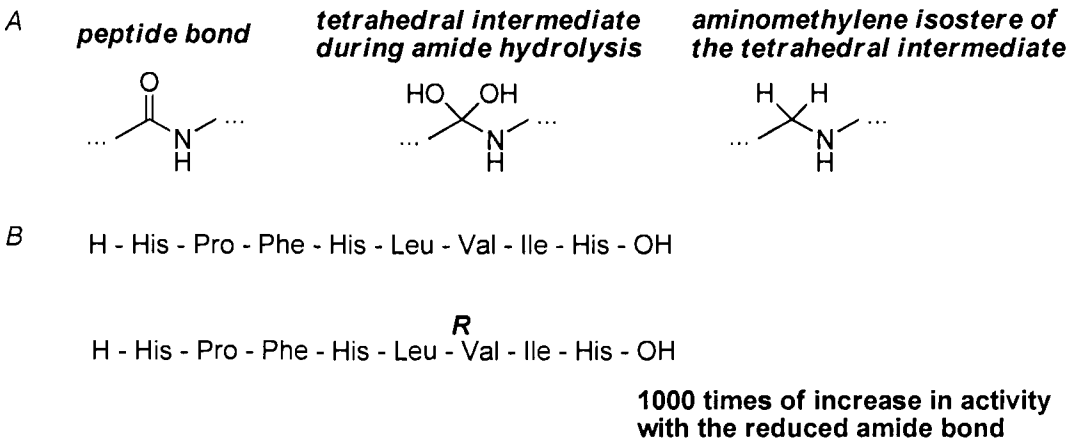

Figure 1.3 Amide bond isosteres and selected protease inhibitors that contain them

The main-chain aliphatic amine functionality, a common structure feature of the aforementioned peptidomimetic protease inhibitors, is protonated at physiological pH. The resulting charge distribution in the vicinity of the amide bond isostere deviates from what would be expected of a natural substrate that has a peptide bond at this position.

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[9] Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305-367.
[10] Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
[11] Giannis, A.; Kolter, T. *Angew. Chem. Int.* **1993**, *32*, 1244-67.
[12] Szelke, M.; Leckie, B.; Hallett, A.; Jones, D. M.; Sueiras, J.; Atrash, B.; Lever, A. F. *Nature* **1982**, *299*, 555-562.

Due to this perturbation, many different modes of binding between proteases and their inhibitors have been observed. The crystallographically characterized structures exhibit hydrogen bonds between the amino and/or hydroxyl groups of the inhibitors and the amino acid residues at the protease active site.[13] The diversity of recognition mechanisms suggests that optimization of the peptidomimetic inhibitor/protease interactions in the vicinity of the active site is an important component of discovering new therapeutic agents. The quest for potent new protease inhibitors hinges upon availability of sophisticated synthetic tools that allow modulation of specificity, particularly of the so-called S1-P1 and S2-P2 interactions.[14]

The most common synthetic route towards making reduced amide bond isosteres is the reductive amination of suitably protected peptide aldehydes with amino acid derivatives.[15] The nitrogen-protected amino acid is first converted into an ester or a Weinreb amide which is then reduced into the corresponding *N*-protected aminoaldehyde by a hydride transfer reagent such as DIBAL. The α-amino aldehydes are sensitive to epimerization at room temperature.[16] In addition, the corresponding imine/enamine intermediates formed during reductive amination can lead to further epimerization on both sides of the aminomethelene linkage.[17] Epimerization is the main challenge facing this synthetic strategy, although the methodology has been widely employed in many

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[13] Wlodawer, A.; Erickson, J. *Annu. Rev. Biochem.* **1993**, *62*, 543-574.
[14] Evans, M. J.; Cravatt, B. F. *Chem. Rev.* **2006**, *106*, 3279-3301; Fonović, M., Bogyo, M. *Curr. Pharm. Design* **2007**, *13*, 253-285.
[15] Gryko, D.; Chalko, J.; Jurczak, J. *Chirality* **2003**, *15*, 514-552.
[16] Potetinova, J. V.; Milgotina, E. I.; Makarov, V. A.; Voyushina, T. L. *Russ. J. Bioorg. Chem.* **2001**, *27*, 141-167.
[17] Epimerizations on both the amine and the aldehyde sides during peptidomimetic synthesis have been documented: (a) Aurelio, L.; Brownlee Robert, T. C.; Hughes Andrew, B. *Chem. Rev.* **2004**, *104*, 5823-5877; (b) Wasserman, H. H.; Berger, G. D.; Cho, K. R. *Tetrahedron Lett.* **1982**, *23*, 465-468; (c) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441-5452; (d) Giannis, A.; Kolter, T. *Angew. Chem. Int. Ed.* **1993**, *32*, 1244-1256; (e) Ho, P. T.; Chang, D.; Zhong, J. W. X.; Musso, G. F. *Peptide Res.* **1993**, *6*, 10-12.

academic and industrial applications.[18]

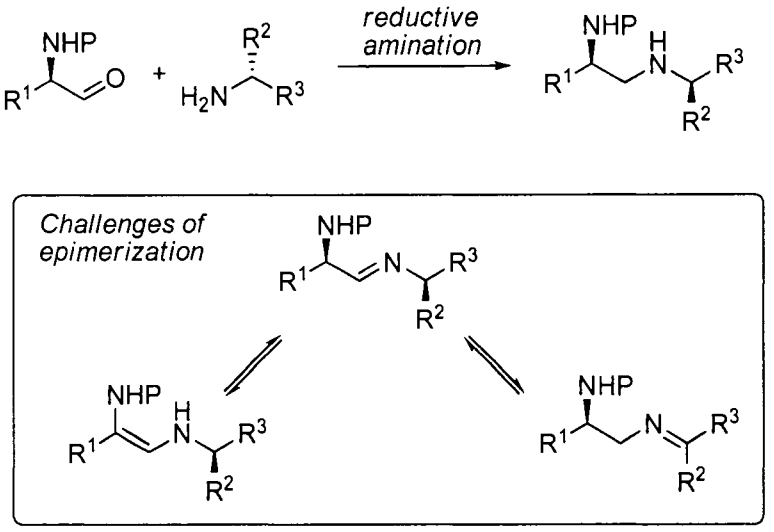

Figure 1.4 The challenges of peptidomimetic construction

Last but not least, reliance on protecting groups at nitrogen in amino aldehydes diminishes synthetic efficiency of these operations. Amino aldehydes without *N*-protecting groups are subject to self-condensation, since the amine is able to react with an aldehyde to form an imine/enamine system (Figure 1.5). As a result, the aldehyde functional group is prematurely destroyed.[19] In order to make it behave as an aldehyde, protection is unavoidable. Even if the unprotected amine and aldehyde groups can co-exist under special conditions,[20] the aldehyde or amine function is unable to react independently.

1.2 Amphoteric molecules

Our recent interest in amphoteric molecules has led to the discovery of stable compounds that contain unprotected amine and aldehyde functionalities. The so-called

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[18] Baxter, E. W.; Reitz, A. B. *Organic Reactions* **2002**, *59*, 1-714.
[19] Rappoport, Z., Ed. *The Chemistry of Enamines*; John Wiley & Sons: New York, 1994; Fischer, E. *Ber.* **1908**, *41*, 956-962; Fischer, E. *Ber.* **1908**, *41*, 1019-1025.
[20] Myers, A. G.; Kung, D. W.; Zhong, B. *J. Am. Chem. Soc.* **2000**, *122*, 3236-3242; Ooi, T.; Saito, A.; Maruoka, J. *J. Am. Chem. Soc.* **2003**, *125*, 3220-3227.

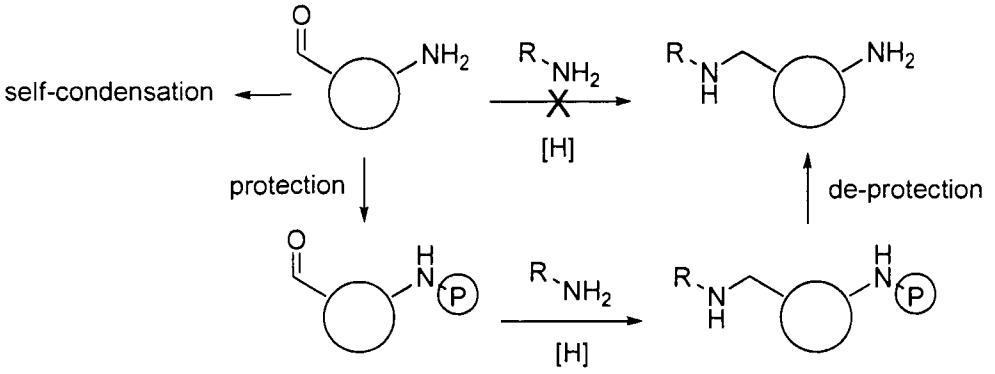


Figure 1.5 Protection groups are necessary for regular amino aldehydes

kinetic amphoterism has been coined in order to describe the co-existence of a secondary amine and aldehyde in such molecules.[21] Due to the aziridine ring strain, the energetically uphill aziridinium ion formation is unlikely to happen, thus ruling out self-condensation (Figure 1.6 A). This orthogonal relationship between aldehyde and aziridine gave us a chance to explore the existence of unprotected amino aldehydes (Figure 1.6 B). This molecule was found to behave as an aldehyde. The amino functional group did not interfere with other amines. Therefore, it is possible for this amino aldehyde to react with another amines without protection of its amino group. If the amine group is part of a

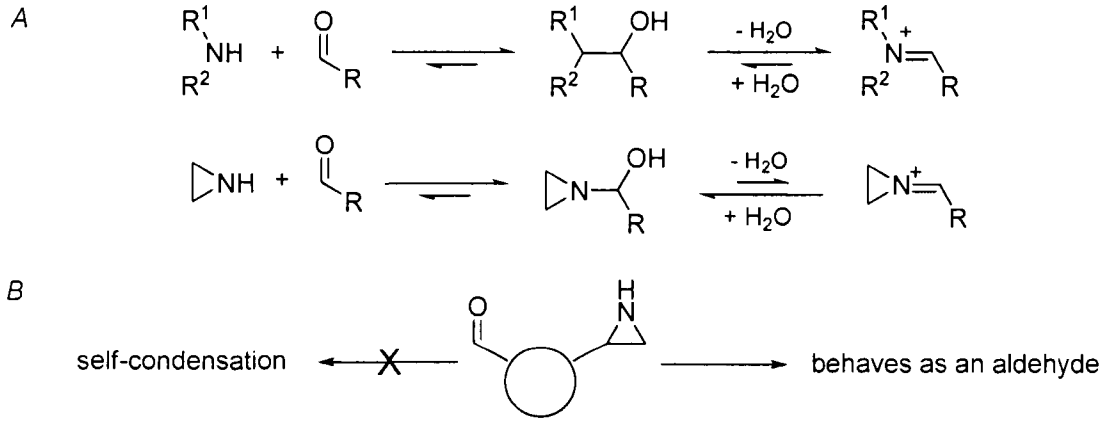


Figure 1.6 The aziridine ring strain gives rise to the co-existence of aziridines and aldehydes

[21] Hili, R.; Yudin, A. K. *J. Am. Chem. Soc.* **2006**, *128*, 14772-12773; Hili, R.; Yudin, A. K. *Chem. Eur. J.* **2007**, *13*, 6538-6543.

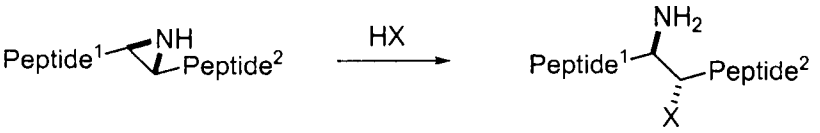


Figure 1.7 Aziridine ring opening can furnish reversible protease inhibitors

peptide of a certain sequence, the corresponding products constitute the central structure of a series of irreversible protease inhibitors.[22] Chemical modification of these conjugates with HX nucleophiles can furnish a series of compounds with α-NH_2 aminoethylene groups (Figure 1.7). This structure has been described as the core of several reversible protease inhibitors.[23] One can expect that by varying the X- substituents a modulated protease inhibitor can be obtained.

[22] Powers, J. C.; Asgian, J. L.; Ekici, O. D.; James, K. E. *Chem. Rev.* **2002**, *102*, 4639-4750.
[23] Sendzik M; Janc J.; Honigberg L.; Mackman R. L.; Magill C.; Waldeck N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3181-3184; Yang W.; Lu W.; Lu Y.; Zhong M.; Fucini R. V.; Jacobs J. W.; McDowell R. S.; Gordon E. M.; Ballinger M. D.; *J. Med. Chem.* **2006**, *49*, 839-842.

Results and Discussions

Part I

Epimerization- and protecting group-free synthesis of versatile peptidomimetic conjugates from amphoteric amino aldehydes

Herein, we describe a general strategy that addresses three critical issues in methodology directed towards peptidomimetic protease inhibitors: (1) the reaction sequence can be used in order to selectively attach an unprotected aziridine electrophile to an amino acid-containing molecule; (2) it delivers a peptidomimetic connection without epimerization on either side of the reduced amide bond; and (3) it may allow for a late-stage peptidomimetic ligation.[24]

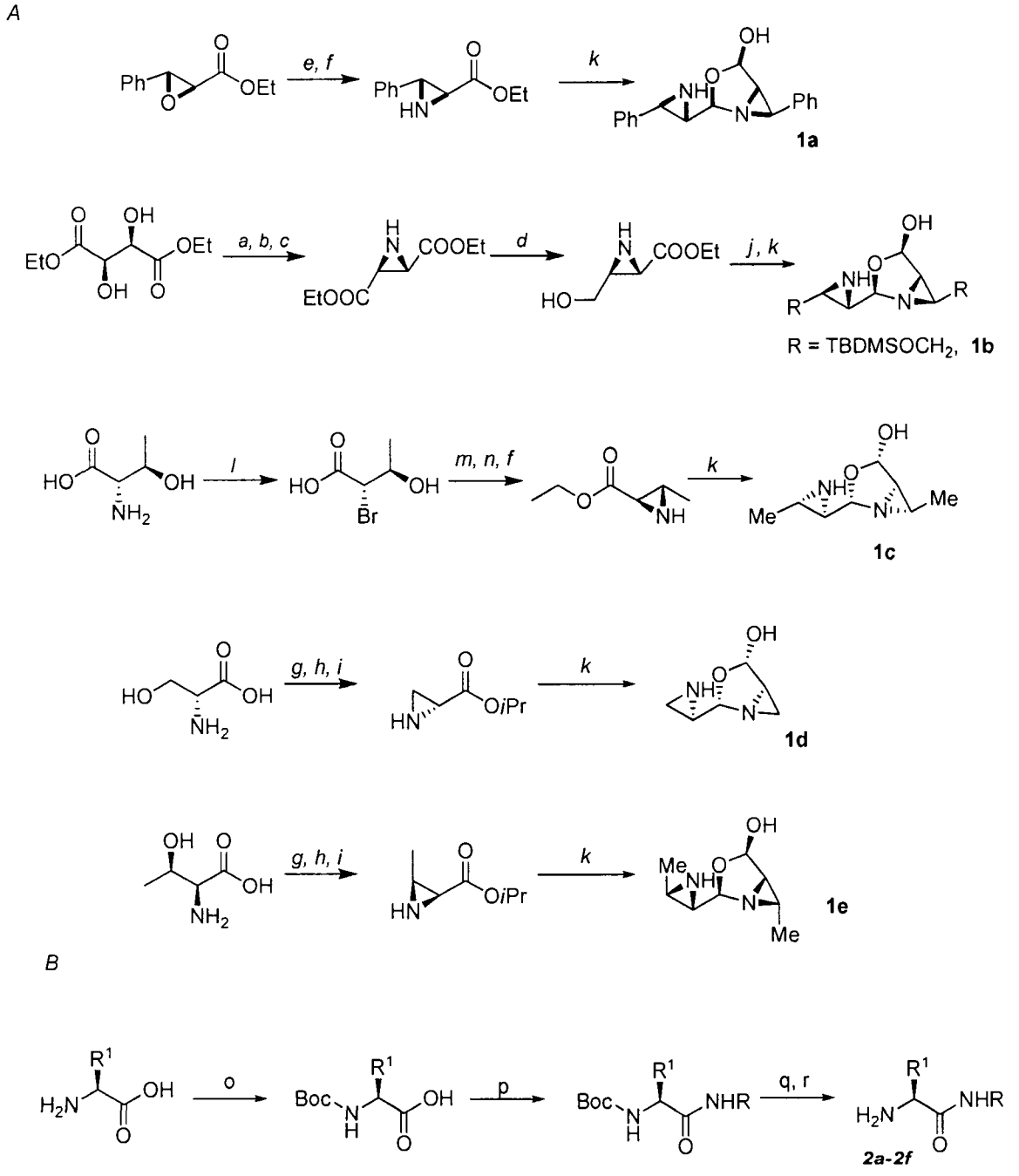
1.1 Synthesis of the model molecules

The model molecules were synthesized according to Scheme 2.1. The aziridine aldehydes were prepared from the corresponding aziridine esters. In the case of **1a**, the readily available epoxy ester was treated with sodium azide followed by triphenylphosphine to afford the corresponding aziridine ester, which underwent

[24] X. Li and A. K. Yudin, *J. Am. Chem. Soc.* **2007**, *129*, 14152

reduction with DIBAL to furnish a

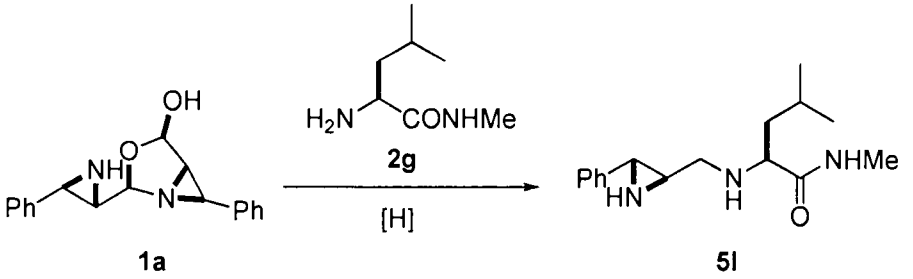
Scheme 2.1 Synthesis of the model molecules



a. $SOCl_2$; b. NaN_3, DMF; c. PPh_3, MeCN; d. $NaBH_4$, MeOH; e. NaN_3, NH_4Cl, EtOH; f. PPh_3, MeCN; g. $SOCl_2$, *i*PrOH; h. NH_4OH; i. PPh_3, DIAD, DCM; j. TBDMSCl, DMAP, DCM; k. DIBAL-H, Toluene, -78°C; l. KBr, H_2SO_4, KNO_2, H_2O; m. NaN_3, DMF, 70°C; n. $SOCl_2$, EtOH; o. $(Boc)_2O$, TEA, THF; p HBTU, DIEA, MeCN; q. TFA, DCM; r. NH_4OH.

bench-stable white solid in a good yield. Starting with naturally abundant amino acid L-serine, after esterification and intramolecular Mitsunobu reaction, the aziridine ester was obtained, which gave the aziridine aldehyde **1d** after the reduction step. **1c** and **1e** are diastereomers, made from L-threonine and D-threonine, respectively. **1e** is a *cis* aziridine and **1c** is a *trans* aziridine. The aziridine esters leading to **1c, 1d** and **1e** are volatile. During work-up steps when vacuum evaporation was applied in order to remove the solvent, most of the product evaporated together with the solvent and low yields were obtained. Alternatively, blowing the solvent by compressed air gave a better yield. Substrates **2a** – **2f** were synthesized according to literature procedures, and good yields were obtained in each case. [25]

1.2 Reductive amination condition studies



Scheme 2.2 Reductive amination between aziridine aldehydes and amino acids

At the beginning of our research, standard reductive amination conditions ($NaCNBH_3$, MeOH, HOAc) were used on aziridine aldehyde dimers and amino acid derivatives, but poor yields were obtained. To optimize the reaction system, different solvents were investigated. $NaCNBH_3$ and $NaHB(OAc)_3$ were tested as the hydride sources. Also, different additives were applied to the reaction in order to achieve the best

[25] Hill, R. R.; Birch, D.; Jeffs, G. E.; North, M. *Organic & Biomolecular Chemistry* **2003**, *1*, 965-972; Katritzky, A. R.; Xu, Y.-J.; He, H.-Y.; Steel, P. J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1767-1770; Kyburz, E.; Els, H.; Majnoni, S.; Englert, G.; Planta, C. v.; Fuerst, A.; Plattner, P. A. *Hel. Chim. Acta* **1966**, *49*, 359-69

results. The results are summarized below (Table 2.1).

Table 2. 1 Reductive amination between **1a** and **2g** under different reaction conditions[a]

Entry	Reduction conditions	Yield (%)
1	$NaCNBH_3$, MeOH, HOAc (1% in MeOH)	7%
2	$NaCNBH_3$, MeOH	3%
3	$NaHB(OAc)_3$, MeOH	no rxn
4	$NaCNBH_3$, TFE	aldehyde reduction product
5	$NaCNBH_3/Ce(SO_4)_2$, MeOH/DCM	<50%
6	$NaCNBH_3/PbBr_2$, MeOH/DCM	<50%
7	$NaCNBH_3/ZnCl_2$, MeOH/DCM	63%
8	$NaCNBH_3/SnCl_4$, MeOH/DCM	<5%
9	$NaCNBH_3/LiOCl_4$, MeOH/DCM	<5%
10	$NaCNBH_3$/LaCl, MeOH/DCM	<5%
11	$NaCNBH_3/Zn(SO_3CF_3)_2$, MeOH/DCM	<5%
12	$NaCNBH_3/AgSO_3CF_3$, MeOH/DCM	<5%
13	$NaCNBH_3/Cu(SO_3CF_3)_2$, MeOH/DCM	<5%
14	$NaCNBH_3/NiCl_2$, MeOH/DCM	<5%
15	$NaCNBH_3/AlCl_3$, MeOH/DCM	<5%
16	$NaCNBH_3/Yb(SO_3CF_3)_3$, MeOH/DCM	<5%
17	$NaCNBH_3/Sc(SO_3CF_3)_3$, MeOH/DCM	<5%
18	$NaCNBH_3/In(SO_3CF_3)_3$, MeOH/DCM	<5%
19	$NaCNBH_3/Zr(i\text{PrO})_4$, MeOH/DCM	<5%
20	$NaCNBH_3/Pb(AcO)_2$, MeOH/DCM	<5%
21	$NaCNBH_3/CaCl_2$, MeOH/DCM	<5%
22	$NaCNBH_3/CeSO_3$, MeOH/DCM	<5%
23	$NaCNBH_3/MgCl_2$, MeOH/DCM	<5%
24	$NaCNBH_3/Ti(i\text{PrO})_4$, MeOH/ DCM	<5%
25	**$NaCNBH_3/ZnCl_2$, MeOH/THF**	**82%**
26	$NaCNBH_3/ZnCl_2$, MeOH/Et_2O	50%
27	$NaCNBH_3/ZnCl_2$, MeOH/Toluene	10%
28	$NaCNBH_3/ZnCl_2$, MeOH/MeCN	<5%

[a] All reactions were carried out using 0.5 eq. of aziridine aldehyde dimer , 1 eq. of *L*-leucine methyl amide in solvent or solvent combination (0.1 mmol/ml) for 12 h.

It should be noted that the protic acid delivered better conversion as well as yield compared to reactions with no acid. However, when the acidic solvent trifluoroethanol (pK_a 12.4) was used, reductive amination did not occur. Instead, the reaction led to preferential aldehyde reduction. In addition, the bulkier reducing reagent sodium triacetoxylborohydride did not promote the reductive amination. Among all the additives, zinc chloride gave the best result. It was reported that $Zn(CNBH_3)_2$ can be formed *in situ* from $NaCNBH_3$. This newly formed metal complex delivered an increase in the reaction rate.[26] In our case, zinc chloride dramatically increased the yield of the reductive amination. To further optimize the condition, different solvent combinations were tested. It was found that THF/MeOH was superior to other solvent system, such as DCM/MeOH, hexane/MeOH, toluene/MeOH, or MeOH.

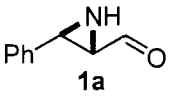
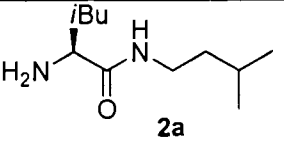
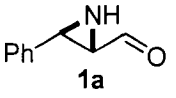
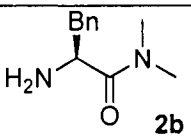
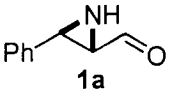
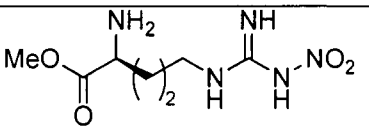
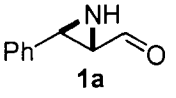
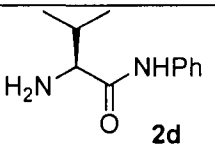
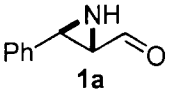
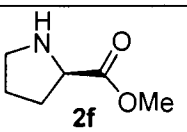
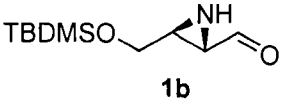
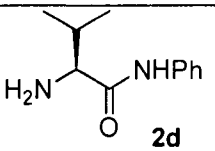
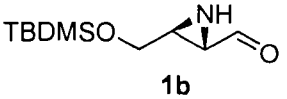
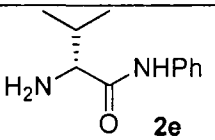
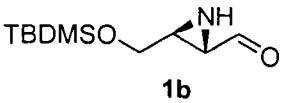
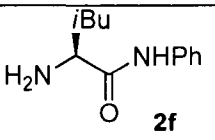
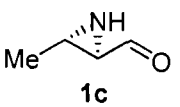
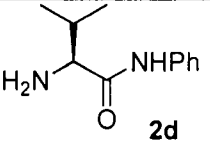
1.3 The reaction scope of peptidomimetic conjugation chemistry

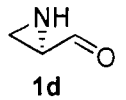
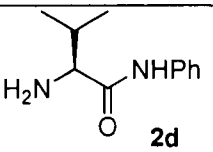
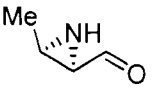
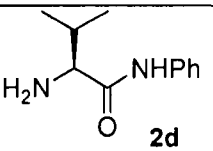
Using this optimized condition, a variety of unprotected amino aldehydes were cleanly conjugated with α-amino acid derivatives (Table 2.2). According to Table 2.2, good to high yields were obtained in most of the cases. There was no evidence of epimerization on the aldehyde side of the aminomethylene linkage, since the energetically uphill enolization of the strained aziridine aldehyde was not occurring. Comparing the results from entries 6 and 7, there was no epimerization on the amine side of the aminomethylene either. This observation will be explained in detail in the mechanistic study section. Interestingly, in entry 5 one single spot was observed on TLC just after the reaction was complete. However, after work-up steps more spots on TLC

[26] Kim, S.; Oh, C. H.; Ko, J. S.; Ahn, K. H.; Kim, Y. J. *J. Org. Chem.* **1985**, *50*, 1927-32.

appeared. The emergence of the newly formed side products was probably because the ester functional group reacted with the amino group and formed a more stable amide bond when heat was applied during work-up steps.

Table 2.2 The scope of peptidomimetic conjugation chemistry[a]

entry	aziridine aldehyde[b]	amino acid derivative	yield[c]
1	 **1a**	 **2a**	85%
2	 **1a**	 **2b**	75%
3	 **1a**	 **2c**	86%
4	 **1a**	 **2d**	81%
5	 **1a**	 **2f**	80%
6	 **1b**	 **2d**	92%
7	 **1b**	 **2e**	92%
8	 **1b**	 **2f**	84%
9	 **1c**	 **2d**	60%

10	 **1d**	 **2d**	51%
11	 **1e**	 **2d**	60%

[a]Unless stated otherwise, the reactions were carried out using 0.5 eq. of the dimer (1 eq. aldehyde), 1.2 eq. amine, 1.5 eq. $NaBH_3CN$, and 1 eq. $ZnCl_2$ in THF and MeOH (1/1) at room temperature; [b]The corresponding monomer; [c]Isolated yield.

Another key observation is that no bis-alkylated product was ever detected. Since the secondary amine is more reactive than the primary amine, over-alkylation is a well-known problem of reductive amination between primary amines and aldehydes.[27] However, from our observation, primary amines showed better reactivity towards aziridine aldehydes than secondary amines under the condition of reductive amination. The application of this special chemical property of aziridine aldehydes will be illustrated in detail in the next chapter dealing with the oligoamine synthesis. In order to compare

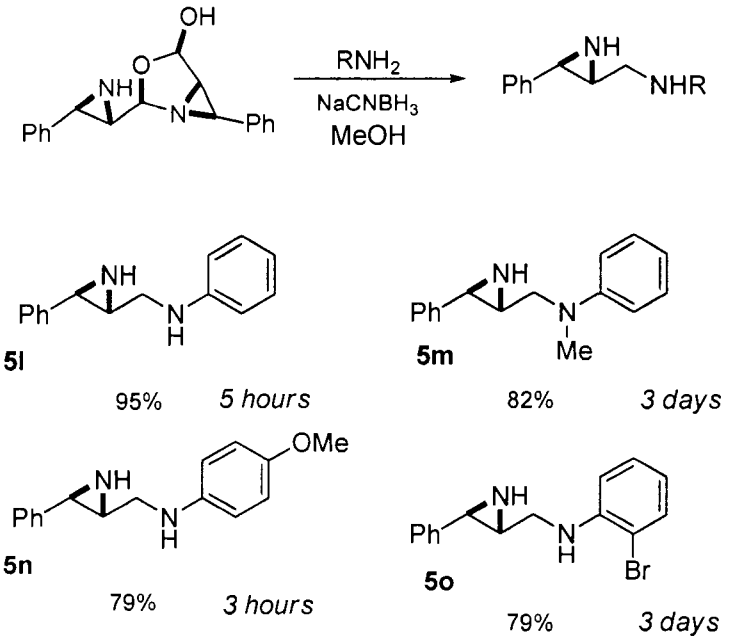


Figure 2.1. Reductive amination with primary and secondary amines

[27] Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849-3862.

the reactivity of aziridine aldehyde dimer with primary and secondary amines, different anilines were subjected to the same reaction condition (Figure 2.1). The reaction time of primary amine (aniline) with the aziridine aldehyde was much shorter than that of secondary amine (*N*-methylaniline). The reaction using more electron-rich primary amine, 4-methoxy-aniline went to completion even faster than *N*-methylaniline, but had a lower yield since more side products were produced. Interestingly, 2-bromoaniline is also a primary amine, but its reaction rate with aziridine aldehydes was as low as that of a secondary amine. This observation indicates that steric effects play a crucial role in the determining the reaction rate.

1.4 Mechanistic studies

We first proposed that the aziridine aldehyde dimer is in unfavorable equilibrium with its monomer form. The resulting aldehyde is then expected to form the imine species by condensation with the amine. After the hydride attack the amination product can be obtained (Figure 2.2). However, the formation of the imine intermediate was never detected by any of our NMR or ESI-MS measurements.

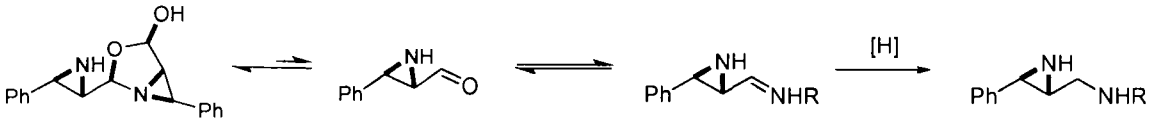


Figure 2.2. The originally proposed mechanism

On the other hand, the adduct **3** was detected by ESI-MS at low concentration, which led us to the mechanism shown on Figure 2.3. According to this proposal, the adduct **3** is formed upon condensation between the amino aldehyde dimer **1** and amine **2.** It participates in an unfavorable equilibrium with its "half-opened" form **4** which is rapidly reduced by the hydride transfer agent to give product **5**, as well as the amino aldehyde

monomer, which rapidly goes back to its dimer form. The short lifetime of **4** ensures that the rates of tautomerization and, therefore, epimerization, are negligible on the amino acid side of the aminomethelene linkage.

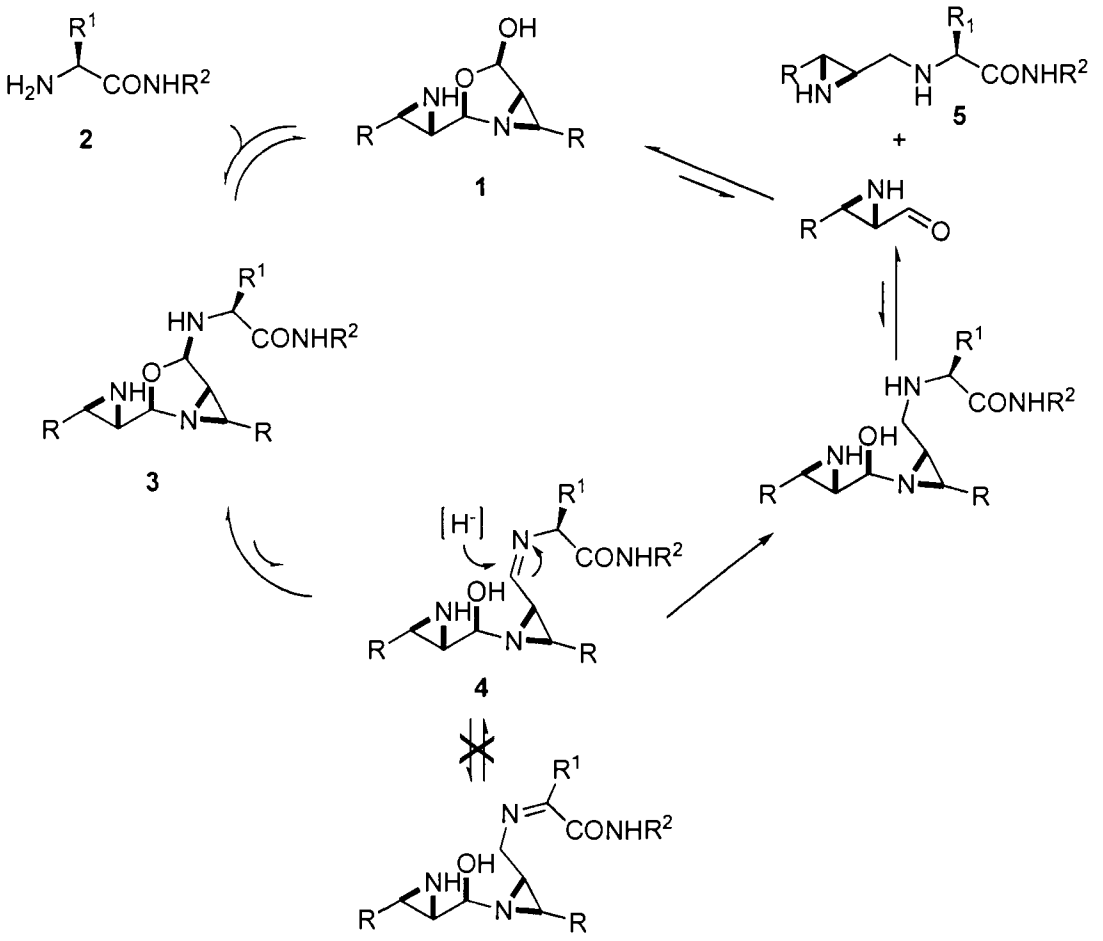


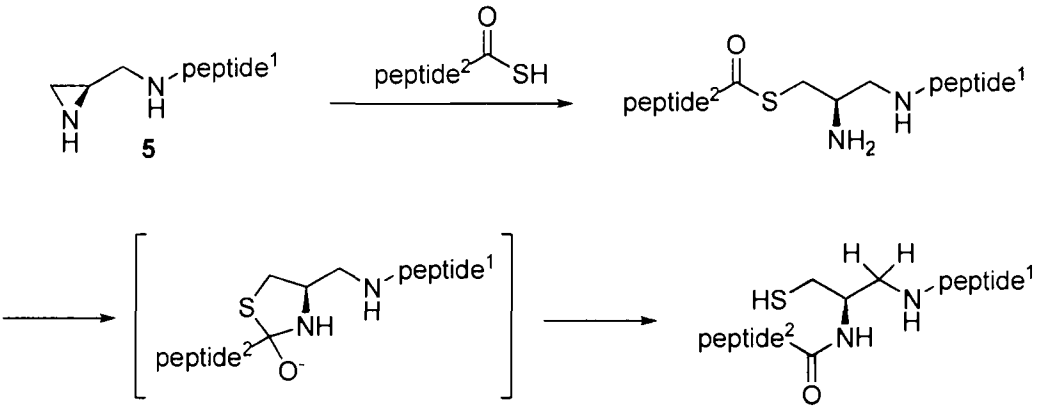
Figure 2.3. Mechanistic underpinnings of epimerization-free synthesis of peptidomimetic conjugates

1.5 The ligation chemistry

These amino acid conjugates **5** can be connected to another amino acid sequence by a thioacid-triggered process (Scheme 2.3). This reaction offers a possibility for a peptidomimetic ligation of two fragments such that a reduced amide bond isostere is specifically introduced next to a cysteine residue with complete stereocontrol of the

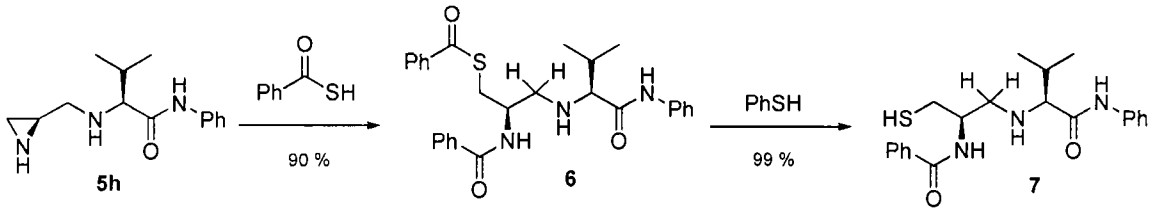
nearby stereocenters.

Scheme 2.3. Ligation of peptide 1 to peptide 2



This chemistry was demonstrated by reacting **5h** with 2 equivalents of thiobenzoic acid in MeOH. This resulted in the benzoylated thioester, which was removed by trans-thioesterification chemistry with thiophenol. A quantitative yield was obtained (Scheme 2.4).

Scheme 2.4. The ligation chemistry was demonstrated by a thio-acid triggered process



In summary, we have developed a general strategy for attaching aziridine electrophiles to a peptide through a peptidomimetic linkage. A high degree of stereocontrol has been achieved during reductive amination due to the unusual properties of the amphoteric amino aldehydes. The resulting products contain the core structures of irreversible protease inhibitors. The reduced amide bond can be easily positioned at the active site using this conjugation technique.

Part II

Oligoamine synthesis via aziridine aldehydes

In the course of studies of the reductive amination between aziridine aldehydes and primary amines, we have found that aziridine aldehyde dimers were more reactive towards primary amines. We then opted to utilize this special property of aziridine aldehyde dimers to make oligoamineswithout protection group manipulation. These oligoamines possess array of biological activities (Figure 3.1).[28] However, there is no example of protection-free synthesis of these compounds.

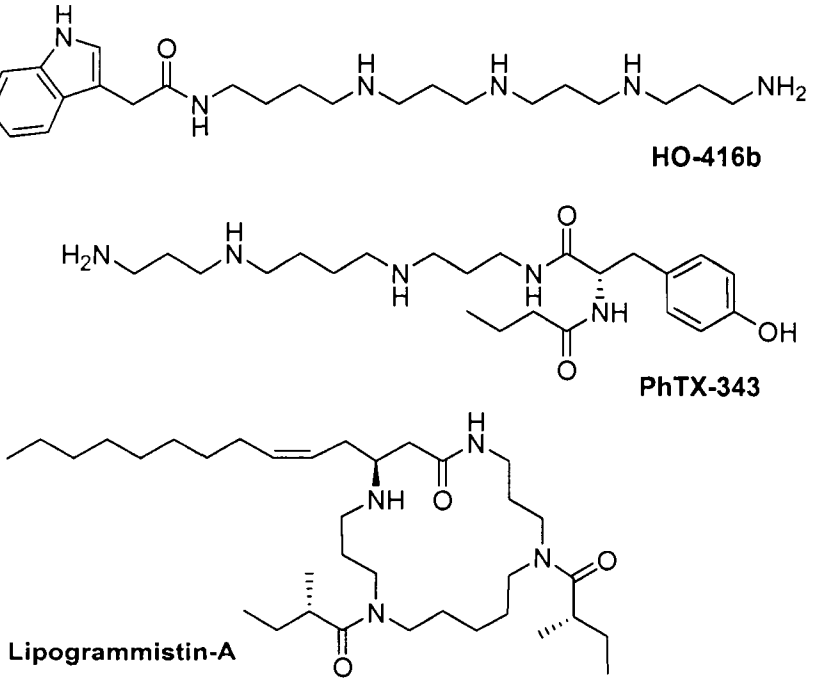


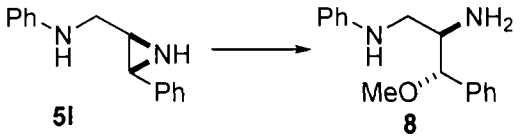
Figure 3.1 Oligoamines with biological activities

[28] Kan T.; Fukuyama T. *Chem. Comm.* **2004,** *10*, 353-359

2.1 Aziridine ring opening chemistry

We first aimed at the ring opening chemistry of the aziridines conjugated with other amines via reductive amination. MeOH was chosen as the nucleophile. Protic acid was employed as the catalyst. (Scheme 3.1). By using one equivalent of HBF_4, several side

Scheme 3.1 Aziridine ring opening chemistry



products were observed. This was due to competitive intramolecular nucleophile attack (Figure 3.2 A). To eliminate this side process, 4 equiv. of HBF_4 were employed (Figure 3.2 B). As a result, the amine was protonated and could no longer act as a nucleophile. Therefore, methanol acted as the only active nucleophile to attack the aziridine. This time no side product was observed and a quantitative yield was obtained.

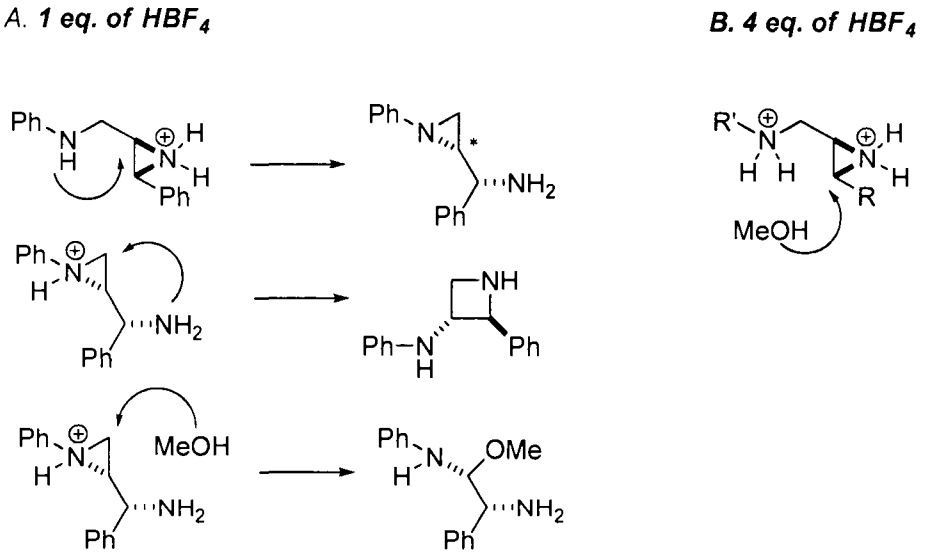
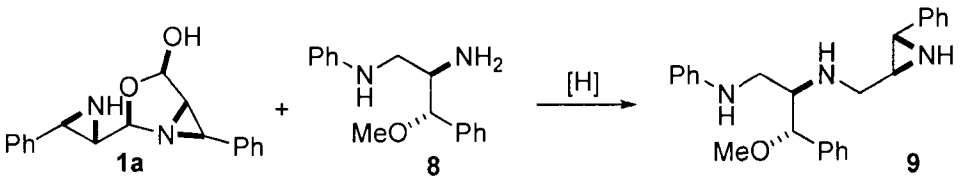


Figure 3.2 The effect of the amount of acid involved

2.2 Investigation of Reductive Amination Conditions

Scheme 3.2 The reductive amination between dimers and diamines



During the investigation of reductive amination between aziridine aldehydes and primary amines, zinc chloride in MeOH/THF combination afforded the optimal condition. In addition, the primary amines were found to give better reactivity towards aziridine aldehydes than the secondary amines. Therefore, we assumed that under the same condition the primary amine of the 1,2-diamine should be the kinetically reactive site and the following transformation should take place (Scheme 3.2). However, several unexpected side products were generated in substantial amounts (Figure 3.3).

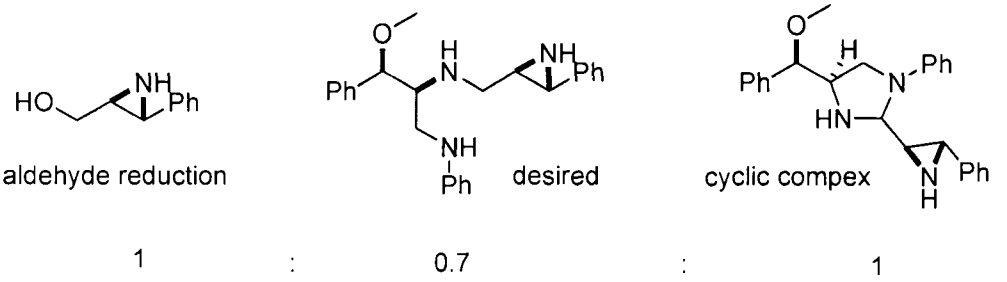


Figure 3.3 The desired and side products during reductive amination

To avoid the formation of undesired products, different solvent systems were investigated. In contrast to our previous findings, $ZnCl_2$ was not helpful in this transformation. The yield did not improve in any of the solvents (Table 3.1). When TFE was employed, there was a big increase in yield, although it was still not satisfactory. Meanwhile, the over-alkylated product was observed under this condition. This project is still under investigation and the main focus is on approaching the optimal condition for chemoselective transformation of primary amine.

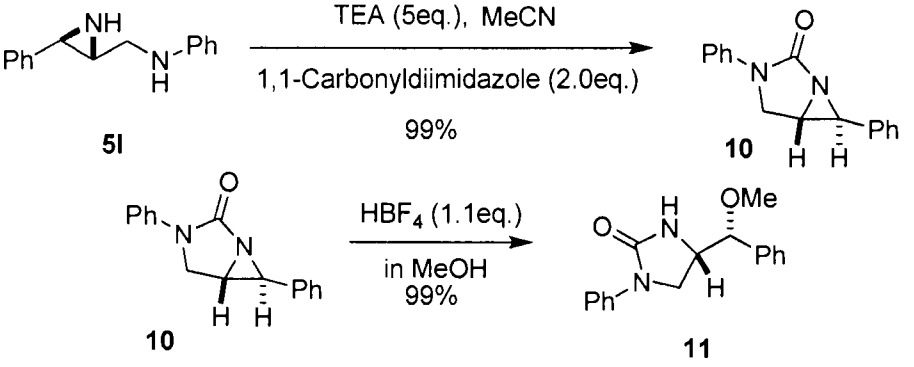
Table 3.1 Reductive amination under different reaction conditions[a]

Entry	Reducing agent	Solvent	Temperature	Additive	Yield
1	$NaCNBH_3$	MeOH	r.t.	$ZnCl_2$	~15%
2	$NaCNBH_3$	DCM	r.t.	$ZnCl_2$	<5%
3	$NaCNBH_3$	Toluene	r.t.	$ZnCl_2$	<5%
4	$NaCNBH_3$	Hexanes	r.t.	$ZnCl_2$	<5%
5	$NaCNBH_3$	TFE	r.t.	$ZnCl_2$	~50%
6	$NaCNBH_3$	Et_2O	r.t.	$ZnCl_2$	<5%
7	$NaCNBH_3$	MeOH	r.t.	-	~15%
8	$NaCNBH_3$	DCM	r.t.	-	<5%
9	$NaCNBH_3$	Toluene	r.t.	-	<5%
10	$NaCNBH_3$	Hexanes	r.t.	-	<5%
11	$NaCNBH_3$	TFE	r.t.	-	<5%
12	$NaCNBH_3$	Et_2O	r.t.	-	<5%
13	Na(OAc)3BH	MeOH	r.t.	-	0%
14	$NaCNBH_3$	MeOH	40 °C	-	0%

[a] All reactions were carried out with 1 eq. of aziridine, 1 eq. of diamine, 1.2 eq. of aziridine aldehyde in solvent (0.1 mmol/ml) for 12 h.

reductive amination barely happened in all the solvents except MeOH and TFE. When

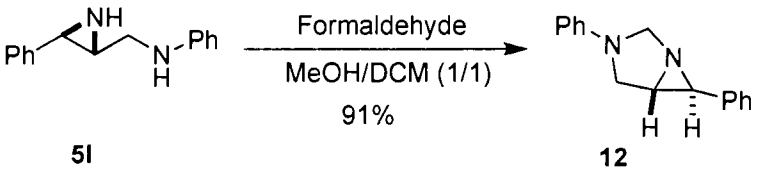
2.3 Synthetic routes towards imidazolidinones and imidazolidines



Scheme 3.3 A synthetic route towards imidazolidinones

We have successfully developed an approach to reductive amination of aziridine aldehydes with primary amines, and a variety of 1,2-diamines were synthesized in moderate to excellent yields. The investigation of synthetic applications of those 1,2-diamines was then carried out.

At the beginning of our investigation, the reaction of oxalyl chloride with 1,2-diamine **10** provided a mixture of products due to the unstability of the activated aziridine. When thionyl chloride was applied, excellent yield was obtained (scheme 3.3). After subsequent ring opening chemistry, the imidazolidinone structure was obtained. To construct the imidazolidine structure, 1,2-diamine **10** was dissolved in a mixture of MeOH (0.5mL), DCM (0.5mL), formaldehyde (38% in MeOH, 0.5mL). After 16 hours at room temperature, compound **12** was obtained in good yield (Scheme 3.4).



5l **12**

Scheme 3.4 The synthetic route towards imidazolidine

In summary, we have investigated a synthetic route towards oligoamines without protection or deprotection steps. As one of the key steps, the ring opening chemistry was first tested, and excellent result were obtained. The second key step, reductive amination of the aziridine aldehyde with the diamine is still under investigation. The design of this synthetic route gains its advantages over the following properties of the aziridine aldehydes: 1) aziridine is a latent primary amine functionality; 2) the co-existence of aziridine and aldehyde functional groups eliminates the cumbersome protection and deprotection steps; 3) the unusual selectivity towards primary amine by aziridine aldehydes can make oligoamine synthesis highly efficient.

Part III

Synthesis of the "shifted" Rolipram

Rolipram, 4-(3-cyclopentyloxy-4-methoxy-phenyl)pyrrolidin-2-one (Figure 4.1), is an inhibitor of phophodiesterase type 4 (PDE4). Like most PDE4-inhibitors, it is an anti-inflammatory drug.[29] Rolipram is being researched as a possible alternative to current antidepressants.[30] Recent studies show that Rolipram may have antipsychotic effects.[31] Other beneficial effects of Rolipram include an increase in long term memory, wakefulness and neuroprotection.[32]

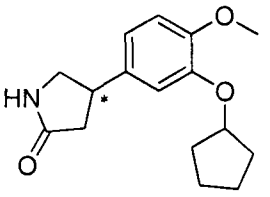


Figure 4.1 The rolipram

3.1 Investigations towards the synthesis of the "shifted" Rolipram

The successful synthesis of compound **13** by electrochemistry encouraged us to investigate the so-called "shifted" Rolipram (Scheme 4.2). The aryl group in this compound has been shifted from 4-position of pyrrolidin-2-one to its 5-position. The

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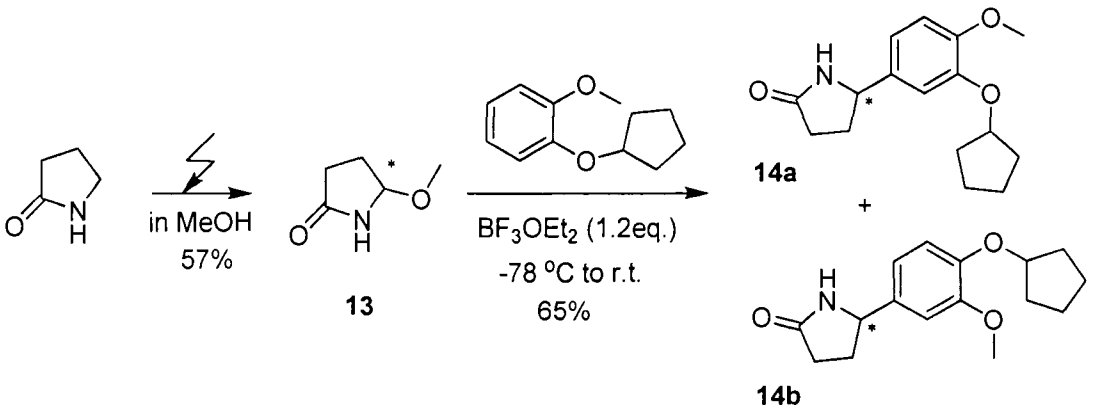
[29] Griswold, D. E.; Breton, J.; White, J. R.; Marshall, P. J.; Torphy, T. J. *Inflammation.* **1993,** *17*, 333–344

[30] Bobon, D.; Breulet, M.; Wachtel, H. *Eur Arch Psychiatry Neurol Sci.* **1988,** *238*, 2–6. Wachtel, H. *Neuropharmacology.* **2003,** *22*, 267–272.

[31] Maxwell, C.R.; Kanes, J.; Abel, T.; Siegel, S.J. *Neuroscience.* **2004,** *194*, 101-107.

[32] Lelkes, Z.; Alföldi, P.; Erdos, A.; Benedek, G. *Pharmacology, Biochemistry and Behaviour.* **1998,** *60,* 835–839. Block, F.; Schmidt, W.; Schwarz, M. *Neuroreport.* **2002**, *7,* 1507–1511. Chen, R. W.; Williams, A.J.; Liao, Z. *Neuroscience Letters.* **2007**, *11*, 165–169.

resulting shifted compound was tested with similar inhibition to PDE4 as the original Rolipram. This was a much simpler and more efficient way than the usual methods to make Rolipram. However, upon activation by the Lewis acid, a mixture of **14a** and **14b** were obtained.

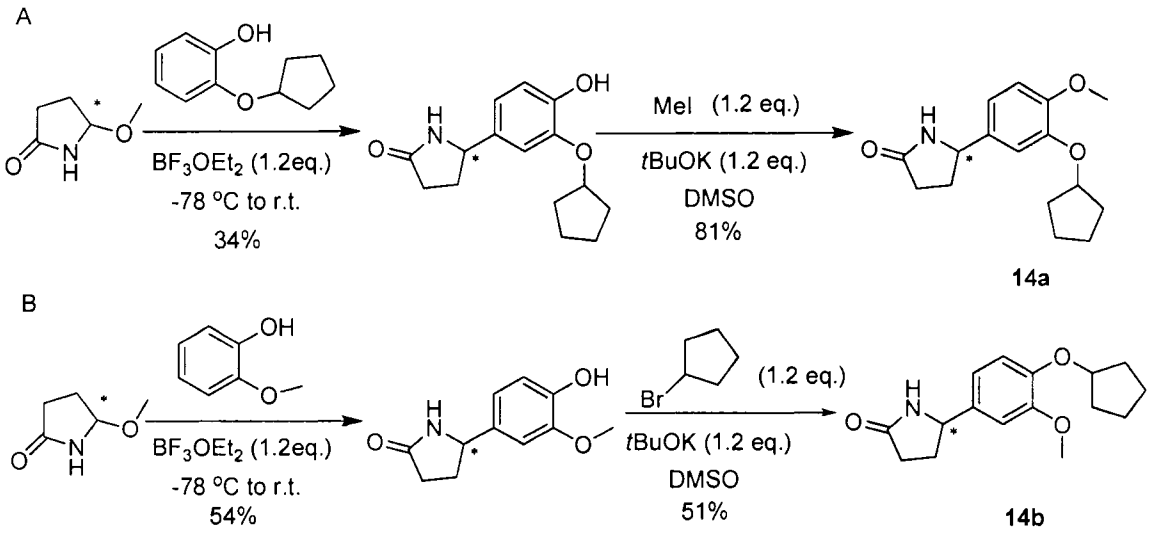


Scheme 4.2 The original synthetic route towards shifted rolipram

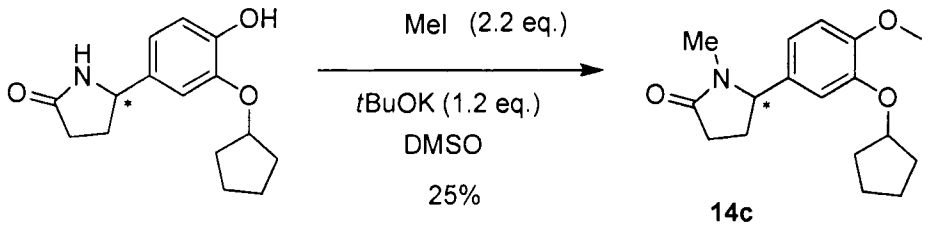
One of the regio-isomers was supposed to have better activity than the other. In order to increase regio-selectivity as well the biological activity, a different synthetic pathway was investigated (Scheme 4.3). Since the hydroxyl group is more electron-donating than the methoxy group, the aromatic substitution chemistry happened at the 4-position of 2-methoxyphenol and selectively afforded **14b**. The same idea was applied to the synthesis of **14a**. In collaboration with Prof. Henming K. of the University of North Carilina, a better inhibition of PDE4 was achieved by **14a** over the mixture of **14a** and **14b**.

Interestingly, when MeI was used in excess amount, a new compound **14c** (Scheme 4.4) was obtained and this new compound was tested sharing better activity than **14a**. This increase in inhibition was probably due to the interaction between the methyl group on **14c** nitrogen and certain hydrophobic groups on the active site of PDE4. Therefore,

different alkyl substitution on **14c** nitrogen should be carried out in order to further increase its activity.



Scheme 4.3 The selective synthesis of the shifted rolipram



Scheme 4.4 The increased activity of the shifted rolipram

In summary, selective synthesis of the "shifted" Rolipram was achieved by utilizing the difference in electron-donating nature of hydroxyl and alkoxyl groups on the aromatic ring. A better biological activity was obtained when methyl substitution was on the nitrogen of **14a**.

Part IV

Arginine-containing bioactive compounds

We have successfully developed an approach to reductive amination between aziridine aldehydes and arginines. Through aziridine opening chemistry we wanted to utilize this special structure (**5C**) to construct a series of arginine-containing bioactive compounds (Figure 5.1). It was reported that a variety of arginine-containing peptides have biological activity towards the enzyme WDR5 involved in histone methylation.[33] Based on structural and binding studies, it was found that arginine plays an important role in interacting with WDR5. The arginine anchored the peptide into the binding pocket. Interestingly, if the amino acid residue adjacent to arginine had a polar functional group, there was an increase in activity. With these important facts in hand, we reasoned that if the nucleophile (Figure 5.1) used at the aziridine ring opening stage is capable of hydrogen bonding, our arginine derivatives can have improved inhibition towards WDR5.

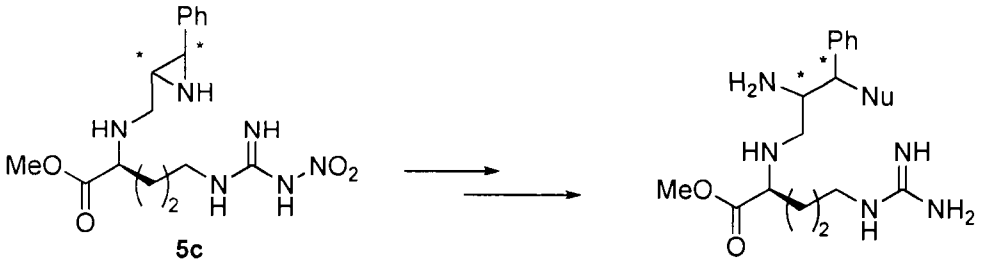
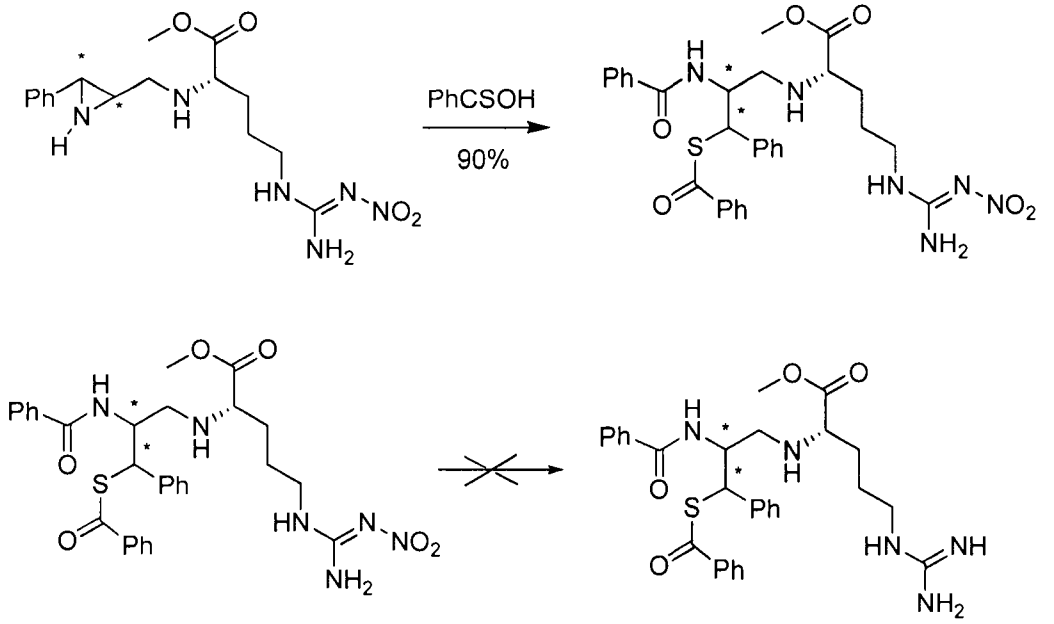


Figure 5.1 Synthesis of arginine-containing derivatives

[33] Schuetz A.; Allali A. A.; Martin F.; Plotnikov N. A.; Arrovowsmith C.; Min J. *EMBO J.* **2006,** *25*, 4245-4252

Our first attempt at the aziridine opening chemistry was achieved with thio-acid ring opening followed by benzoyl group migration (Scheme 5.1). However, the deprotection of nitro group was not successful. Typically, *N*-nitro groups on guanidino groups can be cleaved by reduction.[34] In our case, no desired product was obtained. The reaction always ended up with several by-products which we were unable to characterize.

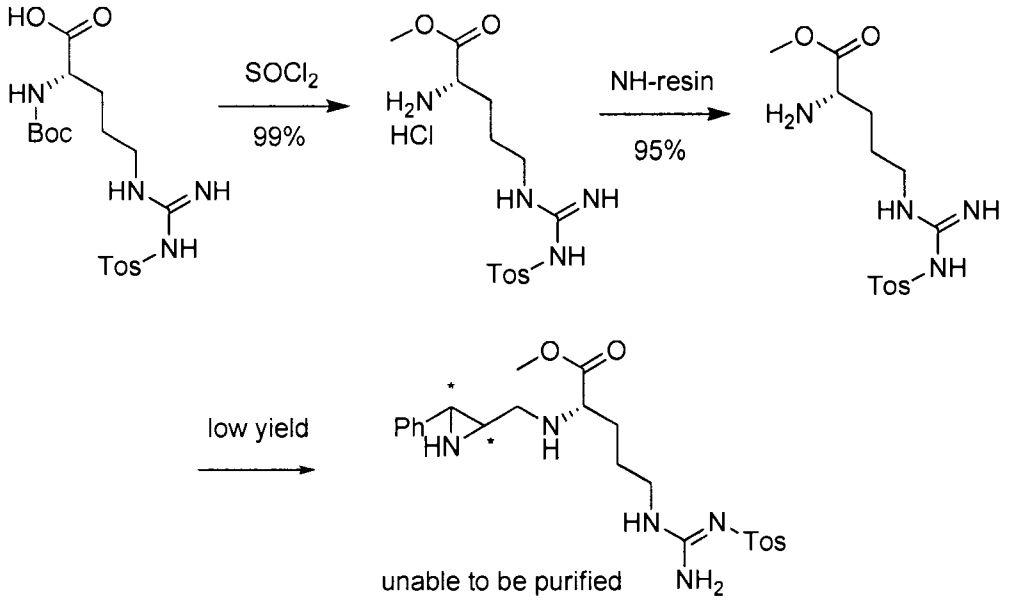
Scheme 5.1 *N*-nitro protection was employed in the transformation



An alternative protecting group was employed. The esterification of the acid group and deprotection of the amino group were successfully carried out. However, the product of the reductive amination could not be obtained (Scheme 5.2). Due to the fact that the arginine substrate is very insoluble in any of the solvents, very low yields were obtained and it was difficult to purify the product by any methods, including normal phase column chromatography, reversed phase column chromatography, and recrystallization. The crude product was subjected to Tos deprotection conditions.[35] However, no desired product was detected by TLC and ESI-MS analysis.

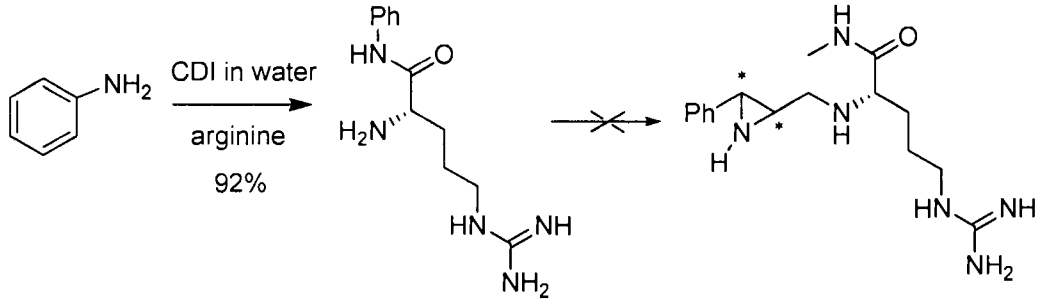
[34] Hofmann K.; Peckham W. D. *J. Am. Chem. Soc.* **1956,** *78,* 238-241.
[35] Zhou W.; Xie N.; Lu Z. *Tetrahedron Lett.* **1996,** *36,* 1291-1297.

Scheme 5.2 Unsuccessful Tosyl deprotection



Unprotected arginine was then employed. Amide bond formation between aniline and arginine was carried out in water (Scheme 5.3). However, the resulting compound could not be dissolved in any solvents except water. There was no other suitable condition found to perform this reductive amination reaction.

Scheme 5.3 No protection group was involved



In summary, the synthesis of arginine-containing derivatives was not achieved. Although many different protecting groups were employed, the main issue was that the arginine group is too polar to be dissolved in any organic solvents. Solid-phase work is a likely avenue to pursue.

Summary

The synthetic potential of aziridine aldehydes was demonstrated. Reductive amination reaction can be achieved upon treatment of aziridine aldehyde with sodium cyanoborohydride and zinc chloride in the solution of MeOH and THF (1/1). This approach was successfully applied to the synthesis of a series of potential protease inhibitors in moderate to high yields. Mechanistic investigations were carried out in order to understand the epimerization-free feature of the reaction.

The special character of the dimer reacting with primary amine under reductive amination was utilized in the construction of oligoamine without protection steps involved.

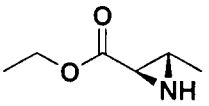
Experimental Section

General: All reactions were carried out under atmosphere otherwise indicated. Anhydrous diethyl ether (Et_2O) and dichloromethane (DCM) were purchased from Aldrich Chemical Co. Tetrahydrofuran (THF) was dried over sodium. Other commercial reagents of high purity were purchased and used without further purification, unless otherwise noted. Analytical thin layer chromatography (TLC) was performed on Macherey Nagel precoated TLC plates (silica gel 60 GF 254, 0.25 mm). Column chromatography was carried out using Silicycle 230-400 mesh silica gel.

Instrumentation: Nuclear magnetic resonance (NMR) spectra were taken on Varian Mercury 400 spectrometer. ^1H NMR spectra were referenced to Me_4Si (δ 0.0 ppm), ^{13}C NMR spectra were referenced to $CDCl_3$ (δ 77.23 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = mutiplet), coupling constants (Hz), integration..

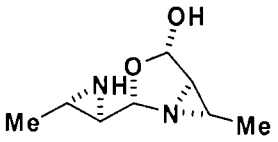
The aziridine aldehydes **1a, 1b** were synthesized using a literature method.[36] The amino acid derivatives **2a, 2b, 2c, 2d, 2e** and **2f** were synthesized using literature methods.[37]

(2R,3S)-ethyl 3-methylaziridine-2-carboxylate



To a solution of 2-Azido-3-hydroxy-butyric acid ethyl ester (1.55g, 9mmol) in DMF (25mL) was added PPh_3 (2.7g, 10 mmol). The mixture was stirred and heated at 85 °C for 12 hours. The reaction mixture was then cooled down to room temperature and 50mL diethyl ether was added while stirring. The mixture was washed with brine 5 times. The organic layer was dried over Mg2SO4 and then concentrated under vacuum. The crude mixture was subjected to silica gel column chromatography (gradient 50% to 100% Et_2O in hexanes) to yield a pale yellow oil (yield 42%). ^1H NMR ($CDCl_3$, 400MHz) δ: 4.20 (m, 2H), 2.20-2.35 (m, 2H), 1.30 (t , 9.0Hz, 3H), 1.24 (d, 6.8Hz, 3H) ppm. ^{13}C NMR (Methanol-d4, 100MHz) δ: 171.0, 61.7, 36.4, 34.9, 18.3, 14.4 ppm.

6-Methyl-2-(3-methyl-aziridin-2-yl)-3-oxa-1-aza-bicyclo[3.1.0]hexan-4-ol (1c)



In a flame dried 100 ml round bottom flask equipped with a magnetic stirring bar was

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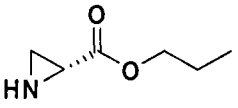
[36] Hili, R.; Yudin, A. K. *J. Am. Chem. Soc.* **2006**, *128*, 14772-14773.

[37] Hill, R. R.; Birch, D.; Jeffs, G. E.; North, M. *Organic & Biomolecular Chemistry* **2003**, *1*, 965-972; Katritzky, A. R.; Xu, Y.-J.; He, H.-Y.; Steel, P. J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1767-1770; Kyburz, E.; Els, H.; Majnoni, S.; Englert, G.; Planta, C. v.; Fuerst, A.; Plattner, P. A. *Hel. Chim. Acta* **1966**, *49*, 359-69; Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-20; Pelagatti, P.; Carcelli, M.; Calbiani, F.; Cassi, C.; Elviri, L.; Pelizzi, C.; Rizzotti, U.; Rogolino, D. *Organometallics* **2005**, *24*, 5836-5844.

placed (*2R,3S*)-ethyl 3-methylaziridine-2-carboxylate (0.37 g, 2.9 mmol) in 4 ml toluene. The solution was stirred at -78 °C for 30 minutes. Subsequent to that, a 1.5M solution of DIBAL in toluene (3.8 ml, 5.7 mmol) was added dropwise. Once the addition was complete, the reaction was allowed to stir at -78 °C for another 2 hours at which point ESI MS showed disappearance of starting material. MeOH was slowly added at -78 °C. The reaction mixture was then allowed to stir for 30 minutes while warming to room temperature. A few drops of saturated aqueous Na_2SO_4 were used to cause precipitation of aluminum salts, which were filtered off after stirring for another 30 minutes. The filtrate was concentrated under reduced pressure to yield a thick clear oil, which was pure enough by NMR for use in subsequent transformations. An analytically pure sample was obtained by subjecting the crude product to flash column chromatography (silica gel; 10% MeOH in DCM) to yield the title compound as a colorless oil in 51% yield. ^{1}H NMR ($CDCl_3$, 400MHz) δ: 5.12 (s, 1H), 4.89 (s, 1 H), 2.37 – 2.35 (m, 2H), 2.11 (m, 1 H), 2.01(m, 1 H), 1.28 (d, J = 6.8 Hz, 3H), 1.17 (d, J = 6.8 Hz, 3H) ppm. ^{13}CNMR ($CDCl_3$, 100 MHz) δ: 96.4, 94.3, 51.3, 39.7, 34.4, 29.6, 18.7, 16.5 ppm.

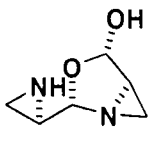
(*R*)-propyl aziridine-2-carboxylate



To a mixture of *D*-serine (10g, 95 mmol) and 250 ml of 20% $SOCl_2$ in *n*-PrOH in a round bottom flask equipped with a water condenser and magnetic stirring bar was added water (100 ml). The reaction mixture was brought to 65 °C and stirred for 48 hours at which point ESI MS analysis showed that the reaction was complete. The mixture was concentrated under reduced pressure. The colorless oil was suspended in 300

ml of DCM. To this stirred mixture 20ml of 30% ammonium hydroxide was added slowly. After stirring for 30 minutes the organic layer separated and the aqueous layer was washed with DCM (X 3). The combined organic portions were dried over sodium sulfate and concentrated under reduced pressure at room temperature to afford the corresponding propyl ester. In a round bottom flask equipped with a magnetic stirring bar was added the ester from above (4.5 g, 165 mmol) dissolved in 200 ml DCM. The reaction mixture was cooled to 0 °C, at which point PPh_3 (7g, 26 mmol) was added followed by DIAD (95%, 5.25 ml, 26mmol) drop wise. The reaction was then brought to room temperature and stirred for 16 hours. The reaction mixture was then concentrated at 60 °C without applying of vacuum. The crude mixture was then dissolved in diethyl ether and placed in the freezer overnight (-15 °C). Any resulting precipitate that formed was filtered off and the filtrate was concentrated and subjected to silica gel column chromatography (gradient 50% to 100% Et_2O in hexanes) to yield a pale yellow oil (yield 41% over three steps). ^1H NMR ($CDCl_3$, 200MHz): δ δ 4.16-4.09 (m, 2H), 2.51 (dd, J = 5.6, 2.8Hz, 1H), 2.01 (m, 1H), 1.85 (d, J = 5.6 Hz, 1H), 1.69(tq, J = 2.7, 6.7Hz, 2H), 0.97(t, J = 6.7 Hz, 3H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 173.4, 67.4, 29.2, 27.5, 22.1, 10.5 ppm. HRMS (ESI+) [M+H]+ calcd. for C6H12N1O2 130.0867, found 130.0871.

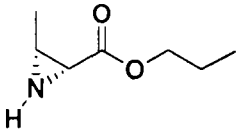
2-Aziridin-2-yl-3-oxa-1-aza-bicyclo[3.1.0]hexan-4-ol (1d)



A similar procedure was followed as described in literature.[1] In a flame dried 100 ml round bottom flask equipped with a magnetic stirring bar was placed the compound from above (0.9 g, 7 mmol) in 18 ml of toluene. The solution was stirred at -

78 °C for 30 min then a 1.5M solution of DIBAL in toluene (9 ml, 13.5 mmol) was added dropwise. Once the addition was complete, the reaction was allowed to stir at -78 °C for another 2 hours at which point ESI MS showed the disappearance of starting material. MeOH was slowly added at -78 °C. The reaction mixture was then allowed to stir for 30 minutes while warming to room temperature. A few drops of saturated aqueous Na_2SO_4 were used to cause precipitation of aluminum salts, which were filtered off after stirring for another 30 minutes. The filtrate was concentrated under reduced pressure to yield a thick clear oil, which was pure enough by NMR to use in subsequent transformations. An analytically pure sample can be obtained by subjecting the crude product to flash column chromatography (silica gel; 10% methanol in DCM) to yield the title compound as a colourless oil in 57% yield. ^{1}H NMR (CDCl3/Methanol-d4, 90/10, 400MHz) δ: 5.27 (s, 1H), 4.90 (s, 1 H), 2.65 (m, 1H), 2.39 (m, 1H), 1.84 (m, 2H), 1.64 (d, J = 5.2 Hz, 1H), 1.32 (d, J = 4 Hz, 1H), 1.26 (d, J = 2.8 Hz, 1H) ppm. ^{13}CNMR (CDCl3/Methanol-d4, 90/10, 100 MHz) δ: 95.9, 95.8, 94.7, 43.6, 43.5, 31.0, 27.6, 20.9, 20.8 ppm.

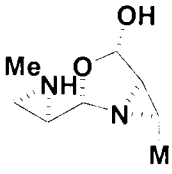
(*2R,3S*)-propyl 3-methylaziridine-2-carboxylate



To a mixture of *D*-threonine (10g, 84 mmol) and 200 ml of 20% $SOCl_2$ in *n*-PrOH in a round bottom flask equipped with a water condenser and magnetic stirring bar was added water (50 ml). The reaction mixture was brought to 65 °C and stirred for 24 hours at which point ESI MS analysis showed that the reaction was complete. The mixture was concentrated under reduced pressure. The colorless oil was suspended in 300 ml of DCM. To this stirring mixture 20ml of 23% ammonium hydroxide was added slowly. After stirring for 30 minutes, the organic layer separated

and the aqueous layer was washed with DCM (X 3). The combined organic portions were dried over sodium sulfate and concentrated to afford the corresponding propyl ester. In a round bottom flask equipped with a magnetic stirring bar was added the ester from above (12 g, 74.5 mmol) dissolved in 200 ml of DCM. The reaction mixture was cooled to 0 °C, at which point PPh_3 (19.5g, 74.5 mmol) was added followed by DIAD (95%, 14.7 ml, 74.5mmol) drop wise. The reaction was then brought to room temperature and stirred for 16 hours. The reaction mixture was concentrated at 60 °C without applying of vacuum. The crude mixture was then dissolved in diethyl ether and filtered. The filtrate was concentrated and subsequently dissolved in pentane and placed in the freezer overnight (-15 °C). Any resulting precipitate that formed was filtered off and the filtrate was concentrated and subjected to silica gel column chromatography (gradient 50% to 100% Et_2O in hexanes) to yield a pale yellow oil (yield 36% over three steps). ^1H NMR ($CDCl_3$, 400MHz) δ: 4.05 (t, J = 9.2 Hz, 2H), 2.56 (d, J = 8 Hz, 1H), 2.22 (t, J = 7.7 Hz, 1H), 1.60 (tq, 9.2, 10Hz, 2H), 1.22 (d, 7.7Hz, 3H), 0.88 (t, J = 10 Hz, 3H) ppm. ^{13}C NMR (Methanol-d4, 100MHz) δ: 171.0, 66.9, 35.0, 33.7, 22.1, 13.1, 10.3 ppm. HRMS (ESI) $[M]^+$ calcd. For C7H13NO2 144.1019, found 144.1022.

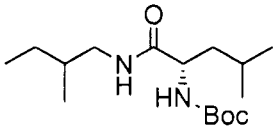
6-Methyl-2-(3-methyl-aziridin-2-yl)-3-oxa-1-aza-bicyclo[3.1.0]hexan-4-ol (1e)



A similar procedure was followed as described in literature.[1] In a flame dried 100 ml round bottom flask equipped with a magnetic stirring bar was placed the compound from above (0.42 g, 2.9 mmol) in 4 ml toluene. The solution was stirred at -78 °C for 30 minutes then a 1.5M solution of DIBAL in toluene (3.8 ml, 5.7 mmol) was added dropwise. Once the addition was complete, the reaction was allowed to stir at -78

°C for another 2 hours at which point ESI MS showed disappearance of starting material. MeOH was slowly added at -78 °C. The reaction mixture was then allowed to stir for 30 minutes while warming to room temperature. A few drops of saturated aqueous Na_2SO_4 were used to cause precipitation of aluminum salts, which were filtered off after stirring for another 30 minutes. The filtrate was concentrated under reduced pressure to yield a thick clear oil, which was pure enough by NMR for use in subsequent transformations. An analytically pure sample was obtained by subjecting the crude product to flash column chromatography (silica gel; 10% MeOH in DCM) to yield the title compound as a colourless oil in 51% yield. ^{1}H NMR ($CDCl_3$, 400MHz) δ: 5.19 (s, 1H), 4.89 (s, 1 H), 2.69 (d, J = 5.4 Hz, 1H), 2.37 – 2.27 (m, 2H), 2.10 (m, 1 H), 1.37 (d, J = 5.5 Hz, 3H), 1.19 (d, J = 6.4 Hz, 3H) ppm. ^{13}CNMR ($CDCl_3$, 100 MHz) δ: 95.8, 95.7, 91.4, 91.3, 47.7, 47.6, 37.2, 33.7, 30.9, 12.4, 7.0 ppm.

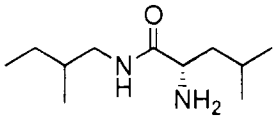
tert-butyl (2S)-4-methyl-1-(2-methylbutylamino)-1-oxopentan-2-ylcarbamate



61.3mg (0.26mmol) of HO-Leu-Boc, 0.046mL(0.40mmol) of 2-methylbutylamine and 0.092mL(0.53mmol) of DIPEA were added into MeCN 3mL. The stirring mixture was added with HBTU 120mg. The reaction mixture was stirred at r.t. until the reaction was complete judged by LCMS. The solvent was evaporated and 5mL diethyl ether was added. The mixture was stirred at room temperature for 1 hour then the ether layer was separated, washed with water (3mL), 1M citric acid (2mL) and brine (2mL), and dried over magnesium sulfate. The mixture was concentrated then under high vacuum to afford the

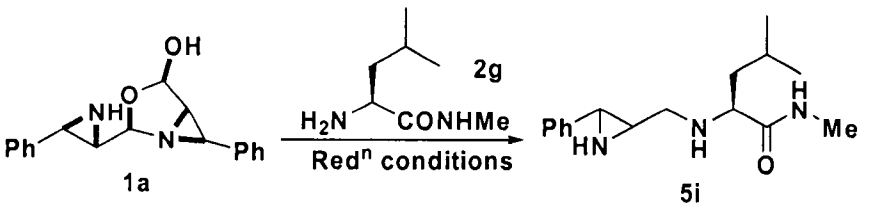
title compound (yield 99%). ^{1}H NMR (CDCl3, 400MHz): δ 6.75 (b, 1H), 5.21(b, 1H), 4.18(b, 1H), 3.17(m, 2H), 1.77-1.07(m, 16H), 0.98(m, 12H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 172.9, 156.0, 53.3, 45.1, 41.4, 35.0, 28.4, 27.1, 27.1, 27.0, 24.9, 23.0, 22.3, 17.2, 17.1, 11.4, 11.3 ppm. HRMS (ESI+) [M+H]+ calcd. for C16H33N2O3 301.2485, found 301.2489.

(2S)-2-amino-4-methyl-N-(2-methylbutyl)pentanamide (2a)



79.2mg (0.26mmol) of Boc protected amine was dissolved into DCM 1mL. TFA was then added at 0 degree. The reaction mixture was stirred over night. 0.2mg of sodium bicarbonate was added and stirred for 2 hours. The mixture was then filtered through celite. The solvent was then evaporated to afford the title compound (yield 99%). ^{1}H NMR (CDCl3, 400MHz): δ 7.36 (b, 1H), 3.41(m, 1H), 3.21(m, 1H), 3.07(m, 1h), 1.73(M, 2H), 1.59(m, 3H), 1.43(m, 2H), 1.26(m, 1H), 0.91(m, 12H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 175.6, 53.8, 44.7, 44.6, 44.3, 35.1, 35.0, 27.2, 27.1, 25.1, 23.6, 21.5, 17.3, 11.5 ppm. HRMS (ESI+) [M+H]+ calcd. for C11H25N2O 201.1961, found 201.1955.

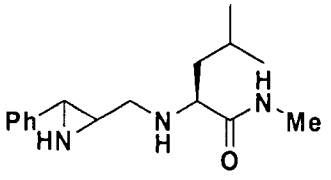
Condition screening.



General procedure for condition screening:

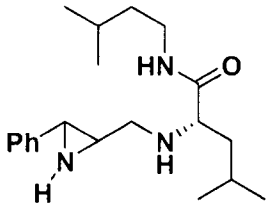
In a flame dried 10 ml round bottom flask equipped with a magnetic stirring bar was placed **1a** (15 mg, 0.05 mmol) and **2g** (17mg, 0.12 mmol) in 1 ml of solvent combination. The solution was stirred at room temperature and $ZnCl_2$ (14 mg, 0.10 mmol) was added. The mixture was stirred for 1 minute at which point $NaCNBH_3$ (10 mg, 0.15 mmol) was added. The reaction was allowed to stir at room temperature overnight at which point ESI MS showed the reaction was complete. The reaction mixture was then filtered through Celite. The filtrate was concentrated under reduced pressure and NMR analysis for the crude product was performed. For Entry 1, 2, 4, 7, 8, the crude product was purified by silica gel column chromatography (DCM/MeOH, 90/10). All operations should be done in the fume hood to avoid evolution of highly toxic HCN.

(*2S*)-*N*,4-dimethyl-2-((3-phenylaziridin-2-yl)methylamino)pentanamide (5i)



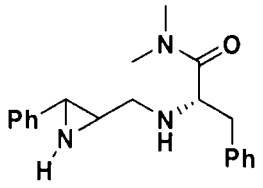
^{1}H NMR (CDCl3, 400MHz): δ 7.32-7.14 ppm (m, 5H), 3.18-3.17(dt, *J* = 8.6, 4.5 Hz, 1H), 2.83-2.57(m, 6H), 2.31(b, 1H), 1.73-1.67(m, 1H), 1.62-1.38(m, 1H), 1.36-1.30(m, 1H), 1.27(b, 1H), 0.92 (m, 6H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 175.6, 175.5, 139.7, 139.6, 129.0, 128.8, 127.6, 127.5, 125.7, 125.6, 61.9, 61.4, 52.2, 51.5, 43.3, 43.2, 41.0, 40.9, 38.6, 38.2, 26.0, 25.3, 23.5, 23.4, 22.2, 22.1 ppm. HRMS (ESI) [M+H]+ calcd. for C16H26N3O 276.2070, found 276.2059.

(*2S*)-4-methyl-*N*-(2-methylbutyl)-2-((3-phenylaziridin-2-yl)methylamino) pentanamide (5a)



In a flame dried 10 ml round bottom flask equipped with a magnetic stirring bar was placed racemic **1a** (30 mg, 0.10 mmol) and racemic **2a** (60mg, 0.30 mmol) in 1 ml of THF and 1 ml of MeOH. The solution was stirred at room temperature and $ZnCl_2$ (27 mg, 0.20 mmol) was added. The mixture was stirred for 1 minute at which point $NaCNBH_3$ (13 mg, 0.3 mmol) was added. The reaction was allowed to stir at room temperature overnight at which point ESI MS showed the reaction was complete. The reaction mixture was then filtered through Celite. The filtrate was concentrated under reduced pressure and subjected to silica gel column chromatography (DCM/MeOH/TEA 95/4/1) to yield a colorless oil. The combined yield was 85% (1:1 mixture of diastereomers). All operations should be done in the fume hood to avoid evolution of highly toxic HCN. ^{1}H NMR ($CDCl_3$, 400MHz) δ:_δ 8.80(b, 2H), 7.95(b, 3H), 7.32-7.19 ppm (m, 5H), 3.74(s, 3H), 3.47(m, 3H), 2.84(m, 2H), 2.41(m, 2H), 1.84-1.75(m, 6H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 175.3, 175.2, 159.6, 129.0, 128.9, 127.7, 125.8, 125.7, 61.1, 60.9, 52.4, 52.3, 51.3, 50.9, 41.3, 41.0, 40.4, 38.4, 25.6 ppm. HRMS (EI+) [M]+ calcd. for C16H24N6O4 331.2624, found 331.2628.

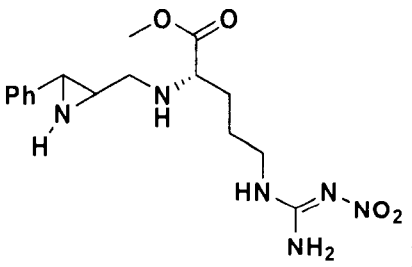
(*2S*)-*N*,*N*-dimethyl-3-phenyl-2-((3-phenylaziridin-2-yl)methylamino)propanamide (5b)



In a flame dried 10 ml round bottom flask equipped with a

magnetic stirring bar was placed racemic **1a** (66 mg, 0.22 mmol) and **2b** (103mg, 0.54 mmol) in 1.5 ml of THF and 1.5 ml of MeOH. The solution was stirred at room temperature and $ZnCl_2$ (61 mg, 0.45 mmol) was added. The mixture was stirred for 1 minute at which point $NaCNBH_3$ (43 mg, 0.70 mmol) was added. The reaction was allowed to stir at room temperature overnight at which point ESI MS showed the reaction was complete. The reaction mixture was then filtered through Celite. The filtrate was concentrated under reduced pressure and subjected to silica gel column chromatography (Gradient: Ethyl acetate/MeOH, 95/5-90-10) to yield colorless oil (1:1 mixture of diastereomers) in 75% yield. All operations should be done in the fume hood to avoid evolution of highly toxic HCN. ^{1}H NMR (CDCl3, 200MHz): δ 7.32-7.19 ppm (m, 10H), 3.82(t, J = 14.4 Hz, 1H), 2.98-2.85(m, 2H), 2.89 (s, 3H), 2.79-2.56(m, 2H), 2.56(s, 3H), 2.24(b, 1H), 1.58(b, 2H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 174.3, 138.1, 129.5, 129.4, 128.7, 128.6, 127.3, 126.9, 125.9, 60.3, 50.6, 40.9, 36.7, 35.8 ppm. HRMS (EI+) [M]+ calcd. for C20H25N3O 323.1998, found 323.1993.

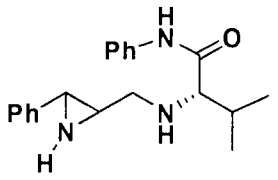
(2*S*)-methyl 5-(2-nitroguanidino)-2-((3-phenylaziridin-2-yl)methylamino) pentanoate (5c)



In a flame dried 10 ml round bottom flask equipped with a magnetic stirring bar was placed racemic **1a** (80 mg, 0.27 mmol) and **2c** (276mg, 1.2 mmol) in 1.5 ml of THF and 1.5 ml of MeOH. The solution was stirred at room

temperature and $ZnCl_2$ (82 mg, 0.6 mmol) was added. The mixture was stirred for 1 minute at which point $NaCNBH_3$ (60 mg, 0.9 mmol) was added. The reaction was allowed to stir at room temperature overnight at which point ESI MS showed the reaction was complete. The reaction mixture was then filtered through Celite. The filtrate was concentrated under reduced pressure and subjected to silica gel column chromatography (gradient DCM/MeOH 95/5 to 80/20) to yield colorless oil (1:1 mixture of diastereomers). The combined yield was 86%. All operations should be done in the fume hood to avoid evolution of highly toxic HCN. ^{1}H NMR (CDCl3, 400MHz): δ 8.80(b, 2H), 7.95(b, 3H), 7.32-7.19 ppm (m, 5H), 3.74(s, 3H), 3.47(m, 3H), 2.84(m, 2H), 2.41(m, 2H), 1.84-1.75(m, 6H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 175.3, 175.2, 159.6, 129.0, 128.9, 127.7, 125.8, 125.7, 61.1, 60.9, 52.4, 52.3, 51.3, 50.9, 41.3, 41.0, 40.4, 38.4, 25.6 ppm. HRMS (EI+) [M]+ calcd. for C16H24N6O4 365.1931, found 365.1984

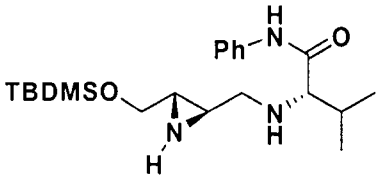
(2*S*)-3-methyl-*N*-phenyl-2-((3-phenylaziridin-2-yl)methylamino)butanamide (5d)



In a flame dried 10 ml round bottom flask equipped with a magnetic stirring bar was placed racemic **1a** (22.5 mg, 0.08 mmol) and **2d** (32.3mg, 0.17 mmol) in 0.7 ml of THF and 0.7 ml of MeOH. The solution was stirred at room temperature and $ZnCl_2$ (20.8 mg, 0.15 mmol) was added. The mixture was stirred for 1 minute at which point $NaCNBH_3$ (14.5 mg, 0.23 mmol) was added. The reaction was allowed to stir at room temperature for overnight at which point ESI MS showed the reaction was complete. The reaction mixture was then filtered through Celite. The filtrate was concentrated under reduced pressure and subjected to silica gel column

chromatography (DCM/MeOH 95/5) to yield colorless oil (1:1 mixture of diastereomers). The combined yield was 81%. All operations should be done in the fume hood to avoid evolution of highly toxic HCN. ^{1}H NMR ($CDCl_3$, 400MHz) δ: 9.47 (s, 0.5H), 9.22(s, 0.5H), 7.61 (m, 2H), 7.33-7.06 (m, 6H), 3.11 (d, J = 4.5Hz, 0.5H), 3.07 (d, J = 4.5Hz, 0.5H), 3.07-2.72 (m, 3H), 2.40 (m, , 1H), 2.24 (m, 1H), 1.07 (b, 2H), 1.00 (d, J = 7.6 Hz, 2H), 0.96(d, J = 7.6 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ: 172.2, 173.1, 139.5, 139.4, 138.0, 137.9, 129.2, 128.9, 127.7, 125.7, 124.3, 124.2, 119.8, 69.4, 69.3, 68.8, 68.2, 52.8, 52.3, 39.0, 38.5, 31.9, 19.9, 19.8, 18.1 ppm. HRMS (ESI) $[M]^+$ calcd. For C20H26N3O 324.2072, found 324.2081.

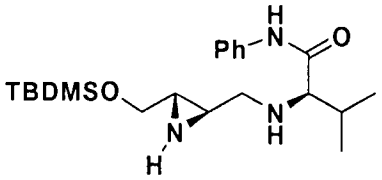
(*S*)-2-(((*2R,3S*)-3-((*tert*-butyldimethylsilyloxy)methyl)aziridin-2-yl)methylamino)-3-methyl-*N*-phenylbutanamide (5e)



In a flame dried 10 ml round bottom flask equipped with a magnetic stirring bar was placed enantiomerically pure **1b** (91 mg, 0.21 mmol) and **2d** (97mg, 0.5 mmol) in 2.5 ml of THF, DCM 1ml and 2.5 ml of MeOH. The solution was stirred at room temperature and $ZnCl_2$ (57 mg, 0.42 mmol) was added. The mixture was stirred for 1 minute at which point $NaCNBH_3$ (40 mg, 0.6 mmol) was added. The reaction was allowed to stir at room temperature overnight at which point ESI MS showed the reaction was complete. The reaction mixture was then filtered through Celite. The filtrate was concentrated under reduced pressure and subjected to silica gel column chromatography (Ethyl acetate/MeOH: 95/5) to yield colorless oil. The combined yield

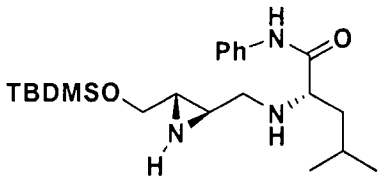
was in 92%. All operations should be done in the fume hood to avoid evolution of highly toxic HCN. ^{1}H NMR (CDCl3, 400MHz): δ 7.65 (d, *J* = 7.6 Hz, 2H), 7.34 (t, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 7.6 Hz 1H), 3.79 (m, 2H), 3.01 (d, *J* = 4.4 Hz, 1H), 2.78 (dd, *J* = 4.4, 12.6 Hz, 1H), 2.59 (dd, *J* = 6.6, 13 Hz, 1H), 2.19(m, 1H), 2.06(m, 1H), 1.91(m, 1H), 1.60(b, 2H), 1.03(d, *J* = 6.7Hz, 3H), 0.96(d, *J* = 6.7Hz, 3H), 0.87(s, 9H), 0.03 (d, *J* = 3.2Hz, 6H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 172.4, 138.1, 129.1, 124.1, 119.6, 69.3, 60.6, 52.1, 36.6, 32.6, 31.8, 26.1, 26.0, 19.8, 18.4, 18.0, -5.2, -5.3 ppm. HRMS (ESI+) [M+H]+ calcd. for C21H38N3O2Si 392.2727, found 392.2746.

(*R*)-2-(((*2R,3S*)-3-((*tert*-butyldimethylsilyloxy)methyl)aziridin-2-yl)methylamino)-3-methyl-*N*-phenylbutanamide (5f)



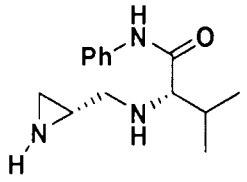
The same procedure was used as that for **5e L** with the exception that **2e** was used instead of **2d**. ^{1}H NMR (CDCl3, 400MHz): δ 7.60 (d, *J* = 7.6 Hz, 2H), 7.30 (t, *J* = 8.0 Hz, 2H), 7.07 (d, *J* = 7.6 Hz 1H), 3.79 (m, 2H), 3.03 (d, *J* = 4.4 Hz, 1H), 2.76 (dd, *J* = 4.3, 12.6 Hz, 1H), 2.50 (dd, *J* = 6.6, 12.6 Hz, 1H), 2.19(m, 1H), 2.06(m, 1H), 1.91(m, 1H), 1.40(b, 2H), 1.01(d, *J* = 6.8 Hz, 3H), 0.89(d, *J* = 6.8 Hz, 3H), 0.80(s, 9H), 0.01(d, *J* = 3.2Hz, 6H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 172.3, 138.0, 129.1, 124.1, 119.6, 68.3, 60.6, 51.2, 37.0, 32.2, 31.7, 26.0, 25.9, 19.9, 18.4, 17.7, -5.2, -5.3 ppm. HRMS (ESI+) [M+H]+ calcd. for C21H38N3O2Si 392.2727, found 392.2735.

(*S*)-2-(((*2R,3S*)-3-((*tert*-butyldimethylsilyloxy)methyl)aziridin-2-yl)methylamino)-4-methyl-*N*-phenylpentanamide (5g)



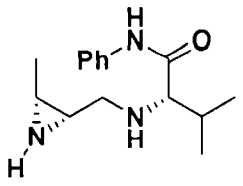
In a flame dried 10 ml round bottom flask equipped with a magnetic stirring bar was placed enantiomerically pure **1b** (38 mg, 0.09 mmol) and **2f** (43.7mg, 0.21 mmol) in 0.8 ml of THF, DCM 0.4ml and 0.8 ml of MeOH. The solution was stirred at room temperature and $ZnCl_2$ (24.1 mg, 0.18 mmol) was added. The mixture was stirred for 1 minute at which point $NaCNBH_3$ (16.7 mg, 0.27 mmol) was added. The reaction was allowed to stir at room temperature overnight at which point ESI MS showed the reaction was complete. The reaction mixture was then filtered through Celite. The filtrate was concentrated under reduced pressure and subjected to silica gel column chromatography (Ethyl acetate/MeOH: 95/5) to yield colorless oil in 84% yield. All operations should be done in the fume hood to avoid evolution of highly toxic HCN. ^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.62 (d, J = 7.9 Hz, 2H), 7.32 (t, J = 7.1 Hz, 2H), 7.09 (t, J = 7.1 Hz, 1H), 3.77(dd, J = 8, 2.8 Hz, 2H), 3.20 (d, J = 4.8 Hz, 1H), 2.78 (d, J = 2.6 Hz, 1H), 2.68-2.58 (m, 2H), 2.11 (m, , 1H), 1.97 (m, , 1H), 1.91 (m, , 3H), 1.67 (m, 1H), 0.94(m, 6H), 0.85(s, 9H), 0.02 (d, J = 6.5 Hz, 6H), ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ: 173.5, 138.4, 129.2, 124.1, 119.6, 62.2, 62.1, 60.5, 51.3, 43.2, 43.1, 36.5, 32.5, 26.1, 25.5, 25.4, 23.4, 22.3, 18.5, -5.3 ppm. HRMS (ESI) $[M+H]^+$ calcd. For C22H40N3O2Si 406.2884, found 406.2903.

(*S*)-2-((*R*)-aziridin-2-ylmethylamino)-3-methyl-*N*-phenylbutanamide (5h)



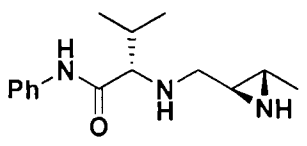
In a flame dried 10 ml round bottom flask equipped with a magnetic stirring bar was placed enantiomerically pure **1d** (24 mg, 0.17 mmol) and **2d** (77mg, 0.4 mmol) in 1.5 ml of THF, 1 ml of DCM and 1.5 ml of MeOH. The solution was stirred at room temperature and $ZnCl_2$ (50 mg, 0.37 mmol) was added. The mixture was stirred for 1 minute at which point $NaCNBH_3$ (35 mg, 0.56 mmol) was added. The reaction was allowed to stir at room temperature overnight at which point ESI MS showed the reaction was complete. The reaction mixture was then filtered through Celite. The filtrate was concentrated under reduced pressure and subjected to silica gel column chromatography (gradient Et2O/MeOH: 90/0-80/20) to yield colorless oil in 51% yield. All operations should be done in the fume hood to avoid evolution of highly toxic HCN. ^{1}H NMR (CDCl3 / Methanol-d4, 97/3 , 400MHz) δ: 7.60-7.09 (m, 5H), 3.33(d, J = 6.4 Hz, 2H), 2.78 (m, 1H), 2.69 (m, 1H), 2.41 (d, J = 5.6 Hz, 1H),2.23 (m, , 1H), 2.03 (d, J = 3.8 Hz, 1H), 1.22 (d, J = 5.6 Hz , 3H), 1.00 (d, J = 5.6 Hz , 3H) ppm. ^{13}C NMR (Methanol-d4, 100MHz) δ: 174.3, 137.0, 128.9, 125.2, 120.4, 66.3, 49.2, 32.9, 29.5, 18.4, 16.3 ppm. HRMS (ESI) $[M+H]^+$ calcd. For C14H22N3O 248.1759, found 248.1761.

(*S*)-3-methyl-2-(((2*S*,3*R*)-3-methylaziridin-2-yl)methylamino)-*N*-phenylbutanamide (5i)



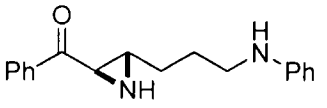
In a flame dried 10 ml round bottom flask equipped with a magnetic stirring bar was placed enantiomerically pure **1c** (29 mg, 0.15 mmol) and **2d** (72mg, 0.37 mmol) in 1 ml of THF 1 ml of MeOH. The solution was stirred at room temperature and $ZnCl_2$ (47 mg, 0.3 mmol) was added. The mixture was stirred for 1 minute at which point $NaCNBH_3$ (32 mg, 0.45 mmol) was added. The reaction was allowed to stir at room temperature overnight at which point ESI MS showed the reaction was complete. The reaction mixture was then filtered through Celite. The filtrate was concentrated under reduced pressure and subjected to silica gel column chromatography (gradient Ethyl acetate/MeOH: 95/5-90/10) to yield colorless oil in 60% yield. All operations should be done in the fume hood to avoid evolution of highly toxic HCN. ^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.62 (dd, J = 1.1, 7.9 Hz, 2H), 7.32 (t, J = 7.1 Hz, 2H), 7.09 (t, J = 7.1 Hz, 1H), 3.10 (d, J = 4.0 Hz, 1H), 2.70 (m, 2H), 2.22-2.19 (m, , 3H), 1.27 (b, 2H), 1.26 (d, J = 4.5 Hz, 2H), 1.17(d, J = 5.6 Hz, 3H), 1.07(d, J = 7.2 Hz, 3H), 0.94(d, J = 7.2 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ: 172.3, 138.0, 129.2, 124.2, 119.7, 68.6, 68.5, 48.4, 34.0, 31.7, 29.9, 20.0, 17.7, 14.1 ppm. HRMS (EI) $[M]^+$ calcd. For C15H23N3O 261.1844, found 261.1844.

(S)-3-methyl-2-(((2S,3S)-3-methylaziridin-2-yl)methylamino)-N-phenylbutanamide (5j)



In a flame dried 10 ml round bottom flask equipped with a magnetic stirring bar was placed enantiomerically pure dimer (29 mg, 0.15 mmol) and NH2-Val-NHPh (72mg, 0.37 mmol) in 1 ml of THF 1 ml of MeOH. The solution was stirred at room temperature and $ZnCl_2$ (47 mg, 0.3 mmol) was added. The mixture was stirred for 1 minute at which point $NaCNBH_3$ (32 mg, 0.45 mmol) was added. The reaction was allowed to stir at room temperature overnight at which point ESI MS showed the reaction was complete. The reaction mixture was then filtered through Celite. The filtrate was concentrated under reduced pressure and subjected to silica gel column chromatography (gradient Ethyl acetate/MeOH: 95/5 to 90/10) to yield colorless oil in 60% yield. All operations should be done in the fume hood to avoid evolution of highly toxic HCN. ^{1}H NMR (CDCl3 / Methanol-d4, 97/3 , 400MHz) δ: 7.52 (dd, J = 1.1, 7.9 Hz, 2H), 7.37 (t, J = 7.1 Hz, 2H), 7.21 (t, J = 7.1 Hz, 1H), 3.70 (b, 3H), 3.50-3.41 (m, 4H), 2.82 (m, 1H), 2.48 (m, 2H), 2.18 (m, 2H), 1.40(d, J = 5.6 Hz, 3H), 1.10-1.05(m, 6H) ppm. ^{13}C NMR (CDCl3 / Methanol-d4, 97/3 , 100MHz) δ: 174.8, 136.1, 129.3, 126.3, 121.1, 67.5, 46.6, 37.5, 32.3, 28.9, 19.5, 17.0 ppm. HRMS (EI) $[M]^+$ calcd. For C15H23N3O 261.1844, found 261.1844.

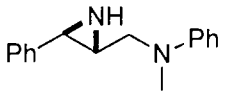
phenyl((2R,3S)-3-(3-(phenylamino)propyl)aziridin-2-yl)methanone (5k)



To a mixture of (2-hydroxy-1-azabicyclo[3.1.0]hex-6-yl) phenylmethanone (35mg, 0.18mmol) and aniline (20mL, 0.22mL) in a round bottom flask equipped with a magnetic stirring bar was added MeOH (1.5 ml). Then 17mg of $NaCNBH_3$ was added. The mixture was stirred at room temperature overnight and

quenched by sat. $NaHCO_3$. The aqueous layer was washed with ethyl acetate (X 3). The combined organic portions were dried over sodium sulfate and concentrated under reduced pressure. The crude mixture was subjected to silica gel column chromatography (ethyl acetate, 100%) to yield a pale yellow oil (yield 95%). ^1H NMR (CDCl3, 400MHz): δ 8.01-8.00 ppm (m, 2H), 7.64-7.48 ppm (m, 3H), 7.19-7.14 ppm (m, 2H), 6.72-6.58 (m, 3H), 3.72 (b, 1H), 3.29 (b, 1H), 3.20 (m, 2H) 2.19-2.15(m, 2H), 1.87-1.61(m, 4H)ppm; ^{13}C NMR (CDCl3, 100MHz): δ 197.2, 148.4, 136.3, 134.0, 129.5, 129.1, 128.4, 117.6, 113.0, 43.6, 42.9, 39.9, 31.0, 27.3 ppm. HRMS (ESI) [M+H]+ calcd. for C18H21N2O 281.1648, found 281.1640.

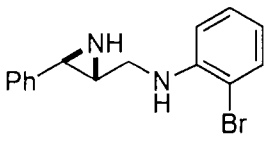
N-methyl-N-(((2R,3R)-3-phenylaziridin-2-yl)methyl)aniline (5m)



To a mixture of 6-Phenyl-2-(3-phenylaziridin-2-yl)-3-oxa-1-azabicyclo[3.1.0.]hexan-4-ol (31mg, 0.11mmol) and *N*-methylanaline (0.027mL, 0.26mmol) in a round bottom flask equipped with a magnetic stirring bar was added MeOH (2 ml). Then 0.25mL of $NaCNBH_3$ (1M in THF) was added. The mixture was stirred at room temperature for 3 days and quenched by sat. $NaHCO_3$. The aqueous layer was washed with ethyl acetate (X 3). The combined organic portions were dried over sodium sulfate and concentrated under reduced pressure. The crude mixture was subjected to silica gel column chromatography (DCM/MeOH, 95/5) to yield a pale yellow oil (yield 82%). ^1H NMR (CDCl3, 400MHz): δ 7.4-7.1 (m, 7H), 6.9-6.7 (m, 3H), 3.6 (d, 2H), 3.1(s, 3H), 2.8 (d, 1H), 2.4(m, 1H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 145.4, 139.9, 129.5, 128.8, 127.4, 125.8, 117.3, 112.9, 55.8, 47.8, 39.6 ppm. HRMS (ESI)

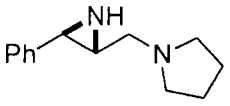
[M+H]+ calcd. for C16H16N 222.1277, found 222.1269.

2-bromo-N-(((2R,3R)-3-phenylaziridin-2-yl)methyl)aniline (5o)



To a mixture of 6-Phenyl-2-(3-phenylaziridin-2-yl)-3-oxa-1-azabicyclo[3.1.0.]hexan-4-ol (57mg, 0.19mmol) and *O*-Bromoanaline (0.80mg, 0.47mmol) in a round bottom flask equipped with a magnetic stirring bar was added MeOH (4 ml). Then 0.42mL of $NaCNBH_3$ (1M in THF) was added. The mixture was stirred at room temperature for 3 days and quenched by sat. $NaHCO_3$. The aqueous layer was washed with ethyl acetate (X 3). The combined organic portions were dried over sodium sulfate and concentrated under reduced pressure. The crude mixture was subjected to silica gel column chromatography (DCM/MeOH, 95/5) to yield a pale yellow oil (yield 79%). ^{1}H NMR (CDCl3, 400MHz): δ 7.4-7.1 (m, 7H), 6.9-6.7 (m, 3H), 4.8 (b, 1H), 3.6 (m, 1H), 3.3(m, 1H), 2.9 (d, 1H), 2.5(m, 1H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 145.2, 139.6, 132.8, 128.9, 128.8, 127.6, 125.8, 118.6, 111.8, 110.2, 46.4 ppm. HRMS (ESI) [M+H]+ calcd. for C15H16N2Br 303.0491, found 303.0492.

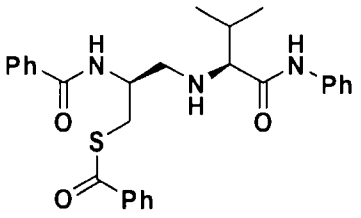
1-(((2R,3R)-3-phenylaziridin-2-yl)methyl)pyrrolidine (5p)



6-Phenyl-2-(3-phenylaziridin-2-yl)-3-oxa-1-azabicyclo[3.1.0.]hexan-4-ol (62mg, 0.21mmol), pyrridine (0.042mL, 0.5mmol) and zinc chloride (69mg, 0.5mmol) were added in the solution of MeOH (0.5mL) and DCM (0.5mL). Then NaCNBH3 (40mg, 0.63mmol) was added into the solution. The mixture was stirred at room

temperature for 2 hours. NaOH solution was added to the mixture and stirred for 10min. The mixture was filtered through celite and concentrated very carefully. The residue was purified by column chromatograph (DCM/MeOH, 90/10, with 2%TEA) (yield 67%). ^{1}H NMR (CDCl3, 400MHz): δ 7.32-7.19 ppm (m, 5H), 2.75-2.70(m, 2H), 2.60(m, 5H), 2.31(b, 1H), 1.84-1.75(m, 4H), 1.50(b, 1H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 140.1, 128.7, 127.3, 125.9, 60.3, 54.8, 40.8, 38.9, 23.7 ppm. HRMS (EI+) [M]+ calcd. for C13H17N2 201.1392, found 201.1396.

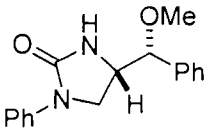
_S_-(_S_)-2-benzamido-3-((_S_)-3-methyl-1-oxo-1-(phenylamino)butan-2-ylamino)propyl benzothioate (6)



In a flame dried 10 ml round bottom flask equipped with a magnetic stirring bar was placed **5i** (16 mg, 0.07 mmol) in 15 ml of MeOH and 0.5 ml of DCM. The solution was cooled down to 0 °C at which point thiobenzoic acid (94%, 21mg, 0.14mmol) was added. The mixture was stirred at 0 °C for 6 hours then brought to room temperature and stirred for 18 hours. The reaction mixture was concentrated under reduced pressure and subjected to silica gel column chromatography (40% ethyl acetate in hexanes) to yield a white solid in 82% yield. ^{1}H NMR ($CDCl_3$, 400MHz) δ: 8.09-7.03 (m, 15H), 4.59 (m, 1H), 3.51(m, 1H), 3.35 (d, J = 4.3 Hz, 1H), 3.10 (d, J = 8.3 Hz, 2H), 2.91 (m, , 1H), 2.21 (m, 1H), 1.04 (d, J = 6.4 Hz , 3H), 0.95 (d, J = 6.4 Hz , 3H) ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ: 193.2, 172.1, 168.1, 137.6, 136.5, 134.1, 133.9, 132.0, 130.3, 129.1, 128.9, 128.7, 127.6, 124.4, 119.8, 69.3, 52.8, 50.7, 31.9, 31.4, 19.8, 18.3 ppm.

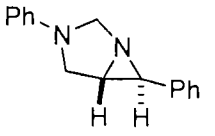
HRMS (ESI) $[M+H]^+$ calcd. For C28H32N3O3S 490.2158, found 490.2179.

(S)-4-((R)-methoxy(phenyl)methyl)-1-phenylimidazolidin-2-one (11)



4-[methoxy(phenyl)methyl]-1-phenylimidazolidin-2-one (10mg, 0.035mmol) was dissolved in MeOH (1.5mL) and DCM (1mL). The mixture was stirred at 0 degree for 30min. 0.015mL of HBF_4 (32%) was added. The reaction mixture was stirred for 1 hour followed by filtration through celite. The filtrate was concentrated and the residue was purified by silica column (hexanes/ethyl acetate, 60/40) to obtain the product (99%). ^1H NMR (CDCl3, 400MHz): δ 7.56-7.54 (m, 2H), 7.43-7.32 (m, 7H), 7.06 (m, 1H), 5.30 (b, 1H), 4.08 (m, 2H), 3.94 (m, 1H), 3.85(m, 1H), 3.25 (s, 3H) ppm; ^{13}C NMR (CDCl3, 100MHz): δ 158.3, 140.1, 137.7, 129.2, 129.1, 129.0, 127.8, 122.9, 117.9, 105.0, 86.1, 57.1, 53.9, 49.1 ppm. HRMS (ESI) [M+H]+ calcd. for C17H19N2O2 283.1441, found 283.1432.

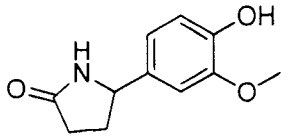
(5S,6S)-3,6-diphenyl-1,3-diazabicyclo[3.1.0]hexane (12)



trans-Phenyl-(3-phenylaziridin-2-ylmethyl)-amine (10mg, 0.05mmol) was dissolved in MeOH (0.5mL), DCM (0.5mL), formaldehyde (38% in MeOH, 0.5mL). The mixture was stirred overnight. When the reaction was complete confirmed by TLC, 2mL DCM and 2mL water were added to the reaction mixture. The aqueous layer was extracted 3 times with DCM. The combined organic layer was concentrated and the residue was purified by silica column (hexanes/ethyl acetate, 80/20) to obtain the product

(yield 91%). ^{1}H NMR (CDCl3, 400MHz): δ 7.36-7.22 (m, 8H), 6.82-6.58 (m, 2H), 4.62 (d, 1H), 4.17 (d, 1H), 4.08 (m, 2H), 3.97 (d, 1H), 3.32(m, 1H), 2.83 (d, 2H) ppm.

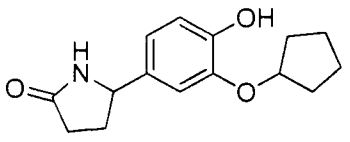
5-(4-hydroxy-3-methoxyphenyl)pyrrolidin-2-one



398mg of 5-Methoxy-2-pyrrolidinone and 433mg of guaiacol was dissolved in 17mL DCM under nitrogen protection. The reaction mixture was stirred at -78°C for 30min. Then 0.52mL of BF_3OEt_2 was added into the reaction mixture in drop wise. The reaction mixture was allowed to warm up to room temperature and stirred overnight. 0.52 mL of water was added to the reaction mixture to quench the reaction. After 20min of stirring, $MgSO_4$ was added to absorb excess water. The mixture was filtrated through celite and concentrated. The crude product was purified through silica column with DCM/MeOH 95/5. 387mg of 5-(4-hydroxy-3-methoxyphenyl)pyrrolidin-2-one was obtained (yield 54%). ^{1}H NMR (400MHz, $CDCl_3$): & 6.89-6.76 (m, 3H), 6.19 (s, 1H), 5.92 (s, 1H), 4.68 (t, 1H), 3.88 (s, 3H), 2.54-2.41 (m, 3H), 1.98-1.93 (m, 1H)ppm.^{13}C NMR (100MHz, $CDCl_3$): & 178.6, 147.2, 145.7, 134.4, 118.9, 114.8, 108.1, 58.3, 56.2, 31.9, 30.7ppm.

Mass: 207.089141

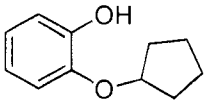
5-(3-(cyclopentyloxy)-4-hydroxyphenyl)pyrrolidin-2-one



255mg of 5-Methoxy-2-pyrrolidinone and 433mg of 2-(cyclopentyloxy)phenol was dissolved in 20mL DCM under nitrogen protection. The

reaction mixture was stirred at -78°C for 30min. Then 0.30mL of BF_3OEt_2 was added into the reaction mixture drop wise. The reaction mixture was allowed to warm up to room temperature and stirred overnight. 0.35 mL of water was added to the reaction mixture to quench the reaction. After 20min of stirring, $MgSO_4$ was added to absorb excess water. The mixture was filtrated through celite and concentrated. The crude product was purified through silica column with DCM/MeOH 95/5. 197mg of 5-[3-(cyclopentyloxy)-4-hydroxyphenyl]pyrrolidin-2-one was obtained (yield 34%). ^1H NMR (400MHz, $CDCl_3$): & 6.9-6.7 (m, 3H), 6.1 (s, 1H), 5.8 (s, 1H), 4.8 (m, 1H), 4.7 (t, 1H), 2.5-2.4 (m, 3H), 1.9-1.6 (m, 9H)ppm.[13] CNMR (100MHz, $CDCl_3$): & 178.6, 147.2, 145.7, 134.4, 118.9, 114.8, 108.1, 58.3, 56.2, 31.9, 30.7ppm. Mass: 261.1435.

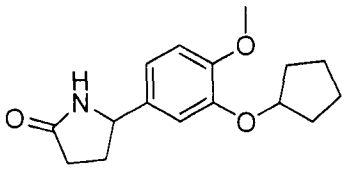
2-(cyclopentyloxy)phenol



To a mixture of catechol (1.10g, 10 mmol) in 35 ml of DMSO was added *t*-BuOK (1.12g, 10 mmol). The mixture was allowed to stir for three hours at room temperature. Bromocyclopentane (1.00 ml, 10 mmol) was then added to the mixture in drop wise. The reaction was then allowed to stir for one hour. The mixture was then quenched with 20ml of cold water, and was twice extracted with DCM. The combined organic layers were then washed three times with brine and further extracted with Claisen's Alkali (6g KOH in 5ml H_2O, diluted with 25 ml of MeOH). Only the alkali layer was kept and neutralized with HCl solution. This aqueous layer was extracted twice with DCM. The organic layer was then dried over magnesium sulfate, filtered, then concentrated and purified by silica gel column (Hexanes/Ethyl Acetate 80/20) to afford a pale yellow oil 930mg (51% yield).

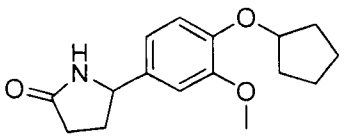
^{1}H NMR (200 MHz. $CDCl_3$) δ: 6.9-6.8 (m, 4H), 5.7 (s, 1H), 4.80-4.77 (m, 1H), 1.91-1.75 (m, 6H), 1.64-1.60 (m, 2H) ppm. ^{13}C NMR (75 MHz. $CDCl_3$) δ: 146.6, 145.2, 121.3, 120.2, 114.7, 113.1, 80.8, 33.2, 24.2 ppm.

5-(3-(cyclopentyloxy)-4-methoxyphenyl)pyrrolidin-2-one (14a)



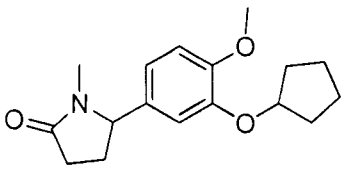
To a mixture of 5-[3-(cyclopentyloxy)-4-hydroxyphenyl]pyrrolidin-2-one (15mg) in 0.5 ml of DMSO was added *t*-BuOK (9mg). The mixture was allowed to stir for three hours at room temperature. Iodomethane (20mg) was then added to the mixture. The reaction was then allowed to stir for one hour. The mixture was then quenched with 5ml of cold water, and was six times extracted with DCM. The combined organic layers were then washed three times with brine and further extracted with Claisen's Alkali (6g KOH in 5ml H_2O, diluted with 25 ml of MeOH). The organic layer was then dried over magnesium sulfate, filtered, then concentrated and purified by silica gel column (DCM/MeOH 95/5) to afford a pale yellow oil 13.2mg (81% yield). ^{1}H NMR (200 MHz. $CDCl_3$) δ: 6.85-6.79 (m, 3H), 6.40 (s, 1H), 4.80-4.77 (m, 1H), 4.77-4.65 (t, 1H), 3.84 (s, 3H), 2.46-2.41 (m, 3H), 1.93-1.81 (m, 8H), 1.61 (m, 1H) ppm. ^{13}C NMR (75 MHz. $CDCl_3$) δ: 178.6, 150.0, 148.3, 135.0, 118.1, 112.4, 112.2, 80.7, 58.2, 56.4, 33.0, 31.9, 30.7, 24.3ppm. Mass: 275.1585

5-(4-(cyclopentyloxy)-3-methoxyphenyl)pyrrolidin-2-one (14b)



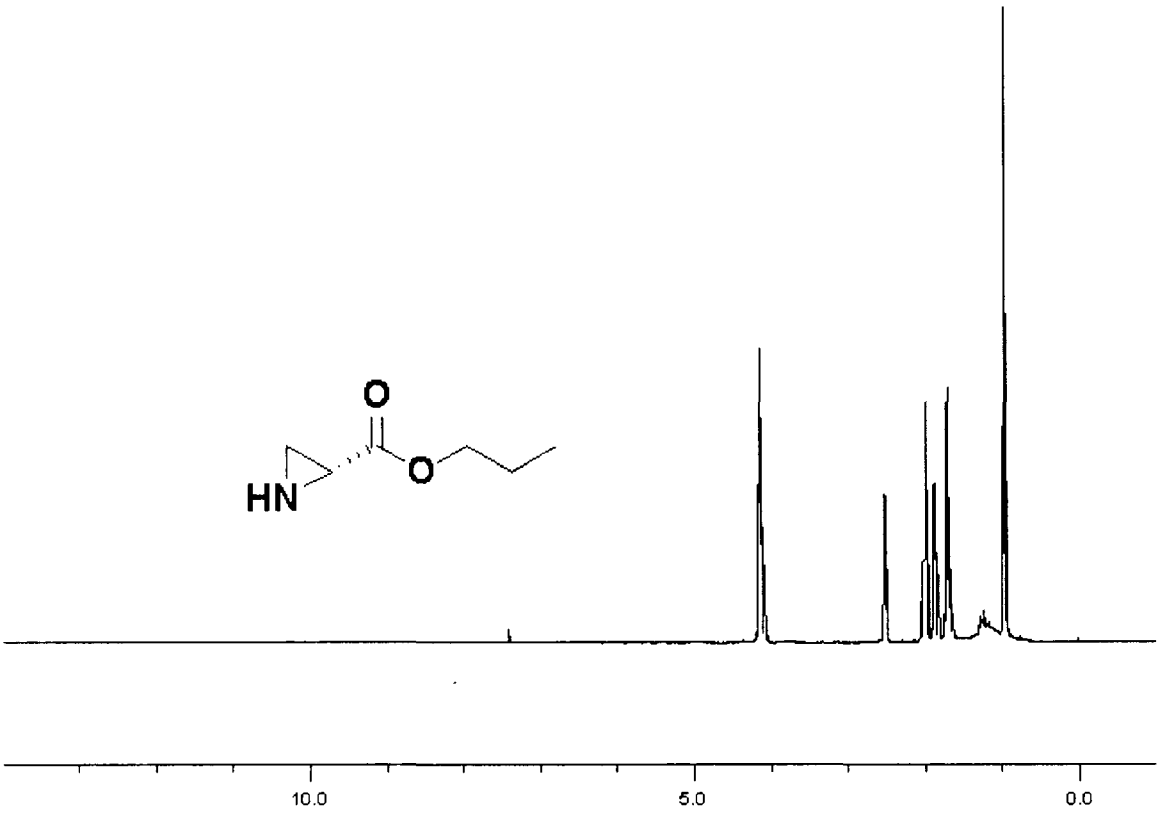
To a mixture of 5-(4-hydroxy-3-methoxyphenyl)pyrrolidin-2-one (100mg, 0.48 mmol) in 1.5 ml of DMSO was added *t*-BuOK (57mg, 0.57 mmol). The mixture was allowed to stir for three hours at room temperature. Bromocyclopentane (0.051 ml, 0.57 mmol) was then added to the mixture, dropwise. The reaction was then allowed to stir for one hour. The mixture was then quenched with 5ml of cold water, and was twice extracted with DCM. The combined organic layers were then washed three times with brine and further extracted with Claisen's Alkali (6g KOH in 5ml H_2O, diluted with 25 ml of MeOH). The organic layer was then dried over magnesium sulfate, filtered, then concentrated and purified by silica gel column (DCM/MeOH 95/5) to afford a pale yellow oil 67mg (51% yield). ^{1}H NMR (200 MHz. $CDCl_3$) δ: 6.85-6.79 (m, 3H), 6.40 (s, 1H), 4.80-4.77 (m, 1H), 4.77-4.65 (t, 1H), 3.84 (s, 3H), 2.46-2.41 (m, 3H), 1.93-1.81 (m, 8H), 1.61 (m, 1H) ppm. ^{13}C NMR (75 MHz. $CDCl_3$) δ: 178.7, 150.6, 147.6, 135.0, 118.1, 115.1, 109.6, 80.7, 58.2, 56.3, 33.0, 31.7, 30.7, 24.3ppm. Mass: 275.1584

5-(3-(cyclopentyloxy)-4-methoxyphenyl)-1-methylpyrrolidin-2-one (14c)



To a mixture of 5-[3-(cyclopentyloxy)-4-hydroxyphenyl]pyrrolidin-2-one (30mg) in 1.0 ml of DMSO was added *t*-BuOK (36mg). The mixture was allowed to stir for three hours at room temperature. Iodomethane (60mg) was then added to the mixture. The reaction

was then allowed to stir for one hour. The mixture was then quenched with 5ml of cold water, and was twice extracted with DCM. The combined organic layers were then washed three times with brine and further extracted with Claisen's Alkali (6g KOH in 5ml H_2O, diluted with 25 ml of MeOH). The organic layer was then dried over magnesium sulfate, filtered, then concentrated and purified by silica gel column (DCM/MeOH 95/5) to afford a pale yellow oil 15mg (50% yield). ^{1}H NMR (200 MHz. $CDCl_3$) δ: 6.85-6.79 (m, 3H), 6.40 (s, 1H), 4.80-4.77 (m, 1H), 4.77-4.65 (t, 1H), 3.84 (s, 3H), 2.66 (s, 3H), 2.46-2.41 (m, 3H), 1.93-1.81 (m, 8H), 1.61 (m, 1H) ppm. ^{13}C NMR (75 MHz. $CDCl_3$) δ: 175.7, 150.1, 148.4, 133.6, 119.1, 112.9, 112.3, 80.7, 64.7, 56.3, 33.0, 30.5, 28.9, 24.2ppm. Mass: 289.1746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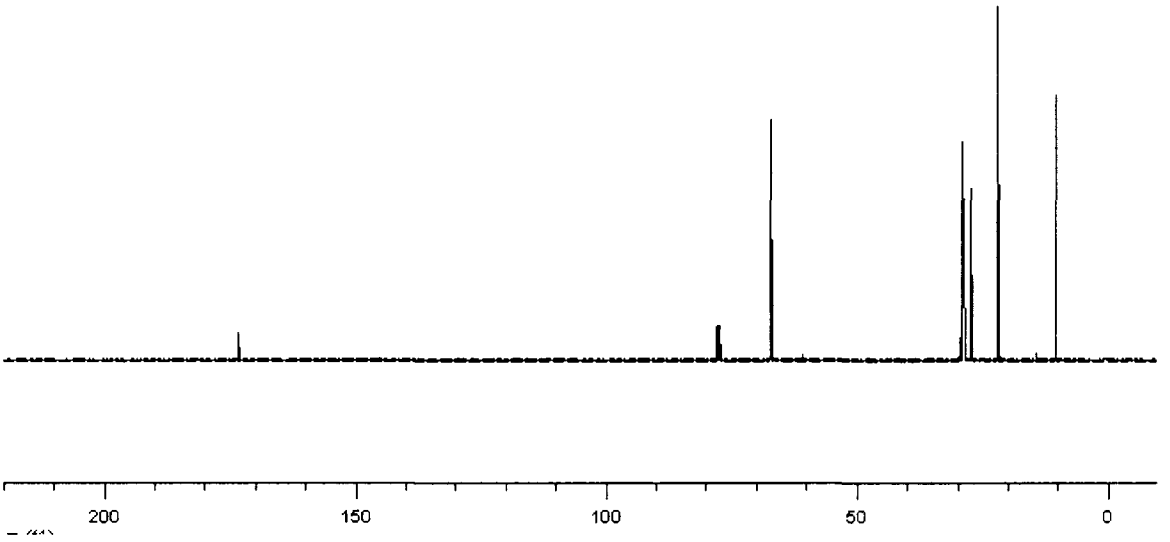
0.0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100
50
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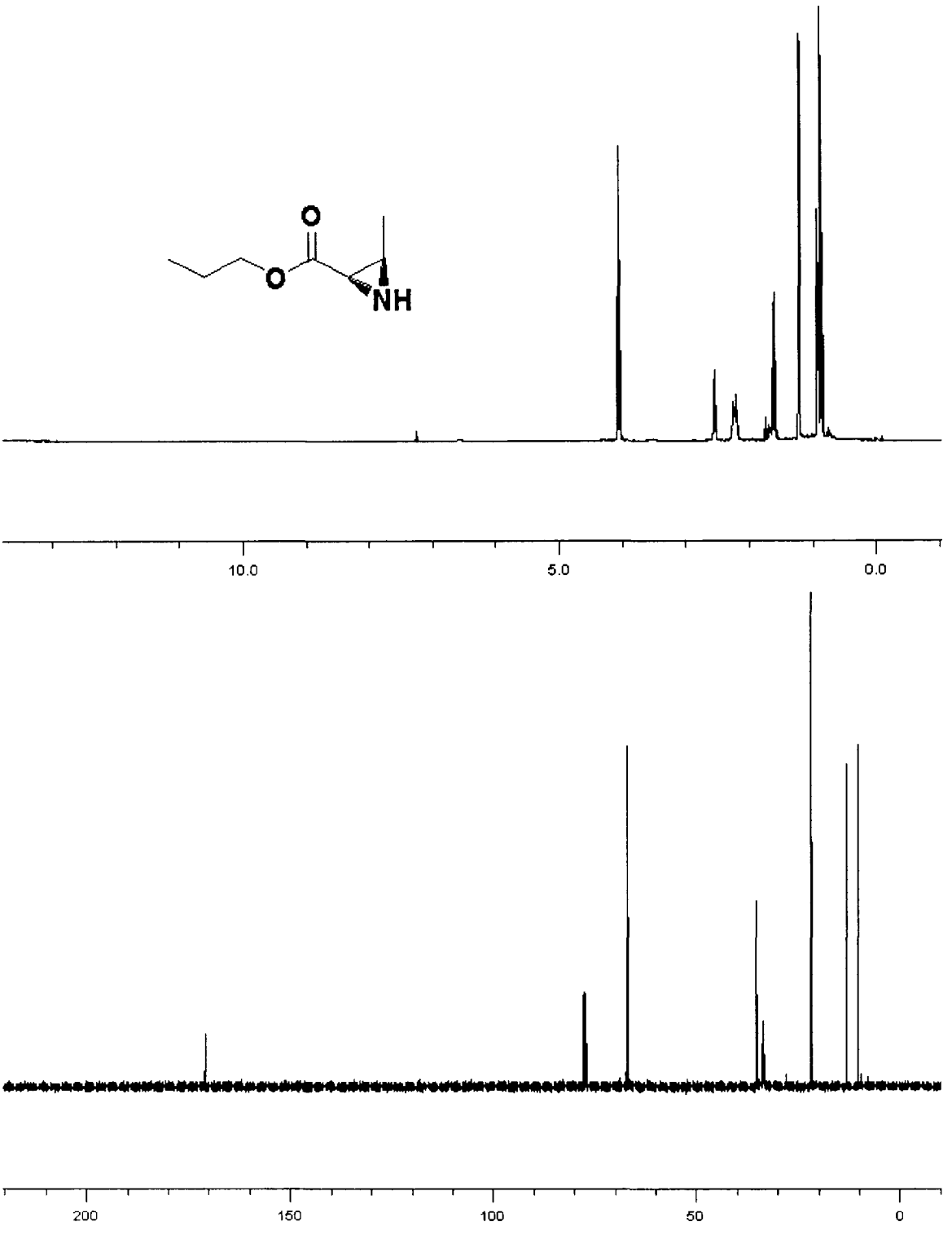
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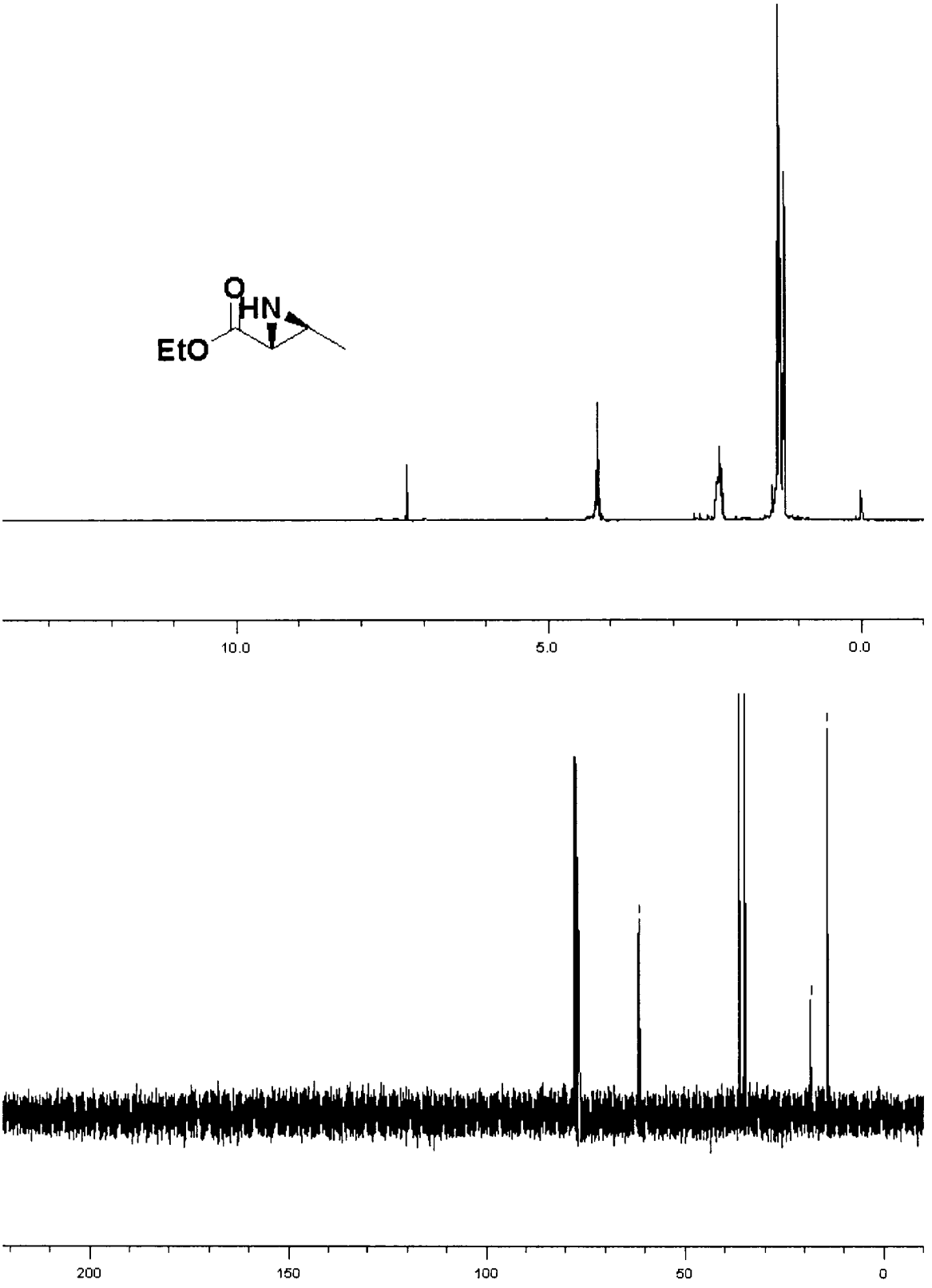
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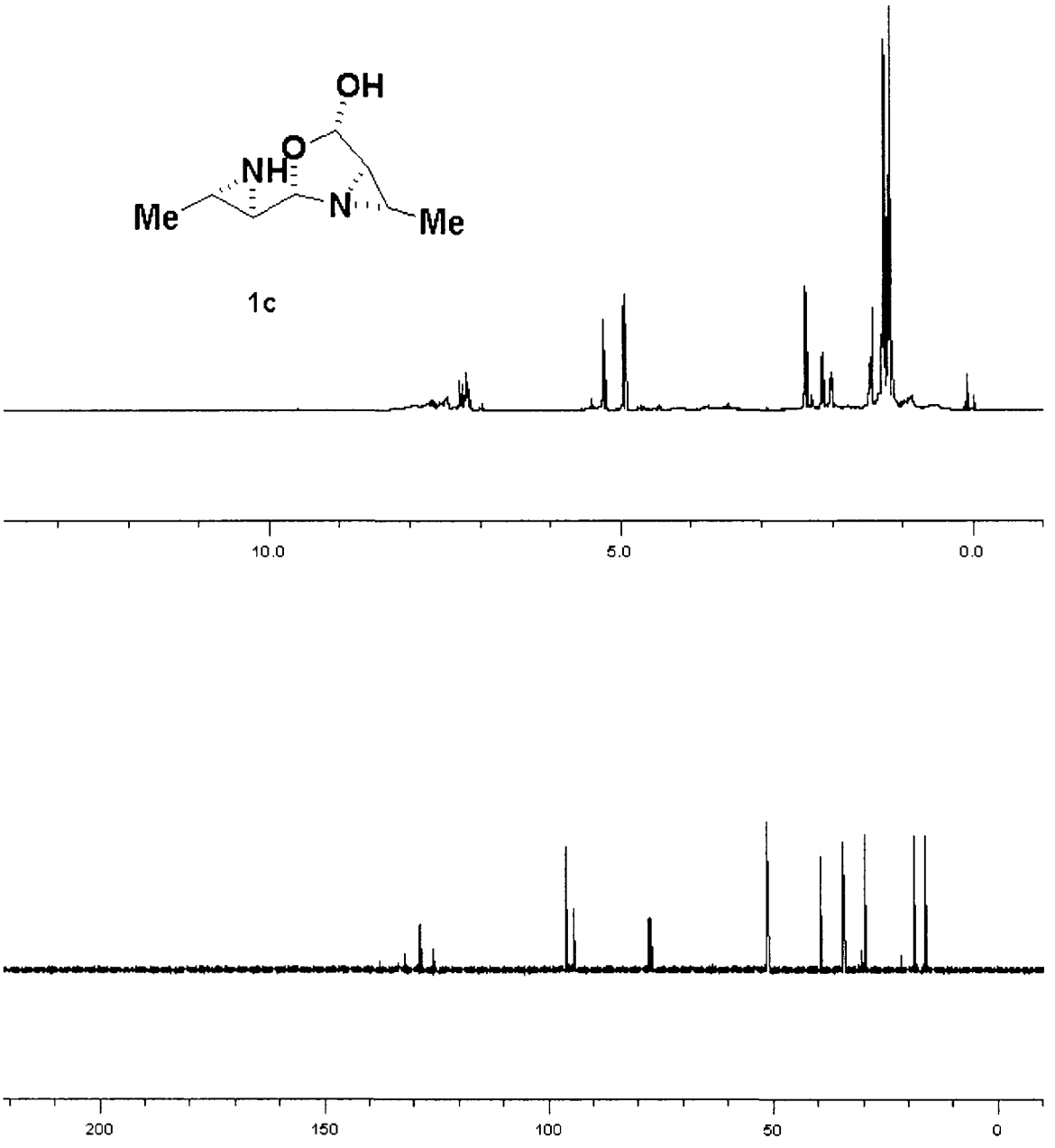
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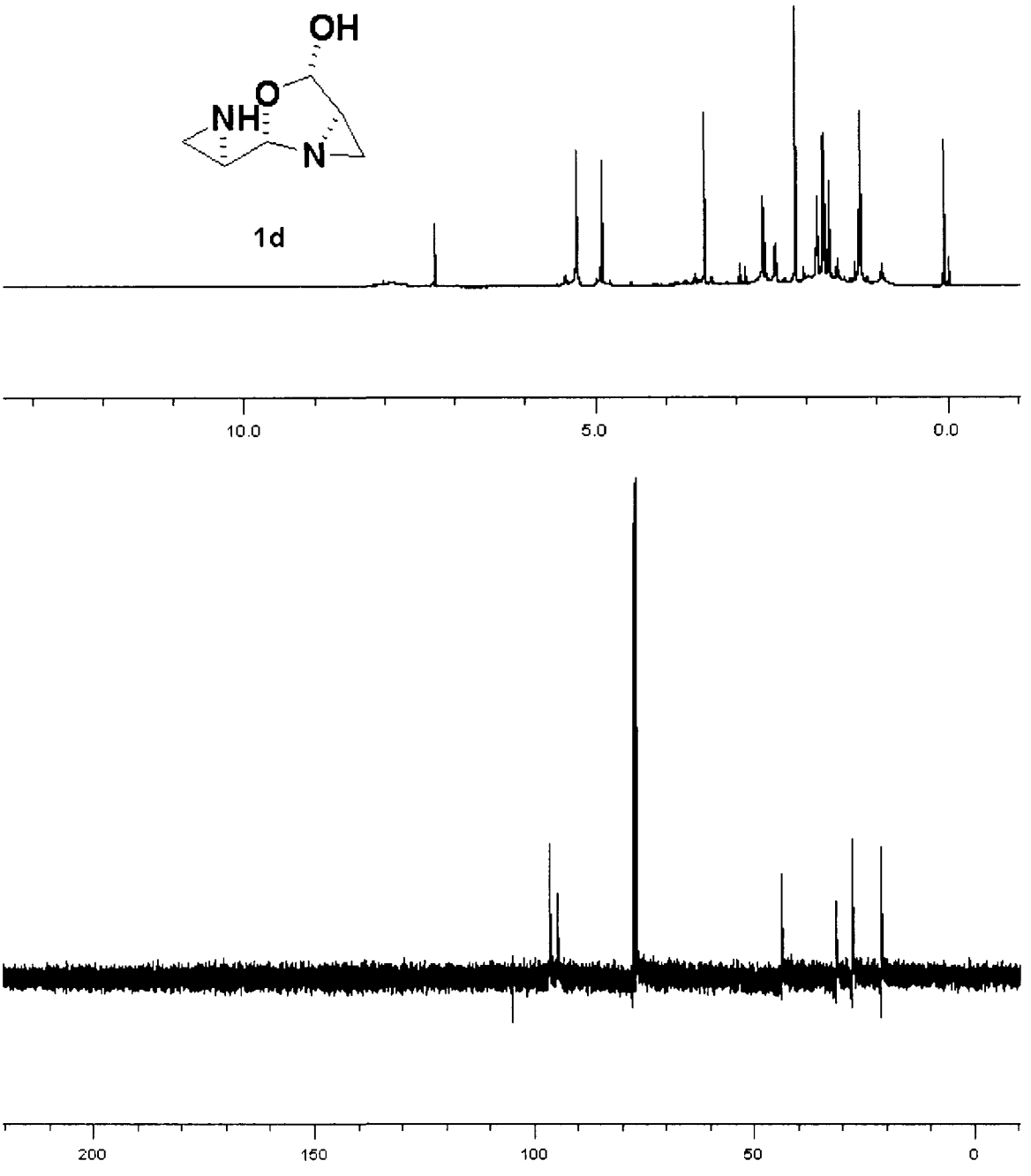
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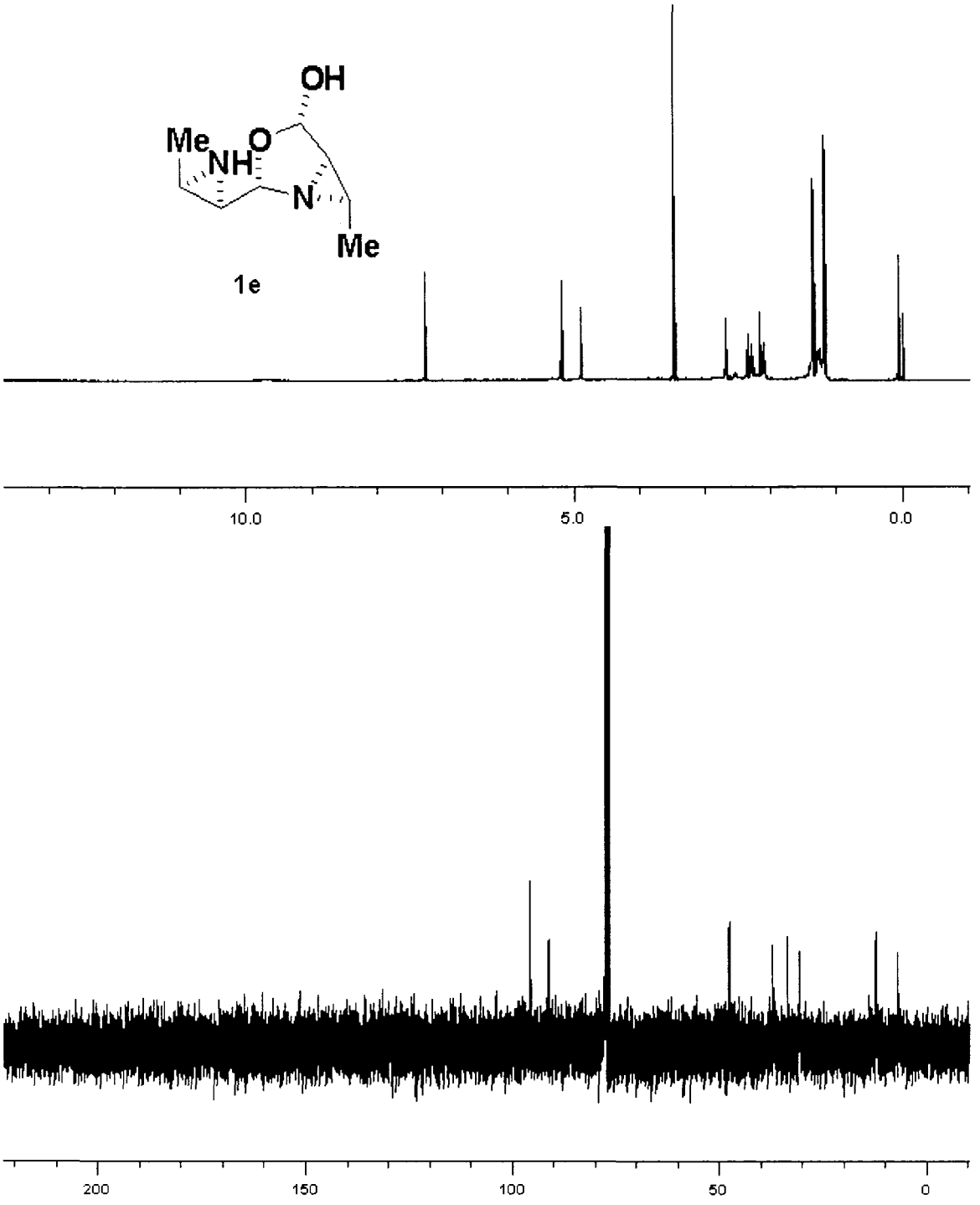
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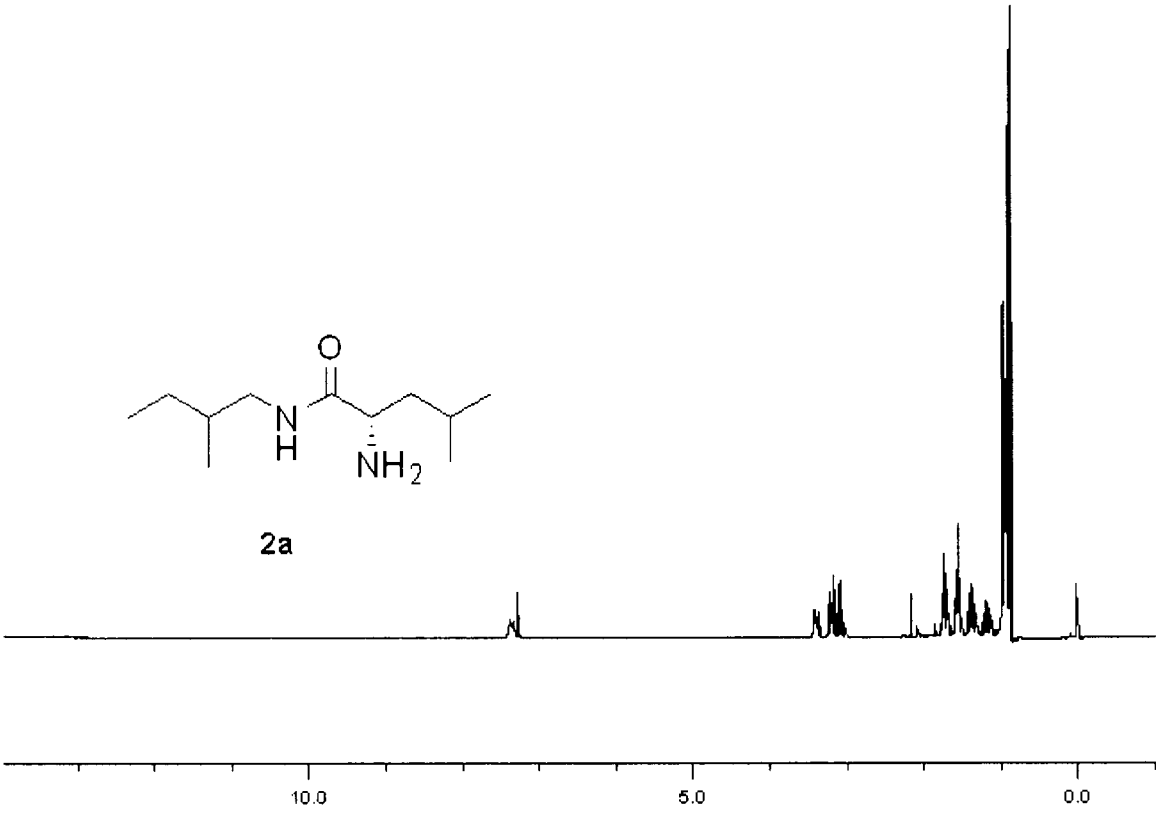
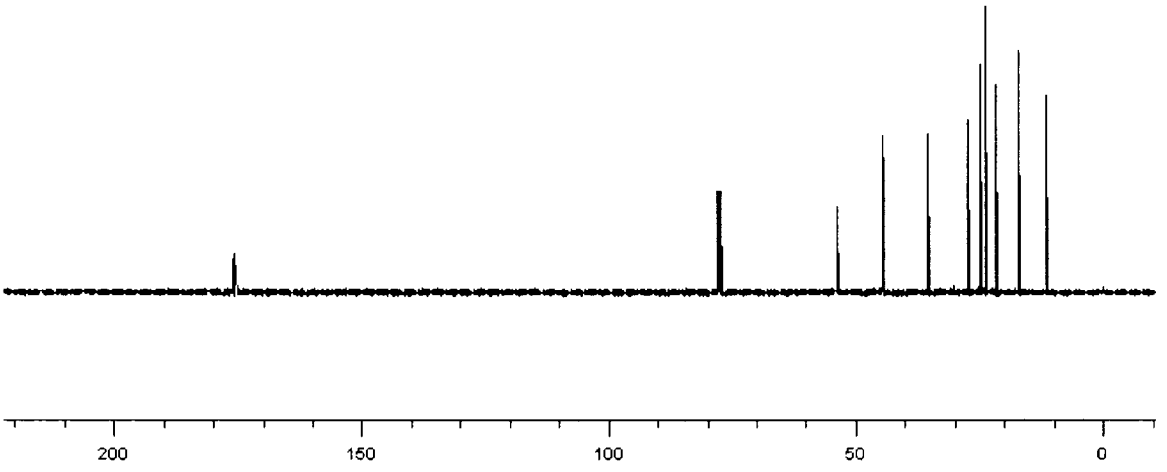
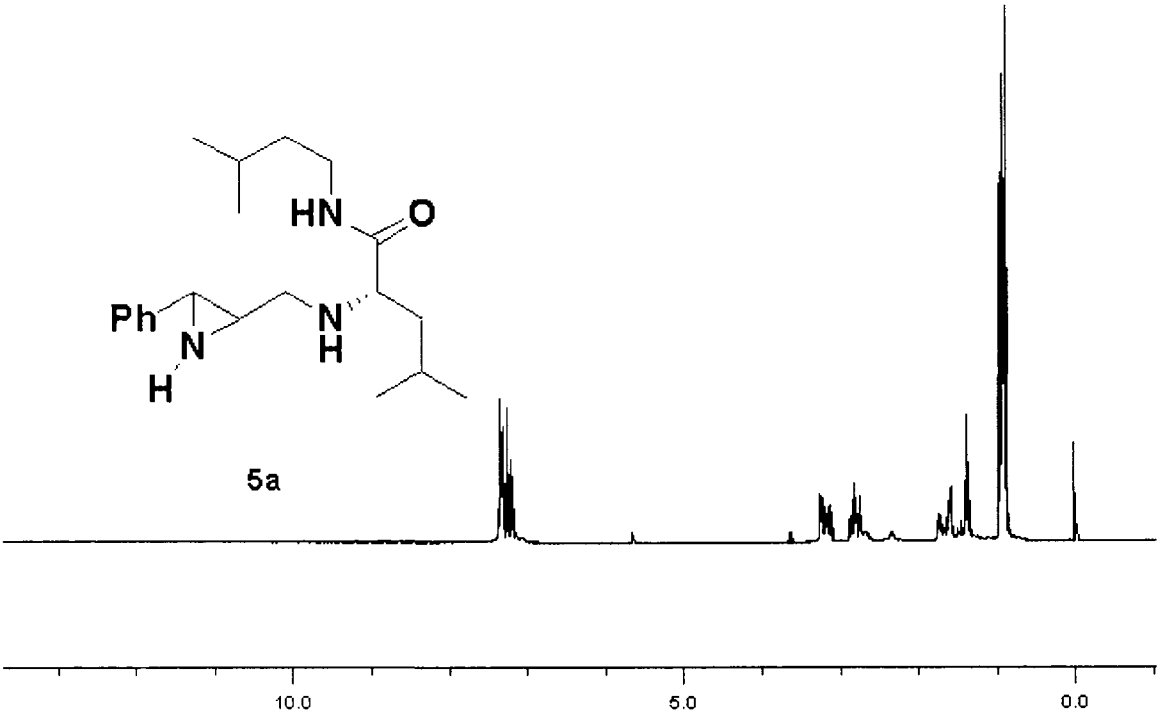


2a




HN
O
Ph
N
H
N
H
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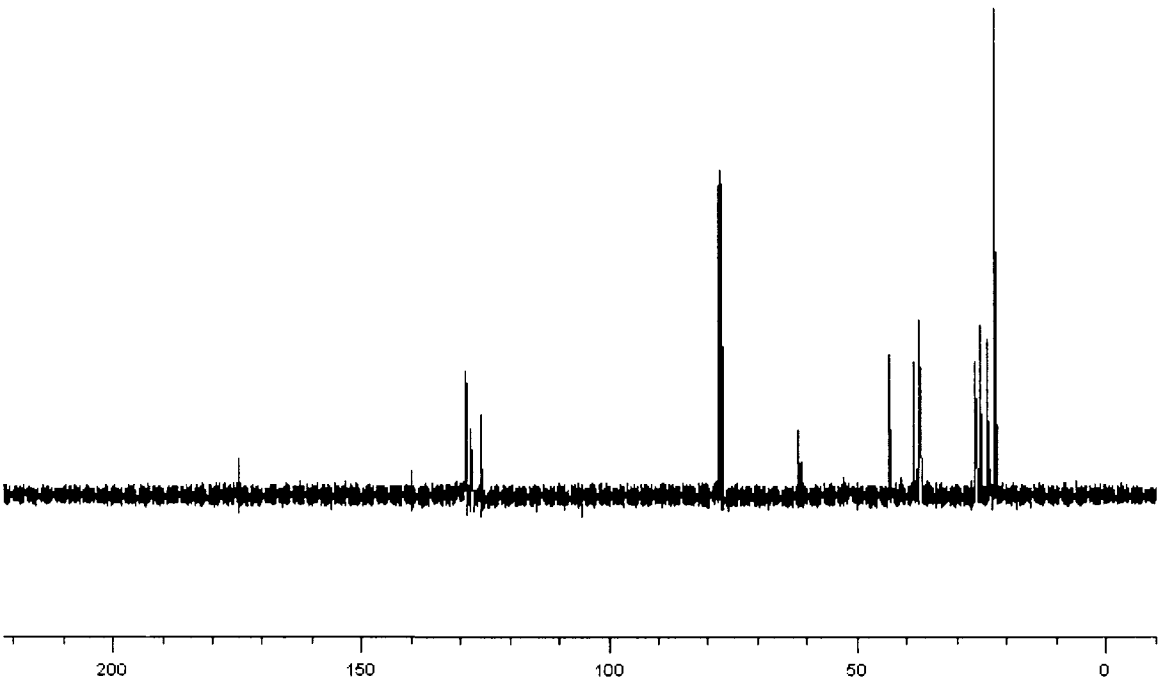
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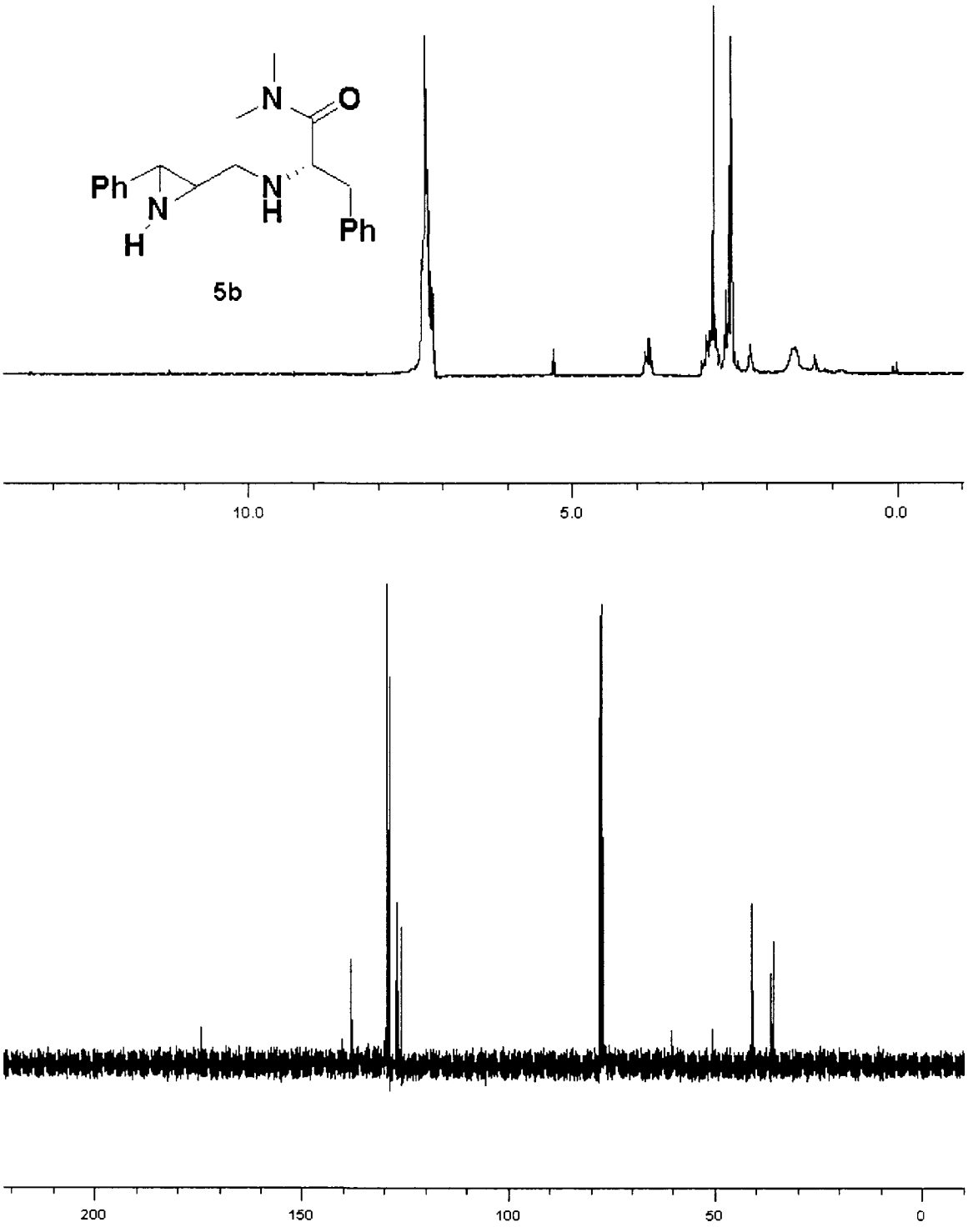
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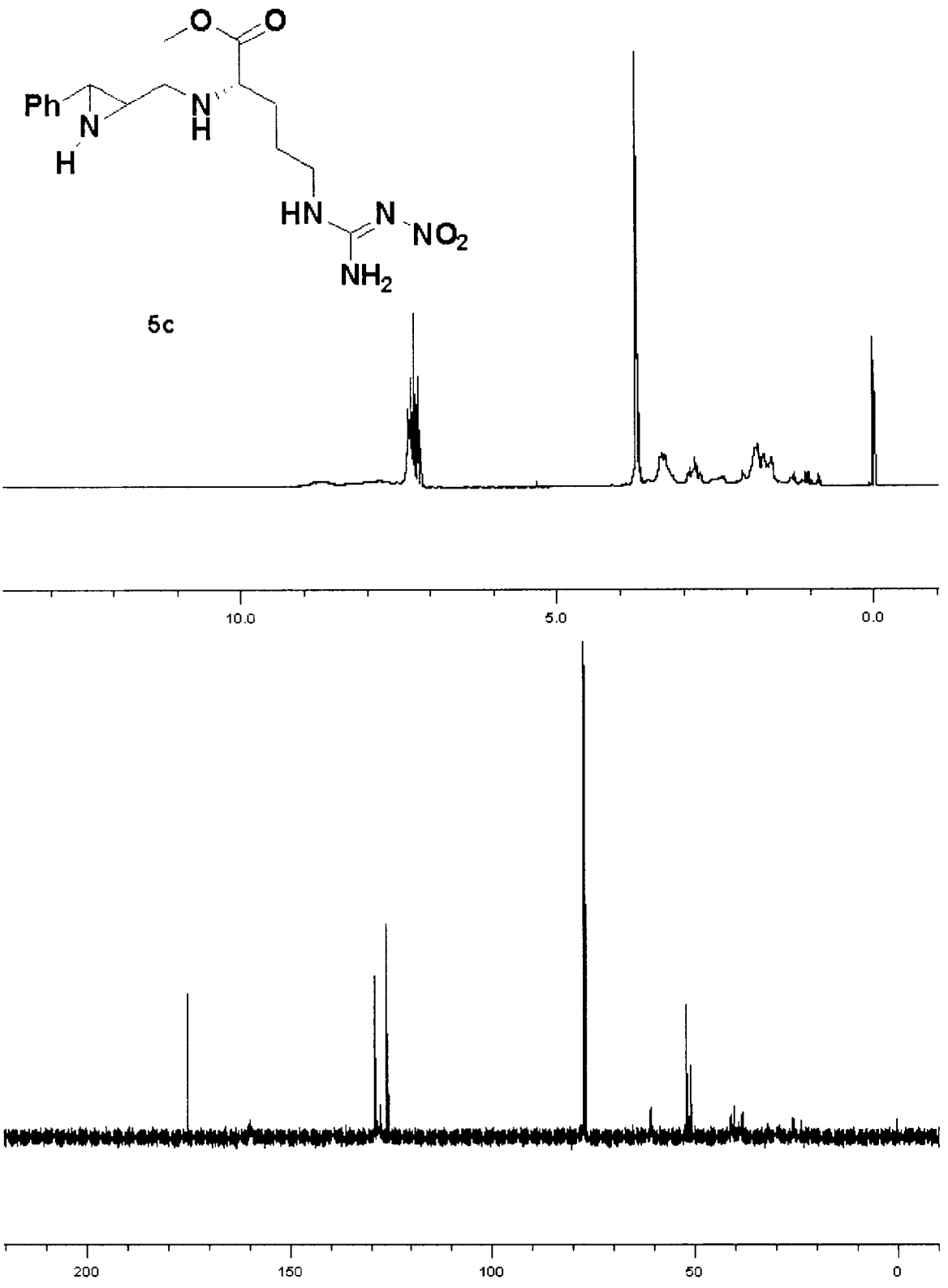
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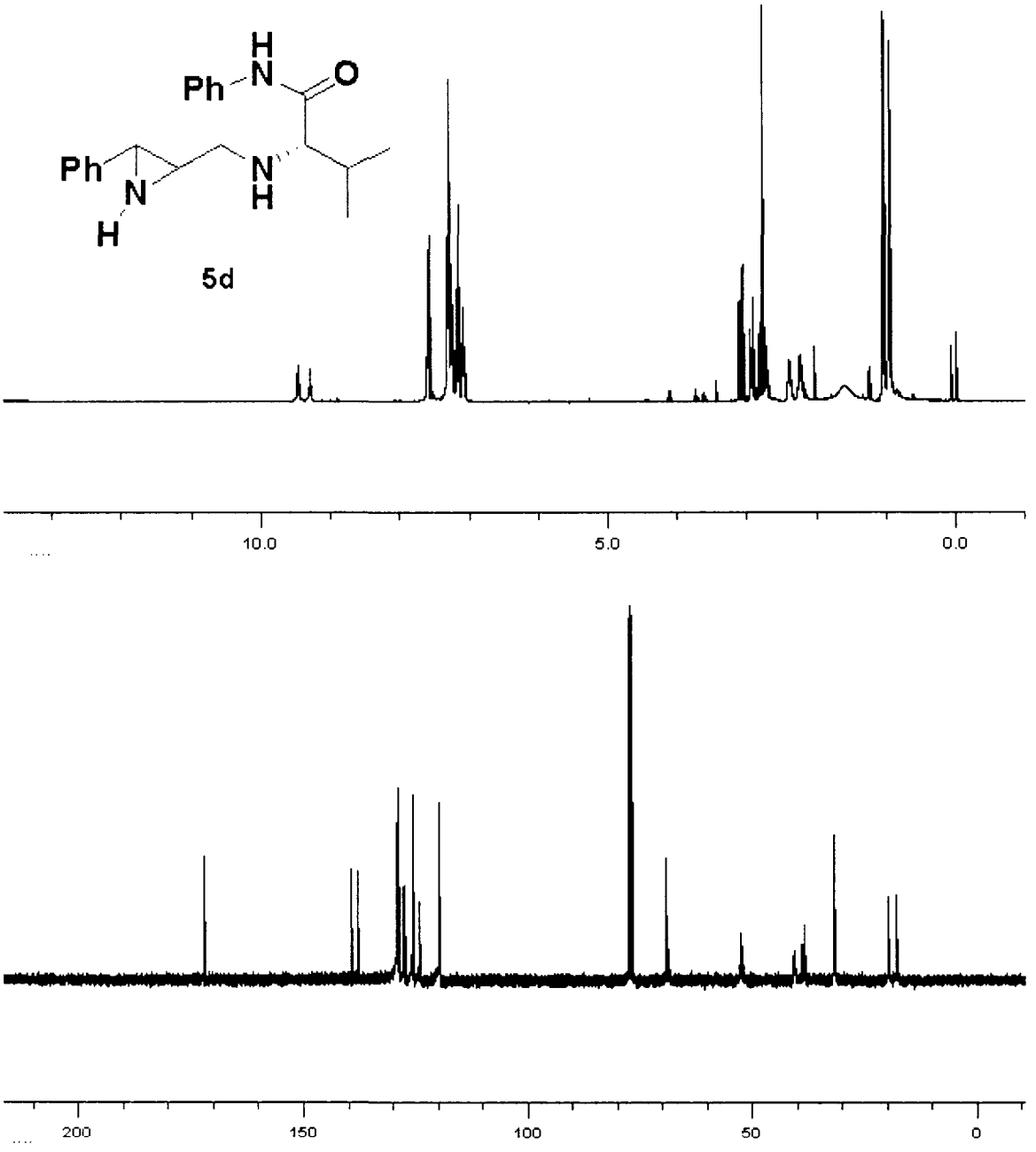
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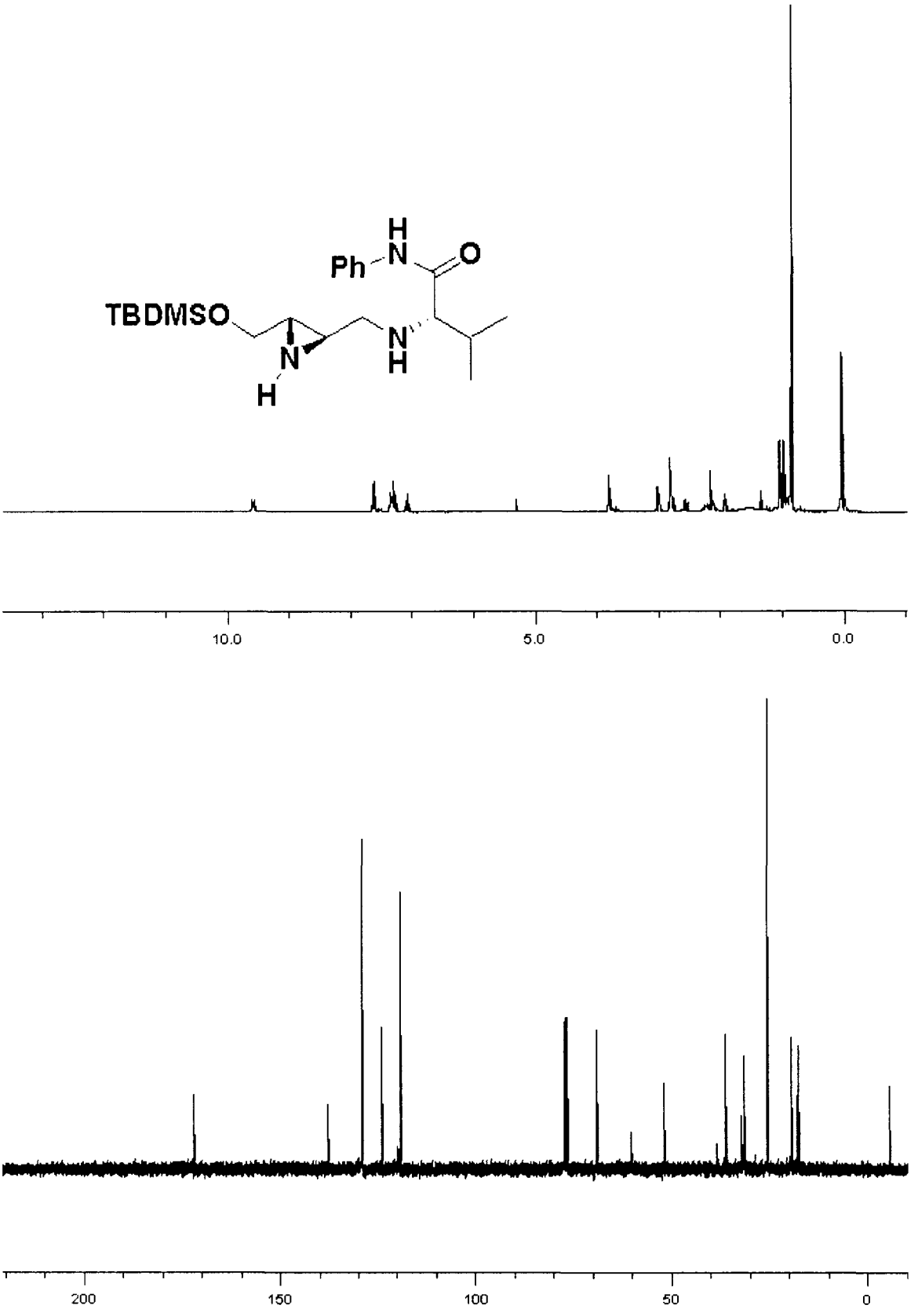
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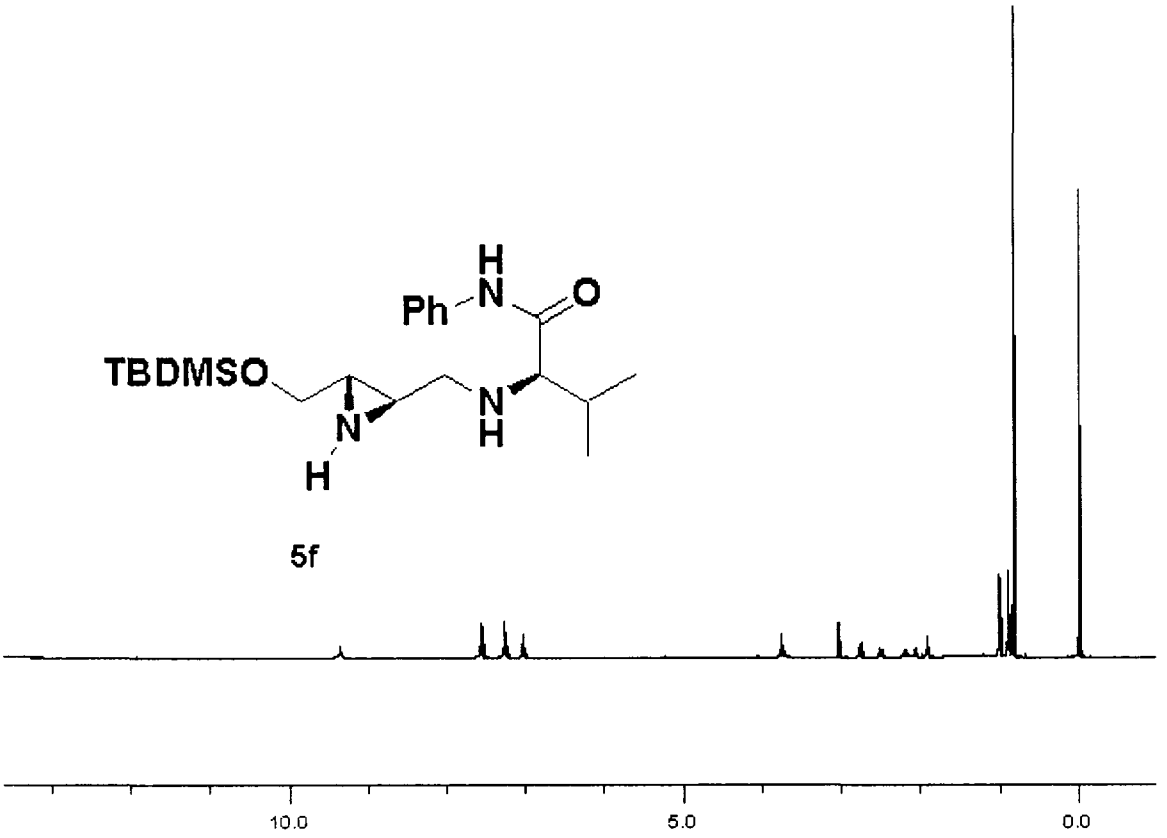

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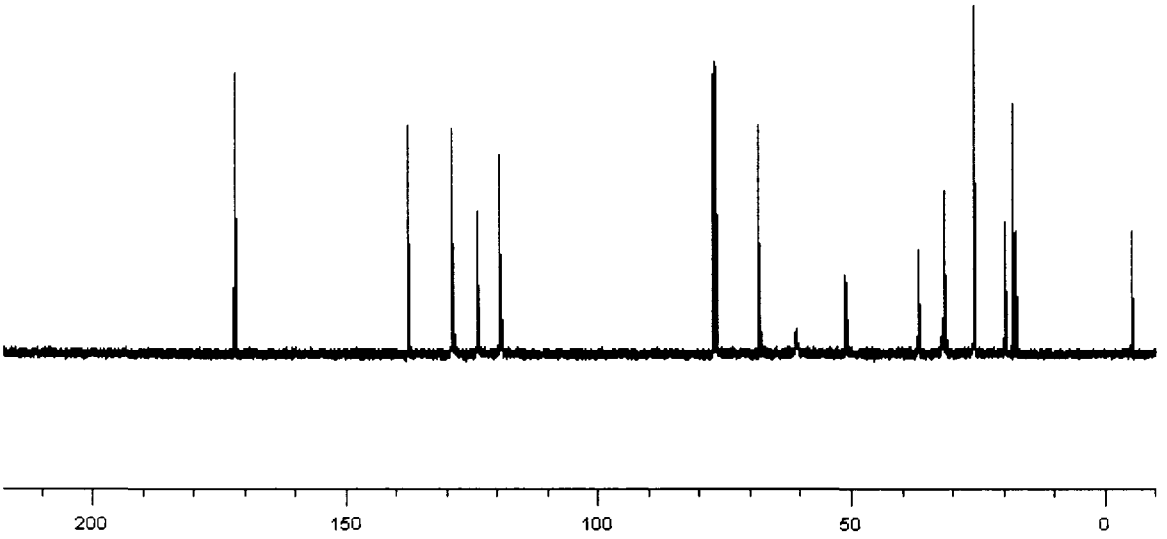

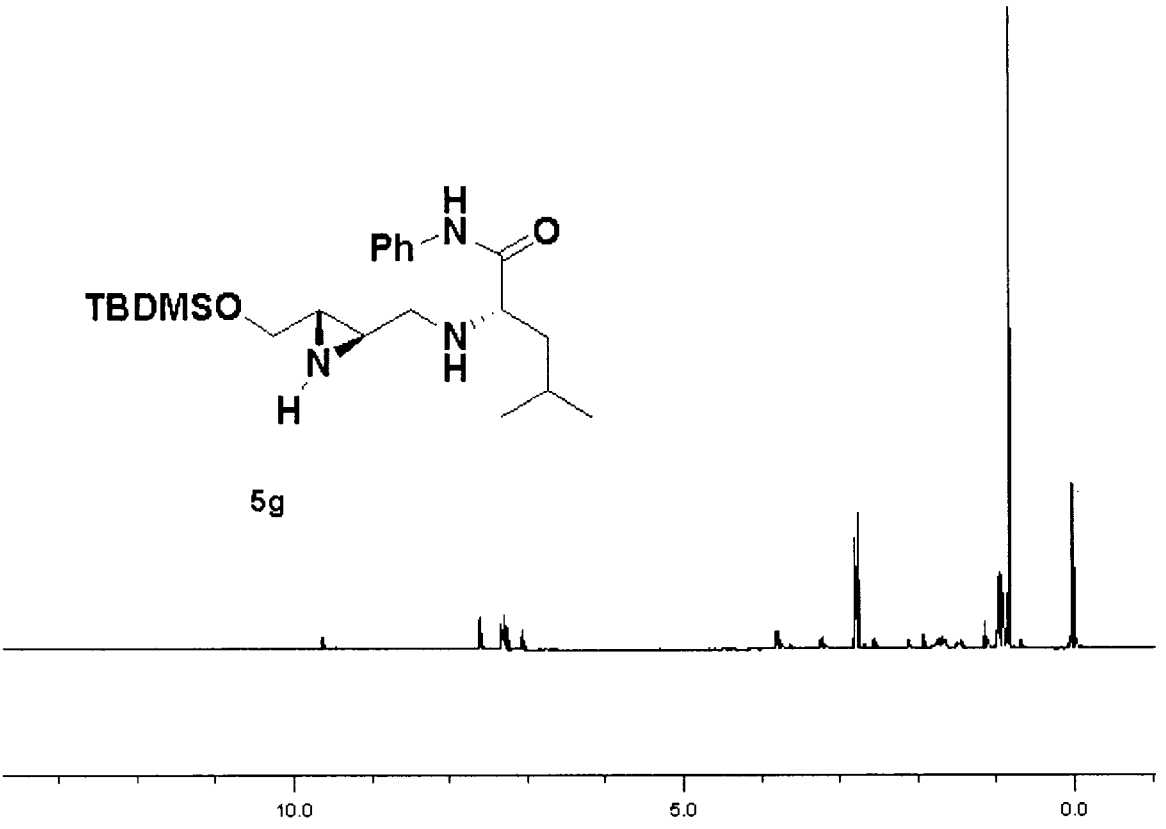

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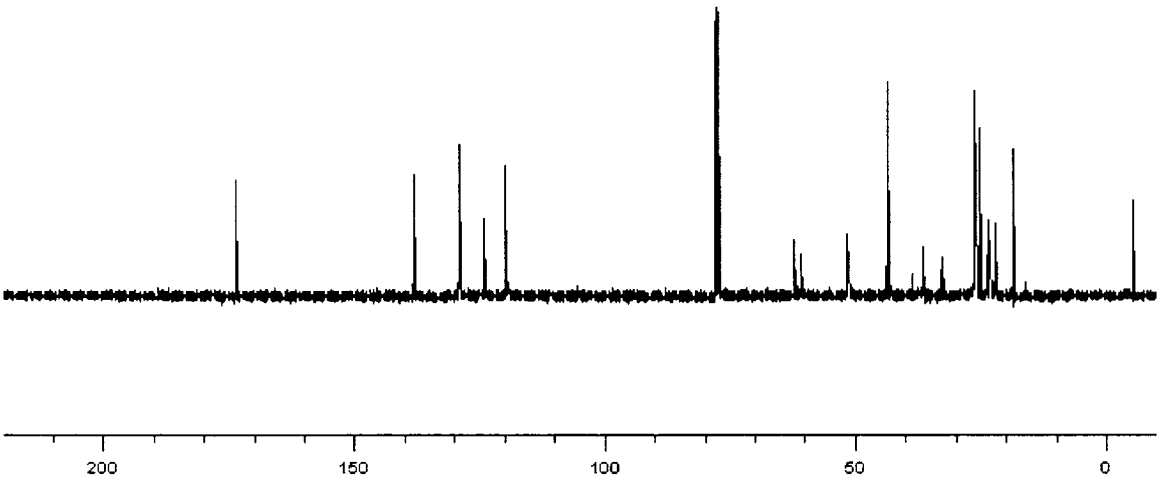

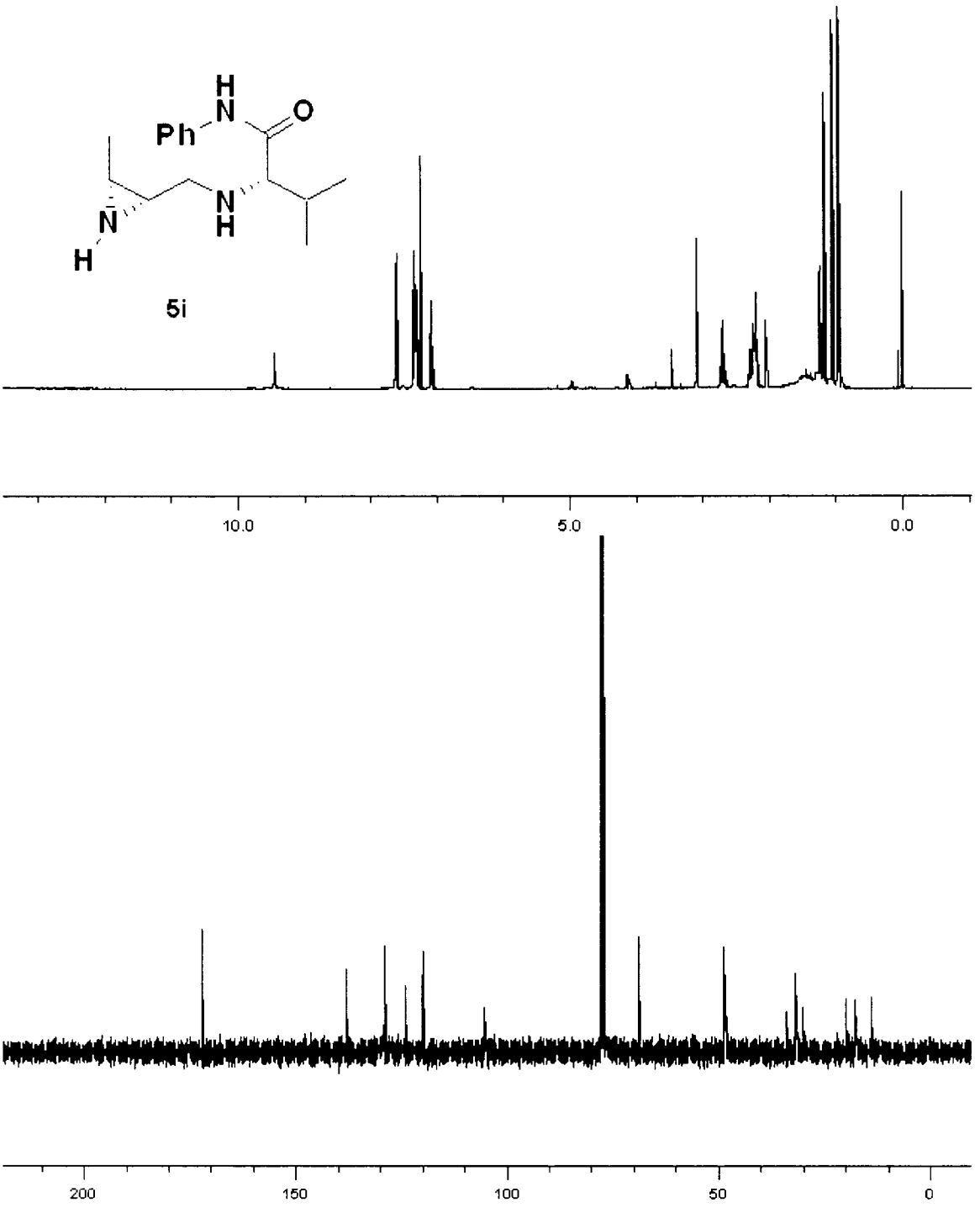
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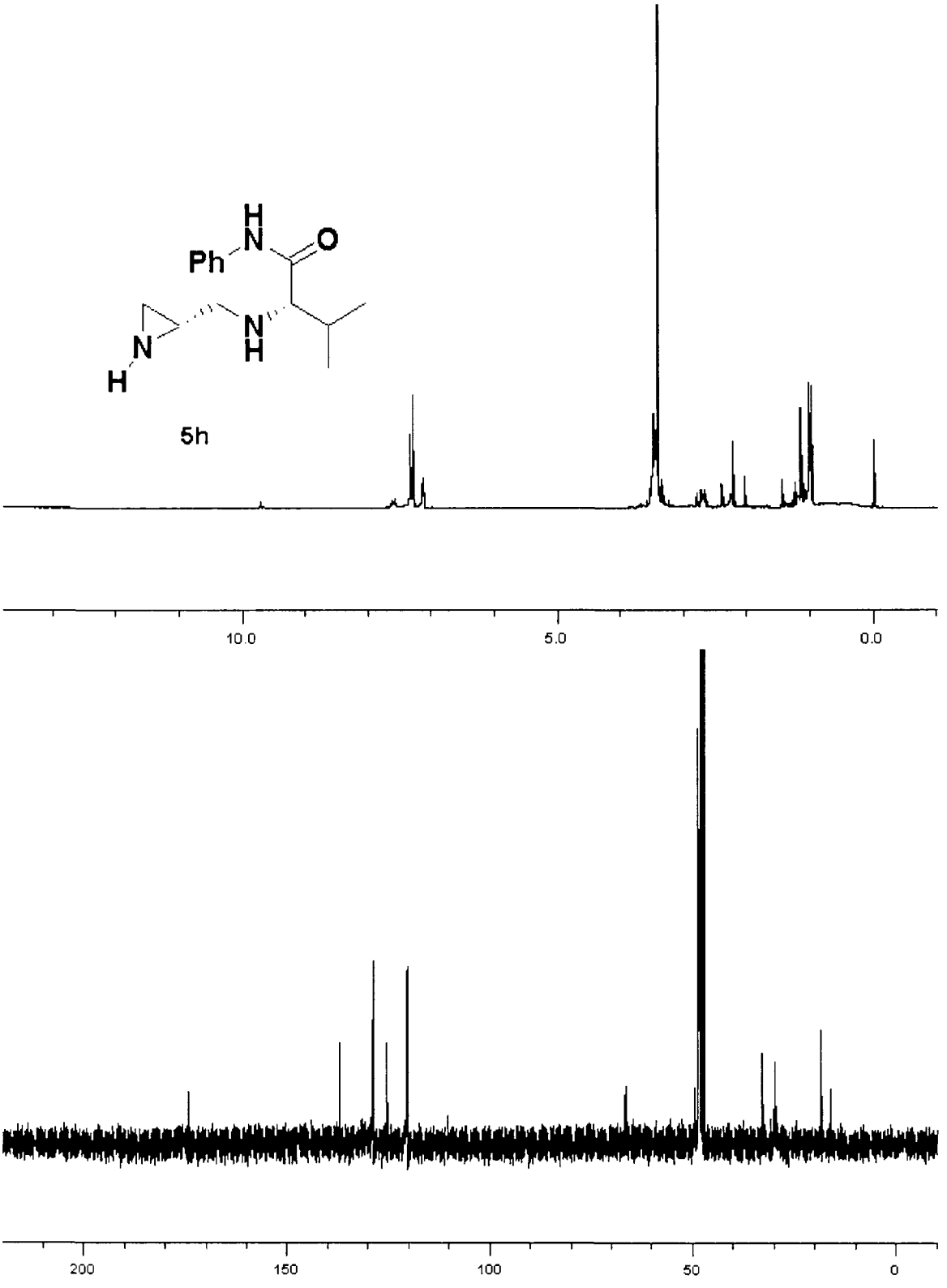
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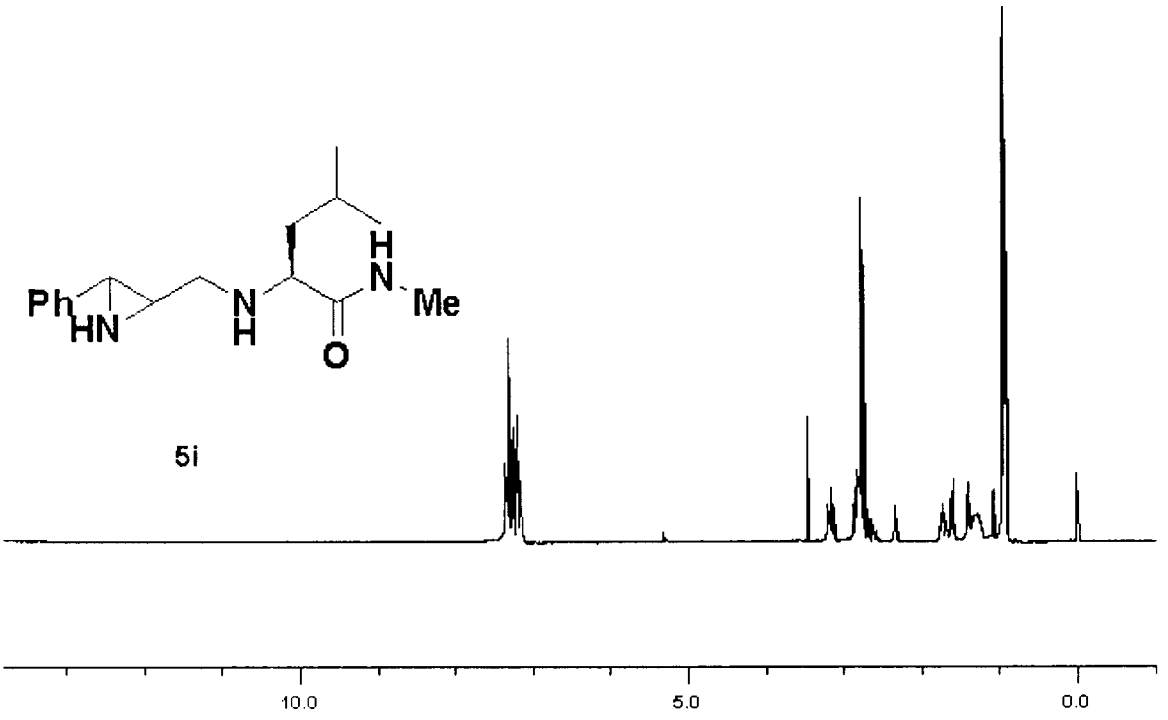

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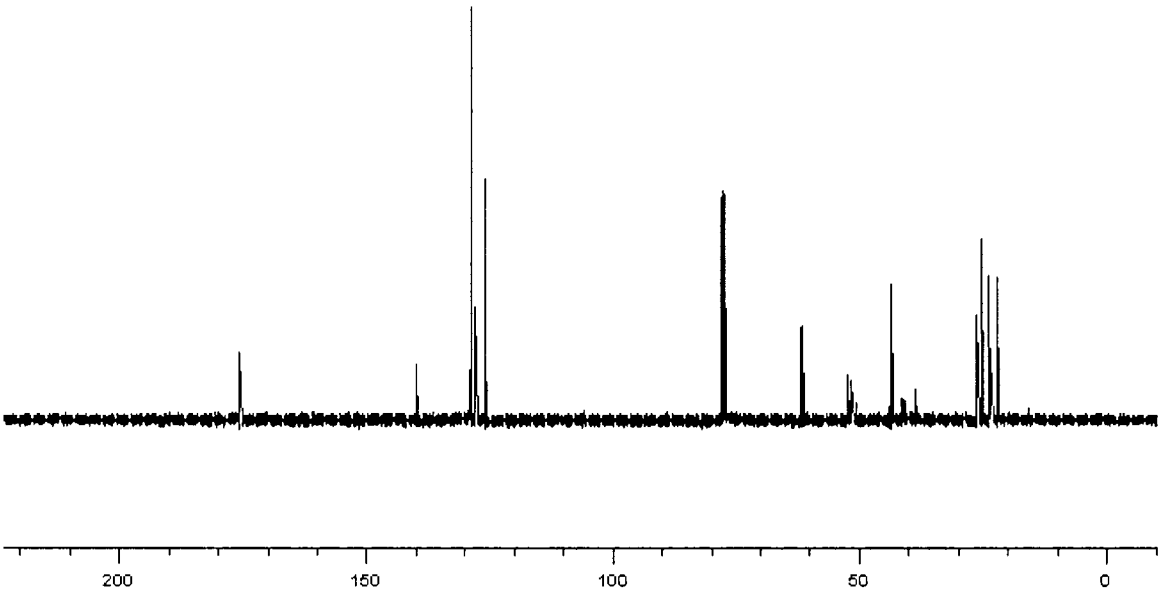

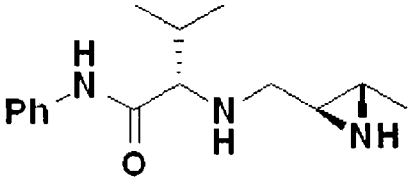

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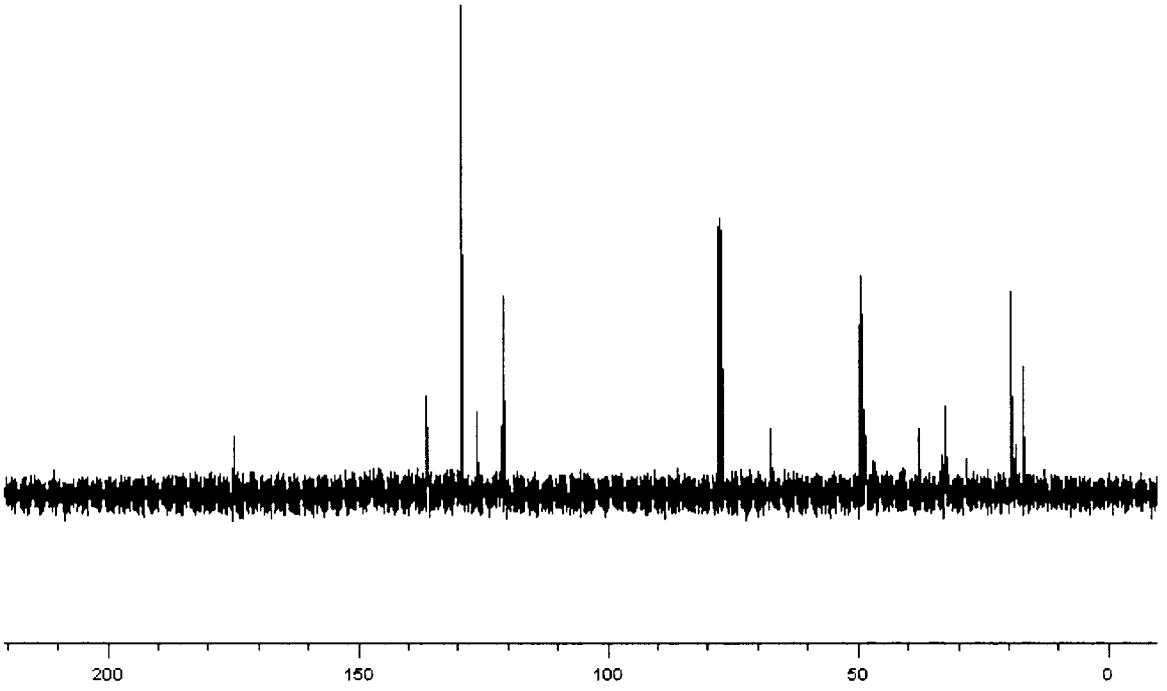

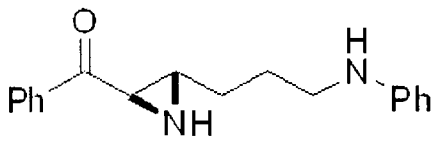

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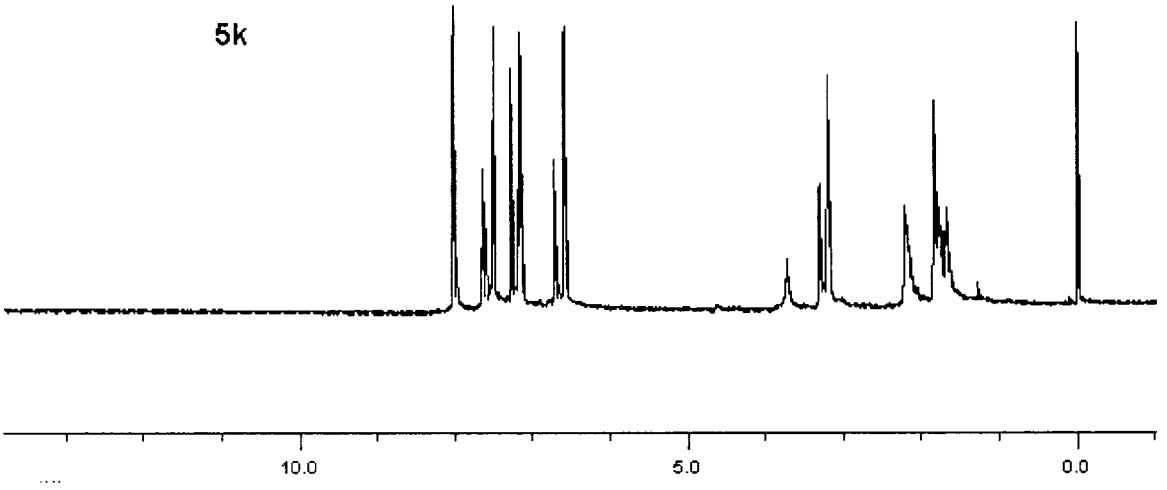

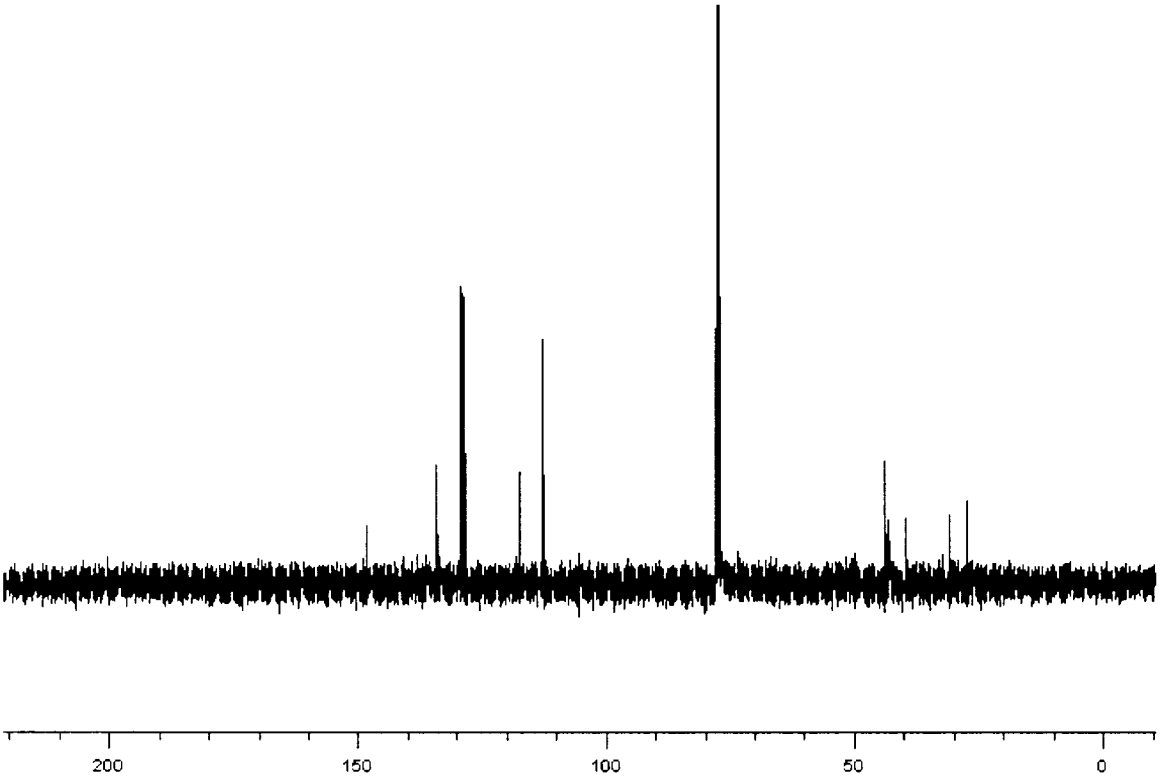

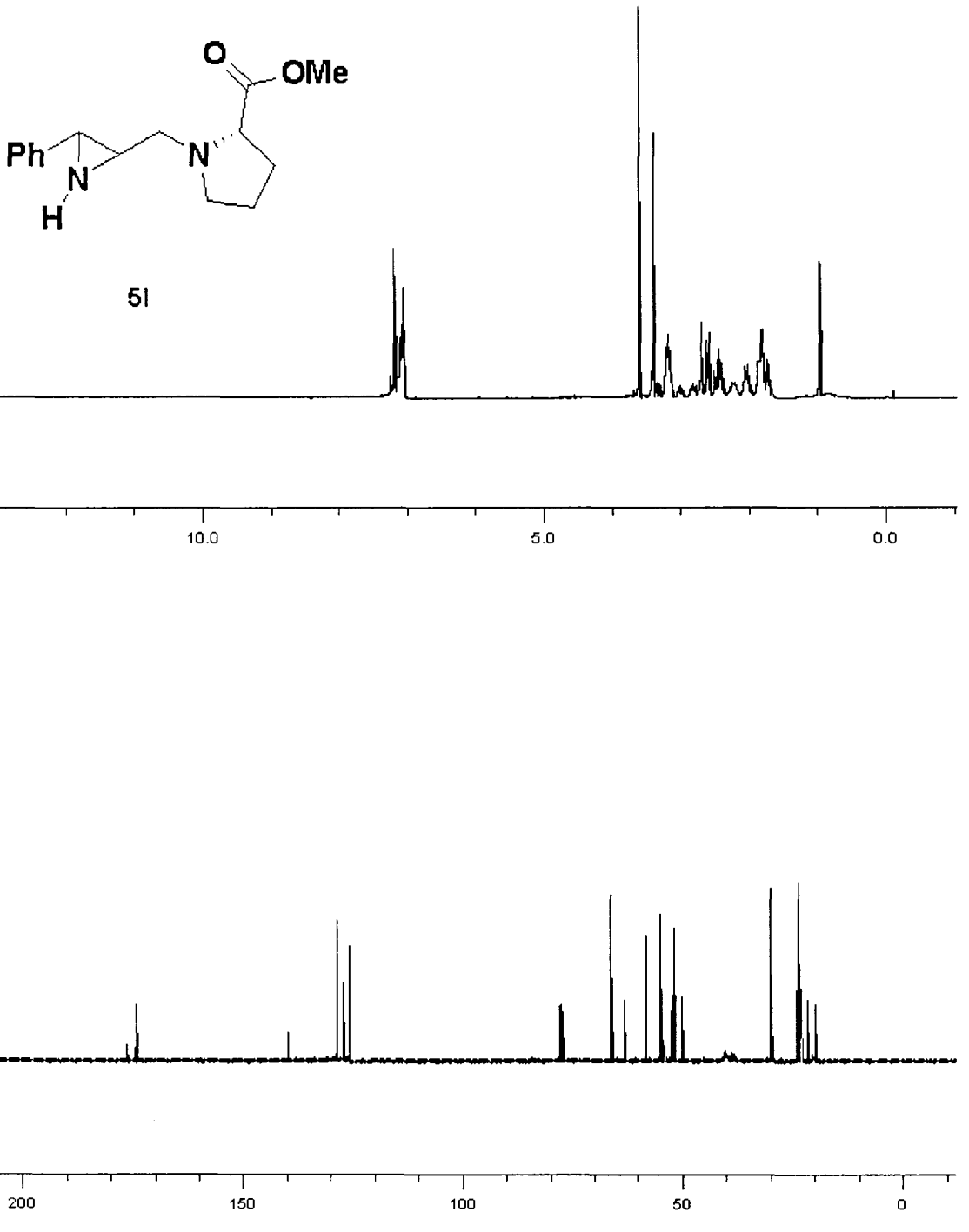
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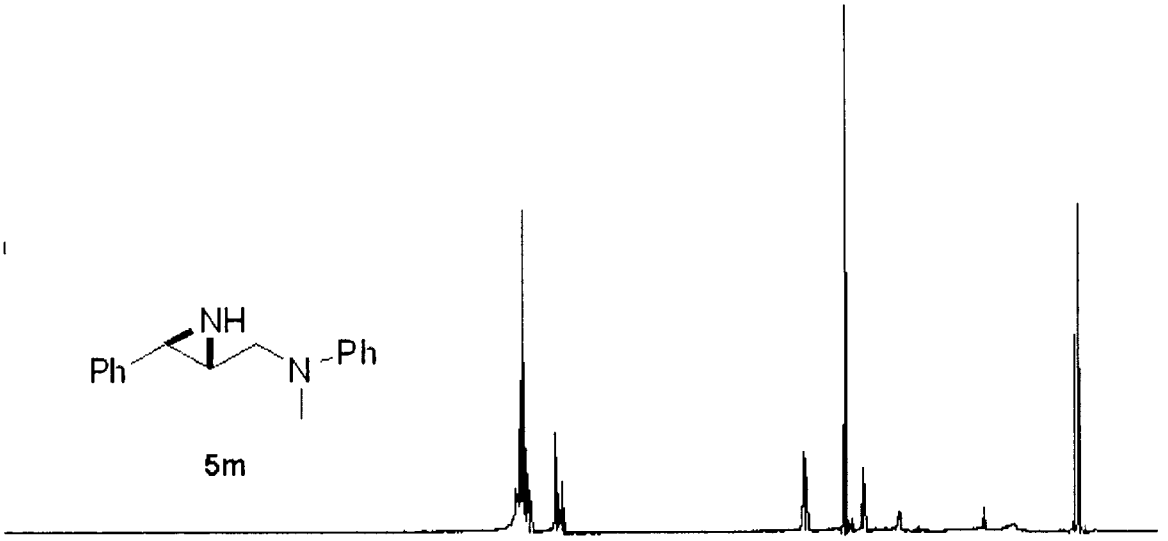
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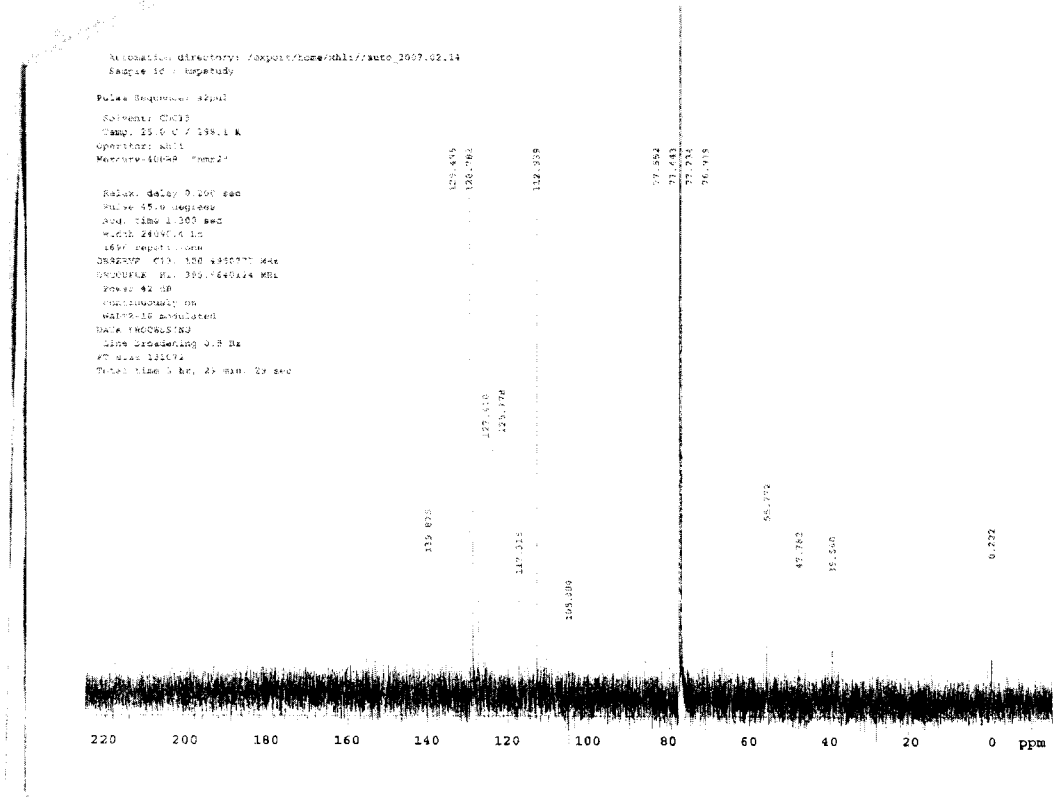
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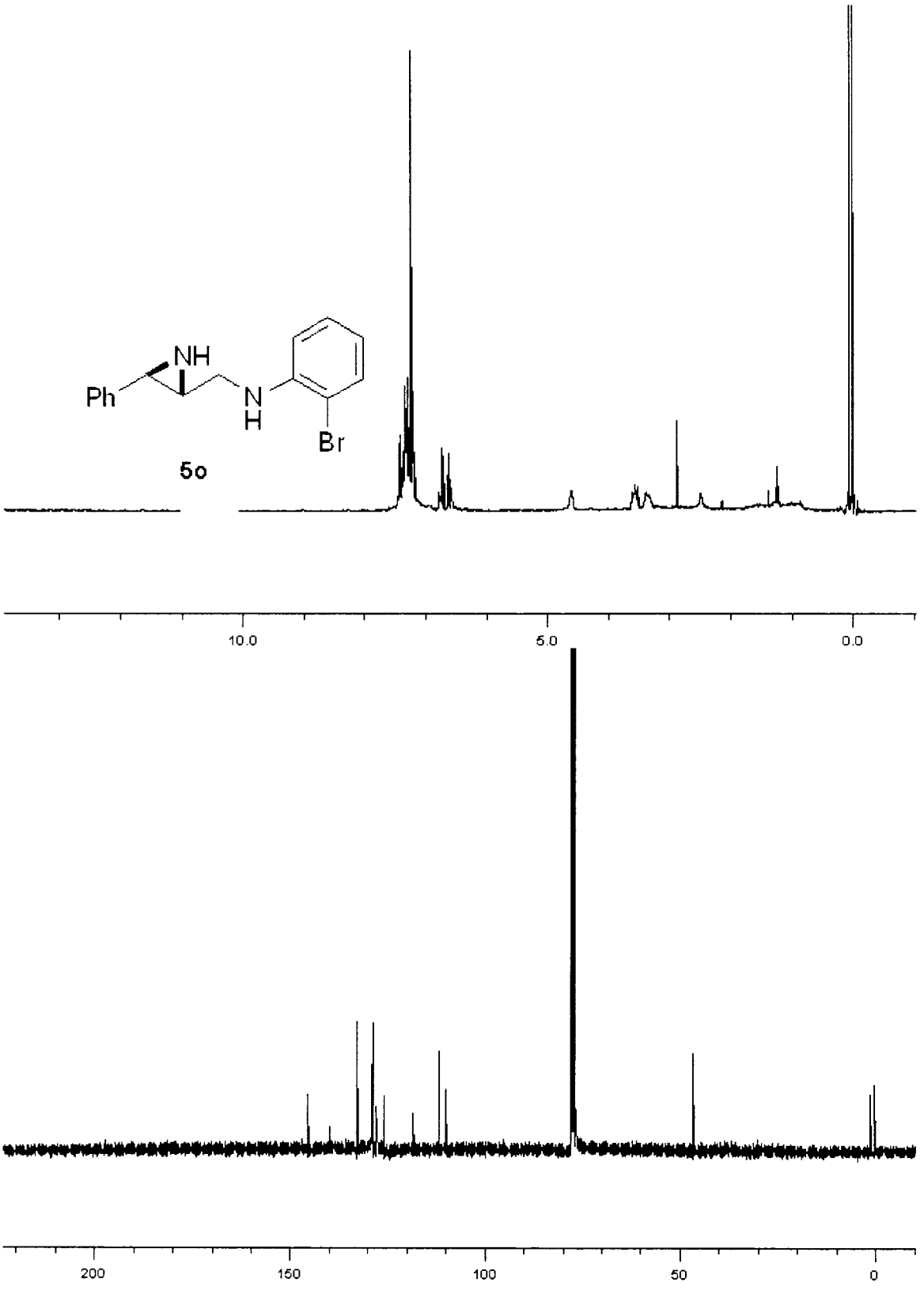
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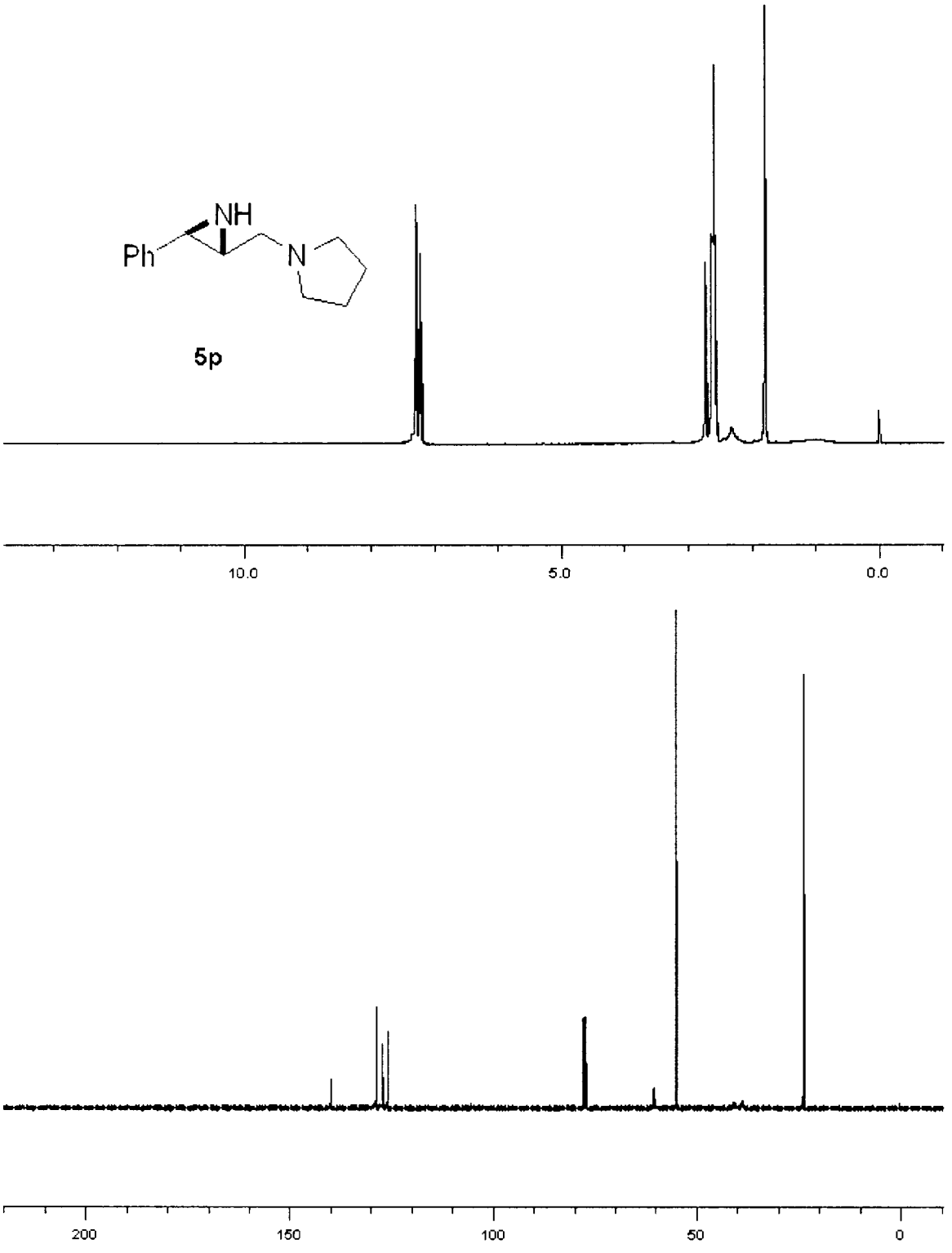
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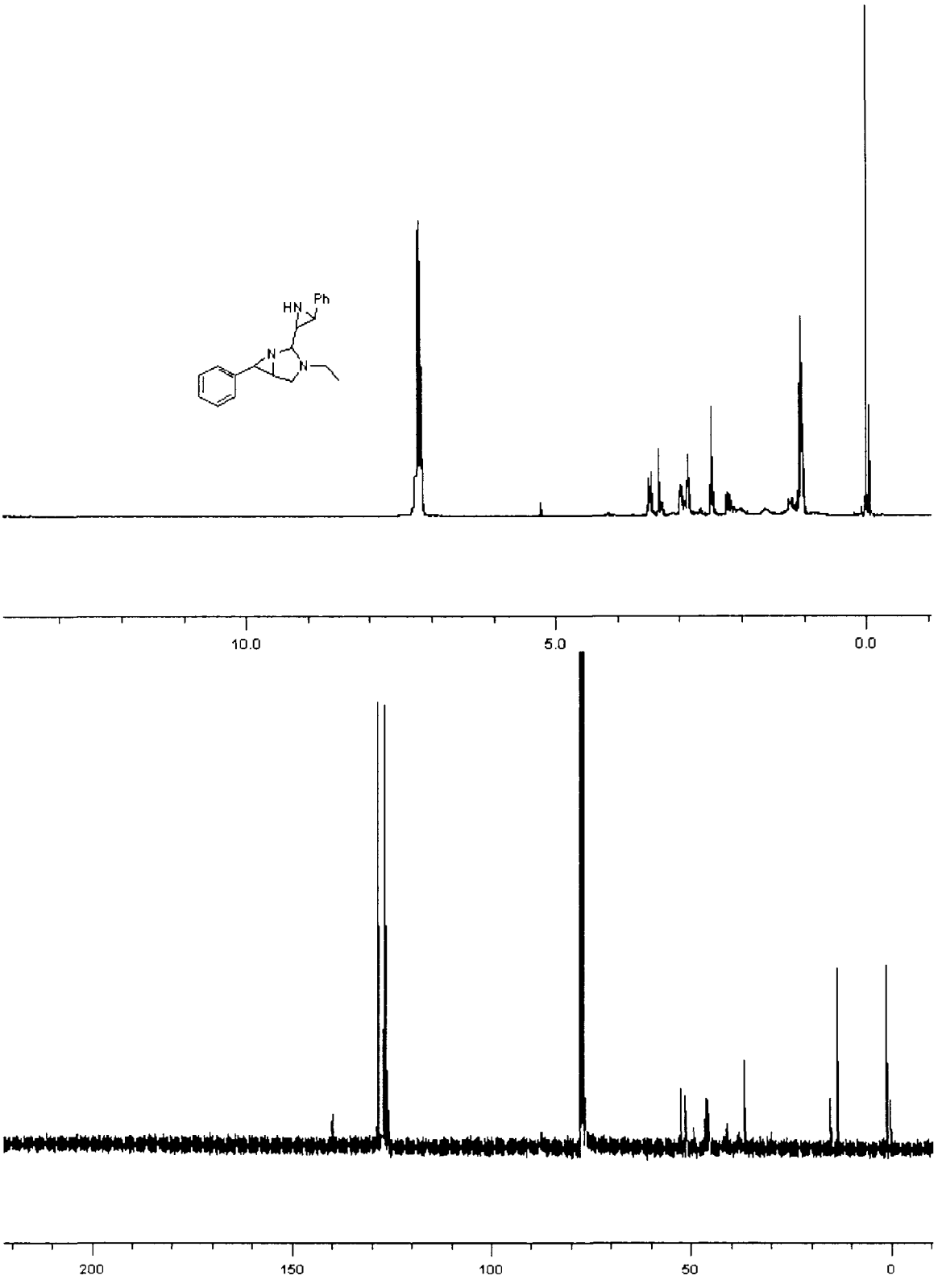
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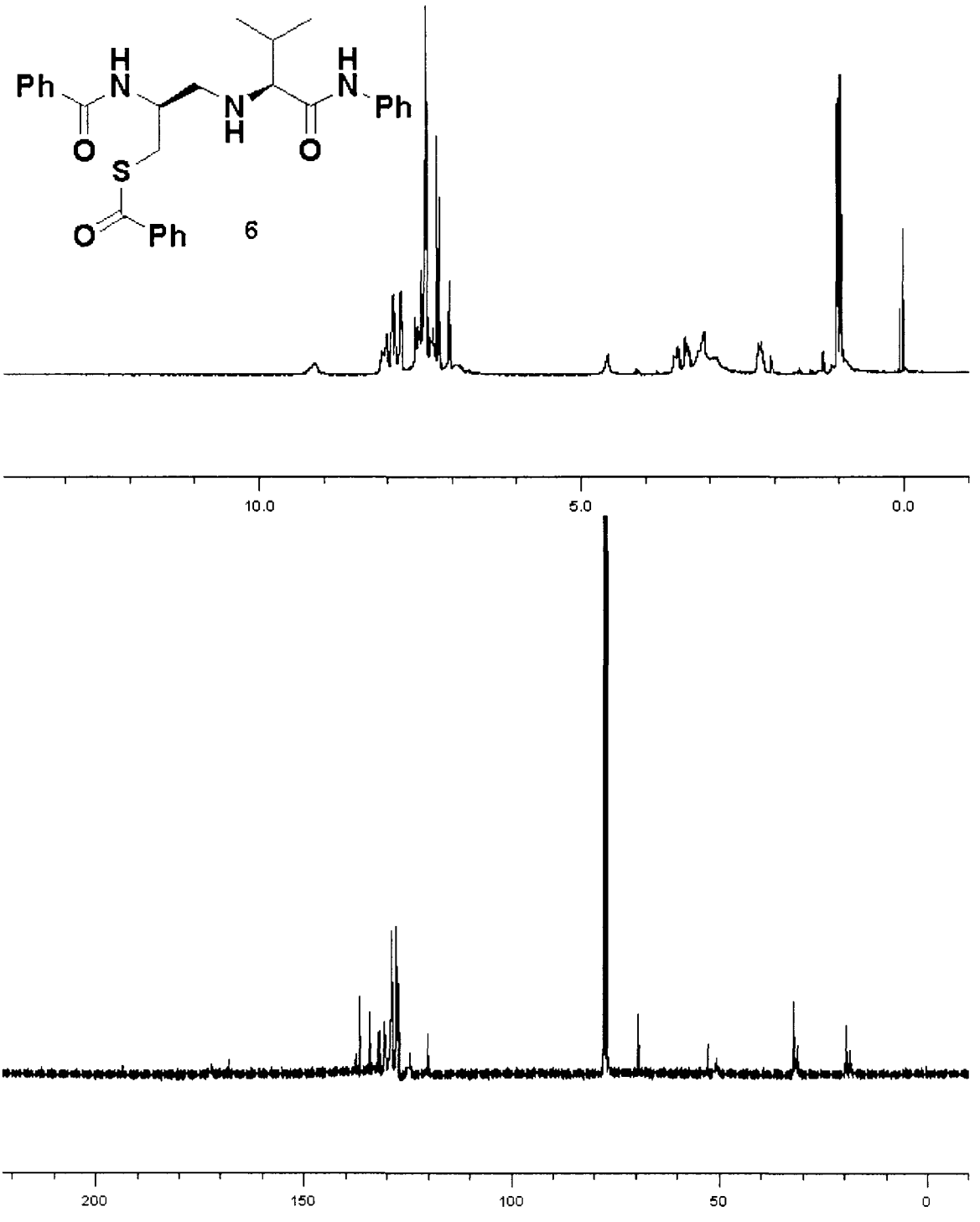
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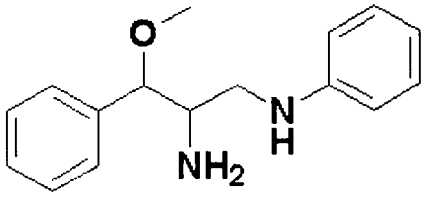

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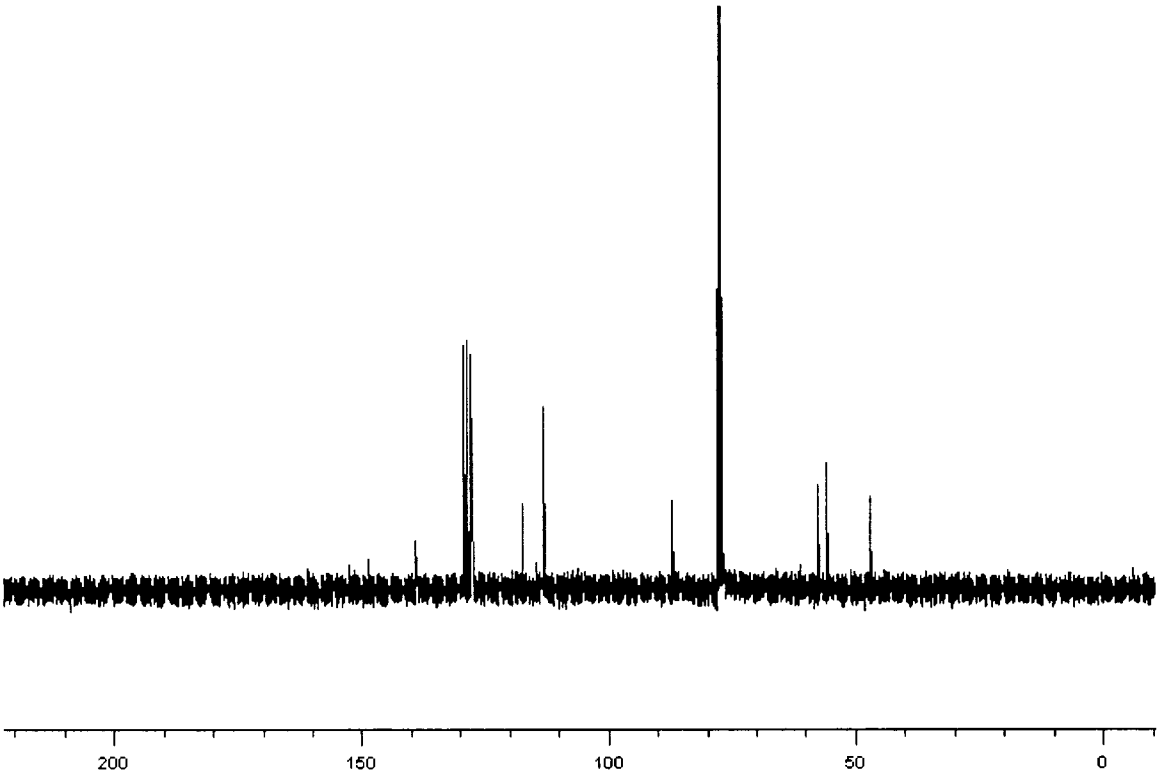

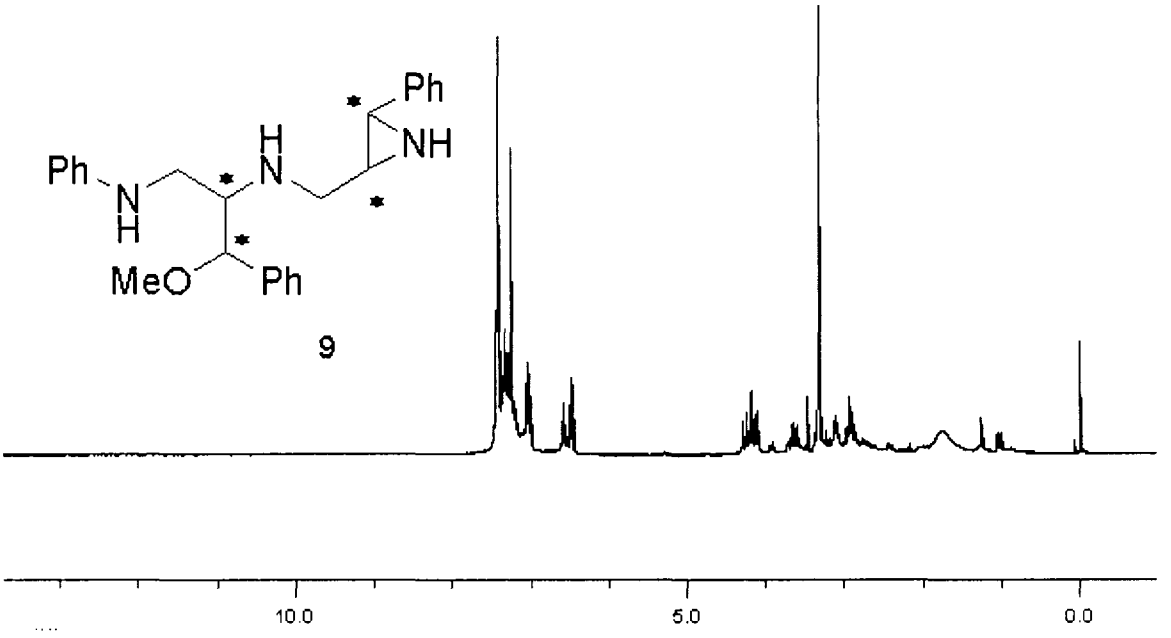

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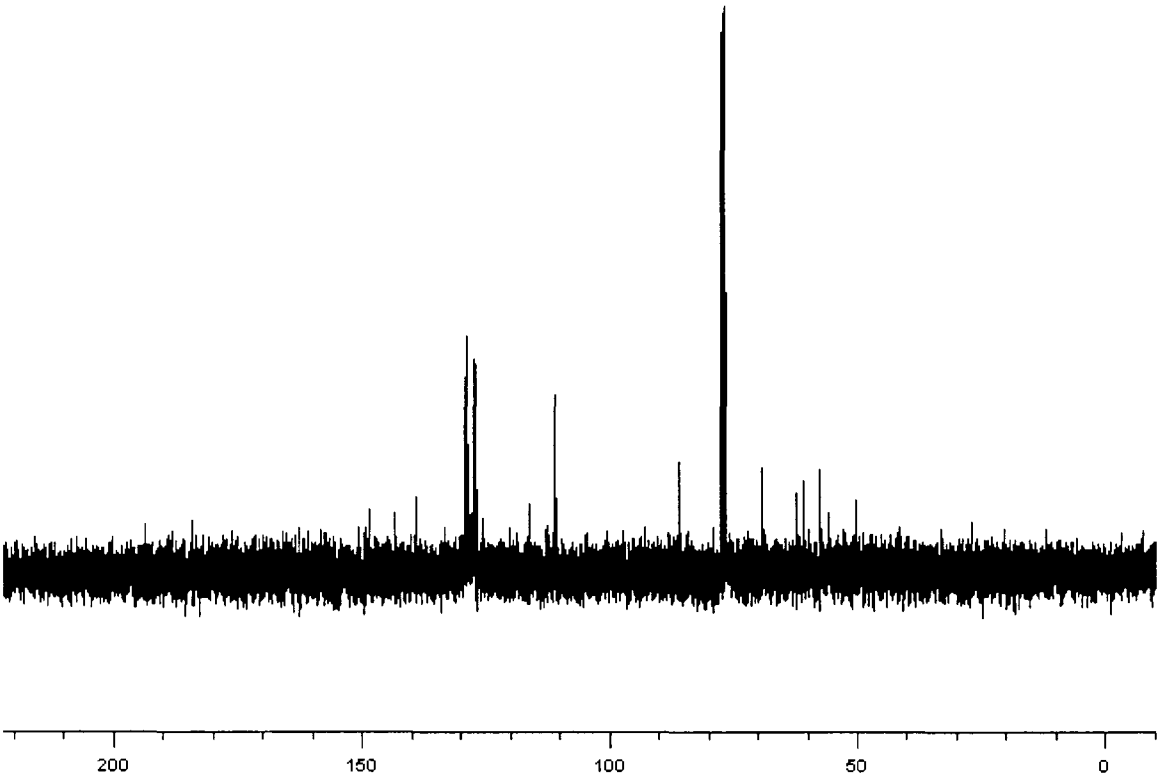

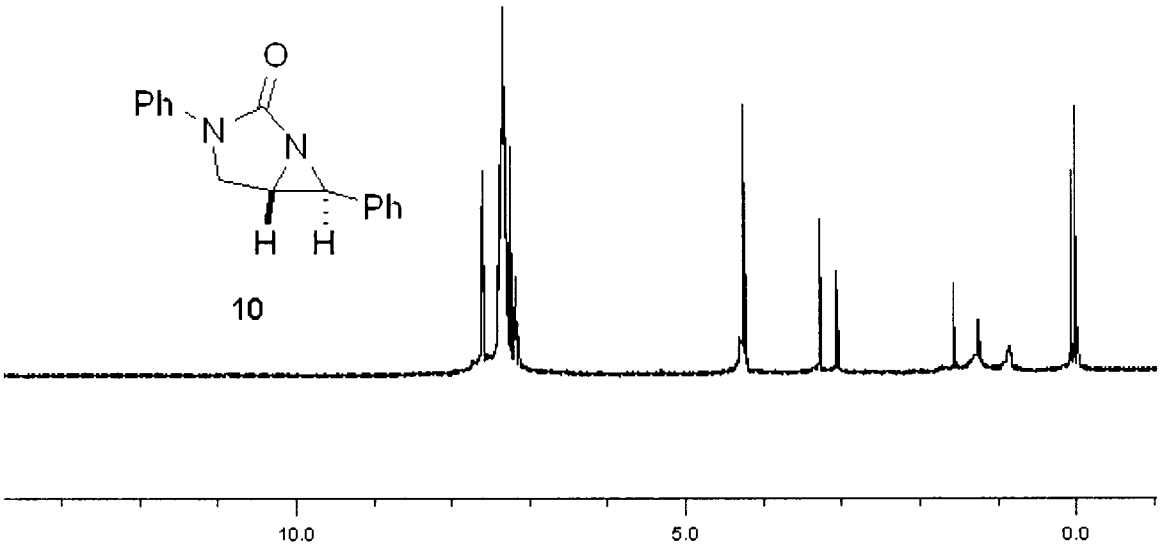
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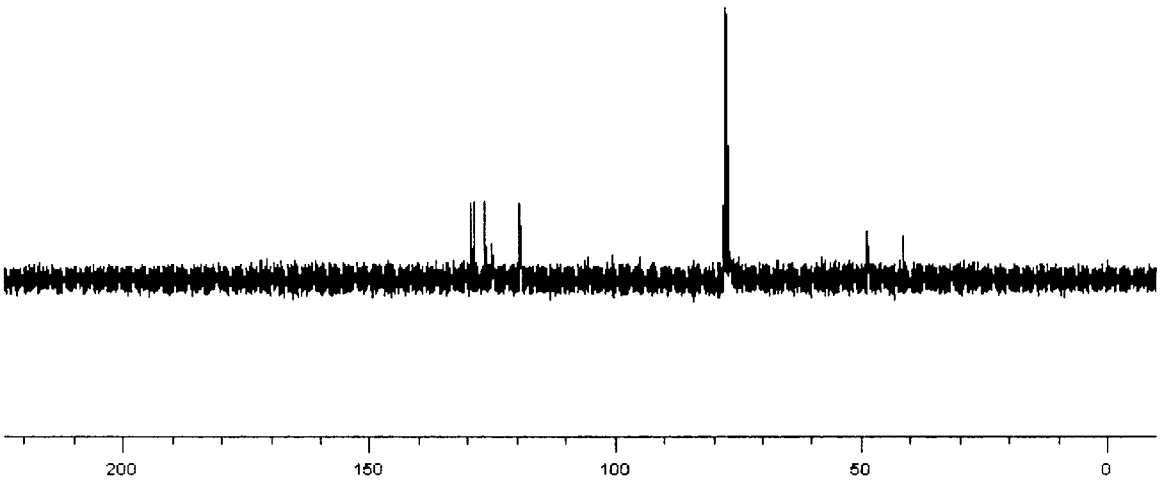
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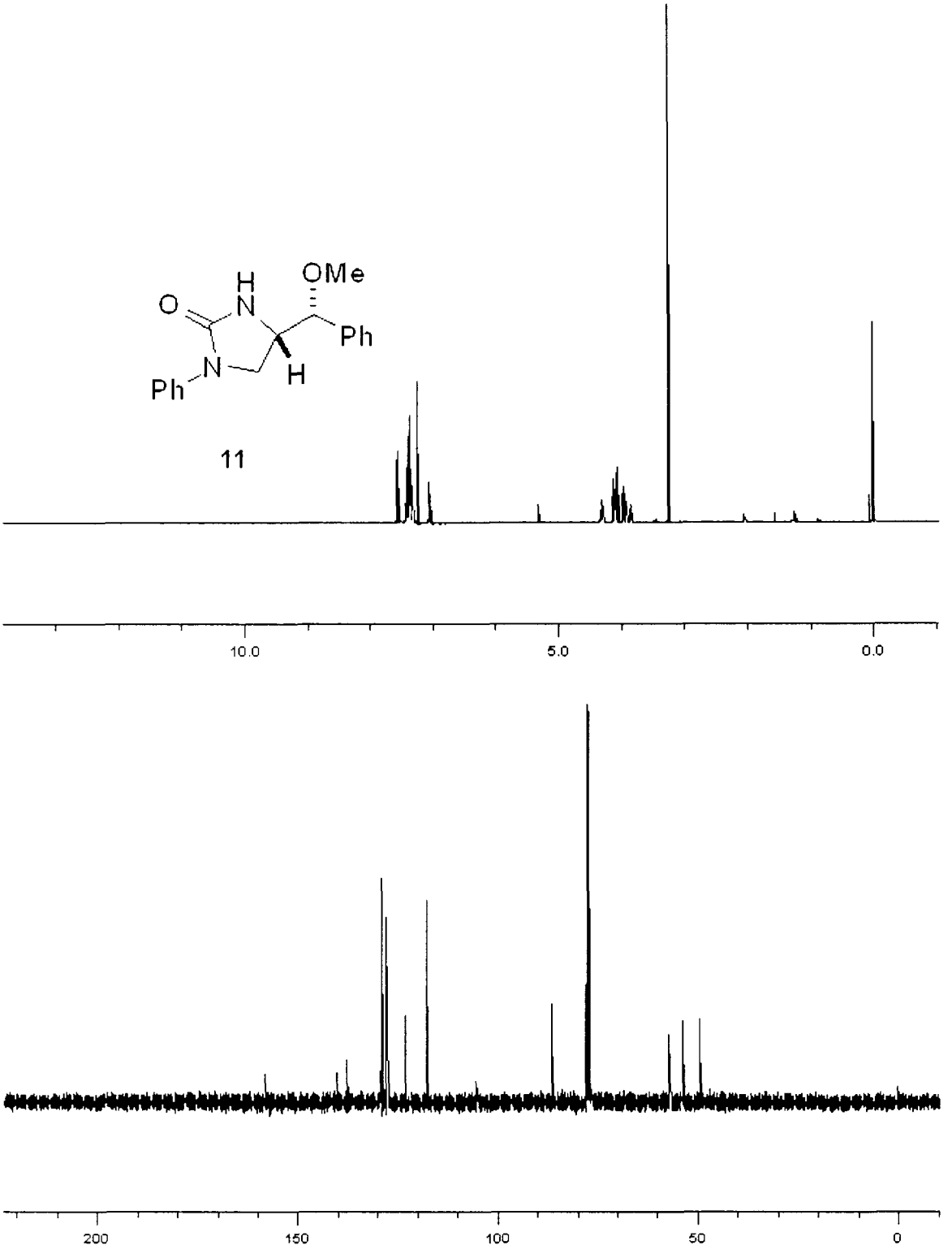
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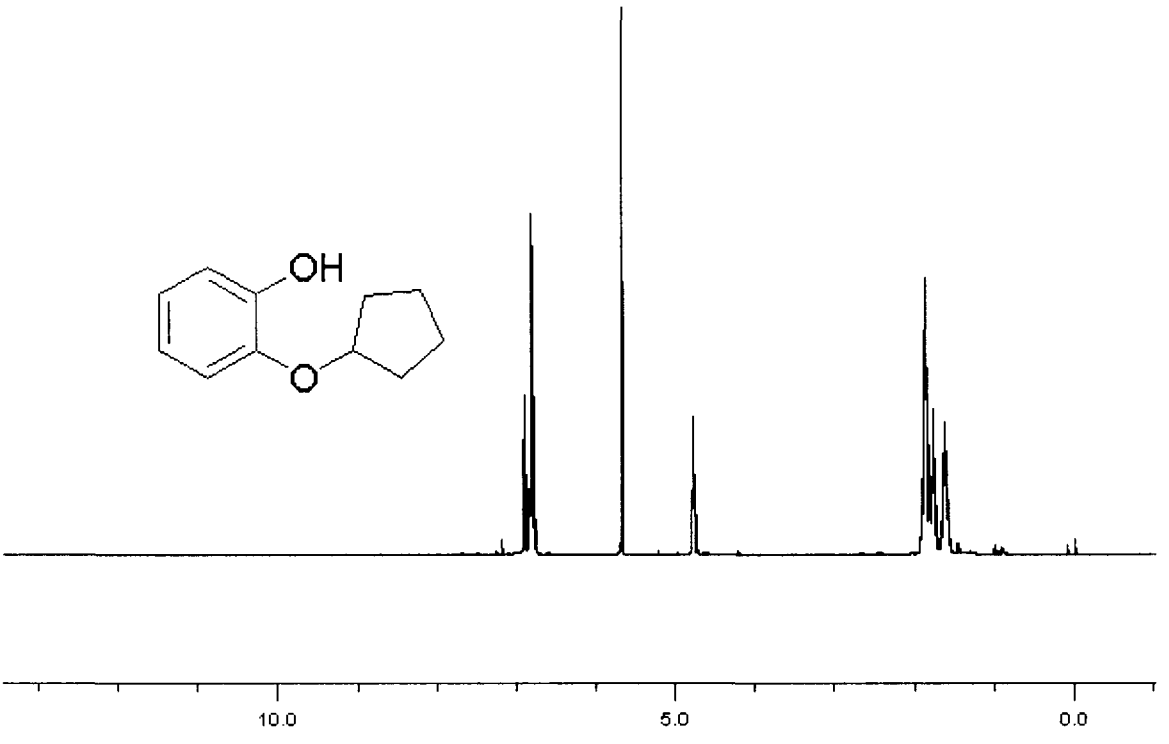
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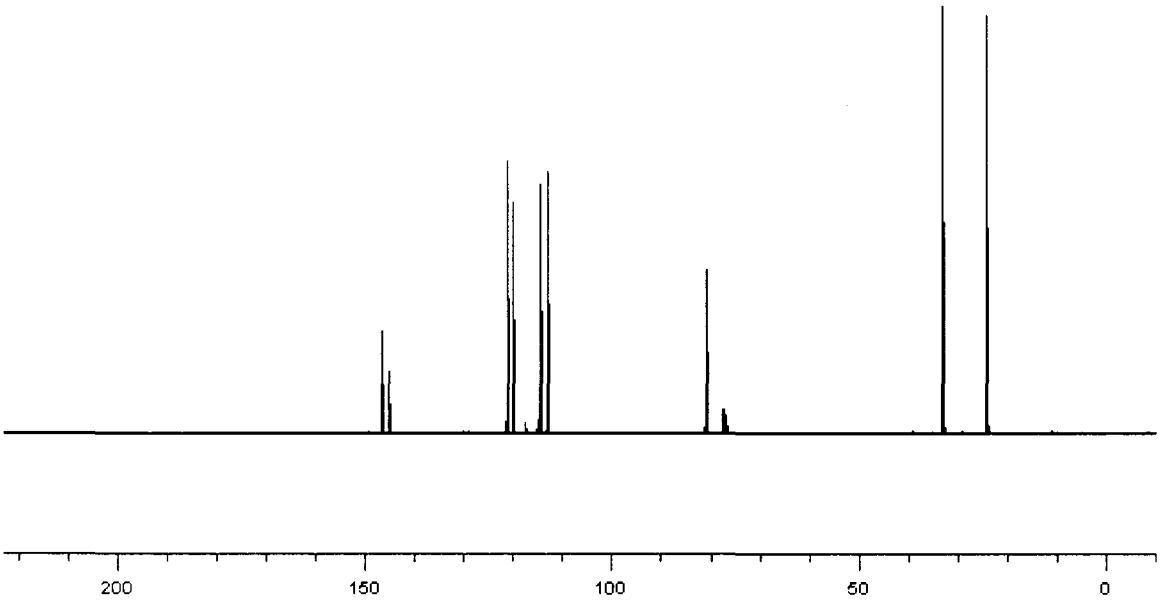
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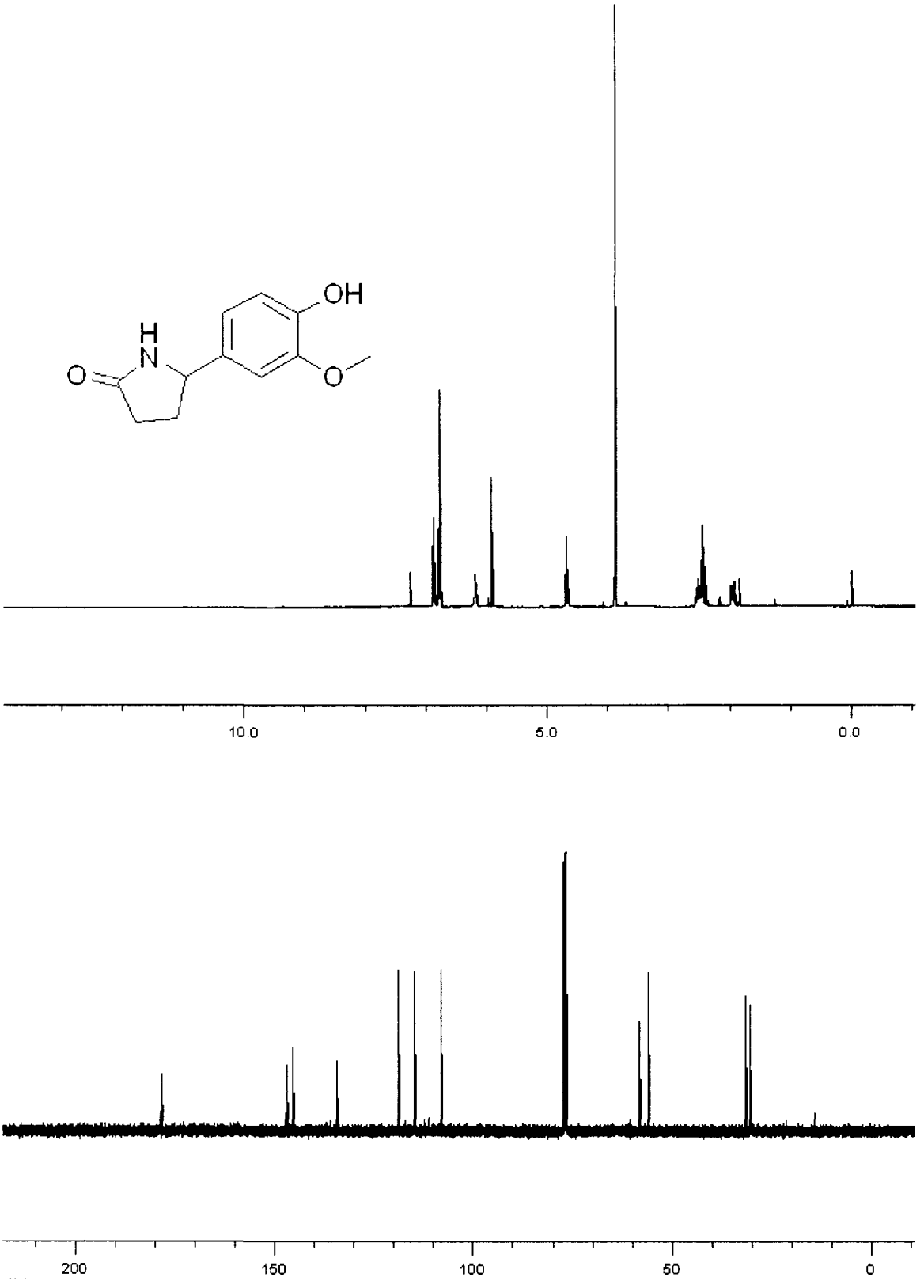
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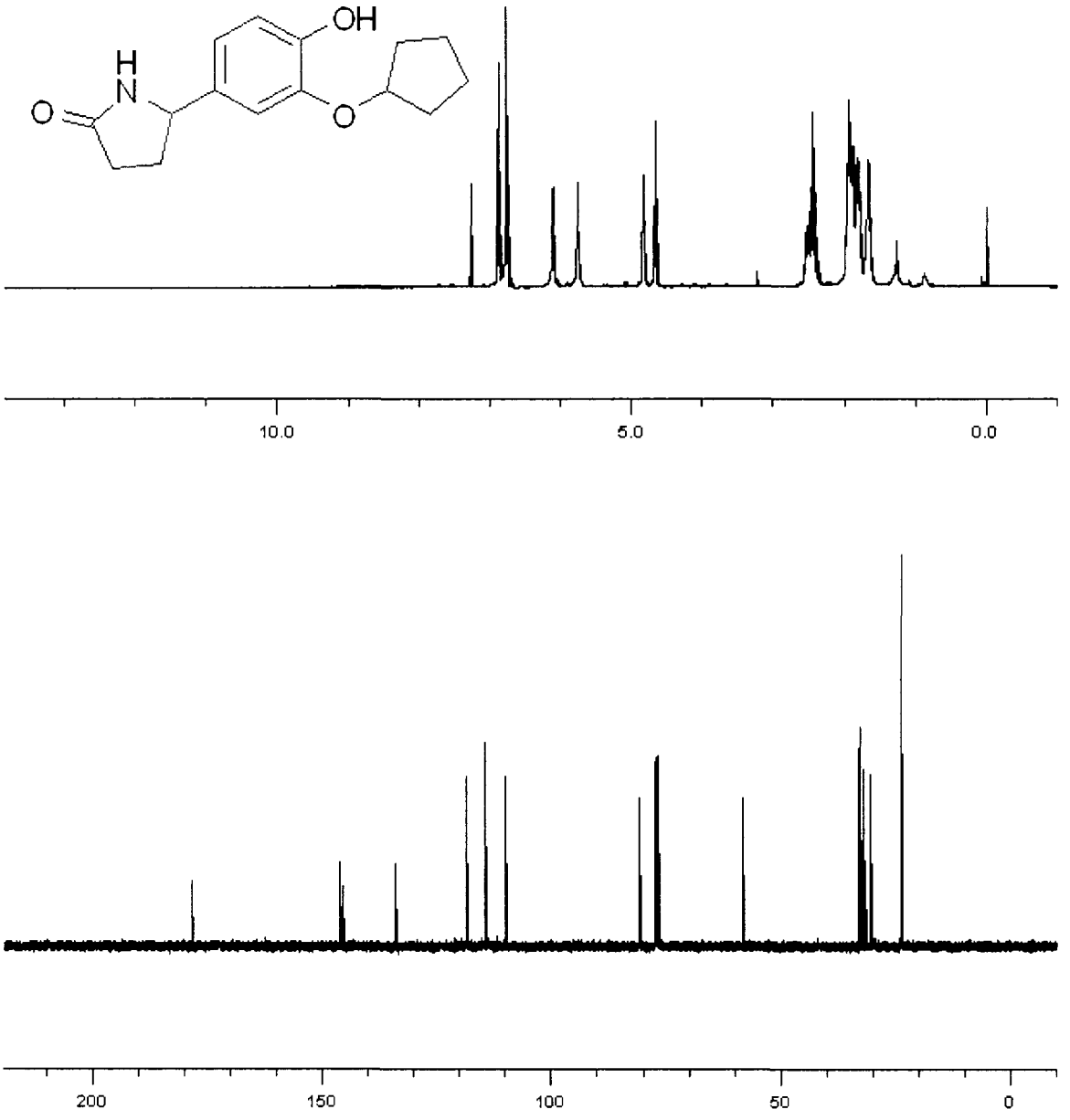
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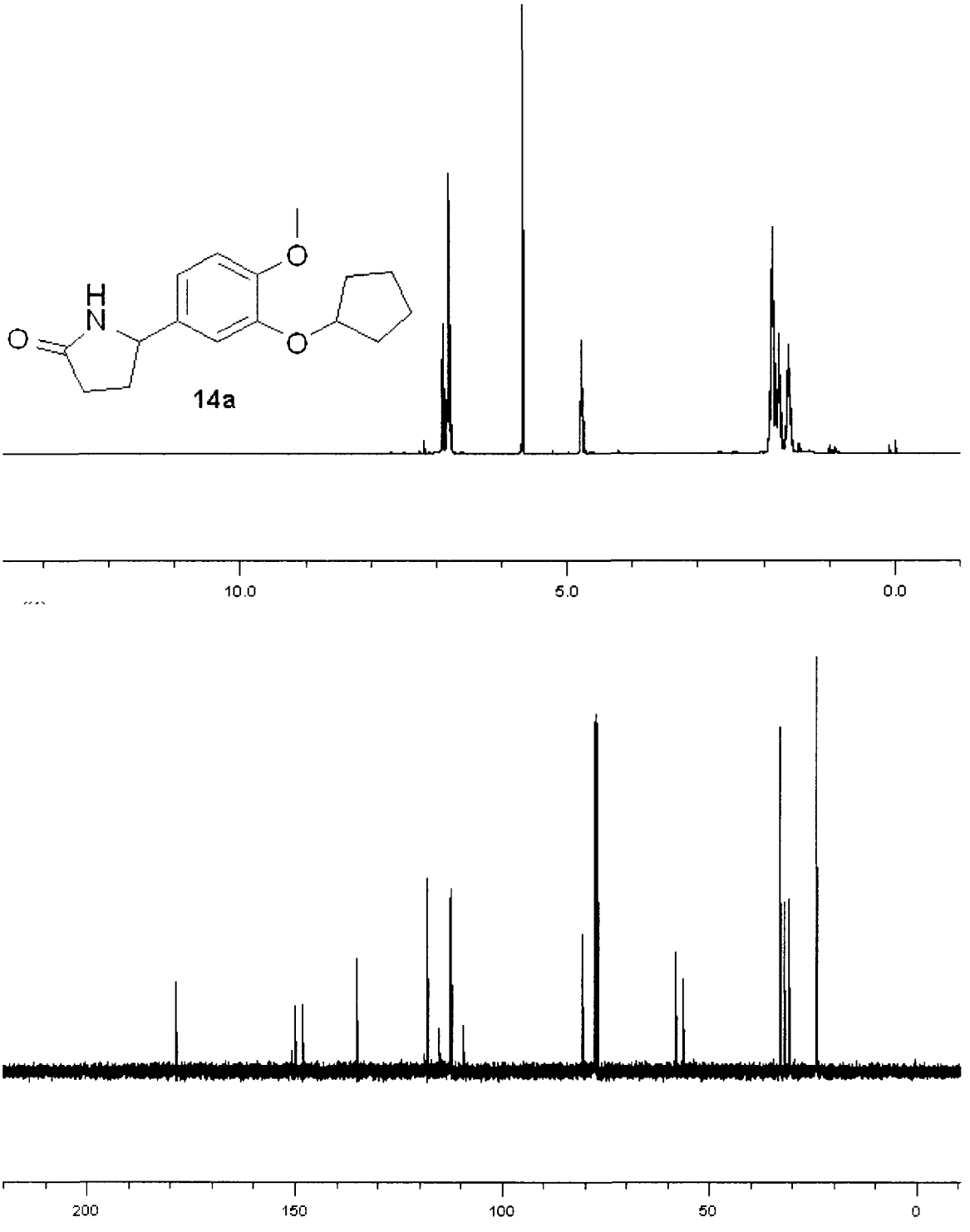
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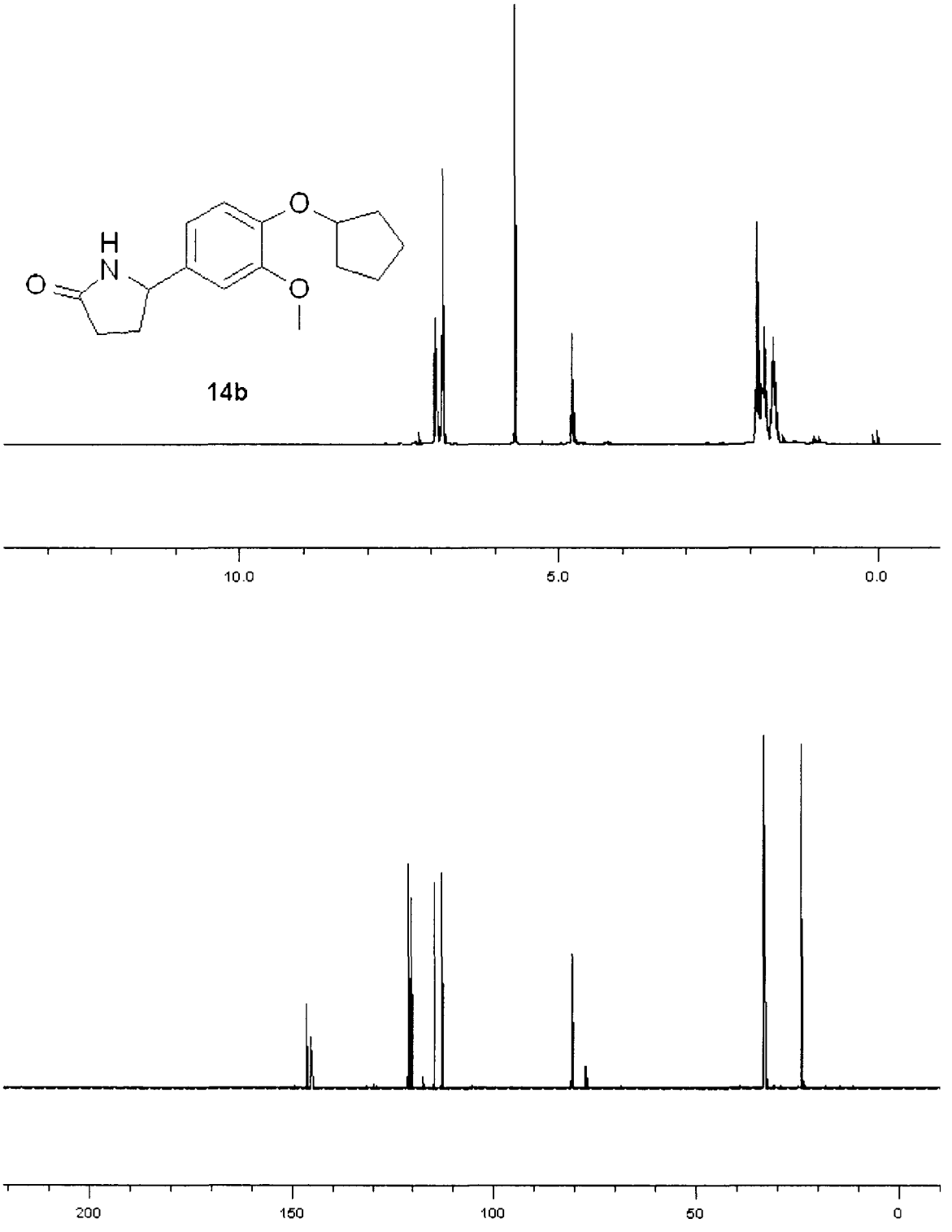
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