CHAPTER 2

SYNTHESIS OF SULFONAMIDE DERIVATIVES AS DPP-IV INHIBITORS

2.1 Introduction:

Dipeptidyl peptidase IV (DPP-IV, E.C.3.4.14.5, CD26) is a widely expressed serine protease in many tissues and body fluids of mammals and exists as either a membrane bound or a soluble enzyme. It is primarily found on vascular endothelium, epithelial cells of kidney, liver, intestine, pancreas, lymphoid and myeloid cells and contributes to the extracellular matrix binding [1, 2]. It functions as a protease, cleaving dipeptides comprising of either proline or alanine at the penultimate position from the amino terminus of the peptide or protein [3, 4].

Glucagon like peptide 1 (GLP-1) [5] and glucose dependent insulinotropic polypeptide (GIP) are incretin hormones released from the gut in response to the food intake and are responsible for the glucose dependent stimulation of insulin secretion through pancreatic β-cells [6-10]. Furthermore, GLP-1 slows gastric emptying, stimulates regeneration and differentiation of pancreatic β-cells while inhibiting glucagon secretion [11-14]. But all these therapeutic effects of both these hormones are lost due to their rapid degradation ($t_{½}$~1 minute) by DPP-IV [5, 14, 15]. Thus inhibition of DPP-IV has emerged as a novel approach for the treatment of type 2 diabetes (T2D) [16-17].

Owing to DPP-IV's substrate specificity, various proline mimetics have been explored as DPP-IV inhibitors as shown in Figure 2.1.

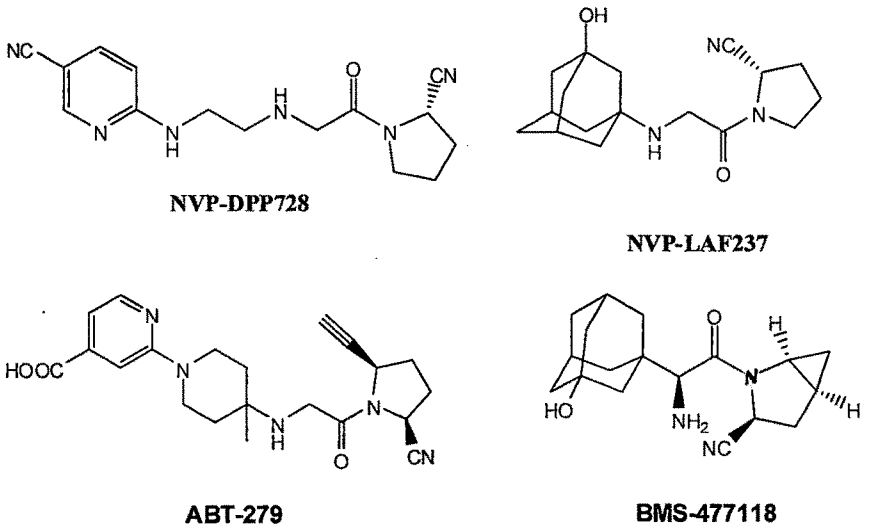


Figure 2.1: Some proline mimetic DPP-IV inhibitors.

Designing of the DPP-IV inhibitors is based on the general structure as shown in Figure 2.2.

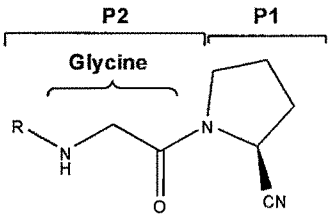


Figure 2.2: General structure of DPP-IV inhibitors.

Most of DPP-IV inhibitors reported till date have been designed taking into account the N-terminal dipeptide residue of enzymatic substrate which comprises of a proline mimic, usually a cyanopyrrolidine at the P1 site, coupled with an additional amino acid or a similar substituted amino acid at the P2 site by formation of an amide bond as shown in Figure 2.2.

Thus, in the design of DPP-IV inhibitor, a common structural motif comprises of an L-amino acid surrogate at the P1 site and a N-substituted glycine with a protonable amine responsible for the enhanced the potency of the inhibitor, at the P2 site [18].

A number of diverse N-substituted glycine when combined with 2S-cyanopyrrolidide at the P1 site have been reported to show better inhibition. Some laboratories have reported potent DPP-IV inhibitors 1(IC_{50} = 6.7 nM) [19] and 2 (K_i = 39 nM) [20] having sulfonamide at the P2 site as shown in Figure 2.3.

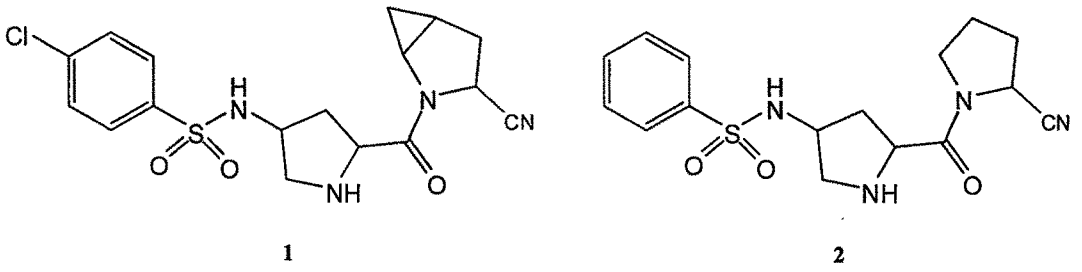


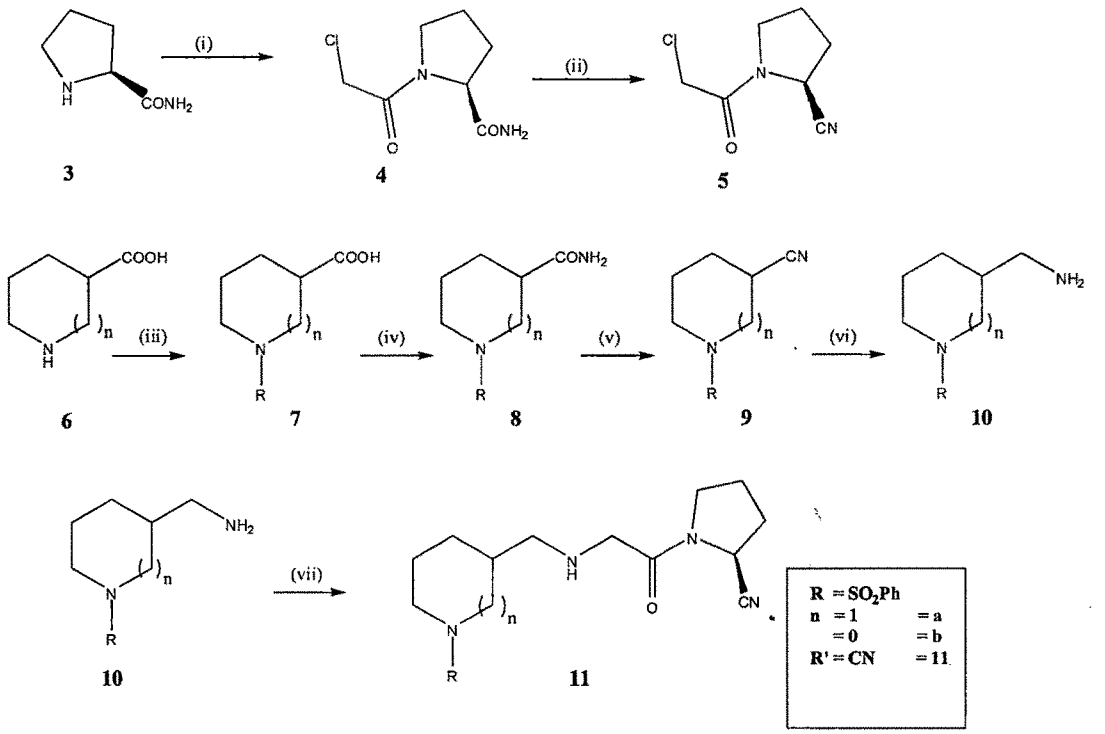
Figure 2.3: Some sulfonamide containing DPP-IV inhibitors.

Thus considering all these structure activity relationship studies, design and synthesis of novel DPP-IV inhibitors with sulfonamide derivatives at the P2 site while keeping the active pharmacophore same has been carried out and reported in this chapter. All the molecules synthesized were screened for *in-vitro* DPP-IV inhibition.

2.2 Results and Discussion

2.2.1 Chemistry

Small molecule DPP-IV inhibitors were synthesized using commercially available amino acids L-proline amide, L-proline and piperidine-3-carboxylic acid.



Scheme 2.1: Reagents (i) $ClCH_2COCl$, K_2CO_3, THF; (ii) TFAA, THF, NH_4HCO_3; (iii) (a) $PhSO_2Cl$, Na_2CO_3, DCM : H_2O (1:1); (b) HCl; (iv) EDCI, NH_4HCO_3, DCM; (v) TFAA, THF, NH_4HCO_3; (vi) LAH, THF, 10% NaOH; (vii) K_2CO_3, **5**, CH_3CN.

L-proline amide **3**, on reaction with chloroacetyl chloride gave *(S)*-1-(2-chloroacetyl)pyrrolidine-2-carboxamide **4** (Scheme 2.1), which on dehydration

with trifluoroacetic anhydride (TFAA) gave *(S)*-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile **5**. The IR spectrum of **5** (Figure 2.4.1) showed strong bands at 2241 and 1656 cm^{-1} for the nitrile and amide groups respectively while its ^1H NMR spectrum (Figure 2.4.2) showed peak at δ 4.076 for the methylene protons of glycine and multiplet at δ 4.69-4.71 for -CH proton of the cyanopyrrolidide and Figures 2.4.3 and 2.4.4 shows ^{13}C NMR and ESI-MS spectra of **5**, thus confirming its structure.

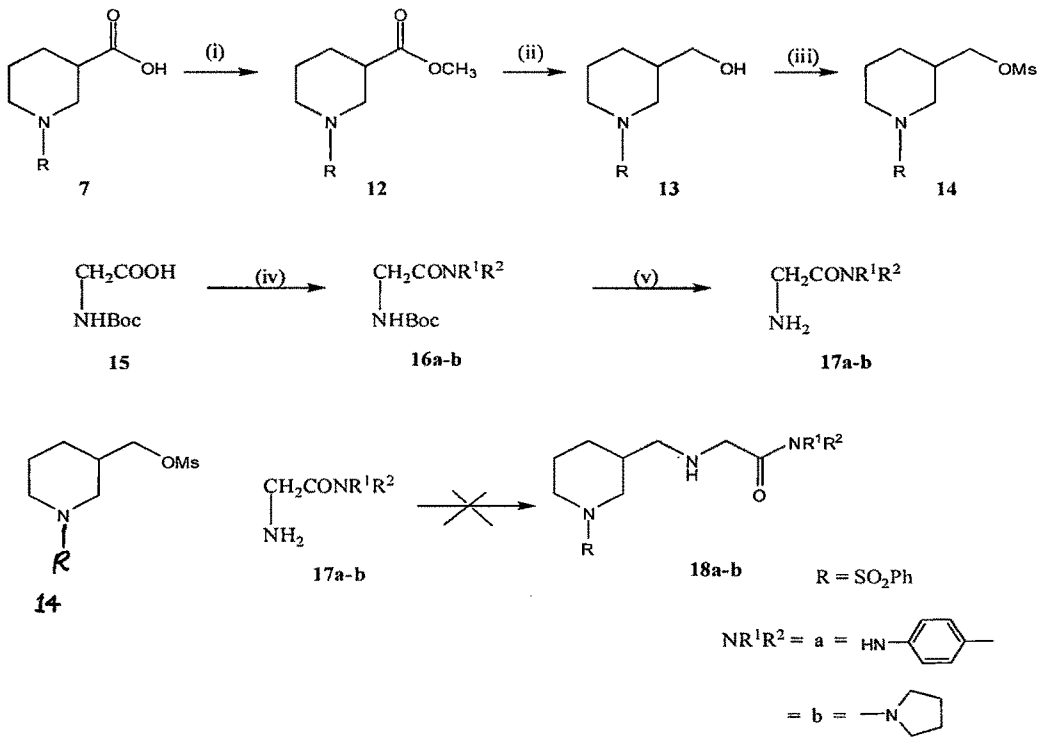
Piperidine-3-carboxylic acid **6a** or L-proline **6b** on reaction with benzene sulfonyl chloride in (1:1) dichloromethane : water (DCM : H_2O), in the presence of sodium carbonate as base gave corresponding sulfonamide **7a** or **7b** as shown in Scheme 2.1. The structures of **7a** or **7b** were confirmed by their IR spectrum (Figure 2.5.1) and ^1H NMR spectrum (Figure 2.5.2) which clearly showed presence of aromatic protons at δ 7.54-7.79 along with aliphatic $-CH_2$ protons of piperidyl or pyrrolidyl ring from δ 1.65-3.83. A broad singlet at δ 8.89 indicated the presence of carboxylic acid proton which disappeared on formation of **8a**. Figures 2.5.3and 2.5.4 shows ^{13}C NMR and ESI-MS spectrum of **7a**. Figures 2.6.1, 2.6.2, 2.6.3 and 2.6.4 show IR, ^1H NMR, ^{13}C NMR and ESI-MS spectra of **7b** respectively. The carboxylic acid group of **7a**, **7b** was then coupled with ammonium bicarbonate in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole (HOBt) in DCM to give corresponding amide **8a, 8b** respectively. The IR spectrum of **8a** (Figure 2.7.1) showed two bands at 3346 and 3173 cm^{-1} for $-NH_2$ group and a band at 1665 cm^{-1} for the carbonyl group of the amide. In the ^1H NMR of **8a** (Figure 2.7.2), disappearance of peak at δ 8.89 for –COOH proton and appearance of two new peaks at δ 6.96 and 7.46 for $-NH_2$ protons which disappeared on D_2O exchange thus confirmed the formation of **8a**.

Figures 2.7.3 and 2.7.4 show ^{13}C NMR and ESI-MS spectrum of **8a** respectively. Similarly, Figures 2.8.1, 2.8.2, 2.8.3 and 2.8.4 respectively show IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of **8b**, thus confirms the structure of **8b**. Addition of trifluoroacetic anhydride (TFAA) to a solution of **8a-b** in tetrahydrofuran (THF) leads to dehydration of amide, thereby yielding nitrile **9a-b** which was confirmed by its IR. The IR spectrum of **9a** (Figure 2.9.1) showed strong band at 2239 cm^{-1} for nitrile group. In the ^{1}H NMR of **9a** (Figure 2.9.2), the two peaks at δ 6.08 and 6.94 for the $-NH_2$ protons of **8a** disappeared thus supported the formation of **9a.** Further, structure of **9a** was confirmed by its ^{13}C NMR (Figure 2.9.3) and ESI-MS (Figure 2.9.4) spectra and Figures 2.10.1, 2.10.2, 2.10.3 and 2.10.4 shows IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of **9b** respectively, thus confirms its structure. **9a-b** was reduced to its corresponding amine **10a-b** by lithium aluminium hydride (LAH). The formation of **10a** was confirmed from its ^{1}H NMR (Figure 2.11.1) and ESI-MS spectrum (Figure 2.11.2). Reaction of amines **10a** and **10b** with **5** gave compounds **11a-b** respectively, as shown in Scheme 2.1. Figure 2.12.1, 2.12.2, 2.12.3 and 2.12.4 shows IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of **11a** respectively.

Attempts were made to synthesize molecules with substitution of amines other than those derived from proline at the P1 site while substituting sulphonamide at the P2 site.

For this purpose compound **7** was at first reacted with oxalyl chloride to give a reactive intermediate, acid chloride which on further reaction with methanol gave corresponding methyl ester **12** as shown in Scheme 2.2. The structure of **12** was confirmed from its IR spectrum (Figure 2.13.1) which showed a band at 1726 cm^{-1} characteristic of carbonyl of the ester with the disappearance of a broad band at 3100-2500 cm^{-1} for the –OH of

carboxyl group and the ^1H NMR spectrum (Figure 2.13.2) wherein a peak at δ 8.98 for the –COOH proton of **7** disappears and a singlet at δ 3.59 for the $-OCH_3$ protons of the ester appears; ^{13}C NMR spectrum (Figure 2.13.3) and also its ESI-MS spectrum (Figure 2.13.4) with a peak at m/z 283.9 for $[M+H]^+$ confirms the formation of methyl ester **12.** The methyl ester **12** on reduction with lithium aluminium hydride (LAH) yielded (1-(phenylsulfonyl)piperidin-3-yl)methanol **13.**



Scheme 2.2: Reagents: (i) (a) $C_2O_2Cl_2$, DCM; (b) CH_3OH; (ii) LAH, THF; (iii) CH_3SO_2Cl, Et_3N, DCM; (iv) EDCI, HOBt, DMAP, various amines, DCM; (v) TFA, DCM.

The IR spectrum of **13** (Figure 2.14.1) showed absence of a band at 1726 cm^{-1} for the carbonyl of ester functionality while a strong band at 3531 cm^{-1} for the –OH group supports the structure of **13** which is also supported by its ^1H NMR spectrum (Figure 2.14.2) wherein a multiplet at δ 3.50-3.60 for the two $-CH_2$ protons of methanol group is observed and ESI-MS spectrum (Figure 2.14.3) with a $[M+H]^+$ peak at *m/z* 256.0 confirmed formation of **13**. Reaction of **13** with methane sulfonyl chloride in the presence of base, triethylamine gave **14**. The formation of **14** could be confirmed from its ^1H NMR spectrum (Figure 2.15.1) wherein a singlet at δ 3.07 for the $-CH_3$ protons of the mesylate group was observed. The reaction of **14** with boc-de-protected glycylamide **17a-b** failed and products **18a-b** could not be isolated. The structure of **17a** has been proved in by its IR, ^1H NMR, ^{13}C NMR and ESI-MS spectra as shown in Figures 5.5.1, 5.5.2, 5.5.3, 5.5.4 **(Chapter 5)** respectively. Also, the IR, ^1H NMR, ^{13}C NMR and ESI-MS spectra: Figures 5.8.1, 5.8.2, 5.8.3, 5.8.4 **(Chapter 5)** respectively, prove the structure of **17b** .

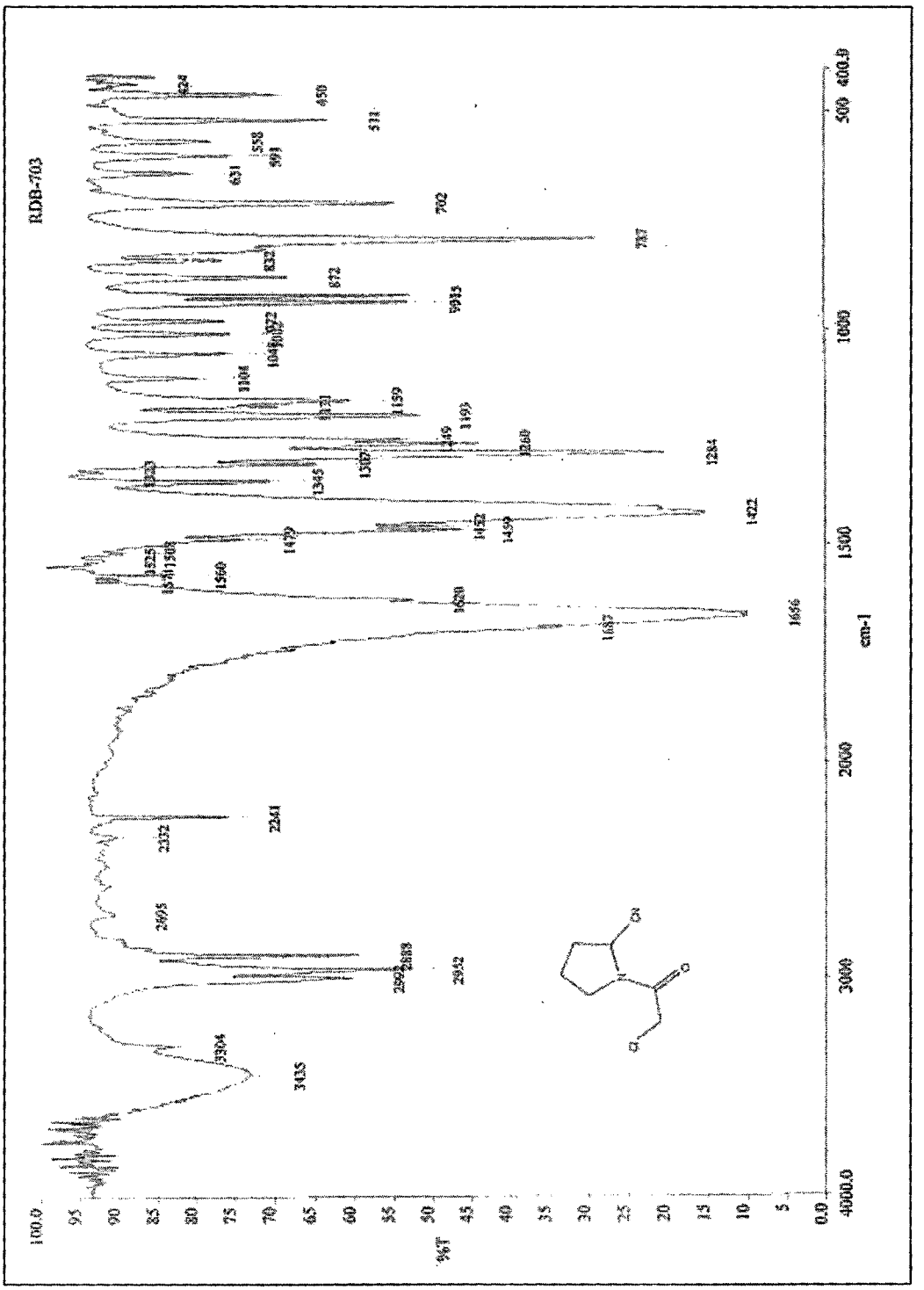


Figure 2.4.1: IR spectrum of *(S)*-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile **5a**

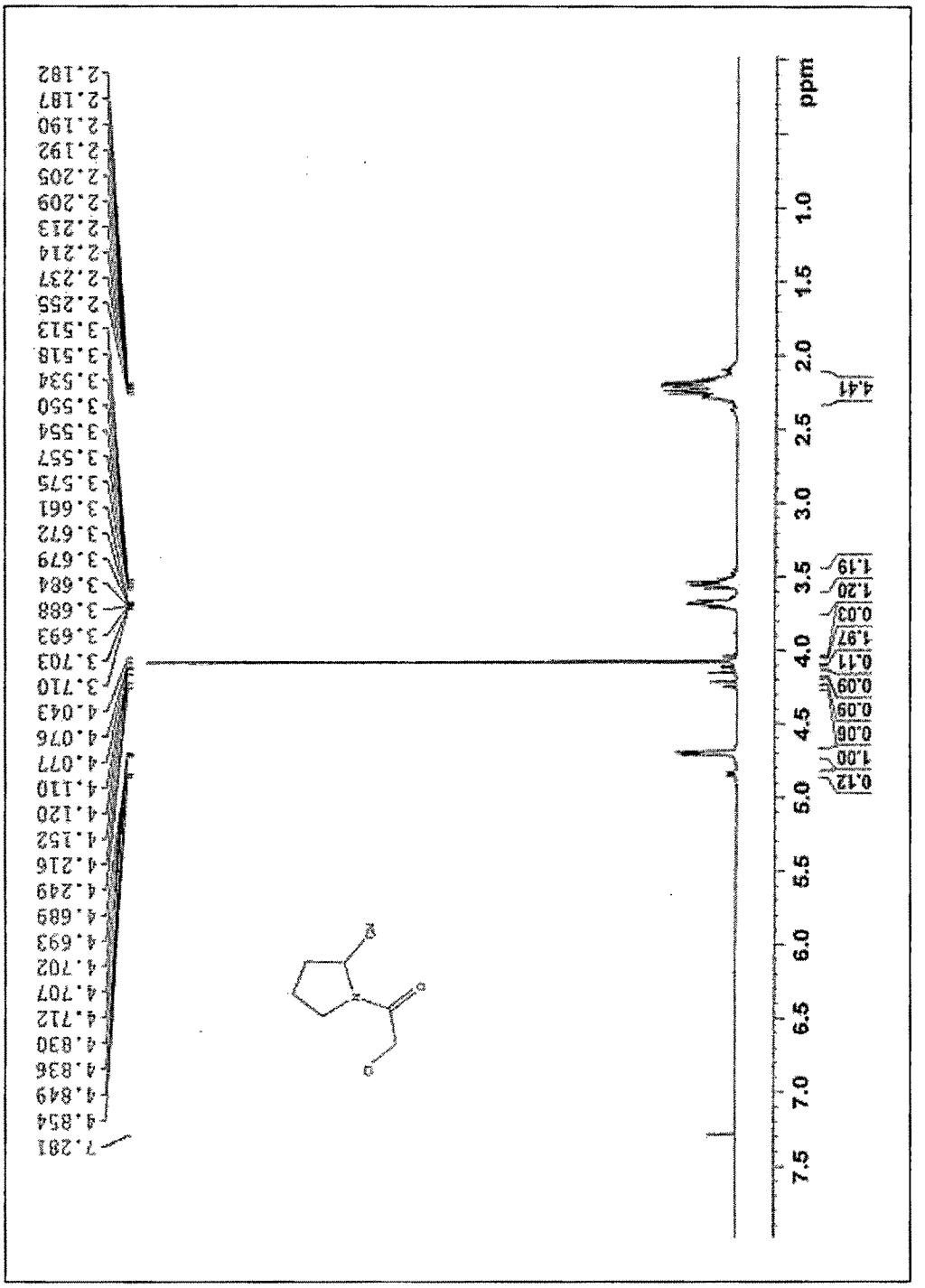


Figure 2.4.2: ^{1}H NMR spectrum of *(S)*-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile **5a**

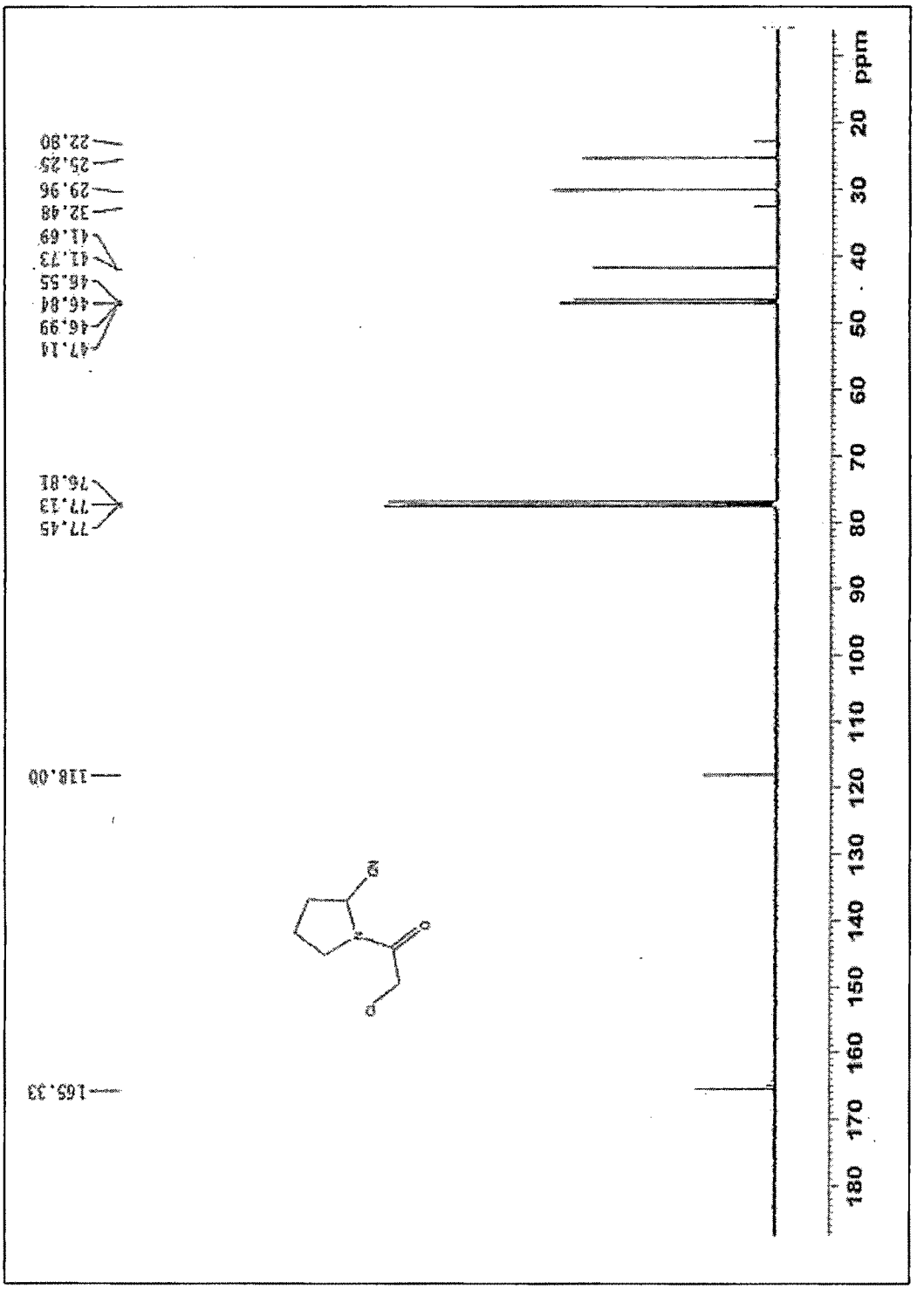


Figure 2.4.3: ^{13}C NMR spectrum of *(S)*-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile **5a**

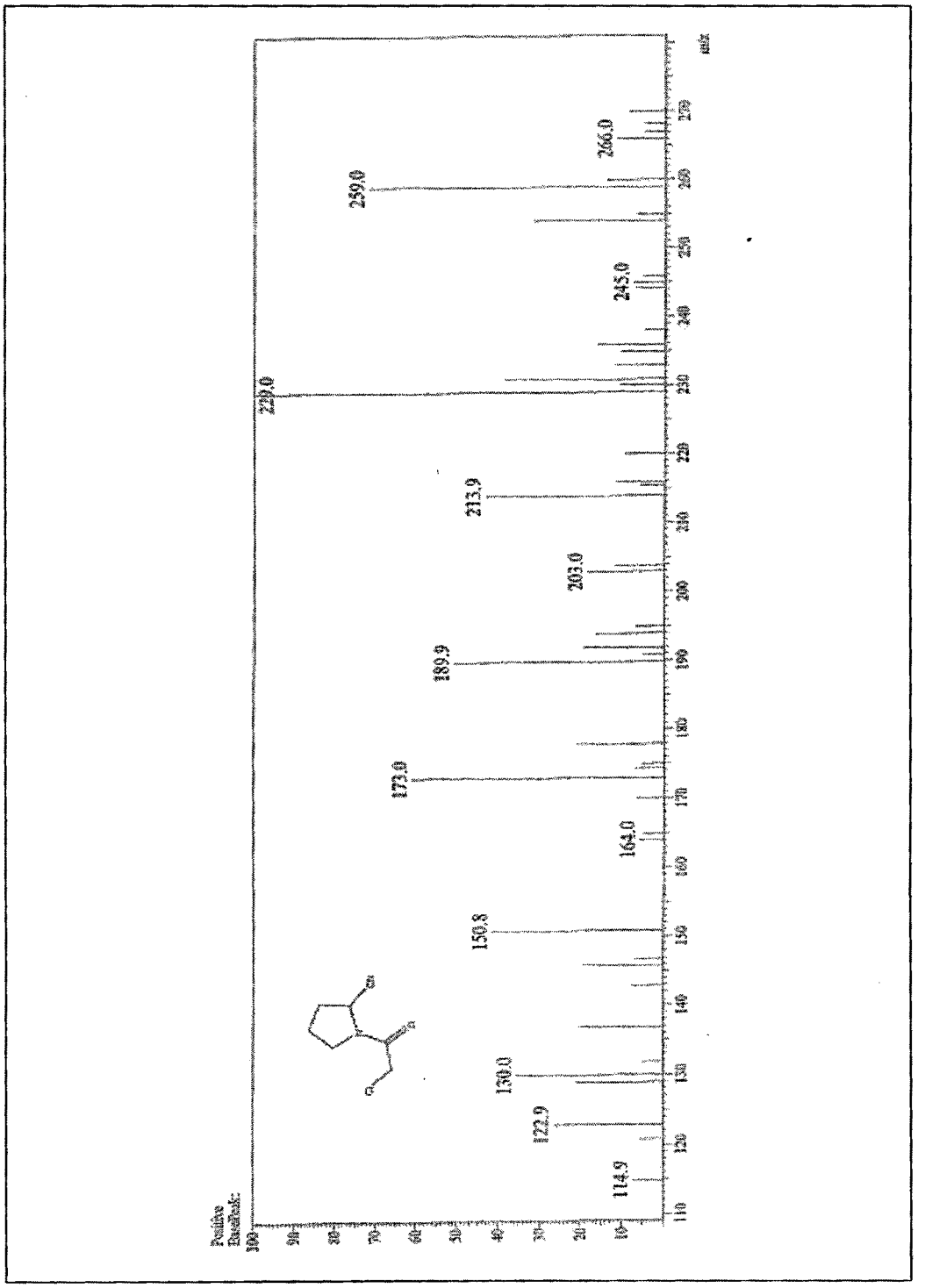


Figure 2.4.4: ESI-MS spectrum of *(S)*-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile **5a**

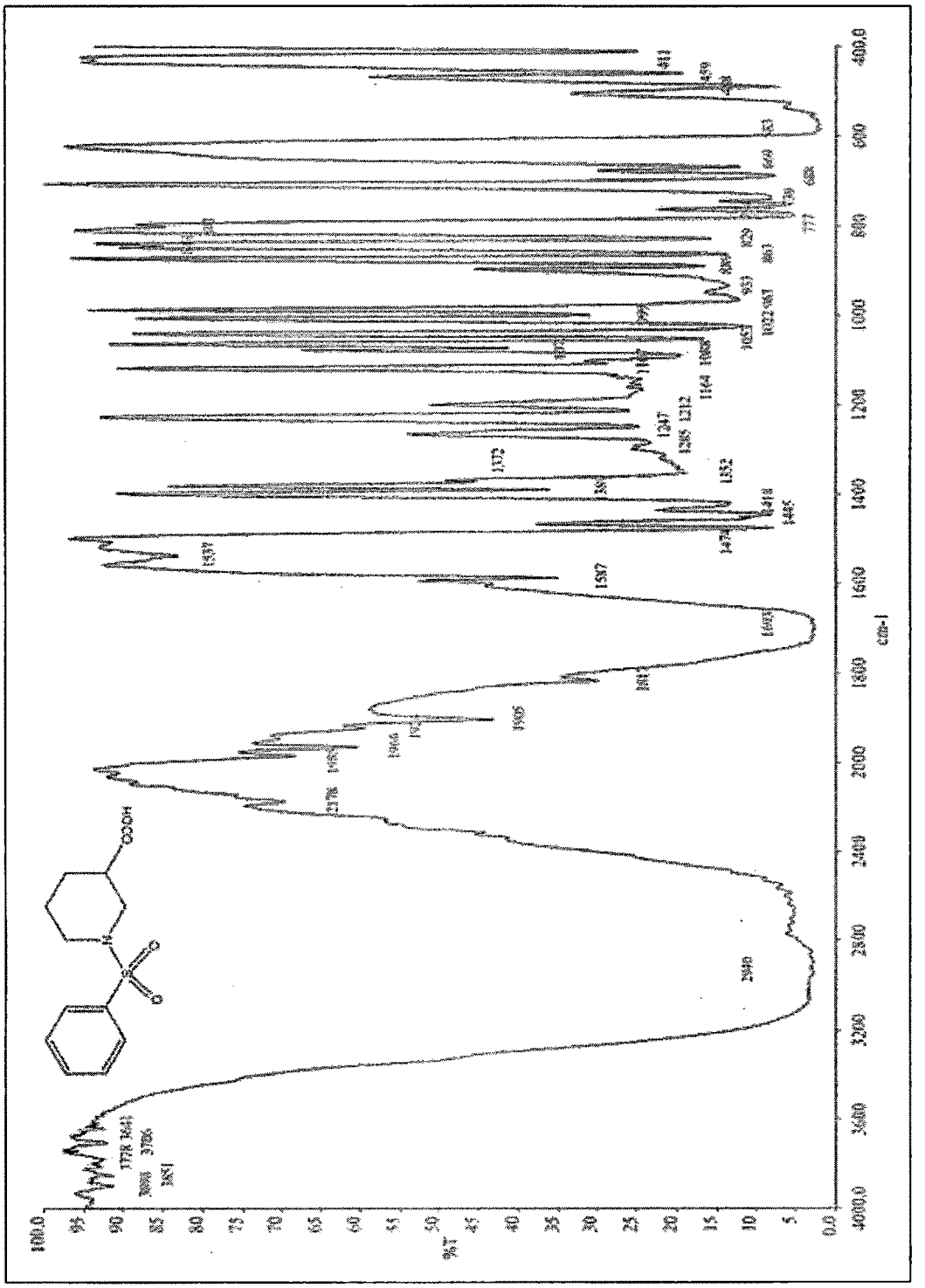


Figure 2.5.1: IR spectrum of 1-(phenylsulfonyl)piperidine-3-carboxylic acid **7a**

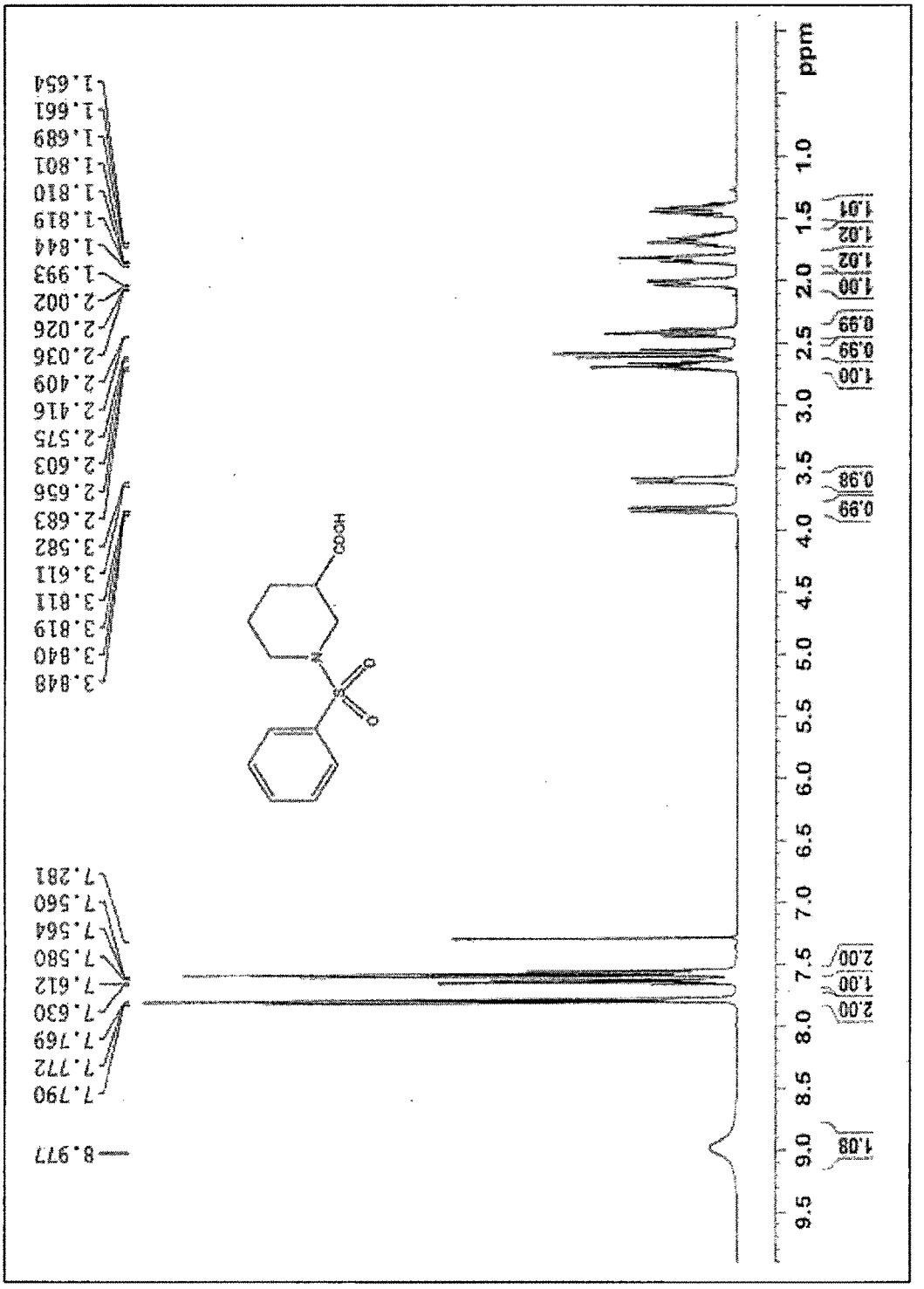


Figure 2.5.2: ^{1}H NMR spectrum of 1-(phenylsulfonyl)piperidine-3-carboxylic acid **7a**

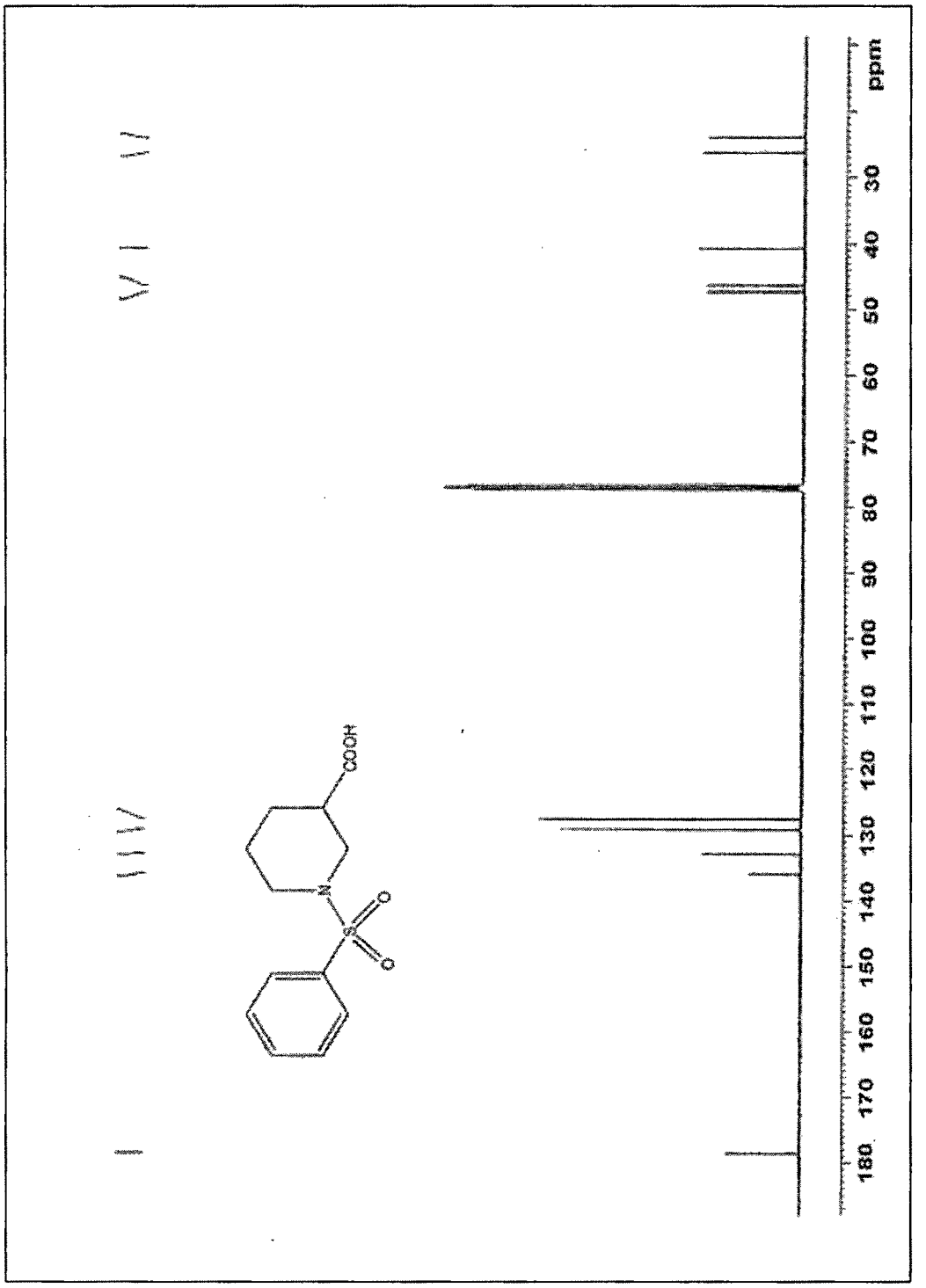


Figure 2.5.3: ^{13}C NMR spectrum of 1-(phenylsulfonyl)piperidine-3-carboxylic acid **7a**

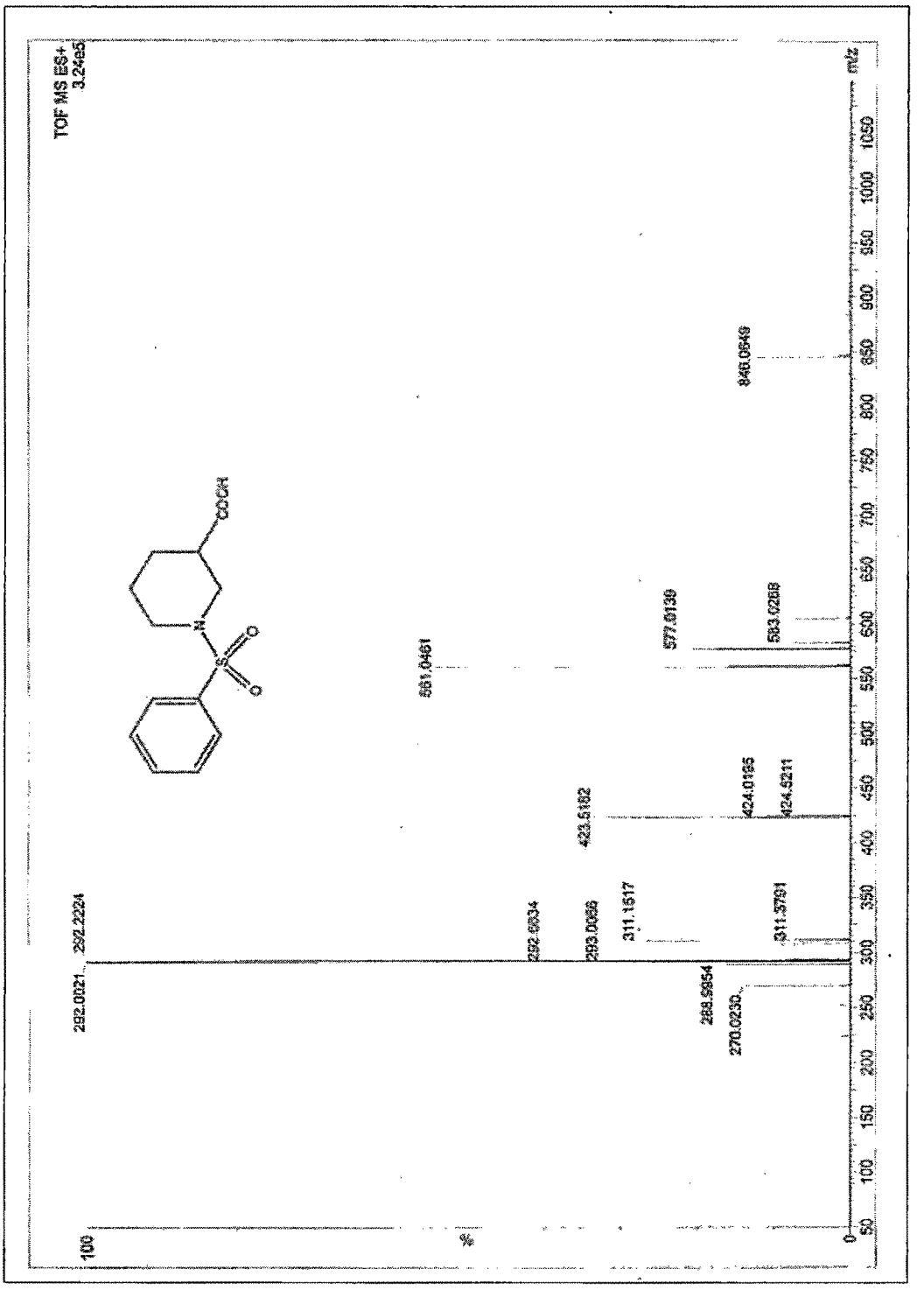


Figure 2.5.4: ESI-MS spectrum of 1-(phenylsulfonyl)piperidine-3-carboxylic acid **7a**

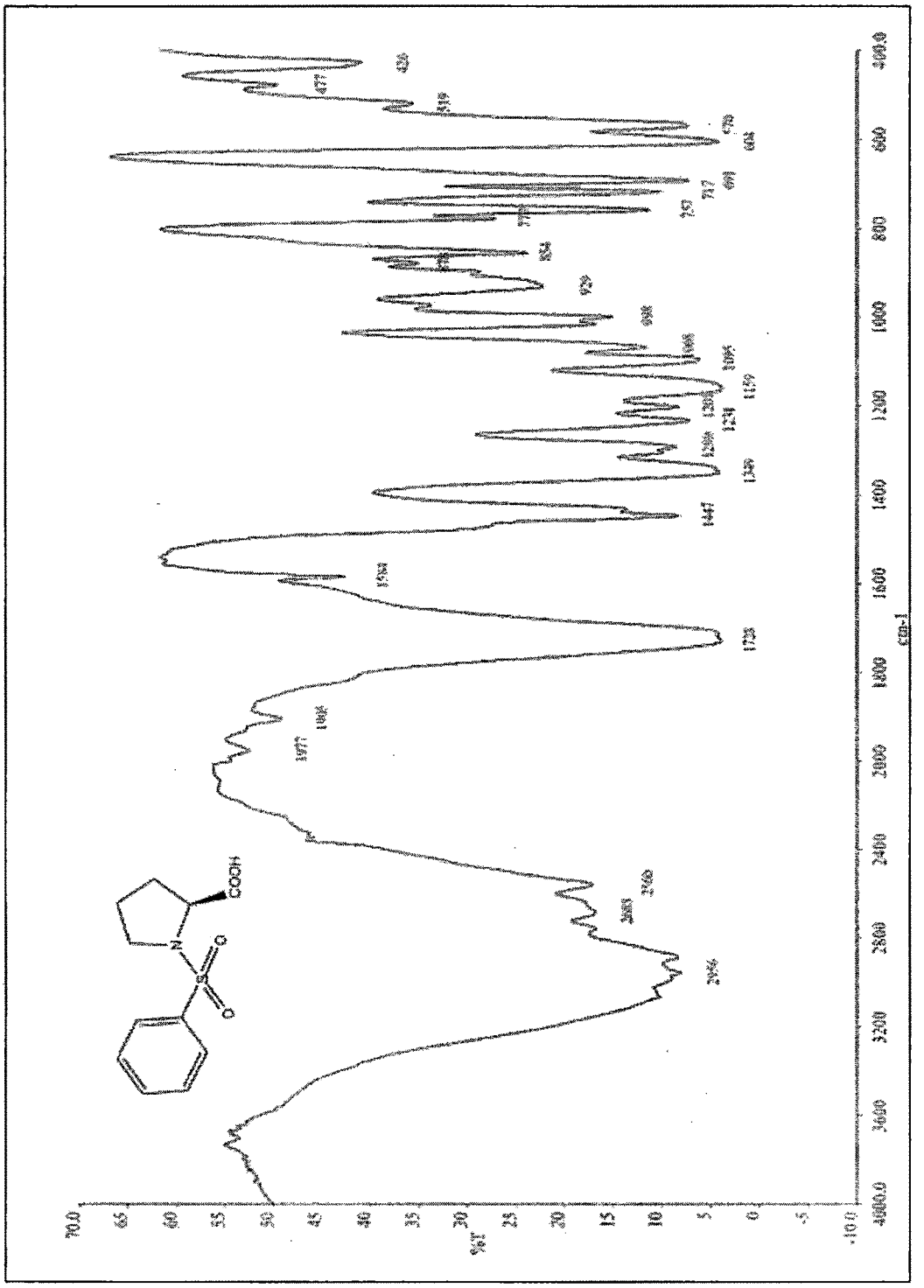


Figure 2.6.1: IR spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carboxylic acid **7b**

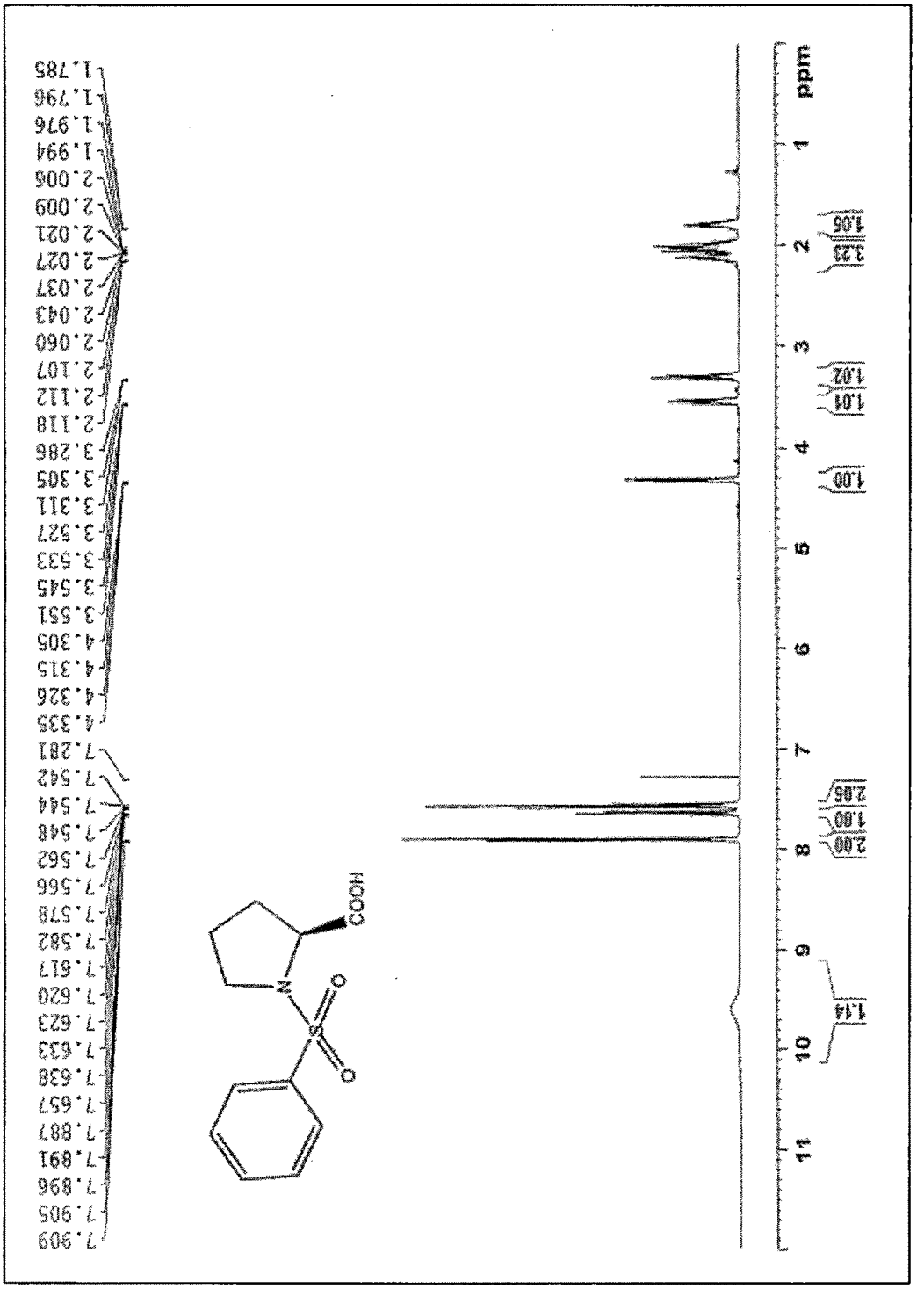


Figure 2.6.2: ^{1}H NMR spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carboxylic acid **7b**

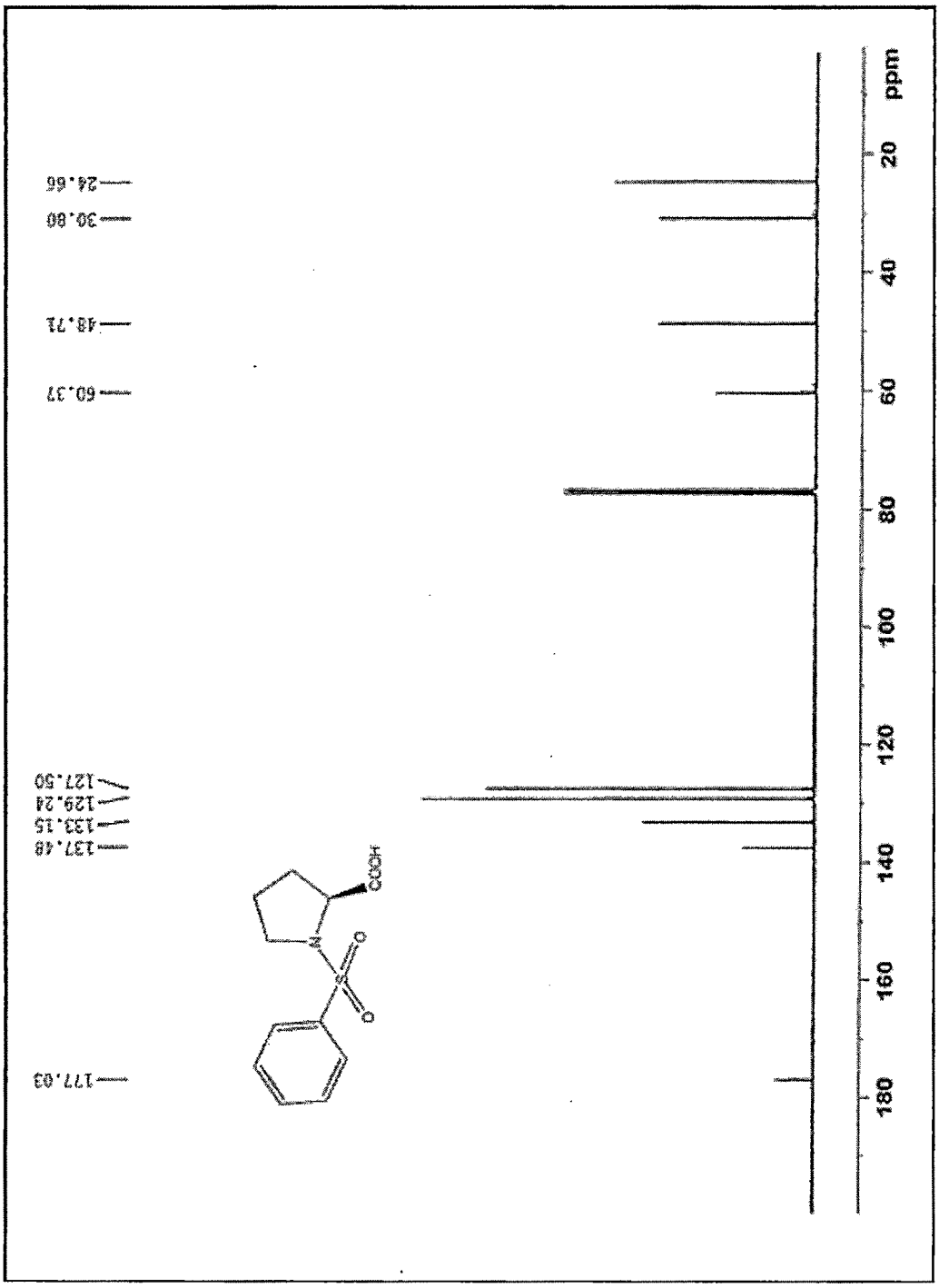


Figure 2.6.3: ^{13}C NMR spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carboxylic acid **7b**

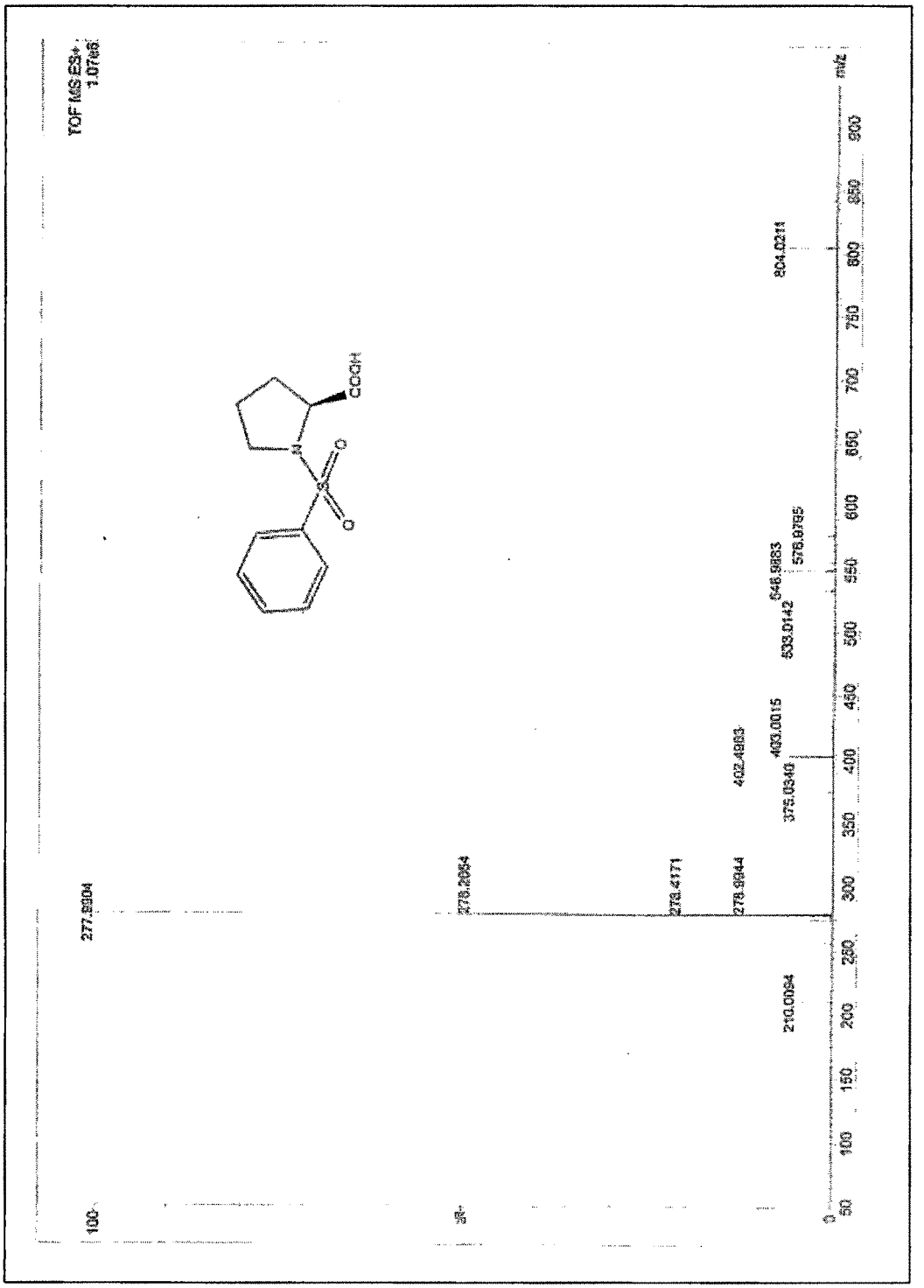


Figure 2.6.4: ESI-MS spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carboxylic acid **7b**

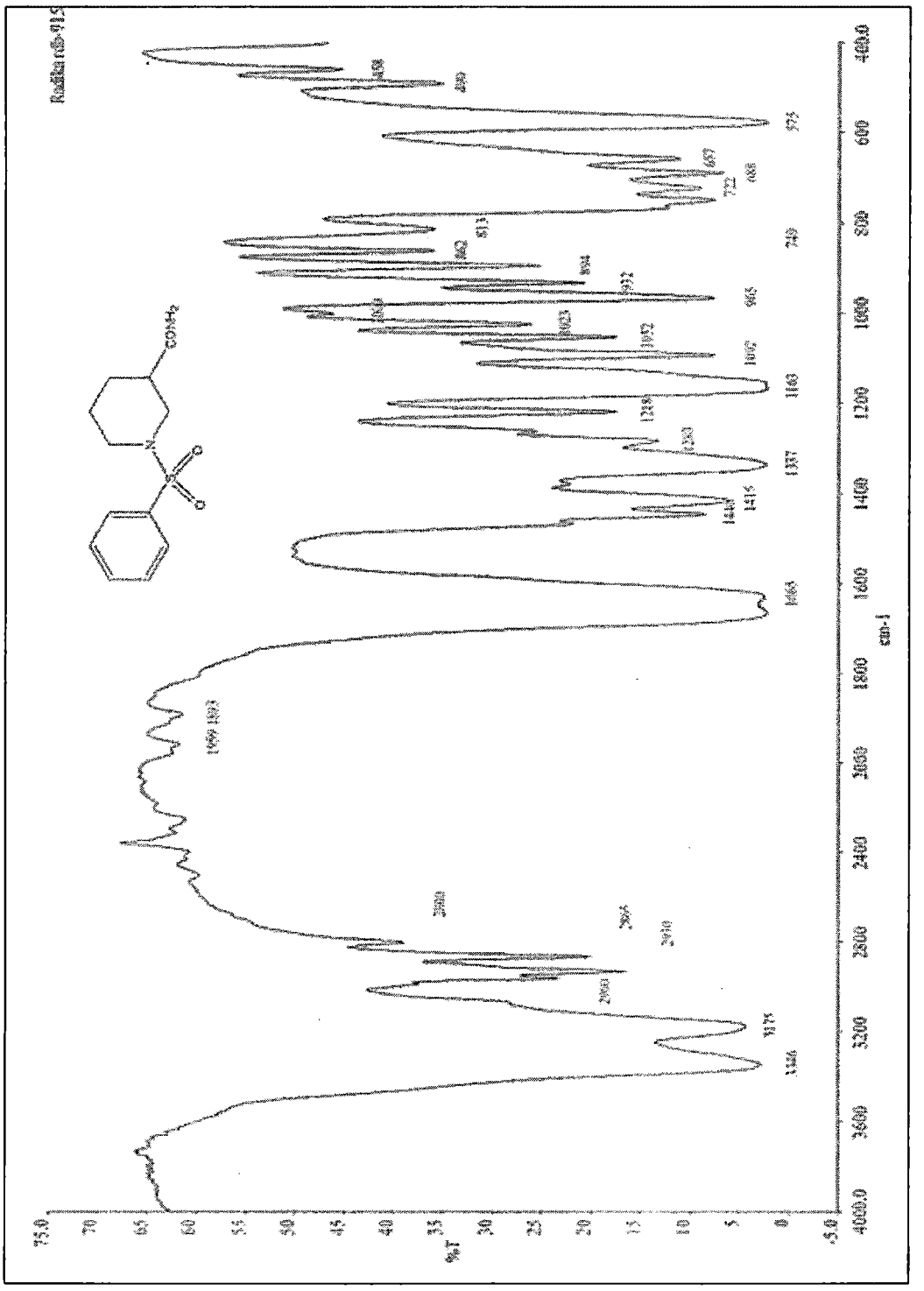


Figure 2.7.1: IR spectrum of 1-(phenylsulfonyl)piperidine-3-carboxamide **8a**

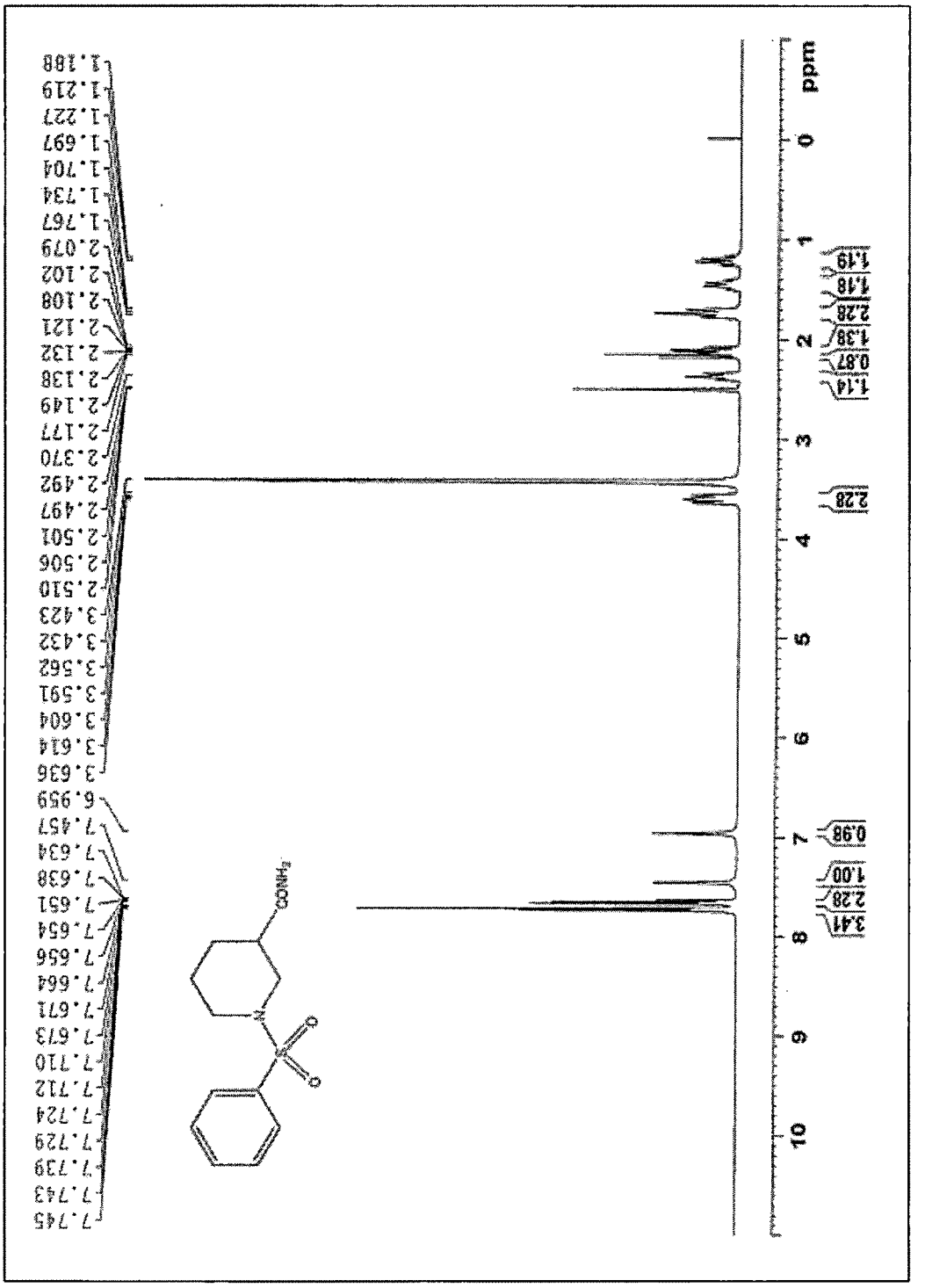


Figure 2.7.2: ^{1}H NMR spectrum of 1-(phenylsulfonyl)piperidine-3-carboxamide **8a**

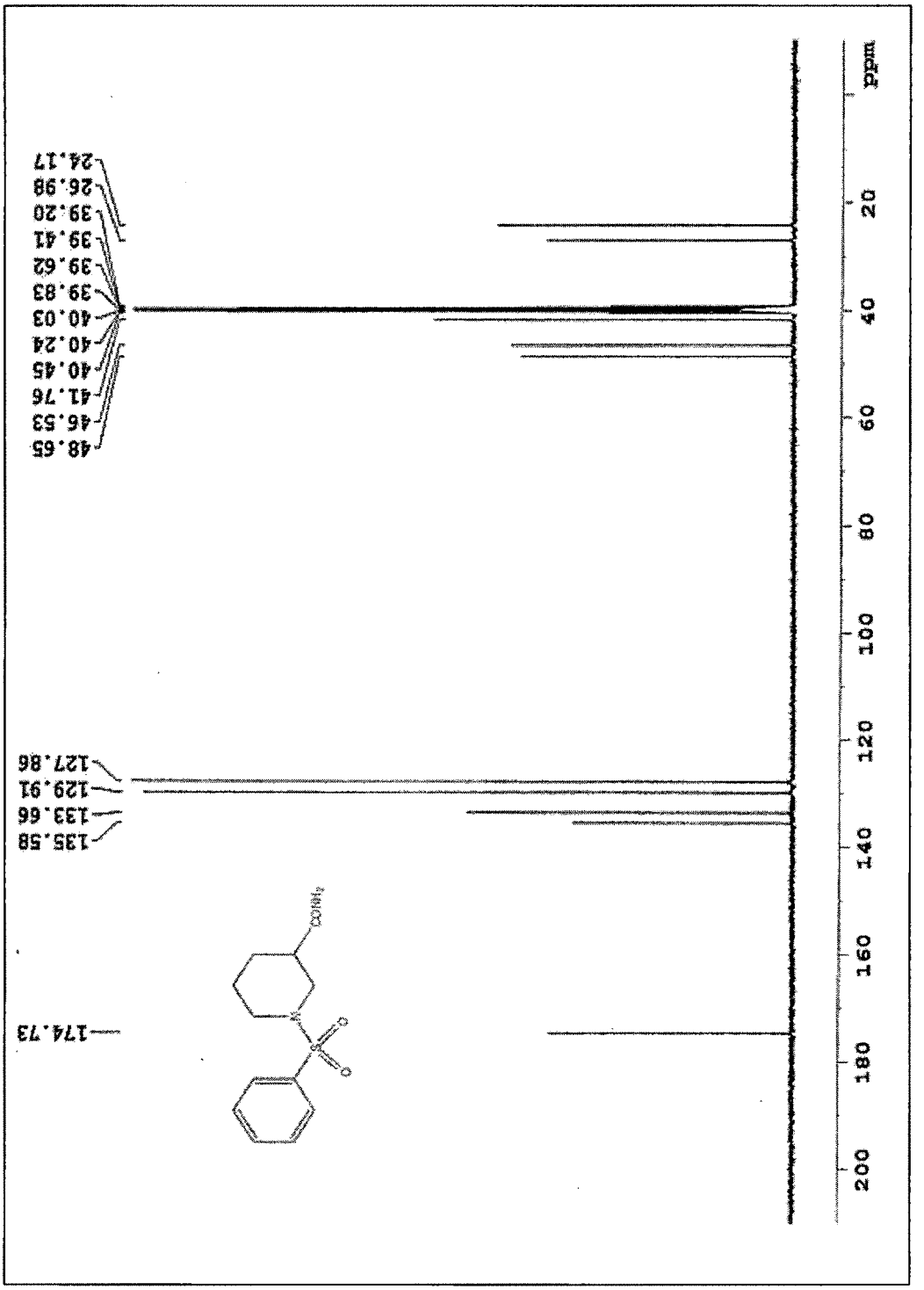


Figure 2.7.3: ^{13}C NMR spectrum of 1-(phenylsulfonyl)piperidine-3-carboxamide **8a**

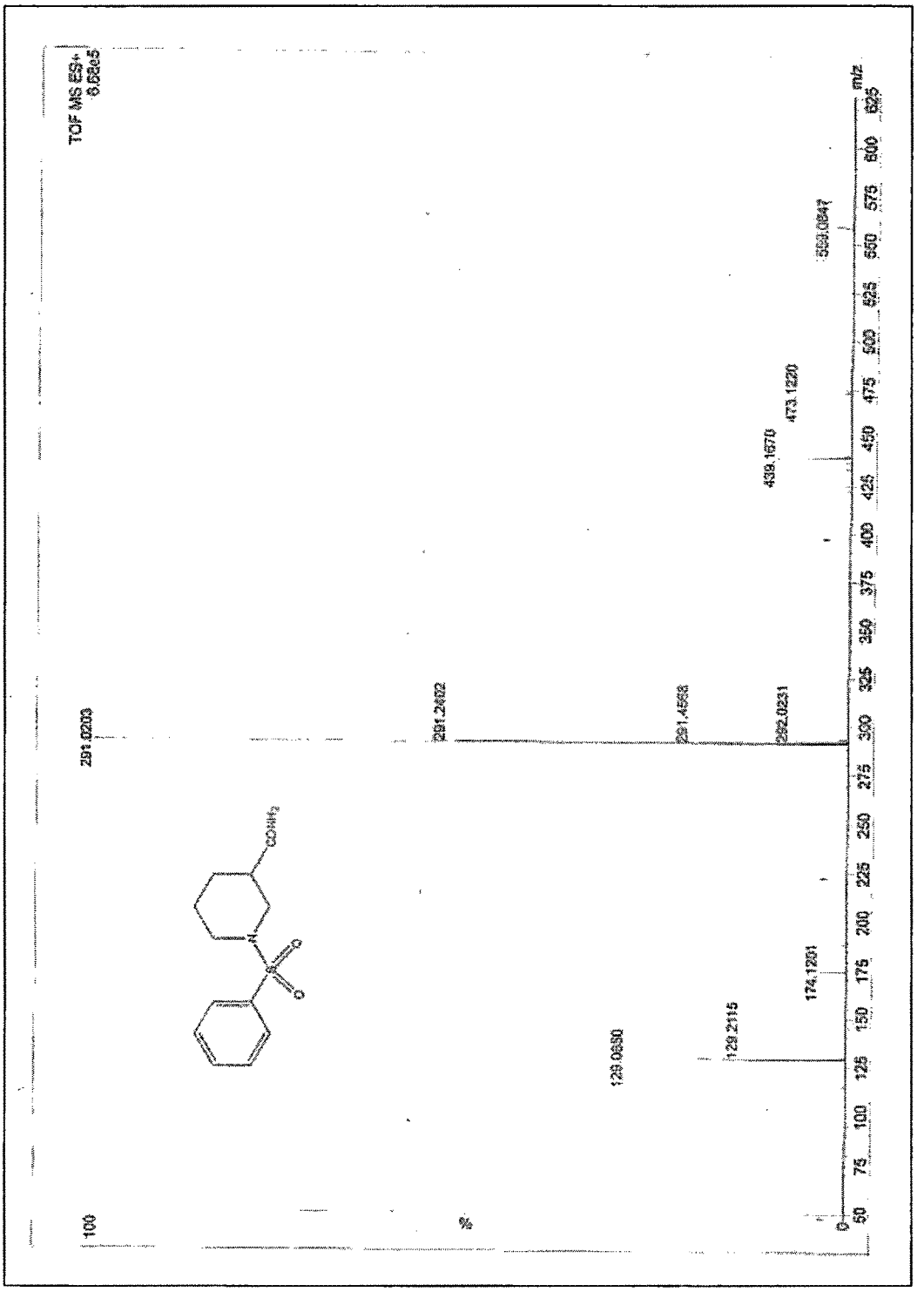


Figure 2.7.4: ESI-MS spectrum of 1-(phenylsulfonyl)piperidine-3-carboxamide **8a**

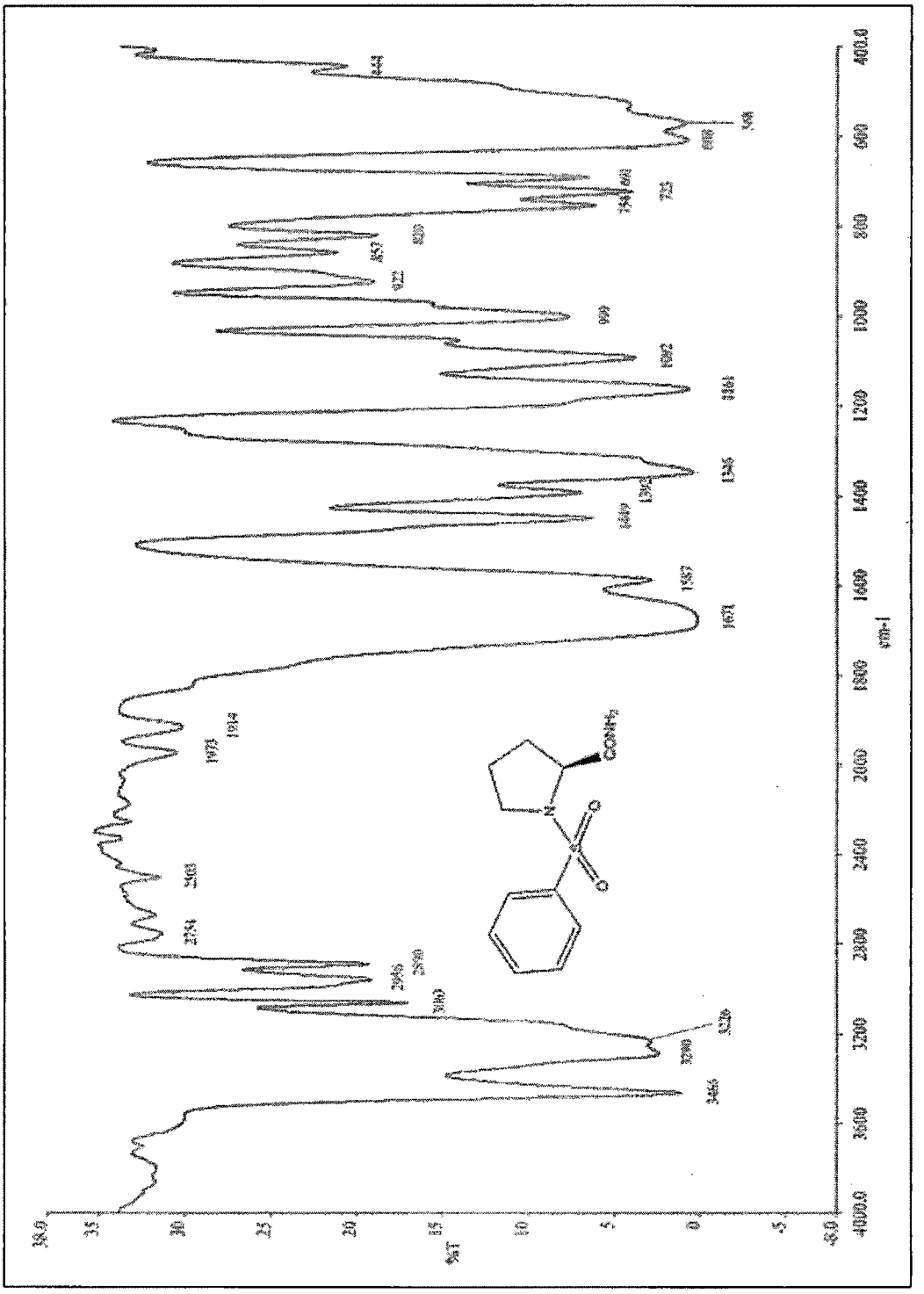


Figure 2.8.1: IR spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carboxamide **8b**

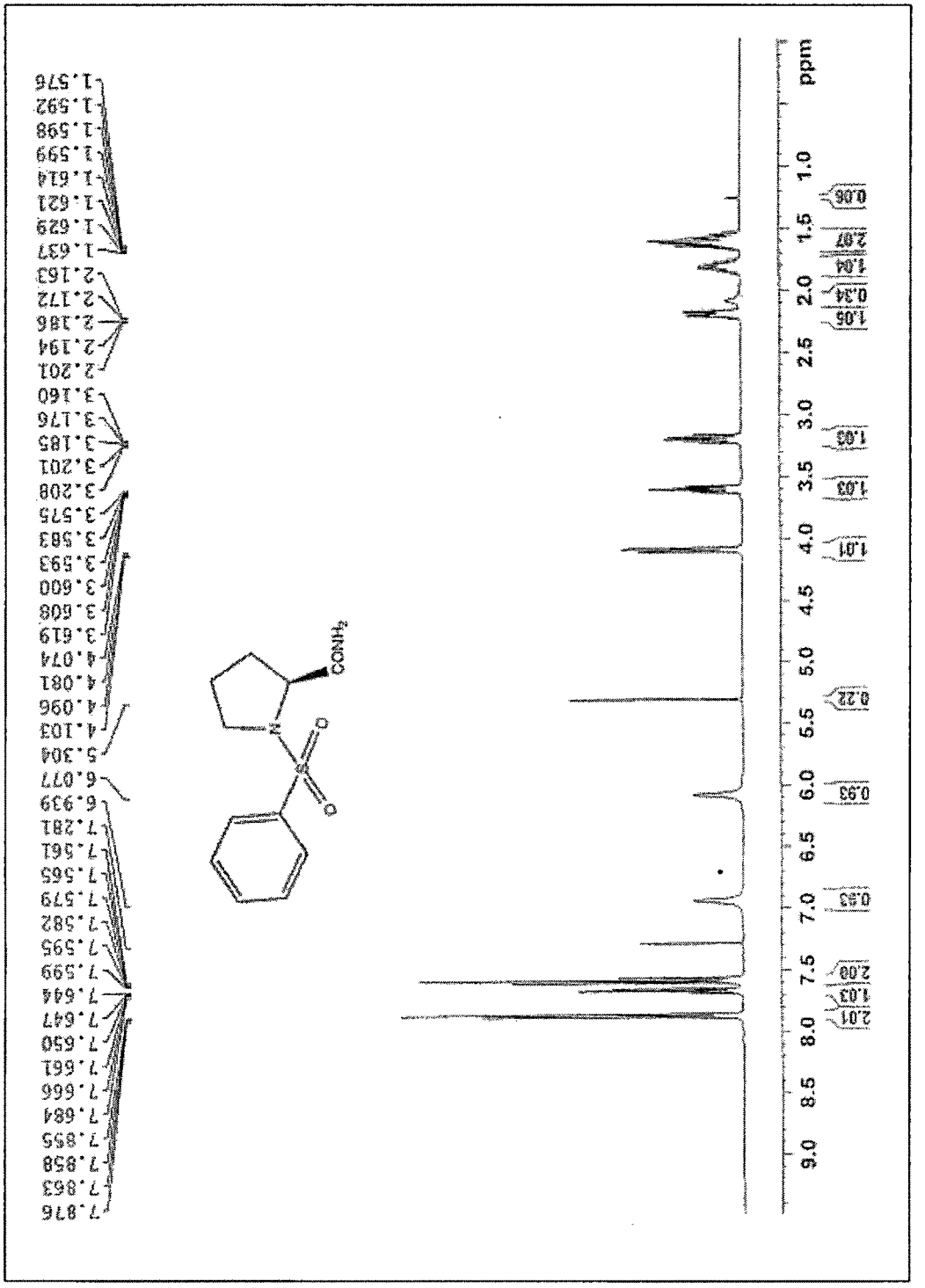


Figure 2.8.2: ^{1}H NMR spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carboxamide **8b**

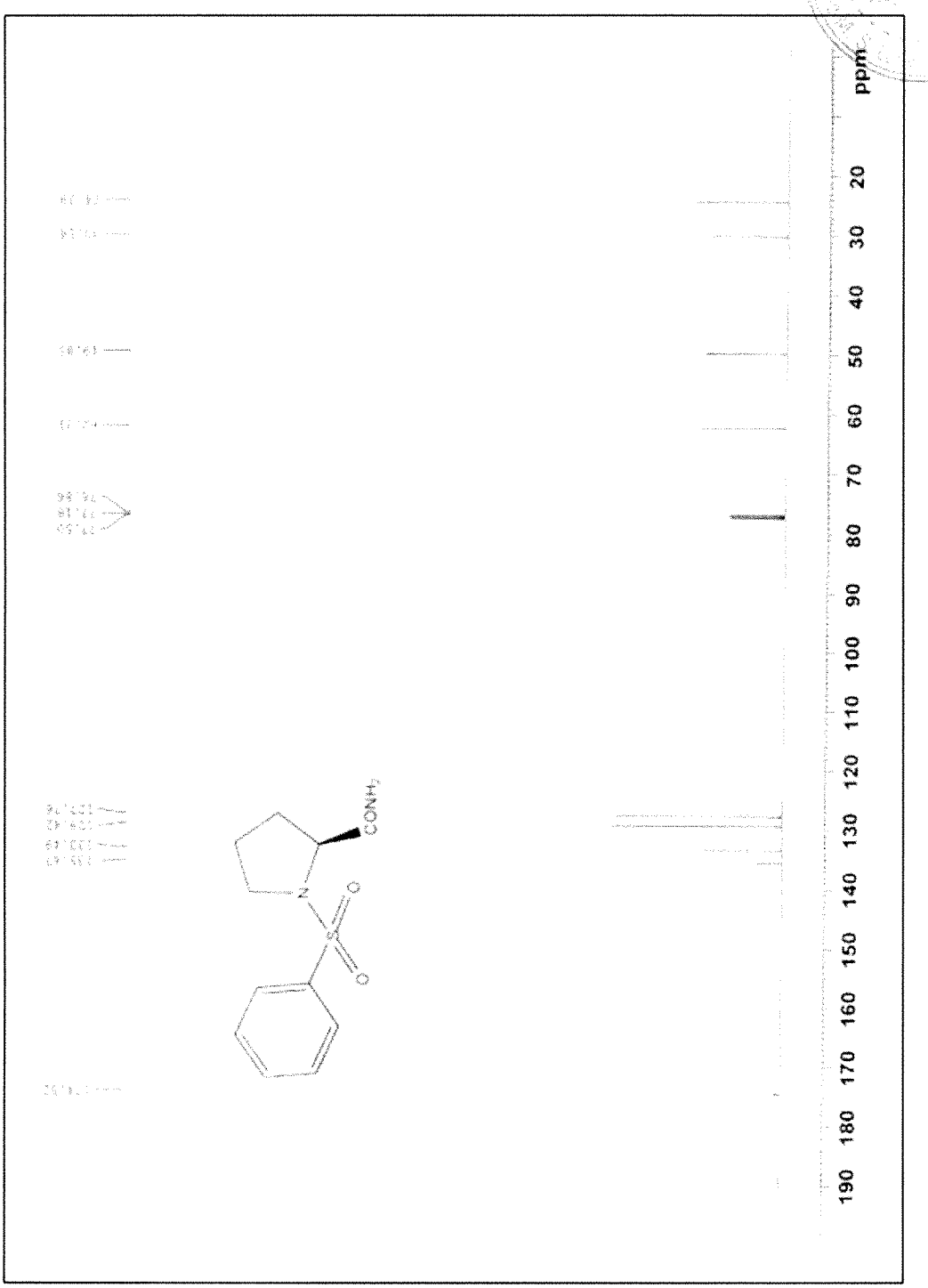


Figure 2.8.3: ^{13}C NMR spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carboxamide **8b**

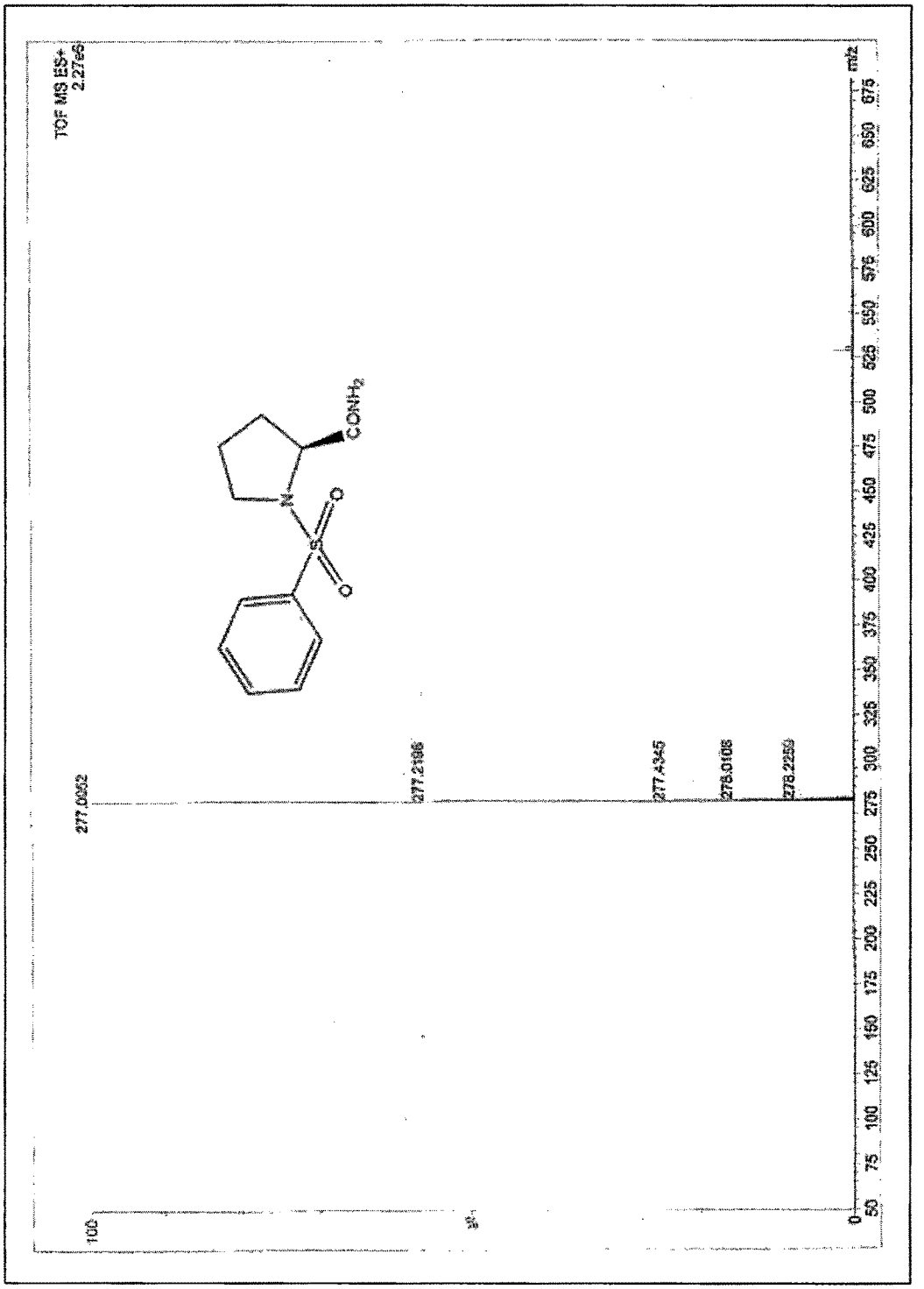


Figure 2.8.4: ESI-MS spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carboxamide **8b**

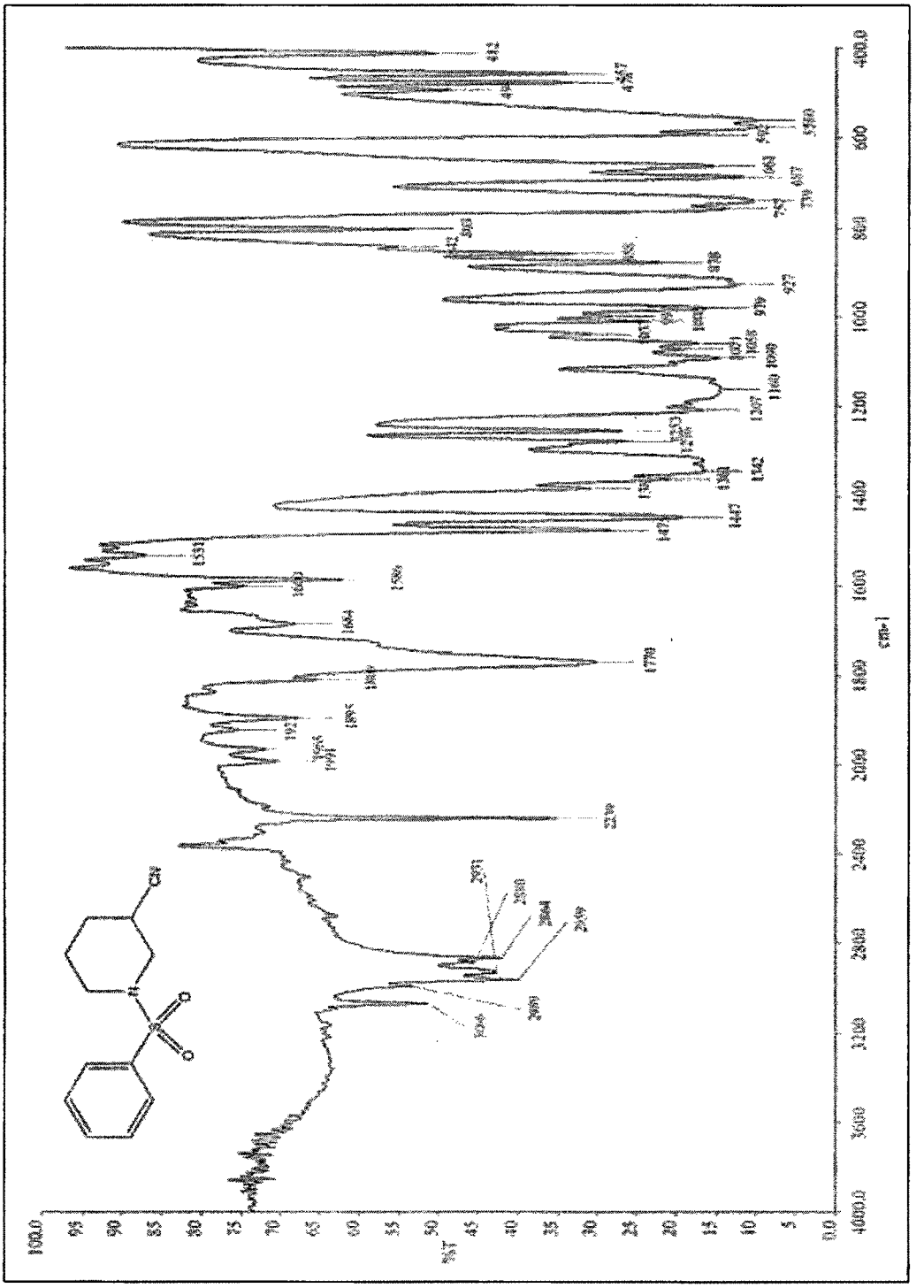


Figure 2.9.1: IR spectrum of 1-(phenylsulfonyl)piperidine-3-carbonitrile **9a**

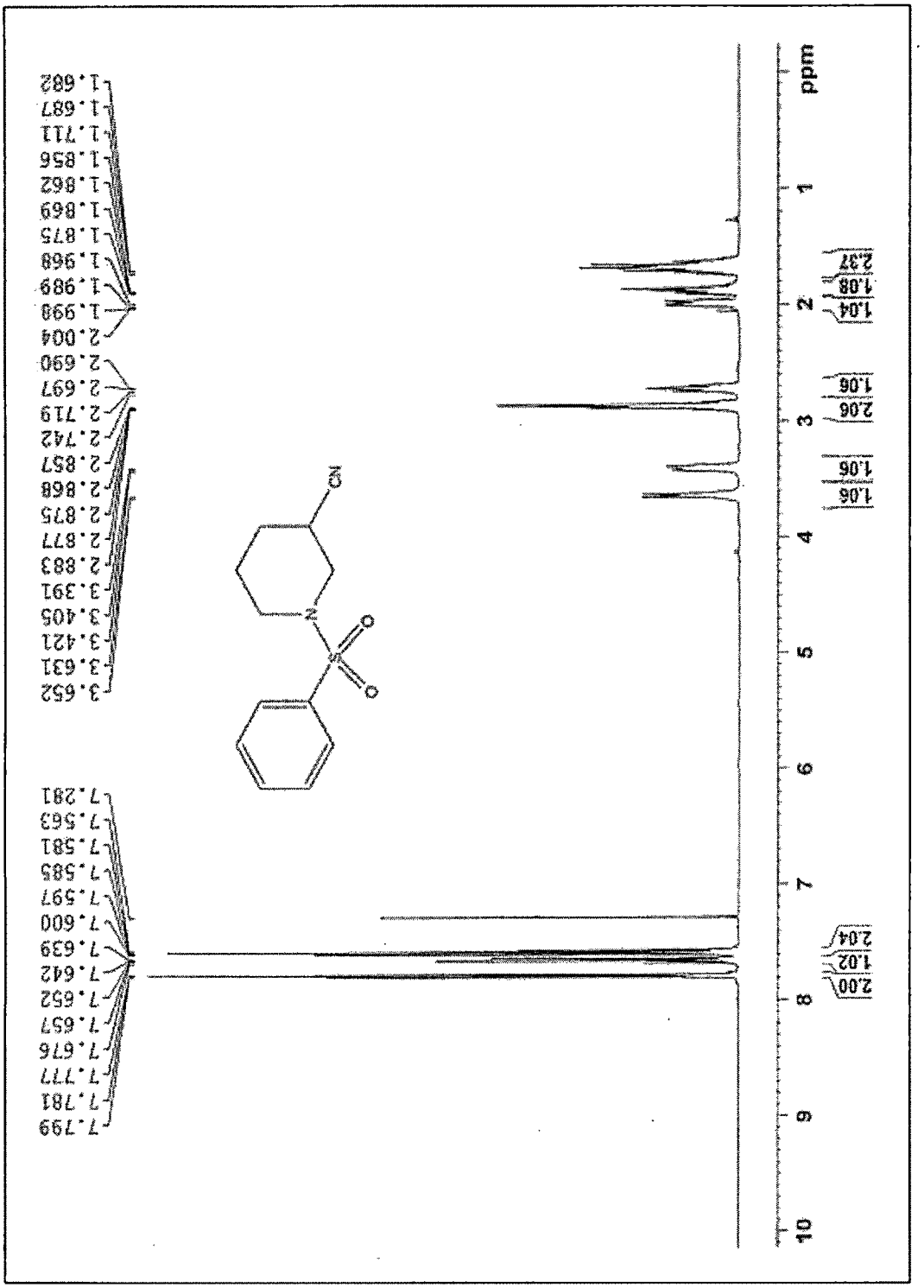


Figure 2.9.2: ^{1}H NMR spectrum of 1-(phenylsulfonyl)piperidine-3-carbonitrile **9a**

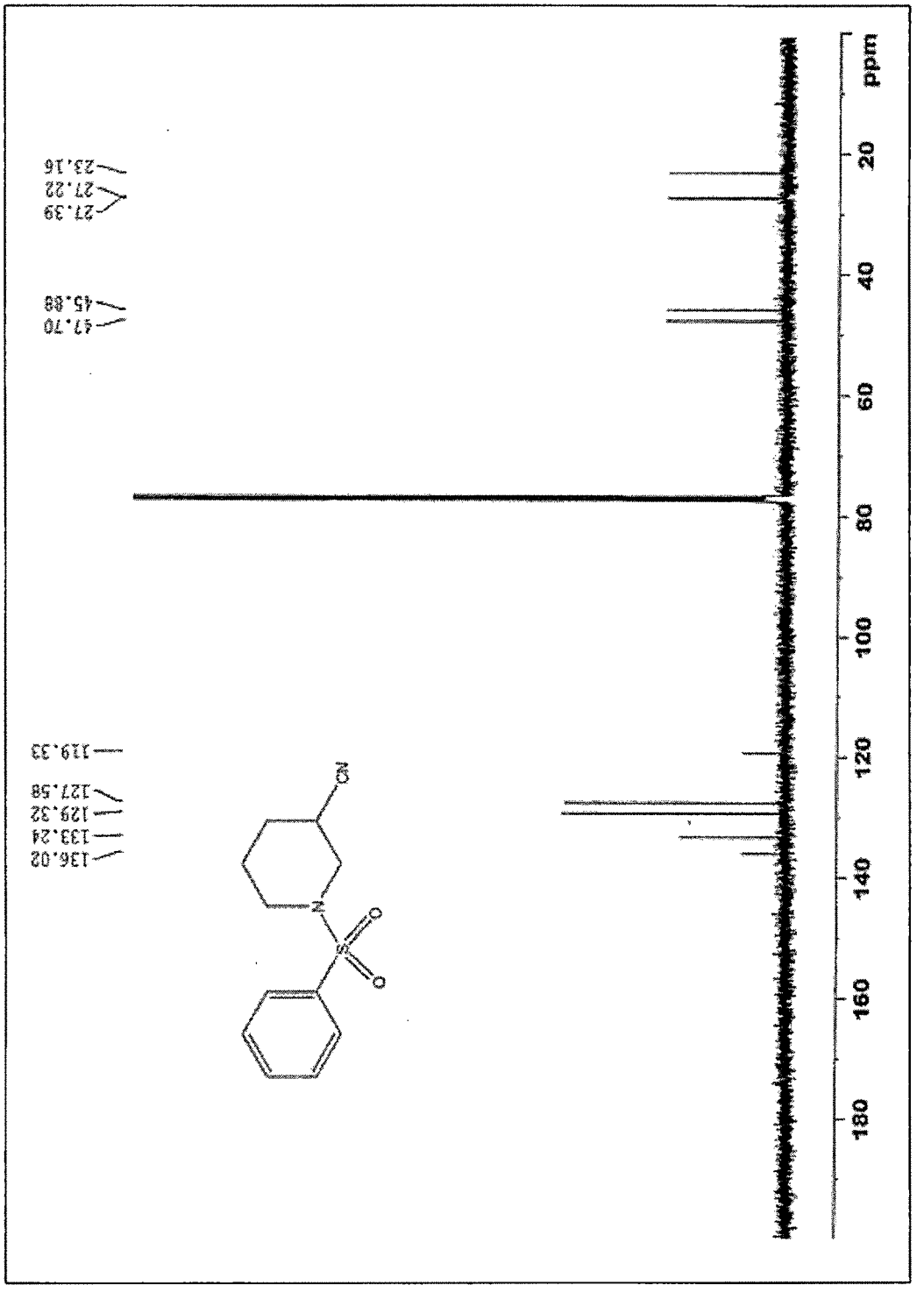


Figure 2.9.3: ^{13}C NMR spectrum of 1-(phenylsulfonyl)piperidine-3-carbonitrile **9a**

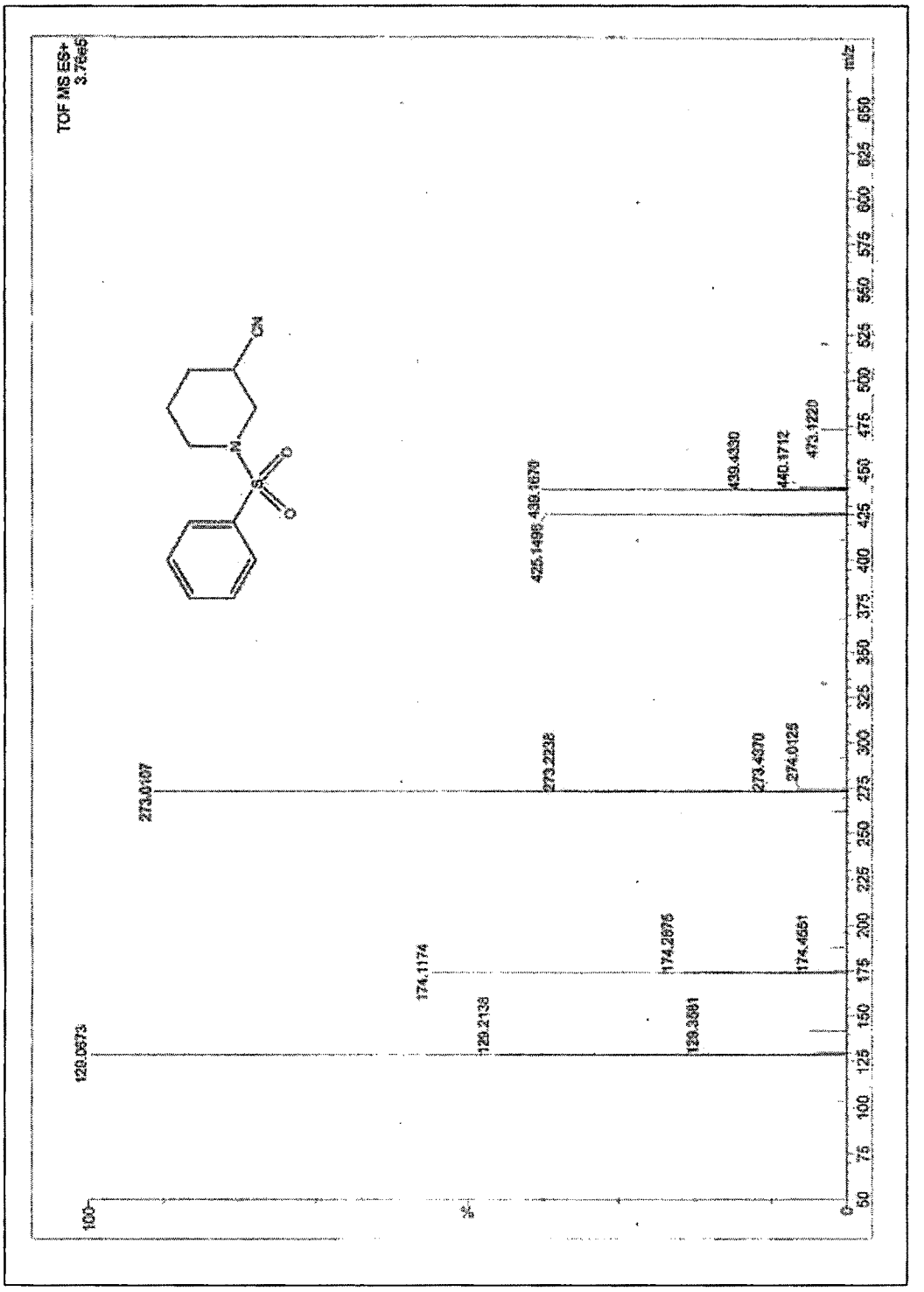


Figure 2.9.4: ESI-MS spectrum of 1-(phenylsulfonyl)piperidine-3-carbonitrile **9a**

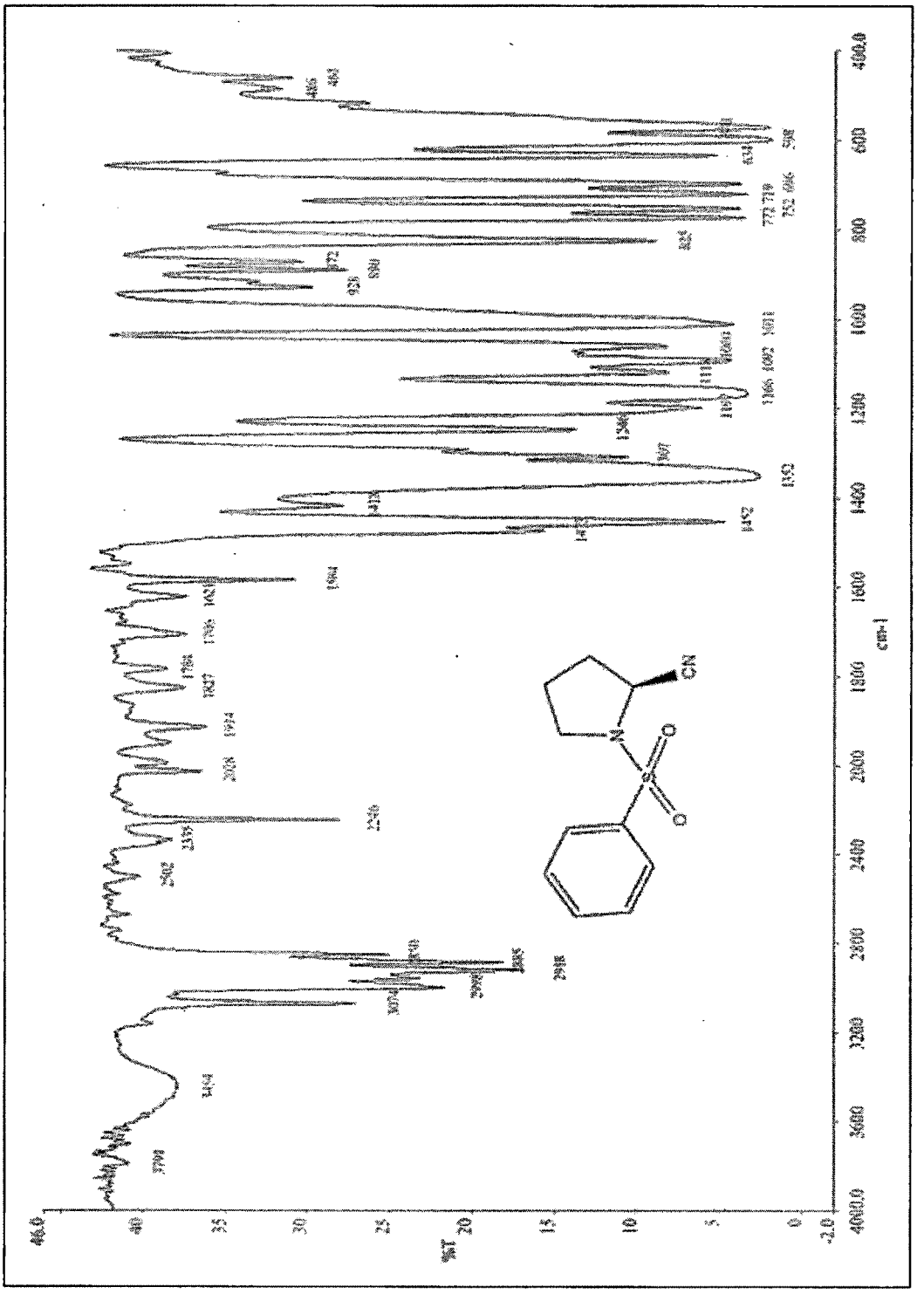


Figure 2.10.1: IR spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carbonitrile **9b**

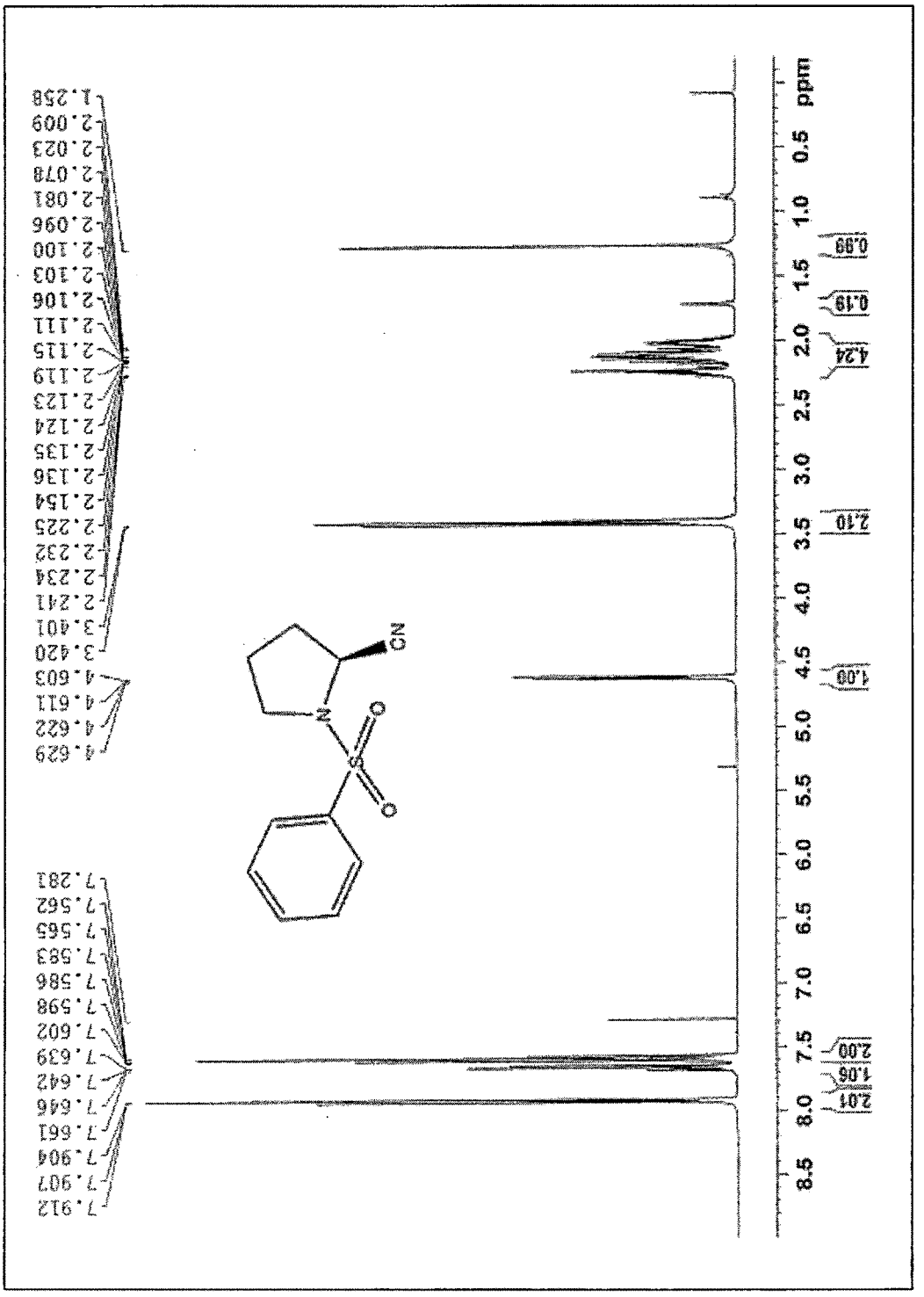


Figure 2.10.2: ^{1}H NMR spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carbonitrile **9b**

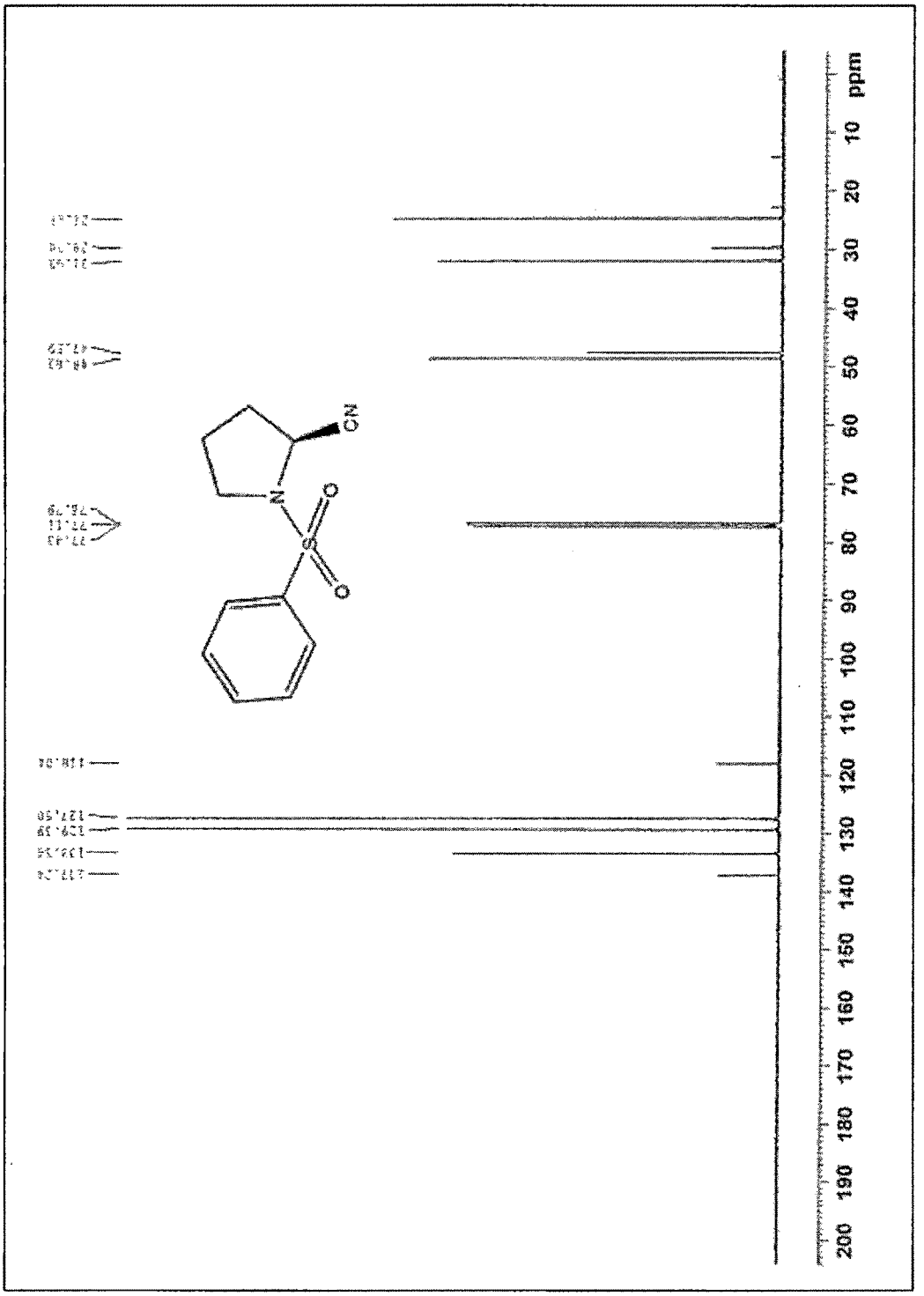


Figure 2.10.3: ^{13}C NMR spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carbonitrile **9b**

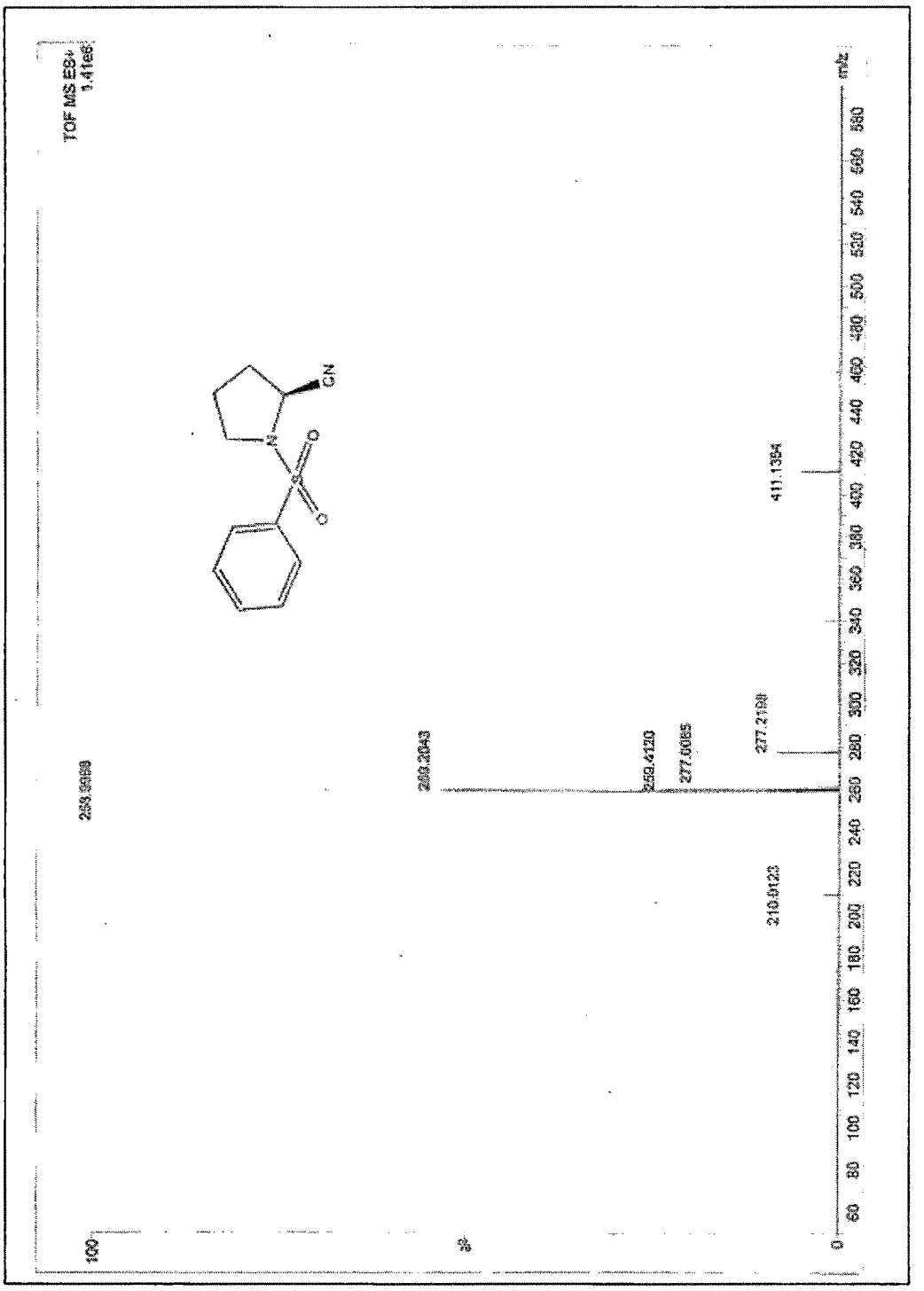


Figure 2.10.4: ESI-MS spectrum of *(S)*-1-(phenylsulfonyl)pyrrolidine-2-carbonitrile **9b**

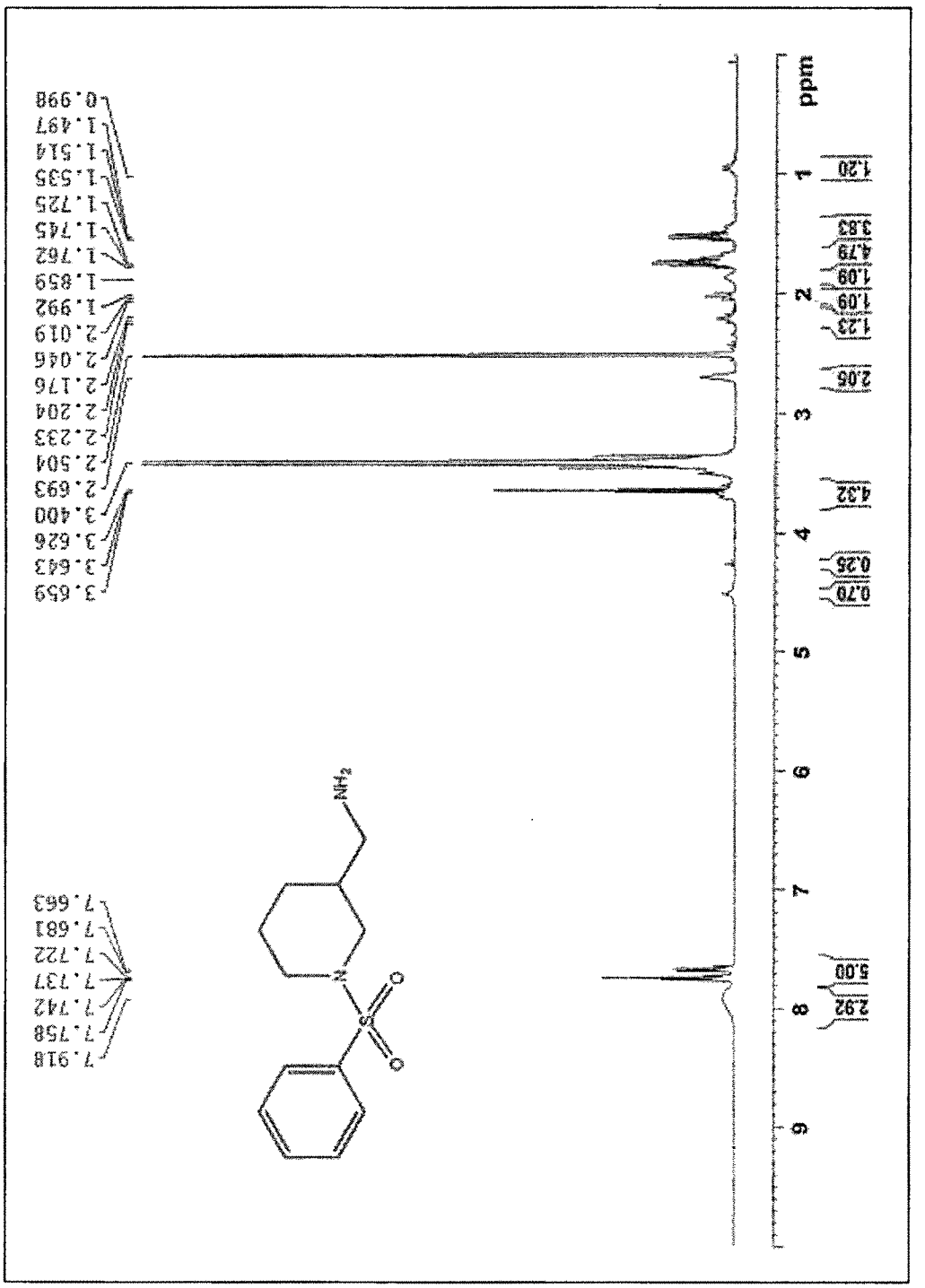


Figure 2.11.1: ^{1}H NMR spectrum of (1-(phenylsulfonyl)piperidin-3-yl)methanamine **10a**

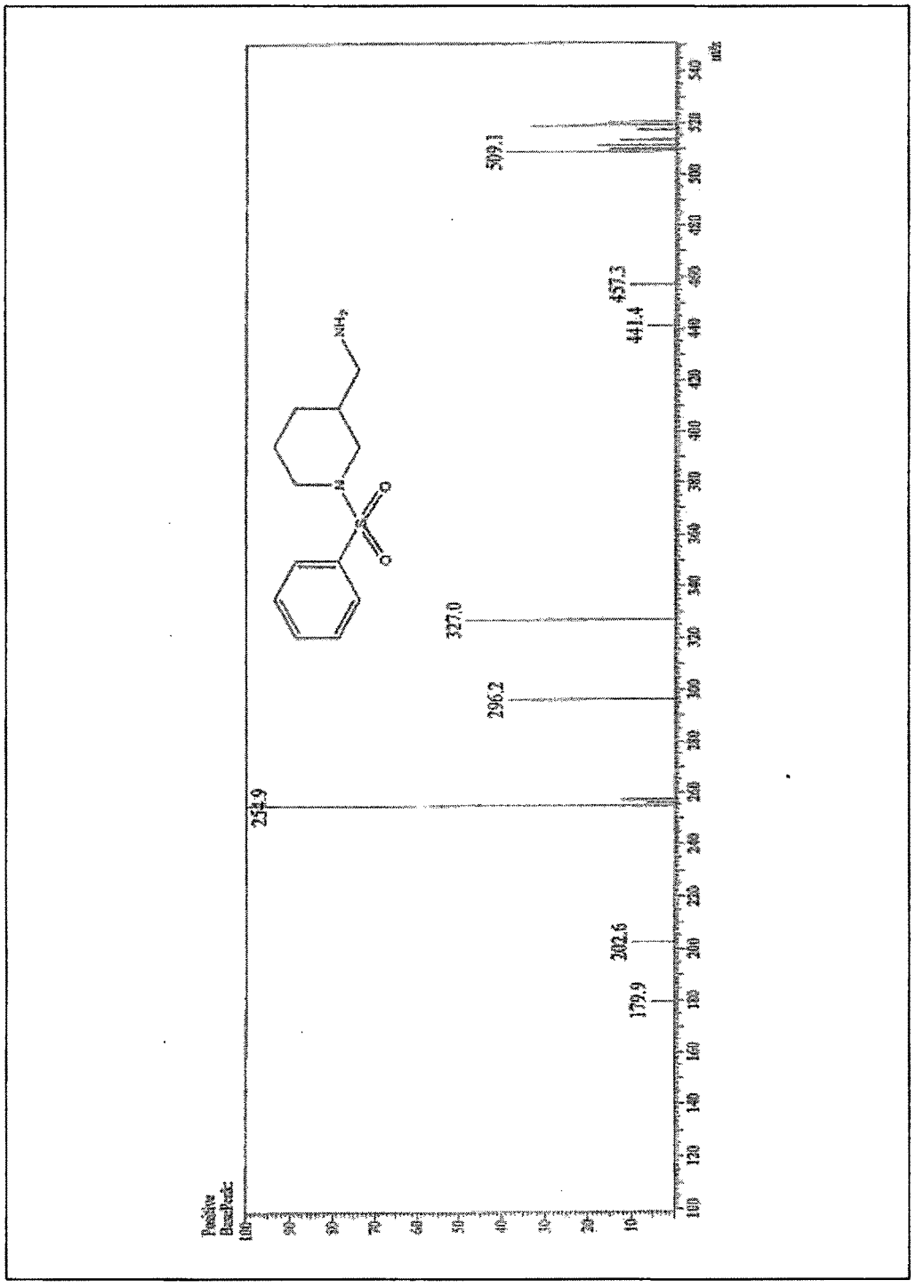


Figure 2.11.2: ESI-MS spectrum of (1-(phenylsulfonyl)piperidin-3-yl)methanamine **10a**

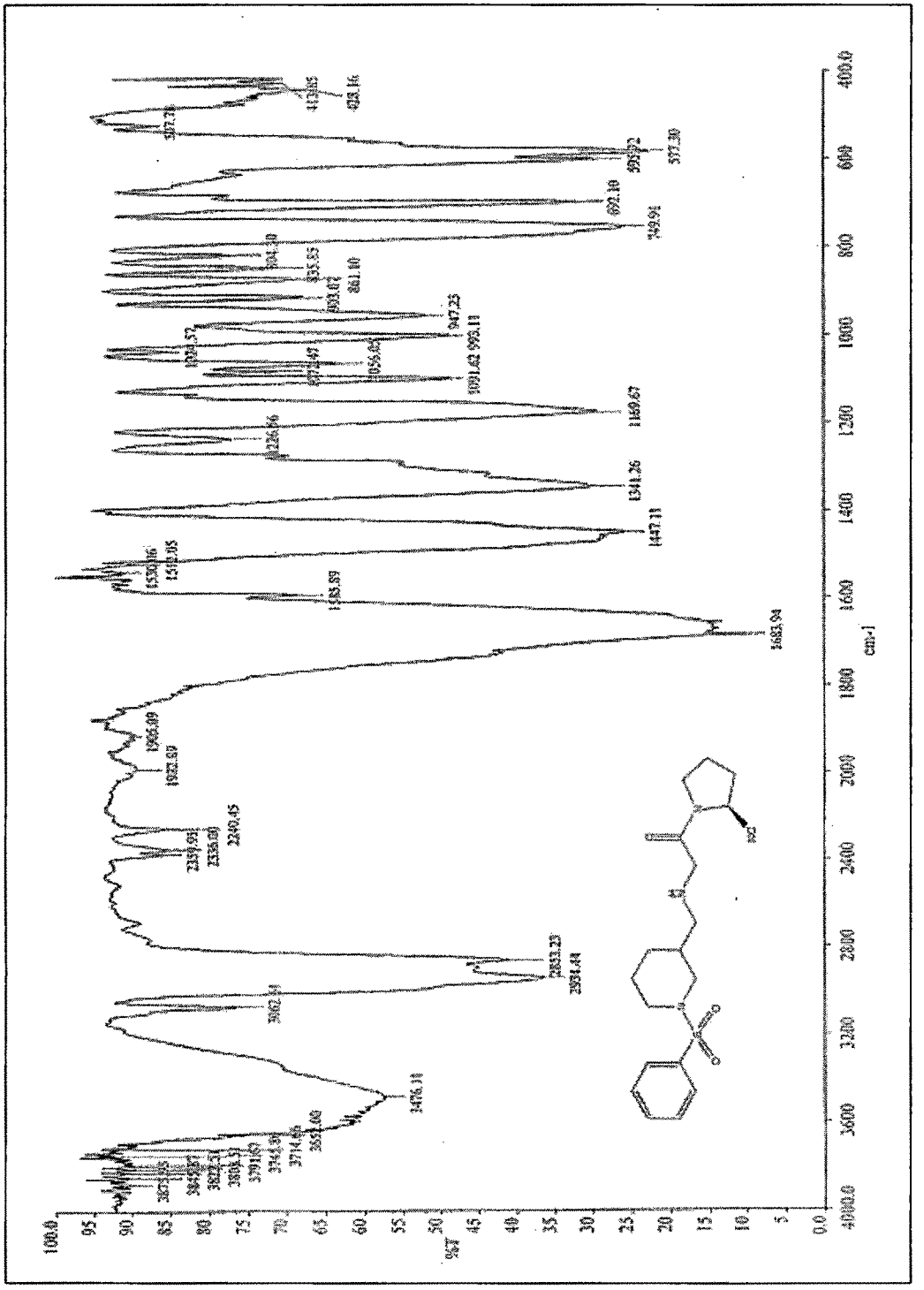


Figure 2.12.1: IR spectrum of (2S)-1-(2-((1-(phenylsulfonyl)piperidin-3-yl) methylamino)acetyl)pyrrolidine-2-carbonitrile **11a**

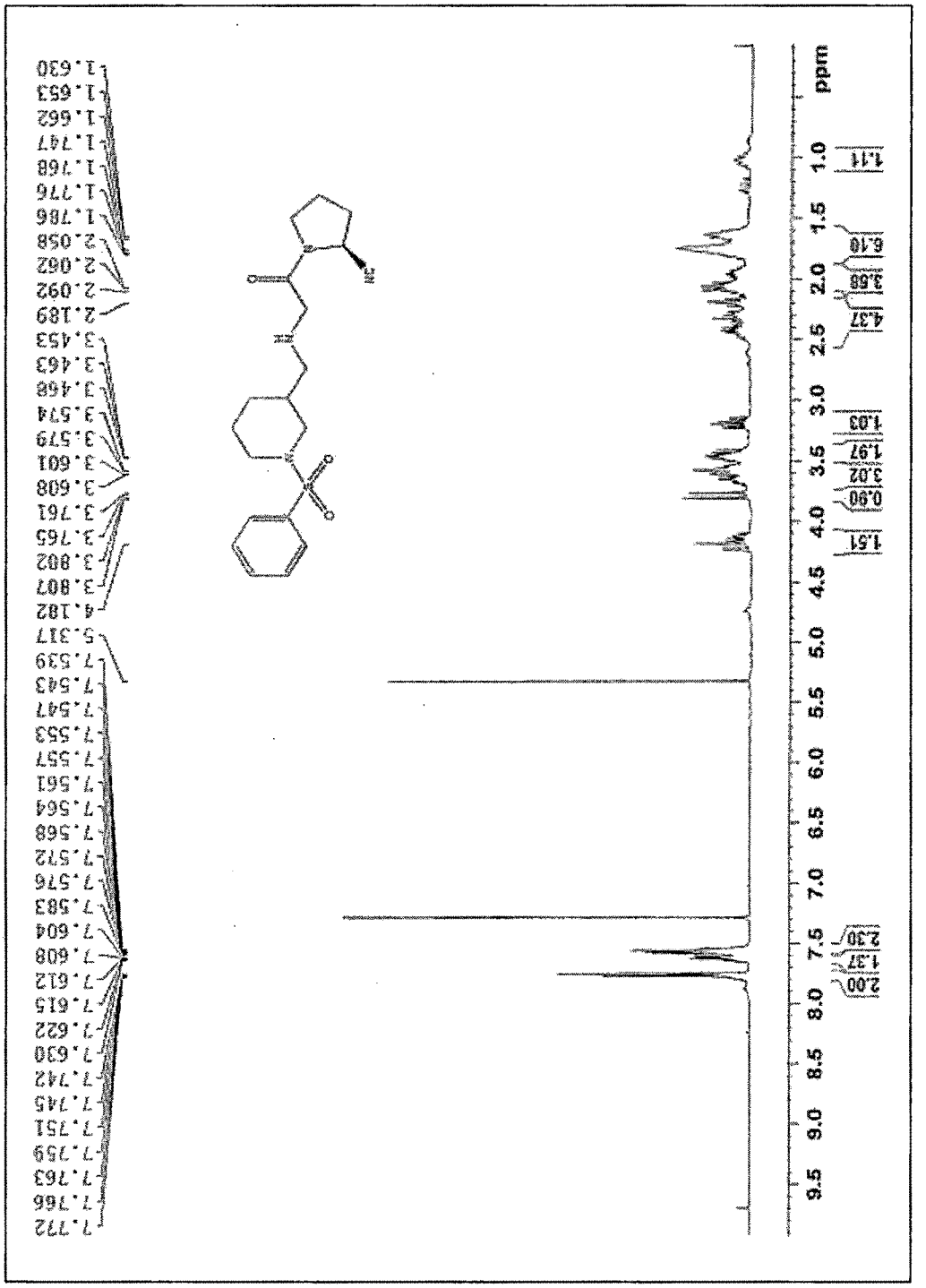


Figure 2.12.2: ^{1}H NMR spectrum of (2S)-1-(2-((1-(phenylsulfonyl)piperidin-3-yl) methylamino)acetyl)pyrrolidine-2-carbonitrile **11a**

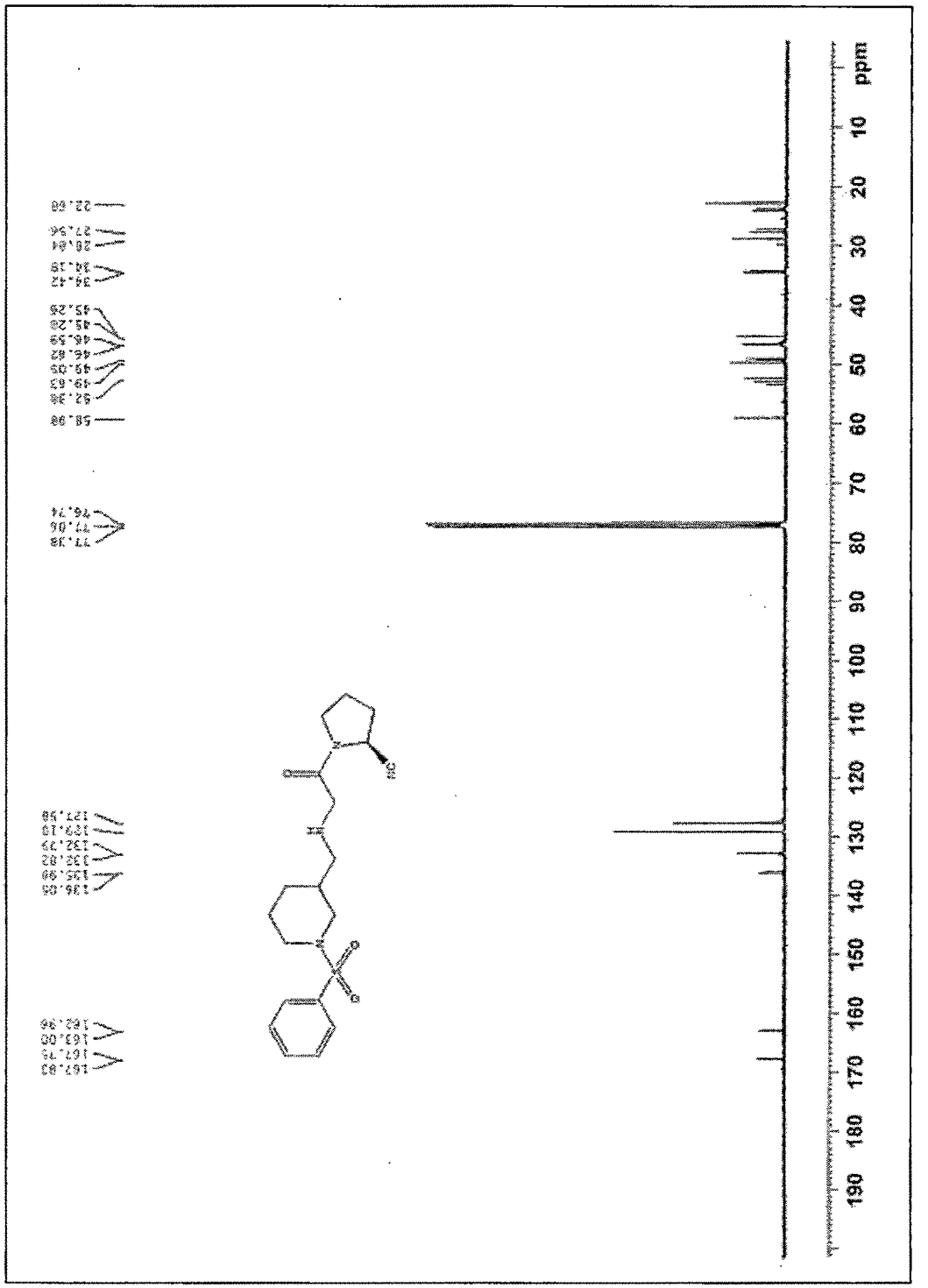


Figure 2.12.3: ^{13}C NMR spectrum of (2S)-1-(2-((1-(phenylsulfonyl)piperidin-3-yl) methylamino)acetyl)pyrrolidine-2-carbonitrile **11a**

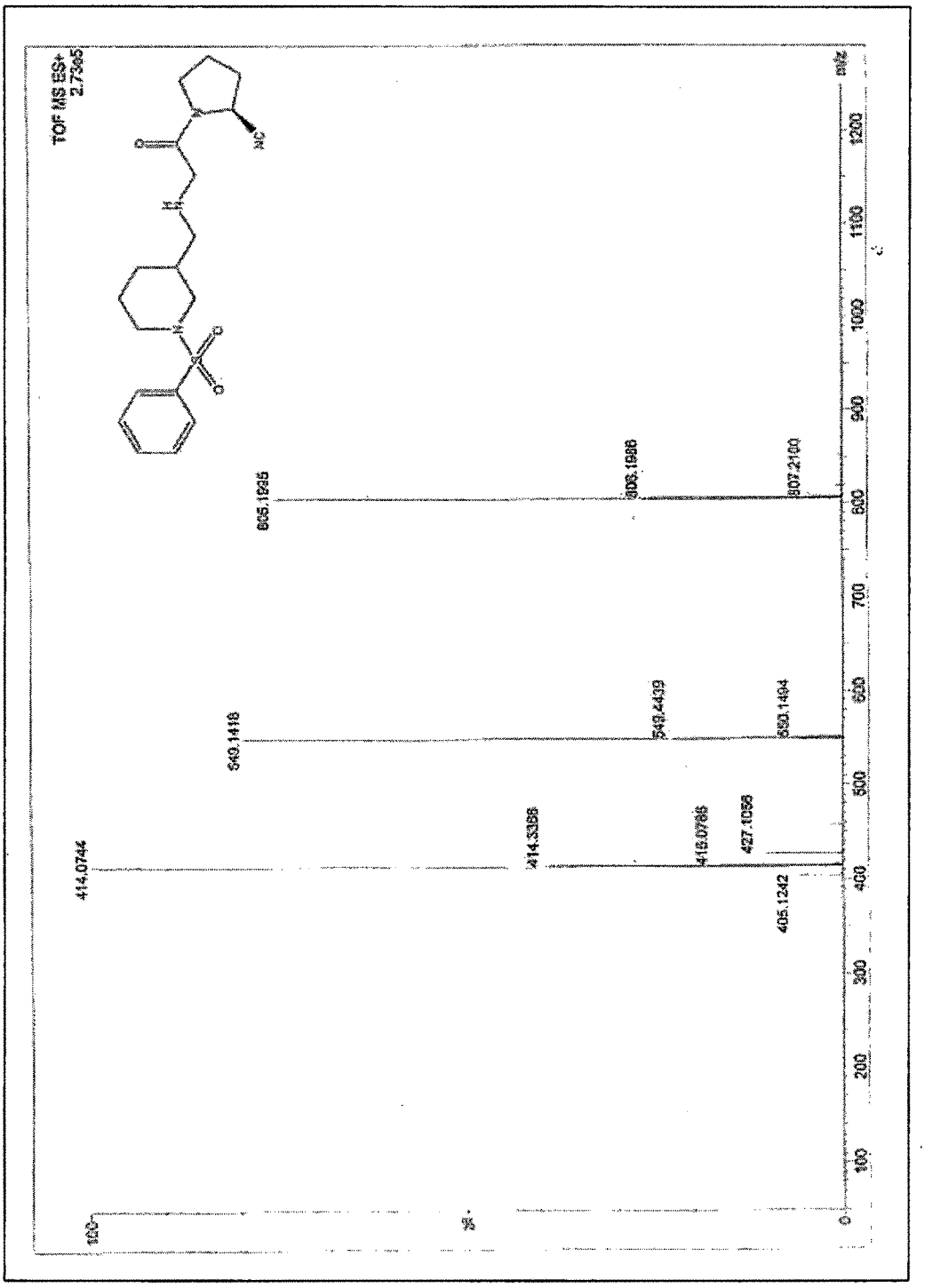


Figure 2.12.4: ESI-MS spectrum of (2S)-1-(2-((1-(phenylsulfonyl)piperidin-3-yl) methylamino)acetyl)pyrrolidine-2-carbonitrile **11a**

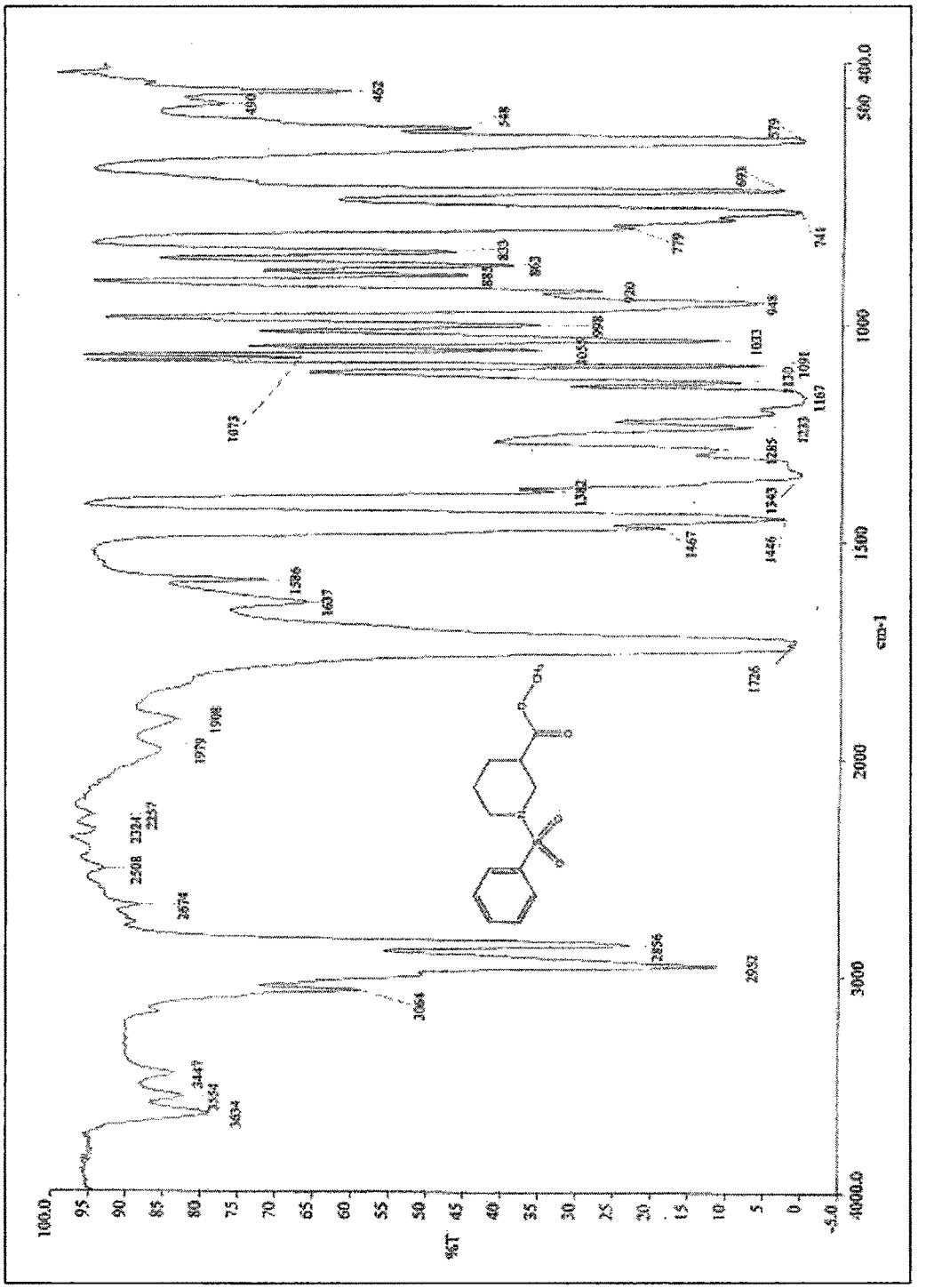


Figure 2.13.1: IR spectrum of 1-(phenylsulfonyl)piperidine-3-carboxylate **12**

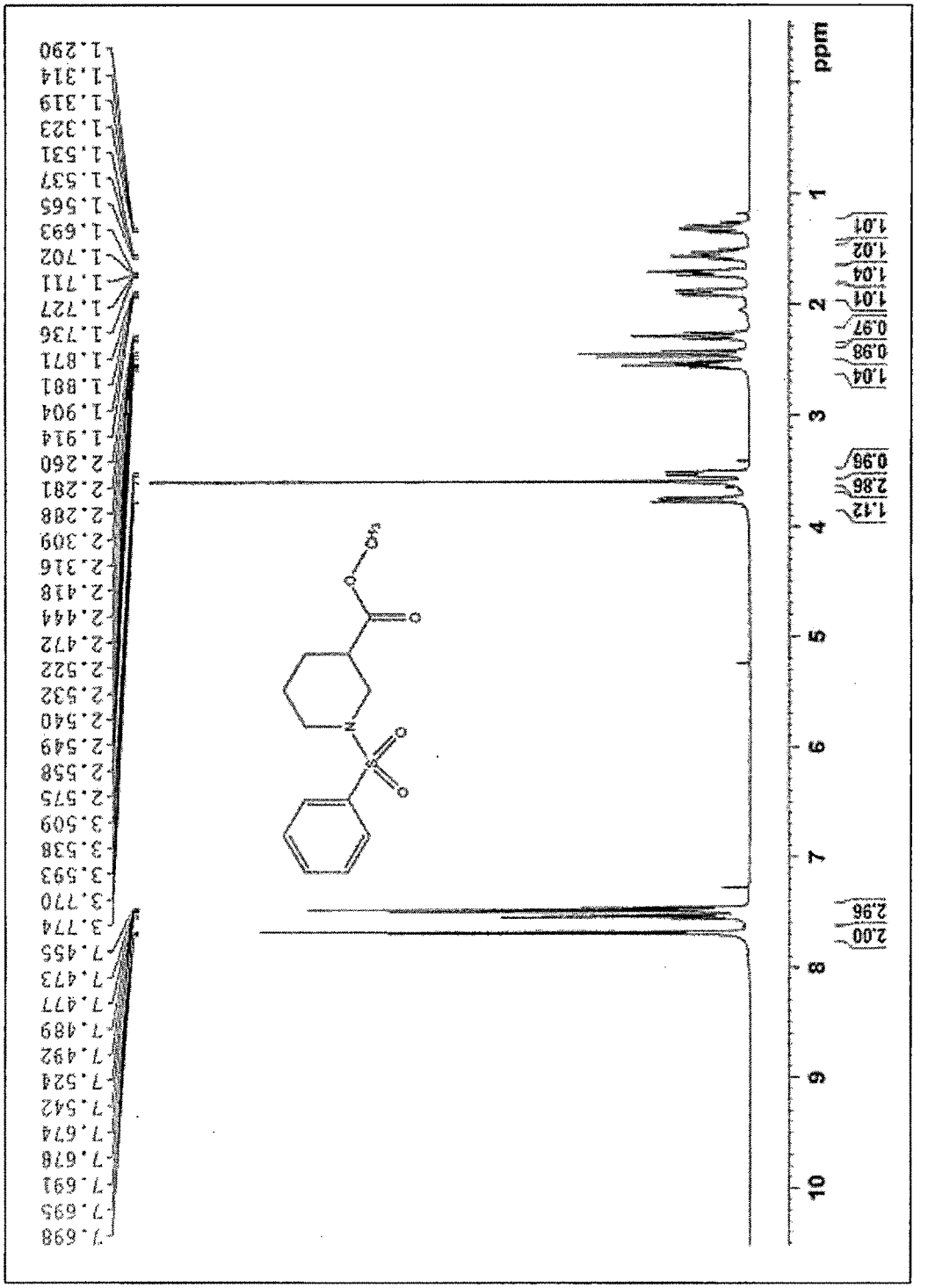


Figure 2.13.1: IR spectrum of 1-(phenylsulfonyl)piperidine-3-carboxylate **12**

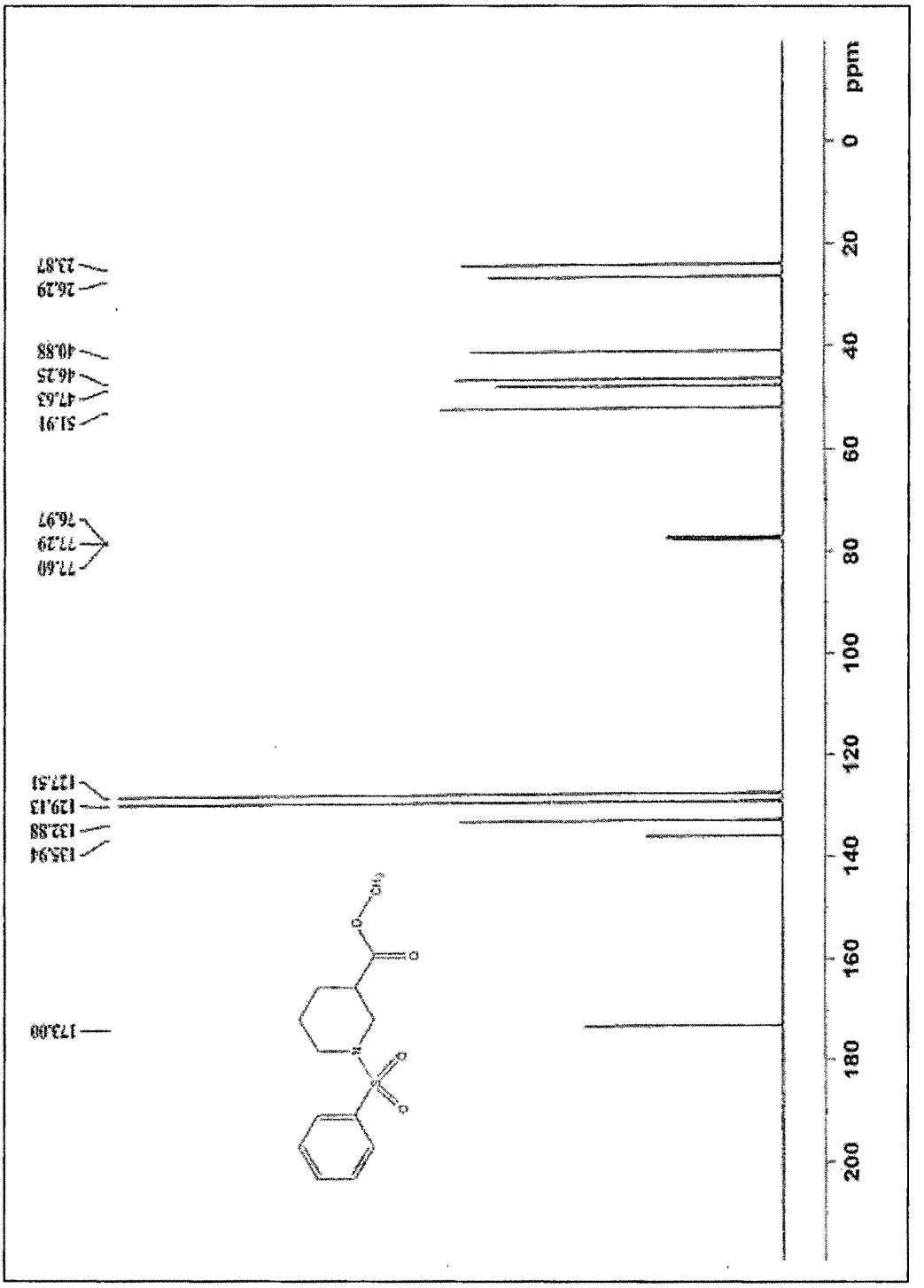


Figure 2.13.3: ^{13}C NMR spectrum of 1-(phenylsulfonyl)piperidine-3-carboxylate **12**

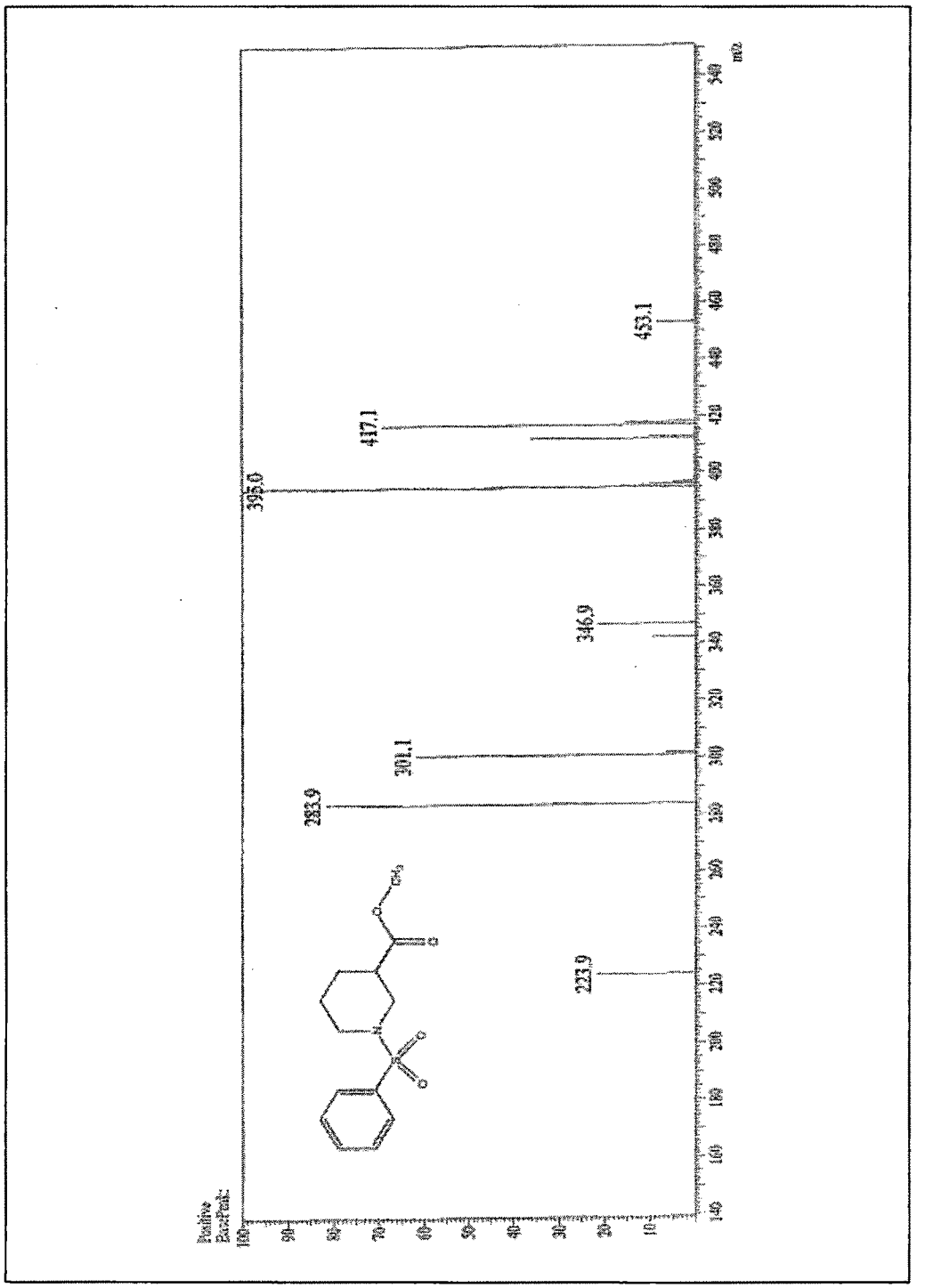


Figure 2.13.4: ESI-MS spectrum of 1-(phenylsulfonyl)piperidine-3-carboxylate **12**

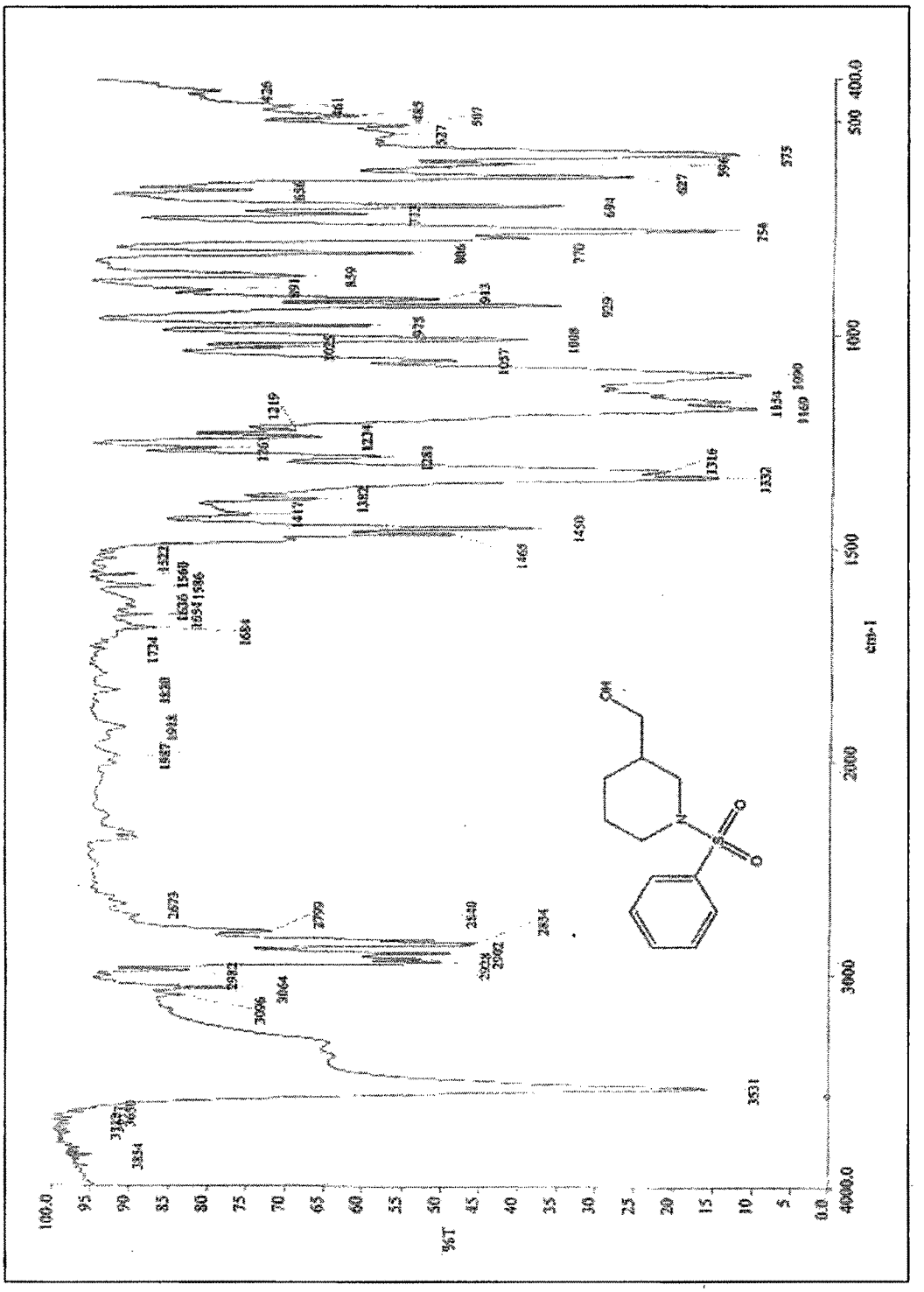


Figure 2.14.1: IR spectrum of (1-(phenylsulfonyl)piperidin-3-yl)methanol **13**

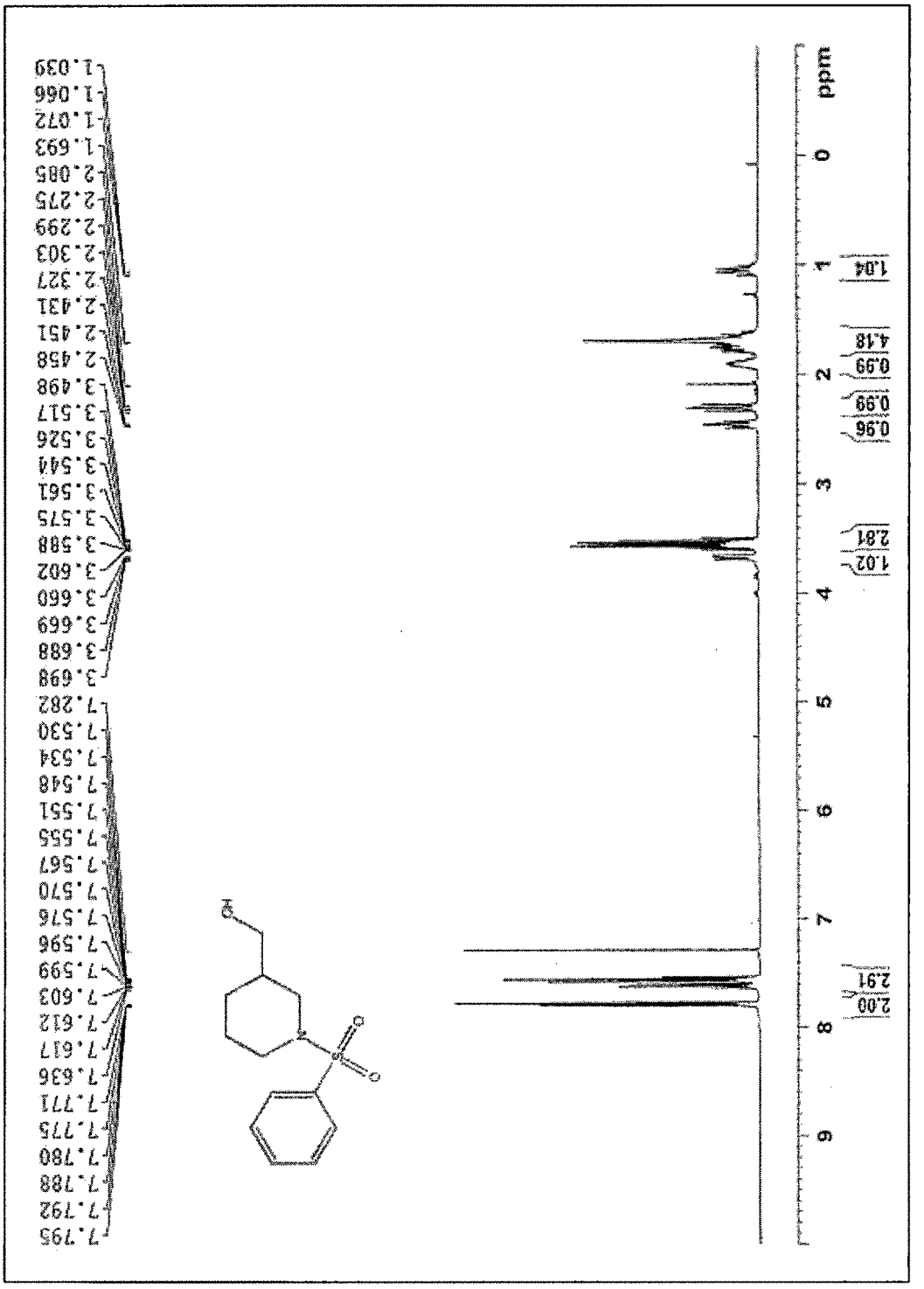


Figure 2.14.2: ^{1}H NMR spectrum of (1-(phenylsulfonyl)piperidin-3-yl)methanol **13**

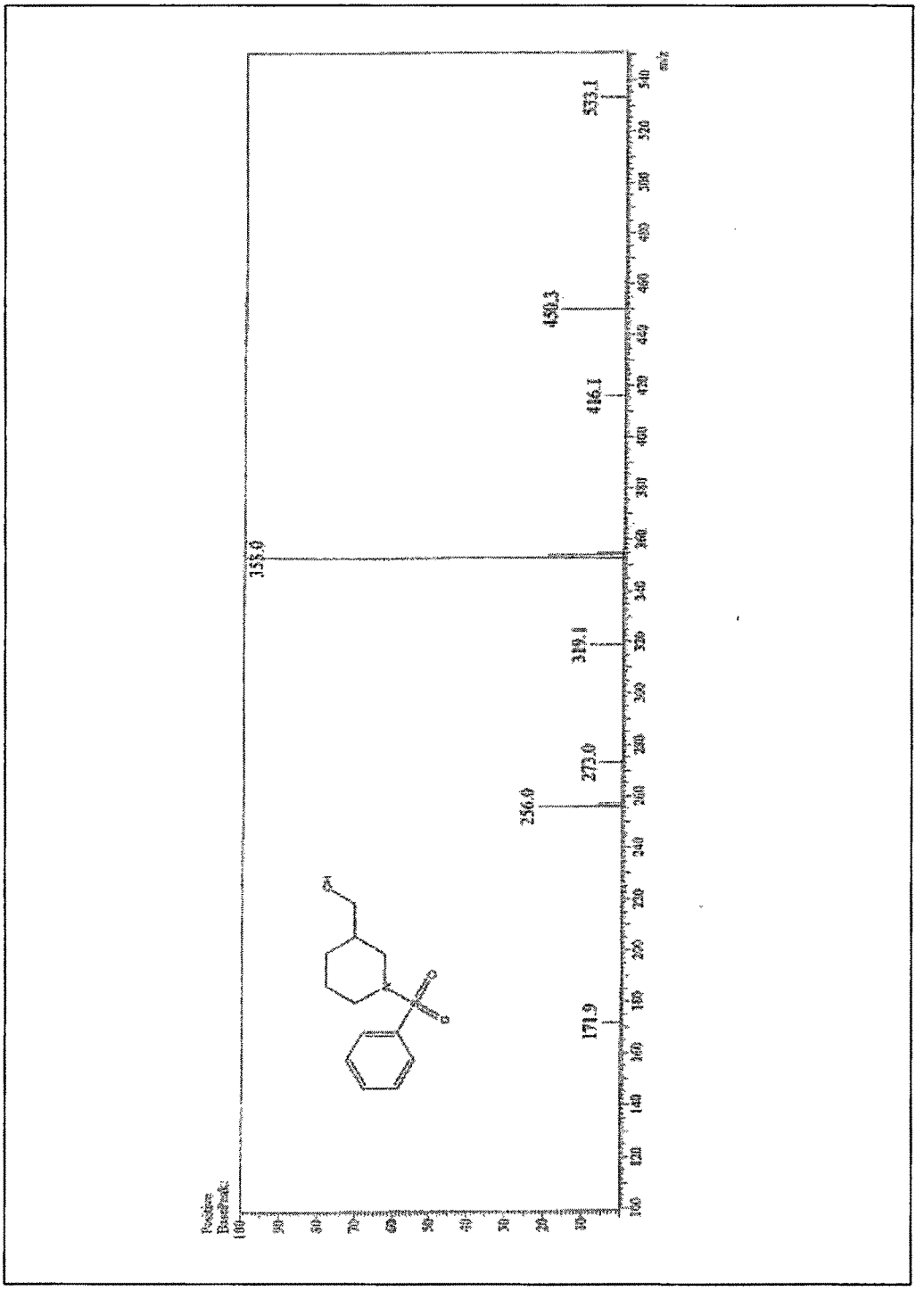


Figure 2.14.3: ESI-MS spectrum of (1-(phenylsulfonyl)piperidin-3-yl)methanol **13**

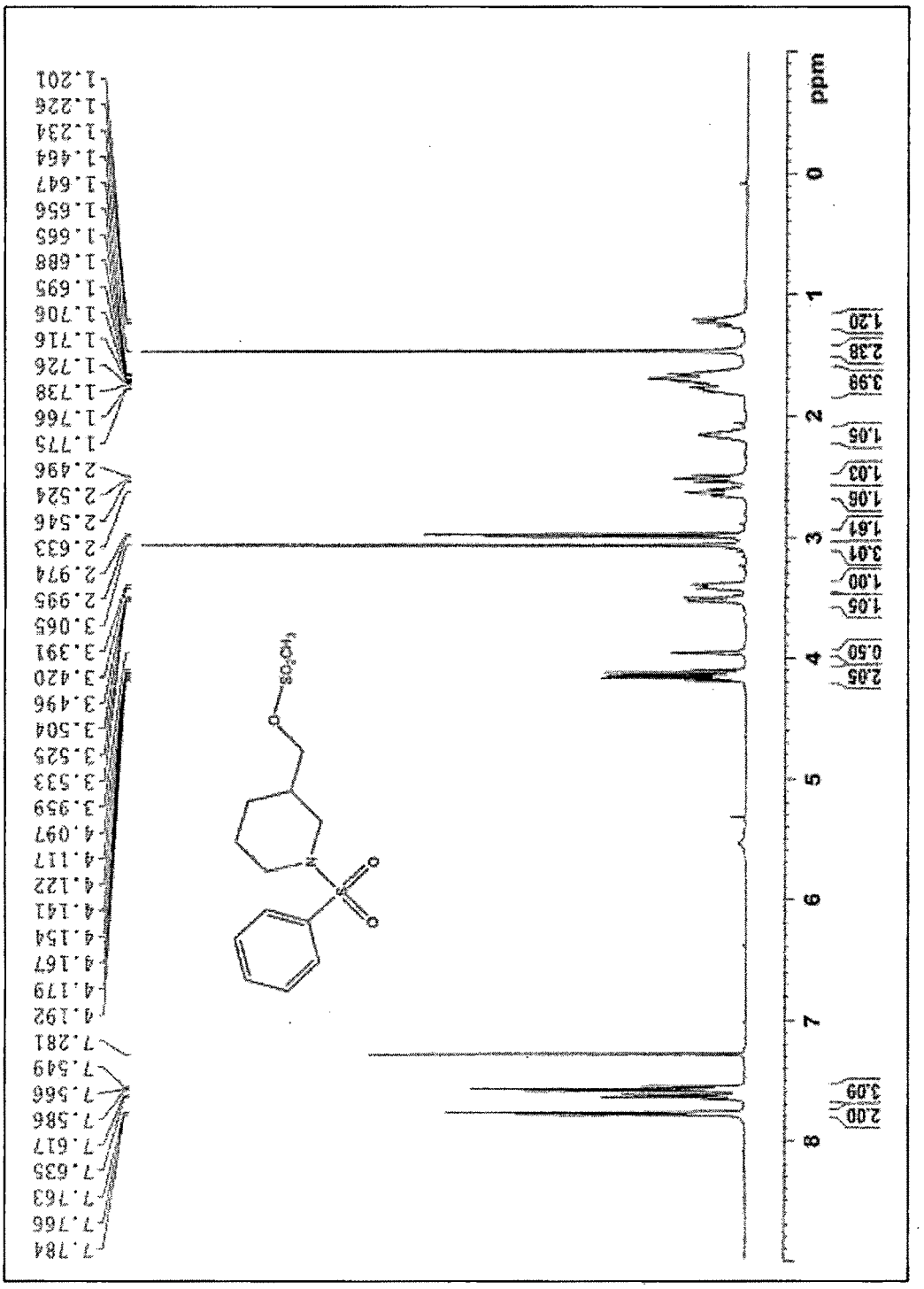


Figure 2.15.1: ^{1}H NMR spectrum of (1-(phenylsulfonyl)piperidin-3-yl)methyl methanesulfonate **14**

2.2.2 Biological Evaluation

DPP-IV inhibition assay uses fluorogenic substrate, Gly-Pro-Aminomethylcoumarin (AMC), to measure DPP-IV activity. Cleavage of the peptide bond by DPP-IV releases the free AMC group, resulting in fluorescence that is analysed using an excitation wavelength of 350-360 nm and emission wavelength of 450-465 nm. Human recombinant DPP-IV enzyme procured from Prospec (**enz-375-b.**), substrate, H-Gly-Pro-AMC procured from Enzo life science (Lot No. : **01221304)** and assay buffer, prepared in-house containing TrisHCl (50 mM), ethylenediamminetetraaceticacid (EDTA) (1mM), sodium chloride (100mM) in deionized water having pH. 7.5 were used in the assay.

DPP-IV activity was measured by mixing reagents in 96-well plate (order of addition of reagents: Assay Buffer, enzyme, solvent/inhibitor and finally substrate). Both the enzyme & 96-well plate was incubated for 30 min and the resulting fluorescence was measured using Spectra Max fluorometer (Molecular Devices, Sunnyvale CA) by exciting at 360 nm and emission at 460 nm. The IC_{50} values were determined for test compounds using Graph Pad prism software.

Calculation:-

(Fluorescence of 100% activity – Fluorescence of test / Fluorescence of 100% activity)*100

Preliminary DPP-IV inhibition assay was performed to screen test compounds **8a-b**, **9a-b**, **11a-b** for their inhibition potential at 2μM concentration as shown in Table 2.1, taking vildagliptin as a standard which exhibited 93.62% enzyme inhibition at the same concentration.

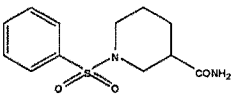
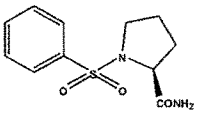
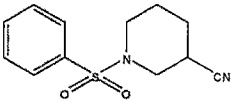
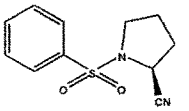
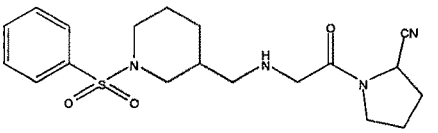
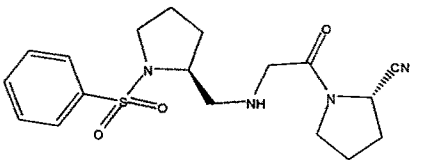
Compound	Structure	% Inhibition of DPP-IV at 2μM
8a		30.20
8b		15.00
9a		59.17
9b		75.13
11a		24.68
11b		67.91

Table 2.1: *DPP-IV inhibition by test compounds at 2μM concentrations*

IC_{50} (nM) values were determined for **9a-b**, **11a-b** as shown in Table 2.2.

Compound	IC_{50} (nM)
9a	41.17
9b	250.4
11a	2367
11b	274.4

Table 2.2: *Inhibition of DPP-IV (IC_{50} nM) of selected compounds*

From the IC_{50} values of the selected compounds **9a**, **9b**, **12a**, **12b**, it can be inferred that nitrile functionality at the P1 site is responsible for the potency of the DPP-IV inhibitors.

2.3 Docking Studies:

In order to perform the docking studies, binding site residues of the A chain of DPP-4 (PDB ID: 3W2T) [21] at a distance of 4.5 Å from **NVP-LAF237** (Vildagliptin) were selected. AutoDock Vina [22] was used for carrying out docking studies. The affinity for the compound **9a** was -7.4 kcal/mol while that of Vildagliptin was shown to be -6.7 kcal/mol. LigPlot [23] was used to observe the interaction of the ligand with the binding site residues as seen in Figure 2.16.

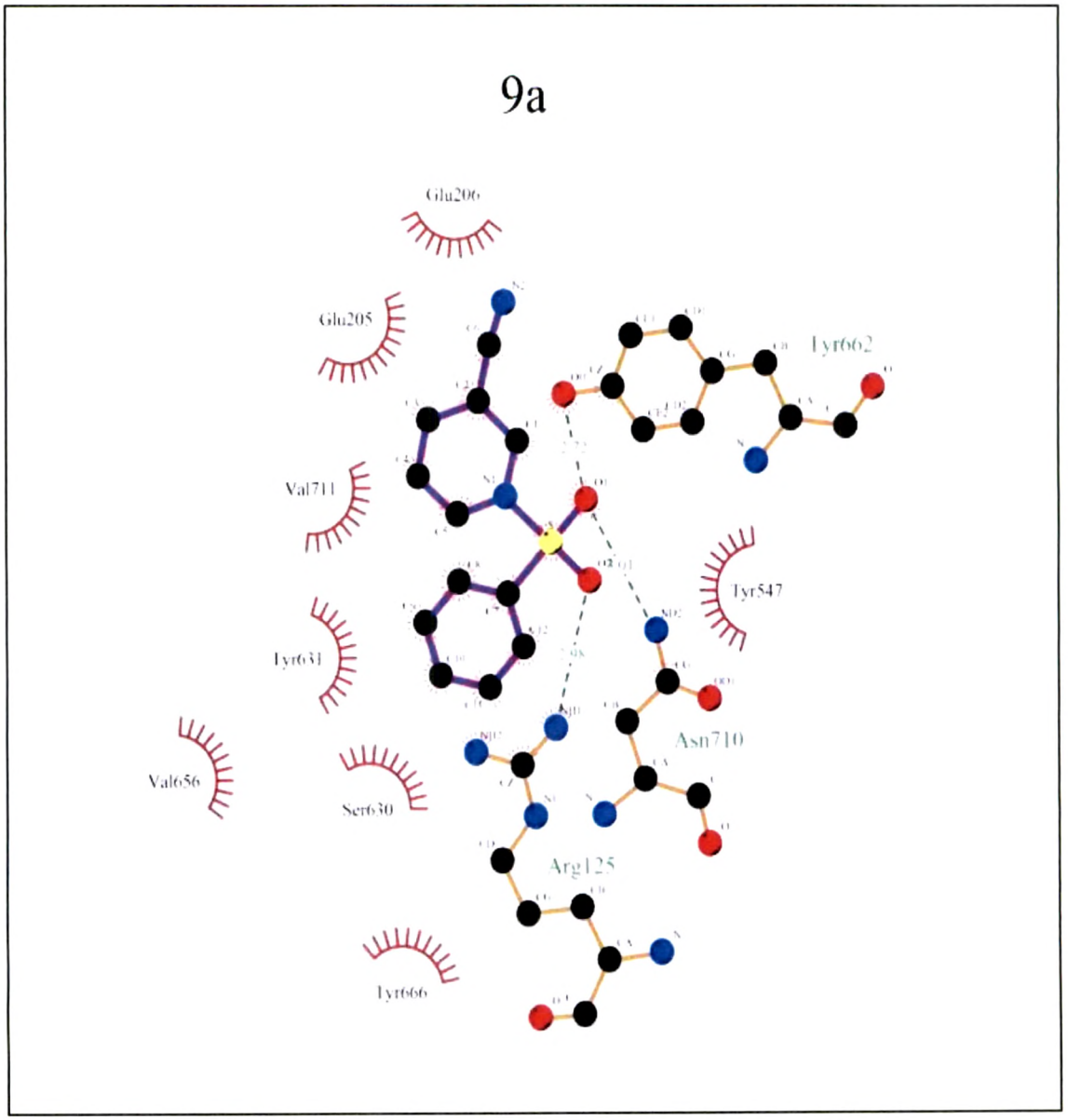


Figure 2.16.1: *LigPlot of compound 1-(phenylsulfonyl)piperidine-3-carbonitrile* ***9a***

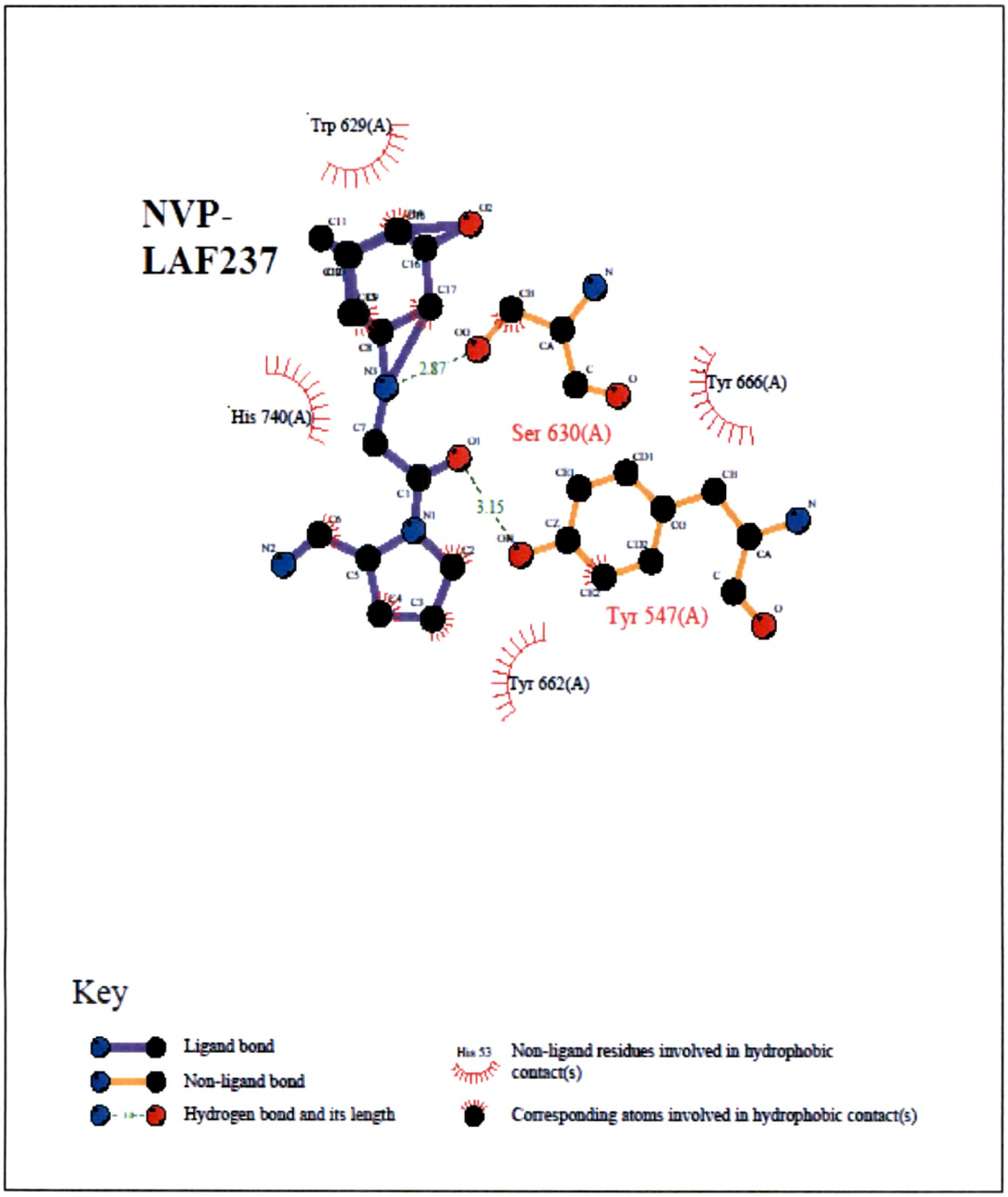
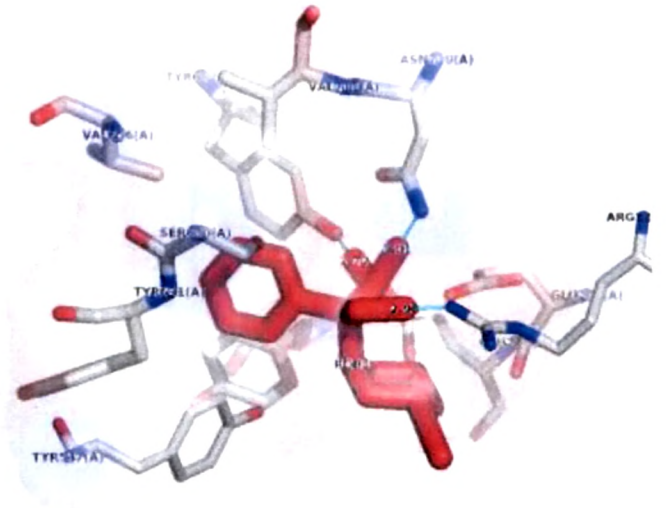
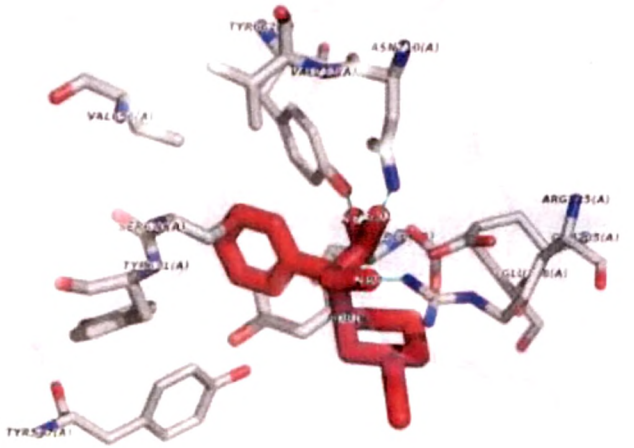


Figure 2.16.2: *LigPlot of compound* ***NVP-LAF237*** *(Vildagliptin)*

Pymol [24] was used to visualize the protein and the docked compound **9a** as seen in Figure 2.17.



A



B

Figure 2.17: *Binding of 1-(phenylsulfonyl)piperidine-3-carbonitrile* ***9a*** *at the active site of DPP-IV*

2.3 Conclusion

Thus from the structure activity relationship study of the compounds **8a-b, 9a-b, 11a-b,** it can be concluded that the presence of nitrile group at the P1 site is inevitable for the DPP-IV inhibition. Small molecules, sulphonamide derivatives of piperidine-3-carbonitrile and pyrrolidine-2-carbonitrile **9a, 9b** showed better inhibition with IC_{50} of 41.17 and 250.4 nM respectively, of which the most potent DPP-IV inhibitor of all the molecules synthesized in the series is **9a.**

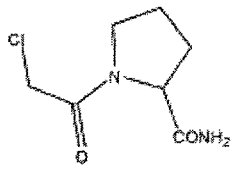
According to the reported literature, proline mimic is essential for DPP-IV enzyme inhibition but an interesting observation is **9a** (derived from piperidine-3-carboxylic acid) showed five-fold greater potency than **9b** (derived from L-proline). Also amide functionality is not desirable at the P1 site as it lead to very low inhibition of the enzyme as is observed from the % inhibition of compounds **8a-b.**

N-substituted glycine with 2-cyanopyrrolidide at the P1 site and sulfonamide derivatives at the P2 site showed good DPP-IV inhibition but are still not as potent as the vildagliptin since their high IC_{50} values indicates the low potency of these compounds.

2.4 Experimental:

Reagent grade chemicals and solvents were purchased from commercial supplier and used after purification. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Advance Bruker 400 spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. Mass spectra were determined by ESI-MS, using a Shimadzu LCMS 2020 apparatus. All the reactions were carried out under nitrogen atmosphere.

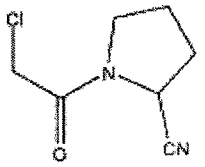
Procedure for the synthesis of (S)-1-(2-chloroacetyl)pyrrolidine-2-carboxamide 4:



To a solution of L-proline amide **3** in chloroacetyl chloride (1.2 mmol) in tetrahydrofuran (THF) (5 mL) and triethylamine (catalytic), a solution of L-proline amide (1.0 mmol) in THF (10 mL), was added drop-wise, at room temperature for 10 minutes and the resulting solution was refluxed for an hour. On completion of reaction, as monitored by TLC, the reaction mixture was concentrated, washed with saturated sodium bicarbonate solution (1X10 mL), dried over anhydrous sodium sulfate and recrystallized from isopropyl alcohol to give the product **4** as white solid.

Yield: 50%; white solid; $[\alpha]_D$ = -64.33; m.p.: 138-140 °C; IR (KBr): 3360, 3158, 2982, 1682, 1657, 1408, 1275, 787 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.99-2.07 (m, 2H), 2.11-2.16 (m, 1H), 2.25-2.29 (m, 1H), 3.52-3.58 (m, 1H), 3.69-3.74 (m, 1H), 4.14 (s, 2H), 4.54 (dd, 1H, J_1 = 2.8 Hz, J_2 = 8.0 Hz), 5.95 (s, 1H), 7.07 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 8.64, 24.93, 28.29, 42.25, 45.81, 47.44, 60.26, 166.21, 173.48; $C_7H_{11}ClN_2O_2$; ESI-MS: *m/z* 213 $[M+Na]^+$.

Procedure for the synthesis of (S)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile 5:



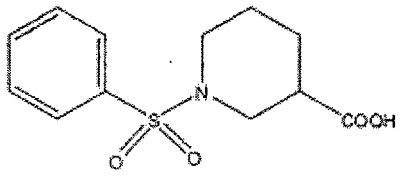
To a suspension of *(S)*-1-(2-chloroacetyl)pyrrolidine-2-carboxamide **4** (1.0 mmol) in THF (10 mL) trifluoroacetic anhydride (2.0 mmol) added at 0–5 °C and the reaction mixture was then stirred at room temperature for 4 h. The reaction was monitored by TLC. On completion of the reaction, to this mixture ammonium bicarbonate (7.0 mmol) added portion wise (over 15 min) while maintaining the temperature of the mixture at 5–10 °C. The mixture again stirred at room temperature for an hour and then concentrated under vacuum at 40 °C to give the product **5**.

Yield: 64%; white solid; $[\alpha]_D$ = -155.21; m.p.: 53-55 °C; IR (KBr): 3435, 2992, 2952, 2888, 2241, 1687, 1656, 1422, 1284, 915, 787 cm^{-1}; ^1H NMR (400 MHz, CDCl3): δ 2.18-2.25 (m, 4H), 3.51-3.57 (m, 1H), 3.66-3.71 (m, 1H), 4.08 (s, 2H), 4.69-4.71 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 22.80, 25.25, 29.96, 32.48, 41.69, 41.73, 46.55, 46.84, 46.99, 47.14, 118.00, 165.33; ESI-MS: *m/z* 173.0 $[M+H]^+$.

General procedure for the preparation of compound 7a, 7b:

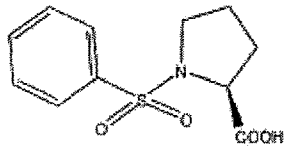
To a mixture amino acid, piperidine-3-carboxylic acid **6a** or L-proline **6b** (1.0 mmol) and sodium carbonate (3.0 mmol) in DCM:water (1:1) benzene sulphonyl chloride (1.1 mmol) added and the reaction mixture stirred at room temperature for 16 hours or till the completion of reaction as monitored by TLC. On completion of reaction, the reaction mixture washed with petroleum ether (20 mL) and then acidified till pH 2 using conc.HCl. The white solid thus separated was filtered, washed with water several times and then dried to yield the desired products **7a-b** as white solid.

***1-(phenylsulfonyl)piperidine-3-carboxylic acid* 7a:**



Yield: 91%; white solid; m.p.: 115-117 °C; IR (KBr): 3100-2500 (b), 2940, 1812, 1693, 1352 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.41-1.50 (m, 1H), 1.65-1.73 (m, 1H), 1.80-1.85 (m, 1H), 1.99-2.04 (m, 1H), 2.41 (dt, 1H, J_1 = 2.8 Hz, J_2 = 11.2 Hz), 2.57 (t, 1H, J = 10.8 Hz), 2.65-2.71 (m, 1H), 3.59 (br d, 1H, J = 11.6 Hz), 3.83 (dd, 1H, J_1 = 3.2 Hz, J_2 = 7.2 Hz), 7.54-7.58 (m, 2H), 7.61-7.63 (m, 1H), 7.77-7.79 (m, 2H), 8.98 (br s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.88, 26.21, 40.73, 46.26, 47.35, 127.62, 129.18, 132.94, 135.96, 178.63; $C_{12}H_{15}NO_4S$; ESI-MS: *m/z* 292.0 $[M+Na]^+$

(S)-1-(phenylsulfonyl)pyrrolidine-2-carboxylic acid 7b:

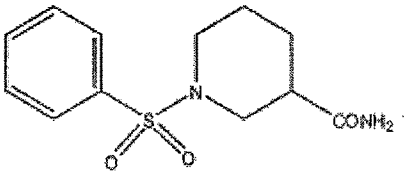


Yield: 90%; white solid; $[\alpha]_D$ = -101.38; m.p.: 86 - 88 °C; IR (KBr): 3180-2560 (b), 2956, 2683, 1728, 1584, 1447, 1349, 1290, 1159, 1095 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.76-1.80 (m, 1H), 1.97-2.14 (m, 3H), 3.26-3.32 (m, 1H), 3.52-3.57 (m, 1H), 4.30-4.33 (m, 1H), 7.54-7.58 (m, 2H), 7.61-7.66 (m, 1H), 7.88-7.91 (m, 2H), 8.65 (br s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.66, 30.80, 48.71, 60.37, 127.50, 129.24, 133.15, 137.48, 177.03; $C_{11}H_{13}NO_4S$; ESI-MS: *m/z* 277.9 $[M+Na]^+$.

General procedure for the preparation of compound 8a, 8b:

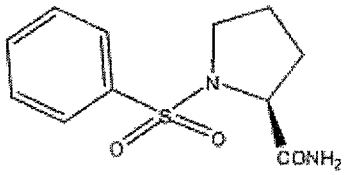
To a solution of compound **7** (1 mmol) in dichloromethane (10 mL), slowly a solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (1.5 mmol) and 1-hydroxybenzotriazole (HOBt) (1.0 mmol) in dichloromethane added at 10–15 °C (duration 5.0 min) and the mixture stirred at room temperature for 5 h. To this ammonium bicarbonate (5.0 mmol) was added and the mixture stirred for 1 h. The reaction was monitored by TLC (5% MeOH-$CHCl_3$, anisaldehyde & I_2). After completion of the reaction, the mixture filtered and the residue washed with DCM. The filtrates were collected and combined, washed with water (2X10 mL), dried over anhydrous sodium sulphate and the solvent evaporated under reduced pressure to give the crude product which was then purified by column chromatography using silica gel employing ethylacetate as eluent to yield desired products **8a-b** as white solid.

1-(phenylsulfonyl)piperidine-3-carboxamide 8a:



Yield: 65 %; white solid; m.p.: 162-164 °C; IR (KBr): 3346, 3173, 2961, 2931, 2865, 1665, 1629, 1446, 1341, 1164, 1151 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.42-1.46 (m, 1H), 1.69-1.77 (m, 2H), 2.08-2.18 (m, 2H), 2.34-2.49 (m, 1H), 2.50 (m, 1H), 3.56-3.64 (m, 2H), 6.96 (s, 1H), 7.46 (s, 1H), 7.63-7.67 (m, 2H), 7.71-7.74 (m, 3H),; ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.75, 26.95, 41.91, 46.42, 48.12, 127.60, 129.24, 133.05, 135.57, 175.16; $C_{12}H_{16}N_2O_3S$; ESI-MS: *m/z* 291.0 $[M+Na]^+$.

(S)-1-(phenylsulfonyl)pyrrolidine-2-carboxamide 8b:

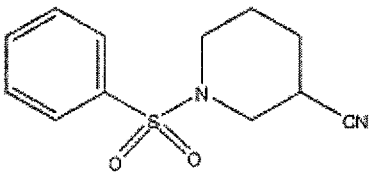


Yield: 55%; white solid; $[\alpha]_D$ = -163.72; m.p.: 78-80 °C; IR (KBr): 3466, 3290, 3226, 2956, 2890, 1671, 1587, 1499, 1346, 1161, 1092 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.54-1.65 (m, 2H), 1.79-1.82 (m, 1H), 2.16-2.21 (m, 1H), 3.16-3.22 (m, 1H), 3.57-3.63 (m, 1H), 4.09 (dd, 1H, J_1 = 2.8 Hz, J_2 = 8.8 Hz), 6.08 (br s, 1H), 6.94 (br s, 1H), 7.56-7.60 (m, 2H), 7.64-7.68 (m, 1H), 7.85-7.88 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.39, 30.14, 49.85, 62.31, 127.76, 129.42, 133.49, 135.67, 174.52; $C_{11}H_{14}N_2O_3S$; ESI-MS: *m/z* 277.0 $[M+Na]^+$.

General procedure for the preparation of compound 9a, 9b:

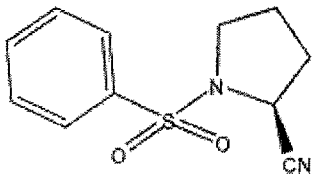
To a suspension of amide **8** (1.0 mmol) in THF (10 mL) trifluoroacetic anhydride (2.0 mmol) added at 0–5 °C and the reaction mixture stirred at room temperature for 4 h. The reaction was monitored by TLC. On completion of the reaction, to this mixture ammonium bicarbonate (7 mmol) added portion wise (over 15 min), maintaining the temperature of the mixture at 5–10 °C and the mixture stirred at room temperature for an hour and then concentrated under vacuum at 40 °C to give the crude product which was then purified by column chromatography using silica gel and employing DCM : methanol (95:5) as eluent to yield desired product **9a-b** as white solid.

1-(phenylsulfonyl)piperidine-3-carbonitrile 9a:



Yield: 75 %; white solid; m.p.: 122-124 °C; IR (KBr): 3066, 2959, 2880, 2864, 2239, 1770, 1586, 1475, 1447, 1342, 1207 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.62-1.72 (m, 2H), 1.86-1.91 (m, 1H), 1.97-2.00 (m, 1H), 2.69-2.74 (m, 1H), 2.86-2.90 (m, 2H), 3.39-3.42 (m, 1H), 3.64 (d, 1H, J = 8.4 Hz), 7.56-7.60 (m, 2H), 7.64-7.68 (m, 1H), 7.78-7.80 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.06, 27.37, 29.50, 45.91, 47.69, 119.40, 127.54, 129.33, 133.25, 136.02; $C_{12}H_{14}N_2O_2S$; ESI-MS: *m/z* 273.0 $[M+Na]^+$.

(S)-1-(phenylsulfonyl)pyrrolidine-2-carbonitrile 9b:



Yield: 70 %; white solid; $[\alpha]_D$ = -116.60; m.p.: 118-120 °C; IR (KBr): 3454, 3074, 2998, 2918, 2885, 2850, 2246, 1584, 1452, 1352, 1246, 1197, 1166, 1092, 1011 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.01-2.24 (m, 4H), 3.40-3.42 (m, 2H), 4.60-4.63 (m, 1H), 7.56-7.60 (m, 2H), 7.64-7.66 (m, 1H), 7.90-7.91 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.68, 29.70, 31.93, 47.59, 48.63, 118.04, 127.50, 129.39, 133.54, 137.24; $C_{11}H_{12}N_2O_2S$; ESI-MS: *m/z* 258.9 $[M+Na]^+$

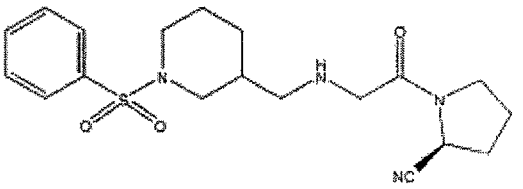
General procedure for the preparation of compound 10a, 10b:

To a solution of compound 9a or 9b (1.0 mmol) in THF (25 mL) at -18 °C, lithium aluminium hydride (LAH) (4.0 mmol) was added portion-wise over a period of 30 minutes and the reaction mixture stirred at room temperature for 4 hours or till the completion of reaction as monitored by TLC. On completion of reaction, 10% aq. NaOH solution, at 5-10 °C, added drop wise to the reaction mixture till effervescence ceases, filtered through hy-flow and extracted with ethylacetate several times. The filtrates were collected and concentrated to give crude product **10a-b**, as viscous liquid which was used directly for the next reaction.

General procedure for the preparation of compound 11a, 11b:

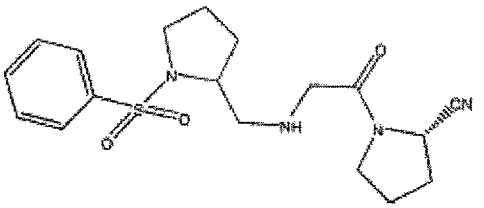
A mixture of anhydrous K_2CO_3 (3.0 mmol), amine **10a** or **10b** (1.7 mmol) and compound **5** (1.5 mmol) was refluxed for 2 hours or till the completion of reaction as detected on the TLC. It was then cooled to room temperature, filtered and the residue was washed with ethylacetate. The filtrate was collected, solvent evaporated under reduced pressure to give the crude product which was purified by column chromatography using neutral alumina as stationary phase and 7:3 ethylacetate : pet.ether as mobile phase to yield product **11a-b** as thick viscous liquids.

(2S)-1-(2-((1-(phenylsulfonyl)piperidin-3-yl)methylamino)acetyl)pyrrolidine-2-carbonitrile 11a:



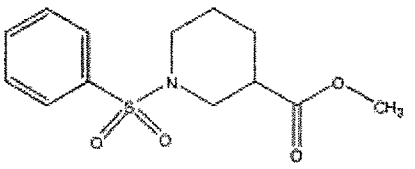
Yield: 45%; viscous liquid; $[\alpha]_D$ = -517.04; IR (Neat): 3476, 3063, 2934, 2853, 1684, 1585, 1447, 1341, 1227, 1170, 1092, 993, 947, 750, 692, 596, 577 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.63-1.79 (m, 6H), 1.95-2.45 (m, 7H), 3.15-3.25 (m, 1H), 3.45-3.47 (m, 2H), 3.57-3.61 (m, 3H), 3.76-3.81 (m, 1H), 4.08-4.18 (m, 1H), 7.54-7.63 (m, 3H), 7.74-7.77 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 22.68, 27.56, 28.84, 34.18, 34.42, 45.26, 45.28, 46.59, 46.62, 49.05, 49.63, 52.38, 58.98, 127.58, 129.10, 132.79, 132.82, 135.98, 136.05, 162.96, 163.00, 167.75, 167.83; $C_{19}H_{26}N_4O_3S$; ESI-MS: *m/z* 414.07 $[M+Na]^+$

(2S)-1-(2-((1-(phenylsulfonyl)pyrrolidin-2-yl)methylamino)acetyl)pyrrolidine-2-carbonitrile 11b:



Yield: 20%; viscous liquid; $[\alpha]_D$ = -116.60; IR (Neat): 3480, 2954, 2879, 1661, 1652, 1645, 1446, 1336, 1162, 1091, 1072, 758, 573 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.26-2.30 (m, 8H), 2.92-3.1 (m, 2H), 3.50-3.73 (m, 8H), 4.74-4.76 (m, 1H), 7.55-7.61 (m, 3H), 7.81-7.84 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 22.73, 23.93, 24.08, 25.36, 29.71, 29.80, 29.88, 32.25, 45.92, 46.09, 46.30, 46.44, 46.53, 46.79, 48.69, 48.77, 56.27, 57.27, 58.88, 59.29, 59.46, 60.19, 60.50, 62.82, 118.50, 119.13, 127.40, 127.48, 127.81, 129.19, 129.27, 132.79, 132.90, 137.07, 137.57, 168.86, 169.28, 169.62; $C_{18}H_{24}N_4O_3S$; ESI-MS: *m/z* 376.8 $[M+H]^+$

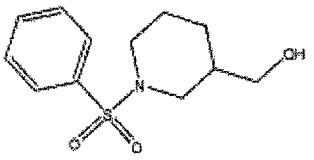
Procedure for the preparation of methyl 1-(phenylsulfonyl)piperidine-3-carboxylate 12:



To a solution of compound **7** (1.0 mmol) in DCM, oxalyl chloride (1.5 mmol) was added at 0-5 °C and stirred at room temperature for an hour and then methanol (1.1 mmol) added and the reaction mixture stirred for another hour or till the completion of reaction as monitored by TLC to yield product **12** as a viscous liquid.

Yield: 90%; viscous liquid; IR (Neat): 3064, 2952, 2856, 1726, 1637, 1586, 1467, 1446, 1343, 1233, 1167, 1130, 1091, 1033, 948, 741, 693, 579 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.25-1.35 (m, 1H), 1.53-1.59 (m, 1H), 1.69-1.75 (m, 1H), 1.87-1.91 (m, 1H), 2.28 (dt, 1H, J_1 = 2.8 Hz, J_2 = 11.2 Hz), 2.45 (t, 1H, J = 11.2 Hz), 2.51-2.58 (m, 1H), 3.52 (d, 1H, J = 11.6 Hz), 3.59 (s, 3H), 3.77 (d, 1H, J = 11.6 Hz), 7.46-7.54 (m, 3H), 7.67-7.70 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.87, 26.29, 40.88, 46.25, 47.63, 51.91, 127.51, 129.13, 132.88, 135.94, 173.00; $C_{13}H_{17}NO_4S$; ESI-MS: *m/z* 283.9 $[M+H]^+$

Procedure for the preparation of (1-(phenylsulfonyl) piperidin-3-yl)methanol 13:

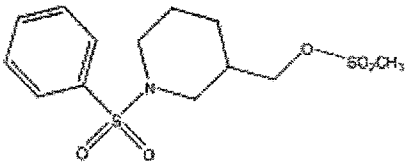


To a solution of 1-(phenylsulfonyl)piperidine-3-carboxylate **12** (1.0 mmol) in THF (25 mL), LAH (2.5 mmol) added portionwise at -18 °C over a period of 15 minutes and the reaction mixture was stirred at room temperature for 4 hours or till the completion of reaction as monitored by TLC. On completion of reaction, 10% aq. NaOH solution, at 5-10 °C, added drop wise to the reaction mixture till effervescence ceases, filtered through hy-flow and extracted with ethylacetate several times. The filtrates were collected and concentrated to give crude product **13**, as viscous liquid.

Yield: 80%; viscous liquid; IR (Neat): 3531, 2928, 2902, 2854, 2840, 1465, 1450, 1332, 1316, 1169, 1154, 1090, 1008, 929, 754, 694, 627, 575 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.01-1.10 (m, 1H), 1.61-1.80 (m, 4H), 1.87-1.94 (m, 1H), 2.30 (t, 1H, J = 10.4 Hz), 2.45 (dt, 1H, J_1 = 2.8 Hz, J_2 = 10.8 Hz), 3.50-3.60 (m, 3H), 3.68 (dd, 1H, J_1 = 4.0

Hz, J_2 = 11.2 Hz), 7.53-7.64 (m, 3H), 7.77-7.80 (m, 2H); $C_{12}H_{17}NO_3S$; ESI-MS: *m/z* 256.0 $[M+H]^+$

Procedure for the preparation of (1-(phenylsulfonyl)piperidin-3-yl)methyl methanesulfonate 14:



(1-(phenylsulfonyl)piperidin-3-yl)methanol **13** (1.0 mmol) mixed with triethyl amine (1.2 mmol) and methane sulfonyl chloride (1.2 mmol) in THF (25 mL) and stirred at room temperature for 2 hours. Then reaction mixture washed with water (1X10 mL), sat. sodium bicarbonate solution (1X25 mL) and solvent evaporated under reduced pressure to give product **14** as viscous liquid.

Yield: 65%; viscous liquid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20-1.26 (m, 1H), 1.46 (s, 1H), 1.65-1.78 (m, 4H), 2.13-2.18 (m, 1H), 2.50-2.55 (m, 1H), 2.61-2.66 (m, 1H), 2.98 (d, 1H, J = 8.4Hz), 3.07 (s, 3H), 3.39-3.42 (m, 1H), 3.50-3.53 (m, 1H), 4.10-4.19 (m, 2H), 7.55-7.65 (m, 3H), 7.76-7.78 (m, 2H).

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