

Synthesis and characterization of impurities of an anticonvulsant drug, Pregabalin

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Abstract

During the process development of Pregabalin **1**, a known anticonvulsant drug, six potential impurities were identified in the final crude material ranging from 0.01 to 0.15% by LCMS. All six impurities were subsequently synthesized and characterized by IR, MS and NMR spectral data. Four of the six related substances are known as 4-isobutylpyrrolidin-2-one **6**, 3-isobutylglutaric acid **2**, (*R*)-(-)-3-carbamoylmethyl-5-methylhexanoic acid **5** and (*R*)-(-)-3-aminomethyl-5-methylhexanoic acid **8**, whilst (*S*)-3-aminomethyl-5-methylhexanoic acid isobutyl ester **9** and (*S*)-3-aminomethyl-5-methylhexanoic acid isopropyl ester **10** are new compounds reported for the first time in our process. The present work describes the formation, synthesis and characterization of these impurities.

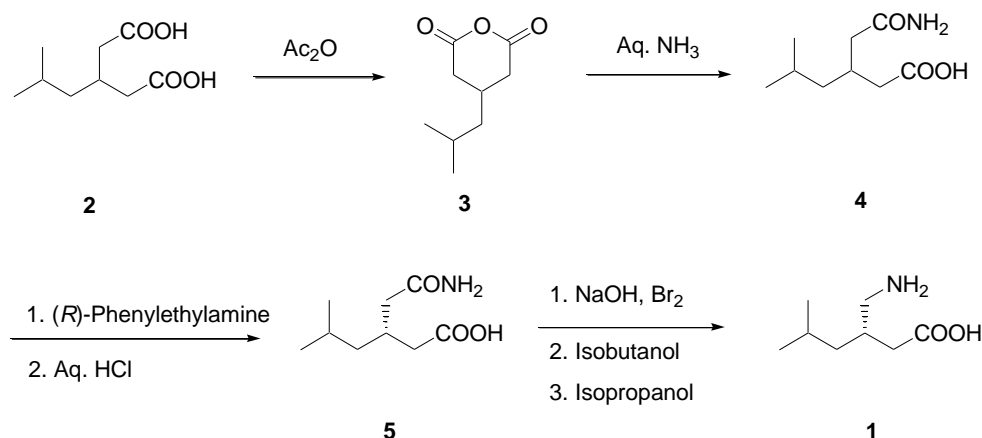
Keywords: Pregabalin, anticonvulsant, impurities, synthesis, characterization

Introduction

Pregabalin **1** ((*S*)-(+)-3-aminomethyl-5-methylhexanoic acid) is a novel and potent anticonvulsant agent for the treatment of epilepsy and pain.¹ It has also been found to be more active than Gabapentin in preclinical models of epilepsy.² It has more potent and robust activity in various models of epilepsy, neuropathic pain and anxiety.³

The presence of impurities in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug products. Therefore, it is necessary to study the impurity profile of the API to be used in the manufacturing of drug substance. International Conference on Harmonization (ICH) guidelines recommended identifying and

characterizing all impurities that are present at a level of 0.10% or more.^{4,5} As Pregabalin **1** is an important drug substance and to obtain information on product profile, a comprehensive study was undertaken on the impurities generated during the process development (Scheme 1).^{6,7}



Scheme 1. Reported synthetic scheme for Pregabalin.

Results and Discussion

During the process development of Pregabalin **1**, HPLC analysis of crude Pregabalin revealed six impurities ranging from 0.01-0.15%. According to ICH (International Conference on Harmonization) guidelines the amount of acceptable level for known and unknown impurities in a final drug candidate must be less than 0.15% and 0.10% respectively. In order to meet the stringent regulatory requirements, the impurities needed to be identified and characterized. Hence, samples of Pregabalin **1** were initially analyzed by LCMS to provide parent ions at m/z 142, 187, 188, 216, 202 for five related impurities and at m/z 160 for the enantiomer of Pregabalin **1**, and thus provide a basis for initial identification. To confirm their proposed structures and complete their characterization, all six substances were individually synthesized and characterized by their respective IR, MS, and NMR spectral data. The structure of these related substances were assigned as 4-isobutylpyrrolidin-2-one **6**, 3-isobutylglutaric acid **2**, (R)-(-)-3-carbamoylmethyl-5-methylhexanoic acid **5**, (S)-3-aminomethyl-5-methylhexanoic acid isobutyl ester **9**, (S)-3-aminomethyl-5-methylhexanoic acid isopropyl ester **10** and (R)-(-)-3-aminomethyl-5-methylhexanoic acid **8** respectively.

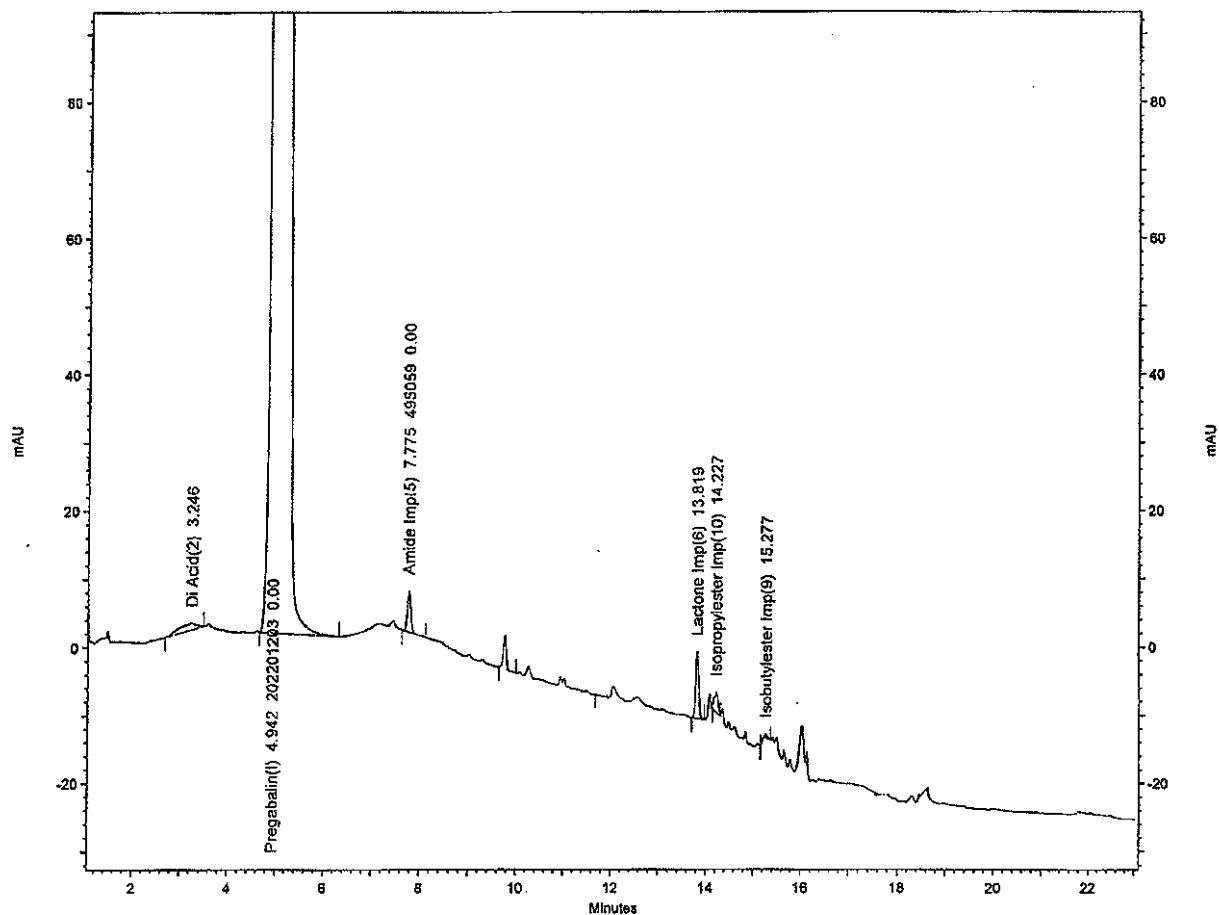
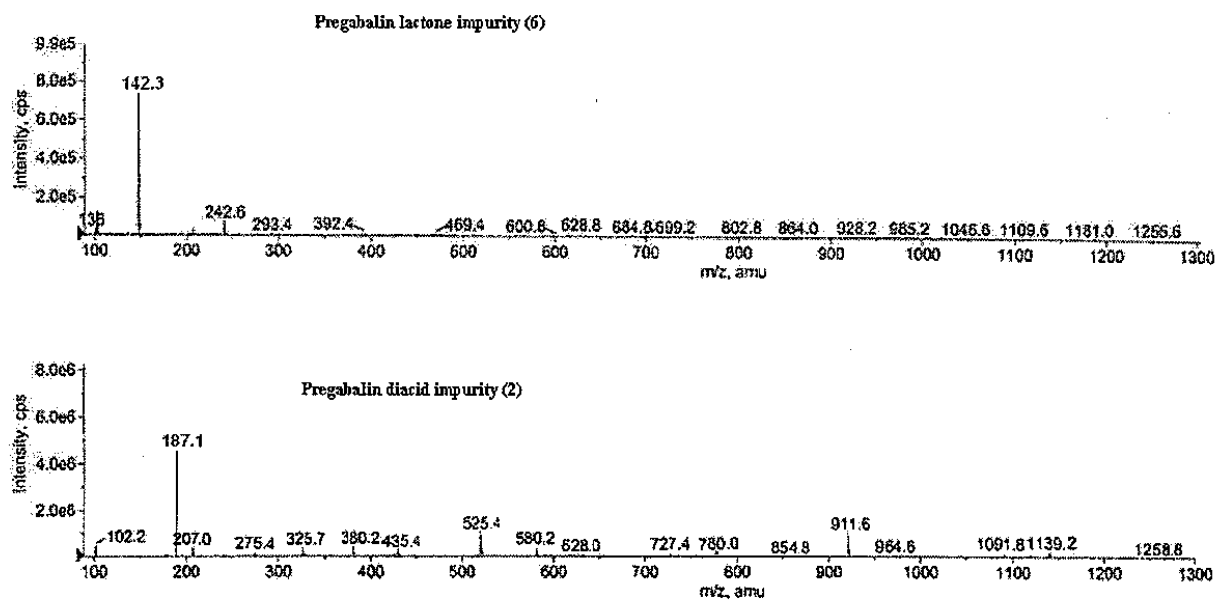


Figure 1. HPLC Chromatogram of pregabalin.



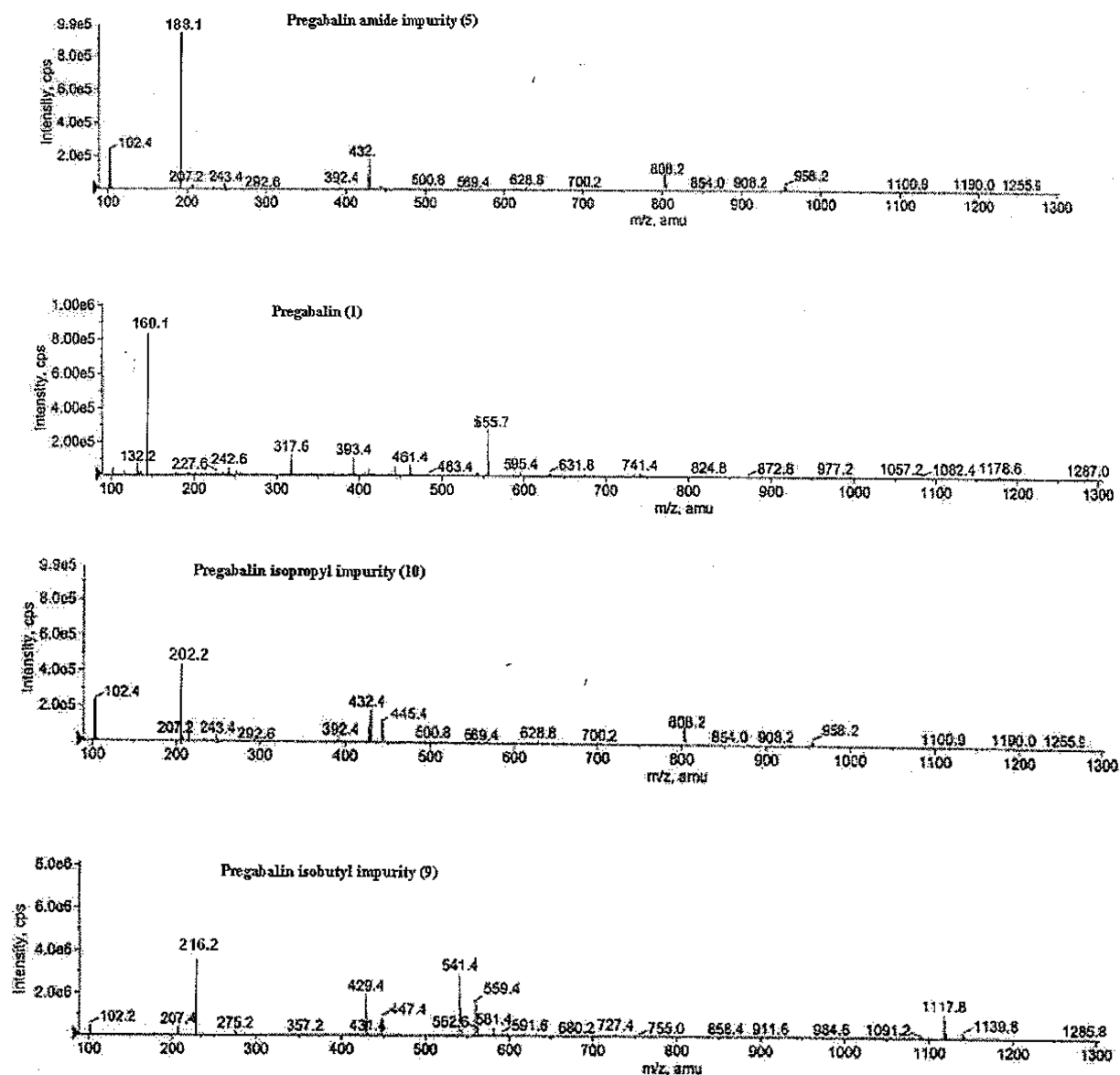
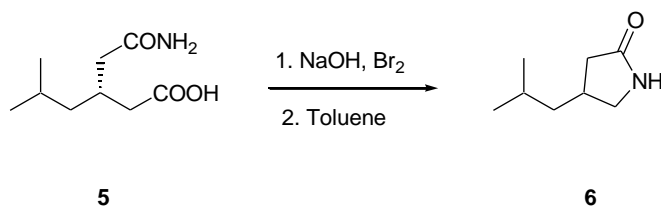


Figure 2. LC-MS Spectrum of pregabalin and its related substances.

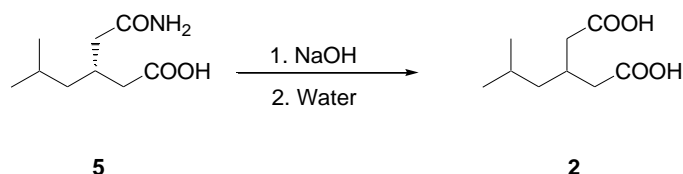
Formation of related compounds

The substance **6** was formed during Hofmann reaction of (*R*)-(-)-3-carbamoylmethyl-5-methylhexanoic acid **5** in the presence of bromine and sodium hydroxide.^{8a} The related compound **6** was synthesized by the reaction of (*R*)-(-)-3-carbamoylmethyl-5-methylhexanoic acid **5** with excess of sodium hydroxide and bromine at elevated temperature (Scheme 2). The mass spectrum displayed a protonated molecular ion at *m/z* 142 and the NMR spectrum showed a peak at δ 7.44 ppm corresponding to cyclic amide –NH proton. Based on the spectral data the structure was confirmed as 4-isobutylpyrrolidin-2-one **6**.



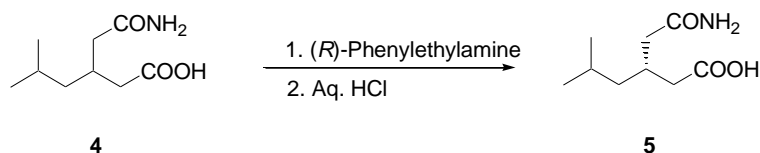
Scheme 2

Related substance **2**, a potential impurity which is base hydrolyzed product during Hofmann reaction,^{8b} was synthesized by the reaction of (*R*)-(-)-3-carbamoylmethyl-5-methylhexanoic acid **5** with excess of sodium hydroxide at elevated temperature (Scheme 3). The mass spectrum of compound **2**, displayed a negative molecular ion at m/z 187 whilst in the IR spectrum a sharp band appeared at 1575 cm^{-1} corresponding to an aliphatic acid carbonyl and the spectral data is consistent with the structure of 3-isobutylglutaric acid **2**.



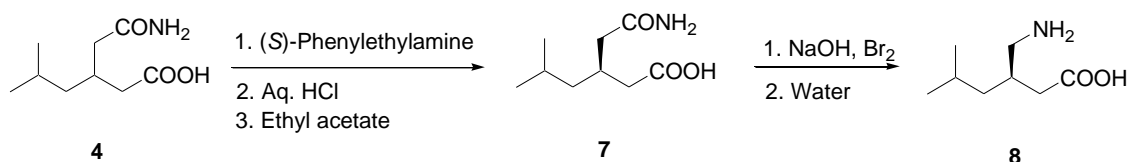
Scheme 3

(*R*)-(-)-3-Carbamoylmethyl-5-methylhexanoic acid **5** is one of the key intermediate in the synthesis of Pregabalin **1**. It was prepared by the resolution of racemic 3-carbamoylmethyl-5-methylhexanoic acid **4** with (*R*)-1-phenylethylamine in the presence of chloroform and ethanol (Scheme 4). The mass spectrum of the substance **5**, showed a protonated molecular ion at m/z 188. The IR spectrum showed a sharp band at 1712 cm^{-1} corresponding to acid carbonyl and at 1644 cm^{-1} corresponding to amide carbonyl. The NMR spectrum displayed a peak at δ 6.76 and δ 7.43 ppm corresponding to amide $-\text{NH}_2$ protons. Based on the spectral data, the structure was confirmed as (*R*)-(-)-3-carbamoylmethyl-5-methylhexanoic acid **5**.



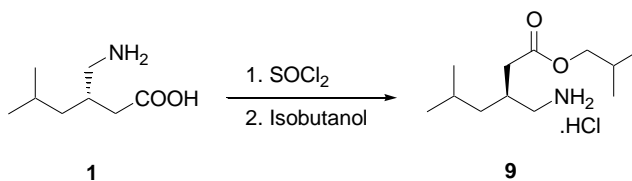
Scheme 4

Any traces of (*S*)-(+)-3-carbamoylmethyl-5-methylhexanoic acid **7** that is present in (*R*)-(-)-3-carbamoylmethyl-5-methylhexanoic acid **5** converts to enantiomer of Pregabalin i.e., (*R*)-(-)-3-aminomethyl-5-methylhexanoic acid **8** during the Hofmann reaction with bromine and sodium hydroxide. Substance **8** was synthesized by the resolution of racemic amide **4** with (*S*)-1-phenylethylamine in chloroform and ethanol followed by Hofmann reaction (Scheme 5). The mass spectrum of the substance **8** displayed a protonated molecular ion at m/z 160. The IR spectrum showed a sharp band at 1644 cm^{-1} corresponding to acid carbonyl and the specific optical rotation of $[\alpha]_{\text{D}}^{25} -10.7^\circ$ confirms the structure of (*R*)-(-)-3-aminomethyl-5-methylhexanoic acid **8**.



Scheme 5

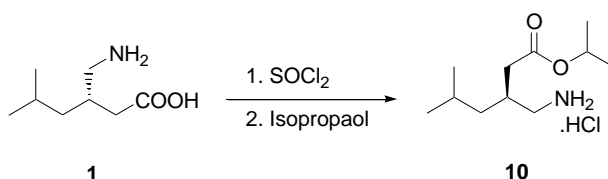
Hofmann reaction of substance **5** with bromine and sodium hydroxide affords Pregabalin **1**. However, as a side reaction isobutanol which is used as solvent for the extraction, reacts with **1** and leads to the formation of isobutyl ester of Pregabalin. This impurity was quantitatively synthesized in the form of hydrochloride salt by the reaction of Pregabalin **1** with thionyl chloride in the presence of isobutanol (Scheme 6). The mass spectrum of the substance **9** displayed a protonated molecular ion at m/z 216 and a sharp band at 1727 cm^{-1} was observed in the IR spectrum which was attributed to ester C=O stretching. The NMR spectrum exhibited 12 protons at δ 0.83-0.90 ppm corresponding to four methyl groups and a doublet at δ 3.8 ppm corresponding to two protons of methylene group adjacent to oxygen atom. Based on the spectral data, the structure of this impurity is assigned as (*S*)-3-aminomethyl-5-methylhexanoic acid isobutyl ester hydrochloride **9**.



Scheme 6

During Hofmann reaction, isopropanol which is used as a solvent for isolation reacts with **1**, as a side reaction and leads to the formation of isopropyl ester of Pregabalin. This impurity was synthesized in the form of hydrochloride salt by the esterification of Pregabalin **1** with thionyl chloride in the presence of isopropanol (Scheme 7). The mass spectrum of the substance **10**

displayed a protonated molecular ion at m/z 202 and a sharp band at 1727 cm^{-1} was observed in the IR spectrum which was attributed to ester C=O stretching. The NMR spectrum displayed a doublet at δ 1.2 ppm corresponding to two methyl groups and a multiplet at δ 4.86-4.95 ppm corresponding to $-\text{CH}$ proton of isopropyl ester. Based on the spectral data the structure of this impurity is assigned as (*S*)-3-aminomethyl-5-methylhexanoic acid isopropyl ester hydrochloride **10**.



Scheme 7

Conclusions

In conclusion, we have identified, synthesized and characterized six potential process related impurities **2**, **5**, **6**, **8**, **9** and **10** of Pregabalin **1**.

Experimental Section

General. The ^1H NMR was recorded in DMSO at 300 MHz on a Bruker 300 MHz FT NMR spectrometer. The chemical shifts were reported in δ ppm relative to TMS. The FT-IR spectra were recorded in the solid state as KBr dispersion using a Perkin-Elmer 1650 FT-IR spectrophotometer. The mass spectrum (70 eV) was recorded on an Agilent-6310 LC-MS spectrometer. The solvents and reagents were used without any purification.

Preparation of 4-isobutylpyrrolidin-2-one (6). To a stirred mixture of water (175 mL) and sodium hydroxide (29.2 g, 0.730 mol) was added bromine (25.5 g, 0.159 mol) slowly for 45-60 min at -10 to $-5\text{ }^\circ\text{C}$. Stirred the reaction mixture for 30 min and added (*R*)-(-)-3-carbamoylmethyl-5-methylhexanoic acid **5** (25.0 g, 0.133 mol) in lot wise for 45-60 min at -10 to $-5\text{ }^\circ\text{C}$. Slowly heated the reaction mixture to 85 - $90\text{ }^\circ\text{C}$ over a period of 5 h and stirred for 8 h at the same temperature. After completion of reaction (TLC), extracted the compound with toluene ($2 \times 75\text{ mL}$) at 80 - $90\text{ }^\circ\text{C}$ and washed the toluene layer with water (50.0 mL), removed the toluene completely under reduced pressure to get light yellow colored oily liquid 4-isobutylpyrrolidin-2-one **6** (5.0 g, yield: 26.2%, purity by HPLC 99%). IR (cm^{-1}): 2924, 1745; ^1H NMR (DMSO- d_6 , δ ppm): 0.84-0.88 (d, 6H, CH_3 , J 6.0 Hz), 1.24-1.29 (t, 2H, CH_2 , J 6.0 Hz), 1.47-1.58 (m, 1H, CH), 1.74-2.23 (dd, 2H, CH_2 , J 6.0 Hz), 2.33-2.44 (m, 1H, CH), 2.78-2.83 (t, CH_2 , J 6.0 Hz), 7.44 (s,

1H, NH); ¹³C NMR (DMSO-*d*₆, ppm): 176.95, 47.89, 43.91, 37.35, 32.86, 26.15, 22.92; HRMS for C₈H₁₅NO [M+H]⁺: *m/z* Calcd: 142.1231; Found: 142.1227.

3-Isobutylglutaric acid (2). To a mixture of water (80 mL) and sodium hydroxide (21.4 g, 0.535 mol) was added (*R*)-(-)-3-carbamoylmethyl-5-methylhexanoic acid **5** (20.0 g, 0.106 mol) and heated to 75-80 °C, stirred for 5 h at the same temperature. After completion of reaction (TLC), cooled the reaction mixture to 25-30 °C and adjusted the pH to ~7.0 with aq. HCl solution, stirred for 30-45 min at 25-30 °C. Filtered the separated solid and washed with water (10.0 mL), dried at 50 °C under vacuum to afford 3-isobutylglutaric acid **2** (17.0 g, yield: 85%, purity by HPLC 99.5%). IR (cm⁻¹): 3436, 2953, 1575; ¹H NMR (D₂O, δ ppm): 0.70 (d, 6H, CH₃, *J* 6.0 Hz), 0.97 (s, 2H, CH₂), 1.49 (s, 1H, CH), 1.95 (s, 4H, CH₂), 2.05 (s, 1H, CH); ¹³C NMR (D₂O, ppm): 182.89, 43.66, 31.90, 24.60, 22.27; MS: *m/z*: 187 (M-H⁺); Analysis calcd. for C₉H₁₆NO₄: C, 57.43; H, 8.57% Found: C, 57.41; H, 8.55%.

(*R*)-(-)-3-Carbamoylmethyl-5-methylhexanoic acid (5). A mixture of 3-carbamoyl-methyl-5-methylhexanoic acid **4** (40.0 g, 0.213 mol), chloroform (464.0 mL), ethanol (9.6 mL) and *R*-(+)-phenylethylamine (18.9 g, 0.156 mol) was heated to 55-60 °C, stirred for 45 min. The reaction mixture was cooled to 25-30 °C in 60 min and stirred for 1 h then cooled to 10-15 °C and stirred for 60 min. Filtered the separated solid and washed with chloroform (20.0 mL), suck dried for 1 h. To the wet cake charged water (200 mL), stirred for 10-15 min and adjusted the pH to 1-2 with aq. HCl at 0-5 °C, stirred for 45 min. Filtered the solid and washed with water (20.0 mL), dried at 50 °C under vacuum to afford (*R*)-(-)-3-carbamoylmethyl-5-methylhexanoic acid **5** (16.0 g yield: 40%, purity by HPLC 99.4%). IR (cm⁻¹): 3436, 3333, 3227, 2959, 1712, 1644; ¹H NMR (DMSO-*d*₆, δ ppm): 0.56-0.92 (d, 6H, CH₃, *J* 6.0 Hz), 1.09 (t, 2H, CH₂, *J* 6.0 Hz), 1.49-1.64 (m, 1H, CH), 1.87-2.34 (m, 5H, CH₂ & CH), 6.76-7.3 (s, 2H, Amide -NH₂), 12.05 (s, 1H, Acid-OH); ¹³C NMR (DMSO-*d*₆, ppm): 174.27, 173.99, 42.96, 29.63, 24.46, 22.5; MS: *m/z*: 188 (M+H⁺); Analysis calcd. for C₉H₁₇NO₃: C, 57.73; H, 9.15; N, 7.48% Found: C, 57.68; H, 9.15; N, 7.69%.

(*R*)-(-)-3-Aminomethyl-5-methylhexanoic acid (8). A mixture of 3-carbamoylmethyl-5-methylhexanoic acid **4** (40.0 g, 0.213 mol), chloroform (464.0 mL), ethanol (9.6 mL) and *S*-(-)-phenylethylamine (18.9 g, 0.156 mol) was heated to 55-60 °C, stirred for 45 min. The reaction mixture was cooled to 25-30 °C in 45-60 min and stirred for 1 h then cooled to 10-15 °C and stirred for 45-60 min. Filtered the separated solid and washed with chloroform (20.0 mL), suck dried for 1 h. To the wet cake charged water (200 mL), stirred for 10-15 min. and adjusted the pH to 1-2 with aq. HCl at 0-5 °C, stirred for 45 min, filtered the solid and washed with water (20.0 mL), suck dried for 2 h to afford (*S*)-(+)-3-carbamoylmethyl-5-methylhexanoic acid **7** (16.0 g). Charged the semi dried compound **7** into a mixture of water (80.0 mL) and sodium hydroxide (18.8 g, 0.47 mol), stirred for 5-10 min. Cooled the reaction mixture to 0-5 °C, added bromine (14.5 g, 0.09) for 1-2 h and stirred for 2 h at 20-25 °C. Poured the mass into aq. HCl (~36.0 mL) solution at below 20 °C. Extracted the compound with isobutanol (80.0 mL) and adjusted the pH to 7.0 with triethyl amine (~12 mL) at 25-30 °C and stirred for 60 min at 0-5 °C. Filtered the isolated solid, washed with isobutanol (8.0 mL) and finally the wet compound was recrystallized from isopropanol and water (1:1) to yield (*R*)-(-)-3-aminomethyl-5-methylhexanoic

acid **8** (7.0 g, yield: 20.6%, purity by HPLC 99.6%). IR (cm^{-1}): 2955, 1644; ^1H NMR (D_2O , δ ppm): 0.83 (d, 6H, CH_3 , J 6.0 Hz), 1.15 (t, 2H, CH_2 , J 6.0 Hz), 1.52-1.63 (m, 1H, CH), 2.05-2.29 (m, 3H, CH_2 & CH), 2.84-2.98 (m, 2H, CH_2); ^{13}C NMR (D_2O , ppm): 181.14, 46.61, 43.67, 40.73, 31.67, 24.37, 21.99; MS: m/z : 160 ($\text{M}+\text{H}^+$); SOR $[\alpha]_{\text{D}}^{25}$: - 10.7 ° ($c=1\%$ water); Analysis calcd. for $\text{C}_8\text{H}_{17}\text{NO}_2$: C, 60.35; H, 10.76; N, 8.80% Found: C, 60.15; H, 10.80; N, 8.72%.

(S)-3-Aminomethyl-5-methylhexanoic acid isobutyl ester hydrochloride (9). To a mixture of Pregabalin (**1**, 10.0 g, 0.062 mol) and isobutanol (50.0 mL) was added thionyl chloride (14.9 g, 0.125 mol) at 10-15 °C and slowly heated to reflux, stirred for 3-4 h at the same temperature. After completion of reaction (TLC), distilled out solvent completely under reduced pressure and purified the crude compound in n-hexane to afford (S)-3-aminomethyl-5-methylhexanoic acid isobutyl ester hydrochloride **9** (9.6 g, yield: 61%, purity by HPLC 99.3%). IR (cm^{-1}): 2957, 1727; ^1H NMR ($\text{DMSO}-d_6$, δ ppm): 0.83-0.90 (d, 6H, CH_3 , J 6.0 Hz), 1.03-1.29 (m, 2H, CH_2), 1.56-1.62 (m, 1H, CH), 1.82-2.18 (m, 2H, CH_2), 2.2-2.34 (m, 1H, CH), 2.54-2.61 (m, 1H, CH), 2.75-2.77 (d, 2H, CH_2 , J 6.0 Hz), 3.81-3.83 (d, 2H, CH_2 , J 6.0 Hz), 8.1 (s, 3H, NH_3^+Cl^-); ^{13}C NMR ($\text{DMSO}-d_6$, ppm): 172.22, 70.25, 42.74, 36.61, 31.44, 27.71, 24.94, 22.94, 20.18, 19.34; MS: m/z : 216 ($\text{M}+\text{H}^+$); Analysis calcd. for $\text{C}_{12}\text{H}_{25}\text{NO}_2$: C, 66.93; H, 11.70; N, 6.50% Found: C, 66.81; H, 11.42; N, 6.40%.

(S)-3-Aminomethyl-5-methylhexanoic acid isopropyl ester hydrochloride (10). To a mixture of Pregabalin (**1**, 10.0 g, 0.062 mol) and isopropanol (50.0 mL) was added thionyl chloride (14.9 g, 0.125 mol) at 10-15 °C and slowly heated to reflux, stirred for 3-4 h at the same temperature. After completion of reaction (TLC), distilled out solvent completely under reduced pressure and purified the crude compound in n-hexane to afford (S)-3-aminomethyl-5-methylhexanoic acid isopropyl ester hydrochloride (**10**, 9.4 g, yield: 63.0%, purity by HPLC 99.3%). IR (cm^{-1}): 2959, 1727; ^1H NMR ($\text{DMSO}-d_6$, δ ppm): 0.83-0.87 (d, 6H, CH_3 , J 6.0 Hz), 1.02-1.11 (m, 1H, CH), 1.17-1.20 (d, 6H, CH_3 , J 6.0 Hz), 1.23-1.64 (m, 2H, CH_2), 2.11-2.27 (m, 2H, CH_2), 2.53-2.55 (m, 1H, CH), 2.74-2.75 (m, 2H, CH_2), 4.86-4.95 (m, 1H, CH), 8.1 (s, 3H, NH_3^+Cl^-); ^{13}C NMR ($\text{DMSO}-d_6$, ppm): 171.13, 67.17, 41.85, 38.6, 36.45, 31.04, 24.46, 22.35, 21.55. MS: m/z : 202 ($\text{M}+\text{H}^+$); Analysis calcd. for $\text{C}_{11}\text{H}_{23}\text{NO}_2$: C, 65.63; H, 11.52; N, 6.96% Found: C, 65.52; H, 11.42; N, 6.68%.

Acknowledgements

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