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Research Article

Synthesis of stable isotopically labelled versions of lamotrigine and its methylated metabolite

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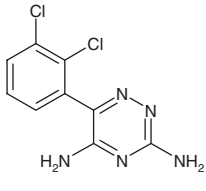
Summary

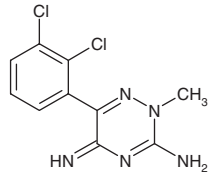
Lamotrigine is a sodium channel antagonist used for the treatment of epilepsy. Synthesis of stable isotopically labelled (SIL) [M + 7] versions of Lamotrigine **(1)** and its *N*-methylated metabolite **(2)** are described. The routes to prepare these compounds used [M + 5] labelled [^{13}C, $^{15}N_4$]-aminoguanidine (obtained from labelled thiourea). The overall yield for the metabolite **(2)** was 34% from [M + 3] labelled [^{13}C, $^{15}N_2$]-thiourea.

Key Words: lamotrigine; mass label; aminoguanidine

Introduction



Lamotrigine **(1)**



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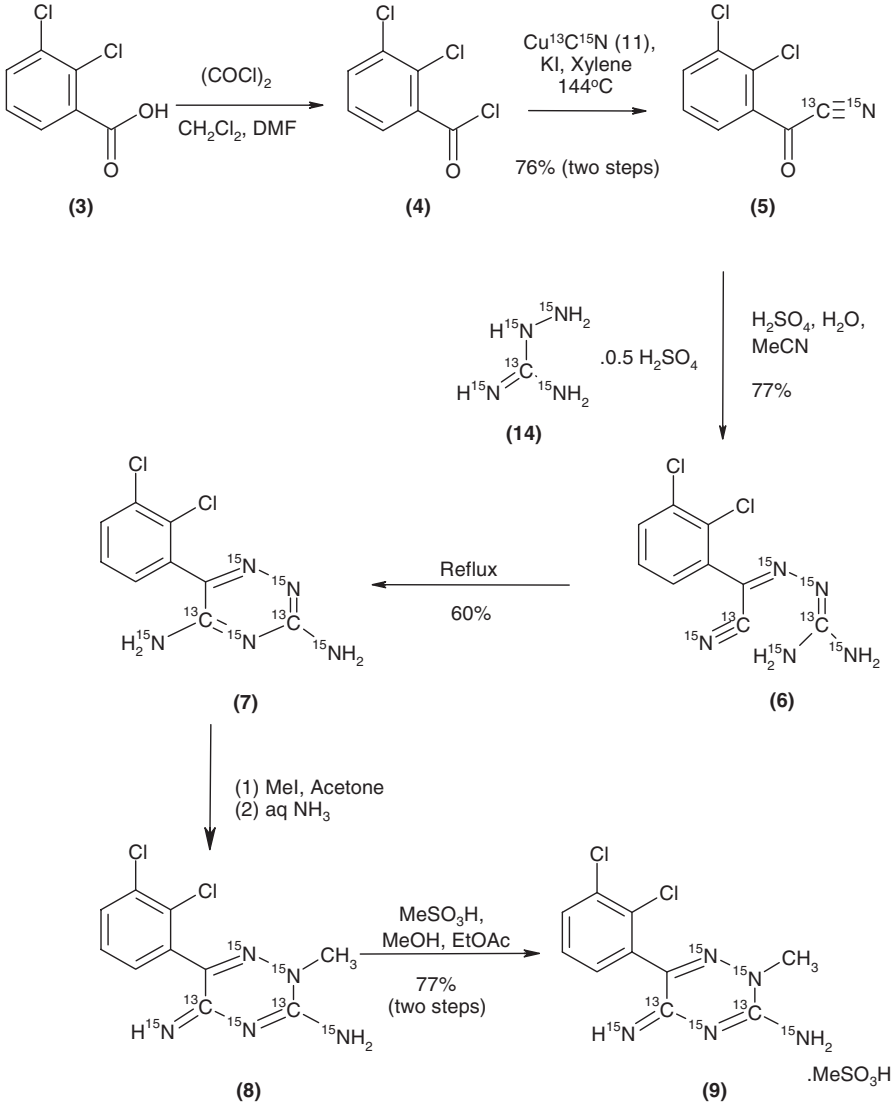
Lamotrigine **(1)** is a sodium channel antagonist marketed as an anticonvulsive agent for the treatment of epilepsy. To enable the quantification of unlabelled parent compound **(1)** and its *N*-2 methylated metabolite **(2)** in biological fluids, mass labelled versions were required for use as internal standards in the mass spectrometric assays. The two chlorine atoms present in the compounds under study meant that an additional seven mass units were required to be incorporated into the SIL versions. This was to ensure absence of interfering molecular ions arising from the natural isotope distribution pattern.

Results and discussion

The SIL versions of Lamotrigine **(1)** and its *N*-2 methylated metabolite **(2)** were synthesized as outlined in Schemes 1, 2 and 3. This allowed both compounds to be prepared using the same route and labelled starting materials.

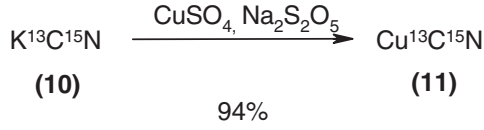
Commercially available 2,3-dichlorobenzoic acid **(3)** was converted in to acid chloride **(4)** and further reacted without isolation with copper [^{13}C, ^{15}N]-cyanide **(11)** to yield [M + 2]-2,3-dichlorobenzoylcyanide **(5)**. The key step involved a condensation of [M + 5]-aminoguanidine **(14)** with [M + 2]-2,3-dichlorobenzoylcyanide **(5)**. In order to maximise incorporation of the more valuable [M + 5]-aminoguanidine sulfate **(14)** four equivalents of the [M + 2]-benzoyl cyanide **(5)** were used to one of [M + 5]-aminoguanidine sulfate **(14)**. The resulting product (Z)-2-(2,3-dichlorophenyl)-2-(guanidinoimino)acetonitrile **(6)** was subsequently cyclized in refluxing propan-1-ol to obtain [M + 7]-Lamotrigine **(7)**. There was a small quantity (3% by HPLC) of the (E)-isomer of **(6)** present prior to the cyclization. This does not cyclize and remained unchanged but was readily removed by chromatography over silica gel. (M + 7)-Lamotrigine **(7)** was methylated with iodomethane to yield the metabolite. The resulting iodide salt was neutralised by slurrying the solid in concentrated aqueous ammonia solution, followed by filtration to give the free base **(8)**, which was converted into the desired mesylate salt **(9)**.

Copper (I) [^{13}C, ^{15}N]-cyanide **(11)** was prepared as shown in Scheme 2 and was obtained from [^{13}C, ^{15}N]-potassium cyanide **(10)** using copper sulfate and sodium metabisulfite as described by Young.[1]

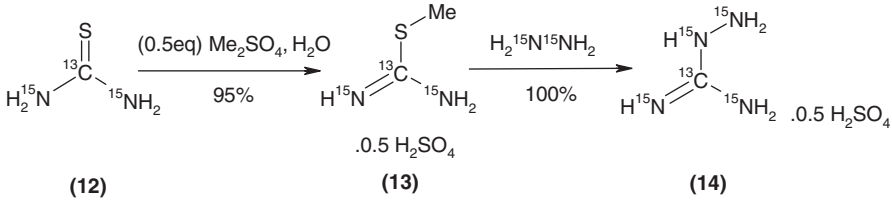


Scheme 1. Synthesis of SIL (Lamotrigine)

[^{13}C, $^{15}N_4$]-Aminoguanidine **(14)** was prepared as shown in Scheme 3. [^{13}C, $^{15}N_2$]-Thiourea **(12)** was treated with 0.5 equivalents of dimethylsulfate in water at reflux to obtain [M + 3]-*S*-methyl isothiouronium sulfate **(13)**.[2] Further reaction with [$^{15}N_2$]-hydrazine liberated methanethiol and gave [M + 5]-aminoguanidine **(14)**.[3] The product was isolated by removal of volatile material under vacuum to give the crude sulfate salt **(14)**, contaminated with sodium sulfate. The yield was essentially



Scheme 2. Preparation of (M + 2) copper cyanide



Scheme 3. Synthesis of (M + 5) aminoguanidine

quantitative (no *S*-methyl isothiourea detected in the mass spectrum) and the salt **(14)** was used directly in the next step.

Conclusion

Syntheses of [M + 7] SIL labelled versions of Lamotrigine and the N^2-methylated metabolite of lamotrigine were developed for their use as internal standards in mass spectrometric assays. During this work, a procedure for preparing SIL [^{13}C, $^{15}N_4$]-aminoguanidine sulfate was developed. This procedure could be modified by using the more expensive [$^{15}N_2$]-hydrazine hydrate (rather than the sulfate) to obtain [^{13}C, $^{15}N_4$]-aminoguanidine free from sodium sulfate.

Experimental

^1H NMR spectra were recorded on a Varian Unity 400 spectrometer. ^{15}N NMR spectra were measured on a Varian 400 spectrometer at 25°C, chemical shifts were referenced to CH_3NO_2 at 0 ppm via external benzamide at −278.4 ppm.[4] To reduce ^{15}N relaxation times, chromium acetylacetonate was added to solutions in DMSO-d[6] and chromium nitrate was added to D_2O solutions. Mass spectra were recorded on a Micromass Platform II mass spectrometer and accurate masses were carried out on a Microman Q-ToF mass spectrometer. Thin layer

chromatography was carried out using Polygram SIL G/UV_{254} plastic sheets. Chromatography was carried out using Merck 9385 silica gel. HPLC analysis was carried out on a Prodigy ODS3 5μ ($150 \times 4.6\,mm^2$) column eluted with a gradient. Eluent A = 0.1% aqueous trifluroacetic acid, eluent B = 95% acetonitrile, 5% water, 0.1% trifluroacetic acid, gradient = 10–80% B over 20 min; UV detection at 235 nm.

The isotopically labelled thiourea and hydrazine sulfate were obtained from Aldrich and the labelled potassium cyanide from Cambridge Isotope Laboratories.

*Copper [^{13}C, ^{15}N]-cyanide (**11**)*

Copper sulfate pentahydrate (8.38 g, 33.6 mmol) and water (20 ml) were stirred at 45°C. The resulting solution at 45°C was treated with a solution of sodium metabisulfite (1.73 g, 9.1 mmol) in water (10 ml). A solution of potassium hydroxide (0.775 g, 13.8 mmol) and potassium cyanide (1.8 g, 26.9 mmol) in water (8.5 ml) was added dropwise over 5 min.

The resulting suspension was stirred at for 1 h at 45°C, followed by 30 min at 23°C.

The suspension was filtered, washed with water (4×30 ml), ethanol (2×30 ml), and ether (2×10 ml) and was dried under vacuum to give the title compound **(11)** as a white solid (2.30 g, 94%).

*S-Methyl [^{13}C, $^{15}N_2$]-isothiouronium sulfate (**13**)*

A stirred suspension of [^{13}C, $^{15}N_2$]-thiourea **(12)** (0.7 g, 8.86 mmol) and water (3 ml) was treated with dimethylsulfate (0.4 2 ml, 4.43 mmol) and heated at reflux for 20 h. The mixture was cooled and concentrated to dryness under vacuum to give the title compound **(13)** as a white solid (1.192 g, 95%); m.p. 243°C ; *m/z* 94 (MH^+, 100%).

*[^{13}C, $^{15}N_4$]-Aminoguanidine sulfate (**14**)*

A stirred suspension of *S*-methyl [^{13}C, $^{15}N_2$] isothiouronium sulfate **(13)** (1.05 g, 7.39 mmol), [$^{15}N_2$] hydrazine sulfate (0.98 g, 7.42 mmol) and water (10 ml) was treated with 2 M aqueous sodium hydroxide (7.8 ml, 15.6 mmol). The resulting mixture was stirred for 16 h at 20°C and then purged with a stream of nitrogen for 30 min to remove the majority of the volatile methanethiol. Two molar aqueous sulfuric acid was added

to obtain pH 2. The volatiles were removed under reduced pressure to give the title compound **(14)** as a white solid (2.272 g); *m/z* 80.0571 (calculated 80.0585); δ_{15N}(400 MHz, DMSO-d_6) −286.6 (1N, d, J_{N-N} = 25.1 Hz), −314.0 (1N, m), 329.4 (2N, t, J = 4 Hz).

*2,3-Dichlorobenzoyl [^{13}C, ^{15}N]-cyanide (**5**)*

2,3-Dichlorobenzoic acid (3.85 g, 20.16 mmol) was stirred in dichloromethane (50 ml). One drop of *N*,*N*-dimethylformamide was added followed by the addition of oxalyl chloride (2.64 ml, 30.26 mmol). The solution was left to stir for 4 h before concentrating under reduced pressure.

The resulting acid chloride was dissolved in anhydrous xylene (60 ml) and treated with copper [^{13}C, ^{15}N]-cyanide (2.3 g, 25.14 mmol) and potassium iodide (4.02 g, 24.22 mmol). The mixture was heated to reflux for 24 h followed by removal of the solvent under reduced pressure. The residue was triturated with isohexane (15 ml), filtered and dried under vacuum at 23°C to give a cream solid (3.075 g, 76%); δ_H (400 MHz, $CDCl_3$) 8.08 (1 H,dd), 7.84 (1 H,dd), 7.49 (1 H, t).

*(Z)-2-(2,3–Dichlorophenyl)-2-([^{13}C,$^{15}N_4$]-guanidinoimino) aceto [^{13}C, ^{15}N]-nitrile (**6**)*

Crude labelled aminoguanidine sulfate **(14)** (1.294 g, ~3.8 mmol) was dissolved in concentrated sulfuric acid (80 g) and water (45 ml). A solution of labelled 2,3-dichlorobenzoyl cyanide **(5)** (3.075 g, 15.22 mmol) in acetonitrile (17 ml) was added dropwise. The resulting suspension was rapidly stirred for 5 days before adding saturated aqueous sodium hydrogen carbonate (1500 ml). The mixture was extracted three times with ethyl acetate (260 ml and 2 × 100 ml). The combined ethyl acetate portions were dried ($MgSO_4$), and concentrated under reduced pressure to give compound **(6)** as a yellow solid (0.767 g, 77%), which was used immediately.

6-(2,3-Dichlorophenyl)-1,2,4-[3,5-$^{13}C_2$,1,2,4-$^{15}N_3$]triazine-3,5-[$^{15}N_2$]diamine (7)

Compound **(6)** (0.767 g) was heated at reflux for 1 h. After concentration under reduced pressure, the crude product was purified by chromatography over silica gel (100 g) eluting with dichloromethane–methanol

(95:5). The fractions containing product were combined and evaporated to dryness to give a white solid (0.603 g). The solid was crystallized from 1-propanol (7 ml) to give [M + 7]-lamotrigine **(7)** as a white solid (0.462 g, 46% from aminoguanidine);

HPLC chemical purity > 99% a/a; *m/z* 263.0074 (calculated 263.0071); δ_H(400 MHz, DMSO-d_6) 7.70(d of d, $J = 8$ Hz, $J = 1.5$ Hz, 1 H, aromatic CH); 7.45(t, $J = 8$ Hz, 1 H, aromatic CH); 7.36(d of d, $J = 8$ Hz, $J = 1.5$ Hz, 1 H, aromatic CH); 6.42(d, $J = 89$ Hz, 2 H, NH_2) 6–7.2(broad singlet, 2 H, NH_2). δ_{15N}(400 MHz, DMSO-d_6) −305.7 (1N, t of d, $J_{H-N} = 89.6$ Hz, $J_{C-N} = 24.5$ Hz), −296.6 (1N, t of d, $J_{H-N} = 86.4$ Hz, $J_{C-N} = 20.0$ Hz), −177.3 (1N, m), −97.5 (1N, d, $J_{N-N} = 21.5$ Hz), 27.0 (1N, d, $J_{N-N} = 21.5$ Hz).

*6-(2,3-Dichlorophenyl)-5-[^{13}C,^{15}N]imino-2-methyl-,2,5-dihydro-[3-^{13}C,$^{15}N_3$]1,2,4-triazin-3-[^{15}N]amine methanesulfonate (**9**)*

To a stirred mixture of [M + 7]-lamotrigene **(7)** (0.2 g, 0.760 mmol) and acetone (30 ml) was added iodomethane (0.2 ml, 3.21 mmol) and left to stir for 24 h. The mixture was concentrated to dryness and stirred with 0.880 ammonia (4 ml) for 4 h (to remove HI). The resulting slurry was filtered and dried under vacuum at 40°C.

The cream solid was stirred with methanol (0.5 ml) and methanesulfonic acid (50 µl). The resulting solution was treated by dropwise addition of ethyl acetate (5.1 ml) and then cooled to 0–5°C for 30 min, filtered and dried under vacuum. This gave the title compound **(9)** as a white solid (0.2182, 77%); HPLC chemical purity 99.3%; *m/z* 277.0227 (calculated 277.0232); δ_H (400 MHz, DMSO-d_6) 9.11(d, $J = 90$ Hz, 1 H, NH_2); 8.15(d of d of t, $J = 91$ Hz, $J = 7$ Hz, $J = 3.5$ Hz, 1 H, NH_2); 7.9–9.0(2 broad doublets, $J = 89$ Hz, 2 H, NH_2); 7.86(d of d, $J = 8$ Hz, $J = 1.5$ Hz, 1 H, aromatic CH), 7.55(t, $J = 8$ Hz, 1 H, aromatic CH); 7.50(d of d, $J = 8$ Hz, $J = 1.5$ Hz, 1 H, aromatic CH); 3.75(apparent quartet, 3 H, N–CH_3); 2.30(s, 3 H, CH_3–SO_3H); δ_{15N}(400 MHz, DMSO-d_6) −290.8 (1N, t of d, $J_{H-N} = 91.1$ Hz, $J_{C-N} = 23.4$ Hz), −272.9 (1N, t of d, $J_{H-N} = 86.8$ Hz, $J_{C-N} = 25.1$ Hz), −218.8 (1N, m), −177.8 (1N, m), 30.9 (1N, d, $J_{N-N} = 12.9$ Hz).

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