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Synthesis of deuterium-labeled pregabalin

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This paper describes the synthesis of deuterium-labeled pregabalin. The stable isotopic-labeled compound was obtained in nine steps starting from the commercially available 4-[$^2H_{11}$]methylvaleric acid as the stable-labeled reagent. It is used as an internal standard for analysis and metabolic studies.
Pregabalin labeled with ^2H was obtained in nine steps using the commercially available 4-[$^2H_{11}$]methylvaleric acid as the stable-labeled reagent. The synthesis prevents deuterium from scrambling and offers the labeled compound with over 99% isotopic enrichment.

Keywords: deuterium; labeled; synthesis; pregabalin

Introduction

Pregabalin, (*S*)-3-(aminomethyl)-5-methylhexanoic acid, is an anticonvulsant drug used for neuropathic pain and as an adjunct therapy for partial seizures.[1] It is marketed by Pfizer (New York, NY, USA) under the trade name Lyrica. As a follow-up compound to gabapentin, pregabalin was found to be more potent and robust in preclinical models of epilepsy and neuropathic pain. Recent studies have shown that pregabalin is effective in treating chronic pain in disorders such as fibromyalgia and spinal cord injury. In June 2007, pregabalin became the first medication approved by the US Food and Drug Administration specifically for the treatment of fibromyalgia. However, as an alkylated analog of γ-aminobutyric acid (GABA), pregabalin is inactive at $GABA_A$ and $GABA_B$ receptors, is not converted metabolically into a $GABA_A$ or a $GABA_B$ antagonist, and does not block GABA uptake or alter GABA degradation.[2] Accordingly, pharmacokinetics and absorption, distribution, metabolism, and excretion investigations on pregabalin should be extensively performed to support clinical studies. Thus, a stable isotope-labeled standard is required. Although A.W. Czarnik has described the pregabalin labeled at different positions with various numbers of deuterium,[3] it lacked detailed procedures and data. The present paper describes the preparation of [$^2H_{10}$]pregabalin in detail (Figure 1).

Results and discussion

Although pregabalin has been readily prepared via several synthetic routes,[4–8] the details of the synthesis of deuterium-labeled pregabalin have not been described. Scheme 1 presents a concise and efficient synthetic scheme for preparing [$^2H_{10}$]pregabalin (**10**).[8] Treatment of compound (**1**) with $SOCl_2$ produced 4-[$^2H_{11}$]methylpentanoyl chloride (**2**). (4*R*,5*S*)-4-methyl-5-phenyl-2-oxazolidinone was treated with *n*-BuLi in dry THF and then alkylated with compound (**2**) to give (4*R*,5*S*)-4-methyl-3-(1-oxo-4-[$^2H_{11}$]methylpentyl)-5-phenyl-2-oxazolidinone (**3**). *n*-BuLi must be added carefully to the reaction mixture and stopped once the red color forms to prevent epimerization and provide high yield for compound (**3**).[8] The anion of (**3**) was readily formed with lithium diisopropylamide below −35°C, which was then alkylated with *tert*-butyl bromoacetate to produce [4*R*-(3*S**,4α,5α)]-4-methyl-β-(2-methylpropyl-γ,2-dioxo-5-phenyl-3-[$^2H_{10}$]oxazolidine butanoic acid, 1,1-dimethylethyl ester (**4**). The one deuterium removed does not scramble the protons on the α position of *t*-butyl ester. The yield for this reaction was found to be highly dependent on the careful temperature control (if the reaction temperature was allowed to rise above −35°C, yields decreased significantly).[8] The retention factor of the starting material (compound **3**) and product (compound **4**) was identical. The reaction can be continued to the next reaction without isolation. Compound (**4**) was treated with the mixture of LiOH and H_2O_2 (30%) to form (*S*)-2-(2-methylpropyl)-1,4-[$^2H_{10}$]butanedioic acid, 4-(1,1-dimethylethyl) ester (**5**). Reduction of compound (**5**) with borane/dimethyl sulfide produced (*S*)-3-(hydroxymethyl)-5-[$^2H_{10}$]methylhexanoic acid, 1,1-dimethylethyl ester (**6**). Compound (**6**) was treated with *p*-toluenesulfonyl chloride to form (*S*)-5-methyl-3-[[[(4-methylphenyl)sulfonyl]oxy]methyl]-[$^2H_{10}$]hexanoic acid, 1,1-dimethyl ester (**7**). Treatment of (**7**) with sodium azide in dry dimethyl sulfoxide gave (*S*)-3-(azidomethyl)-5-[$^2H_{10}$]methylhexanoic acid, 1,1-dimethylethyl ester (**8**). Hydrolysis of compound (**8**) in a mixture of formic acid and sulfuric acid gave (*S*)-3-(azidomethyl)-5-[$^2H_{10}$]methylhexanoic acid (**9**). Reduction of compound (**9**) with hydrogen (50 psi) and Pd/C (10%) afforded [$^2H_{10}$]pregabalin (**10**).

After purification by recrystallization, the desired product (**10**) was obtained with 99.8% chemical purity. Mass spectrometry

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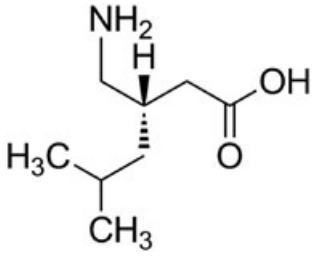


Figure 1. Chemical structure of pregabalin.

analysis of compound (**10**) revealed that the compound has over 99% deuterium enrichment. The compound provided an excellent internal standard for LC-MS-MS studies.

Experimental

General

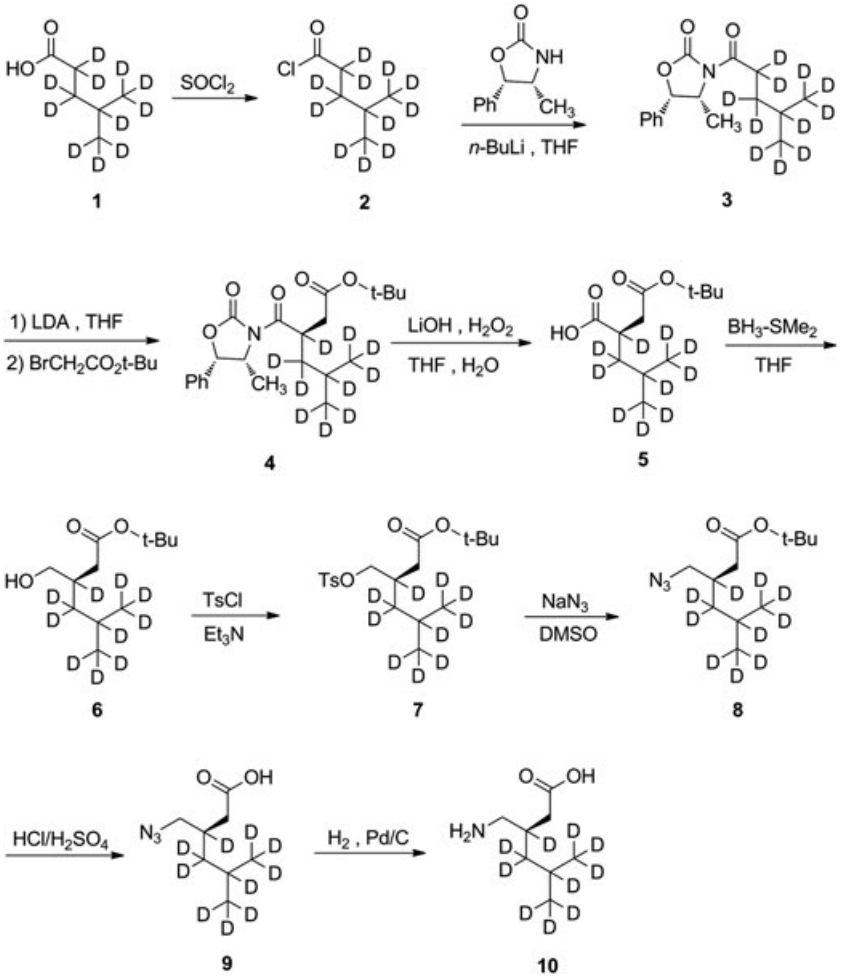
All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) and CDN Isotopes Inc. (Pointe-Claire, Quebec, Canada). Mass spectra were recorded using a Quattro micro API mass spectrometer (Waters Corporation, Milford, MA, USA). ^{1}H NMR spectra were recorded on a Bruker 300-MHz instrument (Bruker Daltonics Inc., Billerica, MA, USA). Chemical purities were determined by an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA) with an XDB-C18 column, 5 μm, 4.6 × 150 mm.

4-[$^{2}H_{11}$]methylpentanoyl chloride (2)

To a solution of $SOCl_2$ (3.12 g, 26.22 mmol) was added 4-[$^{2}H_{11}$] methylvaleric acid (2.50 g, 19.68 mmol) slowly. The resulting solution was stirred at 35°C for 1 h and then stirred at 75°C for 30 min. The reaction mixture was concentrated under reduced pressure to afford (**2**) as a colorless liquid (2.48 g, 86.7%). Crude product (**2**) was used without further purification.

(4*R*,5*S*)-4-methyl-3-(1-oxo-4-[$^{2}H_{11}$]methylpentyl)-5-phenyl-2-oxazolidinone (3)

A solution of oxazolidinone (3.10 g, 17.50 mmol) in dry THF (26 ml) was cooled to −5°C under an argon atmosphere. *n*-BuLi in hexane (7.9 ml 2.2 M, 17.50 mmol) was added while maintaining an internal temperature below −5°C at all times. The solution of compound (**2**) (2.48 g, 17.00 mmol) in dry THF was added slowly. The reaction mixture was stirred at −5°C for 2 h and then quenched with water (6 ml). The product was extracted with heptane (3 × 20 ml). The combined organic layers were washed with saturated $NaHCO_3$ solution (10 ml), water (3 × 10 ml), and brine (10 ml), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a brown oil. The crude product was purified by chromatography on silica gel (120 g)



Scheme 1. Synthesis route of deuterium-labelled pregabalin.

column, eluted with hexanes/EtOAc (20:1) to afford (**3**) as a white solid (4.20 g, 86.0%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42-7.30 (m, 5H), 5.67 (d, 1H), 4.77 (m, 1H), 0.89 (d, 3H).

[4*R*-(3*S,4α,5α)]-4-methyl-β-(2-methylpropyl-γ,2-dioxo-5-phenyl-3-[$^2H_{10}$]oxazolidinebutanoic acid, 1,1-dimethylethyl ester (4)**

Flask A

- The solution of (**3**) (4.20 g, 14.66 mmol) in dry THF (12 ml) was cooled to below −40°C under nitrogen.

Flask B

- The solution of diisopropylamine (1.48 g, 14.66 mmol) in dry THF (2 ml) was cooled to below 0°C. *n*-BuLi in hexane (6.7 ml 2.2 M, 14.66 mmol) was added slowly to the amine/tetrahydrofuran solution. The solution was stirred for 30 min below 0°C. The resulting lithium diisopropylamide solution was transferred slowly to flask A.

After the addition, the resulting solution was stirred for 30 min below −35°C. *tert*-butyl bromoacetate (2.86 g, 14.66 mmol) was added slowly to the solution of flask A. The reaction mixture was stirred for 30 min below −35°C. The temperature was then allowed to rise slowly to −15°C over 2.5–3.5 h. The reaction was quenched with saturated NH_4Cl solution (8 ml). The mixture was warmed up to 25°C and then extracted with EtOAc (3 × 20 ml). The combined organic layers were washed with water (3 × 10 ml) and brine (10 ml), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a light yellow solid. The crude product was purified by chromatography on silica gel (200 g) column, eluted with hexanes/EtOAc (20:1), to afford (**4**) as an off-white solid (3.20 g, 54.6%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61-7.29 (m, 5H), 5.66 (d, 1H), 4.86 (m, 1H), 2.70 (dd, 1H), 2.42 (dd, 1H), 1.41 (s, 9H), 0.91 (d, 3H).

(*S*)-2-(2-methylpropyl)-1,4-[$^2H_{10}$]butanedioic acid, 4-(1,1-dimethylethyl) ester (5)

Compound (**4**) (3.20 g, 8.00 mmol) was dissolved in dry THF (10 ml) and then cooled to 0°C. Separately, a solution of $LiOH{\cdot}H_2O$ (3.36 g, 8.00 mmol) in water (3.5 ml) was cooled to 0°C. H_2O_2 (1.5 ml, 30%) was added slowly to the LiOH solution. The peroxide solution was slowly added to the aforementioned THF solution. The resulting solution was stirred at 0°C for 4 h and then quenched through the addition of saturated Na_2SO_3 solution (9 ml). The mixture was extracted with Et_2O (3 × 20 ml). The aqueous layer was back washed with Et_2O (20 ml). The combined organic layers were washed with brine (30 ml), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a light yellow oil. The crude product was purified by chromatography on silica gel (100 g), eluted with hexanes/EtOAc (15:1) to afford (**5**) as a colorless oil (1.40 g, 72.7%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.58 (dd, 1H), 2.33 (dd, 1H), 1.42 (s, 9H).

(*S*)-3-(hydroxymethyl)-5-[$^2H_{10}$]methylhexanoic acid, 1,1-dimethylethyl ester (6)

After drying by azeotropic distillation with toluene, compound (**5**) (1.40 g, 5.80 mmol) was dissolved in dry Et_2O (3.5 ml), and then cooled to below 5°C in an ice bath. Borane/dimethyl sulfide (3.4 ml 2 M, 6.70 mmol) was added slowly. The reaction mixture was stirred at 5°C for 3 h, warmed to about 25°C and then stirred for overnight. Thin layer chromatography analysis showed that the reaction was complete. The reaction mixture was cooled to 0°C and quenched with water (2 ml). The resulting mixture was diluted with ether (30 ml) and then washed with saturated $NaHCO_3$ solution (3 × 10 ml), water (3 × 10 ml), dried over Na_2SO_4, filtered and concentrated under vacuum to dryness to give an oil (0.70 g, 53.0%), which was used without further purification.

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66 (dd, 1H), 3.50 (dd, 1H), 2.33 (m, 1H), 2.27 (dd, 1H), 1.46 (s, 9H).

(*S*)-5-methyl-3-[[[(4-methylphenyl)sulfonyl]oxy]methyl]-[$^2H_{10}$]hexanoic acid-1,1-dimethyl ester (7)

To a solution of (**6**) (0.70 g, 3.09 mmol) in heptane (10 ml) at 0°C was added *p*-toluenesulfonyl chloride (0.71 g, 3.70 mmol) and triethylamine. The reaction mixture was stirred at 27°C overnight. 1 M HCl (2 ml) was added, and the solution was extracted with Et_2O (3 × 20 ml). The combined organic layers were concentrated under reduced pressure to give a yellow oil. The crude product was purified by chromatography on silica gel (50 g), eluted with hexanes/EtOAc (20:1) to afford (**7**) as an oil (0.25 g, 21.2%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, 2H), 7.36 (t, 2H), 4.01 (dd, 1H), 3.93 (dd, 1H), 2.45 (s, 3H), 2.29 (dd, 1H), 2.16 (dd, 1H), 1.40 (s, 9H).

(*S*)-3-(azidomethyl)-5-[$^2H_{10}$]methylhexanoic acid, 1,1-dimethylethyl ester (8)

To a stirred solution of compound (**7**) (0.25 g, 0.65 mmol) in dry dimethyl sulfoxide (1 ml) was added sodium azide (0.042 g, 0.65 mmol) and stirred at 60°C overnight. The reaction mixture was cooled to 20°C and diluted with water (10 ml) and heptane (20 ml). The organic layer was separated, and the aqueous layer was back extracted with heptane (3 × 20 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4, filtered and concentrated under vacuum to give (**8**) as a light yellow oil. The crude (**8**) (0.12 g, 72.7%) was used without further purification.

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.37 (dd, 1H), 3.29 (dd, 1H), 2.29 (dd, 1H), 2.22 (dd, 1H), 1.46 (m, 9H).

(*S*)-3-(azidomethyl)-5-[$^2H_{10}$]methylhexanoic acid (9)

To a solution of compound (**8**) (0.12 g, 0.48 mmol) in ice bath was added slowly the mixture of formic acid (0.48 g, 10.40 mmol) and sulfuric acid (5 mg, 0.05 mmol). The reaction mixture was stirred at room temperature for 2 h and quenched with saturated NaCl solution (2 ml). The reaction was diluted with Et_2O (20 ml). The organic layer was separated, and the aqueous layer was extracted with Et_2O (3 × 10 ml). The combined organic layers were washed with water (5 ml) and brine (5 ml), dried over Na_2SO_4, filtered and concentrated under vacuum to give a light yellow oil (0.07 g, 75.0%), which was carried directly into the next step.

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.39 (dd, 1H), 3.33 (dd, 1H) 2.36 (dd, 1H) 2.28 (dd, 1H).

[$^2H_{10}$]pregabalin (**10**)

To a solution of (**9**) (0.40 g, 2.00 mmol) in isopropyl alcohol, Pd/C (10%, 40 mg) was added. The solution was stirred at room temperature under 50 psi of hydrogen overnight. The mixture was heated to 80°C and filtered through a bed of Celite to remove catalyst, and then washed with a hot (80°C) solution of water and isopropyl alcohol (1:1). The combined filtrate was concentrated under vacuum to afford a white semi-solid, which was recrystallized from isopropyl alcohol to yield desired product (**10**) as a white solid (0.16 g, 47.0%).

^{1}H NMR (300 MHz, D_2O): δ 2.92 (dd, 1H), 2.90 (dd, 1H), 2.23 (dd, 1H) 2.19 (dd, 1H). MS-EI (m/z): 169.2 (11), 170.2 (M^+, 100), 171.2 (12). HPLC (XDB-C18, CH_3CN/MeOH/10 mmol ammonium acetate = 5/11/84, 1.0 mL/min. The sample was derivatized with 1-fluoro-2,4-dinitrobenzene before HPLC analysis.): t_R 3.0 min (>99.8%). Isotopic enrichment determined by NMR was over 99.0%. $[\alpha]_D^{20} = +10.2^\circ$ (c 1.0, H_2O).

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