THE SYNTHESIS OF 7-CHLORO-5-PENTADEUTERIOPHENYL-1-METHYL-1*H*-1,5-BENZODIAZEPINE-2,4(3*H*, 5*H*)DIONE ([$^2H_5$]CLOBAZAM)

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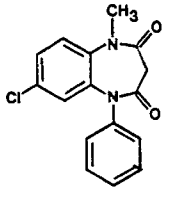
SUMMARY

Pentadeuteriophenyl clobazam **5** was synthesized in essentially quantitative isotopic purity, and characterized by ^{1}H-NMR and mass spectroscopy. The title compound was found to be >98 % pure by HPLC, and its retention time (t_R 6.17 min) was less than that of an authentic clobazam standard (t_R 6.32 min). Of the five steps in the synthesis of **5**, the most susceptible to deuterium exchange was the nucleophilic substitution of 2,4-dichloronitrobenzene by aniline-d_7 to form N-(5-chloro-2-nitrophenyl)pentadeuteriophenylamine **2**. In this step, the isotopic impurity aniline-2,3,4,5,-d_5 introduced protons from nitrogen into the *ortho* and *para* positions of the deuteriophenyl ring of **2**.

KEYWORDS: pentadeuteriophenyl clobazam, aniline-d_7, deuterium, rearrangement, isotope cluster

INTRODUCTION

Clobazam (CLBZ) is a 1,5-benzodiazepine with potent anticonvulsant activity used in the treatment of a variety of seizure types [1]. Although tolerance during long-term therapy has been reported [2], patients with chronic intractable epilepsy have been maintained seizure-free for more than a year on CLBZ [3].



CLOBAZAM

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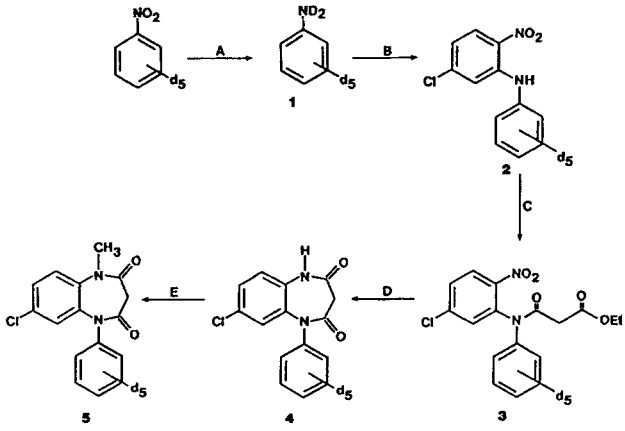
There are limited reports in the literature concerning the metabolism of CLBZ [4,5]. Volz *et al* [5] reported the CLBZ metabolites desmethylclobazam (DMC), and the 4′-hydroxy, 4′-hydroxy-3′-methoxy and dihydrodiol derivatives of both CLBZ and DMC in different animal species, but did not characterize these compounds with published spectral evidence. In order to facilitate our identification of CLBZ metabolites in man and in rats by isotope cluster analysis, we required a stable isotope labelled analogue of the parent drug. The synthesis of trideuteriomethyl CLBZ has been reported [6]; however, since the demethylation of CLBZ is a facile metabolic process, such an analogue would be inappropriate for the identification of labelled metabolites. Instead, we considered pentadeuteriophenyl clobazam ([$^2H_5$]CLBZ) to be an ideal labelled analogue. Deuterium in the 5-phenyl ring is not readily susceptible to exchange, presents no expected major isotope effects, and even with the formation of catechol metabolites at least three $^2H_5$-labels should be retained.

We report in this paper the synthesis of [$^2H_5$]CLBZ and its characterization by ^{1}H-NMR and mass spectroscopy.

DISCUSSION

The synthesis of [$^2H_5$]CLBZ (outlined in Scheme I) was accomplished in essentially quantitative isotopic yield (Figure 1) with appropriate modifications to the procedure of Rossi *et al* (1969) as described by Kuch [4]. In the first step, reduction of nitrobenzene-d_5 (99 atom % D) afforded aniline-d_7 **1** in good yield with an N,N-D_2 isotopic purity of 90 % as determined by ^{2}H-NMR spectroscopy. GCMS was unable to accurately determine the extent of N-deuteration because the labile N-D labels were extensively exchanged on the GC packed column prior to mass spectral detection. Thus, $[M-2]^{+\cdot}$ at m/z 98 appeared as the most intense high mass ion.



Scheme I. The synthesis of [$^2H_5$]CLBZ.

A: DCl, Fe B: 2, 4-dichloronitrobenzene, 170°C

C: ethyl malonyl chloride/dry benzene D: Zn/HCl E: KOH, DMSO/CH_3I

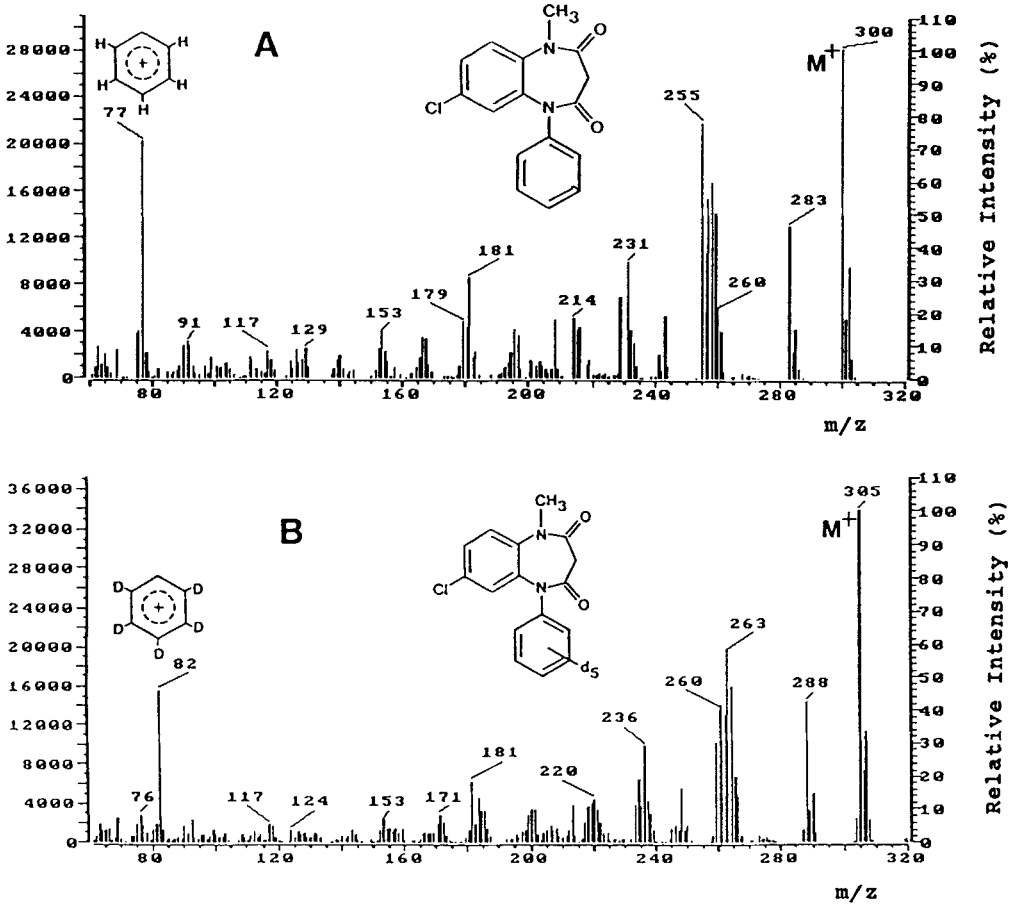


Figure 1. Electron impact mass spectra of (A) CLBZ and (B) [$^2H_5$]CLBZ.

Nucleophilic substitution of 2,4-dichloronitrobenzene by 1 afforded 2 in high yield. The ^{1}H-NMR spectrum of 2 shown in Figure 2A revealed that resonance at the chemical shift of the *meta* protons of the unsubstituted phenyl ring (H-3′/H-5′, 7.48 ppm) was absent, whereas signals appeared in the region characteristic of the *ortho/para* protons (H-2′/6′, H-4′, 7.27 - 7.31). Thus, ^{2}H-labels at the *meta* position were completely retained with minor exchange occurring at the *ortho/para* positions. Integration of the *ortho/para* protons was used to calculate the isotopic purity of 2 at 92 %. Mass (Figure 3A) and ^{13}C-NMR spectra confirmed the identity of the product.

A likely source of the deuteriophenyl protons in 2 could have been the N-H group of aniline-2,3,4,5,-d_5 which occurred as an isotopic impurity in aniline-d_7. Indeed, substantial introduction of protons at the *ortho/para* positions of the deuteriophenyl ring of N-(5-chloro-2-nitrophenyl)deuteriophenylamine 6 occurred when aniline-2,3,4,5,-d_5 was used as the nucleophile (Scheme II, Figure 2B). In order to assign the exact location of deuterium exchange, the *ortho/para* signals were resolved with 0.4 equivalent of Eu(fod)$_3$, and both *ortho* (7.29 ppm) and *para* (7.26 ppm) positions were found to be involved (Figure 2B, Inset). The mass spectrum of 6 (Figure 3B) presented high mass ions at m/z 253, 252, 251 and 250 which demonstrated that incomplete exchange of three deuteriophenyl labels had occurred to afford a mixture of [$^{2}H_5$], [$^{2}H_4$], [$^{2}H_3$] and [$^{2}H_2$] analogues.

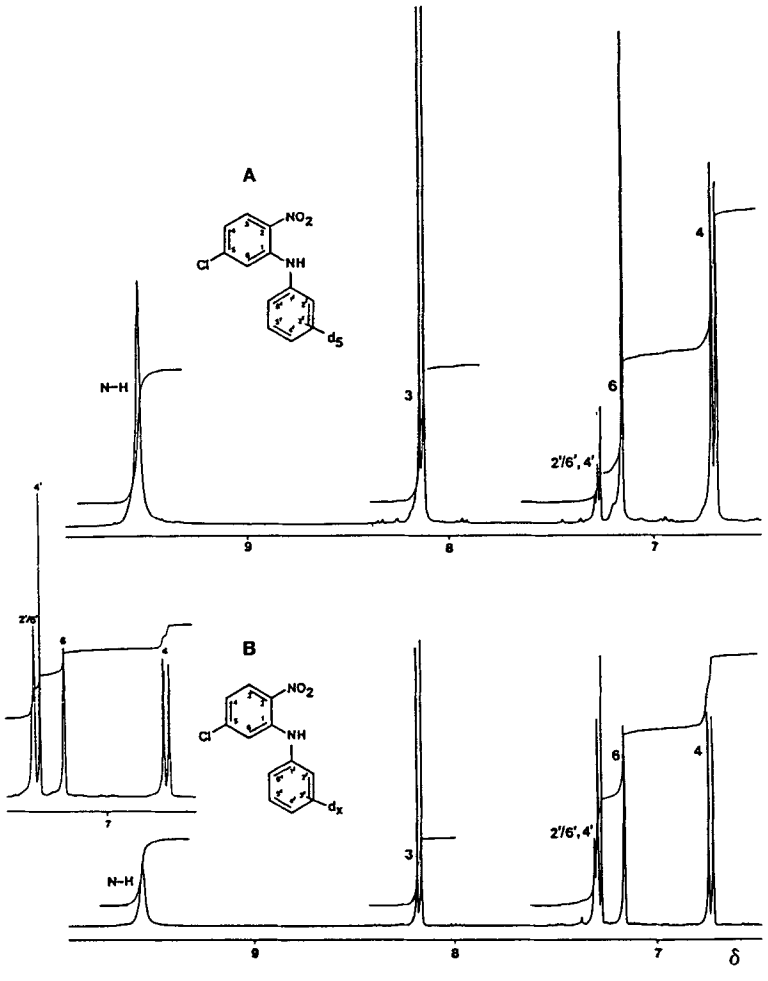


Figure 2. 400 MHz ^{1}H-NMR spectra of (A) 2, and (B) N-(5-chloro-2-nitrophenyl)deuteriophenylamine 6 showing substantial introduction of protons at the *ortho* and *para* positions of the deuteriophenyl ring. Resolution of the *ortho/para* protons with 0.4 equivalent Eu(fod)$_3$ showing that exchange occurred at both *ortho* and *para* positions (Inset).

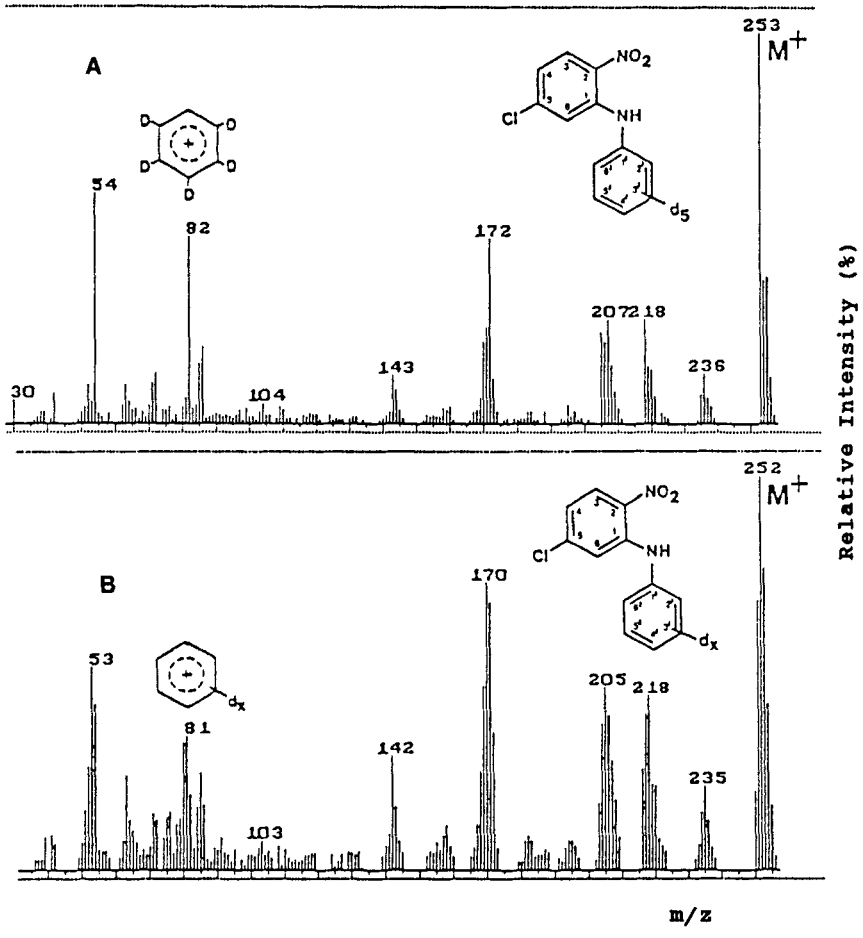
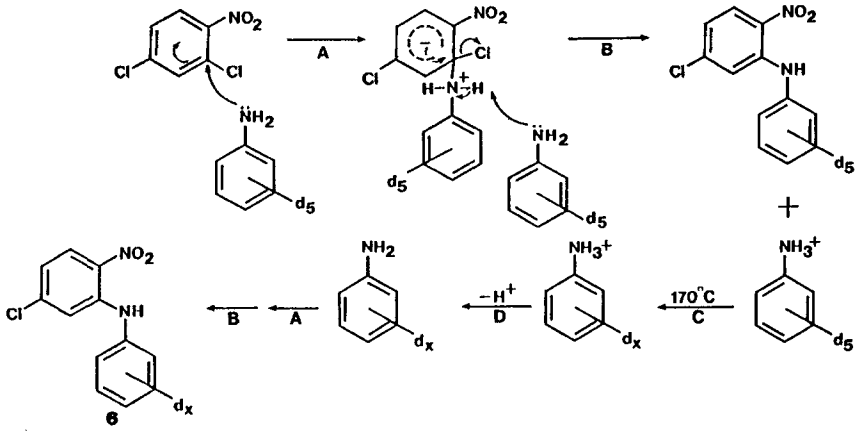


Figure 3. Electron impact mass spectra of (A) 2 showing high isotopic purity with $M^{+\cdot}$, m/z 253, and (B) N-(5-chloro-2-nitrophenyl)deuteriophenylamine 6 showing that it occurred as a mixture of [$^2H_5$] ($M^{+\cdot}$, m/z 253), [$^2H_4$] ($M^{+\cdot}$, 252), [$^2H_3$] ($M^{+\cdot}$, 251) and [$^2H_2$] ($M^{+\cdot}$, 250) analogues.



Scheme II. Mechanism for the selective introduction of protons into the deuteriophenyl nucleus of N-(5-chloro-2-nitrophenyl)-deuteriophenylamine 6. (A) Nucleophilic attack, (B) Proton abstraction and departure of Cl^-, (C) Thermal rearrangement of anilinium cation, x = 2, 3, 4, 5, (D) Proton abstraction to form rearranged aniline nucleophile.

Harada and Titani [7] reported the thermal rearrangement of $C_6H_5NH_2D^+$ to its *ortho* and *para* ring deuterated analogues. Okazaki and Omura [8] proposed that above 150°C and below the melting point, the protons of the NH_3^+ group of anilinium salts are intramolecularly transferred along the π-electron cloud to the carbon atoms of higher electron density. Thus, the *ortho* and *para* positions are selectively targeted for proton exchange. In an analogous fashion, aniline-2,3,4,5,-d_5 could be rearranged to its ring protonated analogues through its anilinium ion as shown in Scheme II.

The substitution of 2,4-dichloronitrobenzene by aniline-2,3,4,5,-d_5 proceeds *via* a 2-step S_NAr mechanism [9]. In the first step aniline acts as a nucleophile attacking the electron deficient 2-position of the substrate to afford a tetrahedral intermediate. In the second step the Cl^- leaving group departs and a proton is abstracted by aniline functioning as a base. At the reaction temperature of 170°C the resulting anilinium cation rearranges with the N-protons being introduced at the *ortho* and *para* positions of the phenyl ring. The resulting conjugate base of the rearranged anilinium cation in

turn, participates as a nucleophile to afford 6. GCMS analysis of the reaction mixture after the formation of 6 revealed that N-D exchanges with N-H (as discussed above) in that the excess aniline was no longer fully deuterated in the phenyl ring but instead consisted of a mixture of -d_5 ($M^{+\cdot}$, m/z 98, 45 %), -d_4 ($M^{+\cdot}$, m/z 97, 100%), -d_3 ($M^{+\cdot}$, m/z 96, 75 %), and -d_2 ($M^{+\cdot}$, m/z 95, 20 %) analogues. On the other hand, when aniline-d_7 was used in the synthesis of 2 the pentadeuteriophenyl nucleus remained essentially intact because of the absence of a proton source with which exchange could occur. GCMS: aniline-d_6 ($M^{+\cdot}$, m/z 99, 2 %), -d_5 ($M^{+\cdot}$, m/z 98, 100%), -d_4 ($M^{+\cdot}$, m/z 97, 25%), and -d_3 ($M^{+\cdot}$, m/z 96, 3 %).

Because the rearrangement of the anilinium cation occurs above 150°C (Scheme II), careful attention to the exclusion of potential proton sources was only required at elevated temperatures. Thus, after the reaction was adjusted to room temperature, the work-up of 2 could be continued under ambient conditions without the possibility of deuterium exchange. Furthermore, the subsequent steps (C -> E) in the synthesis of [$^2H_5$]CLBZ do not result in the loss of ^2H-labels.

In the methylation of 4 to 5, the number of equivalents of base is carefully controlled in order to selectively remove the amide proton and avoid the methylene protons. Use of 1.2 equivalents of KOH and excess CH_3I afforded [$^2H_5$]CLBZ in 37 % yield and virtually no evidence of the dimethyl compound 3-methyl[$^2H_5$]CLBZ (m/z, 319). HPLC analysis revealed that [$^2H_5$]CLBZ was >98 % pure, and chromatographed (t_R 6.17 min) before an authentic standard of CLBZ (t_R 6.32 min). The disparity in the retention times between compounds and their deuterated analogues on reverse phase HPLC has ample precedence in the literature [10], and is not peculiar to CLBZ and [$^2H_5$]CLBZ.

EXPERIMENTAL

All solvents used in synthesis were of analytical reagent grade quality and obtained from BDH Inc. (Vancouver, BC). $CDCl_3$, nitrobenzene-d_5 (99 atom % D), DCl (37 wt. % solution in D_2O, 99 atom % D), deuterium oxide (99.8 atom %

D), 2,4-dichloronitrobenzene and ethyl malonyl chloride were from Aldrich Chemical Co. (Milwaukee, WI) and silica gel either from J.T.Baker (Phillipsburg, NJ) (40 μ particle diameter) or BDH, Inc. (Vancouver, BC) (Silica Gel 60, 230 - 400 mesh).

Purifications involving medium pressure column chromatography were performed according to the method of Still *et al* [11], and the conditions are specified. Melting points were determined in open capillary tubes on a Thomas Hoover Capillary melting point apparatus (Philadelphia, PA) and are uncorrected. All NMR spectroscopy was performed in the Department of Chemistry, University of British Columbia. ^{1}H-NMR and ^{13}C-NMR spectra were determined in $CDCl_3$ solution and the chemical shifts are in parts per million relative to tetramethylsilane. ^{1}H-NMR spectroscopy was performed on either a Bruker WH-400 (400 Mz) or Varian XL-300 (300 Mz) instrument, and ^{13}C-NMR spectroscopy on a Varian XL-300 (75 MHz) instrument with broad-band decoupling on the ^{1}H-frequency and an Attached Proton Test. ^{2}H-NMR spectra were taken in $CHCl_3$ solution on a Bruker WH-400 (84.68 MHz) instrument and chemical shifts measured relative to an external $CDCl_3$ standard. Mass spectroscopy was performed in the electron impact mode on either an HP 5987A GCMS fitted with an HP-1 25 m x 0.32 mm i.d. x 0.52 μ capillary column with a Crosslinked Methyl Silicone Gum stationary phase, or a Varian MAT-111 MS interfaced to an HP 5700A GC fitted with a 1.8 m x 2 mm i.d. glass column packed with 3 % Dexsil 300 on 100/120 mesh Supelcoport (Supelco, Oakville, Ont.). HPLC analysis was done on an HP 1050 Series System consisting of Quaternary Pump and Multiple Wavelength Detector (254 nm) fitted with a Hypersil ODS 5 μ, 20 cm x 4.6 mm i.d. column. The mobile phase consisting of 60:40 (v/v) MeOH:H_2O was controlled at a flow rate of 1 ml/min. IR spectra were recorded on a Michelson BOMEM MB-100 FT-Spectrometer.

Aniline-d_7 (1).

Into a three-necked flask equipped with a mechanical stirrer and a dropping funnel filled with DCl (15 ml, 16.5 mmol) were placed nitrobenzene-d_5 (20.2 g, 15.8 mmol), D_2O (50 ml) and iron powder (24.2 g, 46.9 mmol). The reaction flask was flushed with N_2 and sealed under the slight positive

pressure of a N_2 filled balloon attached to a reflux condenser. To the stirred mixture was added DCl at a rate to maintain the temperature below 80°C. When the addition of DCl was complete the mixture was stirred at 80°C for 12 h. The resulting mixture was cooled in an ice-bath and the pH adjusted to 12 with NaOD while maintaining the reaction mixture under N_2. Under a N_2 atmosphere solids were removed by filtration and the filtrate appeared as a yellowish-green liquid with a suspended pale brown oil. KCl (*ca.* 10 g) was added to the filtrate to "salt out" aqueous aniline which was extracted with ether and concentrated *in vacuuo* to a rust coloured oil. Fractional distillation gave 12.7 g (80 %) of aniline-d_7 as a slightly viscous colourless liquid: bp 28 - 30°C (0.4 torr); ^{2}H-NMR δ 3.47 (s, 1.80D*, -ND_2), 6.60 (s, 2D, *ortho*), 6.78 (s, 1D, *para*), 7.17 (s, 2D, *meta*). (*N,N-D_2 isotopic purity calculated at 90 % from integral).

N-(5-Chloro-2-nitrophenyl)-pentadeuteriophenylamine (2) [12].

Dry dichloronitrobenzene (2.23 g, 11.6 mmol) was dissolved in 1 (4.60 g, 3.6 equiv) to afford an orange solution. The stirred solution was heated at 170 - 180°C under reflux in a N_2 atmosphere for 8.5 h and before cooling, quenched under N_2 with continued stirring by cautious addition of *ca.* 10 ml of dry benzene. The resulting black solution was allowed to reach room temperature and further diluted with an additional 30 ml dry benzene. The crude product was chromatographed on a silica column (15 cm x 5.5 cm) using the following gradient elution: benzene (100 ml); 2:1 (v/v) petroleum ether:ether (240 ml); 3:2 (v/v) petroleum ether:ether (625 ml). The product-containing fractions were pooled and solvent removed *in vacuuo* to afford 2.69 g (91 %) of 2 as bright orange needlelike crystals: mp 109 - 110°C; ^{1}H-NMR δ 6.74 (dd, J = 10 Hz and 2 Hz, 1H, H-4), 7.16 (d, J = 2 Hz, 1H, H-6), 7.27 - 7.31 (m, 0.23H*, H-2′/H-6′ and H-4′), 8.18 (d, J = 10 Hz, 1H, H-3), 9.52 (bs, 1H, N-H); ^{13}C-NMR δ 114.99 (C-4), 117.73 (C-6), 124.26 (t, C-2′/C-6′), 125.77 (t, C-4′), 127.97 (C-3), 129.35 (t, C-3′/C-5′), 131.32 (C-1′), 137.59 (C-1), 142.34 (C-5), 143.75 (C-2), MS: $M^+\cdot(^{35}Cl)$: m/z 253 (100 %), $M^+\cdot(^{37}Cl)$: m/z 255 (37 %); IR (CCl_4 solution) 3345 (N-H), 1487 (NO_2)asymm, 1310 cm^{-1} (NO_2)symm. (*Isotopic purity calculated at 92 % from integral).

Ethyl N-(5-chloro-2-nitrophenyl)-N-pentadeuteriophenyl carbamoylacetate (3).

To a stirred solution of 2 (2.76 g, 10.9 mmol) in *ca.* 25 ml dry benzene was added ethyl malonyl chloride (4.12 g, 27.3 mmol) and the resulting solution heated at 75 - 80°C under reflux for 20 h. The mixture was washed with saturated $NaHCO_3$, dried, and concentrated to a pale brown oil. The crude product was chromatographed on a silica column (15 cm x 5.5 cm) using the following gradient: benzene (100 ml); 2:1 (v/v) petroleum ether:ethyl acetate (450 ml); 3:2 (v/v) petroleum ether:ethyl acetate (250 ml). Product-containing fractions were pooled and concentrated to afford 3.31 g (85 %) of 3 as pale yellow crystals: mp 90 - 92°C; ^{1}H-NMR δ 1.26 (t, 3H, $-CH_2CH_3$), 3.40 (s, 2H, $-C(=O)CH_2C=O$), 4.17 (q, 2H, $-OCH_2CH_3$), 7.21 (d, J = 1 Hz, 1H, H-6), 7.35 (dd, J = 9 Hz and 1 Hz, 1H, H-4), 7.93 (d, J = 9 Hz, 1H, H-3); MS: $[M-114]^{+\cdot}$ (^{35}Cl): m/z 253 (100 %), $[M-114]^{+\cdot}$ (^{37}Cl): m/z 255 (37 %), IR (CCl_4 solution) 1745 (ester carbonyl group), 1693 (amide carbonyl group), 1534 (NO_2)asymm, 1351 cm^{-1} (NO_2)symm.

Pentadeuteriophenyl N-desmethylclobazam (4).

To a stirred suspension of 3 (2.0 g, 5.45 mmol) in 17 ml ethanol and concentrated HCl (10 ml, 110 mmol) was cautiously added Zn dust (1.74 g, 26.6 mmol) over 30 min while maintaining the temperature between 25 - 30°C. The mixture was stirred at 30°C for an additional 2 h to afford 4 as a white precipitate. The product was removed, the supernatant treated with additional Zn (0.58 g, 8.9 mmol) as previously described, and stirring continued overnight at 30°C to afford a second crop of 4. The combined product was washed with cold 3:2 (v/v) acetone: water and dried *in vacuuo* to afford 0.56 g (35 %) of 4 as an amorphous white solid: ^{1}H-NMR δ 3.55 (s, 2H, CH_2), 6.93 (d, J = 3 Hz, 1H, H-6), 7.11 (d, J = 9 Hz, 1H, H-9), 7.21 (dd, J = 9 Hz and 3 Hz, 1H, H-8), 8.21 (bs, 1H, N-H); MS: $M^{+\cdot}$ (^{35}Cl): m/z 291 (62 %), $M^{+\cdot}$ (^{37}Cl): m/z 293 (20 %), $[M-42]^{+\cdot}$ (^{35}Cl): m/z 249 (57 %), $C_6D_5^{+\cdot}$: m/z 82 (41 %); IR (Nujol mull) 3176 (N-H), 1691 and 1673 cm^{-1} (amide carbonyl groups).

Pentadeuteriophenyl clobazam (5).

To an ice-cooled (15°C) solution of 4 (491 mg, 1.68 mmol) and pulverized KOH (114 mg, 2.05 mmol) in 6 ml DMSO was added CH_3I (193 μl, 3.06 mmol) with

stirring. The reaction was maintained at this temperature for 30 min after which it was allowed to reach room temperature with continued stirring for an additional 90 min. The reaction mixture was diluted with 300 ml ethyl acetate, washed with a saturated NaCl solution, dried over anhydrous Na_2SO_4, and concentrated to a yellow oil. The crude product was dissolved in *ca* 10 ml benzene and chromatographed on a silica column (15 cm x 2.5 cm) using 210 ml of 20:1 (v/v) $CHCl_3$:MeOH. Despite several modifications to the chromatographic conditions, unreacted **4** could not be separated from **5** in its latter fractions. Hence, the mixture was concentrated and re-chromatographed. Fractions containing only **5** were concentrated to a pale yellow oil and the product recrystallized from ethyl acetate/petroleum ether to afford 188 mg (37 %) in quantitative isotopic purity as white crystals: mp 182 - 185°C; ^{1}H-NMR δ 3.50 (d, J = 10 Hz, 1H, H-3A*), 3.51 (s, 3H, N-CH_3), 3.56 (d, J = 10 Hz, 1H, H-3B*), 6.93 (d, J = 3 Hz, H-6), 7.25 (dd, J = 10 Hz and 3 Hz, 1H, H-8), 7.31 (d, J = 10 Hz, 1H, H-9); MS: $M^{+\cdot}$ (^{35}Cl): m/z 305 (100 %), $M^{+\cdot}$ (^{37}Cl): m/z 307 (34 %), $[M-17]^{+\cdot}$: m/z 288 (43 %), $C_6D_5^{+\cdot}$: m/z 82 (48 %); IR (CCl_4 solution) 1711 and 1686 cm^{-1} (amide carbonyl groups). The recrystallized **5** was analyzed by HPLC using a mobile phase of 60:40 (v/v) MeOH:H_2O and found to have a retention time of 6.17 min and purity of >98 %. (*Assignments consistent with the conformational structure proposed by Aversa *et al* [13]).

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