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Short communication

Deuterated analogs of verapamil and nifedipine. Synthesis and biological activity

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Summary — The preparations of various deuterium analogs of verapamil and nifedipine have been described. Deuterium is incorporated at specific positions in the molecules in 97% isotopic purity. The deuterated analogs **1d** of verapamil and **2d** of nifedipine lowered blood pressure in spontaneously hypertensive rats with a profile that was, for the most part, similar to the parent compounds. It was therefore concluded that deuterium substitutions fail to significantly alter the metabolism of verapamil or nifedipine *in vivo*.

verapamil / nifedipine / deuterated analogs

Introduction

Both primary and secondary deuterium isotope effects in the metabolism of drugs have been known for a long time [1,2]. In an aliphatic moiety of a drug molecule the replacement of 1 or several carbon-bonded hydrogen atoms with deuterium is expected to impose negligible steric consequences and to cause little change in the physico-chemical properties. Still, the increased carbon–deuterium bond strength may cause a dramatic change in the rate of metabolism, particularly if the mechanism involves cleavage of 1 or more of these bonds. Therefore, by substituting deuterium for hydrogen, the lifetime of a drug in the organism may be increased resulting in an extended duration of drug action, and a lower daily therapeutic dose of the drug may be sufficient for treatment. This is of special interest in chronic diseases such as hypertension, which requires the use of drugs over an extended time period.

The calcium antagonists verapamil **1a** and nifedipine **2a** are commonly used against hypertension [3]. Following oral administration, verapamil is known to undergo substantial first pass metabolism [4]. The major metabolic pathway involves cleavage of carbon–nitrogen bonds with formation of the metabolites **3** and **4**, as shown in scheme 1. In addition, demethylation of the methoxy groups also takes place but to a lesser extent [5]. Therefore, for deuterated verapamil only a secondary deuterium isotope effect would be anticipated. Nifedipine is subject to extensive first pass metabolism as well [6], primarily by undergoing oxidation of the dihydropyridine ring to the inactive metabolites **5** and **6**, as shown in scheme 1 [7, 8]. The oxidation involves cleavage of the tertiary C-H bond and for nifedipine, deuterated at this carbon, a primary deuterium isotope effect is expected. It was therefore of interest to test, particularly for nifedipine, whether specific incorporation of deuterium would cause prolonged duration of biological activity. The present work reports on the synthesis and pharmacological testing of specifically deuterated analogs of verapamil and nifedipine.

Chemistry

For the synthesis of the deuterated verapamil analogs we chose a strategy involving cleavage of the target molecule at the C-N bond leading to the fragments **7** and **8**, which were both derived from homoveratronitrile **9** as shown in scheme 2. Reduction of the

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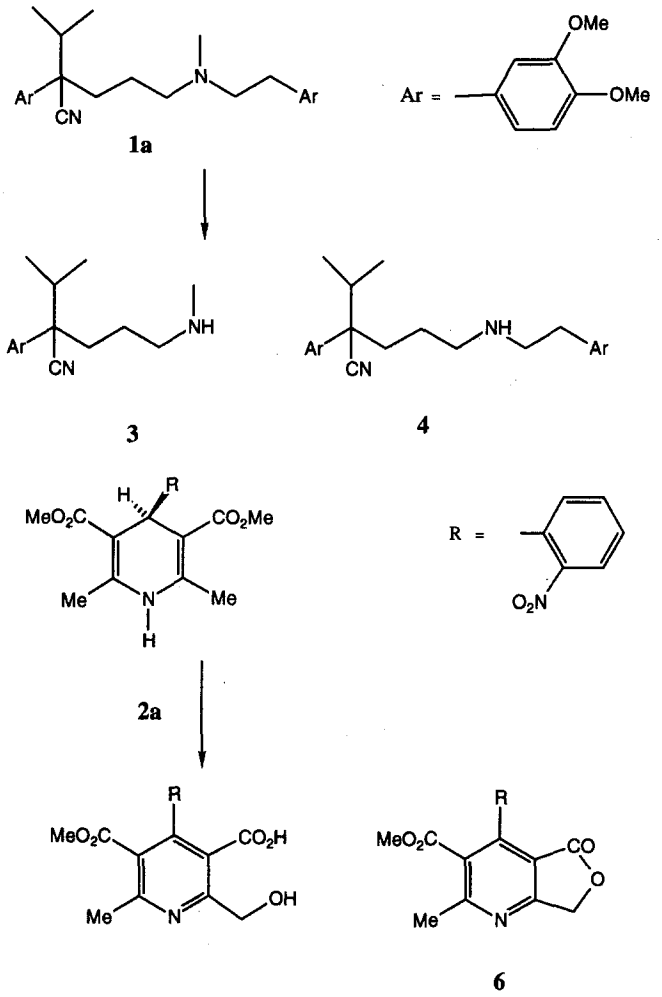
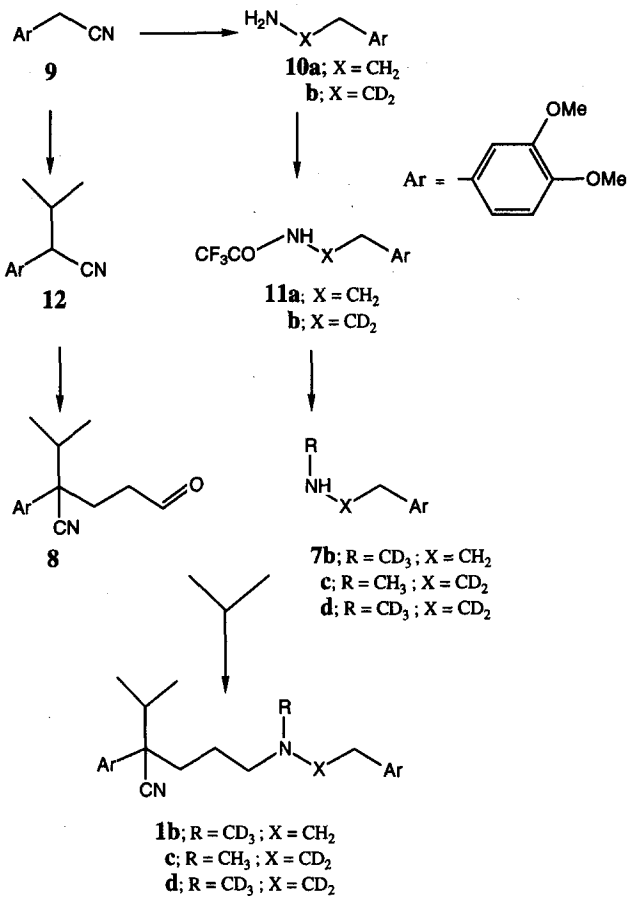


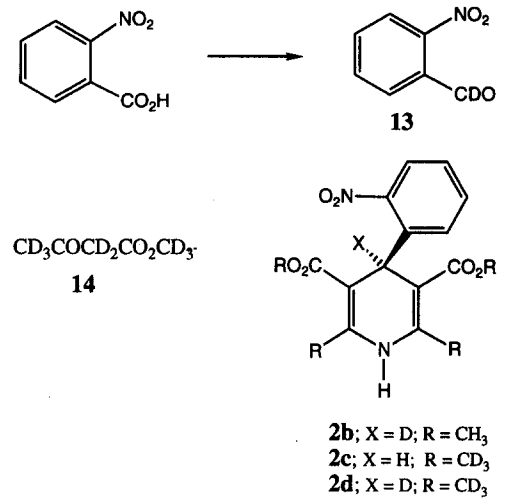
Scheme 1.



Scheme 2.

nitrile by aluminium deuteride in THF [9] afforded in 72% yield the amine **10b**, deuterated in > 98% at the α position. For all the deuterated compounds the positions of the deuterium as well as isotopical purity were determined by high resolution ^{1}H- and ^{2}H-NMR studies. To avoid di- and trialkylations, the amines **10** were converted quantitatively into the trifluoroacetamide derivatives **11a** and **11b** with trifluoroacetic anhydride in refluxing benzene. The subsequent methylation of the amides **11** was achieved in excellent yields using methyl iodide or d_3-methyl iodide and sodium hydride in THF with 12-crown-4 as catalyst. Subsequent hydrolysis with methanolic ammonia provided in good yields the desired deuterated amines **7b–d**, respectively, of > 97% isotopical purity. Preparation of the aldehyde **8** was carried out essentially as described in the literature [10]. Alkylation of homoveratronitrile **9** using isopropyl bromide under phase transfer conditions gave the expected product **12**, albeit in only 26% yield. However, further reactions with 3-chloropropionaldehyde diethylacetal in THF followed by hydrolysis furnished in practically quantitative yields the crystalline aldehyde **8.** Reductive condensation of the aldehyde **8** with the amines **7** was carried out with hydrogen and a catalytic amount of palladium on charcoal. No exchange or scrambling of deuterium took place under these conditions, as shown by the NMR spectra. The deuterated verapamil bases **1b–d** were finally converted with hydrochloric acid in 2-propanol to the corresponding crystalline hydrochlorides, which were used for the biological evaluation.

Starting from α-deutero-2-nitrobenzaldehyde **13** and methyl acetoacetate or perdeutero methyl acetoacetate **14,** the deuterated nifedipine analogs **2b–d** were obtained essentially by the route employed for



Scheme 3.

the synthesis of nifedipine itself [11, 12], as shown in scheme 3. Reduction of 2-nitrobenzoyl chloride with lithium tri-*t*-butoxyaluminium deuteride in diglyme afforded crystalline α-deutero-2-nitrobenzaldehyde **13** in 57% yield [13]. The corresponding benzyl alcohol was formed as a by-product, but was easily separated; use of an excess of the acid chloride or different solvents did not improve the yield of the aldehyde. In the high resolution ^{1}H-NMR spectrum of **13**, the signal due to the aldehyde proton was absent proving that the isotopical purity of the sample was > 98%. The deuterated methyl acetoacetate was obtained by concurrent isotope exchange and *trans*-esterification reactions. Treatment of methyl acetoacetate with sodium d_3 methoxide in a 50 molar excess of perdeuterated methanol gave a product in which > 90% of the hydrogens were replaced with deuterium. A second treatment of this sample under the same con-ditions afforded **14** of > 98% isotopical purity. The methylene hydrogens underwent exchange ≈ 4-fold faster than the enolizable methyl hydrogens, which exchanged at a rate comparable with that of *trans*-esterification. When the condensations of **13** and methyl acetoacetate or the perdeutero analog **14** were carried out with ammonia in methanol, some hydrogen–deuterium exchange at the allylic methyl groups of the product took place. This was avoided by using both perdeuterated ammonia and methanol, and thus the deuterated nifedipine analogs **2b-d** were obtained, each in > 98% isotopical purity. Since the present work was completed the preparation of **2b**, by essentially the same method, has been reported [14].

Pharmacology

Several verapamil metabolites have been described, with *N*-dealkylated and *N*-demethylated species predominating [5, 15]. To assess indirectly what effect deuterium substitution might have on the bioavailability of verapamil **1a**, we examined systolic blood pressure in spontaneously hypertensive rats over a period following oral doses of verapamil or its deuterated analog **1d** (fig 1A). When compared to pretreatment levels, both verapamil and **1d** significantly ($p < 0.05$; ANOVA/Dunnet's *t*-test) lowered systolic blood pressure over the first 12 h. Furthermore, the

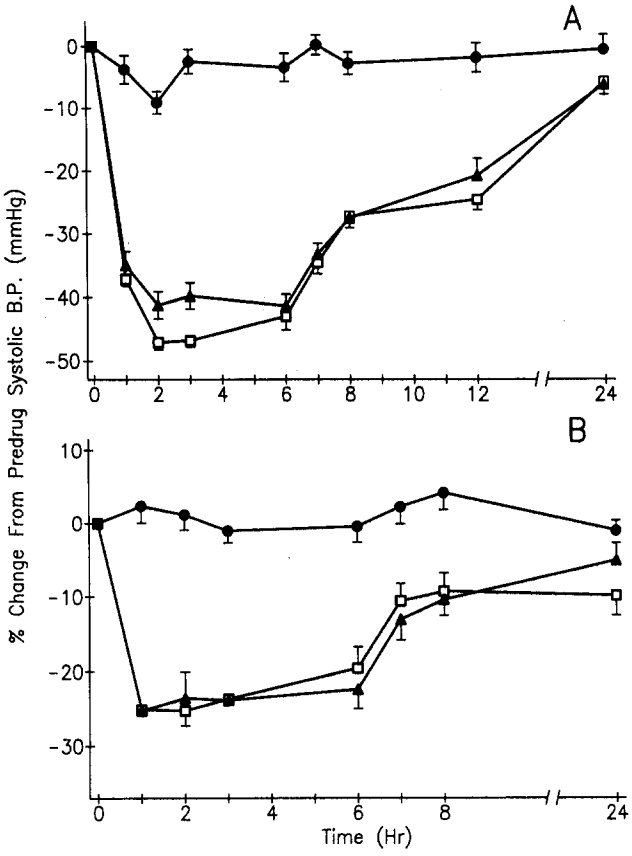


Fig 1. Effects of verapamil, nifedipine, and deuterated analogs on systolic blood pressure in spontaneously hypertensive rats. **A**: Effects of vehicle (●, 0.5% methocel 5 ml/kg *po*), verapamil (▲, 50 mg/kg *po*) and the deuterated analog **1d** (□, 50 mg/kg *po*) on the percent change in blood pressure are shown. Symbols and error bars indicate mean ± SEM (n = 12). **B**: Effects of vehicle (●, 0.5% methocel 5 ml/kg *po*), nifedipine (▲, 3 mg/kg *po*) and the deuterated analog **2d** (□, 3 mg/kg *po*) on the percent change in blood pressure are shown. Symbols and error bars indicate mean ± SEM (n = 12).

deuterated analog **1d** showed slightly but significantly ($p < 0.05$; ANOVA/Dunnet's *t*-test) greater activity at 2 and 3 h when compared to verapamil. With this exception, we found no differences between these 2 compounds with regard to the magnitude or duration of action *in vivo*. These results are consistent with previously reported plasma levels in humans using a trideuterated verapamil analog [16].

Figure 1B illustrates the effects of nifedipine, or its deuterated analog **2d,** on systolic blood pressure in spontaneously hypertensive rats. When compared to pre-drug levels, both compounds significantly ($p < 0.05$; ANOVA/Dunnet's *t*-test) depressed systolic blood pressure over the first 8 h of treatment. Also, systolic blood pressure was still significantly depressed in the animals treated with **2d**, but not in the nifedipine-treated animals, 24 h post-treatment. Except for this time point, no significant difference was noted between groups treated with nifedipine or its analog **2d.**

Conclusions

The deuterated analogs **1d** of verapamil and **2d** of nifedipine lowered blood pressure in spontaneously hypertensive rats. With some minor exceptions the profile of these deuterated compounds was similar to that of the parent molecules. Thus it must be concluded that the deuterium substitutions described here fail to significantly alter the metabolism of verapamil or nifedipine *in vivo* as measured indirectly by their hypotensive effects.

Experimental protocols

Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. The NMR spectra were recorded on a Varian XL-300 spectrometer. The ^{1}H-NMR spectra were obtained in $CDCl_3$ as solvent and with TMS as internal standard. For the ^{2}H-NMR spectra, $CHCl_3$ was used as solvent with $CDCl_3$ ($\delta = 7.25$) as internal standard.

3,4-Dimethoxyphen(1,1-$^2H_2$)ethylamine ***10b***
A suspension of lithium aluminum deuteride (3.02 g, 72 mmol) and beryllium chloride (2.88 g, 36 mmol) in THF (120 ml) was stirred at room temperature for 64 h [17]. The suspension was filtered under nitrogen through a Celite plug, rinsed with THF (2 x 10 ml), and the resulting clear solution of aluminum deuteride was cooled to 0°C. A solution of the nitrile **9** (8.86 g, 50 mmol) in THF (20 ml) was added with vigorous stirring over 1 h, which was continued for 2 h at 0°C and 30 h at room temperature. The mixture was cooled to 0°C and a 50% aqueous solution of THF (12 ml) was added with vigorous stirring for 15 min, followed by 10% aqueous solution of NaOH (40 ml) for 5 min. After 15 min, the resulting suspension was filtered and the precipitate washed with ether (3 x 50 ml). Evaporation of volatile material gave the crude amine, from which water was removed azeotropically using benzene and a Dean–Stark trap. Work-up gave the amine **10b** (6.53 g, 71%), bp: 92°C/0.02 mmHg; ^{1}H-NMR δ 1.14 (s, 2H), 2.68 (s, 2H), 3.85 (s, 3H), 3.87 (s, 3H), 6.71–6.83 (m, 3H); ^{2}H-NMR δ 3.00 (s).

N-3,4-Dimethoxyphen(1,1-$^2H_2$)ethyl trifluoroacetamide ***11b***
TFA anhydride (9.8 ml, 70 mmol) was added through the condenser to a stirred and refluxing solution of the amine **10b** (9.15 g, 50 mmol) in benzene (110 ml) over 45 min. After 30 min, the solvent was evaporated and the residue recrystallized from chloroform/hexane 1:2 to give the amide **11b** (12.0 g, 86%), mp: 83°C; ^{1}H-NMR δ 2.82 (s, 2H), 3.84 (s, 6H), 6.68–6.84 (m, 3H), 6.98 (br s, 1H); ^{2}H-NMR δ 3.55 (s).

N-3,4-Dimethoxyphenethyl trifluoroacetamide ***11a***
Reaction of the amine **10a** and TFA anhydride by the procedure described for **11b** gave the amide **11a** in 89% yield, mp: 83°C; ^{1}H-NMR δ 2.81 (t, J = 6.8 Hz, 2H), 3.58 (dt, J = 5.8, 6.8 Hz, 2H), 3.84 (s, 3H), 3.85 (s, 3H), 6.68–6.84 (m, 3H), 6.98 (br s, 1H).

N-($^2H_3$)Methyl 3,4-dimethoxyphen(1,1-$^2H_2$)ethylamine ***7d***
The amide **11b** (5.59 g, 20 mmol) was added in small portions to a stirred suspension of sodium hydride (600 mg, 25 mmol) in dry THF (60 ml), kept at 15°C. A mixture of d_3-methyl iodide (4.35 g, 30 mmol) and 12-Crown-4 (200 mg) was added all at once, and the mixture stirred at this temperature for 24 h. The solvent was evaporated and the residue partitioned between ether (50 ml) and 1% aqueous HCl (20 ml). Extractive work-up gave the methylated amide in practically quantitative yield, mp: 67–68°C. A solution of this amide (5.33 g, 18 mmol) in concentrated aqueous ammonia (15 ml) and methanol (35 ml) was stirred for 30 h at room temperature. Volatile material was removed *in vacuo* and the residue dissolved in ethyl acetate (50 ml). Extraction with 10% aqueous HCl and work-up in the usual manner gave the amine **7d** (2.59 g, 72%) as a viscous oil that was used without further purification; ^{1}H-NMR δ 1.65 (s, 1H), 2.73 (s, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 6.71–6.81 (m, 3H); ^{2}H-NMR δ 2.62 (s, 3^2H), 3.10 (s, 2^2H).

N-($^2H_3$)Methyl 3,4-dimethoxyphenethylamine ***7b***
Reaction of the amide **11a** with d_3-methyl iodide followed by hydrolysis, as described for **7d**, gave in 81% overall yield the amine **7b**; ^{1}H-NMR δ 2.10 (s, 1H), 2.70–2.86 (m, 4H), 3.83 (s, 3H), 3.85 (s, 3H), 6.71–6.81 (m, 3H); ^{2}H-NMR δ 2.67 (s).

N-Methyl 3,4-dimethoxyphen(1,1-$^2H_2$)ethylamine 7c
Reaction of the amide **11b** with methyl iodide followed by hydrolysis, as described for **7d**, gave in 80% overall yield the amine **7c.** ^{1}H-NMR δ 1.49 (s, 1H), 2.43 (s, 3H), 2.73 (s, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 6.71–6.81 (m, 3H); ^{2}H-NMR δ 3.10 (s).

2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[N-($^2H_3$)methyl-3,4-dimethoxyphen(1,1-$^2H_2$)ethylamino]valeronitrile ***1d***
A solution of the aldehyde **8** (2.76 g, 10 mmol) and the amine **7d** (2.0 g, 10 mmol) in ethanol (5 m) was injected all at once into a vigorously stirred suspension of 10% Pd/C (500 mg) in ethanol (15 ml) kept at 43°C under an atmosphere of hydrogen. The reaction was terminated after 1 h. Filtration and evaporation of ethanol gave a viscous residue that was dissolved in ether (30 ml). Extraction with 10% aqueous HCl (2 x 10 ml) followed by work-up in the usual way gave the verapamil analog **1d** (3.16 g, 88%) as a viscous colorless oil. ^{1}H-NMR

δ 0.79 (d, J = 6.7 Hz, 3H), 1.08–1.22 (m, 1H), 1.19 (d, J = 6.7 Hz, 3H), 1.53 (oct J = 6.7 Hz, lH), 1.84 (dt, J = 4.3, 12.7 Hz, 1H), 2.07 (dq, J = 7.0 Hz, 12.7, 2H), 2.36 (oct J = 7.0 Hz, 2H), 2.65 (s, 2H), 3.84 (s, 3H), 3.86 (s, 3H), 3,87 (s, 3H), 3.88 (s, 3H), 6.67–6.94 (m, 6H); ^{2}H-NMR δ 2.11 (s, 2^{2}H), 2.43 (s, 3^{2}H). It was converted into the hydrochloride, mp: 136–138°C, as described in the literature [10].

2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[N-($^2H_3$)methyl-3,4-dimethoxyphenethylamino] valeronitrile ***1b***
Reaction of the aldehyde **8** with the amine **7b** as described for the preparation of **1b** gave the verapamil analog **1b** in 92% yield; ^{1}H-NMR δ 0.79 (d, J = 6.7 Hz, 3H), 1.08–1.22 (m, 1H), 1.19 (d, J = 6.7 Hz, 3H), 1.53 (oct, J = 6.7 Hz, 1H), 1.84 (dt, J = 4.3 Hz, 12.7, 1H), 2.07 (dq, J = 7.0 Hz, 12.7, 2H), 2.36 (oct J =7.0 Hz, 2H), 2.45–2.55 (m, 2 H), 2.61–2.72 (m, 2 H), 3.84 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 3.88 (s, 3H), 6.67–6.94 (m, 6H); ^{2}H-NMR δ 2.11 (s).

2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[N-methyl-3,4-dimethoxyphen(l,l-$^2H_2$)ethylamino] valeronitrile ***1c***
Reaction of the aldehyde **8** with the amine **7c** as described for the preparation of **1b** gave the verapamil analog **1c** in 94% yield; ^{1}H-NMR δ 0.79 (d, J = 6.7 Hz, 2H), 1.08–1.22 (m, 1H), 1.19 (d, J =6.7 Hz, 3H), 1.53 (oct J = 6.7 Hz, 1H), 1.84 (dt, J = 4.3, 12.7 Hz, 1H), 2.07 (dq, J = 7.0 Hz; 12.7, 2H), 2.19 (s, 3 H), 2.36 (oct J = 7.0 Hz, 2H), 2.65 (s, 2H), 3.84 (s, 3H), 3.86 (s, 3H), 3,87 (s, 3H), 3.88 (s, 3H), 6.67–6.94 (m, 6H); ^{2}H-NMR δ 2.43 (s).

($^2H_3$)Methyl ($^2H_5$)acetoacetate ***14***
To a solution of sodium methoxide prepared from sodium (115 mg, 5 mmol) and d_4-methanol (203 ml, 5.01 mol) was added methyl acetoacetate (11.61 g, 100 mmol). The mixture was heated under reflux for 3 h. Volatile material was distilled *in vacuo* into a cooled receiver. From this material the solvent was removed through an efficient column, and the residue distilled to yield methyl acetoacetate, which was 90% deuterated. The procedure was repeated once more to give **14** (8.19 g, 66%), bp: 61–62°C/15 mmHg, isotopical purity > 98%. ^{2}H-NMR δ 2.17 (s, 3^{2}H), 3.38 (s, 2^{2}H), 3.64 (s, 3^{2}H).

Dimethyl (4-^{2}H)-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) 3,5-pyridinedicarboxylate ***2b***
This compound was prepared in 59% yield, mp: 166°C, from 2-nitro-(^{2}H)benzaldehyde **13** [13] and methyl acetoacetate using perdeuterated ammonia and methanol, as described in the literature for the undeuterated compound [11, 12]. ^{1}H-NMR δ 2.30 (s, 6H), 3.55 (s, 6H), 5.72 (s, 1H), 7.17–7.28 (m, 1H), 7.36–7.52 (m, 2H), 7.64 (d, J = 8.1 Hz, 1H). ^{2}H-NMR δ 5.68 (br, s).

($^2H_6$)Dimethyl 1,4-dihydro-2,6-($^2H_6$)dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate ***2c***
This compound was prepared in 45% yield, mp: 166°C, from 2-nitrobenzaldehyde and methyl acetoacetate **14** using perdeuterated ammonia and methanol, as described in the literature for the undeuterated compound [11, 12]. ^{1}H-NMR δ 5.73 (s, 1 H), 6.32 (s, 1H), 7.20–7.28 (m, 1H), 7.40–7.54 (m, 2H), 7.67 (d, J = 8.1 Hz, 1H). ^{2}H-NMR δ 2.27 (s, 6^{2}H), 3.54 (s, 6^{2}H).

($^2H_6$)Dimethyl (4-^{2}H)-1,4-dihydro-2,6-($^2H_6$)dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate ***2d***
This compound was prepared in 59% yield, mp: 166°C, from 2-nitro-(^{2}H)benzaldehyde **13** [13] and methyl acetoacetate **14** using perdeuterated ammonia and methanol, as described in the literature for the undeuterated compound [11, 12]. ^{1}H-NMR δ 5.64 (s, 1H), 7.20–7.28 (m, 1H), 7.40–7.56 (m, 2H), 7.68 (d, J = 8.1 Hz, 1H), ^{2}H-NMR δ 2.27 (s, 6^{2}H) 3.54 (s, 6^{2}H) 5.68 (br s, 1^{2}H).

Blood pressure measurements
Male spontaneously hypertensive rats (SHR) were used for all blood pressure measurement studies. Rats were divided into groups of 12 animals and systolic blood pressure was obtained from their tails using an indirect method described previously [18]. All drugs were administered by gavage as suspension in 0.5% methylcellulose. Verapamil and its deuterated analog **1d** were administered at 50 mg/kg. Nifedipine and its deuterated analog **2d** were administered at 3 mg/kg. Control animals received 0.5% methylcellulose only (5 ml/kg).

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