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Enantioselective synthesis of (*R*)- and (*S*)-2-methyl-[3,3,2-$^2H_3$] alanines

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The synthesis of optically pure (*R*)- and (*S*)-2-methyl-[3,3,3-$^2H_3$] alanines of biological interest is described. The stereochemistry of the reaction of the lithio derivative of (*R*)-(−)-2,5-dimethoxy-3-benzyl-3-methyl-3,6-dihydropyrazine with alkyl and deuterated alkyl iodides is discussed. The configuration of the newly formed center of chirality in (*R*)- and (*S*)-2-methyl-[3,3,3-$^2H_3$] alanines is derived from ^1H NMR.

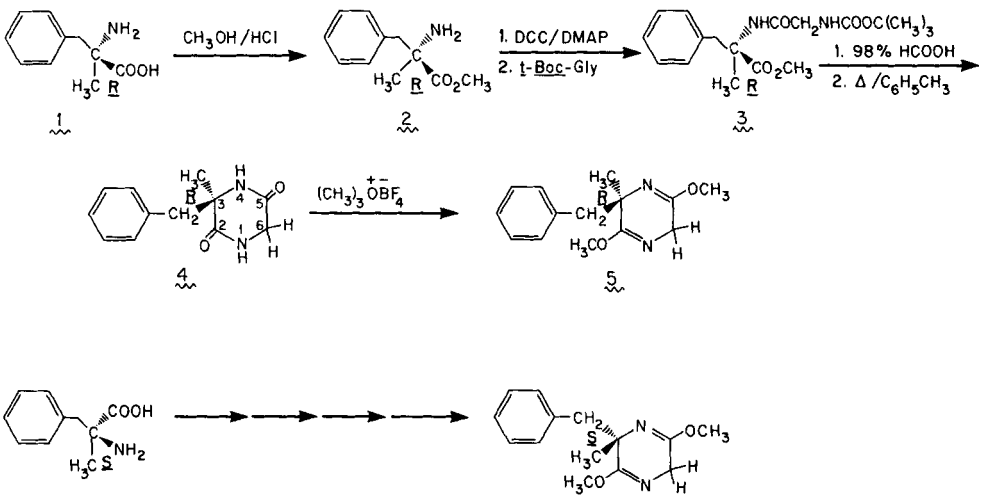
Key words: asymmetric induction; diketopiperazines; α-aminoisobutyric acid; deuterium labeling

Amino acids optically active due to isotopic substitution of deuterium or ^{13}C atoms are gaining increasing importance in research and practice as probes for biosynthesis and interconversion of various amino acids as well as NMR probes for peptide binding and dynamics (1–3).

Schöllkopf *et al.* (4, 5) have recently reported on the enantio-selective synthesis of various nonproteinogenic amino acids starting with bis-lactim ethers of cyclo[L-Val-Gly] and cyclo[(*S*)-*O*,*O*-dimethyl-α-methyldopa-Gly]. These bis-lactim ethers react with butyllithium in tetrahydrofuran at −78°C to give the lithio derivatives which are condensed with alkyl, 2-alkenyl or 2-alkynyl halides, ketones, aldehydes and halomethylbenzyl ethers to form the appropriate adducts in high diastereomeric excess (> 95%). In all cases studied, the lithium derivative of the bis-lactim ethers containing an L(*S*) amino acid center at the C-3 is approached by the electrophiles *trans* to the bulky substituent in the C-3 position to give the *R* configuration at the C-6 position.

We have utilized these methods to prepare (*R*)- and (*S*)-2-methyl-[3,3,3-$^2H_3$] alanines for incorporation into certain peptides in order to restrict their various conformational degrees of freedom (6) and at the same time utilize the deuterated methyl group in ^2H NMR studies to investigate the molecular dynamics of these peptides and their binding to other macromolecular molecules (7).

We chose as our chiral agent (*R*)-(+)-2-methyl-3-phenylalanine **1**, which was prepared from phenylacetone (8) in a convenient four-step sequence in 49% overall yield by a modified asymmetric Strecker synthesis first described by Weinger and coworkers (9). This amino acid was chosen because it bears an α substituent and therefore the cyclo[α-methylphe-gly] derived can be alkylated only at the C-6 position. The phenylalanine derivative was esterified with methanolic HCl (10) and the resulting methyl ester **2** condensed (11) with *N*-*t*-*Boc*-glycine giving the dipeptide methyl ester **3**. Following the procedure of Nitecki *et al.* (12), the dipeptide methyl ester **3** was cyclized,



SCHEME 1
Synthesis of (3*R*)-2,5-dimethoxy-3-benzyl-3-methyl-3,6-dihydropyrazine.

after treating with formic acid, by boiling in a 2:1 mixture of *sec*-butanol and toluene* to yield the 2,5-diketopiperazine **4**. The piperazine **4** was then converted into the bis-lactim ether **5** (see Scheme 1) by treatment with trimethyloxonium tetrafluoroborate (13).

The bis-lactim ether **5** reacted directly with 1 equiv. of butyllithium in tetrahydrofuran (or 1,2-dimethoxyethane) at $-78°C$ to form the lithium derivative of **5**, a resonance-stabilized diazapentadienyl anion, which is regarded as an ion pair. This lithio derivative reacted with alkyl iodides to afford a mixture of diastereomers **6** and **7****. The diastereomer **6a**, in which the alkyl group (CH_3) has entered *trans*, with respect to the benzyl group at C-3, was the predominant isomer (the ratio of *trans*:*cis* approach for alkylation with CH_3I is 80:20 (**6a**:**7a**) in THF, 85:15 in dimethoxyethane and for alkylation with CD_3I, 78:22 (**6b**:**7b**) in THF, 83:17 in dimethoxyethane) and had the (*S*) configuration at the C-6 position.

The (6*S*) configuration of **6a** was deduced from the ^1H NMR spectrum. The bis-lactim ethers **6** and **7** adopt a boat shape for the heterocycle (13) and the folded conformation (14) for the benzyl group. In the ^1H NMR spectra, the hydrogen atom at the C-6 carbon of **6a** appeared at $\delta = 3.10$, while the hydrogen atom of **7a** appeared at $\delta = 3.74$. The upfield shift of 0.7 ppm of the 6-hydrogen is due to the shielding effects of the aromatic ring in the C-3 position (15) in **6a** (compared to that in **7a**). This is consistent with the *trans* addition of the methyl group in the alkylation step, i.e., induction of the *S* configuration at C-6 in **6a**. Additionally, the ^1H NMR spectrum of **6a** showed a signal at $\delta = 1.11$ for the C-6 methyl, whereas the C-6 methyl signal in **7a** appeared at $\delta = 0.24$. The upfield shift of the C-6 methyl in the minor diastereomer **7a** (compared to that in **6a**) is also consistent with the report of Woodard (15) and is in agreement with the 6*S* configuration for **6a**. Similarly, the 6*S* configuration for **6b** (alkylation with CD_3I) can

*One equivalent of morpholine was added to improve the cyclization, otherwise the standard Nitecki conditions gave poor yields.

The reaction of the lithio derivative of **5 with methyl triflate (CH_3OTf or CD_3OTf) gives a 50–50 mixture of diastereomers (6*R* and 6*S*) indicating the total lack of asymmetric induction.

be derived from the ^1H NMR spectra of the hydrogen signals at the C-6.

It is noteworthy to mention that the Rfs of **6a** and **7a** (or **6b** and **7b**) are 0.73 and 0.64 respectively in 4:1 hexane/ethyl acetate and, hence, the two diastereomers **6a** and **7a** (or **6b** and **7b**) are easily separable by either column chromatography or HPLC. The hydrolysis of **6b** and **7b** would give (*S*)- and (*R*)-[$^2H_3$]-alanine, respectively. However, in the present study, the diastereomers did not need to be separated since the configuration at C-6 would be lost on lithiation in the next step.

The mixture of monoalkylated products **6a** and **7a** was further reacted with butyllithium in tetrahydrofuran at $-78°C$ to form a single anion (since the C-6 carbon is now sp^2, there is no difference in the stereochemistry of the anions formed from **6a** and **7a**) which was attacked by [$^2H_3$] methyliodide (see Scheme 2) to give exclusively the (3*R*, 6*S*) diastereomer **8a** (% de $>95\%$ as determined by ^1H NMR). The second alkyl group entered *trans* with respect to the bulky benzyl group in the C-3 position. The (3*R*, 6*R*) diastereomer **8b** was prepared by reversing the order of alkylation, i.e. [$^2H_3$]-methyliodide, followed by unlabeled methyliodide. The ^1H NMR of both **8a** and **8b** showed *only* one C-6 methyl group ($\delta = 0.31$ and $\delta = 1.10$ respectively) and, hence, the diastereomeric excess of both dialkylated products **8a** and **8b** was assumed to be $>95\%$. The characteristic upfield shift (0.8 ppm) of the 6-CH_3 signal ($\delta = 0.31$) in **8a** compared to that ($\delta = 1.1$) in **8b** was in agreement with the (6*S*) configuration in **8a** and the (6*R*) configuration in **8b** (15).

The 3,6-dihydropyrazines **8a** and **8b**, on hydrolysis with 0.25 N HCl (4 equiv.) at room temperature, were cleaved to give (*R*)-2-methyl-3-phenylalanine methyl ester **2** and either (*S*)- or (*R*)-2-methyl-[3,3,3-$^2H_3$] alanine methyl ester (**9a** or **9b**), respectively. The methyl esters were further hydrolyzed by heating them to reflux in 6N hydrochloric acid to the corresponding amino acids. The target (*S*)- and (*R*)-2-methyl-[3,3,3-$^2H_3$] alanines (**10a** and **10b**) were separated by preparative thin layer chromatography from the chiral auxiliary reagent *R*-(+)-2-methyl-3-phenylalanine **1**, which could be reutilized. The (*S*)- and (*R*)-2-methyl-[3-^{13}C] alanines could be synthesized in a similar manner using unlabeled methyliodide and [^{13}C] methyliodide.

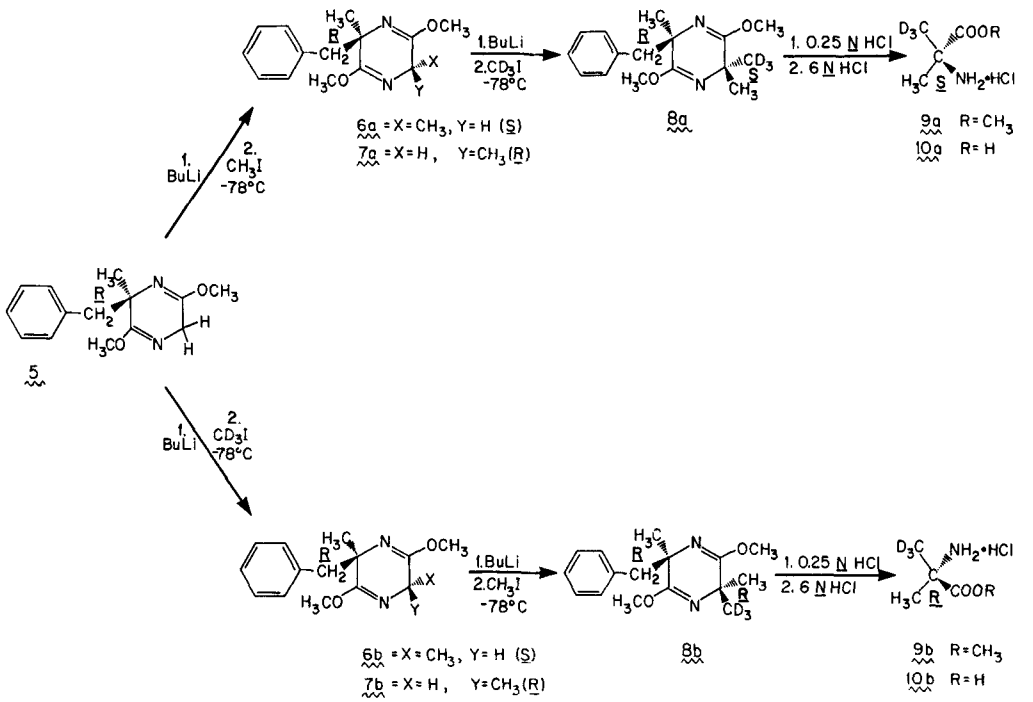
In conclusion, we synthesized the bis-lactim ether (*R*)-(−)-2,5-dimethoxy-3-benzyl-3-methyl-3,6-dihydropyrazine (**5**) in a straightforward manner and utilized it as a chiral auxiliary reagent to prepare the title amino acids, (*R*)- and (*S*)-2-methyl-[3,3,3-$^2H_3$] alanines, in enantiomeric excess of $>95\%$. In addition, the use of the dihydropyrazine (**5**) as a synthon allowed for the determination of the enantiomeric excess at the alkylated center directly by ^1H NMR without the addition of chiral shift reagents.

EXPERIMENTAL PROCEDURES

Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian EM-360 60 MHz, a Bruker WM-360 MHz spectrometer and an IBM WP 270 MHz spectrometer; chemical shift values are reported in ppm downfield from tetramethylsilane. Infrared spectra were recorded on a Perkin-Elmer Model 281 spectrophotometer. Mass spectral data were obtained on a Finnigan 4021 mass spectrometer (Chemistry Department, University of Michigan). Optical rotations were measured in a 1 dm cell at 25°C on a Perkin-Elmer Model 141 polarimeter; c is expressed in g/100 ml.

The CD_3I (99 + atom%) was purchased from Aldrich Chemical and used without further purification.

Column chromatography was performed using E. Merck silica gel 60, 70–230 mesh ASTM, elutions being carried out with 19:1 hexane/ethyl acetate (v:v) followed by 9:1 hexane/ethyl acetate unless otherwise noted. Analytical thin layer chromatography was performed on Analtech, 20 x 20 cm plates precoated with silica gel G. Tetrahydrofuran and 1,2-dimethoxyethane were distilled from $LiAlH_4$ immediately prior to use. Dichloromethane was distilled from P_2O_5 under nitrogen. All organic solvent extractions were dried with Na_2SO_4 and removed *in vacuo* using a rotary evaporator (water aspirator vacuum) unless otherwise stated. Elemental analyses were



SCHEME 2

Synthesis of (2*S*)- and (2*R*)-2-methyl-[3,3,3-$^2H_3$]-alanine.

performed by M-H-W Laboratories, Phoenix, AZ.

(R)-(+)-N-(N-tert.-*butoxycarbonylglycinyl*)-*2-methyl-3-phenylalanine methyl ester* (**3**)(*11*)

To a stirred solution of (*R*)-(−)-2-methyl-3-phenylalanine methyl ester **2** (4.83 g, 25 mmol) in dry dichloromethane (30 ml) at 0°C was added a solution of *N*-2,2-dimethylethyloxycarbonylglycine (4.38 g, 25 mmol) and *N,N'*-dicyclohexylcarbodiimide (5.16 g, 25 mmol) in dry dichloromethane (20 ml) followed by the addition of 4-dimethylaminopyridine (DMAP) (0.61 g, 5 mmol). The reaction mixture was allowed to stir at 0°C for 4 h and at room temperature overnight. The urea which formed was removed by filtration and the filtrate evaporated to yield an oily residue. The residue was dissolved in ether (100 ml) and washed successively with 5% citric acid, a saturated $NaHCO_3$ solution, and water. The organic layer was evaporated to yield the crude peptide **3** as a viscous oil. The oily product was purified by column chromatography with ethyl acetate/hexane (3:2) as eluant to yield 7.2 g (82.2%); $[\alpha]_D = +45.4°$ (c = 1.6, methanol), ^1H NMR (DMSO-d_6) δ 1.21 (s, 3H, C-CH_3), 1.39 (s, 9H, C(CH_3)$_3$), 2.98 and 3.23 (d, AB, J = 13.2 Hz, $C_6H_5CH_2$), 3.53 (dd merged into t, J = 5.82 Hz, 2H, CH_2NH), 3.58 (s, 3H, COOCH_3), 6.96 (t, J = 5.55 Hz, 1H,CH_2N*H*), 7.08–7.30 (m, 5H, C_6H_5), 7.93 (s, 1H, N*H*COCH$_2$).

Anal. calc. for $C_{18}H_{26}N_2O_5$: C, 61.69; H, 7.48. Found: C, 61.59; H, 7.49.

(R)-(−)-*3-Methyl-3-benzyl-2,5-diketopiperazine* (**4**)

The procedure of Nitecki and coworkers (12) for converting *t-Boc*-protected dipeptide methyl ester into cyclic dipeptide was modified as follows: *the t-Boc* dipeptide methyl ester **3** (7.0 g, 20.0 mmol) was dissolved in formic acid (125 mL) and stirred for 2 h under a drying tube. The solution was concentrated *in vacuo* (bath temperature < 30°C) and the residue triturated several times with dry ether to

precipitate the formate salt (5.1 g, 86.1%) which was used without further purification. To a solution of the formate salt in *sec*-butanol (200 mL) and toluene (100 mL) was added 1.5 g (17.2 mmol) of morpholine* and the whole mixture heated at reflux (96°C) for 2½ h. After most of the solvent was removed, the solution was cooled to 0°C and the resulting solid filtered. The crude product was purified by recrystallization from methanol to give 2.8 g (64.1%) of the title compound, mp 302–304°C, $[\alpha]_D = -92.7°$ (c = 0.11, DMF), IR (KBr) 1675 cm^{-1} (NHC = O), ^1H NMR (DMSO-d_6) δ 1.41 (s, 3H, C-C*H*$_3$), 2.51 and 3.34 (d, AB, J = 17.46 Hz, 2H, C*H*$_2$), 2.67 and 3.07 (d, AB, J = 13.02 Hz, $C_6H_5CH_2$), 7.12–7.28 (m, 5H, $C_6H_5CH_2$), 7.79 (bs, 1H, 4-N*H*), 8.25 (bs, 1H, 1-N*H*).

Anal. calc. for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47. Found: C, 66.04; H, 6.47.

(R)-(−)-*2,5-Dimethoxy-3-benzyl-3methyl-3,6-dihydropyrazine* (**5**)

A mixture of the diketopiperazine **4** (4.37 g, 20 mmol) and 7.4 g (50 mmol) of trimethyloxonium tetrafluoroborate in 100 mL of dry dichloromethane was vigorously (mechanically) stirred at 40°C for 72 h. The reaction mixture was cooled to room temperature and a solution of 6 g of potassium carbonate in 25 mL of water added, the organic layer separated and the aqueous layer extracted with dichloromethane (3 x 50 mL). The combined organic extracts were evaporated to give a crude residue which was purified by column chromatography to yield 3.6 g (73%) of **5** as an oil, $[\alpha]_D = -192.3°$ (c = 0.555, ethanol), IR (film) 1695 cm^{-1} (C = N), ^1H NMR (DMSO-d_6) δ 1.42 (s, 3H, C-C*H*$_3$), 2.71 and 3.03 (d, AB, J = 12.77 Hz, 2H, $C_6H_5CH_2$), 2.84 and 3.66 (d, AB, J = 20.53 Hz, 2H, 6-*H*), 3.61 (s, 3H, OC*H*$_3$), 3.63 (s, 3H, OC*H*$_3$), 6.96–7.22 (m, 5H, $C_6H_5CH_2$).

Anal. calc. for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37. Found: C, 68.42; H, 7.28.

(*3*R, *6*S)-*2,5-Dimethoxy-3-benzyl-3,6-dimethyl-3,6-dihydropyrazine* (**6a**)

To a stirred solution of bis-lactim ether **5** (0.5 g, 2.0 mmol) in dry tetrahydrofuran (or better 1,2-dimethoxyethane) (5 mL) under nitrogen at −78°C was added by syringe a 1.6N solution (1.4 mL, 2.2 mmol) of butyllithium in hexane and the mixture stirred for 30–45 min to insure formation of the anion. Then a precooled solution of methyl iodide (310 mg, 2.2 mmol) in dry THF (5 mL) was added and stirring continued for 8 h at −78°C. The mixture was allowed to warm to room temperature and the solvent removed *in vacuo* to give a residue which was dissolved in diethyl ether and washed with Na phosphate buffer, pH = 7 (3 x 25 mL). After evaporation of the solvent, the residual product, 430 mg (82.6%), a mixture of **6a**:**7a** in the ratio of 80:20 (*trans*:*cis* approach) in THF and 85:15 in dimethoxyethane, was purified by column chromatography to yield **6a** as an oil; IR (film) 1695 cm^{-1} (C = N), ^1H NMR (DMSO-d_6) δ 1.11 (d, J = 7.18 Hz, 3H, 6-C*H*$_3$), 1.42 (s, 3H, C-C*H*$_3$), 2.71 and 3.05 (d, AB, J = 12.75 Hz, 2H, $C_6H_5CH_2$), 3.10 (q, J = 7.24 Hz, 1H, 6-*H*), 3.60 (s, 3H, OC*H*$_3$), 3.62 (s, 3H, OC*H*$_3$), 6.93–7.24 (m, 5H, $C_6H_5CH_2$).

Anal. calc. for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74. Found: C, 69.41; H, 7.85.

(*3*R, *6*R)-*2,5-Dimethoxy-3-benzyl-3,6-dimethyl-3,6-dihydropyrazine* (**7a**)

The title compound was obtained from the above reaction mixture as the minor isomer in 10% yield as an oil; IR (film) 1695 cm^{-1} (C = N), ^1H NMR (DMSO-d_6) δ 0.24 (d, J = 7.18 Hz, 3H, 6-C*H*$_3$), 1.42 (s, 3H, C-C*H*$_3$), 2.71 and 3.05 (d, AB, J = 12.75 Hz, 2H, $C_6H_5CH_2$), 3.60 (s, 3H, OC*H*$_3$), 3.63 (s, 3H, OC*H*$_3$), 3.74 (q, J = 7.24 Hz, 1H, 6-*H*), 6.93–7.24 (m, 5H, $C_6H_5CH_2$).

(*3*R, *6*S)-*2,5-Dimethoxy-3-benzyl-3-methyl-6-[$^2H_3$]methyl-3,6-dihydropyrazine* (**6b**)

The bis-lactim ether **5** (0.5 g, 2.0 mmol) was treated with [$^2H_3$]-methyl iodide (320 mg, 2,2 mmol) in the same way as described above for **6a**. Purification by column chromatography yielded 70% (365 mg) of **6b**. IR (film) 1697 cm^{-1} (C = N), ^1H NMR (DMSO-d_6) δ 1.42 (s, 3H, C-C*H*$_3$), 2.71 and 3.05 (d, AB, J = 12.73 Hz, 2H, $C_6H_5CH_2$), 3.08 (s, 1H,

*See footnote on page 580.

6-*H*), 3.60 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 6.93–7.24 (m, 5H, $C_6H_5CH_2$).
Anal. calc. for $C_{15}H_{17}D_3N_2O_2$: C, 69.20; H, 7.74. Found: C, 68.95; H, 7.96.

(*3*R, *6*R)-*2,5-Dimethoxy-3-benzyl-3-methyl-6-*[$^2H_3$]*methyl-3,6-dihydropyrazine* (**7b**)
The title compound was obtained from the above reaction mixture as the minor isomer in 10% yield as an oil; IR (film) 1695 cm^{-1} (C = N), ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 3H, C-CH_3), 2.71 and 3.05 (d, AB, J = 12.73 Hz, 2H, $C_6H_5CH_2$), 3.60 (s, 3H, OCH_3), 3.77 (s, 1H, 6-*H*), 3.62 (s, 3H, OCH_3), 6.93–7.24 (m, 5H, $C_6H_5CH_2$).

(*3*R, *6*S)-*2,5-Dimethoxy-3-benzyl-3,6-dimethyl-6-*[$^2H_3$]*-methyl-3,6-dihydropyrazine* (**8a**)
The dihydropyrazine **6a** or a mixture of **6a** and **7a** (520 mg, 2.0 mmol) in dry tetrahydrofuran (5 mL) was alkylated under standard conditions (BuLi, CD_3I/THF, −78°C, 8 h) to give the dialkylated product **8a** in 74% yield (410 mg) after column chromatography. $[\alpha]_D$ = −137.3° (c = 0.50, ethanol), %de > 95%, IR (film) 1697 cm^{-1} (C = N), ^{1}H NMR (DMSO-d_6) δ 0.31 (s, 3H, 6-CH_3), 1.42 (s, 3H, C-CH_3), 2.68 and 3.058 (d, AB, J = 12.57 Hz, 2H, $C_6H_5CH_2$), 3.59 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 6.95–7.22 (m, 5H, $C_6H_5CH_2$).
Anal. calc. for $C_{16}H_{19}D_3N_2O_2$: C, 70.04; H, 8.08. Found: C, 69.83; H, 8.04.

(*3*R, *6*R)-*2,5-Dimethoxy-3-benzyl-3,6-dimethyl-6-*[$^2H_3$]*-methyl-3,6-dihydropyrazine* (**8b**)
The product **8b** was prepared in 76% yield from 0.52 g (2.0 mmol) of **6b** (or a mixture of **6b** and **7b**) and 0.31 g (2.2 mmol) of methyl iodide following the procedure described above and purified by column chromatography. $[\alpha]_D$ = −137.3° (c = 0.54, ethanol), %de > 95%, IR (film) 1698 cm^{-1} (C = N), ^{1}H NMR (DMSO-d_6) δ 1.10 (s, 3H, 6-CH_3), 1.42 (s, 3H, C-CH_3), 2.68 and 3.08 (d, AB, J = 12.57 Hz, 2H, $C_6H_5CH_2$), 3.59 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 6.95–7.22 (m, 5H, $C_6H_5CH_2$).
Anal. calc. for $C_{16}H_{19}D_3N_2O_2$: C, 70.04; H, 8.08. Found: C, 70.13; H, 8.12

(S)-*2-Methyl-[3,3,3-*$^2H_3$*]alanine* (**10a**)
A mixture of compound **8a** (550 mg, 2 mmol) and 16 mL of 0.25 N hydrochloric acid (4 mmol) was stirred at room temperature for 48 h. The solution was extracted with diethyl ether (2 x 25 mL), the ether layer discarded and the aqueous layer concentrated to dryness *in vacuo*. A 6N HCl solution (5 mL) was added and the mixture heated to reflux for 1 h. After cooling, the solution was loaded onto a column containing Dowex 50W x 8 (H_3O^+ form) and the column eluted with water until the eluant was neutral followed by 100 mL of 1N NH_4OH. Concentration of the ninhydrin-positive fractions of the ammoniacal eluate yielded a crude mixture of **1** (Rf = 0.68) and **10a**, which was separated by preparative thin layer chromatography (silica gel, 2-propanol/ammonia/water, 20:3:1). The (*S*)-2-methyl-[3,3,3-$^2H_3$]alanine (Rf = 0.32) was recrystallized from methanol: mp > 325°C (Lit (16) mp 319–320°C for the nondeuterated analog); yield, 110 mg (51.8%), $[\alpha]_D$ = 0 (c = 0.5, H_2O), ^{1}H NMR (D_2O) δ 1.30 (s, 3H, CH_3); MS (relative intensity), m/e 107(MH^+, 3), 61 (100), 45 (36), 44 (51), 42 (41), 28 (33) 98.66% d_3.

(R)-*2-Methyl-[3,3,3-*$^2H_3$*]alanine* (**10b**)
The 3,6-dihydropyrazine **8b** (0.28 g, 1 mmol) was hydrolyzed to a mixture of amino acids **1** and **10b**, which was separated by preparative thin layer chromatography as described for **10a**. The title compound (Rf = 0.32) after recrystallization from methanol, melted at 320–323°C (dec)(Lit (16) mp 319–320°C for the nondeuterated analog); yield 0.06 g (56.5%), $[\alpha]_D$ = 0 (c = 0.5, H_2O), ^{1}H NMR (D_2O) δ 1.30 (s, 3H, CH_3); MS (relative intensity), m/e 107(MH^+, 16), 61 (100), 45 (12), 42 (11) 98.68% d_3.

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