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THE ENANTIOSPECIFIC SYNTHESIS OF [5-^{2}H]-5-EPI-SHIKIMIC ACID AND OF (6R)[6-^{2}H]-, (6S)[6-^{2}H]- AND [6-$^2H_2$] SHIKIMIC ACID

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SUMMARY

The enantioselective synthesis of [5-^{2}H]-5-epi-shikimic acid starting from commercially available L-shikimic acid has been accomplished in this work. The introduction of the stable isotope was facilitated by an enzymic reduction of a ketone. An interesting stereospecific enolisation was also observed during this reaction resulting in partial deuteration of the 6-equatorial position. In addition, the enantioselective syntheses of methyl(6R)[6-^{2}H]-, and (6S)[6-^{2}H] shikimate are described. The procedure is an adaptation of a reported[1] enantiospecific synthesis of shikimic acid, with the inclusion of an enzymic reduction step.

Key Words : Shikimic acid, epi-shikimic acid, enantioselectivity, deuterium, labelling.

INTRODUCTION

Shikimic acid is a key intermediate in the biosynthesis of aromatic amino acids in microorganisms and plants, as well as of a multitude of other natural products.[2] The study of the stereochemical requirements of the aromatic amino acids requires enantioselective syntheses of labelled shikimate and its diastereomer epi-shikimate. Epi-shikimic acid has been synthesized in racemic[3,4] and optically active form.[5] These interesting syntheses are however not suitable for the synthesis of labelled epi-shikimate. We are reporting

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in this publication the enantioselective synthesis of [5-^{2}H]-5-epi-shikimic acid starting from commercially available L- shikimic acid. The key step was the enzymic reduction of the ketone 4 (Fig. 1).

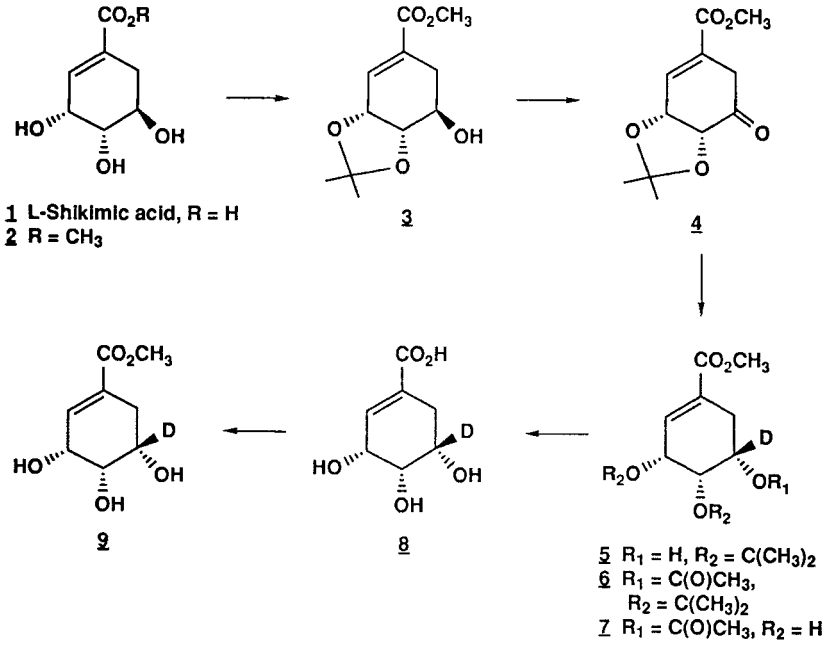


Fig. 1. Synthesis of methyl [5-^{2}H]-5-epi-shikimate

This compound was obtained by oxidation of the alcohol 3 (Fig. 1).[6] Reduction of the ketone 4 with sodium borodeuteride also gave the deuterated epi-shikimate due to the steric approach control. The isopropylidene group of compound 5 was easily removed after protection of the free hydroxyl (at C-5) in the presence of resin at room temperature. Shikimic acid has also been synthesized in racemic and optical active forms.[4] A new facile and elegant synthesis of (+) and (-) shikimic acid as well as enantiospecific synthesis of methyl (6R)- and (6S)-6-deuterioshikimate have been recently reported by Birch and coworkers.[7,8] The approach involves tricarbonyliron complexes of methyl dihydrobenzoate. In the present work, the enantiospecific synthesis of shikimic acid of Fleet and Shing[1] has been adapted and includes an enzymic reduction step (Fig. 2). The main

advantage of the approach of Birch and coworkers[7,8] is that both (+) and (-) shikimic acids are obtained. Resolution of the initial complex is however necessary. In the method of Fleet and Shing since D-mannose is the starting material, the stereochemistry is already fixed. The enzymic reduction is a high yield very facile reaction.

RESULTS AND DISCUSSION

The detailed synthetic steps in the preparation of the deuteriated epi-shikimate and shikimates are outlined in figures 1 and 2. In all these syntheses, the key step was the enzymic reduction with horse liver alcohol dehydrogenase of a keto-functional group.

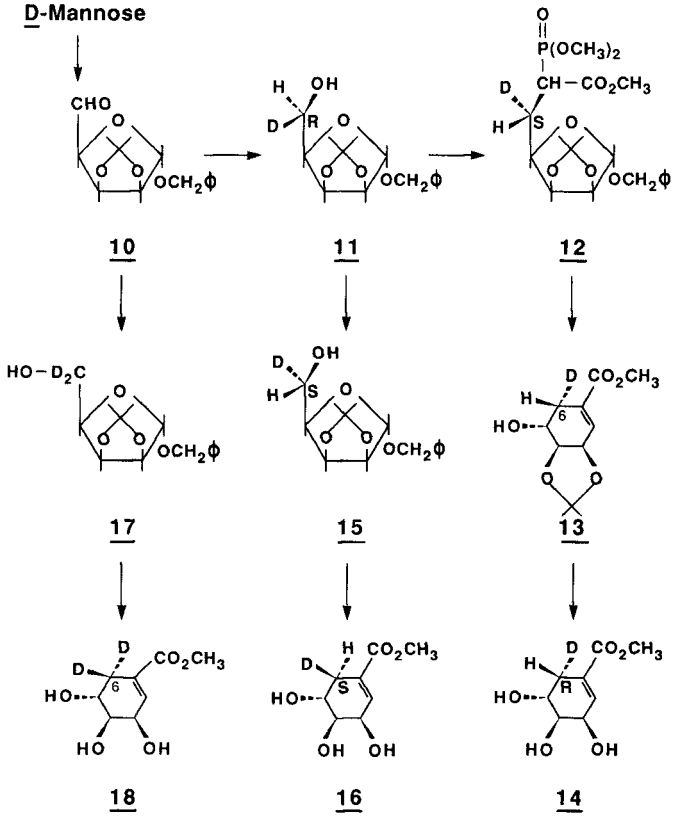


Fig. 2. Synthesis of methyl (6R)[6-^{2}H]-, (6S)[6-^{2}H]- and [6-^{2}H$_2$]shikimates

Synthesis of [5-^{2}H]-5-epi-shikimic acid (Fig. 1)

The commercially available (-)shikimic acid (1) was used as starting material for the synthesis of [5-^{2}H]-5-epi-shikimic acid (Fig. 1). A methanolic solution of shikimic acid was treated with chlorotrimethylsilane to give methyl shikimate which was then readily transformed to the corresponding 3,4-O-O-isopropylidene derivative (3, Fig. 1). The hydroxyl function at C-5 was oxidized with freshly prepared pyridinium chlorochromate to give the ketone 4.[6] Enzymic reduction of this ketone with horse liver alcohol dehydrogenase in ethanol-d_6 gave compound 5. Following the elimination of the protective groups, [5-^{2}H]-5-epi-shikimic acid was obtained in a 26% overall yield starting from shikimic acid.

Syntheses of methyl (6R)[6-^{2}H]-, (6S)[6-^{2}H]- and [6-$^2H_2$]shikimic acids (Fig. 2)

D-Mannose was the starting material of the three shikimic acids deuteriated at position 6. The aldehyde 10 was obtained from D-mannose following the procedure of Fleet and Shing.[1] The enzymic reduction with horse liver alcohol dehydrogenase in ethanol-d_6 gave the alcohol 11 . The chiral alcohol 11 was converted to methyl (6R)[6-^{2}H] shikimate 14 using the reactions reported for unlabeled shikimic acid .

In order to invert the chiral center containing the deuterium, alcohol 11 was reacted with trifluoromethane sulfonic anhydride, potassium acetate and sodium hydroxide to give the enantiomeric alcohol 15 . Using the procedure of Fleet and Shing for the subsequent reactions, compound 15 was converted to methyl (6S)[6-^{2}H] shikimate 16.

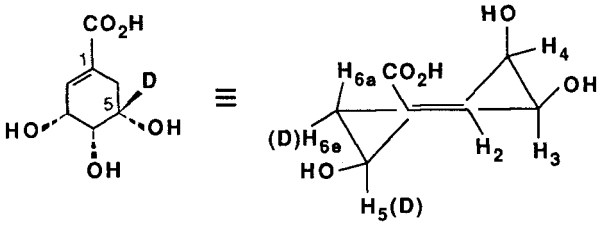
The aldehyde 10 was also oxidized to its corresponding carboxylic acid which was reduced with $LiAlD_4$ to the dideuteriated

alcohol 17. Compound 17 was converted to methyl [6-$^2H_2$] shikimate 18 by the same reactions used for compounds 14 and 16.

^{1}H-, ^{2}H- and ^{13}C-NMR analysis of [5-^{2}H]-5-epi-, (6R)[6-^{2}H]-, (6S)-[6-^{2}H]shikimic acids (Tables I-IV)

The ^{1}H, ^{2}H and ^{13}C-NMR spectra of the deuteriated shikimic and epi-shikimic acids at positions 6 and 5 respectively were acquired in CD_3OD. The incorporation of deuteriums at different positions were monitored by the disappearance of that proton in the ^{1}H-NMR spectrum, the appearance of a deuterium signal at proper chemical shifts in the ^{2}H-NMR and by the presence of a triplet in the ^{13}C-NMR spectra for the carbon coupled to deuterium. In [5-^{2}H]-5-epi-shikimic acid (Table I), the proton at position 5 has disappeared modifying the multiplicity

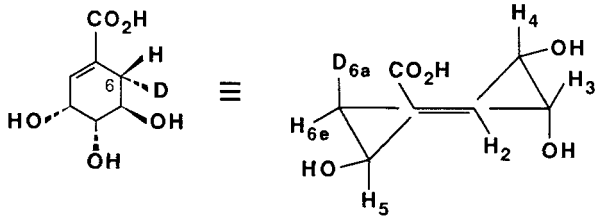
Table I. Proton and deuterium NMR analysis of [5-^{2}H]epi-shikimic acid



Position	ppm (δ)	^{1}H NMR	
		Multiplicity[a]	J (Hz)
2	6.67	t (br)	
3	4.32	q	$J_{2,3}$=2.7; $J_{3,4}$=3.6
4	3.95	d	$J_{4,3}$=3.6
5	----	----	----
5 (D)	3.69		
6a	2.35	dt	
6e	2.51	d (br)	$J_{e,a}$=-16.6
6e (D)	2.48		

a: s, d, t, q, m symbols represent the usual singlet, doublet, triplet, quartet and multiplet. The br symbol stands for broad line.

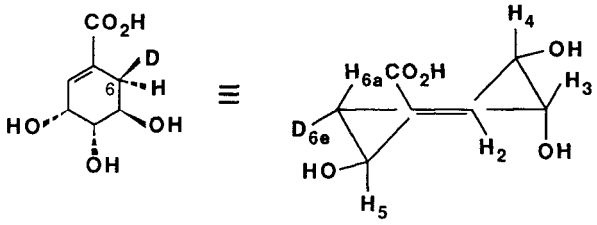
Table II. Proton and deuterium NMR analysis of (6R)[6-^{2}H]shikimic acid



Position	ppm (δ)	^{1}H NMR	
		Multiplicity	J (Hz)
2	6.79	dd	$J_{2,3}$=3.6;$J_{2,6e}$=1.8
3	4.36	td	$J_{3,2}$=3.6
4	3.98	dd	$J_{4,5}$=7.3;$J_{4,3}$=4.5
5	3.67	dd	$J_{5,4}$=7.3;$J_{5,6e}$=4.2
6e	2.66	m(br)	$J_{5,6e}$=4.2
6a(D)	2.20		

of the neighboring protons. The incorporation of deuterium at position 5 is complete. It is interesting to note that NMR analysis indicated that 50% deuterium has been incorporated at position 6 only on the lower field proton (the equatorial position). The higher field proton (axial position at 2.35 ppm) appears as an overlapping doublet of multiplet (for the non-deuteriated species) and as a broad singlet for the partly 6-deuteriated species. The deuteration of position 6 results probably from stereospecific enolisation of compound 4 in the presence of the enzyme horse liver alcohol dehydrogenase in ethanol-d_6. The ^{1}H NMR of the deuteriated methyl (6R)[6-^{2}H]- and (6S)[6-^{2}H]shikimates have been reported.[7,8] It agrees with our values for (6R)[6-^{2}H]- and (6S)[6-^{2}H]shikimic acid. In (6R)[6-^{2}H]shikimic acid, the most shielded proton at position 6 (2.20 ppm) has been selectively deuteriated as can be seen in the proton, deuterium and carbon-13 NMR spectra (Tables II, IV). The proton H_5 is coupled to the remaining H_6 (J = 4.2 Hz) showing that the H_6 proton occupies the pseudo-equatorial

Table III. Proton and deuterium NMR analysis of (6S)[6-^{2}H]shikimic acid



Position	ppm (δ)	^{1}H NMR	
		Multiplicity	J (Hz)
2	6.79	dd	$J_{2,3}$=3.6;$J_{2,6e}$=1.8
3	4.36	td(br)	
4	3.97	dd	$J_{4,5}$=7.3;$J_{4,3}$=4.5
5	3.67	dd	$J_{5,4}$=7.3;$J_{5,6a}$=5.5
6a	2.10	m(br)	
6e(D)	2.72		

Table IV. ^{13}C-NMR analysis of [5-^{2}H]epi-, (6R)[6-^{2}H]- and (6S)[6-^{2}H]shikimic acids (SA)

Carbon	Chemical Shift (ppm)		
	[5-^{2}H]Epi-SA	(6R)[6-^{2}H]-SA	(6S)[6-^{2}H]-SA
CO_2H	169.9	170.1	170.0
C_1	130.2	130.7	130.7
C_2	140.1	139.0	138.8
C_3	69.7	67.4	67.4
C_4	72.8	72.8	72.8
C_5	69.1; t(J_{CD}=20.1)[a]	68.4	68.4
C_6	29.7	31.3; t(J_{CD}=20.0)	31.4; t(J_{CD}=20.0)
C_6(D)[b]	29.4; t(J_{CD}=19.4)[a]		

a: The symbol t following some of the signals stands for triplet signals. These multiplets are due to J_{C-D}splitting (value found in parentheses).

b: The equatorial proton at position 6 is also partly deuterated.

position. In (6S)[6-^{2}H]shikimic acid, the most deshielded proton at position 6 (2.72 ppm), has been deuteriated selectively (Tables III, IV). The proton H_5 is coupled to the remaining H_6 (J = 5.5 Hz) demonstrating that the H_6 proton occupies the pseudo-axial position.

EXPERIMENTAL

Instrumentation

The NMR spectra were obtained on a Varian XL-300 spectrometer equipped with a proton probe and a 5mm broad band probe. The spectrometer was operated at 300 MHz for proton, 75.4 MHz for carbon and 46 MHz for deuterium. The samples (2 - 5 mg) were dissolved in $CDCl_3$ (in $CHCl_3$ for deuterium NMR) and their spectra were recorded at ambient temperature (22°C). Chemical shifts are reported in δ units. The solvents ($CDCl_3$) and ($CHCl_3$) used as an internal reference, were set at 77.0 ppm and 7.26 ppm, respectively. Routine NMR spectra were done on a Varian T-60 instrument. Melting points were obtained on a Büchi 510 melting point apparatus. Analytical thin-layer chromatography was performed using E. Merck glass supported silica gel 60 (F254, 0.25mm) plates. The compounds were visualized using a ceric sulfate/ammonium molybdate solution [for 1 L of developing agent: 100 mL of H_2SO_4 concentrated, 900 mL of water, 25 g of ammonium molydbate, 10g of $Ce(SO_4)_2$ (ceric sulfate)]. All reactions were conducted under a dry nitrogen atmosphere in flame- or oven-dried glassware. The following solvents were purified before use: CH_2Cl_2 and dimethylformamide (DMF) were distilled from CaH_2, toluene was distilled over P_2O5, methanol (MeOH) was distilled over Mg metal, and tetrahydrofuran (THF) was distilled over lithium aluminum hydride. All organic extracts were dried over anhydrous $MgSO_4$.

Synthesis of [5-^{2}H]-5-epi-shikimic acid

Methyl shikimate (2) A mixture of shikimic acid (1) (2g, 11.5 mmol) in dry methanol (80 ml) was treated with chlorotrimethylsilane (2.2

ml, 17.3 mmol), stirred overnight at room temperature and neutralized with Dowex 1 x 8 (OH^-). The resin was removed and the filtrate was concentrated under reduced pressure to yield 2.15 g of 2 (99.5%); mp 113-115°C (Lit[1] mp 115-116.5°C)

3,4-O,O-Isopropylidine-1-methoxycarbonyl-3α,4α,5β-trihydroxycyclohexene (3) A solution of methyl shikimate (2) (2.7 g, 14.3 mmol) in dry acetone (27 ml) treated with p-toluenesulfonic acid (270 mg) and 2,2-dimethoxypropane (8 ml, 65 mmol), was stirred at room temperature for 6h, neutralized ($NaHCO_3$) and concentrated in vacuo. The residue was extracted with ethyl acetate. The extract was washed with water and dried. The crude product was purified on silica[9] using ethyl acetate/hexane (1:1; v/v) as an eluant. The removal of the solvent gave 2.86 g (89%) of 3 as an oil: IR ($CHCl_3$) υ_{max}: 3500, 1710, 1650, 1060 cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz) δ: 1.36, 1.53 (2xs, 6H, $C(CH_3)_2$), 3.70 (s, 3H, OCH_3).

Methyl 3,4-O,O-isopropylidine-3α,4α-dihydroxy-1-cyclohexen-5-one-1-carboxylate (4) A mixture of dry pyridine (8.5 ml, 105.3 mmol), dry dichloromethane (100 ml) and chromium trioxide (4.7 g, 47 mmol) at 5°C, was stirred until (30 min) a deep-red solution was obtained. Compound 3 (2.6 g, 11.4 mmol) in dichloromethane (15 ml) was added to the chromium trioxide solution with stirring at 2-5°C. Acetic anhydride (4.45 ml, 47 mmol) was then added and stirred at 5°C for 2h. The solution was poured into cold ether (250 ml) filtered through celite, washed thoroughly (5% $NaHCO_3$ solution and saturated saline) and dried ($MgSO_4$). The ether was removed and any remaining acetic acid and pyridine were coevaporated with benzene. The residue was purified on silica eluting with ethyl acetate/hexane (3.5:6.5; v/v) to yield the title compound (1.59 g; 61%); mp 75-76°C; IR ($CHCl_3$) υ_{max}: 1730, 1700 cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz) δ: 1.45 (s, 6H, $C(CH_3)_2$), 3.96 (s, 3H, OCH_3).

3,4-O,O-Isopropylidine-1-methoxycarbonyl-3α,4α,5α-trihydroxy-[5-^{2}H] cyclohexene (5)

Method 1: To a solution of ketone 4 (880 mg, 3.9 mmol), in acetonitrile (5 ml), and 0.1 M potassium phosphate buffer (pH 8, 50 ml) was added ethanol-[$^2H_6$] (1 ml, 17 mmol), and NAD (80 mg, 0.11 mmol). The pH of the reaction mixture was readjusted to 8 with 0.1 N NaOH. Horse liver alcohol dehydrogenase (40 mg) was added and the solution was stirred at 20°C overnight, neutralized to pH 7 and extracted with ethyl acetate (5 x 60 ml). The extract was washed with saturated saline and dried ($MgSO_4$). After removal of ethyl acetate, the residue was purified on silica using ethyl acetate/hexane (4:6; v/v) to yield the title compound (480 mg, 55%); IR ($CHCl_3$) υ_{max}: 3540, 1705, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz) δ: 1.28 (s, 6H, C $(CH_3)_2$), 2.20 (2H, H-6), 3.86 (s, 3H, OCH_3).

Method 2: Ketone 4 (735 mg, 3.25 mmol) in ethyl alcohol (10 ml) was reduced by sodium borodeuteride (143 mg, 3.42 mmol) at 0-5°C for 1h, neutralized with 1 N HCl and extracted with ethyl acetate. The ethyl acetate extracts were washed with water, dried ($MgSO_4$) and concentrated. The residue was purified on silica eluting with ethyl acetate/hexane (4:6; v/v) to give the title compound (653 mg, 88%). This compound was identical, spectroscopically and chromatographically, with the compound prepared by method 1.

3,4-O, O-Isopropylidine-5-acetyl-1-methoxycarbonyl-3α,4α,5α-trihydroxy[5-^{2}H] cyclohexene (6) Alcohol (5) (1.47 g, 6.4 mmol) in dry dichloromethane (20 ml) containing 4-dimethylaminopyridine (0.78 g, 6.4 mmol) was treated with acetic anhydride (2.43 ml, 22 mmol). The mixture was stirred at r.t. overnight, poured into ice-water and extracted with dichloromethane which was then washed with water, dried ($MgSO_4$) and evaporated. The oil was chromatographed on silica eluting with ethyl acetate/hexane (3:7; v/v). This gave the title compound as a colourless oil (1.37 g, 79%); IR ($CHCl_3$) υ_{max}: 1735, 1720, 1655 cm^{-1};

^{1}H NMR ($CDCl_3$, 60 MHz) δ: 1.40 (s, 6H, $(CCH_3)_2$), 2.10 (s, 3H, $C(O)CH_3$), 3.80 (s, 3H, OCH_3).

5-Acetyl-1-methoxycarbonyl-3α,4α,5α-trihydroxy[5-^{2}H] cyclohexene (7) A mixture of compound 6 (1.37 g, 5.05 mmol), amberlyst 15 (H^+) ion-exchange resin (1 g) and methanol (40 ml) was stirred at r.t. for 50h and was then filtered to remove the resin. The filtrate was concentrated to yield the title compound (1.07 g, 92%) as an oil; IR ($CHCl_3$) υ_{max}: 3450, 1715, 1648 cm^{-1}; ^{1}H NMR ($CDCl_3$, 60 MHz) δ: 2.12 (s, 3H, $C(O)CH_3$), 3.92 (s, 3H, OCH_3).

[5-^{2}H]-5-Epi-shikimic acid (8) Compound 7 (1 g, 4.3 mmol) in methanol (51 ml), was hydrolyzed by 1 N NaOH (21.6 ml) (at 5°C for 15 min and at room temperature for 2h) and neutralized with amberlite 1R 120(H^+) ion-exchange resin (5.2 g). The resin was removed by filtration and the methanol solution was concentrated under reduced pressure. The residue was freeze dried to give the title compound (0.7 g, 93%).

Methyl [5-^{2}H]-5-epi-shikimate (9) To a methanolic solution (10 ml) of epi-shikimic acid (10 mg) was added ethereal diazomethane until the solution remained yellow. After 20 min, the solvents were removed at reduced pressure to afford the title compound; mp 110-113°C.

Synthesis of (6R)[6-^{2}H] shikimic acid

Benzyl 2,3-O-isopropylidene-(5R)[5-^{2}H]-α-D-lyxofuranoside (11) A solution of compound 10[10] (2.23 g, 8 mmol) in acetonitrile (50 ml) was mixed with 0.1 M aqueous potassium phosphate buffer of pH 8.5 (450 ml). Ethanol-[$^2H_6$] (2 g, 34 mmol), NAD (240 mg, 0.33 mmol) and horse liver alcohol dehydrogenase (120 mg) were added. The resulting emulsion was stirred at room temperature for 96h, neutralized with 1N

HCl and extracted with ethyl acetate (5 x 80 ml). The extract was washed with saturated saline, dried ($MgSO_4$) and concentrated to yield compound 11 (2 g, 89%); mp 81-82°C (Lit[10] mp 87- 87.5°C); IR ($CHCl_3$) υ_{max}: 3520, 1080 cm^{-1}.

Benzyl 2,3-O-isopropylidene-(5R)[5-^{2}H]-5-O-trifluoromethylsulfonyl-α-D-lyxofuranoside. Compound 11 (1 g, 3.5 mmol) in dry dichloromethane (8 ml) was treated with pyridine (0.65 ml, 8.4 mmol). At -25°C a solution of trifluoromethanesulfonic anhydride (0.78 ml, 4.6 mmol) in dichloromethane (4 ml) was added. The reaction was allowed to proceed for 2h at -25°C. Ice-water (2 ml) was then added and the organic layer was washed with water, dried ($MgSO_4$) and evaporated to yield the title compound (1.35 g, 91%) as a syrup which was very unstable. The crude compound was used directly in the next reaction.

Benzyl 2,3-O-isopropylidene-(5S)[5-^{2}H]-5-trimethylphosphonoacetate-α-D-lyxofuranoside. A mixture of sodium hydride (295 mg, 7.37 mmol), 18-crown-6 (199 mg, 0.75 mmol) and DMF (15 ml) was treated with trimethylphosphonoacetate (1.18 ml, 7.3 mmol) at 5°C, and then allowed to warm to 10-15°C for 1h. A solution of the above triflate (2.13 g, 4.78 mmol) in DMF (4 ml) was then added and the reaction mixture was stirred at 50°C for 5h and then poured into ice-water (10 ml) and extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$) and evaporated to give a syrup which was purified on silica eluting with ethyl acetate/hexane (9:1; v/v). A mixture of diastereoisomeric phosphonates was obtained (1.5 g, 65%); IR ($CHCl_3$) υ_{max}: 1715 cm^{-1}.

2,3-O-isopropylidene-(5S)[5-^{2}H]-5-trimethylphosphonoacetate-α-D-lyxofuranoside. A solution of compound 12 (3 g, 6.7 mmol) in methanol (100 ml) and 1N HCl (1 ml) was stirred in the presence of 10% palladium-charcoal (0.5 g) for 48h at a slight overpressure of

hydrogen. The catalyst was filtered off and washed thoroughly with methanol. The combined methanol solution was concentrated to afford the desired lactol (2.08 g, 87%); IR ($CHCl_3$) υ_{max}: 3350, 1735, 1670 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.22, 1.32, 1.33, 1.34 (4 Me singlets).

Methyl 3,4-O-isopropylidene-(6R)[6-^{2}H]shikimate (13) The above lactol (107 mg, 0.3 mmol) in dry methanol (2 ml) was treated with methanolic sodium methoxide (1 N, 1 ml) at 5°C. The reaction mixture was stirred for 2.5 h at r.t., neutralized with acetic acid and extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$) and evaporated. The crude compound was purified on silica eluting with ethyl acetate/hexane (1:1; v/v) to afford the title compound (28 mg, 41%) as an oil; IR ($CHCl_3$) υ_{max}: 3565, 3440, 1700, 1638 cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ: 1.40 (s, 6H, $C(CH_3)_2$), 3.73 (s, 3H, OCH_3).

Methyl (6R)[6-^{2}H] shikimate (14) A mixture of compound 13 (456 mg, 2 mmol), amberlyst 15 (H^+) ion-exchange resin (500 mg) and methanol (50 ml) was stirred at room temperature for 96h. The resin was filtered off and the methanol solution was removed under reduced pressure to get the title compound (335 mg, 89%); mp 111-113°C (Lit[1] mp 115-116.5°C); IR (Nujol)υ_{max}: 3320, 1715, 1656 cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD) δ: 2.69 (s, 1H, H-6), 3.71 (dd, 1H, H-5, J = 6.0 Hz), 3.79 (s, 3H, OCH_3), 4.00 (dd, 1H, H-4, J = 7.0 Hz), 4.40 (m, 1H, H-3), 6.8 (m, 1H, H-2).

(6R)[6-^{2}H] Shikimic acid. A solution of methyl (6R)[6-^{2}H]shikimate (350 mg, 1.9 mmol) in methanol (17 ml) was treated with 1N NaOH (4.7 ml). The reaction mixture was stirred for 4 h. at 5-10°C and neutralized with amberlite IR 120 (H^+). After filtration, the methanol was evaporated and the residue was freeze dried to get the title compound (320 mg, 96%).

Synthesis of methyl (6S)[6-^{2}H]shikimate.

In order to prepare (6S)[6-^{2}H]shikimic acid, the chiral center of the triflate of compound 11 was inverted. The triflate (3.87 g, 9.4 mmol), dissolved in 50 ml MeOH, was refluxed with potassium acetate (4.59 g; 46.8 mmol) for 1h 45 min. The methanol was evaporated and the residue was diluted with ether which was washed with brine, dried ($MgSO_4$) and evaporated to yield 2.31 g of a crude compound.

The crude acetate was hydrolyzed with 1N NaOH (5 ml) in 100 ml MeOH at room temperature for 2h 30 min. The solution was neutralized with 1N HCl (4 ml) and evaporated to dryness. The residue was extracted with ethyl acetate, washed with brine, dried ($MgSO_4$) and evaporated. Purification on silica with ethyl acetate/hexane (1:2; v/v) gave 1.029 g (39% overall yield) of the opposite isomer 15.

Isomer 15 was transformed to methyl (6S)[6-^{2}H] shikimate (16) similarly to the procedure used to prepare methyl 6R[6-^{2}H]shikimate (14) from isomer 11.

Synthesis of methyl [6-$^2H_2$] shikimate

Benzyl 2,3-O-isopropylidene-[5-$^2H_2$]-α-D-lyxofuranoside (17) A solution of aldehyde 10 (12.24 g, 39.5 mmol) in a mixture of acetone:water (900 ml: 48 ml) was treated with a solution of $KMnO_4$ (9.35 g, 59.2 mmol) in water (170 ml), at room temperature for 2h. The mixture was filtered through celite to remove the MnO_2 salts. The filtrate was concentrated, refiltered and evaporated to dryness. The potassium carboxylate was dissolved in water, acidified to pH 3 with amberlite IR 120 (H^+) and the carboxylic acid obtained was extracted into ethyl acetate which was dried ($MgSO_4$) and evaporated to give 7.91 g (64% yield) of the desired compound.

The above carboxylic acid (1.34 g, 4.6 mmol) dissolved in MeOH (30 ml) was treated with chlorotrimethylsilane (0.86 ml, 6.8 mmol) for

3h at r.t., neutralized with dilute NH_4OH and evaporated to dryness. The residue was extracted with ether and washed with water to neutrality. The volatiles were dried ($MgSO_4$) filtered and evaporated. The residue was purified on silica using petroleum ether:ethyl acetate (10:3). The methyl ester was obtained (0.93 g, 69%).

To a solution of $LiAlD_4$ (0.52 g, 12.4 mmol) in 100 ml dry THF at 0°C, was added a solution of the above methyl ester (3.81 g, 12.4 mmol) dissolved in THF (30 ml). The reaction mixture was stirred at 0°C for 1h and quenched with MeOH. The mixture was concentrated in vacuo. The salts were dissolved in ether, filtered through celite and the filtrate was evaporated to dryness. Purification with silica using petroleum ether:ethyl acetate (2:1) gave the dideuteriated alcohol 17 (3.02 g, 79%).

Alcohol 17 was transformed to methyl [6-$^2H_2$]shikimate (18) similarly to the preparation of methyl (6R)[6-^{2}H]shikimate (14) and methyl (6S)[6-^{2}H]shikimate (16) from alcohols 11 and 15, respectively.

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