Synthesis of (−)-Biopterin Using (S)-Ethyl Lactate as a Starting Material

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(−)-Biopterin was synthesized from (1S,2S)-1-(1,3-dithian-2-yl) propane-1,2-diol 5 (= C), which was derived from commercially available (S)-ethyl lactate. Diol 5 (= C) was converted to 15 through a six-step sequence. Ketone 15 was submitted to condensation with 3,5,6-triaminopyrimidinol (TAP, 2), and followed by oxidation to afford isopropylidenebiopterin (16). Finally, 16 was deprotected to give (−)-biopterin (1).

(−)-Biopterin is one of the potent natural pterins isolated from human urine as the growth factor of Crithidia fasciculata by Patterson et al.1) Its 5,6,7,8-tetrahydro form, which can easily be prepared by hydrogenating (−)-biopterin in a basic solvent,2) is known as the coenzyme of aromatic amino acid hydroxylase,3−6) and has attracted much attention in connection with its therapeutic potential for several central nervous system (CNS) disorders such as Parkinson’s disease and depression.7)

Since the first synthesis of (−)-biopterin by Patterson et al. in 1956,8) several reports have appeared to date.9−14) However, the strategies which were employed in those syntheses mostly involved 5-deoxy-L-arabinose as an intermediate.

5-Deoxy-L-arabinose in turn was obtained by the degradation of L-rhamnose, which is a rare sugar and difficult to obtain at a low price for industrial use. The syntheses not involving 5-deoxy-L-arabinose gave either biopterin of low optical rotation10) or that of a racemic form.11) We report here a synthesis of (−)-biopterin employing a non-sugar material, commercially available (S)-ethyl lactate.

Our synthetic plan is shown in Fig. 1. The strategy is to employ diol C as the key intermediate to construct a chiral side chain

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\text{(−)-Biopterin I} \quad \text{TAP 2} \quad \text{A} \quad \text{B} \quad \text{C} \quad \text{D}
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Fig. 1.

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portion A. Hydroxy ketone A will be derived from carboxylic acid B, which will be obtained from C. This diol C has been prepared from diketone D by means of a reduction process with baker's yeast. Construction of the pterin skeleton will be performed by condensing A with 3,5,6-triaminopyrimidinol (2). The first stage of our synthesis was to prepare anti-diol C according to Fujisawa's procedure.

Reduction of 1-(1,3-dithian-2-yl)propane-1,2-dione (D) with baker's yeast gave C in a 82% yield on a small scale. However, on a preparative scale (over 10 g), C was obtained in only a 34% yield, and a hydroxy ketone (4b) was the main product of this reduction. Therefore we had to amend our first plan.

We chose a route which involved the hydride reduction of 1-(1,3-dithian-2-yl)-2-hydroxypropan-1-one (4b) to afford diol C as depicted in Fig. 2. Commercially available (S)-ethyl lactate (3a) was converted to the corresponding THP ether (3b), and treated with 2-lithio-1,3-dithiane. The resulting dithianyl ketone (4a) was deprotected to give 2-hydroxy-1-(1,3-dithian-2-yl) propan-1-one (4b) in a 28% yield from 3a. Recrystallization of 4b yielded colorless needles, mp 91~92°C, [α]D25 +100° (c= 1.10, CHCl3). [lit.15) mp 90°C, [α]D23 +98.4° (c= 1.01, CHCl3)].

The selectivity of the subsequent hydride reduction of ketone 4b has been reported as anti : syn = 64 : 36.15) Accordingly, we tried several reaction conditions in order to improve this selectivity.

After several trials, we finally found that 4b led to diols 5 and 5' in a ratio of 5:1 when treated with sodium borohydride at -15°C in a methanol–water (10:1) solution. The crude product was recrystallized from hexane–ethylacetate (3:2) to give diastereomerically pure anti-diol 5 in a 54% yield from 4b, mp 92~94°C, [α]D25 +11.5° (c=1.10, CHCl3) [lit.15) mp 93°C, [α]D23 +10.5° (c=1.01, CHCl3)]. This method provided a convenient way to afford 5 without employing reduction with baker's yeast.

The second stage of our synthesis was the conversion of 5 (=C) into hydroxy ketone A. Diol 5 was protected as the cyclohexylidene acetal, which was submitted to dethioacetalization according to Fetizon's procedure, and a subsequent Jones oxidation afforded carboxylic acid 7 in a 56% yield from 5. Acid 7 was treated with thionyl chloride in refluxing chloroform and then by diazomethane to give a diazoketone accompanied by a small amount of a chloroketone. After purifying by column chromatography, the diazoketone was treated with acetic acid, acetic anhydride and potassium acetate in the presence of copper(II)
Synthesis of (-)-Biopterin acetate to yield an acetoxy ketone (8), which was a precursor of A, as a colorless oil in a 55% yield. The final stage of synthesis was the construction of a pterin skeleton by condensing A and 3,5,6-triaminopyrimidinol (2). At first, the hydrolysis of 8 by 1 N sodium hydroxide in methanol gave A in situ. This, without isolation, was submitted to a reaction with 3,5,6-triaminopyrimidinol (2) under Viscontini’s conditions. However, after several trials, the reaction gave no desirable product, but afforded instead an unknown more polar substance. This was due to the steric hindrance caused by the cyclohexylidene group, and we therefore changed the protecting group to a less bulky isopropylidene group.

As illustrated in Fig. 3, Diol 5 (=C) was protected as an isopropylidene acetal. Although the dethioacetalization of 6 afforded an aldehyde in an excellent yield, acid-sensitive isopropylidene acetal 11 gave 12 in a 67% yield. Aldehyde 12 was immediately oxidized with Jones reagent to provide 13 in a 65% yield. Thus, the obtained 13 was the optically active form of the intermediate, which has been reported for racemic biopterin by Viscontini et al. Accordingly, the transformation of 13 into (-)-biopterin by a six-step sequence was effected by their procedure. Acid 13 was treated with thionyl chloride and then by diazomethane to afford a diazoketone (14) as the major product contaminated with a small amount of acid anhydride. Because it was difficult to separate the mixture by column chromatography, it was immediately converted to a mixture of the corresponding acetoxy ketone (15) and acid anhydride. After chromatographic separation, 15 was obtained in a pure form in a 60% yield. The construction of the pterin skeleton was achieved by treating A, which had been generated in situ by the hydrolysis of 15, with 2 to afford isopropylidenebiopterin (16) in a 21% yield, mp > 300°C, [α]_D^21 - 114.7° (c = 0.10, 0.1 N NaOH). Finally, the hydrolysis of 16 in a 20% AcOH solution gave the desired (-)-biopterin (1) in a 78% yield, mp > 300°C, [α]_D^24 - 67.2° (c = 0.1, 0.1 N HCl). In summary, a synthesis of (-)-biopterin which does not employ L-rhamnose as a starting material was accomplished, starting from commercially available (S)-ethyl lactate.

**Experimental**

All melting points (mp) and boiling points (bp) are uncorrected. IR spectra were measured on a JASCO IRA-102 spectrometer. 1H-NMR spectra were recorded with TMS as an internal standard at either 60 MHz on a HITACHI R-24A spectrometer or at 200 MHz on a JEOL JNM FX-200 spectrometer in CDCl₃ and with 3-(trimethylsilyl)propionic-d₄ acid sodium salt as an internal standard when DCl-D₂O or NaOD-D₂O solution was used as the solvent at 200 MHz on a JEOL JNM FX-200 spectrometer. Optical rotations were measured on a JASCO DIP-140 polarimeter. HR-mass spectra were mea-
sured on a HITACHI M-80 spectrometer at 70 eV. Merck kieselgel 60 Art 7734 and cellulose powder (100–200 mesh) were used for column chromatography. HPLC analyses were performed on a Nucleosil® 50-5 (25 cm × 4.6 mm) column by detecting at 254 nm.

(2S)-Ethyl 2-tetrahydropyranyloxypropionate (3b). To a solution of (S)-ethyl lactate 3a (101.0 g, 0.881 mol) in dry CH₂Cl₂ (300 ml) was added 2.3-di-hydropropyrene (82.0 g, 0.976 mol) and PPTS (1.00 g, 4 mmol) at room temperature. The mixture was stirred overnight at room temperature, before it was washed with sat. Na₂CO₃ solution and sat. brine, and dried over MgSO₄. The oily layer was concentrated in vacuo. The residue was chromatographed over SiO₂ to give 4b as colorless needles, mp 91-92°C, [α]ν₂ +11.5° (c = 1.09, CHCl₃). 100% d.e., as estimated by HPLC analysis (hexane:CH₂Cl₂:isoPrOH = 700:200:100, 1 ml/min, 254 nm). IR vmax (KBr) cm⁻¹: 3450 (s), 2920 (w), 1270 (m), 1200 (s), 1130 (s), 1022 (s), 985 (s).

1H-NMR (200 MHz): 1.32 (d, 3H, J=7Hz), 1.36, 1.51 (two s, 3H), 4.24 (dd, 1H, J=9Hz, J =5Hz), 4.34 (q, 1H, J=5Hz).

Anal. Found: C, 59.02; H, 8.88.

Calcd. for C₇H₁₄O₂S₂: C, 59.36; H, 8.98%.

(2S)-2-Hydroxy-1-(1,3-dithian-2-yl)propan-1-one (4a). A solution of 1,3-dithiane (56.0 g, 0.467 mol) and PPTS (1.00 g, 4 mmol) at room temperature was stirred overnight at room temperature. The mixture was extracted with CH₂Cl₂, and the extract was concentrated in vacuo. The residue was extracted with CH₂Cl₂, and the extract was washed with sat. Na₂CO₃ solution and sat. brine, dried over MgSO₄ and concentrated in vacuo. The oily residue was distilled under reduced pressure in the presence of Na₂CO₃ to afford 3b (154.1 g, 88.6%) as a colorless oil, bp 86–88°C (1 mmHg). nD²⁰ 1.4342. IR ν₃max (film) cm⁻¹: 2920 (s), 1710 (s), 1438 (m), 1372 (m), 1270 (m), 1200 (s), 1130 (s), 1022 (s), 985 (s).

1H-NMR (60 MHz): 1.28, 1.40 (two t, 3H, J=6Hz), 1.45 (d, 3H, J = 6Hz), 1.5-2.1 (br., 6H), 3.2-4.0 (br., 2H), 4.20 (m, 3H), 4.68 (br. s, 1H). Anal. Found: C, 59.02; H, 8.88.

Calcd. for C₁₂H₂₀O₃S₂: C, 52.15; H, 7.74%.

(2S,2S)-1-(1,3-Dithian-2-yl)propane-1,2-diol (5). A solution of 4b (17.0 g, 0.089 mol) in a mixed solvent of CH₃OH (300 ml) and water (30 ml) was cooled at ~15°C. To the solution was added NaBH₄ (3.00 g, 0.079 mol) portionwise over a 30 min period. After stirring for 1 hr, the temperature was raised to room temperature. The mixture was concentrated in vacuo, and the residue was extracted with CHCl₃. The extract was dried over MgSO₄ and concentrated in vacuo to afford a colorless solid. The crude product was recrystallized from a mixed solvent of CHCl₃ and hexane to give 5 (9.33 g, 54.0% yield) as colorless needles, mp 92–94°C, [α]ν₃ +11.5° (c = 1.09, CHCl₃). 100% d.e., as estimated by HPLC analysis (hexane:CH₂Cl₂:isoPrOH = 700:200:100, 1 ml/min, 254 nm). IR ν₃max (KBr) cm⁻¹: 3430 (s), 2920 (s), 1270 (m), 1200 (s), 1130 (s), 1055 (s), 910 (s), 775 (s).

1H-NMR (200 MHz): 1.26 (d, 3H, J = 6Hz), 1.82 (br. s, 1H), 2.44 (br. s, 1H), 2.7-3.1 (m, 4H, 3.87 (t, 1H, J = 5Hz), 4.00 (d, 1H, J = 7Hz), 4.09 (br., 1H). Anal. Found: C, 43.7; H, 7.11. Calcd. for C₁₀H₁₈O₄S₂: C, 43.28; H, 7.76%.

(4S,5S)-4-(1,3-Dithian-2-yl)-2,2,5-trimethyl-1,3-dioxolane (11). A solution of 5 (8.70 g, 45.0 mmol) and p-TsOH (0.05 g, 0.3 mmol) in 2,2-dimethoxypropane (35 ml) was heated at 70°C for 30 min. The mixture was diluted with ether, washed successively with sat. NaHCO₃ solution, water and sat. brine, dried over MgSO₄ and concentrated in vacuo. The residue was chromatographed over SiO₂ to give 11 (10.2 g, 96.9%) as a colorless oil, nD²⁰ 1.5287, [α]ν₂ +18.4° (c = 0.96, CHCl₃). IR ν₃max (film) cm⁻¹: 2950 (s), 1422 (m), 1380 (s), 1250 (s), 1220 (s), 1170 (m), 1075 (s), 1010 (s), 910 (w), 865 (m), 1H-NMR (200 MHz): 1.32 (d, 3H, J = 7Hz), 1.36, 1.51 (two s, 3H), 1.8–2.2 (m, 2H), 2.90 (m, 4H), 4.07 (d, 1H, J = 9Hz), 2.44 (dd, 1H, J = 9Hz, J' = 5Hz), 4.34 (q, 1H, J = 5Hz). Anal. Found: C, 51.03; H, 7.57. Calcd. for C₁₇H₁₂O₂S₂: C, 51.25; H, 7.74%.

(2S,3S)-2,3-O-Isopropylidene-2,3-dihydroxybutanoic acid (13). To a solution of 11 (10.0 g, 42.7 mmol) in a mixed solvent of CH₃CN (160 ml) and water (16 ml) was added CaCO₃ (42.0 g, 0.42 mol) and CHJ₃ (60.0 g, 0.423 mol). The mixture was stirred vigorously for 1.5 hr under reflux, cooled to room temperature, and then dried over MgSO₄. After filtration, the residual salt was washed with ether. The combined organic layer was washed with sat. brine, dried over MgSO₄ and concentrated in vacuo. The residue was extracted with CH₂Cl₂, and the extract was chromatographed over Florisil to afford 12. This was immediately submitted to the next oxidation.

To a cooled solution of 12 (4.55 g, 28.4 mmol) in acetonitrile (70 ml) was added Jones reagent (10 ml) at 0°C. After stirring for 20 min, to the mixture was added iso-ProH
(25 ml) and NaHCO3 to adjust the pH to 4. The mixture was filtered, the residue was washed with acetone, and the filtrate was concentrated in vacuo. The crude product was chromatographed over SiO2 to give 13 (2.98 g, 43.5%) as a pale yellow oil, n2£ 1.4343, [α]D20 +0.25° (c=1.56, CHCl3). IR νmax (film) cm⁻¹: 3450 (m), 3170 (m), 3000 (w), 2600 (s), 1730 (s), 1380 (s), 1218 (s), 1090 (s), 850 (m). 1H-NMR (60 MHz): 1.25 (d, 3H, J=7Hz), 3.16, 1.57 (two s, 6H), 4.3-4.7 (m, 1H), 4.52 (d, 1H, J=4Hz), 9.28 (br.s, 1H). HR-MS: Found: C10HnO5: C, 55.33; H, 7.32. Calcd. for C10HnO5: C, 55.55; H, 7.49%.

(3S,4S)-3,4-O-Isopropylidene-3,4-dihydroxy-1-di-azopentan-2-one (14). To an ice-cooled solution of 13 (130 g, 7.39 mmol) in CHC13 (26 ml) was added a solution of SOCl2 (1.00 ml, 13.7 mmol) in CHC13 (3 ml) over a 10 min period, before the temperature was raised to 70°C. The mixture was stirred for 2 hr. It was then concentrated in vacuo, and the obtained red-yellow oil was dissolved in ether (11 ml). To the solution was added an ether solution of CH3N2, which was generated from nitrosomethylurea (7.0 g), over a 10 min period. Stirring was continued for 30 min, and the mixture was concentrated in vacuo. The crude product was chromatographed over SiO2 to give 14 (0.80 g, 58.9%) as a yellow oil, n2£ 1.4725, [α]D20 -128.9° (c=1.41, CHCl3). IR νmax (film) cm⁻¹: 3000 (m), 2110 (s), 1750 (s), 1620 (s), 1540 (s), 1290 (m), 1260 (m), 1210 (s), 1060 (s), 855 (m). 1H-NMR (200 MHz, 3n NaOD, TMSCH2CH2COONa-d4): 1.17 (d, 3H, J=6Hz), 1.70 (two s, 6H), 4.45 (m, 2H), 5.75 (s, 1H). HR-MS. Found: 180.0847. Calcd. for C12H15O3N5: 180.0845.

(3S,4S)-3,4-O-Isopropylidene-1-acetoxy-3,4-dihydroxypentan-2-one (15). To a solution of 14 (0.75 g, 4.08 mmol) in AcOH (7.5 ml) and Ac2O (0.38 ml) was added 1 n KOH solution (0.5 ml) under an Ar atmosphere. The mixture was stirred for 2 hr. It was then concentrated in vacuo. The residue was extracted with ether, and the extract was filtered, the residue was washed with acetone, and the filtrate was concentrated in vacuo. The residue was extracted with toluene and concentrated in vacuo, before it was diluted with toluene and concentrated in vacuo. The solution was added to an ice-cooled solution of 15 (0.20 g, 0.93 mmol) in CH3OH (36 ml) containing one drop of 2-mercaptoethanol. The pH of the mixture was adjusted to 7.4 with dil. AcOH solution. After the mixture was heated at 70–80°C for 18 hr under an Ar atmosphere, it was concentrated in vacuo. The residue was dissolved in water (30 ml), and into the solution was bubbled air for 18 hr at room temperature. To the solution was added cellulose powder (1.0 g) and iso-ProOH (5 ml), before the mixture was concentrated in vacuo. The residue was chromatographed over cellulose. Elution with a mixed solvent of iso-ProOH and ammonia water (4:1) gave a pale yellow solid. This was washed with acetone, CH3OH, water and acetone, and dried at 60°C for 4 hr to yield 16 (53.8 mg, 20.4%) as a pale yellow solid, mp >300°C, [α]D20 -114.7° (c=0.10, 0.1 n NaOH). IR νmax (KBr) cm⁻¹: 3450 (m), 3250 (m), 1682 (s), 1540 (m), 1375 (m), 1252 (m), 1100 (m), 862 (m). 1H-NMR (200 MHz, 3 n NaOD, TMSCH2CH2COONa-d4): 0.83 (d, 3H, J=6Hz), 1.53, 1.70 (two s, 6H), 4.8–6.5 (m, 2H), 8.62 (s, 1H). Anal. Found: C, 52.14; H, 5.33, N, 24.66. Calcd. for C12H15O3N5: C, 52.00; H, 5.45; N, 25.27%.

(-)-Biopterin (1). A suspension of 16 (51.0 mg, 0.18 mmol) in 20% AcOH solution (7 ml) was heated at 100°C for 30 min. The yellow solution was concentrated in vacuo, and the pH of the solution was adjusted to 4.0 with Na2CO3 solution. The solution was left to stand at 0°C overnight. The precipitate was filtered and washed with ice-cooled water, ethanol and ether, and then dried at 80°C for 6 hr. (-)-Biopterin was obtained as a pale yellow amorphous solid (33.2 mg, 77.8%), mp >300°C, [α]D20 -67.2° (c=0.2, 0.1 n HCl). IR νmax (KBr) cm⁻¹: 3440 (s), 3270 (s), 2800 (w), 1720 (w), 1680 (s), 1535 (m), 1412 (w), 1292 (m), 1125 (m), 1050 (w), 880 (w). 1H-NMR (200 MHz, 3n DCl, TMSCH2CH2COONa-d4): 1.17 (d, 3H, J=6Hz), 4.22 (q, 1H, J=6Hz), 4.94 (d, 1H, J=5Hz), 8.96 (s, 1H). Anal. Found: C, 52.00; H, 4.91; N, 28.44%.

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