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Synthesis of β-Hydroxywybutines, the Most Probable Alternatives for the Hypermodified Base of Rat Liver Phenylalanine Transfer Ribonucleic Acid†

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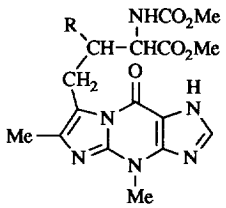
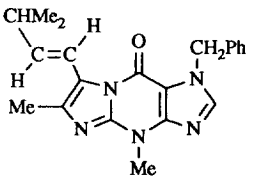
Abstract: Deoxygenation of the 1,2-glycol (±)-**5** was achieved at the position adjacent to the heterocycle through the cyclic carbonate (±)-**14**, providing the monohydroxy compound **6**. This new method of regioselective deoxygenation was employed for the first synthesis of β-hydroxywybutines [[*R*-(*R**,*S**)]- and [*S*-(*R**,*R**)]-**2**]: oxidation of the methyl butenoate **7** with osmium tetroxide, followed by deoxygenation through the cyclic carbonates (**19** and **20**), afforded the two diastereomers of **2**.

Many eukaryotic phenylalanine transfer ribonucleic acids (tRNAs^{Phe}) have fluorescent components at the position next to the 3'-end of the anticodon.[1] The fluorescent base isolated from chicken,[2a] rat,[2a] and bovine liver[2a,b] tRNAs^{Phe} was first reported to be **3** on the basis of comparison of the UV, fluorescent, and MS spectra as well as the chromatographic behavior with those of **1**, the structurally related precedent from yeast tRNA^{Phe}.[3] The base from the plant *Lupinus luteus* was also characterized as **3**.[2c] In this case, the presence of the hydroperoxy group was supported by a specific color test employing ferrous thiocyanate. The structure **3** was suggested to be assigned to the base from wheat germ tRNA^{Phe},[2c] because it had been shown to be indistinguishable from that of beef.[4] Kasai *et al.*, however, reported that the fluorescent base from rat liver tRNA^{Phe} was **2** on the basis of the MS spectral data as well as the negative coloring test for the hydroperoxy group.[5] These authors proposed that **3** might be an artifact formed during storage of the sample of **2**, and suggested that the base from wheat germ tRNA^{Phe} was also **2**. Notwithstanding this report, Mochizuki *et al.* preferred **3** for the fluorescent base isolated from the aquatic fungus *Geotrichum candidum* tRNA^{Phe}.[6] Wiewiórowski's group also reported that the base from tRNAs^{Phe} of wheat germ, yellow lupin seeds, and maize seeds was **3**. They described that **3** was very unstable and decomposed to **2** and **1**:[7] the stability observed for **2** and **3** contradicted that reported by Kasai *et al.*[5] As these bases are accessible from tRNAs^{Phe} in extremely minute quantities, unambiguous solution of the structural problems described above as well as the

† This paper is dedicated to Professor Dr. Tozo Fujii on the occasion of his retirement from Kanazawa University, where he has held the Chair of Synthetic Organic Chemistry since 1967, on March 31, 1995. We have learned a lot from him.



1: R = H
2: R = OH
3: R = O_2H



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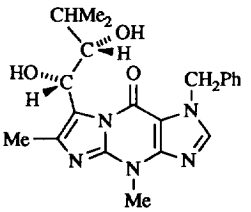
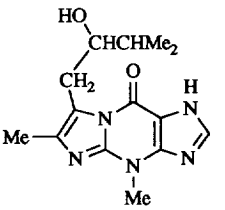
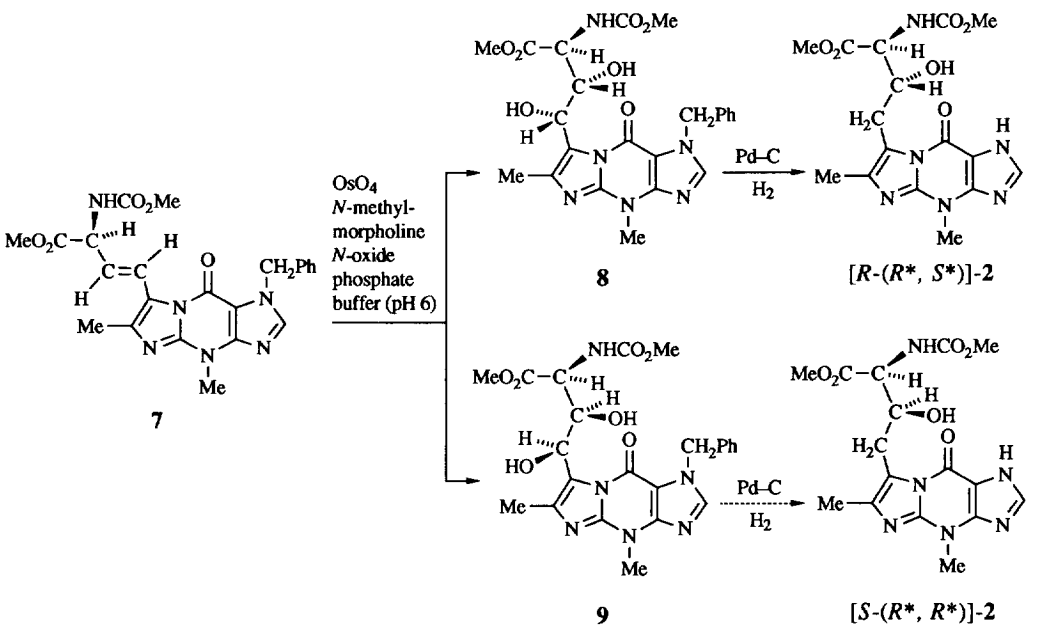


(±)-**5**



6

stereochemistry has to await chemical synthesis. Because *S* configuration has been assigned to wybutine, the fluorescent base from yeast $tRNA^{Phe}$,[1,8] we envisioned [*R*-(*R**, *S**)]- and [*S*-(*R**, *R**)]-**2** as the most probable alternatives for the fluorescent base isolated from rat liver $tRNA^{Phe}$. We present herein a detailed account of the synthesis of these compounds **2**. A preliminary report of this work has been published.[9]



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8

[*R*-(*R**, *S**)]-**2**

9

[*S*-(*R**, *R**)]-**2**

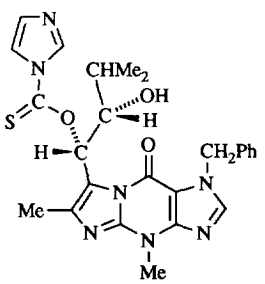
Scheme 1

We have already reported the synthesis of a model compound **6** for **2** through oxidation of **4** with osmium tetroxide, followed by catalytic hydrogenolysis.[10] This method was modified for oxidation of **7**,[1,8b,11] because **7** is susceptible to racemization under alkaline conditions:[1] **7** was treated with osmium tetroxide–*N*-methylmorpholine *N*-oxide in the presence of phosphate buffer (pH 6) to afford **8** as colorless plates and **9** as a colorless glass in 69% and 24% yields, respectively. The relative configuration of **8** has been

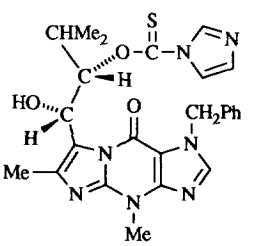
determined by X-ray crystallography.[9] The structure **9** was accordingly assignable to the other isomer in view of the *syn* addition mechanism of osmium tetroxide.[12] The final step to access to **2** was expected to be effectuated by means of hydrogenolysis. Catalytic hydrogenation of **8** over palladium on carbon, however, afforded a complex mixture of fluorescent substances. We experienced similar difficulty in the hydrogenolysis of the model compound (±)-**5**.[10,13] Although [*R*-(*R**, *S**)]-**2** was obtained in 19% yield by careful separation of the mixture, we failed to have [*S*-(*R**, *R**)]-**2** on similar treatment of **9**.

Getting these disappointing results, we turned to model experiments with **4** or (±)-**5**. As treatment of **4** with *m*-chloroperoxybenzoic acid afforded a complex mixture,[10] we attempted epoxidation of **4** with a combination of hydrogen peroxide and acetonitrile in methanol.[14] However, **4** was inert at 50 °C under these conditions. Compound **4** was then treated with sodium borohydride in the presence of tin(IV) chloride[15] to give unidentified products. We already reported that hydrogenolysis of (±)-**5** over palladium on carbon at atmospheric pressure and 60 °C in methanol afforded **6** in a low yield.[10] For the catalytic hydrogenolysis, replacement of the solvent by ethanol or aqueous methanol, addition of perchloric acid, an increase in the hydrogen pressure to 4—5 atm, or use of palladium oxide or palladium black[16] as a catalyst, offered no advantage. The reaction of (±)-**5** with either a combination of triethylsilane and trifluoroborane etherate[17] or sodium borohydride in the presence of trifluoroacetic acid or acetic acid[18] gave a complex mixture of unidentified products.

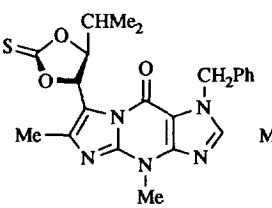
We next undertook to remove the hydroxy group at the 1'-position of (±)-**5** after modifying it. When (±)-**5** was treated with acetic anhydride in pyridine, the product obtained was suggested to be the diacetyl compound according to the ^{1}H NMR and MS spectra.[19] Nevertheless, purification of this compound was difficult and catalytic hydrogenolysis of the crude product over palladium on carbon did not afford **6**. The dimethyl ketal[20] of (±)-**5** was obtained in only a poor yield on treatment of (±)-**5** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid.[21] The starting material (±)-**5** was recovered when it was treated with diphenyl disulfide in the presence of tributylphosphine or triphenylphosphine.[22] The reaction of (±)-**5** with a combination of thiophenol and diethyl azodicaboxylate in the presence of tributylphosphine or triphenylphosphine[23] also resulted in a recovery of (±)-**5**. Heating (±)-**5** with 1,1'-thiocarbonyldiimidazole in tetrahydrofuran (THF)[24] gave (±)-**10** and (±)-**11** in 19% and 35% yields, respectively. Prolonged heating afforded (±)-**12** in 19% yield. If the yield of the desired (±)-**10** or (±)-**12** had been better, we would have attempted to convert it into **6** according to the reported procedure.[24,25]



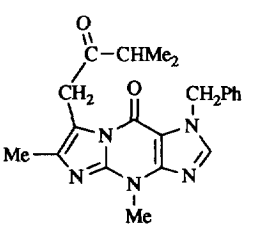
(±)-**10**



(±)-**11**



(±)-**12**



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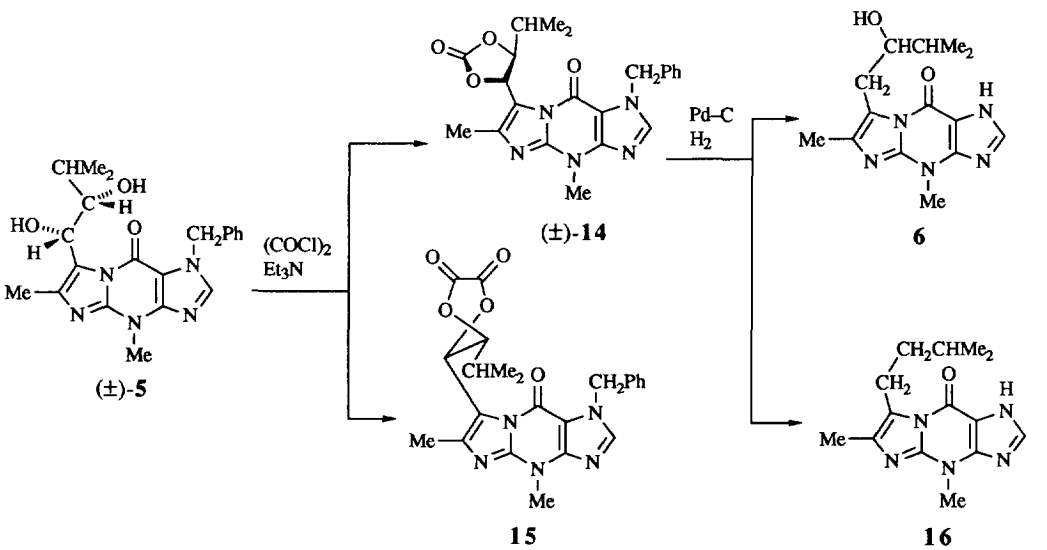
After these unfruitful trials we anchored our hopes on hydrogenolysis of the cyclic carbonate (±)-**14**.[26] When (±)-**5** was treated with phosgene in the presence of triethylamine, (±)-**14** was obtained in 9% yield. Compound **13**, which may be formed through the pinacol rearrangement, was also produced in this reaction. The use of trichloromethyl chloroformate (diphosgene)[27] instead of phosgene provided (±)-**14** in 28% yield. A high yield of (±)-**14** was incidentally attained: when we treated (±)-**5** with oxalyl chloride in the presence of triethylamine, envisioning the cyclic oxalate **15** as an intermediate for deoxygenation of (±)-**5** by stannyl radicals,[28] the product obtained in 63% yield was not the expected oxalate **15**, but the carbonate (±)-**14**. Later on, we disclosed that the formation of the corresponding cyclic carbonates by the action of oxalyl chloride was common for 1,2-glycols, and proposed the reaction mechanism.[29] Small amounts of the olefins **4** and **17**, regenerated (±)-**5**, and the aldehyde **18** were also obtained in the reaction with (±)-**5**. These by-products are most likely degradation products from the unstable cyclic oxalate **15** initially formed.

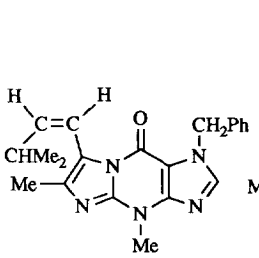


Scheme 2

Successful deoxygenation of (±)-**14** was achieved by catalytic hydrogenolysis over 10% palladium on carbon: we obtained **6** in 46% yield with concomitant formation of the dideoxy compound **16** in 28% yield. Although **16** might be formed through palladium catalyzed reductive elimination[30] and subsequent hydrogenation, (±)-**14** was stable in methanol at 60 °C in the presence of the catalyst without hydrogen.

Now we have established a new method of hydrogenolysis of the model compound (±)-**5** at the 1'-position, the next step required for access to **2** should be modification of **8** and **9** with oxalyl chloride, followed by catalytic hydrogenolysis. Compound **8** was thus treated with oxalyl chloride according to the procedure employed for the model experiment, affording **19** in 66% yield. Catalytic hydrogenolysis of this compound over 10% palladium on carbon provided [*R*-(*R**,*S**)]-**2** and (*S*)-**1** in 48% and 28% yields, respectively. Hydrogenolysis of **19** was more smoothly performed by the use of Pearlman's catalyst. The *R* configuration at the β-position of **2** thus obtained was confirmed by the identity of this product with a sample

prepared by direct hydrogenolysis of **8**. Although the formation of (*S*)-**1** is a drawback of this method from an efficiency standpoint, we can conveniently estimate the optical purity of **2** through evaluation of that of **1**: the HPLC analysis of crude (*S*)-**1** thus obtained, using a chiral column[1] indicated that it was optically pure, supporting that the present sample of [*R*-(*R**, *S**)]-**2** was also enantiomerically pure. A similar sequence of the reactions from **9** *via* **20** afforded [*S*-(*R**, *R**)]-**2** in 20% overall yield.



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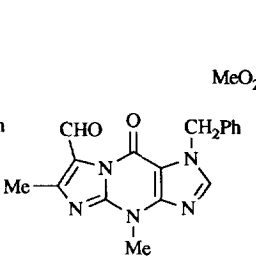

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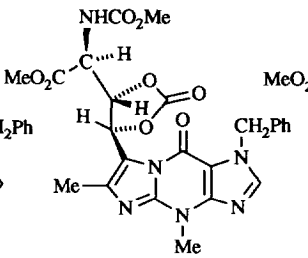

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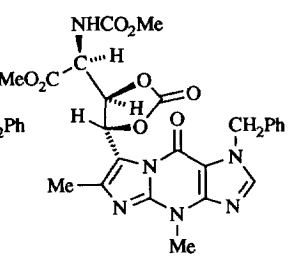

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Thus the two stereoisomers of **2**, the most probable alternatives for the fluorescent base of rat liver $tRNA^{Phe}$, have become available. These compounds are distinguishable from each other by means of HPLC, and IR and ^1H NMR spectroscopy. Therefore, the absolute configurations of the base from natural sources should be disclosed, provided the two-dimensional structure proposed is correct and the amount accessible is sufficient[31] for comparison with the present samples of synthetic **2**.

EXPERIMENTAL

General Notes

All melting points were determined by using a Yamato MP-1 or Büchi 530 capillary melting point apparatus and are collected. UV and mass spectra were recorded on a Hitachi 320 UV spectrophotometer and a Hitachi M-80 mass spectrometer. ^1H NMR spectra were measured with JEOL JNM-FX-100, JEOL JNM-EX-270, and JEOL JNM-GSX-500 NMR spectrometers. Unless otherwise stated, they were recorded at 500 MHz and 25 °C in $CDCl_3$ with tetramethylsilane as internal standard. Optical rotations were measured with a JASCO DIP-181 polarimeter using a 1-dm sample tube. HPLC system was a Waters model 204 ALC which included a 6000A pump, a U6K injector and a model 440 absorbance detector operating at 254 nm. Elemental analyses were performed by Mr. Y. Itatani and his associates at Kanazawa University, and by Mr. M. Teranishi at Hokuriku University. Pre-coated silica gel plates with a fluorescent indicator (Merck) were used for analytical (0.25 mm) and preparative (0.5 mm) thin-layer chromatography. Flash chromatography was performed according to the reported procedure.[32] The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, dddd = doublet-of-doublets-of-doublets-of-doublets, ddt = doublet-of-doublets-of-triplets, m = multiplet, s = singlet, sh = shoulder.

Dihydroxylation of **7** *with Osmium Tetroxide*

Osmium tetroxide (8 mg) was added to a solution of **7**[1] (95 mg, 0.21 mmol) and *N*-methylmorpholine *N*-oxide monohydrate (31.4 mg, 0.232 mmol) in a mixed solvent of acetone (8.4 ml) and 0.5 M phosphate buffer (pH 6.0; 3.1 ml) under nitrogen. The mixture was stirred at 25 °C for 4 h and then concentrated *in vacuo*. The residue was partitioned between chloroform (4 ml) and water (4 ml). The aqueous layer was extracted with chloroform (2 × 4 ml). The organic layers were combined, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was purified by flash chromatography [1,2-dichloroethane–ethanol (7:1, v/v)] to afford [α*S*-(α*R**, β*R**,γ*R**)]-1-benzyl-4,9-dihydro-β,γ-dihydroxy-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine-7-butanoic acid methyl ester (**8**) (53 mg), a mixture (35 mg) of **8** and the [α*S*-(α*R**, β*S**,γ*S**)]-isomer **9**, and pure **9** (13 mg) as colorless glasses. The mixture was further purified by flash chromatography (the same solvent) to afford a second crop of **8** (8 mg), a mixture (23 mg) of **8** and **9**, and **9** (3 mg). The mixture thus obtained was then purified by thin-layer chromatography on silica gel [1,2-dichloroethane–96% (v/v) aqueous ethanol (7:1, v/v)] to afford additional crops of **8** (10 mg; the total yield was 69%) and **9** (9 mg; the total yield was 24%). Compound **9**: a colorless glass; $[\alpha]_D^{25}$ +13° (*c* 0.206, MeOH); ^{1}H NMR δ: 2.41 [3H, s, C(6)Me], 3.53 [1H, s, C(β)OH], 3.58 and 3.72 (3H each, s, two OMe's), 3.96 (3H s, NMe), 4.24 [1H, br d, *J* = 8.3 Hz, C(β)H], 4.28 [1H, br d, *J* = 7.3 Hz, C(α)H], 5.14 [1H, dd, *J* = 8.3 and 11.2 Hz, C(γ)H], 5.27 [1H, d, *J* = 11.2 Hz, C(γ)OH], 5.56 and 5.63 (1H each, d, *J* = 15 Hz, CH_2), 5.73 (1H, d, *J* = 7.3 Hz, NH), 7.35 (5H, m, Ph), 7.73 [1H, s, C(2)H]; MS *m/z*: 498 (M^+).

Crude **8** was crystallized from ethanol. Recrystallization of **8** from ethanol gave an analytical sample of **8** as colorless plates, mp 183—184.5 °C (dec. with somewhat poor reproducibility) (lit.[9] mp 189—192 °C); $[\alpha]_D^{24}$ +14° (*c* 0.200, MeOH); MS *m/z*: 498 (M^+); UV $\lambda_{max}^{95\%\ EtOH}$ 242 nm (ε 35500), 259 (sh) (6100), 318 (5900); ^{1}H NMR δ: 2.26 [3H, s, C(6)Me], 3.50 [1H, s, C(β)OH], 3.67 and 3.72 (3H each, s, two OMe's), 3.91 [1H, d, *J* = 10 Hz, C(α)H], 3.96 (3H s, NMe), 4.53 [1H, d, *J* = 9.6 Hz, C(β)H], 4.80 [1H, dd, *J* = 9.6 and 11.7 Hz, C(γ)H], 5.38 [1H, d, *J* = 11.7 Hz, C(γ)OH], 5.57 and 5.64 (1H each, d, *J* = 15 Hz, CH_2), 5.59 (1H, d, *J* = 10 Hz, NH), 7.36 (5H, m, Ph), 7.75 [1H, s, C(2)H]. *Anal.* Calcd for $C_{23}H_{26}N_6O_7$: C, 55.42; H, 5.26; N, 16.86. Found: C, 55.39; H, 5.20; N, 16.77.

Reaction of (±)-**5** *with* 1,1'-*Thiocarbonyldiimidazole*

A mixture of (±)-**5** (20 mg, 0.05 mmol), 1,1'-thiocarbonyldiimidazole (0.02 g, 0.11 mmol), and dry THF (2 ml) was refluxed under nitrogen in the dark for 72 h and then concentrated *in vacuo*. The residue was purified by thin-layer chromatography on silica gel [chloroform–methanol (10:1, v/v)] to afford (*R**,*R**)-1-benzyl-1,4-dihydro-7-[2-hydroxy-1-[[(imidazol-1-yl)thiocarbonyl]oxy]-3-methylbutyl]-4,6-dimethyl-9*H*-imidazo-[1,2-*a*]-purin-9-one [(±)-**10**] (5 mg, 19%) as a colorless foam, 100 MHz ^{1}H NMR δ: 1.00 and 1.04 (3H each, d, *J* = 7 Hz, CMe_2), 2.55 [3H, s, C(6)Me], 3.88 (3H, s, NMe), *ca.* 4.4 (1H, br, C*H*CHMe_2), 5.57 (2H, s, CH_2), 7.01 and 7.05 (1H each, br s, imidazole protons), 7.26 (5H, s, Ph), 7.63 [1H, s, C(2)H], 7.75 (1H, br s, imidazole proton),[33] and (*R**,*R**)-1-benzyl-1,4-dihydro-7-[1-hydroxy-2-[[(imidazol-1-yl)thiocarbonyl]oxy]-3-methylbutyl]-4,6-dimethyl-9*H*-imidazo[1,2-*a*]purin-9-one [(±)-**11**] (9 mg, 35%) as a slightly brown glass, 270 MHz ^{1}H NMR δ: 0.94 and 1.09 (3H each, d, *J* = 7 Hz, CMe_2), 1.82 (1H, m, C*H*Me_2), 2.46 [3H, s, C(6)Me], 3.92 (3H, s, NMe), 4.66 (1H, m, C*H*CHMe_2), 5.58 and 5.63 (1H each, d, *J* = 14.5 Hz, CH_2), 6.56 [1H, br, C(7)CH], 7.01 and 7.16 (1H each, br s, imidazole protons), 7.40 (5H, s, Ph), 7.71 [1H, s, C(2)H], 7.86 (1H, br s, imidazole proton).

In a separate run, (±)-**5** (24 mg, 0.06 mmol) was heated under reflux with 1,1'-thiocarbonyldiimidazole (21 mg, 0.12 mmol) in THF (3 ml) under argon for 100 h. More 1,1'-thiocarbonyldiimidazole (21 mg) was added and the whole was heated for a further 70 h. The resulting mixture was diluted with dichloromethane (5 ml) and then washed with saturated aqueous sodium chloride. The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The residue was purified by thin-layer chromatography on silica gel [1,2-dichloroethane–ethanol (7:1, v/v)] to afford *trans*-(±)-1-benzyl-1,4-dihydro-7-(5-isopropyl-2-thioxo-1,3-dioxolan-4-yl)-4,6-dimethyl-9*H*-imidazo[1,2-*a*]purin-9-one [(±)-**12**] (5 mg, 19%) as a slightly brown solid, mp 217—222 °C; MS *m/z* : 437 (M^+); 270 MHz ^{1}H NMR δ: 1.04 and 1.06 (3H each, d, J = 6.5 Hz, CMe_2), 2.07 (1H, m, C*H*Me_2), 2.43 [3H, s, C(6)Me], 3.90 (3H, s, NMe), 4.76 [1H, br dd, J = 5 and 6 Hz, C(5')H], 5.58 (2H, s, CH_2), 6.54 [1H, br, C(4')H], 7.37 (5H, s, Ph), 7.68 [1H, s, C(2)H].

trans-(±)-1-*Benzyl*-1,4-*dihydro*-7-(5-*isopropyl*-2-*oxo*-1,3-*dioxolan*-4-*yl*)-4,6-*dimethyl*-9H-*imidazo*[1,2-a]*purin*-9-*one* [(±)-**14**]

(i) *By the Reaction with Oxalyl Chloride.* A solution of oxalyl chloride (0.023 ml, 0.27 mmol) in dry THF (1 ml) was added dropwise to a stirred solution of (±)-**5** (99 mg, 0.25 mmol) and triethylamine (0.15 ml, 1.1 mmol) in dry THF (5 ml) over a period of 5 min in an ice-bath. The mixture was stirred at 0 °C for a further 5 min. The resulting precipitate was filtered off and washed with THF (20 ml). The filtrate and washings were combined and concentrated *in vacuo*. The residue was partitioned between dichloromethane (20 ml) and 10% aqueous sodium carbonate (10 ml). The aqueous layer was extracted with dichloromethane (4 × 10 ml). The organic layers were combined, washed with saturated aqueous sodium chloride (10 ml), and dried over magnesium sulfate. Removal of the solvent by evaporation left a yellow solid, which was washed with benzene (2 ml) to afford (±)-**14** (61 mg), mp 210—212 °C (dec.). The washings were concentrated and the residue was purified by thin-layer chromatography on silica gel [benzene–ethanol (9:1, v/v)] to afford a second crop of (±)-**14** (5 mg; the total yield was 63%). Compound (±)-**5** (3 mg, 3%), which was identical (by comparison of the ^{1}H NMR and IR spectra, and chromatographic behavior) with the starting material, and a mixture (3 mg) of **4**, **17**,[34] and **18** (100 MHz ^{1}H NMR δ: 10.89)[35] (the molar ratio was 17:2:1 judged by ^{1}H NMR spectroscopy) were also obtained. Recrystallization of crude (±)-**14** from ethanol gave an analytical sample as colorless needles, mp 213—215 °C (dec.); MS *m/z*: 421 (M^+); $\lambda_{max}^{95\%\ EtOH}$ 242 nm (ε 36400), 258 (sh) (6400), 307 (7000); ^{1}H NMR δ: 0.94 and 1.08 (3H each, d, J = 6.8 Hz, CMe_2), 2.05 (1H, octet, J = 6.8 Hz, C*H*Me_2), 2.37 [3H, s, C(6)Me], 3.91 (3H, s, NMe), 4.62 [1H, dd, J = 6.8 and 7.8 Hz, C(5')H], 5.59 and 5.61 (1H each, d, J = 14.9 Hz, CH_2], 5.76 [1H, d, J = 7.8 Hz, C(4')H], 7.39 (5H, m, Ph), 7.66 [1H, s, C(2)H]. *Anal.* Calcd for $C_{22}H_{23}N_5O_4$: C, 62.69; H, 5.50; N, 16.62. Found: C, 62.74; H, 5.58; N, 16.76.

(ii) *By the Reaction with Phosgene.* A 1 M solution of phosgene in toluene (0.55 ml, 0.55 mmol) was diluted with dry THF (1 ml), and added dropwise to a stirred solution of (±)-**5** (99 mg, 0.25 mmol) and triethylamine (0.28 ml, 2 mmol) in dry THF (5 ml) at 0 °C. After stirring the mixture at 0 °C for 1 h then at room temperature for 1 h, it was partitioned between dichloromethane (5 ml) and 5% aqueous citric acid (5 ml). The organic layer was washed successively with 5% aqueous citric acid (5 ml) and saturated aqueous sodium bicarbonate (5 ml), dried over magnesium sulfate, and concentrated to leave a yellow oil (109 mg). This was purified by flash chromatography [1,2-dichloroethane–ethanol (7:1, v/v)] to afford crude (±)-**14** and a mixture of more polar substances. The mixture was purified by thin-layer chromatography on silica gel [1,2-

dichloroethane–ethanol (10:1, v/v)] to afford (±)-**5** (40 mg, 40% recovery) and 1-benzyl-1,4-dihydro-4,6-dimethyl-7-(3-methyl-2-oxobutyl)-9*H*-imidazo[1,2-*a*]purin-9-one (**13**) (6 mg, 6%) as colorless needles (recrystallized from ethanol), mp 180—182 °C (dec.); MS *m/z*: 377 (M^+); 270 MHz ^{1}H NMR δ: 1.19 (6H, d, *J* = 6.9 Hz, CMe_2), 2.24 [3H, s, C(6)Me], 2.85 (1H, m, $CHMe_2$), 3.91 (3H, s, NMe), 4.20 [2H, s, $C(7)CH_2$], 5.53 (2H, s, $PhCH_2$), 7.34 (5H, m, Ph), 7.60 [1H, s, C(2)H]. Crude (±)-**14** was recrystallized from ethanol to afford pure (±)-**14** (9 mg, 9%), mp 200.5–202 °C (dec.).

(iii) *By the Reaction with Trichloromethyl Chloroformate.* Diphosgene (0.027 ml, 0.22 mmol) was added to a solution of (±)-**5** (79 mg, 0.2 mmol) and triethylamine (0.22 ml, 1.6 mmol) in THF (4 ml). The mixture was stirred at 0 °C under nitrogen for 1 h. The resulting mixture was partitioned between 5% aqueous citric acid (5 ml) and dichloromethane (10 ml). The organic layer was washed successively with 5% aqueous citric acid (5 ml) and saturated aqueous sodium bicarbonate (5 ml), dried over magnesium sulfate, and concentrated. The residue was purified by flash chromatography [1,2-dichloroethane–ethanol (6:1, v/v)], followed by repeated thin-layer chromatography on silica gel (the same solvent) to afford (±)-**14** (24 mg, 28%), mp 203—205 °C (dec.).

[4S-[4α(R*),5β]]-5-[1-*Benzyl*-4,9-*dihydro*-4,6-*dimethyl*-9-*oxo*-1H-*imidazo*[1,2-a]*purin*-7-*yl*]-α-[(*methoxycarbonyl*)*amino*]-2-*oxo*-1,3-*dioxolane*-4-*acetic Acid Methyl Ester* (**19**)

A solution of oxalyl chloride (0.038 ml, 0.45 mmol) in dry THF (2.5 ml) was added to a solution of **8** (200 mg, 0.401 mmol) and triethylamine (0.34 ml, 24 mmol) in THF (16 ml) at 0 °C over a period of 5 min. The whole was stirred at 0 °C for a further 10 min and then concentrated *in vacuo*. The residue was partitioned between ethyl acetate (20 ml) and water (10 ml). The organic layer was washed successively with each 10 ml portion of 5% aqueous citric acid, water, and saturated aqueous sodium bicarbonate, dried over magnesium sulfate, and concentrated *in vacuo*. Crystallization of the residue from ethanol (15 ml) afforded **19** (138 mg, 66%), mp 196—197.5 °C (dec.). Recrystallization of crude **19** from ethanol afforded an analytical sample of **19** as colorless needles, mp 194.5—196.5 °C (dec. with somewhat poor reproducibility) [lit.[9] mp 204—205 °C (dec.)]; $[\alpha]_D^{26}$ −114° (*c* 0.197, MeOH); MS *m/z*: 524 (M^+); $\lambda_{max}^{95\%\ EtOH}$ 243 nm (ε 38500), 258 (sh) (6800), 307 (7400); ^{1}H NMR δ: 2.40 [3H, s, C(6)Me], 3.799 and 3.801 (3H each, s, two OMe's), 3.93 (3H, s, NMe), 4.68 [1H, d, *J* = 8.8 Hz, C(α)H], 5.38 [1H, dd, *J* = 1 and 7.3 Hz, C(β)H], 5.55 and 5.61 (1H each, d, *J* = 14.7 Hz, CH_2), 5.63 (1H, d, *J* = 8.8 Hz, NH), 5.82 [1H, d, *J* = 7.3 Hz, C(γ)H], 7.37 (5H, m, Ph), 7.67 [1H, s, C(2)H]. *Anal.* Calcd for $C_{24}H_{24}N_6O_8$: C, 54.96; H, 4.61; N, 16.03. Found: C, 55.12; H, 4.61; N, 15.92.

[4R-[4α(S*),5β]]-5-[1-*Benzyl*-4,9-*dihydro*-4,6-*dimethyl*-9-*oxo*-1H-*imidazo*[1,2-a]*purin*-7-*yl*]-α-[(*methoxycarbonyl*)*amino*]-2-*oxo*-1,3-*dioxolane*-4-*acetic Acid Methyl Ester* (**20**)

Compound **9** (139 mg, 0.279 mmol) was treated with oxalyl chloride in a manner similar to that described for the preparation of **19**. Crude **20** thus obtained was crystallized by treating it with ethanol (1 ml). The precipitate that separated was collected by filtration, washed with a little ethanol, and dried to afford **20** (52 mg) as a solid, mp 167—169 °C (dec.). From the combined filtrate and washings, a second crop of **20** (11 mg; the total yield was 43%) was obtained by flash chromatography [1,2-dichloroethane–ethanol (9:1, v/v)]. Recrystallization of **20** from ethanol gave an analytical sample as colorless needles, mp 167.5—169.5 °C

(dec.); $[\alpha]_D^{30}$ +106° (*c* 0.203, MeOH); MS *m/z*: 524 (M^+); $\lambda_{max}^{95\%\ EtOH}$ 243 nm (ε 37400), 260 (sh) (6700), 307 (7200); ^{1}H NMR δ: 2.35 [3H, s, C(6)Me], 3.70 and 3.80 (3H each, s, two OMe's), 3.91 (3H, s, NMe), 4.78 [1H, dd, *J* = 4.4 and 8.8 Hz, C(α)H], 5.12 [1H, dd, *J* = 4.4 and 7.8 Hz, C(β)H], 5.53 (1H, d, *J* = 8.8 Hz, NH), 5.56 and 5.59 (1H each, d, *J* = 14.7 Hz, CH_2), 6.15 [1H, d, *J* = 7.8 Hz, C(γ)H], 7.37 (5H, m, Ph), 7.67 [1H, s, C(2)H]. *Anal.* Calcd for $C_{24}H_{24}N_6O_8$: C, 54.96; H, 4.61; N, 16.03. Found: C, 54.88; H, 4.60; N, 15.94.

Hydrogenolysis of (±)-**14**

A suspension of (±)-**14** (121 mg, 0.287 mmol) and 10% palladium on carbon (0.12 g) in ethanol (80 ml) was hydrogenated at *ca.* 60 °C under atmospheric pressure for 10.5 h. The catalyst was filtered off, and washed successively with hot ethanol (200 ml) and hot chloroform (30 ml). The filtrate and washings were combined, and concentrated *in vacuo* to leave a colorless solid (77 mg). This was dissolved in warm methanol and then silica gel (0.80 g) was added to the solution. The mixture was concentrated *in vacuo* and the dry residue was put on a top of a silica gel column for flash chromatography (column diameter, 10 mm). Elution with chloroform–methanol (11:1, v/v) afforded **16** (22 mg, 28%) as a colorless solid, mp 267—269 °C (dec.). Recrystallization of crude **16** from methanol gave an analytical sample of **16** as colorless prisms, mp 269—271 °C (dec.); 270 MHz ^{1}H NMR δ: 1.00 (6H, d, *J* = 6.3 Hz, CMe_2), 1.59 (2H, m, $CH_2CH_2CHMe_2$), 1.69 (1H, m, $CHMe_2$), 2.29 [3H, s, C(6)Me], 3.12 [2H, m, C(7)CH_2], 3.99 (3H, s, NMe), 7.89 [1H, s, C(2)H], 12.99 (1H, s, NH). *Anal.* Calcd for $C_{14}H_{19}N_5O$: C, 61.52; H, 7.01; N, 25.62. Found: C, 61.19; H, 6.95; N, 25.46.

Further elution of the column with chloroform–methanol (5:1, v/v) afforded **6** (38 mg, 46%) as a colorless solid, mp 278—279 °C (dec.); identical (by comparison of the IR spectrum and chromatographic behavior) with an authentic sample.[10]

[R-(R*, S*)]-4,9-*Dihydro*-β-*hydroxy*-α-[(*methoxycarbonyl*)*amino*]-4,6-*dimethyl*-9-*oxo*-1H-*imidazo*[1,2-a]*purine*-7-*butanoic Acid Methyl Ester* [[R-(R*, S*)]-**2**]

(i) *Hydrogenolysis of* **8**. Compound **8** (20 mg, 0.04 mmol) was dissolved in methanol (10 ml) and hydrogenated over 10% palladium on carbon (20 mg) at *ca.* 60 °C and 6 atm for 12 h. Hydrogenation was continued for a further 10 h by addition of more catalyst (20 mg). The catalyst was collected by filtration and extracted with methanol using a Soxhlet extractor. The filtrate and extracts were combined, and concentrated *in vacuo*. The residue, which contained several fluorescent compounds, was purified by thin-layer chromatography on silica gel [chloroform–methanol–water (7:1:0.1, v/v)]. Compound [*R*-(*R**, *S**)]-**2** (3 mg, 19%) was obtained as a colorless solid from the lowest fluorescent band. Recrystallization from ethanol gave colorless needles, mp *ca.* 235 °C (dec.). This sample was identical (by comparison of the MS, NMR, and UV spectra, and chromatographic behavior) with an analytical sample described below.

(ii) *Hydrogenolysis of* **19** *over 10% Palladium on Carbon.* Compound **19** (100 mg, 0.191 mmol) was suspended in methanol (50 ml), and hydrogenated over 10% palladium on carbon (100 mg) at 60 °C and 5 atm for 16 h. The catalyst was filtered off and continuously extracted with methanol using a Soxhlet extractor. The filtrate and extracts were combined, and concentrated *in vacuo*. The residue was purified by flash chromatography [chloroform–methanol (7:1, v/v)] to afford a mixture (48 mg) of [*R*-(*R**, *S**)]-**2** and (*S*)-**1**,

and [*R*-(*R**,*S**)]-**2** (20 mg), as colorless solids. The mixture was purified by thin-layer chromatography on silica gel [chloroform–methanol (7:1, v/v)] to give a second crop of [*R*-(*R**,*S**)]-**2** (16 mg; the total yield was 48%). From the higher-R_f band, (*S*)-**1** (21 mg, 28% as the monohydrate), whose ^{1}H NMR spectrum was identical with that of an authentic sample.[1]

(iii) *Hydrogenolysis of* **19** *over Pearlman's Catalyst.* Hydrogenolysis of **19** (158 mg, 0.301 mmol) over 20% palladium hydroxide on carbon (0.16 g) was carried out in methanol (80 ml) at *ca.* 60 °C under atmospheric pressure for 7 h. The catalyst was filtered off and extracted with methanol using a Soxhlet extractor. The filtrate and extracts were combined, and concentrated *in vacuo*. The resulting residue was purified by flash chromatography [dichloromethane–methanol–water (100:10:1, v/v)] to afford (*S*)-**1** (47 mg, 39% as the monohydrate[1]) and [*R*-(*R**,*S**)]-**2** (52 mg, 44%) as a colorless solid, mp 224.5—225.5 °C (dec.). Crude (*S*)-**1** thus obtained was optically pure according to HPLC analysis.[1] Recrystallization of crude [*R*-(*R**,*S**)]-**2** from methanol afforded an analytical sample as colorless needles, mp 232—233.5 °C (dec.); $[\alpha]_D^{19}$ −21° (*c* 0.10, MeOH); $\lambda_{max}^{95\%\ EtOH}$ 235 nm (ε 32600), 257 (sh) (5300), 309 (5200); $\lambda_{max}^{H_2O}$ (pH 1) 233 (36200), 286 (7600); $\lambda_{max}^{H_2O}$ (pH 7) 235 (34100), 262 (5300), 311 (5200); $\lambda_{max}^{H_2O}$ (pH 13) 237 (34800), 267 (5400), 304 (7300); MS *m/z*: 392 (M^+); ^{1}H NMR [$(CD_3)_2SO$][36] δ: 2.08 [3H, s, C(6)Me], 3.14 (2H, d, *J* = 6.8 Hz, CH_2), 3.58 and 3.59 (3H each, s, two OMe's), 3.76 (3H, s, NMe), 3.93 (0.1H, m) and 3.99 [0.9H, dd, *J* = 2.4 and 9.3 Hz) [C(α)H], 4.42 [1H, ddt, *J* = 2.4, 6.8, and 7.8 Hz, C(β)H], 4.95 (0.9H, d, *J* = 7.8 Hz) and 5.00 (0.1H, d, *J* = 7.5 Hz) (OH), 6.64 (0.1H, d, *J* = 10 Hz) and 7.12 (0.9H, d, *J* = 9.3 Hz) [C(α)NH], 8.18 [1H, s, C(2)H], 13.57 [1H, s, N(1)H]. *Anal.* Calcd for $C_{16}H_{20}N_6O_6$: C, 48.98; H, 5.14; N, 21.42. Found: C, 48.83; H, 5.06; N, 21.39.

[S-(R*,R*)]-4,9-*Dihydro*-β-*hydroxy*-α-[(*methoxycarbonyl*)*amino*]-4,6-*dimethyl*-9-*oxo*-1H-*imidazo*[1,2-a]*purine*-7-*butanoic Acid Methyl Ester* [[S-(R*,R*)]-**2**]

Compound **20** (125 mg, 0.238 mmol) was hydrogenated over Pearlman's catalyst (125 mg) in a manner similar to that described for the preparation of [*R*-(*R**,*S**)]-**2** and the products were purified by flash chromatography [dichloromethane–methanol (10:1, v/v)] to afford (*S*)-**1** (39 mg, 41% as the monohydrate[1]) and [*S*-(*R**,*R**)]-**2** (44 mg, 46% as the hemihydrate), mp 210—216.5 °C (dec.). Crude (*S*)-**1** thus obtained was optically pure according to HPLC analysis.[1] Recrystallization of crude [*S*-(*R**,*R**)]-**2** from methanol afforded an analytical sample as colorless needles, mp 233—235 °C (dec.); $[\alpha]_D^{21}$ −18° (*c* 0.101, MeOH); $\lambda_{max}^{95\%\ EtOH}$ 236 nm (ε 34700), 261 (6000), 313 (5500); $\lambda_{max}^{H_2O}$ (pH 1) 234 (38500), 287 (8100); $\lambda_{max}^{H_2O}$ (pH 7) 236 (35400), 263 (5800), 314 (5600); $\lambda_{max}^{H_2O}$ (pH 13) 237 (37100), 268 (5900), 305 (7700); MS *m/z*: 392 (M^+); ^{1}H NMR [$(CD_3)_2SO$][36] δ: 2.15 [3H, s, C(6)Me], 3.02 (1H, dd, *J* = 7.8 and 14.6 Hz) and 3.44 (1H, dd, *J* = 4.5 and 14.6 Hz) (CH_2), 3.55 and 3.56 (3H each, s, two OMe's), 3.76 (3H, s, NMe), 4.10 [1H, dddd, *J* = 4.5, 5.0, 5.4, and 7.8 Hz, C(β)H], 4.17 (1H, dd, *J* = 5.0 and 8.3 Hz) [C(α)H], 5.08 (1H, d, *J* = 5.4 Hz, OH), 6.83 (0.1H, br) and 7.24 (0.9H, d, *J* = 8.3 Hz) [C(α)NH], 8.18 [1H, s, C(2)H], 13.60 [1H, s, N(1)H]. *Anal.* Calcd for $C_{16}H_{20}N_6O_6 \cdot 1/2H_2O$: C, 47.88; H, 5.27; N, 20.94. Found: C, 47.74; H, 4.99; N, 20.85.

HPLC of **2**

Complete separation of [*R*-(*R**,*S**)]- and [*S*-(*R**,*R**)]-**2** was attained on a Hibar LiChrosorb® Si-60 column (5 μm) (4 × 250 mm) using chloroform–methanol (95:5, v/v) (retention time: 30.4 and 32.8 min) or 1,2-dichloroethane–ethanol (88:12, v/v) (retention time: 22.8 and 24.8 min) as eluent at the flow rate of 0.5 ml per min and room temperature.

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35. In a separate run, a small amount of a mixture of (±)-**14** and a minor component was obtained. The 270 MHz ^{1}H NMR spectrum [δ: 2.70 (3H, s, CMe), 4.01 (3H, s, NMe), 5.65 (2H, s, CH_2), 7.77 [1H, s C(2)H], 10.89 (1H, s, CHO)] of the latter closely resembled that of **18**.[37]
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