Studies towards the Synthesis of the Fluorescent Bases of Phenylalanine Transfer Ribonucleic Acids: Synthesis of 7-(2-Hydroxy-3-methylbutyl)wye, a Model for the Minor Base Isolated from Rat Liver

Taisuke ITAYA,* Akemi MIZUTANI, and Nobuhide WATANABE

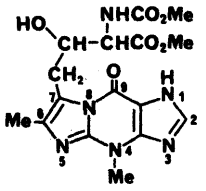
Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi 13-1, Kanazawa 920, Japan. *Received October 24, 1988*

Treatment of 1-benzyl-7-(hydroxymethyl)wye (8) with PBr_3 in the presence of Ph_3P gave the phosphonium bromide 9 in good yield. Heating 9 and Me_2CHCHO (7) in MeOH in the presence of K_2CO_3 provided 1-benzyl-1,4-dihydro-4,7-dimethyl-6-(3-methyl-1-butenyl)-9*H*-imidazo[1,2-*a*]purin-9-one (11), a positional isomer of the objective 1-benzyl-7-(3-methyl-1-butenyl)wye (5), as a major product. When the reaction was conducted in Me_2NCHO at −65 °C using *n*-BuLi as a base, a 7 : 2 mixture of (*E*)- and (*Z*)-5 was obtained in good yield. Nevertheless, neither the protected amino aldehyde 18 nor 21 gave the desired olefin under similar conditions, implying poor applicability of this method to the synthesis of the fluorescent bases of phenylalanine transfer ribonucleic acids ($tRNAs^{Phe}$).

Compound 5 was alternatively synthesized by the Wittig reaction between 1-benzyl-7-formylwye (3) and $Ph_3P^+CH_2CHMe_2\ I^-$ (4) in tetrahydrofuran as an equimolar mixture of the geometrical isomers in 50% yield. When the reaction was carried out in Me_2SO at room temperature using two equivalents each of $NaCH_2SOMe$ and 4, the product, obtained in high yield, was (*E*)-11. The use of an equimolar amount of the base afforded (*E*)-5 in 26% yield. Oxidation of (*E*)-5 with OsO_4 followed by hydrogenolysis over Pd–C gave 7-(2-hydroxy-3-methylbutyl)wye (2), a model for the minor base from rat liver $tRNA^{Phe}$.

Keywords hypermodified base model; 7-alkylwye; 7-substituted 1,4-dihydro-4,6-dimethyl-9*H*-imidazo[1,2-*a*]purin-9-one; Wittig reaction; osmium oxidation; hydrogenolysis; cyclic imidazolide rearrangement; base-catalyzed rearrangement; amino aldehyde

Many eukaryotic phenylalanine transfer ribonucleic acids ($tRNAs^{Phe}$) have fluorescent components at the position adjacent to the 3′-end of an anticodon.[1] One of such bases from rat liver $tRNA^{Phe}$ has been proposed to be hydroxywybutine (**1**),[2,3] although the stereochemistry was not specified. Because of the minute amount available, rigorous identification of the structure of the base, especially the absolute configurations, has had to await chemical synthesis. This paper describes the synthesis of the title compound **2** as a model for **1**.



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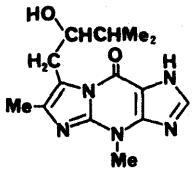

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We have already reported the synthesis of 1-benzyl-7-formylwye (**3**).[4] This compound should be convertible to 1-benzyl-7-(3-methyl-1-butenyl)wye (**5**), which appears to be a good intermediate for the synthesis of **2**. Of two possible routes to **5** depicted in Chart 1, the one *via* the phosphonium bromide **9** was expected to give a better result, subject to easy availability of **9**, since **9** should generate the semistabilized ylide.[5] For the preparation of **9**, the alcohol **8**[4] was first treated with PBr_3 in CH_2Cl_2. Isolation of the desired bromide, however, failed because of its instability. The reaction was then carried out in the presence of Ph_3P to trap the bromide. Thus, **9** was obtained directly from **8** in 90% yield.[6] In the nuclear magnetic resonance (NMR) spectrum of **9**, the C-methyl group giving the signal observed at relatively high magnetic field (δ 2.11 ppm in $CDCl_3$) with a long-range interaction with the phosphorus is assignable to the 6-position rather than the 7-position,[4,7] ruling out the possibility of rearrangement as described below. Treatment of **9** with *n*-BuLi in tetrahydrofuran (THF) at −78 °C, however, resulted in complete recovery of **9**, probably because of its poor solubility. Heating **9** with Me_2CHCHO (**7**) in MeOH in the presence of K_2CO_3[8] gave a mixture of olefins. The relatively deshielded aromatic C-methyl signal (δ 2.73 ppm in $CDCl_3$)[4,7] of the major product shows that it was not the desired **5** but the rearranged product **11**, to which *E* configuration was assigned on the basis of the coupling constant (15 Hz) for the olefinic protons. Compound **11** was probably formed *via* ring-fission of **5** at the N(8)–C(9) bond followed by reclosure between the N(5)- and C(9)-positions, although an alternative possibility of the ring-opening of **9** in preference to the Wittig reaction can not be ruled out at present. 1-Benzyl-7-methylwye (**12**),[4] 1-benzyl-7-(methoxymethyl)wye (**13**), and **14**, a positional isomer of **13**, were also formed in this reaction. The latter two were probably produced *via* metathesis of **9** by methoxide anion that might be formed under the reaction conditions. The reaction at room temperature gave similar results. Finally, **9** was treated with *n*-BuLi in Me_2NCHO at −65 °C followed by addition of **7**, resulting in almost quantitative conversion into a mixture of (*E*)- and (*Z*)-**5**, and **12**. Recrystallization of the mixture from MeOH gave (*E*)-**5** in 28% yield. The minor product **12** obtained in 12% yield had been utilized as a precursor for the synthesis of 7-methylwye,[4] a fluorescent base isolated from archaebacterial tRNAs.[9] Compound **12** was considered to be formed by hydrolysis of the phosphorane generated from **9**. Thus, the phosphorane in Me_2NCHO was quenched with H_2O instead of the aldehyde **7**, resulting in an increase of the yield of **12** to 31%. Hydrogenation of (*E*)-**5** gave 1-benzyl-7-(3-methylbutyl)wye (**10**) whose structure was ascertainable by comparison of the ultraviolet (UV) and NMR spectra with those of **12**[4] or 1-benzyl-7-butylwye,[7b] confirming the correctness of the structure **5**.

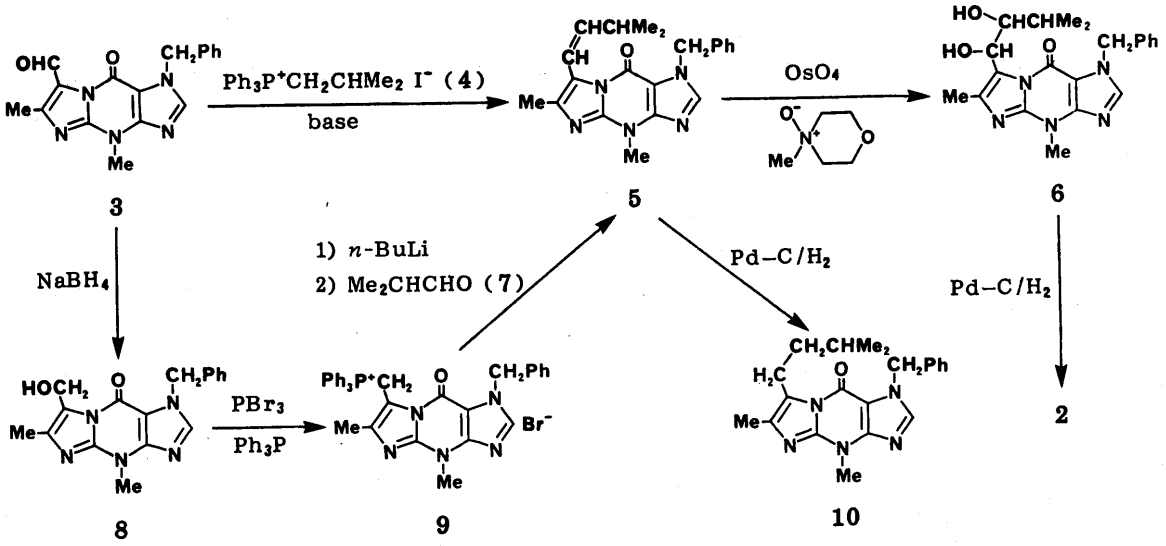
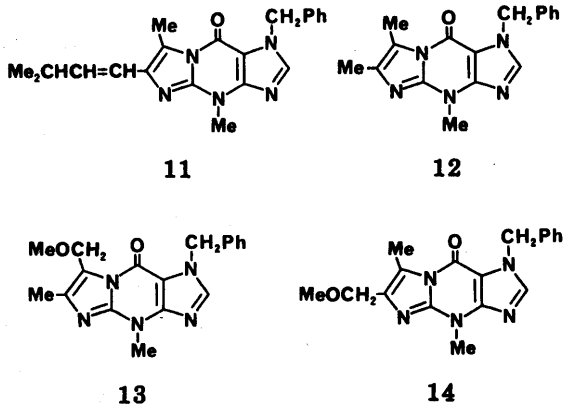


Chart 1

Now that a method of constructing a side chain at the 7-position of the tricycle had been established, an appropriate chiral amino aldehyde was required in the next step of access to the fluorescent bases of $tRNA^{Phe}$. Hamada and Shioiri[10] have achieved a success in applying the method of Parikh and Doering[11] for the preparation of optically pure *N*-protected α-amino aldehydes.[12] However, when *N*-(methoxycarbonyl)- or *N*-(benzyloxycarbonyl)-L-serine methyl ester was treated according to the reported procedure,[10] the desired *N*-protected β-oxoalanine methyl ester was not obtained. The results were not surprising in view of a recent report on the oxidative degradation of a protected serine.[13] Thus, we planned the Wittig reaction with aldehydes such as **18** and **21** followed by conversion of the ether moiety into the ester. Compound **18** was prepared according to the reported procedure[10,14] from *O*-benzyl-*N*-(methoxycarbonyl)-L-serine methyl ester (**16**) *via* the alcohol **17**. The amino aldehyde **21** having a reversed configuration was also prepared from **17** through protection of the hydroxy group followed by hydrogenolysis and oxidation. However, the Wittig reaction of either **18** or **21** with **9** under similar conditions to those described above failed to give the desired olefin. The major products obtained were **12** and Ph_3PO. $PhCH_2OH$ was also formed in the case of the reaction of **18**, suggesting that this type of aldehyde underwent β-elimination in preference to the Wittig reaction. Thus, this route proved unsuitable for access to **1** or its congeners.



We then tried the alternative Wittig reaction between **3** and (2-methylpropyl)triphenylphosphonium iodide (**4**). Unlike the reaction of **9** with **7**, that of **3** with **4** using *n*-BuLi and Me_2NCHO merely gave the ring-opened product **22** in 41% yield along with (2-methylpropyl)diphenylphosphine oxide.[15] The structure **22** was assigned on the basis of the NMR spectrum.[16] When **4** was treated with NaH in Me_2NCHO at room temperature followed by addition of **3**, no olefinic product but (2-methylpropyl)diphenylphosphine oxide was obtained in 77% yield. Treatment of **3** with the phosphorane derived from an equimolar amounts of **4** and $NaCH_2SOMe$ in Me_2SO at room temperature afforded (*E*)-**5** in 26% yield. When the amounts of **4** and the base were increased to two equivalents, the olefinic product, obtained in 74% yield, was (*E*)-**11**. Compound **4** was finally

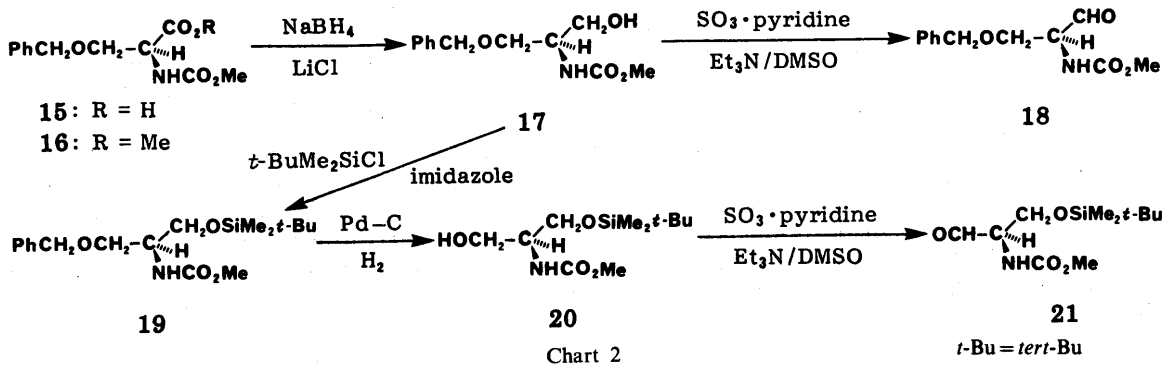
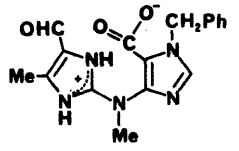


Chart 2

t-Bu = *tert*-Bu



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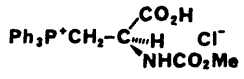

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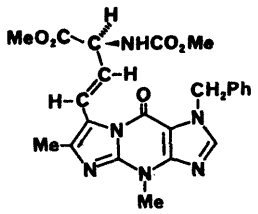

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treated with *n*-BuLi in THF at −78 °C followed by addition of **3** and then the mixture was allowed to warm to 0 °C to afford a mixture of equal amounts of (*E*)- and (*Z*)-**5** in 50% yield. The yield of the mixture **5** was not improved (24% or 14%) when a similar reaction was conducted in benzene or Et_2O at room temperature. These results indicate that this method is inferior to the one through **9** (*vide supra*) for the synthesis of the model compound **5** itself, as was expected. Nevertheless, the Wittig reaction of **3** using the phosphonium salt **23** has been successfully utilized in the first synthesis of **24**, the key intermediate for the synthesis of wybutine[4a] isolated from yeast $tRNA^{Phe}$.

Introduction of a hydroxy group into the side chain of **5** was first attempted by oxidation with a peracid followed by hydrogenolysis. However, treatment of (*E*)-**5** with *m*-chloroperbenzoic acid afforded a complex mixture of products. Successful oxidation of (*E*)-**5** was achieved with *N*-methylmorpholine oxide in the presence of a catalytic amount of OsO_4 in aqueous acetone[17] to give the diol **6** in 79% yield. the *R**,*S**configurations are assignable to this compound in view of the *syn* addition mechanism of OsO_4.[18] Hydrogenolysis of **6** over Pd–C gave **2** in 27% yield. By means of this method, we have achieved the syntheses of two optically active forms of **1**, the most probable alternatives for the structure of hydroxywybutine,[2] from **24**.[19]

Experimental

General Notes All melting points were taken on a Yamato MP-1 capillary melting point apparatus and are corrected. Spectra reported herein were recorded on a Hitachi 320 UV spectrophotometer, a Hitachi M-80 mass (MS) spectrometer, a JEOL JNM-FX-100 NMR spectrometer at 25 °C or a JEOL JNM-PMX 60 NMR spectrometer at 35 °C with Me_4Si as an internal standard. The NMR spectra were taken at 100 MHz in $CDCl_3$ unless otherwise stated. Optical rotations were measured with a JASCO DIP-181 polarimeter. We are indebted to Mr. Y. Itatani and his associates at Kanazawa University for elemental analyses. Flash chromatography was performed on silica gel according to the reported procedure.[20] The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, m = multiplet, s = singlet, sh = shoulder, t = triplet.

[(1-Benzyl-4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purin-7-yl)methyl]triphenylphosphonium Bromide Monohydrate (9·H_2O) A solution of PBr_3 (0.50 ml, 5.3 mmol) in anhydrous CH_2Cl_2 (10 ml) was added to a solution of **8**[4] (3.23 g, 10 mmol) and Ph_3P (5.25 g, 20 mmol) in CH_2Cl_2 (125 ml) over a period of 30 min. The mixture was stirred at room temperature for a further 2 h. It was then neutralized with saturated aqueous $NaHCO_3$. The aqueous layer, which contained a solid precipitate, was extracted with CH_2Cl_2 (200 ml). The aqueous layer was diluted with H_2O (400 ml) and then extracted with $CHCl_3$ (7 × 50 ml). The combined CH_2Cl_2 solutions were dried over $MgSO_4$ and concentrated *in vacuo* to leave a caramel, which was washed with hot toluene (25 ml). The resulting solid was washed with toluene (50 ml) and dried to give 9·H_2O (4.30 g), mp 252—254 °C (dec.). The combined $CHCl_3$ extracts were dried over $MgSO_4$ and concentrated *in vacuo* to give a second crop of 9·H_2O (1.70 g, total yield 90%). Recrystallization from EtOH gave colorless prisms of unchanged melting point. This sample was found to be the monohydrate by NMR spectroscopy, ^{1}H-NMR δ: 2.02 (2H, s, H_2O), 2.11 (3H, d, J = 4 Hz, CMe), 3.80 (3H, s, NMe), 5.39 (2H, s, $PhC\underline{H}_2$), 5.99 [2H, d, J = 11 Hz, C(7)CH_2], 7.30 (5H, m, $\underline{Ph}CH_2$), 7.4—8.0 (15H, m, Ph_3), 7.89 [1H, s, C(2)H], and lost 0.5 mol eq of H_2O on being dried over P_2O_5 at 2 mmHg and 150 °C for 7 h.

(*E*)-1-Benzyl-1,4-dihydro-4,7-dimethyl-6-(3-methyl-1-butenyl)-9*H*-imidazo[1,2-*a*]purin-9-one [(*E*)-11] i) $NaCH_2SOMe$[21] (2 M solution in Me_2SO, 0.5 ml, 1.0 mmol) was added to a solution of **4**[22] (446 mg, 1.0 mmol) in anhydrous Me_2SO (1 ml) and the mixture was stirred at room temperature for 5 min. Compound **3** (161 mg, 0.50 mmol) was added to the mixture and allowed to react at room temperature for 1 h. The mixture was neutralized with 10% aqueous H_3PO_4 and then concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2·(15 ml) and H_2O (15 ml). The aqueous layer was extracted with CH_2Cl_2 (2 × 15 ml). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to leave a solid. This was recrystallized from MeOH to give (*E*)-**11**·1/2H_2O (110 mg), mp 160—165 °C. From the mother liquor, a second crop of **11**·1/2H_2O (28 mg, total yield was 74%) was obtained by flash chromatography [column diameter, 20 mm; eluant, hexane–AcOEt–EtOH (4:2:1, v/v)]. Further recrystallizations from MeOH and drying over P_2O_5 at 2 mmHg and 75 °C for 3 h gave an analytical sample as colorless needles, mp 168–169 °C; UV $\lambda_{max}^{95\%\,EtOH}$ nm (ε): 223 (25500), 251 (25500), 262 (18700), 278 (sh) (10700), 332 (7500); ^{1}H-NMR δ: 1.13 (6H, d, J = 7 Hz, Me_2), 1.68 (1H, s, 1/2H_2O), 2.52 [1H, m, C(3′)H], 2.73 [3H, s, C(7)Me], 3.89 (3H, s, NMe), 5.57 (2H, s, CH_2), 6.31 [1H, J = 15 Hz, C(1′)H], 6.51 [1H, dd, J = 15, 6 Hz, C(2′)H], 7.35 (5H, s, Ph), 7.60 [1H, s, C(2)H]; MS *m/z*: 361 (M^+). *Anal.* Calcd for $C_{21}H_{23}N_5O$·1/2H_2O: C, 68.09; H, 6.53; N, 18.91. Found: C, 68.17; H, 6.46; N, 18.88.

ii) A mixture of 9·H_2O (667 mg, 1.0 mmol), K_2CO_3 (140 mg, 1.01 mmol), **7** (78 mg, 1.1 mmol), and anhydrous MeOH (10 ml) was stirred under reflux for 30 min and then concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 (10 ml) and H_2O (10 ml). The organic layer was washed with H_2O (20 ml), dried over $MgSO_4$, concentrated to a small volume, and purified by flash chromatography [30 mm; AcOEt–EtOH (300 ml : 20 ml and then 300 ml : 30 ml)]. Ph_3P (69 mg, 26%), mp 80—82 °C, was obtained as the least polar substance. From the successive eluates, a mixture (94 mg) of (*Z*)- and (*E*)-**11**, a mixture (169 mg) of (*E*)-**11** and Ph_3PO, Ph_3PO (28 mg, mp 153—156 °C), an approximately 3:2 mixture (16 mg, 4.4%) of (*Z*)- and (*E*)-**5** as a semisolid, **12** (64 mg, 21%, mp 201—202 °C), and an approximately 3:2 mixture (78 mg, 24%, mp 112—150 °C) of **13** {^{1}H-NMR δ: 2.36 [3H, s, C(6)Me], 3.40 (3H, s, OMe), 3.91 (3H, s, NMe), 4.91 [2H, s, C(7)CH_2], 5.63 (2H, s, $PhC\underline{H}_2$), 7.35 (5H, s, Ph), 7.60 [1H, s, C(2)H]} and **14** {^{1}H-NMR δ: 2.75 [3H, s, C(7)Me], 3.43 (3H, s, OMe), 3.89 (3H, s, NMe), 4.43 [2H, s, C(6)CH_2], 5.58 (2H, s, $PhC\underline{H}_2$), 7.35 (5H, s, Ph), 7.63 [1H, s, C(2)H]} were obtained. An attempt to separate **13** from **14** failed.

The mixture of the geometrical isomers of **11** was purified by flash chromatography [10 mm; hexane–AcOEt (2 : 3, v/v)] to give (*Z*)-**11** (21 mg, 5.8%), mp 166—167 °C, and (*E*)-**11**·1/2H_2O (71 mg, mp 163—166 °C. Recrystallizations of (*Z*)-**11** from EtOH gave an analytical sample as colorless needles, mp 166—167 °C; UV $\lambda_{max}^{95\%\,EtOH}$ nm (ε): 228 (28000), 244 (27600), 275 (sh) (9500), 334 (7200); ^{1}H-NMR δ: 1.06 (6H, d, J = 7 Hz, CMe_2), 2.71 [3H, s, C(7)Me], 3.73 [1H, m, C(3′)H], 3.88 (3H, s, NMe), 5.52 [1H, dd, J = 11, 12 Hz, C(2′)H], 5.58 (2H, s, CH_2), 6.11 [1H, d, J = 12 Hz, C(1′)H], 7.34 (5H, s, Ph), 7.61 [1H, s, C(2)H]; MS *m/z*: 361 (M^+). *Anal.* Calcd for $C_{21}H_{23}N_5O$: C, 69.78; H, 6.41; N, 19.38. Found: C, 69.71; H, 6.40; N, 19.52. After storage of pure (*Z*)-**11** in a glass tube, (*E*)-**11** was found by thin-layer chromatography. The (*E*)-isomer was also unstable under similar conditions, but (*Z*)-**11** was not detected among the degradation products. In the dark, both isomers proved to be stable.

The mixture of (*E*)-**11** and Ph_3PO was also purified by flash chromatography (20 mm; AcOEt) to give a second crop of (*E*)-**11**·1/2H_2O (30 mg; the total yield was 27%) and a second crop of Ph_3PO (133 mg; the total yield was 58%). Recrystallization of the crude (*E*)-**11** from MeOH gave colorless needles, mp 168—169 °C, identical with the analytical sample of (*E*)-**11**·1/2H_2O described under item (i).

(*E*)-1-Benzyl-1,4-dihydro-4,6-dimethyl-7-(3-methyl-1-butenyl)-9*H*-imidazo[1,2-*a*]purin-9-one [(*E*)-5] i) *n*-BuLi (1.7 M solution in hexane, 0.30 ml, 0.51 mmol) was added to a suspension of **4**[22] (223 mg, 0.50 mmol) in anhydrous THF (10 ml) at −78 °C under N_2. After 2 min, **3** (161 mg, 0.50 mmol) was added and the mixture was stirred at −78 °C for 2 min. The mixture was then stirred in an ice bath for a further 1 h and

concentrated *in vacuo*. H_2O (10 ml) was added to the residue and the mixture was neutralized with 10% aqueous H_3PO_4. It was then extracted with $CHCl_3$ (3 × 10 ml). The combined extracts were dried over $MgSO_4$ and concentrated *in vacuo* to leave a brown residue (350 mg). This was purified on an alumina (50 g) column [hexane–acetone (6 : 1, v/v)] to give a mixture of approximately equal amounts of (*E*)- and (*Z*)-**5** (90 mg, 50%), mp 135—148 °C; ^{1}H-NMR of (*Z*)-**5** δ: 0.99 (6H, d, J=7 Hz, CMe_2), 2.23 [3H, d, J=1 Hz, C(6)Me], *ca.* 2.5 [1H, m, C(3′)H], 3.90 (3H, s, NMe), 5.58 (2H, s, CH_2), 5.60 [1H, dd, J=12, 7 Hz, C(2′)H], 6.57 [1H, d, J=12 Hz, C(1′)H], 7.34 [5H, s, Ph], 7.60 [1H, s, C(2)H]. Recrystallizations of the mixture six times from MeOH gave an analytical sample of (*E*)-**5** as colorless needles, mp 160—163 °C; UV $\lambda_{max}^{95\%\ EtOH}$ nm (ε): 254 (25500), 282 (sh) (8200), 324 (5200); ^{1}H-NMR δ: 1.13 (6H, d, J=7 Hz, CMe_2), 2.39 [3H, S, C(6)Me], 2.53 [1H, m, C(3′)H], 3.91 (3H, s, NMe), 5.61 (2H, s, CH_2), 5.73 [1H, dd, J=16, 7 Hz, C(2′)H], 7.18 [1H, d, J=16 Hz, C(1′)H], 7.35 (5H, s, Ph), 7.60 [1H, s, C(2)H]; MS *m/z*: 361 (M^+). *Anal.* Calcd for $C_{21}H_{23}N_5O$: C, 69.78; H, 6.41; N, 19.38. Found: C, 69.90; H, 6.41; N, 19.20.

ii) $NaCH_2SOMe$[21] (2 M solution in Me_2SO, 0.25 ml, 0.50 mmol) was added to a solution of **4**[22] (233 mg, 0.50 mmol) in anhydrous Me_2SO (0.5 ml) and the mixture was stirred at room temperature for 20 min. A solution of **3** (161 mg, 0.50 mmol) in anhydrous Me_2SO (12 ml) was added, and the resulting mixture was stirred at room temperature for a further 1 h. It was then neutralized with 10% aqueous H_3PO_4 and concentrated *in vacuo*. The residue was partitioned between H_2O (15 ml) and CH_2Cl_2 (15 ml). The aqueous layer was extracted with CH_2Cl_2 (2 × 15 ml). The combined CH_2Cl_2 extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography [20 mm; hexane–AcOEt–EtOH (4 : 2 : 1, v/v)] to give (*E*)-**5** (46 mg, 26%), mp 147–154 °C, identical with the analytical sample described above on the basis of NMR spectroscopic comparison.

iii) A solution of **9**·1/2H_2O (4.07 g, 6.19 mmol) in anhydrous Me_2NCHO (97 ml) was cooled to −65 °C. *n*-BuLi (1.55 M solution in hexane, 4.00 ml, 6.2 mmol) was added dropwise over a period of 2 min with stirring. After 1 min, **7** (0.69 ml, 7.5 mmol) was added and stirring was continued for a further 15 min at that temperature. The mixture was then allowed to warm to 0 °C in 1 h, neutralized with 10% aqueous H_3PO_4, and concentrated *in vacuo*. The residue was partitioned between H_2O (50 ml) and CH_2Cl_2 (100 ml). The aqueous layer was extracted with CH_2Cl_2 (50 ml). The combined CH_2Cl_2 extracts were concentrated *in vacuo* after drying over $MgSO_4$ to leave a partly crystallized residue. The ratio of (*E*)- and (*Z*)-**5** in this mixture was estimated at *ca.* 7 : 2 on the basis of ^{1}H-NMR spectroscopy. The residue was triturated with MeOH (40 ml). The insoluble solid was collected by filtration, washed with MeOH (10 ml), and dried to afford a 7 : 1 mixture of (*E*)- and (*Z*)-**5** (1.11 g, 50%), mp 140—144 C. The combined filtrate and washings were concentrated and purified by flash chromatography [50 mm; AcOEt–EtOH (10 : 1, v/v)] to give a mixture of almost equal amounts of (*E*)- and (*Z*)-**5** (685 mg, 31%), an equimolar mixture of **5** [*E* : *Z* = 11 : 3] and **12** (160 mg), and **12** (237 mg, 12%). The 7 : 1 mixture of (*E*)- and (*Z*)-**5** described above was recrystallized from MeOH (50 ml) to give almost pure (*E*)-**5** (636 mg, 28%) as slightly yellow needles, mp 148—151 °C. This sample was identical with the analytical sample described under method (i) on the basis of infrared and ^{1}H-NMR spectral comparisons.

1-Benzyl-1,4-dihydro-4,6,7-trimethyl-9*H*-imidazo[1,2-*a*]purin-9-one (12) A solution of **9**·1/2H_2O (699 mg, 1.06 mmol) in anhydrous Me_2NCHO (2 ml) was cooled to −60 °C and *n*-BuLi (1.55 M solution in hexane, 0.70 ml, 1.09 mmol) was added dropwise over a period of 5 min. The mixture was stirred at that temperature for a further 15 min and then allowed to warm to −30 °C in 10 min. H_2O (1 ml) was added to the mixture and stirring was continued for another 10 min. The mixture was neutralized with 10% aqueous H_3PO_4, allowed to warm to room temperature, and concentrated *in vacuo*. H_2O (3 ml) was added to the residue and the mixture was extracted with CH_2Cl_2 (3 × 3 ml). The combined organic layers were dried over $MgSO_4$ then concentrated *in vacuo*. The residue was purified by flash chromatography [40 mm; AcOEt–EtOH (10 : 1, v/v)] to afford **12** (100 mg, 31%), mp 200—202 °C. Recrystallization from MeOH gave colorless needles, mp 202—203 °C, identical with an authentic sample.[4]

1-Benzyl-1,4-dihydro-4,6-dimethyl-7-(3-methylbutyl)-9*H*-imidazo[1,2-*a*]purin-9-one (10) A solution of (*E*)-**5** (90 mg, 0.25 mmol) in EtOH (33 ml) was shaken under H_2 with 10% Pd–C (90 mg) at 50 °C for 5 h. The catalyst was filtered off and washed with hot EtOH (30 ml). The combined filtrate and washings were concentrated *in vacuo* to leave a colorless solid (89 mg). Purification by flash chromatography [20 mm; AcOEt–EtOH (10 : 1, v/v)] gave **10** (66 mg, 73%), mp 138 °C (softened below the melting point). Recrystallizations from MeOH gave an analytical sample as colorless needles, mp 139 °C (softened below the melting point); UV $\lambda_{max}^{95\%\ EtOH}$ nm (ε): 238 (30000), 261 (sh) (6300), 320 (5200); ^{1}H-NMR δ: 0.96 (6H, d, J=6 Hz, Me_2), 1.32 [1H, br, C(3′)H], 1.60 [2H, m, C(2′)H_2], 2.25 [3H, s, C(6)Me], 3.06 [2H, br t, J=7 Hz, C(1′)H_2], 3.88 (3H, s, NMe), 5.61 (2H, s, PhCH_2), 7.35 (5H, s, Ph), 7.59 [1H, s, C(2)H]; MS *m/z*: 363 (M^+), 306 ($M^+ - Me_2CHCH_2$). *Anal.* Calcd for $C_{21}H_{25}N_5O$: C, 69.39; H, 6.93; N, 19.27. Found: C, 69.28; H, 6.95; N, 18.98.

(*R,*S**)-1-Benzyl-1,4-dihydro-7-(1,2-dihydroxy-3-methylbutyl)-4,6-dimethyl-9*H*-imidazo[1,2-*a*]purin-9-one (6)** A mixture of (*E*)-**5** (451 mg, 1.25 mmol), *N*-methylmorpholine oxide monohydrate (179 mg, 1.32 mmol), OsO_4 (4 mg, 0.016 mmol), H_2O (0.5 ml), and acetone (33 ml) was stirred at room temperature for 5 h. The mixture was concentrated *in vacuo*. The residue was neutralized with 10% aqueous H_3PO_4 after addition of H_2O (10 ml), and then extracted with $CHCl_3$ (3 × 10 ml). The combined $CHCl_3$ extracts were dried over $MgSO_4$ and concentrated *in vacuo* to leave a brown oil. This was purified by flash chromatography [40 mm; AcOEt–EtOH (10 : 1, v/v)]. As the less polar substance, **3** (9 mg, 2.2%), mp 221—225 °C, was obtained. Compound **6** (392 mg, 80%) was obtained from the more polar fractions, mp 148—153 °C. Recrystallizations from EtOH gave an analytical sample as colorless needles, mp 156—158 °C; UV $\lambda_{max}^{95\%\ EtOH}$ nm (ε): 243 (33900), 363 (sh) (6100), 319 (5700); ^{1}H-NMR δ: 0.91 (6H, d, J=6 Hz, CMe_2), 1.32 [1H, br, C(3′)H], 2.36 [3H, s, C(6)Me], 3.15 [1H, br, C(2′)OH], 3.77 [1H, m, C(2′)H], 3.95 (3H, s, NMe), 4.72 [1H, dd, J=12, 9 Hz, C(1′)H], 5.21 [1H, d, J=12 Hz, C(1′)OH], 5.60 (2H, s, CH_2), 7.36 (5H, s, Ph), 7.73 [1H, s, C(2)H]. *Anal.* Calcd for $C_{21}H_{25}N_5O_3$: C, 63.78; H, 6.37; N, 17.71. Found: C, 63.72; H, 6.40; N, 17.70.

(±)-1,4-Dihydro-7-(2-hydroxy-3-methylbutyl)-4,6-dimethyl-9*H*-imidazo[1,2-*a*]purin-9-one (2) Compound **6** (200 mg, 0.506 mmol) was hydrogenated in MeOH (15 ml) over 10% Pd–C (400 mg) under atmospheric pressure at *ca.* 60 °C for 10.5 h. The catalyst (400 mg) was added again and the reduction was continued for a further 12 h. The catalyst was filtered off and washed with hot EtOH (200 ml). The combined filtrate and washings were concentrated *in vacuo* to leave a solid. This was washed with $CHCl_3$ (1 ml), giving **2** (15 mg), mp *ca.* 280 °C (softened below the melting point). The $CHCl_3$ washings were concentrated to a small volume and purified by layer chromatography on silica gel [AcOEt–EtOH (5 : 1, v/v)] to furnish a second crop of **2** (19 mg). The catalyst was extracted with EtOH using a Soxhlet extractor and the crude product was similarly purified by layer chromatography to afford a third crop of **2** (6 mg; the total yield was 27%). Recrystallizations from MeOH gave an analytical sample as colorless plates, mp 278—282 °C (dec.); UV $\lambda_{max}^{95\%\ EtOH}$ nm (ε): 236 (34500), 258 (sh) (5800), 313 (5400); $\lambda_{max}^{H_2O}$ (pH 1): 234 (37600), 288 (7700); $\lambda_{max}^{H_2O}$ (pH 7): 236 (35500), 263 (5900), 313 (5300); $\lambda_{max}^{H_2O}$ (pH 13): 236 (36200), 268 (5800), 305 (7500); ^{1}H-NMR [$(CD_3)_2SO$] δ: 0.92 (6H, d, J=6 Hz, CMe_2), 1.57 [1H, m, C(3′)H], 2.14 [3H, s, C(6)Me], 2.61 and 2.85 [1H each, dd, J=14, 9 Hz, C(1′)H_2], 3.50 [1H, m, C(2′)H], 3.76 (3H, s, NMe), 4.19 (1H, d, J=6 Hz, OH), 8.15 [1H, s, C(2)H], 13.52 (1H, br, NH); MS *m/z*: 289 (M^+), 216 ($M^+ - Me_2CHCHOH$). *Anal.* Calcd for $C_{14}H_{19}N_5O_2$: C, 58.11; H, 6.62; N, 24.21. Found: C, 57.81; H, 6.65; N, 24.51.

***O*-Benzyl-*N*-(methoxycarbonyl)-L-serine (15)** MeOCOCl (0.56 g, 5.9 mmol) was added to an ice-cooled suspension of *O*-benzyl-L-serine[23] (976 mg, 5.0 mmol) and $NaHCO_3$ (1.25 g) in H_2O (12.5 ml) with stirring. The mixture was stirred at room temperature for 100 min and MeOCOCl (0.28 g) was added again, and stirring was continued for a further 80 min. The mixture was brought to pH 1 by addition of 10% aqueous HCl and extracted with Et_2O (2 × 20 ml). The Et_2O solution was dried over $MgSO_4$ and concentrated *in vacuo* to leave a colorless solid (1.22 g, 96%), mp 88—91 °C. Recrystallizations from benzene gave an analytical sample as colorless plates, mp 94—95 °C; $[\alpha]_D^{20}$ +23.5 ± 0.1° (c=1.50, MeOH); ^{1}H-NMR δ: 3.70 (3H, s, Me), 3.71 and 3.94 (1H each, dd, J=9, 3 Hz, CH_2CH), 4.51 (1H, m, CH), 4.54 (2H, s, PhCH_2), 5.59 (1H, d, J=8 Hz, NH), 7.30 (5H, m, Ph), 7.57 (1H, br, CO_2H). *Anal.* Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.04; H, 5.97; N, 5.79.

***O*-Benzyl-*N*-(methoxycarbonyl)-L-serine Methyl Ester (16)** A solution of Me_3SiCHN_2 in Et_2O[24] was added to a solution of **15** (507 mg, 2.0 mmol) in a mixture of benzene (14 ml) and MeOH (4 ml) until the yellow color persisted. The resulting solution was concentrated *in vacuo* to leave a colorless solid (531 mg, 99%), mp 31—32 °C; $[\alpha]_D^{22}$ +0.3°, $[\alpha]_{365}^{24}$ +1.61° ± 0.08 (c=1.24, MeOH); ^{1}H-NMR δ: 3.69 and 3.75 (3H, each, two Me's), 3.69 and 3.88 (1H each, dd, J=9, 3 Hz, CH_2CH), 4.44 (1H, m, CH), 4.51 (2H, s, PhCH_2), 5.57 (1H, d, J=8 Hz, NH), 7.30 (5H, m, Ph). Recrystallization of this compound failed.

(*R*)-[1-(Benzyloxymethyl)-2-hydroxyethyl]carbamic Acid Methyl Ester (17) A mixture of **16** (531 mg, 1.99 mmol), $NaBH_4$ (151 mg, 4.0 mmol),

LiCl (170 mg, 4.0 mmol), anhydrous EtOH (16 ml), and anhydrous THF (12 ml) was stirred at room temperature under N_2 for 6 h. After addition of acetone (1 ml), the mixture was neutralized with 10% aqueous H_3PO_4 and then concentrated *in vacuo*. The residue was partitioned between H_2O (10 ml) and Et_2O (20 ml). The aqueous layer was extracted with Et_2O (20 ml). The combined Et_2O layers were dried over $MgSO_4$ and concentrated *in vacuo* to leave **17** (457 mg, 96%) as a colorless oil, $[\alpha]_D^{19}$ $+19.2 \pm 0.09°$ ($c = 1.12$, MeOH); 60 MHz ^{1}H-NMR δ: 2.52 (1H, br, OH), 3.65 (3H, s, Me), 3.4—4.1 [5H, m, $(CH_2)_2CH$], 4.50 (2H, s, $PhCH_2$), 5.30 (1H, br, NH), 7.23 (5H, s, Ph).

(*S*)-[1-(Benzyloxymethyl)-2-(*tert*-butyldimethylsilyloxy)ethyl]carbamic Acid Methyl Ester (19) A mixture of **17** (850 mg, 3.55 mmol), imidazole (604 mg, 8.87 mmol), *tert*-$BuMe_2SiCl$ (642 mg, 4.26 mmol) and anhydrous Me_2NCHO (2 ml)[25] was stirred at room temperature for 1.5 h. H_2O (1 ml) was added and stirring was continued for several minutes. The mixture was then partitioned between H_2O (9 ml) and Et_2O (10 ml). The aqueous layer was extracted with Et_2O (10 ml). The combined Et_2O extracts were washed successively with 10% aqueous citric acid (2 × 10 ml) and saturated aqueous $NaHCO_3$ (10 ml), dried over $MgSO_4$, and concentrated *in vacuo* to leave **19** (1.20 g, 95%) as a slightly yellow oil; 60 MHz ^{1}H-NMR δ: 0.05 (6H, s, $SiMe_2$), 0.87 (9H, s, CMe_3), 3.3—4.0 [5H, m, $(CH_2)_2CH$], 3.60 (3H, s, OMe), 4.43 (2H, s, $PhCH_2$), 4.90 (1H, br, NH), 6.83 (5H, s, Ph).

(*S*)-[1-[(*tert*-Butyldimethylsilyloxy)methyl]-2-hydroxyethyl]carbamic Acid Methyl Ester (20) A solution of **19** (1.61 g, 4.55 mmol) in EtOH (30 ml) was hydrogenated over 10% Pd–C (200 mg) at 50 °C and atmospheric pressure for 6 h. Further catalyst (1.00 g) was added and the reduction was continued for another 3.5 h. The catalyst was filtered off and washed with EtOH (30 ml). The combined EtOH solution was concentrated *in vacuo* to leave **20** (1.16 g, 97%) as a colorless oil, 60 MHz ^{1}H-NMR δ: 0.07 (6H, s, $SiMe_2$), 0.87 (9H, s, CMe_3), 2.50 (1H, br, OH), 3.60 (3H, s, OMe), 3.68 [5H, br, $(CH_2)_2CH$], 5.17 (1H, br, NH).

(*S*)-[1-(Benzyloxymethyl)-2-oxoethyl]carbamic Acid Methyl Ester (18) A solution of SO_3–pyridine complex (1.91 g, 12 mmol) in anhydrous Me_2SO (12 ml) was added to a stirred mixture of **17** (957 mg, 4.0 mmol) and Et_3N (1.21 g, 12 mmol) in anhydrous Me_2SO (12 ml), and the resulting mixture was stirred for 10 min, during which time the temperature was kept at room temperature by occasional cooling. The resulting solution was poured onto crushed ice (*ca.* 120 ml) and the mixture was extracted with CH_2Cl_2 (3 × 80 ml). The combined extracts were washed successively with cold 10% aqueous citric acid (2 × 30 ml), saturated aqueous NaCl (2 × 30 ml), and saturated aqueous $NaHCO_3$, then dried over $MgSO_4$. Removal of the solvent by evaporation gave crude **18** (656 mg, 69% yield) as a slightly yellow oil, $[\alpha]_D^{18}$ $+24.6 \pm 0.2°$ ($c = 1.00$, CH_2Cl_2). Although this type of optically active amino aldehyde is known to easily undergo racemization on silica gel,[10] this product was rapidly purified by flash chromatography [30 mm; AcOEt–hexane (1 : 1, v/v)] at the cost of optical purity, giving chromatographically pure **18** (0.39 g), $[\alpha]_D^{19}$ $+15.5°$ ($c = 1.00$, CH_2Cl_2); 60 MHz ^{1}H-NMR δ: 3.63 (3H, s, Me), 3.5—4.4 (3H, m, CH_2CH), 4.42 (2H, s, $PhCH_2$), 5.52 (1H, d, $J = 8$ Hz, NH), 7.17, (5H, s, Ph), 9.43 (1H, s, CHO).

(*R*)-[1-[(*tert*-Butyldimethylsilyloxy)methyl]-2-oxoethyl]carbamic Acid Methyl Ester (21) Compound **20** (1.096 g, 4.2 mmol) was treated in a manner similar to that described for the preparation of **18** to give **21** (970 mg, 89%) as a slightly yellow oil, $[\alpha]_D^{20}$ $-30.8 \pm 0.5°$ ($c = 1.00$, CH_2Cl_2). The purity of this sample was estimated at 75 mol% on the basis of ^{1}H-NMR spectroscopy. A portion of this sample was purified by repeated flash chromatography [$CHCl_3$–MeOH (200 : 3, v/v) and then hexane–AcOEt (2 : 1, v/v)], giving **21** as a colorless oil, 60 MHz ^{1}H-NMR δ: 0.05 (6H, s, $SiMe_2$), 0.87 (9H, s, CMe_3), 3.67 (3H, s, OMe), 3.7—4.4 (3H, m, CH_2CH), 5.45 (1H, br, NH), 9.50 (1H, s, CHO).

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