Synthesis and Absolute Configuration of Wybutine, the Fluorescent Minor Base from Phenylalanine Transfer Ribonucleic Acids

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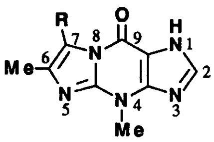
The phosphonium chloride 6 having an optically active amino acid moiety was synthesized from (*S*)-serine benzyl ester tosylate (2b) through a six-step route. The utility of 6 as a reagent for the Wittig reaction was exemplified in the olefination with benzaldehyde, affording the (*E*)-β,γ-unsaturated amino acid derivative 11 as a sole geometrical isomer. This new method of amino acid homologation was successfully employed for the first chiral synthesis of wybutine (1c), the minor base isolated from yeast phenylalanine transfer ribonucleic acids: the Wittig reaction between 6 and the tricyclic aldehyde 16 followed successively by methylation and catalytic reduction afforded 1c. Comparison of wybutine with synthetic 1c has unequivocally established that wybutine has an *S* configuration.

Keywords wybutine; Wittig reaction; β,γ-unsaturated amino acid; chiral synthesis; amino acid homologation; phenylalanine transfer ribonucleic acid; hypermodified base; stereoselective olefination; alanine synthon; absolute configuration

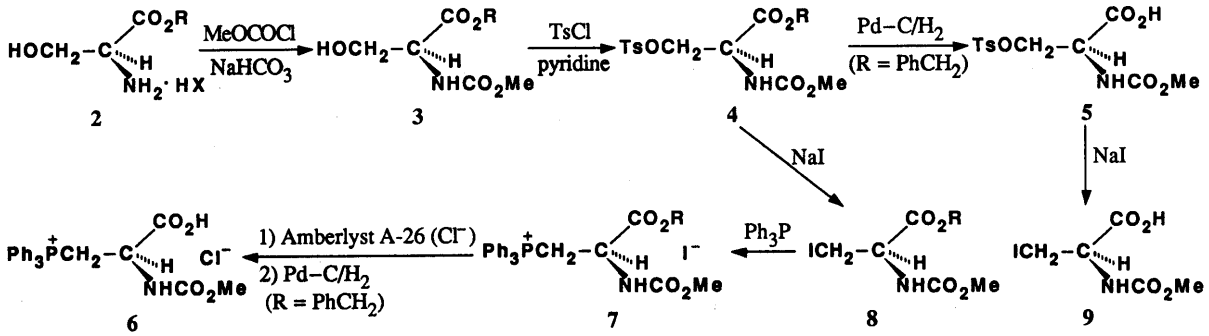
The base sequences of more than 450 transfer ribonucleic acids (tRNAs) have been determined[1] since the publication of the pioneering work by Holley *et al.* on yeast alanine tRNA in 1965.[2] The most prominent feature of tRNAs is a frequent occurrence of modification at the base moiety, and more than 50 modified nucleosides or bases from tRNAs have been characterized.[1,3] RajBhandary *et al.* determined the base sequence of yeast phenylalanine tRNA ($tRNA^{Phe}$)[4] and discovered a fluorescent component at the next position to the 3′-end of the anticodon.[5] The chromophore was isolated as the nucleoside, wybutosine,[5,6] whose *N*-glycosidic bond was shown to be unusually susceptible to acidic hydrolysis by Thiebe and Zachau.[6] Thus the fluorescent base, wybutine, was selectively obtained by mild acid treatment of the tRNA,[6] and the structure **1c** was assigned to this base by Nakanishi's and Zachau's groups without determining the stereochemistry.[7] One of the fluorescent bases isolated from rat and calf liver $tRNAs^{Phe}$ was also reported to be wybutine.[8] The congeners **1a**, **b**, **d**—**f** have subsequently been isolated from $tRNAs^{Phe}$ of various eukaryotic species and unfractionated tRNAs of archaebacteria.[9] The chemical structures of the members of the family **1** are quite unique because they embody not only a 3-methylguanine skeleton but also a condensed tricycle: no other 3-methylguanine derivative has been reported to occur naturally and no condensed tricyclic base other than **1** has been found in nucleic acids. Nakanishi *et al.* achieved a synthesis of the racemic modification of **1c** by cyclocondensation of 3-methylguanine with (±)-5-bromo-2-[(methoxycarbonyl)amino]-6-oxoheptanoate, ascertaining the correctness of the two-dimensional structure of wybutine.[10] They also reported that the reaction of 7-benzyl-3-methylguanine with the bromoketone followed by catalytic hydrogenolysis gave a better result.[11] The absolute configuration of wybutine was reported to be *S* by comparison of the circular dichroism (CD) spectrum of *N*-(methoxycarbonyl)glutamic acid dimethyl ester, obtained from a degradation product of wybutine, with that of an authentic sample derived from (*S*)-glutamic acid.[10] Unfortunately, the dimethyl ester obtained from wybutine was not fully characterized and there is an inconsistency in this report.[12] Since wybutine is available in only a minute quantity from $tRNA^{Phe}$, we planned a synthesis of optically active **1c** for unambiguous determination of the absolute configuration of wybutine. This paper reports the first chiral synthesis of **1c**.[13]



1

a: R = H
b: R = Me
c: R = CH_2CH_2–C(CO_2Me)(H)$NHCO_2$Me (γ, β, α)
d: R = $CH_2CH(OH)CH(NH_2)CO_2H$
e: R = $CH_2CH(OH)CH(NHCO_2Me)CO_2Me$
f: R = $CH_2CH(O_2H)CH(NHCO_2Me)CO_2Me$

In preceding papers, we described some model experiments for construction of a side chain at the 7-position of 1-benzylwye, and found that the Wittig reaction on 1-benzyl-7-formylwye (**16**) was most promising.[14] Along this line, the phosphonium reagent **7a** was required for the preparation of **18**, which appeared to be a good intermediate for the synthesis of not only **1c** but also its congeners **1d**—**f**. We first attempted to synthesize **7a** (TsO^- for I^-) by metathesis of the tosylate **4a**, which is easily accessible from (*S*)-serine methyl ester hydrochloride (**2a**: X = Cl) according to the procedure reported for the *N*-benzyloxycarbonyl analog.[15] The reaction of **4a** with triphenylphosphine in *N*,*N*-dimethylformamide (DMF), however, took place only sluggishly at 40 °C and that at a more elevated temperature failed to afford the pure phosphonium salt. We then converted **4a** into the iodide **8a** by following the general method.[16] The reaction of **8a** with triphenylphosphine in DMF at 50 °C gave a mixture, whose proton nuclear magnetic resonance (^{1}H-NMR) spectrum indicated the presence of **7a** and methyltriphenylphosphonium iodide. When the reaction was conducted in toluene under reflux, 2-[(methoxycarbonyl)amino]-2-propenoic acid methyl ester,[17] a product formed *via* β-elimination,[18] was also obtained. Although the product obtained in the reaction at 80 °C was still contaminated with methyltriphenylphosphonium iodide, prolonged reaction at 50 °C gave pure **7a** in good yield. Nevertheless, treatment of **7a** with *n*-butyllithium in tetrahydrofuran (THF) at −78 °C followed



a: R = Me
b: R = $PhCH_2$

Chart 1

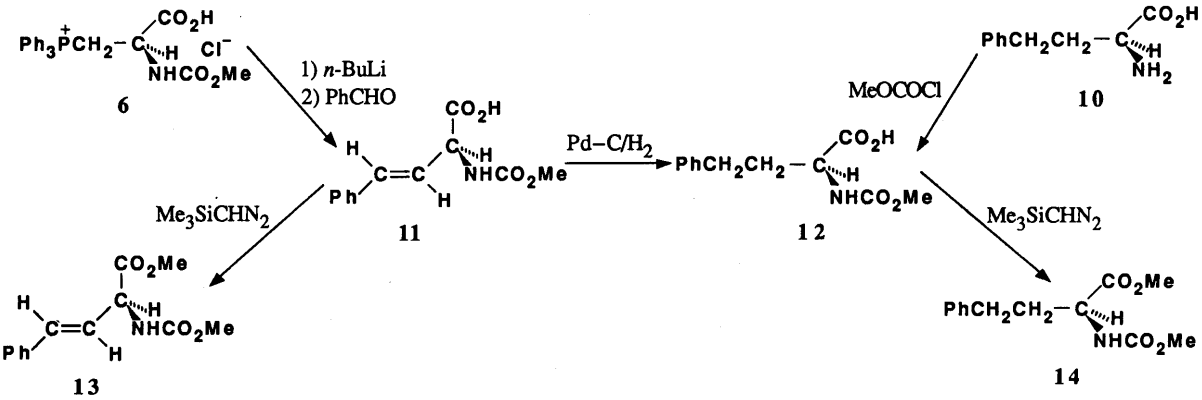
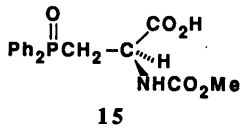


Chart 2

by addition of 1-benzyl-7-formylwye (**16**) resulted in the β-elimination in preference to the Wittig reaction. We considered that the corresponding phosphonium salt **6** with a free carboxy group might not undergo the β-elimination in view of a successful precedent with (2-carboxyethyl)-triphenylphosphonium chloride.[19] An attempt to obtain **6** by treatment of **7a** with sodium hydroxide in aqueous methanol again resulted in the β-elimination. We then prepared **7b** through the iodide **8b**, which was synthesized from (*S*)-serine benzyl ester *p*-toluenesulfonate (**2b**, X=TsO),[20] in a manner similar to that described for **7a**. Although direct hydrogenolysis of **7b** was not achieved, conversion of the iodide **7b** into the chloride followed by hydrogenolysis over Pd–C afforded **6** in 59% overall yield based on (*S*)-serine. We failed in an alternative synthesis of **6** (I^- for Cl^-) by the reaction of triphenylphosphine with (*R*)-3-iodo-*N*-(methoxycarbonyl)alanine (**9**), which was obtained by hydrogenolysis of **4b** followed by metathesis with sodium iodide, because of its poor reactivity.

In order to examine the behavior of **6** in the Wittig reaction, we first performed the reaction of **6** with benzaldehyde using 3 molar eq of *n*-butyllithium in a mixed solvent of THF and hexamethylphosphoric triamide (HMPA), obtaining [*S*-(*E*)]-2-(methoxycarbonyl)amino-4-phenyl-3-butenoic acid (**11**) (isolated as the methyl ester **13** in 28% yield). Even the use of 2 molar eq of the base afforded **11**, but in somewhat lower yield. An *E* configuration of **11** was assignable on the basis of the coupling constant (16 Hz) observed for the olefinic protons. The formation of the (*Z*)-isomer of **11** was not observed by ^{1}H-NMR spectroscopy, although nonstabilized triphenylphosphorus ylides often produce (*Z*)-alkenes preferentially.[21] The observed stereoselectivity may be interpreted by analogy with the preponderance of (*E*)-alkenes reported for similar reactions of nonstabilized ylides bearing an oxido, carboxylato, or amido anion,[21b] but nevertheless, such a marked preference for the (*E*)-isomer in the reaction of **6** is noteworthy. A major side product of this reaction was the phosphine oxide **15**. On hydrogenation over Pd–C **11** afforded (*S*)-2-[(methoxycarbonyl)amino]-4-phenylbutanoic acid (**12**) in 27% overall yield. The specific rotation of this sample was identical with that of an authentic specimen of **12** [$[\alpha]_{365}^{25}$ +30.2° (MeOH)] derived from (*S*)-homophenylalanine (**10**).[22] We can not, however, say whether the configuration of the chiral center was completely retained throughout these transformations, since we can not rule out the possibility that **12** thus obtained from **6** was contaminated by a trace of **15** having a large specific rotation [$[\alpha]_{365}^{24}$ +98.5° (MeOH)] because of the difficulty of purification. Compound **12** was then converted into the methyl ester **14**, from which the methyl ester of **15** was easily removable by chromatography. The specific rotation of **14** thus obtained was 92% of that of an authentic sample derived from **10** *via* **12**.[23]



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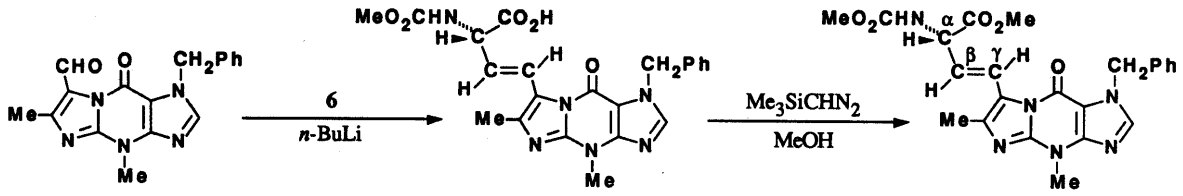
The next step toward access to wybutine should be

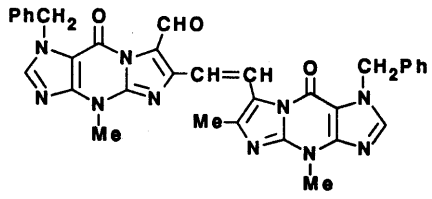


16 17 18

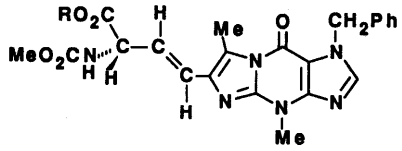
Chart 3

utilization of the aldehyde **16** as a substrate of the present method of constructing optically active β,γ-unsaturated amino acid derivatives. However, the reaction between **16** and the phosphorane generated from **6** gave poorer results than the case of benzaldehyde and proved to depend markedly on subtle changes of the reaction conditions. The best result was obtained when **6** was dried over molecular sieves in a mixture of THF and HMPA, then treated with 3 molar eq of *n*-butyllithium at $-78\,^{\circ}C$, and the resulting phosphorane was then allowed to react with **16** at $-78\,^{\circ}C$ to $-18\,^{\circ}C$. Flash chromatography[24] of the crude product afforded unchanged **16** (28% recovery), a mixture of products, whose ^{1}H-NMR spectrum suggested that the main component was **19**, and a polar fraction containing the desired acid **17**. Compound **19** should be a product formed through vinylogous aldol condensation. For obtaining **17**, it was indispensable to dry the solution of the phosphonium salt **6** over molecular sieves before use; otherwise no **17** was formed, but **15** was produced in 83% yield. When the temperature of the Wittig reaction was raised to room temperature at the final stage, a small amount of the rearranged product **20a** was suggested to be formed. Although we obtained its methyl ester **20b** as a mixture with the methyl ester of **15**, we could not purify it because of the instability of this type of compounds.[14b] Compound **20b**[25] was also obtained as a yellow oil in 3% yield by the Wittig reaction using sodium (methylsulfinyl)methanide in dimethyl sulfoxide at room temperature followed by methylation. We previously reported on this type of rearrangement of a model compound under similar conditions.[14b] Analogous transformations through cleavage of a pyrimidone ring followed by recyclization have been recorded.[26]

Compound **17**, which was contaminated with other polar products, was isolated as the methyl ester **18** by flash chromatography after treatment with trimethylsilyldiazomethane[27] in 16% yield.[28] In this case again, no (*Z*)-isomer of **18** was detected by ^{1}H-NMR spectroscopy. It should be noted that the β,γ-unsaturated amino acid ester **18** underwent racemization when the methylated mixture was allowed to stand at room temperature without acidification. This was probably caused by contaminating bases such as **21**.[29] Indeed, **16**, which should be formed through cyclization of **21**, was detected by means of ^{1}H-NMR spectroscopy in the methylated mixture. The ^{1}H-NMR spectrum of **18** is in accord with that of a model compound, (*E*)-1-benzyl-7-(3-methyl-1-butenyl)wye (**22**),[14b] except for the C(γ)-olefinic proton, which is more deshielded by 0.5 ppm than the corresponding one [C(1′)-H] of **22**. The ultraviolet (UV) spectrum of **18** [$\lambda_{max}^{95\%\ EtOH}$ 232 nm (ε 21200), 260 (21600), 294 (13300), 322 (sh) (7400)], however, exhibited a marked difference from that of **22** [$\lambda_{max}^{95\%\ EtOH}$

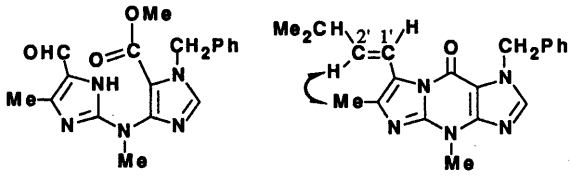


19



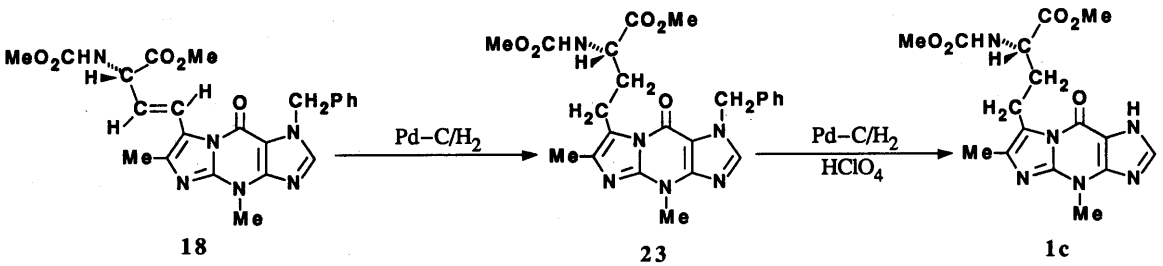
20

a: R = H
b: R = Me



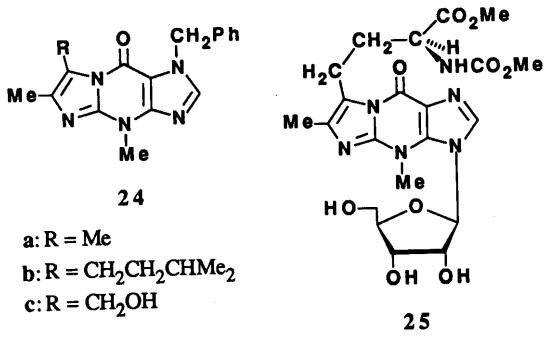
21 22

254 nm (ε 25500), 282 (sh) (8200), 324 (5200).[14b] This should stem from the different extent in conjugation of the exocyclic double bond with the tricycle, because the UV spectrum of compound (±)-**23**[30] with a saturated side chain was practically identical with the spectra of the model compounds **24**.[14] A consideration of a space-filling molecular model for **22** suggested that the exocyclic double bond should be arranged as the s-*cis* form so that the maximum conjugation is attained. This was supported by the nuclear Overhauser effect (NOE)[31]: when the C(6)-Me resonance was irradiated, the enhancement obtained for C(2′)-H was 22%, whereas that for C(1′)-H was only 3%. With **18** also, saturation of the C(6)-Me resonance gave 28% and 2% enhancements of the intensities of the C(β)-H and C(γ)-H signals, respectively, indicating that there was no substantial difference in conformation between **22** and **18**. It is unlikely that the inductive effect of the functional groups of **18** alone brings about the UV spectral difference. We might suppose that the exocyclic double bond in **18** is forced slightly out of the plane of the heterocycle owing to an intramolecular hydrogen bonding between the amino hydrogen at the side chain and the carbonyl oxygen at the 9-position to cause some decrease in the conjugation. However, we found no evidence for such hydrogen bonding in the stretching absorption band due to the carbonyl at the 9-position. A π-stacking interaction between the phenyl



18 23 1c

Chart 4



24

a: R = Me
b: R = $CH_2CH_2CHMe_2$
c: R = CH_2OH

25

of the 1-benzyl group and the carbamate of the side chain might be another factor in the supposed deviation of the coplanarity. In such a defined conformer, the C(γ)-H resonance observed at unexpectedly low magnetic field may be interpreted as a result of the anisotropic effect of the carbamate group.[32]

Now that a key intermediate **18** had become available, saturation of the side chain and removal of the benzyl group were necessary as the next steps for access to **1c**. Although debenzylation of (±)-**23** had been accomplished by Nakanishi's group by hydrogenolysis over Pd–C in 2-propanol in the presence of acetic acid and hydrochloric acid with special care,[11] we had smoothly removed the benzyl group from 1-benzyl-7-methylwye (**24a**) by hydrogenolysis over Pd–C in methanol in the presence of aqueous perchloric acid.[14a] However, we previously experienced the formation of a by-product when 1-benzyl-7-(hydroxymethyl)wye (**24c**) was subjected to hydrogenolysis under similar conditions, probably owing to acid-catalyzed generation of the stabilized carbocation.[14a] To avoid the predictable formation of such a carbocation we first saturated the side chain of **18** over 10% Pd–C in the absence of acid. The reduction was continued by addition of perchloric acid to afford **1c** as the monohydrate [$[\alpha]_D^{23}$ −45° (MeOH)] in 75% yield. The UV, mass (MS), and ^{1}H-NMR spectra of **1c** thus obtained were identical with those of (±)-**1c**[10,33] confirming the correctness of the structure in a two-dimensional sense. We have already assigned the 1,4-dihydro structure [N(1)-H tautomer] to **1a** rather than the alternative 3,4-dihydro structure [N(3)-H tautomer] on the basis of UV spectral comparison with model compounds.[34] This assignment has been supported by recent fluorescence studies on **1a** and related compounds.[35] The UV spectrum of **1c** determined in 95% aqueous ethanol resembles that of (±)-1-benzylwybutine [(±)-**23**] rather than that of 3-β-D-ribofuranosylwybutine (**25**),[36] suggesting that **1c** also exists as the 1,4-dihydro structure [N(1)-H tautomer].

The identity of wybutine had been established by comparison of the MS[7a] and ^{1}H-NMR spectra[7,37] with those of (±)-**1c**.[10] Further evidence in support of the identity was provided by direct comparison of wybutine[38] with the present sample of synthetic **1c** by means of high-performance liquid chromatography (HPLC). Although comparison of the CD spectrum of wybutine[39] with that of synthetic **1c** enabled us to assign an *S* configuration to wybutine, the intensity of the Cotton effect at 235 nm reported for wybutine was only a half of that of the present sample of **1c**. Direct comparison of the CD spectra of natural[38] and synthetic **1c** revealed that they were superimposable except for the larger intensity (1.2 times) of the latter. The same intensity relationship was also recognized in the UV spectra of natural and synthetic **1c**; the low potency of the natural sample was probably due to purification difficulties because of the minute amount available, as had already been noted.[10] Although these results suggested that synthetic **1c** was equivalent to the natural one in optical purity, both samples were ultimately demonstrated to be enantiomerically pure by means of HPLC using a chiral column.

In conclusion, we have established a new method for the synthesis of optically active β,γ-unsaturated α-amino acid derivatives by use of the phosphonium chloride **6** as a synthetic equivalent for the nucleophilic alanine synthon.[40] This method enabled us to perform the first chiral synthesis of **1c** and to assign an *S* configuration to wybutine.

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 JASCO J-500C spectropolarimeter equipped with a JASCO DP-500N data processor, a Hitachi M-80 mass spectrometer, and a JEOL JNM-FX-100 NMR spectrometer at 25 °C with tetramethylsilane as an internal standard. Optical rotations were measured with a JASCO DIP-181 polarimeter using a 1-dm sample tube. The liquid chromatographic 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. Microanalyses were determined by Mr. Y. Itatani and his associates at Kanazawa University. Pre-coated silica gel plates (0.25 mm) with a fluorescent indicator (Merck) were used for analytical thin-layer chromatography (TLC). Flash chromatography was performed on silica gel according to the reported procedure.[24] The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, ddd = doublet-of-doublets-of-doublets, dt = doublet-of-triplets, m = multiplet, q = quartet, s = singlet, sh = shoulder, t = triplet.

(*S*)-*N*-(Methoxycarbonyl)serine Methyl Ester (3a) Methyl chloroformate (11.3 g, 120 mmol) was added to a pre-cooled (10 °C) solution of (*S*)-serine methyl ester hydrochloride (**2a**: X = Cl) (15.56 g, 100 mmol) in water (250 ml) in the presence of sodium bicarbonate (25 g) over a period of 5 min with vigorous stirring on a magnetic stirrer. The mixture was stirred at room temperature for a further 5 min, brought to pH 6 with 10% hydrochloric acid, and concentrated *in vacuo* to *ca.* 70 ml. The resulting solution was extracted with dichloromethane (8 × 50 ml). The

combined organic layers were dried over magnesium sulfate and then concentrated *in vacuo* to leave a colorless heavy oil (16.53 g, 93%), $[\alpha]_D^{19}$ −21.6° (c=1.00, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 2.30 (1H, s, OH), 3.71 and 3.79 (3H each, s, two Me's), 3.91 and 3.99 (1H each, dd, J=10.5, 3.5 Hz, CH_2), 4.42 (1H, m, CH), 5.74 (1H, d, J=7.5 Hz, NH).

(*S*)-*N*-(Methoxycarbonyl)serine Benzyl Ester (3b) (*S*)-Serine benzyl ester *p*-toluenesulfonate (**2b**: X=TsO), which was prepared from (*S*)-serine (25.27 g, 240 mmol) according to the reported procedure,[20] was dissolved in water (600 ml). Sodium bicarbonate (60.0 g, 714 mmol) and methyl chloroformate (22.3 ml, 288 mmol) were successively added to this solution under cooling in ice water over a period of 10 min with vigorous stirring. Stirring was continued for a further 1.5 h at room temperature. The resulting oily precipitate crystallized on being kept in a refrigerator. The crystals were collected by filtration and dried to give a first crop of **3b** (43.45 g), mp 39—44 °C. A second crop [3.27 g; the total yield was 76% based on (*S*)-serine] was obtained by extraction of the mother liquor with dichloromethane (3 × 200 ml) followed successively by drying over magnesium sulfate and concentration *in vacuo*. Recrystallization from 50% (v/v) aqueous methanol followed by drying over phosphorus pentoxide at 2 mmHg and room temperature for 18 h gave an analytical sample as colorless pillars, mp 42—44 °C; $[\alpha]_D^{13}$ −17.8° (c=1.05, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 1.77 (br, 1/5H_2O), 2.38 (1H, br, OH), 3.69 (3H, s, Me), 3.92 and 4.01 (1H each, dd, J=12, 3 Hz, C$\underline{H}_2$CH), 4.46 (1H, m, CH), 5.22 (2H, s, PhC$\underline{H}_2$), 5.71 (1H, brd, J=9 Hz, NH), 7.35 (5H, s, Ph). *Anal.* Calcd for $C_{12}H_{15}NO_5 \cdot 1/5H_2O$: C, 56.11; H, 6.04; N, 5.45. Found: C, 56.23; H, 6.01; N, 5.55.

(*S*)-*N*-(Methoxycarbonyl)-*O*-(*p*-toluenesulfonyl)serine Methyl Ester (4a) *p*-Toluenesulfonyl chloride (13.38 g, 70.1 mmol) was added to a solution of **3a** (10.34 g, 58.4 mmol) in dry pyridine (29 ml) at −10 °C over a period of 20 min with stirring. The mixture was stirred for a further 3.5 h at −10 °C then poured onto crushed ice (170 ml). The solid that separated was collected by filtration, washed with cold water (50 ml), and dried to give **4a** (16.03 g, 83%), mp 83—85 °C. Recrystallization from ethanol afforded an analytical sample as colorless prisms, mp 87—88 °C; $[\alpha]_D^{15}$ +6.6° (c=1.04, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 2.46 (3H, s, CMe), 3.65 and 3.72 (3H each, s, two OMe's), 4.32 (1H, dd, J=10, 3.5 Hz) and 4.41 (1H, dd, J=10, 3 Hz) (CH_2), 4.53 (1H, ddd, J=7, 3.5, 3 Hz, CH), 5.47 (1H, d, J=7 Hz, NH), 7.36 (2H, d, J=8 Hz, phenyl protons *meta* to the sulfonyl group), 7.76 (2H, d, J=8 Hz, phenyl protons *ortho* to the sulfonyl group). *Anal.* Calcd for $C_{13}H_{17}NO_7S$: C, 47.12; H, 5.17; N, 4.23. Found: C, 47.27; H, 5.20; N, 4.33.

(*S*)-*N*-(Methoxycarbonyl)-*O*-(*p*-toluenesulfonyl)serine Benzyl Ester (4b) A solution of **3b**·1/5H_2O (24.83 g, 96.7 mmol) in dry pyridine (120 ml) was cooled to −10 °C and *p*-toluenesulfonyl chloride (32 g, 170 mmol) was added over a period of 20 min. The resulting mixture was stirred at −10±5 °C for 3 h and then poured onto crushed ice (*ca.* 500 ml). The resulting solid was collected by filtration, washed with water (200 ml), and dried to give a slightly brown solid (32.08 g, 81%), mp 75—83 °C. Recrystallization from ethanol (30 ml) gave colorless needles (25.80 g, 65%), mp 89—90.5 °C. Further recrystallization from ethanol gave an analytical sample with unchanged melting point, $[\alpha]_D^{16}$ +2.7° (c=1.08, MeOH); MS m/z: 407 (M^+); ^{1}H-NMR ($CDCl_3$) δ: 2.43 (3H, s, CMe), 3.64 (3H, s, OMe), 4.33 and 4.40 (1H each, dd, J=9, 3 Hz, C$\underline{H}_2$CH), 4.55 (1H, ddd, J=9, 3, 3 Hz, CH), 5.10 and 5.19 (1H each, d, J=12 Hz, PhC$\underline{H}_2$), 5.53 (1H, d, J=9 Hz, NH), 7.31 (2H, d, J=10 Hz, phenyl protons *meta* to the sulfonyl group), 7.34 (5H, s, $\underline{Ph}CH_2$), 7.71 (2H, d, J=10 Hz, phenyl protons *ortho* to the sulfonyl group). *Anal.* Calcd for $C_{19}H_{21}NO_7S$: C, 56.01; H, 5.20; N, 3.44. Found: C, 55.99; H, 5.25; N, 3.23.

(*S*)-*N*-(Methoxycarbonyl)-*O*-(*p*-toluenesulfonyl)serine (5) A solution of **4b** (8.15 g, 20 mmol) in ethanol (280 ml) was hydrogenated over 10% Pd–C (0.82 g) at atmospheric pressure and room temperature for 35 min. The catalyst was filtered off and the filtrate was concentrated *in vacuo* to leave **5** (6.29 g, 99%), mp 127—131 °C. Recrystallization from ethanol gave an analytical sample as colorless pillars, mp 137—139 °C; $[\alpha]_D^{28}$ +19.4° (c=0.500, MeOH); ^{1}H-NMR [$(CD_3)_2SO$] δ: 2.43 (3H, s, CMe), 3.50 (3H, s, OMe), 4.06—4.42 (3H, m, CH_2 CH), 7.49 (2H, d, J=8 Hz, phenyl protons *meta* to the sulfonyl group), 7.60 (1H, d, J=8 Hz, NH), 7.77 (2H, d, J=8 Hz, phenyl protons *ortho* to the sulfonyl group), 13.07 (1H, br, CO_2H). *Anal.* Calcd for $C_{12}H_{15}NO_7S$: C, 45.42; H, 4.76; N, 4.41. Found: C, 45.37; H, 4.71; N, 4.47.

(*R*)-3-Iodo-2-[(methoxycarbonyl)amino]propanoic Acid (9) A solution of sodium iodide (0.68 g, 4.54 mmol) in dry acetone (4 ml) was added to a solution of **5** (1.07 g, 3.37 mmol) in dry acetone (7 ml) and the whole was stirred at 25 °C for 55 h. The resulting precipitate was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in chloroform (30 ml) and the solution was washed with saturated aqueous sodium chloride (2 × 20 ml), dried over magnesium sulfate, and concentrated *in vacuo* to leave a slightly yellow solid (0.706 g, 77%). Recrystallization from benzene gave an analytical sample as colorless prisms, mp 125—127 °C; $[\alpha]_D^{28}$ +22.7° (c=1.00, MeOH); ^{1}H-NMR [$(CD_3)_2SO$] δ: 3.33 (1H, dd, J=10, 8.5 Hz) and 3.54 (1H, dd, J=10, 4.5 Hz) (CH_2), 3.57 (3H, s, Me), 4.19 (1H, ddd, J=8.5, 8.5, 4.5 Hz, CH), 7.55 (1H, d, J=8.5 Hz, NH). *Anal.* Calcd for $C_5H_8INO_4$: C, 22.00; H, 2.95; N, 5.13. Found: C, 22.21; H, 2.93; N, 5.38.

(*R*)-3-Iodo-2-[(methoxycarbonyl)amino]propanoic Acid Methyl Ester (8a) A solution of sodium iodide (16.4 g, 110 mmol) in dry acetone (125 ml) was added to a solution of **4a** (18.22 g, 55 mmol) in dry acetone (25 ml) and the whole was stirred at 25 °C for 5 d. The precipitate that separated was filtered off and washed with acetone. The combined filtrate and washings were concentrated *in vacuo* to leave a solid residue. This was partitioned between chloroform (40 ml) and water (20 ml). The organic layer was washed with water (20 ml), dried over magnesium sulfate, and concentrated *in vacuo* to leave a slightly yellow solid, which was recrystallized from ethanol (20 ml) to afford colorless needles (13.91 g, 88%), mp 105—106 °C. Further recrystallization from ethanol gave an analytical sample with unchanged melting point, $[\alpha]_D^{13}$ +0.5° (c=1.13, MeOH); $[\alpha]_{365}^{13}$ +3.5° (c=1.13, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 3.59 (2H, d, J=4 Hz, CH_2), 3.72 and 3.81 (3H each, s, two OMe's), 4.56 (1H, dt, J=7, 4 Hz, CH), 5.52 (1H, br, NH). *Anal.* Calcd for $C_6H_{10}INO_4$: C, 25.11; H, 3.51; N, 4.88. Found: C, 25.12; H, 3.51; N, 4.72.

(*R*)-3-Iodo-2-[(methoxycarbonyl)amino]propanoic Acid Benzyl Ester (8b) A solution of sodium iodide (12.62 g, 84.2 mmol) in dry acetone (50 ml) was added to a solution of **4b** (25.80 g, 63.3 mmol) in dry acetone (30 ml) and the whole was stirred at room temperature for 3 d. The precipitate that separated was filtered off and washed with dry acetone (3 × 10 ml). The filtrate and the washings were combined and concentrated *in vacuo* to leave a slightly brown oil. This was partitioned between chloroform (100 ml) and water (100 ml). The organic layer was washed with water (2 × 100 ml), dried over magnesium sulfate, and concentrated *in vacuo* to leave a slightly brown solid (22.94 g, 100%), mp 59—61 °C. Recrystallization from methanol gave an analytical sample as colorless plates, mp 60—62 °C; $[\alpha]_D^{17}$ −18.6° (c=1.21, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 3.58 (2H, m, ICH_2), 3.70 (3H, s, Me), 4.57 (1H, m, CH), 5.22 (2H, s, Ph C$\underline{H}_2$), 5.55 (1H, br, NH), 7.37 (5H, s, Ph). *Anal.* Calcd for $C_{12}H_{14}INO_4$: C, 39.69; H, 3.89; N, 3.86. Found: C, 39.99; H, 3.97; N, 3.78.

(*R*)-[2-(Methoxycarbonyl)-2-[(methoxycarbonyl)amino]ethyl]triphenylphosphonium Iodide (7a) A solution of **8a** (5.74 g, 20 mmol) and triphenylphosphine (5.77 g, 22 mmol) in dry toluene (100 ml) was kept at 50 °C for 34 d. The precipitate that separated was collected by filtration, washed with a little toluene, and dried to afford **7a** (8.39 g, 76%) as colorless prisms, mp 147.5—153 °C (dec.); $[\alpha]_D^{18}$ +5.81° (c=3.46, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 3.40 and 3.73 (3H each, s, two Me's), 3.80—4.14 (1H, m, P^+CH), 4.65—5.25 (2H, m, P^+CHCH), 7.33 (1H, d, J=8 Hz, NH), 7.61—8.00 (15H, m, Ph_3).

(*R*)-[2-(Benzyloxycarbonyl)-2-[(methoxycarbonyl)amino]ethyl]triphenylphosphonium Iodide (7b) A solution of **8b** (19.90 g, 54.8 mmol) and triphenylphopsphine (15.81 g, 60.3 mmol) in dry toluene (100 ml) was kept at 50 °C for 5 d. The resulting precipitate was collected by filtration, washed with toluene (40 ml), and dried to afford colorless plates, 21.57 g, mp 149—151 °C; $[\alpha]_D^{18}$ +9.5° (c=2.6, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 3.38 (3H, s, Me), 3.77—4.12 (1H, m, P^+CH), 4.79—5.11 (2H, m, P^+CHCH), 5.16 (2H, s, PhC$\underline{H}_2$), 7.32 (5H, s, overlapped with a broad 1H signal due to NH, $\underline{Ph}CH_2$), 7.90—7.98 (15H, m, Ph_3). The combined filtrate and washings were concentrated *in vacuo* to *ca.* 20 ml and kept at 50 °C for a further 5 d. The precipitate that separated was filtered off, washed with toluene (50 ml), and dried to give a second crop of **7b** (11.80 g) having the same melting point, infrared (IR) spectrum, and specific rotation as those of the first crop described above. The total yield was 97%.

(*R*)-[2-Carboxy-2-[(methoxycarbonyl)amino]ethyl]triphenylphosphonium Chloride (6) A solution of **7b** (24.70 g, 39.5 mmol) in 50% (v/v) aqueous ethanol (720 ml) was passed through a column of Amberlyst A-26 (Cl^-) (330 ml) and the column was eluted with the aqueous ethanol (700 ml). The combined eluate was concentrated *in vacuo* to leave a slightly brown glass (22.80 g). This was dissolved in ethanol (290 ml) and the whole was hydrogenated over 10% Pd–C (13 g) at atmospheric pressure and room temperature for 21 h. The catalyst was filtered off and washed with ethanol (700 ml). The filtrate and the washings were combined and concentrated *in vacuo*. The residue was dried by coevaporation, after dissolving it in a mixture of dry chloroform and dry benzene (1 : 1, v/v), *in vacuo* three times. It was further dried over phosphorus pentoxide at

2 mmHg and 80 °C for 21 h to afford **6** (17.20 g, 98%) as a colorless glass, $[\alpha]_D^{24}$ +52° (c=0.50, $CHCl_3$); ^{1}H-NMR [$(CD_3)_2SO$] δ: 3.30 (3H, s, Me), 3.70—4.50 (3H br, CH_2CH), 7.50 (1H, br, NH), 7.60—8.00 (15H, m, Ph_3). This sample was of *ca.* 95% purity: the ^{1}H-NMR spectrum indicated that it was contaminated by a small amount of benzene and a trace of ethanol.

(*S*)-2-[(Methoxycarbonyl)amino]-4-phenylbutanoic Acid (12) i) From (*S*)-Homophenylalanine (**10**): Methyl chloroformate (0.04 ml, 0.48 mmol) was added to an ice-cooled mixture of (*S*)-2-amino-4-phenylbutanoic acid (**10**)[22] (54 mg, 0.30 mmol) and sodium carbonate (95 mg, 0.90 mmol) in water (6 ml). The whole was stirred at 0 °C for 10 min and then at room temperature for 2 h. The resulting mixture was brought to pH 1 by addition of 10% hydrochloric acid and extracted with dichloromethane (5 × 6 ml). The organic layers were dried over magnesium sulfate and concentrated *in vacuo* to leave **12** (71 mg, 100%), mp 101—103 °C. Recrystallization from benzene gave an analytical sample of colorless plates, mp 103—105 °C; $[\alpha]_{365}^{25}$ +30.2° (c=0.540, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 2.11 [2H, m, C(3)-H_2], 2.73 [2H, dd, J=8 Hz each, C(4)-H_2], 3.71 (3H, s, Me), 4.40 (1H, m, CH), 5.24 (1H, br, NH), 5.48 (1H, br, CO_2H), 7.24 (5H, m, Ph). *Anal.* Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.57; H, 6.43; N, 5.85.

ii) By the Hydrogenation of **11**: A solution of **6** (*ca.* 1.1 mmol as estimated by ^{1}H-NMR spectroscopy) in THF–HMPA (7:3, v/v) (31.5 ml), which had been dried over molecular sieves 4A (2.5 g) at 30 °C for 18 h and filtered in the same way as described for the preparation of **18** (see below), was chilled in a dry ice–acetone bath and *n*-butyllithium (1.55 M solution in hexane, 1.94 ml, 3.01 mmol) was added with stirring under argon over a period of 20 min. Benzaldehyde (0.090 ml, 0.88 mmol) was added to the stirred mixture at −78 °C and the whole was allowed to warm to 0 °C over a period of 17 min. The mixture was neutralized with 10% aqueous phosphoric acid and then concentrated *in vacuo* using a mechanical pump to remove HMPA. The residue was partitioned between saturated aqueous sodium bicarbonate (5 ml) and chloroform (5 ml). The organic layer was extracted with saturated aqueous sodium bicarbonate (5 ml). The combined aqueous layers were brought to pH 2 by addition of 10% hydrochloric acid and then extracted with ether (3 × 10 ml). The ethereal extracts were dried over magnesium sulfate and concentrated *in vacuo* to leave crude **11** as a colorless solid (98 mg). This was dissolved in methanol (8 ml) and hydrogenated over 10% Pd–C (100 mg) at atmospheric pressure and room temperature for 1 h. The catalyst was filtered off and washed with hot methanol (50 ml). The combined filtrate and washings were concentrated *in vacuo* to leave a colorless glass (87 mg), whose ^{1}H-NMR spectrum ($CDCl_3$) showed that this product was a mixture of **12** and **15** (molar ratio, 4:1). This was dissolved in ether (23 ml) and insoluble **15** was filtered off. Recrystallization of crude **15** from ethyl acetate afforded colorless needles, mp 115—125 °C (dec.), $[\alpha]_{365}^{24}$ +98.5° (c=0.466, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3/5H, t, J=7 Hz, $AcOCH_2\underline{Me}$), 2.04 (3/5H, s, $\underline{Ac}OEt$), 2.82—3.32 (2H, m, $C\underline{H}_2CH$), 3.44 (3H, s, CO_2Me), 4.12 (2/5H, q, J=7 Hz, $AcOC\underline{H}_2Me$), 4.38—4.80 (1H, m, $CH_2C\underline{H}$), 5.98 (1H, d, J=5.5 Hz, NH), 7.34—8.01 (11H, m, Ph_2 and CO_2H). *Anal.* Calcd for $C_{17}H_{18}NO_5P\cdot 1/5CH_3CO_2C_2H_5$: C, 58.59; H, 5.41; N, 3.84. Found: C, 58.23; H, 5.41; N, 3.78. Drying at 50 °C and 2 mmHg for 38 h did not remove the ethyl acetate.

The ethereal solution was concentrated and the solid residue was suspended in water (8 ml). The mixture was extracted with ether (5 × 8 ml) after it had been brought to pH 1—2 with 10% hydrochloric acid. The ethereal solution was dried over magnesium sulfate and concentrated *in vacuo* to leave **12** (57 mg, 27%), $[\alpha]_{365}^{18}$ +29.3° (c=0.747, MeOH). Recrystallization from benzene gave colorless plates, mp 102—105 °C; $[\alpha]_{365}^{24}$ +29.9° (c=0.538, MeOH). This sample was identical (IR spectrum and TLC) with the authentic sample described above.

[*S*-(*E*)]-2-[(Methoxycarbonyl)amino]-4-phenyl-3-butenoic Acid Methyl Ester (13) Crude **11** prepared from benzaldehyde (0.254 ml, 2.5 mmol) in a manner similar to that described for **12** under item (ii), was dissolved in a mixture of benzene (7 ml) and methanol (2 ml) and trimethylsilyldiazomethane (*ca.* 10% in hexane)[27] (2 ml) was added. Acetic acid was added to the solution after a delay of 1 min to acidify it. Removal of the solvents by evaporation and purification by flash chromatography [hexane–ethyl acetate (3:1, v/v)] afforded **13** (177 mg, 28%) as a colorless oil, $[\alpha]_D^{26}$ +92° (c=0.20, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 3.72 and 3.79 (3H each, s, two Me's), 5.06 (1H, m, =CHC$\underline{H}$), 5.48 (1H, br d, J=8 Hz, NH), 6.20 (1H, dd, J=6, 16 Hz, =C$\underline{H}$CH), 6.68 (1H, dd, J=1.2, 16 Hz, PhC$\underline{H}$=CH), 7.33 (5H, m, Ph).

(*S*)-2-[(Methoxycarbonyl)amino]-4-phenylbutanoic Acid Methyl Ester (14) i) From **10** through **12**: A solution of trimethylsilyldiazomethane in hexane (*ca.* 10%) (0.8 ml) was added to a solution of **12** (52 mg, 0.22 mmol) in a mixture of methanol (0.4 ml) and benzene (1.4 ml). The resulting solution was concentrated *in vacuo* and the residue was purified by flash chromatography [hexane–ethyl acetate (3:1, v/v)] to afford **14** as a colorless oil (51 mg, 93%), $[\alpha]_D^{29}$ −8.6° (c=2.88, MeOH); $[\alpha]_{365}^{31}$ −14.1° (c=2.88, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 1.76—2.36 (2H, m, $CH_2C\underline{H}_2CH$), 2.68 (2H, m, $PhC\underline{H}_2$), 3.70 and 3.72 (3H each, s, two Me's), 4.38 (1H, m, CH), 5.23 (1H, d, J=8 Hz, NH), 7.24 (5H, m, Ph).

ii) From **11** through **12**: Crude **11**, which was obtained from benzaldehyde (0.276 ml, 2.71 mmol) according to the method described above under item (ii) for the preparation of **12**, was hydrogenated over 10% Pd–C in methanol (35 ml) at room temperature for 1 h. The catalyst was removed by filtration and washed with hot methanol. The combined filtrate and washings were concentrated *in vacuo* and the residue was dissolved in a mixture of methanol (1.6 ml) and benzene (5.6 ml). Trimethylsilyldiazomethane (10% in hexane) (2 ml) was added to this solution and the mixture was concentrated *in vacuo*. The oily residue was purified by flash chromatography [hexane–ethyl acetate (2:1, v/v)] to afford **14** (100 mg, 15%), $[\alpha]_{365}^{28}$ −13.0° (c=3.07, MeOH). This was identical (IR and ^{1}H-NMR spectra) with **14** prepared by method (i).

[*S*-(*E*)]-4-[1-Benzyl-4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo-[1,2-*a*]purin-7-yl]-2-[(methoxycarbonyl)amino]-3-butenoic Acid Methyl Ester (18) The phosphonium chloride **6** (3.3 mmol) was gently stirred with molecular sieves 4A (8.40 g) in a mixture of dry THF (74 ml) and dry HMPA (31 ml) under argon at 30 °C for 23 h. The supernatant of the mixture (96 ml) was withdrawn with a syringe and transferred to a reaction vessel through a funnel with a fritted disk under argon. The solution was cooled to −78 °C and *n*-butyllithium (1.55 M solution in hexane, 5.70 ml, 8.84 mmol) was added over a period of 1 h with stirring, and then **16** (977 mg, 3.04 mmol) was added under argon. The whole was stirred for 1 h and then allowed to warm to −18 °C over a period of 6 h. The resulting mixture was neutralized with 10% aqueous phosphoric acid and concentrated *in vacuo* to a small volume. After addition of water (40 ml), the mixture was brought to pH 3 by addition of 10% aqueous phosphoric acid and extracted with chloroform (4 × 30 ml). The organic layers were combined, dried over magnesium sulfate overnight, and concentrated *in vacuo* to leave a brown oil. This was subjected to flash chromatography [column diameter, 50 mm; ethyl acetate–ethanol (10:1, v/v)] to afford unchanged **16** (254 mg, 28%). Further elution of the column with chloroform–methanol (10:1, v/v) gave a yellow solid (115 mg), whose ^{1}H-NMR spectrum suggested that it was **19** contaminated with small amounts of some components having similar structures, ^{1}H-NMR [$(CD_3)_2SO$] δ: 1.99 (s, CMe), 3.75 and 3.77 (s each, two NMe's), 5.65 (br, two $PhC\underline{H}_2$'s), 7.34 (s overlapped with m, two Ph's and olefinic protons), 8.45 and 8.52 (s each, two heterocyclic protons), 10.54 (s, CHO). Attempts to purify this compound failed. The column was finally eluted with methanol to afford a yellow solid (958 mg) containing **17**. This was dissolved in a mixture of methanol (6 ml) and benzene (21 ml). Trimethylsilyldiazomethane (1.5 M solution in ether, 2.10 ml, 3.2 mmol) was added to the solution and then acetic acid (0.6 ml) was added after a delay of 1 min. The resulting mixture was concentrated *in vacuo* and purified by flash chromatography [column diameter, 30 mm; ethyl acetate–ethanol (10:1, v/v)] to afford **18** (221 mg, 16%) as a slightly yellow solid. Recrystallization from methanol gave slightly yellow needles (158 mg, 11%), mp 179—181 °C; $[\alpha]_D^{19}$ +56.9° (c=0.181, MeOH). Further recrystallization from methanol afforded an analytical sample with unchanged melting point, $[\alpha]_D^{20}$ +58.0° (c=0.207, MeOH); UV: given in the text; MS m/z: 464 (M^+), 405 (M^+-CO_2Me), 373 (M^+-PhCH_2); ^{1}H-NMR ($CDCl_3$) δ: 2.38 [3H, br s, C(6)-Me], 3.72 and 3.81 (3H each, s, two OMe's), 3.89 (3H, s, NMe), 5.08 [1H, m, C(α)-H], 5.53 (1H, br, NH), 5.58 (2H, s, $PhC\underline{H}_2$), 5.77 [1H, dd, J=6.5, 16 Hz, =C(β)-H], 7.35 (5H, s, Ph), 7.64 [1H, s, C(2)-H], 7.67 [1H, ddq, J=16, 1.5, 0.5 Hz, =C(γ)-H]. *Anal.* Calcd for $C_{23}H_{24}N_6O_5$: C, 59.48; H, 5.21; N, 18.09. Found: C, 59.42; H, 5.01; N, 18.38.

(*S*)-4,9-Dihydro-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine-7-butanoic Acid Methyl Ester (1c) A solution of **18** (144 mg, 0.31 mmol) in methanol (110 ml) was hydrogenated over 10% Pd–C (150 mg) at *ca.* 60 °C for 1 h. After confirmation of the consumption of **18** by TLC [ethyl acetate–ethanol (10:1, v/v)], 70% aqueous perchloric acid (0.07 ml) and further 10% Pd–C (312 mg) were added. The reduction was continued for a further 24 h at the same temperature. The catalyst was filtered off and the filtrate was neutralized with saturated aqueous sodium bicarbonate. The mixture was then concentrated *in vacuo* and the residue was treated with water (1 ml). The resulting precipitate was collected by filtration after storage of the mixture in a refrigerator overnight, washed with water (2 ml), and dried. It was dissolved in hot methanol (3 ml) and

insoluble material was removed by filtration while the solution was hot. The solution was concentrated *in vacuo* to give **1c** (79 mg), mp 179—184 °C (dec.). The catalyst was extracted with methanol using a Soxhlet extractor. The methanolic solution was concentrated *in vacuo* and the residue was purified by layer chromatography on silica gel (0.5 mm) [ethyl acetate–ethanol (10 : 1, v/v)] to afford a second crop of **1c** (12 mg; the total yield was 75% as the monohydrate). Recrystallization from methanol gave colorless needles, mp 208—211 °C (dec.). This sample was dried over phosphorus pentoxide at 2 mmHg and 80 °C for 8 h and then exposed to air until constant weight was reached to give an analytical sample as the monohydrate with unchanged melting point, $[\alpha]_D^{23}$ −45° (c = 0.130, MeOH); CD ($c = 2.80 \times 10^{-5}$ M, 10% aqueous MeOH) $[\theta]^{13}$ (nm): −13800 (232) (neg. max.), −4300 (260) (neg. max.); UV $\lambda_{max}^{95\%\ EtOH}$ 236 nm (ε 32000), 260 (sh) (5300), 309 (5300); $\lambda_{max}^{10\%\ MeOH}$ 236 (34500), 261 (6100), 313 (5500); MS m/z (relative intensity): 376 (M^+) (8), 344 (5), 242 (1), 230 (8), 216 (100); ^{1}H-NMR ($CDCl_3$) δ: 2.17 [2H, m, C(β)-H_2], 2.27 [3H, s, C(6)-Me], 3.21 [2H, m, C(γ)-H_2], 3.70 (6H, s, two OMe's), 3.97 (3H, s, NMe), 4.35 [1H, m, C(α)-H], 5.85 [1H, br, C(α)-NH], 7.96 [1H, s, C(2)-H], 11.07 [1H, br, N(1)-H]; [$(CD_3)_2SO$] δ: 1.96 [2H, m, C(β)-H_2], 2.09 [3H, s, C(6)-Me], 3.06 [2H, m, C(γ)-H_2], 3.56 and 3.58 (3H each, s, two OMe's), 3.75 (3H, s, NMe), 3.96 [1H, m, C(α)-H], 7.64 [1H, d, J = 8 Hz, C(α)-NH], 8.16 [1H, d, J = 1 Hz, C(2)-H], 13.55 [1H, br, N(1)-H]. *Anal.* Calcd for $C_{16}H_{20}N_6O_5 \cdot H_2O$: C, 48.73; H, 5.62; N, 21.31. Found: C, 48.81; H, 5.76; N, 21.51. HPLC analyses were performed on pre-packed columns of 4 mm inner diameter and 250 mm length at the flow rate of 0.5 ml per min at room temperature (20 °C). HPLC [column, solvent (ratio by volume), retention time (min)]: Merck LiChrosorb Si-60 (5 μm), chloroform–methanol (95 : 5), 16.4; 1,2-dichloroethane–ethanol (88 : 12), 22.8; Merck LiChrosorb RP-18 (7 μm), acetonitrile–water (20 : 80), 30.8; methanol–water (50 : 50), 18.0. The chiral column used was Sumichiral OA-4600 (5 μm) supplied by Sumika Chemical Analysis Service, Ltd. When eluted with hexane–1,2-dichloroethane–methanol–trifluoroacetic acid (50 : 45 : 5 : 0.2), (±)-**1c**[33] was cleanly resolved to the (*R*)-isomer (retention time, 32.4 min) and the (*S*)-isomer (34.8 min). Analysis of the present sample of **1c** under these conditions indicated that it was free from its antipode.

For comparison of **1c** (39 μg) with wybutine from natural sources (36 μg),[38] both samples were dried over phosphorus pentoxide at 2 mmHg and 50 °C for 6 h and then exposed to air overnight, and separately dissolved in 10% aqueous methanol (4 ml). The UV and CD spectra were taken using these solutions; the results are given in the text. The samples were recovered by evaporation of the solvent and the residual solids were analyzed by HPLC. These behaved in an identical manner under the five different chromatographic conditions defined above.

Acknowledgment This work was supported by a Grant-in-Aid for Scientific Research (No. 59570890) from the Ministry of Education, Science and Culture, Japan, and a grant from the Japan Research Foundation for Optically Active Compounds. We wish to express our gratitude to Professor H. G. Zachau, the University of Munich, for an invaluable sample of wybutine from natural sources, and to Professor K. Nakanishi, Columbia University, for a private communication.

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