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Synthesis and Structure of the Hypermodified Nucleoside of Rat Liver Phenylalanine Transfer Ribonucleic Acid

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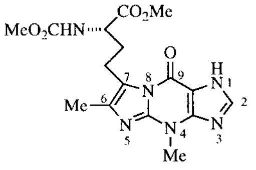
The first synthesis of ($\alpha S,\beta S$)-β-hydroxy-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-β-D-ribofuranosyl-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic acid methyl ester [($\alpha S,\beta S$)-11] has been achieved by OsO$_4$ oxidation of [*S*-(*E*)]-4-[4,6-dimethyl-9-oxo-3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4,9-dihydro-3*H*-imidazo[1,2-*a*]purin-7-yl]-2-[(methoxycarbonyl)amino]-3-butenoic acid methyl ester (13) followed by successive γ-deoxygenation through the cyclocarbonates, separation from the ($\alpha S,\beta R$)-isomer by means of flash chromatography, and deprotection. On the other hand, the minor nucleoside of rat liver tRNA$^{\text{Phe}}$ was isolated on a scale of 100 μg by partial digestion of unfractionated tRNA (1 g) with nuclease P$_1$, followed by reverse-phase column chromatography, complete digestion with nuclease P$_1$/alkaline phosphatase, and reverse-phase HPLC. Comparison of this nucleoside with the synthetic one has unambiguously established its structure to be ($\alpha S,\beta S$)-11.

Key words β-hydroxywybutosine; minor nucleoside; rat liver tRNA$^{\text{Phe}}$; fluorescent nucleoside; condensed tricyclic nucleoside

Many eukaryotic tRNAs$^{\text{Phe}}$ have fluorescent components at the position next to the 3′-end of the anticodon.[1—5] The fluorescent base isolated from chicken, rat, and bovine liver tRNAs$^{\text{Phe}}$ was first reported to be β-hydroperoxywybutine (**3**)[6,7] on the basis of comparison of the UV, fluorescent, and MS spectra as well as the chromatographic behavior with those of wybutine (**1**),[8,9] the structurally related precedent from yeast tRNA$^{\text{Phe}}$. The base from the plant *Lupinus luteus* was also characterized as **3**.[10] In this case, the presence of the hydroperoxy group was supported by a specific color test employing Fe(SCN)$_2$. The structure **3** was suggested to be assigned to the base from wheat germ tRNA$^{\text{Phe}}$,[10] because it had been shown to be indistinguishable from that of beef.[11] Kasai *et al.*, however, reported that the fluorescent base from rat liver tRNA$^{\text{Phe}}$ was β-hydroxywybutine (**2**) on the basis of the MS spectral data as well as the negative coloring test for the hydroperoxy group.[12] Those 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$^{\text{Phe}}$ was also **2**. Notwithstanding that report, Mochizuki *et al.* preferred **3** for the fluorescent base isolated from the aquatic fungus *Geotrichum candidum* tRNA$^{\text{Phe}}$.[13] Wiewiórowski's group also reported that the base from tRNAs$^{\text{Phe}}$ of wheat germ, yellow lupine seeds, and maize seeds was **3**. They described **3** as very unstable and found that it decomposed to **2** and **1**[14]: The stability observed for **2** and **3** contradicted that reported by Kasai *et al.*[12] Although we achieved the synthesis of ($\alpha S,\beta R$)- and ($\alpha S,\beta S$)-**2** as the most probable alternatives for the base isolated from rat liver tRNA$^{\text{Phe}}$,[3,15,16] the lack of a sample of the base from the tRNA$^{\text{Phe}}$ has hampered its structural determination. In the present investigation, we isolated the corresponding nucleoside from rat liver for the first time and determined its structure to be ($\alpha S,\beta S$)-**11**, the first synthesis of which is also described. A preliminary communication of this work has been published.[17,18]



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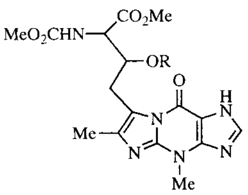

2: R = H
3: R = OH

Results and Discussion

Synthesis The synthesis of the bases ($\alpha S,\beta R$)- and ($\alpha S,\beta S$)-**2** has been accomplished by OsO$_4$ oxidation of the olefin **4** followed by separation of the resulting diastereomers **5** and **7** and hydrogenolysis through the cyclic carbonates **6** and **8**, as shown in Chart 1.[3,15,16] In the present study, we first examined the applicability of this reaction sequence to the nucleoside level. Thus the Heck reaction between 2′,3′,5′-tri-*O*-acetyl-7-iodowyosine (**9**)[19] and (±)-2-[(methoxycarbonyl)amino]-3-butenoic acid[20] was conducted according to the procedure reported for the synthesis of 3-β-D-ribofuranosylwybutine (**12**).[19] The product was treated with Me$_3$SiCHN$_2$ to give a mixture of diastereomers **10**. This was subjected to OsO$_4$ oxidation followed by cyclocondensation with triphosgene, catalytic hydrogenolysis, purification by preparative TLC, and deprotection to afford a mixture of four diastereomers **11**, as shown in Chart 2. These were separated by HPLC. Two were ($\alpha S,\beta R$)- and ($\alpha S,\beta S$)-**11**, of which unambiguous syntheses are described below. The structures of the others [($\alpha R,\beta S$)- and ($\alpha R,\beta R$)-**11**] were assignable by comparison of the ^{1}H-NMR patterns of their amino acid moieties with those of ($\alpha S,\beta R$)- and ($\alpha S,\beta S$)-**11**. Assuming that **2** is the correct two-dimensional expression of the structure of the hypermodified base of rat liver tRNA$^{\text{Phe}}$, one of the four diastereomers **11** is likely the correct structure of the corresponding nucleoside. We preferred ($\alpha S,\beta R$)-**11** and ($\alpha S,\beta S$)-**11** as the most probable alternatives because structurally related wybutosine (**12**) from yeast tRNA$^{\text{Phe}}$ had been determined by us to have the (αS) configuration.[2]

Because it is difficult to obtain stereochemically pure (αS)-**10** in a large quantity,[19] we selected **13**[2] as a key intermediate for the stereospecific synthesis of ($\alpha S,\beta R$)- and ($\alpha S,\beta S$)-**11**. OsO$_4$ oxidation of the olefin **13** in the presence of *N*-methylmorpholine *N*-oxide in acetone–phosphate buffer (pH 6) at room temperature, followed by HPLC on silica gel

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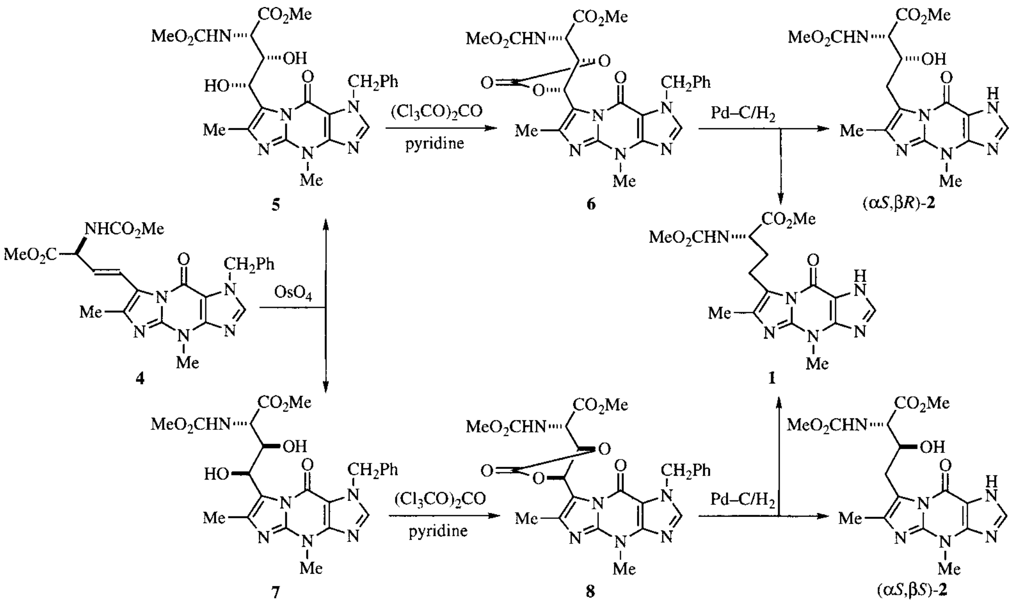


Chart 1

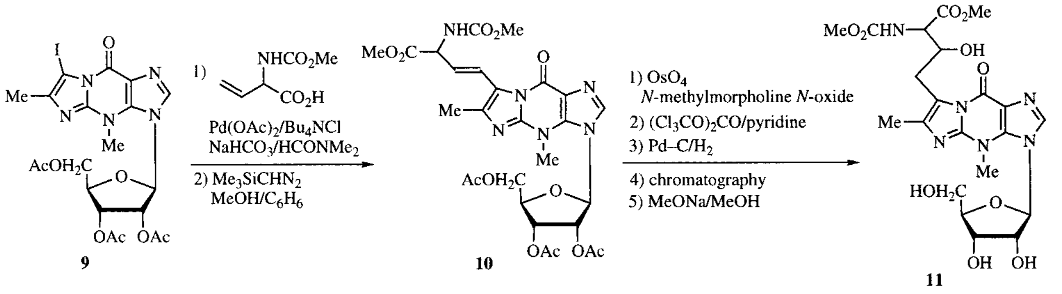
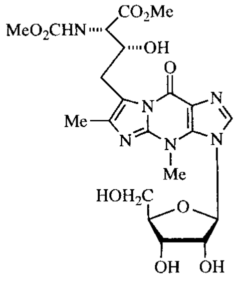
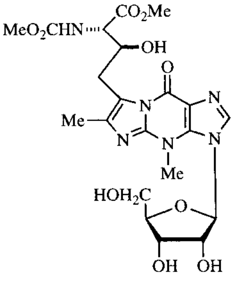


Chart 2



(αS,βR)-11



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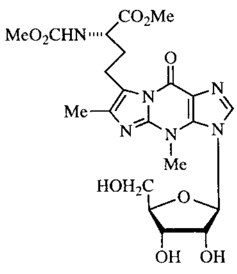

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afforded the diols **14** and **16** in 51% and 30% yields, respectively. The configurations of these compounds were assignable by comparison of their ^{1}H-NMR spectra with those of the bases **5** and **7**.[3] Treatment of the major isomer **14** with an excess of triphosgene in CH_2Cl_2 in the presence of pyridine at 0 °C afforded the cyclic carbonate **15** in 80% yield. Catalytic hydrogenolysis of **15** over Pearlman's catalyst afforded the β-hydroxy compound **17** in 28% yield together with the dideoxy compound **18**[2] (23%). The analogous concomitant formation of the dideoxy compounds has already been recognized in the reaction of the model compound (±)-**19**[3,16] and in the reactions involved in Charts 1, 2. As illustrated in Chart 4, hydrogenolysis of (±)-**19** should produce the intermediate **20**. While hydrolysis of the hydrogen carbonate **20** would provide the desired monohydroxy compound **21**, elimination of H_2CO_3 followed by hydrogenation would form the dideoxy compound **23** through the olefin **22**. Analogous examples of the formation of saturated compounds and olefins have already been reported for the electrochemical reduction of cyclic carbonates of *meso*-hydrobenzoin, (±)-hydrobenzoin, and (*E*)-2,3-diphenylbutane-1,2-diol.[21] Although the undesirable dideoxygenation of (±)-**19** could be suppressed to some extent by the use of Pt instead of Pd–C,[16] the Pt catalyst was found in the present investigation to be inferior in view of reproducibility for hydrogenolysis of **15**. Deprotection of **17** was accomplished by treatment with Bu_4NF in aqueous THF in the presence of pyridine at room temperature[22] without cleaving the extraordinarily labile glycosyl

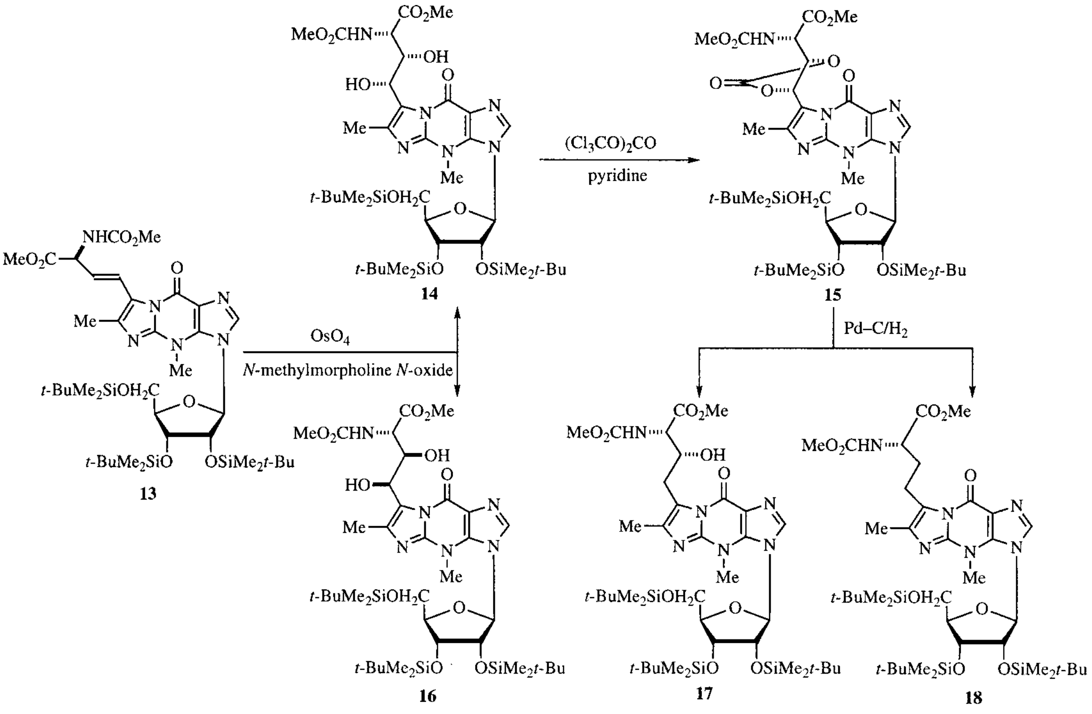


Chart 3

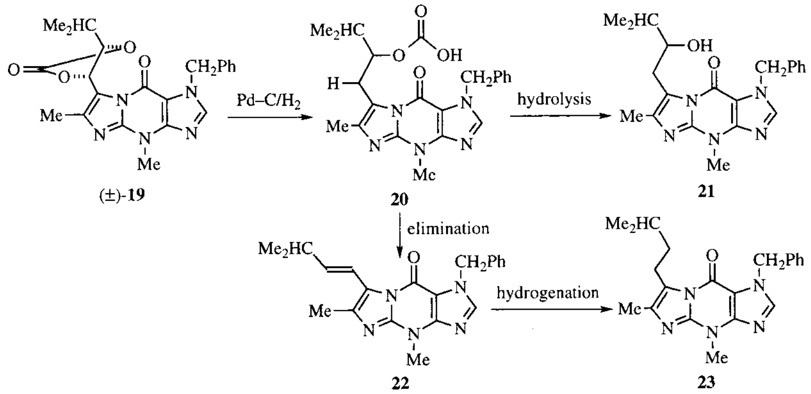
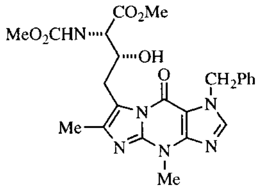
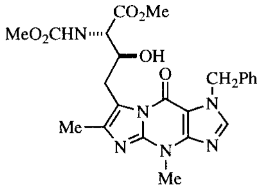


Chart 4



(±)-**24**



(±)-**25**

bond to provide the desired nucleoside in 86% yield. The correctness of the assignment of the structure (*αS*,*βR*)-**11** to this product was established by its hydrolysis with 0.1 N aqueous HCl, leading to optically pure (*αS*,*βR*)-**2**. The ^{1}H-NMR [$(CD_3)_2SO$ or $(CD_3)_2CO$] spectral patterns of signals arising from the side chains of these two compounds closely resemble each other. This is also the case with the ^{1}H-NMR spectra of **17** and (*R**,*S**)-1-benzyl-*β*-hydroxy-*α*-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-4,9-dihydro-1*H*-imidazo[1,2-*a*]purine-7-butanoic acid methyl ester [(±)-**24**][16] measured in $CDCl_3$, as shown in Table 1. However, (*αS*,*βR*)-**2** shows a somewhat different ^{1}H-NMR spectrum from that of **17** in $CDCl_3$ (Table 1), suggesting that N(1)-H of (*αS*,*βR*)-**2** affects the conformation of its side chain through hydrogen bonding in this solvent.

Contrary to the successful conversion of the diol **14** into the carbonate **15**, the minor isomer **16** with (*αS*,*βR*,*γR*) configurations did not produce the cyclic carbonate **26** at all upon treatment with triphosgene in a manner similar to that employed for the preparation of **15**. The starting material **16** was recovered in *ca.* 80% yield. This is an unbelievably strange result in view of the positive reaction of the base **7**[3] (Chart 1) having the same configurations and the reaction of the (*αS*,*βR*,*γR*)-nucleoside involved in the reaction sequence shown in Chart 2. The latter case indicated what should be done to obtain the desired carbonate **26**. When a mixture of **16** and **14**, accessible in 91% yield in a ratio of 1 : 2 in the above OsO_4 oxidation of **13**, was subjected to the reaction with triphosgene, **26** was obtained as a mixture with **15** in a

Table 1. ^{1}H-NMR Spectral Data for ($\alpha S,\beta R$)-, ($\alpha S,\beta S$)-**2**, and Related Compounds Measured in $CDCl_3$[a]

Proton	Chemical shift (δ)						
	($\alpha S,\beta R$)-**2**[b]	**17**[c]	(±)-**24**[d]	($\alpha S,\beta S$)-**2**[b]		**27**[c]	(±)-**25**[d]
				Species 1	Species 2		
CO_2CH_3	3.68 s	3.72	3.74	3.69 s	3.69 s	3.70	3.72
	3.73 s	3.76	3.77	3.82 s	3.87 s	3.80	3.73
C(α)-H	4.56 d (9.8)[e]	4.46	4.49[f]	4.78 m	4.19 m	4.50	4.52[f]
C(β)-H	4.47 m	4.39	4.37	4.27 m	4.58 m	4.16	4.15
C(γ)-H_2	3.19 dd (15.6, 4.4)	3.15	3.22	3.10 m	3.44 dd (15, 10.7)	3.41	3.36
	3.75 dd (15.6, 8.8)	3.57	3.46	3.70 m	3.55 dd (15, 1.5)		3.41
C(β)-OH	4.94 d (5.9)[g]	4.17	4.15	4.78 br	4.10 br	3.83	3.80
C(α)-NH	6.39 d (9.5)[h]	5.66	5.65[f]	6.90 br	8.00 br	5.88	5.90[f]
C(6)-CH_3	2.32 s	2.25	2.27	2.26 s	2.44 s	2.25	2.26
NCH_3	3.97 s	4.11	3.92	3.95 s	3.97 s	4.11	3.90
C(2)-H	7.90 s	7.94	7.66	7.93 s	7.89 s	7.94	7.66
N(1)-H	11.51 s[i]	—	—	11.46 br	13.52 br	—	—

a) Figures in parentheses denote coupling constants (*J*) in Hz. *b*) Measued for a 2.4 mM solution. *c*) See Experimental for complete data. *d*) Taken from ref. 16. *e*) Accompanied by a small broad signal at 4.35. *f*) Accompanied by a minor signal. *g*) Accompanied by a small broad signal at 5.03. *h*) Accompanied by a small broad signal at 6.29. *i*) Accompanied by a small broad signal at 11.74.

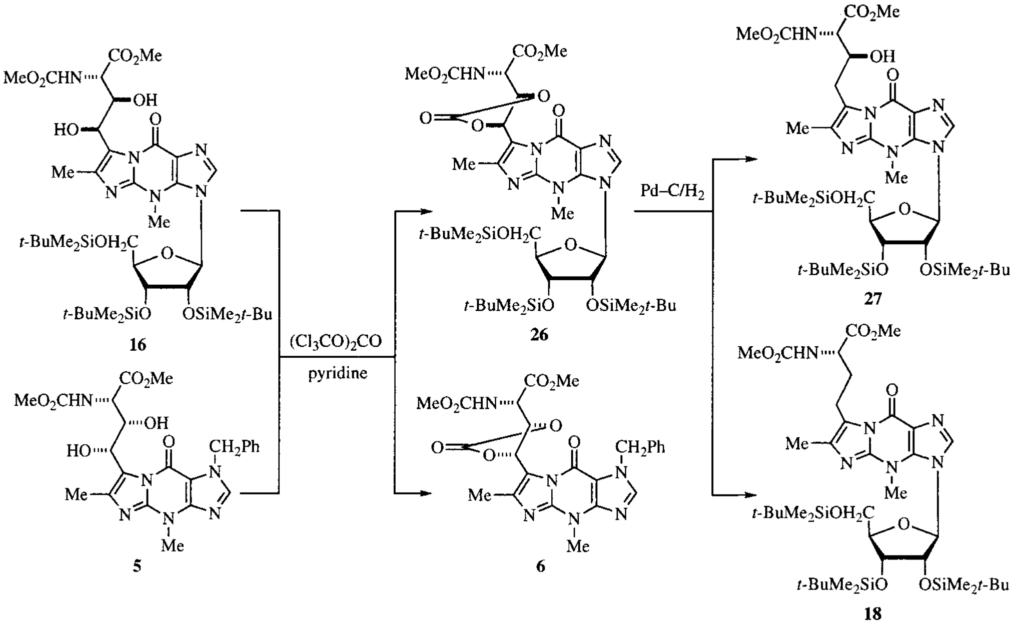


Chart 5

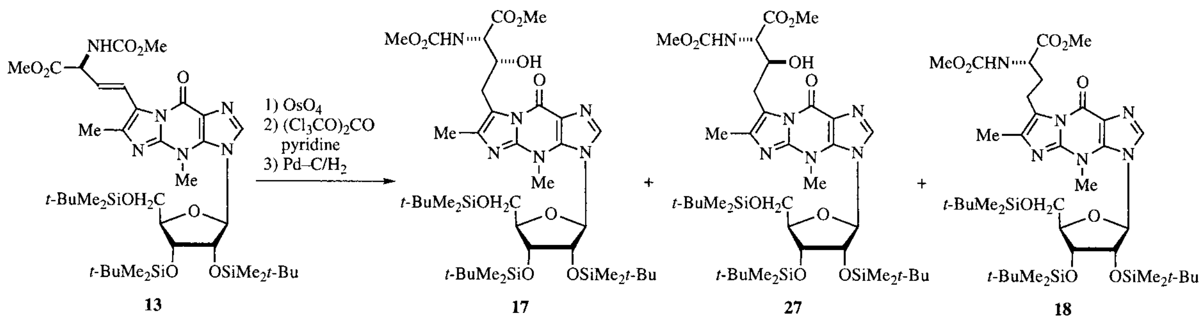


Chart 6

ratio of 1 : 2.9. However, **26** was not formed when **16** was added after the reaction of **14** with triphosgene was completed even in the presence of excess reagents. These results suggest that an intermolecular interaction between the side chains of **14** and **16** is important for the cyclocondensation of **16**. Although separation of the mixture of **15** and **26** was difficult, **16** underwent cyclocondensation as well with triphosgene in the presence of the base **5** having the ($\alpha S,\beta S,\gamma S$)

configurations, giving a mixture of the carbonates (**26** and **6**) as shown in Chart 5. Compound **26** was easily obtained from this mixture by flash chromatography in 57% yield. Hydrogenolysis of **26** using Pearlman's catalyst afforded the monohydroxy compound **27** in 39% yield together with the dideoxy compound **18** (19%). As the two diastereomers **17** and **27** can be separated by flash chromatography, these were more conveniently obtained by hydrogenolysis of the 1 : 2.9 mixture of **15** and **26** described above in 21% and 8% yields, respectively, based on the olefin **13** (Chart 6). Desilylation of **27** afforded ($\alpha S,\beta S$)-**11** in 86% yield. Hydrolysis of ($\alpha S,\beta S$)-**11** produced optically pure ($\alpha S,\beta S$)-**2** in 98% yield.

It should be noted that the ^{1}H-NMR spectrum arising from the side chain of the base ($\alpha S,\beta S$)-**2** in $CDCl_3$ does not resemble those of (±)-**25**[16] and **27**. Interestingly, ($\alpha S,\beta S$)-**2** shows two sets of signals (species 1 and 2 in Table 1) in $CDCl_3$, while the diastereomer ($\alpha S,\beta R$)-**2** exists as a single species. This complexity of the signals disappeared in the spectra taken in $(CD_3)_2SO$,[3] $(CD_3)_2CO$, CD_3CN, and pyridine-d_5, all of which are stronger hydrogen bond acceptors than $CDCl_3$. Furthermore, the molar ratio of the two species estimated on the basis of relative areas of the C(6)-Me signals depended on the total concentration, as shown in Table 2. These results suggest that ($\alpha S,\beta S$)-**2** molecules associate themselves in part through intermolecular hydrogen bond(s) in $CDCl_3$.

Compounds ($\alpha S,\beta R$)- and ($\alpha S,\beta S$)-**11** thus obtained were converted into their tetraacetates-d_{12} **28** and **29** according to the strategy that we had used to determine the structures of wyosine[1] from torula yeast tRNA and wybutosine (**12**)[2] from baker's yeast tRNA. Compounds **28** and **29** could be distinguished by comparison of their ^{1}H-NMR spectra taken in $CDCl_3$.

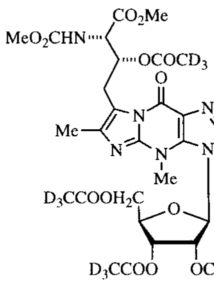
Isolation and Identification of the Minor Nucleoside and Its Base from Rat Liver tRNA We had already determined the structures of wyosine[1] and wybutosine (**12**)[2] by isolating these nucleosides on a scale of 70—80 μg. To determine how many rats are required for isolation of a comparable amount of the target nucleoside, we first attempted isolation of the corresponding base as a preliminary experiment. Unfractionated tRNA obtained from the livers of *ca.* 10 rats was subjected to mild acid treatment[23] (pH 2.9 at 40 °C for 16 h), and polynucleotides were removed by precipitation with EtOH. The minor base was obtained on a scale of a few micrograms from the soluble part by means of TLC on silica gel. The HPLC behavior of the base thus obtained was identical with that[3] of ($\alpha S,\beta S$)-**2**, and the correctness of its absolute configurations was established by chiral HPLC analysis. Furthermore, the assignable signals appearing in the ^{1}H-NMR spectrum [$(CD_3)_2CO$] of this compound corresponded to those of ($\alpha S,\beta S$)-**2**. No trace of wybutine (**1**) was detected in contrast to the results reported by Kasai *et al.*[12] It follows that ($\alpha S,\beta S$)-**2** is unlikely to be an artifact of hydroperoxywybutine (**3**), because **3** was reported to decompose to **2** and **1**.[14] Thus we concluded that the formula ($\alpha S,\beta S$)-**2** is a complete expression of the hypermodified base of rat liver $tRNA^{Phe}$. It should be noted that ($\alpha S,\beta S$)-**2** was stable during storage at room temperature, contrary to the contention of Kasai *et al.*[12]

To isolate the nucleoside on a scale of some 50 μg, we next started with the same grade of tRNA (1 g) obtained from 100 rats. Because this material resisted digestion with nuclease P_1, it was extracted with aqueous Me_2CHOH and then with H_2O. The tRNA in the combined solution was purified by ion-exchange chromatography to give unfractionated tRNA (350 mg). This was partially digested with nuclease P_1 followed successively by reverse-phase column chromatography, complete digestion with nuclease P_1, dephosphorylation with alkaline phosphatase, and HPLC according to the reported procedure[1,2] to give the target nucleoside (100 μg). We did not find any trace of wybutosine (**12**). The HPLC behavior of the nucleoside was identical to that of ($\alpha S,\beta S$)-**11** but different from that of the diastereomer ($\alpha R,\beta R$)-**11**, ruling out the 3-β-L-ribofuranosyl structure **30** for this nucleoside because ($\alpha R,\beta R$)-**11** is the enantiomer of **30**. The minor nucleoside thus obtained was converted into the tetraacetate-d_{12}, which was identical to **29** on the basis of MS and ^{1}H-NMR spectroscopy. Finally the tetraacetate-d_{12} of natural origin was treated with MeONa–MeOH and then with dilute aqueous HCl to give the base, the identity of which with ($\alpha S,\beta S$)-**2** was confirmed by comparison of their HPLC behavior and ^{1}H-NMR spectra. The structure of the hypermodified nucleoside of rat liver $tRNA^{Phe}$ was hereby determined unambiguously to be ($\alpha S,\beta S$)-**11**.

Table 2. Effect of Total Concentrations on Ratios of Two Species of ($\alpha S,\beta S$)-**2** in $CDCl_3$ as Determined by ^{1}H-NMR Spectroscopy

Total concentration of ($\alpha S,\beta S$)-**2** (mM)	Mole fraction of species 1[a] (%)
0.038	81
0.075	71
0.15	63
0.30	59
0.60	52
1.2	49
2.4	46

[a] Estimated on the basis of relative areas of the C(6)-Me signals of the two species.



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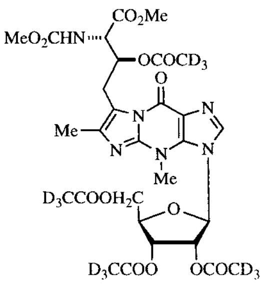

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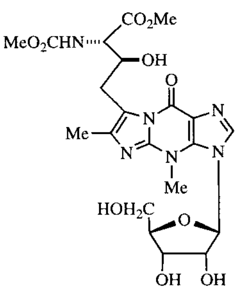

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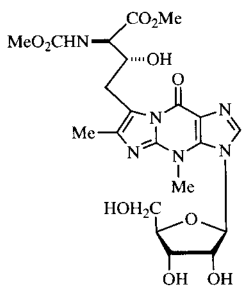

($\alpha R,\beta R$)-**11**

Experimental

General Notes Spectra reported herein were recorded on a JEOL JMS-SX102A mass spectrometer, a Hitachi U-3010 spectrophotometer, or a JEOL JNM-GSX-500 NMR spectrometer (measured at 25 °C with Me_4Si as an internal standard unless otherwise stated). $CDCl_3$ for measurements of small samples was treated with alumina according to the reported procedure.[1] MS measurements were performed by Dr. M. Takani and her associates at Kanazawa University. The optical rotation was measured with a Horiba SEPA-300 polarimeter using a 10-cm sample tube. The HPLC system employed consisted of a Tosoh CCPD pump, an injection valve unit, a UV-8020 detector, and a Chromatocorder 21 integrator or a Waters 6000A pump, a U6K injector, and a model 440 absorbance detector. The following abbreviations are used: br=broad, d=doublet, dd=doublet-of-doublets, ddd=doublet-of-doublets-of-doublets, dddd=doublet-of-doublets-of-doublets-of-doublets, ddt=doublet-of-doublets-of-triplets, m=multiplet, and s=singlet.

Synthesis of Four Diastereomers of β-Hydroxy-3-β-D-ribofuranosylwybutine (11) A mixture of $Pd(OAc)_2$ (6.6 mg, 0.029 mmol), $NaHCO_3$ (201 mg, 2.39 mmol), **9**[19] (468 mg, 0.797 mmol), Bu_4NCl (222 mg, 0.799 mmol), and Me_2NCHO (12 ml) was stirred at 60 °C for 10 min. (±)-2-[(Methoxycarbonyl)amino]-3-butenoic acid[20] (191 mg, 1.20 mmol) was added to the mixture, and the whole was stirred at 60—65 °C for 8 h. After H_2O (30 ml) was added, the resulting mixture was brought to pH 3 by the addition of 10% aqueous H_3PO_4 and extracted with $CHCl_3$ (2×30 ml). The organic layers were combined and extracted with saturated aqueous $NaHCO_3$ (3×20 ml). The aqueous layers were combined, brought to pH 3 with 10% aqueous H_3PO_4, and extracted with $CHCl_3$ (4×20 ml). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The oily residue was dissolved in a mixture of MeOH (1 ml) and benzene (4 ml), and 2.0 M Me_3SiCHN_2 solution in hexane (0.3 ml) was added. The resulting solution was concentrated *in vacuo*, and the residue was purified by flash chromatography [AcOEt–EtOH (10 : 1, v/v)] to give **10** (115 mg, 23%) as a yellow glass. The diastereomeric mixture thus obtained was dissolved in Me_2CO (15 ml). After a solution of *N*-methylmorpholine *N*-oxide monohydrate (42 mg, 0.31 mmol) in 0.5 M phosphate buffer (pH 6, 15 ml) and a 1.6% (w/v) OsO_4 solution in Me_3COH (0.4 ml) were added, the mixture was stirred at room temperature for 5 h and then for a further 30 min after the addition of $Na_2S_2O_5$ (57 mg, 0.30 mmol). The resulting suspension was concentrated to half the initial volume and extracted with $CHCl_3$ (2×20 ml). The organic layers were combined, washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated *in vacuo* to leave a colorless glass (109 mg). This was purified by flash chromatography [$CHCl_3$–MeOH (10:1, v/v)], providing a colorless glass (57 mg). A portion (20 mg) of this mixture of the diols was treated with triphosgene (18 mg) in CH_2Cl_2 (2 ml) in the presence of pyridine (0.03 ml) at 0 °C for 20 min. The resulting solution was washed successively with H_2O (3 ml), 5% aqueous citric acid (2×3 ml), and H_2O (2×3 ml), dried over $MgSO_4$, and concentrated *in vacuo*, leaving a colorless glass (7 mg). A portion (5 mg) of this material was hydrogenated over Pearlman's catalyst (15 mg) in MeOH (4 ml) at 40 °C for 2 h. The catalyst was removed by filtration and washed with hot MeOH (50 ml). The filtrate and washings were combined and concentrated *in vacuo*. The residue was purified by TLC on silica gel [$CHCl_3$–MeOH (10 : 1, v/v)] to give a diastereomeric mixture of the monohydroxy compounds (2 mg) as a colorless glass. After a solution of this product in 0.1 M MeONa–MeOH (0.15 ml) was stored at 0 °C for 5 min, 0.1 M aqueous NaH_2PO_4 (0.3 ml) was added at once. The resulting mixture was concentrated *in vacuo*, and the residue was purified by TLC on silica gel [$CHCl_3$–MeOH (5 : 1, v/v)], giving a colorless glass (1 mg). Separation of the diastereomers thus obtained was accomplished by HPLC [LiChrosorb RP18 (7 μm, 250×10 mm) (Merck); MeOH–H_2O (30 : 70, v/v) at the rate of 5 ml/min] in two portions. The molar ratio of the diastereomers was 1 : 2 : 2 : 1 in the order of elution. The isomer that was eluted the fastest was identical (by comparison of the ^{1}H-NMR spectrum and HPLC mobility) to the authentic (*αS*,*βS*)-**11** described below. (*αR*,*βS*)-β-Hydroxy-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-β-D-ribofuranosyl-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic acid methyl ester [(*αR*,*βS*)-**11**] was obtained from the second fraction, ^{1}H-NMR [$(CD_3)_2CO$] δ[24]: 2.19 [3H, s, C(6)-Me], 3.28 (dd, *J*=14.2, 5.5 Hz), 3.36 (dd, *J*=14.2, 7 Hz) [1H each, C(γ)-H_2], 3.68, 3.69 (3H each, s, CCO_2Me and NCO_2Me), 3.85 (ddd, *J*=12.2, 5.4, 2.9 Hz), 3.94 (ddd, *J*=12.2, 4.9, 2.9 Hz) [1H each, C(5′)-H_2], 4.21 [1H, m, C(4′)-H], 4.22 (3H, s, NMe), 4.26 [1H, dd, *J*=1.5, 9.3 Hz, C(α)-H], 4.49 [2H, m, C(5′)-OH, C(3′)-H], 4.57—4.67 [2H, m, C(β)-OH, C(β)-H], 4.74 [1H, m, C(2′)-H], 4.78 [1H, br, C(3′)-OH], 5.13 [1H, br, C(2′)-OH], 6.19 [1H, d, *J*=9.3 Hz, C(α)-NH], 6.30 [1H, d, *J*=4.9 Hz, C(1′)-H], 8.21 [1H, s, C(2)-H]. The isomer obtained from the third fraction was identical (by comparison of the ^{1}H-NMR spectrum and HPLC mobility) with authentic (*αS*,*βR*)-**11** described below. (*αR*,*βR*)-β-Hydroxy-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-β-D-ribofuranosyl-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic acid methyl ester [(*αR*,*βR*)-**11**] was obtained from the fourth fraction, ^{1}H-NMR [$(CD_3)_2CO$] δ[24]: 2.24 [3H, s, C(6)-Me], 3.18 [1H, dd, *J*=14.7, 8.3 Hz, one C(γ)-H_2], 3.64, 3.67 [3H each, s, overlapping with a 1H signal arising from one C(γ)-H_2, CCO_2Me and NCO_2Me], 3.84, 3.92 [1H each, m, C(5′)-H_2], 4.21 [1H, m, C(4′)-H], 4.24 (3H, s, NMe), 4.28 [1H, m, C(β)-H], 4.37 [1H, m, C(α)-H], 4.49 [2H, m, C(5′)-OH and C(3′)-H], 4.58 [1H, br, C(β)-OH], 4.74 [1H, m, C(2′)-H], 4.90 [1H, br, C(3′)-OH], 5.25 [1H, br, C(2′)-OH], 6.30 [1H, d, *J*=4.9 Hz, C(1′)-H], 6.68 [1H, d, *J*=7.8 Hz, C(α)-NH], 8.21 [1H, s, C(2)-H].

Dihydroxylation of 13 A solution of *N*-methylmorpholine *N*-oxide monohydrate (90 mg, 0.67 mmol) in 0.5 M phosphate buffer (pH 6, 33 ml) and a 1.1% (w/v) OsO_4 solution in Me_3COH (1.2 ml, 0.05 mmol) were added to a solution of **13**[2] (333 mg, 0.392 mmol) in Me_2CO (33 ml). The resulting mixture was stirred at room temperature for 4 h and then for a further 30 min after the addition of $Na_2S_2O_5$ (124 mg, 0.652 mmol). The mixture was concentrated to half the initial volume and extracted with CH_2Cl_2 (3×20 ml). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo* to leave a foam (341 mg). This was dissolved in hexane (4 ml) and the solution was subjected to HPLC [LiChrosorb Si-60 (7 μm, 250×10 mm) (Merck); hexane–$CHCl_3$–MeOH (50 : 48 : 2, v/v)] in eight portions, providing (*αS*,*βS*,*γS*)-β,γ-dihydroxy-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic acid methyl ester monohydrate (**14**·H_2O) (176 mg, 50%), mp 117—121 °C. Recrystallization of this sample from 90% (v/v) aqueous MeOH and drying over P_2O_5 at 2 mmHg and 50 °C for 17 h afforded an analytical sample of **14**·H_2O as colorless needles, mp 115 °C (softened) 207—209.5 °C. $[\alpha]_D^{20}$ −14.2° (*c*=0.456, MeOH). FAB-MS *m/z*: 905 (MNa^+), 865 (MH^+-18). UV $\lambda_{max}^{95\%\,EtOH}$ nm (ε): 240 (35600), 297 (7000). IR ν_{max}^{Nujol} cm^{-1}: 1746, 1725, 1684 (C=O). ^{1}H-NMR ($CDCl_3$) δ: −0.29, −0.02, 0.13, 0.15, 0.16, 0.17 (3H each, s, three $SiMe_2$), 0.75, 0.95, 0.97 (9H each, s, three *tert*-Bu), 1.56 (s, H_2O), 2.24 [3H, s, C(6)-Me], 3.44 [1H, br s, C(β)-OH], 3.66, 3.72 (3H each, s, CCO_2Me and NCO_2Me), 3.80 [1H, dd, *J*=11.7, 1.5 Hz, one C(5′)-H_2], 3.88 [1H, d, *J*=10 Hz, C(α)-H], 3.89 [1H, dd, *J*=11.7, 2.5 Hz, one C(5′)-H_2], 4.13 [1H, dd, *J*=1.5, 2.5 Hz, C(4′)-H], 4.18 (3H, s, NMe), 4.20 [1H, d, *J*=4.4 Hz, C(3′)-H], 4.42 [1H, dd, *J*=4.4, 7.8 Hz, C(2′)-H], 4.53 (0.1H), 4.57 (0.9H) [d each, *J*=9.8 Hz, C(β)-H], 4.79 [1H, dd, *J*=9.8, 11.7 Hz, C(γ)-H], 5.46 (0.1H), 5.61 (0.9H) [d each, *J*=10 Hz, C(α)-NH], 5.62 [1H, d, *J*=11.7 Hz, C(γ)-OH], 6.25 [1H, d, *J*=7.8 Hz, C(1′)-H], 8.04 [1H, s, C(2)-H]. *Anal.* Calcd for $C_{39}H_{70}N_6O_{11}Si_3\cdot H_2O$: C, 51.97; H, 8.05; N, 9.32. Found: C, 52.12; H, 7.94; N, 9.38. (*αS*,*βR*,*γR*)-β,γ-Dihydroxy-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic acid methyl ester (**16**) (103 mg, 30%) was obtained from a later fraction as a colorless glass, $[\alpha]_D^{26}$ −13.9° (*c*=0.512, MeOH). FAB-MS *m/z*: 905 (MNa^+), 865 (MH^+-18). ^{1}H-NMR ($CDCl_3$) δ: −0.23, −0.01, 0.13, 0.14 (3H each), 0.15 (6H) (s, three $SiMe_2$), 0.75, 0.949, 0.953 (9H each, s, three *tert*-Bu), 2.39 [3H, s, C(6)-Me], 3.51 [1H, br, C(β)-OH], 3.55, 3.77 (3H each, s, NCO_2Me and CCO_2Me), 3.79 (dd, *J*=11.5, 1.5 Hz), 3.88 (dd, *J*=11.7, 2.5 Hz) [1H each, C(5′)-H_2], 4.13 [1H, dd, *J*=1.5, 2.5 Hz, C(4′)-H], 4.17 (3H, s, NMe), 4.21 [1H, d, *J*=4.4 Hz, C(3′)-H], 4.22 [1H, m, C(α)-H], 4.30 [1H, dd, *J*=2.4, 8.3 Hz, C(β)-H], 4.44 [1H, dd, *J*=4.4, 7.3 Hz, C(2′)-H], 5.16 [1H, dd, *J*=8.3, 11.2 Hz, C(γ)-H], 5.45 [1H, d, *J*=11.2 Hz, C(γ)-OH], 5.62 (0.1H, d, *J*=10 Hz), 5.72 (0.9H, d, *J*=7.8 Hz) [C(α)-NH], 6.22 [1H, d, *J*=7.3 Hz, C(1′)-H], 8.00 [1H, s, C(2)-H].

(αS,4S,5S)-5-[4,6-Dimethyl-9-oxo-3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4,9-dihydro-3*H*-imidazo[1,2-*a*]purin-7-yl]-α-[(methoxycarbonyl)amino]-2-oxo-1,3-dioxolane-4-acetic Acid Methyl Ester (15) A solution of **14**·H_2O (65 mg, 0.072 mmol) in benzene (5 ml) was dried over $MgSO_4$ and concentrated *in vacuo*. The residual foam was dried over P_2O_5 at 2 mmHg at room temperature for 3 h. This was dissolved in CH_2Cl_2 (2 ml), and pyridine (0.06 ml, 0.7 mmol) was added. A solution of triphosgene (16 mg, 0.054 mmol) in CH_2Cl_2 (2 ml) was then added dropwise at 0 °C over a period of 3 min, and the mixture was stirred at 0 °C for a further 15 min. The reaction mixture was diluted with CH_2Cl_2 (5 ml), washed successively with H_2O (5 ml), 5% aqueous citric acid (2×5 ml), and saturated aqueous $NaHCO_3$ (5 ml), dried over $MgSO_4$, and concentrated *in vacuo*, leaving a yellow glass. This was purified by flash chromatography [hexane–AcOEt (2 : 3, v/v)] to give **15** (53 mg, 80%) as a faintly yellow glass, $[\alpha]_D^{18}$ −50.6° (*c*=0.425, MeOH). FAB-MS *m/z*: 909 (MH^+). ^{1}H-NMR ($CDCl_3$) δ: −0.27, −0.02, 0.13 (3H each), 0.15 (6H), 0.16 (3H), (s, three $SiMe_2$), 0.76, 0.95, 0.96 (9H each, s, three *tert*-Bu), 2.38 [3H, s, C(6)-Me], 3.78, 3.79 (3H each, s, two CO$_2$Me), 3.79 (dd, *J*=11.7, 2 Hz), 3.87 (dd, *J*=11.7, 2.9 Hz) [1H each, C(5′)-H$_2$], 4.12 [1H, dd, *J*=2, 2.9 Hz, C(4′)-H], 4.14 (3H, s, NMe), 4.20 [1H, d, *J*=4 Hz, C(3′)-H], 4.42 [1H, dd, *J*=4, 7.5 Hz, C(2′)-H], 4.67 [1H, dd, *J*=1, 9 Hz, C(α)-H], 5.41 [1H, dd, *J*=1, 7.3 Hz, C(β)-H], 5.62 [1H, d, *J*=9 Hz, C(α)-NH], 5.83 [1H, d, *J*=7.3 Hz, C(γ)-H], 6.23 [1H, d, *J*=7.5 Hz, C(1′)-H], 8.00 [1H, s, C(2)-H].

(α*S*,4*R*,5*R*)-5-[4,6-Dimethyl-9-oxo-3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4,9-dihydro-3*H*-imidazo[1,2-*a*]purin-7-yl]-α-[(methoxycarbonyl)amino]-2-oxo-1,3-dioxolane-4-acetic Acid Methyl Ester (26) A solution of triphosgene (6 mg, 0.02 mmol) in dry CH$_2$Cl$_2$ (0.6 ml) was added dropwise to a solution of **16** (7.8 mg, 0.0088 mmol), **5**[3,16] (9.0 mg, 0.018 mmol), and pyridine (0.03 ml) in CH$_2$Cl$_2$ (0.6 ml) at 0 °C under N$_2$ over a period of 3 min, and the mixture was stirred at 0 °C for a further 15 min. The reaction mixture was diluted with CH$_2$Cl$_2$ (5 ml), washed successively with H$_2$O, 5% aqueous citric acid, and saturated aqueous NaHCO$_3$ (5 ml each), dried over MgSO$_4$, and concentrated *in vacuo*, leaving a yellow glass. This was purified by column chromatography on silica gel (AcOEt) to give **26** (4.6 mg, 57%) as a faintly yellow glass, ^{1}H-NMR (CDCl$_3$) δ: −0.26, −0.01, 0.13 (3H each), 0.14 (6H), 0.15 (3H), (s, three SiMe$_2$), 0.76, 0.947, 0.953 (9H each, s, three *tert*-Bu), 2.36 [3H, s, C(6)-Me], 3.69, 3.77 (3H each, s, CCO$_2$Me and NCO$_2$Me), 3.79 (dd, *J*=11.7, 2 Hz), 3.88 (dd, *J*=11.7, 2.9 Hz) [1H each, C(5′)-H$_2$], 4.12 [1H, dd, *J*=2, 2.9 Hz, C(4′)-H], 4.13 (3H, s, NMe), 4.20 [1H, d, *J*=4.4 Hz, C(3′)-H], 4.41 [1H, dd, *J*=4.4, 7.8 Hz, C(2′)-H], 4.86 [1H, dd, *J*=3.4, 8.8 Hz, C(α)-H], 5.20 [1H, dd, *J*=3.4, 7.3 Hz, C(β)-H], 5.69 [a total of 1H with a small broad signal at 5.35, br, C(α)-NH], 6.21 [1H, d, *J*=7.8 Hz, C(1′)-H], 6.43 [1H, d, *J*=7.3 Hz, C(γ)-H], 7.98 [1H, s, C(2)-H]. Compound **6**[3,16] (5.9 mg, 62%) was obtained from the later fraction.

(α*S*,β*R*)-β-Hydroxy-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic Acid Methyl Ester (17) Compound **15** (52 mg, 0.057 mmol) was hydrogenated over Pearlman's catalyst (52 mg) in MeOH (20 ml) at 40 °C under atmospheric pressure for 3 h. The catalyst was filtered off and washed with hot MeOH (100 ml). The filtrate and washings were combined and concentrated *in vacuo* to leave a colorless glass. This was purified by TLC on silica gel [CHCl$_3$–MeOH (40 : 1, v/v)] to give **18**[2] (11.2 mg, 23%) and **17** (14.3 mg, 29%) as a colorless glass, $[\alpha]_D^{18}$ −28.6° (*c*=0.500, MeOH). FAB-MS *m/z*: 889 (MNa$^+$), 867 (MH$^+$). ^{1}H-NMR (CDCl$_3$) δ: −0.28, −0.03, 0.12 (3H each), 0.14 (6H), 0.15 (3H) (s, three SiMe$_2$), 0.74, 0.94, 0.95 (9H each, s, three *tert*-Bu), 2.25 [3H, s, C(6)-Me], 3.15 (dd, *J*=15, 3.5 Hz), 3.57 (dd, *J*=15, 8.5 Hz) [1H each, C(γ)-H$_2$], 3.72, 3.76 (3H each, s, two CO$_2$Me), 3.79 (dd, *J*=11.7, 1.5 Hz), 3.87 (dd, *J*=11.7, 2.9 Hz) [1H each, C(5′)-H$_2$], 4.11 [3H, s, overlapping with a 1H signal arising from C(4′)-H, NMe], 4.17 [1H, d, *J*=5.4 Hz, C(β)-OH], 4.19 [1H, d, *J*=4.4 Hz, C(3′)-H], 4.39 [1H, dd, *J*=4.4, 7.5 Hz, overlapping with a 1H signal arising from C(β)-H, C(2′)-H], 4.46 [1H, d, *J*=9 Hz, C(α)-H], 5.66 [1H, d, *J*=9 Hz, C(α)-NH], 6.21 [1H, d, *J*=7.5 Hz, C(1′)-H], 7.94 [1H, s, C(2)-H].

(α*S*,β*S*)-β-Hydroxy-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic Acid Methyl Ester (27) i) Dihydroxylation of **13**[2] (300 mg, 0.353 mmol) was conducted in a manner similar to that described above, and the crude product was purified by flash chromatography [CHCl$_3$–MeOH (30 : 1, v/v)] to give a 2.1 : 1 (estimated by ^{1}H-NMR spectroscopy) mixture (288 mg) of **14** and **16**. A portion (277 mg, 0.314 mmol) of this product was treated with triphosgene (70 mg, 0.24 mmol) in a manner similar to that described above for the preparation of **15**. The crude product was purified by flash chromatography [hexane–AcOEt (2 : 3, v/v)] to give a 2.9 : 1 (estimated by ^{1}H-NMR spectroscopy) mixture (195 mg) of **15** and **26**. The mixture (194 mg) was shaken in MeOH (77 ml) under H$_2$ in the presence of Pearlman's catalyst (194 mg) at 40 °C for 2 h. The catalyst was filtered off and washed with hot MeOH (100 ml). The filtrate and washings were combined and concentrated *in vacuo*. The residue was purified by repeated flash chromatography and TLC on silica gel [CHCl$_3$–MeOH (40 : 1, v/v)], providing **18**[2] (79 mg, 28%), **17** (61 mg, 21%), and **27** (23 mg, 8%) as a colorless glass, $[\alpha]_D^{18}$ −21.5° (*c*=0.413, MeOH). FAB-MS *m/z*: 889 (MNa$^+$), 867 (MH$^+$). ^{1}H-NMR (CDCl$_3$) δ: −0.27, −0.03, 0.12 (3H each), 0.14 (6H), 0.15 (3H) (s, three SiMe$_2$), 0.75, 0.94, 0.96 (9H each, s, three *tert*-Bu), 2.25 [3H, s, C(6)-Me], 3.41 [2H, d, *J*=5.9 Hz, C(γ)-H$_2$], 3.70 (3H, s, CCO$_2$Me), 3.79 [1H, dd, *J*=11.5, 1.5 Hz, one C(5′)-H$_2$], 3.80 (3H, s, NCO$_2$Me), 3.83 [1H, d, *J*=6.8 Hz, C(β)-OH], 3.87 [1H, dd, *J*=11.5, 2.5 Hz, one C(5′)-H$_2$], 4.11 [3H, s, overlapping with a 1H signal arising from C(4′)-H, NMe], 4.16 [1H, m, C(β)-H], 4.20 [1H, d, *J*=4.4 Hz, C(3′)-H], 4.41 [1H, dd, *J*=4.4, 7.8 Hz, C(2′)-H], 4.50 [1H, m, C(α)-H], 5.88 [1H, d, *J*=7.3 Hz, C(α)-NH], 6.21 [1H, d, *J*=7.8 Hz, C(1′)-H], 7.94 [1H, s, C(2)-H].

ii) Compound **26** (4 mg, 0.004 mmol) was hydrogenated over Pearlman's catalyst (4 mg) in MeOH (2 ml) at 40 °C for 2 h and then at 50 °C for a further 2 h. The catalyst was filtered off and washed with hot MeOH (100 ml). The filtrate and washings were combined and concentrated *in vacuo*. The residue was purified by TLC on silica gel [CHCl$_3$–MeOH (40 : 1, v/v)] to give **18**[2] (0.7 mg) and **27** (1.5 mg) as a colorless glass.

(α*S*,β*R*)-β-Hydroxy-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-β-D-ribofuranosyl-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic Acid Methyl Ester [(α*S*,β*R*)-11] A 1 M Bu$_4$NF solution (0.7 ml, 0.7 mmol) in THF was added to a solution of **17** (58 mg, 0.067 mmol) in pyridine–THF–H$_2$O (1 : 8 : 1, v/v) (3.3 ml), and the mixture was stirred at room temperature for 18 h. The resulting solution was concentrated *in vacuo*. The residue was purified by flash chromatography [CH$_2$Cl$_2$–MeOH (5 : 1, v/v)] to give (α*S*,β*R*)-**11**·H$_2$O (31 mg, 86%), mp 173—183 °C. Recrystallization of this compound from 80% (v/v) aqueous MeOH and drying over P$_2$O$_5$ at 2 mmHg and 50 °C for 10 h afforded colorless plates, mp 205—210 °C. These were exposed to air at room temperature until a constant weight was reached, providing (α*S*,β*R*)-**11**·H$_2$O: mp 198—210 °C (dec.). $[\alpha]_D^{30}$ −72.8° (*c*=0.167, H$_2$O). FAB-MS *m/z*: 547 (MNa$^+$), 525 (MH$^+$). ^{1}H-NMR [(CD$_3$)$_2$SO] δ: 2.07 [3H, s, C(6)-Me], 3.10 (dd, *J*=14.2, 6.8 Hz), 3.16 (dd, *J*=14.2, 7 Hz) [1H each, C(γ)-H$_2$], 3.53 (0.3H, s), 3.59 [5.7H, s, overlapping with a 1H signal arising from one C(5′)-H$_2$] (two CO$_2$Me), 3.69 [1H, ddd, *J*=12.2, 3.4, 4.9 Hz, one C(5′)-H$_2$], 3.89 (0.1H, br), 3.94 (0.9H, dd, *J*=2.4, 8.8 Hz) [C(α)-H], 3.99 [1H, ddd, *J*=3.4, 3.4, 4.9 Hz, C(4′)-H], 4.03 (3H, s, NMe), 4.13 [1H, ddd, *J*=4.9, 4.9, 5.9 Hz, C(3′)-H], 4.41 [1H, dddd, *J*=7, 7, 2.4, 7.8 Hz, C(β)-H], 4.45 [1H, ddd, *J*=4.9, 5.9, 4.9 Hz, C(2′)-H], 4.97 (0.9H), 5.01 (0.1H) [d each, *J*=7.8 Hz, C(β)-OH], 5.12 [1H, dd, *J*=5.4, 4.9 Hz, C(5′)-OH], 5.32 [1H, d, *J*=5.9 Hz, C(3′)-OH], 5.71 [1H, d, *J*=5.9 Hz, C(2′)-OH], 6.10 [1H, d, *J*=4.9 Hz, C(1′)-H], 6.63 (0.1H), 7.11 (0.9H) [d each, *J*=8.8 Hz, C(α)-NH], 8.22 [1H, s, C(2)-H]. ^{1}H-NMR [(CD$_3$)$_2$CO] δ[24]: 2.19 [3H, s, C(6)-Me], 3.28 (dd, *J*=14.7, 5.9 Hz), 3.36 (dd, *J*=14.7, 7 Hz) [1H each, C(γ)-H$_2$], 3.68, 3.69 (3H each, s, CCO$_2$Me and NCO$_2$Me), 3.85 (ddd, *J*=12.2, 5.4, 2.9 Hz), 3.94 (ddd, *J*=12.2, 4.9, 2.9 Hz) [1H each, C(5′)-H$_2$], 4.21 [1H, m, C(4′)-H], 4.22 (3H, s, NMe), 4.26 [1H, dd, *J*=1.5, 9.3 Hz, C(α)-H], 4.49 [2H, m, C(5′)-OH, C(3′)-H], 4.57—4.67 [2H, m, C(β)-OH, C(β)-H], 4.74 [1H, m, C(2′)-H], 4.78 [1H, br, C(3′)-OH], 5.13 [1H, br, C(2′)-OH], 6.19 [1H, d, *J*=9.3 Hz, C(α)-NH], 6.30 [1H, d, *J*=4.9 Hz, C(1′)-H], 8.21 [1H, s, C(2)-H]. *Anal.* Calcd for C$_{21}$H$_{28}$N$_6$O$_{10}$·H$_2$O: C, 46.49; H, 5.57; N, 15.49. Found: C, 46.40; H, 5.38; N, 15.41.

(α*S*,β*S*)-β-Hydroxy-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-β-D-ribofuranosyl-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic Acid Methyl Ester [(α*S*,β*S*)-11] Treatment of **27** (37 mg, 0.043 mmol) with Bu$_4$NF and purification of the product were performed in manners similar to those described above for the preparation of (α*S*,β*R*)-**11**, giving (α*S*,β*S*)-**11** (19 mg, 86%) as a colorless glass. This was subjected to HPLC [LiChrosorb RP18 (7 μm, 250×10 mm) (Merck); MeOH–H$_2$O (30 : 70, v/v) at the rate of 1.5 ml/min] in seven portions to give (α*S*,β*S*)-**11**·3/2H$_2$O (12 mg, 50%), mp 183—185 °C. Recrystallization of this compound from 90% (v/v) aqueous MeOH, drying over P$_2$O$_5$ at 2 mmHg and 50 °C for 10 h, and exposure to air at room temperature until a constant weight was reached, provided an analytical sample of (α*S*,β*S*)-**11**·3/2H$_2$O as colorless needles, mp 189 °C (softened), 200—203 °C (dec.). $[\alpha]_D^{29}$ −38.3° (*c*=0.174, H$_2$O). FAB-MS *m/z*: 547 (MNa$^+$), 525 (MH$^+$). UV $\lambda_{\max}^{95\%\,\mathrm{EtOH}}$ nm (ε): 239.5 (32300), 280 (sh) (5000), 298 (6500). ^{1}H-NMR [(CD$_3$)$_2$SO] δ: 2.14 [3H, s, C(6)-Me], 3.02 (dd, *J*=14.7, 7.5 Hz), 3.41 (dd, *J*=14.7, 4.9 Hz) [1H each, C(γ)-H$_2$], 3.55, 3.58 [3H each, s, overlapping with a 1H signal arising from one C(5′)-H$_2$, NCO$_2$Me and CCO$_2$Me], 3.68 [1H, ddd, *J*=12, 3.4, 5.4 Hz, one C(5′)-H$_2$], 3.99 [1H, ddd, *J*=3.4, 3.4, 4.4 Hz, C(4′)-H], 4.04 (3H, s, NMe), 4.09 [1H, m, C(β)-H], 4.11—4.16 [2H, m, C(α)-H and C(3′)-H], 4.45 [1H, ddd, *J*=4.4, 5.9, 4.9 Hz, C(2′)-H], 5.06 [1H, d, *J*=5.4 Hz, C(β)-OH], 5.12 [1H, dd, *J*=5.4 Hz each, C(5′)-OH], 5.31 [1H, d, *J*=5.4 Hz, C(3′)-OH], 5.70 [1H, d, *J*=5.9 Hz, C(2′)-OH], 6.11 [1H, d, *J*=4.9 Hz, C(1′)-H], 6.86 (0.1H, br), 7.28 (0.9H, d, *J*=8.3 Hz) [C(α)-NH], 8.22 [1H, s, C(2)-H]. ^{1}H-NMR [(CD$_3$)$_2$CO] δ[24]: 2.24 [3H, s, C(6)-Me], 3.18 [1H, dd, *J*=14.5, 8.5 Hz, one C(γ)-H$_2$], 3.65, 3.66 [3H each, s, overlapping with a 1H signal arising from one C(γ)-H$_2$, CCO$_2$Me and NCO$_2$Me], 3.84 (ddd, *J*=12.2, 5.4, 2.9 Hz), 3.94 (ddd, *J*=12.2, 4.9, 2.9 Hz) [1H each, C(5′)-H$_2$], 4.22 [1H, m, C(4′)-H], 4.24 (3H, s, NMe), 4.27 [1H, m, C(β)-H], 4.37 [1H, m, C(α)-H], 4.44 [1H, br, C(5′)-OH], 4.49 [1H, m, C(3′)-H], 4.54 [1H, d, *J*=5.9 Hz, C(β)-OH], 4.65 [1H, br, C(3′)-OH], 4.76 [1H, m, C(2′)-H], 5.01 [1H, br, C(2′)-OH], 6.29 [1H, d, *J*=4.9 Hz, C(1′)-H], 6.67 [1H, d, *J*=9.8 Hz, C(α)-NH], 8.19 [1H, s, C(2)-H]. *Anal.* Calcd for C$_{21}$H$_{28}$N$_6$O$_{10}$·3/2H$_2$O: C, 45.73; H, 5.67; N, 15.24. Found: C, 45.51; H, 5.38; N, 15.29.

Hydrolysis of ($\alpha S,\beta R$)-11 Leading to ($\alpha S,\beta R$)-2 A solution of ($\alpha S,\beta R$)-**11**·H_2O (7 mg) in 0.1 N aqueous HCl (2 ml) was stored at room temperature for 1 h, neutralized with 0.2 M aqueous Na_2HPO_4 (2 ml), and concentrated *in vacuo*. The residue was purified by TLC on silica gel [$CHCl_3$–MeOH (10 : 1, v/v)], providing ($\alpha S,\beta R$)-**2** (5 mg), mp 232—235 °C (dec.) [lit.[3] mp 232—233.5 °C (dec.)]. ^1H-NMR ($CDCl_3$) (Table 1). ^1H-NMR [$(CD_3)_2CO$] δ[24]: 2.21 [3H, s, C(6)-Me], 3.33 [2H, d, J=6.8 Hz, C(γ)-H_2], 3.68, 3.69 (3H each, s, CCO_2Me and NCO_2Me), 3.88 (3H, s, NMe), 4.19 (0.1H, br), 4.30 (0.9H, dd, J=2.0, 9.3 Hz) [C(α)-H], 4.51 (0.9H, d, J=5.9 Hz), 4.58 (0.1H, br) [C(β)-OH], 4.62 [1H, ddt, J=2.0, 5.9, 6.8 Hz, C(β)-H], 5.74 (0.1H, br), 6.17 (0.9H, d, J=9.3 Hz) [C(α)-NH], 8.17 [1H, d, J=1.0 Hz, C(2)-H], 12.42 [1H, br, N(1)-H]. This sample was identical (by comparison of the IR and ^1H-NMR spectra and TLC mobility) to an authentic sample[3] and optically pure on the basis of chiral HPLC analysis.[16]

Hydrolysis of ($\alpha S,\beta S$)-11 leading to ($\alpha S,\beta S$)-2 Hydrolysis of ($\alpha S,\beta S$)-**11**·3/2H_2O (2.1 mg) and purification of the product were performed in manners similar to those described above for the preparation of ($\alpha S,\beta R$)-**2**, providing ($\alpha S,\beta S$)-**2**·1/2H_2O (1.5 mg), mp 230—233 °C (dec.) [lit.[3] mp 233—235 °C (dec.)]. ^1H-NMR ($CDCl_3$) (Table 1). ^1H-NMR [$(CD_3)_2CO$] δ[24]: 2.25 [3H, s, C(6)-Me], 3.17 [1H, dd, J=14.6, 7.8 Hz, one C(γ)-H_2], 3.63, 3.65 (3H each, s, two CO_2Me), 3.67 [1H, dd, J=14.6, 4.4 Hz, one C(γ)-H_2], 3.90 (3H, s, NMe), 4.28 [1H, dddd, J=7.8, 4.4, 5.9, 6.4 Hz, C(β)-H], 4.38 [1H, dd, J=5.9, 8.3 Hz, C(α)-H], 4.49 [1H, d, J=6.4 Hz, C(β)-OH], 6.25 (0.1H, br), 6.64 (0.9H, d, J=8.3 Hz) [C(α)-NH], 8.20 [1H, d, J=1.0 Hz, C(2)-H], 12.50 [1H, br, N(1)-H]. ^1H-NMR [CD_3CN] δ[25]: 2.20 [3H, s, C(6)-Me], 3.08 (1H, dd, J=15.1, 8.3 Hz), 3.54 (1H, dd, J=15.1, 4.4 Hz), [C(γ)-H_2], 3.63, 3.65 (3H each, s, CCO_2Me and NCO_2Me), 3.71 [1H, d, J=6.4 Hz, C(β)-OH], 3.84 (3H, s, NMe), 4.15 [1H, m, C(β)-H], 4.29 [1H, m, C(α)-H], 6.32 [1H, br, C(α)-NH], 7.94 [1H, s, C(2)-H], 11.09 [1H, br, N(1)-H]. ^1H-NMR (pyridine-d_5) δ[26]: 2.47 [3H, s, C(6)-Me], 3.61 [1H, dd, J=14.7, 8.3 Hz, one C(γ)-H_2], 3.64, 3.66 (3H each, s, two CO_2Me), 3.70 [1H, m, C(β)-H], 3.91 (3H, s, NMe), 4.14 [1H, dd, J=14.7, 3.9 Hz, one C(γ)-H_2], 5.16 [1H, dd, J=6.4, 7.8 Hz, C(α)-H], 8.33 [1H, s, C(2)-H], 8.57 [1H, d, J=7.8 Hz, C(α)-NH]. This sample was identical (by comparison of the IR and ^1H-NMR spectra and TLC mobility) to an authentic sample[3] and optically pure on the basis of chiral HPLC analysis.[16]

($\alpha S,\beta R$)-β-(Acetoxy-d_3)-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-[2,3,5-tri-*O*-(acetyl-d_3)-β-D-ribofuranosyl]-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic Acid Methyl Ester (28) Compound ($\alpha S,\beta R$)-**11** (5 mg) was treated in a manner similar to that described below for the preparation of **29**, giving **28** (6 mg) as a colorless glass, MS *m/z*: 704 (M^+). ^1H-NMR ($CDCl_3$) δ: 2.21 [3H, s, C(6)-Me], 3.09 [1H, dd, J=15.1, 9.8 Hz, one C(γ)-H_2], 3.71, 3.75 (3H each, s, NCO_2Me and CCO_2Me), 3.78 [1H, dd, J=15.1, 2.9 Hz, one C(γ)-H_2], 4.12 (3H, s, NMe), 4.32 [2H, d, J=2.4 Hz, C(5′)-H_2], 4.49 [1H, dt, J=2.4, 2.9 Hz, C(4′)-H], 4.63 (0.1H, br), 4.67 (0.9H, dd, J=9.8, 2.9 Hz) [C(α)-H], 5.28 (0.1H, br), 5.54 (0.9H, d, J=9.8 Hz) [C(α)-NH], 5.50 [1H, dd, J=2.9, 5.4 Hz, C(3′)-H], 5.80 [1H, ddd, J=9.8, 2.9, 2.9 Hz, C(β)-H], 5.85 [1H, dd, J=5.4, 6.3 Hz, C(2′)-H], 6.18 [1H, d, J=6.3 Hz, C(1′)-H], 7.74 [1H, s, C(2)-H].

($\alpha S,\beta S$)-β-(Acetoxy-d_3)-α-[(methoxycarbonyl)amino]-4,6-dimethyl-9-oxo-3-[2,3,5-tri-*O*-(acetyl-d_3)-β-D-ribofuranosyl]-4,9-dihydro-3*H*-imidazo[1,2-*a*]purine-7-butanoic Acid Methyl Ester (29) A solution of ($\alpha S,\beta S$)-**11** (5 mg) in a mixture of Ac_2O-d_6 (47 mg) and pyridine (102 mg) was allowed to stand at room temperature for 1.5 h and then concentrated *in vacuo*. The residue was purified by TLC on silica gel [$CHCl_3$–MeOH (30 : 1, v/v)], giving **29** (6 mg) as a colorless glass, MS *m/z*: 704 (M^+). ^1H-NMR ($CDCl_3$) δ: 2.24 [3H, s, C(6)-Me], 3.01 [1H, dd, J=15.1, 8.8 Hz, one C(γ)-H_2], 3.69, 3.70 (3H each, s, NCO_2Me and CCO_2Me), 3.89 [1H, dd, J=15.1, 4.4 Hz, one C(γ)-H_2], 4.11 (3H, s, NMe), 4.32 [2H, m, C(5′)-H_2], 4.50 [1H, m, C(4′)-H], 4.71 [1H, dd, J=5.4, 8.8 Hz, C(α)-H], 5.48 [1H, dd, J=3.4, 5.4 Hz, C(3′)-H], 5.50 [1H, m, C(β)-H], 5.83 [1H, dd, J=5.4, 6.4 Hz, C(2′)-H], 6.12 [1H, d, J=8.8 Hz, C(α)-NH], 6.19 [1H, d, J=6.4 Hz, C(1′)-H], 7.74 [1H, s, C(2)-H].

Isolation of β-Hydroxywybutine Unfractionated tRNA was obtained as described below according to the reported procedure.[27] Twenty female Wistar rats were decapitated and their livers (160 g) removed and quickly chilled in ice. Batches of 9 g were homogenized with 88% (v/v) aqueous phenol (14 ml) and Tris buffer [0.02 M $(HOCH_2)_3CNH_2$–HCl (pH 7.5) containing 1 M NaCl–0.5 mM ethylenediaminetetraacetic acid–0.01 M $MgCl_2$] (14 ml) in a homogenizer at high speed for 2—3 min. The combined homogenate was shaken at room temperature for 2 h and centrifuged (8000 rpm, 40 min) at 4 °C. The precipitate was mixed with the Tris buffer (150 ml) and centrifuged. EtOH (1.4 l) was added to the combined supernatant (*ca.* 700 ml), and the whole was stored at 4 °C overnight. The precipitate that separated was collected by centrifugation at 4 °C and suspended in 0.3 M aqueous AcONa (140 ml). The mixture was stirred at room temperature for 10 min after the addition of Me_2CHOH (56 ml) and centrifuged at 20 °C. The precipitate was again treated with 0.3 M aqueous AcONa (80 ml) and Me_2CHOH (40 ml), and the mixture was centrifuged at 20 °C. The combined supernatant was cooled with ice for 1 h after the addition of Me_2CHOH (130 ml), and the precipitate was collected by centrifugation. The precipitate thus obtained was washed successively with 67% (v/v) aqueous EtOH (containing 0.1 M NaCl–0.005 M $MgCl_2$), EtOH, and Et_2O (10 ml each), and dried over P_2O_5 under reduced pressure, giving crude tRNA (184 mg). A portion of this product (100 mg, 1000 A_{260} units) was dissolved in H_2O (15 ml), and the solution (pH 8.9) was brought to pH 2.9 by the addition of dilute aqueous HCl. The resulting mixture was stirred at 40 °C for 16 h and centrifuged at 4 °C. After the addition of EtOH (30 ml) the supernatant was centrifuged at 4 °C. The supernatant was neutralized with $NaHCO_3$, and the precipitate that resulted was removed by centrifugation at 4 °C. The supernatant was concentrated *in vacuo*, and the residue was purified by TLC on silica gel [$CHCl_3$–MeOH (10 : 1, v/v)], giving the minor base. The HPLC [LiChrosorb Si 60 (Merck); $CHCl_3$–MeOH (95 : 5, v/v)] and chiral HPLC[16] behavior of this substance was identical to that of ($\alpha S,\beta S$)-**2**. The observable ^1H-NMR signals [[$(CD_3)_2CO$] δ[24]: 2.25 (s), 3.63 (s), 3.65 (s), 3.90 (s), 4.48 (d, J=6.4 Hz), 6.64 (d, J=8 Hz), 8.20 (s), 12.50 (br)] were superimposable on those obtained for a dilute solution of ($\alpha S,\beta S$)-**2**.

Isolation and Identification of β-Hydroxywybutosine Crude tRNA (1 g, 10000 A_{260} units) was produced in five batches of 20 rats in a manner similar to that described above. This was extracted with a mixture of 0.3 M aqueous AcONa (75 ml) and Me_2CHOH (30 ml) and then with a mixture of 0.3 M aqueous AcONa (42 ml) and Me_2CHOH (21 ml). The extracts were combined, centrifuged, and diluted with Me_2CHOH (70 ml). The resulting precipitate was collected by centrifugation at 4 °C after cooling with ice, washed successively with EtOH and Et_2O, and dried to give the first crop of tRNA (520 mg). The residue, which remained undissolved on extraction with a mixture of aqueous AcONa and Me_2CHOH, was further extracted with 0.3 M aqueous AcONa (2×28 ml). The extracts were collected by centrifugation, diluted with Me_2CHOH (58 ml), and chilled with ice. The resulting precipitate was collected by centrifugation at 4 °C, providing a second crop of tRNA (210 mg). These crops were combined and extracted twice with the Tris buffer (20 ml and 10 ml) described above. The extracts were collected by centrifugation at 20 °C and subjected to a column packed with ion-exchange cellulose (Whatman DE 32) (wet volume 50 ml) which had been equilibrated with the Tris buffer. The column was washed with the Tris buffer (140 ml) followed by elution with the Tris buffer (800 ml) containing 1 M NaCl. EtOH (1.5 l) was added to the eluate and the mixture was stored at 4 °C overnight. The resulting precipitate was collected by centrifugation at 4 °C, washed successively with 67% (v/v) aqueous EtOH (containing 0.1 M NaCl–0.005 M $MgCl_2$), EtOH, and Et_2O (30 ml each), and dried over P_2O_5 under reduced pressure, providing tRNA (355 mg).

A portion of this product (350 mg, 5300 A_{260} units) was treated with nuclease P_1 (Yamasa) (500 units) in 0.02 M acetate buffer (pH 5.35, 30 ml) at 50 °C for 3 h. The hydrolysate was subjected to column chromatography on Cosmosil 140C_{18}-OPN (Nacalai Tesque) (10 g) [H_2O (80 ml) and then MeOH–H_2O (30 : 70, v/v)] in two portions. The MeOH–H_2O fractions (80 ml) were combined and concentrated *in vacuo* to leave a yellow glass (210 A_{260} units). It was dissolved in H_2O (3.9 ml), and a solution of nuclease P_1 (2000 units) in 0.02 M acetate buffer (pH 5.35, 4.7 ml) was added. The whole was stored at 50 °C for 3 h and then 0.02 N aqueous NaOH (1.05 ml) and *Escherichia coli* alkaline phosphatase (Takara Shuzo) (6.4 units) were added. The mixture (pH 9) was stored at 50 °C for 1 h and concentrated *in vacuo*. This was dissolved in H_2O (8 ml) and purified by HPLC [LiChrosorb RP18 (7 μm, 250×10 mm) (Merck); MeOH–H_2O (30 : 70, v/v)] in seven portions, providing the target nucleoside (1.5 A_{310} unit, *ca.* 100 μg), of which HPLC behavior was identical to that of ($\alpha S,\beta S$)-**11**.

The nucleoside was dissolved in a mixture of Ac_2O-d_6 (29 mg) and pyridine (69 mg), stored at room temperature for 1.5 h, and purified by TLC on silica gel [CH_2Cl_2–MeOH (30 : 1, v/v)] to afford the tetraacetate-d_{12}, MS *m/z* (%): 704 (M^+) (7), 641 (3), 609 (18), 582 (12), 550 (2), 483 (18), 437 (3), 374 (2), 342 (22), 315 (14), 283 (12), 268 (9), 216 (100), 142 (26). ^1H-NMR ($CDCl_3$) δ: 2.24 (3H, s), 3.02 (1H, dd), 3.697, 3.704 (3H each, s), 4.11 (3H, s), 4.33 (2H, m), 4.50 (1H, m), 4.71 (1H, dd), 5.48 (1H, dd), 5.51 (1H, m), 5.83 (1H, dd), 6.12 (1H, d), 6.19 (1H, d), 7.74 (1H, s). This compound was identical (by comparison of the MS and ^1H-NMR spectra) to **29**.

A solution of half of the tetraacetate-d_{12} in 0.1 M MeONa–MeOH (0.1 ml) was stored at 0 °C for 5 min and 0.1 N aqueous HCl (0.2 ml) was added at once. The resulting solution was stored at room temperature for 1 h, neutralized by the addition of 0.2 M Na_2HPO_4 (0.1 ml), and concentrated *in vacuo*. The residue was purified by TLC on silica gel [$CHCl_3$–MeOH (10 : 1, v/v)], giving the base, ^{1}H-NMR [$(CD_3)_2CO$] δ[24]: 2.25 (3H, s), 3.18 (1H, dd), 3.64, 3.65 (3H each, s), 3.67 (1H, dd), 3.90 (3H, s), 4.28 (m), 4.39 (1H, m), 4.47 (1H, d), 6.62 (d), 8.20 (1H, s), 12.50 (1H, br). This compound was identical (by comparison of the ^{1}H-NMR spectrum and chiral HPLC[16] mobility) to ($\alpha S,\beta S$)-**2**.

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