Pergamon

*Tetrahedron Letters*, Vol. 38, No. 11, pp. 1979-1982, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00253-0

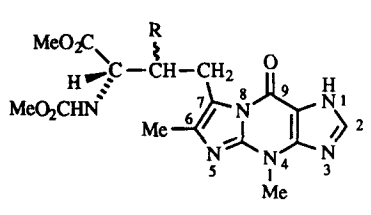
# Synthesis of [*R*-(*R**,*S**)]- and [*S*-(*R**,*R**)]-β-Hydroxy-3-(β-D-ribofuranosyl)-wybutines, the Most Probable Alternatives for the Hypermodified Nucleoside of Rat Liver Phenylalanine Transfer Ribonucleic Acid

**Taisuke Itaya,* Tae Kanai, and Takehiko Iida**

Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan

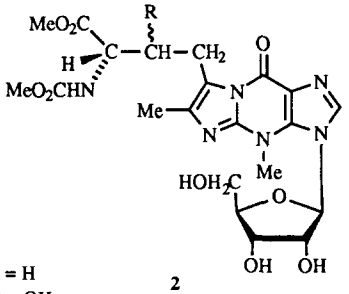
***Abstract:*** The synthesis of the title compounds started with the Vilsmeier reaction of 3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]wye (**5b**) and proceeded through the Wittig reaction with (*R*)-*N*-(methoxycarbonyl)-3-(triphenylphosphonio)alaninate (**4**), methylation with trimethylsilyldiazomethane, $OsO_4$ oxidation, cyclocondensation with triphosgene, and catalytic hydrogenolysis. Chromatographic separation of the resulting diastereomeric mixture and subsequent deprotection afforded the two desired nucleosides [[*R*-(*R**,*S**)]- and [*S*-(*R**,*R**)]-**2b**] for the first time.


An *S* configuration has been assigned to wybutine (**1a**) from yeast phenylalanine transfer ribonucleic acid ($tRNA^{Phe}$) on the basis of our chiral synthesis utilizing the Wittig reaction with **3**.[1] Therefore, we envisioned [*R*-(*R**,*S**)]- and [*S*-(*R**,*R**)]-**1b** as the most probable alternatives for β-hydroxywybutine[2] from rat liver $tRNA^{Phe}$ and have already synthesized these two candidates.[3] However, lack of a sample of β-hydroxywybutine from the $tRNA^{Phe}$ has hampered determination of its three-dimensional structure. Although the structure of the parent nucleoside of β-hydroxywybutine is considered to be **2b**, rigorous identification of the position of glycosylation and the structure of the sugar moiety has had to await isolation of the nucleoside from the $tRNA^{Phe}$. We wish to report herein the first synthesis of **2b**, which should help toward isolation of the nucleoside under consideration and hence determination of its complete structure.

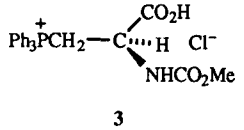


**1**

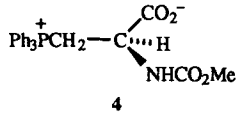
**a**: R = H
**b**: R = OH



**2**



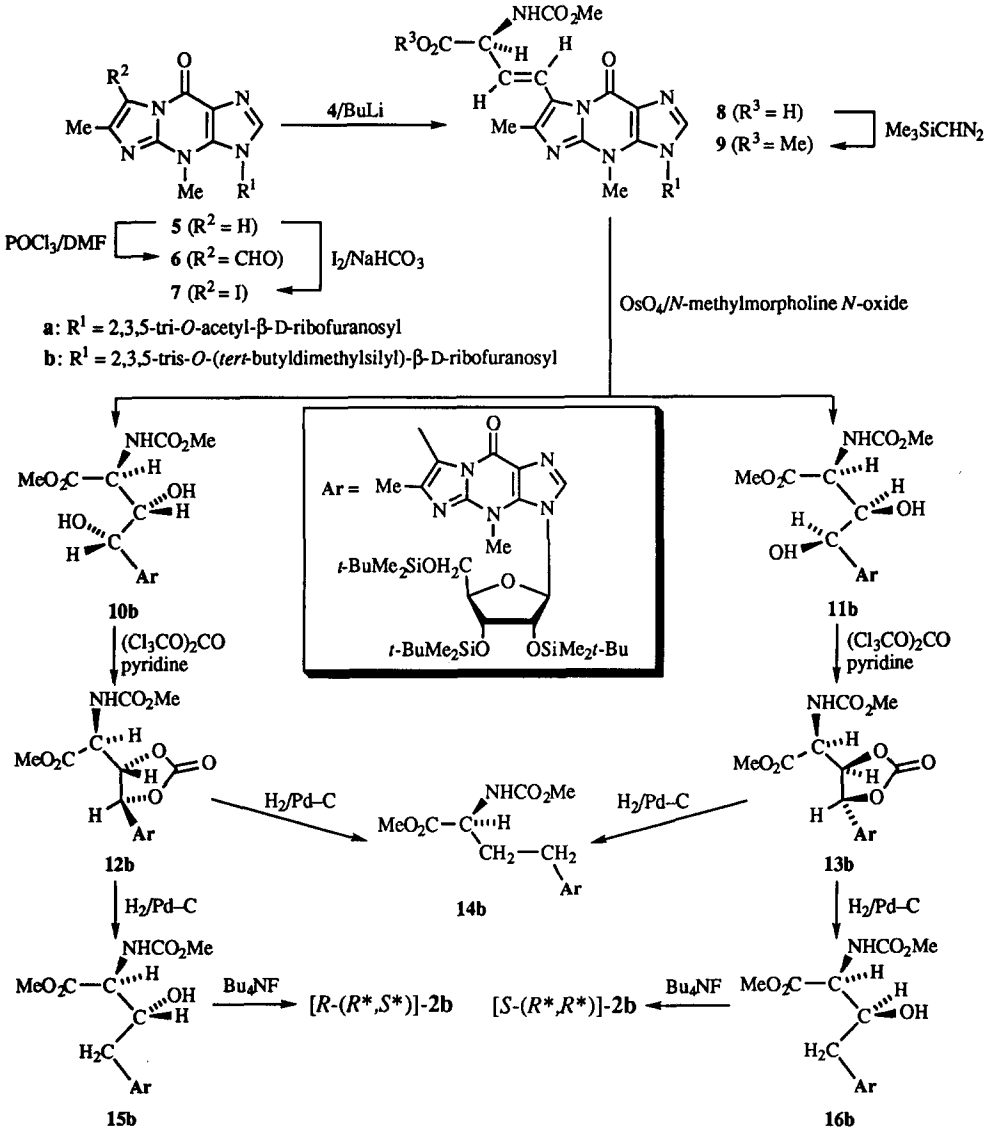
**3**



**4**

We have already synthesized **2a**, the putative structure for the nucleoside isolated from yeast $tRNA^{Phe}$, through **8a** employing the Heck reaction between **7a** and (*S*)-*N*-(methoxycarbonyl)vinylglycine as the key step.[4] Compound **8a** is also a desirable intermediate for the synthesis of **2b**. The Heck reaction, however,

was accompanied by partial epimerization at the amino acid moiety, and **8a** was obtained in only a small quantity after repeated HPLC.[4] We accordingly attempted the Wittig reaction between the triacetate **6a**[5] and **3**,[1] however, no trace of the desired product **8a** was obtained, probably owing to the acidity and/or electrophilicity of the acetoxy groups of **6a**.[6] In the present study, the silyl ether **6b** [$[\alpha]_D^{34}$ –38.9° (*c* 1.00, MeOH)], which was obtainable as a colorless glass in 64% yield by the Vilsmeier reaction of **5b**[7] in DMF at –30 °C for 1 h, was adopted for the Wittig reaction. The Wittig reaction (*n*-BuLi/THF–HMPA; –70—0 °C) between **6b** and the inner salt **4**, which was shown to be a better reagent than the phosphonium chloride **3**,[8] and subsequent methylation with trimethylsilyldiazomethane afforded **9b**[9] in 22% yield.



With the key intermediate **9b** in hand, we envisioned it would be possible to obtain the protected nucleosides (**15b** and **16b**) by following the reaction sequence analogous to that employed for the synthesis of **1b**.[3] Thus, $OsO_4$ oxidation of **9b** [acetone–phosphate buffer (pH 6) (1:1, v/v)] in the presence of *N*-methylmorpholine *N*-oxide at room temperature for 4 h, followed by HPLC on silica gel [hexane–$CHCl_3$–MeOH (50:48:2, v/v)] afforded **10b**·$H_2O$ [mp 207—209.5 °C (softened at 115 °C); $[\alpha]_D^{20}$ –14.2° (*c* 0.456, MeOH)] and **11b** [a colorless glass; $[\alpha]_D^{26}$ –13.9° (*c* 0.512, MeOH)] in 50% and 30% yields, respectively. For the preparation of cyclic carbonates, we found in the present study that triphosgene–pyridine was better than oxalyl chloride–triethylamine[3] and obtained **12b** [a slightly yellow glass; $[\alpha]_D^{18}$ –50.6° (*c* 0.425, MeOH)] in 80% yield by treatment of **10b** with an excess of triphosgene in $CH_2Cl_2$ in the presence of pyridine at 0 °C for 15 min. Catalytic hydrogenolysis of **12b** over Pearlman's catalyst afforded **15b** [$[\alpha]_D^{18}$ –28.6° (*c* 0.500, MeOH)] in 28% yield, together with **14b** (23%).

Surprisingly, no trace of **13b** was produced on treatment of the minor diastereomer **11b** with triphosgene under conditions similar to those described above for the preparation of **12b**. More surprisingly, when a mixture of **11b** and **10b**, accessible in 91% yield in a ratio of 1:2 in the above $OsO_4$ oxidation of **9b**, was subjected to the reaction with triphosgene, **13b** was obtained as a mixture with **12b** in a ratio of 1:3. The resulting diastereomeric mixture was hydrogenated over Pearlman's catalyst, followed by flash chromatography [$CHCl_3$–MeOH (40:1, v/v)], affording **16b** [$[\alpha]_D^{18}$ –21.5° (*c* 0.413, MeOH)], **15b**, and **14b**, each as a colorless glass, in 8%, 21%, and 27% yields (based on **9b**), respectively. Deprotection of **15b** and **16b** was accomplished by treatment with $Bu_4NF$ in aqueous THF in the presence of pyridine at room temperature without cleaving the extraordinarily labile glycosyl bonds to provide [*R*-(*R**,*S**)]- and [*S*(*R**,*R**)]-**2b** in 86% yield each.

The target nucleosides thus obtained are distinguishable from each other by $^1H$ NMR spectroscopy[10] and HPLC.[11] The structures [*R*-(*R**,*S**)]- and [*S*-(*R**,*R**)]-**2b** were assigned to them by comparison of their $^1H$ NMR spectra with those of [*R*-(*R**,*S**)]- and [*S*-(*R**,*R**)]-**1b**.[3] Final identification of [*R*-(*R**,*S**)]-**2b** rested on its hydrolysis with 0.1 N aqueous HCl leading to [*R*-(*R**,*S**)]-**1b**.[3]

In conclusion, the present synthesis of **9b** has greatly facilitated syntheses of stereochemically pure nucleosides **2**, providing authentic samples of the title compounds **2b**. We are now investigating the chemical properties of **2b** in order to establish an efficient procedure for the isolation of the hypermodified nucleoside from natural sources.

***Acknowledgment***

This work was supported by a grant from the Japan Research Foundation for Optically Active Compounds and Grant-in-Aid for Scientific Research (No. 07557289) from the Ministry of Education, Science, Sports and Culture, Japan.

## REFERENCES AND NOTES

1. Itaya, T.; Mizutani, A.; Iida, T. *Chem. Pharm. Bull.* **1991**, *39*, 1407—1414.
2. Kasai, H.; Yamaizumi, Z.; Kuchino, Y.; Nishimura, S. *Nucleic Acids Res.* **1979**, *6*, 993—999.
3. Itaya, T.; Watanabe, N.; Iida, T.; Kanai, T.; Mizutani, A. *Tetrahedron* **1995**, *51*, 6419—6430.

4. Itaya, T.; Morisue, M.; Shimomichi, M.; Ozasa, M.; Shimizu, S.; Nakagawa, S. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2759—2765.
5. Itaya, T.; Morisue, M.; Takeda, M.; Kumazawa, Y. *Chem. Pharm. Bull.* **1990**, *38*, 2656—266
6. The Wittig reaction with the corresponding 2',3',5'-tri-*O*-benzyl derivative also afforded no desired product.[4]
7. Glemarec, C.; Wu, J.-C.; Remaud, G.; Bazin, H.; Oivanen, M.; Lönnberg, H.; Chattopadhyaya, J. *Tetrahedron* **1988**, *44*, 1273—1290.
8. Itaya, T.; Iida, T.; Shimizu, S.; Mizutani, A.; Morisue, M.; Sugimoto, Y.; Tachinaka, M. *Chem. Pharm. Bull.* **1993**, *41*, 252—261.
9. Compound **9b**: a yellow foam; $[\alpha]_D^{17}$ +16.4° (*c* 1.00, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ: –0.29, –0.04, 0.12 (3H each), 0.137 (6H), and 0.144 (3H) (s each, three $SiMe_2$'s), 0.73, 0.94, and 0.95 (9H each, s, three $CMe_3$'s), 2.37 [3H, s, C(6)-Me], 3.73 (3H, s, $NCO_2Me$), 3.78 (1H, dd, *J* = 11.5 and 2 Hz) and 3.87 (1H, dd, *J* = 11.5 and 2.9 Hz) [C(5')-$H_2$], 3.82 (3H, s, $CCO_2Me$), 4.10 [3H, s, overlapping with a 1H signal arising from C(4')-H at 4.11, N(4)-Me], 4.19 [1H, d, *J* = 4 Hz, C(3')-H], 4.37 [1H, dd, *J* = 4 and 7.5 Hz, C(2')-H], 5.12 [1H, br, C(α)-H], 5.53 (1H, br, NH), 5.85 [1H, br d, *J* = 16 Hz, C(β)-H], 6.21 [1H, d, *J* = 7.5 Hz, C(1')-H], 7.74 [1H, d, *J* = 16 Hz, C(γ)-H], 7.91 [1H, s, C(2)-H].
10. Compound [*R*-(*R**,*S**)]-**2b**·$H_2O$: mp 198—210 °C (dec.); $^1H$ NMR ($Me_2SO$-$d_6$) δ: 2.07 [3H, s, C(6)-Me], 3.10 and 3.16 [1H each, dd, *J* = 14.2 and 7 Hz, C(γ)-$H_2$], 3.59 [6H, s, overlapping with a 1H signal arising from one of C(5')-$H_2$, two $CO_2Me$'s], 3.69 [1H, ddd, *J* = 12.2, 3.4, and 4.9 Hz, one of C(5')-$H_2$], 3.89 (1/10H, br) and 3.94 (9/10H, dd, *J* = 2.4 and 8.8 Hz) [C(α)-H], 3.99 [1H, ddd, *J* = 3.4, 3.4, and 4.9 Hz, C(4')-H], 4.03 [3H, s, N(4)-Me], 4.13 [1H, ddd, *J* = 4.9, 4.9, and 5.9 Hz, C(3')-H], 4.41 [1H, dddd, *J* = 7, 7, 2.4, and 7.8 Hz, C(β)-H], 4.45 [1H, ddd, *J* = 4.9, 5.9, and 4.9 Hz, C(2')-H], 4.97 (9/10H) and 5.01 (1/10H) [d each, *J* = 7.8 Hz, C(β)-OH], 5.12 [1H, dd, *J* = 5.4 and 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 (1/10H) and 7.11 (9/10H) (d each, *J* = 8.8 Hz, NH), 8.22 [1H, s, C(2)-H].

    Compound [*S*-(*R**,*R**)]-**2b**: a colorless glass; $^1H$ NMR ($Me_2SO$-$d_6$) δ: 2.14 [3H, s, C(6)-Me], 3.02 (1H, dd, *J* = 14.7 and 7.5 Hz) and 3.41 (1H, dd, *J* = 14.7 and 4.9 Hz) [C(γ)-$H_2$], 3.55 and 3.58 [3H each, s, overlapping with a 1H signal arising from one of C(5')-$H_2$, two $CO_2Me$'s], 3.68 [1H, ddd, *J* = 12.5, 3.4, and 5 Hz, one of C(5')-$H_2$], 3.99 [1H, ddd, *J* = 3.4, 3.4, and 4.9 Hz, C(4')-H], 4.04 [3H, s, N(4)-Me], 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, and 4.9 Hz, C(2')-H], 5.06 [1H, d, *J* = 5.9 Hz, C(β)-OH], 5.12 [1H, dd, *J* = 5 Hz each, C(5')-OH], 5.32 [1H, d, *J* = 5.4 Hz, C(3')-OH], 5.71 [1H, d, *J* = 5.9 Hz, C(2')-OH], 6.11 [1H, d, *J* = 4.9 Hz, C(1')-H], 7.28 (1H, d, *J* = 8.3 Hz, NH), 8.22 [1H, s, C(2)-H].
11. Complete separation of [*R*-(*R**,*S**)]- and [*S*-(*R**,*R**)]-**2b** was attained on a Hibar LiChrosorb® RP-18 column (7 μm) (4 × 250 mm) using $H_2O$–MeOH (70:30, v/v) (retention time: 21.9 and 17.8 min) as eluent at the flow rate of 0.5 ml per min and room temperature.

(*Received in Japan* 9 *January* 1997; *accepted* 7 *February* 1997)