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SYNTHESIS OF OPTICALLY ACTIVE FORMS OF HYDROXY-Y BASE, THE MINOR COMPONENT OF RAT LIVER PHENYLALANINE TRANSFER RIBONUCLEIC ACID

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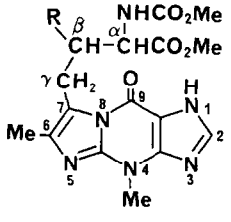
Abstract —— [*R*-(*R**,*S**)]-β-Hydroxywybutine (**6**) and its [*S*-(*R**,*R**)]-isomer (**9**) have been synthesized as the most probable alternatives for hydroxy-Y base isolated from rat liver phenylalanine tRNA.

The structure of wybutine (Y base), the fluorescent minor base from yeast $tRNA^{Phe}$ has been elucidated as **1a**.[1] The base isolated from $tRNAs^{Phe}$ of chicken, rat, and bovine livers,[2] the plant Lupinus luteus,[3] and an aquatic fungus Geotrichum candidum[4] has been proposed to be peroxy-Y base (**1c**), whereas Kasai et al. have determined the structure of the base from rat liver $tRNA^{Phe}$ to be hydroxy-Y base (**1b**) on the basis of mass spectral evidence.[5] They suggested that the base[6] from wheat germ $tRNA^{Phe}$ was also **1b** and described that **1c** might be an artefact formed during storage of a sample of **1b**. An under-modified base **2** has been also isolated from tumor-specific $tRNAs^{Phe}$ in place of normal **1b**.[7] As these bases were isolated in extremely minute quantities, unambiguous solution of the structural problem, especially the stereochemistry, has to rest on chemical synthesis. Since the configuration of wybutine has established to be S,[8] it can be assumed that hydroxy-Y base also has an S configuration at the α-position. We now report here the first synthesis of [*R*-(*R**,*S**)]- and [*S*-(*R**,*R**)]-β-hydroxywybutine (**6** and **9**, respectively), one of which is most likely hydroxy-Y base.

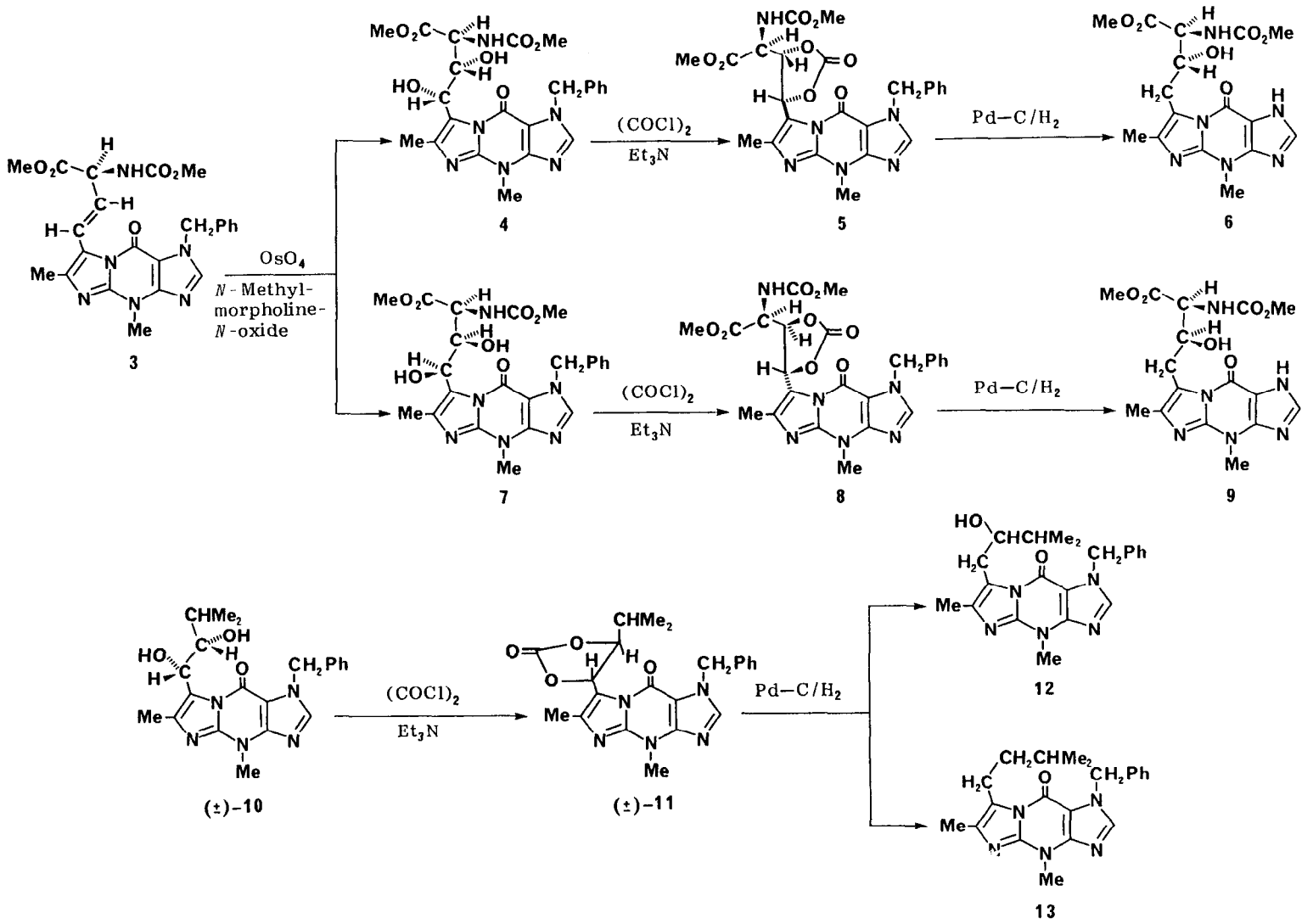


1

a: R = H
b: R = OH
c: R = O_2H

2

Oxidation of **3**[8] with OsO_4 according to a slightly modified procedure of VanRheenen et al.[9] provided a mixture of two dihydroxy compounds. These were separated on a silica gel column using 1,2-dichloroethane–ethanol as an eluent. Compound **4**, mp 189 – 192°C,[10] which was eluted earlier, was obtained in 69% yield, and the relative configurations were determined by X-ray crystallography.[11] The structure **7** was thus assigned to the other isomer, which was


OsO4
N-Methyl-morpholine-N-oxide
(COCl)2
Et3N
Pd–C/H2
3
4
5
6
7
8
9
(±)-10
(±)-11
12
13

obtained as a glass in 24% yield. Catalytic hydrogenolysis of **4** over Pd−C gave **6**, mp 234−235°C (dec.); ^{1}HNMR (Me_2SO-$\underline{d}_6$) δ: 2.08 (s, CMe), 3.14 (d, $\underline{J}$ = 8 Hz, CH_2), 3.59 (s, two OMe's), 3.76 (s, NMe), 4.00 (d-d, $\underline{J}$ = 9 and 2 Hz, $C_{(\alpha)}$-H), 4.41 (m, $C_{(\beta)}$-H), 4.95 (d, $\underline{J}$ = 8 Hz, OH), 7.10 (d, $\underline{J}$ = 9 Hz, $C_{(\alpha)}$-NH), 8.18 (s, $C_{(2)}$-H), 13.56 (br, $N_{(1)}$-H); MS m/e: 392 (M^+); CD (H_2O) $\Delta\varepsilon_{225}$ -3.2, $\Delta\varepsilon_{236}$ -2.9, $\Delta\varepsilon_{259}$ -1.7, in only 18% yield with a complex mixture of yet unidentified fluorescent products. Similar transformation of **7** into **9** failed.[12]

Our effort was then focussed on development of a method of deoxygenation at the γ-position using a model compound (±)-**10**.[13] After many unfruitful trials, **10** was treated with oxalyl chloride in the presence of triethylamine at 0°C to give (±)-**11** in 41% yield. The correctness of the structure **11** was supported by the formation (14% yield) of the same compound in the reaction of **10** and trichloromethyl chloroformate under similar conditions. Catalytic hydrogenolysis of **11** over Pd−C gave **12** (56% yield) and **13** (12% yield).[14]

Compound **4** was converted into **5**,[15] mp 204−205°C (dec.), in 46% yield according to the model experiment. Prolonged reduction of **5** using H_2 and Pd−C caused fission of the carbonate ester followed by debenzylation to provide **6** (45% yield) and (*S*)-**1a**[8] (28% yield). Identity of the product **6** with that obtained by the direct hydrogenolysis of **4** supports that the configuration is retained at the β-position during the formation of the cyclic carbonate **5**. The other diastereomer **9**, mp 233−234°C (dec.); ^{1}HNMR (Me_2SO-$\underline{d}_6$) δ: 2.15 (s, CMe), 3.01 (m, CH_2), 3.55 and 3.56 (s each, two OMe's), 3.76 (s, NMe), 4.12 (m, two CH's), 5.07 (d, $\underline{J}$ = 4 Hz, OH), 7.22 (d, $\underline{J}$ = 9 Hz, $C_{(\alpha)}$-NH), 8.17 (s, $C_{(2)}$-H), 13.61 (br, $N_{(1)}$-H); MS m/e: 392 (M^+); CD (H_2O) $\Delta\varepsilon_{239}$ -2.0, was obtained from **7** through **8**, mp 177−179°C (dec.), in 19% overall yield as well as (*S*)-**1a** (11% yield) in a similar manner.

Showing that synthetic hydroxywybutines, **6** and **9**, are distinguishable from each other by ^{1}HNMR and CD spectroscopies, the present work should help towards structural determination of hydroxy-Y base accessible from the tRNA in only a minute quantity.

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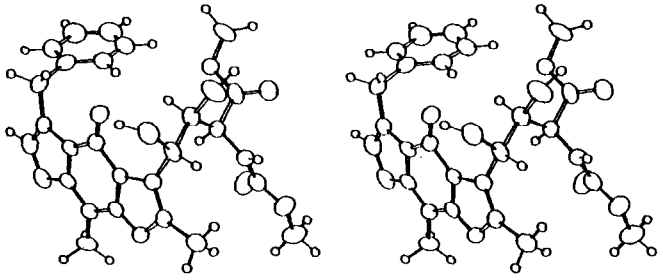
10) Complete analytical and/or spectral data were obtained for all new compounds reported.

11) The crystals of **4** from methanol belong to the orthorhombic system of space group $P2_12_12_1$ with four molecules in a unit cell of dimensions a = 21.547(3), b = 20.720(3), c = 5.346(1) Å. The structure was solved by the direct method using the programs MULTAN and X-ray System, and refined by block diagonal least-squares method. Anisotropic temperature factors were given for all nonhydrogen atoms, followed by introduction of hydrogen atoms, to yield R = 6.8%.



Stereoscopic drawing of **4**

12) The failure of the hydrogenolysis at the highly reactive benzylic position is analogous to the recently reported case [T. Fujii, M. Ohba, T. Tachinami, H. Miyajima, M. Koch, and E. Seguin, Heterocycles, 24, 1215 (1986)].

13) Details for the synthesis of this compound will be reported elsewhere.

14) The deoxygenation proceeded in preference to debenzylation at the 1-position.

15) The configuration at the γ-position was tentatively assigned by analogy to that of (±)-**11**.

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