STUDIES ON UROTHION. SYNTHESIS OF (*R*)-DEPHOSPHO FORM B, A DEGRADATION PRODUCT OF THE MOLYBDENUM COFACTOR

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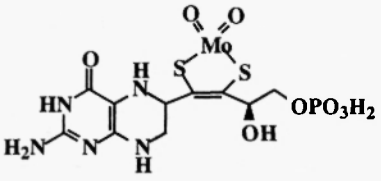
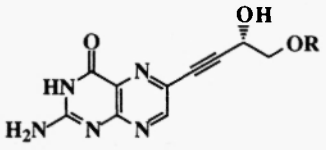
Abstract: In order to determine the absolute configuration of the dephospho Form-B (**3b**) derived from urothion (**4**), 7-amino-2-[(1*R*)-1,2-dihydroxyethyl]thieno[3,2-g]pteridin-5(6H)-one ((*R*)-**3b**) and its (1*S*)-enantiomer ((*S*)-**3b**) were synthesized. Comparing their CD spectra with that of **3b**, the *R*-configuration was concluded for the secondary hydroxyl group on the side chain of **3b**, as well as **4**.

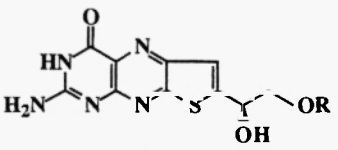
The molybdenum cofactor, which exists in most molybdenum-containing enzymes, is a complex of Mo^{VI} with molybdopterin, a sulfur-containing reduced pterin, for which structure **1** has been suggested.[1,2] Several oxidative degradation products of **1** has been isolated. Structure **2a** has been assigned to Form A derived from **1**, including the absolute configuration of *S* at the chiral center on the side chain, on the basis



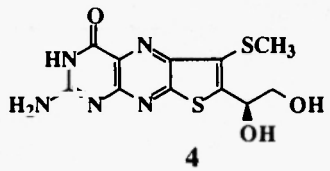
1



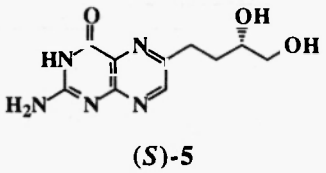
2a : R=PO_3H_2
2b : R=H



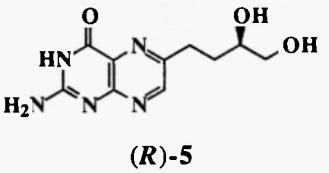
3a : R=PO_3H_2
3b : R=H



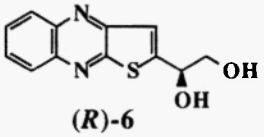
4



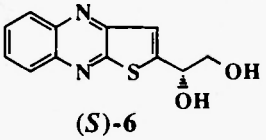
(*S*)-**5**



(*R*)-**5**



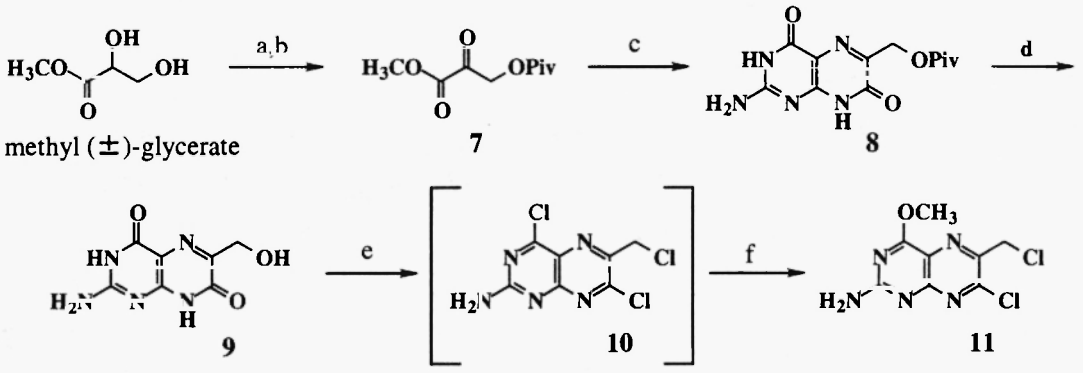
(*R*)-**6**



(*S*)-**6**

of the asymmetric synthesis of the dephospho Form A (**2b**) and its CD spectroscopic analysis.[3-5] While Form B, which possesses a fused thiophene ring and retains one of the original sulfur atoms, has been assigned to structure **3a**,[3,4,6] the absolute configuration of **3a** remained ambiguous. However, the determination of structure **2b** suggested that **1**, as well as **3a**, has the *R*-configuration at the chiral center on their side chains. The structure of urothion,[7,8] a pteridine pigment in urine, has been determined as structure **4** by total syntheses.[9-11] Furthermore, on the basis of the metabolic study of the molybdenum cofactor, urothion was presumed to be a urinary metabolite of **1**.[4] Asymmetric synthesis of desulfurization products (***S***)-**5** and (***R***)-**5** of **4** and their CD spectroscopic analysis indicated that the absolute configuration at the chiral center of **4** had the *R*-configuration.[12,13]

We recently attempted the asymmetric synthesis of the thienoquinoxaline derivatives (***R***)-**6** and (***S***)-**6**, which were model compounds of dephospho Form B (**3b**), and obtained important information on the asymmetric synthesis of **3b**.[14] The asymmetric synthesis of **3b** carried out according to this method, is described below, which establishes the absolute configuration at the chiral center of urothion, as depicted in structure **4**.

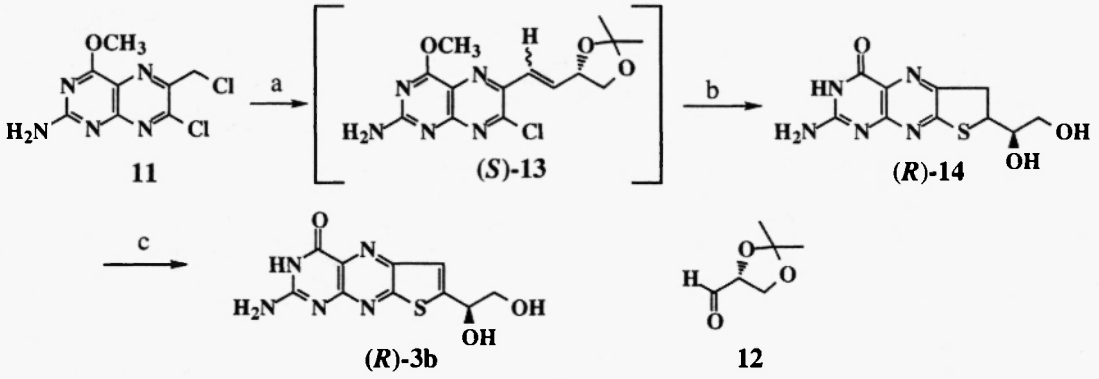


Scheme 1: a. 1.1 eq. pivalic anhydride, 2.5 eq. pyridine, 0.1 eq. DMAP/CH_2Cl_2, Ar, reflux, 5h, 55%; b. excess RuO_4/CCl_4, rt, 0.5h, 90%; c. 1.1 eq. 2,4,5-triamino-6-hydroxypyrimidine hydrochloride, 1.75 eq. $NaHCO_3$/ H_2O-MeOH (1:1), reflux, 0.5h, 73%; d. excess 1M NaOH/H_2O-MeOH (1:1), rt, 4h; AcOH (pH 4), 94%; e. 6 eq. $PCl_5/POCl_3$, Ar, reflux, 12h; f. MeOH, rt, 1h, 27% from **9**.

The dichloride **11** was prepared by chlorination of the isoxanthopterin derivative **9**, followed by methanolysis (Scheme 1).[15-17] (*R*)-DephosphoForm B ((***R***)-**3b**) was synthesized *via* the dihydrothienopterin (***R***)-**14**, by utilizing the Wittig reaction of the dichloride **11** with a chiral synthon of D-glyceraldehyde

acetonide **12** which was prepared from D-mannitol according to Bear's method.[18] (*S*)-Dephospho Form B ((*S*)-**3b**) was synthesized in the same method as described above, by utilizing the Wittig reaction with a chiral synthon of L-glyceraldehyde acetonide which was prepared from L-ascorbic acid according to Jung's method.[19]

The resulting alkenes (*S*)-**13** and its (*R*)-enantiomer were used for the syntheses of (*R*)-**14** and its (*S*)-enantiomer after removal of excess amounts of triphenylphosphine with a small size of silica-gel column, respectively. The dihydrothienopterin (*R*)-**14** and its (*S*)-enantiomer were purified by ion exchange chromatography (Dowex 1X8, acetate form, eluent: 0.05M NH_4OAc). The thienopterin (*R*)-**3b** and (*S*)-**3b** were purified by ODS reverse phase chromatography (Merck, LiChroprep RP-8, size B, eluent: 20% MeOH).



Scheme 2: a. 6 eq. PPh_3/CH_3CN, Ar, reflux, 12h; 1.5 eq. Et_3N, 1.1 eq. **12**/THF, rt, 12h; b. 8eq. NaSH/EtOH, Ar, reflux, 3h, ; HCl/H_2O (pH 2), rt, 6h, 21% from **11**; c. 2 eq. SeO_2/AcOH, Ar, 80°C, 2h, 91%.

For the determination of the absolute configuration, CD spectra in 1% NH_3 of (*R*)-**3b** and (*S*)-**3b** (λ_{ext} 260 nm, $\Delta\varepsilon$ -1.3 and λ_{ext} 260 nm, $\Delta\varepsilon$ +1.3, respectively) were compared with that of **3b**[20] derived from **4** by desulfurization with Raney-Ni, followed by SeO_2 oxidation.[4,7,8] The spectrum of **3b**[14] was found superimposable with that of (*R*)-**3b**. From these observations, **3b** was confirmed to have the *R*-configuration, as was suggested from the asymmetric syntheses of (*S*)-**5** and (*R*)-**5**.[12,13] Thus it follows that **4** has the *R*-configuration, which supports the suggestion that **4** might be a urinary metabolite of **1**.

References and notes

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(15) Compound **7**; ^{1}H NMR ($CDCl_3$, δ ppm): 1.27 (9H, s, $C(C\underline{H}_3)_3$), 3.91 (3H, s, $OC\underline{H}_3$), and 5.13 (2H, s, $C\underline{H}_2$). ^{13}C NMR ($CDCl_3$, δ ppm): 27.1 ($C(\underline{C}H_3)_3$), 38.8 ($\underline{C}(CH_3)_3$), 53.2 ($O\underline{C}H_3$), 66.4 ($\underline{C}H_2O$), 159.7 ($\underline{C}O_2CH_3$), 178.2 ($\underline{C}O_2C(CH_3)_3$), and 186.4 ($\underline{C}O$).

(16) Compound **8**; ^{1}H NMR ($CDCl_3$, δ ppm): 1.19 (9H, s, $C(C\underline{H}_3)_3$), 5.25 (2H, s, $C\underline{H}_2$), 5.85 (2H, brs, $N\underline{H}_2$), 12.90 (1H, brs, $N\underline{H}CO$), and 13.55 (1H, brs, $N\underline{H}CO$).

(17) Compound **11**; ^{1}H NMR ($CDCl_3$, δ ppm): 4.21 (3H, s, $OC\underline{H}_3$), 4.90 (2H, s, $C\underline{H}_2Cl$), and 5.80 (2H, brs, $N\underline{H}_2$).

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(20) Compound **3b**; ^{1}H NMR (DMSO-d_6, δ ppm): 3.61 (2H, m, $C\underline{H}_2OH$), 4.90 (1H, q, J=5.0Hz, $C\underline{H}OH$), 5.04 (1H, t, J=6.0Hz, $CH_2O\underline{H}$), 6.10 (1H, d, J=5.0Hz, $CHO\underline{H}$), 6.95 (2H, brs, $N\underline{H}_2$), 7.38 (1H, s, $=C\underline{H}$), and 8.45 (1H, brs, $N\underline{H}CO$). UV (0.1M NaOH): λ_{max} 242 (ε 23800), 268 (28600), and 395 nm (12700). CD (1% NH_3): λ_{ext} 260 nm ($\Delta\varepsilon$ -1.3).

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