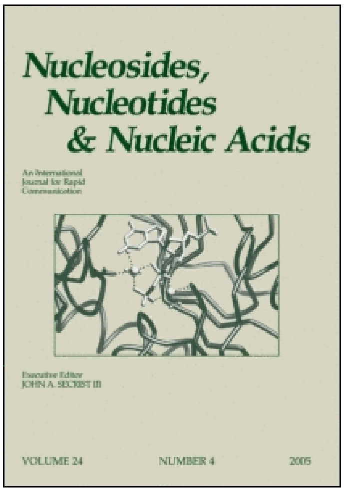

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A Convenient and Stereoselective Synthesis of 2'-Deoxy-β-L-Ribonucleosides

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A CONVENIENT AND STEREOSELECTIVE SYNTHESIS OF 2'-DEOXY-β-L-RIBONUCLEOSIDES**

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ABSTRACT: 2'-Deoxy-β-L-ribonucleosides containing usual bases which are useful as synthons for modified oligodeoxyribonucleotides, were conveniently synthesized by a stereoselective glycosylation procedure. The method is suitable for large-scale preparations.

Antisense oligonucleotides and their analogues have been attracting much attention as designed/tailored chemotherapeutic agents and biochemical tools[1]. We have recently found that a hexamer of *enantio*-deoxyadenylic acid interacted with complementary poly(U) rather than poly(dT) and seemed to be useful as an RNA specific antisense oligodeoxynucleotide analogue[2]. For further studies of *enantio*-oligodeoxyribonucleotides, large quantities of 2'-deoxy-β-L-nucleosides (nucleosides containing 2-deoxy-L-*erythro*-pentofuranose as the sugar moiety) are required as starting materials.

Various syntheses of 2'-deoxy-β-L-nucleosides have been reported; 2'-deoxy-L-adenosine and guanosine were first prepared by a fusion method[3], and L-thymidine was synthesized by a mercuri procedure[4] and subsequently *via* a stereospecific aminoxazoline intermediate from L-arabinose, and 2'-deoxy-L-cytidine was also prepared by the latter

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** This paper is dedicated to the late Professor Tohru Ueda.

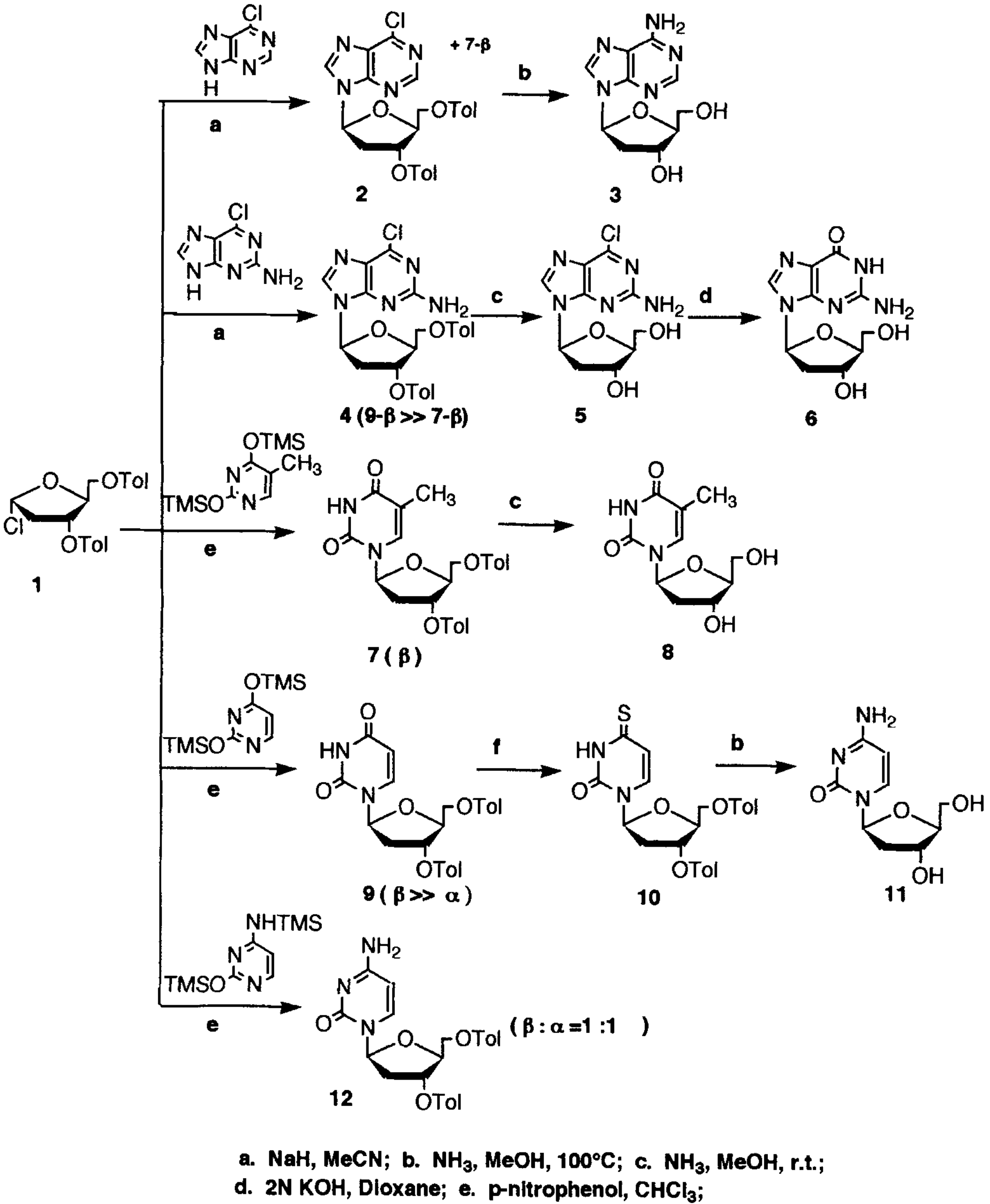
method[5]. However, these methods are limited to small-scale preparations and provide rather low yields due to formation of anomeric and positional isomers. They do not represent general procedures for both pyrimidine and purine type nucleosides.

Recently, several stereoselective glycosylation methods have been developed to prepare natural nucleosides and their analogues[6,7,8], but these methods have not been applied to the preparation of *enantio*-nucleosides. Now we report a series of facile preparations of 2'-deoxy-β-L-nucleosides by stereoselective glycosylation with a 3,5-protected 2-deoxy-α-L-chlorosugar (**1**) obtained from 2-deoxy-L-ribose (Scheme).

The preparation of 2-deoxy-β-L-*erythro*-pentose (2-deoxy-L-ribose) was performed according to the procedure of Deriaz et al[9] from L-arabinose with some modifications. The free 2-deoxy-L-ribose was converted into 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-α-L-*erythro*-pentofuranose (**1**) as a common glycosylating unit by using Hoffer's method[10] for the D enantiomer.

In the case of purine nucleoside, the reaction of the sodium salt of 6-chloropurine with the chloro-sugar (**1**) gave a 57% yield of 6-chloro-9-(2-deoxy-3,5-di-O-p-toluoyl-β-L-*erythro*-pentofuranosyl)purine (**2**) together with the corresponding N-7 glycosyl isomer in 7% yield after silica gel column chromatography. Subsequent treatment of **2** with NH_3/MeOH at 100°C resulted in deprotection and nucleophilic displacement of the 6-chloro moiety to give 6-amino-9-(2-deoxy-β-L-*erythro*-pentofuranosyl)purine (2'-deoxy-L-adenosine, **3**) in 77.6% yield in essentially the same manner as for the D-isomer[6]. Similarly, glycosylation of the sodium salt of 2-amino-6-chloropurine with **1** gave a 50% yield of 2-amino-6-chloro-(2-deoxy-3,5-di-O-p-toluoyl-β-L-*erythro*-pentofuranosyl)purine (**4**) and a trace (<<1%) of the corresponding N-7 glycosyl isomer, though the isomer was obtained in 9% yield by Hanna et al[11] in the case of the D-isomer. After deprotection of **4** with NH_3/MeOH, alkaline hydrolysis of the 6-chloro moiety gave 2-amino-9-(2-deoxy-β-L-*erythro*-pentofuranosyl)purin-6-one (2'-deoxy-L-guanosine, **6**) in good yield.

For the synthesis of enantiomeric pyrimidine nucleosides, it is favorable to employ β-selective glycosylation in the presence of Brönsted acid, as reported by Aoyama[7]. Thus, 5-methyl-2,4-bis(trimethysilyloxy)pyrimidine was reacted with **1** in the presence of



a. NaH, MeCN; b. NH_3, MeOH, 100°C; c. NH_3, MeOH, r.t.;
d. 2N KOH, Dioxane; e. p-nitrophenol, $CHCl_3$;
f. Lawesson's reagent, Dioxane.

0.3 eq. of p-nitrophenol to give 1-(3,5-di-O-p-toluoyl-2-β-L-*erythro*-pentofuranosyl)-5-methyl-2,4(1H,3H)-pyrimidinedione (**7**); the α-anomer was not detected. Deprotection gave 1-(2-deoxy-β-L-*erythro*-pentofuranosyl)-5-methyl-2,4(1H,3H)-pyrimidinedione (2'-deoxy-L-thymidine, **8**) in an overall yield of 67%. An attempt to obtain deoxycytidine by the reaction of O,N-bis(trimethylsilyl)cytosine[12] with **1** failed due to the formation of an anomeric mixture (**12**) in a nearly equal ratio. Thus, the treatment of 2,4-bis(trimethylsilyloxy)pyrimidine with **1** gave exclusively 1-(3,5-di-O-p-toluoyl-2-deoxy-β-L-*erythro*-pentofuranosyl)-2,4(1H,3H)-pyrimidinedione (**9**) in 77% yield (the α-anomer was not detected until the third crop of crystals). The nucleoside (**9**) was easily converted quantitatively to the 4-thioxo compound (**10**) by the action of Lawesson's reagent[13] (the reagent is preferable to commonly used P_2S_5 in terms of handling and yield of the product). Subsequent treatment of **10** with NH_3/MeOH at 100°C gave 4-amino-1-(2-deoxy-β-L-*erythro*-pentofuranosyl)-2(1H)-pyrimidinone (2'-deoxy-L-cytidine, **11**) in 67% yield.

These 2'-deoxy-β-L-ribonucleosides were found to exhibit identical NMR spectra with their corresponding D enantiomers and opposite optical rotatons (see Experimental section).

Thus, convenient syntheses of four common base-deoxy-L-nucleosides were achieved by stereoselective glycosylation in excellent yield. The results are reproducible, and the procedures are convenient. These procedures will be suitable for large-scale preparation of enantio-deoxynucleosides for the synthesis of oligodeoxynucleotide analogues containing L-deoxyribose.

EXPERIMENTAL

Melting points were determined by using a Yanagimoto hot-stage melting point apparatus. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo. NMR spectra were recorded on JEOL GX 400-MHz NMR spectrometers. Chemical shifts are expressed in ppm relative to TMS or TSP. Optical rotations were measured in a 1-dm cell with a JASCO DIP 370 polarimeter at 25°C. Column chromatography was done with Merck silica gel 60, 35-70 mesh for open and 230-400 mesh for flash chromatography. Thin-layer chromatography was performed with Merck silicagel 60 F_{254} TLC plate, and spots were visualized by UV light, iodine or spraying with 5% p-anisaldehyde in EtOH containing H_2SO_4, followed by heating.

2-Deoxy-L-ribose. L-Arabinose was manipulated as described in the literature[9] with some modifications: α-tetraacetyl-L-arabinose was brominated with commercial 25% HBr-AcOH to obtain crystalline β-acetobromo-L-arabinose, then reduced and deprotected to give L-arabinal, which was hydrated and purified as the anilide. Crystals of the free product were obtained in 5 % overall yield: $[\alpha]_D$ + 55.9° (C=1.58, H_2O).

1-Chloro-2-deoxy-3,5-di-O-p-toluoyl-α-L-*erythro*-pentofuranose(1). Using Hoffer's method[10], 2'-deoxy-L-ribose was converted to **1** in an overall yield of 76%, mp 118-120°C, $[\alpha]_D$ -125.7° (C=0.811, DMF). ^{1}H-NMR ($CDCl_3$) δ 6.47 (d, 1H, J=5.1 Hz, 1'-H).

6-Chloro-9-(2-deoxy-3,5-di-O-p-toluoyl-*β*-L-*erythro*-pentofuranosyl)purine (2). A mixture of 6-chloropurine (7.48 g, 48.4 mmol, Aldrich) and NaH (60% in oil, 2.2 g, 55 mmol, washed with n-hexane) in anhydrous MeCN (400 ml) was stirred for 40 min at room temperature under argon, then freshly prepared **1** (19.4 g, 49.9 mmol) was added portionwise during 20 min. Stirring was continued for 15 h. A small amount of insoluble material was removed by filtration, then evaporation of the solvent gave an oily residue which was purified on a silica gel column (6 X 23 cm) with benzene:MeCN (8:1, 5:1, 3:1 in that order) as the eluent. The fraction of Rf 0.58 (benzene:MeCN=4:1) was collected and the residue on evaporation was crystallized from EtOH to give 14.1 g (57.3%) of **2**, mp 111.5-113°C. ^{1}H-NMR (DMSO-d_6): δ 6.70 (t, 1H, J=7.0 Hz, 1'-H), 8.73 (S, 1H, 2-H) 8.91 (s, 1H, 8-H). Anal. Calcd. for $C_{26}H_{23}ClN_4O_5$: C, 61.60; H, 4.57; N, 11.05. Found: C, 61.35; H, 4.54; N, 10.84.

The N-7 glycosyl isomer was isolated from the fraction of Rf 0.37 and crystallized from EtOH to yield 1.71 g (7.0%), mp 147-149°C. ^{1}H-NMR (DMSO-d_6) δ 6.90 (t, 1H, J=6.6 Hz, 1'-H).

2'-Deoxy-L-adenosine (3). A solution of **2** (4.06 g, 18 mmol) and NH_3-saturated MeOH (720 ml) in a sealed, stainless steel cylinder was heated at 100°C for 12 h, then evaporated. An aqueous solution of the residue was washed with $CHCl_3$ and ether, and then evaporated to dryness. The residue was crystallized from H_2O to give 1.56 g (77.6%)

of **3**, mp 188-189°C, $[\alpha]_D$ +26.5° (C=1.52, H_2O), (lit.[3] mp 184-185°C, $[\alpha]_D$ +23.2°). ^{1}H-NMR (D_2O) δ 6.37 (t, 1H, J=7.0 Hz, 1'-H), 8.04 (s, 1H, 2-H), 8.22 (s, 1H, 3'-H). Anal. Cald for $C_{10}H_{13}N_5O_3$: C,47.80; H,5.21; N,27.88. Found: C, 47.78; H,5.21; N.28.11.

2-Amino-6-chloro-9-(2-deoxy-3,5-di-O-p-toluoyl-*β*-L-*erythro*-pentofuranosyl)purine (4). In a manner similar to that described in the literature[11], a sodium salt was prepared with 2-amino-6-chloropurine (3.92 g, 23.1 mmol, Aldrich) and 60% NaH in oil (1.11 g, 30 mmol, washed with n-hexane) in anhydrous MeCN (400 ml) with stirring for 2 h at 60°C under argon. The mixture was coold to room temperature, then freshly prepared **1** (9.41 g, 24.2 mmol) was added with stirring during 5 min, and stirring was continued overnight. After removal of insoluble material by filtration, the filtrate was evaporated to give an oily residue, which was purified by flash column chromatography using AcOEt:n-hexane (1:2, 2:1, in that order). The fraction of Rf 0.3 (AcOEt:n-hexane=2:3, development twice) was evaporated to give 5.99 g (50%) of **4**, mp 180-182°C (lit.[11] for D-isomer mp 173-175°C). ^{1}H-NMR (DMSO-d_6) δ 6.40 (t, 1H, J=6.6 Hz, 1'-H), 8.34 (s, 1H, 8-H). Anal. Calcd. for $C_{26}H_{24}ClN_5O_5$: C,59.83; H,4.63; N,13.42. Found: C,59.85; H,4.73; N,13.20.

The N-7 glycosyl isomer was not isolated but a trace quantity was detected in the subsequently eluted fraction (δ 6.68 is assigned to the 1'-H proton of the 7-*β* isomer[11]).

2-Amino-6-chloro-9-(2-deoxy-*β*-L-*erythro*-pentofuranosyl)-purine (5). Compound **4** (5.99 g, 115 mmol) was dissolved in NH_3-saturated MeOH (300 ml) and stirred for 12 h at room temperature, then evaporated. The residue was crystallized from EtOH to give 3.15 g (96%) of **5**, mp 156-158°C. ^{1}H-NMR (DMSO-d_6) δ6.22 (t, 1H, J=7.0 Hz, 1'-H), 6.95 (s, 2H, NH_2), 8.34 (s, 1H, 8-H).

2'-Deoxy-L-guanosine (6). A mixture of **5** (3.14 g, 11 mmol), 2 N KOH (95 ml) and dioxane (95 ml) was refluxed for 13 h and evaporated, and the residue was dissolved in H_2O. The aqueous solution was neutralized with Dowex-50(H^+) resin and filtered. The filtrate was evaporated to dryness, and the residue was triturated with EtOH to give **6** as a powder, 1.39 g (44%), mp >300°C (lit.[3], mp >300°). ^{1}H-NMR (DMSO-d_6) δ 6.11 (dd, 1H, J= 6.2, 8.1 Hz, 1'-H), 6.46 (s, 2H, NH_2), 7.91 (s, 1H, 8-H), 10.56 (bs, 1H, CONH). Further assignment of **6** was done by derivatizing it to the 2-isobutylyl compound; $[\alpha]_D$ +6.93° (C=1.01, DMF); Anal. Calcd. for $C_{14}H_{19}N_5O_5$: C,49.84; H,5.68; N,20.76. Found: C,49.50; H,5.61; N,20.70.

1-(3,5-Di-O-p-toluoyl-2-deoxy-β-L-*erythro*-pentofuranosyl)-5-methyl-2,4(1H,3H)-pyrimidinedione (7). The chlorosugar **1** (17.7 g, 46 mmol) was added to a stirred mixture of 5-methyl-2,4-bis(trimethylsilyloxy)pyrimidine[7] (15.7 g, 58.1 mmol), p-nitrophenol and dry $CHCl_3$ (175 ml) and the whole was stirred overnight at room temperature. After addition of a small amount of EtOH, the resulting insoluble material was removed by filtration through Celite, and the filtrate was concentrated. The crystalline residue was recrystallized from EtOH to give 16.9 g (76.9%) of **7**, mp 199-201°C, $[\alpha]_D$ +71.9° (C=0.528, $CHCl_3$). ^1H-NMR ($CDCl_3$) δ 1.64 (dd, 3H, J=1.1 Hz, 5-Me), 6.47 (dd, 1H, J=5.5, 9.2 Hz, 1'-H), 7.28 (d,1H, J=1.1 Hz, 6-H), 8.48 (s, 1H, NH). Anal. Calcd. for $C_{26}H_{26}N_2O_7$: C,65.26; H,5,48; N,5.86. Found: C,65.27; H,5.51; N,5.78.

2'-Deoxy-L-thymidine (8). Compound **7** (16.7 g, 34.9 mmol) was added to NH_3-saturated MeOH (300 ml) and the mixture was stirred for 6 h, then allowed to stand overnight. After evaporation of the solvent, the residue was dissolved in H_2O and the solution was filtered. The filtrate was washed with $CHCl_3$ and ether, and evaporated to dryness with toluene, then the residue was triturated with EtOH to give a crystalline powder, 7.31 g (86.8%), mp 188.5-189°C (lit.[5] 189°). $[\alpha]_D$ -18.9° (C=0.996, H_2O) (lit.[4] -20.3°). ^1H-NMR (D_2O) δ 1.90 (d, 3H, J=1.1 Hz, 5-Me), 6.30 (t, 1H, J=7.0 Hz, 1'-H), 7.66 (d, 1H, J=0.47 Hz, 6-H). Anal. Calcd. for $C_{10}H_{14}N_2O_5$: C,49.58; H,5.83; N,11.57. Found: C,49.34; H,5.78; N,11.44.

1-(3,5-Di-O-p-toluoyl-2-deoxy-β-L-*erythro*-pentofuranosyl)-2,4(1H,3H)-pyrimidinedione (9). In the same manner as described for **7**, the reaction of 2,4-bis(trimethylsilyloxy)pyrimidine[7] (6.92 g, 27 mmol) and **1** (9.35 g, 24 mmol) in dry $CHCl_3$ (80 ml) in the presence of p-nitrophenol (1.17 g, 8.4 mmol) gave 7.36 g (66.3%) of crystalline product (from AcOH), mp 215-216°C. $[\alpha]_D$ +46.3° (C=0.736, $CHCl_3$). ^1H-NMR ($CDCl_3$) δ 6.41 (dd, 1H, J=5.5, 8.4 Hz, 1'-H), 5.60 (d, 1H, J=7.6 Hz, 6-H), 7.53 (d, 1H, J=8.0 Hz, 5'-H), 8.75 (s, 1H, NH). Anal. Calcd. for $C_{25}H_{24}N_2O_7$: C,64.64; H,5.21; N,6.03. Found: C,64.43; H,5.20; N,5.92.

1-(3,5-Di-O-p-toluoyl-2-deoxy-β-L-*erythro*-pentofuranosyl)-3,4-dihydro-4-thioxo-2(1H)-pyrimidinone (10). A mixture of **9** (7.98 g, 17.2 mmol) and Lawesson's reagent (4.17 g, 10.3 mmol) in dioxane (150 ml) was refluxed for 2 h and, after cooling, insoluble material was filtered off. Evaporation of the solvent gave a crystalline residue, which was washed with EtOH to afford pure **10** (7.69 g, 93%), mp 188-189°C (from EtOH). $[\alpha]_D$ +74.0° (C=0.572, $CHCl_3$). ^1H-NMR ($CDCl_3$) δ 6.26 (d, 1H, J=7.7 Hz, 6-H), 6.34 (dd, 1H, J=3.9, 8.4 Hz, 1'-H), 7.38 (d, 1H, J=7.7 Hz, 5-H), 9.49 (s, 1H, NH). Anal. Calcd. for $C_{25}H_{24}N_2O_6S$: C,62.48; H,5.03; N,5.83. Found: C,62.34; H,5.00; N,5.71.

2'-Deoxy-L-cytidine (11). A solution of **10** (7.28 g, 15.1 mM) and NH_3-saturated (at 0°C) MeOH (200 ml) in a sealed, stainless steel cylinder was heated with stirring at 100°C for 10 h, then evaporated to dryness. An aqueous solution of the residue was washed with AcOEt and ether, then evaporated to dryness with EtOH. The residue was dissolved in hot MeOH (30 ml), treated with Norit and filtered. The filtrate was diluted with MeCN (100 ml) and cooled to give **11** as prisms (2.35 g, 68.5%), mp 196-198°C (lit.[5] 214°). $[\alpha]_D$ -60.4° (C=0.682, H_2O) (lit.[14] +57.6 (H_2O) for D-isomer). ^1H-NMR (D_2O) δ 6.05 (d, 1H, J=7.7 Hz, 5-H), 6.27 (t, 1H, J=6.6 Hz, 1'-H), 7.83 (d, 1H, J=7.4 Hz, 6-H). Anal. Calcd. for $C_9H_{13}N_3O_4$: C,47.57; H,5.77; N,18.49. Found: C,47.29; H,5.32, N,18.21.

REFERENCES

1. For reviews, see: (a) Uhlmann,E.;Peyman,A., Chem.Rev.,**1990**, *90*, 543. (b) Héléne,C.;Toulmé,J-J., Biochim. Biophys. Acta, **1990**, *1049* 99.
2. Fujimori,S.;Shudo,K.;Hashimoto,Y., J. Am. Chem. Soc., **1990**, *112*, 7436.
3. Robins,M.J.;Khwaja,T.A.;Robins,R.K., J. Org. Chem., **1970**, *35*, *636*.
4. Smejkal,J.;Sorm,F., Collect. Czech. Chem. Commun. **1964**, *29*, 2809.
5. Holy, A., Collect. Czech. Chem. Commun. **1972**, *37*, 4072.
6. Kazimierczuk,Z.;Cottam,H.B.;Revankar,G.R.;Robins,R.K., J. Am. Chem. Soc., **1984**, *106*, 6379.
7. Aoyama,H., Bull. Chem. Soc. Jpn., **1987**, *60*, 2073.

8. Baud,M.V.;Chavis,C.;Lucas,M.;Imbach,J.L., Tetrahedron Lett., **1990**, *31*, 4437.

9. Deriaz,R.E.;Overend,W.G.;Stacey,M.;Teece,E.G.;Wiggins,L.F., J. Chem. Soc., **1949**, 1879.

10. Hoffer,H., Chem. Ber., **1960**, *93*, 2777.

11. Hanna,N.B.;Ramasamy,K.;Robins,R.K.;Revankar,G.R., J. Heterocyclic Chem., **1988**, *25*, 1899.

12. Martin,J.A.;Bushnell,D.J.;Duncan,I.B.;Dunsdon,S.J.;Hall,M.T.; Machin,P.J.;Marrett,J.H.;Parkes,K.E.B.;Pokerts,N.A.;Thomas,G.J.; Galpin,S.A.;Kinchington,D., J. Med. Chem., **1990**, *33*, 2137.

13. Kaneko,K.;Katayama,H.;Wakabayashi,T.;Kumonaka,T., Synthesis, **1988**, 152.

14. Macnutt,W.S., Biochem.J., **1952**, *50*, 384.

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