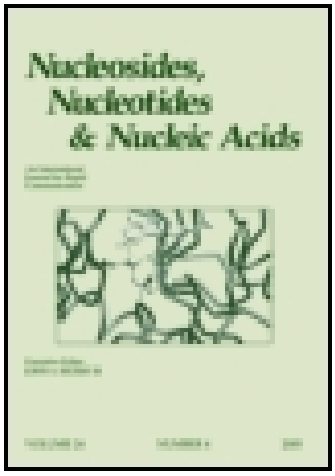


Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

Nucleoside Analogues on the Basis of 4(R),5(R)-Dihydroxymethyl-2-methyl-1,3-dioxolane

Sergey N. Mikhailov [a], Ekaterina V. Efimtseva [a], Sergey V. Meshkov [b] & Earl R. Kern [c]

[a] Engelgardt Institute of Molecular Biology, the Russian Academy of Sciences, Vavilov str. 32, Moscow, 117984, Russia

[b] Semyonov Institute of Chemical Physics, the Russian Academy of Sciences, Kosygin str. 4, Moscow, 117977, Russia

[c] Department of Pediatrics, University of Alabama, 401 Volker Hall 1670, University Blvd., Birmingham, Alabama, 35294, USA

Published online: 23 Sep 2006.

To cite this article: Sergey N. Mikhailov, Ekaterina V. Efimtseva, Sergey V. Meshkov & Earl R. Kern (1994) Nucleoside Analogues on the Basis of 4(R),5(R)-Dihydroxymethyl-2-methyl-1,3-dioxolane, Nucleosides and Nucleotides, 13:1-3, 615-623, DOI: 10.1080/15257779408013267

To link to this article: http://dx.doi.org/10.1080/15257779408013267

NUCLEOSIDES & NUCLEOTIDES, 13(1-3), 615-623 (1994)

NUCLEOSIDE ANALOGUES ON THE BASIS OF 4(R),5(R)-DIHYDROXYMETHYL-2-METHYL-1,3-DIOXOLANE

Sergey N. Mikhailov[1], Ekaterina V. Efimtseva[1], Sergey V. Meshkov[2] and Earl R. Kern[3]

[1] Engelgardt Institute of Molecular Biology, the Russian Academy of Sciences, Vavilov str. 32, Moscow 117984, Russia;

[2] Semyonov Institute of Chemical Physics, the Russian Academy of Sciences, Kosygin str. 4, Moscow 117977, Russia;

[3] Department of Pediatrics of the University of Alabama, 401 Volker Hall 1670, University Blvd., Birmingham, Alabama, 35294, USA.

ABSTRACT. New nucleoside analogues on the basis of 4(*R*),5(*R*)-dihydroxymethyl-2-methyl-1,3-dioxolane 2 have been prepared. Alkylation of thymine, adenine and N^2-palmitoyl-guanine with 2-bromomethyl-4(*R*),5(*R*)-dibenzyloxymethyl-1,3-dioxolane followed by separation of regio isomers by adsorption chromatography and deprotection yielded the desired chiral nucleoside analogues. The structures of thus prepared compounds were confirmed by UV and PMR spectroscopy. The obtained compounds 11 have no anti-HIV and antiherpetic activity and are not cytotoxic.

INTRODUCTION

The discovery of 2',3'-dideoxynucleosides[1,2] as potent inhibitors of the HIV virus has stimulated intensive efforts in this field (for a recent review see[3]). Recent studies of a number of nucleosides in which the ribose moiety was replaced by five membered rings such as dioxolane 1[4-7] or oxathiolane 2[8-10] have demonstrated the ability of these analogs to inhibit HIV replication[6,9,10].

Recently we have synthesized two series of dioxolane nucleoside homologs 3 and 4[11,12]. The most laborious step in their preparation was the separation of *cis* and *trans* isomers. To overcome this we decided to synthesize chiral nuc-

---

Dedicated to the memory of Professor R.K.Robins.

leoside analogues on the basis of 4(*R*),5(*R*)-dihydroxymethyl-2-methyl-1,3-dioxolane. Optically active tartaric acids and their derivatives with the symmetry axis of second order C_2 are frequently used in organic synthesis for similar purposes[13] and this property may be transfered to their dioxolane derivatives too. It should be mentioned that **11** may be considered as analogues of 2',3'-dideoxy-3'-C-hydroxymethylnucleosides (**5**), exhibiting anti-HIV activity[14,15].

RESULTS AND DISCUSSION

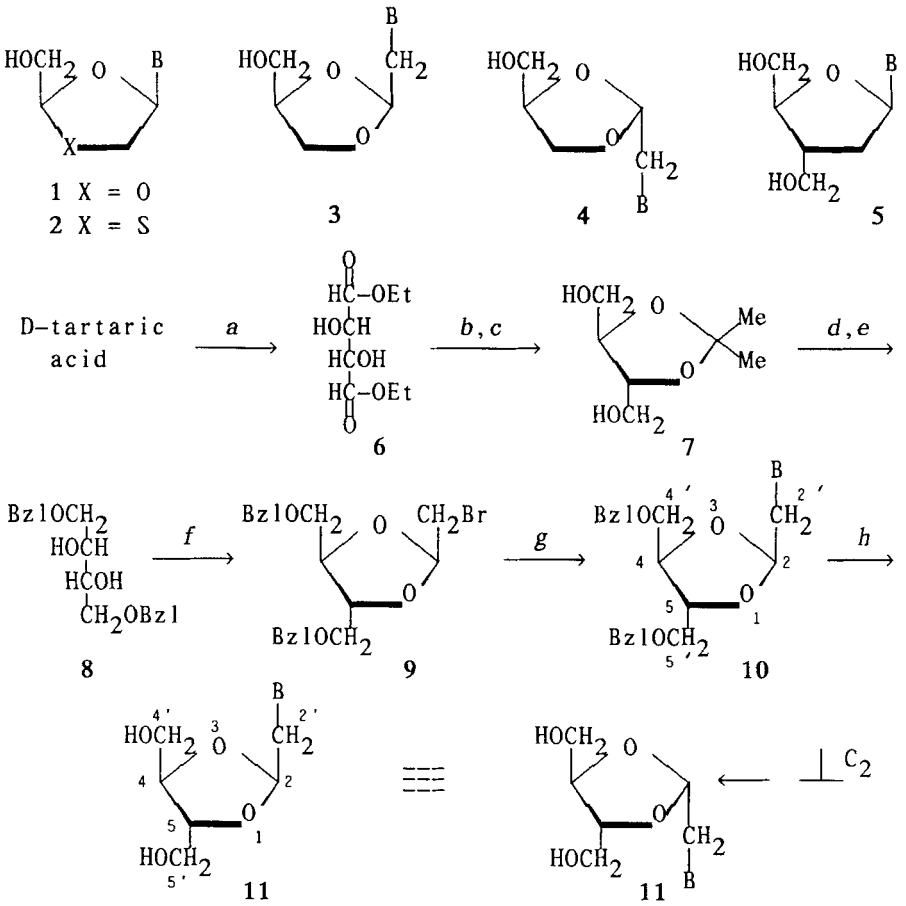
Starting from D-tartaric acid the known **6**[16] was prepared and then transformed to **7**[18]. 1,4-O-Dibenzyl-D-threitol[18] was prepared in a good overall yield. Acid-catalyzed transacetalization of bromoacetaldehyde diethylacetal gave **9** in 74% yield. An attempt to use 1,4-O-dibenzoyl-D-threitol in this reaction failed due to migration of O-benzoyl groups and formation of a complex reaction mixture.

Alkylation of the sodium salt of thymine with bromide **9** gave N^1,N^3-bis-alkylated and N^1-alkylated bases as main products[12]. With the sodium salt of adenine the alkylation took place at N^9 and N^3 positions[11]. Reaction of N^2-palmitoylguanine with **9** in the presence of potassium carbonate gave N^9 and N^7 isomers[19]. The regio isomers were separated by adsorption chromatography on silica gel.

The benzyl protecting groups in **10** were removed by treatment with an excess of 10% palladium on carbon and ammonium formate in boiling methanol[20]. For **10c**, the palmitoyl group was removed by treatment with methanolic ammonia. The yields of 4(*R*),5(*R*)-dihydroxymethyl-2-methyl-1,3-dioxolane nucleosides **11** were moderate.

The structure of the compounds prepared was verified by ^{1}H NMR spectroscopy (tables 1 and 2). Rather complicated ^{1}H NMR spectra of **10** and **11** were analyzed using the reported data for 2,4-disubstituted 1,3-dioxolane nucleosides **3**, **4**[11,12].

The signals of methylene groups H2'a,b, H4'a,b, H5'a,b and protons H4 and H5 were unambiguously assigned by the NOE difference spectroscopy. Saturation of H2 in **10a** exerted a rather strong NOE on the neighbouring protons H2'a,2'b (total effect > 4%). The *cis* oriented H5'a,5'b (1.2%) and H4 (2.9%) were also influenced significantly, while the effect on the



B = **a**) Thy, **b**) Ade, **c**) Gua (in **10** B = N^2-Pal-Gua)

a: EtOH/HCl, Δ; *b*: acetone/$MeC(OEt)_2Me$/HCl; *c*: $LiAlH_4$; *d*: 60% NaH, BzlCl/THF; *e*: 0,5 N HCl, MeOH, Δ; *f*: $BrCH_2CH(OEt)_2$/p-TsOH; *g*: sodium salts of thymine and adenine, N^2-palmitoylguanine/K_2CO_3, DMF; *h*: 10% Pd/C, $HCOONH_4$, MeOH.

SCHEME

TABLE 1. ^1H NMR chemical shifts (δ) for the dioxolane nucleoside analogues **10** in $CDCl_3$ and **11** in D_2O at 300 K.

Compd.	Base		Dioxolane moiety								
			H2	H2'a	H2'b	H4	H4'a	H4'b	H5	H5'a	H5'b
10a	7.03 q	1.80 d	5.23 t	3.88 dd	3.81 dd	4.08 ddd	3.50 dd	3.47 dd	4.02 ddd	3.52 dd	3.51 dd
10b	8.30 s	7.88 s	5.37 t	4.37 dd	4.35 dd	4.08 ddd	3.28 dd	3.28 dd	3.86 ddd	3.51 dd	3.47 dd
10c	8.31 s		5.38 t	4.34 brs		4.41 ddd	3.36 dd	3.29 dd	3.90 ddd	3.55 dd	3.51 dd
11a	7.36 q	1.74 d	5.25 t	3.94 dd	3.87 dd	3.88 ddd	3.56 dd	3.41 dd	3.78 ddd	3.60 dd	3.53 dd
11b	8.58 s	8.51 s	5.89 t	4.86 d	4.86 d	4.34 ddd	3.76 dd	3.59 dd	4.07	– m	3.95
11c	7.95 s		5.58 t	4.45 dd	4.41 dd	4.09 ddd	3.57 dd	3.43 dd	3.82 ddd	3.79 dd	3.74 dd

Benzyl group: 7.30m (10H,2Ph), 4.48s (2H,CH_2), 4.45s (2H,CH_2)
Palmitoyl group: 2.48t (2H, CH_2), 1.70m (2H, CH_2), 1.30brs (24H, CH_2), 0.87t (3H, Me).

TABLE 2. Coupling constants (J in Hz) for **10** and **11** at 300 K.

	2,2'a	2,2'b	2'a,2'b	4,4'a	4,4'b	4'a,4'b	4,5	5,5'a	5,5'b	5'a,5'b
10a	3.5	3.7	-14.3	4.6	4.8	-10.3	6.3	4.6	5.1	-10.3
10b	3.0	3.0	-14.4	5.0	5.0	-10.4	6.3	4.2	4.8	-10.4
10c	2.5	2.5	–	4.3	5.5	-10.3	6.2	4.0	4.9	-10.5
11a	2.9	2.9	-14.9	3.6	5.7	-12.3	6.6	3.5	5.7	-12.5
11b	2.5	2.5	–	3.7	5.9	-12.1	6.4	–	–	–
11c	2.6	2.6	-15.2	4.0	6.0	-12.1	6.4	4.7	5.9	-12.3

$J_{5,6}$ 1.2 (Thy).

trans oriented 4'a,4'b and H5 remained near zero. The assignment in the other derivatives **10b** and **10c** was carried out similarly.

It should be noted that the geminal constant $J_{2'a,2'b}$ in CH_2N group is greater than that in CH_2O group ($J_{4'a,4'b}$ and $J_{5'a,5'b}$) (tabl. 2). The vicinal coupling constants $J_{4,5}$ in **10** and **11** were in the range of 6.2-6.6 Hz.

The deprotected nucleoside analogues **11** were UV spectroscopically almost identical with their parent nucleosides thymidine, adenosine and guanosine. A low negative Cotton effect with λ_{max} 268 nm ($\Delta\varepsilon$ -0.22) was observed in CD spectra of thymine nucleoside **11a**.

The above described nucleoside analogues were found to be inactive against HIV-1 (CEM cells) and HSV-1, HSV-2, HCMV, VZV (HFF cells) at concentration up to 100 μg/ml and were nontoxic towards CEM and HFF cells. The absence of activity is probably due to the fact that 4(*R*),5(*R*)-dihydroxymethyl-2-methyl-1,3-dioxolane structures are not being recognized by selective cellular and viral kinases.

EXPERIMENTAL

General methods. Melting points (uncorrected) were determined with a TP (USSR) instrument. UV spectra were recorded on a Specord UV-vis spectrometer. Silica gel L (40-100 μm) (Czechoslovakia) was used for adsorption column chromatography. TLC was carried out on Silufol UV_{254} (Kavalier, Czechoslovakia) and Kieselgel 60 F_{254} (Merck, Germany) using A $CHCl_3$, B 95:5 $CHCl_3$-EtOH, C 9:1 $CHCl_3$-EtOH, and D 8:2 $CHCl_3$-EtOH and detection by UV light. All crystalline compounds gave correct elemental analyses ($\pm$0.4%).

^1H NMR spectra were recorded using a Bruker AMX 400 spectrometer at 300 K. Chemical shifts were measured relative to solvent signals. The signals were assigned by the double resonance techniques. The NOE measurements in $CDCl_3$ were performed under identical spectral and processing conditions by applying an NOEDIFF pulse sequence of Bruker software package UXNMR (release version 911101) for steady-state NOE measurements. For compounds **10** and **11**, the values of chemical shifts and coupling constants were calculated by means of a DAISY programme on a Aspect X32/2 computer.

2-Bromomethyl-4(R),5(R)-dibenzyloxymethyl-1,3-dioxolane (**9**). A mixture of 1,4-di-O-benzyl-D-threitol **5** (6.1 g, 20.2 mmol), bromoacetaldehyde diethyl acetal (2.42 mL, 20.2 mmol) and p-toluenesulfonic acid (0.2 g) in dry acetonitrile (20 mL) was gently heated for 2 h and evaporated in vacuo to dryness. The residue was diluted with chloroform (150 mL), washed successively with saturated aqueous sodium hydrogencarbonate (30 mL) and water (2x30 mL), dried with Na_2SO_4, filtered and evaporated in vacuo to dryness. The residue was chromatographed on silica gel (50 g), using a chloroform-hexane (1:4 v/v) mixture. The pooled fractions were evaporated to a syrup. Yield 6.1 g (74%). R_F 0.93 (A). ^1H NMR ($CDCl_3$): 7.26 brs (10H, $2CH_2\underline{Ph}$), 5.27 t (1H, J 4.0, $\underline{CH}CH_2$), 4.55 brs (2H, $\underline{CH_2}Ph$), 4.54 brs (2H, $\underline{CH_2}Ph$), 4.20-4.08 m (2H, 2CH), 3.66-3.56 m (4H, $2CH_2$), 3.39 d (2H, J 4.0, $CH\underline{CH_2}$).

4(R),5(R)-Dibenzyloxymethyl-2-(thymin-1-ylmethyl)-1,3-dioxolane (**10a**). To a suspension of dry thymine (1.04 g, 8.25 mmol) in dry dimethylformamide (DMF, 30 mL) was added sodium hydride (0.4 g, 10.3 mmol, 60% in oil) and the mixture was stirred for 1 h at 20°C. Then the mixture was heated to 110°C and a solution of **9** (3.05 g, 7.5 mmol) in DMF (10 mL) was added in several portions over 2 h. The mixture was heated over 10 h, cooled to 20°C, filtered and the combined filtrate and DMF washings were evaporated in vacuo to dryness. The residue was dissolved in chloroform (150 mL), the organic layer was washed with water (2x30 mL), dried with Na_2SO_4, filtered, evaporated to dryness and chromatographed on silica gel (100 g). Elution with a chloroform-hexane (1:1 v/v) mixture gave the starting bromide **8** and with system A gave N^1,N^3-bis-product (yield 0.32 g, 5%). Further elution with system A gave **10a** as a syrup. Yield 0.37 g (10%). R_F 0.42 (B). The ^1H NMR data are given in Tables 1 and 2.

4(R),5(R)-Dibenzyloxymethyl-2-(adenin-9-ylmethyl)-1,3-dioxolane (**10b**). This was prepared analogously by alkylation of sodium salt of adenine (4.73 mmol) with **9** (1.75 g, 4.3 mmol) in dry DMF (15 mL). The products were separated on silica gel (system B) to give **10b**. Yield 0.9 g (41%). R_F 0.57 (B). M.p. 117-119°C (ethanol). Further elution with the same solvent gave the corresponding N^3-isomer. Yield 0.20 g (4%). R_F 0.35 (B).

4(R),5(R)-Dibenzyloxymethyl-2-(N^2-palmitoylguanin-9-ylmethyl)-1,3-dioxolane (**10c**). To a stirred suspension of N^2-palmitoylguanine (1.45 g, 3.72 mmol) and K_2CO_3 (0.75 g, 5.4 mmol) in dry DMF (200 mL) a solution of **9** (3 g, 7.37 mmol) in dry DMF (20 mL) was added in several portions over 4 h at 20°C. The mixture was stirred for 3 days at 20°C and evaporated in vacuo to dryness. The residue was dissolved in chloroform (300 mL), the organic layer was washed with water (2x50 mL), dried with Na_2SO_4, filtered through Hyflo Super Cel, evaporated to dryness and chromatographed on silica gel (100 g). A column was washed with system A (400 mL), further elution with a chloroform–ethanol (98.5:1.5 v/v) mixture gave N-7 isomer. Yield 0.35 g (13%). R_F 0.60 (B). Further elution with the same mixture gave **10c** as a syrup. Yield 0.35 g (13%). R_F 0.47 (B).

4(R),5(R)-Dihydroxymethyl-2-(thymin-1-ylmethyl)-1,3-dioxolane (**11a**). To a solution of **10a** (0.2 g, 0.44 mmol) in methanol (20 mL) $HCOONH_4$ (250 mg) and 10% Pd/C (Fluka, 154 mg, 350 mg for 1 mmol) were added, the mixture was refluxed for 3 h, filtered through Hyflo Super Cel and the combined filtrate and methanol washings were evaporated to dryness. The residue was dissolved in water (70 mL), the aqueous layer was washed with ethyl acetate (2x20 mL) and evaporated to dryness. Product **11a** slowly crystallized from ethanol. Yield 78 mg (66%). R_F 0.23 (B). M.p. 135–136°C. UV: λ_{max}^{pH1-7} 271 nm (ε 8700); λ_{max}^{pH13} 271 nm (ε 6500).

4(R),5(R)-Dihydroxymethyl-2-(adenin-9-ylmethyl)-1,3-dioxolane (**11b**). This was prepared analogously starting from **10b** (0.2 g, 0.43 mmol). Product **11b** slowly crystallized from acetone. Yield 90 mg (70%). R_F 0.10 (B). M.p. 145–147°C. UV: λ_{max}^{pH1} 258 nm (ε 12520); λ_{max}^{pH7-13} 261 nm (ε 12860).

4(R),5(R)-Dihydroxymethyl-2-(guanin-9-ylmethyl)-1,3-dioxolane (**11c**). A mixture of **10c** (0.32 g, 0.45 mmol), $HCOONH_4$ (250 mg) and 10% Pd/C (154 mg) in methanol (20 mL) was refluxed for 2 days, filtered through Hyflo Super Cel and the combined filtrate and methanol washings were evaporated to dryness. The residue was dissolved in methanolic 5M ammonia (20 mL), kept for 3 days at 20°C and then concentrated to dry-

ness. The residue was partitioned between water (50 mL) and chloroform (20 mL), and the organic layer was washed with water (20 mL). The combined aqueous extracts were washed with chloroform (20 mL), concentrated to dryness, and the residue was recrystallized from ethanol. Yield 53 mg (40%). R_F 0.08 (B). M.p. 187–189°C. UV: $\lambda_{max}^{pH\ 1}$ 258 nm (ε 10790); $\lambda_{max}^{pH\ 7}$ 252 nm (ε 11090); $\lambda_{max}^{pH\ 13}$ 268 nm (ε 9500).

ACKNOWLEDGEMENTS

This research was supported by the Russian Fund of Fundamental Sciences, project 93-04-7607(S.N.M.) and NIH, NIAD grant No NO1 AI 82518 (E.R.K.). We would like to thank "Bruker Analytische Messtechnick GmbH" (Karlsruhe, Germany) for providing an opportunity to perform our measurements on NMR spectrometer AMX400 in the Demo Center in Moscow. We are greatful to Dr. C.Tseng from the National Institute of Allergy and Infectious Diseases (NIAID) and Dr. R.Schultz from the National Cancer Institute (NCI) for the antiviral screening.

REFERENCES

1. Mitsuya H., Weinhold K.J., Furman P.A., St.Clair M.H., Lerman N.S., Gallo R.G., Bolognesi D., Barry D.W., Broder S. *Proc. Natl. Acad. Sci. USA*, **1985**, 82, 7096.
2. Mitsuya H., Broder S. *Proc. Natl. Acad. Sci. USA*, **1986**, 83, 1911.
3. *Design of Anti-AIDS Drug. (Edited by E. De Clercq).* Amsterdam, Elsevier, Pharmac. Library, **1990**, 14, 372p.
4. Wainberg M.A., Stern M., Martel R., Belleau B., Soudeyns H. *Abstr. Papers, Vth Inter. Confer. on AIDS*, Montreal, Ontario, **1989**, M.C.P. 63.
5. Norbeck D.W., Spanton S., Broder S., Mitsuya H. *Tetrahedron Lett.*, **1989**, 30, 6263.
6. Kim H.O., Ahn S.K., Alves A.J., Beach W.J., Jeong L.S., Choi B.G., Van Roey P., Schinazi R.F., Chu C.K. *J. Med. Chem.*, **1992**, 35, 1987.
7. Kim H.O., Schinazi R.F., Shanmuganathan K., Jeong L.S., Beach J.W., Nampalli S., Cannon D.L., Chu C.K. *J. Med. Chem.*, **1993**, 36, 519.
8. Belleau B., Dixit D., Nguyen-Ba N., Kraus J-L. *Abstr. Papers, Vth Inter. Confer. on AIDS*, Montreal, Ontario, 1989, T.C.O.1.

9. Beach J.W., Jeong L.S., Alves A.J., Pohl D., Kim H.O., Chang C-N., Doong S-L., Schinazi R.F., Cheng Y-C., Chu C.K. *J. Org. Chem.*, **1992**, 57, 2217.
10. Jeong L.S., Schinazi R.F., Beach J.W., Kim H.O., Nampalli S., Shanmuganathan K., Alves A.J., McMillan A., Chu C.K., Mathis R. *J. Med. Chem.*, **1993**, 36, 181.
11. Efimtseva E.V., Mikhailov S.N., Meshkov S.V., Lonnberg H. *Acta Chem. Scand.* **1992**, 46, 1122.
12. Efimtseva E.V., Mikhailov S.N., Meshkov S.V., Bochkarev A.V., Gurskaya G.V. *Bioorg. Khim.(Russia)*, **1993**, 19, 103.
13. Mash E.A. *Homochiral ketals and acetals in organic synthesis. In: Studies in Natural Products Chemistry*, **1988**, p. 577-653.
14. Tseng C.K.-H., Marques V.E., Milne G.W., Wisocki R.J.Jr., Mitsuya H., Shirasaki T., Driscoll J.S. *J. Med.Chem.*, **1991**, 34, 343.
15. Svansson L., Kvarnstrom I., Classon B., Samuelsson B. *J. Org. Chem.*, **1991**, 56, 2993.
16. Kuhn P., Wagner-Jauregg O. *Chem. Ber.*, **1928**, 61, 483.
17. Holy A. *Collect. Czech. Chem. Commun.*, **1979**, 44, 593.
18. Ando N., Yamamoto Y., Oda J., Inouye Y. *Synthesis*, **1978**, 9, 688.
19. Kjellberg J., Johansson N.G. *Nucleos. Nucleot.* **1989**, 8, 225.
20. Bieg T., Szeja W. *Synthesis*, **1985**, N 1, 76.

Received 8/17/93
Accepted 10/21/93