Novel Carbocyclic Nucleoside Containing a Cyclopentyl Ring and Uracil

Carmen BALO, Franco FERNÁNDEZ, Evangelina LENS, and Carmen LÓPEZ*

Departamento de Química Orgánica, Facultade de Farmacia, Universidade de Santiago, E-15706-Santiago de Compostela, Spain. Received September 17, 1997; accepted November 17, 1997

A carbocyclic analogue of uridine, (±)-1-[*t*-2,*t*-3-dihydroxy-*c*-4-(hydroxymethyl)-*r*-1-cyclopentylmethyl]-1,2,3,4-tetrahydropyrimidine-2,4-dione (13), was synthesized with a view to studying its structure–activity relationships.

Key words carbocyclic nucleoside; antiviral activity; uracil; aminocyclopentylmethanol

In carbocyclic analogues of nucleosides (CANs), the bond linking the base to the pseudosugar is less labile than the glycosidic bond of natural nucleosides. Most CANs nevertheless have conformations similar to those of natural nucleosides and are thus activated *in vivo* by phosphorylases for antiviral or antineoplastic action through their incorporation into nucleic acids or as inhibitors of enzymes regulating viral or tumor-cell activity.[1,2] Two promising antiviral and antitumor agents are the naturally occurring antibiotics aristeromycin (**1**)[3] and neplanocin A (**2**),[3,4] and these, together with **3a** and **3b**, and their enantiomers, are all substrates of human immunodeficiency virus (HIV)-1 thymidylate kinase.[5]

The synthesis and the biological properties of carbocyclic homo-nucleosides have recently become of interest.[6,7] As part of our research on the structure–activity relationships of CANs,[7] we required the uridine analogue **13**, whose hydroxylated pseudosugar ring and base nitrogen are separated by an additional methylene, thus altering their spatial relationship and increasing the overall lipophilicity and flexibility of the molecule with respect to natural nucleosides.

The synthesis of CANs usually involves construction of the purine or pyrimidine base on an appropriate amino alcohol precursor.[8] As preliminary biological tests were to be performed with racemic **13**, its precursor **11** was prepared by a non-enantioselective route.

Results and Discussion

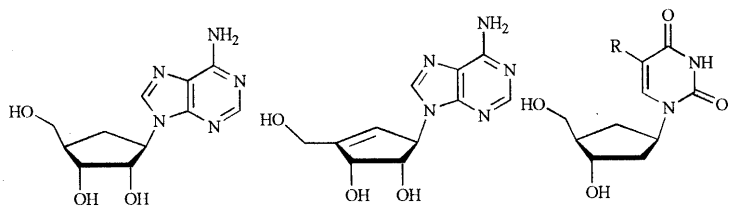
Synthesis of the amino alcohol (±)-**11** was carried out in seven steps from commercially available norbornadiene (**4**), as outlined in Chart 1.

Syn hydroxylation of **4** was performed by direct oxidation with potassium permanganate at −70 °C, avoiding over oxidation by the use of excess **4**. In the first instance, the cyclic manganese intermediate was destroyed with aqueous sodium sulfite/sodium hydroxide, and the unsaturated diol was worked up *via* the laborious extraction–decantation sequence described by Shealy and Clayton.[8] Chromatography of the crude product afforded **5** in similar yield to the 25—28% (from $KMnO_4$) reported by them. However, we also obtained a product not mentioned by Shealy and Clayton, and identified it as 4-hydroxy-4-methyl-2-pentanone (diacetone alcohol). Since this compound almost certainly resulted from aldol condensation of the solvent (acetone) during the basic hydrolysis; the reaction was run again in dichloromethane, in an effort to improve the yield. However, under these conditions **5** was obtained in only 10% yield.

To avoid formation of the by-product derived from the acetone, the hydrolysis was performed without adding sodium hydroxide, by using excess aqueous sodium metabisulfite ($Na_2S_2O_5$). In addition, the work-up and isolation procedures were simplified and made cleaner (see Experimental). Under these conditions, the yield of **5** was 27%.

Next, protection of the diol **5** as its isopropylidene derivative **6**, followed by one-pot ozonolysis and then reduction with sodium borohydride,[9] gave the diol **7** in 91% yield. Conversion of **7** into the amino alcohol **11** involved firstly acylating one of the two hydroxyl groups, which gave the racemic monoacetate **8**, and then activating the other by allowing it to react with tosyl chloride under anhydrous conditions.[10] The resulting racemic tosyl monoacetate **9** was treated with sodium azide in a heterogeneous system containing methyltrioctylammonium chloride as a phase-transfer catalyst. Finally, reduction of the azide **10** with lithium aluminium hydride[11] gave the desired precursor, the amino alcohol **11**.

With **11** in hand, we proceeded to construct the pyrimidine base on the primary amino group, using the traditional approach outlined in Chart 2.[12,13] Thus, a solution of **11** in dimethylformamide was allowed to react with a benzene solution of β-methoxyacryloyl isocyanate, which was freshly prepared from methyl β-methoxyacrylate *via* the sodium salt and chloride of the corresponding acid.[12] Cyclization of the resulting acryloyl urea **12** in sulfuric acid medium caused concomitant



1 **2** **3a** R = I **3b** R = CH=CHBr

* To whom correspondence should be addressed.

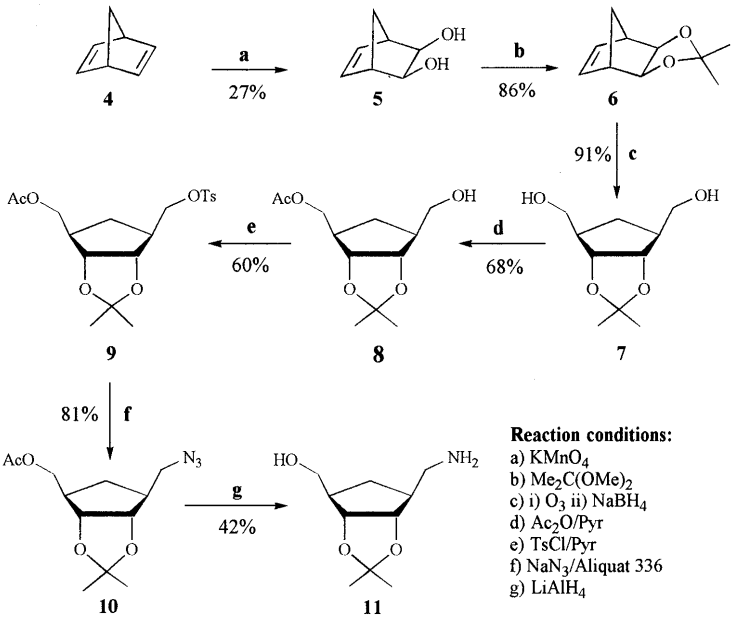


Chart 1

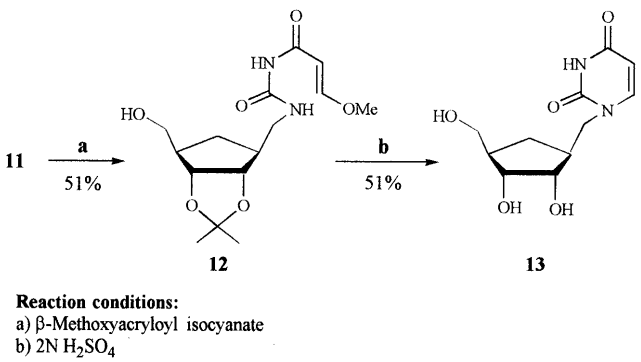


Chart 2

hydrolysis of the isopropylidene protecting group, thus affording the desired homouridine analogue **13**.

Experimental

Melting points are uncorrected and were determined in a Reichert Kofler thermopan. IR spectra of samples in KBr disc (for solids) or as films between NaCl plates (for oils) were obtained in a Perkin Elmer FT-IR 1640 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded in a Bruker AMX-300 spectrometer, at 300 and 75 MHz, respectively, with tetramethylsilane (TMS) as an internal standard. Microanalyses were performed by a Perkin Elmer 240B Elemental Analyser. Column chromatography was done on Solvants Documentation Syntheses (SDS) silica chromagel 60 (70—230 mesh). Thin layer chromatography was performed on Merck GF_{254} chromatoplates. Reagents and solvents were of commercial grade and were all supplied by Aldrich Chemical Co.

***exo-cis*-5-Norbornene-2,3-diol (5)** Finely powdered $KMnO_4$ (15.8 g, 100 mmol) was slowly added in several portions, to a cooled (−65 °C), vigorously stirred solution of norbornadiene (25 ml, 232 mmol) in acetone (300 ml), so that the reaction temperature did not exceed −60 °C. After addition was complete, the reaction mixture was stirred at −65 °C for 1 h, then treated with an ice-cold solution of $Na_2S_2O_5$ (21.75 g, 114 mmol) in water (100 ml) and stirred at −65 °C for 30 min and at +40 °C for 15 min. The cooled mixture was filtered through a bed of silica gel, which was then washed with 5 : 1 (v/v) acetone/water, and the combined filtrates and washings were concentrated under reduced pressure to eliminate acetone. The resulting aqueous solution was extracted with dichloromethane, and the organic layer was dried over anhydrous Na_2SO_4 and evaporated to leave a crystalline solid **5** (3.0 g, 27%), mp (cyclohexane) 114—116 °C (lit.[8] 114—116 °C). IR (KBr) ν (cm^{-1}): 3410, 3245, 2983, 1570, 1078. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.64 (1H, dt, J_d = 9.29, J_t = 1.62 Hz, 7-HH), 1.90 (1H, d, J = 9.29 Hz, 7-HH), 2.70 (2H, br s, OH, D_2O exch.), 2.73 (2H, dd, J = 3.47, 1.42 Hz, 1-H + 4-H), 3.72 (2H, d, J = 1.32 Hz, 2-H + 3-H), 6.04 (2H, t, J = 1.73 Hz, 5-H + 6-H). ^{13}C-NMR δ (ppm): 136.74, 69.35, 48.37, 42.56.

***exo-cis*-5,6-(Isopropylidenedioxy)bicyclo[2.2.1]hept-2-ene (6)** A solution of the diol **5** (5.00 g, 39.6 mmol) and a catalytic amount of *p*-toluenesulfonic acid in 2,2-dimethoxypropane was stirred until the diol was no longer detectable by TLC (*ca.* 3 h), whereupon the reaction mixture was poured into 5% (w/v) aqueous $NaHCO_3$ (200 ml). The whole was extracted with CH_2Cl_2, and the organic phase was separated, washed with water, and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, and the resulting oily residue (5.95 g) was chromatographed on silica gel with CH_2Cl_2 to afford **6** a colorless oil (5.67 g, 86%). IR (film) ν (cm^{-1}): 3064, 2985, 1635, 1531, 1299, 1234. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.23 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.63 (1H, dt, J_d = 8.98, J_t = 1.50 Hz, 7*anti*-H), 1.93 (1H, d, J = 8.98 Hz, 7*syn*-H), 2.71 (2H, t, J = 1.48 Hz, 1-H + 4-H), 4.14 (2H, d, J = 1.31 Hz, 5-H + 6-H), 6.04 (2H, t, J = 1.67 Hz, 2-H + 3-H).

***t*-4,*t*-5-(Isopropylidenedioxy)-*r*-1,*c*-3-cyclopentane-1,3-dimethanol (7)** In a Fischer 503 ozonolysis apparatus, a solution of **6** (4.00 g, 24.1 mmol) in 2 : 1 methanol/CH_2Cl_2 was cooled (−78 °C) and vigorously stirred while O_3 (6 g h^{-1}) was bubbled through it for 1 h (the O_3 was generated from 60 l h^{-1} of O_2, in accordance with the technical specifications of the apparatus). Solid $NaBH_4$ (0.22 g, 5.79 mmol) was added portionwise while maintaining the reaction temperature at around −78 °C, and then the temperature was raised to −5 °C and further $NaBH_4$ (0.93 g, 24.6 mmol) was added. The mixture was stirred for 4 h, warmed to room temperature, and concentrated to afford a white, solid residue. This was triturated in ethyl acetate, collected by filtration, washed with more ethyl acetate, and then dissolved in methanol. This solution was adjusted to pH 7 with concentrated HCl, the solvent was evaporated, and the residue was chromatographed on silical gel with 2 : 1 CH_2Cl_2/ethyl acetate to afford **7** as a colorless oil (4.44 g, 91%). IR (film) ν (cm^{-1}): 3392, 2931, 1377, 1058. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.19—1.29 (1H, m, 2-HH), 1.26 (3H, s, CH_3), 1.45 (3H, s, CH_3), 2.04 (1H, dt, J_d = 12.96, J_t = 7.64 Hz, 2-HH), 2.18—2.22 (2H, m, 1-H + 3-H), 3.39 (2H, br s, 2 × OH, D_2O exch.), 3.59 (2H, part A of a ABM system; J_{AB} = 10.60, J_{AM} = 6.18 Hz, 1,3-(CHH)$_2$), 3.61 (2H, part B of a ABM system, J_{BA} = 10.60, J_{BM} = 5.85 Hz, 1,3-(CHH)$_2$), 4.35 (2H, d, J = 3.05 Hz, 4-H + 5-H). *Anal.* Calcd for $C_{10}H_{18}O_4$: C, 59.40; H, 8.97. Found: C, 59.25; H, 9.03.

(±)-*c*-4-(Hydroxymethyl)-*t*-2,*t*-3-(isopropylidenedioxy)-*r*-1-cyclopentylmethyl Acetate (8) A solution of **7** (3.95 g, 19.5 mmol) in pyridine (25 ml) and acetic anhydride (1.85 ml, 19.6 mmol) was stirred overnight at room temperature. The solvent was removed *in vacuo*, and the oily residue (4.40 g) was chromatographed on silica gel with 1 : 1 hexane/ethyl

acetate and then ethyl acetate. The diacetate of **7** (0.99 g, 25%) eluted first, and then the racemic monoacetate **8** (2.05 g, 52%); in the more polar eluent, some diol **7** (0.75 g, 19%) was recovered. For **8** (68%, on the basis of unrecovered **7**), IR (film), ν (cm^{-1}): 3462, 2986, 2937, 1737. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.28 (3H, s, CH_3), 1.19—1.30 (1H, m, 5-HH), 1.48 (3H, s, CH_3), 2.05 (3H, s, $COCH_3$), 1.99—2.10 (1H, m, 5-HH), 2.18—2.29 (1H, m, 4-H), 2.31—2.41 (1H, m, 1-H), 3.61—3.69 (2H, m, 4-CH_2), 4.08 (2H, d, J=6.38 Hz, 1-CH_2), 4.13—4.44 (2H, m, 2-H+3-H).

(±)-*t*-2,*t*-3-(Isopropylidenedioxy)-*c*-4-(tosyloxymethyl)-*r*-1-cyclopentylmethyl Acetate (9) *p*-Toluenesulfonyl chloride (5.00 g, 26.5 mmol) was added to a cooled (0 °C) solution of **8** (4.56 g, 18.7 mmol) in dry pyridine (11 ml) under argon. The mixture was stirred at room temperature for 24 h, then poured into 2 N HCl (70 ml) and extracted with diethyl ether (3×70 ml), and the combined extracts were washed with 5% (w/v) $NaHCO_3$ and water, then dried over anhydrous Na_2SO_4. Removal of the solvent *in vacuo* gave an oil, which was chromatographed on silica gel with 9:1 (v/v) toluene/ethyl acetate to afford **9** as a colorless oil (4.41 g, 60%). IR (film) ν (cm^{-1}): 2936, 1740, 1189, 1177, 1070. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.25 (3H, s, CH_3), 1.22—1.34 (1H, m, 5-HH), 1.45 (3H, s, CH_3), 2.05 (3H, s, $COCH_3$), 2.01—2.10 (1H, m, 5-HH), 2.28—2.35 (2H, m, 1-H+4-H), 2.45 (3H, s, 4′-CH_3), 3.99—4.12 (4H, m, 1-CH_2+4-CH_2), 4.23—4.31 (2H, m, 2-H+3-H), 7.34 (2H, d, J=8.16 Hz, 3′-H+5′-H), 7.78 (2H, d, J=8.16 Hz, 2′-H+6′-H).

(±)-*c*-4-(Azidomethyl)-*t*-2,*t*-3-(isopropylidenedioxy)-*r*-1-cyclopentylmethyl Acetate (10) Compound **9** (2.92 g, 7.33 mmol) was added to a solution of sodium azide (0.96 g, 14.8 mmol) and methyltrioctylammonium chloride (Aliquat 336) (0.30 g, 0.72 mmol) in water (4 ml), and the mixture was stirred at 75 °C for 5 h. The cooled mixture was diluted with ethyl acetate (16 ml), and the organic layer was separated, washed with saturated NaCl solution and dried over anhydrous Na_2SO_4. Removal of the solvent *in vacuo* gave an oil (1.90 g), which was chromatographed on silica gel with ethyl acetate. Evaporation of the early eluate fractions gave **10** as a colorless oil (1.7 g, 81%). IR (film) ν (cm^{-1}): 2986, 2935, 1743, 1278, 1246. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.30 (3H, s, CH_3), 1.21—1.33 (1H, m, 5-HH), 1.49 (3H, s, CH_3), 2.07 (3H, s, $COCH_3$), 2.04—2.15 (1H, m, 5-HH), 2.24—2.31 (1H, m, 4-H), 2.33—2.43 (1H, m, 1-H), 3.40 (1H, part A of a ABM system, J_{AB}=12.22, J_{AM}=6.35 Hz, 4-HH), 3.42 (1H, part B of a ABM system, J_{BA}=12.22, J_{BM}=6.59 Hz, 4-CHH), 4.10 (2H, d, J=6.20 Hz, 1-CH_2), 4.27—4.39 (2H, m, 2-H+3-H). *Anal.* Calcd for $C_{12}H_{19}N_3O_4$: C, 53.35; H, 7.11; N, 15.60. Found: C, 53.07; H, 7.21; N, 15.48

(±)-*c*-4-(Aminomethyl)-*t*-2,*t*-3-(isopropylidenedioxy)-*r*-1-cyclopentylmethanol (11) A solution of $LiAlH_4$ (1.86 g, 48.8 mmol) in tetrahydrofuran (THF) (60 ml) was carefully added to a cooled (0 °C) solution of **10** (1.60 g, 5.94 mmol) in THF (100 ml). The mixture was warmed and left at reflux for 6 h, then cooled and treated with water (50 ml), and most of the THF was evaporated *in vacuo*. The remaining aqueous mixture was extracted with CH_2Cl_2 (2×50 ml), and the combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated to a brown oil, which was chromatographed on silica gel with 1:1 (v/v) methanol/ethyl acetate to afford **11** as an oil (0.50 g, 42%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.07—1.23 (1H, m, 5-HH), 1.31 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.96—2.02 (1H, m, 5-HH), 2.03—2.17 (1H, m, 4-H), 2.20—2.30 (1H, m, 1-H), 2.78 (2H, d, J=6.19 Hz; 4-CH_2), 3.67 (1H, part A of a ABM system, J_{AB}=10.62, J_{AM}=6.52 Hz, 1-CHH), 3.69 (1H, part B of a ABM system, J_{BA}=10.62, J_{BM}=5.65 Hz, 1-CHH), 4.30 (1H, dd, J=6.89, 4.89 Hz, 3-H), 4.41 (1H, dd, J=6.89, 5.20 Hz, 2-H). *Anal.* Calcd for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.52; N, 6.96. Found: C, 59.45; H, 9.65; N, 7.18.

(±)-*N*-[*c*-4-(Hydroxymethyl)-*t*-2,*t*-3-(isopropylidenedioxy)-*r*-1-cyclopentylmethyl]-*N*′-(3-methoxyacryloyl)urea (12) A freshly prepared toluene solution of β-methoxyacryloyl isocyanate (1.9 ml, *ca.* 1.2 mmol),[12,13] was added to a cooled (−15 °C) solution of **11** (0.17 g, 0.84 mmol) in dimethylformamide (4.0 ml) with stirring under argon, at such a rate that the reaction temperature remained < −10 °C. The mixture was allowed to warm to room temperature and further stirred overnight. The solvent was removed *in vacuo* (ethanol was added so as to form a low-boiling ternary azeotrope), and the resulting residue (0.24 g) was chromatographed on silica gel with 95:5 (v/v) $CHCl_3$/methanol. Concentration of the eluate gave **12** as an oil (0.14 g, 51%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.29 (3H, s, CH_3), 1.21—1.31 (1H, m, 5′-HH), 1.48 (3H, s, CH_3), 2.02—2.21 (3H, m, 1′-H+4′-H+5′-HH), 2.25 (1H, br s, OH, D_2O exch.), 3.45—3.54 (2H, m, CH_2N), 3.70 (3H, s, OCH_3), 3.69—3.75 (2H, m, CH_2O), 4.23—4.27 (1H, m, 2′-H), 4.41—4.45 (1H, m, 3′-H), 5.30 (1H, d, J=12.52 Hz, OC–CH=), 7.68 (1H, d, J=12.52 Hz, =CH–O–), 8.72 (1H, s, NH, D_2O exch.), 9.34 (1H, s, N′H, D_2O exch.).

(±)-1-[*t*-2,*t*-3-Dihydroxy-*c*-4-(hydroxymethyl)-*r*-1-cyclopentylmethyl]-1,2,3,4-tetrahydropyrimidine-2,4-dione (13) A mixture of **12** (100 mg, 0.30 mmol) and 2 N H_2SO_4 (1.7 ml) was refluxed for 3 h, then the hot mixture was treated with a small amount of activated carbon and filtered. The filtrate was adjusted to pH 7 with 2 N NaOH, the solvent was evaporated, and the residue was extracted with ethanol. The ethanolic extracts were concentrated *in vacuo*, and the resulting oil was chromatographed on silica gel with 9:1 (v/v) $CHCl_3$/methanol. Concentration of the eluate gave **13** as an oil (40 mg, 51%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.85—0.95 (1H, m, 5′-HH), 1.71—2.02 (3H, m, 1′-H+4′-H+5′-HH), 3.30 (3H, br s, 3×OH, D_2O exch.), 3.45—3.57 (2H, m, CH_2O), 4.07—4.21 (2H, m, CH_2N), 4.40—4.46 (1H, m, 3′-H), 4.62—4.67 (1H, m, 2′-H), 5.43 (1H, d, J=7.64 Hz, 5-H), 7.37 (1H, d, J=7.64 Hz, 6-H), 10.97 (1H, br s, NH, D_2O exch.). UV λ_{max} (MeOH) nm ($\log\varepsilon$): 269.6 (4.05). HR-EI-MS *m/z*: Calcd for $C_{11}H_{16}N_2O_5$: 256.1059. Found: 256.1048.

Acknowledgments The authors thank the Spanish Ministry of Education and Science (MEC-DGICYT, PB94-0617) and the Xunta de Galicia (XUGA 20307B94) for financial support for this work.

References

1) De Clercq E., *J. Med. Chem.*, **38**, 2491—2517 (1995).
2) De Clercq E., *Rev. Med. Virology*, **5**, 149—164 (1995).
3) Kishi T., Muroi M., Kusaka T., Nishikewa M., Kamiya K., Mizuno K., *Chem. Pharm. Bull.*, **20**, 940—946 (1972).
4) Kusaka T., Yamamoto H., Shibata M., Muroi M., Kishi T., Mizuno K., *J. Antibiotic*, **21**, 255—259 (1968).
5) Balzarini J., Baumgartner H., Bodenteich B., De Clercq E., Griengl H., *J. Med. Chem.*, **32**, 1861—1865 (1989).
6) Katagiri N., Makino M., Nakajima H., Kaneko C., *Nucleic Acids, Symp. Ser.*, **34**, 157—158 (1995).
7) Balo C., Fernández F., Lens E., López C., De Clercq E., Andrei G., Snoeck R., Balzarini J., *Nucleosides & Nucleotides*, **15**, 1335—1346 (1996).
8) Shealy Y. F., Clayton J. D., *J. Am. Chem. Soc.*, **91**, 3075—3083 (1969).
9) Grob C. A., Pfaenler H. R., *Helv. Chim. Acta*, **53**, 2156—2159 (1970).
10) Yoder L., *J. Org. Chem.*, **20**, 1317—1321 (1955).
11) Powell J., James N., Smith S. J., *Synthesis*, **1986**, 338—340.
12) Shaw G., Warrener R. N., *J. Chem. Soc.*, **1958**, 153—156.
13) Shealy Y. F., O'Dell C. A., *J. Heterocyclic Chem.*, **13**, 1015—1020 (1976).