MODEL SYNTHESIS OF SIX-MEMBERED CARBOCYCLIC SPIRONUCLEOSIDES

Radim NENCKA[1], Hubert HŘEBABECKÝ[2,*] and Martin DRAČÍNSKÝ[3]

Centre for New Antivirals and Antineoplastics, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Gilead Sciences & IOCB Research Centre, 166 10 Prague 6, Czech Republic; e-mail: [1] nencka@uochb.cas.cz, [2] hubert@uochb.cas.cz, [3] dracinsky@uochb.cas.cz

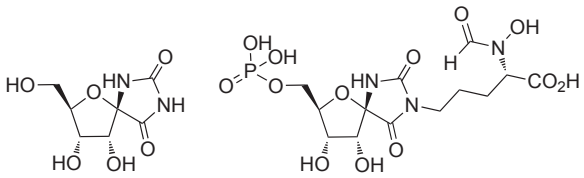
Received August 16, 2010
Accepted September 27, 2010
Published online November 24, 2010

Model study focusing on the synthesis of carbocyclic spironucleosides is presented. Hydantoin base was built on the easily accessible ketone precursors by Bucherer–Bergs reaction. On saturated substrates the reaction proceeded smoothly. On α,β-unsaturated ketones, however, the Bucherer–Bergs reaction competed with Michael addition of cyanide ion. We showed that the equilibrium of the reaction could be significantly shifted depending on the applied reaction conditions. Significant diastereoselectivity was observed for tandem Michael addition/Bucherer–Bergs reaction.

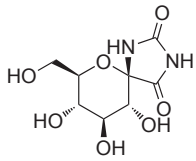
Keywords: Spironucleosides; Carbocyclic; Bucherer–Bergs reaction; Michaels addition; Tandem reaction; Diastereoselective synthesis; Ultrasound sonication.

Spironucleosides, a relatively new class of nucleoside analogues, are characterized by structurally constrained conformation, which is locked by sharing the one common atom between the nucleobase and the sugar moiety. The primary interest in this kind of compounds arose from the discovery of hydantocidin **1**, a natural spironucleoside originally isolated from the culture broth of *Streptomyces hygroscopicus*[1]. Hydantocidin exhibits high nonselective herbicidal activity with very low toxicity for mammals[2]. Its mechanism of action is based on inhibitory activity of its 5′-phosphorylated metabolite against adenylsuccinate synthase (AdSS) where it mimics the molecule of inosine monophosphate[3]. Extensive research in this field based on rational design, molecular modeling and simulated docking experiments led to very potent inhibitors of AdSS, e.g., the bisubstrate inhibitor **2** [4]. In addition, antitumor activity of several antibiotics, including hadacidin and alanosine, involves inhibition of AdSS, and thus, the derivatives of hydantocidin might also find a therapeutic application[5].

Collect. Czech. Chem. Commun. 2010, Vol. 75, No. 12, pp. 1259–1272
© 2010 Institute of Organic Chemistry and Biochemistry
doi:10.1135/cccc2010103



1 **2**



3

The glucopyranosylidene-spiro-heterocycles such as **3** were found to significantly inhibit glycogen phosphorylases and α-amylase[6]. Furthermore, various other spirocyclic hydantoin derivatives possess wide variety of biological activities[7] and therefore, the importance of design and synthesis of novel derivatives of these compounds is obvious.

The term carbocyclic spironucleosides is used for spironucleosides that contain a carbocyclic pseudosugar moiety instead of a natural furanose or pyranose ring.

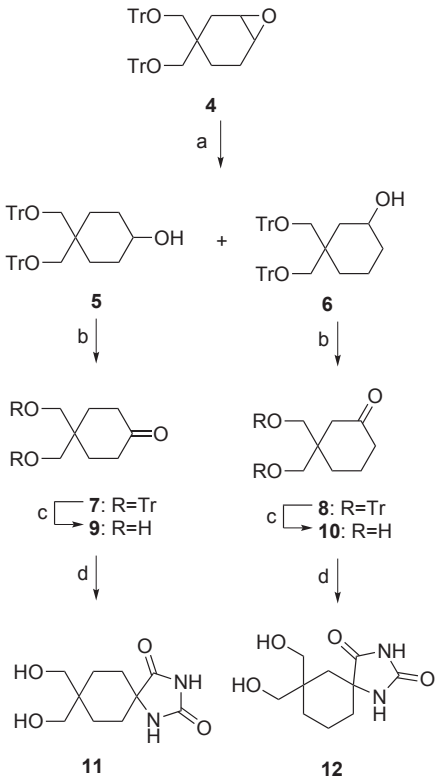
Although the findings of Sano and Sugai[8] clearly indicate that the oxygen atom of the hydantocidin's furanose ring can be replaced by a methylene bridge without loss of herbicidal activity, only very few examples of carbocyclic spironucleosides were synthesized, so far. Several approaches towards four-[9] and five-membered[10] analogues of hydantocidine were presented in the past few years, as far as we know however, there are no examples of carbocyclic spironucleoside analogues with substituted cyclohexane or cyclohexene pseudosugar moiety in the literature. Since the permanent interest of our laboratory is focused on the synthesis of conformationally locked nucleosides with six-membered rings[11], we also decided to prepare several spironucleosides with six-membered carbocyclic moiety bonded directly to hydantoin ring.

RESULTS AND DISCUSSION

First, we focused on the simple saturated cyclohexane derivatives bearing two hydroxymethyl groups that should mimic the sugar ring of naturally occurring nucleosides. The synthetic route started from cyclohexanols **5**

and **6** synthesized by reduction of epoxide **4** with $LiAlH_4$ in ether. The reduction proceeded smoothly with good yield and the resolution of isomers **5** and **6** was achieved without complications by column chromatography. Further oxidation to ketones **7** and **8** was performed by PDC in DMF. The ketones **7** and **8** were deprotected on treatment with 80% acetic acid at 70 °C to afford corresponding bishydroxymethyl ketones **9** and **10** in good yields, respectively. Using traditional conditions for Bucherer–Bergs reaction[12], the hydantoin ring was built from the carbonyl functionality, and the achiral nucleoside derivative **11** and racemic spironucleoside **12** were obtained (Scheme 1).

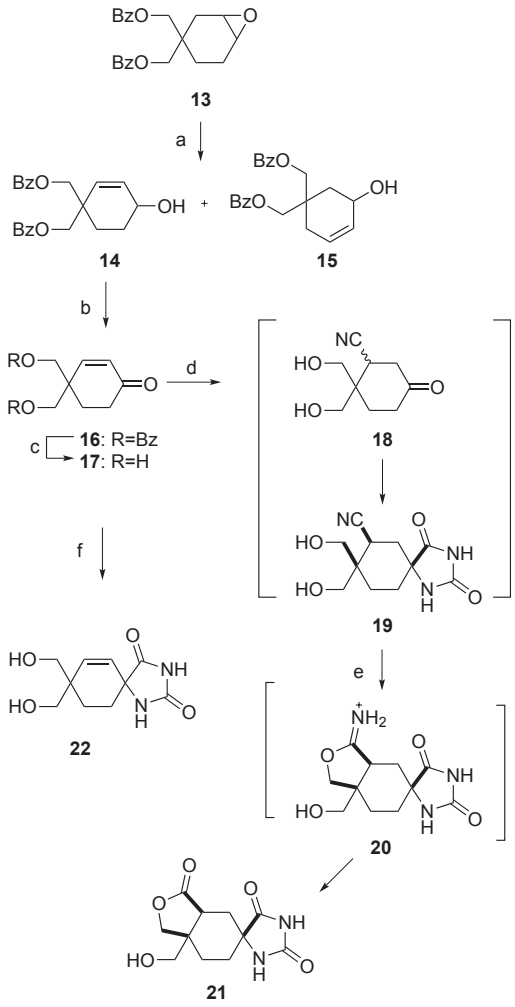
Our further goal was to investigate the applicability of the Bucherer–Bergs reaction on the unsaturated substrates.



a) $LiAlH_4$, Et_2O, 37% of **5**, 40% of **6**; b) PDC, DMF, 86% of **7**, 88% of **8**; c) 80% AcOH, 70 °C, 80% for **9**, 74% for **10**; d) i. KCN, $(NH_4)_2CO_3$, NH_4Cl, EtOH, H_2O, 65 °C; ii. 6 M HCl, 72% for **11**, 22% for **12**.

SCHEME 1

For this model study, we prepared allylic intermediates **14** and **15** from the epoxide **13** by Lewis acid-induced rearrangement[13], on treatment with TMSOTf and DBU, and subsequent hydrolysis of formed trimethylsilylether by diluted hydrochloric acid. The allylic alcohols **14** and **15** were easily separable by column chromatography and were obtained in good yields in almost 1:1 ratio (Scheme 2).



a) i.TMSOTf, DBU, toluene; ii. aq. HCl, MeOH, 31% of **14**, 34% of **15**; b) PDC, DMF, 68%; c) K_2CO_3, MeOH, 72%; d) KCN, $(NH_4)_2CO_3$, EtOH, H_2O, 65 °C; e) 6 M HCl, 5% (2 steps); f) KCN, $(NH_4)_2CO_3$, EtOH, H_2O, sonication, 45 °C; 32%.

SCHEME 2

The starting allylic alcohol **14** was then oxidized by PDC in DMF to yield the ketone **16**, which was subsequently deprotected by treatment with K_2CO_3 in methanol to afford the intermediate **17** in a good yield. The initially applied traditional Bucherer–Bergs procedure afforded a complex mixture of product, where the tricyclic derivative **21** was the major product and the only isolable product. This interesting product results most likely from tandem Michael addition/Bucherer–Bergs reaction followed by acid catalyzed intramolecular cyclization of hydroxy group to nitrile group, and the whole proposed sequence is finished by hydrolysis of the iminium salt **20** (see Scheme 2). Relative configuration of **21** was determined from ^{1}H NMR spectrum and from 2D-ROESY spectrum. Hydrogen H-3a had a high coupling constant with H-4ax (9.8 Hz), from which we could conclude that these two hydrogens are both axial. Hydrogen H-4eq was equatorial and it had a small coupling constant to H-6eq, which indicated that H-6eq is also equatorial. Amide NH had a cross-peak in ROESY spectrum with H-3ax, from which results that this amide is *cis* with respect to H-3ax. Hydrogens of CH_2O group (1) had, among others, ROESY spectrum cross-peaks to H-3ax, H-4eq which is only possible for *cis*-annulation of the five-membered ring (Fig. 1).

Although the expected product **22** was also present in the reaction mixture, the yield of this compound was neglectable. After a series of optimization experiments, we found out that the abundance of this simple carbocylic spironucleoside in the reaction mixture could be significantly increased by reduction of the reaction temperature and time, and especially by the application of ultrasound sonication originally used by Li and co-

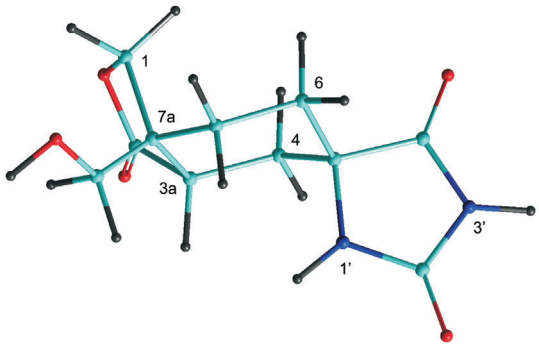
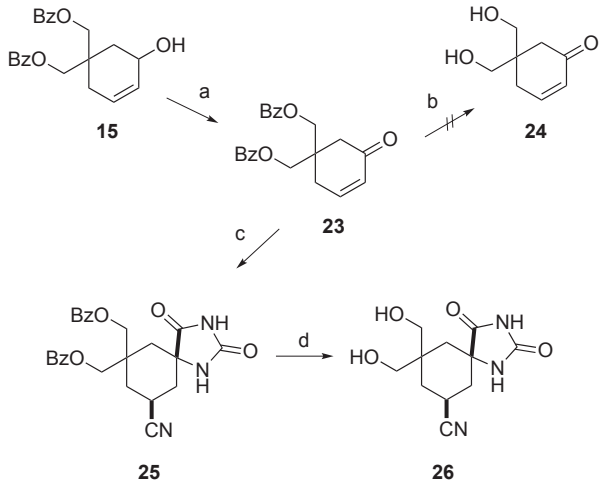


FIG. 1
Model of the tricyclic derivative **21**

workers[14] to accelerate this type of reactions. Nevertheless, even our best results did not exceed 32% yield.

We tried to follow the similar pathway with the second regioisomeric alcohol **15**, which was oxidized by PDC to ketone **23** (Scheme 3). Unfortunately, our efforts to deprotect this compound were unsuccessful. After treatment with K_2CO_3 in methanol for 16 h, no significant products were



a) PDC, DMF, 77%; b) K_2CO_3, MeOH, 0%; c) KCN, $(NH_4)_2CO_3$, formamide, DMF, sonication, 45 °C, 41%; d) NH_3, MeOH, 69%.

SCHEME 3

apparent on TLC after detection with $KMnO_4$. The GC-MS analysis of the mixture showed a complex mixture of at least 6 different products. Therefore, we decided to overcome this trouble by building-up the hydantoin ring before the removal of the benzoyl groups. However, the elevated hydrofobicity of the benzoylated intermediate prohibited application of the optimized procedure described above. For this purpose, we tried to follow the modified Bucherer–Bergs procedure of hydantoin formation according to Sarges et al.[15]. The benzoyl derivative **23** was, however, insoluble in formamide unless a certain portion of DMF was added. We also avoided use of $NaHSO_3$ and applied sonication in ultrasound bath.

The main product of the reaction was the diastereomericaly pure racemate **25** (see Scheme 3). The relative configuration was successively determined by 2D-ROESY NMR experiment from the NOE contacts depicted in Fig. 2.

Obviously, the observed diastereoselectivity arose from the initial Michael addition of the cyanide anion to the unsaturated ketone and subsequent reorientation of the hydatoin ring according to the newly formed nitrile group. It is noteworthy that the orientation of the hydantoin ring corresponds with its orientation in compound **21**, which suggest that this tandem Michael addition/Bucherer–Bergs reaction generally proceeds with significant diastereoselectivity. The final product **26** was obtained by mild debenzoylation with ammonia in methanol (see Scheme 3), which proceeded in acceptable yield.

In conclusion, the carbocyclic spironucleosides bearing hydantoin ring were prepared as model compounds for bioactivity screening. Our results show that the classic conditions for Bucherer–Bergs reaction can be applied to simple saturated ketones but their applicability to unsaturated ketones is rather questionable. Moreover, the application of the ultrasound sonication has, without a doubt, positive effect on the reaction speed and can significantly affect the distribution of the reaction products. In addition, we have proved that the Bucherer–Bergs reaction can be performed also in the mixture of formamide and DMF, which significantly enlarges the group of possible reaction substrates. Most importantly, it seems that the tandem Michael addition/Bucherer–Bergs reaction may proceed with significant diastereoselectivity. Although diastereoselective Bucherer–Bergs reaction have been previously reported[16], this is the first example of diastereoselective tandem Michaels addition/Bucherer–Bergs reaction. Unfortunately, the preliminary cytotoxicity assay did not bring any interesting outcomes, which gives little chance for further development of this group of nucleoside analogues.

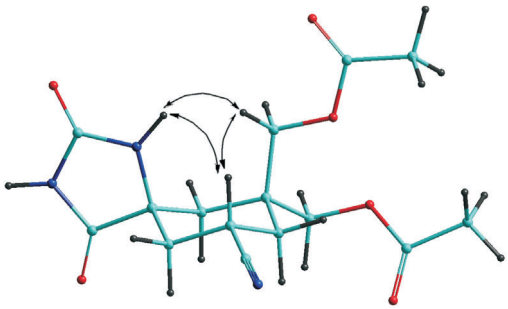


FIG. 2
NOE contacts important for relative configuration determination of the derivative **25**. Benzene rings are omitted for clarity

EXPERIMENTAL

Melting points were determined on a Büchi melting point B-540 apparatus. NMR spectra (δ, ppm; *J*, Hz) were measured on Bruker Avance 500 (^{1}H at 500 MHz, ^{13}C at 125.8 MHz) or Bruker Avance II 600 (^{1}H at 600 MHz, ^{13}C at 151 MHz) spectrometers in DMSO-d_6 or $CDCl_3$ with TMS as an internal standart or referenced to the residual solvent signal (DMSO-d_6 – 2.5 ppm for ^{1}H NMR and 39.7 ppm for ^{13}C NMR and $CDCl_3$ – 7.26 ppm for ^{1}H NMR and 77.0 ppm for ^{13}C NMR). Mass spectra were measured on a LTQ Orbitrap XL (Thermo Fischer Scientific) using electrospray ionization (ESI) and a GCT Premier (Waters) using EI. The elemental analyses were obtained on a Perkin Elmer CHN Analyzer 2400, Series II Sys (Perkin Elmer). IR specra (ν, cm^{-1}) were recorded with FT IR Bruker Equinox IFS 55 spectrometer in KBr pellets or in $CHCl_3$. Column chromatography and thin-layer chromatography (TLC) were performed using Silica gel 60 (Fluka) and Silufol Silica gel 60 F_{254} foils (Merck), respectively. Solvents were evaporated at 2 kPa and bath temperature 30–60 °C. The compounds were dried at 13 Pa and 50 °C.

Preparation of 4,4-Bis(trityloxymethyl)cyclohexanol (**5**) and 3,3-Bis(trityloxymethyl)cyclohexanol (**6**)

Epoxide **4** (3 g, 4.7 mmol) was added to the ice-cooled suspension of $LiAlH_4$ (0.5 g, 13.2 mmol) in Et_2O (35 ml) and stirred at room temperature for 2 days. The reaction mixture was carefully quenched with water and filtered through Celite. The Celite pad was washed with CH_2Cl_2 (25 ml). The filtrate was diluted with CH_2Cl_2 (50 ml), washed with water (30 ml), dried over Na_2SO_4 and evaporated to dryness. Chromatography on silica gel (toluene:EtOAc, 20:1) gave alcohols **5** (1.2 g, 40%) and **6** (1.1 g, 37%).

4,4-Bis(trityloxymethyl)cyclohexanol (**5**). White foam. HRMS (FAB, M + Na$^+$) calculated 667.31882, found 667.31827. ^{1}H NMR (600.13 MHz, DMSO): 0.82 m, 2 H (H-2ax and H-6ax); 1.08 m, 2 H (H-3ax and H-5ax); 1.25 m, 2 H (H-2eq and H-6eq); 1.58 m, 2 H (H-3eq and H-5eq); 3.05 s, 2 H and 3.13 s, 2 H (OCH_2); 3.23 m, 1 H (H-1); 4.32 d, 1 H, *J*(OH,1) = 3.4 (OH); 7.22–7.34 m, 30 H (Ph). ^{13}C NMR (150.92 MHz, DMSO): 27.96 (C-3 and C-5); 29.81 (C-2 and C-6); 37.96 (C-4); 63.54 and 66.96 (OCH_2); 67.78 (C-1); 85.71 and 85.75 (**C**-Ph_3); 127.09 (C-4′); 127.98 (C-3′); 128.59 and 128.62 (C-2′); 144.16 (C-1′). IR ($CHCl_3$): 3612, 3453, 3088, 3062, 3035, 1597, 1585, 1491, 1449, 1318, 1184, 1175, 1160, 1153, 1089, 1069, 1053, 1033, 1000, 946, 900, 708, 700, 644, 633, 617. For $C_{46}H_{44}O_3$ (644.8) calculated: 85.68% C, 6.88% H; found: 85.48% C, 6.99% H.

3,3-Bis(trityloxymethyl)cyclohexanol (**6**). White foam. HRMS (FAB, M + Na$^+$) calculated 667.31882, found 667.32127. ^{1}H NMR (499.95 MHz, DMSO): 0.65 m, 1 H (H-5a); 0.79–0.92 m, 3 H (H-2a, H-4a and H-6a); 1.26 m, 1 H (H-5b); 1.50 m, 2 H (H-4b and H-6b); 1.89 m, 1 H (H-2b); 3.02 m, 1 H (H-1); 2.97 d, 1 H, J_{gem} = 8.2, 3.07 d, 1 H, J_{gem} = 8.8, 3.11 d, 1 H, J_{gem} = 8.2 and 3.14 d, 1 H, J_{gem} = 8.7 (OCH_2); 4.34 d, 1 H, *J*(OH,1) = 4.2 (OH); 7.20–7.38 m, 30 H (Ph). ^{13}C NMR (125.73 MHz, DMSO): 19.37 (C-5); 29.53 (C-4); 35.44 (C-6); 40.52 (C-3); 40.80 (C-2); 62.42 (OCH_2); 64.98 (C-1); 68.95 (OCH_2); 85.73 and 85.75 (**C**-Ph_3); 127.07 and 127.12 (C-4′); 127.92 and 127.99 (C-3′); 128.55 and 128.60 (C-2′); 144.07 and 144.14 (C-1′). IR ($CHCl_3$): 3608, 3447, 3088, 3061, 3035, 1597, 1585, 1491, 1449, 1318, 1184, 1177, 1160, 1153, 1090, 1070, 1054, 1032, 1002, 946, 899, 709, 700, 646, 641, 632, 617. For $C_{46}H_{44}O_3$ (644.8) calculated: 85.68% C, 6.88% H; found: 85.58% C, 6.98% H.

Preparation of 4,4-Bis(trityloxymethyl)cyclohexanone (**7**) and 3,3-Bis(trityloxymethyl)cyclohexanone (**8**)

PDC (1.94 g, 5.1 mmol) was added to the alcohol **5** or **6** (1.1 g, 1.7 mmol) in DMF (35 ml) and stirred for 24 h. The reaction mixture was poured in saturated aqueous solution of $KHCO_3$ and extracted with diethylether (2 × 200 ml) and toluene (150 ml). The combined organic layers were dried over Na_2SO_4, evaporated and chromatographed on silica gel (light petroleum:EtOAc, 40:7) to yield ketone **7** or **8**.

4,4-Bis(trityloxymethyl)cyclohexanone (**7**). Prepared from **5**. Yield 86%, white crystals, m.p. 209–210.5 °C (EtOAc). HRMS (FAB, M + Na^+) calculated 665.30316, found 665.30479. ^{1}H NMR (600.13 MHz, $CDCl_3$): 1.73 t, 4 H, *J*(3,2) = 6.9 (H-3 and H-5); 1.85 t, 4 H, *J*(2,3) = 6.9 (H-2 and H-6); 3.35 s, 4 H (OCH_2); 7.24 m, 18 H (H-3′ and H-4′); 7.40 m, 12 H (H-2′). ^{13}C NMR (150.92 MHz, $CDCl_3$): 30.08 (C-3 and C-5); 36.63 (C-2 and C-6); 38.30 (C-4); 64.51 (OCH_2); 86.34 (**C**-Ph_3); 126.96 (C-4′); 127.77 (C-3′); 128.81 (C-2′); 143.93 (C-1′); 212.55 (C-1). IR ($CHCl_3$): 3088, 3062, 3034, 1710, 1597, 1585, 1491, 1449, 1317, 1185, 1160, 1153, 1072, 1033, 944, 900, 707, 699, 649, 633, 617.

3,3-Bis(trityloxymethyl)cyclohexanone (**8**). Prepared from **6**. Yield 88%, white crystals, m.p. 165–166 °C (EtOH). HRMS (FAB, M + Na^+) calculated 665.30317, found 665.30232. ^{1}H NMR (600.13 MHz, $CDCl_3$): 1.42 m, 2 H (H-5); 1.64 m, 2 H (H-4); 2.10 m, 2 H (H-6); 2.28 m, 2 H (H-2); 3.12 d, 2 H and 3.18 d, 2 H, J_{gem} = 8.8 (OCH_2); 7.23 m, 18 H (H-3′ and H- 4′); 7.35 m, 12 H (H-2′). ^{13}C NMR (150.92 MHz, $CDCl_3$): 20.72 (C-5); 28.49 (C-4); 40.75 (C-6); 44.03 (C-3); 47.31 (C-2); 65.31 (OCH_2); 86.22 (**C**-Ph_3); 126.81 (C-4′); 127.64 (C-3′); 128.68 (C-2′); 143.71 (C-1′); 211.20 (C-1). IR ($CHCl_3$): 3089, 3062, 3035, 1704, 1597, 1585, 1491, 1449, 1318, 1184, 1160, 1153, 1090, 1033, 946, 900, 708, 700, 650, 642, 633, 618.

Preparation of Cyclohexanone Derivatives **9** and **10**

Mixture of the trityl derivative **7** or **8** (1.56 mmol) and 80% acetic acid (20 ml) was heated at 70 °C for 4 h with occasional sonication in ultrasound bath until the complete dissolution. Volatiles were evaporated and the residue was portioned between water (2 × 50 ml) and the mixture of light petroleum:toluene (40 ml, 3:1). The aqueous parts were evaporated adsorbed onto a small amount of silica gel and chromatoraphed (ethyl acetate:actetone: EtOH:H_2O, 19:3:2:1) on silica gel column to afford the product.

4,4-Bis(hydroxymethyl)cyclohexanone (**9**). Yield 80%, white solid. HRMS (ESI, M + Na^+) calculated 181.0835, found 181.0835. ^{1}H NMR (499.95 MHz, DMSO): 1.59 t, 4 H, *J*(3,2) = 7.0 (H-3); 2.22 t, 4 H, *J*(2,3) = 7.0 (H-2); 3.37 d, 4 H, *J*(CH_2,OH) = 5.2 (CH_2O); 4.53 t, 2 H, *J*(OH,CH_2) = 5.4 (OH). ^{13}C NMR (125.73 MHz, DMSO): 28.15 (C-3); 36.86 (C-2); 38.66 (C-4); 64.10 (CH_2OH); 212.38 (C-1).

3,3-Bis(hydroxymethyl)cyclohexanone (**10**). Yield 74%, colorless oil. HRMS (ESI, M + Na^+) calculated 181.0835, found 181.0835. ^{1}H NMR (499.95 MHz, DMSO): 1.56 m, 2 H (H-4); 1.77 m, 2 H (H-5); 2.09 bs, 2 H (H-2); 2.18 m, 2 H (H-6); 3.19 m, 4 H (CH_2O); 4.57 m, 2 H (OH). ^{13}C NMR (125.73 MHz, DMSO): 21.16 (C-5); 27.43 (C-4); 40.68 (C-6); 44.78 (C-3); 45.52 (C-2); 64.69 (CH_2OH); 211.60 (C-1).

8,8-Bis(hydroxymethyl)-1,3-diazaspiro[4.5]decane-2,4-dione (**11**)

Mixture of ketone **9** (220 mg, 1.39 mmol), KCN (111 mg, 1.67 mmol), $(NH_4)_2CO_3$ (295 mg, 3.06 mmol) and NH_4Cl (84 mg, 1.53 mmol) in EtOH (4 ml) and H_2O (4 ml) was heated at

65 °C for 16 h. After cooling, the reaction mixture was diluted with the aqueous 6 M HCl (6 ml). About half of the solvents were evaporated and the residue was crystallized in refrigerator, the precipitate was collected by filtration and dried in vacuo to give the product **11** (145 mg). Yield 72%, white crystals, m.p. 295.5–296.5 °C. HRMS (ESI, M + Na^+) calculated 251.1002, found 251.1002. ^{1}H NMR (600 MHz, DMSO-d_6): 1.25 td, 2 H, J_{gem} = 14.1, J_{vic} = 13.0, 4.2 (H-3,5ax); 1.35 dt, 2 H, J_{gem} = 13.3, J_{vic} = 4.2, 3.3 (H-2,6eq); 1.50 dt, 2 H, J_{gem} = 14.1, J_{vic} = 4.3, 3.3 (H-3.5eq); 1.69 td, 2 H, J_{gem} = 13.3, J_{vic} = 13.0, 4.3 (H-2,6ax); 3.18 and 3.35 2 × s, 2 × 2 H (CH_2O); 8.42 bs, 1 H (NH-amide); 10.54 bs, 1 H (NH-imide). ^{13}C NMR (151 MHz, DMSO-d_6): 24.10 (CH_2-3,5); 28.93 (CH_2-2,6); 37.76 (C-4); 60.35 (CH_2O); 62.36 (C-1); 66.83 (CH_2O); 156.55 (CO-imide); 179.04 (CO-amide). For $C_{11}H_{15}N_3O_4$ (228.2) calculated: 52.62% C, 7.07% H, 12.27% N; found: 52.56% C, 7.14% H, 11.93% N.

7,7-Bis(hydroxymethyl)-1,3-diazaspiro[4.5]decane-2,4-dione (**12**)

Mixture of ketone **10** (165 mg, 1.04 mmol), KCN (204 mg, 3.07 mmol), $(NH_4)_2CO_3$ (310 mg, 3.21 mmol) and NH_4Cl (92 mg, 1.68 mmol) in EtOH (5 ml) and H_2O (5 ml) was heated at 65 °C for 24 h. After acidification by aqueous 6 M HCl (8 ml), the mixture was evaporated and chromatographed on silica gel column (ethyl acetate:acetone:EtOH:H_2O, 20:3:1:1). The crystallization of resulted solid from water afforded product **12** (53 mg). Yield 22%, white crystals, m.p. 227–227.5 °C. HRMS (ESI, M + Na^+) calculated 251.1002, found 251.1002. ^{1}H NMR (600 MHz, DMSO): 1.16 m, 1 H (H-4a); 1.39 d, 2 H, J_{gem} = 14.2 (H-2a); 1.41–1.52 m, 4 H (H-6a, H-6b, H-4b and H-5a); 1.61 d, 1 H, J_{gem} = 14.1 (H-2b); 1.64 m, 1 H (H-5b); 3.09 dd, 1 H, J_{gem} = 10.5, J(CH_2-OH) = 5.4 (CH_2O); 3.16 dd, 1 H, J_{gem} = 10.5, J(CH_2-OH) = 5.6 (CH_2O); 3.39 d, 2 H, J(CH_2-OH) = 5.1 (CH_2O); 4.47 t, 1 H, J(OH-CH_2) = 5.5 (OH); 4.51 t, 1 H, J(OH-CH_2) = 5.1 (OH); 8.07 s, 1 H (NH-amide); 10.61 brs, 1 H (NH-imide). ^{13}C NMR (151 MHz, DMSO): 17.31 (C-5); 27.71 (C-4); 33.56 (C-6); 36.11 (C-2); 38.89 (C-3); 62.16 (C-1); 63.38 and 68.10 (2 × CH_2O); 156.85 (CO-imide); 179.38 (CO-amide). For $C_{11}H_{15}N_3O_4$ (228.2) calculated: 52.62% C, 7.07% H, 12.27% N; found: 52.40% C, 7.22% H, 11.89% N.

Preparation of Allylic Alcohols **14** and **15**

Mixture of epoxide **13** [17] (1 g, 2.7 mmol) and DBU (0.5 ml, 3.3 mmol) in dry toluene (4 ml) was added by syringe to a solution of TMSOTf (0.54 ml, 3 mmol) in toluene (3.5 ml). The resulting mixture was stirred for 20 h. DBU (0.5 ml, 3.3 mmol) and TMSOTf (0.54 ml, 3 mmol) were added and the mixture was stirred for additional 20 h. The resulting solution was diluted with toluene (70 ml) and washed with aqueous 0.1 M HCl (40 ml), saturated solution of $NaHCO_3$ (30 ml) and water (30 ml). The organic layer was dried over Na_2SO_4, evaporated and dissolved in MeOH (30 ml). Aqueous 2 M HCl (10 ml) was added and the mixture was stirred at room temperature for 5 h. The solution was alkalinized (pH 8) and extracted with CH_2Cl_2 (2 × 70 ml). Organic layers were washed with water (3 × 70 ml), dried over Na_2SO_4 and evaporated to minimum volume. Column chromatography (light petroleum:ethyl acetate, 15:7) afforded products **14** (302 mg, 31%) and **15** (331 mg, 34%).

(4-Hydroxycyclohex-2-ene-1,1-diyl)bis(methylene) dibenzoate (**14**). White foam. HRMS (FAB, M + H^+) calculated 389.1359, found 389.1356. ^{1}H NMR (600.13 MHz, DMSO): 1.59 m, 2 H (H-5a and H-6a); 1.86 m, 2 H (H-5b and H-6b); 4.05 m, 1 H (H-4); 4.28 m, 4 H (OCH_2); 4.87 d, 1 H, J(OH,4) = 5.5 (OH); 5.66 dd, 1 H, J(2,3) = 10.3, J(2,4) = 1.9 (H-2); 5.92 dd, 1 H, J(3,2) = 10.3, J(3,4) = 2.7 (H-3); 7.51 m, 4 H (H-3′); 7.65 m, 2 H (H-4′); 7.97 m, 4 H (H-2′). ^{13}C NMR (150.92 MHz, DMSO): 24.39 (C-6); 28.06 (C-5); 39.22 (C-1); 64.39 (C-4); 66.84 and

67.61 (OCH_2); 127.71 (C-2); 129.02 and 129.03 (C-3′); 129.40 and 129.45 (C-2′); 129.67 and 129.69 (C-1′); 133.68 (C-4′); 136.30 (C-3); 165.72 and 165.74 (C=O).

(5-Hydroxycyclohex-3-ene-1,1-diyl)bis(methylene) dibenzoate (**15**). White foam. HRMS (FAB, M + H^+) calculated 389.1359, found 389.1356. ^{1}H NMR (DMSO, 600.13 MHz): 1.59 dd, 1 H, J_{gem} = 13.0, J(6a,5) = 8.1 (H-6a); 2.05 m, 2 H (H-2a and H-6b); 2.17 dm, 1 H, J_{gem} = 18.2 (H-2b); 4.16 m, 1 H (H-5); 4.30 m, 4 H (OCH_2); 4.89 d, 1 H, J(OH,5) = 5.0 (OH); 5.68 m, 1 H (H-3); 5.73 dm, 1 H, J(4,3) = 10.3 (H-4); 7.51 m, 4 H (H-3′); 7.65 m, 2 H (H-4′); 7.97 m, 4 H (H-2′). ^{13}C NMR (150.92 MHz, DMSO): 29.32 (C-2); 35.67 (C-6); 37.57 (C-1); 62.40 (C-5); 66.08 and 68.51 (OCH_2); 124.85 (C-3); 129.01 and 129.04 (C-3′); 129.41 (C-2′); 129.69 and 129.82 (C-1′); 131.42 (C-4); 133.62 and 133.69 (C-4′); 165.77 (C=O).

(4-Oxocyclohex-2-ene-1,1-diyl)bismethylene Dibenzoate (**16**)

The alcohol **14** (1.1 g, 3.11 mmol) in DMF (60 ml) was treated with PDC (3.5 g, 9.3 mmol) overnight. The mixture was partitioned between ethyl acetate (3 × 250 ml) and saturated aqueous $KHCO_3$ (200 ml). The organic phase was washed with several portions of water, dried over Na_2SO_4 and evaporated to dryness. Crystallization from aqueous methanol gave ketone **16** (771 mg, 68%) as white cubes, m.p. 85–86 °C. HRMS (ESI, M + Na^+) calculated 387.12029, found 387.12127. ^{1}H NMR (499.95 MHz, $CDCl_3$): 2.20 m, 2 H (H-6); 2.64 m, 2 H (H-5); 4.43 d, 2 H and 4.57 d, 2 H, J_{gem} = 11.2 (CH_2O); 6.21 d, 1 H, J(3,2) = 10.3 (H-3); 6.91 dt, 1 H, J(2,3) = 10.3, J(2,6) = 0.8 (H-2); 7.45 m, 4 H (H-3′); 7.59 m, 2 H (H-4′); 8.02 m, 2 H (H-2′). ^{13}C NMR (125.73 MHz, $CDCl_3$): 26.98 (C-6); 33.45 (C-5); 40.51 (C-1); 66.06 (CH_2O); 128.58 (C-3′); 129.36 (C-1′); 129.62 (C-2′); 131.91 (C-3); 133.46 (C-4′); 148.57 (C-2); 166.07 (C=O); 197.93 (C-4).

4,4-Bis(hydroxymethyl)cyclohex-2-en-1-one (**17**)

The ketone **16** (500 mg, 1.4 mmol) was dissolved in MeOH (25 ml). Powdered K_2CO_3 (0.76 g, 5.5 mmol) was added and the suspension was stirred overnight. Neutralization with diluted HCl was followed by evaporation to dryness. The residue was partitioned between ethyl acetate (5 × 50 ml) and brine (25 ml). The organic layer was dried over Na_2SO_4 and evaporated. Chromatography on silica gel (ethyl acetate:acetone:EtOH:H_2O, 19:3:2:1) gave ketone **17** (153 mg, 72%) as a white solid. The analytical sample was recrystallized from ethyl acetate. M.p. 95.5–96.5 °C HRMS (ESI, M+Na^+) calculated 179.0679, found 179.0679. ^{1}H NMR (499.95 MHz, DMSO): 1.79 m, 2 H (H-6); 2.37 m, 2 H (H-5); 3.36 dd, 2 H, J_{gem} = 10.7, J(CH_2,OH) = 5.7 (CH_2Oa); 3.44 dd, 2 H, J_{gem} = 10.7, J(CH_2,OH) = 5.4 (CH_2Ob); 4.78 t, 2 H, J(OH,CH_2) = 5.5 (OH); 5.91 d, 1 H, J(3,2) = 10.3 (H-3); 6.82 dt, 1 H, J(2,3) = 10.3, J(2,6) = 0.8 (H-2). ^{13}C NMR (DMSO, 125.73 MHz): 25.82 (C-6); 33.74 (C-5); 43.47 (C-1); 63.79 (CH_2O); 129.66 (C-3); 154.83 (C-2); 199.12 (C-4).

(3a*R**,5*S**,7a*S**)-7a-(Hydroxymethyl)hexahydro-2′*H*,3*H*,5′*H*-spiro-[2-benzofuran-5,4′-imidazolidine]-2′,3,5′-trione (**21**)

Potassium cyanide (85 mg, 1.3 mmol) and ammonium carbonate (250 mg, 2.6 mmol) were added to the solution of ketone **17** (139 mg, 0.9 mmol) in EtOH (5 ml) and water (5 ml). The mixture was heated at 65 °C for 16 h, poured into 6 M HCl (8 ml) and evaporated. Chromatography of the residue (ethyl acetate:acetone:EtOH:H_2O, 20:3:1:1) afforded the product **21** (10 mg, 5%) as white crystals, m.p. 266 °C. HRMS (ESI, M+Na^+) calculated

277.0795, found 277.0796. ^{1}H NMR (600 MHz, DMSO): 1.52 m, 2 H (H-7ax and H-6ax); 1.75 m, 3 H (H-4ax, H-7eq and H-6eq); 1.82 ddd, 1 H, J_{gem} = 14.0, J(4eq-3a) = 7.1, J(4eq-6eq) = 1.0 (H-4eq); 2.40 dd, 1 H, J(3a-4ax) = 9.8, J(3a-4eq) = 7.2 (H-3a); 3.36 m, 2 H (CH_2OH); 4.13 s, 2 H (CH_2O); 5.06 t, 1 H, J(OH-CH_2) = 5.3 (OH); 8.30 s, 1 H (NH-amide); 10.66 brs, 1 H (NH-imide). ^{13}C NMR (151 MHz, DMSO): 22.33 (C-7); 28.70 (C-6); 30.31 (C-4); 38.90 (C-3a); 41.21 (C-7a); 60.02 (C-5); 66.08 (CH_2OH); 71.58 (CH_2O); 156.44 (CO-amide); 177.82 (CO-imide); 178.00 (CO).

8,8-Bis(hydroxymethyl)-1,3-diazaspiro[4.5]dec-6-ene-2,4-dione (**22**)

Potassium cyanide (214 mg, 3.3 mmol) and ammonium carbonate (1.05 g, 11 mmol) were added to the solution of ketone **17** (170 mg, 1.1 mmol) in EtOH (5 ml) and water (5 ml). The mixture was sonicated in ultrasonic bath at 45 °C for 2 h and then left at room temperature overnight. The solution was evaporated, adsorbed onto silica gel and chromatographed (ethyl acetate:acetone:EtOH:H_2O, 21:3:1:1) to give the product **22** (80 mg). Yield 32%, white crystals, m.p. 219–221 °C. HRMS (ESI, M + Na^+) calculated 249.0846, found 249.0846. ^{1}H NMR (499.95 MHz, DMSO): 1.38 m, 1 H (H-9a); 1.60–1.71 m, 2 H (H-9b and H-10a); 1.89 m, 1 H (H-10b); 3.20–3.35 m, 4 H (CH_2O); 4.54 bt, 1 H, J(OH,CH_2) = 5.3 (OH); 4.57 bt, 1 H, J(OH,CH_2) = 5.3 (OH); 5.44 d, 1 H, J(6,7) = 10.0 (H-6); 5.83 d, 1 H, J(7,6) = 10.0 (H-7); 8.15 s, 1 H (H-1); 10.62 bs, 1 H (H-3). ^{13}C NMR (125.73 MHz, DMSO): 22.33 (C-9); 28.71 (C-10); 41.16 (C-8); 61.64 (C-5); 63.35 and 64.12 (CH_2O); 125.99 (C-6); 136.20 (C-7); 156.49 (C-2); 177.50 (C-4). For $C_{11}H_{15}N_3O_4$ (226.2) calculated: 53.09% C, 6.24% H, 12.38% N; found: 52.89% C, 6.19% H, 12.05% N.

(5-Oxocyclohex-3-ene-1,1-diyl)dimethanediyl Dibenzoate (**23**)

The alcohol **15** (1.5 g, 4.1 mmol) in DMF (80 ml) was treated with PDC (4.6 g, 12.2 mmol) overnight. The solvent was partly removed and the residue was partitioned between ethyl acetate (3 × 250 ml) and saturated solution of $KHCO_3$ (200 ml). The organic layer was washed with water (5 × 150 ml), dried over Na_2CO_3 and evaporated. Chromatography on silica gel (hexane:ethyl acetate, 6:1) afforded ketone **23** (1.2 g, 77%) as a colorless oil. HR-MS (ESI, M + Na^+) calculated 387.1203, found 387.1199. ^{1}H NMR (499.95 MHz, $CDCl_3$): 2.60 dd, 2 H, J(2,3) = 4.2, J(2,4) = 2.1 (H-2); 2.69 s, 2 H (H-6); 4.38 d, 2 H and 4.43 d, 2 H, J_{gem} = 11.3 (CH_2O); 6.16 dt, 1 H, J(4,3) = 10.2, J(4,2) = 2.1 (H-4); 6.92 dt, 1 H, J(3,4) = 10.2, J(3,2) = 4.2 (H-3); 7.44 m, 4 H (H-3′); 7.58 m, 2 H (H-4′); 8.01 m, 4 H (H-2′). ^{13}C NMR (125.73 MHz, $CDCl_3$): 30.31 (C-2); 40.91 (C-1); 42.42 (C-6); 66.74 (CH_2O); 128.53 (C-3′); 129.44 (C-1′); 129.62 (C-2′); 129.64 (C-4); 133.38 (C-4′); 146.36 (C-3); 166.09 (C=O); 196.46 (C-5).

[(5*R**,9*S**)-9-Cyano-2,4-dioxo-1,3-diazaspiro[4.5]decane-7,7-diyl]bismethylene Dibenzoate (**25**)

Potassium cyanide (130 mg, 2 mmol) and ammonium carbonate (1.3 g, 14 mmol) were added to the solution of ketone **23** (365 mg, 1 mmol) in formamide (6 ml) and DMF (2 ml). The suspension was sonicated in an ultrasonic bath at 40–50 °C for 2 h and subsequently partitioned between ethyl acetate (3 × 50 ml) and water (25 ml). The organic phase was dried over Na_2SO_4, evaporated and chromatographed on silica gel (ethyl acetate:toluene, 7:1) to yield the crude product as white crystals. Recrystallization from the mixture of ethyl acetate and methanol afforded the hydantoin **25** (190 mg, 41%) as white crystals, m.p.

234.5 °C. HRMS (ESI, M + Na^+) calculated 484.1479, found 484.1474. ^{1}H NMR (600.13 MHz, DMSO): 1.85 d, 1 H, J_{gem} = 14.5 (H-6ax); 1.86 t, 1 H, J_{gem} = J(8ax,9) = 13.4 (H-8ax); 1.96 t, 1 H, J_{gem} = J(10ax,9) = 13.2 (H-10ax); 1.96 dt, 1 H, J_{gem} = 14.5, J(6eq,8eq) = J(6eq,10eq) = 1.9 (H-6eq); 2.12 dm, 1 H, J_{gem} = 13.3 (H-10eq); 2.31 dm, 1 H, J_{gem} = 13.8 (H-8eq); 3.29 tt, 1 H, J(9,8ax) = J(9,10ax) = 13.1, J(9,8eq) = J(9,10eq) = 3.4 (H-9); 4.20 d, 1 H and 4.25 d, 1 H, J_{gem} = 11.0 (CH_2O); 4.41 d, 1 H and 4.52 d, 1 H, J_{gem} = 11.9 (CH_2O); 7.48 m, 2 H and 7.50 m, 2 H (H-3′); 7.64 m, 1 H and 7.65 m, 1 H (H-4′); 7.96 m, 2 H and 7.98 m, 2 H (H-2′); 8.42 s, 1 H (H-1); 11.0 bs, 1 H (H-3). ^{13}C NMR (150.92 MHz, DMSO): 20.61 (C-9); 30.53 (C-8); 35.17 (C-10); 35.76 (C-6); 37.36 (C-7); 60.98 (C-5); 63.79 and 69.55 (CH_2O); 121.95 (CN); 129.00 and 129.07 (C-2′); 129.48 (C-1′); 129.49 and 129.56 (C-3′); 133.73 (C-4′); 157.29 (C-2); 165.61 and 165.65 (COO); 177.30 (C-4).

(5*R**,7*S**)-9,9-Bis(hydroxymethyl)-2,4-dioxo-1,3-diazaspiro[4.5]decane-7-carbonitrile (**26**)

Mixture of benzoylated derivative **25** (170 mg, 1.1 mmol) and ammonia in 3.5 M methanol (10 ml) was stirred for 2 days. Volatiles were evaporated and the residue was chromatographed on silica gel (ethyl acetate:acetone:EtOH:H_2O, 21:3:2:1) to give the final hydantoin derivative **26** (38 mg, 69%). Analytical sample was crystallized from water. White crystals, m.p. 251–251.5 °C. HRMS (ESI, M + Na^+) calculated 276.0955, found 276.0955. ^{1}H NMR (499.95 MHz, DMSO): 1.36 dm, 1 H, J_{gem} = 14.3 (H-10ax); 1.49 t, 1 H, J_{gem} = J(8ax,7) = 13.2 (H-8ax); 1.66–1.72 m, 2 H (H-10eq and H-6ax); 1.85 dm, 1 H, J_{gem} = 12.9 (H-8eq); 1.96 dm, 1 H, J_{gem} = 12.7 (H-6eq); 3.14 m, 1 H (H-7); 3.12 m, 2 H and 3.39 m, 2 H (CH_2O); 4.72 bt, 1 H, J(OH,CH_2) = 5.2 (OH); 4.93 bs, 1 H (OH); 8.07 bs, 1 H (H-1); 10.84 bs, 1 H (H-3). ^{13}C NMR (125.73 MHz, DMSO): 21.04 (C-7); 31.08 (C-8); 35.60 (C-6); 35.83 (C-10); 39.42 (C-9); 61.20 (C-5); 63.33 and 67.15 (CH_2O); 122.56 (CN); 156.72 (C-2); 177.85 (C-4). For $C_{11}H_{15}N_3O_4$ (253.3) calculated: 52.17% C, 5.97% H, 16.59% N; found: 51.80% C, 5.89% H, 16.23% N.

Our thanks are due to Ms J. Sklenářová for excellent technical assistance. This study, a part of the research project No. Z4 055 0506, was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Centre for New Antivirals and Antineoplastics No. 1M0508), and by Gilead Sciences, Inc., Foster City (CA), USA.

REFERENCES

1. Nakajima M., Itoi K., Takamatsu Y., Kinoshita T., Okazaki T., Kawakubo K., Shindo M., Honma T., Tohjigamori M., Haneishi T.: *J. Antibiot.* **1991**, *44*, 293.
2. Lamberth C.: *Heterocycles* **2005**, *65*, 667.
3. Siehl D. L., Subramanian M. V., Walters E. W., Lee S. F., Anderson R. J., Toschi A. G.: *Plant Physiol.* **1996**, *110*, 753.
4. Hanessian S., Lu P. P., Sancéau J. Y., Chemla P., Gohda K., Fonne-Pfister R., Prade L., Cowan-Jacob S. W.: *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 3159.
5. Stayton M. M., Rudolph F. B., Fromm H. J.: *Curr. Top. Cell Reg.* **1983**, *22*, 103.
6. a) Somsák L., Nagy V., Hadady Z., Docsa T., Gergely P.: *Curr. Pharm. Des.* **2003**, *9*, 1177; b) Oikonomakos N. G., Somsák L.: *Curr. Opin. Investig. Drugs* **2008**, *9*, 379.
7. Meusel M., Gütschow M.: *Org. Prep. Proced. Int.* **2004**, *36*, 391.

8. Sano H., Sugai S.: *Tetrahedron* **1995**, *51*, 4635.
9. Martarello L., McConathy J., Camp V. M., Malveaux E. J., Simpson N. E., Simpson C. P., Olson J. J., Bowers G. D., Goodman M. M.: *J. Med. Chem.* **2002**, *45*, 2250.
10. a) Sano H., Sugai S.: *Tetrahedron: Asymmetry* **1995**, *6*, 1143; b) Pham T. Q., Pyne S. G., Skelton B. W., White A. H.: *J. Org. Chem.* **2005**, *70*, 6369; c) Renard A., Lhomme J., Kotera M.: *J. Org. Chem.* **2002**, *67*, 1302.
11. a) Šála M., Hřebabecký H., Dračínský M., Masojídková M., De Palma A. M., Neyts J., Holý A.: *Collect. Czech. Chem. Commun.* **2010**, *75*, 1; b) Šála M., De Palma A. M., Hřebabecký H., Nencka R., Dračínský M., Masojídková M., Leyssen P., Neyts J., Holý A.: *Bioorg. Med. Chem.* **2010**, *18*, 4374; c) Šála M., Hřebabecký H., Dračínský M., Masojídková M., De Palma A. M., Neyts J., Holý A.: *Tetrahedron* **2009**, *65*, 9291; d) Hřebabecký H., Dračínský M., De Palma A. M., Neyts J., Holý A.: *Collect. Czech. Chem. Commun.* **2009**, *74*, 487; e) Hřebabecký H., Dračínský M., Holý A.: *Collect. Czech. Chem. Commun.* **2009**, *74*, 1425; f) Dejmek M., Hřebabecký H., Dračínský M., Holý A.: *Collect. Czech. Chem. Commun.* **2007**, *72*, 1523; g) Hřebabecký H., Dračínský M., Holý A.: *Collect. Czech. Chem. Commun.* **2008**, *73*, 44.
12. Ware E.: *Chem. Rev.* **1950**, *46*, 403.
13. Rosenquist A., Kvarnström I., Classon B., Samuelsson B.: *J. Org. Chem.* **1996**, *61*, 6282.
14. Li J. T., Li L. J., Li T. S., Li H. Z., Liu J. K.: *Ultrason. Sonochem.* **1996**, *3*, S141.
15. Sarges R., Goldstein S. W., Welch W. M., Swindell A. C., Siegel T. W.: *J. Med. Chem.* **1990**, *33*, 1859.
16. a) Lee W., Miller M. J.: *J. Org. Chem.* **2004**, *69*, 4516; b) Mičová J., Steiner B., Kooš M., Langer V., Gyepesová D.: *Carbohydr. Res.* **2004**, *339*, 2187; c) Kooš M., Steiner B., Mičová J., Langer V., Ďurík M., Gyepesová D.: *Carbohydr. Res.* **2001**, *332*, 351.
17. Nencka R., Šála M., Dejmek M., Dračínský M., Holý A., Hřebabecký H.: *Synthesis* **2010**, *23*, 4119.