Synthesis of Phospholipid–*Ribavirin* Conjugates

by **Ilona A. Oleynikova**, **Tamara I. Kulak***, **Dmitry A. Bolibrukh**, and **Elena N. Kalinichenko**

Institute of Bioorganic Chemisrty, National Academy of Sciences of Belarus, 220141 Minsk, Belarus (e-mail: kulak@iboch.bas-net.by)

New conjugates of antiviral nucleoside *Ribavirin* (=1-(β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide; **1**) with 1,2- and 1,3-diacyl glycerophosphates have been synthesized by the phosphoramidite method. A combination of 2′,3′-phenylboronate protecting group for the sugar moiety of the ribonucleoside **1** and 2-cyanoethyl protection for the phosphate fragment ensured the preparation of the desired compounds with reasonable yields *via* a small number of synthetic steps.

1. Introduction. – Nucleoside and nucleotide analogs are successfully used in the therapy of various malignances as well as for the treatment of viral infections [1][2]. However, the usage of such compounds is often restricted by their low bioavailability and pure pharmacokinetic properties, which necessitate the application of these drugs at high, often toxic, doses. One of the ways to overcome this disadvantage is a chemical modification of biologically active nucleosides and nucleotides aimed at the preparation of their prodrugs, including the lipid derivatives [3]. The type of an active-compound modification is dictated both by the expected point of its action (intestine, brain, lymphatic system, *etc.*) and the knowledge on possible metabolic pathways of lipoconjugates [4].

This approach was successfully employed for preparing the prodrugs of various biologically active nucleosides and nucleotides such as 3′-azido-3′-deoxythymidine (AZT) [5–8], 2′,3′-dideoxyinosine (ddI) [6], 2′,3′-didehydro-3′-deoxythymidine (d4T) [9], 1-(β-D-arabinofuranosyl)cytosine (*Cytarabine*) [10–12], 2-fluoro-9-(β-D-arabinofuranosyl)adenine (*Fludarabine*) [13], 2′,2′-difluoro-2′-deoxycytidine (dFdC, *Gemcitabine*) [12][14].

One of the important nucleosides used in medical practice is *Ribavirin* (=1-(β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide; **1**), a drug exhibiting pronounced activity against various RNA and DNA viruses [15]. Oral *Ribavirin* is approved (in a combination with recombinant human interferon 2α) for the therapy of chronic hepatitis C in adults [16], and its nebulized form – for the treatment of diseases caused by respiratory sincitial virus (RSV) in children [17]. Intravenous *Ribavirin* was effectively used against RSV viral infections in adults after lung transplantation [18], and for treatment of viral hemorrhagic fevers [19][20]. In recent studies on rats, it was demonstrated that intraperitoneal (i.p.) *Ribavirin* induced amelioration of experimental autoimmune encephalomyelitis, a prototypical animal model of human multiple sclerosis [21][22]. The use of *Ribavirin* is often limited by the toxicity of the drug

connected mainly with its property to accumulate in erythrocytes causing severe hemolytic anemia. At present, the studies are aimed at the development of reasonable schemes for the application of *Ribavirin* prodrug, *Viramidine* (=1-*β*-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamidine), which, being transformed to *Ribavirin* in the liver, does not accumulate in the erythrocytes and, consequently, does not cause severe hemolysis, thus reducing the risks of side-effects connected with anemia [23]. In animal model of viral hepatitis, targeted delivery of *Ribavirin* to liver was accomplished using *Ribavirin* conjugates with macromolecular carriers such as lactosaminated poly-L-lysine [24] and hemoglobin [25]. This approach was associated with reduced systemic toxicity, increased chemotherapeutic index of the drug, and amelioration of liver injury. The efficacy of liposome-encapsulated *Ribavirin* in liver targeting has also been demonstrated [26][27].

In the literature, there are few data on the synthesis and biological properties of lipophilic ribavirin derivatives. For example, in the *in vivo* experiments it was shown that 2′,3′,5′-tri-*O*-acetate derivative of *Ribavirin* exhibited essentially higher activity against encephalitis caused by *Dengue* virus type 2 in mice [28] and viral hemorrhagic fewer in monkeys [29]. On the other hand, in several studies on animals, it was demonstrated that 1,2-diacylphosphatidyl derivatives of antiviral dideoxynucleosides (dideoxycytidine, dideoxyguanosine) were characterized by enhanced hepatic uptake after i.p. administration of their liposomal preparations [30][31], and were substantially more effective in the treatment of experimental hepatitis viral infection as compared with neat nucleoside [31].

The present paper is devoted to the preparation of previously unknown lipid derivatives of *Ribavirin*, namely, the conjugates of a given nucleoside with 1,2- and 1,3-diacyl glycerophosphates.

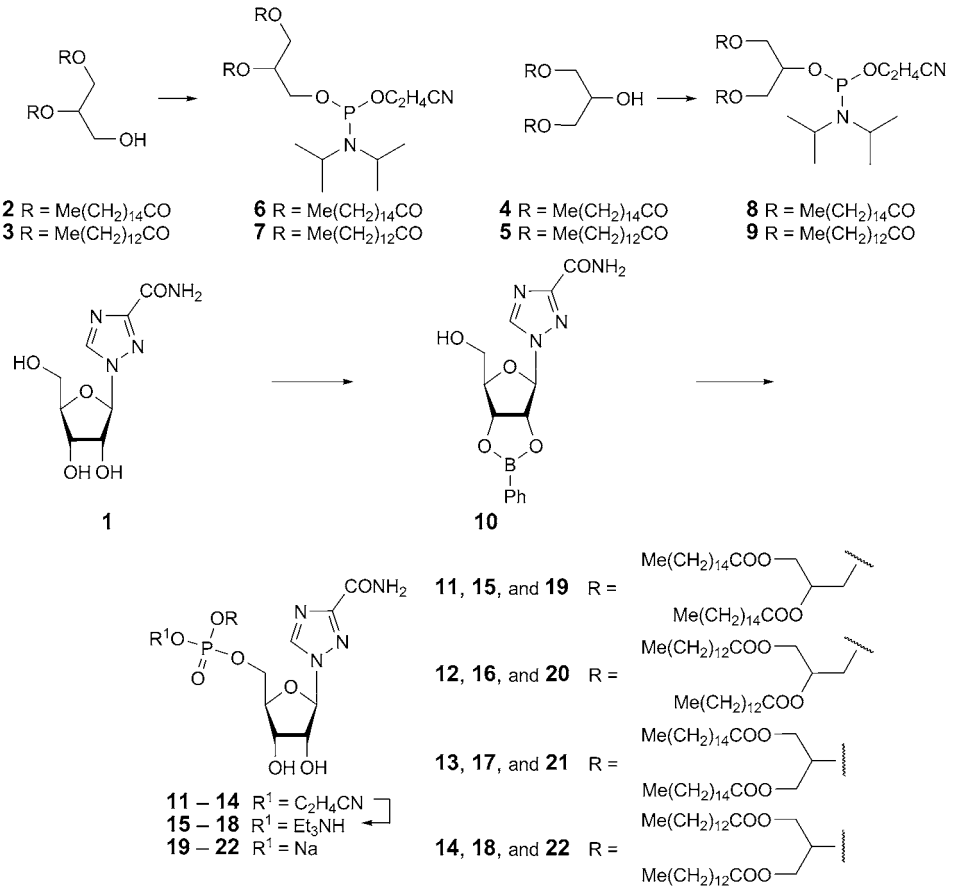
2. Synthesis. – To conjugate the lipids with the nucleosides, one can use the approaches similar to those employed in oligonucleotide chemistry, including phosphodiester [9][13], *H*-phosphonate [6][7][9][32], phosphotriester [8], phosphoramidite [5][12][14][33], and enzymatic [34] methods. For the synthesis of conjugates, we have chosen the method based on the preparation of phosphoramidite derivatives of the lipids, followed by their condensation with the selectively protected nucleoside with a free OH group at C(5′).

1,2-Diacyl-*sn*-glyceroles **2** and **3** containing palmitoyl or myristoyl groups were prepared by the known methods starting from D-mannitol [35][36]. 1,3-Diacyl derivatives **4** and **5** were obtained by the thermal isomerization of compounds **2** and **3**, respectively, as described in [37]. The treatment of lipids **2**–**5** with commercial chloro(diisopropylamino)(2-cyanoethoxy)phosphane and EtN^iPr_2 [38] in CH_2Cl_2 led to phosphoramidite derivatives **6**–**9**, respectively (*Scheme*). After purification by chromatography on alumina, phosphoramidites **6**–**9** were isolated in 81–87% yields.

The structures of phosphoramidites **6**–**9** have been confirmed by ^{1}H-NMR spectroscopy. ^{31}P-NMR Spectra of compounds **6**–**9** exhibit signals at δ(P) 149–150 ppm, characteristic for both nucleoside and lipid phosphoramidite derivatives [5][38].

For the preparation of *Ribavirin* building block with free OH group at C(5′), suitable for the condensation with lipid phosphoramidites **6**–**9**, an easily removable

Scheme



2′,3′-*O*-phenylboronylidene protecting group was chosen. It is well-known that a wide range of molecules with *cis*, or coaxial 1,2- or 1,3-diol functionalities, including sugars and nucleosides, interact with phenylboronic acid ($PhB(OH)_2$) to give the corresponding phenylboronates, which usually can be hydrolyzed under mild conditions, *e.g.*, in the case of nucleosides, by a simple aqueous workup or treatment with i-PrOH in anhydrous media [39].

2′,3′-*O*-phenylboronate **10** has been synthesized by refluxing *Ribavirin* (**1**) with equimolar $PhB(OH)_2$ in anhydrous pyridine, as described for the preparation of phenylboronate derivatives of other ribonucleosides [39]. After crystallization, **10** was separated from the reaction mixture in 84% yield. The presence of phenylboronate protecting group in nucleoside **10** is confirmed by characteristic signals of five aromatic H-atoms at δ(H) 7.88 – 7.32 ppm in its ^{1}H-NMR spectrum.

The condensation of nucleoside **10** with lipid phosphoramidites **6** – **9** in MeCN in the presence of 1*H*-tetrazole, followed by I_2/H_2O oxidation, afforded the phosphotriesters **11** – **14**. The oxidation step was accompanied by the hydrolysis of 2′,3′-phenylboronate

protecting group that was evident from the absence of phenyl H-atoms signals in ^{1}H-NMR spectra of the compounds **11**–**14** (data not shown). The 3-phosphoglyceride derivatives **11** and **12** were obtained in 79 and 71% yield, respectively, while the yields of isomeric 2-phosphoglyceride-nucleoside conjugates **13** and **14** did not exceed 56–57%, probably due to the steric hindrances in the course of nucleoside coupling with phosphoramidite functionality adjacent to two long-chain acyl groups.

The removal of 2-cyanoethyl protecting group from the phosphate moiety of **11**–**14** has been performed by their treatment with Py/Et_3N 1:1. The triethylammonium salts, **15**–**18**, of nucleoside–lipid conjugates were isolated by column chromatography on silica gel in 56–76% yields and then converted to the Na salts **19**–**22**, respectively, by the treatment of their MeOH solutions with NaI/acetone.

3. Physical Data. – All newly synthesized lipid derivatives were characterized by TLC, ^{1}H- and ^{31}P-NMR, and elemental analysis, and conjugates **19**–**22** also by UV and ^{13}C-NMR.

The ^{1}H- and ^{13}C-NMR spectra of the synthesized *Ribavirin*–lipid conjugates contained the signals of the H- and C-atoms, respectively, of every structural fragment including sugar, heterocyclic base, glycerol residue, and acyl groups. The ^{31}P-NMR spectra of **19**–**22** exhibited the peaks near -0.7 ppm, *i.e.*, in the field characteristic for nucleoside phosphodiesters [40]. The two-bond C, P couplings observed in ^{13}C-NMR spectra of isomeric conjugates **19** and **21** correspond to C-atoms in both nucleoside and glycerol fragments attached to the phosphate group (^2J(C(5′),P) = 5 and ^2J(C(1),P) = 5 Hz for 2,3-diacyl glycerophosphate derivative **19**, and ^2J(C(5′),P) = 5 and ^2J(C(2),P) = 4.5 Hz for their 1,3-diacyl isomer **21**). The ^{13}C-NMR spectra of synthesized conjugates also showed the expected three-bond C, P coupling involving C(4′)-atom of carbohydrate moiety (^3J(C(4′),P) = 7 and ^3J(C(4′),P) = 6.5 Hz for **19** and **21**, resp.). Besides, in the ^{13}C-NMR spectra of 2,3-diacyl glycerophosphate derivative **19**, a coupling between P- and C(2)-atom of glycerol fragment was observed (^3J(C(2),P) = 5 Hz), whereas a distinctive feature of 1,3-diacyl isomer **21** was the three-bond C,P couplings involving C(1)- and C(3)-atoms of glycerol skeleton (^3J(C(1),P) ≈ ^3J(C(3),P) ≈ 5 Hz; the *doublet*s were not completely resolved).

4. Enzymatic Hydrolysis by Pancreatic Phospholipase A_2. – The well-known investigations on the antiretroviral activity of phosphatidyl derivatives of AZT clearly demonstrated that phosphatidyl-AZT exerted its antiviral action by metabolic conversion to AZT-5′-triphosphate [41]. It has been shown that, in the cells, these conjugates are metabolized by sequential deacylation catalyzed by phospholipases A and lysophospholipases, followed by the hydrolysis of the produced glycerophospho-AZT by cellular phosphodiesterases to release the nucleoside/5′-nucleotide moiety which are further phosphorylated by kinases to an active metabolite [41].

To verify the possibility for entering the first stage of the above described metabolic pathway by the regioisomeric *Ribavirin*–lipid conjugates **19**, **20** and **21**, **22**, we have conducted preliminary experiments on the hydrolysis of these compounds by porcine pancreatic phospholipase A_2, the most common enzyme of phospholipase A_2 (PLA_2) superfamily. The enzymatic reactions were carried out by incubation of **19**–**22** with PLA_2 (4.8 μg per 1 μmol of substrate) at 37° in 0.05M *Tris*·HCl buffer (pH 8.0)

containing sodium deoxycholate and Ca^{2+} ions. In all cases, the formation of lysophospholipid derivatives with lower mobility than the corresponding parent compound was observed by TLC. In the control mixtures (without PLA_2), only initial **19**–**22** were detected in the same periods of time; hence, the formation of the compounds with low R_f was attributable to the enzymatic hydrolysis. The initial rates of hydrolytic cleavage (V_0) and half-time of hydrolysis ($\tau_{1/2}$) were determined at the 0.6-mM concentration of **19**–**22**. The probes of the reaction mixtures were collected in fixed periods of time (t), and hydrolysis was stopped by addition of ethylenediaminetetraacetic acid (EDTA). The components of the mixtures were resolved by TLC, the phosphate derivatives were visualized by the formation of phosphomolybdenum blue. The content of non-hydrolyzed **19**–**22** was determined by the measurement of the absorbance (D) at 820–830 nm as described in [42]. The values of V_0 and $\tau_{1/2}$ for each of **19**–**22** were calculated from the corresponding D–t plot.

Under the above mentioned experimental conditions, the initial rates of hydrolysis of phosphatidyl derivatives **19** and **20** were 29 and 16 μmol min^{-1} mg^{-1}, respectively. The hydrolytic cleavage proceeded much more slowly in the case of regioisomeric 1,3-diacylglycerophosphate–ribavirin conjugates **21** and **22** for which the values of V_0 were 1.7 and 1.5 μmol min^{-1} mg^{-1}, respectively. This dependency was also reflected in $\tau_{1/2}$ values (5 and 15 min for compounds **19** and **20**, resp.; 80 and 150 min for conjugates **21** and **22**, resp.).

The ability of PLA_2 to hydrolyze not only 2-acyl group of naturally occuring 1,2-diacyl-*sn*-glycerophospholipids but also 1-acyl residue of their artificial 1,3-diacyl regioisomers was established with the use of diacylglycerophosphocholines as substrates [43]. It has been shown that the affinity of 1,3-diacylglycerophosphocholines to phospholipases A_2 are similar to that of natural 1,2-diacyl derivatives, whereas their hydrolysis is essentially decelerated [44]. The enzyme-kinetic study revealed that, in the case of porcine pancreatic PLA_2, the maximum rates ($V_{max,app}$) were lower by the factor 13–20 for 1,3-diacylglycerophosphocholines with different chain lengths compared to the corresponding 1,2-diacyl-*sn*-glycerophosphocholines [44].

The data collected in our work show a similar tendency. The possibility of the hydrolysis of both 1,2-diacyl-*sn*- and 1,3-diacylglycerophosphate–*Ribavirin* conjugates by PLA_2 indicates in principle that these compounds can enter the first stage of metabolic deacylation pathways under the action of cellular phospholipases.

5. Conclusions. – The described approach to the preparation of phospholipid *Ribavirin* derivatives by the phosphoramidite technique, implying a combination of easily removable 2′,3′-*O*-phenylboranylidene protecting group for the carbohydrate moiety of the ribonucleoside and cyanoethyl group for the phosphate fragment of conjugate molecule, allows the preparation of the desired compounds with reasonable yields *via* a small number of synthetic steps. Besides, it ensures a stability of the acyl groups at glycerol moiety in the course of all synthetic stages, avoiding impure structural isomers in the intermediate compounds and final nucleoside–phospholipid conjugates.

The glycerophosphate derivatives **19**–**22** can be considered as *Ribavirin* prodrugs potentially useful for medicinal purposes. The study of the biochemical and antiviral properties of the synthesized conjugates is in progress.

Experimental Part

General. Porcine pancreatic PLA_2 for enzymatic hydrolysis from *Sigma-Aldrich*, (cat. No P6534). TLC: Precoated Al_2O_3 thin-layer sheets (*Fluka*) and SiO_2 thin-layer sheets (*60 F254*; *Merck*); lipid derivatives visualized by spraying with 30% H_2SO_4 and charring. Prep. column chromatography (CC): Al_2O_3 (*Brockmann* activity II, neutral; *Reanal*), SiO_2 (*60*, 63–200 μm; *Merck*). M.p.: *Barnstead Electrothermal IA9300*; uncorrected. UV/VIS Spectra: *Cary 100* (*Varian*); λ_{max} (log ε) in nm. ^{1}H- and ^{13}C-NMR spectra: *Avance 500* (*Bruker*); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz, assignments carried out with the use of ^{1}H,^{1}H and ^{1}H,^{13}C correlation spectroscopy. ^{31}P-NMR: *Avance 500* (*Bruker*); δ in ppm rel. to external H_3PO_4. Elemental analysis: *CHNS-O Analyser EA-3000* (*EuroVector*).

Lipid Phosphoramidites **6**–**9**. *General Procedure*. To a soln. of a lipid, **2**–**5** (1 mmol) in CH_2Cl_2 (5 ml), EtN^iPr_2 (0.52 g, 0.68 ml, 4 mmol) and chloro(diisopropylamino)(2-cyanoethoxy)phosphane (0.47 g, 0.45 ml, 2 mmol) were added under Ar. After stirring for 1 h at r.t., the mixture was diluted with CH_2Cl_2 (30 ml) and hexane (60 ml), and extracted with a mixture of sat. NaCl and $NaHCO_3$ solns. 1:1 (*v*/*v*; 5 × 15 ml). The org. layer was dried (Na_2SO_4) and evaporated. The residue was purified by CC (Al_2O_3 (2.5 × 4 cm); hexane, hexane/Et_2O 9:1, and then hexane/Et_2O 7:3). After evaporation of the appropriate fractions, the phosphoramidites **6**–**9** were isolated as white powders.

3-({[Bis(1-methylethyl)amino](2-cyanoethoxy)phosphanyl}oxy)propane-1,2-diyl Dihexadecanoate (**6**; mixture of diastereoisomers). From **2** (0.6 g, 1.05 mmol): 0.69 g (85%) of **6**. R_f (Al_2O_3; hexane/Et_2O 1:2) 0.72. ^{1}H-NMR ($CDCl_3$): 5.21–5.17 (*m*, H–C(2)); 4.36 (*dd*, *J*(1,2) = 4, *J*(1,1) = 12, CH_2(1)); 4.32 (*dd*, *J*(1,2) = 4, *J*(1,1) = 12, CH_2(1)); 4.18 (*dd*, *J*(1,2) = 6, *J*(1,1) = 12, CH_2(1)); 4.15 (*dd*, *J*(1,2) = 6.5, *J*(1,1) = 12, CH_2(1)); 3.88–3.75 (*m*, CH_2(3), CH_2CH_2CN); 3.73–3.66 (*m*, CH_2(3)); 3.63–3.56 (*m*, 2 Me_2CH); 2.65–2.62 (*m*, CH_2CH_2CN); 2.33–2.29 (*m*, 2 CH_2CO); 1.64–1.58 (*m*, 2 CH_2CH_2CO); 1.30–1.25 (*m*, 2 $Me(CH_2)_{12}$); 1.19–1.17 (*m*, 2 Me_2CH); 0.89–0.87 (*m*, 2 $Me(CH_2)_{14}$). ^{31}P-NMR ($CDCl_3$): 149.7; 149.6. Anal. calc. for $C_{44}H_{85}N_2O_6P$ (769.1): C 68.71, H 11.14, N 3.64; found: C 69.11, H 10.94, N 3.59.

3-({[Bis(1-methylethyl)amino](2-cyanoethoxy)phosphanyl}oxy)propane-1,2-diyl Ditetradecanoate (**7**; mixture of diastereoisomers). From **3** (0.6 g, 1.17 mmol): 0.77 g (92%) of **7**. R_f (Al_2O_3; hexane/Et_2O 1:2) 0.72. ^{1}H-NMR ($CDCl_3$): 5.22–5.17 (*m*, CH(2)); 4.36 (*dd*, *J*(1,2) = 4, *J*(1,1) = 12, CH_2(1)); 4.32 (*dd*, *J*(1,2) = 4, *J*(1,1) = 12, CH_2(1)); 4.18 (*dd*, *J*(1,2) = 6.5, *J*(1,1) = 12, CH_2(1)); 4.15 (*dd*, *J*(1,2) = 6.5, *J*(1,1) = 12, CH_2(1)); 3.88–3.75 (*m*, CH_2(3), CH_2CH_2CN); 3.72–3.65 (*m*, CH_2(3)); 3.63–3.55 (*m*, 2 Me_2CH); 2.65–2.62 (*m*, CH_2CH_2CN); 2.33–2.30 (*m*, 2 CH_2CO); 1.64–1.58 (*m*, 2 CH_2CH_2CO); 1.30–1.25 (*m*, 2 $Me(CH_2)_{10}$); 1.19–1.16 (*m*, 2 Me_2CH); 0.89–0.87 (*m*, 2 $Me(CH_2)_{12}$). ^{31}P-NMR ($CDCl_3$): 149.7; 149.6. Anal. calc. for $C_{40}H_{77}N_2O_6P$ (713.0): C 67.38, H 10.88, N 3.93; found: C 67.67, H 11.12, N 3.75.

2-({[Bis(1-methylethyl)amino](2-cyanoethoxy)phosphanyl}oxy)propane-1,3-diyl Dihexadecanoate (**8**). From **4** (0.6 g, 1.05 mmol): 0.71 g (88%) of **8**. R_f (SiO_2; hexane/AcOEt 9:1) 0.52. ^{1}H-NMR ($CDCl_3$): 4.26–4.12 (*m*, CH_2(1)CH(2)CH_2(3)); 3.89–3.76 (*m*, CH_2CH_2CN); 3.65–3.55 (*m*, 2 Me_2CH); 2.65–2.63 (*m*, CH_2CH_2CN); 2.33–2.29 (*m*, 2 CH_2CO); 1.65–1.58 (*m*, 2 CH_2CH_2CO); 1.31–1.25 (*m*, 2 $Me(CH_2)_{12}$); 1.19–1.17 (*m*, 2 Me_2CH); 0.89–0.87 (*m*, 2 $Me(CH_2)_{14}$). ^{31}P-NMR: 150.2. Anal. calc. for $C_{44}H_{85}N_2O_6P$ (769.1): C 68.71, H 11.14, N 3.64; found: C 69.08, H 10.99, N 3.65.

2-({[Bis(1-methylethyl)amino](2-cyanoethoxy)phosphanyl}oxy)propane-1,3-diyl Ditetradecanoate (**9**). From **5** (0.6 g, 1.17 mmol): 0.66 g (79%) of **9**. R_f (SiO_2, hexane/AcOEt 9:1) 0.52. ^{1}H-NMR ($CDCl_3$): 4.26–4.12 (*m*, CH_2(1)CH(2)CH_2(3)); 3.89–3.77 (*m*, CH_2CH_2CN); 3.65–3.54 (*m*, 2 Me_2CH); 2.65–2.63 (*m*, CH_2CH_2CN); 2.34–2.29 (*m*, 2 CH_2CO); 1.65–1.59 (*m*, 2 CH_2CH_2CO); 1.31–1.25 (*m*, 2 $Me(CH_2)_{10}$); 1.19–1.17 (*m*, 2 Me_2CH); 0.89–0.87 (*m*, 2 $Me(CH_2)_{12}$). ^{31}P-NMR: 150.2. Anal. calc. for $C_{40}H_{77}N_2O_6P$ (713.0): C 67.38, H 10.88, N 3.93; found: C 67.59, H 10.69, N 3.71.

*1-(2,3-Di-*O*-phenylboranylidene-β-*D*-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide* (*=1-[Tetrahydro-6-(hydroxymethyl)-2-phenylfuro[3,4-*d*][1,3,2]dioxaborol-4-yl]-1*H*-1,2,4-triazole-3-carboxamide*; **10**). To a soln. of *Ribavirin* (**1**; 0.2 g, 0.82 mmol) in pyridine (40 ml), a soln. of $PhB(OH)_2$ (0.1 g, 0.82 mmol) in pyridine (20 ml) was added under stirring for 5 min at r.t. The mixture was refluxed for 2 h under anh. conditions and then evaporated. The residue was dissolved in dioxane (5 ml), and Et_2O (25 ml) was added. After keeping the mixture for 16 h at +4°, the precipitate was filtered and dried in vacuum to yield crystalline **10** (0.23 g, 85%). M.p. 213–215°. ^{1}H-NMR ((D_5)pyridine): 9.12 (*s*, H–C(5)); 8.85, 8.80 (2*s*, $CONH_2$); 7.88–7.32 (*m*, 5 arom. H); 6.58 (*d*, *J*(1′,2′) = 1.5, H–C(1′)); 5.37 (*dd*, *J*(2′,3′) = 6,

H–C(2′)); 5.11 (*dd*, *J*(3′,4′) = 2.5, H–C(3′)); 4.88 – 4.86 (*m*, H–C(4′)); 4.16 (*dd*, *J*((5′a,4′) = 5, *J*(5′a,5′b) = 11.5, H_a–C(5′)); 4.08 (*dd*, *J*(5′b,4′) = 5.5, H_b–C(5′)). Anal. calc. for $C_{14}H_{15}BN_4O_5$ (330.1): C 50.94, H 4.58, N 16.97; found: C 51.12, H 4.75, N 16.73.

*Sodium 1-(5-*O*-{[2,3-Bis(hexadecanoyloxy)propoxy]phosphinato}-β-*D*-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide* (**19**). A soln. of **10** (72 mg, 0.22 mmol) in MeCN (15 ml) and 0.45M soln. of 1*H*-tetrazole (1.47 mmol) in MeCN (3.3 ml) were added to **6** (261 mg, 0.34 mmol) under Ar. After stirring for 16 h, a soln. of I_2 (86 mg, 0.34 mmol) in pyridine/H_2O/CH_2Cl_2 3 : 1 : 1 (1.29 ml) [5] was added. The mixture was diluted with $CHCl_3$ (200 ml) and extracted with 2% soln. of $Na_2S_2O_3$ in sat. NaCl (60 ml), and the org. layer was dried (Na_2SO_4) and evaporated. The residue was purified by CC (SiO_2 (1.8 × 20 cm); $CHCl_3$ and then $CHCl_3$/MeOH 15 : 1) to give 144 mg (71%) of **11**. Colorless syrup. R_f (SiO_2; $CHCl_3$/MeOH 9 : 1) 0.31.

A soln. of **11** (100 mg, 0.11 mmol) in pyridine/Et_3N 1 : 1 (2.2 ml) was kept at r.t. for 24 h and evaporated. The residue was evaporated with toluene (2 ml) and purified by CC (SiO_2 (2 × 20 cm); $CHCl_3$/MeOH/Et_3N 99 : 1 : 0 → 50 : 50 : 1) to give **15** (74 mg, 70%). Colorless syrup. The salt **15** was dissolved in a small volume of $CHCl_3$, and treated with 1M NaI/acetone (0.15 ml) and acetone (10 ml). The precipitate was filtered and dried in vacuum dessicator to give 46 mg (67%) of **19**. White powder. R_f (SiO_2, $CHCl_3$/MeOH 2 : 1) 0.26. UV (MeOH): 207 (4.06). ^{1}H-NMR ((D$_6$)DMSO): 8.90 (*s*, H–C(5)); 7.98, 7.54 (2*s*, $CONH_2$); 5.78 (*d*, *J* (1′,2′) = 4, H–C(1′)); 5.62 – 5.51 (*m*, HO–C(2′), HO–C(3′)); 5.06 – 5.01 (*m*, CH_2CHCH_2OP); 4.40 – 4.37 (*m*, H–C(2′)); 4.31 – 4.24 (*m*, CH_2CHCH_2OP); 4.22 – 4.19 (*m*, H–C(3′)); 4.10 – 4.03 (*m*, CH_2CHCH_2OP); 4.04 – 4.01 (*m*, 1 H–C(4′)); 3.83 – 3.79 (*m*, 1 H–C(5′)); 3.77 – 3.72 (*m*, H–C(5′)); 3.73 – 3.66 (*m*, CH_2CHCH_2OP); 2.23 (*t*, *J* = 7; 2 CH_2CO); 1.53 – 1.43 (*m*, 2 CH_2CH_2CO); 1.28 – 1.21 (*m*, 2 Me(CH_2)$_{12}$); 0.84 (*t*, *J* = 7, 2 *Me*(CH_2)$_{14}$). ^{13}C-NMR ((D$_6$)DMSO): 172.53; 172.28 (2 Me(CH_2)$_{14}$*C*O); 160.51 ($CONH_2$); 157.04 (C(3)); 144.81 (C(5)); 92.08 (C(1′)); 84.15 (*d*, ^{3}*J*(C(4′),P) = 7, C(4′)); 74.77 (C(2′)); 70.50 (C(3′)); 70.45 (*d*, ^{3}*J*(C,P) = 5, CH_2CHCH_2OP); 64.04 (*d*, ^{2}*J*(C(5′),P) = 5, C(5′)); 62.44 (*d*, ^{2}*J*(C,P) = 5, CH_2CHCH_2OP); 62.36 (CH_2CHCH_2OP); 33.55; 33.38 (2 Me(CH_2)$_{13}$$CH_2$); 31.25 (2 Me$CH_2CH_2$); 29.01; 28.97; 28.90; 28.88; 28.65; 28.40; 28.37 (2 Me(CH_2)$_2$(CH_2)$_9$); 28.73; 28.70 (2 Me(CH_2)$_{11}$$CH_2$); 24.43, 24.37 (2 Me($CH_2$)$_{12}$$CH_2$); 22.04 (2 Me$CH_2$); 13.87 (2 *Me*($CH_2$)$_{14}$). ^{31}P-NMR ((D$_6$)DMSO): −0.73. Anal. calc. for $C_{43}H_{78}N_4NaO_{12}P \cdot H_2O$ (915.1): C 56.44, H 8.81, N 6.12; found: C 56.31, H 8.49, N 5.90.

*Sodium 1-(5-*O*-{[2,3-Bis(tetradecanoyloxy)propoxy]phosphinato}-β-*D*-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide* (**20**). As described for **11**, with **10** (30 mg, 0.09 mmol), **7** (100 mg, 0.14 mmol), MeCN (7 ml), 0.45M soln. of 1*H*-tetrazole in MeCN (1.34 ml, 0.60 mmol), I_2 (36 mg, 0.14 mmol), and pyridine/H_2O/CH_2Cl_2 3 : 1 : 1 (0.54 ml); CC afforded 66 mg (79%) of **12**. Colorless syrup. R_f (SiO_2, $CHCl_3$/MeOH 9 : 1) 0.31.

As described for **15**, with **12** (53 mg, 0.06 mmol) and pyridine/Et_3N 1 : 1 (1.2 ml); after CC, **16** (36 mg, 64%) was obtained. Treatment of **16** in $CHCl_3$ with 1M NaI/acetone (0.08 ml) and acetone (6 ml) gave **20** (25 mg, 76%). White powder. R_f (SiO_2; $CHCl_3$/MeOH 2 : 1) 0.26. UV (MeOH): 207 (4.06). ^{1}H-NMR ((D$_6$)DMSO): 8.90 (*s*, H–C(5)); 7.99, 7.59 (2*s*, $CONH_2$); 5.80 (*d*, *J*(1′,2′) = 4, H–C(1′)); 5.64 (*d*, *J*(HO–C(2′),2′) = 5, HO–C(2′)); 5.58 (*d*, *J*(HO–C(3′),3′) = 4.5, HO–C(3′)); 5.06 – 5.02 (*m*, CH_2CHCH_2OP); 4.41 – 4.38 (*m*, H–C(2′)); 4.31 – 4.24 (*m*, CH_2CHCH_2OP); 4.23 – 4.20 (*m*, H–C(3′)); 4.10 – 4.03 (*m*, CH_2CHCH_2OP); 4.05 – 4.02 (*m*, H–C(4′)); 3.84 – 3.80 (*m*, 1 H–C(5′)); 3.78 – 3.73 (*m*, 1 H–C(5′)); 3.74 – 3.67 (*m*, CH_2CHCH_2OP); 2.24 (*t*, *J* = 7, 2 CH_2CO); 1.54 – 1.44 (*m*, 2 CH_2CH_2CO); 1.28 – 1.21 (*m*, 2 Me(CH_2)$_{10}$); 0.85 (*t*, *J* = 7, 2 *Me*(CH_2)$_{12}$). ^{31}P-NMR ((D$_6$)DMSO): −0.72. Anal. calc. for $C_{39}H_{70}N_4NaO_{12}P \cdot 2\ H_2O$ (877.0): C 53.41, H 8.50, N 6.39; found: C 53.15, H 8.30, N 6.16.

*Sodium 1-[5-*O*-({[1,3-Bis(hexadecanoyloxy)propan-2-yl]oxy}phosphinato)-β-*D*-ribofuranosyl]-1*H*-1,2,4-triazole-3-carboxamide* (**21**). As described for **11**, with **10** (49 mg, 0.15 mmol), **8** (177 mg, 0.23 mmol), MeCN (13 ml), 0.45M soln. of 1*H*-tetrazole in MeCN (2.2 ml, 0.99 mmol), I_2 (58 mg, 0.23 mmol), and pyridine/H_2O/CH_2Cl_2 3 : 1 : 1 (0.87 ml); CC gave 77 mg (56%) of **13**. Colorless syrup. R_f (SiO_2; $CHCl_3$/MeOH 9 : 1) 0.31.

As described for **15**, with **13** (68 mg, 0.073 mmol) and pyridine/Et_3N 1 : 1 (1.46 ml), after CC, **17** (40 mg, 56%) was obtained. Treatment of **17** in $CHCl_3$ with 1M NaI/acetone (0.082 ml) and acetone (7 ml) gave **21** (26 mg, 70%). White powder. R_f (SiO_2; $CHCl_3$/MeOH 2 : 1) 0.31. UV (MeOH): 207 (4.06). ^{1}H-NMR ((D$_6$)DMSO): 8.84 (*s*, H–C(5)); 7.72, 7.32 (2*s*, $CONH_2$); 5.79 (*d*, *J*(1′,2′) = 4, H–C(1′)); 5.36 –

5.31 (*m*, HO–C(2′), HO–C(3′)); 4.42 – 4.39 (*m*, H–C(2′)); 4.34 – 4.29 (*m*, $(CH_2)_2CHOP$); 4.24 – 4.21 (*m*, H–C(3′)); 4.13 – 4.05 (*m*, $(CH_2)_2CHOP$, H–C(4′)); 3.90 – 3.84 (*m*, 1 H–C(5′)); 3.87 – 3.80 (*m*, 1 H–C(5′)); 2.25 (*t*, *J* = 7, 2 CH_2CO); 1.52 – 1.46 (*m*, 2 CH_2CH_2CO); 1.29 – 1.23 (*m*, 2 $Me(CH_2)_{12}$); 0.84 (*t*, *J* = 7, 2 $Me(CH_2)_{14}$). ^{13}C-NMR ((D_6)DMSO): 172.50 (2 $Me(CH_2)_{14}CO$); 160.49 ($CONH_2$); 157.07 (C(3)); 144.71 (C(5)); 92.22 (C(1′)); 84.27 (*d*, ^3J(C(4′),P) = 6.5, C(4′)); 74.82 (C(2′)); 70.58 (C(3′)); 69.21 (*d*, ^2J(C,P) = 4.5, $(CH_2)_2CHOP$); 64.26 (*d*, ^2J(C(5′),P) = 5, C(5′)); 63.09 (*d*, ^3J(C,P) = 5), 63.05 (*d*, ^3J(C,P) = 5) ($(CH_2)_2CHOP$); 33.39 (2 CH_2CO); 31.16 (2 $MeCH_2CH_2$); 28.90; 28.86; 28.74; 28.58; 28.54; 28.36 (2 $Me(CH_2)_2(CH_2)_9$); 24.31 (2 $Me(CH_2)_{12}CH_2$); 21.93 (2 $MeCH_2$); 13.73 (2 $Me(CH_2)_{14}$). ^{31}P-NMR ((D_6)DMSO): −0.73. Anal. calc. for $C_{43}H_{78}N_4NaO_{12}P \cdot 2\ H_2O$ (933.1): C 55.35, H 8.86, N 6.00; found: C 55.22, H 8.45, N 6.02.

*Sodium 1-[5-*O*-({[1,3-Bis(tetradecanoyloxy)propan-2-yl]oxy}phosphinato)-β-*D*-ribofuranosyl]-1*H*-1,2,4-triazole-3-carboxamide* (**22**). As described for **11**, with **10** (33 mg, 0.10 mmol), **9** (111 mg, 0.16 mmol), MeCN (8 ml), 0.45M soln. of 1*H*-tetrazole in MeCN (1.49 ml, 0.67 mmol), I_2 (41 mg, 0.16 mmol), pyridine/H_2O/CH_2Cl_2 3 : 1 : 1 (0.62 ml); CC gave 50 mg (57%) of **14**. Colorless syrup. R_f (SiO_2, $CHCl_3$/MeOH 9 : 1) 0.31.

As described for **15**, with **14** (36 mg, 0.041 mmol) and pyridine/Et_3N 1 : 1 (0.83 ml); after CC, **18** (29 mg, 76%) was obtained. Treatment of **18** in $CHCl_3$ with 1M NaI/acetone (0.063 ml) and acetone (5 ml) gave **22** (19 mg, 70%). White powder. R_f (SiO_2, $CHCl_3$/MeOH 2 : 1) 0.31. UV (MeOH): 207 (4.06). ^{1}H-NMR ((D_6)DMSO): 8.85 (*s*, H–C(5)); 7.73, 7.34 (2*s*, $CONH_2$); 5.80 (*d*, *J* (1′,2′) = 4, H–C(1′)); 5.37 – 5.32 (*m*, HO–C(2′), HO–C(3′)); 4.43 – 4.40 (*m*, H–C(2′)); 4.35 – 4.30 (*m*, $(CH_2)_2CHOP$); 4.25 – 4.22 (*m*, H–C(3′)); 4.13 – 4.06 (*m*, $(CH_2)_2CHOP$), H–C(4′)); 3.91 – 3.85 (*m*, 1 H–C(5′)); 3.88 – 3.81 (*m*, 1 H–C(5′)); 2.26 (*t*, *J* = 7, 2 CH_2CO); 1.53 – 1.47 (*m*, 2 CH_2CH_2CO); 1.28 – 1.23 (*m*, 2 $Me(CH_2)_{10}$); 0.85 (*t*, *J* = 7, 2 $Me(CH_2)_{12}$). ^{31}P-NMR ((D_6)DMSO): −0.73. Anal. calc. for $C_{39}H_{70}N_4NaO_{12}P \cdot 2\ H_2O$ (877.0): C 53.41, H 8.50, N 6.39; found: C 53.23, H 8.33, N 6.22.

Enzymatic Hydrolysis of **19** – **22** *by PLA*$_2$. To 1.2 μmol of *Ribavirin*–lipid conjugate, **19** – **22**, 10 mM sodium deoxycholate (0.36 ml) and 0.05M *Tris* · HCl buffer (pH 8.0, 1 mM Ca^{2+}; 1.64 ml) were added. The mixture was sonicated in *Elmasonic S 10 H* untrasonic bath (4 × 10 min) until obtaining clear one-phase dispersion. The reaction was started at 37° by addition of PLA_2 soln. containing 5.8 μg of the protein. The probes of the reaction mixture (0.25 ml) were collected in fixed periods of time (*t*) and the hydrolysis was stopped by the addition of 10 mM EDTA (0.62 ml). Then, the probes were extracted by vortexing with $CHCl_3$/MeOH 2 : 1 (2 × 0.5 ml), centrifuged at 1800 rpm for 15 min, the lower layers separated and evaporated to dryness, the residues were dissolved in $CHCl_3$/MeOH 2 : 1 (60 μl) and applied on TCL plate, which was further developed with $CHCl_3$/MeOH 2 : 1. After subsequent workup in accordance with the method of *Vaskovsky et. al.* [42], the content of the phospholipid derivatives in each probe was determined by the measurement of the absorbance (*D*) at 820 – 830 nm. The values of V_0 and $\tau_{1/2}$ for each of **19** – **22** were calculated from the corresponding *D*–*t* plot. The data presented are the average of at least two experiments.

REFERENCES

[1] E. De Clercq, *J. Clin. Virol.* **2004**, *30*, 115.
[2] T. Robak, A. Korycka, E. Lech-Maranda, P. Robak, *Molecules* **2009**, *14*, 1183.
[3] R. J. Jones, N. Bischofberger, *Antiviral Res.* **1995**, *27*, 1.
[4] D. M. Lambert, *Eur. J. Pharm. Sci.* **2000**, *11*, S15.
[5] H. Sigmund, W. Pfleiderer, *Helv. Chim. Acta* **1996**, *79*, 426.
[6] E. L. Vodovozova, Yu. B. Pavlova, M. A. Polushkina, A. A. Rzhaninova, M. M. Garaev, Jul. G. Molotkovsky, *Russian J. Bioorg. Chem.* **1996**, *22*, 451.
[7] D. V. Lonshakov, E. O. Baranova, A. I. Lyutik, N. S. Shastina, V. I. Shvets, *Pharm. Chem. J.* **2011**, *44*, 557.
[8] T. Mavromoustakos, T. Calogeropoupou, M. Koufaki, A. Kolocouris, I. Daliani, C. Demetzos, Z. Meng, A. Makriyannis, J. Balzarini, E. De Clercq, *J. Med. Chem.* **2001**, *44*, 1702.

[9] S. I. Malekin, Yu. L. Kruglyak, N. Yu. Khromova, M. Yu. Aksenova, V. P. Sokolov, A. V. Kisin, T. I. Novozhilova, S. G. Popova, V. K. Kurochkin, G. G. Miller, L. N. Pokidysheva, *Russian J. Bioorg. Chem*. **1997**, *23*, 648.
[10] T. Matsushita, E. K. Ryu, C. I. Hong, M. MacCoss, *Cancer Res.* **1981**, *41*, 2707.
[11] C. I. Hong, A. J. Kirisits, A. Nechaev, D. J. Buchheit, C. R. West, *J. Med. Chem*. **1990**, *33*, 1380.
[12] R. L. Alexander, S. L. Morris-Natschke, K. S. Ishaq, R. A. Fleming, G. L. Kucera, *J. Med. Chem*. **2003**, *46*, 4205.
[13] H. Brachwitz, J. Bergmann, Y. Thomas, T. Wollny, P. Langen, *Bioorg. Med. Chem*. **1999**, *7*, 1195.
[14] S. M. Ali, A. R. Khan, M. U. Ahmad, P. Chen, S. Sheikh, I. Ahmad, *Bioorg. Med. Chem. Lett*. **2005**, *15*, 2571.
[15] R. W. Sidwell, J. H. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, R. K. Robins, *Science* **1972**, *177*, 705.
[16] M. W. Fried, M. L. Shiffman, K. R. Reddy, C. Smith, G. Marinos, F. L. Gonçales Jr., D. Häussinger, M. Diago, G. Carosi, D. Dhumeaux, A. Craxi, A. Lin, J. Hoffman, J. Yu, *N. Engl. J. Med*. **2002**, *347*, 975.
[17] J. D. Flynn, W. S. Akers, M. Jones, N. Stevkovic, T. Waid, T. Mullett, S. Jahania, *Pharmacotherapy* **2004**, *24*, 932.
[18] A. R. Glanville, A. I. R. Scott, J. M. Morton, C. L. Aboyoun, M. L. Plit, I. W. Carter, M. A. Malouf, *J. Heart Lung Transplant*. **2005**, *24*, 2114.
[19] J. B. McCormick, I. J. King, P. A. Webb, C. L. Scribner, R. B. Craven, K. M. Johnson, L. H. Elliott, R. Belmont-Williams, *N. Eng. J. Med*. **1986**, *314*, 20.
[20] J. W. Huggins, C. M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Zh. O. Wu, J. W. LeDuc, Zh. M. Zheng, J. M. Meegan, Q. N. Wang, D. D. Oland, X. E. Gui, P. H. Gibbs, G. H. Yuan, T. M. Zhang, *J. Infect. Dis.* **1991**, *164*, 1119
[21] I. Milicevic, S. Pecovic, S. Subasic, M. Mostarica-Stojkovic, S. Stosic-Grujicic, L. Medic-Mijcevic, V. Pejanovic, L. Rakic, M. Stojiljkovic, *J. Neurosci. Res.* **2003**, *72*, 268.
[22] I. Lavrnja, D. Stojkov, I. Bjelobaba, S. Pecovic, S. Dacic, N. Nedeljkovic, M. Mostarica-Stojkovic, S. Stosic-Grujicic, L. Rakic, M. Stojiljkovic, *Int. Immunopharmacol*. **2008**, *8*, 1282.
[23] F. Poordad, E. Lawitz, M. L. Shiffman, T. Hassanein, A. J. Muir, B. R. Bacon, J. Heise, D. Halliman, E. Chun, J. Hammond, *Hepatology* **2010**, *52*, 1208.
[24] G. Di Stefano, F. P. Colonna, A. Bongini, C. Busi, A. Mattioli, L. Fiume, *Biochem. Pharmacol*. **1997**, *54*, 357.
[25] G. A. Levy, G. Adamson, M. J. Phillips, L. A. Scrocchi, L. Fung, P. Biessels, N. F. Ng, A. Ghaneker, A. Rowe, M. X. Ma, A. Levy, C. Koscik, W. He, R. Gorczynski, S. Brookes, C. Woods, I. D. McGilvray, D. Bell, *Hepatology* **2006**, *43*, 581.
[26] M. Kende, C. R. Alving, W. L. Rill, G. M. Swartz Jr., P. G. Canonico, *Antimicrob. Agents Chemother*. **1985**, *27*, 903.
[27] F. Hashim, M. El-Ridy, M. Nasr, Y. Abdallah, *Drug Delivery* **2010**, *17*, 282.
[28] W. C. Koff, R. D. Pratt, J. L. Elm, C. N. Venkateshan, S. B. Halstead, *Antimicrob. Agents Chemother*. **1983**, *24*, 134.
[29] E. L. Stephen, J. S. Walker, J. W. Dominik, H. W. Young, R. F. Berendt, *Ann. N.Y. Acad. Sci*. **1977**, *284*, 264.
[30] K. Y. Hostetler, B. E. Korba, C. N. Sridhar, M. F. Gardner, *Antiviral Res.* **1994**, *24*, 59.
[31] B. E. Korba, H. Xie, K. N. Wright, W. E. Hornbuckle, J. L. Gerin, B. C. Tennant, K. Y. Hostetler, *Hepatology* **1996**, *23*, 958.
[32] I. Lindh, J. Stawinski, *J. Org. Chem*. **1989**, *54*, 1338.
[33] S. Khiati, N. Pierre, S. Andriamanarivo, M. W. Grinstaff, N. Arazam, F. Nallet, L. Navailles, P. Barthelemy, *Bioconjugate Chem*. **2009**, *20*, 1765.
[34] S. Shuto, S. Ueda, S. Imamura, K. Fukukawa, A. Matsuda, T. Ueda, *Tetrahedron Lett*. **1987**, *28*, 199.
[35] H. Eibl, *Chem. Phys. Lipids* **1981**, *28*, 1.
[36] R. J. Howe, T. Malkin, *J. Chem. Soc*. **1951**, 2663.
[37] A. Crossley, I. P. Freeman, B. J. F. Hudson, J. H. Pierce, *J. Chem. Soc*. **1959**, 760.
[38] N. D. Sinha, J. Biernat, J. McManus, H. Köster, *Nucl. Acids Res.* **1984**, *12*, 4539.

[39] A. M. Yurkevich, I. I. Kolodkina, L. S. Varshavskaya, V. I. Borodulina-Shvetz, I. P. Rudakova, N. A. Preobrazhenski, *Tetrahedron* **1969**, *25*, 477.
[40] A. V. Lebedev, A. I. Rezvukhin, *Nucleic Acids Res.* **1984**, *12*, 5547.
[41] K. Y. Hostetler, D. A. Carson, D. D. Richman, *J. Biol. Chem.* **1991**, *266*, 11714.
[42] V. E. Vaskovsky, E. V. Kostevsky, L. N. Vasendin, *J. Chromatogr., A* **1975**, *114*, 129.
[43] G. H. De Haas, L. L. Van Deenen, *Biochim. Biophys. Acta* **1964**, *84*, 469.
[44] J. Mansfeld, W. Brandt, R. Haftendorn, R. Schöps, R. Ulbrich-Hofmann, *Chem. Phys. Lipids* **2011**, *164*, 196.

Received April 19, 2012