Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2022, **32**, 433–435

Mendeleev Communications

Oligoglycol carbonate prodrugs of 5-modified 2′-deoxyuridines: synthesis and antibacterial activity

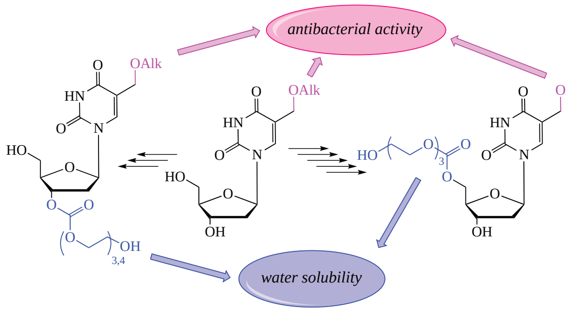
Sergey D. Negrya,[a] Maxim V. Jasko,[a] Dmitriy A. Makarov,[a] Inna L. Karpenko,[a] Pavel N. Solyev,[a] Vladimir O. Chekhov,[a] Olga V. Efremenkova,[b] Byasilya F. Vasilieva,[b] Tatiana A. Efimenko,[b] Sergey N. Kochetkov[a] and Liudmila A. Alexandrova*[a]

[a] *V. A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russian Federation. E-mail: ala2004_07@mail.ru*

[b] *G. F. Gause Institute of New Antibiotics, 119021 Moscow, Russian Federation*

DOI: 10.1016/j.mencom.2022.07.002

In order to develop a new generation of antibacterial nucleosides, a representative set of novel 3′- and 5′-tri- or tetraethylene glycol prodrug forms of 5-alkyloxymethyl-2′-deoxyuridines was synthesized. These compounds were at least two orders of magnitude more soluble than the parent nucleosides, possessed significant inhibitory activity against a set of bacteria including resistant strains of *Staphylococcus aureus* and *Mycobacterium smegmatis*, and showed low cytotoxicity. The obtained data indicate that glycol carbonates are convenient and prospective for usage in prodrugs of nucleoside derivatives with antibacterial activity.



Keywords: modified nucleosides, glycol carbonates, prodrugs, solubility, hydrolysis, antibacterial activity, cytotoxicity, bioavailability.

Nucleoside analogues are promising in the development of antimicrobial agents. Among the modified nucleosides, the C-5 modified 2′-deoxyuridine derivatives possess significant antitubercular activity.[1–6] However, their low solubility in water is a serious problem, which impedes investigations of their activity against bacterial strains. The most common method of modulating the needed pharmacological parameters is the prodrug approach which involves synthesis of a modified compound containing the parental drug that undergoes some *in vivo* biotransformation releasing the active molecule.[7–13]

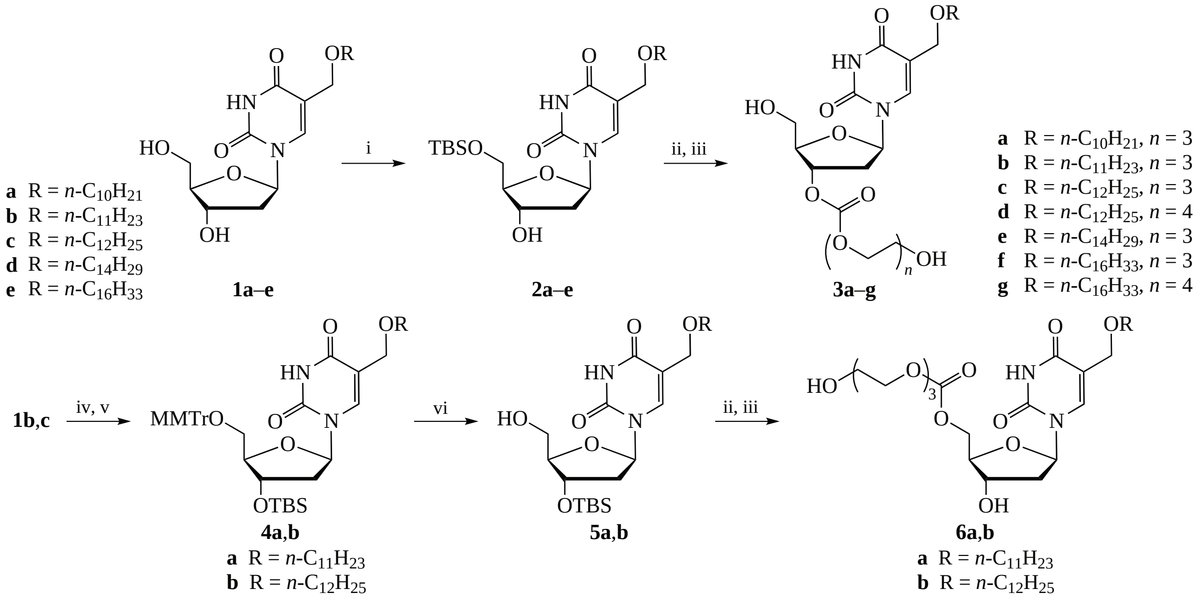
Previously, we obtained glycol-containing prodrugs of 5-alkyltriazolylmethyl-2′-deoxyuridines[14] that were at least two orders of magnitude more soluble in water and possessed significant inhibitory activity against Gram-positive bacteria, which were not inhibited by the parent compounds. Encouraged by these results, we transferred this approach to another series of antibacterial 2′-deoxyuridines. Herein, we report the synthesis of novel 3′- and 5′-tri- or tetraethylene glycol forms of 5-alkyloxymethyl-2′-deoxyuridines and the study of their water solubility, stability, cytotoxicity and antibacterial activity.

The starting 5-modified 2′-deoxyuridines **1a**–**e** (Scheme 1) were synthesized as described previously.[5] For the 3′-modified nucleoside derivatives **3a**–**g** we proposed the following synthetic sequence.[14] The first step was the selective protection of the 5′-hydroxy group with the (*tert*-butyl)dimethylsilyl (TBS) one. Further condensation of the 3′-hydroxyl group of compounds **2a**–**e** with *N*,*N*′-carbonyldiimidazole (CDI) was carried out followed by the reaction with tri- or tetraethylene glycol. Final deprotection afforded the products **3a**–**g** with an overall yield of 34–49% (for synthetic details, see Online Supplementary Materials).

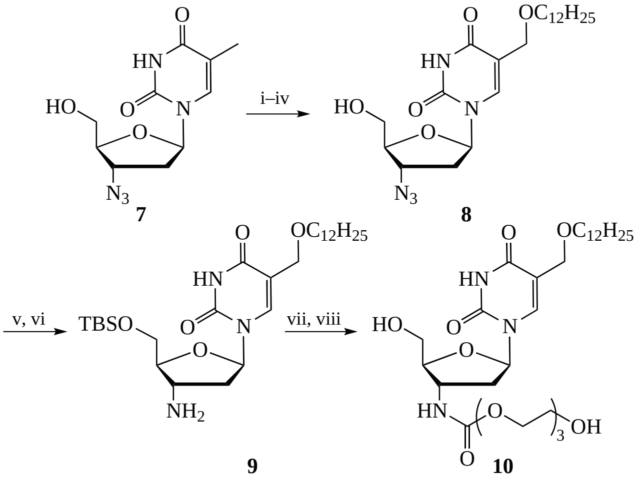
Previously,[14,15] it was shown that nucleoside prodrugs containing a 5′-positioned glycol fragment do not significantly differ in antibacterial activity or water solubility from the corresponding 3′-derivatives, however, their synthesis is more complex compared to their 3′-analogues. Nevertheless, it seemed rational to compare the impact of the glycol fragment position on chemical and biological properties of the most promising compounds. To synthesize 5′-triethylene glycol derivatives **6a**,**b**, we used a combination of protecting groups (see Scheme 1). First, a monomethoxytrityl (MMTr) group was introduced at the 5′-position of the nucleoside. Then the 3′-hydroxy group was TBS-protected, and the double-protected nucleosides **4a**,**b** were treated by 80% AcOH to liberate 5′-hydroxy group. The obtained compounds **5a**,**b** were converted into 5′-triethylene glycol derivatives **6a**,**b** with overall yields of 40–43%.

In order to prove our concept that hydrolysis of the carbonate moiety and release of the glycol-free 5-modified nucleoside is essential for antibacterial activity exhibition, we obtained a poorly hydrolysable derivative **10** which structure included carbamate linker that can possibly hamper the esterase action. The synthesis was performed from 3′-azido-3′-deoxythymidine **7** (Scheme 2). Its 5-dodecyloxymethyl derivative **8** was obtained by the method described before.[5] Then the 5′-hydroxy group was TBS-protected, and the 3′-azido group was reduced with dithiothreitol. The obtained amine **9** was subsequently treated with CDI, triethylene glycol and $Bu_4NF \cdot 3H_2O$ giving the desired product **10**.

To check the relationship between antibacterial activity and hydrolytic stability of the prodrugs, we also obtained 5-dodecyloxymethyl-2′-deoxyuridine bearing two triethylene glycol moieties **11**, similarly to the method described previously[16] (Scheme 3).

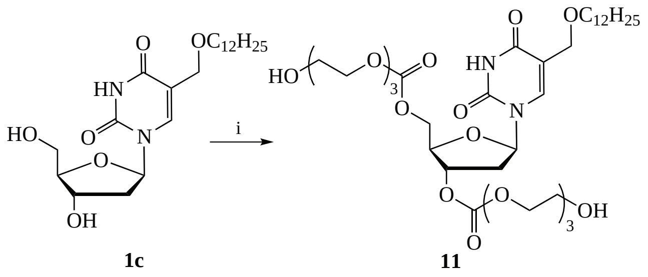


Scheme 1 *Reagents and conditions*: i, TBSCl, Py, 25 °C, 16 h; ii, 1) CDI, DMF, 37 °C, 24 h, 2) triethylene glycol (for **3a**–**c**,**e**,**f**, **6a**,**b**) or tetraethylene glycol (for **3d**,**g**), dioxane, 37 °C, 24 h; iii, $Bu_4NF{\cdot}3H_2O$, dioxane, 25 °C, 8 h; iv, MMTrCl, Py, 37 °C, 18 h; v, TBSCl, imidazole, DMF, 25 °C, 8 h; vi, AcOH, H_2O, 37 °C, 25 min.



Scheme 2 *Reagents and conditions*: i, Ac_2O, Py, 25 °C, 16 h; ii, NBS, AIBN, DCE, reflux, 3 h; iii, n-$C_{12}H_{25}OH$, DMF, 37 °C, 24 h; iv, NH_3 (aq.), EtOH, 25 °C, 16 h; v, TBSCl, Py, 25 °C, 16 h; vi, dithiothreitol, NH_3 (aq.), EtOH, 25 °C, 18 h; vii, 1) CDI, DMF, 37 °C, 24 h, 2) triethylene glycol, dioxane, 37 °C, 24 h; viii, $Bu_4NF{\cdot}3H_2O$, dioxane, 25 °C, 24 h.

This work broadens previous water solubility studies[14,15] with more lipophilic derivatives **1d**,**e** and their glycol forms **3e**–**g** as well as amide-containing deoxyuridine **10** and bis-*O*-carbonyltriethylene glycol derivative **11**. Confirming the previous data, the presence of tri- or tetraethylene glycol group leads to more than two orders of magnitude rise in solubility (0.15–4.46 mg ml^{-1}) compared to the parent compounds (0.001–0.028 mg ml^{-1}, see Table S1 in Online Supplementary Materials). Tetraethylene glycol group allows increasing solubility approximately 1.2–2 times greater in comparison with triethylene glycol group. However, the isolation of the intermediates with longer glycol chain was more difficult. The 5′-triethylene glycol analogues **6a**,**b** appeared to be slightly less soluble than the corresponding 3′-analogues **3b**,**c**. Insertion of an additional triethylene glycol group as in compound **11** leads to at least 5-fold increase in water solubility compared to the monoderivatives.



Scheme 3 *Reagents and conditions*: i, 1) CDI, DMF, 37 °C, 24 h, 2) triethylene glycol, dioxane, 37 °C, 24 h.

The hydrolysis time of the obtained tri- and tetraethylene glycol prodrugs of 5-alkyloxymethyl-2′-deoxyuridines in human blood serum was estimated using a method described previously.[15] Most of the obtained glycol derivatives showed optimal[17] hydrolysis half-life time from 3 to 12 h (see Table S2 in Online Supplementary Materials) giving the only parent nucleosides with the exception of poorly hydrolysable 3′,5′-disubstituted *O*-carbonyltriethylene glycol derivative **11** and amide-containing nucleoside **10**. All the derivatives were slightly hydrolyzed in the buffer solution at pH 9 after 24 h and were completely stable at pH 2.2 and 7.0. In addition, the tested compound **3c** was efficiently hydrolyzed by carboxylesterase, which indicates the enzymatic nature of the hydrolysis. The hydrolysis time increases with the length of glycol or alkyl chain, as well as with incorporation of two glycol substituents. During incubation of compounds **3c** and **6b** in a medium containing *S. aureus*, the only parental nucleoside **1c** was formed with a 30% conversion.

The antibacterial effect of the obtained compounds (Table 1) was studied based on their ability to inhibit *in vitro* growth of a number of microorganisms according to the previously reported procedures.[15] The glycol-containing nucleosides effectively inhibited the growth of Gram-positive bacteria but did not affect Gram-negative bacteria, baking yeast and fungi. The most active compounds appeared to be nucleosides with dodecyl fragment at the uracil residue. It should be noted that their effect on different strains of microorganisms is alike but not the same: the 5′-derivative **6b** is two times more effective in inhibiting *S. aureus* and *M. smegmatis* growth than the 3′-derivative **3c**. At the same time, replacement of the triethylene glycol residue in the position C-5′ with tetraethylene glycol leads to a decrease in inhibitory activity, namely, **3c** is, in most cases, 1.5 times more active than **3d**. As expected, poorly hydrolysable amide **10** and

Table 1 Minimal inhibitory concentrations (μM) of the synthesized compounds.

Compound/Strain	*Leuconostoc mesenteroides* VKPM B-4177	*Mycobacterium smegmatis* mc^2 155	*Mycobacterium smegmatis* VKPM Ac 1339	*Staphylococcus aureus* FDA 209P (MSSA)	*Staphylococcus aureus* INA 00761 (MRSA)	*Micrococcus luteus* NCTC 8340
3b	>340	>340	42	>340	>340	>340
3c	166	41.5	41.5	83	83	83
3d	>310	77	>77	77	155	>310
3e	79	63	79	79	79	40
3a,f,g, 10, 11	>700	>700	>700	>700	>700	>700
6a	>680	>680	42	>680	>680	>680
6b	21	21	21	42	42	21
Antibiotics used in clinics[a]	VA > 275, CIP 6	AN 50, CIP 12, RF 10, INZ 2	AN 50, CIP 12, RF 5, INZ 3	AN 3, CIP 12, OX 3	AN 50, CIP 12, OX 80	AN 2

[a] Amikacin (AN), Ciprofloxacin (CIP), Isoniazid (INZ), Rifampicin (RF), Oxacillin (OX), Vancomycin (VA).

bis-carbonate derivative **11** did not exhibit antibacterial activity. The obtained data allows us to suggest that the inhibitory activity of 2′-deoxyuridine analogues depends on the substitute at the C-5 position of the nucleobase and alters respectively: $C_{10}H_{21}$, $C_{16}H_{33}$ (**3a**,**f**,**g**, not active) $\ll$ $C_{11}H_{23}$ (**3b**, **6a**) $<$ $C_{14}H_{29}$ (**3e**) $<$ $<$ $C_{12}H_{25}$ (**3c**,**d**, **6b**).

The cytotoxicity of the synthesized compounds was evaluated using the MTT test[18] on Jurkat and A549 cell lines. For the obtained glycol derivatives CD_{50} value was equal to or more than 100 μg ml^{-1} (128–174 μM).

In conclusion, new tri- and tetraethylene glycol prodrugs of 5-alkyloxymethyl-2′-deoxyuridines demonstrated antibacterial activity against several Gram-positive bacteria strains. The presence of glycol group was shown to significantly increase water solubility of the prodrugs. Most of the obtained derivatives were hydrolyzed in human blood serum with optimal hydrolysis half-life time and were stable in buffer solutions, which indicates the enzymatic nature of the hydrolysis. The most active compounds were the triethylene glycol derivatives of 5-dodecyloxymethyl-2′-deoxyuridine **3c** and **6b** which exhibited activity against a range of Gram-positive bacteria including resistant strains of *S. aureus* and *M. smegmatis* and were low cytotoxic for human cell lines. Our results are consistent with those of recent studies.[19–21]

This work was supported by the Russian Foundation for Basic Research (grants nos. 18-29-08010 and 20-04-00094). The authors are grateful to Dr. R. A. Novikov (Engelhardt Institute of Molecular Biology RAS) for NMR investigations. S.D.N. and M.V.J. contributed equally to this work.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.07.002.

References

1 M. Serpi, V. Ferrari and F. Pertusati, *J. Med. Chem*., 2016, **59**, 10343.

2 M. Johar, T. Manning, D. Y. Kunimoto and R. Kumar, *Bioorg. Med. Chem*., 2005, **13**, 6663.

3 D. Rai, M. Johar, T. Manning, B. Agrawal, D. Y. Kunimoto and R. Kumar, *J. Med. Chem*., 2005, **48**, 7012.

4 E. R. Shmalenyuk, S. N. Kochetkov and L. A. Alexandrova, *Russ. Chem. Rev*., 2013, **82**, 896.

5 E. R. Shmalenyuk, L. N. Chernousova, I. L. Karpenko, S. N. Kochetkov, T. G. Smirnova, S. N. Andreevskaya, O. V. Efremenkova and L. A. Alexandrova, *Bioorg. Med. Chem*., 2013, **21**, 4874.

6 E. R. Shmalenyuk, I. L. Karpenko, L. N. Chernousova, A. O. Chizhov, T. G. Smirnova, S. N. Andreevskaya and L. A. Alexandrova, *Russ. Chem. Bull.*, 2014, **63**, 119.

7 B. M. A. Sanches and E. I. Ferreira, *Int. J. Pharm*., 2019, **568**, 118498.

8 D. H. Jornada, G. F. Dos Santos Fernandes, D. E. Chiba, T. R. F. De Melo, J. L. Dos Santos and M. C. Chung, *Molecules*, 2016, **21**, 42.

9 K. Huttunen, H. Raunio and J. Rautio, *Pharmacol. Rev.*, 2011, **63**, 750.

10 J. Rautio, H. Kumpulainen, T. Heimbach, R. Oliyai, D. Oh, T. Järvinen and J. Savolainen, *J. Nat. Rev. Drug Discov*., 2008, **7**, 255.

11 A. Khandazhinskaya, M. Yasko and E. Shirokova, *Curr. Med. Chem*., 2006, **13**, 2953.

12 V. J. Stella and K. W. Nti-Addae, *Adv. Drug Deliv. Rev*., 2007, **59**, 677.

13 G. Mori, L. R. Chiarelli, G. Riccardi and M. R. Pasca, *Drug Discov. Today*, 2017, **22**, 519.

14 S. D. Negrya, M. V. Jasko, P. N. Solyev, I. L. Karpenko, O. V. Efremenkova, B. F. Vasilyeva, I. G. Sumarukova, S. N. Kochetkov and L. A. Alexandrova, *J. Antibiot.*, 2020, **73**, 236.

15 S. D. Negrya, M. V. Jasko, D. A. Makarov, P. N. Solyev, I. L. Karpenko, O. V. Shevchenko, V. O. Chekhov, A. A. Glukhova, B. F. Vasilyeva, T. A. Efimenko, I. G. Sumarukova, O. V. Efremenkova, S. N. Kochetkov and L. A. Alexandrova, *Mol. Biol.*, 2021, **55**, 143 (*Molekulyar. Biologiya*, 2021, **55**, 164).

16 L. Alexandrova, S. Zicari, E. Matyugina, A. Khandazhinskaya, T. Smirnova, S. Andreevskaya, L. Chernousova, C. Vanpouille, S. Kochetkov and L. Margolis, *Antiviral Res*., 2017, **145**, 175.

17 E. De Clercq, *J. Clin. Virol*., 2001, **22**, 73.

18 M. Niks and M. Otto, *J. Immunol. Methods*, 1990, **130**, 149.

19 E. S. Matyugina, V. T. Valuev-Elliston, A. O. Chizhov, S. N. Kochetkov and A. L. Khandazhinskaya, *Mendeleev Commun*., 2016, **26**, 114.

20 M. S. Gaman, E. S. Matyugina, P. N. Solyev, S. N. Kochetkov, A. L. Khandazhinskaya, M. S. Novikov and D. A. Babkov, *Mendeleev Commun*., 2017, **27**, 346.

21 M. P. Paramonova, A. A. Ozerov, A. O. Chizhov, R. Snoeck, G. Andrei, A. L. Khandazhinskaya and M. S. Novikov, *Mendeleev Commun*., 2019, **29**, 638.

Received: 29th December 2021; Com. 21/6781