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5-Alkyloxymethyl Derivatives of 2′-Deoxyuridine Bearing 2,4-Dinitrophenyl and Dansyl Groups: Synthesis and Antibacterial Activity

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Abstract—Condensation of 3′,5′-di-*O*-acetyl-5-bromomethyl-2′-deoxyuridine with 6-trifluoroacetylaminohexan-1-ol yielded 5-(6-trifluoroacetylaminohexyl-1-oxymethyl)-3′,5′-di- *O*-acetyl-2′-deoxyuridine. Its deblocking with an aqueous alcoholic solution of ammonia gave 5-(6-aminohexyl-1-oxymethyl)-2′-deoxyuridine, and condensation with triazole and 2-chlorophenyl phosphorodichloridate followed by treatment with an aqueous solution of ammonia led to the formation of 5-(6-aminohexyl-1-oxymethyl)-2′-deoxycytidine. The interaction of the obtained compounds with 2,4-dinitrofluorobenzene or *N*-hydroxysuccinimide esters of *N*-2,4-dinitrophenylaminohexanoic acid or 5-dimethylaminonaphthalene-1-sulfonyl glycine was used to synthesize DNP- and DNS-derivatives of 2′-deoxyuridine as well as 5-(6-DNP-aminohexanoylaminohexyl-1-oxymethyl)-2′-deoxycytidine. DNP derivatives of 2′-deoxyuridine were shown to inhibit the growth of *Micrococcus luteus*.

Keywords: nucleosides, condensation, *N*-bromosuccinimide, azobisisobutyronitrile, *N*-hydroxysuccinimide, dinitrophenyl, 5-dimethylaminonaphthalene-1-sulfonyl groups, antibacterial activity

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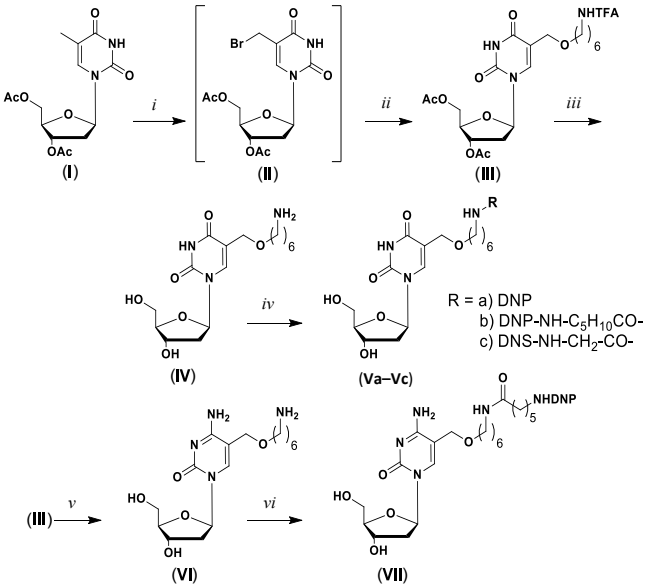
INTRODUCTION

The development of entirely new antibacterial drugs that act on new pathogen targets and are active against resistant strains of microorganisms is currently of great interest. At the beginning of the 21st century, the first reports have appeared on the synthesis of several groups of modified nucleosides that demonstrated antibacterial (primarily antitubercular) activity *in vitro* [1–6], which opens up prospects for the creation of drugs based on them.

The mechanism of the antibacterial action of modified pyrimidine 2′-deoxynucleosides containing extended alkyl substituents in the 5th position of the base [2, 3, 6–8] has not yet been established. On the one hand, it has been shown that their 5′-monophosphates effectively inhibit a unique enzyme—flavin-dependent thymidylate synthase ThyX of *Mycobacterium tuberculosis*, which catalyzes the biosynthesis of the thymidine 5′-monophosphate and is absent in most bacteria and eukaryotes. At the same time, the main thymidylate synthase of *M. tuberculosis,* ThyA, remains virtually unaffected [9–12]. On the other hand, we have demonstrated that incapable of phosphorylation 5′-iodo-, azido- and amino derivatives of 5-dodecyloxymethyl-2′-deoxyuridine [8], as well as carbocyclic d4-5′-nor-5-(1-alkinyl)uridines with extended 1-alkinyl [13], alkyloxymethyl, and alkyltriazolylmethyl [14] substituents possess a high inhibitory activity against *M. tuberculosis in vitro*. This activity is most likely related to the destruction of the cell wall of mycobacteria [14, 15].

To develop a convenient method of introduction of tags in 5′-modified pyrimidine 2′-deoxynucleosides, needed to further elucidate their cellular localization,

Abbreviations: DNP, dinitrophenyl; DNS, 5-dimethylaminonaphthalene-1-sulfonyl; NBS, *N*-bromosuccinimide; AIBN, azobisisobutyronitrile; TFAc, trifluoroacetyl; OSu, *N*-hydroxysuccinimide ester.



Scheme 1. Reagents and conditions: *i*, *N*-bromosuccinimide, azobisisobutyronitrile, dichloroethane, Δ, 3 h; *ii*, TFAcNH-$(CH_2)_6$-OH, DIEA, DMF, 37°C, 20 h; *iii*, NH_3 (aq), ethanol, 25°C, 20 h; *iv*, DNP-F, DIEA, DMF, 20 h, 20°C (for (**Va**)); DNP-NH-$(CH_2)_5$-CO(O)-NOSu, *N*-MeIm, DMF, 20 h, 20°C (for (**Vb**)); DNS-NH-CH_2-CO(O)-NOSu, *N*-MeIm, DMF, 20 h, 20°C (for (**Vc**)); *v*, 1) 1,2,4-triazole, 2-chlorophenyldichlorophosphate, pyridine, 20°C, 20 h; 2) aq 34% NH_3, dioxane, 20°C, 20 h; *vi*, DNP-NH-$(CH_2)_5$-CO(O)-NOSu, *N*-MeIm, DMF, 20 h, 20°C.

we have synthesized 5-(6-aminohexyl-1-oxymethyl)-2′-deoxyuridine and -cytidine that allow to obtain tagged compounds in one step. In this article, we describe the synthesis of 2,4-dinitrophenyl (DNP) and 5-dimethylaminonaphthalene-1-sylfonyl (dansyl, DNS) derivatives of 5-modified pyrimidine 2′-deoxynucleosides and show the data on their action against a range of microorganisms.

RESULTS AND DISCUSSION

Chemical Synthesis

The starting compound for the synthesis of compounds (**Va–Vc** and **VII**) (Scheme 1) was 3′,5′-di-*O*-acetyl-5-bromomethyl-2′-deoxyuridine (**II**) [16] that was obtained by radical bromination according to the method described in [17, 18] and adapted to the stereoselective bromination of 3′,5′-di-*O*-acetyl thymidine [19]. The nucleophilic substitution of bromine in (**II**) to 6-trifluoroacetylaminohexan-1-ol, according to a simple and rational method [20, 21] developed by us earlier, allowed obtaining 3′,5′-di-*O*-acetyl-5-(6-trifluoroacetylaminohexyl-1-oxymethyl)-2′-deoxyuridine (**III**). Further deblocking of protective groups with an aqueous alcoholic solution of ammonia and purification on a column with silica gel have led to the obtaining of the derivative (**IV**) with the yield of 69%. The interaction of the latter with 2,4-dinitrofluorobenzene or with *N*-hydroxysuccininide esters of *N*-2,4-dinitrophenylaminohexanoic acid or 5-dimethylaminonaphthalene-1-sulfonyl glycine in the presence of *N*-methylimidazole has caused the synthesis of 2′-deoxyuridine derivatives (**Va–Vc**) that contain a dinitrophenyl (DNP) or a dansyl (DNS) group.

To synthesize the derivatives of 2′-deoxycytidine, we have obtained 5-(6-aminohexyl-1-oxymethyl)-2′-deoxycytidine (**VI**) according to the method by Divakar and Reese [22] in its more recent modification [23]. Thus, condensation of the compound (**III**) with 2-chlorophenyldichlorophosphate and 1,2,4-triazole, followed by treatment with an aqueous solution of ammonia resulted in the synthesis of the compound (**VI**) with the yield of 54%. Condensation of the latter with the *N*-hydroxysuccininide ester of *N*-2,4-dinitrophenylaminohexanoic acid in dioxane in the presence of *N*-methylimidazole allowed obtaining 5-(6-DNP-aminohexanoylaminohexyl-1-oxymethyl)-2′-deoxycytidine (**VII**).

The structure of all synthesized compounds was confirmed by UV, ^{1}H, and ^{13}C NMR-spectroscopy, as well as by high-resolution mass spectrometry.

Study of the Antibacterial Activity of Obtained Compounds

Antibacterial activity of obtained compounds was studied by their ability to inhibit the growth of a range of microorganisms *in vitro*, including seven Gram-positive and two Gram-negative bacteria [24]. The list of microorganisms is presented in the Experimental.

Due to the low solubility of the studied compounds in water, they have been dissolved in a solvent system methanol-water (3 : 7, v/v). The addition of 10% of the used solvent system to the incubation mixture did not have a toxic effect on the cells of the test strains of microorganisms. The obtained compounds did not inhibit the growth of most microorganisms at maximum concentrations (0.2–0.65 mM), with the exception of (**Va**) and (**Vb**) that inhibited the growth of *Micrococcus luteus* in the concentrations of 0.35 and 0.65 mM, respectively.

EXPERIMENTAL

In this work, commercial reagents supplied by Fluka (Germany), Sigma-Aldrich (USA), and Acros Organics (USA) were used. The solvents were purified according to the standard methods. Column chromatography was conducted using silica gel Kieselgel 60 (40–63 μM) (Merck, Germany). NMR spectra (δ, ppm, SSCS, Hz) were registered in DMSO-d_6 and D_2O using the Avance III spectrometer (Bruker, USA) with the working frequency of 300 MHz for ^{1}H NMR (internal standard—Me_4Si), 75 MHz for ^{13}C NMR (internal standard—Me_4Si). TLC was conducted using Merck plates in systems ethyl acetate-hexane (2 : 1) (**A**), ethyl acetate-hexane (1 : 3) (**B**), chloroform-ethanol (20 : 1) (**C**), chloroform-ethanol (9 : 1) (**D**), chloroform-ethanol (4 : 1) (**E**), chloroform-NH_3/MeOH (9 : 1). Preparative chromatography was conducted on 20 × 20 cm Merck PLC Silica gel 60 F_{254} glass plates, 1 mm, with a 20 × 4 cm concentrating zone. UV-spectra were registered on the Perkin Elmer lambda 25 spectrophotometer (Perkin Elmer, USA) in methanol. High-resolution mass spectra were registered on the Bruker Daltonics micrOTOF-Q II using the electrospray ionization (ESI) method. Measurements were performed on positive ions in accordance with previously developed conditions [24].

3′,5′-Di-*O*-acetyl-5-(6-trifluoroacetylaminohexyl)-oxymethyl-2′-deoxyuridine (III). 6-Trifluoroacetyl-aminohexan-1-ol (1.88 g, 10 mmol) and diisopropylethylamine (0.65 g, 5 mmol) were added to the DMF solution of 3′,5′-di-*O*-acetyl-5-bromomethyl-2′-deoxyuridine ((**II**), 10 mL), obtained by the method [19] from 3′,5′-di-*O*-acetyl thymidine ((**I**), 1.4 g, 4.2 mmol). After stirring at 37°C for 20 h in an argon atmosphere, the solvent was removed under vacuum, the residue was dissolved in ethyl acetate and applied to a silica gel column (3 × 15 cm). The product was eluted with a solvent system ethyl acetate-hexane (1 : 4) with subsequent change in the ratio of solvents to 2 : 1. The target fractions were concentrated. The product was dried in vacuum. Yield 1.57 g (73%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.21–1.34 (m, 4H, –$OCH_2CH_2(C\underline{H}_2)_2CH_2CH_2NHC(O)CF_3$), 1.38–1.58 (m, 4H,–$OCH_2C\underline{H}_2(CH_2)_2C\underline{H}_2CH_2NHC(O)CF_3$), 2.04–2.05 (m, 6H, $OCOC\underline{H}_3$), 2.26–2.35 (m, 2H, H-2′), 3.11–3.22 (m, 2H, –$C\underline{H}_2NHC(O)CF_3$), 3.35–3.46 (m, 2H, 5-$CH_2OC\underline{H}_2$), 4.11 (s, 2H, 5-$C\underline{H}_2OCH_2$), 4.17–4.29 (m, 3H, H-4′,5′), 5.16–5.25 (m, 1H, H-3′), 6.19 (dd, 1H, J = 8.09, 6.24 Hz, H-1′), 7.64 (s, 1H, H-6), 9.34–9.46 (m, 1H, $N\underline{H}OCOCF_3$), 11.41 (s, 1H, 3-NH).

5-(6-Aminohexyl)-oxymethyl-2′-deoxyuridine (IV). 3′,5′-Di-*O*-acetyl-5-(6-trifluoroacetylaminohexyl)-oxymethyl-2′-deoxyuridine (**III**) 1.53 g (3 mmol) was dissolved in ethanol (20 mL) with the addition of an aqueous solution of ammonia (20 mL) and was left overnight at room temperature. The obtained reaction mixture was concentrated under vacuum, the residue was dissolved in the system chloroform-ethanol (5–10 mL) and applied to a silica gel column (3 × 20 cm). The product was eluted with a solvent system chloroform-ethanol 20 : 1, then chloroform-NH_3/MeOH (9 : 1). The target fractions were dried in vacuum. Yield 0.9 g (2.5 mmol) (84%); UV: λ_{max} 262 nm (ε 9800); ^{1}H NMR (300 MHz, D_2O): δ 1.35 (m, 4H, –$OCH_2CH_2(C\underline{H}_2)_2CH_2CH_2NH_2$), 1.53–1.60 (m, 4H, –$OCH_2C\underline{H}_2(CH_2)_2C\underline{H}_2CH_2NH_2$), 2.27–2.39 (m, 2H, H-2′), 2.84–3.00 (m, 2H, –$OCH_2CH_2(CH_2)_2CH_2C\underline{H}_2NH_2$), 3.51 (t, J = 6.51 Hz, 2H, –$OC\underline{H}_2CH_2(CH_2)_2CH_2CH_2NH_2$), 3.69–3.88 (m, 2H, H-5′), 3.97–4.02 (m, 1H, H-4′), 4.11 (s, 2H, –$C\underline{H}_2OCH_2$), 4.43 (m, 1H, H-3′), 6.11 (t, J = 6.69 Hz, 1H, H-1′), 7.60 (s, 1H, H-6); ^{13}C NMR (75 MHz, D_2O): 25.31, 26.06, 28.52, 29.11 (–$OCH_2(\underline{C}H_2)_4CH_2NH_2$), 40.31 (C-2′), 42.80 (–$OCH_2CH_2(CH_2)_2CH_2\underline{C}H_2NH_2$), 61.41 (C-5′), 65.24 (–$O\underline{C}H_2CH_2(CH_2)_3CH_2NH_2$), 70.21 (C-3′), 71.10 (5-$\underline{C}H_2OCH_2$), 89.80 (C-4′), 94.50 (C-1′), 112.40 (C-5), 115.10 (C-6), 150.80 (C-2), 162.60 (C-4); MS (ESI): $[M + H]^+$ Calculated: 358.1973; Found: 358.1972.

5-[6-(2,4-Dinitrophenyl)aminohexyl]-oxymethyl-2′-deoxyuridine (Va). 2,4-Dinitrofluorobenzene (13 mg, 70 μmol) and diisopropylethylamine (9 mg, 12 μL, 70 μmol) were added to the DMF solution of 5-(6-amino-hexyl)-oxymethyl-2′-deoxyuridine ((**IV**), 21 mg, 59 μmol). After stirring at 20°C for 20 h, the solvent was evaporated, the residue was dissolved in 0.3 mL of the system **E** and applied to the preparative Merck plate, which was eluted with the system **D**. The target fraction was eluted with the system **E**, then dried in vacuum. Yield 24 mg (80%). UV: λ_{max} 272 nm (ε 9800), 363 nm (ε 17500); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.30–1.44 (m, 4H, $OCH_2CH_2(C\underline{H}_2)_2CH_2CH_2NHDNP$), 1.46-1.57 (m, 2H, $OCH_2CH_2(CH_2)_2C\underline{H}_2CH_2NHDNP$), 1.59–1.70 (m, 2H, $OCH_2C\underline{H}_2(CH_2)_2CH_2CH_2NHDNP$), 2.02–2.18 (m, 2H, H-2′), 3.39 (t, J = 6.40 Hz, 2H, 5-$CH_2OC\underline{H}_2$), 3.43–3.52 (m, 2H, –$OCH_2CH_2(CH_2)_2CH_2C\underline{H}_2NHDNP$), 3.52–3.60 (m, 2H, H-5′), 3.79 (td, J = 3.62, 3.57 Hz, 1H, H-4′), 4.08 (s, 2H, 5-$C\underline{H}_2OCH_2$), 4.20–4.28 (m, 1H, H-3′), 4.98 (t, J = 5.14 Hz, 1H, 5′-OH), 5.23 (d, J = 4.27 Hz,

1H, 3′-OH), 6.16 (dd, J = 7.29, 6.24 Hz, 1H, H-1′), 7.21 (d, J = 9.64 Hz, 1H, DNP H-6), 7.86 (s, 1H, H-6), 8.25 (ddd, J = 9.67, 2.79, 0.72 Hz, 1H, DNP H-5), 8.82 (t, J = 5.78 Hz, 1H, $CH_2N\underline{H}$DNP), 8.85 (d, J = 2.73 Hz, 1H, DNP H-3), 11.33 (s, 1H, 3-NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 25.29, 26.04, 28.01, 28.99, (–$OCH_2(\underline{C}H_2)_4CH_2$NHDNP), 39.67 (C-2′), 42.75 (–$OCH_2CH_2(CH_2)_2CH_2\underline{C}H_2$NHDNP), 61.30 (C-5′), 64.48 (–$O\underline{C}H_2CH_2(CH_2)_3CH_2$NHDNP), 69.47 (C-3′), 70.40 (–$\underline{C}H_2OCH_2$), 84.14 (C-1′), 87.40 (C-4′), 110.70 (C-5), 115.20 (DNP C-6), 123.66 (DNP C-3), 129.58, 129.95 (DNP C-2, C-5), 134.63 (DNP C-4), 138.67 (C-6), 148.13 (DNP C-1), 150.27 (C-2), 162.64 (C-4); MS (ESI): [M + H]$^+$; Calculated: 524.1987; Found: 524.1985.

General method for the synthesis of the derivatives of 2′-deoxyuridine (Vb–Vc) and 2′-deoxycytidine (VII) containing dinitrophenyl (DNP) or dansyl (DNS) groups. Two portions of the corresponding *N*-hydroxysuccinimide ester (0.2 mmol) and *N*-methylimidazole (16 mg, 0.12 mmol) were added to the solution of the nucleoside (**IV**) or (**VI**) (44 mg, 0.12 mmol) in the mixture of dioxane (2 mL) and water (0.1 mL). After stirring at 20°C for 20 h, the solvent was evaporated in vacuum, the residue was dissolved in the system **E** and applied to the preparative Merck plate. The mixture was separated using the system **D**. The target fraction was eluted in the system **E**, then dried in vacuum.

5-[6-(2,4-Dinitrophenyl)aminohexanoylaminohexyl]-oxymethyl-2′-deoxyuridine (Vb) was obtained by the interaction of the nucleoside (**IV**) and *N*-hydroxysuccinimide ester of *N*-2,4-dinitrophenylaminohexanoic acid with the yield of 48 mg (78%); UV: λ_{max} 272 nm (ε 9800), 363 nm (ε 17500); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.21–1.43 (m, 6H, $OCH_2CH_2(C\underline{H}_2)_2CH_2CH_2NHC(O)\cdot CH_2CH_2C\underline{H}_2CH_2CH_2NH$DNP), 1.44–1.72 (m, 6H, $OCH_2C\underline{H}_2(CH_2)_2C\underline{H}_2CH_2NHC(O)\cdot(CH_2)_3C\underline{H}_2CH_2NH$DNP), 2.01–2.18 (m, 4H, $O(CH_2)_6NHC(O)CH_2C\underline{H}_2CH_2CH_2CH_2NH$DNP, H-2′), 2.97–3.06 (m, 2H, $O(CH_2)_6NHC(O)C\underline{H}_2CH_2CH_2CH_2CH_2NH$DNP), 3.27–3.32 (m, 2H, 3.37, $O(CH_2)_5C\underline{H}_2NHC(O)(CH_2)_5NH$DNP), 3.34–3.41 (t, J = 6.53 Hz, 2H, 5-$CH_2OC\underline{H}_2$), 3.42–3.52 (m, 2H, $O(CH_2)_6NHC(O)CH_2CH_2CH_2CH_2C\underline{H}_2NH$DNP), 3.54–3.63 (m, 2H, H-5′), 3.79 (td, J = 3.63, 3.59 Hz, 1H, H-4′), 4.07 (s, 2H, 5-$C\underline{H}_2OCH_2$), 4.24 (m, 1H), 4.97 (t, J = 5.08 Hz, 1H, 5′-OH), 5.22 (d, J = 4.21 Hz, 1H, 3′-OH), 6.16 (t, J = 6.73 Hz, 1H, H-1′), 7.21 (dd, J = 9.65, 2.09 Hz, 1H, DNP H-6), 7.69 (t, J = 5.62 Hz, 1H, $O(CH_2)_6N\underline{H}C(O)(CH_2)_5NH$DNP), 7.86 (s, 1H, H-6), 8.24 (dd, J = 9.65, 2.70 Hz, 1H, DNP H-5), 8.77–8.83 (m, 1H, $CH_2N\underline{H}$DNP), 8.85 (d, J = 2.71 Hz, 1H, DNP H-3), 11.31 (s, 1H, 3-NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.06, 24.88, 25.85, 27.82, 28.85, 35.23, 38.29 ($OCH_2(\underline{C}H_2)_5NHC(O)(\underline{C}H_2)_4CH_2$NHDNP), 39.65 (C-2′), 42.67 ($O(CH_2)_6NHC(O)(CH_2)_4\underline{C}H_2$NHDNP), 61.29 (C-5′), 64.45 (5-$CH_2O\underline{C}H_2$), 69.53(C-3′), 70.37(5-$\underline{C}H_2OCH_2$), 84.14 (C-1′), 87.37 (C-4′), 110.67 (C-5), 115.18 (DNP C-6), 123.61 (DNP C-3), 129.57, 129.89 (DNP C-2, C-5), 134.62 (DNP C-4), 138.60 (C-6), 148.09 (DNP C-1), 150.24 (C-2), 162.59 (C-4), 171.75 ($NH\underline{C}(O)CH_2$); MS (ESI): [M + H]$^+$; Calculated: 637.2828; Found: 637.2829.

5-(6-Aminohexyl)-oxymethyl-2′-deoxycytidine (VI). 1,2,4-Triazole (200 mg, 3 mmol) and 2-chlorophenyldichlorophosphate (188 mg, 0.77 mmol) were added to a cooled to 0°C solution of 3′,5′-di-*O*-acetyl-5-(6-trifluoroacetylaminohexyl)-oxymethyl-2′-deoxyuridine ((**III**), 256 mg, 0.5 mmol) in absolute pyridine (5 mL). The mixture was kept at room temperature for 20 h, then the solvent was removed in vacuum. The residue was distributed between chloroform and 0.5 M sodium bicarbonate. The organic layer was washed with water, dried over Na_2SO_4, and concentrated. The residue was dissolved in dioxane, cooled to 0°C, then 32% aqueous solution of ammonia was added. The mixture was kept at room temperature for 40 h, then concentrated in vacuum. The derivative (**VII**) was purified on a silica gel column (2x15 cm), the product was eluted with a system chloroform-ethanol (9 : 1), then chloroform–NH_3/MeOH (9 : 1). The yield of the target product (**VII**) was 220 mg (85%). UV: λ_{max} 271 nm (ε 9110), λ_{max} (pH 2.0) 283.3 nm (ε 13400); ^{1}H NMR (300 MHz, D_2O): 1.35 (m, 4H, $-OCH_2CH_2(C\underline{H}_2)_2CH_2CH_2NH_2$), 1.53–1.60 (m, 4H, $-OCH_2C\underline{H}_2(CH_2)_2C\underline{H}_2CH_2NH_2$) 2.27–2.39 (m, 2H, H-2′), 2.84-3.00 (m, 2H, $-OCH_2CH_2(CH_2)_2CH_2C\underline{H}_2NH_2$), 3.51 (t, J = 6.51 Hz, 2H, $-OC\underline{H}_2CH_2(CH_2)_2CH_2CH_2NH_2$), 3.69–3.88 (m, 2H, H-5′), 3.97–4.02 (m, 1H, H-4′), 4.11 (s, 2H, $-C\underline{H}_2OCH_2$), 4.43 (m, 1H, H-3′), 6.11 (t, J = 6.69 Hz, 1H, H-1′), 7.60 (s, 1H, H-6); ^{13}C NMR (75 MHz, D_2O): 25.31, 26.06, 28.52, 29.11 ($-OCH_2(\underline{C}H_2)_4CH_2NH_2$), 40.3 (C-2′), 42.8 ($-OCH_2CH_2(CH_2)_2CH_2\underline{C}H_2NH_2$), 61.4 (C-5′), 65.2 ($-O\underline{C}H_2CH_2(CH_2)_3CH_2NH_2$), 70.2 (C-3′), 71.1 ($-\underline{C}H_2OCH_2$), 89.8 (C-4′), 94.5 (C-1′), 112.4 (C-5), 115.1 (C-6), 141.8 (C-2), 165.6 (C-4); MS (ESI): [M + H]$^+$; Calculated: 357.2132; Found: 357.2134.

5-{6-[6-(2,4-Dinitrophenyl)aminohexanoyl]-aminohexyl}-oxymethyl-2′-deoxycytidine (VII). The product was obtained by the interaction of the derivative (**VI**) and *N*-hydroxysuccinimide ester of *N*-2,4-dinitrophenylaminohexanoic acid with the yield of 45 mg (73%); UV: λ_{max} 272 nm (ε 9800), 362 nm (ε 17500); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24–1.38 (m, 8H, $OCH_2CH_2(C\underline{H}_2)_2CH_2CH_2NHC(O)CH_2CH_2C\underline{H}_2CH_2CH_2NHDNP$), 1.47–1.67 (m, 8H, $OCH_2C\underline{H}_2(CH_2)_2C\underline{H}_2CH_2NHC(O)(CH_2)_3C\underline{H}_2CH_2NHDNP$), 1.95–2.15 (m, 4H, $O(CH_2)_6NHC(O)CH_2C\underline{H}_2CH_2CH_2CH_2NHDNP$, H-2′), 2.97–3.04 (m, 2H, $O(CH_2)_6NHC(O)\cdot C\underline{H}_2(CH_2)_4NHDNP$), 3.33–3.39 (m, 2H, 5-$CH_2OC\underline{H}_2$), 3.42–3.51 (m, 2H, $O(CH_2)_6NHC(O)\cdot CH_2CH_2CH_2CH_2C\underline{H}_2NHDNP$), 3.53–3.65 (m, 2H, H-5′), 3.79 (t, J = 3.60 Hz, 1H, H-4′), 4.10–4.27 (m, 3H, 5-$C\underline{H}_2OCH_2$, H-3′), 5.00 (br. s, 1H, 5′-OH), 5.20 (br. s, 1H, 3′-OH), 6.14 (dd, J = 7.18, 6.00 Hz, 1H, H-1′), 7.22 (d, J = 9.70 Hz, 1H, DNP H-6), 7.72 (t, J = 5.62 Hz, 1H, $O(CH_2)_6N\underline{H}C(O)(CH_2)_5NHDNP$), 7.88 (s, 1H, H-6), 8.24 (dd, J = 9.63, 2.76 Hz, 1H, DNP H-5), 8.82 (t, J = 6.18 Hz, 1H, DNP H-3), 8.85 (d, J = 2.76 Hz, 1H, DNP H-3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.11, 24.92, 25.87, 27.84, 28.73, 28.98, 35.24 ($OCH_2(\underline{C}H_2)_5NHC(O)(\underline{C}H_2)_4CH_2NHDNP$), 38.30 (C-2′), 45.70 ($O(CH_2)_6NHC(O)(CH_2)_4\underline{C}H_2NHDNP$), 61.21(C-5′), 65.07 (5-$CH_2O\underline{C}H_2$), 68.88 (C-3′), 70.19 (5-$\underline{C}H_2OCH_2$), 84.95 (C-1′), 87.27 (C-4′), 102.44 (C-5), 115.25 (DNP C-6), 123.65 (DNP C-3), 129.57, 129.93 (DNP C-2, C-5), 134.61(DNP C-4), 140.76(C-6), 148.11(DNP C-1), 154.48 (C-2), 164.08 (C-4), 171.75 ($NH\underline{C}(O)CH_2$); MS (ESI): $[M + H]^+$; Calculated: 636.2988; Found: 636.2985.

Study of the antibacterial activity. The following test strains were used: Gram-positive bacteria *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* FDA 209P (MRSA), and methicillin-resistant *Staphylococcus aureus* INA 00761 (MRSA); *Leuconostoc mesenteroides* VKPM B-4177, *Micrococcus luteus* NCTC 8340, *Mycobacterium smegmatis* mc^2 155, and VKPM Ac 1339; Gram-negative bacteria *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. The strains were obtained from the collection of the FSBI Gause Institute of New Antibiotics.

Test strains were incubated in the modified Gause's no. 2 medium of the following composition (%): glucose —1, peptone—0.5, tryptone—0.3, NaCl—0.5, water, pH 7.2–7.4. The cell concentration used in the experiments was 10^6 cells/mL.

The studied compounds were dissolved in the solvent system water-MeOH (7 : 3). 10% (v/v) compounds were added to the medium. Double control of the growth of the test culture was conducted: without the compounds and with the addition of the mixture used for the dissolution of the compounds. *L. mesenteroides* was incubated at 28°C, while all of the other strains were incubated at 37.

CONCLUSIONS

Convenient synthons, namely 6-(6-aminohexyl)-oxymethyl-2′-deoxyuridine (**IV**) and 5-(6-aminohexyl)-oxymethyl-2′-deoxycitidine (**VI**), for tagging 5-modified pyrimidine 2′-deoxynucleosides have been synthesized. Compounds 5-[6-(DNP)aminohexyl]-oxymethyl-2′-deoxyuridine (**Va**) and 5-{6-[6-(DNP)aminohexanoil]-aminohexyl}oxymethyl-2′-deoxyuridine (**Vb**) have been shown to inhibit the growth of *Micrococcus luteus* at concentrations of 0.35 and 0.65 mM, respectively. Given the relatively large size of *M. luteus* (around 1 μM), DNP derivatives of 2′-deoxyuridine can be used to study their cellular localization in Gram-positive bacteria.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATES

This article does not contain any studies involving patients or animals as test objects.

Informed consent was not required for this article.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

AUTHOR CONTRIBUTION

Authors DAM, IAO—chemical synthesis; authors DAM, LAA—selected the literature on the review topic; authors MVJ, LAA—manuscript preparation, proofreading and conceptualization; authors MVD, OVE, and BFV—study of antibacterial effect; author PNS—registration of mass-spectra; author SNK—manuscript editing, conceptualization.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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