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First Macrocyclic 1,2,3-Triazolyl Uridine Analogues

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Two macrocyclic 1,2,3-triazolyl uridine analogues were obtained for the first time by a copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. A 29-membered macrocycle consisted of one uracil molecule, two D-ribofuranose molecules and four 1,2,3-triazole molecules. A 58-membered macrocycle consisted of two uracil molecules, four D-ribofuranose molecules and eight 1,2,3-triazole molecules. Both macrocycles demonstrated moderate cytotoxicity against eight human cancer cell lines and normal cell line WI-38.

Keywords: Nucleoside analogues, macrocycles, uridine, 1,2,3-triazole, click chemistry, CuAAC reaction.

Первые макроциклические 1,2,3-триазоловые аналоги уридина

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Катализируемой медью реакцией азид-алкинового циклоприсоединения (CuAAC) впервые получены два макроциклических 1,2,3-триазоловых аналогов уридина. 29-Членный макроцикл состоял из одной молекулы урацила, двух молекул D-рибофуранозы и четырех молекул 1,2,3-триазола. 58-Членный макроцикл состоял из двух молекул урацила, четырех молекул D-рибофуранозы и восьми молекул 1,2,3-триазола. Оба макроцикла продемонстрировали умеренную цитотоксичность в отношении восьми линий раковых клеток человека и линии нормальных клеток WI-38.

Ключевые слова: Аналоги нуклеозидов, макроциклы, уридин, 1,2,3-триазол, клик химия, CuAAC реакция.

Introduction

Almost immediately after the discovery of the fundamental role of nucleic acids in the vital activity of the cell, a large-scale study of the properties of not only nucleosides as essential components of nucleic acids, but also their various derivatives, called nucleoside analogues, began. Hundreds of nucleoside analogues have been synthesized, many of which were approved for use as antiviral and anticancer drugs.[1,2] Numerous approaches to obtaining nucleoside analogues were described in the literature: the introduction of various substituents into the pyrimidine (purine) moiety of a native nucleoside or into a ribofuranose residue; the replacement of a nucleic base with another heterocycle; the replacement of a ribofuranose residue with a cyclopentane (cyclopentene) ring or a heterocyclic ring containing sulfur or nitrogen, and so on.[3]

Cyclic derivatives of native nucleosides are very interesting representatives of nucleoside analogues. They can be classified into four families: (i) spironucleosides in which a carbocyclic/heterocyclic ring is inserted into the ribofuranose residue of the nucleoside, usually at the C4′

position (Figure 1a); (ii) bicyclic nucleoside analogues in which two atoms of the ribofuranose residue are connected by an alkyl or ether bridge (Figure 1b); (iii) cyclic nucleoside analogues in which one atom of the ribofuranose residue and one atom of the nucleic base are connected by an alkyl or ether bridge (Figure 1c); (iv) macrocyclic nucleoside analogues in which several nucleic bases are connected by any linkers (Figure 1d,e,f).

Chemists' interest in the synthesis of spirocyclic nucleoside analogues (Figure 1a) arose after the discovery in 1961 of the naturally occurring spirocyclic nucleoside analogue hydantocidine which showed potent herbicidal activity and in which the imidizolidine-2,5-dione cycle was inserted into the ribofuranose residue at the anomeric carbon.[4] Since then, various series of spirocyclic nucleoside analogues, not only $C^{4'}$-spironucleosides, but also anomeric spiro-, $C^{2'}$-spiro- and $C^{3'}$-spironucleosides have been synthesized and some of them have shown excellent antiviral activities.[5]

Bicyclonucleoside analogues (Figure 1b) possessing a bicyclic sugar ring instead of the natural D-ribofuranose ring have attracted the spotlight of nucleoside chemists worldwide due to their conformationally restricted structure of the ribofuranose ring[6,7] that increases binding affinity to complementary RNA or DNA which allows them to be used for the synthesis of antisense oligonucleotides.[8-10] For the past time a large series of bicyclic nucleoside analogues possessing a cycloprorane ring,[6,11,12] an oxirane ring,[6,13,14] an oxetane ring,[15] a cyclobutane ring[16] fused to the $C^{2'}$-$C^{3'}$ bond of various 2′,3′-dideoxynucleoside analogues, 2′-*C*,4′-*C*-bridged bicyclonucleosides,[17] nucleoside analogues containing a 2′-*O*,4′-*C*-methylene-linked bicyclic furanose moiety[18-20] has been synthesized. Several bicyclic nucleoside analogues showed anti-HIV activity [11-13,15] and good growth inhibition activity against human tumor cell lines.[9]

Cyclic nucleoside analogues (Figure 1c), in which a pyrimidine or purine nucleic base is connected to a ribofuranose residue, in addition to a glycoside bond, by a polymethylene or ether chain, mainly through the $C^{5'}$ atom, have been synthesized and studied for more than 50 years.[21-23] The first cyclic nucleoside analog, 2′,3′-*O*-isopropylidene-*N*3,5′-cycloadenosine, was accidentally obtained by Todd's group in 1951.[24] Of course, such compound aroused the interest of chemists with its unusual structure, and in 1969 the Ikehara's group for the first time prepared a large series of various 8-cyclopurine nucleosides.[25] Interestingly, *N*3,5′-cycloxanthosine synthesized by the Holmes group in 1963[26] appeared to be a natural compound that was isolated from the sea sponge *Eryus sp*. from the Great Australian Bight by the Trotter group in 2005.[27] Note that many cyclic nucleoside analogues have shown antiviral activity, as a rule, against hepatitis C virus.[21-23,28-30]

In order to study supramolecular assemblies formed by amphiphilic molecular systems containing nucleic bases due to hydrogen bonds, stacking and hydrophobic interactions, a small series of macrocyclic nucleoside analogues (Figure 1d,e,f) was synthesized.[31-34] The literature provides data on the preparation of 12- and 13-membered macrocycles containing two uridine or guanidine moieties (Figure 1d),[32-34] 12-, 16-, 17- membered macrocycles containing three uridine moieties (Figure 1e),[31-33] and a 21-membered macrocycle containing four uridine moieties (Figure 1f).[33] Polymethylene chains,[31,33] urea, thiourea, carbodiimide moieties,[34] and isoxazolidine cycles[32] were used as linkers in the listed macrocyclic nucleoside analogues.

Recently, we synthesized a series of 1,2,3-triazole nucleoside analogues[35,36] and being interested in the work on the synthesis of macrocyclic nucleoside analogues,[31-34] we also decided to prepare several macrocycles, but to use for this aim not the ring-closing metathesis strategy,[33] but the strategy of a copper-catalyzed 1,3-dipolar cycloaddition.[37] Herein, we report on the synthesis of a 29-membered macrocycle formed from one uracil molecule, two D-ribofuranose molecules and four 1,2,3-triazole molecules as well as a 58-membered macrocycle formed from two uracil molecules, four D-ribofuranose molecules and eight 1,2,3-triazole molecules.

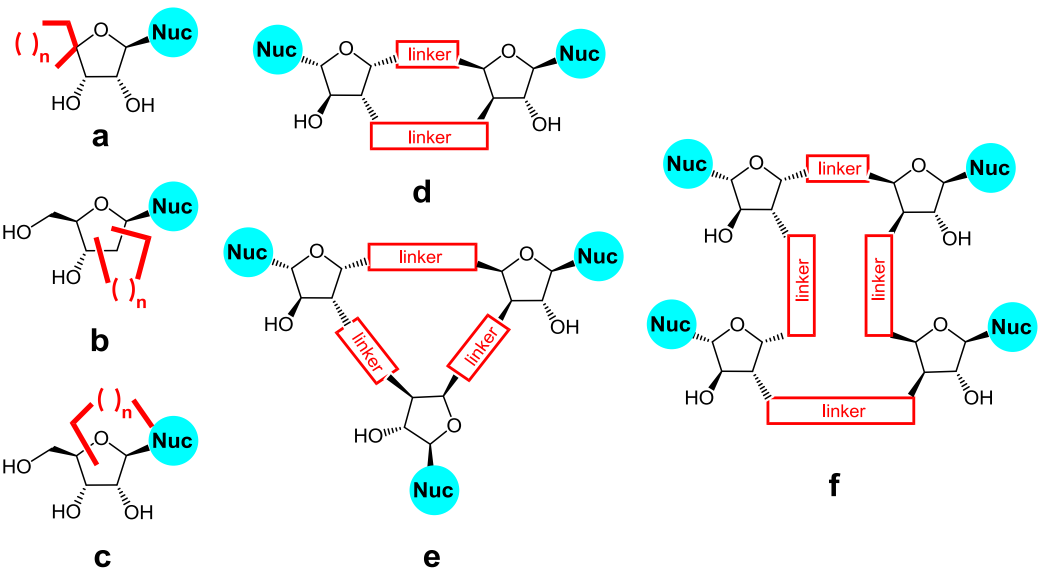


Figure 1. Schematic representation of families of cyclic nucleoside analogues (**Nuc** stands for a nucleic base).

Experimental

Chemistry

General Methods

^{1}H and ^{13}C spectra were recorded on 400 MHz, 500 MHz and 600 MHz Brucker Avance. ^{13}C NMR spectra were obtained in the above instrument operating at 100.6 MHz. Mass spectra (MALDI) were recorded in a positive ion mode on a Bruker Ultraflex III TOF/TOF mass spectrometer for 10^{-3} mg/mL solutions in $CHCl_3$. Flash chromatography was performed on silica gel 60 (40-63 μm, Buchi, Sepacore). Thin-layer chromatography was carried out on plates with silica gel (Sorbfil, Russia). Spots of compounds were visualized by using ultraviolent fluorescence under a short wavelength (254 nm) followed by heating the plates (at ca. 150 °C) after immersion in a solution of 5% H_2SO_4 and 95% H_2O. All reactions sensitive to air and/or moisture were carried out under argon atmosphere with anhydrous solvents. Anhydrous solvents were purified and dried (where appropriate) according to standard procedures.

Uracil and propargyl bromide were purchased from Sigma-Aldrich; D-ribose was purchased from Acros. Compounds **3**, **5-9** were prepared as described earlier.[36,38] Their spectral data were in keeping with published ones.[36,38]

Synthesis

Azido 5-mesyloxy-2,3-O-isopropylidene-β-D-ribofuranoside (***10***). Azide **9** (2.23 g, 10.4 mmol) was dissolved in anhydrous methylene chloride (50 mL) and triethylamine (3 mL). The reaction mixture was cooled to 0^{o}C and methanesulfonyl chloride (1.8 g, 15.7 mmol) was added dropwise. The reaction was stirred at room temperature for 16 hours, then washed with water, saturated solution of sodium bicarbonate, water again, dried over anhydrous sodium sulfate and evaporated under vacuum to afford **10** as a yellow syrup (2.64 g, 87%). Found, %: C 36.82, H 5.21, N 14.39, S 10.89. $C_9H_{15}N_3O_6S$ requires, %: C 36.86, H 5.16, N 14.33, S 10.93. m/z ESI MS: [$(M+H)^+$] calcd for $C_9H_{16}N_3O_6S$: 294.08, found 294.90 ^{1}H NMR ($CDCl_3$) δ_H ppm: 5.54 (1H, s, H1), 4.7 (1H, dd, *J* = 5.9, 0.9 Hz, H-3), 4.45-4.51 (2H, m, H-2, H-4), 4.23-4.31 (2H, m, H-5^{a,b}), 3.06 (3H, s, Ms), 1.46 (3H, s, CH_3), 1.29 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$) δ_C ppm: 113.50 (C^6), 97.06 (C^1), 85.11 (C^4), 84.56 (C^3), 81.34 (C^2), 67.72 (C^5), 37.57 (C^{Ms}), 26.36, 24.88 (C^7, C^8).

*1,3-Bis{1-(5-mesyloxy-2,3-O-isopropylidene-β- D-ribofuranosyl)-1H-1,2,3-triazol-4-yl]methyl}pyrimidine-2,4-dion (**11**).* To a solution of 1,3-bis(propargyl)uracil **3** (0.3 g, 1.6 mmol) and azide **10** (0.94 g, 3.2 mmol) in a mixture of 1:1 *tert*-butanol/water (50 mL) was added freshly prepared solution of sodium ascorbate (0.63 g, 3.2 mmol) in 2 mL water and $CuSO_4 \times 5H_2O$ (0.79 g, 3.2 mmol) in 2 mL water. The reaction mixture was stirred at 40^{o}C for 48 h, then was concentrated under reduced pressure. The residue was taken up in methylene chloride, washed successively with water, dried over anhydrous sodium sulphate and concentrated under vacuum. The residue was purified by flash chromatography on a silica gel (eluent chloroform:methanol from 200:1 to 20:1) to afford **11** as a white foam (0.6 g, 49%). Found, %: C 43.38, H 4.99, N 14.48, S 8.25. $C_{28}H_{38}N_8O_{14}S_2$ requires, %: C 43.41, H 4.94, N 14.46, S 8.28. MALDI MS: calcd for [$(M+H)^+$]: 775.2, found 775.1. ^{1}H NMR ($CDCl_3$) δ_H ppm: 7.90 (1H, s, H-9 (H-12)), 7.77 (1H, s, H-12 (H-9)), 7.43 (1H, d, *J* = 8.1 Hz, H-6), 6.14 (1H, d, *J* = 0.8 Hz, H-1′ (H-1″)),6.09 (1H, s, H-1″ (H-1′)),5.74 (1H, d, *J* = 7.8 Hz, H-5), 5.49-5.45 (2H, m, H-3′, H-3″), 5.24-5.18 (2H, m, 2H-10), 5.05-4.95 (4H, m, 2H-7, H-2′, H-2″), 4.6-4.53 (2H, m, H-4′, H-4″), 4.21-4.08 (4H, m, H-5′^{a,b}, H-5″^{a,b}), 2.91 (3H, s, Ms), 2.85 (3H, s, Ms), 1.57 (3H, s, CH_3), 1.56 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.37 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$) δ_C ppm: 162.51 (C^4), 151.21 (C^2), 143.76, 142.63 (C^8, C^{11}), 142.49 (C^6), 123.79, 123,65 (C^9, C^{12}), 114.32, 114.11 ($C^{6'}$, $C^{6''}$), 102.03 (C^5), 93.63, 93.38 ($C^{1'}$, $C^{1''}$), 86.03, 85.95 ($C^{4'}$, $C^{4''}$), 84.36, 84.31 ($C^{3'}$, $C^{3''}$), 81.48, 81.37 ($C^{2'}$, $C^{2''}$), 67.91 ($C^{5'}$, $C^{5''}$), 44.10, 36.08 (C^7, C^{10}), 37.58, 37.37 ($2C^{Ms}$), 26.82, 26.79, 25.14, 25.13 ($4CH_3$).

1,3-Bis{1-(5-azido-5-deoxy-2,3-O-isopropylidene-β-D-ribofuranosyl)-1H-1,2,3-triazol-4-yl]methyl}pyrimidine-2,4-dion (***12***) To a solution of **11** (1 g, 1.3 mmol) in dry DMF sodium azide (0.7 g, 10.7 mmol) was added. The mixture was heated at 90^{o}C 20 h., then cooled, diluted with acetone, washed with water, saturated solution of $NaHCO_3$, water again. The resulting solution was dried over anhydrous sodium sulphate and concentrated under vacuum. The residue was purified by flash chromatography on a silica gel (eluent chloroform:methanol from pure chloroform to 50:1) to afford **12** as a white foam (0.43 g, 50%). Found, %: C 46.69, H 4.86, N 29.39. $C_{26}H_{32}N_{14}O_8$ requires, %: C 46.71, H 4.82, N 29.33. MALDI MS: calcd for [$(M+H)^+$]: 670.6, found 670.6. ^{1}H NMR ($CDCl_3$) δ_H ppm: 7.90 (1H, s, H-9 (H-12)), 7.78 (1H, s, H-12 (H-9)), 7.40 (1H, d, *J* = 7.8 Hz, H-6), 6.12 (1H, s, H-1′ (H-1″)), 6.07 (1H, s, H-1″ (H-1′)),5.71 (1H, d, *J* = 7.8 Hz, H-5), 5.49-5.45 (2H, m, H-3′, H-3″), 5.23-5.16 (2H, m, 2H-10), 5.02-4.93 (2H, m, 2H-7), 4.87-4.82 (2H, m, H-2′, H-2″), 4.46-433 (2H, m, H-4′, H-4″), 3.37-3.17 (4H, m, H-5′^{a,b}, H-5″^{a,b}), 1.55 (3H, s, CH_3), 1.54 (3H, s, CH_3), 1.35 (3H, s, CH_3), 1.35 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$) δ_C ppm: 162.35 (C^4), 151.12 (C^2), 143.73, 142.61, 142.24 (C^8, C^{11}, C^6), 124.16, 124,06 (C^9, C^{12}), 114.18, 113.96 ($C^{6'}$, $C^{6''}$), 102.11 (C^5), 93.49, 93.11 ($C^{1'}$, $C^{1''}$), 87.45, 87.29 ($C^{4'}$, $C^{4''}$), 84.35, 84.31 ($C^{3'}$, $C^{3''}$), 82.38, 82.22 ($C^{2'}$, $C^{2''}$), 52.40 ($C^{5'}$, $C^{5''}$), 44.08, 35.83 (C^7, C^{10}), 26.77, 26.74, 25.11 ($4CH_3$).

Synthesis of macrocyclic 1,2,3-triazolyl uridine analogues. To a solution of sodium ascorbate (0.24 g, 1.2 mmol) and $CuSO_4 \times 5H_2O$ (0.3 g, 1.2 mmol) in 100 mL mixture of 1:1 *tert*-butanol/water **12** (0.41 g, 0.6 mmol) in 50 mL mixture of 1:1 *tert*-butanol/water and 1,7-octadiyne (0.065 g, 0.6 mmol) in 50 mL mixture of 1:1 *tert*-butanol/water were contemporaneously dropped. The reaction mixture was stirred at 40^{o}C for 60 h, then it was concentrated under reduced pressure. The residue was taken up in methylene chloride, washed successively with water, dried over anhydrous sodium sulphate and concentrated under vacuum. The residue was purified by flash chromatography on a silica gel (eluent chloroform:methanol from pure chloroform to 1:1)

*4^2,4^3,11^2,11^3-di-O-isopropylideno-1(3,1)-uracila-3(4,1),5(1,4), 10(4,1),12(1,4)-tetratriazola-4(1,5),11(5,1)-di-β-D-ribofuranosacyclotriskaidecaphan (**13**).* A white foam (0.1 g, 21%). Found, %: C 52.78, H 5.42. N 25.28. $C_{34}H_{42}N_{14}O_8$ requires, %: C 52.71, H 5.46, N 25.31. MALDI MS: calcd for [$(M+Na)^+$]: 797.8, found 797.9. ^{1}H NMR ($CDCl_3$) δ_H ppm: 7.68 (1H, s, H-9 (H-12)), 7.59 (1H, s, H-12 (H-9)), 7.46 (1H, d, *J* = 7.7 Hz, H-6), 6.88 (1H, s, H-19 (H-21)), 6.56 (1H, s, H-21 (H-19)), 6.13 (1H, d, *J* = 1.4 Hz H-1′ (H-1″)), 6.07 (1H, s, H-1″ (H-1′)), 5.83 (1H, d, *J* = 8.1 Hz, H-5), 5.51 (1H, d, *J* = 5.9 Hz H-3′ (H-3″)), 5.37-4.92 (7H, m, H-3″ (H-3′), 2H-10, 2H-7, H-2′, H-2″), 4.76-4.67 (2H, m, H-4′, H-4″), 4.52-4.43 (2H, m, H-5′^{a}, H-5″^{a}), 4.38-4.25 (2H, m, H-5′^{b}, H-5″^{b}), 2.78-2.66 (4H, m, 2H-23, 2H-26), 1.69-1.6 (4H, m, 2H-24, 2H-25), 1.57 (3H, s, CH_3), 1.55 (3H, s, CH_3), 1.39 (6H, s, $2CH_3$). ^{13}C NMR ($CDCl_3$) δ_C ppm: 162.36 (C^4), 151.34 (C^2), 147.87, 147.62, 144.04, 142.93, 142.53 (C^6, C^8, C^{11}, C^{20}, C^{22}), 123.49, 123.14, 122.47, 122.31 (C^9, C^{12}, C^{19}, C^{21}), 114.74, 114.31 (C^{13}, C^{16}), 102.25 (C^5), 93.48, 93.15 ($C^{1'}$, $C^{1''}$), 86.63, 85.83 ($C^{4'}$, $C^{4''}$), 84.06, 84.03 ($C^{3'}$, $C^{3''}$), 81.98, 81.66 ($C^{2'}$, $C^{2''}$), 51.09, 51.03 ($C^{5'}$, $C^{5''}$), 44.22, 36.21 (C^7, C^{10}), 28.36, 28.29, 26.82, 26.76 (C^{23}, C^{24}, C^{25}, C^{26}), 25.18, 24.91 ($4CH_3$).

4^2,4^3,11^2,11^3,17^2,17^3,24^2,24^3-tetra-O-isopropylideno-1(3,1), 14(3,1)-diuracila-3(4,1),5(1,4),10(1,4),12(1,4),16(4,1),18(1,4), 23(4,1)-octatriazola-4(1,5),11(5,1),17(1,5),24(5,1)-tetra-β-D-ribofuranosacyclotetracosahexaphan (***14***). A white foam (0.11 g, 23%). Found, %: C 52.73, H 5.41, N 25.36. $C_{68}H_{84}N_{28}O_{16}$ requires, %: C 52.71, H 5.46, N 25.31. MALDI MS: calcd for [$(M+H)^+$]: 1550.61, found 1550.6. ^{1}H NMR ($CDCl_3$) δ_H ppm: 7.94, 7.93, 7.79, 7.78 (4H, 4s, H-9, H-12, H-39, H-42), 5.521 (1H, d, *J* = 7.7 Hz, H-6 (H-36)), 5.519 (1H, d, *J* = 7.7 Hz, H-36 (H-6)), 6.94,

6.93, 6.81, 6.80 (4H, 4s, H-19, H-21, H-49, H-51), 6.13 (2H, d, *J* = 2.2 Hz, H-1′, H-1‴ (H-1″, H-1⁗)), 6.07 (2H, s, H-1″, H-1⁗ (H-1′, H-1‴)), 5.75 (1H, d, *J* = 8.1 Hz, H-5 (H-35)), 5.74 (1H, d, *J* = 8.1 Hz, H-35 (H-5)), 5.46-5.41 (4H, m, H-3′, H-3″, H-3‴, H-3⁗), 5.27-5.14 (4H, m, H-2′, H-2″, H-2‴, H-2⁗), 5.05-4.96 (8H, m, 2H-10, 2H-40, 2H-7, 2H-37), 4.72-4.64 (4H, m, H-4′, H-4″, H-4‴, H-4⁗), 4.53-4.44 (4H, m, H-5′^{a}, H-5″^{a}, H-5‴^{a}, H-5⁗^{a}), 4.31-4.20 (4H, m, H-5′^{b}, H-5″^{b}, H-5‴^{b}, H-5⁗^{b}), 2.68-2.57 (8H, m, 2H-23, 2H-26, 2H-27, 2H-30), 1.65-1.58 (8H, m, 2H-24, 2H-25, 2H-28, 2H-29), 1.54 (6H, s, 2CH_3), 1.53 (6H, s, 2CH_3), 1.37 (6H, s, 2CH_3), 1.36 (6H, s, 2CH_3). ^{13}C NMR ($CDCl_3$) δ_C ppm: 162.43 (C^{4}, C^{34}), 151.27 (C^{2}, C^{32}), 147.87, 147.81, 147.70, 147.65 (C^{8}, C^{11}, C^{38}, C^{41}), 143.79 (C^{6}, C^{36}), 142.87, 142.85, 142.74 (C^{20}, C^{22}, C^{50}, C^{52}), 124.43, 124.39, 124.05, 124.04 (C^{9}, C^{12}, C^{39}, C^{42}), 122.12, 122.10, 121.79, 121.75 (C^{19}, C^{21}, C^{49}, C^{51}), 114.37, 114.18 (C^{13}, C^{16}, C^{43}, C^{46}), 101.97 (C^{5}, C^{35}), 93.15, 93.12, 93.04, 93.02 ($C^{1'}$, $C^{1''}$, $C^{1'''}$, $C^{1''''}$), 87.11, 86.89, 86.87 ($C^{4'}$, $C^{4''}$, $C^{4'''}$, $C^{4''''}$), 84.37, 84.34, 84.27, 84.24 ($C^{3'}$, $C^{3''}$, $C^{3'''}$, $C^{3''''}$), 82.20, 82.07 ($C^{2'}$, $C^{2''}$, $C^{2'''}$, $C^{2''''}$), 51.47, 51.43 ($C^{5'}$, $C^{5''}$, $C^{5'''}$, $C^{5''''}$), 44.17, 44.15, 36.04 (C^{7}, C^{37}, C^{10}, C^{40}), 28.61, 28.53, 26.75, 26.74 (C^{23}, C^{24}, C^{25}, C^{26}, C^{27}, C^{28}, C^{29}, C^{30}), 25.15, 25.13, 25.10 (8CH_3).

Biological Study

Cells and Materials

For experiments, we used tumor cell cultures M-HeLa clone 11 (epithelioid carcinoma of the cervix, subline HeLa., clone M-HeLa), HuTu 80, human duodenal adenocarcinoma; PC3 – prostate adenocarcinoma cell line from ATCC (American Type Cell Collection, USA; CRL 1435); MCF7 – human breast adenocarcinoma (pleural fluid); Hep G2, human liver carcinoma; PANC-1, human pancreatic carcinoma; A549, human lung carcinoma; A375, human amelanotic melanoma cell line; WI38, VA 13 subline 2RA, human embryonic lung from the collection of the Institute of Cytology, Russian Academy of Sciences (St. Petersburg).

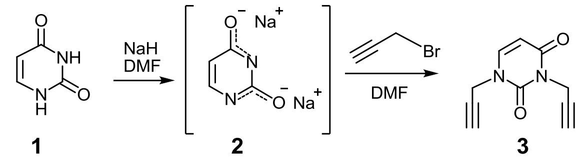
MTT assay

The cytotoxic effect on cells was determined using the colorimetric method of cell proliferation – the MTT test. NADP-H-dependent cellular oxidoreductase enzymes can, under certain conditions, reflect the number of viable cells. These enzymes are capable of reducing the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) to insoluble blue-violet formazan, crystallizes inside the cell. The amount of formazan formed is proportional to the number of metabolically-active cells. Cells were seeded on a 96-well Nunc plate at a concentration of $5\cdot10^{3}$ cells per well in a volume of 100 μL of medium and cultured in a CO_2 incubator at 37 °C until a monolayer was formed. Then the nutrient medium was removed and 100 μL of solutions of the test drug in given dilutions were added to the wells, which were prepared directly in the nutrient medium with the addition of DMSO (5% v/v) to improve solubility. After 48 h incubation of cells with the tested compounds, the nutrient medium was removed from the plates, and 100 μL of the nutrient medium without serum containing MTT at a concentration of 0.5 mg/mL was added and incubated for 4 h at 37 °C. Formazan crystals were added 100 μL of DMSO to each well. Absorbance was recorded at 540 nm on an Invitrologic microplate reader (Russia). Experiments for all compounds were repeated three times.

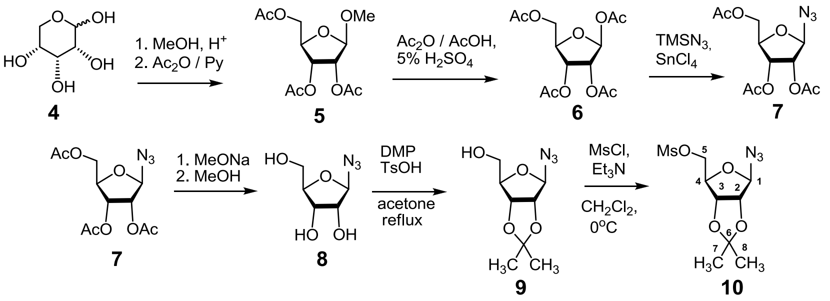
Results and Discussion

Synthesis

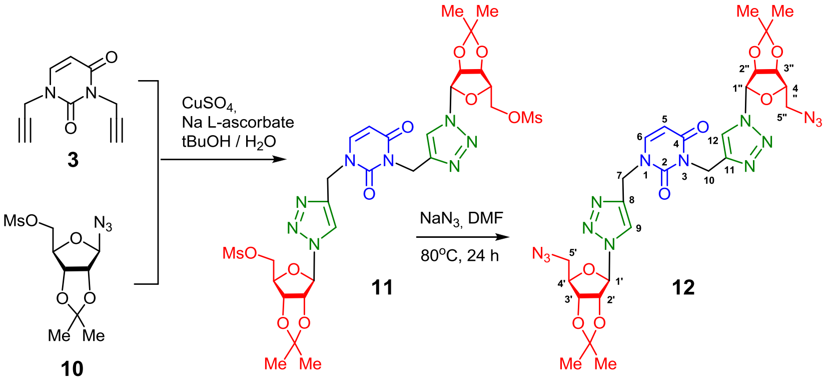
The synthesis of target macrocycles was carried out in four stages. At the first stage (Scheme 1), in accordance with the procedure already described,[36] uracil **1** was converted by a reaction with an excess of sodium hydride in dimethylformamide (DMF) into disodium salt **2**, which was alkylated in situ with propargyl bromide to afford 1,3-bis(propargyl)uracil **3** in 70% yield. At the second stage (Scheme 2), using the methods previously reported,[36] firstly, commercial D-ribose **4** was converted to tetraacetyl D-ribofuranose **6**, which was involved in a reaction with trimethylsilylazide ($TMSN_3$) in the presence of tin tetrachloride to obtain azide 2,3,5-triacetyl-β-D-ribofuranose **7**. Secondly, the acetyl protection of monosaccharide **7** was removed by the treatment with sodium methylate in methanol and a 2,3-*O*-isopropylidene protecting group was introduced by the reaction with 2,2-dimethoxypropane (DMP) in boiling acetone in the presence of *p*-toluenesulfonic acid (TsOH) according to a known procedure[38] to afford compound **9**. Then 5-mesyloxy-1-azido-1-deoxy-2,3-*O*-isopropylidene-β-D-glucofuranose **10** was obtained by the reaction of azide **9** with mesyl chloride (MsCl) in the presence of Et_3N (Scheme 2). On the third stage, a copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction was utilized to couple 1,3-bis(propargyl)uracil **3** with azide **10** (Scheme 3) by analogy with the procedure previously described.[36] The 1,3-disubstituted uracil derivative **11** obtained in 49% yield was treated with sodium azide in DMF at 80 °C for 12 h to give the 5′,5′′-diazide **12** in moderate yield (50%). The ^{1}H and ^{13}C NMR spectra of **12** confirmed the presence of 1,2,3-triasole rings. The signals within the range 7.78 – 7.9 ppm in the ^{1}H NMR spectrum of **12** belonged to the triazolyl protons H-9 and H-12 in accordance with the literature data.[35,36] The ^{13}C NMR spectrum of **11** showed signals within the range 142.24 – 143.73 ppm and signals within the range 124.06 – 124.16 ppm attributing to the 1.2.3-triazolyl C^{8}, C^{11} carbons and C^{9}, C^{12} carbons, respectively, in keeping with known data.[35,36] At the fourth stage, the CuAAC reaction of 1,7-octadiine with 5′,5′′-bisazide **12** was carried out to obtaine target macrocycles (Scheme 4). The reagents were introduced into the reaction in equimolar quantities in the presence of two moles of $CuSO_4$ and sodium ascorbate in a 1:1 tBuOH / H_2O solution at a concentration of 10^{-3} mol/L. The reaction mixture was stirred for 60 hours at a temperature of 40 °C. Macrocycles **13** and **14** were isolated by preparative flash chromatography on silica gel in 21 and 23% yields respectively. The structure of the obtained macrocycles **13** and **14** was confirmed by ^{1}H and ^{13}C NMR spectroscopy and MALDI mass spectrometry. In the ^{1}H NMR spectrum of macrocycle **13**, there are four signals for the triazolyl protons at 6.56, 6.88, 7.59, 7.68 ppm, two sets of signals for the protons of the ribofuranose residue and the signals for the protons at the C^{5} and C^{6} atoms of the uracil moiety in the corresponding integral intensities. The protons at the $C^{1'}$ and $C^{1''}$ atoms of the D-ribofuranose residues resonated at 6.07 and 6.13 ppm as singlets, that indicated β-orientation of the glycosidic bonds in macrocycle **13**. All the above signals also presented in the ^{1}H NMR spectrum of macrocycle **14** but with the doubled quantity. Two anomeric protons of macrocycle **14** resonated in the ^{1}H NMR spectrum as doublets at 6.13 ppm with vitinal constants of 3.3 Hz, and two other anomeric protons of macrocycle **14** resonated as a broad singlet with an integral intensity corresponding to two protons. This indicated the β-orientation of the four glycosidic bonds. The ^{13}C NMR spectra of both macrocycles are also consistent with the assumed structures. In the MALDI spectrum of macrocycle **13**, there is only one signal of 797 *m/z* which corresponds to the mass of $[M+Na]^+$. In the MALDI spectrum of macrocycle **14**, there is only one signal of 1550 m/z, which corresponds to the mass $[M+H]^+$.



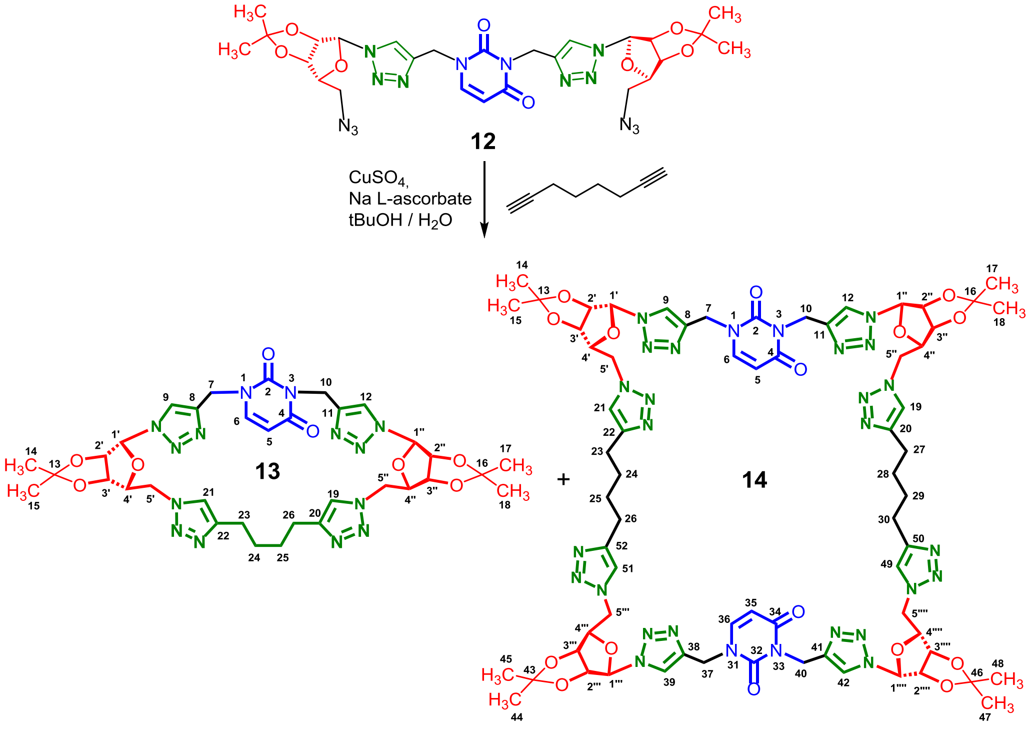
Scheme 1. Synthesis of 1,3-dipropargyluracil.



Scheme 2. Synthesis of 5-methanesulfonyloxy-1-azido-1-deoxy-2,3-*O*-isopropylidene-β-D-glucofuranose.



Scheme 3. Synthesis of compound **12** possessing the uracil moiety coupled to two 1,2,3-triazol-4-yl-β-D-ribofaranosyl fragments.



Scheme 4. Synthesis of 29- and 58-membered macrocyclic 1,2,3-triazolyl uridine analogues.

Table 1. Cytotoxicity (IC$_{50}$, μM) of macrocycles **13** and **14**.

Compounds	IC$_{50}$,* μM								
	Cancer cell lines								Normal cell line
	M-HeLa	HuTu80	PC3	MCF-7	HepG2	PANC-1	A549	A375	WI-38
13	70.0±5.5	57.7±4.5	86.7±6.6	69.0±5.3	>100	100±8.7	>100	43.4±3.5	40.3±3.1
14	54.6±4.3	68.8±5.4	100±8.2	55.4±4.3	>100	59.1±4.6	>100	39.5±3.1	45.3±5.5

*IC$_{50}$ represents the concentration at which a compound exerts half of its maximal inhibitory effect. The experiments were performed in triplicate. Results are expressed as the mean ± standard deviation.

Cytotoxicity

Macrocycles **13** and **14** were evaluated for *in vitro* cytotoxicity against eight human cancer cell lines: M-HeLa cervical epitheloid carcinoma, HuTu-80 duodenal adenocarcinoma, PC3 prostate adenocarcinoma, MCF-7 breast adenocarcinoma, Hep G2 human liver carcinoma, PANC-1 pancreatic carcinoma, A549 pulmonary adenocarcinoma, A375, human amelanotic melanoma cell line, as well as a diploid human cell strain WI-38 composed of fibroblasts. The resulting data expressed as concentrations causing the inhibition of the growth of 50% of cells in the experimental population (IC$_{50}$) are presented in Table 1. As one can see, the tested compounds demonstrated moderate activity in the range of IC$_{50}$ values of 39-87 μM against most of the cell lines used, including the normal cell line WI-38. The exception was HepG2 and A549 cell lines, for which both macrocycles were completely inactive.

Conclusions

Two macrocyclic 1,2,3-triazolyl uridine analogues were obtained for the first time by a copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. A 29-membered macrocycle consisted of one uracil molecule, two D-ribofuranose molecules and four 1,2,3-triazole molecules. A 58-membered macrocycle consisted of two uracil molecules, four D-ribofuranose molecules and eight 1,2,3-triazole molecules. The ability of the macrocycles to form supramolecular assemblies is in progress and will be reported in due course. Both macrocycles demonstrated moderate cytotoxicity against eight human cancer cell lines and normal cell line WI-38.

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