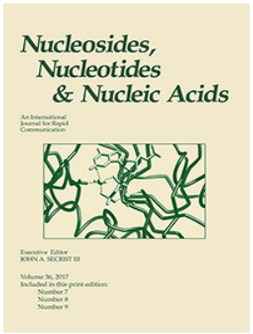

Nucleosides, Nucleotides and Nucleic Acids

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Syntheses of 1,2,3-triazole-bridged pyranose sugars with purine and pyrimidine nucleobases and evaluation of their anticancer potential

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ABSTRACT

With the aim to create a library of compounds with potential bioactivities by combining special characteristics of two important groups such as nucleobases and carbohydrates, twenty 1,4-disubstituted-triazole nucleosides were synthesized in good yields (80-94%) using the copper catalyzed 'Click' reaction between azido-modified pento- or hexopyranoses and alkyne-bearing pyrimidine or purine nucleobases. Structural elucidation was made with the assistance of spectroscopic techniques such as FTIR, 1D-, 2D-NMR, and ESI-TOFMS. All the synthesized triazole nucleosides were evaluated for their cytotoxic activity against three human cancer cell lines (MDA-MB-231, Hep3B, PC-3) by using the MTT assay. Particularly, compounds **3a** and **1b** were identified as potential hits against Hep3B cell.

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Click chemistry; 1,2,3-Triazoles; azido sugars; nucleobases; nucleosides; anticancer activity

Introduction

Click chemistry was developed to be a basic tool in organic and/or bioorganic chemistry for the preparation of different macromolecules.[1] To reach this aim, many techniques have been developed including reaction types and methods with various catalysts and solvent systems.[1,2] The results obtained by any of these methods are generally excellent and they can be improved with a simple fine-tuning. Among these methods; CuAAC (Cu(I)-catalyzed azide-alkyne cycloaddition), the modified form of Huisgen reaction and on the contrary, copper-free type SPAAC (strain-promoted azide-alkyne cycloaddition) reactions have been the most common approaches which have been applied for the coupling of azido-modified

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Dedication

We dedicated this work to Prof. Dr. Levent YÜCEER on account of his 72th birthday and his endless help during the doctorate research of Kadir AY.

Supplemental data for this article can be accessed on the publisher's website.

sugars and alkyne containing molecules to produce 1,2,3-triazole derivatives with improved properties for drug discovery, bioconjugation, fluorescent probing, proteomic profiling and cellular target identification.[3–6]

Both carbohydrates and nucleobases have an important role in diverse fundamental biological processes and have hence been in the centre of attention for chemists and biologists. According to chemists, azide derivatives of carbohydrates are an important group of compounds that serves as building blocks for complex synthetic operations. The anomeric position is usually preferred for linking azide functionality, though the rest of the sugar ring positions (especially C-3 and free methylene carbon) have also been used for this aim.[7–9]

Nucleobases are nitrogen-containing heterocyclic compounds with single ring (pyrimidine) or fused bicyclic (purine) structures serving as basic units of nucleotides which form DNA and RNA. Nucleobases have at least as much importance as the carbohydrates, that making modified nucleosides desirable targets.[10] Of course the compounds within this scope have also been tested for their various biological activities.[11] As an example, in a similar to ours, a series of novel nucleoside compounds were synthesized *via* the CuAAC reaction of azido sugars with propynylated pyrimidines, and resulting triazole compounds were evaluated for the inhibition of α-glucosidase.[12]

From this point, 1,2,3-triazole nucleosides and their attractive analogues with assorted biological activities such as anticancer,[13–16] antifungal,[17–19] antibacterial,[20–22] antiviral,[23–28] antitubercular,[29–31] antiproliferative,[32–34] are potentially pharmaceutically important molecules.[35] Among the drugs based on 1,2,3-triazoles, carboxyamidotriazole and cefatrizine with anticancer activity, tazobactam (β-lactamase inhibitory), *tert*-butyldimethylsilylspiroamino oxathioledioxide with anti-HIV activity are currently in clinical trials and have potential to be used as a pharmacophore in the coming years.[36,37]

The facts mentioned above prompted us to continue our exploration of triazole analogues for better understanding the relationship between structural changes and the activities. As an application of the most powerful click reaction to date, CuAAC was used for designing series of nucleosides containing a 1,2,3-triazole ring. All synthesized compounds were evaluated for their anticancer potential.

Results and discussion

Chemistry

1,4-Disubstituted-1,2,3-triazole bridged nucleosides (**1a-5a**, **1b-5b**, **1c-5c**, **1d-5d**) were synthesized in good yield (80-94%) *via* the CuAAC reaction in the presence of $CuSO_4$/Na ascorbate from monopropynylated uracil, thymine, 5-fluorouracil (5-FU), adenine (**a**-**d**) nucleobases with azidosugars obtained according to the literature[38–43], which have a pyranose ring with D-*galacto*-,[38,39] D-*fructo*-,[40,41] D-*gluco*-,[42] D-*xylo*,[39,43] configurations (Figure 1). Propargyl derived nucleobases (**a-d**) were obtained in moderate yields due to the formation of dipropargylated

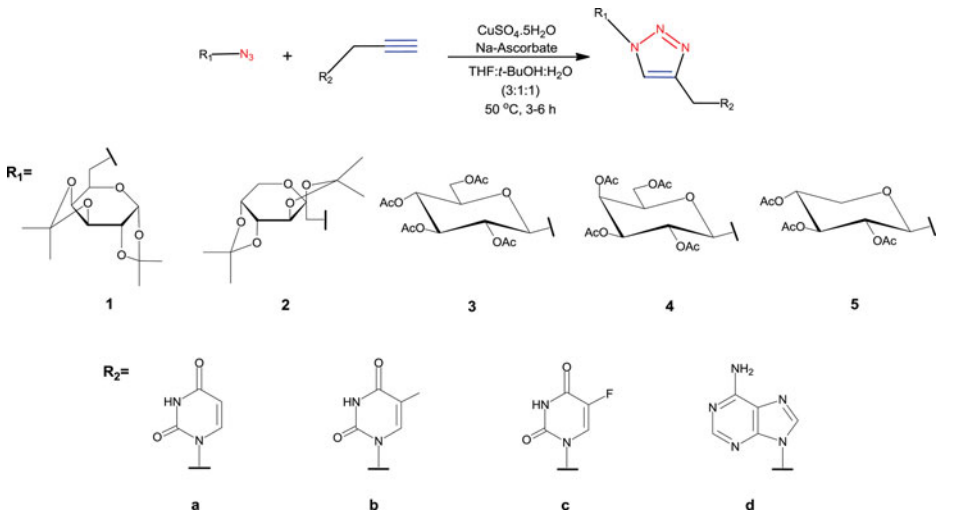

Figure 1. Synthesis of 1,2,3-triazole-bridged purine and pyrimidine nucleosides.

by-products and spectral data for their structural elucidation are consistent with the literature.[44] The coupling patterns for the alkynyl group for **a-d** were observed as a triplet for ≡CH at δ 3.35–3.40 ppm (J = 1.2–2.4 Hz) whereas they were observed as a doublet for –CH_2C≡, at δ 4.43–5.01 ppm in ^{1}H NMR spectra. ^{13}C NMR spectra were also confirmed the structure of monoalkynated nucleobases with the evidence of signals at about δ 79.0 (–CH_2<u>C</u>≡), 76.0 (≡CH) and 33.0 (–<u>C</u>H_2C≡) ppm, which are in agreement with literature.[44]

The occurrence of the click reaction was proved by the disappearance of terminal alkyne proton signals (δ 3.35–3.40 ppm) and the presence of triazole olefinic -CH- peaks at δ 7.85–8.39 ppm in ^{1}H NMR spectra. The appearance of a sharp singlet for H-5′at about δ 7.85–8.39 ppm was also confirmed the completion of click reaction for all nucleoside products (**1a**-**5a**, **1b**-**5b**, **1c**-**5c**, **1d**-**5d**). (*see* Supplementary material, Table S1, S3, S5, S8). The diastereotopic *geminal* methylene protons (N–CH_2-) between triazole ring and nucleobases appear as AB quartet, J_{AB} = 15.2 Hz, at about δ 4.84–5.08 ppm for all products except adenine derivatives. For the adenine derivatives (**1d-5d)** that methylene protons were established as a singlet at about δ 5.42–5.44 ppm (*see* Supplementary material, Table S8). The signal of those methylene carbons resonated at δ 38.2–43.0 ppm in ^{13}C NMR spectra. Furthermore, ^{13}C NMR spectra exhibit characteristic signals for C-4′and C-5′of the triazole ring at δ 141.6–143.7 and 122.8–126.6 ppm, respectively (*see* Supplementary material, Table S2, S4, S6, S9). The large difference of chemical shifts between C4′ and C5′ (about δ 20 ppm) indicated the formation of 1,4-regioisomers.

With regard to 5-FU derivatives (**1c**-**5c**), the signals at between δ −169.0–−169.3 ppm corresponding to fluorine atom were determined in the ^{19}F NMR. Moreover, adjacent F-H ($J_{H6,F}$ = ~6.8–7.2 H z) and F-C ($J_{C4,F}$ = ~33.0, $J_{C5,F}$ = ~229.0, $J_{C6,F}$ = ~26.0 H z) coupling interactions were observed in ^{1}H– and ^{13}C– NMR spectra respectively (*see* Supplementary material, Table S5, S6, S7).

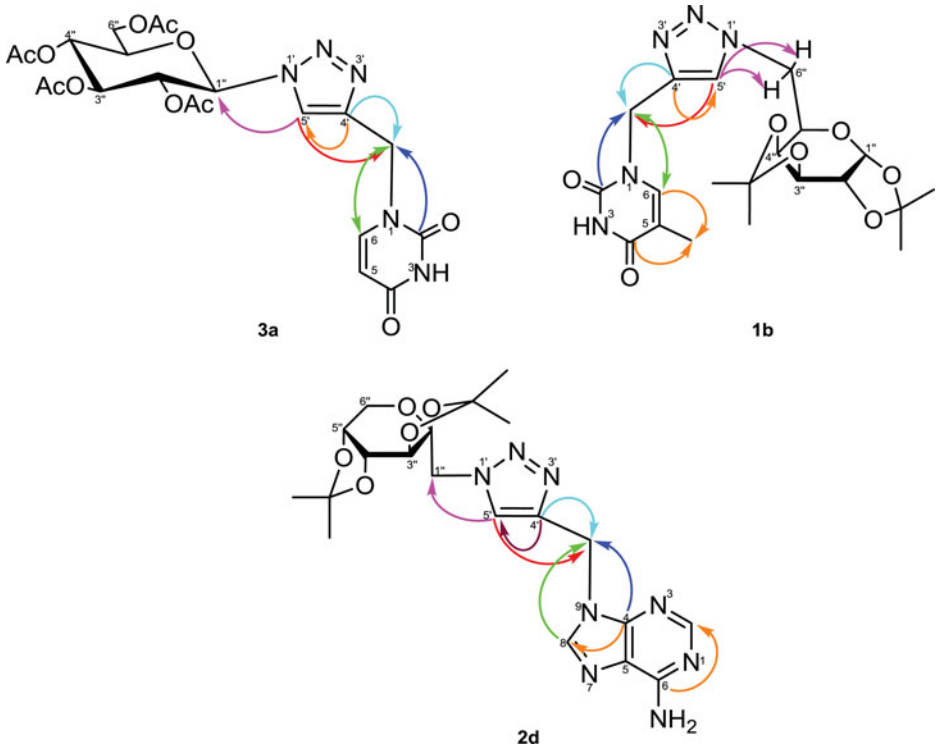

Figure 2. Significant HMBC Correlations of Some Uracil, Thymine and Adenine Nucleosides.

HSQC and HMBC NMR techniques were also used for the detailed structural assignments of all 5-FU derivatives (**1c-5c**) (see Supplementary material).

For all synthesized nucleosides, there are two different methylene groups one of which is in nucleobase-triazole bridges and the others is in the sugar moieties, and determination of those related methylene carbons and protons was made with the assistance of HSQC correlations. In this regard, the diastereotopic *geminal* methylene protons (δ_H = 4.84–5.08 ppm) were correlated with methylene carbon (δ_C = 38.2–43.2 ppm) and these correlations confirmed identification of methylenes. Similarly, the location of all carbon and hydrogen atoms for remaining nucleosides were verified on the basis of the $^1H/^{13}C$ correlations in HSQC spectra.

Significant HMBC correlations of uracil (**3a**), thymine (**1b**), adenine (**2d**) and 5-FU (**1c-5c)** nucleosides are given in Figure 2 and **3**. C-5′ of the triazole ring shows long-range couplings ($^3J_{C,H}$) with the H1″ or H6″ protons as well as with the bridge methylene group. Concordantly, C-4′ did not show any correlations with H1″/H6″ while this olefinic carbon had long-range correlation with bridged methylene protons both confirming the formation of the 4-substituted-1,2,3-triazole part. Those long-range HMBC correlations that gived ^2J and $^3J_{CH}$ coupling constants allowed the assignment of the bridged N–CH_2- protons correlated with both nucleobase ring carbons and triazole-C-4′.

In addition to all these, the characteristic ^1H– and ^{13}C–NMR peaks of sugar skeleton were also observed as expected. The ^1H-NMR spectra of target nucleosides exhibited doublets with coupling constants between *vicinal* H-1″ and H-2″ protons.

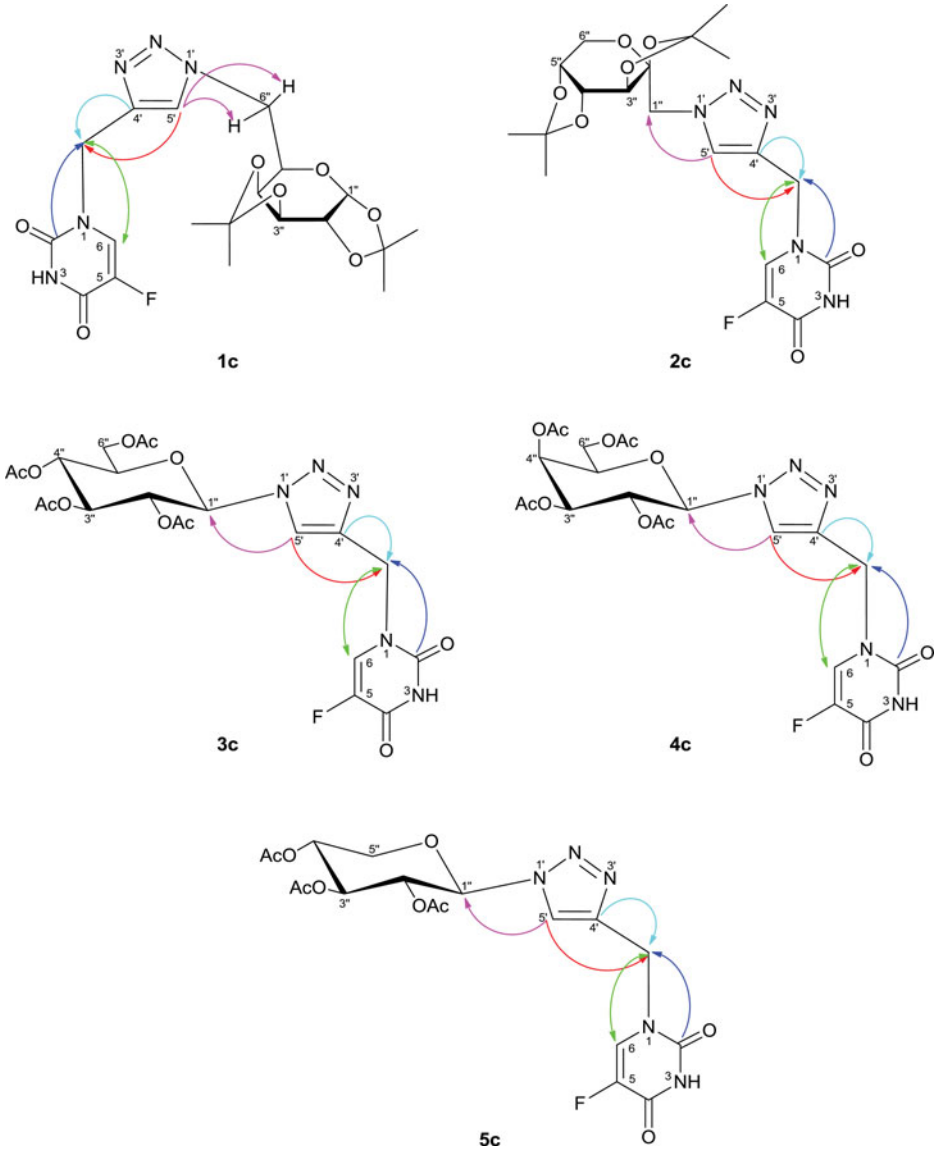

Figure 3. Significant HMBC Correlations of 5-Fluorouracil Nucleosides.

These corresponding large couplings (J = over 6.0 Hz) in the sugar moiety for compounds **3a-d, 4a-d** and **5a-d** referred to 1,2-*trans* relationship and β-anomeric configuration.

Finally, the predicted structures of all triazole nucleosides given in Figure 1 were confirmed by molecular ion peaks such as $[M + H]^+$ or $[M + Na]^+$ observed in positive ion mode TOF-MS (ESI) spectra (see Supplementary material).

Biological activity

The synthesized twenty compounds (**1a-5a**, **1b-5b**, **1c-5c**, **1d-5d**) as well as alkynated nucleobase intermediates (**a-d**) were screened for their *in vitro* cytotoxic

activities against human breast (MDA-MB-231), human hepatoma (Hep3B) and human prostate (PC-3) cancer cell lines by employing the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay at 200, 100, 50, 25, and 10 μM concentrations. The concentrations that cause 50% inhibition of cancer cell growth are expressed as IC_{50} values and these resulting values of compounds are summarized in Table 1.

Firstly, because of the importance of selective killing of cancer cells, all compounds were also tested for their possible cytotoxicities towards the L929 control cell line. The growth of cell lines was not affected by any of the compounds and this means that none of the compounds exhibited a cytotoxic effect.

From the IC_{50} results shown in Table 1, we concluded the following;

(1) Based on the detailed IC_{50} analysis, among nucleobase alkynes, propargyl substitute 5-FU (**c**) was the most potent, especially with potential hits against Hep3B and PC-3 cells. On the other hand, adenine alkyne displayed much more cytotoxic potency against MDA-MB-231 cells than other alkynated nucleobases.

(2) Compounds **3a**, **1b**, **2b**, **5b**, **5c**, **3d**, and **5d** possessed the strongest inhibitory activity among all the investigated cancer cells with IC_{50} value range of 59.3-82.6 μM. Specifically, compound **3a** exhibited more potent inhibitory activity (59.3 μM) against Hep3B, compound **1c** exhibited more potency (112.6 μM) against MDA-MB-231 cells and compound **1a** along with **4d** were more active (106.6 and 107.0 μM, respectively) than other pyranonucleosides.

(3) In the light of the results, Hep3B was defined as the most liable to be influenced by the synthesized pyranonucleosides. The highest activity in this all

Table 1. IC_{50} Values of synthesized compounds (μM).

	MDA-MB-231	HEP3B	PC-3	L929
a	>200	177.9 ± 0.13	>200	ND
1a	115.6 ± 0.08	89.38 ± 0.16	106.6 ± 0.10	ND
2a	>200	99.06 ± 0.14	>200	ND
3a	177.9 ± 0.18	59.39 ± 0.15	130.7 ± 0.15	ND
4a	121.3 ± 0.07	110.2 ± 0.12	179.0 ± 0.14	ND
5a	120.2 ± 0.08	98.53 ± 0.11	138.8 ± 0.15	ND
b	196.7 ± 0.15	73.58 ± 0.17	118.5 ± 0.14	ND
1b	156.4 ± 0.13	65.42 ± 0.15	195.4 ± 0.13	ND
2b	149.3 ± 0.18	77.12 ± 0.18	156.8 ± 0.10	ND
3b	168.1 ± 0.15	98.9 ± 0.11	127.3 ± 0.14	ND
4b	188.0 ± 0.13	137.2 ± 0.16	>200	ND
5b	135.6 ± 0.06	80.11 ± 0.17	171.7 ± 0.16	ND
c	125.4 ± 0.18	70.42 ± 0.15	92.74 ± 0.13	ND
1c	112.6 ± 0.07	112.6 ± 0.13	109.0 ± 0.09	ND
2c	137.4 ± 0.10	107.1 ± 0.13	131.5 ± 0.14	ND
3c	140.5 ± 0.11	109.6 ± 0.13	130.0 ± 0.18	ND
4c	199.5 ± 0.10	106.3 ± 0.13	133.0 ± 0.13	ND
5c	135.0 ± 0.10	80.18 ± 0.16	113.5 ± 0.10	ND
d	108.4 ± 0.08	81.95 ± 0.17	167.5 ± 0.11	ND
1d	182.9 ± 0.06	139.1 ± 0.16	129.2 ± 0.05	ND
2d	163.6 ± 0.10	103.4 ± 0.11	>200	ND
3d	158.0 ± 0.19	74.35 ± 0.09	111.3 ± 0.09	ND
4d	135.0 ± 0.15	166.8 ± 0.17	107.0 ± 0.13	ND
5d	128.6 ± 0.09	82.63 ± 0.17	163.1 ± 0.15	ND

Data presented the mean ± standard deviation (S.D.) of each compound from three independent experiments.
ND: not detected.

line was displayed by the uracil derived, acetyl substituted pyranonucleoside **3a** (59.3 μM), with a threefold greater potency than the intermediate uracil alkyne compound **a** (177.9 μM). Moreover, compounds **1b**, **2b**, **5b**, **5c**, **3d**, and **5d** exhibited more potent cytotoxic effects (65.4–82.6 μM) than other compounds. Contrary to this, higher concentrations of the candidate compounds were essential for the inhibition of other two cancer cells, MDA-MB-231 and PC-3.

Based on these findings, although integration of sugar and nucleobase scaffolds to form 1,2,3-triazole skeleton have demonstrated their usefulness by playing specific roles in the exhibition of cytotoxicity, no considerable potency has been detected in MDA-MB-231 and PC-3 cells (IC_{50} ranging from 103.4 to > 200 μM). But, it is worth noting that the cytotoxicity data obtained from this study indicate that different carbohydrate and/or nucleobase scaffolds, position of linkage and so steric hindrance markedly influence the activity potential of compounds. In this context, we recognize that anomeric glycosidic linkage to the triazole ring is important for keeping the cytotoxic efficacy of compounds and as mentioned above, compound **3a** is the most potent molecule with IC_{50} value of 59.3 μM. Besides, galactose derived nucleosides with the glycosidic linkage to the C-6″ position displayed less activity (65.4 > IC_{50} > 200) than anomeric glycoside derived nucleosides while fructose derived nucleosides linking to the triazole at C-2″ position were defined to be the least potent (77.1 > IC_{50} > 200) among all synthesized pyranonucleosides.

Conclusion

In conclusion, we have presented fourteen new triazole-bridged purine and pyrimidine nucleosides (**2a**, **5a**, **2b**, **5b**, **1c**-**5c**, **1d**-**5d**) were synthesized along with six known compounds (**1a**, **3a**, **4a**, **1b**, **3b**, **4b**) *via* the highly efficient CuAAC reaction of pyrano-sugar azides and terminal alkynated nucleobases. The structures of all these products were confirmed by FTIR, ^{1}H, ^{13}C, ^{19}F, HSQC, HMBC spectroscopy and MS data. All 1,4-disubstituted triazole compounds were tested for their biological efficacy against human cancer cell lines (MDA-MB-231, Hep3B and PC-3). It was determined that nucleoside **3a** had the best potency against Hep3B cells with IC_{50} value of 59.3 μM and Hep3B was the most convenient cell line. Based on these *in vitro* assays, it has been aimed to start creating a nucleoside library to see activity-structure relationship and provide other scientists an opportunity for accession to these structures throwing light on the future works.

Experimental

General methods

Melting points were determined using a Gallenkamp Electrothermal melting point apparatus and are uncorrected. IR spectroscopy was performed on a Perkin Elmer Spectrum 100 FTIR spectrophotometer equipped with ATR apparatus. Optical rotations were measured on a Rudolph Research Analytical Autopol II automatic

polarimeter. All 1D- and 2D-NMR spectra (^{1}H, ^{13}C, ^{19}F, HSQC and HMBC) were recorded on a Varian AS 400 MHz spectrometer in $CDCl_3$ or DMSO-d_6 using TMS as internal standard. High resolution mass spectra (HRMS) analyses were performed on an Agilent 6230 TOF-MS equipped with Jet Stream ESI source operated in positive ion mode. All reactions were monitored by thin layer chromatography using precoated silica gel G-60 F_{254} (Merck 5554) aluminum plates with detection under UV light (254 and 366 nm) and sprayed with 20% H_2SO_4 solution in water followed by heating at 120°C or basic aqueously $KMnO_4$ solution. While 70 × 230 mesh silica gel (Merck 7734) was used for open column chromatographic studies, flash chromatography was performed using 230 × 400 mesh silica gel (Merck 9385) with the indicated solvent mixture. All starting materials, solvents and other chemicals were purchased from Merck, Fluka, Carlo Erba, Alfa Aesar or Sigma-Aldrich and were used without purification.

Synthesis

General procedure for the syntheses of propargylated nucleobases

To a stirred suspension of nucleobase (uracil, thymine, 5-fluorouracil and adenine, 1.0 eq.) in anhydrous DMF, K_2CO_3 (1.0 eq.) was added and stirred for 30 min. Then propargyl bromide (1.1 eq.) was added to this mixture and allowed to stir at 50°C for 8–12 hours with the assistance of TLC (CH_2Cl_2:MeOH, 9:0.4) control. After the removal of the solvent by evaporating under reduced pressure, the residue was purified by silica gel column chromatography and afforded the pure monopropynylated products (**a**-**d**).[44]

N-1-Propargyl uracil (a)

Following the general procedure with uracil (5.0 g, 44.6 mmol) and propargyl bromide (5.5 mL, 61.7 mmol) in DMF, the residue was then subjected to column chromatography using CH_2Cl_2:MeOH (100:0→100:1.5) solvent system and white solid (**a**) was obtained. Yield: 50%; mp 168–169°C; FTIR (ATR, cm^{-1}): 3243 (–NH), 3114 (C=CH), 2923 and 2851 (–CH), 2120 (C≡C), 1715 and 1688 (C=O), 1620 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H, NH), 7.66 (d, 1H, $J_{5,6}$ = 8.0 Hz, H-6), 5.60 (d, 1H, H-5), 4.49 (d, 2H, $J_{CH2,C\equiv CH}$ = 2.4 Hz, –C$\underline{H}_2$–C≡CH), 3.35 (t, 1H, –CH$_2$–C≡C$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ164.2 (C-4), 151.0 (C-2), 145.1 (C-6), 102.3 (C-5), 79.0 (–CH$_2$–$\underline{C}$≡CH), 76.4 (–CH$_2$–C≡$\underline{C}$H), 37.3 (–$\underline{C}$H$_2$–C≡CH).

N-1-Propargyl thymine (b)

Following the general procedure with thymine (5.0 g, 39.6 mmol) and propargyl bromide (4.8 mL, 53.8 mmol) in DMF, the residue was then subjected to column chromatography using CH_2Cl_2:MeOH (100:0→100:2) solvent system and white solid (**b**) was obtained. Yield: 65%; mp 158–160°C; FTIR (ATR, cm^{-1}): 3254 (–NH), 3160 (C=CH), 2932, 2893, 2833 (–CH), 2124 (C≡C), 1704 and 1680 (C=O); ^{1}H NMR

(400 MHz, DMSO-d_6): δ 11.30 (s, 1H, NH), 7.52 (t, 1H, $J_{6,CH3}$ = 1.2 Hz, H-6), 4.45 (dd, 2H, $J_{CH2,C\equiv CH}$ = 1.2 Hz, −C<u>H</u>$_2$−C≡CH), 3.29 (d, 1H, −CH$_2$−C≡C<u>H</u>), 1.74 (d, 3H, CH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.8 (C-4), 151.0 (C-2), 140.7 (C-6), 110.1 (C-5), 79.3 (−CH$_2$−<u>C</u>≡CH), 76.2 (−CH$_2$−C≡<u>C</u>H), 36.9 (−<u>C</u>H$_2$−C≡CH), 12.5 (CH$_3$).

N-1-Propargyl 5-fluorouracil (c)

Following the general procedure with 5-fluorouracil (5.0 g, 38.4 mmol) and propargyl bromide (4.7 mL, 52.7 mmol) in DMF, the residue was then subjected to column chromatography using CH_2Cl_2:MeOH (100:0→100:1) solvent system and white solid (**c**) was obtained. Yield: 40%; mp 147–148°C; FTIR (ATR, cm^{-1}): 3225 (–NH), 3030 (C=CH), 2825 (–CH), 2132 (C≡C), 1705 (C=O), 1651 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.86 (bs, 1H, NH), 8.08 (d, 1H, J = 6.4 Hz, H-6), 4.43 (d, 2H, $J_{CH2,C\equiv CH}$ = 2.0 Hz, −C<u>H</u>$_2$−C≡CH), 3.36 (t, 1H, −CH$_2$−C≡C<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.1, 157.9 (C-4), 149.7 (C-2), 141.6, 139.3 (C-5), 129.6, 129.4 (C-6), 78.7 (−CH$_2$−<u>C</u>≡CH), 76.7 (−CH$_2$−C≡<u>C</u>H), 37.6 (−<u>C</u>H$_2$−C≡CH).

N-9-Propargyl adenine (d)

Following the general procedure with adenine (5.0 g, 37.0 mmol) and propargyl bromide (4.5 mL, 50.5 mmol) in DMF, the residue was then subjected to column chromatography using CH_2Cl_2:MeOH (100:0→100:3.5) solvent system and white solid (d) was obtained. Yield: 69%; mp 214-215°C; FTIR (ATR, cm^{-1}): 3372 and 3245 (−NH$_2$), 3139 (C=CH), 2910 and 2863 (−CH), 2110 (C≡C), 1686 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.17 (d, 1H, $J_{2,8}$ = 0.8 Hz, H-2), 8.15 (d, 1H, H-8), 7.24 (bs, 2H, NH$_2$), 5.01 (d, 2H, $J_{CH2,C\equiv CH}$ = 2.0 Hz, −C<u>H</u>$_2$−C≡CH), 3.40 (t, 1H, −CH$_2$−C≡C<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.6 (C-6), 153.4 (C-2), 149.7 (C-4), 140.8 (C-8), 119.1 (C-5), 78.9 (−CH$_2$−<u>C</u>≡CH), 76.4 (−CH$_2$−C≡<u>C</u>H), 32.9 (−<u>C</u>H$_2$−C≡CH).

General procedure for the CuAAC reactions

To a well stirred solution of azido-sugar (**1**-**5**) (1.1 eq) and propargylated nucleobase (**a**-**d**) (1.0 eq) in THF:*t*-BuOH:H$_2$O (3:1:1) solvent system, CuSO$_4$.5H$_2$O (0.11 eq) and sodium ascorbate (0.55 eq) was added.[45] The mixture was then allowed to stir at 50°C for 3-6 hours along with TLC monitoring. After completion, the reaction mixture was diluted with water and extracted with $CHCl_3$ (3 × 30 mL). The combined organic fractions were dried over anhydrous Na_2SO_4 and solvent evaporated under reduced pressure to afford crude residue. After all, the residue was subjected to silica gel flash column chromatography using CH_2Cl_2:MeOH system and afforded the pure products (**1a**-**5a**, **1b**-**5b**, **1c**-**5c**, **1d**-**5d**).

1-[{1′-(6″-Deoxy-1″,2″:3″,4″-di-O-isopropylidene-α-D-galactopyranos-6″-yl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]uracil (1a)

According to the general CuAAC procedure, compound **1a**[12] (678 mg, 82%) was obtained from the reaction of azido sugar **1** (600 mg, 2.1 mmol) with propargyl

uracil **a** (287 mg, 1.9 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:1.5 *v*/*v*). White foam; lit mp 177–179°C, mp 189–191°C; $[\alpha]_D^{20}$-32 (*c* 0.25, $CHCl_3$); FTIR (ATR, cm^{-1}): 3138 (–NH-), 3051 (C=CH), 2987, 2937 and 2835 (–CH), 1697 (C=O), 1620 (C=C), 1385 (C–N), 1065 (C-O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.86 (bs, 1H, NH), 7.87 (s, 1H, H-5′), 7.51 (d, 1H, $J_{5,6}$ = 7.6 Hz, H-6), 5.69 (d, 1H, H-5), 5.50 (d, 1H, $J_{1'',2''}$= 4.8 Hz, H-1″), 5.07, 4.96 (q, AB-system, 2H, J = 15.2 Hz, CH_2), 4.65 (dd, 1H, $J_{2'',3''}$= 2.8 Hz, $J_{3'',4''}$= 8.0 Hz, H-3″), 4.61 (dd, 1H, $J_{5'',6''a}$ = 3.6 Hz, $J_{6''a,6''b}$ = 14.0 Hz, H-6″a), 4.46 (dd, 1H, $J_{5'',6''b}$ = 8.8 Hz H-6″b), 4.33 (dd, 1H, H-2″), 4.21 (dd, 1H, $J_{4'',5''}$= 8.0 Hz H-4″), 4.19-4.17 (m, 1H, H-5″), 1.48, 1.37, 1.36 and 1.28 (s, 12H, 4 × C$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.1 (C-4), 151.1 (C-2), 144.4 (C-6), 141.6 (C-4′), 125.1 (C-5′), 110.1 and 109.2 (2 × $(CH_3)_2\underline{C}$), 102.7 (C-5), 96.3 (C-1″), 71.3 (C-4″), 70.9 (C-3″), 70.4 (C-2″), 67.3 (C-5″), 50.8 (C-6″), 43.1 ($\underline{C}H_2$–N), 26.1, 26.0, 25.0 and 24.6 (4 × $\underline{C}H_3$); TOF-ESIMS calcd for $C_{19}H_{26}N_5O_7$ $[M + H]^+$: 436.1832; found: 436.1912.

1-[{1′-(1‴-Deoxy-2″,3″:4″,5″-di-O-isopropylidene-β-D-fructopyranos-1″-yl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]uracil (2a)

According to the general CuAAC procedure, **2a** (703 mg, 85%) was obtained from the reaction of azido sugar **2** (600 mg, 2.1 mmol) with propargyl uracil **a** (287 mg, 1.9 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:1.5 *v*/*v*). White foam; mp 150–152°C; $[\alpha]_D^{20}$-56 (*c* 0.25, $CHCl_3$); FTIR (ATR, cm^{-1}): 3208 (–NH), 3060 (C=CH), 2990, 2940 (–CH), 1683 (C=O), 1382 (C–N), 1068 (C–O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.73 (bs, 1H, NH), 7.89 (s, 1H, H-5′), 7.53 (d, 1H, $J_{5,6}$ = 8.0 Hz, H-6), 5.69 (d, 1H, H-5), 5.06, 4.96 (q, AB-system, 2H, J = 15.2 Hz, CH_2), 4.73 (d, 1H, $J_{1''a,1''b}$ = 14.0 Hz, H-1″a), 4.65 (dd, 1H, $J_{4'',5''}$= 8.0 Hz, $J_{3'',4''}$= 2.8 Hz, H-4″), 4.56 (d, 1H, H-1″b), 4.43 (d, 1H, H-3″), 4.25 (dd, 1H, $J_{5'',6''a}$ = 1.2 Hz, H-5″), 3.91 (dd, 1H, $J_{6''a,6''b}$ = 13.2 Hz, H-6″a), 3.79 (d, 1H, H-6″b), 1.50, 1.45, 1.36 and 0.74 (s, 12H, 4 × C$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.0 (C-4), 151.1 (C-2), 144.4 (C-6), 141.7 (C-4′), 126.6 (C-5′), 109.7 and 109.5 (2 × $(CH_3)_2\underline{C}$), 102.8 (C-5), 100.8 (C-2″), 70.9 (C-3″), 70.7 (C-5″), 70.2 (C-4″), 62.0 (C-6″), 55.3 (C-1″), 43.0 ($\underline{C}H_2$–N), 26.5, 26.1, 24.3 and 24.2 (4 × $\underline{C}H_3$); TOF-ESIMS calcd for $C_{19}H_{26}N_5O_7$ $[M + H]^+$: 436.1832; found: 436.1929.

1-[{1′-(2″,3″,4″,6″-Tetra-O-acetyl-β-D-glucopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]uracil (3a)

According to the general CuAAC procedure, **3a**[12] (659 mg, 90%) was obtained from the reaction of azido sugar **3** (600 mg, 1.6 mmol) with propargyl uracil **a** (219 mg, 1.4 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:1.5 *v*/*v*). White foam; mp 228–229°C; $[\alpha]_D^{20}$ + 21 (*c* 0.25, $CHCl_3$); FTIR (ATR, cm^{-1}): 3055 (C=CH), 1753, 1688 (C=O), 1630 (C=C), 1371 (C–N), 1043 (C-O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.79 (bs, 1H, NH), 8.10 (s, 1H, H-5′), 7.48 (d, 1H, $J_{5,6}$ = 8.0 Hz, H-6), 5.91 (m, 1H, H-1″), 5.73 (d, 1H, H-5), 5.48-5.42 (m, 1H, H-2″), 5.45 (dd, 1H, $J_{2'',3''}$= 6.6 Hz, $J_{3'',4''}$= 2.4 Hz, H-3″), 5.31 (dd, 1H, $J_{4'',5''}$= 9.8 Hz, H-4″), 5.06, 5.00 (q, AB-system, 2H, J = 15.2 Hz, CH_2), 4.31

(dd, 1H, $J_{5'',6''a}$ = 4.8 Hz, $J_{6''a,6''b}$ = 12.6 Hz, H-6″a), 4.18 (dd, 1H, $J_{5'',6''b}$ = 1.6 Hz H-6″b), 4.05 (ddd, 1H, H-5″), 2.07, 2.07, 2.01 and 1.82 (s, 12H, 4 × C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.1, 169.6, 168.9 (4 × Acetyl–<u>C</u>=O), 163.9 (C-4), 151.3 (C-2), 144.2 (C-6), 142.7 (C-4′), 123.0 (C-5′), 103.0 (C-5), 86.0 (C-1″), 75.3 (C-5″), 72.6 (C-3″), 70.7 (C-2″), 67.9 (C-4″), 61.8 (C-6″), 43.2 (<u>C</u>H$_2$–N), 20.8, 20.7, 20.6 and 20.2 (4 × <u>C</u>H$_3$); TOF-ESIMS calcd for $C_{21}H_{26}N_5O_{11}$ $[M+H]^+$: 524.1628; found: 524.1714.

1-[{1′-(2″,3″,4″,6″-Tetra-O-acetyl-β-D-galactopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]uracil (4a)

According to the general CuAAC procedure, **4a**[12] (674 mg, 92%) was obtained from the reaction of azido sugar **4** (600 mg, 1.6 mmol) with propargyl uracil **a** (219 mg, 1.4 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:1.5 *v*/*v*). White foam; mp 165–167°C; $[\alpha]_D^{20}$ + 32 (*c* 0.25, $CHCl_3$); FTIR (ATR, cm^{-1}): 3180 (C=CH), 2970 (–CH), 1746 and 1687 (C=O), 1630 (C=C), 1374 (C–N), 1042 (C-O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76 (bs, 1H, NH), 7.99 (s, 1H, H-5′), 7.45 (d, 1H, $J_{5,6}$ = 8.0 Hz, H-6), 5.83 (d, 1H, $J_{1'',2''}$= 9.2 Hz, H-1″), 5.69 (d, 1H, H-5), 5.52 (d, 1H, $J_{3'',4''}$= 3.2 Hz, $J_{4'',5''}$= 0.0 Hz, H-4″), 5.46 (dd, 1H, $J_{2'',3''}$= 10.0 Hz, H-2″), 5.25 (dd, 1H, H-3″), 5.08, 4.90 (q, AB-system, 2H, *J* = 15.2 Hz, CH_2), 4.25–4.16 (m, 2H, H-5″ and H-6″a), 4.11 (dd, 1H, $J_{5'',6''b}$ =6.4 Hz, $J_{6''a,6''b}$ =11.2 Hz, H-6″b), 2.20, 2.00, 1.97 and 1.82 (s, 12H, 4 × C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.0, 169.8 and 168.9 (4 × Acetyl–<u>C</u>=O), 163.8 (C-4), 150.8 (C-2), 144.1 (C-6), 142.4 (C-4′), 122.5 (C-5′), 102.6 (C-5), 86.3 (C-1″), 74.1 (C-5″), 70.5 (C-3″), 68.1 (C-2″), 66.7 (C-4″), 61.2 (C-6″), 42.7 (<u>C</u>H$_2$–N), 20.6, 20.6, 20.4 and 20.1 (4 × <u>C</u>H$_3$); TOF-ESIMS calcd for $C_{21}H_{26}N_5O_{11}$ $[M+H]^+$: 524.1628; found: 524.1609.

1-[{1′-(2″,3″,4″-Tri-O-acetyl-β-D-xylopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]uracil (5a)

According to the general CuAAC procedure, **5a** (666 mg, 82%) was obtained from the reaction of azido sugar **5** (600 mg, 2 mmol) with propargyl uracil **a** (271 mg, 1.8 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:1.5 *v*/*v*). White foam; mp 110–112°C; $[\alpha]_D^{20}$ + 29 (*c* 0.25, $CHCl_3$); FTIR (ATR, cm^{-1}): 2956, 2937, 2899 and 2867 (–CH), 1596 (C=C), 1372 (C–N), 1037 (C-O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.66 (bs, 1H, NH), 8.04 (s, 1H, H-5′), 7.43 (d, 1H, $J_{5,6}$ = 7.6 Hz, H-6), 5.77 (dd, 1H, $J_{1'',2''}$= 8.0 Hz, H-1″), 5.70 (d, 1H, H-5), 5.43-5.38 (m, 1H, H-3″), 5.39 (d, 1H, H-2″), 5.21–5.16 (m, 1H, H-4″), 5.05, 4.92 (q, AB-system, 2H, *J* = 15.2 Hz, CH_2), 4.27 (dd, 1H, $J_{4'',5''a}$ = 6.0 Hz, $J_{5''a,5''b}$ = 11.2 Hz, H-5″a), 3.58 (dd, 1H, $J_{4'',5''b}$ = 11.2 Hz, H-5″b), 2.05, 2.02 and 1.82 (s, 9H, 3 × C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 169.7, 168.8 (3 × Acetyl–<u>C</u>=O), 163.6 (C-4), 151.0 (C-2), 144.0 (C-6), 142.4 (C-4′), 122.8 (C-5′), 102.8 (C-5), 86.3 (C-1″), 71.9 (C-3″), 70.5 (C-2″), 68.2 (C-4″), 65.5 (C-5″), 43.1 (<u>C</u>H$_2$–N), 20.6, 20.5 and 20.1 (3 × <u>C</u>H$_3$); TOF-ESIMS calcd for $C_{18}H_{21}N_5O_9Na[M+Na]^+$: 474.1237; found: 474.1192.

1-[{1′-(6″-Deoxy-1″,2″:3‴,4″-di-O-isopropylidene-α-D-galactopyranos-6″-yl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]thymine (1b)

According to the general CuAAC procedure, **1b**[12] (785 mg, 92%) was obtained from the reaction of azido sugar **1** (600 mg, 2.1 mmol) with propargyl thymine **b** (313 mg, 1.9 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:2.0 *v*/*v*). White foam; mp 238–240°C; $[\alpha]_D^{20}$-24 (*c* 0.25, $CHCl_3$); FTIR (ATR, cm^{-1}): 3129 (C=CH), 2995, 2933 and 2828 (–CH), 1680 (C=O), 1651 (C=C) 1368 (C–N), 1050 (C-O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.30 (bs, 1H, NH), 7.85 (s, 1H, H-5′), 7.32 (d, 1H, $J_{6,\text{thymine-CH3}} = 0.8$ Hz, H-6), 5.49 (d, 1H, $J_{1'',2''} = 4.8$ Hz, H-1″), 5.04, 4.91 (q, AB-system, 2H, $J = 15.2$ Hz, CH_2), 4.64 (dd, 1H, $J_{2'',3''} = 2.4$ Hz, $J_{3'',4''} = 7.8$ Hz, H-3″), 4.60 (dd, 1H, $J_{5'',6''a} = 4.0$ Hz, $J_{6''a,6''b} = 10.8$ Hz, H-6″a), 4.45 (dd, 1H, $J_{5'',6''b} = 8.4$ Hz, H-6″b), 4.33 (dd, 1H, H-2″), 4.20 (dd, 1H, $J_{4'',5''} = 2.0$ Hz, H-4″), 4.18 (ddd, 1H, H-5″), 1.89 (d, 3H, thymine-C$\underline{H}_3$),1.48, 1.37, 1.35 and 1.28 (s, 12H, 4 × C$\underline{H}_3$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 164.4 (C-4), 151.1 (C-2), 141.9 (C-4′), 140.3 (C-6), 125.0 (C-5′), 111.2 (C-5), 110.1 and 109.2 (2 × $(CH_3)_2\underline{C}$), 96.4 (C-1″), 71.3 (C-4″), 70.9 (C-3″), 70.5 (C-2″), 67.3 (C-5″), 50.8 (C-6″), 42.9 ($\underline{C}H_2$–N), 26.0, 26.0, 25.0 and 24.6 (4 × $\underline{C}H_3$), 12.4 (thymine–$\underline{C}H_3$); TOF-ESIMS calcd for $C_{20}H_{28}N_5O_7$ $[M + H]^+$: 450.1988; found: 450.2091.

1-[{1′-(1″-Deoxy-2″,3″:4″,5″-di-O-isopropylidene-β-D-fructopyranos-1″-yl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]thymine (2b)

According to the general CuAAC procedure, **2b** (785 mg, 92%) was obtained from the reaction of azido sugar **2** (600 mg, 2.1 mmol) with propargyl thymine **b** (313 mg, 1.9 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:2.0 *v*/*v*). White foam; mp 105–107°C; $[\alpha]_D^{20}$-24 (*c* 0.25, $CHCl_3$); FTIR (ATR, cm^{-1}): 3147 (C=CH), 2988, 2916 (–CH), 1674 (C=O), 1372 (C–N), 1067 (C-O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ9.80 (bs, 1H, NH), 7.90 (s, 1H, H-5′), 7.35 (d, 1H, $J_{6,CH3} = 1.2$ Hz, H-6), 5.04, 4.93 (q, AB-system, 2H, $J = 15.2$ Hz, CH_2), 4.73 (d, 1H, $J_{1''a,1''b} = 14.4$ Hz, H-1″a), 4.65 (dd, 1H, $J_{3'',4''} = 2.8$ Hz, $J_{4'',5''} = 7.8$ Hz, H-4″), 4.64 (d, 1H, H-1″b), 4.45 (d, 1H, H-3″), 4.25 (dd, 1H, $J_{5'',6''a} = 1.6$ Hz, $J_{5'',6''b} = 0.0$ Hz, H-5″), 3.91 (dd, 1H, $J_{6''a,6''b} = 12.8$ Hz, H-6″a), 3.79 (d, 2H, H-6″b), 1.88 (s, 3H, thymine-C$\underline{H}_3$), 1.50, 1.45, 1.36 and 0.72 (s, 12H, 4 × C$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.6 (C-4), 151.2 (C-2), 142.2 (C-4′), 140.3 (C-6), 126.6 (C-5′), 111.3 (C-5), 109.7 and 109.5 (2 × $(CH_3)_2\underline{C}$), 100.8 (C-2″), 70.8 (C-3″), 70.7 (C-5″), 70.2 (C-4″), 62.0 (C-6″), 55.2 (C-1″), 42.7 ($\underline{C}H_2$–N), 26.5, 26.1, 24.3 and 24.2 (4 × $\underline{C}H_3$), 12.3 (thymine–$\underline{C}H_3$); TOF-ESIMS calcd for $C_{20}H_{28}N_5O_7$ $[M + H]^+$: 450.1988; found: 450.1987.

1-[{1′-(2″,3″,4″,6‴-Tetra-O-acetyl-β-D-glucopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]thymine (3b)

According to the general CuAAC procedure, **3b**[12] (654 mg, 87%) was obtained from the reaction of azido sugar **3** (600 mg, 1.6 mmol) with propargyl thymine **b** (239 mg, 1.4 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:2.0 *v*/*v*). White foam; mp 242–244°C; $[\alpha]_D^{20}$ + 27 (*c* 0.25, $CHCl_3$); FTIR

(ATR, cm^{-1}): 3043 (C=CH), 2815 (–CH), 1749, 1739, 1696 and 1678 (C=O), 1369 (C–N), 1038 (C-O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76 (bs, 1H, NH), 8.04 (s, 1H, H-5′), 7.24 (s, 1H, H-6), 5.87 (dd, 1H, $J_{1'',2''}$ = 8.4 Hz, H-1″), 5.45–5.37 (m, 2H, H-2″ and H-3″), 5.23 (dd, 1H, $J_{3'',4''} = J_{4'',5''}$ =9.6 Hz, H-4″), 4.99, 4.89 (q, AB-system, 2H, J = 15.2 Hz, CH_2), 4.25 (dd, 1H, $J_{5'',6''a}$ = 4.8 Hz, $J_{6''a,6''b}$ = 12.4 Hz, H-6″a), 4.12 (d, 1H, $J_{5'',6''b}$ = 0.0 Hz, H-6″b), 4.05 (dd, 1H, H-5″), 2.01, 2.01, 1.96 and 1.77 (s, 12H, 4 × C<u>H</u>$_3$), 1.84 (s, 3H, thymine-C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.1, 169.5, 168.9 (4 × Acetyl–<u>C</u> = O), 164.4 (C-4), 151.3 (C-2), 143.0 (C-4′), 140.1 (C-6), 123.0 (C-5′), 111.5 (C-5), 85.9 (C-1″), 75.3 (C-5″), 72.7 (C-3″), 70.7 (C-2″), 67.9 (C-4″), 61.8 (C-6″), 42.8 (<u>C</u>H$_2$–N), 20.8, 20.6, 20.6 and 20.2 (4 × <u>C</u>H$_3$), 12.3 (thymine–<u>C</u>H$_3$); TOF-ESIMS calcd for $C_{22}H_{28}N_5O_{11}$ $[M + H]^+$: 538.1785; found: 538.1896.

1-[{1′-(2″,3″:4″,6″-Tetra-O-acetyl-β-D-galactopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]thymine (4b)

According to the general CuAAC procedure, **4b**[12] (677 mg, 90%) was obtained from the reaction of azido sugar **4** (600 mg, 1.6 mmol) with propargyl thymine **b** (239 mg, 1.4 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:2.0 *v*/*v*). White foam; mp 211–213°C; $[\alpha]_D^{20}$ + 16 (*c* 0.25, $CHCl_3$); FTIR (ATR, cm^{-1}): 3034 (C=CH), 2836 (–CH), 1747, 1732, 1708 and 1682 (C=O), 1367 (C–N), 1044 (C-O–C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.87 (s, 1H, NH), 7.98 (s, 1H, H-5′), 7.25 (s, 1H, H-6), 5.82 (d, 1H, $J_{1'',2''}$= 9.2 Hz, H-1″), 5.49 (d, 1H, $J_{3'',4''}$= 3.2 Hz, H-4″), 5.44 (t, 1H, $J_{2'',3''}$= 10.0 Hz, H-2″), 5.24 (dd, 1H, H-3″), 5.05, 4.84 (q, AB-system, 2H, J = 15.2 Hz, CH_2), 4.23 (t, 1H, $J_{5'',6''b}$ = 6.8 Hz, H-5″), 4.15 (dd, 1H, $J_{5'',6''a}$ = 6.0 Hz, H-6″a), 4.08 (dd, 1H, $J_{6''a,6''b}$ = 11.2 Hz, H-6″b), 2.18, 1.98, 1.95 and 1.80 (s, 12H, 4 × C<u>H</u>$_3$), 1.84 (s, 3H, thymine-C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.0, 169.8 and 168.9 (4 × Acetyl–<u>C</u> = O), 164.5 (C-4), 151.0 (C-2), 142.6 (C-4′), 140.0 (C-6), 122.5 (C-5′), 111.1 (C-5), 86.2 (C-1″), 74.0 (C-5″), 70.5 (C-3″), 68.1 (C-2″), 66.8 (C-4″), 61.2 (C-6″), 42.5 (<u>C</u>H$_2$–N), 20.6, 20.5, 20.4 and 20.1 (4 × <u>C</u>H$_3$), 12.2 (thymine–<u>C</u>H$_3$); TOF-ESIMS calcd for $C_{22}H_{28}N_5O_{11}$ $[M + H]^+$: 538.1785; found: 538.1888.

1-[{1′-(2″,3″,4″-Tri-O-acetyl-β-D-xylopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]thymine (5b)

According to the general CuAAC procedure, **5b** (703 mg, 84%) was obtained from the reaction of azido sugar **5** (600 mg, 2 mmol) with propargyl thymine **b** (297 mg, 1.8 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:2.0 *v*/*v*). White foam; mp 122–124°C; $[\alpha]_D^{20}$ + 38 (*c* 0.25, DMSO); FTIR (ATR, cm^{-1}): 3154, 3045 (C=CH), 1744, 1683 (C=O), 1215 (C–N), 1038 (C-O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.35 (s, 1H, H-5′), 7.50 (s, 1H, H-6), 6.17 (d, 1H, $J_{1'',2''}$= 9.2 Hz, H-1″), 5.57 (t, 1H, $J_{2'',3''}$= 9.6 Hz, H-3″), 5.45 (t, 1H, H-2″), 5.09 (td, 1H, $J_{4'',5''a}$ = 5.6 Hz, H-4″), 4.91, 4.86 (q, AB-system, 2H, J = 15.2 Hz, CH_2), 4.06 (dd, 1H, $J_{5''a,5''b}$ = 11.2 Hz, H-5″a), 3.80 (t, 1H, H-5″b), 1.99, 1.96 and 1.75 (s, 9H, 3 ×

$C\underline{H}_3$), 1.73 (s, 3H, thymine-$C\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.9, 169.9 and 168.9 (3 × Acetyl–$\underline{C}$ = O), 164.6 (C-4), 151.1 (C-2), 143.7 (C-4′), 141.2 (C-6), 123.0 (C-5′), 109.4 (C-5), 85.1 (C-1″), 72.2 (C-3″), 70.7 (C-2″), 68.3 (C-4″), 64.5 (C-5″), 42.4 ($\underline{C}H_2$–N), 20.8, 20.7 and 20.3 (3 × $\underline{C}H_3$), 12.3 (thymine–$\underline{C}H_3$); TOF-ESIMS calcd for $C_{19}H_{23}N_5O_9Na$ $[M + Na]^+$: 488.1393; found: 488.1411.

1-[{1′-(6″-Deoxy-1″,2″:3″,4″-di-O-isopropylidene-α-D-galactopyranos-6″-yl)-1′H-1′, 2′,3′-triazol-4′-yl}methyl]5-fluorouracil (1c)

According to the general CuAAC procedure, **1c** (809 mg, 94%) was obtained from the reaction of azido sugar **1** (600 mg, 2.1 mmol) with propargyl 5-fluorouracil **c** (321 mg, 1.9 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:1.0 *v*/*v*). White foam; mp 213–215°C; $[\alpha]_D^{20}$-32 (*c* 0.25, DMSO); FTIR (ATR, cm^{-1}): 3168 (C=CH), 2981, 2828 (–CH), 1718, 1693 (C=O), 1659 (C=C), 1382 (C–N), 1223 (–NH), 1052 (C-O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.81 (bs, 1H, NH), 8.11 (d, 1H, $J_{6,F}$ = 6.4 Hz, H-6), 8.06 (s, 1H, H-5′), 5.38 (d, 1H, $J_{1'',2''}$ = 4.8 Hz, H-1″), 4.89 (s, 2H, $C\underline{H}_2$–N), 4.64 (dd, 1H, $J_{2'',3''}$ = 2.4 Hz, $J_{3'',4''}$ = 8.0 Hz, H-3″), 4.54 (dd, 1H, $J_{5'',6''a}$ = 2.8 Hz, $J_{6''a,6''b}$ = 14.0 Hz, H-6″a), 4.36 (dd, 1H, $J_{5'',6''b}$ = 9.6 Hz, H-6″b), 4.34 (dd, 1H, H-2″), 4.29 (dd, 1H, $J_{4'',5''}$ = 8.0 Hz, H-4″), 4.15 (bd, 1H, H-5″), 1.38, 1.30, 1.32 and 1.21 (s, 12H, 4 × $C\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ157.9 and 157.7 (C-4, J = 25.6 Hz), 149.7 (C-2), 142.3 (C-4′), 141.2 and 138.9 (C-5, J = 228.6 Hz), 130.3 and 129.9 (C-6, J = 33.3 Hz), 124.4 (C-5′), 109.2 and 108.5 (2 × $(CH_3)_2\underline{C}$), 95.9 (C-1″), 71.0 (C-4″), 70.6 (C-3″), 70.1 (C-2″), 67.2 (C-5″), 50.6 (C-6″), 43.0 ($\underline{C}H_2$–N), 26.3, 26.0, 25.2 and 24.7 (4 × $\underline{C}H_3$); ^{19}F NMR (376 MHz, DMSO-d_6): δ−169.1 (d, 1F, $J_{F,H6}$ = 6.8 Hz, $C\underline{F}$ = CH–N); TOF-ESIMS calcd for $C_{19}H_{25}FN_5O_7$ $[M + H]^+$: 454.1738; found: 454.1802.

1-[{1′-(1″-Deoxy-2″,3″:4″,5″-di-O-isopropylidene-β-D-fructopyranos-1″-yl)-1′H-1′, 2′,3′-triazol-4′-yl}methyl]5-fluorouracil (2c)

According to the general CuAAC procedure, **2c** (800 mg, 93%) was obtained from the reaction of azido sugar **2** (600 mg, 2.1 mmol) with propargyl 5-fluorouracil **c** (321 mg, 1.9 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:1.0 *v*/*v*). White foam; mp 129–131°C; $[\alpha]_D^{20}$-24 (*c* 0.25, DMSO); FTIR (ATR, cm^{-1}): 3120 (C=CH), 2987, 2948, 2834 (–CH), 1691 (C=O), 1644 (C=C), 1373 (C–N), 1211 (–NH), 1065 (C-O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (bs, 1H, NH), 8.14 (d, 1H, $J_{6,F}$ = 6.8 Hz, H-6), 8.04 (s, 1H, H-5′), 4.90, 4.86 (q, AB-system, 2H, J = 15.2 Hz, CH_2), 4.62 (dd, 1H, $J_{3'',4''}$ = 2.8 Hz, $J_{4'',5''}$ = 8.0 Hz, H-4″), 4.58 (s, 2H, H-1″), 4.38 (d, 1H, H-3″), 4.23 (bd, 1H, $J_{5'',6''a}$ = 1.2 Hz, H-5″), 3.73 (dd, 1H, $J_{6''a,6''b}$ = 12.8 Hz, H-6″a), 3.60 (d, 1H, H-6″b), 1.37, 1.37, 1.28 and 0.73 (s, 12H, 4 × $C\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.9 and 157.6 (C-4, J = 26.6 Hz), 149.8 (C-2), 142.2 (C-4′), 141.1 and 138.9 (C-5, J = 228.6 Hz), 130.4 and 130.1 (C-6, J = 33.4 Hz), 126.5 (C-5′), 109.0 and 108.7 (2 × $(CH_3)_2\underline{C}$), 100.9 (C-2″), 70.6 (C-3″), 70.2 (C-5″), 69.7 (C-4″), 61.4 (C-6″), 55.0 (C-1″), 42.9 ($\underline{C}H_2$–N), 26.5, 26.2, 24.4 and 24.3 (4 × $\underline{C}H_3$); ^{19}F NMR (376 MHz, DMSO-d_6): δ−169.3 (d, 1F, $J_{F,H6}$ =

7.2 Hz, C<u>F</u> = CH–N); TOF-ESIMS calcd for $C_{19}H_{25}FN_5O_7$ $[M + H]^+$: 454.1738; found: 454.1763.

1-[{1′-(2″,3″,4″,6″-Tetra-O-acetyl-β-D-glucopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl} methyl]5-fluorouracil (3c)

According to the general CuAAC procedure, **3c** (682 mg, 90%) was obtained from the reaction of azido sugar **3** (600 mg, 1.6 mmol) with propargyl 5-fluorouracil **c** (245 mg, 1.4 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:1.0 *v*/*v*). White foam; mp 229–230°C; $[\alpha]_D^{20}$ + 8 (*c* 0.25, DMSO); FTIR (ATR, cm^{-1}): 3150, 3075 (C=CH), 1740, 1689 (C=O), 1668 (C=C), 1370 (C–N), 1226 (–NH), 1042 (C-O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ11.81 (bs, 1H, NH), 8.39 (s, 1H, H-5′), 8.09 (d, 1H, $J_{6,F}$ = 6.4 Hz, H-6), 6.30 (d, 1H, $J_{1'',2''}$= 9.2 Hz, H-1″), 5.59 (t, 1H, $J_{2'',3''}$= 9.2 Hz, H-2″), 5.52 (t, 1H, $J_{3'',4''}$= 9.6 Hz, H-3″), 5.15 (t, 1H, $J_{4'',5''}$= 9.6 Hz, H-4″), 4.90 (s, 2H, C<u>H</u>$_2$–N), 4.34 (m, 1H, H-5″), 4.14–4.04 (m, 2H, $J_{5'',6''a}$ = 4.8 Hz, $J_{6''a,6''b}$ = 14.8 Hz, H-6″a and H-6″b), 2.01, 1.98, 1.94 and 1.76 (s, 12H, 4 × C<u>H</u>$_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.4, 169.9, 169.7, 168.8 (4 × Acetyl–<u>C</u> = O), 157.9 and 157.7 (C-4, *J* = 25.6 Hz), 149.7 (C-2), 143.4 (C-4′), 141.3 and 139.0 (C-5, *J* = 228.6 Hz), 130.3 and 129.9 (C-6, *J* = 33.3 Hz), 123.1 (C-5′), 84.3 (C-1″), 73.7 (C-5″), 72.5 (C-3″), 70.6 (C-2″), 67.9 (C-4″), 62.2 (C-6″), 43.0 (<u>C</u>H$_2$–N), 20.9, 20.7, 20.6 and 20.2 (4 × <u>C</u>H$_3$); ^{19}F NMR (376 MHz, DMSO-d_6): δ−169.1 (d, 1F, $J_{F,H6}$ = 7.2 Hz, C<u>F</u> = CH–N); TOF-ESIMS calcd for $C_{21}H_{24}FN_5O_{11}Na$ $[M + Na]^+$: 564.1354; found: 564.1371.

1-[{1′-(2″,3″,4″,6″-Tetra-O-acetyl-β-D-galactopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl} methyl]5-fluorouracil (4c)

According to the general CuAAC procedure, **4c** (644 mg, 85%) was obtained from the reaction of azido sugar **4** (600 mg, 1.6 mmol) with propargyl 5-fluorouracil **c** (245 mg, 1.4 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:1.0 *v*/*v*). White foam; mp 109–111°C; $[\alpha]_D^{20}$ + 11 (*c* 0.25, DMSO); FTIR (ATR, cm^{-1}): 3088 (C=CH), 2840 (–CH), 1746, 1697 (C=O), 1367 (C–N), 1211 (–NH), 1043 (C-O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ11.82 (bs, 1H, NH), 8.34 (s, 1H, H-5′), 8.11 (d, 1H, $J_{6,F}$ = 6.8 Hz, H-6), 6.23 (d, 1H, $J_{1'',2''}$= 9.2 Hz, H-1″), 5.54 (t, 1H, $J_{2'',3''}$= 10.0 Hz, H-2″), 5.43 (dd, 1H, $J_{3'',4''}$= 3.6 Hz, H-4″), 5.41 (t, 1H, H-3″), 4.93, 4.89 (q, AB-system, 2H, *J* = 15.6 Hz, CH_2), 4.56 (t, 1H, $J_{5'',6''b}$ = 6.4 Hz, H-5″), 4.12 (dd, 1H, $J_{5'',6''a}$ = 5.2 Hz, $J_{6''a,6''b}$ = 11.6 Hz, H-6″a), 4.01 (dd, 1H, H-6″b), 2.16, 1.96, 1.92 and 1.78 (s, 12H, 4 × C<u>H</u>$_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ170.4, 170.3, 169.9, 168.9 (4 × Acetyl–<u>C</u> = O), 158.0 and 157.7 (C-4, *J* = 25.6 Hz), 149.8 (C-2), 143.2 (C-4′), 141.3 and 139.0 (C-5, *J* = 228.6 Hz), 130.3 and 130.0 (C-6, *J* = 33.3 Hz), 123.5 (C-5′), 84.7 (C-1″), 73.4 (C-5″), 70.7 (C-3″), 68.2 (C-2″), 67.7 (C-4″), 61.9 (C-6″), 42.9 (<u>C</u>H$_2$–N), 20.8, 20.8, 20.7 and 20.3 (4 × <u>C</u>H$_3$); ^{19}F NMR (376 MHz, DMSO-d_6): δ−169.1 (d, 1F, $J_{F,H6}$ = 7.2 Hz, C<u>F</u> = CH–N); TOF-ESIMS calcd for $C_{21}H_{24}FN_5O_{11}Na$ $[M + Na]^+$: 564.1354; found: 564.1367.

1-[{1′-(2″,3″,4‴-Tri-O-acetyl-β-D-xylopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl}methyl] 5-fluorouracil (5c)

According to the general CuAAC procedure, **5c** (675 mg, 80%) was obtained from the reaction of azido sugar **5** (600 mg, 2 mmol) with propargyl 5-fluorouracil **c** (304 mg, 1.8 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:1.0 *v*/*v*). White foam; mp 137–139°C; $[\alpha]_D^{20}$ + 29 (*c* 0.25, DMSO); FTIR (ATR, cm^{-1}): 3066 (C=CH), 1747, 1688 (C=O), 1367 (C–N), 1212 (–NH), 1043 (C-O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.38 (s, 1H, H-5′), 8.07 (d, 1H, $J_{6,F}$ = 6.8 Hz, H-6), 6.18 (d, 1H, $J_{1'',2''}$= 8.8 Hz, H-1″), 5.55 (t, 1H, $J_{2'',3''}$= 9.6 Hz, H-2″), 5.45 (t, 1H, H-3″), 5.08 (td, 1H, $J_{4'',5''a}$ = 5.6 Hz, H-4″), 4.89 (s, 2H, C$\underline{H}_2$–N), 4.06 (dd, 1H, H-5″a), 3.81 (t, 1H, $J_{5''a,5''b}$ = 10.8 Hz, H-5″b); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.9, 169.9 and 168.9 (3 × Acetyl–$\underline{C}$ = O), 158.2 and 157.9 (C-4, *J* = 25.2 Hz), 149.9 (C-2), 143.4 (C-4′), 141.3 and 139.0 (C-5, *J* = 228.7 Hz), 130.1 and 129.8 (C-6, *J* = 33.6 Hz), 123.1 (C-5′), 86.1 (C-1″), 72.2 (C-3″), 70.7 (C-2″), 68.4 (C-4″), 64.5 (C-5″), 43.0 ($\underline{C}H_2$–N), 20.8, 20.7 and 20.3 (3 × $\underline{C}H_3$); ^{19}F NMR (376 MHz, DMSO-d_6): δ−169.0 (d, 1F, $J_{F,H6}$ = 7.2 Hz, C$\underline{F}$ = CH–N); TOF-ESIMS calcd for $C_{18}H_{20}FN_5O_9Na$ $[M + Na]^+$: 492.1142; found: 492.1152.

9-[{1′-(6″-Deoxy-1″,2″:3″,4″-di-O-isopropylidene-α-D-galactopyranos-6‴-yl)-1′H-1′, 2′,3′-triazol-4′-yl}methyl]adenine (1d)

According to the general CuAAC procedure, **1d** (757 mg, 87%) was obtained from the reaction of azido sugar **1** (600 mg, 2.1 mmol) with propargyl adenine **d** (331 mg, 1.9 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:4.0 *v*/*v*). White foam; mp 132–134°C; $[\alpha]_D^{20}$-36 (*c* 0.25, DMSO); FTIR (ATR, cm^{-1}): 3314, 3153 (–NH_2), 2990, 2936 (–CH), 1651 (C=N), 1600 (C=C), 1578 (–NH_2), 1212 (C–N), 1068 (C-O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ8.16 (s, 2H, H-2 and H-8), 8.04 (s, 1H, H-5′), 7.32 (bs, 2H, NH_2), 5.43 (s, 2H, C$\underline{H}_2$–N), 5.35 (d, 1H, $J_{1'',2''}$= 5.2 Hz, H-1″), 4.62 (dd, 1H, $J_{2'',3''}$= 2.4 Hz, $J_{3'',4''}$= 8.0 Hz, H-3″), 4.52 (dd, 1H, $J_{5'',6''a}$ = 2.8 Hz, $J_{6''a,6''b}$ = 14.4 Hz, H-6″a), 4.35 (dd, 1H, $J_{5'',6''b}$ = 9.6 Hz, H-6″b), 4.32 (dd, 1H, H-2″), 4.26 (dd, 1H, $J_{4'',5''}$= 8.0 Hz, H-4″), 4.12 (bd, 1H, H-5″), 1.36, 1.27, 1.24 and 1.19 (s, 12H, 4 × C$\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ156.2 (C-6), 152.7 (C-2), 149.9 (C-4), 143.0 (C-4′), 141.3 (C-8), 124.6 (C-5′), 119.2 (C-5), 109.5 and 108.7 (2 × $(CH_3)_2\underline{C}$), 96.1 (C-1″), 71.2 (C-4″), 70.8 (C-3″), 70.3 (C-2″), 67.4 (C-5″), 50.8 (C-6″), 38.8 ($\underline{C}H_2$–N), 26.5, 26.1, 25.4 and 24.9 (4 × $\underline{C}H_3$); TOF-ESIMS calcd for $C_{20}H_{27}N_8O_5$ $[M + H]^+$: 459.2104; found: 459.2098.

9-[{1′-(1″-Deoxy-2″,3″:4″,5″-di-O-isopropylidene-β-D-fructopyranos-1″-yl)-1′H-1′, 2′,3′-triazol-4′-yl}methyl]adenine (2d)

According to the general CuAAC procedure, **2d** (792 mg, 91%) was obtained from the reaction of azido sugar **2** (600 mg, 2.1 mmol) with propargyl adenine **d** (331 mg, 1.9 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:4.0 *v*/*v*). White foam; mp 128–129°C; $[\alpha]_D^{20}$-24 (*c* 0.25, DMSO); FTIR

(ATR, cm^{-1}): 3311, 3154 ($-NH_2$), 2987, 2937 (−CH), 1644 (C=N), 1597 (C=C), 1575 ($-NH_2$), 1211 (C–N), 1067 (C-O−C); ^1H NMR (400 MHz, DMSO-d_6): δ8.17 (s, 1H, H-8), 8.13 (s, 1H, H-2), 8.05 (s, 1H, H-5′), 7.21 (s, 2H, NH_2), 5.42 (s, 2H, C$\underline{H}_2$–N), 4.59 (dd, 1H, $J_{3'',4''}$= 2.4 Hz, $J_{4'',5''}$= 8.0 Hz, H-4′′), 4.57 (s, 1H, H-1′′), 4.35 (d, 1H, H-3′′), 4.21 (d, 1H, H-5′′), 3.70 (d, 1H, $J_{6''a,6''b}$ = 12.8 Hz, H-6′′a), 3.59 (d, 1H, H-6′′b), 1.34, 1.32, 1.26 and 0.63 (s, 12H, 4 × C$\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ156.3 (C-6), 152.9 (C-2), 149.7 (C-4), 142.7 (C-4′), 141.0 (C-8), 126.4 (C-5′), 119.0 (C-5), 109.0 and 108.7 (2 × $(CH_3)_2\underline{C}$), 100.8 (C-2′′), 70.6 (C-3′′), 70.2 (C-5′′), 69.7 (C-4′′), 61.4 (C-6′′), 55.0 (C-1′′), 38.2 (C$\underline{H}_2$–N), 26.4, 26.1, 24.4 and 24.2 (4 × C$\underline{H}_3$); TOF-ESIMS calcd for $C_{20}H_{27}N_8O_5$ $[M + H]^+$: 459.2104; found: 459.2106.

9-[{1′-(2′′,3′′,4′′,6′′-Tetra-O-acetyl-β-D-glucopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl} methyl]adenine (3d)

According to the general CuAAC procedure, **3d** (711 mg, 93%) was obtained from the reaction of azido sugar **3** (600 mg, 1.6 mmol) with propargyl adenine **d** (253 mg, 1.4 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:4.0 *v*/*v*). White foam; mp 213–215°C; $[\alpha]_D^{20}$ + 18 (*c* 0.25, DMSO); FTIR (ATR, cm^{-1}): 3403, 3322 ($-NH_2$), 3197 (C=CH), 1751, 1708 (C=O), 1647 (C=N), 1601 (C=C), 1579 ($-NH_2$), 1227 (C–N), 1060 (C-O−C); ^1H NMR (400 MHz, DMSO-d_6): δ8.37 (s, 1H, H-5′), 8.12 (s, 2H, H-2, H-8), 7.20 (s, 2H, NH_2), 6.30 (d, 1H, $J_{1'',2''}$= 9.2 Hz, H-1′′), 5.59 (t, 1H, $J_{2'',3''}$= 9.2 Hz, H-2′′), 5.50 (t, 1H, $J_{3'',4''}$= 9.6 Hz, H-3′′), 5.44 (s, 2H, C$\underline{H}_2$–N), 5.14 (t, 1H, H-4′′), 4.32 (m, 1H, $J_{5'',6''a}$ = 2.0 Hz, H-6′′a), 4.10 (dd, 1H, $J_{6''a,6''b}$ = 12.8, H-6′′b), 4.05 (td, 1H, $J_{5'',6''b}$ = 4.8, H-5′′), 1.99, 1.96, 1.93 and 1.72 (s, 12H, 4 × C$\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ170.6, 170.2, 170.0, 169.1 (4 × Acetyl–$\underline{C}$ = O), 156.6 (C-6), 153.3 (C-2), 150.0 (C-4), 143.9 (C-4′), 141.2 (C-8), 123.4 (C-5′), 119.2 (C-5), 84.6 (C-1′′), 74.0 (C-5′′), 72.8 (C-3′′), 70.8 (C-2′′), 68.2 (C-4′′), 62.4 (C-6′′), 38.6 (C$\underline{H}_2$–N), 21.1, 21.0, 20.8 and 20.4 (4 × C$\underline{H}_3$); TOF-ESIMS calcd for $C_{22}H_{27}N_8O_9$ $[M + H]^+$: 547.1901; found 547.1893.

9-[{1′-(2′′,3′′:4′′,6′′-Tetra-O-acetyl-β-D-galactopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl} methyl]adenine (4d)

According to the general CuAAC procedure, **4d** (681 mg, 89%) was obtained from the reaction of azido sugar **4** (600 mg, 1.6 mmol) with propargyl adenine **d** (253 mg, 1.4 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:4.0 *v*/*v*). White foam; mp 105–108°C; $[\alpha]_D^{20}$-8 (*c* 0.25, DMSO); FTIR (ATR, cm^{-1}): 3399, 3331 ($-NH_2$), 3148 (C=CH), 1744 (C=O), 1636 (C=N), 1596 (C=C), 1579 ($-NH_2$), 1211 (C–N), 1046 (C-O−C); ^1H NMR (400 MHz, DMSO-d_6): δ8.36 (s, 1H, H-5′), 8.14 (s, 1H, H-8), 8.12 (s, 1H, H-2), 7.22 (s, 2H, NH_2), 6.22 (d, 1H, $J_{1'',2''}$= 9.2 Hz, H-1′′), 5.54 (t, 1H, $J_{2'',3''}$= 9.6 Hz, H-2′′), 5.44 (s, 2H, C$\underline{H}_2$–N), 5.42 (dd, 1H, $J_{3'',4''}$= 3.2 Hz, H-3′′), 5.40 (dd, 1H, H-4′′), 4.54 (t, 1H, $J_{5'',6''a}$ = 5.6 Hz, H-5′′), 4.10 (dd, 1H, $J_{6''a,6''b}$ = 11.6 Hz, H-6′′a), 3.99 (dd, 1H, $J_{5'',6''b}$ = 7.2 Hz, H-6′′b), 2.16, 1.95, 1.92 and 1.74 (s, 12H, 4 × C$\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ170.4, 170.3,

169.9, 168.9 (4 × Acetyl–C = O), 156.4 (C-6), 153.0 (C-2), 149.7 (C-4), 143.5 (C-4′), 140.9 (C-8), 123.7 (C-5′), 119.0 (C-5), 84.7 (C-1′′), 73.4 (C-5′′), 70.7 (C-3′′), 68.2 (C-2′′), 67.7 (C-4′′), 61.9 (C-6′′), 38.2 ($\underline{C}H_2$–N), 20.9, 20.8, 20.7 and 20.3 (4 × $\underline{C}H_3$); TOF-ESIMS calcd for $C_{22}H_{27}N_8O_9$ $[M + H]^+$: 547.1901; found 547.1897.

9-[{1′-(2′′,3′′,4′′-Tri-O-acetyl-β-D-xylopyranosyl)-1′H-1′,2′,3′-triazol-4′-yl}methyl] adenine (5d)

According to the general CuAAC procedure, **5d** (682 mg, 80%) was obtained from the reaction of azido sugar **5** (600 mg, 2 mmol) with propargyl adenine **d** (313 mg, 1.8 mmol) and purified *via* flash chromatography (CH_2Cl_2:MeOH, 100:0→100:4.0 *v*/*v*). White foam; mp 122–124°C; $[\alpha]_D^{20}$ + 52 (*c* 0.25, DMSO); FTIR (ATR, cm^{-1}): 3360, 3240 (–NH_2), 3103 (C=CH), 1739 (C=O), 1683 (C=N), 1608 (C=C), 1579 (–NH_2), 1220 (C–N), 1041 (C-O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.35 (s, 1H, H-5′), 8.11 (s, 2H, H-8 and H-2), 7.19 (s, 2H, NH_2), 6.16 (d, 1H, $J_{1'',2''}$= 9.2 Hz, H-1′′), 5.54 (t, 1H, H-2′′), 5.44 (t, 1H, H-3′′), 5.43 (s, 2H, $C\underline{H}_2$–N), 5.08 (td, 1H, $J_{4'',5''a}$ = 5.6 Hz, H-4′′), 4.04 (dd, 1H, $J_{5''a,5''b}$ = 11.2 Hz, H-5′′a), 3.78 (t, 1H, $J_{4'',5''b}$ = 10.8 Hz, H-5′′b), 1.99, 1.76 and 1.73 (s, 9H, 3 × $C\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): 169.9, 169.9, 168.8 (3 × Acetyl–C = O), 156.3 (C-6), 153.0 (C-2), 149.7 (C-4), 143.6 (C-4′), 140.9 (C-8), 123.1 (C-5′), 118.9 (C-5), 85.1 (C-1′′), 72.2 (C-3′′), 70.7 (C-2′′), 68.3 (C-4′′), 64.5 (C-5′′), 38.3 ($\underline{C}H_2$–N), 20.8, 20.7 and 20.2 (3 ×$\underline{C}H_3$); TOF-ESIMS calcd for $C_{19}H_{22}N_8O_7Na[M + Na]^+$: 497.1509; found 497.1526.

Cytotoxic Activity Assay

3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (Cell Proliferation Kit I, Roche Diagnostic, Germany) was performed for the evaluation of cytotoxic properties and *in vitro* anticancer potentials of the synthesized compounds.[46]

The cell lines used for testing the *in vitro* cytotoxicity, and cell viability including MDA-MB-231 (Human breast cancer, ATCC HTB-26), Hep3B (Human hepatoma cancer, ATCC HB-8064), PC-3 (Human prostate cancer, ATCC CRL-1435) and L929 (control cell line, ATCC CRL-6364) were obtained from the American Type Culture Collection (USA) and maintained in Dulbecco's Modified Eagles's Medium (DMEM) supplemented with 10% (*v*/*v*) of fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA). Cells were routinely grown in 25 and 75 cm^2 flasks in an environment containing 5% CO_2 and passed every 3 days. Cells were seeded in 96 multiwell plate at a density of 5 × 10^3 cells/well for 24 h before treating with the compounds to allow attachment of cells. The compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma D2650) and the final concentration of DMSO was 0.1%. After 24 h of growth, the medium was replaced with fresh medium containing compounds at the different concentrations (10, 25, 50, 100 and 200 µM). Triplicate wells were prepared for each individual concentration. Cells were incubated with the compounds for 48 h at 37°C, under a 5% CO_2 atmosphere. After the incubation period, 10 µL

MTT was added to each well and incubated overnight. MTT containing medium was removed and 100 µL solubilization buffer (10% SDS in 0.01 M HCl) was added to the formazan crystal formed by live cells. Plates were incubated for overnight and absorbances were measured at 550 and 600 nm wavelength using a multimode microplate reader (BioTek, USA).

The percentage cell viability was calculated by expressing the absorbance of treated cells as a percentage of untreated control cells. Three independent experiments with triplicate data were performed to obtain mean cell viability. The IC_{50} value is the concentration (μM) of a compound was able to cause 50% cell death with respect to the control culture. The IC_{50} values of the compounds were calculated using GraphPad Prism 7.02 software according to the inhibition ratios.

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