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FULL PAPER

Synthesis and Conformational Analysis of 2-*O*-Silyl Protected Nucleosides from Unprotected Nucleobases and Sugar epoxides

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Abstract: Synthesis of orthogonally protected 2-silyl nucleosides were achieved by trans opening of sugar epoxides with nucleobases catalyzed by trimethylsilyltrifluoromethanesulfonate using hexamethyldisilazane both as solvent and silylating agent. Both α and β nucleosides were obtained with complete stereocontrol only by changing the epoxide stereochemistry. The synthesized nucleosides exist in different chair conformations depending upon the nature of protecting groups present in the sugar moiety as determined from coupling constants in the proton NMR. All the synthesized nucleosides were screened for biofilm inhibition activity against four bacterial pathogens and compound **7c** was found to be most potent against *Salmonella typhimurium* with 1.22 µM biofilm inhibitory activity taking ciprofloxacin as control.

Introduction

Nucleosides are involved in various cellular processes and therefore represent a unique starting point for drug discovery. They are important building blocks for nucleic acid synthesis (DNA and RNA)[1] and many analogues have been in clinical use as anticancer and antiviral drugs, out of these several leads has shown biological activity against worldwide pandemic covid-19 infections.[2,3] Nucleosides can be either modified in the heterocyclic bases or sugar moiety to give nonnatural nucleosides with modified biological properties.[4] They are often varied in the sugar part by introducing either pyranosyl or carbocyclic (Fig. 1a, entries **1**, **2**) rings without disturbing the heterocyclic base so as to preserve base pairing functionalities.[5] Introduction of silicon strategically in the sugar part of a nucleoside often modulates its drug like properties as the sugar part tend to have higher lipophilicity which improves the bio-efficacy through better cell penetration. For example, 2,3-*O*-TBS-protected ureidoadenosine (Fig. 1b, entry **3**) has been found to have better antiproliferative effect than its free hydroxyl counterpart.[6a-c] Since silyl ethers can be cleaved to hydroxyl groups at physiological pH, such compounds can also be used as prodrugs (Fig 1b, entry **4**).[6d-e] Synthetically, orthogonally protected 2-silyl ether nucleosides can be an attractive target as it can be easily converted into the corresponding 2-OH sugar derivatives selectively which are precursors for medicinally important 2-deoxy nucleosides.

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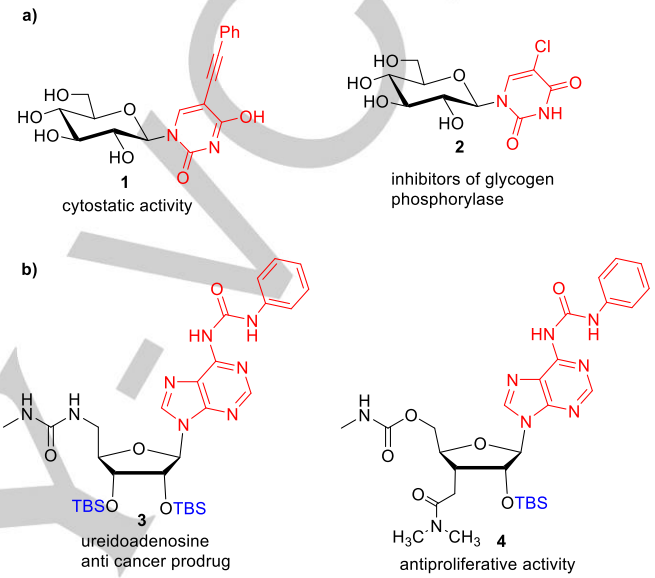

Figure 1. (a) Pyran based biologically important nucleosides; (b) Utility of Silylated Nucleosides in medicinal chemistry

Nucleosides are mostly synthesized from protected electrophilic sugars like anomeric acetates, chlorides etc. and presilylated bases through the Vorbrüggen and its modified methods[7] (Scheme 1) where a participating group is prerequisite to control the anomeric selectivity. While synthetically useful, these methods have certain drawbacks like requirement of stoichiometric amounts of metal salts under inert atmosphere, presilylated heterocyclic bases, limited substrate scope and requirement of further protecting group manipulation to make the C-2 OH available for the synthesis of DNA and 2-fluoro nucleosides. There is no general method reported by which both α and β-nucleosides can be synthesized without altering the reaction condition. Therefore, new methods are required for direct access to orthogonally protected silylated nucleosides to get both α and β-nucleosides from unactivated nucleobases. In this context we thought that both α and β-nucleosides having free 2-OH group can be accessed directly from 1,2-anhydro sugars in a straightforward way using silylated bases. In continuation of our interest in nucleoside and sugar epoxide chemistry,[8] herein we report a TMSOTf-catalyzed one-pot synthesis of orthogonally protected nucleosides from sugar epoxides and non-silylated heterocyclic bases in hexamethyldisilazane used as both solvent and silicon source (Scheme 1).

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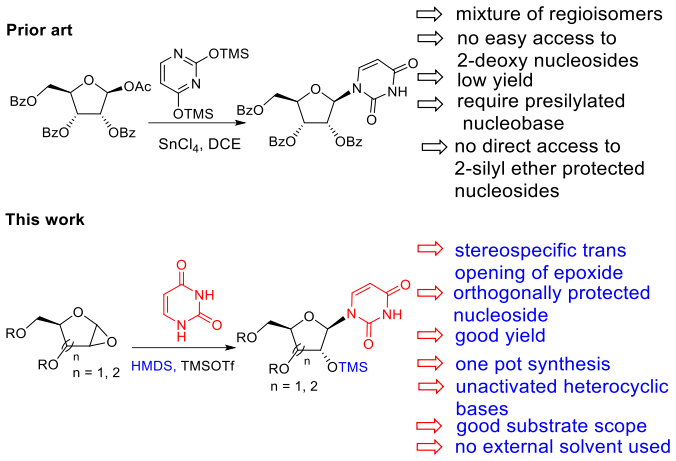
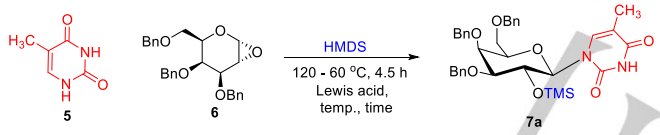


Scheme 1. Previous art and current work for synthesizing orthogonally protected nucleoside derivatives

Results and Discussion

We have started the optimization study using unprotected thymine **5** and benzyl protected galactal epoxide **6**.[9] Galactal epoxide **6** (0.3 equiv) and TMSOTf (1 equiv) were added to a preheated solution of thymine in HMDS at 120 °C and resulting mixture was further heated at 100 °C for 3 h only to get **7a** as a diastereomeric mixture in the ratio 1:0.3 (Table 1, entry 1).

Table 1. Optimization of the reaction conditions[a]

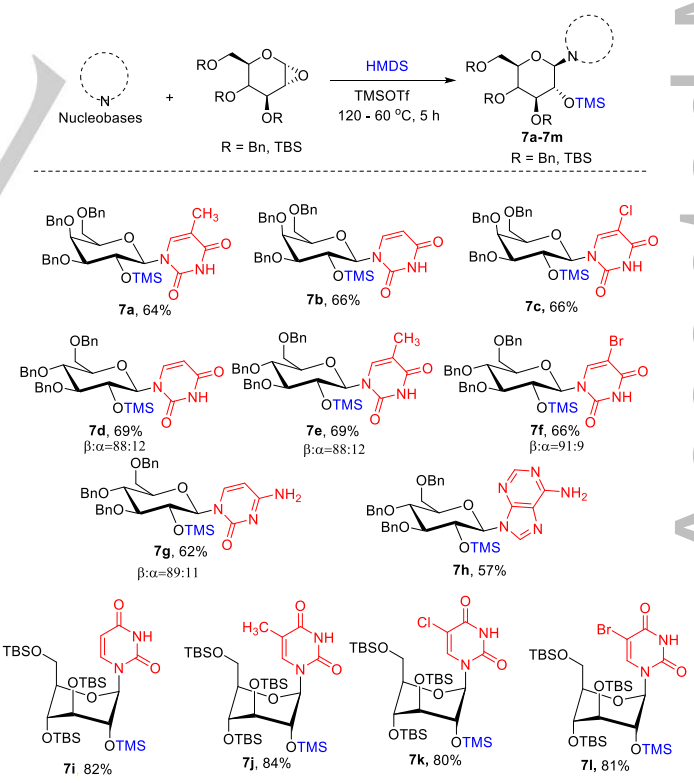


entry	Lewis acid (equiv)	temp. (°C)	time (h)	Yield[b] (%)	β/α[c]
1	TMSOTf (1.0)	100	2	55	1:0.3
2	TMSOTf (1.0)	80	3	58	1:0.2
3	TMSOTf (1.0)	60	3	65	1:0
4	TMSOTf (1.0)	40	5	35	1:0
5	TMSOTf (0.5)	60	3	63	1:0
6	TMSOTf (0.1)	60	3	67	1:0
7	$BF_3(OEt)_2$ (0.1)	60	3	ND	ND
8	TfOH (0.1)	60	3	ND	ND
9	$AlCl_3$(0.1)	60	3	ND	ND

[a] Reaction conditions: In all cases reaction was carried out by heating **5** (1 equiv) in HMDS (1 mL) at 120 °C for 1.5 h followed by the addition of **6** (0.3 equiv) and Lewis acids at the temperature mentioned in the table. [b] Isolated yields. [c] Diastereomeric ratio.

Temperature played a critical role on the selectivity and yield of the reaction. Decreasing the temperature to 60 °C not only affected the yield of reaction but also increased the selectivity of the reaction giving β-nucleoside as the sole product (Table 1, entries 2-4). The high coupling constants indicated that the nucleoside exists in $^4C_1$ conformation. Changing the proportion of TMSOTf from stoichiometric amount to catalytic amount has little effect on the yield of reaction (Table 1, entries 5-6). Screening of other Lewis acids confirmed that TMSOTf was the best catalyst for this coupling reaction (Table 1, entries 6-9). Consolidating the outcome of our optimization study, using 1 equiv of thymine **5** in HMDS (20 equiv) at 120 °C for 1.5 h followed by the addition of 0.3 equiv of **6** and TMSOTf (0.1 equiv) after cooling the reaction mixture down to 60 °C and allowing the reaction mixture to stir for 3 h at the same temperature gives the desired nucleoside in good yield. There is no requirement of external solvent as HMDS acts both as solvent and silylating agent. Other uracils were explored under the optimized reaction conditions to give the desired nucleosides (Scheme 2, **7b**, **7c**) in good yield. Other sugar-epoxides like glucal epoxide also gave good results under the standard reaction conditions giving the desired nucleosides with 88:12 to 91:9 β:α ratio (Scheme 2, **7d**, **7e**, **7f**) in good yield. Further the pyrimidine base cytosine undergoes the reaction smoothly to give the desired product with 89:11 β:α ratio (Scheme 2, **7g**). Considering the biological importance of purine nucleosides,[6] purine base such as adenine was allowed to react with 3,4,6-tri-*O*-benzyl glucal epoxide **6** under optimized conditions and to our satisfaction, the desired product (Scheme 2 **7h**) was obtained in good yield. The functional group tolerance of the method was checked by treating 3,4,6-tri-*O*-tert-butyldimethylsilyl-1,2-anhydro sugars with various substituted

Scheme 2. Substrate scope of reaction using sugar-epoxide as donors and different uracils



Reaction conditions: Reaction was carried out by heating nucleobase (1 equiv) in HMDS (1 mL) at 120 °C for 1.5 h before the addition of **6** (0.3 equiv) and TMSOTf (10 mol %) at 60 °C for 3 h.

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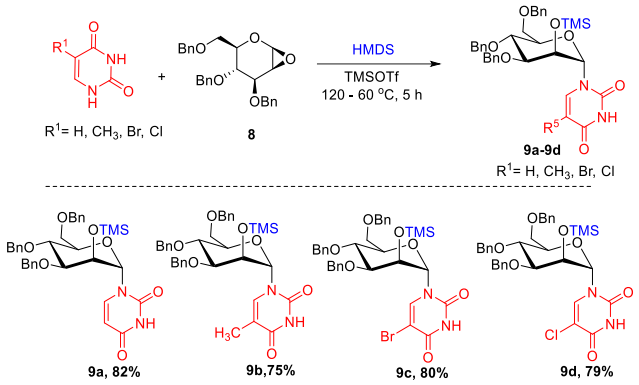
uracil under the optimized reaction conditions to get the β-nucleoside selectively with good yield (Scheme 2, **7i**, **7j**, **7k**, **7l**).

Scheme 3: Reaction of uracil and substituted uracils with β-sugar-epoxides



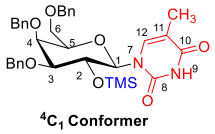
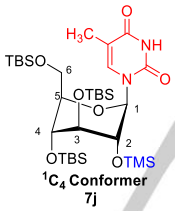
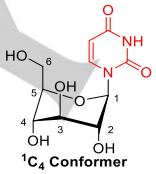
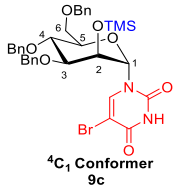
Reaction conditions: Reaction was carried out by heating nucleobase (1 equiv) in HMDS (1 mL) at 120 °C for 1.5 h before the addition of **8** (0.3 equiv) and TMSOTf (10 mol %) at 60 °C for 3 h.

Keeping in mind the importance of α-nucleosides we carried out the reaction of thymine **5** with β-sugar-epoxide **8**[8] under the optimized reaction conditions and to our delight we got the desired α-nucleoside (Scheme 3, **9b**) in good yield. The broad singlet of anomeric proton confirmed the $^4C_1$ conformation with H-1 and H-2 being diequatorial. Other substituted uracils reacted with the β-sugar-epoxide to give the respective nucleosides in good yields (Scheme 3, **9a**, **9c**, **9d**).

Structure and Conformation

Since pyranose sugars exist in two flexible chair conformations[11] namely $^4C_1$ and $^1C_4$, we wanted to investigate the effect of substituents on the conformation of the synthesized nucleosides. Each of the two chair conformations of the pyranose nucleosides (β-anomeric) can be characterized by the coupling constants (*J*). A large *J* value of vicinal sugars protons is characteristic of $^4C_1$ whereas $^1C_4$ is characterized by a small *J* value (Table 2) due to the presence of all equatorial protons (Table 2).

Table 2: Comparison of chemical shifts and coupling constants data of different sugar ring protons in different conformation of pyran based nucleosides

entry	 $^4C_1$ Conformer 7a		 $^1C_4$ Conformer 7j		 $^1C_4$ Conformer 16		 $^4C_1$ Conformer 9c	
1	H-1 = 5.61 (d, *J* = 8.5 Hz, 1H)	C-1 = 83.1	H-1 = 6.01 (d, *J* = 2.0 Hz, 1H)	C-1 = 77.9	H-1 = 6.11 (d, *J* = 2.8 Hz, 1H)	C-1 = 77.8	H-1 = 5.67 (s, 1H)	C-1 = 82.0
2	H-2 = 4.05 (t, *J* = 8.9 Hz, 1H),	C-2 = 71.0	H-2 = 3.82 (d, J = 2.4 Hz, 1H)	C2 = 74.5	H-2 = 4.00 – 3.94 (m, H)	C-2 = 70.4	H-2 = 4.15 (s, 1H)	C-2 = 69.1
3	H-3 = 3.77 (t, *J* = 6.4 Hz, 1H)	C-3 = 76.0	H-3 = 3.96 (s, 1H)	C-3 = 69.2	H-3 = 3.93 – 3.89 (m, 1H)	C-3 = 74.0	H-3, H-4, H-5, H-6a, H-6b, and (–CH_2-Bn)$_3$ = (4.82-3.60)	
4	H-4 = 3.94 (d, *J* = 1.7 Hz, 1H)	C-4 = 73.6	H-4 = 3.67 (d, *J* = 5.4 Hz, 1H)	C-4 = 70.6	H-4 = 3.63 (t, *J* = 5.0 Hz, 1H)	C-4 = 71.5		C-4 = 73.4
5	H-5 = 3.57 – 3.52 (m, 1H)	C-5 = 83.3	H-5 = 4.17 (d, *J* = 5.3 Hz, 1H)	C-5 = 80.1	H-5 = 4.10 – 4.02 (m, 1H)	C-5 = 81.1		C-5 = 81.8
6	H-6a = 3.57 – 3.52 (m, 1H)	C-6 = 68.2	H-6a = 3.91 (dd, *J* = 10.7, 4.2 Hz, 1H)	C-6 = 62.9	H-6a = 4.00 – 3.94 (m, 1H)	C-6 = 61.8		C-6 = 69.0
7	H-6b = 3.57 – 3.52 (m, 1H)	C-6 = 68.2	H-6b = 3.77 (dd, *J* = 10.6, 6.9 Hz, 1H)	C-6 = 62.9	H-6b = 3.75 (dd, *J* = 12.2, 2.8 Hz, 1H)	C-6 = 61.8		C-6 = 69.0

The reaction of TBS-protected pyranose sugars with different heterocyclic bases indeed resulted in nucleosides with $^1C_4$ conformations. (Scheme 2, **7i**-**7l**). Moreover, the conformations of resulting nucleosides were determined by two-dimensional nuclear magnetic resonance (2D NMR) (COSY, NOESY, HSQC, and HMBC, (See SI). A strong correlation of H-1 with H-3 and H-5 in the NOESY spectrum of **7a** along with its large coupling constant in ^{1}HNMR confirms its $^4C_1$ conformation with α-axial

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configuration of anomeric proton (Fig. 2, A). The $^1C_4$ conformation in case of **7j** is confirmed from the small coupling constant of anomeric proton with H-2 along with absence of any cross peaks in NOESY spectrum (Fig. 2, B). However, in case of **9c**, a broad singlet of anomeric proton in the ^{1}HNMR and no correlation of H-1 with either H-3 or H-5 in the NOESY spectrum confirms its $^4C_1$ conformation with the β-equatorial configuration of H-1 (Fig. 2, C).

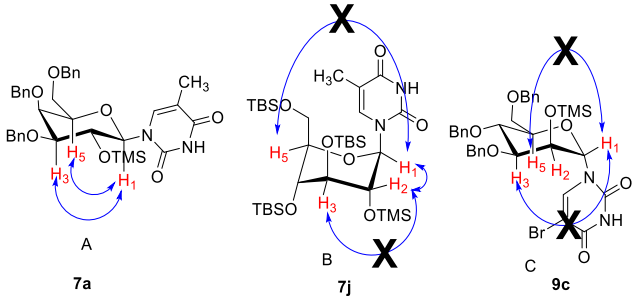
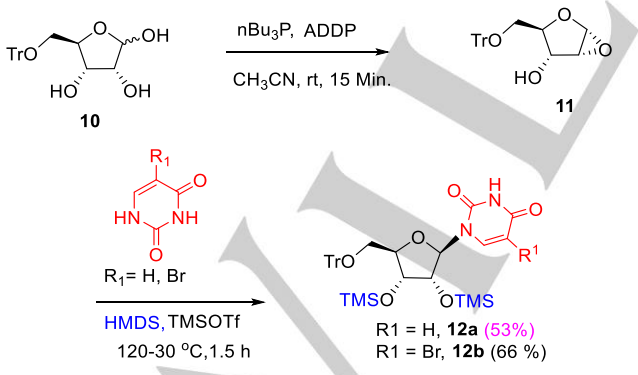


Figure 2: NOESY Correlation of compound **7a** (A), **7j** (B) and **9c** (C).

In view of the biological importance of nucleosides with furanose sugars, the success achieved with the pyranoid sugar-epoxides prompted us to extend it to furanoid sugar-epoxides. It is pertinent to mention that a protecting group at allylic 3-OH of furanoside sugars directs the stereochemistry of 1,2-oxiranes which in turn controls anomeric selectivity with stoichiometric amounts of $ZnCl_2$ which limits its synthetic utility as observed by Danishefsky and co-workers.[12] Thus, we synthesized ribose sugar epoxide **11**[13] and allowed it to react with uracil. We were delighted to obtain 2,3-di-*O*-silyl ribofuranosyl nucleosides (Scheme 4, **12a**) in good yield with complete β-selectivity. Similar products without silicon protection were obtained by Hocek group while working only with furanoside system in the presence of strong base like NaH. Similar result was obtained with 5-bromo uracil (Scheme 4, **12b**).

Scheme 4. Synthesis of 1,2-anhydroribosugar and its conversion to silyl protected natural nucleoside

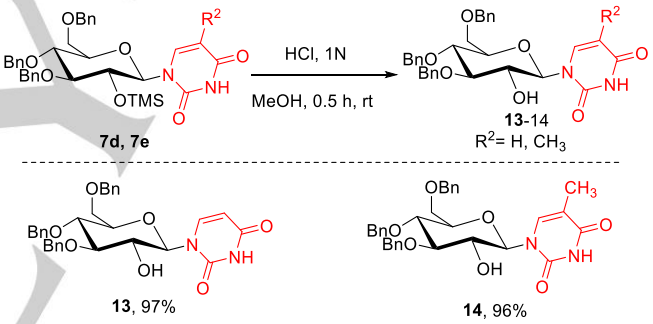


Reaction conditions: Step 1) Reaction was carried out by taking **10** (1 equiv) in CH_3CN (3 mL) then nBu_3P (1.6 equiv) and ADDP (1.5 equiv) were added successively at 25 °C for 15 minutes. Step 2) Reaction was carried out by heating nucleobase (1 equiv) in HMDS (1 mL) at 120 °C for 1.5 h followed by the addition of **11** (0.3 equiv) and TMSOTf (10 mol%) and heating at 30 °C for 3 h.

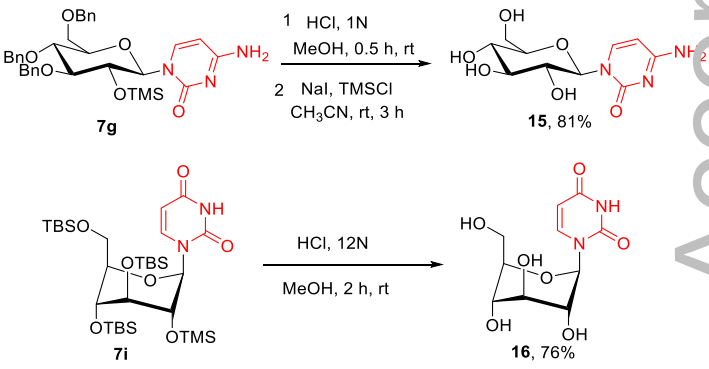
Biological Activity

The widespread use of antibiotics has resulted in rapid propagation of antibiotic-resistant pathogens, which require new antibacterial drugs that are efficient against the development of resistance.[14] One innovative way is to combine antibiofilm treatments with antibiotics thereby, lowering the tolerance of antibiotic-resistant pathogenic bacteria.[15] Above mentioned approach has the ability to inhibit bacterial biofilm growth without killing the pathogens thereby has less probability of developing resistance. Small molecules like nucleosides[16] that inhibit bacterial virulence and biofilm formation by controlling the quorum sensing could be useful for the discovery of new antibiotics. To date, there are no drugs in clinical use that are developed specifically to target biofilm formation. In order to test the effect of silyl substitution of nucleosides on their biofilm inhibitory activity, deprotection of some of the synthesized nucleosides (Scheme 2, **7d**, **7e**, **7g**, **7i**) also was carried out (Scheme 5 and 6, **13**, **14**, **15**, **16**) All the synthesized nucleosides were screened for biofilm inhibition against four bacterial pathogens viz *Salmonella typhimurium* (MTCC 98), *Pseudomonas aeruginosa* (MTCC 424),

Scheme 5. Conversion of 2-silyl nucleosides to 2-hydroxy nucleosides



Scheme 6: Deprotection of synthesised nucleosides by different methods for the biological evaluation.



Bacillus subtilis (MTCC 121), and *Staphylococcus aureus* (MTCC 737). The result showed that *O*-silylated nucleosides showed more potency than unprotected ones (Table 3, **7d**, **7e**, **7g**, **7i** and **13**, **14**, **15**, **16**), this may be due to the greater cell permeability of silyl-protected nucleosides. Galactal derived nucleosides were more potent than glucal derived compounds (Table 3, **7a, 7b, 7c,** **7g** and **7d**, **7e**, **7f**). Among all, compound **7c** containing silicon at

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C-2 position (Table 2, **7c**) was the most active against all four pathogens (Fig. 3).

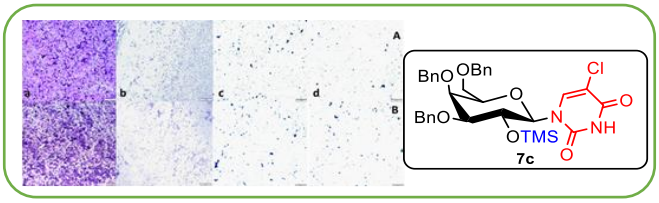


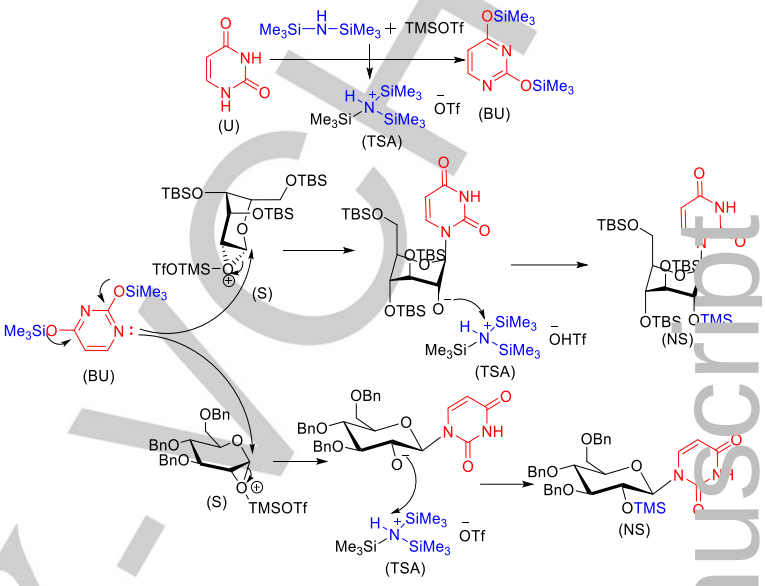
Figure 3. Microscopic documentation of inhibition in biofilm-formation using the compound **7c** and Ciprofloxacin against *B. subtilis* (row1, A) and *S. aureus* (row2, B) a) bacterial cells with no compound b) with compound **7c** at MBIC50 c) compound **7c** at MBIC90 d) Ciprofloxacin displayed promising antibiofilm activity particularly against *B. subtilis* (MTCC 121).

Further nucleosides with substituted uracil were more active than nucleoside with simple uracil (Table 3, **7a**, **7b**, **7c**, **7d**, **7e**, **7f**, **7g**). Also, α-nucleosides obtained from β-epoxide were more active than the β-nucleosides synthesized from α-epoxides (Table 3, **9a**, **9b**, **9c**, **9d** and **7d**, **7e**, **7f**). Also, furanose nucleosides (Table 3, **12a**, **12b**) displayed promising antibiofilm activity particularly against *B. subtilis* (MTCC 121).

MECHANSIM

Stereo-selective synthesis of 2-*O*-silylated nucleoside from sugar epoxide and HMDS in the presence of catalytic amount of TMSOTf can be explained in the following way (Scheme 7). HMDS act as silylating agent as well as solvent. HMDS in the presence of catalytic TMSOTf forms trisilyl ammonium salt[17] (TSA) which activates uracil (U) to nucleophilic bis-silylated uracil (BU). BU attacks 1,2-anhydrosugars (S) from the opposite side results in trans opening. The 2-OH group thus formed get silylated by excess TSA present in the medium to form final product (NS). Conformation of the NS depends on the conformation of sugar epoxide (S) which further depend on the nature of substituent present in the sugar epoxide.

Scheme 7: Explanation for the synthesis of 2-*O*-silylated nucleosides



CONCLUSION

In summary we have developed a direct route to access differentially protected nucleosides from unprotected nucleobases and sugar-epoxides via trans opening as new biofilm inhibitors. Both α and β nucleosides can be synthesized by trans opening of sugar-epoxides with catalytic amount of Lewis acid. Further work to explore this current method for the synthesis of medicinally important 2-deoxy nucleosides are in progress and will be reported in due course.

Table 3: Biofilm inhibition activity (MBIC in µM) of the synthesised nucleosides against human bacterial pathogens [1]

S. No.	Molecule Code	*S. typhimurium*	*P. aeruginosa*	*B. subtilis*	*S. aureus*
1	**7a**	441.454±0.014	165.765±0.009	431.394±0.026	239.28±0.011
2	**7b**	1.712±0.01	2.532±0.022	1.912±0.016	2.658±0.034
3	**7c**	1.22±0.065	1.805±0.004	1.449±0.014	1.164±0.022
4	**7d**	314.905±0.012	297.584±0.021	231.506±0.008	160.082±0.034
5	**7e**	69.266±0.027	99.907±0.013	297.387±0.032	273.592±0.019
6	**7f**	>500	>500	>500	>500
7	**7g**	20.265±0.02	18.874±0.025	12.594±0.021	27.953±0.028
8	**7i**	433.542±0.034	66.774±0.03	500	39.512±0.015
9	**7j**	106.758±0.016	196±0.025	222.243±0.022	421.626±0.004
10	**7k**	96.713±0.021	318.55±0.014	374.407±0.034	>500
11	**7l**	49.913±0.017	80.049±0.009	19.034±0.027	88.7±0.055

12	**9a**	100.458±0.05	212.54±0.023	125.425±0.025	483.953±0.022
13	**9b**	35.679±0.033	104.786±0.026	91.086±0.015	109.775±0.022
14	**9c**	27.059±0.015	21.996±0.025	20.684±0.025	44.19±0.03
15	**9d**	32.448±0.027	21.064±0.013	12.954±0.016	57.226±0.099
16	**12a**	11.259±0.014	16.708±0.012	4.49±0.035	10.031±0.0604
17	**12b**	64.852±0.032	182.108±0.013	363.47±0.043	260.789±0.056
18	**13**	>500	>500	>500	>500
19	**14**	>500	>500	>500	>500
20	**15**	>500	>500	>500	>500
21	**16**	>500	>500	>500	>500
22	Ciprofloxacin	0.962±0.026	0.134±0.032	0.187±0.016	0.145±0.019

[1] The values represent the minimum biofilm inhibition concentrations (MBIC50) in µM against four different bacterial pathogens using ciprofloxacin as positive control. Each value corresponds to the average ± Standard deviation of three independent experiments.

EXPERIMENTAL SECTION

General information: ^{1}H and ^{13}C NMR spectra were recorded on 400, 101 and 126 MHz. spectrometers with TMS as internal standard. Chemical shifts are expressed in parts per million (δ ppm). Silica gel coated aluminium plates were used for TLC. The products were purified by column chromatography on silica gel (60-120/100-200 mesh) using petroleum ether–ethyl acetate and dichloromethane-methanol as the eluent to obtain the pure products. Exact mass of all products was analyzed by using HRMS having QTOF analyzer. Reagents used were mostly purchased from Sigma Aldrich and TCI Chemicals.

Preparation of starting materials

General procedure for the synthesis of α-sugar-epoxides (6)[9]

The glycal (3.60 mmol) was dissolved in CH_2Cl_2 (30 mL), acetone (3 mL) and saturated aqueous solution of $NaHCO_3$ (50 mL) were added at 0 °C. The mixture was vigorously stirred and a solution of Oxone (6 g) in H_2O (25 mL) was added dropwise over 15 min. The crude reaction was vigorously stirred at 0 °C for 30 min and was then allowed to warm to room temperature until TLC indicated complete consumption of the starting material. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to afford the sugar-epoxide. The crude could not be purified due the decomposition of the product.

General procedure for the synthesis of β-sugar-epoxides (8)[10]

Synthesized according to the procedure available in literature where KOtBu (3.05 g, 27.14 mmol) was added pinch wise to a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannosyl chloride (12.61 g, 24.67 mmol) in THF (300 mL). The mixture was heated at 70 °C for 1 h. After the completion of the reaction the reaction mixture was extracted with ethyl acetate and washed with brine, dried over Na_2SO_4. Solvent was evaporated. The residue was washed with Et_2O/hexane to give β-sugar-epoxides as a white powder.

General procedure for the synthesis of furanoid-epoxide (11)[13]

Synthesized according to the available procedure in literature as, a 25 mL round bottom flask containing a stir bar was charged with 5-*O*-trityl-D-ribose (100 mg, 0.25 mmol) and fitted with a rubber septum and a nitrogen filled balloon. To this flask was added CH_3CN (3 mL) via syringe. To this stirring solution was added $P(nBu)_3$ (101 mg, 0.41 mmol,) via syringe followed by 1,1-(azodicarbonyl) dipiperidine (ADDP) (97 mg, 0.38 mmol,) at rt. After 15 minutes white precipitate formed indicate the synthesis of epoxide. Diluted with CH_3CN and used in next step without workup or purification.

Typical procedure for the synthesis of nucleosides from glycal-epoxides

In an atmosphere of nitrogen (1 equiv) of nucleobase (NB) was added in Hexamethyldisilazane (HMDS) (20 equiv) in a round bottom flask at 120 °C and stirred the mixture for 1.5 h at the same temperature. The reaction mixture was cooled to 60 °C, then charged with glycal-epoxide (0.3 equiv) and Trimethylsilyltrifluoromethanesulfonate (TMSOTf) (10 mol %) successively. After the completion of the reaction the reaction mixture was extracted with ethyl acetate and washed with brine, dried over Na_2SO_4. The residue was purified by column chromatography.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-β-D-galactopyranosyl}-5-methyl-2,4(1H,3H)-pyrimidinedione (7a). 94 mg, 64%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J = 9.5 Hz, 1H, H-9), 7.30 – 7.18 (m, 15H), 7.06 (s, 1H, H-12), 5.51 (d, J = 8.7 Hz, 1H, H-1),4.84 (d, J = 11.3 Hz, 1H,OBn), 4.62 (s, 2H,OBn), 4.49 – 4.34 (m, 3H,OBn), 3.98 (t, J = 8.9 Hz, 1H, H-2), 3.86 (s, 1H, H-4), 3.69 (t, J = 6.4 Hz, 1H, H-3), 3.56 – 3.45 (m, 3H,H-5, H-6a, H-6b), 1.85 (s, 3H, methyl), -0.08 (s, 2H, TMS).^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6 (C-10), 150.9 (C-8) 138.7, 137.9, 137.7 (quaternary aromatic) 135.7 (C-12), 128.5, 128.5, 128.4, 128.0, 127.9, 127.9, 127.8, 127. 8, 127.7 (aromatic), 111.6 (C-11), 83.3 (C-5), 83.12 (C-1), 76.0 (C-3) 74.8 (OBn), 73.6 (C-4), 73.6 (OBn), 72.9 (OBn), 71.0 (C-2), 68.2 (C-6) 12.4 (methyl), 0.3 (TMS). HRMS (ESI+): m/z calcd. for $C_{35}H_{43}N_2O_7Si$ $(M+H)^+$ 631.2840, found 631.2841.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-β-D-galactopyranosyl}-2,4(1H,3H)-pyrimidinedione (7b).95 mg, 66%, gummy solid;^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H, H-9), 7.31 – 7.17 (m, 16H, H-12, aromatic), 5.69 (d, J = 7.5 Hz, 1H, H-11), 5.54 (d, J = 8.7 Hz, 1H, H-1), 4.82 (d, J = 11.3 Hz, 1H,OBn), 4.63 (s, 2H,OBn), 4.43-4.39 (m, 3H,OBn), 3.97 (t, J = 8.9 Hz, 1H, H-2), 3.86 (s, 1H, H-4), 3.70 (t, J = 6.3 Hz, 1H, H-3), 3.53-3.47

(m, 3H, H-5,H-6a, H-6b), -0.06 (s, 9H, TMS).^{13}C NMR (101 MHz, $CDCl_3$) δ 162.7 (C-10), 150.50 (C-8), 140.1 (C-12), 138.6, 137.8, 137.7(quaternary aromatic), 128.5, 128.5, 128.3, 127.9, 127.9, 127.8, 127.7, 127.6(aromatic),104.0 (C-11), 83.3 (C-1), 83.2 (C-5), 76.0 (C-3), 74.9(OBn), 73.6 (C-4), 73.6 (OBn), 72.9 (OBn), 71.1 (C-2), 68.2 (C-6), 0.3 (TMS). (ESI+): m/z calcd for $C_{34}H_{41}N_2O_7Si$ $(M+H)^+$ 617.2683, found 617.2669.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-β-D-galactopyranosyl}-5-chloro-2,4(1H,3H)-pyrimidinedione (7c).99 mg, 66%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (d, J = 11.1 Hz, 1H, H-9), 7.47 (s, 1H, H-12), 7.30 – 7.19 (m, 15H, aromatic), 5.52 (d, J = 8.7 Hz, 1H, H-1), 4.83 (d, J = 11.4 Hz, 1H, OBn), 4.62 (s, 1H, OBn), 4.50 – 4.33 (m, 3H, OBn), 3.96 (t, J = 8.9 Hz, 1H, H-2), 3.85 (s, 1H, H-4), 3.71 (t, J = 6.3 Hz, 1H, H-3), 3.51 – 3.46 (m, 3H, H-5, H-6a, H-6b), -0.06 (s, 9H, TMS).^{13}C NMR (101 MHz, $CDCl_3$) δ 158.5(C-10), 149.7 (C-8), 138.4, 137. 8, 137.6,(quaternary aromatic), 137.0 (C-12), 128.5, 128.4, 128.0, 127.9, 127.9, 127.8, 127.7 (aromatic), 109.8(C-11), 83.7(C-1), 83.1(C-5), 76.3(C-3), 74.8(OBn), 73.6 (C-4), 73.5(OBn), 72.9(OBn), 71.1(C-2), 68.3(C-6), 0.3 (TMS). HRMS (ESI+): m/z calcd. for $C_{34}H_{43}ClN_3O_7Si$ $(M+NH_4)^+$ 668.2559, found 668.2522.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-β-D-glucopyranosyl}-2,4(1H,3H)-pyrimidinedione (7d). 96 mg, 67%, gummy solid, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (s, 1H,H-9), 7.62 (d, J = 8.2 Hz, 0.17H, H-12(α-nucleoside)), 7.39 – 7.26 (m, 17H, H-12(β-nucleoside), aromatic), 7.15-7.09 (m, 2H, aromatic), 6.10 (d, J = 2.7 Hz, 0.16H, H-1(α-nucleoside)), 5.85 (d, J = 8.1 Hz, 1H, H-11(β-nucleoside)), 5.72 – 5.64 (m, 1.11H, H-1(β-nucleoside), H-11(α-nucleoside)), 4.91 (q, J = 11.4 Hz, 2H, OBn), 4.78 (d, J = 10.7 Hz, 1H, OBn), 4.67 (s, 0.19H), 4.60 – 4.49 (m, 4H, OBn,), 4.43 (s, 0.38H), 4.36 (s, 0.19H), 3.80 – 3.67 (m, 7H,H-2, H-3, H-4, H-5, H-6a, H-6b)), 0.02 (s, 9H, TMS(β-nucleoside)), 0.00 (s, 1.52H, TMS(α-nucleoside)).^{13}C NMR (101 MHz, $CDCl_3$) δ 162.9 (C-10), 150.7 (C-8), 139.8 (C-12), 138.4, 137.9, 137.8 (quaternary aromatic), 128.5, 128.5, 128.4, 127.9, 127.9, 127.8, 127.7, 127.5, 127.2 (aromatic), 103.3 (C-11), 85.7 (C-1), 77.7 (C-5), 77.5 (OBn), 75.6 (C-3), 75.1 (OBn), 74.0 (OBn), 73.5 (C-4, C-2), 68.4 (C-6), 0.3 (TMS). HRMS (ESI+): m/z calcd. for $C_{34}H_{41}N_2O_7Si$ $(M+H)^+$ 617.2683, found 617.2669.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-β-D-glucopyranosyl}-5-methyl-2,4(1H,3H)-pyrimidinedione (7e). 101 mg, 69%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H,H-9),), 7.48 (d, J = 1.0 Hz, 0.16H, H-12(α-nucleoside), 7.40 – 7.28 (m, 16H, H-12(β-nucleoside), aromatic), 7.21 (s, 1H, aromatic), 7.16 – 7.09 (m, 2H, aromatic), 6.12 (d, J = 2.9 Hz, 0.16H, H-1(α-nucleoside)), 5.68 (d, J = 6.3 Hz, 1H, H-1(β-nucleoside)), 4.93 (q, J = 11.5 Hz, 2H, OBn), 4.80 (d, J = 10.8 Hz, 1H, OBn), 4.64-4.54 (m, 4H, OBn), 4.47 (s, 0.16H), 4.35 (t, J = 2.9 Hz, 0.16H), 4.17 (q, J = 7.1 Hz, 0.33H).3.83 – 3.67 (m, 7H, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.01 (d, J = 0.7 Hz, 3H, methyl(β-nucleoside)), 1.90 (d, J = 0.5 Hz, 0.48H, methyl(α-nucleoside)), 0.03 (s, 9H, TMS(β-nucleoside)), 0.01 (s, 1.29H, TMS(α-nucleoside)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5 (C-10), 150.8 (C-8), 138.5, 137.8 (quaternary aromatic), 135.4 (C-12), 128.6, 128.5, 128.4, 128.3, 127.9, 127.9, 127.8, 127.5, 127.1 (aromatic), 111.7 (C-11), 85.8 (C-1), 77.6 (C-5), 77.5 (OBn), 75.5 (C-3, OBn), 75.1 (OBn), 73.8 (C-4), 73.5 (C-2), 68.4 (C-6), 12.4 (methyl), 0.3 (TMS). HRMS (ESI+): m/z calcd. for $C_{35}H_{43}N_2O_7Si$ $(M+H)^+$ 631.2840, found 631.2819.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-β-D-glucopyranosyl}-5-bromo-2,4(1H,3H)-pyrimidinedione (7f). 107 mg, 66%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H, NH), 7.67 (s, 1H, H-12), 7.38 – 7.26 (m, 16H, aromatic), 7.12 – 7.07 (m, 2H, aromatic), 6.06 (d, J = 2.7 Hz, 0.10H, H-1(α-nucleoside)), 5.60 (d, J = 7.3 Hz, 1H, H-1(β-nucleoside)), 4.89 (dd, J = 27.5, 11.5 Hz, 2H, OBn), 4.74 (d, J = 10.7 Hz, 1H, OBn), 4.54 (q, J = 12.1 Hz, 4H, OBn), 3.75 – 3.62 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 0.00 (s, 9H, TMS(β-nucleoside)), -0.01 (s, 0.88H, TMS(α-nucleoside)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.3 (C-10), 149.6 (C-8), 139.3 (C-12), 138.3, 137.6, 137.6 (quaternary aromatic), 128.5, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.5, 127.0 (aromatic), 97.6 (C-11), 85.5 (C-1), 77.8 (C-5), 77.3 (OBn), 75.5 (C-3), 75.1 (OBn), 74.0 (OBn), 73.5 (C-4, C-2), 68.2 (C-6), 0.3 (TMS). HRMS (ESI+): m/z calcd. for $C_{34}H_{39}BrN_2O_7Si$ $(M+H+2)^+$ 697.1788, found 697.1749.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-β-D-glucopyranosyl}-4-amino-2(lH,3H)-pyrimidinone (7g). 89 mg, 62%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 7.3 Hz, 0.15H), 7.41 – 7.26 (m, 16H, H-12, aromatic), 7.17 – 7.13 (m, 2H, aromatic), 6.17 (d, J = 2.5 Hz, 0.13H, H-1(α-nucleoside)), 5.95 (d, J = 6.6 Hz, 1H, H-1), 5.87 (s, 1H, H-11), 4.91 (s, 2H, OBn), 4.80 (d, J = 10.8 Hz, 1H, OBn), 4.61 – 4.49 (m, 4H, OBn), 3.80 – 3.59 (m, 7H, H-2, H-3, H-4, H-5, H-6a, H-6b), 0.00 (s, 9H, TMS(β-nucleoside)), -0.01 (s, 1.41H, TMS(α-nucleoside).^{13}C NMR (101 MHz, $CDCl_3$) δ 165.2 (C-10), 156.0 (C-8), 140.8 (C-12), 138.6, 138.0, 137.9 (quaternary aromatic), 128.5, 128.4, 128.3, 127.91, 127.8, 127.8, 127.8, 127.4, 127.2 (aromatic), 95.9 (C-11), 86.1 (C-1), 77.7 (C-5), 77.6 (OBn), 77.3 (C-3), 75.6 (OBn), 75.0 (OBn), 74.6 (C-4), 73.5 (C-2), 68.6 (C-6), 0.3 (TMS). HRMS (ESI+): m/z calcd. for $C_{34}H_{42}N_3O_6Si$ $(M+H)^+$ 616.2843, found 616.2833.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-β-D-glucopyranosyl}-9-purine-6-amine (7h).85 mg, 57%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.47 (s, 1H), 7.38 – 7.24 (m, 13H, aromatic), 7.15 – 7.11 (m, 2H, aromatic), 6.00 – 5.90 (m, 2H, H-1, NH), 5.00 – 4.78 (m, 3H, OBn), 4.54 (d, J = 11.0 Hz, 1H, OBn), 4.46 (s, 2H, OBn), 4.30 (dd, J = 9.4, 4.9 Hz, 1H, H-5), 4.09 (t, J = 8.9 Hz, 1H, H-2), 3.76 (t, J = 8.9 Hz, 1H), 3.66 (dd, J = 10.9, 4.6 Hz, 1H), 3.61 – 3.55 (m, 1H), 3.50 (d, J = 9.5 Hz, 1H, NH), 0.10 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.4, 153.4, 151.7, 144.9, 137.6, 137.5, 137.2, 128. 8, 128.6, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 128.0, 127.9, 127.7, 127.0, 111.6, 83.2 (C-1), 81.5 (C-5), 75.8 (OBn), 75.0 (C-3), 73.4 (OBn), 72.6 (OBn, C-2), 67.5 (C-6), -0.0. HRMS (ESI+): m/z calcd. for $C_{35}H_{42}N_5O_5Si$ $(M+H)^+$ 640.3000, found 640.3012.

1-{3',4',6'-Tri-*O*-(tert-butyldimethylsilyl)-2-*O*-trimethylsilyl-β-D-glucopyranosyl)}-2,4(1H,3H)-pyrimidinedione (7i). 110 mg, 82%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H, H-9), 7.62 (d, J = 8.1 Hz, 1H, H-12), 5.97 (d, J = 2.6 Hz, 1H, H-1), 5.67 (d, J = 7.9 Hz, 1H, H-9), 4.20 – 4.14 (m, 1H, H-5), 4.09 – 4.06 (m, 1H, H-2), 3.89 (dd, J = 10.8, 3.8 Hz, 1H, H-6a), 3.82 (d, J = 3.1 Hz, 1H, H-3), 3.74 (dd, J = 10.8, 7.0 Hz, 1H, H-6b), 3.66 (d, J = 6.2 Hz, 1H, H-4), 0.92 (s, 7H, TBS), 0.91-0.87 (m, 20H, TBS), 0.17 – 0.05 (m, 18H, TBS), 0.02 (s, 9H, TMS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.1 (C-10), 150.0 (C-8), 142.4 (C-12), 100.4 (C-11), 79.6 (C-5), 79.0 (C-1), 74.6 (C-3), 71.0 (C-4), 69.0 (C-2), 62.9 (C-6), 25.8, 25.7, 25.7, 18.2, 17.8 (TBS), -0.4 (TMS), -4.1, -4.5, -4.8, -5.0, -5.3, -5.3 (TBS). HRMS (ESI+): m/z calcd. for $C_{31}H_{65}N_2O_7Si_4$ $(M+H)^+$ 689.3869, found 689.3879.

1-{3',4',6'-Tri-*O*-(tert-butyldimethylsilyl)-2-*O*-trimethylsilyl-β-D-glucopyranosyl}-5-methyl-2,4(1H,3H)-pyrimidinedione (7j). 115 mg, 84%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (s, 1H, H-9), 7.41 (s, 1H, H-12), 6.01 (d, J = 2.0 Hz, 1H, H-1), 4.17 (d, J = 5.0 Hz, 1H, H-5), 3.96 (s, 1H, H-3), 3.91 (dd, J = 10.7, 4.2 Hz, 1H, H6a), 3.82 (d, J = 2.4 Hz, 1H, H-2), 3.77 (dd, J = 10.6, 6.9 Hz, 1H, H-6b), 3.67 (d, J = 5.3 Hz, 1H, H-4), 1.94 (s, 3H, methyl), 0.92 (d, J = 3.7 Hz, 18H, TBS), 0.90 (s, 9H, TBS), 0.16 (s, 3H, TBS), 0.13 – 0.09 (m, 11H, TBS), 0.06 (d, J = 3.0 Hz, 4H, TBS), 0.00 (s, 9H, TMS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.3 (C-10), 149.9 (C-8), 138.6 (C-12), 108.5 (C-11), 80.1 (C-5), 77.9 (C-1), 74.5 (C-2), 70.6 (C-4), 69.2 (C-3), 62.9 (C-6), 25.9, 25.8, 25.7, 18.3, 17.9, 17.8 (TBS), 12.4 (methyl), -0.4 (TMS), -4.1, -4.5, -4.8, -4.9, -5.2, -5.3 (TBS). HRMS (ESI+): m/z calcd. for $C_{32}H_{67}N_2O_7Si_4$ $(M+H)^+$ 703.4025, found 703.4037.

1-{3',4',6'-Tri-*O*-(tert-butyldimethylsilyl)-2-*O*-trimethylsilyl-β-D-glucopyranosyl}-5-chloro-2,4(1H,3H)-pyrimidinedione (7k). 113 mg, 80%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (s, 1H, H-9), 7.79 (s, 1H, H-12), 6.00 (d, J = 2.4 Hz, 1H, H-1), 4.18 (dd, J = 10.2, 6.4 Hz, 1H, H-5), 4.02 (s, 1H, H-2), 3.90 (dd, J = 10.8, 3.9 Hz, 1H, H-6a), 3.83 (d, J = 2.6 Hz, 1H, H-3), 3.76 (dd, J = 10.8, 7.0 Hz, 1H, H-6b), 3.67 (d, J = 5.8 Hz, 1H, H-4), 0.91 (d, J = 3.8 Hz, 18H, TBS), 0.90 (s, 9H, TBS), 0.17 (s, 3H, TBS), 0.13 – 0.09 (m, 11H, TBS), 0.07 (d, J = 1.6 Hz, 4H, TBS), 0.03 (s, 9H,

TMS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.4 (C-10), 149.2 (C-8), 139.4 (C-12), 107.5 (C-11), 80.0 (C-5), 79.0 (C-1), 74.4 (C-3), 70.8 (C-4), 69.0 (C-2), 63.0 (C-6), 25.9, 25.8, 25.6, 18.4, 17.9, 17.8 (TBS), -0.4 (TMS), -4.1, -4.4, -4.9, -5.0, -5.2, -5.3 (TBS). HRMS (ESI+): m/z calcd. for $C_{31}H_{64}ClN_2O_7Si_4$ $(M+H)^+$ 723.3479, found 723.3459.

1-{3',4',6'-Tri-O-(tert-butyldimethylsilyl)-2-O-trimethylsilyl-β-D-glucopyranosyl}-5-bromo-2,4(1H,3H)-pyrimidinedione (7l). 121 mg, 81%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H, H-9), 7.88 (s, 1H, H-12), 6.01 (d, *J* = 2.2 Hz, 1H, H-1), 4.18 (dd, *J* = 10.5, 5.9 Hz, 1H, H-5), 3.98 (s, 1H, H-2), 3.90 (dd, *J* = 10.8, 4.1 Hz, 1H, H-6a), 3.83 (d, *J* = 2.7 Hz, 1H, H-3), 3.77 (dd, *J* = 10.8, 6.9 Hz, 1H, H-6b), 3.67 (d, *J* = 5.5 Hz, 1H, H-4), 0.92 (d, *J* = 2.8 Hz, 18H, TBS), 0.90 (s, 9H, TBS), 0.16 (s, 3H, TBS), 0.13 – 0.09 (m, 11H, TBS), 0.07 (d, *J* = 0.9 Hz, 4H, TBS), 0.03 (s, 9H, TMS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.4 (C-10), 149.3 (C-8), 142.2 (C-12), 94.9 (C-11), 80.2 (C-5), 78.8 (C-1), 74.4 (C-3), 70.6 (C-4), 69.0 (C-2), 62.9 (C-6), 26.0, 25.8, 25.6, 18.4, 17.9, 17.8(TBS), -0.4 (TMS), -4.1, -4.5, -4.9, -4.9, -5.2, -5.3 (TBS). HRMS (ESI+): m/z calcd. for $C_{31}H_{63}BrN_2O_7Si_4$ (M+2) 768.2896, found 768.2891.

1-{3',4',6'-Tri-O-(benzyl)-2-O-trimethylsilyl-α-D-glucopyranosyl}-2,4(1H,3H)-pyrimidinedione (9a). 112 mg, 78%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H, H-9), 7.51 (d, *J* = 8.2 Hz, 1H, H-12), 7.29 – 7.21 (m, 13H, aromatic), 7.13 – 7.10 (m, 2H, aromatic), 5.61 (d, *J* = 8.0 Hz, 1H, H-11), 5.54 (s, 1H, H-1), 4.79 (d, *J* = 10.9 Hz, 1H, OBn), 4.71 – 4.63 (m, 2H, OBn), 4.53 – 4.48 (m, 3H, OBn), 4.17 (s, 1H, H-2), 3.78 (t, *J* = 9.6 Hz, 1H, H-6a), 3.66-3.64 (m, 2H, H-3, H-4), 3.59-3.55 (m, 2H, H-5, H-6b), -0.03 (s, 9H, TMS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.0 (C-10), 149.8 (C-8), 142.7 (C-12), 138.0, 137.8, 137.6 (quaternary aromatic), 128.5, 128.4, 128.2, 128.1, 127.9, 127.9, 127.8, 127.8, 127.7 (aromatic), 100.9 (C-11), 82.1 (C-5), 81.9 (C-1), 78.3 (C-3), 75.2 (OBn), 73.5 (C-4), 73.3 (OBn), 73.3 (OBn), 69.6 (C-2), 68.9 (C-6), 0.5 (TMS). HRMS (ESI+): m/z calcd. for $C_{34}H_{41}N_2O_7Si$ $(M+H)^+$ 617.2683, found 617.2683.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-α-D-glucopyranosyl}-5-methyl-2,4(1H,3H)-pyrimidinedione (9b).110 mg, 75%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H, H-9), 7.44 (s, 1H, H-12), 7.37 – 7.28 (m, 13H, aromatic), 7.21 – 7.17 (m, 2H, aromatic), 5.65 (s, 1H, H-1), 4.87 (d, *J* = 10.9 Hz, 1H, OBn), 4.78 – 4.69 (m, 2H, OBn), 4.63 – 4.57 (m, 3H, OBn), 4.23 (s, 1H, H-2), 3.84 (t, *J* = 9.4 Hz, 1H, H-6a), 3.76 – 3.61 (m, 4H, H-3, H-4, H-5, H-6b), 1.91 (s, 3H, methyl), 0.04 (s, 9H, TMS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.9 (C-10), 150.0 (C-8), 138.6 (C-12), 138.0, 137.9, 137.6 (quaternary aromatic), 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6 (aromatic), 108.9 (C-11), 82.2 (C-1), 81.6 (C-5), 78.2 (C-3), 75.1 (OBn), 73.5 (C-4), 73.2 (OBn), 73.2 (OBn), 69.8 (C-2), 69.0 (C-6), 12.4 (methyl), 0.4 (TMS). HRMS (ESI+): m/z calcd. for $C_{35}H_{43}N_2O_7Si$ $(M+H)^+$ 631.2840, found 631.2800.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-α-D-glucopyranosyl}-5-bromo-2,4(1H,3H)-pyrimidinedione (9c). 123 mg, 76%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H, H-9), 7.91 (s, 1H, H-12), 7.40 – 7.31 (m, 13H, aromatic), 7.22 – 7.19 (m, 2H, aromatic), 5.67 (s, 1H, H-1), 4.88 (d, *J* = 11.0 Hz, 1H, OBn), 4.80 – 4.71 (m, 2H, OBn), 4.64 (d, *J* = 3.6 Hz, 2H, OBn), 4.58 (d, *J* = 11.0 Hz, 1H, OBn), 4.15 (s, 1H, H-2), 3.84 – 3.74 (m, 3H, H-3, H-4 ,H-6a), 3.72 – 3.65 (m, 2H, H-5, H-6b), 0.08 (s, 9H, TMS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.0 (C-10), 149.3 (C-8), 142.1 (C-12), 137.7, 137.5, 137.5 (quaternary aromatic), 128.5, 128.5, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8 (aromatic), 95.7 (C-11), 82.0 (C-1), 81.8 (C-5), 78.2 (C-3), 75.1 (OBn), 73.4 (C-4), 73.4 (OBn), 73.2 (OBn), 69.7 (C-2), 69.0 (C-6), 0.5 (TMS). HRMS (ESI+): m/z calcd for $C_{34}H_{40}BrN_2O_7Si$ $(M+2+H)^+$ 697.1788, found 697.1734.

1-{3',4',6'-Tri-*O*-(benzyl)-2-*O*-trimethylsilyl-α-D-glucopyranosyl}-5-chloro-2,4(1H,3H)-pyrimidinedione (9d). 119 mg, 79%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.91 (s, 1H, H-9), 7.81 (s, 1H, H-12), 7.39 – 7.32 (m, 13H, aromatic), 7.22 – 7.19 (m, 2H, aromatic), 5.67 (s, 1H, H-1), 4.88 (d, *J* = 11.0 Hz, 1H, OBn), 4.80 – 4.71 (m, 2H, OBn), 4.63 (d, *J* = 1.5 Hz, 2H, OBn), 4.62-4.59 (m, 1H, OBn,), 4.23 (s, 1H, H-2), 3.83 – 3.66 (m, 5H, H-3, H-4, H-5, H-6a, H-6b) ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.0 (C-10), 149.1 (C-8), 139.6 (C-12), 137.7, 137.7, 137.5 (quaternary aromatic), 128.5, 128.5, 128.3, 128.2, 128.0, 127.9, 127.9, 127.8, 127.8 (aromatic), 107.9 (C-11), 82.0 (C-1), 81.8 (C-5), 78.2 (C-3), 75.1 (OBn), 73.4 (C-4), 73.4 (OBn), 73.2 (OBn), 69.7 (C-2), 69.0 (C-6), 0.4 (TMS). HRMS (ESI+): m/z calcd. for $C_{34}H_{40}ClN_2O_7Si$ $(M+H)^+$ 651.2293, found 651.2275.

1-{3,4-bis(trimethylsilyl)oxy}-5{(trityloxy)methyl) tetrahydrofuran-2-yl}pyrimidine-2,4(1H,3H)-dione (12a).90 mg, 53%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (s, 1H, H-8), 7.66 (d, *J* = 8.1 Hz, 1H, H-12), 7.47-7.43 (m, 6H, aromatic), 7.39-7.31 (m, 9H, aromatic), 6.29 (d, *J* = 3.8 Hz, 1H, H-1), 5.73 (d, *J* = 8.1 Hz, 1H, H-11), 4.40 (t, *J* = 3.8 Hz, 1H), 4.22 (s, 1H), 4.20 – 4.16 (m, 1H), 3.46 (d, *J* = 10.6 Hz, 1H, H-5a), 3.08 (dd, *J* = 10.5, 3.9 Hz, 1H, H-5b), 0.05 (s, 9H, TMS), -0.03 (s, 9H, TMS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.6 (C-9), 150.6 (C-7), 143.8 (C11), 143.6, 143.0 (OTr), 128.7, 128.6, 127.9, 127.2 (aromatic), 100.4 (C-10), 86.9 (C-4), 85.7 (C-1), 82.5, 72.6, 72.2, 63.1, 0.1 (TMS), 0.0 (TMS). HRMS (ESI+): m/z calcd. for $C_{34}H_{43}N_2O_6Si_2$ $(M+H)^+$ 631.2660, found 631.2665.

1-{3,4-bis((trimethylsilyl)oxy}-5{(trityloxy)methyl) tetrahydrofuran-2-yl}-5-bromopyrimidine-2,4(1H,3H)-dione (12b). 115 mg, 68%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H, H-11), 7.47-7.43 (m, 6H, aromatic), 7.37-7.31 (m, 9H, aromatic), 6.34 (d, *J* = 4.7 Hz, 1H, H-1), 4.48 (t, *J* = 4.5 Hz, 1H), 4.26 (s, 1H), 4.17 – 4.13 (m, 1H), 3.47 (dd, *J* = 10.6, 2.8 Hz, 1H, H-5a), 3.09 (dd, *J* = 10.7, 3.6 Hz, 1H, H-5b), 0.07 (s, 9H, TMS), 0.01 (s, 9H, TMS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.2 (C-9), 150.0 (C-7), 143.5 (C-11), 143.1 (OTr), 128.7, 128.0, 127.3 (aromatic), 94.8 (C-10), 87.1 (C-4), 85.8 (C-1), 83.8, 72.5, 63.1 (C-5), 0.1 (TMS), -0.1 (TMS). HRMS (ESI+): m/z calcd. for $C_{34}H_{42}BrN_2O_6Si_2$ $(M+2+H)^+$ 711.1765, found 7011.1781.

General procedure for synthesis of compounds 13, 14

To a solution of **7a** (1 equiv) in 1 mL of methanol was added 1N hydrochloric acid (3 mL) and stirred for 0.5 h at room temperature. After complete consumption of starting material, reaction mixture was neutralized with saturated aqueous solution of sodium bicarbonate. The reaction mixture was extracted with ethyl acetate and washed with brine, dried with Na_2SO_4 and evaporated in vacuo to get product.

1-{3',4',6'-Tri-*O*-(benzyl)-β-D-glucopyranosyl}-2,4(lH,3H)-pyrimidinedione (13). 60 mg, 97%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 7.1 Hz, 1H), 7.34 – 7.27 (m, 13H), 7.23 – 7.19 (m, 2H), 5.70 (d, *J* = 8.1 Hz, 1H), 5.67 (d, *J* = 9.3 Hz, 1H), 5.02 (d, *J* = 11.4 Hz, 1H), 4.89 (d, *J* = 12.2 Hz, 2H), 4.63 – 4.48 (m, 4H), 3.86 – 3.80 (m, 1H), 3.72 – 3.67 (m, 4H), 2.18 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.4, 151.2, 140.0, 138.5, 138.0, 137.8, 128.6, 128.5, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.8, 103.2, 85.2, 82.6, 77.7, 75.5(2C), 75.0, 73.5, 73.3, 68.5. HRMS (ESI+): m/z calcd. for $C_{31}H_{33}N_2O_7$ $(M+H)^+$ 545.2288, found 545.2269.

1-{3',4',6'-Tri-*O*-(benzyl)-β-D-glucopyranosyl}-5-methyl-2,4-(1H,3H)-pyrimidinedione (14). 51 mg, 96%, gummy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.18 (s, 1H), 7.40 – 7.29 (m, 13H), 7.23-7.19 (m, 3H), 5.72 (d, *J* = 9.2 Hz, 1H), 5.03 (d, *J* = 11.3 Hz, 1H), 4.92 – 4.85 (m, 2H), 4.63 – 4.48 (m, 3H), 3.89 – 3.83 (m, 1H), 3.78 – 3.69 (m, 5H), 1.89 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.1, 151.5, 138.6, 138.2, 137.9, 128.4, 128.2, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.5, 111.5, 85.4, 82.6, 77.7, 75.6, 75.0, 73.5, 73.5, 73.4, 68.6, 12.5. HRMS (ESI+): m/z calcd. for $C_{32}H_{35}N_2O_7$ $(M+H)^+$ 559.2444, found 559.2457.

Procedure for the Synthesis of compound 15

1-(β-D-glucopyranosyl)-4-amino-2-(lH,3H)-pyrimidinone (15). To a solution of **7g** (0.16 mmol, 100 mg) in ethyl acetate (1 mL) taken in a round bottom flask was added 1N hydrochloric acid (2 mL) and stirred for 0.5 h at rt. After complete consumption of starting material, reaction was

neutralized with saturated solution of sodium bicarbonate, extracted with ethyl acetate, washed with brine, dried with Na_2SO_4 and evaporated in vacuo to get 2-hydroxy of **3g** (85 mg, 96%). without purification dissolved in Acetonitrile (1 mL) and charged sodium iodide (0.47 mmol, 70 mg) and trimethyl silyl chloride (0.31 mmol, 40 µL) successively and stirred for 1 h then triturated with hexane and ethyl acetate and concentrated in vacuo to get **15** as a gummy product (35 mg, 81%); ^{1}H NMR (400 MHz, MeOD) δ 7.75 (d, J = 8.1 Hz, 1H), 5.79 (d, J = 8.1 Hz, 1H), 5.57 (d, J = 8.8 Hz, 1H), 3.90 (dd, J = 12.1, 1.7 Hz, 1H), 3.75 (dd, J = 12.1, 4.9 Hz, 1H), 3.61 – 3.55 (m, 1H), 3.53 – 3.46 (m, 2H), 3.38 (s, 1H), 3.34 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 164.5, 151.4, 141.4, 101.9, 83.0, 79.6, 77.2, 71.6, 69.6, 61.0. HRMS (ESI+): m/z calcd. for $C_{10}H_{16}N_3O_6$ $(M+H)^+$ 274.1039,found 274.1047.

Procedure for the Synthesis of compound 16

1-(β-D-glucopyranosyl)-2,4(1H,3H)-pyrimidinedione (16). To a solution of the **7i** (50 mg) in MeOH (2 mL) was added conc. HCl (12N, 1 mL) at rt. The reaction mixture was stirred for 2 h, after which time it was concentrated to remove MeOH. The residue was triturated with hexane (10 mL) to provide the product as whitish solid. 17 mg, 76%; ^{1}H NMR (400 MHz, MeOD) δ 7.89 (d, J = 8.1 Hz, 1H, H-12), 6.11 (d, J = 2.8 Hz, 1H, H-1), 5.68 (d, J = 8.1 Hz, 1H, H-11), 4.09 – 4.04 (m, 1H, H-5), 4.00 – 3.93 (m, 2H, H-2, H-6a), 3.93 – 3.89 (m, 1H, H-3), 3.75 (dd, J = 12.2, 2.8 Hz, 1H, H-6b), 3.63 (t, J = 5.0 Hz, 1H, H-4). ^{13}C NMR (101 MHz, MeOD) δ 166.4 (C-10), 152.3 (C-8), 145.1 (C-12), 102.0 (C-11), 82.0 (C-5), 79.3 (C-1), 74.0 (C-3), 71.5 (C-4), 70.4(C-2), 61.8 (C-6). HRMS (ESI+): m/z calcd. for $C_{10}H_{15}N_2O_7$ $(M+H)^+$ 275.0879, found 275.0888.

BIOFILM FORMATION ASSAY

Inhibition of biofilm formation was determined by following protocol adopted by[18] using tissue culture plate with minor modifications. The bacterial strains used in this study were *S. typhimurium* (MTCC 98), *P. aeruginosa* (MTCC 424), *B. subtilis* (MTCC 121), and *S. aureus* (MTCC 737). All bacterial cultures were maintained on Muller Hinton agar media (MHA). The in vitro biofilm formation assay was performed using round bottom 96 well microtiter plate. For this study, different bacterial cultures were grown in tryptone soya broth (TSB) supplemented with 2% sucrose for 24 hrs at 37 ºC. The overnight culture was diluted 1:1000 in TSB broth media, which corresponds to 1x105 CFU/mL. This suspension was added to each well of 96-well plate containing a different concentration of test compound (range 500-0.061 µM) and incubated for 24 h at 37 ºC under stationary condition. The well without test compound was served as the negative control. After incubation, the planktonic/unattached cells were discarded by aspirating the supernatant and wells were washed thrice with an equal volume of distilled water. To quantify the biofilm formed, 100 µL of 0.4% (w/v) crystal violet dye was added for 30 min. The excess stain was removed by washing four times with distilled water and immediately discoloured with 200 µL of 95% ethanol. The optical density at 570 nm (OD 570) of the stained biofilm was measured using a microtiter plate reader (Molecular Devices, Sunnyvale, CA, USA).[19] The percentage of biofilm formed was calculated comparing the absorbance of the compound treated wells versus untreated control wells. The Minimum Biofilm Inhibition Concentration (MBIC) was defined as the minimum concentration required for reducing the biofilm formation by 90% as compared to the control. Experiments were performed in triplicate, and the mean data were presented.

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Title

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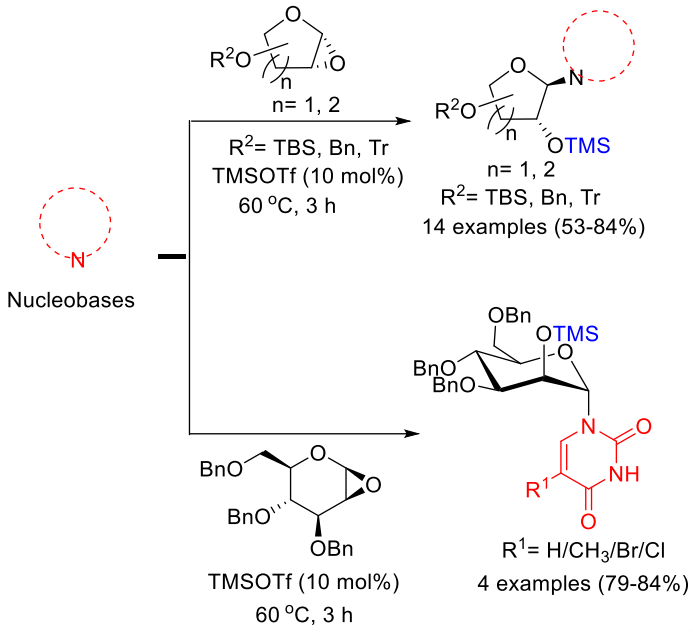

Silyl protected nucleosides possess better biological activity. 2-*O*-silylated nucleosides were synthesized by trans opening of 1,2-anhydrosugar with nucleobases where HMDS acts both as activating and silylating agent. Conformational analysis and biofilm inhibition activity of synthesized nucleosides were performed.

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