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# A Facile Synthesis of 8-Bromo-3-methyl-7-(β-D-ribofuranosyl)purine-2,6-dione with $SnCl_4$ under Vorbruggen Glycosylation Conditions

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In course of the synthetic studies of 8-bromo-3-methyl based nucleosides, we have developed a high regeo selective synthetic route to acquire *N*-7 isomer in the presence of different Lewis acids. The glycosylation reaction was performed with tin(IV) chloride as a Lewis acid instead of TMSOTf to avoid the emulsion formation, cross contamination of trifluoromethanesulfonic acid (TfOH) in the isolated product with similar yields (70 %). The concentration of sodium methoxide solution, reaction time and reaction temperature was studied for the successful deprotection of sugars. The consistent heavy metal (tin) lower limit in the range of 10-15 ppm obtained at various scales in isolated nucleosides, which can be pharmaceutically accepted as per ICH guidelines.

**Keywords: 8-Bromo-3-methyl-1H-purine-2,6-(3H,7H)-dione, D-ribofuranose, Vorbruggen glycosilylation.**

## INTRODUCTION

A convenient two step synthesis was described for the synthesis of 8-bromo-3-methyl-7-(β-D ribofuranosyl)purine-2,6-dione (throughout this article, purine numbering system is only used) in the presence of tin(IV) chloride ($SnCl_4$) as Lewis acid in ethyl aceate according to Vorbruggen glycosylation method. Earlier studies [1-3] for the synthesis of nucleosides *via* Vorbruggen glycosylation have been concerned with the basic condition by $SN^1$-like pathway of nucleophilic substitution reactions. Among them some studies were reported by preparing the sodium salt or mercury salt [4] of purines in the presence of inert solvent.

## EXPERIMENTAL

All the solvents were distilled from $P_2O_5$, sugars (**6a**, **6b**) were purchased from Sigma and silylated bromoxanthine was prepared according to reported method [1-3]. TLC analysis was performed on Merck silica gel 60 $F_{254}$ plates and visualized under UV illumination at 254 nm in DCM-MeOH (99:1).

All glycosylation reactions were carried out under argon atmosphere. Melting points were measured on a Buchi capillary apparatus and are uncorrected. UV spectra were recorded on a 2300 spectrophotometer. IR spectra were recorded on Perkin-Elmer 1420 spectrophotometer. Optical rotations were determined on a Perkin-Elmer Inc. Model 341 polarimeter and are average of at least three measurements.

HPLC analyses were carried out on an HP Agilent 1260 system, composed of quaternary pump, auto sampler, photodiode array detector (DAD) and HP Chemstation software. The separation was carried out on a C18 Zorbax extend column 50 × 4.6 mm, 5 µm particle size. Mobile phase was prepared as ammonium bicarbonate (solvent A) - 0.1 % in water and acetonitrile (solvent B) at a flow rate of 1 mL/min at 20 °C.

Chiral purity analysis were carried out on waters HPLC 6000 system composed of quaternary pump, auto sampler, photodiode array detector Multi 1/281 nm 4 nm. The separation was carried out on CHIRAL- PAK AD-H (250 X 4.6 mm, 5 µ particle size). Mobile phase was comprised of 0.1 % diethyl amine (DEA) in hexane-ethanol (30:70) at a flow rate of 1.0 mL/min.

LC-MS analysis was performed on Agilent 1200 system, operating in ESI mode with detection of positive and negative ions which was equipped with Zorbax XDB column. The eluent was of 0.1 % ammonium carbonate in water with a gradient of solution in acetonitrile. Mass spectrometric analyses were performed using XCT ion trap mass spectrometers with ESI interface at chromatographic peaks were detected with evaporating light scattering detector (ELSD) and pulsed amperometric detection (PAD).

For the chromatographic separations of compound **7** and **8**, Agilent 1100 system with Zorbax XDB column (4.6 × 50 mm, 1.8 µm) was used with a flow rate of 200 µL/min. The mobile phase was prepared of 0.1 % if ammonium bicarbonate

(solvent A), acetonitrile (solvent B). Ionization analysis was carried out using electron spray ionization (ESI). The capillary temperature was maintained at 275°C and ion source voltage was set at 5000 V and the nebulizer gas was set at 300 units. The capillary voltage was set on positive mode at 15 V and negative ionization mode at -8 V. The average scan time was set as 0.01 min while the average is 0.02 min to change the polarity. The collision energy was maintained about 35 % abundance of the precursor ion.

$^1$H NMR spectra were recorded at Bruker AMX Ultra shield 400 MHz (with residual solvent signals as internal standards DMSO-$d_6$, δ = 2.50 and $CDCl_3$, δ = 7.20) and $^{13}$C NMR at 100 MHz with Bruker spectrometer using the DMSO-$d_6$. Preparative HPLC was carried out with Waters Model 6000 pump and Model 405 differential refractometer detector.

Purine base 8-bromo-3-methyl-3,7-dihydro-1H-purine-2,6-dione (**4**) was suspended in neat 1,1,1,3,3,3-hexamethyldisilazane (HMDS) with catalytic amount of anhydrous ammonium sulphate crystals and refluxed for 3 h. Thus obtained clear solution was distilled *in vacuo* and taken up with sugar in EtOAc. Anhydrous $SnCl_4$ was added drop wise and stirred for 2 h at room temperature. Samples were taken at different intervals, satd. $NaHCO_3$ solution used for quenching the sample and then analyzed by HPLC.

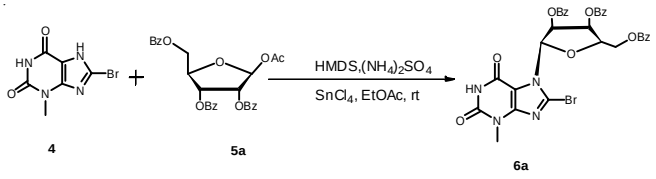
**Silylation-method A (with neat HMDS):** 8-Bromo-3-methyl-3,7-dihydro-1H-purine-2,6-dione (**4**) (1.0 g) and catalytic amount (≈ 10 mg) of anhydrous $(NH_4)_2SO_4$ were suspended in HMDS (10 mL) and heated to reflux for 3 h and the resulting dark brown solution was evaporated *in vacuo*.

**Silylation-method B (combination of solvents with HMDS):** 8-Bromo-3-methyl-3,7-dihydro-1H-purine-2,6-dione (**4**) (1.0 g) and catalytic amount (≈ 10 mg) of anhydrous $(NH_4)_2SO_4$ were suspended HMDS(10 mL), toluene (10 mL) and heated to reflux for 3 h. Thus obtained clear brown solution was evaporated *in vacuo*.

**Silylation-method C (with BSA):** 8-Bromo-3-methyl-3,7-dihydro-1H-purine-2,6-dione (**4**) (1.0 g) was added to N,O-*bis*-trimethylsilyl-acetamide (BSA) (10 mL), 1,2-dichloroethane (DCE) (10 mL) and refluxed at 80 °C for 2 h. Excess of BSA (5 mL) was added to dissolve the solid and evaporated the complete BSA *in vacuo*.

**Synthesis**

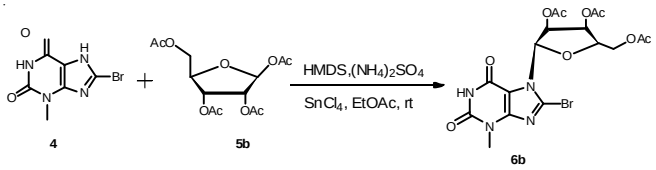
**3-Methyl-8-bromo-7-(2,3,5-tri-O-benzoyl-β-D-ribofurnaosyl)-purine-2,6-dione (6a):** A suspension of 8-bromo-3-methyl-3,7-dihydro-1H-purine-2,6-dione 4 (5.34 g, 21.82 mmol) and a catalytic amount of $(NH_4)_2SO_4$ (20 mg) in HMDS (25 mL) was heated to reflux (≈ 125 °C) in an inert atmosphere under argon. A clear solution obtained after 30 min. Reflux continued for 3 h and cooled to room temperature. Excess HMDS was removed *in vacuo* to obtain white solid which is further dried at 90 °C under vacuum for 2 h to remove traces of HMDS. Thus obtained white solid and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**5a**) (10.0 g, 19.83 mmol) were taken up in dry EtOAc (100 mL) under argon atmosphere. The reaction mixture is cooled to 0 °C and $SnCl_4$ (3.5 mL) was added drop wise in 15 min under argon atmosphere. After completion of the addition, reaction mixture was stirred for 15 min at 0 °C before raised to room temperature. After 1 h stirring at room temperature, $Na_2CO_3$ (2.0 g), $NaHCO_3$ (2.0 g) were added to the reaction mixture. Reaction mixture was cooled to 5 °C and purified water (25 mL) was added by maintaining the temperature at 5 °C to 10 °C. Reaction mixture was filtered after 10 min and filtrate was washed with satd. $NaHCO_3$, $Na_2CO_3$ and brine solution. Organic layer was dried over $MgSO_4$ and distilled out *in vacuo* at 50 °C to obtain white foam. Thus obtained product was dissolved in hot EtOAc (25 mL) (50 °C) and silica gel (15.0 g) (60-120 mesh), activated charcoal (2.0 g) was added. Stirred for 10 min at 50 °C and filtered through the celite pad and washed the celite pad with hot EtOAc (100 mL). Distilled out the complete solvent to dryness to obtain crude **6a** (11.6 g, 85 % yield) as white foam (**Scheme-I**). The crude product chromatographed (hexane-EtOAc, 6:4) to give the pure **6a** (9.54 g, 70 % yield) as off-white solid. m.p. >155 °C; $[\alpha]_D^{20}$= -33° (C = 1.0 in DCM), $^1$H NMR (400 MHz, $CDCl_3$,) δ ppm 8.55 (s, 1H, N-H), 7.80-8.07 (m, 6H, o-Ar-H), 7.40-7.55 (m, 6H, p-Ar-H), 7.25-7.29(m, 3H, m-Ar-H), 6.30 (d, 1H, H-1′), 6.13 (m, 1H, H-2′), 4.95-5.01 (t, 2H, H-3′ & 4′), 4.75 (t, 2H, -$CH_2$), 3.55 (s, 3H, N-$CH_3$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, ppm) 165.1, 153.9, 151.2, 150.8, 135.0, 134.1, 133.0, 129.0, 128.2, 108.4, 90.2, 79.3, 30.1. MS (ES+) *m/z*: 687-689 ($^{81}$Br). Anal. calcd. for $C_{32}H_{25}BrN_4O_9$: C, 55.74; H, 3.65; Br, 11.59; N, 8.13. Found: C, 55.81; H, 3.71; Br, 11.65; N, 8.15.




**Scheme-I**

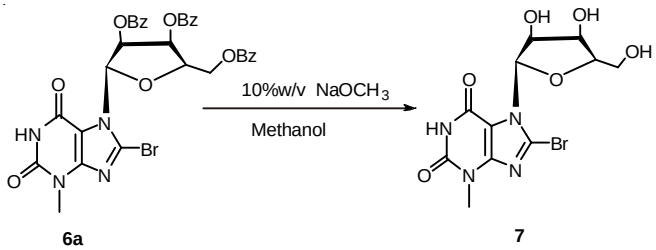
**3-Methyl-8-bromo-7-(2,3,5-tri-O-acetyl-β-D-ribofurnaosyl)-purine-2,6-dione (6b):** To a suspension of 8-bromo-3-methyl-3,7-dihydro-1H-purine-2,6-dione 4 (8.47 g, 34.58 mmol) and a catalytic amount of $(NH_4)_2SO_4$ (20 mg) was suspended in HMDS (25 mL) and DCE (25 mL) was heated to reflux in inert atmosphere under argon. A clear solution obtained after 0.5 h, reflux continued for 3 h and cooled to room temperature. Excess solvent was removed *in vacuo* to obtain white solid which is further dried at 90 °C for 1 h to remove traces of HMDS. Thus obtained white solid and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (**5b**) (10 g, 31.44 mmol) were taken up in dry EtOAc (100 mL) under argon atmosphere. The reaction mixture is cooled to 0 °C and $SnCl_4$ (6 mL) was added drop wise in 15 min under argon. After completion of the addition, stirred for 15 min at 0 °C and raised the temperature to room temperature. After 1 h stirring $Na_2CO_3$ (2 g), $NaHCO_3$ (2 g) were added to the reaction mixture. Reaction mixture was cooled to 5° C and added purified water (25.0 mL) by maintaining the temperature at 5 °C to 10 °C. Reaction mixture was filtered after 10 min and filtrate was washed with satd. $NaHCO_3$, $Na_2CO_3$ and brine solution. Organic layer was dried over $MgSO_4$ and distilled out *in vacuo* at 50 °C to obtain white foam. Thus obtained product was dissolved in hot EtOAc (20 mL) (50 °C) and silica gel (15.0 g) (60-120 mesh), activated charcoal (2.0 g) was added. Reaction mixture was stirred for

10 min at 50 °C and filtered through the celite pad and washed the celite pad with hot EtOAc (100 mL). Distilled out the complete solvent to dryness to obtain crude **6b** (12.62 g, 80 % yield) as yellow foam (**Scheme-II**). The crude product chromatographed (hexane-EtOAc, 6:4) to give the pure **6b** (9.47g, 60 % yield) as off-white solid. m.p. >128 °C; $[\alpha]_D^{20}$ = -29° (C = 1.0 in DCM), $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.55 (s, 1H, N-H), 6.1 (d, 1H, H-1′), 5.72 (s, 1H, H-4′), 5.48 (s, 1H, H-2′), 4.45 (m, 1H, H-3′),4.19-4.33 (m, 2H, -$CH_2$), 3.34 (s, 3H, N-$CH_3$), 2.10 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.01 (s, 3H, Ac). $^{13}$C NMR (100 MHz, DMSO-$d_6$, ppm) δ: 170.1, 161.8, 150.8, 141.1, 117.3, 116.1, 85.9, 82.7, 73.1, 63.5, 31.5, 21.0, 21.1, 20.9. MS (ES+) *m/z*: 502-505 ($^{81}$Br). Anal. calcd. for $C_{17}H_{19}BrN_4O_9$: C, 40.57; H, 3.81; Br, 15.88; N, 11.13. Found: C, 41.90; H, 3.90; Br, 16.23; N, 12.38.



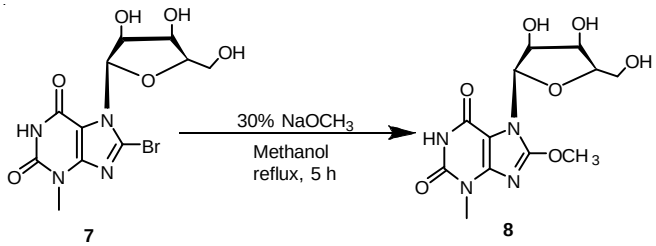

**Scheme-II**

**3-Methyl-8-bromo-7-(β-D-ribofuranosyl)-purine-2,6-dione (7)**: To a suspension of 3-methyl-8-bromo-7-(2,3,5-tri-O-benzoyl-β-D-ribofurnaosyl)-purine-2,6-dione **(6a)** (10.0 g, 14.53 mmol) in methanol (50 mL) was heated to 35 °C and added sodium methoxide solution (5.0 mL) (10 % w/v in MeOH) and the reaction mixture was stirred for 2 h at 35 °C to get a clear solution. Reaction monitoring was carried out by HPLC analysis. The reaction mixture was cooled to 5-10 °C and glacial acetic acid (0.2 mL) was added drop wise to neutralize the reaction mixture. Then *n*-heptane (100 mL) was added and stirred for 24 h at room temperature. The methanol layer was collected by separation and evaporated *in vacuo* to give crude **7** (90 %) (**Scheme-III**). Crude **7** was loaded to prep HPLC and collected pure fractions were evaporated *in vacuo* to obtain pure **7** (4.38 g, 80 %) as white solid. (Purity by HPLC 98 %). m.p.: 137 °C, $[\alpha]_D^{20}$= -20° (C = 1.0 in $H_2O$), $^1$H NMR (400 MHz, $D_2O$,) δ ppm 5.87 (d, 1H, H-1), 5.40 (d, 1H, H-4), 5.20 (d, 1H, H-2), 3.71-3.84 (m, 2H, -$CH_2$), 3.34 (S, 3H, N-$CH_3$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, ppm) δ: 29.17, 62.1, 70.6, 72.7, 86.8, 91.3, 108.6, 130.1, 150.7, 151.3, 153.6. MS ($ES^+$) *m/z*: 376-379($^{81}$Br).Anal. calcd. for $C_{11}H_{13}BrN_4O_6$: C, 35.03; H, 3.47; Br, 21.19; N, 14.86. Found: C, 35.00; H, 3.40; Br, 21.21; N, 14.90.




**Scheme-III**

**3-Methyl-8-methoxy-7-(β-D-ribofuranosyl)-purine-2,6-dione (8):** To a solution of 3-methyl-8-bromo-7-(β-D-ribofuranosyl)-purine-2,6-dione **7** (1.0 g, 2.65 mmol) in methanol (10 mL) was added sodium methoxide (30 % w/v, 2.5 mL) and heated to 60 °C for 5 h. Reaction mixture was cooled to room temperature and adjusted the pH to 3.7 with glacial acetic acid and *n*-heptane (50 mL) was added. Reaction mixture was stirred for 24 h and bottom methanol layer was collected by separation. Distilled out methanol *in vacuo* to give 3-methyl-8-methoxy-7-(β-D-ribofuranosyl)-purine-2,6-dione **8** (0.63 g, 80 % w/w) (**Scheme-IV**). m.p.: 158 °C, $[\alpha]_D^{20}$ = -37° (C = 1.0 in $H_2O$), $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 11.1 (s. 1H, -NH), 5.81 (d, 1H, H-12), 4.5 (d, 1H, H-2′), 4.0 (d, 1H, H-3′), 3.79 (m, 1H, H-4′), 3.56-3.60 (m, 2H, -$CH_2$), 3.34 (s, 3H, N-$CH_3$). $^{13}$C NMR (100 MHz, DMSO-$d_6$,ppm) δ: 29.17, 62.3, 70.3, 72.5, 85.9, 87.5, 102.9, 129.4, 149.0, 151.1, 154.3, 156.9. MS ($ES^+$) *m/z*: 328.1. Anal. Cald for $C_{12}H_{16}N_4O_7$: C, 43.90; H, 4.91; N, 17.07. Found C, 43.98; H, 4.97; N, 17.10.




**Scheme-IV**

## RESULTS AND DISCUSSION

In the present work, we have used 8-bromo-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione **4,** as purine for the synthesis of corresponding traditional key-intermediates **6a** and **6b**. Thus obtained intermediates eventually converted in to the desired 7-β-substituted nucleosides by the direct nucleophilic displacement reactions. By the careful examination of the literature [5] the purine **4** was silylated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in the presence of ammonium sulphate to obtain the intermediates **6a**, **6b**. According to the reported literature [6], we have investigated different solvents such as toluene, xylene and acetonitrile in the presence of HMDS. There was no much difference in the yield (≈ 50 %) when trimethylsilylchloride (TMS-Cl) used with the combination of HMDS for the silylation. The best result was obtained (yield 70 % of **6a** and 60 % of **6b**) when HMDS was used for the silylation of **4** with TMS-Cl and $(NH_4)_2SO_4$ (entries 1-2, Table-1).

We thoroughly studied the effect of silylating reagent by performing the silylation of purine **4** with different silylating reagents such as N,O-*bis*(trimethylsilyl)acetamide (BSA), HMDS, sodium*bis*(trimethylsilyl)amide (NaHMDS) and lithium*bis*(trimethylsilyl)amide (LiHMDS) with different catalyst such as TMS-Cl, $(NH_4)_2SO_4$, iodine and trimehtylsilyliodide (TMS-I). Surprisingly we observed the differences in purity and yield of **6a** and **6b**. Even though BSA worked as a better silylating reagent in the absence of catalyst. Kim *et al.* [6] and others [7] reported that HMDS is a better silylating reagent than BSA in the presence of non-aromatic solvents such as dichloromethane (DCM), 1,2-dichloroethane (DCE) and acetonitrile (ACN) to get desired 7-β-isomer. By considering

TABLE-1
DIFFERENT SILYLATING REAGENTS AND CATALYSTS FOR THE SILYLATION OF 8-BROMO-3-METHYL-1H- PURINE-2,6-(3H,7H)- DIONE (**4**)

| Entries | Salylating reagent | Temp. (°C) | Catalyst | Time (min) | Yield[d] (%) of **6a** | Yield[d] (%) of **6b** |
|---|---|---|---|---|---|---|
| 1 | HMDS | 125 | TMSCl | 180 | 70 | 61 |
| 2 | HMDS | 125 | $(NH_4)_2SO_4$ | 180 | 70 | 60 |
| 3 | HMDS | 125 | $I_2$ | 180 | 58 | 55 |
| 4 | HMDS | 125 | TMSI | 180 | 65 | 55 |
| 5 | HMDS[a] | 125 | TMSCl | 180 | 50 | 50 |
| 6 | HMDS[b] | 125 | TMSCl | 180 | 52 | 48 |
| 7 | HMDS[c] | 125 | TMSCl | 180 | 60 | 55 |
| 8 | HMDS[a] | 125 | $(NH_4)_2SO_4$ | 180 | 55 | 52 |
| 9 | HMDS[b] | 125 | $(NH_4)_2SO_4$ | 180 | 50 | 52 |
| 10 | BSA | 80 | – | 180 | 71 | 60 |

Reaction conditions: [a]1:1 v/v toluene was used for the reaction; [b]1:1 v/v xylene was used for the reaction; [c]1:1 v/v acetonitrile used for the reaction; [d]Isolated yield.

this result HMDS was used for the silylation with different catalyst like TMS-Cl, $(NH_4)_2SO_4$ and $I_2$ (Table-1).

The excess HMDS was removed *in vacuo* after 3 h of heating at 125 °C to give an off-white solid. However, since excess HMDS had remarkable effect on glycosylation [7,8] thus obtained off-white solid further dried under high vacuum for another 2 h at 90 °C to remove traces of HMDS. Thus obtained solid was used for the coupling with activated sugar under an inert atmosphere. The pre-silylated **4** was then coupled with 0.90 equiv of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose **5a** and 1,2,3,5-O-tetra-O-acetyl-β-D-ribofuranose **5b** in the presence of 1.5 equiv. of $SnCl_4$ as a Lewis acid in EtOAc at 0 °C in separate experiments. This classical reaction conditions lead us to obtain a 70 % and 60 % yields of **6a** and **6b** respectively (entries 7-8, Table-2). Careful analysis of the mother liquor by HPLC has shown very less amount of positional isomer (≤ 4 %).

Even though there are majority of the literature reported for the direct glycosylation by using trimethylsilyl- trifluoromethanesulfonate (TMSOTf), the quantity of TMSOTf is a critical parameter to obtain pure nucleoside. Bobek and Huang [8] was reported that when the excess quantity of TMSOTf (more than 5 equiv.) was used for the coupling, TMSOTf is blocking the *N*-7 isomer formation and in other side, *N*-1 isomer is obtained with good yields. Being a superior silylating reagent, TMSOTf can enhance the ring cleavage of sugars, leading to obtain lower purity as well as lower yields [9]. The byproduct of TMSOTf (TfOH) [10] is also cross contaminating with the isolated product of nucleosides (identified by $^1$H NMR). The resulting TfOH was removed by the aqueous workup, silica gel purification. Bookser and Raffaele [11] reported some reagents in basic media and methods to remove the traces of TfOH. This phenomenon led us to search for another suitable Lewis acid for the glycosylation such as $SnCl_4$, borontriflouride dietherate ($BF_3{\cdot}Et_2O$), aluminum chloride, silver perchlorate for the direct glycosylation of the silylated purines with an activated sugar. But there was no **6a** or **6b** product formation was observed when $AlCl_3$ and $AgClO_4$ were used even at 60 °C (entries 1-2, Table-2). The coupled product formation was obtained when $BF_3.Et_2O$ was used at 0 °C and -78 °C temperature for the glycosylation with **5a** and **5b** in DCM with very poor yields (25 % of **6a** and 20 % of **6b**) (entry 3-4, Table-2).

Ionescu and Blumbergs [12] reported that there is a lack of control on heavy metal (tin) levels when $SnCl_4$ is used as Lewis acid catalyst for the glycosylation. Praveen and Satyanarayana [13] recently reported the process for the preparation of active pharmaceutical ingredient of nucleosides by using $SnCl_4$ to control the heavy metal (tin) levels. We optimized the process to get consistent heavy metal content (tin ≈ 10-15 ppm) in various scale up batches. While we were need of large amount of quantity of **7** for the biological activity studies we have performed synthesis of **7** on higher scale (5-500 g) and achieved the same yield and heavy metal content limit (15

TABLE-2
DIFFERENT LEWIS ACIDS AND SOLVENTS USED FOR THE PREPARATION OF **6a**, **6b** (**Scheme-I**)

| Entries | Lewis acid | Lewis acid equiv. | Sugar | Temp. (°C) | Solvent | Time (min) | Purity by HPLC | Yield[a] (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | $AlCl_3$ | 2.5 | **5a** | 60 | EtOAc | 120 | 0 | 0 |
| 2 | $AgClO_4$ | 2.5 | **5b** | 60 | EtOAc | 120 | 0 | 0 |
| 3 | $BF_3{\cdot}Et_2O$ | 2.5 | **5a** | 0 | EtOAc | 90 | 30 | 25 |
| 4 | $BF_3{\cdot}Et_2O$ | 2.5 | **5b** | -78 | EtOAc | 90 | 27 | 20 |
| 5 | $SnCl_4$ | 2.5 | **5a** | 25 | EtOAc | 90 | 98 | 70 |
| 6 | $SnCl_4$ | 2.5 | **5b** | 25 | EtOAc | 90 | 97 | 60 |
| 7 | $SnCl_4$ | 1.5 | **5a** | 25 | EtOAc | 90 | 98 | 70 |
| 8 | $SnCl_4$ | 1.5 | **5a** | 25 | EtOAc | 90 | 96 | 60 |
| 9 | $SnCl_4$ | 1.5 | **5b** | 25 | EtOAc | 90 | 50 | 40 |
| 10 | $SnCl_4$ | 1.0 | **5a** | 25 | EtOAc | 90 | 49 | 35 |
| 11 | $SnCl_4$ | 1.0 | **5b** | 25 | EtOAc | 90 | 50 | 40 |
| 12 | TMSOTf | 1.5 | **5a** | 25 | EtOAc | 90 | 97 | 65 |
| 13 | TMSOTf | 1.5 | **5b** | 25 | DCE | 90 | 97 | 62 |

[a]Isolated yield of **6a** and **6b**

ppm) without using any expensive heavy metal scavengers (Table-3).

The amount of $SnCl_4$ is an important and critical parameter for the control of heavy metal (tin) level at final product **7**. So we have optimized the required quantity of $SnCl_4$ for the coupling reaction. When glycosylation performed with 1.0 equiv. to 2.5 equiv. of $SnCl_4$, same yield was obtained in both cases. While using 1.5 equiv. of $SnCl_4$ for the coupling, there was no acyclic nucleoside or silylated acyclic nucleoside were detected which was a deviation from Pedersen *et al.* [14,15] observation. Increasing the equiv. of $SnCl_4$ up to 2.5 equiv. neither altered the purity nor yield of the product (entries 5-8, Table-2). But the tin content level is high (27 ppm) in 2.5 equiv of $SnCl_4$ reactions due to the higher equiv. of $SnCl_4$ and in other hand lower value of tin content (15 ppm) obtained at 1.5 equiv. reactions (entries 1-4, Table-3). Under these optimized reaction conditions we have been successful to obtain the consistent heavy metal limit (10-15 ppm) in different scale-up batches (Table-3).

Glycosylation reaction progress was analyzed by analytical HPLC, which indicated that the reaction completed in 120 min at ambient temperature. The reaction progress in EtOAc is much more similar to the acetonitrile and DCE solvent mediated reactions by the HPLC analysis. We therefore, carefully studied the product formation of desired nucleoside with pre-silylated purine and activated sugar at 0 °C to room temperature. Glycosylation reaction samples were analyzed in equal time of intervals to observe the reaction progress. The required time to complete the reaction was calculated by plotting % of consumption of sugars *versus* reaction time. Equal amount of sample was (≈ 5 µL) analyzed in different time intervals to observe the progress of the reaction.

The deprotection of **6a**, **6b** and isolation, purification of **7** from the solvent presented a number of challenges. First, when the methanolic ammonia (15 %) used for the deprotection, the instability of methanolic ammonia concentration is a major problem at higher temperature. Secondly, liquor ammonia took longer time (≥ 16 h) to complete the reaction and decomposition of the product with poor yield was obtained (≈ 35 %). Third, surprisingly DBU also worked as a deprotecting reagent when heated with methanol at reflux temperature. But there was no satisfactory amount of recrystallized product obtained from the methanol (≈ 30 %). Benzoyl (**6a**), acetyl (**6b**) intermediates are deprotected with different bases such as sodium methoxide ($NaOCH_3$), 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU), lithium hydroxide monohydrate, methanolic ammonia (20 %), aqueous ammonia, sodium hydroxide and HCl solution at different temperatures to obtain **7**. The Best result was obtained when the $NaOCH_3$ (10 % w/v) was used for the deprotecting reagent in methanol at ambient temperature. The complete deprotection of both **6a** and **6b** achieved in 2 h at ambient temperature.

We like to eliminate the aqueous workup at deprotection step of intermediates **6a**, **6b**, due to the degradation of the nucleoside **7** which is lead to the decreased yields of product. We searched for the alternative non-aqueous work up methods such as extraction with non-polar solvents like pet. ether, *n*-hexane and *n*-heptane. The by-product methylbenzoate resulting deprotection of **6a** was removed by the extraction with *n*-heptane, thanks to its solubility in *n*-heptane at room temperature. Thereby, the methanol was evaporated to obtain the product as white solid. Thus obtained product was chromatographed and pure fractions were collected from the preparative HPLC. These fractions were evaporated *in vacuo* to obtain pure **7** as a white solid (entry 7, **Table 2**) with HPLC purity ≈ 98 % and chiral purity ≈ 98 %. Thus obtained solid was stored at -5 °C to 0 °C under nitrogen.

Next, another interesting and important observation is that bromine of **7** was replaced when 8-bromo-3-methyl-7-(β-D-ribofuranosyl)purin-2,6-dione (**7**) was treated with excess $NaOCH_3$ (30 % w/v) in methanol at 60 °C to obtain **8** with 80 % yield. The reaction completion is mainly depends on the concentration of the $NaOCH_3$ and reaction temperature. Thus excess $NaOCH_3$ was used for the successful bromine replacement.

The structures of all compounds were determined by mass spectrum, IR and $^1H$ NMR and $^{13}C$ NMR, COSY and NOESY spectral data. We were interested on *N*-$CH_3$ proton spacial interaction with H-12 and H-52 protons of sugar, so that we have irradiated the concern *N*-$CH_3$ proton to find out the NOE. As we expected there was no significant signal enhancement of other protons, which indicates that there is no spacial interaction between the *N*-$CH_3$ proton and other protons of sugar. This phenomenon confirms the N-**7** isomer orientation. The quantitative $^1H$ NMR was performed with Brooker 400 MHz in $CDCl_3$ by using tetrachloronitrobenzene (TCNB (δ 8.46 [1H])) as an internal standard to obtain 89.5 % of NMR assay. The $pK_a$ of compound **7** were determined by the spectrophotometric titration to obtain log $pK_a$ value as 9.6 which is in full agreement with the traditional nucleosides $pK_a$ values.

## Conclusions

We have established a easy, convenient, industrially adoptable and cost-effective synthetic method for the new 8-bromo-3-methyl-7-(β-D-ribofuranosyl)purine-2,6-dione with number

TABLE-3
HEAVY METAL (TIN) LEVELS IN 7 AT DIFFERENT BATCHES

| Entries | Batch size (**5a,g**) | $SnCl_4$ equiv. | Solvents for glycosylation | Purity by HPLC, **6a** | Purity by HPLC, **7** | Tin level[a] in **7** |
|---|---|---|---|---|---|---|
| 1 | 5.0 | 1.0 | DCE & EtOAc | 50 | 80 | 10 |
| 2 | 5.0 | 1.5 | DCE & EtOAc | 98 | 98 | 15 |
| 3 | 5.0 | 2.0 | DCE & EtOAc | 98 | 98 | 20 |
| 4 | 5.0 | 2.5 | DCE & EtOAc | 98 | 98 | 27 |
| 5 | 10.0 | 1.5 | DCE & EtOAc | 97 | 98 | 14 |
| 6 | 100.0 | 1.5 | DCE & EtOAc | 98 | 98 | 15 |
| 7 | 500.0 | 1.5 | DCE & EtOAc | 98 | 98 | 15 |

[a]In isolated product.

of glycosylation reagents. The key features of the present methodology are as follows:

• A convenient two-step process for the synthesis of pure 7-β-D-nucleoside with $SnCl_4$ in EtOAc.

• Quenching of $SnCl_4$ with sodium carbonate and sodium bicarbonate.

• Shorten duration of reaction time for the silylation and glycosylation reactions.

• Simple process for the deprotection of sugars.

• Isolation and purification of product from methanol.

• Removal of methyl-benzoate with *n*-heptane extraction.

• Consistent heavy metal (tin) with lower level in the pure nucleosides which can be acceptable for pharmaceutical use.

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