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An efficient convergent synthesis of adenosine-5′-*N*-alkyluronamides

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Abstract

Herein we report an efficient synthesis of adenosine-5′-*N*-alkyluronamides in which an enzyme-mediated deacetylation reaction is a key to the selective modification of the 5′-*N*-position, prior to coupling the ribose and purine components via a microwave-assisted Vorbrüggen coupling. This approach provides access to highly functionalised adenosines with 2- and N^6-substitutents, which can be incorporated before or after the ribose-coupling step. In all cases the microwave-assisted Vorbrüggen coupling conditions afforded anomerically pure purine ribosides in good to excellent yields.

1. Introduction

Adenosine is an endogenous purine nucleoside, which regulates a number of physiological processes in the cardiac, nervous and immune systems.[1] Adenosine's effects are mediated by four receptor subtypes, namely A_1, A_{2A}, A_{2B} and A_3, which are members of the G-protein coupled receptor superfamily. Agonists with high adenosine receptor subtype selectivity have therapeutic potential in a range of areas, including the treatment of cardiovascular disease, pain, wound healing, rheumatoid arthritis and colorectal cancer.[2] One modification which has proven beneficial in the development of selective agonists for the A_1, A_{2A} and A_3 adenosine receptors (ARs) is the installation of a 5′-*N*-alkyluronamide moiety. Selodenoson (**1**) is an N^6-substituted adenosine-5′-*N*-ethyl uronamide, which acts as a potent and selective agonist at the A_1AR.[3] A controlled release formulation of this compound has been patented for the treatment of atrial flutter and atrial fibrillation.[4] A range of adenosine-5′-*N*-ethyl uronamides with additional C2 substitution have proven to be potent and selective agonists of the A_{2A}AR. For example, apadenoson (ATL-146e, **2**)[5] is a 2-alkynyl derivative of *N*-ethylcarboxamidoadenosine, which acts as an anti-inflammatory agent and has a range of potential therapeutic applications.[1,6] Furthermore, the prototypical A_3AR agonist, Cl-IB-MECA (**3**), is an adenosine analogue with a 5′-methyl carboxamido group and a 3-chlorobenzyl substituent in the N^6-position.[7] This compound has been shown to have cardioprotective effects against myocardial ischemia/reperfusion injury as well as potential therapeutic applications in the treatment of liver cancer.[8,9]

In general, the preparation of functionalised adenosine-5′-*N*-uronamides has involved relatively lengthy syntheses in which modifications are made to a nucleoside precursor such as inosine in a linear fashion. Alternatively, convergent approaches where the appropriately functionalised purines and sugars are coupled together have also been employed. The latter are generally more efficient for the synthesis of highly functionalised adenosine-5′-*N*-uronamides. Glycosylation conditions developed by Vorbrüggen generally afford the desired β-anomer in reasonable to good yield.[10] This coupling approach requires the presence of an anomeric acyloxy moiety and ester protection of the 2′-hydroxy as the reaction proceeds via a cyclic 1,2-acyloxonium intermediate upon treatment with a Lewis acid. These structural features are incompatible with the typical reaction conditions required for the introduction of the carboxamido group in the 5′-position, which generally necessitates the use of a convoluted protecting group strategy. For example, the original synthesis of 2-chloro-N^6-(3-iodobenzyl)adenosine-5′-*N*-methyl uronamide (Cl-IB-MECA, **3**)[11] was achieved in a 10-step sequence in which the modified

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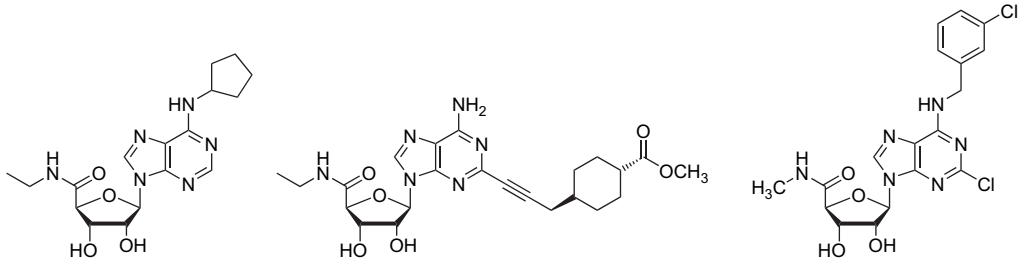


Selodenoson (RG14202, **1**) Apadenoson (ATL-146e, **2**) Cl-IB-MECA (**3**)

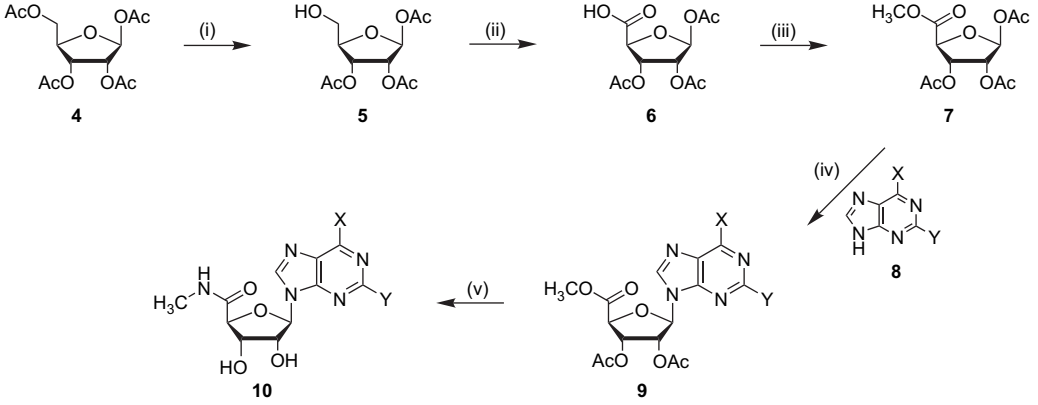
sugar, *N*-methyl-1-*O*-acetyl-2,3-dibenzoyl-D-ribofuranuronamide, was prepared in 8 steps and coupled to the appropriate substituted purine prior to deprotection. The coupling step was performed using the conditions developed by Vorbrüggen and proceeded in quite modest yield (33%). Moreover, the overall yield of this approach was 1.7%, highlighting the need for a more efficient synthetic route. The same research team subsequently developed an improved approach in which methyl 1,2,3-tri-*O*-acetyl-β-D-ribofuronate was prepared from D-ribose in four steps (17% yield) and coupled with the purine base prior to 5′-carboxamide formation and deprotection.[11] This coupling reaction afforded a mixture of isomers, which necessitated the use of chromatography to isolate the desired β-anomer in 52% yield. Overall, this six-step sequence provided Cl-IB-MECA in 5.6% yield.

We now report an improved synthetic approach to adenosine-5′-*N*-alkyluronamides in which an enzyme-mediated deprotection provides selective access to the 5′-position of the ribose and a microwave-assisted coupling affords the desired nucleosides in good to excellent yield.

2. Results and discussion

In our approach the desired 5-modified sugar, methyl 1,2,3-tri-*O*-acetyl-β-D-ribofuronate (**7**), was prepared in three steps from the commercially available starting material, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranoside (**4**) (Scheme 1). This initially involved selective deacylation at the 5-position using the lipase, *Candida rugosa* (CRL Type VII, Sigma 1754), under the conditions that were reported by Fernandez-Lorente et al.[12] and subsequently optimised by Chien and Chern.[13] Selective deprotection of primary acetyl esters of carbohydrates and nucleosides has also been achieved with iodine and methanol.[14] In our case, these reaction conditions failed to produce any detectable amounts of the desired deacetylated product. Oxidation of the alcohol **5** with TEMPO and BAIB in a 1:1 mixture of MeCN/H_2O[15] afforded the corresponding 5-carboxylic acid **6** in good yield. This acid was treated without purification with EDCI and DMAP in methanol to give methyl 1,2,3-tri-*O*-acetyl-β-D-ribofuronate (**7**). The overall yield of this strategy from the tetraacetyl ribose was 43%, which is an improvement over previous methods.

Methyl 1,2,3-tri-*O*-acetyl-β-D-ribofuronate (**7**) was subsequently coupled with a range of purine bases using the general reaction conditions developed by Vorbrüggen. The purines **8a**–**8g** were heated under reflux in HMDS with a catalytic amount of $(NH_4)_2SO_4$ and the excess HMDS was evaporated at reduced pressure (Scheme 1). The silylated purines were then coupled to the triacetylated ribofuronate **7** in DCE with TMSOTf in a Biotage microwave reactor for 20 min at 90 °C. After the reaction a pale yellow solution is formed, which was then carefully treated with saturated $NaHCO_3$ and extracted into CH_2Cl_2 to give the coupled products **9a**–**9g**. Adenine coupled with **7** to form the corresponding adenosine analogue **9a** in good yield (71%) (entry 1, Table 1). N^6-Substituted adenosines could also be efficiently prepared using



Scheme 1. Reagents and conditions: (i) *Candida rugosa* lipase, 9:1 (0.1 M sodium phosphate buffer (ph 7.0)/DMF), 25 °C, 50–80%; (ii) TEMPO, $C_6H_5I(OAc)_2$, 25 °C; (iii) EDCI, DMAP, MeOH, 25 °C, 63% (over two steps); (iv) (a) **8**, HMDS, $(NH_4)_2SO_4$, 125 °C; (b) **7**, TMSOTf, DCE, 90 °C [MW], 63–91%; (v) for **9a**–**9e**: $MeNH_2$, THF, 110 °C [MW], 58–69%.

Table 1
Efficiency of microwave-assisted ribose–purine coupling

Entry	Product (**9**)	X	Y	Yield %[a]
1	**9a**	NH_2	H	71
2	**9b**	NHBn	H	75
3	**9c**	NH_2	Cl	89
4	**9d**	NH_2	C≡CPh	63
5	**9e**	NHBn	Cl	71
6	**9f**	Cl	H	81
7	**9g**	Cl	Cl	91
8	**9h**	Cl	I	89

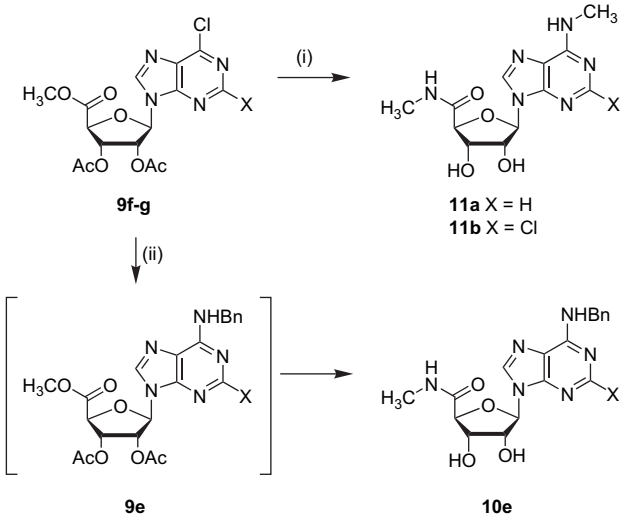
[a] Isolated yields following column chromatography.

this approach, as exemplified by the coupling of N^6-benzyladenine with **7**, which proceeded in 75% yield (entry 2, Table 1). A substituent in the 2-position was well tolerated as evidenced by the coupling of 2-chloroadenine and 2-(2-phenylethynyl)-adenine in 89% and 63% yield, respectively (entries 3 and 4, Table 1). One example of a 2,N^6-disubstituted adenine was also included (2-chloro-N^6-benzyladenine, entry 5, Table 1) and proceeded in the identical yield to adenine itself (71%). In all cases there was no sign of the unwanted α-anomer apparent in the ^{1}H NMR spectrum of the crude reaction products. This reaction was significantly more efficient than closely related couplings of 2,N^6-disubstituted adenines performed with conventional heating. For example, the coupling of 2-chloro-N^6-(3-iodobenzyl)adenine with **7** was heated at reflux for 48 h and afforded a 52% yield of the desired product along with 12% of the corresponding α-anomer.[11] The improved efficiency of the microwave-assisted process may have resulted from more effective generation of the initial acetoxonium ion intermediate (thereby reducing the formation of the α-anomer) and/or the shorter reaction time minimising the amount of decomposition of material relative to an extended period of reflux. The halopurines **8f–8h** were also converted to the corresponding purine ribosides in excellent yields ranging from 81% to 91% (entries 6–8, Table 1).

During the course of this research a related microwave-assisted glycosylation reaction was reported in the literature.[16] In this case the Vorbrüggen coupling was adapted to a one-step microwave-assisted protocol, which proceeded at 130 °C for 5 min. Although this approach was quite efficient for the generation of nucleoside libraries, it was generally low yielding and therefore less useful for preparative applications. For example, the coupling of N^6-benzyladenine and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose afforded the corresponding adenosine in 11% yield, along with 9% of the *N*7-glycosylated product. Likewise, 6-chloropurine was coupled with the same sugar to afford 6-chloropurine riboside in 29% yield.

The coupled products **9a–9e** were subsequently treated with methylamine in THF, which introduced the 5′-carboxamide with concomitant deprotection of 2′- and 3′-hydroxyls to afford the desired adenosine-5′-*N*-methyl uronamides (**10a–10e**) in good yield (Scheme 1).

The halogenated purine ribosides **9f–9h** are versatile synthetic intermediates, which can be readily functionalised in the 2-, 5′- and/or 6-positions via nucleophilic or organometallic coupling protocols. This was exemplified by the treatment of **9f** and **9g** with excess methylamine, which simultaneously effected N^6-substitution and *N*-methylcarboxamide formation at C5′ as well as deprotection of the 2′- and 3′-hydroxyls to form the corresponding N^6-methyladenosine-5′-methyl uronamides in 68% and 65% yield, respectively (Scheme 2). Alternatively, the use of 1 molar equiv of amine allowed the N^6-substituent to be installed selectively, followed by 5′-carboxamide formation with concomitant deprotections with a different amine. The use of 1 equiv of benzylamine followed by a 20-fold excess of methylamine afforded 2-chloro-N^6-benzyladenosine-5′-*N*-methyl uronamide (**10e**) in an overall yield of 47% from **9g**.



Scheme 2. Reagents and conditions: (i) $MeNH_2$ (excess), THF, 110 °C [MW], 68% (X=H), 65% (X=Cl); (ii) from **9g**: (a) NH_2Bn (1 equiv), DIPEA, MeOH, 80 °C [MW], 79%; (b) $MeNH_2$ (excess) in THF, 110 °C [MW], 60%.

3. Conclusions

An efficient and high yielding synthesis of adenosine-5′-uronamides as well as 2- and/or N^6-substituted adenosine-5′-uronamides has been developed. In this work, methyl 1,2,3-tri-*O*-acetyl-β-D-ribofuronate (**7**) was prepared in three steps from commercially available sugar, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**4**) and then coupled to a range of purine bases. The coupling reactions were achieved in good to high yield (63–91%) using a microwave-assisted Vorbrüggen reaction. Subsequent 5′-carboxamide formation with concomitant 2′,3′-deprotection afforded the desired target products. When 2,6-dihalopurine ribofuronates were prepared via this approach it was also possible to functionalise these positions post-coupling.

4. Experimental

4.1. General

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. All microwave reactions took place in a Biotage Initiator Microwave Synthesiser. All NMR spectra were recorded on a Bruker Avance DPX 300

spectrometer and ^{1}H and ^{13}C NMR spectra were recorded at 300.13 MHz and 75.4 MHz, respectively. Infrared spectra were recorded with a Scimitar Series Varian 800 FT-IR Spectrometer fitted with a PIKE Technologies MIRacle ATR and samples were run as pure solids. High Resolution Mass Spectrometry analyses were collected on a Waters Micromass LCT Premier XE TOF mass spectrometer fitted with an ESI ion source. Optical rotations were measured on an Atago polarimeter. Thin layer chromatography was conducted on 0.2 mm plates using Merck silica gel 60 F_{254}. Column chromatography was achieved using Merck silica gel 60 (particle size 0.063–0.200 μm, 70–230 mesh). Adenine was purchased from the Sigma–Aldrich Chemical company, while 6-chloropurine and 2,6-dichloropurine were obtained from Advanced Molecular Technologies. The other purines that were used were prepared by literature methodology.[17]

4.1.1. 1,2,3-Tri-O-acetyl-β-D-ribofuranose (5)

A solution of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**4**) (3.82 g, 12 mmol) in *N*,*N*-dimethylformamide (30 mL) was diluted with 0.1 M pH 7.0 sodium phosphate buffer (270 mL) and *C. rugosa* lipase [5.93 g, lipase/ribose=1.5:1 (w/w)] was stirred very slowly at room temperature for 24 h. The mixture was filtered and the filtrate extracted with EtOAc (3×500 mL), washed with brine (2×500 mL), dried with $MgSO_4$ and evaporated under reduced pressure. The subsequent oil was purified by silica column chromatography (Et_2O, R_f=0.35) to give **5** as a colourless oil in yields of 50–80%. $[\alpha]_D^{20}$ −8.61 (lit.[13] −10.44). ^{1}H NMR ($CDCl_3$): δ 2.06 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.12 (s, 3H, OAc), 3.61–3.67 (m, 1H, H-5), 3.79–3.84 (m, 1H, H-5), 4.22–4.26 (m, 1H, H-4), 5.32–5.40 (m, 2H, H-2, H-3), 6.12 (s, 1H, H-1).

4.1.2. Methyl 1,2,3-tri-O-acetyl-β-D-ribofuronate (7)

A solution of **5** (1.68 g, 6.08 mmol), TEMPO (238 mg, 1.52 mmol) and BAIB (4.11 g, 12.77 mmol) in MeCN/H_2O (1:1) was stirred at room temperature for 16 h and reduced in vacuo to a third of its volume. The residue was then extracted with DCM (3×50 mL) and the combined organic fractions were washed with water, then brine, dried ($MgSO_4$), filtered and the filtrate was evaporated to dryness. A solution of the resultant oil (1.63 g) in MeOH (50 mL) was treated with EDCI (2.69 g, 14.05 mmol) and DMAP (172 mg, 1.41 mmol) and then stirred at room temperature for 2 h. The MeOH was evaporated and the residue extracted with DCM (3×50 mL), washed with water (50 mL), then brine (2×50 mL). The organic extracts were dried over $MgSO_4$, filtered and concentrated to dryness. The yellow oil was then purified by silica column chromatography (hexane/EtOAc, 1:1, R_f=0.30) to give **7** as a colourless oil (805 mg, 63%). ^{1}H NMR ($CDCl_3$): δ 2.07 (s, 6H, 2×OAc), 2.11 (s, 3H, OAc), 3.76 (s, 3H, OMe), 4.58 (d, *J*=6.3 Hz, 1H), 5.33 (d, *J*=4.8 Hz, 1H), 5.61 (dd, *J*=6.3, 4.8 Hz, 1H), 6.17 (s, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ 20.3, 20.4, 20.8, 52.7, 72.5, 73.8, 79.1, 98.5, 168.9, 169.3, 169.4, 169.7.

4.2. General procedure for the ribose–purine coupling

A mixture of purine (**8**, 1.2 molar equiv), ammonium sulfate (0.25 molar equiv) and hexamethyldisilazane was heated under reflux for 16 h under nitrogen. The solution was concentrated to dryness and dissolved in anhydrous DCE and a solution of methyl 1,2,3-tri-*O*-acetyl-β-D-ribofuronate (**7**) (1 molar equiv) in anhydrous DCE and TMS triflate (2.0 molar equiv) was added. In the cases of **9a**, **9c** and **9d**, anhydrous MeCN was added to solubilise the silylation step. The mixture was then heated in the microwave (90 °C, 20 min) under nitrogen. A saturated solution of $NaHCO_3$ was added and the whole was stirred for 15 min. The aqueous layer was then extracted with DCM (×2), washed with brine (×2), dried over $MgSO_4$, filtered and concentrated to dryness to afford **9** as a foamy residue, which was routinely reacted further without purification.

4.2.1. Methyl 1-[adenin-9-yl]-2,3-di-O-acetyl-β-D-ribofuronate (9a)

Adenine (**8a**) (30 mg, 0.224 mmol), $(NH_4)_2SO_4$ (6 mg, 0.047 mmol), anhydrous MeCN (2 mL) in HMDS (2 mL), and then **7** (57 mg, 0.187 mmol), TMSOTf (68 μL, 0.375 mmol) in DCE (4 mL) gave **9a** (hexane/EtOAc, 1:1, R_f=0.05) as a colourless foam (50 mg, 71%). ^{1}H NMR ($CDCl_3$): δ 2.09 (s, 3H, OAc), 2.23 (s, 3H, OAc), 3.89 (s, 3H, OMe), 4.80 (s, 1H, H-4′), 5.84–5.89 (m, 2H, H-2′, H-3′), 6.07 (br s, 2H, NH_2), 6.50 (d, *J*=6.0 Hz, 1H, H-1′), 8.39 (s, 1H), 8.46 (s, 1H). ESMS calcd for $C_{15}H_{18}N_5O_7^+$ (M+H) 380.1, found 380.4.

4.2.2. Methyl 1-[N^6-(benzyl)adenin-9-yl]-2,3-di-O-acetyl-β-D-ribofuronate (9b)

N^6-Benzylaminoadenine (**8b**) (57 mg, 0.253 mmol), $(NH_4)_2SO_4$ (7 mg, 0.053 mmol) in HMDS (2 mL), and then **7** (64 mg, 0.211 mmol), TMSOTf (76 μL, 0.421 mmol) in DCE (4 mL) gave **9b** (hexane/EtOAc, 1:1, R_f=0.15) as a yellow foam (74 mg, 75%). ^{1}H NMR ($CDCl_3$): δ 2.06 (s, 3H, OAc), 2.21 (s, 3H, OAc), 3.87 (s, 3H, OMe), 4.77 (s, 1H, H-4′), 4.92 (br s, 2H, CH_2), 5.79–5.83 (m, 2H, H-2′, H-3′), 6.33 (s, 1H, NH), 6.47 (d, *J*=6.3 Hz, 1H, H-1′), 7.29–7.42 (m, 5H, ArH), 8.35 (s, 1H), 8.42 (s, 1H). ESMS calcd for $C_{22}H_{24}N_5O_7^+$ (M+H) 470.2, found 470.2.

4.2.3. Methyl 1-[2-chloroadenin-9-yl]-2,3-di-O-acetyl-β-D-ribofuronate (9c)

2-Chloroadenine (**8c**) (33 mg, 0.197 mmol), $(NH_4)_2SO_4$ (5 mg, 0.041 mmol), anhydrous MeCN (2 mL) in HMDS (2 mL), and then **7** (50 mg, 0.164 mmol), TMSOTf (59 μL, 0.329 mmol) in DCE (4 mL) gave **9c** (hexane/EtOAc, 1:1, R_f=0.05) as a colourless foam (60 mg, 89%). ^{1}H NMR ($CDCl_3$): δ 2.10 (s, 3H, OAc), 2.26 (s, 3H, OAc), 3.92 (s, 3H, OMe), 4.80 (s, 1H, H-4′), 5.77–5.81 (m, 2H, H-2′, H-3′), 6.31 (br s, 2H, NH_2), 6.45 (d, *J*=6.6 Hz, 1H, H-1′), 8.47 (s, 1H, H-8). ESMS calcd for $C_{15}H_{17}ClN_5O_7^+$ (M+H) 414.1, 416.1, found 413.2.

4.2.4. Methyl 1-[2-(phenylethynyl)adenin-9-yl]-2,3-di-O-acetyl-β-D-ribofuronate (**9d**)

2-(2-Phenylethynyl)adenine (**8d**) (60 mg, 0.257 mmol), $(NH_4)_2SO_4$ (7 mg, 0.053 mmol), anhydrous MeCN (2 mL) in HMDS (2 mL), and then **7** (65 mg, 0.214 mmol), TMSOTf (77 μL, 0.428 mmol) in DCE (4 mL) gave **9d** (hexane/EtOAc, 1:1, R_f=0.05) as a yellow foam (65 mg, 63%). ^{1}H NMR ($CDCl_3$): δ 2.11 (s, 3H, OAc), 2.26 (s, 3H, OAc), 3.92 (s, 3H, OMe), 4.80 (s, 1H, H-4′), 5.68–5.87 (m, 2H, H-2′, H-3′), 6.00 (br s, 2H, NH_2), 6.60 (d, J=6.6 Hz, 1H, H-1′), 7.41–7.43 (m, 3H, ArH), 7.70–7.72 (m, 2H, ArH), 8.59 (s, 1H, H-8). ESMS calcd for $C_{23}H_{22}N_5O_7^+$ (M+H) 480.2, found 480.3.

4.2.5. Methyl 1-[N^6-benzyl-2-chloroadenin-9-yl]-2,3-di-O-acetyl-β-D-ribofuronate (**9e**)

N^6-Benzyl-2-chloroadenine (**8e**) (53 mg, 0.205 mmol), $(NH_4)_2SO_4$ (6 mg, 0.043 mmol) in HMDS (2 mL), and then **7** (52 mg, 0.171 mmol), TMSOTf (62 μL, 0.342 mmol) in DCE (4 mL) gave **9e** (hexane/EtOAc, 1:1, R_f=0.05) as a yellow foam (65 mg, 71%). ^{1}H NMR ($CDCl_3$): δ 2.11 (s, 3H, OAc), 2.27 (s, 3H, OAc), 3.93 (s, 3H, OMe), 4.79 (s, 1H, H-4′), 4.88 (br s, 2H, CH_2), 5.76–5.80 (m, 2H, H-2′, H-3′), 6.24 (s, 1H, NH), 6.48 (d, J=6.0 Hz, 1H, H-1′), 7.36–7.43 (m, 5H, ArH), 8.40 (s, 1H, H-8). ESMS calcd for $C_{22}H_{23}ClN_5O_7^+$ (M+H) 504.1, 506.1, found 504.2, 506.2.

4.2.6. Methyl 1-[6-chloropurin-9-yl]-2,3-di-O-acetyl-β-D-ribofuronate (**9f**)

6-Chloropurine (**8f**) (31 mg, 0.197 mmol), $(NH_4)_2SO_4$ (5 mg, 0.041 mmol) in HMDS (2 mL), and then **7** (50 mg, 0.164 mmol), TMSOTf (59 μL, 0.329 mmol) in DCE (4 mL) gave **9f** (hexane/EtOAc, 1:1, R_f=0.15) as a yellow foam (53 mg, 81%). ^{1}H NMR ($CDCl_3$): δ 2.10 (s, 3H, OAc), 2.27 (s, 3H, OAc), 3.93 (s, 3H, OMe), 4.83 (s, 1H, H-4′), 5.80–5.90 (m, 2H, H-2′, H-3′), 6.56 (d, J=6.6 Hz, 1H, H-1′), 8.81 (s, 1H), 8.86 (s, 1H). ESMS calcd for $C_{15}H_{15}N_4O_7^+$ (M+H) 399.8, found 399.3.

4.2.7. Methyl 1-[2,6-dichloropurin-9-yl]-2,3-di-O-acetyl-β-D-ribofuronate (**9g**)

2,6-Dichloropurine (**8g**) (45 mg, 0.237 mmol), $(NH_4)_2SO_4$ (7 mg, 0.049 mmol) in HMDS (2 mL), and then **7** (60 mg, 0.197 mmol), TMSOTf (71 μL, 0.395 mmol) in DCE (4 mL) gave **9g** (hexane/EtOAc, 1:1, R_f=0.15) as a yellow foam (78 mg, 91%). ^{1}H NMR ($CDCl_3$): δ 2.09 (s, 3H, OAc), 2.26 (s, 3H, OAc), 3.91 (s, 3H, OMe), 4.83 (s, 1H, H-4′), 5.75–5.81 (m, 2H, H-2′, H-3′), 6.50 (d, J=6.3 Hz, 1H, H-1′), 8.90 (s, 1H, H-8). ESMS calcd for $C_{15}H_{15}Cl_2N_4O_7^+$ (M+H) 433.0, 435.0, found 433.3, 435.2.

4.2.8. Methyl 1-[6-chloro-2-iodopurin-9-yl]-2,3-di-O-acetyl-β-D-ribofuronate (**9h**)

2-Iodo-6-chloropurine (**8h**) (46 mg, 0.167 mmol), $(NH_4)_2SO_4$ (5 mg, 0.035 mmol) in HMDS (2 mL), and then **7** (42 mg, 0.138 mmol), TMSOTf (50 μL, 0.276 mmol) in DCE (4 mL) gave **9h** as a colourless foam onto SiO_2 (hexane/EtOAc, 1:1, R_f=0.15) as a yellow foam (64 mg, 89%). IR ν 1748, 1583, 1547, 1340, 1201, 729. ^{1}H NMR ($CDCl_3$): δ 2.09 (s, 3H, OAc), 2.26 (s, 3H, OAc), 3.90 (s, 3H, OMe), 4.81 (s, 1H, H-4′), 5.76–5.80 (m, 2H, H-2′, H-3′), 6.47 (d, J=6.3 Hz, 1H, H-1′), 8.77 (s, 1H, H-8). HRMS (ESI) calcd for $C_{15}H_{15}ClIN_4O_7^+$ (M+H) 524.9669, found 524.9678.

4.3. General procedure for carboxamide formation and deprotection

4.3.1. Adenosine-5′-N-methyl uronamide (**10a**)

$MeNH_2$ (2.0 M) in THF (1 mL) was added to the foamy residue (**9a**) (45 mg, 0.119 mmol) in THF (1 mL) and heated in a Biotage microwave reactor in a 2–5 mL microwave vial (110 °C, 20 min). The solution was then evaporated onto SiO_2 and purified by column chromatography to give **10a** ($CHCl_3$/MeOH/Et_3N, 89:10:1, R_f=0.05) as a colourless solid (24 mg, 69%). ^{1}H NMR (CD_3OD): δ 2.90 (s, 3H, NHCH_3), 4.35 (dd, J=4.8, 1.2 Hz, 1H, H-3′), 4.51 (s, 1H, H-4′), 4.77 (dd, J=7.5, 4.8 Hz, 1H, H-2′), 6.05 (d, J=7.5 Hz, 1H, H-1′), 8.29 (s, 1H), 8.31 (s, 1H). ^{13}C NMR (CD_3OD): δ 26.2, 73.6, 75.1, 86.7, 90.7, 120.9, 142.8, 151.7, 154.0, 157.2, 173.0. ESMS calcd for $C_{11}H_{15}N_6O_4^+$ (M+H) 295.1, found 295.2.

4.3.2. N^6-Benzyladenosine-5′-N-methyl uronamide (**10b**)

Compound **9b** (74 mg, 0.158 mmol) in THF (1 mL) and $MeNH_2$ (2.0 M) in THF (1 mL) gave **10b** ($CHCl_3$/MeOH/Et_3N, 89:10:1, R_f=0.20) as a colourless solid (37 mg, 61%). ^{1}H NMR (CD_3OD): δ 2.89 (s, 3H, NHCH_3), 4.35 (d, J=4.8 Hz, 1H, H-3′), 4.51 (s, 1H, H-4′), 4.77 (dd, J=7.8, 4.8 Hz, 1H, H-2′), 4.85 (br s, 2H, CH_2), 6.04 (d, J=7.8 Hz, 1H, H-1′), 7.23–7.41 (m, 5H, ArH), 8.25 (s, 1H), 8.33 (s, 1H). ^{13}C NMR (CD_3OD): δ 24.7, 43.6, 72.2, 73.6, 85.0, 89.1, 120.2, 126.9, 127.4, 128.4, 135.3, 138.8, 140.7, 152.5, 154.9, 171.3. ESMS calcd for $C_{18}H_{21}N_6O_4^+$ (M+H) 385.2, found 385.2.

4.3.3. 2-Chloroadenosine-5′-N-methyl uronamide (**10c**)

Compound **9c** (50 mg, 0.121 mmol) in THF (1 mL) and $MeNH_2$ (2.0 M) in THF (1 mL) gave **10c** ($CHCl_3$/MeOH/Et_3N, 89:10:1, R_f=0.05) as a colourless solid (26 mg, 65%). ^{1}H NMR (CD_3OD): δ 2.96 (s, 3H, NHCH_3), 4.34 (d, J=4.8 Hz, 1H, H-3′), 4.50 (s, 1H, H-4′), 4.71 (dd, J=7.8, 4.8 Hz, 1H, H-2′), 5.98 (d, J=7.8 Hz, 1H, H-1′), 8.25 (s, 1H, H-8). ^{13}C NMR (CD_3OD): δ 25.1, 71.8, 73.4, 85.0, 89.2, 120.2, 141.5, 149.9, 153.8, 157.0, 171.0. ESMS calcd for $C_{11}H_{14}ClN_6O_4^+$ (M+H) 329.1, 331.1, found 329.0, 330.9.

4.3.4. 2-(Phenylethynyl)adenosine-5′-N-methyl uronamide (**10d**)

Compound **9d** (55 mg, 0.115 mmol) in THF (1 mL) and $MeNH_2$ (2.0 M) in THF (1 mL) gave **10d** ($CHCl_3$/MeOH/Et_3N, 89:10:1, R_f=0.20) as a colourless solid (26 mg, 58%). ^{1}H NMR (CD_3OD): δ 3.00 (s, 3H, NHCH_3), 4.36 (d, J=4.8 Hz, 1H, H-3′), 4.52 (s, 1H, H-4′), 4.71 (dd, J=7.8, 4.8 Hz, 1H, H-2′), 6.03 (d, J=7.8 Hz, 1H, H-1′), 7.41–7.47 (m, 3H, ArH), 7.61–7.64 (m, 2H, ArH), 8.32 (s, 1H, H-8). ^{13}C NMR (CD_3OD): δ 25.9, 72.2, 74.0, 85.3, 85.7, 89.8, 97.6,

120.1, 121.8, 129.0, 129.9, 132.2, 142.8, 146.7, 149.4, 156.9, 171.9. ESMS calcd for $C_{19}H_{19}N_6O_4^+$ (M+H) 395.1, found 395.2.

4.3.5. N^6-Benzyl-2-chloroadenosine-5′-N-methyl uronamide (**10e**)

Method A. Compound **9e** (62 mg, 0.123 mmol) in THF (1 mL) and $MeNH_2$ (2.0 M) in THF (1 mL) gave **10e** ($CHCl_3$/MeOH/Et_3N, 89:10:1, R_f=0.20) as a pale yellow solid (31 mg, 60%). ^{1}H NMR (CD_3OD): δ 2.96 (s, 3H, NHCH_3), 4.34 (d, J=4.8 Hz, 1H, H-3′), 4.50 (s, 1H, H-4′), 4.71 (dd, J=7.5, 4.8 Hz, 1H, H-2′), 4.80 (br s, 2H, CH_2), 5.96 (d, J=7.5 Hz, 1H, H-1′), 7.27–7.43 (m, 5H, ArH), 8.20 (s, 1H, H-8). ^{13}C NMR (CD_3OD): δ 25.1, 43.8, 71.8, 73.5, 85.1, 89.2, 119.5, 127.0, 127.4, 127.7, 128.2, 138.5, 149.0, 153.8, 155.3, 171.5. HRMS (ESI) calcd for $C_{18}H_{19}ClN_6O_4\cdot Na^+$ (M+Na) 441.1049, found 441.1054.

Method B. A solution of **9g** (56 mg, 0.130 mmol), $BnNH_2$ (14 μL, 0.130 mmol) and DIPEA (25 μL, 0.143 mmol) in MeOH (2 mL) was heated in a microwave reactor at 80 °C for 10 min. The solution was then evaporated onto SiO_2 and purification by column chromatography (hexane/EtOAc, 1:1, R_f=0.20) gave **9e** as a colourless foam (46 mg, 79%). This was then dissolved in THF (1 mL) and 2.0 M $MeNH_2$ in THF (1 mL) was added and heated at 110 °C for 20 min in a microwave reactor. The solution was evaporated onto SiO_2 and purified by column chromatography, which gave **10e** ($CHCl_3$/MeOH/Et_3N, 89:10:1, R_f=0.20) as a pale yellow solid (23 mg, 60%), which had an identical ^{1}H NMR spectrum to the previous preparation.

4.3.6. N^6-Methyladenosine-5′-N-methyl uronamide (**11a**)

Compound **9f** (57 mg, 0.143 mmol) in THF (1 mL) and $MeNH_2$ (2.0 M) in THF (1 mL) gave **11a** ($CHCl_3$/MeOH/Et_3N, 89:10:1, R_f=0.10) as a colourless solid (30 mg, 68%). IR ν 3234, 3095, 2941, 1630, 1580, 1356, 1305, 1223, 1055, 865, 633. ^{1}H NMR (CD_3OD): δ 2.97 (s, 3H, CONHCH_3), 3.11 (br s, 3H, NHCH_3), 4.33 (d, J=4.8 Hz, 1H, H-3′), 4.49 (s, 1H, H-4′), 4.70 (dd, J=7.8, 4.8 Hz, 1H, H-2′), 5.97 (d, J=7.8 Hz, 1H, H-1′), 8.18 (s, 1H), 8.30 (s, 1H). ^{13}C NMR (CD_3OD): δ 24.7, 26.3, 72.1, 73.6, 85.1, 89.1, 120.3, 140.6, 152.5, 155.5, 161.9, 171.4. ESMS calcd for $C_{12}H_{18}N_6O_4^+$ (M+H) 309.1, found 309.4.

4.3.7. 2-Chloro-N^6-methyladenosine-5′-N-methyl uronamide (**11b**)

Compound **9g** (51 mg, 0.118 mmol) in THF (1 mL) and $MeNH_2$ (2.0 M) in THF (1 mL) gave **11b** ($CHCl_3$/MeOH/Et_3N, 89:10:1, R_f=0.15) as a colourless solid (26 mg, 65%). IR ν 3361, 3287, 3127, 2857, 1618, 1582, 1352, 1310, 1225, 1046, 869, 621. ^{1}H NMR (CD_3OD): δ 2.97 (s, 3H, CONHCH_3), 3.11 (br s, 3H, NHCH_3), 4.33 (d, J=4.8 Hz, 1H, H-3′), 4.49 (s, 1H, H-4′), 4.70 (dd, J=7.8, 4.8 Hz, 1H, H-2′), 5.97 (d, J=7.8 Hz, 1H, H-1′), 8.18 (s, 1H, H-8). ^{13}C NMR (CD_3OD): δ 25.1, 26.3, 71.8, 73.5, 85.1, 89.2, 119.5, 141.0, 149.9, 153.7, 155.9, 171.4. HRMS (ESI) calcd for $C_{12}H_{15}ClN_6O_4\cdot Na^+$ (M+Na) 365.0736, found 365.0741.

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