A Convenient Synthesis of 8-Alkyl-2' (or 3')-azido (or amino)-2' (or 3')-deoxyadenosine as Diverse Synthetic Precursors of Cyclic Adenosine Diphosphate Ribose (cADPR)

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As key nucleoside intermediates for the preparation of cyclic adenosine diphosphate ribose (cADPR, **1**) analogues, 8-alkyl-2' (or 3')-azido(or amino)-adenosine derivatives (**16-19**) were successfully prepared by alkylating selectively protected adenosine derivatives (**12**, **13**) *via* Pd(0) catalyzed cross-coupling reaction with tetraalkyltin reagents, followed by the sugar modification of these 8-alkyl-adenosine derivatives according to our precedent procedure. Compared to other precedent procedures, our 8-alkylation methodology using selectively TBDMS-protected 8-alkyl adenosine derivatives as starting materials will be utilized very conveniently to prepare highly functionalized adenosine analogues, which will be serve as key intermediates for the cADPR.

Key Words : Cyclic adenosine diphosphate ribose (cADPR), 8-Alkyl-2' (or 3')-azido (or amino)-2' (or 3')-deoxyadenosine

Introduction

Cyclic adenosine diphosphate ribose (cADPR, **1**),[1] a naturally occurring cyclic metabolite of NAD^+, has been known to play an important role as a Ca^{2+}-mobilizing second messenger in various cellular events, such as glucose-dependent insulin secretion in pancreatic β-cells, proliferation of human T-lymphocytes, arrhythmogenic oscillations of intracellular Ca^{2+} in cardiac muscles.[2] However, since the understanding of the role of cADPR in Ca^{2+} signaling network is only just emerging, there have been continuous needs for the preparation of cADPR analogues having diverse structural modification to investigate the mechanism of cADPR-mediated signaling pathways in diverse cell systems and the therapeutic potentials of those compounds.[3] The structural modifications, mostly occurring on the adenine or ribose sugar moieties of cADPR, give rise to remarkable changes in their activity profiles; namely, showing considerable change in agonistic activity (**2**, **5**, **6**), or showing no activity (**3**, **4**), or even reversed activity (**7-9**) (Figure 1).[3d] Particularly, the cADPR analogues showing antagonistic activity in vertebrate cell systems have common and characteristic structural feature, *i.e.* the substitution at 8-position of purine ring with diverse substituents, such as amino group (**7**), methoxy (**8**), bromine (**9**).[4] This indicates that a substituent at 8-position of purine ring seems to be essential for antagonistic activity, even though this observation is based on the limited number of cADPR analogues. By considering the remarkable reversion of activity by chemical modifications on sugar or purine moieties of cADPR molecule and not many analogues found on our literature survey, we determined to prepare another type of cADPR analogues having chemical modifications on both of purine and sugar moieties.

As shown in Figure 1, the general synthetic strategy to prepare cADPR analogues includes intracyclization of NAD^+ analogues, which in turn should be prepared from the elaborately pre-modified nucleosides. Therefore, the synthetic priority should be given to the step to develop new and diverse nucleosides.[5] In the meantime, we have already reported the convenient synthetic route to prepare sugar-modified adenosine derivatives having an azido group and an amine group at 2' (or 3')-position of ribose moiety as key precursors to NAD^+ derivatives.[6] So, we decided to synthesize adenosine derivatives on which the chemical modifications occur at both of sugar and purine moiety, *i.e.* 8-alkyl-2' (or 3')-azido (or amino)-2' (or 3')-deoxyadenosine adenosine derivatives (**16-19**, in Scheme 1). This synthetic work would make it possible to extend the chemical diversity of the adenosine derivatives in hand, and could ultimately contribute to scrutinize structure-activity relationship of cADPR derivatives, which would be prepared from these nucleosides. In this paper, our synthetic efforts for sugar/base-modified adenosine derivatives utilizing our previous synthetic methodology will be reported.

Results and Discussion

The precedent literature revealed that the introduction of alkyl substituents to 8-position of adenine moiety has been conventionally accomplished through halogenation followed by nucleophilic displacement,[7] or lithiation of the 8-position followed by reaction with suitable electrophiles,[8] or palladium(0) catalyzed cross-coupling reaction with diverse organotin reagents.[9] After scrutinizing the literatures, the last methodology is much likely to be promising over others in

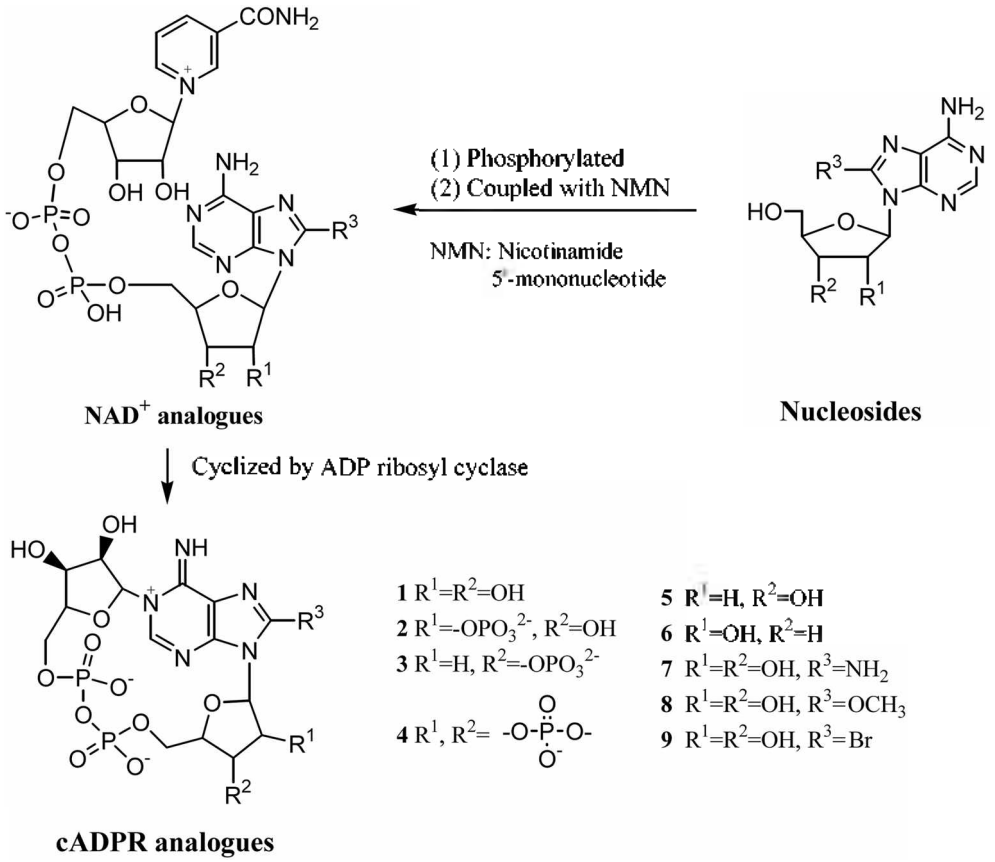


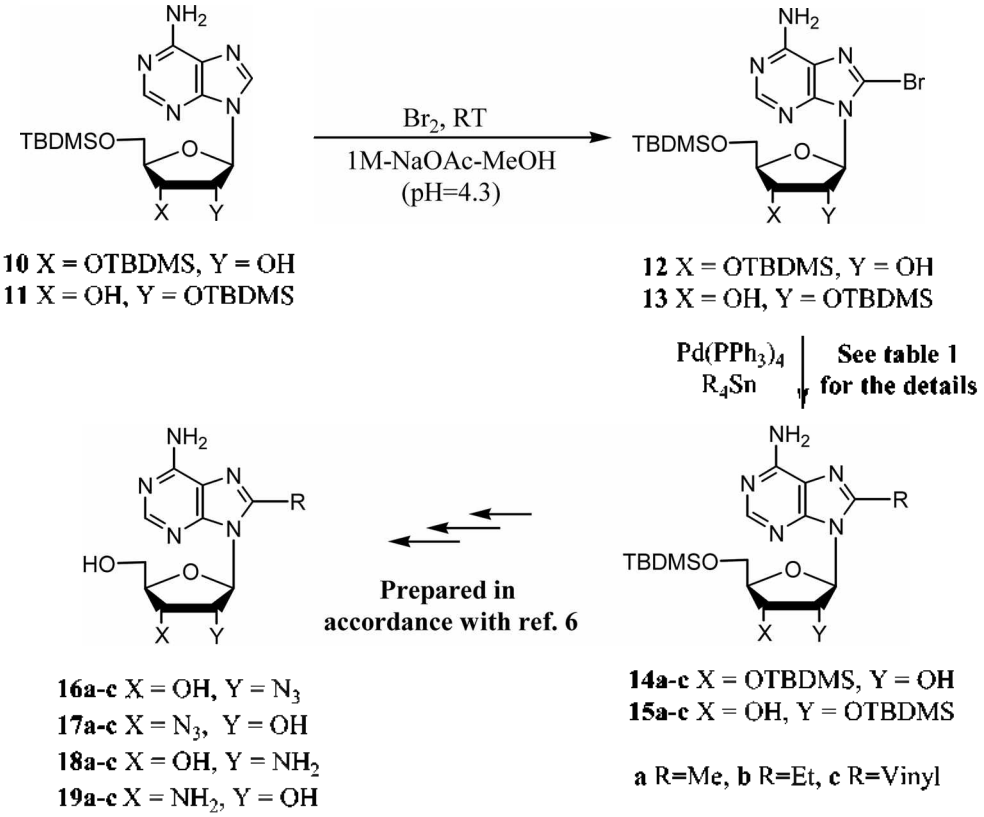
Figure 1

terms of yields and handling reactions, so it was utilized for the preparation of 8-alkyl adenosine derivatives.

8-Bromoadenosine derivatives (**12**, **13**), the precursors for the 8-alkylation reaction, are easily obtained from selectively TBDMS-protected adenosine derivatives (**10**, **11**)[6] with usual bromination condition (Br_2, 1 M acetate buffer (pH 4.3)-MeOH, rt) in high yields (> 90%, Scheme 1). For the successful 8-alkylation step, the optimum reaction condition was obtained by varying the concentrations of palladium(0) catalyst [$Pd(PPh_3)_4$] and tetraalkyltin (alkyl = methyl, ethyl, and vinyl), and range of temperature (shown in Table 1). The high reaction temperature around 100 °C is required, while the reactions occurred very slowly or not at all at low reaction temperature (30-80 °C).[9] Under the applied reaction condition, the 8-alkylated adenosine derivatives having selectively protected sugar (**14a-c** and **15a-c**) were successfully prepared in acceptable yields (70-90%, Table 1).

With those compounds in hand, we moved on to prepare our target compounds, 8-alkyl-2' (or 3')-azido (or amino)-2' (or 3')-deoxyadenosine derivatives (**16-19**), simply by modifying the sugar moiety according to our already reported procedure.[6] That is, the initial step was the conversion of sugar hydroxyl group at the 2' (and 3')-position of **14** (and **15**) to azido group *via* arbino- or xylofuranosyl nucleosides, which provide the selectively TBDMS-protected 2' (or 3')-azido-2' (or 3')-deoxyadenosine derivatives in good yields in each step (data not shown). From these compounds, the 2' (or 3')-azido compounds (**16** and **17**) were directly prepared with no incidence by the simple desilylation reaction condition (NH_4F/MeOH at 60 °C).[10] The desilylation step is apparently occurred in two-steps on the analysis of TLC, and so the selective desilylation of our particular compounds is possible upon needed. The next efforts were the reduction of azido group to amino group to prepare the 2' (or 3')-amino compounds (**18** and **19**). 8-Methyl (and ethyl)-2' (or 3')-aminoadenosine derivatives (**18a**, **18b** and **19a**, **19b**) were easily obtained with the conventional hydrogenation condition (H_2, Pd/C, rt) and subsequent desilylation. For the preparation of 8-vinyl-2' (or 3')-aminoadenosine derivatives (**18c** and **19c**), however, we have attempted several selective reduction methods with no success. In all the cases tried ($NaBH_4$, $LiAlH_4$, $NaBH_3CN$ etc.),[11] the unwanted reduction of vinyl group occurred.

In summary, 8-alkyl-2' (or 3')-azido(or amino)-adenosine derivatives (**16-19**) were successfully prepared from the 8-alkylation of selectively protected adenosine derivatives (**12**, **13**) using Pd(0) catalyzed cross-coupling reaction with tetraalkyltin reagents, followed by the sugar modification of these 8-alkyl-adenosine derivatives according to our precedent procedure. More importantly, compared to other precedent procedures, our 8-alkylation reaction system using selectively TBDMS-protected 8-alkyl adenosine derivatives as starting materials is meaningful in terms of yields and the



Scheme 1

Table 1.

Entry	Starting material	Reaction condition			Product/ yield (%)
		Pd(0) catalyst (%)	R_4Sn (equiv.)	Reaction temp. (°C)/time (h)	
1	**12**	10	R=Me (2)	100/1	**14a** (90)
2	**13**	10	R=Me (2)	100/2	**15a** (92)
3	**12**	15	R=Et (4)	100/1	**14b** (91)
4	**13**	12	R=Et (4)	100/3	**15b** (75)
5	**12**	15	R=vinyl (2)	100/5	**14c** (67)[a]
6	**13**	15	R=vinyl (4)	100/1.5	**15c** (77)

[a]10% of starting material recovered

reaction manipulation. With these important adenosine derivatives, the transformation of them to NAD^+ analogues and then to cADPR is now underway.

Experimental Section

Melting points were recorded on Electrothermal melting point apparatus and are uncorrected. Mass and NMR spectra were recorded on Applied Biosystems Qstar XL and Jeol 400 MHz spectrometer, respectively.

A typical procedure for the 8-bromination. A solution of **10**[6] (1.3 g, 2.62 mmol) in MeOH (120 mL)/1 M sodium acetate (20 mL) was added bromine (0.28 mL, 5.50 mmol) at rt and stirred for a half-hour. The mixture was diluted with 50 mL of saturated sodium metabisulfite solution and stirred until the red color was disappeared. The volatile was evaporated and the resulting aqueous layer was extracted with EtOAc (100 mL × 3). The combined organic layer was washed with water (100 mL), dried over $MgSO_4$, and evaporated to give pale yellowish foam. This residue was purified by column chromatography to give **12** as pale yellow solid (1.38 g, 92%). **12**: ^{1}H-NMR ($CDCl_3$) δ 8.13 (s, 1H, H2), 6.05 (s, 1H, H1), 5.89 (s, 2H, NH_2), 5.29 (t, 1H, J = 5.2 Hz, H2), 4.95 (t, 1H, J = 5.2 Hz, H3), 3.95 (m, 1H, H4), 3.87 (dd, 1H, J = 11.6, 4.4 Hz, H5a), 3.64 (dd, 1H, J = 11.2, 4.4 Hz, H5b), 0.92 (s, 9H, *t*-butyl), 0.71 (s, 9H, *t*-butyl), 0.17 (s, 3H, methyl), 0.16 (s, 3H, methyl), –0.09 (s, 3H, methyl), –0.19 (s, 3H, methyl); ^{13}C-NMR δ 154.14, 152.21, 150.48, 128.25, 120.05, 90.76, 84.34, 71.85, 71.07, 61.66, 25.78, 25.68, 18.23, 18.06, –4.62, –4.85, –5.52, –5.59. **13**: ^{1}H-NMR ($CDCl_3$) δ 8.22 (s, 1H, H2), 5.89 (d, 1H, J = 5.2 Hz, H1), 5.74 (s, 2H, NH_2), 5.52 (t, 1H, J = 5.2 Hz, H2), 4.44 (s, 1H, H3), 4.06 (m, 1H, H4), 3.97 (dd, 1H, J = 11.2, 6 Hz, H5a), 3.77 (dd, 1H, J = 11.2, 5.2 Hz, H5b), 2.72 (s, 1H, –OH), 0.85 (s, 9H, *t*-butyl), 0.81 (s, 9H, *t*-butyl), 0.01 (s, 3H, methyl), 0.00 (s, 3H, methyl), –0.06 (s, 3H, methyl), –0.23 (s, 3H, methyl); ^{13}C-NMR δ 154.21, 152.81, 150.99, 128.30, 120.39, 90.79, 85.30, 72.19, 70.85, 62.78, 25.91, 25.56, 18.41, 17.88, 0.00, –5.00, –5.09, –5.37.

Typical procedure of 8-alkylated adenosine derivatives (14 and 15). To a solution of **12** (0.1 g, 0.17 mmol) and tetrakis(triphenylphosphine)palladium(0) (20 mg, 0.017 mmol) in 1 mL of *N*-methyl-2-pyrrolidinone (NMP) was

added tetramethyltin (0.05 mL, 0.35 mmol) under Ar atmosphere. The mixture was stirred for 1 hour at 100 °C. The mixture was partitioned between EtOAc (50 mL) and water (25 mL). The aqueous layer was extracted with EtOAc once more (50 mL). The combined organic layer was washed with water (50 mL × 2) and brine (50 mL), dried over $MgSO_4$, evaporated to give pale yellow oily residue. The residue was applied to column chromatography (2 × 10 cm) and eluted with EtOAc-hexane (7 : 3). The appropriate fractions were collected and evaporated to give **14a** (80 mg, 90%) as a white solid. **14a**: ^{1}H-NMR ($CDCl_3$) δ 8.01 (s, 1H, H2), 5.60 (d, 1H, J = 4.0 Hz, H1'), 5.26 (br, 2H, NH_2), 5.02 (t, 1H, J = 4.0 Hz, H2'), 4.73 (t, 1H, J = 5.2 Hz, H3'), 3.78 (dd, 1H, J = 4.4, 9.6 Hz, H4'), 3.68 (dd, 1H, J = 4.0, 11.2 Hz, H5'a), 3.49 (dd, 1H, J = 4.0, 11.2 Hz, H5'b), 3.00 (br, 1H, OH2'), 2.43 (s, 3H, 8-methyl), 0.77 (s, 9H, *t*-butyl), 0.55 (s, 9H, *t*-butyl), 0.02 (s, 3H, methyl), −0.22 (s, 3H, methyl), −0.25 (s, 3H, methyl), −0.35 (s, 3H, methyl); ^{13}C-NMR δ 151.80, 132.06, 131.97, 128.47, 128.35, 89.12, 84.42, 72.33, 71.22, 62.04, 25.87, 25.76, 18.31, 18.17, 14.84, −4.56, −4.72, −5.47, −5.53; TOFMS: *m/z* (M^++H) 510. **14b**: ^{1}H-NMR ($CDCl_3$) δ 7.98 (s, 1H, H2), 5.7 (s, 2H, NH_2), 5.59 (d, 1H, J = 4.4 Hz, H1), 5.06 (t, 1H, J = 4.4 Hz, H2), 4.74 (t, 1H, J = 5.2, H3), 3.78 (t, 1H, J = 4.4 Hz, H4), 3.69 (dd, 1H, J = 11.2, 4.4 Hz, H5a), 3.49 (dd, 1H, J = 11.6, 4.4 Hz, H5b), 2.73 (dd, 2H, J = 15.2, 7.2 Hz, ethyl), 2.4 (s, 1H, OH), 1.19 (t, 3H, J = 7.2 Hz, ethyl), 0.74 (s, 9H, *t*-butyl), 0.56 (s, 9H, *t*-butyl), 0.01 (s, 3H, methyl), 0.00 (s, 3H, methyl), −0.25 (s, 3H, methyl), −0.34 (s, 3H, methyl); ^{13}C-NMR δ 154.82, 154.45, 151.69, 150.59, 118.56, 88.88, 84.37, 72.24, 71.33, 62.09, 25.86, 25.75, 21.53, 18.30, 18.16, 11.92, −4.57, −4.73, −5.47, −5.53; TOFMS: *m/z* (M^++H) 524. **14c**: ^{1}H-NMR ($CDCl_3$) δ 8.03 (s, 1H, H2), 6.74 (dd, 1H, J = 16.8, 11.2 Hz, Ha), 6.24 (d, 1H, J = 16.8 Hz, Hc), 5.74 (d, 1H, J = 4.4 Hz, H1), 5.51 (s, 2H, NH_2), 5.47 (d, 1H, J = 4.4 Hz, Hb), 4.89 (s, 1H, H2), 4.66 (t, 1H, J = 4.4 Hz, H3), 3.78 (t, 1H, J = 4.4 Hz, H4), 3.69 (dd, 1H, J = 11.2, 3.6 Hz, H5a), 3.52 (dd, 1H, J = 11.2, 3.6 Hz, H5b), 3.06 (s, 1H, OH), 0.76 (s, 9H, *t*-butyl), 0.58 (s, 9H, *t*-butyl), 0.00 (s, 3H, methyl), 0.01 (s, 3H, methyl), −0.23 (s, 3H, methyl), −0.32 (s, 3H, methyl); ^{13}C-NMR δ 154.78, 152.21, 150.40, 149.12, 123.80, 123.70, 119.14, 88.35, 84.63, 72.62, 70.87, 61.87, 25.78, 25.71, 18.26, 18.09, −4.63, −4.78, −5.55, −5.57; TOFMS: *m/z* (M^++H) 522. **15a**: ^{1}H-NMR ($CDCl_3$) δ 8.20 (s, 1H, H2), 5.78 (d, 1H, J = 5.6 Hz, H1'), 5.78 (s, 2H, NH_2), 5.37 (t, 1H, J = 6.0 Hz, H2'), 4.31 (t, 1H, J = 2.4 Hz, H3'), 4.06 (m, 1H, H4'), 3.94 (dd, 1H, J = 11.2 Hz, 5.2 Hz, H5'a), 3.75 (dd, 1H, J = 10.8 Hz, 4.4 Hz, H5'b), 2.57 (s, 3H, methyl), 0.83 (s, 9H, *t*-butyl), 0.75 (s, 9H, *t*-butyl), 0.00 (s, 3H, methyl), −0.00 (s, 3H, methyl), −0.12 (s, 3H, methyl), −0.33 (s, 3H, methyl); ^{13}C-NMR δ 154.40, 152.14, 151.15, 150.15, 118.66, 88.49, 85.04, 72.40, 70.88, 62.90, 25.89, 25.51, 18.38, 17.83, 14.87, −5.15, −5.21, −5.42, −5.44; TOFMS: *m/z* (M^++H) 510. **15b**: ^{1}H-NMR ($CDCl_3$) δ 8.21 (s, 1H, H2), 5.77 (d, 1H, J = 6.0 Hz, H1), 5.72 (s, 2H, NH_2), 5.58 (t, 1H, J = 6.0 Hz, H2), 4.32 (d, 1H, J = 2.4, H3), 4.08 (s, 1H, H4), 3.96 (dd, 1H, J = 10.8, 6.0 Hz, H5a), 3.74 (dd, 1H, J = 10.8, 4.8 Hz, H5b), 2.88 (dd, 2H, J = 7.6, 3.6 Hz, ethyl), 2.85 (s, 1H, OH), 1.36 (t, 3H, J = 7.6 Hz, ethyl), 0.84 (s, 9H, *t*-butyl), 0.76 (s, 9H, *t*-butyl), 0.00 (s, 6H, dimethyl), −0.13 (s, 3H, methyl), −0.34 (s, 3H, methyl); ^{13}C-NMR δ 154.80, 154.51, 151.90, 151.01, 118.80, 88.26, 84.96, 71.96, 71.10, 62.86, 25.94, 25.58, 21.63, 18.43, 17.90, 12.29, −5.04, −5.13, −5.32; TOFMS: *m/z* (M^++H) 524. **15c**: ^{1}H-NMR ($CDCl_3$) δ 8.21 (s, 1H, H2), 6.97 (dd, 1H, J = 17.2, 11.2 Hz, Ha), 6.41 (d, 1H, J = 17.2 Hz, Hc), 5.98 (d, 1H, J = 6.4 Hz, H1), 5.59 (d, 1H, J = 11.2 Hz, Hb), 5.53 (s, 2H, NH_2), 4.96 (t, 1H, J = 6.4 Hz, H2), 4.24 (t, 1H, J = 3.2 Hz, H3), 4.05 (d, 1H, J = 3.2 Hz, H4), 3.92 (dd, 1H, J = 11.2, 3.2 Hz, H5a), 3.77 (dd, 1H, J = 11.2, 3.2 Hz, H5b), 2.76 (d, 1H, OH), 0.83 (s, 9H, *t*-butyl), 0.68 (s, 9H, *t*-butyl), 0.00 (s, 6H, dimethyl), −0.22 (s, 3H, methyl), −0.43 (s, 3H, methyl); ^{13}C-NMR δ 154.82, 152.59, 151.08, 148.98, 124.09, 123.94, 119.26, 87.33, 85.19, 73.55, 70.35, 62.95, 26.01, 25.54, 18.52, 17.89, −5.16, −5.23, −5.28, −5.31; TOFMS: *m/z* (M^++H) 522.

The preparation of 8-Alkyl-2'(or 3')-azido (or amino)-2' (or 3')-deoxyadenosine derivatives (16-19): Compounds (**16-19**) were prepared by applying the same procedures described in the reference 6. **16a**: mp 200 °C (decomp.); ^{1}H-NMR (DMSO) δ 8.07 (s, 1H, H2), 7.26 (s, 1H, NH_2), 6.02 (d, 1H, -OH), 5.91 (d, 1H, J = 6.8 Hz, H1), 5.45 (dd, 1H, J = 7.6, 4.4 Hz, -OH), 4.91 (t, 1H, J = 6.0 Hz, H2), 4.57 (dd, 1H, J = 9.2, 6.0 Hz, H3), 3.97 (dd, 1H, J = 7.2, 3.2 Hz, H4), 3.34-3.69 (m, 2H, H5), 2.55 (s, 3H, methyl); ^{13}C-NMR; δ 155.35, 151.70, 149.88, 148.63, 117.85, 86.21, 85.80, 71.13, 62.61, 61.34, 14.34; TOFMS: *m/z* (M^++H) 307. **16b**: mp 190 °C; ^{1}H-NMR (DMSO) δ 7.89 (s, 1H, H2), 7.10 (s, 2H, NH_2), 5.85 (d, 1H, OH), 5.72 (d, 1H, J = 6.8 Hz, H1), 5.30 (dd, 1H, OH), 4.81 (t, 1H, J = 6.4 Hz, H2), 4.40 (dd, 1H, J = 8.8, 5.2 Hz, H3), 3.80 (t, 1H, J = 3.2 Hz, H4), 3.48 (m, 1H, H5a), 3.35 (m, 1H, H5b), 2.72 (dd, 2H, J = 14.4, 7.2 Hz, ethyl), 1.13 (t, 3H, J = 7.2 Hz, ethyl); ^{13}C-NMR δ 155.34, 152.95, 151.56, 149.75, 117.92, 86.32, 85.64, 71.21, 62.42, 61.50, 20.79, 11.96; TOFMS: m/z (M^++H) 321. **16c**: mp 190 °C (decomp.); ^{1}H-NMR (DMSO) δ 8.09 (s, 1H, H2), 7.44 (s, 2H, NH_2), 7.10 (dd, 1H, J = 17.2, 10.8 Hz, Ha), 6.34 (dd, 1H, J = 17.2, 2.0 Hz, Hc), 6.15 (s, 1H, OH), 5.92 (d, 1H, J = 6.8 Hz, H1), 5.78 (s, 1H, OH), 5.69 (s, 1H, J = 10.8, 2 Hz, Hb), 5.17 (t, 1H, J = 6.4 Hz, H2), 4.36 (q, 1H, J = 2.8 Hz, H3), 3.95 (t, 1H, J = 2.8 Hz, H4), 3.66 (d, 1H, J = 12.0 Hz, H5a), 3.58 (d, 1H, J = 8.0 Hz, H5b); ^{13}C-NMR δ 155.78, 152.02, 149.53, 147.51, 123.89, 123.17, 118.75, 87.52, 82.95, 72.62, 62.05, 61.77; TOFMS: *m/z* (M^++H) 319. **17a**: mp 178 °C ; ^{1}H-NMR (DMSO) δ 8.00 (s, 1H, H2), 7.23 (s, 2H, NH_2), 6.09 (d, 1H, -OH), 5.89 (dd, 1H, -OH), 5.69 (d, 1H, J = 6.8 Hz, H1), 5.13 (dd, 1H, J = 12.4, 6 Hz, H2), 4.31 (dd, 1H, J = 5.6, 2.4 Hz, H3), 3.90 (dd, 1H, J = 6.0, 2.4 Hz, H4), 3.59 (m, 1H, H5a), 3.28 (m, 1H, H5b), 2.49 (s, 3H, methyl); ^{13}C-NMR δ 155.45, 151.43, 149.56, 148.92, 118.04, 88.38, 83.15, 72.47, 62.42, 62.00, 14.30; TOFMS: *m/z* (M^++H) 307. **17b**: mp 208 °C (decomp.); ^{1}H-NMR (DMSO) δ 8.06 (s, 1H, H2), 7.30 (br, 2H, NH_2), 6.24 (d, 1H, J = 5.6 Hz, -OH), 6.02 (dd, 1H, J = 3.6, 9.2 Hz, H1'), 5.74 (d, 1H, J = 6.8 Hz, -OH), 5.26 (dd, 1H, J = 6.0, 12.4 Hz, H2'),

4.39 (dd, 1H, J = 2.8, 6.0 Hz, H3'), 3.96 (d, 1H, J = 2.8 Hz, H4'), 3.66 (m, 1H, H5'a), 3.55 (m, 1H, H5'b), 2.89 (q, 2H, J = 7.6 Hz, ethyl), 1.29 (t, 3H, J = 7.6 Hz, ethyl); ^{13}C-NMR δ 155.44, 153.30, 151.32, 149.48, 118.08, 88.13, 83.21, 72.31, 62.54, 62.10, 20.85, 12.18; TOFMS: m/z (M^++H) 321. **17c**: mp 170 °C; ^{1}H-NMR (DMSO) δ 8.09 (s, 1H, H2), 7.44 (s, 2H, NH_2), 7.10 (dd, 1H, J = 17.2, 10.8 Hz, Ha), 6.34 (dd, 1H, J = 17.2, 2 Hz, Hc), 6.15 (s, 1H, -OH), 5.92 (d, 1H, J = 6.8 Hz, H1), 5.78 (s, 1H, -OH), 5.69 (s, 1H, J = 10.8, 2.0 Hz, Hb), 5.17 (t, 1H, J = 6.4 Hz, H2), 4.36 (q, 1H, J = 2.8 Hz, H3), 3.95 (t, 1H, J = 2.8 Hz, H4), 3.66 (d, 1H, J = 12.0 Hz, H5a), 3.58 (d, 1H, J = 8.0 Hz, H5b); ^{13}C-NMR δ 155.78, 152.02, 149.53, 147.51, 123.89, 123.17, 118.75, 87.52, 82.95, 72.62, 62.05, 61.77; TOFMS: *m/z* (M^++H) 319. **18a**: mp 114 °C; ^{1}H-NMR (DMSO) δ 8.06 (s, 1H, H2), 5.92 (d, 1H, J = 8.4 Hz, H1), 4.54 (dd, 1H, J = 8.0, 5.2 Hz, H2), 4.38 (d, 1H, J = 5.2 Hz, H3), 4.16 (d, 1H, J = 1.2 Hz, H4), 3.82 (dd, 1H, J = 12.8, 2.8, H5a), 3.69 (dd, 1H, J = 12.8, 2.4 Hz, H5b), 3.14 (s, 3H, methyl); ^{13}C-NMR δ 156.51, 152.49, 151.85, 150.88, 119.45, 89.80, 89.56, 72.76, 63.89, 56.73, 14.57; TOFMS: *m/z* (M^++H) 281. **18b**: mp 188 °C; ^{1}H-NMR (DMSO) δ 8.09 (s, 1H, H2), 7.29 (s, 2H, NH_2), 6.15 (s, 1H, -OH), 5.62 (d, 1H, J = 8.0 Hz, H1), 4.52 (s, 1H, -OH), 4.24 (d, 1H, J = 8.0 Hz, H2), 4.08 (d, 2H, H3, 4), 3.73-3.61 (m, 2H, H5a,b), 2.96 (d, 2H, J = 7.2 Hz, ethyl), 1.37 (t, 3H, J = 7.2 Hz, ethyl); ^{13}C-NMR δ 155.36, 153.45, 150.90, 149.42, 118.15, 89.50, 87.42, 71.97, 62.74, 56.04, 20.88, 12.14; TOFMS: *m/z* (M^++H) 295. **19a**: mp 136 °C; ^{1}H-NMR (DMSO) δ 8.00 (s, 1H, H2), 7.16 (s, 2H, NH_2), 5.79 (d, 1H, J = 5.2 Hz, H1), 4.71 (t, 1H, J = 5.6 Hz, H2), 3.78 (s, 1H, -OH), 3.65 (d, 1H, J = 12 Hz, H3), 3.58 (t, 1H, J = 5.2 Hz, H4), 3.46 (dd, 2H, J = 2.8, 12.4 Hz, H5), 2.49 (s, 3H, methyl); ^{13}C-NMR δ 155.18, 151.30, 149.58, 148.59, 117.85, 89.46, 85.37, 72.07, 62.14, 53.08, 14.43; TOFMS: *m/z* (M^++H) 281. **19b**: mp 195 °C; ^{1}H-NMR (DMSO) δ 7.80 (s, 1H, H2), 5.80 (d, 1H, J = 5.2 Hz, H1), 4.82 (t, 1H, H2), 3.77-3.59 (m, 4H, H3, 4, 5a,b), 2.71 (d, 2H, J = 8.4 Hz, ethyl), 1.12 (t, 3H, J = 7.6 Hz, ethyl); ^{13}C-NMR δ 156.22, 155.76, 152.14, 151.85, 117.87, 89.79, 84.71, 72.36, 60.99, 52.96, 21.58, 11.62; TOFMS: *m/z* (M^++H) 295.

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