A Novel and Facile Reaction to N^6-Alkylated Adenosine *via* Benzotriazole as a Synthetic Auxiliary

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Received May 18, 1999

The reaction of benzotriazole with aliphatic, aromatic or heteroaromatic aldehyde and adenosine leads to a benzotriazole adduct which is reduced with sodium borohydride to the corresponding N^6-alkylated adenosine derivatives. This procedure is also utilized in a new route to N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide (IB-MECA) which is considered an important adenosine agonist at $\mathbf{A_3}$ adenosine receptors.

J. Heterocyclic Chem., **37**, 339 (2000).

The potential of adenosine receptors as drug targets has been reviewed [1,2]. The regulatory role for adenosine implicates adenosine-based drugs as therapeutic targets. An improved understanding of the physiology, pharmacology and molecular biology of adenosine and adenosine receptors is fundamental to the realization of the therapeutic potential for adenosine nucleosides and provides a solid foundation for the continuation of active research in the adenosine field [3]. Adenosine is a ubiquitous chemical messenger or "local hormone" involved in regulation of many physiological functions [2]. There are three classes of adenosine receptors: $\mathbf{A_1}$, $\mathbf{A_2}$, and $\mathbf{A_3}$, and tremendous advances have been made in recent years in the synthesis of selective agents acting at subtypes of adenosine receptors [4]. Selective adenosine antagonists are under development for use in cognitive diseases ($\mathbf{A_1}$), [5,6] renal failure ($\mathbf{A_1}$), [7] Parkinson's and Huntington's diseases ($\mathbf{A_2}$), [8] and cardiac arrhythmias ($\mathbf{A_1}$) [9]. Adenosine agonists ($\mathbf{A_1}$ and $\mathbf{A_3}$) are likewise of potential therapeutic interest as cerebroprotective agents antiepileptic drugs *etc.* [6].

The structure-activity relationships (SAR) for adenosine and xanthine derivatives at rat $\mathbf{A_3}$ receptors have been reported [10,11]. Highly selective agonists have recently been synthesized [11,12]. The combination of N^6-benzyl- and 5'-*N*-alkyluronamide modifications of adenosine increased $\mathbf{A_3}$ receptor binding affinity and selectivity *versus* $\mathbf{A_1}$ and $\mathbf{A_{2a}}$ receptors [10]. Optimization of substituents resulted in the compound N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide (**6**, IB-MECA) which is 50-fold selective in binding assays for rat $\mathbf{A_3}$ *versus* either $\mathbf{A_1}$ or $\mathbf{A_{2a}}$ receptors [11]. Further modification indicated that 2-substitution, such as chloro, methylamino or methylthio, in combination with the IB-MECA structure enhanced $\mathbf{A_3}$ selectivity [13].

The N^6-alkylated adenosine-5'-uronamides are typically synthesized in a multistep linear synthesis from inosine or adenosine [11,13,14]. Alternatively, a convergent synthesis has been devised by condensing an N^6-alkyladenine with an *N*-alkyl 1-*O*-acetyl-2,3-dibenzoyl-α-D-ribofuranuronamide [12].

In a recent synthesis of N^6-alkylated 2'-deoxyadenosines [15], the strategy was based on direct alkylation at the exocyclic amino group of 2'-deoxyadenosine using benzotriazole as commerically available auxiliary avoiding side reactions, *e.g.* bisalkylation or alkylation at other sites in the adenosine. In the present work the N^6-alkylated adenosines **3a-k** and N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide (**6**, IB-MECA) were prepared by the reduction of the corresponding benzotriazole adducts **2a-k** as well as the adduct **5** using 6 molar equivalents of sodium borohydride in dry tetrahydrofuran under reflux for 6-8 hours. The adducts **2a-k** and **5** were prepared according to Katritzky methodology [16] using 1 mole of adenosine, 1.25 moles of benzotriazole and 1.5 moles of the appropriate aldehyde. The reaction of adenosine **1**, as well as 5'-uronamide **4**, with benzotriazole and the appropriate aldehyde was carried out in refluxing ethanol in the presence of a catalytic amount of acetic acid, using a soxhlet extractor equipped with a thimble filled with molecular sieves (4 Å) to trap the water formed during the reaction. By this modification of the reaction conditions and after the appropriate reaction time, the formed adducts **2a-k** and **5** were obtained in good yields (Table 1). The purification of the obtained adduct was carried out using methanol:chloroform (0-10%, v/v) on chromatographic silica gel to yield the adducts **2a-k** and **5** in a pure form. The ^1H and ^{13}C nmr assignment of the adduct structures prove that all the adducts **2a-k** and **5** are diastereomeric mixtures.

The target N^6-alkylated adenosine **3a-k** and **6** were obtained in good yields through the reduction of the adducts **2a-k** and **5** directly on the diastereomeric mixture using sodium borohydride in dry tetrahydrofuran without

Scheme I

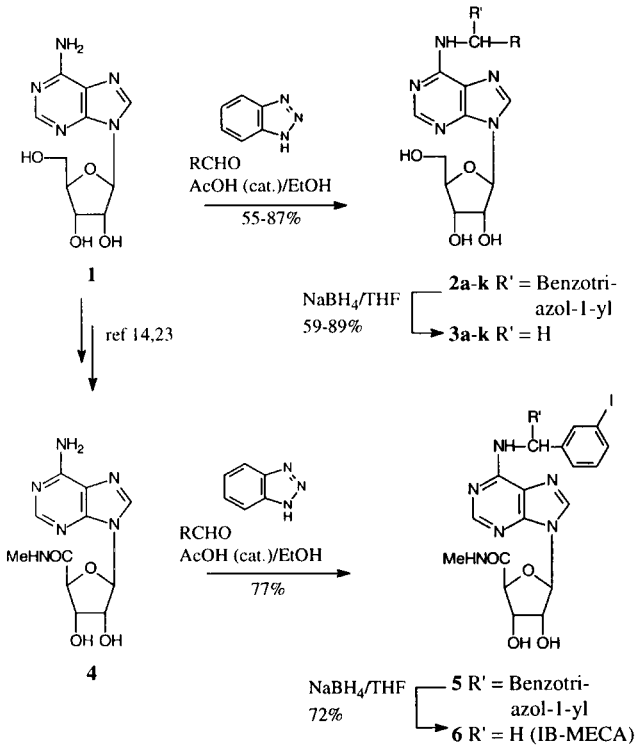

Table 1
Yield of the Obtained Adducts (**2** and **5**) and their Reduction Products (**3** and **6**)

Entry	R	Yield of **2** and **5** (%)	Yield of **3** and **6** (%)	Mp of **3** and **6** (°)
a	3-BrC_6H_4	81	84	oil
b	2-BrC_6H_4	77	82	163-165 [a]
c	4-$AcNHC_6H_4$	68	69	170-173 [a]
d	3-$O_2NC_6H_4$	71	74	oil [21]
e	2,4-$Cl_2C_6H_3$	65	76	186-188 (187-188 [17])
f	2-Pyridyl	55	59	oil [17]
g	2-Furyl	82	85	146-147 (148-150 [22])
h	3-Furyl	82	84	oil
i	2-Ethylbutyl	87	89	oil
j	3-Phenylpropyl	83	87	128-130 (124-126 [18])
k	2-Phenylpropyl	86	86	91-93 (93-95 [19])
5 and 6		77	72	176-177 (177-178 [11])

[a] Recrystallized from methanol.

any trials to separate each of the diastereomeric compounds. For the synthesis of IB-MECA (**6**) this is an attractive route since the starting material **4** is easily obtained from adenosine [14,23]. The structures of the obtained N^6-alkylated adenosine have been confirmed by ^1H and ^{13}C nmr, FAB ms, and microanalysis, and their spectra were found to be in accordance with previously reported ^1H nmr and ^{13}C nmr chemical shift values [11,17-22].

EXPERIMENTAL

Nmr Spectra were recorded at 300 MHz for ^1H and 75.5 MHz for ^{13}C nmr on a Varian Gemini 2000 300 MHz spectrometer; δ values are in ppm relative to tetramethylsilane as an internal standard. Fast atom bombardment mass spectra (FAB ms) were recorded on a Kratos 50TC spectrometer. Analytical silica gel tlc was performed on Merck precoated 60 F_{254} plates. The silica gel (0.040-0.063 mm) used for column chromatography was purchased from Merck.

Adenosine Benzotriazole Adducts **2a-k** and **5**.

Benzotriazole (1.25 mmoles) and the appropriate aldehyde (1.5 mmoles) were added to a solution of the adenosine **1** or **4** (1 mmole) in ethanol (40 ml). The suspension was refluxed in the presence of a few drops of acetic acid as a catalyst for 6 hours using a soxhlet extractor with 4 Å molecular sieves in the thimble. The mixture was filtered and evaporated to dryness. The adduct was chromatographed on a silica gel using methanol:-chloroform (0-10%, v/v) as the eluent to give **2a-k** and **5**.

N^6-Benzyladenosines **3a-k** and **6**.

The adducts **2a-k** or **5** (0.5 mmole) were dissolved in dry tetrahydrofuran (20 ml) and refluxed with excess of sodium borohydride (3 mmoles) for 8 hours. The solution was cooled to room temperature, poured onto ice water, neutralized with acetic acid and extracted with chloroform. After evaporating the solvent the compounds were purified by column chromatography using methanol:chloroform (0-10%, v/v) as the eluent to give **3a-k** and **6**.

N^6-(3-Bromobenzyl)adenosine (**3a**).

^1H nmr (DMSO-d_6): δ 8.25 (s, H-8), 8.01 (s, H-2), 7.91-7.16 (m, Ar), 7.86 (t, NH), 6.35 (br, 2'-OH), 5.93 (d, H-1'), 5.28 (br, 5'-OH, 3'-OH), 4.82 (t, H-2'), 4.37 (d, $NHCH_2$) 4.23 (m, H-3'), 3.91 (m, H-4'), 3.72-3.58 (m, H-5').

^{13}C nmr (DMSO-d_6): δ 154.10 (C-6), 151.34 (C-2), 147.13 (C-4), 141.15 (C-8), 139.46, 129.42, 129.20, 129.06, 125.37, 121.34 (Ar), 120.21 (C-5), 89.65(C-1'), 86.52 (C-4'), 72.93 (C-2'), 70.85 (C-3'), 61.87 (C-5'), 42.61 (CH_2); FAB ms: (DMSO+3-nitrobenzyl alcohol) m/z 436 (M).

Anal. Calcd. for $C_{17}H_{18}BrN_5O_4$ (436): C, 46.79; H, 4.13; N, 16.05. Found: C, 47.18; H, 4.17; N, 16.06.

N^6-(2-Bromobenzyl)adenosine (**3b**).

^1H nmr (DMSO-d_6): δ 8.27 (s, H-8), 8.01 (s, H-2), 7.85 (t, NH), 7.61-7.13 (m, Ar), 6.32 (br, 2'-OH), 5.92 (d, H-1'), 5.21 (br, 5'-OH, 3'-OH), 4.87 (t, H-2'), 4.40 (d, $NHCH_2$), 4.25 (m, H-3'), 3.91 (m, H-4'), 3.73-3.63 (m, H-5').

^{13}C nmr (DMSO-d_6): δ 154.30 (C-6), 151.57 (C-2), 147.91 (C-4), 139.77 (C-8), 137.02, 131.88, 128.37, 128.07, 126.78,

122.40 (Ar), 120.34 (C-5), 90.00 (C-1'), 86.79 (C-4'), 73.17 (C-2'), 71.10 (C-3'), 62.10 (C-5'), 43.55 (CH_2); FAB ms: ($CDCl_3$+3-nitrobenzyl alcohol) m/z 436 (M).

Anal. Calcd. for $C_{17}H_{18}BrN_5O_4$ (436): C, 46.79; H, 4.13; N, 16.05. Found: C, 47.11; H, 4.28; N, 16.28.

N^6-(4-Acetamidobenzyl)adenosine (**3c**).

^{1}H nmr (DMSO-d_6): δ 8.29 (s, H-8), 8.20 (s, H-2), 7.76 (t, NH), 7.33-7.28 (m, Ar), 6.32 (d, H-1'), 6.18 (br, 2'-OH), 5.60 (br, 5'-OH, 3'-OH), 4.80 (t, H-2'), 4.48 (d, NHCH_2), 4.21 (m, H-3'), 3.92 (m, H-4'), 3.73-3.48 (m, H-5'), 2.02 (s, CH_3).

^{13}C nmr (DMSO-d_6): δ 168.56 (C=O), 152.67 (C-6), 151.64 (C-2), 150.28 (C-4), 143.55 (C-8), 139.46 (Ar), 137.24 (Ar), 127.69 (Ar), 123.15 (C-5), 119.90 (Ar), 90.05 (C-1'), 87.38 (C-4'), 74.00 (C-2'), 70.70 (C-3'), 63.97 (C-5'), 45.11 (CH_2), 24.28 (CH_3); FAB ms: (DMSO+3-nitrobenzyl alcohol) m/z 415 ($M+H^+$).

Anal. Calcd. for $C_{19}H_{22}N_6O_5$ (414): C, 55.07; H, 5.31; N, 20.28. Found: C, 54.89; H, 5.28; N, 20.19.

N^6-(3-Furylmethyl)adenosine (**3h**).

^{1}H nmr (DMSO-d_6): δ 8.25 (s, H-8), 8.10 (s, H-2), 7.74 (t, NH), 7.48 (m, H-2 furan), 7.40 (m, H-5 furan), 6.48 (m, H-4 furan), 6.13 (br, 2'-OH), 5.93 (d, H-1'), 5.29 (5'-OH), 4.65 (br, 3'-OH), 4.30 (t, H-2'), 4.17 (H-3'), 3.87 (m, H-4'), 3.69-3.55 (m, H-5'), 3.53 (d, NHCH_2).

^{13}C nmr (DMSO-d_6): δ 153.87 (C-6), 150.91 (C-2), 150.84 (C-4), 141.71 (C-8), 141.56 (C-5 furan), 138.74 (C-2 furan), 121.99 (C-3 furan), 121.89 (C-5), 109.34 (C-4 furan), 88.60 (C-1'), 85.72 (C-4'), 72.56 (C-2'), 70.13 (C-3'), 61.14 (C-5'), 36.54 (CH_2); FAB ms: (DMSO+3-nitrobenzyl alcohol) m/z 348 ($M+H^+$).

N^6-(2-Ethylbutyryl)adenosine (**3i**).

^{1}H nmr (DMSO-d_6): δ 8.32 (s, H-8), 8.04 (s, H-2), 6.80 (t, NH), 6.40 (br, 2'-OH), 5.92 (d, H-1'), 5.36 (br, 5'-OH, 3'-OH), 4.93 (t, H-2'), 4.41 (NHCH_2), 4.22 (m, H-3'), 3.90 (m, H-4'), 3.73-3.54 (m, H-5'), 1.66 (m, CH), 1.64-1.18 (m, CH_2), 0.99 (t, CH_3).

^{13}C nmr (DMSO-d_6) δ 154.48 (C-6), 151.19 (C-2), 150.51 (C-4), 138.97 (C-8), 124.19 (C-5), 89.44 (C-1'), 86.38 (C-4'), 72.81 (C-2'), 70.71 (C-3'), 61.72 (C-5'), 41.85 (NCH_2), 40.82 (CH), 22.25 (CH_2), 9.64 (CH_3); FAB ms: (DMSO+3-nitrobenzyl alcohol) m/z 352 ($M+H^+$).

Anal. Calcd. for $C_{16}H_{25}N_5O_4$ (351): C, 54.70; H, 7.12; N, 19.94. Found: C, 54.61; H, 7.17; N, 19.88.

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