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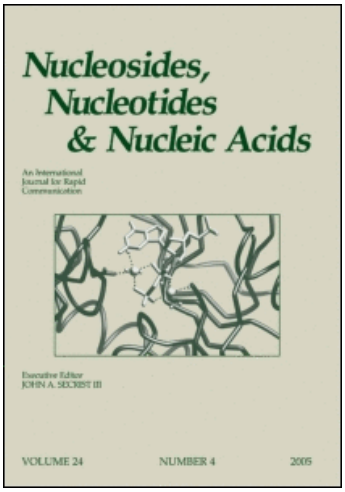

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Synthesis of Iodo-aryl-azido Adenosine Analogs as Affinity Ligands for Adenylyl Cyclase

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NUCLEOSIDES & NUCLEOTIDES, 13(9), 1977-1989 (1994)

SYNTHESIS OF IODO-ARYL-AZIDO ADENOSINE ANALOGS AS AFFINITY LIGANDS FOR ADENYLYL CYCLASE

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ABSTRACT: Potential affinity probes for adenylyl cyclase were synthesized that take advantage of the enzyme's sensitivity to "P"-site-mediated inhibition by 2',5'-dideoxyadenosine analogs and its tolerance for large 3'-ribose substitutions. We report the synthesis of a series of 3'-substituted 2',5'-dideoxyadenosine analogs. The syntheses involved the intermediate formation of symmetric anhydrides that were then coupled to 2',5'-dideoxyadenosine by base-catalyzed esterification at the 3'-ribose position.

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INTRODUCTION

Adenylyl cyclase is potently and directly inhibited by analogs of adenosine via a domain referred to as the "P"-site from its evident requirement for an intact purine moiety.[1-4] Evidence suggests that inhibition of adenylyl cyclases via the "P"-site may mediate regulatory control of the enzyme by nucleic acid metabolites.[5] "P"-site-mediated inhibition is non-competitive with respect to substrate and may be characterized pharmacologically by: (a) a requirement for an intact adenine moiety, including N^6, N(3), N(7), and N(9) positions (unpublished observations); (b) a requirement for a β-glycosidic linkage at the ribosyl moiety; (c) substantially increased inhibitory potency of 2'-deoxy- and especially 2',5'-dideoxyribosyl moieties; (d) a strong preference for phosphate at the 3'-position; and (e) a tolerance for large substitutions at the 3'-position.[3,4,6] Available evidence suggests that the "P"-site is distinct from, yet homologous with, the catalytic domain.[6-9]

As an approach towards elucidating the structure-function relationships of adenylyl cyclase, covalent affinity ligands could be particularly useful.[10] We previously

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reported the synthesis of 3'-(*p*-fluorosulfonylbenzoyl)-2',5'-dideoxyadenosine[7] to take advantage of "P"-site tolerance for large substitutions at the 3'-ribose position. This affinity ligand irreversibly inactivated adenylyl cyclase.[7] As alternative covalent affinity probes arylazido analogs have been used for many nucleoside binding domains[10,11] and might be effective in studies of adenylyl cyclase. While the synthesis of 3'-substituted arylazido analogs of 2',5'-dideoxyadenosine should be straightforward based on procedures used for other nucleotides,[11,12] in practice we encountered poor coupling efficiencies with these methods and substantial difficulties due to the insolubility of the 2',5'-dideoxyadenosine in the solvents that are typically used for these reactions. To circumvent these problems an alternative synthetic approach was developed that involves the intermediate formation of symmetric aryl anhydrides. In this paper we report the synthesis of a series of 3'-substituted-2',5'-dideoxyadenosine affinity probes.

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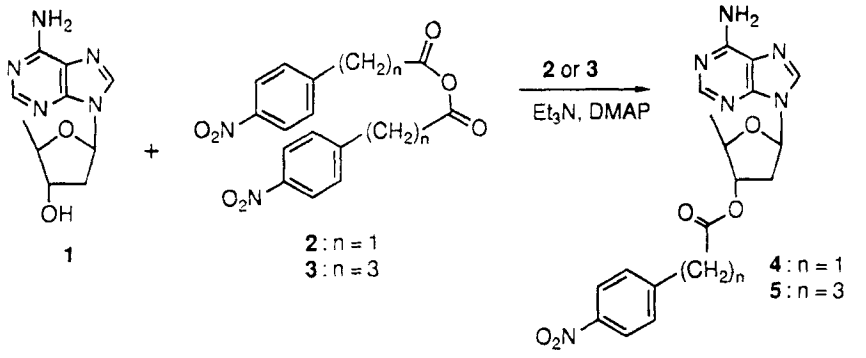
RESULTS AND DISCUSSION

The previously described synthesis of 3'-(*p*-fluorosulfonylbenzoyl)-2',5'-dideoxyadenosine relied on acylation of the 3'-hydroxyl of 2',5'-dideoxyadenosine by fluorosulfonylbenzoyl-chloride and carbodiimidazole in DMF.[7] While this procedure was successful, yields were typically low (5 to 10%). This approach was similar to that used by Chen and Guillory[12] in the preparation of 3'-substituted analogs of NAD, NMN, and 5'AMP. However, when we attempted to apply this method to the synthesis of 3'-substituted aryl-azido analogs of 2',5'-dideoxyadenosine, unworkably low yields were encountered. The low yields may have been due to two factors: a) the 3'-hydroxyl group of 2',5'-dideoxyadenosine being a poor nucleophile; and b) poor solubility of 2',5'-dideoxyadenosine. To improve acylation at the 3'-hydroxyl position two approaches were considered. First, reaction at the 3'-ribose could be improved by the use of DMAP.[13] Second, a coupling solvent other than DMF could be used to increase solubility of 2',5'-dideoxyadenosine. To improve solubility of 2',5'-dideoxyadenosine several solvents were tried. Optimal reaction occurred when acetonitrile[14] was used with DMF.

In pilot experiments to validate this approach, commercially available (4-nitrophenyl)-acetic and (4-nitrophenyl)-butyric acids were reacted with DCC to form the symmetric anhydrides.[15,16] The symmetric anhydride of (4-nitrophenyl)-acetic anhydride **2** was coupled with 2',5'-dideoxyadenosine (**1**) in the presence of a catalytic



SCHEME 1

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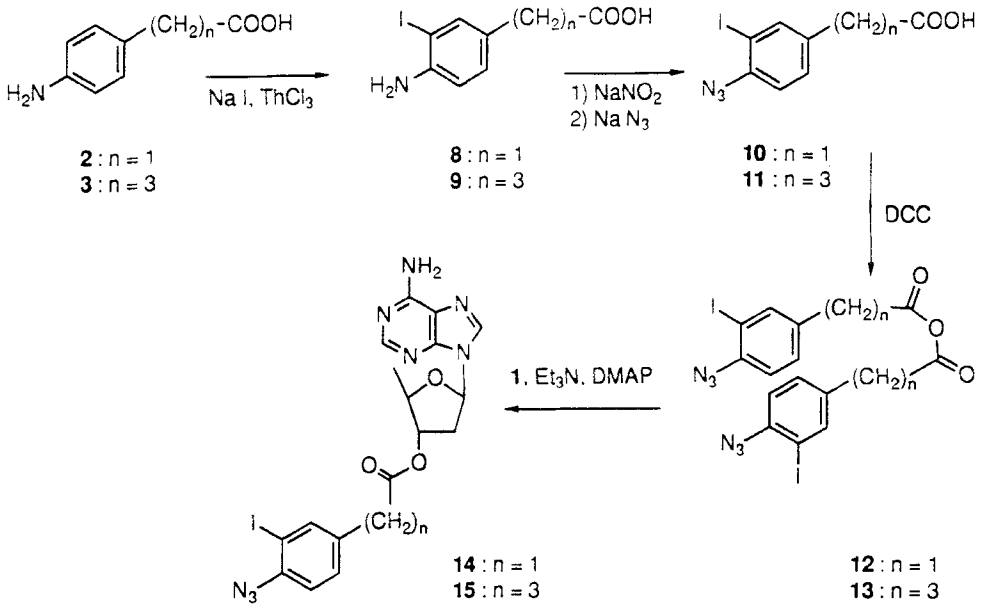
amount of DMAP in acetonitrile (*Scheme 1*). However, the solubility of 2',5'-dideoxyadenosine was found to be low and it did not completely dissolve. Purification by flash chromatography gave the desired 3'-[(4-nitrophenyl)-acetyl]-2',5'-dideoxyadenosine **4**, in 7% yield.

The same approach was applied to the synthesis of the ester **5**. Acylation with this anhydride gave an encouraging yield of 40% (*Scheme 1*). The use of DMAP[13,14] as catalyst and of DMF and acetonitrile as protic solvent seemed to eliminate the limitations of solubility and reaction efficiency.

The above approach was then used in the synthesis of the esters **14** and **15** according to *Scheme 2*. (4-Aminophenyl)-acetic acid reacted with sodium iodide in the presence of thallium trichloride and sodium acetate to form **8**.[17] The 4-amino group was then converted to 4-diazonium salt by reaction with sodium nitrite in an acidic medium, then to the 4-azido- compound **10** by reaction with sodium azide.[17] All reactions in which azido-analogs are formed or used were conducted under subdued light. Activation of **10** by DCC generated the desired anhydride **12**. Coupling of **12** with 2',5'-dideoxyadenosine **1** in acetonitrile and DMF in the presence of triethylamine (Et_3N) gave **14** with a 49% yield after purification.

The butyryl-analog **15** was prepared by the same approach as **14**. The coupling of **13** with 2',5'-dideoxyadenosine yielded **15** (90%) after purification by flash chromatography.

The nitrophenyl analogs (**4**,**5**) served both as precursors in the synthetic scheme and as control reagents for the experiments with adenylyl cyclase. The azidoiodophenyl



SCHEME 2

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analogs (**14,15**) were *uv*-labile, as noted by the characteristic shifts in *uv*-spectra following exposure at 254 nm (Fig. 1, *panels B and D*). These difference spectra are more clearly seen with the precursor acetic- and butyric acid analogs, in the absence of the strongly absorbing adenine moiety (Fig. 1, *panels A and C*). All four compounds (**4,5,14,15**) inhibited adenylyl cyclase (Table 1) in the absence of *uv*-irradiation and the azido-iodo- compounds (**14,15**) caused light-dependent and irreversible inactivation of the enzyme (not shown). The nitro-analogs were typically more potent than were the respective azido-iodo analogs, though with this enzyme and under these assay conditions potency was substantially less than we reported previously with 3'-(*p*-fluorosulfonylbenzoyl)-2',5'-dideoxyadenosine.[7] Another consideration is that different types of covalent affinity probes will exhibit different properties with the several adenylyl cyclase isozymes, since there are striking differences in their "P"-site sensitivities.[18] Efforts are underway to synthesize and identify additional covalent affinity ligands for the "P"-site domain on the known adenylyl cyclases isozymes.

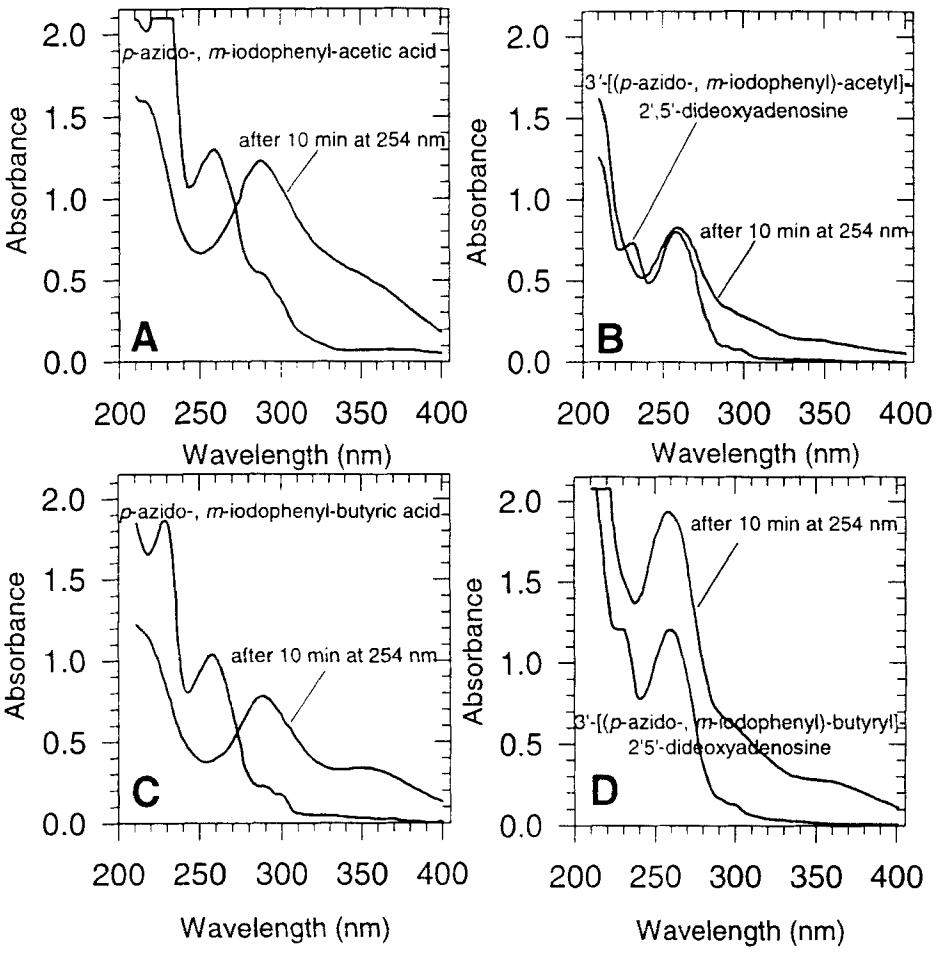

Figure 1

Table 1. Inhibition of detergent-dispersed adenylyl cyclase from rat brain by several 3'-substituted analogs of 2',5'-dideoxyadenosine.

NUCLEOSIDE	IC_{50} (μM)
3'-(*p*-Fluorosulfonylbenzoyl)-2',5'-dideoxyadenosine[7]	30
3'-[(4-Nitrophenyl)-acetyl]-2',5'-dideoxyadenosine	225
3'-[(4-Nitrophenyl)-butyryl]-2',5'-dideoxy adenosine	150
3'-[(4-Azido-3-iodophenyl)-acetyl]-2',5'-dideoxyadenosine	275
3'-[(4-Azido-3-iodophenyl)-butyryl]-2',5'-dideoxyadenosine	880

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EXPERIMENTAL PROCEDURES

Adenylyl cyclase. Detergent-dispersed adenylyl cyclase was prepared from rat brain and was assayed in the presence of 100 μM ATP and 10 mM $MnCl_2$ as described previously.[4-7] To remove the dithiothreitol present during the extraction of the enzyme with Lubrol-PX, the enzyme was dialyzed overnight against 10 mM Hepes, pH 7.5, 1 mM $MgCl_2$, 0.1% Lubrol-PX, and 5% glycerol. All assays were conducted in subdued light.

General. 2',5'-Dideoxyadenosine was synthesized by methods described by Beacham[19] and Wang et al.[20] (4-Nitrophenyl)-acetic acid, 4-(4-nitrophenyl)-butyric acid, (4-aminophenyl)-acetic acid, 4-(4-aminophenyl)-butyric acid, thallium (III) chloride tetrahydrate, Et_3N, 4-dimethylaminopyridine, 1,3-dicyclohexylcarbodiimide and deuterated (d_6) dimethylsulfoxide (99.9 atom %) were purchased from Aldrich Chemical in the purest forms available. The solvents used in the syntheses, acetonitrile, dichloromethane, and Et_3N were redistilled over calcium hydride and were stored under dry nitrogen. Dimethylformamide was dried over molecular sieves. All solid starting materials used in syntheses were dried under vacuum over P_2O_5 for several days before use. Flash chromatography was conducted on silica gel 60 (40-63 micron; 230-400 mesh ASTM) from EM Science. Thin-layer chromatography plates wher Silica Gel 60F254 from EM Science or Silica Gel GF from Analtech). Irradiation of compounds shown in Fig. 1 was in quartz tubes for 10 min at room temperature in a Rayonet Photochemical Reactor is from The Southern New England Ultraviolet Company with 254 nm *uv* lamps.

^1H NMR (300 MHz) and ^{13}C NMR spectra were obtained from a General Electric QE-300 spectrometer with tetramethylsilane as internal standard. Fast atom bombardment mass spectra (FAB-MS) were obtained on a Kratos MS-890/DS-90 instrument with a 50% mixture of glycerol + thioglycerol as matrix. A 1600 series FTIR instrument from Perkin Elmer was used for infrared spectra. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

(4-Nitrophenyl)-acetic anhydride (2). (4-Nitrophenyl)-acetic acid (1.27 g; 7 mmol) was dissolved in 50 mL of dichloromethane. The solution was cooled in an ice/water bath, 0.721 g (3.5 mmol) of DCC was added, and the mixture was stirred at

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0°C under dry N_2 for an hour. The insoluble urea formed during the reaction was removed by filtration under N_2 and the solvent was evaporated under reduced pressure, yielding 0.91 g. The anhydride **2** was employed without further purification. IR ($CHCl_3$): 1750, 1798 cm^{-1}.

3'-[(4-Nitrophenyl)-acetyl]-2',5'-dideoxyadenosine (4). 2',5'-Dideoxyadenosine **1** (200 mg; 0.85 mmole), 0.156 mL (1.12 mmole) of Et_3N, and 7.9 mg (0.065 mmole) of DMAP were suspended in 15 mL of acetonitrile. (4-Nitrophenyl)-acetic anhydride **2** (350 mg; 1.02 mmole) was added in one portion. As the reaction was incomplete after 3 hr, additional 15 mL of acetonitrile, 7.9 mg of DMAP, and 350 mg of (4-nitrophenyl)-acetic anhydride were added. The reaction mixture was stirred at room temperature for an additional 48 hr. The reaction showed substantial residual insoluble 2'5'-dideoxyadenosine by TLC. The precipitate was removed by filtration and ethanol was added to destroy the excess (4-nitrophenyl)-acetic anhydride. The soluble material was subjected to flash chromatography on silica gel eluting with ethyl acetate:ethanol 9:2 as solvent to give **4** (26 mg) as a yellow oil: Rf: 0.42, (ethyl acetate:ethanol, 9:2); 0.65 (butanol:acetic acid:water, 8.5:0.5:1). ^1H NMR (DMSO-d_6): δ 1.33(d, 3H, J=6.5 Hz), 2.50(m, 1H), 3.33(m, 1H), 3.99(s, 2H), 4.15(m, 1H), 5.22(m, H), 6.29(m, 1H), 7.31(s, 2H, exchangeable with D_2O), 7.62(d, J=8.6 Hz, 2H), 8.15(s, 1H), 8.21(d, J=8.7 Hz, 2H), 8.38(s, 1H). ^{13}C NMR (DMSO-d_6): δ 169.8, 155.9, 152.4, 149.1, 146.5, 139.4, 130.8, 123.1, 121.5, 119.2, 83.0, 80.0, 78.6, 35.0, 19.0. *UV* max (EtOH): 262, 397 nm. IR ($CHCl_3$): 3280, 3120, 1730, 1673, 1604, 1515, 1346, 1214, 1150, 1114 cm^{-1}. FAB-MS (m/z): 399 [$(M+H)^+$, 28%]; peak matching [$(M+H)^+$] calcd 399.1417, found 399.1654.

(4-Nitrophenyl)-butyric anhydride (3). (4-Nitrophenyl)-butyric acid (1.47 g; 7 mmol) was dissolved in 30 mL of dichloromethane. The solution was cooled to O°C, DCC (0.721 g; 3.5 mmol) was added, and the mixture was stirred under dry N_2 for 1.5 hr. The insoluble urea formed during the reaction was removed by filtration under N_2 and the solvent was evaporated under reduced pressure, yielding 0.63 g. The anhydride **3** was employed as such without further purification. IR ($CHCl_3$): 1745, 1815 cm^{-1}.

3'-[(4-Nitrophenyl)-butyryl]-2',5'-dideoxy adenosine (5). 2',5'-Dideoxyadenosine **1** (210 mg; 0.894 mmole), 0.130 mL (0.93 mmole) of Et_3N, and

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17.8 mg (0.146 mmole) of DMAP were added to 15 mL of acetonitrile. DMF (4 mL) was added to effect complete solution of **1**. 4-(4-Nitrophenyl)-butyric anhydride **3** (410 mg; 1.02 mmole) in 14 mL of acetonitrile was then added in one portion and the reaction mixture was stirred overnight at room temperature. The white precipitate that formed was filtered and identified as **5**. Column purification of the filtrate yielded an additional 15 mg of **5**. Total yield 40%. Rf: 0.55 (ethyl acetate:ethanol, 9:2). ^{1}H NMR (DMSO-d_6): δ 1.34(d, J=6.3 Hz, 3H), 1.90(m, 2H), 2.42(m, 1H), 2.51(m, 2H), 2.78(m, 2H), 3.20(m, 1H), 4.12(m, 1H), 5.187(m, H), 6.0(m, 1H), 7.33(s, 2H, exchangeable with D_2O), 7.54(d, J=7.89 Hz, 2H), 8.18(m, 3H), 8.39(s, 1H). ^{13}C NMR: δ 172.0, 156.0, 152.4, 149.7, 149.1, 145.9, 139.4, 129.5, 123.3, 119.2, 83.1, 80.1, 77.9, 35.0, 33.9, 32.7, 25.4, 19.1. *UV* max (EtOH): 216, 327 nm (sh). IR ($CHCl_3$): 3282, 3126, 3020, 2973, 2674, 2295, 1920, 1732, 1678, 1632, 1604, 1569, 1506, 1472, 1458 cm^{-1}. FAB-MS (m/z): 427 [$(M+H)^+$], 73%]; Anal. calcd for $C_{20}H_{22}N_6O_5$: C, 56.34; H, 5.16; N, 19.72. Found C, 56.26; H, 5.20; N, 19.55.

(4-Amino-3-iodophenyl)-acetic acid (8). (4-Aminophenyl)-acetic acid **6** (0.907 g; 6 mmol) was suspended along with 6 mmol of sodium iodide (0.9 g) in 180 mL of 0.1 M sodium acetate buffer, pH 4.2, in a 500 mL three-port round-bottom flask.[17] The mixture was heated at reflux on a steam bath and agitated with both a gentle stream of nitrogen gas and with magnetic stirring. Sodium acetate buffer (50 mL) containing 2.21 g of thallium trichloride was added dropwise over a 1 hr period. After an additional 1 hr the reaction was stopped by the addition of 0.69 g of sodium metabisulfite in 15 mL of water and the mixture was then cooled to room temperature and extracted three times with 100 mL ethyl acetate.[17] The combined extracts were concentrated by rotary evaporation and the oily residue was subjected to flash chromatography on silica gel with chloroform:acetic acid (20:1) as solvent. Fractions containing product were pooled and solvent was removed by rotary evaporation. Approximately 100 mL water was added, which became cloudy, and then enough methanol was added to suspend the oily residue on the bottom of the flask. The desired compound precipitated out of solution. Water and methanol were removed by rotary evaporation and yielded **8** (0.76 g, 46%). Rf: 0.45 ($CHCl_3$:acetic acid, 20:1). ^{1}H NMR (DMSO-d_6): δ 3.34(s 2H), 5.07(s, 2H), 6.68(d, J= 8.2 Hz, 1H), 6.94(dd, J=8.1 & 1.5 Hz, 1H), 7.42 (d, J=1.5 Hz, 1H).

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4-(4-Amino-3-iodophenyl)-butyric acid (9). The same experimental procedure was used as in the case of **8**. The desired iodinated derivative **9** was purified by flash chromatography with chloroform:acetic acid 40:1 as the eluant, giving 1.09 g of **9** (yield 60%). Rf: 0.34 on silica gel TLC (chloroform:acetic acid, 20:1); Rf: 0.44 (toluene:acetonitrile:acetic acid, 20:1:2). ^{1}H NMR (DMSO-d_6): δ 1.7(m, 2H), 2.15(t, J=7.37 Hz, 2H), 2.38(t, J=7.69 Hz, 2H), 4.98(s, 2H), 6.67(d, J= 8.17 Hz, 1H), 6.89(dd, J= 8.2 & 1.5 Hz, 1H), 7.35(d, J=1.54 Hz, 1H).

(4-Azido-3-iodophenyl)-acetic acid (10). Under subdued light **8** (0.7 g; 2.53 mmol) was suspended in 3% sulfuric acid (50 mL) and cooled to 4°C.[17] Sodium nitrite (0.35 g, 5.06 mmol) in ice/water (25 mL) was added dropwise and the reaction was stirred at 4°C for 30 min. Sodium azide (0.33 g, 5.06 mmol) in ice-cold water (4.2 mL) was added dropwise and was stirred at 4°C for an additional 45 min. The desired product **10** was extracted with chloroform (three times with 50 mL), which was subsequently removed by rotary evaporation. The residue was washed twice with methanol, which was subsequently removed by rotary evaporation. Water (100 mL) was added and enough methanol was added to dissolve the oily residue. The solution was evaporated to remove the methanol which caused **10** to precipitate. The water was then removed under reduced pressure. The solid, 0.613 g (80% yield), was chromatographically pure and was used without further purification. Rf: 0.37 (toluene:acetonitrile:acetic acid, 20:1:2). ^{1}H NMR (DMSO-d_6): δ 3.55(s, 2H), 7.27(d, J=8.2 Hz, 2H), 7.33(dd, J= 8.2 & 1.9 Hz, 1H), 7.72(d, J=1.8 Hz, 1H). IR ($CHCl_3$): 2123 cm^{-1} (aryl azido group). *UV* max (EtOH): 370, 320 (sh), 300 (sh), 290 (sh), max peak at 258 and 226 nm.

(4-Azido-3-iodophenyl)-butyric acid (11). The procedure used was the same as in the case of **10**. Yield of **11** was 0.734 g (87%). Rf: 0.56 (toluene:acetonitrile:acetic acid = 20:1:2). ^{1}H NMR (DMSO-d_6): δ 1.74(m, 2H), 2.17(t, J=7.35 Hz), 2.53(t, J=7.42 Hz, 2H), 7.24(d, J=8.14 Hz 1H), 7.28(dd, J=8.2 Hz & 1.6 Hz), 7.65(d, J=1.6 Hz, 1H). IR ($CHCl_3$): 2119 cm^{-1} (aryl azido group). *UV* max (EtOH): 340 (br), 300 (sh), 290 (sh), peak at 257.5 and 228 nm.

(4-Azido-3-iodophenyl)-acetic anhydride (12). (4-Azido-3-iodophenyl)-acetic acid **10** (1.00 g; 3.3 mmol) was dissolved in 20 mL of dichloromethane. The solution

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was cooled to 0°C, DCC (0.34 g; 1.65 mmol) was added, and the mixture was stirred under dry N_2 for 1 hr. The insoluble N,N'-dicyclohexylurea was removed by filtration under N_2 and the solvent was evaporated under reduced pressure. The crude anhydride was used as such for further reactions. IR ($CHCl_3$): 1752, 1819 cm^{-1}.

(4-Azido-3-iodophenyl)-butyric anhydride (13). The procedure used was the same as in the case of **12**. (4-Azido-3-iodophenyl)-butyric acid **11** (1.06 g; 3.21 mmol) was dissolved in 15 mL of dichloromethane. The solution was cooled to 0°C, DCC (0.34 g; 1.65 mmol) was added, and the mixture was stirred under dry N_2 for 2 hr. The insoluble N,N'-dicyclohexylurea was removed by filtration under N_2 and the solvent was evaporated under reduced pressure. The oily residue was dried over P_2O_5 under vacuum overnight. The anhydride was used as such for further reactions. IR ($CHCl_3$): 1745, 1815 cm^{-1}.

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3'-[(4-Azido-3-iodophenyl)-acetyl]-2',5'-dideoxyadenosine (14). 2',5'-Dideoxyadenosine **1** (300 mg; 1.275 mmol), Et_3N (0.195 mL (1.4 mmole), and DMAP (100 mg) were added together in 10 mL of acetonitrile. DMF (6 mL) was added to dissolve completely the 2',5'-dideoxyadenosine **1**. Compound **12** (1.059 mg; 1.8 mmole) dissolved in 10 mL of acetonitrile was added. The reaction mixture was then stirred at room temperature for 5 days and thereafter the solvent was removed at reduced pressure. Purification of the reaction mixture by flash chromatography gave **14** as an oil (330 mg; yield 49%). Rf: 0.45 (ethyl acetate:ethanol, 9:2). ^1H NMR (DMSO-d_6): δ 1.33(d, 3H, J=6.3 Hz), 2.49(m, 1H), 3.23(m, 1H), 3.76(s, 2H), 4.16(m, 1H), 5.20(s, 1H), 6.31(m, 1H), 7.35(m, 4H), 7.80(s, 1H), 8.16(s, 1H), 8.37(s, 1H). ^{13}C NMR: δ 170.4, 156.0, 152.5, 149.2, 140.3, 139.7, 139.5, 132.6, 130.9, 119.2, 118.8, 87.7, 83.2, 80.1, 78.5, 38.6, 35.1, 19.1. IR ($CHCl_3$): 3313, 3156, 2979, 2930, 2122, 1734, 1646, 1596, 1481, 1299 cm^{-1}. *UV* max (EtOH): 320 (sh), 300 (sh), 290 (sh), max 259, 231 nm. FAB-MS (m/z): 521 [$(M+H)^+$, 46%]. Peak matching [$(M+H)^+$] calcd 521.0547, found 521.0847.

3'-[(4-Azido-3-iodophenyl)-butyryl]-2',5'-dideoxyadenosine (15). The procedure used was the same as for the preparation of **14**, though the formation of **15** was 90% complete within two hours. The reaction was allowed to continue overnight.

Flash chromatography yielded **15** as an oil (yield 90%). Rf: 0.61 (ethyl acetate:ethanol, 9:2). ^{1}H NMR (DMSO-d_6): δ 1.34(d, J=6.6 Hz, 3H), 1.84(t, J=7.3 Hz, 2H), 2.36(t, J=7.3 Hz, 2H), 2.39(m, 1H), 2.59(t, J=7.3, 2H), 3.21(m, 1H), 4.12(m,1H), 4.12(m, 1H), 5.16(m, 1H), 6.29(t, J=6.6 Hz, 1H), 7.26(m, 4H), 7.7(s, 1H), 8.16(s, 1H), 8.38(s, 1H). ^{13}C NMR: δ 172.2, 155.9, 152.5, 149.2, 140.1, 139.5, 139.1, 138.7, 129.8, 119.2, 118.9, 88.0, 83.2, 80.2, 77.9, 35.1, 33.0, 32.7, 25.8, 19.1. *UV* max (EtOH): 300 (sh), 290 (sh), 258, maximum at 230 nm. IR ($CHCl_3$): 3314, 3174, 2974, 2433, 2119 (aryl azido group), 1731.9, 1619, 1575, 1483, 1301 cm^{-1}. FAB-MS (m/z): 549 [$(M+H)^+$, 35%]. Peak matching [$(M+H)^+$] calcd 549.0860, found 549.1629.

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