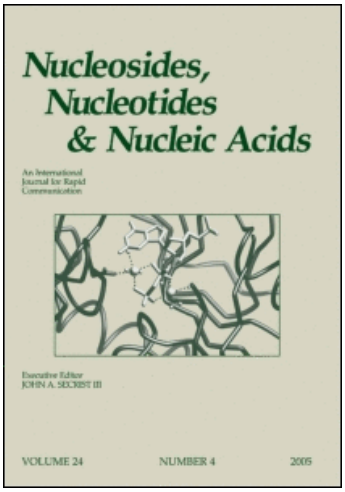

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Design, Synthesis, and Antiviral Evaluation of Some 3'-Carboxymethyl-3'-Deoxyadenosine Derivatives

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DESIGN, SYNTHESIS, AND ANTIVIRAL EVALUATION OF SOME 3′-CARBOXYMETHYL-3′-DEOXYADENOSINE DERIVATIVES

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Brenda R. McDougall and W. Edward Robinson, Jr. □ *Department of Pathology and Laboratory Medicine, University of California, Irvine, California, USA*

□ *3′-Carboxymethyl-3′-deoxyadenosine derivatives were prepared from 2′-O-TBDMS-3′-[(ethoxycarbonyl)methyl]-3′-deoxyadenosine (**1**) via simple and efficient procedures. Conversion of **1** to its 5′-azido-5′-deoxy derivative **5** was accomplished via a novel one-pot method employing 5′-activation (TosCl) followed by efficient nucleophilic displacement with tetramethylguanidinium azide. Compound **5** was converted to 5′-[(*N*-methylcarbamoyl)amino] derivative **8** via one-pot reduction/acylation employing H_2/Pd-C followed by treatment with* p*-nitrophenyl* N*-methylcarbamate.* N^6*-phenylcarbamoyl groups were introduced by treatment with phenylisocyanate, and an efficient new method for lactonization of 2′-O-TBDMS-3′-[(ethoxycarbonyl)methyl]-3′-deoxyadenosines to give corresponding 2′,3′-lactones was also developed. Target compounds were evaluated for anti-HIV and anti-HIV integrase activities, but were not active at the concentrations tested.*

Keywords HIV; HIV integrase; Antivirals; 3′-C-Branched nucleotides

INTRODUCTION

Branched chain 2′(3′)-deoxy- or 2′,3′-dideoxynucleosides have long been of interest due to their possession of unique biological activities relative to those of unbranched parent nucleosides.[1–7] Antiviral,[8–10] antitumor,[11–13] and/or antitubercular[14] activities for these compounds have been reported, and conformational effects induced by sugar branching have also been investigated.[15] Recently, we became interested by the possibility that 3′-carboxymethyl-3′-deoxyadenosine[4,7] derivatives could serve

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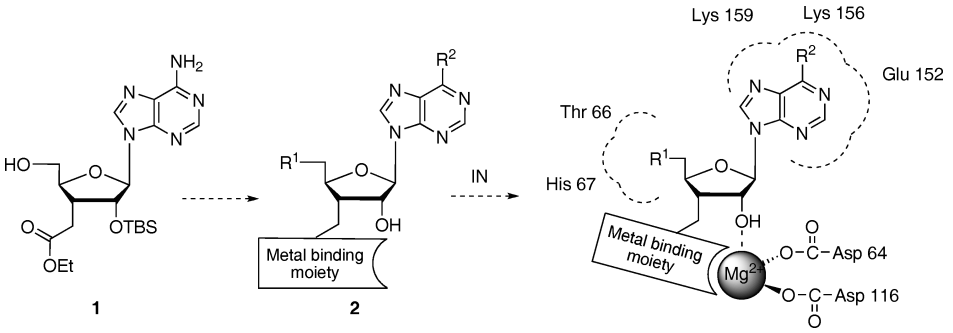


FIGURE 1

as ideal scaffolds on which to build inhibitors of HIV integrase (Figure 1). HIV integrase (IN) is one of three enzymes involved in HIV replication and is essential for covalently joining the viral DNA into the host genome. IN is a multimeric enzyme (at least tetrameric and probably octameric)[16] and belongs to a superfamily of polynucleotidyl phosphotransferases in which a metal dication (Mg^{2+} or Mn^{2+}) is required for catalysis.[17] The metal dication plays a critical role in both 3′-end processing of viral DNA and in strand transfer (Figure 2). In the 3′-end processing reaction, IN catalyzes cleavage of a GT dinucleotide from each 3′-end of the viral DNA via magnesium-mediated phosphodiester hydrolysis. In the subsequent strand transfer reaction, each free 3′-OH on the 3′-processed viral cDNA undergoes magnesium-mediated transesterification with host DNA, ultimately giving rise to fully integrated provirus (Figure 2). We reasoned that appropriately functionalized 3′-carboxymethyl-3′-deoxyadenosine derivatives might bind to the active-site Mg^{2+} and active-site amino acid residues, and, thus, potentially inhibit IN. Binding interactions between the 3′-terminal deoxyadenosine of viral DNA and IN had been indicated by ligand-docking calculations[18] and DNA photo-crosslinking experiments,[19] and these studies supported the notion that 3′-carboxymethyl-3′-deoxyadenosine derivatives **2** might bind to the active site and provide significant levels of IN inhibition. Molecular mechanics calculations (MMFF, MacSpartan Pro, Wavefunction, Inc., Irvine, CA, USA) had also indicated that 3′-carboxymethyl-3′-deoxyadenosine derivative **2a** coordinates with $Mg(OH)_4$ to give a complex with octahedral geometry (Figure 3). Carboxylates are known ligands[20] for Mg^{2+}, and hydroxide had been used as a solvent surrogate in related model complexes.[21]

The foregoing considerations suggested that 3′-carboxymethyl-3′-deoxyadenosine framework **2** (metal binding moiety = COO^-) might be a useful construct from which to conduct a structure-based lead discovery program. Accordingly, an approximately 49,000 member virtual library was screened in silico for derivatives **2** that exhibited tight binding against IN crystal structure 1BIU (Protein Data Bank). Crystal structure 1BIU is one

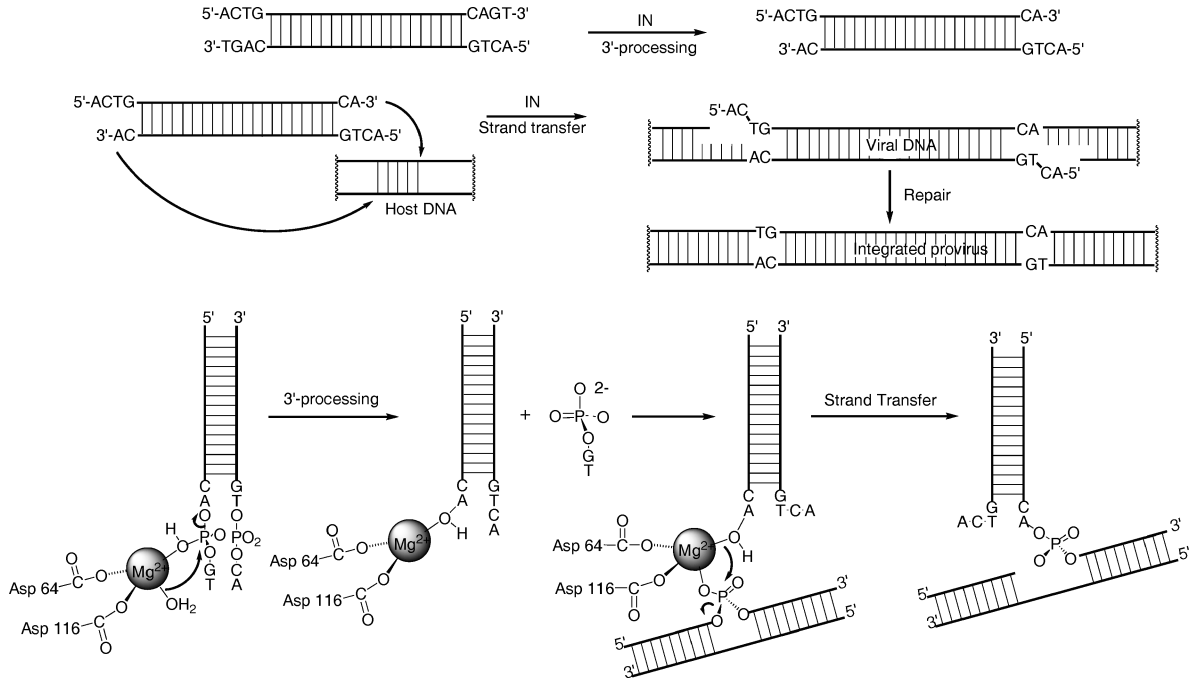


FIGURE 2

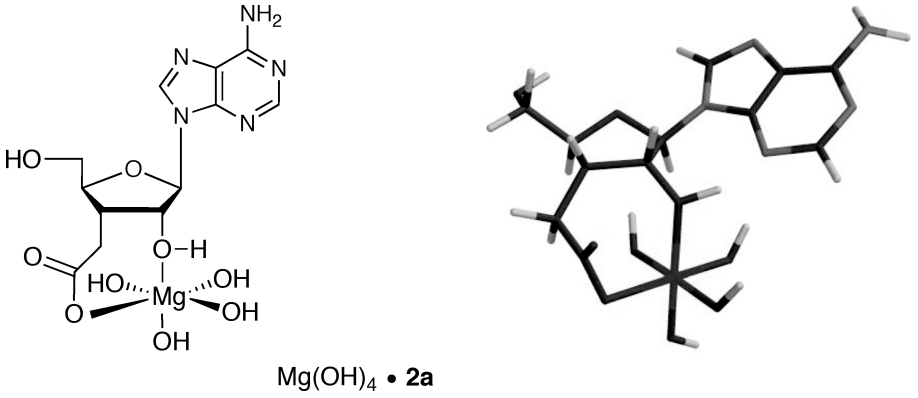


FIGURE 3

of only two crystal structures for IN catalytic core domains that contain an intact active-site Mg^{2+} ion.[22,23] A vast combinatorial library of compounds **2** (metal binding moiety = COO^-) varying at R^1 and R^2 (222 variations at each position generating a 222 × 222 combinatorial library) was docked against known active-site residues Asp 64, Thr 66, His 67, Asp 116, Glu 152, Lys 156, Lys 159, and the active-site Mg^{2+} ion. The lowest energy binding solution for each of the 49,000 compounds was determined (FlexX, Sybyl 6.9, Tripos, Inc., St. Louis, MO, USA), and the highest ranked (lowest FlexX score) ligand from the entire library was identified (Figure 4). Hydrogen bonding interactions for the top hit (**2b**) occurred between His 67 and the 5′-urea moiety, Thr 66 and adenine N3, Glu 152 and the C6 urea, and between the 2′-OH and the carbonyl oxygen of Asp 64. The 3′-carboxymethyl group coordinated with Mg^{2+} in an equatorial relationship relative to the 2′-OH, in harmony with the calculated geometry for the model complex $Mg(OH)_4$·**2a** (Figure 3). His 67 underwent a σ-π interaction with the 5′-urea, and Asn 155 underwent a similar interaction with the adenine heterocycle.

RESULTS AND DISCUSSION

In order to establish the validity of the binding interactions indicated by the virtual library screening, we prepared compounds **2b–h** (Figure 5). Compounds **2c–h** were designed to probe the effects of various groups on binding activity (structure activity relationship), and **2b** had been selected as the top hit from the virtual library. Compounds **2b–d** shared in common the 5′-*N*-methyl and 6-*N*-phenyl urea moieties postulated to bind with His 67/Lys 159 and Glu 152, respectively. The 5′-azido group had been identified as a lower ranking 5′-substituent from the library docking calculations, and **2e** and **2f** were readily derived from intermediates needed to prepare 5′-*N*-methylurea derivatives **2b–d**. Compounds **2g** and **2h** were included to

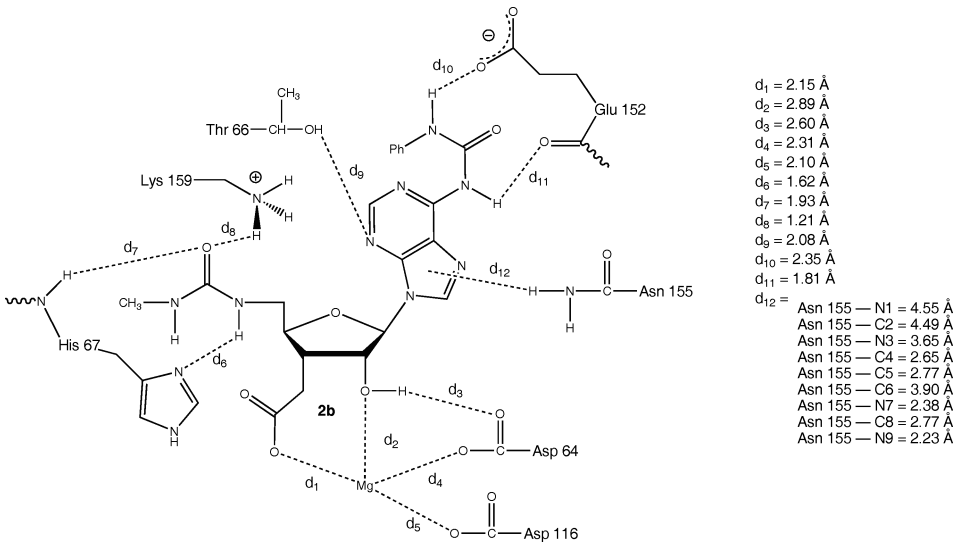
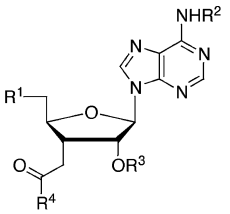
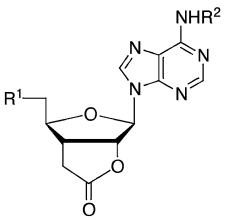


FIGURE 4

test the possibility that lactones might undergo IN-catalyzed saponification to generate carboxylates **2b** and **2e** in the active site of IN.

The synthesis began with compound **1** which was readily prepared[24] in four steps from 2′,5′-bis-*O*-TBDMS adenosine (Scheme 1). Attempts to prepare 5′-chloro-5′-deoxyadenosine derivative **3** using standard chlorination conditions ($SOCl_2$/Pyr/CH_2Cl_2) gave desired product in low yields (30–40%). Significant amounts of an unisolated polar byproduct (baseline by TLC) were formed, which we assume derived from the N^3,5′-cyclonucleoside salt.[25] Treatment of **1** with TsCl/DMAP[26] in ice-cold CH_2Cl_2 gave compound **4** in excellent isolated yields (72–90%). Conversion of compounds **3** (**4**) to 5′-azido-5′-deoxyadenosine derivative **5** using standard conditions (NaN_3/DMF)[27] was complicated by decomposition, and yields for the desired product ranged from 20–40%. Since it has





2a R^1 = OH; R^2 = H; R^3 = H; R^4 = OH
2b R^1 = $CH_3NHCONH$; R^2 = $CONHC_6H_5$; R^3 = H; R^4 = ONa
2c R^1 = $CH_3NHCONH$; R^2 = $CONHC_6H_5$; R^3 = TBDMS; R^4 = OEt
2d R^1 = $CH_3NHCONH$; R^2 = $CONHC_6H_5$; R^3 = TBDMS; R^4 = $NCH_3(OCH_3)$
2e R^1 = N_3; R^2 = $CONHC_6H_5$; R^3 = H; R^4 = ONa
2f R^1 = N_3; R^2 = H; R^3 = H; R^4 = OH

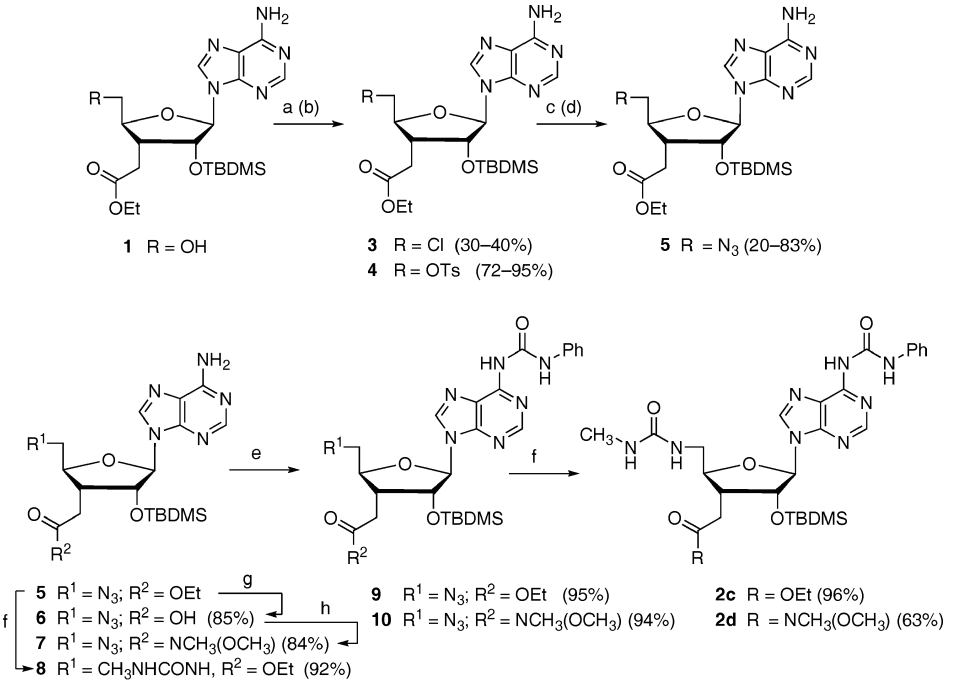
2g R^1 = N_3; R^2 = $CONHC_6H_5$
2h R^1 = $CH_3NHCONH$; R^2 = $CONHC_6H$

FIGURE 5

long been recognized that *intramolecular* alkylation of N3 competes with *intermolecular* nucleophilic substitution of 5′-activated adenosine derivatives to give cyclonucleosides[28] and derived rearrangement products,[29] we reasoned that yields for the desired intermolecular substitution reaction might be enhanced if higher concentrations of the poorly soluble azide nucleophile could be achieved. This approach represented a fundamental departure from the more generally employed strategies for suppressing cyclonucleoside formation which typically involve reducing the nucleophilicity of N3 via N6 acylation[27,28] or disfavoring the required *syn* conformation by employing sterically encumbered N6 protection.[30] While these latter strategies had proven successful in a number of previous cases, we wished to avoid any protection/deprotection steps that might unnecessarily lengthen the synthesis. Accordingly, we undertook an optimization study and were ultimately delighted to find that treatment of crude **4** with 7 equiv. of tetramethylguanidinium azide (TMGA; $[(Me_2N)_2CNH_2]N_3$) in DMF (65°C) gave **5** in 83% yield from compound **1**. The relatively high solubility of TMGA in polar aprotic organic solvents has been exploited for high-yield preparations of amino acids and other target compounds. To the best of our knowledge, the present report represents the first application of TMGA to problems involving adenosine cyclonucleoside formation. This new strategy may provide a generally useful alternative to two-step N6-protection/deprotection strategies more commonly employed for suppressing N^3,5′-cyclonucleoside formation. [Caution: TMGA is known to form explosive byproducts in halogenated solvents—e.g., diazidomethane from dichloromethane—thus, halogenated solvents should be avoided.][31]

Compound **5** was saponified to give compound **6** (85%). Introduction of the metal-coordinating *N*-methoxy-*N*-methylcarboxamide group was accomplished by treating **6** with carbonyldiimidazole and *N,O*-dimethylhydroxylamine to give **7** (84%). Hydrogenation of **5** (H_2/Pd–C/EtOH) followed by treatment of the resulting 5′-amino-5′-deoxyadenosine intermediate with *p*-nitrophenyl *N*-methylcarbamate gave compound **8** (92%). Conversion of **5** (**7**) to **9** (**10**) was accomplished by treatment of **5** (**7**) with phenylisocyanate in CH_2Cl_2 (95 and 94%, respectively). One-pot reduction/acylation of **9** (**10**) using the same conditions employed for **5** → **8** gave targets **2c** and **2d** (96 and 63%, respectively). Our recently reported high-yield synthesis of *p*-nitrophenyl *N*-methylcarbamate[32] makes this method an attractive alternative to reported methods for introducing the 5′-(*N*-methylcarbamoyl) group which employ highly toxic methylisocyanate as the acylating reagent.[33]

Conversion of 3′-carboxymethyl-3′-deoxy derivatives **1**, **2c**, **5**, **8**, and **9** to the corresponding 2′,3′-lactones using conditions we had previously found effective for preparing compound **11** and a related uridine-derived 2′,3′-lactone (TBAF/THF),[24] gave products that were very difficult to

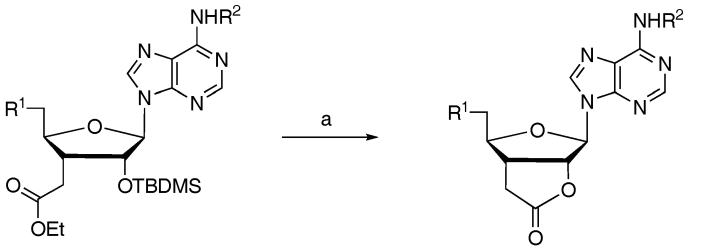


Reagents: (a) $SOCl_2/Pyr/CH_2Cl_2$. (b) TsCl/DMAP/CH_2Cl_2. (c) NaN_3/DMF. (d) TMGA/DMF. (e) $PhNCO/CH_2Cl_2$. (f) i. H_2/Pd-C/EtOAc, ii. *p*-NO_2-$C_6H_4OCONHCH_3/Na_2CO_3$. (g) $NaOH/H_2O$/MeOH/THF. (h) Carbonyldiimidazole/ $CH_3NHOCH_3 \cdot HCl/Et_3N$

SCHEME 1

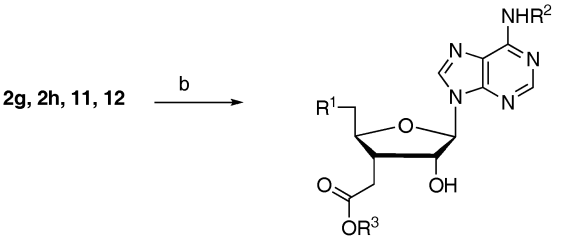
purify due to co-elution of tetrabutylammonium salts with the desired target compounds (Scheme 2). Multiple column chromatographies employing careful gradient elutions were required to obtain 2′,3′-lactone derivatives that were free from contaminating tetrabutylammonium impurities. An evaluation of alternative desilylating reagents ultimately revealed that a phase transfer catalyzed biphasic mixture consisting of $KF/PhCH_2N(Et)_3Cl/CH_3CN/H_2O$ gave clean conversion to the 2′,3′-lactone products, which could be purified via a single pass through a silica gel column using an appropriate eluting solvent. Treatment of compounds **1**, **2c**, **5**, **8**, and **9** with $KF/PhCH_2N(Et)_3Cl/CH_3CN/H_2O$ gave **11–13** and **2g** (**2h**) in excellent yields (79–92%). Saponification of 2′,3′-lactones **2g**, **2h**, **11**, and **12** gave compounds **2a**, **2b**, **2e**, and **2f** (81–100%).

With compounds **2b–h** in hand, we next turned our attention to an evaluation of their anti-HIV and IN inhibitory activities (Table 1). Promising activities were not exhibited by any of the compounds tested. The failure of these compounds to exhibit IN inhibitory activities may derive from their possible affinity for binding sites remote from the active-site, or may



1 $R^1 = OH$; $R^2 = H$
5 $R^1 = N_3$; $R^2 = H$
8 $R^1 = CH_3NHCONH$; $R^2 = H$
9 $R^1 = N_3$; $R^2 = CONHC_6H_5$
2c $R^1 = CH_3NHCONH$; $R^2 = CONHC_6H_5$

11 $R^1 = OH$; $R^2 = H$ (80%)
12 $R^1 = N_3$; $R^2 = H$ (81%)
13 $R^1 = CH_3NHCONH$; $R^2 = H$ (79%)
2g $R^1 = N_3$; $R^2 = CONHC_6H_5$ (86%)
2h $R^1 = CH_3NHCONH$; $R^2 = CONHC_6H_5$ (92%)



2a $R^1 = OH$; $R^2 = R^3 = H$ (81%)
2b $R^1 = CH_3NHCONH$; $R^2 = CONHC_6H_5$; R^3 = Na (quant)
2e $R^1 = N_3$; $R^2 = CONHC_6H_5$; R^3 = Na (quant)
2f $R^1 = N_3$; $R^2 = R^3 = H$ (85%)

Reagents: (a) $KF/PhCH_2N(Et)_3Cl/CH_3CN/H_2O$. (b) $NaOH/H_2O/DMSO(MeOH/THF)$.

SCHEME 2

also possibly reflect weaknesses inherent in the incremental construction algorithm forming the basis of the FlexX ligand docking calculations.[34,35] Entropic and enthalpic contributions of dissociating water ligands from the active site Mg^{2+} are not taken into consideration by the FlexX algorithm, and ligand conformational energy terms are not included in the FlexX scoring function.[34] Thus, whereas the docking experiments point to favorable binding interactions for compound **2b** (Figure 4), free energy contributions from the dissociation of water ligands and/or from the conformation of the inhibitor may actually disfavor binding as indicated by the docking. In addition, the underlying success of FlexX docking experiments strongly depends upon initial placement of base fragments that serve as anchors for iterative incremental construction and docking of increasingly more complex ligand fragments.[34] Incorrect selection or placement of the base fragment can skew ensuing iterative placements of the more complex fragments generated during the incremental construction, and heavy weighting of charged ligand/receptor interactions has been shown to give "false positives" in some instances.[36] It is also true that no

TABLE 1 Activities of Test Compounds in Biochemical Assays

Compd	ED_{50}[a] (μM)	CT_{50}[b] (μM)	CT_5[c] (μM)	IC_{50}[d] (μM)	
				EP[e]	ST[f]
2b	>98	385	143	>10	>10
2c	>13	37.8	6.2	>10	>10
2d	>17	22.6	11.3	>10	>10
2e	>149	812	162	>10	>10
2f	>62	175	21	>10	>10
2g	>19	21.9	9.3	>10	>10
2h	>34	58.5	23.2	>10	>10

[a]Inhibitory concentration required to protect MT-2 cells from 50% viral induced cell death.
[b]Cytotoxic concentration required to inhibit cell growth by 50%.
[c]Cytotoxic concentration required to inhibit cell growth by 5%.
[d]Inhibitory concentration required to inhibit IN 3′-end processing (EP) or strand transfer (ST) by 50%.
[e]3′-End processing.
[f]Strand transfer.

full-length IN structure and no structure of an IN-DNA substrate complex have been reported; thus, our lack of success in identifying potent lead IN inhibitors may be due to insufficient structural information. Coordination of a second active-site Mg^{2+} cation by the third member of the catalytic triad (Glu 152) has been invoked as being critical for the mechanism of full-length integrase enzymes.[37] Such coordination was not present in the two-domain X-ray structure used in this study (1BIU). Furthermore, Glu 152 resides in what has been shown to be a flexible loop in the IN primary structure;[38] thus, it is possible that the position of Glu 152 in crystal structure 1BIU does not accurately reflect its position in solution. X-ray data for partial IN structures such as 1BIU suggest that for the full-length enzyme the active site may be relatively solvent accessible and could thus potentially possess a local dielectric constant >4. Theoretical calculations of the free energies for dissociating water ligands from Mg^{2+} aqua complexes support an inverse relationship between the polarity of the local environment and the favorability of the displacement of a water ligand by a charged carboxylate (threshold value $\varepsilon \leq 4$).[20] If the local dielectric constant of IN is substantially > 4, the free energies for binding carboxylate ligands to the active-site Mg^{2+} of IN may be unfavorable.[20]

CONCLUSION

We have prepared a small library of compounds designed to test the hypothesis that appropriately derivatized 3′-carboxymethyl-3′-deoxyadenosine derivatives might bind to the active-site Mg^{2+} and active-site amino acid residues of IN and thus competitively inhibit the enzyme. Although biological activities were not promising, several important results derive

from the synthetic effort: (1) TMGA-promoted nucleophilic substitution of 5′-*O*-*p*-toluenesulfonyl adenosine derivative **4** gave high yields of 5′-azido-5′-deoxyadenosine derivative **5**, thus demonstrating a potentially general alternative to two-step N6-protection/deprotection strategies currently used for high yield preparations of 5′-azido-5′-deoxyadenosine derivatives from corresponding 5′-activated adenosine precursors; (2) the biphasic reagent/solvent system $KF/PhCH_2N(Et)_3Cl/CH_3CN/H_2O$ gives enhanced yields of adenosine 2′,3′-lactone nucleosides with greatly simplified work-up procedures relative to previously reported conditions (TBAF/THF; painstaking chromatography); and (3) conversion of 5′-azido-5′-deoxyadenosine derivatives **5**, **9** and **10** to *N*-methylurea derivatives **8**, **2c**, and **2d** (respectively) was achieved via an efficient one-pot reduction/acylation procedure employing *p*-nitrophenyl *N*-methylcarbamate as a safer alternative to methylisocyanate.

EXPERIMENTAL

Moisture sensitive reactions were performed in flame-dried flasks under an inert atmosphere (N_2 or Ar) at ambient temperature unless indicated otherwise. Solvents (CH_2Cl_2, pyridine, EtOAc, DMF, Et_3N) were dried by passing through columns of activated alumina under Ar. TLC was performed using Merck Kieselgel 60 F_{254} sheets, and Flash[39] chromatography was performed with silica gel (230–400 mesh) and reagent grade solvents. "Solvent A" for chromatography consisted of the separated organic phase of EtOAc/*i*-PrOH/H_2O (4:1:2). UV spectra were determined with solutions in MeOH or H_2O. ^1H NMR spectra were determined using internal references at δ 7.27 ($CDCl_3$), and 2.50 (DMSO-d_6), and ^{13}C NMR spectra were measured using internal references at δ 77.3 ($CDCl_3$), and 39.5 (DMSO-d_6). High resolution mass spectra were obtained using fast atom bombardment (FAB, NaOAc/thioglycerol or thioglycerol matrix) or electrospray (ES) ionization techniques. Commercially available reagents were used as supplied, and tetramethylguanidinium azide[40] and compound **1**[24] were prepared as previously reported.

2′-*O*-(*tert*-Butyldimethylsilyl)-5′-chloro-3′,5′-dideoxy-3′-[(ethoxycarbonyl) methyl]adenosine (3). To a stirred solution of **1** (200 mg, 0.443 mmol) and pyridine (100 mg, 1.27 mmol) in CH_2Cl_2 (3.0 mL) at 0°C was added thionyl chloride (2 M in CH_2Cl_2, 1.0 mL, 2.0 mmol). The mixture was stirred for 30 minutes, then allowed to warm to room temperature and stirred overnight. Volatiles were removed under reduced pressure and the residue was partitioned (EtOAc//$NaHCO_3$(aq)). The organic layer was dried (Na_2SO_4), filtered, and volatiles were removed under reduced pressure. Chromatography (5% MeOH/CH_2Cl_2) gave **3** (62 mg, 30%): UV (MeOH) λ max 260 nm, λ min 230 nm; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.35

(s, 1H), 8.18 (s, 1H), 5.97 (s, 1H), 5.59 (br s, 2H), 4.94 (d, $J = 4.5$ Hz, 1H), 4.37–4.34 (m, 1H), 4.12 (q, $J = 7.4$ Hz, 2H), 4.01 (dd, $J = 3.0$, 12.5 Hz, 1H), 3.78 (dd, $J = 4.3$, 12.8 Hz, 1H), 2.85–2.82 (m, 1H), 2.70 (dd, $J = 9.0$, 17.0 Hz, 1H), 2.42 (dd, $J = 5.8$, 16.8 Hz, 1H), 1.26 (t, $J = 7.3$ Hz, 3 H), 0.90 (s, 9 H), 0.15 (s, 3 H), 0.07 (s, 3 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.9, 155.8, 153.2, 138.2, 120.4, 91.3, 82.9, 77.5, 61.1, 45.2, 40.7, 30.1, 25.9, 18.1, 14.3, −4.4, −5.4; MS (FAB) m/z 492.1805 (MNa^+ [$C_{20}H_{32}{}^{35}ClN_5O_4SiNa$] = 492.1810).

2′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-3′-[(ethoxycarbonyl)methyl]-5′-*O*-(*p*-toluenesulfonyl)adenosine (4). To a chilled (0°C) flame-dried flask containing 1 (378 mg, 0.837 mmol; azeotropically dried via evaporation of benzene, 5 × 20 mL), *p*-toluenesulfonylchloride (278 mg, 1.46 mmol), and DMAP (218 mg, 1.78 mmol) was added ice-cold CH_2Cl_2 (4.0 mL). The solution was stirred for 24 hours at 0°C, then applied directly to a chromatography column and eluted (80% EtOAc/hexanes → EtOAc). Appropriate fractions were pooled and volatiles were removed under reduced pressure (≤20°C) to give **4** (390 mg, 77%). Compound **4** was not stable at ambient temperature and underwent decomposition upon standing either in solution or as a solid amorphous glass. Characterization was therefore accomplished immediately following isolation, and maximum purities obtained in this way were approximately 90%. Unambiguous characterization by ^{13}C NMR was complicated by compound instability: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.30 (s, 1H), 7.95 (s, 1H), 7.77–7.75 (m, 2H), 7.29–7.28 (m, 2H), 5.91 (d, $J = 1.0$ Hz, 1H), 5.56 (br s, 2H), 4.85 (d, $J = 4.0$ Hz, 1H), 4.37 (dd, $J = 2.0$, 8.5 Hz, 1H), 4.27–4.20 (m, 2H), 4.11 (q, $J = 7.2$ Hz, 2H), 2.82–2.76 (m, 1H), 2.64 (dd, $J = 8.8$, 16.8 Hz, 1H), 2.42 (s, 3 H), 2.32 (dd, $J = 5.5$, 17.0 Hz, 1H), 1.19 (t, $J = 7.2$ Hz, 3H), 0.89 (s, 9 H), 0.14 (s, 3 H), 0.03 (s, 3 H); MS (FAB) *m/z* 606.2417 (MH^+ [$C_{27}H_{40}N_5O_7SSi$] = 606.2418).

5′-Azido-2′-*O*-(*tert*-butyldimethylsilyl)-3′,5′-dideoxy-3′-[(ethoxycarbonyl) methyl]adenosine (5). To a chilled (0°C) flame-dried flask containing **1** (360 mg, 0.797 mmol; azeotropically dried via evaporation of benzene, 5 × 20 mL), *p*-toluenesulfonylchloride (208 mg, 1.10 mmol), and DMAP (208 mg, 1.70 mmol) was added ice-cold CH_2Cl_2 (16 mL). The solution was stirred for 24 hours at 0°C, after which volatiles were removed under reduced pressure (≤20°C). Tetramethylguanidinium azide (880 mg, 5.56 mmol) and DMF (4 mL) were *immediately* added and the solution was heated at 65°C for 7 hours. The mixture was cooled to ambient temperature and then vigorously stirred while anhydrous Et_2O (100 mL) was slowly added. Precipitated TMGA was removed by filtering through celite. The white solid mass was triturated, and the filter cake was washed with anhydrous Et_2O to ensure complete transfer of product. Volatiles were removed under reduced pressure (40°C) and the residue chromatographed (90% EtOAc/hexanes → EtOAc) to give **5** (315 mg, 83%): UV (MeOH) λmax 262 nm, λmin

233 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.36 (s, 1H), 8.16 (s, 1H), 5.98 (s, 1H), 5.54 (br s, 2H), 4.86 (d, J = 5.0 Hz, 1H), 4.22–4.20 (m, 1H), 4.14 (q, J = 7.0 Hz, 2H), 3.78 (dd, J = 3.3, 13.8 Hz, 1H), 3.61 (dd, J = 4.8, 13.8 Hz, 1H), 2.85–2.77 (m, 1H), 2.69 (dd, J = 8.3, 16.8 Hz, 1H), 2.37 (dd, J = 5.8, 16.8 Hz, 1H), 1.26 (t, J = 7.3 Hz, 3 H), 0.91 (s, 9 H), 0.17 (s, 3 H), 0.07 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.6, 155.4, 153.0, 149.4, 138.7, 120.2, 91.1, 82.2, 77.3, 60.9, 52.2, 40.0, 29.9, 25.7, 17.9, 14.1, −4.5, −5.5; MS (FAB) m/z 499.2214 (MNa^+ [$C_{20}H_{32}N_8O_4SiNa$] = 499.2214).

5′-Azido-2′-*O*-(*tert*-butyldimethylsilyl)-3′-(carboxymethyl)-3′,5′-dideoxyadenosine (6). To a stirred solution of **5** (150 mg, 0.315 mmol) in THF (2 mL) was added NaOH (200 μL, 5.0 M, 1.0 mmol), and MeOH (400 μL). The mixture was stirred at ambient temperature until starting material had been converted to baseline product (6 hours, TLC). Volatiles were removed under reduced pressure (≤20°C) and the crude material was partitioned (CH_2Cl_2//H_2O). Ice was added and the pH was carefully adjusted to ≈3 via dropwise addition of 1% HCl (aq). The aqueous layer was washed (CH_2Cl_2, 5X) until the organic layer was UV transparent (TLC). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated under reduced pressure (≤20°C) to give **6** (120 mg, 85%): UV (MeOH) λmax 260 nm, λmin 233 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.32 (s, 1H), 8.25 (s, 1H), 7.27 (br s, 2H), 6.02 (s, 1H), 4.76 (d, J = 4.0 Hz, 1H), 4.25 (dd, J = 6.5, 10.5 Hz, 1H), 3.86 (d, J = 13.0 Hz, 1H), 3.63 (dd, J = 3.5, 13.5 Hz, 1H), 2.83–2.80 (m, 1H), 2.71 (dd, J = 8.5, 17.0 Hz, 1H), 2.42 (dd, J = 4.8, 17.3 Hz, 1H), 0.93 (s, 9H), 0.21 (s, 3H), 0.10 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.1, 155.4, 151.8, 148.9, 138.8, 118.9, 91.1, 82.5, 77.9, 51.9, 39.8, 30.2, 29.7, 25.7, 18.0, −4.5, −5.5; MS (FAB) m/z 471.1902 (MNa^+ [$C_{18}H_{28}N_8O_4SiNa$] = 471.1901).

5′-Azido-2′-*O*-(*tert*-butyldimethylsilyl)-3′,5′-dideoxy-3′-[(*N*-methoxy-*N*-methylcarboxamido)methyl]adenosine (7). To a stirred solution of **6** (50 mg, 0.112 mmol) in CH_2Cl_2 (1.0 mL) at 0°C was added carbonyl diimidazole (500 μL of 0.36 M solution in CH_2Cl_2, 29 mg, 0.18 mol). The ice-bath was removed and the reaction was allowed to warm to ambient temperature for 1 hour. *N*,*O*-Dimethylhydroxylamine hydrochloride (18 mg, 0.19 mmol), and Et_3N (82 mg, 0.82 mmol), were added and the reaction was followed by TLC (24 hours). Chromatography (5%MeOH/EtOAc) gave **7** (46 mg, 84%): UV (MeOH) λmax 260 nm, λmin 230 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.35 (s, 1H), 8.16 (s, 1H), 5.99 (d, J = 2.0 Hz, 1H), 5.67 (br s, 2H), 4.87–4.86 (m, 1H), 4.25–4.22 (m, 1H), 3.77 (dd, J = 2.8, 13.3 Hz, 1H), 3.70 (s, 3 H), 3.65 (dd, J = 4.5, 13.5 Hz, 1H), 3.16 (s, 3 H), 2.85–2.83 (m, 2H), 2.60–2.52 (m, 1H), 0.90 (s, 9 H), 0.11 (s, 3 H), 0.02 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.6, 155.7, 153.2, 149.8, 138.8, 120.3, 91.0, 82.9, 77.8, 61.5, 53.0, 39.9, 32.5, 28.4, 26.0, 18.2, −4.40, −5.10; MS (FAB) m/z 514.2327 (MNa^+ [$C_{20}H_{33}N_9O_4SiNa$] = 514.2323).

2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-dideoxy-3′-[(ethoxycarbonyl)methyl]-5′-[(*N*-methylcarbamoyl)amino]adenosine (8). A solution of **5** (613 mg, 1.29 mmol) and 10% Pd–C (220 mg) in EtOAc (11 mL) was vigorously stirred overnight under an atmosphere of H_2 (balloon pressures). *p*-Nitrophenyl *N*-methylcarbamate (440 mg, 2.24 mmol) and anhydrous Na_2CO_3 (440 mg, 4.15 mmol) were added, and the resulting mixture was stirred for 5 hours under N_2. Solids were filtered (celite), the filter cake washed with EtOAc, and volatiles were evaporated under reduced pressure. Chromatography (10% MeOH/CH_2Cl_2) gave **8** (600 mg, 92%): UV (MeOH) λmax 260 nm, λmin 229 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.37 (s, 1H), 7.88 (s, 1H), 6.02 (br s, 1H), 5.78 (d, J = 4.0 Hz, 1H), 5.57 (br s, 2H), 4.95–4.93 (m, 1H), 4.51–4.38 (m, 1H), 4.24–4.22 (m, 1H), 4.15 (q, J = 7.2 Hz, 2H), 3.71–3.66 (m, 1H), 3.49 (dd, J = 4.0, 15.0 Hz, 1H), 2.84–2.80 (m, 1H), 2.80 (d, J = 5.0 Hz, 3 H), 2.69 (dd, J = 6.8, 17.3 Hz, 1H), 2.49 (dd, J = 6.8, 17.3 Hz, 1H), 1.28 (t, J = 7.0 Hz, 3 H), 0.84 (s, 9 H), −0.07 (s, 3 H), −0.14 (s, 3 H); ^{13} C NMR ($CDCl_3$, 125 MHz) δ 172.1, 159.5, 155.8, 152.8, 149.2, 139.4, 120.4, 91.4, 83.7, 76.2, 60.7, 41.9, 39.7, 30.4, 27.1, 25.6, 17.8, 14.1, −4.80, −5.40; MS (ES) *m/z* 508.2699 (MH^+ [$C_{22}H_{38}N_7O_5Si$] = 508.2698).

5′-Azido-2′-*O*-(*tert*-butyldimethylsilyl)-3′,5′-dideoxy-3′-[(ethoxycarbonyl) methyl]-N^6-(*N*-phenylcarbamoyl)adenosine (9). To a stirred solution of **5** (633 mg, 1.33 mmol) in CH_2Cl_2 (16 mL) was added phenylisocyanate (190 mg, 1.60 mmol). The mixture was stirred at ambient temperature until TLC indicated complete conversion of **5** to desired product (5 days). The mixture was added directly to a chromatography column and eluted (10 → 40% EtOAc/hexanes) to give **9** (755 mg, 95%): UV (MeOH) λmax 279 nm, λmin 243 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.74 (s, 1H), 8.62 (s, 1H), 8.39 (s, 1H), 8.11 (s, 1H), 7.65 (d, J = 8.5 Hz, 2H), 7.39–7.36 (m, 2H), 7.14–7.12 (m, 1H), 6.04 (s, 1H), 4.86 (d, J = 5.0 Hz, 1H), 4.24–4.22 (m, 1H), 4.14 (q, J = 7.2 Hz, 2H), 3.81 (dd, J = 2.8, 13.3 Hz, 1H), 3.63 (dd, J = 4.3, 13.3 Hz, 1H), 2.81–2.79 (m, 1H), 2.69 (dd, J = 8.5, 17.0 Hz, 1H), 2.39 (dd, J = 5.3, 17.3 Hz, 1H), 1.26 (t, J = 7.3 Hz, 3 H), 0.93 (s, 9 H), 0.19 (s, 3 H), 0.07 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.5, 151.4, 150.8, 150.0, 149.9, 141.5, 138.1, 129.0, 123.8, 120.2, 91.3, 82.5, 77.5, 60.9, 52.2, 40.1, 29.7, 25.7, 18.0, 14.1, −4.5, −5.5; MS (FAB) *m/z* 596.2772 (MH^+ [$C_{27}H_{38}N_9O_5Si$] = 596.2765).

5′-Azido-2′-*O*-(*tert*-butyldimethylsilyl)-3′,5′-dideoxy-3′-[(*N*-methoxy-*N*-methylcarboxamido)methyl]-N^6-(*N*-phenylcarbamoyl)adenosine (10). To a solution of **7** (46 mg, 0.094 mmol) in CH_2Cl_2 (1.0 mL) was added phenylisocyanate (12 mg, 0.10 mmol). The mixture was stirred at ambient temperature until TLC indicated complete conversion of **7** to desired product (7 days). The mixture was added directly to a chromatography column and eluted (80% EtOAc/hexanes → EtOAc) to give **10** (54 mg, 94%): UV (MeOH) λmax 279 nm, λmin 242 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.77 (s, 1H), 8.63 (s, 1H), 8.40 (s, 1H), 8.13 (s, 1H), 7.66 (d,

J = 8.0 Hz, 2H), 7.40–7.37 (m, 2H), 7.15–7.11 (m, 1H), 6.05 (s, 1H), 4.88 (m, 1H), 4.28–4.26 (m, 1H), 3.82 (d, J = 10.5 Hz, 1H), 3.71–3.66 (m, 1H), 3.70 (s, 3 H), 3.17 (s, 3 H), 2.86–2.53 (m, 2H), 2.56–2.53 (m, 1H), 0.90 (s, 9 H), 0.15 (s, 3 H), 0.08 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.1, 151.2, 150.8, 149.9, 141.1, 138.0, 129.0, 123.8, 120.8, 120.3, 91.0, 82.8, 77.7, 61.2, 52.5, 39.6, 32.2, 29.7, 27.9, 25.7, 17.9, −4.6, −5.4; MS (ES) m/z 633.2695 (MNa^+ [$C_{27}H_{38}N_{10}O_5SiNa$] = 633.2694).

3′-(Carboxymethyl)-3′-deoxyadenosine-2′,3′-lactone (11). To a stirred solution of **1** (50 mg, 0.11 mmol) in CH_3CN (1.0 mL) were added $PhCH_2N(Et)_3Cl$ (5 mg, 0.022 mmol), KF (15 mg, 0.26 mmol), and H_2O (40 μL). The mixture was vigorously stirred at ambient temperature until TLC indicated that **1** had been consumed (42 hours). Silica gel was added and volatiles were evaporated under reduced pressure (≤20°C). The dried silica gel was poured onto the top of a chromatography column packed with CH_2Cl_2 and eluted (5 → 10% MeOH/CH_2Cl_2). Evaporation of pooled fractions gave **11** (26 mg, 80%). ^{1}H and ^{13}C NMR and UV data agreed with reported values.[24]

5′-Azido-3′-(carboxymethyl)-3′,5′-dideoxyadenosine-2′,3′-lactone (12). To a stirred solution of **5** (50 mg, 0.105 mmol) in CH_3CN (1.0 mL) were added $PhCH_2N(Et)_3Cl$ (5 mg, 0.022 mmol), KF (15 mg, 0.26 mmol), and H_2O (80 μL). The mixture was vigorously stirred at ambient temperature until TLC indicated that **5** had been consumed (72 hours). Silica gel was added and volatiles were evaporated under reduced pressure (≤20°C). The dried silica gel was poured onto the top of a chromatography column packed with CH_2Cl_2 and eluted (2.5 → 10% MeOH/CH_2Cl_2). Evaporation of pooled fractions gave **12** (27 mg, 81%): UV (MeOH) λmax 259 nm, λmin 236 nm; ^{1}H ($CDCl_3$, 500 MHz,) δ 8.35 (s, 1H), 7.91 (s, 1H), 6.17 (s, 1H), 5.61 (dd, J = 1.0, 6.5 Hz, 1H), 5.54 (br s, 2H), 4.14–4.10 (m, 1H), 3.82–3.79 (m, 1H), 3.61 (dd, J = 4.8, 12.8 Hz, 1H), 3.55 (dd, J = 5.5, 13.0 Hz, 1H), 2.96 (dd, J = 8.8, 18.3 Hz, 1H), 2.55 (dd, J = 1.0, 18.0 Hz, 1H); ^{13} C NMR (DMSO-d_6, 125 MHz) δ 175.6, 156.2, 152.9, 148.8, 139.9, 119.1, 88.0, 86.6, 84.1, 51.8, 40.8, 31.6; MS (ES) m/z 317.1110 (MH^+ [$C_{12}H_{13}N_8O_3$] = 317.1111).

3′-(Carboxymethyl)-3′,5′-dideoxy-5′-[(*N*-methylcarbamoyl)amino]adenosine-2′,3′-lactone (13). To a stirred solution of **8** (26 mg, 0.051 mmol) in CH_3CN (1.0 mL) were added $PhCH_2N(Et)_3Cl$ (30 mg, 0.13 mmol), KF (15 mg, 0.26 mmol), and H_2O (80 μL). The mixture was vigorously stirred at ambient temperature until TLC indicated that **8** had been consumed (9 hours). The reaction mixture was added directly to a column and chromatographed (solvent A) to give **13** (14 mg, 79%): UV (MeOH) λmax 260 nm, λmin 239 nm; ^{1}H (DMSO-d_6, 500 MHz) δ 8.32 (s, 1H), 8.17 (s, 1H), 7.34 (br s, 2H), 6.24 (d, J = 1.5 Hz, 1H), 6.07 (t, J = 5.8 Hz, 1H), 5.78 (q, J = 4.7 Hz, 1H), 5.51 (dd, J = 2.3, 7.3 Hz, 1H), 3.97–3.93 (m, 1H), 3.28–3.24 (m, 1H), 2.94 (dd, J = 8.5, 18.0 Hz, 1H), 2.53 (d, J = 5.0 Hz, 3 H), 2.51–2.46 (m, 2H); ^{13} C NMR (DMSO-d_6, 125 MHz) δ 175.7, 158.6,

156.1, 152.8, 148.8, 139.6, 119.1, 87.8, 86.8, 84.5, 41.8, 40.9, 31.8, 26.3; MS (ES) *m/z* 348.1416 (MH^+ [$C_{14}H_{18}N_7O_4$] = 348.1420).

3′-(Carboxymethyl)-3′,5′-dideoxyadenosine (2 a). To a solution of **11** (21 mg, 0.072 mmol) in THF:MeOH [0.6 mL, (5:1)] was added NaOH (80 μL of 1.0 M, 0.080 mmol). The mixture was stirred at 65°C until TLC showed conversion of **11** to baseline product. Volatiles were removed under reduced pressure to give **2a** (24 mg, quant). The crude residue was dissolved in H_2O (100 μL). Silica gel and solvent A were added, and volatiles were evaporated under reduced pressure (≤20°C). The dried silica gel was added to a column and chromatographed (solvent A) to give **2a** (18 mg, 81%): UV (MeOH) λmax 261 nm, λmin 229 nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.57 (br s, 1H), 8.42 (s, 1H), 8.12 (s, 1H), 7.22 (br s, 2H), 5.84 (d, J = 2.5 Hz, 1H), 5.52 (br s, 1H), 4.32 (d, J = 4.5 Hz, 1H), 4.01–3.98 (m, 1H), 3.69 (d, J = 12.0 Hz, 1H), 3.62–3.59 (m, 1H), 3.50 (d, J = 12.0 Hz, 1H), 2.24 (dd, J = 7.5, 14.5 Hz, 1H), 2.17 (dd, J = 5.3, 14.8 Hz, 1H), 1.77–1.75 (m, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.4, 156.0, 152.4, 148.6, 138.6, 119.1, 90.4, 84.3, 75.4, 60.7, 37.5, 29.6; MS (ES) *m/z* 310.1144 (MH^+ [$C_{12}H_{16}N_5O_5$] = 310.1151).

3′-(Carboxymethyl)-3′,5′-dideoxy-5′-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine sodium salt (2b). To a solution of **2h** (54 mg, 0.12 mmol) in DMSO (0.5 mL) was added NaOH (0.20 mL of 1.0 M, 0.20 mmol). The mixture was stirred at ambient temperature until TLC showed conversion of **2h** to baseline product. Volatiles were removed under reduced pressure to give **2b** (64 mg, quant). This material was >98% pure as determined by reverse phase HPLC and ^{1}H NMR: UV (MeOH) λmax 279 nm, λmin 243 nm; UV (H_2O) λmax 278 nm (ε 22,600); ^{1}H NMR (D_2O:DMSO-d_6 (1:9), 500 MHz) δ 8.25 (s, 1H), 8.11 (s, 1H), 7.55 (d, J = 8.0 Hz, 2H), 7.22 (t, J = 7.8 Hz, 2H), 6.90–6.87 (m, 1H), 5.83 (d, J = 3.0 Hz, 1H), 4.50 (dd, J = 2.0, 6.0 Hz, 1H), 3.90–3.87 (m, overlaps with solvent), 3.41 (dd, J = 3.0, 14.5 Hz, 1H), 3.16 (dd, J = 6.5, 14.0 Hz, 1H), 2.52 (s, 3 H), 2.42–2.38 (m, 1H), 2.31 (dd, J = 8.0, 14.8 Hz, 1H), 2.15 (dd, J = 5.3, 14.8 Hz, 1H); ^{13} C NMR (D_2 O:DMSO-d_6 (1:9), 125 MHz) δ 177.5, 161.6, 159.8, 159.1, 152.3, 148.5, 141.5, 138.2, 129.3, 124.7, 121.8, 119.2, 90.3, 83.8, 76.8, 42.8, 42.0, 35.0, 26.9; MS (ES) *m/z* 507.1711 (MH^+ [$C_{21}H_{24}N_8O_6Na$] = 507.1717).

2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-dideoxy-3′-[(ethoxycarbonyl)methyl]-5′-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (2c). A solution of **9** (100 mg, 0.168 mmol) and 10% Pd–C (50 mg) in EtOAc (2 mL) was vigorously stirred for 15 hours under an atmosphere of H_2 (balloon pressures). *p*-Nitrophenyl *N*-methylcarbamate (45 mg, 0.23 mmol) and anhydrous Na_2CO_3 (45 mg, 0.42 mmol) were added, and the resulting mixture was stirred for 4 hours under N_2. Solids were removed via filtration (celite/EtOAc), and volatiles were evaporated under reduced pressure. The crude residue was chromatographed (5 → 10% MeOH/CH_2Cl_2) to

give **2c** (101 mg, 96%): UV (MeOH) λmax 279 nm (ε 22,700), λmin 242 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.31 (s, 1H), 10.13 (br s, 1H), 8.86 (s, 1H), 8.64 (s, 1H), 7.57 (d, J = 7.5 Hz, 2H), 7.42–7.39 (m, 2H), 7.21–7.18 (m, 1H), 5.94 (s, 1H), 5.78 (t, J = 6.3 Hz, 1H), 5.06–5.03 (m, 2H), 4.20 (d, J = 10.5 Hz, 1H), 4.11–4.07 (m, 2H), 3.85–3.83 (m, 1H), 3.49 (d, J = 13.0 Hz, 1H), 2.79 (dd, J = 4.5, 17.0 Hz, 1H), 2.62 (d, J = 5.0 Hz, 3 H), 2.62–2.50 (m, 1H), 2.49–2.48 (m, 1H), 1.24 (t, J = 7.0 Hz, 3 H), 0.94 (s, 9 H), 0.27 (s, 3 H), 0.11 (s, 3 H); ^{13} C NMR ($CDCl_3$, 125 MHz) δ 172.0, 159.4, 153.3, 149.9, 149.8, 142.8, 137.3, 129.1, 124.6, 121.2, 92.0, 84.7, 77.2, 60.3, 39.7, 38.5, 28.8, 26.7, 25.7, 17.9, 14.0, −4.3, −5.8; MS (FAB) m/z 649.2899 (MNa^+ [$C_{29}H_{42}N_8O_6SiNa$] = 649.2894).

2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-dideoxy-3′-[(*N*-methoxy-*N*-methylcarboxamido) methyl]-5′-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl) adenosine (2d). A solution of **10** (50 mg, 0.082 mmol) and 10% Pd–C (50 mg) in EtOAc (1 mL) was vigorously stirred for 18 hours under an atmosphere of H_2 (balloon pressures). *p*-Nitrophenyl *N*-methylcarbamate (25 mg, 0.13 mmol) and anhydrous Na_2CO_3 (50 mg, 0.47 mmol) were added, and the resulting mixture was stirred for 4 hours under N_2. Solids were removed via filtration (celite/EtOAc), volatiles were evaporated under reduced pressure, and the residue was chromatographed (10% MeOH/EtOAc) to give **2d** (33 mg, 63%): UV (MeOH) λmax 279 nm (ε 22,200), λmin 245 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.32 (s, 1H), 10.14 (br s, 1H), 8.90 (s, 1H), 8.61 (s, 1H), 7.58 (d, J = 7.5 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.19–7.16 (m, 1H), 5.96 (s, 1H), 5.85 (br s, 1H), 5.07 (d, J = 4.0 Hz, 1H), 5.02 (d, J = 3.5 Hz, 1H), 4.25 (d, J = 10.5 Hz, 1H), 3.78–3.75 (m, 1H), 3.73 (s, 3 H), 3.58 (d, J = 11.5 Hz, 1H), 3.13 (s, 3 H), 2.78 (d, J = 5.0 Hz, 2H), 2.61 (d, J = 4.5 Hz, 3 H), 2.50–2.46 (m, 1H), 0.94 (s, 9 H), 0.28 (s, 3 H), 0.10 (s, 3 H); ^{13} C NMR ($CDCl_3$, 125 MHz) δ 172.7, 159.3, 153.2, 150.04, 150.01, 149.9, 142.8, 137.5, 129.1, 124.5, 121.2, 92.1, 84.8, 77.6, 61.1, 40.3, 38.4, 32.1, 29.7, 26.8, 25.8, 18.0, −4.4, −5.5; MS (ES) m/z 642.3182 (MH^+ [$C_{29}H_{44}N_9O_6Si$] = 642.3184).

5′-Azido-3′-(carboxymethyl)-3′,5′-dideoxy-N^6-(*N*-phenylcarbamoyl) adenosine sodium salt (2e). To a solution of **2g** (29 mg, 0.067 mmol) in DMSO (0.5 mL) was added NaOH (0.10 mL of 1.0 M, 0.10 mmol). The mixture was stirred at ambient temperature until TLC showed conversion of **2g** to baseline product. Volatiles were removed under reduced pressure to give **2g** (32 mg, quant). This material was >98% pure as determined by reverse phase HPLC and ^{1}H NMR: UV (MeOH) λmax 279 nm, λmin 241 nm; UV (H_2O) λmax 278 nm (ε 22, 100); ^{1}H NMR (D_2 O:DMSO-d_6 (1:9), 500 MHz) δ 8.24 (s, 1H), 8.13 (s, 1H), 7.49 (dd, J = 1.8, 7.3 Hz, 2H), 7.26–7.16 (m, 2H), 6.87–6.83 (m, 1H), 5.86 (d, J = 2.5 Hz, 1H), 4.54 (dd, J = 2.0, 6.0 Hz, 1H), 4.03–4.00 (m, overlaps with solvent), 3.54 (dd, J = 2.3, 13.8 Hz, 1H), 3.42 (dd, J = 5.8, 13.8 Hz, 1H), 2.45–2.44 (m, 1H),

2.26 (dd, J = 7.8, 15.3 Hz, 1H), 2.08 (dd, J = 5.5, 15.0 Hz, 1H); ^{13} C NMR (D_2 O:DMSO-d_6 (1:9), 125 MHz) δ 177.3, 160.7, 158.3, 152.4, 148.9, 141.2, 138.7, 138.6, 129.4, 124.2, 122.2, 119.5, 90.3, 83.3, 76.5, 53.1, 41.8, 34.4; MS (ES) m/z 476.1404 (MH^+ [$C_{19}H_{19}N_9O_5Na$] = 476.1407).

5′-Azido-3′-(carboxymethyl)-3′,5′-dideoxyadenosine (2f). To a solution of **12** (22 mg, 0.070 mmol) in THF:MeOH [0.6 mL, (5:1)] was added NaOH (80 μL of 1.0 M, 0.080 mmol). The mixture was stirred at 65°C until TLC showed conversion of **12** to baseline product. The mixture was added directly to a chromatography column and chromatographed (5 → 10%MeOH/CH_2Cl_2; 25 → 50% solvent A/EtOAc). Pooled fractions were evaporated under reduced pressure (≤20°C) to give **2f** (20 mg, 85%): UV (MeOH) λmax 260 nm, λmin 228 nm; UV (H_2O) λmax 260 nm (ε 14,500); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.27 (s, 1H), 8.17 (s, 1H), 7.30 (br s, 2H), 5.96 (d, J = 2.0 Hz, 1H), 4.64 (dd, J = 2.0, 5.5 Hz, 1H), 4.10–4.07 (m, 1H), 3.70–3.66 (m, 2H), 3.33 (br s, 1H), 2.77–2.71 (m, 1H), 2.57 (dd, J = 8.8, 17.3 Hz, 1H), 2.43 (dd, J = 5.3, 17.3 Hz, 1H); ^{13} C NMR (DMSO-d_6, 125 MHz) δ 173.3, 156.1, 152.6, 149.0, 138.7, 119.1, 90.4, 82.2, 74.8, 52.2, 39.8, 29.6; MS (ES) m/z 335.1230 (MH^+ [$C_{12}H_{15}N_8O_4$] = 335.1216).

5′-Azido-3′-(carboxymethyl)-3′,5′-dideoxy-N^6-(*N*-phenylcarbamoyl) adenosine-2′,3′-lactone (2 g). To a stirred solution of **9** (73 mg, 0.123 mmol) in CH_3CN (2.0 mL) were added $PhCH_2$ $N(Et)_3Cl$ (5 mg, 0.022 mmol), KF (15 mg, 0.26 mmol), and H_2O (40 μL). The mixture was vigorously stirred at ambient temperature until TLC indicated that **9** had been consumed (4 days). Silica gel was added and volatiles were evaporated under reduced pressure (≤20°C). The dried silica gel was poured onto the top of a column packed with 75% EtOAc/hexanes and product was eluted (75% EtOAc/hexanes → EtOAc). Evaporation of pooled fractions gave **2g** (46 mg, 86%): UV (MeOH) λmax 279, λmin 240; UV (H_2O) λmax 278 nm (ε 20,500); ^{1}H (DMSO-d_6, 500 MHz,) δ 11.70 (s, 1H), 10.21 (s, 1H), 8.72 (s, 1H), 8.66 (s, 1H), 7.63 (d, J = 7.5 Hz, 2H), 7.38–7.35 (m, 2H), 7.08 (t, J = 7.5 Hz, 1H), 6.43 (d, J = 2.0 Hz, 1H), 5.65 (dd, J = 1.8, 6.8 Hz, 1H), 4.28–4.24 (m, 1H), 3.73 (dd, J = 3.0, 13.5 Hz, 1H), 3.55–3.49 (m, 2H), 2.98 (dd, J = 8.5, 18.0 Hz, 1H), 2.69 (dd, J = 1.5, 18.0 Hz, 1H); ^{13} C NMR (DMSO-d_6, 125 MHz) δ 175.3, 151.0, 150.7, 150.0, 142.6, 138.4, 128.9, 123.2, 120.5, 119.4, 88.2, 86.4, 84.3, 51.7, 40.6, 31.5; MS (ES) m/z 436.1483 (MH^+ [$C_{19}H_{18}N_9O_4$] = 436.1482).

3′-(Carboxymethyl)-3′,5′-dideoxy-5′-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine-2′,3′-lactone (2h). To a stirred solution of **2c** (82 mg, 0.131 mmol) in CH_3CN (3.0 mL) were added $PhCH_2$ $N(Et)_3Cl$ (50 mg, 0.22 mmol), KF (22 mg, 0.38 mmol), and H_2O (80 μL). The mixture was vigorously stirred at ambient temperature until TLC indicated that **2c** had been consumed (60 hours). Silica gel was added and volatiles were evaporated under reduced pressure (≤20°C). The dried silica gel was

poured onto the top of a column packed with 5% MeOH/CH_2Cl_2 and eluted (5 → 10% MeOH/CH_2Cl_2). Evaporation of pooled fractions gave **2h** (56 mg, 92%): UV (MeOH) λmax 279 nm (ε 23,200), λmin 240 nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.74 (s, 1H), 10.18 (br s, 1H), 8.71 (s, 1H), 8.66 (s, 1H), 7.63 (d, J = 8.0 Hz, 2H), 7.38–7.35 (m, 2H), 7.09 (t, J = 7.5 Hz, 1H), 6.37 (d, J = 2.0 Hz, 1H), 6.05 (t, J = 6.0 Hz, 1H), 5.77 (dd, J = 4.5, 8.5 Hz, 1H), 5.57 (dd, J = 1.8, 7.3 Hz, 1H), 4.03–3.99 (m, 1H), 3.41–3.36 (m, 2H), 2.98 (dd, J = 8.5, 18.0 Hz, 1H), 2.55 (d, J = 5.0 Hz, 3 H); ^{13} C NMR (DMSO-d_6, 125 MHz) δ 176.3, 159.3, 151.8, 151.6, 150.8, 143.3, 139.2, 129.7, 123.9, 121.4, 120.1, 88.8, 87.5, 85.7, 42.4, 41.5, 40.7, 32.5, 27.1; MS (ES) m/z 467.1795 (MH^+ [$C_{21}H_{23}N_8O_5$] = 467.1791).

CELLS AND VIRUS

MT2 and H9 cells, both $CD4^+$ lymphoblastoid cell lines, were cultured in RPMI-1640 containing HEPES and supplemented with 11.5% heat-inactivated fetal bovine serum and 2 mM L-glutamine. HIV-containing supernatant fluids were clarified of cells by low-speed centrifugation followed by filtration through 0.45 μm cellulose acetate filters.

50% Cytotoxic and 50% Effective Concentrations

The 50% cytotoxic/cytostatic concentration (CT_{50}) of each compound was determined as described, previously.[41–43] Briefly, in triplicate wells of a 96 well plate each compound was serially diluted. MT2 cells were added to each well and the cells plus compound were incubated for 72 hours at 37°C. Cells were resuspended, transferred to a poly-L-lysine coated plate, and stained with Finter's neutral red dye, a vital dye. Cells were incubated for 1 hour at 37°C to stain and to adhere. Cells were washed with phosphate buffered saline and lysed in acid alcohol. A_{540} was determined and the percent viable cells were calculated relative to 8 replicates without compound (100% viable) and 8 blank wells (0% viable). The CT_{50} was calculated using CalcuSyn for Windows software. The 5% cytotoxic/cytostatic dose (CT5), a nontoxic concentration where 95% of the cells were viable, was also calculated for each of the compounds.

The 50% effective concentration (EC_{50}) was determined in essentially the same manner as the CT_{50} and as described, previously.[41–43] However, after a one hour incubation of cells and compounds, HIV_{LAI} was added to each well. The cells were harvested at 72 hours and the percentage of protection from HIV-induced cytopathic effects was calculated using Finter's neutral red dye. The percentage of viable cells relative to 8 cell control replicates (cells but no virus or compound, 100% viable) and 8 virus control

replicates (cells and virus but no compound, 0% viable) was determined. The EC_{50} was calculated using CalcuSyn for Windows software.

Inhibition of HIV Integrase

We previously have documented the practical application of screening compounds at 10 μM for inhibition of integrase in vitro.[44] Compounds which inhibit HIV replication by 50% or more at 10 μM are further subjected to dilution from 10 μM to 30 nM in 1/2 $\log_{10}$ dilutions to calculate the 50% inhibitory concentration (IC_{50}). None of the compounds were active at 10 μM in the 3′-endprocessing or strand transfer reactions; therefore, IC_{50} analyses were not performed. Recombinant integrase from HIV_{NL4-3} was expressed in and purified from *Escherichia coli*. Recombinant integrase was incubated with compound and ^{32}P-labeled oligonucleotide substrate homologous to the HIV long terminal repeat DNA for 1 hour at 37°C. Reactions were stopped by the addition of EDTA to a final concentration of 18 mM. The reaction products were separated from substrate by denaturing polyacrylamide gel electrophoresis. The percent conversion of substrate to products was quantified using a Storm phosphorimager (Molecular Dynamics, Sunnyvale, CA, USA). The conditions for these reactions have been well-described, previously.[44–48] Controls in all experiments include 25 μM L-chicoric acid (100% inhibition) and 25 μM L-tartaric acid (0% inhibition).

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