AMP Deaminase Inhibitors. 2. Initial Discovery of a Non-Nucleotide Transition-State Inhibitor Series[1]

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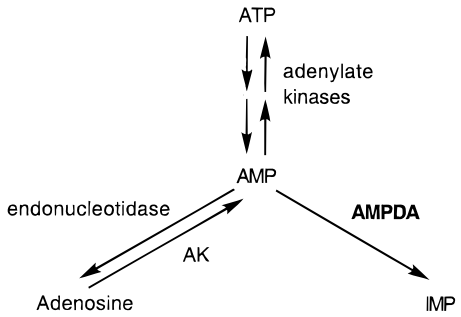
A series of N3-substituted coformycin aglycon analogues are described that inhibit adenosine 5′-monophosphate deaminase (AMPDA) or adenosine deaminase (ADA). The key steps involved in the preparation of these compounds are (1) treating the sodium salt of 6,7-dihydroimidazo-[4,5-*d*][1,3]diazepin-8(3*H*)-one (**4**) with an alkyl bromide or an alkyl mesylate to generate the N3-alkylated compound **5** and (2) reducing **5** with $NaBH_4$. Selective inhibition of AMPDA was realized when the N3-substituent contained a carboxylic acid moiety. For example, compound **7b** which has a hexanoic acid side chain inhibited AMPDA with a $K_i = 4.2\ \mu$M and ADA with a $K_i = 280\ \mu$M. Substitution of large lipophilic groups α to the carboxylate provided a moderate potency increase with maintained selectivity as exemplified by the α-benzyl analogue **7j** (AMPDA $K_i = 0.41\ \mu$M and ADA $K_i > 1000\ \mu$M). These compounds, as well as others described in this series of papers, are the first compounds suitable for testing whether selective inhibition of AMPDA can protect tissue from ischemic damage by increasing local adenosine concentrations at the site of injury and/or by minimizing adenylate loss.

Introduction

AMP deaminase (AMPDA) is a potential new target for the treatment of ischemia-related diseases based on initial studies showing that AMPDA is important in the regulation of the production of adenosine and sparing of adenylates specifically during periods of net ATP breakdown.[1] AMPDA catalyzes the hydrolytic deamination of AMP to IMP ($K_m \sim 1$ mM) (Scheme 1). With this pathway blocked, AMP may be dephosphorylated to adenosine via an endonucleotidase or reverted back to ATP via adenylate kinase. Moreover, flux through the enzyme is reported to be significant only during periods of net ATP breakdown when the concentration of AMP is increased manyfold.[2] Hence, inhibitors of this enzyme represent potential site- and event-specific cardio- and neuroprotective agents since they would only affect purine metabolism during periods of ischemia and would be pharmacologically silent otherwise.[3] A recent published study further supports this contention.[4] Patients diagnosed with congestive heart failure (CHF) were found to have a significantly longer time period of survival without heart transplant (7.6 vs 3.2 years; $P < 0.001$) if they had a genetic deficiency in skeletal muscle AMPDA. This suggests a potential use of AMPDA inhibitors for the treatment of CHF.[5]

AMPDA, however, represents a particularly difficult target for medicinal chemists since no compounds are known that can bind with high affinity and specificity to AMP binding sites and also diffuse into cells. In general, a phosphate or phosphonate functionality is the crucial component necessary for a compound to have affinity to a nucleotide binding site, but this feature also prevents cell penetration. Also, compounds containing a phosphate are subject to rapid dephosphorylation in vivo by ectonucleotidases. Nevertheless, when a nucleotide is the active drug, a common approach is administration of nucleotide precursors (e.g. nucleosides and bases) and to rely on intracellular metabolic conversion to the active nucleotide form.[6] Alternatively, phosphate mimetics and prodrug strategies that mask charge or combinations thereof are used.[7] However, these strategies posed significant challenges when applied to AMPDA, and the historically poor results from such strategies forced us to look for alternatives.

Scheme 1. AMP Metabolic Pathway



Coformycin (**1**)[8] and coformycin 5′-monophosphate (**2**) are potent AMPDA inhibitors with K_is of 3 and 0.0001 μM,[9] respectively. Coformycin (**1**) has been used to evaluate the role of AMPDA in purine metabolism,[10] but since it is a much more potent ADA inhibitor ($K_i = 0.00001\ \mu$M)[11] it has limited utility for defining the importance of AMPDA in nucleotide metabolism. We also sought to avoid dual inhibitors because of the association of ADA deficiency with severe combined immunodeficiency.[12] Coformycin 5′-monophosphate (**2**) is completely selective, but its phosphate substituent makes it an unsuitable tool compound (vida supra).

The potency of coformycin (**1**) at AMPDA is notable since it does not require the highly charged phosphate group normally necessary for affinity to nucleotide binding sites. Coformycin (**1**) can be defined as a transition-state inhibitor based on the similarity of its

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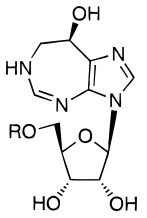


1, R = H
2, R = PO_3H_2

structure to the structure of the tetrahedral intermediate of the deamination reaction (Chart 1). Coformycin (**1**) compensates for the binding not accessed in the phosphate pocket by having good transition-state (TS) mimicry from the aglycon portion of the molecule.[13] In the active site of these enzymes the aglycon moiety of coformycin (**1**) can be positioned to have close interactions with the zinc and other catalytic residues which stabilize the TS of the deamination.[1,14] The aglycon binding regions of AMPDA and ADA are likely to be very similar because of the high active site residue homology they share.[1] TS mimicry is also enzyme specific so coformycin (**1**) or analogues containing the aglycon moiety are unlikely to interact with other adenosine- or AMP-binding proteins. Thus, we reasoned that analogues of coformycin (**1**) could potentially be selective cell-penetrable inhibitors. Still, the challenge was to find a replacement for the ribose group which would allow discriminatory binding to AMPDA relative to ADA.

In Figure 1 is shown our inhibitor design strategy which entailed replacing the ribose monophosphate of **2** with a lipophilic group capped with an anionic substituent Y.[1] We envisioned that the anionic group would interact with the cationic, phosphate binding site of AMPDA and thereby provide potent and selective inhibition of AMPDA vs ADA. The anionic Y group was envisioned to have physicochemical properties which would allow the molecule to cross cell membranes. Thus a carboxylate was chosen as the Y group.

Herein we report that replacement of the ribose 5′-monophosphate of **2** with an alkylcarboxy group resulted in a series of potent and selective AMPDA inhibitors. These compounds and additional efforts to optimize the series[15] for the first time enable investigation of inhibition of AMPDA as a potential route to drugs targeting ischemia-related diseases.

Chemistry

Scheme 2 illustrates the general method of coformycin aglycon analogue synthesis.[16] The known heterocycle

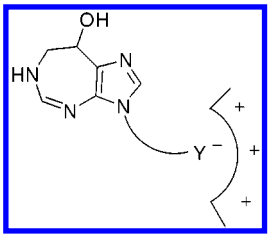
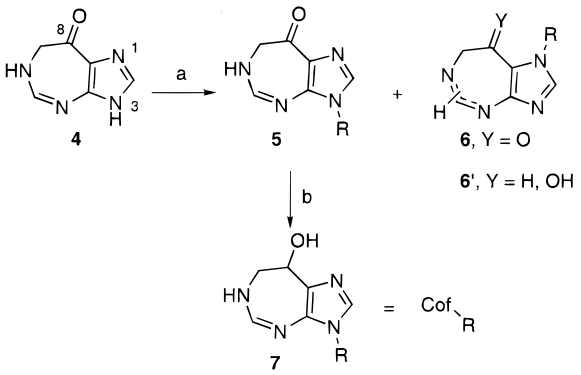


Figure 1. AMPDA inhibitor design strategy.

Scheme 2. General Method for Coformycin Aglycon Analogue Preparation[a]

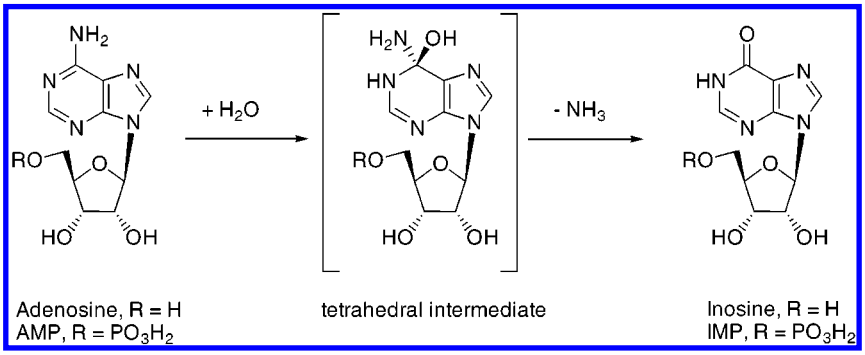


[a] (a) NaH, DMF, 60 °C, 30 min; R-X, NaI, 90 °C; (b) $NaBH_4$, MeOH, CH_2Cl_2.

6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**4**; prepared in eight steps from 4-methylimidazole)[16a] was alkylated employing sodium hydride as a base and an alkyl bromide or mesylate as the electrophile in the presence of sodium iodide in hot DMF. This produced mainly a chromatographically separable mixture of *N*3- and *N*1-alkyl products **5** and **6** in yields typically on the order of 30% and 15%, respectively. Products of dialkylation were occasionally obtained, especially if the base and electrophile were in excess of the heterocycle. The ketone **5** was not stable to aqueous acid or aqueous base.[16,17] It was typically reduced as the next step with $NaBH_4$ to provide a 60−90% yield of the *N*3-alkylcoformycin aglycon product **7** as a 1:1 mixture of C8 epimers. In general, for reactivity considerations, the alcohol **7** had good stability to aqueous base but had a limited half-life in mild aqueous acid.[18,19] Tables 1 and 2 list analogues **7**.

The regiochemistry of the alkylation was proven in part by chemical means as shown in Scheme 3. The analogue **5f′** could be prepared either by alkylation of **4** with benzyl bromide or by cyclization of compound **8** (a precursor in the synthesis of compound **4**) with

Chart 1. AMPDA- and ADA-Catalyzed Deamination Reaction

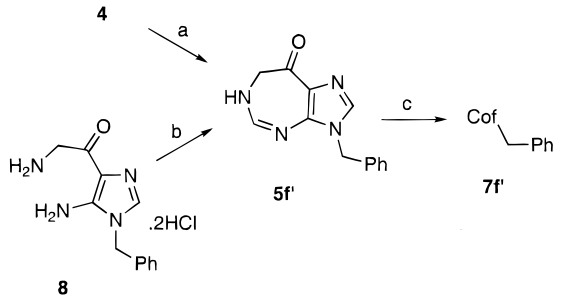


Adenosine, R = H
AMP, R = PO_3H_2

tetrahedral intermediate

Inosine, R = H
IMP, R = PO_3H_2

Scheme 3. Regiochemistry of Major Alkylation Product Defined[a]



[a] (a) NaH, DMF; NaI, BnBr; (b) $(EtO)_3CH$, DMSO, 70 °C; (c) $NaBH_4$.

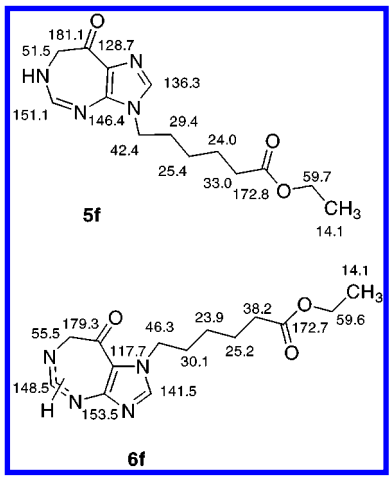
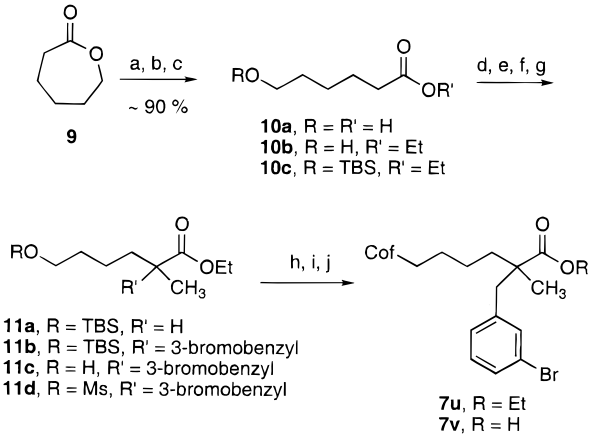


Figure 2. ^{13}C NMR (DMSO-d_6) assignment of compounds **5f** and **6f**.

triethyl orthoformate. Since the structure of **8** was previously defined[16a] it follows that the alkylation of **4** provides **5** as the primary product.

The structure assignments were further confirmed by ^{13}C NMR (Figure 2), ^1H NMR, and UV/vis spectral analysis. $^1H-^1H$ and $^1H-^{13}C$ 2-dimensional NMR correlation experiments in DMSO-d_6 enabled carbon and proton shift assignments for compounds **5f** and **6f**. The bridgehead carbons C8a and C3a were particularly diagnostic for each isomer. The bridgehead carbon resonance shifts upfield when the neighboring nitrogen is substituted. N1-Substitution results in C8a being shielded by 11 ppm in compound **6f** compared to **5f.** N3-Substitution results in a 7.1 ppm upfield shift in the C3a resonance in **5f** compared to **6f**. This parallels ^{13}C NMR shift variances seen with *N*9- vs *N*7-methyl-substituted adenines.[20] The ^1H NMR in DMSO-d_6 also exhibits distinctly different spectra for the isomers. Most notable are those protons connected to the diazepinone section of the molecule. For the N3-isomer **5f**, protons on C5 and C7 are sharp doublets with coupling constants of $J = 4$ Hz for each and the N6-proton is well-defined with a broad peak at δ 8.4−8.5. By contrast the C5- and C7-protons of **6f** are defined by broad singlets. This line broadening can be explained by considering **6f** as an equilibrating 1:1 tautomeric mixture of N4═C5−N6 and N4−C5═N6 forms in DMSO-d_6. Comparison of the N6-H (δ 8.2) integration for the former to the N4-H (δ 10.6) integration for the latter supports this ratio assignment. Further evidence for this equilibration is the broad ^{13}C NMR resonances for those carbons close

Scheme 4. Representative Substituted Hexanoate Electrophile Preparation and Representative Final Product Ester Hydrolysis[a]



[a] (a) NaOH, H_2O, dioxane; (b) EtI, K_2CO_3, DMF; (c) TBSCl, DMF, imidazole; (d) LDA, MeI, THF, DMPU; (e) LDA, (3-BrPh)CH_2Br, THF, DMPU; (f) Bu_4NF, THF; (g) MsCl, NEt_3, CH_2Cl_2; (h) **4**, NaH, DMF; **11d**, NaI; (i) $NaBH_4$, MeOH, CH_2Cl_2; (j) 0.5 M NaOH, dioxane; DOWEX 1×8-400 acetate. DMPU = *N*,*N*′-dimethylpropyleneurea.

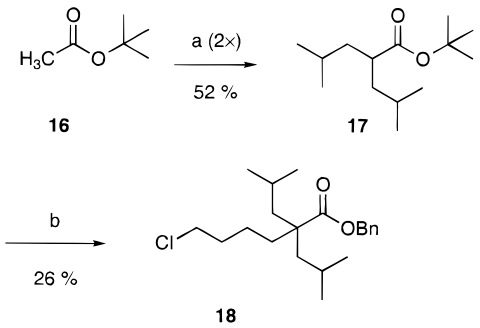
to this triad, especially C3a and C7. Thus structure **6** in Scheme 2 is drawn with a partial double bond between the three atoms N4, C5, and N6. Finally, since the UV/vis λ_{max} value for **7f** (R = $(CH_2)_5CO_2Et$), λ_{max}(methanol) 283 nm (ϵ 6545), is nearly identical to that reported for coformycin (**1**),[8a] $\lambda_{max}(H_2O)$ 282 nm (ϵ 8250), it must be the *N*3-alkyl analogue. In comparison the value for the *N*1-alkyl analogue **6f′** (R = $(CH_2)_5CO_2Et$) is significantly shifted, λ_{max}(methanol) 278 nm (ϵ 4680).

Some of the electrophiles, R-X, used in the coupling reaction of Scheme 2 were commercially available alkyl bromides or were mesylates of available alcohols.[21] Scheme 4 shows the general method for construction of some of the α-substituted hexanoate electrophiles as exemplified by the preparation of **11d**. ϵ-Caprolactone (**9**) was hydrolyzed and protected to provide the acyclic ester **10c** (92% for three steps). Two enolate alkylations were followed by conversion of the resulting silyl ether **11b** to the mesylate **11d** (47% for four steps). Ketone **4** was alkylated with **11d** to provide a 31% yield of **5u** (not shown) which was reduced to analogue **7u** (74%).

Scheme 4 also shows the final step used to prepare many of the carboxylic acids in Table 1. Sodium hydroxide hydrolysis of ester **7u** followed by purification of the product with DOWEX 1×8-400 acetate ion-exchange resin provided carboxylate **7v** (69%). Some sodium carboxylates in the series (compounds **7a**, **7b**, and **7i**) and free acids (**7c** and **7d**) were obtained by treatment of the resulting hydrolysis solution with Amberlite CG-50 resin to absorb excess sodium hydroxide. However, some of these sodium salts were prohibitively deliquescent, and this necessitated the use of DOWEX 1×8-400 acetate ion-exchange resin to isolate the more stable free acids. Alternatively, some carboxylates (i.e. **7y**, **7z**, and **7a′**) were prepared in good yields by hydrogenolysis of their respective benzyl esters.

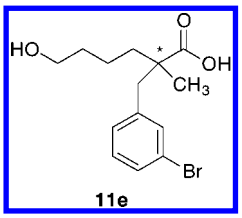
Electrophile **11d** is a racemic mixture. To evaluate whether one enantiomeric form of this side chain has a higher affinity for AMPDA, the undefined single enantiomer **11e** was prepared by crystallization of the (−)-

Scheme 5. Preparation of a Hindered Ester Electrophile[a]

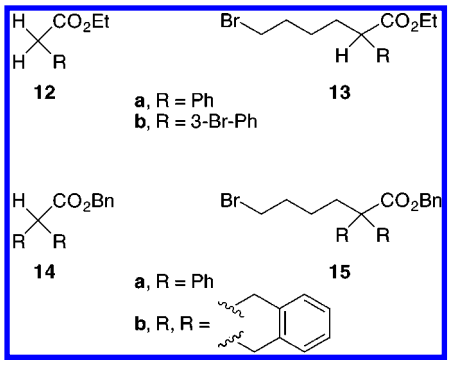


[a] (a) LICA, THF, −78 °C; $(CH_3)_2CHCH_2I$, DMSO, 23 °C; (b) 3 equiv LDA, THF, DMPU, 0 °C; 4 equiv $I(CH_3)_4Cl$, 55 °C; TFA, CH_2Cl_2, reflux; BnBr, K_2CO_3, DMF, 23 °C.

brucine salt from acetonitrile (34% yield). Compound **11e** was judged to be >95% ee on the basis of the ^{1}H NMR ($CDCl_3$) of the complex of with (*S*)-(−)-α-methylbenzylamine. This in turn was used to prepare **7w**, which is a 1:1 8*R*/*S*-diastereomeric mixture of two of the four compounds that make up compound **7v**.

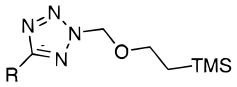


The hexanoate electrophiles **13** and **15** were prepared by reacting precursors **12** and **14**[22] with LDA and 2 equiv of 1,4-dibromobutane in THF/DMPU.

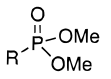


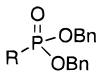
The hindered ester **17** was prepared using successive alkylations of *tert*-butyl acetate (**16**) as shown in Scheme 5.[23,24] α-Alkylation under conditions of excess LDA in THF−DMPU (2−1) at 0 °C for deprotonation and then addition of 4-chloro-1-iodobutane at elevated temperatures[25] provided the desired hindered ester which after ester exchange provided the electrophile **18**.

Other desired electrophiles were also prepared by monoalkylating 1,ω-dibromoalkanes. Tetrazole was treated with NaH and then N-protected with SEM-Cl (SEM = $(CH_3)_3SiCH_2CH_2OCH_2$). This produced a separable 1:1 mixture of N1- and N2-protected tetrazoles. The N1-protected compound decomposed upon treatment with *t*-BuLi, whereas the N2-protected compound **19a** upon successive treatment with *t*-BuLi and 1,5-dibromopentane produced the desired electrophile **19b**.[26] Alkylation of the base **4** with this electrophile followed by reduction and finally SEM group removal with fluoride ion in DMF provided the analogue **7b′**. The phosphonates **20b**, **21b**, and **21c** were prepared by reacting the individual phosphites **20a** and **21a** with NaH and then with the specific 1,ω-dibromoalkane. These electrophiles were used to prepare **7c′**, **7d′**, and **7e′**.



19a, R = H
19b, R = $(CH_2)_5Br$





20a, R = H
20b, R = $(CH_2)_6Br$

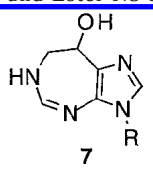
21a, R = H
21b, R = $(CH_2)_5Br$
21c, R = $(CH_2)_6Br$

Results

Tables 1 and 2 list the coformycin aglycon analogues **7** and their K_i values for inhibition of either porcine heart or human L-type AMPDA and calf intestinal ADA. A cross-comparison of AMPDA K_i values determined from both enzyme sources indicated the values have ≤10% variation. K_i values of the 8*R*-isomers are likely one-half the indicated value since the compounds are 1:1 8*R*/*S*-isomer mixtures and only the 8*R*-isomer is likely to be inhibitory based on earlier work with (8*S*)-2′-deoxycoformycin monophosphate[27] and (8*S*)-2′-deoxycoformycin.[28] The inhibitor initial screening concentrations in general were chosen so that the upper limits of the K_i values determined for AMPDA and ADA inhibition were 125 and 7.5 μM, respectively. In select examples, to evaluate the full extent of the selectivity of the enzyme inhibition, ADA inhibition was evaluated at higher concentrations. Table 1 shows that potency and selectivity can be achieved when the carbon tether connecting the aglycon to the CO_2H was at least five atoms in length (e.g. for compound **7b**, AMPDA K_i = 4.2 μM and ADA K_i = 280 μM). Longer chain lengths provided similar potency and selectivity (i.e. compounds **7c** and **7d**), but the CO_2H group was crucial to selectivity and potency as analysis of the various esters demonstrates. For example, the ethyl ester **7f** was approximately a 10-fold weaker AMPDA inhibitor and a 100-fold better ADA inhibitor compared to its derivative carboxylate **7b**. Examination of other anionic groups revealed the tetrazole as in analogue **7b′** to be a satisfactory replacement for the carboxylate, but phosphonates **7c′**, **7d′**, and **7e′** were at least 10-fold weaker than the corresponding carboxylate analogues.

Substitution of large hydrophobic groups α to the carboxylate group resulted in increased potency and maintained selectivity (see **7j**: AMPDA K_i 0.41 μM and ADA K_i > 1000 μM). This substitution also introduced a second chiral center into the molecule in addition to the C8 center. Thus it was of interest to examine a resolved α-substituted analogue for evidence of a preferred configuration. In this regard, compound **7w** showed little difference from the corresponding diastereomeric mixture **7v**. Since little preference was evident, α,α-disubstituted analogues **7y**, **7z**, and **7a′** were ex-

Table 1. Coformycin Aglycon Analogues with Anionic and Ester N3-Substituents



7

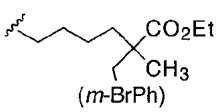
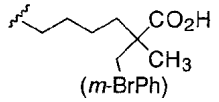
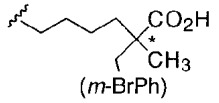
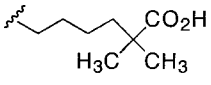
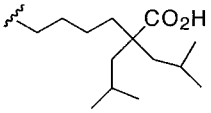
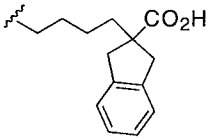
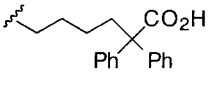
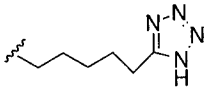
cmpd	R-group	mp (°C)	formula[a]	AMPDA K_i (μM)	ADA K_i (μM)
7a	-$(CH_2)_4CO_2Na$	250	$C_{11}H_{15}N_4O_3Na\cdot0.5H_2O\cdot0.25(C_2H_5)_2O$	> 125	> 7.5
7b	-$(CH_2)_5CO_2Na$	118-121	$C_{12}H_{17}N_4O_3Na\cdot0.5H_2O$	4.2	280
7c	-$(CH_2)_6CO_2H$	165-168	$C_{13}H_{20}N_4O_3\cdot0.5H_2O$[b]	10	250
7d	-$(CH_2)_7CO_2H$	166	$C_{14}H_{22}N_4O_3$	8	> 7.5
7e	-$(CH_2)_4CO_2Me$	136-139	$C_{12}H_{18}N_4O_3$	63	3.5
7f	-$(CH_2)_5CO_2Et$	115-118	$C_{14}H_{22}N_4O_3$	44	3
7g	-$(CH_2)_6CO_2Et$	117-118	$C_{15}H_{24}N_4O_3$	55	0.9_6
7h	-$(CH_2)_7CO_2Me$	131-133	$C_{15}H_{24}N_4O_3$	20	0.9_5
7i	-$CH_2O(CH_2)_3CO_2Na$	nd[c]	$C_{11}H_{15}N_4O_4Na\cdot H_2O$	85	> 500
7j	-$(CH_2)_4CH(Bn)CO_2H$	58-61	$C_{19}H_{24}N_4O_3\cdot H_2O\cdot0.2CH_3CO_2H$	0.41	> 1000
7k	-$(CH_2)_4CH(o\text{-}BrBn)CO_2H$	111	$C_{19}H_{23}N_4O_3Br\cdot H_2O$	0.30	> 7.5
7l	-$(CH_2)_4CH(m\text{-}BrBn)CO_2Et$	125-127	$C_{21}H_{27}N_4O_3Br$	3.8	> 7.5
7m	-$(CH_2)_4CH(m\text{-}BrBn)CO_2H$	98	$C_{19}H_{23}N_4O_3Br\cdot H_2O$	0.79	> 7.5
7n	-$(CH_2)_4CH(p\text{-}BrBn)CO_2H$	116	$C_{19}H_{23}N_4O_3Br\cdot1.25H_2O$	1.1	> 7.5
7o	-$(CH_2)_4CH(m\text{-}ClBn)CO_2H$	83	$C_{19}H_{23}N_4O_3Cl\cdot H_2O\cdot0.25CH_3CO_2H$	1.4	> 7.5
7p	-$(CH_2)_4CH(p\text{-}ClBn)CO_2H$	86	$C_{19}H_{23}N_4O_3Cl\cdot H_2O\cdot0.25CH_3CO_2H$	1.7	> 7.5
7q	-$(CH_2)_4CH(Ph)CO_2Et$	110-114	$C_{20}H_{26}N_4O_3$	9.6	> 7.5
7r	-$(CH_2)_4CH(Ph)CO_2H$	106	$C_{18}H_{22}N_4O_3\cdot0.75H_2O\cdot0.27CH_3CO_2H$	2.1	> 7.5
7s	-$(CH_2)_4CH(m\text{-}BrPh)CO_2Et$	110-114	$C_{20}H_{25}N_4O_3Br$	9.7	> 7.5
7t	-$(CH_2)_4CH(m\text{-}BrPh)CO_2H$	90-95	$C_{18}H_{21}N_4O_3Br\cdot0.75H_2O\cdot0.36CH_3CO_2H$	2.4	> 7.5
7u	 (*m*-BrPh)	133-136	$C_{22}H_{29}N_4O_3Br$	3	> 7.5
7v	 (*m*-BrPh)	83-88	$C_{20}H_{25}N_4O_3Br\cdot H_2O\cdot0.42CH_3CO_2H$[d]	0.8	> 7.5
7w	 (*m*-BrPh)	115	$C_{20}H_{25}N_4O_3Br\cdot2H_2O\cdot0.1CH_3CO_2H$[e]	0.64	> 7.5

Table 1 (Continued)

cmpd	R-group	mp (°C)	formula[a]	AMPDA K_i (μM)	ADA K_i (μM)
7x		70-80	$C_{14}H_{22}N_4O_3 \cdot 0.6H_2O \cdot 0.25CH_3CO_2H$	8.2	> 7.5
7y		120	$C_{20}H_{34}N_4O_3 \cdot 2H_2O \cdot C_{12}H_{23}N$[f]	0.86	120
7z		190	$C_{20}H_{24}N_4O_3 \cdot 1H_2O$[g]	0.97	> 7.5
7a'		200-201	$C_{24}H_{26}N_4O_3 \cdot 2.75H_2O$[h]	0.92	> 7.5
7b'		220	$C_{24}H_{41}N_9O \cdot 2H_2O \cdot C_{12}H_{23}N$[i]	6.0	> 7.5
7c'	$-(CH_2)_6P(O)(OMe)OH$	67-68	$C_{13}H_{23}N_4O_4P \cdot 2H_2O$	140	> 500
7d'	$-(CH_2)_5PO_3H_2$	119-120	$C_{11}H_{19}N_4O_4P \cdot 1.75H_2O$	35	> 500
7e'	$-(CH_2)_6PO_3H_2$	210-211	$C_{12}H_{21}N_4O_4P \cdot 2H_2O$	55	> 7.5

[a] Analyses for C, H, N were correct within ±0.4% unless otherwise stated. [b] H, N; C: calcd, 53.97; found, 54.38. [c] Not determined, very deliquescent compound. [d] C, H; N: calcd, 11.37; found, 10.57. [e] C; H: calcd, 6.03; found, 5.56; N: calcd, 11.40; found, 10.74. [f] C; H: calcd, 10.32; found, 9.79; N: calcd, 11.75; found, 12.35. [g] C, H; N: calcd, 14.50; found, 14.02. [h] C, N; H: calcd, 6.78; found, 6.33. [i] C, H; N: calcd, 24.83; found, 23.80.

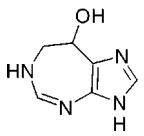
amined and found to be modest and selective inhibitors of AMPDA with K_is of ca. 1 μM each.

Inhibitory potency of analogues with neutral or non-anionic N3-substituents are described in Table 2. These compounds with alkyl, alkylhydroxy, alkylcarboxamide, and alkylcyano N3-substituents were in general much weaker inhibitors of AMPDA than the carboxylates and nonselective vs ADA. A few *n*-alkyl-substituted analogues did inhibit AMPDA modestly, but these compounds also proved to be good ADA inhibitors (e.g. **7k'** with an AMPDA K_i of 10 μM and an ADA K_i of 0.35 μM).

The carboxylate **7m** was evaluated for its ability to penetrate cells in vitro. This compound was able to equilibrate rapidly across cell membranes ($t_{1/2} \leq 5$ min) for a variety of cell types which included rat hepatocytes, rabbit erythrocytes, and cultured bovine endothelial cells.

Discussion

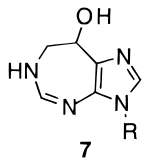
The first potent, selective, and cell-penetrating inhibitors of AMPDA have been prepared by (1) utilizing the affinity and selectivity that the coformycin aglycon moiety exhibits presumably as a result of it TS mimicry and (2) rationalizing that an N3-anionic group, namely an alkyl-CO_2H group, would provide preferred affinity to AMPDA by interacting with its phosphate binding site. For example, compound **7j**, the most potent of this series, has an AMPDA $K_i = 0.41$ μM and an ADA $K_i > 1000$ μM. As a point of reference, the aglycon itself (**22**) is not an AMPDA inhibitor ($K_i > 1000$ μM) and a very weak ADA inhibitor ($K_i = 50$ μM).[1] Thus the N3-substituent is very important for proper positioning of the aglycon moiety in the active site as a TS inhibitor, and importantly, it does not have to be a ribose analogue. Prior to this work, no inhibitors of AMPDA were described with properties appropriate to study their effects on cellular function.[29] In addition to having poor affinity to AMPDA, those compounds all contain a phosphate or phosphonate functionality and thus are relatively impermeable to cells. Coformycin (**1**) was the best non-nucleotide with an AMPDA $K_i = 3$ μM; however, it is also a very potent ADA inhibitor ($K_i = 0.00001$ μM) and thus is preempted as a tool to study the biological effects of selective AMPDA inhibition.



22

We propose that the CO_2H group of these AMPDA inhibitors does not lie inside the phosphate-binding

Table 2. Coformycin Aglycon Analogues with Nonanionic N3-Substituents



7

cmpd	R-group	mp (°C)	formula[a]	AMPDA K_i (μM)	ADA K_i (μM)
7f'	$-CH_2Ph$	179-182	$C_{13}H_{14}N_4O$[b]	> 125	6
7g'	$-CH_2CH_3$	189-190	$C_8H_{12}N_4O$	> 125	28
7h'	$-(CH_2)_2CH_3$	159-160	$C_9H_{14}N_4O$	> 125	> 20
7i'	$-(CH_2)_3CH_3$	163-164	$C_{10}H_{16}N_4O$	84	17
7j'	$-(CH_2)_5CH_3$	159-160	$C_{12}H_{20}N_4O$	20	0.48
7k'	$-(CH_2)_6CH_3$	152-153	$C_{13}H_{22}N_4O$	10	0.35
7l'	$-(CH_2)_7CH_3$	151-152	$C_{14}H_{24}N_4O \cdot 0.25H_2O$	18	0.45
7m'	$-(CH_2)_2OH$	220-221	$C_8H_{12}N_4O_2$[c, d]	150	> 7.5
7n'	$-(CH_2)_3OH$	210-211	$C_9H_{14}N_4O_2$[c, e]	68	> 7.5
7o'	$-(CH_2)_4OH$	176-177	$C_{10}H_{16}N_4O_2$[c, b]	53	6.3
7p'	$-(CH_2)_5OH$	135-136	$C_{11}H_{18}N_4O_2$[c, f]	36	4.7
7q'	$-(CH_2)_6OH$	138-139	$C_{12}H_{20}N_4O_2 \cdot 0.5H_2O$[c, g]	64	3.6
7r'	$-(CH_2)_5C(O)NH_2$	159-163	$C_{12}H_{19}N_5O_2 \cdot 0.4H_2O$	180	5.9
7s'	$-(CH_2)_3CN$	171-173	$C_{10}H_{13}N_5O$	> 125	> 7.5
7t'	$-(CH_2)_4CN$	146-149	$C_{11}H_{15}N_5O$	240	14
7u'	$-(CH_2)_6CN$	129-132	$C_{13}H_{19}N_5O$	170	1

[a] Analyses for C, H, N were correct within ±0.4% unless otherwise stated. [b] Elemental analysis not determined. [c] >95% HPLC pure, detector wavelength set at 280 nM. [d] Calcd: C, 48.97; H, 6.17; N, 28.55. Found: C, 47.09; H, 5.96; N, 25.70. [e] Calcd: C, 51.41; H, 6.70; N, 26.65. Found: C, 47.25; H, 6.47; N, 21.74. [f] Calcd: C, 55.41; H, 7.61; N, 23.51. Found: C, 52.58; H, 7.19; N, 21.09. [g] Calcd: C, 55.17; H, 8.04; N, 21.45. Found: C, 56.00; H, 8.13; N, 20.98.

pocket of the active site but rather that it provides binding to the pocket with a through-space electrostatic interaction. A modeling study comparing the energy-minimized structure of a more advanced AMPDA inhibitor to the crystal structure of AMP in the anti conformation supports this assertion.[1] This study revealed the critical CO_2H to overlay in close proximity to the 5′-phosphate of AMP. The inhibitor could not assume a low-energy conformation with direct CO_2H/PO_4H_2 overlay.[30] Neutral N3-substituents containing groups such as alkyl esters, alkyls, alkylphenyls, alkylhydroxys, alkyl-$C(O)NH_2$, and alkyl-CN are generally nonselective inhibitors of AMPDA which underlines the importance of the CO_2H to potency and selectivity. Other anionic groups such as tetrazole and phosphonate, while not improving potency relative to the CO_2H analogues, also show selective inhibition of AMPDA relative to ADA. A tether length of at least five atoms was optimal (compare **7b**, **7c**, and **7d** to **7a**) possibly because shorter tethers cannot position the CO_2H close enough to the phosphate binding site. This minimum five-atom tether length further reinforces the similarity of these inhibitors to AMP since it equals the shortest atom-chain path from N9 of AMP to its 5′-phosphate.

It is useful to note when considering the further optimization of this series that the active site allows large hydrophobic substituents close to the CO_2H group

which incidentally might not be expected if the CO_2H was inside the very hydrophilic phosphate binding site. Compound **7j**, with an α-benzyl substituent, is 10-fold more potent than the initial lead **7b** and still AMPDA selective. Even more hydrophobic substitutions near the CO_2H are tolerated by AMPDA. The α,α-diisobutyl and α,α-diphenyl analogues **7y** and **7a′** were moderate inhibitors (AMPDA K_i = 0.86 and 0.92, respectively). The moderate increases in potency realized from α-substituents suggest that some hydrophobic binding is occurring and much more optimization might be possible by a different approach. Further work on compound optimization can be found in the following papers.[15]

Summary

The coformycin aglycon is an excellent template from which to construct potent, selective, and cell-penetrating AMPDA inhibitors. The best compounds result from substitution of an alkylcarboxy group at N3 of the aglycon, wherein the alkyl chain is at least five atoms long. The CO_2H group is critical to potency and selectivity vs ADA. We propose that the CO_2H group has through-space electrostatic interactions with the phosphate binding site of AMPDA. Affinity gained through TS mimicry from the inhibitors probably compensates for the affinity not accessed from direct binding into the phosphate pocket. TS mimicry also makes the compounds selective vs other AMP- and adenosine-binding proteins.[1] These compounds provide an entry point from which to examine the effects of AMPDA inhibition on cellular function. Inhibitors of AMPDA in this series have been shown to increase both intra- and extracellular adenosine and preserve the adenylate pool in hepatocytes subjected to conditions that induce ATP breakdown.[1] The inhibitors did not alter adenosine and adenylate concentrations under unstressed or normal physiological conditions. This indicates that these compounds may be potential site- and event-specific antiischemic drugs.

Experimental Section

General Methods. Glassware for moisture-sensitive reactions was flame-dried and cooled to room temperature in a desiccator and all reactions were carried out under an atmosphere of nitrogen. Anhydrous solvents were purchased from Aldrich and stored over 4A molecular seives. THF was freshly distilled from Na/benzophenone ketyl under nitrogen. Flash chromatography was performed on 230−400 mesh EM Science silica gel 60. Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR were obtained on a Varian Gemini-200 operating at 200 and 50 MHz, respectively. ^{1}H and ^{13}C NMR spectra were recorded in units δ with tetramethylsilane (δ 0.00) and $CDCl_3$ (δ 77.0) or DMSO-d_6 (δ 39.9) as reference line internal standards, respectively. UV spectra (200−350 nM) of methanolic solutions were recorded on a Kontron Uvikon 860. Analytical HPLC was performed on a 4.6- × 250-mm YMC ODS-AQ 5-μm column eluting with a 0.1% aqueous AcOH/MeOH gradient and the detector set at 280 nM. C, H, N microanalyses were performed by NuMega Resonance Labs, Inc., San Diego, CA, or by Robertson Microlit Laboratories, Inc., Madison, NJ. Low-resolution mass spectral (LRMS) analyses were performed at Mass Consortium, San Diego, CA.

Enzyme Assays. The AMPDA and ADA K_i determinations were performed as previously described.[1]

DOWEX 1×8-400 Acetate Ion-Exchange Resin. DOWEX 1×8-400 ion-exchange resin (chloride form) was first slurried with about 3−4 volumes of water and the fine particles which did not settle rapidly were decanted off and discarded. This was repeated until no significant suspension of fine particles was evident (3−4 times). The resin was transferred as an aqueous slurry to a coarse glass fritted funnel and gravity eluted with 10 volumes of 3 M aqueous NaOH, 10 volumes of 3 M aqueous acetic acid and 20 volumes of water (final pH 6−7). The resin was stored at 5 °C in water.

Ethyl 2-(3-Bromophenylmethyl)-6-methanesulfonoxy-2-methylhexanoate (11d). Step 1. To ϵ-caprolactone (50.0 g, 0.44 mol) in 1.7 L of dioxane was added NaOH (0.88 L of a 1 M aqueous solution) and the mixture stirred at 25−35 °C for 16 h. The mixture was diluted with 1 L of water and extracted with EtOAc (3 × 0.7 L). Under ice bath cooling, the pH of the aqueous layer was lowered to 1 with the addition of 96 mL of concentrated HCl then extracted with EtOAc (5 × 0.7 L). The combined extracts were dried ($MgSO_4$) and evaporated to provide 53.4 g (95%) of 6-hydroxyhexanoic acid (**10a**) as an oil: ^{1}H NMR (D_2O/NaOD with 3-(trimethylsilyl)proprionic acid, sodium salt as internal standard) 1.25−1.45 (m, 2), 1.5−1.7 (m, 4), 2.20 (t, 2, J = 7 Hz), 3.60 (t, 2, J = 7 Hz).

Step 2. A mixture of 6-hydroxyhexanoic acid (27.9 g, 0.21 mol), iodoethane (20.8 mL, 0.26 mol) and K_2CO_3 (43.7 g, 0.32 mol) in 0.4 L of DMF was stirred at 80 °C for 4 h, cooled, diluted with 1 L of water and extracted with EtOAc (3 × 0.5 L). The combined extracts were dried ($MgSO_4$) and evaporated to provide 40.0 g of ethyl 6-hydroxyhexanoate (**10b**) as an oil contaminated by residual DMF. It was used directly in the next reaction without further purification: ^{1}H NMR (DMSO-d_6) 1.17 (t, 3, J = 7 Hz), 1.2−1.6 (m, 6), 2.26 (t, 2, J = 7 Hz), 3.37 (q, 2, J = 7 Hz), 4.04 (q, 2, J = 7 Hz), 4.36 (t, 1, J = 7 Hz).

Step 3. A mixture of ethyl 6-hydroxyhexanoate (6.12 g, 38 mmol), *tert*-butyldimethylsilyl chloride (5.76 g, 0.38 mmol) and imidazole (2.72 g, 39.9 mmol) in 100 mL of DMF was stirred at 23 °C for 16 h, then diluted with 300 mL of Et_2O and washed with 500 mL of water. The aqueous layer was separated and extracted with 100 mL of Et_2O. The combined organic extracts were washed with 5% aqueous HCl, water, and brine, dried ($MgSO_4$) and evaporated to provide 10.09 g (97%) of ethyl 6-*tert*-butyldimethylsilyloxyhexanoate (**10c**) as an oil: ^{1}H NMR (DMSO-d_6) 0.01 (s, 6), 0.85 (s, 9), 1.13 (t, 3, J = 7 Hz), 1.2−1.6 (m, 6), 2.26 (t, 2, J = 7 Hz), 3.56 (t, 2, J = 7 Hz), 4.03 (q, 2, J = 7 Hz).

Step 4. To a solution of diisopropylamine (10.0 mL, 71.2 mmol) in 77 mL of THF at −78 °C was added *n*-BuLi (7.12 mL of a 10 M solution in hexanes, 71.2 mmol) and the mixture stirred at −78 °C for 15 min. To this solution was added a solution of ethyl 6-*tert*-butyldimethylsilyloxyhexanoate (17 g, 61.9 mmol) in 30 mL of THF over a period of 40 min by syringe pump injection. After stirring for an additional 45 min at −78 °C, 9 mL of DMPU was added followed by the addition of iodomethane (4.8 mL, 77 mmol) and then the temperature raised to −15 °C and stirring continued for 1 h when it was quenched with 4 M NH_4Cl. The solvent was concentrated to ca. 40 mL by rotary evaporation and then diluted with 200 mL of Et_2O and extracted with water. The aqueous layer was separated and extracted with 100 mL of Et_2O. The combined ether extracts were washed with 5% aqueous HCl, water, and brine, dried ($MgSO_4$) and evaporated to provide 17.2 g (96%) of ethyl 6-*tert*-butyldimethylsilyloxy-2-methylhexanoate **(11a)** as an oil: ^{1}H NMR (DMSO-d_6) 0.01 (s, 6), 0.85 (s, 9), 1.05 (d, 3, J = 7 Hz), 1.17 (t, 3, J = 7 Hz), 1.2−1.7 (m, 6), 2.38 (q, 2, J = 7 Hz), 3.55 (t, 2, J = 7 Hz), 4.04 (q, 2, J = 7 Hz).

Step 5. In a manner identical to step 4, ethyl 6-*tert*-butyldimethylsilyloxy-2-methylhexanoate (4.47 g, 15.5 mmol) and 3-bromobenzyl bromide (4.65 g, 18.6 mmol) were combined to produce 7.36 g of crude ethyl 2-(3-bromobenzyl)-6-*tert*-butyldimethylsilyloxy-2-methylhexanoate **(11b)** as an oil which was used directly in the next reaction: ^{1}H NMR (DMSO-d_6) 0.01 (s, 6), 0.85 (s, 9), 0.98 (s, 3), 1.17 (t, 3, J = 7 Hz), 1.2−1.7 (m, 6), 2.66 (d, 1, J = 13 Hz), 2.93 (d, 1, J = 13 Hz), 3.56 (t, 2, J = 6 Hz), 4.04 (q, 2, J = 7 Hz), 7.09 (d, 1, J = 8 Hz), 7.19 (t, 1, J = 8 Hz), 7.27 (s, 1), 7.36 (d, 1, J = 8 Hz).

Step 6. A mixture of crude ethyl 2-(3-bromobenzyl)-6-*tert*-butyldimethylsilyloxy-2-methylhexanoate (7.36 g, ca. 15.5 mmol) and tetrabutylammonium fluoride (24 mL of a 1 M solution in THF) in 55 mL THF was stirred at 23 °C for 4 h and then diluted with Et_2O, washed with water and brine, dried ($MgSO_4$) and evaporated. Chromatography of the residue and elution with a hexane/EtOAc gradient of 5:1, 4:1, 3:1 and 2:1 provided 2.63 g (49%) of ethyl 2-(3-bromobenzyl)-6-hydroxy-2-methylhexanoate **(11c)** as an oil: ^{1}H NMR (DMSO-d_6) 1.02 (s, 3), 1.21 (t, 3, J = 7 Hz), 1.1−1.8 (m, 6), 2.69 (d, 1, J = 13 Hz), 2.98 (d, 1, J = 13 Hz), 3.42 (q, 2, J = 7 Hz), 4.09 (q, 2, J = 7 Hz), 4.41 (t, 1, J = 7 Hz), 7.14 (d, 1, J = 8 Hz), 7.28 (t, 1, J = 8 Hz), 7.32 (s, 1), 7.45 (d, 1, J = 8 Hz).

Step 7. To an ice bath-cooled solution of ethyl 2-(3-bromobenzyl)-6-hydroxy-2-methylhexanoate (2.5 g, 7.3 mmol) and triethylamine (1.4 mL, 9.8 mmol) in 10 mL of CH_2Cl_2 was added methanesulfonyl chloride (0.71 mL, 9.1 mmol). The mixture was then stirred at 23 °C for 1 h, diluted with CH_2Cl_2, washed with water and brine, dried ($MgSO_4$) and evaporated to provide 3.06 g (99%) of ethyl 2-(3-bromophenylmethyl)-6-methanesulfonoxy-2-methylhexanoate **(11d)** as an oil: ^{1}H NMR (DMSO-d_6) 1.03 (s, 3), 1.20 (t, 3, J = 7 Hz), 1.2−1.8 (m, 6), 2.70 (d, 1, J = 13 Hz), 3.00 (d, 1, J = 13 Hz), 4.04 (q, 2, J = 7 Hz), 4.16 (t, 2, J = 7 Hz), 7.15 (d, 1, J = 8 Hz), 7.27 (t, 1, J = 8 Hz), 7.30 (s, 1H), 7.47 (d, 1, J = 8 Hz).

This general method was also used to prepare the electrophiles used as coupling partners for the preparation of compounds **7j**−**7p** and **7u**−**7x**.

In a manner identical to step 4 of the preparation of compound **11d**, compounds **12a**, **12b**, **14a** and **14b** were combined with 2 equiv of 1,4-dibromobutane to provide respectively compounds **13a**, **13b**, **15a** and **15b.**

Resolution of 2-(3-Bromophenylmethyl)-6-hydroxy-2-methylhexanoic Acid. (2*R* or *S*)-2-(3-Bromophenylmethyl)-6-hydroxy-2-methylhexanoic Acid (11e). A solution of methyl 2-(3-bromophenylmethyl)-6-hydroxy-2-methylhexanoate (12.6 g, 33 mmol, prepared as in the preparation of **11d**) in 130 mL of dioxane and 66 mL of 2 M aqueous NaOH was stirred for 16 h at 100 °C, then cooled and diluted with water and extracted with EtOAc. The pH of the aqueous layer was lowered to 2 with aqueous HCl and extracted with EtOAc (4 × 0.3 L) and the organic layer dried ($MgSO_4$) and evaporated to provide 6.60 g (63%) of 2-(3-bromophenylmethyl)-6-hydroxy-2-methylhexanoic acid: ^{1}H NMR ($CDCl_3$) 1.10 (s, 3, α-CH_3), 1.1−1.8 (m, 6, C3, C4 and C5 Hs), 2.69 and 3.02 (d, 1 each, J = 14 Hz, benzylic Hs), 3.66 (t, 2, J = 7 Hz, C6 Hs), 5.37 (br s, 1, O−H), 7.0−7.4 (m, 4, Ar Hs).

A mixture of 2-(3-bromophenylmethyl)-6-hydroxy-2-methylhexanoic acid (2.07 g, 6.6 mmol) and (−)-brucine (2.46 g, 6.24 mmol) in 35 mL of CH_3CN was heated at 80 °C until solution was obtained and then cooled to 30 °C and the resulting crystals were collected by filtration to provide 1.6 g of the partially resolved carboxylate (−)-brucine salt. This was redissolved in 30 mL of CH_3CN at 80 °C and allowed to gradually cool to 30 °C to provide a white powder (mp 167−169 °C) which was collected and dissolved in water and the pH lowered to 2. Extraction of the aqueous mixture with EtOAc, drying ($MgSO_4$) the organic extract and evaporation provided 701 mg (34%) of the resolved carboxylate **11e** as a gum. ^{1}H NMR ($CDCl_3$) of a 1:1 complex of this acid with (*S*)-(−)-α-methylbenzylamine, characteristic shifts: 0.96 (s, 3, α-CH_3), 2.57 and 2.94 (d, 1 each, J = 13 Hz, benzylic Hs). The first CH_3CN filtrate was processed in a manner similar to provide a sample enriched in the opposite enantiomer. ^{1}H NMR ($CDCl_3$) of a 1:1 complex of this acid mixture with (*S*)-(−)-α-methylbenzylamine showed characteristic shifts of the enantiomer which does not crystallize first with (−)-brucine: 0.98 (s, 3, α-CH_3), 2.54 and 2.90 (d, 1 each, J = 13 Hz, benzylic Hs).

This compound was converted to the resolved analogue of **11d** by standard procedures (step 2 with iodomethane and step 7 in the preparation of compound **11d**) and used to synthesize compound **7w**.

5-Carbobenzyloxy-1-chloro-7-methyl-5-(2-methylpropyl)octane (18). **Step 1**. A mixture of isopropylcyclohexylamine (5.1 mL, 31.1 mmol) and *n*-BuLi (3.1 mL of a 10 M solution in hexanes, 31 mmol) in 30 mL of THF was stirred at −78 °C for 15 min (preparation of LICA) and then *tert*-butyl acetate **16** (4 mL, 29.6 mmol) was added over a period of 30 min by syringe pump injection. This mixture was stirred for an additional 30 min at −78 °C while the solution went from cloudy to pale yellow and homogeneous. This solution was cannulated into a solution of 1-iodo-2-methylpropane (3.6 mL, 31.1 mmol) in 30 mL of DMSO at room temperature which produced a white suspension which was stirred for 50 min and then diluted with 20 mL of 4 M NH_4Cl. This mixture was extracted with ether (2 × 200 mL) and the ether extract washed with 5% aqueous HCl, water, and brine, dried ($MgSO_4$) and evaporated to provide 3.13 g (61%) of *tert*-butyl 4-methylpentanoate as an oil: ^{1}H NMR ($CDCl_3$) 0.86 (d, 6, J = 7 Hz), 1.4−1.6 (m, 3), 1.41 (s, 9), 2.1−2.25 (m, 2).

Step 2. This reaction was conducted as for step 1 except that *tert*-butyl 4-methylpentanoate (2.87 g, 16.6 mmol) was added as a solution in 6 mL of THF slowly to the LICA (17.5 mmol) in 16 mL of THF at −78 °C and after stirring this for 30 min it was added to the 1-iodo-2-methylpropane (2.0 mL, 17.5 mmol) in 16 mL of DMSO. Workup as for step 1 provided 3.25 g (86%) of *tert*-butyl 2-(2-methylpropyl)-4-methylpentanoate **(17)** as an oil sufficiently pure for the next step: ^{1}H NMR ($CDCl_3$) 0.87 (t, 12, J = 7 Hz), 1.4−1.6 (m, 6), 1.41 (s, 9), 2.38 (m, 1).

Step 3. To a mixture of diisopropylamine (1.7 mL, 12 mmol) in 12 mL of THF and 6 mL of DMPU at 5 °C was added *n*-BuLi (1.16 mL of a 10 M solution in hexanes, 11.64 mmol). To this solution was added slowly a solution of *tert*-butyl 2-(2-methylpropyl)-4-methylpentanoate (886 mg, 3.88 mmol) in 3 mL of THF and this mixture was allowed to stir at 5 °C for 2 h to provide an orange solution. Then the solution was warmed to 55 °C and 4-chloro-1-iodobutane (1.9 mL, 15.52 mmol) was added which initiated a reflux and dispersed the color to yellow. The temperature was maintained at 55 °C for 4 h and then the mixture was cooled to room temperature, diluted with aqueous 4 M NH_4Cl and extracted with ether (2 × 100 mL); the ether extract was washed with 5% aqueous HCl, water, and brine, dried ($MgSO_4$) and evaporated to provide 1.17 g of residue which by ^{1}H NMR appeared to be a 3:1 mixture of product to starting ester. This residue was refluxed in a mixture of 19 mL of CH_2Cl_2 and 1.5 mL of TFA for 3 h and the mixture cooled, evaporated, diluted with EtOAc and extracted with aqueous 10% K_2CO_3; the aqueous extract was washed with EtOAc. The pH of the basic aqueous layer was lowered to pH 2 with aqueous 5% HCl and then extracted with EtOAc (3 × 100 mL). These combined EtOAc extracts were washed with brine, dried ($MgSO_4$) and evaporated to provide 940 mg of a residue predominantly 5-carboxy-1-chloro-7-methyl-5-(2-methylpropyl)octane: ^{1}H NMR ($CDCl_3$) 0.85 (d, 6, J = 6 Hz), 0.88 (d, 6, J = 6 Hz), 1.1−1.9 (m, 12), 3.55 (t, 2, J = 7 Hz). This residue (0.94 g) was mixed with benzyl bromide (0.64 mL, 5.4 mmol) and K_2CO_3 (1 g, 7.2 mmol) in 20 mL of DMF for 18 h at room temperature. Then the mixture was diluted with water and extracted with ether (2 × 100 mL) and the ether extract washed with water and brine, dried ($MgSO_4$) and evaporated to provide 1.03 g of residue which was subjected to chromatography eluting with hexane/EtOAc mixtures of 100:0 (300 mL), 100:1 (450 mL) and 75:1 (500 mL) which provided 360 mg (26% from **17**) of the title ester **18** as an oil: ^{1}H NMR ($CDCl_3$) 0.78 (d, 6, J = 6 Hz), 0.86 (d, 6, J = 6 Hz), 1.4−1.8 (m, 12), 3.52 (t, 2, J = 7 Hz), 5.05 (s, 2), 7.36 (m, 5); MS found [MH^+] 353/355. Anal. ($C_{21}H_{33}ClO_2$) C, H.

5-(5-Bromopentyl)-2-(2-(trimethylsilyl)ethoxymethyl)-tetrazole (19b). **Step 1**. A 60% suspension of NaH in mineral oil (1.26 g, 31.4 mmol) was slurried with 4 mL of dry hexane, the hexane decanted by syringe under N_2 (2×) and the solid dried under a high vacuum. To the solid was added 100 mL of DMF and while the suspension stirred at 5 °C a solution of tetrazole (2.00 g, 28.5 mmol) in 40 mL of DMF was

added. After this mixture stirred for 30 min, H_2 evolution had ceased. The reagent 2-(trimethylsilyl)ethoxymethyl chloride (5.30 mL, 29.9 mmol) was added and the mixture allowed to stir at room temperature for 16 h. Then the mixture was diluted with 250 mL of ether, washed with water (2 × 200 mL) and brine, dried ($MgSO_4$) and evaporated to provide an oil which was subjected to chromatography eluting with a hexane/EtOAc gradient of 20:1, 15:1, and 10:1 to provide 1.3 g (23%) of the less polar regioisomer assigned as 2-(2-(trimethylsilyl)ethoxymethyl)tetrazole, **19a**: ^{1}H NMR ($CDCl_3$) 0.04 (s, 9), 0.96 (t, 2, J = 7 Hz), 3.72 (t, 2, J = 7 Hz), 5.95 (s, 2), 8.60 (s, 1).

Step 2. To a solution of **19a** (500 mg, 2.5 mmol) in 10 mL of THF and 10 mL of DMPU at −78 °C was added *t*-BuLi (3.67 mL of a 1.7 M solution in hexanes, 6.24 mmol) which produced an orange suspension. This was allowed to stir at −78 °C for 5 min and then 1,5-dibromopentane (1.71 mL, 12.5 mmol) was added; this mixture stirred at −23 °C for 7 h to produce a yellow solution. Aqueous NH_4Cl was added and the mixture extracted with ether (2 × 100 mL). The combined ether extracts were washed with water (2 × 200 mL) and brine, dried ($MgSO_4$) and evaporated to provide an oil which was subjected to chromatography eluting with a hexane/EtOAc gradient of 20:0, 20:1, 10:1 and 5:1 which provided 468 mg of 75% pure **19b** with the 25% impurity being **19a**. This material was used to alkylate compound **4** without further purification. Compound **19b**: ^{1}H NMR ($CDCl_3$) 0.02 (s, 9), 0.97 (t, 2, J = 7 Hz), 1.57 (quintet, 2, J = 7 Hz), 1.8−2.1 (m, 4), 2.98 (t, 2, J = 7 Hz), 3.45 (t, 2, J = 7 Hz), 3.71 (t, 2, J = 7 Hz), 5.86 (s, 2).

Dibenzyl 5-Bromopentylphosphonate (21b). A 60% suspension of NaH in mineral oil (2.00 g, 50 mmol) was slurried with 5 mL of dry hexane, the hexane decanted by syringe under N_2 (2×) and the solid dried under a high vacuum. To the solid was added 250 mL of DMF and while this suspension stirred at 5 °C, dibenzyl phosphite **(21a)** (10.0 mL, 45.5 mmol) was added slowly. After this mixture stirred for 1 h, 1,5-dibromopentane (7.5 mL, 55 mmol) was added at 5 °C and the resulting mixture allowed to stir at room temperature for 16 h, then it was diluted with ether, washed with water (2×) and brine, dried ($MgSO_4$) and evaporated to provide an oil which was subjected to chromatography eluting first with CH_2Cl_2 and then with 18:1 CH_2Cl_2/MeOH to provide 9.03 g (49%) of the title compound as an oil: ^{1}H NMR ($CDCl_3$) 1.3−2.0 (m, 8), 3.46 (t, 2, J = 7 Hz), 4.9−5.1 (m, 4), 7.25−7.45 (m, 10).

This method was also used to prepare the phosphonate electrophiles **20b** and **21c**.

3-(6-(3-Bromophenyl)-5-carbethoxy-5-methylhexyl)coformycin Aglycon (7u). Step 1, Alkylation. According to the method previously described[1] ketone **4** (1.09 g, 7.3 mmol) was alkylated with **11d** (3.07 g, 7.3 mmol). Chromatography on SiO_2 with a CH_2Cl_2/methanol gradient of 20:1, 18:1 and 16:1 provided 1.08 g (31%) of 3-(6-(3-bromophenyl)-5-carbethoxy-5-methylhexyl)-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one **(5u)** as a light yellow gum: ^{1}H NMR (DMSO-d_6) 0.96 (s, 3), 1.0−1.6 (m, 4), 1.13 (t, 3H, J = 7 Hz), 1.70 (quin, 2, J = 7 Hz), 2.65 (d, 1, J = 13 Hz), 2.92 (d, 1, J = 13 Hz), 3.73 (d, 2, J = 4 Hz), 3.94 (t, 2, J = 7 Hz), 4.02 (q, 2, J = 7 Hz), 7.08 (d, 1, J = 8 Hz), 7.23 (t, 1, J = 8 Hz), 7.27 (s, 1), 7.42 (d, 1, J = 8 Hz), 7.43 (d, 1, J = 4 Hz), 7.62 (s, 1), 8.34 (br m, 1).

Step 2, Reduction. According to the method previously described[1] **5u** (1.08 g, 2.27 mmol)was reduced with $NaBH_4$ (106 mg, 2.72 mmol). Chromatography on SiO_2 with a CH_2Cl_2/methanol/triethylamine gradient of 25:1:0.25 and 20:1:0.2 provided a fraction which was reduced to a minimal volume and diluted with ether. The resulting solid was collected to provide 802 mg (74%) of **7u** as a white solid: mp 133−136 °C; ^{1}H NMR (DMSO-d_6) 0.97 (s, 3), 1.15 (t, 3H, J = 7 Hz), 1.2−1.8 (m, 6), 2.65 (d, 1, J = 13 Hz), 2.93 (d, 1, J = 13 Hz), 3.15 (br s, 2), 3.84 (t, 2, J = 7 Hz), 4.03 (q, 2, J = 7 Hz), 4.80 (br s, 1), 4.90 (br s, 1), 6.96 (d, 1, J = 4 Hz), 7.08 (d, 1, J = 8 Hz), 7.24 (t, 1, J = 8 Hz), 7.28 (s, 1), 7.42 (d, 1, J = 8 Hz), 7.45 (br s, 1). Anal. ($C_{22}H_{29}BrN_4O_3$) C, H, N.

This method was used to prepare all compounds **7** or the ester precursors of compounds **7** and compound **6f′** (the reduced product of **6f** in Figure 2).

Compound **7f**: mp 115−118 °C; ^{1}H NMR (DMSO-d_6) 1.17 (t, 3, J = 7 Hz), 1.2−1.8 (m, 6), 2.26 (t, 2, J = 7 Hz), 3.15 (br s, 2), 3.84 (t, 2, J = 7 Hz), 4.03 (q, 2, J = 7 Hz), 4.80 (br s, 1), 4.91 (d, 1, J = 6 Hz), 6.95 (d, 1, J = 4 Hz), 7.28 (s, 1), 7.45 (d, 1, J = 4 Hz); UV (methanol) λ_{max} 283 nm (ϵ 6545). Anal. ($C_{14}H_{22}N_4O_3$) C, H, N.

Compound **6f′**: ^{1}H NMR (DMSO-d_6) 1.17 (t, 3, J = 7 Hz), 1.2−1.8 (m, 6), 2.27 (t, 2, J = 7 Hz), 3.36 (br s, 2), 3.79 (t, 2, J = 7 Hz), 4.04 (q, 2, J = 7 Hz), 4.62 (br s, 1), 5.30 (br s, 1), 7.12 (s, 1), 8.00 (s, 1); UV (methanol) λ_{max} 278 nm (ϵ 4680); APCI MS (positive mode) M + 1 calcd 295, found m/z 295.

Isolation of Representative N1- and N3-Alkylation Products. 3-(5-Carbethoxypentyl)-6,7-dihydroimidazo-[4,5-*d*][1,3]diazepin-8(3*H*)-one (5f) and 1-(5-Carbethoxypentyl)-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(1*H*)-one (6f). Reaction of the base **4** (37.8 mmol) with ethyl 6-bromohexanoate (37.8 mmol) as previously described[1] provided an N3/N1-alkylation mixture which was subjected to chromatography on SiO_2 eluting with a CH_2Cl_2/methanol gradient of 20:1, 17.5:1 and 15:1 to provide a fraction which was reduced to a minimal volume and diluted with ether. The resulting solid was collected to provide 1.30 g of the N3-isomer **5f** as a tan solid. The ^{1}H and ^{13}C NMR assignments were made based in part on ^{1}H−^{1}H coupling (COSY) and ^{1}H−^{13}C coupling (HETCOR) two-dimensional NMR experiments. Compound **5f**: mp 128−131 °C; ^{1}H NMR (DMSO-d_6) 1.17 (t, 3, J = 7 Hz, ester CH_3), 1.25 (quin, 2, J = 7 Hz, C3′ Hs), 1.55 (quin, 2, J = 7 Hz, C4′ Hs), 1.71 (quin, 2, J = 7 Hz, C2′ Hs), 2.28 (t, 2, J = 7 Hz, C5′ Hs), 3.78 (d, 2, J = 4 Hz, C7 Hs), 3.96 (t, 2, J = 7 Hz, C1′ Hs), 4.04 (q, 2, J = 7 Hz, ester CH_2), 7.45 (d, 1, J = 4 Hz, H5), 7.67 (s, 1, H2), 8.4−8.5 (m, 1, H6); ^{13}C NMR (DMSO-d_6) 14.1 (ester CH_3), 24.0 (C4′), 25.4 (C3′), 29.4 (C2′), 33.3 (C5′), 42.4 (C1′), 51.5 (C7), 59.7 (ester CH_2), 128.7 (C8a), 136.3 (C2), 146.4 (C3a), 151.1 (C5), 172.8 (C6′), 181.1 (C8); UV (methanol) λ_{max} 231 nm (ϵ 14300), 235 (14300), 302 (4,700). Anal. ($C_{14}H_{20}N_4O_3$) C, H, N.

Further elution with 15:1 CH_2Cl_2/methanol provided 2.04 g of a mixture of N3- and N1-isomers (**5f** and **6f**). This was subjected to MPLC eluting with a CH_2Cl_2/methanol gradient of 17.5:1 and 15:1 which provided 875 mg of additional **5f**. The total yield of **5f** was 2.17 g (20%). Further elution with 12.5:1 provided a fraction which was reduced to a minimal volume and diluted with ether. The resulting solid was collected to provide 676 mg (6%) of the N1-isomer **6f** as orange crystals. The ^{1}H and ^{13}C NMR assignments were made based in part on ^{1}H−^{1}H coupling (COSY) and ^{1}H−^{13}C coupling (HETCOR) two-dimensional NMR experiments. Compound **6f**: mp 113−116 °C; ^{1}H NMR (DMSO-d_6) 1.17 (t, 3, J = 7 Hz, ester CH_3), 1.25 (quin, 2, J = 7 Hz, C3′ Hs), 1.53 (quin, 2, J = 7 Hz, C4′ Hs), 1.67 (quin, 2, J = 7 Hz, C2′ Hs), 2.27 (t, 2, J = 7 Hz, C5′ Hs), 3.8 (br s, 2, C7 Hs), 4.04 (q, 2, J = 7 Hz, ester CH_2), 4.18 (t, 2, J = 7 Hz, C1′ Hs), 7.37 (br s, 1, H5), 7.84 (s, 1, H2), 8.2 (br s, 1/2, H6), 10.6 (br s, 1/2, H4); ^{13}C NMR (DMSO-d_6) 14.1 (ester CH_3), 23.9 (C4′), 25.1 (C3′), 30.1 (C2′), 33.3 (C5′), 46.3 (C1′), 55.5 (br s, C7), 59.6 (ester CH_2), 117.7 (C8a), 141.5 (C2), 153.5 (br s, C3a), 148.5 (C5), 172.7 (C6′), 181.2 (C8); UV (methanol) λ_{max} 231 nm (ϵ 17100), 235 (16900), 301 (5020). Anal. ($C_{14}H_{20}N_4O_3$) C, H, N.

Alternate Method for the Preparation of 3-Alkyl-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-ones. 3-Benzyl-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (5f′). To a suspension of 2-amino-1-(1-benzyl-5-amino-1*H*-imidazol-4-yl)ethanone dihydrochloride **(8)**[16a] (1.00 g, 3.3 mmol) in 16.5 mL of DMSO at 70 °C was added triethyl orthoformate (2.74 mL, 16.5 mmol). After 15 min the resulting solution was cooled to room temperature and stirred with Darco G-60 charcoal and filtered. The filtrate was concentrated under high vacuum distillation to a 5 mL volume and then poured onto ether. The solvent was decanted off of the resulting red oil and it was mixed with methanol. The resulting white precipitate was filtered and the filtrate concentrated and then diluted

with CH_2Cl_2 and applied to the head of a chromatography column previously eluted with 10:1 CH_2Cl_2/methanol. Elution with 10:1 CH_2Cl_2/methanol provided 150 mg (19%) of **5f′** as a tan solid: ^{1}H NMR (DMSO-d_6) 3.76 (d, 2, J = 4 Hz), 5.20 (s, 2), 7.1−7.4 (m, 5), 7.44 (d, 1, J = 4 Hz), 7.74 (s, 1), 8.41 (br s, 1).

3-(5-Carboxy-5-methyl-6-(3-bromophenyl)hexyl)coformycin Aglycon (7v). The ester **7u** (614 mg, 1.29 mmol) was hydrolyzed and isolated with DOWEX 1×8-400 acetate ion-exchange resin as previously described[1] to provide 440 mg (69%) of the carboxylate **7v** as a pink solid: R_f 0.51 (3:1 CH_3CN/0.2 N NH_4Cl); mp 83−88 °C; ^{1}H NMR (DMSO-d_6) 0.92 (s, 3), 1.0−1.7 (m, 6), 2.61 (d, 1, J = 12 Hz), 2.92 (d, 1, J = 12 Hz), 3.16 (br s, 2), 3.84 (t, 2, J = 7 Hz,), 4.81 (br s, 1), 6.97 (d, 1, J = 4 Hz), 7.13 (d, 1, J = 8 Hz), 7.21 (t, 1, J = 8 Hz), 7.33 (s, 1,), 7.29 (s, 1), 7.40 (d, 1, J = 8 Hz). Anal. ($C_{20}H_{25}BrN_4O_3\cdot0.8CH_3CO_2H\cdot1H_2O$) C, H, N.

This method was also used to prepare carboxylates **7j**, **7k**, **7m**−**7p**, **7r**, **7t** and **7v**−**7x**. Note: in general, hydrolysis of methyl esters was more rapid.

Alternatively, in a limited number of examples the aqueous layer was neutralized with Amberlite CG-50 resin and filtered and the filtrate lyophilized to provide the sodium salt of the acid as a deliquescent solid. This solid was handled as a suspension under ether or acetone; then the solvent was decanted off and the solid dried under a high vacuum. This method was used to prepare **7a**, **7b** and **7i**. This method produced compounds **7c** and **7d** as methanol-insoluble free acids.

Representative Benzyl Ester Hydrogenolysis Procedure. Preparation of 3-(5-Carboxy-5,5-diphenylpentyl)-coformycin Aglycon (7a′). 3-(5-Carbobenzyloxy-5,5-diphenylpentyl)coformycin aglycon (181 mg, 0.36 mmol) was hydrogenated (30 psi H_2) in 5 mL of methanol in the presence of 10% $Pd(OH)_2$ on carbon (25 mg) for 4 h. The mixture was filtered over Celite and solvent concentrated and then diluted with ether. The resulting solid was collected to provide 124 mg (74%) of **7a′** as a white solid: mp 200−201 °C; ^{1}H NMR (DMSO-d_6) 0.9−1.1 (m, 2), 1.5−1.7 (m, 2), 2.2−2.4 (m, 2), 3.13 (br s, 2), 3.76 (t, 2, J = 7 Hz), 4.77 (br s, 1), 6.93 (d, 1, J = 4 Hz), 7.1−7.5 (M, 11), 7.50 (m, 1). Anal. ($C_{24}H_{26}N_4O_3\cdot2.75H_2O$) C, H, N.

This method was also used to prepare carboxylates **7y** and **7z** and the phosphonates **7d′** and **7e′**.

3-(5-(Tetrazol-5-yl)pentyl)coformycin Aglycon Dicyclohexylammonium Salt (7b′). A mixture of 3-(5-(2-(2-(trimethylsilyl)ethoxymethyl)tetrazol-5-yl)pentyl)coformycin aglycon (14 mg, 0.033 mmol) (prepared according to the method described for the preparation of **7u**), n-Bu_4NF (0.05 mL of a 1 M solution in THF) and CsF (10 mg, 0.066 mmol) in 0.5 mL of DMF was stirred at 50 °C for 2 h and then the solvent removed by rotary evaporation. The residue was dissolved in water and slurried with DOWEX 1×8-400 acetate for 1 h and filtered. The resin was washed with water (3×) and then slurried with 0.05 M AcOH for 15 min and filtered (3×). The combined filtrates were lyophilized; the residue was dissolved in methanol and dicyclohexylamine (13 μL, 0.065 mmol) added. The mixture was evaporated and then suspended in acetone, the acetone decanted and suspended in ether, the ether decanted and the resulting solid dried under vacuum to provide 6 mg (39%) of **7b′** as a pale orange solid: mp 220 °C; ^{1}H NMR (DMSO-d_6) 1.0−2.0 (m, 26), 2.64 (t, 2, J = 7 Hz), 2.96 (m, 2), 3.15 (br s, 2), 3.82 (t, 2, J = 7 Hz), 4.79 (br s, 1), 6.96 (d, 1, J = 4 Hz), 7.27 (s, 1), 7.43 (m, 1). Anal. ($C_{12}H_{18}N_8O\cdot1C_{12}H_{23}N\cdot2H_2O$) C, H; N: calcd, 24.83; found, 23.80.

3-(6-(Monomethylphosphonoxy)hexyl)coformycin Aglycon (7c′). A mixture of 3-(6-(dimethylphosphonoxy)-hexyl)coformycin aglycon (98 mg, 0.28 mmol) and lithium methoxide (162 mg, 4.3 mmol) was stirred for 72 h at 50 °C and then the pH reduced to 1−2 with the addition of methanolic HCl. Reverse-phase (C-18) silica gel (450 mg) was added and the solvent evaporated. The resulting powder was loaded onto a column packed with 5 g of reverse phase silica gel in water. Elution with water and then with 4% methanolic water provided a fraction which was lyophilized to provide 20 mg (22%) of the desired monoacid **7c′** as a solid: mp 67−68 °C; ^{1}H NMR (DMSO-d_6) 1.2−1.8 (m, 10), 3.2−3.4 (m, 2), 3.53 (d, 3, J = 10 Hz), 4.19 (t, 2, J = 7 Hz), 4.9−5.0 (m, 1), 7.36 (d, 1, J = 4 Hz), 9.03 (s, 1). Anal. ($C_{13}H_{23}N_4O_4P\cdot2H_2O$) C, H, N.

3-(6-Hydroxyhexyl)coformycin Aglycon (7q′). To 3-(6-acetoxyhexyl)coformycin aglycon (90 mg, 0.32 mmol) was added 1.6 mL of a freshly prepared 0.5 M MeONa solution in MeOH (prepared by dissolving 230 mg of sodium in 10 mL of MeOH) and the mixture stirred for 30 min at room temperature, then neutralized with Amberlite CG-50 resin, and filtered; the filtrate was evaporated. The residual water was removed by lyophilization and the residue precipitated from a concentrate in methanol by the addition of ether. The solid was collected by filtration to provide 12 mg (15%) of the alcohol **7q′** as a white solid: mp 138−139 °C; ^{1}H NMR (DMSO-d_6) 1.2−1.7 (m, 8), 3.17 (m, 2), 3.85 (t, 2, J = 7 Hz), 4.35 (br s, 2), 4.82 (br s), 4.91 (d, 1, J = 6 Hz), 6.97 (d, 1, J = 4 Hz), 7.29 (s, 1), 7.4−7.5 (m, 1).

This method was also used to prepare compounds **7m′**−**7p′**.

3-(5-(Carbamoyl)pentyl)coformycin Aglycon (7r′). A solution of 3-(5-carbethoxypentyl)coformycin aglycon **(7f)** (60 mg, 0.20 mmol) in 2 mL of 15% NH_3/MeOH was heated in a closed bomb apparatus at 100 °C for 72 h, then cooled and evaporated. The product was precipitated from a concentrate in methanol by the addition of ether. The resulting solid was collected to provide 48 mg (87%) of the primary amide **7r′** as a tan solid: mp 159−163 °C; ^{1}H NMR (DMSO-d_6) 1.21 (quin, 2, J = 7 Hz), 1.48 (quin, 2, J = 7 Hz), 1.64 (quin, 2, J = 7 Hz), 2.02 (t, 2, J = 7 Hz), 3.1−3.2 (m, 2), 3.83 (t, 2, J = 7 Hz), 4.80 (t, 1, J = 3 Hz), 4.9 (br s, 1), 6.7 (br s, 1), 6.96 (d, 1, J = 4 Hz), 7.23 (br s, 1), 7.28 (s, 1), 7.45 (br d, 1, J = 4 Hz). Anal. ($C_{12}H_{19}N_5O_2\cdot0.4H_2O$) C, H, N.

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Supporting Information Available: Lists of key intermediates and electrophiles and ^{1}H NMR data of key intermediates and electrophiles. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For AMP Deaminase Inhibitors. 1, see: Erion, M. D.; Kasibhatla, S. R.; Bookser, B. C.; van Poelje, P. D.; Reddy, M. R.; Gruber, H. E.; Appleman, J. R. Discovery of AMP Mimetics that Exhibit High Inhibitory Potency and Specificity for AMP Deaminase. *J. Am. Chem. Soc.* **1999**, *121*, 308−319.

(2) Skladanowski, A. C. In *Myocardial Energy Metabolism*; de Jong, J. W., Ed.; Martinus Nijhoff: Dordrecht, 1988; pp 53−65.

(3) For lead references on adenosine-related therapeutics research, see: (a) *Adenosine and Adenosine Receptors*; Williams, M., Ed.; Humana Press: Clifton, NJ, 1990. (b) *Purinergic Approaches in Experimental Therapeutics*; Jacobson, K. A., Jarvis, M. F., Eds.; Wiley-Liss: New York, 1997.

(4) Loh, E.; Rebbeck, T. R.; Mahoney, P. D.; DeNofrio, D.; Swain, J. L.; Holmes, E. W. Common Variant in AMPD1 Gene Predicts Improved Clinical Outcome in Patients With Heart Failure. *Circulation* **1999**, *99*, 1422−1425.

(5) In addition to its potential antiischemic uses, recent reports indicate AMPDA inhibitors may be worthwhile to investigate as herbicidal and anti-Alzheimer's agents: (a) Lindell, S. D.; Moloney, B. A.; Hewitt, B. D.; Earnshaw, C. G.; Dudfield, P. J.; Dancer, J. E. The Design and Synthesis of Inhibitors of Adenosine-5′-Phosphate Deaminase. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1985−1990. (b) Sims, B.; Powers, R. E.; Sabina, R. L.; Theibert, A. B. Elevated Adenosine Monophosphate Deaminase Activity in Alzheimer's Disease Brain. *Neurobiol. Aging* **1998**, *19*, 385−391.

(6) (a) Hapke, D. M.; Stegmann, A. P. A.; Mitchell, B. S. Retroviral Transfer of Deoxycytidine Kinase into Tumor Cell Lines Enhances Nucleoside Toxicity. *Cancer Res.* **1996**, *56*, 2343−2347. (b) Maehara, Y.; Kusumoto, T.; Yasunori, E.; Anai, H.; Sakaguchi, Y.; Kohnoe, S.; Sugimachi, K. 5-Fluorouracil Is Converted to F−Nucleotides More Extensively and is More Cytotoxic in Poorly Differentiated than in Well Differentiated Human Gastric Carcinoma. *Anticancer Res.* **1990**, *10*, 1091−1094.

(7) (a) Starrett, J. E., Jr.; Tortolani, D. R.; Hitchcock, M. J. M.; Martin, J. C.; Mansuri, M. M. Synthesis and in vitro evaluation of a phosphonate prodrug: bis(pivaloyloxymethyl) 9-(2-phosphonylmethoxyethyl)adenine. *Antiviral Res.* **1992**, *19*, 267−273. (b) Srivastva, D. N.; Farquhar, D. Bioreversible Phosphate Protective Groups: Synthesis and Stability of Model Acyloxymethyl Phosphates. *Bioorg. Chem.* **1984**, *12*, 118−129. (c) Krise, J. P.; Stella, V. J. Prodrugs of phosphates, phosphonates and phosphinates. *Adv. Drug Delivery Rev.* **1996**, *19*, 287−310. (d) Jones, R. J.; Bischofberger, N. Minireview: nucleotide prodrugs. *Antiviral Res.* **1995**, *27*, 1−17.

(8) Isolation and identification: (a) Ohno, M.; Yagisawa, N.; Shibahara, S.; Kondo, S.; Maeda, K.; Umezawa, H. Structure of Coformycin, an Unusual Nucleoside of Microbial Origin. *J. Am. Chem. Soc.* **1974**, *96*, 4327−4328. The 8*R*/*S*-isomer was prepared for this study according to literature methods: (b) Hawkins, L. D.; Hanvey, J. C.; Boyd, F. L. Jr.; Baker, D. C. Inhibitors of Adenosine Deaminase. Synthesis of Coformycin and 3′-Deoxycoformycin. *Nucleosides Nucleotides* **1983**, *2*, 479−494. (c) Showalter, H. D. H.; Putt, S. R.; Baker, D. C. In *Nucleic Acid Chemistry*; Townsend, L. B., Tipson, R. S., Eds.; Wiley: New York, 1991; pp 308−313.

(9) Frieden, C.; Kurz, L. C.; Gilbert, H. R. Adenosine Deaminase and Adenylate Deaminase: Comparative Kinetic Studies with Transition State and Ground-State Analogue Inhibitors. *Biochemistry* **1980**, *19*, 5303−5309.

(10) (a) Hohl, C. M.; Wimsatt, D. K.; Brierley, G. P.; Altschuld IMP Production by ATP-Depleted Adult Rat Heart Cells Effects of Glycolysis and α_1-Adrenergic Stimulation. *Circ. Res.* **1989**, *65*, 754−760. (b) Bennett, L. L., Jr.; Brockman, R. W.; Allan, P. W.; Rose, L. M.; Shaddix, S. C. Alterations in Nucleotide Pools Induced by 3-Deazaadenosine and Related Compounds Role of Adenylate Deaminase. *Biochem. Pharmacol.* **1988**, *37*, 1233−1244. (c) Zoref-Shani, E.; Shainberg, A.; Sperling, O. Pathways of adenine nucleotide catabolism in primary rat muscle cultures. *Biochim. Biophys. Acta* **1987**, *926*, 287−295. (d) Ashby, B.; Wernick, E.; Holmsen, H. Coformycin Inhibition of Platelet AMP Deaminase Has No Effect on Thrombin-induced Platelet Secretion nor on Glycolysis or Glycogenolysis. *J. Biol. Chem.* **1983**, *258*, 321−325. (e) Fishbein, W. N.; Davis, J. I.; Winkert, J. W.; Strong, D. M. Levels of Adenosine Deaminase, AMP Deaminase, and Adenylate Kinase in Cultured Human Lymphoblast Lines: Exquisite Sensitivity of AMP Deaminase to Adenosine Deaminase Inhibitors. *Biochem. Med.* **1981**, *26*, 377−386. (f) Van den Berghe, G.; Bontemps, F.; Hers, H.-G. Purine Catabolism in Isolated Rat Hepatocytes Influence of Coformycin. *Biochem. J.* **1980**, *188*, 913−920.

(11) Cha, S.; Agarwal, R. P.; Parks, R. E., Jr. Tight Binding Inhibitors-II Non-Steady-State Nature of Inhibition of Milk Xanthine Oxidase by Allopurinol and Alloxanthine and of Human Erythrocytic Adenosine Deaminase by Coformycin. *Biochem. Pharmacol.* **1975**, *24*, 2187−2197.

(12) Kredich, N. M.; Hershfield, M. S. In *The Metabolic Basis of Inherited Disease*; Scrivner et al., Eds.; McGraw-Hill: New York, 1989; pp 1045−1075.

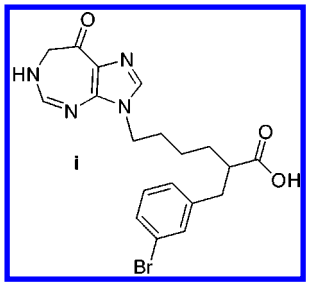
(13) Wolfenden, R. Analogue Approaches to the Structure of the Transition State in Enzyme Reactions. *Acc. Chem. Res.* **1972**, *5*, 10−18.

(14) (a) Marrone, T. J.; Straatsma, T. P.; Briggs, J. M.; Wilson, D. K.; Quiocho, F. A.; McCammon, J. A. Theoretical Study of Inhibition of Adenosine Deaminase by (8*R*)-Coformycin and (8*R*)-Deoxycoformycin. *J. Med. Chem.* **1996**, *39*, 277−284. (b) Kline, P. C.; Schramm, V. L. Electrostatic Potential Surfaces of the Transition State for AMP Deaminase and for (*R*)-Coformycin, a Transition State Inhibitor. *J. Biol. Chem.* **1994**, *269*, 22385−22390. (c) Kati, W. M.; Acheson, S. A.; Wolfenden, R. A Transition State in Pieces: Major Contributions of Entropic Effects to Ligand Binding by Adenosine Deaminase. *Biochemistry* **1992**, *31*, 7356−7366.

(15) See the following papers in this issue: (a) Kasibhatla, S. R.; Bookser, B. C.; Probst, G.; Appleman, J. R.; Erion, M. D. AMP Deaminase Inhibitors. 3. SAR of 3-(Carboxyarylalkyl)coformycin Aglycon Analogues. *J. Med. Chem.* **2000**, *43*, 1508−1518. (b) Bookser, B. C.; Kasibhatla, S. R.; Erion, M. D. AMP Deaminase Inhibitors. 4. Further N3-Substituted Coformycin Aglycon Analogues. N3-Alkylmalonates as Ribose 5'-Monophosphate Mimetics. *J. Med. Chem.* **2000**, *43*, 1519−1524.

(16) For literature relating to the synthesis of coformycin analogues, see ref 8 and: (a) Chan, E.; Putt, S. R.; Showalter, H. D. H.; Baker, D. C. Total Synthesis of (8*R*)-3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol (Pentostatin), the Potent Inhibitor of Adenosine Deaminase. *J. Org. Chem.* **1982**, *47*, 3457−3464. (b) Showalter, H. D. H.; Putt, S. R.; Borondy, P. E.; Shillis, J. L. Adenosine Deaminase Inhibitors. Synthesis and Biological Evaluation of (±)-3,6,7,8-Tetrahydro-3-[(2-hydroxyethoxy)methyl]imidazo[4,5-*d*][1,3]-diazepin-8-ol and Some Selected C-5 Homologues of Pentostatin. *J. Med. Chem.* **1983**, *26*, 1478−1482. (c) Smal, E. Synthesis, Biosynthesis and Studies Related to Compounds of Interest in Cancer Chemotherapy. Ph.D. Dissertation, University of Alabama, 1985. (d) Thomas, H. J.; Riordan, J. M.; Montgomery, J. A. The Synthesis of Coformycin from 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamide. *Nucleosides Nucleotides* **1986**, *5*, 431−439. (e) Saville-Stones, E. A.; Turner, R. M.; Lindell, S. D.; Jennings, N. S.; Head, J. C.; Carver, D. S. Total Synthesis of Carbocyclic Analogues of Coformycin. *Tetrahedron* **1994**, *50*, 6695−6704. (f) Truong, T. V.; Rapoport, H. Chirospecific Synthesis of the Tetrahydroimidazodiazepinol Aglycon of Pentostatin and Its Analogues. *J. Org. Chem.* **1993**, *58*, 6090−6096. (g) Truong, T. V. Chirospecific Synthesis of Pentostatin and Its Analogues. Ph.D. Dissertation, University of California, Berkeley, 1989.

(17) The $t_{1/2}$ values for the decomposition of example ketone **i** were determined at low pHs at $T = 30$ °C by following the change in UV absorbance: pH ($t_{1/2}$ min) 1 (0.3), 2 (3).



i

(18) Al-Razzak, L. A.; Benedetti, A. E.; Waugh, W. N.; Stella, V. J. Chemical Stability of Pentostatin (NSC-218321), a Cytotoxic and Immunosuppressant Agent. *Pharm. Res.* **1990**, *7*, 452−460.

(19) The $t_{1/2}$ values for the decomposition of example alcohol **7k′** were determined at low pHs at $T = 35$ °C by following the change in UV absorbance: pH ($t_{1/2}$ min) 1 (23), 2 (47). The pK_as for bisprotonated **7k′** were determined to be 6.5 and 2.1.

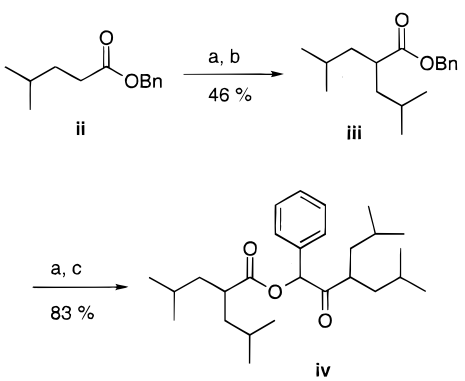
(20) (a) Iyer, R. S.; Voehler, M. W.; Harris, T. M. Adenine Adduct of Aflatoxin B_1 Epoxide. *J. Am. Chem. Soc.* **1994**, *116*, 8863−8869. (b) Pugmire, R. J.; Grant, D. M.; Townsend, L. B.; Robins, R. K. Carbon-13 Magnetic Resonance. XXII. The *N*-Methylpurines. *J. Am. Chem. Soc.* **1973**, *95*, 2791−2796.

(21) The electrophile used to prepare **7i** was synthesized according to a literature method: Shchaveleva, I. L.; Kochetkova, S. V.; Tsilevich, T. L.; Vladyko, G. V.; Korobchenko, L. V.; Boreko, E. I.; Smirnov, I. P.; Khorlin, A. A.; Gottikh, B. P.; Florent'ev, V. L. Preparation of Chloromethylalkylcarboxy Esters. *Sov. J. Bioorg. Chem.* **1990**, *15*, 523−527.

(22) Compound **14b** was prepared according to the method described by: (a) Chen, B.-C.; Lue, P. A Convenient Preparation of 5,5-Dialkyl Meldrum's Acids. *Org. Prep. Proced. Int.* **1992**, *24*, 185−188 and (b) Oikawa, Y.; Hirasawa, H.; Yonemitsu, O. Meldrum's Acid in Organic Synthesis. 1. A Convenient One-Pot Synthesis of Ethyl Indolepropionates. *Tetrahedron Lett.* **1978**, 1759−1762.

(23) Rathke, M. W.; Lindert, A. The Reaction of Lithium *N*-Isopropylcyclohexylamide with Esters. A Method for the Formation and Alkylation of Ester Enolates. *J. Am. Chem. Soc.* **1971**, *93*, 2318−2320.

(24) The application of the method used to prepare compounds **13** and **15** to the very hindered ester **iii** resulted in apparent deprotonation of the benzylic methylene, and the resulting anion Claisen-condensed with another molecule of ester to produce the unusual product **iv** in high yield[a]:



ii iii iv

[a] (a) 1.1 eq LDA, THF, -78 °C; (b) 1.1 eq $(CH_3)_2CHCH_2I$, DMPU, -5 °C; (c) 2 eq $Br(CH_2)_4Br$, DMPU, -5 °C.

Compound **iv**: ^{1}H NMR ($CDCl_3$) 0.8−1.0 (m, 24, methyls), 1.1−1.4 (m, 4, *i*-Bu methines), 1.5−1.8 (m, 8, methylenes), 2.55−2.8 (m, 2, C-α methines); MS found [MH^+] 417, [MNa^+] 439, [MK^+] 455. For a similar observation see: Upton, C. L.; Beak, P. Dipole Stabilized Carbanions. Reactions of Benzoate Esters with Lithium 2,2,6,6-Tetramethylpiperidide. *J. Org. Chem.* **1975**, *40*, 1094−1098.

(25) MacPhee, J. A.; Dubois, J.-E. Steric Limits to Ester Alkylation; Synthesis of Highly Hindered Esters via Hexamethylphosphoramide-favoured Enolization. *J. Chem. Soc., Perkin Trans. 1* **1977**, 694−696.

(26) For prior 5-lithiotetrazole chemistry see: (a) Yi, K. Y.; Yoo, S.-e. Synthesis of 5-Aryl and Vinyl Tetrazoles by the Palladium-Catalyzed Cross-Coupling Reaction. *Tetrahedron Lett.* **1995**, *36*, 1679−1682. (b) Satoh, Y.; Marcopulos, N. Application of 5-Lithiotetrazoles in Organic Synthesis. *Tetrahedron Lett.* **1995**, *36*, 1759−1762.

(27) Merkler, D. J.; Brenowitz, M.; Schramm, V. L. The Rate Constant Describing Slow-Onset Inhibition of Yeast AMP Deaminase by Coformycin Analogues Is Independent of Inhibitor Structure. *Biochemistry* **1990**, *29*, 8358−8364.

(28) Schramm, V. L.; Baker, D. C. Spontaneous Epimerization of (*S*)-Deoxycoformycin and Interaction of (*R*)-Deoxycoformycin, (*S*)-Deoxycoformycin, and 8-Ketodeoxycoformycin with Adenosine Deaminase. *Biochemistry* **1985**, *24*, 641−646.

(29) (a) Hampton, A.; Sasaki, T.; Perini, F.; Slotin, L. A.; Kappler, F. Design of Substrate-Site-Directed Irreversible Inhibitors of Adenosine 5′-Phosphate Aminohydrolase. Effect of Substrate Substituents on Affinity for the Substrate Site. *J. Med. Chem.* **1976**, *19*, 1029−1033. (b) Merkler, D. J.; Schramm, V. L. Catalytic Mechanism of Yeast Adenosine 5′-Monophosphate Deaminase. Zinc Content, Substrate Specificity, pH Studies and Solvent Isotope Effects. *Biochemistry* **1993**, *32*, 5792−5799.

(30) For X-ray crystallographic examples of this type of interaction, see: (a) Erion, M. D.; Niwas, S.; Rose, J. D.; Ananthan, S.; Allen, M.; Secrist, III, J. A.; Babu, Y. S.; Bugg, C. E.; Guida, W. C.; Ealick, S. E.; Montgomery, J. A. Structure-Based Design of Inhibitors of Purine Nucleoside Phosphorylase. 3. 9-Arylmethyl Derivatives of 9-Deazaguanine Substituted on the Methylene Group. *J. Med. Chem.* **1993**, *36*, 3771−3783. (b) Guida, W. C.; Elliot, R. D.; Thomas, H. J.; Secrist, III, J. A.; Babu, Y. S.; Bugg, C. S.; Erion, M. D.; Ealick, S. E.; Montgomery, J. A. Structure-Based Design of Inhibitors of Purine Nucleoside Phosphorylase. 4. A Study of Phosphate Mimics. *J. Med. Chem.* **1994**, *37*, 1109−1114.

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