AMP Deaminase Inhibitors. 4. Further N3-Substituted Coformycin Aglycon Analogues: N3-Alkylmalonates as Ribose 5′-Monophosphate Mimetics

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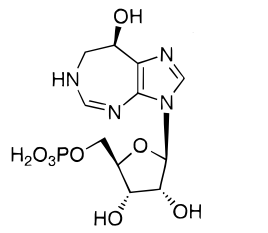
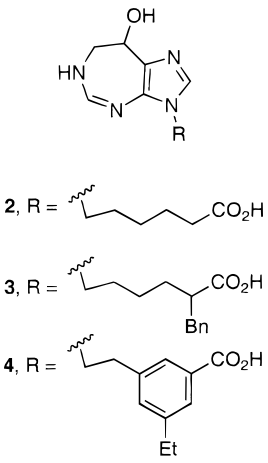
AMP deaminase (AMPDA) inhibitors increase the levels of extracellular adenosine and preserve intracellular adenylate pools in cellular models of ATP depletion and therefore represent a potential new class of antiischemic drugs. Recently we reported that replacement of the ribose 5′-monophosphate component of the very potent transition-state analogue AMPDA inhibitor coformycin monophosphate (**1**) with a simple alkylcarboxy group resulted in potent, selective, and cell-penetrating AMPDA inhibitors. Here we report that replacement of this alkylcarboxy group with an α-substituted alkylmalonic acid resulted in enhanced inhibitor potency. The lead compound, 3-(5,5-dicarboxy-6-(3-(trifluoromethyl)phenyl)-*n*-hexyl)coformycin aglycon (**21**), exhibited an AMPDA K_i of 0.029 μM which is (3×10^5)-fold lower than the K_M for the natural substrate AMP. A comparison of inhibitory potencies shows that the diacid analogues with α-benzyl substituents are 2−10-fold more inhibitory than similar monoacid−monoester, monoester−monoamide, or diester derivatives. Finally, these diacid analogues are 2−40-fold more potent inhibitors than the corresponding monocarboxylates.

Introduction

Recently, we reported the discovery of the first potent, selective, and cell-penetrating AMP deaminase (AMPDA) inhibitors.[1] They are of particular interest as a new class of antiischemic agents. In cellular models of ATP depletion, increases in adenosine and conservation of adenylate pools were observed in the presence of these compounds. Both effects are expected to enhance tissue preservation during ischemia.[1] These compounds are analogues of the potent transition-state analogue inhibitor coformycin monophosphate (**1**) (K_i = 0.00006 μM).[2] Initial work demonstrated that the aglycon of **1** is an excellent template for the construction of inhibitors.[3] Simple N3-alkylcarboxylates were inhibitors selective for AMPDA, as exemplified by the prototype compound **2** (K_i = 4.2 μM). Furthermore, by positioning hydrophobic substitution close to the carboxylate, a 10-fold increase in potency was realized (see compound **3**, K_i = 0.41 μM). Finally, incorporation of a substituted benzene ring into the tether between N3 and the CO_2H resulted in hydrophobic binding combined with optimized conformational restriction (see compound **4**, K_i = 0.060 μM).[4] The potency and selectivity of these compounds were rationalized based on a model wherein the N3-side chain helps to position the aglycon to receive a full complement of binding interactions with the protein as a transition-state mimic.[1] The N3-side chain itself replaces the ribose monophosphate of **1,** and it was postulated that selectivity over adenosine deaminase (ADA) and enhancement in binding are gained as a result of the CO_2H group interacting with the phosphate binding site. Historically, the use of CO_2H to replace PO_3H_2 has been met with limited success because of geometric and charge density differences.[5] In a case where CO_2H was an effective PO_3H_2 mimetic, crystal structure data of complexes support the interpretation that the CO_2H interacts indirectly with residues in the phosphate binding site using electrostatic through-space interactions.[5a,b]



1, Coformycin monophosphate



Malonic acid derivatives have also been tested as phosphate replacements because a doubly-negative-charged group might mimic phosphate better than a monocarboxylate.[6] As part of the inhibitor design strategy (Figure 1), we sought to use the malonate group to improve the interaction with the phosphate binding site. We report herein the successful use of this approach to produce potent inhibitors of AMPDA.

Chemistry

The N3-substituted coformycin aglycon analogues **7** were typically prepared by a sequence of reactions which consisted of (1) NaH/DMF-mediated N3-alkylation[3] of the heterocycle **5**[7] with an electrophile **6**, (2) $NaBH_4$ reduction of the C8 ketone to a 1:1 *R*/*S* alcohol mixture, (3) ester hydrolysis or hydrogenolysis, and (4) other

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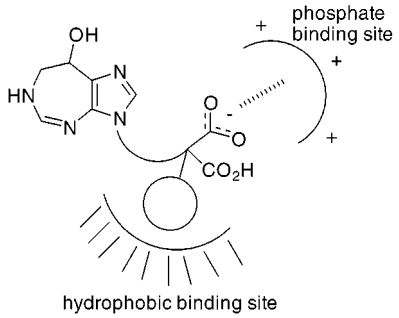
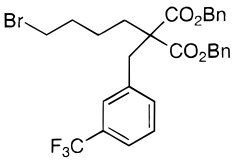


Figure 1. AMPDA inhibitor design strategy.

modifications, if necessary (Scheme 1). The electrophiles were prepared by sequential NaH/DMF-mediated alkylations of a malonate diester. For example, compound **8** was derived from alkylation of dibenzyl malonate with α′-bromo-α,α,α-trifluoro-*m*-xylene followed by alkylation with 1,4-dibromobutane.



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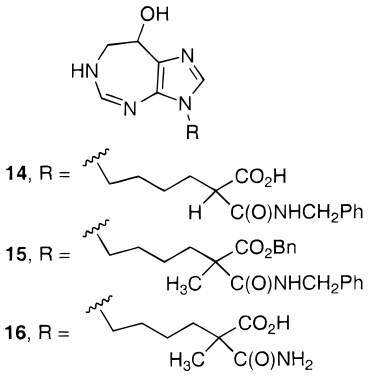
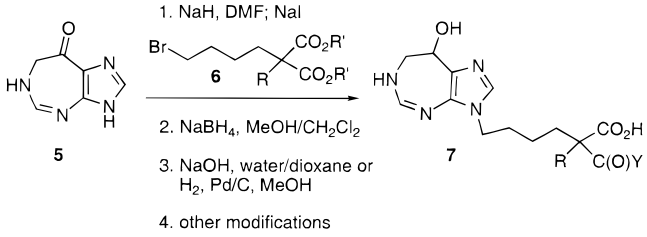
The parent monosubstituted malonates **12** and **13** were prepared from the tricarboxylate **9** wherein the third CO_2Bn group served to mask the acidic α-proton and thus prevent any competitive alkylation chemistry which would limit the success of the sequence (Scheme 2). Tricarboxylate **9**[8] required elevated temperatures for alkylation to provide electrophile **10**[9] in 54% yield. Compound **10** was then used in the synthesis of the coformycin aglycon analogue **11** which after NaOH hydrolysis yielded a mixture of the monoacid **12** and diacid **13**. These compounds were purified using ion-exchange chromatography (for **12**) and preparative C-18 HPLC (for **13**). Monoacid−monoamides were typically prepared using the diphenyl phosphorazidate (DPPA) amide synthesis method[10] followed by NaOH ester hydrolysis (e.g. **12** to **14** conversion). Last, phenylamide **15** was converted to the primary amide **16** by hydrogenolysis.



Results

The compounds were evaluated as inhibitors of porcine heart or recombinant human E-type AMPDA and calf intestinal ADA (Tables 1−3).[1] 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. Every compound examined from this series showed good AMPDA specificity relative to ADA. In each case, the ADA K_is were >7.5 μM. In select examples, to evaluate the full extent of the selectivity of the enzyme inhibition, ADA inhibition was evaluated at higher concentrations.

Scheme 1. General Analogue Synthesis



The dicarboxylate series of AMPDA inhibitor potencies ranged from 0.029 to 6 μM (Table 1). Adding a CO_2H group to the initial lead compound **2** (AMPDA K_i = 4.2 μM) does not affect potency as shown by compound **13** (K_i = 5.1 μM). However, AMPDA inhibitory potency was enhanced when the second CO_2H group was combined with a large hydrophobic substituent in the α-position. For example, a modest boost in affinity was realized when a second CO_2H was added to the potent monocarboxylate **3** (K_i = 0.41 μM) as shown with dicarboxylate **18** (K_i = 0.17 μM). The most dramatic potency increase was realized in the study of the *o*-, *m*-, and *p*-CF_3 analogues **20**−**22**. The most potent example, compound **21** with an α-CO_2H (K_i = 0.029 μM), is 40-fold more potent than compound **19**,[11] which has an α-methyl group (K_i = 1.2 μM).

Malonyl monocarboxylates were less potent than the dicarboxylates (see Table 2). The closest compounds in inhibitory potency were the monoacid−monoesters **12** and **23** (K_i = 0.36 and 0.90 μM, respectively). Monoacid−monoamide derivatives were only modest inhibitors of AMPDA with K_is of 1−10 μM (compounds **14**, **16**, and **24**−**26**). Finally, examination of the SAR of the diesters demonstrates how the hydrophobic component of the N3-side chain can be very important to binding affinity (Table 3). The small α-substituent, methyl, combined with two small esters (ethyl) provided only a weak AMPDA inhibitor, **27** (K_i = 44.8 μM). However, increasing the size of the α-substituent or the ester resulted in the ca. 10-fold more potent inhibitors **28** and **29**. The most potent neutral compounds were esters of the potent diacid **20** (K_i = 0.041 μM). Masking the diacid **20** as a diester resulted in compounds that were only 6−9-fold weaker enzyme inhibitors than the diacid (i.e. compounds **30** and **31**, K_i = 0.36 and 0.25 μM, respectively).

Discussion

Exploration of the malonic acid substituent as a phosphate mimetic on the N3-side chain of the coformycin aglycon analogue series resulted in a very potent and selective series of AMPDA inhibitors. The most potent compound of the series, compound **21** (K_i = 0.029 μM, Table 1), underscores the α-substituent as being essential for high potency. This compound, with an α-(3-trifluoromethylbenzyl), is nearly 200-fold more potent

Scheme 2. Preparation of Monosubstituted Malonate Derivatives

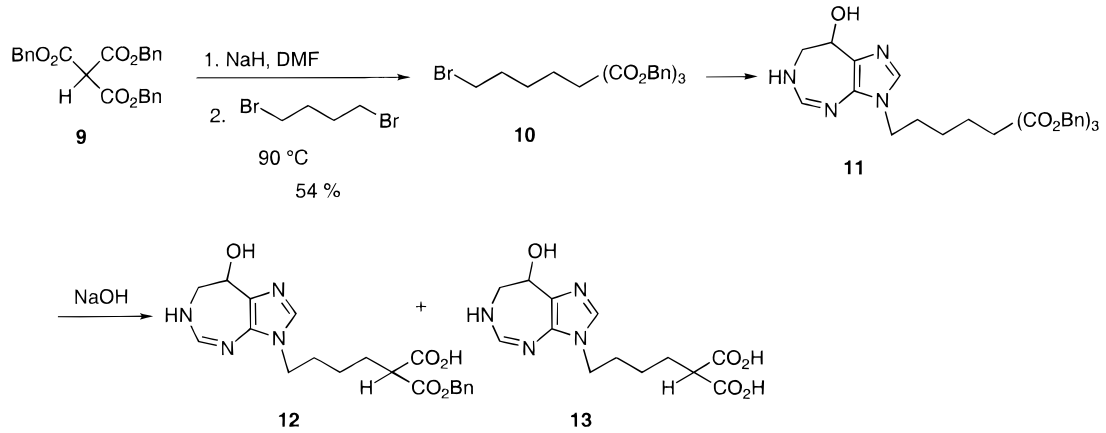
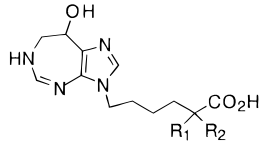


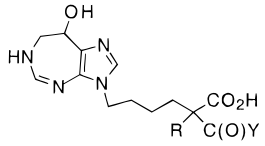
Table 1. Coformycin Aglycon Analogues: Dicarboxylates



compd	R_1	R_2	mp (°C)	formula[a]	AMPDA K_i (μM)	ADA K_i (μM)
2	H	H	*b*	*b*	4.2	280
13	H	CO_2H	85−90	$C_{13}H_{18}N_4O_5 \cdot 1.33H_2O$	5.1	>7.5
17	CH_3	CO_2H	155	$C_{14}H_{20}N_4O_5 \cdot 1H_2O \cdot 1(C_6H_{11})_2NH$	6	>7.5
3	CH_2Ph	H	*b*	*b*	0.41	>1000
18	CH_2Ph	CO_2H	200	$C_{20}H_{24}N_4O_5 \cdot 0.5H_2O \cdot 0.5(C_2H_5)_2O$[c]	0.17	>7.5
19	CH_2(3-CF_3Ph)	CH_3	100−104	$C_{21}H_{25}F_3N_4O_3 \cdot 1H_2O \cdot 0.5CH_3CO_2H$[d]	1.2	>7.5
20	CH_2(2-CF_3Ph)	CO_2H	179−181	$C_{21}H_{23}N_4O_5F_3 \cdot 3H_2O$[e]	0.041	>7.5
21	CH_2(3-CF_3Ph)	CO_2H	122−123	$C_{21}H_{23}N_4O_5F_3 \cdot 1(C_2H_5)_3N \cdot 1.5H_2O$	0.029	>7.5
22	CH_2(4-CF_3Ph)	CO_2H	170−171	$C_{21}H_{23}N_4O_5F_3 \cdot 2H_2O$	0.040	>7.5

[a] Analyses for C, H, N were correct within ±0.4% unless otherwise stated. [b] See ref 3. [c] C: calcd, 59.18; found, 58.43; H; N: calcd, 12.55; found, 13.01. [d] C, H; N: calcd, 11.52; found, 11.05. [e] C, H; N: calcd, 10.72; found, 10.20.

Table 2. Coformycin Aglycon Analogues: Monoacid−Monoesters and Monoacid−Monoamides



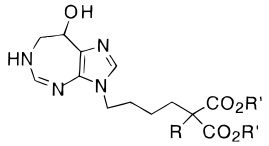
compd	R	Y	mp (°C)	formula[a]	AMPDA K_i (μM)	ADA K_i (μM)
12	H	OCH_2Ph	53−58	$C_{20}H_{24}N_4O_5 \cdot 1H_2O$	0.36	>7.5
14	H	$NHCH_2Ph$	138	$C_{20}H_{25}N_5O_4 \cdot 1.5H_2O \cdot 0.15CH_3CO_2H$[b]	2.4	>7.5
16	CH_3	NH_2	170	$C_{14}H_{21}N_5O_4 \cdot 0.75H_2O \cdot 0.25CH_3CO_2H$[c]	7.8	>7.5
23	CH_3	OCH_2Ph	158−160	$C_{23}H_{26}N_4O_5 \cdot 1.33H_2O$[d]	0.9	>7.5
24	CH_3	NH-cyclohexyl	199−203	$C_{20}H_{31}N_5O_4 \cdot 2H_2O \cdot 0.5CH_3CO_2H$	5.2	>7.5
25	H	$NHCH_2$(4-ClPh)	184	$C_{21}H_{26}ClN_5O_4 \cdot 1H_2O \cdot 0.25CH_3CO_2H$	1.3	>7.5
26	CH_3	$NH(CH_2)_2Ph$	93	$C_{22}H_{29}N_5O_4 \cdot 0.75H_2O \cdot 0.3CH_3CO_2H$	2.1	>7.5

[a] Analyses for C, H, N were correct within ±0.4% unless otherwise stated. [b] C; H: calcd, 6.62; found, 6.17; N. [c] C: calcd, 49.49; found, 50.18; H, N. [d] C, H; N: calcd, 13.14; found, 12.60.

than the α-H and α-CH_3 analogues **13** and **17**, respectively. The presence of a key hydrophobic binding site, close to the phosphate binding site, was first evidenced by compounds such as **3** and **4**. It was probably also accessed with the potent compounds from the malonate monoacid and diester series (Tables 2 and 3). All of the more potent compounds from these two series have at least one benzyl substituent present as either an ester or an α-substituent. Surprisingly, the diester **31**, with a total of three benzyl groups, has inhibitory potency only 6-fold less than its parent diacid **20**. From a practical standpoint, this implies that prodrug diesters may be useful for achieving oral bioavailability, cell penetration, and then efficacy additive to the parent carboxylic acids to which they are metabolized in vivo. From a binding perspective, it cannot be ruled out that bulky esters, such as those present on compound **31**, might be binding in a hydrophobic region away from the electropositive phosphate binding site.

Given the structural differences between a phosphate group and a malonate group it is unlikely both carboxylates reside inside the phosphate binding site of AMPDA. Thus an exact comparison taking into consideration the two pK_a values of a malonic diacid (pK_as of ca. 2.9 and 5.8) vs the natural substrate AMP (pK_as of 3.8 and 6.2) is not explicitly possible. Nevertheless, it is apparent from the SAR presented here that the second CO_2H group is making a significant contribution to the bind-

Table 3. Coformycin Aglycon Analogues: Diesters



compd	R	R′	mp (°C)	formula[a]	AMPDA K_i (μM)	ADA K_i (μM)
27	CH_3	CH_2CH_3	122−125	$C_{18}H_{28}N_4O_5$	44.8	>500
28	CH_2Ph	CH_2CH_3	139−140	$C_{24}H_{32}N_4O_5 \cdot 0.5H_2O$	2.6	>7.5
29	CH_3	CH_2Ph	113−116	$C_{28}H_{32}N_4O_5$	5.2	>7.5
30	CH_2(2-CF_3Ph)	CH_2CH_3	110−111	$C_{25}H_{31}F_3N_4O_5$	0.36	>7.5
31	CH_2(2-CF_3Ph)	CH_2Ph	104−105	$C_{35}H_{35}F_3N_4O_5$	0.25	>7.5

[a] Analyses for C, H, N were correct within ±0.4%.

ing. This effect is most dramatic when a CH_3 is substituted for the second CO_2H which resulted in a 40-fold loss in enzyme affinity (i.e. compare compound **19**, $K_i = 1.2$ μM, with **21**, $K_i = 0.029$ μM). Recent crystal structure determinations coupled with thermodynamic calculations of malonates binding to phosphotyrosine binding sites support a model wherein one CO_2H interacts with residues in the phosphate binding site whereas the other CO_2H interacts with residues located outside the phosphate binding pocket.[6b,c] In these studies it was found that even though the malonate group is more readily desolvated, only one CO_2H group can bind well because the group is 21% larger than the phosphate group. Consequently, there is an entropic cost, with the phosphate binding site having to reorganize to neutralize its remaining net positive charge.[6b] In one of these studies, the second CO_2H was found to make additional H-bonding interactions by replacing a water molecule outside of the pocket which may have been responsible for it being a 37-fold better inhibitor than the corresponding monocarboxylate.[6c] Lacking structural data and considering the SAR presented in this study, this model of malonate binding seems possible in the case of AMPDA. This model can be used to explain certain SAR. For example, comparison of monoacid−monoester **12** with monoester−monoamide **14** supports the "outside the pocket" carboxylate group interacting as a H-bond acceptor since the amide, a less efficient acceptor, is a 6-fold weaker AMPDA inhibitor.

Experimental Section

General Methods. Glassware for moisture-sensitive reactions was flame-dried and cooled to room temperature in a desiccator prior to use and all reactions were carried out under an atmosphere of nitrogen. Anhydrous solvents were purchased from Aldrich and stored over 4A molecular sieves. 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 NMR were obtained on a Varian Gemini-200 operating at 200 MHz. ^{1}H NMR spectra were recorded in units δ with tetramethylsilane (δ 0.00) or DMSO-d_6 (δ 2.50) as reference line internal standards. C, H, N microanalyses were performed at NuMega Resonance Labs, Inc., San Diego, CA. 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]

Dibenzyl 2-(4-Bromobutyl)-2-(3-trifluoromethylbenzyl)-malonate (8). Dibenzyl malonate (4.75 g, 16.7 mmol) was added to a suspension of NaH (670 mg of a 60% dispersion in mineral oil prewashed with hexanes and dried under vacuum, 16.7 mmol) in 70 mL DMF at 0 °C and the mixture stirred for 30 min. Then α′-bromo-α,α,α-trifluoro-*m*-xylene (6.00 g, 25 mmol) was added; the resulting mixture stirred at room temperature for 2 h and then it was diluted with 5% NH_4Cl and ether. The ether layer was separated, washed with water and brine, dried ($MgSO_4$) and evaporated. The resulting oil was subjected to flash chromatography eluting with hexane:EtOAc gradients of 20:1 and 10:1 which provided 3.84 g (52%) of dibenzyl 2-(3-trifluoromethylphenylmethyl)malonate as an oil: ^{1}H NMR (DMSO-d_6) 3.24 (d, 2, $J = 8$ Hz), 4.19 (t, 1, $J = 8$ Hz), 5.09 (s, 4), 7.1−7.7 (m, 14).

This compound (3.8 g, 8.6 mmol) was added to a suspension of NaH (343 mg of a 60% dispersion in mineral oil prewashed with hexanes and dried under vacuum, 8.6 mmol) in 45 mL DMF at 0 °C and the mixture stirred for 30 min. Then 1,4-dibromobutane (3.71 g, 17.2 mmol) was added; the resulting mixture stirred at 50 °C for 1 h and then it was diluted with 5% NH_4Cl and ether. The ether layer was separated, washed with water and brine, dried ($MgSO_4$) and evaporated. The resulting oil was subjected to flash chromatography eluting with hexane:EtOAc gradients of 20:1 and 10:1 which provided 3.2 g (64%) of the desired compound **8** as an oil: ^{1}H NMR (DMSO-d_6) 1.2−1.8 (m, 6), 3.28 (s, 2), 3.44 (t, 2, $J = 7$ Hz), 5.07 (d, 2, $J = 12$ Hz), 5.15 (d, 2, $J = 12$ Hz), 7.2−7.6 (m, 14).

Tribenzyl Methanetricarboxylate (9). Dibenzyl malonate (5.00 g, 17.6 mmol) was slowly added to a suspension of NaH (774 mg of a 60% dispersion in mineral oil prewashed with hexanes and dried under vacuum, 19.3 mmol) in 180 mL DMF at 0 °C and the resulting mixture stirred at 0 °C for 15 min and then at room temperature for 1 h. This mixture was recooled to 0 °C and benzyl chloroformate (2.76 mL, 19.3 mmol) was slowly added which resulted in an immediate white precipitate. This mixture was stirred at 0 °C for 1 h and then it was diluted with 5% NH_4Cl and ether. The ether layer was separated, washed with water and brine, dried ($MgSO_4$) and evaporated. The resulting oil was subjected to flash chromatography eluting with hexane:EtOAc gradients of 10:1 and 7.5:1 which provided first recovered dibenzyl malonate and then 2.60 g (35%) of the triester **9** as an oil: ^{1}H NMR ($CDCl_3$) 4.56 (s, 1), 5.19 (s, 6), 7.2−7.4 (m, 15). Anal. ($C_{25}H_{22}O_6$) C, H.

1-Bromo-5,5,5-tris(carbobenzyloxy)-*n*-pentane (10). A solution of triester **9** (2.89 g, 6.9 mmol) in 20 mL DMF was added via cannula needle to a suspension of NaH (386 mg of a 60% dispersion in mineral oil prewashed with hexanes and dried under vacuum, 9.7 mmol) in 50 mL DMF at 0 °C and the resulting mixture stirred at room temperature for 1 h. Then 1,4-dibromobutane (1.64 mL, 13.8 mmol) was added and the mixture stirred at 90 °C for 16 h, cooled to room temperature, and diluted with ether and water. The ether layer was separated, washed with water and brine, dried ($MgSO_4$) and evaporated. The resulting oil was subjected to flash chromatography eluting with a hexane:EtOAc gradient of 25:1 which provided 1.95 g (51%) of the desired compound **10** as an oil: ^{1}H NMR ($CDCl_3$) 1.53 (m, 2), 1.79 (m, 2), 2.1−2.2 (m, 2), 3.26 (t, 2, $J = 7$ Hz), 5.15 (s, 6), 7.1−7.4 (m, 15). Anal. ($C_{29}H_{31}BrO_6$) C: calcd, 62.94; found, 63.71; H.

3-(5,5-Dicarboxy-6-(3-(trifluoromethyl)phenyl-*n*-hexyl)coformycin Aglycon (21). According to the method previ-

ously described[1] heterocycle **5** (1.12 g, 7.4 mmol) was alkylated with diester **8** (4.3 g, 7.4 mmol). The residue was adsorbed to silica gel from a solution in CH_2Cl_2/MeOH and subjected to flash chromatography eluting with CH_2Cl_2:MeOH mixtures of 25:1 and 20:1 which provided 1.6 g (32%) of 3-(5,5-dicarbobenzyloxy-6-(3-(trifluoromethyl)phenyl)hexyl)-6,7-dihydroimidazo-[4,5-*d*]-[1,3]diazepin-8(3*H*)-one as a viscous oil: ^{1}H NMR (DMSO-d_6) 1.1−1.3 (m, 2), 1.5−1.8 (m, 4), 3.26 (s, 2), 3.72 (d, 2, J = 4 Hz), 3.88 (t, 2, J = 7 Hz), 5.08 (s, 4), 7.2−7.5 (m, 15), 7.60 (s, 1), 8.35 (m, 1).

This ketone (1.55 g, 2.4 mmol) was reduced with $NaBH_4$ (91 mg, 2.4 mmol) as previously described[1] to provide after chromatography 1.2 g (77%) of 3-(5,5-dicarbobenzyloxy-6-(3-(trifluoromethyl)phenyl)hexyl)coformycin aglycon as a white solid: mp 104−105 °C; ^{1}H NMR (DMSO-d_6) 1.1−1.3 (m, 2), 1.5−1.8 (m, 4), 3.15 (br s, 2), 3.26 (s, 2), 3.78 (t, 2, J = 7 Hz), 4.82 (br s, 1), 4.93 (d, 1, J = 5 Hz), 5.09 (s, 4), 6.95 (d, 1, J = 4 Hz), 7.2−7.5 (m, 15), 7.60 (d, 1, J = 8 Hz). Anal. ($C_{35}H_{35}F_3N_4O_5$) C, H, N.

This method was also used to prepare diesters **27**−**31**.

A mixture of 3-(5,5-dicarbobenzyloxy-6-(3-(trifluoromethyl)-phenyl)-*n*-hexyl)coformycin aglycon (1.2 g, 1.85 mmol), NEt_3 (0.5 mL, 3.7 mmol) and 1.2 g of 20% $Pd(OH)_2$ on carbon in 20 mL MeOH was shaken under 50 psi H_2 for 10 min and then it was filtered over Celite and evaporated. The residue was dissolved in water and filtered over Celite. The filtrate was frozen and lyophilized to provide 450 mg (40%) of the dicarboxylate **21** as a white powder: mp 122−123 °C; ^{1}H NMR (DMSO-d_6) 1.15 (t, 9, J = 7 Hz), 1.1−1.3 (m, 2), 1.5−1.8 (m, 4), 3.05 (q, 6, J = 7 Hz), 3.10 (br s, 2), 3.17 (s, 2), 3.82 (t, 2, J = 7 Hz), 4.79 (br s, 1), 5.05 (br s, 1), 7.01 (d, 1, J = 4 Hz), 7.3−7.6 (m, 6). Anal. ($C_{21}H_{23}F_3N_4O_5 \cdot 1.5H_2O \cdot 1N(CH_2CH_3)_3$) C, H, N.

This method was also used (with the exclusion of NEt_3) to prepare diacids **17**, **18**, **20,** and **22**.

3-(5,5,5-Tris(carbobenzyloxy)-*n*-pentyl)coformycin Aglycon (11). Prepared as described[1] from heterocycle **5** and electrophile **10**: ^{1}H NMR (DMSO-d_6) 1.2−1.5 (m, 2), 1.60 (m, 2), 2.0−2.2 (m, 2), 3.14 (br s, 2), 3.76 (t, 2, J = 7 Hz), 4.79 (m, 1), 4.88 (m, 1), 5.16 (s, 6), 6.95 (d, 1, J = 4 Hz), 7.15−7.4 (m, 15), 7.23 (s,1), 7.44 (m, 1).

3-(5-Carbobenzyloxy-5-carboxy-*n*-pentyl)coformycin Aglycon (12) and 3-(5,5-Dicarboxy-*n*-pentyl)coformycin Aglycon (13). A mixture of triester **11** (5.98 g, 9.6 mmol), 48 mL 0.5 M NaOH and 50 mL dioxane was stirred at 0 °C for 2 h and then at room temperature for 6 h and then passed through a 25-mL column of DOWEX 1×8-400 acetate ion-exchange resin[3] (column 1). Column 1 was further eluted with 50 mL MeOH. These initial eluates were combined and concentrated to about 20 mL and passed through another 25-mL column of DOWEX 1×8-400 acetate ion-exchange resin (column 2) followed by 50 mL MeOH. These initial eluates were made basic with 0.5 M NaOH (pH 10) and extracted with CH_2Cl_2. The aqueous fraction was applied to another 25-mL column of DOWEX 1×8-400 acetate ion-exchange resin (column 3) followed by 50 mL MeOH. Columns 1−3 were gravity eluted with water and then 0.1 N AcOH. Column 1 eluted an early fraction of pure monoester **12**, a mixture of monoester **12** and diacid **13,** and a later fraction of pure monoester **12**. Columns 2 and 3 both eluted pure monoester **12**. The fractions of monoester from columns 1−3 were combined and lyophilized to provide 1.67 g (43%) of monoester **12** as a white powder: mp 53−58 °C; ^{1}H NMR (DMSO-d_6) 1.1−1.4 (m, 2), 1.5−1.9 (m, 4), 3.15 (dt, 1, J = 11, 6 Hz), 3.28 (d, 1, J = 11 Hz), 3.42 (t, 1, J = 6 Hz), 3.92 (t, 2, J = 7 Hz), 4.81 (m, 1), 5.14 (s, 2), 5.45 (br s, 1), 7.07 (d, 1, J = 4 Hz), 7.2−7.5 (m, 5), 7.82 (br s,1), 7.93 (s, 1). Anal. ($C_{20}H_{24}N_4O_5 \cdot 1H_2O$) C, H, N.

This method was also used to prepare acid **23**.

A sample of the **12**/**13** mixture was subjected to preparative reverse-phase C-18 HPLC and eluted with 50:50 MeOH:0.01 M AcOH to provide after lyophilization of the early fractions the diacid **13** as a white powder: mp 85−90 °C; ^{1}H NMR (DMSO-d_6) 1.1−1.4 (m, 2), 1.5−1.9 (m, 4), 2.95 (t, 1, J = 6 Hz), 3.14 (dt, 1, J = 11, 6 Hz), 3.31 (d, 1, J = 11 Hz), 3.94 (t, 2, J = 7 Hz), 4.82 (br s, 1), 7.10 (d, 1, J = 4 Hz), 7.90 (br s, 1), 8.05 (s, 1). Anal. ($C_{13}H_{18}N_4O_5 \cdot 1.33H_2O$) C, H, N.

3-(5-*N*-Benzylcarbamoyl-5-carboxy-*n*-pentyl)coformycin Aglycon (14). To a mixture of monoester **12** (80 mg, 0.20 mmol), benzylamine (44 μL, 0.40 mmol) and NEt_3 (83 μL) in 2 mL DMF at 0 °C was added DPPA (65 μL, 0.3 mmol) and the mixture stirred for 8 h at room temperature. After sovent evaporation the residue was subjected to flash chromatography eluting with CH_2Cl_2:MeOH:NEt_3 mixtures of 25:1:0.25 and 20:1:0.2 which provided 74 mg (76%) of 3-(5-*N*-benzylcarbamoyl-5-carbobenzyloxy-*n*-pentyl)coformycin aglycon as an amorphous white solid.

A mixture of this ester (70 mg, 0.14 mmol) in 1 mL dioxane and 1 mL 0.5 M NaOH was stirred at room temperature for 16 h and then diluted with 5 mL water and extracted with CH_2Cl_2 (2 × 10 mL). Approximately 1 g of DOWEX 1×8-400 acetate ion-exchange resin was added to the aqueous layer; the mixture stirred for 30 min and was filtered. The resin was washed with water (2 × 10 mL) and then suspended in 10 mL of 0.1 N AcOH for 30 min at 0 °C and filtered (2×). The combined 0.1 N AcOH filtrates were lyophilized to provide 34 mg (61%) of the amide **14** as a white powder: mp 138 °C; ^{1}H NMR (DMSO-d_6) 1.1−1.3 (m, 2), 1.5−1.8 (m, 4), **2.63** (t, 1, J = 6 Hz), 3.17 (m, 2), 3.81 (t, 2, J = 7 Hz), 4.28 (d, 2, J = 6 Hz), 4.80 (br s, 1), 6.96 (d, 1, J = 4 Hz), 7.2−7.4 (m, 5), 7.29 (s,1), 7.45 (br s, 1), 8.65 (t, 1, J = 6 Hz). Anal. ($C_{20}H_{25}N_5O_4 \cdot 1.5H_2O \cdot 0.15CH_3CO_2H$) C; H: calcd, 6.62; found, 6.17; N.

This method was used to prepare the ester−amide **15** and the acid−amides **24**−**26**.

3-(5-Carbamoyl-5-carboxy-*n*-hexyl)coformycin Aglycon (16). A mixture of ester−amide **15** (86 mg, 0.18 mmol) and 30 mg of 10% Pd/C in 5 mL MeOH was shaken on a Parr shaker under 30 psi H_2 for 16 h and then filtered over Celite. The filtrate was evaporated and the residue dissolved in 2 mL 0.1 N NaOH, mixed with ca. 0.5 g of DOWEX 1×8-400 acetate ion-exchange resin for 30 min and then filtered. The resin was washed with water (2 × 10 mL) and then suspended in 10 mL 0.1 N AcOH for 30 min at 0 °C and filtered (2×). The combined 0.1 N AcOH filtrates were lyophilized to provide 18 mg (28%) of the amide **16** as a glassy solid: mp 170 °C; ^{1}H NMR (DMSO-d_6) 1.1−1.3 (m, 2), 1.22 (s, 3), 1.5−1.9 (m, 4), 3.16 (br s, 2), 3.83 (t, 2, J = 7 Hz), 4.80 (br s, 1), 6.97 (d, 1, J = 4 Hz),7.06 (br s, 1), 7.30 (s, 1), 7.34 (br s, 1), 7.5 (br s, 1). Anal. ($C_{14}H_{21}N_5O_4 \cdot 0.75H_2O \cdot 0.25CH_3CO_2H$) C: calcd, 49.49; found, 50.18; H, N.

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