Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 9, pp 1853–1857, 1997

PII: S0968-0896(97)00124-7
0968-0896/97 $17.00 + 0.00

Multisubstrate Analogue Based on 5,8,10-Trideazafolate

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Abstract—The preparation and evaluation of the multisubstrate analogue **3** based on the 5,8,10-trideazafolate core for GAR and AICAR Tfase inhibition is detailed. © 1997 Elsevier Science Ltd.

Introduction

In preceding articles[1–3] we detailed the preparation and evaluation of 10-formyl-5,8,10-trideazafolate (**2**)[4] and a series of functionalized analogues as potential inhibitors of glycinamide ribonucleotide transformylase (GAR Tfase) and aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase). Both are folate-dependent enzymes utilizing **1** as the cofactor and are responsible for the transfer of a formyl group to GAR and AICAR in the de novo synthesis of purines (Fig. 1).[5–21] Here we report the preparation and evaluation of the simplified multisubstrate adduct **3** derived from further functionalization of this core for comparison with the multisubstrate adduct inhibitors (MAI) **4**[22] and **5**[23] that have been shown to be exceptionally potent GAR Tfase inhibitors with the former being capable of enzyme assembly from appropriate precursors (Fig. 2).[24]

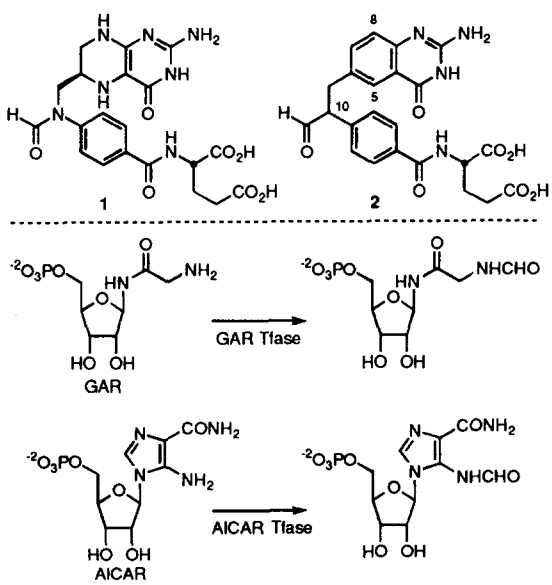


Figure 1.

Chemistry

The multisubstrate adduct **3** was prepared by acylation of the primary alcohol **6**[2] employed as a 1:1 mixture of diastereomers with 8-(*tert*-butyldimethylsilyloxy)-octanoic acid (**7**)[25] which was found to work best when promoted by DCC (1 equiv) in the presence of DMAP (0.5 equiv, CH_2Cl_2, 0 °C, 12 h, 75%; Scheme 1). Acylation at the primary alcohol versus quinazolinone C2 amine was established by the diagnostic downfield ^1H NMR chemical shift of the alcohol α-CH_2 (0.57 ppm) on acylation and the unaffected C-2

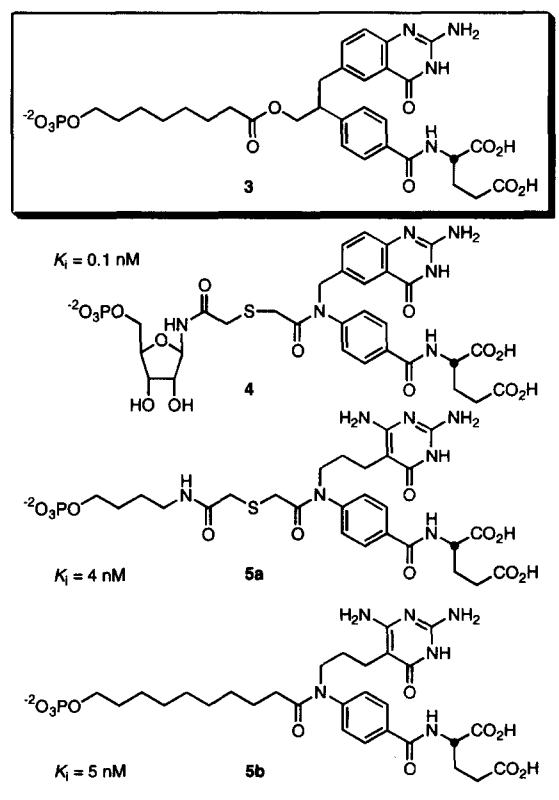


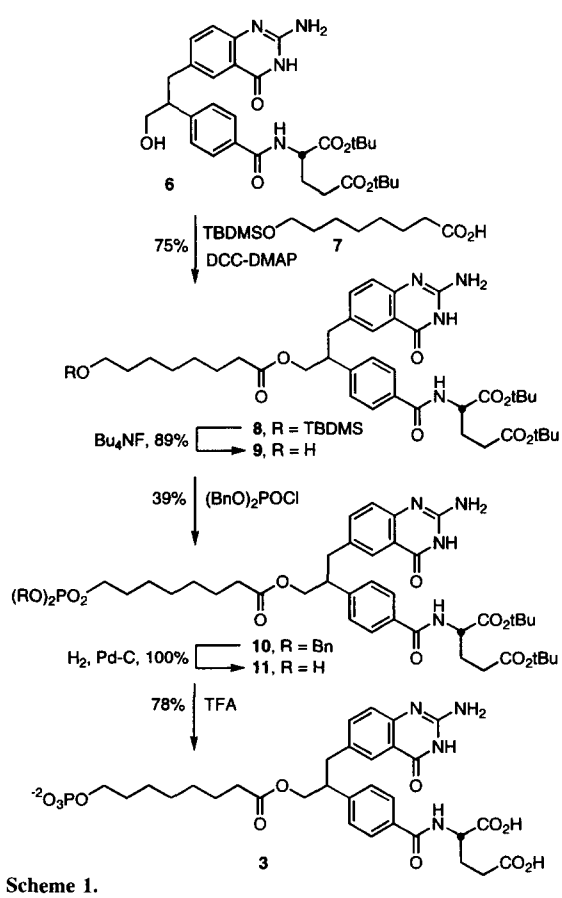
Figure 2.

amine (2H, 6.43 versus 6.48 ppm). Deprotection of the TBDMS ether **8** (2 equiv Bu_4NF, THF, 25 °C, 4 h, 89%) followed by conversion of the alcohol **9** to the dibenzyl phosphate ($(BnO)_2POCl$, pyridine, −78 to 25 °C, 24 h, 39%) provided the key intermediate **10**. That phosphorylation occurred on the primary alcohol was established by the diagnostic downfield ^1H NMR chemical shift of the alcohol D-CH_2 (0.54 ppm) and the unaffected C2 amine (2H, 6.45 ppm). Sequential deprotection of the phosphate by hydrogenolysis (10% Pd–C, H_2, EtOH, 25 °C, 4 h, 100%) followed by acid-catalyzed deprotection of the *tert*-butyl esters (20% TFA–$CHCl_3$, 0–25 °C, 15 h, 78%) provided **3** as a 1:1 mixture of diastereomers.

Initial attempts to prepare **3** through use of the diphenylphosphate **12** proved to be much less successful and we were unable to convert **12** to **11** by a variety of established procedures (Scheme 2).[23,26]

Inhibition studies

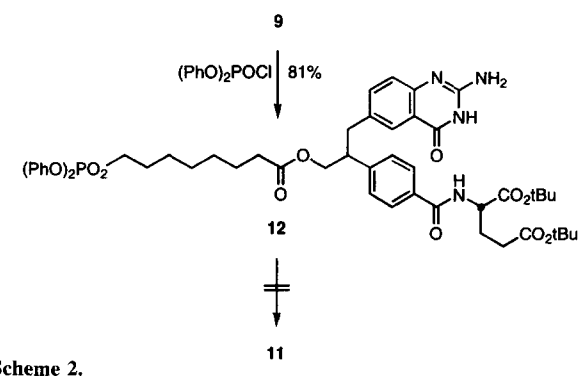
Compound **3** was tested for inhibition of GAR Tfase and AICAR Tfase. In neither case did the binding of **3** show any improvement over that of the aldehyde **2**. For AICAR Tfase, the K_i of **3** is 6.5 ± 1.6 μM, essentially identical to the value of 7.6 ± 1.5 μM measured for **2**. For GAR Tfase, the K_i of **3** is 2.4 ± 0.3 μM while that of **2** is 0.26 ± 0.05 μM. Prolonged incubation up to 30 min of **3** with GAR Tfase prior to the addition of substrate GAR had no effect on the K_i. Thus, the simple aldehyde **2** was an order of magnitude more effective than the multisubstrate analogue of **3**. Moreover, the properties of **3** more closely approach those of the free alcohol of **2** which exhibits K_i = 3.1 ± 0.8 μM for GAR Tfase and K_i = 30 ± 7 μM for AICAR Tfase, and is only one order of magnitude more effective at binding to GAR Tfase than the 10-formyl-5,8-dideazafolate (fDDAF) cofactor employed in the enzymatic assays (K_m = 17 μM). Consequently, the appended phosphate of **3** appears to contribute less to binding than was observed with the preceding examples of **4** and **5**. The source of these distinctions is not clear and one would expect that the binding of **3** would be similar to that of the multisubstrate adduct **5b**, which exhibits a K_i = 5 nM, nearly three orders of magnitude tighter than that of **3**. Moreover, the comparisons of **3** with the aldehyde **2** serve to highlight again the special properties of this latter inhibitor within the series of inhibitors based on the trideazafolate core.



Scheme 1.

Cytotoxic properties

The cytotoxic activity of **3** was examined in both the L1210 and CCRF-CEM cell lines in the presence and absence of purines (hypoxanthine). Under these conditions, **3** exhibited a cytotoxic potency about an order of magnitude or more greater than that of its enzymatic inhibitory properties with no perceptible sensitivity in the absence of purines (hypoxanthine) (Table 2). Consequently, the cytotoxic properties of **3** do not appear to be derived from selective inhibition of purine synthesis via GAR or AICAR Tfase inhibition. Such observations are analogous to preceding studies with **4** and **5** where despite the exceptional enzyme inhibitory properties, they exhibited much more modest cytotoxic activity. In part, this has been attributed to poor cell



Scheme 2.

Table 1. GAR and AICAR Tfase Inhibition, K_i (μM)

Agent	GAR Tfase[a]	AICAR Tfase[b]
3	2.4 ± 0.3 μM	6.5 ± 1.6 μM
4[22]	0.1 nM	>100 nM
5a[3]	4 nM	ND

[a] *pur*N GAR Tfase.
[b] Avian AICAR Tfase.

Table 2. Cytotoxic activity (IC_{50}, μM)[a]

Agent	L1210[b]	CCRF-CEM[b]
(6*R*)-DDATHF	>225, 0.07	>225, 0.05
Methotrexate	0.05, 0.05	0.06, 0.07
3	35, 20	>100, 100

[a]Dialyzed FBS, RPMI-1640 medium.
[b]With + hypoxanthine, − hypoxanthine.

penetration and low intracellular concentrations of the anionic multisubstrate adducts.[22,23]

Experimental

Di-*tert*-butyl (2*RS*)-*N*-[4-{3-(2-amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-((8-(*tert*-butyldimethyl)silyl-oxy)-1-(octanoyloxy))prop-2-yl}benzoyl]-L-glutamate (8). A solution of **7**[26] (5.8 mg, 0.02 mmol) in CH_2Cl_2 (106 μL) cooled to 0 °C was treated with DCC (5.2 mg, 0.02 mmol). The solution was stirred at 0 °C for 5 min at which time DMAP (1.3 mg, 0.009 mmol) followed by **6** (12.3 mg, 0.02 mmol) in CH_2Cl_2 were added. The mixture was stirred at 0 °C for 4 h before the solvent was removed under reduced pressure. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) afforded **8** (13.3 mg, 75%) as a yellow oil: ^1H NMR (DMF-d_7, 400 MHz) δ 10.93 (1H, br s, NH), 7.90 (2H, d, J = 8.1 Hz), 7.75 (1H, d, J = 1.3 Hz, H-5), 7.43 (2H, d, J = 8.1 Hz), 7.38 (1H, dd, J = 1.8, 8.4 Hz, H-7), 7.08 (1H, d, J = 8.3 Hz, H-8), 6.43 (2H, br s, NH_2), 4.49 (1H, m, NHC*H*$CO_2C(CH_3)_3$), 4.29 (2H, d, J = 6.5 Hz, CH_2), 3.60 (2H, q, J = 6.5 Hz, CH_2), 3.45 (1H, m, CH), 3.17 (1H, dd, J = 6.8, 13.9 Hz, C*H*H), 3.04 (1H, dd, J = 9.0, 13.8 Hz, C*H*H), 2.42 (2H, t, J = 7.2 Hz, CH_2), 2.25 (2H, q, J = 7.7 Hz, CH_2), 2.13 (1H, m, C*H*H), 2.01 (1H, m, CH*H*), 1.55 (2H, br m, CH_2), 1.47 (9H, s, $CO_2C(CH_3)_3$), 1.45 (2H, br m, CH_2), 1.39 (9H, s, $CO_2C(CH_3)_3$), 1.3 (6H, br s, $(CH_2)_3$), 0.86 (9H, s, $SiC(CH_3)_3$), 0.03 (6H, s, $Si(CH_3)_2$); ^{13}C NMR (DMF-d_7, 100 MHz) δ 175.1, 173.5, 172.5, 172.0, 167.2, 162.8, 152.8, 146.1, 135.7, 133.6, 133.5, 128.7, 128.1, 127.0, 126.5, 124.6, 118.0, 81.3, 80.4, 67.5, 63.3, 53.6, 47.0, 38.3, 34.3, 34.2, 33.2, 32.2, 29.6, 29.4, 28.0, 27.0, 26.2, 26.1, 25.5, 25.3, 18.6, −5.1, −5.2; IR (neat) ν_{max} 3328, 2920, 2845, 1731, 1661 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 969.3831 (M^+ + Cs, $C_{45}H_{68}N_4SiO_9$ requires 969.3831).

Di-*tert*-butyl (2*RS*)-*N*-[4-{3-(2-amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-((8-hydroxy)-1-octanoyloxy)-prop-2-yl}benzoyl]-L-glutamate (9). A solution of **8** (4.7 mg, 0.005 mmol) in THF (100 μL) was treated with Bu_4NF (11.2 μL, 0.01 mmol, 1 M in THF) and the reaction mixture was stirred at 25 °C for 1 h. Additional Bu_4NF (11.2 μL, 0.01 mmol) was added and the solution was stirred at 25 °C for 4 h before the solvent was removed under reduced pressure. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) afforded **9** (3.6 mg, 89%) as a yellow oil: ^1H NMR (DMF-d_7, 400 MHz) δ 11.37 (1H, br s, NH), 7.90 (2H, d, J = 8.2 Hz), 7.75 (1H, d, J = 1.8 Hz, H-5), 7.43 (2H, d, J = 8.2 Hz), 7.35 (1H, dd, J = 2.0, 8.4 Hz, H-7), 7.06 (1H, d, J = 8.3 Hz, H-8), 6.72 (2H, br s, NH_2), 4.55 (1H, br s, OH), 4.48 (1H, m, NHC*H*$CO_2C(CH_3)_3$), 4.28 (2H, d, J = 6.6 Hz, CH_2), 3.47 (2H, q, J = 6.5 Hz, CH_2), 3.45 (1H, m, CH), 3.16 (1H, dd, J = 6.8, 13.7 Hz, C*H*H), 3.03 (1H, dd, J = 8.5, 13.8 Hz, CH*H*), 2.42 (2H, t, J = 7.4 Hz, CH_2), 2.22 (2H, t, J = 7.3 Hz, CH_2), 2.13 (1H, m, C*H*H), 2.03 (1H, m, CH*H*), 1.46 (4H, m, CH_2CH_2), 1.42 (s, 9H, $CO_2C(CH_3)_3$), 1.39 (9H, s, $CO_2C(CH_3)_3$), 1.24 (6H, m, $(CH_2)_3$); ^{13}C NMR (DMF-d_7, 100 MHz) δ 173.5, 172.4, 172.0, 167.2, 162.8, 146.1, 135.7, 135.6, 133.2, 128.7, 128.1, 126.9, 126.4, 125.0, 118.0, 81.2, 80.4, 67.5, 61.9, 53.5, 47.0, 38.3, 34.2, 33.5, 32.2, 29.4, 27.9, 27.0, 26.2, 25.3; IR (neat) ν_{max} 3337, 2920, 2845, 1731, 1638, 1480, 1364, 1258, 1150, 1095 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 855.2965 (M^+ + Cs, $C_{39}H_{54}O_9N_4$ requires 855.2945).

Di-*tert*-butyl (2*RS*)-*N*-[4-{3-(2-amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-((8-di(benzyloxy)phosphinoyl-oxy)-1-(octanoyloxy)prop-2-yl}benzoyl-L-glutamate (10). A solution of sulfuryl chloride (3.2 μL, 0.03 mmol) and dibenzyl phosphite (8 μL, 0.03 mmol) in CCl_4 (24 μL) was stirred at 25 °C for 15 min. The reaction was purged with N_2 for 1 h and concentrated in vacuo. A solution of **9** (18.5 mg, 0.02 mmol) in pyridine (24 μL) at −78 °C was treated with the dibenzylphosphorochloridate. The reaction mixture was warmed to 25 °C, shaken to homogeneity, and recooled to −78 °C. The reaction was allowed to warm to 4 °C and stirred for 20 h and concentrated in vacuo. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) afforded **10** (9.8 mg, 39%) as a yellow oil: ^1H NMR (DMF-d_7, 400 MHz) δ 11.02 (1H, br s, NH), 7.90 (2H, d, J = 8.3 Hz), 7.76 (1H, s, H-5), 7.40 (13H, m, Ar-H and H-7), 7.08 (1H, d, J = 8.4 Hz, H-8), 6.45 (2H, br s, NH_2), 5.11 (2H, s, CH_2), 5.09 (2H, s, CH_2), 4.48 (1H, m, NHC*H*$CO_2C(CH_3)_3$), 4.29 (2H, d, J = 6.6 Hz, CH_2), 4.01 (2H, q, J = 7.0 Hz, CH_2), 3.44 (1H, m, CH), 3.17 (1H, dd, J = 6.7, 13.8 Hz, C*H*H), 3.03 (1H, dd, J = 8.6, 13.8 Hz, CH*H*), 2.42 (2H, t, J = 7.1 Hz, CH_2), 2.22 (2H, t, J = 7.4 Hz, CH_2), 2.14 (1H, m, C*H*H), 2.03 (1H, m, CH*H*), 1.57 (2H, m, CH_2), 1.45 (2H, m, CH_2), 1.42 (9H, s, $CO_2C(CH_3)_3$), 1.39 (9H, s, $CO_2C(CH_3)_3$), 1.26–1.18 (6H, m, $(CH_2)_3$); ^{13}C NMR (DMF-d_7, 100 MHz) δ 173.5, 172.5, 172.0, 167.2, 162.8, 152.5, 151.0, 146.2, 137.2, 135.8, 133.3, 129.2, 129.0, 128.7, 128.5, 128.1, 126.4, 125.0, 118.0, 81.3, 80.4, 76.3, 70.9, 69.37, 69.32, 68.26, 68.21, 67.5, 66.0, 53.6, 47.0, 38.3, 34.2, 32.2, 28.0, 27.0, 25.7, 25.2; IR (neat) ν_{max} 3366, 2920, 2848, 1728, 1713 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 1115.3512 (M^+ + Cs, $C_{53}H_{67}O_{12}N_4P$ requires 1115.3547).

Di-*tert*-butyl (2*RS*)-*N*-[4-{3-(2-amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-((8-phosphono)-1-(octanoyloxy))-prop-2-yl}benzoyl]-L-glutamate (11). A solution of **10** (2.6 mg, 0.002 mmol) in EtOH was treated with 10% Pd–C (1 mg). The reaction mixture was stirred under H_2 for 4 h, filtered through a plug of Celite, and concentrated to give **11**·CF_3CO_2H (2.7 mg, quant) as a

white solid: mp >300 °C; ^{1}H NMR (DMF-d_7, 400 MHz) δ 9.36 (2H, br s, NH_2), 7.94 (2H, d, J = 8.3 Hz), 7.90 (1H, br s, H-5), 7.67 (1H, dd, J = 1.9, 6.5 Hz, H-7), 7.50 (2H, d, J = 8.2 Hz), 7.32 (1H, d, J = 8.3 Hz, H-8), 4.49 (1H, m, NHC*H*$CO_2C(CH_3)_3$), 4.25 (2H, m, CH_2), 3.91 (2H, m, CH_2), 3.51 (1H, m, CH), 3.26 (1H, dd, J = 7.7, 13.4 Hz, C*H*H), 3.15 (1H, dd, J = 7.6, 13.8 Hz, CH*H*), 2.42 (2H, t, J = 7.4 Hz, CH_2), 2.21 (2H, t, J = 7.4 Hz, CH_2), 2.16 (1H, m, C*H*H), 2.03 (1H, m, CH*H*), 1.58 (2H, m, CH_2), 1.42 (9H, s, $C(CH_3)_3$), 1.40 (9H, s, $C(CH_3)_3$), 1.46–1.20 (8H, m, $(CH_2)_4$); IR (KBr) ν_{max} 2932, 1787, 1720, 1500, 1204, 1146 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 935.2632 (M^+ + Cs, $C_{39}H_{55}O_{12}N_4P$ requires 935.2608).

(2*RS*)-*N*-[4-{3-(2-Amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-((8-phosphono)-1-(octanoyloxy))prop-2-yl}-benzoyl]-L-glutamate acid (3). A solution of **11** (3.8 mg, 0.004 mmol) in $CHCl_3$ (387 μL) cooled to 0 °C was treated with 97 μL of trifluoroacetic acid and the solution stirred at 0 °C for 3 h and at 25 °C for 12 h. Et_2O (1 mL) was added to the reaction mixture and a white precipitate formed. The precipitate was triturated with Et_2O (3 × 1 mL) and dried in vacuo to give **3**·CF_3CO_2H (3 mg, 78%) as a white solid: mp >300 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.79 (2H, d, J = 8.2 Hz), 7.75 (1H, s, H-5), 7.39 (2H, d, J = 7.9 Hz), 7.39 (1H, m, H-7), 7.13 (1H, d, J = 8.1 Hz, H-8), 4.37 (1H, m, NHC*H*CO_2H), 4.21 (2H, m, CH_2), 3.77 (2H, br s, CH_2), 3.36 (1H, m, CH), 3.07 (1H, m, C*H*H), 2.99 (1H, m, CH*H*), 2.33 (2H, t, J = 7.2 Hz, CH_2), 2.17 (1H, m, C*H*H), 2.06 (2H, t, J = 6.8 Hz, CH_2), 1.98 (1H, m, CH*H*), 1.50 (2H, m, CH_2), 1.31–1.06 (8H, m, $(CH_2)_4$); IR (KBr) ν_{max} 3444, 1719, 1096 cm^{-1}; Ionspray MS (NBA–CsI) *m/z* 689 ([M-H]-, $C_{31}H_{39}O_{12}N_4P$ requires 689).

Di-*tert*-butyl (2*RS*)-*N*-[4-{3-(2-amino-3,4-dihydro-4-oxo-quinazolin-6-yl)-((8-di(phenyloxy)phosphinoyloxy)-1-(octanoyloxy))prop-2-yl}benzoyl]-L-glutamate (12). A solution of **9** (4.3 mg, 0.005 mmol) in pyridine (37 μL) at −78 °C was treated with the diphenylphosphorochloridate (3.7 μL, 0.01 mmol). The reaction mixture was warmed to 25 °C, shaken to homogeneity, and recooled to −78 °C. The reaction mixture was allowed to warm to 4 °C and stirred for 20 h and concentrated in vacuo. PCTLC (SiO_2, 1 mm plate, 10% CH_3OH–$CHCl_3$) afforded **12** (4.5 mg, 81%) as a yellow oil: ^{1}H NMR (DMF-d_7, 400 MHz) δ 11.00 (1H, br s, NH), 7.90 (2H, d, J = 8.2 Hz), 7.75 (1H, d, J = 1.4 Hz, H-5), 7.44 (6H, m), 7.38 (1H, dd, J = 1.8, 8.3 Hz, H-7), 7.28 (6H, m), 7.08 (1H, d, J = 8.3 Hz, H-8), 6.45 (2H, br s, NH_2), 4.48 (1H, m, NHC*H*$CO_2C(CH_3)_3$), 4.30 (4H, m), 3.46 (1H, m, CH), 3.17 (1H, dd, J = 6.9, 13.8 Hz, C*H*H), 3.03 (1H, dd, J = 8.6, 13.8 Hz, CH*H*), 2.42 (2H, d, J = 7.1 Hz, CH_2), 2.22 (2H, t, J = 7.4 Hz, CH_2), 2.13 (1H, m, C*H*H), 2.01 (1H, m, CH*H*), 1.67 (2H, p, J = 7.0 Hz, CH_2), 1.44 (2H, m, CH_2), 1.42 (9H, s, $CO_2C(CH_3)_3$), 1.39 (9H, s, $CO_2C(CH_3)_3$), 1.31–1.15 (6H, m, $(CH_2)_3$); IR (neat) ν_{max} 3335, 3131, 2926, 1725, 1651 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 1087.3279 (M^+ + Cs, $C_{51}H_{63}O_{12}N_4P$ requires 108.3234).

Enzyme inhibition studies

The study of the inhibition of *pur*N GAR Tfase and avian AICAR Tfase by **3** was conducted as detailed.[1]

Cytotoxic testing

The testing of **3** was conducted following protocols described in detail.[1]

Acknowledgments

We gratefully acknowledge the financial support of the National Institutes of Health (CA63536), The Skaggs Institute for Chemical Biology, and the award of an ACS Medicinal Chemistry Graduate Fellowship (NEH, 1994–1995) sponsored by Parke–Davis Pharmaceutical Research.

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(*Received in U.S.A.* 5 *December* 1997; *accepted* 29 *April* 1997)