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Registry No. 1, 36821-85-9; 2, 53808-87-0; 3, 738-70-5; 4, 56066-63-8; 5, 56518-41-3; 6, 68902-57-8; 7, 69194-91-8; 8, 73090-70-7; 9, 78025-72-6; 10, 77113-54-3; 11, 20344-69-8; 12, 100515-03-5; 13, 107698-02-2; 14, 107698-00-0; 15, 100515-04-6; 16, 71525-05-8; 17, 69945-54-6; 18, 73356-41-9; 19, 13932-40-6; 20, 5355-16-8; 21, 83166-76-1; 22, 107698-03-3; 23, 73356-40-8; 24, 107697-99-4; 25, 83158-06-9; 26, 107698-04-4; 27, 78233-99-5; 28, 50823-96-6; 29, 98612-09-0; 30, 98612-08-9; 31, 80407-58-5; 32, 77113-56-5; 33, 77113-58-7; 34, 77113-57-6; 35, 77113-55-4; 36, 80416-29-1; 37, 59481-28-6; 38, 69945-57-9; 39, 69945-56-8; 40, 69945-58-0; 41, 69945-59-1; 42, 50823-94-4; 43, 77113-61-2; 44, 30077-60-2; 45, 77113-63-4; 46, 69945-60-4; 47, 77113-62-3; 48, 80407-62-1; 49, 77113-60-1; 50, 69945-50-2; 51, 69945-53-5; 52, 107698-01-1; 53, 30077-67-9; 54, 80407-59-6; 55, 69945-52-4; 56, 20285-70-5; 57, 836-06-6; 58, 69945-51-3; 59, 46726-70-9; 60, 18588-43-7; 61, 69945-55-7; 62, 20285-70-5; 63, 77113-59-8; 64, 49873-11-2; 65, 80407-61-0; 66, 80407-60-9; 67, 93317-64-7; 68, 7319-45-1; 4-phenylbenzaldehyde, 3218-36-8; β-anilinopropionitrile, 1075-76-9; 4-phenyl-β-cyano-N-phenylcinnamylaniline, 121269-12-3; guanidine, 113-00-8; 2,4-diamino-5-[3,4-bis(hydroxymethyl)-5-ethylbenzyl]pyrimidine, 121269-13-4; 2,4-diamino-5-(3-acetamido-4-(hydroxymethyl)-5-ethylbenzyl)pyrimidine, 121269-14-5; dihydrofolate reductase, 9002-03-3.

Nucleoside Peptides. 10. Synthesis and T-Cell Immunostimulatory Properties of Certain Peptide Derivatives of 6-Azacadeguomycin[1]

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Several amino acid and peptide conjugates of 6-azacadeguomycin (6-amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxylic acid, **2**) have been prepared in good yields, via a two-step procedure involving 1-hydroxybenzotriazole and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride mediated coupling of **2** with an appropriately protected amino acid or peptide, followed by ammonolysis. Thus, condensation of **2** with L-phenylalanine methyl ester, glycine ethyl ester, and L-glutamic acid diethyl ester gave the corresponding protected linear nucleoside peptides (**3**, **5**, and **7**, respectively). Subsequent ammonolysis of **3**, **5**, and **7** furnished L-phenylalanine amide (**4**), glycine amide (**6**) and L-glutamic acid diamide (**8**) conjugates of 6-azacadeguomycin, respectively. Saponification of **7** gave the corresponding L-glutamic acid derivative **9**. A similar coupling of **2** with L-phenylalaninyl-N^t-nitro-L-arginine methyl ester trifluoroacetate and subsequent ammonolysis (after catalytic hydrogenation) gave L-phenylalaninyl-L-arginine amide conjugate (**12**) of 6-azacadeguomycin. Compounds **2**, **4**, **6**, **8**, **9**, and **12** were evaluated for their ability to potentiate T-cell responses to plant mitogens, in comparison with cadeguomycin (**1**). Compounds **4**, **6**, and **9** exhibited an increase in the T-cell proliferation in a dose-dependent manner.

Interest in the nucleoside peptides was rekindled largely due to the recent isolation of several new naturally occurring peptidyl nucleoside antibiotics, e.g. arginomycin,[2] chryscandin,[3] and A201A.[4] The nucleoside and nucleotide peptides isolated from various sources differ markedly in structure and length of nucleotide and peptide chain, as well as in the nature of the peptide linkage.[5-8] Such variance of type of linkage and position of peptide attachment may be correlated with different reactivity and biological function.[5] Certain nucleotide peptides which readily bind to DNA and inhibit nucleic acid synthesis are suggestive of a regulatory function.[9] Gabbay and coworkers[10] have shown that peptides containing aromatic amino acids readily interact with DNA and the aromatic residue of the peptide is partially inserted between base pairs. This intercalation is rather specific and shows an affinity for A:T binding sites.[11] Sequence-specific DNA binding proteins regulate gene expression and also serve structural and catalytic functions in other cellular processes.[12,13] Considerable evidence is now accumulating indicating that various peptides and proteins are linked to certain types of viral DNA[14] and RNA.[15,16] It is of particular interest that certain DNA-binding oligopeptides exhibit remarkable antiviral activity,[17] e.g. netropsin[18] and

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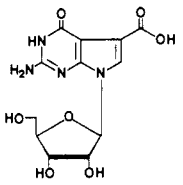
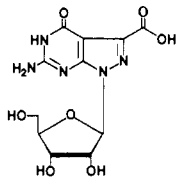
distamycin.[19,20] However, both netropsin and distamycin are too toxic for clinical use.[19,21]

Cadeguomycin is a novel nucleoside antibiotic isolated recently[22] from the culture broth of *Streptomyces hygroscopicus* IM7912T, as a minor component together with tubercidin, and characterized as 2-amino-3,4-dihydro-4-oxo-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid (1).[23] This interesting antibiotic inhibits



1
Cadeguomycin

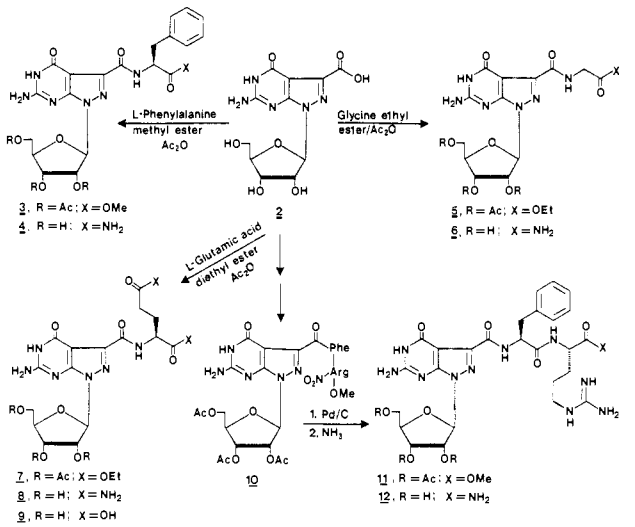


2
6-Azacadeguomycin

the growth of solid IMC carcinoma and pulmonary metastatis of Lewis lung carcinoma in mice in association with modification of the immune system.[24] It enhances cell-mediated immunity and macrophage activity.[24] Cadeguomycin displays a unique property of enhancing uptake of pyrimidine nucleosides into K562 human myelogenous leukemic cells and YAC-1 murine lymphoma cells, and it potentiates cytotoxicity of *ara*-C[24-26] as well as 5-fluoro-2′-deoxycytidine[27] both in vitro and in vivo.

The immune system is important in the host defense against tumors and microbial infections including viruses. T-cell-mediated specific, as well as nonspecific, host immune responses have been shown to be involved in providing protection against various pathogens and malignant diseases.[28-34] Breakdown in the immune defense system can result in the induction of malignancy and various microbial and parasitic infections as evident by the acquired immune deficiency syndrome (AIDS).[35]

Scheme I



Qualitative/quantitative abnormalities in the helper T-cells ($CD4^+$)[36,37] appear to be the predominant cause for the induction of this immunodeficiency disorder as T-cells are important in the regulation of various immune functions. In an effort to evaluate the nucleoside peptides for their ability to potentiate immune functions, especially the T-cell responses to plant mitogens, we have now synthesized certain amino acid and peptide derivatives of the readily available aza congener of **1**, 6-azacadeguomycin[38] [6-amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo-[3,4-*d*]pyrimidine-3-carboxylic acid, **2**] in which the peptide linkage is on the carboxylic acid group of the aglycon moiety. We hoped that the COOH group of **2** could be altered to a –CONH– function by coupling amino acids or peptides with 6-azacadeguomycin, which could impart greater selectivity of binding. The representative amino acids were selected on the basis of their ability to form hydrogen bonds with either the A:T or the G:C base pair.[12] An aromatic amino acid was chosen because of its intercalation behavior with DNA.[10] These nucleoside peptides could permit a quick assessment of the role of the nature and size of the charged end group in potentiating the ability of immune function.

Results and Discussion

The synthesis of these amino acid and peptide conjugates of 6-azacadeguomycin (**2**) was accomplished in good yields, via a two-step procedure involving the coupling of **2** with an appropriately protected amino acid or peptide ester. Since the purification of these coupling products was found to be rather difficult, due primarily to the coelution of unreacted **2**, acetylation of the reaction product was found to be beneficial. 6-Azacadeguomycin (**2**) was prepared as reported from our laboratory.[38] Compound **2** was coupled to L-phenylalanine methyl ester hydrochloride in anhydrous DMF with 1-hydroxybenzotriazole monohydrate (HOBT) and the water-soluble carbodiimide 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC), in the presence of triethylamine (TEA)[39] (Scheme I). Without extensive pu-

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Table I. Effect of 6-Azacadeguomycin Peptides on Mitogen-Induced Human Lymphocyte Proliferation in Comparison with Cadeguomycin (1)[a]

			[^{3}H]thymidine incorporation ± SD,[b] cpm					
				with compd (μM)				
compd	mitogen	concn, μg	without compd	0.1	1	10	50	400
1	PHA	0.125	15708 ± 3076	13682 ± 2851	14865 ± 3069	17242 ± 2347	15707 ± 2463	1268 ± 235
		1.0	40349 ± 4799	38824 ± 2675	45537 ± 5020	42486 ± 6661	36835 ± 1927	55547 ± 7324
	Con A	0.25	42670 ± 4484	45121 ± 1409	43757 ± 2757	42403 ± 1796	12990 ± 1399	9782 ± 387
	PWM	1/1250[c]	52418 ± 5526	51152 ± 2766	53594 ± 3713	54392 ± 902	50597 ± 1913	14118 ± 3068
		1/5000[c]	40053 ± 2385	47896 ± 631	44790 ± 1392	43600 ± 2826	42735 ± 1637	10034 ± 275
2	PHA	0.25	28436 ± 2939	25106 ± 2787	28393 ± 3296	32955 ± 6358	31662 ± 2843	29655 ± 3419
	Con A	0.25	21143 ± 3507	20186 ± 2429	21319 ± 4946	27385 ± 3584	22446 ± 2645	22619 ± 3464
	PWM	1/2500[c]	35867 ± 1098	35813 ± 1385	39189 ± 1813	36867 ± 1014	34808 ± 1857	34537 ± 4433
4	PHA	0.125	38473 ± 7059	26149 ± 1298	35042 ± 8558	54960 ± 3648	53121 ± 1779	42771 ± 2580
	Con A	0.25	27326 ± 6186	24317 ± 2514	26510 ± 3748	29989 ± 4590	27642 ± 4552	30812 ± 2340
	PWM	1/2500[c]	31047 ± 5442	39180 ± 2960	41073 ± 2046	44634 ± 5017	40511 ± 2014	37204 ± 2862
6	PHA	0.125	35075 ± 4557	27956 ± 2713	32413 ± 9867	47343 ± 3912	50056 ± 4327	66017 ± 3127
	Con A	0.50	43401 ± 5213	43954 ± 1406	40503 ± 6329	46662 ± 5133	44360 ± 1844	43649 ± 3480
	PWM	1/2500[c]	39468 ± 7653	44670 ± 1244	42288 ± 1032	44370 ± 1321	39719 ± 5563	40023 ± 1980
8	PHA	0.25	57799 ± 7256	49448 ± 2935	50331 ± 6228	48588 ± 10203	43877 ± 2633	39824 ± 7089
	Con A	0.25	25758 ± 7853	26181 ± 4583	30068 ± 5101	31799 ± 7591	28119 ± 4244	24167 ± 2546
	PWM	1/2500[c]	40326 ± 6052	46067 ± 1903	39260 ± 9360	45049 ± 2150	44234 ± 4140	29854 ± 2274
9	PHA	0.25	6506 ± 1506	8002 ± 2358	12995 ± 4310	16224 ± 3121	14187 ± 1533	13781 ± 2725
		1.0	56641 ± 10295	49176 ± 12072	50330 ± 3073	50830 ± 4077	50246 ± 6160	44427 ± 5941
	Con A	0.50	26393 ± 5216	43786 ± 2456	49196 ± 7377	47573 ± 8386	41114 ± 3807	45681 ± 13441
	PWM	1/1250[c]	40443 ± 2120	51918 ± 11290	56187 ± 4579	49105 ± 2482	53140 ± 3592	49556 ± 2959
12	PHA	0.125	23120 ± 3157	22401 ± 3538	19743 ± 2556	36645 ± 6639	21841 ± 3817	26854 ± 3307
		0.25	35768 ± 5894	38673 ± 4702	46087 ± 9968	36688 ± 6112	37174 ± 11289	33862 ± 4852
	Con A	0.50	35226 ± 5317	43850 ± 2542	36088 ± 2997	41908 ± 5680	36977 ± 4838	30792 ± 4437
	PWM	1/2500[c]	15723 ± 2987	17344 ± 1980	18470 ± 1632	18784 ± 2382	19755 ± 1347	18394 ± 2165
		1/5000[c]	7043 ± 667	11906 ± 1531	8437 ± 990	8734 ± 2025	9960 ± 1669	8214 ± 2695

[a] Peripheral blood lymphocytes (1×10^5/0.2 mL) from healthy donors were incubated with the plant mitogens in the presence or absence of compounds for 3–4 days. Lymphocyte proliferation induced by the mitogens was measured by incorporation of [^{3}H]thymidine in lymphocytes. The background [^{3}H]thymidine incorporation without mitogen were usually in the hundreds (cpm). [b] Standard deviation. [c] Dilution of PWM from GIBCO.

rification, the resulting reaction product was acetylated with acetic anhydride in DMF/pyridine to furnish *N*-[[6-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl]carbonyl]-L-phenylalanine methyl ester (**3**), which was isolated as a crystalline material in 58% yield. Treatment of **3** with MeOH/NH_3 at room temperature resulted in the ammonolysis of the ester function with concomitant deacetylation to give the desired *N*-[(6-amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl)-carbonyl]-L-phenylalanine amide (**4**) in excellent yield.

This general synthetic procedure was found to be applicable equally well to the preparation of other linear nucleoside peptides. Thus, condensation of **2** with glycine ethyl ester hydrochloride or L-glutamic acid diethyl ester hydrochloride in the presence of HOBT, EDC, and TEA gave the corresponding protected nucleoside peptide ester (**5** and **7**, respectively) in good yields. Ammonolysis of **5** and **7** with MeOH/NH_3 gave the desired glycine amide (**6**) and L-glutamic acid diamide (**8**) derivatives of 6-azacadeguomycin. Hydrolysis of the ester function of **7** with 1 N NaOH in MeOH/acetone at room temperature gave *N*-[(6-amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo-[3,4-*d*]pyrimidin-3-yl)carbonyl]-L-glutamic acid (**9**), which was isolated in 90% yield.

Following the similar coupling procedure an appropriately protected dipeptide was also reacted with **2**. When **2** was subjected to the above coupling conditions with the trifluoroacetate salt of L-phenylalaninyl-N^ϵ-nitro-L-arginine methyl ester in the presence of *N*-methylmorpholine, followed by acetylation, [*N*-[[6-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl]carbonyl]-L-phenylalaninyl]-N^ϵ-nitro-L-arginine methyl ester (**10**) was formed (Scheme I). The yield of crystalline **10** by this procedure was 70%. Direct coupling of 2′,3′,5′-tri-*O*-acetyl-6-azacadeguomycin with L-phenylalaninyl-N^ϵ-nitro-L-arginine methyl ester trifluoroacetate under identical conditions also gave **10** in 70% yield. Subsequent catalytic (Pd/C) hydrogenation of **10**, followed by ammonolysis of the reaction product (**11**) with MeOH/NH_3, gave [*N*-[(6-amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl)carbonyl]-L-phenylalaninyl]-L-arginine amide (**12**), in an overall yield of 72%. All the 6-azacadeguomycin peptides synthesized during this study were fully characterized by spectroscopic and elemental analyses. Confirmation that little or no racemization of the amino acid moieties had occurred was ascertained by TLC and HPLC studies.[40]

Effect of the Nucleosides on Mitogen-Induced Human Lymphocyte Proliferation. Human peripheral blood lymphocytes isolated over a Ficoll–Hypaque gradient were cultured with phytohemagglutinin (PHA), concanavalin A (Con A), or pokeweed mitogen (PWM) in the presence or absence of the test compounds. The results are shown in Table I. Cadeguomycin (**1**) significantly increased the lymphocyte proliferation induced by 1 μg of PHA at 400 μM concentration ($P < 0.05$). There was, however, no sigificant potentiation of Con A induced and PWM-induced lymphocyte proliferation by cadeguomycin. Compound **9** had no significant effect when the PHA-induced T-cell proliferation (without **9**) was high (56641 CPM). However, it showed a significant increase (up to a maximum increase of 140%, $P < 0.05$) in the T-cell proliferation in a dose-dependent manner when the response without **9** was relatively low (6506 CPM). In contrast to cadeguomycin, compound **9** also enhanced responses to T-cell mitogen Con A and T- and B-cell mitogen

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PWM when compared with the response to mitogens alone ($P < 0.05$). Similarly, compounds 4 and 6 were able to potentiate PHA-induced T-cell proliferation by 43% and 88%, respectively (maximal increases), which were greater than the proliferation induced by PHA alone ($P < 0.05$, $P = 0.01$). Again, the optimal enhancement was noted when the control (without compounds 4 or 6) response was moderate. Compound 4 also significantly augmented the proliferative response to PWM ($P < 0.05$), whereas compound 6 had a marginal effect. 6-Azacadeguomycin (2) and the nucleoside peptides 8 and 12 exhibited no significant increases in lymphocyte proliferation.

In summary, several selected amino acid and peptide derivatives of 6-azacadeguomycin have been prepared in good yield. The peptide linkage is on the carboxylic group of the pyrazolo[3,4-*d*]pyrimidine moiety, which mimics the substituted carbamoyl group at position 5 of cadeguomycin. Compounds 4, 6, and 9 showed an increase in the T-cell proliferation in a dose-dependent manner, whereas 6-azacadeguomycin (2) did not show such an increase.

Experimental Section

General Procedures. Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting point apparatus. Elemental analyses were performed by Robertson Laboratory, Madison, NJ. Thin-layer chromatography (TLC) was performed on plates of silica gel 60F-254 (EM Reagents). Silica gel (E. Merck; 230–400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of nucleoside components in TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30 °C. Infrared (IR) spectra were recorded in KBr with a Perkin-Elmer 1420-spectrophotometer and ultraviolet (UV) spectra were recorded with a Beckman DU-50 spectrophotometer (sh = shoulder). Nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz with an IBM NR/300 spectrometer. The chemical shift values were expressed in δ values (parts per million) relative to Me_4Si as the internal standard. The signals are described as s (singlet), d (doublet), t (triplet), and m (multiplet). The presence of solvent as indicated by elemental analysis was verified by ^{1}H NMR spectroscopy. The L-amino acids and coupling reagents used in this study were commercially available. The dipeptide was prepared by a standard solution phase method. THF was distilled prior to use from sodium benzophenone ketyl. Dichloromethane was distilled from P_2O_5 and stored over Linde 3A molecular sieves. Dimethylformamide was distilled from CaH_2.

***N*-[[6-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl]carbonyl]-L-phenylalanine Methyl Ester (3).** A mixture of 6-amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxylic acid[38] (6-azacadeguomycin, **2**, 0.98 g, 3 mmol), L-phenylalanine methyl ester hydrochloride (0.65 g, 3 mmol), and 1-hydroxybenzotriazole monohydrate (HOBT, 0.42 g, 3.1 mmol) in dry DMF (50 mL) was cooled to 0 °C. To this cold, stirred solution was added triethylamine (TEA, 0.42 g, 4.1 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC, 0.62 g, 3.2 mmol). The reaction mixture was stirred at 0 °C for 2 h and then at room temperature for 12 h. The solvent was evaporated, and the oily residue was dried over P_2O_5 under vacuum for 1 day. This dry material was used as such for the acetylation reaction without characterization.

A solution of the above residue in dry DMF/pyridine (1:1, 40 mL) was treated with acetic anhydride (1.23 g, 12 mmol) and the mixture was stirred at 0 °C for 8 h. The reaction mixture was evaporated to dryness and the residue was purified by flash chromatography over silica gel using CH_2Cl_2/acetone (7:3, v/v) as the eulent. The homogeneous fractions were pooled, evaporated to dryness and the residue was crystallized from CH_2Cl_2 containing acetone to give 1.07 g (58%) of **3**: mp 243–245 °C; IR ν_{max} 1630, 1670 (amide), 1740 (ester), 3200–3400 (NH_2) cm^{-1}; UV (pH 1) λ_{max} 232 (ϵ 18800), 260 (sh) nm (11700); UV (pH 7) λ_{max} 230 (ϵ 22100), 260 (sh) (13500), 285 (sh) nm (5800); UV (pH 11) λ_{max} 230 (ϵ 26200), 265 (sh) (11700), 288 (sh) nm (6700); ^{1}H NMR (Me_2SO-d_6) δ 2.03–2.09 (3 s, 9 H, 3 COCH_3), 2.97–3.22 (m, 2 H, Phe-CH_2), 3.63 (s, 3 H, COOCH_3), 4.04 (m, 1 H, Phe-α-*H*), 6.16 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, $C_1'H$), 7.0–7.30 (m, 7 H, Ph-*H* + NH_2), 10.63 (d, 1 H, N*H*), and 11.50 (br s, 1 H, N*H*). Anal. ($C_{27}H_{30}N_6O_{11}$, MW 614.51) C, H, N.

***N*-[(6-Amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl)carbonyl]-L-phenylalanine Amide (4).** A solution of **3** (0.65 g, 1.06 mmol) in MeOH/NH_3 (70 mL, saturated at 0 °C) was stirred at room temperature in a pressure bottle for 12 h. The bottle was cooled to 0 °C and opened and the NH_3 was allowed to evaporate. The MeOH was evaporated to dryness and the residue on crystallization from aqueous EtOH gave 0.35 g (70%) of **4**: mp 274–276 °C; IR ν_{max} 1650 (C=O), 3200–3400 (NH_2, OH) cm^{-1}; UV (pH 1) λ_{max} 234 (ϵ 29700), 260 (sh) (18900), 286 nm (7200); UV (pH 7) λ_{max} 234 (ϵ 32400), 260 (sh) (19900), 285 nm (8100); UV (pH 11) λ_{max} 230 (ϵ 38000), 265 (sh) (16500), 287 nm (9200); ^{1}H NMR (Me_2SO-d_6) δ 2.75–3.18 (m, 2 H, Phe-CH_2), 4.12 (m, 1 H, Phe-α-*H*), 5.89 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, $C_1'H$), 6.90–7.62 (m, 9 H, Ph-*H* + NH_2 + CONH_2), 10.36 (d, 1 H, N*H*), and 11.27 (s, 1 H, N*H*). Anal. ($C_{20}H_{23}N_7O_7 \cdot H_2O$, MW 491.40) C, H, N.

***N*-[(6-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl)carbonyl]-glycine Ethyl Ester (5).** By following the procedure as described for the preparation of **3**, the title compound was prepared by using **2** (0.98 g, 3 mmol), glycine ethyl ester hydrochloride (0.46 g, 3.4 mmol), HOBT (0.42 g, 3.1 mmol), EDC (0.62 g, 3.2 mmol), and TEA (0.34 g, 3.4 mmol) in dry DMF (50 mL). Acetylation of the crude reaction product with Ac_2O (1.23 g, 12 mmol) in DMF/pyridine (1:1, 100 mL) and purification by flash chromatography over silica gel using CH_2Cl_2/acetone (7:3, v/v), followed by crystallization from a mixture of CH_2Cl_2 and acetone gave 0.85 g (53%) of **5**: mp 202–205 °C; IR ν_{max} 1630, 1680 (C=O of amide), 1750 (C=O of ester), 3200–3400 (NH_2) cm^{-1}; UV (pH 1) λ_{max} 232 (ϵ 16600), 259 (sh) (9700), 286 (sh) nm (4200); UV (pH 7) λ_{max} 228 (ϵ 19400), 260 (sh) (10800), 284 (sh) nm (5400); UV (pH 11) λ_{max} 230 (ϵ 22100), 288 nm (5900); ^{1}H NMR (Me_2SO-d_6) δ 1.20 (t, 3 H, CH_2CH_3), 2.05–2.10 (3 s, 9 H, 3 COCH_3), 4.11 (m, 2 H, Gly-CH_2), 6.18 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, $C_1'H$), 7.20 (br s, 2 H, NH_2), 10.48 (m, 1 H, N*H*), and 11.45 (br s, 1 H, N*H*). Anal. ($C_{21}H_{26}N_6O_{11}$, MW 538.42) C, H, N.

***N*-[(6-Amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl)carbonyl]glycine Amide (6).** The title compound was prepared in a similar manner as described for **4** with **5** (0.60 g, 1.12 mmol) and MeOH/NH_3 (70 mL). The product was crystallized from 95% aqueous EtOH to yield 0.27 g (63%) of **6**: mp >250 °C dec; IR ν_{max} 1650, 1670 (C=O of amide), 3200–3400 (NH_2, OH) cm^{-1}; UV (pH 1) λ_{max} 235 (ϵ 5500), 260 (sh) nm (3400); UV (pH 7) λ_{max} 234 (ϵ6000), 260 nm (3400); UV (pH 11) λ_{max} 230 (ϵ 13200), 265 (sh) nm (5400); ^{1}H NMR (Me_2SO-d_6) δ 3.89 (m, 2 H, Gly-CH_2), 5.92 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, $C_1'H$), 6.96 (br s, 2 H, NH_2), 7.08 and 7.48 (2 s, 2 H, CONH_2), 10.25 (m, 1 H, N*H*), and 11.25 (br s, 1 H, N*H*). Anal. ($C_{13}H_{17}N_7O_7 \cdot {}^1/_2H_2O$, MW 401.29) C, H, N.

***N*-[[6-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl]carbonyl]-L-glutamic Acid Diethyl Ester (7).** In a similar manner as for **3**, the title compound was prepared by using **2** (1.65 g, 5 mmol), L-glutamic acid diethyl ester hydrochloride (1.30 g, 5.5 mmol), HOBT (0.65 g, 5 mmol), EDC (0.96 g, 5 mmol), and TEA (0.60 g, 6 mmol) in dry DMF (50 mL). Acetylation of the crude reaction product with Ac_2O (2.04 g, 20 mmol) in dry DMF (30 mL) and pyridine (20 mL) and purification by flash chromatography over silica gel using CH_2Cl_2/acetone (7:3, v/v), followed by crystallization from CH_2Cl_2 containing MeOH gave 2.6 g (81%) of **7**: mp 220–224 °C; IR ν_{max} 1680 (C=O of amide), 1740 (C=O of ester), 3200–3400 (NH_2) cm^{-1}; UV (EtOH) 228 (ϵ 21700), 257 (13800), 286 (sh) nm (5400); ^{1}H NMR (Me_2SO-d_6) δ 1.14–1.23 (m, 6 H, 2 CH_2CH_3), 1.90–2.43 (m, 13 H, 3 COCH_3 + Glu-CH_2), 4.01–4.17 (m, 5 H, 2 CH_2CH$_3$ + Glu-α-*H*), 6.19 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, $C_1'H$), 7.12 (br s, 2 H, NH_2), 10.54 (d, 1 H, N*H*) and 11.40 (s, 1 H, N*H*). Anal. ($C_{26}H_{34}N_6O_{13}$, MW 638.53) C, H, N.

***N*-[(6-Amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl)carbonyl]-L-glutamic Acid**

Diamide (8). This compound was prepared by following the procedure as described for the synthesis of 4, with compound 7 (1.40 g, 2.2 mmol) and MeOH/NH_3 (100 mL). The residue after crystallization from 95% aqueous EtOH gave 0.70 g (70%) of the title compound: mp 263–265 °C; IR ν_{max} 1670 (C=O of amide), 3200–3400 (NH_2, OH) cm^{-1}; UV (pH 1) λ_{max} 234 (ϵ 23 400), 260 (sh) (15 000), 290 (sh) nm (6800); UV (pH 7) λ_{max} 232 (ϵ 24 900), 260 (sh) (16 800), 287 (sh) nm (8200); UV (pH 11) λ_{max} 230 (ϵ 29 000), 262 (sh) (14 100), 287 (sh) nm (8600); ^1H NMR (Me_2SO-d_6) δ 1.80–2.18 (m, 4 H, Glu-CH_2), 4.16 (m, 1 H, Glu-α-H), 5.92 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, $C_{1'}H$), 6.76 and 7.29 (2 s, 2 H, CONH_2), 7.06 and 7.50 (2 s, 2 H, CONH_2), 7.00 (br s, 2 H, NH_2), 10.26 (d, 1 H, NH), and 11.25 (br s, 1 H, NH). Anal. ($C_{16}H_{22}N_8O_8 \cdot {}^1/_2CH_3OH \cdot H_2O$, MW 488.42) C, H, N.

N-[(6-Amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl)carbonyl]-L-glutamic Acid (9). A solution of 7 (1.40 g, 2.2 mmol) in MeOH/acetone (10 mL each) and 1 N NaOH (15.4 mL, 15.4 mmol) was stirred at ambient temperature for 6 h. The MeOH/acetone was evaporated and the aqueous solution was diluted with water (50 mL). The pH of the aqueous solution was adjusted to 4 with Dowex-50 (H^+) resin. The resin was removed by filtration and washed with water (2 × 10 mL), and the combined filtrates were evaporated to dryness. The residue was crystallized from 95% aqueous EtOH to give 0.9 g (90%) of 9: mp >230 °C dec; IR ν_{max} 1670 (C=O of amide), 1730 (C=O of COOH), 3200–3400 (NH_2, OH) cm^{-1}; UV (pH 1) λ_{max} 234 (ϵ 29 800), 260 (sh) (17 300), 284 nm (7400); UV (pH 7) λ_{max} 229 (ϵ 29 600), 257 (sh) (20 500), 284 (sh) nm (7300); UV (pH 11) λ_{max} 230 (ϵ 30 000), 258 (sh) (20 000), 285 (sh) nm (7000); ^1H NMR (Me_2SO-d_6) δ 1.87–2.28 (m, 4 H, Glu-CH_2), 4.16 (m, 1 H, Glu-α-H), 5.93 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, $C_{1'}H$), 7.05 (br s, 2 H, NH_2), 10.44 (d, 1 H, NH), 11.32 (s, 1 H, NH), and 12.50 (br s, 2 H, 2 COOH). Anal. ($C_{16}H_{20}N_6O_{10} \cdot {}^1/_2H_2O$, MW 465.33) C, H, N.

[N-[[6-Amino-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl]carbonyl]-L-phenylalaninyl]-N^{ϵ}-nitro-L-arginine Methyl Ester (10). Method A. In the same manner as for 3, reaction of 2 (0.82 g, 2.5 mmol), L-phenylalaninyl-N^{ϵ}-nitro-L-arginine methyl ester trifluoroacetate (1.24 g, 2.5 mmol), *N*-methylmorpholine (0.25 g, 2.5 mmol), HOBT (0.33 g, 2.5 mmol), and EDC (0.48 g, 2.5 mmol) in dry DMF (50 mL) and subsequent acetylation of the reaction product with Ac_2O (1.78 g, 17.5 mmol) in anhydrous pyridine (30 mL) and DMF (30 mL) gave the crude product. Purification of the crude product by flash chromatography over silica gel using CH_2Cl_2/acetone (7:3, v/v) as the eluent, followed by crystallization from a mixture of CH_2Cl_2/acetone/MeOH, gave 1.0 g (70%) of 10: mp 135–137 °C; IR ν_{max} 1650 (C=O of amide), 1750 (C=O of ester), 3200–3400 (NH_2) cm^{-1}; UV (pH 1) λ_{max} 225 (sh) (ϵ 24 100), 259 nm (24 500); (pH 7) λ_{max} 229 (sh) (ϵ 27 500), 260 nm (27 100); UV (pH 11) λ_{max} 230 (ϵ 31 500), 263 nm (25 000); ^1H NMR (Me_2SO-d_6) δ 1.48–1.90 (m, 4 H, Arg-CH_2), 2.03–2.09 (3 s, 9 H, 3 COCH_3), 2.82–3.30 (m, 4 H, Phe and Arg-CH_2), 3.62 (s, 3 H, OCH_3), 4.02 (m, 1 H, Phe-α-H), 4.25 (m, 1 H, Arg-α-H), 6.15 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, $C_{1'}H$), 6.90–7.35 (m, 7 H, Phe-H + NH_2), 7.70–8.40 (m, 3 H, NH + NH_2), 8.64 (d, 1 H, NH), 10.40 (d, 1 H, NH), and 11.37 (s, 1 H, NH). Anal. ($C_{33}H_{41}N_{11}O_{14}$, MW 815.66) C, H, N.

Method B. A solution of 6-azacadeguomycin[38] (2, 2.5 g, 7.65 mmol) in dry DMF (50 mL), anhydrous pyridine (20 mL), and Ac_2O (3.90, 38.2 mmol) was stirred at room temperature for 12 h and then evaporated to dryness. The residue was suspended in a mixture of water (50 mL) and EtOAc (75 mL) and stirred for 1 h. The aqueous phase was separated and again extracted with EtOAc (2 × 25 mL). The organic layers were combined and washed with saturated brine solution (2 × 25 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness. Crystallization of the residue from a mixture of CH_2Cl_2 and MeOH gave 2.8 g (81%) of 6-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxylic acid (2′,3′,5′-tri-*O*-acetyl-6-azacadeguomycin): mp 159–161 °C; IR ν_{max} 1640 (C=O), 1750 (C=O of COOH), 3300–3400 (NH_2) cm^{-1}; UV (pH 1) λ_{max} 230 (ϵ 18 900), 259 (8700), 288 nm (4100); UV (pH 7) λ_{max} 255 nm (ϵ 11 800); UV (pH 11) λ_{max} 222 (sh) (ϵ 24 500), 262 nm (8800); ^1H NMR (Me_2SO-d_6) δ 1.97–2.09 (3 s, 9 H, 3 COCH_3), 6.17 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, $C_{1'}H$), 7.30 (br s, 2 H, NH_2), and 11.90 (br s, 1 H, NH). Anal. ($C_{17}H_{19}N_5O_{10}$, MW 453.36) C, H, N.

2′,3′,5′-Tri-*O*-acetyl-6-azacadeguomycin (0.91 g, 2 mmol) was coupled with L-phenylalaninyl-N^{ϵ}-nitro-L-arginine methyl ester trifluoroacetate (0.86 g, 1.76 mmol) in the presence of *N*-methylmorpholine (0.20 g, 2 mmol), HOBT (0.26 g, 2 mmol), and EDC (0.48 g, 2.5 mmol) in a mixture of dry DMF (30 mL) and CH_2Cl_2 (70 mL) as described in method A. The crude product was purified by flash chromatography using CH_2Cl_2/acetone (7:3, v/v) as the eluent to give 1.0 g (70%) of 10: mp 135–137 °C and was identical with 10 prepared by method A.

[N-[[6-Amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidin-3-yl]carbonyl]-L-phenylalaninyl]-L-arginine Amide (12). To a solution of 10 (1.5 g, 1.84 mmol) in EtOH (100 mL) and water (25 mL) was added Pd/C (10%, 0.5 g) and the mixture was hydrogenated on a Parr hydrogenator at 40 psi for 10 h. The catalyst was removed by filtration on a Celite pad and washed with hot aqueous EtOH (2 × 25 mL), and the combined filtrates were evaporated to dryness. The residue (11), after drying over P_2O_5 under vacuum for 5 h, was used as such for the next step without characterization.

The above residue (11, 1.1 g, 1.43 mmol) was stirred with MeOH/NH_3 (saturated at 0 °C, 120 mL) at ambient temperature for 24 h in a pressure bottle. The bottle was cooled and opened, and the solvents were evaporated to dryness. The residue was crystallized from aqueous EtOH to yield 0.65 g (72%) of 12: mp 253–255 °C; IR ν_{max} 1650 (C=O of amide), 3200–3400 (NH_2, OH) cm^{-1}; UV (pH 1) λ_{max} 233 (ϵ 17 600), 260 (sh) (10 700), 290 (sh) nm (3200); (pH 7) λ_{max} 233 (ϵ 20 200), 260 (sh) (11 300), 290 (sh) nm (4400); (pH 11) λ_{max} 229 (ϵ 22 500), 260 (sh) (9400), 287 (sh) nm (5100); ^1H NMR (Me_2SO-d_6) δ 1.10–1.70 (m, 4 H, Arg-CH_2), 2.99–3.15 (m, 4 H, Arg- and Phe-CH_2), 4.12–4.30 (m, 2 H, Arg- and Phe-α-Hs), 5.92 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, $C_{1'}H$), 6.07 (br s, 2 H, NH_2), 7.03–7.30 (m, 11 H, Ph-H + 2NH + NH_2 + CONH_2), 7.56 (d, 1 H, NH), 8.88 (br, s, 1 H, NH), and 11.50 (d, 1, NH). Anal. ($C_{26}H_{35}N_{11}O_{18} \cdot 6H_2O$, MW 629.54) C, H, N.

Effect of 6-Azacadeguomycin Peptides on Mitogen-Induced Human Lymphocyte Proliferation. 6-Azacadeguomycin peptides were tested for their ability to modulate lymphocyte proliferation to T and B plant mitogens in vitro. The inhibition of, or the increase in, [^3H]thymidine incorporation in mitogen-stimulated lymphocytes was used as the measure of immunomodulatory activity of these compounds. Lymphocytes were isolated from heparinized peripheral blood of normal healthy human donors over Ficoll–Hypaque as previously described.[41,42] In brief, the blood was diluted with (1:1) Hanks balanced salt solution (HBSS), layered over a Ficoll–Hypaque gradient, and centrifuged at 500*g* for 20 min. The lymphocytes recovered from the interface were washed and resuspended in complete RPMI-1640 medium containing 10% heat-inactivated human AB serum (CM). Lymphocytes (1×10^5) suspended in 100 mL of CM were cultured with plant mitogens with or without various concentrations of cadeguomycin or its aza analogues in microculture plates. The total volume was 200 μL. The incubation was carried out at 37 °C in a 5% CO_2, humid atmosphere for 72–96 h. All cultures were incubated in quadruplicate. Sixteen hours before the end of incubation period, each sample was pulsed with 1 μCi of [^3H]thymidine, and the cells were harvested by filtration on glass-fiber filter paper using a cell harvester and washed with distilled water. [^3H]Thymidine incorporation was determined by counting in a liquid-scintillation counter. The data was analyzed using Student's *t* test.

Registry No. 2, 96555-48-5; 2 (tri-*O*-acetyl derivative), 121176-42-9; 3, 121176-29-2; deacetyl-3, 121176-38-3; 4, 121176-30-5; 5, 121176-31-6; deacetyl-5, 121176-39-4; 6, 121176-32-7; 7, 121176-33-8; deacetyl-7, 121176-40-7; 8, 121176-34-9; 9, 121176-35-0; 10, 121191-54-6; deacetyl-10, 121176-41-8; 11, 121176-36-1; 12, 121176-37-2; H-Phe-OMe·HCl, 7524-50-7; H-Gly-OEt·HCl, 623-33-6; H-Glu(OEt)-OEt·HCl, 1118-89-4; H-Phe-Arg(NO_2)-OMe·TFA, 30668-59-8.

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