Benzylated 1,2,3-Triazoles as Anticoccidiostats

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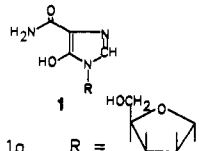
Substituted 5-amino-4-carbamoyl-1,2,3-triazoles **3a–w** were prepared by two synthetic schemes and evaluated in vivo for anticoccidial activity. Both schemes proceeded by brominating appropriately substituted toluenes **4a–s,v** to **5a–s,v**. In Scheme I, the brominated benzyl analogues **5** were converted to the corresponding benzyl azides **6**, which were treated with cyanoacetamide to yield 1-substituted-5-amino-4-carbamoyl-1,2,3-triazoles **3**. In Scheme II, the benzyl halides **5** were employed to alkylate the sodium salt of 5-amino-4-carbamoyl-1,2,3-triazole (**7**). Preliminary screening data against *Eimeria acervulina* and *E. tenella* in chickens suggested structural requirements for maximizing activity. Further evaluation against a relatively resistant series of eight *Eimeria* field isolates revealed L-651,582 (**3a**) to be a highly effective coccidiostat. However, unacceptable tissue residues precluded further development. Mechanistic studies on this series of 5-amino-4-carbamoyl-1,2,3-triazoles and, in particular, on L-651,582 (**3a**) revealed that its mode of action does not involve inhibition of IMP dehydrogenase, but probably interferes with host cell calcium entry. In addition, L-651,582 has been found to have antiproliferative activity in several disease models and was recently reported to possess antimetastatic activity in a model of ovarian cancer progression.

Coccidiosis is the most important disease affecting the economics of the broiler chicken industry. Athough species of the causative organisms of coccidiosis, protozoa of the genus *Eimeria*, are known to infect many animal species, massive losses from the disease occur mainly in the poultry industry. Under current broiler husbandry conditions, large numbers of young chickens are reared in close confinement, making them extremely susceptible to infection. Anticoccidial drugs are given continuously in the feed as a prophylactic measure to avoid potentially large losses. A successful drug must be effective against all eight major *Eimeria* species, not retard growth due to subacute toxicity, and leave a negligible residue in the edible part of the carcass. The ready development of drug resistance to coccidiostats has resulted in a continuing search for new drugs preferably with different mechanisms of action.

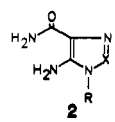
A starting point in our laboratories was the antiviral natural product bredinin (**1a**), which is known to inhibit inosine monophosphate (IMP) dehydrogenase,[1] a potential target enzyme in protozoa. Since bredinin aglycon **1b** is known to be glycosylated into bredinin in vivo, the aglycon was synthesized and found to be moderately active against coccidia in chickens.[2]



1

1a R = 

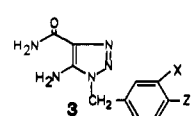
1b R = H



2

2a X = C–H, R = H

2b X = N, R = H

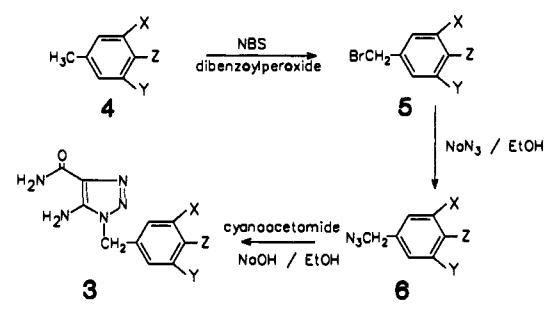


3

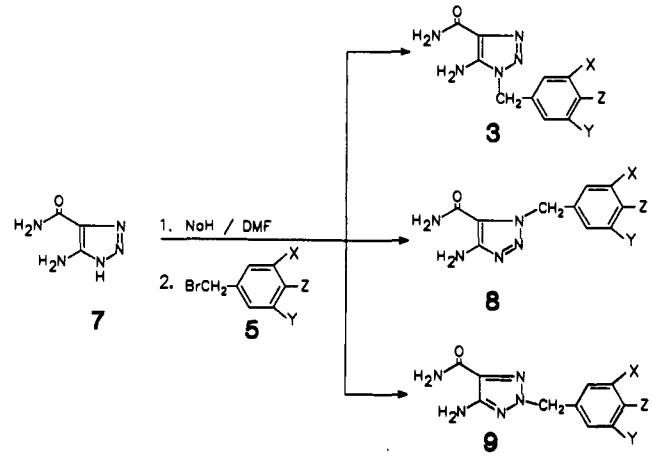
2c X = C–H, R = $-CH_2-C_6H_3-3,4,Cl_2$

Anticipated resistance development, similar to that found with purine and pyrimidine analogues, which must also be activated by in vivo conversion into nucleosides and nucleotides, precluded development of the aglycon into an anticoccidial drug.[3] However, in view of observations that halobenzyl groups can mimic ribose moieties in some cases, the syntheses of a series of 1-benzyl-5-hydroxy-4-carbamoylimidazoles was initiated.[4] Straightforward benzylation of this substrate, **1b**, was not immediately successful, so the readily available isosteric 5-amino-4-carbamoylimidazole (**2a**) was chosen as a starting point for syntheses. The synthesis of a series of 1-benzyl-5-aminoimidazole-4-

Scheme I



Scheme II



(1) Sakaguchi, K.; Tsujino, M.; Yoshizawa, M.; Mizuno, K.; Hayano, K. Action of Bredinin on Mammalian Cells. *Cancer Res.* **1975**, *35*, 1643–1648.

(2) T. Tamas, unpublished results.

(3) (a) Brockman, R. W. Resistance to Purine Antagonists in Experimental Leukemia Systems. *Cancer Res.* **1965**, *25*, 1596–1605. (b) Tomizawa, S.; Aronow, L. Studies on Drug Resistance in Mammalian Cells. II. 6-Mercaptopurine Resistance in Mouse Fibroblasts. *J. Pharmacol. Exp. Therap.* **1960**, *128*, 107–114.

(4) (a) Chabala, J. C.; Fisher, M. H.; Patchett, A. A. U.S. Patent 4,659,720, 1987. (b) Mylari, B. L.; Miller, M. W.; Howes, H. L., Jr.; Figdor, S. K.; Lynch, J. E.; Koch, R. C. Anticoccidial Derivatives of 6-Azauracil. 1. Enhancement of Activity by Benzylation of Nitrogen-1. Observations of the Dwsign of Nucleotide Analogues in Chemotherapy. *J. Med. Chem.* **1977**, *20*, 475–483.

0022-2623/91/1834-2843$02.50/0

carboxamides and their activity against the protozoan parasite, *Trypanosoma cruzi*, has recently been described.[5] These compounds also possessed weak to moderate anticoccidial activity.[2] An important observation was made early in the program. Replacement of the imidazole moiety in **2c** by a 1,2,3-triazole ring, **2b**, as in **3w**, resulted in an increase in anticoccidial potency. This report describes the synthesis and biological activity of a series of 1-substituted-5-amino-4-carbamoyl-1,2,3-triazoles, **3**.

Chemistry

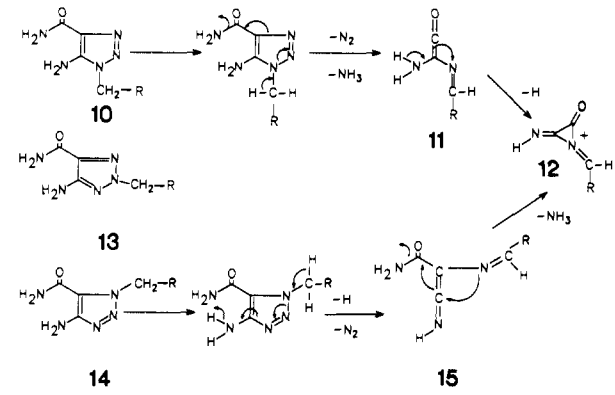
The desired 5-amino-4-carbamoyl-1,2,3-triazoles **3a–w** were prepared by two general synthetic pathways shown in Schemes I and II.[6a,b]

Both synthetic schemes proceeded from the appropriately substituted toluenes, **4a–s,v** (Table I), which were brominated under radical conditions using NBS to yield **5a–s,v** (Table II). In Scheme I, the brominated benzyl analogues **5** were converted to the corresponding benzyl azides **6** (Table III) with sodium azide in refluxing ethanol. The reaction of the azides under basic conditions with cyanoacetamide yielded the desired 1-substituted-5-amino-4-carbamoyl-1,2,3-triazoles **3**. The details of the azide cyclizations to triazoles are summarized in Table IV. Scheme II employed the benzyl halides **5** to alkylate the sodium salt of the parent 5-amino-4-carbamoyl-1,2,3-triazole (**7**) in dimethylformamide. These triazole alkylation reactions are summarized in Table V. This method was useful when an azide proved unstable to base, due to the enhanced acidity of the benzylic protons such as in **6n**. Although such cases resulted in low yields of the desired triazole, a good yield of corresponding aryl aldehyde was obtained. Presumably the aryl aldehyde was formed by α-elimination of N_2 from the benzyl azide and subsequent hydrolysis of the resulting imine during workup. Also, in some examples the 4″-substituent of the methylbenzophenone analogue proved susceptible to replacement by methoxide during attempted cyclization. ^{1}H NMR spectra indicated that as much as 30% of the 4″-OCH_3 analogue was generated by the reaction of **6g** and cyanoacetamide using conditions outlined in Scheme I.

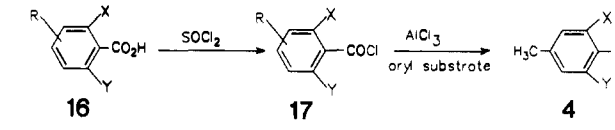
Examination of the ^{1}H NMR chemical shifts of the benzylic and 5-amino protons in DMSO solutions of comparable analogues prepared by the methods of Schemes I and II indicated that in all cases, the Scheme II method generated a mixture of 1-substituted 1,2,3-triazole **3** and an isomeric monobenzyl analogue, **8** or **9**, as the major products. While a small chemical shift differences in the benzyl protons were observed, more dramatic were the 0.8–0.9 ppm downfield shifts of the amino protons in the 1-substituted analogues. These isomeric analogues were usually easily separated by column chromatography.

Structure elucidation of the isomeric benzyl compounds **8** or **9** proved more difficult than expected. The 4-carbamoyl group interfered with efforts to examine nuclear Overhauser effects in both isomers. Proton and ^{15}N NMR studies and efforts to prepare suitable crystals of the new isomers for X-ray analysis proved fruitless. However, the mass spectra of both the 1-substituted series and the isomeric analogues exhibit a strong M – 46 fragment. High resolution mass spectral comparisons of both isomers indicated that this fragment corresponded to the loss of H_4N_3. Although fragmentations involving loss of H_4N_3 can

Scheme III

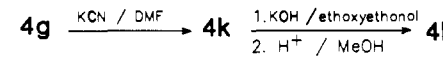


Scheme IV



16a X = Y = Cl; R = 4-CH_3
16b X = Cl; Y = Br; R = 4-CH_3
16c X = Y = Cl; R = 4-Cl
16d X = Cl; Y = H; R = 4-CH_3
16e X = Y = H; R = 4-CH_3
16f X = Cl; Y = H; R = 4-Cl
16g X = Y = H; R = 3-CH_3
16h X = Y = Cl; R = H
16i X = Cl; Y = H; R = 5-CH_3

Scheme V



easily be envisioned to proceed from **10** through **11** to **12** and from **14** through **15** to **12** as outlined in Scheme III, it is difficult to envision a similar fragmentation for **13**. On this basis, structure **8** is tentatively assigned to the new isomeric product rather than the 2-substituted isomer **9**, which would have been predicted on the basis of literature precedent.[7] Since compounds in the isomeric series were devoid of significant anticoccidial activity, further efforts in their structure determination were not pursued.

Preparation of Substituted Toluenes 4a–x. The benzyl side chains utilized were either substituted benzophenones or substituted benzyl analogues. The benzophenone analogues were prepared according to Scheme IV, from the appropriate carboxylic acids **16a–e**, which were then converted to the acid chlorides **17a–e** and directly reacted with an aryl substrate under Friedel–Crafts conditions. The results of these acylations are summarized in Table I. The acid chlorides were obtained by treatment of the benzoic acids with thionyl chloride in dichloromethane with dimethylformamide as a catalyst and were used without purification. Compounds **4k** and **4l** were prepared by a method that utilized the lability of the 4″-substituent mentioned above. Scheme V outlines the displacement of fluorine from **4g** with KCN to provide the

(5) Chabala, J. C.; Waits, V. B.; Ikeler, T.; Patchett, A. A.; Payne, L.; Peterson, L. H.; Reamer, R. A.; Hoogsteen, K.; Wyvratt, M.; Hanson, W. L.; Fisher, M. H. 1-(Substituted)benzyl-5-aminoimidazole-4-carboxamides Are Potent Orally Active Inhibitors of *Trypanosoma cruzi* in Mice. *Experientia* **1991**, *47*, 51–53.

(6) (a) For review of the preparation and chemistry of 1,2,3-triazoles, see: Gilchrist, T. L.; Gymer, G. E. 1,2,3-Triazoles. In *Advances in Heterocyclic Chemistry*, Vol. 16; Katritzky, A. R., Boulton, A. J., Eds.; Academic Press: New York, 1974; pp 33–45. (b) Grimmett, M. R. Diazoles, Triazoles, Tetrazoles and Their Benzoanalogues. In *Comprehensive Organic Chemistry*, Vol. 4; Sammes, P. G., Ed.; Pergamen Press: Oxford, 1979; pp 357–410.

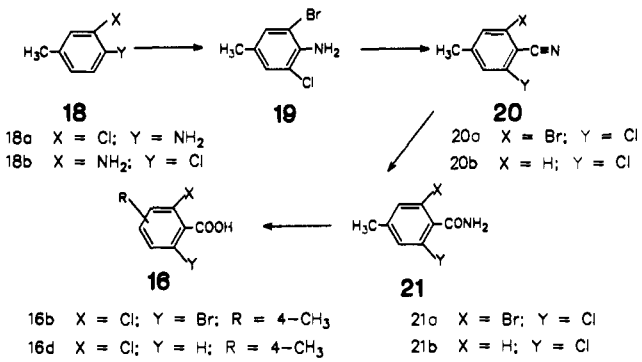
(7) See ref 6b, p 377.

Table I. Preparation of Methylbenzophenones 4

SM #	MOL 16	SUBSTRATE	SUBSTRATE Vol (mL)	COSOLVENT Vol (mL)	$AlCl_3$ MOL	PROD #	X	Z	Y	YlD 4 %
16a	0.0380	Chlorobenzene	8.5	235[a]	0.0410	4a	Cl	-COC_6H_4-4-Cl	Cl	63.2
16b	0.0205	Chlorobenzene	15	[b]	0.0210	4b	Cl	-COC_6H_4-4-Cl	Br	68.9
16d	0.0386	Chlorobenzene	10	[b]	0.0380	4d	Cl	-COC_6H_4-4-Cl	H	78.1
16e	0.646	Chlorobenzene	15	[b]	0.0647	4e	H	-COC_6H_4-4-Cl	H	50.8
16a	0.024	Benzene	30	[b]	0.0258	4f	Cl	-COC_6H_5	Cl	72.3
16a	0.094	Fluorobenzene	105	[b]	0.098	4g	Cl	-COC_6H_4-4-F	Cl	74.1
16a	0.0294	[c]	[d]	40[e]	0.0281	4j	Cl	-COC_6H_4-4-CCl=CCl_2	Cl	43.5
16a	0.0313	*o*-Dichlorobenzene	7	35[e]	0.0328	4m	Cl	-COC_6H_3-3,4-Cl_2	Cl	66.0
16h	0.0826	Toluene	30	[b]	0.082	4n	H	-COC_6H_3-2,6-Cl_2	H	57.6
16f	0.012	3-Clorotoluene	150	[b]	0.129	4o	Cl	-COC_6H_3-2,4-Cl_2	H	60.6
16c	0.031	Toluene	3.5	30[e]	0.0210	4q	H	-COC_6H_2-2,4,6-Cl_3	H	71.9
16g	0.0647	Chlorobenzene	14	[b]	0.0647	4r	-COC_6H_4-4-Cl	-H	H	63.1
16i	0.0551	Chlorobenzene	14	[b]	0.0548	4s	-COC_6H_4-4-Cl	-Cl	H	70.9

[a] Carbon tetrachloride. [b] No added solvent. [c] Trichlorovinylbenzene (Scherer, O.; Hahn, H. *Liebigs Ann. Chem.* **1964**, *677*, 83. [d] Used 6.83 g (32.9 mmol). [e] Tetrachloroethylene.

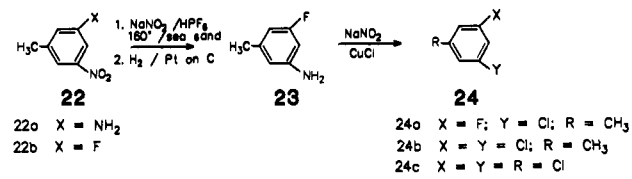
Scheme VI



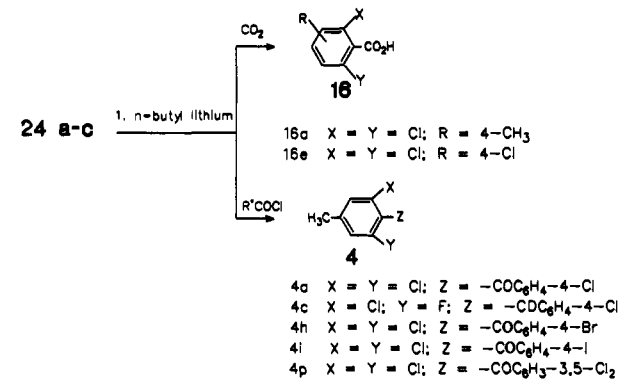
4″-cyano analogue **4k**. Basic hydrolysis of the cyano group in **4k** and esterification of the resulting 4″-carboxylic acid yielded the 4″-carbomethoxy synthon **4l**.

Preparation of Carboxylic Acids 16a–d. Where needed, the carboxylic acid synthons were prepared as outlined in Scheme VI. Direct bromination of the unprotected aniline **18** yielded the 2,6-dihaloaniline **19**. This sterically hindered, weakly basic aniline was converted to the nitrile **20** by using nitrosylsulfuric acid and KCN. Subsequent hydration of the nitrile **20** and hydrolysis of the benzamide **21** yielded the bromochlorotoluic acid **16b**. The prerequisite 1,3,5-trisubstituted fluoro analogue **24a** was obtained from **22a** as outlined in Scheme VII. The sequence proceeded via a modified Scheimann reaction, yielding **22b**, which was subjected to catalytic reduction to yield **23**. Standard Sandmeyer conditions on **23** yielded **24a**. Carbonation of the lithiated 1,3,5-trisubstituted benzenes **24a–c** as shown in Scheme VIII, was employed to prepare the corresponding benzoic acids **16a** and **16d**. Alternative treatment of the lithiated substituted benzene **24a** with an acid chloride yielded **4g**, **4h**, **4i**, and **4p**.

Scheme VII



Scheme VIII



Acylation of lithiated **24b** with *p*-chlorobenzoyl chloride also provided **4a**.

Preparation of α-Bromotoluenes 5a–m. Treatment of the toluene analogues **4a–s,v** in a benzene solution at reflux with *N*-bromosuccinimide and benzoyl peroxide as catalyst afforded mixtures of starting toluenes **4**, α-monobrominated **5a–s,w** and α,α-dibrominated products. The stoichiometry and reaction times were adjusted on the basis of small-scale reactions where the compounds were heated with a 10% molar excess of NBS until a negative reaction to starch iodide paper was observed. After evaluation of the reaction mixtures by ^1H NMR and as-

Table II. Preparation of α-Bromotoluenes 5

SM #	MOL RATIO NBS/4[a]	MOL % PEROXIDE	C_6H_6 mL	RXN TIME, h	PROD #	X	Z	Y	% YIELD 5[b]
4a	1.76	3.61	247	24	5a	Cl	-COC_6H_4-4-Cl	Cl	51.4
4b	1.55	6.12	85	23	5b	Cl	-COC_6H_4-4-Cl	Br	43.5
4c	1.74	5.94	90	23	5c	Cl	-COC_6H_4-4-Cl	F	53.4
4d	1.19	6.97	190	1.5	5d	Cl	-COC_6H_4-4-Cl	H	45.1
4e	1.08	1.61	[c]	2	5e	H	-COC_6H_4-4-Cl	H	62.9
4f	1.29	8.66	130	3	5f	Cl	-COC_6H_5	Cl	62.5
4g	1.75	5.97	190	25	5g	Cl	-COC_6H_4-4-F	Cl	41.7
4h	1.74	10.3	30	24	5h	Cl	-COC_6H_4-4-Br	Cl	44.1
4i	1.74	10.3	30	18	5i	Cl	-$COC_6H_4$4-I	Cl	43.7
4j	1.80	5.92	90	25	5j	Cl	-COC_6H_4-4-CCl=CCl_2	Cl	38.2
4k	1.72	6.16	115	26	5k	Cl	-COC_6H_4-4-C≡N	Cl	35.0
4l	1.75	7.13	60	24	5l	Cl	-COC_6H_4-4-CO_2CH_3	Cl	63.7
4m	1.77	3.91	150	24	5m	Cl	-COC_6H_3-3,4-Cl_2	Cl	36.5
4n	1.11	3.73	90	1.5	5n	H	-COC_6H_3-2,6-Cl_2	H	48.4
4o	1.04	10.57	280	7	5o	Cl	-COC_6H_3-2,4-Cl_2	H	40.2
4p	1.04	10.5	30	2	5p	Cl	-COC_6H_3-3,5-Cl_2	Cl	52.1
4q	1.08	10.54	250	3	5q	H	-COC_6H_2-2,4,6-Cl_3	H	51.6
4r	1.10	1.58	45	3.5	5r	-COC_6H_4-4-Cl	H	H	71.7
4s	1.24	3.33	200	1.5	5s	-COC_6H_4-4-Cl	Cl	H	50.4
4t	[d]				5t	H	H	H	----
4u	[d]				5u	H	Cl	H	----
4v	1.23	1.12	50	4	5v	Cl	H	Cl	[e]

[a] NBS was recrystallized from H_2O and dried over P_2O_5 to constant weight at room temperature. [b] Isolated yields (not corrected for recovered starting material). [c] 70 mL of carbon tetrachloride as solvent. [d] Commercially available bromide. [e] An extremely potent lachrymator, not isolated but directly converted to the azide. ^1H NMR analysis indicated ca. 50% monobromination.

sessment of the separability of the nonbrominated, α-bromo, and the α,α-dibromo components by thin layer chromatography, the amount of NBS was adjusted to optimize reaction conditions for the preparative scale reaction. The reaction conditions and stoichiometry for these brominations are summarized in Table II. Clearly, the substituents greatly influenced the reaction times as well as product composition. In the case of the 4″-iodo analogue **4g**, the iodine proved labile under reaction conditions leading to halogen exchange to afford approximately 20% of the 4″-bromo derivative as confirmed by ^1H NMR. This partial replacement resulted in an inseparable mixture of 4″-iodo and 4″-bromo benzyl bromides and consequently of impure triazole product. In all other cases, separation by column chromatography proved adequate for partial purifications. Due to the highly lachrymatory properties of the benzyl bromides **5a-w**, only ^1H NMR was used as structural confirmation of the isolated benzyl bromides.

Preparation of Substituted α-Azidotoluenes 6. The desired azides **6** were cleanly obtained by treatment of the appropriate benzyl halide **5** with sodium azide in refluxing ethanol (Scheme I), which were carried forward without further characterization to the desired 1,2,3-triazoles. The azide to triazole conversions are summarized in Table III.

Biological Assay. Preliminary anticoccidal testing in chickens against *E. tenella* and *E. acervulina* was carried out by using the procedure described by Tamas et al.[8] The assay consists of feeding the test compound in the feed and expressed at the indicated rates to 3-day-old chicks that were independently infected with either *E. acervulina*

(8) Tamas, T; Chabala, J.; Bochis, R. J.; Nicolich, S.; Wyvratt, M.; Ostlind, D.; Fisher, M. H. Step 1 Evaluation of An Anticoccidial Candidte in Chickens. *Poul. Sci.* **1990**, *69*, 134.

Table III. Preparation of α-Azidotoluenes **6**

SM 5	mMOL 5	mMOL NaN_3	MOL RATIO NaN_3/5	EtOH VOL(mL)	RXN TIME (H)	Prod 6	X	Z	Y	% Yld Crude
5a	8.45	19.9	2.36	32	5	6a	Cl	-COC_6H_4-4-Cl	Cl	98.0
5d	8.43	19.2	2.28	30	5	6d	Cl	-COC_6H_4-4-Cl	H	99.6
5e	14.0	20.3	1.45	40	5	6e	H	-COC_6H_4-4-Cl	H	94.8
5f	6.88	20.3	2.95	30	5	6f	Cl	-COC_6H_5	H	92.6
5g	7.2	11.4	1.58	35	2	6g	Cl	-COC_6H_4-4-F	H	98.0
5k	11.1	23.1	2.08	30	3	6k	Cl	-COC_6H_4-4-C≡N	Cl	39.5
5m	5.47	11.5	2.11	30	3	6m	Cl	-COC_6H_3-3,4-Cl_2	Cl	84.8
5n	21.7	64.4	2.97	100	5	6n	H	-COC_6H_3-2,6-Cl_2	H	99.0
5p	2.79	5.58	2.00	15	3	6p	Cl	-COC_6H_3-3,5-Cl_2	Cl	98.2
5r	13.8	20.3	1.50	40	5	6r	-COC_6H_4-4-Cl	-H	H	99.2
5s	7.76	19.2	2.47	25	5	6s	-COC_6H_4-4-Cl	-Cl	H	83.1
5t	300	600	2.00	300	5	6t	H	-H	H	76.9
5u	7.2	14.4	2.00	125	3	6u	H	-Cl	H	95
5v	30	45	1.50	50	3	6v	Cl	-H	Cl	60
5w[a]	64	101	1.50	100	3	6w	Cl	-Cl	H	99

[a] Used commercially available benzyl chloride.

Table IV. Preparation of 1-(Substituted-benzyl) 1,2,3-Triazoles **3** from Benzyl Azides **6** (Scheme I)

SM #	mMOL CyanAc	mMOL 6	Solvent (mL)	Molarity[a]	Rxn Time(H)	Rxn Temp	PROD #	X	Z	Y	% Yld 3
6a	1.76	1.32	5E[b]	0.344	1	REF	3a	Cl	-COC_6H_4-4-Cl	Cl	41
6d	4.49	3.36	15E	0.291	2	REF	3d	Cl	-COC_6H_4-4-Cl	H	30.6
6e	6.26	4.69	20M[c]	0.412	2	REF	3e	H	-COC_6H_4-4-Cl	H	10.9
6f	13.2	4.36	5E	0.451	2	60	3f	Cl	-COC_6H_5	Cl	31.9
6k	2.99	2	20M	0.155	3	60	3k	Cl	-COC_6H_4-4-C≡N	Cl	19.3
6m	2.99	1.99	20M	0.152	5	REF	3m	Cl	-COC_6H_3-3,4-Cl_2	Cl	24.4
6n	6.2	7.3	20E	0.305	2	REF	3n	H	-COC_6H_3-2,6-Cl_2	H	14.1
6p	2.99	1.99	15E	0.15	5	REF	3p	Cl	-COC_6H_3-3,5-Cl_2	Cl	17.6
6r	6.41	4.9	20E	0.32	2	REF	3r	-COC_6H_5-4-Cl	-H	H	62.5
6s	6.41	4.9	20E	0.32	2	REF	3s	-COC_6H_5-4-Cl	-Cl	H	60
6t	178	173	450E	0.397	1	REF	3t	H	-H	H	73.3
6v	17.8	17.8	14E	0.32	0.75	REF	3v	Cl	-H	Cl	59
6w	18	9.0	40E	0.43	1	REF	3w	Cl	-Cl	H	67.2

[a] Prepared by addition of metallic sodium, commercial sodium methoxide, or solid NaOH to the alcohol. [b] E = ethanol. [c] M = methanol.

or *E. tenella*. The activity is recorded as the percent suppression of oocyst production compared to infected, unmediated controls. Toxicity was assessed by average weight gains relative to uninfected controls. Compounds

Table V. Preparation of Substituted-Benzyl 1,2,3-triazoles 3 from 7 via Alkylations with Benzyl Bromides 5 (Scheme II)[a]

SM #	mMol 7	mMol 5	Mol Ratio NaH/7	DMF ml	Rxn Time,h	Prod #	X	Z	Y	%Yld 3	%Yld 8
5a	4.96	4.99	1.05	20	1	3a	Cl	-COC_6H_4-4-Cl	Cl	16.6	33.0
5b	3.8	3.54	0.99	15	2	3b	Cl	-COC_6H_4-4-Cl	Br	30.8	43.2
5c	4.56	4.14	0.95	17	2	3c	Cl	-COC_6H_4-4-Cl	F	27.8	33.1
5h	4.56	4.14	0.95	17	2	3h	Cl	-COC_6H_4-4-Br	Cl	17.1	29.3
5i	4.56	4.14	0.95	17	2	3i	Cl	-COC_6H_4-4-I	Cl	20.1	35.6
5g	8.5	7.7	0.95	31	1.5	3g	Cl	-COC_6H_4-4-F	Cl	27.1	21.3
5j	2.75	2.74	1.07	11	1.5	3j	Cl	-COC_6H_4-4-CCl=CCl_2	Cl	25.5	52.9
5l	3.47	3.73	1.06	15	0.75	3l	Cl	-COC_6H_4-4-CO_2CH_3	Cl	14.1	55.1
5o	5.0	4.4	1.05	20	2	3o	Cl	-COC_6H_3-2,4-Cl_2	H	16.8	21.9
5q	5.0	5.0	1.05	20	1	3q	H	-COC_6H_2-2,4,6-Cl_3	H	18.1	23
	See Preparation of 3a above					8a	Cl	-COC_6H_4-4-Cl	Cl		

[a] All reactions were carried out at room temperature.

that resulted in weight gains less than 25% were deemed toxic.

Results and Discussion

Table VI lists the minimum level of compound given as ppm in the diet at which controlled infection was detected.

Replacement of the imidazole ring in **2c** as in **3w** resulted in a significant reduction in oocyst production relative to controls. Entries **3t–w** reveal that in the simple benzyl series, activity is maximized with two chlorines in the 3,4 positions of the benzyl ring, **3w**. Replacement of one of the chlorines of **3w** with unsubstituted or substituted benzoyl group as in **3d** or **3s** offered no immediate improvement in potency. However, a substantial gain is achieved by flanking the carbonyl of the benzophenone moiety with halogens as demonstrated by entries **3a** through **3f**. This need for 3′,5′ disubstitution is demonstrated again in analogues **3n**, **3o**, and **3p**. Retention of the 3′,5′-dichloro substitution pattern while varying the substituent in the second ring with a series of lipophilic, electron-withdrawing substituents as in **3f** through **3m** and **3p** yielded a series of compounds with interesting activity against both *Eimeria* species. While high potency generally correlated reasonably well with high lipophilicity, geometrical factors in the benzophenone strutural unit as demonstrated by the 3′,5′-dichloro substitution requirement also had an important effect on biological activity. Compound **3a** containing a 3′,5′,4″-trichlorobenzophenone was the most potent compound synthesized and was selected for further study.

Compound **3a**, L-651,582, was tested against a relatively resistant series of *Eimeria* field isolates. It has been found that the "DP" series is partially resistant to known coccidiostats. The "FS" series is obtained from a commercial pathology laboratory. The "LS" series was isolated at Merck. Table VII records dose–response in the reduction in oocyst production in chickens by **3a** as compared to several commercial coccidiostats at their recommended use level. L-651,582 (**3a**) was extremely effective against the economically important strains of *Eimeria* and, in particular, fully effective against *E. maxima*, an organism that is particularly resistant to the commercially employed ionophores, monensin and salinomycin.

In spite of its exceptional anticoccidial activity and safety, compound **3a** was not developed as an anticoccidial drug because tissue residue studies in chickens at the anticipated use levels and withdrawal period revealed residues in chicken livers that were judged unacceptable for prophylatic use.

Mechanistic studies on this series of 5-amino-4-carbamoyl-1,2,3-triazoles and, in particular, on L-651,582 (**3a**) revealed that its mode of action does not involve inhibition of IMP dehydrogenase but most likely L-651,582 interferes with calcium entry into the host cell.[9] In addition, L-651,582 has been found to have antiproliferative activity in a number of disease models. Most importantly, it has been recently reported to possess antimetastatic activity in a model of ovarian cancer progression.[10] Further research on this novel anticancer drug is currently underway at the National Cancer Institute.

Experimental Section

General Comments. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian XL-200 instrument.

2,6-Dichloro-4-methylbenzoic Acid (16a). A solution of **24b** (5.15 g, 0.0319 mol) in dry THF (50 mL) at −50 °C was treated with 1.6 M *n*-butyllithium in hexane (20 mL, 0.032 mol). The reaction mixture was stirred at −50 °C for 1.5 h and then poured onto a slurry of pulverized solid CO_2 in 100 mL of Et_2O. After warming to room temperature, the solvent was removed in vacuo and the residue was dissolved in 10% aqueous NaOH (30 mL). The solution was extracted with Et_2O, and the aqueous layer was

(9) (a) Hupe, D. J.; Behrens, N. D.; Boltz, R. Anti-Proliferative Activity of L-651,582 Correlates With Calcium-Mediated Regulation of Nucleotide Metabolism at Phosphoribosyl Pyrophosphate Synthetase. *J. Cell. Physiology* **1990**, *144*(3), 456–466. (b) Hupe, D. J.; Pfefferkorn, E. R.; Behrens, N. D.; Peters, K. L-651,582 Inhibition of Intracellular Parasitic Protozoal Growth Correlates with Host-Cell Directed Effects. *J. Pharm. Exp. Ther.* In press.

(10) Kohn, E. C.; Liotta, L. A. L651,582: A Novel Antiproliferative and Antimetastasis Agent. *J. Nat. Cancer Inst.* **1990**, *82*, 54–60.

Table VI. Activity of 1,2,3-Triazoles **3** against *E. acervulina* and *E. tenella* in Chickens[a]

Entry	X	Z	Y	E.a.[b]	E.t.[c]
3a	Cl	-COC_6H_4-4-Cl	Cl	ma 7.5	sa 7.5
3b	Cl	-COC_6H_4-4-Cl	Br	ma 15	ma 60
3c	Cl	-COC_6H_4-4-Cl	F	ma 30	ma 60
3d	Cl	-COC_6H_4-4-Cl	H	ma 500	>500
3e	H	-COC_6H_4-4-Cl	H	>500	>500
3f	Cl	-COC_6H_5	Cl	ma 60	ma 60
3g	Cl	-COC_6H_4-4-F	Cl	ma 30	ma 60
3h	Cl	-COC_6H_4-4-Br	Cl	sa 7.5	ma 30
3i	Cl	-COC_6H_4-4-I	Cl	ma 15	fa 60
3j	Cl	-COC_6H_4-4-CCl=CCl_2	Cl	ma 30	sa 30
3k	Cl	-COC_6H_4-4-C≡N	Cl	ma 60	ma250
3l	Cl	-COC_6H_4-4-CO_2CH_3	Cl	>250	>250
3m	Cl	-COC_6H_3-3,4-Cl_2	Cl	ma 12.5	ma 60
3n	H	-COC_6H_3-2,6-Cl_2	H	>250	>250
3o	Cl	-COC_6H_3-2,4-Cl_2	H	>250	ma 250
3p	Cl	-COC_6H_3-3,5-Cl_2	Cl	fa 30	fa 60
3q	H	-COC_6H_2-2,4,6-Cl_3	H	ma 250	>250
3r	-COC_6H_4-4-Cl	-H	H	ma 500	>500
3s	-COC_6H_4-4-Cl	-Cl	H	ma 60	ma 250
3t	H	-H	H	inactive	
3u	H	-Cl	H	sa 1000	>1000
3v	Cl	-H	Cl	>125	>125
3w	Cl	-Cl	H	sa 250	sa 60
8a	Cl	-COC_6H_4-4-Cl	Cl	>500(TOX)	>500(TOX)
2c[d]				fa 500	fa 500
Robenidine[e]				sa 8	sa 4
Arprinocid[f]				sa 15	sa 20
Amprolium[g]				sa 40	sa 20
Nicarbazin[h]				sa 20	sa 20
Monensin[i]				sa 40	sa 40
Lasolicid[j]				sa 40	sa 30

[a] Doses are ppm in diet; activity is expressed as oocyst reduction inactive = <80% reduction; sa = 80–89% reduction; ma = 90–99% reduction; (fa = >99% reduction, TOX = toxic. [b] E.a. = *E. acervulina*. [c] E.t. = *E. tenella*. [d] See ref 5. [e] Kantor, S.; Kennett, R. L., Jr.; Waletzky, E.; Tomcuflf, A. S. *Science* **1970**, *168*, 373. [f] Olson, G.; Tamas, T.; Smith, D. A.; Weppleman, R. M.; Schleim, K.; McManus, E. *Poult. Sci. 57*, 1245. [g] Rogers, E. F.; Clark, R. L.; Becker, H. J.; Pessolano, A. A.; Leanza, W. J.; McManus, E. C.; Andriuli, F. J.; Cuckler, A. C. *Proc. Soc. Exp. Biol. Med.* **1964**, *117*, 488. [h] Cuckler, A. C.; Malanga, C. M.; Ott, W. H. *Poult. Sci.* **1956**, *35*, 98. [i] Shumard, R. F.; Callender, M. E. *Antimicrob. Agents Chemother.* **1968**, 369. [j] Mitrovic, M.; Schlidknecht, E. G. *Poult. Sci.* **1974**, *53*, 1448.

separated and acidified with concentrated HCl. The precipitate was removed by filtration and dissolved in CH_2Cl_2. After drying over $MgSO_4$, the solvent was removed in vacuo to yield 3.51 g (53.6%) of **16a**, mp 153–155 °C (H_2O): ^1H NMR ($CDCl_3$) δ 2.36 (s, 3 H, Ar-CH_3), 7.20 (s, 2 H, Ar-3,5 H). Anal. (C, H, N, Cl).

2,4,6-Trichlorobenzoic Acid (16c). The reaction of **24c** (18.1 g, 0.1 mol) and 2.5 M *n*-butyllithium in hexane (40 mL, 0.1 mol) with pulverized solid CO_2 as above afforded **16c** (21.1 g, 93.7%), mp 162–164 °C (H_2O) (lit.[11] mp 164–164 °C): ^1H NMR (DMSO-d_6) δ 7.8 (s, 2 H, Ar-3,5 H).

2-Bromo-6-chloro-4-methylaniline (19). A solution of Br_2 (37.6 g, 0.234 mol) in HOAc (100 mL) was added dropwise to **18** (33.1 g, 0.234 mol) dissolved in HOAc (500 mL) at 10 °C. The resultant thick suspension was allowed to warm to room temperature and stirred for 30 min. The mixture was diluted with Et_2O (1 L), and the precipitated HBr salt was removed by filtration and washed with Et_2O. The air-dried solids were added to a stirred solution of 50% NaOH (20 mL) in H_2O (150 mL). The free base was extracted with CH_2Cl_2 and the extracts were combined, washed with brine, and dried over $MgSO_4$. Evaporation of the solvent yielded 40.6 g (79%) of **19**, mp 62.5–64 °C (lit.[12]

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(12) Cohen, J. B.; Murray, W. J. XCII. Studies in Orientation. Nitration Products of 3-Chloro-5-bromotoluene. *J. Chem. Soc.* **1915**, *107*, 847–851.

Table VII. Percent Oocyst Reduction of **3a** vs Various *Eimeria* Strains in Chickens Relative to Commercial Coccidiostats

Entry	ppm	E.a.[a]	E.t.[b]	E.m.[c]	E.n.[d]	E.b.[e]	E.mi.[f]	E.p.[g]	E.h.[h]
3a	7.5	21	60	99.9	12	58		72[i]	
3a	15	98	83	99.9	88	95		98[i]	
3a	30	99.9	98	99.9	100	100		100[i]	
3a	45	99.9	99.9	99.9	100	100		100[i]	
3a	60	99.9	99.9	99.9	100	100		100[i]	
amprolium	125	7	100	0	NA[j]	79	36	18	0
lasalocid	100	16	100	100	NA[j]	100	100	34	74
monensin	121	4	100	73	NA[j]	100	72	19	19
nicarbazin	125	0	100	21	NA[j]	100	100	34	0
robenidine	33	99.9	0	21	NA[j]	100	100	54	0
salinomycin[j]	60	NA[j]	85	0	NA[j]	74	91	62	0
controls		(292.9)[k]	(11.7)[k]	(40.9)[k]	(0.17)[k]	(11.1)[k]		(410.6)[k,l]	

[a] **E.a.** = *E. acervulina* (DP-761). [b] **E.t.** = *E. tenella* (DP-761). [c] **E.m.** = *E. maxima* (DP-776). [d] **E.n.** = *E. necatrix* (Aquet). [e] **E.b.** = *E. Brunetti* (FS-103). [f] **E.mi.** = *E. mitis* (LS-84). [g] **E.p.** = *E. praecox* (DP-785). [h] **E.h.** = *E. hagani* (DP-786). [i] NA = not assayed. [j] Miyazaki, Y.; Shibuya, M.; Suganara, H.; Kawaguchi, O.; Hirose, C.; Nagatsu, J.; Esumi, S. *J. Antibiot.* **1974**, *27*, 814. [k] Number of oocysts × 10^6 per bird; assay utilized 3 replications of 5 broilers per level. [l] Pooled oocyst count of **E.mi.**, **E.p.**, and **E.h.**

mp 63 °C): ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 3 H, Ar-CH_3), 7.16 (s, 1 H, Ar-5 H), 7.26 (s, 1 H, Ar-3 H).

2-Bromo-6-chloro-4-methylbenzonitrile (20). The method was similar to that described for the diazotization of weakly basic, sterically hindered anilines.[13] A solution of nitrosylsulfuric acid was prepared as follows: Concentrated H_2SO_4 (118 mL) was cooled to 5 °C and powdered $NaNO_2$ (16.9 g, 0.245 mol) and added portionwise, with vigorous stirring. After addition was complete, the suspension was warmed at 50 °C until a solution was obtained (30 min). The flask was cooled to 0 °C and a solution of **19** (47.67 g, 0.214 mol) in HOAc (128 mL) was added dropwise over 45 min while maintaining a temperature of 3–5 °C. After addition was complete, the reaction mixture was stirred at 15 °C for 3 h. The cold diazonium solution was poured into a cooled (15 °C), vigorously stirred suspension prepared from KCN (76.04 g, 1.16 mol) in H_2O (216 mL). The reaction mixture was warmed to 35–40 °C for 1 h, cooled, and extracted with CH_2Cl_2. The combined extracts were successively washed with H_2O and aqueous $NaHCO_3$ (to pH 7), dried over $MgSO_4$, and evaporated in vacuo. The residue (46.7 g) was partitioned between CH_2Cl_2 (200 mL) and *n*-hexane (300 mL) and then filtered. The filtrate was passed over 600 g of SiO_2 (60–200 mesh); elution with CH_2Cl_2–*n*-hexane (60:40 v/v) yielded 16 g (32.4%) of **20**, mp 125–127 °C (*n*-hexane): ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3 H, Ar-CH_3), 7.29 (s, 1 H, Ar-5 H), 7.42 (s, 1 H, Ar-3 H). Anal. (C, H, N, Cl, Br).

2-Bromo-6-chloro-4-methylbenzamide (21). Purified **20** (15.0 g, 0.065 mol) was added to concentrated H_2SO_4 and the resultant suspension was heated on the steam bath for 3 h. The reaction mixture was cooled, poured onto an ice/water mixture, and filtered. The wet cake was taken up in CH_2Cl_2 (400 mL) and the resulting solution was washed with H_2O and dried over $MgSO_4$. The solvent was removed in vacuo to yield 11.8 g of crude **20**, which was chromatographed over SiO_2 (500 g) and eluted with CH_2Cl_2–Et_2O (90:10 v/v) to yield 6.6 g (40.8%) of **20**, mp 118–121 °C (EtOH/H_2O): ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3 H, Ar-CH_3), 7.35 (s, 1 H, Ar-5 H), 7.46 (s, 1 H, Ar-3 H), 7.73 and 7.98 (br s, 2 H, amide H). Anal. (C, H, N, Cl, Br).

2-Chloro-6-bromo-4-methylbenzoic Acid (16b). A solution of **20** (6.6 g, 0.26 mol) in HOAc (70 mL) and concentrated HCl (35 mL) was cooled to 10 °C and treated dropwise with a solution of $NaNO_2$ (12.0 g, 0.173 mol) in H_2O (27 mL) while maintaining the temperature at 0–4 °C. After addition was complete, the reaction mixture was stirred for 30 min at 5 °C, allowed to warm to room temperature, and cautiously heated at 85 °C for 1 h. The reaction mixture was concentrated in vacuo and diluted with H_2O (100 mL). The solids were collected by filtration, washed with H_2O, and dried over P_2O_5 in vacuo at room temperature to yield **16b** (5.13 g, 77.4%), mp 161–163 °C (H_2O). Anal. (C, H, N, Cl, Br).

2,6-Dichloro-4-methylbenzoyl Chloride (17a). A solution of **16a** (34.7 g, 0.169 mol) in $SOCl_2$ (646 mL) was treated with DMF (2 mL) and heated at reflux for 3 h. The excess $SOCl_2$ was removed in vacuo and the residue was flushed with C_6H_6 (2 × 100 mL). The oily residue was taken up in petroleum ether (250 mL), filtered through diatomaceous earth, and evaporated in vacuo. The crude acid chloride, 37.63 g (99.7%), was used without further purification. Acid chlorides **17a–i** were prepared in a similar manner.

General Procedure for Benzophenone Formation. As an example, **4g** was prepared by the following procedure: A vigorously stirred solution of **17a** (21.0 g, 0.094 mol) in fluorobenzene (105 mL) was treated portionwise with $AlCl_3$ (13.06 g, 0.098 mol) at 85 °C. After addition was complete, heating was continued for 15 h. The cooled reaction mixture was poured onto an ice/concentrated HCl mixture and then extracted with EtO_2. The combined EtO_2 extracts were washed with brine, dried over $MgSO_4$, and evaporated in vacuo to yield crude **4g** (14.8 g, 54.4%), mp 99–101 °C (*n*-hexane): ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3 H, ar-CH_3), 7.15 (t, 2 H, 3″,5″, Ar-H, *J* = 8.8 Hz), 7.23 (s, 2 H, 2′,6′ Ar-H), 7.85 (dd, 2 H, 2″,6″ Ar-H, *J* = 8.8, 5.53 Hz). Anal. (C, H, N, Cl, F). The mother liquors were passed over SiO_2 and eluted with CH_2Cl_2–*n*-hexane (80:20 v/v) to yield an additional 5.27 g (19.9%) of **4g**. The other benzophenones were prepared in a similar manner from the appropriate acid chloride and aryl substrate. The stoichiometry, reaction conditions, and results are given in Table I.

2′,6′-Dichloro-4′-methyl-4″-cyanobenzophenone (4k). A mixture of **4g** (8.4 g, 0.030 mol) and KCN (3.45 g, 0.053 mol) in DMF (100 mL) was heated at reflux for 18 h. The reaction was mixture cooled, diluted with H_2O (1000 mL), and extracted with CH_2Cl_2. The combined extracts were washed with H_2O, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed over SiO_2 (300 g) and eluted with *n*-hexane–CH_2Cl_2 (60:40 v/v) to yield **4k** (3.51 g, 40.5%), mp 150–152 °C (*n*-hexane): ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3 H, Ar-CH_3), 7.23 (s, 2 H, 2′,6′ Ar-H), 7.78 (d, 2 H, 3″,5″ Ar-H, *J* = 8 Hz), 7.92 (d, 2 H, 2″,6″ Ar-H, *J* = 8 Hz). Anal. (C, H, Cl, N). Further elution yielded 1.2 g of a monochloro cyano analogue.

2′,6′-Dichloro-4′-methyl-4″-carbethoxybenzophenone (4l). A solution of **4k** (2.64 g, 9.98 mmol) in methoxyethanol (18 mL) and 3 N aqueous KOH (18 mL) was heated at reflux for 30 min. The reaction mixture was cooled, diluted with H_2O, and acidified with concentrated HCl. The solids were collected by filtration, washed with H_2O, and dried over P_2O_5 in vacuo. The residue (2.75 g) was dissolved in MeOH (100 mL) and treated with concentrated H_2SO_4 (2.0 mL), and the solution was heated at reflux for 18 h. After the volume was reduced in vacuo, the oily mixture was diluted with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The extracts were washed with H_2O and evaporated in vacuo. The crude ester was passed over SiO_2 (100 g) and eluted with CH_2Cl_2 to yield **4l** (2.8 g, 86.8%), mp 99–100.5 °C (*n*-hexane): ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3 H, Ar-CH_3), 3.95 (s, 3 H, OCH_3), 7.22 (s, 2 H, 2′,6′ Ar-H), 7.88 (d, 2 H, 3″,5″ Ar-H, *J* = 8.6 Hz), 8.14 (d, 2 H, 2″,6″ Ar-H, *J* = 8.0 Hz). Anal. (C, H, Cl, N).

3-Fluoro-5-nitrotoluene (22b). A suspension of **22a** (32.7 g, 0.215 mol), prepared from *p*-acetotoluidide by the method of Emokpae et al.[14] in concentrated HCl (172 mL) at 0 °C, was treated dropwise with $NaNO_2$ (18.06 g, 0.26 mol) in H_2O (172 mL) over 30 min while maintaining the temperature at 0–4 °C. After 30 min of stirring, the cold solution was filtered and the filtrate was treated with 65% HPF_6 (70 mL) with vigorous shaking. The heavy precipitate was collected by filtration and washed with H_2O.

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(14) Emokpae, T. A., Eguavoen, O.; Hirst, J. The Kinetics of the Reactions of Picryl Chloride with Some Substituted Anilines. Part 5. *J. Chem. Soc., Perkins Trans. II* **1980**, 829–834.

The solids were dried at room temperature over P_2O_5 in vacuo to constant weight to yield 59.9 g (85%) of crude diazonium hexafluoroborate salt.

A vigorously stirred, intimate mixture of the dried diazonium hexafluoroborate salt (10 g, 30 mmol) and sea sand (90 g) was immersed in an oil bath at 160–165 °C. Within a few minutes a violent, but controlled reaction occurred. After an additional 10 min of heating, the reaction mixture was cooled, purged with N_2, and diluted with 300 mL of CH_2Cl_2. The mixture was filtered and the cake was washed with CH_2Cl_2. A total of 57 g of the diazonium hexafluoroborate salt were processed as above, and the combined CH_2Cl_2 extracts were washed with saturated aqueous $NaHCO_3$ and H_2O, dried over $MgSO_4$, and evaporated in vacuo. The residue was taken up in CH_2Cl_2–*n*-hexane (40:60 v/v) and passed over SiO_2 (500 g). Elution with CH_2Cl_2–*n*-hexane (40:60 v/v) yielded **22b** (16.1 g, 57.1%), mp 38–40 °C (lit.[15] mp 40–40.5 °C).

3-Chloro-5-fluorotoluene (24a). A solution of **22b** (15.17 g, 0.1 mol) in MeOH (400 mL) and 5% Pt on carbon (1.0 g) was shaken under 40 psi H_2. When the uptake of H_2 was complete (1 h), the reaction mixture was filtered and the filtrate was evaporated to dryness in vacuo to yield 12.3 g of **23**. After distillation, **23** (13.0 g, 0.103 mol) was added to concentrated HCl (26 mL) and H_2O (26 mL) and cooled to 0 °C. A solution of $NaNO_2$ (7.22 g, 0.104 mol) dissolved in H_2O (15 mL) was added dropwise through a dropping funnel immersed below the surface of the liquid while the reaction temperature was maintained between 0 and 5 °C. After 10 min of stirring, the cold diazonium solution was poured slowly into a stirred suspension, at 5 °C, of concentrated HCl (50 mL) and CuCl, prepared from 40 g of $CuSO_4 \cdot 5H_2O$ according to the method described by Vogel.[16] The reaction mixture was allowed to come to room temperature and then warmed at 60 °C for 5 min. The cooled reaction mixture was extracted with Et_2O. The combined extracts were washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The crude tolyl compound **24a** (12.3 g) was taken up in petroleum ether (bp 30–60 °C) and chromatographed over SiO_2 (300 g). Elution with petroleum ether (bp 30–60 °C) afforded 8.92 (63%) of **24a**, bp 110–112 °C/152 mm. Anal. (C, H, N).

2′,6′,4″-Trichloro-4′-methylbenzophenone (4a). A solution of **24b** (337.1 g, 2.09 mol) in THF (4 L) at −70 °C was treated with *n*-butyllithium (2.5 M in hexane) (876.4 mL, 2.19 mol) over 45 min. The reaction mixture was stirred at −70 °C for 30 min and a solution of *p*-chlorobenzoyl chloride (377 g, 2.09 mol) in THF (2 L) was added over 45 min. After stirring at −70 °C for 4 h, saturated NH_4Cl (458 mL) was added. The reaction mixture was stirred overnight at room temperature, Na_2SO_4 (458 g) was added, and the mixture was filtered. The solids were throughly washed with Et_2O. After drying again over Na_2SO_4, the filtrate was evaporated in vacuo and the residue was recrystallized from EtOH to yield 384.4 g (61.5%) of **4a** in two crops, mp 125–127 °C: ^1H NMR ($CDCl_3$) δ 2.38 (s, 3 H, Ar-CH3), 7.23 (s, 2 H, 2′,6′ Ar-H), 7.46 (d, 2 H, 3″,5″ Ar-H, J = 8 Hz), 7.77 (d, 2 H, 2″,6″ Ar-H, J = 8 Hz). Anal. (C, H, N, Cl). The material was identical with product obtained from the Friedel–Crafts acylation of chlorobenzene with **16a** and $AlCl_3$ (see Table I).

2′,4″-Dichloro-6′-fluoro-4′-methylbenzophenone (4c). A solution of **24a** (2.8 g, 0.02 mol) in dry THF (20 mL) under N_2 was cooled to −50 °C was treated with *n*-butyllithium (2.5 M in hexane) (8.0 mL, 0.02 mol). The solution was stirred at −40 °C for 30 min and treated with a solution of *p*-chlorobenzoyl chloride (3.5 g, 0.02 mol) in THF (4 mL). The reaction was stirred at −40 °C for 1 h, at which point an aliquot indicated that no **24a** remained. Saturated aqueous NH_4Cl (2.2 mL) was added to the cold reaction mixture, and the mixture was allowed to warm to room temperature. The reaction mixture was partitioned between EtO_2 and H_2O, dried over $MgSO_4$, and evaporated in vacuo. The residue was recrystallized from *n*-hexane to yield **4c** (3.17 g, 56.6%), mp 81.5–82.8 °C: ^1H NMR ($CDCl_3$) δ 2.43 (s, 3 H, Ar-CH_3), 6.75 (d, 1 H, Ar-2′H, J = 10 Hz), 7.14 (s, 1 H, Ar-6′ H), 7.49 (d, 2 H, 3″,5″ H, J = 8 Hz), 7.82 (d, 2 H, 2″,6″ H, J = 8 Hz). Anal. (C, H, Cl, N).

The reaction of *p*-bromo-, *p*-iodo-, and 3,5-dichlorobenzoyl chlorides as described above provided **4h**, **4i**, and **4p** in 46%, 9%, and 43% yields, respectively.

General Procedure for the Bromination of Substituted Toluenes 4. Preparation of 5h. A solution of **4h** (3.44 g, 10 mmmol) in C_6H_6 (100 mL) containing dibenzoyl peroxide (242 mg, 1 mmol) was treated with NBS (3.14 g, 17.5 mmol). The reaction mixture was heated until a starch iodide test was negative (24 h). The reaction mixture was cooled, filtered to remove succinimide, and evaporated in vacuo. The ^1H NMR spectrum ($CDCl_3$) of the residue exhibited three singlets: δ 2.40, Ar-CH_3 (11.5%), δ 4.35 Ar-CH_2Br (57%), and δ 6.58 Ar-$CHBr_2$ (20%). Chromatography over SiO_2 and elution with *n*-hexane–CH_2Cl_2 (80:20 v/v) yielded early fractions containing mixtures of starting **4h** and α,α-dibrominated product. Further elution yielded **5h** (1.8 g, 44.1%): ^1H NMR ($CDCl_3$) δ 2.40 (s, 3 H, Ar-CH_3), 7.21 (s, 2 H, 2′,6′ Ar-H), 7.64 (d, 2 H, 3″,5″ Ar-H, J = 8 Hz), 7.66 (d, 2 H, 2″6″Ar-H, J = 8 Hz). The stoichiometry, conditions, and results for other analogues, **5a–w**, are recorded in Table II.

General Procedure for the Formation of α-Azidotoluenes 6. A solution of the α-bromomethylbenzophenone **5q** (4.35 g, 0.011 mol) in EtOH (40 mL) was treated with NaN_3 (1.65 g, 0.025 mol) and heated under reflux for 5 h. The solution evaporated to dryness in vacuo and the residue was triturated with Et_2O. The suspension was filtered and the filtrate was evaporated in vacuo to yield 3.88 g (99.2%) of nearly pure azide **6q**, mp 74–76 °C. Other benzyl halides were converted in a similar manner. The reaction conditions, stoichiometry and results are given in Table III.

5-Amino-4-carbamoyl-1,2,3-triazole (7). The parent compound **7** was prepared in 63% yield by debenzylation of the 1-benzyl analogue **3t**, using Na in liquid NH_3, mp 224–225 °C (H_2O) (lit.[18] mp 224–225 °C).

General Procedure for the Formation of 1-Substituted-4-carbamoyl-5-amino-1,2,3-triazoles. A. Scheme I. Preparation of 3a. A suspension of 2-cyanoacetamide (149 mg, 1.76 mmol) in EtOH (5.0 mL) was treated with $NaOCH_3$ (92 mg, 1.72 mmol) and heated at reflux for 20 min. The reaction mixture was cooled, **6a** (450 mg, 1.32 mmol) was added, and the solution was heated at 60 °C for 1 h. The solvent was removed in vacuo and the residue was chromatographed over SiO_2 (50 g). Elution with CH_2Cl_2–MeOH (19:1 v/v) afforded **3a** (231 mg, 41%), mp 194–195.5 °C (EtOH): high resolution MS M^+ 423.0055; ^1H NMR (DMSO-d_6) δ 5.28 (s, 2 H, benzyl CH_2), 5.26, (br s, 2 H, 5-NH_2), 7.15, and 7.5, (br s, 2 H, amide H), 7.46, (s, 2 H, 2′,6′ Ar-H), 7.67 (d, 2 H, 3″,5″ Ar-H, J = 8 Hz), 7.79, (d, 2 H 2″,6″ Ar-H, J = 8 Hz). Anal. (C, H, N, Cl). The stoichiometry, reaction conditions, and results of azide cyclizations are recorded in Table IV.

B. Scheme II. A cooled solution of **7** (0.633 g, 4.98 mmol) in dry DMF (20 mL) was treated portionwise with 50% NaH (0.250 mg, 1.0 mmol). After stirring at room temperature for 20 min, **5a** (1.89 g, 4.99 mmol) was added and the resultant solution stirred at room temperature until TLC indicated no remaining starting material (1 h). The reaction was slowly poured onto ice and acidified with HOAc. The solids were collected by filtration, washed with H_2O, and dried.[19] The solids were dissolved in CH_2Cl_2–MeOH (97:3 v/v) and chromatographed SiO_2 (20 g). Elution with CH_2Cl_2–MeOH (97:3) provided **8a** (0.7 g, 33%), mp 173–174.5 °C (EtOH): high resolution MS M^+ 423.0055; ^1H NMR (DMSO-d_6) δ 5.55 (s, 2 H, benzyl CH_2), 5.71 (br s, 2 H, 5-NH_2),

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7.35 and 7.52 (br s, 2 H, amide H), 7.52 (s, 2 H 2',6' Ar-H), 7.67, (d, 2 H, 3'',5'' Ar-H, J = 8.6 Hz), 7.79 (d, 2 H, 2'',6'' H, J = 8 Hz). Anal. (C, H, N, Cl). Further elution yielded **3a** (0.351 g, 16.6%), which was identical with that prepared via Scheme I. Table V summarizes the reaction conditions, stoichiometry, and results of the triazole alkylations.

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Registry No. **2c**, 93272-19-6; **3a**, 99519-84-3; **3b**, 135365-03-6; **3c**, 135340-66-8; **3d**, 99519-83-2; **3e**, 99519-81-0; **3f**, 99880-60-1; **3g**, 135340-67-9; **3h**, 135340-68-0; **3i**, 135340-69-1; **3j**, 135340-70-4; **3k**, 135340-71-5; **3l**, 135340-72-6; **3m**, 135340-73-7; **3n**, 99519-91-2; **3o**, 135340-74-8; **3p**, 135340-75-9; **3q**, 135340-76-0; **3r**, 99519-82-1; **3s**, 99533-74-1; **3t**, 4342-08-9; **3u**, 132269-38-6; **3v**, 133992-52-6; **3w**, 99613-59-9; **4a**, 99508-23-3; **4b**, 135340-37-3; **4c**, 135340-38-4; **4d**, 99519-97-8; **4e**, 5395-79-9; **4f**, 99508-46-0; **4g**, 135340-39-5; **4h**, 135340-40-8; **4i**, 135340-41-9; **4j**, 135340-42-0; **4k**, 135340-43-1; **4l**, 135340-44-2; **4m**, 135340-45-3; **4n**, 99508-49-3; **4o**, 135340-46-4; **4p**, 135340-47-5; **4q**, 135340-48-6; **4r**, 35256-82-7; **4s**, 99520-00-0; **4t**, 108-88-3; **4u**, 106-43-4; **4v**, 25186-47-4; **5a**, 99508-24-4; **5b**, 135340-49-7; **5c**, 135340-50-0; **5d**, 99519-98-9; **5e**, 91457-11-3; **5f**, 99508-47-1; **5g**, 135340-51-1; **5h**, 135340-52-2; **5i**, 135340-53-3; **5j**, 135340-54-4; **5k**, 135340-55-5; **5l**, 135340-56-6; **5m**, 135340-57-7; **5n**, 99508-50-6; **5o**, 135340-58-8; **5p**, 135340-59-9; **5q**, 135340-60-2; **5r**, 35278-62-7; **5s**, 99520-01-1; **5t**, 100-39-0; **5u**, 622-95-7; **5v**, 7778-01-0; **5w**, 18880-04-1; **6a**, 99508-26-6; **6d**, 99519-99-0; **6e**, 99519-95-6; **6f**, 135340-61-3; **6g**, 135340-62-4; **6k**, 135340-63-5; **6m**, 135340-64-6; **6n**, 99508-51-7; **6p**, 135340-65-7; **6q**, 135340-83-9; **6r**, 99519-96-7; **6s**, 99520-02-2; **6t**, 622-79-7; **6u**, 27032-10-6; **6v**, 133992-55-9; **6w**, 99613-63-5; **7**, 4342-07-8; **8a**, 135340-77-1; **16a**, 99520-05-5; **16b**, 135340-36-2; **16c**, 50-43-1; **16d**, 7697-25-8; **16e**, 99-94-5; **16f**, 50-84-0; **16g**, 99-04-7; **16h**, 50-30-6; **16i**, 6342-60-5; **17a**, 99508-22-2; **17b**, 135365-02-5; **17c**, 4136-95-2; **17d**, 21900-53-8; **17e**, 874-60-2; **17f**, 89-75-8; **17g**, 1711-06-4; **17h**, 4659-45-4; **17i**, 21900-50-5; **18a**, 615-65-6; **18b**, 95-81-8; **19**, 135340-78-2; **20a**, 135340-79-3; **29b**, 21423-84-7; **21a**, 135340-80-6; **21b**, 64597-37-1; **22a**, 618-61-1; **22b**, 499-08-1; **23**, 52215-41-5; **24a**, 93857-90-0; **24b**, 25186-47-4; **24c**, 108-70-3; 3-diazonium-5-nitrotoluene hexafluorophosphate salt, 135340-82-8; chlorobenzene, 108-90-7; benzene, 71-43-2; fluorobenzene, 462-06-6; trichlorovinylbenzene, 700-60-7; *o*-dichlorobenzene, 95-50-1; toluene, 108-88-3; 3-chlorotoluene, 108-41-8; 2-cyanoacetamide, 107-91-5; 4-chlorobenzoyl chloride, 122-01-0; 4-bromobenzoyl chloride, 586-75-4; 4-iodobenzoyl chloride, 1711-02-0; 3,5-dichlorobenzoyl chloride, 2905-62-6.

Design and Synthesis of HIV Protease Inhibitors. Variations of the Carboxy Terminus of the HIV Protease Inhibitor L-682,679

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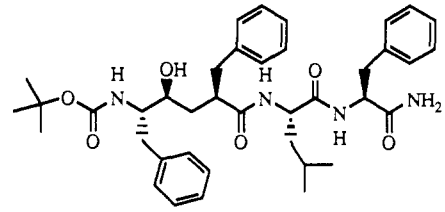
A series of tetrapeptide analogues of 1 (L-682,679), in which the carboxy terminus has been shortened and modified, was prepared and their inhibitory activity measured against the HIV protease in a peptide cleavage assay. Selected examples were tested as inhibitors of virus spread in cell culture. Compound 12 was a 10-fold more potent enzyme inhibitor than 1 in vitro and 30-fold more potent in inhibiting the viral spread in cells.

The HIV protease has been characterized biochemically and structurally as a member of the aspartyl protease family of enzymes. Inactivation of this protease results in the production of noninfectious virons and consequent inhibition of the spread of viral infection in susceptible cells.[1] Inhibition of this enzyme should provide an attractive therapeutic goal in the treatment of AIDS. Our strategy, like that of others,[2-4] was based on the transition-state mimetic concept which proved successful in the design of renin inhibitors. We found early on[5] that incorporation of a hydroxyethylene isostere as a dipeptide mimic provided compounds which were potent and selective inhibitors of HIV protease. The prototype, L-682,679 (1), essentially a pentapeptide, is a potent and

P_1 P_1' P_2' P_3'



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