Novel Bis[1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamines] as Antitrypanosomal Agents[1,2]

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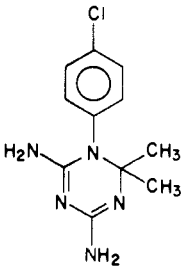
A series of novel 1,1'-(4,1-phenylene)bis[1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamines] was prepared and evaluated for activity against *Trypanosoma rhodesiense* in mice. The importance of the bis structure and the nature of the spacer between the two phenyl rings for optimal activity have been revealed. The potent parenteral activity of several analogues within this series as well as preliminary indication of oral activity lends encouragement to further development of this structural class.

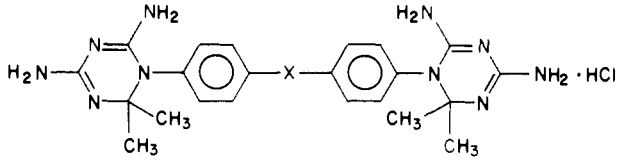
Among a group of hemoflagellate protozoa known as trypanosomes, three species, *Trypanosoma cruzi* in South America and *Trypanosoma rhodesiense* and *Trypanosoma gambiense* in Africa, are pathogenic in humans. Neither control of the disease via elimination of the intermediate insect host nor its treatment with the several classes of available drugs have been adequate—thus the need for new discovery is great.

A large number of 1,6-dihydro-6,6-dimethyl-1-phenyl-1,3,5-triazine-2,4-diamines related to cycloguanil (I), a potent antimalarial drug, were subjected to screening in



I

mice against *T. rhodesiense* infections in mice by the Walter Reed Army Institute of Research, and no trypanocidal activity was detected. In contrast three members of a small group of 1,1'-(4,1-phenylene)bis[1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamines] (IIa-c) synthesized in our laboratories some years ago[3] exhibited curvative activity after administration of single subcutaneous doses.

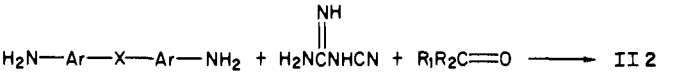


IIa, X = CH_2
IIb, X = $(CH_2)_2$
IIc, X = O

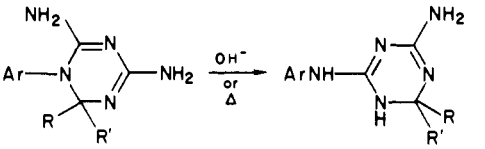
This unique activity required the expansion and systematic examination of this structural class, and it is this effort that is the subject of the current paper.

Chemistry. Three major routes exist for the synthesis of 1-aryl-1,6-dihydro-1,3,5-triazine-2,4-diamines.[4] Generally the three-component synthesis of Modest[5] was utilized to provide the target compounds (Scheme I). However, a number of the diamines reacted very slowly and incompletely under the standard conditions to provide the desired dihydrotriazines. In most cases this difficulty has been overcome by using *N,N*-dimethylformamide as a cosolvent and heating for prolonged periods. We were mindful of the potential for rearrangement of the dihydrotriazines (Scheme II); however, this has not been a problem when the modified reaction conditions are used.

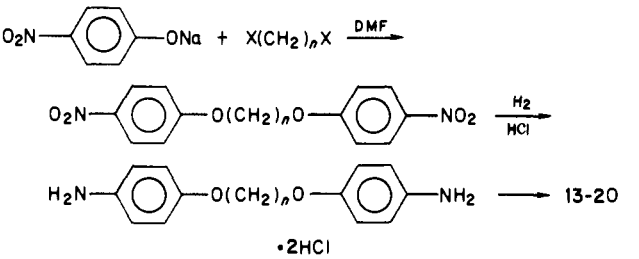
Scheme I



Scheme II



Scheme III



Our primary interest was varying the spacer between the two aryl rings. A variety of the needed diamines were available commercially and application of Scheme I led directly to the target compounds (Tables I and II delineate the dihydrotriazines and their properties prepared in these studies).

In many other instances the requisite bis(arylamines) had to be prepared. The bis ether spacers were synthesized as depicted in Scheme III via a method adapted from the work of Cope.[6]

The urea spacers were prepared as shown in Scheme IV on the basis of work described previously[7-9] and led to dihydrotriazines **26** and **33-35**.

(1) For the last paper of this series on antiparasitic drugs, see: Colbry, N. L.; Elslager, E. F.; Werbel, L. M. *J. Med. Chem.* **1985**, *28*, 248.
(2) This investigation was supported by U.S. Army Medical Research and Development Command Contract DAMD-17-79-6-9028. This is Contribution No. 1747 to the Army Research Program on Antiparasitic Drugs.
(3) Capps, D. B.; unpublished results.
(4) Capps, D. B.; Bird, O. D.; Elslager, E. F.; Gavrilis, Z. B.; Roush, J. A.; Thompson, P. E.; Vaitkus, J. W. *J. Heterocycl. Chem.* **1969**, *5*, 355.
(5) Modest, E. J. *J. Org. Chem.* **1956**, *21*, 1.
(6) Cope, A. C. *J. Am. Chem. Soc.* **1935**, *57*, 572.
(7) U.S. Patent 2503797, April 11, 1950 (*Chem. Abstr.* **1950** 6582a).
(8) Kutepov, A.; Rozanova, N. S. *Zh. Obsch. Khim.* **1957**, *27*, 2532.
(9) Scott, F. L; O'Donovan, D. G.; Kennedy, M. R.; Reilly, J. *J. Org. Chem.* **1957**, *22*, 820.

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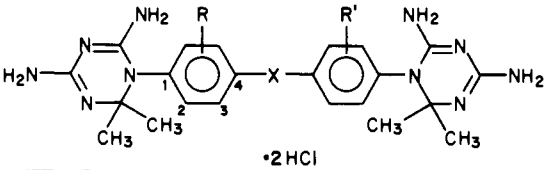
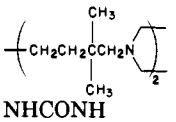
Table I. Properties of Dihydrotriazines

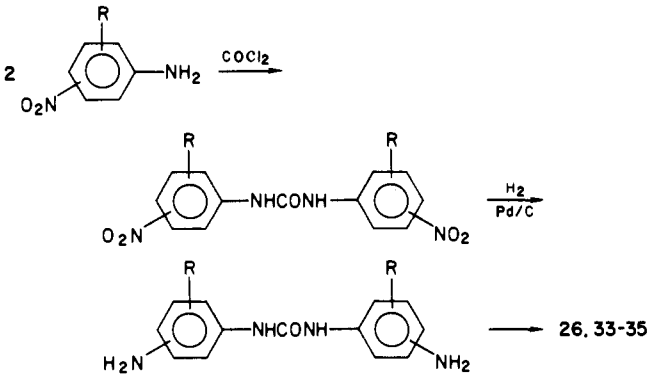


•2HCl

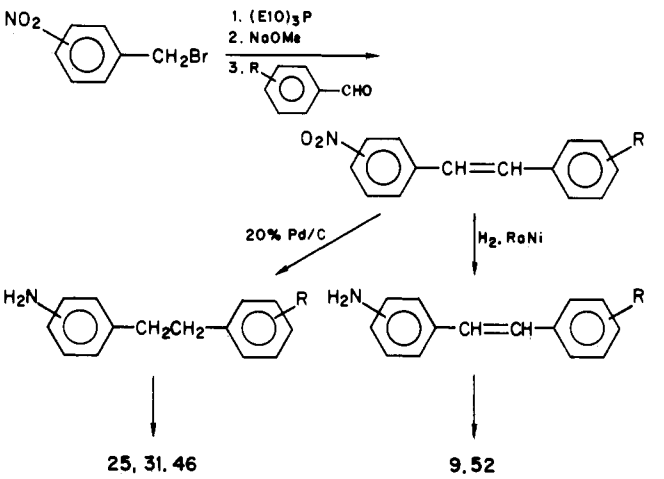
no.	X	R	R′	rctn solvent–ketone/aldehyde (time, days)	recrystn (trituration) solvent	mp, °C	yield, %	formula	anal.
1		H	H	DMF–Me_2CO (2.8)	(2-PrOH)	255–282 dec	55	$C_{22}H_{28}N_{10}$·2HCl·0.6H_2O	C, H, N, Cl, H_2O
8	CH_2CH_2	3-Me	3′-Me	DMF–Me_2CO (1.5)	MeOH–Et_2O	280–282 dec	31	$C_{26}H_{36}N_{10}$·2HCl·0.25H_2O	C, H, N, Cl, H_2O
9	CH=CH	H	H	DMF–Me_2CO (2.8)	(MeOH)	268–270 dec	77	$C_{24}H_{30}N_{10}$·2HCl·1.1H_2O	C, H, N, Cl, H_2O
12	OC_6H_4O (1,4)	H	H	Me_2CO–MeOH $Me_2C(OMe)_2$ (1)	MeOH–Et_2O	254–255 dec	21	$C_{28}H_{32}N_{10}O_2$·2HCl·1.3H_2O	C, H, N, Cl, H_2O
13	OCH_2O	H	H	EtOH–Me_2CO (2)	(MeOH)	231–235 dec	29	$C_{23}H_{30}N_{10}O_2$·2HCl·0.3H_2O	C, H, N, Cl, H_2O[a]
14	$O(CH_2)_2O$	H	H	MeOH–Me_2CO (5.5)	MeOH–Et_2O	252–255 dec	43	$C_{24}H_{32}N_{10}O_2$·2HCl·0.5H_2O	C, H, N, Cl, H_2O
15	$O(CH_2)_3O$	H	H	MeOH–Me_2CO (3)	MeOH–Et_2O	243.5–244.5 dec	33	$C_{25}H_{34}N_{10}O_2$·2HCl	C, H, N, Cl[b]
16	$O(CH_2)_4O$	H	H	MeOH–Me_2CO	MeOH–Et_2O	240–241 dec	24	$C_{26}H_{36}N_{10}O_2$·2HCl·1.3H_2O	C, H, N, Cl, H_2O
17	$O(CH_2)_5O$	H	H	MeOH–Me_2CO (2)	MeOH–Me_2CO	226–227 dec	33	$C_{27}H_{38}N_{10}O_2$·2HCl·0.9H_2O	C, H, N, Cl, H_2O
18	$O(CH_2)_6O$	H	H	MeOH–Me_2CO (8)	MeOH–2-PrOH	225–227 dec	4	$C_{28}H_{40}N_{10}O_2$·2HCl·0.5H_2O	C, H, N, Cl, H_2O[c]
19	$O(CH_2)_{10}O$	H	H	MeOH–Me_2CO (8)	MeOH–2-PrOH	226–227 dec	17	$C_{32}H_{48}N_{10}O_2$·2HCl·H_2O	C, H, N, Cl, H_2O
20	$O(CH_2)_{12}O$	H	H	Me_2CO (3)	(MeOH)	204–210 dec	11	$C_{34}H_{52}N_{10}O_2$·2HCl·0.7H_2O	C, H, N, Cl, H_2O[d]
21	N=N	H	H	DMF–Me_2CO (3.5)	MeOH–Et_2O	268–269 dec	25	$C_{22}H_{28}N_{12}$·2HCl·0.6H_2O	C, H, N, Cl, H_2O[e]
25		H	H	MeOH–Me_2CO (4)	MeOH–Me_2CO	232–238 dec	38	$C_{38}H_{60}N_{12}$·4HCl·3.5H_2O	C, H, N, Cl, H_2O
26	NHCONH	H	H	DMF–Me_2CO (3)	(Me_2CO; MeOH)	292–294 dec	29	$C_{23}H_{30}N_{12}O$·2HCl·1.8H_2O	C, H, N, Cl, H_2O

[a] N: calcd, 25.15; found, 24.56. [b] N: calcd, 24.17; found, 23.21. [c] N: calcd, 22.12; found, 21.42. [d] N: calcd, 19.5; found, 18.04. Cl: calcd, 9.87; found, 10.35. H_2O: calcd, 1.76; found, 2.66. [e] H_2O: calcd, 1.99; found, 1.08 (insoluble in KF reagent).

Scheme IV



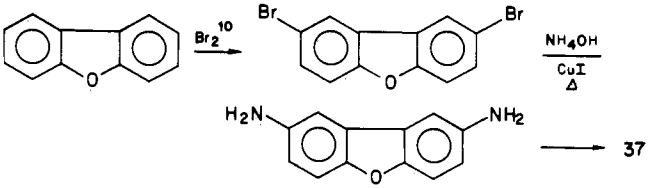
Scheme V



Unsaturated and saturated two-carbon spacers were prepared as shown in Scheme V and led to dihydrotriazines **9**, **25**, **31**, **46**, and **52**.

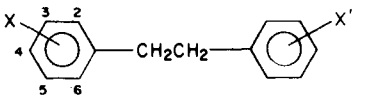
The bridged O analogue in which the two aromatic rings were tied together (**37**) was prepared from dibenzofuran as shown in Scheme VI.

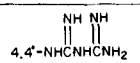
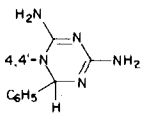
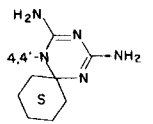
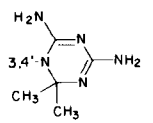
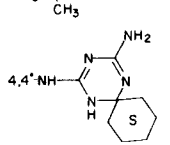
Scheme VI

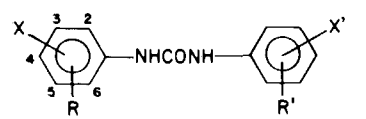


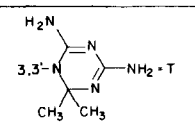
Several analogues with more severe structural variation were also prepared to probe the structural requirements for antitrypanosomal activity. The synthetic methodology for these analogues generally followed that described previously. Thus removal of the bridging group X and attachment of both dihydrotriazine rings to the same

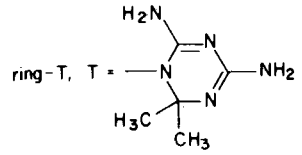
Table II. Properties of Miscellaneous Dihydrotriazines and Related Structures



no.	X, X'	rctn solvent-ketone/ aldehyde (time, days)	recrystn (trituration) solvent	mp, °C	yield, %	formula	anal.
28		EtOH-none (0.63)	MeOH	263–264 dec	23	$C_{18}H_{24}N_{10}\cdot 2HCl\cdot 0.1H_2O$	C, H, N, Cl, H_2O
29		EtOH-C_6H_5CHO (0.67)	(MeOH)	268–271 dec	48	$C_{32}H_{32}N_{10}\cdot 2HCl$	C, H, N, Cl
30		EtOH-$C_7H_{10}O$ (0.63)	(MeOH)	263–266 dec	27	$C_{30}H_{40}N_{10}\cdot 2HCl\cdot 0.5H_2O$	C, H, N, Cl, H_2O[a]
31		DMF-Me_2CO (1.5)	(MeOH)	234–238 dec	44	$C_{24}H_{32}N_{10}\cdot 2HCl\cdot 0.75H_2O$	C, H, N, Cl, H_2O
32		DMF-$C_6H_{10}O$ (2.0)	MeOH-ether	>290	6	$C_{30}H_{40}N_{10}\cdot 2HCl\cdot 0.4H_2O$	C, H, N, Cl, H_2O



no.	X, X'	R, R'	rctn solvent-ketone/ aldehyde (time, days)	recrystn (trituration) solvent	mp, °C	yield, %	formula	anal.
33		H	DMF-Me_2CO (3)	(1) MeOH-2-PrOH, (2) (MeOH)	259–269 dec	22	$C_{23}H_{30}N_{12}O\cdot 2HCl\cdot H_2O$	C, H, N, Cl, H_2O[b]
34	5,5'-T	2,2'-CH_3	DMF-Me_2CO (2.3)	MeOH-Me_2CO	248–251 dec	7	$C_{25}H_{34}N_{12}O\cdot 2HCl\cdot 1.1H_2O$	C, H, N, Cl, H_2O
35	3,3'-T	4,4'-T	DMF-Me_2CO (2.6)	MeOH-Me_2CO	254–257 dec	4	$C_{25}H_{34}N_{12}O\cdot 2HCl\cdot 1.3H_2O$	C, H, N, Cl, H_2O



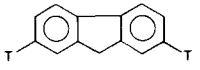
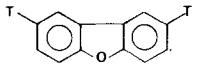
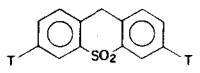
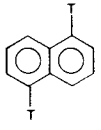
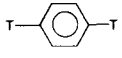
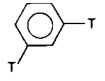
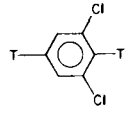
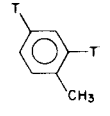
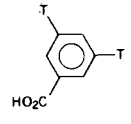
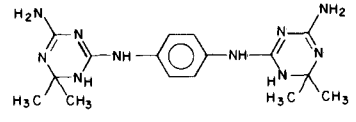
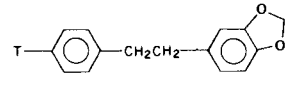
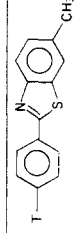
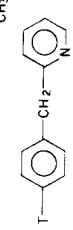
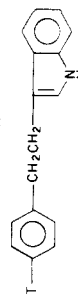
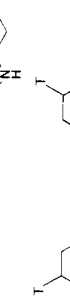
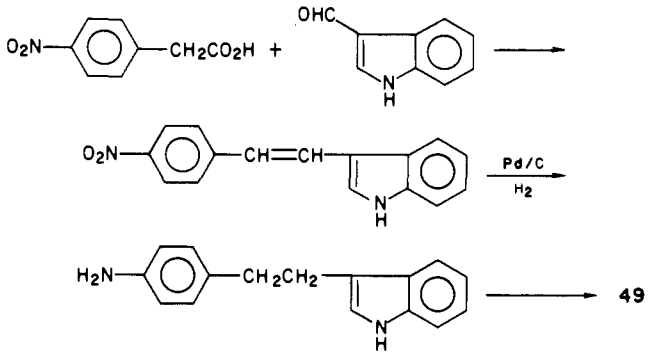
no.	ring-T	rctn solvent–ketone/ aldehyde (time, days)	recrystn (trituration) solvent	mp, °C	yield, %	formula	anal.
36		DMF–Me_2CO (3)	MeOH–Me_2CO, Et_2O	293–297 dec	24	$C_{23}H_{28}N_{10}\cdot 2HCl\cdot 0.5H_2O$	C, H, N, Cl, H_2O[c]
37		DMF–Me_2CO (1)	MeOH–Et_2O	264–270 dec	8	$C_{22}H_{26}N_{10}O\cdot 2HCl\cdot 1.5H_2O$	C, H, N, Cl, H_2O[d]
38		MeOH–Me_2CO (0.7)	(Me_2CO–MeOH)	277–281 dec	28	$C_{23}H_{28}N_{10}O_2S\cdot 2HCl\cdot 1.6H_2O$	C, H, N, Cl, H_2O
39		DMF–Me_2CO (3)	(H_2O–MeOH)	>300	12	$C_{20}H_{26}N_{10}\cdot 2HCl\cdot H_2O$	C, H, N, Cl, H_2O[e]
40		EtOH–Me_2CO (1)	(MeOH)	267–271 dec	32	$C_{16}H_{26}Cl_2N_{10}\cdot 2HCl\cdot 0.5H_2O$	C, H, N, H_2O[f]
41		MeOH–Me_2CO (1)	MeOH	234–238 dec	19	$C_{16}H_{24}N_{10}\cdot 2HCl\cdot 1.5H_2O$	C, H, N, H_2O[g]
42		Me_2CO (0.75)	(1) H_2O, (2) MeOH–2-PrOH, (3) (Me_2CO)	266–270 dec	0.4	$C_{16}H_{22}Cl_2N_{10}\cdot 2HCl\cdot 1.9H_2O$	C, H, N, Cl, H_2O
43		MeOH–Me_2CO (0.83)	MeOH–Et_2O	238–241 dec	11	$C_{17}H_{26}N_{10}\cdot 2HCl\cdot 1.2H_2O$	C, H, N, Cl, H_2O
44		Me_2CO (1.9)	H_2O–2-PrOH	276 dec	27	$C_{17}H_{24}N_{10}O_2\cdot 2HCl\cdot 1.4H_2O$	C, H, N, Cl, H_2O
45		H_2O–KOH	(1) H_2O, (2) MeOH–ether	204–207 dec	51	$C_{16}H_{24}N_{10}\cdot 1.1H_2O$	C, H, N, H_2O
46		DMF–Me_2CO (1)	MeOH	228–231 dec	40	$C_{20}H_{23}N_5O_2\cdot HCl$	C, H, N, Cl

Table II (*Continued*)

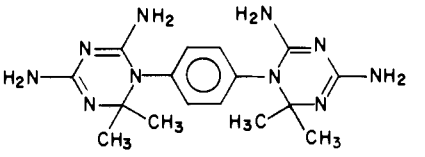
no.	ring-T	rctn solvent-ketone/ aldehyde (time, days)	recrystn (trituration) solvent	mp, °C	yield, %	formula	anal.
47		MeOH-Me_2CO (0.67)	(MeOH)	244–247 dec	39	$C_{19}H_{20}N_6S\cdot 2HCl$	C, H, N, Cl, S
48		MeOH-Me_2CO (1)	MeOH-Et_2O	235–237 dec	57	$C_{17}H_{20}N_6\cdot 2HCl$	C, H, N, Cl
49		MeOH-Me_2CO (1)	(MeOH)	223–226 dec	38	$C_{21}H_{24}N_6\cdot HCl\cdot {}^1/_8H_2O$	C, H, N, Cl, H_2O
52		Me_2CO (3)	(1) H_2O–2-PrOH, (2) MeOH-Et_2O, (3) EtOH	256 dec	9	$C_{24}H_{30}N_{10}\cdot 2HCl\cdot 1.4H_2O$	C, H, N, Cl, H_2O[h]

[a] H_2O: calcd, 1.45; found, 0.8. [b] H_2O: calcd, 3.10; found, 1.84. [c] N: calcd, 26.60; found, 25.95. [d] H_2O: calcd, 4.94; found, 3.58. [e] N: calcd, 28.16; found, 27.49. H_2O: calcd, 3.62; found, 2.28. [f] C: calcd, 43.83; found, 43.33. N: calcd, 31.95; found, 31.53. [g] C: calcd, 42.10; found, 41.49. H: calcd, 6.40; found, 6.95. [h] H: calcd, 6.30; found, 6.77. Cl: calcd, 12.74; found, 13.38.

Scheme VII



benzene ring led to compound **40** as well as analogues **41–45**. In addition several mono(dihydrotriazines) hooked

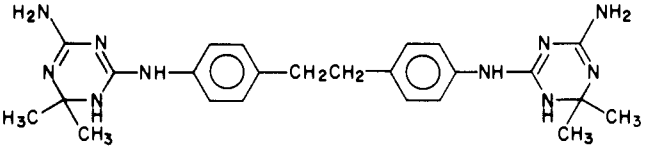


40

to a heterocyclic amine (**46–49**) were prepared. The unique indole starting material was prepared according to Scheme VII.

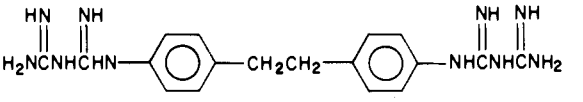
Finally the unique tris[triazine] **50** derived from the corresponding tris(aminophenyl)methane was prepared, and the bis[dihydrotriazine] **51** wherein the triazines were linked at their 6-positions through a 1,4-cyclohexanediyl bridge.

To determine the effect of the facile dihydrotriazine → anilinotriazine rearrangement on biological activity, the rearrangement product (compound **27**) of the active lead compound IIb was also prepared. In addition the effect



27

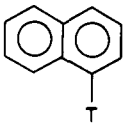
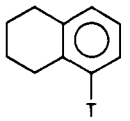
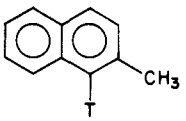
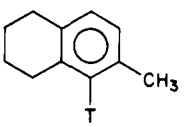
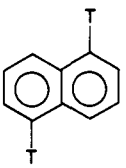
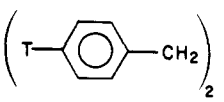
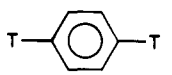
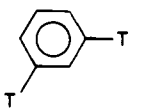
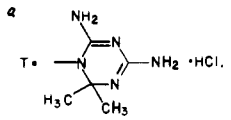
of opening the dihydrotriazine ring to the parent biguanide was also explored with the preparation of the bis[biguanide] compound **28**.



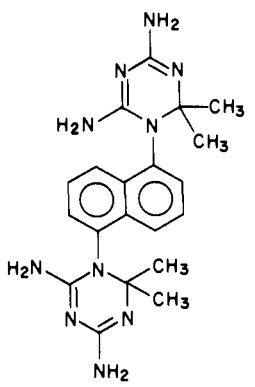
28

A brief comment on our observations regarding the spectral data of certain of the dihydrotriazines is appropriate since it may bear on the biological activity of these moieties. The proton NMR spectrum of compound **39** showed two widely sepd. resonances ($\Delta\delta$ = 0.56 ppm) for the 6,6-methyl groups of the dihydrotriazine ring. This prompted further studies to explore the possibility of rotational hindrance as a cause for this effect. Temperature studies with a sample of the mononaphthyldihydrotriazine III obtained from our files revealed that apparently in this compound a barrier to rotation exists that is sufficient to prevent rotation even at 100° in dimethyl sulfoxide. After heating was continued for 24 h at 100 °C, it was observed that the methyl resonances had coalesced into a single resonance, which persisted after cooling to room temperature. This may be the result of a thermal rearrangement of the dihydrotriazine to the anilinotriazine structure IV,

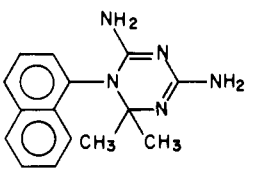
Chart I. Chemical Shifts of the 2-Methyl Groups of Selected 1,6-Dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamines[a]

structure	solvent	δ (integration)
	Me_2SO-d_6	1. 1.04 (3), 1.62 (3) (room temperature) 2. 1.10, 1.48, 1.62 (100 °C) 3. 1.10 (1.5), 1.48 (3.0), 1.62 (1.5) (80 °C) 4. 1.09, 1.48, 1.64 (60 °C) 5. 1.11 (1.2), 1.49 (3.6), 1.63 (1.2) (100 °C) 6. 1.05 (1.2), 1.46 (3.6), 1.62 (1.2) (room temperature) 7. 1.43 (6) (~18 h @ 100 °C, then room temperature)
	TFA Me_2SO-d_6	1.60 (3), 2.02 (3) 1.18 (3), 1.58 (3) (100 °C) 1.08 (3), 1.52 (3) (room temperature)
	TFA	1.88 (6)
	Me_2SO-d_6	1.35 (3), 1.40 (3) (100 °C) 1.29 (3), 1.34 (3) (room temp)
	TFA	1.57 (6), 2.13 (6)
	TFA	1.77 (12)
	TFA	1.85 (12)
	TFA	1.93 (6), 1.83 (6)
[a] 		

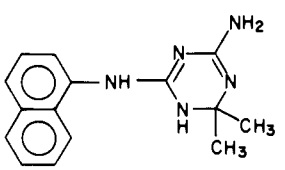
which might have much less hindrance to rotation.



39



III

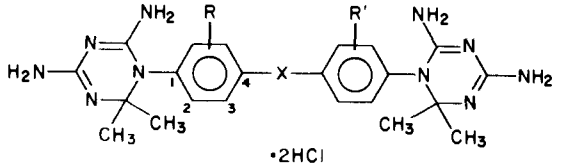


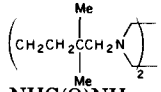
IV

Similar nonequivalence of the methyl resonances at 100 °C was also observed for other 5,6,7,8-tetrahydronaphthalene analogues as well as for a number of ortho-substituted phenyl analogues (Chart I). The bis[dihydrotriazine] from 1,3-phenylenediamine (41) showed nonequivalent methyl resonances. However, at higher temperature, equivalence was observed, indicating that in this orientation there is a lower effect on the freedom of rotation. Compounds in which there is no ortho or meta substitution on the aromatic rings result in all cases in equivalence of the methyl resonances at room temperature. Examination of models of the possible conformers of the dihydrotriazines supports the possibility of the existence of preferred stereochemical configurations because of restricted rotation as a cause of the spectral phenomenon. Methyl groups situated over the aromatic ring will experience more shielding due to ring current effects and should therefore resonate at higher fields.

It may be of interest to determine whether such conformational effects can be related to the biological activity of the compounds. It has been speculated previously that the presence of bulky substituents in the ortho position of aryldihydrotriazines was related to anthelmintic activity.[4]

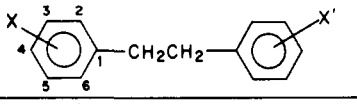
Table III. Activity of Dihydrotriazines against *Trypanosoma rhodesiense* Infections in Mice

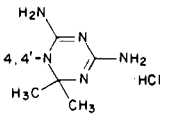
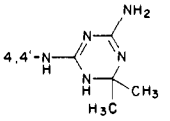
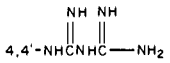
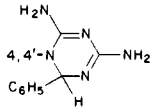
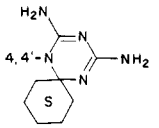
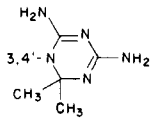
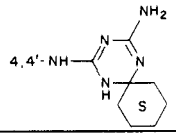


				ΔMST; T and/or C after single dose (mg/kg, sc or *po)[a]								
no.	X	R	R′	424	212	106	53	26	13	6.6	3.3	1.6
1		H	H		T-5	T-5	T-5	T-5	T-5	7.8 C-4	9.2 C-1	3.7 C-1
2		2′-OMe	2′-OMe	T-5	3.2 C-2	11.2 C-2	4.9 C-3	8.9 C-4	3.0 C-3	13.5 C-3	7.2	2.2
3	CH_2	H	H	T-5	T-5	8.0 C-2, T-2	6.5 C-2	4.0 C-1	3.0			
4	$C(CH_3)_2$	H	H	T-5		1.8 T-2		1.3 T-1	1.5 T-1	0.9		
5	CHPh	H	H	T-5	4.3 T-2	4.3 T-2	2.3	2.8	0.9			
6	CH_2	3-NO_2	3′-NO_2	T-5		T-5		0.6				
7	CH_2CH_2	H	H	0.2 T-3	3.8 C-3	C-5	7.8 C-4	7.7 C-4	6.8 C-4	3.4	3.2	
8	CH_2CH_2	3-CH_3	3′-CH_3	T-5		T-5		1.4 T-3	1.8	1.0	0.4	0.8
9	CH=CH	H	H		6.6 C-4	3.4 C-3	C-5	10.9 C-3	C-5	1.4 C-3	1.7	1.2 C-1
10	$CH_2C(CO_2H)HCH_2$	H	H	0.0 T-2		0.2		0.0				
11	O	H	H	T-5		T-5		C-5	10, C-2	5.3 C-1	4.2	
12	OC_6H_4O (1,4)	H	H	*T-5		*1.9 T-4		*0.5				
13	OCH_2O	H	H	T-5		T-5	9.0 C-1, T-2	1.8 C-3	2.0 C-3	2.3 C-1	1.2	1.0
14	$O(CH_2)_2O$	H	H	T-5		T-5		8.9 T-4	5.2	2.6	1.0	2.8
15	$O(CH_2)_3O$	H	H	T-5		T-5		T-5	T-5	T-5	0.0 T-1	0.0
16	$O(CH_2)_4O$	H	H	T-5		1.0 T-4		0.3 T-1	0.2	0.0	0.2	0.0
17	$O(CH_2)_5O$	H	H	T-5		T-5		T-5	T-5	0.4	0.2	0.0
18	$O(CH_2)_6O$	H	H	T-5		T-5		T-5	T-5	0.2 T-1	−0.2	−0.2
19	$O(CH_2)_{10}O$	H	H	0.1		0.1		−0.1				
20	$O(CH_2)_{12}O$	H	H	0.8	0.8	0.8	0.4	0.2	0.0			
21	N=N	H	H	*0.1 T-2, C-4, T-1	0.9 C-4	*5.9 C-4, C-5	C-5	*1.9 C-4, 1.0 C-5	1.6	1.0	0.2	0.0
22	S	H	H	T-5		T-5		2.7 T-1	2.2	0.6	0.6	
23	SS	H	H	0.5		0.1		0.1				
24	SO_2	H	2-CH_3	T-5		T-5		0.7 T-4	0.2	0.4	0.2	
25		H	H	T-5		T-5		T-5	0.0	0.2	0.0	0.0
26	NHC(O)NH	H	H	T-5		T-5	T-5	1.0 C-1, T-1	3.2	0.2	0.0	0.0

[a] ΔMST is the difference in mean survival time between test and control animals; T, toxic; C, cures.

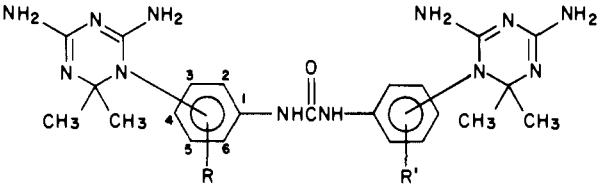
Table IV. Activity of Analogues of Compound **7** against *Trypanosoma rhodesiense* Infections in Mice



no.	X, X′	ΔMST; T and/or C after single dose (mg/kg, sc or *po)[a] 424	212	106	53	26	13	6.6	3.3
7		0.2 T-3	3.8 C-3	C-5	7.8 C-4	7.7 C-4	6.8 C-4	3.4	3.2
27		0.0 T-3		0.8		0.2			
28		T-5	12.0 C-2. T-2	8.0 C-3, T-1	1.4	3.0 C-1	0.6		
29		C-3, T-2	2.2 C-4	C-5	C-5	4.1 C-1	1.8	0.0	0.4
30		C-4, T-1	11.0 C-3, T-1	12.0 C-3	9.0 C-2	1.3 C-2	0.8		
31		T-5, *3.7 C-1, T-1	*1.8	T-5, *0.4	*0.2	T-5, *0.0	1.5 T-1, *0.0	1.7 C-1, T-1	1.0
32		0.2		0.2		0.2			

[a] See Table III.

Table V. Activity of Analogues of Compound **26** against *Trypanosoma rhodesiense* Infections in Mice



no.	triazine positions	R, R′	ΔMST; T and/or C after single dose (mg/kg, sc)[a] 424	212	106	53	26	13	6.6	3.3	1.6	0.8
26	4,4′	H	T-5		T-5		T-5	1.0 C-1, T-1	3.2	0.2	0.0	0.0
33	3,3′	H	T-5		T-5		T-5	0.0 T-3	0.0	0.0	0.0	0.2
34	5,5′	2,2′-CH_3	−0.1 T-3		−0.1		−0.1					
35	3,3′	4,4′-CH_3	T-5		0.0 T-2		0.0 T-2	0.0				

[a] See Table III.

Biology. Biological data are tabulated in Tables III–VI. Included for comparison purposes are data on compounds **2–7**, **10**, **11**, **22**, **23**, **24**, **50**, and **51**, prepared previously and obtained from our files.

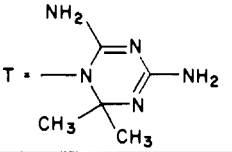
Although specific structure–activity relationship (SAR) conclusions are difficult to draw from such a broadly based exploratory survey, several trends may be seen. It seems clear that as in the malaria area activity resides in the dihydrotriazines and not in the rearranged anilino triazines (compare **7** and **27**, Table IV). Moreover the intermediate biguanide (**28**) although retaining some activity is clearly of much less interest than the parent dihydrotriazines.

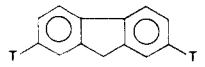
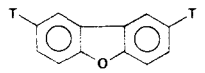
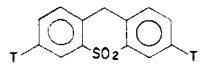
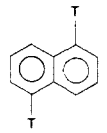
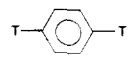
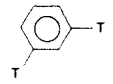
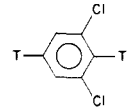
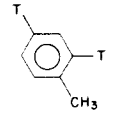
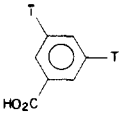
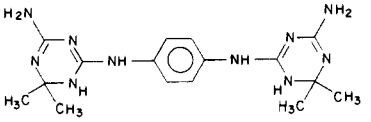
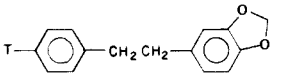
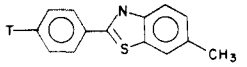
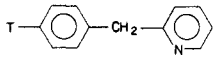
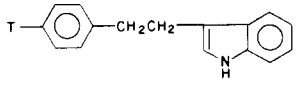
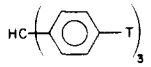
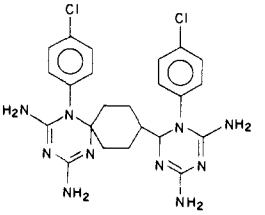
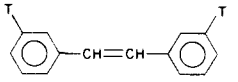
Clearly the presence of two dihydrotriazine units *and* a spacer appropriate both in size and electronic make-up is vital to antiparasitic activity. Since the SARs are clearly different in the present antitrypanosomal activity and the historical antimalarial activity, relationships to the binding sites and receptor geometry of the classic dihydrofolate reductase enzyme surface cannot be invoked at this time. Further biochemical evidence must be obtained before any such relationships become clear. What can be done presently, however, is to firm up the SARs within the active series and point out directions that future research should take.

Thus the presence of a simple phenyl spacer between the two dihydrotriazine units (i.e., compounds **40–44**) is clearly not adequate for biological activity.

The fragile limitations of the receptor geometry is further indicated by the lack of activity of the 3,4′-ditriazine analogue (**31**) as well as the removal of activity caused by

Table VI. Activity of Bridged Analogues against *Trypanosoma rhodesiense* Infections in Mice



no.	compound	ΔMST; T and/or C after single dose (mg/kg, sc)[a]								
		424	212	106	53	26	13	6.6	3.3	1.6
36		T-5	26.0 T-4	2.0 C-1, T-3	C-4, T-1	4.5 C-3	4.5 C-3	4.3 C-2	4.0 C-1	3.0 C-1
37		T-5		T-5		−0.2	−0.2	−0.2	−0.2	0.2
38		T-5		−0.3 T-4		0.0	0.0	0.2	0.0	0.0
39		−0.3 T-3		−0.3		1.1				
40		1.0 T-3		0.2		0.0	0.1	0.1	−0.1	0.1
41		T-5		T-5		0.0 T-1, 0.0	0.0	0.0	0.0	0.0
42			−0.2 T-4		−0.2		−0.2			
43		T-5		T-5		−0.1 T-2, 0.0	0.2	0.0	0.2	0.2
44		−0.1		0.1		−0.1				
45		T-5		1.9 T-4		1.3				
46			0.6		0.4		0.2			
47			0.4		0.6		0.2			
48		T-5		T-5		−0.2				
49		0.0, *C-2, T-2		0.2 *C-3		0.2				
50		T-5		T-5		T-5	0.1	−0.1	−0.1	0.1
51		T-5		T-4		1.0				
52		T-5		T-5		T-5	0.0 T-1	0.0	0.0	0.0

[a] See Table III.

the insertion of two methyl groups in the intervening phenyl rings (8).

Interestingly, the presence of a single methylene between the two phenyl rings (3–6) lowers or eliminates activity as does a sulfur atom (22), while an oxygen (11) restores activity. Whether this is a steric or electronic effect is not at all clear at this time.

More interesting antitrypanosomal activity results from a somewhat longer spacer. Thus the ethylene spacer (7) and the corresponding vinyl analogue (9) exhibit perhaps the broadest range of activity. Attempts to expand the oxygen spacer via a series of methylene groups (13–20) resulted in good activity with a three-atom spacer with rapid fall-off of activity with increasing chain length. Interestingly, the azo spacer (21) demonstrated good activity across a wide dose range, somewhat lower toxicity at higher doses, and only slightly lowered potency when compared with the ethylene spacer (7).

Removal of the spacer between the two phenyl rings (1) results in irregular activity at low doses but also seems to induce an increase in toxicity. Surprisingly, in only one analogue examined (2) toxicity was lowered and the range of activity broadened by the presence of substituents on the biphenyl rings. This may suggest that further improvement might be available through exploration of both electronic and restricted rotation effects within the biphenyl system.

Only one example of a three-carbon bridge culled from our files was examined (10), but its inactivity together with the results on 13, 14, and urea spacers 26, 33–35 do not encourage further effort in this direction.

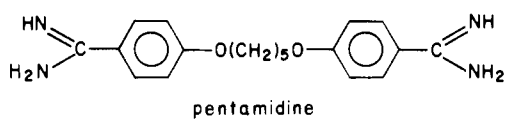
One further finding of interest was the activity exhibited by the fluorenyl analogue 36, a compound that contains both a methylene spacer and the biphenyl system, held however in a unique planar geometric relationship.

Conclusions

Potent antitrypanosomal activity was revealed in several modifications of a unique series of 1,1′-(4,1-phenylene)-bis[1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamines]. Should further test data and particularly demonstration of oral efficacy reveal reason for continued interest in these compounds, it will be most appropriate to explore further the biochemical target, the electronic and steric factors related to activity (possibly via a molecular modelling approach), and additional synthetic effort designed to further optimize the structural features revealed in this preliminary work as responsible for antitrypanosomal activity.

Discussion

Efforts to relate these structures to other systems of known antitrypanosomal activity lead to very little. Thus some resemblance to the phenamidines, i.e., pentamidine,

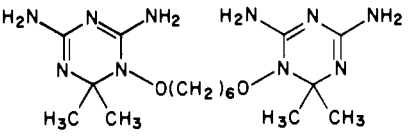


pentamidine

may be claimed although an essential difference lies in the attachment of nitrogen instead of carbon directly to the aromatic ring, and moreover activity is improved rather than diminished by the presence of a long $O(CH_2)_xO$ grouping.

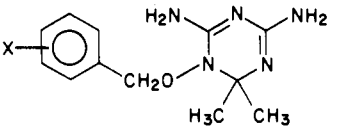
Perhaps a closer analogy might be with 1,1′-[1,6-hexanediyl-bis(oxy)]bis[1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamine] (V), which has been reported to be trypanocidal.[12]

A concern with these compounds would be efficacy upon oral administration, since this would be a preferred dosage



V

form. Clearly the 1-aryl-1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamines and the corresponding ether derivatives (VI) are absorbed from the gastrointestinal tract, since both classes have demonstrated antimalarial activity after oral dosing.[13-15]



VI

Thus far only very limited oral data have been available for this series. However compound 21 with the azo spacer has been shown to have equivalent activity orally and parenterally at doses of 106 and 26 mg/kg. Moreover compound 49 was curative orally, albeit at very high dose levels (424 and 106 mg/kg), and was inactive subcutaneously at the same dose levels. This lends substantial encouragement for the potential oral activity of the class.

Experimental Section

Melting points were obtained using a Thomas-Hoover melting point apparatus and are uncorrected. The ^1H NMR 90-MHz spectra were obtained with a Varian EM390 or Brüker WH90 spectrometer at 90 MHz or a Varian XL-200 spectrophotometer at 200 MHz. The IR spectra were obtained on Digilab DP-1-15 or Beckman IR-9 spectrophotometers. The IR and NMR spectra of all compounds and intermediates were consistent with the assigned structures. Analyses for C, H, N, and Cl were within 0.4% and for H_2O (method of Karl Fisher) within 0.5% of calculated values, unless otherwise noted.

Compounds were evaluated in a life span in IUR/HA Swiss mice infected with the Wellcome CT strain of *T. rhodesiense*.[11] Six-week-old mice (30–32 g) received an intraperitoneal injection of 0.5 mL of a 1:50 000 dilution of heparinized heart blood drawn from donor mice infected 3 days earlier. Compounds were given as a single subcutaneous dose in peanut oil about 2 h after parasite inoculation. Five animal groups were used at each dose level, and positive controls are mice treated with 40 mg/kg of stilbamidine isethionate or 2-hydroxystilbamadine isethionate. In this test a uniform disease was produced fatal to all untreated mice within 4–6 days with a mean survival time of 4.45 ± 0.25 days. Deaths prior to the fourth day were regarded as nonparasitic and were tabulated as "toxic deaths". Treated animals were observed for 30 days, and survivals at the end of this time period were considered "cured".

Sample Preparation of Dihydrotriazine (Scheme I). (a) 1,1′-(Azodi-4,1-phenylene)bis[1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamine] Dihydrochloride Hydrate (5:3) (21). A mixture of 2.0 g (0.009 mol) of 4,4′-azobis[benzenamine], 1.7 g (0.02 mol) of cyanoguanidine, 30 mL of DMF, 10 mL of Me_2CO, and 1.9 mL (2.3 equiv) of concentrated HCl was heated at reflux for 3.5 days. The mixture became very dark and re-

(10) McCombie, H.; MacMillan, W. G.; Scarborough, H. A. *J. Chem. Soc.* **1931**, 529.
(11) Rane, L.; Rane, D. S.; Kinnamon, K. E. *Am. J. Trop. Med. Hyg.* **1976**, *25*, 395.
(12) Mamalis, P. *Xenobiotica* **1971**, *1*, 569.
(13) Schmidt, L. H.; Loo, T. L.; Friedkin, R.; Hughes, H. B. *Proc. Soc. Exp. Biol. Med.* **1952**, *80*, 367.
(14) Smith, C. C.; Ihrig, J.; Menne, R. *Am. J. Trop. Med. Hyg.* **1961**, *10*, 694.
(15) Mamalis, P. U.S. Patent 3 682 912, 1972.

mained nonhomogeneous, although most of the solid had dissolved. The mixture was cooled to room temperature and poured into 350 mL of Me_2CO. The orange-tan precipitate was collected, washed with Me_2CO, and recrystallized from MeOH–Et_2O. The product was obtained as a light yellow-tan powder: 1.27 g (25%); ^1H NMR (CF_3CO_2H) δ 1.9 (s, 12 H), 7.75 (d, 4 H, $J \simeq 9$ Hz), 8.30 (d, 4 H, $J \simeq 9$ Hz); UV [λ_{max} nm (log ϵ)] (pH 2, HCl) 323 (4.42), 235 (4.42), (0.1 M NaOH) 398 (4.46), 248 (4.35). Similarly prepared were **1**, **8**, **9**, **12**, and **39**.

(b) 1,1′-(1,2-Ethanediyldi-4,1-phenylene)bis[1,6-dihydro-6-phenyl-1,3,5-triazine-2,4-diamine] Dihydrochloride (29). To a suspension of 4,4′-diaminobibenzyl (10.5 g, 50 mmol) and cyanoguanidine (8.4 g, 100 mmol) in EtOH (100 mL) was added concentrated HCl (8.5 mL) and then benzaldehyde (10.5 g, 100 mmol). The mixture, which remained nonhomogeneous, was heated under reflux for 18 h. The mixture was cooled to about 25 °C and the product was collected, washed with MeOH until the filtrate was colorless, and dried. The title compound was obtained in 48% yield (14.8 g): ^1H NMR (Me_2SO-d_6) δ 2.73 (br s, 4 H), 6.0 (s, 2 H), 6.9–7.6 (~26 H, NH resonances overlay δ 6.95, 7.12, and 7.30), 6.95 (d, ~4 H, J = 8 Hz), 7.12 (d, ~4 H, J = 8 Hz), 7.30 (s, ~10 H), 9.3 (s, 2 H); UV [λ_{max} nm (log ϵ)] (pH 2, HCl) 249 (4.23), (0.1 M NaOH) (Δ at 80–90 °C, 18 h) 245 (4.55).

Preparation of Intermediates [Scheme Number (Target Compound)]. **1,1′-[1,6-Hexanediylbis(oxy)]bis[4-nitrobenzene] [III (18a)].** A solution of 59.1 g (0.3 mol) of sodium 4-nitrophenolate dihydrate in 150 mL of DMF was treated with 36.6 g (0.15 mol) of 1,6-dibromohexane. The solution was heated on a steam bath for 10 h and then allowed to cool to room temperature. The mixture was poured into 700 mL of 1.5% NaOH. The yellowish solid was collected and washed successively with H_2O and MeOH until the filtrate was no longer yellow. After air-drying, the precipitate was recrystallized from C_6H_6–MeOH to give 39.1 g (72%) of the product: mp 102.5–104.5 °C. Anal. $C_{18}H_{20}N_2O_6$ (C, H, N).

4,4′-[1,6-Hexanediylbis(oxy)]bis[benzenamine] Dihydrochloride Hydrate (10:1) [III (18)].[16,17] A solution of 20.0 g (0.055 mol) of 1,1′-[1,6-hexanediylbis(oxy)]bis[4-nitrobenzene] in 150 mL of tetrahydrofuran and 50 mL of MeOH was reduced over 0.5 g of 5% Pd on carbon under an average of ca. 3 atm. of hydrogen. Some product precipitated from the reaction mixture. The contents of the bomb were warmed to 45 °C, and concentrated HCl and 50 mL of H_2O were added. The mixture was filtered and the filter cake was washed well with H_2O. The filtrate was concentrated at reduced pressure at about 40 °C and H_2O was removed by successive additions of 2-PrOH followed by evaporation to give a solid product. Trituration of the residue with 100 mL of 2-PrOH and 150 mL of Et_2O afforded the title compound in 92% yield (19.2 g): mp 238–241 °C dec. Anal. $C_{18}H_{24}N_2O_2 \cdot 2HCl \cdot 0.1H_2O$ (C, H, N, Cl, H_2O).

***N,N′*-Bis(2-methyl-5-nitrophenyl)urea [IV (34)].** To a solution of 30.4 g (0.20 mol) of 2-methyl-5-nitrobenzenamine in 150 mL of 1,4-dioxane and 50 mL of triethylamine was added dropwise 80 g (~0.10 mol) of a 12.5% solution of phosgene in C_6H_6. The reaction was very exothermic and a heavy precipitate was formed. After addition of an additional 100 mL of 1,4-dioxane to dilute the mixture, it was heated under reflux for 12 h. After cooling, the mixture was poured into 500 mL of ice–H_2O and the product was collected and washed with H_2O. The material was recrystallized from a mixture of DMF–MeOH to provide 21.3 g of the product. Addition of H_2O to the mother liquors afforded a second crop, which was recrystallized from the same solvent system to afford an additional 2.8 g. Total yield 24.1 g (73%) of the title compound: mp 291–293 °C dec. Anal. $C_{15}H_{14}N_4O_5$ (C, H, N).

***N,N′*-Bis(5-amino-2-methylphenyl)urea Hydrate (4:1) [IV (34)].** A solution of 15.0 g (0.045 mol) of *N,N′*-bis(2-methyl-5-nitrophenyl)urea in 200 mL of DMF was reduced at 25 °C in the presence of 0.5 g of 10% Pd on carbon at an average pressure of ca. 3 atm of hydrogen. The dark solution was filtered and poured into 800 mL of ice–H_2O. The product was collected and dissolved in 300 mL of DMF, and the solution was decolorized with carbon. The addition of 200 mL of MeOH and cooling for 18 h at 0 °C afforded the title compound: 8.9 g (71%); mp 233–235 °C dec. Anal. $C_{15}H_{18}N_4O \cdot 0.25H_2O$ (C, H, N; H_2O: calcd, 1.64; found, 0.93, insoluble).

1-Nitro-3-[2-(4-nitrophenyl)ethenyl]benzene [V (31)]. A mixture of 1-(bromomethyl)-4-nitrobenzene (64.8 g, 0.3 mol) and triethyl phosphite (49.8 g, 0.3 mol) was heated at about 140 °C for 2 h, resulting in the evolution of bromoethane. This was added to NaOMe (prepared from 11.8 g of Na in 150 mL of MeOH and removal of solvent) in 60 mL of DMF. The light brown solution became dark purple. A solution of 3-nitrobenzaldehyde (40.8 g, 0.3 mol), in 240 mL of DMF, was added in 3–5-mL aliquots. The temperature was kept between 5 and 35 °C by using an ice bath. The solution was allowed to stand for 24 h, and then 300 mL of H_2O was added. After cooling, an orange-red solid was collected and washed with H_2O to provide 30 g (37%) of material. The crude product (8 g) was purified by trituration in hot EtOH to give 6.5 g of a bright orange solid: mp 207–209 °C. Anal. $C_{14}H_{10}N_2O_4$ (C, H, N).

3-[2-(4-Aminophenyl)ethyl]benzenamine Dihydrochloride [V (33)]. A solution of 1-nitro-3-[2-(4-nitrophenyl)ethenyl]-benzene (7.1 g, 26 mmol in 2-PrOH (100 mL) and tetrahydrofuran (20 mL) was hydrogenated over 20% Pd on carbon with an average of 3 atm of hydrogen. The mixture was filtered into concentrated HCl (5 mL) and the pale pink filtrate was evaporated under reduced pressure (40 °C) to give a semisolid residue. Trituration with 2-PrOH (100 mL) afforded the title compound: 4.6 g (61%); mp 287–291 °C dec. Anal. $C_{14}H_{16}N_2 \cdot 2HCl$ (C, H, N, Cl).

1,4-Bis[2,2-dimethyl-4-(4-nitrophenyl)-3-butenyl]-piperazine Hydrate (4:1) (*E,E*) [V (25)]. A mixture of 1-(bromomethyl)-4-nitrobenzene (43.0 g, 0.2 mol) and triethyl phosphite (33.2 g, 0.2 mol) was heated under reflux for 1 h. The resulting red solution was added slowly to a suspension of NaOMe (0.2 mol) in 50 mL of DMF. The temperature of the mixture was maintained at 10–15 °C by cooling in an ice bath. Then a solution of 3,3′-(1,4-piperazinediyl)bis[2,2-dimethylpropanal] (25.4 g, 0.1 mol) in 400 mL of DMF was added at such a rate that the temperature of the mixture remained below 25 °C. The mixture was stirred at 15–20 °C for 15 min and then at 25–30 °C for 18 h. MeOH (100 mL) and H_2O (100 mL) were added, and the mixture was cooled at 0 °C for 2 h. The solid was collected, recrystallized from DMF–2-PrOH–H_2O, and triturated with 2-PrOH to afford the title compound: 8.7 g (18%); mp 160–161 °C. The ^1H NMR spectrum in $CHCl_3$-d showed two doublets at δ 6.23 and 6.45 ($J_{CH=CH} \cong 17$ Hz). The large coupling is indicative of the *E* configuration. Anal. $C_{28}H_{36}N_4O_4 \cdot 0.25H_2O$ (C, H, N, H_2O).

4,4′-[1,4-Piperazinediylbis(3,3-dimethyl-4,1-butanediyl)]-bis[benzenamine] Tetrahydrochloride Hemihydrate [V (25)]. A solution of 1,4-bis[2,2-dimethyl-4-(4-nitrophenyl)-3-butenyl]-piperazine hydrate (**25a**; 7.5 g, 15 mmol) in tetrahydrofuran (75 mL) and MeOH (25 mL) was catalytically reduced over Raney nickel (3.5 g) with an average of 3 atm of hydrogen. The solution was then filtered and the filtrate was evaporated under reduced pressure (<40 °C). The solid residue was triturated with 2-PrOH (15 mL) and then dissolved in EtOH (75 mL). Addition of dry HCl (4 equiv) in 2-PrOH and then Et_2O gave a white solid, which was collected, washed with Et_2O, and dried to afford the title compound: 7.5 g (83%); mp 293–297 °C dec. Anal. $C_{28}H_{44}N_4 \cdot 4HCl \cdot 0.5H_2O$ (C, H, N, Cl, H_2O).

1,1′-[1,4-Piperazinediylbis[(3,3-dimethyl-4,1-butanediyl)-4,1-phenylene]]bis[1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamine] Tetrahydrochloride Hydrate (2:7) [V (25)]. To a suspension of 4,4′-[1,4-piperazinediylbis(3,3-dimethyl-4,1-butanediyl)]bis[benzenamine] tetrahydrochloride (hemihydrate (5.0 g, 8.5 mmol) and cyanoguanidine (1.5 g, 18 mmol) in MeOH (100 mL) was added concentrated HCl (0.5 mL) and then Me_2CO (50 mL). The mixture was heated under reflux for 4 days, cooled to about 25 °C, and then poured into a 2:1 mixture of Et_2O–Me_2CO (300 mL). The mixture was cooled to 0 °C and the product was collected, triturated with MeOH (10 mL), and recrystallized from MeOH–Me_2CO to afford the title compound: 2.9 g (38%); ^1H NMR (D_2O) δ 1.13 (s, 12 H), 1.43 (s, 12 H), 1.5–1.8 (m, 4 H), 2.5–2.9 (m, 4 H), 3.10 (s, 4 H), 3.55 (s, 8 H), 7.23 (d, 4 H, $J \simeq 8$ Hz), 7.40 (d, 4 H, $J \simeq 8$ Hz); UV [λ_{max} nm (log ϵ)] (pH 2, HCl) 240 (4.31), (0.1 M NaOH) 256 (4.24).

5-[2-(4-Nitrophenyl)ethenyl]-1,3-benzodioxole [V (46)]. To

(16) Ashley, J. N.; Collins, R. F.; Davis, M.; Sirett, N. E. *J. Chem. Soc.* **1958**, 3298.
(17) Partridge, M. W.; Short, W. F. *J. Chem. Soc.* **1947**, 382.

triethyl phosphite (34.2 g, 0.206 mol) was added 1-(bromomethyl)-4-nitrobenzene (44.5 g, 0.206 mol) and the mixture was heated at ca. 140 °C for 2 h. Bromoethane gas was evolved. After cooling, NaOMe (prepared from 11.8 g of Na in 150 mL MeOH and removal of the solvent) in 60 mL of DMF was added. 1,3-Benzodioxole-5-carboxaldehyde (piperonal) (30.9 g, 0.206 mol), dissolved in 240 mL of DMF, was added in 5–10-mL aliquots, with swirling. The temperature was maintained between 5 and 35 °C by using an ice bath. The solution was allowed to stand at room temperature for 24 h, and then 300 mL of H_2O was added. A dark orange solid was collected and washed with H_2O. The product was triturated with hot EtOH twice to provide a yellow-orange solid: 38 g (68.5%); mp 184–186 °C. Anal. $C_{15}H_{11}NO_4$ (N, H; C: calcd, 66.90; found, 66.46).

4-[2-(1,3-Benzodioxol-5-yl)ethyl]benzenamine [V (46)]. A solution of 33.1 g of 5-[2-(4-nitrophenyl)ethenyl]-1,3-benzodioxole in 300 mL of DMF was reduced over 2.0 g of 10% Pd on carbon with an average of ca. 3 atm. of hydrogen. The mixture was then filtered and the filtrate was added to 400 mL of ice–H_2O. The product was collected and dissolved in MeOH. The solution was decolorized with activated carbon, diluted with about one-third its volume of H_2O, and cooled to 0 °C. The title compound was obtained as a beige solid in 70% (20.9 g) yield: mp 80 °C. Anal. $C_{15}H_{15}NO_2$ (C, H, N).

3-[2-(4-Nitrophenyl)ethenyl]-1*H*-indole [VII (49)]. A mixture of 4-nitrobenzeneacetic acid (17.0 g, 0.094 mol), 1*H*-indole-3-carboxaldehyde (13.1 g, 0.090 mol), and piperidine (4 mL) was heated at 110 °C until bubbling and evolution of water had ceased (~2.5 h). The deep red, solid mixture was then heated at 140 °C for 2 h. After cooling to ~25 °C, the mixture was boiled with EtOH (150 mL) and the solid was broken up. The red solid was collected, washed with EtOH, triturated with MeOH, and dried to yield 13.5 g (57%), mp 182–185 °C. Although the product was not analytical, it was pure by TLC (9:1 $CHCl_3$–Et_2O) and its infrared and NMR spectra were consistent with the proposed structure.

4-[2-1*H*-Indol-3-yl)ethyl]benzenamine [VII (49)]. A solution of 3-[2-(4-nitrophenyl)ethenyl]-1*H*-indole (5.01 g, 0.019 mol) in tetrahydrofuran (50 mL) and MeOH (50 mL) was catalytically hydrogenated over 5% Pd on carbon with an average of 3 atm of hydrogen. The initial deep red solution became pale yellow. The catalyst was removed by filtration and 1 equiv of dry HCl in 2-PrOH was added. The solution immediately turned red. After treatment with activated carbon, the solution was evaporated and the red solid was dissolved in 1:1 MeOH–H_2O. The solution was made slightly basic with 28% NH_4OH solution (pH ~8) and the pink precipitate was collected and dried to give the title compound in 85% yield (3.80 g): mp 134–136 °C. Anal. $C_{16}H_{16}N_2$ (C, H, N).

2,8-Dibromodibenzofuran [VI (37)].[10] To a solution of 33.6 g (0.2 mol) of dibenzofuran in 100 mL of HOAc was added a solution of 64.0 g (0.4 mol) of Br_2 in 100 mL of HOAc. The solution immediately became hot, and crystals were deposited after ca. 5 min. The mixture was allowed to remain at room temperature 18 h and then heated under reflux for 6 h, cooled to room temperature, and poured into 1000 mL of ice–H_2O. The product was collected and recrystallized from C_6H_6–MeOH to give the title compound: 22.0 g (34%); mp 186–188 °C. Anal. $C_{12}H_6Br_2O$ (C, H, Br).

2,8-Dibenzofurandiamine [VI (37)].[18] A mixture of 25.2 g (0.077 mol) of 2,8-dibromodibenzofuran, 35.7 g (0.25 mol) of CuBr, and 510 mL of 28% NH_4OH was heated at 205 °C for 12 h in a steel bomb. The mixture was then cooled to 15 °C and the grey solid was collected and dissolved in 200 mL of DMF and 300 mL of EtOH. Decolorization of the solution with activated carbon, followed by addition of 750 mL of H_2O, gave 8.8 g (63%) of the title compound as shiny beige needles: mp 210–212 °C. Anal. $C_{12}H_{10}N_2O$ (C, H, N).

Sample Preparation of Rearranged Dihydrotriazine. $N^2,N^{2\prime}$-(1,4-Phenylene)bis[1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamine] Hydrate (10:11) (45). To a suspension of 5.0 g (0.011 mol) of 1,1′-(1,4-phenylene)bis[1,6-dihydro-6,6-dimethyl-1,3,5-triazine-2,4-diamine] dihydrochloride hemihydrate in 120 mL of H_2O and 100 mL of EtOH was added 3.5 equiv of NaOH (2.8 g of 50% NaOH in 50 mL of H_2O). The mixture was heated on a steam bath for 2 h. After about 45 min of heating, all the material had dissolved. The solution was then evaporated at reduced pressure and the yellow, semisolid residue was triturated with 20 mL of H_2O. The gummy residue was recrystallized first from H_2O and then from MeOH–Et_2O to afford 2.2 g (51%) of the title compound as a dull, off-white solid: UV λ_{max} (H_2O) 258 nm (log ϵ 4.35). The unrearranged starting material **39** showed λ_{max} (0.1 M NaOH) 240 nm (sh) (log ϵ 4.18), which shifted on heating to λ_{max} (0.1 M NaOH) 254 nm (log ϵ 4.21), thus confirming the structure of the title compound:[5] ^1H NMR (Me_2SO-d_6, 200 MHz) δ 1.19 (s, 12 H), 6.95–7.85 (m, 4 H); UV [λ_{max} nm (log ϵ)] (pH 2, HCl) 258 (4.35), (0.1 M NaOH) 262 (4.32).

4-(2-Pyridinylmethyl)benzenamine Dihydrochloride [I (48)].[19] A solution of 2-[(4-nitrophenyl)methyl]pyridine (15.1 g, 71 mmol) in tetrahydrofuran (100 mL) and MeOH (100 mL) was hydrogenated over Raney nickel (2.0 g) with an average of ca. 3 atm of hydrogen. The catalyst was removed by filtration and the filtrate was evaporated to a dark red oil. A solution of this oil in 2-PrOH was treated with activated carbon and then 2 equiv of dry HCl in 2-PrOH was added. Precipitation of the product was completed by the addition of Et_2O. The product was collected and triturated with 2-PrOH, thus, affording the title compound: 13.5 g (74.5%); mp 263–266 °C dec. Anal. $C_{12}H_{12}N_2$·HCl (C, H, N; Cl: calcd, 27.57; found, 27.09.

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Registry No. 1, 96632-93-8; 1·2HCl, 96615-44-0; 8, 96615-45-1; 8·2HCl, 96615-46-2; **9**, 96615-47-3; **9**·2HCl, 96615-48-4; **12**, 96615-49-5; **12**·2HCl, 96615-50-8; **13**, 96615-51-9; **13**·2HCl, 96615-52-0; 14, 96615-53-1; 14·2HCl, 96615-54-2; **15**, 96615-55-3; **15**·2HCl, 96615-56-4; **16**, 96615-57-5; **16**·2HCl, 96615-58-6; **17**, 96615-59-7; **17**·2HCl, 96615-60-0; 18, 96615-61-1; 18·2HCl, 96615-62-2; **19**, 96615-63-3; **19**·2HCl, 96615-64-4; **20**, 96615-65-5; **20**·2HCl, 96615-66-6; **21**, 96615-67-7; **21**·2HCl, 96615-68-8; **25**, 96615-69-9; **25**·4HCl, 96615-70-2; **26**, 96615-71-3; **26**·2HCl, 96615-72-4; **28**, 96615-73-5; **28**·2HCl, 96615-74-6; **29**, 96615-75-7; **29**·2HCl, 96615-76-8; **30**, 96632-94-9; **30**·2HCl, 96615-77-9; **31**, 96615-78-0; **31**·2HCl, 96615-79-1; **32**, 96615-80-4; **32**·2HCl, 96632-95-0; **33**, 96615-81-5; **33**·2HCl, 96615-82-6; **34**, 96615-83-7; **34**·2HCl, 96615-84-8; **35**, 96615-85-9; **35**·2HCl, 96615-86-0; **36**, 96615-87-1; **36**·2HCl, 96615-88-2; **37**, 96615-89-3; **37**·2HCl, 96615-90-6; **38**, 96615-91-7; **38**·2HCl, 96615-92-8; **39**, 96615-93-9; **39**·2HCl, 96615-94-0; **40**, 96615-95-1; **40**·2HCl, 96615-96-2; **41**, 96615-97-3; **41**·2HCl, 96615-98-4; **42**, 96615-99-5; **42**·2HCl, 96616-00-1; **43**, 96616-01-2; **43**·2HCl, 96616-02-3; **44**, 96616-03-4; **44**·2HCl, 96616-04-5; **45**, 96616-05-6; **46**, 96616-07-8; **46**·HCl, 96616-06-7; **47**, 96616-09-0; **47**·2HCl, 96616-08-9; **48**, 96616-11-4; **48**·2HCl, 96616-10-3; **49**, 96616-13-6; **49**·HCl, 96616-12-5; **52**, 96616-15-8; **52**·2HCl, 96616-14-7; Me_2CO, 67-64-1; 4,4′-azobisbenzenamine, 538-41-0; cyanoguanidine, 461-58-5; 4,4′-diaminobibenzyl, 621-95-4; benzaldehyde, 100-52-7; 1,1′-[1,6-hexanediylbis(oxy)]bis[4-nitrobenzene], 7511-70-8; sodium 4-nitrophenolate, 824-78-2; 1,6-dibromohexane, 629-03-8; 4,4′-[1,6-hexanediylbis(oxy)]bis[benzenamine] dihydrochloride, 6889-02-7; *N*,*N*′-bis(2-methyl-5-nitrophenyl)urea, 96616-16-9; 2-methyl-5-nitrobenzenamine, 99-55-8; *N*,*N*′-bis(5-amino-2-methylphenyl)-urea, 96616-17-0; 1-nitro-3-[2-(4-nitrophenyl)ethenyl]benzene, 27892-99-5; 1-(bromomethyl)-4-nitrobenzene, 100-11-8; 3-nitrobenzaldehyde, 99-61-6; 3-[2-(4-aminophenyl)ethyl]benzenamine dihydrochloride, 96616-18-1; (*E*,*E*)-1,4-bis[2,2-dimethyl-4-(4-nitrophenyl)-3-butenyl]piperazine, 96616-19-2; 3,3′-(1,4-piperazinediyl)bis[2,2-dimethylpropanal], 92370-25-7; 4,4′-[1,4-piperazinediylbis(3,3-dimethyl-4,1-butanediyl)]bis[benzenamine] tetrahydrochloride, 96616-20-5; 5-[2-(4-nitrophenyl)ethenyl]-1,3-benzodioxole, 3001-09-0; 1,3-benzodioxole-5-carboxaldehyde, 120-57-0; 4-[2-(1,3-benzodioxol-5-yl)ethyl]benzenamine, 96632-96-1; 3-[2-(4-nitrophenyl)ethenyl]-1*H*-indole, 96616-21-6; 4-

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nitrobenzeneacetic acid, 104-03-0; 1*H*-indole-3-carboxaldehyde, 487-89-8; 4-[2-(1*H*-indol-3-yl)ethyl]benzenamine, 96616-22-7; 2,5-dibromodibenzofuran, 10016-52-1; dibenzofuran, 132-64-9; 2,8-dibenzofurandiamine, 25295-66-3; 4-(2-pyridinylmethyl)-benzenamine dihydrochloride, 96616-23-8; 2-[(4-nitrophenyl)-methyl]pyridine, 620-87-1.

Notes

2-Fluoroformycin and 2-Aminoformycin. Synthesis and Biological Activity

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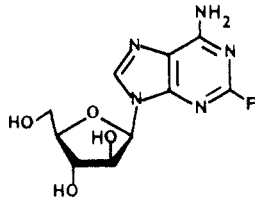
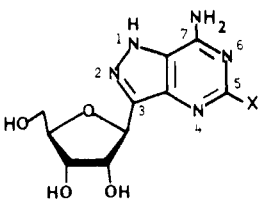
Syntheses of 2-fluoroformycin [7-amino-5-fluoro-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine] (**2b**) and 2-aminoformycin [5,7-diamino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine] (**2c**) are described. Cytotoxicity data are given for **2b** and **2c** alone as well as with added pentostatin. Kinetic parameters for adenosine deaminase are also provided. 2-Fluoroformycin, although a much poorer substrate for adenosine deaminase than formycin A, is not nearly as cytotoxic to cells in culture.

The anticancer activity of 9-(β-D-arabinosyl)-2-fluoroadenine (2-F-araA, **1**)[1] has led us to synthesize various other ring fluorinated purine nucleosides. These have

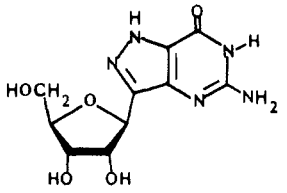


1



2

a) X = H
b) X = F
c) X = NH_2



3

included, recently, 2-fluoro-2′-deoxyadenosine,[2] 2-fluoro-8-azaadenosine,[3] and 8-amino-6-fluoro-9-(β-D-ribofuranosyl)purine.[4] A nucleoside antibiotic that has attracted considerable interest is formycin A (**2a**), a C-nucleoside closely resembling adenosine (N-9 and C-8 of adenosine are juxtaposed). Formycin A has a wide variety of biological effects, including some in vivo anticancer activity.[5-7] In terms of metabolism in mammalian cells, it is phosphorylated by adenosine kinase, carried all the way to the triphosphate level, and incorporated to some extent into nucleic acids. Formycin A is also deaminated rapidly by adenosine deaminase to formycin B, which is a modest inhibitor of purine nucleoside phosphorylase but has little toxicity to cells in culture.[8] It thus appears that any anticancer activity seen with formycin A probably can be attributed to its phosphorylation.

Insertion of a fluorine into the 2-position of a purine or purine analogue is known to greatly reduce the ability of the compound to serve as a substrate for adenosine deaminase.[9] In addition, the 2-fluoro substituent does not seriously impair phosphorylation by adenosine kinase, though bigger groups at C-2 largely prevent phosphorylation by this enzyme.[9] Consideration of the information presented above suggests 2-fluoroformycin [7-amino-5-fluoro-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine] as an attractive synthetic target. Herein we report the synthesis of 2-fluoroformycin as well as 2-aminoformycin, together with some biological data on these two compounds.

Functionalization at C-5 of formycin (corresponding to C-2 in the purine system) has been accomplished by ring opening of the pyrimidine after *N*-oxide formation at N-6 (purine N-1) followed by reclosure of an appropriately substituted precursor.[10] Our approach involved starting with the pyrazolo[4,3-*d*]pyrimidine nucleoside analogue (**3**) of guanosine[10] and converting it in several steps to the diamino compound **2c**, which was then converted to the desired fluoro compound **2b** by a method used successfully in other related systems.[4,11] Acetylation of crude **3** under standard conditions afforded a mixture of acetylated compounds that contained both a triacetate and a tetraacetate, as judged by mass spectral analysis. The crude

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