Bridged Isocytosine-Adenosine Compounds: Synthesis and Antibacterial Evaluation

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In an approach to novel antibacterial agents, we synthesized a series of 5-nitrosoisocytosines in which a 6-alkylamino substituent is bridged, through an amide linkage at the terminus of the alkyl chain, to the 5'-position of a 5'-deoxyadenosine moiety. A corresponding series of 5-nitro analogues were also prepared. None of the bridged compounds showed significant antibacterial activity.

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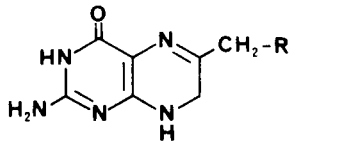
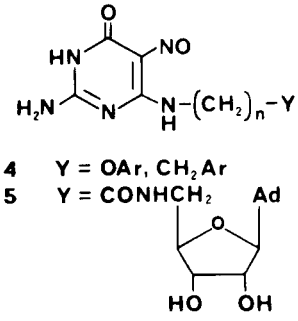
Two important stages of dihydrofolic acid biosynthesis involve: (a) the pyrophosphorylation of 6-hydroxymethyl-7, 8-dihydropterin (**1**), carried out by the enzyme hydroxymethyldihydropterin pyrophosphokinase; and (b) the subsequent formation of 7,8-dihydropteroic acid (**3**) through condensation of pyrophosphate **2** with *p*-aminobenzoic acid, a reaction catalyzed by dihydropteroate synthase [2]. We have recently found [3] that the dihydropteroate synthase of *Escherichia coli* is potently inhibited *in vitro* by certain 6-(ω-arylalkyl)amino-5-nitrosoisocytosines (*cf.* **4**), and that the terminal aryl substituent of these inhibitors may be of considerable size. We thus considered the possibility that compounds such as **5**, into which an adenine nucleoside moiety is incorporated, might also inhibit the pyrophosphokinase by bridging the kinase binding sites for **1** and ATP, and might be effective antibacterial agents. To explore this possibility, we synthesized a series of 5-nitrosoisocytosines in which a 6-alkylamino substituent is bridged, through an amide linkage at the terminus of the alkyl chain, to a 5'-deoxyadenosine moiety. A corresponding series of 5-nitro analogues were also prepared.





The bridged compounds listed in Table II were synthesized as outlined in Scheme I, and the properties of intermediates are provided in Table I. The ω-amino acids **6** were chosen as the starting materials as they are endowed with an electrophilic center at one terminus and a nucleophilic center at the other terminus and the termini are readily manipulated. The amino acids were initially protected at the amino terminus as their carbobenzyloxy de-

Scheme 1

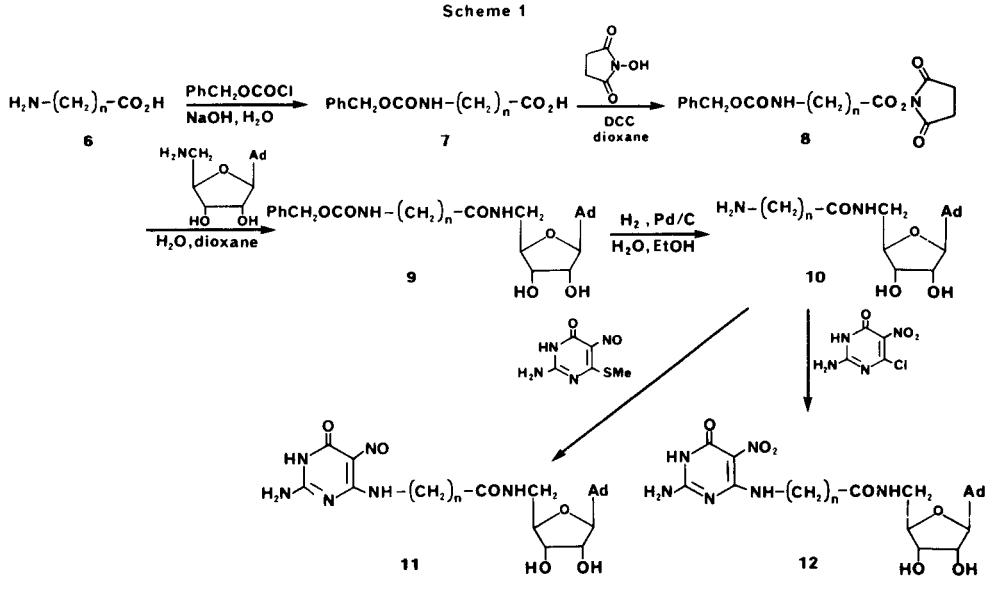


Table I

N-5′-Deoxy-5′-adenosyl Amides **9**

No.	n	Mp, (°C)	Yield (%)	Molecular Formula	Analysis % Calcd./Found C	H	N
9a	2	189-192	90	$C_{21}H_{25}N_7O_6$	53.49	5.34	20.80
					53.37	5.37	20.59
9b	3	157-158	44	$C_{22}H_{27}N_7O_6$	54.42	5.61	20.20
					54.56	5.65	20.02
9c	4	159-161	76	$C_{23}H_{29}N_7O_6$	55.30	5.85	19.63
					55.09	5.92	19.48
9d	5	149-151	52	$C_{24}H_{31}N_7O_6$	56.13	6.09	19.09
					56.07	6.11	18.96
9e	6	151-154	77	$C_{25}H_{33}N_7O_6$	56.91	6.30	18.59
					56.75	6.29	18.50
9f	7	143-144	18	$C_{26}H_{35}N_7O_6$	57.66	6.51	18.11
					57.70	6.55	17.96

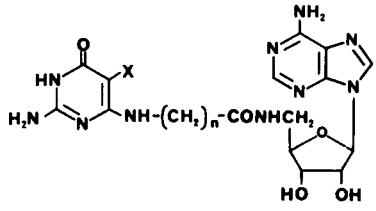
rivatives **7**, and were then converted to the *N*-hydroxysuccinimide esters **8** with dicyclohexylcarbodiimide/*N*-hydroxysuccinimide in dioxane. The active esters **8** were coupled in aqueous dioxane with 5′-amino-5′-deoxyadenosine [4] to provide the functionalized nucleosides **9**, which were reductively deprotected (Pd/C, aqueous ethanol) to afford the primary amines **10**. The amines were condensed either with 6-methylthio-5-nitrosoisocytosine [3] to provide the 5-nitroso series **11**, or with 6-chloro-5-nitroisocytosine [5] to provide the 5-nitro series **12**. In antibacterial evaluation, none of the bridged compounds had significant antibacterial activity when tested *in vitro* against twenty-two bacterial strains at 30 μg/ml [6].

EXPERIMENTAL

Melting points were determined with a Buchi melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. The ^{1}H-nmr spectra (Varian T-60, XL-100, and CFT20 and Hitachi Perkin-Elmer R-24B spectrometers) obtained for all target compounds and intermediates were consistent with the assigned structures. All amino acids **6** were obtained commercially. Individual

Table II

Physical Data for the Isocytosine-Adenosine Bridged Compounds **11** and **12**



No.	X	n	Mp, (°C)	Yield (%)	Molecular Formula	Analysis % Calcd./Found C	H	N
11a	NO	2	>240	72	$C_{17}H_{21}N_{11}O_6$	42.94	4.45	32.41
						42.66	4.44	32.19
11b	NO	3	>230	57	$C_{18}H_{23}N_{11}O_6$	44.17	4.74	31.48
						43.93	4.81	31.38
11c	NO	4	>230	76	$C_{19}H_{25}N_{11}O_6 \cdot .3\ H_2O$	44.84	5.07	30.28
						44.85	4.78	30.53
11d	NO	5	>225	72	$C_{20}H_{27}N_{11}O_6$	46.41	5.26	29.78
						46.33	5.29	29.70
11e	NO	6	227-228 dec	53	$C_{21}H_{29}N_{11}O_6$	47.45	5.50	28.99
						47.21	5.62	28.82
11f	NO	7	217-218 dec	74	$C_{22}H_{31}N_{11}O_6 \cdot 0.5\ H_2O$	47.64	5.82	27.79
						47.34	5.69	27.59
12a	NO_2	2	206 dec	61	$C_{17}H_{21}N_{11}O_7 \cdot 0.5\ H_2O$	40.80	4.43	30.79
						40.57	4.48	30.66
12b	NO_2	3	196-208 dec	39	$C_{18}H_{23}N_{11}O_7 \cdot 0.5\ H_2O$	42.02	4.70	29.95
						41.80	4.82	29.73
12c	NO_2	4	250-251 dec	50	$C_{19}H_{25}N_{11}O_7 \cdot 0.5\ H_2O$	43.17	4.95	29.15
						42.86	4.92	29.08
12d	NO_2	5	210 dec	45	$C_{20}H_{27}N_{11}O_7$	45.02	5.10	28.88
						44.76	5.19	28.65
12e	NO_2	6	240-242 dec	53	$C_{21}H_{29}N_{11}O_7$	46.06	5.34	28.14
						46.32	5.41	27.98
12f	NO_2	7	195 dec	47	$C_{22}H_{31}N_{11}O_7 \cdot 0.5\ H_2O$	46.31	5.65	27.01
						46.29	5.59	26.85

synthetic steps (Scheme I) were carried out in similar fashion for each member of the homologous polymethylene series. The general methods are described below and significant deviations are noted therein or in Tables I and II.

Methods for Conversion of ω-Amino Acids **6** to the Bridged Isocytosine-Adenosine Compounds **11** and **12**, as Outlined in Scheme I.

The *N*-benyloxycarbonyl derivatives **7** (Table I) were prepared by dropwise addition of benzyl chloroformate (1 equivalent) to an ice cold solution of the ω-amino acid **6** (1 equivalent) in aqueous sodium hydroxide (2 equivalent). Typically, the mixture was stirred overnight, acidified to *ca.* *p*H 3 with aqueous hydrochloric acid, and the product **7** was isolated by filtration and/or by ether extraction of the acidified aqueous solution. The protected amino acids **7** have been previously reported in the literature (n = 2 [7]; n = 3 [8]; n = 4 [9]; n = 5 [10]; n = 6 [11]; n = 7 [10]).

Active esters **8** were obtained by addition of dicyclohexylcarbodiimide (1 equivalent), either as a solid or dissolved in dioxane, to an ice cold solution of **7** (1 equivalent) and *N*-hydroxysuccinimide (1 equivalent) in dioxane. After stirring overnight, the mixture was filtered and the filtrate was concentrated to dryness under reduced pressure. The residue was dissolved in ether-benzene, and the resulting solution was filtered, washed with aqueous sodium bicarbonate, dried (magnesium sulfate), and concentrated under reduced pressure. Active esters **8** were then coupled with 5′-amino-5′-deoxyadenosine without further purification.

A dioxane solution of the appropriate active ester **8** was added to an aqueous solution of 5′-amino-5′-deoxyadenosine [4], and additional water or dioxane was added if necessary to provide a homogeneous reaction mixture. The solution was stirred for a period of 1-4 days and the solvents were then removed under reduced pressure. The residue was recrystallized from water (**9a**, **9c**, **9d**) or from aqueous ethanol (**9b**, **9e**, **9f**) to provide the analytically pure nucleoside derivatives **9** (Table I).

Nucleosides **9** were converted to primary amines **10** by catalytic hydrogenation (5% Pd/C, 4-6 hours at *ca.* 30-40 psi) in aqueous ethanol (1:1). The catalyst was removed by filtration through Celite, and the filtrate was concentrated under reduced pressure. Complete deprotection was verified by *tlc* and by nmr (absence of the benzyloxy methylene), and the amines were immediately condensed with the appropriate isocytosine as described below.

The 5-nitroso analogues **11** were obtained by adding 6-methylthio-5-nitrosoisocytosine [3] (1 equivalent) in one portion to a refluxing solution of the appropriate amine **10** (1 equivalent) in either ethanol (**10e**, **10f**), 30% aqueous ethanol (**10a**, **10d**) or 17% aqueous ethanol (**10b**, **10c**). After a reflux period of *ca.* 4 hours (for **10a-10c** and **10e**) or 24 hours (for **10d** and **10f**), the mixture was cooled and the product was collected by filtration and either recrystallized from water (**11b**) or triturated with ethanol (**11c**) or with hot water (**11a**, **11d-f**) to provide analytically pure material (Table II).

The 5-nitro analogues **17** were obtained by adding 6-chloro-5-nitroisocytosine [5] (1 equivalent) in one porton to a refluxing solution of the appropriate amine **10** (1 equivalent) and triethylamine (1 equivalent) in absolute ethanol. After a reflux period of 1-3 hours, the mixture was cooled and the crude product was collected by filtration and then triturated with hot water (**12c-12e**), or recrystallized from water (**12a**, **12b**) or from ethanol (**12f**) to provide analytically pure material (Table II).

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