An Application of the Mannich Reaction Using Hydroxylamine and Some of its Derivatives. 6-Substituted-2,4-diamino-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidines (1)

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Hydroxylamine and some of its *O*-substituted derivatives (**2**) have been used as the amine component in Mannich reactions with 2,4,6-triaminopyrimidine (**1**). The resulting 6-substituted tetrahydropyrimido[4,5-*d*]-pyrimidines (**3**) contain an N-O bond linking the substituent to the ring. These results extend the utility of this modified Mannich reaction to otherwise inaccessible substituents. Reaction conditions, spectral data and certain limitations of the reaction are discussed.

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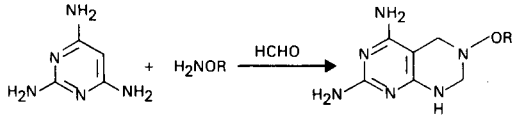
A recent review of the Mannich reaction has detailed many applications of this useful synthetic tool (2). Although the use of a wide variety of substrates and amines is described very little work appears to have been carried out either with hydroxylamine or *O*-substituted hydroxylamines. Irikura and Kasuga reported the use of hydroxylamine derivatives in a Mannich reaction with paraformaldehyde and substituted acetophenones to provide the corresponding hydroxylaminoethylphenyl ketones (3). In their work the substituent on oxygen was either a benzyl group or a short chain alkyl group. In a few cases a second substituent was located on nitrogen. Yu and coworkers describe a similar example in which hydroxylamine participates in a Mannich reaction on the active methyl group of a complex acetate ester (4).

Despite the demonstrated participation of hydroxylamine and some of its derivatives in the Mannich reaction the lack of many other examples may be related to two differences between hydroxylamines and alkyl amines. Hydroxylamines are weaker bases than amines (5) and both the N-O and C-O bonds are more labile than the N-C bond.

SCHEME I



1 2 3

R	Compound
H	a
4-Cl-C_6H_4	b
4-Cl-$C_6H_4CH_2$	c
2,4-Cl_2-$C_6H_3CH_2$	d
2,6-Cl_2-$C_6H_3CH_2$	e
3-CF_3-$C_6H_4CH_2$	f
2,4-Cl_2-$C_6H_3CH_2CH_2$	g
2,4-Cl_2-C_6H_3-OCH_2CH_2	h

In connection with our earlier work in which benzyl and phenethyl amines were incorporated into partially reduced pyrimido[4,5-*d*]pyrimidines (6) we explored the reaction of hydroxylamine, 4-chlorophenoxyamine, several *O*-benzylhydroxylamines and two *O*-ethylhydroxylamine derivatives with 2,4,6-triaminopyrimidine.

Initially we viewed 2,4-diamino-6-hydroxy-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidine, **3a**, as a useful intermediate in the preparation of a wide variety of N-O substituted pyrimido[4,5-*d*]pyrimidines. Early attempts to prepare this compound by altering the *p*H of the medium, ratio of reactants and the order of addition while maintaining the reaction mixture under reflux led to complex mixtures. These results are in contrast to our earlier work (6) where order of addition was important to obtain clean reaction products. The hplc analysis of these mixtures indicated as many as eleven products under such conditions. To be sure, however, a significant number of these byproducts arose solely from various reactions between formaldehyde and hydroxylamine (7).

The best yield of **3a** was obtained when the three components were allowed to stir at room temperature for one week. Thus, **1**, **2a**·HCl, and formaldehyde in a ratio of 1:2:3 in ethanol provided a 70% yield of **3a**·HCl. We were able to follow the formation of **3a** by means of hplc which clearly showed the presence of an intermediate substance, which we have not yet identified. Very careful neutralization of this salt with aqueous base was required to obtain **3a** as the free base. The product was characterized by means of ^{1}H pmr and elemental analysis (Table 1). The free base decomposes rapidly in even slightly alkaline solution. While we have not examined the products of this decomposition, ring opening of pyrimido[4,5-*d*]pyrimidines by nucleophiles has been reported (8).

Table I

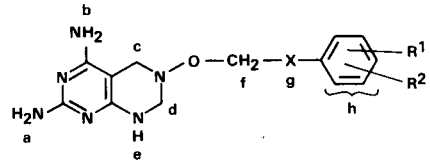
Physical and Chemical Data for **3**

Compound	R	Yield, %	Crystallization Solvent	Mp °C	Empirical Formula	Calculated Mole Weight	Analysis Calcd./Found C	H	N
3a	H	70	—	>219 dec	$C_6H_{10}N_6O$	182.1	39.55	5.53	46.14
							39.44	5.50	46.21
3c	4-Cl-C_6H_4-CH_2-O-	56	Methanol	205-206.5	$C_{13}H_{15}N_6ClO$	306.8 (a)	50.90	4.89	27.40
							51.02	5.08	27.41
3d	2,4-Cl_2-C_6H_3-CH_2-O-	43	Ethanol	208-209.5	$C_{13}H_{14}N_6Cl_2O$	341.2 (a)	45.75	4.11	24.63
							45.76	4.09	24.67
3e	2,6-Cl_2-C_6H_3-CH_2-O-	42	Methanol	230-231.5	$C_{13}H_{14}N_6Cl_2O$	341.2 (a)	45.75	4.11	24.63
							45.64	4.14	24.58
3f	3-CF_3-C_6H_4-CH_2-O-	37	Chloroform-methanol	200-202	$C_{14}H_{15}N_6F_3O$	340.3 (a)	49.41	4.41	24.70
							49.64	4.61	24.78
3g	4-Cl-C_6H_4-CH_2CH_2-O-	62	Methanol-water	187-189	$C_{14}H_{17}N_6ClO$	320.5 (a)	52.46	5.30	26.21
							52.48	5.31	26.25
3h	2,4-Cl_2-C_6H_3-O-CH_2CH_2-O-	22	Methanol	180-182.5	$C_{14}H_{16}N_6Cl_2O_2$	371.0 (a)	45.28	4.31	22.64
							45.04	4.55	22.13

(a) Chemical Ionization mass spectra data are consistent with the molecular weights shown.

Table II

NMR Data for **3**



	a(a)	b(a)	c(a)	d(a)	e(b)	f	g	h	i(b,c)
3a	5.27	5.52	3.60	4.00	6.27	—	—	—	8.13
3c	5.25	5.55	3.75	4.15	6.25	4.68	—	7.4	—
3d	5.20	5.50	3.75	4.16	6.30	4.75	—	7.3-7.6	—
3e	5.30	5.58	3.80	4.20	6.30	4.95	—	7.42	—
3f	5.35	5.60	3.75	4.18	6.35	4.80	—	7.6	—
3g	5.30	5.56	3.77	4.17	6.33	3.87	2.77	7.30	—
3h	5.26	5.53	3.73	(d)	6.26	(d)	—	7.03-7.50	—

(a) All values are singlets. (b) All values are broad singlets. (c) Represents H in place of grouping **f-h**. (d) The methylene groups at position 7 and in the substituent at position 6 are indistinguishable and are found at 4.97-5.17 ppm.

Because of the facile decomposition of **3a** in basic media we abandoned our goal of using this substance as an intermediate in the formation of other derivatives. However, in an effort to extend the use of hydroxylamine derivatives in this reaction, we considered next *O*-phenylhydroxylamine derivatives. A survey of the literature indicated that there was no general method for the synthesis of these compounds. Isolated examples were reported for a few compounds which either were in error or proceeded in poor yield. We chose as a model 4-chlorophenoxyamine (**2b**) which we obtained in 36% yield following the method of Cadogan and Rowley (9).

Upon treatment of **1** with **2b** under a variety of conditions, the cleavage product 4-chlorophenol, was the major isolable compound. Since it was not clear from the product mixture at which stage the cleavage of the N-O bond occurred **2b** was treated separately with acid and with base in refluxing ethanol in the absence of **1**. In both circumstances 4-chlorophenol was obtained as the major product. This observation dissuaded us from using *O*-arylhydroxylamines as reasonable reactants in these studies.

More encouraging results were obtained with *O*-benzylhydroxylamine derivatives (**2c-f**). Treatment of **1** with **2c-f** and either formalin or polyoxymethylene under acid-catalyzed conditions provided moderate yields of the corresponding 6-substituted benzyloxy-2,4-diamino-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidines (**3c-f**) (See Table 1). The formation of an insoluble product thought to be the bismethylene-2,4,6-triaminopyrimidine (6, ref 12 cited therein) and some **3a**, which could only have arisen from

cleavage of the O-C bond, served to lower the yields of the desired products. We did not attempt to ascertain the stage at which the O-C cleavage occurred. The required *O*-benzylhydroxylamines (**2c-f**) were usually prepared from the corresponding benzyl halides and *N*-hydroxyphthalimide, followed by hydrazinolysis.

As a further extension of the scope of hydroxylamine derivatives, two other compounds were employed. The addition of a second methylene unit between the aromatic ring and the oxygen is illustrated with **2g**. Reaction of **1** with **2g** and formaldehyde was carried out successfully to provide **3g** in 62% yield. When an additional oxygen was interposed between the aryl group and the alkyl chain a poor yield of **3h** was obtained.

The generally lower yields obtained with hydroxylamine derivatives compared with benzyl and phenethylamines (6), coupled with the cleavage associated with 4-chlorophenoxyamine, suggests that the lower basicity and more facile cleavage mentioned at the outset may limit the usefulness of this class of amines. In all the experiments reported here significant quantities of the bis-methylene-2,4,6-triaminopyrimidine were isolated. In view of these results and the anticipated difficulty in the synthesis of more complex *O*-substituted hydroxylamines, we did not pursue extension of this series.

Compounds **3c-f,h** have been screened for antimalarial activity (10) and **3c-e,h** have been screened for antitrypanosomal activity (11). None of the compounds exhibited any inhibitory activity in either test system.

EXPERIMENTAL

Melting points are uncorrected. The ^{1}H nmr spectra were recorded in DMSO-d_6 on a Varian T-60 spectrometer with TMS as internal standard. Mass spectra were performed by the Walter Reed Army Institute of Research. The benzylhydroxylamines are known and were prepared according to a known general procedure (12).

6-Hydroxy-2,4-diamino-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidine (**3a**).

In a 100 ml rb flask fitted with a magnetic stirrer was placed 2,4,6-triaminopyrimidine (1.25 g, 0.01 mole), hydroxylamine hydrochloride (1.39 g, 0.02 mole), paraformaldehyde (0.90 g, 0.03 mole) and 95% ethanol (70 ml). The mixture was stirred at room temperature for one week. The precipitated white solid was collected and determined to be largely a hydrochloride of **3a** by ^{1}H nmr and hplc. This product was dissolved in water and the solution carefully made alkaline with dilute sodium hydroxide. The free base of **3a** was filtered and dried, mp >219° dec.

General Method for the Preparation of 6-Aralkoxy-2,4-diamino-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidines (**3c-h**).

A mixture of two equivalents of the appropriate hydroxylamine hydrochloride derivative and two equivalents of polyoxymethylene was refluxed in 95% ethanol while stirring for 5 hours. The oxime solution thus obtained was treated with a solution of one equivalent of 2,4,6-triaminopyrimidine in ethanol. After refluxing and stirring for 2 hours, a white precipitate separated from the reaction medium. Refluxing and stirring was continued for 26 hours. The cold (room temperature) reaction mixture was made alkaline by stirring with sodium hydroxide pellets at room temperature and the insoluble precipitate was filtered. This included the methylene bis-2,4,6-triaminopyrimidine. The ethanol filtrate was evaporated under reduced pressure and the residue obtained was washed with ether then purified either directly by several crystallizations or by chromatography on a silica gel column (chloroform-methanol, 9:1) followed by crystallization from an appropriate solvent (see Table 1) to give the title compounds.

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