

Synthesis of Bis(imides) and Bis(half amides) of *NN'*-Ethylenebis(iminodiacetic Acid)

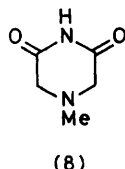
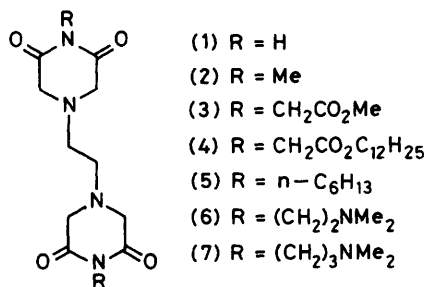
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NN'-Disubstituted derivatives of the bis-(imide) of *NN'*-ethylenebis(iminodiacetic acid) were prepared by alkylation of the di-sodium derivative of the bis(imide). The hydrolysis and methanolysis of the bis(imide) and some of its *NN'*-disubstituted derivatives were promoted by copper(II) and afforded copper complexes from which the expected bis(half amides) of *NN'*-ethylenebis(iminodiacetic acid) were obtained by the action of hydrogen sulphide.

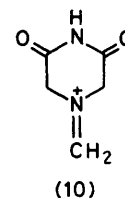
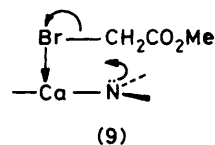
FOLLOWING the observation by Furst¹ that most of the useful anti-tumour drugs are actual or potential chelating agents and the suggestion² that *NN'*-ethylenebis(iminodiacetic acid) is ineffective as an anti-tumour agent because its high polarity prevents penetration of cell walls, Creighton *et al.*³ examined derivatives of this acid which have low polarity and which afford the acid on hydrolysis. This work led to the discovery of the anti-tumour properties of a group of bis(2,6-dioxopiperazines) of which the bis(imide) (1) is the parent member. During this work it was observed also that the hydrolysis of the bis(imide) (1) is strongly promoted by copper(II) acetate.⁴ In this paper we describe the preparation of some *NN'*-disubstituted derivatives of the bis(imide) (1) and the use of copper(II) for converting some of these derivatives and also the bis(imide) (1) into bis(half amides) of *NN'*-ethylenebis(iminodiacetic acid) under relatively mild conditions.

The bis(imides) (1) and (2) were prepared from *NN'*-ethylenebis(iminodiacetic acid) by the action of formamide⁴ and methylformamide⁵ respectively, and the

Initial attempts to *NN'*-dialkylate the bis(imide) (1) with alkyl halides using the conditions normally used in the Gabriel reaction (potassium carbonate in hot dimethylformamide) gave very low yields of the expected products, largely because of the insolubility of the starting imide. Even with the reactive halide, methyl bromoacetate, only a 10% yield of the diester (3) was obtained after a reaction time of 20 h. Substantially higher yields were obtained, however, by initially converting the imide into its di-sodium derivative by the action of sodium methoxide in methanol, and then treating this derivative with the alkyl halide in dimethylformamide at 60–85 °C. This route was used to prepare the *NN'*-disubstituted imides (2)–(7) (see Table 1) of which the first was identical (i.r., ¹H n.m.r.) with the sample prepared from *NN'*-ethylenebis(iminodiacetic acid) and methylformamide. The bis(imides) (3) and (5) were also prepared by the reaction between the appropriate alkyl bromide and the calcium derivative of the bis(imide) (1). Interestingly, in spite of the higher electronegativity of the metal involved, both these reactions proceeded at a much faster rate and gave higher yields of product than the corresponding reactions with the di-sodium derivative. It is possible that the higher rates of reaction were due to the alkylations of the calcium derivative proceeding partly by a relatively fast intramolecular route⁷ [see partial structure (9)] which was not available to the sodium systems. The mass spectra of all the bis(imides) (1)–(7) showed a peak corresponding to the molecular ion, but while the base peak in the spectra of (6) and (7) had *m/z* 58 (H₂C=ĊNMe₂), that in the spectra of (1)–(5) had *m/z* 127 and was assigned to the fragment (10).



mono-imide (8) was prepared similarly from *N*-methyliminodiacetic acid⁶ and formamide. This method for preparing the mono-imide was found to be far more successful for large-scale preparations (>20 g) than that described by Chase and Downes⁶ in which the imide sublimes from a fused mixture of *N*-methyliminodiacetic acid and urea.



The bis(imide) (1) was highly insoluble in water and, as expected, hydrolysis proceeded very slowly in this medium. When an aqueous suspension of the bis(imide) was heated under reflux for 8 h, 79% of the bis(imide) was recovered unchanged. Dissolution was complete after

24 h of heating, and removal of the solvent afforded *NN'*-ethylenebis(iminodiacetic acid) which contained no detectable amount (^1H n.m.r.) of the intermediate bis(half amide) (17). In contrast to the slow hydrolysis in water, when the bis(imide) was heated under reflux in aqueous copper(II) acetate a clear solution was obtained after only 5 min, and after a further 4 min the colour of this solution had changed from royal blue to dark blue. Removal of the solvent afforded a copper complex which gave analytical data consistent with a dihydrate of the

cause of ^1H - ^2H exchange⁹ when the solution was basified (pD 8.5) and heated at 60 °C for 45 min.

In aqueous copper(II) acetate the hydrolysis of the two substituted bis(imides) (2) and (3) and the mono-imide (8) also proceeded extremely rapidly to the half-amide stage, with the ester group of (3) remaining intact. Under reflux conditions all three hydrolyses were complete within 5 min and afforded copper complexes formulated as (14), (15), and (16) respectively. The parent half-amides were generated from these complexes by the

TABLE I
Preparation of imides of *NN'*-ethylenebis(iminodiacetic acid)

Compd.	Temp. (°C)	Time (h)	Yield (%)	M.p. (°C)	Found ^a				Formula	Required ^a			
					C	H	N	<i>M</i> ⁺		C	N	N	<i>M</i>
(2)	40	2	56 ^b	176–180 ^c									
(4)	60	4	61 ^b	95–96	64.5	9.9	7.8	282	$\text{C}_{38}\text{H}_{66}\text{N}_4\text{O}_8$	64.4	9.7	7.9	282
(5)	65	14	69 ^b	97–101	62.1	9.5	12.9	422.2888	$\text{C}_{22}\text{H}_{38}\text{N}_4\text{O}_4$	62.5	9.1	13.3	422.2893
(6)	85	14	20 ^d	93–96	54.7	8.0	20.9	396.2484	$\text{C}_{18}\text{H}_{32}\text{N}_6\text{O}_4$	54.5	8.1	21.2	396.2485
(7)	85	12	40 ^d	91–94	56.5	9.0	19.9	424.2790	$\text{C}_{20}\text{H}_{36}\text{N}_6\text{O}_4$	56.6	8.55	19.8	424.2798

^a C, H, and N values expressed as percentages.

^b Using the alkyl bromide as the alkylating agent.

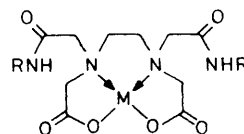
^d Using the alkyl chloride as the alkylating agent.

bis(half amide) complex (11) and which gave the bis(half amide) (17) when the copper was removed by the action of hydrogen sulphide. Esterification of this bis(half amide) with methanol gave the corresponding bis(half ester) (20) which was identical (i.r., ^1H n.m.r.) with the sample described below.

The amide groups in the copper complex (11) were less susceptible to hydrolysis than those in the parent bis(half amide) (17), and while the latter compound was completely hydrolysed to *NN'*-ethylenebis(iminodiacetic acid) when heated for 8 h in water under reflux, the copper complex (and also the zinc one described below) was recovered unchanged when subjected to this treatment. Presumably, the carboxylic acid groups in the bis(half amide) (17) were able to promote the hydrolysis of the amide groups by participating in the reaction (*cf.* the hydrolysis of phthalamic acid⁹).

The divalent cations of cobalt, nickel, and zinc also promoted the hydrolysis of the bis(imide) to the bis(half amide) stage, but were less effective than that of copper. On the basis of the weights of unchanged bis(imide) recovered by filtration after reaction times of 8 h, the efficiencies of these cations decreased in the order Ni^{2+} , Co^{2+} , Zn^{2+} . With the cobalt system the hydrolysis product was a violet gum which could not be induced to crystallise, but the nickel and zinc systems afforded crystalline complexes which on the basis of analytical data were formulated as a dihydrate of compound (12) and a tetrahydrate of compound (13) respectively. The i.r. spectra of these crystalline complexes were almost identical in the 'carbonyl stretching' region with that of the copper complex, and showed strong absorption bands at 1 680, 1 645, and 1 590 cm^{-1} . The ^1H n.m.r. spectrum of the zinc complex in D_2O showed a broad signal centred on δ 2.93 due to the protons of the $-\text{CH}_2\text{CH}_2-$ group, and signals at δ 3.44 and 3.67. The last signal was assigned to the $-\text{CH}_2\text{CO}_2-$ groups, as the signal disappeared be-

cause of hydrogen sulphide, but in all three systems the half-amide was a hygroscopic gum which resisted crystallisation and whose ^1H n.m.r. spectrum [like that of (17)] indicated that in D_2O the α -amino-acid moiety was present as a zwitterion. For example, while the protons of the CH_2CH_2 and CH_2CO groups in the bis(imides) (1)–(3) gave rise to signals in the ranges δ 2.60–2.75 and 3.35–3.75, respectively [the former signal was also present in the spectra of the two methyl esters (20) and (21)], the corresponding protons in the three bis(half amides) obtained from the bis(imides) by hydrolysis gave no signal lower than δ 3.3.



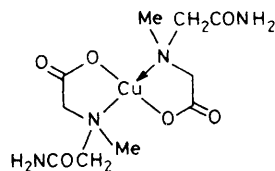
(11) *M* = Cu, *R* = H

(12) *M* = Ni, *R* = H

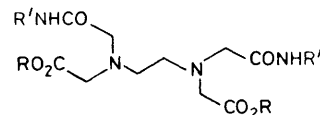
(13) *M* = Zn, *R* = H

(14) *M* = Cu, *R* = Me

(15) *M* = Cu, *R* = $\text{CH}_2\text{CO}_2\text{Me}$



(16)



(17) *R* = H, *R*¹ = H

(18) *R* = H, *R*¹ = Me

(19) *R* = H, *R*¹ = $\text{CH}_2\text{CO}_2\text{Me}$

(20) *R* = Me, *R*¹ = H

(21) *R* = Me, *R*¹ = Me

The bis(imide) (1) was highly insoluble in methanol and was recovered unchanged after being heated under reflux in this solvent for 48 h, but this was also the case with the *NN'*-dimethyl derivative (2) which was soluble in methanol. Ring-opened products were obtained, however, when methanolysis was carried out in the

presence of copper(II) chloride. Thus, when heated with the chloride (2 equivalents) in methanol, the bis(imide) (1) gave a copper complex from which the bis(half ester-half primary amide) (20) was isolated by the initial action of hydrogen sulphide followed by treatment of the resultant hydrochloride with ammonia in chloroform. The bis(half ester-half secondary amide) (21) was obtained in a similar manner. The mass spectra of the bis(half ester) (20) and the parent bis(half acid) (17) showed no molecular ion and were almost identical with that of the bis(imide) (1). Presumably, this was due to ring-closure occurring before fragmentation in the spectrometer.

EXPERIMENTAL

I.r. spectra were recorded in Nujol on a Pye Unicam SP 200 spectrometer. ^1H N.m.r. spectra were obtained on a Varian R14 (100 MHz) spectrometer, using tetramethylsilane as internal standard. Mass spectra were recorded with A.E.I. MS9 or Varian CH 5D instruments.

1-Methylpiperazine-3,5-dione (8).—A mixture of *N*-methyliminodiacetic acid ⁶ (20.6 g, 0.14 mol) and formamide

Other *NN'*-disubstituted imides prepared by alkylation of the di-sodium derivative of the imide (1) are listed in Table 1.

(b) The imide (1) (5.04 g) was stirred and heated under reflux for 20 h with calcium methoxide [from calcium (0.80 g) in dry methanol (120 ml)] and the resultant calcium salt (5.8 g) was filtered off, washed with dry methanol, dried *in vacuo*, and suspended in dry dimethylformamide (130 ml) at 60 °C. Methyl bromoacetate (6.12 g) was added during 5 min to the stirred suspension and the mixture was stirred and heated for a further 25 min. Isolation of the organic product as described above gave the di-ester (6.2 g, 78%) which was identical (i.r., n.m.r., m.p. and mixed m.p.) with the sample described above.

A similar alkylation of the calcium salt (2.92 g) of the imide (1) in dimethylformamide (40 ml) at 60 °C with 1-bromohexane (3.30 g) gave the imide (5) (74%) after a reaction time of 45 min (*cf.* Table 1).

{*NN'*-Ethylenebis[N-(aminocarbonylmethyl)glycinate]}-copper(II) (11).—The imide (1) (2.54 g) was stirred and heated under reflux for 10 min with copper(II) acetate (2.00 g) in water (60 ml). The mixture was filtered and the filtrate was concentrated to give the hydrated complex (2.50 g, 80%) as royal blue crystals, m.p. 216–217 °C (Found:

TABLE 2
Preparation of the metal complexes (11)–(15)

Compd.	Reaction time	Yield (%)	M.p. (°C)	Found (%)			Formula	Required (%)		
				C	H	N		C	H	N
(12)	4 h	82	>295	30.9	5.5	14.5	$\text{C}_{10}\text{H}_{16}\text{N}_4\text{NiO}_6 \cdot 2\text{H}_2\text{O}$	31.25	5.25	14.6
(13)	8 h	81	>295	27.5	5.2	13.3	$\text{C}_{10}\text{H}_{16}\text{N}_4\text{O}_6\text{Zn} \cdot 4\text{H}_2\text{O}$	28.15	5.7	13.1
(14)	5 min	72	267–268	38.0	6.0	15.0	$\text{C}_{12}\text{H}_{22}\text{CuN}_4\text{O}_8$	38.0	5.3	14.8
(15)	5 min	93	258–263	36.1	5.0	10.6	$\text{C}_{16}\text{H}_{26}\text{CuN}_4\text{O}_{10} \cdot 2\text{H}_2\text{O}$	36.1	5.3	10.5
(16)	5 min	80	>295	30.6	6.2	14.45	$\text{C}_{10}\text{H}_{22}\text{CuN}_4\text{O}_8$	30.8	5.7	14.4

(150 ml) under a pressure of 100 mmHg was heated at 120 °C for 1.25 h and then at 150 °C for 3.75 h. The excess of formamide was removed under reduced pressure, and the residue was crystallised from propanol to give the imide (11.6 g, 65%), m.p. 102–103 °C (lit.,⁶ 103–104 °C); ν_{max} 1 700 and 1 697 cm^{-1} ; $\delta(\text{CDCl}_3)$ 2.45 (3 H, s) and 3.55 (4 H, s).

Similarly prepared were the imides (1) [78% yield from *NN'*-ethylenebis(iminodiacetic acid) and formamide], m.p. 296–299 °C (lit.,⁴ 297–300 °C), $\delta[(\text{CD}_3)_2\text{SO}]$ 11.0 (2 H, s, 2 \times NH), and (2) [83% yield from *NN'*-ethylenebis(iminodiacetic acid) and methylformamide], m.p. 182–184 °C (lit.,⁵ 181–184 °C), $\delta(\text{CDCl}_3)$ 3.15 (6 H, s, 2 \times NCH_3).

Dimethyl 4,4'-Ethylenebis[(2,6-dioxopiperazin-1-yl)acetate] (3).—(a) The imide (1) (7.62 g), sodium methoxide (from sodium, 1.38 g), and dry methanol (75 ml) were stirred and heated under reflux for 1.5 h after which the solvent was removed under reduced pressure to leave the di-sodium derivative (8.9 g) of the starting imide as a white solid. This solid was suspended in dry dimethylformamide at 60 °C and methyl bromoacetate (9.2 g) was added dropwise with stirring over 1 h. The mixture was stirred and heated at 60 °C for a further 2 h after which the solvent was removed under reduced pressure. The organic material was extracted (chloroform) and crystallised from dichloromethane to give the di-ester (4.91 g, 41%), m.p. 145–147 °C; ν_{max} 1 765, 1 740, and 1 690 cm^{-1} ; $\delta(\text{CDCl}_3)$ 4.50 (4 H, s, 2 \times $\text{CH}_2\text{CO}_2\text{Me}$) and 3.75 (6 H, s, 2 \times OCH_3) (Found: C, 48.3; H, 5.8; N, 14.0. $\text{C}_{16}\text{H}_{22}\text{N}_4\text{O}_8$ requires C, 48.2; H, 5.6; N, 14.1%).

C, 30.9; H, 5.3; N, 14.5. $\text{C}_{10}\text{H}_{16}\text{CuN}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$ requires C, 31.0; H, 5.2; N, 14.4%).

Other complexes prepared in a similar manner are listed in Table 2.

NN'-Ethylenebis[N-(aminocarbonylmethyl)glycine] (17).—The preceding complex (0.77 g) in water (25 ml) was treated with hydrogen sulphide and the resultant precipitate was filtered off. Concentration of the filtrate afforded the bis-(amide) (0.33 g, 57%), m.p. 206–207 °C; $\delta(\text{D}_2\text{O})$ 3.9 (4 H, s), 3.75 (4 H, s), and 3.35 (4 H, s, $\text{CH}_2\text{CH}_2\text{ND}$) (Found: C, 41.35; H, 6.2; N, 18.8. $\text{C}_{10}\text{H}_{18}\text{N}_4\text{O}_6$ requires C, 41.4; H, 6.25; N, 19.3%).

The following bis(amides), which were obtained in a similar manner in yields of 71–98%, afforded (in D_2O) the n.m.r. data indicated (all signals occurred as singlets): (18), δ 3.8 (4H), 3.7 (4 H), 3.30 (4H, $\text{CH}_2\text{CH}_2\text{ND}$), and 2.8 (6 H, NDCH_3); (19), δ 4.1 (4 H, $\text{CH}_2\text{CO}_2\text{Me}$), 3.9 (4 H), 3.8 (4 H), 3.7 (6 H, CO_2CH_3), and 3.3 (4 H, $\text{CH}_2\text{CH}_2\text{ND}$); *N*-(aminocarbonylmethyl)-sarcosine, δ 4.15 (2 H), 3.95 (2 H), and 3.05 (3 H, NCH_3).

Dimethyl *NN'*-Ethylenebis[N-(aminocarbonylmethyl)glycinate] (20).—The bis(imide) (1) (0.46 g) was stirred and heated under reflux for 24 h with anhydrous copper(II) chloride (0.49 g) in dry methanol (12 ml) and then the mixture was filtered and the solvent was removed from the filtrate under reduced pressure. The residue was dissolved in water (25 ml) and the solution was treated with hydrogen sulphide. The mixture was filtered and the solvent was removed from the filtrate under reduced pressure to leave a

hydrochloride which showed δ (D₂O) 4.1 (4 H, s) 4.0 (4 H, s), 3.8 (6 H, s, CO₂CH₃), and 3.5 (4 H, s, CH₂CH₂⁺ND). Treatment of this hydrochloride with ammonia in chloroform in the usual manner¹⁰ gave the *bis(ester)* (0.51 g, 88%), m.p. 142–144 °C; ν_{\max} 1 735 and 1 645 cm⁻¹; δ (CDCl₃) 3.7 (6 H, s, CO₂CH₃), 3.4 (4 H, s), 3.3 (4 H, s), and 2.8 (4 H, s, CH₂CH₂) (Found: C, 44.6; H, 6.7; N, 17.8. C₁₂H₂₂N₄O₆ requires C, 45.3; H, 7.0; N, 17.6%).

The *bis(ester)* (21), m.p. 123–124 °C, was prepared in a similar manner (Found: *M*⁺, 346.1851. C₁₄H₂₆N₄O₆ requires *M*, 346.1852), ν_{\max} 1 740 and 1 665 cm⁻¹; δ (CDCl₃) 3.7 (6 H, s, 2 × CO₂CH₃), 3.4 (4 H, s), 3.25 (4 H, s), 2.85 (6 H, d, 2 × NHCH₃), and 2.75 (4 H, s, CH₂CH₂).

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