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# *N*-(Carboxymethyl)-*N*-[2-(2,6-diisopropylphenyl)amino]-2-oxoethylglycine and Analogues: Synthesis and Characterization

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*Abstract*

*N*-(Carboxymethyl)-*N*-[2-(2,6-diisopropylphenyl)amino]-2-oxoethylglycine (DIS-IDA)* (2a) and two analogues have been successfully synthesized. The synthesis involved a modified one-pot reaction with specific reaction conditions to maximize yields. A general procedure for the isolation of the products has been set out and the stability of compounds (2) is briefly discussed. Characterization is reported with a view for use with $^{99m}$Tc as radiopharmaceuticals.

## Introduction

The use of *N*-(carboxymethyl)-*N*-[2-(2,6-dimethylphenyl)amino]-2-oxoethylglycine (2; $R^1 = Me$, $R^2 = H$; HIDA*)[1] as a chelating agent for complexing $^{99m}$Tc has stimulated investigation of this type of structure for use in radiopharmaceuticals in recent years.[2–6] The clinical value of $^{99m}$Tc–HIDA in hepatobiliary imaging is severely limited by the degree of jaundice. Images lose resolution and visual interference results from the altered elimination kinetics in the presence of high levels of bilirubin. Several analogues have been shown to have superior kinetics, in particular $^{99m}$Tc–(2a)[7–9] with its high hepatic extraction efficiency and relatively short transit time.[10–12]

* Although Chemical Abstracts nomenclature is used in the paper the compounds described are more commonly known as derivatives of iminodiacetic acid (IDA) and denoted by acronyms based thereon.

[1] Callery, P. S., Faith, W. C., Loberg, M. D., Fields, A. T., Harvey, E. B., and Cooper, M. D., *J. Med. Chem.*, 1976, **19**, 962.
[2] Fonseca, C., Rosenthal, L., Greenberg, D., Mernandez, M., and Arzoumaman, A., *Clin. Nucl. Med.*, 1979, **4**, 135.
[3] Wistow, B. W., Subramanian, G., and Grossman, G. M., *Radiology*, 1978, **128**, 793.
[4] Tu, H. W., Chandler, R. P., Moon, W. H., and Howard, L. M., *IEEE Trans. Nucl. Sci.*, 1979, NS-26, 599.
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[7] Green, A., Rosenberg, N., and Sheahan, M., *J. Nucl. Med.*, 1980, **21**, P18.
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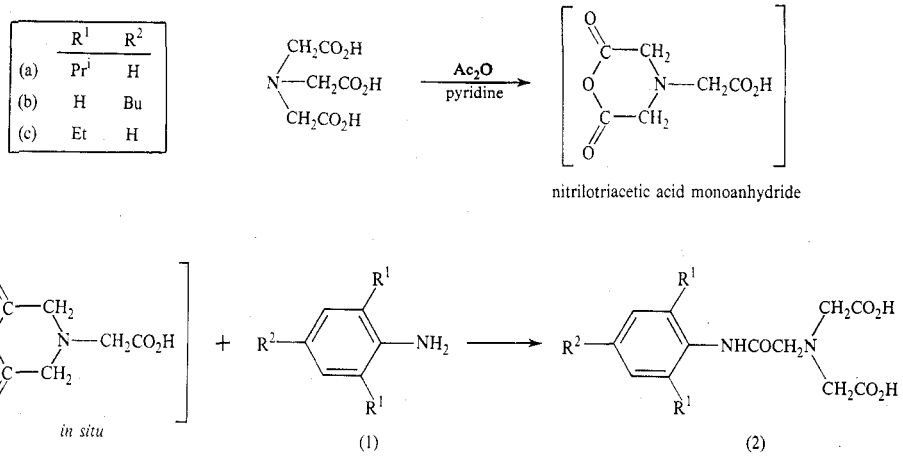
[9] Rosenthal, L., Division of Nuclear Medicine, The Montreal General Hospital, Montreal, Quebec, Canada, personal communication.
[10] Ronai, P. M., *J. Nucl. Med.*, 1977, **18**, 488.
[11] Wistow, B. W., Subramanian, G., Van Heertum, R. L., Henderson, R. W., Gagne, G. M., Hall, R. C., and McAfee, J. G., *J. Nucl. Med.*, 1977, **18**, 455.
[12] Pors Nielsen, S., Trap-Jensen, J., Linderberg, J., and Nielsen, J., *J. Nucl. Med.*, 1978, **19**, 452.

We report details of the synthesis and characterization for three derivatives (2) obtained by using a single-pot reaction, with good yields, according to Scheme 1.




Scheme 1

## Discussion

*Yields of Products in Reactions*

The method of Callery *et al.*[1] has been widely used to synthesize derivatives of (2). It is a two-step reaction and gives a low yield (about 20% for HIDA), but has the advantage of yielding a pure product which can be easily isolated from the reaction mixture after the unchanged starting materials have been extracted with ether.

**Table 1. Reaction conditions and yield of products (2)**

| Run | Compound | Temp. (°C) | Time (h) | Nitrilotriacetic acid/$Ac_2O$ | Yield (%) |
|---|---|---|---|---|---|
| 1 | (2a) | 70 | 4·5 | 1:1·1 | 55 |
| 2 | (2a) | 75 | 4 | 1:1 | 72 |
| 3 | (2b) | 100 | 1·5 | 1:1·2 | 78 |
| 4 | (2b) | 80 | 3 | 1:1 | 89 |
| 5 | (2c) | 80 | 2 | 1:1 | 83 |

A modified method of Burns *et al.*[13] has been used in the present work and it gives high yields but suffers from side product formation (acetanilides) giving rise to difficulties in the purification process. However, the side product formation may be reduced by decreasing the proportion of acetic anhydride ($Ac_2O$) used. Table 1 shows the reaction conditions and yields of products. Runs 1 and 3 were lower-yielding, and used a higher proportion of acetic anhydride than in runs 2 and 4 respectively. For the reaction temperature, we found that at 75° better yields of (2a) were obtained. Steric effects do not seem to affect the yield to any great extent as a high yield has been obtained for (2a) (run 2).

[13] Burns, H. D., Sowa, D. T., and Marzilli, L. G., *J. Pharm. Sci.*, 1978, **67**, 1434.

*Stability of Compounds* (2)

Stability of compounds (2) towards acid or base catalysed hydrolysis is important since products formed may be toxic. The amide bond might be expected to hydrolyse to form the corresponding aniline which is known to be highly toxic.[14] This is particularly relevant in the preparation of radiopharmaceuticals where pH adjustment for dissolution and stannous reduction of the technetium to be used for labelling is carried out. Hydrolysis products may also alter biodistribution and labelling efficiency.

No kinetic data for hydrolysis of compounds (2) are available but the structural similarity to lignocaine [2-(diethylamino)-*N*-(2,6-dimethylphenyl)acetamide], for which the resistance of the amide linkage to hydrolysis is striking,[15] may be noted. Steric hindrance of the amide bond towards hydrolysis by *ortho* methyl groups has been demonstrated to be responsible for this stability by Sekera *et al.*[16] By analogy the stability of compounds (2) towards hydrolysis might even be greater than in lignocaine for (2a) while (2b) would be less stable.

## Experimental

For general procedure see refs.[17-19]

*Starting materials*

Commercially available aniline derivatives (1a,b,c), recently purchased, were used without further treatment. Acetic anhydride was distilled before use, anhydrous pyridine was obtained by distillation of the commercial grade after refluxing over sodium hydroxide pellets for 2 h and nitrilotriacetic acid was dried in a vacuum desiccator for 4 h over self-indicating silica gel.

*Thin-Layer Chromatography*

Activated silica gel G (Merck) (0·25 mm) was used on glass plates. The developing solvent was the organic phase separated from the mixture (butan-1-ol/acetic acid/water 4:1:5) after periodic shaking for 1 h. The developed plates were dried and spots were detected by spraying first with nickel sulfate (0·1%) in methanol followed by dimethylglyoxime (0·1%) in ethanol. The position of derivatives (2) was revealed by a white spot on a pink background. For other organic impurities the spray reagent was ceric sulfate (5%) in sulfuric acid (10%) and the plate was heated at 120° for 10 min following its application.

*Identification*

Infrared spectra were recorded for solids in potassium bromide discs and for liquids as films between sodium chloride windows; a Shimadzu IR-400 spectrometer was used. $^1$H n.m.r. spectra were recorded with a Varian T60 spectrometer with $SiMe_4$ as the internal standard and $(CD_3)_2SO$ as the solvent. Mass spectra were obtained on a Varian CH7 spectrometer. Melting points were determined with an Electrothermal melting-point apparatus 1A 6304 Mark II.

[14] Jacobson, K. H., *Toxicol. Appl. Pharmacol.*, 1972, **22**, 153.
[15] Bullock, K., and Grundy, J., *J. Pharm. Pharmacol.*, 1955, **7**, 755.
[16] Sekera, A., Sova, J., and Vrba, Č., *Experientia*, 1955, **11**, 275.
[17] McKeown, R. H., *J. Chem. Soc., Perkin Trans. 2*, 1980, 504.
[18] McKeown, R. H., and Prankerd, R. J., *J. Chem. Soc., Perkin Trans. 2*, 1981, 481.
[19] Wong Ooi, 'The Synthesis and Characterization of N-(2,6-Diisopropylphenylcarbamoylmethyl)-iminodiacetic acid and Analogues for Use in Hepatobiliary Imaging Agents' Rep. submitted to the Otago Medical Research Foundation, 1981.

*Isolation and Purification Procedure*

The products (2) formed may be easily isolated by acidifying the residue from the reaction with dilute hydrochloric acid until a pH of 3 is obtained. Further careful addition of hydrochloric acid precipitates most of the product. Where a coloured product is obtained it may be decolorized with activated charcoal by boiling in ethanol for 20 minutes, filtering the hot solution and removal of the solvent.

The general synthetic procedure is described in detail for (2a).

N-(*Carboxymethyl*)-N-[*2-(2,6-diisopropylphenyl)amino*]-*2-oxoethylglycine* (*2a*)

A three-necked round-bottomed flask (500 ml) was fitted with a thermometer, condenser, pressure-equalizing dropping funnel, magnetic follower and drying tubes containing silica gel at all outlets. The apparatus was flushed with a slow stream of dry nitrogen for 1 h. Nitrilotriacetic acid (25 g, 0·13 mol) was placed in the flask, anhydrous pyridine (200 ml) was slowly added from the dropping funnel and the suspension was heated and stirred at 50° for 15 min. Acetic anhydride (13·5 g, 0·13 mol) was then added dropwise and the temperature of the reaction mixture raised to 70°C for 2 h. The reaction mixture was then cooled to room temperature, (1a) (23·2 g, 0·13 mol) was added and the mixture was heated at 75°C for 4 h. The pyridine was removed by rotary evaporation at reduced pressure and the residue remaining dissolved in 250 ml of water and extracted with ether. The aqueous alkaline solution was cooled in an ice bath and adjusted to about pH 2, with dilute hydrochloric acid, whereupon a copious white precipitate formed which was filtered off. Further solid was recovered by reducing the volume of the filtrate and this was combined with that previously obtained to give 34 g of product (72% yield). The pure *product* (2a), m.p. 191–192·5°, was obtained after two recrystallizations from water (Found: C, 61·8; H, 7·5; N, 8·1. $C_{18}H_{26}N_2O_5$ requires C, 61·7; H, 7·5; N, 8·0%). $\nu_{max}$: 3450 (OH); 3250 (NH); 2975, 2945, 2875 (alkyl); 1690 (C=O); 1550 (C=C) $cm^{-1}$. $^1H$ n.m.r. $\delta$ [$(CD_3)_2SO$]: 1·10, d, *J* 7 Hz, $(CH_3)_2CH$; 3·05, sept, *J* 7 Hz, $(CH_3)_2CH$; 3·52, s, $COCH_2N$; 3·58, s, $NCH_2CO_2H$; 7·17, s, aromatic protons; 9·35, br s, NH ($D_2O$ exch.). *m/e* 350 (M).

N-(*Carboxymethyl*)-N-[*2-(4-butylphenyl)amino*]-*2-oxoethylglycine* (*2b*)

The following quantities of reagents were used: nitrilotriacetic acid (5 g, 0·026 mol), acetic anhydride (3·2 g, 0·031 mol), anhydrous pyridine (40 ml) and (1b) (3·9 g, 0·026 mol). After addition of the aniline derivative (1b), the reaction mixture was heated with stirring at 100° for 1·5 h and the usual workup followed to give (2b) (6·5 g, 78% yield). The pure *product* (2b), m.p. 206·5–207·5°, was obtained after two recrystallizations from ethanol/water (1:4) (Found: C, 59·6; H, 6·6; N, 8·8. $C_{16}H_{22}N_2O_5$ requires C, 59·6; H, 6·9; N, 8·7%). $\nu_{max}$: 3450 (OH); 3250, 3200 (NH); 2975, 2950, 2870 (alkyl); 1730, 1700 (C=O); 1620, 1565 (C=C) $cm^{-1}$. $^1H$ n.m.r. $\delta$ [$(CD_3)_2SO$]: 0·90, t, *J* 5 Hz, $CH_3CH_2CH_2CH_2$; 1·43, overlapping multiplets, $CH_3CH_2CH_2CH_2$; 2·52, t, 5 Hz, $CH_3CH_2CH_2CH_2$; 3·47, s, $COCH_2N$; 3·53, s, $NCH_2CO_2H$; 7·25, symmetry for *p*-substituted aromatic protons; 10·05, br s, NH ($D_2O$ exch.). *m/e* 322 (M).

N-(*4-Butylphenyl*)*acetamide* (*4-Butylacetanilide*)

The following quantities of reagents were used: (1b) (3 g, 0·020 mol), acetic anhydride (2·5 g, 0·025 mol) and anhydrous pyridine (20 ml). The reaction mixture was heated with stirring at 80° for 3·5 h and this was followed by the usual workup to give 4-butylacetanilide (3·2 g, 84%). Two recrystallizations from water afforded the pure product with m.p. 108–109° (lit.[20] 103°). The 4-butylacetanilide thus obtained was used in t.l.c. to identify the byproduct formed in the preparation of (2b).

N-(*Carboxymethyl*)-N-[*2-(2,6-diethylphenyl)amino*]-*2-oxoethylglycine* (*2c*)

The following quantities of reagents were used: nitrilotriacetic acid (5·0 g, 0·026 mol), acetic anhydride (2·7 g, 0·026 mol), anhydrous pyridine (40 ml) and (1c) (3 g, 0·026 mol). The reaction

[20] Itaya, M., Takai, Y., and Suzuki, K., *Yakugaku Zasshi*, 1961, **81**, 1629 (*Chem. Abstr.*, 1962, **56**, 10006c).

mixture was heated and stirred at 80° for 2 h and this was followed by the usual workup to give (2c) (7 g, 83%). Two recrystallizations from ethanol/water (1:4) afforded (2c) with m.p. 185·5–187° (dec.). A further two recrystallizations from water gave the pure *product*, m.p. 193·5–195° (dec.) (Found: C, 59·9; H, 7·1; N, 8·9. $C_{16}H_{22}N_2O_5$ requires C, 59·6; H, 6·9; N, 8·7%). $\nu_{max}$: 3450 (OH); 3335 (NH); 2985, 2960, 2900 (alkyl); 1710, 1670 (C=O); 1540 (C=C) $cm^{-1}$. $^1H$ n.m.r. $\delta$ [$(CD_3)_2SO$]: 1·10, t, *J* 7 Hz, $CH_3CH_2$; 2·50, q, *J* 7 Hz, $CH_3CH_2$; 3·50, s, $COCH_2N$; 3·57, s, $NCH_2CO_2H$; 7·03, aromatic protons; 9·35, br s, NH ($D_2O$ exch.). *m/e* 322 (M).

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