N,N'-bis(*S*-Benzoylmercaptoacetamido) Ethylenediamine and Propylenediamine Ligands as Renal Function Imaging Agents. I. Alternate Synthetic Methods

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A new method was developed to synthesize tetradentate ligands containing the *N,N'*-bis(*S*-benzoylmercaptoacetyl) ethylenediamine and propylenediamine moieties (DADS compounds). Methods are also represented with which to synthesize some of the positional isomers of the above compounds. These isomers represent a new class of compounds. A total of 21 different compounds were prepared. These will be used in an effort to establish a relationship between structure and renal imaging properties.

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Recently a number of tetradentate ligands (DADS compounds) of the general structure shown in Fig. 1 have been reported (*1–5*). These ligands, when complexed with Tc-99m, have been proposed as possible substitutes for I-131 *o*-iodohippuric acid (OIH) for renal function studies (*1–5*). The original published method of synthesis of these ligands starts with the corresponding substituted ethylene or propylene diamine and by a series of reactions converts them to DADS compounds (*1–3*).

We have developed a more convenient alternate synthetic procedure (Scheme I, Fig. 2) consisting of three steps. However, active ester 2 can be prepared in large quantity and stored, thus reducing the final synthetic effort to a simple one-step procedure. An additional advantage of this route is that the starting diamines (which in some cases are expensive or must be synthesized by multistep procedures whose overall yield is not always high) are exposed to only one synthetic step, thereby minimizing their losses. With this method in hand, we synthesized a variety of ligands in an effort to gain some insight as to the relationship of ligand and ligand-complex structure to renal imaging properties.

MATERIALS AND METHODS

General. All melting points were obtained and were uncorrected. Infrared spectra were determined on a spectrophotometer using the Nujol null method. HPLC analyses were performed using a chromatograph equipped with a Spheresorb S-5-ODS-2 C-18 reverse-phase column and a mobile phase consisting of 60% acetonitrile, with a flow rate of 1.4 ml/min. Peak detection was by UV set at 254 nm. Elemental analyses were performed commercially.* Unless otherwise stated, yields are expressed for dry, finished material. The diamines used to synthesize 17–19 and 24–32, were used as purchased, whereas those used to prepare 20–23 were synthesized by procedures described in the experimental section of this paper under compounds 11 to 16.

***S*-Benzoylthioglycolic acid (1).** Sodium hydroxide pellets, 88.8

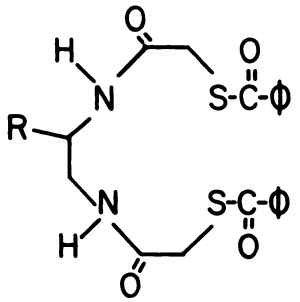

FIG. 1. Structures of *N, N'*-bis (*S*-benzoylmercaptoacetyl) ethylenediamine (DADS) and *N, N'*-bis (*S*-benzoylmercaptoacetyl)-2,3-diaminopropionic acid ethyl ester (carboxy DADS) (*1–5*).

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SCHEME I

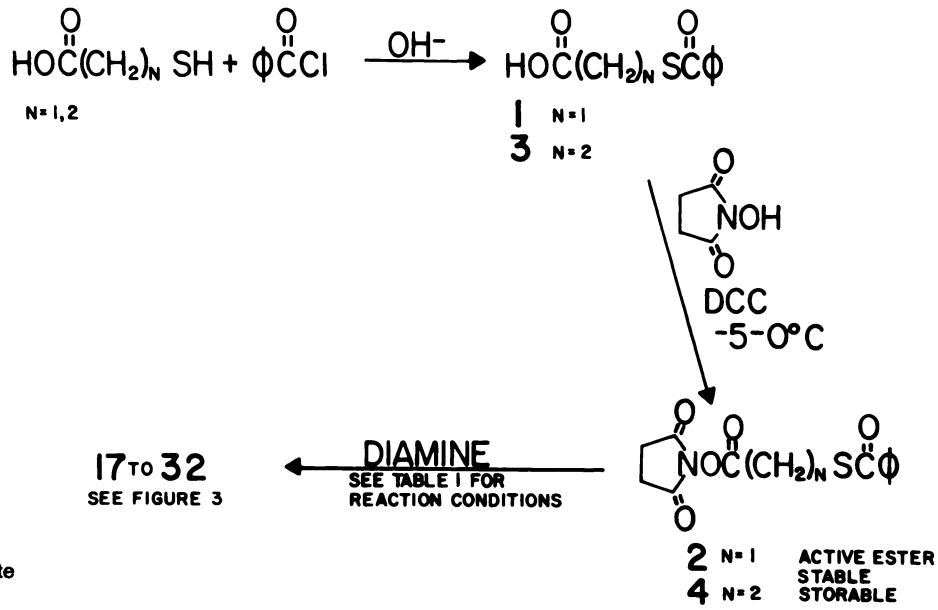

FIG. 2. Scheme I: Synthetic route for Compounds **17** to **32**.

g (2.2 mole) were dissolved in a mixture of 750 ml water and 750 ml of benzene. Benzoyl chloride 92.0 ml (1.0 mole) was added over 30 min, maintaining the temperature at 5-15°C.

Stirring was continued for an additional 30 min at 5-15°C, then at room temperature for 30 min. The reaction mixture was diluted to twice its volume with water, and the layers separated. The aqueous layer was washed twice with 200-ml portions of benzene, which were discarded. The pH of the aqueous layer was adjusted to 1.0-1.5 with concentrated hydrochloric acid. After chilling, the precipitate was removed by filtration, washed with cold water, and dried. The product was recrystallized twice using ethyl acetate. The yield was 155.4 g (79.2%) of white crystalline material with a melting point of 102.0-103.0°C (lit: 102-103°C, Ref. *6*). The infrared spectrum showed absorptions at 3400 (OH), 1700 (COOH), 1660 (SC=O), 1590 and 1580 (ø) cm^{-1}.

Succinimidyl-*S*-benzoylthioglycolate (2). **1**,155.4 g (0.792 mole) and *N*-hydroxysuccinimide, 91.3 g (0.792 mole) were dissolved in 900 ml tetrahydrofuran. The temperature of the reaction mixture was lowered to −5 to 0°C.

Over a 30-min period, dicyclohexylcarbodiimide, 196.5 g (0.95 mole) dissolved in 200 ml tetrahydrofuran was added. After the addition, the temperature was maintained at −5 to 0°C for 2 hr, then at room temperature for 18 hr. Glacial acetic acid, 3.1 ml, was added and stirring continued for an additional hour. The reaction mixture was filtered and the filter cake extracted twice with hot tetrahydrofuran. All of the filtrates were combined and evaporated to a pasty mass, which was recrystallized twice from ethyl acetate to obtain 131.7 g (71.6%) of white crystals with a melting point (mp) of 135.0-137.0°C. The infrared spectrum showed absorptions at 1820, 1780, 1750, (typical of active esters), 1670 (S—C=O), 1630 (NC=O), 1595 and 1585 (ø) cm^{-1}.

***S*-Benzoyl-3-mercaptopropionic acid (3).** The procedure used to prepare **1** was used, except that the reagent and solvent usages were reduced to one half, and **3** was recrystallized twice from acetone/water to give 41.5 g (39.5%) of finished material, mp 76.5-78.5°C (Ref. 7 crude prep: 47-51°). Infrared: 3120 (OH), 1710 (COOH), 1665 (S—C=O), 1600, 1590 (ø) cm^{-1}.

Succinimidyl-*S*-benzoyl-3-mercaptopropionate (4). The procedure used to prepare **3** was used, except that the amounts of reagent and solvent were reduced to one quarter. 28.5 g (47%) of **4** were obtained with mp 102.5-104.°C. Infrared: 3180 (OH), 1820, 1790, 1735 (active ester), 1660 (SC=O), 1595, 1580 (ø) cm^{-1}.

***N*-(*S*-benzoylmercaptoacetyl) glycine (5).** Compound **2**, 20.00 g (0.068 mole), and 390 ml of absolute ethanol were placed into a 500-ml flask. The reaction mixture was heated to 50-55°C and a solution consisting of glycine, 3.40 g (0.045 mole), dissolved in 45 ml of water, was added in one portion. The reaction mixture was refluxed for 2.5 hr, then stirred at room temperature for 16 hr. The solvent was removed and the residue recrystallized from acetonitrile to yield 6.78 g (59.1%) of V with mp 138.0-139.5°C. The infrared showed absorptions at 3360 (NH), 1740 (COOH), 1660 (S—C=O), 1620 (NC=O), 1500, 1580 (ø), 1530 (Amide II) cm^{-1}.

***N*-(*S*-Benzoylmercaptoacetyl)-β-alanine (6).** Compound **2**, 30.0 g (0.102 mole), and 510 ml of absolute ethanol were warmed to 50°C and a mixture consisting of β-alanine 6.07 g, (0.068 mole), water (68 ml), and 68 ml of absolute ethanol was added. The mixture was refluxed for 2.5 hr, then allowed to stand at room temperature for 16 hr. Evaporation of the solvent gave an oil which, upon addition of acetonitrile, formed a precipitate. Recrystallization with acetonitrile gave 15.4 g (84.5%) of the desired product with mp of 124.0-126.0°C. Infrared: 3280 (NH), 1700 (HOC=O), 1650 (NC=O, SC=O), 1590, 1580 (ø), 1545 (Amide II) cm^{-1}. High performance liquid chromatography (HPLC) indicated a purity of 99.8%.

Succinimidyl-*N*-(*S*-benzoylmercaptoacetyl glycinate (7). Compound **5**, 6.78 g (0.027 mole), and *N*-hydroxysuccinimide 3.10 g (0.027 mole), were dissolved in 80 ml of tetrahydrofuran. Then over 30 min a solution was added consisting of dicychohexylcarbodiimide, 5.75 g (0.028 mole), dissolved in 10 ml of tetrahydrofuran. The reaction mixture was stirred at 0-5°C for 2 hr, then at room temperature for 16 hr. Glacial acetic acid, 0.2 ml, was added and the reaction mixture stirred an additional hour. It was filtered and the dicyclohexylurea cake was extracted twice with hot tetrahydrofuran. The tetrahydrofuran filtrates were combined and evaporated to a gummy residue. Two recrystallizations from ethyl acetate yielded 5.60 g (59.6%) of 7 with mp 161.5-163.5°C. The infrared spectrum showed absorptions at 3260 (NH), 3080

(O), 1840, 1790, 1730 (characteristic of active esters), 1660 (S—C=O), 1590, 1580 (ø), 1550 (amide II) cm^{-1}.

Succinimidyl-*N*-(*S*-benzoylmercaptoacetyl)-β-alanate (8). Compound (**6**), 15.4 g (0.058 mole), and *N*-hydroxysuccinimide 6.63 g, (0.058 mole), were dissolved in 175 ml tetrahydrofuran and placed into an ice bath. Then over 30 min a mixture consisting of dicyclohexylcarbodiimide, 13.07 g (0.063 mole), dissolved in 20 ml dry tetrahydrofuran was added. The mixture was stirred for 2.0 hr at ice-bath temperature, then at room temperature for 16 hr. Glacial acetic acid, 0.4 ml, was added and stirring was continued for another 30 min. The reaction mixture was filtered and the filter cake washed with hot tetrahydrofuran. Evaporation yielded a residue that was recrystallized twice from ethyl acetate and finally from acetonitrile to yield 11.2 g (53.4%) of (**8**). The mp was 124.5-127.0°C. Infrared: 3280 (NH), 1820, 1780, 1720 (characteristic of active esters), 1650 (NC=O) SC=O, 1590, 1580 (ø), 1540 (amide II) cm^{-1}.

S-Benzoyl-2-mercaptoethylamine hydrochloride (9). 2-Mercaptoethylamine hydrochloride, 7.83 g, (0.069 mole), and benzoyl chloride, 9.7 g (0.069 mole), were dissolved in 47 ml of trifluoroacetic acid and refluxed for 5 hr. The trifluoracetic acid was removed under vacuum and the residue recrystallized twice from aqueous hydrochloric acid (~ 15%). The crystalline product was stirred in boiling ethyl acetate. After cooling and filtering there were obtained 8.9 g (59%) of the product with mp 172.5-175.0°C. Infrared: 3320-2040 (amine hydrochloride), 1670 (S—C=O), 1600, 1590, 1580 (ø) cm^{-1}.

S-Benzoylcysteine ethyl ester hydrochloride (10). Cysteine ethyl ester hydrochloride, 25 g (0.135 mole), and benzoyl chloride, 15.8 ml (0.136 mole), were dissolved in 95 ml of trifluoroacetic acid and refluxed for 4.5 hr. Excess trifluoroacetic acid was removed under vacuum, and the oily residue was taken up with approximately one volume of water. Then sufficient concentrated hydrochloric acid was added to give about a 15% final acid concentration. After chilling for 16 hr at −15°C, a precipitate was collected that had a syrupy consistency. A second crop was obtained by adding more acid and chilling. This was combined with the first crop. Addition of ethyl acetate gave a granular precipitate which, after chilling, was removed. The precipitate was dissolved in water, filtered to remove any insolubles, and extracted twice with ethyl acetate. Concentrated hydrochloric acid was added to the aqueous phase to give a final acid concentration of 10-15%. Chilling and filtration yielded tiny white needles weighing 9.0 g (23.0%) with mp 156.0-157.5°C. Infrared showed absorptions at 2730-2020 (amine hydrochloride), 1755 (ester), 1670 (S—C=O), 1600, 1590, 1575 (ø) cm^{-1}.

Ethyl-2,3-diaminopropionate dihydrochloride (11). 2,3,-Diaminopropionic acid hydrochloride, 7.1 g (0.051 mole), and absolute ethanol (1300 ml) were placed in a 2 liter flask. Dry hydrogen chloride was then bubbled in at a moderate rate for 5 min. The reaction mixture was refluxed for 24 hr, stirred at room temperature for 65 hr. The ester was removed and partial evaporation of the mother liquor yielded a second crop. A total of 9.3 g (88.9%) of the ester were recovered with mp of 163.5-165.5°C (Ref. *8*: 164.5-165.0°. Infrared showed absorptions at 3470, 3470-2000 (amine hydrochloride), 1750 (ester) cm^{-1}.

Diethyl-2,3,-diaminosuccinate dihydrochloride (12). Diglycine hydrochloride, 18.65 g (0.1 mole), and 1200 ml absolute ethanol were placed in a 2000-ml flask. Hydrogen hydrochloride was then bubbled in at a moderate rate for 5 min and there resulted a clear solution. After refluxing for 48 hr, the solvent was evaporated and the residue, after washing with ethyl acetate, was recrystallized from ethanol. 18.93 g (67.8%) of **12** were recovered with mp of 135.0-138.0°C. The infrared spectrum showed absorptions at 2780-2000 (amine hydrochloride), 1735 (ester) cm^{-1}.

α-Phenylglycinonitrile hydrochloride (13). This material was synthesized according to Steiger's method (*9*). The yield was 37.5% with mp of 164.0-167.0°C (Ref. *9*: 171-172°). Infrared showed absorptions at 2260 (C N weak), 1660 (amine hydrochloride), 1580 (ø) cm^{-1}.

TABLE 1. REACTION DATA FOR THE PREPARATION OF 17 TO 32 FROM THE REACTION OF 2 WITH VARIOUS DIAMINES

Compound	2 (mole)	Diamine (mole)	Triethyl amine (mole)	T	t (hr)	Yield %	Solvent	Recrystallization solvent
17	0.01	0.005	0	Reflux	2	72.1	THF	MEK
18	0.01	0.005	0	Reflux	2	63.3	THF	MEK
19	0.01	0.005	0	Reflux	3	57.1	THF	MEK
20	0.01	0.005	0.011	Reflux	3	68.6	THF	Benzene or isopropanol
21	0.02	0.01	0.022	Reflux	16	56.2	THF	MEK/heptane
22	0.01	0.005	0.01	Reflux	16	69.0	THF	MEK
23	0.01	0.005	0.01	Reflux	18	39.5	THF	CH_3CN
24	0.01	0.005	0	Reflux	2.5	60.6	THF	CH_3CN
25	0.01	0.005	0	R.T.	16	83.5	THF	MEK
26	0.01	0.005	0	Reflux	16	43.0	THF	MEK
27	0.015	0.005	0.011	Reflux R.T.	1.5 16	31.5	THF	MEK
28	0.01	0.005*	0	Reflux	18	22.1	THF	MEK
29	0.01	0.005	0	Reflux	71	14.9	THF	CH_3CN
30	0.01	0.005	0	Reflux	44	48.7	THF	CH_3CN
31	0.01†	0.005	0	Reflux	24	84.6	THF	MEK
32	0.01†	0.005	0	Reflux	24	78.5	THF	MEK

* Mix cis and trans isomers.

† Used **4** instead of **2**.

TABLE 2. ANALYTICAL DATA FOR COMPOUNDS 17 TO 37

		mp °C	Combustion analysis C	H	N	S	Infrared cm^{-1}	HPLC % Purity*	Ret. vol.
17	Lit.	192–194	57.67	4.84	6.73	15.40	3270 (NH), 1640 C-O,	98.8	4.05
	Fd.	202.5–204	57.67	4.67	6.72	15.34	1650 (AMIDE II)		
18	Lit.		58.58	5.15	6.51	14.90	3280 (NH), 1660 (SC═O),	97.6	3.69
	Fd.	191.0–193.0	58.47	4.90	6.55	15.03	1640 (NC═O), 1550 (AMIDE II)		
19	Lit.		59.43	5.44	6.30	14.43	1550 (AMIDE (II), 3300 NH),	97.2	6.11
	Fd.	118.0–119.5	59.35	5.61	6.60	14.14	1670 (SC═O), 1650 (NC═O), 1530 (AMIDE II)		
20	Lit.	129.5–131.0	56.54	4.95	5.74	13.13	3280 (NH), 1740 (ESTER), 1670 (NC═O),	99.0	4.90
	Fd.	133.0–136.0	56.43	5.22	5.61	13.19	1650 (SC═O)		
21	Lit.	52.0–53.0	55.70	5.03	5.00	11.44	3300 (NH), 1740 (OC═O),	99.0	3.94
	Fd.		55.48	5.29	4.91	11.59	1670 (SC═), 1640 (NC═O), 1550 (AMIDE II)		
22	Lit.		63.39	4.91	5.69	13.02	3300 (NH), 1670 (C═O), 1640 (C═O),	99.2	5.49
	Fd.	193.0–197.0	63.65	5.18	5.57	13.02	1540 (AMIDE II)		
23	Lit.		62.05	5.02	5.36	12.27	3360 (OH), 3260 (NH), 1640 (C═O),	97.5	3.85
	Fd.	101.0–104.0	61.85	5.15	5.45	12.20	1540 (AMIDE II)		
24	Lit.		59.70	5.01	6.33	14.49	1660 (SC═O), 1640 (NC═O), 1595, 1585 (ø)	99.9	4.69
	Fd.	187.5–189.0	59.58	5.10	6.31	14.48			
25	Lit.	179–180	58.58	5.15	6.51	14.90	1550 (AMIDE II), 3270 (NH), 1660 (SC═O),	99.1	4.82
	Fd.	185.5–187.0	58.32	5.21	6.56	14.91	1630 (NC═O), 1540 (AMIDE II)		
26	Lit.		56.48	4.97	6.27	14.36	3460 (OH), 3290 (NH), 1660 (C═O),	96.3	3.99
	Fd.	173.0–175.0	56.38	5.26	6.13	14.34	1550 (AMIDE II)		
27	Lit.		56.74	4.54	6.30	14.43	3310 (NH), 1730 (C═O), 1550 (AMIDE II)	99.5	4.31
	Fd.	185.5–187.5	56.81	4.60	6.27	14.45			
28	Lit.		61.25	5.57	5.95	13.63	3380, 3260, 3170 (NH), 1670 (SC═O),	20.88/ 77.9	4.81/ 5.45†
	Fd.	190.0–198.0	61.35	5.75	6.03	13.55	1640 (NC═O)		
29	Lit.	164–165	62.05	4.34	6.03	13.80	3220 (NH), 1650 (C═O), 1530 (AMIDE II)	97.2	6.45‡
	Fd.	155.0–156.5	62.20	4.52	6.32	13.53			
30	Lit.		63.39	4.91	5.69	13.02	3300 (NH), 1690 (HC═O), 1670 (SC═O)	99.0	8.11
	Fd.	179.0–180.5	63.10	5.07	5.61	12.90	1520 (AMIDE II)		
31	Lit.	201.0–202.5	59.43	5.44	6.30	14.43	3300, 3200 (NH), 1670 (SC═O), 1640 (NC═O)	99.8	4.15
	Fd.	206.0–209.0	59.15	5.53	6.31	14.34	1550 (AMIDE II)		
32	Lit.	151–152	60.23	5.72	6.11	13.98	3300, 3200 (NH), 1670 (SC═O), 1640 (NC═O)	98.6	4.38
	Fd.	148.5–150.0	59.94	5.75	6.14	13.91	1550 (AMIDE II)		
33	Lit.		57.67	4.84	6.73	15.40	3290 (NH), 1660 (SC═O), 1640 (NC═O),	99.5	4.66
	Fd.	140.5–143.0	58.58	5.51	7.01	14.28	1540 (AMIDE II)		
34	Lit.		56.54	4.95	5.74	13.13	3320 (NH), 1740 (ESTER), 1670 (SC═O),	98.6	5.24
	Fd.	145.0–146.0	56.62	5.08	5.83	13.26	1640 (NC═O), 1530 (AMIDE II).		
35	Lit.		58.58	5.15	6.51	14.90	3290 (NH), 1660 (C═O), 1530 (AMIDE II)	100.0	3.66
	Fd.	163.0–166.0	58.59	5.20	6.60	15.16			
36	Lit.		57.35	5.21	5.58	12.76	3280 (NH), 3080 (ø), 1730 (ESTER),	99.0	5.20
	Fd.	147.5–149.5	57.63	5.33	5.54	12.93	1670 (SC═O), 1640 (NC═O), 1550 AMIDE II		
37	Lit.		57.67	4.84	6.73	15.40	3300 (NH), 1660 (C═O), 1530 (AMIDE II)	99.6	4.69
	Fd.	181.0–182.0	57.39	5.05	6.46	15.46			

* Spheresorb S-5-ODS-2 C-18 reverse-phase column, 60% acetonitrile, 1.4 ml/min flow rate.

† Mixture of cis and trans isomers.

‡ Std. for normalization of HPLC retention volumes.

***N,N'*-Diacetyl-α-phenylethylenediamine (14).** This was prepared by the method of Sundberg (*10*). The yield was 41.5%, with mp 151.5–153.0°C after two recrystallizations from ethylacetate (Ref. *10*: 155–156°). Infrared 3270, 3200 (NH), 1640 (N—C═O), 1550 (amide II) cm^{-1}.

α-Phenylethylenediamine dihydrochloride (15). Compound **15**, 5.4 g (0.025 mole), glacial acetic acid, 33 ml, and 49 ml of concentrated hydrochloric acid were refluxed for 24 hr. Then 250 ml of ethyl acetate were added and, after chilling in ice, the precipitate was collected. 4.2 g of **15** (76.9%) were recovered with mp of greater than 300°C (Ref. *11*: >260°). The infrared spectrum showed absorptions at 2740–2150 (amine hydrochloride), 1600, 1590, 1570, 1520 (ø) cm^{-1}.

α-(*p*-Hydroxybenzyl ethylenediamine dihydrochloride (16). This

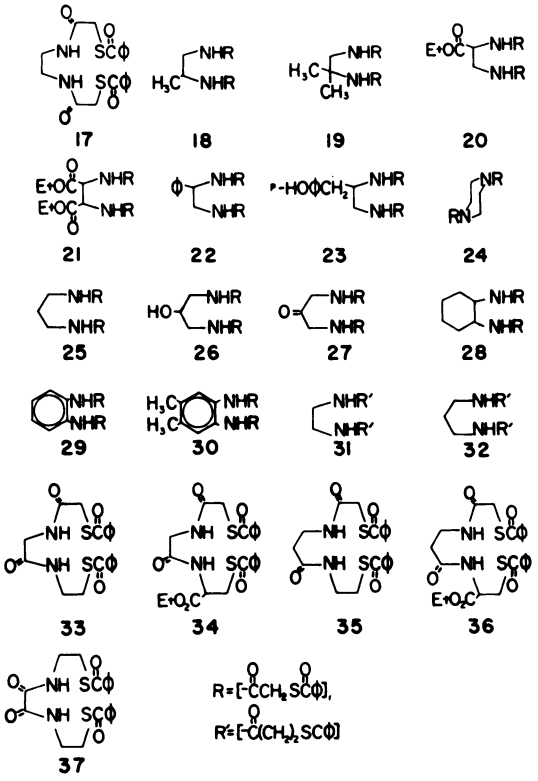

FIG. 3. Structures of Compounds **17** to **37**.

material was prepared by a method described by Yeh, Sherman, and Meares (*12*). The yield was 59.8%. The infrared spectrum showed absorptions at 3280 (OH), 2760-1990 (amine hydrochloride) 1630, 1610, 1510, 1500 (ø) cm^{-1}.

General procedure for preparation of DADS compounds (17–32). Compound **2** (0.01 mole) was dissolved in 70 ml of dry tetrahydrofuran. To this was added a solution of the diamine (0.005 mole) dissolved in 20 ml of tetrahydrofuran. The data presented in Table 1 list the other reaction conditions used. In the preparation of **20–23** and **27**, triethylamine was added as well to trap hydrogen chloride, since the precusor amines were used as their hydrochlorides. Finally, in the preparation of **27**, an extra 0.005 mole of **2** was added to compensate for the water of hydration of the starting amine, which could be expected to hydrolyze **2** and render it unavailable for reaction with the amine. The analytical data for **17–32** are presented in Table 2.

***N*-[*N*-(*S*-benzoylmercaptoacetylglycyl)]-*S*-benzoylmercaptoethyl amine 33.** Compound **7**, 5.60 g (0.016 mole), 185 ml of tetrahydrofuran, 296 g of **9** (0.0136 mole), and 1.92 ml of triethylamine were refluxed for 16 hr. The reaction mixture was concentrated to about 25% of its original volume, then six volumes of water were added. After stirring 0.5 hr, the precipitate that formed was removed and recrystallized twice from methylethyl ketone. 2.90 g (51.2%) of 22 were obtained. Analytical data for this material are given in Table 2.

***S*-Benzoylmercaptoacetyl-glycyl-*S*-benzoyl-L-cysteine ethyl ester (34).** Compound **7**, 10.5 g, (0.03 mole), was dissolved 350 ml of tetrahydrofuran by warming to 45–50°C. Then **10**, 7.42 g (0.026 mole), and triethylamine, 3.6 ml (0.026 mole), were added and the mixture refluxed for 16 hr. Any triethylamine hydrochloride was removed and the filtrate evaporated to an oil that yielded a precipitate upon addition of acetonitrile. Analysis by HPLC at this point indicated that about 5.0 g (40.2%) of **34** was present.

Final purification was by preparative HPLC using a reverse-phase (C-18) column,† with 50% acetonitrile as eluant. 3.0 g (24.0%) of **34** were recovered. See Table 2 for further analytical data.

***N*-[*N*-(*S*-benzoylmercaptoacetyl-β-alanyl)]-*S*-benzoylmercaptoethyl amine 35.** Compound **8**, 12.8 g (0.0352 mole), **9**, 6.51 g (0.030 mole), triethylamine 4.21 ml, and 400 ml tetrahydrofuran were refluxed for 16 hr. Triethylamine hydrochloride was removed and the filtrate was concentrated to about one fourth its volume, then six volumes of water were added. After filtration and two recrystallizations of the precipitate from methylethyl ketone, 6.40 g (49.7%) of product were obtained. See Table 2 for further analytical data.

***S*-Benzoylmercaptoacetyl-β-alanyl-*S*-benzoyl-L-cysteine ethyl ester 36.** Compound **8**, 7.6 g, (0.021 mole), **10**, 5.15 g (0.018 mole), triethylamine, 1.82 g (0.018 mole), and 240 ml of tetrahydrofuran were refluxed gently for 16 hr. Triethylamine hydrochloride was removed and the filtrate was concentrated to 25% of its volume, then diluted with six volumes of water and stirred for 1 hr. The sticky precipitate was removed and recrystallized twice from acetonitrile and once from ethyl acetate. Isolation by preparative HPLC under conditions used to isolate **34** yielded 1.3 g of **36** (14.3%). See Table 2 for further analytical data.

1,2-dioxo-*N*,*N*′-bis(2-benzoylthioethyl ethylenediamine (37). Dry acetone, 100 ml, *S*-benzoylmercaptoethylamine hydrochloride, 5.0 g (0.023 mole), and triethylamine, 4.2 g (0.042 mole), were placed into a 125-ml Erylenmeyer flask equipped with an efficient stirrer and placed in an ice/salt bath. Then over about 3 min were added oxalylchloride, 1.22 g (0.0096 mole). The reaction mixture was stirred at −5–0°C for 1 hr, then at room temperature for 1 hr; it was then poured into 300 ml of water, chilled and filtered. The product was recrystallized from chloroform/heptane to yield 0.6 g (15%) of **36.** See Table 2 for the pertinent analytical data.

TABLE 3. COMPARISON OF YIELDS: SYNTHESIS VIA SCHEME I COMPARED WITH EXISTING LITERATURE METHODS

Compound	Via Scheme I yield %	Literature yield %	Ref
7	72.1	85.0	2
25	83.5	64.0	2
20	68.6	52.0	3
31	84.6	78.0	2
32	78.5	83.0	2
29	14.9	42.0	2
Average yield	67.0	68.0	

RESULTS AND DISCUSSION

Compounds **17** to **32** (Fig. 3) were prepared as indicated by Scheme I, Fig. 2. Compound **1** was prepared by the well-known Schotten-Baumann reaction (*13*). It was condensed to active ester **2** with *N*-hydroxysuccinimide in the presence of dicycohexylcarbodiimide, another well-documented reaction (*14,15*). This active ester **2**, contains all of the components of the desired DADS except the diamine backbone. It is susceptible to nucleophilic attack, especially by amines. This property was used to advantage by the reaction of two equivalents of **2** with one equivalent of the diamine to yield products **27–30**. Substitution of 3-mercaptopropionic acid for

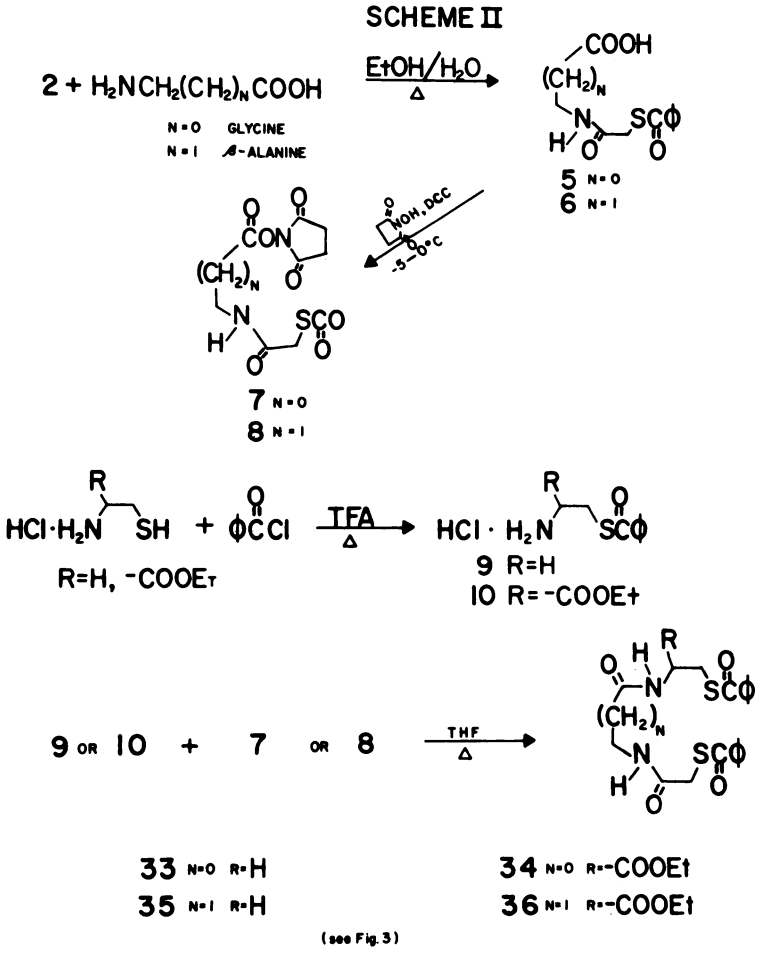

FIG. 4. Scheme II: Synthetic route for Compounds **33** to **36**.

mercaptoacetic acid in Scheme I produces the series **31** and **32.**

From the data in Table 3, the average yield for the six compounds synthesized by Scheme I (67%) compares favorably with the value of 68% for the earlier published methods. Equally important is the fact that active ester **2** or **4** may be prepared and stored in quantity until ready to react with the desired diamine. Thus, in effect, we have reduced the synthetic method to an essentially one-step procedure. Table 2 shows that compounds **17** to **32** also were prepared with acceptable purity.

The yield of **29** from *o*-phenylenediamine, even with a prolonged reaction time of 71 hr, was only 14.9%, probably due to the inhibiting steric effect of the ortho-amino groups and the decreased nucleophilicity of the amino groups towards the active ester induced by the phenyl ring. Methyl substitution on the 4 and 5 positions of *o*-phenylenediamine (**30**), which would be expected to increase the electron density on the amino groups, improved the yield to 48.7% with a shorter reaction time of 44 hr. Electron-withdrawing nitro or carboethoxy groups resulted in only a sluggish reaction toward **2**, giving only the mono-substituted derivatives. For DADS compounds with an unsubstituted *o*-phenylenediamine precursor (or one substituted by electron-withdrawing group), we conclude that the existing published procedure described in Ref. *2* is the method of choice.

Asymmetrical DADS Compounds (**33** to **36**), while isomerically related to the symmetrical DADS, actually represent a new class of compounds, and can be considered as derivatized dipeptides. The synthetic route leading to these compounds is shown in Scheme II, Fig. 4. The key to their synthesis was the selective benzoylation of the mercapto group of 2-mercaptoethylamine hydrochloride, or L-cysteine ethyl ester hydrochloride, in trifluoroacetic acid. Besides being an excellent solvent, trifluoroacetic acid suppresses the ionization of the protonated amino group, thus preventing it from competing with the mercapto group for benzoyl chloride. The remaining portion of the synthetic procedure is based upon standard polypeptide-building techniques, and needs no further comments.

Compound **37**, which represents another isomeric DADS compound, was also synthesized as indicated in

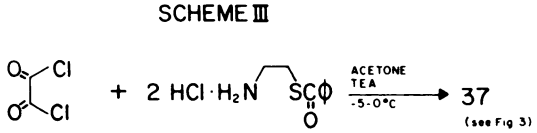

FIG. 5. Scheme III: Synthetic route for Compound **37**.

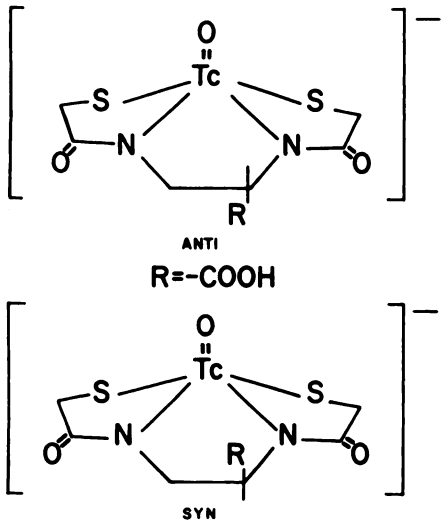

FIG. 6. Geometrical isomers formed when **20** is complexed with Tc-99m.

Scheme III, Fig. 5. The yield of this material was rather low. *N*-Benzoyl-2-mercaptoethyl amine was found among the reaction products, suggesting that considerable S → N benzoyl exchange is occurring in competition with the desired reaction.

Compounds **18, 20, 26, 22,** and **23** have an asymmetrically substituted α carbon on the backbone, and when complexed with technetium will give rise to two different geometrical isomeric complexes, depending upon whether the substituent is syn or anti to the oxo group present on the technetium core (Fig. 6). A similar situation exists for the Tc-99m complexes of **34** and **36,** where the substituent is present on the α position of one of the side chains. Compound **21** has two asymmetric centers and would be expected to give rise to three geometrical isomeric technetium complexes. In this case the carboxyl substituents could be syn-syn, anti-anti, or syn-anti to the oxo group. This type of isomerism was previously reported by Fritzberg, Kuni, Klingensmith et al. (*3*). They found that Compound **20** (synthesized by a different route), when labeled, gave two components that they termed "A" and "B." "A" had a lower hepatic uptake and faster renal clearance than "B." Unfortunately Component "B" degraded the images and slowed the renal clearances to such an extent as to require its removal by HPLC after labeling—an undesirable feature for clinical use. It is desirable, then, that when this type of isomer formation is possible, all of the isomers should have similar renal clearance properties, which would remove the necessity of including an HPLC separation step in the labeling procedure. Evaluation studies are being carried out in order to see whether or not some of the compounds that we have synthesized, which give rise to isomeric complexes, solve this problem. Radionuclide labeling, biodistribution, and imaging procedures performed with these compounds are currently being pursued.

FOOTNOTES

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† Whatman Magnum 20.

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