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# Synthesis of a New Diaminodithiol Bifunctional Chelator for Radiolabeling Biomolecules with Indium(III)

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**Abstract**—The synthesis of a new bifunctional ligand 1-(*p*-carboxybenzyl)-*N*,*N*′-bis-[1,1-dimethyl-1-(*p*-methoxybenzylthio)ethyl]-ethylenediamine-*N*,*N*′-diacetic acid, di-*t*-butyl ester (**1**, **nbi6ss**) is described. It consists of a carboxybenzyl group substituted on a carbon atom of the ethylenediamine moiety of a hexadentate ligand, which has been found to have a very high affinity for In(III). The *gem*-dimethylthiol groups and the carboxylic acid groups of the ligand were protected by groups that can be removed under mild conditions after conjugation to a peptide or protein. 

## Introduction

Meares et al. in 1974[1] synthesized the first bifunctional chelator (BFC). In this ligand a linkage of L-1 (see Chart 1) is used to combine a chelating agent, EDTA (ethylenedinitrilotetraacetic acid) to protein by amidation through an α-bromoacetamido group (in $BrCH_2CONH–C_6H_4–CH_2–$ group), which was formed from catalytic hydrogenation of the nitro group and then acylation with bromoacetyl chloride. This linkage was further modified by Meares et al. (L-2,[2] L-3[3]). Since then, L-3 is the most widely used intermediate for the design and synthesis of BFC. Altman et al. (1984) synthesized L-4[4] as the linkage for the BFC of EDTA. Here an aliphatic carboxylic acid is used for conjugating to protein. Gansow et al. (1986) designed L-5[5] for the BFC of DTPA (diethylenetrinitrilopentaacetic acid). The Meares' group also successfully prepared the 1,4,7,10-tetraazacyclododecane (cyclen) and 1,4,7-triazacyclononane (tacn) BFC linkage with the aromatic amine for conjugation (L-6[6] and L-7[7]). Parker's group prepared the L-8,[8] L-9[8] with aliphatic amines for conjugation. Other compounds are of interest for the design of BFC appeared in the recent literatures are: L-10,[9] with a thioester group for conjugation; L-11,[10] with a protected ethyleniminyl group to form the ethylenediamine moiety, and L-12,[11] use a *trans*-1,2-diaminocyclohexyl group as linkage.

Indium-111 is a radioisotope whose physical characteristics are perfectly suitable for diagnostic applications, many of the In-111-radiopharmaceuticals are nevertheless limited by a high liver uptake. One of the reasons is probably due to the large formation constant of In(III)-transferrin ($\log K_1 = 18.74$)[12] and the high plasma concentration of this protein (0.25 g/100 mL) thermodynamically favor the in vivo exchange of many In(III) complexes with transferrin.

The design and synthesis of new chelating agents for effective coordination of In(III) has long been an important objective of this research group. Not long ago, DTPA was considered to be the best candidate for the design of bifunctional chelators for In(III) (see Table 1). Since 1995, several ligands containing aminoethanethiol donor groups were found to have very high affinity for In(III).[14–16] Examples are the two EDTA analogues: **6ss**, *N*,*N*′-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine-*N*,*N*′-diacetic acid (**2**, Chart 2, pM=30.9)[14] and **EDDASS**, *N*,*N*′-bis(2-mercaptoethyl)ethylenediamine-*N*,*N*′-diacetic acid (**3**, Chart 2, pM=30.4).[15] A logical approach to the use of these complexes is to prepare their BFCs. The challenge for the design and synthesis of bifunctional chelators containing thiol groups are: thiol groups are not as stable as carboxylic acid or amines (in EDTA, DTPA or polyazamacrocycles); and the α-bromoacetamido group ($BrCH_2CONH–C_6H_4–CH_2–$) which is widely used for the amidation reaction to bond proteins, also reacts with the thiol groups of the ligand.[17] Based on the structure of **6ss**, a bifunctional ligand: 1-(4-carboxymethoxybenzyl)-*N*,*N*′-bis-[(2-mercapto-2,2-dimethyl)ethyl]-1,2-ethylenediamine-*N*,*N*′-diacetic acid (**4**, **bi6ss** Chart 2) was designed and synthesized in this laboratory.[18] In this ligand a carboxymethoxy group was used for conjugation. However, it was found that the labeled complexes to be difficult to conjugate to proteins. One reason may be due to the fact that carboxymethoxy

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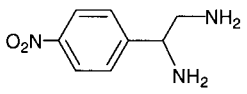
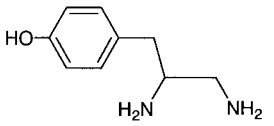
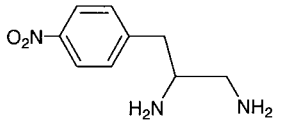


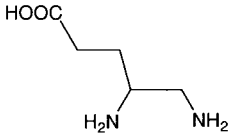

C. F. Meares 1974, L-1[1]



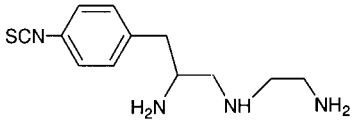

C. F. Meares 1979, L-2[2]



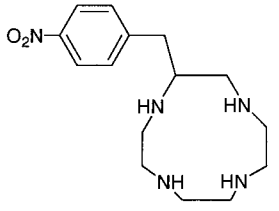

C. F. Meares 1980, L-3[3]



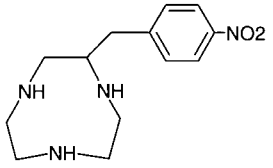

J. Altman 1984, L-4[4]



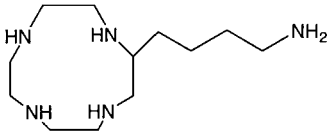

O. A. Gansow 1986, L-5[5]



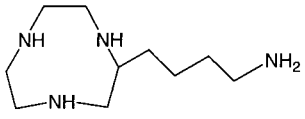

C. F. Meares 1988, L-6[6]



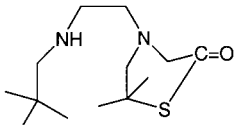

C. F. Meares 1992, L-7[7]



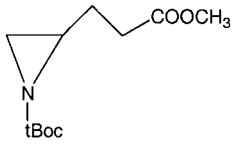

D. Parker 1989, L-8[8]



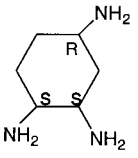

D.Parker 1990, L-9[8]



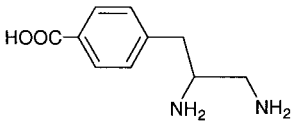

S. Z. Lever 1998, L-10[9]




A. Srinivasan 1991, L-11[10]




J. F. Gestin 1997, L-12[11]




This work **(7)**

**Chart 1.** Some intermediates for the preparation of BFC.

**Table 1.** Formation constants (log $K_{ML}$) and pM (pM is −log [M] of In(III) at pH=7.4 with 100% excess of the ligand) of In(III) (Ref. 13)

| Ligand | log $K_{ML}$ | pM |
|---|---|---|
| EDTA | 24.9 | 22.1 |
| DTPA | 29.0 | 24.9 |
| DOTA | 23.9 | 17.8 |
| NOTA | 26.2 | 21.6 |
| HBED | 27.9 | 17.9 |
| 6ss | 39.8 | 30.9 |
| EDDASS | 37.0 | 30.4 |
| Transferrin[a] | 18.74, 16.86 | 18.3 |

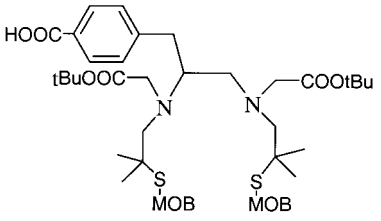
[a] Ref. 12.

group may be involved in a dissociation mechanism before the ligand can be bonded to the amino groups of a protein. Rogers et al.[19] published a paper on two bifunctional polyazamacrocycles (see Chart 2, **CPTA**, **5** and **PCBA**, **6**), in which benzoic acid groups were used for conjugation. Here we design and synthesize a bifunctional ligand of **6ss** with the chelating donor groups protected and with a benzoic acid group as the linkage for conjugation. After conjugation, the protecting groups, *tert*-butyl and 4-methoxybenzyl, can be removed under mild conditions.

## Results and Discussion

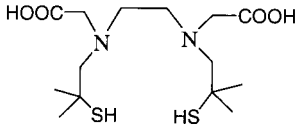
1-(*p*-Carboxybenzyl)-ethylenediamine (**7**, see Scheme 1) is a simple and very useful intermediate for the synthesis of bifunctional ligands for covalently bonded metal ions to protein. However this intermediate has not been described in the literature. Kidwai[20] published 'A New Route for the Synthesis of Substituted Ethylenediamines', including 1-benzylethylenediamine, by catalytic hydrogenation of the azalactones. (See Scheme 2). Following his idea, a *para*-substituted azalactone was prepared and hydrogenated. All that could be obtained is the glycinamide and the 2-(*p*-carboxybenzyl)glycine. It was concluded that it is impossible to reduce the amide by catalytic hydrogenation under such mild conditions as Kidwai described in his paper.[20] The reaction probably requires very high pressure and temperature. However, it is well known that cyano groups can be reduced to amines by hydrogenation with Raney Nickel under low pressure and room temperature. Thus, 2-benzamidoacetonitrile (**9**) was used instead of *N*-benzoylglycine ethyl ester, to prepare the ethylenediamine derivative: compound **7**.

For the preparation of the new bifunctional ligand, the strategy similar to that used for the previous bifunctional

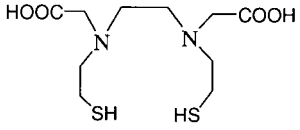



**nbi6ss (1)**

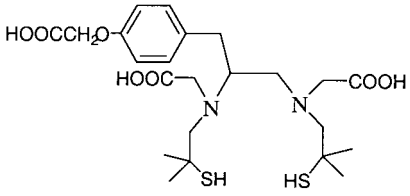
(MOB=methoxybenzyl)



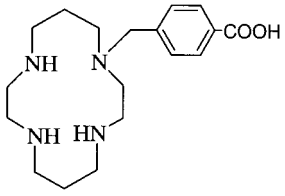

**6ss(2)**



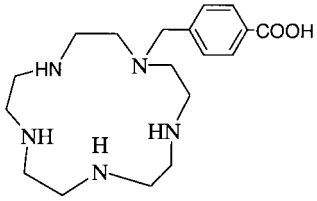

**EDDASS(3)**




**bi6ss(4)**

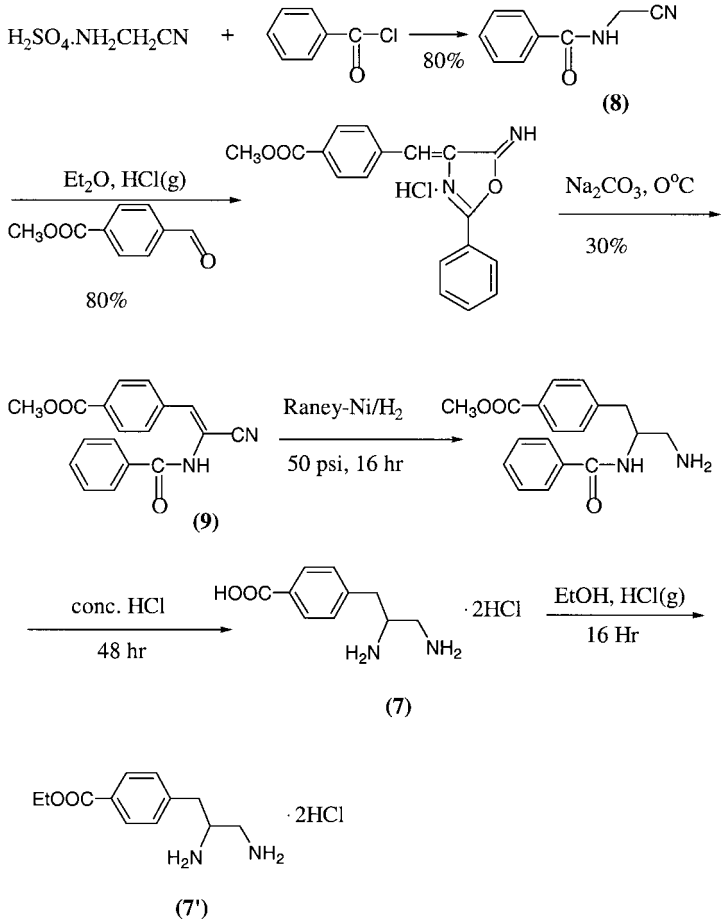



**CPTA(5)**




**PCBA(6)**

**Chart 2.**




**Scheme 1.**

ligand, **bi6ss**: 1-(4-carboxymethoxybenzyl)-*N*,*N*′-bis[(2-mercapto-2,2-dimethyl)ethyl]-1,2-ethylenediamine-*N*,*N*′-diacetic acid[18] was employed (see Scheme 3). The first four steps of this route run smoothly (see Experimental). However, in the last step: the opening of the disulfide bond by $Na/NH_{3\ liq.}$, the aromatic ring of the benzyl group is also hydrogenated by Birch reduction.[21] Although aerial oxidation of the cyclohexadiene may allow reformation of the aryl ring, the thiol groups will also be oxidized. The *gem*-dimethyl disulfide bond is much more stable than the unsubstituted disulfide. Several other reducing reagents (Na metal, $NaBH_4$, and $LiBH_4$) were tried; none of them gave a clean product. At this time, another route, Scheme 4, was designed.

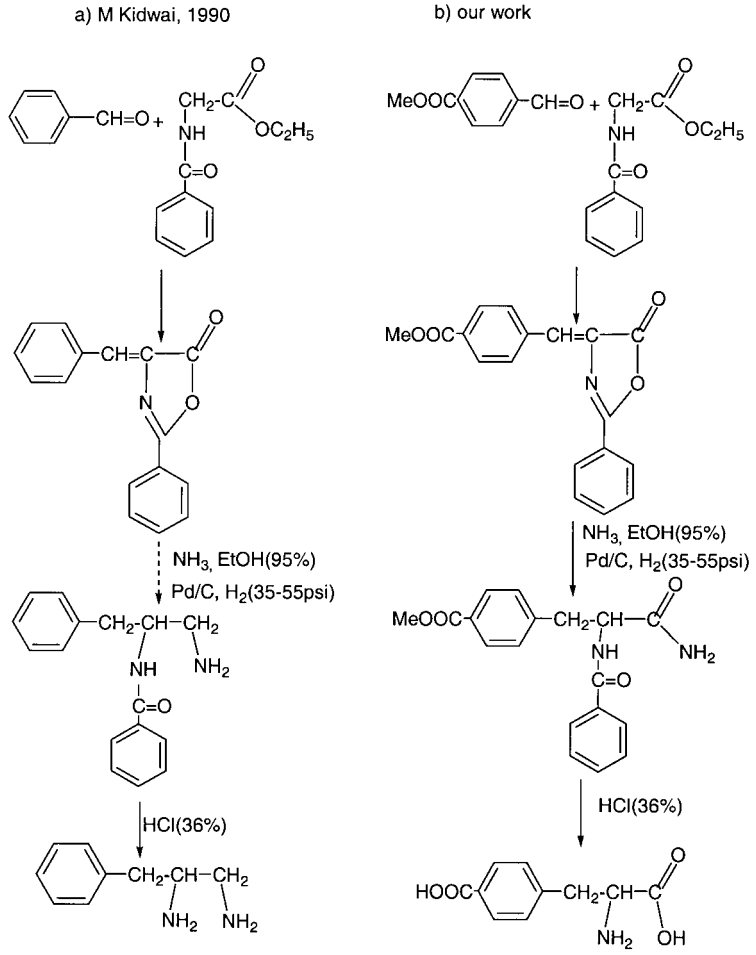
For the preparation of multidentate ligands containing gem-dimethyl thiol groups, compound **10**: 2-(*p*-methoxybenzylthio)-2-methylpropanal is a very useful intermediate, since the condition for the deprotection of the methoxybenzyl group is much milder than that of the benzyl group.[22] Koji Yoneda et al. 1993[23] published a three step route for the synthesis of this aldehyde (see Scheme 5). The starting material is not commercially available. Jones and Elmaleh[24] (1990) reported a one-pot synthesis of the benzyl-protected aldehyde, 2-benzylthio-2-methylpropanal (Scheme 6). However, due to the fact that the aldehyde group also can be attacked, the yield is low. The Jones and Elmalehs' route was modified by protecting the aldehyde group by Schiff base formation before chlorination with *N*-chlorosuccinimide. The remaining steps are simple (see Scheme 7), and the overall yield is above 70%.

This new BFC (**nbi6ss**, **1**) is designed as a pre-conjugation type bifunctional ligand. After conjugation, the protecting groups (*t*-butyl and methoxybenzyl) will be removed.[22] This strategy may work when conjugating small peptides or non-biological macromolecules. For conjugating bio-macramolecules, during the removal of these protecting groups, acid treatments may compromise the biological activity of biomolecules irreversibly. This new BFC (**nbi6ss**, **1**) may also be used as the precursor of a precomplexation type BFC, i.e. to remove the *t*-butyl and methoxybenzyl protecting groups and prepare its In-111 complexes, then conjugate to proteins.

## Experimental

### Materials and methods

Aminoacetonitrile disulfate, benzoyl chloride, methyl 4-formylbenzoate, *iso*-butylaldehyde, *sec*-butylamine, *N*-chlorosuccinimide, *p*-methoxybenzylthiol, *t*-butyl bromoacetate, sodium cyanoborohydride and bis-trimethylsilyl acetamide (BSA), were obtained from Aldrich Chemical Co. and were used as supplied.




**Scheme 2.**

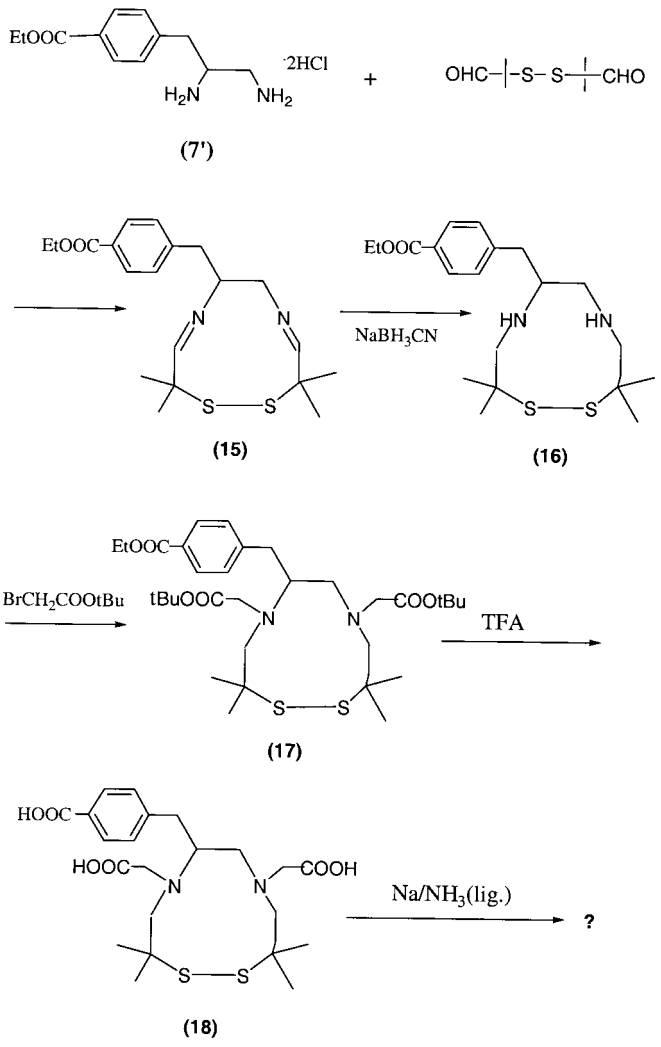
The proton and carbon-13 NMR were recorded on a Varian XL-200 spectrometer operating at 200 MHz, and the chemical shifts are reported in ppm relative to tetramethylsilane. The mass spectra were obtained with a VG analytical 70S high resolution double focusing magnetic sector spectrometer with an attached VG analytical 11/250J data system. Fast atom bombardment (FAB) technique was used for ionization. The C, H, N analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. The melting point was determined with a Fisher-Johns melting point apparatus and was uncorrected.

## Synthetic procedures

The route for the synthesis of 1-(*p*-carboxybenzyl)-ethylenediamine dihydrochloride (**7**) and 1-(*p*-carbethoxybenzyl)-ethylenediamine dihydrochloride (**7′**) involving intermediates **8** and **9** is shown in Scheme 1

**2-Benzamidoacetonitrile (8).** The procedure of Li, Gang et al.[25] was followed and modified. A solution of 60 g (0.39 mol) of aminoacetonitrile disulfate in 150 mL water was placed in a 1 L three-necked flask in a 5–6°C bath with mechanical stirring. A solution of 60 g (0.43 mol) of benzoyl chloride in 200 mL of benzene was added dropwise. At the same time anhydrous $Na_2CO_3$, 82 g (0.78 mol) was added portionwise. After the addition was finished, the reaction mixture was stirred at room temperature for 20 h. The crude product was taken out by filtration and washed with 3×100 mL of benzene and 3×100 mL of ethyl ether and was vacuum dried over $P_2O_5$ for 16 h. To 49 g of this crude product 150 mL of methanol was added and heated to reflux for 20 min. The hot suspension was filtered and cooled at 0°C for 16 h. A large amount of crystalline material was separated. The product was collected by filtration and washed with dry ethanol and vacuum drying; 44 g of crystalline product was obtained, yield 80%. Mp=146–148°C (lit. [25] 140°C). $^1$H NMR (in $CDCl_3$): 7.80 (d, 2H, arom.); 7.51 (m, 3H, arom.); 6.6 (b,H, CONH); 4.40 (d, 2H, $-CH_2-$).

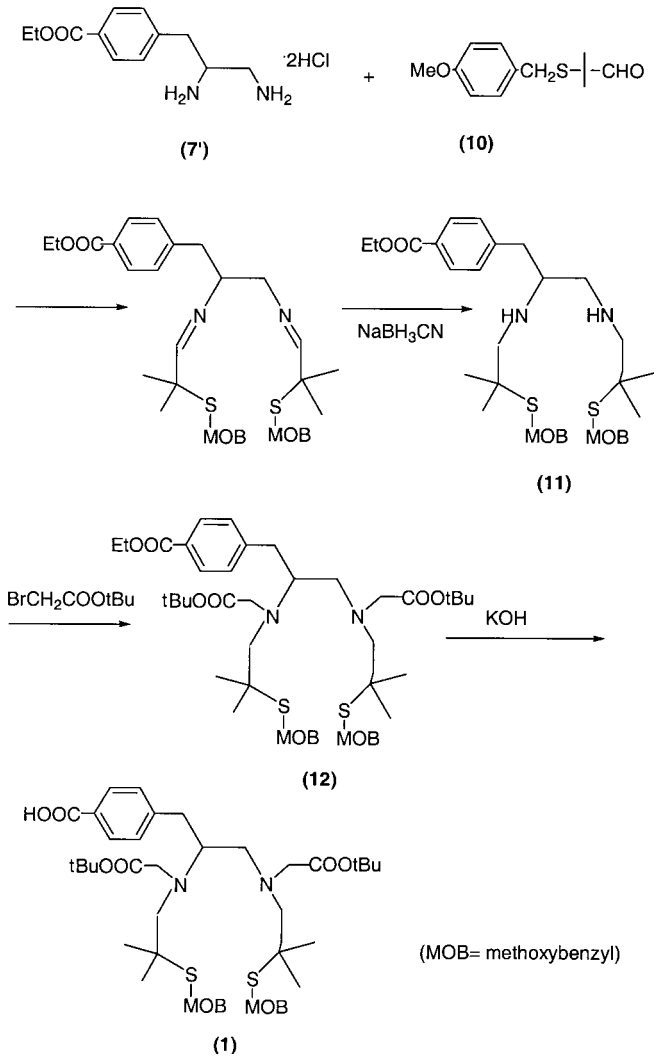
**2-(*p*-Carbomethoxybenzylidine)-2-benzamidoacetonitrile (9).** A 500 mL three-necked flask was equipped with a mechanical stirrer and $HCl_g$ inlet and outlet tubes. A solution of 4.26 g (0.026 mol) methyl 4-formylbenzoate in 250 mL of dry ethyl ether and 4 g (0.025 mol) of *N*-benzamidoacetonitrile (**8**) was added to the flask. The reaction mixture was cooled to about −10°C by a NaCl–ice-water bath. Dry HCl gas was bubbled through the reaction mixture for about 30 min. The mixture was then stirred at −5°C for another half hour. It was filtered with a sintered glass funnel and washed with 2×100 mL of benzene. The wet residue was suspended in about 50 mL of benzene. This suspension was evaporated under reduced pressure to nearly dryness.




**Scheme 3.**

More benzene was added and the solid material was separated by filtration and washed with benzene and ethyl ether. It was air-dried and 6.1 g of yellow solid was obtained. This yellow solid was suspended in 50 mL 5% $Na_2CO_3$ in aqueous solution and was stirred in an ice-water bath for 2 h. The insoluble material was separated by filtration and washed with 3×50 mL cold 2% $Na_2CO_3$ solution and 5×60 mL cold water, until the washings became neutral. This solid was vacuum dried over $P_2O_5$ at room temperature for 2 days; 4.8 g crude product was obtained. It was dissolved in 70 mL boiling toluene. After hot filtration, the clear filtrate was allowed to stand at 0°C, 2.9 g white pure product was obtained, yield 30%. $^1$H NMR (in $CDCl_3$): 8.03 (d, 2H, arom.); 7.79 (d, 2H, arom.); 7.55–7.47 (m, 5H, arom.); 7.85 (b, H, CONH); 6.84 (s, H, CH═C–); 3.88 (s, 3H, $CH_3OOC$–). FAB. MS: $[M+H^+]$=307. Anal. Calcd for: $C_{18}H_{14}N_2O_3$: C, 70.36; H, 4.56; N, 9.12. Found: C, 70.00; H, 4.64; N, 9.36.

**1-(*p*-Carbethoxybenzyl)-ethylenediamine dihydrochloride (7′)** Compound **9**, 5 g (16 mmol) was added to a 500 mL Parr glass bottle containing 55 mL of absolute ethanol saturated with $NH_3$ gas in an ice-water bath. Raney Nickel (CAUTION! dry solid is flammable), which was prewashed with water, 90% ethanol and absolute ethanol, 3–4 g, were added and the reaction mixture was hydrogenated at 50–55 psi and room temperature for 20 h. The reaction mixture was degassed (remove $H_2$ and $NH_3$ by aspiration), and then filtered through decolorized charcoal. The insoluble material was washed with 6×50 mL of absolute ethanol. The filtrate and washings were combined and acidified with 6 M HCl to pH<1. The $NH_4Cl$ that was separated was removed by filtration. The filtrate was concentrated under reduced pressure until a yellow residue was obtained. To this solid, 140 mL of conc. HCl was added and heated in a 120°C bath for 48 h. After cooling, it was filtered and the clear filtrate was evaporated under reduced pressure to nearly dryness. Then it was further dried with 250 mL of benzene by azeotropic distillation, and 4.5 g of yellow powder was obtained. This is the crude product of 1-(*p*-carboxybenzyl)-ethylenediamine dihydrochloride (**7**). $^1$H NMR (in $D_2O$, pD=1.5, *t*-BuOH as internal standard, 1.29 ppm): 7.88 (d. 2H, arom.); 7.33 (d, 2H, arom.); 3.83 (quint. H, –CH–); 3.25–2.94 (m, 4H, $-CH_2CHCH_2$–). $^{13}$C NMR (in $D_2O$, pD=1.5, *t*-BuOH as internal standard, 31.1 ppm): 169.3 (–C═O), 138.9, 129.7, 128.8, 128.5 (arom.); 49.51 (–CH–); 39.81 and 35.30 ($-CH_2CHCH_2$–). FAB. MS.: $[M+H^+]$=195.

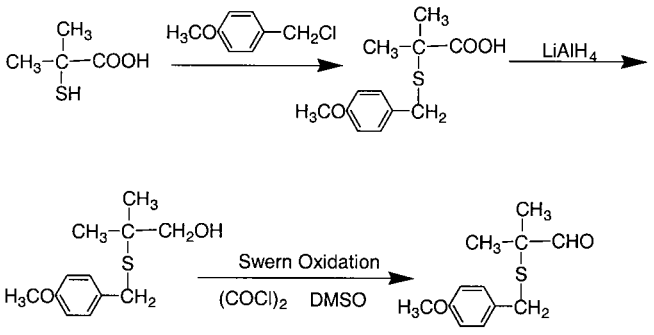



**Scheme 4.**

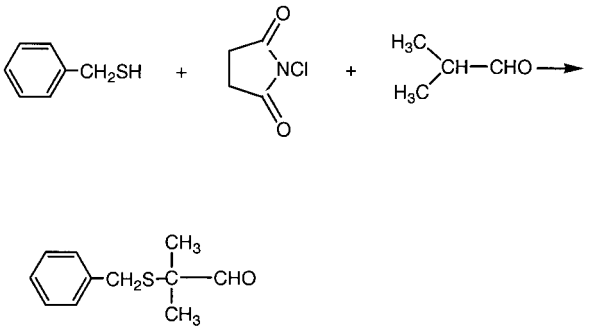
The 4.5 g yellow powder of the above crude product was esterified with 600 mL of absolute ethanol and $HCl_g$. The product was recrystallized with 10–15 mL of absolute ethanol, and 1.98 g of the ethyl ester (**7′**) of compound **7** was obtained, yield 41%. $^1$H NMR (in $D_2O$, pD=1.5, *t*-BuOH as internal standard, 1.29 ppm): 7.88 (d. 2H, arom.); 7.33 (d, 2H, arom.); 4.24 (quat. 2H, $-CH_2CH_3$); 3.82 (quint. H, –CH–); 3.25–2.96 (m, 4H, $CH_2CHCH_2$–); 1.22 (t, 3H, $-CH_3$). $^{13}$C NMR (in $D_2O$, pD=1.5, *t*-BuOH as internal standard, 31.1 ppm): 167.9 (–C=O), 139.0, 129.8, 129.0, 128.8 (arom.); 61.68 ($-CH_2CH_3$); 49.66 (–CH–); 39.96 and 35.45 ($-CH_2CHCH_2-$); 12.71 ($-CH_3$). FAB. MS: $[M+H^+]$=223. Anal. Calcd for: $C_{12}H_{18}N_2O_2 \cdot 2HCl.NH_4Cl \cdot 3/4H_2O$: C, 39.78; H, 7.04; N, 11.60. Found: C, 39.77; H, 6.70; N, 11.40.

The route used for the synthesis of **nbi6ss (1)** involving intermediates **11** and **12** is shown in Scheme 4.

**1-(*p*-Carbethoxybenzyl)-*N*,*N*′-[1,1-dimethyl-1-(*p*-methoxybenzylthio) ethyl]ethylenediamine (11).** A sodium ethanolate solution was prepared by dissolving 0.14 g (6 mmol) of

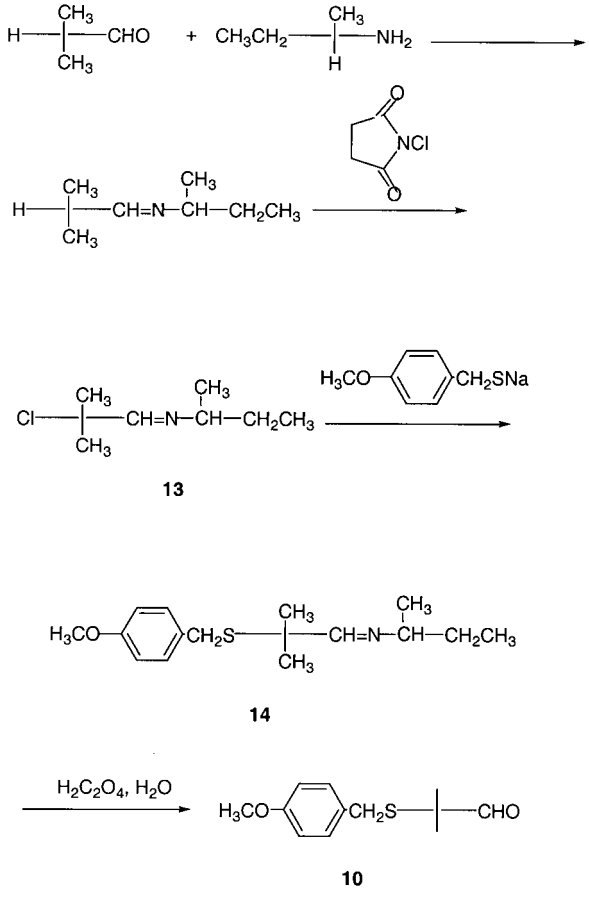



**Scheme 5.** Koji Yoneda et al., 1993.



**Scheme 6.** Jones and Elmaleh, 1990.

sodium into 4.5 mL of absolute ethanol. This solution was added to a suspension of 0.89 g (3 mmol) of compound **7′** in 3 mL of absolute ethanol; then a solution of 1.49 g (6 mmol) of compound **10** (see the procedures bellow) in 10 mL of $CCl_4$ was added. This reaction mixture was stirred at room temperature for 10 min. The solvents were removed by evaporation under reduced pressure. To the residue 10 mL of $CCl_4$ and 3–4 g of anhydrous $MgSO_4$ were added and the mixture was stirred at room temperature for another 20 h. The insoluble material was removed by filtration and the solvents were removed by evaporation under reduced pressure. To the residue 12 mL of methanol was added; 0.35 g of $NaBH_3CN$ was added portionwise, while the pH of the solution was maintained at about 4. The reaction was stirred at room temperature for 1.5 h. After the methanol was removed, a solution of 1.2 g of 50% NaOH in 15 mL of saturated NaCl solution was added; 3×30 mL of ethyl ether were used to extract the product. The combined ether solutions were dried with anhydrous $MgSO_4$ for 16 h. After the solvents and drying agent were removed, 1.7 g pale yellow oil was obtained. It was purified by flash chromatography; 0.76 g pure product was obtained, yield 40%. $^1$H NMR (in $CDCl_3$): 7.95 (d. 2H, arom.); 7.25–7.16 (m, 6H, arom.); 6.81–6.77 (d, 4H, arom.); 4.35 (quat. 2H, $CH_2CH_3$); 3.75 (s, 6H, $CH_3O$–); 3.63–3.56 (two singlet, 4H, $CH_2S$–); 2.8–2.2 (m. 9H, $-CH_2CHCH_2$, $-CH_2C(CH_3)_2$); 1.9 (b, 2H, –NH–); 1.4–1.3 (m, 15H, $-CH_3$). $^{13}$C NMR (in $CDCl_3$): 166.2 (–C═O of benzoate), 158.1, 144.5, 130.1, 129.5, 129.2, 128.0 (arom.);113.5 (C-3 of methoxybenzyl); 60.4($-CH_2CH_3$); 59.1 and 58.9(–CH–); 56.8, 54.8, 52.7, 46.6, ($-CH_2CHCH_2$–, NH–$CH_2$–, $CH_3O$–); 39.3 ($-SCH_2$–); 31.7 ($-C(CH_3)_2$); 26.9 ($-C(CH_3)_2$); 13.9 ($-CH_2CH_3$). FAB. MS: $[M+H^+]$=639. Anal. Calcd for:$C_{36}H_{50}N_2O_4S_2$: C, 67.71; H, 8.14; N, 4.39. Found: C, 67.60; H, 8.04; N, 4.48.



**Scheme 7.**

**1-(*p*-Carbethoxybenzyl)-*N*,*N′*-[1,1-dimethyl-1-(*p*-methoxybenzylthio)ethyl]ethylenediamine-*N*,*N′*-diacetic acid, di-*t*-butyl ester (12).** Compound **11**, 0.64 g (1 mmol), *t*-butyl bromoacetate, 3.9 g (20 mmol) and 2,6-lutidine, 0.24 g (2 mmol) were mixed and stirred at room temperature for 24 h. The excess reactants were removed by distillation under reduced pressure. The crude product was dried at 40°C (0.1 mm Hg) for 6 h, and 0.9 g of pale yellow oil was obtained. It was purified by flash chromatography, the product was eluted by hexane/ethyl acetate=95:5; and 0.14 g pure product was obtained, yield 16%. $^1$H NMR (in $CDCl_3$): 7.90 (d, 2H, arom.); 7.26–7.19 (m, 6H, arom.); 6.82–6.79 (d, 4H, arom.); 4.33 (quat. 2H, $CH_2CH_3$); 3.77 (s, 6H, $CH_3O$–); 3.63–3.58 (two singlet, 4H, $CH_2S$–); 3.5–3.0 (m, 9H, $-CH_2CO$–, $-CH_2CHCH_2$–); 2.8–2.3 (m, 4H, $CH_2C(CH_3)_2$); 1.5–1.1 (m, 33H, $CH_2CH_3$, $-C(CH_3)_2$ and $-C(CH_3)_3$). $^{13}$C NMR (in $CDCl_3$): 171.8 and 171.1 (C═O of acetate) 166.4 (–C═O of benzoate), 158.1, 146.1, 130.0, 129.7, 129.2, 127.8 (arom.); 113.6 (C-3 of methoxybenzyl); 80.6 and 80.4 ($-CH_2-$ of the two acetate); 60.4 ($CH_2CH_3$); 58.0 and 57.3 (–CH–); 66.7, 65.5, 63.2, 54.9, 53.4, 47.7, ($-CH_2CHCH_2$–, NH–$CH_2$–, $CH_3O$–); 36.4 ($-SCH_2$–); 31.7 ($C(CH_3)_2$); 27.8 ($-CH_3$ of *t*-butyl); 26.9($-C(CH_3)_2$); 14.0 ($CH_2CH_3$). FAB. MS.: $[M+H^+]$=867. Anal. Calcd for: $C_{48}H_{70}N_2O_8S_2{\cdot}1.5H_2O$: C, 64.50; H, 8.17; N, 3.14. Found: C, 64.66; H, 8.15; N, 3.01.

**1-(*p*-Carboxybenzyl)-*N*,*N′*-[1,1-dimethyl-1-(*p*-methoxybenzylthio) ethyl]ethylenediamine-*N*,*N′*-diacetic acid, di-*t*-butyl ester (1).** Compound **2**, 0.135 g (0.156 mmol) was dissolved in 6.8 g of KOH–$H_2O$–1, 2-dimethoxyethane (0.12 g KOH (87%), 3.8 g $H_2O$ and 5.6 g of 1,2-dimethoxyethane) solution, and was heated to reflux under Ar for 1 h. After the solution was cooled to rt the solvents were removed by evaporation under reduced pressure. To the residue 10 mL of water was added and 2.5 mL HCl was used to acidify the potassium salt. Dichloromethane was used to extract the product. After it was worked up and dried, 0.12 g of pure product (pale yellow oil) was obtained, yield 80%. $^1$H NMR (in $CDCl_3$): 7.90(d. 2H, arom.); 7.26–7.19 (m, 6H, arom.); 6.82–6.79 (d, 4H, arom.); 3.75 (s, 6H, $CH_3O$–); 3.63–3.58 (two singlet, 4H, $CH_2S$–); 3.5–2.5 (m,

13H, $-CH_2CO-$, $-CH_2CHCH_2-$, $-CH_2C(CH_3)_2$); 1.5–1.1 (m, 30H, $-C(CH_3)_3$ and $-CH_2C(CH_3)_2$). $^{13}C$ NMR (in $CDCl_3$): 171.9 and 171.2 (C=O of acetate); 158.2 (–C=O of benzoic acid), 147.2, 129.8, 127.8 126.7 (arom.); 114.0 (C-3 of methoxybenzyl); 80.7 and 80.6 ($-CH_2-$ of the two acetate); 58.0 and 57.3 (–CH–); 66.6, 65.4, 63.4, 54.9, 53.6, 47.7, ($-CH_2CHCH_2-$, NH–$CH_2-$, $CH_3O-$); 36.5 ($-SCH_2-$); 31.8 ($-C(CH_3)_2$); 27.9 ($-C(CH_3)_3$); 26.8 ($-C(CH_3)_2$); FAB. MS: $[M+H^+]$=839. Anal. Calcd For $C_{46}H_{66}N_2O_8S_2 2.5H_2O$: C, 62.51; H, 8.04; N, 3.17. Found: C, 62.77; H, 7.62; N, 3.18.

The route used for the synthesis of 2-(4-methoxybenzylthio)-2-methylpropanal (**10**) involving intermediates **13** and **14** is shown in Scheme 7.

***N*-(2-Chloro-isobutylidene)-*sec*-butylamine (13).** A mixture of 7.2 g (0.1 mol) of *iso*-butylaldehyde and 7.3 g (0.1 mol) of *sec*-butylamine was stirred at room temperature for 5 min. To the reaction mixture 150 mL of carbon tetrachloride and anhydrous $MgSO_4$ were added; this suspension was stirred at room temperature for another 2 h. The insoluble material was removed by filtration. To the filtrate 14.7 g (0.11 mol) of *N*-chlorosuccinimide was added portionwise while the temperature was maintained at rt by means of a water bath. After it was stirred thoroughly for 3 h, the reaction mixture was filtered and washed with a small amount of $CCl_4$. The solvent was then removed in vacuo and the turbid colorless oil was distilled under reduced pressure; 11.8 g pure product was obtained, bp=63– 64°C/30 mmHg. $^1H$ NMR (in $CDCl_3$): 7.65 (s, H, CH=N–); 3.03 (quint. H, N–CH–); 1.69 (s, 6H, $(CH_3)_2CCl-$); 1.50 (quat. 2H, $-CH_2-$), 1.13 and 1.15 (d, 3H, CH–$CH_3$) 0.78 (t. 3H, $CH_2CH_3$). $^{13}C$ NMR (in $CDCl_3$): 162.5(–CH=N–); 67.76 (–CCl–), 66.52 (=N–CH–); 29.87 ($-CH_2CH_3$); 29.08 ($(CH_3)_2CCl$); 21.53 (CH–$CH_3$); 10.43 ($CH_2CH_3$).

***N*-[2-(*p*-methoxybenzylthio)-*iso*-butylidene]-*sec*-butylamine (14).** A sodium methanolate solution was prepared by dissolving 0.8 g (0.033 mol) of Na in 17 mL of methanol. To this solution, 12 g (0.035 mol) of 4-methoxybenzylthiol was added dropwise. After the addition was complete, 5.3 g (0.033 mol) of compound **13** was added slowly. The reaction mixture was heated to 85°C for 30 min. The cooled mixture was poured into 70 mL of water and extracted with dichloromethane. The organic extractions were combined and dried with anhydrous $MgSO_4$ for 16 h. After the solvent and drying agent were removed, 10 g of colorless oil was obtained. It was crystallized after cooling to about -20°C. $^1H$ NMR (in $CDCl_3$): 7.48(s, H, –CH=N–); 7.16 (d, 2H, arom.); 6.80 (d, 2H, arom.); 3.74 (d, 3H, methoxy); 3.58 (s, 2H, $-CH_2$-S–); 3.04 (hexad. H, N–CH–); 1.50 (quint. 2H, $CH_2CH_3$); 1.41 (s, 6H, $(CH_3)_2CS-$).

**2-(*p*-Methoxybenzylthio)-2-methylpropanal (10).** A warm solution of 3.78 g (0.03 mol) of oxalic acid ($C_2H_2O_4 2H_2O$) was added to a solution of 5.6 g of compound **14** in 20 mL of dichloromethane with vigorous stirring at rt. for 1 h. The organic phase was separated and the aqueous phase was extracted with 2×15 mL of dichloromethane. The combined dichloromethane solutions were dried with anhydrous $MgSO_4$. After the solvent and drying agent were removed, 5.1 g of colorless oil was obtained, which was crystallized after cooling to about −20°C. $^1H$ NMR (in $CDCl_3$): 9.12 (s, H, CH=O); 7.18 (d, 2H, arom.); 6.82 (d. 2H, arom.); 3.78 (s, 3H, methoxy-); 3.47 (s, 2H, $-CH_2S-$); 1.38 (s, 6H, two methyl groups). $^{13}C$ NMR (in $CDCl_3$): 193.7 (–CH=O); 158.8, 130.5, 128.6, 113,9 (arom.); 55.22 (methoxy-), 42.76 ($-C-$ $(CH_3)_2-$); 32.81 ($-CH_2S-$); 21.08 (the two methyl groups).

The synthetic procedures of the new compounds in Scheme 3 are shown as follows:

**6-(*p*-Carbethoxybenzyl)-3,3,10,10-tetramethyl-1,2-dithia-5,8-diaza-cyclodeca-4,8-diene (15)** Compound **7′**, 1.77 g (6 mmol), was placed in a 50 mL two-necked flask, which was connected with a trap and vacuum distillation equipment. The whole system was flushed with Ar for three times before BSA, 15 mL (60 mmol) were added. The reaction mixture was heated to 50°C bath for 1 h. Then vacuum at 30–40 mm Hg for 30 min was used to remove the excess BSA. A solution of 2,2′-dithio-bis(2-methylpropanal),[26] 1.26 g (6.12 mmol) in 8 mL of benzene was added, and the reaction mixture was heated to 70–80°C for 3 h. The solvents and by-products were removed by distillation under reduced pressure. About 40 mL of benzene and 40 mL of water were added to extract the product and deprotect the trimethylsilyl groups. The organic phase was filtered and dried with anhydrous $MgSO_4$ for 16 h. The crude product was loaded on 45 g of silica gel 60 and eluted with benzene, $CH_2Cl_2$ and $CH_2Cl_2$–MeOH (v/v=98/2); 1.63 g of pure product was obtained, yield 68%. $^1H$ NMR (in $CDCl_3$): 7.95 and 7.92 (d, 2H, 3,5-arom.) 7.25 and 7.23(d, 2H, 2,6-arom.); 6.90 and 6.78 (two s, 2H, –CH=N–); 4.37–4.35 (quadri. 2H, $-CH_2CH_3$); 4.12–4.07 (m, 1H, 1 of ethylene); 3.4 and 3.1–2.8 (m, 4H, $-CH_2-$ of benzyl and ethylene); 1.43–1.22 (m, 15H, methyl). $^{13}C$ NMR (in $CDCl_3$): 167.9 and 166.0 (–CH=N–); 160.4 (–CO–O); 144.4 (4-benzene); 129.3–129.2 (2,3,5,6-benzene); 128.4 (1-benzene); 73.27 (1 of ethylene); 66.52 (2 of ethylene); 60.76 ($-CH_2CH_3$); 52.7 ($-CH_2-$ of benzyl); 39.98 ($-C(CH_3)_3$); 24.4–21.1 (methyl). FAB.MS: [M+H]=393.

**6-(*p*-Carbethoxybenzyl)-3,3,10,10-tetramethyl-1,2-dithia-5.8-diaza-cyclodecane (16).** A 1.2 M HCl–$CH_3OH$ solution (conc. HCl/$CH_3OH$=1:9, v/v) was added to a solution of 1.6 g (4.1 mmol) of compound **15** in 25 mL of methanol until pH was about 5. Sodium cyanoborohydride ($NaBH_3CN$), 0.51 g (8.2 mmol) was added portionwise within 2 h. The pH of the reaction mixture was maintained at 5.0–5.3 with 1.2 M HCl–$CH_3OH$. More acid was added until pH was 1.8. About 5 mL of water was added and the methanol was removed by distillation under reduced pressure. To the resulting mixture 16 mL of water were added, and 7.5 M of $NH_3$–$H_2O$ was added until pH is about 9.5. The product was extracted with 32 mL of $CH_2Cl_2$, which was then filtered and dried, with anhydrous $MgSO_4$ for 2 days. After working up, 1.6 g of yellow oil was obtained. It was purified by flash chromatography; 0.99 g of pure product was obtained from $CH_2Cl_2$/MeOH=98:2, 9:1 and $CH_2Cl_2$/MeOH/conc.$NH_3$–$H_2O$=100:15:2, yield 62%. $^1H$ NMR (in $CDCl_3$): 7.97 (m, 2H, 3,5-benzene); 7.26 (m, 2H, 2,6-benzene); 4.37 (quadri, 2H, $-CH_2CH_3$); 3.2–2.2

(m, 9H, –NH–$CH_2$, –NH–$CH$ and $CH_2$– of benzyl); 2.1–1.8 (b, 2H, –NH–); 1.4–1.1 (m, 15H, methyl). $^{13}$C NMR (in $CDCl_3$): 160.2 (–$CO$O); 144.6 (4 of benzene); 129.5, 128.9 and 128.7 (1,2,3,5,6 of benzene); 61.6 (–$CH_2$CH$_3$); 60.6–50.4 (–NH–$CH_2$, –NH–$CH$); 40.9 (–$CH_2$– of benzyl); 29.3–23.7 (–$C$(C$H_3$)$_2$, –$C$(C$H_3$)$_3$); 14.1 (–CH$_2$$C$H$_3$). FAB MS: [M+H]=397. Anal. Calcd for $C_{20}H_{32}N_2O_2S_2\cdot 1/2H_2O$: C, 59.26; H, 8.15; N, 6.91. Found: C, 59.47; H, 8.33; N, 6.86.

**6-(*p*-Carbethoxybenzyl)-3,3,10,10-tetramethyl-1,2-dithia-5,8-diaza-cyclodecane-*N*,*N*′-diacetic acid, di-*tert*-butyl) ester (17).** A mixture of 0.24 g (0.61 mmol) of compound **16**, 0.72 g (3.7 mmol) of *t*-butyl bromoacetate; 0.25 g (1.8 mmol) $K_2CO_3$ and 0.1 g (0.61 mmol) of KI was prepared and was stirred at rt for 19 h. It was filtered and washed with $CH_2Cl_2$. The filtrate and washings were combined and the solvents and excess BrCH$_2$COO–*t*-Bu were removed by distillation under reduced pressure. After vacuum drying at 1 mmHg/50°C for 6 h, 0.45 g of pale yellow oil was obtained. It was purified with silica gel 60 and was eluted by benzene and benzene–ethyl ether; 0.3 g of pure product was obtained, yield 79%. $^1$H NMR (in $CDCl_3$): 7.97 (d, 2H, 3,5-benzene); 7.26 (d, 2H, 2,6-benzene); 4.36 (quadri, 2H, –$CH_2$CH$_3$); 4.33.8 and 3.4–2.8 (m, 9H, –N–CH$_2$C–, –N–$CH$ and CH$_2$– of benzyl); 2.2–2.5 (m, 4H, –$CH_2$–COO*t*-Bu); 1.5–1.1 (m, 33H, methyl). $^{13}$C NMR (in $CDCl_3$): 171.5 and 170.7 (–C═O of the ester); 166.6 (C═O– of the benzoate); 145.6 (4 of benzene); 129.5, 129.4 and 128.3 (1,2,3, 5,6 of benzene); 80.0 (–$CH_2$– of the acetate); 60.7, 58.8, 56.0, 53.7, 50.1 (–$CH_2$CH$_3$, –N–$CH_2$CH–, –N–$CH_2$C–, –CH$_2$– of benzyl); 38.3 (–$C$(CH$_3$)$_3$); 33.2 and 32.3 (–$C$(CH$_3$)$_2$); 28.1 (–C(CH$_3$)$_3$); 27.0 and 25.3 (–C(CH$_3$)$_2$); 14.3 (–CH$_2$CH$_3$). FAB. MS: [M+H$^+$]=625. Anal. Calcd for $C_{32}H_{52}N_2O_6S_2\cdot 1/2H_2O$: C, 60.06; H, 8.37; N, 4.42. Found: C, 60.23; H, 8.36; N, 4.26.

**6-(*p*-Carboxybenzyl)-3,3,10,10-tetramethyl-1,2-dithia-5,8-diaza-cyclodecane-*N*,*N*′-diacetic acid (18).** In an ice-water bath, 0.35 mL of water was added to a suspension of 7.85 g (0.070 mmol) of $K^+OC(CH_3)_3^-$ in 77 mL of ethyl ether. A solution of 1.7 g (2.7 mmol) of compound **17** in 50 mL of ethyl ether was added dropwise. This reaction mixture was stirred at rt for 27 h. After 10 mL of water was added, it became two phases. The yellow aqueous phase was separated and the ether phase was washed with water. The aqueous phase and washings were combined and neutralized with 6 M HCl until pH=2–3. A large amount of yellow precipitate was obtained. It was taken out, washed and redissolved in dilute KOH aqueous solution and reprecipitated with dilute HCl at pH=1.8. After vacuum drying, 0.92 g pale yellow powder-like product was obtained; another 0.1 g pure product was obtained from the filtrate; total yield 1.02 g, 77%. $^1$H NMR (in D$_2$O–NaOD; CH$_3$OD as internal standard: 3.34 ppm; pD=12): 7.81 (d, 2H, 3,5-benzene); 7.23 (d, 2H, 2,6-benzene); 3.4–2.1 (m, 13H, –N–CH$_2$–, –N–CH and CH$_2$– of benzyl); 1.4–1.2 (m, 12H, methyl). $^{13}$C NMR (in D$_2$O–NaOD; CH$_3$OD as internal standard: 49.0 ppm; pD=12): 179.4 and 179.0 (–C═O of the di-acetate); 174.9 (–C═O– of the benzoate); 143.7 (4 of benzene); 134.4, 129.5, 129.4 and 128.9 (1,2,3,5,6 of benzene); 62.7, 53.2, 51.8, 51.1, 50.8 (CH$_2$CH$_3$, –N–CH$_2$CH–, –N–CH$_2$C–, –CH$_2$– of benzyl); 32.3, 31.7, (–C(CH$_3$)$_2$); 30.8, 29.0 27.7, 27.0 (–C(CH$_3$)$_2$); FAB. MS: [M+H$^+$]=484. Anal. Calcd for $C_{22}H_{32}N_2O_6S_2\cdot 1.5H_2O$: C, 51.66; H, 6.85; N, 5.48. Found: C, 51.37; H, 6.34; N, 5.20.

## Preliminary experiment about conjugation

About 0.11 g (0.13 mmol) of compound **1** and 11.6 mg (0.13 mmol) of dl-alanine in of $CH_2Cl_2$ (0.5 mL) were treated with a solution of 26.8 mg (0.13 mmol) of DCC (*N*,*N*′-dicyclohexylcarbodiimide) in 0.5 mL of $CH_2Cl_2$. After 15 min., the conjugated precipitate was filtered and washed with small amount of ethyl acetate. The $^{13}$C NMR of this product shows a benzamide peak at 162 ppm.

## Acknowledgements

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