An Efficient Route for the Synthesis of New Analogues of Polyaminocarboxylic Acid Incorporating O, N, and S Atoms

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We have synthesised three rigid polycarboxylated hexadentate ligands that differ only in the nature of their heteroatom, either oxygen (**1**), nitrogen (**2**), or sulfur (**3**). These compounds were obtained in four or five steps from substituted orthoanilines after protection/deprotection sequences. An aromatic backbone was chosen to stiffen the structure of the ligands and, thus, facilitate complexation (preorganization concept). A preliminary chelation test revealed a marked selectivity of the nitrogen-containing ligand for indium-111. In accordance with the hard and soft acid and base (HSAB) theory, the choice of ligand should result in notable differences in behaviour during complexation and, thus, augment selectivity with regard to the metal.

Introduction

Oxygenated, nitrogenated, and sulfurated polydentate ligands that bear carboxylic (or phosphonic) acid functions are known for their ability to complex transition and non-transition metal cations.[1−6] According to the nature of the metal used, many different applications can be considered, most notably in medical fields. For example, gadolinium complexes are employed as contrast agents in MRI and are currently used in clinical practice.[7−11] Similarly, polyaminocarboxylic derivatives are classically used to complex radionuclides for diagnostic applications (e.g., the gamma emitters ^{111}In, ^{64}Cu, ^{67}Ga, and ^{99m}Tc)[12−14] or in radioimmunotherapy (e.g., the alpha or beta emitters ^{90}Y, ^{153}Sm, and ^{212}Bi).[15−21] In all these applications, in vivo stability of the ligand−metal complex is highly desirable. Such stability requires ligands that are suitable for the chosen metal. Other examples include the use of polyaminocarboxylated ligands for trapping heavy metals, such as lead, cadmium, and mercury.[22,23] Finally, there has been increasing interest during the last ten years in using lanthanide complexes for energy transfer processes in numerous photoluminescence applications.[24−27]

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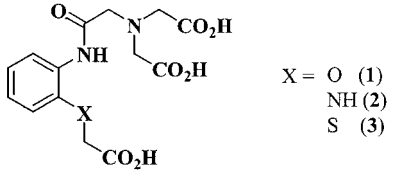

Figure 1. Ligands prepared in the study

These considerations, and our experience in the synthesis of polyaminopolycarboxylated molecules,[28−33] led us to develop three new analogous ligands (Figure 1), whose structures differ only in the nature of their heteroatom, which is either oxygen (**1**), nitrogen (**2**), or sulfur (**3**). Thus, as a function of the "hard/soft" nature of the metal, the choice of ligand should lead to a notable differences in complexation and, thus, increase the selectivity with regard to the metal. Our ligands possess an aromatic ring to induce rigidity into the cavity and to facilitate complexation (most notably by reducing the entropy of the reaction). It is known that a semi-rigid or rigid structure provides a significant increase for the stability of its complexes. In fact, rigidification of the molecular skeleton reduces the freedom of donor atoms, and this spatial preorganisation is conducive to more-efficient complexation.[34−38]

Polydentate ligands based on an aromatic design have stable geometries and are also easily functionalisable, which is an essential feature for grafting them onto any type of support (e.g., antibody, peptide, or silica). This paper describes the synthesis of three potential hexadentate ligands that are all derived from a substituted orthoaniline. The nature of the heteroatom is responsible for the notable varia-

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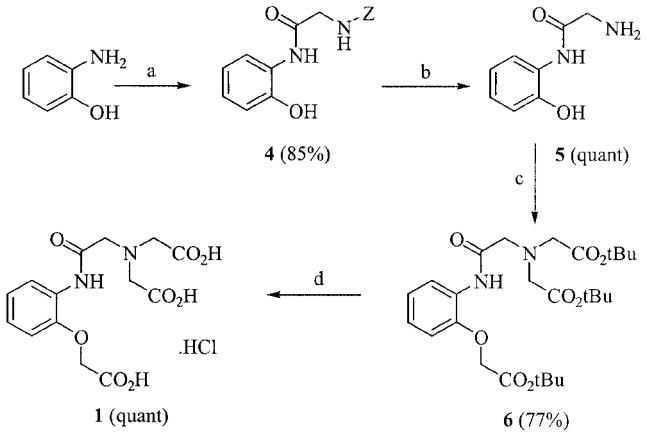
tions in the syntheses. We also checked whether selectivity during complexation was affected by the nature of the heteroatom. To do so, we performed a preliminary complexation test using radioactive indium (^{111}In).

Result and Discussion

1. Synthesis of Ligands

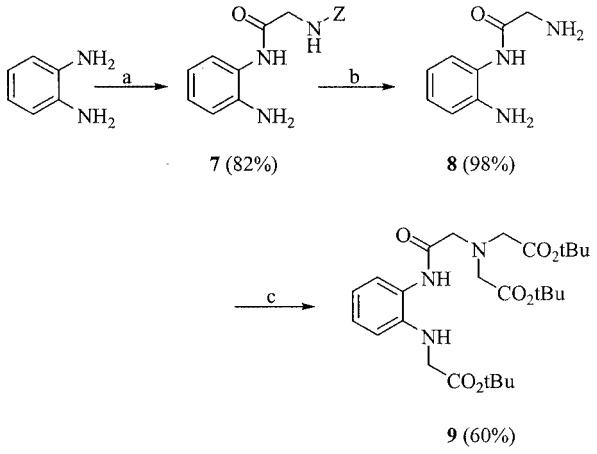
The synthesis of compound **1** was performed in four steps with an overall yield of 65% (Scheme 1). The first step involved a conventional dicyclohexylcarbodiimide-mediated amide coupling in THF between *N*-carbobenzyloxyglycine and *ortho*-aminophenol.[39] This coupling reaction was entirely chemoselective; no acylation was observed to occur on the oxygen atom. The benzyloxycarbonyl protecting group was removed by the action of 10% palladium on charcoal as a catalyst under a hydrogen atmosphere, which gave **5**[39] in quantitative yield without the need for further purification.



Scheme 1. Synthetic route to ligand **1**. *Reagents and conditions:* (a) DCC, Z-glycine, THF, reflux, 4 h; (b) EtOH, Pd/C 10%, H_2, room temp., 18 h; (c) $BrCH_2CO_2tBu$, K_2CO_3, KI, CH_3CN, room temp., 24 h; (d) TFA, room temp., 24 h, then HCl (1 M)

An alkylation reaction at ambient temperature using *tert*-butyl bromoacetate in the presence of a catalytic amount of potassium iodide and an excess of potassium carbonate in acetonitrile[40,41] gave the trialkylated molecule **6** in 77% yield. Final product **1** was obtained after hydrolysis of the esters in neat trifluoroacetic acid at ambient temperature. After 24 h of shaking, and then treatment with 1 M HCl, the triacid was precipitated as its corresponding hydrochloride salt as a white solid that was isolated in quantitative yield simply by filtration.

The synthesis of **2**, a nitrogenous homologue of **1**, began in the same manner from phenylene-1,2-diamine (Scheme 2). Monosubstitution by *N*-carbobenzyloxyglycine provided intermediary **7** in 82% yield.[42] Deprotection of the glycine amino group occurred in this case using hydrogen gas under pressure to give **8** in 98% yield. This method proved to be more efficient than the use of cyclohexene[39,43] or deprotection in an acidic medium, which gave a benzimidazole quantitatively by intramolecular cyclisation.[30] The trialkylation of **8** with *tert*-butyl bromoacetate to give **9** proved to be difficult because it was necessary to avoid polyalkylation as completely as possible.



Scheme 2. Synthesis of the intermediate **9**. *Reagents and conditions:* (a) DCC, Z-glycine, THF, reflux, 4 h; (b) EtOH, Pd/C 10%, H_2, room temp., 24 h; (c) $BrCH_2CO_2tBu$, K_2CO_3, KI, THF, 35 °C, 4 days

The best results were achieved in THF at 35 °C using 3.4 equivalents of alkylating agent. Thus, compound **9** was obtained in 60% yield by minimising the formation of the secondary products (**10**, **11**, and **12**) as much as possible (Figure 2). Alkylation of the amide (compound **10**, 15%) occurred preferentially over double substitution of the aromatic amino group (compound **11**, 7%).

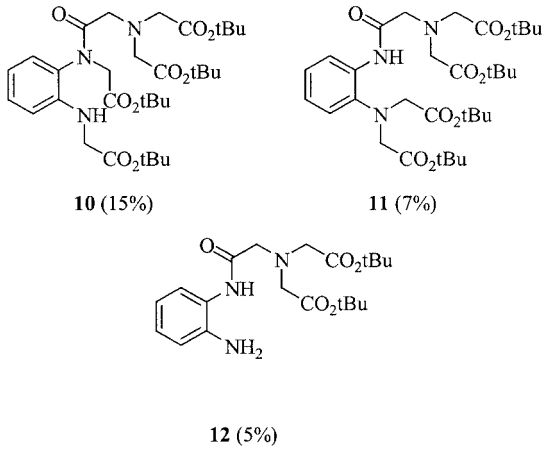
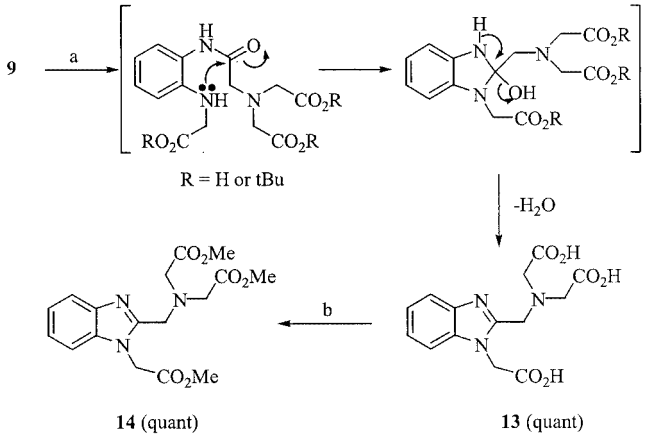


Figure 2. Secondary products obtained during alkylation

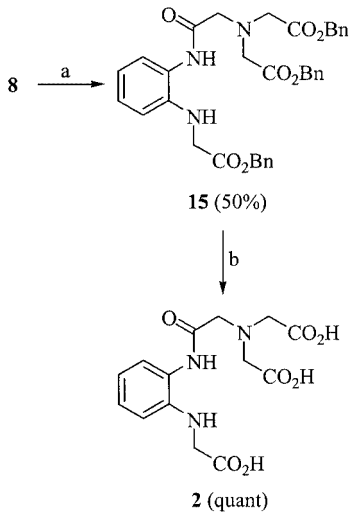
The structure of compound **9** was determined unequivocally by complementary 2D ^{1}H NMR spectroscopy techniques (COSY, HMQC, and HMBC). At this stage, the hydrolysis of the *tert*-butyl esters using trifluoroacetic acid[44,45] did not lead to the formation of the expected

compound **2**, but instead it gave a heterocyclic molecule having a molar mass that corresponded to the loss of a water molecule. The structure of compound **13**, which possesses three carboxylic acid functions, was revealed unequivocally by the action of diazomethane,[46] which provided the corresponding methyl ester **14** (Scheme 3). Because no such intramolecular cyclisation was observed with the corresponding oxygenated and sulfurated compounds, it is logical to suppose that the acidic medium favoured attack on the carbonyl group by the electron lone-pair of the aromatic amino group. The benzoimidazole structure proposed for **13**, and confirmed by spectroscopic analyses, was probably obtained according to the mechanism shown in Scheme 5.



Scheme 3. Formation of benzoimidazole **13**. *Reagents and conditions:* (a) MeOH, 6 M HCl, 60 °C, 4 days; (b) MeOH, CH_2N_2, 5 min

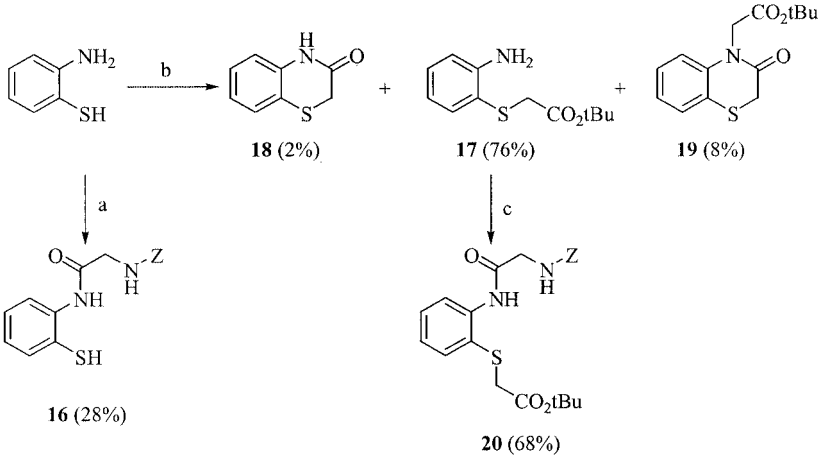
The synthesis of compound **2** was then resumed from **8** by using benzyl bromoacetate as the alkylating agent. This approach introduces ester functions that are cleavable by hydrogenolysis in a neutral medium. The intermediate **15** was then obtained in a relatively modest yield of 50% under



Scheme 4. Synthesis of ligand **2**. *Reagents and conditions:* (a) $BrCH_2CO_2Bn$, K_2CO_3, KI, THF, 35 °C, 3 days; (b) MeOH, $Pd(OH)_2$, H_2, room temp., 1.5 h

the conditions described for the synthesis of **9** (Scheme 4). The benzyl groups were then removed by hydrogenolysis using Pearlman's catalyst [$Pd(OH)_2$] in methanol.[47] Ligand **2** was obtained in this manner as a white solid in an overall yield of 40%.

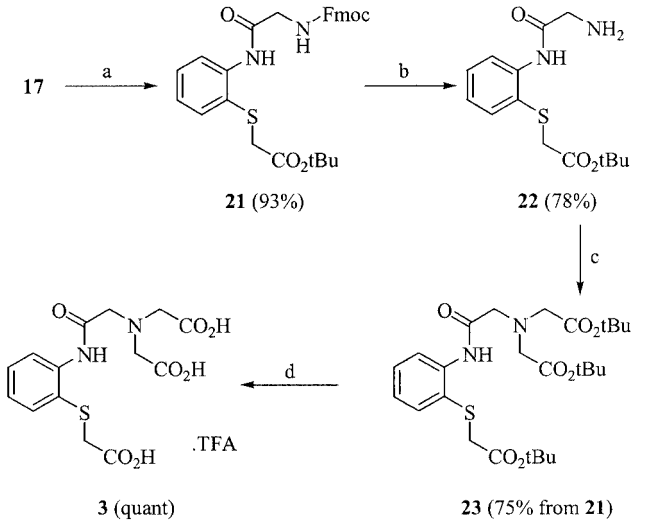
The preparation of the sulfurated ligand **3** was conducted in a manner different from that of the other two ligands. The peptide coupling between *ortho*-aminothiophenol and *N*-carbobenzyloxyglycine allowed compound **16** to be isolated in a modest yield of 28% (probably because of competitive acylation on the sulfur atom). To overcome this problem, the sulfur atom (the most nucleophilic site) was alkylated initially using *tert*-butyl bromoacetate; product **17** was isolated in 76% yield (Scheme 5). Despite total chemoselectivity, the by-products **18** (5%) and **19** (8%) were observed to result from intramolecular attack by the nitrogen atom on the ester carbonyl group. Once the thiol was protected, it was reacted with Z-glycine. The best results were obtained by activating the carboxylic acid function of gly-



Scheme 5. Synthesis of intermediate **20**. *Reagents and conditions:* (a) Z-glycine, DCC, THF, room temp., 4 h; (b) $BrCH_2CO_2tBu$, K_2CO_3, KI, THF, 60 °C, 18 h; (c) $(COCl)_2$, DMF, Z-glycine, CH_2Cl_2, 0 °C, 2 h then **17**, Et_3N, CH_2Cl_2, room temp., 18 h

cine as its acid chloride prepared in situ by the addition of oxalyl chloride in the presence of a catalytic amount of DMF.[48−50] After peptide coupling on **17**, the intermediate **20** was isolated in 68% yield.

The next step — the selective deprotection of the amino group — gave poor results, regardless of the conditions used. Classically, heterogeneous catalysts, such as palladium on charcoal, are inactivated by slight traces of sulfur derivatives. Thus, it was not possible to obtain compound **22** using catalytic hydrogenation. Lewis acids, which have been used previously to deprotect benzyloxycarbonyl groups, also gave unsatisfactory results.[51,52] We decided to avoid this step by repeating the synthesis procedure using the glycine derivative whose nitrogen atom is protected by the 9-fluorenylmethoxycarbonyl (Fmoc) group, which can be cleaved readily in basic media (Scheme 6). The acylation reaction gave the intermediate **21** in 93% yield. The Fmoc function was then removed by the action of piperidine in dichloromethane,[53] which led to the primary amine **22**. Because this synthon proved relatively unstable on the column, it seemed preferable to cleave the Fmoc group and allow the reaction mixture to be reacted directly with *tert*-butyl bromoacetate. Thus, the triester **23** was obtained from **21** in 75% yield. The corresponding triacid was isolated quantitatively by hydrolysis of the *tert*-butyl groups in neat trifluoroacetic acid at ambient temperature. Thus, ligand **3** was synthesised, as its trifluoroacetate salt, in five steps with an overall yield of 53%.



Scheme 6. Synthetic route to ligand **3**. *Reagents and conditions:* (a) $(COCl)_2$, DMF, Fmoc-glycine, THF, 0 °C, 30 min, then **17**, *N*-ethyldiisopropylamine, THF, room temp., 1 h; (b) piperidine, CH_2Cl_2, room temp., 20 min; (c) $BrCH_2CO_2tBu$, K_2CO_3, KI, THF, 70 °C, 5 h; (d) TFA, room temp., 2 days

2. Testing Complexation

Preliminary complexation tests were performed with all three ligands **1**, **2**, and **3** using indium-111, which appears to be an appropriate isotope for the first chelation test because its size (R = 0.80 Å) and coordination geometry seem suited for complexation with the O-, N-, and S-containing molecules. Moreover, we chose indium-111 because its physical properties [half-life of 67.4 h, γ emission with an energy of 173 keV (83%), 247 keV (94%)] allow the preparation of complexes that can be used in immunoscintigraphy, which is a widely used diagnostic method. To form the ^{111}In-chelated complexes, a fixed quantity (13 μCi) of an ^{111}In stock solution was added to each chelating agent (**1**, **2**, and **3**; 1 or 10 equiv). The volume of each sample was adjusted to 150 mL with 0.1 M sodium acetate, and the solutions were incubated at 37 °C for 1 h. The final pH of each solution ranged from 5.6 to 5.8, with final ^{111}In concentrations of 48 pM. The chelation yield was measured on a Phosphorimager apparatus after TLC on cellulose plates.

The experimental results, summarised in Table 1, display a marked difference in the reactivity of the three ligands. Indeed, only the nitrogen-containing ligand **2** is able to complex efficiently with indium: no chelated indium was observed for the oxygen- and sulfur-containing molecules, even when using 1000 equivalents of the ligands. Therefore, this first test clearly demonstrates that the selectivity of compound **2** toward indium is related to the nature of its heteroatom.

Table 1. Effect of ligand concentration on the efficiency of chelator-labelling ([^{111}In] = 1.5 nmol·mL^{-1}; reaction period = 1 h; pH = 5.6−5.8; temperature = 37 °C; final chelation volume = 150 μL; 0.1 M sodium acetate buffer)

	% ^{111}In-chelated		
Mol equivalents	Ligand **1**	Ligand **2**	Ligand **3**
1	0	57	0
10	0	75	0

Conclusion

We have synthesised three new hexacoordinate polydentate ligands that differ only in the nature of their heteroatom. The three analogues, **1**, **2**, and **3**, which contain O, NH, and S units, respectively, were synthesised in four or five steps in yields of 65, 40, and 53%, respectively. A preliminary complexation test using radioactive indium demonstrated differences in the behaviour of the three analogues. Indeed, only ligand **2**, which incorporates a nitrogen atom, is able to chelate this metal efficiently. The complexation properties of this molecule with indium still need to be quantified (e.g., its stability in serum and its complexation constant). To establish a correlation between the nature of the ligand and the HSAB theory, a range of more or less hard metals must be tested. This study should allow us to gain greater insight into the potential of such chelates. Depending on the end application, extra functional groups may be added readily to ligands by introducing an amino function via a nitro group under mild conditions.[54]

Experimental Section

General Remarks: All reagents were purchased either from Acros Organics or Sigma−Aldrich. The C.N.R.S. Analysis Laboratory (Vernaison) performed the elemental analyses. Column chromatography was conducted over silica gel 60 (40−63 μm) purchased from E. Merck. Thin-layer chromatography was performed on 0.5 mm × 20 cm × 20 cm E. Merck silica gel plates (60 F-254). Melting points were measured using a Reichert microscope and are uncorrected. The ^{13}C and ^{1}H NMR spectra were recorded at room temperature using Bruker AC 200 and ARX 400 spectrometers. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as internal standard, and coupling constants (*J*) are given in Hertz (Hz). Multiplicities were listed as s (singlet), d (doublet), t (triplet), q (quadruplet), and m (multiplet). Mass spectra were determined using Hewlett−Packard 5989 and NERMAG R 10−10 mass spectrometers. HRMS spectra were recorded at the CRMPO (Centre régional de Mesures physiques de l'ouest de Rennes). IR spectra in the range 4000−400 cm^{-1} were obtained using a Bruker Vector 22 spectrometer. All chemicals were reagent grade and used without further purification. Tetrahydrofuran was freshly distilled from Na/benzophenone ketyl, while dichloromethane was distilled over CaH_2. All reactions were carried out under an N_2 atmosphere.

Di-*tert*-butyl *N*-[*N*-*o*-(*tert*-Butoxycarbonylmethoxy)phenylcarbamoylmethyl]iminodiacetate (6): Potassium carbonate (8.31 g, 60.2 mmol) and potassium iodide (0.20 g, 1.2 mmol) were added to a solution of 2-amino-*N*-(2-hydroxyphenyl)ethanamide **5** (1.00 g, 6.02 mmol) in freshly distilled CH_3CN (30 mL). *tert*-Butyl bromoacetate (2.92 mL, 3.9 g, 20 mmol) was then added dropwise and the resulting solution was stirred at room temperature for 24 h. The mixture was filtered and the filtrate was concentrated to dryness. The resulting residue was partitioned between CH_2Cl_2 (100 mL) and water (80 mL). The organic extract was dried ($MgSO_4$) and filtered and then the solvents were evaporated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/heptane, 1:1) to give **6** as a yellow oil (2.35 g, 4.63 mmol, 77%). IR (KBr): $\tilde{\nu}$ = 3276, 2976, 1750, 1737, 1685, 1601, 1535, 1295, 1240, 1146, 759 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.42 (s, 9 H), 1.43 (s, 18 H), 3.51 (s, 6 H), 4.56 (s, 2 H), 6.75 (m, 1 H), 6.98 (m, 2 H), 8.35 (m, 1 H), 10.03 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 28.1(9), 56.5(2), 59.1, 66.9, 81.5(2), 82.2, 112.3, 120.6, 122.1, 123.7, 128.4, 147.5, 167.8, 169.3, 170.0(2) ppm. MS: *m*/*z* = 509 $[M + H]^+$ (100), 395. $C_{26}H_{40}N_2O_8$: calcd. C 61.42, H 7.87, N 5.51; found C 61.39, H 7.93, N 5.52.

***N*-[*N*-*o*-(Carboxymethoxy)phenylcarbamoylmethyl]iminodiacetic Acid Hydrochloride (1):** Compound **6** (0.6 g, 1.18 mmol) was stirred for 24 h at room temperature in trifluoroacetic acid (5 mL) and then the solution was concentrated to dryness. The resulting residue was precipitated by trituration with 1 M hydrochloric acid to afford a white amorphous solid (0.401 g, 1.18 mmol, 100%). M.p. 198−200 °C. IR (KBr): $\tilde{\nu}$ = 3371, 1731, 1682, 1559, 1351, 1247 cm^{-1}. ^{1}H NMR ([D$_6$]DMSO, 200 MHz, 25 °C): δ = 3.49 (s, 2 H), 3.58 (s, 4 H), 4.73 (s, 2 H), 6.98 (m, 3 H), 8.19 (d, *J* = 6.4 Hz, 1 H), 9.94 (s, 1 H) ppm. ^{13}C NMR ([D$_6$]DMSO, 50 MHz, 25 °C): δ = 54.8(2), 58.4, 65.3, 112.5, 119.6, 121.1, 123.6, 127.6, 147.2, 168.6, 170.0, 171.8(2) ppm. MS (LSIMS+): *m*/*z* = 362.9 $[M + Na]^+$ (100), 378.9 $[M + K]^+$. MS (LSIMS−): *m*/*z* = 339.1 $[M - H]^-$ (100), 361.1 $[M + Na - 2H]$; $C_{14}H_{17}ClN_2O_8$: calcd. C 44.62, H 4.24, N 7.43; found C 44.89, H 4.14, N 7.35.

Di-*tert*-butyl *N*-[*N*-*o*-(*tert*-Butoxycarbonylmethylamino)phenylcarbamoylmethyl]iminodiacetate (9): Potassium carbonate (1.28 g, 9.3 mmol) and potassium iodide (0.048 g, 0.29 mmol) were added to a solution of 2-amino-*N*-(2-aminophenyl)ethanamide **8** (0.15 g, 0.91 mmol) in freshly distilled THF (10 mL). *tert*-Butyl bromoacetate (452 μL, 605 mg, 3.1 mmol) was then, added slowly by syringe and the resulting solution was heated at 35 °C for 4 d. After cooling to room temperature, the mixture was concentrated to dryness. The resulting residue was partitioned between CH_2Cl_2 (30 mL) and water (20 mL). The organic extract was dried ($MgSO_4$) and filtered and then the solvents were evaporated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/hexane/Et_3N, 5:93:2) to give **9** as a colourless oil (280 mg, 0.55 mmol, 60%). Without modifying the elution conditions, compounds **11**, **10**, and **12** were isolated in that order. IR (KBr): $\tilde{\nu}$ = 3288, 2978, 1733, 1368, 1150 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.45 (s, 9 H), 1.47 (s, 18 H), 3.53 (s, 6 H), 3.83 (d, *J* = 5.7 Hz, 2 H), 5.13 (m, 1 H), 6.59 (d, *J* = 7.9 Hz, 1 H), 6.77 (t, *J* = 7.5 Hz, 1 H), 7.10 (t, *J* = 7.9 Hz, 1 H), 7.43 (d, *J* = 7.9 Hz, 1 H), 9.62 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 28.3(9), 46.9, 57.4(2), 59.5, 81.7, 82.1(2), 112.2, 118.1, 124.0, 125.2, 126.8, 141.3, 170.2, 170.4, 170.8(2) ppm. MS: *m*/*z* = 508 $[M + H]^+$ (100), 394, 246, 202. HRMS (LSIMS): calcd. for $C_{26}H_{42}N_3O_7$ $[M + H]^+$ *m*/*z* = 508.3023, found 508.3021.

Di-*tert*-butyl *N*-[*N*-*o*-(*tert*-Butoxycarbonylmethylamino)phenyl-*N*-*tert*-butoxycarbonylmethylcarbamoylmethyl]iminodiacetate (10): Colourless oil (87 mg, 0.14 mmol, 15%). IR (KBr): $\tilde{\nu}$ = 3337, 2978, 2933, 1739, 1671, 1606, 1524, 1458, 1368, 1224, 1155 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.41 (s, 18 H), 1.43 (s, 9 H), 1.46 (s, 9 H), 3.43 (m, 6 H), 3.83 (d, *J* = 6.2 Hz, 2 H), 3.94 (d, *J* = 16.8 Hz, 1 H), 4.28 (d, *J* = 16.8 Hz, 1 H), 6.01 (t, *J* = 8.1 Hz, 1 H), 6.52 (d, *J* = 8.1 Hz, 1 H), 6.67 (t, *J* = 7.5 Hz, 1 H), 7.15 (m, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 28.2(12), 45.9(2), 51.8(2), 55.4(2), 56.2(4), 80.8(2), 81.6, 82.1, 111.4, 117.5, 127.8, 129.3, 129.8, 144.6, 169.4, 169.9, 170.7(2), 172.0 ppm. MS: *m*/*z* = 622 $[M + H]^+$ (100), 508, 380, 246. HRMS (LSIMS): calcd. for $C_{32}H_{52}N_3O_9$ $[M + H]^+$ *m*/*z* = 622.3704, found 622.3706.

Di-*tert*-butyl *N*-[*N*-*o*-(Di-*tert*-butoxycarbonylmethylamino)phenylcarbamoylmethyl]iminodiacetate (11): Colourless oil (40 mg, 0.06 mmol, 7%). IR (KBr): $\tilde{\nu}$ = 3282, 2978, 2931, 1738, 1685, 1589, 1522, 1457, 1368, 1223, 1147 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.36 (s, 18 H), 1.44 (s, 18 H), 3.55 (s, 2 H), 3.60 (s, 4 H), 3.88 (s, 4 H), 7.00 (td, *J* = 7.6, 1.6 Hz, 1 H), 7.13 (td, *J* = 7.9, 1.5 Hz, 1 H), 7.41 (dd, *J* = 7.6, 1.5 Hz, 1 H), 8.40 (dd, *J* = 7.9, 1.6 Hz, 1 H), 10.34 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 28.2(6), 28.3(6), 55.8(4), 56.4(4), 58.8(2), 81.2(2), 81.4(2), 119.8, 123.5, 125.6, 125.9, 135.0, 139.0, 169.7(2), 170.1(3) ppm. MS: *m*/*z* = 622 $[M + H]^+$ (100), 508, 246, 147. HRMS (LSIMS): calcd. for $C_{32}H_{52}N_3O_9$ $[M + H]^+$ *m*/*z* = 622.3704, found 622.3707.

Di-*tert*-butyl *N*-(*N*-*o*-Aminophenylcarbamoylmethyl)iminodiacetate (12): Colourless oil (18 mg, 0.05 mmol, 5%). IR (KBr): $\tilde{\nu}$ = 3285, 2977, 2933, 1730, 1685, 1526, 1459, 1368, 1227, 1147 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.47 (s, 18 H), 3.51 (s, 6 H), 6.78 (m, 2 H), 7.01 (m, 1 H), 7.51 (m, 1 H), 9.70 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 28.3(6), 57.4(2), 59.7, 82.2(2), 117.2, 118.9, 124.2, 126.3, 139.8 (2), 169.6, 170.8(2) ppm. MS: *m*/*z* = 393 ($M^{+\bullet}$), 236, 146, 121, 57. HRMS (LSIMS): calcd. for $C_{20}H_{32}N_3O_5$ $[M + H]^+$ *m*/*z* = 394.2342, found 394.2345.

***N*-(1-Carboxymethylbenzimidazol-2-ylmethyl)iminodiacetic Acid (13):** 6 M Hydrochloric acid (18 mL) was added dropwise to a mixture of **9** (0.6 g, 1.18 mmol) in MeOH (12 mL). The solution was heated at 60 °C and stirred for 2 days. The mixture was concentrated in vacuo, 6 M hydrochloric acid (20 mL) was added, and the solution was heated at 60 °C for 2 days. The water was evaporated

to give triacid **13** as a hygroscopic yellow powdery solid that was dried under vacuum and kept under a nitrogen atmosphere (381 mg, 1.18 mmol, 100%). M.p. 108−110 °C. IR (KBr): $\tilde{\nu}$ = 3424, 2937, 1735, 1621, 1529, 1465, 1412, 1219 cm^{-1}. ^1H NMR (D_2O, 200 MHz, 25 °C): δ = 3.63 (s, 4 H), 4.48 (s, 2 H), 5.45 (s, 2 H), 7.57 (m, 2 H), 7.70 (m, 2 H) ppm. ^{13}C NMR (D_2O, 50 MHz, 25 °C): δ = 50.5, 53.9, 62.3(2), 113.8, 121.9, 126.1, 126.7, 138.9, 144.3, 156.1, 178.7, 182.6(2) ppm. MS (LSIMS+): *m/z* = 322.0 [M + H]$^+$ (100), 344.1 [M + Na$^+$]. MS (LSIMS−): *m/z* = 320.2 [M − H]$^-$ (100), 342.1 [M + Na − H].

Dimethyl *N*-(1-Methoxycarbonylmethylbenzimidazol-2-ylmethyl)-iminodiacetate (14): A solution of **13** (0.03 g, 0.093 mmol) in MeOH (1 mL) was treated with an excess of diazomethane (CAUTION), and the reaction mixture was stirred for 5 min at room temperature. The solution was concentrated under reduced pressure to afford the trimethyl ester **14** as an oil (0.034 mg, 0.093 mmol, 100%). IR (KBr): $\tilde{\nu}$ = 2955, 1751, 1734, 1521, 1465, 1419, 1208 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 3.48 (s, 4 H), 3.69 (s, 6 H), 3.76 (s, 3 H), 4.22 (s, 2 H), 5.46 (s, 2 H), 7.26 (m, 4 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 45.2, 51.7, 51.8(2), 52.7, 54.5(2), 109.1, 120.1, 122.5, 123.4, 136.3, 142.3, 150.8, 168.9, 171.1(2) ppm. MS: *m/z* = 364 [M + H]$^+$ (100), 204. HRMS (LSIMS): calcd. for $C_{17}H_{22}N_3O_6$ [M + H]$^+$ *m/z* = 364.1509, found 364.1513.

Dibenzyl *N*-[*N*-*o*-(Benzoxycarbonylmethylamino)phenylcarbamoylmethyl]iminodiacetate (15): Potassium carbonate (1.84 g, 13 mmol) and potassium iodide (0.070 g, 0.422 mmol) were added to a solution of 2-amino-*N*-(2-aminophenyl)ethanamide **8** (0.217 g, 1.315 mmol) in freshly distilled THF (15 mL). Benzyl bromoacetate (690 μL, 994 mg, 4.34 mmol) was then added dropwise and the resulting solution was heated at 35 °C for 3 days under argon. After cooling to room temperature, the mixture was concentrated to dryness. The resulting residue was partitioned between CH_2Cl_2 (45 mL) and water (30 mL). The organic extract was dried ($MgSO_4$) and filtered and then the solvents were evaporated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2) to give **15** as a colourless oil (400 mg, 0.656 mmol, 50%). IR (KBr): $\tilde{\nu}$ = 3288, 3033, 2954, 1740, 1684, 1606, 1525, 1456, 1190 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 3.55 (s, 2 H), 3.67 (s, 4 H), 3.96 (d, *J* = 5.6 Hz, 2 H), 5.01 (t, *J* = 4.9 Hz, 1 H), 5.15 (s, 4 H), 5.16 (s, 2 H), 6.58 (d, *J* = 8.1 Hz, 1 H), 6.78 (t, *J* = 7.6 Hz, 1 H), 7.08 (t, *J* = 7.8 Hz, 1 H), 7.31 (s, 2 H), 7.33 (s, 3 H), 7.42 (d, *J* = 7.9 Hz, 1 H), 9.51 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 46.3, 56.5(2), 59.6, 66.9, 67.1(2), 112.5, 118.6, 124.2, 125.0, 126.8, 128.6(15), 135.3(2), 135.6, 140.8, 169.5, 171.1, 171.2(2) ppm. MS: *m/z* = 610 [M + H]$^+$, 592, 502, 435, 364, 236, 91 (100). HRMS (ESI): calcd. for $C_{35}H_{35}N_3O_7Na$ [M + Na]$^+$ *m/z* = 632.23727, found 632.2370.

***N*-[*N*-*o*-(Carboxymethylamino)phenylcarbamoylmethyl]iminodiacetic Acid (2):** A mixture of **15** (0.126 g, 0.2 mmol), MeOH (12 mL), and 20% palladium hydroxide on charcoal (Pearlman's catalyst; 19 mg, 0.03 mmol) was stirred under hydrogen gas (1 atm) for 1.5 h at room temperature. The catalyst was filtered off and the filtrate concentrated to dryness to afford a white amorphous solid (0.068 mg, 0.20 mmol, 100%). M.p. 178−180 °C. IR (KBr): $\tilde{\nu}$ = 3436, 1746, 1664, 1612, 1513, 1355, 1242 cm^{-1}. ^1H NMR (CD_3OD, 200 MHz, 25 °C): δ = 3.66 (s, 2 H), 3.69 (s, 4 H), 3.93 (s, 2 H), 6.62 (d, *J* = 7.8 Hz, 1 H), 6.71 (t, *J* = 7.7 Hz, 1 H), 7.12 (t, *J* = 7.7 Hz, 1 H), 7.25 (d, *J* = 7.8 Hz, 1 H) ppm. ^{13}C NMR (CD_3OD, 50 MHz, 25 °C): δ = 46.2, 57.6(2), 59.7, 112.8, 118.5, 124.0, 127.7, 128.8, 143.9, 172.6, 174.5(2), 175.2 ppm. MS (ES): *m/z* = 338 [M − H]$^+$, 320 (100), 302, 276, 232. $C_{14}H_{17}N_3O_7$: calcd. C 49.56, H 5.05, N 12.38; found C 49.74, H 5.16, N 12.59.

***N*-*o*-Mercaptophenyl-2-benzoxycarbonylaminoethanamide (16):** *o*-Aminothiophenol (0.25 g, 2 mmol) was added into a solution of *N*-(benzyloxycarbonyl)glycine (0.418 g, 2 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (0.446 g, 2 mmol) in THF (8 mL) and the resulting mixture was stirred for 4 h at room temperature under a nitrogen atmosphere. The insoluble *N*,*N*′-dicyclohexylurea was filtered off and the filtrate was concentrated to dryness. The resulting solid was diluted with EtOAc (5 mL) and heated under reflux for 5 min. The resulting precipitate was filtered off to give compound **16** as a white solid that was dried under vacuum (0.185 g, 0.56 mmol, 28%). M.p. 134−136 °C. IR (KBr): $\tilde{\nu}$ = 3352, 3265, 1700, 1681, 1538, 1289 cm^{-1}. ^1H NMR ([D$_6$]DMSO, 200 MHz, 25 °C): δ = 3.38 (br. s, 1 H), 3.87 (d, *J* = 6.0 Hz, 2 H), 5.07 (s, 2 H), 7.47 (m, 9 H), 7.68 (t, *J* = 6.0 Hz, 1 H), 9.77 (s, 1 H) ppm. ^{13}C NMR ([D$_6$]DMSO, 50 MHz, 25 °C): δ = 43.9, 65.6, 125.5, 126.4, 127.2, 127.8(3), 128.4(2), 129.5, 130.6, 136.0, 137.0, 156.6, 168.7 ppm. MS: *m/z* = 317 [M + H]$^+$ (100), 209. $C_{16}H_{16}N_2O_3S$: calcd. C 60.74, H 5.10, N 8.85; found C 60.82, H 4.98, N 8.62.

***tert*-Butyl *o*-Aminophenylsulfanyl Acetate (17):** Potassium carbonate (6.63 g, 47.9 mmol) and potassium iodide (0.025 g, 0.15 mmol) were added to a solution of *o*-aminothiophenol (1 g, 8 mmol) in freshly distilled THF (40 mL). After stirring the mixture for 15 min, *tert*-butyl bromoacetate (1.18 mL, 8 mmol) was added dropwise under nitrogen. The reaction mixture was kept at 60 °C over a period of 18 h prior to cooling to room temperature, filtration, and concentration under reduced pressure. The resulting residue was partitioned between CH_2Cl_2 (80 mL) and water (80 mL). The organic extract was dried ($MgSO_4$) and filtered and then the solvents were evaporated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/hexane/Et_3N, 5:93:2) to give **19** (178 mg, 0.64 mmol, 8%) and **17** as a colourless oil (1.45 g, 6 mmol, 76%), and then **18** (26 mg, 0.16 mmol, 5%) by using CH_2Cl_2/Et_3N (98:2) as the eluent. IR (KBr): $\tilde{\nu}$ = 3456, 3357, 1722, 1298, 1137 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.39 (s, 9 H), 3.39 (s, 2 H), 6.70 (m, 2 H), 7.13 (td, *J* = 1.6, 8.1 Hz, 1 H), 7.44 (dd, *J* = 1.6, *J* = 7.6 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 30.0(3), 38.2, 81.8, 115.1(3), 116.7, 118.5, 130.5(3), 136.6, 148.7, 169.4 ppm. MS: *m/z* = 239 [M + H]$^+$, 183, 124, 93 (100), 57. $C_{12}H_{17}NO_2S$: calcd. C 60.22, H 7.16, N 5.85; found C 60.13, H 7.25, N 5.95.

4*H*-1,4-Benzothiazin-3-one (18): White solid. M.p. 176−178 °C. IR (KBr): $\tilde{\nu}$ = 3197, 3063, 1662, 1593, 1479, 1386, 740 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 3.45 (s, 2 H), 6.93 (dd, *J* = 1.4, 7.9 Hz, 1 H), 7.04 (td, *J* = 1.4, 7.6 Hz, 1 H), 7.18 (td, *J* = 1.5, 7.8 Hz, 1 H), 7.32 (d, *J* = 7.8 Hz, 1 H), 9.43 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 28.9, 117.2, 119.0, 122.9, 126.9, 127.3, 137.4, 165.2 ppm. MS: *m/z* = 165 [M]$^+$ (100), 136, 120, 96. HRMS (ESI): *m/z* calcd. for C_8H_7NNaOS [M + Na]$^+$ 188.0147, found 188.0151.

4-(*tert*-Butoxycarbonylmethyl)-4*H*-1,4-benzothiazin-3-one (19): Colourless oil. IR (KBr): $\tilde{\nu}$ = 2978, 1743, 1679, 1586, 1480, 1367, 1154, 752 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.47 (s, 9 H), 3.45 (s, 2 H), 4.58 (s, 2 H), 6.87 (dd, *J* = 1.2, 8.1 Hz, 1 H), 7.03 (td, *J* = 1.1, 7.5 Hz, 1 H), 7.23 (td, *J* = 1.5, 7.8 Hz, 1 H), 7.38 (dd, *J* = 1.5, 7.6 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 28.1(3), 31.3, 47.8, 82.6, 117.3, 123.8, 127.5, 128.5, 139.7, 165.8, 167.6 ppm. MS: *m/z* = 297 [M + NH$_4$]$^+$, 280, 241 (100), 224. HRMS (ESI): calcd. for $C_{14}H_{17}NNaO_3S$ [M + Na]$^+$ *m/z* = 302.0827, found 302.0830.

***N*-[*o*-(*tert*-Butoxycarbonylmethylsulfanyl)phenyl]-2-benzyloxycarbonylaminoethanamide (20):** Oxalyl chloride (601 μL, 888 mg, 7 mmol) and dimethylformamide (40 μL, 38 mg, 0.52 mmol) were added under nitrogen at 0 °C to a suspension of *N*-(benzyloxycarbonyl)glycine (1.37 g, 6.54 mmol) in anhydrous CH_2Cl_2 (20 mL). The ice-water bath was removed and stirring was continued at ambient temperature for 2 h. The mixture was added to a solution of **17** (0.6 g, 2.5 mmol) and Et_3N (2.86 mL, 20 mmol) in CH_2Cl_2 (20 mL). After being stirred at room temperature for 18 h, the solvent was evaporated in vacuo. The resulting residue was partitioned between CH_2Cl_2 (60 mL) and water (2 × 40 mL). The organic extract was dried ($MgSO_4$) and filtered and then the solvents were evaporated. The residual oil was crystallised by trituration with Et_2O to give **20** as a white solid that was dried under vacuum (0.734 g, 1.7 mmol, 68%). M.p. 73−75 °C. IR (KBr): $\tilde{\nu}$ = 3428, 3324, 2982, 2932, 2252, 1719, 1582, 1521, 1304, 1147, 910, 732 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.34 (s, 9 H), 3.35 (s, 2 H), 4.14 (d, *J* = 5.8 Hz, 2 H), 5.18 (s, 2 H), 5.82 (br. s, 1 H), 7.05 (td, *J* = 1.4, 7.6 Hz, 1 H), 7.36 (m, 7 H), 7.57 (dd, *J* = 1.5, 7.8 Hz, 1 H), 8.35 (d, *J* = 7.9 Hz, 1 H), 9.60 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 28.0(3), 40.4, 45.5, 67.3, 82.8, 120.7, 122.4, 124.6, 128.2, 128.6(2), 130.8, 136.4, 136.6, 139.9, 156.6, 167.4, 169.7 ppm. MS: *m*/*z* = 448 $[M + NH_4]^+$, 431, 392, 375(100). HRMS (ESI): calcd. for $C_{22}H_{26}N_2NaO_5S$ $[M + Na]^+$ *m*/*z* = 453.1460, found 453.1465.

2-(9-Fluorenylmethoxycarbonyl)amino-*N*-[*o*-(*tert*-butoxycarbonylmethylsulfanyl)phenyl]ethanamide (21): Oxalyl chloride (100 μL, 148 mg, 1.16 mmol) and dimethylformamide (10 μL, 9.5 mg, 0.13 mmol) were added under nitrogen at 0 °C to a suspension of *N*-(9-*f*luorenylmethoxycarbonyl)glycine (0.3 g, 1.01 mmol) in anhydrous THF (5 mL). The ice-water bath was removed and stirring was continued at ambient temperature for 30 min. The mixture was added to a solution of **17** (0.2 g, 0.84 mmol) and diisopropylethylamine (541 μL, 3.16 mmol) in THF (5 mL). After being stirred at room temperature for 1 h, the solvent was evaporated in vacuo. The resulting residue was partitioned between CH_2Cl_2 (20 mL) and water (2 × 20 mL). The organic extract was dried ($MgSO_4$) and filtered and then the solvents were evaporated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2 then CH_2Cl_2/EtOAc, 5:95) to give **21** as a white solid (0.404 g, 0.78 mmol, 93%). M.p. 37−39 °C. IR (KBr): $\tilde{\nu}$ = 3054, 1723, 1507, 1437, 1265, 739 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.34 (s, 9 H), 3.38 (s, 2 H), 4.23 (m, 2 H), 4.44 (s, 1 H), 4.48 (s, 1 H), 5.86 (br. s, 1 H), 7.07 (td, *J* = 1.2, 7.6 Hz, 1 H), 7.31−7.67 (m, 9 H), 7.77 (d, *J* = 7.0 Hz, 1 H), 8.39 (d, *J* = 8.1 Hz, 1 H), 9.68 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 28.0(3), 40.6, 45.5, 47.2, 67.5, 82.9, 120.1, 120.7, 122.3, 124.6, 125.2, 127.2, 127.8, 130.9, 136.7, 139.9, 141.4, 143.9, 156.6, 167.5, 169.9 ppm. MS: *m*/*z* = 537 $[M + NH_4]^+$, 519, 297, 214 (100), 179. HRMS (ESI): calcd. for $C_{29}H_{30}N_2NaO_5S$ $[M + Na]^+$ *m*/*z* = 541.17731, found 541.17730.

2-Amino-*N*-[*o*-(*tert*-butoxycarbonylmethylsulfanyl)phenyl]ethanamide (22): Compound **21** (0.456 g, 0.88 mmol) was dissolved in CH_2Cl_2 (20 mL) and treated with piperidine (4 mL, 4 mmol). The reaction mixture was stirred for 20 min under a nitrogen atmosphere and then concentrated in vacuo to afford a solid. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc/Et_3N, 83:15:2) to give amine **22** as a colourless oil (0.203 g, 0.68 mmol, 78%). IR (KBr): $\tilde{\nu}$ = 3255, 2979, 1724, 1684, 1518, 1297, 1132, 736 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.33 (s, 9 H), 3.43 (s, 2 H), 3.57 (s, 2 H), 7.05 (td, *J* = 1.4, 7.5 Hz, 1 H), 7.34 (m, 1 H), 7.58 (dd, *J* = 1.6, 7.8 Hz, 1 H), 8.47 (d, *J* = 7.0 Hz, 1 H), 10.46 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz, 25 °C): δ = 28.8(3), 39.2, 45.9, 82.1, 120.2, 122.0, 124.0, 130.2, 135.7, 139.6, 168.7, 171.3 ppm. MS: *m*/*z* = 314 $[M + NH_4]^+$, 297(100), 241, 84. HRMS (ESI): calcd. for $C_{14}H_{20}N_2NaO_3S$ $[M + Na]^+$ *m*/*z* = 319.10923, found 319.1092.

Di-*tert*-butyl *N*-[*N*-*o*-(*tert*-Butoxycarbonylmethylsulfanyl)phenylcarbamoylmethyl]iminodiacetate (23): A mixture of **21** (0.4 g, 0.77 mmol) and piperidine (4 mL, 4 mmol) in freshly distilled CH_2Cl_2 (20 mL) was stirred under a hydrogen atmosphere for 20 min and then concentrated in vacuo. The crude product was dissolved in anhydrous THF (10 mL) and then potassium carbonate (1.07 g, 7.74 mmol), potassium iodide (0.025 g, 0.15 mmol), and *tert*-butyl bromoacetate (1.443 mL, 1.93 g, 9.9 mmol) were added. The resulting solution was heated at 70 °C for 5 h. After cooling to room temperature, the mixture was concentrated to dryness. The resulting residue was partitioned between CH_2Cl_2 (30 mL) and water (20 mL). The organic extract was dried ($MgSO_4$) and filtered and then the solvents were evaporated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc, 96:4) to give **23** as a colourless oil (0.302 g, 0.58 mmol, 75%). IR (KBr): $\tilde{\nu}$ = 3055, 2984, 1731, 1686, 1518, 1266, 739 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 1.36 (s, 9 H), 1.45 (s, 18 H), 3.43 (s, 2 H), 3.55 (s, 2 H), 3.58 (s, 2 H), 7.05 (td, *J* = 1.4, 7.5 Hz, 1 H), 7.35 (t, *J* = 7.6 Hz, 1 H), 7.58 (dd, *J* = 1.4, 7.8 Hz, 1 H), 8.40 (d, *J* = 8.2 Hz, 1 H), 10.43 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 27.8(3), 28.2(6), 38.8, 56.5(2), 59.1, 81.7(2), 81.8, 120.8, 122.9, 124.1, 130.0, 135.6, 139.4, 168.6, 169.5, 169.8 ppm. MS: *m*/*z* = 525 $[M + H]^+$ (100), 216, 150. HRMS (LSIMS): calcd. for $C_{26}H_{40}N_2O_7S$ $[M]^+$ *m*/*z* = 524.25562, found 524.2560.

***N*-[*N*-*o*-(Carboxymethylsulfanyl)phenylcarbamoylmethyl]iminodiacetic Acid Trifluoroacetate Salt (3):** Compound **23** (0.129 g, 0.25 mmol) was stirred for 5 h at room temperature in trifluoroacetic acid (1 mL) and then concentrated in vacuo. The crude product was dissolved again in trifluoroacetic acid (1 mL) and was stirred at room temperature for 20 h. This protocol was repeated twice. The solution was concentrated to dryness to afford a white amorphous solid (0.117 g, 0.25 mmol, 100%). M.p. 52−54 °C. IR (KBr): $\tilde{\nu}$ = 3434, 3016, 1730, 1696, 1582, 1539, 1440, 1260, 1193 cm^{-1}. ^1H NMR (CF_3CO_2D, 400 MHz, 25 °C): δ = 3.77 (s, 2 H), 4.74 (s, 4 H), 4.91 (s, 2 H), 7.40−7.70 (m, 4 H) ppm. ^{13}C NMR (CD_3OD, 100 MHz, 25 °C): δ = 38.8, 56.4(2), 59.5, 123.3, 126.4, 126.6, 130.5, 136.0, 139.8, 171.1, 173.1, 173.4(2) ppm. MS (LSIMS+): *m*/*z* = 357.1 $[M + H]^+$, 378.9 $[M + Na]^+$, 395 $[M + K]^+$. MS (LSIMS−): *m*/*z* = 355.1 $[M - H]^+$ (100), 377.0 $[M + Na - H]$. $C_{16}H_{17}F_3N_2O_9S$: calcd. C 40.85, H 3.64, N 5.96; found C 40.82, H 3.91, N 6.17.

Complexation Studies with ^{111}In: Stock solutions of each chelate (2500, 250, 25, and 2.5 nmol·mL^{-1}) were prepared in 0.1 M sodium acetate (pH 5.7). To form the ^{111}In-chelated complexes, a radioactive solution of $^{111}InCl_3$ (10 mCi·mL^{-1}; 10 μL, 0.62 pmol) and a solution of $InCl_3$ (48 pmol·μL^{-1} in 0.05 M hydrochloric acid; 4.2 μL, 200 pmol) was added to each chelating agent **1**, **2**, or **3** (1 or 10 equiv) or **1** and **3** (1000 equivalents), before adjustment of the volume (to 150 μL). The nonradioactive solution of $InCl_3$ was used to maintain a fixed concentration of indium during the experiment. The final pH of each solution ranged from 5.6 to 5.8, with a final ^{111}In concentration of 48 nmol·mL^{-1}. After incubation at 37 °C for 1 h and thin-layer chromatography on cellulose plates (Merck 5552/0025; elution with 0.1 M sodium acetate, pH 5.7), complexation was measured using a 445SI phosphoimager.

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