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Synthesis and properties of a neutral derivative of diethylenetriaminepentaacetic acid (DTPA)

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Abstract—A neutral bifunctional derivative of diethylenetriaminepentaacetic acid europium(III) (**11**) was synthesized and its suitability to dissociation-enhanced lanthanide fluorescence immunoassay was investigated.

Because of its excellent metal-chelating properties diethylenetriaminepentaacetic acid (DTPA) is one of the most widely used organic ligands in magnetic resonance imaging (MRI) and positron emission tomography (PET).[1–3] Indeed, the first FDA-approved contrast agent in clinical use is the Gd^{3+} DTPA chelate.[4] The corresponding ^{111}In and ^{68}Ga chelates, in turn, are suitable for PET applications,[5] while Eu^{3+}, Tb^{3+}, Sm^{3+}, and Dy^{3+} chelates can be used in applications based on dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA).[6] ^{99m}Tc, in turn, is suitable for single positron emission computed tomography (SPECT).[7,8] Bioactive molecules labeled with ^{111}In or ^{117m}Sn DTPA may find applications as target-specific radiopharmaceuticals.[9]

In several applications, covalent conjugation of DTPA to bioactive molecules is required. This can be performed in solution by allowing an amino or mercapto group of a bioactive molecule to react with isothiocyanato, haloacetyl or 3,5-dichloro-2,4,6-triazinyl derivatives of the label molecules.[10] Several bifunctional DTPA derivatives are currently commercially available. Also a solid-phase method for the introduction of DTPA to synthetic oligonucleotides and oligopeptides has been demonstrated.[11]

The net charge of DTPA chelates is most commonly −2, which may cause problems in several applications. The most commonly used MRI contrast agent Gd-DTPA (Magnevist) distributes throughout the extracellular and intravascular fluid spaces, but does not cross an intact blood–brain barrier. Naturally, bioactive molecules labeled with this type of chelates have lower cell permeability than the corresponding intact molecules.[12] This diminishes the suitability of DTPA chelates to in vivo applications. Furthermore, the negatively charged chelates may bind unselectively to positively charged binding sites of target molecules, such as antibodies, via electrostatic interactions which may result in low recoveries.[13] Naturally, all these above-mentioned problems will be even more serious when the target molecule is labeled with several charged chelates.[14]

Several of the above-mentioned problems can be avoided by neutralizing the net charge of the chelate by substituting two of the DTPA acetates with carboxamido functions. Indeed, several this type of chelators have been synthesized[15,16] and one of them, Gd[DTPA-bis(ethylamide)][17] (gadodiamide; Omniscan), is currently in clinical use. However, it has been shown that if one of the acetic acid groups of DTPA is used for conjugation, the resulting chelate is less stable than the parent DTPA molecule.[18] This may be a serious problem especially in in vivo applications if toxic metal ions have to be used.

We present here synthesis of neutral derivatives of DTPA chelate which allow covalent conjugation of bioactive molecules. Also their suitability to DELFIA-based assays is demonstrated.

Synthesis of the neutral DTPA chelate is depicted in Scheme 1. Initially, 2-amino-*N*-(2-aminoethyl)-3-(4-nitrophenylphenyl)propanamide[19] (**1**) was converted to the Schiff base, **2**, by treatment with benzaldehyde, borane reduction of which yielded the amine **3**. It was then alkylated with *tert*-butyl bromoacetate to give **4** in good

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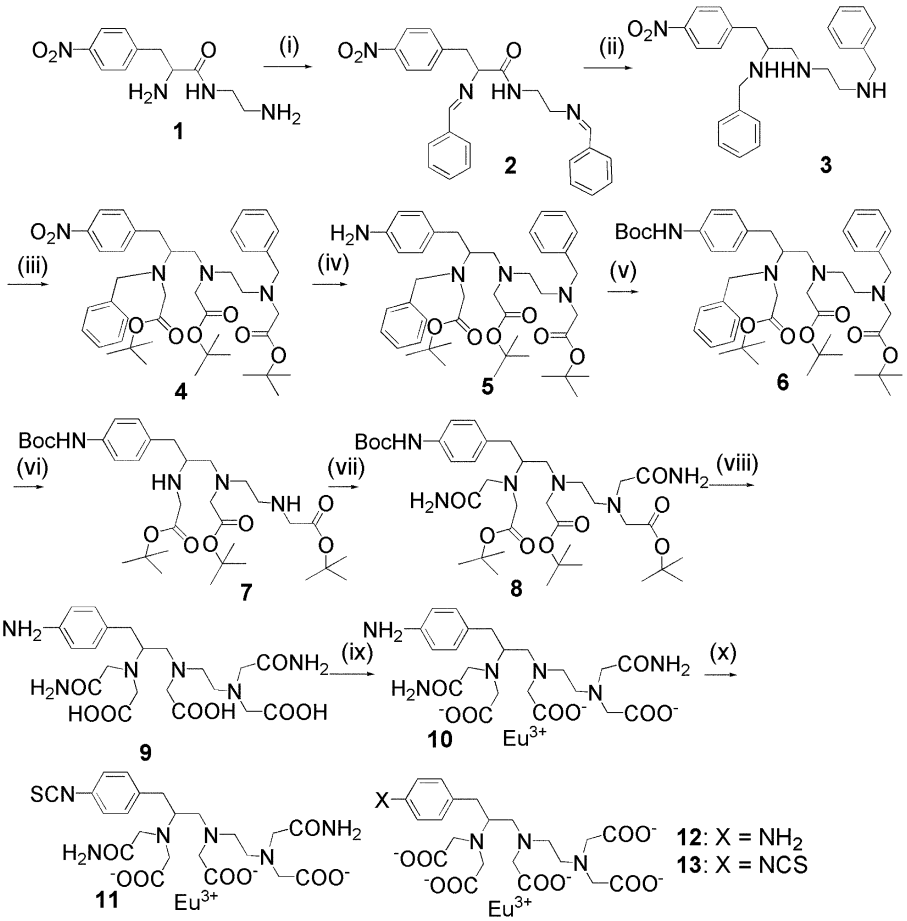
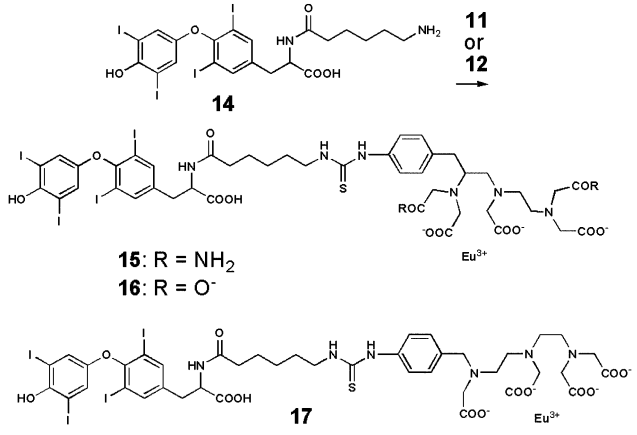


Scheme 1. Reagents and conditions: (i) PhCHO in EtOH; (ii) a—$BH_3 \cdot THF$, o/n at reflux, b—concd HCl, 3 h at reflux, c—aq ammonia, d—purification on Al_2O_3; (iii) $BrCH_2COO$-*t*-Bu, DIPEA in DMF, o/n at rt; purification on silica gel (79%); (iv) Pd/C, $NaBH_4$, in MeOH, 30 min at rt; purification on silica gel (58%); (v) Boc_2O, TEA, in MeCN, 2 h at rt, purification on silica gel (92%); (vi) Pd/C, ammonium formate, in MeOH, 15 min at reflux; purification on silica gel (75%); (vii) iodoacetamide, K_2CO_3 in MeCN, 2 h at reflux, purification on silica gel (86%); (viii) TFA 4 h at rt; (ix) $EuCl_3$; pH 6–7; 2 h at rt; (x) thiophosgene, $CHCl_3$, aq $NaHCO_3$, 1 h at rt.

yield. Selective reduction of the nitro group to the amino function using the mixture of Pd/C and $NaBH_4$ in methanol[20] gave rise to **5**. After protection of the aromatic amino group as *tert*-butyl carbamate, the benzyl groups were removed by hydrogenation with Pd/C in the presence of ammonium formate.[21] The secondary amines of **7** were alkylated with iodoacetamide to give the protected ligand **8**. After cleavage of the protecting groups by acidolysis, the free ligand **9** was converted to the corresponding europium(III) chelate **10** by treatment with europium(III) chloride.[22] Finally, reaction of **10** with thiophosgene[22] gave the activated chelate **11**.

To study the applicability of neutral DTPA derivatives to DELFIA assays, the stability of **10** and the corresponding charged chelate **12** in DELFIA Enhancement Solution and Inducer was compared. Accordingly, the stabilities of **10** and **12** was equal, giving times needed for complete dissociation <5 and 30 min for Inducer and DELFIA Enhancement Solution, respectively.[23]

Next, comparison of the performance of the tracer **15** and the corresponding DTPA derivative **16** to tracer used in AutoDELFIA Neonatal T_4 (thyroxine) kit **17** was performed. Tracer synthesis is outlined in Scheme 2.[24]



Scheme 2.

The assay conditions were optimized for each tracer individually, and the analytical sensitivities of the optimized standard curves were defined. The correlation between the methods was studied with a small sample panel. The on-board stability was tested up to one week in instrument-like conditions. Also the sensitivity to the

Table 1. Comparison of the performance of the tracer **15** and the corresponding DTPA derivative **16**–**17**

Tracer	**15**	**16**	**17**
Analytical sensitivity[a]	0.42 μL/dL	0.35 μL/dL	0.63 μL/dL
Correlation to AutoDELFIA neonatal T4 assay	$y = 1.02 \times -0.37$, $R = 0.87$, $n = 27$	$y = 1.2 \times -3.22$, $R = 0.94$, $n = 27$	
Mean bias	−0.7%	−0.1%	
Interference with EDTA[b]	No	No	Yes

[a] Analytical sensitivity was detected as 2 SD below the mean of the zero standard measurement value.
[b] Tested with EDTA concentration up to 12 mg/mL.

interference of EDTA-containing samples was studied. The results are summarized in Table 1.

The shapes of the calibration curves obtained with optimized amounts of tracer and antiserum were slightly different with the three tracers. All tracers were sensitive enough at clinically important range. Assays with the tested tracers compared well to the AutoDELFIA Neonatal T_4 assay and no significant level differences were obtained. In strict contrast to tracer **17**, neither **15** nor **16** was sensitive to EDTA. Also their stabilities under acidic conditions were similar.

Accordingly, two of the DTPA acetates can be substituted with carboxamido functions without loss of the desired chelate stability. The neutral DTPA chelates synthesized here are as stable as the corresponding charged derivatives. Since the preliminary results are promising we are currently synthesizing tracers labeled with several neutral DTPA derivatives.

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23. The chelates (ca. 1 mg) were dissolved either in Inducer or Enhanchement Solution (PerkinElmer), and the dissociation of europium at 25 °C was followed using a time-resolved fluorometer (Victor^{2}V).
24. Compound **14** was synthesized by allowing L-thyroxine to react with Fmoc-aminohexanoic acid *N*-hydroxysuccinate (1.1 equiv) in dry DMF in dark (1 h at rt). Piperidine was then added (removal of Fmoc group), and the reaction was allowed to proceed for 30 min at rt before being concentrated in vacuo. The title compound was obtained by precipitation from methanol.