Using the Ugi multicomponent condensation reaction to prepare families of chromophore appended azamacrocycles and their complexes†‡

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The Ugi reaction offers an effective method for preparing chromophore-appended DOTA-monoamide ligands, which can readily be elaborated to their lanthanide complexes.

The paramagnetic and relaxometric properties of lanthanide complexes have made them the subject of considerable current interest, owing to their potential as agents for imaging[1] and assay[2] in biological systems. For application *in vivo*, such complexes need to be kinetically robust to avoid dissociation in solution and to minimise the toxicity associated with lanthanide ions.[3] A wide variety of such complexes have been prepared, and most of these are derived from polydentate ligands such as DTPA, or from azamacrocycles such as cyclen.[1,3,4] Many such complexes have been attached to monoclonal antibodies or low molecular weight targeting vectors that are designed to deliver them to tissue associated with specific disease states.[5]

Two techniques dominate the field. In MRI (magnetic resonance imaging), the paramagnetic properties of gadolinium ions are used to generate proton contrast in whole body imaging;[1a,b] kinetically stable gadolinium complexes are widely employed in this role, but are of limited utility in targeted imaging due to the high concentrations required.[6] By contrast, luminescent complexes can be detected at the low concentrations associated with cell-surface receptors (allowing targeted imaging) as a result of the ease with which their long lived emission can be separated from biological background,[7] but poor tissue penetration by light means that whole body imaging is prohibitively difficult. Our own studies,[8] and those of others,[9] have sought to overcome these difficulties by using long wavelength excitation and emission to optimise tissue penetration. We have also begun to prepare polymetallic complexes that can be used to combine the advantages of MRI with those of luminescence.[10]

All the useful systems described above present considerable synthetic challenges to the interested chemist. In particular, lengthy and sometimes troublesome syntheses can limit the range of complexes that can be accessed, as well as limiting their potential application. New methods which simplify the preparation of such systems and provide a 'toolkit' for the biomedical sciences are desirable.

The Ugi four-component condensation,[11] in which an acid, an aldehyde, an amine and an isocyanide react to afford an acylaminoamide is a powerful tool to obtain peptide-like sequences. This reaction has been extensively used, both in solution and on the solid phase, to prepare large libraries of compounds with interesting biological properties.[12,13] We now present the synthesis and study of a series of novel DOTA monoamide derivatives and their complexes, using the Ugi reaction as a key step. This approach potentially gives rapid access to a variety of DOTA labelled compounds, while the strategy can be varied to incorporate chromophores for sensitised luminescence and/or targeting vectors as appropriate.

Scheme 1 shows our synthetic approach. Reaction of cyclen **1** with ethyl bromoacetate in the presence of sodium hydrogencarbonate yielded the ethyl triester **2**, which was elaborated further by reaction with *tert*-butylbromoacetate to give the orthogonally protected tetraester **3**. Subsequent exposure to TFA to cleave the *tert*-butyl ester and purification by HPLC gave the monoacid **4** in good overall yield.

We then investigated the suitability of **4** as a carboxylic acid substrate for the Ugi reaction. Multi-component reaction with Ph_2CHNH_2 (chosen as a representatively bulky amine), benzaldehyde and benzylisocyanide yielded the protected ligand **5** in good yield, while variation of the aldehyde component yielded **6–8**, showing that the procedure appears generally applicable. Ester hydrolysis yielded the deprotected ligands **9–12**.

These ligands were reacted with one equivalent of the lanthanide triflate in methanol (Ln = Eu, Tb, Yb) to afford the corresponding complexes in quantitative yield. ^{1}H NMR spectroscopy of the paramagnetic complexes gave rise to spectra in which the protons close to the metal centre were shifted well outside the 0–10 ppm region of the spectrum; Fig. 1 shows the NMR spectrum of Tb.**12**. This spectrum shows groups of four resonances spread over a broad chemical shift range. The spectrum is much more complex than that of $[Tb.DOTA]^-$,[14] as loss of the fourfold symmetry in the monoamide complexes making all the proton environments around the macrocycle ring inequivalent and splitting each peak in the $[Tb.DOTA]^-$ into four separate resonances. There is also another difference between the spectra of Tb.**12** and of $[Tb.DOTA]^-$, in that the NMR spectrum of the DOTA complex shows the presence of two isomers, usually assigned to square antiprismatic (SAP) and

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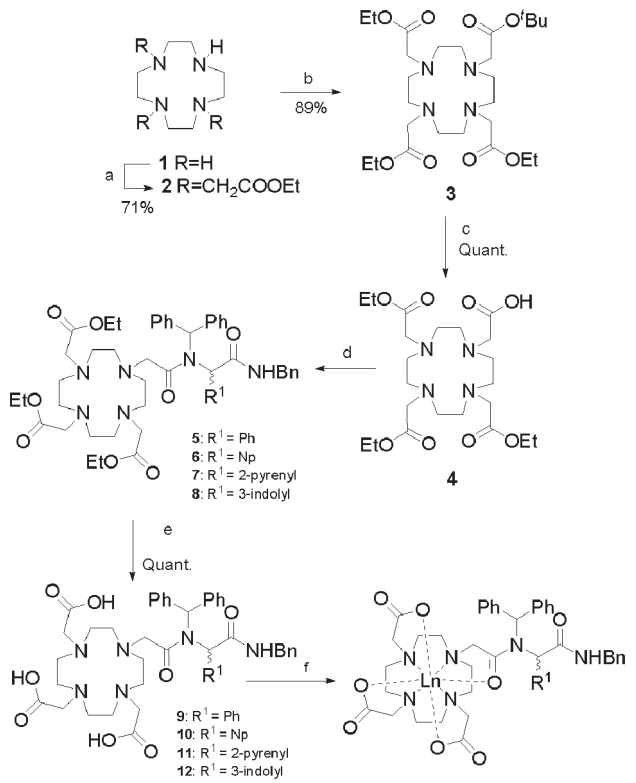
‡ This paper is dedicated to Dr Josephine Peach on her retirement.



Scheme 1 Synthesis of ligands and complexes. *Reagents and conditions*: (a) $BrCH_2COOEt$, $NaHCO_3$, $CHCl_3$, rt; (b) $BrCH_2COOtBu$, Et_3N, THF, rt (c) TFA CH_2Cl_2 1 : 1, rt; (d) R^1CHO, Ph_2CHNH_2, BnNC, EtOH, 65 °C; (e) LiOH, THF/H_2O (1 : 1), rt; (f) $Ln(OTf)_3$, CH_3OH, 40 °C, 3 days.

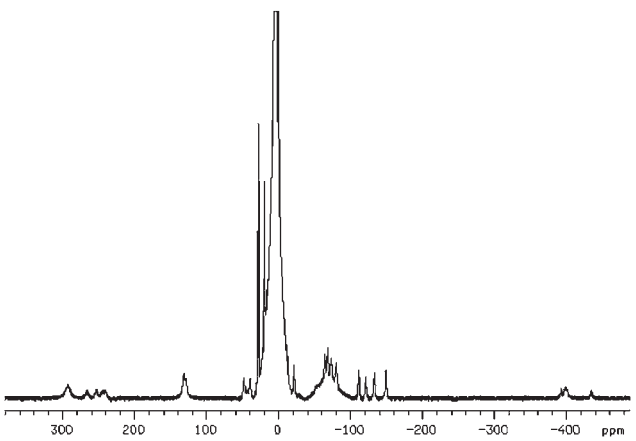


Fig. 1 ^{1}H NMR spectrum of Tb.**12**.

twisted square antiprismatic (TSAP) geometry of donor atoms around the metal centre,[15] while the spectrum of Tb.**12** indicates the presence of a single isomer. These resonances are observed at similar frequencies to those of the SAP isomer in $[Tb.DOTA]^-$, suggesting either that any minor isomer is present as an undetectably small percentage of the mixture, or that conversion between the isomers is rapid on the NMR timescale (which is unlikely). By contrast, the spectrum of the complex Eu.**9** shows the presence of resonances in groups of eight (Fig. 2), with roughly equal intensities comparable to, and with shifts similar to those of the SAP isomer in $[Eu.DOTA]^-$. It is likely that this

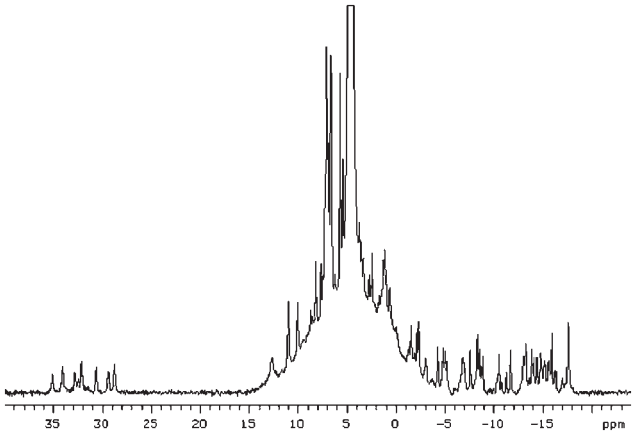


Fig. 2 ^{1}H NMR spectrum of Eu.**9**.

additional complexity arises as a consequence of stereoisomerism on the NMR timescale. In DOTA complexes, both the SAP and TSAP isomers exists as pairs of enantiomers.[15] However, in the case of the complexes formed from Ugi products, the stereogenic centre in the amide pendent arm means that the major form will exist as two enantiomeric pairs of diastereoisomers. In the case of Eu.**9**, these are clearly in slow exchange on the NMR timescale. In fact HPLC suggests that they are also in slow exchange under the conditions used for purification. The HPLC traces for complexes showed two well separated peaks, in ratios varying between 1 : 1 and 3 : 1; the separated fractions gave identical mass spectra showing the expected molecular ion for the complex. ^{1}H NMR of the separated fractions revealed both to have identical spectra, suggesting that the isomers re-equilibrate relatively rapidly in aqueous solution. To confirm this, the two fractions were separately injected onto the HPLC column at six minute intervals over a four hour period. A slow equilibration between the two species was observed, with a 1 : 1 ratio achieved after *ca.* 2 h. Once the original equilibrium ratio had been reached, the sample remained unchanged after 24 h.

The luminescence of the complexes was also studied, and the luminescence lifetimes for a range of complexes are shown in Table 1. Most of the data on these systems was collected in aqueous solution, though we found that the increased lipophilicity conferred by the presence of the pyrene group alters the solubility of Ln.**11** to the point that studies could only be carried out in alcoholic solvents.

A chromophore can act as a sensitiser to a lanthanide ion only if the donor state (commonly the T_1 state) is significantly higher in energy than the lanthanide emissive state. This relationship between triplet energy and sensitisation is illustrated by the behaviour of three ions in different complexes. For the Tb^{3+} ion, the emissive state ($^5D_4$) is relatively high in energy ($\sim$20 490 cm^{-1}) and can only be sensitised by chromophores with high triplet energies;[16] in the case of this study, only Tb.**9** and Tb.**12** were found to be highly luminescent. Tb.**10** was weakly luminescent in aerated solution as a consequence of facile thermal repopulation of the ligand triplet state, while Tb.**11** showed no metal-centred luminescence at all. In the case of the europium complexes, the emissive state ($^5D_0$, $\sim$17 250 cm^{-1})[1] is significantly lower in energy and

Table 1 Photophysical properties of emissive complexes[a]

	R[1]	λ_{ex}/nm	λ_{em}/nm	τ_H/μs	τ_D/μs	q
Tb.**9**[b]	Ph	250	545	1750	2480	0.5
Tb.**12**[b]	Indolyl	280	545	1740	2150	0.2
Eu.**9**[b]	Ph	250	617	550	2070	1.3
Eu.**10**[b]	Np	280	617	640	2340	1.1
Eu.**12**[b]	Indolyl	280	617	500	1430	1.3
Yb.**10**[b]	Np	337	980	0.81	6.13	1.0
Yb.**11**[c]	Pyrenyl	337	980	2.24	9.91	0.6

[a] The terbium and europium complexes of the pyrenyl-bearing ligand were non-emissive owing to the mismatch between triplet and emissive states, while the terbium complex with the naphthyl-bearing ligand exhibits weak emission as the result of efficient back energy transfer. The emission of the Yb phenyl system was not studied in the absence of a suitable excitation source. Lifetimes are ±10%. [b] Measured in H_2O–D_2O. [c] Measured in CH_3OH–CD_3OD.

sensitised emission was observed for Eu.**9**, Eu.**10** and Eu.**12**, though not for Eu.**11**. The pyrene chromophore did, however, sensitise emission from the ytterbium $^2F_{5/2}$ state at 980 nm ($\sim$10 200 cm^{-1}).

The luminescence lifetimes of the emissive complexes were used to probe the local environment and the solvation at the metal centre. The number of inner sphere solvent molecules, q, can be calculated using the equation

$$q = A(1/\tau_H - 1/\tau_D - B)$$

where A and B are constants for a given metal ion and solvent, and τ_H and τ_D are the observed luminescence lifetimes in protiated and deuteriated media respectively.[17]§ Table 1 also shows these calculated values for q, which illustrate that the metal binding pocket changes little across the series. The lower values of q obtained for Tb.**9** and Tb.**12** relative to their Eu analogues may reflect the structure break commonly observed around gadolinium as a consequence of the lanthanide contraction.

This study on a range of related complexes suggest that all adopt very similar structures in solution, in which a single isomer predominates around the metal centre.

The approach described above illustrates how luminescent complexes can be prepared from simple building blocks by using a multi-component synthesis approach. We are currently investigating how this approach can be combined with peptide vectors and other substrates to allow the use of a flexible molecular toolkit in "smart" bioassay and imaging applications.

Notes and references

§ For Tb^{3+} in water, A = 5 ms, B = 0.06 ms^{-1}; for Eu^{3+} in water, A = 1.2 ms, B = (0.25 + 0.075x) ms^{-1}, where x is the number of exchangeable amide N–H oscillators; for Yb^{3+} in water, A = 1 μs, B = 0.1 μs^{-1}; for Yb^{3+} in methanol, A = *2* μs, B = 0.05 μs^{-1}.

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