Click chemistry with lanthanide complexes: a word of caution†

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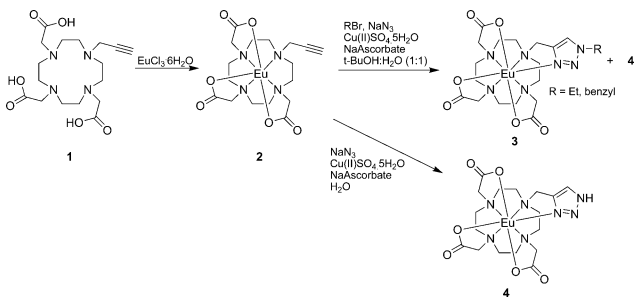
A propargyl-appended Eu(III) complex shows unanticipated reactivity towards NaN_3 in the Cu(I) catalysed click reaction yielding an unsubstituted 1,2,3-triazole. The resulting complex exhibits pH-responsive ^{1}H NMR and Eu(III) luminescence behaviour in the physiological pH range consistent with deprotonation of the NH-acidic bound triazole.

In order to overcome the inherent sensitivity problem of Magnetic Resonance (MR) imaging,[1] our recent studies have been geared towards developing Gd(III) complexes as contrast agents that respond to physiological changes such as enzyme activity to amplify the signal.[2] We are now looking at ways to improve the sensitivity of MR contrast agents by 'increasing the payload', *i.e.* by delivering multiple Gd(III) chelates. We were attracted to the apparent perfection of the Cu(I) catalyzed azide–alkyne cycloaddition reaction in order append multiple lanthanide chelates from a central 'hub'. In recent years this 'click' reaction to form 1,2,3-triazoles[3] has been applied to a plethora of applications.[4] Recent reports document the use of this synthetic strategy to attach Eu(III) chelates to dansyl chromophores and Gd(III) chelates to *e.g.* a cyclodextrin hub.[5] In both cases an N-(2-propynyl)acetamide appended from a DOTA-based chelate was utilized. In order to avoid the use of amides due to slowing of the water exchange rate on Gd(III) complexes, we chose to use the DOTA-based proligand **1**. As a Ln(III) complex we have demonstrated facile conversion of **2** to the corresponding triazole *via* the Cu(I) catalyzed click reaction with organic azides.[6] Due to the number of azides per molecule required to synthesize multimeric assemblies of Gd(III) complexes, we were wary of isolation of the potentially explosive azide precursors. Conventional wisdom suggests that reaction of alkynes with *in situ* generated azides (*via* alkyl halides in the presence of NaN_3) is a clean, facile reaction and residual NaN_3 does not interfere with the cycloaddition reaction.[7] We believed that this attractive synthetic trick would allay our safety concerns, circumventing the need to isolate compounds containing multiple azides. Herein, we report the unexpected reactivity of **2** with NaN_3, to yield a Eu(III) complex containing an unsubstituted NH-triazole **4**, and detail its pH responsive ^{1}H NMR and luminescent behavior.

2 was synthesized *via* standard methods.† Our initial attempts to react **2** with simple *in situ* generated alkyl azides (*e.g. via* ethyl or benzyl bromide) under standard room temperature click conditions did not give the clean high-yielding reaction that we expected and two products were formed: the desired alkyl triazole **3** and the unsubstituted NH-triazole **4** (Scheme 1). Whilst reports of the synthesis of unsubstituted triazoles have appeared, these tend to be formed at elevated temperatures, not under ambient conditions.[8] There is currently much interest in the generation of unsubstituted 1,2,3-triazoles due to their topological and electronic similarities to amides; their biological activity is being explored as peptide bond mimics.[4d] Synthesis of NH-triazoles usually requires the use of a 'protected' organic azide, such as azidomethyl pivalates and carbamates that can be easily removed once the triazole is formed.[9] The alternative is the highly undesirable reaction with hydrazoic acid at high temperature.[10]



Scheme 1 Synthesis of **4**.

Due to the unexpected reactivity of **2** with NaN_3, we attempted the click reaction of **2** in water in the presence of $CuSO_4$, sodium ascorbate and NaN_3. Under these conditions complete conversion to **4** was seen after stirring at room temperature for 12 hours. It is evident that Ln(III) complexes based on proligand **1** are unsuitable for use in click reactions where the azide is generated *in situ*, due to their propensity to react with NaN_3. We have observed this effect with a variety of lanthanides (*e.g.* Sm–Dy) and suspect that it is linked to the close proximity of the alkyne to the Ln(III) ion. There are numerous postulated intermediates in the proposed mechanism of the Cu(I) catalyzed 1,3 Huissgen cycloaddition reaction, in all cases an organic azide is deemed essential for the reaction to proceed.[4,7] One mechanism suggests the Cu-acetylide that is initially formed, and the azide, are bound to different copper atoms in a doubly bridged assembly. This may offer some clues to the unusual reactivity observed in **2** as the copper acetylide must be in close proximity to the central Eu(III) ion. It is likely that N_3^- bound to Eu(III) is amenable to cycloaddition to the activated alkyne.[11]

4 was characterized by ^{1}H NMR and luminescence spectroscopy and displays interesting pH-responsive behaviour in the physiological pH range. The ^{1}H NMR spectrum (400 MHz, pD 9.0, 278K) is typical of a rigid 8-coordinate unsymmetrical Eu(III) complex exhibiting a broad range of resonances from −18 to 33.5 ppm. This is indicative of triazole coordination to Eu(III).

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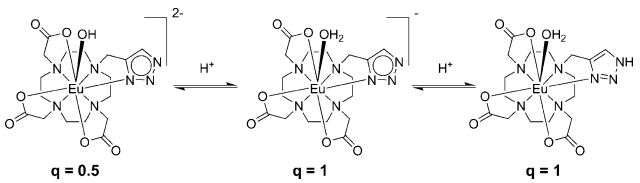
The restricted arm rotation in 8-coordinate (*cf.* 7-coordinate) complexes slows the exchange between the various stereoisomers, resulting in resolution of the axial and equatorial cyclen ring hydrogens. The four axial resonances shifted to high frequency are typical of a square antiprismatic coordination geometry. The presence of the minor twisted square antiprismatic isomer is also demonstrated by four resonances at slightly lower frequency (9.9 to 13.6 ppm).

At basic pH the triazole is bound to Eu(III) as a monoanionic donor *i.e.* as the 1,2,3-triazolide (Scheme 2).[12] NH-triazoles are acidic; their pK_as typically ~ 5–9.[13] This anionic coordination mode is different to the coordination behavior of N-substituted triazoles that coordinate *via* a 'pyridinic' nitrogen atom[6] (the pK_a of this nitrogen is typically < 1). The ^{1}H NMR spectrum of **4** exhibits pH-dependent behavior (Fig. 1). On titrating from base to acid, three of the four most shifted axial resonances (33.4, 33.1 and 32.6 ppm) broaden, moving to lower frequency, the fourth (31.1 ppm) moves to higher frequency. On moving to acidic media, the resonances are once more resolved. The resonance that shifts to higher frequency now appears at 32.1 ppm; the three resonances that shift to lower frequency now appear at 29.8, 29.2 and 28.6 ppm. This is a clear indication of a protonation event, *i.e.* of one of the triazolide nitrogens. The subtle change from effectively N$^-$ coordination to pyridinic N-coordination results in a marked change in spectral form. Similar movements can be traced for other resonances in the spectrum for both the square and the twisted square antiprismatic isomers. The change in chemical shift of these resonances $\Delta\delta$ enable the determination of a protonation constant (logK_{MLH} or pK_a) of 7.5 ± 0.1 *i.e.* **4** exhibits pH-responsive behavior in the physiological pH range. Given the apparent rigidity demonstrated in the ^{1}H NMR spectrum after protonation, we conclude that the triazole remains coordinated to Eu(III) after protonation, but *via* the pyridinic nitrogen, indeed the form of the spectrum in acid media is very similar to related coordinated N-substituted triazoles.[6]



Scheme 2 Protonation sequence for **4**.

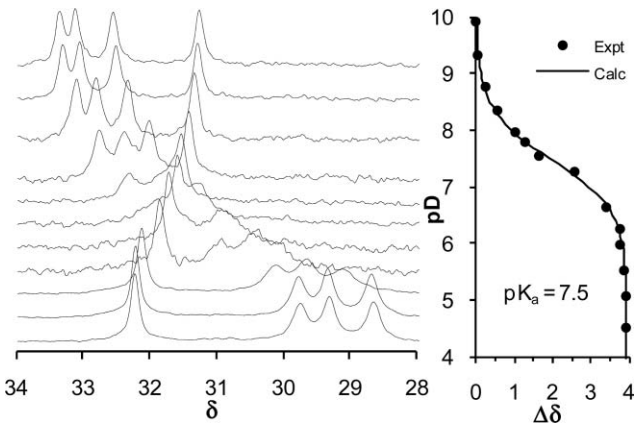


Fig. 1 ^{1}H NMR *vs.* pD for **4** (400 MHz, 278K).

The luminescent emission spectrum of **4** is typical of an 8-coordinate DOTA-based Eu(III) complex. At pH 5.5 the form of the spectrum is reminiscent of DOTA-based Eu(III) complexes bearing pendant coordinated pyridyls[14] (and indeed of the N-substituted triazoles)[6] as are the relative intensities of the $\Delta J = 1$ ($^5D_0 \rightarrow {}^7F_1$) and $\Delta J = 2$ ($^5D_0 \rightarrow {}^7F_2$) transitions. On raising the pH of the solution, one of the transitions in the $\Delta J = 1$ manifold shifts from 592 to 597 nm. The ratio of intensities of these peaks enables a protonation constant of 6.9 (±0.1) to be extracted from the data (an excited-state pK_a recorded at 298 K), *i.e.* ratiometric concentration independent pH response. This is consistent with the changes observed in the ^{1}H NMR spectrum (pK_a = 7.5, a ground-state pK_a recorded at 278 K). This behavior supports the idea of a subtle change in coordination mode of the triazole from neutral 'pyridyl' to anionic triazolide. The titration is complicated by a second deprotonation event occurring at pH >8. This is deprotonation of the bound water molecule in the 9th coordination site of **4** and is characteristic of such complexes: there is a change in spectral form of $\Delta J = 1$ and an increase in intensity of the hypersensitive $\Delta J = 2$ transition. This transition is particularly sensitive to the polarisability of the axial donor atom.

Luminescent lifetime measurements confirm these conclusions. The lifetime of the Eu(III) excited state is susceptible to quenching from O–H oscillators. Luminescent lifetime measurements on **4** in H_2O and D_2O enable the determination of the hydration state (q) of the complex. At pH 10.0 $q = 0.4$ ($k_{H_2O} = 1.81$, $k_{D_2O} = 1.20$ ms^{-1}), consistent with deprotonation of the bound water molecule, *i.e.* OH$^-$ is bound. At pH 8.5, before hydrolysis of the bound water, when the triazole is bound in an anionic manner, $q = 1.1$ ($k_{H_2O} = 2.27$, $k_{D_2O} = 1.11$ ms^{-1}). At pH 5.5 when bound in a pyridinic manner, the apparent hydration state is slightly higher than unity $q = 1.4$ ($k_{H_2O} = 2.51$, $k_{D_2O} = 1.06$ ms^{-1}).[15]

Conclusions

In conclusion this study has demonstrated that a seemingly small alteration, substituting an N-(2-propynyl)acetamide for propargyl as a means of coupling lanthanide chelates *via* click chemistry is only suitable if the organic azide involved in the click reaction is pre-formed and the solution is free of NaN_3. The *in situ* generation of azides to avoid the isolation of potentially hazardous multiple azide-containing precursors is incompatible with **2** due to the unprecedented reactivity of NaN_3 with propargyl-appended Eu(III) complex. This has implications if complexes such as these are to be used to make multimeric contrast agents (as Gd(III)) where precursors containing multiple azides are required.

The unexpected product of this click reaction **4** showed interesting protonation behavior, switching from triazole bound to Eu(III) in an N-deprotonated anionic form in basic media, to an N–H protonated pyridyl form in acidic media. This pH-dependent switch, demonstrated by both ^{1}H NMR and luminescence studies occurs in the physiological pH range. We are currently probing the origins of this unusual reactivity and exploring the potential

applications of this pH-responsive behavior for a variety of Ln(III) ions.

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