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Simple, high yielding synthesis of trifunctional fluorescent lanthanide chelates

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Abstract—In an effort to produce strong fluorophores which are conjugated to biologically active substrates, we report a facile, high yielding synthesis of conjugable lanthanide chelates based on the cyclen (1,4,7,10-tetraazacyclododecane) macrocycle.

It is the goal of this study to produce bioconjugable, highly fluorescent, strong binding lanthanide chelates in a simple and efficient manner. Existing synthetic methodology for producing conjugable lanthanide chelates has had the drawback of either requiring a long and labored synthesis, production of multiple active sites for conjugation[1] or ligands lacking a suitable light harvesting moiety.[2–4]

The ability to link fluorophores to biologically active species is a critical step toward making contrast agents that can help elucidate cellular mechanisms and aid in the identification of disease.[5–7] While having shown great utility, most of the currently available conjugable fluorophores still have limitations regarding their spectroscopic properties.[8,9] Photobleaching, overlapping excitation and emission, and short lived fluorescence limit these fluorophores adding to increased background noise and making gated detection rather expensive and complicated. In addition, fluorophores with more than one active conjugation site often give poor yields of the desired product through cross-linking or require multiple steps with protecting groups, increasing the total time and cost of product production.[10] Lanthanide chelates have been recently exploited as optical imaging agents, due in part to the fact that they have excellent spectroscopic properties as a result of a unique luminescent pathway.[11] By employing a molecular antenna to serve as a light harvesting moiety, through which intramolecular energy transfer then conveys the absorbed energy to a chelated lanthanide ion, ionic fluorescence from the chelated lanthanide can be obtained. This pathway not only results in emission that is far removed from the absorption ($\Delta\lambda \geq 280$ nm) but is also responsible for their extremely long fluorescent lifetimes (0.5–3.5 ms), thus allowing detection with inexpensive instrumentation and at very low levels of background signal.[11–14]

We report here the efficient, inexpensive synthesis, in high yields, of a lanthanide chelating ligand based on the cyclen (1,4,7,10-tetraazacyclododecnae) nucleus which possesses a single carboxyl group for conjugation, a 6-substituted quinaldine light harvesting moiety, and two phosphonic acid pendant arms for strong, thermodynamically stable lanthanide complexation (Fig. 1).

Selective substitution is paramount in this synthesis in order to yield the type of molecule in Fig. 1 with just one nitrogen linked to a light-harvesting moiety and a

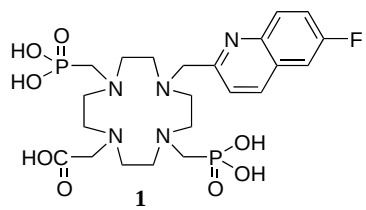

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Figure 1. Lanthanide chelating ligand possessing a single conjugable acetic acid side-chain, two opposing phosphonic acids for strong lanthanide complexation, and a 6-fluoroquinaldine light harvesting moiety for efficient lanthanide sensitization.

Keywords: 1,4,7,10-tetraazacyclododecane; lanthanide; fluorescence; conjugates; chelate.

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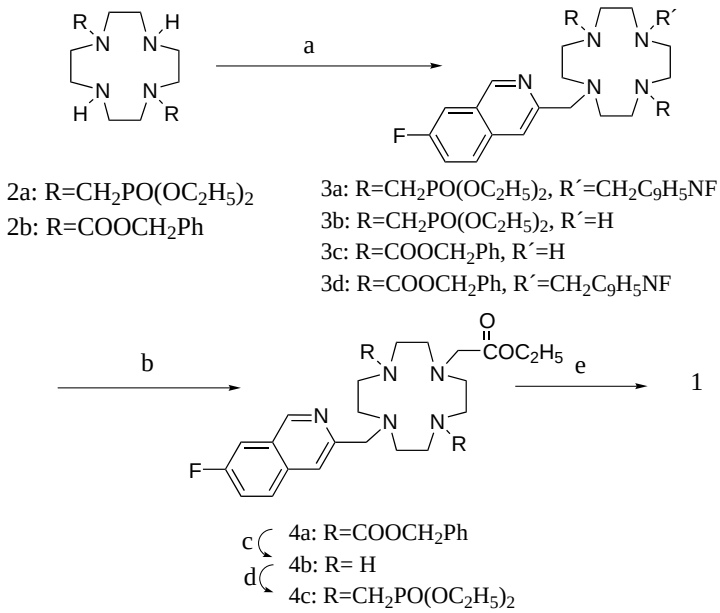
single carboxyl group on the opposite nitrogen. The first attempt at monoalkylation with the quinaldine derivative[15a–c] utilized 1,7-bis (methylene phosphonic acid diethyl ester)-1,4,7,10-tetraazacyclododecane, **2a**, first described by Sherry,[16] as a starting point (Scheme 1). However, the main product isolated was the undesired disubstituted compound **3a** with yields of the monosubstituted compound **3b** of 30%.[17] Even when employing two equivalents of **2a** with one equivalent of quinaldine antenna, the main product obtained was still the disubstituted compound **3a**.

Surprisingly, when utilizing 1,7-bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane, **2b**,[16] as the starting point for the monosubtitution with the quinaldine derivative, the desired monosubstituted product was isolated as the main product. Selective monoalkylation with the quinaldine light-harvesting moiety was performed under fairly high dilution (0.04 M) conditions with a 1:1 molar ratio of reactants. Simple chromatography gave the monosubstituted product **3c** in 70% yield and the disubstituted biproduct **3d** in 10% yield.[18a,b] This apparent high selectivity when using benzyloxycarbonyl (BOC) protecting groups in the 1,7-positions is likely due to a conformation that renders one of the nitrogens inaccessible either before or after the first substitution. Starting materials, reagents and conditions and resulting percentage yield of monosubstituted versus disubstituted products are summarized in Table 1.

Alkylation of the remaining macrocyclic nitrogen in **3c** with α-chloro ethyl acetate placed the acetic acid conjugation unit on the ring to produce **4a**. Deprotection of the BOC groups was accomplished by catalytic transfer hydrogenation utilizing 5% Pd/C in ethanol with cyclohexene as the hydrogen donor to give **4b** in up to 92% yield. These conditions were much superior to Sherry's[16] original conditions for similar deprotections. Potential reduction of the quinoline ring[19,20] was also avoided.

Next the bis methylene phosphonic acid pendant arms were added via reaction of **4b** with paraformaldehyde and triethyl phosphite to produce **4c**. Finally, acid hydrolysis of **4c** with 6 M HCl gave the desired product **1**. Purification consisted of azeotropic distillation with water to remove excess HCl. The resulting solid was then lypholized to produce a floculant white solid with a yield of 95%.[21]

The commercial availability and low cost of cyclen coupled with the relative simplicity of this synthesis (effective monoalkylation, soft/efficient deprotection



Scheme 1. (a) $ClCH_2C_9H_5NF$, CH_3CN, K_2CO_3; (b) $BrCH_2COOC_2H_5$, CH_3CN, K_2CO_3; (c) cyclohexene, Pd/C 5%, ethanol reflux; (d) paraformaldehyde, triethyl phosphite; (e) 6 M HCl; reflux, 4 days.

Table 1. Monosubstituted and disubstituted quinaldine derivatives **3a–d** via Scheme 1

Entry	Reagents and conditions	Ratio α:β[a]	% Yield monosubstituted	% Yield disubstituted
2a	25°C, CH_3CN, 24 h, 0.16 M	1:1	25	60
2a	25°C, CH_3CN, 24 h, 0.16 M, K_2CO_3	1:1	17	64
2a	25°C, CH_3CN, 48 h, 0.04 M	2:1	30	55
2c	40°C, CH_3CN, 48 h, 0.04 M, K_2CO_3	1:1	70	10

[a] α: substrate listed under entry; β: quinaldine antenna.

and high yield) make this an attractive route to bioconjugable lanthanide chelates that require a lanthanide sensitizer, opposing pendant arms for strong lanthanide complexation and a single conjugation moiety.

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17. Compound **3a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.16 (t 3H, CH_3), 2.74 (br d 12H, NCH_2CH_2N), 2.89 (br 8H, NCH_2CH_2N and PCH_2N), 3.86 (d 4H, CH_2Ar), 3.93 (q 8H, OCH_2), 7.24–7.42 (m 4H, Ar), 7.84 (d 2H, Ar), 8.05–8.09 (m 4H, Ar); ^{31}P NMR (300 MHz, $CDCl_3$) δ: 26.26. Compound **3b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.16 (t 12H, CH_3), 2.49–2.82 (br m 19H, NCH_2CH_2N, and $HNCH_2CH_2N$), 3.39 (s 4H, NCH_2P), 3.83–3.98 (m 10H, OCH_2 and CH_2Ar), 7.24–7.42 (m 2H, Ar), 7.85 (d 1H, Ar), 7.96 (t 1H, Ar), 8.03 (d 1H, Ar); ^{31}P NMR (300 MHz, $CDCl_3$) δ: 26.19.
18. (a) Experimental details for **3c**: To a stirring solution of **2c** (1 g, 2.27 mmol) and K_2CO_3 (325 mesh, 0.941 g, 6.51 mmol) in dry acetonitrile (57 mL, 0.04 M) was added 2-(chloromethyl)-6-fluoroquinoline (0.444 g, 2.27 mmol). The solution was then heated to 40°C and allowed to stir for 48 hours. After reaction completion the K_2CO_3 was filtered through Celite then the reaction volume was reduced producing a slightly yellow viscous oil. The crude product was then purified on silica using a 30:4:1 ethyl acetate:methanol:$NH_4OH_{(aq)}$ eluent system. Fractions containing monosubstituted product were collected, the volume was reduced then redissolved in CH_2Cl_2 and washed with $NH_4OH_{(aq)}$. The organic layer was dried over $MgSO_4$ and the volume was reduced producing a viscous colorless oil; (b) ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.65–2.88 (br d 8H, NCH_2CH_2N), 3.23–3.61 (br 8H, NCH_2CH_2N), 3.85–4.05 (br d 2H, NCH_2Ar), 4.53 (d 2H, CH_2Ar), 4.81–4.97 (br d 2H, CH_2Ar), 6.85–7.35 (m 10H, Ar), 7.30–7.53 (m 3H, Ar), 7.72–7.94 (m 2H, Ar).
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21. Compound **1** was isolated in its fully protonated form and showed a complex proton NMR spectrum in D_2O. The phosphorous NMR spectrum showed a single peak and high resolution MALDI produced a mass accuracy within −3.7 ppm (theoretical [M+1]: 578.19394; experimental [M+1]: 578.1918). ^{1}H NMR (500 MHz, D_2O) δ: 2.84–3.89 (br m 24H, NCH_2CH_2N, NCH_2P, NCH_2CO, and NCH_2Ar), 7.82–7.88 (m 2H, Ar), 8.05 (d 1H, Ar), 8.36 (m 1H, Ar), 8.97 (d 1H, Ar); ^{31}P NMR (500 MHz, D_2O) δ: 6.85 singlet.