Synthesis of a Europium Complex for Anion-Sensing Involving Regioselective Substitution of Cyclen

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A regioselective synthetic route that allows the construction of tri-functionally substituted cyclen is described. This facilitates the synthesis of a europium complex that exhibits a good photoresponse to malate, forming the basis of a chemoselective sensor.

Introduction

Complexes of europium as well as other emissive lanthanides are being applied widely as probes in the life, chemical and earth sciences.[1] The characteristic luminescence of the Eu^{3+} ion can subtly respond to its coordination environment by changes in its emission fine structure, luminescence lifetime and even the intensity ratio of the luminescence. Recently, we have employed such features to probe the binding of certain anions, for example, hydrogen carbonate, citrate and phospho anions.[2] A successful probe based on europium luminescence should possess a high quantum yield of luminescence. This can be generated through an efficient energy-transfer process from the triplet-excited chromophore to europium, as well as the appropriate construction of its coordination environment. This means that the ligand should possess not only sufficient donors (≥ 6) to achieve complex stability, but also the chromophore needs to be integrated into the ligand structure to allow both sensitization of Eu and allow scope for the introduction of targeting vectors.

Cyclen (1,4,7,10-tetraazacyclododecane) is the pre-eminent ligand framework upon which to construct the lanthanide complexes,[3] and a series of methods has been established to achieve its selective mono-, di- and tri-substitution.[4] Whereas most of them have concentrated on the synthesis of mono- and tri-substituted systems, a second functional group can also be attached to the remaining N-position(s) of cyclen afterwards. 1,4- and 1,7-Disubstitution can be achieved by the reaction of diethyl oxalate and benzyl chloroformate with cyclen, respectively, releasing two free N positions.[5] Reports on the controlled mono-substitution of these disubstituted ligands are rare. Handel has prepared such tri-substituted ligands by the reflux of "cyclenoxamide" in acrylonitrile before its deprotection in a good yield. This is limited to the use of acrylonitrile.[5a] Bornhop has used 1,7-di-protected cyclen to react with 2-(chloromethyl)-6-fluoroquinoline in the presence of potassium carbonate to get a tri-substituted ligand.[6] However, a tetra-substituted ligand might be the main product in this method. Wong also applied this method but using 1,4-di-protected cyclen to react with alkyl halides; only "less reactive" haloalkanes resulted in adequate yields, whereas for activated electrophiles such as benzyl halides, the undesired bis-substituted product predominated.[7] Thus a more regioselective synthetic route needs to be developed.

Results and Discussion

The construction of our target ligands requires the regioselective synthesis of trifunctional pendants. In previous work, such a system was built up with two chelating arms, a chelating chromophore and a free amine on cyclen ring, by a similar reaction between 3-chloromethyl-4-pyridothioxanthone and the cyclen derivative **1a**,[2c] as reported by Bornhop.[6] In this reaction, the major product was mono-substituted cyclen rather than the di-substituted. When 3-chloromethyl-4-pyridothioxanthone was replaced with 7-bromomethyl-4-pyridothioxanthone,[8] the proportion of the di-substituted product increased dramatically.[9] This may result from the higher activity of the alkyl bromide compared to the chloride which minimises the selectivity.

Surprisingly, when this reaction was conducted in refluxing ethanol between the bromoalkyl compound and the cyclen derivative (just 20% excess, Scheme 1), very little of the di-substituted was detected by electrospray mass spectroscopy in the reaction mixture, whereas the mono-substituted (**1**) was the dominant species (see Supporting Infor-

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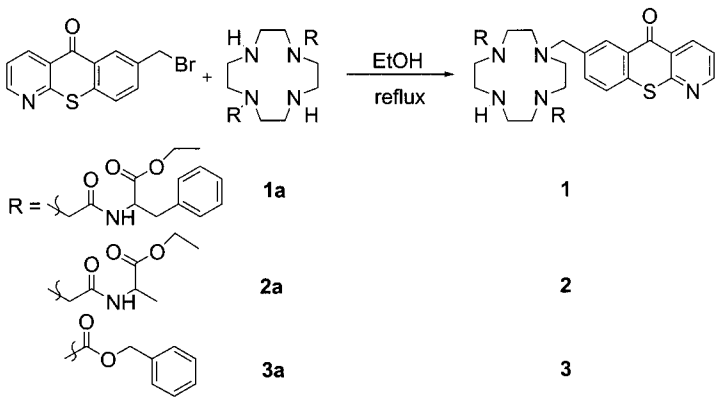

Scheme 1. Regioselective synthesis of trifunctionalised cyclen derivatives.

mation; see also the footnote on the first page of this article). This high selectivity is quite useful for the preparation of trifunctional cyclen derivatives, and facilitates the purification process.

Such a reaction as a general procedure for the monoalkylation of 1,7-di-protected cyclen by an activated electrophile has been corroborated by reactions with two other cyclen derivatives: **2a** and **3a**. Among them, the synthesis of **3a** exhibited the highest yield (34%). The moderate yield may be partially attributed to the high reactivity of 7-bromomethyl-4-pyridothioxanthone which underwent side-reactions.

The reason why the fourth N position would not undergo alkylation may be that the first alkylation of the 1,7-di-protected cyclen produces a proton which may protonate the last free nitrogen, and consequently prevent it from alkylation. The single-crystal X-ray structure of tri-substituted cyclen hydrochloride also supports this argument, which shows that the unalkylated N atom on the cyclen ring is protonated, and forms a bifurcated H bond.[10]

The complex **Eu1** was prepared by refluxing **1** and $Eu(CF_3SO_3)_3$ in acetonitrile overnight. This complex exhibits a distinctive luminescence emission profile in the presence of malate. Malate plays a central role in metabolism. It is not only an intermediate of the Krebs cycle, but also a key component of mitochondrial malate-aspartate shuttle which involves the movement of electrons from NADH across the mitochondrial membranes to balance the mitochondrial metabolism and cytosolic redox state.[11] However, the determination of malate needs elaborate biological processes, e. g., the use of enzymes and requiring special apparatus.[12] Particularly, these methods cannot be applied for living cell imaging. The application of this complex in vitro or in vivo may significantly facilitate the measurement process.

Titration of malate to an aqueous solution of $[\mathbf{Eu1}]^{3+}$ (20 μM) resulted in very significant changes in its luminescence spectrum at low added malate concentrations, with the intensity of the $\Delta J = 2$ bands (centred at 616 nm) increasing while that of the $\Delta J = 0$ transition at 579 nm decreased (see Supporting Information). This made it possible to take advantage of a ratiometric measurement to probe the concentration of malate (Figure 1) with the intensity ratio of 616/579 nm increasing with malate concentration. Analysis of the binding isotherm was consistent with an apparent binding constant of $5.7 \times 10^5\ M^{-1}$ (± 20%). A separate Job plot confirmed the 1:1 stoichiometry of complexation. However, this complex has a similar photoresponse with added citrate that may limit its widely biological application. The selectivity needs to be improved in the future work. The presence of some other common biological anions, such as phosphate, lactate, acetate, and hydrogen carbonate, led to increases in the overall emission intensity with increasing anion concentration. However, for each of these anions, the emission changes did not significantly influence the intensity ratio of the 616 nm to 579 nm bands, which remained around 4.5 in each case.

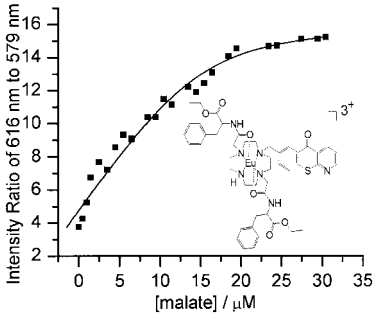

Figure 1. The intensity ratio of the 616 nm to 579 nm bands for an aqueous solution of $\mathbf{[Eu1](CF_3SO_3)_3}$ (20 μM) upon titration with sodium malate; λ_{exc} = 382 nm. The curve (solid line) represents the best fit using non-linear least-squares fitting to the experimental data.

Conclusions

In conclusion, a pragmatic regioselective method has been developed for the synthesis of trifunctional cyclen derivatives. Using this method, a pyridothioxanthone-sensitized europium complex has been made, which not only exhibits high affinity to malate, but also presents a useful intensity ratio change of the europium luminescence which is potentially valuable for the ratiometric measurement of malate in dilute solution.

Experimental Section

General Remarks: All the solvents were distilled before use. All the other reagents (Aldrich) were used as received. UV/Vis spectra and luminescence spectra were recorded with a Perkin–Elmer Spectrometer (UV/Vis/NIR Lambda 900) and Fluorolog-3 Spectrometer (HORIBA Jobin Yvon) in a 1-cm optical path quartz cell at room temperature, respectively. Silica gel (TLC standard grade, Aldrich) was used for column chromatography. ESI-MS mass spectra were recorded using a VG Platform II electrospray mass spectrometer. Accurate masses were determined with a Thermo-Finnigan LTQ FT mass spectrometer. ^{1}H and ^{13}C NMR was recorded with a Bruker Avance-400 (400 MHz), Varian Mercury-200 (200 MHz) or Varian Unity-300 (300 MHz) machine.

General Methods: Under argon, cyclen derivatives (1.7 mmol), 7-bromo-4-pyridothioxanthone (1.4 mmol), and ethanol (80 mL) were boiled under reflux overnight. The solvent was then evaporated and DCM (300 mL) was added. The mixture was washed with water, dried with sodium sulfate, and purified by column chromatography on silica gel, with DCM/MeOH/Et_3N as eluent, yielding a light yellow solid.

1: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.81 [d, $^3J_{H,H}$ = 5.1 Hz, 1 H, Ar-H$_3$], 8.79 (d, $^3J_{H,H}$ = 4.5 Hz, 1 H, Ar-H$_1$), 8.44 (s, 1 H, Ar-H$_8$), 7.64 (dd, $^3J_{H,H}$ = 7.5, 1.2 Hz, 1 H, Ar-H$_6$), 7.57 (d, $^3J_{H,H}$ = 7.5 Hz, 1 H, Ar-H$_5$), 7.46 (dd, $^3J_{H,H}$ = 5.1, 4.5 Hz, 1 H, Ar-H$_2$), 7.09–7.23 (m, 10 H, Ph-H$_2$), 4.79 (m, 2 H, α-H), 4.12 (q, $^3J_{H,H}$ = 6.8 Hz, 4 H, C*H*$_2$CH3), 3.14 (br. s, 6 H, Ph-CH$_2$, Ph-CH$_2$, Ar-CH$_2$), 3.11 (s, 4 H, N–CH$_2$), 3.01 (br. s, 8 H, cyclen), 2.77 (br. s, 4 H, cyclen), 2.70 (br. s, 4 H, cyclen), 1.21 (t, $^3J_{H,H}$ = 6.8 Hz, 6 H, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 180.6, 171.7, 170.2, 158.7, 153.5, 137.8, 136.6, 136.3, 134.1, 130.3, 129.3, 128.5, 127.2, 126.3, 121.8, 61.6, 59.4, 53.9, 53.4, 53.3, 52.6, 50.5, 46.9, 37.6, 14.1 ppm. HRMS (ES$^+$) (*m*/*z*), found 864.4110. $C_{47}H_{58}N_7O_7S$ requires 864.4113.

2: ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 8.83 (dd, $^3J_{H,H}$ = 4.2, 1.6 Hz, 1 H, Ar-H$_1$), 8.81 (dd, $^3J_{H,H}$ = 8.1, 1.6 Hz, 1 H, Ar-H$_3$), 8.58 (s, 1 H, Ar-H$_8$), 7.80 (d, $^3J_{H,H}$ = 2.0 Hz, 1 H, Ar-H$_6$), 7.79 (d, $^3J_{H,H}$ = 2.0 Hz, 1 H, Ar-H$_5$), 7.59 (dd, $^3J_{H,H}$ = 8.1, 4.2 Hz, 1 H, Ar-H$_2$), 4.32 (m, 2 H, α-H), 4.12 (q, $^3J_{H,H}$ = 7.2 Hz, 4 H, C*H*$_2$CH3), 3.71 (s, 2 H, Ar-CH$_2$), 3.39 (s, 4 H, N–CH$_2$CO), 3.28 (m, 4 H, cyclen), 3.00 (m, 4 H, cyclen), 2.88 (m, 4 H, cyclen), 2.74 (m, 4 H, cyclen), 1.43 (d, $^3J_{H,H}$ = 7.2 Hz, 2 H, α-CH$_3$), 1.36 (d, $^3J_{H,H}$ = 7.2 Hz, 4 H, α-CH$_3$), 1.28 (t, $^3J_{H,H}$ = 7.2 Hz, 6 H, CH$_2$C*H*$_3$) ppm. MS (ES$^+$): *m*/*z* = 712.6 [M + H]$^+$.

3: ^{1}H NMR (200 MHz, CD_3OD, 25 °C): δ = 8.83 (m, 2 H, Ar-H$_1$ + H$_3$), 8.16 (s, 1 H, Ar-H$_8$), 7.69 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, Ar-H$_6$), 7.59 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, Ar-H$_5$), 7.49 (m, 1 H, Ar-H$_2$), 7.15 (m, 10 H, Ph-H), 5.06–5.23 (m, 1 H, ArCH$_2$O), 5.4.80–4.96 (m, 1 H, ArCH$_2$O), 4.67 (d, $^3J_{H,H}$ = 12.0 Hz, 1 H, ArCH$_2$O), 4.45 (d, $^3J_{H,H}$ = 12.0 Hz, 1 H, ArCH$_2$O), 3.92 (s, 2 H, ArCH$_2$N), 3.65 (m, 4 H, cyclen), 3.50 (m, 4 H, cyclen), 3.25 (m, 8 H, cyclen) ppm. MS (ES$^+$): *m*/*z* = 666.1 [M + H]$^+$.

[Eu1]: Under argon, **1** (54 mg, 0.06 mmol), Eu(CF_3SO_3)$_3$ (200 mg, 0.33 mmol), and acetonitrile (4 mL) were boiled under reflux for 24 h. The mixture was then added dropwise into ethyl ether (30 mL) and stirred for at least 2 h, resulting in a precipitate. This precipitate was separated and re-dissolved into acetonitrile (2 mL) and the same procedure was repeated five times. All the ethyl ether was collected and evaporated, yielding a light yellow solid (65 mg, 67%). HRMS-ES$^+$ (*m*/*z*), found 1314.2269. $C_{49}H_{57}EuF_6N_7O_{13}S_3$ ([ML + 2CF SO_3]$^+$) requires 1314.2288.

Supporting Information: The ESMS spectra of reaction mixtures, emission spectra of [**Eu1**] in the presence of varied concentrations of citrate as well as in the presence of some other common biological anions. For supporting information see also the footnote on the first page of this article.

Acknowledgments

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[8] a) Experimental details for 7-bromomethyl-4-pyridothioxanthone: under argon, a mixture of 7-methyl-4-pyridothioxanthone (2.5 g, 11.0 mmol), *N*-bromosuccinimide (2.4 g, 13.4 mmol), benzoyl peroxide (30 mg, 0.1 mmol), and carbon tetrachloride (40 mL) was refluxed overnight under illumination of a 100 W tungsten lamp. The solvents were evaporated and the crude product was purified by column chromatography on silica gel, with toluene as the eluent to yield a yellow solid, 7-bromo-4-pyridothioxanthone (2.2 g, 64%); b) ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.84 (dd, $^3J_{H,H}$ = 8.1, 1.8 Hz, 1 H, H^1), 8.81 (dd, $^3J_{H,H}$ = 4.5, 1.8 Hz, 1 H, H^3), 8.59 (d, $^3J_{H,H}$ = 1.8 Hz, 1 H, H^8), 8.74 (dd, $^3J_{H,H}$ = 8.1, 1.8 Hz, 1 H, H^6), 7.66 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, H^5), 7.47 (dd, $^3J_{H,H}$ = 8.1, 4.5 Hz, 1 H, H^2), 4.62 (s, 2 H, ArCH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 180.2, 158.5, 137.9, 137.6, 136.8, 133.6, 129.8, 129.0, 127.2, 126.4, 121.9, 32.1 ppm. MS(ES$^+$): *m*/*z* = 306 [M + H]$^+$. $C_{13}H_8BrNOS$ (306.2): calcd. C 51.00, H 2.62, N 4.57; found C 51.04, H 2.57, N 4.41.
[9] The reaction was conducted in acetonitrile (40 mL) under argon with the cyclen derivative (2.2 mmol), alkyl halide (1.1 mmol) and potassium carbonate (5 g) stirred at room temp. for 48 h. The electrospray mass spectrum showed that the peak at 1111 (the di-substituted compound combining a sodium ion) was of much greater intensity than that of target (864 [M + H]$^+$). The relative peak intensities in the ES-MS spectra were within ± 20% of the intensities revealed by TLC and column chromatography by which about a 50:50 ratio of mono- and di-substituted products was obtained.
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