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# Specific circularly polarized luminescence of Eu(III), Sm(III), and Er(III) induced by *N*-acetylneuraminic acid†

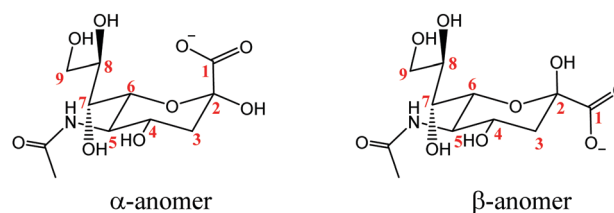
Tao Wu \* and Petr Bouř 

Recently, we found that the Raman optical activity (ROA) technique can be used to monitor even a weak lanthanide luminescence including circular polarization. In the present study we compare circularly polarized luminescence (CPL) spectra of Eu(III), Sm(III), and Er(III) induced by aqueous solution of sialic acid. For Eu providing the strongest signal a chelation model was proposed where the carboxyl adopts the axial conformation and carboxyl oxygen is attached to Eu(III).

In modern biology and medicine cost-efficient analytical and imaging methods are used to acquire knowledge on the structure and function of living systems. High-contrast images are used for the diagnosis and treatment of cancer.<sup>1</sup> *N*-Acetylneuraminic acid (Neu5Ac) can serve as a convenient marker molecule because its presence in mammalian cells depends on the metabolic intensity. It can also be found in glycol-conjugates such as glycolipids and glycoproteins, where it is important for their selective binding affinity. The concentration of this sialic acid released in blood serum thus may indicate malignant health conditions.<sup>2,3</sup>

The carboxyl group can be at an axial or equatorial position, Neu5Ac thus exists in the  $\alpha$  and  $\beta$  anomers (Scheme 1). In solution, the  $\beta$ -anomer is prevalent (over 90%).<sup>4,5</sup>

Lanthanide(III) ions are used as fluorescent probes designed for analysis and imaging of biological materials because of their unique electronic structures and sensitivity to the environment.<sup>6,7</sup> They are also relatively non-toxic and stable under irradiation. Lanthanide probes were found to be useful for the detection of sialic acid by MRI<sup>8–10</sup> and luminescence spectroscopic methods.<sup>11,12</sup> Recently, circularly polarized luminescence (CPL) was used to indicate the presence of sialic acid, upon chelation with a racemic europium(III) complex.<sup>5</sup>

Scheme 1 Two forms of the *N*-acetylneuraminic ("sialic") acid anion.

Commercial CPL instruments use relatively weak excitation sources, which weakens the sensitivity of this method.<sup>13–16</sup> Raman instruments with a stronger laser excitation are usually not suitable for luminescence measurements on organic molecules, because of their limited spectral range. For europium(III), however we found that CPL recording using the Raman optical activity (ROA) spectrometer is quite convenient.<sup>17</sup> The metal provides narrow spectral lines that fit into the spectrometer operational range. ROA-based identification of luminescent achiral lanthanide compounds when the chirality is induced by a magnetic field is also possible.<sup>18</sup> This was shown for Na<sub>3</sub>[Ln(DPA)<sub>3</sub>] complexes, Ln = Ce, Pr, Nd, Sm, Eu, Tb, Dy, Ho, and Er. The ROA/CPL spectroscopy thus seems quite versatile and very promising for future applications in biospectroscopy.

The (vibrational) ROA signal itself is equally useful in molecular structural studies,<sup>19,20</sup> but lanthanide CPL is often much stronger. Sugar-based compounds were indicated as particularly suitable for Ln/CPL spectroscopy, providing multiple metal binding groups (mostly OH) and very specific signals<sup>21</sup> suitable for their recognition.<sup>22</sup> However, many spectral features are not understood, and suitability of different metals for the CPL probing is not systematically explored. In the present study, we compare the optical activity of Eu(III), Sm(III), and Er(III) induced by Neu5Ac. A europium(III) complex was identified as the most promising indicator of the acid.

The Raman/TL and ROA/CPL spectra of plain Neu5Ac solution and mixtures with EuCl<sub>3</sub>, Sm(NO<sub>3</sub>)<sub>3</sub> and ErCl<sub>3</sub> are plotted in Fig. 1. The Neu5Ac Raman spectrum is very rich, reflecting the number of vibrational normal modes; however,

Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Flemingovo náměstí 2, 16610 Prague 6, Czech Republic. E-mail: wu@uochb.cas.cz

† Electronic supplementary information (ESI) available: Details of the experimental method, the temperature dependent CID ratio of the band at 1901 cm<sup>-1</sup> of Neu5Ac:EuCl<sub>3</sub>; the concentration dependent CID ratio of the band at 1901 cm<sup>-1</sup> of Neu5Ac:EuCl<sub>3</sub> in aqueous solution; and the Neu5Ac:Sm(NO<sub>3</sub>)<sub>3</sub> spectrum. See DOI: 10.1039/c7cc09463a



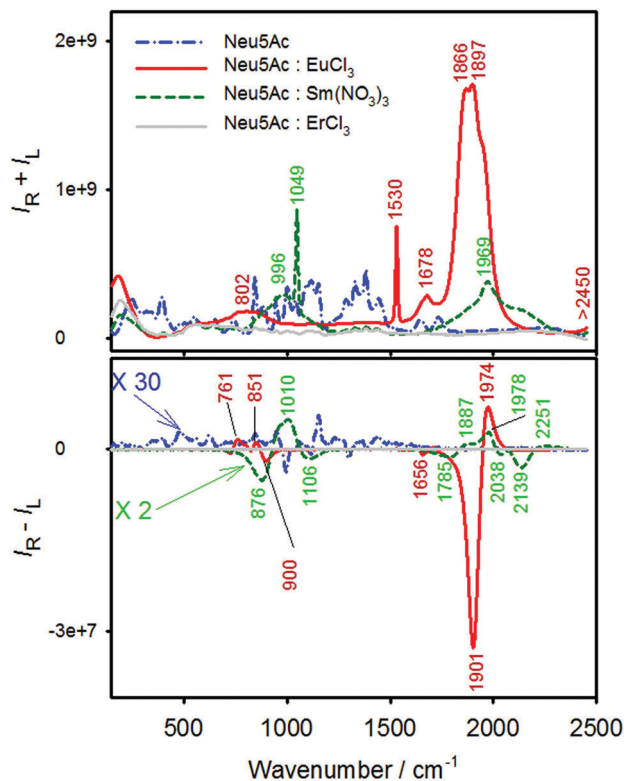


Fig. 1 Raman/TL and ROA/CPL spectra of Neu5Ac (300 mM) and its complexes with  $\text{EuCl}_3$  (red, 4 mM:4 mM),  $\text{Sm}(\text{NO}_3)_3$  (green, 30 mM:30 mM), and  $\text{ErCl}_3$  (grey, 30 mM:30 mM).

its ROA spectrum is rather weak; the ratio of the ROA and Raman intensity (CID, circular intensity difference) is about  $\sim 10^{-4}$ .

Compared to this, the CPL signal induced in  $\text{Eu}(\text{III})$  and  $\text{Sm}(\text{III})$  is quite remarkable,  $\text{CID} \sim 10^{-2}$ – $10^{-3}$  (Table 1). On the other hand,  $\text{Er}(\text{III})$  provides no signal even at high concentrations. This is somewhat unexpected, because at least the  $^2\text{G}_{9/2} \rightarrow ^4\text{I}_{13/2}$  erbium transition previously did provide measurable CPL induced by a magnetic field.<sup>18</sup> Thus the interaction with Neu5Ac is either weak or does not provide the magnetic and quadrupole components necessary for CPL.

Both TL and CPL of the  $\text{EuCl}_3$ –Neu5Ac system are very different from spectral shapes observed for  $\text{EuCl}_3$  in monosaccharide solutions,<sup>21</sup> indicating a specific binding mode. Although the Eu concentration is rather low (4 mM), CPL is comfortably measurable as the relative intensity is about 30 times stronger than the ROA intensity of Neu5Ac. For the ROA acquisition, a much larger concentration (300 mM) was needed. The highest CID ratio is  $-1.92 \times 10^{-2}$  at  $1901 \text{ cm}^{-1}$ , corresponding to the  $^5\text{D}_0 \rightarrow ^7\text{F}_1$  Eu transition.

The interaction of Neu5Ac with europium was examined in more detail. Both the TL and CPL spectra depend on temperature; colder temperature is favorable for the larger CID ratio (Fig. S1, top, ESI†). Spectral shapes also exhibit some dependence on the concentration, as follows from titration results shown in Fig. 2. The usually extremely weak  $^5\text{D}_1 \rightarrow ^7\text{F}_2$  transition is apparent both in TL ( $802 \text{ cm}^{-1}$ ) and CPL spectra ( $900, 851, 761$ , and  $725 \text{ cm}^{-1}$ , Fig. 2).<sup>23</sup>

Table 1 Assignment of observed  $\text{Eu}(\text{III})$  and  $\text{Sm}(\text{III})$  luminescent bands in the Neu5Ac solution selected CID ratio, the Raman shift from the  $532 \text{ nm}$  laser frequency (in  $\text{cm}^{-1}$ ) and the corresponding wavelength (in nm)

| Neu5Ac:Ln                  | Raman shift ( $\text{cm}^{-1}$ ) | Wavelength (nm) | CID ratio              | Transition                                      |
|----------------------------|----------------------------------|-----------------|------------------------|---|
| $\text{EuCl}_3$            | > 2450                           | 611.7           |                        | $^5\text{D}_0 \rightarrow ^7\text{F}_2$         |
|                            | 1974                             | 594.4           | $7.38 \times 10^{-3}$  | $^5\text{D}_0 \rightarrow ^7\text{F}_1$         |
|                            | 1901                             | 591.9           | $-1.92 \times 10^{-2}$ | $^5\text{D}_1 \rightarrow ^7\text{F}_3$         |
|                            | 1897                             | 591.7           |                        |   |
|                            | 1866                             | 590.6           |                        |   |
|                            | 1678                             | 584.1           |                        |   |
|                            | 1656                             | 583.4           | $-3.47 \times 10^{-3}$ |   |
|                            | 1530                             | 579.1           |                        | $^5\text{D}_0 \rightarrow ^7\text{F}_0$         |
|                            | 900                              | 558.8           | $-1.33 \times 10^{-2}$ | $^5\text{D}_1 \rightarrow ^7\text{F}_2$         |
|                            | 851                              | 557.2           | $8.66 \times 10^{-3}$  |   |
|                            | 802                              | 555.7           |                        |   |
|                            | 761                              | 554.4           | $9.29 \times 10^{-3}$  |   |
|                            | 725                              | 553.3           | $-3.94 \times 10^{-3}$ |   |
| $\text{Sm}(\text{NO}_3)_3$ | 2251                             | 604.4           |                        | $^4\text{G}_{5/2} \rightarrow ^6\text{H}_{7/2}$ |
|                            | 2139                             | 600.3           | $-9.11 \times 10^{-3}$ |   |
|                            | 2038                             | 596.7           |                        | $^4\text{F}_{3/2} \rightarrow ^6\text{H}_{9/2}$ |
|                            | 1978                             | 594.6           | $3.93 \times 10^{-3}$  |   |
|                            | 1969                             | 594.2           |                        |   |
|                            | 1887                             | 591.4           |                        |   |
|                            | 1785                             | 587.8           |                        |   |
|                            | 1106                             | 565.3           | $-4.13 \times 10^{-3}$ | $^4\text{G}_{5/2} \rightarrow ^6\text{H}_{5/2}$ |
|                            | 1010                             | 562.2           | $7.58 \times 10^{-3}$  | $^4\text{F}_{3/2} \rightarrow ^6\text{H}_{7/2}$ |
|                            | 996                              | 561.8           |                        |   |
|                            | 876                              | 558.0           | $-1.14 \times 10^{-2}$ |   |

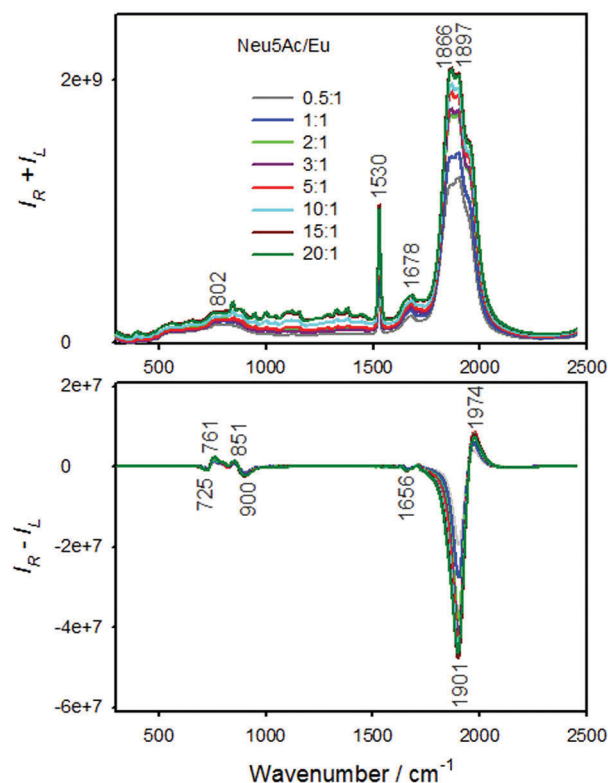


Fig. 2 Raman/TL and ROA/CPL spectra of  $\text{EuCl}_3$  (4 mM) for different concentrations of Neu5Ac.



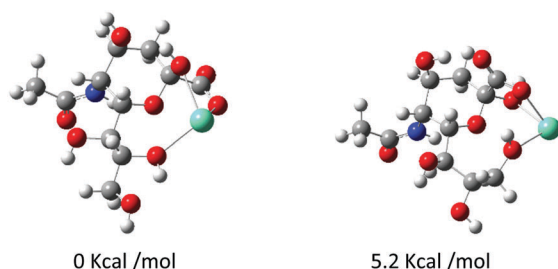


Fig. 3 Optimized geometries of Eu with  $\alpha$ - (left) and  $\beta$ - (right) Neu5Ac anomers, relatively free energy is indicated.

Table 2 Calculated coordination bond lengths in the Eu–Neu5Ac (see carbon numbering in Scheme 1)

| Bond       | $\alpha$ -Anomer | $\beta$ -Anomer |
|------------|------------------|-----------------|
| Eu–OO(C1)– | 218 pm           | 226 pm          |
| Eu–OH(C2)– | 262 pm           | 257 pm          |
| Eu–OH(C8)– | 241 pm           | 247 pm          |

High CID values at  $900\text{ cm}^{-1}$  ( $-1.33 \times 10^{-2}$ ) and elsewhere suggest a coordination binding between Eu(III) and Neu5Ac. The titration curve (Fig. 2) indicates that one europium ion might bind two Neu5Ac molecules. However, more complex intermolecular interactions may be involved; because the largest CID was obtained for the five to one Neu5Ac/EuCl<sub>3</sub> ratio (Fig. S1, ESI<sup>†</sup>). After this, the CID values slightly decrease, although the intensity of both TL and CPL increases slightly.

The TL intensity of the Sm(NO<sub>3</sub>)<sub>3</sub>–Neu5Ac system is much weaker and a higher concentration is needed to obtain good spectra when compared to EuCl<sub>3</sub>. In accord with a previous study,<sup>18</sup> the Sm(NO<sub>3</sub>)<sub>3</sub> nitrate ion provides the peak at  $1049\text{ cm}^{-1}$  and two broad Sm(III) luminescence bands ( $1969$  and  $996\text{ cm}^{-1}$ ) are observed (Fig. S2, ESI<sup>†</sup>). As for Eu, Neu5Ac binding to the Sm(III) core is accompanied by induced CPL spectra, composed of more bands compared to magnetic Sm(NO<sub>3</sub>)<sub>3</sub> CPL.<sup>18</sup> The largest CID ratio gives the peak at  $876\text{ cm}^{-1}$  ( $-1.14 \times 10^{-2}$ , Table 1), corresponding to the  $^4\text{G}_{5/2} \rightarrow ^6\text{H}_{5/2}$  transition; the ratio is slightly bigger than that for the magnetic CPL.<sup>18</sup>

Possible binding model geometries of the Neu5Ac and Eu(III) complexes were obtained by the DFT method (Fig. 3). Apparently, both  $\alpha$  and  $\beta$ -anomers may form three Eu–O coordination bonds, *i.e.* with the participation of one COO<sup>–</sup> (in C1) and two OH (at C2 and C8, respectively) groups. The bond lengths are exemplified in Table 2. The calculation indicates that europium binding to the carboxylic group is stronger than that to the OH groups, and the binding of Eu to the  $\alpha$ -anomer is preferred by a relatively high energy margin ( $5.2\text{ kcal mol}^{-1}$ ).

The CPL spectra of three lanthanides induced by the sialic acid solution were acquired using the ROA technique and analyzed. No usable signal was detected for erbium, but the

induced optical activity of europium and samarium appeared useful for Neu5Ac detection. The titration indicated that one europium ion binds about two ligands. The possible structure of the complex was proposed with the aid of DFT calculation. The rich signal (*e.g.*  $^5\text{D}_1 \rightarrow ^7\text{F}_2$ ,  $^5\text{D}_0 \rightarrow ^7\text{F}_0$ , and  $^5\text{D}_0 \rightarrow ^7\text{F}_1$  europium transitions) of simple chlorides that can be measured on the ROA spectrometer is convenient for many applications, avoiding a relatively expensive synthesis of organic Eu(III) complexes.

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## Conflicts of interest

There are no conflicts to declare.

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