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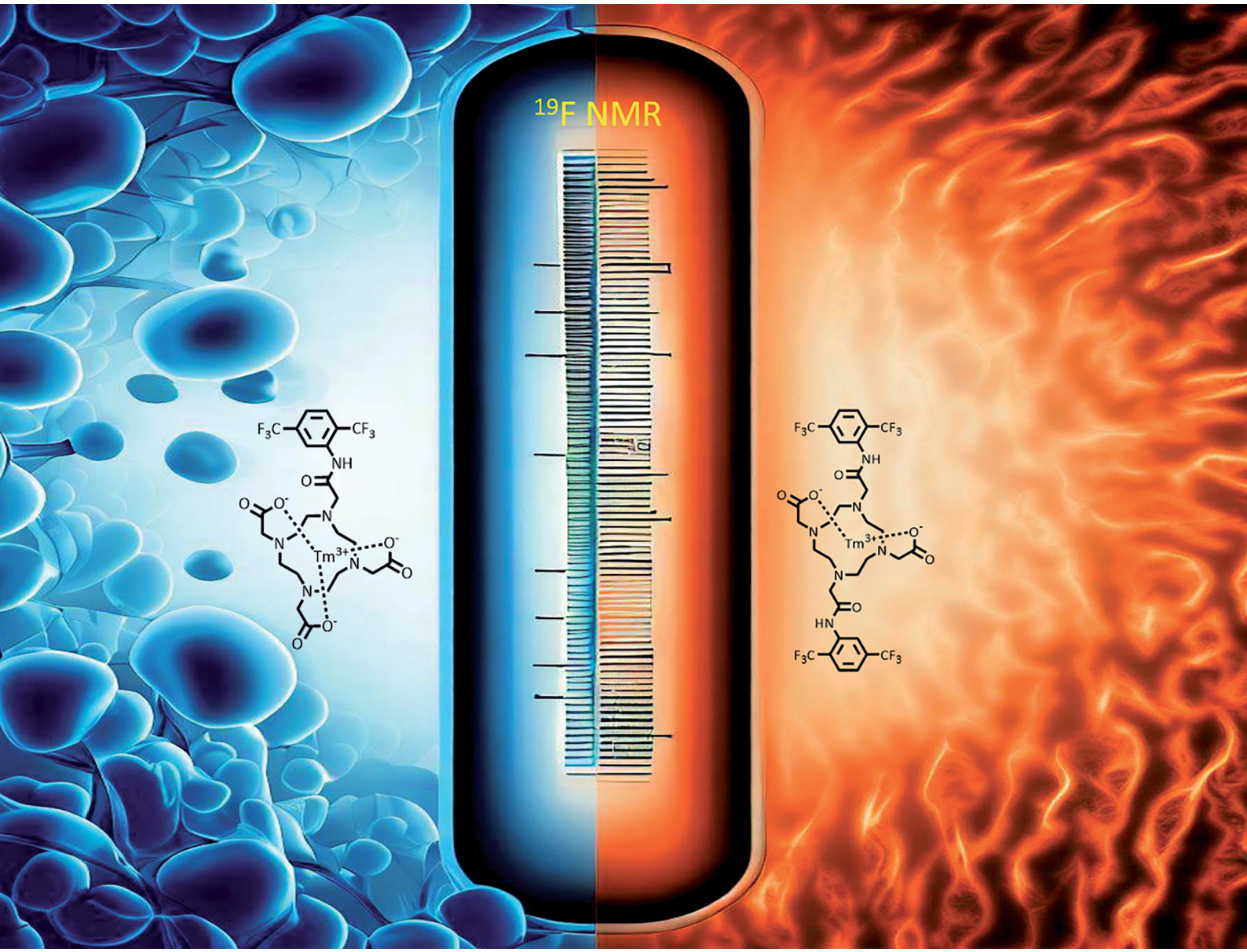

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Felix Mysegaes, Markus Plaumann *et al.*
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Two fluorinated thulium complexes as molecular temperature sensors in MR applications†

Felix Mysegaes,*[ab] Pauline Voigt,[b] Peter Spiteller,[b] Isabell Prediger,[a] Johannes Bernarding[a] and Markus Plaumann *[a]

^{19}F-based magnetic resonance is a powerful tool to overcome several difficulties of standard ^{1}H MR. We present the syntheses and characterization (including cell viability and stability tests) of two Tm^{3+} complexes. Both complexes allow the detection of temperature (ΔC_T = −0.2319 ppm K^{-1} and −0.2122 ppm K^{-1}) without a reference compound.

MR thermometry enables the detection of non-invasive temperature distributions within extended objects.[1] Temperature plays an important role in various biochemical processes, where it can fluctuate within a range of 1–5 K over the course of a day.[2] Therefore, medical applications such as discrimination of healthy and abnormal tissue or progressing of diseases are also of interest.[2a,3] In heat treatments such as hypothermia, monitoring local temperature helps prevent damage to healthy tissue.[3,4]

There are some MR parameters of water that are temperature-sensitive, including T_1 and T_2 relaxation times and proton resonance frequency (PRF).[2a,3,4b] The PRF is currently the most widely used method for temperature detection and imaging. It involves measuring the resonance frequencies of water protons using a gradient-echo-based pulse sequence and calculating the phase coefficient from the measured phase shift as a function of temperature.[3,5] However, when measuring the water protons using this technique, only a low temperature sensitivity of 0.01 ppm K^{-1} is measured.[4b,5b–d,6]

Therefore, the development of substances with highly temperature-sensitive MR signals is necessary. For this purpose, paramagnetic lanthanoid[1b,6,7] (with C_T-values up to 1.45 ppm K^{-1})[7f] and transition metal[8] complexes are coming into focus. The lanthanoid complexes $TmDOTMA^{-}$ and $TmDOTP^{-}$ are probably the most promising examples, with temperature sensitivities of 0.57 ppm K^{-1} and 1.0 ppm K^{-1}, respectively.[1b,6,7] However, $TmDOTP^{-}$ has a high affinity towards Ca^{2+}-ions and is strongly affected by pH value.[1b,7c–e,9] Tsitovich *et al.* synthesized some Fe- and Co-complexes with temperature sensitivities up to 0.52 ppm K^{-1}.[8]

These MR probes show a significant advantage in comparison to the PRF method, but are limited by the inherent Curie temperature dependence of chemical shift in paramagnetic complexes.[3] ^{19}F NMR and MRI are gaining more and more interest for diagnostic studies due to the fact that they have some benefits compared to other nuclei. There is only one natural isotope of fluorine (^{19}F). Additionally, it has a similar gyromagnetic ratio close to ^{1}H. The most important advantage is the absence of fluorine signals in the body, which makes detection of ^{19}F MR probes without background signals possible.[2a,3,10]

To the best of our knowledge, the list of molecular paramagnetic ^{19}F MR temperature sensors is limited to only a few numbers of compounds.[3,11] Other examples include perfluorocarbons or organofluorine compounds, but they are limited in their temperature sensitivity.[2a,12] Lee *et al.* and Li *et al.* recently published a study in which they examined the temperature sensitivity of organofluorine compounds. The synthesized compounds contained several fluorine cores and use the difference between the strongest shifting signals while increasing temperature to determine the temperature sensitivity.[2a,13] These compounds had a temperature sensitivity of 0.0195 ppm K^{-1}, which is almost two times higher than that of PFCs.[2a,13]

The spin-crossover complexes of Thorarinsdottir *et al.* showed much greater potential than ^{19}F MR thermosensitive probes. These results showed a temperature sensitivity of up to 0.45 ppm K^{-1} in FBS (0.67 ppm K^{-1} in MeCN-d_3) and, to our knowledge, are the highest values for ^{19}F.[3] In this paper, we present the synthesis of two Tm^{3+} complexes (**TmL1** and **TmL2**, see Fig. 1) that have high temperature sensitivity. Both complexes possess two CF_3 groups with different chemical shifts. Accordingly, the temperature is determined by the difference

[a] *Institute of Biometry and Medical Informatics, Otto von Guericke University Magdeburg, Medical Faculty, Leipziger Str. 44, Magdeburg 39120, Germany. E-mail: markus.plaumann@med.ovgu.de*

[b] *Instrumental Analytics, University of Bremen, Leobener Str. 7, Bremen 28359, Germany. E-mail: s_zoc4hg@uni-bremen.de*

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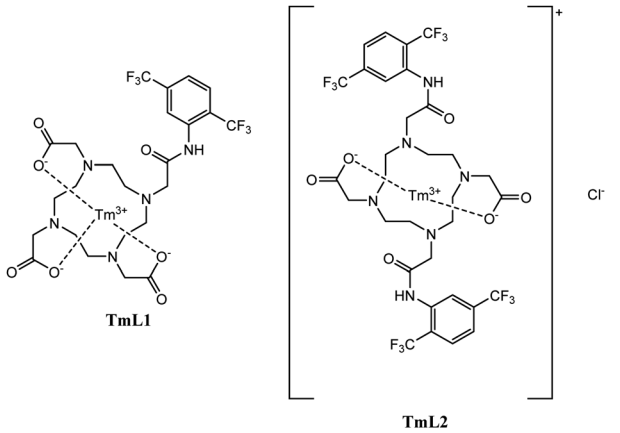

Fig. 1 Molecular structures of the synthesized complexes.

between the two signals, and no added reference substance is required.

Both complexes were synthesized according to a previously published method.[14] First, the tri- or bis-alkylated DO3A-*t*Bu or DO2A-*t*Bu and the bromoacetamide **2** were synthesized according to a literature procedure.[15] The free amine(s) of DO3A-*t*Bu and DO2A-*t*Bu were substituted, following a slightly modified procedure from literature.[14] The compounds were solved in $CHCl_3$ and Na_2CO_3 was added, with stirring taking place over a period of 7 days. Deprotection can be carried out in two different ways: one way involves deprotection with TFA and the other involves a hydrolysis of the esters using formic acid. Due to difficulties in removing residues of TFA, hydrolysis with formic acid was the method of choice. Both complexes **L1** and **L2** were obtained in high yields (89% respectively 83%).

L2 had a higher hydrophobicity than **L1**, the usage of different complexation methods was necessary. Complexation with $TmCl_3$ of **L1** was carried out in water at 45 °C, stirred for one day, while complexation of **L2** was carried out in MeOH at room temperature, stirred for three days. The purification step of the complexation started with precipitation of $Tm(OH)_3$ at pH 10. After filtration of the salt, the solution was neutralized and the solvent was evaporated. The resulting solid was suspended in EtOH and centrifuged to remove NaCl, which yielded the pure complexes (92% for **TmL1** and 85% for **TmL2**). Starting from DO3A-*t*Bu or DO2A-*t*Bu, both complexes were obtained with an overall yield of 75% for **TmL1** and 34% for **TmL2** (see Fig. 2).

Stability experiments were performed with **TmL1**, here, the complex was dissolved in D_2O and two equivalents of $ZnCl_2$ were added. Control of the pH-value showed a slight decrease after 48 h from 3.35 (before the addition of $ZnCl_2$) to 2.83. The stability was verified by recording ^{19}F NMR spectra after five minutes, thirty minutes, seven hours and twenty-four hours. For a long-term study, the probe was measured again after six months, and no transmetalation was observed. Additionally, the mixture was heated to 323.15 K and no changes in ^{19}F NMR spectrum were observable. Finally, **TmL1** showed a high stability towards Zn^{2+}-ions (see Fig. 3).

A solution of 0.1 mmol mL^{-1} of the complexes in 0.5 mL D_2O was used in the experiments. The temperature range used

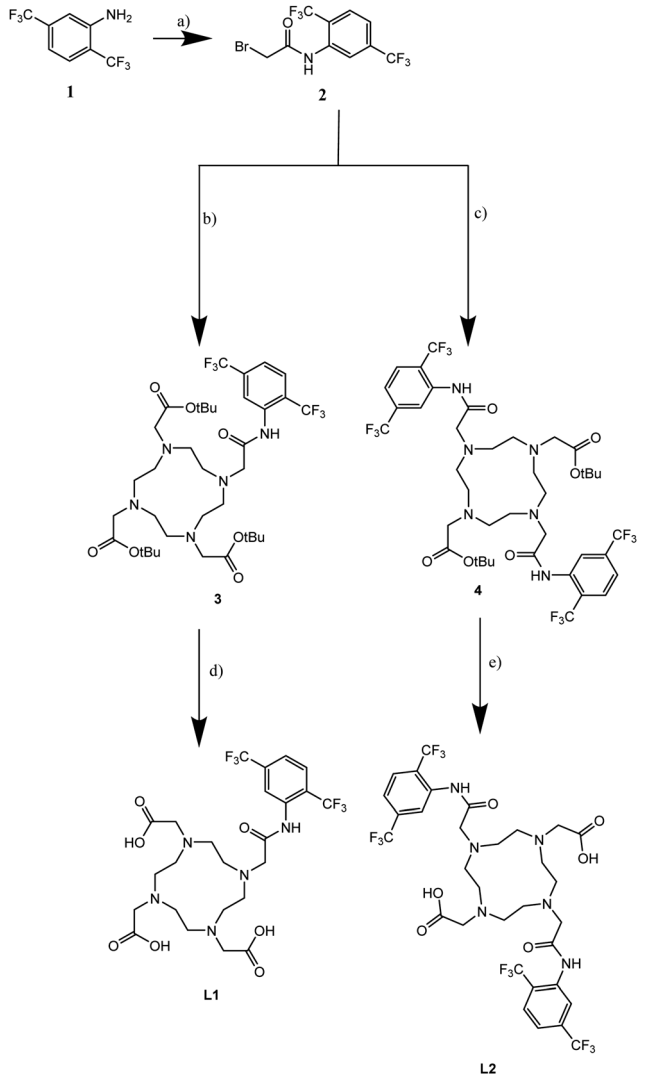

Fig. 2 Synthesis and reagents of **L1** and **L2**. (a) Bromoacetyl bromide (2 eq.), K_2CO_3 (2 eq.), DCM, 30 min at 273.15 K (0 °C) to 90 min at rt, quant., (b) DO3A-*t*Bu (0.94 eq.), Na_2CO_3 (2.2 eq.), $CHCl_3$, 7 d, 55 °C, 92%, (c) DO2A-*t*Bu (0.42 eq.), Na_2CO_3 (2.2 eq.), $CHCl_3$, 7 d, 55 °C, 35%, (d) formic acid, 48 h, 111 °C, 89% for **L1** and 83% for **L2**.

was between 298.15 K and 323.15 K. The chemical shift changes of the two fluorine signals and the difference between these were determined. The difference between two fluorine signals allows the determination of the absolute temperature without the need for an internal or external reference. Complex **TmL1** showed a decrease in the difference between the chemical shifts of both CF_3-groups with rising temperature (see Fig. 4).

The signal at −34.5 ppm (298.15 K) shifted to −37.5 ppm (323.15 K) at higher temperatures, resulting in a C_T-value of −0.1058 ppm K^{-1}. The other signal shifted from −78.7 ppm to −75.6 ppm, resulting in a C_T-value of 0.1261 ppm K^{-1}. This change in the difference between both signals resulted in a temperature coefficient ΔC_T of −0.2319 ppm K^{-1}, which is over 20 times higher than the temperature coefficient of water (C_T = 0.01 ppm K^{-1}).[6b] In the following step, the influence of Zn^{2+}-ions on the temperature sensitivity of **TmL1** was

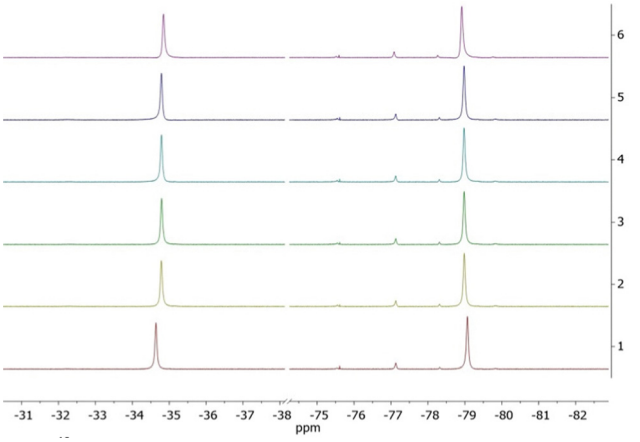

Fig. 3 ^{19}F NMR spectra of **TmL1** after addition of two equivalents of $ZnCl_2$ at different times: 1 : ^{19}F NMR spectrum before addition, 2 : ^{19}F NMR spectrum after 5 min, 3 : ^{19}F NMR spectrum after 30 min, 4 : ^{19}F NMR spectrum after 7 h, 5 : ^{19}F NMR spectrum after 24 h and 6 : ^{19}F NMR spectrum after 6 months.

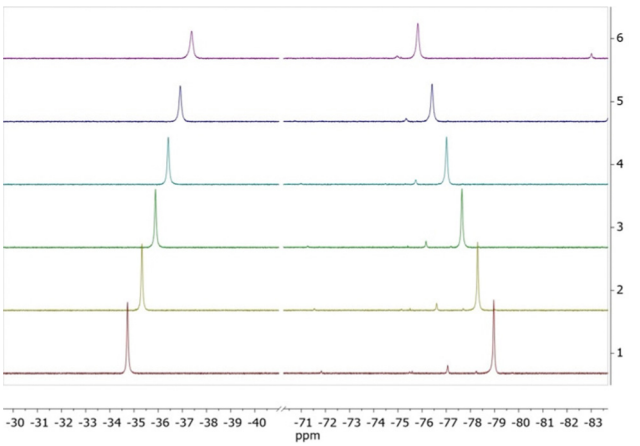

Fig. 4 Variable-temperature ^{19}F NMR spectra of **TmL1** in D_2O. The temperature range was chosen as 298 K to 323 K. 1: T = 298 K, 2: T = 303 K, 3: T = 308 K, 4: T = 313 K, 5: T = 318 K and 6: T = 323 K.

examined. A slight increase to C_T = −0.2370 ppm K^{-1} was observed. We assumed that changes in the C_T-value of ± 0.005 ppm K^{-1} were measurement errors. Thus, the presence of Zn^{2+}-ions does not affect the temperature sensitivity.

Complex **TmL2** showed a slightly lower overall temperature sensitivity compared to **TmL1** (see ESI†). The CF_3-groups displayed C_T-values of 0.0904 ppm K^{-1} and −0.1218 ppm K^{-1}, respectively. This resulted in a ΔC_T between both signals of −0.2122 ppm K^{-1}. Structural differences, such as the positive charge or higher hydrophobicity of **TmL2**, are suggested to have no significant influence on the sensitivity.

The work of Pujales-Paradela *et al.* proved that these types of complexes can be used for paraCEST (paramagnetic Chemical Exchange Saturation Transfer) imaging and fluorine imaging.[14] In addition to their high temperature sensitivity, the synthesis of two multifunctional contrast agents was achieved.

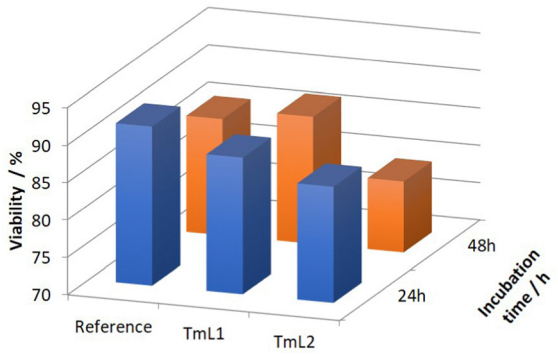

Fig. 5 Results of the viability tests after an incubation time of 24 h and 48 h.

Compared to the results of Thorarinsdottir *et al.*, a lower temperature sensitivity (−0.2319 ppm K^{-1} *vs.* 0.52 ppm K^{-1}) was obtained.[3] However, the use of two different CF_3-groups for the determination of the temperature enables the calculation of the absolute temperature by determining the difference between the two signals. Also, the usage of one CF_3-group gives these complexes an advantage for future imaging experiments. Furthermore, these complexes can be used as multifunctional contrast agents.

After conducting successful ^{19}F VT NMR measurements, the toxicity of the synthesized complexes **TmL1** and **TmL2** was evaluated using fibroblasts (L929) in cell culture experiments. The method used is described in the experimental section. The complexes were dissolved in a cell culture medium at concentrations of 0.19 mM for **TmL1** and 0.24 mM for **TmL2** and added to the cells. The cell viability was determined after 24 h and 48 h of incubation, and the results were compared to a control sample containing only the cells and the cell culture medium. Both complexes showed no significant toxicity (Fig. 5). Complex **TmL1** had a cell viability of 88.56% after 24 h and 87% after 48 h, while complex **TmL2** had slightly lower viability at 85.5% and 79.5%, respectively. These results are comparable to the control samples (91.25% and 85.56%, respectively) and indicate that the complexes can be used in future in *in vivo* experiments (Fig. 5).

We presented the synthesis of two multifunctional complexes, **TmL1** and **TmL2**, with a high ^{19}F MR signal temperature sensitivity and no significant toxicity towards fibroblasts (L929). **TmL1** had a slightly higher temperature sensitivity than **TmL2**, with a ΔC_T of -02319 ppm K^{-1} (and -0.2122 ppm K^{-1}), which is over 20 times higher than the temperature coefficient of water. The presence of Zn^{2+}-ions did not affect the temperature sensitivity of **TmL1**. Based on the previously published complexes,[14] these complexes could be used for paraCEST imaging and ^{19}F imaging. Furthermore, the two different CF_3-groups in each complex enables the determination of the absolute temperature without an internal or external reference. The promising complexes **TmL1** and **TmL2** will be examined in future MR imaging experiments.

Conflicts of interest

There are no conflicts to declare.

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