# Oxygen-17 Dynamic NMR Study of the Pr-DOTA Complex.

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### Contents

Temperature dependence of the $O_1$ chemical shift.		
Temperature dependence of the O <sub>2</sub> chemical shift.	3	
Best-fit analysis of <sup>17</sup> O linewidth data observed for diamagnetic systems.	4	
<sup>17</sup> O NMR spectra recorded before and after addition of NaCl.	5	
Estimation of the TSAP/SAP ratio at low temperature.	6	
Variable temperature <sup>1</sup> H NMR spectra.	7	
<sup>17</sup> O NMR lineshape observed for $O_2$ at variable temperatures.	9	
Estimation of $\Delta \mathbf{G}^{\ddagger}$ characterizing the TSAP-SAP exchange process.	10	
Full line-shape analysis of the $O_2$ spectral region.	11	



#### Temperature dependence of the O<sub>1</sub> chemical shift.

Figure 1: <sup>17</sup>O chemical shift of the  $O_1$  resonance line measured at pH 2.5 (a), 5.9 (b), 9.8 (c), 12.4 (d), as well as at pH 6.8 with 1 equivalent of excess PrCl<sub>3</sub> (e).



#### Temperature dependence of the O<sub>2</sub> chemical shift.

Figure 2: <sup>17</sup>O chemical shift of the O<sub>2</sub> resonance line(s) measured at pH 2.5 (a), 5.9 (b), 9.8 (c), 12.4 (d), as well as at pH 6.8 with 1 equivalent of excess  $PrCl_3$  (e) and at pH 9.8 with 2 equivalents of excess NaCl (f).



Best-fit analysis of <sup>17</sup>O linewidth data observed for diamagnetic systems.

Figure 3: Temperature dependence of the <sup>17</sup>O NMR full linewidth at half-height (LW) observed for the La-DOTA chelate at pH 11.8 (•) and for the free DOTA ligand at pH 12.4 ( $\blacksquare$ ). The spectra were recorded at 14.1 T for <sup>17</sup>O-enriched samples in aqueous solution. The confidence intervals correspond to ±5% for the La-DOTA data and to ±10% for the free DOTA.

The signal narrowing observed for increasing temperatures is properly accounted for by a single Arrhenius-type expression :

$$LW = LW_{nat} = LW_{a}^{298} e^{\frac{E_a}{R} \left[\frac{1}{T} - \frac{1}{298}\right]}$$
(1)

where the pre-exponential factor is the natural linewidth due to quadrupole relaxation at 298 K and  $E_a$  is the corresponding activation energy. The best-fit parameters are :

	La-DOTA	free DOTA
$LW_q^{298}$ (kHz)	$2.63 \pm 0.08$	$1.9 \pm 0.3$
$E_a$ (kJ mol <sup>-1</sup> )	$17.5 \pm 0.7$	$18\pm2$

## <sup>17</sup>O NMR spectra recorded before and after addition of NaCl.



Figure 4: <sup>17</sup>O NMR spectra recorded at 14.1 T and 95 °C for a sample <sup>17</sup>O-enriched Pr-DOTA in aqueous solution at pH 9.8 (a) in the presence of a slight excess of free DOTA and after addition of (b) 1.0 and (c) 2.0 equivalents of NaCl. No apodization of the free induction decay was used. The asterisk indicates the peak of free DOTA ( $O_F$ ).



### Estimation of the TSAP/SAP ratio at low temperature.

Figure 5: (a) Low temperature <sup>1</sup>H NMR spectrum of the Pr-DOTA complex and (b) region of the <sup>17</sup>O NMR spectrum showing the signals of the coordinated oxygen atoms (O<sub>2</sub>). Both the major TSAP ( $\blacksquare$ ) and minor SAP ( $\bullet$ ) conformations are detected (Aime et al., *Inorg. Chem.* **1997**, *36*, 2059-2068). The spectra were recorded at 14.1 T and 3 °C for an <sup>17</sup>O-enriched aqueous sample of Pr-DOTA at pH 9.8 with 2 equivalents of excess NaCl.

	Integral (%) TSAP	Integral (%) SAP
$^{1}H 0 ^{\circ}C$	76	24
<sup>1</sup> H 3 °C	74	26
$^{1}H$ 5 $^{\circ}C$	77	23
<sup>1</sup> H 10 °C	74	26
<sup>17</sup> O 3 °C	71	29

Table 1: Proportion of the TSAP and SAP conformations of the Pr-DOTA complex. The integrated intensity of the <sup>1</sup>H NMR signals (-27 ppm for TSAP and -49 ppm for SAP at 3 °C; see SI Figure 5) and  $O_2$  <sup>17</sup>O NMR resonance lines were determined by Lorentzian deconvolution.



# Variable temperature <sup>1</sup>H NMR spectra.

Figure 6: Variable temperature <sup>1</sup>H NMR spectra recorded for aqueous samples of the Pr-DOTA complex at pH 5.9 (a), 9.8 (b), 9.8 with 2 equivalents of excess NaCl (c) and at pH 12.4 (d).



Figure 7: Variable temperature <sup>1</sup>H NMR spectra recorded for aqueous samples of the Pr-DOTA complex at pH 5.9 (a), 9.8 (b), 9.8 with 2 equivalents of excess NaCl (c) and at pH 12.4 (d). The vertical scale is increased with respect to SI Figure 6 to better show the coalescence of the TSAP and SAP resonance lines observed at low frequency.



## <sup>17</sup>O NMR lineshape observed for $O_2$ at variable temperatures.

Figure 8: Region of variable temperature <sup>17</sup>O NMR spectra of the Pr-DOTA complex showing the signal(s) of the coordinated oxygen atoms (O<sub>2</sub>). The spectra were recorded at 14.1 T for an <sup>17</sup>O-enriched aqueous sample of Pr-DOTA at pH 9.8 with 2 equivalents of excess NaCl.

### Estimation of $\Delta G^{\ddagger}$ characterizing the TSAP-SAP exchange process.

The equilibrium between the TSAP and SAP conformations is an unequally populated two-sites exchange process. If the frequency difference between the resonance lines  $(\Delta v)$  and the population difference  $(\Delta p)$  are known, the rate constant  $(k_c)$  at the coalescence temperature  $(T_c)$  can be estimated using the following equations (Pons, M. and Millet, O., *Prog. Nucl. Magn. Reson. Spectrosc.* **2001**, 38 267-324):

$$k_c = \pi \frac{\Delta \nu}{X} \tag{2}$$

where *X* is the real solution of the polynomial :

$$X^{6} - 6X^{4} + [12 - 27(\Delta p)^{2}]X^{2} - 8 = 0$$
(3)

 $\Delta G^{\ddagger}$  can be determined from Eq. 5, which is obtained by combining Eq. 2 with the Eyring equation (Eq. 4 where *R* is the the universal gas constant, *h* is the Planck constant and *N*<sub>A</sub> is the Avogadro number).

$$k_c = \frac{RT_c}{hN_A} \times exp\left(\frac{-\Delta G^{\ddagger}}{RT_c}\right) \tag{4}$$

$$\Delta G^{\ddagger} = RT_c \left( ln \frac{RX}{\pi h N_A} + ln \frac{T_c}{\Delta \nu} \right)$$
(5)

The  $\Delta G^{\ddagger}$  values estimated from several coalescence events observed either in the <sup>1</sup>H NMR spectra (SI Figure 6 and 7) or in the <sup>17</sup>O NMR spectra (SI Figure 8) are in excellent agreement (see SI Table 2). They are smaller by about 10 kJ/mol than the values reported in the literature, which were determined by <sup>1</sup>H NMR at much lower magnetic field and pH 10-11 (Desreux, J. F. *Inorg. Chem.* **1980**, 19, 1319-1324).

	$\delta_{TSAP}$	$\delta_{SAP}$	$\Delta v$	T <sub>coal</sub> .	k <sub>c</sub>	$\Delta G^{\ddagger}$
	(ppm)	(ppm)	(kHz)	(K)	$(10^3 \ s^{-1})$	(kJ/mol)
$^{1}\mathrm{H}$	22.9	19.4	2.11	283	2.88	50.4
$^{17}O$	-734	-845	8.98	298	12.3	49.7
$^{1}\mathrm{H}$	-27.3	-48.8	12.9	308	17.6	50.5

Table 2: Rate constant and activation free energy determined at various coalescence temperatures for the exchange between the TSAP and SAP conformations of the Pr-DOTA complex (aqueous solution at pH 9.8 with 2 equivalents of excess NaCl). These results were obtained using Eq. 2 and 4 with  $\Delta p$ =0.50 (p<sub>TSAP</sub>=0.75, p<sub>SAP</sub>=0.25) and X=2.30.  $\Delta v$  was measured at 0 °C and 3 °C for the <sup>1</sup>H and <sup>17</sup>O resonance lines, respectively. These data were assumed to be temperature independent.

#### Full line-shape analysis of the O<sub>2</sub> spectral region.

The series of <sup>17</sup>O NMR spectra shown in SI Figure 8 were submitted to a full line-shape analysis (see SI Figure 9). The chemical shift of the O<sub>1</sub> signal, which is temperature independent (see SI Figure 1), was fixed to 321.5 ppm. The natural linewidth of both the TSAP and SAP O<sub>2</sub> signals were constrained to the average value observed for O<sub>1</sub> at pH 5.9 and 9.8 : LW<sup>298</sup><sub>nat</sub> = 1.87 kHz and E<sub>a</sub> = 16.4 kJ mol<sup>-1</sup>. Similarly, the activation enthalpy characterizing the carboxylate rotation was fixed to the average value determined via the O<sub>1</sub> linedwidth data :  $\Delta H^{\ddagger} = 77.0$  kJ mol<sup>-1</sup>. The TSAP/SAP ratio was assumed to be temperature independent and fixed to the average value determined by <sup>1</sup>H NMR, *i.e.* 0.75/0.25 (see SI Table 1). The activation entropy characterizing the TSAP-SAP exchange was assumed to be negligible :  $\Delta S^{\ddagger} = 0$ ; hence  $\Delta G^{\ddagger} = \Delta H^{\ddagger}$ . The chemical shift of both the the TSAP and SAP O<sub>2</sub> signals, which are temperature dependent (see SI Figure 2), were described by the following equation:

$$\delta = \delta_R \left( 1 + \alpha (1 - T_R/T) \right) \tag{6}$$

where  $\delta_R$  is the chemical shift at a reference temperature  $T_R$ , which was chosen to be 276 K (3 °C), and  $\alpha$  accounts for the linear variation of  $\delta$  with 1/T.

The best-fit parameters are:  $\delta_R = 731$  ppm and  $\alpha = -0.602$  for the TSAP O<sub>2</sub> signal,  $\delta_R = 840$  ppm and  $\alpha = -0.817$  for the SAP O<sub>2</sub> signal, and the activation enthalpy characterizing the TSAP-SAP exchange process is found to be 50.5 kJ mol<sup>-1</sup>, in excellent agreement with the estimations based on the detection of coalescence events (see SI Table 2). This model properly accounts for the linewidth of the average O<sub>2</sub> signal observed above 30 °C (see SI Figure 9) and, consequently, it can be concluded that it is the exchange between the TSAP and SAP conformations which is responsible for additional broadening of the O<sub>2</sub> signal and not PRE effects.



Figure 9: Full line-shape analysis of variable temperature <sup>17</sup>O NMR spectra showing the signal(s) of the coordinated oxygen atoms ( $O_2$ ) of the Pr-DOTA complex. The spectra were recorded at 14.1 T for an <sup>17</sup>O-enriched aqueous sample of Pr-DOTA at pH 9.8 with 2 equivalents of excess NaCl.