Evaluation of the Equilibrium Constants, Chemical Shifts and Stoichiometries for Reactions of Lanthanide Shift Reagents containing β-Diketones with Carbohydrate Derivatives

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The interactions between lanthanide shift reagents containing β-diketone ligands and a number of methyl 2,3-anhydro-4,6-*O*-benzylidene-D-hexopyranosides have been investigated using NMR spectroscopy. Chemical shift data have been used to determine the stoichiometries of the reactions and to evaluate the equilibrium constants. Various models are evaluated. The data show that the carbohydrate moiety may be bound to the lanthanide shift reagent in either a monodentate or chelated fashion.

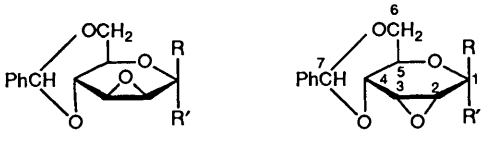
Lanthanide complexes of certain β-diketones have found much use as shift reagents in NMR spectroscopy. Initially they were used to aid in the interpretation of the NMR spectra of a wide range of molecules including carbohydrates.[1–5] Shift reagents (M) have been used to investigate the solution conformations of a range of carbohydrate molecules such as 2,3-anhydro-4,6-*O*-benzylidene-D-aldohexopyranosides and related compounds.[6–13] Subsequently, the solution conformations of a range of 2,3-anhydro-4,6-*O*-benzylidene-D-hexopyranosides (**1–3**, Fig. 1) were investigated using lanthanide shift reagents.[14] In the case of the α-*manno* epoxide (**1**), the observed shift data were consistent with a model in which only the epoxide oxygen was bound to the shift reagent. The carbohydrate was present in a half-chair $^{O}H_5$ conformation. In the case of other molecules such as the α-*allo* (**2**), the shift data could not be satisfactorily fitted to a single-site model.

In a previous study, Armitage *et al.*[15] have attempted to evaluate the binding constants for reaction of lanthanide shift reagents (M) with six carbohydrate derivatives (L). However, in these studies the substrate (carbohydrate) was always present in considerable excess so that it was most unlikely that complexes in which more than one molecule of M was bound to the carbohydrate moiety would be formed. Additionally, the calculation procedures used placed considerable limitations on the amount of information that could be extracted. Using the program EQNMR[16] for the determination of equilibrium constants from NMR chemical shift data, we have investigated some of the systems previously studied[14] in order to determine the stoichiometries and stabilities of the complexes formed.

Experimental

The 2,3-anhydro compounds **1–3** were prepared as previously described.[17,18] NMR spectra in $CDCl_3$ were recorded on JEOL MH100 and JEOL JNM GX270 spectrometers with internal Me_4Si as reference.

Since all the shift reagent–carbohydrate adducts were in rapid equilibrium under the experimental conditions used, only a single ^1H resonance was observed for each of the carbohydrate protons. The chemical shift of this resonance, δ_{calc}, is the weighted average of the chemical shifts of the various adducts present M_mL_n where M represents the lanthanide shift reagent, L represents the carbohydrate, and i and j represent the maximum values of m and n, respectively, eqn. (1). Since $[M_mL_n] = \beta_{mn}[M]^m[L]^n$ eqn. (1) can be written as eqn. (2).



1 R = H, R′ = OMe
3 R = OMe, R′ = H

2 R = H, R′ = OMe

Fig. 1 Structures of carbohydrates used: **1** α-D-mannopyranoside; **2** α-D-allopyranoside; **3** β-D-mannopyranoside

$$\delta_{calc} = \sum_{\substack{m=1\\n=0}}^{\substack{m=i\\n=j}} \delta_{mn} m[M_mL_n]/[L]_{total} \quad (1)$$

$$\delta_{calc} = \sum_{\substack{m=1\\n=0}}^{\substack{m=i\\n=j}} \delta_{mn}\beta_{mn} m[M]^m[L]^n/[L]_{total} \quad (2)$$

Thus the problem resolves itself into determining the optimum values for the various δ_{mn} and β_{mn} values which best fit the experimental chemical shift data.

The non-linear least-squares program EQNMR[16] was used to calculate these parameters. The program contains three main sections. Section one reads the input data. This consists of (*i*) the concentrations of the various reagents together with the measured chemical shifts, (*ii*) details of the model in the form of the stoichiometric coefficients of the complex species present and (*iii*) initial guesses for the various parameters (stability constants and chemical shifts) to be fitted, together with values for any parameters which are to be held constant. Section two contains the non-linear least-squares subroutines which carry out the refinement of the various parameters using the Levenberg–Marquardt method. Section three contains the output routines. Output consists of printed tables of the input data and best-fit values of the calculated chemical shifts and formation constants, together with estimates of their errors. A table of the concentrations of all species present at each experimental point in the titration is also produced. Graphical output is also provided in the form of three plots. The first of these displays plots of the variation in the concentrations of the various species present as the titration proceeds. The second plot consists of plots of the experimental and calculated chemical shifts against the concentration of the titrant. The experimental points are shown as circles while the fitted curve

Table 1 Equilibrium constants and chemical shifts for reaction of lanthanide complexes of β-diketones with α-D-mannopyranoside (1) in $CDCl_3$

Shift Reagent	Proton	K_1/dm^3 mol^{-1}	δ(L)/ppm	δM(L)/ppm	R(%)[a]
$Eu(fod)_3$	1	5.42	4.89	17.9	1.34
$Eu(fod)_3$	2	9.66	3.17	19.2	2.04
$Eu(fod)_3$	3	7.08	3.45	21.8	1.87
$Eu(fod)_3$	4	8.85	3.66	17.4	1.68
$Eu(fod)_3$	OMe	5.38	3.42	6.37	0.63
$Pr(fod)_3$	1	5.18	4.83	−18.1	0.84
$Pr(fod)_3$	4	4.67	3.50	−20.5	3.19
$Yb(fod)_3$	1	10.3	4.99	31.6	1.53
$Yb(fod)_3$	2	9.46	3.37	48.5	3.87
$Yb(fod)_3$	3	9.16	3.62	48.7	4.77

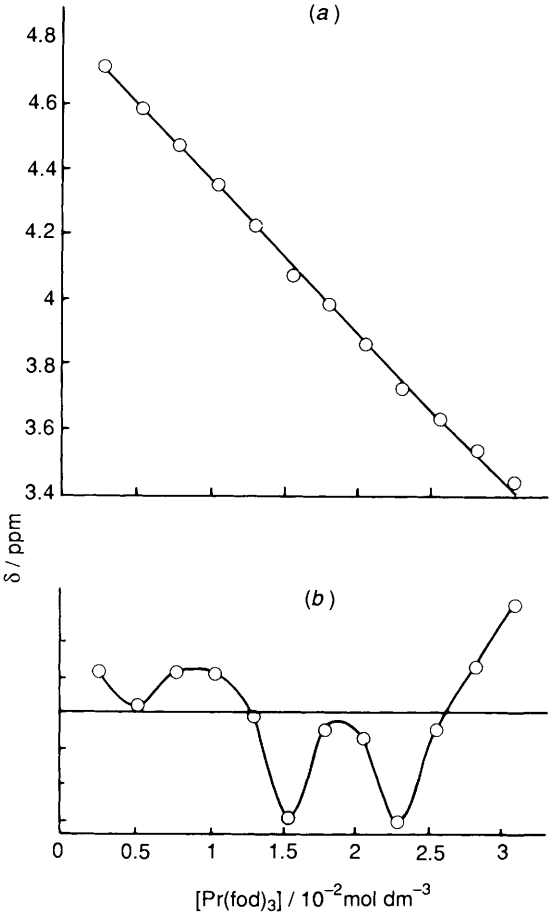


Fig. 2 (*a*) Plot of δ_H for 1-H of α-D-mannopyranoside *vs.* $[Pr(fod)_3]$ in $CDCl_3$. Open circles (○) represent experimental points, solid line (——) represents shifts calculated using the 'best-fit' parameters; (*b*) residuals plot.

obtained using the 'best-fit' parameters is shown as a solid curve. A third plot shows a plot of the residuals (in magnified form) against the titrant concentration.

EQNMR can deal with chemical shift data from a wide variety of reactions where the equilibria can be expressed in terms of formation constants. These include acid dissociation constants, metal-ion hydrolysis and complex formation reactions. Starting values for the equilibrium constants can be input as either K or log K.

With the aid of EQNMR various models could be readily evaluated *e.g.* models containing only species ML, ML_2 or M_2L, or any combination of these. In most instances visual inspection of the graphical output was sufficient to determine the 'best-fit' model. Any systematic deviation between the experimental chemical shifts and those calculated using the 'best-fit' parameters was highlighted by the residuals plot. In addition, quantitative comparison of the fits was carried out using the function in eqn. (3) where W_i is the weight attributed

$$R = 100\left(\frac{W_i(\delta_{obs} - \delta_{calc})^2}{W_i\delta_{obs}}\right)^{\frac{1}{2}} \quad (3)$$

to observation i. Where the chemical shifts are of similar magnitude, this function usually enables a choice to be made between potential models. In the present work unit weights were used at all times.

Results and Discussion

Tables 1–3 show the results obtained. The shift data used to evaluate the results in these Tables are the same data used in the previous investigation.[14]

The NMR chemical shift data for reaction of α-D-mannopyranoside (**1**) with the shift reagents $[Eu(fod)_3]$, $[Pr(fod)_3]$ and $[Yb(fod)_3]$* are consistent with a model in which only a 1:1 complex is formed between the shift reagent M and the carbohydrate. This is in agreement with the results previously obtained in the conformation studies[14] in which it was proposed that the chemical shift data were consistent with a single-site model. The equilibrium constants calculated from the chemical shifts of the various protons are given in Table 1, together with the calculated chemical shifts for both the uncoordinated carbohydrate and the complex. The shifts calculated for the uncoordinated carbohydrate are in excellent agreement with those directly determined. Fig. 2 shows a plot of the chemical shift against the concentration of $[Pr(fod)_3]$ added for α-D-mannopyranoside (**1**). The solid line represents the chemical shifts calculated on the basis of the equilibrium constants and chemical shifts in Table 1.

In the case of α-D-allopyranoside (**2**), the chemical shift data are inconsistent with the presence of only a 1:1 complex. Fig. 3 shows the plot of chemical shift against shift reagent concentration when a 1:1 model is assumed. The residuals plot clearly shows that the model is incorrect. Fig. 4 shows the plot obtained when two complexes having the stoichiometries ML and M_2L (L = carbohydrate) are assumed to be present. The agreement between the experimental data and the values calculated using the 'best-fit' values for the equilibrium constants and chemical shifts shows that this model gives an accurate description of the experimental data. The plot of the residuals highlights the fact that there are no systematic differences between the experimental and the calculated values. It should be noted that the ML complex actually represents the average of two 1:1 complexes in which the shift reagent may be bound to either of

* fod = 6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato.

Table 2 Equilibrium constants ($dm^3\ mol^{-1}$) and chemical shifts (ppm) for reaction of lanthanide complexes of β-diketones with α-D-allopyranoside (**2**) in $CDCl_3$

Shift Reagent	Proton	K_1	K_2	δ(L)	δ(ML)	δ(M_2L)	R(%)
Eu(fod)$_3$	1	7.83	40.3	4.94	12.8	6.89	0.39
Eu(fod)$_3$	2	7.55	73.8	3.49	19.8	7.23	0.69
Eu(fod)$_3$	3	8.89	43.8	3.58	13.4	6.79	0.83
Pr(fod)$_3$	1	4.26	*a*	4.86	−19.6	*a*	2.24
Pr(fod)$_3$	2	6.98	*a*	3.43	−23.0	*a*	4.05
Pr(fod)$_3$	3	5.39	*a*	3.45	−23.1	*a*	3.37
Yb(fod)$_3$	1	2.26	1.40	4.84	18.6	2.3	0.52
Yb(fod)$_3$	2	3.32	1.67	3.42	22.0	43.5	0.97
Yb(fod)$_3$	3	2.61	0.55	3.48	20.0	68.2	1.29

[a] Insufficient M_2L present under the experimental conditions to warrant its inclusion

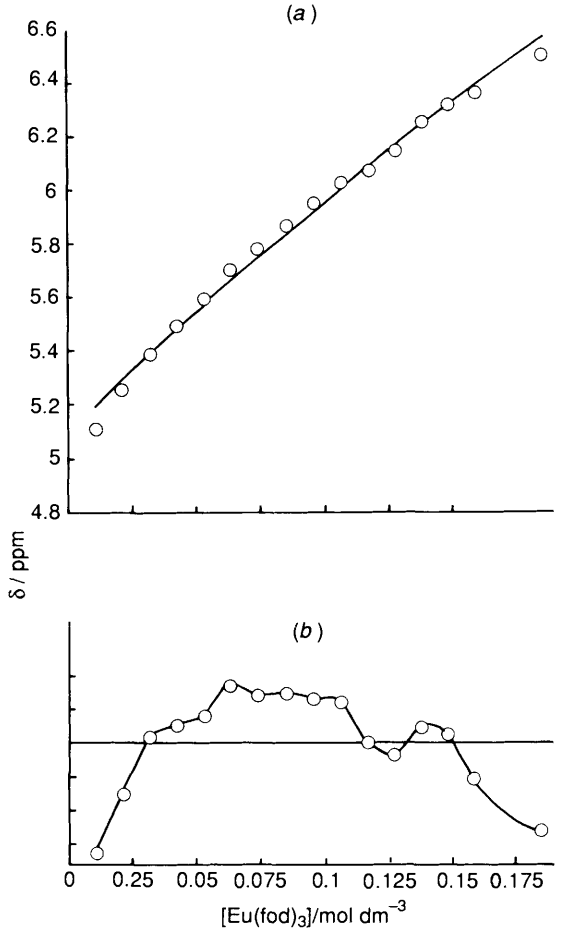


Fig. 3 (*a*) Plot of δ_H for 1-H of α-D-allopyranoside *vs.* [Eu(fod)$_3$] in $CDCl_3$ assuming that only a 1:1 complex is formed. Open circles (○) represent experimental points, the solid line (——) represents shifts calculated using the 'best-fit' parameters; (*b*) residuals plot.

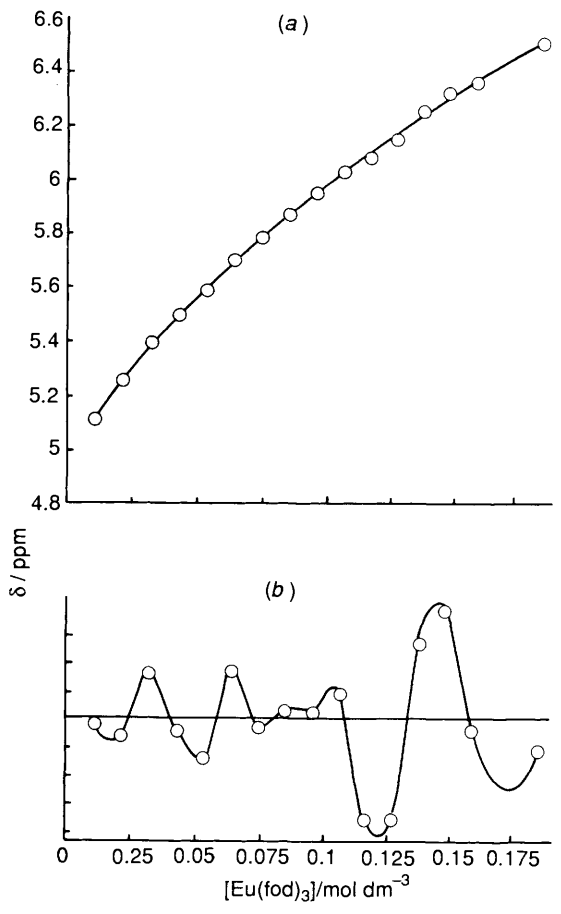


Fig. 4 (*a*) Plot of δ_H for 1-H of α-D-allopyranoside *vs.* [Eu(fod)$_3$] in $CDCl_3$ assuming that both 1:1 and 2:1 (M:L) complexes are formed. Open circles (○) represent experimental points, the solid line (——) represents shifts calculated using the 'best-fit' parameters; (*b*) residuals plot.

the two donor atoms on the carbohydrate species. The two complexes are of course in rapid equilibrium on the NMR time scale. Fig. 5 shows the relative concentrations of the various species present during the course of the titration. The data for this system could also be fitted to a model in which ML and ML_2 species were present. However, this model was rejected in favour of the model containing ML and M_2L species on the basis that the R value was significantly smaller in the case of the latter model.

In a previous investigation,[14] attempts to fit the chemical shift data for β-D-mannopyranoside (**3**) to a model in which the carbohydrate was coordinated to the shift reagent in a monodentate fashion through the expoxide oxygen proved unsuccessful and it was proposed that both the epoxide and methoxy oxygens were in an ideal orientation for chelation to the lanthanide complex. In agreement with this, a reasonable fit was obtained when the shift data were fitted to the $^oH_5$ conformation using a chelation model.[14] The present results partly support this hypothesis.

During the course of the fitting procedure, it became obvious that the equilibrium constants involved in this system were considerably greater than those listed in Tables 1 and 2. It was apparent that under the experimental conditions, virtually all of the shift reagent was complexed to the carbohydrate, so large was the equilibrium constant. Consequently, only lower limits for the equilibrium constants could be evaluated. These were calculated by constraining the equilibrium constant used in the program EQNMR while allowing the shift values to vary. The

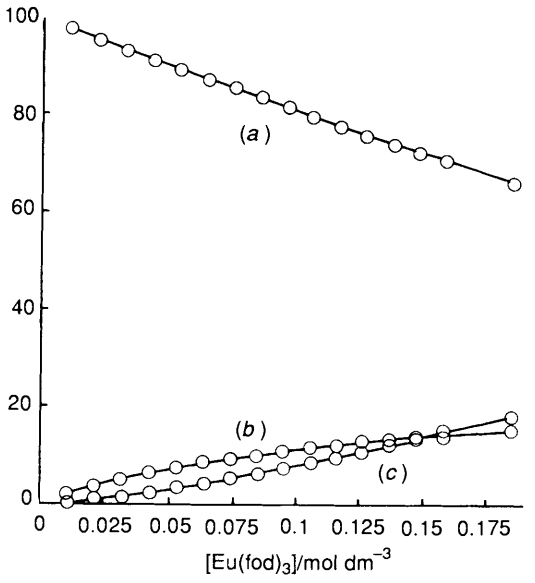


Fig. 5 Species distribution plot for reaction of α-D-allopyranoside with $[Eu(fod)_3]$ in $CDCl_3$. (*a*), free L; (*b*), ML; (*c*), M_2L.

Table 3 Equilibrium constants ($dm^3\ mol^{-1}$) and chemical shifts (ppm) for reaction of lanthanide complexes of β-diketones with β-D-mannopyranoside (**3**) in $CDCl_3$

Shift Reagent	Proton	K_1	δ(L)[a]	δ M(L)[a]	R(%)[a]
$Pr(fod)_3$	1	>100			
$Pr(fod)_3$	2	>100			
$Yb(fod)_3$	1	>100			
$Yb(fod)_3$	2	>100			

[a] Could not be reliably determined.

limits quoted in Table 3 represent the situation where serious differences between the experimental and calculated shift values become apparent.

This work demonstrates the validity of the single-site model previously used in the conformation studies of α-mannopyranoside (**1**).[14] It also highlights possible reasons for the difficulties encountered by these investigators in attempting to fit the shift data for α-allopyranoside (**2**) to a single-site model. There are at least two complexes present, one of which contains two lanthanide shift reagents bound to the carbohydrate substrate.

The present investigations are also consistent with the hypothesis[14] that the shift reagent is bound to β-mannopyranoside in a chelated fashion. The relatively large values of the equilibrium constants compared to those obtained for monodentate complexation support this view.

In a previous investigation, Rackham measured the binding constants for reaction of the lanthanide shift reagent $[La(thd)_3]$* with alicyclic ketones, ethers and epoxides in $CDCl_3$.[19] He also investigated the reactions of fully deuteriated $[Eu(fod)_3]$ and $[Yb(fod)_3]$ with three epoxides. He noted that non-sterically hindered epoxides were comparable to alicyclic ketones in their binding power to $[Eu(thd)_3]$. He also noted that fluorinated lanthanide shift reagents give more pronounced chemical shifts and have higher equilibrium constants than their thd analogues. In addition $[Yb(fod)_3]$ has higher binding constants than $[Eu(fod)_3]$. The binding constants obtained for reaction of shift reagents with 2,3-anhydrohexopyranosides when the shift reagent is bound in a monodentate fashion are broadly similar to those obtained by Rackham. However, there are a number of significant differences. Firstly, the values reported in this investigation are more representative of those obtained by Rackham for interactions with alicyclic ketones than with epoxides. Secondly, the discrimination exhibited by the epoxides towards the various shift reagents is not evident. The differences in the binding constants obtained for reaction of the various shift reagents with the carbohydrates are hardly significant.

It is apparent from the results in Table 2 that the proton chemical shift obtained on coordination of a second shift reagent is less than that obtained when only a single shift reagent is coordinated. Similar observations have been previously made when two carbohydrate molecules were bound to a single molecule of shift reagent.[15] It was proposed that binding of the second molecule of substrate results in 'crowding' of the first so that both of them end up further away from the shift reagent. It is highly likely that a similar situation pertains when two shift reagents are bound to a single carbohydrate molecule.

* thd = 2,2,6,6-tetramethyl-3,5-heptanedionato.

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