# Determination of the Binding Constants, Bound Chemical Shifts, And Stoichiometry of Lanthanide-Substrate Complexes

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Summary: Analysis of the experimental results of the equilibrium between a lanthanide shift reagent, L, and a substrate, S, is presented. When different methods were used, evaluation of equilibrium binding constants ($K_B$), bound chemical shift ($\Delta_B$), and stoichiometry (n) of lanthanide (L) -substrate (S) complexes brought about the same numerical values under the conditions of $[S]_o \gg [L]_o$. Finally, it is shown that the association between the lanthanide and the substrate (i.e. 1- (X-benzo [b] thienyl- ethyl acetate derivatives) has 1:1 stoichiometry.

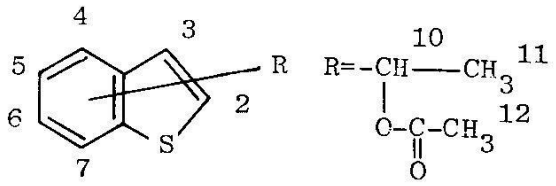
## Introduction

Lanthanide shift reagent (LSR'S) have proved to be of considerable value for evaluation of molecular structure using nuclear magnetic resonance, both as a qualitative aid in simplifying spectra and as a quantitative means of correlating molecular structures by means of the pseudocontact equation[1]. In this paper we present experimental data for several compounds which demonstrate that the different methods lead to the same value $K_B$ binding constants, $\Delta_B$ bound chemical shifts and the stoichiometry of lanthanide-substrate complexes were also determined.

Addition of Eu $(FOD)_3$ to the chloroform solution of benzo [b] thiophene under the same condition used for (benzo [b] thienyl) ethyl acetate derivatives show no appreciable proton shifts, but only broad peaks were observed. This indicates that the induced shifts are not consistent with complexing involving the sulphur atom; on the other hand, addition of Eu $(FOD)_3$ to the (benzo [b] - thienyl) ethyl acetate derivatives was very useful. Ester group is the only site of substrate able to react with the induced shift reagents. Earlier results confirmed that thioamides involve the sulphur atom, when forming complexes with shift reagents[2,3]. But sulfoxides and sulfones complex readily via the oxygen atom[4].

## Results and Discussion

Esters are weaker Lewis bases than ketones toward LSR[5], the preferred coordination site being the carbonyl oxygen. For this reason, the stronger Lewis acid Eu $(FOD)_3$ was initially employed for the simplification of the spectra of simple esters[6]. Analysis of the chemical shifts of the rings and the ring side chain protons spectra after the addition of Eu $(FOD)_3$ to compounds 1-(X-benzo [b] thienyl) ethyl acetate derivatives, X = 2 to 7 are listed in Table 1 with the induced downfield shifts.




Observed chemical shifts of the ring side chain protons ($H_{10}$ , $H_{11}$ & $H_{12}$) illustrate that $H_{10}$ is strongly affected by LSR than $H_{12}$ while $H_{11}$ is less affected by LSR than $H_{12}$. The same results were obtained by addition of Eu $(FOD)_3$ to 1-phenylethyl acetate and 1-phenylethyl alcohol. This may indicate that the oxygen atom of the ester group is the favoured binding site than the carbonyl oxygen[7].

Much progress has been made in applying LSR's to the study of molecular geometry in solution and excellent techniques for this are now available[8]. However, structural determinations have nearly all been derived from observed lanthanide induced shifts (LIS)

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Table 1. Lanthanide-Induced Downfield Shifts (Hz) in $^1$H NMR Spectra of 1-(X-Benzo [b] theinylethyl Acetate Derivatives[a].

| Compound | Substituent | | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ | $H_7$ | $H_{10}$ | $H_{11}$ | $H_{12}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 2–R | δ | — | 8.38 | 7.90 | 7.45 | 7.41 | 8.09 | 10.2 | 3.03 | 5.04 |
| | | Δδ | — | 115 | 22 | 16 | 14 | 33 | 400 | 136 | 297 |
| 3 | 3–R | | 8.46 | — | 9.24 | 7.68 | 7.56 | 8.19 | 10.5 | 3.21 | 5.1 |
| | | | 107 | — | 144 | 34 | 26 | 35 | 419 | 153 | 303 |
| 4 | 4–R | | 7.66 | 8.79 | — | 8.52 | 7.71 | 8.17 | 10.23 | 2.97 | 4.74 |
| | | | 20 | 125 | — | 115 | 42 | 36 | 389 | 133 | 267 |
| 5 | 5–R | | 7.58 | 7.52 | 9.00 | — | 8.57 | 8.15 | 9.98 | 2.95 | 4.93 |
| | | | 20 | 28 | 128 | — | 133 | 35 | 398 | 139 | 289 |
| 6 | 6–R | | 7.58 | 7.47 | 8.14 | 8.51 | — | 9.05 | 9.75 | 2.87 | 4.83 |
| | | | 18 | 19 | 39 | 120 | — | 119 | 375 | 128 | 275 |
| 7 | 7–R | | 7.80 | 7.71 | 8.18 | 7.84 | 8.61 | — | 10.44 | 3.13 | 5.08 |
| | | | 38 | 33 | 45 | 45 | 135 | — | 428 | 146 | 296 |
| | | | | | | | | | 400 | 139 | 288 |
| 8 | 1-phenylethyl-acetate | | Ho | Hm | Hp | — | — | — | 11.61 | 3.50 | 6.27 |
| | | | 9.12 | 7.93 | 7.85 | | | | | | |
| | | | 183 | 64 | 56 | | | | 574 | 205 | 426 |
| 9 | 1-phenylethyl-alcohol | | Ho | Hm | Hp | — | — | — | 10.86 | 4.88 | —OH |
| | | | 10.30 | 8.21 | 8.02 | | | | | | 7.26 |
| | | | 296 | 87 | 68 | | | | 579 | 339 | 541 |

= In chloroform-d 0.1M solution. Δδ = is the differences in Hz before and after the addition of $Eu(FOD)_3$.

rather than from the intrinsic parameters, the limiting incremental shifts. Also the equilibrium constants are of some importance since they give information on the stability of a complex. It has been reported by Armitage et al[9] , and of Kelsey[10] that the LIS obey an equation of the following form, under the conditions So ≫ Lo (see experimental method 1).

$$[S]_o = [L]_o \Delta_B (1/\Delta\delta) - ((1/K_B) + [L]_o) \ldots 1$$

Thus equation 1 gives the important fact that a plot of $[S]_o$ vs $1/\Delta\delta$ (at constant $L_o$) gives a straight line whose slope is $[L]_o \Delta_B$, and whose Y-intercept is $-((1/K_B) + [L]_o)$. Such a plot thus yield both $\Delta_B$ and $K_B$ unambiguously. This equation was derived under the assumption of a 1:1 complex formation and is very similar to the Scott[11] modification of the Benesi-Hilderbrand equation[12].

The fits[9,10] so far obtained to eq. 1 have yielded excellent straight lines and this has been used as evidence for the simple one-step mechanism. The results are shown in Fig. 1. Here are plotted the substrate concentration $[S]_o$ of 1-phenylethyl acetate vs $1/\Delta\delta$ 1-phenylethyl acetate. This compound was chosen here because of the fact that the protons are not obscured by fused ring protons as in (benzo [b] thienyl) ethyl acetate derivatives. The values of $K_B$ and $\Delta_B$ were obtained from these plots following the relationship given in eq. 1 ; the numerical values for these parameters are shown in Table 2. It is expected that the same value should be obtained for $K_B$ regardless of which proton is used for its determination. This may be confirmed

Table 2. Calculated Values of Bound Chemical Shifts ($^{\triangle}$B), Binding Constants ($K_B$) and Stoichiometry for Complexes of Organic Substrates with Eu $(FOD)_3$.

| Substrate | | $^{\triangle}B$* (1) | $^{\triangle}B$* (2) | $K_B^*$ (1,2) | Stoichiometry (2) | Stoichiometry (3) | |
|---|---|---|---|---|---|---|---|
| 1-phenylethyl– | $H_{10}$ | 8.4 | 8.8 | ⩾ 100 | | 1.2 | 0.7 |
| acetate | $H_{11}$ | 2.9 | 3.1 | ⩾ 100 | | 1.1 | |
| | $H_{12}$ | 5.9 | 6.5 | ⩾ 100 | | 1.1 | |
| 1-(4-Benzo[b]– | $H_{10}$ | 6.0 | | ⩾ 100 | 1.1 | | |
| thienyl–ethyl– | $H_{11}$ | 2.0 | | ⩾ 100 | 1.1 | | |
| acetate | $H_{12}$ | 4.5 | | ⩾ 100 | 1.1 | | |

*Values of $^{\triangle}$B(ppm) and $K_B$(1 $mol^{-1}$) derived from method (1) and method(2).

(1) Method 1, (see experimental)

(2) Method 2, (see experimental)

(3) This value should be regarded with some skepticism (See text and eq.2.).

experimentally by noting that the plots for protons $H_{10}$, $H_{11}$ and $H_{12}$ in Fig. 1, all intercept the Y-axis at the same point. It is worthnoting that the $\Delta_B$ values obtained by this method vary inversely with distance from the coordination site, as expected for a pseudo-contact shift. $K_B$ was too large to be measured, the Y-intercept depends on $(1/K_B)$, and when $K_B$ is large, the Y-intercept is so close to zero that $K_B$ cannot be determined with any confidence; in this case only a lower limit for $K_B$ may be deduced. It is well known that Eu $(FOD)_3$ form a strong binding complex with the substrate, compared with Eu $(DPM)_3$ complex[9].

The calculated $\Delta_B$ are consistent with $\Delta_B$ i/$\Delta_B$j slopes, e.g., $\Delta_B$ for $H_{10}$ and $H_{12}$ are calculated to be 8.4 and 5.9 $ppm^{-1}$; ratio 1.4. The plot of $\Delta_{obsd}$ $H_{10}$ vs $\Delta_{obsed}$ $H_{12}$ gives slope 1.4 (see Fig. 2). This analysis allows convenient estimations of $\Delta_B$ and $K_B$.

In other experiments (method 2), in which $[L]_o$ is varied at constant $[S]_o$ and $S_o \gg L_o$, for strong binding or high substrate concentration, a derived equation was obtained[9].

$$\delta = \Delta_B [L]_o/[S]_o \quad \ldots\ldots\ldots\ldots 2$$

Thus plots of $\delta$ vs $[L]_o$ / $[S]_o$ , gives a slope of $\Delta_B$. Fig. 3 illustrates these observed shifts and Fig. 4 shows the same shifts as a function of the sum of the observed shifts of all the signals in the spectrum. Clearly, the plotting of shift vs shift has the effect of linearizing the data. The numerical values of $\Delta_B$ obtained from Fig. 3 are nearly the same of that obtained by method 1 (see Table 2).

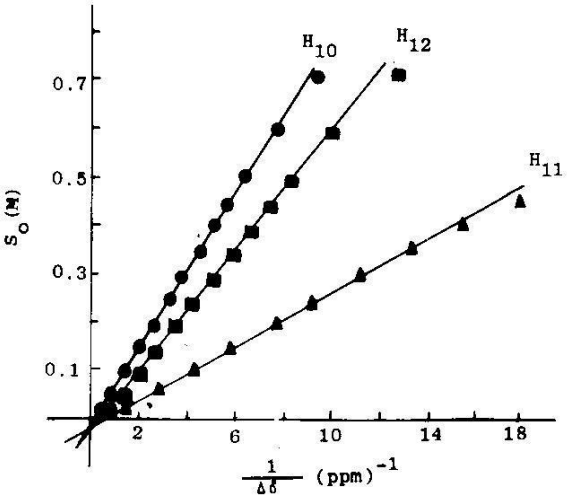


Fig.1

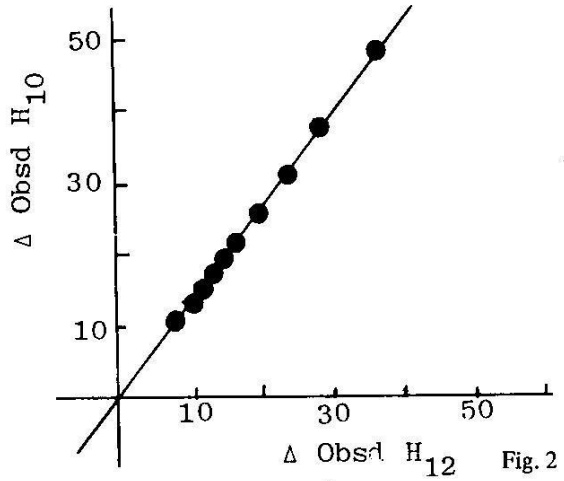


Fig. 2

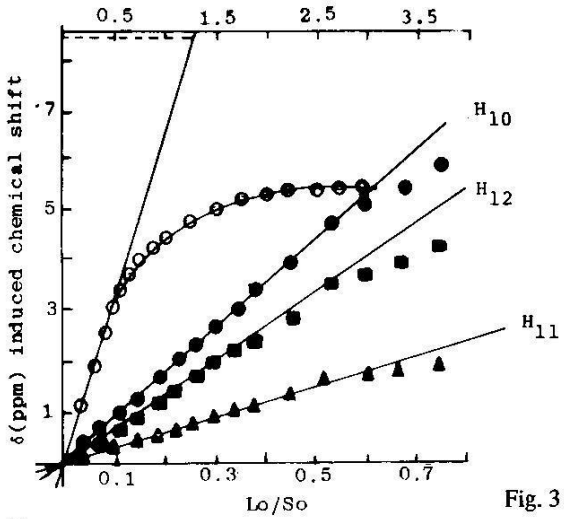


Fig. 3

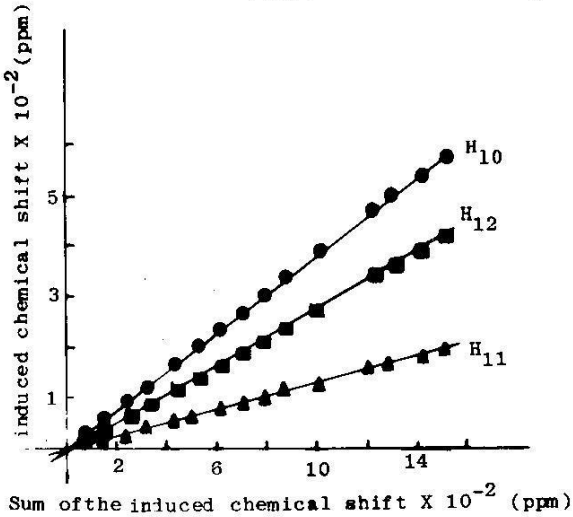


Fig. 4

**Stoichiometry of the Complex**

The stoichiometry of the complex can be estimated graphically from the position of maximum curvature of the $[L]_O$ / $[S]_O$ axis of a shift diagram[9] (Fig. 3). In this case, the limiting line (the left-most points in Fig. 3) extrapolated to the known $\Delta_B$ value. The stoichiometry appears to be 1:1. Further evidence for the stoichiometry of 1:1 gained from the straight lines in Fig. 1 (see text).

More precise method have been described for the determination of the stoichiometry n, using eq. 3[13].

$$\text{Log } [S] = 1/n \, [\log ([LS] / [L]) - \log K] \quad \ldots 3$$

Thus a plot of log [S] vs log ( [LS] / [L] ) will have a slope 1/n and log K from intercept, giving a direct measurement of the stoichiometry of the complex (see Table 2). However, for large values of K, eq. 2 gives uncertain values of n[13]

**Experimental:**

All NMR spectra were measured on JEOL JNM FX-100 spectrometer operating at 100 MHz in the Fourier Transform Mode. All the spectra were recorded at ambient temperature 25° and over 1000 and 1500 Hz sweep width using 16 K data points. Chemical shifts are in δ units (part per million) from internal TMS. Heterocyclic compounds of the title were prepared by known methods[14,15]. The LSR used in these studies was Europium (III) - tris- (1,1,1,2,2,3,3,- heptafluoro-7, 7-dimethyl-4, 6-octadionate). Eu $(FOD)_3$, was supplied by FLUKA AG. The solutions of shift reagent were prepared freshly prior to use but generally no further precautions were taken to exclude moisture.

The NMR runs for data were fit to Fig. 1 to 4 were performed in the following manner:

Method 1: *Experiments in which* $[S]_O$ *is varied at constant* $[L]_O$.

The lanthanide reagent (5.3 mg, 0.01 M) of Eu $(FOD)_3$ was dissolved in chloroform-d (0.5 ml), in a clean, ovendried NMR tube with 0.01 ml TMS. The substrate was injected into the NMR tube with thorough mixing through the plastic cap using a syringe graduated in 1μl increments. The NMR tube was inserted to the NMR probe and scan was done. More substrate was added as before, and scan were repeated until

excess of substrate 60 $\mu$l were added (ca. 0.7 M) and spectra were recorded for a total of 13 different concentrations of substrate (Data were fit to Fig. 1 and 2).

Method: 2 *Experiments in which* $[L]_o$ *is varied at constant* $[S]_o$:

(A) 0.5 M of substrate in chloroform-d (0.5 ml) was prepared in a clean, oven-dried NMR tube with 0.01 ml TMS. The NMR tube was inserted to the NMR probe and first scan was recorded. The lanthanide reagent of 10 mg increments were added with thorough mixing to the NMR tube and scans were repeated until a total of 200 mg were added (15 different concentrations of lanthanide reagents), data were fit to Fig. 3 and 4).

(B) The initial sample was prepared in a clean, ovendried NMR tube by first putting in $\sim$ 155 mg of Eu $(FOD)_3$ (0.3 M) in chloroform-d solution (0.5 ml) with 8.2 mg (0.1 M) of substrate in the NMR tube. The original spectrum was recorded. The incremental dilution method[16] was used, in which the initial sample was successively diluted with a 0.1 M chloroform-d solution of the substrate. Thus the concentration of the substrate remains constant at 0.1 M while the concentration of the shift reagent decreases with each dilution. Spectra were recorded for a total of 25 different concentrations of shift reagent (data were fit to Fig. 3, the broken curve).

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**References:**

1. D.J. Raber, M.D. Johnston, C.M. Campbell, C.M. Tanks and P. Sutton, *Org. Magn. Reson.*, 11, 323 (1978) and references cited therein.
2. W. Walter, R.F. Becker and J. Thiem, *Tetrahedron Lett.*, 1971 (1971).
3. A.H. Lewin, *Tetrahedron Lett.*, 3583 (1971).
4. K.K. Anderson and J.J. Uebel, *Tetrahedron lett.*, 5253 (1971).
5. J.K.M. Sanders and D.H. Williams, *J. Amer. Chem. Soc.*, **93**, 641 (1971).
6. R.G. Rondeau and R.E. Sievers, *J. Amer. Chem. Soc.*, **93** 1522 (1971).
7. H.B. Amin, S.S. Al-Showiman and I.M. Al-Najjar, *J. Chem. Soc. Pak.* **4 (3)**, 155, (1982).
8. A.F. Cockerill, G.L. Odavies, R.C. Harden and D.M. Rackham, *Chem. Rev.* **73 (6)** 553, (1973).
9. I. Armitage, G. Dunsmore, L.D. Hall and A.G. Marshall, *Canad. J. Chem.*, **50**, 2119 (1972).
10. D.R. Kelsey, *J. Amer. Chem. Soc.*, **94**, 1764 (1972).
11. R.L. Scott, *ibid*, **75**, 787 (1956).
12. H.A. Benesi and J.H. Hildebrand, *ibid*, **71**, 2703 (1949).
13. I. Armitage, G. Dunsmore, L.D. Hall and A.G. Marshall, *Chem. Ind.* (London), **79** (1972).
14. R. Taylor, *J. Chem. Soc.* **(B)**, 2382 (1971).
15. H.B. Amin and R. Taylor, *J. Chem. Soc. Perkin* **II**, 1053, (1978).