Tetrahedron: *Asymmetry* 20 (2009) 1099–1102

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Use of sub-stoichiometric amounts of chiral auxiliaries for enantiodifferentiation by NMR; caveats and potential utility

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ARTICLE INFO

Article history:
Received 23 February 2009
Accepted 10 March 2009
Available online 6 May 2009

ABSTRACT

Chemical shift (δ) order reversal of the population-weighted averaged NMR signals for enantiomers when using sub-stoichiometric levels of a chiral auxiliary (CA) can occur when the δ difference between the enantiomer–CA complexes and the signal of the free enantiomers is greater for the less-stable complex. The potential utility of CA titration curves with regard to ΔG evaluation, configuration determination, and modeling validation is considered.

1. Introduction

Chiral discrimination for the purposes of enantiodifferentiation (enantiomer identification, quantification of enantiomer percent,[1] and/or determination of absolute configuration) by chromatographic means relies on an energy difference existing between the diastereomeric complexes formed from the analyte enantiomers (*R*) and (*S*) and the chiral selector of the stationary phase. By contrast, enantiodifferentiation by NMR[2] using a chiral auxiliary (CA), whether it be a chiral solvating/encapsulating agent or chiral lanthanide shift reagent, can result from either a difference in complex stability or a chemical shift difference between pertinent signals of the diastereomeric complexes (*R*)·CA and (*S*)·CA. Thus the appeal of NMR methods, in addition to circumventing the procurement of expensive columns, is that differentiation can arise from multiple sources, though presumably in most cases of enantiomeric resolution both properties might be in effect. In NMR, the application of sub-stoichiometric amounts of the CA does not necessarily lead to a compromise in optimality since quite often rapid exchange on the NMR timescale in the equilibrium,

$$S + R\cdot\mathrm{CA} \rightleftharpoons R + S\cdot\mathrm{CA} \qquad (1)$$

yields a population-weighted average of the complexed and free enantiomer signals to result in distinct signals for the two enantiomers without affording additional spectral complexity. Indeed, it is often beneficial to apply sub-stoichiometric amounts of CA, aside from economical considerations, since the resolution attained can be improved and of course it is absolutely necessary in the case of a non-existent chemical shift difference between the two diastereomeric complexes. As a consequence, the intentional application of sub-stoichiometric amounts of CA in NMR enantiodifferentiation work is commonplace. However a problem can potentially arise in such situations if for the less-stable diastereomeric complex, the NMR signal selected for observation is positioned further from the corresponding free enantiomer signal than for the more-stable diastereomeric complex. In symbolic terms, for the nucleus signal under scrutiny:

$$|\,\delta_{R\cdot\mathrm{CA}} - \delta_{\mathrm{free}}\,| > |\,\delta_{S\cdot\mathrm{CA}} - \delta_{\mathrm{free}}\,| \quad \text{and} \quad \Delta G(S-R) < 0 \qquad (2)$$

Under such conditions, the resulting relative positional order of the population-weighted averaged signals representing each of the enantiomers is dependent on a number of factors including, not only the relative chemical shift order of the two enantiomer–CA complexes relative to the free enantiomer, but also the chemical shift difference between these two signals, the relative stabilities of the two enantiomer–CA complexes, and the amount of CA present.

2. Results and discussion

Consider firstly the arbitrary case where an 80:20 ratio (*R*:*S*, respectively) of a mixture of enantiomers forms a strongly bound but rapidly exchanging dynamic complex with a CA, and the chemical shifts of the species are, for the sake of convenience, 2.2 [for the (*R*)·CA complex], 2.0 [for the (*S*)·CA complex], and 1 (for the free enantiomers) ppm. With an equilibrium constant of 1.20 favoring the (*S*) complex, the plots that result[†] for the estimated chemical shift for each enantiomer versus a titration from 0% to 100% mol fraction of CA are presented in Figure 1.

The plots are in accord with expectations and the lines diverge, almost bereft of discernible curvature despite the strong content bias toward the unfavored *R*·CA complex, as the content of CA is increased. However, for a modest larger value of *K*, viz. 1.50, the problem of chemical shift order reversal manifests itself (Fig. 2) wherein it can be seen that the chemical shift order of the complexes is dependent on the amount of CA.

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[†] After solving the equilibrium equation and substituting in appropriate values.

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doi:10.1016/j.tetasy.2009.03.036

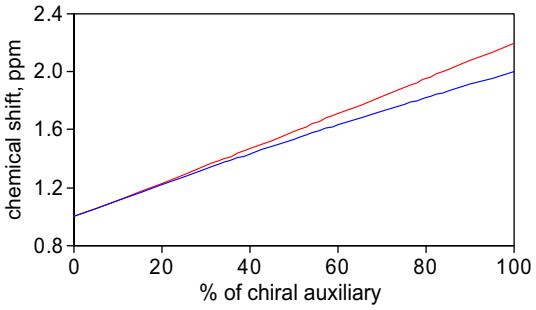

Figure 1. Plots of the calculated δs for the (*R*) (red trace) and (*S*) (blue trace) enantiomers versus mol % of CA in a dynamic equilibrium consisting of free enantiomers and complexed enantiomers (*R*)·CA and (*S*)·CA. Parameters: enantiomer ratio = 80:20 (*R*:*S*); $\delta_{R\cdot CA} = 2.2$, $\delta_{S\cdot CA} = 2.0$, and $\delta_{free} = 1$ ppm; $K = 1.20$.

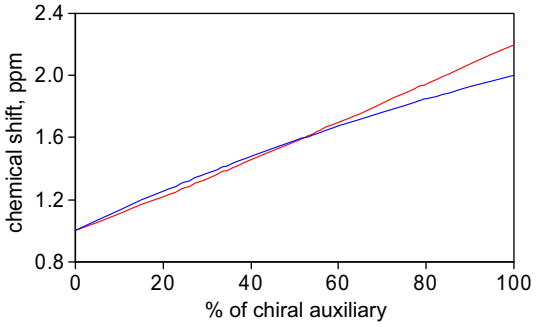

Figure 2. Analogous titration curves as for Figure 1 but with $K = 1.50$. Maximum divergence (0.03 ppm) of the curves (below δ equivalence) occurs at 25 mol % and δ equivalence occurs at 52 mol %. (*R*)-enantiomer, red trace; (*S*)-enantiomer, blue trace.

The ramifications of the plot in Figure 2 are quite evident and if one is not aware of the implicit consequences for the case described by Eq. 2 then it is possible to either incorrectly assign the major enantiomer, to record the state of enantiopurity in reverse order, or infer that the sample is enantiopure when it is not. A further implication is that additional CA does not necessarily lead to improved resolution unless one is already past the point of equivalence (or below the point of maximum divergence); in other words, additional CA can even reduce the resolution at hand. The dangers that such conditions represent are particularly acute when using small amounts of analyte, as happens frequently in the isolation of natural products. The preliminary measurements on the racemate that have been performed using sub-stoichiometric amounts of CA run the risk that a different (greater or less or stoichiometric) amount of CA is then applied to the analytical sample, or conversely, if a stoichiometric amount of CA has been applied in the preliminary measurements, there is a risk that only sub-stoichiometric amounts of CA are unwittingly applied to the analytical sample. Thus as a consequence it is a necessity that correctly measured amounts of the CA are utilized irrespective of whether sub-stoichiometric amounts are used for the control sample or not. Simply relying on a single measurement on the racemate and then applying this to the analytical sample are insufficient as the chemical shift order is also dependent on the enantiomer ratio (vide infra). Thus, simply taking a racemate, adding a sub-stoichiometric amount of a CA, obtaining resolution, spiking the sample with a pure enantiomer of known configuration to determine the relative order and then measuring the analytical sample does not guarantee correct interpretation of the results. In other words, even if consistent chemical shift divergence may appear to occur across a titration for one particular set of parameters, a change in enantiomer ratio may result in chemical shift reversal occurring during an otherwise identical titration.

Certainly, performing a titration on the racemate (assuming it to be more readily available than other sample formulations though other ratios may be more relevant) with the selected CA should allow a better appreciation of the situation and allude to the stability order and the chemical shift order, as exemplified by the plots in Figures 3 and 4.

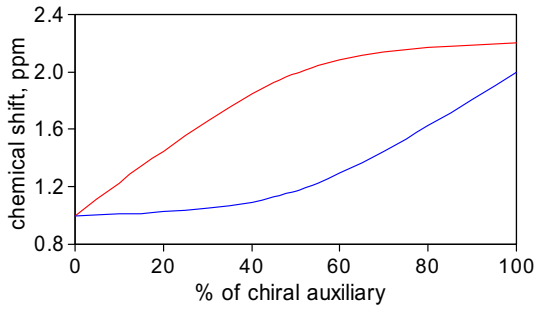

Figure 3. Titration curves with parameters: enantiomer ratio = 50:50; $\delta_{R\cdot CA} = 2.2$, $\delta_{S\cdot CA} = 2.0$, and $\delta_{free} = 1$ ppm; $K = 0.0431$; max. δ divergence 0.82 ppm at 52 mol %. (*R*)-enantiomer, red trace; (*S*)-enantiomer, blue trace.

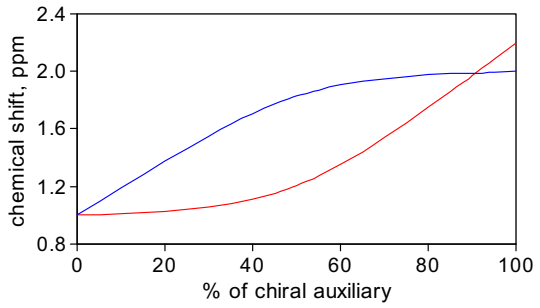

Figure 4. Titration curves with parameters: enantiomer ratio = 50:50; $\delta_{R\cdot CA} = 2.2$, $\delta_{S\cdot CA} = 2.0$, and $\delta_{free} = 1$ ppm; $K = 23.2$; max. δ divergence 0.62 ppm at 48 mol %; δ equivalence at 91 mol %. (*R*)-enantiomer, red trace; (*S*)-enantiomer, blue trace.

Interestingly, quite distinct curves (e.g., Figs. 5 and 6) can arise depending on the value of the parameters in effect though the interpretations remain intact.

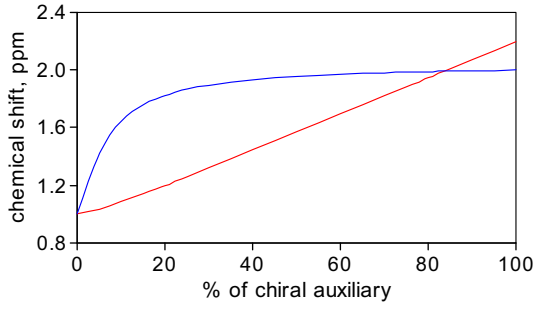

Figure 5. Titration curves with parameters: enantiomer ratio = 95:5 (*R*:*S*); $\delta_{R\cdot CA} = 2.2$, $\delta_{S\cdot CA} = 2.0$, and $\delta_{free} = 1$ ppm; $K = 23.2$; max. δ divergence 0.62 ppm at 19 mol %; δ equivalence at 83 mol %. (*R*)-enantiomer, red trace; (*S*)-enantiomer, blue trace.

The results for a broader range of parameter sets are presented in Table 1. One clear consequence is that for different enantiomer ratios the equivalence point varies, thus finding that resolution is attained at a certain mole percentage of CA on, say, a racemic sample is not a guarantee that this can be applied to the analytical

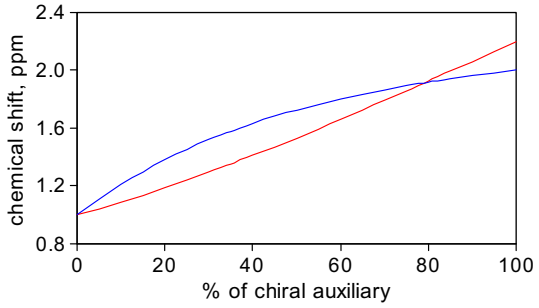

Figure 6. Titration curves with parameters: enantiomer ratio = 80:20 (*R*:*S*); $\delta_{R\cdot CA} = 2.2$, $\delta_{S\cdot CA} = 2.0$, and $\delta_{free} = 1$ ppm; $K = 3.33$; max. δ divergence 0.23 ppm at 34 mol %; δ equivalence at 79 mol %. (*R*)-enantiomer, red trace; (*S*)-enantiomer, blue trace.

Table 1
Characterization parameters for various systems

(*R*:*S*) ratio	*K*	Max. δ diver.[a] (ppm)	Point max. δ diver. (mol %)	Pt. δ equiv (mol %)
50:50	0.043	0.66[b]	50	—
50:50	23.2	0.62	48	91
60:40	23.2	0.62	41	89
40:60	23.2	0.62	55	92
95:5	23.2	0.62	19	83
5:95	23.2	0.62	77	98
80:20	23.2	0.62	28	86
20:80	23.2	0.62	68	96
80:20	3.33	0.23	34	79
20:80	3.33	0.23	51	88
80:20	1.50	0.03	25	52
80:20	1.30	0.01	14	29

[a] For δs of 2.2 [(*R*)·CA], 2.0 [(*S*)·CA], and 1 (free enantiomer) ppm.
[b] For δs (*R*)·CA = (*S*)·CA = 2 ppm.

sample of unknown enantiomeric percentage without recourse to a full examination of the properties of the system by a complete titration and examination of the effect of varying enantiomer ratios. Thus, an analytical sample which may appear to be enantiopure can instead be just exhibiting a lack of resolution. The conundrum of course is that until resolution is attained, the true state of the enantiomer ratio in the analytical sample remains uncertain. In any case, with several factors influencing the final result, the behavior of a system for any particular set of parameters is unlikely to be intuitive. Thus, irrespective of whether it may be self-evident or not that equivalence and hence reversal of chemical shift occur, the location of the equivalence point and maximum chemical shift divergence are not self-evident.

Although the results may appear disconcerting, at least for large *K* values the equivalence point is located at large mol % values, though caution is still advised regarding the titration of suitable samples to provide the support necessary to exclude misinterpretation. If the signal under scrutiny is more distant from the free enantiomer for the more-stable complex, then there is no possibility for misinterpretation, and the titration curves can unequivocally indicate this. Most complexes are likely to differ only by minor amounts of energy and thus most plots will appear to simply diverge linearly with a finite chemical shift difference (cf. Fig. 1). Such a condition, though, can be deceptive unless closely scrutinized.

To manage the problem for the case of Eq. 2 with no other recourse available, obviously performing measurements using stoichiometric amounts of CA can be a solution, though this may be neither desirable (e.g., in the limit when the chemical shift difference is zero, resolution is not effected) nor convenient for all situations. In principle, at least two measurements are required for the preliminary sample (to determine if chemical shift reversal occurs) and also for the analytical sample (to ascertain that chemical shift equivalence is not in effect) wherein the amount of CA needs to be appropriately measured. However, because both the amount of the added CA and the enantiomer ratio affect the behavior of the system an expansive titration is advised. Moreover, the problem of an unknown enantiomer ratio of the analytical sample (at least until resolution is attained) needs to be addressed by comparable measurements on standard samples of appropriate enantiomer ratio, that is, a set of samples spanning a range of enantiomer ratio values also needs to be evaluated.

Experimentally, the situation can quickly become convoluted, however, the potential liability that such circumstances purport to can also represent an opportunity as the ensuing results provide an inference toward the value of ΔG for the equilibrium. This can be garnered from the titration plot as the shape of the curves is distinct and dependent on the value of the equilibrium constant (amongst other parameters). This may be accomplished most simply by comparison of experimental titration curves with generated curves by varying the mole percentage of CA for known values of the chemical shifts and enantiomer ratios (all evident from the spectra) and for various values of *K*. Such an approach has proved to be qualitatively useful in the NMR analysis[3] of the phenomenon of enantiomer self-disproportionation over achiral-phase chromatography (ESDAC).[4] Thus, in addition to alluding to potential dangers, a comprehensive titration can also be implicit in assessing the value of ΔG. It may in any case be desirable to confirm the validity of any modeling that has been performed, and the evaluation of ΔG expands the perspective for accomplishing this, whether this concerns simply the appropriate level of theory or model conditions for the solvent, but more specific to the chemistry, the correct conformation/number of conformations and their weighting as well as the very structure of the purported complex itself. Furthermore, with the present capability to reliably calculate both ΔG[5] and relative chemical shifts,[6] particularly for stereochemical systems,[7] with only an enantiomerically impure analytical sample or a racemate and an enantiopure sample in hand, the determination of the actual configuration of the analytical sample is feasible by the construction of simulated plots and comparison to experimental results.

3. Conclusions

In summary, whether the problem of chemical shift order reversal is widely appreciated or not is unclear, although with NMR another formidable attribute is that there can often be a number of signals to select from, thus one or more signals may provide a non-problematic chemical shift order though this still needs to be ascertained by evaluating titration curves. However, by conducting a comprehensive titration on a sample of the racemate or other formulation with the CA to obtain well-defined curves and by comparing with simulated curves, one can attest to the validity of calculations that have been performed and/or provide additional support for the assignment of configuration rather than just relying solely on the chemical shift difference. Certainly workers are advised to take due care and caution in the interpretation of results when applying sub-stoichiometric amounts of CA in NMR differentiation work or where there is the possibility for such to occur. Importantly, it should not be assumed that resolution of a racemate will ensure the resolution of non-racemic mixtures or that the chemical shift order remains invariant. A compilation of plots for various parameter sets is available upon request from the author.

Acknowledgment

Financial support of this work by Turun Yliopistosäätiö is gratefully acknowledged.

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