ORGANIC LETTERS

2008
Vol. 10, No. 23
5433−5436

"Pure by NMR"?

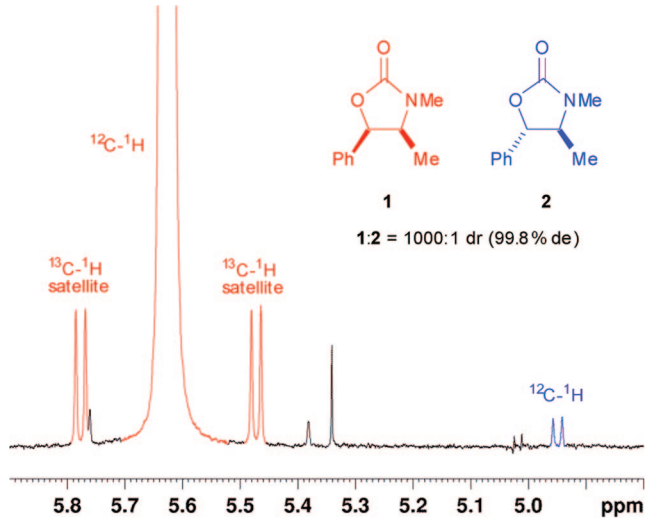
Timothy D. W. Claridge, Stephen G. Davies,* Mario E. C. Polywka, Paul M. Roberts, Angela J. Russell, Edward D. Savory, and Andrew D. Smith

Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford OX1 3TA, U.K.

steve.davies@chem.ox.ac.uk

Received September 22, 2008

ABSTRACT



Integration of a $^{13}C-^{1}H$ satellite peak of a given $^{12}C-^{1}H$ parent resonance within a quantitative ^{1}H NMR spectrum and comparison to the minor component represents a simple protocol for the accurate determination of diastereoisomeric ratios of up to 1000:1 (i.e., 99.8% de).

The determination of ratios of components in a mixture is an everyday task within organic chemistry, which is usually tackled by the application of ^{1}H NMR spectroscopy or HPLC analysis. ^{1}H NMR spectroscopy is arguably the most widely used tool for this analysis, as examination of the relative integration of resonances rapidly allows access to product ratios. When applied to routine analysis of ^{1}H NMR spectra, the term "pure by NMR" is generally understood to indicate a purity (diastereoisomeric or otherwise) at the level of 95% or above, although the accuracy to which such figures are reported varies widely.[1] A number of quantitative NMR techniques have been introduced in order to improve the precision with which the minor components in a mixture can be quantified,[2] including doping of samples with a reference standard.[3] However, signals in ^{1}H NMR spectra corresponding to protons directly bound to

(1) For examples, see: Bray, B. L.; Muchowski, J. M. *Can. J. Chem.* **1990**, *68*, 1305. MacKeith, R. A.; McCague, R.; Olivo, H. F.; Palmer, C. F.; Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **1993**, 313. Wimmer, R.; Müller, N. *Monatsh. Chem.* **1998**, *129*, 1161. Jain, R. P.; Williams, R. M. *Tetrahedron Lett.* **2001**, *42*, 4437. Hutin, M.; Bernardinelli, G.; Nitschke, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 17655.

(2) Hollis, D. P. *Anal. Chem.* **1963**, *35*, 1682. Thakur, K. A. M.; Kean, R. T.; Hall, E. S. *Anal. Chem.* **1997**, *69*, 4303. Forshed, J.; Erlandsson, B.; Jacobsson, S. P. *Anal. Chim. Acta* **2005**, *552*, 160. For reviews, see: (a) Holzgrabe, U.; Diehl, B. W. K.; Wawer, I. *J. Pharm. Biomed. Anal.* **1998**, *17*, 557. (b) Malz, F. *NMR Spectroscopy in Pharmaceutical Analysis*; Holzgrabe, U., Wawer, I., Diehl, B., Eds.; Elsevier: New York, 2008; pp 43−62. (c) Holzgrabe, U. *NMR Spectroscopy in Pharmaceutical Analysis*; Holzgrabe, U., Wawer, I., Diehl, B., Eds.; Elsevier: New York, 2008; pp 131−137. (d) Wells, R. J.; Cheung, J.; Hook, J. M. *NMR Spectroscopy in Pharmaceutical Analysis*; Holzgrabe, U., Wawer, I., Diehl, B., Eds.; Elsevier: New York, 2008; pp 291−315.

(3) O'Neill, I. K.; Pringuer, M. A.; Prosser, H. J. *J. Pharm. Pharmacol.* **1975**, *27*, 222. Lacroix, P. M.; Dawson, B. A.; Sears, R. W.; Black, D. B.; Cyr, T. D.; Ethier, J.-C. *J. Pharm. Biomed. Anal.* **1998**, *18*, 383. Malz, F.; Jancke, H. *J. Pharm. Biomed. Anal.* **2005**, *38*, 813.

10.1021/ol802211p CCC: $40.75
Published on Web 11/01/2008

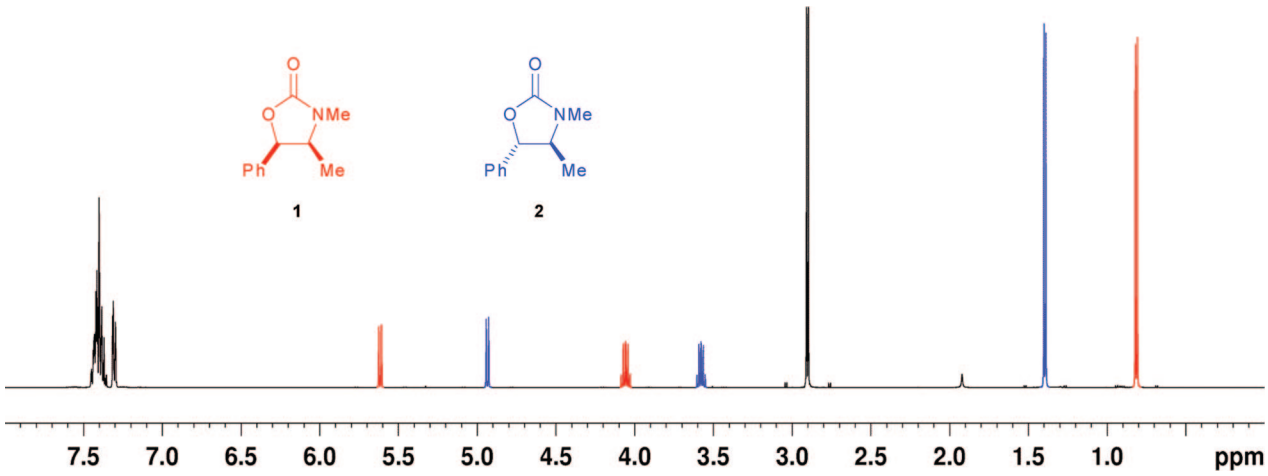


Figure 1. 500 MHz ^{1}H NMR spectrum of a 1:1 mixture of oxazolidinones **1** and **2**, recorded in $CDCl_3$ at ambient temperature [signals in black correspond to resonances attributable to both diastereoisomers].

carbon have two satellite peaks (one on either side) of the "parent" ^{12}C−^{1}H peak due to coupling to the 1.108% of naturally occurring, NMR-active carbon isotope ^{13}C (for which $I = 1/2$). This produces a ratio of 178.5:1 for each ^{13}C−^{1}H satellite peak versus the corresponding ^{12}C−^{1}H parent resonance. Provided that the resonances of interest are well resolved and the signal-to-noise ratio in the sample is acceptable (>10:1 for the smallest signal),[4] a satellite resonance may be used as an intrinsic internal standard for accurate quantification of minor components of a mixture.[4,5] For this procedure to represent a general method for the accurate determination of very high diastereoisomeric ratios or purities, there are a number of specific experimental parameters that must be considered. In this manuscript we describe a simple experimental NMR procedure to facilitate quantitative analysis of the ^{13}C−^{1}H satellite resonances of a given ^{12}C−^{1}H parent resonance of the major component in a ^{1}H NMR spectrum, which allows diastereoisomeric ratios of up to 1000:1 (i.e., 99.8% de) to be accurately assessed; this procedure is equally applicable to analysis of product ratios or purity.

The methodology follows the well-understood principles for achieving quantitative NMR analysis.[6] Although ^{13}C-decoupling during proton observation has been recommended as a means of simplifying proton spectra for quantification purposes,[6b,7] the use of the ^{13}C−^{1}H satellites as an internal reference is advantageous as it requires the employment of only the simpler, standard technique of single-pulse, direct proton acquisition. Nevertheless, for the accurate quantification of an NMR spectrum it is necessary to avoid (differential) saturation of resonances caused by too rapid signal averaging and this requires the complete equilibration of magnetization between each transient. The recovery times are dictated by the longitudinal relaxation time constants (T_1 values) of the NMR nuclei and delays of $5T_1$ are required to re-establish essentially complete recovery (>99%) after full excitation of the NMR response. In the context of this methodology, it is important to note that a ^{13}C nucleus acts as an efficient relaxation source for a directly attached proton, unlike the NMR inactive ^{12}C nucleus, implying that recovery rates for the ^{13}C satellites will be significantly faster, and hence T_1 values shorter, than for the parent ^{12}C resonance. Thus, recycle delays must be dictated by the relaxation time

Table 1. Proton Longitudinal Relaxation Time Constants (T_1 Values) for the C(5)*H* Resonances of **1** and **2**, where the Proton is Attached to Either ^{12}C or ^{13}C

compound	δ ^{12}C−^{1}H (ppm)	T_1 ^{12}C−^{1}H (s)	T_1 ^{13}C−^{1}H (s)
1	5.607	3.10	1.84
2	4.929	3.49	1.97

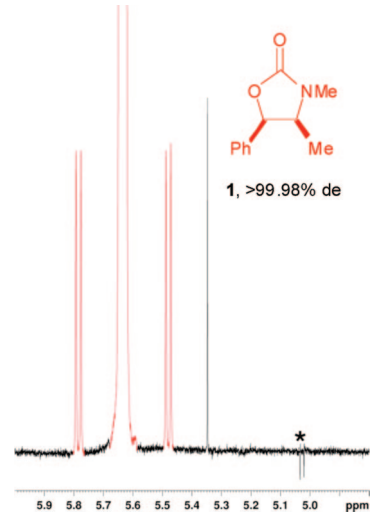


Figure 2. Partial quantitative 500 MHz ^{1}H NMR spectrum for **1**, showing the region containing the C(5)*H* resonances. The asterisk denotes low level spectrometer receiver artifacts.

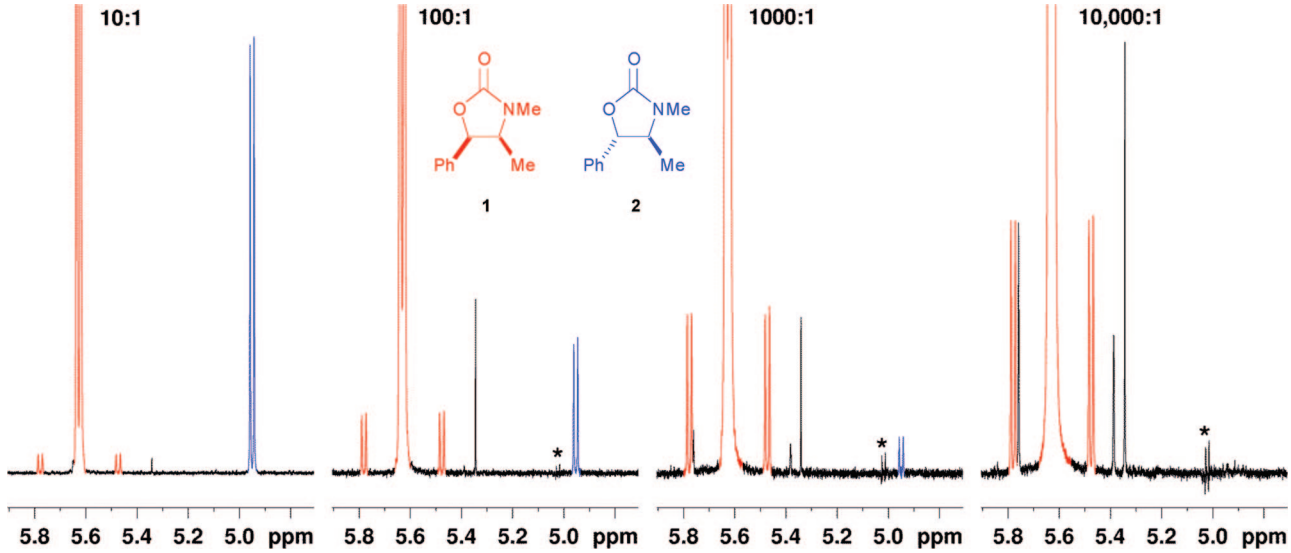


Figure 3. Partial quantitative 500 MHz ^{1}H NMR spectra for diastereoisomeric mixtures of **1** and **2**, showing the region containing the C(5)*H* resonances. The asterix denotes low level spectrometer receiver artifacts.

constant of the slowest relaxing parent ^{12}C resonances; it is this that would usually be measured when determining T_1 values with, for example, the well-known inversion−recovery method.[8] Failure to avoid saturation effects will lead to a relative enhancement of the ^{13}C satellite resonance intensities and will result in an overestimation of the diastereoisomeric or product ratio. For many small organic molecules studied in (nondegassed) low-viscosity solvents such as chloroform, proton T_1 values are below 5 s, dictating recycle delays of up to 25 s. The use of conservative recycle delays should avoid the need for direct T_1 measurement on every sample analyzed. While relaxation reagents can be used to reduce these requirements, the addition of these to precious samples is often undesirable, and may lead to unacceptable line-broadening.

To fully resolve the ^{13}C satellites from the parent resonance, its line shape must be sufficiently narrow at the baseline, a condition that is usually met on modern, well-shimmed spectrometers. In this context gradient shimming routines can be advantageous in the avoidance of low-level broadening or asymmetrical humps that may arise from inadequate shim optimization. It is also likely to be beneficial to avoid sample rotation and eliminate the possible appearance of undesirable "spinning sidebands" either side of the parent resonances.[6b] Furthermore, it is advantageous to consider measurements derived from the simplest and narrowest available multiplet structures such that the satellite resonances remain resolved: singlets are to be preferred. Likewise, the use of only moderate sensitivity enhancement window (apodization) functions when processing the FID is recommended such that the base of the parent resonance is not made so broad that it extends to the satellites. In situations when the signal-to-noise ratio of the minor isomer resonance is sufficient, such enhancement functions may be avoided altogether. Spectra should also be baseline corrected, at least in the region of the peaks of interest, prior to integration.

For ^{1}H NMR spectra recorded under these experimental conditions, it is apparent that when the height of the ^{13}C−^{1}H satellite resonances associated with the major diastereoisomer are greater than that of the ^{12}C−^{1}H resonance of the minor diastereoisomer, the diastereoisomeric ratio is >180:1 (>98.9% de), thus allowing an approximate, yet ready, quantitative assessment of the diastereoisomeric ratio by direct inspection of the ^{1}H NMR spectrum.

As a model system to demonstrate the effectiveness and ease with which this protocol may be applied, the known oxazolidinones **1** and **2**, derived from (1*R*,2*S*)-ephedrine and (1*S*,2*S*)-pseudoephedrine, respectively, were prepared and recrystallized twice from CH_2Cl_2/hexane.[9] Stock solutions of **1** (1.675 g in 25 mL of $CDCl_3$) and **2** (685 mg in 10 mL of $CDCl_3$) were prepared. ^{1}H NMR spectroscopic analysis of a 1:1 mixture of diastereoisomers **1** and **2** (prepared from the stock solutions) indicated excellent resonance dispersion in $CDCl_3$, with the resonances due to C(4)*H* (1H, dq), C(5)*H* (1H, d), and C(4)*Me* (3H, d) being well resolved within each diastereoisomer, and from each other (Figure 1).

The inversion−recovery sequence was used to determine T_1 values for the doublet resonances associated with C(5)*H*, for both the parent ^{12}C−^{1}H resonances and their corresponding ^{13}C−^{1}H satellites, within both **1** and **2**. Fourteen ^{1}H aquisitions were collected, with inversion recovery delays ranging from 0−20 s, and the individual recovery curves

(4) Maniara, G.; Rajamoorthu, K.; Rajan, S.; Stockton, G. W. *Anal. Chem.* **1998**, *70*, 4921.

(5) Dewey, R. S.; Schoenewaldt, E. F.; Joshua, H.; Paleveda, W. J., Jr.; Schwam, H.; Barkemeyer, H.; Arison, B. H.; Veber, D. F.; Denkewalter, R. G.; Hirschmann, R. *J. Am. Chem. Soc.* **1968**, *90*, 3254. Boate, A. R.; Eaton, D. R. *Can. J. Chem.* **1976**, *54*, 3895. Brown, S. L.; Davies, S. G.; Foster, D. F.; Seeman, J. I.; Warner, P. *Tetrahedron Lett.* **1986**, *27*, 623. Fux, P. *Analyst* **1990**, *115*, 179. Vedejs, E.; Wang, J.; Lin, S.; Klapars, A. U. S. Patent 5,900,427, 1999. Al-Deen, T. S.; Hibbert, D. B.; Hook, J. M.; Wells, R. J. *Anal. Chim. Acta* **2002**, *474*, 125. Wells, R. J.; Hook, J. M.; Al-Deen, T. S.; Hibbert, D. B. *J. Agric. Food Chem.* **2002**, *50*, 3366. Forshed, J.; Andersson, F. O.; Jacobsson, S. V. *J. Pharm. Biomed. Anal.* **2005**, *29*, 495. For a review see ref 2.

were fitted to extract T_1 values.[10] These data demonstrated significant differences between the values for the parent and satellite resonances, as well as differences in values between the diastereoisomers **1** and **2** (Table 1). Based on the T_1 values, recovery delays of 20 s were adopted for all subsequent analysis of diastereoisomers **1** and **2** by quantitative ^{1}H NMR.

The diastereoisomeric purity of **1** was determined by ^{1}H NMR to be >10,000:1 (>99.98% de), since **2** could not be detected under conditions of high signal-to-noise ratio (s/n >70:1) for the ^{13}C−^{1}H satellites of **1** (Figure 2).

In order to investigate the levels at which the minor diastereoisomeric component could be accurately determined using this technique, diastereoisomeric mixtures of **1** and **2** in 10:1, 100:1, 1000:1, and 10,000:1 ratios, respectively, were prepared by serial dilution of the stock solution of **2**. Quantitative ^{1}H NMR spectra of these diastereoisomeric mixtures were acquired using 90° pulse tip angles (7.5 μs) with recovery delays of 20 s and acquisitions times of 3.2 s and were processed without apodization functions (Figure 3).

When the spectra were integrated over identical regions, good correlation between the calculated and observed diastereoisomeric ratios was noted for the 10:1, 100:1, and 1000:1 mixtures (Table 2). In the case of the 10,000:1 mixture, the resonance attributable to C(5)*H* of **2** could not be observed above baseline noise. These results therefore demonstrate that this technique may be applied to the accurate determination of diastereoisomeric ratios of up to 1000:1 (i.e., 99.8% de), signal-to-noise ratio permitting.

Table 2. Correlation between Calculated and Experimentally Observed Diastereoisomeric Excess Values for Solutions of **1** and **2** in $CDCl_3$

ratio **1**:**2**	calculated de	peak	relative integration	s/n ratio	observed de
1000:1	99.80	**1** (^{13}C−^{1}H)	1.0000	162	99.82
		2 (^{12}C−^{1}H)	0.1603	11	
100:1	98.02	**1** (^{13}C−^{1}H)	1.0000	48	98.04
		2 (^{12}C−^{1}H)	1.7696	82	
10:1	81.82	**1** (^{13}C−^{1}H)	1.0000	44	81.35
		2 (^{12}C−^{1}H)	18.3561	270	

In conclusion, the technique described in this manuscript offers a robust method for accurate measurement of very high diastereoisomeric ratios (>95% de), which may be applied routinely to the analysis of product ratios or purity. The application of this protocol to the determination of the levels of selectivity resulting from a very highly diastereoselective olefination protocol are reported in the following manuscript.

Acknowledgment. The authors wish to thank New College, Oxford for a Junior Research Fellowship (A.D.S.).

OL802211P

(6) (a) Claridge, T. D. W. *High-Resolution NMR Techniques in Organic Chemistry*; Elsevier Science Ltd.: Oxford, 1999; pp 114−116. (b) Pauli, G. F.; Jaki, B. U.; Lankin, D. C. *J. Nat. Prod.* **2007**, *70*, 589.

(7) Li, S.; Wilson, W. K.; Schroepfer, G. J., Jr. *J. Lipid Res.* **1999**, *40*, 764. Soininen, P.; Haarala, J.; Vepsäläinen, J.; Niemitz, M.; Laatikainen, R. *Anal. Chim. Acta* **2005**, *542*, 178. Pauli, G. F.; Jaki, B.; Lankin, D. World Patent WO 2008/051857, 2008.

(8) Claridge, T. D. W. *High-Resolution NMR Techniques in Organic Chemistry*; Elsevier Science Ltd.: Oxford, 1999; pp 25−30.

(9) Oxazolidinones **1** and **2** were prepared according to the procedure of Cutugno, S.; Martelli, G.; Negro, L.; Savoia, D. *Eur. J. Org. Chem.* **2001**, 517 *via* treatment of either (1*R*,2*S*)-ephedrine hydrochloride or (1*S*,2*S*)-pseudoephedrine hydrochloride with CDI.

(10) Curves were fitted using the T_1/T_2 relaxation module of Bruker TOPSPIN software.