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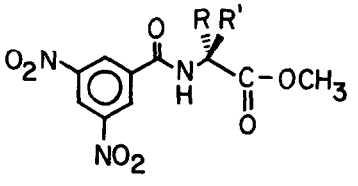
3,5-DINITROBENZOYL AMINO ACID ESTERS. BROADLY APPLICABLE CHIRAL SOLVATING AGENTS FOR NMR DETERMINATION OF ENANTIOMERIC PURITY.

William H. Pirkle* and Athanasios Tsipouras

School of Chemical Sciences
University of Illinois
Urbana, IL 61801, USA

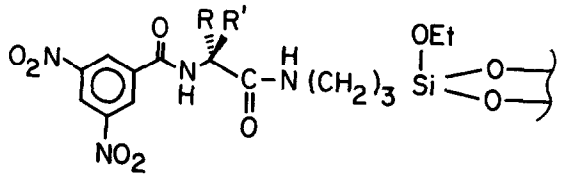
Abstract: Methyl esters of N-(3,5-dinitrobenzoyl)-amino acids have been used as chiral solvating agents to induce NMR spectral nonequivalence between the enantiomers of a broad array of solutes.

Some years ago, we introduced the use of chiral solvating agents (CSA) for the NMR determination of enantiomeric purity and absolute configuration.[1,2] Subsequent studies of the mode of operation of these CSAs led to the design of a number of chiral stationary phases (CSPs) on which one may effect the liquid chromatographic separation of many enantiomers.[3] In the course of detailed investigations of the chiral recognition mechanisms responsible for the enantioselectivities of these CSPs, we have conducted NMR studies of the interactions between soluble analogs of the CSPs and the enantiomers of resolvable compounds. For example, (R)-methyl-N-3,5-dinitrobenzoylphenylglycinate, **1a**, closely resembles chiral stationary phase, **2a**, and induces NMR spectral nonequivalence between enantiomers which are separable upon bonded phase **2a**. It should be noted that Kagan et al have reported the use of N-3,5-dinitrobenzoyl-α-methylbenzylamine as a CSA for the NMR determination of enantiomeric purity.[4] This compound can be considered a soluble analog of CSPs **3a-b**, prepared and evaluated in our laboratories.[5] For enantiomer separations, CSPs **3a-b** are generally inferior to amino acid-derived CSPs **2a-b**.



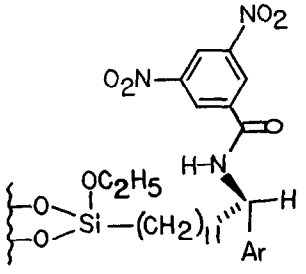
1a R = Ph, R' = ^{1}H **1b** R = H, R' = $CH_2CH(CH_3)_2$

Owing to the ready availability of **1a** and its leucine-derived counterpart, **1b**, these compounds are deemed to be useful CSAs for the NMR determination of enantiomeric purity.[6] Generalized structures **4-7** represent a few of the classes of compounds whose enantiomers

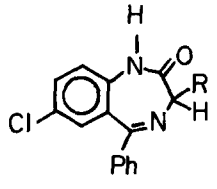


2a = Ph, R' = H **2b** R = H, R' = $CH_2CH(CH_3)_2$

may be chromatographically separated upon CSPs **2a-b.** In all cases so far examined, the NMR spectra of deuterochloroform solutions of racemates of generalized structures **4, 5, 6** or **7** and CSA **1a** (or **1b**) show enantiomeric spectral nonequivalence. The magnitude of these chemical-shift differences usually ranges between 0.05 to 0.10 ppm and nonequivalence is frequently noted for more than one set of resonances in the analyte. Nonequivalence magnitudes as large as 0.5 ppm have been observed for protons participating in hydrogen bonding (e.g. the NH protons in **4**). Equivalent concentrations of analyte and CSA are usually satisfactory, although $\Delta\delta$s can be increased by using a 3 to 4-fold excess of CSA, or by lowering the probe temperature. Carbon-13 nonequivalence has also been noted.



3a, Ar = Phenyl
3b, Ar = 6,7-$(CH_3)_2$-1-Naph



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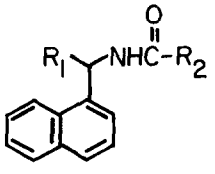

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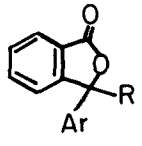

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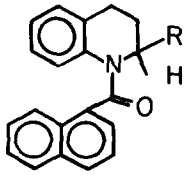

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The NMR nonequivalence of enantiomers results from the formation of transient diastereomeric solvates which may differ intrinsically in chemical shift and which may form to different extents.[2] It seems probable that for many solutes, the observed senses

of nonequivalence can be related to chiral recognition models not unlike those used to account for the separability of the racemates on CSPs **2a-b.**[9] It may be anticipated that CSAs **1a-b** will afford NMR spectral nonequivalence for a broad range of enantiomeric solutes, just as CSPs **2a-b** suffice for the separation of a broad range of enantiomeric analytes.

Fifteen enantiomerically enriched (by chromatography on CSP **2a-b**) type **4** benzodiazepinones have been examined; R = $-O(CH_2)_nCH_3$ n = 3-6, $-OCH_2CH_2CH(CH_3)_2$; $-OCOCH_3$; -OCOPh; $-OCOCH_2CH_3$; $-OCO(CH_2)_3Ph$; $-OCOCH(CH_3)_2$; $-CH_2(CH_2)_nCH_3$ n = 0-2; $-CH(CH_3)_2$; $-N(CH_2CH_2)_2O$.[7] It has been found that the enantiomer most retained by CSP (R)-**2a** (i.e. the (S)-enantiomer) invariably undergoes the greatest spectral shift by CSA (R)-**1a**.[8] For example, signals of the 3-protons of both enantiomers are shifted upfield, but the upfield shift is much greater for the (S)-enantiomer (0.05-0.10 ppm) than it is for the (R)-enantiomer (0.01-0.02 ppm). The uniform sense of nonequivalence observed allows one to confidently assign absolute configuration to other type **4** benzodiazepinones. These assignments could be verified by the observed elution order from CSP **2a**. Figure **1** shows an expansion of the 3-H signals from a 200 MHz spectrum of an (R)-enriched sample of 3-pentoxybenzodiazepinone. Enantiomeric purities, when determined for the preceding benzodiazepinones (60-80% major enantiomer) by both the NMR and HPLC methods agreed closely, the average difference between the two techniques being 1.0%.[10] In general, HPLC methods are the more accurate, particularly when enantiomeric purities are high.

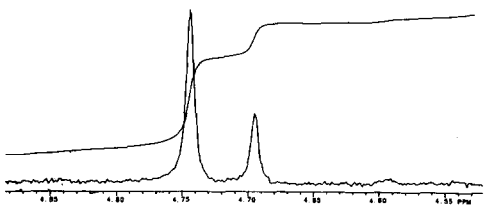

Figure 1: 3-H signals from a 200 MHz spectrum of an (R)-enriched sample of 3-pentoxy benzodiazepinone (**4**, R = $O(CH_2)_4CH_3$) in the presence of (R)-**1a** (1 eq).

Although the CSP method has an advantage in terms of sensitivity, accuracy, and instrumentation requirements, it does require that the transiently formed diastereomeric adsorbates differ in stability. It is not essential to the NMR method that the diastereomeric solvate differ in stability although, of course, they may. In the presence of CSA **1a**, the methyl signals of dimethylsulfoxide are anisochronus, a $\Delta\delta$ of 0.021 ppm being observed. This clearly demonstrates that no stability difference is required for observation of NMR nonequivalence. Hence, the NMR-CSA approach can solve problems (e.g., determining the enantiomeric purity of compounds chiral by virtue of isotopic substitution) beyond the scope of the HPLC-CSP method.

The mechanistic origin of the nmr nonequivalence engendered by CSA's **1a-b** is being studied, owing to its presumed pertinence to chromatographically-derived chiral recognition models.

Acknowledgment

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References and Notes

1. Pirkle, W. H. J. Am. Chem. Soc. 1966, **88**, 1837.
2. For a review of CSAs see: Pirkle, W. H.; Hoover, D. J. Top. Stereochem. 1982, 263.
3. Pirkle, W. H.; Finn, J. M; Hamper, B. C.; Schreiner, J.; Pribish, J. R. in "Asymmetric Reactions and Processes in Chemistry" ACS Symposium Series, Washington, 1982, p. 245.
4. Deshmukh, M.; Dunach, E.; Juge, S.; Kagan, H. B. Tetrahedron Lett. 1984, 3467.
5. Pirkle, W. H.; Hyun, M. H., manuscript in preparation
6. The NMR spectra of CSA **1a** and **1b** are relatively simple. In fact alternate use of **1a** and **1b** make almost the whole NMR range available for study. The acid precursors of **1a** and **1b** are commercially available (Aldrich, Sigma) or may be prepared as described: W. H. Pirkle and M. H. Hyun, J. Org. Chem., 1984, **49**, 3043. Esters **1a** and **1b** were prepared using hydrogen chloride-saturated methanol.
7. Synthesis and resolution of these compounds can be found in: Pirkle, W. H.; Tsipouras, A. J. Chromatogr. 1984, **291**, 291.
8. Assignment of absolute configuration for the benzodiazepinones in this series was made by the Snatzke method, which is based on the nonempirical theoretical prediction of CD spectra: Snatzke, G.; Konowall, A.; Sablij, A.; Blazevic, N.; Sunjic, V. Croat Chem. Acta 1982, **55**, 435.
9. Detailed studies of the structure of these diastereomeric solvates is underway and will be reported at a later date.
10. Because of the enantiodependence of the physiological activity of these Valium ^{R} analogs, there is a need for simple methods for evaluation of enantiomeric purity. See for example: Simonyi, M.; Fitos, I.; Kajtar, J.; Kajtar, M. Biochem. Biophys. Res. Commun. 1982, **109**, 851.

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