Use of Spin Echo Magic Angle Spinning ^{1}H NMR in Reaction Monitoring in Combinatorial Organic Synthesis

Ravi S. Garigipati,† Bruce Adams,‡ Jerry L. Adams,† and Susanta K. Sarkar*,§

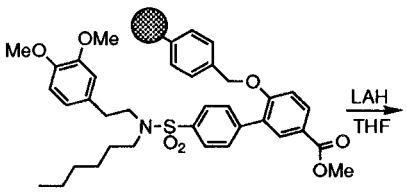
Departments of Medicinal Chemistry and Physical and Structural Chemistry, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, Pennsylvania 19406, and Varian Instruments, 25 Hanover Road, Florham Park, New Jersey 07932

Received December 4, 1995

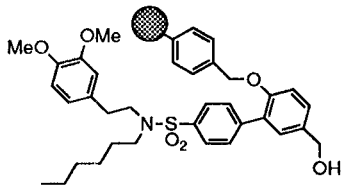
The avidity to attain molecular diversity, as it relates to lead compound generation in medicinal chemistry, has greatly increased in the past few years.[1] Merrifield's solid phase peptide synthesis has been coupled with combinatorial methods, resulting in generation of peptide libraries containing millions of oligomers.[2] Given the inherent limitations of peptides as drug candidates, recent efforts have been focused on the construction and evaluation of small molecule nonoligomeric libraries[3] containing the structural complexity that is typically encountered in a medicinal chemistry effort. However, for the successful synthesis of high-quality nonpeptide combinatorial libraries, it is essential to monitor reactions on a solid phase. Unfortunately, the conventional analytical tools for following reactions in solution, e.g., TLC, NMR, and MS, are no longer applicable to the resin-bound compounds. Therefore, in order to monitor the chemistry and characterize the reaction products, one must cleave the bound compound from the solid support and then use standard techniques for analysis. This process is both inefficient and can give rise to artifacts during the cleavage of reactive intermediates. Although FT-IR has been successfully used in reaction monitoring, it is of limited utility since many reactions do not involve a clear infrared detectable functional group change. It is therefore important to develop a rapid and nondestructive analytical technique applicable to solid phase chemistry.

Recently, it has been shown that the resolution of ^{1}H NMR spectra of organic molecules covalently attached to resins (suspended in organic solvents) is significantly improved by magic angle spinning.[4] However, the resulting spectrum may still be complicated by the presence of large peaks resulting from the polystyrene matrix of the beads. These resonances are much broader presumably because of short T_2 and, thus, could be reduced to a significant extent by judicious choice of τ values in the spin echo pulse sequence[5] ($90° - \tau - 180° - \tau$). The use of spin echo sequence to distinguish between narrow and broad lines has been reported.[6,7]

Scheme 1



1



2

We report here an extension of the MAS ^{1}H NMR technique[4] by demonstrating the utility of the combination of MAS along with a spin echo sequence to follow the completion of a chemical reaction carried out in combinatorial organic synthesis. The reaction chosen for this study was the LAH reduction of a resin-bound methyl benzoate **1** to the corresponding benzyl alcohol **2** (Scheme 1). In this example, the structural complexity in the reactant and product is typical of a chemical transformation during library construction.

Experimental Section

Materials and Sample Preparation. Merrifield resin (1.5% cross linked, 1.4 mmol/g) was obtained from Nova Biochem. The FTIR spectra were collected on a Nicolet Impact 400D spectrophotometer.

Preparation[8] of the ester **3**: To a suspension of the Merrifield resin (4.5 g) in dimethyl acetamide (25 mL) was added sodium methoxide (0.58 g, 10.5 mmol) and methyl 3-bromo-4-hydroxybenzoate[9] (2.4 g). The mixture was stirred under inert atmosphere at 85 °C for 26 h. The reaction mixture was filtered and washed with DMF, dioxane, CH_2Cl_2, MeOH, 1:1/MeOH/H_2O, and finally methanol to afford resin **3** (5.0 g) (Scheme 2). Elemental analysis indicated a stoichiometric amount of bromine.

Preparation of the ester **1**: To a suspension of the resin **3** (2.0 g) in toluene (20 mL) was added tetrakis(triphenylphosphine)palladium (0.4 g), the boronic acid **4**[10] (2.5 g, 6 mmol) in ethanol (6 mL), and 2 M K_2CO_3 (4 mL). This heterogeneous mixture was stirred at 80 °C for 24 h. The mixture was cooled to room temperature and was filtered to afford a black resin. The resulting resin was treated with a solution of KCN in DMSO until the black color due to adsorbed palladium disappeared. The resin was carefully filtered and washed with DMSO, water, methanol, CH_2Cl_2, and finally with methanol to afford the resin

* To whom correspondence should be addressed. Phone: (610) 270-6652. Fax: (610) 270−6608. E-mail: sarkar@sbphrd.com.
† Department of Medicinal Chemistry.
‡ Department of Physical and Structural Chemistry.
§ Varian Instruments.

(1) (a) Moos W. H.; Green G. D. Annual Reports in *Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press, Inc.: San Diego, 1993; p 315. (b) Pavia, M. R.; Sawyer, T. K., Moos, W. *J. Bioorg. Med. Chem. Lett.* **1993**, 3, 387.
(2) Houghten, R. A.; Pinnila, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84.
(3) Gallop, M. A.; Barret, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon E. M. *J. Med. Chem.* **1994**, *37*, 1233. (b) Gordon, E. M.; Barret, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385. (c) Ecker, D. J.; Crooke, S. T. *Biotechnology* **1995**, *13*, 351.
(4) Fitch, W. L.; Detre, G; Holmes, C. P.; Shoolery, J. N.; Keifer P. A. *J. Org. Chem.* **1994**, *59*, 7955.
(5) Hahn, E. L. *Phys. Rev.* **1950**, *80*, 580.
(6) Rabenstein, D. L.; Mills, K. K.; Strauss, E. J. *Anal. Chem.* **1988**, *60*, 1380A.
(7) Agris, P. F.; Campbell, I. D. *Science* **1982**, *216*, 1325.
(8) Wang, S. S. *J. Am. Chem. Soc.* **1973**, *95*, 1328.
(9) Brink, M. *Chem. Abstr.* **1967**, *67*, 21571*k*.
(10) Morgan, J.; Pinhey, J. T. *J. Chem. Soc.* **1990**, 715.

0022-3263/96/1961-2911$12.00/0

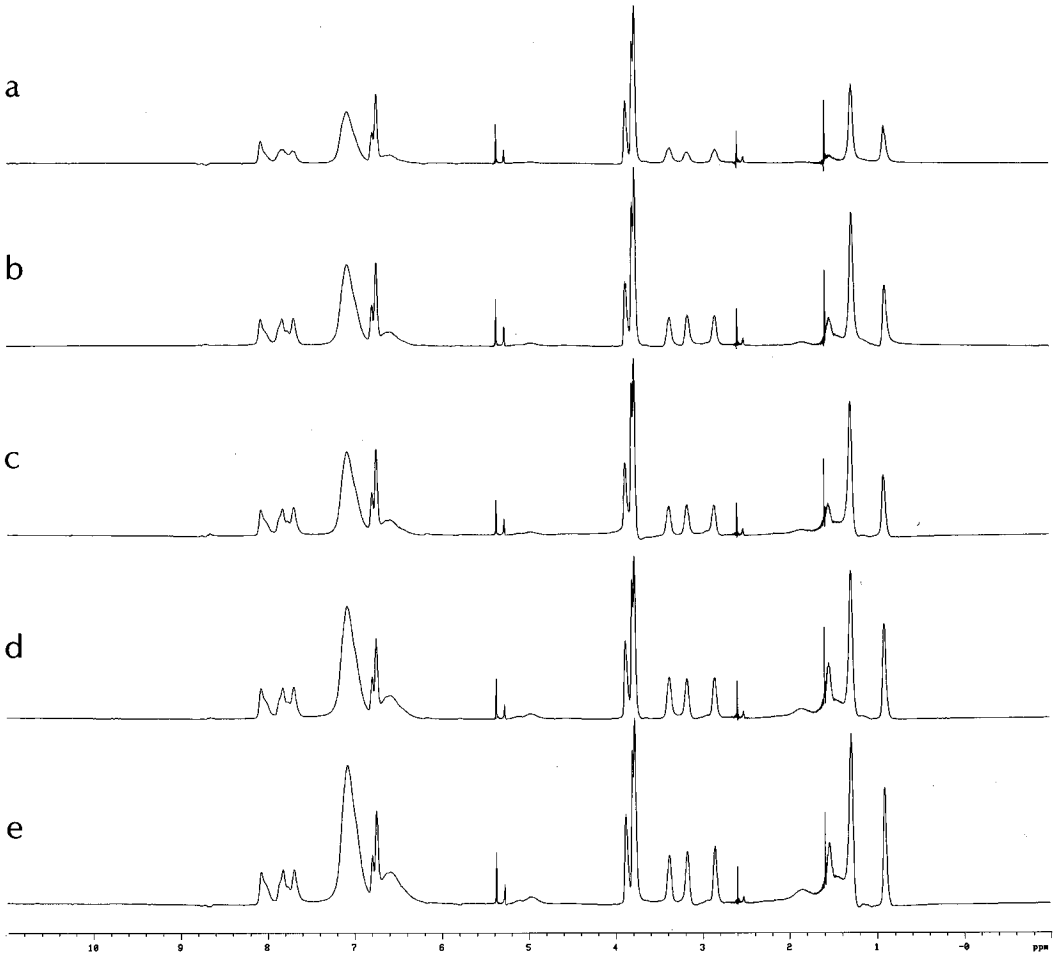
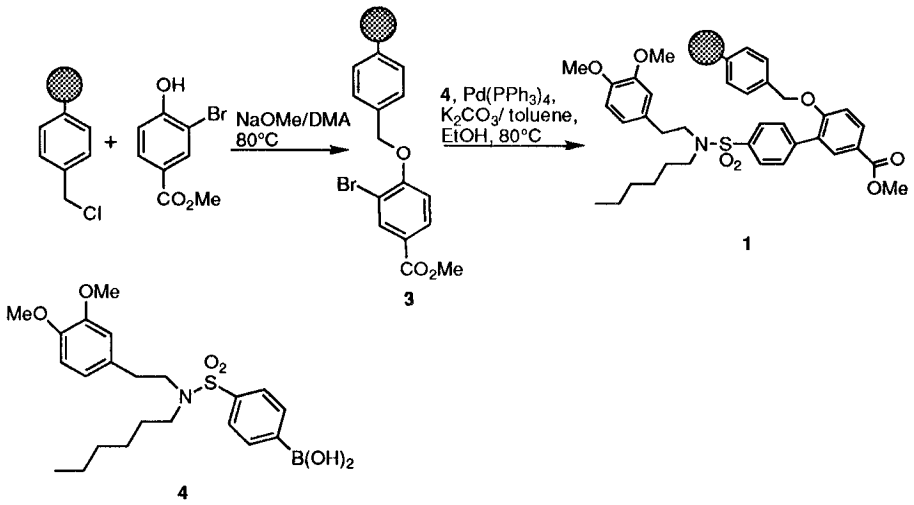


Figure 1. 500 MHz ^{1}H MAS NMR spectrum of **1** on the resin in CD_2Cl_2 using the spin echo sequence with different τ values: (a) $\tau = 16$ ms; (b) $\tau = 8$ ms; (c) $\tau = 4$ ms; (d) $\tau = 2$ ms; (e) $\tau = 1$ ms. All the spectra are plotted on same vertical scale.

Scheme 2



1 (2.5 g). FTIR (KBr): 3058, 3024, 1730, 1678, 1513, 1492, 1451, 1338, 1246, 1155, 1028, 754. Elemental analysis indicated adequate amounts of sulfur and nitrogen and <0.5% bromine.

Reduction of ester **1**: To a suspension of the ester **1** (2.0 g) in THF (20 mL) was added LAH (0.22 g, 5.8 mmol), and the mixture was stirred at room temperature for 6 h. The excess LAH was carefully destroyed by adding ethyl acetate (10 mL) and then methanol (10 mL). The resin was filtered and washed with methanol (100 mL). The gray resin was treated with 100 mL of 1:1 dioxane/1 N H_2SO_4 for 36 h. The resin **2** was collected and washed with dioxane (200 mL) and methanol (200 mL) to afford the alcohol **2** (1.9 g). FTIR (KBr): 3500 (br.), 3058, 3024, 1677, 1513, 1492, 1450, 1338, 1320, 1298, 1180, 1155, 780.

NMR Spectroscopy. All the NMR spectra of compounds attached to the Wang resin were collected using a Varian UnityPlus spectrometer operating at ^{1}H frequency of 500 MHz using a Nano.NMR probe.[11] Five to six mg of beads containing

(11) Barbara, T. M. *J. Magn. Reson. A* **1994**, *109*, 265.

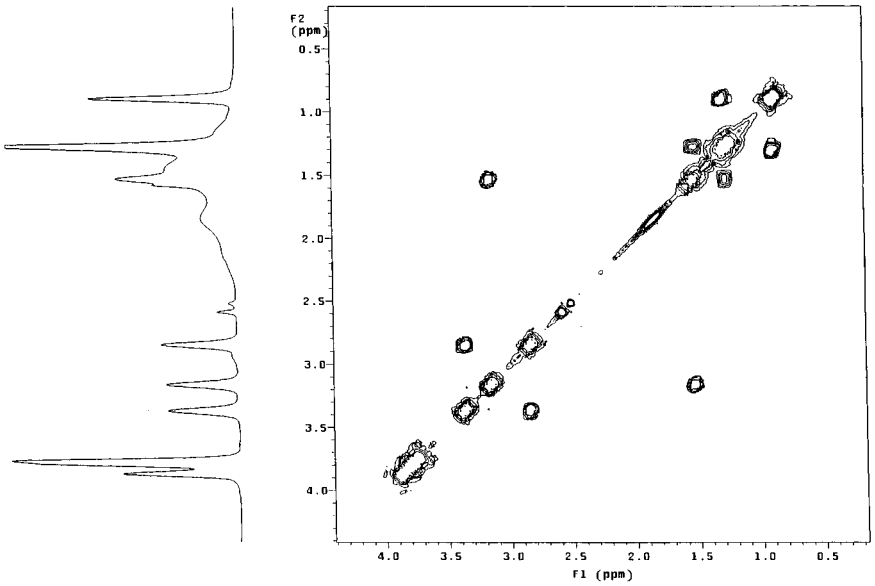


Figure 2. 500 MHz ^{1}H MAS NMR COSY spectrum (expansion of the aliphatic region) of **1** on the resin in CD_2Cl_2.

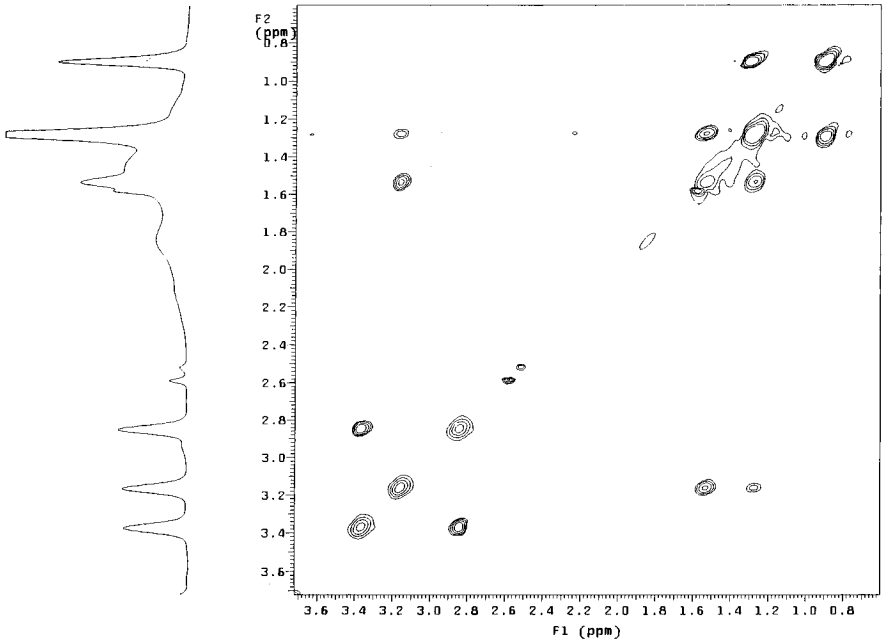


Figure 3. 500 MHz ^{1}H MAS NMR TOCSY spectrum (expansion of aliphatic region) of 1 on the resin in CD_2Cl_2.

5−6 μmol of compounds was transferred into a Nano.NMR probe cell, and 40 μL of CD_2Cl_2 was then added. The spectra were collected using the spin echo sequence ($90° - \tau - 180° - \tau$), with different τ values, along with magic angle spinning at a speed of 2.0 KHz.

A 2D COSY[12,13] spectrum of the resin-bound compound **1** was collected using a Nano.NMR probe by spinning the sample at magic angle at a speed of 2.0 KHz. The data were collected in magnitude mode using 16 scans for each 128 t_1 increments with a recycle delay of 1 s, spectral widths of 6 kHz in both the dimensions, and an acquisition time of 0.171 s. Data were processed with unshifted sine-bell weighting and symmetrized after zero-filling to a 2K × 2K matrix.

A TOCSY spectrum[14,15] of the resin-bound compound **1** was collected using a Nano.NMR probe by spinning the sample at magic angle at a speed of 2.0 KHz. The phase sensitive data were collected using 16 scans for each of the 128 hypercomplex t_1 increments, a 2.0 s recycle delay, spectral widths of 6 kHz in both dimensions, an acquisition time of 0.171 s, and a 20 ms 7 kHz MLEV-16 spin lock field. Data were processed with Gaussian weighting and zero-filled to a 2K × 1K matrix.

Results and Discussion

The utility of the spin echo sequence along with magic angle spinning in suppressing signals from the polysty-

(12) Aue, W. P., Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229.
(13) Bax, A. *Two-Diemnsional Nuclear Magnetic Resonance in Liquids*; Delft Univ. Press: Delft, 1982.
(14) Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521.
(15) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355

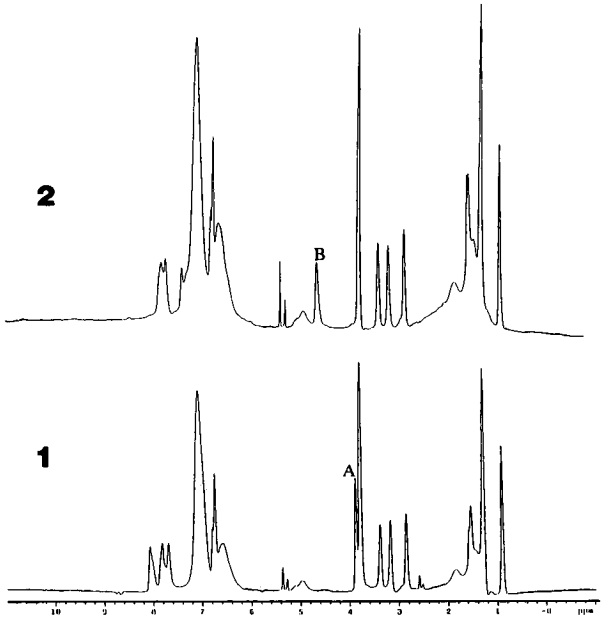


Figure 4. 500 MHz ^{1}H MAS NMR spectra of **1** and **2** on the resin in CD_2Cl_2 using the spin echo sequence with $\tau = 4$ ms.

rene backbone of the resin is shown in Figure 1. Figure 1 shows a series of ^{1}H MAS NMR spectra of compound **1** covalently bound to polystyrene-based resin, swollen by the addition of CD_2Cl_2, as a function of different τ values. It is clear from Figure 1 that by the appropriate choice of τ value one can significantly minimize the effect due to these polystyrene peaks. The relevant peaks in the spectrum of the resin-bound compound **1** were assigned independently via two-dimensional techniques. Expanded regions of COSY and TOCSY spectra of resin-bound compound **1** are shown in Figures 2 and 3, respectively.

Figure 4 shows ^{1}H MAS NMR ($\tau = 4$ ms) spectra of the ester **1** and the alcohol **2**, illustrating the utility of this technique to monitor progress of the reaction. The diagnostic signals for the methyl ester (peak A) and the alcohol (peak B) are clearly discernable.

In conclusion, we have demonstrated that spin echo magic angle spinning (MAS) ^{1}H NMR can be used as a nondestructive analytical technique to follow the course of chemical reactions on solid support. It is also demonstrated that standard 2D techniques like COSY, TOCSY, etc. can be used to assign the spectra of compounds bound to resin. The ability to monitor reactions on resins without cleaving will have a significant role in enhancing the development of combinatorial chemistry for the drug discovery process.

JO952153O

Additions and Corrections

Vol. 60, 1995

Kevin L. McLaren. Total Synthesis of the Cyclic Depsipeptide Leualacin.

Page 6082. The author apologizes for the omission of an important reference, which reports the first total synthesis of leualacin: Schmidt, U.; Langer, J. *J. Chem. Soc., Chem. Commun.* **1994**, 2381−2.

JO964003Z

Dennis G. Hall and Pierre Deslongchamps*. Transannular Diels−Alder/Intramolecular Aldol Tandem Reaction as a Stereocontrolled Route to (+)-Aphidicolin and its Isosteric C8-Epimer.

Page 7798, Figure 1, caption should read "Superimposition of geometry-optimized ...".

Page 7798, column 2, line 12, should read "**Trienes 42 and 46.**"

JO9640027