An NMR Method To Identify Nondestructively Chemical Compounds Bound to a Single Solid-Phase-Synthesis Bead for Combinatorial Chemistry Applications

Susanta K. Sarkar,*,† Ravi S. Garigipati,†
Jerry L. Adams,† and Paul A. Keifer‡

SmithKline Beecham Pharmaceuticals
709 Swedeland Road
King of Prussia, Pennsylvania 19406
Varian NMR Instruments, 3120 Hansen Way
Palo Alto, California 94304

Received July 21, 1995

As part of our program to develop analytical methods for combinatorial chemistry applications, we report here an NMR technique for the selective identification of a chemical compound still bound to *a single bead* of a solid-phase-synthesis resin. In this method, the ^{13}C-labeled compound is selectively detected using isotope-filtered ^{1}H NMR (HMQC[1]) in a two-coil high-resolution magic-angle spinning (MAS) probe.[2] This technique suppresses signals from the unlabeled portions of the resin bead, thus allowing an unambiguous identification of the bound compound.

Solid-phase-synthesis (SPS) techniques,[3] combined with the growing field of combinatorial chemistry,[4] are currently having a very large impact upon drug discovery[5,6] and chemistry in general.[7] Unfortunately, the analytical techniques for SPS samples are not as highly developed as the synthetic techniques.[8] Currently, the samples are cleaved from the resins, either chemically or photolytically,[9] and analyzed by conventional structure determination techniques; however, there is considerable interest in developing analytical techniques that can analyze samples while they are still bound to the polymer matrix. If the technique was also nondestructive, it would prove particularly useful in the rapidly expanding field of "one-bead, one-compound" combinatorial chemistry techniques.[10,11] In these experiments, a reaction mixture containing a diverse library of possibly millions of individual beads (representing millions of different structures) may be tested. From this mixture, only one bead may be isolated which has the desired activity, and the success of this approach critically depends upon determination of the structure of the 0.1−1 nmol of sample bound to that single bead.[5]

In classic peptide combinatorial chemistry, the peptide would be cleaved from the bead and sequenced by either Edman degradation or mass spectrometry. This strategy fails for nonpeptide samples which are not built up from simple repeating units, so "encoding" methods have been developed.[12] To encode a resin library, an additional "tag" compound is added to each bead to document its synthetic history, creating a unique "bar code" [13] which can be cleaved and identified (by GC or GC/MS) to indirectly identify the primary sample. Examples of reporter tags that have been used include nucleotides (which were cleaved, amplified by PCR, and sequenced),[12,14] peptides (sequenced by Edman degradation),[15] or a combined series of hydrocarbon homologues and polychlorinated aromatics (analyzed by GC).[16] In all cases, the use of reporter tags complicates synthetic strategies, increases the risk of side reactions and byproducts, and yields only indirect evidence of structure. The presence of the encoding structure may also affect the results of the binding assays.[11] While new MS techniques have recently been developed that can analyze single-bead samples of peptides[17] and peptoids[18] without the use of encoding ligands, all such methods are currently destructive.

Although NMR spectroscopy is routinely used to analyze samples that have been cleaved from SPS resins, the NMR spectra of resin-bound samples consist of very broad lines, even if the resin is swollen by the addition of solvents. While relatively low-resolution ^{1}H and ^{13}C NMR spectra have been reported,[19] ^{13}C NMR has proven more useful because its larger spectral dispersion at least partially compensates for the broad line widths. The incorporation of site-specific ^{13}C labels in resin-bound samples has been shown to facilitate the use of ^{13}C NMR to monitor the progress of synthetic SPS reactions.[20]

It has been shown that magic-angle spinning[21] can narrow the ^{13}C NMR line widths of resin samples[22] and other heterogeneous mixtures[23] due to the removal of magnetic susceptibility line-broadening.[24] This was recently extended to ^{1}H NMR by using a combination of MAS and high-resolution probe technology (into what is called a Nano·NMR probe), which allowed high-resolution ^{1}H NMR data to be obtained directly on samples still bound to the resin beads.[25] Spectra having line widths as narrow as 4−5 Hz were easily obtained on 5 mg of beads.[26] We chose to investigate the sensitivity of this technique and, after acquiring spectra on progressively smaller amounts of resin, found that the ^{1}H signals from a

† SmithKline Beecham Pharmaceuticals.
‡ Varian NMR Instruments.

(1) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565−569.
(2) Barbara, T. M. *J. Magn. Reson., Ser. A* **1994**, *109*, 265−269.
(3) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154.
(4) Gallop, M. A.; Barret, R. W.; Dower, W. J.; Foder, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233−1251. (4b) Gordon, E. M.; Barret, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385−1401.
(5) Madden, D.; Krchnak, V.; Lebl, M. *Perspect. Drug Discovery Des.* **1994**, *2*, 269−285.
(6) Ecker, D. J.; Crooke, S. T. *Biotechnology* **1995**, *13*, 351−360.
(7) Parlow, J. J. *Tetrahedron Lett.* **1995**, *36*, 1395−1396.
(8) Jung, G.; Beck-Sickinger, A. G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 367−383.
(9) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49* (48), 11065−11133.
(10) Lam, K. S.; Salmon, S. E.; Hersch, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82−84.
(11) Lebl, M.; Krchnak, V.; Sepetov, N. F.; Seligmann, B.; Strop, P.; Felder, S.; Lam, K. S. *Biopolymers* **1995**, *37*, 177−198.
(12) Brenner, S.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5381−5383.
(13) Chabala, J. C.; Baldwin, J. J.; et al. *Perspect. Drug Discovery Des.* **1994**, *2*, 305−318.
(14) Needels, M. C.; Jones, D. G.; Tate, E. H.; Heinkel, G. L.; Kochersperger, L. M.; Dower, W. J.; Barrett, R. W.; Gallop, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10700−10704.
(15) Kerr, J. M.; Banville, S. C.; Zuckermann, R. N. *J. Am. Chem. Soc.* **1993**, *115*, 2529−2531.
(16) Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922−10926. (16b) Borchardt, A.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 373−374. (16c) Baldwin, J. J.; Burbaum, J. J.; Henderson, I.; Ohlmeyer, M. H. J. *J. Am. Chem. Soc.* **1995**, *117*, 5588−5589.
(17) Youngquist, R. S.; Fuentes, G. R.; Lacey, M. P.; Keough, T. *J. Am. Chem. Soc.* **1995**, *117*, 3900−3906. (17b) Egner, B. J.; Langley, G. J.; Bradley, M. *J. Org. Chem.* **1995**, *60*, 2652−2653. (17c) Brummel, C. L.; Lee, I. N. W.; Zhou, Y.; Benkovic, S. J.; Winograd, N. *Science* **1994**, *264*, 399−402.
(18) Zambias, R. A.; Boulton, D. A.; Griffin, P. R. *Tetrahedron Lett.* **1994**, *35*, 4283−4286.
(19) Giralt, E.; Rizo, J.; Pedroso, E. *Tetrahedron* **1984**, *40*, 4141−4152. (19b) Albericio, F.; Pons, M.; Pedroso, E.; Giralt, E. *J. Org. Chem.* **1989**, *54*, 360−366. (19c) Mazure, M.; Calas, B.; Cavé, A.; Parello, J. *C. R. Acad. Sci., Ser. 2* **1986**, *303*, 553−556.
(20) Look, G. C.; Holmes, C. P.; Chinn, J. P.; Gallop, M. A. *J. Org. Chem.* **1994**, *59*, 7588−7590.
(21) VanderHart, D. L.; Earl, W. L.; Garroway, A. N. *J. Magn. Reson.* **1981**, *44*, 361−401.
(22) Stöver, H. D. H.; Fréchet, J. M. J. *Macromolecules* **1991**, *24*, 883−888.
(23) Garroway, A. N. *J. Magn. Reson.* **1982**, *49*, 168−171.
(24) Springer, C. S., Jr. In *NMR in Physiology and Biomedicine*; Gillies, R. J., Ed.; Academic Press, Inc.: San Diego, CA, 1994; Chapter 5.
(25) Fitch, W. L.; Detre, G.; Holmes, C. P.; Shoolery, J. N.; Keifer, P. A. *J. Org. Chem.* **1994**, *59*, 7955−6. (b) Garigipati, R. S.; Adams, B.; Adams, J. L.; Sarkar, S. K. *J. Org. Chem.*, in press.
(26) Keifer, P. A.; Baltusis, L.; Rice, D. M.; Tymiak, A. A.; Shoolery, J. N. *J. Magn. Reson.*, in press.

0002-7863/96/1518-2305$12.00/0

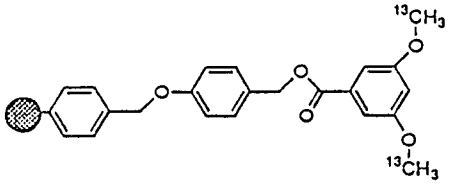

Figure 1. [3,5-dimethoxy-^{13}C]Benzoic acid bound to Wang resin (**1**).

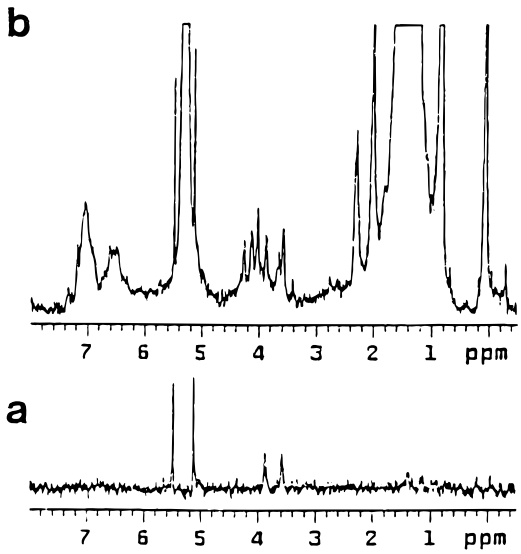

Figure 2. (a) 500-MHz ^{13}C-filtered ^{1}H NMR spectrum of **1** coupled to Wang resin, obtained on a single bead using the decoupler coil of a standard commercially-available heteronuclear Nano·NMR probe. The single bead was suspended in 30 μL of dichloromethane-d_2 in a standard (40 μL) Nanoprobe cell and spun at the magic angle (54.7°) at ~2 kHz. This proton spectrum was acquired using a one-dimensional version of the HMQC sequence[1] run without ^{13}C decoupling but with presaturation (11-Hz field strength for 1 s) of the residual protonated dichloromethane resonance at 5.32 ppm. Total experiment time was 170 min (5000 transients, 1.02-s acquisition time, 1.0-s presaturation delay). (b) ^{1}H NMR spectrum obtained using a one-pulse experiment. Total experiment time was 250 min (5000 transients, 2.05-s acquisition time, 1.0-s presaturation delay). Both spectra were processed with 4 Hz of exponential weighting (the dichloromethane ^{13}C satellites have 2-Hz line widths, while the less mobile 3,5-dimethoxy-^{13}C resonances have 20-Hz line widths).

sample attached to *only a single bead* could be reproducibly detected.[27] This limit of detection is sufficient to now allow NMR to be used in one-bead, one-compound techniques.

Although we determined that it is possible to get a ^{1}H NMR spectrum on such a small amount of material bound to a single bead, the spectrum is typically complicated by large signals arising from solvent backgrounds, impurities, and fingerprints on the outside of the sample cell, as well as smaller peaks due to the polystyrene backbone of the bead itself. To circumvent these problems, we synthesized [3,5-dimethoxy-^{13}C]benzoic acid (**1**, Figure 1), coupled it to 100-μm-diameter Wang resin beads,[28] and utilized isotope-filtered NMR to selectively observe the proton resonances of the 3,5-dimethoxy-^{13}C group. A 1D ^{13}C-filtered ^{1}H NMR spectrum of **1** is shown in Figure 2a. The protons of the 3,5-dimethoxy-^{13}C moiety (at 3.7 ppm) and the ^{13}C satellites of the solvent (at 5.32 ppm) are clearly observable. The corresponding unfiltered spectrum is shown in Figure 2b, and it is clear from the spectrum that assignment of the proton

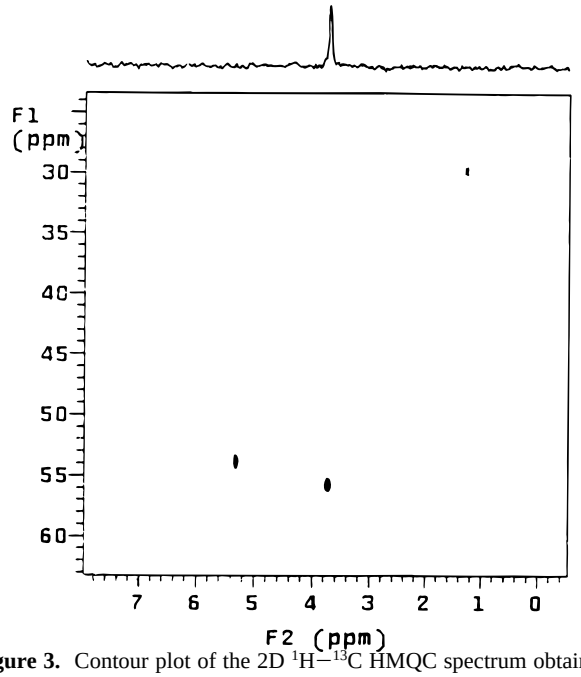

Figure 3. Contour plot of the 2D ^{1}H−^{13}C HMQC spectrum obtained on the same single-bead sample. An f_1 cross section at the f_2 frequency of the $^{13}CH_3O$-protons is also shown, indicating both the high sensitivity and the excellent suppression of the protons attached to ^{12}C. The 17.5-h experiment used broadband (3.5 kHz) ^{13}C GARP-1 decoupling[31] during the 128-ms acquisition time, a 1.13-s presaturation delay, 512 scans for each of the 49 hypercomplex t_1 data points, and 5- and 8-kHz spectral widths and cosine and 10-Hz exponential weightings in t_1 and t_2, respectively.

resonances is difficult due to the presence of large background signals. These signals, as well as the non-^{13}C-labeled peaks from the polystyrene resin itself, are completely suppressed in the ^{13}C-filtered spectrum. Since complete structural characterization of chemical compounds relies on through-bond and through-space proton−proton or proton−carbon connectivities, which are generally obtained from 2D NMR data, we show data from a 2D version of ^{1}H−^{13}C HMQC in Figure 3. Again, correlations arising from the protons of both the 3,5-dimethoxy-^{13}C group and the ^{13}C satellites of the solvent are clearly visible.[29] These data verify that the sensitivity and selectivity are sufficient to observe any unique ^{13}C-labeled substrate still bound to a single resin bead using 1D NMR and that 2D NMR is feasible if mixture analysis is required.

In summary, we report here a novel method for the identification of isotopically-labeled chemical compounds bound to a single resin bead. Although we used only one ^{13}C-labeled moiety on the resin, the synthesis of uniformly labeled molecules and their subsequent analysis by multidimensional isotope-filtered NMR experiments (like HMQC) are generally applicable and could facilitate the complete structural characterization of any unknown compound bound to a single resin bead. We estimate that ~800 pmol of material was used in our experiments.[30] While the outer ^{1}H decoupler coil of the heteronuclear Nano·NMR probe used was sufficient for this study, we are finding that more efficient inverse-geometry probe designs (having an inner proton coil, analogous to conventional indirect-detection NMR probes) may allow sensitivity sufficient to detect as little as 100 pmol of material, bound to a single bead, using this method which requires isotopically-labeled compounds.

JA952434D

(27) Three different single-bead samples were tested to verify the reproducibility of this technique. We also found that 1D ^{13}C NMR spectra could be obtained on the ^{13}C-labeled single-bead samples used in this study. The Nanoprobe sample cell restricts 100% of the sample (40 μL maximum) within the active region of the receiver coil to ensure that even a single bead would always be detected.

(28) 100−200 mesh Merrifield resin obtained from Calbiochem was functionalized according to: Wang, S. S. *J. Am. Chem. Soc.* **1973**, *95*, 1328−1333. The beads selected for NMR were measured by a light microscope to have a 100 μm diameter.

(29) In addition, the upfield region contains a lower intensity correlation arising from the large aliphatic proton resonance at 1.3 ppm, which was eventually shown to arise from contaminants, presumably fingerprint oils, located on the outside of the sample cell. The other large proton resonance at 1.6 ppm shows no such ^{13}C correlation and is presumably absorbed water.

(30) The stated loading of 1.4 mmol/g and an estimated 1 700 000 beads/g, gives ~800 pmol of sample per bead.

(31) Shaka, A. J.; Barker, P. B.; Freeman, R. J. *J. Magn. Reson.* **1985**, *64*, 547−552.