Methods for Combinatorial Organic Synthesis: The Use of Fast ^{13}C NMR Analysis for Gel Phase Reaction Monitoring

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Received October 26, 1994®

Summary: A method for the rapid monitoring by ^{13}C NMR spectroscopy of reactions performed on solid supports has been developed which utilizes ^{13}C-enriched building blocks to enhance the NMR spectral signals.

The advent of the field of combinatorial chemistry, especially pertaining to lead identification and drug discovery, has renewed interest within the chemical community toward organic synthesis on solid supports.[1] Whereas methods for the formation of combinatorial libraries of peptides and oligonucleotides are now well established, the preparation of libraries of small organic molecules remains an emerging area of research.[1b] During the course of our work in the area of small molecule combinatorial organic synthesis we established the need for a nondestructive method for assessing the progress of nonpeptide coupling reactions during synthesis optimization. While numerous methods exist in the arena of peptide and oligonucleotide synthesis for the monitoring and quantification of reactions, relatively little work has been performed in the area of nonpeptide reaction visualization on solid supports.[2,3] Infrared spectral monitoring of reactions on resin has been used successfully in a number of instances, especially for cases where changes in hybridization or functional group interconversions are involved.[3] However, this method of visualization is somewhat limited as it requires a change in an IR-distinctive chromophore, and it remains a destructive technique because a portion of the solid support is consumed. The use of ^{13}C NMR to visualize compounds bound to a solid support has been well documented and is, indeed, a useful method for the characterization of such compounds.[2] However, typical ^{13}C NMR experiments involving resin require thousands of transients and are thus impractical for the monitoring of a reaction. We describe herein a technique now routinely used in our laboratories that allows for the monitoring of the progress of reactions on as little as 20 mg of resin containing less than 1 mg of compound through the use of commercially available NMR tube inserts and ^{13}C-enriched building blocks.[4]

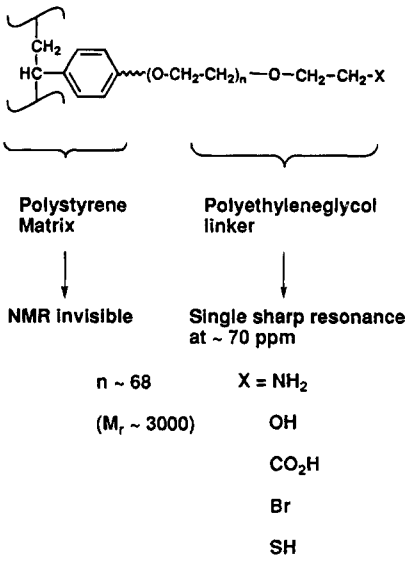

Figure 1. Physical characteristics and NMR properties of TentaGel resin.

As part of our strategy for the design of small molecule combinatorial libraries we have chosen to target molecules constructed from diverse and readily available building blocks. This strategy permits one to exploit an abundant pool of commercially available ^{13}C labeled compounds. Although labeled compounds can be quite costly, solid supported synthesis frequently requires only tens of milligrams of a given building block for reaction optimization studies. Furthermore, we have found that adapting and optimizing novel chemistries on resins often can be accomplished by following the fate of a few key ^{13}C-enriched carbon atoms.

A key component in this analytical technique is the use of readily available NMR tube inserts which permits the positioning of small amounts of resin within the observation coils of the NMR instrument.[5] For these studies, the resin[6] is easily applied to and withdrawn from the insert as a slurry with a widebore syringe needle.

We have found that the poly(ethylene glycol)-grafted polystyrene TentaGel resins are particularly suitable for fast ^{13}C NMR (Figure 1). Bayer and co-workers have also found that poly(ethylene glycol)-grafted polystyrene resin

® Abstract published in *Advance ACS Abstracts,* November 15, 1994.

(1) For reviews on the use of combinatorial technology for drug discovery, see: (a) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233. (b) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385.

(2) For examples of NMR spectra obtained for resin bound compounds, see: (a) Fréchet, J. M. J. *Tetrahedron* **1981**, *37*, 663. (b) Barany, G.; Kneib-Cordonier, N.; Mullen, D. G. *Int. J. Peptide Protein Res.* **1987**, *30*, 705. (c) Epton, R.; Goddard, P.; Irvin, K. *J. Polymer* **1980**, *21*, 1367. (d) Giralt, E.; Rizo, J.; Pedroso, E. *Tetrahedron* **1984**, *40*, 4141. (e) Feliz, M.; Giralt, E.; Ribó, J. M.; Trull, F. R. *Makromol. Chem.* **1988**, *189*, 1551. (f) Giralt, E.; Albericio, F.; Bardella, F.; Eritja, R.; Feliz, M.; Pedroso, E.; Pons, M.; Rizo, J. In *Innovation and Perspectives in Solid Phase Synthesis*; Epton, R., Ed.; SPCC Ltd: Birmingham, UK, 1990; p 111. (g) Bayer, E.; Albert, K.; Willisch, H.; Rapp, W.; Hemmasi, B. *Macromolecules* **1990**, *23*, 1937.

(3) For examples on the use of IR for the monitoring of resin supported reactions, see: (a) Chen, C.; Ahlberg Randell, L. A.; Miller, R. B.; Jones, A. D.; Kurth, M. J. *J. Am. Chem. Soc.* **1994**, *116*, 2661. (b) Fréchet, J. M.; Schuerch, C. *J. Am. Chem. Soc.* **1971**, *93*, 492.

(4) For recent examples of the use of ^{13}C-enriched compounds for increasing NMR signal response, see: (a) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511. (b) Rychnovsky, S. D.; Skalitzky, D. J.; Pathirana, C.; Jensen, P. R.; Fenical, W. *J. Am. Chem. Soc.* **1992**, *114*, 671.

(5) The NMR tube inserts were 20 cm long with a 3 mm widebore neck and were obtained from Wilmad, Vineland, NJ.

(6) The resins that were used for these experiments were TentaGel 90 μm diameter polystyrene-based resins functionalized with poly(ethylene glycol) and were purchased from Rapp Polymere, Tübingen, Germany. The reported loadings of these resins are approximately 300 μmol/g. We have also obtained satisfactory fast ^{13}C NMR spectra from compounds bound to Sasrin resin which was obtained from BACHEM Bioscience, Inc., Philidelphia, PA 19104.

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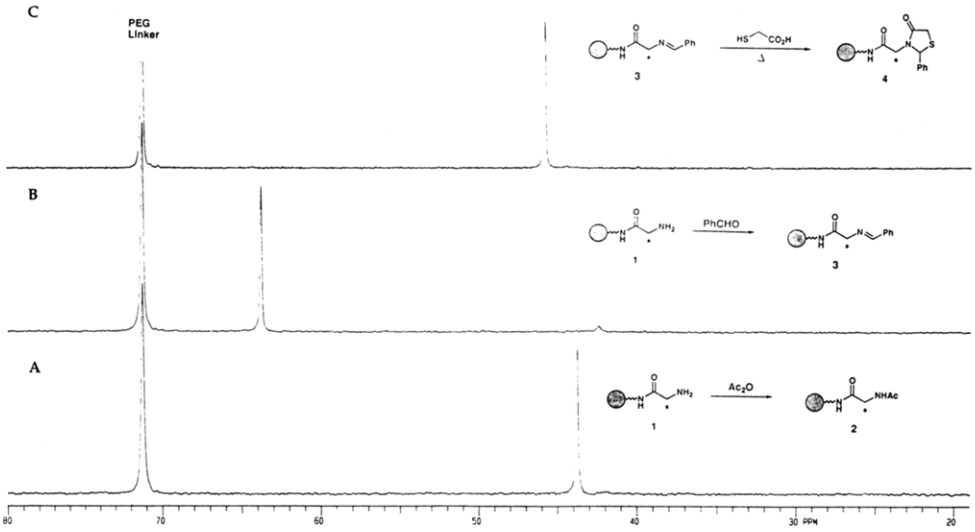

Figure 2. Fast ^{13}C NMR monitoring of thiazolidinone formation. The spectra were acquired in benzene-d_6.

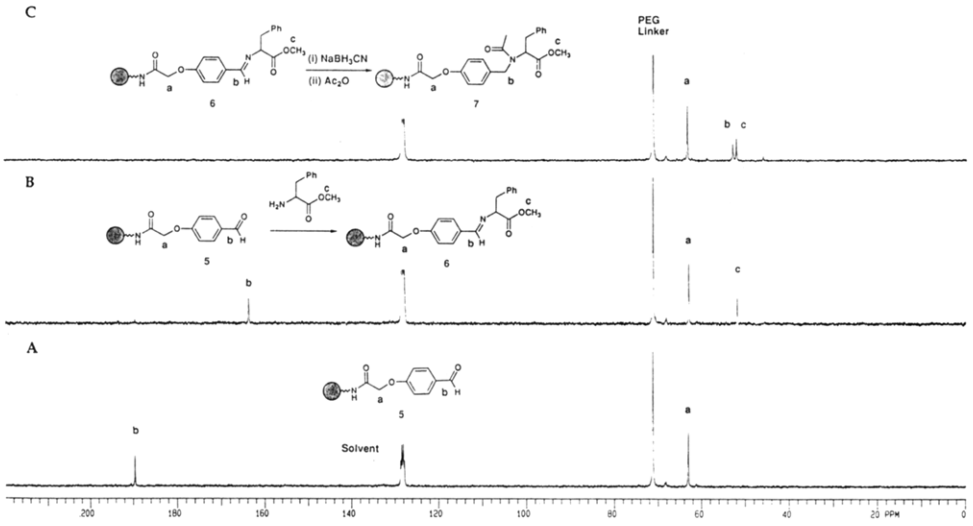

Figure 3. Fast ^{13}C NMR monitoring of a stepwise reductive amination sequence. Labels a–c indicate ^{13}C enriched centers. The spectra were acquired in benzene-d_6.

is a particularly useful support for obtaining ^{13}C NMR spectra.[2g] While workers in this field have noted that the swelling of the resin and the degree of crosslinking are critical to the quality of the spectra obtained, we have found that no preswelling for TentaGel is necessary. The resin is suspended in the NMR solvent of choice and administered to the tube insert for immediate observation. The quality of the spectra does not change upon extended periods of resin exposure to solvents.

The use of ^{13}C enriched building blocks enables one to obtain high-quality spectra at the center of interest without interference from unenriched peaks and solvent. We have consistently obtained meaningful data with minimum background noise in as few as 64 transients. Typical turnaround times from the preparation of the resin to the recovery of the beads for subsequent reactions is from 15 to 30 min with most of the time involved with the drying of the resin.[7] As can be seen by the representative examples presented in Figures 2 and 3, the enriched center need not be directly involved in the reaction that is monitored since changes in hybridization as well as electron density can be easily detected.

In the first experiment (Figure 2), the stepwise formation of a 4-thiazolidinone[8] was examined by fast ^{13}C monitoring.[9] Thus, α-^{13}C-labeled Fmoc-glycine was coupled to the resin and deprotected with piperidine to afford the free amine. A portion of this resin was capped with acetic anhydride to provide the amine standard (Figure 2A). A second portion was treated with benzaldehyde in THF containing molecular sieves followed by washing and capping to form imine **3** which showed a dramatic chemical shift change from 44 to 64 ppm for the labeled carbon (Figure 2B). The absence of any **2** indicated that complete conversion to imine **3** had taken place. Condensation of resin-bound imine **3** with mer-

(7) Typically, the resin is washed three to four times with the reaction solvent and then several times with absolute ethanol and/or diethyl ether. The resin is filtered and dried under a high vacuum for 10–15 min. A slurry of the resin is then prepared in the NMR solvent and the slurry is placed into the insert via syringe. The ^{13}C NMR spectra are obtained on a 300 MHz instrument with 64 transients using the customary solution phase ^{13}C NMR acquisition parameters. The resin may be extracted from the insert by syringe. For studies using benzene-d_6 as the NMR solvent, the spectra are referenced to resonance for the ethylene glycol linker of the resin at δ 71.27 ppm downfield from TMS.

(8) Work performed at Affymax concerning the synthesis of thiazolidinone combinatorial libraries on solid support will be reported in due course.

(9) For the described experiments, the resin was rinsed and dried between each reaction step prior to ^{13}C NMR observation. We have also successfully monitored a reductive amination process directly in a tube insert without isolation and drying of the resin.

captoacetic acid in THF containing molecular sieves followed by washing and capping gave 4-thiazolidinone **4**, which showed a shift from 64 to 46 ppm for the labeled carbon atom (Figure 2C). The observed chemical shifts of the resin-bound compounds were in excellent agreement with the *solution* phase ^{13}C NMR spectra of the soluble analogs.

The second experiment involved a stepwise reductive amination and subsequent acylation reaction sequence (Figure 3).[9] The support-bound benzaldehyde derivative **5**[10] which contained an internal ^{13}C reference (Figure 3A) was treated with phenylalanine methyl ester to form imine **6** as indicated by the shift of the aldehyde ^{13}C resonance at 190 ppm to that of an imine at 164 ppm (Figure 3B). Additionally, a labeled methyl ester was signified by the appearance of a new peak at 52 ppm. Imine **6** was reduced with sodium cyanoborohydride (2 × 5 equiv) in ethanol to afford an intermediate amine in which only two ^{13}C-enriched peaks were observed, one at 64 ppm for carbon a and one at 53 ppm. Treatment of the resin-bound compound with acetic anhydride resolved the peak at 53 ppm into two peaks at 53 and 52 ppm (Figure 3C). Gel phase APT experiments indicated that the peak at 52 ppm corresponded to a methyl group resonance.

As with unenriched gel phase NMR experiments, other routine ^{13}C NMR methods can be run on the enriched compounds. We have been able to integrate the signals from multiply labeled compounds on resin to ascertain the extent of a given reaction[11] and have run two dimensional C−H correlation spectra.[2f] The presence of an additional labeled carbon as an internal reference also facilitates assessment of the progress of a reaction without the need to accurately weigh the amount of resin used. For example, in the 4-thiazolidinone work, we have used a labeled internal reference which was attached to a resin *before* a cleavable linker was attached in order to monitor the extent of release of a labeled material from a solid support.[8]

In summary, the use of fast ^{13}C NMR spectroscopy has been invaluable for the monitoring of a variety of transformations on resins. This method should be of broad applicability and utility to those interested in the optimization of small molecule chemistries on solid supports.[12]

(10) Resin **5** is prepared by the cesium carbonate-promoted *O*-alkylation of 4-hydroxybenzaldehyde (^{13}C labeled at the carbonyl) with ^{13}C-labeled bromoacetamide resin. The aldehyde is prepared by the quenching of the anion generated from 4-bromo-*O*-(*tert*-butyldiphenylsilyl)-phenol with ^{13}C-labeled DMF followed by fluoride-mediated removal of the silyl group.

(11) We have performed T_1 experiments on resin-bound compounds and determined that most carbon centers relax within 500 ms or less. Thus, we are able to integrate the resonance peaks of the labeled centers in order to judge the extent of various reactions. These results are in good correlation with those of Bayer et al. (ref 2g).

(12) The authors wish to thank George Detre for helpful discussions regarding the use of ^{13}C NMR for the quantification of resonances from compounds on solid support and for assistance in T_1 and C−H correlation experiments. We would like to thank Khehyong Ngu for the preparation of some of the labeled amino acid building blocks.