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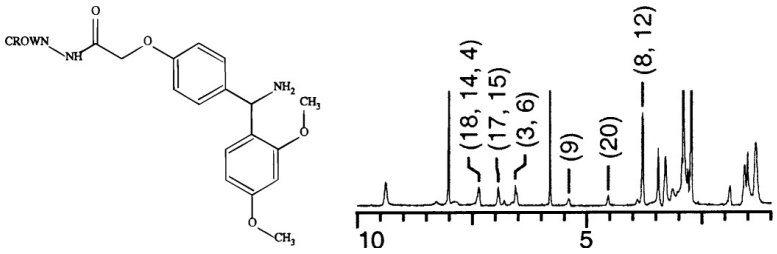

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Using One- and Two-Dimensional NMR Techniques to Characterize Reaction Products Bound to Chiron SynPhase Crowns

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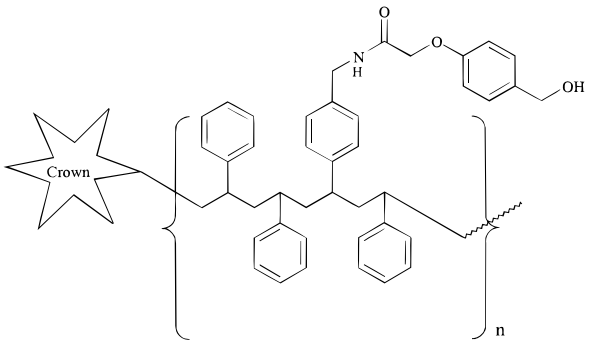
Solid-phase MAS techniques have proved to be very useful in characterizing compounds bound to resin; however, little has been reported on using NMR to characterize compounds attached to Chiron SynPhase crowns. We have used proton, carbon, and COSY spectra obtained with a Varian Nano•nmr probe to characterize products from a published reaction sequence attached to MD (methacrylic acid/dimethylacrylamide copolymer) crowns. We have also performed solvent surveys to determine the best solvents for acquiring spectra of materials bound to both MD and PS (polystyrene) crowns.

Introduction

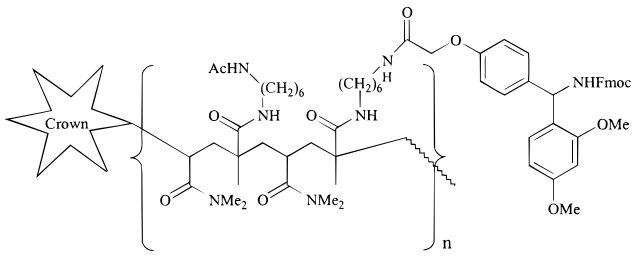
In recent years, solid-phase synthesis has become a routine and indispensable tool for organic and medicinal chemistry. Rapid biological screening methodology has created the need for larger numbers of compounds to screen, thereby providing an incentive to improve parallel synthesis and combinatorial chemistry techniques. While solid-phase synthesis has made it possible to rapidly produce large chemical libraries, it has provided numerous challenges to analytical chemistry. IR, MS, NMR, and LC techniques have long been used by synthetic chemists to optimize reaction conditions, determine the extent of completion of a reaction, evaluate the success or failure of new reactions, and determine the identity of a reaction product or impurity. It is often necessary to repeat these analyses and reoptimize reactions when performing them on the solid phase because the solution-phase conditions are not always directly transferable.

All of the standard analytical techniques are available to the solid-phase chemist when the material is cleaved from the solid support; however, the typically harsh conditions for cleavage can often introduce new impurities and side products. This can make it difficult to identify the source of the impurity. Also, cleaving material after each step is inefficient, especially if enough material for NMR analysis is desired. For these reasons, it is advantageous to directly analyze the bound reaction products, for which both IR[1,2] and NMR[3] have proven to be useful analytical techniques. Gallop and Fitch have recently reviewed the use of these methodologies for resin-bound compounds.[4]

Although most references for solid-phase chemistry and analytical techniques refer to resins, there are other useful solid supports. In particular, Chiron has developed the SynPhase crowns which have many advantages.[5,6] The crowns have polymer chains to which the compounds of interest are linked, much like resins; however, the ends of the polymer chains are grafted at one end to a small, dimensionally stable polyethylene or polypropylene rodlike structure. Because most of the reactive sites on resin are inside the bead, the reaction solvent must efficiently swell the bead for efficient transfer of reagents to occur. In contrast, the reactive sites of crowns are on the surface, leading to improved reaction kinetics and a wide range of solvent compatibility.[7,8] These same surface characteristics facilitate the removal of byproducts and unreacted reagents. Finally, the crowns are physically more robust than resin, and the individual crowns easily provide the spatial separation necessary for parallel synthesis. Examples of the uses of



SynPhase-PS crown with an HMP (4-(Hydroxymethyl)phenoxyacetamido) linker



SynPhase-MD crown with a RAM (Rink amide) linker

Figure 1. Comparison of the HMP and RAM linkers.

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Table 1. Spectral Quality[a] for Two Types of Crowns in Various Solvents

solvent	SynPhase-MD crowns with RAM linker	SynPhase-PS crowns with HMP linker
acetone-d_6	D	C
acetonitrile-d_3	E	E
benzene-d_6	E	D
$CDCl_3$	C	D
D_2O	E	E
dichloroethane-d_4	E	C
CD_2Cl_2	D	D
DMF-d_7	A	D
DMSO-d_6	B	D
dioxane-d_8	E	D
CD_3OD	C	E
N-methyl-pyrrolidinone-d_9	B	B
nitromethane-d_3	E	E
pyridine-d_5	B	A
THF-d_8	C	B

[a] Spectral quality on a scale of A−E, where A = this solvent generates the best spectrum for this resin, B = acceptable spectra, C = fair, D = poor, E = no visible resin signal. The scale only compares the performance of different solvents for each crown individually and does not accurately compare the two crowns against one another.

crowns in synthesis include the optimization of the synthesis of 4-aminoproline analogues,[9] large-scale synthesis of peptides,[7] and the syntheses of trisubstituted guanidines,[10] quinazolines,[11] and spiropiperidinehydantoins.[12]

Despite the importance and usefulness of crowns for solid-phase synthesis, to our knowledge there has only been one report of obtaining NMR spectra of materials attached to crowns, in contrast to the numerous papers on the NMR of resins. Chin and co-workers described in a communication a method whereby an entire crown was placed in a large liquid-capable rotor with DMF-d_7.[13] The data were obtained in a wide-bore NMR equipped with a 7 mm MAS probe, and a CPMG sequence was used to suppress the broader signals from the crown. The method yielded reasonable spectra; however, it is somewhat impractical as few organic chemistry NMR labs are equipped with expensive wide-bore magnets and 7 mm MAS probes. In addition, Keifer and co-workers performed a careful study and showed that for resin samples the Nano•nmr probes, which are relatively inexpensive and fit a standard bore magnet, give better quality spectra than a conventional solids CP/MAS probe.[14] Herein we report a series of NMR techniques for following reactions on crowns using Nano•nmr probes.

Results and Discussion

Sample Preparation and Solvent Selection. Because the entire crown will not fit into a probe rotor, it was necessary to determine a suitable method of sample preparation. Unlike resin, the reactive sites are grafted on the surface of the crown, so the interior polyethylene base is not of interest for NMR and only contributes to unwanted background signal. Thus we found it best to shave small, thin pieces off the surface of the crown with a razor blade and place these pieces in the rotor. Although crown surfaces are inhomogeneous, we have learned through parallel studies using IR microscopy that a small shaving of crown material is representative of the whole sample, as these inhomogeneities are microscopic as compared to the size of the shaving.[15] All crowns used were SynPhase PS or MD crowns, I-Series, with loading ranges of 8−12 μmol per crown for PS and 5−10 μmol for the MD crowns.[16] For ^{1}H spectra, we shaved ∼1−1.5 mg (∼0.5 μmol of compound)[17] of crown material and ∼9 mg (∼3.0 μmol of compound) of crown material for ^{13}C spectra. The rotor was then filled with the deuterated solvent of choice. In general, it took approximately 1−2 min to prepare samples for proton spectra and 5 min to prepare samples for carbon spectra. All NMR spectra were acquired on a Varian UnityPlus 500 MHz spectrometer and processed with VNMR 6.1a software.

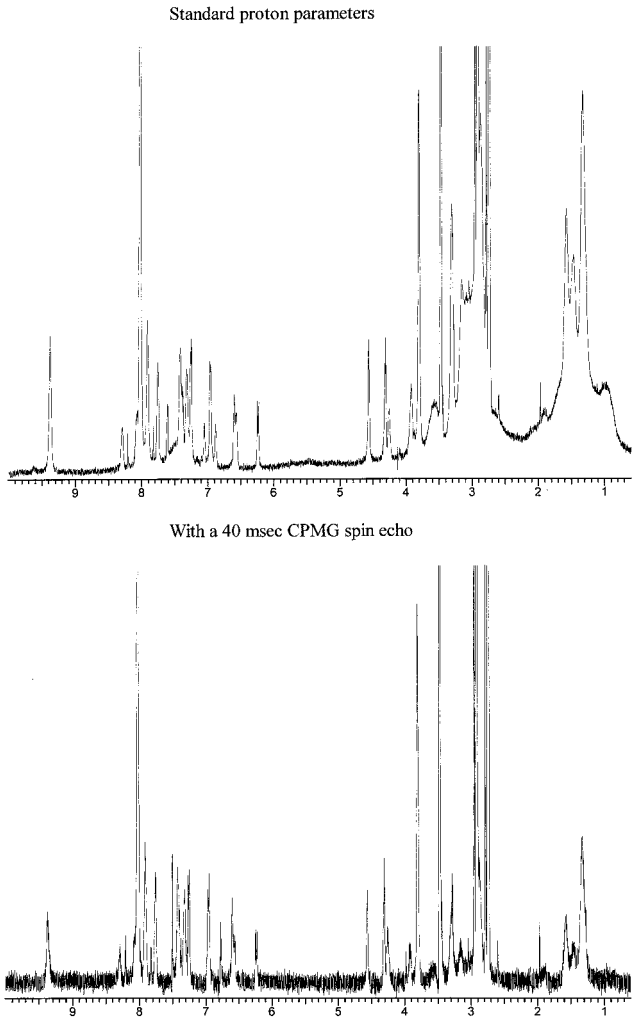
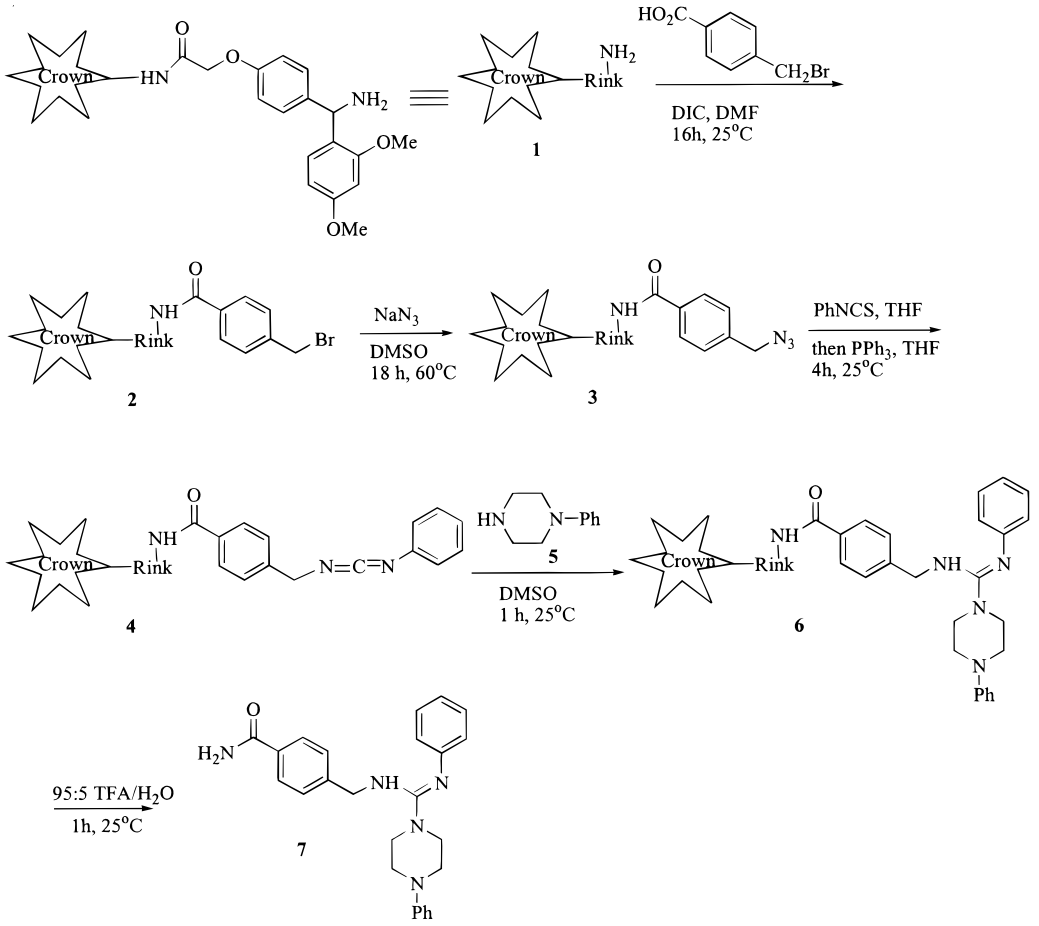


Figure 2. Comparison of spectra with and without CPMG suppression of broad lines.

Keifer performed a solvent survey for various resin samples and found the spectral quality to be highly dependent on solvent.[18] Chin et al. prepared all their crown samples with DMF-d_7, without providing further details, so we wished to study the effects of solvent on the quality of the crown spectra obtained. We chose a wide variety of solvents and performed the studies with two different types of crowns, the SynPhase-PS and the SynPhase-MD, with different linkers. These crowns have different grafted reaction surfaces (see Figure 1) and might be expected to produce different results as the surface of the PS crown is hydrophobic and

Scheme 1



the MD crown is hydrophilic. Table 1 summarizes the results of the solvent survey. In general, we found that DMF-d_7 gave the best results for the MD crowns, however, DMSO-d_6 also yielded acceptable spectra if solvent cost is an issue. For the PS crowns, pyridine-d_5 and THF-d_8 were the best solvent choices. It is interesting that the quality of the crown spectra shows such a strong solvent dependence. Resins have vastly different swelling properties in different solvents, and the better quality NMR spectra are generally obtained in solvents which swell the resin well;[18] however, crowns do not show these large differences in swelling. It is possible that the quality of NMR spectra obtained for crown-linked materials is dependent on how well the grafted material is solvated. The observation that the hydrophilic MD crowns give reasonable spectra in methanol-d_4 in contrast to the hydrophobic PS crowns supports this theory. Because of this observation, we were also concerned that the best solvent choice may vary with the compound attached to the solid support, so we repeated the solvent survey with a number of different compounds attached to the solid support with varying hydrophobicities and functional groups. Although the absolute quality (i.e. line widths) of the NMR spectra obtained did vary in these studies, the *best* solvent choice did not and the overall trend of spectral quality vs solvent shown in Table 1 remained constant.

One-Dimensional Proton Techniques. Chin and coworkers reported the usage of a CPMG pulse sequence to suppress the broad background signals from the crown, and we also found this method to be very useful for the MD crowns. Figure 2 shows the spectrum of an MD crown with the commercially available Rink amide linker acquired with standard proton parameters and a 40 ms CPMG spin−echo. These spectra also illustrate the sensitivity of the Nano•nmr probe as they were acquired with 64 transients on a single crown shaving, which corresponds to ~0.2 μmol of compound. This method has been less useful suppressing the polystyrene signals of the PS, possibly because the line widths of the signals of interest are broader, approaching that of the background. The broader line widths of the PS crowns may be due to the absence of the long hexamethylenediamine linker of the MD crowns (see Figure 1) and/or nonaveraged magnetic susceptibility differences from the polystyrene.[19]

To illustrate the utility of MAS NMR for following reaction sequences, we chose a recently published sequence for the solid-phase synthesis of trisubstituted guanidines, as shown in Scheme 1.[10]

Briefly, SynPhase MD crowns with the Rink amide linker were coupled with α-bromo-*p*-toluic acid with DIC in DMF (although in the original synthesis HOBt was used in the coupling reaction,[10] we have since discovered that HOBt is not necessary). The bromide was treated with NaN_3 to provide the azide, which was reacted with phenyl isothiocyanate and triphenylphosphine to give the carbodiimide **4**. The carbodiimide was reacted with amine **5** to yield the final guanidine product, which was cleaved from the crown with 95:5 trifluoroacetic acid/water and analyzed. After each reaction step, samples were prepared for proton NMR as

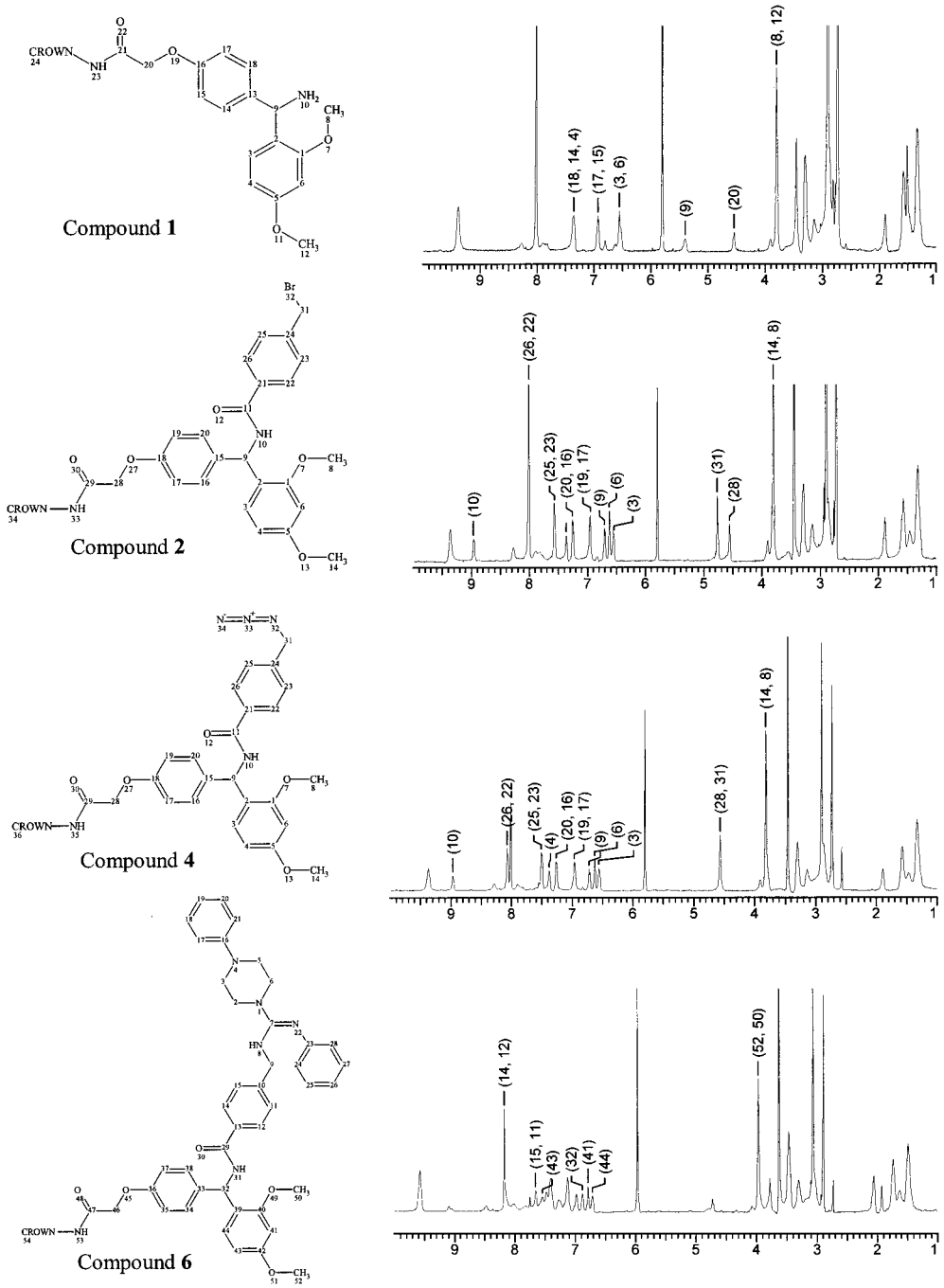


Figure 3. CPMG spectra of the reaction products.

described above and spectra were obtained both with and without a 40 ms CPMG spin−echo. Figure 3 shows the CPMG data with key resonances labeled. The spectra indicate that each reaction is proceeding cleanly to a product with the expected new proton resonances.

Because the grafted reaction surface material and linker contain many exchangeable NH protons, we attempted to perform a deuterium exchange experiment to simplify the spectrum. Methanol-d_4 was chosen as the exchange solvent because the MD crowns give reasonably well-resolved spectra in this solvent as opposed to D_2O. A small amount (∼2−3 μL) of DMF-d_7 was removed from the rotor of the previously prepared sample, and the rotor was refilled with methanol-d_4. Figure 4 shows the both initial spectrum and the spectrum 3 h after addition of the methanol-d_4. In general, we found the kinetics of the exchange to be slow. However, the exchange did eventually go to completion with only the aromatic protons and residual DMF (8.2 ppm) from washings remaining in the downfield region of the spectrum.

One-Dimensional Carbon Techniques. In some cases it may be easier to follow reactions by carbon NMR because the proton signals of interest may occur in a region of the spectrum crowded with linker resonances or the transformation may not add or significantly shift the proton resonances. Thus we wished to test our ability to obtain carbon spectra of crowns. We found it necessary to shave as much surface material off a single crown to acquire interpretable carbon spectra. The amount of material used essentially filled the

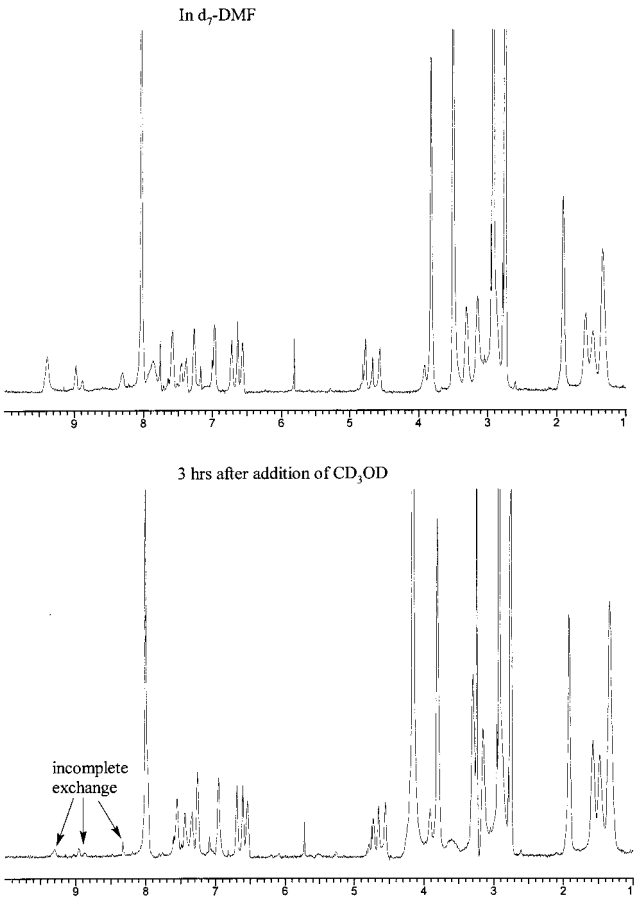


Figure 4. Deuterium exchange with compound **2**.

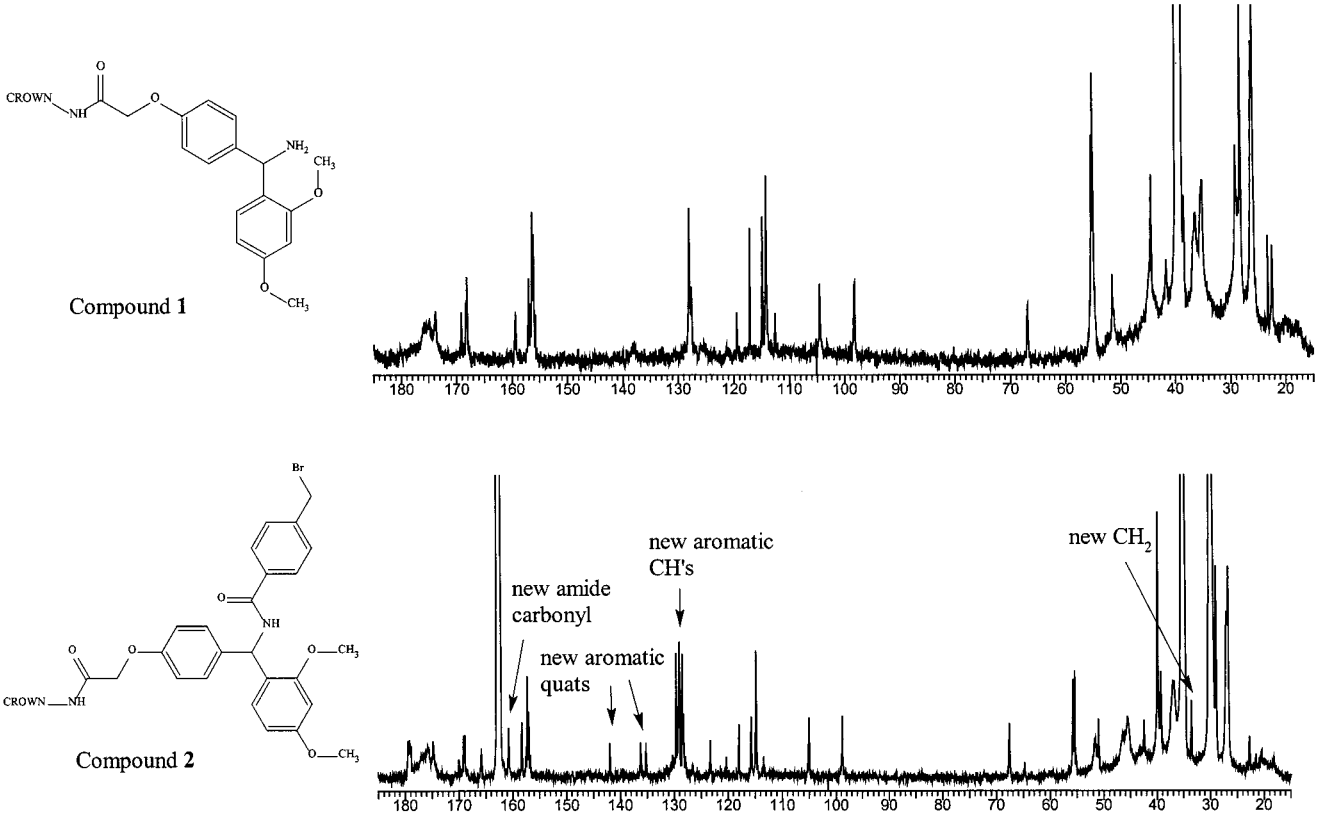


Figure 5. Carbon spectra of the starting amine and bromide product.

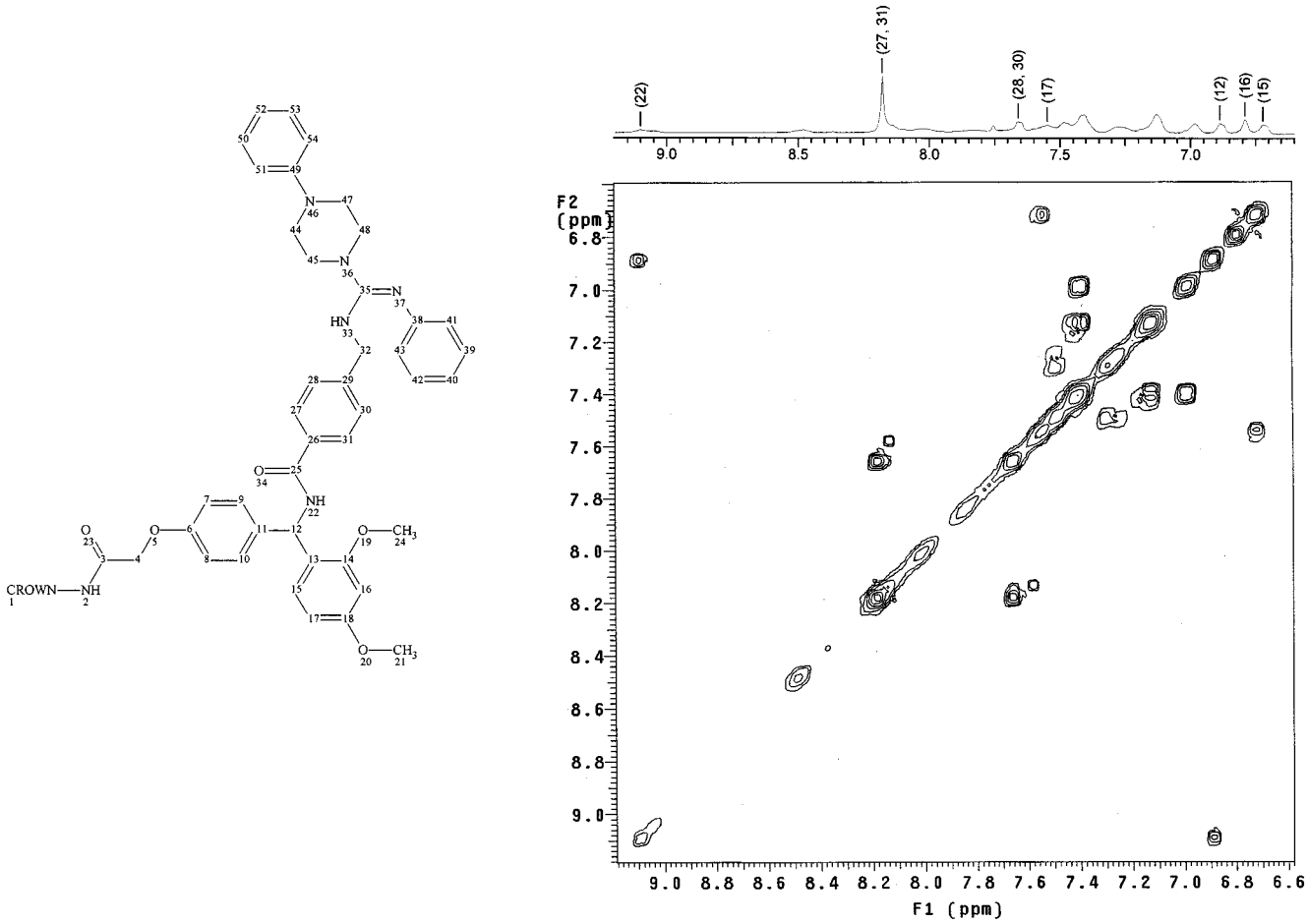


Figure 6. COSY of compound **6**.

cavity of the rotor with only enough solvent to obtain lock. Even so, it was necessary to acquire for 12 h to obtain spectra with a reasonable signal-to-noise ratio. Examples of the starting amine and the bromide carbon spectra are shown in Figure 5 with key resonances labeled; these spectra are interpretable and show that the desired reaction products are forming. We were also able to obtain DEPT spectra to aid in the assignment of the carbon peaks.

Two-Dimensional Techniques. Finally, we wished to acquire a COSY spectrum of the final reaction product before cleavage to confirm the assignments. Figure 6 shows COSY spectra of the final product attached to the crown. As can been seen, the MAS COSY contains reasonably sharp peaks and is quite interpretable. Most other common 2D experiments have been reported with resins,[14] including inverse experiments such as HMQC,[20,21] so these experiments should also work with crown samples.

Conclusions

We have developed simple techniques for obtaining NMR spectra of crowns with Varian Nano•nmr MAS probes. We have also shown that many of the common NMR experiments performed by organic chemists to analyze reaction products work well, and we have used these experiments to follow a reaction sequence on crowns. These techniques should prove invaluable for developing new chemistry on crowns.

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