Report

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Reports

Improved Procedure for Routine ^{13}C NMR Analysis of Merrifield Resin Bound Molecules

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Merrifield's solid-phase synthesis (SPS) concept, first developed for biopolymer synthesis, has spread in every field where organic synthesis is involved. Many laboratories and companies focused on the development of technologies and chemistry suitable to SPS. This resulted in the spectacular outburst of automated high-throughput synthesis and combinatorial chemistry, which profoundly changed the approach for new drugs, new catalysts, or new materials discovery.[1] However, most laboratories have not yet integrated SPS as a common tool.

One of the major reasons for this is the lack of available routine analytical methods for the characterization of compounds bound to the resin. In classical chemistry numerous powerful spectroscopic techniques, especially NMR, are widespread and readily available. For solid-phase chemistry the appropriate analytical methods like mass spectrometry[2] and magic angle spinning (MAS) NMR[3] are often not on-site or not devoted to routine analyses.

Available classical NMR techniques proved to be useless for the analysis of resin bound molecules or restricted to very specific problems. For instance, ^{19}F and ^{31}P NMR[4] and the use of ^{13}C and ^{15}N enriched substrates[5] enabled the successful monitoring of reactions performed on solid phase.

FT-IR is thus the most handy analytical tool in SPS; however, it remains limited in terms of structural discrimination.[6] Therefore, in the optimization phase of reactions procedures, it is usually required for each experiment to release the product from the solid support to be able to analyze it. This is a tedious and time-consuming process.

In this paper we report our investigations to determine NMR conditions and parameters that allow the recording of ^{13}C spectra of molecules bound to Merrifield resin. We obtained satisfactory resolution in 7.5 min using a conventional 300 MHz spectrometer.

Previously reported results showed that it is possible to record ^{13}C spectra of nonenriched molecules bound to Merrifield resin with a classical spectrometer. The utilization of highly functionalized (63% substituted, 4.7 mequiv/g)[7] or important quantities of resins (0.75 g in 2.5 mL of deuterated solvent) in a 10 or a 25 mm o.d. NMR tube is described.[8] Other studies showed that it is possible to carry out ^{13}C NMR analysis in 5 mm o.d. tubes.[9] However, neither data-processing parameters nor acquisition/relaxation delays were optimized. This resulted in a very long experiment time (up to 58 h).[10] Thus none of the reported conditions are compatible with routine analysis of classical Merrifield resin.

We carried out a systematic study by varying different parameters to determine the best compromise between the increase of the signal, the line broadening, and the total experiment time. We recorded a series of ^{13}C spectra using a classical Merrifield resin (1 mequiv/g, 1% cross-linked, 200−400 mesh) derivatized with triethyleneglycol spacer linked to 3,3-dimethyl acrylate ester. Among many molecules tested, dimethylacrylate appeared to be a representative substrate to illustrate the behavior of different NMR signals (C═O, HC═, ═C, CH_3). The NMR samples were prepared using 130 mg of resin suspended in 0.7 mL of solvent. The resin which tended to float was kept down in the tube by a small piece of cotton.[11]

We first set a total experiment time of 7.5 min, and we shortened all delays. As a consequence, the number of scans (ns) in the given experiment time increased. In a preliminary set of experiments we varied the pulse length (pl) from 10 to 90°, the acquisition time (aq) from 150 down to 25 ms, and the relaxation delay (dl) from 600 down to 10 ms.

The optimum balance between the enhancement of the signal to noise ratio and the decrease of the resolution is obtained with a pl of 30°, an aq of 75 ms, and a dl of 100 ms. Increasing or decreasing any of these parameters had as a consequence a loss in resolution (peak broadening) or a lowering of the signal to noise ratio. The receiver gain (rg) was set to be the value automatically determined by the spectrometer (rga).

Compared to spectra obtained with described parameters, the use of the above-mentioned conditions resulted in a dramatic improvement (spectra 1 and 2, Figure 1). The signal of the carbon nuclei of the methyl groups and the alkene's monosubstituted carbon could be visualized without any doubt.

An additional improvement was observed when using mixtures of 20% v/v of deuterated solvent (benzene-d_6, dioxan-d_8, dimethylsulfoxide-d_6) in carbon tetrachloride (spectrum 3, Figure 1). CCl_4 was chosen because its carbon nucleus is known to have a long relaxation time and the choice of the deuterated cosolvent is dependent upon what is bound to the resin. This resulted in a decrease of the intensity of the solvent.

In another study we evaluated the effect of paramagnetic relaxation agents: $Cr(acac)_3$, $Cu(acac)_2$, $Cu(tmhd)_2$, $Mn(acac)_2$, $Gd(acac)_3$. In solution, paramagnetic relaxation

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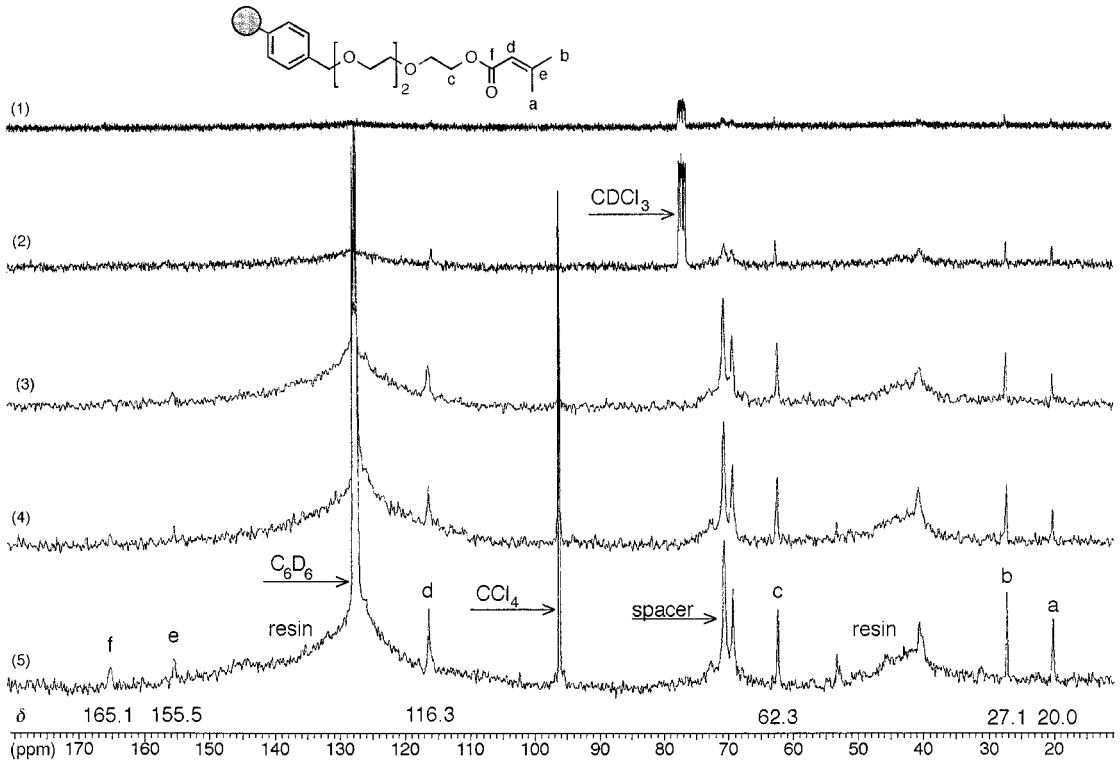

Figure 1. ^{13}C NMR spectra were recorded at 75.5 MHz using 130 mg of resin swelled in 0.7 mL of solvent in a 5 mm o.d. tube, within a total experiment time of 7.5 min (spectra 1−4) or 20 min (spectrum 5) with the following conditions: (1) $CDCl_3$, dl = 500 ms, pl = 5.5 μs (≡ 30°), aq = 688 ms; (2) $CDCl_3$, dl = 100 ms, pl = 5.5 μs, aq = 75 ms; (3) CCl_4/C_6D_6:80/20 (v/v), dl = 100 ms, pl = 5.5 μs, aq = 75 ms; (4) CCl_4/C_6D_6:80/20 (v/v), $[Cu(acac)_2] = 5 \times 10^{-3}$ M, dl = 100 ms, pl = 5.5 μs, aq = 75 ms; (5) CCl_4/C_6D_6:80/20 (v/v), dl = 400 ms, pl = 5.5 μs, aq = 75 ms. Data were acquired on 32 768 (spectrum 1) or 3568 points (spectra 2−5), zero-filled to 65 536 (spectrum 1) or 8192 points (spectra 2−5), and multiplied by a $\pi/2$ shifted square sine-bell window function before Fourier transform. Spectra were plotted keeping the background noise signal constant.

agents are used to increase the intensity of quaternary ^{13}C nuclei signals which suffer from long spin lattice relaxation time T_1. The addition of increasing amounts of these agents resulted in two opposite effects: the shortening of the relaxation times T_1 and the increase in peak width.[12]

We varied the concentration of the relaxation agent from 10^{-3} to 10^{-1} M. The optimum was found to be 5×10^{-3} M. At this concentration the slight peak broadening due to the relaxation reagent is compensated by the increase of the signal, allowing the quaternary carbon from the double bond to be distinguished (spectrum 4, Figure 1). At higher concentration the peak broadening became the predominant effect. To recover the starting material, the relaxation agent can be eliminated by washing the resin with methylene chloride.

For the visualization of carbonyl or quaternary carbon without addition of relaxation agent, a slightly different sequence can be used (spectrum 5, Figure 1). Lengthening the dl to 400 ms and the experiment time to 20 min resulted in the appearance of the carbonyl signal in conditions still compatible with routine analysis.

In an additional study the free induction decays (FIDs) from spectra 1, 3, and 5 were reprocessed to improve the sensitivity with fixed weighting function and exponential decay. The spectra were plotted as 1′, 3′, and 5′ in Figure 2. Whereas in spectrum 1 no signal can be clearly attributed, in spectrum 1′ the methyl signals (a and b) as well as the sp2 carbon (d) can be attributed without any doubt. However, applying the same mathematical treatment to FIDs from spectra 3 and 5 did not lead to such significant changes, even though a slight improvement for carbon (e) in spectrum 3′ is observed.

The reproducibility of the analysis was assessed by preparing three tubes with an identical resin sample. The recorded spectra were very similar with, however, some slight differences in the relative peak broadening of some carbon. The broadening of the methyl signal from spectrum 3 to 4 is a representative example of such variations. These slight differences are unavoidable considering the inhomogeneity of the tube.

This analytical method might not allow one to determine the exact structure of complex products bound to Merrifield resin, but it represents a powerful complement to IR for routine nondestructive analysis. ^{13}C allows one to probe specific structural modifications or to use groups such as methyl, *tert*-butyl, methoxy, trimethylsilyl, and *tert*-butyldimethylsilyl as tracers for reactions or side reactions monitoring.

In summary, we found NMR parameters (ns = 2500, pl = 30°, aq = 75 ms, dl = 100 ms, rg = rga) and conditions (mixture of CCl_4 and 20% of deuterated solvent) that allow one to record the ^{13}C spectra of molecules bound to Merrifield resin via a triethyleneglycol spacer with a classical 300 MHz spectrometer in only 7.5 min acquisition time. For these optimized conditions, different FID treatments gave comparable results. The above-mentioned ^{13}C NMR condi-

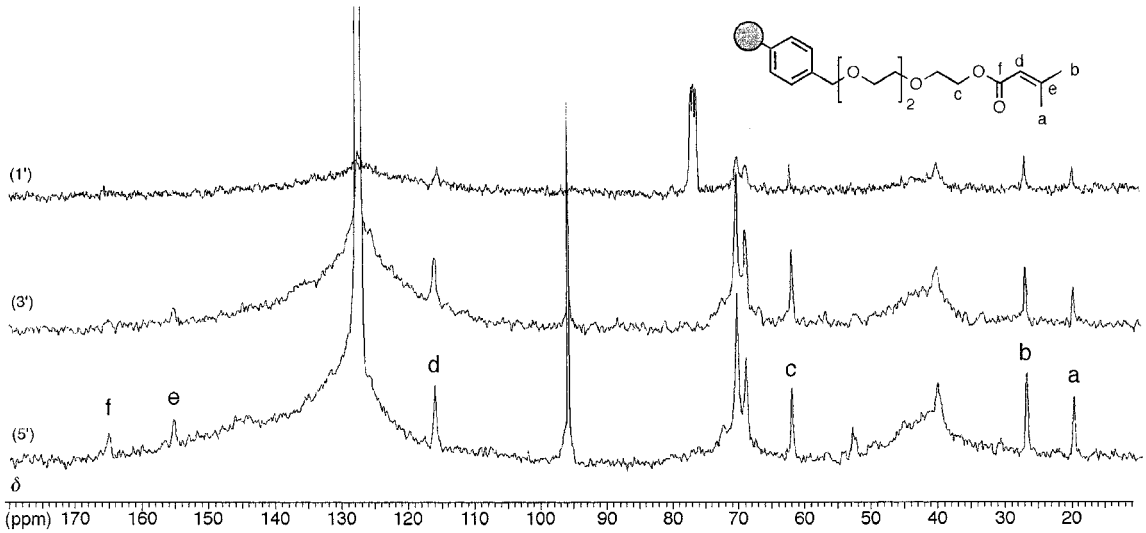

Figure 2. Spectra 1′, 3′, and 5′ were obtained from the same FIDs as spectra 1, 3, and 5 (Figure 1), respectively. Data were zero-filled and multiplied by both an exponential decay (LB = 10.00 Hz) and a $\pi/2$ shifted square sine-bell window function.

tions are presently used in our laboratory for routine analyses and enable us to assess complex chemical transformations on solid support by direct structural analysis or by using appropriate tracers.

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(11) The NMR samples were prepared as follows: resins were dried under vacuum at room temperature until constant weight. A sample (130 mg) was put into a 5 mm o.d. NMR tube. A freshly prepared solution (0.7 mL) of 20% C_6D_6 in CCl_4 (purity 99.9%) was added, after filtration, into the tube. Homogeneous slurries were obtained by swirling the tubes with a vortex shaker and allowing them to equilibrate with the solvent for 30 min. ^{13}C NMR spectra were recorded at 75.5 MHz, with broad band decoupling, on a Brüker DPX 300 spectrometer, equipped with a $^1H/^{13}C$ dual 5 mm probe. The ^2H resonance of C_6D_6 was used for the field frequency lock. Tubes were spinning at 25 Hz, and temperature was set to 300 K. Peak positions were referenced to the center $CDCl_3$ peak taken as 77.00 ppm or to the CCl_4 peak taken as 96.10 ppm. Data were acquired and processed as specified in Figures 1 and 2.

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