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Influence of Resin Structure, Tether Length, and Solvent upon the High-Resolution ^{1}H NMR Spectra of Solid-Phase-Synthesis Resins

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Solid-phase synthesis (SPS) is playing an ever increasing role in organic and combinatorial chemistries;[1] however, the lack of good analytical techniques for samples still bound to the resin has been a long-standing problem.[2] Since it was first shown that high-resolution ^{1}H NMR spectra of samples still bound to SPS resins can be obtained by using a Nanoprobe,[3] we have observed that the NMR line widths obtained can vary significantly from sample to sample. We have conducted a systematic study of nine different SPS resins and seven different solvents to evaluate the ability of various combinations to generate ^{1}H NMR spectra having narrow line widths. The results show that the choice of both the SPS resin and the solvent can radically affect the NMR spectral quality.

A resin sample may have up to four distinct sections: the polymer matrix, a variable-length tether, a linker, and an organic moiety. For this study we kept the moiety as "constant" as possible and varied both the length of the tether and the composition of the polymer matrix. Nine commercially-available resins, all bearing a protected aspartic acid group, were used: Fmoc-Asp(OtBu)-Wang (**1**), Fmoc-Asp(OtBu) NovaSyn KA 100 (**2**), Fmoc-Asp(OtBu)-NovaSyn PA 500 (**3**), Fmoc-Asp(OtBu)-NovaSyn TGA (**4**), Fmoc-Asp(OtBu)-NovaSyn TGT (**5**), Boc-Asp(OcHx)-Merrifield (**6**), Boc-Asp(OBzl)-PAM (**7**), Boc-Asp(OcHx)-MBHA (**8**), and Fmoc-Asp(Rink amide MBHA)-OtBu (**9**).[4] The solvents used were the following: CD_2Cl_2 (**10**), DMF-d_7 (**11**), DMSO-d_6 (**12**), acetone-d_6 (**13**), benzene-d_6 (**14**), CD_3OD (**15**), and D_2O (**16**).

It has previously been shown that the quality of the NMR spectra of SPS resins is determined primarily by the achievable line widths[5] and that sensitivity is not a limiting factor (since the Nanoprobe can obtain NMR spectra on even a single 90 μm bead).[6] This suggested that the ^{1}H NMR line width of a resonance *from the organic moiety* could serve as a measure of spectral quality; the readily recognizable *tert*-butyl singlet near 1.4 ppm was chosen. Its line width, in addition to a measure of the overall quality of the NMR spectrum, is summarized in Table 1 for each different resin−solvent combination. The extent of resin swelling upon addition of solvent is also reported to demonstrate that it has little direct correlation to spectral quality.

Table 1 shows that the resin structure is the dominant factor influencing ^{1}H line widths, with the solvent playing a secondary role. Tentagel resins (**4** and **5**), which have the longest tethers and the most mobile moieties, provided the narrowest line widths and the best overall data in the widest variety of solvents. Wang (**1**), PA-500 (**3**), and to a lesser extent Rink MBHA (**9**) resins, which have only short tether/linker structures and hence more restricted mobility, sometimes produced suitable spectra but clearly required a proper choice of solvent. The more conventional polystyrene resins (**6**−**8**) have no tethers and little regional mobility, and never generated high-quality spectra. The more solid-state Kieselguhr-based resin (**2**) produced more numerous and intense spinning sidebands which required higher spin rates (>3 kHz) to produce good spectra.[7]

Table 1. Spectral Quality,[a] Line Width,[b] and Solvent Swelling Factor[c] for each Combination of Resin (1−9) and Solvent (10−16)

	10	11	12	13	14	15	16
1	A[d]	B	E	B	B	F	F
	10.0	8.7	nm	10.2	13.9	nm	nm
	×3	×4	×1	×2	×4	×1	×1
2	C	C	C	E	E	B	E
	14.9	14.3	16.0	nm	nm	20.6	nm
	×2	×1	×1	×1	×1	×1	×1
3	B	A[d]	A	C	E	B	E
	13.0	11.6	11.5	28.8	nm	23.4	nm
	×3	×1	×1	×1	×1	×2	×1
4	A[d]	A[d]	A	B	C	B	E
	8.4	10.0	8.8	12.5	25.1	13.2	nm
	×3	×1.25	×2	×1	×1.5	×1.75	×1
5	A	A	A[d]	B	C	B	E
	4.1	5.9	5.8	11.0	19.4	3.8	nm
	×4	×4	×3	×3	×3	×2	×2
6	D[d]	D	F	D	D	F	F
	12.6	17.3	nm	16.9	19.4	nm	nm
	×4	×3	×2	×2.5	×4.5	×1	×1
7	D[d]	D	D	D	D	E	F
	12.1	10.7	16.8	13.5	15.1	nm	nm
	×3	×3	×1	×1.5	×1	×1	×1
8	D	D	F	D	E	F	F
	25.7	27.1	16.8	28.0	26.2	nm	nm
	×4	×4	×1	×2	×6	×1	×1
9	B	A[d]	E	D	E	F	F
	12.5	8.0	24	16	nm	nm	nm
	×4	×3	×1.5	×2	×3	×1	×1

[a] Spectral quality on a scale of A−F, where A = this solvent generates the best spectrum for this resin, B = acceptable spectra, C = fair, D = poor, E = bad spectra, F = no visible resin signal. The scale only compares the performance of different solvents for any one resin and does not accurately compare different resins against each other. [b] Line width (in Hz; ± 0.12 Hz) of either the *β-O-tert*-butyl singlet (resins **1**−**5**, **9**) or the *N*-α-Boc-*tert*-butyl singlet (resins **6**−**8**), both located near 1.4 ppm. Line widths are measured under high-throughput conditions and do not necessarily represent the minimum attainable line width (nm = not measurable). [c] Volume increase induced by solvent swelling (measured qualitatively on 10 μL aliquots of dry resin in a 40 μL cavity; ×2 indicates a doubling in resin volume; ×1 indicates no swelling). [d] A presaturation or one-pulse spectrum of this resin−solvent combination is shown in Figure 1; one-pulse spectra of all combinations are shown in the supporting information.

Each resin also exhibited a characteristic set of multiple broad resonances arising from the polymer matrix and the tether. While presaturation can reduce these background signals,[5] the residual signals still complicate spectral assignments. Presaturation was particularly effective for the Tentagel resins; however, in that saturation of the relatively narrow methylene singlet at 3.5 ppm

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(1) (a) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49(48)*, 11065−11133. (b) Madden, D.; Krchnák, V.; Lebl, M. *Perspect. Drug Discovery Des.* **1994**, *2*, 269−285.

(2) Lebl, M., Krchnák, V.; Sepetov, N. F.; Seligmann, B.; Strop, P.; Felder, S.; Lam, K. S. *Biopolymers* **1995**, *37*, 177−198.

(3) Fitch, W. L.; Detre, G.; Holmes, C. P.; Shoolery, J. N.; Keifer, P. A. *J. Org. Chem.* **1994**, *59*, 7955−6.

(4) All obtained from Calbiochem-Novabiochem (La Jolla, CA).

(5) Keifer, P. A.; Baltusis, L.; Rice, D. M.; Tymiak, A. A.; Shoolery, J. N. *J. Magn. Reson., Ser. A,* **1996**, in press.

(6) Sarkar, S. K.; Garigipati, R. S.; Adams, J. L.; Keifer, P. A. *J. Am. Chem. Soc.* **1996**, in press.

(7) All other resins produced roughly equivalent spectra at any spin rate > 1 kHz, although (< 0.5%) sidebands of particularly large peaks could often be observed. All spectra shown here used spin rates of approximately 2 kHz.

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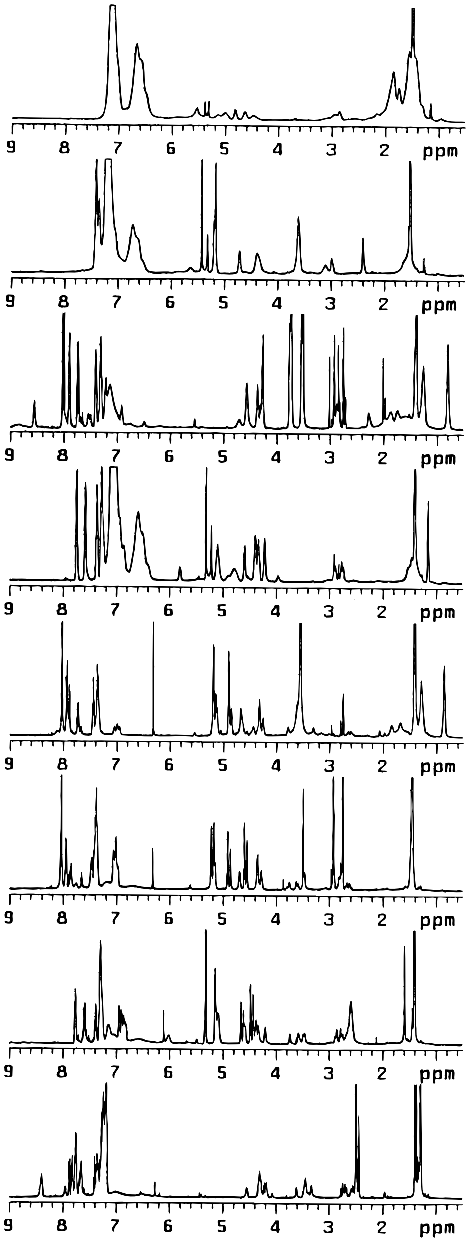

Figure 1. 500 MHz 1D spectra of (top-to-bottom): (a) Resin **6** (Merrifield) in CD_2Cl_2 (no presaturation); (b) Resin **7** (PAM) in CD_2Cl_2 (presaturated at 1.92 ppm); (c) Resin **9** (Rink) in DMF-d_7 (presaturated at 6.59 ppm); (d) Resin **1** (Wang) in CD_2Cl_2 (presaturated at 1.81 ppm); (e) Resin **3** (PA 500) in DMF-d_7 (presaturated at 2.89 ppm); (f) Resin **4** (TGA) in DMF-d_7 (presaturated at 3.61 ppm); (g) Resin **4** (TGA) in CD_2Cl_2 (presaturated at 3.50 ppm); (h) Resin **5** (TGT) in DMSO-d_6 (presaturated at 3.47 ppm).

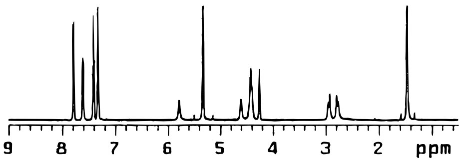

Figure 2. A 500 MHz solution-state ^{1}H NMR spectrum of N-FMOC-L-aspartic acid β-*tert*-butyl ester (Sigma) dissolved in CD_2Cl_2.

reduced all of the resonances arising from the resin and tether, including the broad aromatic resonances.[8] This phenonenon was considerably less effective in all other resins. This indicates that not only the location of all background signals, but also their susceptibility to presaturation, should influence the choice of resin.

The solvents DMF, CD_2Cl_2, and DMSO (in order) typically generated the best NMR data. The solvent of choice was CD_2Cl_2 since its volatility facilitated sample recovery, but DMF sometimes provided better spectra (i.e. resins **3** and **9**). DMSO was sometimes useful (resin **3**) and sometimes not (resins **1** and **9**). Whereas acetone, benzene, and methanol were all intermediate in performance, D_2O was always unsuitable.

The ^{1}H NMR spectra for several resin–solvent combinations are shown in Figure 1. Figure 1a shows that a Merrifield resin has numerous broad background signals in both the aromatic and aliphatic regions of the spectrum and provides an overall poor-quality spectrum. Figures 1, parts b–d, show that even after presaturation of Pam (**7**), Rink (**9**), and Wang (**1**) resins, some of the broad background peaks still remain (i.e. between 6–8 and 1–2 ppm). Presaturation of PA 500 resin (**3**; Figure 1e) almost completely removed its large broad background signals at 3.6, 3.0, and 1.7 ppm. Presaturation spectra of Tentagel resins (**4** or **5**) in either DMF, CD_2Cl_2, or DMSO (Figures 1, parts f–h) produced spectra having the narrowest resonances (see Table 1) and the smallest polymer background signals. These spectra can be compared to the solution-state spectrum of free FMOC-Asp-β-(O-*tert*-butyl)aspartic acid dissolved in CD_2Cl_2 (Figure 2). The line width of the *tert*-butyl resonance in Figure 2 (2.2 Hz) compares quite favorably with the line width observed for the *tert*-butyl group of resin **5** in CD_2Cl_2 (4.1 Hz). This verifies that the spectral resolution of bound samples can approach that of solution-state NMR.

The line widths reported in Table 1 are limited by the molecular mobility of the organic moiety (which is controlled by the length of the tether) and not by probe construction as would be typical for conventional magic-angle spinning (MAS) probes.[9] The importance of using high-resolution Nanoprobe MAS technology for the acquisition of ^{1}H NMR spectra of SPS resins, especially at high field, has previously been documented, as has the influence of spin rate, temperature, and experimental techniques.[5] This communication has now shown how various combinations of resins and solvents will also influence spectral quality. While high-field ^{1}H NMR spectra are the most demanding test of high-resolution MAS technology, the conclusions reached here are equally valid for ^{13}C NMR spectroscopy.

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Supporting Information Available: A complete collection of 63 one-dimensional ^{1}H NMR spectra acquired without presaturation for compounds **1**–**9** in solvents **10**–**16** as well as experimental conditions and definitions (8 pages).

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[8] This is presumably due to spin diffusion throughout the regions of the resin which have very different correlation times from the organic moeity.

[9] The ^{1}H NMR lineshape ($CHCl_3$ in acetone-d_6) was measured several times during this study; a typical result was 1.2, 12.9, and 16.5 Hz (50%, 0.55%, and 0.11% levels, respectively).