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Quality control of solid-phase synthesis: ^{13}C PST/MAS NMR analysis on non-destructed SynPhase lantern

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Abstract—Pulse saturation transfer (PST)/MAS was highly effective for enhancing a magic angle spinning (MAS) ^{13}C NMR of the inter-mobile region of polymer supported organic compounds. Direct monitoring of solid-phase synthesis on non-destructed SynPhase lantern was demonstrated using a 7 mm probe on the ^{13}C PST/MAS NMR study.

Combinatorial chemistry, the science of molecular diversity, has provided dramatic results from rational approaches to exploring biologically active compounds toward pharmaceuticals and medicine.[1,2] Compared with conventional solid-phase syntheses performed on resins typically less than 100 μm in size and with a loading ratio of 1–10 nmol per bead, recent advance using macroscopic supports makes split-pool syntheses much easier. Among the macroscopic supports, 'SynPhase lantern' shows remarkable advantages such as (i) easy handling, (ii) easy 'split and mix' synthesis using a colored stem, and (iii) cost effectiveness.[3] In addition, the high loading ratio per lantern is also fascinating for further experimentation using the resulting stock solutions after releasing the diverged compounds from the lantern support. Due to these advantages, the SynPhase lanterns are now being used worldwide.

While a solid-phase combinatorial approach facilitates the synthesis of large numbers of compounds, the analysis of the chemical transformation on each solid support is arduous compared with that of solution-phase synthesis. The following two reports on the application of magic angle spinning (MAS) NMR are noteworthy for analyzing such macroscopic solid supports. The first report operated ^{1}H MAS NMR of a crown, so-called prototype lantern, for the whole body of the crown using a 7 mm MAS probe.[4] The second one focused on the analysis of the new platform of SynPhase lantern itself.[5] In the latter, high-resolution (HR) MAS NMR of the lantern was operated using a 4 mm HRMAS nanoprobe after cutting the lantern into pieces. Because in the combinatorial stepwise synthesis, especially in the split-pool synthesis, non-destructive analyses of the macroscopic supports are highly desired to do monitoring through several reaction steps, here, we present a useful ^{13}C NMR analysis of the whole SynPhase lantern without breaking it into pieces.[6]

The present study initially tested several experimental modes of MAS NMR to acquire a satisfactory ^{13}C NMR spectrum of the backbone amide linker (BAL) anchored to the SynPhase lantern (**1**). The NMR experiments were carried out in a 7 mm sample tube to include the whole body of the lantern (6 mm diameter).[7]

Cross-polarization (CP)/MAS NMR,[8] the most conventional method for solid samples, provided intense background signals of the SynPhase lantern and negligible aromatic aldehyde peaks (Fig. 1A). This was expected, because the enhancement of ^{13}C magnetization in the CP mode is effective only for immobile regions, resulting in detection of the backbone polymer only due to its high content in the sample. Next, a high-resolution (HR) technique as the application of solution phase MAS NMR was applied in the experiment using 7 mm probe for the selective enhancement of ^{13}C magnetization of the mobile regions after swelling in $CDCl_3$

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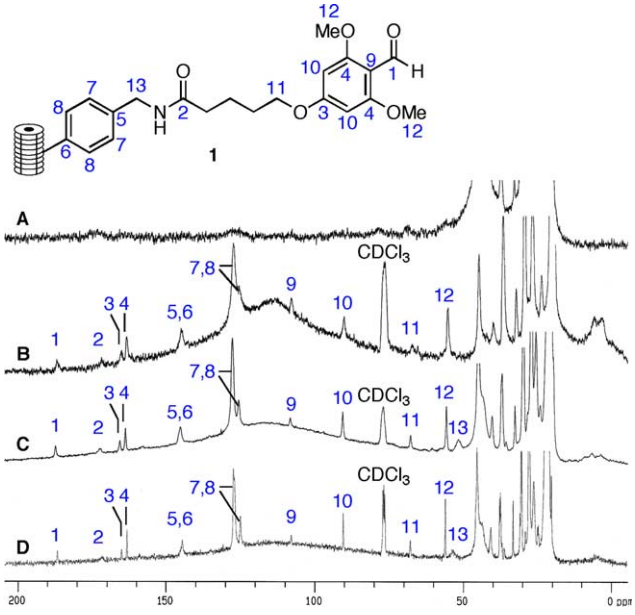

Figure 1. ^{13}C MAS NMR spectra of BAL anchored SynPhase lantern (**1**). **A**: CP/MAS (10,000 accumulation), **B**: HR/MAS (10,000 accumulation), **C**: PST/MAS (10,000 accumulation), and **D**: PST/MAS (1000 accumulation).

(Fig. 1B).[9,10] Although the spectrum of the mobile regions obviously improved by the swelling, the intensity was still not satisfactory even after 10,000 accumulations. Because the HR/MAS harvests only the signals of completely swollen and highly mobile regions, information on the main part, the remaining inter-mobile region, would be lost.

Although the nuclear Overhauser effect (NOE) can be considered effective in enhancing the ^{13}C magnetization in HR/MAS, the proton pre-saturation-derived NOE for the inter-mobile region requires irradiation using a very powerful continuous radio frequency (RF) wave, which would cause serious thermal damage to the sample on some probes. One way to reduce the thermal heating on larger diameter MAS probes (i.e., >5 mm) is to use pulse saturation transfer (PST) protocol.[11] The PST/MAS mode adopts a reasonably small duty cycle (< 0.1%) of 90° comb pulses to achieve the full proton pre-saturation (Fig. 2).[12]

The ca. 10 μs pulse width (PW) and ca. 10 ms pulse interval (PI) of the typical PST/MAS would reduce the

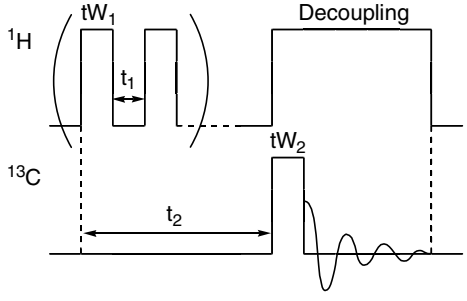

Figure 2. Pulse sequence of pulse saturation transfer method.

exo-thermal energy to 1/1000 using RF pulses. It is noteworthy that similar periods of the longitudinal relaxation time (T_1) and the transverse relaxation time (T_2) in highly mobile target samples do not allow the application of PST protocol in the solution phase NMR. When T_1 has a significantly longer period than that of T_2, as observed in low mobility samples, the PST method is possible.

The PST/MAS operation dramatically improved the signal-to-noise ratio (Fig. 1C). Although the intense background signal due to the base polymer of the SynPhase lantern concealed several peaks in the 0–50 ppm range, chemically important peaks of functionalized carbons are clearly observed in the PST/MAS method. To show the idea of rapid analysis toward quality control in solid synthesis, a spectrum obtained with 1000 accumulations was also provided as Figure 1D.

The advantage of the PST/MAS method for selective identification of the inter-mobile region becomes obvious with an insufficiently swelled lantern sample as shown in Figure 3. With an adequate length for the flexible linker, both HR/MAS and PST/MAS provided useful spectra for analyzing the functionalities attached to lantern **2** (Fig. 3A and B). Nevertheless, for lantern **3**, lack of a flexible linker restricted the mobility of the surface functional groups resulting in poor intensity in HR/MAS. For example, the α-carbon peak of the L-Phe methyl ester was not observed in HR/MAS (Fig. 3C) but was clearly monitored in PST/MAS (Fig. 3D).

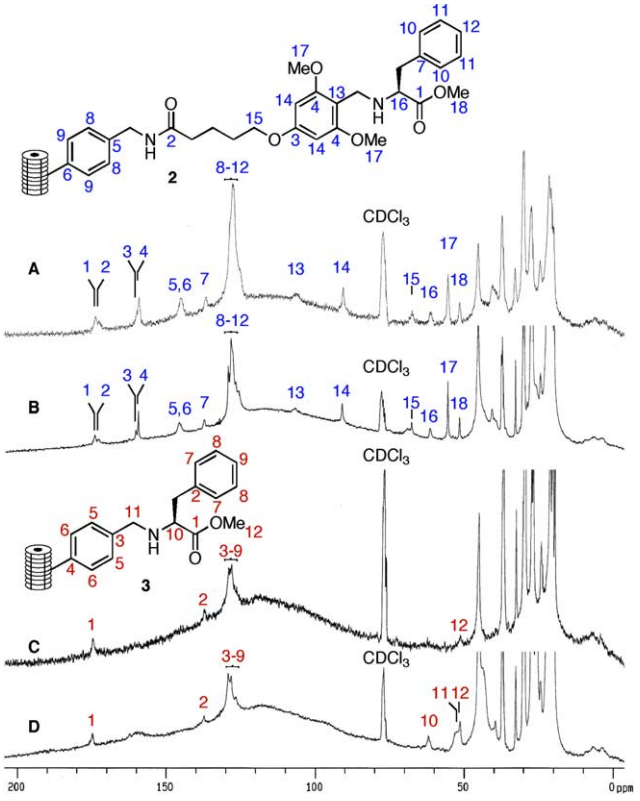

Figure 3. ^{13}C MAS NMR spectra of L-Phe methyl ester connecting SynPhase Lanterns in 10,000 times accumulation. **A**: HR/MAS of **2**, **B**: PST/MAS of **2**, **C**: HR/MAS of **3**, and **D**: PST/MAS of **3**.

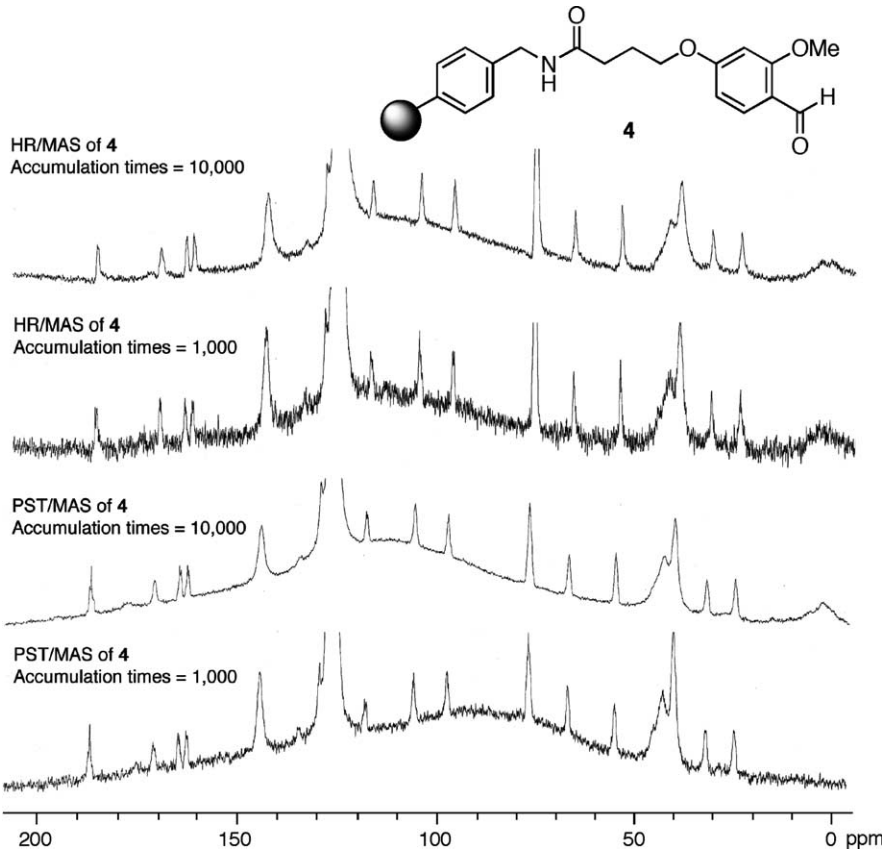

Figure 4. ^{13}C MAS NMR spectra of resin **4**.

It is obvious that PST/MAS offers a versatile technique for analyzing chemically modified lanterns. The advantage of PST/MAS NMR was also evaluated in the analysis of conventional polystyrene beads of 4-(4-formyl-3-methoxyphenoxy)butyryl amino-methylated resin **4** purchased from Novabiochem (Fig. 4).

The newly established PST/MAS analysis for the SynPhase lantern using the 7 mm sample tube was applied for monitoring through sequential synthesis. Figure 5 represents the results when isoxazoline **6** was prepared from **1** in two steps. PST/MAS NMR succeeded in identifying the processes of an oxime formation followed by the nitrile oxide cycloaddition reaction.

Moreover, a 1,3-dipolar cycloaddition of azomethine ylide was examined as shown in Figure 6.[13] The one-pot three component coupling reaction of **1**, phenylalanine methyl ester, and *N*-methylmaleimide proceeded smoothly in toluene at 110 °C, and the conversion on

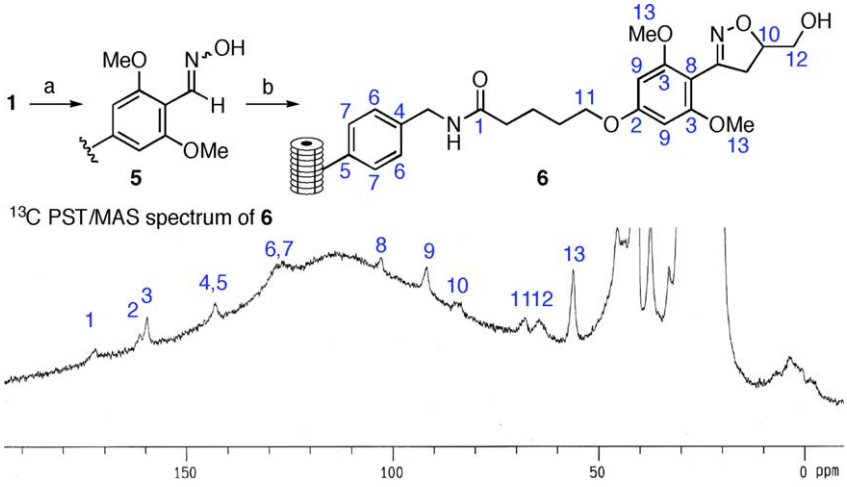

Figure 5. PST/MAS analyses of isoxazoline synthesis on a single SynPhase lantern (DMSO as the swelling solvent). (a) H_2NOH–HCl, Py, EtOH, reflux; (b) NaOCl aq, allyl alcohol, CH_2Cl_2, rt.

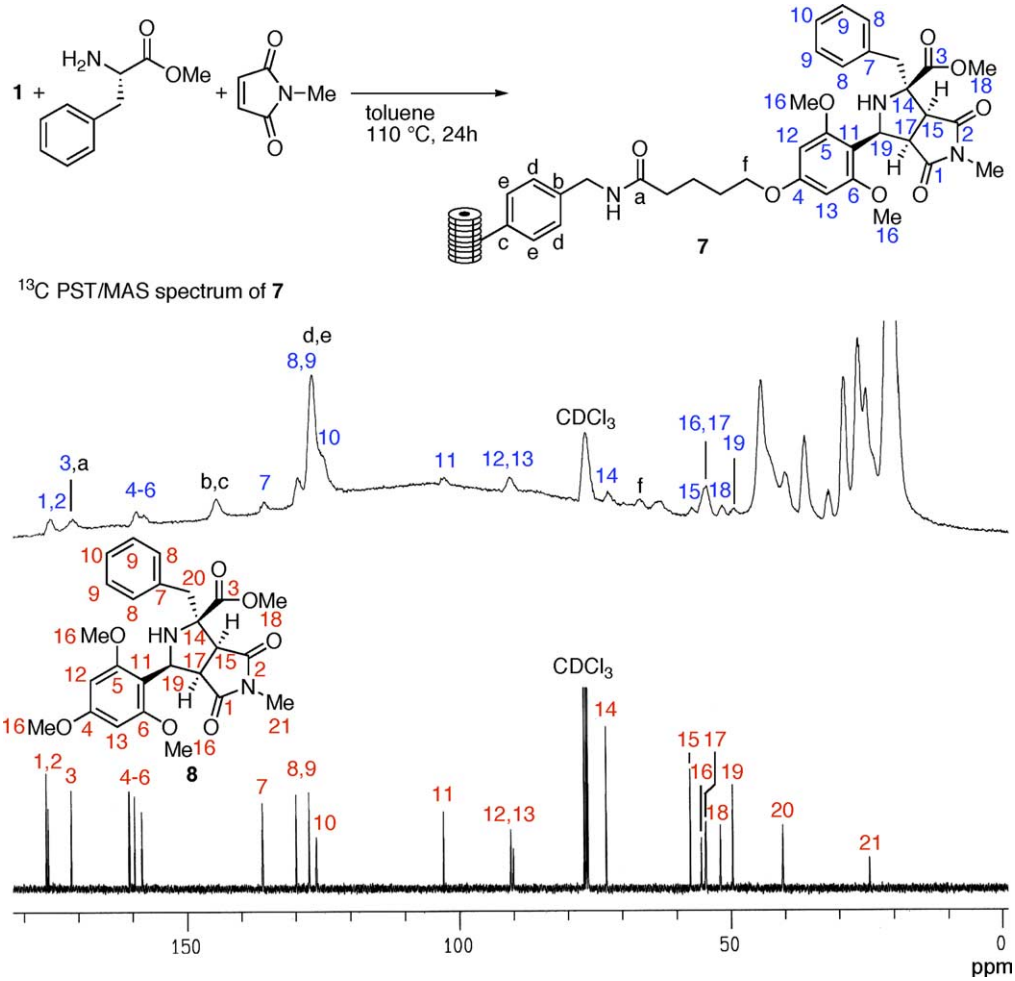

Figure 6. PST/MAS analysis of 1,3-dipolar cycloaddition of azomethine ylide on a single SynPhase lantern ($CDCl_3$ as the swelling solvent).

the lantern was monitored by the ^{13}C PST/MAS NMR. The highly stereoselective construction of the target product **7** was also confirmed by a comparison with the conventional solution-phase ^{13}C NMR spectrum of authentic sample **8**, which was synthesized in solution phase.

In conclusion, we have demonstrated a useful experimental mode of PST for selective signal enhancement in ^{13}C MAS NMR analyses. This enhancement method would have great advantages for analyzing small content in large volume samples. Although the use of the nanoprobe has enabled recent progress in MAS NMR, the development of a new probe with enough volume is apparently fascinating for analyzing versatile macroscopic objects. The implications of the PST/MAS NMR-assisted solid-phase combinatorial synthesis are now being investigated.

Acknowledgements

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