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Magic Angle Spinning NMR: A Valuable tool for Monitoring the Progress of Reactions in Solid Phase Synthesis

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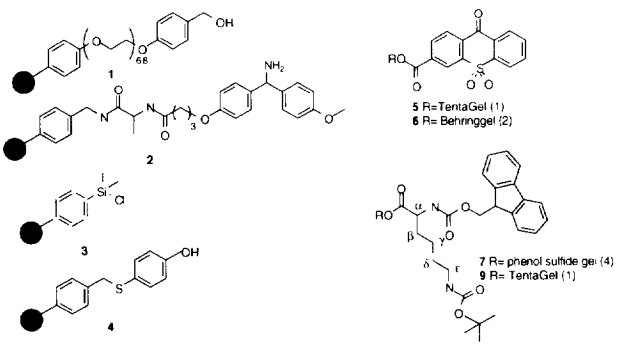
Abstract: Proton NMR-spectra of four different solid supports were obtained using the magic angle spinning Nano NMR probe to evaluate their utility in solid phase chemistry. It was possible to analyse the spectra in respect to structure and quantity. An intramolecular cyclisation reaction on the solid support in the Nano tube could be followed directly.

Combinatorial chemistry is one of the important new methodologies developed for making "chemical libraries" as potential sources of new leads for drug discovery[1]. The solid phase chemistry has been optimized in the areas of peptides and oligonucleotides[2], and been used for several years. However, one problem with the solid phase chemistry is the analysis of the chemical outcome of the synthesis when the compounds are attached to solid support, especially when it is not possible to rely on coloring reaction[3]. The use of NMR techniques for structure determination on solid supports have been recently described[4-9]. Unfortunately these reports mostly discuss the resolution of ^{13}C-NMR on a standard ID-probe and Magic angle spinning (MAS) ^{1}H-NMR on the poly(ethylene glycol)-grafted polystyrene TentaGel resin. One disadvantage with the TentaGel resin is the low loading capacity (0.2-0.3 mmol/g gel) compared to, for example, the Merrifield resin (1.0-4.0 mmol/g gel). We have therefore studied different solid supports for their NMR properties. They exhibit some differences in the NMR spectra, but we found all of them suitable for NMR analysis.

Solid support resin spectra obtained at standard ^{1}H-NMR conditions are dominated by broad signals of the solid support. This would exclude a confident monitoring of solid phase synthesis by liquid NMR spectroscopy. However, it has been recently demonstrated that the Varian Nano NMR probe dramatically improves resolution and sensitivity of proton signals obtained from support bounded compounds[7]. The Nano probe is very sensitive and spins very small liquid NMR samples, *i.e.* 40 µL, around the magic angle to remove bulk magnetic susceptibility discontinuities and line broadening caused by chemical shift anisotropy, which would be a result from restricted molecular motion.

In this study the Nano probe was used on a 600 MHz Varian Unity plus spectrometer to evaluate NMR properties of different solid supports that we use in solid phase synthesis. We tested four different resins: the commercially available TentaGel S PHB and Behring resin, a modified Merrifield resin with phenol sulfide linker and a polystyrene resin with dimethyl silyl chloride linker[10], nonsubstituted and substituted with compounds **5-7,9**, respectively. All resins were swollen in deuturated chloroform prior to NMR analysis. Even with the use of the Nano probe, signals from the solid support are broad and exhibit line widths of more than 120 Hz. As a consequence, all spectra are overlapped with broad signals, of which position and shape are depending on the nature of the linker on the solid support.



It is therefore advisable to decrease those signals to obtain cleaner and simpler spectra to interpret. Some improvement could be achieved by presaturation of an upfield linker signal and/or by a 180-t-90 double pulse experiment. Fig. 1 shows the aromatic part of the spectra from a TentaGel substituted with compound **5**, where the two techniques were applied. Presaturation eliminated spinning artefacts as well as reduced the remaining inner aromatic signals to about half the original height. The signal to noise ratio was improved up to 30%. The double pulse experiment, however, was difficult to optimize for the intensity of the scaffold signals and variation of signal intensities. But in some cases, when studying the progress of compound **4**[10] production for example, the use of double pulse experiments was the only way to obtain the signals from the phenolic protons, which will otherwise be drowned in the broad inner aromatic signals. TentaGel linker signals became narrower along the large poly-(ethylene glycol) side chain and signals from the end of the side chain had line widths of about 5 Hz. This is also reflected in the signals from the support bounded compounds. Where scaffold signals on the TentaGel have a line width of less than 5 Hz, the other solid supports exhibit scaffold signals line widths of around 25 Hz. This was still acceptable for an analysis of the spectra (see Fig 2.). The spectra quality allowed a quantification of the amount of support bounded compounds on the resin relative to linker signals of the outer aromatic linker signals.

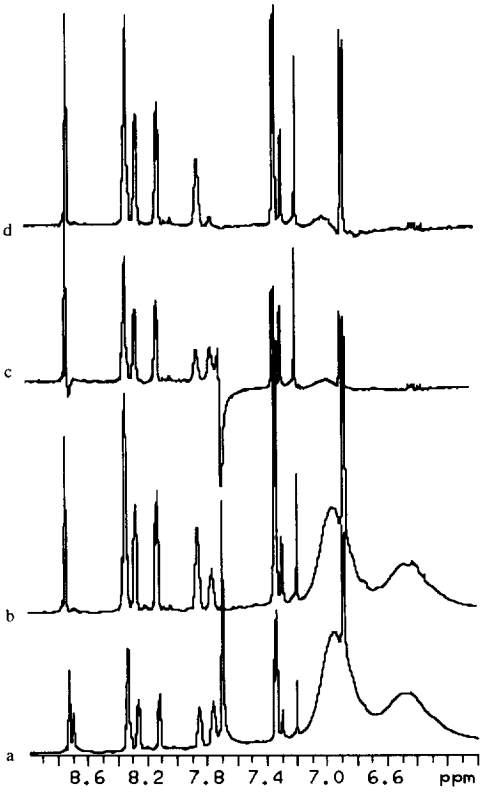


Fig 1. 600 MHz MAS NMR spectra of the aromatic region of compound **5** a, with standard proton NMR conditions b, with presaturation at 3.55 ppm c, with a double-pulse experiment and d, both with presaturation and double-pulse.

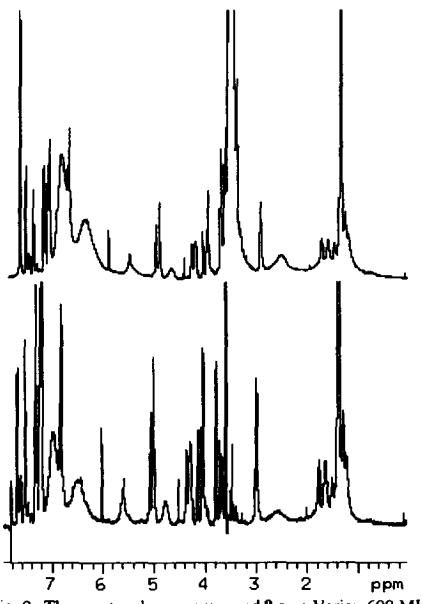


Fig. 2a.The spectra shows compound **9** on a Varian 600 MHz NMR using the Nano NMR probe. The lower spectra is pre-saturated at 3.55 ppm showing that not only the broad peak from the resin becomes smaller but that spinning artefacts disappears.

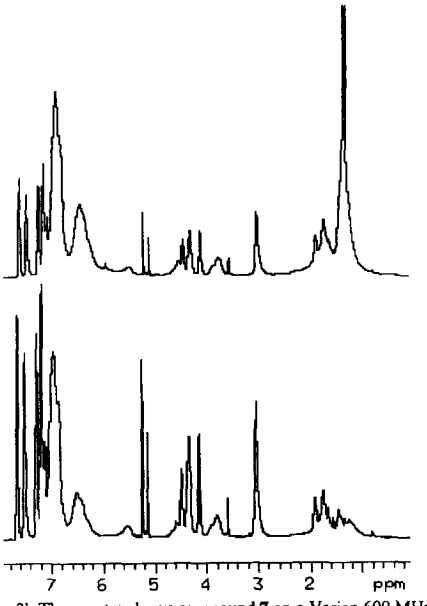


Fig. 2b.The spectra shows compound **7** on a Varian 600 MHz NMR using the Nano NMR probe. The lower spectra is pre-saturated at 1.4 ppm. This decreases the intensity of the aromatic linker signals but also the Boc protecting group disappears.

Assignments of the scaffold signals could be done with the aid of 2D experiments. Figure 3 shows as an example the aromatic region of a TOCSY spectrum of compound **5** substituted on the TentaGel.

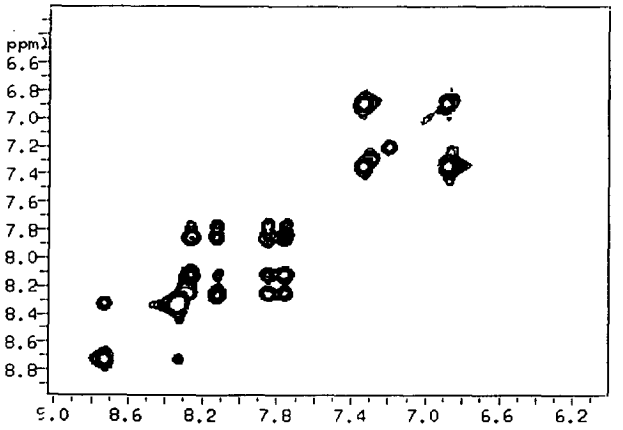


Fig 3. Part of the aromatic region in the TOCSY NMR spectrum for compound **5**

The Merrifield phenol sulfide resin (**4**) was substituted with N-α–Fmoc-N-ε-Boc-Lysine-OH[11] (**7**) (Fig 4). This resin has the property that the compound can be cleaved from the solid support by the presence of a primary amine. After cleavage of the Boc protection group with trifluoroacetic acid and neutralization with triethylamine[12] an intramolecular cyclisation was studied directly inside the Nano tube. The formation of the

cyclic product could be followed by studying the increase of narrow signals in the aromatic region, allowing the direct study of reaction kinetics from solid supports.

In summary, even if the TentaGel has been proven to possess the best NMR properties in respect to line width and spectral purity, other solid supports such as the Merrifield resin and the Behring resin are suitable resins for NMR analysis. Disturbing signals from the solid support could be decreased with presaturation of a signal from the solid support. The spectral quality was sufficient to make quantifications, obtain 2D spectra and follow reactions inside the NMR tube. It is therefore possible to monitor solid phase synthesis on different resins with the Nano NMR probe.

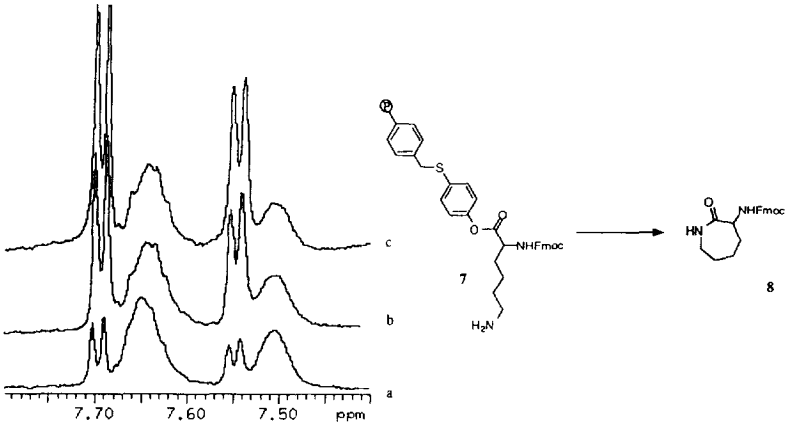


Fig 4. The spectra show peaks from the aromatic region of the Fmoc-protecting group for the spontanous intramolecular transformation **7** to **8** in the 40µL NMR tube. The broad peaks are from compound **7** when still substituted on the solid support while the narrow peaks are from compound **8** free in solution. Spectra were taken after a, 0.5 h. b, 1h. c, 1.5 h.

Reference and notes

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10. Experimental procedure for **3-4**: The silyl ether resin (3) was produced from 1% divinyl-benzene polystyrene and dimethyl-dichloro silane via lithiation of the phenyl groups (Randolph J.,McClure K. F., Danishefsky S. J.*J. Am. Chem. Soc.* **1995**, 117, 5712-5719. The phenol sulfide gel (4) was produced from Merrifield resin and 4-mercapto phenol (Marshall D. L., Liener I. E. *J. Org. Chem.* **1970**, 35, 867-868).
11. Experimental procedure for compound **5-7,9**: Samples 5,6 were produced by coupling between the solid support and 9-Oxo-9H-thioxanthene-2-carboxylic acid 10,10 dioxide with water-soluble carbodiimide hydrochloride as coupling reagent and 4-pyrrolidinopyridine. Samples 7 and 9 were produced by coupling the solid support to Fmoc-Lys(Boc)-OH with water-soluble carbodiimide hydrochloride as coupling reagent and 4- pyrrolidinopyridine.
12. Experimental procedure for compound **8:** Compound **7** was treated with 50% Tfa in CH_2Cl_2 at room temperature for 30 min. The resin was washed several times with CH_2Cl_2 and EtOH. The obtained amin salt was neutralized with Et_3N. The liberated amine then cleaves the compound from the solid support via an intramolecular cyclization.

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