# CP/MAS $^{13}C$ NMR Spectroscopy of Epoxypropyl-Activated Agarose Functionalized with Pyridine Derivatives

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**Introduction.** Recently, the reaction of a series of pyridine derivatives with *n*-butyl glycidyl ether was investigated as a model system for the functionalization of glycidyl ether (2,3-epoxypropyl-) activated agarose (Figure 1).[1] These new materials have been shown to facilitate the selective separation of immunoglobulins and $\alpha$-2-macroglobulin from serum.[2,3] Since in principle the reaction of the pyridine derivatives with the activated agarose may result in two regioisomers with markedly different separation characteristics,[2,3] depending upon whether the reaction occurs via the ring nitrogen or the exocyclic heteroatom,[1] it is important to be able to identify these isomers. For the soluble model compounds it was shown that an unambiguous distinction is possible from the $^{13}C$ NMR chemical shifts, once these have been assigned to the respective isomer.[1] On the basis of the observed high regioselectivity of the model reactions and with some additional evidence from IR spectra, it was then concluded that the corresponding isomers were formed from the activated agarose as well.[1] However, a more direct proof would be desirable. This is not obtainable from solution NMR, since the materials form insoluble, solid gels. An alternative technique might therefore be $^{13}C$ CP/MAS solid-state NMR. We report here the feasibility of this approach.

**Results and Discussion.** The samples studied (Figure 1; R = 2,3-epoxypropyl-activated agarose) were prepared as described in ref 4. $^{13}C$ cross-polarization and magic angle spinning (CP/MAS) NMR spectra were recorded at 75 MHz at room temperature on a Bruker MSL-300 spectrometer. The samples were packed in 4-mm-o.d. zirconium oxide rotors, and the spinning rate was 5.8–7.5 kHz. The $^1H$ and $^{13}C$ radio-frequency field strength during cross polarization and decoupling corresponded to $\gamma B_1/2\pi$ = 56 kHz. A total of 4000–6000 transients was accumulated for each spectrum, with a cross-polarization contact time of 2 ms and a recycle delay between 1.5 and 3 s.

The $^{13}C$ CP/MAS NMR spectra obtained for the solid agarose derivatives as well as for 2,3-epoxypropyl-activated agarose itself are shown in Figure 2 and are compared with the line positions of the solution spectra of model compounds based on *n*-butyl glycidyl ether.[1] The dominant feature in all of the solid-state spectra is the contribution from the agarose carbons. A major double peak around 74 ppm, a smaller peak around 100 ppm, and a shoulder around 60 ppm are quite similar to published

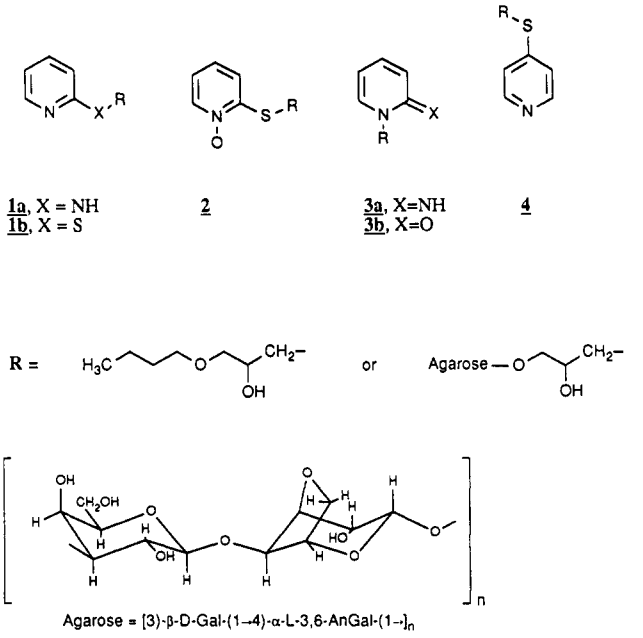


**Figure 1.** Materials studied.

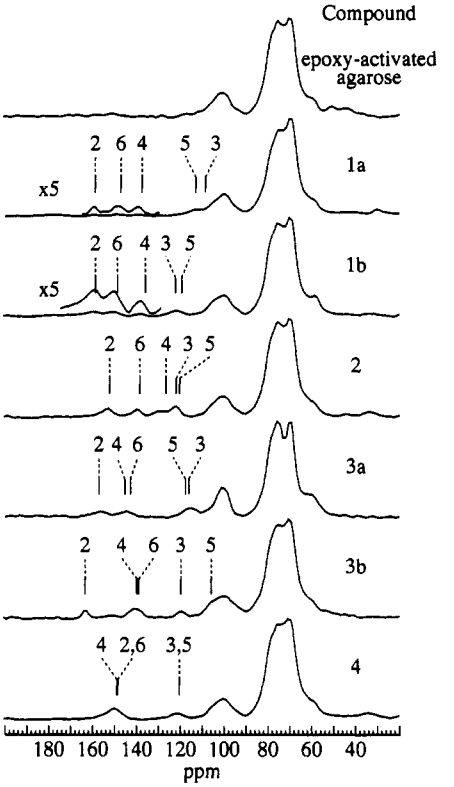


**Figure 2.** 75-MHz CP/MAS $^{13}C$ NMR spectra of 2,3-epoxypropyl-activated agarose functionalized with pyridine derivatives.[4] For comparison the spectrum of epoxy-activated agarose itself is included (top spectrum). Vertical lines indicate spectral line positions of the pyridine ring carbons in the solution spectra of model compounds obtained from *n*-butyl glycidyl ether.[1]

spectra of other amorphous polysaccharides, such as dextran[5] or starches.[6] As is expected for molecules in such noncrystalline gel-type materials, which exhibit a distribution of chemical environments,[7] all spectral lines are fairly broad. Nevertheless, distinct lines are observed for all the pyridine ring carbons in the spectra of the functionalized agarose. All these lines are located close to the positions of the lines in the solution spectra of the corresponding model compound (Figure 2). One particularly

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interesting case is provided by comparison between compounds **1a** and **3a**, both of which are derived from 2-aminopyridine. Depending on the reaction conditions,[1,4] the 2-aminopyridine is attached to the agarose framework via the amino nitrogen (**1a**) or via the ring nitrogen (**3a**). The solid-state $^{13}C$ spectra allow an unambiguous distinction between these two cases.

**Conclusion.** Solid-state CP/MAS $^{13}C$ spectra are a useful tool for the identification of gel-type pyridine derivatives of 2,3-epoxypropyl-activated agarose. The spectra provide additional support to the idea[1] that corresponding compounds are formed when *n*-butyl glycidyl ether or 2,3-epoxypropyl-activated agarose is reacted with pyridine derivatives under identical conditions.

## References and Notes

(1) Gogoll, A.; Oscarsson, S. *Tetrahedron* **1990**, *46*, 2539.
(2) Porath, J.; Oscarsson, S. *J. Makromol. Chem., Macromol. Symp.* **1988**, *17*, 359.
(3) Oscarsson, S.; Porath, J. *Anal. Biochem.* **1989**, *176*, 330.
(4) Oscarsson, S.; Porath, J. *J. Chromatogr.* **1990**, *499*, 235.
(5) Hirai, A.; Ito, T.; Horii, F.; Kitamaru, R.; Kobayashi, K.; Sumitomo, H. *Macromolecules* **1990**, *23*, 1837.
(6) Horii, F.; Hirai, A.; Kitamaru, R. *Macromolecules* **1986**, *19*, 930.
(7) Arnott, S.; Fulmer, A.; Scola, W. E.; Dea, I. C. M.; Moorhouse, R.; Rees, D. A. *J. Mol. Biol.* **1974**, *90*, 269.