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Monitoring of a Three-Step Solid Phase Synthesis Involving a Heck Reaction Using Magic Angle Spinning NMR Spectroscopy

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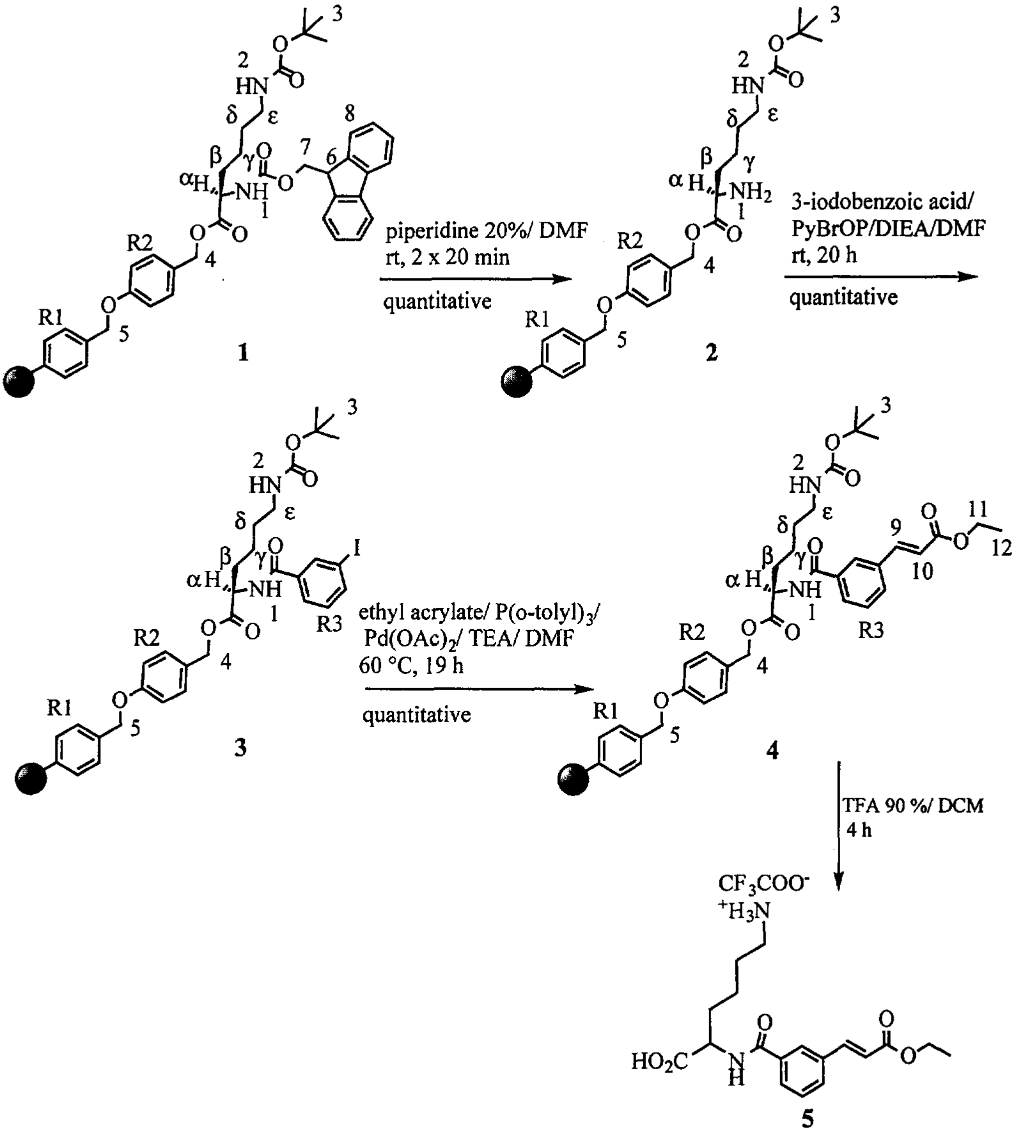
Abstract: MAS NMR technique with standard equipment was used to characterize the intermediate products in a three step reaction scheme leading to disubstituted olefins on solid support. The step-by-step analysis of the reaction presented here demonstrates that the goal of reaction characterization and optimization *in situ* can be obtained in an entirely satisfying manner using conventional MAS NMR. Some of the experimental parameters that should be taken into consideration to obtain workable spectra are examined. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Organic synthesis by solid phase methods is emerging as a powerful tool for the combinatorial and parallel simultaneous multiple synthesis of large arrays of small molecules. An important research topic is therefore the development of straightforward and robust chemical reactions that offer the possibility of exploiting a large variety of building blocks and that can be used in automated synthesis protocols. A second important topic is the development of rapid and reliable protocols for the characterization and optimization of such reactions. Problems of significant losses of both time and material when using the traditional « cleave and analyse » strategies, as well as difficulties encountered due to alteration of fragile products at the cleavage step or to limited solubility of the intermediates have been overcome by Magic Angle Spinning (MAS)[1, 2] NMR techniques of organic molecules while still attached to their solid support[3-7]. Indeed, whereas the standard high resolution NMR of compounds tethered to the insoluble matrix is impractical due to the important line broadening caused by bulk magnetic susceptibility and homonuclear dipolar interactions, spinning the macroscopic sample under an angle of 54.7 ° (the « magic angle ») has overcome some of these limitations, and very promising results have been presented both with conventional solid-state MAS equipment[3, 4] and with specially designed probes[5-7]. However, whereas the latter magnetic-susceptibility-matching probe technology convincingly leads to improved line widths, it is not clear at this point what resolution is needed for routine structure assignment in the framework of reaction characterization and optimization in combinatorial chemistry.

We describe here the use of MAS with standard equipment to characterize the intermediate products in a three step reaction scheme that leads to disubstituted olefines on solid support (Scheme 1).



Scheme 1

The Heck reaction chosen for this purpose has been previously described in solid phase synthesis[8, 9]. This process of generating carbon-carbon bonds presents a great interest for the combinatorial synthesis, as it offers the possibility of exploiting a large variety of building blocks. Moreover, the Heck reaction generally proceeds under very mild conditions, thus being readily amenable to automation. The step-by-step analysis of the reaction presented here demonstrates that the goal of reaction characterization and optimization *in situ* can be obtained in an entirely satisfying manner using conventional MAS NMR. We further examine some of the experimental parameters that should be taken into consideration in order to obtain high quality spectra.

RESULTS AND DISCUSSION

The solvent motion in the polymer network leads to chain expansion observable on a macroscopic scale as a swelling of the bead and is accompanied by an enhanced local mobility of the attached molecules at the microscopic level. As a consequence of the enhanced molecular mobility the various interactions that govern the line width of the NMR signal will be motionally averaged, including the homonuclear dipolar interaction that can broaden proton signals up to values of 20 kHz in a static solid state sample. This motional averaging may be incomplete, due to the anisotropic environment of the solvent molecules in the polymer. The resulting residual broadening due to the local anisotropy can be further reduced by spinning the sample at the magic angle at a spinning rate higher than the non-spinning residual line width. This last condition on the spinning rate can be advantageously exploited to suppress the lines of the more rigid supporting polymer while obtaining a liquid-like spectrum for the more mobile attached molecules. Furthermore, MAS will also eliminate the magnetic-susceptibility broadening caused by the difference in magnetic susceptibility between the polymer and the pure solvent, combined with the irregular shape of the solvent/polymer interface[3-7]. The contribution of magnetic-susceptibility discontinuities within the materials used for the probe construction, however, will not be eliminated by MAS, and can only be eliminated by a careful probe design.

The theoretical considerations mentioned above lead to the conclusion that the resin sample preparation will be crucial to obtain workable data. Recent work along these lines came to the conclusion that the resin structure is the dominant factor influencing the line widths, with the solvent playing only a secondary role[6]. These results would promote resins with relatively long linkers and hence inherently mobile moieties in view of the subsequent NMR analysis. In the solid phase organic chemistry however, requirements such as reactivity, ease of synthesis, availability and price of the resin are often more determining factors for the choice of the resin. When the resin under consideration has only short linkers, the quality of the NMR spectra will be largely influenced by the choice of the solvent used to swell the beads.

This is demonstrated here by our results on a 1 % divinylbenzene crosslinked polystyrene Wang-Lys(Boc)-Fmoc resin (**1**). A first physicochemical test concerned the ability of six different solvents to swell the resin. In this context, two aspects have to be taken into account: the macroscopic swelling of a collection of beads, and the microscopic swelling of a single bead. At the macroscopic level, the swelling ability of a solvent can be estimated by measuring the volume increase of a given resin sample upon the addition of the solvent. This volume increase, however, will depend on both the increase of the bead diameter due to the penetration of the solvent inside the polymer network and the amount of interstitial liquid among the polymeric beads. This latter highly depends on the relative density of the resin bead and the solvent. Therefore, the correlation between the macroscopic volume increase of a resin sample and the swelling ability of the solvent is not obvious. A direct correlation between the extent of the volume increase of a resin sample upon addition of solvent and the spectral quality could not be established[6].

A more precise manner to evaluate the swelling ability of a solvent is to determine the diameter increase of a single resin bead, as this parameter will only be influenced by the amount of solvent that has penetrated inside the bead. The volume of a single polymeric bead swollen in different solvents was determined by direct measurement of the bead diameter under a light microscope provided with a micrometric ocular. The averaged diameter of the dry bead was 59.5 μm. The swelling ability of the solvents is expressed on the basis of this latter criterium as the ratio of solvated bead volume to the volume of the dry bead (Table 1). The pyridine possesses the best swelling ability and it is closely followed by dichloromethane (DCM), as can be seen from the radius and volume ratio. Chloroform and benzene possess neighbouring swelling properties while N,N-dimethylformamide (DMF) follows with a rather poor swelling ability. Regarding the dimethylsulfoxide (DMSO), the volume ratio shows that it does not efficiently penetrate into the polymer network.

In order to correlate the physicochemical data of Table 1 with the resulting NMR results, MAS NMR TOCSY[10] and ^{1}H-^{13}C HMQC[11] experiments were performed on the starting polymer **1**, using resin beads swollen with the six different deuterated solvents. A spinning rate of 2 kHz was sufficient to obtain line narrowing and to remove all spinning side bands outside the interesting spectral window. For spinning rates superior to 3 kHz, the rotation of the sample was harder to stabilize, and for DMSO-*d6* and chloroform-*d3* the solvent tightness of the rotor proved to be a limiting factor. However, for samples where the higher spinning rate could be used, no significant improvement of the spectral quality was observed when operating at 3 kHz or higher spinning rates. Therefore, all further studies were performed at a 2 kHz spinning rate.

Table 1. Solvation and MAS NMR ^{1}H line width for the Wang-Lys(Boc)-Fmoc Resin (**1**)

entry	solvent	averaged diameter (μm)	radius ratio	volume ratio[a]	MAS NMR ^{1}H line width[b] (Hz)
1	pyridine	109.6	1.84	6.3	21
2	DCM	107.5	1.81	5.9	21
3	chloroform	103.4	1.74	5.4	21
4	benzene	102.9	1.73	5.2	32
5	DMF	92.7	1.56	3.8	21
6	DMSO	85.1	1.43	2.9	-

[a] the ratio of solvated volume and the volume of dry resin as calculated from the averaged diameters.

[b] the MAS NMR line width was measured as the width at half height of the lysine ε-protons signal.

No workable data could be collected for DMSO-*d6*. This can be correlated to the poor swelling ability of this solvent, as a good swelling of the bead is likely to be related to a wide relaxation of the polymeric framework and an improved mobility of the tethered molecules. The motional freedom for the molecules located within the polymer network, which represent more than 99 % of the tethered molecules, is probably more restricted when using DMSO than for the other solvents. The resulting MAS NMR spectra of the resin **1** showed very similar line widths for DCM-*d2*, chloroform-*d3*, benzene-*d6* and pyridine-*d5* as well as for DMF-*d7*, despite the lesser degree of swelling ability of the latter. This indicates that the mobility of the tethered molecules in all five solvents is very similar (see Table 1). Probably, a threshold exists for the solvation volume above which the mobility of the tethered molecules becomes sufficient to reduce the residual dipolar coupling, resulting in liquid-like spectra for the ^{1}H resonances of the protected lysine bound to the solvent swollen beads. Although a proton line width of 21 Hz can be considered as broad for high resolution studies, this resolution proved to be entirely satisfactory for the extraction of structural information through two-dimensional NMR spectra.

It should be remarked here that 2D spectra are not limited by the sensitivity, as the amount of resin is not the limiting factor when one tries, as it is the case here, to characterize and eventually optimize reaction conditions.

As recently reported[7], MAS NMR can also be used for the completely different goal of identifying the product on a single bead. In this perspective, the required sensitivity to do a full structural analysis by multidimensional NMR becomes a real problem, and will require specially designed probe heads. In the perspective of chemical research or parallel simultaneous multiple synthesis of individual compounds, however, such indentification of the molecules on individual beads is not directly necessary, and multidimensional spectra can be obtained in a couple of hours. Moreover, a statistical collection of beads to be analysed will present the averaged properties of the whole of the resin thus being more representative for the reaction advancement than a single bead. Complete ^{1}H and ^{13}C NMR assignments and structural identification for the resin **1** could readily be obtained using standard TOCSY and ^{1}H-^{13}C HMQC experiments. These results for the pyridine-*d5* swollen beads are shown in Fig. 1 and Fig. 2. Results for the other solvents (excepting DMSO-*d6*) were very similar.

The identification of the ^{1}H spin system of the protected lysine was unambiguous using the pattern of the relayed conectivities in the TOCSY spectrum as depicted in Fig. 1. The chain can be traced from the backbone amide proton through the α-proton and the different side chain protons up to the ε-protons, as was shown by Shapiro *et al*[13].

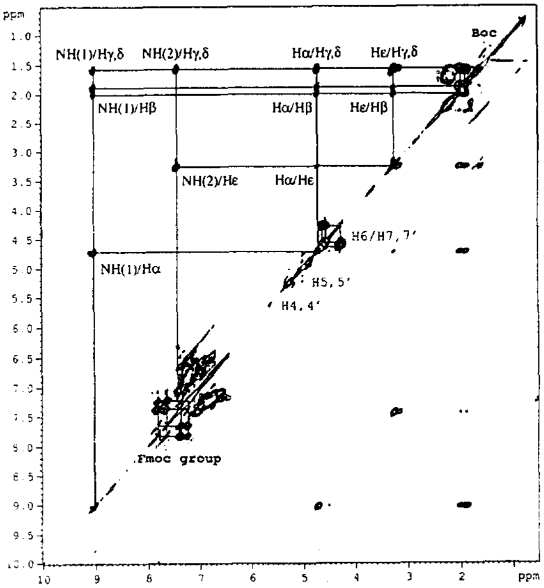


Figure 1. 2D Phase sensitive TOCSY spectrum for resin **1** swollen in pyridine-*d5*

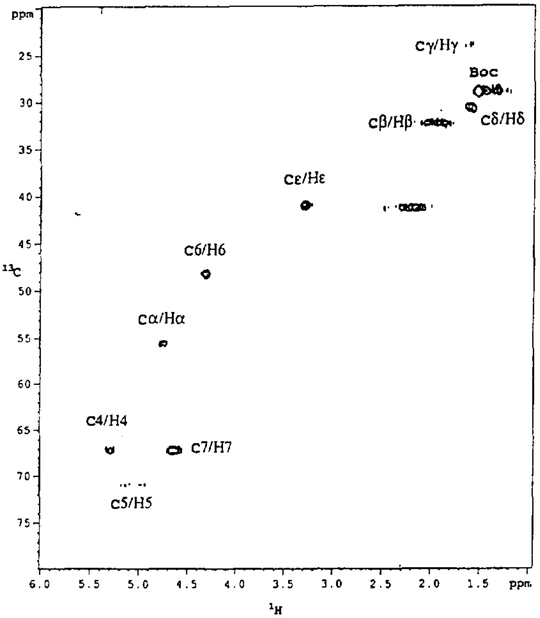


Figure 2. Aliphatic region of the ^{1}H-^{13}C HMQC spectrum for resin **1** swollen in pyridine-*d5*

The reverse pathway from the *tert*-butoxycarbonyl (Boc) protected amine proton through the ε-protons and all side chain protons until the α-proton can also be identified. The 9-fluorenylmethyloxycarbonyl (Fmoc) proton resonances are characterized by a typical pattern of connectivities in the aromatic region. The H6 and H7 protons connecting the Fmoc group to the lysine residue were assigned as a group of three interconnected protons, whereas the H4 and H5 protons form isolated proton pairs and could not be unambiguously assigned at this stage. The carbon assignments follow directly from the ^{1}H-^{13}C correlation peaks as shown in Fig. 2. Complete ^{1}H and ^{13}C NMR assignments for resin **1** swollen in the pyridine-*d5* are provided in Table 2. The chemical shift assignment obtained here agrees well with the values reported by Shapiro *et al.* for the Wang-Lys(Boc)-Fmoc resin[3].

Whereas the 2 kHz Magic Angle Spinning removes the residual static line broadening caused by the magnetic susceptibilities and the proton dipolar coupling, the dipolar cross-relaxation mechanism is unaffected by this process[12]. Therefore, a NOESY[13] experiment yielded intense cross peaks between the protons spins of the attached molecules. Fig. 3A shows the TOCSY spectrum for the resin **1** swollen in benzene-*d6*, which yields results very comparable to those obtained in pyridine-*d5*. A part of the corresponding NOESY spectrum is shown in Fig. 3B. NOE connectivities between the H6 and H7 proton resonances and the H8 proton resonance of the Fmoc group can be observed. The NOESY spectrum allows further an unambiguous identification of the H4 and H5 resonances. A sharp NOE peak was observed between H4 and two aromatic protons of the ring noted R2, whereas a broad NOE peak was observed between H5 and two aromatic protons of the ring noted R1 which already belongs to the polystyrene backbone.

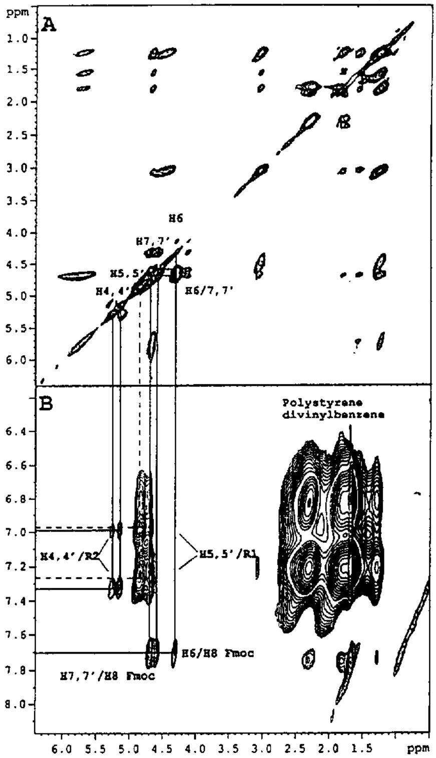


Figure 3. 2D Phase sensitive TOCSY (A) and (B) NOESY spectrum for resin **1** swollen in benzene-*d6*

The line width read from these NOE connectivities is directly correlated to the motional freedom of the protons H4 and H5 in the molecule: H5 is closer to the crosslinked polymer than H4, and presents a broader line width. The difference in line width clearly indicates the mobility gradient for the two aromatic rings R1 and R2: whereas the proton resonances of the R1 ring of the polystyrene are large, the presence of the ether linker confers an important degree of motional freedom to the R2 ring, resulting in a narrow line width for protons on the latter. In the TOCSY spectrum, the resonances of the resin are effectively suppressed by relaxation during the spin-lock field, but they show up as intense and broad connectivities between the different proton spins belonging to the polystyrene in the NOESY spectrum.

The complete assignment of all ^{1}H and ^{13}C resonances of the Wang-Lys(Boc)-Fmoc forms the base for the step-by-step characterization by MAS NMR of the reaction depicted in the Scheme 1. MAS TOCSY and HMQC NMR spectra were recorded using the experimental conditions previously determined for the Wang-Lys(Boc)-Fmoc resin. Pyridine was found to be the solvent of choice to swell the polymeric beads for the resins in all the three reaction steps. Whereas this facilitated our analysis, as previous resonance assignments could readily be used to identify the products in the next reaction step, it should be kept in mind that swelling properties of a resin depend on the nature of the growing molecule fixed on the polymer. Therefore, it might be necessary to perform a systematic study of the swelling after each step of the synthesis.

TOCSY spectra collected using pyridine-*d5* swollen beads are presented in Fig. 4, Fig. 5 and Fig.6. In Table 2, the assignment of the protons for each new molecular entity formed on the polymer is also presented.

The first reaction step involves the deprotection of the Fmoc protecting group. Indeed, for resin **2**, peaks corresponding to the Fmoc protecting group disappear (Fig. 4). Simultaneously, the shift of the α-proton toward high field values confirms the elimination of the carbamate moiety which was present in the proximity of the Cα position. We further remarked the absence of proton resonances for the αNH_2 protons in the spectrum. This inability to observe amino proton resonances in MAS NMR was previously reported in the case

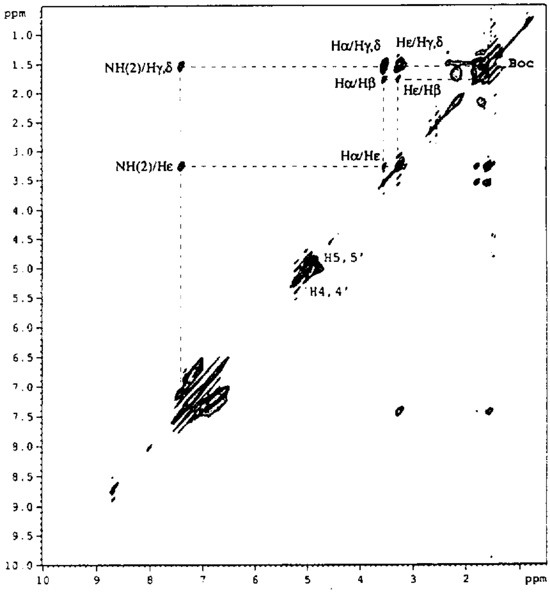


Figure 4. 2D Phase sensitive TOCSY spectrum for resin 2 swollen in pyridine-*d5*

of the NH^+_3 of L-alanine[14]. In that case, it was explained by an interference phenomenon between the molecular dynamics and the characteristic radiofrequency field strength of homonuclear pulse trains employed in the NMR experiments leading to dramatic broadening of lines of amino protons. Here, we expect that line broadening due to intermediate exchange of the NH_2 protons is at the origin of the absence of the expected lines.

The second reaction step involves the coupling of the 3-iodobenzoic acid to the liberated αNH_2 group. For the acylated resin **3**, peaks that appeared in the aromatic zone of the TOCSY NMR chart could be attributed to the 3-iodobenzoyl group newly attached to the resin, but we could not identify which peak corresponded to a defined proton. At this step, the important downfield shift noted for the α-proton confirms the presence of the benzene ring in the proximity of the Cα position (Fig. 5).

Finally, the aryl iodide on the resin was reacted with ethyl acrylate under the conditions of the Heck reaction, to generate the desired disubstituted olefin on the polymeric support. For the resin **4**, apparition of peaks attributed to the ethyl acrylate group on the TOCSY NMR chart confirmed the formation of the *trans* disubstituted olefin on resin, *via* the Heck reaction.

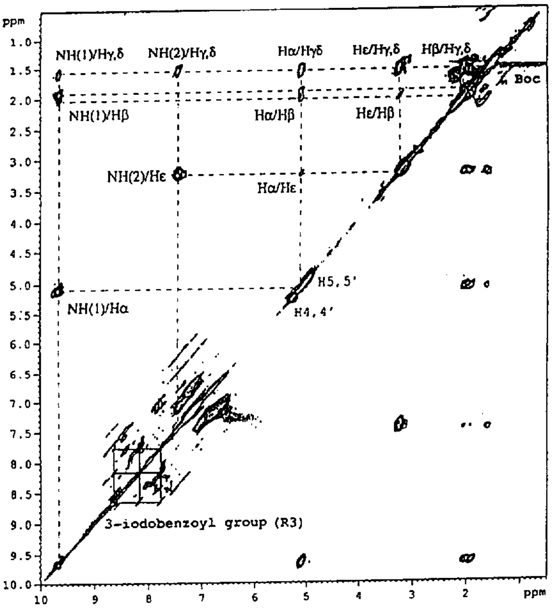


Figure 5. 2D Phase sensitive TOCSY spectrum for resin **3** swollen in pyridine-*d5*

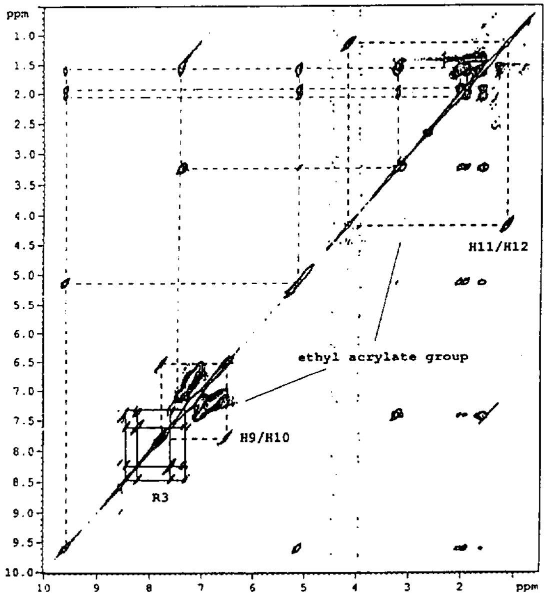


Figure 6. 2D Phase sensitive TOCSY spectrum for resin **4** swollen in pyridine-*d5*

This time, the four protons corresponding to the R3 aromatic ring are much better resolved than in compound **3** but once more we could not identify which peak corresponded to a defined proton (Fig. 6).

Table 2. ^{13}C Assignments for the resin **1** and ^{1}H assignments for the resins **1**, **2**, **3** and **4**

Assignment	^{13}C shift	^{1}H shift			
	resin **1**	resin **1**	resin **2**	resin **3**	resin **4**
a	55.2	4.73	3.55	5.11	5.14
b	32.14	1.89, 2.03	1.80	1.94, 2.05	1.94, 2.06
g	23.85	1.59	1.57	1.56	1.54
d	30.31	1.59	1.57	1.56	1.54
e	41.13	3.26	3.28	3.24	3.25
NH(1)	-	9.03	not determined	9.64	9.91
NH(2)	-	7.43	7.41	7.45	7.42
3	28.73	1.53	1.52	1.50	1.49
4	66.80	5.27	5.22	5.27	5.27
5	70.46	5.00	5.04	4.95	4.98
Fmoc group aromatics	not determined	7.83, 7.65 7.34, 7.24	-	-	-
6	47.89	4.3	-	-	-
7	66.80	4.57, 4.66	-	-	-
R3	-	-	-	8.63, 8.17, 7.76	8.47, 8.23, 7.60, 7.34
9	-	-	-	-	7.78
10	-	-	-	-	6.52
11	-	-	-	-	4.18
12					1.15

For each reaction step, no proton resonance corresponding to the starting compound could be detected. In this context, two possibilities had to be taken into account. Either the starting species on the resin completely reacted, or it only partially reacted but the quantity still existing on the polymer can no more be detected by this technique. To determine which was the case in our experiment, resin **4** was cleaved in presence of trifluoroacetic acid (TFA) and analysed by High Pressure Liquid Chromatography (HPLC), NMR and mass spectroscopy.

We confirmed thus the structure of the compound **5** and we also proved that reactions were quantitative, as a simple peak was detected in HPLC (Fig. 7).

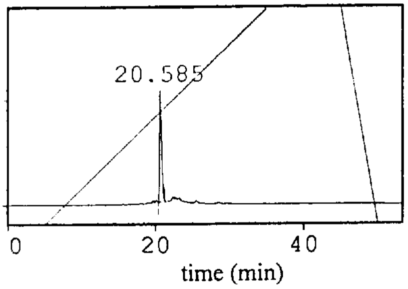


Figure 7. HPLC Profile of crude **5** (215 nm)

In summary, the Magic Angle Spinning (MAS) NMR technique applied to solvent swollen resins was successfully used to follow a multi-step solid phase synthesis without cleavage of the products from the polymeric support. A spinning rate of 2 kHz, easily obtainable on a standard solid state probe, proved to be sufficient to obtain high quality spectra, when a good swelling of the resin beads was obtained. Application of these simple rules allows the complete attribution of structurally complex molecules attached to a polymeric support, using 1D and 2D homonuclear and heteronuclear spectra, as is demonstrated here for the disubstituted olefin obtained *via* a Heck reaction. We believe that this technique is very promising in the characterization and optimization of organic reactions on solid support.

EXPERIMENTAL

Materials and Synthetic Reaction

The starting material in the reaction scheme previously presented was a 1 % divinylbenzene crosslinked polystyrene Wang-Lys(Boc)-Fmoc resin (200-400 mesh) purchased from NovaBiochem (1.5 g, 0.49 mmol/g). Fmoc groups were cleaved by treatement with a 20 % piperidine solution in DMF, twice, for 20 min each time and the resin was washed with DMF (5 times). 3-iodobenzoic acid (0.364 g, 1.47 mmol) was dissolved in DMF in presence of bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (0.685 g, 1.47 mmol) and then diisopropylethyamine was added (0.512 mL, 2.94 mmol). A final solution volume of 3.7 mL was obtained. Resin **2** (1.3 g, 0.49 mmol) was treated with this solution and the mixture was stirred at ambient temperature for 15 h. The resin was then washed with DMF (5 times), with DCM (3 times) and dried under vacuum. The completion of the reaction was determined by quantification of the residual amino groups on the resin, according to the quantitative ninhydrin procedure[15]. Palladium acetate (0.112 g, 0.5 mmol) was dissolved into 3 mL of DMF into which nitrogen was bubbled. The tris(2-tolyl)phosphine (0.304 g, 1 mmol) was dissolved into 4 mL of DMF. These two solutions were added to the resin **3** (0.6 g, 0.239 mmol). Ethyl acrylate (0.324 mL, 3 mmol) and triethylamine (1 mL, 7.2 mmol) were then added to this mixture. After the reaction mixture was stirred under nitrogen at 60 °C for 19 h, the resin was washed several times with DMF, water/di-sodium hydrogen phosphate solution, again DMF, DCM and was dried under vacuum. Resin **4** (0.2 g, 0.081 mmol) was treated with a 90 % TFA solution in DCM for 4 h. The crude product was analysed by HPLC using a column Vydac C18, 5 µ, 25x4 mm and H_2O/ 80 % acetonitrile in H_2O (grad) as eluent, at 1 mL/ min flowing rate (100 % conversion determined in HPLC). The crude product was purified by preparative reversed phase HPLC using a column Vydac C18, 5 µ, 500x20 mm and H_2O/ 80 % acetonitrile in H_2O (grad) as eluent, at 4 mL/ min flowing rate, to give 16 mg of **5** (43 % yield with respect to the 0.081 mmol of resin **4** submitted to the TFA treatment). ^{1}H NMR δ (DMSO-*d6*): 1.26-1.31 (t, 3 H, *J* = 7.09 Hz), 1.45 (m, 2 H), 1.59 (m, 2 H), 1.84 (m, 2H), 2.79-2.81 (m, 2 H), 4.19-4.26 (q, 2 H, *J* = 7.09 Hz), 4.43 (m, 1 H), 6.74-6.80 (d, 1H, *J* = 16.05 Hz), 7.53-7.58 (t, 1 H, *J* = 7.74 Hz), 7.69-7.75 (d, 1 H, *J* = 16.05 Hz), 7.72 (2.5 H), 7.89-7.95 (m, 2 H), 8.27 (s, 1 H), 8.69-8.71 (d, 1 H, *J* = 7.85 Hz); ^{13}C NMR δ (DMSO-*d6*):15.0, 23.7, 27.4, 31.0, 39.6, 53.2, 61.1, 120.1, 127.3, 129.9, 130.4, 132.4, 134.9, 135.2, 144.5, 166.6, 166.9, 174.5. Found: MH^+, 349.174. Calcd for $C_{18}H_{25}N_2O_5$: MH, 349.175.

Determination of Swelling Ability

The individual polymeric bead volumes were determined by direct measurement of their diameters under a light microscope provided with a micrometric ocular. Beads preswollen in the solvent were spread over a slide with a coverglass to minimize solvent evaporation. The resin beads proved to have even diameters so a total count of about 100 beads for each determination was enough to determine an averaged diameter.

MAS NMR Experiments

TMS was added as internal reference to the deuterated solvent before the resin swelling. The resins submitted to the MAS NMR experiments were swollen with the minimal volume of deuterated solvent before introducing them into the rotor. Data were collected using a conventional 7 mm MAS solid state probe on a Bruker DRX-300 specrometer at a spinning rate of 2 kHz. The ^2H resonance of the solvent was used for the field frequency lock during the homonuclear data collection. Heteronuclear spectra were run without field locking. Field drifting was not important during the reasonably short duration of the HMQC spectra. For the TOCSY experiments, a 70 ms MLEV-17 mixing time was used with a field strength of 10 kHz and appropriated delays to compensate for ROESY effects (clean-TOCSY)[11]. The TOCSY and HMQC data were collected as a data matrix of 1024*256 complex points with 16 scans per increment. The NOESY experiment was performed with a 600 ms mixing time. The total time for each 2D experiment was approximately 3 hours.

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