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A general approach to the quantification of resin-bound functional groups by NMR

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Received (in Toulouse, France) 29th April 2004, Accepted 24th June 2004
First published as an Advance Article on the web 8th October 2004

There has been a continuing need for sensitive, accurate and rapid methods to monitor functional loading of insoluble supports for solid phase synthesis. The present articles reports our findings regarding functional group loading quantification using ^{1}H NMR. Results obtained for supported amino, hydroxyl and NH-Fmoc groups are in agreement with those calculated using well-established methods and demonstrate that the strategy of looking, either at the excess reagent left in solution (NH_2 and OH), or at the protecting group derivatives released from the polymer (Fmoc), is a viable approach to resin loading quantification.

Introduction

Solid phase synthesis is routinely used for the preparation of combinatorial libraries of small organic molecules. The clear advantage of this biphasic method is in the area of purification as simple filtration generally leads to the desired products in high purity. However, evaluation of resin loadings and reaction yields, as well as reaction monitoring, are complicated and may require unusual and expensive instrumentation such as a combustion analysis device,[1] mass spectrometer,[2] high-resolution magic angle spinning NMR apparatus[3] or infrared microspectroscopy device.[4,5] Therefore, there is a continuing need for sensitive, accurate and rapid methods to monitor functional loading of insoluble supports for solid phase synthesis.[6,7]

In connection with a project in which we design a range of functionalized solid supports for organic synthesis, we undertook to develop ^{1}H NMR strategies for the quantification of resins loadings. The present article reports our progress in this matter.

Results and discussion

Primary amine quantification

Detection of amino groups is of basic importance in solid phase synthesis. Indeed, monitoring and quantification of primary amino groups is a well-established part of solid phase peptide synthesis,[6,7] and a reliable strategy has also been developed for qualitative detection of secondary amines on solid phases.[8]

First, we decided to evaluate the amount of supported amino groups using the quantitative reaction of amines with Boc_2O. We knew from experience that Boc_2O readily reacts with supported primary amines when the reaction is carried out in $CDCl_3$. No catalyst or base is required. We found that the integration of the ^{1}H NMR signal of *t*BuOH formed in the process (1.30 ppm, 9H) does not give reliable loading values, almost certainly because *t*BuOH is volatile. However, we were able to evaluate loadings of the resins by integration of the signal of unreacted Boc_2O (1.55 ppm, 18H). The loadings found using this strategy are sometimes somewhat lower than those provided by the suppliers, especially for the highly loaded resins. This may result from the fact that some amino groups are imprisoned within the polymer core and may not be sufficiently accessible for large electrophile trapping, for example, for amino acid grafting.

As can be seen from Table 1, the loadings were reproducibly quantified with as little as 6 mg of resin using picric acid (9.23 ppm, 2H) instead of Boc_2O. On such a very small scale, accurate results were obtained by dilution of larger measurable amounts of picric acid and pentamethylbenzene (2.18–2.29 ppm, 15H, NMR internal standard) in $CDCl_3$. Another clear advantage of this picric acid method is in the area of supported amine recovery as simple washing with a tertiary amine base leads to deprotonation of the ammonium and release of the picric acid salt.

Obviously, the picric acid strategy generates more reliable results, although we believe that Boc_2O loading quantifications might reflect the amount of accessible functional groups within the polymer core. Therefore, both strategies may be complementary for quantifying supported free amines and evaluating the efficient loading in amino groups.

Hydroxyl group quantification

Three color tests are currently available for qualitative detection of resin-bound hydroxyl groups. However, none of these have yet been adapted to quantitative evaluation of free alcohol loadings. The first of these tests involves reaction of the polymer-supported alcohol with cyanuric chloride to form an active ether, which in turn is coupled with a dye (Mordant Orange 1, Fuchsin or Fluorescein).[9] The second involves conversion of the supported alcohol into the corresponding tosylate, and this leaving group is further displaced with 4-(4-nitrobenzyl)pyridine, yielding a blue to purple resin by treatment with base.[10] The third method was specifically designed for detection of polymer-supported tertiary alcohols and relies upon the formation of an intermediate diphenylsilylchloride ether, which is subsequently treated with Methyl Red to afford the tethered dye.[11] In addition to these visual hydroxyl group detection methods, Yan and coworkers have

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DOI: 10.1039/b406513a

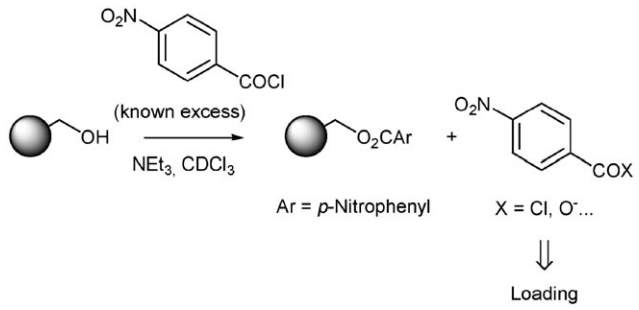
Table 1 Polymer-bound amine quantification

Resin	Loading/mmol g^{-1}	Loading (Boc_2O)[a]/mmol g^{-1}	Loading (picric acid)[a]/mmol g^{-1}
Aminomethyl PS resin (Senn Chemicals)	1.3[b]	1.16	1.31 (1.38[c])
Aminomethyl PS resin (Novabiochem)	0.46[d]	0.44	0.49

[a] Loadings were measured 3 times with each resin. Estimated uncertainty based on these 3 measurements is 10% on small scale (<10 mg of resin) and 5% on large scale (>50 mg of resin). [b] Determined by HCl titration. [c] Loading determined with 6–7 mg of resin. [d] Determined by elemental analysis of chlorine, after derivatization with 3,4-dichlorophenylisocyanate.

described the only method published so far for rapid UV quantification of the amount of supported hydroxyl groups. Their method involves trapping of 9-anthroylnitrile and subsequent UV analysis of the supernatant.[12]

Our approach to hydroxyl group quantification relies upon the high yielding formation of supported esters when reacting hydroxyl resins with a known excess of *para*-nitrobenzoyl chloride (Scheme 1). The later is a very good NMR probe considering that the aromatic signals of the acid chloride and the corresponding carboxylic acid are far from interfering with those of pentamethylbenzene (NMR internal standard) or triethylamine.

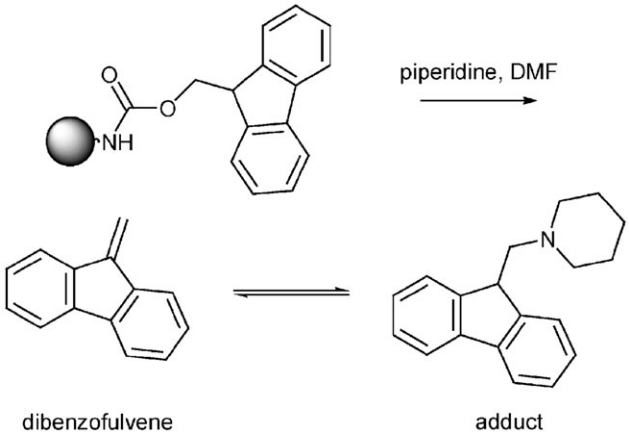


Scheme 1

The amount of aromatic protons resulting from *p*-nitrobenzoyl chloride derivatives left in solution (8.20–8.50 ppm, 4H) provided us with a reading of the loading in hydroxyl groups by subtraction (Table 2). Of course, this method will also give positive results with other nucleophiles such as amines. Therefore, if there is a doubt, this test should be associated with an amine-specific quantification (Boc_2O or picric acid scavenging) or at least with a qualitative color test for detection of amines (Kaiser test...).

Fmoc quantification

Base-catalyzed cleavage of the 9-fluorenylmethyloxycarbonyl (Fmoc) group and subsequent UV analysis is commonly employed to evaluate the amount of supported Fmoc protected functional groups.[6,7] However, piperidine does not generally scavenge dibenzofulvene quantitatively, and both the piperidine-dibenzofulvene adduct and the free dibenzofulvene are observable by NMR after cleavage (Scheme 2). This leads to varying UV absorbances, depending on the relative proportion of the two compounds. Inaccuracy of UV quantification of Fmoc derivatives in solution has been discussed in the past, but this approach remains the most widely used method to evaluate loading in NH-Fmoc subunits. However, the non-nucleophilic amidine 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) has been utilized recently as an alternative to piperidine for semi-quantitative and quantitative analysis.[13] Adduct formation is prevented under these conditions. Therefore, only dibenzofulvene is formed and accurate loading values can be obtained from a UV absorbance determination.



Scheme 2

In our case, upon evaporation of the cleavage solution, the adduct was, if not absent, a very minor component in the mixture because of the reversibility of the scavenging reaction. Addition of a ^1H NMR internal standard, followed by homogenization in $CDCl_3$, allowed a direct NMR reading of the amount of dibenzofulvene (6.05 ppm, 2H) and possibly dibenzofulvene-piperidine adduct (4.19 ppm, 1H) released from the resin (Table 3). We discovered that if pentamethylbenzene was to be used as internal standard, it really should be added after evaporation of DMF for fear of being almost entirely co-evaporated with the solvent. However, on a small scale, we found it more convenient to use methyl *ortho*-aminobenzoate as the NMR internal standard because the ester signal we used as reference (3.83 ppm, 3H) only integrates for 3 hydrogens. Again, accurate results were obtained with this

Table 2 Polymer-bound hydroxyl group quantification

Resin	Loading/mmol g^{-1}	Loading[a]/mmol g^{-1}
Wang resin (Novabiochem)	0.72[b]	0.71
Wang resin (Novabiochem)	0.86[b]	0.89

[a] Loadings were measured 3 times with each resin. Estimated uncertainty based on these 3 measurements is 5% with 50 mg of resin or more. [b] Determined by elemental analysis of sulfur, after derivatization with thiophenecarbonyl chloride.

Table 3 Polymer-bound fluorenylmethyl carbamate quantification

Resin	Loading/ mmol g^{-1}	Loading[a]/ mmol g^{-1}
Rink amide AM PS resin (Senn Chemicals)	0.87[b]	0.93
Rink amide (Novabiochem)	0.51[c]	0.54

[a] Loadings were measured 3 times with each resin. Estimated uncertainty based on these 3 measurements is 5% with 50 mg of resin or more. [b] Determined by UV quantitation upon cleavage with piperidine. [c] Determined by dibenzofulvene UV quantitation upon cleavage with DBU.[13]

technique as compared to loading values measured by standard UV titration.

General conclusions

In conclusion, we have developed a new general strategy for the determination of resin loadings. Since, the method relies upon a quantitative conversion of the supported functional groups, we believe that this technique may be carried out with other reagents than those used in our experiments, provided that important ^{1}H NMR signals from reagent, internal standard and catalyst do not overlap. Overall, results obtained for supported amino, hydroxyl and NH-Fmoc groups are in agreement with those calculated using well-established methods and demonstrate that the strategy of looking, either at the excess reagent left in solution (NH_2 and OH), or at the protecting group derivatives released from the polymer (Fmoc), is a viable approach to resin loading quantification. Careful measurement of the weight of resin, reagent and NMR internal standard is the only prerequisite for the success of our technique.

Experimental

^{1}H NMR spectra were recorded at 200 MHz, and chemical shifts (δ) are in ppm downfield from tetramethylsilane.

Primary amine quantification

Amino resin (200 mg) was allowed to react with Boc_2O (1.5 equiv.) in $CDCl_3$ (1 mL) in the presence of pentamethylbenzene (0.5 equiv., NMR internal standard). The reaction mixture was stirred overnight at room temperature. Filtration and ^{1}H NMR analysis of the filtrate allowed the amount of Boc_2O left in solution to be determined.

Amino resin (100 mg) was treated with picric acid (1.5 equiv.) in $CDCl_3$ (1 mL) in the presence of pentamethylbenzene (0.5 equiv., NMR internal standard). The reaction mixture was stirred overnight at room temperature. Filtration and ^{1}H NMR analysis of the filtrate allowed the amount of picric acid left in solution to be determined.

Small scale: picric acid (30.5 mg, 0.13 mmol) and pentamethylbenzene (6.8 mg, 0.046 mmol, NMR internal standard) were dissolved in $CDCl_3$ (5 mL). An aliquot of solution (0.5 mL) was taken and added to the resin (6–7 mg). The mixture was stirred overnight at room temperature, filtered, and ^{1}H NMR analysis of the filtrate allowed evaluation of the amount of untrapped picric acid.

Hydroxyl group quantification

Hydroxy resin (50 mg) was allowed to react with *para*-nitrobenzoyl chloride (1.5 equiv.) in $CDCl_3$ (1 mL) in the presence of pentamethylbenzene (0.5 equiv., NMR internal standard). The reaction vessel was sealed and the reaction mixture was stirred overnight at 65 °C. After cooling and filtration, ^{1}H NMR analysis of the filtrate allowed one to determine the amount of *para*-nitrobenzoyl chloride derivatives left in solution. Since *para*-nitrobenzoyl chloride may generate several species, depending on the quality of the solvent, we recommend to integrate together all signals appearing in the 8.20–8.50 ppm region (4H).

Fmoc quantification

Fmoc-protected amino resin (50 mg) was treated twice (5 min and 20 min) with a solution of piperidine in DMF (1 : 5, 2 mL). The combined filtrates were concentrated and a known amount of methyl anthranilate (NMR internal standard) was added. The mixture was homogenized in $CDCl_3$ and an aliquot was submitted to ^{1}H NMR. The relative integration of signals belonging to methyl anthranilate, dibenzofulvene and possibly dibenzofulvene-piperidine adduct allowed the NH-Fmoc loading of the resin to be determined.

Acknowledgements

We wish to gratefully acknowledge Dr. Bodo Baumeister (Novabiochem) and Dr. Christof Senn (Senn Chemicals) for kindly responding to our mails and providing us with precious information and comments regarding commercial resin loading determinations. We also want to thank Dr. Florine Cavelier for helpful discussions.

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