$^{1}H,^{13}C$-HSQC HR-MAS NMR as a Tool for Investigating the Quality of Fmoc-AA-Wang Resins for SPPS

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Introduction

The use of high quality starting materials in SPPS is a prerequisite to obtain peptides of high purity and with high yields [1]. Beside AA derivatives and reagents, the quality of employed resins like Fmoc-AA-Wang is also of crucial importance. Due to the insolubility of resins, the range of analytical methods suitable to assess their quality directly is restricted. Many attempts have been described in literature to overcome this difficulty [2,3,4]. Nevertheless, the quality control of resin beads still remains challenging compared to building blocks and reagents.
MAS NMR enables direct analysis of resin beads without prior cleavage and sample preparations. 1D ^{13}C MAS NMR with conventional MAS probes has already been used successfully to determine quantitatively the loading of resins using internal references [5]. The significantly better resolution of HR-MAS probes enabled quantitative results from 1D ^{1}H MAS NMR spectra as well [6,7]. Owing to overlap of resonances, the 1D NMR methods cannot always be successfully applied, particularly in cases with weak resonances. Herein, results obtained with the much more powerful HSQC tool on the generic example Fmoc-Ala-Wang resin are presented.

Results and Discussion

Wang resin (1.3 mmol/g) was loaded with a substoichiometric amount of Fmoc-Ala-OH via the standard esterification method (DCCI/DMAP in DMF/THF at 0°C), aiming at an incomplete loading. The resulting Fmoc-Ala-Wang resin (0.43 mmol/g, corresponding to approx. 33% of the theoretical full loading) was subsequently treated with benzoyl-chloride/pyridine in THF to cap the remaining active sites leading to a 33:67 mixture of Fmoc-Ala-Wang resin and benzoyl-Wang resin. This resin shows characteristic signals in the ^{13}C MAS NMR spectrum which can be assigned to the corresponding structures (Fig.1).
Characteristic signals of Fmoc-Ala-Wang can be identified at 172.2 ppm (C-(11)), 155.0 ppm (C-(14)), 66.4 ppm (C-(10)), 49.1 ppm (C-(12)) and 18.0 ppm (C-(13)). As a result of endcapping the residual hydroxyl groups of Wang to benzoyl-Wang, a second signal of carboxyl C-(11) can be observed at 165.8 ppm. The integral ratio of the Fmoc-Ala-Wang signals C-(11), (12), (13) and (14) to C-(11) of benzoyl-Wang enables to determine quantitatively the proportions of Fmoc-Ala- and benzoyl-Wang. For the resin shown in Fig. 1, a content of 34% Fmoc-Ala-Wang versus 66% benzoyl-Wang has been determined which corresponds well with the substitution determined by Fmoc cleavage / UV absorption.

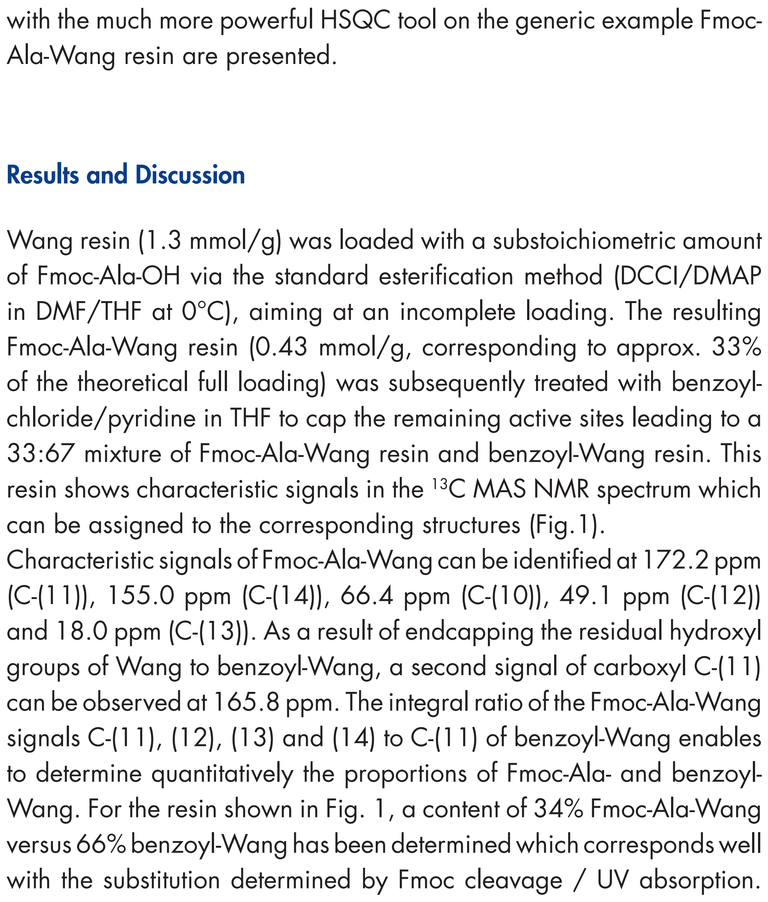

Fig. 1: Structure and 1D ^{13}C MAS NMR spectrum of Fmoc-Ala-Wang resin with benzoyl endcapped residual Wang sites recorded at 100.6 MHz (Bruker Avance 400, MAS rate 2 kHz) with assignment of characteristic signals.

In order to avoid free hydroxyl groups of Wang and thus the formation of side products, endcapping of Fmoc-AA loaded Wang resins is an important synthesis step. Therefore, a direct and sensitive detection method to verify the completeness of the endcapping is of high interest. C-(10) of unloaded Wang resin (Fig. 2a) shows a significantly lower chemical shift (64.1 ppm) compared to the corresponding C-(10) of Fmoc-Ala-Wang (66.4 ppm) and benzoyl-Wang (65.9 ppm) (Fig.2b). Potentially, the characteristic signal at 64.1 ppm enables to determine quantitatively the content of residual unreacted hydroxyl groups in Fmoc-AA-Wang resins. However 10% Wang resin or more had to be spiked to Fmoc-Ala-Wang in order to receive a Wang-C-(10) signal using 1D ^{13}C MAS NMR, even at recording times of 12 hours (Fig. 2c).

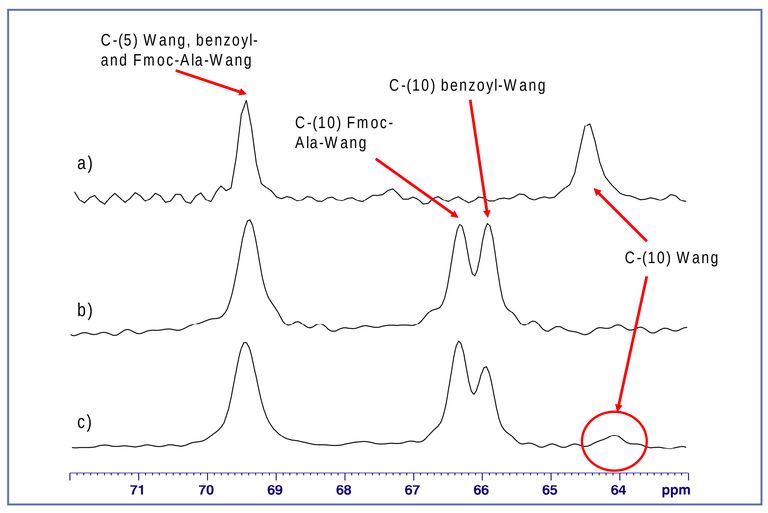

Fig. 2: Expansions of ^{13}C MAS NMR spectra of a) Wang resin, b) benzoyl endcapped Fmoc-Ala-Wang resin and c) benzoyl endcapped Fmoc-Ala-Wang resin spiked with 10% Wang (100.6 MHz, Bruker Avance 400, MAS rate 2 kHz).

A significantly lower LOD of unreacted Wang hydroxyl groups can be achieved by $^{1}H,^{13}C$-HSQC HR-MAS NMR. The 2D spectrum of Fmoc-Ala-Wang spiked with 10% Wang resin shows a characteristic cross signal of H-(10) / C-(10) at 4.7/64.1 ppm (Fig. 3). The same Fmoc-Ala-Wang lot spiked with only 2% Wang resin still shows a clear cross signal (Fig. 4). The intensity of the cross signal depends directly on the amount of Wang resin spiked. Quantitative determinations using calibration curves are therefore possible.

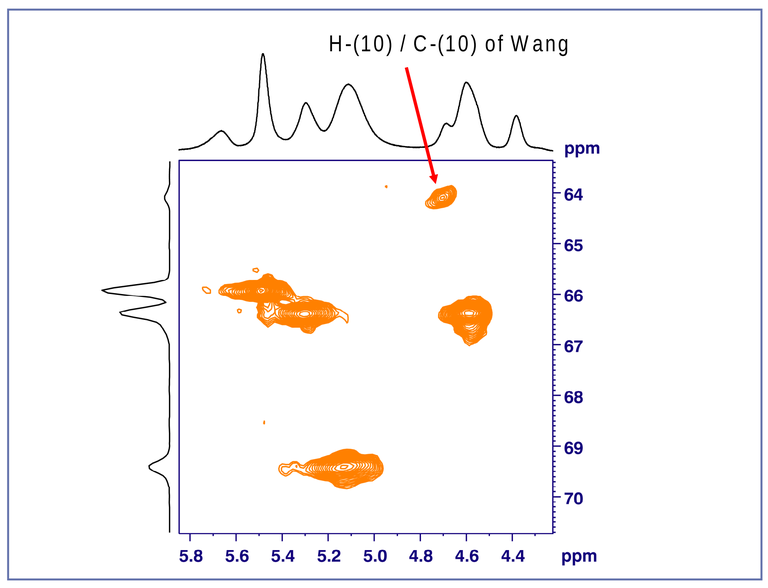

Fig. 3: Expansion of the $^{1}H,^{13}C$-HSQC HR-MAS NMR spectrum of benzoyl endcapped Fmoc-Ala-Wang spiked with 10% Wang (Bruker Avance 400, MAS rate 4 kHz).

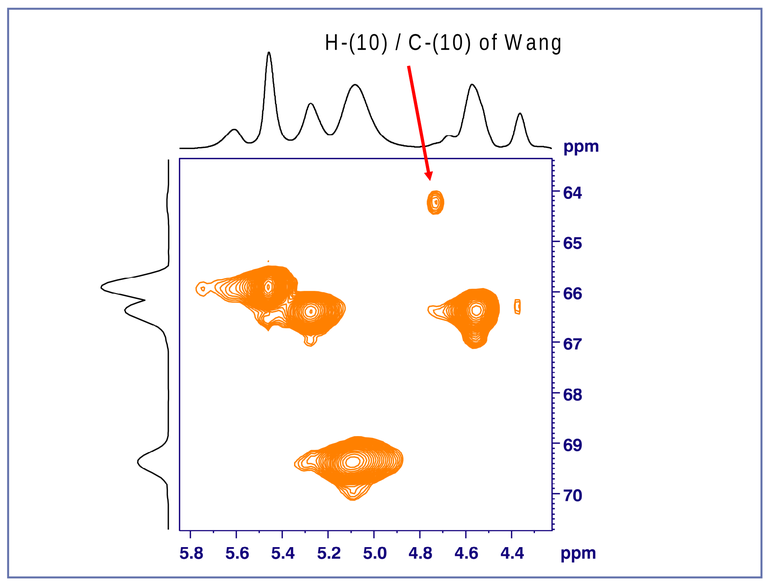

Fig. 4: Expansion of the $^{1}H,^{13}C$-HSQC HR-MAS NMR spectrum of benzoyl endcapped Fmoc-Ala-Wang spiked with 2% Wang (Bruker Avance 400, MAS rate 4 kHz).

Conclusion and Outlook

The NMR method presented here is considered to be a powerful tool for investigating quality aspects of resins used in SPPS. The main advantage of the method is that significant structural information can be acquired directly from the resins without the need for cleavage and sample preparation. The quantitative determination of residual unreacted Wang in Fmoc-Ala-Wang resin is shown as an example.
Further investigations will be performed with the aim to improve the analytical method (lower LOD for unreacted Wang) and to extend its application to other resins used in SPPS.

Abbreviations

AA	amino acid
DCCI	1,3-dicyclohexylcarbodiimide
DMAP	4-(N,N-dimethylamino)pyridine
DMF	N,N-dimethylformamide
HR	high resolution
HSQC	heteronuclear single quantum coherence
IPE	diisopropylether
LOD	limit of detection
MAS	magic angle spinning
SPPS	solid phase peptide synthesis
THF	tetrahydrofuran
TKS	tetrakis(trimethylsilyl)silane

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