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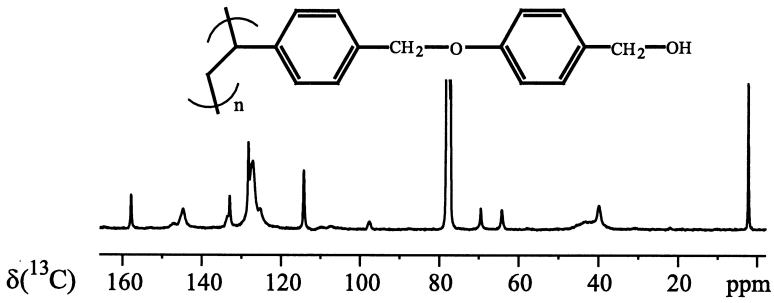

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Quantitative Determination of Resin Loading in Solid-Phase Organic Synthesis Using ^{13}C MAS NMR

Roland Hany,*,† Daniel Rentsch,† Boopathy Dhanapal,‡ and Daniel Obrecht§

Swiss Federal Laboratories for Materials Testing and Research (EMPA), Überlandstr. 129, CH-8600 Dübendorf, Switzerland, Lipal Biochemicals, Winterthurerstr. 190, CH-8057 Zürich, Switzerland, and Polyphor Ltd., Gewerbestr. 14, CH-4123 Allschwil, Switzerland

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The use of quantitative carbon nuclear magnetic resonance spectroscopy (^{13}C NMR) for the determination of resin loadings has been investigated. Magic angle spinning (MAS) NMR spectra have been obtained for solvent-swollen resins on a conventional 7 mm CP/MAS probe using the two pulse phase modulation (TPPM) proton decoupling sequence. Loadings of resin-bound organic compounds were evaluated via addition of tetrakis(trimethylsilyl)silane as reference or using the carbon resonances of the polymeric resin material as an internal standard. Results for several functionalized Wang and trityl resins are consistent with those obtained using well-established analytical methods. The ^{13}C NMR method has interesting applications in the field of solid-phase organic synthesis (SPOS), since no functional group acting as a support for the attachment of a quantifiable chromophore must be available in the material of interest.

Introduction

The rapid growth in the field of solid-phase organic synthesis (SPOS) is reflected in the exponentially increasing number of publications in recent years.[1,2] By proper selection of resins and reaction conditions, a wide variety of solution organic reactions is successfully adapted to the solid phase.[3−5] The arsenal of analytical methods to monitor and optimize these reactions has been reviewed recently.[6] To circumvent the undesired "cleave-and-analyze" step, analytical developments are directed to the on-resin analysis of the solid-supported compounds, both at intermediate stages and of the final product. Recent examples of these efforts involve single-bead fluorescence and NMR spectroscopy[7,8] or IR and Raman methods.[9] These methods do not necessarily yield direct information on the resin loading but are tailored for the rapid monitoring of the conversion of a chemical reaction through the appearance or disappearance of a signal and include examples using ^{19}F,[10,11] ^{13}C,[12,13] and ^{31}P[14] NMR spectroscopy or ATR FT-IR.[15] On-resin reaction yields have been quantitatively determined by ESR,[16] fluorescence,[17] IR and UV spectroscopy,[18,19] combustion elemental analysis,[20] or with the Fmoc couple and cleavage method.[21−23] Although gel-phase NMR spectroscopy in combination with magic angle spinning has advanced to the point where liquid-like spectra can be obtained for most resin-bound molecules,[24] this method is not often used for the quantification of resin loadings. Polymer loadings and product conversion were determined after cleavage of resin-bound molecules and measurement of ^{1}H NMR spectra with an internal standard.[25] Examples of ^{19}F gel-phase NMR in conjunction with a polymeric support bearing ^{19}F[26] or fluorobenzene as an internal standard[27] have been reported. ^{13}C NMR has been used for the analysis of the degree of chlorination of polystyrenes,[28,29] and optimized experimental parameters have been presented for the rapid accumulation of ^{13}C gel-phase NMR spectra.[30] A ^{13}C MAS NMR method for solvent-swollen resins for quantification purposes is presented here. A set of modified polystyrene (PS) resins used as starting materials for SPOS has been selected to evaluate and asses a quantitative method. The Wang resins **1a**−**c**[31] with three different degrees of loadings were prepared in our laboratories and served to set up the experimental protocol. The investigations have been extended to derivatives of the PS Wang resin (**2a**−**c**, **3a**−**c**),[32] to the 2-chloro-trityl resin and its precursor (**4** and **5**), and to the more complex structure of a Fmoc/Pmc protected arginine Wang resin (**6**).

Results and Discussion

The chemical structures of resins **1**−**5** are shown in Figure 1. A necessary condition for obtaining quantifiable ^{13}C NMR spectra is knowledge of the T_1 relaxation behavior of the individual components. For the reference standard tetrakis-(trimethylsilyl)silane (TKS, see below), the T_1 value is approximately 15 s, resulting in prohibitively long measurement times, since quantitative spectra require a recycle delay between individual pulses of $>5T_1$ of the slowest relaxing nucleus. After the addition of 0.1 M chromium(III)acetylacetonate ($Cr(acac)_3$) as relaxation reagent, the T_1 values of TKS and the solvent $CDCl_3$ decreased to 800 ms and 340 ms, respectively. The T_1 values of the carbon atoms of the Wang resin **1a** were in the range of 80 ms for C-(10) and

* Correspondence to Dr. Roland Hany, EMPA, Überlandstr. 129, CH-8600 Dübendorf, Switzerland. Tel: ++41 1 823 40 84. Fax: ++41 1 823 40 38. E-mail: roland.hany@empa.ch.

† Swiss Federal Laboratories for Materials Testing and Research (EMPA).

‡ Lipal Biochemicals.

§ Polyphor Ltd.

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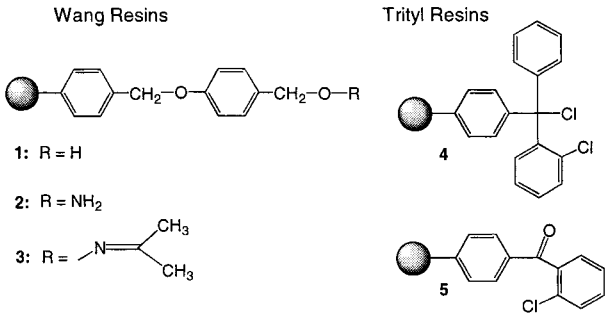

Figure 1. Structure formula of the Wang and Trityl Resins **1**−**5**.

430 ms for the quaternary carbon atom C-(1) under these conditions (see Figure 2a).

Therefore, a recycle delay of 5 s is sufficient for quantification purposes and was chosen whenever $Cr(acac)_3$, as the relaxation reagent, and TKS were present. If the resin loading was determined with the PS framework acting as internal standard (see below), the relaxation delay can be set to 2.5 s which halves the analysis time. The measurement time for the ^{13}C NMR spectra of **1a** shown in Figure 2a was 3 h, and the blank spectrum of the empty rotor equipped with Teflon spacer was subtracted (for sample preparation see Experimental Section). This sample had a good swelling capacity in $CDCl_3$, and the 7 mm circonia rotor with a working volume of approximately 310 μL was filled with 53 mg of **1a**, 8.6 mg of TKS, and 360 mg of solvent. This corresponds to a swelling capacity of approximately 4.5 mL/g. The MAS rate was chosen to prevent overlapping between spinning sidebands of PS (*) and isotropic signals. Although the line widths vary, the relative intensities are consistent between all Wang carbon resonances (e.g., C-(4−7, 9, 10)) free from signals of the PS backbone.

In Figure 2b, the difference spectrum between $^{13}C\{^1H\}$ spectra using (i) the two pulse phase modulation (TPPM) and (ii) WALTZ-16 ^1H decoupling is shown. This illustrates that satisfactory ^1H decoupling performance is an essential experimental requirement. The TPPM ^1H decoupling sequence[33] has been developed for solids, but works also for the gel-phase samples studied here. The 30.5 kHz decoupling strength corresponds to the maximum power of the proton amplifier used for experiments in the liquid phase, and we found that even higher decoupling strengths (56 kHz) using the high-power amplifier did not improve the quality of the spectra. Similar spectra have been obtained by using the recently presented SPINAL-64 ^1H decoupling sequence.[34] The WALTZ-16 sequence used for liquid applications with decoupling strengths of around 3 kHz does not produce quantifiable ^{13}C NMR spectra (Figure 2b), even for resins with good swelling capacities, and the relative intensities of C-(7), of the methylene carbons C-(5 and 10), and of the PS carbon signals are too low. Increasing the WALTZ decoupling strength to the 30.5 kHz used for TPPM yields correct intensities for C-(5, 7, 10) of the Wang resin but still underestimates the carbon intensities of the PS framework (spectrum not shown).

Polymer loadings have been evaluated via comparison of integrals of carbon resonances of known amounts of resin and a reference compound (method A, eq 1). The reference

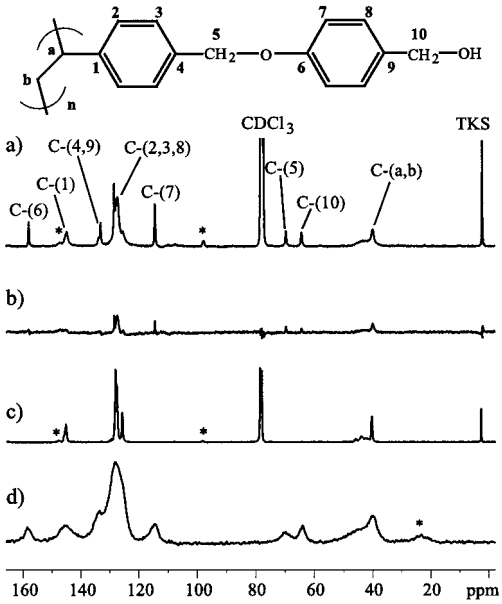

Figure 2. (a) Quantitative $^{13}C\{^1H\}$ NMR spectrum of $CDCl_3$-swollen Wang resin **1a** with the assignment of carbon resonances, MAS rate 2000 Hz, 2048 transients, 30.5 kHz TPPM ^1H decoupling, 0.1 M $Cr(acac)_3$, relaxation delay 5 s. The asterisk (*) denotes spinning sidebands. (b) Difference of carbon spectra using TPPM and WALTZ ^1H decoupling (30.5 and 3.1 kHz decoupling field strengths, respectively). (c) Same as (a), but of pure PS resin. (d) Solid-state ^{13}C NMR spectrum of **1c**, MAS rate 10 500 Hz, 1500 transients, 48.0 kHz TPPM ^1H decoupling, relaxation delay 30 s.

standard TKS has been chosen since this compound is chemically inert and soluble in organic solvents, and its ^{13}C NMR signal at 2.4 ppm usually does not overlap with carbons of organic compounds. Alternatively, the signals of the solvent chloroform may be used as the reference, since it is weighed directly into the circonia rotor. The resin loading l_i is given by the mass m_{ref}, the molecular weight MG_{ref}, and the number of carbon atoms $n_{C(ref)}$ (= 12 for TKS) contributing to the signal intensity I_{ref} of the reference material, the mass m_{Resin}, and the average signal intensity I_i of one carbon atom of the resin-bound molecule.

$$\text{Method A:}\quad l_i = 1000\,\frac{m_{ref}\; n_{C(ref)}\;\; I_i}{MG_{ref}\;\; I_{ref}\;\; m_{Resin}}\quad [\text{mmol/g}] \quad (1)$$

The application of method A to unfunctionalized PS (see Figure 2c) yielded a loading $l_i = 9.72 \pm 0.16$ mmol/g. The theoretical value is $l_i = 9.60$ mmol/g with the molecular formula C_8H_8 for PS and neglecting the approximately 1% level of cross-linking. This means that the PS framework itself may be used as an internal reference for the determination of resin loadings (method B, eq 2). The molar fraction $x_i = I_i/(I_i + I_{PS})$ is obtained from NMR signal intensities I_{PS} of PS and I_i of the organic residue i (see above); the loading l_i may then be calculated with the known molecular weights MG of the organic residue and PS. The formula (method B) is given for the simultaneous determination of n various organic molecules linked to a resin. For the individual MG_j values, it has to be noted that the skeleton of the resin also has to be included, e.g., for the Wang resin **1** the molecular formula $C_{16}H_{16}O_2$ is used.

$$\text{Method B:}\quad l_i = 1000\,\frac{x_i}{\sum_{j=1}^{n} x_j MG_j}\ \text{[mmol/g]} \qquad (2)$$

Method B is advantageous since the amount of sample does not need to be known. The method works also for resins with low swelling capacity, in cases where the sample is studied by solid-state NMR (see below). Method B is expected to fail for very high loaded resins ($x_{PS} \rightarrow 0$), when the inaccuracy of the NMR signal integration leads to large relative errors of the results. Care has to be taken if chemical reactions are not quantitative and small amounts of products from side reactions or incomplete reactions are formed. These impurities might not be detectable by NMR spectroscopy; however, they add weight to the resin which is not accounted for using only the quantity of PS as the internal reference. This causes method B to give increased loading values by underestimating the weight of the resin matrix relative to the desired product. No such restrictions exist for method A.

The loadings of the individual organic residues attached to PS resins are summarized in Table 1. Method A has been applied either with TKS or $CDCl_3$ as reference standards. Wang resins **1a**−**c** with three different degrees of loadings were investigated. For **1a** and **1b**, both methods A and B resulted in identical loadings (2.40−2.51 and 1.03−1.05 mmol/g, respectively). The loading of 2.5 mmol/g for **1a** evaluated by the chemical method agrees with the NMR result; however, the loading for **1b** (0.8 mmol/g) is somewhat lower. This discrepancy may be due to the different analytical methods used. The chemically evaluated loadings have been obtained by converting the Wang resin to the Fmoc derivative and then measuring photometrically the amount of Fmoc chromophore released upon treatment with piperidine/DMF. This method depends highly on the completeness of both reactions steps (Fmoc derivatization and cleavage)[19] and determines the loading of functional groups accessible for this derivatization reaction,[21] in contrast to the total amount of available hydroxymethylene groups obtained from the NMR method.

Methods A and B yielded markedly different resin loadings for **1c**. In contrast to **1a** and **1b**, the swelling capacity of **1c** was low (<2 mL/g) in chloroform and other solvents. The NMR spectrum showed quite broad resonances, e.g., 65 Hz for C-(7), whereas a line width of 30 Hz has been found for **1a**. We also studied **1c** as a solid using ^{13}C single pulse excitation (see Figure 2d). A loading of (3.1 ± 0.3) mmol/g was found, in agreement with the values obtained from the Fmoc release (3.3 mmol/g) and via method B from the gel-phase spectra (3.21 mmol/g). Likewise, consistent loadings for **1a** (2.5 mmol/g) and **1b** (1.1 mmol/g) were obtained from solid-state ^{13}C NMR experiments. Therefore, the results from method A for **1c** (1.90, 2.11 mmol/g) are questionable. It seems that due to excessive broadening of the carbon resonance signals of highly rigid fractions of the Wang resin, their contribution to the NMR spectral intensity is considerably reduced with the consequence that any quantification fails. For regional rigid polymers, a MAS rate of 2−3 kHz is not sufficient to substantially reduce anisotropic magnetic interactions responsible for this line broadening, and thus method A is applicable only for fairly good swelling resins (swelling capacity = 3−4 mL/g).

Table 1. Quantitation of Resin Loadings [mmol/g][a]

compd	NMR data: method A (TKS)	NMR data: method A ($CDCl_3$)	NMR data: method B	data from chemical methods
1a	2.43 ± 0.09	2.40 ± 0.08	2.51 ± 0.12	2.5[b]
1b	1.05 ± 0.04	1.04 ± 0.05	1.03 ± 0.07	0.8[b]
1c	1.90 ± 0.20	2.11 ± 0.24	3.21 ± 0.35	3.3[b]
2a			1.09	1.0[c]
3a			0.88	0.96[c,d]
3b	1.97 ± 0.16	2.03 ± 0.11	2.00 ± 0.08	1.96[c,d]
3c			2.07 ± 0.09	2.02[c,d]
4	2.46[e]	2.36[e]	2.49[e]	2.0[f]
5	3.72 ± 0.25	3.70 ± 0.14	3.80 ± 0.28	3.0[g]
6	0.60 ± 0.05	0.56 ± 0.03	0.58 ± 0.09	0.53[h]

[a] Mean values are the results from independent NMR experiments. [b] Determined by the Fmoc couple and cleavage method. [c] Mean value from Fmoc determination and nitrogen combustion elemental analysis. [d] Calculated from the loadings of **2a**, **2b**, and **2c**, respectively. [e] Calculated from the loading of the methoxy substituted compound (see text). [f] From the amount of released product after the coupling with Pbf protected glycine or arginine derivatives. [g] From chlorine and/or from nitrogen combustion elemental analysis after conversion with hydroxylamine to the corresponding oxime. [h] Determined by UV after Fmoc release.

Due to the high chemical reactivity of the amino substituted resins **2a**−**c**, $Cr(acac)_3$ could not be added as relaxation reagent. This resulted in long measurement times, and the ^{13}C NMR spectrum of **2a** was measured only once using a relaxation delay of 30 s between pulses. The NMR result for **2a** (loading = 1.09 mmol/g) agrees with the value of 1.0 mmol/g determined by the Fmoc couple and cleave method with UV detection and combustion elemental analysis of nitrogen. Taking advantage of their chemical reactivity, acetone was added and the resins **2a**−**c** quantitatively converted to the Schiff bases **3a**−**c**, which are stable in the presence of $Cr(acac)_3$. Also for **3a**−**c**, the NMR and Fmoc/cleavage results agree. In analogy, the highly reactive chloro-trityl resin **4** was quantitatively converted to the methoxy substituted compound by adding methanol. For **4** and its precursor resin **5**, loadings evaluated via methods A and B agree and are approximately 20% higher than the chemically determined values.

In Figure 3, the quantitative ^{13}C NMR spectrum of the more complex Fmoc/Pmc derived arginine derivative **6** of the amino substituted Wang resin **2a** is shown. The best swelling of the beads was obtained with the solvent mixture $CDCl_3$/DMF. The evaluated loading (average of 0.58 mmol/g) corresponds to an initial loading of 0.92 mmol/g of the precursor compound **2a**. Most of the NMR signals of **6** have been assigned with the help of ^{13}C chemical shift increment calculations and comparison with compounds of similar chemical structure. The nonassignable carbon signals have chemical shifts in the well-separated aromatic (>100 ppm) or aliphatic (<80 ppm) regions of the ^{13}C NMR spectrum, and the loading of this resin could still be calculated via method B. The structure of the hydroxamic arginine residue has been confirmed by classical analytical methods after cleavage from the solid support.

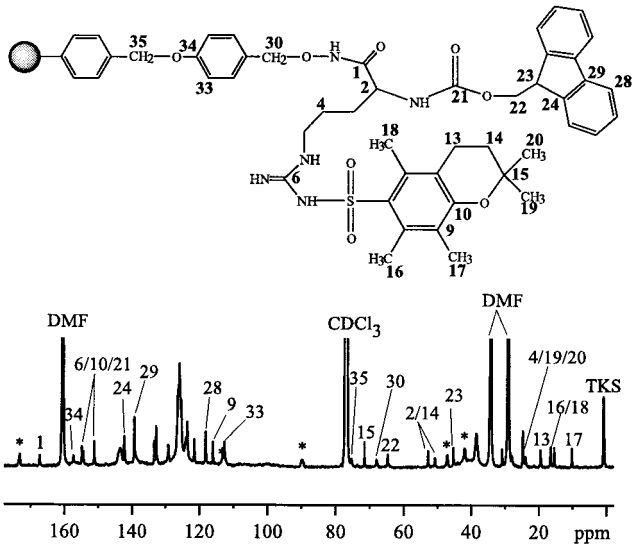

Figure 3. Structure and quantitative $^{13}C\{^1H\}$ MAS NMR spectrum of the Fmoc/Pmc protected arginine Wang resin **6** with assignment of carbon resonances, swollen in DMF/$CDCl_3$, doped with 0.1 M $Cr(acac)_3$, 4096 transients, 1200 Hz MAS rate.

Conclusions

The nature of size, shape, and functional groups of resin beads can have significant effects on the reproducibility and yield of solid-phase organic reactions. These properties vary considerably within and among batches of commercially available beads, and quantifiable analytical methods to study them are required. The NMR method presented here has a wide range of applications, since no functional group acting as a support for the attachment of a quantifiable chromophore must be available in the material of interest. Loadings may be evaluated by integration of NMR intensities of selected resin resonances and signals of an external reference standard (method A) or by using the signals of the resin material as internal standard (method B). Method A is applicable only for resins with a fairly high swelling capacity ($\gtrsim$4 mL/g). Otherwise, method B can be used if well-defined on-resin compounds are studied. The quality of the NMR spectra of SPOS resins is determined by the achievable line widths, and it has been demonstrated for a variety of resins that, by proper choice of the solvent, acceptable spectra can be obtained in most cases.[35] Therefore, the NMR methods are not restricted to the resins studied here. In any case, the complete ^1H decoupling during data acquisition is an important experimental aspect.

A measurement time of 2−3 h per sample is certainly not acceptable for use as a high-throughput analytical method; however, it can be used for batch control purposes of starting resins or cases where other methods are difficult to apply. A similar quantitative experimental protocol based on ^1H NMR spectroscopy would allow an increased sample turnover. Special high-resolution MAS probes should be used for this purpose; however, resolution in ^1H NMR spectra is still an issue. These HR MAS probes can probably not be used for the method presented here, since they do not allow the application of a sufficient proton decoupling field strength and, due to the small sample volume, measurement times for ^{13}C data collection might be too long.[36]

Experimental Section

The ^{13}C MAS NMR spectra were recorded on a Bruker ASX-400 MHz NMR spectrometer at ambient temperature using a conventional 7 mm CP/MAS broadband probe. The samples were prepared directly in the zirconia rotors by weighing known amounts of the resin (50−100 mg, depending on the swelling capacity), the internal reference tetrakis-(trimethylsilyl)silane (4−8 mg), and the solvent (≈350 mg), doped with 0.1 M chromium(III)acetylacetonate. (The use of deuterated solvents is not really necessary, since the spectra were measured without ^2H lock.) Preswelling of the beads in the appropriate solvents before filling the rotor did not improve the NMR spectra. A Teflon spacer was inserted to prevent the drop off of the keflar rotor cap during MAS. Varying rotation rates (0.9−2.7 kHz) have been used to prevent overlapping of spinning sidebands with isotropic carbon signals. ^{13}C single-pulse spectra were acquired in the inverse gated mode at 100.61 MHz with 4.8 μs 90° pulse lengths and a proton decoupling field of 30.5 kHz. The TPPM[33] pulse decoupling has been applied for most of the experiments; the SPINAL-64[34] and WALTZ-16[37] (with variable decoupling strength) decoupling sequences have been tested in several cases. The following parameters generally have been applied: 40 000 Hz spectral widths, 8k data points, 1024 to 4096 transients, and 5 s (30 s) relaxation delays for experiments with (without) relaxation reagent. The T_1 spin lattice relaxation times were determined with the inversion recovery experiment;[38] the results are given as mean of an area and intensity fit of 16 ascending delays (200 μs−14 s). The ^{13}C chemical shifts are given in parts per million (ppm) relative to the signals of chloroform (77.0 ppm) or the reference material TKS (2.4 ppm) as internal standard. All FID's were treated with 5 Hz exponential line broadening. Prior to integration, the blank ^{13}C NMR spectrum of the empty rotor plus Teflon insert obtained with identical experimental parameters was subtracted, and a baseline correction of the spectrum was performed. ^{13}C NMR solid-state spectra of resins **1a**−**c** were recorded on a 4 mm CP/MAS probe with MAS rates of 10 500 Hz and TPPM ^1H decoupling (48 kHz) using a 4.0 μs 90° ^{13}C pulse length, a spectral with of 70 400 Hz, 4k data points, 1500 transients, and 30s relaxation delays. Again, the background signal of the empty rotor was subtracted before integration.

Chloroform-*d* (99.8 atom % D) was purchased from Dr. Glaser AG, CH-Basel; tetrakis(trimethylsilyl)silane (purum, >97%), chromium(III)acetylacetonate (pract., 97%), and dimethylformamide (DMF, puriss p.a., >99.8%) were from Fluka Chemie AG, CH-Buchs. To remove trace amounts of dimethylamine, argon was bubbled through DMF prior to use.

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