Report

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J. Comb. Chem., **2000**, 2 (1), 8-9• DOI: 10.1021/cc990057l • Publication Date (Web): 15 December 1999

A Method for Quantitation of Solid-Phase Synthesis Using ^{19}F NMR Spectroscopy

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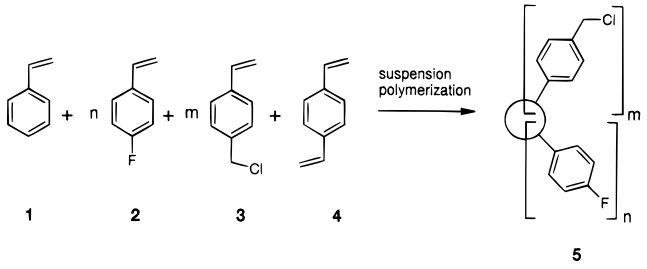
Received October 4, 1999

The past decade has seen a resurgence of interest in solid-phase synthesis (SPS) initiated largely by the emergence of combinatorial and parallel synthesis strategies directed at drug discovery endeavors.[1−3] In solid-phase synthesis of chemical libraries the most time intensive phase of library generation is often the optimization (or validation) of each chemical reaction for all of the library members. Presently, SPS chemistry is commonly analyzed quantitatively after cleavage from the solid support by methods such as HPLC, gravimetry, and ^{1}H NMR.[4] SPS can be qualitatively monitored by techniques such as infrared, ^{1}H, and ^{13}C spectroscopies.[5] However, there have been relatively few reports on techniques for quantitating chemical reactions on polymer-bound moieties.[6] Hence, the development of new methodologies to accelerate the analysis of solid-phase chemistry is critically important. In this communication we describe a simple approach to quantify resin-bound chemistry using ^{19}F NMR spectroscopy in conjunction with a polymeric support bearing ^{19}F as an internal standard.

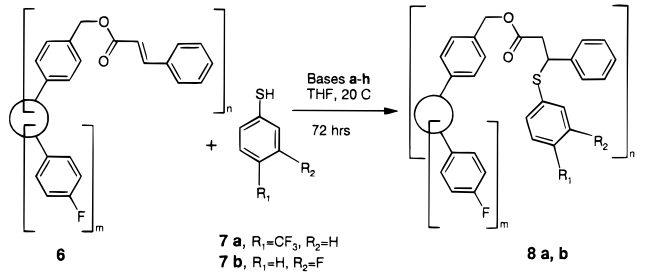
Advantages of using ^{19}F NMR to monitor solid-phase syntheses include high sensitivity (the spin $^1/_2$ nucleus ^{19}F is 100% abundant), wide spectral dispersion (∼200 ppm), simple spectra (one line per nonequivalent F), and nondestructive analysis. Furthermore, the internal standard renders the analysis independent of the resin sample mass. In addition, since nonfluorinated residual solvents are transparent to ^{19}F NMR, the product resin can be analyzed immediately after washing to remove any excess fluorine-containing reagents, and hence time-consuming drying of the resin prior to analysis is unnecessary. While there have been a few recent reports discussing analytical applications of ^{19}F NMR in SPS,[7] to our knowledge quantitation of resin-bound chemical reactions using fluorine as an internal standard has not previously been disclosed.

In practice, SPS quantitation using this methodology requires a solid support having a well-characterized, chemically robust fluorine internal standard and an additional functional group (or linker) for covalent attachment of the desired reactants. We incorporated an internal standard by copolymerization with a fluorinated monomer as depicted in Scheme 1. The fluorinated chloromethylpolystyrene **5** was prepared by copolymerization of styrene (**1**), 4-fluorostyrene (**2**), 4-vinylbenzyl chloride (**3**), and 1,4-divinylbenzene (**4**).[8]

Scheme 1



Scheme 2[a]



[a] Bases: **a**, Cs_2CO_3; **b**, K_2CO_3; **c**, $NaOCH_3$; **d**, LiOH; **e**, $(C_2H_5)_3N$; **f**, $LiN[Si(CH_3)_3]_2$; **g**, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU); **h**, $(n\text{-}C_4H_9)_4NOH$.

It is possible to use alternate approaches such as modification of preformed resins either with a fluorinated linker[7c] or by fractional refunctionalization with a fluorinated capping reagent for this purpose.[7f] The loading of the fluorine internal standard in **5** was determined by combustion and subsequent fluoride ion chromatography.[9]

When a fluorine-containing reactant is bound to the solid phase during a synthetic step, the reaction yield is determined from the ^{19}F NMR peak integration values of the product and internal standard on the resin. This method permits rapid yield measurements at any step in a reaction sequence when a fluorine-labeled reactant is incorporated in the synthetic step. We determined practical levels of internal standard by considering cost of materials and spectral acquisition time. By varying the 4-fluorostyrene content of **5**, we found that an internal standard loading of ∼0.3 mmol F/g resin was sufficient to give good spectra (signal-to-noise ≥ 15) in a reasonable amount of spectrometer time. Thus, using ∼50 mg of resin **5** (loading of 0.32 mmol F/g resin) swollen in $CDCl_3$ in a standard 5 mm NMR tube, the spectral acquisition time was 6 min with a 200 MHz (188.23 MHz at ^{19}F) spectrometer and 1 min with a 500 MHz (470.15 MHz at ^{19}F) spectrometer.[10] The application of magic angle spinning in combination with Nanoprobe technology improves spectral resolution and minimizes sample requirements.

To exemplify this methodology we investigated optimization of the solid-phase Michael addition reactions outlined in Scheme 2. The thiophenols **7a** and **7b** were reacted with the resin-bound cinnamic acid ester **6** using parallel conditions and varying the basic catalyst. The reaction yields given in Figure 1 for product resins **8a** and **8b** were determined by ^{19}F NMR in DMF-d_7.[10] Quantitative reactions occurred with bases **d** (lithium hydroxide) and **f** (lithium hexamethyl-

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10.1021/cc990057l CCC: $19.00
Published on Web 12/15/1999

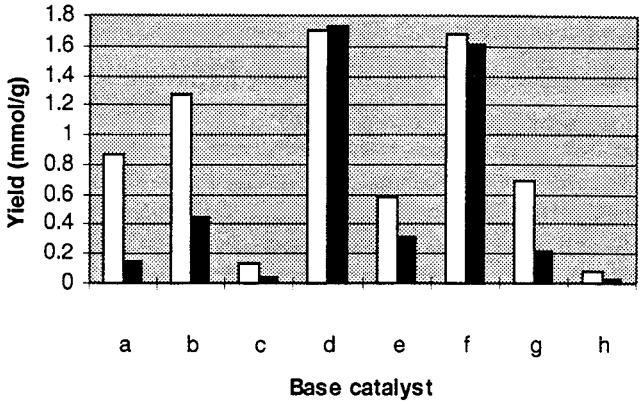

Figure 1. Yields (mmol/g) of resin-bound Michael adducts according to Scheme 2 for resins **8a** (black) and **8b** (white). Base catalysts are as defined in Scheme 2.

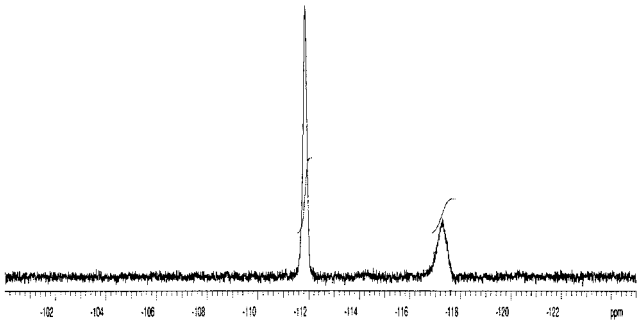

Figure 2. Nanoprobe ^{19}F NMR spectrum of product resin **8b** from base catalyst **g** (DBU). Integration values (ppm): 217 (−111.8); 100 (−117.3).

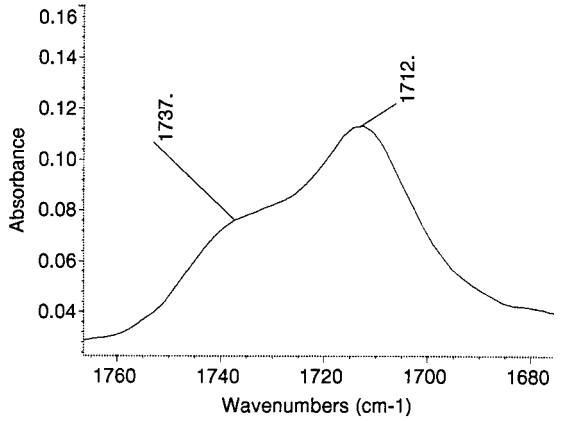

Figure 3. Ester carbonyl stretching absorption of product resin **8b** from base catalyst **g** (DBU).

disilylamide). Figure 2 shows the ^{19}F NMR spectrum of resin **8b** resulting from base catalyst **g** (DBU). The internal standard resonance is at −117.3 ppm and the Michael adduct at −111.9 ppm. On the basis of the internal standard loading of 0.32 mmol F/g resin and the ^{19}F integration values, the product loading was calculated to be 0.69 mmol/g. In contrast, the corresponding infrared spectrum[11] of **8b** shown in Figure 3 clearly indicates an incomplete Michael reaction with base **g** ($\nu_{C=O}$ **6** at 1712 cm^{-1} and $\nu_{C=O}$ **8b** at 1737 cm^{-1}), but affords no quantitative information. In addition, for reactions in which the yield was below 0.25 mmol/g the product was not detectable by IR, but was quantifiable by ^{19}F NMR to a limit of approximately 0.05 mmol/g.[12]

In conclusion, ^{19}F NMR used in conjunction with a fluorine-labeled solid support and fluorine-containing reactants presents a versatile and facile approach to determining reaction yields of solid-supported chemistry. We believe this approach will prove very useful in optimizing solid-phase chemistry for the generation of chemical libraries. The ^{19}F NMR spectra can be acquired rapidly using standard ^{1}H NMR probes and low field strength (e.g., 188 MHz) spectrometers, although the use of high field spectrometers with magic angle spinning and a Nanoprobe affords higher resolution with smaller sample requirements.

Acknowledgment. The authors thank Drs. Andrew Coffey and Graham Margetts of Polymer Laboratories Ltd. (U.K.) for the preparation of polymer resin **5**.

Supporting Information Available. Experimental procedures for the preparations of **5**, **6**, **8a**, and **8b**; analytical data for compounds **5** and **6**; Nanoprobe ^{19}F NMR spectrum of resin **8b** from base catalyst **h**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Arshady, A.; Ledwith, A. *React. Polym.* **1983**, *1*, 159−174 and references therein. See Supporting Information for a typical synthesis of resin **5**. The polymer resin **5** used in this study was prepared by Polymer Laboratories Ltd., U.K.
(9) For leading references on fluoride ion chromatography, see: Dorsey, J. G.; Cooper, W. T.; Siles, B. A.; Foley, J. P.; Barth, H. G. *Anal. Chem.* **1996**, 68, 515R−568R. The fluoride ion chromatography analyses were performed by Quantitative Technologies Inc., NJ. The analysis was replicated five times on each of three different days.
(10) Most of the ^{19}F NMR spectra in the present study were acquired using a Varian 500 MHz Inova spectrometer and a ^{1}H Nanoprobe tuned to ^{19}F nucleus. A relaxation delay of 5 s was used to minimize artifacts due to relaxation in quantitation, and no apodization was applied. Chemical shifts were referenced to $CFCl_3$. Typical samples consisted of 2−4 mg of resin swollen in DMF. The sample was spun at 1500−1700 Hz. We also showed the feasibility of using gel-phase samples in standard 5 mm probes at different field strengths (200, 300, and 500 MHz).
(11) Infrared spectra were acquired at 4 cm^{-1} resolution on a Nicolet model 460 FTIR interfaced to an InspectIR microscope with a Si ATR objective.
(12) Refer to Supporting Information.

CC990057L