JOURNAL OF combinatorial CHEMISTRY

Report

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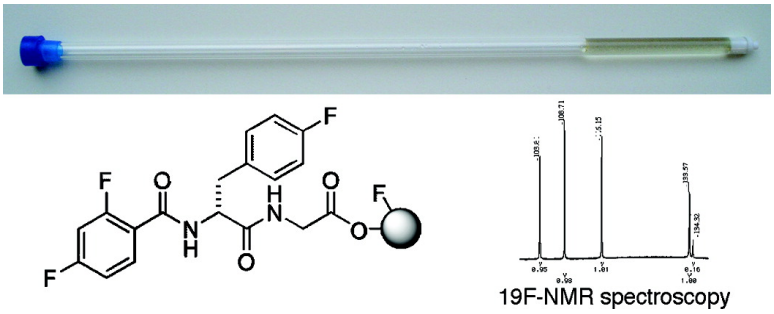
J. Comb. Chem., **2006**, 8 (2), 150-152• DOI: 10.1021/cc050144f • Publication Date (Web): 24 December 2005



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NMR Tube Filter Reactor for Solid-Phase Synthesis and Gel-Phase ^{19}F NMR Spectroscopy

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Received October 28, 2005

With the expansion of solid-phase organic synthesis of nonpeptidic compounds, the need for on-resin monitoring techniques has become apparent. Due to its high information content and fast acquisition on standard NMR spectrometers, gel-phase ^{19}F NMR spectroscopy shows promise to answer that need and is being increasingly used. When performing solid-phase reactions in ordinary reactors, the transfer of resin to and from NMR tubes and preparation of the gel phase constitute an impractical and time-consuming step that might result in loss of resin. In combination with the general trend for miniaturizations in organic chemistry, this made us envision a solid-phase reactor made out of an NMR tube. Herein, we report on the construction of such a reactor and its use.

Gel-phase ^{19}F NMR spectroscopy has been used in several studies to monitor and optimize solid-phase reactions, using fluorine-containing protecting groups,[1−7] when the target molecules themselves contain fluorine,[8−18] or when only the linker,[8,9,13,14,19−22] starting materials,[23,24] or a capping reagent[10] contain fluorine. In addition, ^{19}F NMR spectroscopy has been used to encode a chemical library[25] as well as to monitor reactions on soluble polymers.[26] Fluorinated compounds have received increasing interest in medicinal chemistry over the last 20 years and are now commonly used.[27] Addition of fluorine can influence the reactivity;[28] metabolic stability; and physiochemical, conformational, and binding properties of a compound[27] as well as the conditions used to introduce and remove protective groups.[28] The presence of a fluorine atom also permits the use of ^{19}F NMR spectroscopy to study interactions between small molecules and proteins.[29−33]

One of the advantages with solid-phase synthesis is the possibility to drive the reactions to completion using a large excess of the reactants; however, sometimes the reactants are expensive or require extensive work to prepare, and minimal consumption is therefore desired.[34] By performing the reactions on a smaller scale, this drawback becomes less critical. Moreover, for miniaturized biological screening techniques, such as microarrays, the amount of compound needed for testing has diminished, and often, amounts in the low micromole range are sufficient.[35] Another important factor is the ability to abort reactions that do not work to avoid spending reactants in vain. Here, gel-phase ^{19}F NMR spectroscopy is an excellent tool, since failure is directly observed in the ^{19}F NMR spectrum.

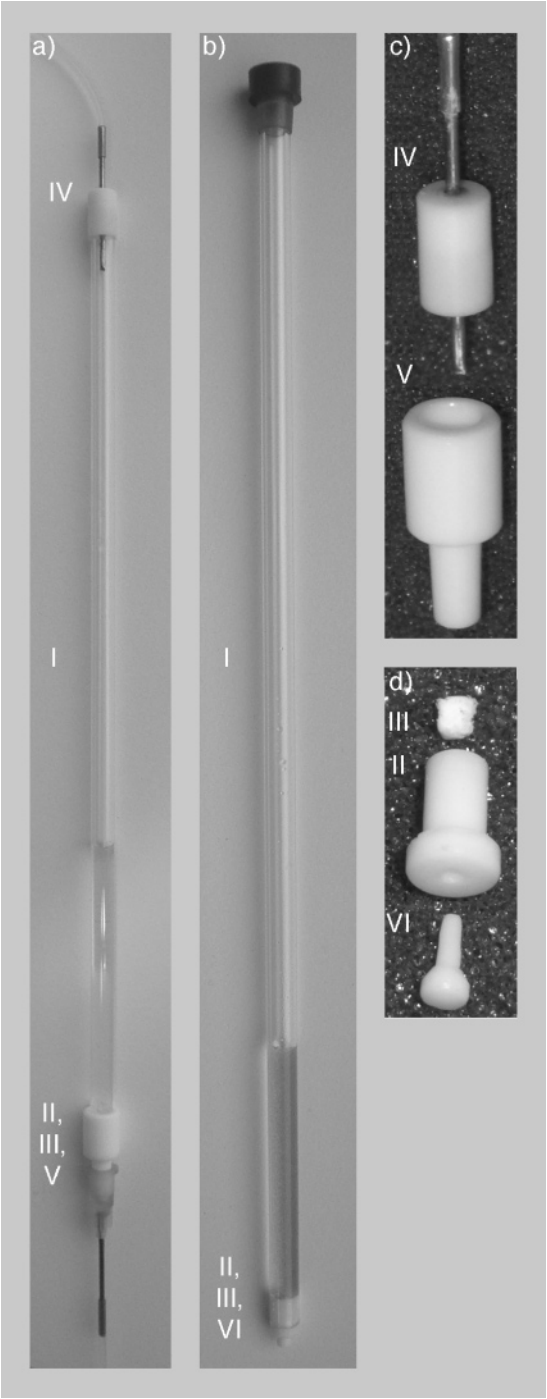

Figure 1. NMR-compatible solid-phase reactor. (a) The reactor during a synthesis. (b) The reactor ready for NMR spectroscopy with an ordinary NMR tube cap at the top. (c) Close-up of the Teflon adaptors IV and V. (d) Close-up of the hollow Teflon plug (II), Teflon filter (III), and Teflon stopper (VI).

To facilitate gel-phase NMR spectroscopy and to decrease the scale, we have designed a modified NMR tube that is suitable for solid-phase synthesis (Figure 1 and Supporting Information). A hollow Teflon plug (II) with a small Teflon

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10.1021/cc050144f CCC: $33.50
Published on Web 12/24/2005

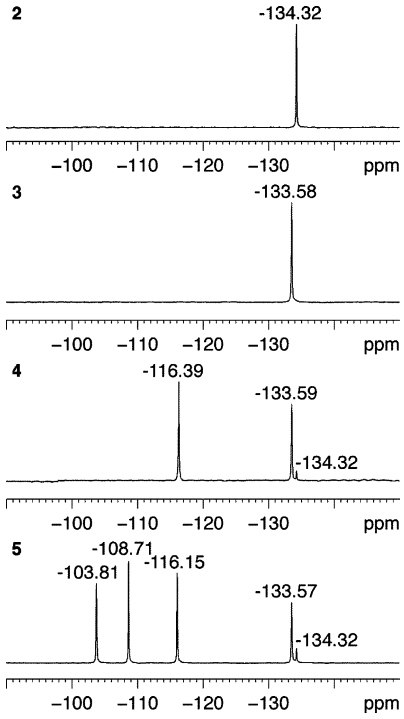
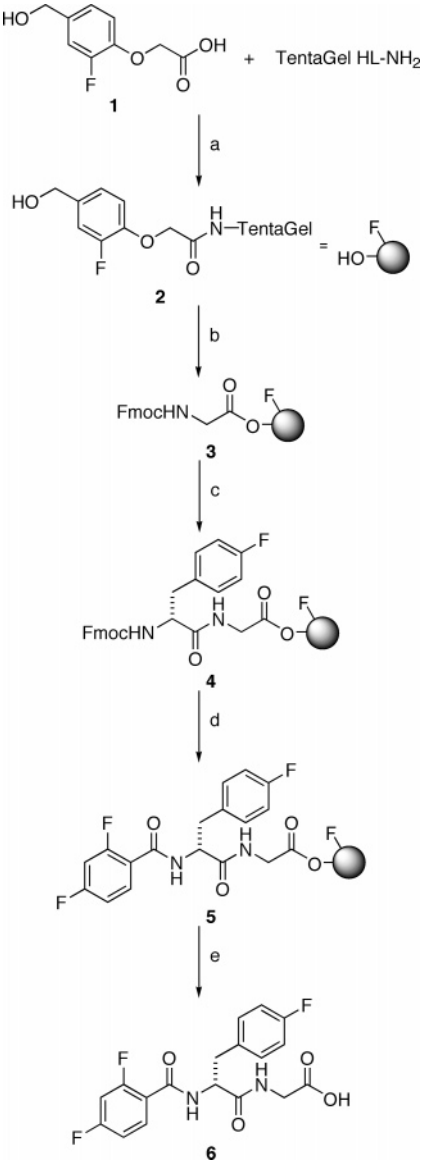


Figure 2. Gel-phase ^{19}F NMR spectra for resins **2**−**5**.

filter (III, 100 μm, cut to fit in II) was inserted into a medium-thick-walled standard borosilicate glass tube (I). By using standard borosilicate glass tubes, cost is reduced and the extra thick walls confer sufficient stability to avoid cracking during reactor manipulations, as compared to standard NMR tubes that are relatively fragile. The so-formed reactor was then connected to a pump through additional Teflon adapters (IV and V). The inlet to the reactor is constructed from a steel tube, connected to a Teflon hose, inserted through the adaptor IV on the top of the tube. Adaptor V is a Luer lock adaptor, made to fit over II and the bottom end of the NMR tube. The Luer lock adaptor (V) is connected to the pump inlet through an ordinary Luer syringe needle, connected to a Teflon hose. With this setup, reactions and washings can easily be performed using continuous flow. After the completion of a reaction, the Teflon adapters (IV and V) can be removed, the hole in the Teflon plug is sealed with the Teflon stoppper VI, and the reactor can be inserted into an ordinary NMR spectrometer. The Teflon plug and filter were adjusted to fit under the coils in the probe in order not to interfere with the signals. Gel-phase ^{19}F NMR spectra recorded showed line widths (around 30−50 Hz, cf. Figure 2 and Supporting Information) similar to those spectra recorded in normal NMR tubes.[6]

To test the reactor, peptide **6** was synthesized (Scheme 1, Figure 2 and Supporting Information). Tentagel HL-NH_2 (0.42 mmol/g, 85 mg, 36 μmol) was added to the reactor and swelled in circulating distilled DMF. The linker **1**,[7] *N*,*N*′-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBT), and bromophenol blue in DMF (1.5 mL) were then circulated through the filtered resin. After completion of the reaction, according to bromophenol blue, the resin was washed with DMF. Any unreacted amines were capped with acetic anhydride and pyridine, and all formed esters were cleaved with sodium methoxide. After extensive washings,

Scheme 1. Solid-Phase Peptide Synthesis



(a) (i) DIC, HOBt; (ii) Ac_2O, pyridine; (iii) NaOMe, MeOH. (b) Fmoc-gly-OH, MSNT, Me-Im. (c) (i) Piperidine, 20% in DMF; (ii) Fmoc-*p*-F-phe-OH, DIC, HOBt. (d) (i) Piperidine, 20% in DMF; (ii) *o*,*p*-difluorobenzoic acid, DIC, HOBt. (e) TFA/water (9:1), 60 °C.

$CDCl_3$ with 0.1% $CFCl_3$ was added to the resin until an even gel was formed. The excess $CDCl_3$ was then filtered off, the reactor was disconnected from the pump and stopped, and a gel-phase ^{19}F NMR spectrum was recorded. This showed a single peak, confirming pure resin-bound linker. With the linker-equipped resin **2** in hand, the peptide was synthesized. Fmoc-Glycine was attached to the benzylic alcohol with 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) and 1-methylimidazole in CH_2Cl_2. Gel-phase ^{19}F NMR spectroscopy showed quantitative yield. Continued amide formations with *N*-Fmoc-*p*-fluorophenylalanine and *o*,*p*-difluorobenzoic acid proceeded in quantitative yield according to gel-phase ^{19}F NMR spectroscopy. However, for each reaction step (Fmoc deprotections as well as couplings), partial cleavage from the resin was observed, resulting in ∼20% free linker. Throughout the whole synthesis and NMR monitoring, the resin was never taken out of the reactor. Circulation of the reaction solution was

essential for completion of the reaction. Peptide couplings performed with only shaking of the reactor became extremely slow, according to analysis with bromophenol blue, probably due to limited diffusion in the narrow reactor. With circulation, the amide bond formations were rapid and essentially complete in less than 1 h.

The resin was then removed from the reactor to allow heating, and the peptide was cleaved from the resin with trifluoroacetic acid (TFA) and water (9:1) at 60 °C for 6 h. Preparative LC/MS gave peptide **6** (4.5 mg, 12 μmol), in 37% yield based on the loading of the resin, with the main byproduct showing a mass corresponding to *N*-(2,4-difluorobenzoyl)-*p*-fluorophenylalanine.

To conclude, we have designed a solid-phase reactor that can be inserted in a standard NMR spectrometer. In addition, we have demonstrated its usefulness by synthesizing a small fluorine-containing peptide. Despite the small scale, a sufficient amount of purified material for characterization and biological testing is obtained with standard resins. During the synthesis, loss of peptide from the resin was detected by ^{19}F NMR spectroscopy, something that would go unnoticed in a nonmonitored synthesis. This study further reinforces the strength of gel-phase ^{19}F NMR spectroscopy as an analytical tool. Our new reactor simplifies resin handling, decreases the time required for synthesis, and opens up possibilities to apply automation.

Acknowledgment. This work was supported by the Swedish Research Council. We thank Svante Jonsson for excellent technical assistance in the construction of the reactor.

Supporting Information Available. Experimental procedures as well as ^{19}F NMR spectra for compounds **2**−**6**. Schematic drawings of the reactor. This material is available free of charge via the Internet at http://pubs.acs.org.

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CC050144F