Report

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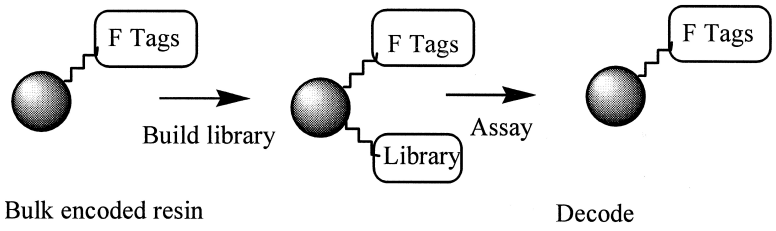
J. Comb. Chem., **1999**, 1 (4), 291-293• DOI: 10.1021/cc9900145 • Publication Date (Web): 08 June 1999



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Received April 14, 1999

Fluorine-19 nuclear magnetic resonance spectroscopy (F-19 NMR) offers an excellent tool for the generation of an encoding method for single beads in combinatorial libraries produced by the mix and split synthetic method. The 100% natural abundance of the spin $^1/_2$ nucleus and the high gyromagnetic ratio of fluorine-19 make this a sensitive nucleus to detect, at approximately 83% the relative sensitivity of ^{1}H NMR. Fluorine is sensitive to its chemical environment, and hence a wide range of chemical shifts are observed for closely related fluorine-containing compounds. This means that numerous fluorine "tags", including many from commercial sources, are available for use. The relative stability of these fluorine tags is also an important positive characteristic, as large batches of bulk encoded resin may be generated and subsequently used for the synthesis of libraries with virtually any chemistry desired. Additionally, the reading of fluorine codes is relatively straightforward as little or no background signal is encountered due to support or, in general, library compounds. If a library member contains a fluorine, the chemical environment and hence chemical shift for the compound is usually different from any encoding tags used, thus distinguishing it from code. Moreover, library compounds are usually fully removed before decoding occurs. We have employed a fluorine encoding method for the first position monomers in our combinatorial chemistry mix and split libraries for the purpose of assistance to mass spectrometry in deconvolution of library hits.[1,2] The ability to acquire NMR spectra of bead-bound compounds by MAS (magic angle spinning) NMR has been widely reported upon,[3] and reaction monitoring in combinatorial chemistry by fluorine NMR has been successfully employed in the past.[4,5]

Our combinatorial chemistry group synthesizes large mix and split libraries containing three sites of diversity, in a format of 25−100 diversity elements per site. As a matter of protocol, we do not mix the pools after the last diversity step, hence the libraries are screened as individual pools of 625 (25 × 25) to 10000 (100 × 100) compounds each. Mass spectrometry is employed as the primary structural deconvolution tool. However, due to mass redundancies in the screened pools, an approach using solely mass spectral analysis leaves multiple compounds which would need to be resynthesized in the absence of any additional structural information, such as a code. Thus we decided to employ a hybrid structural determination strategy which would rely on mass spectral analysis of the active bead coupled with the on-bead analysis of a first position nonreleasable code. Utilizing both of these methods reduces the number of structural possibilities to a single structure in every case. To

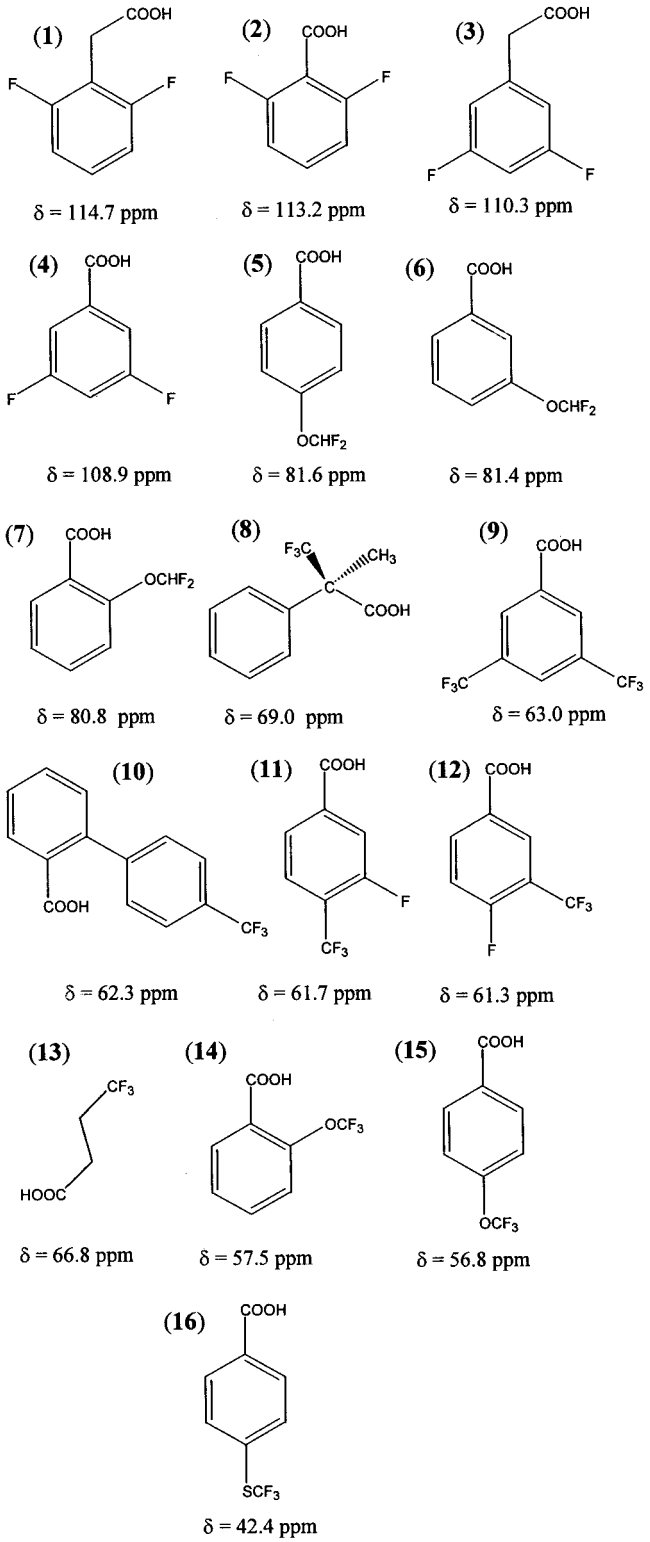
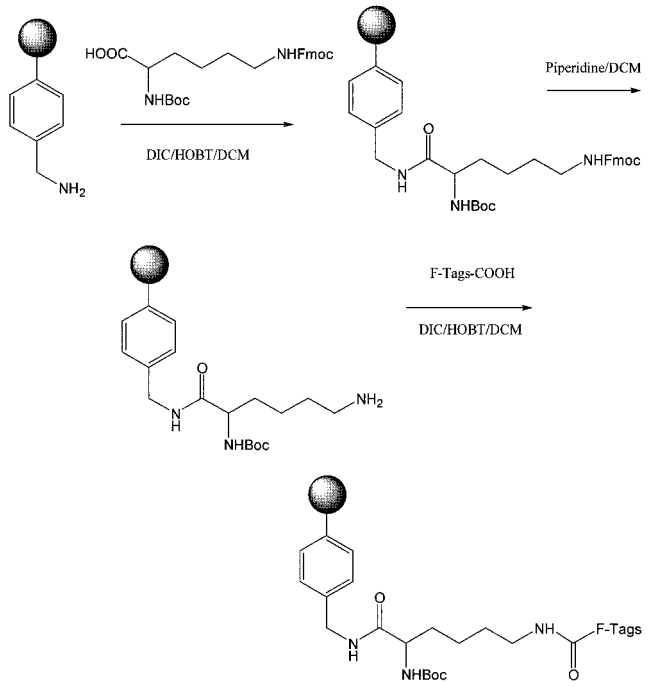


Figure 1. Fluorine-19 NMR tags for library encoding (chemical shifts observed, in DMF-d_7, when attached to resin via an amide linkage).

10.1021/cc9900145 CCC: $18.00
Published on Web 06/08/1999

Scheme 1. Synthesis of Bulk Fluorine-19 Encoded Resin



date, we have successfully employed this technology for the decoding of approximately 10 combinatorial chemistry libraries.

A series of commercially available fluorine-carboxylic acids such as those shown in Figure 1 are used to generate an encoding system for mix and split libraries. Attached to resin via an amide linkage, each tag has a unique, distinguishable fluorine chemical shift. A preference has been made for fluorine-carboxylic acids containing multiple equivalent fluorine atoms as this increases the code signal and hence reduces the spectral acquisition time required for single bead decoding.

Large numbers of codes may be generated from a small set of differentiable fluorine carboxylic acid tags. For a set of N tags, used two at a time, there are $N!/(2!(N-2)!)$ unique combinations (i.e., for 11 tags there are $11!/2!9! = 55$, two-tag combinations, for 15 tags there are $15!/2!13! = 105$, two-tag combinations, etc.). For a set of N tags used M at a time, there are $N!/(M!(N-M)!)$ unique combinations (i.e., for 10 tags there are $10!/3!7! = 120$, three-tag combinations). Typically, we use combinations of two fluorine tags for this purpose and synthesize 50−100 first position encoded resin pools, requiring 11−15 individual tags.

Large batches of encoded resin are initially synthesized as outlined in Scheme 1.

To aminomethylpolystyrene (AMPS) is attached the orthogonally protected linker α-Boc-ϵ-Fmoc-lysine. The ϵ-amine is then Fmoc deprotected under standard piperidine/dichloromethane conditions to allow irreversible amide-linked attachment of our fluorine encoding tags at this site. Premixed combinations of various fluorine-carboxylic acids are attached using the standard peptide coupling method of 1,3-diisopropylcarbodiimide/1-hydroxybenzotriazole catalyzed amide formation for each individual pool. Multiple bead fluorine NMR spectra from these encoded resin pools are acquired and stored as a standard data set for future decoding. Figure 2 depicts a few selected examples of these multiple bead spectra.

These pools of bulk encoded resin are available for the subsequent synthesis of individual combinatorial mix and split libraries. Upon beginning the synthesis of a combinatorial mix and split library, the chemist weighs out resin from the required number of encoded pools, deprotects the α-amine position, and attaches the desired linker and library core at this site. A typical mix and split synthesis then proceeds from this point, and the synthetic chemist does not have to invest any effort into the decoding process.

When active beads from an encoded library are identified, single bead F-19 NMR spectra are acquired with sample swelled in DMF-d_7, in a 10 μL volume, custom-made glass tube[6] (Figure 3) in a MAS NMR probe. A second, single

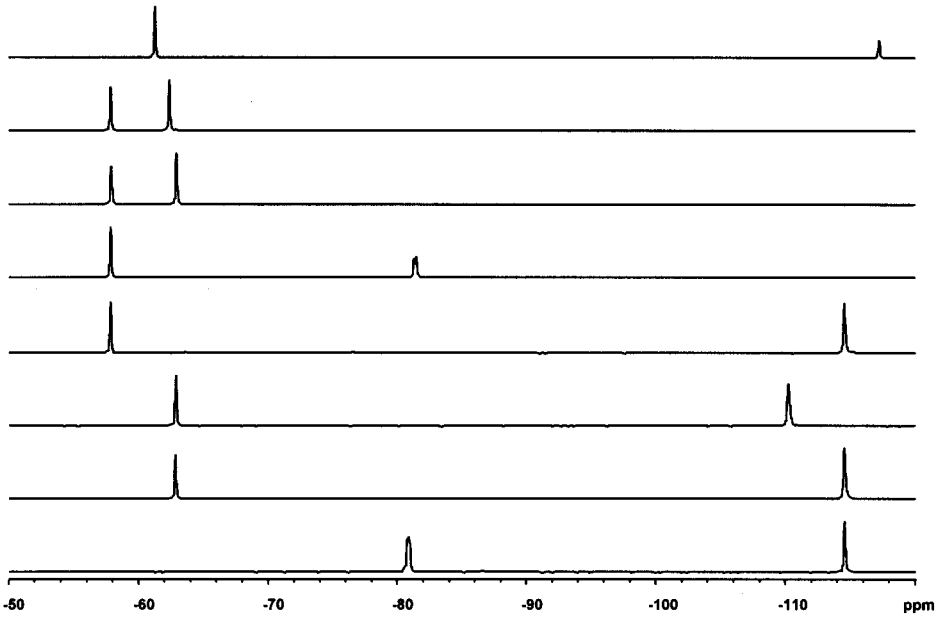


Figure 2. Selected F-19 NMR codes (containing tags, from bottom to top spectrum, of 1 and 7, 1 and 9, 3 and 9, 1 and 14, 5 and 14, 9 and 14, 10 and 14, 11).

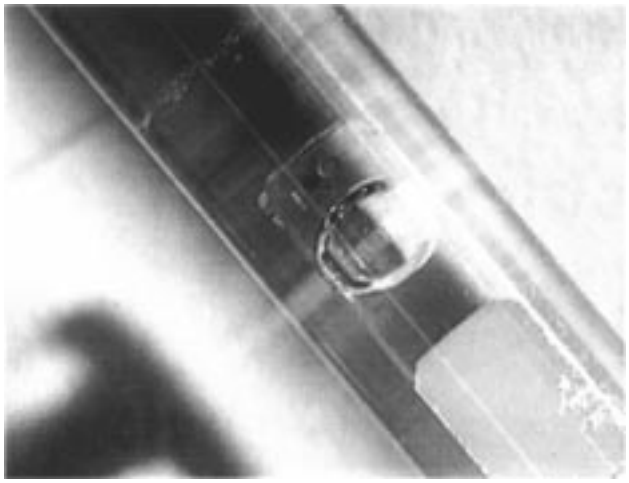
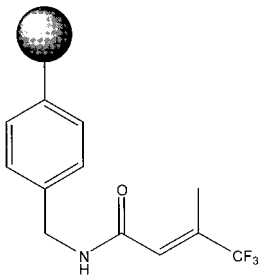


Figure 3. A 10 μL tube for gel phase MAS NMR.

control bead containing **17** is placed along with the bead to be decoded, both as a chemical shift reference and as a check on the progress of signal acquisition. (Note that in Figure 3, the clear bead is the control bead and the darkened bead is that being decoded, the characteristic color change normally being observed when library compound is cleaved from linker.) The time required to obtain a readable F-19 NMR code from a single bead for the typical sample (130 μm aminomethylpolystyrene resin with a library and tag loading capacity of 1.2 mmol/g each) is approximately 1−4 h.



(17)

The effect of solvent on line width was evaluated (Keifer has reviewed the effect of solvent on line width for various SPS resins[7]) both for encoded resin pools before library synthesis and beads to be decoded after library compound was cleaved. As would be anticipated, line widths were substantially broader for beads used for our libraries employing an acid-cleavable linker after sample release with TFA. Presumably the resultant further cross-linking of resin instills more rigidity, hence hinders movement of the fluorine tags. Evaluation of a series of library compounds cleaved from beads demonstrates that this additional rigidity is also somewhat dependent upon the specifics of compound cleaved from bead. Widths at $^1/_2$ height observed for **2** left on bead as a tag after cleavage of six widely varying library compounds range from 71 to 115 Hz in CD_2Cl_2, from 56 to 61 Hz in DMF-d_7, and from 65 to 73 Hz in CD_3OD. Two solvents good for swelling beads after cleavage of library compounds under various cleavage conditions were found to be DMF-d_7 and CD_3COOD. Single bead spectra are compared to the "spectral library" of coded resin batches

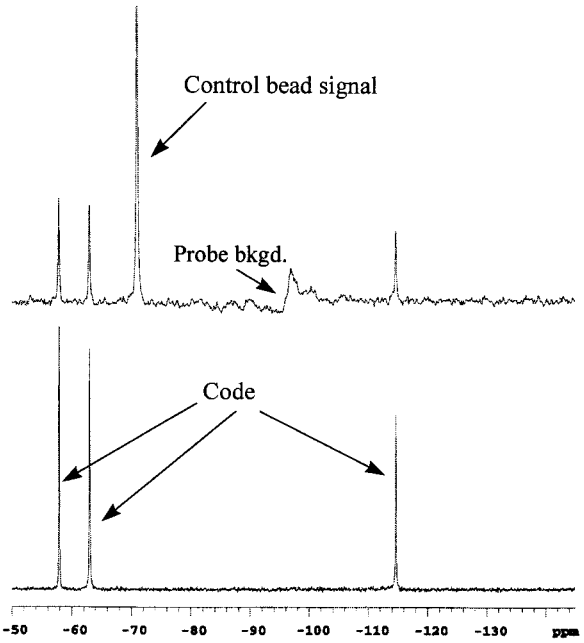


Figure 4. Decoding of a combinatorial chemistry library bead by ^{19}F NMR.

which were generated on the large resin batch as multiple bead spectra as shown in Figure 4.

The coding for first position monomers in our combinatorial libraries offers an excellent adjunct to the deconvolution of library hits with mass spectrometry. While this technique offers a rather low throughput, the "spent bead" after sample release is queued for analysis simultaneous to mass spectral analysis. Additionally, automation is employed for the decoding step allowing analysis of 10−20 samples/day. Efforts are currently underway to further reduce the decoding time per bead.

Acknowledgment. The authors thank Dr. Warren Wade and Dr. Daniel Norbeck for valuable input and suggestions.

Supporting Information Available. Experimental details and ^{19}F MAS NMR spectra of encoded resin pools. This material is available free of charge via the Internet at http://pubs.acs.org.

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