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Discovery of Selective Metal-Binding Peptoids Using ^{19}F Encoded Combinatorial Libraries

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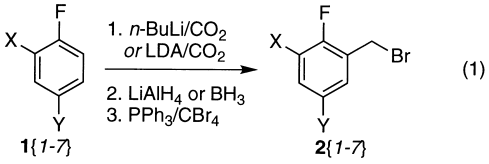
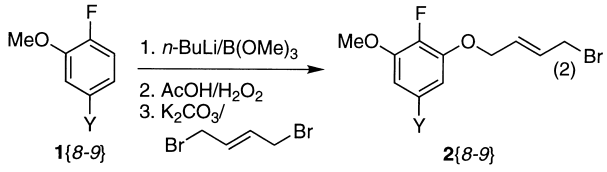
Abstract—A method for encoding solid-phase split/mix combinatorial libraries using the chemical shift of synthetic fluoroarenes ('F-codes') has been developed. They have wide chemical shift dispersion and are detectable at the sub-μmol level. ^{19}F NMR is used for decoding. Nine fluoroarenes bearing linkers for attachment to solid-phase synthesis supports through a photocleavable group were prepared. A library of 90 *N*-alkylglycines bearing substituted succinamides was prepared on solid phase from nine amines, in which the amine is encoded by the fluorinated tag, and 10 anhydrides. Metal binding studies followed by decoding identified unique, specific binders of copper(II) and iron(III) with μM K_Ds. © 2000 Elsevier Science Ltd. All rights reserved.

The power of split-pool synthesis in the preparation of 1-bead/1-compound combinatorial libraries has been made manifest through a wide range of studies.[1] In specialized cases such as peptides, beads identified as bearing a library member with a desired property can be subjected to direct structural analysis.[2] More often, a code molecule is added simultaneously with molecular building blocks during solid-phase synthesis, and a binary encoding methodology[3] is used to identify the structures of compounds derived from single beads through an easily read molecular code. Encoding molecules include secondary amines,[4] isotopes,[5] and α,ω-diols bearing electron-deficient aromatics,[6] and decoding methodologies include fluorescence-detected HPLC, mass spectrometry, and gas chromatography with electron-capture detection. These methods require specialized techniques or instrumentation not broadly available.

Our goal was to develop an encoding method using the most broadly available structural analysis tool, NMR. Previous discussion of this concept has been sparse,[7] but a related study using high-resolution MAS NMR techniques has recently appeared.[8] We chose ^{19}F NMR because of fluorine's large chemical shift dispersion (~1000 ppm overall, ~200 ppm in organofluorine compounds), so that it should be possible to analyze many tags that are unambiguously separated by a significant chemical shift difference. There is also essentially no background of fluorine in the chemical laboratory environment, making ^{19}F NMR a high-sensitivity analytical technique. The newly-synthesized molecular tags are aryl fluorides whose chemical shifts are exquisitely sensitive to substitution. They have an *ortho* link to the support that eliminates one large H–F coupling. The other *ortho* position is also substituted. The *para* position is available for substitutions that also affect the fluorine chemical shift.





A series of nine fluorinated aromatics (**2**) bearing a linker for attachment during solid-phase synthesis was prepared by fluorine-directed metalation (eqs 1 and 2).[9] The ^{19}F NMR chemical shift dispersion of this set of tags (determined at the stage of the alcohols, the state in which they are read) is >62 δ, and the minimum chemical shift separation between tags is 4.7 δ (Fig. 1). This synthetic route makes readily accessible up to 16 fluorinated tags with (calculated[10]) chemical shift dispersion of 80 δ and minimum separation of 4 δ. Tags can be

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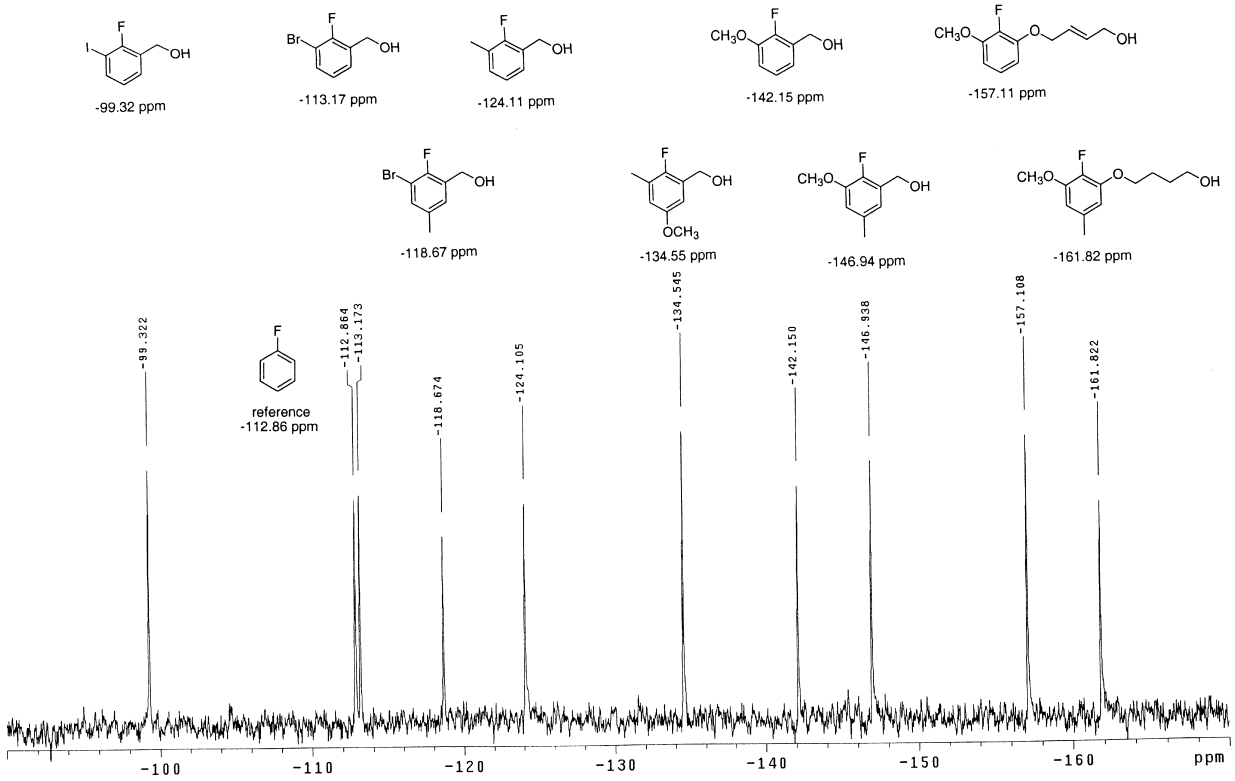
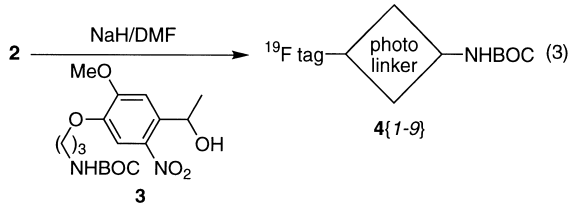


Figure 1. ^{19}F NMR spectrum of nine F-codes released by irradiation from encoded synthesis beads.

detected at 376 MHz with 10:1 S/N ratio in a capillary microcell at a concentration of 5 mM in 13 μL $CDCl_3$ (65 nmol) in a 40 min acquisition. Tags were adapted for peptoid synthesis[11] and photochemical release by alkylation with **3**, obtained from the Holmes photolinker[12] through a Curtius reaction.[13]



A peptoid library was designed using a set of nine R_1 amines (Chart 1) and ten R_2 anhydrides (Chart 2). Synthesis was performed on 3 g (2.85 mmol) 500 μM polystyrene benzylamine beads[14] (0.95 mmol/g, 83 nmol/bead), which were bromoacetylated.[15] Nine groups of beads were formed and individual ^{19}F-encoded tags were added to each as amines (**5**) (0.7 M in DMSO, rt, 2 days), obtained by TFA treatment of **4**. After bromoacetylation, the amine diversity components (**6**) were added (1 M in DMSO, rt, 12 h) and all groups of beads (**7**) were pooled. These beads were split into 10 pools and an anhydride (**8**) was added to each pool (DMF, rt, 12 h). The protecting groups were removed from amino alcohol building blocks by treatment with *n*-Bu_4NF (1 M, THF, rt, 10 h). Pools (**9**) were kept separate for screening.

Bead pools were tested for metal binding by treatment with $Cu(OTf)_2$ in acetonitrile (rt, 48 h) at concentrations increasing from 0.1 mM. One pool (No. 6) showed many sky-blue-colored beads at 4 mM, and no other pools were colored at concentrations up to 100 mM.[16] Ten blue beads were selected from this pool and subjected to irradiation in THF at 350 nm for 12 h. The released tags were isolated by filtration/evaporation and

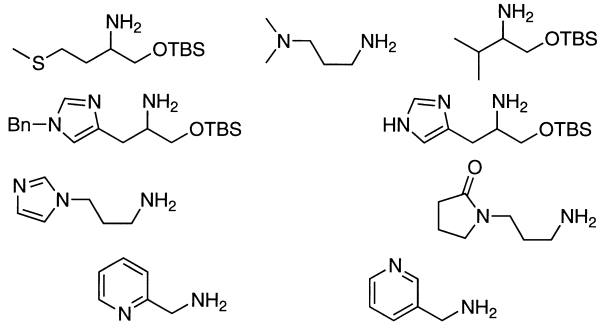


Chart 1.

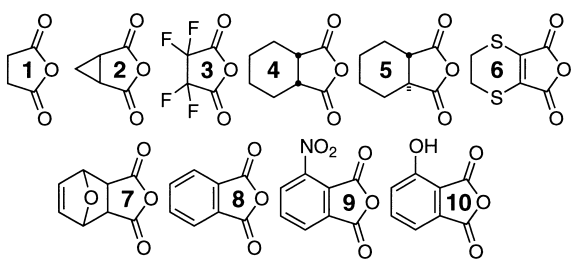
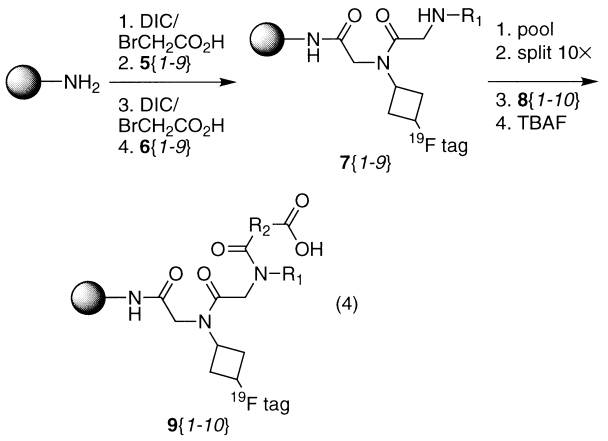


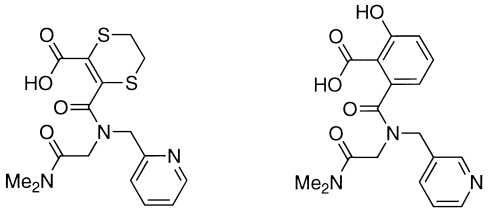
Chart 2.

submitted to ^{19}F NMR analysis in the presence of an internal standard. A single peak was observed (Fig. 2) corresponding to a single amine. The structure of the optimum copper-binding library member was thereby deduced. Determination of its copper-binding properties in solution required significantly larger quantities of the compound than were prepared on solid phase. In order to simplify the synthesis compared to the initial mono-*N*-alkylamide peptoid, a dimethylamide peptoid with the same binding groups was prepared by conventional synthesis.



Compound **10** was obtained as its triethylammonium salt, which shows a 44 μM K_D for copper triflate as measured by spectrophotometric titration[17] at 390 nm. While the affinity of this peptoid for copper might be slightly modified by the change from the mono-*N*-alkylamide to a dimethylamide peptoid, the selectivity should be similar.

Screening of this same library for binding to a second metal ion, iron (as Fe(2-ethylhexanoate)$_3$ in THF), led to staining of only one pool (No. 10 — 3-hydroxyphthalic). A gradation of intensity permitted the binding beads to be stratified into four groups. Decoding of the most intensely stained group or a frequency-weighted analysis of the appearance of individual building blocks led to the same optimum amine. Preparation by conventional synthesis of the 3-pyridylmethylamine/3-hydroxyphthalamide peptoid **11** led to a triethylammonium salt whose iron binding was evaluated (485 nm). It shows a 31 μM K_D for iron 2-ethylhexanoate.



10 **11**

This study establishes a novel method for decoding of combinatorial libraries using NMR instrumentation present in essentially all chemical laboratories and fluoroaromatic tags that are resistant to many chemical reaction conditions. In addition, because this library incorporates a photolinker to the tags, they are readily released for NMR analysis in solution, rather than requiring a solid-phase NMR method. In this application, the fluoroaromatics perform as analogue codes in that they directly represent building blocks in the library. The frequency of appearance of a tag is therefore a direct measure of the performance of a building

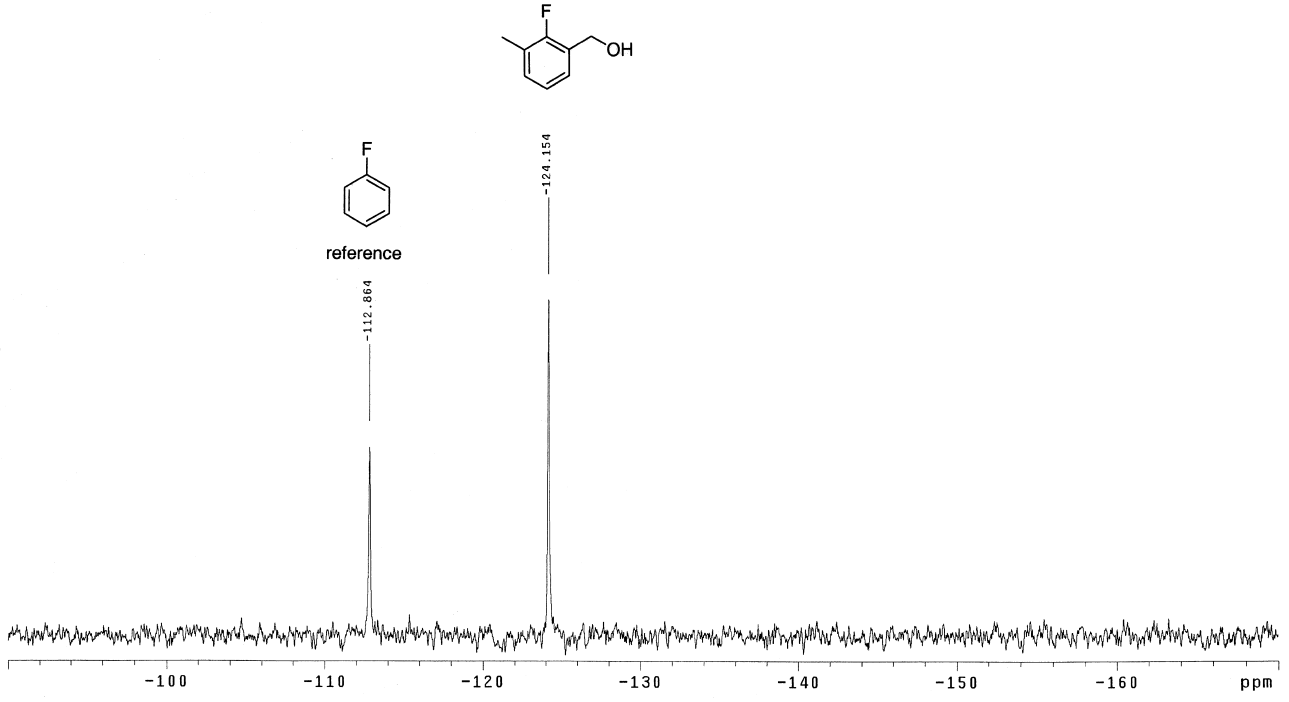


Figure 2. ^{19}F NMR spectrum of one F-code released by irradiation from copper-binding encoded synthesis beads.

block in the assay. This concept is distinguished from earlier binary (digital) encoding methods, and should be useful for kinetic studies and the like. The structural variation leading to selective binding of iron or copper are non-intuitive (e.g., 2- vs 3-pyridylmethylamine) and could only be discovered empirically. This method has been used here in organic solvents. Direct screening for biological activity would require aqueous solvents, which should be compatible with this method using hydrophilic, PEG-grafted support beads.

Acknowledgements

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