A General Method for Molecular Tagging of Encoded Combinatorial Chemistry Libraries

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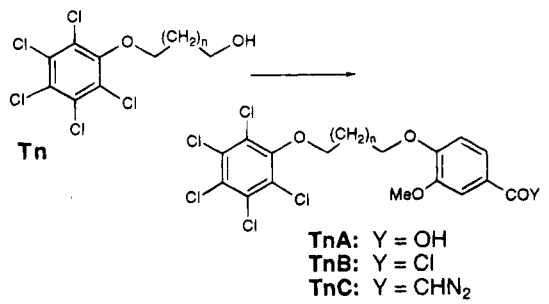
Summary: Acylcarbenes can be used to attach chemically inert, vanillin-linked tagging molecules directly to an unfunctionalized Merrifield polystyrene solid support and thus to allow encoding of combinatorial libraries prepared by a wide range of chemistries.

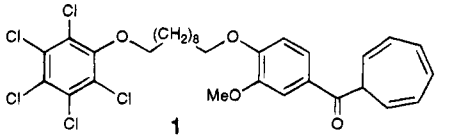
For complex problems in molecular design, screening massive libraries of diverse molecules prepared by combinatorial chemistry offers a promising alternative to deterministic design. Until recently, such libraries having $>10^4$ members were limited to libraries of oligonucleotides and peptides because direct structure elucidation is generally problematic on the available (picomolar) quantities of individual library members.[1,2] The breakthrough came with *encoding*, a technique that labels each individual library member with a unique array of readily analyzable molecules called *tags*.[3] Each tag array forms a kind of molecular barcode that can be read to determine the structure of associated library members. In all previously described procedures for encoded combinatorial synthesis, tags are added to reactive functional groups (*e.g.*, amines) that are attached either directly or indirectly to the library member.[4,5] However, handling tag-attaching functionality not only complicates library synthesis but can also place undesirable constraints on the reagents and reaction conditions that can be used. In this paper, we describe a new technique for tagging polystyrene-supported combinatorial libraries that requires no particular tag-attaching functional groups other than those (*e.g.*, phenyls) which make up the polymer matrix.

To tag unfunctionalized organic material, we have developed a new type of tagging reagent that is composed of a tag plus a linker bearing a precursor of a reactive intermediate that reacts with a variety of common organic moieties. The tags themselves have been described previously.[5] They are halophenol derivatives (*e.g.*, **Tn**) which are both chemically inert and conveniently analyzed at subpicomolar levels by electron capture gas chromatography (ECGC). The linker is used to attach the tag to the solid support and to facilitate its subsequent detachment for ECGC analysis. In this work, the linker is a derivative of vanillic acid (3-methoxy-4-hydroxybenzoic acid)—the complete tagging reagent is shown above as **TnC**. This construct incorporates diazoketone functionality that can be converted to a reactive acylcarbene for direct attachment to a polymer matrix and a catechol diether moiety that can be cleaved oxidatively to effect tag release for analysis.



Synthesis of the tagging reagent is straightforward. Starting with a tag alcohol (**Tn**), a Mitsunobu reaction is used to attach the linker precursor, methyl vanillate, yielding **TnA** (~60% yield) after saponification (LiOH, MeOH). Oxalyl chloride then gives acid chloride **TnB**, and excess diazomethane yields the tagging reagent **TnC** (~75% from **TnA**). These reagents are stable, yellow solids that can be stored for periods of months at room temperature. Experimental details are provided in the supplementary material.

Our first experiments with tagging reagent **T8C** (**TnC** where $n = 8$) were directed toward finding a suitable catalyst for diazoketone decomposition and a solvent which would both swell the polymer and react only slowly with the generated acylcarbene. We began with 4:1 mixture of CH_2Cl_2:benzene where the benzene was taken as a soluble analog of polystyrene. We found that **T8C** reacted rapidly and cleanly with $Rh_2(OAc)_4$ or $Rh_2(O_2CCF_3)_4$[6] to give a new material that we characterized as cycloheptatriene **1**. Benzene/acylcarbene adducts such as **1** have been described previously.[7] Though both Rh(II) catalysts gave **1** cleanly, we prefer $Rh_2(O_2CCF_3)_4$ because of its high solubility in CH_2Cl_2.



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Our new tagging reagents (**TnC**) are also able to bond directly to polystyrene synthesis beads. However, be-

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(1) Review: Pavia, M. R.; Sawyer, T. K.; Moos, W. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 387 and other papers in that volume. Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233.

(2) Mass spectroscopy has recently been used to analyze simple peptides on single synthesis particles: Brummel, C. L.; Lee, I. N. W.; Zhou, Y.; Benkovic, S. J.; Winograd, N. *Science* **1994**, *264*, 399. It is not clear how such a direct approach would deal with isomers or the impure products which typically result from the synthesis of non-oligomeric compounds.

(3) Brenner, S.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5381.

(4) Tagging using biopolymers: (a) Kerr, J. M.; Banville, S. C.; Zuckermann, R. N. *J. Am. Chem. Soc.* **1993**, *115*, 2529. (b) Nikolaiev, V.; Stierandova, A.; Krchnak, V.; Seligmann, B.; Lam, K. S.; Salmon, S. E.; Lebl, M. *Peptide Res.* **1993**, *6*, 161. (c) Nielsen, J.; Brenner, S.; Janda, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 9812.

(5) Tagging using chemically inert organic small molecules: Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922.

(6) Johnson, S. A.; Hunt, H. R.; Neuman, H. M. *Inorg. Chem.* **1963**, *2*, 960.

(7) McKervey, M. A.; Russell, D. N.; Twohig, M. F. *J. Chem. Soc., Chem. Commun.* **1985**, 491.

(8) Tags appear chemically bound to the solid support particles as judged by comparisons of tag loading before and after 100 washings with CH_2Cl_2 (each washing: 15 min agitation on a wrist action shaker).

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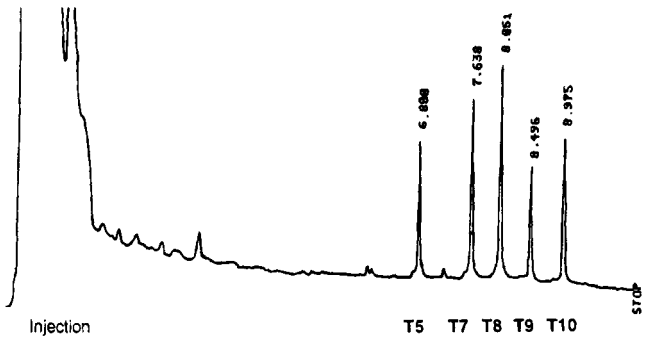
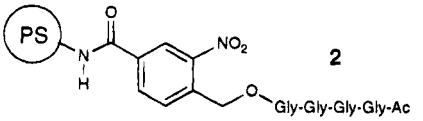


Figure 1. Electron capture gas chromatogram of tags **Tn** from a single synthesis bead.

cause the polystyrene is localized in the form of solid particles, the acylcarbene/arene coupling is less efficient and is accompanied by substantial dimerization yielding a stilbene-like byproduct. Though wasteful of tag, this dimerization is not a serious problem because the soluble dimer is readily removed from the synthesis beads by washing. No other soluble products appear to be formed, and 10–20% of the original **TnC** ends up bonded to the solid support. The best procedure we have found involves thoroughly mixing 50–100 μm Merrifield polystyrene beads with a CH_2Cl_2 solution containing 0.002% of the $Rh_2(O_2CCF_3)_4$ catalyst. Next, a reagent-coding mixture[5] of diazoketone tags (50 mg **TnC** per gram of beads) in CH_2Cl_2 is added with vigorous agitation in three to four equal portions. The diazoketone decomposition and coupling reactions are very fast, provided that any basic groups on the beads (*e.g.*, amines) are either protonated or otherwise protected. This procedure leads to almost uniform tagging of the solid support at a loading corresponding to ~1 pmol of tag per bead.[8]

To read the synthetic information encoded by the vanillin-linked tags, the tags on a particular solid support particle are released oxidatively. Thus, the tags on a single bead are read by first sonicating the bead in a melting point capillary containing 3 μL of hexane and 1 μL of 0.5M ceric ammonium nitrate in 1:1 water:acetonitrile. After centrifugation and removal of the aqueous phase, silylation (*bis*-trimethylsilacetamide) of the released tag alcohols (**Tn**) gives the sample for ECGC analysis. Control experiments with polystyrene supports tagged by an amide bond at the known loading of 0.8 mmol/gm of support using **T8A** indicate that oxidative release occurs in >90% yield. Using the preceding recipes for tag addition and release, we find that tag arrays can be read unambigously from single beads in ≥95% of the cases. For example, Figure 1 shows an ECGC analysis of tags **T5**, **T7**, **T8**, **T9**, and **T10** which were released oxidatively from a single Merrifield synthesis bead (loading ~1 pmol **Tn**/bead).

Because of the extraordinary sensitivity of ECGC of our halogenated tagging molecules, beads need to be tagged at a level (0.5–1 pmol/bead) corresponding to only 0.5–1% of the loading of the library member being synthesized (~100 pmol/bead). Consequently, the tagging procedure does not interfere with the library sythesis, whether the tagging acylcarbene adds to polymer matrix or to the attached library member. Nevertheless, we carried out an experiment to establish the selectivity of our acylcarbenes for attachment to polymer or library member in a simple example. To this end, we prepared the polystyrene-bound, *o*-nitrobenzyl ester-linked tetrapeptide **2**. We tagged this material using reagent **T7C**



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as described above. By photolysis of the tagged polymer in acetonitrile (λ = 350 nm, 18 h), we were able to release the acetyltetraglycine fragment into solution and to remove the polymer beads. We then treated the tetraglycine fragment solution and the beads separately with ceric ammonium nitrate to release the tag alcohol **T7**. ECGC analysis of these two oxidation products indicated a ratio of tag from the tetraglycine solution to tag from the solid support to be ~1:8. Since our support was functionalized by tetraglycine at a loading of ~0.8 mmol/gm of polystyrene, this ratio corresponds approximately to the relative weights of the polymer matrix and the tetraglycine in **2**. This result suggests that our acylcarbenes add to both polymer and library member with little discrimination. Thus, a typical combinatorial synthesis using 25 tags can be expected to be accompanied by an overall tag-induced destruction of ≤5% of the library member on each bead. Even if the tagging reagents sought out the compound being synthesized and attacked it exclusively, a combinatorial synthesis encoded with 25 tags would still yield 75–85% of tag-free product.

The acylcarbene tagging method described here is a major advance over previous tagging procedures. Not only are no specific functional groups required for tag attachment, but the tags and linkers are generally inert to the often vigorous reaction conditions that are commonly used in organic synthesis. Reagent insensitivity is one of the key advantages of the tagging scheme outlined above, especially in comparison to alternative tagging methods based on biopolymers.[4] Thus, the problem of structure determination in encoded combinatorial libraries is effectively solved by the method described above in a way that is compatible with a wide range of chemistries. The challenge now is to develop solid phase synthetic reactions leading to valuable classes of molecules and screening methods that allow the most interesting compounds to be selected from a combinatorial library.[9]

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Supplementary Material Available: General experimental procedures and characterization data (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(9) *E.g.*: Ellman, J. A.; Bunin, B. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997.