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OBOC Small-Molecule Combinatorial Library Encoded by Halogenated Mass-Tags

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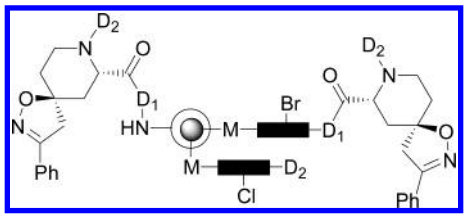
ABSTRACT



A bromine-/chlorine-containing mass-tag encoding strategy for a small-molecule OBOC combinatorial library is reported. The resulting MALDI FTMS isotope pattern of each tag clearly defines the component building blocks of each "hit" bead in an 1890-member demonstration library screened on-bead for binding against streptavidin via both enzyme-linked colorimetric and Quantum Dot/COPAS assays.

While "one-bead one-compound" (OBOC) combinatorial strategies[1] have been successfully applied to the discovery of target ligands,[2] a continuing objective in this area is the development of encoding strategies for the rapid and reliable determination of the identity of a single bead's ligand. Various encoding methods have been reported,[3] with Edman degradation and MALDI FTMS analysis of the coding tag in topologically segregated bifunctional (TSB) beads proving to be particularly advantageous in decoding peptide-based OBOC libraries.[4] That said, each method still has its limitations. For example, for peptide-encoded libraries, decoding with Edman degradation requires HPLC retention time information for all possible PTH derivatives. MALDI FTMS employing nonhalogenated mass-tags do not always unambiguously define each building block, and because some mass tags may contain building blocks that are either difficult to ionize or easily fragmented, impurity and/or fragment peaks often confuse the encoding analysis. Recently, a ladder synthesis approach using brominated tags has been employed to partially address these issues.[5] In this paper, we fully address these limitations with a novel Br-/Cl-MS tagging strategy. This is manifested in the preparation of an 1890-member library from which streptavidin-specific ligands were identified using a Quantum Dot (Qdot 605 streptavidin

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[||] Contribution for MALDI-FTMS.
[#] Contribution for X-ray crystallographic data.

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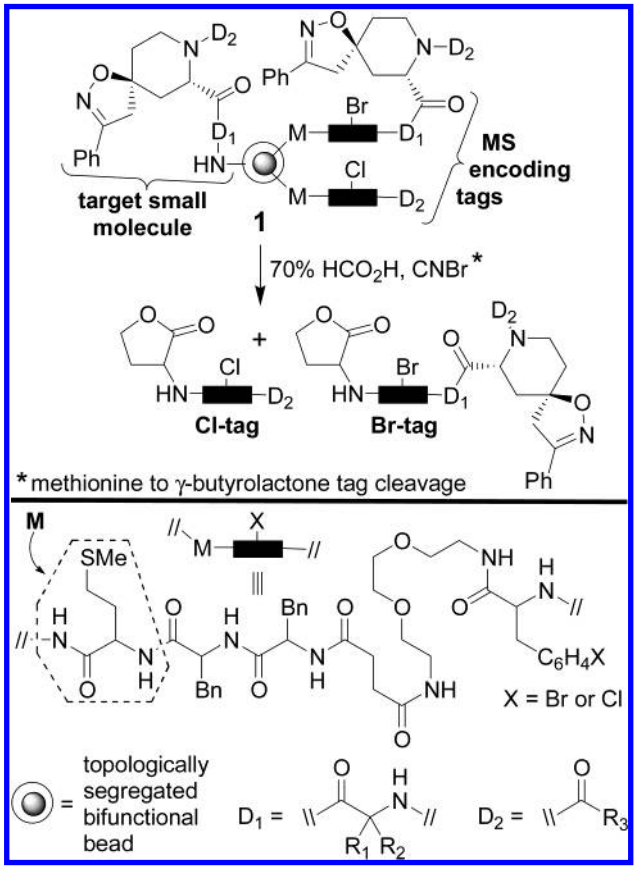


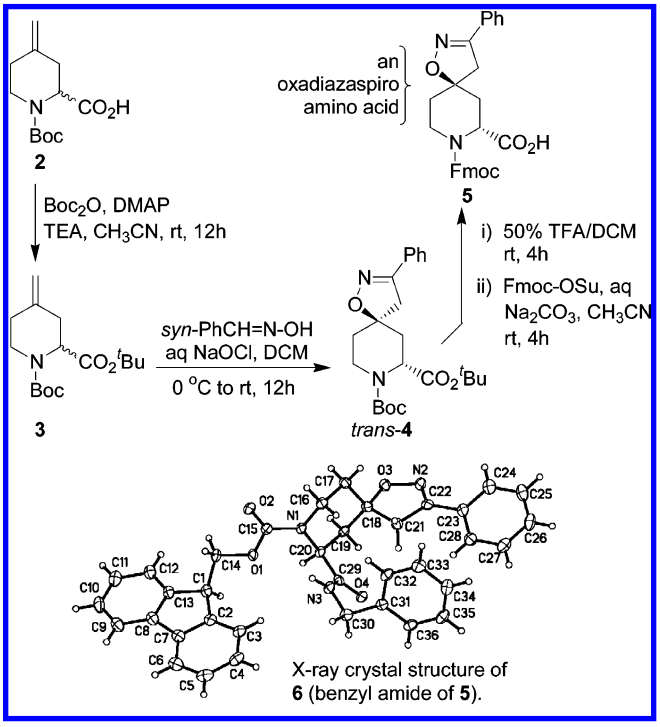
Figure 1. Br-/Cl-MS coding tagging strategy.

conjugates)[6]/COPAS on-bead assay as well as a more traditional on-bead enzyme-linked colorimetric assay. Quantum Dot nanoparticles are emerging as an alternative to organic fluorescence probes in cell biology and biomedicine, offering several advantages.[7,8] COPAS is an automated fluorescent-activated bead sorter.

Our coding strategy is outlined in Figure 1. Topologically segregated bifunctional (TSB) beads were manipulated to deliver bead **1** with Br- and Cl-tags on the bead's interior and the small-molecule compound on the bead's exterior. Subsequent cyanogen bromide treatment liberates the mass tags (methionine → γ-butyrolactone) and MALDI FTMS then defines the building blocks employed.

This method offers several advantages. First, each mass tag is easily differentiated by its unique isotope pattern (e.g., the Cl-tag isotope pattern is unique from the Br−tag isotope pattern; see Figure 2). Second, each diversity element can be associated with a specific isotope pattern; therefore, the diversity inputs are isotope resolved from one another. Third, confusion caused by fragment peaks is avoided since isotope labeling uniquely associates these fragments with one

Scheme 1. Preparation of Oxadiazaspiro Amino Acid **5**



diversity input (in non- or monoisotope labeling strategies, a given fragment peak can potentially arise from any diversity input). To establish this methodology, we prepared an 1890-member library with two diversity inputs: see D_1 and D_2 in Figure 1. By way of an orthogonal protecting group strategy, the Cl-tag (Dde protected;[9] introduced via **8** in Scheme 2) captures only the D_2 diversity element, while the Br-tag (Boc protected; introduced via **9** in Scheme 2) sequentially incorporates the building block inputs for the entire small molecule target. By subtraction {mass (Br-tag) − mass (Cl-tag) − mass ($C_{14}H_{14}N_2O_2$ from **5** [see Scheme 1]) − mass (bromine minus chlorine)}, the Br-tag unambiguously defines the D_1 input. We chose this strategy so that MALDI FTMS analysis would evidence complete and successful construction of the target small molecule. One can easily envision Cl- and Br-tagging strategies where each tag directly codes for one diversity element, as is demonstrated here with the Cl-tag.

The unnatural oxadiazaspiro amino acid **5** was prepared as outlined in Scheme 1. Amino acid **2** was *t*butyl ester-protected[10] to **3**. Subsequent 1,3-dipolar cycloaddition gave **4** as a 2.5:1 mixture of diastereomers with the major isomer having trans stereochemistry as established by X-ray crystallographic analysis of the corresponding *N*-Fmoc benzyl amide **6**. Amino acid **5** was obtained by TFA cleavage of both protecting groups in *trans*-**4** followed by Fmoc protection. Starting amino acid **2** was prepared by modified

(6) Commercially available from Quantum Dot Corporation (http://www.qdots.com).

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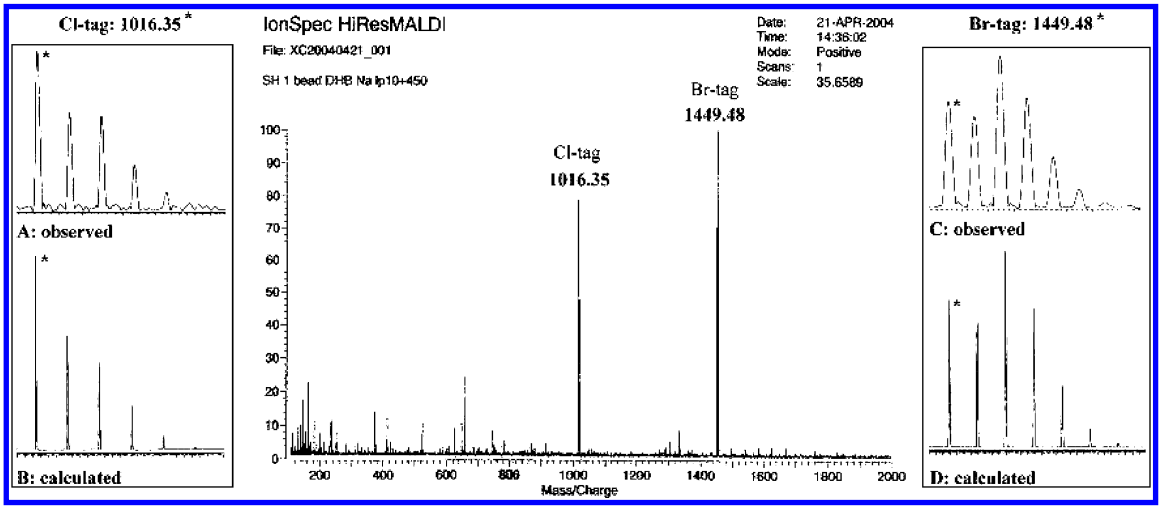
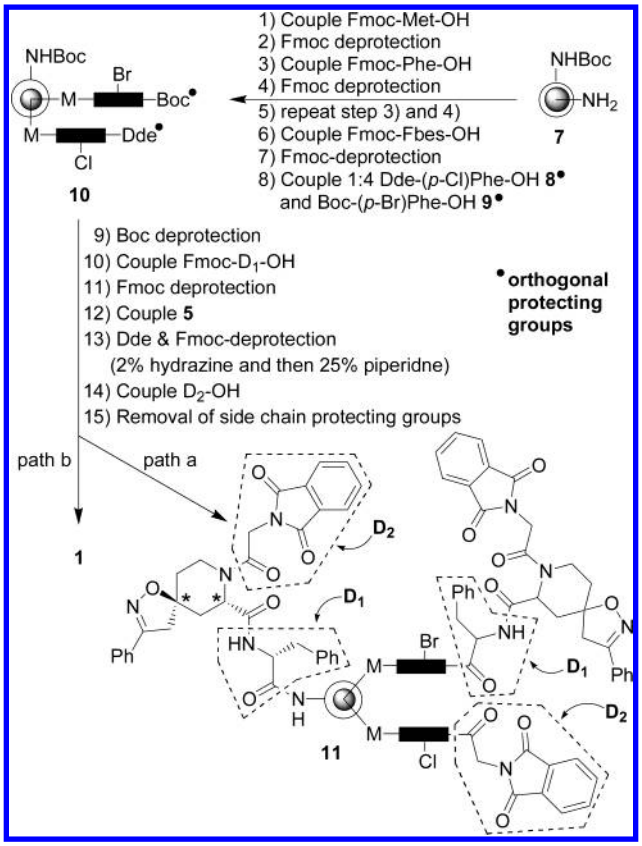


Figure 2. MALDI FTMS decoding spectrum for bead **11** (A and C = observed isotope clusters; B and D = calculated isotope clusters; peaks in A−D are separated by 1 mass unit).

literature procedures[11] from commercially available 4-bromo-1-butene.

Scheme 2. Test Case (**11**) and Library (**1**) Construction[a]



[a] Path a gives test case **11** by employing Fmoc-Phe-OH in step 10 and *N*-phthaloylglycine in step 14. Path b gives library **1** by employing 42 and 45 diversity reagents (see Supporting Information) in these steps, respectively. Note: **5** (step 12) is racemic, so the asterisks (*) indicate (±).

With oxadiazaspiro amino acid **5** in hand, we set out to demonstrate the TSB bead/MS-tagging protocol with the preparation of on-bead small-molecule compound **11** (Scheme 2, path a). Boc-protected TSB bead **7** was converted to tag-loaded TSB bead **10** using standard Fmoc-peptide chemistry. As evidenced by later MS studies, we found that step 8 required a 1:4 mixture of amino acids **8** [Dde-(Cl)Phe-OH] and **9** [Boc-(Br)Phe-OH] because of different reactivity. Bead **10** was elaborated to the target (**11**) by a series of Boc/Fmoc/Dde deprotections, couplings, and acylations. It should be noted that we expected the Dde and Fmoc protecting groups to be simultaneously deprotected when the beads were treated with 2% hydrazine. However, further treatment with 25% piperidine was necessary to completely deprotect the Fmoc group.

As illustrated in Figure 2, we were pleased to find that tag cleavage (0.25 M CNBr in 70% aq HCO_2H)[4b] of *a single bead* **11** followed by MALDI FTMS clearly and unambiguously defined the two building blocks leading to **11** as D_1 = Fmoc-Phe-OH and D_2 = *N*-phthaloylglycine by isotope clusters at 1016.35 for the Cl-tag (M + sodium) and 1449.48 for the Br-tag (M + sodium). Moreover, as shown in Figure 2, the *observed* isotope clusters for the molecular ions of these two MS tags (inset A shows the Cl-tag and inset C shows the Br-tag) match identically the *calculated* isotope clusters (inset B shows the Cl-tag and inset D shows the Br-tag).

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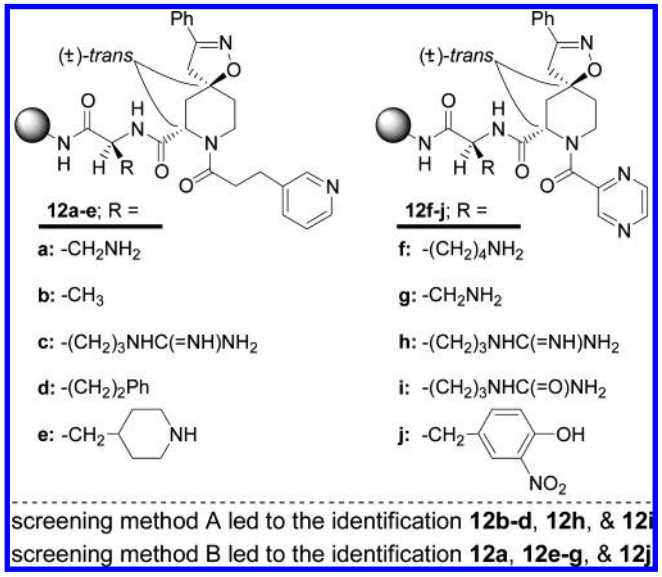

Figure 3. Oxadiazaspiro compounds with streptavidin binding.

Having thus validated this tagging procedure, we set out to prepare Br-/Cl-MS encoded library **1** (Figure 1). As illustrated in Scheme 2, path b, steps 10 and 14 were modified to include 42 and 45 diversity reagents, respectively (see Supplementary Information, Tables 1 and 2, for a list of these diversity elements). The resulting library was screened for streptavidin binding employing both a Quantum Dot (Qdot 605 streptavidin conjugate)/COPAS on-bead assay (method A; see Supplementary Information) as well as a more traditional on-bead enzyme-linked colorimetric assay[12] (method B; see Supplementary Information). The results of these assays and subsequent decodings are presented in Figure 3. Interestingly, all hit beads were derived from step 14 (Scheme 2) diversity inputs 3-(3′-pyridyl)propanoic acid (**12a**−**e**) or 2-pyrazinecarboxylic acid (**12f**−**j**). We found no actives containing any of the other 43 acids. In contrast, step 10 amino acid diversity showed little specificity (i.e., streptavidin binding accommodates at least 10 of the 42 amino acids employed).

Resynthesis of **12e** and **12f** on TentaGel beads allowed us to confirm the streptavidin binding of these two ligands: one containing the pyridine moiety (**12e**) and one containing the pyrazine moiety (**12f**). Each set of beads was retested for streptavidin binding using the on-bead enzyme-linked colorimetric assay (method B).

Figure 4 shows 12 microtiter wells where columns 1 and 2 contain **12e** and columns 3 and 4 contain **12f**. Beads with bound streptavidin-alkaline phosphatase (STAP) turn blue in the presence of BCIP substrate.[13] Wells X1 (incubated with 0.15 μg of STAP), Y1 (incubated with 0.015 μg of STAP), and Z1 (no STAP) provide visual evidence of **12e** binding to streptavidin. Column 2 is a repeat of column 1 except that biotin was added in a preincubation step. The resulting lack of blue color in all column 2 (vs column 1) wells establishes that biotin preincubation blocks binding by **12e** and suggests that ligand **12e** binds streptavidin in the biotin binding pocket. Columns 3 and 4 are repeats of columns 1 and 2, respectively, substituting bead **12f** for bead **12e**. The color intensity of well X3 was very weak, indicating that the binding affinity of **12f** toward streptavidin is much weaker than that of **12e**.

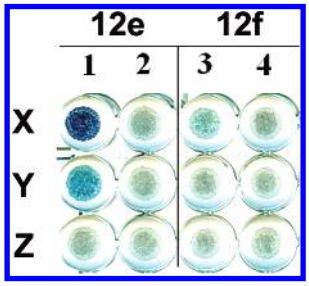

Figure 4. Method B verification of **12e**/**12f** biotin binding site specificity.

In conclusion, effective streptavidin-binding ligands were identified from library **1** using a novel halogenated mass-tag encoding strategy. Two advantages of our encoding strategy are that halogenated mass-tags are easily differentiated due to their unique isotope patterns and that synthesis of the target small molecule was confirmed (here by the bromine-containing mass-tag). In addition, a streptavidin-binding assay method employing streptavidin-coated Qdots 605 has been developed that, compared to the nonautomated enzyme-linked colorimetric assay, facilitates isolation of hit beads by automated COPAS sorting.

This halogenated mass-tag strategy is currently being used to generate a larger and more varied library that will be screened against drug targets using the Qdots detection method

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Supporting Information Available: Detailed experimental procedures, structures of diversity reagents used in library synthesis, molecular weights of the corresponding halogenated mass-coding tags, screening procedures, and a CIF X-ray data file for **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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