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1,3,5-Triazine-Based Mass Spectral Tagging of One-Bead One-Compound Libraries

Lori I. Robins and Mark J. Kurth*

Department of Chemistry, One Shields Avenue, University of California, Davis, California 95616

mjkurth@ucdavis.edu

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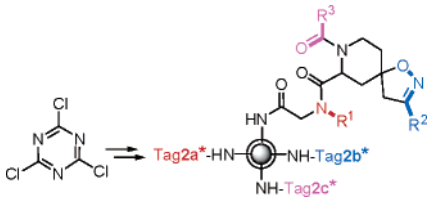
ABSTRACT



A triazine-based mass encoding strategy that accommodates cleavable linker, isotopic labeling, and diversity receptor moieties is reported. The resulting triazine-based tags, which are coupled to bifunctionalized TentaGel resin in a one-pot transformation, enable the construction of a 1-oxa-2,8-diazaspiro[4.5]dec-2-ene-7-carboxamide library and facilitate decoding by equalizing the ionization potential of the liberated tags in single bead MALDI-TOF experiments as well as balancing the reactivity of the starting tags in the resin coupling step.

Small molecules are ideal tools for probing biological systems and combinatorial chemistry provides the opportunity to synthesize many hundreds of these compounds.[1] One-bead one-compound[2] (OBOC) solid-phase techniques eliminate many of the inherent drawbacks of solution-phase synthesis and additional benefits are derived from the ease of on-bead screening—in both binding and functional assays—of OBOC libraries.[3]

On-bead screening requires the isolation of "hit" beads and the subsequent identification of the hit compound. While a variety of techniques including Edman degradation, mass spectrometry, and molecular tagging protocols have been developed for the identification of single bead compounds,[2c,4] application of these encoding techniques remains challenging.

Mass spectrometry offers a fast, highly sensitive, and reliable method for detection and this tool has been successfully applied to OBOC small molecules by using topologically segregated bifunctionalized TentaGel resin.[2b] Differentiating the inner and outer portion of the bead allows for construction of the target molecule (**TM**) on the outer portion while devoting the inner portion to the cleavable encoding tags (Figure 1). In a recent report from our laboratory, we designed an 1890 member OBOC mass tag-encoded 1-oxa-2,8-diazaspiro[4.5]dec-2-ene-7-carboxamide library that employed two diversity inputs (e.g., **TM** in Figure 1 where **D1** and **D3** were diversity inputs; **D2** was held constant).[5] Expanding this work to include **D2** diversity revealed two critical limitations: (i) highly variable tag-to-tag response in the MALDI-TOF experiment (i.e., signal intensities were quite disparate and often only 2 of the 3 tags were detectable)

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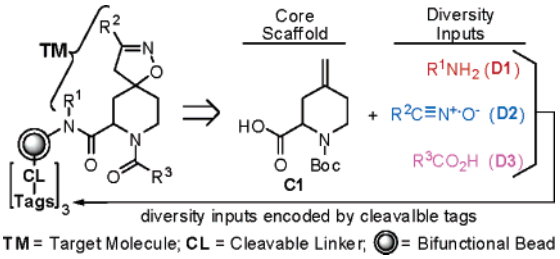
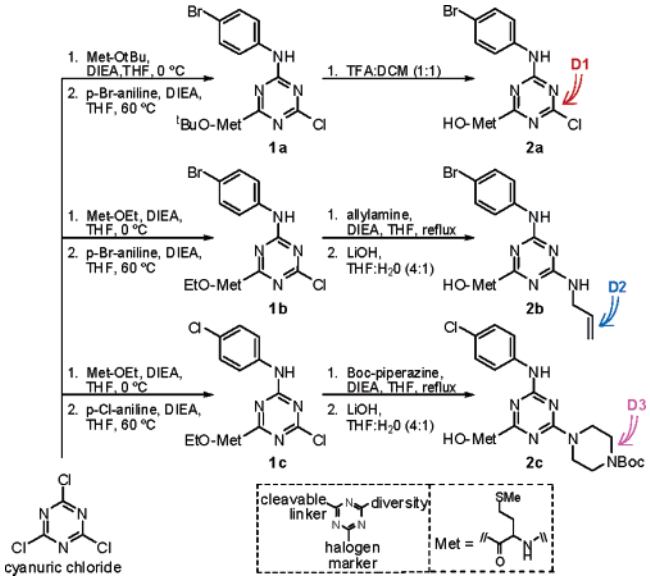


Figure 1. An OBOC mass tag-encoded 1-oxa-2,8-diazaspiro-[4.5]dec-2-ene-7-carboxamide library.

and (ii) the coupling reactivity of the three tags to bifunctionalized TentaGel resin varied significantly (i.e., we often needed to utilize one tag in considerable excess relative to the other two tags in these coupling reactions). These observations led to the redesigned encoding strategy reported here where the cleavable linker (**CL**) is simplified (-Met-Phe-Phe-NH[CH_2CH_2O]$_2CH_2CH_2$NH- → -Met-) and the tags are standardized by incorporation of a 1,3,5-triazine core (see Scheme 1).

Scheme 1. Synthesis of Cyanuric Based Tags for Capturing OBOC Diversity Elements



The requisite triazine-based tags were constructed in solution phase as outlined in Scheme 1. By controlling the stoichiometry and reaction conditions, cyanuric chloride, which affords an attractive mass tag core, led to "diversity-ready" tag precursors **2a**−**c** by sequential displacement of the first chloride rapidly at 0 °C and the second chloride rapidly at room temperature (60 °C for anilines) to give intermediates **1a**−**c**. The third *ipso* substitution requires relatively harsher conditions (∼80 ° C for multiple hours).[6] The resulting tag precursors vary in the aniline halogen (Cl vs Br), which serves as a mass marker, and the diversity (e.g., **D1**−**D3**) "receptor" element. By fixing the cleavable linker directly to the triazine core, the number of steps necessary for tag construction is greatly reduced vis-à-vis other mass tag strategies.[4,5] As illustrated in Scheme 1, diversity receptor elements of **2a**−**c** are positioned to selectively intercept inputs **D1**−**D3**: **2a** by ipso substitution, **2b** by 1,3-dipoar cycloaddition, and **2c** by *N*-acylation. Moreover, these tags have significant structural homology, which we hoped would translate into similar ionization potentials and similar responses to mass spectral analysis.[7]

As an initial test of this concept, **B1** was prepared by coupling **2b** to TentaGel resin (HOBt/DIC). An individual bead was then subjected to cleavage (methionine → γ-butyrolactone; Figure 2) by treatment with cyanogen

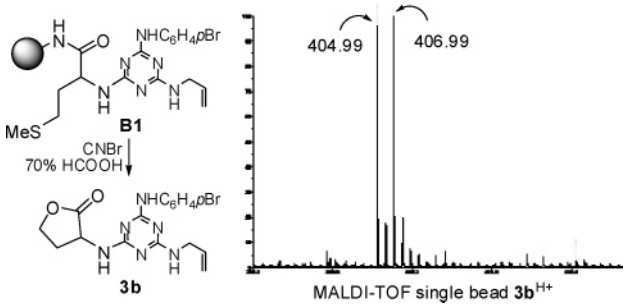


Figure 2. Resin cleavage of triazine **3b** and subsequent single bead MALDI-TOF spectral analysis.

bromide and formic acid.[8] Analysis of the resulting triazine (**3b**) by both ESI and MALDI-TOF gave the anticipated data. In our hands, analysis by ESI proved more difficult and more sample dependent whereas MALDI-TOF proved more robust as well as more sample-to-sample reliable.

Next, in a one-pot reaction, we coupled **2a**−**c** to TentaGel resin (1:1:1 **2a**:**2b**:**2c** + HOBt/HBTU/DIEA). Treating these beads with benzylamine (10 equiv + 10 equiv DIEA; microwave irradiation for 20 min at 80 °C) to effect *ipso* substitution of the remaining triazine chloride in tag **2a** followed by cleavage of a 50 mg batch of this resin with CNBr/HCO_2H delivered an essentially equal mixture of lactones **3a**−**c** (Figure 3) and established that tags **2a**−**c** couple with approximately equal reactivity.

With these results in hand, the stage was set for OBOC library production. Following the synthetic route outlined in Scheme 2, Fmoc protection of the outer portion (∼10%) of TentaGel resin was accomplished (→**B2**) according to literature procedures;[2b] Fmoc selection was driven by the need for orthogonal protection vis-à-vis tag **2c** (Boc protected). Additionally, standard Fmoc deprotection with a 20−

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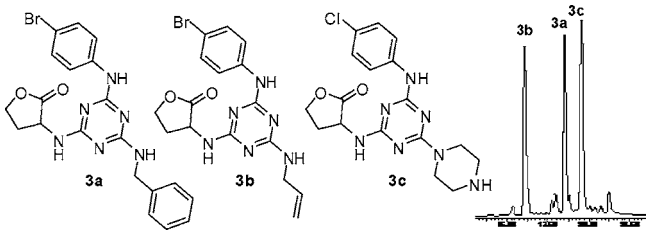

Figure 3. HPLC analysis of resin-released lactones **3a**−**c**.

25% solution of piperidine in DMF would prove incompatible with the reactivity[6a] of tag **2a** and alternative Fmoc deprotection methods—including TBAF[9] and DBU[10]—were tested. DBU (2% in DMF) was found to be compatible with **2a**. By using standard peptide coupling protocols, tags **2a**−**c** were one-pot coupled to **B2** to give **B3**. Fmoc deprotection and bromoacetic acid coupling gave resin, which was properly positioned for OBOC library production. Specifically, microwave-mediated S_N2 displacement of bromide introduced **D1** (nineteen primary amines) on the bead's periphery with simultaneous *ipso* displacement of chloride in tag **2a** gave tag **2a***. Core scaffold **C1** was next coupled to the growing **TM** (e.g., via the secondary amine **D1**), but not to any of the secondary amines in tags **2a***, **2b**, or **2c**—a consequence of the lack of nucleophilicity in these triazine-conjugated amines. The second diversity element was introduced through in situ nitrile oxide formation (seven oxymoyl chlorides)[11] and concomitant 1,3-dipolar cycloaddition to the exo-methylene of the growing **TM** and the alkene of tag **2b** (→**2b***). Boc deprotection of the growing **TM** (see **C1** in Figure 1) and tag **2c** followed by coupling of the third diversity element (19 carboxylic acids) completed the OBOC library synthesis.

Fifteen beads were randomly selected from this 2527-compound library for analysis. CNBr/HCO_2H treatment of each individual bead and subsequent MALDI-TOF analysis successfully decoded 14 beads—a 93% success rate. A representative mass spectrum from this sampling is depicted in Scheme 2: this target molecule **TM-1** was constructed from **D1** = 4-phenylbutylamine → **4a** ($R^1 = (CH_2)_4Ph$), **D2** = *N*-hydroxybenzimidoyl chloride → **4b** (R^2 = Ph), and **D3** = 2,4-hexadienoic acid → **4c** (R^3 = *E,E*-CH═CHCH═$CHCH_3$).

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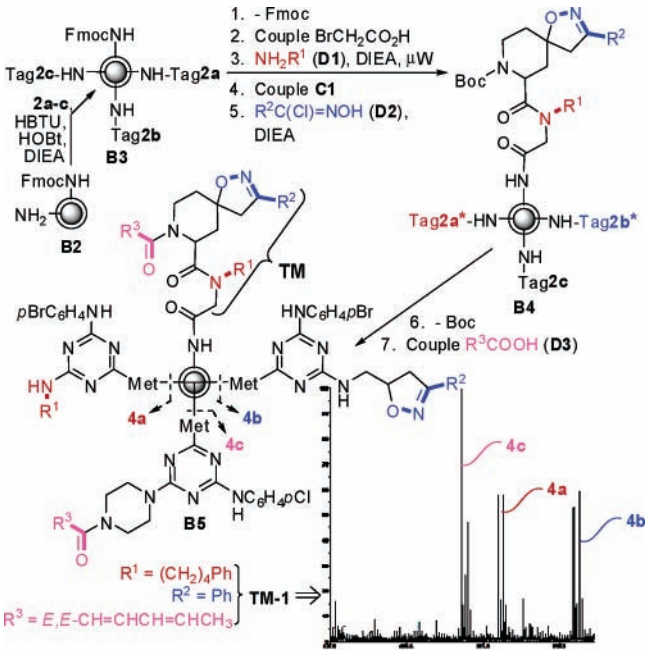
Scheme 2. OBOC Triazine Based Encoding Tag and Library Synthesis



In conclusion, we have developed a triazine-based mass encoding strategy for OBOC libraries. As depicted in Scheme 1, the triazine core accommodates cleavable linker, halogen marker, and diversity receptor moieties and, for the chemistry required to construct this 1-oxa-2,8-diazaspiro[4.5]dec-2-ene-7-carboxamide library, leads to tags (**2a**−**c**) which are coupled to bifunctionalized TentaGel resin in a one-pot transformation. These triazine-based tags facilitate decoding by (i) equalizing the ionization potential of liberated tags **4a**−**c** in single bead MALDI-TOF experiments and (ii) by equalizing the reactivity of tags **2a**−**c** in the bifunctionalized TentaGel resin coupling step. Applications of this tagging strategy to the discovery of chorismate and aldose reductase inhibitors are underway and will be reported in due course.

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Supporting Information Available: Experimental details for **2a**−**c**/**B5** and representative **TM** MALDI-TOF results. This material is available free of charge via the Internet at http://pubs.acs.org.

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