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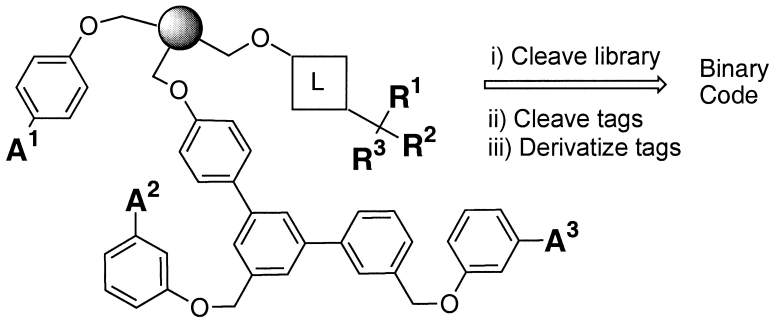

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Novel Chemical Tagging Method for Combinatorial Synthesis Utilizing Suzuki Chemistry and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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We describe a novel chemical tagging strategy for combinatorial solid-phase chemistry. The tags used are para-substituted alkyl phenols, with the first tags attached directly to the chloromethyl polystyrene and subsequent tags attached via Suzuki couplings using either aryl diboronic acids or aryl iodides. The identities of the tags attached to a single bead are discovered by the high-resolution, accurate mass technique of Fourier transform ion cyclotron resonance mass spectrometry. The method is exemplified for the coded assembly of a tripeptide.

Introduction

Several methods have been described in recent years for the identification of individual compounds of interest from combinatorial libraries.[1] The chemical history of a bead may be recorded in a number of ways, such as spatially addressable synthesis,[2] radio frequency encoding,[3−5] or chemical tagging of beads.[6] Chemical tags attached to beads must be inert so that they do not interfere with library chemistry, should be attached to the support under mild conditions, and should be easily and reliably identified at low concentration. To date, chemical tagging methods have involved inter alia the use of peptides,[7] oligonucleotides,[8] and small molecules[9,10] as the tags.

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) has emerged as a powerful tool for the accurate mass analysis of compounds with high sensitivity.[11,12] This is an ideal instrument for the detection of chemical tags owing to the potential for accurate mass analysis of mixtures of compounds, thus allowing for a measure of certainty in the decoding of a binary-type tagging system from a mass spectrum. Further, the levels of detection are more than adequate for detection of tags from a low percentage of sites from a single bead in a combinatorial synthesis.

We elected to use para-substituted alkyl phenols as tags because of their inertness particularly when attached to resin as a benzyl ether, their hydrophobic nature (which is well-suited to chemistry on polystyrene beads), and the possibility of the introduction of a polar group on the phenolic oxygen after cleavage from the support to encourage the tags to fly in the mass spectrometer. The introduction of a functional group after cleavage is common to chemical tagging methods, be that group a dansyl group (for fluorescence detection)[9] or a trimethylsilyl group for improved electron capture gas chromatography.[10]

For the introduction of the tags to the support during synthesis, we were interested in developing a Suzuki coupling step. Palladium-catalyzed C−C bond formation on solid support is characterized by the mild conditions often used, the reliably high yields obtained, and the tolerance of a variety of functional groups.[13] It was thought that the use of aromatic diboronic acids, carrying molecules attached to them via a benzyl ether, would afford a facile route to the iterative introduction of tags (a method employing aromatic monoboronic acids to introduce tags to a similar resin has been described in the patent literature).[14] The proposed method is outlined in Scheme 1. A solid support is loaded with the first tag, an alkyl phenol, and 4-iodophenol at low levels such that only approximately 1−5% of the sites on the resin are displaced by these phenols. The remaining sites on the support are displaced by a linker molecule. It was decided that the Wang linker would be used for the present example. These resins (**1**) are therefore the starting point for library synthesis. These can be made in bulk and stored. The initial library element is then attached to the linker, and this is already coded by the first tag. A split-and-mix step is performed prior to the second library synthesis step, which could be either the addition of a second library component or simply a chemical transformation. This second step is coded by the addition of the second tag element **2**, a diboronic acid carrying an alkyl phenol tag. This diboronic acid couples to the aryl iodide attached in the first step and *leaves a pendant boronic acid group*. A split-and-mix step is then performed on the reaction mixture, and the third tag is introduced by a second Suzuki coupling between the support-bound boronic acid and the third tagging component **3**, an aryl iodide carrying another alkyl phenol tag. This third tag codes for the third library synthesis step, which is performed at the end of the scheme. Use is therefore made of the fact that tagging may be performed before or after the library step for which it codes. Furthermore, because the first tag is introduced when the resin is prepared and the second and third tags effectively are in the same step (without workup), the incorporation of the three tags only adds one step to the synthesis.

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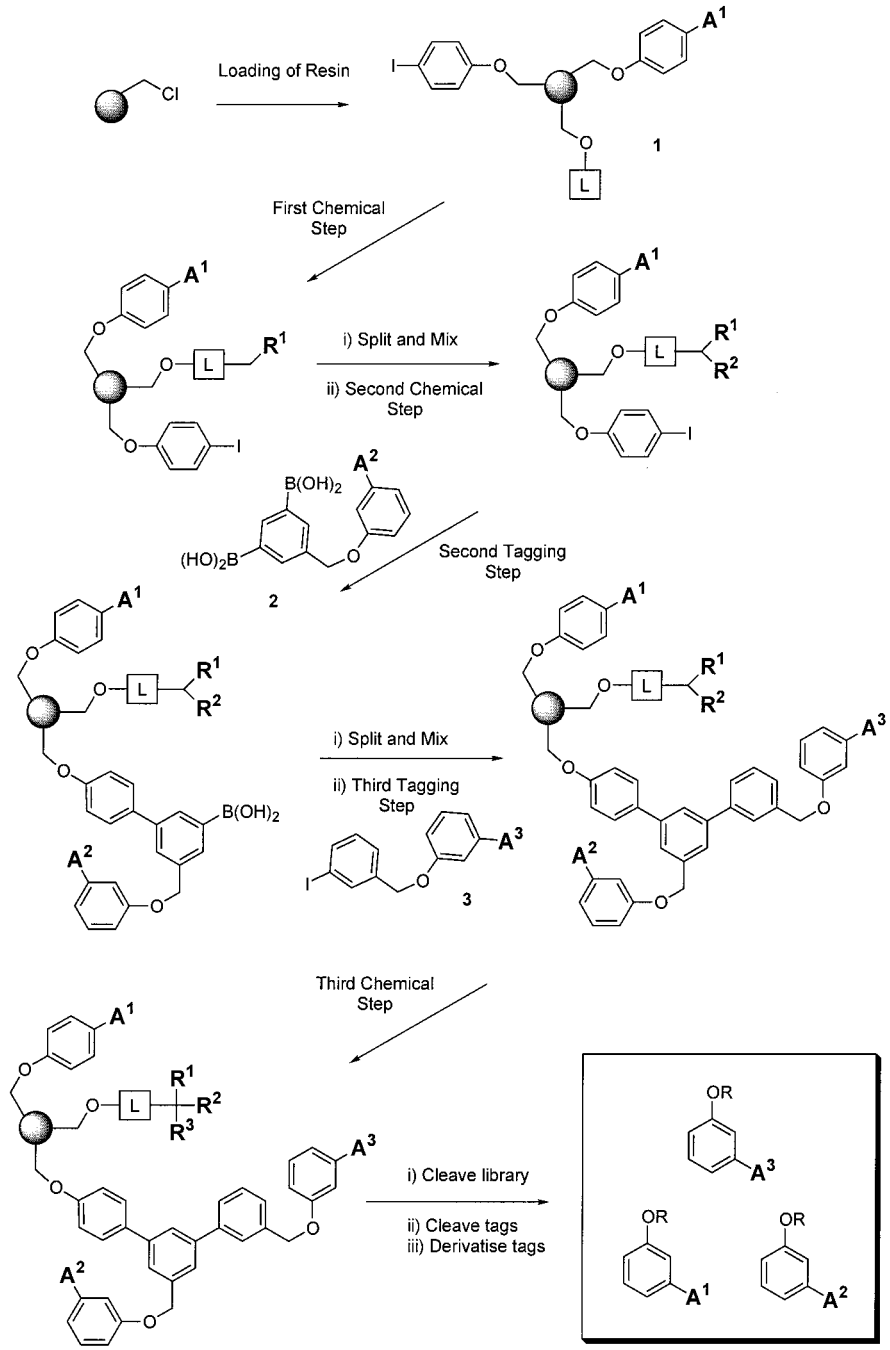
Scheme 1. Outline of the Proposed Tagging Strategy (A = Alkyl Group, R = Library Element, L = Linker Element)



The library can be cleaved from the support, leaving the tags attached to the support via their robust ether linkages. Beads furnishing compounds of interest can then be decoded by harsher cleavage conditions to liberate the tags, followed by mass spectrometry to identify the tags. A binary code is used, formed by the absence ("0") or presence ("1") of a tag in the mass spectrum, with no meaning being ascribed to a blank (where no tags are detected for a step of the sequence, i.e., '00′) due to the ambiguity involved in such a result.[10] It was decided to produce a set of six tags that could in theory encode a 27-member tripeptide library. This paper describes such a three-stage sequence, specifically the construction of a single coded tripeptide on solid support to exemplify the approach. Thus, two phenols would code for three different elements of chemical diversity, R^1 (01, 10, 11). Likewise, boronic acid tagging elements would encode three possible R^2s and similarly two monoiodo tagging elements for different R^3s. Were a diiodo version of **3** (analogous to the diboronate **2**) to be used at this stage, then clearly the coding could continue further in an iterative manner, allowing for the introduction of multiple tags for larger libraries.

Development of Mass Spectral Tag Detection System

An orthogonal tagging system requires that the tags be inert and therefore have the minimum of reactive functional groups. For the reliable detection of molecular ions with high sensitivity in mass spectrometry, however, a functional group that is easily protonated or one that chelates metal ions is usually required. Since these two requirements are likely to

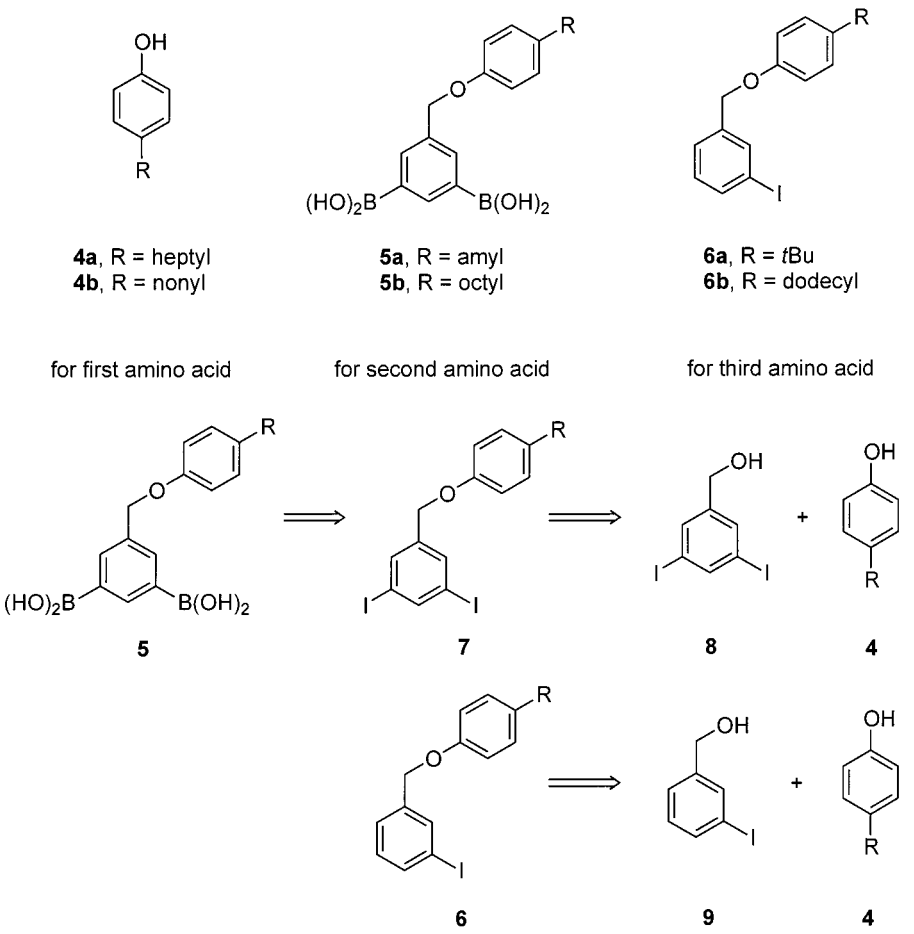


Figure 1. Required tags and their retrosyntheses.

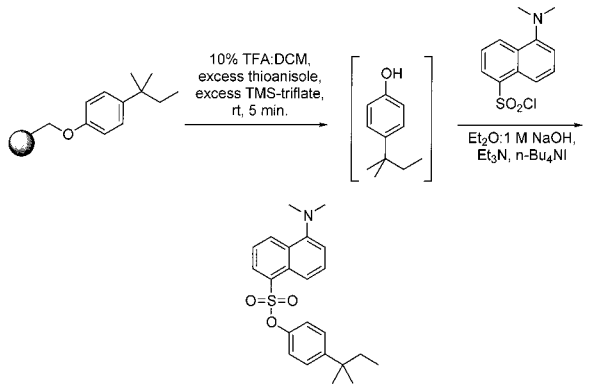
be mutually exclusive, a chemical derivatization of the tags prior to detection seemed inevitable. Indeed, derivatization of some sort has been used in all previous small-molecule tagging methods published. We synthesized several of the alkyl phenol tags intended for use in the present tagging strategy and were not surprised to find that they did not fly well underivatized under electrospray conditions.

We have previously reported investigations into the loading of phenols onto solid support and their single-bead cleavage and detection using FT-ICR-MS.[15] The method employed for the cleavage was a thioanisole-based system, which achieved rapid, room-temperature removal of the alkyl phenols from resin. The derivatization method employed was that of an in situ dansylation designed for ease and automation (Scheme 2). Excellent mass spectra from single beads were obtained using this method and allowed for the unequivocal identification of the phenols attached to the support via a process (cleavage, derivatization and analysis) that took no more than an hour. The dimethylamino moiety of the dansyl group provides an excellent site for protonation of the dansylated tag, and the extra mass gained by addition of this group places the molecular ions in a less noisy portion of the mass spectrum.

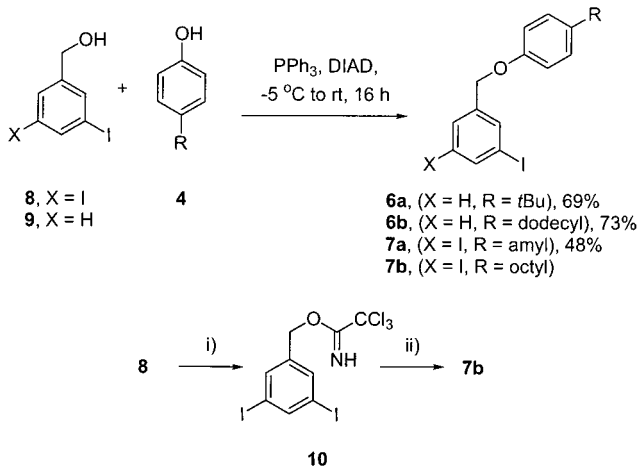
Synthesis of Novel Diboronic Acid-Based Tags

The tagging elements required to allow the full encoding of a split and mix tripeptide library are shown in Figure 1. It was decided (arbitrarily) that the first amino acid was to be encoded by heptylphenol (**4a**) or nonylphenol (**4b**) (or both). These phenols were to be attached to the solid support at the outset. The second amino acid would be encoded by amylphenol or octylphenol (or both). Thus, tagging constructs **5a** and **5b** respectively were required. These diboronic acids could be formed from the corresponding diiodides **7**, which in turn could be synthesized from 3,5-diiodobenzyl alcohol (**8**) and the appropriate phenol. The third amino acid was to be encoded by butylphenol or dodecylphenol (or both), which requires the mono-iodo tagging constructs **6a** and **6b** respectively. These are clearly similar to the diiodo constructs and may be synthesized with similar chemistry (from 3-iodobenzyl alcohol (**9**) and the appropriate phenol). This degeneracy is an attractive feature of the scheme.

Scheme 2. Cleavage−Derivatization Scheme Employed for the Mass Spectral Detection of Alkyl Phenol Tags



Scheme 3. Synthesis of Tags I: Mitsunobu and Trichloroacetimidate Chemistry



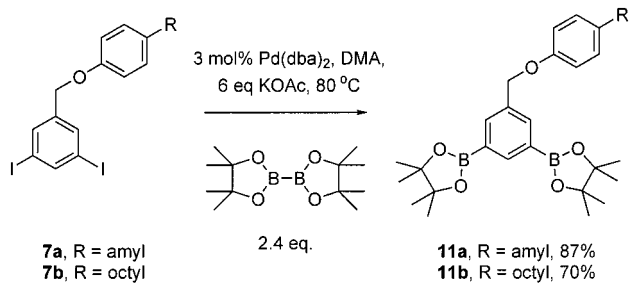
i) Cl_3CCN, KOH(aq), CH_2Cl_2, n-Bu_4NHSO_4, 0 °C to rt, 2 h, 94%
ii) 1.6 eq octylphenol, TMS-OTf, CH_2Cl_2, 0 °C to rt, 4 h, 53%

3-Iodobenzyl alcohol is commercially available, and 3,5-diiodobenzyl alcohol may be synthesized according to literature methods from methyl-4-aminobenzoate.[16−19] Common to the syntheses of all four desired molecules **5a**−**6b** is the attachment of the tag phenol to a benzyl alcohol via the formation of a benzyl ether. There are a number of ways of carrying out this transformation, and a Mitsunobu reaction between the benzyl alcohol and the phenol was used (Scheme 3). It is known that both the solvent used and the order of addition of reagents are important to the success of the Mitsunobu reaction.[20,21] In the present case, carrying out the reaction in toluene (but not THF) gave good results, and mixing the triphenylphosphine and DIAD first followed by the addition of the benzyl alcohol and finally addition of the acid (in this case, the phenol) was also beneficial. Any other order of addition of the reagents failed to give good yields of the desired products. The novel compounds **6a** and **6b** were synthesized in this way, as was the amylphenol-based diiodo molecule **7a**, albeit in a lower yield of 48%.

The use of the Mitsunobu reaction for the synthesis of the corresponding octylphenol-based diiodide gave persistent difficulties, generating mixtures of products with similar retention times. Consequently the diiodobenzyl alcohol was converted to its trichloroacetimidate **10** in 94% yield, from which the appropriate tag construct **7b** was readily synthesized using a catalytic quantity of a Lewis acid (TMS-triflate).

With both diiodo constructs **7a** and **7b** in hand, their conversion to the corresponding diboronic acids **5a** and **5b** was attempted. During our previous investigations[22] into simple aryl diboronic acids, the synthesis of 1,4-benzene diboronic acid was found to be significantly easier than that of the 1,3-isomer. Indeed the conversion of 1,3-dibromobenzene to the diboronic acid tended to require higher reaction temperature and to produce a number of other products that were not separable from the desired diacid. In the present case, attempts to convert the diiodo compounds **7a** and **7b** into the desired diboronic acids using the standard method[23] of butyllithium followed by quenching with trialkylborate and finally hydrolysis failed.

Scheme 4. Synthesis of Tags II: Conversion of Diiodo Constructs (**7**) to Tags (**11**) Using Pinacolatodiboronate

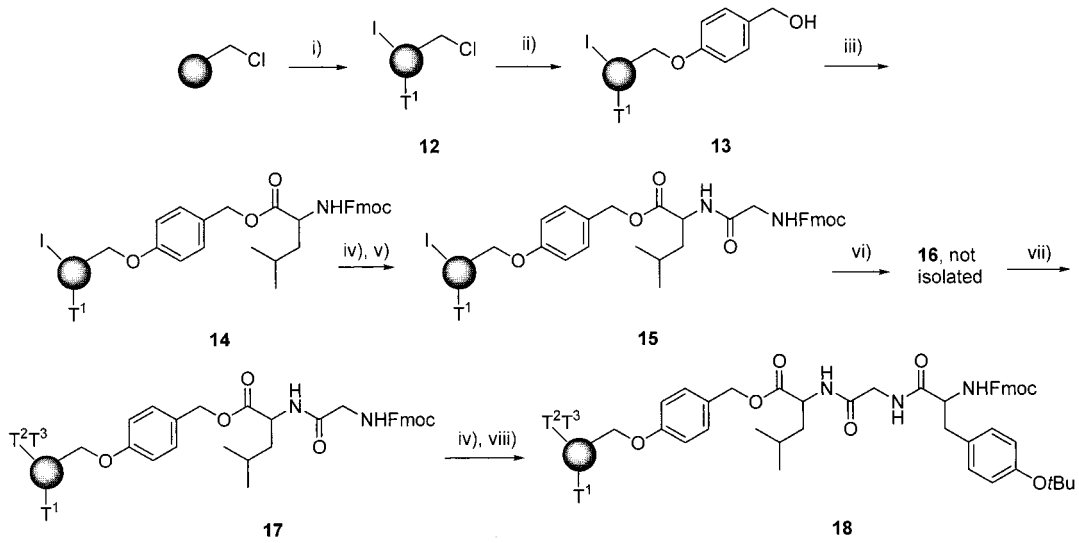


An alternative route was adopted for this key transformation. Miyaura reported the synthesis of boronic esters directly from the corresponding halides by a palladium(0)-catalyzed reaction with bispinacolatodiboron.[24] This chemistry has been further adapted for the synthesis of aryl boronates from aryl triflates[25] and the platinum(0)-catalyzed diboration of alkenes.[26] Subjection of the diiodo precursor molecules to these conditions led to the smooth production of the desired diboronic ester tag constructs **11a** and **11b** (Scheme 4). To our knowledge, this is the first synthesis of aromatic diboronates via this strategy. Diboronic esters are easier to work with than the corresponding diacids, and because they are seemingly stable compounds under ambient conditions, they are useful as tagging constructs, where prolonged storage may be necessary. The tags for the second amino acid are therefore attached to the resin as the esters rather than the acids. The synthesis of a complete set of tagging constructs was thus completed. We then turned our attention to the coding of a single tripeptide, without the split and mix steps for simplicity, requiring three of the above tags.

Successful Single-Bead Decoding of Tripeptide

The synthesis of a single encoded tripeptide on solid support was performed (Scheme 5). It was decided arbitrarily to construct the tripeptide leucine−glycine−tyrosine and to code for this with nonylphenol−amylphenol−butylphenol. Chloromethyl polystyrene resin (1.8 mmol g^{-1}) was loaded with low levels of nonylphenol (<15% loading) and iodophenol (<25% loading) to give **12**. This resin was loaded with the Wang linker via a double coupling to produce **13**.[27] The first amino acid was attached to this resin using the 2,6-dichlorobenzoyl chloride/pyridine method[28] to give **14**. The second amino acid was attached to the first via Fmoc deprotection and standard peptide coupling chemistry to give **15**. This second amino acid was coded by the attachment of the amylphenol-based diboronate under mild conditions. Without isolation or even washing of this resin, the final coding step was performed by palladium-catalyzed attachment of the butylphenol-based iodide to give **17**. Approximately 2−3 equiv of diboronate and iodide tags were found to be necessary for these coupling reactions. Control experiments were performed, indicating that cross-linking of the resin did not interfere with the iterative Suzuki

Scheme 5. Synthesis of Encoded Tripeptide on Solid Phase



i) 0.15 eq nonylphenol, 0.25 eq 4-iodophenol, 0.4 eq NaOMe, DMA, 50 °C, 16 h; ii) 8 eq 4-hydroxybenzylalcohol, 8 eq NaOMe, DMA, 50 °C, 16 h (2 couplings); iii) Fmoc-Leu-OH, DMA, pyr., 2,6-dichlorobenzoyl chloride, rt 16 h; (2 couplings); iv) piperidine/DMF; v) Fmoc-Gly-OH, DMA, PyBOP, HOBt, DIPEA (2 couplings); vi) 2 eq **11a**, 10 mol% $Pd(PPh_3)_4$, DMA, 12 eq K_2CO_3, 50 °C, 6 h; vii) 2 eq **6a**, 10 mol% $Pd(PPh_3)_4$, DMA, 12 eq K_2CO_3, 50 °C, 5 h; viii) Fmoc-Tyr-(O-*t*Bu)-OH, DMA, PyBOP, HOBt, DIPEA (2 couplings)

couplings at this loading level.[29] This final tag coded for tyrosine, which was then attached in the final step to yield **18**.

The peptide was readily identifiable under low-resolution ES-MS after cleavage of the Wang linker with 10% TFA/DCM at room temperature for 10 min. Cleavage with this mixture was also used to verify that each step in the peptide synthesis had gone to completion.

After peptide removal, single beads were treated using the thioanisole−dansyl chloride cleavage−derivatization sequence. The FT-ICR mass spectrum reveals clearly the three expected tags (Figure 2). The three tags not used in the synthesis were not present. The masses of the molecular ions observed for the dansylated tags were all correct to within 4 ppm. While it would be desirable to improve the signal-to-noise ratio observed in these spectra, the encoding strategy is exemplified by this result because the three expected tags are unequivocally identified (at high resolution) while those tags not included are absent. This provides the appropriate binary code for this peptide. Further, no chromatography was

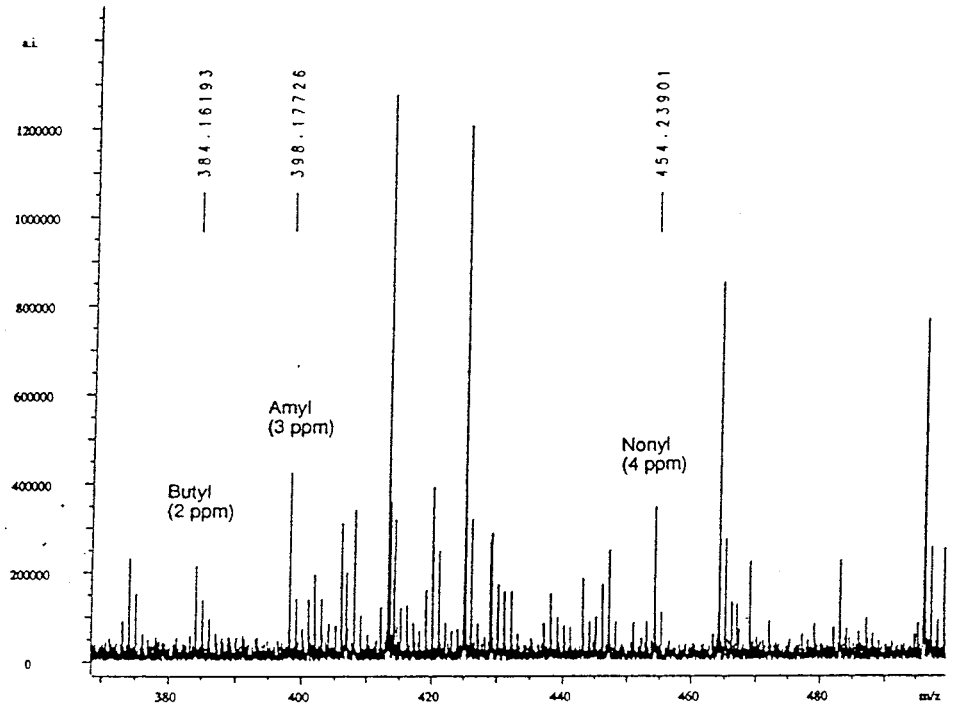


Figure 2. FT-ICR mass spectrum showing the three required tags obtained from treatment of a single bead of resin **18**, after removal of peptide, according to the cleavage-derivatization method (Scheme 2).

required prior to injection of this mixture into the mass spectrometer.

Conclusion

We have described a novel approach for the use of high-resolution mass spectrometry for the decoding of a chemical compound attached to a single resin bead via the use of chemical tags. We have described the synthesis of novel tags based on aryl diboronic esters and aryl iodides, which allow for the coupling of inert molecules to solid support under mild conditions. The steps required for both the encoding and decoding are facile, mild, and rapid. The tags were unequivocally identified by accurate mass spectrometry, and their presence or absence furnished the appropriate binary code for the corresponding library molecule. We have illustrated the technique for a single tripeptide on solid phase, which represents a typical library compound. Investigations and optimizations of this new method for the encoding of formal split-and-mix libraries are in progress.

Experimental Section

General Procedures. Melting points were determined on a Gallenkamp 889339 electrothermal melting point apparatus or on a Griffin melting point apparatus and are uncorrected. Infrared spectra were recorded on an ATI Mattson Genesis FTIR apparatus. NMR spectra were recorded using either a Bruker DRX250 or a DRX400 instrument. Mass spectra were recorded using a Kratos MS890 double-focusing magnetic sector apparatus (for EI and FAB) or a Kratos MS50 instrument (for FIB). Low-resolution ES-MS spectra were recorded using a VGBIO-Q or Micromass instrument, with MassLynx software and using PEG-NH_4 as calibrant. Fourier transform ion cyclotron resonance mass spectra were obtained on a Bruker BioApex 47e Fourier transform ion cyclotron resonance mass spectrometer, typically using 150 V capillary voltage, 8−32 scans, 60 mL h^{-1} injection rate, and 10 V cone at a gas temperature of 300 °C. Analytical TLC was carried out on commercially prepared silica plates coated to a thickness of 220−250 mm and visualized with ultraviolet light at 254 nm, or a ceric molybdate stain. Flash column chromatography was performed using Kieselgel 60 with mesh size 230−400 ASTM. All reactions were carried out under anhydrous conditions using oven-dried apparatus and under argon unless otherwise stated. All solvents were distilled prior to use. Anhydrous DMA was purchased from Aldrich and used from the bottle. Anhydrous DMF was either purchased from Aldrich and used from the bottle, or nonanhydrous DMF was dried over 3 Å molecular sieves for 72 h.

Resin washing was performed by bubbling nitrogen through solvent-suspended resin for 5 min per wash. FT-IR spectra of resin samples were performed by swelling several milligrams of resin in CH_2Cl_2 on a sodium chloride plate, then compressing the sample between two plates as for normal samples; peaks listed are usually those that differ from the polystyrene backbone. Gel-phase NMR samples were prepared by allowing the resin to swell in CD_2Cl_2 (usually for 20 min), with shimming being performed on a similar height of $CDCl_3$ in a separate NMR tube. Typically all relaxation delays were reduced to zero, and the acquisition time was set at 0.1 s with each spectrum acquired overnight (ca. 400K scans). An increased line broadening was not generally applied to these spectra. Resin reaction vessels were shaken in an Innova instrument with variable temperature water bath at 180 rpm, and vessels were sealed (to exclude water) by fitting with a ground-glass stopper.

3-Iodo-4′-*tert*-butylphenoxybenzyl Ether (6a). To triphenylphosphine (560 mg, 1 equiv) in toluene (25 mL) at −7 °C was added diisopropylazodicarboxylate (0.42 mL, 1 equiv) dropwise. After the mixture was stirred for several minutes, 3-iodobenzyl alcohol (500 mg, 2.1 mmol) in toluene (2 mL) was added dropwise followed by 4-*tert*-butylphenol (640 mg, 2 equiv) in toluene (2 mL) dropwise. After being stirred for an additional 5 min, the reaction mixture was allowed to warm to room temperature for 16 h, diluted with diethyl ether (10 mL), and washed with 1 N NaOH (8 mL). This aqueous wash was extracted with diethyl ether (5 mL), and the combined organic portions were washed with brine (8 mL), dried ($MgSO_4$), and concentrated under reduced pressure to yield a clear, colorless oil containing large, clear, colorless crystals. This residue (oil and crystals) was purified by flash column chromatography in 5:95 EtOAc−light petroleum (bp 60−80 °C) to give the benzyl ether as a white solid (539 mg, 69%). Elemental analysis of this solid indicated a slight impurity (found: C, 57.82; H, 5.25), and so the solid was recrystallized from light petroleum (bp 60−80 °C) to give the *benzyl ether* as white needles, with improved elemental analysis. Mp 80−82 °C; R_f (EtOAc−light petroleum (bp 60−80 °C), 5:95) 0.45; IR (mull) 1605, 1577, 1566, 1511 cm^{-1}; ^1H NMR δ 7.79 (1H, s), 7.65 (1H, d, J = 7.8), 7.39 (1H, d, J = 7.6), 7.31 (2H, d, J = 9.0), 7.11 (1H, t, J = 7.8), 6.89 (2H, d, J = 8.5), 4.98 (2H, s), 1.30 (9H, s); ^{13}C NMR δ 156.21, 143.84, 139.63, 136.87, 136.21, 130.24, 126.50, 126.29, 114.18, 94.44, 68.95, 34.07, 31.49; *m*/*z* (FAB) 366.1 (M^+, 67%), 217.0 (IBn^+, 100); HRMS $C_{17}H_{19}IO$ (M^+) requires 366.04807, found 366.04877. Anal. Calcd for $C_{17}H_{19}IO$: C, 55.75; H, 5.23. Found: C, 55.64; H, 5.24.

3-Iodo-4′-dodecylphenoxybenzyl Ether (6b). To triphenylphosphine (560 mg, 1 equiv) in toluene (20 mL) cooled to −5 °C was added diisopropylazodicarboxylate (420 μL, 1 equiv) dropwise. After the mixture was stirred for several minutes, 3-iodobenzyl alcohol (500 mg, 2.1 mmol) in toluene (2 mL) was added dropwise followed by 4-dodecylphenol (1.12 g, 2 equiv) in toluene (2 mL) dropwise. After a being stirred a further 10 min, the reaction mixture was allowed to warm to room temperature over 16 h and was diluted with diethyl ether (30 mL) and washed with 1 N NaOH (15 mL). This aqueous wash was extracted with a further portion of diethyl ether (10 mL), and the combined organic portions were washed with brine (10 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a yellow oil. This was purified by flash column chromatography in 5:95 EtOAc−light petroleum (bp 60−80 °C) to give the benzyl ether as a clear, colorless oil (740 mg, 73%). Elemental analysis on this oil indicated a slight impurity (found: C, 63.46; H, 7.57), so a second purification was performed by flash column chromatography in hexane to give the *benzyl*

ether as a clear, colorless oil with improved elemental analysis. R_f (EtOAc−light petroleum (bp 60−80 °C), 5:95) 0.52; IR (film) 1608, 1595, 1581, 1568, 1511 cm^{-1}; ^1H NMR δ 7.82 (1H, s), 7.66 (1H, d, $J = 7.8$), 7.41 (1H, d, $J = 7.5$), 7.23 (2H, m), 7.12 (1H, t, $J = 7.7$), 6.90 (2H, m), 4.99 (2H, s), 0.40−1.80 (25H, m); ^{13}C NMR δ 156.08, 139.75, 136.89, 136.35, 136.30, 130.24, 127.02 (m), 114.07 (m), 94.43, 69.00, 29 (br m); *m/z* (EI-MS) 478.2 (M^+, 19%), 449.1 ($M^+ - Et$, 16), 435.1 ($M^+ - Pr$, 15), 421.1 ($M^+ - Bu$, 15), 407.1 ($M^+ -$ pentyl, 15), 393.1 ($M^+ -$ hexyl, 16), 379.1 ($M^+ -$ heptyl, 36), 365.0 ($M^+ -$ octyl, 100); HRMS $C_{25}H_{35}IO$ (M^+) requires 478.17211, found 478.17328. Anal. Calcd for $C_{25}H_{35}IO$: C, 62.76; H, 7.37. Found: C, 62.76; H, 7.29.

3,5-Diiodo-4′-*tert*-amylphenoxybenzyl Ether (7a). To triphenylphosphine (4.40 g, 1 equiv) in toluene (150 mL) at 0 °C was added DIAD (3.3 mL, 1 equiv) dropwise. To this was added 3,5-diiodobenzyl alcohol (6.00 g, 16.7 mmol) in toluene−THF (8 mL:17 mL) dropwise followed by *tert*-amylphenol in toluene−THF (8 mL:4 mL). The reaction mixture was allowed to warm to room temperature for 16 h and was washed with 1 N NaOH (50 mL) and brine (15 mL) and dried ($MgSO_4$). Removal of solvent under reduced pressure gave a thick yellow oil, which was dissolved in the minimum quantity of 1:1 EtOAc−light petroleum (bp 60−80 °C) and passed through a short plug of silica to remove baseline material. Concentration of the eluent gave a light-yellow oil that was purified by flash column chromatography in light petroleum (bp 60−80 °C) to yield the *benzyl ether* as bright-white plates (4.05 g, 48%). Mp 48−50 °C; R_f (EtOAc−light petroleum (bp 60−80 °C), 5:95) 0.48; IR (mull) 1606, 1578, 1543, 1512, 1549, 1420 cm^{-1}; ^1H NMR δ 7.99 (1H, s), 7.74 (2H, s), 7.25 (2H, d, $J = 8.6$), 6.87 (2H, d, $J = 8.6$), 4.91 (2H, s), 1.61 (2H, q, $J = 7.4$), 1.26 (6H, s), 0.67 (3H, t, $J = 7.1$); ^{13}C NMR δ 155.88, 144.45, 142.40, 141.41, 135.41, 127.04, 114.11, 94.87, 68.06, 37.31, 36.91, 28.56, 9.14; *m/z* (FAB-MS) 506.0 (M^+, 16%), 505.0 ($M^+ - H$, 22), 342.9 (I_2Bn^+, 100); HRMS $C_{18}H_{20}I_2O$ (M^+) requires 505.96036, found 505.95721. Anal. Calcd for $C_{18}H_{20}I_2O$: C, 42.71; H, 3.98. Found: C, 42.65; H, 3.89.

Di-(3,5-diiodobenzyl) Ether. From the purification of 3,5-diiodo-4′-*tert*-amylphenoxybenzyl ether (**7a**) could be isolated *di-(3,5-diiodobenzyl) ether* as white needles (quantities varied, max 5%). Mp 172 °C (dec); R_f (EtOAc−light petroleum (bp 60−80 °C), 5:95) 0.32; IR (mull) 1578, 1545, 1419 cm^{-1}; ^1H NMR δ 7.99 (2H, t, $J = 1.6$), 7.64 (4H, dd, $J = 1.6$ & 0.7), 4.42 (4H, s); ^{13}C NMR δ 144.52, 141.76, 135.81, 94.85, 70.77; *m/z* (FAB) 701.8 (M^+, 1%), 548.0(3), 476.9(64), 343.0 (I_2Bn^+, 100).

3,5-Diiodobenzyl-1,1,1-trichloroacetimidate (10). 3,5-Diiodobenzyl alcohol (2.0 g, 5.6 mmol) was suspended in CH_2Cl_2 (50 mL), and 50% KOH solution (10 mL) and (n-Bu)$_4NHSO_4$ (45 mg, 2 mol % with respect to nitrile) were added. The reaction mixture was cooled to 0 °C, and then trichloroacetonitrile (668 μL, 1.2 equiv) was added dropwise such that the temperature of the reaction mixture did not rise above 0 °C. The stirring was continued for 30 min and then allowed to warm to room temperature for 90 min. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and H_2O (20 mL), and the layers separated. The aqueous phase was extracted with a further quantity of CH_2Cl_2 (10 mL) and the combined organic portions were washed with brine (15 mL), dried ($MgSO_4$), and concentrated under reduced pressure to yield a beige solid (2.8 g) that was purified by flash column chromatography in 1:4 CH_2Cl_2−hexane to give the *acetimidate* as a white solid (2.6 g, 94%). This solid could be recrystallized from CH_2Cl_2−hexane to yield white needles. Mp 65−67 °C; R_f (EtOAc−light petroleum (bp 60−80 °C), 1:9) 0.33; IR (mull) 3321, 1658, 1582, 1544, 1455 cm^{-1}; ^1H NMR δ 8.44 (1H, br s), 8.01 (1H, t, $J = 1.5$), 7.72 (2H, d, $J = 1.5$), 5.21 (2H, s); ^{13}C NMR δ 162.13, 144.97, 139.42, 135.86, 94.81, 90.99, 68.43; *m/z* (FIB-MS) 503.7 (M^+, 13%), 342.9 ($I_2ArCH_2^+$, 68); HRMS $C_9H_6I_2NOCl_3$ (MH^+) requiress 503.7680, found 503.7660. Anal. Calcd for $C_9H_6Cl_3I_2NO$: C, 21.43; H, 1.20; N, 2.78; Cl, 21.09. Found: C, 21.31; H, 1.18; N, 2.68; Cl, 21.35.

3,5-Diiodo-4′-*tert*-octylphenoxybenzyl Ether (7b). 3,5-Diiodobenzyltrichloroacetimidate (**10**, 1.38 g, 2.7 mmol) and *tert*-octylphenol (899 mg, 4.4 mmol, 1.6 equiv) were dissolved in CH_2Cl_2 (40 mL), and this solution was cooled in an ice−salt bath for 20 min. To this was added TMS-triflate (198 μL, approximately 40 mol %) dropwise, and the reaction mixture was allowed to warm to room temperature. After being stirred for 3.5 h, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with 1 M NaOH (15 mL). This aqueous wash was re-extracted with CH_2Cl_2 (10 mL), and the combined organic portions were washed with saturated $NaHCO_3$ solution (10 mL) and were dried ($MgSO_4$). The solvent was removed under reduced pressure to yield a white solid (1.9 g) that was purified by flash column chromatography in light petroleum (bp 60−80 °C) to give the *benzyl ether* as white needles (833 mg, 56%). Mp 57−59 °C; R_f (EtOAc−light petroleum (bp 60−80 °C), 5:95) 0.52; IR (mull) 1602, 1579, 1547, 1515, 1421 cm^{-1}; ^1H NMR δ 8.00 (1H, s), 7.74 (2H, s), 7.29 (2H, d, $J = 8.5$), 6.85 (2H, d, $J = 8.4$), 4.91 (2H, s), 1.71 (2H, s), 1.35 (6H, s), 0.72 (9H, s); ^{13}C NMR δ 155.80, 144.40, 143.01, 141.36, 135.42, 127.18, 113.93, 94.84, 68.01, 56.95, 37.96, 32.30, 31.75, 31.62; *m/z* (FAB-MS) 548.0 (M^+, 20%), 477.0 ($M^+ - t$BuCH$_2$, 99), 342.9 (I_2Bn^+, 100); HRMS $C_{21}H_{26}I_2O$ (MH^+) requires 548.00732, found 548.00714. Anal. Calcd for $C_{21}H_{26}I_2O$: C, 46.01; H, 4.78. Found: C, 45.94; H, 4.74.

(4′-*tert*-Amylphenoxy)benzyl-3,5-pinacolatodiboronate (11a). To 3,5-diiodo-4′-*tert*-amylphenoxybenzyl ether (**7a**, 231 mg, 0.46 mmol) in DMA (10 mL) was added bispinacolatodiboron (280 mg, 2.4 equiv), KOAc (290 mg, 6 equiv), and palladium bisdibenzylidene acetone (16 mg, 3 mol %). Light was excluded from this dark-brown suspension, and it was stirred at 80 °C for 2 h, then allowed to cool to room temperature. The reaction mixture was diluted with ether (10 mL) and water (10 mL), and the layers separated. The aqueous phase was extracted with ether (3 × 10 mL) and the combined organic portions were washed with brine (8 mL), dried ($MgSO_4$), and concentrated in vacuo to yield a yellow oil. This residue was purified by flash column chromatography in 1:9 EtOAc−light petroleum (bp 60−80 °C) rising to neat EtOAc to yield the *diboronate* as a white solid (201 mg, 87%). R_f (EtOAc−light petroleum (bp 60−80 °C), 1:4) 0.42; IR (mull) 1603, 1513, 1458, 1406, 1372

s, 1323 s cm^{-1}; ^1H NMR δ 8.26 (1H, s), 8.00 (2H, s), 7.24 (2H, dt, $J = 8.9$ & 2.2), 6.94 (2H, dt, $J = 8.9$ & 3.2), 5.03 (2H, s), 1.62 (2H, q, $J = 7.4$), 1.35 (24H, s), 1.27 (6H, s), 0.69 (3H, t, $J = 7.4$); ^{13}C NMR δ 156.69, 141.70, 141.00, 137.11, 135.75, 126.85, 114.23, 83.83, 70.15, 37.27, 36.99, 28.61, 24.89, 9.15; *m/z* (FAB-MS) 506.1 (M^+, 43%), 505.1 (M^+, 100), 504.1 (M^+, 38), 491.0 ($M^+ - CH_3$, 38), 432.9 (74), 342.6 (M^+ − amylphenoxy, 100); HRMS $C_{30}H_{44}B_2O_5$ (M^+) requires 506.33749, found 506.33791.

(4′-*tert*-Octylphenoxy)benzyl-3,5-pinacolatodiboronate (11b). To 3,5-diiodo-4′-*tert*-octylphenoxybenzyl ether (**7b**, 250 mg, 0.46 mmol) in DMA (10 mL) was added bispinacolatodiboron (280 mg, 2.4 equiv), KOAc (290 mg, 6 equiv), and palladium bisdibenzylidene acetone (16 mg, 3 mol %). The procedure of the reaction and purification of the compound were as for (4′-*tert*-amylphenoxy)benzyl-3,5-pinacolatodiboronate save for the reaction time being 16 h. This gave the *diboronate* as a white solid (174 mg, 70%). R_f (EtOAc−light petroleum (bp 60−80 °C), 1:4) 0.44; IR (mull) 1617, 1512, 1457, 1407, 1370 s, 1333 cm^{-1}; ^1H NMR δ 8.25 (1H, s), 7.99 (2H, s), 7.27 (2H, dt, $J = 8.6$ and 3.1), 6.90 (2H, dt, $J = 8.8$ and 3.1), 5.01 (2H, s), 1.70 (2H, s), 1.34 (30H, s), 0.72 (9H, s); ^{13}C NMR δ 156.64, 142.30, 140.96, 137.13, 135.71, 127.01, 114.01, 83.83, 70.10, 57.04, 37.94, 32.33, 31.77, 31.67, 24.88; *m/z* (FAB-MS) 547.6 (M^+, 8%), 477.5 ($M^+ - t$BuCH$_2$, 6), 343.2 (M^+ − octylphenoxy, 100); HRMS $C_{33}H_{50}B_2O_5$ (M^+) requires 548.38692, found 548.38446.

General Phenol Resin Loading Procedure. To chloromethylpolystyrene resin (1.8 mequiv) were added DMA, the required phenol (3 equiv), and sodium methoxide (3 equiv), and the reaction vessel was fitted with a ground-glass stopper. This suspension was shaken at 50 °C for 16 h, filtered, and washed according to the general washing procedure.

General Resin Washing Procedure. Resin was washed in fritted polypropylene tubes. Each washing cycle consisted of bubbling with nitrogen for 2 min and filtering with a water aspirator. Washes were typically with the following sequence: CH_2Cl_2 (2 × 4 mL), dioxane−H_2O (1:1, 2 × 4 mL), CH_2Cl_2 (2 × 4 mL), toluene (2 × 4 mL), CH_2Cl_2 (2 × 4 mL), CH_2Cl_2−MeOH (2 × 4 mL), MeOH−H_2O (2 × 4 mL), CH_2Cl_2 (4 × 4 mL). The resin was then dried in vacuo for 24 h.

Synthesis of Encoded Tripeptide. 1. Doping of Chloromethyl Resin with First Tag and Iodophenol (12). To low-mesh chloromethyl resin (1.8 mequiv, 400 mg, 0.72 mmol sites) swollen in DMA (4 mL each) were added nonylphenol (24 mg in DMA (1 mL), 0.15 equiv), iodophenol (40 mg, 0.25 equiv), and sodium methoxide (16 mg, 0.4 equiv), and this suspension shaken and washed as described above to give **12** as a white powder (432 mg, implies approximately 15% iodophenol loading and 9% tag loading). Found: C, 83.37; H, 7.15; Cl, 4.39. Initial resin found Cl: 6.3%. This implies 30% reaction, implying approximately 19% iodophenol loading level and 11% tag loading level. IR (CH_2Cl_2 gel): 1510, 1487 cm^{-1}.

2. Loading of Tagged Resin with Wang Linker (13). To resin **12** (355 mg, 1.21 mequiv, approximately 0.43 mmol sites) swollen in DMA (5 mL) were added recrystallized 4-hydroxybenzyl alcohol (410 mg, 8 equiv) and sodium methoxide (173 mg, 8 equiv). The reaction mixture was shaken and washed as described above. The reaction was repeated to give **13** as a beige powder (421 mg, implies 153% yield by mass). Found: Cl, 1.01, implying 77% yield for this step (incomplete reaction always observed for this step). IR (CH_2Cl_2 gel): 3400 br, 1610, 1583, 1512 cm^{-1}.

3. Attachment of First Fmoc Amino Acid to Wang Resin (14). Resin **13** (350 mg, 0.88 mequiv, 0.31 mmol sites) was suspended in DMF (2 mL) and Fmoc−LEU−OH (277 mg, 2.5 equiv) was added. This suspension was shaken at room temperature for 20 min, and then pyridine (104 μL, 4 equiv) and 2,6-dichlorobenzoyl chloride (112 μL, 2.5 equiv) were added and the reaction mixture shaken at room temperature for 24 h. The resin was filtered and washed with CH_2Cl_2, then resubjected to the reaction conditions. After filtration and washing, the resin was suspended in CH_2Cl_2 (4 mL) and to this was added benzoyl chloride (167 μL, excess) and pyridine (116 μL, excess) to cap unreacted sites. This suspension was shaken at room temperature for 2 h, filtered, and washed as described above to yield resin **14** as a straw-colored powder (518 mg). IR (CH_2Cl_2 gel): 3400 br, several 1745−1715, 1612, 1585 cm^{-1}. Fmoc quantitation gave an amino acid loading of 0.92 mequiv.

4. Attachment of Second Amino Acid (15). Resin **14** (358 mg) was treated with a 20% piperidine−DMF solution (3 mL) for 10 min, drained, and washed with DMF, 2-propanol, and DMF (3 × 3 mL each). The resin was suspended in DMF (3 mL), and a preprepared solution of approximately 2 equiv each of Fmoc−GLY−OH (270 mg), PyBOP (470 mg), and HOBt (140 mg) in DMF (3 mL) was added. To this suspension was added DIPEA (157 μL, approximately 4 equiv). These suspensions were agitated by bubbling with nitrogen for 60 min and then drained and the resins washed with DMF (3 × 3 mL). The resins were resubjected to the reaction conditions, washed in the usual way, and dried in vacuo to yield the dipeptide resin **15** as a yellow powder (354 mg). IR (CH_2Cl_2 gel): 3420, 3340, several 1750−1660, 1612−1601 cm^{-1}. Fmoc quantitation gave an amino acid loading of 0.87 mequiv.

5. Diboronate Coding Step (Second Tagging Step) (16). To the dipeptide-loaded resin **15** (50 mg) swollen in DMA (1.5 mL) was added (4′-*tert*-amylphenoxy)benzyl-3,5-pinacolatodiboronate (27 mg, approximately 3 equiv with respect to iodo-loaded sites). Tetrakis(triphenylphosphine)palladium-(0) (3 mg, approximately 10 mol %) and 2 M K_2CO_3 (100 μL, 12 equiv) were added, and the reaction mixture was shaken at 50 °C for 16 h. The resin was filtered and used directly in the next step.

6. Mono-iodide Coding Step (Third Tagging Step) (17). The boronate resin **16** was swollen in DMA (1.5 mL), and 3-iodo-4′-*tert*-butylphenoxybenzyl ether (27 mg, 3 equiv) was added followed by tetrakis(triphenylphosphine)palladium-(0) (3 mg, approximately 10 mol %) and 2 M K_2CO_3 (100 μL, 12 equiv). This suspension was shaken at 50 °C for 16 h, filtered, and washed in the usual way to give the fully tagged resin **17** as a beige powder (50 mg).

7. Attachment of Final Amino Acid (18). The procedure was exactly as for step 4 save for the use of Fmoc−TYR-

(O*t*-Bu)−OH in place of Fmoc−GLY−OH. Resin **17** (42 mg) gave final resin **18** (55 mg) as a light-yellow powder.

Typical Procedure for the Thioanisole-Based Cleavage of Tags. To a single bead of **18** was added 10% TFA in CH_2Cl_2 (250 μL) containing TMS-triflate (4.5 mM) and thioanisole (7 mM). The suspension was allowed to stand, with occasional agitation, for 30 min, and then the supernatant was removed under a stream of nitrogen. To the residue was added diethyl ether (0.5 mL) containing dansyl chloride (15 μM) and triethylamine (5.7 mM), followed by aqueous 1 M NaOH (0.5 mL) containing tetra-*n*-butylammonium iodide (0.25 mM). This biphase was stirred vigorously for 30 min, and the organic phase was separated and concentrated under a stream of nitrogen, resuspended in acetonitrile containing 0.1% TFA (250 μL), and then filtered and injected into an FT-ICR mass spectrometer.

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