Versatile Approach To Encoding Combinatorial Organic Syntheses Using Chemically Robust Secondary Amine Tags†

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Received January 12, 1996[⊗]

Encoded combinatorial organic synthesis has recently emerged as a powerful tool for the discovery of biologically active compounds from complex chemical libraries. This report describes a new encoding methodology that uses chemically robust secondary amines as tags. These amines are incorporated into an *N*-[(dialkylcarbamoyl)methyl]glycine-coding oligomer through simple chemistry that is compatible with a wide range of polymer-supported transformations useful in combinatorial synthesis. In the decoding process acidic hydrolysis of the tagging polymer regenerates the secondary amines, which after dansylation are resolved and detected at sub-picomole levels by reversed-phase HPLC. The versatility of this strategy is demonstrated here by encoded syntheses of members of several representative heterocyclic compound classes, including β-lactams, 4-thiazolidinones, and pyrrolidines.

Introduction

Synthetic chemical libraries produced by combinatorial synthesis have rapidly become important tools for pharmaceutical lead discovery and compound optimization.[1] Many of the approaches devised to prepare such libraries rely on solid-phase synthesis techniques and exploit the efficient "split/pool" method to assemble a statistical sampling of all possible combinations of a set of chemical building blocks.[2] Determination of the chemical structure of biologically active library members has represented a major challenge because the quantity of material available from a complex library is frequently insufficient for conventional chemical analysis. A general solution to this structure elucidation problem has been described that exploits a set of surrogate analytes, or identifier tags, which can be detected with either greater ease or sensitivity than the chemical entities which they represent.[3] Through their concurrent attachment to the synthesis supports, these tags provide an unambiguous record of the chemical reaction history, or chronology of monomer (building block) additions to each support in the library. This method, which has become known as encoded combinatorial synthesis,[4] has broad scope and utility, and conceptually may be applied to the construction of any collection of compounds that can be produced through a multistep synthesis on solid supports.[5−7]

One essential requirement with any scheme of encoded combinatorial synthesis using molecular tags[8] is that the chemistries employed in the ligand synthesis and tag addition steps be mutually compatible. An ideal tagging medium would, therefore, be completely chemically inert and amenable to rapid and straightforward analysis at trace levels. Still and co-workers have described one useful approach[7] in which a series of chromatographically resolvable halocarbon derivatives are appended to synthesis resin via acylcarbene insertion chemistry following a binary coding strategy[9] and subsequently liberated oxidatively for analysis by electron capture gas chromatography. We disclose in this report a versatile alternative encoding strategy that is particularly suited to the automated combinatorial synthesis of small organic molecule libraries. This is a binary coding methodology using chemically robust secondary amine tags, which are incorporated into a polyamide supporting backbone on a differentially functionalized synthesis resin (Figure 1a). The site of tag addition to the resin is unambiguously controlled through the use of orthogonal protecting group chemistry, avoiding the potential for uncertain levels of ligand corruption through interaction with reactive tagging intermediates.[7b]

Results and Discussion

Several important design criteria were satisfied through our choice of dialkylamines as coding moieties. We wished to couple the tags to synthesis supports using simple and reliable chemistry that would lend itself readily to automation using custom-built synthesizer hardware. The tag recovery and decoding process also needed to be straightforward and provide for highly sensitive tag detection at the level of individual resin beads. Finally, the tags needed to be chemically resilient and the whole encoding process compatible with a wide array of ligand chemistries. This has been accomplished by incorporating the tagging amines into an *N*-[(dialkylcarbamoyl)methyl]glycine polymer using an amine-based synthesis resin that is differentially functionalized with sites for ligand synthesis and tag addition, shown schematically in Figure 1. The tagging monomer units are obtained simply by treating an *N*-protected iminodiacetic anhydride with the desired secondary amine, the tagging monomer protecting group (PG^T) chosen so that coding polymer can be extended without compromising the integrity of the final ligand or its precursor intermediates. Tag addition to the resin is accomplished in one of two ways, shown in Scheme 1. In the first method (a), set mixtures of *N*-protected *N*-[(dialkylcarbamoyl)methyl]glycines are coupled using HATU or other peptide-coupling agents to the tag attachment site. Alternatively, the tagging monomer is elaborated by a three-step process (b) in which the amino group of the tag addition site is acylated by the reactive iminodiacetic anhydride, the pendant carboxy-

† Abbreviations: HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate; DIEA,diisopropylethylamine; Alloc,allyloxycarbonyl; NMP, *N*-methylpyrrolidinone.

⊗ Abstract published in *Advance ACS Abstracts,* April 1, 1996.

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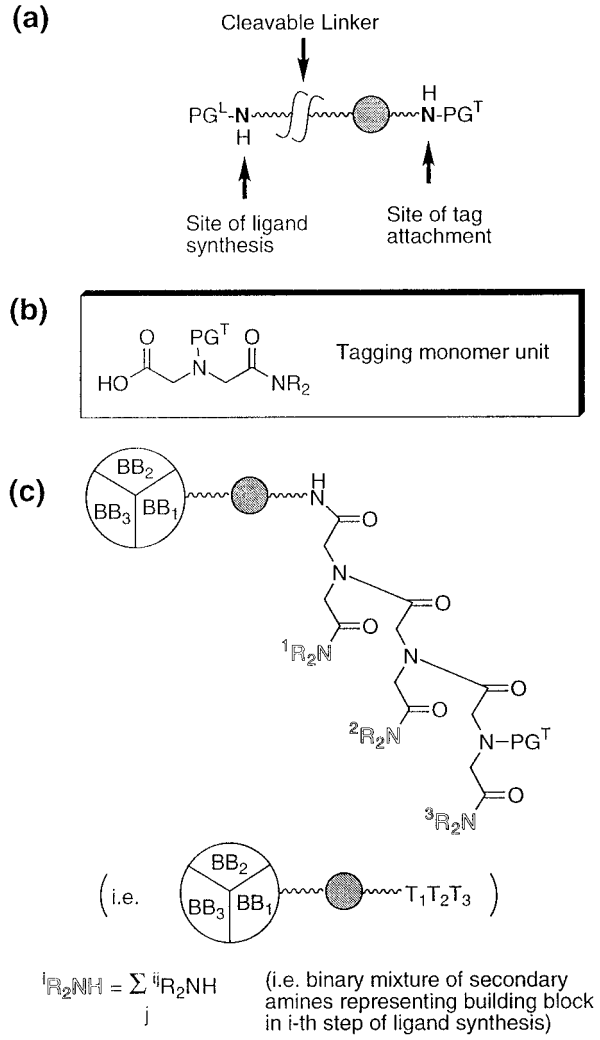


Figure 1. Binary encoded synthesis using secondary amine tags on a differentially functionalized polymer support; PG = protecting group: (a) functionalized resin, (b) structure of *N*-protected tagging monomer, and (c) schematic representation of the product of three-step encoded synthesis.

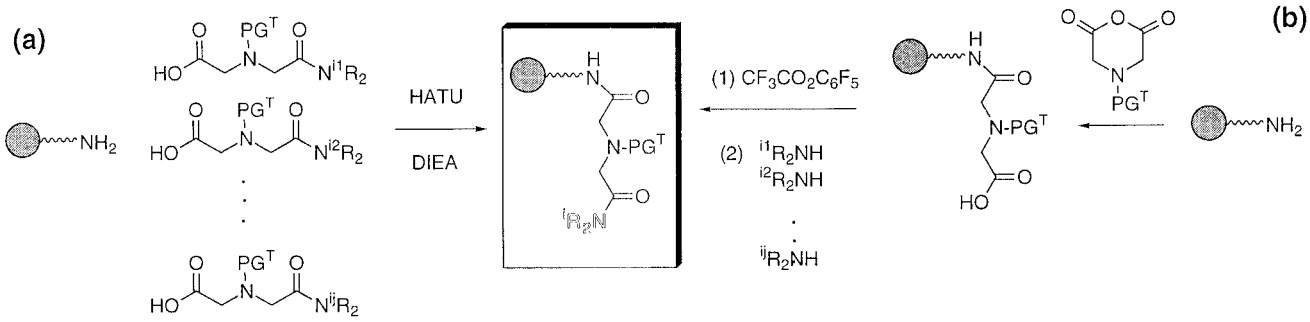
late group activated by conversion to its pentafluorophenyl ester derivative, and the active ester reacted with a binary mixture of secondary amines leading to net elongation of the tag polymer. Application of this tagging procedure at each step of a "split" synthesis either before or after addition of a ligand monomer, but prior to pooling of the resin, permits the chemical reaction history of each solid support to be recorded unambiguously.

The tag decoding process is outlined in Scheme 2. Secondary amine tags are released from individual selected beads upon exposure to standard polypeptide acidic hydrolysis conditions. We had initially anticipated that tag analysis would be most conveniently accomplished using electrospray mass spectrometry: The tags selected would provide ammonium ions having distinctive molecular masses, and a tag mixture would be decoded by direct infusion into the spectrometer without the need for prior chromatographic resolution. While MS-based decoding has indeed been feasible, a complementary approach that relies on HPLC analysis of highly fluorescent dansyl sulfonamide derivatives of the amine tags has been adopted as a more robust decoding strategy (*vide infra*).

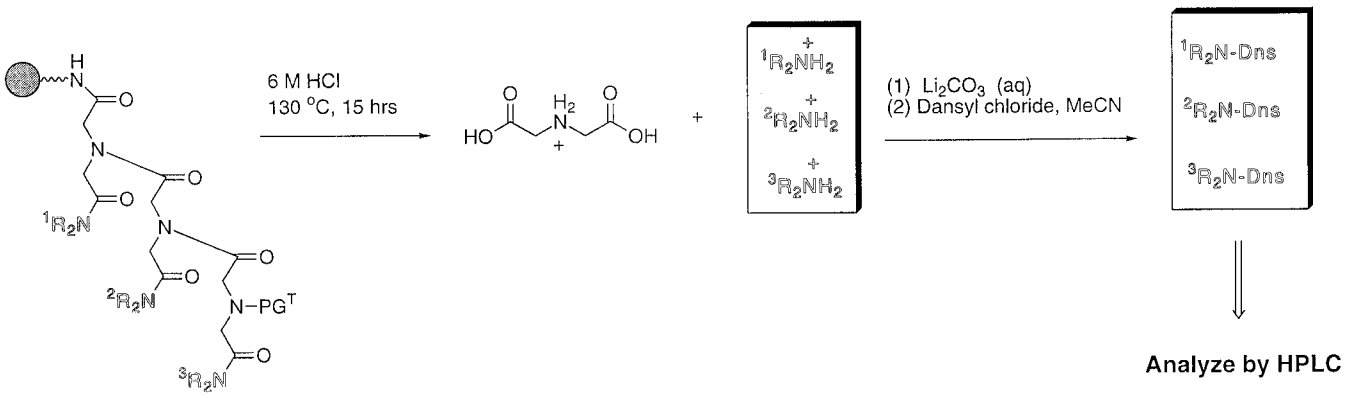
Preliminary experiments indicated that a series of dialkylamines could be derivatized by dansylation with equal efficiency to give a group of highly fluorescent adducts with distinctive and reproducible chromatographic retention characteristics. Using a conventional narrow-bore HPLC system equipped with a fluorescence detector, dilution experiments with dansyl dihexylamide as a standard indicated that as little as 10−20 fmol of the sulfonamide could be reliably detected (data not shown). The synthesis support routinely used in our work is the 130 μm diameter Tenta Gel S resin (Rapp Polymere) with an approximate loading of 300 pmol of NH_2/bead. Thus, by reserving around 10% of these reactive groups (i.e., ~30 pmol) as sites for tag addition, we ensure that each bead carries sufficient tag to permit facile detection. The remaining ~270 pmol of NH_2/bead are available as sites for ligand synthesis, and typically these groups are first derivatized with a linker moiety that permits cleavage of the assembled ligand from the resin under mild conditions (*vide infra*). Liberation of the ligand from a single bead of this type into a volume of 100 μL (e.g., in a microtiter well) provides a useful concentration of compound for biological assay (i.e., ~1.4 μM, assuming a 50% overall yield for the synthesis).

The power of a binary coding method is that a great many compounds can be unambiguously represented as binary combinations of just a few tags.[7] For example, with six tags one can code for $2^6 - 1 = 63$ reactions or structures (the "null" tag combination is not used), and thus a three-component synthesis using 63 building blocks at each step can be encoded using $3 \times 6 = 18$ tags and gives rise to $63^3 \sim 250\,000$ compounds. A set of 18 secondary dialkylamines, selected so that their dansyl derivatives have distinctive retention times when analyzed by RP-HPLC, have been used as tags in this work.[10] The corresponding *N*-Boc-*N*-[(dialkylcarbamoyl)methyl]glycine derivatives of these amines were prepared as shown in Scheme 3. To demonstrate the efficient coupling, recovery, derivatization, and resolu-

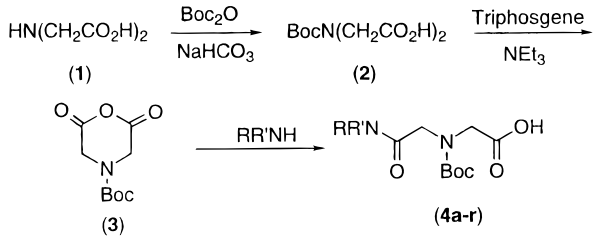
Scheme 1. Encoding



Scheme 2. Decoding



Scheme 3. Preparation of *N*-Boc-*N*-[(dialkylcarbamoyl)methyl]glycine Tag Monomers



(**4a**) N(Et)(Bu)
(**4b**) NMe(C_6H_{13})
(**4c**) NBu_2
(**4d**) NMe(C_7H_{15})
(**4e**) NBu(C_5H_{11})
(**4f**) $N(C_5H_{11})_2$
(**4g**) NBu(C_7H_{15})
(**4h**) $N(C_6H_{13})_2$
(**4i**) $N(C_5H_{11})(C_8H_{17})$
(**4j**) $N(C_3H_7)(C_{10}H_{21})$
(**4k**) NMe($C_{12}H_{25}$)
(**4l**) $N[CH_2CH(Et)C_4H_9]_2$
(**4m**) $N(C_8H_{17})_2$
(**4n**) NBu($C_{12}H_{25}$)
(**4o**) $N(C_5H_{11})(C_{12}H_{25})$
(**4p**) $N(C_6H_{13})(C_{12}H_{25})$
(**4q**) $N(C_7H_{15})(C_{12}H_{25})$
(**4r**) $N(C_{10}H_{21})_2$

tion of this tag set, an equimolar mixture of the 18 monomers **4a**−**r** was appended to Tenta Gel S resin by treatment with HATU and DIEA as coupling reagents. After thorough washing, individual resin beads were selected at random (using a microscope) and heated at 130−140 °C in a sealed melting point capillary tube with 6 M HCl (100 μL) for 15 h.[11] The excess liquid was removed by lyophilization, the residue neutralized with aqueous Li_2CO_3, and the amine mixture derivatized with dansyl chloride in MeCN. RP-HPLC analysis of a small aliquot (2%) of the dansylated hydrolysate from a single bead (Figure 2) shows 18 well-resolved peaks of approximately equal intensity.

Differential functionalization of an amino resin support for encoded synthesis is accomplished by coupling a mixture of orthogonally protected amino acids (e.g., Fmoc-Gly-OH, Boc-Gly-OH) or by treatment with a mixture of the appropriate chloroformates (e.g., Fmoc-Cl, Alloc-Cl). We have found that four orthogonally compatible protecting group and cleavable linker chemistries (base-sensitive, acid-sensitive, Pd(0)-sensitive, and photosensitive) can be blended to provide robust and highly flexible strategies for encoded solid-phase organic synthesis. Scheme 4, for example, shows the construction of a bifunctional resin (**5**) that incorporates Boc-protected amino groups as sites for tag addition, together with Fmoc-protected amines for ligand elaboration (the ratio of Boc:Fmoc groups is ~1:9). Here, the ligand synthesis sites are derived from a photolabile linker group (based on α-methylnitroveratrylamine, abbreviated PL) that permits the liberation of the assembled ligand from the support upon photolysis at 365 nm.[12] The synthesis protocol is recorded by stepwise extension of the coding polymer via TFA deprotection of the Boc groups with subsequent addition of mixtures of *N*-Boc-*N*-[(dialkylcarbamoyl)methyl]glycine tags. A useful variation of this approach utilizes allyloxycarbonyl (Alloc) protection of the tagging sites and reserves base-labile (e.g., Fmoc) and acid-labile (e.g., Boc) chemistries for protection of the ligand monomers.

The versatility of this encoding strategy has been established by demonstrating its compatibility with a variety of polymer-supported heterocyclic chemistries. We have previously described solid-phase approaches to β-lactam,[13] pyrrolidine,[14] and thiazolidinone[15] templates through cycloaddition and condensation reactions of resin-bound imines. These chemistries may be performed directly using resin (**5**), with encoded syntheses of an individual representative β-lactam (**7**) and a thiazolidinone (**8**) illustrated in Scheme 5. The amino acid (glycine) and aldehyde (PhCHO) building blocks of these heterocycles are encoded by tags derived from mixtures of *N*-methyl-*N*-heptylamine/*N*,*N*-dipentylamine (i.e., **4d**/**4f**) and *N*,*N*-dihexylamine/*N*-methyl-*N*-dodecy-

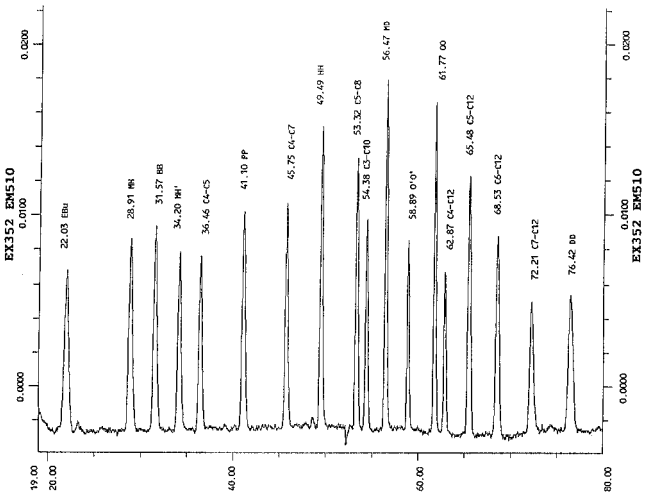
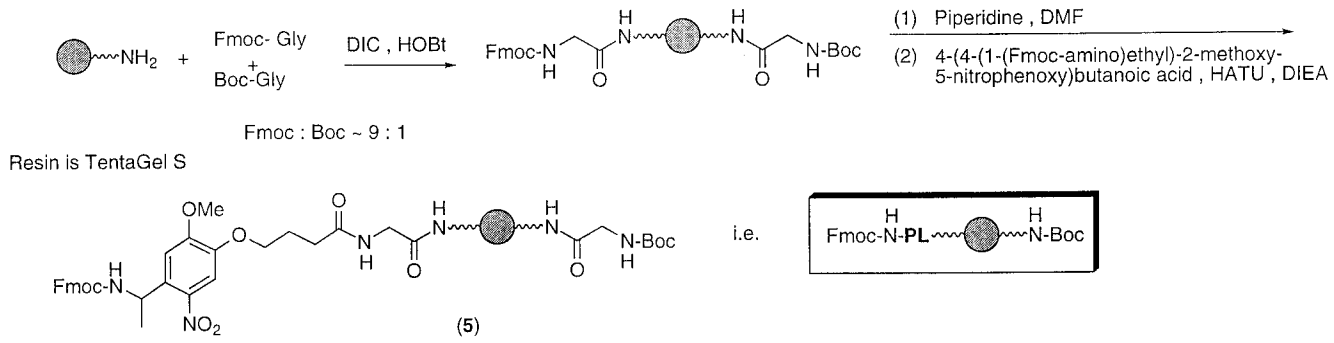


Figure 2. HPLC analysis of an equimolar mixture of 18 dansylated secondary amine tags recovered from a single resin bead.

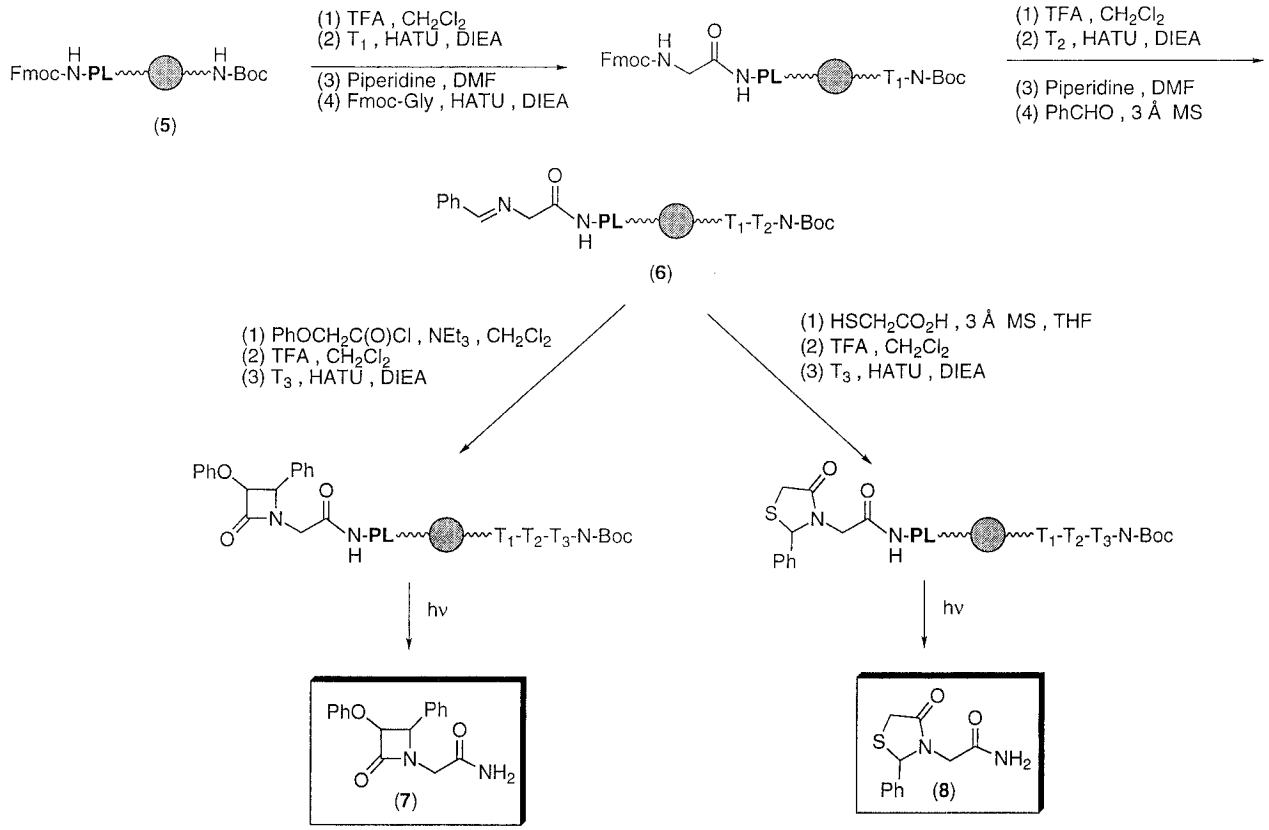
Scheme 4. Preparation of a Differentially Functionalized Synthesis Resin



lamine (i.e., **4h**/**4k**), respectively. The third monomer of the β-lactam (i.e., ketene) and thiazolidinone (i.e., α-mercapto acid) compounds is encoded by a mixture of tags obtained from *N*,*N*-bis[(2-ethyl)hexyl]amine/*N*,*N*-dioctylamine (i.e., **4l**/**4m**). Note that elongation of the tagging polymer can occur either immediately before or after addition of each ligand building block and that this order can be varied, if necessary, to preserve the integrity of labile intermediates in the ligand synthesis (e.g., the second tagging step occurs prior to imine formation, while the third set of tags is coupled to resin upon addition of the final ligand building block so as to avoid imine decomposition during the Boc deprotection step). Figure 3 illustrates that the purity of racemic cis β-lactam product obtained after photolysis of an aliquot of resin from the encoded synthesis is equivalent to that from a control solid-phase preparation and that analysis of tags recovered from a single bead reveal six dansyl amide peaks as expected. Products from the encoded and unencoded control thiazolidinone syntheses are equally satisfactory (data not shown).

A strategy for encoding the preparation of functionalized pyrrolidines via 1,3-dipolar cycloaddition of resin-bound azomethine ylides to olefins, as shown in Scheme 6, features the three-step approach to building *N*-[(dialkylcarbamoyl)methyl]glycine tags described previously. In this example, pyrrolidine synthesis occurs on an acid-cleavable alkoxybenzyl ester linker (MPB) and the tag addition sites are defined as Alloc-protected amines. The first ligand monomer (an Fmoc-protected amino acid) is coupled to the resin, and then removal of the Alloc group from the tag site is accomplished cleanly with tetrabutylammonium azide in the presence of 20 mol % $Pd(PPh_3)_4$.[16] Stepwise elaboration of the tagging polymer using *N*-Alloc-iminodiacetic anhydride, pentafluorophenyl trifluoroacetate, and a set of secondary amines (^iR_2NH) is used to encode both the amino acid and subsequent aldehyde monomer addition reactions as shown. Silver(I)-promoted metalloazomethine ylide formation and cycloaddition with an electron deficient olefin then provides the pyrrolidine product. Importantly, the newly formed secondary amine func-

Scheme 5. Encoded Syntheses of a Representative β-Lactam and 4-Thiazolidinone



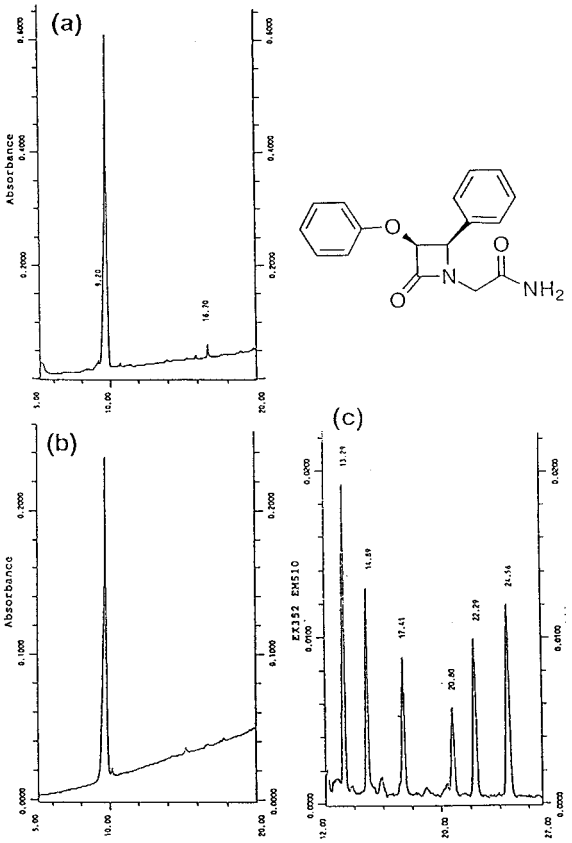


Figure 3. Characterization of a representative β-lactam from encoded synthesis (NB product is a racemic mixture): (a) HPLC analysis of crude product from unencoded solid-phase (control) synthesis, (b) HPLC analysis of crude product from encoded synthesis, and (c) tag analysis from a single encoded bead.

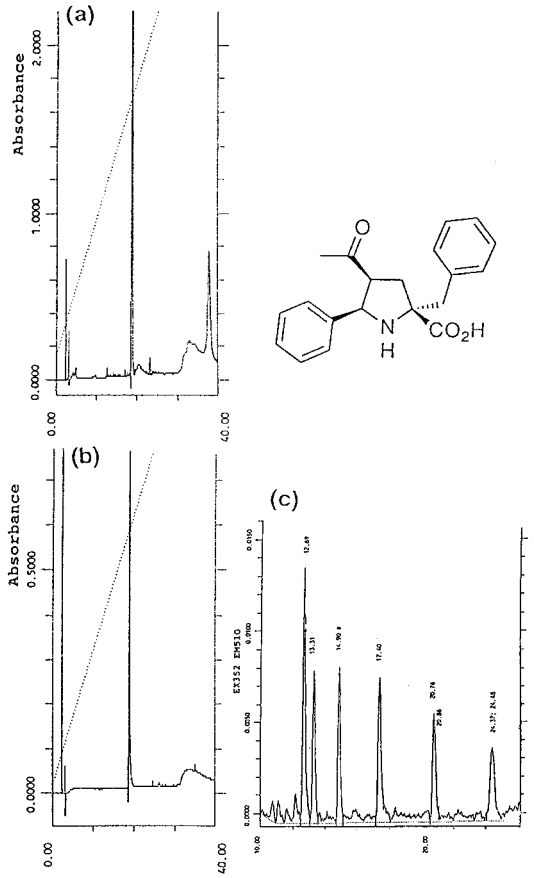


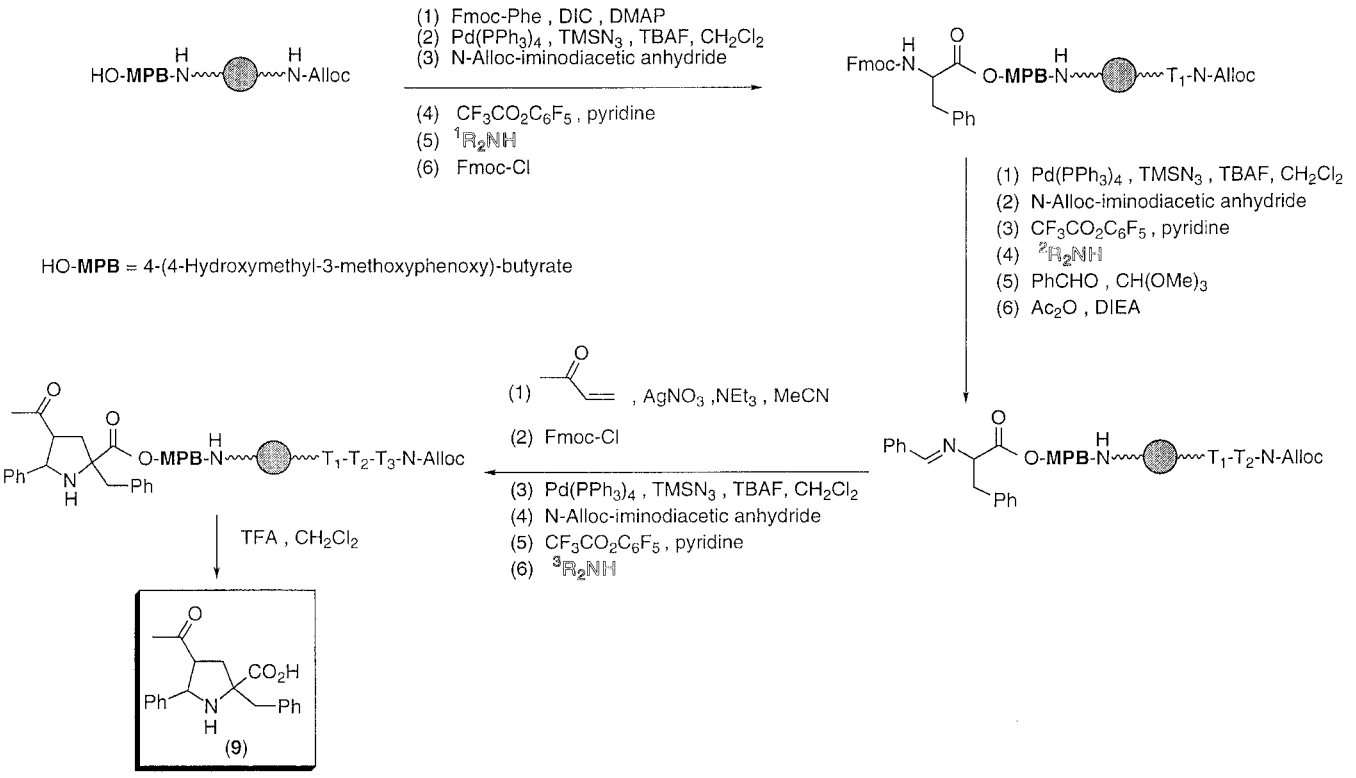
Figure 4. Characterization of a representative pyrrolidine from encoded synthesis (NB product is a racemic mixture): (a) HPLC analysis of crude product from unencoded solid-phase (control) synthesis, (b) HPLC analysis of crude product from encoded synthesis, and (c) tag analysis from a single encoded bead.

tionality of the ligand must be transiently protected prior to the final olefin-coding step; this is conveniently accomplished by treatment with Fmoc-Cl, and this protecting group is removed quantitatively on addition of the third set of amine tags. Product analysis from a representative encoded pyrrolidine synthesis using two tagging amines per synthesis step (Figure 4) again demonstrates mutual compatibility of the ligand and coding chemistries as well as facile tag identification.

From a comparison of the two alternative approaches for elongation of the coding polymer described above (i.e., coupling of preformed *N*-protected *N*-[(dialkylcarbamoyl)methyl]glycine monomers vs the three-step elaboration process), it is evident that the former strategy is generally favored since the number of chemical steps required for any encoded synthesis is significantly fewer using this method. Moreover, the rates of acylation of polymer-supported amines by active esters of the preformed monomers (**4a**−**r**) are not especially sensitive to the identity of the dialkylamino moiety, which facilitates coupling of approximately equivalent amounts of differing tags from binary mixtures.[17] The *in situ* tag elaboration process obviates the requirement for prior synthesis of a set of protected tagging monomers, however. This latter strategy could be particularly advantageous for encoding a synthesis in which a specialized group was required for protection of the tag addition site at a specific step in the synthetic pathway (e.g., to preserve the integrity of an intermediate in the ligand synthesis). Here, only the appropriately protected iminodiacetic anhydride rather than a special set of protected tag monomers need be prepared.

In summary, this report demonstrates that dialkylamines can be used to construct a simple binary code for recording the identity of a wide variety of polymer-supported synthetic transformations useful in combinatorial organic chemistry. When contrasted with the encoding approach of Still and co-workers, attractive features of the present method include (i) the use of rapid, reliable chemistry for tag addition that is readily automated and (ii) the greater degree of control over sites of tag addition that is afforded by use of an orthogonally protected synthesis resin. The process of ligand synthesis should, in general, be unaffected by the chemistry used to construct the encoding polymer (a potential limitation of any molecular coding strategy), which in turn is sufficiently robust to withstand all but the harshest reaction conditions that would degrade a polymeric tertiary amide (e.g., use of a powerful hydride donor such as $LiAlH_4$ would not be compatible with this coding scheme). Note that the exclusive use of secondary amines in building this tagging molecule ensures that there are no readily ionizable protons present in the polymer, and thus this strategy is compatible with the use of powerful nonnucleophilic bases (e.g., LDA) in ligand synthesis if desired (data not shown).[18] This approach is directly amenable to constructing encoded libraries of small organic molecules by incorporating resin splitting and pooling cycles at intermediate steps

Scheme 6. Encoded Synthesis of a Representative Pyrrolidine



in the synthesis, and we have been applying this technique in conjunction with custom-built synthesis automation hardware to generate large (~50 000 member) encoded libraries of various heterocyclic compounds for use in lead discovery. Results obtained from the screening of an encoded library of pyrrolidine derivatives will be presented in a forthcoming communication.

Experimental Section

General Methods. Reagents were bought from Aldrich, Bachem Biosciences and Rapp Polymere and used without further purification. NMR spectra were obtained on a Varian Gemini 300 instrument with DMSO-d_6 as solvent unless noted. ^{1}H NMR spectral data are reported as follows: chemical shift relative to tetramethylsilane (0.00 ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad), coupling, integration. ^{13}C NMR signals are reported in ppm relative to DMSO-d_6 (39.51 ppm).

***N*-Boc-iminodiacetic Anhydride (3).** To a solution of iminodiacetic acid (**1**) (5.0 g, 37.6 mmol) in 50% (v/v) aqueous THF (100 mL) was slowly added sodium bicarbonate (12.6 g, 150 mmol). After 10 min, di-*tert*-butyl dicarbonate (9.85 g, 45.1 mmol) was added. The solution was stirred for 2 days. After the THF was removed, the aqueous layer was extracted twice with ether, acidified with 6 N HCl (30 mL) to pH 1, and extracted with ethyl acetate. The organic layers were washed with brine, dried over sodium sulfate, and concentrated to give 8.1 g (92%) of *N*-Boc-iminodiacetic acid (**2**), which was recrystallized from hot ethyl acetate: mp 144−145 °C.

To a solution of **2** (2.0 g, 8.58 mmol) in ethyl acetate (100 mL) at 0 °C were added NEt_3 (2.43 mL, 17.4 mmol) and powdered triphosgene (0.84 g, 2.83 mmol). The mixture was stirred at 0 °C for 15 min and at room temperature for 15 min and then washed with brine, 0.1 N HCl, aqueous sodium bicarbonate (5%), and brine. The solution was dried over magnesium sulfate and concentrated to give a solid, which was recrystallized from ether to give 1.41 g (76%) of anhydride **3** as a white crystalline solid: mp 110−111 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.42 (s, 4 H), 1.48 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.13, 152.48, 83.10, 44.61, 28.05. Anal. ($C_9H_{13}NO_5$) C, H, N.

General Procedure for the Preparation of Tags 4 from Anhydride 3 and Secondary Amines: *N*-Boc-*N*-[(butylpentylcarbamoyl)methyl]glycine (4e). To a solution of the *N*-Boc anhydride (**3**) (2.0 g, 9.30 mmol) in ethyl acetate (50 mL) at room temperature was added *N*-butyl *N*-pentylamine (1.33 g, 9.30 mmol) in one portion. The solution was stirred overnight and concentrated to give a residue, which was subjected to column chromatography on silica gel eluting with 10% hexane in ethyl acetate to give 3.2 g (96%) of butylpentyl tag monomer **4e** as a white solid: mp 84−85 °C; ^{1}H NMR (300 MHz) δ 4.14 (d, 2H, J = 8.5 Hz), 3.88 (d, 2H, J = 9.7 Hz), 3.26−3.19 (m, 4H), 1.60−1.18 (m, 19H), 0.95−0.83 (m, 6H); ^{13}C NMR (75 MHz) δ 171.06, 169.08, 154.75, 154.53, 79.72, 79.55, 50.91, 50.72, 49.75, 49.21, 46.49, 46.38, 46.33, 46.20, 45.90, 45.60, 45.37, 30.53, 30.32, 29.46, 29.31, 28.49, 28.33, 28.28, 28.05, 27.76, 26.96, 26.81, 21.92, 19.59, 19.43, 13.87, 13.82, 13.72, 13.67; MS (ESI) m/z 359 [(M + H)$^+$]. Anal. ($C_{18}H_{34}N_2O_5$) C, H, N.

All 18 tags **4a**−**r** were prepared in a similar fashion in good yields and characterized by NMR, MS, and combustion analyses. *N*-Alloc protected tag monomers can be prepared in an analogous manner from the *N*-Alloc iminodiacetic anhydride.

Coupling of an Equimolar Mixture of the 18 Tag Monomers 4a−r to Tenta Gel Resin. Tenta Gel S-NH_2 resin (130 μm diameter, 0.29 mmol of NH_2/g, 20 mg, 5.8 μmol; Rapp Polymere), in a specially designed vessel was washed three times with DMF. An equimolar solution of the 18 tag monomers **4a**−**r** (0.18 mmol total), HATU (68 mg, 0.18 mmol), and DIEA (30 μL, 0.18 mmol) in DMF (0.2 mL) was prepared and mixed for 2 min before being added to the resin. After agitation for 20 min, the resin was washed three times each with DMF, MeOH, and CH_2Cl_2 and then dried under vacuum.

General Procedure for Recovery, Derivatization, and Analysis of Secondary Amine Tags from Encoded Resin. An individual resin bead was selected using forceps under a low-power microscope and placed in a capillary tube. After adding 6 N HCl (0.1 mL), the capillary was sealed and heated on a heating block at 130−140°C for 15 h. The tube was opened, and excess liquid was removed by lyophilization. To the tube was added aqueous lithium carbonate (54 mM, 40 μL) and MeCN (40 μL). After mixing for 10 min, dansyl chloride solution (0.74 mM in MeCN, 40 μL) was added. The

mixture was agitated for 10 min, diluted to 1.0 mL with MeCN, and filtered through a 0.4 μm filter. The derivatized tag solution (20 μL) was injected onto a reversed-phase HPLC column for analysis.

The following chromatographic and detection conditions were used with a Beckman System Gold HPLC instrument to separate and detect individual dansyl amides from encoded beads: detector, Model RT-551 Shimadzu fluorescence monitor relayed to the HPLC system through a Beckman Analog Interface Module (Model 406), with excitation wavelength at 352 nm, emission wavelength at 510 nm, and sensitivity at high level; column, Alltech Adsorbosphere narrow-bore C18 solvent miser column (5 μm) with 2.1 mm diameter and 250 mm length; flow rate: 0.25 mL/min; eluents: (A) water and (B) MeCN; gradient, 55% eluent B to 59% in 5 min, 59% to 100% eluent B over 50 min, and 100% eluent B for 30 min.

Note that a more rapid analysis of the dansyl amides is possible when a sub-set of the 18 secondary amines are used as tags, as in the β-lactam, thiazolidinone, and pyrrolidine examples below. The eluents and chromatographic gradients are modified as follows: eluents, (A) 50 mM aqueous NH_4OAc, pH 7.5, and (B) 2% eluent A in MeCN; gradient, 65% eluent B for 1 min, 65% to 100% eluent B over 10 min with curve 3, and 100% eluent B for 24 min.

Preparation of an Orthogonally Differentiated (Fmoc/Boc) Photolabile Resin (Scheme 4). Tenta Gel S-NH_2 resin (130 μm diameter, 0.3 mmol of NH_2/g, 1.0 g, 0.3 mmol), in a specially designed vessel, was washed three times with DMF. To the resin was added a solution of Fmoc-Gly-OH (200 mg, 0.675 mmol), Boc-Gly-OH (13 mg, 0.075 mmol), 1-hydroxybenzotriazole (HOBt; 101 mg, 0.75 mmol), and 1,3-diisopropylcarbodiimide (DIC; 120 μL, 0.75 mmol) in DMF (3 mL). The resin beads were shaken for 3 h and washed three times with DMF. Acetic anhydride (2 mL) and pyridine (2 mL) were added to cap any free amino groups. After 10 min, the beads were washed three times each with DMF, MeOH, and CH_2Cl_2 and then dried under vacuum.

A portion of the beads (0.4 g, 0.12 mmol) was washed three times with DMF. To the beads was added a 20% (v/v) solution of piperidine in DMF (2 mL). After 30 minutes, the beads were washed three times each with DMF, MeOH, and DMF. A solution of the photolabile linker 4-[4-[1-(Fmoc-amino)ethyl]-2-methoxy-5-nitrophenoxy]butanoic acid[12] (187 mg, 0.36 mmol), HATU (134 mg, 0.36 mmol) and DIEA (60 μL, 0.36 mmol) in DMF (0.6 mL) was added. After agitation for 20 min, the resulting resin **5** was washed with DMF, MeOH, and CH_2Cl_2, three times each.

Preparation of an Encoded 4-Thiazolidinone Resin (Scheme 5). To the resin **5** prepared above (0.2 g, 0.06 mmol) was added a 50% (v/v) solution of TFA in CH_2Cl_2 (2 mL) to remove the Boc protecting group. The resin was shaken for 30 min and washed three times with CH_2Cl_2; 50% (v/v) DIEA in CH_2Cl_2 (2 mL) was added, and after agitation for 30 min, the resin was washed three times each with CH_2Cl_2, MeOH, and DMF. A solution of tag monomers **4d**,**f** (0.18 mmol total), HATU (68 mg, 0.18 mmol), and DIEA (30 μL, 0.18 mmol) in DMF (0.2 mL) was prepared and mixed for 2 min before being added to the resin. After agitation for 20 min, the resin was washed three times with DMF. A 20% (v/v) solution of piperidine in DMF (1 mL) was then added to the resin to remove the Fmoc protecting group. After 20 min, the resin was washed three times each with DMF, MeOH, and DMF. A solution of Fmoc-Gly-OH (54 mg, 0.18 mmol), HATU (68 mg, 0.18 mmol) and DIEA (50 μL, 0.3 mmol) in DMF (0.3 mL) was prepared and mixed for 2 min before being added to the resin. After agitation for 20 min, the resin was washed three times each with DMF, MeOH, and CH_2Cl_2. The cycle of Boc deprotection, tag monomer coupling (**4h**,**k**), and Fmoc deprotection was repeated prior to imine formation. The resin was then suspended in THF (2 mL) and treated with 3 Å molecular sieves (0.5 g) and benzaldehyde (183 μL, 1.8 mmol). The mixture was heated at 70 °C for 3 h, and the sieves were removed. The beads were washed three times with CH_2Cl_2 to give encoded imine resin **6**.

The resin **6** was suspended in THF (2 mL) together with 3 Å molecular sieves (0.5 g) and mercaptoacetic acid (100 μL, 1.4 mmol). The mixture was heated at 70 °C for 3 h, and the sieves were again removed. The resin was washed three times each with CH_2Cl_2, MeOH, and CH_2Cl_2. The process of Boc deprotection and tag monomer coupling (**4l**,**m**) was repeated to afford the encoded thiazolidinone resin. The thiazolidinone product **8** was cleaved from a 10 mg aliquot of this resin by photolysis (365 nm) for 2 h in DMSO containing 0.1% (v/v) hydrazine (100 μL) as previously described[12] and then characterized by HPLC and spectroscopic analysis: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.27 (m, 5H), 5.85 (s, 1H), 4.26 (d, 1H, J = 16.3 Hz), 3.80 (s, 1H), 3.39−3.37 (m, 1H), and 3.21 (d, 1H, J = 16.3 Hz); MS (ESI) m/z 237 [$(M + H)^+$].

Individual resin beads were selected at random for decoding as described above.

Preparation of an Encoded β-Lactam Resin (Scheme 5). To resin **6** (0.2 g) suspended in CH_2Cl_2 (6 mL) at −78 °C were added triethylamine (167 μL, 1.20 mmol) and phenoxyacetyl chloride (124 μL, 0.90 mmol). The mixture was warmed to room temperature overnight and washed three times each with CH_2Cl_2, MeOH, and CH_2Cl_2. The process of Boc deprotection and tag monomer coupling (**4l,m**) was repeated to afford the encoded β-lactam resin. The β-lactam product **7** was cleaved from a 10 mg aliquot of this resin by photolysis (365 nm) for 2 h in DMSO containing 0.1% (v/v) hydrazine (100 μL) as previously described[12] and then characterized by HPLC and spectroscopic analysis: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30−6.66 (m, 10H), 5.55 (d, 1H, J = 4.6 Hz), 5.18 (d, 1H, J = 4.6 Hz), 4.28 (d, 1H, J = 17.1 Hz), 3.40 (d, 1H, J = 17.1 Hz); MS (ESI) m/z 297 [$(M + H)^+$].

Individual resin beads were selected at random for decoding as described above.

Elongation of an *N*-Alloc-Protected Tagging Polymer. Alloc-protected tag monomers can be used analogously to the Boc compounds except that the conditions for protecting group removal are modified.[16] To Alloc-protected amino resin (0.1 mmol, ca. 0.25 mmol/g) was added a solution of tetrakis(triphenylphosphine)palladium(0) (0.023 g, 0.02 mmol) in CH_2Cl_2 (2 mL). A solution of tetrabutylammonium fluoride (0.078 g, 0.3 mmol) and azidotrimethylsilane (0.106 mL, 0.092 g, 0.8 mmol) in CH_2Cl_2 (2 mL) was prepared and allowed to stand at room temperature for 5 min before addition to the resin mixture. The mixture was flushed with nitrogen and gently shaken. After 30 min, the mixture was drained and the resin washed with CH_2Cl_2 (2 × 5 mL). The deprotection procedure was then repeated. The resin was subsequently treated with a 3% (w/v) solution of sodium diethyldithiocarbamate in *N*-methylpyrrolidine (NMP) containing 5% (v/v) DIEA (5 mL) to remove any residual palladium. After shaking for 10 min, the mixture was drained and the resin washed with NMP (3 × 5 mL).

Coupling of a mixture of Alloc-protected tag monomers to the resin can then be achieved using HATU according to the protocol described above.

Preparation of an Encoded Pyrrolidine Resin (Scheme 6): (a) Preparation of an Orthogonally Differentiated (Fmoc/Alloc) Acid-Labile Resin. Tenta Gel S-NH_2 resin (130 μm diameter, 0.29 mmol of NH_2/g, 1.0 g, 0.29 mmol) was treated with a mixture of 9-fluorenylmethyl chloroformate (Fmoc-Cl; 0.54 g, 2.09 mmol) and allyl chloroformate (Alloc-Cl; 24.6 μL, 0.232 mmol) in NMP (10 mL). DIEA (0.4 mL, 2.32 mmol) was added and the suspension shaken at room temperature for 90 min. The reaction was shown to be complete by negative Kaiser test. Spectrophotometric determination of dibenzofulvene released from a small aliquot of resin upon treatment with piperidine indicated an approximate derivatization ratio of 5:1 Fmoc:Alloc.

The resin was treated with 20% piperidine in NMP (10 mL) for 20 min and then with 4-[4-(hydroxymethyl)-3-methoxyphenoxy]butyric acid (HMPB; 0.278 g, 1.16 mmol) and DIC (182 μL, 1.16 mmol) in NMP (10 mL). A second treatment with HMPB/DIC resulted in complete coupling. Fmoc-Phe-OH (0.432 g, 1.16 mmol) and DIC (0.182 mL, 1.16 mmol) were mixed in NMP (10 mL) and then added to the resin with DMAP (0.014g, 0.116 mmol). The suspension was shaken at room temperature for 2 h and then drained and the resin washed with NMP. A second coupling with Fmoc-Phe-OH/DIC was followed by treatment of the resin with a large excess

of acetic anhydride and pyridine (each 10%, v/v, in NMP, 10 mL). The resin was thoroughly washed with NMP and CH_2Cl_2 and then subjected to one round of the tag addition cycle described below.

(b) Three-Step Tag Elaboration Process (Scheme 1b). The Alloc protecting group was removed from the tag addition sites by treatment of the resin with tetrabutylammonium azide and Pd(0) as described above. After reaction with *N*-Alloc-iminodiacetic anhydride (0.1 g, 0.5 mmol, 10 equiv relative to free NH_2) and DIEA (0.087 mL, 0.5 mmol) in NMP (5 mL) for 1 h, the resin suspension was drained and then treated with an excess of pentafluorophenyl trifluoroacetate and pyridine in NMP (1:1:1, total volume 3 mL) for 1 h. Subsequent treatment of the polymer-supported OPfp ester with an appropriate mixture of secondary amines completed one round of the tagging cycle.

After treatment with Fmoc-Cl (0.6 g, 2.32 mmol) and DIEA (0.4 mL, 2.32 mmol), a second repetition of the tag elongation cycle gave phenylalanine resin encoded with a tag dimer. This resin was then gently agitated with a 1.0 M solution of benzaldehyde in trimethyl orthoformate (4 mL) for 4 h. The resin was again filtered and washed with CH_2Cl_2 (2 × 3 mL). [2 + 3] Cycloaddition of the imine was effected by addition of a solution containing 1.0 M each of silver(I) nitrate, 3-buten-2-one, and NEt_3 in MeCN. The solution turned black after 5 min with plating of silver upon the walls of the vessel occurring after 2 h. After 8 h the resin was filtered and washed with saturated ammonium chloride (2 × 3 mL), MeOH (2 × 3 mL), and CH_2Cl_2 (2 × 3 mL). The newly formed pyrrolidine amino group was protected by 2 × 1 h treatments with an NMP solution containing Fmoc-Cl and DIEA (2.0 M each) followed by 2 × 1 h treatments with a pyridine solution containing Fmoc-Cl (2.0 M). One further cycle of tag elongation gave the fully encoded pyrrolidine resin. Spectrophotometric determination of dibenzofulvene released from a small aliquot of this resin upon treatment with piperidine indicated a loading of the Fmoc-protected pyrrolidine that was equivalent to the initial loading of Fmoc-Phe. The pyrrolidine **9** was cleaved from an aliquot of the resin upon treatment with a 10% solution of trifluoroacetic acid in CH_2Cl_2 (2 mL) for 30 min and characterized by HPLC and spectroscopic analysis: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80−7.01 (m, 10H), 4.54 (d, J = 6.9 Hz, 1H), 3.41−3.31 (m, 1H), 3.34 (ab q, J = 14.0 Hz, 2H), 2.90 (dd, J = 3.5, 14.3 Hz, 1H), 2.50 (dd, J = 7.7, 14.3 Hz, 1H), 2.08 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.715, 152.754, 133.769, 132.851, 129.926, 129.911, 129.855, 129.782, 128.954, 127,601, 127.498, 126.955, 126.814, 120.662, 61.734, 55.725, 51.499, 49.348, 38.475, 29.694; MS (ESI) m/z 324 [(M + H)$^+$].

Individual resin beads were selected at random for decoding as described above.

Acknowledgment. We thank Jung Lee and Caryn Schunk for assistance in the preparation of several tagging monomers.

Supporting Information Available: Spectroscopic and analytical data characterizing the 18 *N*-Boc-*N*-[(dialkylcarbamoyl)methyl]glycine tag monomers **4a**−**r** (38 pages). Ordering information is given on any current masthead page.

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(10) The 18 amines, listed in order of increasing retention time of their dansyl amides, are *N*-ethyl-*N*-butylamine, *N*-methyl-*N*-hexylamine, *N*,*N*-dibutylamine, *N*-methyl-*N*-heptylamine, *N*-butyl-*N*-pentylamine, *N*,*N*-dipentylamine; *N*-butyl-*N*-heptylamine, *N*,*N*-dihexylamine, *N*-pentyl-*N*-octylamine, *N*-propyl-*N*-decylamine, *N*-methyl-*N*-dodecylamine, *N*,*N*-bis[(2-ethyl)hexyl]-amine, *N*,*N*-dioctylamine, *N*-butyl-*N*-dodecylamine, *N*-pentyl-*N*-dodecylamine, *N*-hexyl-*N*-dodecylamine, *N*-heptyl-*N*-dodecylamine, and *N*,*N*-didecylamine.

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(18) This is a significant limitation with the amino acid-based coding schemes previously described.

JM960043J