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A Divergent Strategy for Attaching Polypyridyl Ligands to Peptides

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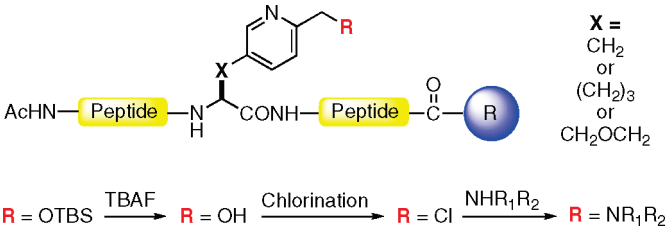
Received October 12, 2009



A divergent method for incorporating polypyridyl ligands into peptides is reported. Three *N*-Fmoc unnatural amino acids (**1**−**3**) that contain varying linkers between the α-carbon and a 2-(hydroxymethyl)pyridyl group were synthesized in enantioenriched form. These amino acids were used as anchors for incorporating multidentate ligands onto a peptide chain in a site-specific fashion. Multiple peptide−ligand conjugates were synthesized from single precursors by solution- or solid-phase methods. Peptides containing more than one metal-binding unit can be produced by this method.

Introduction

The attachment of metal-binding ligands is a powerful method for creating peptides with remarkable properties. Besides the obvious enhancement of metal-binding affinity that a strong ligand confers, the conjugation of unnatural metal-binding motifs creates peptides for a variety of applications.[1] For instance, peptide−metal complex conjugates containing radioactive metal isotopes can act as radiopharmaceuticals, particularly for anticancer applications.[2−4] Appending metal centers that are catalytically active or possess labile coordination sites has produced peptides with the ability to selectively bind to or target DNA and proteins.[5−7] In addition, peptides can be used to deliver metal complexes to specific locations in cells.[8,9] Metal−peptide constructs have been used extensively in de novo protein design[10−13] and in the creation of artificial enzymes that catalyze organic transformations.[14,15]

A variety of different synthetic methods have been reported for constructing peptide−metal complex conjugates. In particular, solid-phase methodologies have been sought that can take advantage of the combinatorial approach in order to produce large libraries of metal-binding peptides for screening and optimization purposes. Metal complexes have been attached to numerous positions on peptides via solid-phase synthesis, including the *N*-terminus,[9,16] side chains,[2,17]

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Published on Web 01/05/2010 DOI: 10.1021/jo9021953

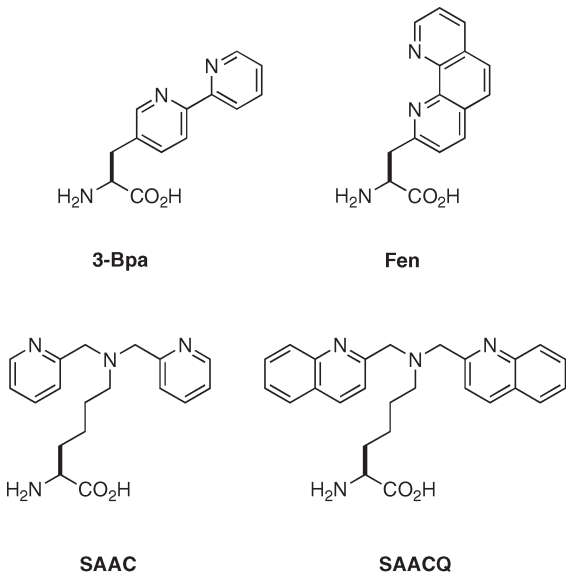

FIGURE 1. Unnatural amino acids containing bi- or tridentate polypyridyl ligands.

or even as part of the peptide backbone itself.[18,19] Of these three strategies, attaching metal complexes to side chains via a metal-binding amino acid is perhaps the most ideal because ligands can be placed site-specifically at a desired location anywhere on the peptide chain, rather than just on the *N*-terminus or through conjugation with Cys or Lys residues. Selected examples of amino acids used in the site-specific strategy include Ala derivatives which contain bidentate ligands such as 2,2-bipyridyl (Bpa) or 1,10-phenanthroline (Fen),[20−24] as well as the single amino acid chelates derived from Lys that contain di(2-pyridylmethylene)amine (SAAC) or di(2-quinolinemethylene)amine (SAACQ) units,[2,17,25,26] which act as tridentate chelating groups (Figure 1). Despite major synthetic efforts directed toward producing peptide-metal ligand conjugates, there is a paucity of divergent synthetic strategies that can be used to adorn a single, resin-bound peptide with multiple metal-binding units in a site-specific fashion. This is unfortunate because the metal-binding unit represents an additional handle for achieving diversity in peptide−metal complex conjugates.

In addition to the bidentate and tridentate *N*-donor ligands shown in Figure 1, polypyridyl ligands with higher denticity have properties that make them attractive for incorporating into peptides (Figure 2). By virtue of the additional ligating group, the tetradentate ligand TPA (tri(2-pyridylmethylene)amine) binds more strongly, by

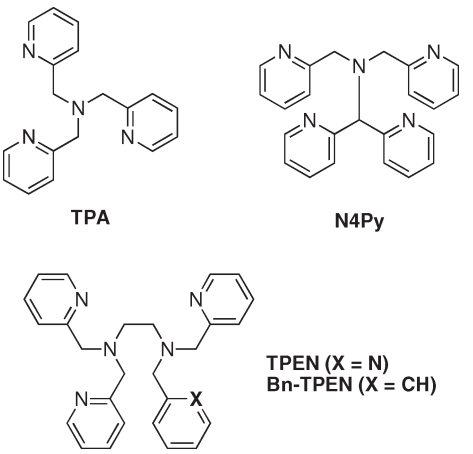

FIGURE 2. Tetra-, penta-, and hexadentate polypyridyl ligands.

several orders of magnitude, to transition metals than its tridentate counterpart di(2-pyridylmethylene)amine, the core element of SAAC (Figure 1).[27,28] This trend of greater affinity continues with ligands of higher denticity to the hexadentate ligand TPEN, which binds to first-row transition metals with dissociation constants in the pM to fM range.[28] Besides their greater affinity, ligands such as TPA and N4Py have been used to create catalysts and active-site models of enzymes in nature. Complexes derived from these multidentate ligands and their derivatives containing Fe, Cu, and Mn bind to and activate O_2,[29−31] oxidize organic substrates including the strong C−H bonds found in hydrocarbons,[32−37] bind to and release NO,[38] and cleave DNA under oxidizing conditions.[39−41] Furthermore, recent work published from our laboratory demonstrated that the iron(IV)-oxo species derived from N4Py oxidizes amino acids selectively,[42,43] and that $[Fe^{IV}(O)(N4Py)]^{2+}$ can be incorporated into a short peptide.[44] The latter publication provided

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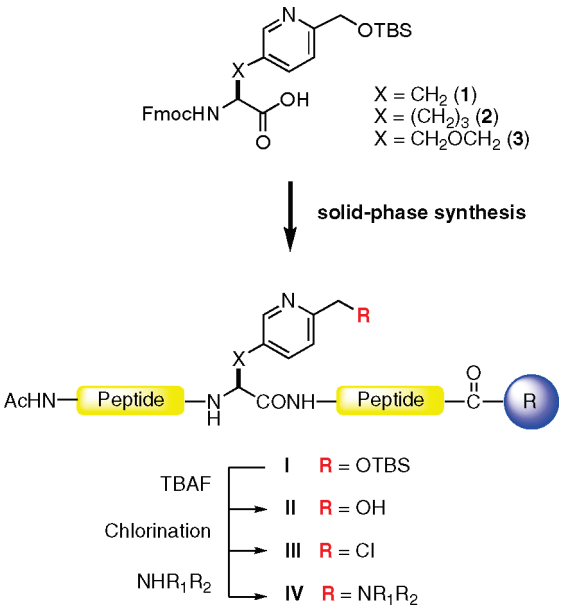


FIGURE 3. Schematic diagram for incorporating metal-binding units into peptide chains using a divergent strategy.

proof of concept that these polypyridyl ligands can be attached to peptides, bind metal ions, and produce reactive intermediates much like their parent ligands.

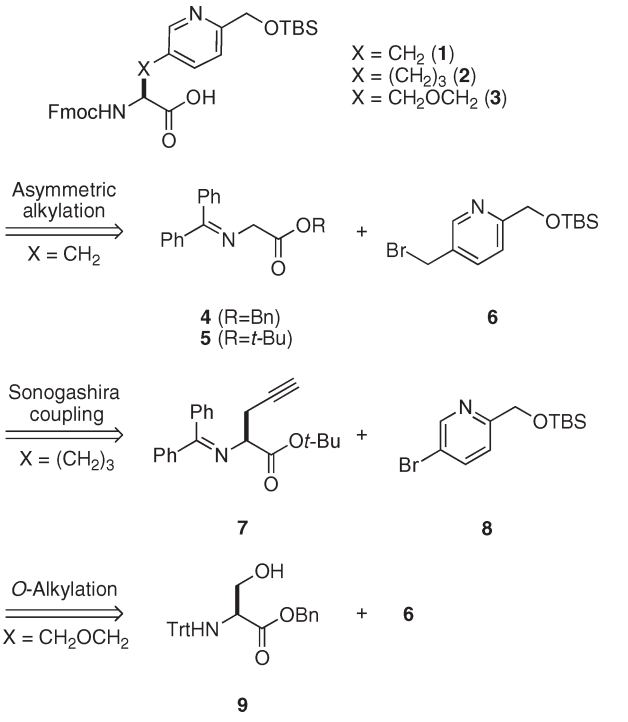
Herein we report a divergent method for preparing peptide-ligand conjugates based on the polypyridyl amine scaffold. Recognizing that the pyridyl ring is a core element in the ligands shown in Figure 2, three unnatural amino acids containing the pyridylmethyl group were designed to act as anchors for the site-specific incorporation of polypyridyl ligands into peptides. The enantioselective syntheses of these amino acids are discussed, as well as a synthetic strategy that creates multiple metal-binding peptides with varying denticity from a single peptide precursor, both in solution and on solid phase.

Results and Discussion

The strategy envisioned for incorporating polypyridyl ligands into peptide chains is shown in Figure 3. Unnatural amino acids **1**−**3** were designed so that the 2-pyridylmethylene group, a common fragment of several nonheme ligands (Figure 2), is bound at the end of the side chain. After introducing these amino acids into a peptide chain, transformation of the silyloxy functionality into a leaving group, followed by *N*-alkylation with secondary amines, could be used to attach various ligands to the peptide in a divergent fashion. In addition, changing the position of the unnatural amino acid within the peptide chain could facilitate the insertion of ligands at various positions. Furthermore, availability of three amino acids with various linkers, between the α-position and 2-(hydroxymethyl)pyridyl group, would provide additional flexibility in designing peptide−ligand conjugates. This combination of structural, positional, and metal-binding ligand flexibilities would expedite the synthesis of libraries of metal-binding peptides.

Design and Retrosynthesis of Unnatural Amino Acids 1−3. Three unnatural amino acids were designed to provide variation in linker composition and length by connecting

SCHEME 1. Retrosyntheses of Unnatural Amino Acids 1−3



the α-position of an amino acid with a 2-(hydroxymethyl)-pyridyl group. Retrosynthetic analyses envisioned for the preparation of these three unnatural amino acids are presented in Scheme 1. The unnatural amino acid **1** (2-hydroxymethyl-5-pyridyl alanine, HPA) was designed so that the 2-(hydroxymethyl)pyridyl unit is separated from the α-position by a one-carbon spacer and hence resembles phenylalanine. Retrosynthetically, the unnatural amino acid **1** was disconnected to protected glycine derivatives **4**[45] or **5**[45] and bromide **6**. Next, to aid the flexibility in designing of peptide-ligand conjugates, amino acid **2** was envisioned possessing a three-carbon linker between the α-position and 2-(hydroxymethyl)pyridyl group. This amino acid contains norvaline, and hence it is abbreviated as HPN (2-hydroxymethyl-5-pyridyl norvaline). Sonogashira coupling between propargylglycine derivative **7**[46] and precursor **8**[47,48] was considered as the most plausible synthetic route for the preparation of HPN (**3**). The third unnatural amino acid **3** [(2-hydroxymethyl-5-pyridyl methyloxy)-3-alanine, HPMA] was envisioned as an oxygen-containing variant of HPN. The synthetic disconnection for HPMA involved *O*-alkylation of serine derivative **9**[49] by bromide **6**. This route holds the advantage that the asymmetric center in **3** would be derived from Ser.

Synthesis of Unnatural Amino Acids 1−3. To begin the synthesis of unnatural amino acids **1**−**3**, precursors **4**,[45] **5**,[45] **7**,[46] **8**,[47,48] and **9**[49] were prepared by previously reported

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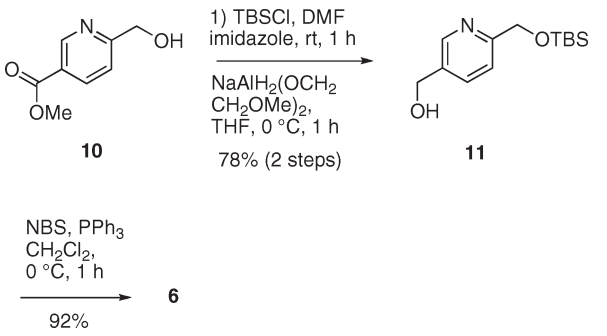
SCHEME 2. Synthesis of Pyridylmethyl Bromide 6



procedures. The route to compound **6** began from alcohol **10**, which is a known compound available in one step from the commercially available starting material dimethyl pyridine-2,5-dicarboxylate (Scheme 2). Compound **10** was accessed by selective reduction of the diester in the presence of $NaBH_4/CaCl_2$ using a slight modification of the previously described procedure.[50] Upon scale-up using the previously reported procedure, in which THF/EtOH was used as a solvent mixture, transesterification to form mixtures of methyl and ethyl esters was observed. Use of THF/MeOH as a solvent mixture under otherwise identical reaction conditions furnished **10** with better yields. After optimizing conditions to provide **10** on multigram scale, protection of alcohol **10** with TBSCl, followed by reduction of C-5 ester moiety with sodium bis(2-methoxyethoxy)aluminum hydride gave the corresponding alcohol **11** in 78% yield over two steps. Importantly, no chromatography was required in this three-step sequence. Transformation of alcohol **11** to pyridylmethyl bromide **6** was achieved using NBS and PPh_3, which provided **6** in 92% yield from **11**.[44]

With all precursors in hand, the synthesis of **1** was developed using an asymmetric alkylation[51] as the key step (Scheme 3). First, the alkylation reaction was carried out to furnish racemic product. Reaction of **4** with bromide **6** using the achiral phase-transfer catalyst (PTC) Bu_4NHSO_4 furnished racemic **12** in 82% yield (Scheme 3).[21,52] Incorporating the benzyl protecting group was advantageous because deprotection of **12** by hydrogenolysis[53] could be used to access **14**, which was converted into Fmoc-HPA(OTBS)-OH (**1**) in a separate step. Unfortunately alkylation reactions between **4** and **6** with chiral PTCs gave **12** in good yield (74−88%) but low to moderate ee (11−72%) under a variety of conditions (Table 1, entries 3 and 6). Initial optimization studies established that use of substrate **5** (R = *t*-Bu) instead of **4** (R = Bn)[52] along with *N*-(9-methylanthracenyl)-*O*-allylcinchonidine bromide (**16**) as a chiral PTC and $CsOH\cdot H_2O$ as a base at −30 °C furnished **13** in 87% yield and 92% ee (entry 7).[54] Although the reaction conditions could be used to prepare gram quantities of **13**, the reaction was further optimized to obtain conditions that were more easily amenable to scale up. Toward this goal, the solid $CsOH\cdot H_2O$ base was replaced with 50% aq NaOH to furnish **13** with similar yields and ee when the reaction was performed in toluene: CH_2Cl_2 (7:3) at 0 °C (entry 8). Using these conditions, the catalyst loading could be lowered to 5 mol % (Entry 9). Deprotection of the *C*- and *N*-termini of **13** by treatment with $NH_2OH\cdot HCl$[55] followed by TMSOTf/2,6-lutidine[56] gave **14**, albeit in low isolated yield. This required us to adopt an alternative strategy. Global deprotection of **13** with 6N HCl, followed by replacement of the TBS group and Fmoc protection gave **1** in 80% yield over the three steps, without the need for isolating **14**.

SCHEME 3. Synthesis of Fmoc-HPA(OTBS)-OH (1)

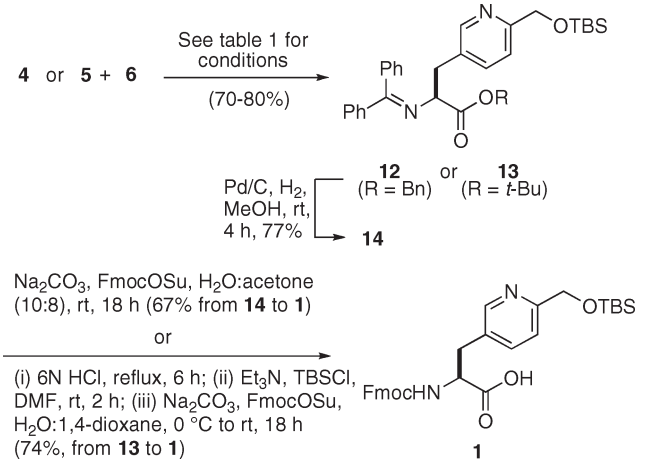


TABLE 1. Optimization of Asymmetric Alkylation Reaction

entry	PTC[a,b] (mol %)	R	temp (°C)	% yield (% ee[b,c])
1	TBAH[d] (20)	Bn	0	82 (0)[f]
2	TBAI (20)	*t*-Bu	0	70 (0)[f]
3	15 (20)	Bn	0	88 (11)[f]
4	15 (20)	Bn	−78	10 (nd[e])[g]
5	16 (20)	Bn	−78	46 (79)[g]
6	16 (20)	Bn	−30	74 (72)[g]
7	16 (20)	*t*-Bu	−30	87 (92)[g]
8	16 (20)	*t*-Bu	0	80 (93)[f]
9	16 (5)	*t*-Bu	0	70 (93)[f]

[a]Phase-transfer catalyst **15** = *N*-benzylcinchonidine chloride; **16** = *N*-(9-methylanthracenyl)-*O*-allylcinchonidine bromide. [b]See the Supporting Information for structures of **15** and **16** and determination of ee. [c]Absolute configuration of major enantiomers of **12** and **13** is based on literature precedent (refs 22, 52, and 54,). [d]Tetrabutylammonium hydrogensulphate (Bu_4NHSO_4). [e]nd = not determined. [f]50% aq NaOH, toluene/CH_2Cl_2 (7:3). [g]$CsOH\cdot H_2O$, CH_2Cl_2.

Sonogashira coupling was chosen as the key step toward the synthesis of HPN amino acid **2** because previous studies indicated that propargylglycine derivatives had been used in arylation reactions.[57,58] In order to minimize cumbersome protecting group manipulations, alkyne **7** was used directly as the coupling partner. Although alkyne **7** is available in one step and 93% ee from propargyl bromide and imine **5**,[46] two

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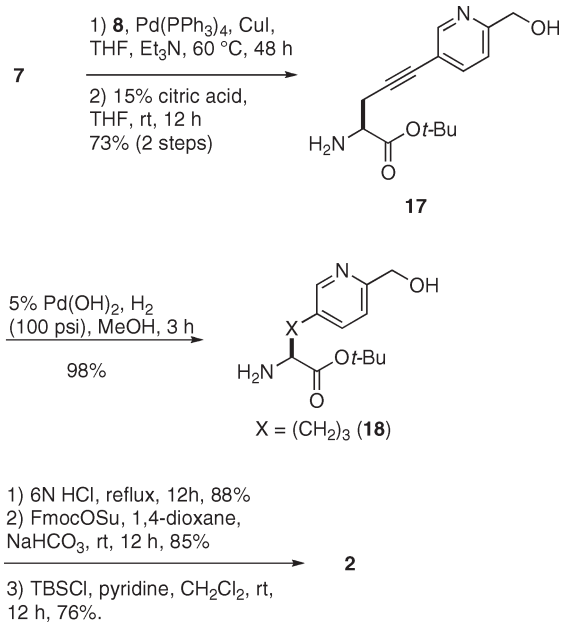
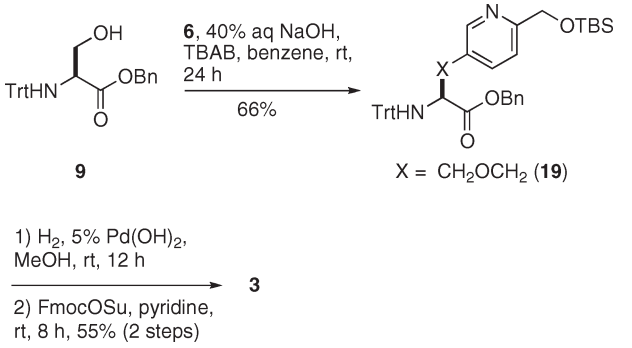
SCHEME 4. Synthesis of Fmoc-HPN(OTBS)-OH (2)



commercially available starting materials, Sonogashira coupling reactions with **7** had not been reported in the past. Gratifyingly, the arylation of **7** with bromopyridine **8**[47,48] proceeded smoothly at 60 °C in the presence of catalytic amounts of CuI (16 mol %) and $Pd(PPh_3)_4$ (10 mol %), furnishing the coupled product (Scheme 4). Purification at this stage proved difficult due to catalyst decomposition products that could not be separated from the desired product, so the benzophenone imine and TBS group were removed in one step by treatment with dilute aqueous citric acid, giving **17** in 73% yield over the two steps. At this stage, the ee of **17** was determined to be 93%, identical to compound **7**, confirming that no epimerization took place during the coupling or deprotection sequence. Subsequent reduction of the alkyne was carried out using 5% $Pd(OH)_2$ and H_2 (100 psi) in MeOH, giving **18** in 98% yield. To complete the synthesis of Fmoc-HPN(OTBS)-OH (**2**), hydrolysis of the *tert*-butyl ester using 6 N HCl, followed by Fmoc protection and replacement of the TBS group, furnished **2** in 57% yield over the three steps.[59]

In order to gain access to the amino acid HPMA, the *O*-alkylation of Ser with bromide **6** was chosen because it has been demonstrated in the literature,[60,61] and the corresponding precursors are easily accessed. Initial trials for *O*-alkylation of *N*-Boc-, *N*-Cbz-, or *N*-Trt-protected Ser derivatives with **6** in the presence of a variety of strong or weak bases such as NaH, NaHMDS, KHMDS, Et_3N, *i*-Pr_2EtN, or K_2CO_3 gave either decomposition products or no reaction. With strong bases, bromide **6** was found to decompose. Alkylation under phase-transfer conditions was pursued as an alternate method because bromide **6** was demonstrated to be stable under these conditions during the synthesis of **1**. The *O*-alkylation reaction was attempted by treating *N*-Boc,

SCHEME 5. Synthesis of Fmoc-HPMA(OTBS)-OH (3)



N-Cbz, or *N*-Trt protected Ser derivatives with **6** in biphasic solvent mixtures. Optimum yields were obtained when Trt-Ser-OBn (**9**) was treated with **6** in the presence of TBAB as a phase-transfer catalyst in a mixture of benzene and 40% aqueous NaOH, giving alkylation product **19** in 66% yield (Scheme 5). The synthetic route for HPMA was advantageous not only because installation of a new chiral center was not necessary, as in the syntheses of **1** and **2**, but also because hydrogenolysis could be used to remove both the *N*- and *C*-terminal protecting groups in **19** in a single step while leaving the TBS protecting group intact. Subsequent Fmoc protection furnished **3** in 55% over the two steps.

Incorporation of Metal-Binding Units into a Peptide via a Solution-Phase Method. Having developed scalable syntheses of amino acids **1**−**3**, we sought to develop a divergent method for ligand incorporation. Toward this goal, the model dipeptide **20** was synthesized in order to develop conditions for attaching ligands using solution-phase methods (Scheme 6). Dipeptide **20**, prepared in racemic form for these studies, was accessed by the acetylation of **14** followed by coupling of the resultant acid with H-Gly-OBn using EDAC and HOBt, which gave **20** in 60% yield over the two steps. The silyl ether group of **20** was transformed into chloride **21** in a two-step sequence involving deprotection with a mixture of TBAF and AcOH in THF, followed by treatment with a combination of TsCl and excess LiCl in MeCN. Attachment of the amine side chains by *N*-alkylation was carried out by heating mixtures of chloride **21**, 3.0 equiv of the appropriate secondary amine (NHR_1R_2),[40,62] and excess *i*-Pr_2EtN in acetonitrile at 55 °C. These conditions furnished products **22** (TPA derivative) and **23** (Bn-TPEN derivative) in good to excellent yields (79−95%). However, formation of the N4Py derivative **24** occurred in low yield under these conditions, presumably due to the steric hindrance of the amine nucleophile.[14] Addition of a catalytic amount of NaI improved the yield dramatically and provided **24** in 90% yield.

Extension of the Methodology to Resin-Bound Peptides. After successful incorporation of metal-binding units into model dipeptide **20** via solution-phase methods, the transition to solid-phase synthesis was made. Starting from Fmoc-Gly bound to Rink amide resin, the sequence began by deprotection of the Fmoc group, coupling with Fmoc amino acids **1**, **2**, or **3** using reagents DCC and HOBt or alternatively HBTU, followed by Fmoc deprotection and

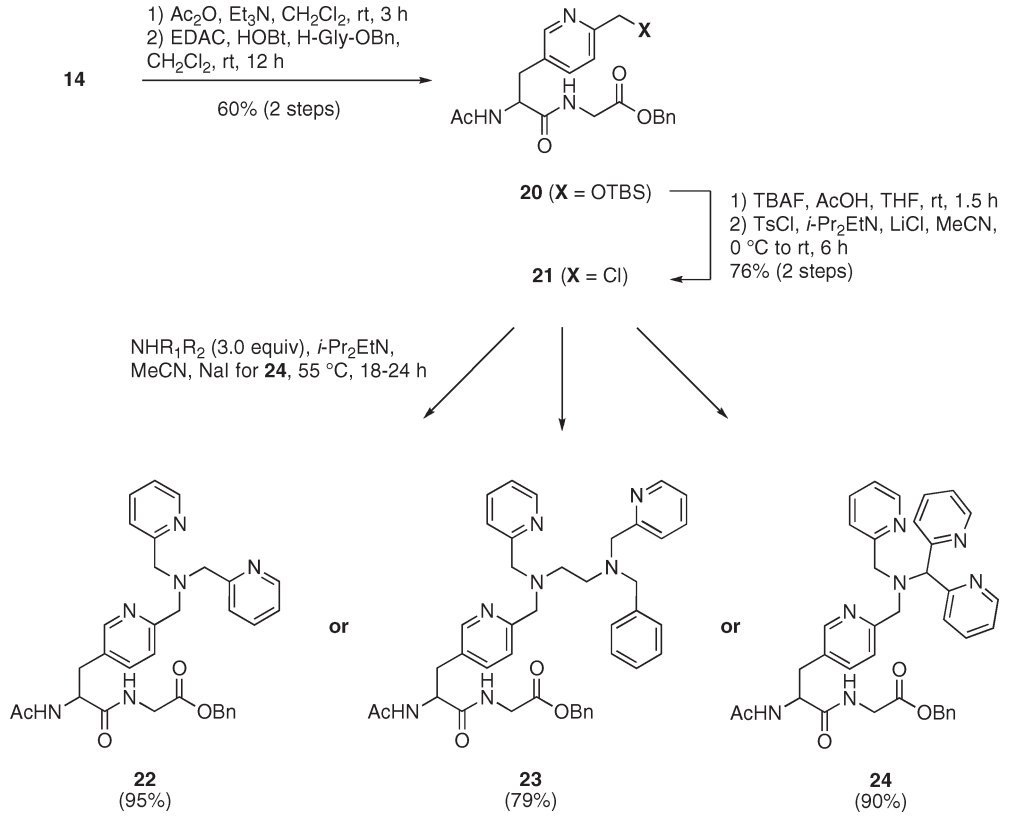
(59) Attempts were made to deprotect the *t*-Bu ester under mild conditions that would preserve the TBS group in moving from **7** to **2**, but overall yields were considerably lower.

(60) Palmer, M. J.; Danilewicz, J. C.; Vuong, H. *Synlett* **1994**, 171–2.

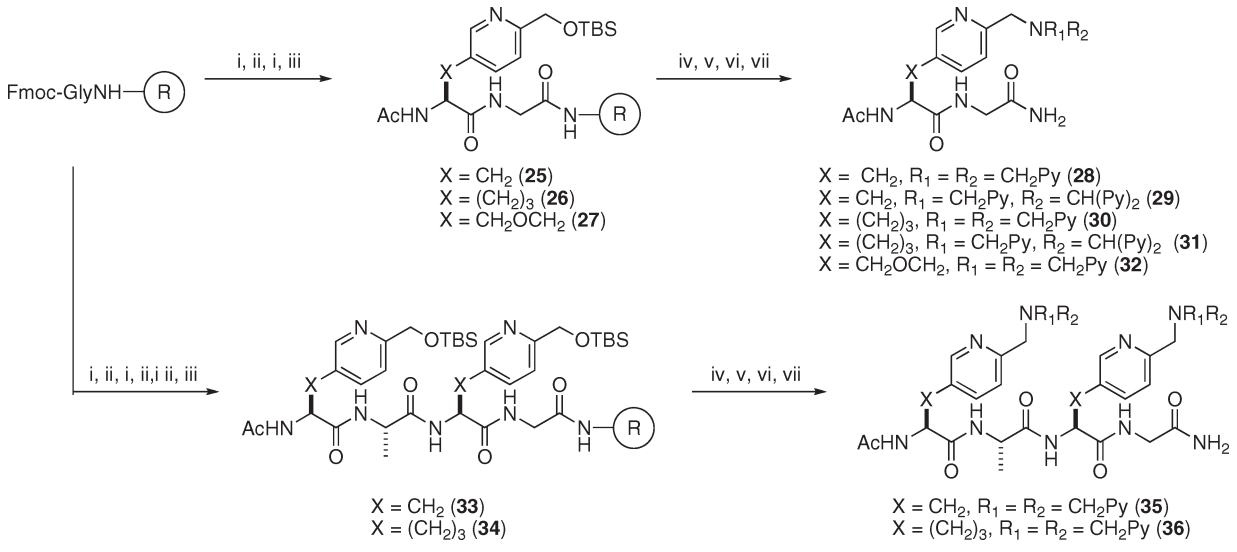
(61) Faul, M. M.; Winneroski, L. L.; York, J. S.; Reinhard, M. R.; Hoying, R. C.; Gritton, W. H.; Dominianni, S. J. *Heterocycles* **2001**, *55*, 689–704.

(62) Baffert, C.; Collomb, M.-N.; Deronzier, A.; Kjaergaard-Knudsen, S.; Latour, J.-M.; Lund, K. H.; McKenzie, C. J.; Mortensen, M.; Nielsen, L. P.; Thorup, N. *Dalton Trans.* **2003**, 1765–1772.

SCHEME 6. Incorporation of Metal Binding Units Using Solution-Phase Synthesis



SCHEME 7. Incorporation of Metal Binding Units Using SPPS[a]

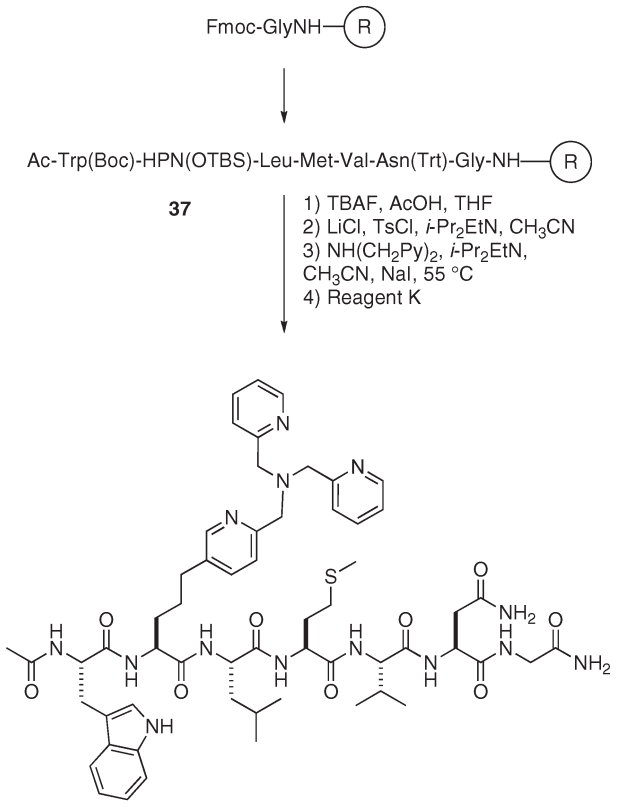


[a]Conditions: (i) 20% piperidine, rt, 20 min (two cycles); (ii) Fmoc-AA-OH, DCC, HOBt or HBTU, *i*-Pr_2EtN, DMF, rt, 3−4 h; (iii) Ac_2O, *i*-Pr_2EtN, DMF, rt, 1 h; (iv) TBAF, AcOH, THF, rt, 1 h (two cycles); (v) LiCl, TsCl, *i*-Pr_2EtN, MeCN, rt, 6 h; (vi) NHR_1R_2, *i*-Pr_2EtN, MeCN, NaI, 55 °C, 24 h; (vii) TFA/H_2O (9:1), rt, 1 h.

N-acetylation with Ac_2O, which gave resin-bound dipeptides **25**−**27**. Next, the silyloxy ether groups of dipeptides (**25**−**27**) were converted into the corresponding chlorides using the conditions shown in Scheme 6.[63] Using the appropriate secondary amines, five dipeptide derivatives (**28**−**32**) were obtained from these chlorides after cleavage from the resin with TFA/H_2O (95:5). HPLC analysis indicated that products obtained from the resin were of >80% purity.

(63) In some cases, it was necessary to resubject pyridylmethyl alcohols to the chlorination conditions (LiCl, TsCl, *i*-Pr_2EtN) to observe complete conversion to the chloride. See the Supporting Information for further details.

SCHEME 8. Synthesis of Heptapeptide 38



Ac-Trp-HPN(TPA)-Leu-Met-Val-Asn-Gly-NH_2 (**38**)

All combinations, except the N4Py derivative derived from HPMA, were prepared by this method. Solution-phase studies (not shown) proved that complex mixtures resulted from attempts to aminate the chloride derived from HPMA with hindered amine nucleophiles. Presumably, an elimination pathway to form dehydroalanine competes with the substitution reaction with bulky nucleophiles. To illustrate the utility of our method further, two tetrapeptides (**33** and **34**) were constructed in a similar manner each containing two unnatural amino acids (HPA or HPN). Both unnatural amino acids were converted into TPA ligands using the aforementioned reaction conditions (**35** and **36**). All three steps, (i) TBS cleavage, (ii) chlorination, and (iii) *N*-alkylation, proceeded cleanly (Scheme 7). Success in the syntheses of peptides **35** and **36** confirms that reaction conditions used for attaching ligands to peptides were not causing epimerization. Overall, the divergent methodology described herein is not limited to attaching one ligand onto a peptide chain but can be used to link multiple ligands to the same peptide chain.

In order to demonstrate the scope of this divergent method, the heptapeptide **37** was prepared (Scheme 8). First the pentapeptide Fmoc-Leu-Met-Val-Asn(Trt)-Gly bound to Rink amide resin was synthesized using a standard protocol on an automated peptide synthesizer. After deprotection of the Fmoc group, coupling of the amino acids **2** and Fmoc-Trp(Boc)-OH was carried out manually. Cleavage of the Fmoc group and capping of the *N*-terminus with Ac_2O furnished resin-bound peptide **37**. Selective removal of the TBS protecting group, chlorination, and substitution with bis(pyridin-2-ylmethyl)amine was used to install the full metal-binding unit TPA. Global deprotection and cleavage from the resin using Reagent K furnished the heptapeptide **38**. These results confirm that peptides with a rich array of functional groups can be prepared by this method.

Conclusion

In summary, we have presented a divergent strategy for attaching polypyridyl ligands to peptides. Three unnatural amino acids (**1**−**3**) were synthesized in enantioenriched form that can be used in SPPS. All together, the availability of three unnatural amino acids, flexibility to incorporate them anywhere into a peptide chain, and the ability to attach a variety of ligands by varying the secondary amine nucleophiles, makes this methodology highly divergent and ideal for the synthesis of peptide−ligand conjugate libraries. These studies are now underway in our laboratory.

Experimental Section

General Considerations. All reagents were purchased from commercial suppliers and used as received. HPLC was performed on a preparative purification system equipped with a multiwavelength detector. Column purifications were performed using silica gel flash chromatography unless mentioned otherwise. Compounds **4**,[45] **5**,[45] **6**,[44] **7**,[46] **8**,[47,48] **9**,[49] **16**,[54] **20**,[44] **21**,[44] and **24**[44] used in this report were synthesized according to previously reported literature procedures. All reactions were performed under ambient atmosphere unless otherwise noted. Anaerobic reactions were performed in Schlenk tubes. These reactions were deoxygenated by performing five vacuum-backfill cycles with Ar and were run under a constant purge of Ar. For anaerobic reactions, Et_3N and DMF were distilled over CaH_2. THF was deoxygenated by bubbling Ar through a submerged needle, before use. Toluene and dichloromethane were dried over 4 Å molecular sieves before use.

General Procedures for Preparation of 12 and 13. General Procedure A. A mixture of **4** or **5** (16.7 mmol), PTC (See Table 1), and 7:3 toluene/CH_2Cl_2 (72 mL) was cooled to 0 °C under a nitrogen atmosphere. A 50% aqueous NaOH solution (24.5 mL) was added at 0 °C. 5-(Bromomethyl)-2-((*tert*-butyldimethylsilyloxy)methyl)pyridine **6** (5.30 g, 16.7 mmol) was dissolved in 7:3 toluene/CH_2Cl_2 (11 mL), and this solution was added dropwise to the reaction mixture at 0 °C over 10 min. The reaction mixture was stirred vigorously at 0 °C for 3−12 h. After consumption of the starting material as judged by TLC analysis (20% EtOAc/hexanes), the reaction mixture was diluted with cold H_2O (40 mL). The organic layer was separated and the aqueous layer was extracted using CH_2Cl_2 (3 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain a brown viscous oil. The crude product was purified by silica gel chromatography (5% to 15% EtOAc/hexanes) to afford corresponding product **12** or **13**.

General Procedure B. A mixture of **4** or **5** (0.79 mmol), **15** or **16** (See Table 1), 5-(bromomethyl)-2-((*tert*-butyldimethylsilyloxy)-methyl)pyridine **6** (250 mg, 0.79 mmol), and CH_2Cl_2 (2.3 mL) was cooled to the appropriate temperature (see Table 1) under a nitrogen atmosphere. Solid $CsOH \cdot H_2O$ (1.32 g, 7.89 mmol) was added, and the reaction mixture was stirred vigorously for 3−4 h. After consumption of the starting material as judged by TLC analysis (20% EtOAc/hexanes), the reaction mixture was diluted with diethyl ether (20 mL) and then combined with cold water (30 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and

concentrated to get brown viscous oil. The crude product was purified by silica gel chromatography (5% to 15% EtOAc/hexanes) to afford **12** or **13**.

(*S*)-Benzyl 3-(6-((*tert*-Butyldimethylsilyloxy)methyl)pyridin-3-yl)-2(diphenylmethyleneamino)propanoate (12). Compound **12** was synthesized in racemic form by general procedure A while the corresponding enantioenriched form was prepared using general procedure B: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.55 (d, J = 7.3 Hz, 2H), 7.38−7.22 (m, 13H), 6.63 (d, J = 7.3 Hz, 2H), 5.19 (d, J = 13.0 Hz, 1H), 5.12 (d, J = 13.0 Hz, 1H), 4.76 (s, 2H), 4.26 (dd, J = 8.9, 4.9 Hz, 1H), 3.27−3.16 (m, 2H), 0.91 (s, 9H), 0.72 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 171.1, 159.3, 149.7, 139.0, 138.0, 135.9, 135.7, 131.4, 130.5, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.4, 119.5, 66.7, 66.6, 65.9, 36.4, 25.9, 18.3, −5.4; IR (thin film) 3061, 3032, 2954, 2928, 2885, 2856, 1741, 1622, 1599, 1574, 1489, 1471, 1446, 1397, 1372, 1314, 1287, 1252, 1163, 1128, 1104, 1029, 1004, 939, 910, 838, 779, 696, 640 cm^{-1}; LRMS (ESMS) calcd for $C_{35}H_{41}N_2O_3Si$ $(M + H)^+$ 565, found 565.

(*S*)-*tert*-Butyl 3-(6-((*tert*-Butyldimethylsilyloxy)methyl)pyridin-3-yl)-2-(diphenylmethyleneamino)propanoate (13). Compound **13** was synthesized in racemic form by general procedure A and in enantioenriched form using either general procedure A or B (see Table 1): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.58 (d, J = 7.3 Hz, 2H), 7.43−7.26 (m, 8H), 6.72 (d, J = 6.5 Hz, 2H), 4.79 (s, 2H), 4.13−4.10 (m, 1H), 3.20−3.14 (m, 2H), 1.43 (s, 9H), 0.92 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 170.0, 158.8, 149.4, 138.9, 137.7, 135.9, 131.5, 129.9, 128.4, 128.0, 127.9, 127.6, 127.1, 119.1, 88.0, 66.9, 65.6, 36.1, 27.6, 25.5, 18.0, −5.7; IR (thin film) 3059, 2955, 2929, 2885, 2856, 1734, 1624, 1599, 1574, 1488, 1472, 1462, 1446, 1394, 1368, 1314, 1288, 1253, 1151, 1104, 1030, 1006, 977, 938, 910, 840, 815, 779, 731, 696, 670, 641 cm^{-1}; $[\alpha] = -132$ (c = 1.0, MeOH); HRMS (ESMS) calcd for $C_{32}H_{43}N_2O_3Si$ $(M + H)^+$ 531.3043, found 531.3037.

(*S*)-2-Amino-3-(6-((*tert*-butyldimethylsilyloxy)methyl)pyridin-3-yl)propanoic Acid (14). A mixture of **12** (6.15 g, 10.9 mmol), MeOH (123 mL), and 5% Pd/C (615 mg) was stirred at rt under H_2 (1 atm) for 4 h. After consumption of the starting material, as judged by TLC analysis, the reaction mixture was filtered through Celite. The filtrate was concentrated to obtain a sticky colorless solid, which upon triturating with Et_2O for 30 min furnished **14** as a colorless solid. The colorless solid was isolated by filtration and washed with Et_2O (2.60 g, 77%): mp = 165 °C dec; ^{1}H NMR (400 MHz, CD_3OD) δ 8.38 (s, 1H), 7.81 (dd, J = 8.1, 2.4 Hz, 1H), 7.54 (d, J = 7.3 Hz, 1H), 4.78 (s, 2H), 3.81 (dd, J = 8.1, 4.9 Hz, 1H), 3.30−3.10 (m, 2H), 0.96 (s, 9H), 0.13 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.2, 161.1, 150.0, 139.8, 132.0, 121.9, 66.5, 57.0, 35.0, 26.3, 19.2, −5.3; IR (KBr) 3405 (b), 2956, 2930, 2887, 2858, 1604, 1524, 1492, 1472, 1463, 1442, 1398, 1361, 1333, 1254, 1108, 838, 778 cm^{-1}; LRMS (ESMS) calcd for $C_{15}H_{27}N_2O_3Si$ $(M + H)^+$ 311, found: 311.

(*S*)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-(6-((*tert*-butyldimethylsilyloxy)methyl)pyridin-3-yl)propanoic Acid (1). FromCompound 14. A mixture of compound **14** (1.00 g, 3.22 mmol), Na_2CO_3 (324 mg, 3.06 mmol), and H_2O (10 mL) was cooled at 0 °C. A suspension of FmocOSu (1.09 mg, 3.22 mmol) in acetone (8 mL) was added, and the reaction mixture was maintained at 0 °C for 1 h. The reaction mixture was warmed to rt and maintained overnight. The reaction mixture was maintained at pH 7−8 during this period. After completion of the reaction, as judged by TLC analysis, the reaction mixture was combined with H_2O (130 mL) and the aqueous layer was washed with hexanes (2 × 30 mL). The aqueous layer was acidified to pH 4−5 by slow addition of 10% aqueous citric acid and extracted with EtOAc (5 × 30 mL). The combined EtOAc layers were washed with NaCl (satd aq). The EtOAc layer was dried over anhydrous Na_2SO_4 and concentrated to obtain crude product (1.14 g, 67%).

From Compound 13. A solution of compound **13** (2.70 g, 5.09 mmol) and 6 N HCl (13.5 mL) was refluxed for 6 h. The reaction mixture was combined with H_2O (30 mL) and extracted with diethyl ether (3 × 10 mL). The aqueous layer was concentrated under high vacuum to obtain a crude product. The crude product was dissolved in H_2O (20 mL) and concentrated under high vacuum (twice) to remove excess HCl. The crude product was dried on high vacuum yielding an off-white solid, which was found to be hygroscopic and stored under nitrogen.

A mixture of the off-white solid (1.10 g, 4.00 mmol), Et_3N (8.3 mL, 60.0 mmol), and DMF (22 mL) was maintained at rt under nitrogen atmosphere for 5 min. A suspension of TBSCl (6.20 g, 40.0 mmol) in DMF (6 mL) was added under nitrogen atmosphere, and the reaction mixture was maintained for 2 h. The reaction mixture was combined with Na_2CO_3 (0.1% aq, 150 mL) and washed with diethyl ether (3 × 20 mL). A colorless solid formed that was isolated and combined with the aqueous layer, which was cooled to 0 °C, and a solution of FmocOSu (1.50 g, 4.50 mmol) in 1,4-dioxane (12 mL) was added over 5 min. The reaction mixture was maintained at 0 °C for 1 h and then warmed to rt. The reaction mixture was maintained at rt overnight, combined with H_2O (160 mL), and extracted with hexanes (2 × 200 mL). The aqueous solution was acidified to pH 4−5 by slow addition of 10% aqueous citric acid. The aqueous solution was extracted with EtOAc (4 × 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain the product. The crude product was further purified by silica gel chromatography (0−5% MeOH/CH_2Cl_2) (2.00 g, 74%). A small sample was recrystallized from MTBE/hexanes (1:2) for analysis: mp = 81−83 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (s, 1H), 7.76−7.70 (m, 3H), 7.57−7.43 (m, 3H), 7.38−7.24 (m, 4H), 4.72 (s, 2H), 4.46−4.41 (m, 1H), 4.34−4.28 (m, 1H), 4.19−4.07 (m, 2H), 3.30−3.20 (m, 1H), 3.03−2.93 (m, 1H), 0.91 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.2, 160.2, 158.2, 149.8, 145.2, 142.5 139.8, 133.7, 128.7, 128.1, 126.3, 126.2, 121.5, 120.9, 67.9, 66.3, 56.6, 35.7, 26.3, 19.2, −5.3; IR (KBr) 3419 (b), 3065, 2953, 2928, 2887, 2856, 1713, 1606, 1536, 1450, 1401, 1335, 1253, 1106, 1053, 1007, 839, 779, 759, 740, 671, 621 cm^{-1}; $[\alpha] = -2.5°$ (c = 1.1, MeOH); HRMS (ESMS) calcd for $C_{30}H_{37}N_2O_5Si$ $(M + H)^+$ 533.2472, found 533.2481.

(*S*)-*tert*-Butyl 2-Amino-5-(6-(hydroxymethyl)pyridin-3-yl)-pent-4-ynoate (17). (*S*)-*tert*-Butyl 2-(diphenylmethyleneamino)-pent-4-ynoate **7** (2.81 g, 8.44 mmol), 5-bromo-2-((*tert*-butyldimethylsilyloxy)methyl)pyridine **8** (3.03 g, 10.1 mmol), $Pd(PPh_3)_4$ (975 mg, 0.84 mmol), CuI (257 mg, 1.35 mmol), Et_3N (8.53 g, 84.4 mmol), and THF (85.0 mL) were combined in a sealed tube under nitrogen atmosphere in a drybox, resulting the formation of a dark brown solution. The sealed tube was heated at 60 °C. Over time, formation of a colorless precipitate was observed. After 48 h, THF was removed under vacuum. The resulting residue was dissolved in water (50 mL) and extracted with EtOAc (3 × 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography (0% to 10% EtOAc/hexanes) to give the product as a yellow oil (3.75 g, 80%), which contained trace amounts of PPh_3 from decomposition of the Pd catalyst.

A mixture of yellow oil (2.36 g, 4.24 mmol), 15% aqueous citric acid (14 mL), and THF (27 mL) was maintained at rt for 12 h. The organic layer was concentrated under vacuum, and the resulting residue was dissolved with 1 N HCl (5 mL). The aqueous solution was extracted with diethyl ether (3 × 75 mL). The aqueous layer was basified using Na_2CO_3 (aq., pH = 9−10) and extracted with EtOAc (3 × 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to give **17** as a yellow oil (1.07 g, 91%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.47 (s, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 4.67 (s, 2H), 3.59−3.30 (m, 1H), 2.85−2.80

(dd, J = 16.2, 4.9 Hz, 2H). 1.47 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.0, 161.6, 151.8, 121.5, 120.4, 89.7, 82.8, 80.5, 65.3, 54.6, 28.3, 26.4; IR (thin film) 3342, 3293, 2977, 1916, 1732, 1701, 1594, 1556, 1487, 1459, 1420, 1393, 1338, 1302, 1238, 1157, 1064, 1028, 954, 846 cm^{-1}; [α] = −6.9 (c = 1.0, MeOH); LRMS (ESMS) calcd for $C_{15}H_{21}N_2O_3$ $(M + H)^+$ 277, found 277.

(*S*)-*tert*-Butyl 2-Amino-3-(6-(hydroxymethyl)pyridin-3-yl)pentanoate (18). A mixture of (*S*)-*tert*-butyl 2-amino-5-(6-(hydroxymethyl)pyridin-3-yl)pent-4-ynoate **17** (1.12 g, 4.06 mmol), $Pd(OH)_2/C$ (112 mg, 5% w/w), and MeOH (25 mL) was stirred at rt under H_2 (100 psi) for 3 h. The reaction mixture was filtered through a Celite bed to remove Pd/C, and the filtrate was concentrated to give **18** (1.10 g, 98%) as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (s, 1H), 7.66 (dd, J = 8.1, 1.6 Hz, 1H), 7.44 (dd, J = 8.1, 1.6 Hz, 1H), 4.61 (s, 2H), 2.64−2.60 (m, 2H), 1.69−1.53 (m, 5H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.9, 159.8, 149.3, 138.8, 137.8, 121.9, 82.2, 65.3, 55.5, 35.2, 33.1, 28.3, 28.1; IR (KBr) 3353, 2976, 2931, 2863, 1726, 1601, 1572, 1479, 1460, 1393, 1368, 1251, 1155, 1068, 846 cm^{-1}; [α] = +3.6 (c = 1.0, MeOH); LRMS (ESMS) calcd for $C_{15}H_{25}N_2O_3$ $(M + H)^+$ 281, found 281.

(*S*)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-(6-(hydroxymethyl)pyridin-3-yl)pentanoic Acid (2). A mixture of (*S*)-*tert*-butyl 2-amino-3-(6-(hydroxymethyl)pyridin-3-yl)propanoate **18** (1.10 g, 3.9 mmol) and 6 N HCl (4 mL) was refluxed for 12 h. The reaction mixture was combined with H_2O (10 mL) and extracted with diethyl ether (2 × 50 mL). The aqueous layer was concentrated under high vacuum to obtain a crude product. The crude product was dissolved in H_2O (10 mL) and concentrated under high vacuum (twice) to remove excess HCl. The reaction mixture was concentrated to give the product as a yellow solid (1.02 g, 88%).

A mixture of the yellow solid (300 mg, 1.01 mmol), aqueous Na_2CO_3 (pH = 8−9), Fmoc-OSu, and 1,4-dioxane (2 mL) was maintained at rt. After 12 h, the reaction mixture was acidified with 15% aqueous citric acid solution to pH 4−5 and the aqueous layer was extracted with EtOAc (3 × 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography (0−10% MeOH/CH_2Cl_2) to give the corresponding product as a colorless solid (385 mg, 85%).

A mixture of the colorless solid (248 mg, 0.56 mmol), TBSCl (500 mg, 3.33 mmol), pyridine (482 mg, 6.11 mmol), and CH_2Cl_2 (5 mL) was maintained at rt under a nitrogen atmosphere. After 12 h, the reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with water (3 × 50 mL). The aqueous layer was extracted using EtOAc (3 × 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography (0−10% MeOH/CH_2Cl_2) to give **2** as a colorless solid (233 mg, 76%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.29 (s, 1H), 7.77 (dd, J = 7.3, 3.2 Hz, 2H), 7.75−7.55 (m, 3H), 7.48 (d, J = 7.3 Hz, 1H), 7.36−7.27 (m, 4H), 4.89 (s, 2H), 4.39−4.34 (m, 2H), 4.20 (t, J = 6.9 Hz, 2H), 2.75−2.59 (m, 2H), 1.90−1.80 (m, 1H), 1.75−1.65 (m, 3H), 0.95 (s, 9H), 0.13 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.4, 157.5, 155.6, 144.2, 143.9, 143.6, 142.8, 141.4, 139.1, 127.6, 126.9, 125.1, 122.7, 119.8, 66.7, 63.1, 53.7, 31.4, 30.9, 27.0, 25.2, 18.1, −6.5; IR (thin film) 3318, 3018, 2953, 2929, 2857, 2360, 2342, 1716, 1610, 1533, 1450, 1399, 1259, 1174, 1106, 1084, 1052, 839, 780, 758, 740, 667 cm^{-1}; [α] = +16.9 (c = 1.0, $CHCl_3$); HRMS (ESMS) calcd for $C_{32}H_{40}N_2O_5SiNa$ $(M + Na)^+$ 583.2604, found 583.2598.

(*S*)-Benzyl 3-((6-((2,3,3-Trimethylbutan-2-yloxy)methyl)pyridin-3-yl)methoxy)-2-(tritylamino)propanoate (19). A mixture of compound **9** (95 mg, 0.30 mmol), benzene (1.0 mL), 40% aqueous NaOH (300 mg, 3.00 mmol), TBAB (97 mg, 0.30 mmol), and compound **6** (131 mg, 0.30 mmol) was maintained at rt for 24 h. The reaction mixture was combined with water (2 mL), and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain crude product. The resulting crude was purified by silica gel chromatography (2−10% EtOAc/hexanes) to obtain **19** as a colorless oil (132 mg, 66%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.30 (d, J = 1.6 Hz, 1H), 7.61 (dd, J = 8.1, 1.6 Hz, 1H), 7.46−7.45 (m, 7H), 7.23−7.13 (m, 14H), 4.78 (s, 2H), 4.74 (d, J = 13.0 Hz, 1H), 4.52 (d, J = 13.0 Hz, 1H), 4.42 (d, J = 7.3 Hz, 2H), 3.70 (dd, J = 8.9, 4.1 Hz, 1H), 3.56−3.47 (m, 2H), 0.96 (s, 9H), 0.12 (s, 6H); ^{13}CNMR (100 MHz, CD_3OD) δ 173.9, 160.3, 147.3, 146.0, 137.1, 135.9, 132.8, 128.7, 128.2, 128.0, 127.9, 127.8, 126.5, 120.4, 72.6, 71.0, 70.0, 66.5, 65.4, 56.7, 25.2, 18.0, −6.4; IR (thin film) 3061, 3032, 2955, 2928, 2887, 2854, 1734, 1601, 1489, 1471, 1456, 1448, 1371, 1362, 1254, 1182, 1174, 1128, 1101, 837, 777, 746, 706 cm^{-1}; [α] = +23.6° (c = 1.7, MeOH); HRMS (ESMS) calcd for $C_{42}H_{49}N_2O_4Si$ $(M + H)^+$ 673.3462, found 673.3459.

(*S*)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-((6-((*tert*-butyldimethylsilyloxy)methyl)pyridin-3-yl)methoxy)propanoic Acid (3). A mixture of compound **19** (114 mg, 0.170 mmol), $Pd(OH)_2/C$ (28 mg, 5% w/w), and MeOH (10 mL) was stirred at rt under H_2 (1 atm) for 12 h. The reaction mixture was filtered through a Celite bed to remove Pd/C, and the filtrate was concentrated to give a colorless solid.

A mixture of the colorless solid (100 mg, 0.170 mmol), FmocOSu (69 mg, 0.21 mmol), and pyridine (2.8 mL) was maintained at rt for 12 h. The reaction mixture was concentrated to obtain crude product. The crude product was dissolved in water (10 mL) and acidified with 5% aqueous citric acid (pH = 5−6). The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain crude product. The resulting crude was purified by silica gel chromatography (0−10% MeOH/CH_2Cl_2) to obtain **3** as a colorless amorphous solid (53 mg, 55%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.40 (s, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.76 (d, J = 7.3 Hz, 2H), 7.63 (dd, J = 6.9, 3.2 Hz, 2H), 7.47 (d, J = 8.1 Hz, 1H), 7.36 (t, J = 7.3 Hz, 2H), 7.26 (t, J = 6.9 Hz, 2H), 4.75 (s, 2H), 7.58−7.51 (m, 2H), 4.38 (dd, J = 10.5, 7.3 Hz, 2H), 4.31−4.23 (m, 2H), 4.18 (t, J = 6.5 Hz, 1H), 3.91−3.79 (m, 2H), 0.93 (s, 9H), 0.10 (s, 6H); ^{13}CNMR (100 MHz CD_3OD) δ 175.7, 160.2, 157.2, 147.4, 144.2, 144.1, 141.4, 137.2, 127.6, 127.0, 125.1, 125.0, 120.4, 119.8, 70.9, 70.0, 66.8, 65.3, 47.2, 25.2, 18.0, −6.5; IR (thin film) 3340 (b) 2953, 2856, 1716, 1606, 1506, 1450, 1253, 1105, 910, 839, 779, 759, 738 cm^{-1}; [α] = +11.3 (c = 0.97, MeOH); HRMS (ESMS) calcd for $C_{31}H_{37}N_2O_6SiNa_2$ $(M - H + 2Na)^+$, 607.2216, found 607.2209.

General Procedure for *N*-Alkylation in Solution-Phase Synthesis. A mixture of compound **21**, corresponding secondary amine (see the Supporting Information, part A), MeCN, and *i*-Pr_2EtN was heated at 55 °C for 18−20 h. The reaction mixture was evaporated, and crude product was dissolved in CH_2Cl_2. The organic layer was extracted with $NaHCO_3$ (5% aq, 2 × 5 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain the crude product. The yield was calculated by ^{1}H NMR analysis using methyl *m*-toluate as an internal standard. Yields for compounds **22** and **23** are 95% and 79%, respectively.

Benzyl 2-(2-Acetamido-3-(6-((bis(pyridin-2-ylmethyl)amino)-methyl)pyridin-3-yl)propanamido)acetate (22). Compound **22** was prepared by general procedure for *N*-alkylation using **21** (33 mg, 81.6 μmol), MeCN (1.5 mL), *i*-Pr_2EtN (22 μL, 122 μmol), and bis(pyridin-2-ylmethyl)amine (21 mg, 106 μmol). A sample was purified by column chromatography on alumina (0−5% MeOH/EtOAc) for analysis: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, J = 4.0 Hz, 2H), 8.29 (s, 1H), 7.63−7.59 (m, 2H), 7.53−7.46 (m, 4H), 7.33−7.26 (m, 5H), 7.11−7.02 (m, 3H), 6.52 (d, J = 8.1 Hz, 1H), 5.07 (s, 2H), 4.73 (dd, J = 14.6, 6.5 Hz, 1H), 4.06−3.91 (m, 2H), 3.82−3.80 (m, 6H), 3.07 (dd, J = 13.6, 7.3 Hz, 1H), 2.94 (dd, J = 13.6, 7.3 Hz, 1H), 1.88 (s, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.4, 169.2, 159.2, 158.0, 149.6, 149.0, 137.3, 136.4, 135.0, 130.3, 128.6, 128.5, 128.3, 122.9, 122.7, 122.0, 67.2, 60.0, 59.7, 53.6, 41.2, 34.8, 29.6, 23.0; IR (thin film) 3283 (b), 2926, 1749, 1654, 1590, 1569, 1434, 1370, 1188, 994, 756, 698 cm^{-1}; HRMS (ESMS) calcd for $C_{32}H_{35}N_6O_4$ $(M + H)^+$ 567.2720, found 567.2706.

Benzyl 2-(2-Acetamido-3-(6-(((2-(benzyl(pyridin-2-ylmethyl)-amino)ethyl)(pyridin-2-ylmethyl)amino)methyl)pyridin-3-yl)propanamido)acetate (23). Compound **23** was prepared by general procedure for *N*-alkylation using compound **21** (20 mg, 49.5 μmol), MeCN (0.9 mL), *i*-Pr_2EtN (35 μL, 198 μmol), and N^1-benzyl-N^1,N^2-bis(pyridin-2-ylmethyl)ethane-1,2-diamine (50 mg, 148 μmol). A sample was purified by preparative HPLC for analysis. HPLC column: Zorbax XDB-C18, 21.2 × 150 mm, 5 μm equipped with guard column, Zorbax XDB-C18, 21.2 mm, 5 μm; flow rate = 20 mL/min; gradient elution 0−5 min 5% MeCN in 0.1% aq TFA, 5−15 min 5−95% MeCN in 0.1% aq TFA, rt = 10.2 min. After HPLC purification, desired fractions were combined and the pH of the mixture was adjusted between 8 and 9 using satd Na_2CO_3. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain the product: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 4.0 Hz, 2H), 8.26 (s, 1H), 7.58−7.53 (m, 2H), 7.46−7.41 (m, 3H), 7.34−7.08 (m, 14H), 6.44 (b, 1H), 5.10 (s, 2H), 4.70−4.65 (m, 1H), 3.95 (dd, J = 13.0, 5.7 Hz, 2H), 3.73−3.69 (m, 4H), 3.58−3.52 (m, 4H), 3.05 (dd, J = 13.9, 6.5 Hz, 1H), 2.96 (dd, J = 13.9, 6.5 Hz, 1H), 2.69−2.55 (m, 4H), 1.90 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.3, 169.1, 160.2, 159.6, 158.5, 149.5, 148.9, 148.8, 139.1, 137.2, 136.5, 136.4, 135.1, 130.2, 128.6, 128.5, 128.3, 128.2, 126.9, 122.8, 122.6, 121.9, 67.2, 60.8, 60.5, 60.1, 58.9, 53.9, 51.7, 41.2, 34.9, 23.0; IR (thin film) 3286(b), 2917, 2849, 1749, 1653, 1591, 1539, 1435, 1373, 1260, 1188, 1029, 756, 699, 665 cm^{-1}; HRMS (ESMS) calcd for $C_{41}H_{46}N_7O_4$ $(M + H)^+$ 700.3611, found 700.3604.

Acknowledgment. We thank Prof. Barbara Imperiali for helpful discussions, Ms. Alyssa Yousif for preparation of synthetic intermediates, Zhongwu Guo for use of a peptide synthesizier, and Wayne State University and the American Chemical Society Petroleum Research Fund (46714-G1) for their generous support of this research.

Supporting Information Available: Experimental procedures for preparation of **10**, **11**, **28**−**32**, **35**, **36**, and **38** including characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.