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Investigation of Unanticipated Alkylation at the N(π) Position of a Histidyl Residue Under Mitsunobu Conditions and Synthesis of Orthogonally Protected Histidine Analogues

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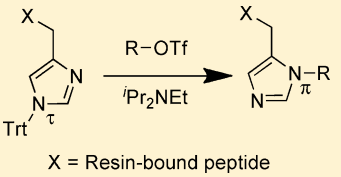
Supporting Information

ABSTRACT: We had previously reported that Mitsunobu-based introduction of alkyl substituents onto the imidazole N(π)-position of a key histidine residue in phosphothreonine-containing peptides can impart high binding affinity against the polo-box domain of polo-like kinase 1. Our current paper investigates the mechanism leading to this N(π)-alkylation and provides synthetic methodologies that permit the facile synthesis of histidine N(π)-modified peptides. These agents represent new and potentially important tools for biological studies.



INTRODUCTION

The serine/threonine specific polo-like kinase 1 (Plk1) is one of four Plks involved in cell cycle regulation. Plk1 is of particular interest as a therapeutic target, due to its ability to promote oncogenic transformation.[1−3] Pharmacological inhibition of Plk1 is being approached through kinase catalytic site-directed agents.[4−11] Inhibitors of Plk1 function that interfere with the actions of its C-terminal polo-box domains (PBDs) are also being investigated. These latter homologous protein modules facilitate subcellular localization of Plk1 by recognizing and binding to phosphoserine (pSer)/phosphothreonine (pThr)-containing sequences.[12,13]

The polo-box interacting protein 1 (PBIP1) is a Plk1 substrate, which undergoes phosphorylation at its T78 residue to form a Plk1 PBD-binding site that is critical for recruiting Plk1 to the interphase and mitotic kinetochores.[14] We had previously shown that the wild-type PBIP1-derived five-mer sequence, 74-PLHSpT-78, represents a minimal peptide (**1**) that specifically interacts with the Plk1 PBD with high affinity (K_d = 0.45 μM).[15] More recently, in an effort to investigate whether reduction in the phosphoryl anionic charge could also be accomplished with retention of binding affinity, we performed synthetic experiments to esterify one of the two pThr phosphoryl hydroxyls of the parent peptide **1**.[16] Our approach was to conduct solid-phase Mitsunobu coupling[17] of a series of alcohols with the protected resin-bound peptide, Ac-Pro-Leu-His[$N(\tau)$Trt]-Ser(OBu^t)-Thr[OPO(OBn)(OH)]-amide,[18] which contained a single free phosphoryl hydroxy group (Figure 1). Applications of Mitsunbu chemistries to resin-supported substrates and to the synthesis of phosphoryl esters are known.[19,20]

Following coupling reactions, peptides were cleaved from the resin under mild acid conditions. In addition to the expected phosphoryl esters, unanticipated synthetic peptide byproducts were isolated having the same molecular weights as the intended products. These byproducts exhibited exceptionally high affinities in Plk1 PBD-binding assays, and in some, these affinities were approximately 3 orders of magnitude greater than the parent peptide (**1**). A Plk1 PBD cocrystal structure of the highest affinity peptide byproduct, which was obtained in a small amount along with the expected major product (**2**) following Mitsunobu reaction with $C_6H_5(CH_2)_8$-OH, showed it to be the modified peptide **3**, in which alkylation had occurred on the histidine imidazole N(π) group (Figure 2).[16] The high affinity and unexpected nature of this histidine adduct prompted us to investigate potential mechanisms leading to its formation and to develop synthetic methodologies to prepare related peptide analogues.

RESULTS AND DISCUSSION

We rationalized that alkylation at the His N(π) of peptide **3** could potentially have occurred either indirectly by intramolecular alkyl transfer of the phosphoryl ester group (mechanism 1, Figure 3) or directly through alkylation by the alcohol reactants under Mitsunobu conditions (mechanism 2, Figure 3). There are literature precedents for both mechanisms. For example, in 1-substituted imidazoles, alkylation of the 3-nitrogen [corresponding to the N(π)-position of histitine] can be effected by phosphoryl esters. However, these reactions require elevated temperatures.[21,22] Similarly, alkylation of imidazole by alcohols can be achieved using Mitsunobu chemistries.[23−25]

To examine whether phosphoryl ester-mediated transfer was involved in the formation of **3** (mechanism 1), Mistunobu esterification of the free phosphoryl hydroxyl of resin-bound N(α)-Fmoc-*O*-(benzyl phosphoryl)-threonine using $C_6H_5(CH_2)_8$-OH was conducted prior to coupling of the

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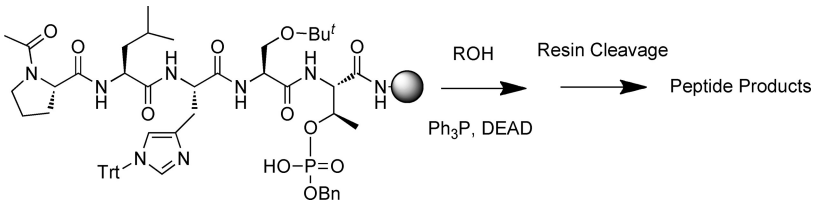
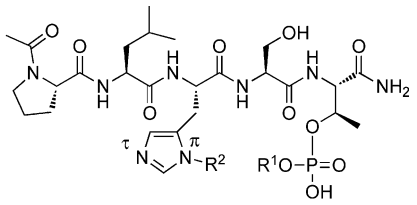


Figure 1. Solid-phase Mistunobu reaction.



1 R^1 = H; R^2 = H
2 R^2 = H; R^1 = $C_6H_5(CH_2)_8$ –
3 R^1 = H; R^2 = $C_6H_5(CH_2)_8$ –
4 R^1 = $C_6H_5(CH_2)_8$ –; R^2 = $C_6H_5(CH_2)_8$ –
5 R^1 = $C_6H_5(CH_2)_8$ –; R^2 = $C_6H_5(CH_2)_7$ –

Figure 2. Structures of peptides discussed in the text.

$N(\alpha)$-Fmoc-[$N(\tau)$-Trt]-His residue. Following Mitsunobu phosphoryl ester formation, the resin was washed, and peptide synthesis was completed in standard fashion using Fmoc protocols. A portion of the resulting finished resin was cleaved and examined by HPLC. The chromatogram showed the presence of the peptide phosphoryl ester **2** with no evidence of product **3**. This indicated that N(π)-alkylation did not occur by intramolecular transfer from the phosphoryl ester.

Direct Mitsunobu-induced N(π)-alkylation (mechanism 2) was examined next by again subjecting a portion of the above, fully formed resin-bound peptide to a second Mitsunobu coupling with $C_6H_5(CH_2)_8$-OH prior to resin cleavage. HPLC analysis of the resulting peptide products showed that in addition to **2**, a new product was obtained having a mass consistent with the bis-alkylated product (**4**, Figure 2). This indicated that histidine alkylation had occurred during the second Mitsunobu reaction. To unambiguously discriminate whether the additional alkyl adduct originated directly from the alcohol used in the Mitsunobu reacton or from preformed phosphoryl ester under Mitsunobu catalysis, the above reaction was repeated using a homologous alcohol having a shorter chain length [$C_6H_5(CH_2)_7$-OH] as compared with the $C_6H_5(CH_2)_8$-OH used in the second Mitsunobu reaction. This allowed differentiation between sources of the alkylating species. In this case, it was found that the mass of the bis-adduct was consistent with the addition of $C_6H_5(CH_2)_7$– (peptide **5**, Figure 2), indicating that histidine alkylation occurred directly from the alcohol used in the second Mitsunobu reaction (mechanism 2). We concluded from these studies that the high affinity byproduct **3** was formed by direct alkylation with $C_6H_5(CH_2)_8$–OH during the Mitsunobu phosphoryl esertification reaction.

As exemplified by **3**, $N(\pi)$-alkyl-histidine residues can impart exceptional Plk1 PBD-binding affinity.[16] However, the adducts in the referenced study were obtained as minor reaction byproducts. To facilitate a broader application of $N(\pi)$-alkyl-histidine analogues, we undertook the preparation of $N(\alpha)$-Fmoc-[$N(\pi)$-R]-His [**8** for R = $C_6H_5(CH_2)_8$–], which is suitably protected for incorporation of histidine derivatives into peptides using standard Fmoc protocols. We employed Hodges' regiospecific synthesis of N(π)-substituted histidines, which relies on the use of N(τ)-Boc-protected histidines and alkyl triflates as the alkyting species.[26] Our approach started with $N(\alpha)$,$N(\tau)$-bis(*tert*-butoxycarbonyl)-L-histidine methyl ester (**6**).[26] A solution of 8-phenyloctan-1-ol in CH_2Cl_2 at −75 °C was treated with triflic anhydride and diisopropylethylamine (DIEPA, 1 equiv) to form the triflate ester in situ. This was then reacted with the $N(\tau)$-Boc histidine derivative **6** to form the corresponding N(π)-adduct **7** in 60% yield (Scheme

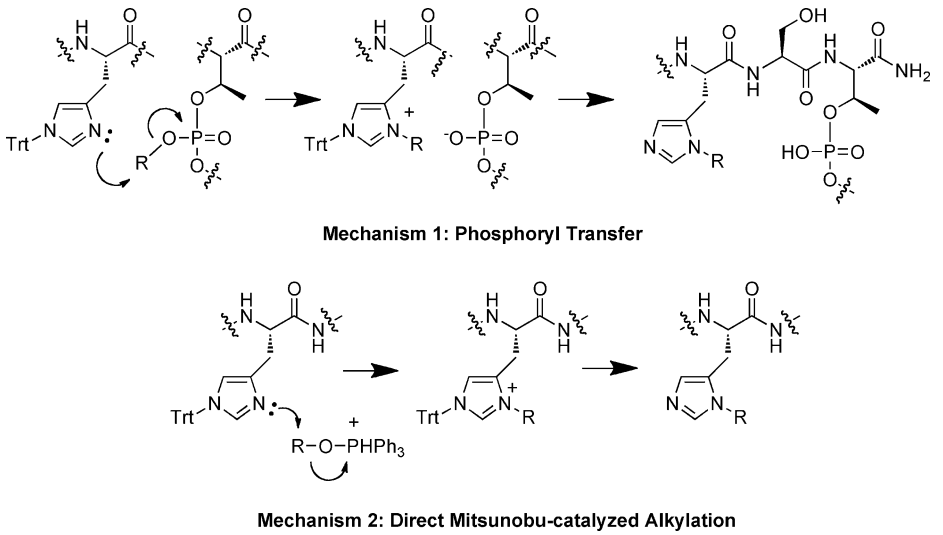


Figure 3. Two possible mechanisms of histidine alkylation.

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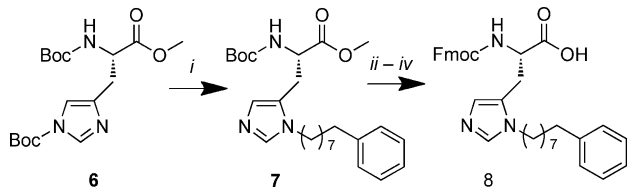
Scheme 1. [a]

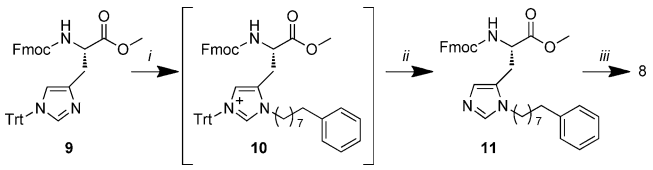


[a]Reagents and conditions: (i) $Ph(CH_2)_7CH_2OH$ (1.1 equiv), Tf_2O (1.1 equiv), DIPEA (1.1 equiv), CH_2Cl_2, −75 °C−rt, 16−18 h, 60% yield. (ii) $LiOH·H_2O$ (2.0 equiv), THF, H_2O (v/v 4:1), 0 °C−rt, 1 h. (iii) 4 M HCl in dioxane (10 equiv), rt, 1 h. (iv) Fmoc-OSu (1.5 equiv), $NaHCO_3$ (4.0 equiv), THF − H_2O (v/v 1:1), rt, 12 h (82% over three steps).

1). Without purification, global deprotection was performed, and the crude product was reacted with 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu) and aqueous $NaHCO_3$ in tetrahydrofuran to yield the final, protected amino acid analogue, **8** (83% from **7**, Scheme 1).

N(π)-Derivatization in the presence of $N(\tau)$-Trt protection has also previously been reported using alkyl halides as the alkylating species; however, these conditions either involve elevated temperatures, prolonged reaction times, or large excesses of reagent.[27−29] In model reactions on $N(\alpha)$-Fmoc-[$N(\tau)$-Trt]-His methyl ester (**9**)[30] (prepared from commercially available $N(\tau)$-trityl-L-histidine methyl ester), we found that N(π)-alkylated product (**11**) could be obtained in high yield at room temperature using in situ-generated alkyl triflate. The crude product from this reaction contained a mixture of the $N(\tau)$-trityl, $N(\pi)$-alkyl bis-adduct (**10**) as well as the desired product (**11**) (Scheme 2). Treatment of this mixture

Scheme 2. [a]



[a]Reagents and conditions: (i) $Ph(CH_2)_7CH_2OH$ (1.1 equiv), Tf_2O (1.1 equiv), DIPEA (1.1 equiv), CH_2Cl_2, −75 °C−rt, 16−18 h. (ii) TFA (10 equiv), triisopropylsilane (1.1 equiv), CH_2Cl_2, rt, 2 h, (90% over two steps). (iii) LiI (6.0 equiv), EtOAc, reflux, 20 h, 80%.

with TFA gave **11** in 90% yield from **9**, and demethylation under nucleophilic conditions gave **8** in good yield. This approach represents a more direct synthesis of **8** than that described above using starting material **6** (Scheme 1).

Traditionally, during peptide synthesis, protection of the histidine imidazole ring is important to avoid side product formation arising from ring acylation and subsequent migration of acyl species to other regions of the peptide. Protection also minimizes intramolecular base-catalyzed racemization of activated histidine by the N(π)-group. Acid-labile $N(\tau)$-Trt derivatization is commonly employed for this purpose in combination with $N(\alpha)$-Fmoc protection.[31] Analogue **8** is somewhat unique in that its N(π)-adduct serves a dual capacity of protecting the imidazole ring during peptide synthesis and providing potentially important biological functionality. To verify the synthetic utility of **8**, we employed the reagent for the solid-phase synthesis of peptide **3**, which had previously been obtained only as a minor byproduct from the synthesis of intended product **2**. The successful completion of this synthesis confirmed the utility of **8** as providing a much improved route to peptides containing N(π)-modified histidine residues.

The general solid-phase methodology outline above using modified histidine analogue **8** is inherently limited because it requires that a new histidine reagent be prepared for each unique N(π)-substituent installed. A more useful approach that would allow the generation of multiple peptides containing unique N(π)-substituents could be realized through on-resin modification of the histidyl residue in postpeptide synthesis fashion. Scheme 3 shows the application of such a protocol to the synthesis of peptide **13**. Peptide **13** is a variant of **3** having the labile pThr residue replaced by (2*S*,3*R*)-2-amino-3-methyl-4-phosphonobutyric acid (Pmab), which is a phosphatase-stable pThr mimetic[32] that retains the PBD-binding affinity of parent pThr-containing peptides.[15,16] A key feature of the route outlined in Scheme 3 is its use of commercially available $N(\alpha)$-Fmoc-[$N(\tau)$-Trt]-His for the incorporation of an $N(\tau)$-Trt-protected histidine residue. Although $N(\alpha)$-Fmoc-[$N(\tau)$-Boc]-His is also commercially available, the $N(\tau)$-Boc group suffers from a lack of stability against prolonged or repeated exposure to piperidine used in Fmoc-based protocols.[31]

In conclusion, our current paper provides evidence that the formation of high affinity Plk1 PBD-binding peptide by-products previously observed as minor components following on-resin Mitsunobu esterification result from direct alkylation at the N(π)-position. We detail the high yield preparation of $N(\alpha)$-Fmoc-$N(\pi)$-modified histidine reagents from $N(\alpha)$-Boc- and $N(\alpha)$-Fmoc-protected precursors, which permit facile Fmoc-based solid-phase synthesis of N(π)-modified peptides. We extend the utility of this chemistry by demonstrating on-resin triflate-mediated derivatization of fully formed peptides that should permit the ready preparation of libraries of $N(\pi)$-alkyl peptides. Although only a very narrow range of alcohols is specifically examined in our current report, it is anticipated that the approach should provide access to a broad range of N(π)-modified histidine analogues that may be useful in a variety of biological contexts.

EXPERIMENTAL SECTION

Synthesis of *N*(α)-Fmoc-[*N*(π)-(8-phenyloctyl)]-histidine (8 from 6) . *N(α),N(τ)-Bis[(1,1-dimethylethoxy)carbonyl]-L-histidine Methyl Ester (6).*[26] Triethylamine (11.51 mL, 83 mmmol) was added to a stirred suspension of L-histidine methyl ester hydrochloride (10.0 g, 41.3 mmol) in MeOH (98 mL), and stirring was continued at room temperature until dissolution was complete (30 min). A solution of di-*tert*-butyl dicarbonate (18.03 g, 83 mmol) in MeOH (49 mL) was then added dropwise over 30 min, and stirring was continued at room temperature (48 h). Solvent was removed in vacuo, and the residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with 10% citric acid, dried ($MgSO_4$), concentrated, and purified by gel column chromatography (EtOAc:hexanes; from = 4:1 to 1.5:1) to afford known **6** as a white amorphous solid (11.06 g, 72% yield).

N(α)-[(1,1-Dimethylethoxy)carbonyl]-N(π)-(8-phenyloctyl)-L-histidine Methyl Ester (7). To a stirred solution of triflic anhydride (1.51 mL, 8.93 mmol) in CH_2Cl_2 (18 mL) under nitrogen at −75 °C was added a solution of 8-phenyloctan-1-ol (1.88 mL, 8.93 mmol) and diisopropylethylamine (DIEPA) (1.56 mL, 8.93 mmol) in CH_2Cl_2 (12.0 mL) dropwise over 10 min. Stirring was continued at −75 °C (20 min), then a solution of **6** (3.0 g, 8.12 mmol) in CH_2Cl_2 (21 mL) was added dropwise, and the mixture was allowed to gradually warm to room temperature over a period of 16−18 h. The mixture was poured into a solution of aqueous $NaHCO_3$ and stirred vigorously (30 min).

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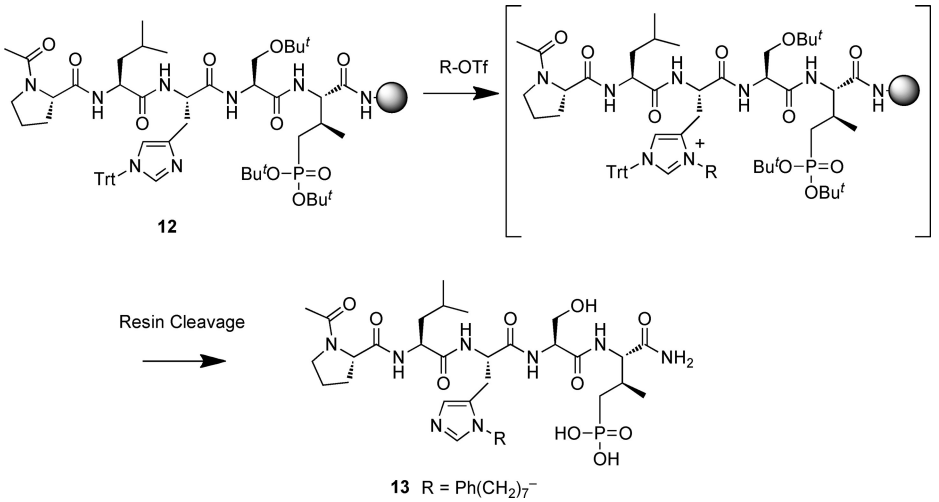
Scheme 3. Postpeptide Synthesis On-Resin N(π)-Alkylation



The organic layer was diluted with CH_2Cl_2 and washed with aqueous $NaHCO_3$ and brine, then dried (Na_2SO_4), and concentrated to a gum. Purification by silica gel flash chromatography ($CHCl_3$:MeOH; from 100:1 to 100:2) provided **7** as colorless gum (2.23 g, 60% yield). $[\alpha]_D^{21}$ 23.99 (*c* 1.045, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (s, 1H), 7.30−7.24 (m, 2H), 7.20−7.14 (m, 3H), 6.78 (s, 1H), 5.18 (d, *J* = 8 Hz, 1H), 4.54 (q, *J* = 4 Hz, 1H), 3.82 (t, *J* = 8 Hz, 2H), 3.74 (s, 3H), 3.16−2.96 (m, 2H), 2.60 (t, *J* = 8 Hz, 2H), 1.83 (brs, 1H), 1.77−1.65 (m, 2H), 1.65−1.55 (m, 2H), 1.43 (s, 9H), 1.31 (brs, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 155.0, 142.7, 137.4, 128.3, 128.2, 128.0, 125.8, 125.5, 80.2, 53.0, 52.5, 44.7, 35.9. 31.3, 30.9, 29.3, 29.1, 29.0, 28.2, 26.9, 26.6. IR ν_{max} 2929, 2361, 1705, 1164 cm^{-1}. HR-ESI MS calcd for $C_{26}H_{40}N_3O_4$ (M + H)$^+$, 458.3013; found, 458.3023.

*N(α)-[(9H-Fluoren-9-ylmethoxy)carbonyl]-N(π)-(8-phenyloctyl)-L-histidine (**8**).* To a solution of **7** (0.7 g, 1.53 mmol) in dissolved in THF (10 mL) and H_2O (2.5 mL) at 0 °C was added LiOH·H_2O (128 mg, 3.06 mmol), and the mixture was stirred at 0 °C (1.5 h). The solution was acidified to pH 1−2 (aqueous HCl), then THF was removed in vacuo, the residue was extracted (EtOAc), and the organic layer was dried and concentrated in vacuo. The residue was dissolved in a solution of 4 M HCl in dioxane (4 mL) and EtOAc (4 mL), stirred at room temperature (1 h), and then concentrated in vacuo. The residue was dissolved in solution of THF (7 mL) and H_2O (7 mL), and 9-fluorenylmethyl-succinimidyl carbonate (Fmoc-OSu) (774 mg, 2.30 mmol) and $NaHCO_3$ (514 mg, 6.12 mmol) were added, and the mixture was stirred at room temperature (overnight). The mixture was neutralized by the addition of saturated NH_4Cl and extracted (EtOAc), and the organic layer was dried and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography first by elutiom with ($CHCl_3$:MeOH; from 100:1 to 100:3) to remove byproduct and then ($CHCl_3$:MeOH; from 10:1 to 3:1) to provide the desire product **8** as a white wax (716 mg, 83% yield from **7**). $[\alpha]_D^{21}$ 38.78 (*c* 0.90, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.39 (s, 1H), 7.89 (d, *J* = 8 Hz, 2H), 7.85 (d, *J* = 8 Hz, 1H), 7.67 (d, *J* = 8 Hz, 2H), 7.41 (t, *J* = 8 Hz, 2H), 7.32 (t, *J* = 8 Hz, 2H), 7.25 (t, *J* = 8 Hz, 2H), 7.20−7.10 (m, 3H), 7.8 (s, 1H), 4.35−4.15 (m, 4H), 4.10−3.90 (m, 2H), 3.20−2.90 (m, 2H), 2.60 (s, 1H), 2.55−2.47 (m, 2H), 1.75−1.60 (m, 2H), 1.60−1.45 (m, 2H), 1.23 (brs, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.4, 155.9, 143.7, 142.2, 140.7, 135.9, 129.1, 128.20, 128.16, 127.6, 127.1, 125.5, 125.2, 121.8, 120.1, 65.7, 53.0, 46.6, 45.0, 35.1, 30.9, 29.8, 28.7, 28.5, 28.4, 25.8, 25.2. IR ν_{max} 2930, 2361, 1713, 1452, 1255 cm^{-1}. HR-ESI MS calcd for $C_{35}H_{40}N_3O_4$ (M + H)$^+$, 566.3013; found, 566.3022.

Synthesis of *N(α)*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl-[*N(π)*-(8-phenyloctyl)]-histidine (8 from 9). *N(α)-[(9H-Fluoren-9-ylmethoxy)carbonyl]-N(τ)-(triphenylmethyl)-L-histidine Methyl Ester (**9**).* To a solution of *N*(τ)-(triphenylmethyl)-L-histidine methyl ester hydrochloride (0.8 g, 1.78 mmol) and $NaHCO_3$ (0.45 g, 5.3 mmol) in THF:H_2O (v/v 1:1) at 0 °C was added Fmoc-OSu (0.9 g, 2.67 mmol), and the mixture was allowed to come to room temperature and stirred (overnight). The organic layer was collected, the aqueous layer was extracted (EtOAc), and the combined organic layers were washed with H_2O and brine and dried (Na_2SO_4). Concentration in vacuo provided a residue, which was purified by silica gel column chromatography (EtOAc:petroleum ether; from 1:10 to 1:4) to yield known **9**[30] as a white foam (84% yield). $[\alpha]_D^{23}$ 9.55 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 8.0 Hz, 2H), 7.64 (t, *J* = 8.0 Hz, 2H), 7.44 (s, 1H), 7.40 (t, *J* = 8.0 Hz, 2H), 7.37−7.32 (m, 9H), 7.30 (t, *J* = 8.0 Hz, 2H), 7.16−7.10 (m, 6H), 6.60 (s, 1H), 6.55 (d, *J* = 8.0 Hz, 1H), 4.68−4.63 (m, 1H), 4.40−4.24 (m, 3H), 3.65 (s, 3H), 3.14−3.17 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.3, 156.4, 144.3, 144.1, 142.5, 141.4, 139.0, 136.6, 129.9, 128.26, 128.25, 127.8, 127.2, 125.6, 125.5, 120.1, 119.8, 75.5, 67.4, 54.5, 52.4, 47.4, 30.3. IR ν_{max} 2361, 1719, 1492, 1445, 1248 cm^{-1}. HR-ESI MS calcd for $C_{41}H_{35}N_3O_4Na$ (M + Na)$^+$, 656.2525; found, 656.2538.

*N(α)-[(9H-Fluoren-9-ylmethoxy)carbonyl]-N(τ)-(8-phenyloctyl)-L-histidine Methyl Ester (**11**).* To a stirred solution of triflic anhydride (0.101 mL, 0.60 mmol) in CH_2Cl_2 (3.8 mL) under nitrogen at −75 °C was added a solution of 8-phenyloctan-1-ol (124 mg, 0.60 mmol) and DIEA (0.104 mL, 0.60 mmol) in CH_2Cl_2 (2.6 mL) dropwise over 10 min at −75 °C. Stirring was continued at −75 °C (20 min), then a solution of **9** (0.34 g, 0.54 mmol) in CH_2Cl_2 (4.5 mL) was added dropwise, and the mixture was allowed to gradually warm to room temperature and stirred (16 −18 h). The mixture was poured into aqueous $NaHCO_3$ and stirred vigorously (30 min) and then extracted with CH_2Cl_2, and the organic extract washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. To a solution of the resulting gum in CH_2Cl_2 (4.2 mL) was added trifluoroacetic acid (0.42 mL, 5.4 mmol) and triisopropylsilane (TIS) (0.12 mL, 0.60 mmol), and the mixture was stirred at room temperature until reaction was complete as shown by TLC (2 h). The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH; from 100:1 to 20:1) to provide **11** as a white foam (90% yield from **9**). $[\alpha]_D^{22.4}$ 1.35 (*c* 1.01, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.62 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.39 (t, *J* = 8.0 Hz, 3H), 7.40−7.23 (m, 4H), 7.22−7.10 (m, 4H), 6.05 (d, *J* = 8.0 Hz, 1H), 4.56 (d, *J* = 4.0 Hz, 1H), 4.38 (d, *J* = 8.0 Hz, 2H), 4.18 (d, *J* = 8.0 Hz, 2H), 4.06−4.01 (m, 2H), 3.77 (s, 3H), 3.25−3.14 (m, 2H), 2.58 (t, *J* = 8.0 Hz, 2H), 1.81−1.73 (m 2H,), 1.63−1.53 (m, 2H), 1.28 (s, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 156.2, 143.79, 143.74, 142.9, 141.5, 134.9, 129.8, 128.6, 128.4, 128.0, 127.4, 125.8, 125.2, 120.2, 119.1, 67.5, 53.3, 53.1, 47.2, 36.1, 31.5, 30.2, 29.4, 29.3, 29.0, 26.5, 26.3. IR ν_{max} 2928, 2361, 1718, 1166, 1028 cm^{-1}. HR-ESI MS calcd for $C_{36}H_{42}N_3O_4$ (M + H)$^+$, 580.3175; found, 580.3178.

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N(α)-[(9H-Fluoren-9-ylmethoxy)carbonyl]-N(τ)-(8-phenyloctyl)-L-histidine (8). Lithium iodide (1.13 g, 8.45 mmol) was added to a stirred solution of **11** (0.82 g, 1.41 mmol) in dry EtOAc (28 mL) under nitrogen, and the mixture was heated at reflux (20 h). The mixture was brought to room temperature, and the reaction was quenched by the addition of 10% aqueous HCl and extracted (EtOAc). The organic extract was dried (Na_2SO_4) and concentrated in vacuo, and the residue was purified by silica gel flash chromatography (CH_2Cl_2:MeOH; from 95:5 to 80:20) to provide **8** as a colorless gum (80% yield).

General Solid-Phase Peptide Synthesis. Protected amino acids used were Fmoc-Thr(PO(OBzl)OH)-OH, Fmoc-Ser(O^{t}Bu), *N*(τ)-Trt-*N*(α)-Fmoc-His, Fmoc-Leu, and Fmoc-Pro (purchased from Novabiochem). Peptides were synthesized on NovaSynTGR resin (Novabiochem, catalog no. 01-64-0060) using standard Fmoc solid-phase protocols in *N*-methyl-2-pyrrolidone (NMP). 1-*O*-Benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (5.0 equiv), hydroxybenzotriazole (HOBT) (5.0 equiv), and *N*,*N*-diisopropylethylamine (DIPEA) (10.0 equiv) were used as coupling reagents. Amino terminal acetylation was achieved using 1-acetylimidazole. Finished resins were washed with *N*,*N*-dimethylformamide (DMF), MeOH, CH_2Cl_2, and Et_2O, then dried under vacuum (overnight), and then cleaved by treatment with a solution of TFA:H_2O:TIS (95:2.5:2.5) (4 h). The resin was removed by filtration, and the filtrate was concentrated under vacuum and then precipitated with ether, and the precipitate was washed with ether. The resulting solid was dissolved in 50% aqueous MeCN (5 mL) and purified by reverse phase preparative HPLC using a Phenomenex C18 column (21 mm × 250 mm, catalog no. 00G- 4436-P0) with a linear gradient from 0% aqueous MeCN (0.1% TFA) to 65% MeCN (0.1% TFA) over 30 min at a flow rate of 10.0 mL/min (detection at 220 nm). Lyophilization provided products as white powders. For the synthesis of Pmab-containing peptides, (2*S*,3*R*)-4-[di(*tert*-butyl)-oxyphosphinyl]-*N*-Fmoc-L-valine[32] was used in place Fmoc-Thr(PO(OBzl)OH)-OH.

Synthesis of Peptide 3 using *N*(α)-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*(τ)-(8-phenyloctyl)-L-histidine (8). The synthesis of peptide **3** was accomplished according to the procedures listed under General Solid-Phase Synthesis, except that reagent **8** was used in place of *N*(τ)-Trt-*N*(α)-Fmoc-His.

On-Resin *N*(π)-Alkylation Using Mitsunobu Reaction Conditions. Peptides **2** and **3** were prepared as previously reported.[16] For the synthesis of peptides **4** and **5**, resin-bound Fmoc-Thr[(OP(OBn)-OH]-amide, prepared as indicated above (400 mg, 0.1 mmol), was swelled in CH_2Cl_2 (15 min) and then treated with triphenylphosphine (262 mg, 1.0 mmol), diethyl azidodicarboxylate (DEAD) (0.46 mL, 40% solution in toluene, 1.0 mmol), and 8-phenyloctan-1-ol (206 mg, 1.0 mmol) in dry CH_2Cl_2 at room temperature (2 h). Coupling of the remaining peptide residues was conducted as described above in General Solid-Phase Synthesis. The resulting resin bond Ac-Pro-Leu-[(τ)-Trt-His]-Ser(O^{t}Bu)-Thr[(OP(OBn)(O(CH_2)$_8$Ph)]-amide was subjected to Mitsunobu coupling using triphenylphosphine (262 mg, 1.0 mmol), DEAD (0.46 mL, 40% solution in toluene, 1.0 mmol), and the appropriate alchohol [8-phenyloctan-1-ol (206 mg, 1.0 mmol) for peptide **4** and 7-phenylheptan-1-ol (192 mg, 1.0 mmol) for peptide **5**] in dry CH_2Cl_2 at room temperature (2 h). The resins were then washed (CH_2Cl_2), dried under vacuum (2 h), and cleaved by treatment with [TFA:H_2O:TIS (95:2.5:2.5) (4 h)]. Peptides **4** and **5** were following procedures indictated in General Solid-Phase Synthesis. HPLC and mass spectral data are provided in Table 1.

Synthesis of Peptide 13 by On-Resin N(π)-Alkylation. To a stirred solution of triflic anhydride (42 μL, 0.25 mmol) in CH_2Cl_2 (3 mL) under nitrogen at −75 °C was added a solution of 8-phenyloctan-1-ol (52 mg, 0.25 mmol) and DIEA (45 μL, 0.26 mmol) in CH_2Cl_2 (2.0 mL) dropwise over 10 min. Stirring was continued at −75 °C (20 min), and then, the mixture was warmed to room temperature and added to peptide resin **12**[16] (200 mg, 0.05 mmol) that had been swelled in CH_2Cl_2 for 15 min. Coupling was conducted at room temperature (12 h), and then, the resin was washed (CH_2Cl_2 and Et_2O), dried under vacuum (2 h), and cleaved (TFA:H_2O:TIS; 95:2.5:2.5) (4 h). Peptide **13** was obtained following work up and HPLC purification as indicated in General Solid-Phase Synthesis. HPLC and mass spectral data are provided in Table 1.

Table 1. Peptide ESI-MS Spectral Data and HPLC Retention Times

no.	expected (M + H)$^+$	observed (M + H)$^+$	retention (min)	HPLC[a]
2[16]	863.4	863.4	25.2	II
3[16]	863.4	863.4	24.3	II
4	1051.6	1051.5	29.8	I
5	1037.6	1037.4	29.2	I
13[16]	861.5	861.4	23.3	I

[a]HPLC method: λ = 220 nm; flow rate, 10 mL/min. Solvent A: H_2O, 0.1% TFA. Solvent B: MeCN, 0.1% TFA. Gradients as indicated below:

I				
time	0	30	30.1	35
B%	0	100	100	100
II				
time	0	30	30.1	35
B%	0	90	100	100

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of **7**−**9** and **11**, including 1D NOESY assignment of N(π)-alkylation in **11** and HPLC charts of peptide **2**−**5** and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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