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N(π)-2-Naphthylmethoxymethyl-Protected Histidines: Scalable, Racemization-Free Building Blocks for Peptide Synthesis

Kohei Torikai,*,† Ryota Yanagimoto,† and Louis A. Watanabe‡

† Department of Chemistry, Faculty and Graduate School of Science, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan

‡ Watanabe Chemical Industries, Ltd., 2-2-5 Sakaimachi, Naka-ku, Hiroshima 730-0853, Japan

Supporting Information Placeholder

ABSTRACT: Histidine (His) racemizes with relative ease during peptide synthesis. One strategy to suppress this racemization is the protection of the nitrogen atom of the imidazole moiety in His with a suitable protecting group. Among the numerous protecting groups that have already been tested, the *p*-methoxybenzyloxymethyl (PMBOM) group on the π-nitrogen atom effectively suppresses the racemization. However, a large-scale synthesis of *N*(π)-PMBOM-protected derivatives has hitherto been hampered by the requirement of a freshly prepared unstable reagent. Herein, we report the synthesis of *N*(π)-2-naphthylmethoxymethyl (NAPOM)-protected His derivatives, which can be prepared on a gram scale and do not suffer from the aforementioned instability problems. Furthermore, these NAPOM-protected His derivatives suppress the racemization in Boc- and Fmoc-based peptide synthesis.

KEY WORDS: histidine, *N*(π), NAPOM, protecting group, racemization, peptide synthesis

INTRODUCTION

Histidine (His; **1** in Scheme 1), one of the 20 naturally occurring amino acids, contains a basic imidazole moiety with two nitrogen atoms. The nitrogen atom that is in closer proximity to the stereocenter will henceforth be denoted *N*(π) (from the Latin word "pros" – "in front"), whereas the other will be referred to as *N*(τ) (from the Greek word "tele" – "far, far off"). In biologically active peptides and proteins, the His imidazole moiety often serves as a hydrogen-bonding and/or metal-binding site.[1] Given these significant roles, the controlled chemical synthesis of His-containing peptides has become an important task in the synthetic community.

Yet, the basicity of the imidazole moiety causes difficulties in the peptide synthesis.[2] Specifically, the His residue exhibits a high propensity toward epimerization, especially when its carboxylic moiety is activated in e.g. acid-halides or acid-anhydrides (**2**; Scheme 1), which is usually the case for the subsequent coupling with an elongating peptide chain. The epimerization is tentatively triggered via the abstraction of a Cα-H by the π-nitrogen to generate enolate **3**, which affords L-His derivative **2** and its enantiomer ***ent*-2** at the same rate.[3] In order to suppress this racemization, protection of the more nucleophilic and hence more easily and selectively protectable τ-nitrogen atom with bulky and/or electron-withdrawing groups such as triphenylmethyl (Trt) in e.g. **4** (Chart 1)[4] was first attempted to reduce the basicity of the π-nitrogen atom indirectly.[5] In fact, this *N*(τ)-protection considerably suppressed the racemization, albeit that it was still unsatisfactory given the presence of the free π-nitrogen atom, which is considered to act directly as a base. Therefore, currently developed His building blocks bear protecting groups such as phenacyl,[6] allyl,[7] benzyloxymethyl (BOM),[8] *t*-butyloxymethyl (Bum)[9] and *p*-methoxybenzyloxymethyl (PMBOM)[10ab] on the π-nitrogen atom,[5] as these groups suppress the racemization more efficiently. Among these, *N*(α)-fluorenylmethoxycarbonyl (Fmoc)- and *N*(π)-PMBOM-protected His derivative **5** (Chart 1) is particularly useful, considering that the PMBOM group can be cleaved readily with trifluoroacetic acid (TFA) following the Fmoc-based peptide synthesis. Despite its synthetic utility, **5** has not found widespread applications, which is probably due to two main reasons: i) difficulties associated with generating **5** on a large scale, which requires substantial amounts of the relatively unstable protecting agent PMBOMCl, and ii) the requirement for low temperature reactions and chromatographic purification during the synthesis of PMBOMCl.[10c] It should also be noted that the application of the PMBOM group to the Boc-based strategy may be difficult due to its lability under acidic conditions, which are required to remove the Boc group.

We have recently reported that the 2-naphthylmethoxymethyl (NAPOM) group (Chart 1) may serve as an alternative and also orthogonally to PMB(OM).[11] We discovered that the NAPOM group is moderately stable in acidic media, i.e., it tolerates e.g. 1 equivalent of camphorsulfonic acid in MeOH,[11] and a catalytic amount of trifluoromethanesulfonic acid at −20 °C,[12] while it is cleaved with CBr_4 in refluxing MeOH.[11] We envisaged that this lability of the NAPOM group could potentially offer the chance of a selective removal of the Boc group in the presence of NAPOM group. Furthermore, NAPOMCl, an introducing agent for the NAPOM group, is a stable and storable solid that can be prepared at 0 °C without chromatographic purification.[11] Herein, we report the chromatography- and epimerization-free synthesis of *N*(α)-Fmoc- (**6**) or *N*(α)-Boc- and *N*(π)-NAPOM-protected His (**7**), and their application to the racemization-free synthesis of peptides.

RESULTS AND DISCUSSION

The first task of our project was the chromatography-free synthesis of *N*(π)-NAPOM-protected **6** and **7** under retention of the high enantiomer excess (ee). The synthesis of *N*(α)-Boc derivative **7** commenced with the esterification of inexpensive **1** using $SOCl_2$ and MeOH to afford **8** (Scheme 2). Since this esterification is very clean, crude **8**, after complete evaporation of all volatiles, was used in the next reaction, i.e., the selective introduction of the Boc group at the τ-nitrogen atom of **8**.

Scheme 1. Main Epimerization Pathway of the Histidine Residue During Chemical Peptide Synthesis.

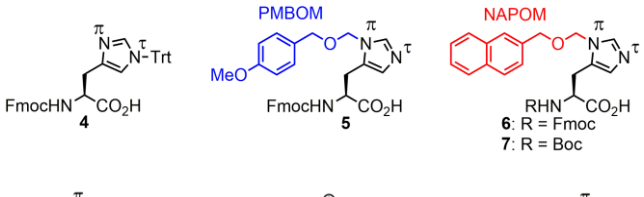


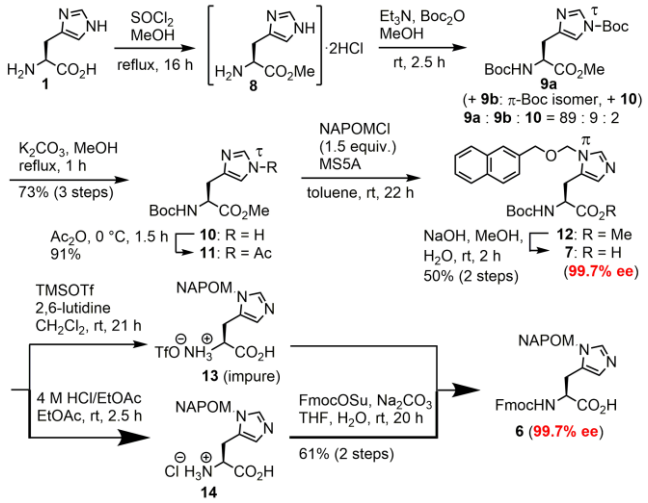
Chart 1. Structures of protected histidines 4-7.

Initially, we very slowly added Boc_2O to a mixture of **8** and Et_3N at room temperature (6 h), followed by stirring for further 18 h at room temperature. This approach afforded **9a** in excellent selectivity (**9a** : **9b** : **10** ≈ 97 : 3 : 0), but low ee, judging from the fact that **11** obtained from this lot exhibited only 33% ee. We envisaged that the racemization could potentially occur during the prolonged exposure of the *N*(π)-unprotected materials such as **8** and **9a** to basic conditions. Thus, **8** was subsequently exposed to Et_3N and Boc_2O for only 2.5 h at room temperature,[13] which furnished a mixture of **9a**, **9b**, and **10** in a 89 : 9 : 2 ratio. The ensuing treatment of this mixture with K_2CO_3 in MeOH to cleave any unnecessary Boc groups on the imidazole rings of **9a** and **9b**, generated Boc-His-OMe **10** in 73% over three steps as the sole product. Subsequently, **10** was protected using Ac_2O to afford acetate **11** (91% yield) as a colorless crystalline solid after reprecipitation. It should be noted that attempts to purify **11** by recrystallization resulted in considerable racemization.

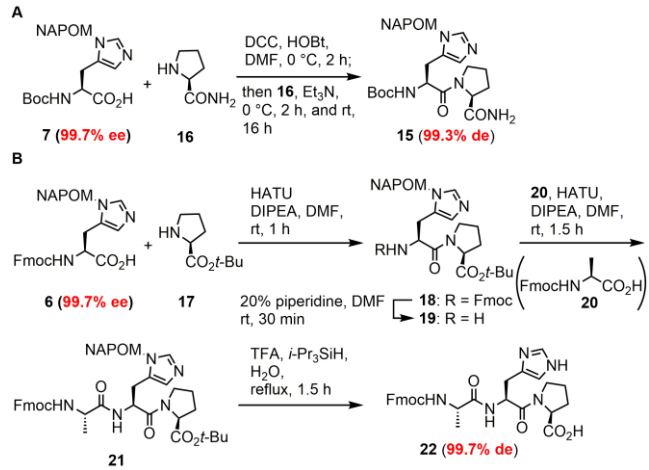
The introduction of the NAPOM group at the π-nitrogen atom was achieved by treating **11** with NAPOMCl[14] in the presence of MS5A at room temperature, and a subsequent saponification afforded *N*(α)-Boc-, *N*(π)-NAPOM-protected His **7** in 50% yield over two steps with 99.7% ee[15] after recrystallization. Here, a special purification process was required to obtain a pure specimen of **7**: i) After concentrating the organic solvent mixture from the saponification *in vacuo*, the reagent-derived organic impurities were removed by washing the aqueous layer with Et_2O under basic conditions, followed by ii) a back extraction at pH ≈ 4, which recovered **7** in the organic layer; iii) the organic layer was then evaporated to dryness; iv) the residual crude material (84% purity, 90.2% ee) was dissolved in acetone and the undissolved solid, which is mainly a racemate of **7**, was removed by filtration; v) the filtrate was evaporated *in vacuo*, and the residue was recrystallized from hexane/acetone.

Next, we moved on to the synthesis of Fmoc-protected His **6**. The remaining task to prepare **6** was a seemingly easy manipulation of the protecting group (Boc→Fmoc), which turned out to be more complicated than anticipated. For instance, a known procedure using TMSOTf/2,6-lutidine for the removal of Boc was not suitable given that it requires the use of a halogenated solvent (CH_2Cl_2) and gave impure **13** and thus **6** after introducing the Fmoc group, which were difficult to purify; nevertheless, this procedure could be successfully applied to the synthesis of the PMBOM counterpart **5**.[10ab] After considerable experimentation, we ultimately found that the use of HCl in ethyl acetate to give **14** and the use of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (FmocOSu) with Na_2CO_3 furnishes **6** (61% over 2 steps) without any byproducts detectable by NMR analysis after workup. Indeed, this material showed, without further purification, 97.7% purity and 99.7% ee[15] via HPLC analysis.[16]

Scheme 2. Chromatography-free synthesis of *N*(π)-NAPOM-protected histidines 6 and 7.



Scheme 3. Racemization test: Liquid-phase synthesis of (A) Boc-His(π-NAPOM)-Pro-NH_2 and (B) Fmoc-Ala-His-Pro.



As chromatographic purification steps and halogenic solvents are not required in this approach, our synthesis of *N*(π)-NAPOM-protected His **6** and **7** could potentially be useful for the large-scale (e.g. industrial) synthesis of such synthons. Indeed, the reproducibility of this process was very good, at least on the gram scale. With building blocks **6** and **7** in hand, we turned our attention to applying them to the Boc- and Fmoc-based synthesis of peptides. To examine the racemization rate during Boc-based peptide synthesis (Scheme 3A), we selected Boc-His(π-NAPOM)-Pro-NH_2 (**15**) as a model peptide, considering that the His-Pro-NH_2 sequence has previously been reported to racemize easily at the His residue during its synthesis.[6,7] When *N*(α)-Z-*N*(τ)-phenacyl-His (Z: benzyloxycarbonyl) or *N*(α)-Z-*N*(π)-phenacyl-His were coupled with Pro-NH_2 (**16**) using dicyclohexylcarbodiimide (DCC) in the presence of Et_3N by Fletcher and co-workers, the target dipeptides were obtained in 30% and 96% de, respectively.[6] In contrast, our *N*-(π)-NAPOM-protected His derivative **7** suppresses the racemization significantly and affords the corresponding dipeptide **15** with 99.3% de.[15]

For a demonstration of the synthetic utility in Fmoc-based liquid-phase peptide synthesis (Scheme 3B), we chose Ala-His-Pro-OH as a model peptide, as this sequence has previously been used in a study to examine the racemization of PMBOM counterpart **5**.[10ab]

Fmoc-His(π)-NAPOM-OH **6** and Pro-O-*t*-Bu **17** were coupled using hexafluorophosphate azabenzotriazole tetramethyl uranium

(HATU) and diisopropylethylamine (DIPEA) to give Fmoc-protected dipeptide **18**. After deprotection, free amine **19** was coupled with Fmoc-Ala-OH **20** to yield protected tripeptide **21**.

Finally, removal of both the NAPOM and *t*-Bu groups from **21** via treatment with refluxing TFA in the presence of *i*-Pr_3SiH and water furnished tripeptide **22**. Judging from the observed 99.7% de of **22**,[15] which was identical to the ee of amino-acid building block **6**, *N*(π)-NAPOM-protected His perfectly prevents the racemization, even in the Fmoc-based strategy. It is noteworthy that when *N*(τ)-Trt-protected His counterpart **4** was subjected to the same peptide synthesis conditions, the de of **22** decreased to 98.2% de (data not shown).

CONCLUSION

Herein, we have reported a chromatography-free synthesis of *N*(π)-NAPOM-protected histidine derivatives and demonstrated their extremely high ability to prevent racemization, both in the Boc- and Fmoc-based synthesis of peptides. So far only very few *N*(π)-protecting groups are known to be compatible with both the Boc and Fmoc strategies, despite continued demand of the Boc-based strategy in the base-sensitive synthesis of peptides.[17] The BOM group is one of these rare examples, albeit that it requires potentially explosive hydrogenolysis or corrosive HF, which may be problematic in the context of a large-scale synthetic operation. Based on the results shown herein, it is feasible to expect a significantly increased number of applications for the NAPOM protecting group.

EXPERIMENTAL SECTION

Amino acids, derivatives of amino acids, protecting and condensation agents were purchased from Watanabe Chemical Industries, LTD, and used as supplied. Dehydrated toluene, MeOH, and *N*,*N*-dimethylformamide (DMF) were purchased from Kanto Chemical Co. Inc. or Wako Pure Chemical Industries Ltd., and used without further dehydration. Molecular sieves 5A (MS5A) were preactivated by heating *in vacuo*. All other chemicals were obtained from local venders, and used as supplied. Thin-layer chromatography (TLC) was performed using precoated TLC grass plates (silica gel 60 F_{254}, 0.25 mm thickness) for the reaction analyses. Silica gel (spherical, neutral, 100–210 μm) was used for column chromatography. IR spectra were recorded on an FT/IR equipment. ^1H and ^{13}C NMR spectra were recorded at 400 MHz. Chemical shifts are reported in ppm from tetramethylsilane (TMS) with reference to internal residual solvent [^1H NMR, $CDCl_3$ (7.26), CD_3OD (3.31), DMSO-d6 (2.50); ^{13}C NMR, $CDCl_3$ (77.16), CD_3OD (49.00), DMSO-d6 (39.52)]. The following abbreviations are used to designate the multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet. High resolution mass spectra (HRMS) were recorded on an ESI-TOF equipment.

Boc-L-His-OMe 10. In the air, $SOCl_2$ (9.00 mL, 125 mmol) was added to a solution of L-histidine **1** (15.0 g, 96.7 mmol) in dehydrated MeOH (390 mL) at 0 °C. After being stirred at 80 °C for 16 h, the reaction mixture was concentrated, and the residue was co-evaporated with MeOH for 3 times to give crude methyl ester **8** (23.42 g, colorless solid). The crude material was used directly in the next reaction without further purification. In the air, to a solution of the above crude methyl ester **8** (23.42 g) in dehydrated MeOH (390 mL), $(Boc)_2O$ (46.4 g, 212 mmol) and triethylamine (27.0 mL, 193 mmol) were added at 0 °C. After stirred at rt for 2.5 h, full consumption of methyl ester **8** was confirmed by TLC. The reaction was quenched with saturated aqueous solution of NH_4Cl (150 mL). The mixture was concentrated, and the residue was extracted with EtOAc. The organic layer was washed with saturated aqueous solution of NH_4Cl, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a crude mixture (33.5 g) of diBOC compounds **9a** and **9b**, and a monoBoc compound **10**. This crude material was used directly in the next reaction without further purification. In the air, K_2CO_3 (1.26 g, 9.12 mmol) was added to the above crude mixture (33.5 g) in MeOH (360 mL) at rt. After stirred under reflux for 1 h, the reaction mixture was cooled to 0 °C, and quenched with saturated aqueous solution of $KHSO_4$ (7.50 mL). The mixture was concentrated and H_2O (150 mL) was added. The mixture was extracted with EtOAc (140 mL × 9 times). The organic layer was washed with H_2O for 3 times, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give crude Boc-His-OMe **10** as a gum. To this gummy crude material, a mixture of hexane and EtOAc (10:1) was added and triturated to form powder material in the solvent. The powder was collected by suction filteration to afford Boc-L-His-OMe **10** (19.08 g, 70.9 mmol, 73% for 3 steps) as a colorless powder. Despite the absence of a purification process, the obtained powder showed fully identical physical data to those reported in the literature,[18] except for optical rotation. In our case, **10** showed $[\alpha]_D^{25}$ +13.1 (*c* 1.02, $CHCl_3$), whereas the literature value was $[\alpha]_D^{25}$ −9.70 (*c* 1.00, $CHCl_3$).[18] We also measured the $[\alpha]_D$ value of a commercially available Boc-L-His-OMe (Watanabe Chemical Industries, LTD., Japan), whose ee had been confirmed as >99.9% ee by HPLC (personal communication). The result was $[\alpha]_D^{26}$ +13.0 (*c* 1.00, $CHCl_3$), which is almost identical to ours.

Boc-L-His(τ-Ac)-OMe 11. In the air, Boc-L-His-OMe **10** (18.0 g, 66.8 mmol) was added to Ac_2O (90 mL) at 0 °C. After being stirred at 0 °C for 1 h, the reaction mixture was concentrated, co-evaporated with toluene (5 times), and triturated in Et_2O/hexane = 1/5. The powder was collected by suction filtration to afford **11** (18.9 g, 60.7 mmol, 91%) as a colorless powder. Despite the absence of a purification process, the obtained powder showed clean ^1H NMR. $[\alpha]_D^{29}$ +28.8 (*c* 1.02, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 1.6 Hz, 1H), 7.25 (s, 1H), 5.65 (broad d, J = 7.6 Hz, 1H) 4.58–4.61 (m, 1H), 3.73 (s, 3H), 3.02–3.07 (m, 2H), 2.56 (s, 3H), 1.43 (s, 9H); HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $C_{14}H_{21}N_3NaO_5^+$ 334.1373, found 334.1359. **Comment:** An attempt to recrystallize **11**, via a procedure accompanied by heating, resulted in the severe racemization. Because compound **11** seemed unstable, it was soon used in the next reaction.

Boc-L-His(π-NAPOM)-OH 7. After the moisture was removed from Boc-L-His(τ-Ac)-OMe **11** (5.25 g, 16.9 mmol) by azeotropy with dehydrated toluene (3 times), under argon atmosphere, dehydrated toluene (79.1 g) and MS5A (1.70 g, powder) were added and the reaction mixture was cooled to 0 °C. To the mixture was added NAPOMCl (5.23 g, 25.3 mmol) and the reaction was stirred at 0 °C for 10 min, and at rt for 22 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The solid residue, which was poorly soluble in Et_2O, was washed with Et_2O (4 times) to give crude Boc-L-His(π-NAPOM)-OMe **12** (8.03 g) as a colorless solid. The crude material was used directly in the next reaction without further purification. In a stoppered flask, 2 M aqueous solution of NaOH (21.1 mL) was added to a solution of the above crude Boc-L-His(π-NAPOM)-OMe **12** (8.03 g) in MeOH (68 mL) at rt. After stirred at rt for 1.5 h, the reaction mixture was diluted with H_2O (68 mL), and concentrated under reduced pressure to remove MeOH. The residual aqueous mixture was washed with Et_2O (70 mL × 3 times), and the pH of the aqueous layer was adjusted to 4 using saturated aqueous solution of $KHSO_4$ (6.2 mL). The acidified aqueous mixture was extracted with AcOEt (70 mL × 5 times). The combined organic layers were washed with H_2O (100 mL × 2 times) and dried over anhydrous Na_2SO_4. The mixture was filtered and the filtrate was concentrated under reduced pressure to give crude Boc-L-His(π-NAPOM)-OH **7** (6.76 g). To the crude material was added acetone (338 mL) and the insoluble material after sonication was filtered off. The filtrate was evaporated and the resultant gummy residue was recrystallized from hexane / acetone (2/3, 250 mL) to give Boc-L-His(π-NAPOM)-OH **7** (3.63

g, 8.53 mmol, 50% for 2 steps) as colorless needles. The purity and the enantiomeric excess of **7** at this stage were determined by HPLC as 97.7% (Figure S1) and 99.7% ee (Figure S2), respectively. **Comment:** Although these purity and enantiomeric excess were satisfactory to us, to ensure its commercialization, another recrystallization using hexane / acetone (1/4, 150 mL) was examined. As a result, purer specimen of **7** (2.80 g, 39%) was obtained as colorless needles, whose purity and ee were 99.5% and 99.7% ee, respectively (charts not shown). M.p. 148–149 °C; $[\alpha]_D^{22}$ +35.9 (*c* 0.80, $CHCl_3$); IR (neat) 3319 (broad), 3123, 2976, 2931, 1696 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.18 (s, 1H), 7.87–7.77 (m, 4H), 7.50–7.40 (m, 3H), 7.00 (s, 1H), 5.63 (d, *J* = 11.2 Hz, 1H), 5.59 (d, *J* = 11.2 Hz, 1H), 4.75 (d, *J* = 12.0 Hz, 1H), 4.71 (d, *J* = 12.0 Hz, 1H), 4.39 (broad m, 1H), ca. 3.38–3.30 (m, 1H, overlapped with a solvent peak); 3.08 (dd, *J* = 15.6, 8.4 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.4, 157.6, 138.4, 135.5, 134.7, 134.6, 131.1, 129.4, 129.0, 128.7, 127.9, 127.3, 127.2, 126.7, 125.3, 80.5, 76.1, 72.2, 54.8, 28.7, 27.7; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{23}H_{28}N_3O_5^+$ 426.2023, found 426.2027. Through an identical method, ***ent*-7** was synthesized from D-histidine. The physical data of ***ent*-7** were completely identical, except that it showed negative optical rotation with keeping the absolute value.

Fmoc-L-His(π-NAPOM)-OH 6. In the air, 4 M HCl in EtOAc (31.0 mL, 124 mmol) was added to a solution of Boc-L-His(π-NAPOM)-OH **7** (2.00 g, 4.70 mmol) in EtOAc (16 mL) at 0 °C. After stirred at rt for 1.5 h, the reaction mixture was concentrated under reduced pressure. The residue was washed with EtOAc, and concentrated to give crude L-His(π-NAPOM)-OH·HCl **14** (2.03 g), which was used directly in the next reaction without further purification. In the air, saturated aqueous solution of Na_2CO_3 (11.8 mL, 24.4 mmol) and FmocOSu (2.39 g, 7.05 mmol) were added to a solution of the above-mentioned crude L-His(π-NAPOM)-OH·HCl **14** (2.03 g) in THF (11.8 mL) at 0 °C. After stirred at rt for 16 h, the reaction mixture was concentrated under reduced pressure. The resulting aqueous mixture was washed with EtOAc (5 times), and the pH was adjusted to 5.0 with saturated aqueous solution of $KHSO_4$. The acidified solution was extracted with EtOAc (10 times). The combined organic layers were washed with H_2O (4 times) and dried over anhydrous Na_2SO_4. The mixture was filtered and the filtrate was concentrated under reduced pressure to give crude Fmoc-L-His(π-NAPOM)-OH (2.65 g, colorless amorphus). The crude material was triturated in Et_2O and the formed precipitate was collected by suction filtration to furnish Fmoc-L-His(π-NAPOM)-OH **6** (1.57 g, 2.87 mmol, 61%, 2 steps). The purity and the ee were determined to be 97.7% and 99.7% ee, respectively, via HPLC analyses (Figures S3 and S4). M.p. 171–172 °C; $[\alpha]_D^{20}$ −14.2 (*c* 0.30, DMF); IR (neat) 3407 (broad), 3132, 3056, 1710 cm^{-1}; ^1H NMR (400 MHz, DMSO-d6) δ 7.92–7.65 (m, 9H), 7.54–7.28 (m, 7H), 6.80 (s, 1H), 5.51 (d, *J* = 11.0 Hz, 1H); 5.46 (d, *J* = 11.0 Hz, 1H), 4.59 (s, 2H), 4.39–4.16 (m, 3H), 3.45–3.27 (broad, 1H), 3.24–3.11 (m, 1H), 3.01 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d6) δ 173.0, 155.9, 143.8, 140.7, 138.5, 134.8, 132.8, 132.5, 127.9, 127.8, 127.7, 127.6, 127.1, 126.2, 126.0, 125.8, 125.3 × 2, 120.1, 73.4, 69.4, 65.7, 53.4, 46.6, 25.3; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{33}H_{30}N_3O_5^+$ 548.2180, found 548.2196. Through an identical method, ***ent*-6** was synthesized from D-histidine. The physical data of ***ent*-6** were completely identical, except that it showed positive optical rotation with keeping the absolute value.

Boc-His(π-NAPOM)-Pro-NH_2 15. In a stoppered flask, Pro-NH_2 (13.9 mg, 0.117 mmol) was added to a solution of Boc-His (π-NAPOM)-OH **7** (50.1 mg, 0.117 mmol), anhydrous HOBt (16.0 mg, 0.117 mmol) and DCC (24.4 mg, 0.117 mmol) in dehydrated DMF (1.8 mL) at 0 °C. After stirred at rt for 16 h, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. After added water, the mixture was extracted with $CHCl_3$. The organic portions were combined and washed with saturated aqueous Na_2CO_3. The organic layer was dried over Na_2SO_4 and filtered, and the filtrate was evaporated *in vacuo* to give crude Boc-His(π-NAPOM)-Pro-NH_2 **15** (100.0 mg). After keeping a part of this crude material (ca. 20 mg) for racemization analysis, the residue was roughly purified by silica gel column chromatography, using $CHCl_3$ and MeOH (50/1 to 10/1) as eluents, to give impure Boc-His(π-NAPOM)-Pro-NH_2 **15** to use as a reference of **15** in HPLC analysis. In the same procedure, ***epi*-15**, whose His residue has D-configuration, was synthesized from ***ent*-7**, and used in HPLC analysis (Figure S5A).

Fmoc-Ala-His-Pro-OH 22. In a stoppered flask, a solution of Pro-O*t*Bu **17** (94.0 mg, 0.548 mmol) in dehydrated DMF (1.65 mL) was added to a solution of Fmoc-L-His(π-NAPOM)-OH **6** (200 mg, 0.365 mmol), HATU (278 mg, 0.731 mmol) and DIEA (63.6 μL, 0.365 mmol) in dehydrated DMF (2.0 mL) at rt. After stirred at rt for 1 h, the reaction mixture was diluted with $CHCl_3$. The resultant solution was washed with saturated aqueous solution of $KHSO_4$, and then with that of Na_2CO_3. The organic layer was separated and dried over anhydrous Na_2SO_4. The mixture was filtered and the filtrate was concentrated under reduced pressure to give crude Fmoc-His(π-NAPOM)-Pro-O*t*Bu **18** (281 mg), which was used directly in the next reaction without further purification. 20% piperidine solution in dehydrated DMF (3.7 mL) was added to a solution of the above crude Fmoc-His(π-NAPOM)-Pro-O*t*Bu **18** (281 mg) in dehydrated DMF (0.3 mL) at rt. After stirred for 30 min, the reaction mixture was concentrated under reduced pressure to give crude His(π-NAPOM)-Pro-O*t*Bu **19** (244 mg), which was used directly in the next reaction without further purification. A solution of the above crude His(π-NAPOM)-Pro-O*t*Bu **19** (244 mg) in dehydrated DMF (1.8 mL) was added to a solution of Fmoc-Ala-OH **20** (171 mg, 0.548 mmol), HATU (277.5 mg, 0.730 mmol) and DIEA (63.6 μL, 0.365 mmol) in dehydrated DMF (1.8 mL) at rt. After being stirred at room temperature for 1.5 h, the reaction mixture was diluted with $CHCl_3$. The resultant solution was washed with saturated aqueous solution of $KHSO_4$, and then with that of Na_2CO_3. The organic layer was separated and dried over anhydrous Na_2SO_4. The mixture was filtered and the filtrate was concentrated under reduced pressure to give crude Fmoc-Ala-His(π-NAPOM)-Pro-O*t*Bu **21** (305 mg), which was used in the next reaction without further purification. To the above crude material **21** (69.5 mg) was added a cocktail of *i*-Pr_3SiH (0.05 mL), H_2O (0.05 mL) and TFA (0.90 mL) at rt. After stirred under reflux for 1.5 h, the reaction was cooled to rt and evaporated. The residual amorphus was washed with Et_2O (3 times), to give crude Fmoc-Ala-His-Pro-OH **22** (48.0 mg). In the same procedure, crude material of ***epi*-22**, whose His residue has D-configuration, was synthesized from ***ent*-6**, and used as a reference in HPLC analysis (Figure S6A).

ASSOCIATED CONTENT

Supporting Information

HPLC charts to determine ee values, ^1H and ^{13}C NMR charts of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: torikai@chem.kyushu-univ.jp

ORCID

Kohei Torikai: 0000-0002-9928-4300

Notes

The authors declare that a patent regarding the use of NAPOMCl has been granted in the USA (Kyushu University, Agent for Introducing Protecting Group for Hydroxy group and/or Mercapto

Group, Patent No. US10160694, December 25, 2018), and is pending in several other countries (Kyushu University, Agent for Introducing Protecting Group for Hydroxy group and/or Mercapto Group, Publication No. 20170305809, October 26, 2017).

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(15) For details of the analysis, see the SI.

(16) Recrystallization could be achieved using $CHCl_3$/MeOH/Et_2O = 4/1/1 (v/v) to give **6** in 23% over two steps, although the purified material showed almost identical purity and ee to the crude material.

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