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Introduction of histidine units using benzotriazolide activation

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***N*$^{\alpha}$-Boc-*N*^{im}-(4-toluenesulfonyl-L-histidylbenzotriazole) enables convenient acylation of *N*-, *O*-, *S*-, and *C*-nucleophiles with no detectable racemization. We report efficient syntheses of novel histidine-containing di-, tri-, and tetra-peptides and models for the preparation of potentially biologically active histidine *N*-, *O*-, *S*-, and *C*-conjugates. Copyright © 2013 European Peptide Society and John Wiley & Sons, Ltd.**

Supporting information can be found in the online version of this article.

Keywords: histidine; conjugates; benzotriazole; acylation

Introduction

Histidine is of prime importance as an essential component of the active site of many enzymes [1], which is widely distributed in nature, and its derivatives exhibit diverse biological activities [2,3]: for example, naphthalene-substituted histidine derivatives act as useful antihypertensive agents [4], a histidine-estrone conjugate has pronounced anti-tumor activity [5], and histidine-tocopherol conjugates are antioxidants and prodrug delivery vehicles [6]. Carnosine (β-alanyl-L-histidine) and carcinine (β-alanyl-histamine) occur in the non-protein fraction of mammalian tissues [7]. In proteins, histidine residues play an important role in interactions with metal ions because of the presence of three coordinating groups. Histidine coordinates strongly and forms six membered rings in which a metal is attached to an imidazole nitrogen atom and the α-amino group [8]. For example, the N-terminus of the protein bovine serum albumin (Asp-Thr-His-Lys-OH) specifically binds the Cu(II) ion because of the histidine residue [9]. Histidine-containing peptides act as catalysts in Michael addition of nitroalkanes [10], direct asymmetric aldol reactions [11], and asymmetric synthesis of cyanohydrins [12].

Histidine residues are often involved in neutral hydrolytic metalloenzyme active centers and have the potential to bind DNA. The unprotonated imidazole ($pH > 7$) is nucleophilic, whereas the protonated form ($pH < 7$) serves as a proton donor.

Histidine is often susceptible to racemization during solid-phase synthesis (2.1% racemization detected for the L-His-containing peptide and 1.9% detected for the D-His-containing peptide) [13]. Ishiguro *et al.* reported unexpected chain-terminating side reactions caused by histidine in solid-phase peptide synthesis [14]. Racemization during the synthesis of polypeptides could be a serious problem as a high degree of steric homogeneity is usually required for biological or physical studies. The detection of small amounts of diastereoisomeric impurities and their removal from synthetic polypeptide preparations are often difficult; thus, synthetic procedures that minimize the risk of racemization are commonly used. In a histidine unit, the presence of the imidazole ring and an amino group has complicated the development of synthetic methods to prepare histidine C-terminus derivatives. The nucleophilic imidazole ring of histidine easily undergoes *N*-acylation in absence of appropriate protecting groups, which can result in side reactions such as acyl transfer. In particular, histidine derivatives are known to be prone to racemization during activating and coupling processes involving the π-nitrogen of the imidazole ring [15]. Thus, several protecting groups as well as activating reagents have been introduced in attempts to solve problems such as the regioselective protection of the π-nitrogen, and this enables avoidance of racemization. For this purpose, the N^{π}-benzyloxymethyl (Bom) group has been widely employed in Boc chemistry [16], and the N^{τ}-Trt protecting group [17], used in combination with N^{α}-Fmoc protection [17,18]. Although the steric hindrance of the Trt group may help reduce racemization to a certain extent, N^{τ}-protecting groups cannot inherently prevent racemization. Other N^{π}-protecting groups including N^{π}-*t*-butoxymethylhistidine [His(Bum)] [19], N^{π}-1-adamantyloxymethylhistidine [His(1-Adom)] [20], and 4-methoxybenzyloxymethyl (MBom) [21,22] have been introduced. The use of several activating agents has been reported in literature including (i) solution phase synthesis of a histidine-containing dipeptide derivative from Z-Phe-OH and H-His-OMe using isobutyl chloroformate [12], (ii) preparation of the histidine dipeptide (Boc-His(Tos)-Leu-OH) from Boc-His(Tos)-

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OSu and H-Leu-OH [23], (iii) dipeptide (Boc-Ala-His(Tos)-OMe) from protected histidine and alanine in the presence of EDCI and Et_3N [24], and (iv) dipeptide (Boc-His(MBS)-Gly-OBzl) utilizing *p*-TsOH, and diethyl phosphorocyanidate as a coupling agent [25]. Literature routes to C-terminus histidine conjugates include the use of the coupling reagents (i) DCC with DMAP [26,27], (ii) EDCI-HOBt [28], and (iii) isobutyl chloroformate [29]. These methods showed good yields, but products needed column purification. Additionally, some reports indicate that histidine derivatives racemize on activation with DCC [30].

We have extensively applied *N*-acylbenzotriazoles for *N*-acylation, *C*-acylation, *O*-acylation, and *S*-acylation [31]. In these reactions, *N*-(Z-aminoacyl)benzotriazoles coupled with unprotected amino acids to produce peptides in 85–95% yield with minimal racemization [32]. *N*-(Z-α-aminoacyl)benzotriazoles prepared from *N*-protected α-amino acids with unfunctionalized side chains such as Ala, Val, and Phe coupled successfully with unprotected amino acids (Ala, Val, Phe, Ser, Trp) in the presence of Et_3N in partially aqueous solution (CH_3CN/H_2O). We have also prepared *N*-(Z- or Fmoc-α-aminoacyl)benzotriazoles derived from Tyr, Trp, Cys, Met, and Gln containing unprotected side-chain functionality [33], and successfully coupled them with α-amino acids (L-Ala, L-Phe) in partially aqueous medium to form the expected peptides. NMR and HPLC analysis supported the preservation of the original chirality. These findings were further confirmed by NMR and HPLC comparisons of the diastereomeric dipeptides prepared by coupling *N*-(Z-Tyr, Trp, Cys, Met, Gln and Fmoc-Trp, Met acyl)benzotriazole with H-DL-Ala-OH, H-DL-Phe-OH [33]. We have also reported successful benzotriazole activation in racemization free tri- and tetra-peptides [31]. Herein, we report another method to introduce histidine residues at the N-terminus in solution phase peptide synthesis by utilization of benzotriazole-activated histidine intermediate **3a** and **3b** (Scheme 1). Compound **3a** was also used to synthesize novel histidine conjugates by *N*-, *O*-, *S*-, and *C*-acylations. There is a previous reference [23] for the preparation of **5c** (Scheme 2), but no experimental details are given.

Materials and Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in $CDCl_3$, DMSO-d_6, or CD_3OD-d_4 on Mercury, Gemini, or Varian NMR spectrometers operating at 300 MHz for ^1H (with TMS as an internal standard) and 75 MHz for ^{13}C NMR. Elemental analyses were performed on a Carlo Erba-EA1108 instrument. All microwave-assisted reactions were carried out with a single mode cavity Discover Microwave Synthesizer (CEM Corporation, NC, USA). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum, and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 s; PowerMax-cooling mode).

Preparation of N^{α}-Boc-N^{im}-protected-L-histidylbenzotriazole (3a,b)

Compounds **3a**,**b** were synthesized by irradiating an equimolar amount of N^{α}-Boc-N^{im}-protected-L-histidine with 1-(methylsulfonyl)-1*H*-benzo[*d*][1,2,3]triazole ($BtSO_2Me$) in the presence of 1.0 eq. of Et_3N in anhydrous DMF for 30 min at 50 °C and 20 W irradiation power. Completion of the reaction was monitored by TLC. After completion, the reaction mixture was quenched with water. The precipitate obtained was washed with saturated solution of Na_2CO_3 to afford the desired products, which were further crystallized from methylene chloride/hexane.

(*S*)-*tert*-Butyl-(1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-oxo-3-(1-tosyl-1*H*-imidazol-4-yl)propan-2-yl)carbamate (Boc-His(Tos)-Bt, 3a)

White microcrystals (65%); mp 150–152 °C; ^1H NMR ($CDCl_3$) δ 8.18 (d, J = 8.4 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 1.5 Hz, 1H), 7.75 (d, J = 8.4 Hz, 2H), 7.66 (t, J = 7.5 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.34 (d, J = 8.4 Hz, 2H), 7.01 (s, 1H), 6.12–6.06 (m, 1H), 5.96–5.84 (m, 1H), 3.40–3.22 (m, 2H), 2.44 (s, 3H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.7, 155.6, 146.5, 146.1, 139.6, 136.8, 134.9, 131.3, 130.8, 130.6, 127.5, 126.6, 120.5, 115.1, 114.6, 80.6, 54.3, 30.8, 28.5, 22.0. HRMS (+ESI-TOF): *m/z* for $C_{24}H_{26}N_6O_5S$ $[M+H]^+$ calcd 511.1758, found 511.1749.

(*S*)-*tert*-Butyl-1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-3-(1-benzyl-1*H*-imidazol-4-yl)-1-oxopropan-2-ylcarbamate (Boc-His(Bzl)-Bt, 3b)

White microcrystals (62%); mp 85–87 °C; ^1H NMR ($CDCl_3$) δ 8.16 (d, J = 8.2 Hz, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.59 (t, J = 7.9 Hz, 1H), 7.50–7.45 (m, 2H), 7.32–7.26 (m, 3H), 7.05–7.03 (m, 2H), 6.63 (d, J = 7.7 Hz, 1H), 6.55 (s, 1H), 5.94–5.87 (m, 1H), 4.98 (s, 2H), 3.48–3.27 (m, 2H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.9, 155.6, 145.7, 137.4, 137.0, 135.9, 131.1, 130.4, 128.8, 128.1, 127.1, 126.2, 120.0, 117.2, 114.3, 79.9, 54.8, 50.7, 30.4, 28.3.

General Procedure for the Preparation of Histidine-containing di-, tri-, and tetra-peptides (5a–f)

To a solution of compound **3** (1.0 eq.) in acetonitrile, a solution of amino acid, dipeptide or tripeptide (1.0 eq.), and Et_3N (1.2 eq.) in water was added dropwise, and the reaction mixture was stirred at room temperature for 3–4 h. After complete consumption of compound **3** (TLC), the solution was concentrated under reduced pressure. The solution was neutralized by 4N HCl and extracted with ethyl acetate, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to afford a white solid, which was either crystallized from diethyl ether or purified by flash column chromatography with 30% ethyl acetate–hexane solution as eluent.

(*S*)-2-((*S*)-2-((tert-Butoxycarbonyl)amino)-3-(1-tosyl-1*H*-imidazol-4-yl)propanamido)-3-phenylpropanoic acid (Boc-L-His(Tos)-L-Phe-OH, 5a)

White microcrystals (79%); mp 133–134 °C; ^1H NMR ($CDCl_3$) δ 7.98 (s, 1H), 7.81 (d, J = 6.9 Hz, 2H), 7.38–7.29 (m, 3H), 7.26–7.18 (m, 5H), 7.09 (s, 1H), 5.39 (d, J = 8.7 Hz, 1H), 4.76–4.68 (m, 1H), 4.45 (t, J = 9.0 Hz, 1H), 3.20–2.92 (m, 3H), 2.53–2.41 (m, 1H), 2.42 (s, 3H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 174.3, 170.9, 155.2, 146.9, 139.9, 136.5, 134.6, 130.7, 129.9, 128.5, 127.7, 127.1, 115.6, 79.6, 54.2, 53.7, 38.7, 32.8, 28.4, 22.0; Anal. calcd for $C_{27}H_{32}N_4O_7S$: C, 58.26; H, 5.79; N, 10.07; found: C, 58.15; H, 6.13; N, 10.09.

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2-((*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(1-tosyl-1*H*-imidazol-4-yl)propanamido)-3-phenylpropanoic acid (Boc-His(Tos)-DL-Phe-OH, 5a+5a′)

White microcrystals (72%); mp 175–177 °C; ^{1}H NMR ($CDCl_3$) δ 8.97–8.96 (d, J = 4.4 Hz, 1H), 8.16–8.07 (m, 1H), 7.50–7.47 (m, 2H), 7.27–6.98 (m, 8H), 4.51–4.43 (m, 1H), 4.32–4.25 (m, 1H), 3.19–2.64 (m, 4H), 2.29 (s, 3H), 1.32 (s, 9H).^{13}C NMR ($CDCl_3$) δ 172.6, 170.3, 170.1, 155.1, 145.5, 137.7, 137.3, 133.7, 129.5, 129.5, 129.2, 128.2, 128.1, 126.5, 125.5, 116.8, 116.6, 78.5, 53.3, 53.0, 36.8, 36.6, 28.1, 27.1, 20.8; Anal. calcd for $C_{27}H_{32}N_4O_7S$: C, 58.36; H, 5.79; N, 10.07; found: C, 57.87; H, 6.02; N, 9.83.

(*S*)-2-((*S*)-3-(1-Benzyl-1*H*-imidazol-4-yl)-2-(tert-butoxycarbonylamino)propanamido)-3-methylbutanoic acid (Boc-His(Tos)-L-Val-OH, 5b)

Oil (72%); ^{1}H NMR ($CDCl_3$) δ 8.04 (s, 1H), 7.82 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.1 Hz, 1H), 7.11 (s, 1H), 5.52 (d, J = 8.7 Hz, 1H), 4.58–4.49 (m, 2H), 3.13 (d, J = 14.1 Hz, 1H), 2.61–2.50 (m, 1H), 2.43 (s, 3H), 2.25–2.19 (m, 1H), 1.31 (s, 9H), 0.98–0.92 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 174.4, 171.5, 155.4, 146.9, 139.9, 136.6, 134.6, 130.8, 130.7, 127.8, 115.6, 79.7, 57.8, 53.9, 32.4, 31.8, 28.4, 22.0, 18.8, 18.1. HRMS (+ESI-TOF): m/z for $C_{23}H_{32}N_4O_5$ $[M+H]^+$ calcd 507.1919, found 507.1926.

2-((*S*)-3-(1-Benzyl-1*H*-imidazol-4-yl)-2-(tert-butoxycarbonylamino)propanamido)-3-methylbutanoic acid (Boc-His(Tos)-DL-Val-OH, 5b+5b′)

Oil (70%); ^{1}H NMR ($CDCl_3$) δ 8.03 (s, 1H), 7.83 (d, J = 8.1 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 7.21–7.14 (m, 1H), 5.86–5.84 (m, 1H), 5.65–5.62 (m, 1H), 4.68–4.50 (m, 2H), 3.18–3.05 (m, 1H), 2.74–2.64 (m, 1H), 2.43 (s, 3H), 2.28–2.19 (m, 1H), 1.31 (d, J = 12.0 Hz, 9H), 0.96–0.90 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 174.6, 174.3, 171.6, 171.4, 155.6, 155.4, 146.8, 139.9, 136.5, 134.6, 130.7, 127.7, 115.6, 79.8, 79.7, 66.0, 57.7, 57.3, 53.9, 31.7, 31.6, 28.4, 21.9, 18.9, 18.8, 18.0, 17.9, 15.4. HRMS (+ESI-TOF): m/z for $C_{23}H_{32}N_4O_5$ $[M+H]^+$ calcd 507.1919, found 507.1932.

(*S*)-2-((*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(1-tosyl-1*H*-imidazol-4-yl)propanamido)-4-methylpentanoic acid (Boc-L-His(Tos)-L-Leu-OH, 5c)

White microcrystals (89%); mp 121–123 °C [lit. mp 146–148 °C][16]; ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 7.5 Hz, 1H), 7.36 (d, J = 8.4 Hz, 2H), 7.15 (s, 1H), 5.60 (d, J = 9.0 Hz, 1H), 4.64–4.52 (m, 2H), 3.14–3.05 (m, 1H), 2.68–2.56 (m, 1H), 2.43 (s, 3H), 1.78–1.56 (m, 3H), 1.32–1.22 (m, 9H), 0.96 (d, J = 6.3 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 175.6, 171.4, 155.4, 146.8, 139.9, 136.5, 134.6, 130.7, 127.7, 115.7, 79.7, 53.7, 51.5, 42.6, 32.3, 31.8, 28.3, 25.0, 22.8, 22.6, 21.9, 14.3.

2-((*S*)-2-(*tert*-Butoxycarbonylamino)-3-(1-tosyl-1*H*-imidazol-4-yl)propanamido)propanoic acid (Boc-L-His(Tos)-L-Ala-OH, 5d)

White microcrystals (92%); mp 151–153 °C; ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1H), 7.84–7.82 (d, J = 8.1 Hz, 2H), 7.38–7.35 (d, J = 8.1 Hz, 2H), 7.12 (s, 1H), 5.66–5.63 (d, J = 8.2 Hz, 1H), 5.46–5.44 (d, J = 7.9 Hz, 1H), 4.63–4.50 (m, 1H), 3.19–3.10 (m, 1H), 2.70–2.48 (m, 2H), 2.44 (s, 3H), 1.51–1.27 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 157.9, 171.3, 155.3, 146.9, 139.8, 136.5, 134.5, 130.8, 127.8, 115.7, 79.7, 53.6, 49.2, 48.8, 32.5, 28.3, 22.0, 18.9. HRMS (+ESI-TOF): m/z for $C_{21}H_{28}N_4O_7S$ $[M+Na]^+$ calcd 503.1571, found 503.1571.

(6*S*,12*S*)-12-Isopropyl-2,2-dimethyl-4,7,10-trioxo-6-((1-tosyl-1*H*-imidazol-4-yl)methyl)-3-oxa-5,8,11-triazatridecan-13-oic acid (Boc-L-His(Tos)-Gly-L-Val-OH, 5e)

White microcrystals (68%); mp 113–115 °C; ^{1}H NMR ($CDCl_3$) δ 8.02–8.00 (m, 1H), 7.87–7.74 (m, 3H), 7.45–7.14 (m, 5H), 5.95–5.87 (m, 1H), 4.49–4.39 (m, 1H), 4.11–3.78 (m, 2H), 3.08–2.93 (m, 2H), 2.42 (s, 3H), 2.42–2.13 (m, 1H), 1.33 (s, 9H), 0.94–0.90 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 174.7, 172.6, 169.8, 155.8, 146.8, 139.9, 136.8, 134.6, 130.7, 127.7, 126.0, 115.4, 80.3, 58.3, 58.1, 54.2, 43.4, 30.7, 28.4, 21.9, 19.4, 18.1. Anal. calcd for $C_{25}H_{35}N_5O_8S$: C, 53.08; H, 6.24; N, 12.38; found: C, 52.68; H, 6.43; N, 12.32.

(6*S*,15*S*)-15-((*S*)-*sec*-Butyl)-2,2-dimethyl-4,7,10,13-tetraoxo-6-((1-tosyl-1*H*-imidazol-4-yl)methyl)-3-oxa-5,8,11,14-tetraazahexadecan-16-oic acid (Boc-L-His(Tos)-Gly-Gly-L-Ile-OH, 5f)

White microcrystals (60%); mp 140–142 °C; ^{1}H NMR (DMSO-d_6) δ 12.62 (s, 1H), 8.28 (s, 1H), 8.22–8.10 (m, 2H), 7.92 (d, J = 8.1 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.37 (s, 1H), 6.88 (d, J = 8.1 Hz, 1H), 4.23–4.16 (m, 2H), 3.79 (t, J = 5.7 Hz, 2H), 3.66 (d, J = 5.4 Hz, 2H), 2.92–2.80 (m, 1H), 2.75–2.65 (m, 1H), 2.40 (s, 3H), 1.82–1.70 (m, 1H), 1.30 (s, 9H), 1.24–1.11 (m, 2H), 0.85–0.78 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 172.8, 171.5, 168.9, 168.6, 155.1, 146.3, 140.7, 136.7, 134.4, 130.6, 127.2, 114.7, 78.3, 56.2, 53.5, 42.2, 41.6, 36.4, 30.3, 28.1, 24.6, 21.2, 15.5, 11.3. Anal. calcd for $C_{28}H_{40}N_6O_9S$: C, 52.82; H, 6.33; N, 13.20; found: C, 52.88; H, 6.70; N, 13.09.

Procedure for Preparation of Compound 7

To a solution of compound **3b** (1.0 eq.) in acetonitrile, a solution of D-H-Phe-OMe (1.0 eq.) and Et_3N (1.2 eq.) in water was added dropwise, and the reaction mixture was stirred at room temperature for 3 h. After complete consumption of compound **3b** (TLC), the solution was concentrated under reduced pressure. The residue was extracted with ethyl acetate, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to afford the desired product as an oil.

Methyl 2-((*S*)-3-(1-benzyl-1*H*-imidazol-4-yl)-2-(tert-butoxycarbonylamino)propanamido)-3-phenylpropanoate (Boc-His(Bzl)-Phe-OMe, 7)

Oil (69%); ^{1}H NMR ($CDCl_3$) δ 7.42–7.38 (m, 1H), 7.34–7.16 (m, 7H), 7.13–7.06 (m, 3H), 6.96–6.94 (m, 1H), 6.71–6.63 (m, 1H), 6.39–6.27 (m, 1H), 4.97 (s, 2H), 4.79–4.75 (m, 1H), 3.69 (s, 1H), 3.63 (s, 1H), 3.59 (s, 1H), 3.06–2.84 (m, 4H), 1.40 (d, J = 4.0 Hz, 9H). ^{13}C NMR ($CDCl_3$) δ 175.4, 171.6, 171.5, 171.4, 155.6, 138.5, 138.2, 136.7, 136.0, 135.8, 129.2, 128.9, 128.5, 128.4, 128.3, 128.2, 127.4, 127.3, 126.9, 126.8. HRMS (+ESI-TOF): m/z for $C_{28}H_{34}N_4O_5$ $[M+H]^+$ calcd 507.2602, found 507.2604.

Procedure for Preparation of Compound 8a

N-(3-Aminopropyl)-imidazole (1.0 eq.) was dissolved in THF and Et_3N (1.5 eq.). N^{α}-Boc-N^{im}-4-toluenesylfonyl-histidylbenzotriazole **3a** (1.0 eq.) was added to the solution, and the mixture was stirred at room temperature for 3 h. The mixture was acidified with 4N HCl, concentrated and then diluted with ethyl acetate. The organic layer was washed with 10% Na_2CO_3 solution, water and dried over anhydrous $MgSO_4$, filtered, and evaporated to give a white solid, which was further washed with diethyl ether to give the desired compound.

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(*S*)-*tert*-Butyl-(1-((3-(1*H*-imidazol-1-yl)propyl)amino)-1-oxo-3-(1-tosyl-1*H*-imidazol-4-yl) propan-2-yl)carbamate (8a)

White microcrystals (92%); mp 64–66 °C; ^{1}H NMR ($CDCl_3$) δ 7.93 (s, 1H), 7.80 (d, J = 8.4 Hz, 2H), 7.46 (s, 1H), 7.35 (d, J = 8.1 Hz, 2H), 7.10 (s, 1H), 7.04 (s, 1H), 6.91 (br s, 2H), 5.93 (d, J = 7.2 Hz, 1H), 4.40–4.33 (m, 1H), 3.87 (t, J = 6.9 Hz, 2H), 3.14 (q, J = 6.3 Hz, 2H), 3.05 (dd, J = 14.7, 5.4 Hz, 1H), 2.90 (dd, J = 14.7, 5.4 Hz, 1H), 2.43 (s, 3H), 1.92–1.84 (m, 2H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.7, 155.9, 146.7, 140.7, 137.3, 136.3, 134.8, 130.6, 129.7, 127.6, 119.0, 115.1, 80.5, 54.4, 44.2, 36.4, 31.2, 30.3, 28.5, 21.9; HRMS (+ESI-TOF): *m/z* for $C_{24}H_{33}N_6O_5S$ $[M+H]^+$ calcd 517.2228, found 517.2229.

Procedure for Preparation of Compound 8b

A dried heavy-walled Pyrex tube containing a small stir bar was mixed with compound **3a** (0.3 g, 0.587 mmol) and 2-aminopyridine (0.056 g, 0.587 mmol) in dry DMF (5 mL) for 30 min at 50 °C and 20 W irradiation power. After completion (TLC), the reaction mixture was quenched with water. The precipitate obtained was washed with saturated Na_2CO_3 solution and water to afford a solid, which was flash chromatograhed with 30% ethyl acetate/hexane solution to give compound **8b**.

(*S*)-*tert*-Butyl-(1-oxo-1-(pyridin-2-ylamino)-3-(1-tosyl-1*H*-imidazol-4-yl)propan-2-yl)carbamate (8b)

White microcrystals (91%); mp 87–88 °C; ^{1}H NMR ($CDCl_3$) δ 9.00 (br s, 1H), 8.24 (d, J = 4.5 Hz, 1H), 8.14 (d, J = 8.4 Hz, 1H), 7.94 (s, 1H), 7.73 (d, J = 8.1 Hz, 2H), 7.67 (t, J = 8.0 Hz, 1H), 7.25 (d, J = 8.4 Hz, 2H), 7.10 (s, 1H), 7.08–7.00 (m, 1H), 6.18–6.04 (m, 1H), 4.62–4.54 (m, 1H), 3.22–3.12 (m, 1H), 2.99 (dd, J = 15.0, 5.1 Hz, 1H), 2.39 (s, 3H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.3, 155.4, 151.1, 147.9, 146.4, 140.4, 138.5, 136.6, 134.9, 130.5, 127.4, 120.0, 115.1, 114.2, 80.7, 55.1, 30.2, 28.4, 21.9; HRMS (+ESI-TOF): *m/z* for $C_{23}H_{28}N_5O_5S$ for $[M+H]^+$ calcd 486.1806, found 486.1796.

General Procedure for Preparation of Compound 8c,d

The *O*-nuceophile (0.783 mmol) was added to a solution of compound **3a** and DMAP (0.05 g, 0.392 mmol) in dry THF (2 mL). The mixture was irradiated in microwave at 60 °C for 60 min and 100 W. The solvent was evaporated, and the residue was purified over flash chromatography to give **8c** or **8d**.

(*S*)-4-Ethylphenyl 2-(*tert*-butoxycarbonylamino)-3-(1-tosyl-1*H*-imidazol-4-yl)propanoate (8c)

Oil (50%), ^{1}H NMR ($CDCl_3$) δ 7.96 (d, J = 1.3 Hz, 1H), 7.79 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 8.1 Hz, 3H), 6.87 (d, J = 8.4 Hz, 2H), 5.77 (d, J = 8.1 Hz, 1H), 4.77 (br s, 1H), 3.26–3.07 (m, 2H), 2.62 (q, J = 7.6 Hz, 2H), 2.42 (s, 3H), 1.43 (s, 9H), 1.22 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.6, 155.6, 148.6, 146.4, 142.0, 140.2, 136.8, 135.0, 130.6, 128.8,127.5, 121.2, 115.0, 80.1, 53.2, 30.5, 30.4, 28.4, 21.9, 15.7. HRMS (+ESI-TOF) *m/z* for $C_{26}H_{32}N_3O_6S$ $[M+H]^+$ calcd 514.2006, found 514.2025.

(*S*)-Naphthalen-2-yl 2-(tert-butoxycarbonylamino)-3-(1-tosyl-1*H*-imidazol-4-yl)propanoate (8d)

Oil (55%). ^{1}H NMR ($CDCl_3$) δ 8.00 (d, J = 1.2 Hz, 1H), 7.84–7.76 (m, 5H), 7.51–7.46 (m, 3H), 7.27 (d, J = 7.8 Hz, 2H), 7.18 (s, 1H), 7.12 (dd, J = 8.9, 2.3 Hz, 1H), 5.78 (d, J = 8.1 Hz, 1H), 4.86–4.83 (m, 1H), 3.33–3.12 (m, 2H), 2.37 (s, 3H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.6, 155.6, 148.3, 146.5, 140.2, 136.9, 135.0, 133.8, 131.7, 130.6, 129.6, 127.9, 127.5, 126.8, 126.0, 121.0, 118.6, 115.2, 80.3, 53.4, 30.5, 28.5, 21.9. HRMS (+ESI-TOF) *m/z* for $C_{28}H_{29}N_3O_6S$ $[M+H]^+$ calcd 536.1850, found 536.1850.

General Procedure for Preparation of Compound 8e,f

The mercapto nucleophile (1.0 eq.) was dissolved in THF and Et_3N (1.5 eq.). N^{α}-Boc-N^{im}-4-toluenesylfonyl-L-histidinylbenzotriazle **3a** (1.0 eq.) was added to the solution, and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure, then diluted with ethyl acetate. The organic layer was washed with 10% Na_2CO_3 solution, water and dried over anhydrous $MgSO_4$, filtered, and evaporated to give the desired compound.

(*S*)-*S*-Benzyl 2-((tert-butoxycarbonyl)amino)-3-(1-tosyl-1*H*-imidazol-4-yl)propanethioate (8e)

White microcrystals (80%); mp 60–61 °C; ^{1}H NMR ($CDCl_3$) δ 7.91 (s, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 7.8 Hz, 2H), 7.28–7.13 (m, 5H), 7.05 (s, 1H), 6.10 (d, J = 8.7 Hz, 1H), 4.70–4.57 (m, 1H), 3.97 (s, 2H), 3.09 (dd, J = 15.0, 6.0 Hz, 1H), 2.95 (dd, J = 13.2, 5.1 Hz, 1H), 2.40 (s, 3H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 201.0, 155.3, 146.4, 139.9, 136.8, 136.5, 134.8, 130.5, 128.8, 128.6, 127.4, 127.3, 115.0, 80.3, 59.7, 33.3, 30.0, 28.4, 21.8; Anal. calcd for $C_{25}H_{29}N_3O_5S_2$: C, 58.23; H, 5.67; N, 8.15; found: C, 58.11; H, 6.00; N, 8.54.

(*S*)–Methyl 2-((2-((tert-butoxycarbonyl)amino)-3-(1-tosyl-1*H*-imidazol-4-yl)propanoyl) thio)acetate (8f)

White microcrystals (78%); mp 110–111 °C; ^{1}H NMR ($CDCl_3$) δ 7.94 (d, J = 1.2 Hz, 1H), 7.81 (d, J = 8.1 Hz, 2H), 7.36 (dd, J = 8.1, 0.6 Hz, 2H), 7.10 (s, 1H), 6.10 (d, J = 8.7 Hz, 1H), 4.68–4.58 (m, 1H), 3.70 (s, 3H), 3.58 (d, J = 10.5 Hz, A part of AB system, 1H), 3.53 (d, J = 10.5 Hz, B part of AB system, 1H), 3.09 (dd, J = 14.7, 6.2 Hz, 1H), 2.97 (dd, J = 14.7, 4.5 Hz, 1H), 2.44 (s, 3H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 200.3, 169.1, 155.4, 155.3, 146.5, 139.6, 136.5, 134.9, 130.5, 127.5, 115.3, 80.5, 59.7, 52.8, 31.2, 29.9, 28.4, 21.8; Anal. calcd for $C_{21}H_{27}N_3O_7S_2$: C, 50.69; H, 5.47; N, 8.44; found: C, 51.04; H, 5.89; N, 8.08.

Procedure for Preparation of Compound 8g

To a solution of **3a** (0.07 g, 0.137 mmol) and malononitrile (0.02 g, 0.274 mmol) in dry THF (1 mL), diisopropylethylamine (0.026 mL, 0.151 mmol) was added. The mixture was irradiated in microwave at 60 °C for 30 min and 100 W. The solvent was evaporated, and the residue was purified by flash chromatography to give **8g**.

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(*S*)-*tert*-Butyl 4,4-dicyano-3-oxo-1-(1-tosyl-1*H*-imidazol-4-yl) butan-2-ylcarbamate (8g)

Yellow microcrystal (52%), mp 75–77 °C. ^{1}H NMR (CD_3OD-d_4) δ 8.05 (s, 1H), 7.84 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.23 (s, 1H), 4.61 (br s, 1H), 3.27–3.24 (m, 1H), 2.86–2.66 (m, 2H), 2.38 (s, 3H), 1.31 (s, 9H); ^{13}C NMR (CD_3OD) δ 194.2, 157.2, 148.1, 141.8, 138.0, 136.2, 131.8, 128.7, 121.7, 120.5, 116.5, 80.5, 55.5, 32.7, 28.8, 21.8. HRMS (+ESI-TOF) m/z for $C_{21}H_{23}N_5O_5S$ $[M+H]^+$ calcd 480.1320, found: 480.1312.

Procedure for Preparation of Compound 8h

Thiophenol (1.0 eq.) was dissolved in THF and Et_3N (1.5 eq.). N^{α}-Boc-N^{im}-4-benzyl-L-histidylbenzotriazole **3b** (1.0 eq.) was added to the solution, and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and then diluted with ethyl acetate. The organic layer was washed with 10% Na_2CO_3 solution, water and dried over anhydrous $MgSO_4$, filtered, and evaporated to give the desired compound.

(*S*)-*S*-Phenyl 3-(1-benzyl-1*H*-imidazol-4-yl)-2-(*tert*-butoxycarbonylamino)propanethioate (8h)

Yellow microcrystals (60%); mp 130–132 °C; ^{1}H NMR ($CDCl_3$) δ 7.55–7.51 (br s, 1H), 7.42–7.31 (m, 10H), 7.00 (s, 1H), 5.34 (br s, 2H), 4.74–4.62 (m, 1H), 4.02 (d, J = 6.3 Hz, 1H), 3.37–3.34 (m, 2H), 1.39 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 199.2, 169.7, 155.7, 134.8, 134.7, 133.2, 131.3, 129.6, 129.3, 129.1, 128.7, 127.4, 118.6, 80.4, 73.9, 53.0, 28.3, 18.9. HRMS (+ESI-TOF): m/z for $C_{24}H_{27}N_3O_3S$ $[M+H]^+$ calcd 438.1846, found 438.1850.

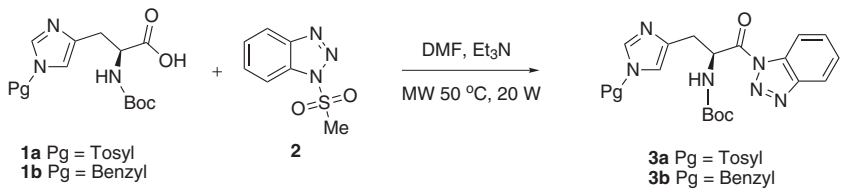
Results and Discussion

Histidine **1** protected by a tosyl/benzyl group on the imidazole nitrogen and a Boc group on the primary amine, on treatment with 1-(methylsulfonyl)-1*H*-benzotriazole (**2**) under microwave irradiation for 30 min, in the presence of Et_3N, gave N^{α}-Boc-N^{im}-protected-L-histidylbenzotriazole (**3a**,**b**) in 62–65% yield (Scheme 1), which we used for the preparation of diverse histidine derivatives.

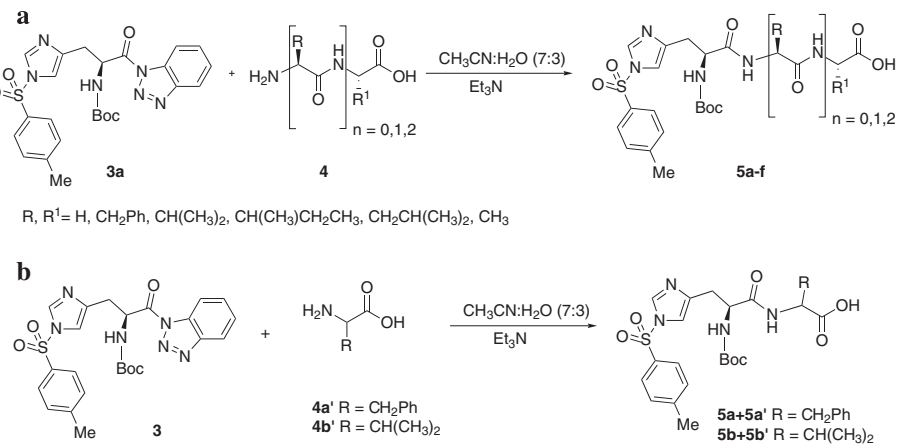
N^{α}-Boc-N^{im}-4-toluenesylfonyl-L-histidyl-benzotriazole (**3a**) couples readily with free amino acids (**4a–d**, **4a**+**4a′**, **4b**+**4b′**), dipeptide **4e**, and tripeptide **4f** at 20 °C in the presence of 1.2 eq. of Et_3N to give the corresponding peptides (**5a–f**, **5a**+**5a′**, **5b**+**5b′**) (Scheme 2a and b, Table 1).

The enantiomeric purity of compound **5a** was confirmed by HPLC analysis; thus, using a Chiralcel-OD column, **5a** showed a single peak (11.36 min), whereas the racemic mixture **5a**+**5a′** showed two peaks (10.16 and 10.60 min), one of which had nearly the same retention time as the single peak from **5a**. To justify the small difference in retention times, we ran the HPLC of the 1 : 1 ratio mixture of **5a** and **5a**+**5a′** and observed the two peaks at 10.48 and 11.01 min in a ratio of 1 : 3, respectively, which confirmed the previous analysis. We have also observed duplication of peaks in ^{13}C NMR of **5a**+**5a′** and **5b**+**5b′**, whereas in the case of **5a** and **5b**, extra peaks were not observed (Figure 1a and 1b). However, duplications of peaks in ^{13}C NMR were observed when N^{α}-Boc-N^{im}-4-benzyl-L-histidyl-benzotriazole **3b** was coupled with D-phenylalanine methyl ester (Scheme 3). This indicates that tosyl-protected histidine is less prone to racemization as compared with benzyl-protected histidine. Similar results were also observed in optical activity.

N^{α}-Boc-N^{im}-4-toluenesylfonyl-L-histidyl-benzotriazole **3a** and **3b** were reacted with different *N*-, *O*-, *S*-, and *C*-nucleophiles of biological importance in the presence of base to prepare the



Scheme 1. Synthesis of N^{α}-Boc-N^{im}-protected-L-histidylbenzotriazole **3a**,**b**.



Scheme 2. (a) Syntheses of histidine-containing di-, tri- and tetra-peptides **5a–f**. (b) Syntheses of histidine-containing dipeptides **5a**+**5a′**, **5b**+**5b′**.

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Table 1. Syntheses of histidine containing di-, tri-, and tetra-peptides **5a–f**, **5a**+**5a′**, **5b**+**5b′**

Product	Reactant **4**	Yield (%)	mp (°C) (lit. mp)	$[\alpha]_D^{23}$
5a	H-L-Phe-OH **4a**	79	133–134	−11
5a+**5a′**	H-DL-Phe-OH **4a′**	72	175–177	Racemic
5b	H-L-Val-OH **4c**	72	Oil	−44
5b+**5b′**	H-DL-Val-OH **4c′**	70	Oil	Racemic
5c	H-L-Leu-OH **4c**	89	121–123 (146–148) [21]	−15
5d	H-L-Ala-OH **4d**	92	151–153	−8
5e	H-Gly-L-Val-OH **4e**	68	113–115	−12
5f	H-Gly-Gly-L-Ile-OH **4f**	60	140–142	−9

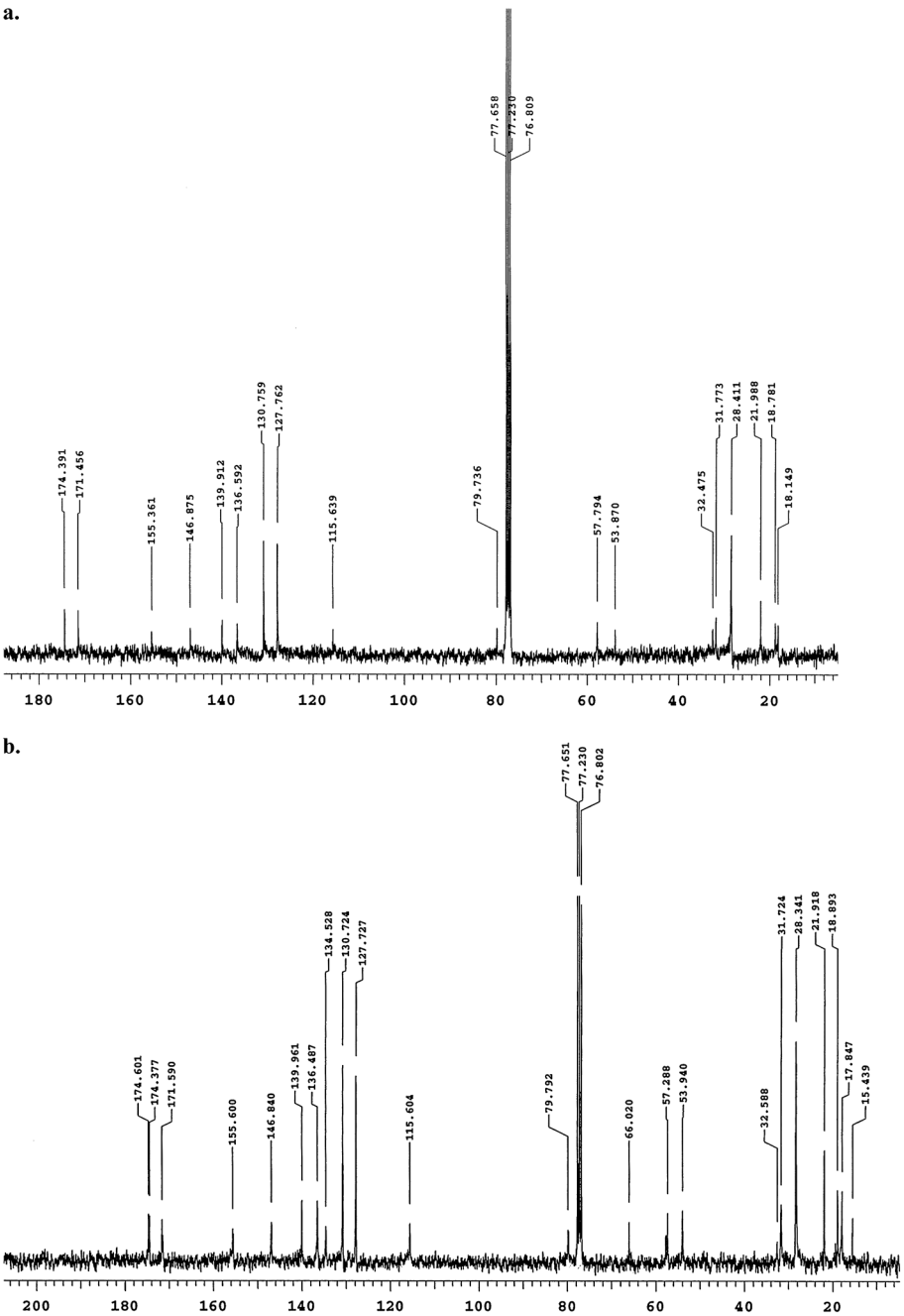

Figure 1. (a) ^{13}C NMR spectra of compound **5b**. (b) ^{13}C NMR spectra of compound **5b**+**5b′**.

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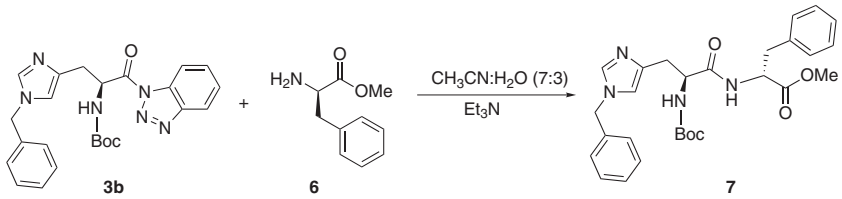
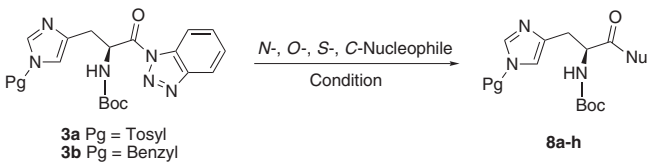


Scheme 3. Syntheses of histidine-containing dipeptide **7**.



Scheme 4. *N*-, *O*-, *S*-, and *C*-acylations with N^{α}-Boc-N^{im}-protected-L-histidylbenzotriazole **3a**,**b**.

Table 2. *N*-, *O*-, *S*-, and *C*-acylation with N^{α}-Boc-N^{im}-protected-L-histidyl-benzotriazole **8a–h**

Product	Reactant	Nucleophile	Conditions	Yield >(%)	Mp (°C)
8a	**3a**	*N*-(3-Aminopropyl) imidazole	A	92	64–66
8b	**3a**	2-Aminopyridine	B	91	87–88
8c	**3a**	Ethylphenol	C	50	Oil
8d	**3a**	*β*-Napthol	C	55	Oil
8e	**3a**	Benzylmercaptan	A	80	60–61
8f	**3a**	Methyl mercaptoacetate	A	78	110–111
8g	**3a**	Malononitrile	D	52	75–77
8h	**3b**	Thiophenol	A	60	130–132

A: THF, TEA, 2 h, 20 °C; B: DMF, 0.5 h, MW (50 °C, 20 W); C: THF, DMAP, 1 h MW (60 °C, 100 W); D: THF, DIPEA, 0.5 h, MW (60 °C, 100 W).

corresponding pure derivatives **8a–h** in good yields (Scheme 4, Table 2). HPLC analysis of these conjugates shows racemization in case of **8h**, which again indicates that benzyl-protected histidine is more prone to racemization than tosyl-protected histidine.

Conclusion

In conclusion, N^{α}-Boc-N^{im}-4-toluenesylfonyl-L-histidyl-benzotriazole **3a** is a novel, crystalline coupling reagent that (i) is sufficiently reactive to form amide and ester bonds at ambient temperature, (ii) provides good to excellent yields without detectable racemization, and (iii) is inexpensive to prepare. Hence, N^{α}-Boc-N^{im}-4-toluenesylfonyl-L-histidyl-benzotriazole **3a** offers an efficient preparative route to prepare *N*-protected histidine-containing peptides and conjugates with *N*-, *O*-, *S*-, and *C*-nucleophiles in synthetically useful yields without racemization.

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