Int J Pept Res Ther (2014) 20:377–384
DOI 10.1007/s10989-014-9397-9

Facile Synthesis of N^{α}-Protected Amino/Peptide Hydroxamic Acids Mediated by COMU

Nageswara Rao Panguluri · Basavaprabhu · Vommina V. Sureshbabu

Accepted: 29 January 2014 / Published online: 19 February 2014

Abstract One-pot preparation of N^{α}-protected amino/peptide hydroxamic acids from corresponding carboxylic acids is described using uronium-type coupling reagent COMU. The present protocol is simple and mild conditions are used. Thus the resulting hydroxamic acids are obtained in good yields without racemization.

Keywords N^{α}-protected amino/peptide acids · COMU · Hydroxamic acids

Introduction

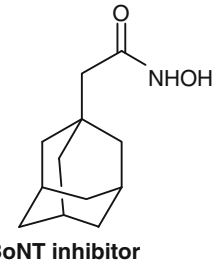
Hydroxamic acids have received much attention as biologically active compounds (Miller 1989; Kwak et al. 2011) and are well known to bind with hard metal ions (Jung 2001; Dhungana et al. 2004). Molecules containing this functional group serve as botulinum neurotoxin (BoNT), tyrosinase and histone deacetylase (HDAC) inhibitors. Adamantane hydroxamates were found to be BoNT inhibitors, thus serve as valuable therapeutic tool for the treatment of strabismus, migraines and even facial wrinkles (Silhar et al. 2013) (Fig. 1). Hydroxycinnamoyl phenylalanyl/prolyl hydroxamic acid derivatives have emerged as tyrosinase inhibitors (Kwak et al. 2013) and good antioxidants, which are useful in the fields of medicine, agriculture and cosmetic industry (Fig. 2). HDAC inhibitors were proven to fight against cancer and other human afflictions including psychiatric, metabolic and infectious diseases (Librizzi et al. 2012; Wagner et al. 2013) (Fig. 3).

Hydroxamic acids are the essential precursors for the Lossen rearrangement to prepare carbamates, thiocarbamates and ureas (Yoganathan and Miller 2013; Vasantha et al. 2010; Yadav et al. 2012; Narendra et al. 2009). The reported methods for the preparation of hydroxamic acids include the reaction of *O*/*N*-protected hydroxylamines with activated carboxylic acids (Tamaki et al. 1993; Altenburger et al. 1992; Ando and Tsumaki 1983; Lee and Miller 1983; Anilkumar et al. 2000). Coupling of carboxylic acids with hydroxylamine in presence of cyanuric chloride (TCT) (Giacomelli et al. 2003) and cyclic phosphonic anhydride (PPAA or T3P) (Ech-Chahad et al. 2005; Basavaprabhu et al. 2013) require longer duration (6–12 h) for completion. A one-step approach using ethyl chloroformate as carboxylic activator is limited as its vapor is irritant to skin and eyes (Reddy et al. 2000). Vasanthakumar and Sureshbabu (2003) reported MgO mediated synthesis of N^{α}-Fmoc protected amino acid hydroxamates from acid chlorides but due to instability of the acid chloride, the protocol could not be extended to *N*-Boc/Cbz-protected amino acids. Competent methods for the synthesis of *O*-alkyl hydroxamic acids include the treatment of carboxylic acids with the coupling agent (phosphoric acid diethyl ester 2-phenyl-benzimidazol-1-yl ester) (Kokare et al. 2007) and the reaction of *N*-acyloxazolidinones with hydroxylamine using samarium triflate (Sibi et al. 2002). In case of *O*-alkyl hydroxamic acids, deprotection is necessary after acylation, which limits their synthetic utility in multistep synthesis. In order to develop an alternative to the existing

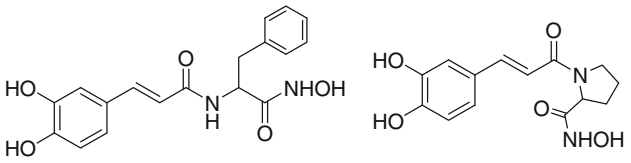
Electronic supplementary material The online version of this article (doi:10.1007/s10989-014-9397-9) contains supplementary material, which is available to authorized users.

N. R. Panguluri · Basavaprabhu · V. V. Sureshbabu (✉)
No. 109, Peptide Research Laboratory, Department of Studies in Chemistry, Central College Campus, Bangalore University, Dr. B. R. Ambedkar Veedhi, Bangalore 560 001, India
e-mail: sureshbabuvommina@rediffmail.com; hariccb@hotmail.com; hariccb@gmail.com

Fig. 1 BoNT inhibitor

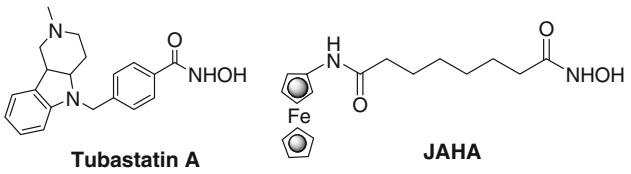


BoNT inhibitor



Tyrosinase inhibitors

Fig. 2 Tyrosinase inhibitors



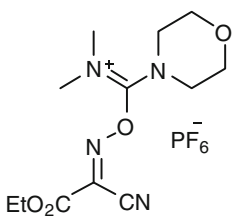
HDAC inhibitors

Fig. 3 HDAC inhibitors

protocols 1-[(1-(cyano-2-ethoxy-2-oxoethylidenaminooxy)-dimethylamino-morpholinomethylene)] methaneaminium hexafluorophosphate (COMU, Fig. 4) (El-Faham and Albericio 2010, 2011) as an acid activator has been envisaged for the one-pot conversion of N^{α}-protected amino/peptide acids to hydroxamic acids.

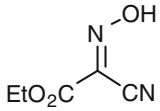
El-Faham et al. (2009) investigated the third generation uronium-type coupling reagent COMU, based on Oxyma [ethyl 2-cyano-2-(hydroxyimino)acetate, Fig. 5]. Due to the presence of morpholino group in conjugation with an oxime derivative, COMU shows a less hazardous safety profile and offers better results than benzotriazole based reagents such as *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-b]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) and *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU). It shows excellent solubility and remarkable stability in DMF and NMP, which makes it ideally suited for solid phase peptide synthesis (Malik et al. 2010; Chantell et al. 2012; Hjorringgaard et al. 2012). It is equally prominent for solution phase synthesis since by-products formed by COMU are water soluble and can be separated by simple extraction. Calorimetry assays (DSC and ARC data) indicate that COMU is non-explosive. COMU has been employed as an excellent choice of coupling reagent in solution phase and solid phase peptide synthesis. Tyrrell et al. (2011) synthesized Weinreb amides employing COMU as a coupling reagent. Zhang et al. (2011) used the similar protocol for the synthesis of rubescesin S. Synthesis of *O*-acyl isodipeptides had been reported by our group using COMU (Samarasimhareddy et al. 2012). The combination of microwave irradiation with COMU for the synthesis of Aib-enkephalin pentapeptide (Subiros-Funosas et al. 2009) and cyclic RGD peptides (Yamada et al. 2012) were reported. Hjelmgaard et al. (2011a, b) reported arylopeptoids with both free acids and free amides at the C-terminus and *N*-methylated cyclic peptides using COMU. The use of COMU and Oxyma offer high-yield couplings for the synthesis of *N*-methylated cyclic peptide (NMe-IB-01212) (Marcucci et al. 2012). Deprotected, hydrophobic peptide cross-linked polystyrene nanoparticles were synthesized in inverse miniemulsion using COMU (Maier et al. 2011).

Fig. 4 COMU



COMU

Fig. 5 Oxyma



Oxyma

Experimental

Materials and Methods

All solvents were freshly distilled before use. Amino acids were used as received from Sigma-Aldrich Company. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 300 and 75 MHz respectively, with DMSO-d_6 as an internal standard. Mass spectra were recorded using high resolution mass spectrometer (HRMS) Q-T of mass spectrometer. Melting points were measured with Veego (Model: VMP-DS) melting point apparatus and the samples were dried under vacuum before analysis. All the reactions were monitored using TLCs with precoated silica gel plates purchased from Merck. Chiral HPLC analysis of isomers was carried out by Agilent 1100 series, Lux 5u Amylose-2, 250 × 4.60 mm.

General Procedure for the Synthesis of N^{α}-Urethane Protected Amino/Peptide Hydroxamic Acids 2, 4

To a solution of N^{α}-urethane protected amino/peptide acid (1.0 mmol) in DMF, DIPEA (1.0 mmol), COMU (1.1 mmol) and NH_2OH (1.3 mmol) were added at 0 °C. After completion of reaction as monitored by TLC (1–1.5 h), the product was extracted into ethyl acetate. The organic layer was washed with water (2 × 10 mL), saturated NaCl solution (2 × 10 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to afford the product.

Spectral Data of Compounds 2, 4

*N^{α}-Cbz-Val-NHOH (**2a**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.20 (d, 6H, J = 6.6 Hz, CH(**CH_3)$_2$**), 2.58 (m, 1H, **CH**$(CH_3)_2$), 4.30 (d, 1H, J = 5.8 Hz, **CH**CH$(CH_3)_2$), 5.14 (s, 2H, **$CH_2C_6H_5$**), 5.72 (s, 1H, NH Cbz), 7.16–7.28 (m, 5H, C_6H_5), 8.44 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz,.DMSO-d_6): δ (ppm) = 16.2, 33.0, 56.3, 61.6, 127.1, 128.6, 129.4, 140.8, 158.0, 164.6. HRMS *m/z* calcd for $C_{13}H_{18}N_2O_4$: 289.1164 $[M+Na]^+$, found: 289.1160 $[M+Na]^+$.

*N^{α}-Cbz-Ser-NHOH (**2b**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 3.20 (s, 1H, CH_2**OH**), 3.75–3.77 (m, 2H, **CH_2**OH), 4.18 (m, 1H, **CH**CH_2OH), 5.01 (s, 2H, **$CH_2C_6H_5$**), 5.42 (s, 1H, NH Cbz), 7.21–7.26 (m, 5H, C_6H_5), 8.22 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 51.6, 63.8, 66.0, 127.4, 127.9, 128.8, 140.8, 162.8, 176.7. HRMS *m/z* calcd for $C_{11}H_{14}N_2O_5$: 277.08 $[M+Na]^+$, found : 277.0805 $[M+Na]^+$.

*N^{α}-Cbz-Ala-NHOH (**2c**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.44 (d, 3H, J = 5.8 Hz, CH**CH_3**), 4.14 (m, 1H, **CH**CH_3), 5.16 (s, 2H, **$CH_2C_6H_5$**), 6.22 (s, 1H, NH Cbz), 7.20–7.36 (m, 5H, C_6H_5), 8.04 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 19.8, 42.6, 74.4, 128.4, 128.9, 129.8, 148.6, 168.8, 184.6. HRMS *m/z* calcd for $C_{11}H_{14}N_2O_4$: 261.0851 $[M+Na]^+$, found: 261.0848 $[M+Na]^+$.

*N^{α}-Cbz-Thr-NHOH (**2d**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 0.98 (d, 3H, J = 5.8 Hz, CHCH(OH)**CH_3**), 3.52 (s, 1H, CHCH(**OH**)CH_3), 3.85 (m, 1H, CH**CH**(OH)CH_3), 4.28 (d, 1H, J = 6.1 Hz, **CH**CH(OH)CH_3), 5.48 (s, 2H, **$CH_2C_6H_5$**), 5.96 (s, 1H, NH Cbz), 7.12–7.26 (m, 5H, C_6H_5), 8.56 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 19.4, 54.4, 68.4, 69.6, 125.2, 125.7, 127.0, 143.2, 158.2, 162.2. HRMS *m/z* calcd for $C_{12}H_{16}N_2O_5$: 291.0957 $[M+Na]^+$, found: 291.0962 $[M+Na]^+$.

*N^{α}-Boc-Pro-NHOH (**2e**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.32 (s, 9H, C$(CH_3)_3$), 1.80–1.89 (m, 4H, **–N$CH_2$$CH_2$$CH_2$–**), 3.24–3.44 (m, 2H, **–NCH_2**CH_2CH_2–), 4.16 (m, 1H, –CH_2**CH**N–), 8.22 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 26.8, 32.6, 34.0, 49.6, 66.8, 77.6, 161.0, 176.0. HRMS *m/z* calcd for $C_{10}H_{18}N_2O_4$: 253.1164 $[M+Na]^+$, found: 253.1167 $[M+Na]^+$.

*N^{α}-Boc-Asp(OBzl)-NHOH (**2f**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.34 (s, 9H, C$(CH_3)_3$), 3.21 (d, 2H, J = 5.8 Hz, CH**CH_2**COOBzl), 4.42 (m, 1H, **CH**CH_2COOBzl), 5.04 (s, 2H, **$CH_2C_6H_5$**), 5.72 (s, 1H, NH Boc), 7.22–7.32 (m, 5H, C_6H_5), 8.22 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 26.8, 36.6, 47.6, 67.8, 77.6, 125.4, 125.8, 127.6, 142.4, 156.4, 167.8, 176.2. HRMS *m/z* calcd for $C_{16}H_{22}N_2O_6$: 361.1376 $[M+Na]^+$, found: 361.1379 $[M+Na]^+$.

*N^{α}-Boc-Phe-NHOH (**2g**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.38 (s, 9H, C$(CH_3)_3$), 2.80–2.92 (d, 2H, J = 5.8 Hz, **$CH_2C_6H_5$**), 3.58 (m, 1H, **CH**$CH_2C_6H_5$), 5.92 (s, 1H, NH Boc), 7.22–7.38 (m, 5H, C_6H_5), 8.12 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 27.4, 34.8, 55.4, 75.4, 124.0, 126.0, 127.6, 141.4, 166.8, 171.2. HRMS *m/z* calcd for $C_{14}H_{20}N_2O_4$: 303.1321 $[M+Na]^+$, found: 303.1326 $[M+Na]^+$.

*N^{α}-Boc-Leu-NHOH (**2h**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 0.92 (d, 6H, J = 6.8 Hz, $CHCH_2CH$(**CH_3)$_2$**), 1.43 (s, 9H, C$(CH_3)_3$), 1.72 (m, 2H, CH**CH_2**$CH(CH_3)_2$), 1.78 (m, 1H, $CHCH_2$ **CH**$(CH_3)_2$), 4.58 (m, 1H, **CH**$CH_2CH(CH_3)_2$), 5.96 (s, 1H, NH Boc), 8.34 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 21.6, 22.2, 27.4, 41.4, 48.4, 75.5, 154.0, 164.6. HRMS *m/z* calcd for $C_{11}H_{22}N_2O_4$: 269.1477 $[M+Na]^+$, found: 269.1482 $[M+Na]^+$.

*N^{α}-Fmoc-Tyr-NHOH (**2i**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 3.12–3.26 (d, 2H, J = 5.8 Hz, CH**CH_2**C_6H_4OH), 4.08 (t, 1H, J = 6.6 Hz, CH Fmoc), 4.26 (m, 1H, **CH**$CH_2C_6H_4OH$), 4.38 (d, 2H,

$J = 6.6$ Hz, CH_2 Fmoc), 5.26 (s, 1H, $CH_2C_6H_4\mathbf{OH}$), 5.66 (brs, 1H, NH Fmoc), 6.46 (d, 2H, $J = 6.2$ Hz, $CH_2\mathbf{C_6H_4}$ OH), 6.74 (d, 2H, $J = 6.2$ Hz, $CH_2\mathbf{C_6H_4}$OH), 7.16–7.94 (m, 8H, Fmoc), 7.86 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 36.8, 46.6, 51.8, 67.8, 115.6, 126.6, 128.6, 128.8, 129.0, 129.6, 131.8, 141.8, 143.4, 155.2, 156.8, 168.2. HRMS *m/z* calcd for $C_{24}H_{22}N_2O_5$: 441.1426 $[M+Na]^+$, found: 441.1422 $[M+Na]^+$.

*N^{α}-Fmoc-Ile-NHOH (**2j**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 0.83–0.87 (m, 6H, CHCH(**CH_3**)CH_2**CH_3**), 1.20 (m, 2H, CHCH(CH_3) **CH_2**CH_3), 2.06 (m, 1H, CH**CH**(CH_3)CH_2CH_3), 4.02 (m, 1H, **CH**CH(CH_3)CH_2CH_3), 4.14 (t, 1H, $J = 6.0$ Hz, CH Fmoc), 4.26 (d, 2H, $J = 6.0$ Hz, CH_2 Fmoc), 5.30 (s, 1H, NH Fmoc), 7.19–7.71 (m, 8H, Fmoc), 8.48 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 11.2, 13.9, 25.8, 35.6, 45.0, 53.6, 68.4, 127.4, 129.2, 129.4, 129.8, 141.6, 143.8, 157.4, 167.8. HRMS *m/z* calcd for $C_{21}H_{24}N_2O_4$: 391.1634 $[M+Na]^+$, found: 391.1639 $[M+Na]^+$.

*N^{α}-Fmoc-Glu(O^{t}Bu)-NHOH (**2k**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.37 (s, 9H, $C(CH_3)_3$), 1.77–1.80 (m, 2H, –NCH**CH_2**CH_2–), 2.17–2.23 (m, 2H, –$NCHCH_2$**CH_2**–), 3.84 (t, 1H, $J = 6.3$ Hz, CH Fmoc) 4.18–4.36 (m, 3H, CH_2, Fmoc & –N**CH**CH_2CH_2–), 6.34 (s, 1H, NH Fmoc), 7.30–7.89 (m, 8H, Fmoc), 8.83 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 26.8, 27.4, 27.8, 43.0, 52.2, 65.6, 79.4, 127.4, 129.2, 129.4, 129.8, 139.8, 146.2, 155.5, 166.6, 174.2. HRMS *m/z* calcd for $C_{24}H_{28}N_2O_6$: 463.1845 $[M+Na]^+$, found: 463.1851 $[M+Na]^+$.

*N^{α}-Fmoc-Phg-NHOH (**2l**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 4.12 (t, 1H, $J = 6.0$ Hz, CH, Fmoc), 4.20 (d, 2H, $J = 6.0$ Hz, CH_2, Fmoc), 4.68 (s, 1H, **CH**C_6H_5), 5.17 (s, 1H, NH Fmoc), 7.18–7.69 (m, 13H, Fmoc & Ph), 8.75 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 48.2, 56.2, 68.2, 124.8, 125.5, 126.2, 126.4, 126.8, 127.3, 127.7, 135.8, 141.6, 142.8, 155.6, 169.2. HRMS *m/z* calcd for $C_{23}H_{20}N_2O_4$: 411.1321 $[M+Na]^+$, found: 411.1324 $[M+Na]^+$.

*N^{α}-Fmoc-Ala-Ile-NHOH (**4a**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 0.86–1.02 (m, 6H, $2CH_3$, Ile), 1.28 (m, 2H, CHCH(CH_3)**CH_2**CH_3), 1.32 (d, 3H, $J = 5.6$ Hz, CH**CH_3**, Ala), 2.14 (m, 1H, CH**CH**(CH_3)CH_2CH_3), 4.18 (t, 1H, $J = 6.4$ Hz, CH, Fmoc), 4.41–4.58 (m, 3H **CH**CH(CH_3)CH_2CH_3, CH_2 Fmoc), 4.68 (m, 1H, **CH**CH_3), 5.34 (brs, 1H, NH Fmoc), 6.51 (s, 1H, NH amide), 7.22–7.86 (m, 8H, Fmoc), 7.96 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 11.2, 14.4, 18.2, 23.8, 34.6, 46.8, 52.6, 53.8, 67.8, 125.6, 127.4, 128.6, 128.8, 141.8, 142.8, 154.0, 162.2, 174.6 . HRMS *m/z* calcd for $C_{24}H_{29}N_3O_5$: 462.2005 $[M+Na]^+$, found: 462.2003 $[M+Na]^+$.

*Boc-(L)-Phe-(D)-Phg-NHOH (**4b**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.30 (s, 9H, $C(CH_3)_3$), 3.36 (d, 2H, $J = 5.9$ Hz, **CH_2**C_6H_5), 4.06 (m, 1H, **CH**$CH_2C_6H_5$), 4.34 (s, 1H, **CH**C_6H_5), 5.32 (d, 1H, $J = 8.2$ Hz, NH Boc), 7.06 (d, 1H, $J = 8.2$ Hz, NH amide), 7.18–7.36 (m, 10H, $2C_6H_5$), 8.68 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 27.9, 37.2, 53.6, 56.0, 78.1, 126.1, 126.6, 127.5, 127.8, 128.1, 129.3, 138.0, 138.9, 155.5, 166.2, 171.5. HRMS *m/z* calcd for $C_{22}H_{27}N_3O_5$: 436.1848 $[M+Na]^+$, found: 436.1846 $[M+Na]^+$.

*Boc-(L)-Phe-(L)-Phg-NHOH (**4c**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.32 (s, 9H, $C(CH_3)_3$), 3.38 (d, 2H, $J = 5.9$ Hz, **CH_2**C_6H_5), 4.02 (m, 1H, **CH**$CH_2C_6H_5$), 4.31 (s, 1H, **CH**C_6H_5), 5.37 (d, 1H, $J = 8.2$ Hz, NH Boc), 7.02 (d, 1H, $J = 8.2$ Hz, NH amide), 7.20–7.36 (m, 10H, $2C_6H_5$), 8.74 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 27.7, 37.2, 53.5, 56.3, 78.3, 126.4, 126.6, 127.5, 127.8, 128.1, 129.3, 138.2, 138.9, 155.4, 166.3, 171.6. HRMS *m/z* calcd for $C_{22}H_{27}N_3O$: 436.1848 $[M+Na]^+$, found: 436.1845 $[M+Na]^+$.

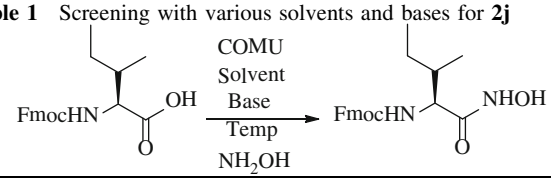
*Boc-Val-Leu-NHOH (**4d**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 0.85-0.90 (m, 12H, $4CH_3$, Val, Ile), 1.35 (s, 9H, $C(CH_3)_3$), 1.43–1.57 (m, 2H, CH**CH_2**$CH(CH_3)_2$, 1.90 (m, 1H, $CHCH_2$**CH**$(CH_3)_2$), 3.76 (t, 1H, $J = 7.2$ Hz, **CH**$CH_2CH(CH_3)_2$), 4.23 (m, 1H, **CH**$CH(CH_3)_2$), 6.76 (d, 1H, $J = 9.1$ Hz, NH Boc), 7.78 (d, 1H, $J = 8.3$ Hz, NH amide), 8.84 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 16.1, 22.5, 24.8, 28.0, 33.1, 44.5, 48.9, 58.2, 78.0, 156.1, 168.2, 170.5. HRMS *m/z* calcd for $C_{16}H_{31}N_3O_5$: 368.2161 $[M+Na]^+$, found: 368.2164 $[M+Na]^+$.

*N^{α}-Cbz-Phe-Ala-NHOH (**4e**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 1.26 (d, 3H, $J = 6.4$ Hz, CH**CH3**), 2.86–3.14 (d, 2H, $J = 5.8$ Hz, CH**CH_2**C_6H_5), 4.50 (m, 1H, **CH**$CH_2C_6H_5$), 4.66 (m, 1H, **CH**CH_3), 5.16 (s, 2H, CH_2 Cbz), 6.2 (brs, 1H, NH Cbz), 6.8 (s, 1H, NH amide), 7.04–7.26 (m, 10H, $2C_6H_5$), 7.62 (s, 1H, **NH**OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ

Table 1 Screening with various solvents and bases for **2j**



Entry	Solvent	Base	Yield (%)[c]
1	CH_3CN	TEA[a]	70
2	DMF	TEA[b]	85
3	DMF	NMM[a]	80
4	DMF	DIPEA[a]	94
5	**DMF**	**DIPEA**[b]	**96**
6	THF	DIPEA[a]	75
7	THF	TEA[b]	70

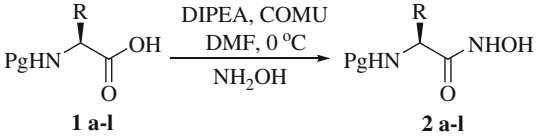
Bold entries indicate that the reaction gave maximum yield at that condition

[a] 2.0 eq of base was used

[b] 1.0 eq of base was used

[c] Reaction was monitored for 1.0–1.5 h at 0 °C using COMU

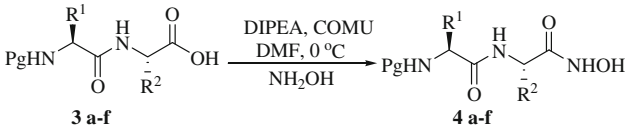
(ppm) = 16.4, 32.4, 47.4, 53.8, 63.4, 126.2, 127.4, 127.8, 127.9, 128.4, 129.2, 136.4, 140.4, 153.0, 167.4, 168.6. HRMS *m/z* calcd for $C_{20}H_{23}N_3O_5$: 408.1535 $[M+Na]^+$, found: 408.1539 $[M+Na]^+$.



1 a-l **2 a-l**

Pg= Fmoc/Boc/Cbz, R=amino acid side chain

Scheme 1 Synthesis of amino hydroxamic acids **2**



3 a-f **4 a-f**

Pg= Fmoc/Boc/Cbz, R^1,R^2=amino acid side chain

Scheme 2 Synthesis of peptide hydroxamic acids **4**

*N^{α}-Cbz-Ala-Ile-NHOH (**4f**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 0.64–0.98 (m, 6H, $2CH_3$, Ile), 1.14 (d, 3H, J = 5.8 Hz, CH**CH_3**), 1.42 (m, 2H, $CHCH(CH_3)$**CH_2**CH_3), 1.64 (m, 1H, **CH**$CH(CH_3)$-CH_2CH_3), 4.02 (m, 1H, **CH**CH_3), 4.18 (m, 1H, **CH**$CH(CH_3)CH_2CH_3$), 4.98 (s, 2H, **CH_2**C_6H_5), 6.92 (s, 1H, NH Cbz), 7.26–7.34 (m, 5H, C_6H_5), 7.54 (d, 1H, J = 8.3 Hz, NH amide), 8.94 (s, 1H, **NH**OH); ^{13}C NMR

Table 2 List of amino hydroxamic acids **2**

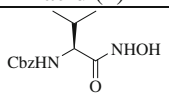
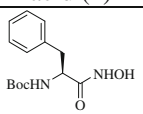
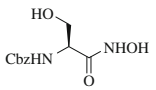
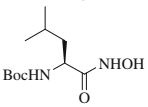
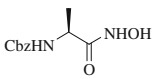
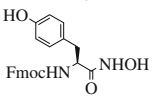
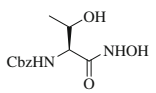
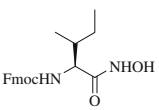
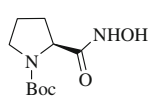
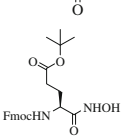
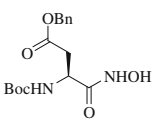
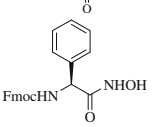
S. No	Hydroxamic acid (2)	m.p. (°C)	Yield (%)	S. No	Hydroxamic acid (2)	m.p. (°C)	Yield (%)
a		Oil	94	g		oil	96
b		Oil	91	h		gum	92
c		Oil	93	i		gum	92
d		Oil	90	j		195	96
e		Gum	95	k		96	94
f		82-83	90	l		132	95

Table 3 List of peptide hydroxamic acids **4**

Entry	Pg	R^1	R^2	Yield (%)	% of epimerization
4a	Fmoc	CH_3	$CHCH_3CH_2CH_3$	91	
4b	Boc	(L)-$CH_2C_6H_5$	(D)-C_6H_5	89	1.0
4c	Boc	(L)-$CH_2C_6H_5$	(L)-C_6H_5	90	0.8
4d	Boc	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	89	–
4e	Cbz	$CH_2C_6H_5$	CH_3	86	–
4f	Cbz	CH_3	$CHCH_3CH_2CH_3$	87	–

(75 MHz, DMSO-d_6): δ (ppm) = 9.6, 14.2, 16.8, 23.8, 35.8, 51.2, 53.2, 68.4, 125.2, 125.7, 127.2, 143.6, 153.2, 164.2, 176.2. HRMS *m/z* calcd for $C_{17}H_{25}N_3O_5$: 374.1692 $[M+Na]^+$, found: 374.1695 $[M+Na]^+$.

Results and Discussion

Attracted by the wider utility, we investigated the use of COMU for the one pot conversion of carboxylic acid to hydroxamic acid. In a typical study, to a solution of Fmoc-Ile-OH (**1j**) in CH_3CN, TEA (2.0 eq), COMU (1.1 eq) were added at 0 °C. Then the activated carboxylic acid was treated with a neutralized solution of $NH_2OH{\cdot}HCl$ (prepared by neutralizing $NH_2OH{\cdot}HCl$ with methanolic KOH) to obtain corresponding hydroxamic acid (70 %). In order to improve the yield and to optimize the reaction condition, various solvents and bases were screened. Delightly, DMF and DIPEA found to be efficient reaction condition to afford the desired hydroxamates in 96 % at 0 °C (Table 1, entry 5). Compared to existing protocols where 2–3 eq base was required, in the present protocol one equivalent of base was sufficient with COMU (El-Faham et al. 2009), as the polar morpholino group in the reagent contributes as internal base. The product Fmoc-Ile-NHOH (**2j**) was extracted into ethyl acetate and isolated after simple workup. One-pot synthesis of hydroxamic acids using COMU is expedient and averts the ex-situ activation of carboxylic acid processes (acid halides, esters etc.). Unlike one-pot preparation of hydroxamic acids using TCT (Giacomelli et al. 2003) and PPAA (Ech-Chahad et al. 2005) it proceeds in shorter duration (1–1.5 h).

Interestingly, when N^{α}-protected Ser, Thr and Tyr were subjected to hydroxyamidation under optimized conditions, these amino acids afforded corresponding hydroxamates in good yields without affecting free –OH (**2b, 2d, 2i**). It is also endurable for the protecting groups like Fmoc/Boc/Cbz as well as bifunctional amino acids. The generality of the protocol was demonstrated for the synthesis of a series of N^{α}-urethane protected amino hydroxamic acids from corresponding carboxylic acids (Scheme 1, Table 2).

The racemization study of the hydroxamates prepared (Fmoc-L and D-Phg-NHOH, **2l** and **2l***) using above optimized conditions was undertaken as model compounds and they showed peaks at R_t = 12.03 min (**2l**) and R_t = 17.10 min (**2l***) respectively. Also, intentionally prepared equimolar mixture of L and D-Phg-NHOH exhibited distinct peaks at R_t = 12.55 and 17.60 min (**2l** and **2l***). These observations inferred that the protocol is free from racemization and the hydroxamates were obtained as optically pure isomers (method: n-hexane: 2-propanol (7:3); flow rate: 0.5 ml/min, 30 min).

Subsequently the similar protocol was exemplified for the synthesis of peptide hydroxamic acids. Under the optimized reaction conditions, Fmoc-Ala-Ile-OH **4a** was treated with DIPEA, COMU and NH_2OH in DMF, the formation of corresponding peptide hydroxamic acid was observed within 1.5 h. Even though literature shows that dipeptides in general tend to racemize much more than amino acids, HPLC analysis of L,D and L,L-*N*-(Boc)phenylalanyl-phenylglycine hydroxamates **4b** and **4c** prepared from the above protocol showed that the dipetide hydroxamic acids were of >99 % diastereomeric ratio. This indicates that no racemization has occurred at the α-centre during the hydroxamates synthesis. Employing this protocol **4d, 4e** and **4f** were also obtained in good yields. (Scheme 2, Table 3).

Conclusion

In outline, we have developed one-pot conversion of N^{α}-protected amino/peptide acids to hydroxamic acids employing COMU as an acid activator and the protocol is racemization free as evidenced by HPLC analysis. Unlike other protocols for the preparation of hydroxamic acid, one equivalent of base is sufficient as the morpholino group in the reagent itself serves as internal base. In the present protocol, the products were obtained in short duration, the byproducts produced were all water soluble which makes isolation of the products with high purity easier. The reaction can be monitored visually by color change during the reaction and is applicable for the synthesis of simple, bifunctional and sterically hindered α-amino/peptide hydroxamic acids.

HPLC chromatographs of **2l**, **2l*** and mixture of **2l** and **2l*** and NMR (^1H, ^{13}C) spectra of **2**, **4** were available in the supporting information.

Acknowledgments We thank Department of Science and Technology (DST) (Grant No. SR/S1/OC-52/2011), New Delhi, India for financial assistance.

Conflict of interest Nageswara Rao Panguluri, Basavaprabhu, Vommina V. Sureshbabu declare that they have no conflict of interest.

Statement of informed consent/Human and animal rights Authors declare that there is no informed consent in the article. This article does not contain any studies with human or animal subjects performed by any of the authors.

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