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
© Springer Science+Business Media New York 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 **Fig. 2** Tyrosinase inhibitors **Fig. 3** HDAC inhibitors

protocols 1-[(1-(cyano-2-ethoxy-2-oxoethylidenaminoxy)-dimethylamino-morpholinomethylene)] methanaminium hexafluorophosphate (COMU, Fig. 4) (El-Faham and Albericio 2010, 2011) as an acid activator has been envisaged for the one-pot conversion of N^z -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 -methylme-

Fig. 4 COMU **Fig. 5** Oxyma

thanaminium 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 rubescensin 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).

Experimental

Materials and Methods

All solvents were freshly distilled before use. Amino acids were used as received from Sigma-Aldrich Company. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX 300 and 75 MHz respectively, with $\text{DMSO-}d_6$ 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 NH₂OH (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 Na₂SO₄. The solvent was removed under reduced pressure to afford the product.

Spectral Data of Compounds 2, 4

N^α-Cbz-Val-NHOH (2a)

¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 1.20 (d, 6H, *J* = 6.6 Hz, CH(CH₃)₂), 2.58 (m, 1H, CH(CH₃)₂), 4.30 (d, 1H, *J* = 5.8 Hz, CHCH(CH₃)₂), 5.14 (s, 2H, CH₂C₆H₅), 5.72 (s, 1H, NH Cbz), 7.16–7.28 (m, 5H, C₆H₅), 8.44 (s, 1H, NHOH); ¹³C 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 C₁₃H₁₈N₂O₄: 289.1164 [M+Na]⁺, found: 289.1160 [M+Na]⁺.

N^α-Cbz-Ser-NHOH (2b)

¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 3.20 (s, 1H, CH₂OH), 3.75–3.77 (m, 2H, CH₂OH), 4.18 (m, 1H, CHCH₂OH), 5.01 (s, 2H, CH₂C₆H₅), 5.42 (s, 1H, NH Cbz), 7.21–7.26 (m, 5H, C₆H₅), 8.22 (s, 1H, NHOH); ¹³C 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 C₁₁H₁₄N₂O₅: 277.08 [M+Na]⁺, found : 277.0805 [M+Na]⁺.

N^α-Cbz-Ala-NHOH (2c)

¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 1.44 (d, 3H, *J* = 5.8 Hz, CHCH₃), 4.14 (m, 1H, CHCH₃), 5.16 (s, 2H, CH₂C₆H₅), 6.22 (s, 1H, NH Cbz), 7.20–7.36 (m, 5H, C₆H₅), 8.04 (s, 1H, NHOH); ¹³C 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 C₁₁H₁₄N₂O₄: 261.0851 [M+Na]⁺, found: 261.0848 [M+Na]⁺.

N^α-Cbz-Thr-NHOH (2d)

¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 0.98 (d, 3H, *J* = 5.8 Hz, CHCH(OH)CH₃), 3.52 (s, 1H, CHCH(OH)CH₃), 3.85 (m, 1H, CHCH(OH)CH₃), 4.28 (d, 1H, *J* = 6.1 Hz, CHCH(OH)CH₃), 5.48 (s, 2H, CH₂C₆H₅),

5.96 (s, 1H, NH Cbz), 7.12–7.26 (m, 5H, C₆H₅), 8.56 (s, 1H, NHOH); ¹³C 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 C₁₂H₁₆N₂O₅: 291.0957 [M+Na]⁺, found: 291.0962 [M+Na]⁺.

N^α-Boc-Pro-NHOH (2e)

¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 1.32 (s, 9H, C(CH₃)₃), 1.80–1.89 (m, 4H, –NCH₂CH₂CH₂–), 3.24–3.44 (m, 2H, –NCH₂CH₂CH₂–), 4.16 (m, 1H, –CH₂CHN–), 8.22 (s, 1H, NHOH); ¹³C 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 C₁₀H₁₈N₂O₄: 253.1164 [M+Na]⁺, found: 253.1167 [M+Na]⁺.

N^α-Boc-Asp(OBzl)-NHOH (2f)

¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 1.34 (s, 9H, C(CH₃)₃), 3.21 (d, 2H, *J* = 5.8 Hz, CHCH₂COOBzl), 4.42 (m, 1H, CHCH₂COOBzl), 5.04 (s, 2H, CH₂C₆H₅), 5.72 (s, 1H, NH Boc), 7.22–7.32 (m, 5H, C₆H₅), 8.22 (s, 1H, NHOH); ¹³C 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 C₁₆H₂₂N₂O₆: 361.1376 [M+Na]⁺, found: 361.1379 [M+Na]⁺.

N^α-Boc-Phe-NHOH (2g)

¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 1.38 (s, 9H, C(CH₃)₃), 2.80–2.92 (d, 2H, *J* = 5.8 Hz, CH₂C₆H₅), 3.58 (m, 1H, CHCH₂C₆H₅), 5.92 (s, 1H, NH Boc), 7.22–7.38 (m, 5H, C₆H₅), 8.12 (s, 1H, NHOH); ¹³C 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 C₁₄H₂₀N₂O₄: 303.1321 [M+Na]⁺, found: 303.1326 [M+Na]⁺.

N^α-Boc-Leu-NHOH (2h)

¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 0.92 (d, 6H, *J* = 6.8 Hz, CHCH₂CH(CH₃)₂), 1.43 (s, 9H, C(CH₃)₃), 1.72 (m, 2H, CHCH₂CH(CH₃)₂), 1.78 (m, 1H, CHCH₂CH(CH₃)₂), 4.58 (m, 1H, CHCH₂CH(CH₃)₂), 5.96 (s, 1H, NH Boc), 8.34 (s, 1H, NHOH); ¹³C 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 C₁₁H₂₂N₂O₄: 269.1477 [M+Na]⁺, found: 269.1482 [M+Na]⁺.

N^α-Fmoc-Tyr-NHOH (2i)

¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 3.12–3.26 (d, 2H, *J* = 5.8 Hz, CHCH₂C₆H₄OH), 4.08 (t, 1H, *J* = 6.6 Hz, CH Fmoc), 4.26 (m, 1H, CHCH₂C₆H₄OH), 4.38 (d, 2H,

$J = 6.6$ Hz, CH_2 Fmoc), 5.26 (s, 1H, $\text{CH}_2\text{C}_6\text{H}_4\text{OH}$), 5.66 (brs, 1H, NH Fmoc), 6.46 (d, 2H, $J = 6.2$ Hz, $\text{CH}_2\text{C}_6\text{H}_4\text{OH}$), 6.74 (d, 2H, $J = 6.2$ Hz, $\text{CH}_2\text{C}_6\text{H}_4\text{OH}$), 7.16–7.94 (m, 8H, Fmoc), 7.86 (s, 1H, NHOH); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{24}\text{H}_{22}\text{N}_2\text{O}_5$: 441.1426 $[\text{M}+\text{Na}]^+$, found: 441.1422 $[\text{M}+\text{Na}]^+$.

N^α -Fmoc-Ile-NHOH (**2j**)

^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ (ppm) = 0.83–0.87 (m, 6H, $\text{CHCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$), 1.20 (m, 2H, $\text{CHCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$), 2.06 (m, 1H, $\text{CHCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$), 4.02 (m, 1H, $\text{CHCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$), 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, NHOH); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_4$: 391.1634 $[\text{M}+\text{Na}]^+$, found: 391.1639 $[\text{M}+\text{Na}]^+$.

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

^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ (ppm) = 1.37 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.77–1.80 (m, 2H, $-\text{NCHCH}_2\text{CH}_2-$), 2.17–2.23 (m, 2H, $-\text{NCHCH}_2\text{CH}_2-$), 3.84 (t, 1H, $J = 6.3$ Hz, CH Fmoc), 4.18–4.36 (m, 3H, CH_2 , Fmoc & $-\text{NCHCH}_2\text{CH}_2-$), 6.34 (s, 1H, NH Fmoc), 7.30–7.89 (m, 8H, Fmoc), 8.83 (s, 1H, NHOH); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{24}\text{H}_{28}\text{N}_2\text{O}_6$: 463.1845 $[\text{M}+\text{Na}]^+$, found: 463.1851 $[\text{M}+\text{Na}]^+$.

N^α -Fmoc-Phg-NHOH (**2l**)

^1H NMR (300 MHz, $\text{DMSO}-d_6$): 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, CHC_6H_5), 5.17 (s, 1H, NH Fmoc), 7.18–7.69 (m, 13H, Fmoc & Ph), 8.75 (s, 1H, NHOH); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{23}\text{H}_{20}\text{N}_2\text{O}_4$: 411.1321 $[\text{M}+\text{Na}]^+$, found: 411.1324 $[\text{M}+\text{Na}]^+$.

N^α -Fmoc-Ala-Ile-NHOH (**4a**)

^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ (ppm) = 0.86–1.02 (m, 6H, 2 CH_3 , Ile), 1.28 (m, 2H, $\text{CHCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$), 1.32 (d, 3H, $J = 5.6$ Hz, CHCH_3 , Ala), 2.14 (m, 1H, $\text{CHCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$), 4.18 (t, 1H, $J = 6.4$ Hz, CH, Fmoc), 4.41–4.58 (m, 3H $\text{CHCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$, CH_2

Fmoc), 4.68 (m, 1H, CHCH_3), 5.34 (brs, 1H, NH Fmoc), 6.51 (s, 1H, NH amide), 7.22–7.86 (m, 8H, Fmoc), 7.96 (s, 1H, NHOH); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{24}\text{H}_{29}\text{N}_3\text{O}_5$: 462.2005 $[\text{M}+\text{Na}]^+$, found: 462.2003 $[\text{M}+\text{Na}]^+$.

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

^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ (ppm) = 1.30 (s, 9H, $\text{C}(\text{CH}_3)_3$), 3.36 (d, 2H, $J = 5.9$ Hz, $\text{CH}_2\text{C}_6\text{H}_5$), 4.06 (m, 1H, $\text{CHCH}_2\text{C}_6\text{H}_5$), 4.34 (s, 1H, CHC_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, 2 C_6H_5), 8.68 (s, 1H, NHOH); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{22}\text{H}_{27}\text{N}_3\text{O}_5$: 436.1848 $[\text{M}+\text{Na}]^+$, found: 436.1846 $[\text{M}+\text{Na}]^+$.

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

^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ (ppm) = 1.32 (s, 9H, $\text{C}(\text{CH}_3)_3$), 3.38 (d, 2H, $J = 5.9$ Hz, $\text{CH}_2\text{C}_6\text{H}_5$), 4.02 (m, 1H, $\text{CHCH}_2\text{C}_6\text{H}_5$), 4.31 (s, 1H, CHC_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, 2 C_6H_5), 8.74 (s, 1H, NHOH); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{22}\text{H}_{27}\text{N}_3\text{O}_5$: 436.1848 $[\text{M}+\text{Na}]^+$, found: 436.1845 $[\text{M}+\text{Na}]^+$.

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

^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ (ppm) = 0.85–0.90 (m, 12H, 4 CH_3 , Val, Ile), 1.35 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.43–1.57 (m, 2H, $\text{CHCH}_2\text{CH}(\text{CH}_3)_2$), 1.90 (m, 1H, $\text{CHCH}_2\text{CH}(\text{CH}_3)_2$), 3.76 (t, 1H, $J = 7.2$ Hz, $\text{CHCH}_2\text{CH}(\text{CH}_3)_2$), 4.23 (m, 1H, $\text{CHCH}(\text{CH}_3)_2$), 6.76 (d, 1H, $J = 9.1$ Hz, NH Boc), 7.78 (d, 1H, $J = 8.3$ Hz, NH amide), 8.84 (s, 1H, NHOH); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{16}\text{H}_{31}\text{N}_3\text{O}_5$: 368.2161 $[\text{M}+\text{Na}]^+$, found: 368.2164 $[\text{M}+\text{Na}]^+$.

N^α -Cbz-Phe-Ala-NHOH (**4e**)

^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ (ppm) = 1.26 (d, 3H, $J = 6.4$ Hz, CHCH_3), 2.86–3.14 (d, 2H, $J = 5.8$ Hz, $\text{CHCH}_2\text{C}_6\text{H}_5$), 4.50 (m, 1H, $\text{CHCH}_2\text{C}_6\text{H}_5$), 4.66 (m, 1H, CHCH_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, 2 C_6H_5), 7.62 (s, 1H, NHOH); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ

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

Entry	Solvent	Base	Yield (%) ^c
1	CH ₃ CN	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 C₂₀H₂₃N₃O₅: 408.1535 [M+Na]⁺, found: 408.1539 [M+Na]⁺.

Scheme 1 Synthesis of amino hydroxamic acids **2** **Scheme 2** Synthesis of peptide hydroxamic acids **4**

*N*²-Cbz-Ala-Ile-NHOH (**4f**)

¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 0.64–0.98 (m, 6H, 2CH₃, Ile), 1.14 (d, 3H, *J* = 5.8 Hz, CHCH₃), 1.42 (m, 2H, CHCH(CH₃)CH₂CH₃), 1.64 (m, 1H, CHCH(CH₃)CH₂CH₃), 4.02 (m, 1H, CHCH₃), 4.18 (m, 1H, CHCH(CH₃)CH₂CH₃), 4.98 (s, 2H, CH₂C₆H₅), 6.92 (s, 1H, NH Cbz), 7.26–7.34 (m, 5H, C₆H₅), 7.54 (d, 1H, *J* = 8.3 Hz, NH amide), 8.94 (s, 1H, NHOH); ¹³C 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 ₃ CH ₂ CH ₃	91	
4b	Boc	(L)-CH ₂ C ₆ H ₅	(D)-C ₆ H ₅	89	1.0
4c	Boc	(L)-CH ₂ C ₆ H ₅	(L)-C ₆ H ₅	90	0.8
4d	Boc	CH(CH ₃) ₂	CH ₂ CH(CH ₃) ₂	89	–
4e	Cbz	CH ₂ C ₆ H ₅	CH ₃	86	–
4f	Cbz	CH ₃	CHCH ₃ CH ₂ CH 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 C₁₇H₂₅N₃O₅: 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 CH₃CN, 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 NH₂OH·HCl (prepared by neutralizing NH₂OH·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. Delightfully, 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 NH₂OH 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, ¹³C) 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.

References

- Altenburger JM, Mioskowski C, d'Orchymont H, Schirlin D, Schalk C, Tarnus C (1992) Useful hydroxylamine derivatives for the synthesis of hydroxamic acids. *Tetrahedron Lett* 33:5055–5058
- Ando W, Tsumaki H (1983) Facile preparation of aliphatic hydroxamic acid from *N,N,O*-tris(trimethylsilyl)hydroxylamine and acid chloride. *Synth Commun* 13:1053–1056
- Anilkumar R, Chandrasekhar S, Sridhar M (2000) *N,O*-Bis-(ethoxycarbonyl)hydroxylamine: a convenient reagent for the Lossen transformation. *Tetrahedron Lett* 41:5291–5293
- Basavaprabhu, Vishwanatha TM, Panguluri NR, Sureshbabu VV (2013) Propanephosphonic acid anhydride (T3P®)—a benign reagent for diverse applications inclusive of large-scale synthesis. *Synthesis* 45:1569–1601
- Chantell CA, Onaiyekan MA, Menakuru M (2012) Fast conventional Fmoc solid-phase peptide synthesis: a comparative study of different activators. *J Pept Sci* 18:88–91
- Dhungana S, Miller MJ, Dong L, Ratledge C, Crumbliss AL (2004) Iron chelation properties of an extracellular siderophore exochelin MN. *J Am Chem Soc* 125:7654–7663
- Ech-Chahad A, Minassi A, Berton L, Appendino G (2005) An expeditious hydroxyamidation of carboxylic acids. *Tetrahedron Lett* 46:5113–5115
- El-Faham A, Albericio F (2010) COMU: a third generation of uronium-type coupling reagents. *J Pept Sci* 16:6–9
- El-Faham A, Albericio F (2011) Peptide coupling reagents, more than a letter soup. *Chem Rev* 111:6557–6602
- El-Faham A, Funosas RS, Prohens R, Albericio F (2009) COMU: a safer and more effective replacement for benzotriazole-based uronium coupling reagents. *Chem Eur J* 15:9404–9416
- Giacomelli G, Porcheddu A, Salaris M (2003) A simple preparation of ketones: *N*-protected α -amino ketones from α -amino acids. *Org Lett* 5:2715–2717
- Hjelmgaard T, Faure S, Staerk D, Taillefumier C, Nielsen J (2011a) Efficient and versatile COMU-mediated solid-phase submonomer synthesis of arylopeptides (oligomeric *N*-substituted aminomethyl benzamides). *Org Biomol Chem* 9:6832–6843
- Hjelmgaard T, Faure S, Staerk D, Taillefumier C, Nielsen J (2011b) Expedient solution-phase synthesis and NMR studies of arylopeptides. *Eur J Org Chem*. doi:10.1002/ejoc.201100232
- Hjorringgaard CU, Brust A, Alewood PF (2012) Evaluation of COMU as a coupling reagent for in situ neutralization Boc solid phase peptide synthesis. *J Pept Sci* 18:199–207
- Jung M (2001) Inhibitors of histone deacetylase as new anticancer agents. *Curr Med Chem* 8:1505–1511
- Kokare ND, Nagawade RR, Rane VP, Shinde DB (2007) Design, synthesis and utilization of a novel coupling reagent for the preparation of *O*-alkyl hydroxamic acids. *Tetrahedron Lett* 48:4437–4440
- Kwak SY, Lee S, Choi HR, Park KC, Lee YS (2011) Dual effects of caffeoyl-amino acidyl-hydroxamic acid as an antioxidant and depigmenting agent. *Bioorg Med Chem Lett* b21:5155–5158
- Kwak SY, Yang JK, Choi HR, Park KC, Kim YB, Lee YS (2013) Synthesis and dual biological effects of hydroxycinnamoyl phenylalanyl/prolyl hydroxamic acid derivatives as tyrosinase inhibitor and antioxidant. *Bioorg Med Chem Lett* 23:b1136–b1142
- Lee BH, Miller MJ (1983) Natural ferric ionophores: total synthesis of schizokinen, schizokinen A, and arthrobactin. *J Org Chem* 48:24–31
- Librizzi M, Longo A, Chiarelli R, Amin J, Spencer J, Luparello C (2012) Cytotoxic effects of jay amin hydroxamic acid (JAHA), a ferrocene-based class I histone deacetylase inhibitor, on triple-negative MDA-MB231 breast cancer cells. *Chem Res Toxicol* 25:2608–2616
- Maier M, Kotman N, Friedrichs C, Andrieu J, Wagner M, Graf R, Strauss WSL, Mailander V, Weiss CK, Landfester K (2011) Highly site specific, protease cleavable, hydrophobic peptide-polymer nanoparticles. *Macromolecules* 44:6258–6267
- Malik L, Tofteng AP, Pedersen SL, Sorensen KK, Jensen KJ (2010) Automated 'X-Y' robot for peptide synthesis with microwave heating: application to difficult peptide sequences and protein domains. *J Pept Sci* 16:506–512
- Marcucci E, Tulla-Puche J, Albericio F (2012) Solid-phase synthesis of NME-IB-01212, a highly *N*-methylated cyclic peptide. *Org Lett* 14:612–615
- Miller MJ (1989) Syntheses and therapeutic potential of hydroxamic acid based siderophores and analogs. *Chem Rev* 89:1563–1579
- Narendra N, Chennakrishnaireddy G, Sureshbabu VV (2009) Application of carbodiimides mediated Lossen rearrangement for the synthesis of α -ureidopeptides and peptidyl ureas employing *N*-urethane α -amino/peptidyl hydroxamic acids. *Org Biomol Chem* 7:3520–3526
- Reddy AS, Kumar MS, Reddy GV (2000) A convenient method for the preparation of hydroxamic acids. *Tetrahedron Lett* 41:6285–6288
- Samarasimhareddy M, Hemantha HP, Ananda K, Sureshbabu VV (2012) Epimerization free synthesis of *O*-acyl isodipeptides employing COMU. *Protein Pept Lett* 19:1281–1287
- Sibi MP, Hasegawa H, Ghorpade SR (2002) A convenient method for the conversion of *N*-acyloxazolidinones to hydroxamic acids. *Org Lett* 4:3343–3346
- Silhar P, Silvaggi NR, Pellett S, Capkova K, Johnson EA, Allen KN, Janda KD (2013) Evaluation of adamantane hydroxamates as botulinum neurotoxin inhibitors: synthesis, crystallography, modeling, kinetic and cellular based studies. *Bioorg Med Chem* 21:1344–1348
- Subiros-Funosas R, Acosta GA, El-Faham A, Albericio F (2009) Microwave irradiation and COMU: a potent combination for solid-phase peptide synthesis. *Tetrahedron Lett* 50:6200–6202
- Tamaki K, Ogita T, Tanzawa K, Sugimura Y (1993) Synthesis and determination of the absolute configuration of matlystatin B. *Tetrahedron Lett* 34:683–686
- Tyrrell E, Brawn P, Carew M, Greenwood I (2011) An expedient conversion of α -amino acids into Weinreb amides using COMU® as a coupling agent. *Tetrahedron Lett* 52:369–372
- Vasanth B, Hemantha HP, Sureshbabu VV (2010) 1-Propanephosphonic acid cyclic anhydride (T3P) as an efficient promoter for the Lossen rearrangement: application to the synthesis of urea and carbamate derivatives. *Synthesis* 17:2990–2996
- Vasanthakumar GR, Sureshbabu VV (2003) Direct synthesis of Fmoc protected amino acid hydroxamates from acid chlorides mediated by magnesium oxide. *Tetrahedron Lett* 44:4099–4101
- Wagner FF, Olson DE, Gale JP, Kaya T, Weiwer M, Aidoud N, Thomas M, Davoine EL, Lemercier BC, Zhang YL, Holson EB (2013) Potent and selective inhibition of histone deacetylase 6 (HDAC6) does not require a surface-binding motif. *J Med Chem* 56:1772–1776

- Yadav DK, Yadav AK, Srivastava VP, Watal G, Yadav LS (2012) Bromodimethylsulfonium bromide (BDMS)-mediated Lossen rearrangement: synthesis of unsymmetrical ureas. *Tetrahedron Lett* 53:2890–2893
- Yamada K, Nagashima I, Hachisu M, Matsuo I, Shimizu H (2012) Efficient solid-phase synthesis of cyclic RGD peptides under controlled microwave heating. *Tetrahedron Lett* 53:1066–1070
- Yoganathan S, Miller SJ (2013) *N*-Methylimidazole-catalyzed synthesis of carbamates from hydroxamic acids via the Lossen rearrangement. *Org Lett* 15:602–605
- Zhang M, Zhang Y, Lu W, Nan FJ (2011) Synthesis and revision of stereochemistry of rubescensin S. *Org Biomol Chem* 9:4436–4439