A Convenient Procedure for the Preparation of Amino Acid Hydroxamates from Esters

Michael C. Pirrung*,[1] and Joseph H.-L. Chau

Department of Chemistry, P. M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27708-0346

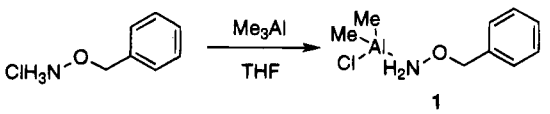
Received July 27, 1995

Introduction

Hydroxamic acids are well-known to have high affinity for metal ions.[2] As such, they have recently been used to provide a point of attachment for inhibitors of metalloproteases such as thermolysin,[3] matrix metalloprotease,[4] endothelin-converting enzyme,[5] angiotensin-converting enzyme,[6] and enkephalinase.[7] Preparations of the hydroxamic acids in these inhibitors have usually involved activation of a carboxylic acid and reaction with hydroxylamine. On the basis of Weinreb's reports of the direct conversion of esters to *O*-methyl *N*-methylhydroxamates (Weinreb amides)[8] with the corresponding *O*-methyl-*N*-methylhydroxylamine aluminum reagent, we expected that a similar reagent derived from *O*-benzylhydroxylamine would permit a simple preparation of a benzylhydroxamate that could be readily hydrogenolyzed to the hydroxamate.[9] We report herein details of this process as applied to amino acid esters to prepare amino acid hydroxamates, which may find use as inhibitors of metalloenzymes[10] and other enzymes.[11]

Results

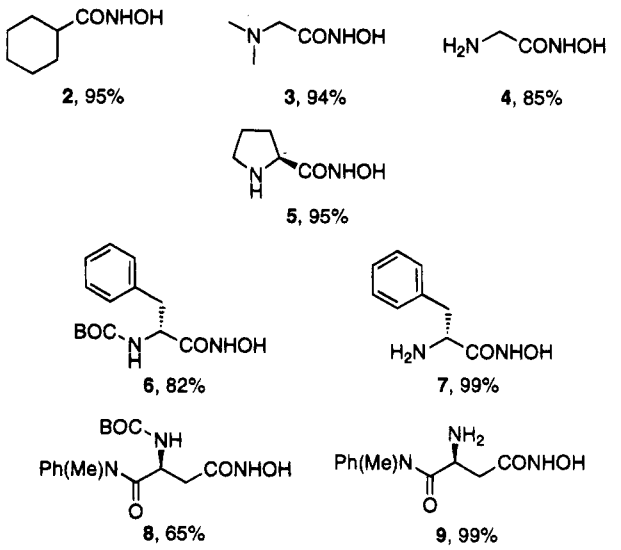
O-Benzylhydroxylamine hydrochloride was treated with trimethylaluminum in toluene to provide a reagent for which we suggest structure **1** on the basis of analogy.[12]



1

This substance was then treated *in situ* with various esters under reflux in benzene to provide the hydroxamates. For initial development of the method, methyl cyclohexanecarboxylate was used as a model. It was converted in 96% yield to the *O*-benzyl hydroxamate. On palladium-catalyzed hydrogenation at atmospheric pressure, the benzyl group is removed to provide the hydroxamate **2** in 95% overall yield. Ferrous chloride staining of thin layer chromatography plates readily confirms the production of the hydroxamate: a distinctive purple spot develops. Spectroscopic studies of this material also confirm its structure. This encouraging initial result was followed up using a number of amino acid esters as starting materials, with the results collected in Chart 1. *N,N*-Dimethylglycine methyl ester gives an overall conversion to hydroxamate **3** that is comparable to the results obtained with cyclohexanecarboxylate. With methyl CBZ-glycinate, inclusion of an additional equivalent of trimethylaluminum is necessary to prevent the acidic proton of the carbamate from destroying the aminating reagent. Protected secondary amino acids can be hydroxamated without the requirement for an additional equivalent of aluminum reagent, as evidenced in methyl L-CBZ-prolinate. Subsequent catalytic reduction also deprotects the benzyl carbamate in these cases, producing glycine hydroxamate **4** and L-proline hydroxamate **5**, both of which were identical to commercial samples. For comparison to a classical method, CBZ-glycine was activated as its NHS ester and treated with aqueous hydroxylamine. After deprotection, this protocol delivers **4** in only 71% overall yield.

Chart 1



The nitrogen protecting group can be retained by using BOC in place of CBZ, such as in hydroxamate **6**. The issue of racemization was then addressed with **7**. Thus, after deprotection of **6** to **7**, in 99% overall yield, it was compared to an authentic sample of the commercial racemic material using chiral HPLC, establishing that only one stereoisomer was present (>99% ee). Discrimination between carboxyl groups in the amidation is possible, since an aspartic acid β-methyl ester derivative gives hydroxamate **8** in moderate conversion and with exclusive selectivity for the ester over the anilide. Sub-

(1) Fellow of the John Simon Guggenheim Memorial Foundation, 1994–95.

(2) Kurzak, B.; Kozlowski, H.; Farkas, E. *Coord. Chem. Rev.* **1992**, *114*, 169–200.

(3) Nishino, N.; Powers, J. C. *Biochemistry* **1978**, *17*, 2846–50.

(4) Gowravaram, M. R.; Tomczuk, B. E.; Johnson, J. S.; Delecki, D.; Cook, E. R.; Ghose, A. K.; Mathiowetz, A. M.; Spurlino, J. C.; Rubin, B.; Smith, D. L.; Pulvino, T.; Wahl, R. C. *J. Med. Chem.* **1995**, *38*, 2570–2581.

(5) Doherty, A. M.; Kornberg, B. E.; Nikam, S. P. PCT Int. Appl. WO 9428012 A1 941208; *Chem. Abstr.* **1995**, *122*, 155223.

(6) Harris, R. B.; Strong, P. D. M.; Wilson, I. B. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 394–9.

(7) Fournie-Zaluski, M. C.; Hernandez, J. F.; Soleilhac, J. M.; Renwart, N.; Peyroux, J.; Xie, J.; Roques, B. P. *Int. J. Pept. Protein Res.* **1989**, *33*, 146–53. Hernandez, J. F.; Soleilhac, J. M.; Roques, B. P.; Fournie-Zaluski, M. C. *J. Med. Chem.* **1988**, *31*, 1825–31.

(8) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989–993.

(9) A novel procedure for hydrogenolysis of *O*-benzylhydroxamates using $Pd/BaSO_4$ was recently reported: Nikam, S. S.; Kornberg, B. E.; Johnson, D. R.; Doherty, A. M. *Tetrahedron Lett.* **1995**, *36*, 197–200.

(10) See, for example: Pirrung, M. C.; Cao, J.; Chen, J. *J. Org. Chem.* **1995**, *60*, 5790.

(11) Silverman, R. B.; Olson, G. T. *BioMed. Chem.* **1995**, *3*, 11–18.

(12) Sidler, D. R.; Lovelace, T. C.; McNamara, J. M.; Reider, P. J. *J. Org. Chem.* **1994**, *59*, 1231–1233.

0022-3263/95/1960-8084$09.00/0

sequent deprotection of this compound affords amine **9** in 64% overall yield.

Experimental Section

Chiral HPLC anaylsis was conducted using a Resolvosil BSA-7 column (150 × 4.4 mm) and eluting with a 5% isopropyl alcohol–95% 0.04 M phosphate buffer (pH 7.0) solution at a flow rate of 1.0 mL/min and oven temperature of 35 °C. Compounds **5**, **7**, and **9** all showed only one peak in the HPLC trace, whereas authentic racemic hydroxamates showed two.

General Procedure for the Preparation of (*O*-Benzylhydroxylamine)methyl aluminum chloride (Reagent 1) and Its Reaction with Methyl Esters. Solid *O*-benzylhydroxylamine hydrochloride (159 mg, 1.00 mmol), dried overnight, in a flame-dried 10 mL round bottom flask was suspended in 5 mL of benzene at 5 °C and treated with 0.550 mL of a 2.0 M solution (1.10 mmol) of trimethylaluminum in toluene. After 5 min, the mixture was warmed to room temperature and aged for 1.5 h, the ester was added in 20 mL of benzene, and the reaction mixture was heated at reflux under argon for 3–4 h. The reaction mixture was poured into 50 mL of ethyl acetate, quenched with an aqueous 5% HCl solution, and successively washed with aqueous 5% HCl, saturated Na_2CO_3, and saturated NaCl. The organic phase was dried ($MgSO_4$) and evaporated *in vacuo* and the residue purified by preparative TLC (1:1 EtOAc:hexanes).

The benzyl hydroxamate (0.500 mmol) was dissolved in 20 mL of methanol, and 25 mg of 5% Pd/C was added. A balloon of hydrogen was placed on the flask, and the reaction mixture was stirred at rt until TLC (1:1 EtOAc:hexanes) showed that the reaction was complete (4–10 h). The reaction mixture was filtered through Celite and evaporated to provide the hydroxamate.

***O*-Benzylcyclohexyl Hydroxamate.** This compound was obtained after amidation in a 96% yield on a 1.00 mmol scale. The white crystalline product had mp ($CHCl_3$) 69–70 °C. IR (film): 3187, 1659, 1455, 1027, 748, 702 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.50–7.25 (m, 5 H), 4.89 (s, 2 H), 4.69 (s, 1 H, exchanged with D_2O), 2.78 (d, J = 6 Hz, 1 H), 2.05–1.05 (m, 11 H). FAB-MS (M + H): 234. Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 71.66; H, 8.60; N, 6.14.

Cyclohexanehydroxamate (2). The above compound was reduced in 99% yield to give a material with ^1H NMR and IR data in concert with the literature (mp 125–129 °C dec (lit.[13] 128–129 °C)).

***N,N*-Dimethyl-*O*-Benzylglycine Hydroxamate.** This compound was obtained as a yellow oil in 95% yield on a 1.00 mmol scale. IR (film): 3337, 1649 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.51–7.26 (m, 5 H), 5.18 (s, 2 H), 3.23 (s, 2 H), 2.36 (s, 6 H). FAB-MS (M + H): 209. Anal. Calcd for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45. Found: C, 63.35; H, 7.57; N, 13.77.

***N,N*-Dimethylglycine Hydroxamate (3).** Reduction of the above *O*-benzyl protected hydroxamate gave **3**, which had spectroscopic and physical properties identical to those described in the literature, in 99% yield.[14]

***N*-CBZ-*O*-Benzylglycine Hydroxamate.** This compound was obtained as white crystals in 86% yield on a 1.00 mmol scale, mp ($CHCl_3$) 115–116 °C. IR (film): 3318, 3240, 1684 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.48–7.24 (m, 10 H), 6.00 (s, 1 H, exchanged with D_2O), 5.09 (s, 2 H), 4.65 (s, 2 H), 2.71 (d, J = 6 Hz, 2 H). FAB-MS (M + H): 315. Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91. Found: C, 65.22; H, 5.94; N, 9.02.

Glycine Hydroxamate (4). Reduction of the above *O*-benzyl-protected hydroxamate gave in 99% yield compound **4**, which was identical to a commercial sample.[15]

***N*-CBZ-*O*-Benzylproline Hydroxamate.** This compound was obtained as a brown oil in 96% yield on a 1.00 mmol scale. IR (film): 3417, 1656 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.51–7.26 (s, 10 H), 5.17 (m, 3 H, 2 H when exchanged with D_2O), 4.71 (s, 2 H), 4.40 (m, 1 H), 3.59 (m superimposed on s, 3 H total), 2.40–1.80 (m, 4 H). FAB-MS (M + H): 355. Anal. Calcd for $C_{20}H_{22}N_2O_4$: C, 67.78; H, 6.26; N, 7.90. Found. C, 68.16; H, 6.10; N, 7.70.

Proline Hydroxamate (5). Reduction of the above *O*-benzyl-protected hydroxamate gave **5**, which had spectroscopic and physical properties identical to those described in the literature, in 99% yield.[16]

***N*-*t*-BOC-*O*-Benzylphenylalanine Hydroxamate.** This compound was obtained as a yellow oil in 82% yield on a 1.00 mmol scale. IR (film): 3325, 1762, 1668 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.40–7.00 (m, 10 H), 5.72 (s, 1 H, exchanged with D_2O), 4.71 (d, J = 6 Hz, 2 H), 4.15 (s, 1 H, exchanged with D_2O), 3.04, (dd, J = 9, 12 Hz, 1 H), 2.74 (dd, J = 9, 12 Hz, 2 H), 1.41 (s, 9 H). FAB-MS (M + H): 371. Anal. Calcd for $C_{21}H_{26}N_2O_4$: C, 68.09; H, 7.07; N, 7.56. Found: C, 68.18; H, 7.02; N, 7.35.

Phenylalanine Hydroxamate (7). Reduction of the above *O*-benzyl-protected hydroxamate gave *N*-*t*-BOC-phenylalanine hydroxamate (**6**), identified by its ^1H NMR (D_2O): δ 7.40–7.05 (m, 5 H), 3.04 (dd, J = 9,12 Hz, 1 H), 2.74 (dd, J = 9, 12 Hz, 2 H), 1.40 (s, 9 H). This compound was further deprotected with trifluoroacetic acid to afford **7**, which had spectroscopic properties identical to a commercial sample, in 99% yield over the two steps.[15]

***N*-*t*-BOC-aspartoyl-α-*N*-methylanilide β-Hydroxamate (8).** This compound was obtained as a yellow oil in 65% yield on a 1.00 mmol scale. IR (film): 3266, 1708, 1648 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.50–7.05 (m, 10 H), 5.60 (s, 1 H, exchanged with D_2O), 4.81 (s, 2 H), 4.60 (s, 1 H, exchanged with D_2O), 3.28–3.20 (d superimposed on s, 4 H total), 2.38 (d, J = 9 Hz, 2 H), 1.37 (s, 9 H). FAB-MS (M + H): 428. Anal. Calcd for $C_{23}H_{29}N_3O_5$: C, 64.62; H, 6.84; N, 9.83. Found: C, 64.43; H, 7.07; N, 9.85.

Aspartoyl-α-*N*-methylanilide β-Hydroxamate (9). Reduction of the above *O*-benzyl-protected hydroxamate gave the *N*-*t*-BOC-aspartic hydroxamate. This compound was further deprotected with trifluoroacetic acid to afford **9** in 99% yield over the two steps, mp (MeOH) 214–215 °C. IR (film): 3410–2980, 1655, 1639 cm^{-1}. ^1H NMR (D_2O): δ 7.62–7.30 (m, 5 H), 3.05 (s, 3 H), 2.78 (t, J = 6 Hz, 1 H), 2.22 (d, J = 6 Hz, 2 H). FAB-MS (M + H): 238. Anal. Calcd for $C_{11}H_{15}N_3O_3$: C, 55.69; H, 6.37; N, 17.71. Found: C, 55.97; H, 6.68; N, 17.93.

Acknowledgment. Financial support provided by NIH GM-43816 is acknowledged.

JO9513774

(13) Nagarajan, K.; Rajappa, S.; Rajagopalan, P.; Talwalkar, T. *Ind. J. Chem.* **1991**, *30B*, 222–229.

(14) Ivanov, V. A.; Syrbu, V. A; Vember, P. A. *Metallkhelat. Ikh Svoistva* **1985**, 20–25.

(15) Available from Sigma Chemical Co.

(16) Menzel, M.; Zinner, G. *Chem.-Ztg.* **1980**, *104*, 277–278.