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Synthesis of L-Canaline and γ-Functional 2-Aminobutyric Acid Derivatives

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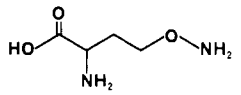
The convergent synthesis of L-canaline (1) from L-homoserine (3) or L-methionine by the common intermediate 2-[(benzyloxycarbonyl)amino]-L-4-butyrolactone **5a** is described. Functional group protection of L-homoserine (3) is examined, and procedures are reported which overcome the tendency for lactone formation, to provide protected 2-amino-4-hydroxybutyric acid derivatives with γ-hydroxyl groups which are suitable for further γ-functionalization.

L-Canaline (1), 2-amino-4-(aminooxy)butyric acid, is the toxic 5-oxa structural analogue of L-ornithine, and it is the only known naturally occurring amino acid that contains a free aminooxy group in its side chain. The aminooxy group rapidly forms Schiff base adducts with α-keto acids[1] and with the pyridoxal phosphate (B_6) cofactor[2,3] of many B_6-dependent enzymes. The resulting potent inhibition of catalytic action[4-6] is an important basis for the antimetabolic activity of canaline (1).[7]



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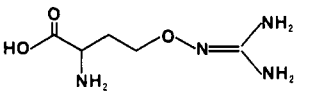

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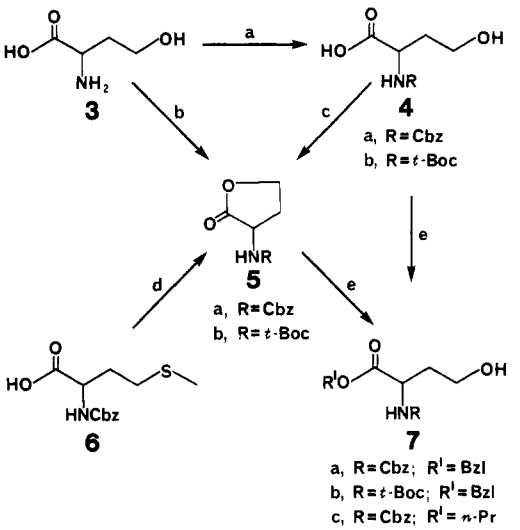
Canaline (1) is found in leguminous plants which contain the metabolically related non-protein amino acid canavanine (2), the 5-oxa analogue of arginine.[8] Over 500 species of leguminous plants are known to produce canavanine (2),[8] and since enzymatic cleavage of 2 by arginase results in canaline (1),[9-13] these plants are potential sources of 1. The jack bean has become the most useful preparative source of 1.[14] A synthesis of L-canaline (1) from L-homoserine (3)[13] provided structural proof of 1, although certain experimental details and yields were omitted. Features of this procedure were incorporated into subsequent syntheses of racemic canaline (1) from γ-butyrolactone[15,16] and acrolein.[17] Central to these procedures is the hydrohalogenation of 2-benzamido-4-butyrolactone to form halogenated homoserine derivatives. We required more versatile procedures for the synthesis of the L-isomer of canaline (1). This report describes the synthesis of L-canaline (1) from L-homoserine (3) or *N*-Cbz-L-methionine (6). The synthetic procedures from both starting materials 3 and 6 are related by the common intermediate *N*-Cbz-*L*-homoserine lactone (**5a**). A general approach is described, involving the preparation of tri-

Scheme I[a]



[a] (a) **4a**: $ClCOOCH_2Ph/NaHCO_3/H_2O$; **4b**: $(Me_3CO)_2CO/NaOH/H_2O/THF$; (b) $ClCOOCH_2Ph/NaHCO_3/H_2O$, citric acid/H_2O/pH 2/Δ; (c) DCC or H^+; (d) $ICH_2CONH_2/EtOH/H_2O$, citric acid/H_2O/pH 2/Δ; (e) $NaOH/EtOH/H_2O$, evaporate, RBr/DMF.

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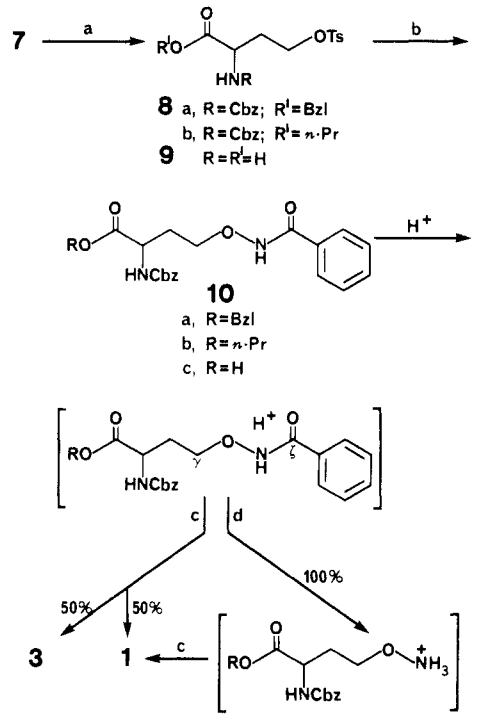
functional 2-aminobutyric acid derivatives containing γ-hydroxyl groups, which are useful for the preparation of various γ-functionalized derivatives of **3**.

N-Cbz-homoserine (**4a**) and *N*-*t*-Boc-homoserine (**4b**) were prepared from **3** in 82% and 86% yield, respectively (Scheme I).[18] The susceptibility of N-substituted homoserine derivatives to facile lactonization was demonstrated by cyclization of **4a** with DCC, even in the presence of primary alcohols, to provide lactone **5a** in quantitative yield. Treatment of the reaction mixture containing **4a** with hot citric acid produced lactone **5a** in 80% yield. Reaction of *N*-*t*-Boc-homoserine (**4b**) with DCC afforded lactone **5b** in 84% yield.[19] Lactone **5a** was prepared alternatively from *N*-Cbz-L-methionine (**6**),[20] since S-alkylated methionine derivatives readily cyclize to the corresponding homoserine lactone derivatives.[21] Alkylation of **6** with iodoacetamide and subsequent lactonization in hot citric acid solution gave lactone **5a** in 70–80% yield. This lactone, available from homoserine (**3**) or methionine,[20] was useful in the subsequently described reaction sequence for canaline (**1**) synthesis.

Esterification of **4a** and **4b** required formation of the corresponding sodium salts first in ethanol, followed by removal of solvent, and then reaction of the salts with benzyl bromide in DMF to produce esters **7a** and **7b**, respectively, in 80% yield each (Scheme I). This reaction sequence was also applied after saponification of lactone **5b** with sodium hydroxide in absolute ethanol, and esterification of the resulting sodium salt with benzyl bromide in DMF gave **7b** in 80% yield. The presence of 1 equiv of sodium hydroxide in an absolute ethanol solution of lactone **5a** gave quantitative cleavage of the N-protective group and resulted in ring-opening of the lactone to the sodium salt of homoserine (**3**). In ethanol–water (17:3), lactone **5a** was converted rapidly to the corresponding sodium salt, which, after solvent removal, was esterified in DMF with either benzyl bromide or with *n*-propyl bromide to give esters **7a** and **7c**, respectively, in 92% yield each.

The side chain hydroxyl groups of the homoserine derivatives **7a** and **7c** were functionalized with *p*-toluenesulfonyl chloride to yield tosylates **8a** and **8b**, in 87% and 82% yields, respectively (Scheme II). The aminooxy function was introduced by reaction of tosylates **8a** and **8b** with sodium benzohydroxamate in DMF to give the fully protected canaline derivatives **10a** and **10b**, respectively, in 80% yield each. Quenching the reaction mixture with water resulted in concomitant cleavage of the benzyl ester to afford the free acid **10c** in 75% yield. Analogously, the tosyl group of **8** was successfully replaced with the sodium salt of *syn*-benzaldoxime, and the resulting benzyloxy Schiff-base was remarkably stable to attempted acidic deprotection. In an alternative reaction sequence to introduce the aminooxy group, the amine and carboxyl protective groups of **8a** were removed by hydrogenation over palladium on carbon to provide the tosylate **9** in 80% yield, which was resistant to the introduction of the desired aminooxy function by the use of various N-substituted hydroxylamines.

Scheme II[a]



[a] (a) *p*-TsCl/Et_3N/THF; (b) HONHCO*Ph*/NaH/DMF; (c) 4 N HCl/H_2O/Δ; (d) HCl/EtOH (19%, w/w)/Δ.

Hydrolysis of the benzamidooxy derivatives **10a–c** in refluxing aqueous acid[16] resulted in a 1:1 mixture of canaline (**1**) and homoserine (**3**).[22] Homoserine **3** did not form by cleavage of **1** under these conditions, since there was no detectable decomposition of authentic **1** upon several hours exposure to refluxing 4 N HCl. Instead, the canaline precursors **10a** and **10b** appeared to be equally susceptible in aqueous acid to cleavage at the γ-carbon and at the benzamido ζ-carbonyl on protonation of the aminooxy ether oxygen and the carbonyl oxygen, respectively (Scheme II). This event was unexpected since it was reported previously[16] that a similar canaline derivative bearing a γ-benzamidooxy group was deprotected in refluxing aqueous acid to give canaline (**1**) in 85–92% yield.

The benzamidooxy groups of **10a** and **10b** were cleaved selectively and quantitatively at the benzamido ζ-carbonyl in anhydrous ethanolic HCl,[23] resulting in the free aminooxy group (Scheme II). The stability of the γ-carbon–ether bond in ethanolic HCl, in contrast to its cleavage in

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(18) The synthesis of N-substituted homoserines required longer reaction times than N-substitution of most amino acids, most likely due to the intramolecular hydrogen bonding between the amino and the γ-hydroxyl groups of **3**, as indicated in the IR spectrum. See: Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; p 885.
(19) A purer preparation of **5b** was obtained by lactonization of a 1 M chloroform solution of **4b** to **5b** in 95% yield over a 2–3-week period, with a half-life of about 4 days. The apparent trace of spontaneously formed hydrogen chloride present in chloroform was sufficient for slow catalysis of lactone formation but insufficient for appreciable cleavage of the acid-sensitive *t*-Boc protective group.
(20) *N*-Cbz-L-methionine (**4**) is readily available commercially, and can be prepared routinely from L-methionine in 85% yield: Dekker, C. A.; Fruton, J. S. *J. Biol. Chem.* **1948**, *173*, 471.
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(22) While **1** and **3** were indistinguishable in aqueous solution by routine NMR spectroscopy, spectra of their hydrochloride salts were clearly differentiated. The presence of homoserine (**3**) in the reaction mixture was further demonstrated by TLC comparison with authentic **3**.
(23) Frankel, M.; Knobler, Y.; Zvilichovsky, G. *J. Chem. Soc.* **1963**, 3127.

aqueous HCl, must reflect the weaker nucleophilicity of ethanol with respect to that of water. Consequently, **10a** and **10b** were deprotected sequentially without isolation of intermediates by treatment first with hot anhydrous ethanolic HCl and then with refluxing aqueous HCl. The HCl salt of canaline (1) was obtained quantitatively as a sticky solid by ether precipitation from an ethanol solution. The free base 1 was precipitated upon treatment of a hot ethanol solution of the salt with triethylamine, and crystalline canaline (1) subsequently was obtained in 80% yield.

The canaline (1) synthesis reported here results in a 51% overall yield of 1 from L-homoserine (3) and in a 35–41% overall yield from *N*-Cbz-L-methionine (6). The common intermediate lactone **5a** can be used conveniently to prepare canaline (1) from either 3 or 6 by convergent synthetic pathways. The procedure for L-canaline (1) synthesis can be extended to canavanine (2) synthesis in a single step in nearly quantitative yield by condensation of canaline (1) and cyanamide in the presence of Zn^{2+}.[24] In addition to the usefulness of the amine and carboxyl protected γ-functional homoserine derivatives in the synthesis of 1, they may also allow the selective, higher yield synthesis of a variety of interesting O-substituted homoserines[25] and other derivatives.

Experimental Section

L-2-[(Benzyloxycarbonyl)amino]-4-hydroxybutyric Acid (4a). To a solution of 3 (119 mg, 1.0 mmol) in 5 ml of 1 N $NaHCO_3$ was added 188 mg (1.1 mol) of benzyl chloroformate. The reaction mixture was stirred at 23 °C for 24 h and then extracted with ether (2 × 10 mL). The aqeuous phase was ice cooled, carefully acidified to pH 2–3 (paper) with 3 N HCl, and extracted with ethyl acetate (4 × 6 mL). The extract was dried over Na_2SO_4, filtered, and evaporated. The resultant oil was crystallized from ethyl acetate/hexane to afford 208 mg (82%) of **4a**: mp 99–100 °C; NMR (Me_2CO-d_6) δ 7.35 (s, 5 H, C_6H_5), 6.50 (br, 1 H, NH), 5.08 (s, 2 H, CH_2Ph), 4.80 (br, 1 H, OH) 4.40 (m, 1 H, CH), 3.69 (dd, 2 H, CH_2O), 1.95 (m, 2 H, $CHCH_2$); IR (KBr) 3380 (s), 3329 (s), 2560 (w), 1690 (s), 1263 (s), 741 (m), 698 (w) cm^{-1}. Anal. Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.97; H, 5.97; N, 5.52.

L-2-(*tert*-Butyloxycarbonylamino)-4-hydroxybutyric Acid (4b). To a solution of 119 mg (1.0 mmol) of 3 in 3 mL of ethanol–water (2:1, v/v) was added 1 mL of 1 N NaOH, followed by the addition of 240 mg (1.1 mmol) of di-*tert*-butyl dicarbonate and 1 mL of THF. After being stirred at 23 °C for 16 h, the reaction mixture was extracted with ether (2 × 10 mL). The aqueous layer was cooled to 0 °C, acidified with 1 N HCl to pH 2 (paper), and extracted with ethyl acetate (4 × 6 mL). The extract was dried over sodium sulfate, filtered, and evaporated. The resulting pure oil crystallized to yield 188 mg (86%) of **4b**: mp 138–140 °C; NMR (Me_2CO-d_6) δ 4.40 (m, 2 H, CH, OH), 3.67 (m, 2 H, CH_2OH), 2.0 (m, 2 H, $CHCH_2$), 1.40 (m, 10 H, *t*-Bu, NH); IR (film) 1050 (br, s), 900 (m), 860 (s), 780 (s), 750 (w) cm^{-1}.

L-2-[(Benzyloxycarbonyl)amino]-4-butyrolactone (5a). Method A. A solution of 6 (283 mg, 1.0 mmol) and iodoacetamide (555 mg, 3.0 mmol) in 6 mL of ethanol/water (1:1) was stirred at 45 °C for 72 h. To the reaction mixture was added 3 mL of 0.1 M citric acid, and the solution was refluxed (ca. 105 °C) for 4 h. The ethanol was evaporated, and the aqueous solution was extracted with ethyl acetate (4 × 6 mL). The extract was washed with 0.5 N HCl (3 × 10 mL), water, saturated NaCl, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography on silica gel with ethyl acetate/hexane (3:2, v/v) to give 160–190 mg (70–80%) of **5a**, which crystallized upon standing. It was recrystallized from ethyl acetate/hexane: mp 129–130 °C; NMR ($CDCl_3$) δ 7.35 (s, 5 H, C_6H_5), 5.39 (br, 1 H, NH), 5.12 (s, 2 H, CH_2Ph), 4.30 (m, 3 H, CH, CH_2O), 2.0–2.9 (m, 2 H, $CHCH_2$); IR (KBr) 3320 (s), 1778 (s), 1180 (m), 742 (m), 693 (m) cm^{-1}. Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.04; H, 5.34; N, 5.97.

Method B. To the ether-extracted aqueous phase of the reaction mixture to form **4a** from 3 was added 3 mL of 0.1 M citric acid. This mixture was refluxed and worked up as described in method A, yielding 190 mg (80%) of **5a**. Identity was determined by comparison of TLC mobility with authentic **5a**.

Method C. A mixture containing 51 mg (0.2 mmol) of **4a** and 27 mg (0.22 mmol) of 4-(dimethylamino)pyridine in 1 mL of CH_2Cl_2 was treated with 42 mg (0.2 mmol) of 1,3-dicyclohexylcarbodiimide (DCC) and stirred at 23 °C for 16 h. The solvent was evaporated, and the residue was treated with 5 mL of ethyl acetate and filtered. The ethyl acetate was washed with 0.1 M citric acid (2 × 5 mL), water, and saturated NaCl, dried over Na_2SO_4, filtered, and evaporated. Purification by flash chromatography as described in method A afforded 47 mg (100%) of **5a**.[26]

L-2-[(*tert*-Butyloxycarbonyl)amino]-4-butyrolactone (5b). A solution was prepared containing 50 mg (0.23 mmol) of **4b**, 35 mg (0.26 mmol) of 1-hydroxybenzotriazole, and a few small crystals of 4-(dimethylamino)pyridine in 1 mL of tetrahydrofuran. The reaction was initiated by the addition of 52 mg (0.25 mmol) of DCC, and the reaction mixture was stirred at 23 °C for 16 h. One drop of acetic acid was added, and after 5 min the solvent was removed. The residue was dissolved in 5 mL of ethyl acetate and filtered. The ethyl acetate was washed with 0.1 M citric acid (3 × 5 mL), washed with saturated NaCl, dried over Na_2SO_4, filtered, and evaporated. Crystallization from ethyl acetate/hexane afforded 39 mg (84%) of **5b**. The analytical sample was obtained after recrystallization: mp 141–142 °C; NMR (Me_2CO-d_6) δ 6.33 (br, 1 H, NH), 4.30 (m, 3 H, CH, CH_2O), 2.40 (m, 2 H, $CHCH_2$), 1.41 (s, 9 H, *t*-Bu); IR (KBr) 3350 (s), 1777 (s), 1160 (m) cm^{-1}. Anal. Calcd for $C_9H_{15}NO_4$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.72; H, 7.64; N, 6.96.

Benzyl L-2-[(Carbobenzyloxy)amino]-4-hydroxybutyrate (7a). Method A. To a mixture containing 290 mg (1.23 mmol) of **5a** in 4 mL of ethanol was added 53 mg (1.3 mmol) of NaOH in 0.7 mL of water, and the resulting solution was stirred at 23 °C for 0.5 h. The solvent was evaporated, and the residue was evaporated again from 4 mL of ethanol and dried under vacuum. The residue was dissolved in 2 mL of DMF, and 182 mg (1.5 mmol) of benzyl bromide was added. The reaction mixture was stirred in the dark at 23 °C for 48 h, diluted with 10 mL of ethyl acetate, washed with water, saturated $NaHCO_3$ (2 × 5 mL), and saturated NaCl, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by silica gel flash chromatography with ethyl acetate/hexane (3:2, v/v), yielding 390 mg (92%) of **7a** as a pure oil, which crystallized upon standing. The analytical sample was obtained after two recrystallizations from ethyl acetate/hexane: mp 65–70 °C; NMR ($CDCl_3$) δ 7.25 (s, 10 H, *Ar*), 5.68 (br, 1 H, NH), 5.08 (s, 2 H, CH_2Ph), 5.03 (s, 2 H, CH_2Ph), 4.35 (m, 2 H, CH, OH), 3.59 (m, 2 H, CH_2OH), 1.95 (m, 2 H, $CHCH_2$); IR (KBr) 3530 (m), 3332 (s), 1748 (br, m), 1672 (s), 1058 (s) 748 (m) cm^{-1}. Anal. Calcd for $C_{19}H_{21}NO_5$: C, 66.46; H, 6.16; N, 4.08. Found: C, 66.25; H, 6.18; N, 4.14.

Method B. A mixture of 253 mg (1.0 mmol) of **4a**, 3 mL of ethanol, and 40 mg (1.0 mmol) of NaOH in 0.5 mL of water was stirred at 23 °C for 24 h. After evaporation, esterification with 190 mg (1.1 mmol) of benzyl bromide, and workup as described in method A, 278 mg (80%) of **7a** was obtained.[26]

Benzyl L-2-[(*tert*-Butyloxycarbonyl)amino]-4-hydroxybutyrate (7b). Method A. A solution containing 22 mg (0.1 mmol) of **5b** in 1.0 mL of 0.1 N ethanolic NaOH was stirred at 23 °C for 16 h. The reaction mixture was evaporated and dried under vacuum. To the residue was added 0.3 mL of DMF, followed by 22 mg (0.13 mmol) of benzyl bromide. The mixture was stirred at 23 °C for 24 h, quenched with 5 mL of 1 N $NaHCO_3$, and extracted with ethyl acetate (4 × 6 mL). The extract was washed with 1 N $NaHCO_3$, water, and saturated NaCl, dried over Na_2SO_4, filtered, and evaporated. Silica gel flash chromatography

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(26) Identity was established by NMR and TLC comparison with authentic material.

of the residue using ethyl acetate/hexane (1:1, v/v) resulted in 28 mg (80%) of **7b** as an oil: NMR ($CDCl_3$) δ 7.33 (s, 5 H, C_6H_5), 5.40 (br, 1 H, NH), 5.17 (s, 2 H, CH_2Ph), 4.40 (m, 2 H, CH, OH), 3.65 (m, 2 H, CH_2OH), 2.15 (m, 2 H, $CHCH_2$), 1.45 (s, 9 H, *t*-Bu); IR (film) 3480 (br, s), 2977 (s), 1700 (br, s), 907 (m), 864 (m), 697 (s) cm^{-1}.

Method B. A solution of 207 mg (1.0 mmol) of **4b**, 3 mL of ethanol, and 40 mg (1 mmol) of NaOH in 0.5 mL of water was stirred at 23 °C for 24 h. After evaporation, esterification with 190 mg (1.1 mmol) of benzyl bromide, and workup as described in method A, 237 mg (80%) of **7b** was obtained.[26]

Propyl L-2-[(Carbobenzyloxy)amino]-4-hydroxybutyrate (7c). The sodium salt of **5a** was prepared with the procedure used for synthesis of **7a**, using 720 mg (3.06 mmol) of **5a** in 11 mL of ethanol and 123 mg (3.06 mmol) of NaOH in 1.5 mL of water. After solvent removal and drying of the residue as for **7a**, it was dissolved in 3 mL of DMF, and 443 mg (3.6 mmol) of *n*-propyl bromide was added. The reaction mixture was stirred at 60 °C for 48 h. Aqueous workup and flash chromatography were done with the procedures described for the synthesis of **7a**, resulting in 833 mg (92%) of **7c**, which solidified upon standing: mp 58–64 °C; NMR ($CDCl_3$) δ 7.40 (s, 5 H, C_6H_5), 6.24 (br, 1 H, NH), 5.15 (s, 2 H, CH_2Ph), 4.50 (m, 1 H, CH), 4.10 (t, 2 H, CH_2OCO), 3.67 (m, 3 H, CH_2OH), 1.2–2.2 (m, 4 H, $CHCH_2$, CH_2CH_3), 0.88 (t, 3 H, CH_3); IR (KBr) 3479 (br, m), 3317 (s), 1725 (s), 1690 (s), 738 (m), 698 (m) cm^{-1}. Anal. Calcd for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 61.15; H, 7.16; N, 4.76.

Benzyl L-2-[(Carbobenzyloxy)amino]-4-[(*p*-tolylsulfonyl)oxy]butyrate (8a). A solution containing 1.029 g (2.97 mmol) of **7a**, 1.21 g (12 mmol) of triethylamine, and 2 mL of THF (filtered through alumina) was cooled to −10 °C and treated with 1.14 g (6.0 mmol) of *p*-toluenesulfonyl chloride in 2 mL of THF. The solution was stirred at 0 °C for 24 h. The reaction mixture was evaporated with a stream of N_2 in a hood. The residue was taken up in 15 mL of ethyl acetate, the mixture was filtered, and the ethyl acetate was washed with 0.5 N HCl (2 × 10 mL), saturated $NaHCO_3$, and saturated NaCl, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by silica gel flash chromatography with ethyl acetate/hexane (1:3), resulting in 1.29 g (87%) of **8a** as an oil: NMR ($CDCl_3$) δ 7.5 (m, 14 H, *Ar*), 5.35 (br, 1 H, NH), 5.08 (s, 2 H, CH_2Ph), 5.02 (s, 2 H, CH_2Ph), 4.40 (m, 1 H, CH), 4.05 (m, 2 H, CH_2OTs), 2.37 (s, 3 H, CH_3), 2.20 (m, 2 H, $CHCH_2$); IR (film) 3360 (br, m), 1725 (br, s), 1360 (s), 750 (m), 699 (m), 664 (m) cm^{-1}. Anal. Calcd for $C_{26}H_{27}NO_7S$: C, 62.76; H, 5.47; N, 2.82. Found: C, 63.01; H, 5.45; N, 2.87.

Propyl L-2-[(Carbobenzyloxy)amino]-4-[(*p*-tolylsulfonyl)oxy]butyrate (8b). Tosylate **8b** was prepared with the same procedure used for preparation of tosylate **8a**, starting with 300 mg (1.02 mmol) of **7c**, 4 mL of CH_2Cl_2, 404 mg (4.0 mmol) of triethylamine, and 388 mg (2.03 mmol) of *p*-toluenesulfonyl chloride. Silica gel flash chromatography was done with ethyl acetate/hexane (3:8, v/v) to give 386 mg (85%) of **8b** as an oil: NMR ($CDCl_3$) δ 7.2–8.0 (m, 9 H, *Ar*), 5.76 (br, 1 H, NH), 5.12 (s, 2 H, CH_2Ph), 4.40 (m, 1 H, CH), 4.12 (m, 4 H, CH_2OTs, CH_2OCO), 2.40 (s, 3 H, CH_3Ph), 2.15 (m, 2 H, $CHCH_2$), 1.55 (m, 2 H, CH_2CH_3), 0.88 (t, 3 H, CH_2CH_3); IR (film) 3350 (br, m), 2968 (s), 1720 (br, s), 1599 (w), 817 (m), 699 (m), 664 (m) cm^{-1}. Anal. Calcd for $C_{22}H_{27}NO_7S$: C, 58.78; H, 6.05; N, 3.12. Found: C, 58.22; H, 6.03; N, 3.13.

L-2-Amino-4-[(*p*-tolylsulfonyl)oxy]butyric Acid (9). To 312 mg (0.63 mmol) of **8a** in 9 mL of methanol and 3 mL of tetrahydrofuran was added 150 mg of palladium black, and H_2 was bubbled through the stirred mixture at 25 °C. After 2 h, 50 mg of palladium black was added, and H_2 was bubbled through the stirred mixture for an additional 1 h. The reaction mixture was filtered and evaporated, and the residue was partitioned between water and ether. The aqueous phase was extracted with ether (4 × 6 mL) and evaporated. The resultant oil was dissolved in 5 mL of ethanol and evaporated to give 137 mg (80%) of **9** as a white powder: mp 208–210 °C; NMR (D_2O, DSS internal reference) δ 7.50 (m, 4 H, *Ar*), 4.50 (m, 3 H, CH, CH_2OTs), 2.70 (m, 2 H, $CHCH_2$), 2.38 (CH_3); IR (KBr) 3420 (br, w), 2990 (br, m), 1772 (s), 1212 (s), 1009 (s), 819 (m), 684 (s) cm^{-1}.

Benzyl L-2-[(Carbobenzyloxy)amino]-4-(benzamidooxy)butyrate (10a). To 225 mg (1.64 mmol) of benzohydroxamic acid in 2 mL of DMF was added 36 mg (1.48 mmol) of NaH (added as a mineral oil dispersion) with vigorous stirring, continued for 10 min. To this suspension was added 370 mg (0.74 mmol) of **8a** in 2 mL of DMF. The reaction mixture was stirred at 55 °C for 24 h. It was then diluted with 15 mL of ethyl acetate, washed with 0.5 N HCl (3 × 10 mL) and saturated NaCl, dried over Na_2SO_4, filtered, and evaporated. Purification by silica gel flash chromatography using ethyl acetate/hexane (4:5) resulted in 275 mg (80%) of **10a** as an oil: NMR ($CDCl_3$) δ 9.62 (br, 1 H, $NHCOPh$), 7.2–7.8 (m, 15 H, *Ar*), 6.38 (br, 1 H, $NHCH$), 5.11 (s, 2 H, CH_2Ph), 5.06 (s, 2 H, CH_2Ph), 4.60 (m, 1 H, CH), 4.05 (dd, 2 H, CH_2ON), 2.13 (m, 2 H, $CHCH_2$); IR (film) 3250 (br, m), 2950 (m), 1715 (br, s), 1049 (s), 902 (w), 739 (shoulder, m), 698 (s) cm^{-1}. Anal. Calcd for $C_{26}H_{26}N_2O_6$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.42; H, 5.82; N, 6.04.

Propyl L-2-[(Carbobenzyloxy)amino]-4-(benzamidooxy)butyrate (10b). The procedure described for the preparation of **10a** was used, starting with 78 mg (0.57 mmol) of benzohydroxamic acid, 14 mg (0.58 mmol) of NaH, 100 mg (0.286 mmol) of **8b**, and 1.5 mL of DMF. Aqueous workup was done as described for **10a**, and silica gel flash chromatography was done with ethyl acetate/hexane (1:1, v/v), resulting in 75 mg (82%) of **10b** as an oil: NMR ($CDCl_3$) δ 9.72 (br, 1 H, $NHCOPh$), 7.2–7.8 (m, 10 H, *Ar*), 6.20 (br, 1 H, $NHCH$), 5.08 (s, 2 H, CH_2Ph), 4.55 (m, 1 H, CH), 4.05 (m, 4 H, CH_2ON, CH_2OCO), 2.12 (m, 2 H, $CHCH_2$), 1.65 (m, 2 H, CH_2CH_3), 0.89 (t, 3 H, CH_3); IR (film) 3260 (br, s), 2970 (s), 1646 (br, s), 1064 (br, s), 897 (m), 738 (shoulder, m), 695 (s) cm^{-1}. Anal. Calcd for $C_{22}H_{26}N_2O_6$: C, 63.76; H, 6.32; N, 6.76. Found: C, 63.76; H, 6.36; N, 6.80.

L-2-[(Carbobenzyloxy)amino]-4-(benzamidooxy)butyric Acid (10c). Synthesis of **10c** was done with the procedure described for **10a**, starting with 70 mg (0.51 mmol) of benzohydroxamic acid, 7 mg (0.28 mmol) of NaH, and 70 mg (0.14 mmol) of **8a**. The reaction mixture was quenched with 5 mL of water, adjusted to pH 4 with 1 N HCl, extracted with ethyl acetate (4 × 5 mL), dried over Na_2SO_4, filtered, and evaporated. Silica gel flash chromatography with ethyl acetate/hexane (7:6) yielded 40 mg (75%) of **10c** as an oil: NMR ($CDCl_3$) δ 9.48 (s, 1 H, $NHCOPh$), 7.2–7.8 (m, 10 H, *Ar*), 6.05 (br, 1 H, $NHCH$), 5.08 (s, 2 H, CH_2Ph), 4.55 (m, 1 H, CH), 4.17 (m, 2 H, CH_2ON), 2.12 (m, 2 H, $CHCH_2$); IR (film) 3252 (br, s), 2958 (m), 1700 (br, s), 1379 (m), 1060 (br, s), 905 (m), 694 (s) cm^{-1}.

L-2-Amino-4-(aminooxy)butyric Acid (Canaline, 1). A solution of 176 mg (0.38 mmol) of **10a** in 3 mL of ethanolic HCl (19%, w/w) was refluxed (105 °C) for 4 h. The solvent was removed, and the residue was dissolved in 3 mL of 3 N HCl and refluxed for 3 h. The solvent was evaporated, and the residue was evaporated from water (3 × 4 mL) and dried at 35 °C at high vacuum for 1 h. The crude product was dissolved in 0.5 mL of ethanol and precipitated with ether, yielding 189 mg (100%) of crude canaline–1.5HCl. The canaline HCl salt was dissolved in the minimum of ethanol and warmed to 70 °C, and sufficient triethylamine was added with mixing to result in pH 7, thus precipitating canaline as the free base. The canaline was dissolved in 1 mL of water, stirred with 3 mg of charcoal for 10 min, filtered, and evaporated to a viscous oil. Canaline (**1**) was crystallized by the addition of cold ethanol with scratching, resulting in 44 mg (80%). The analytical sample was obtained by recrystallization from water–ethanol as described above: mp 192–194 °C; NMR (D_2O, DSS internal reference) δ 3.85 (m, 3 H, CH, CH_2O), 2.18 (m, 2 H, $CHCH_2$); IR (KBr) 3420 (br, w), 2930 (br, m), 1581 (s), 1500 (m), 1405 (m), 1320 (w), 1037 (w) cm^{-1}. Anal. Calcd. for $C_4H_{10}N_2O_3$: C, 35.81; H, 7.51; N, 20.88. Found: C, 35.77; H, 7.49; N, 19.78.

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