Synthesis of N^{ϵ}-(*p*-Bromophenyl)-L-lysine and N^{τ}-(*p*-Bromophenyl)-L-histidine as Models for Adducts of Bromobenzene 3,4-Oxide to Protein. Observation of an Unusual Pd-Catalyzed N^{τ}- to N^{π}-Aryl Substituent Migration

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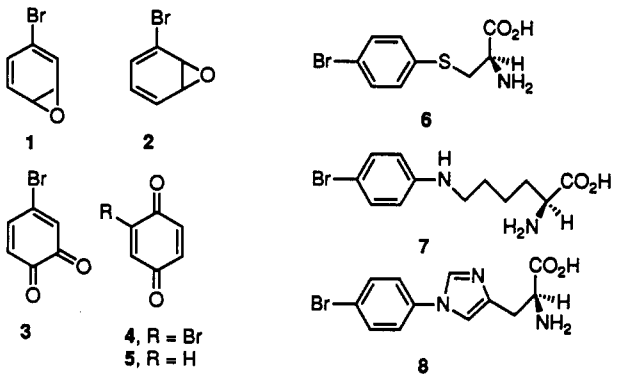
Bromobenzene 3,4-oxide (1), the putative toxic metabolite of bromobenzene, is known to alkylate protein sulfur nucleophiles in vivo and is postulated to alkylate protein nitrogen nucleophiles, the expected products of which would include, after hydrolysis, N^{τ}-(*p*-bromophenyl)-L-histidine (8) and N^{ϵ}-(*p*-bromophenyl)-L-lysine (7). These non-proteinogenic amino acids have now been synthesized by unambiguous routes and their stability under protein hydrolysis conditions demonstrated. Treatment of N^{α}-Cbz-lysine with sodium nitroprusside gave the ϵ-lysinol derivative, which by successive treatment with CBr_4/Ph_3P and excess *p*-bromoaniline and deprotection (6.0 M HCl, 110 °C) afforded an overall 14% yield of 7. Alkylation of N^{α}-Ac-L-histidine methyl ester with *p*-fluoronitrobenzene, followed by reduction, a modified Sandmeyer bromo-dediazotization (*tert*-BuONO/$CuBr_2$), and deprotection afforded 8 in 10% overall yield. An unexpected N^{τ}- to N^{π}-aryl migration was observed during hydrogenation of a N^{τ}-(*p*-nitrophenyl)histidine derivative over Pd/charcoal; it was avoided by use of $SnCl_2$/ethanol for nitro reduction. The N^{α}-acetyl derivatives of 7 and 8, of interest as haptens for use in raising antibodies against proteins alkylated by epoxide 1, were also prepared and characterized.

Aryl halides such as bromobenzene (BB) are known to cause organ-selective tissue injury in mammals, particularly to liver,[1-3] kidney,[4] and lung.[5] The hepatotoxicity of BB has been extensively studied as a model for cellular injury induced by chemicals and has been strongly associated with and generally attributed to the formation of chemically-reactive metabolites which then covalently bind to cellular protein nucleophiles.[3,6-8] Adducts of BB to sulfur nucleophiles of rat liver proteins have been shown to arise from several different reactive metabolites, including epoxides 1 and 2 and quinones 3–5.[9,10] Since S-nucleophiles account for only about 10% of the total amount of protein covalent binding observed in vivo, other protein nucleophiles such as lysine or histidine must also be involved.[10] Indeed, in vitro studies have implicated histidine residues as targets for reactive metabolites of ^{14}C-BB,[11] although the structures of the adducts formed and the reactive metabolites responsible were not identified.

The availability[12] of synthetic *S*-(bromophenyl)cysteine isomers (e.g. 6) as chromatographic standards proved extremely helpful in the initial detection of adducts of epoxides 1 and 2 to protein sulfur nucleophiles.[9] To test the hypothesis that 1 can also alkylate histidine and/or lysine residues in proteins in vivo, we sought to obtain compounds 7 and 8 as reference standards for a chromatographic search through the hydrolysate of liver proteins from rats treated with ^{14}C-BB. Compounds 7 and/or 8 could be expected to arise from metabolite 1 as shown in Scheme 1, which is well-precedented for both protein-[9,10] and non-protein[13-15] sulfur nucleophiles. Compounds 7 and 8 would also be useful as haptens for induction of antibodies capable of recognizing proteins covalently modified with BB metabolites, as we have recently done with 6.[16]

A search of the literature revealed no prior synthesis of 7 or 8. We did find two reports[17,18] claiming the synthesis of N^{im}-(2,4-dinitrophenyl)histidine from N^{α}-acetylhisti-



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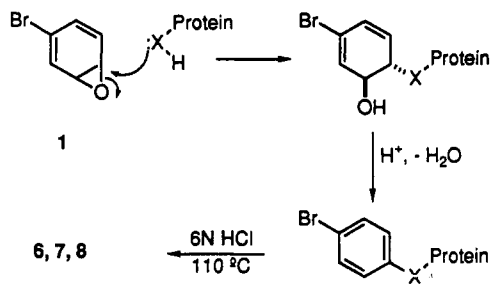
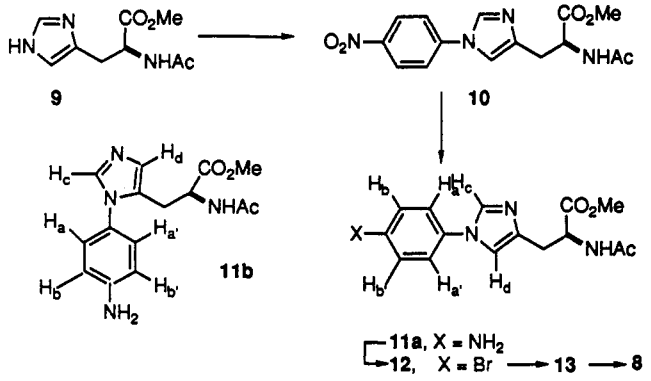
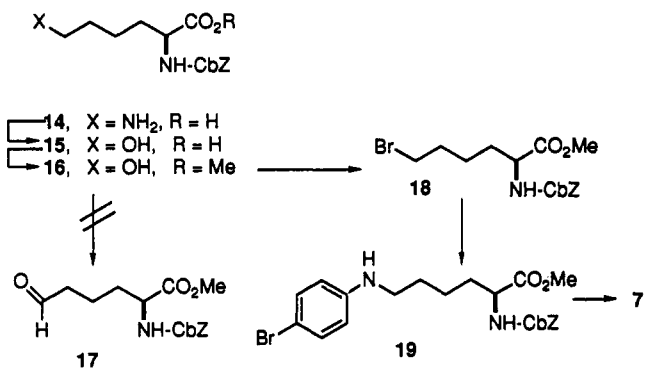
Scheme 1



Scheme 2



Scheme 3



dine and 2,4-dinitrofluorobenzene, but in neither case was the product well-characterized. Nevertheless, this approach seemed reasonable for the introduction of an aryl substituent onto the τ-nitrogen of histidine, so we adapted it for the synthesis of **8**, which was ultimately achieved as shown in Scheme 2. Compound **7** was obtained as outlined in Scheme 3. Both of these routes have the advantage of starting with the natural amino acids and preserving their chirality, an important consideration in view of their intended immunological applications.

Synthesis of N^{τ}-(*p*-Bromophenyl)histidine (8). *N*-Acetyl-L-histidine methyl ester (**9**) was prepared as reported[19] by passing HCl gas into a solution of *N*-acetyl-L-histidine in dry methanol. This procedure was much more efficient than the acetylation of L-His-OMe with acetic anhydride and pyridine; the latter gave mixtures from which **9** could only be obtained in low yield after repeated chromatography. *p*-Fluoronitrobenzene reacted smoothly with **9** in DMF over anhydrous potassium carbonate to produce **10** in good yield. As expected[20] electrophilic attack on **9** occurred exclusively at the less-hindered N^{τ} imidazole nitrogen giving rise to a single regioisomer of **10**. Catalytic hydrogenation of **10** over Pd/charcoal gave a 3:1 mixture of two products which were readily separated by silica chromatography. The major (higher R_f) product recrystallized easily from ethyl acetate with addition of pentane, while the minor (lower R_f) product was a hygroscopic solid which could be recrystallized as an oxalate salt. Both products gave similar but clearly not identical ^{1}H-NMR spectra which indicated the presence of a *p*-nitrophenyl moiety and an N-Ac-His-OMe moiety in each. Their mass spectra, which were nearly identical, confirmed that they were indeed isomers of N^{α}-acetyl-N^{im}-(*p*-nitrophenyl)histidine methyl ester. Assignment of structure **11a** to the major isomer and structure **11b** to the minor isomer was based on NOE studies. In the case of the major isomer **11a** the phenyl protons $H_{aa'}$ gave a nearly equal NOE to both imidazole protons, whereas with the minor isomer **11b** the same protons gave a 4-fold greater NOE to H_c than to H_d.

The apparent rearrangement of **10** during catalytic hydrogenation, involving the apparent migration of a phenyl ring from the τ-nitrogen to the π-nitrogen of the imidazole ring, was unexpected. As far as we could determine such a rearrangement is unprecedented in the literature. To verify that **10** was in fact a single regioisomer, and that the formation of **11b** during catalytic hydrogenation involved a molecular rearrangement, **10** was subjected to reduction with stannous chloride in ethanol.[21] This gave a single product which was identical to **11a** as judged by TLC, ^{13}C-NMR, and ^{1}H-NMR including NOESY. To investigate the rearrangement process further, **10** and **11a** were exposed separately to Pd/charcoal in ethanol in the absence of hydrogen gas for several hours, but TLC and NMR analysis indicated no change. Reexposure of **11a** to the hydrogenation conditions for several hours also failed to result in formation of isomer **11b**. Thus the rearrangement occurred at some intermediate stage of the hydrogenation process.

Conversion of **11a** to the bromophenyl derivative **12** was readily achieved by a deaminative-substitution procedure using *tert*-butyl nitrite and cupric bromide.[22] Hydrolysis of **12** in 1.0 M NaOH, followed by product isolation and purification by C-18 reverse-phase chromatography, afforded N^{α}-acetyl-N^{τ}-(*p*-bromophenyl)histidine (**13**) in good yield.[23] Deprotection of **13** in 6.0 M HCl at 110 °C for 12 h afforded amino acid **8** in 92% yield, which also demonstrated that **8** is stable under protein hydrolysis conditions.

Synthesis of N^{ϵ}-(*p*-Bromophenyl)-L-lysine (7). Our first approach to **7** was based on reduction of the Schiff base obtained by condensation of *p*-bromoaniline with ϵ-lysinal (**17**) as suggested in Scheme 3. To this end N^{α}-Cbz-L-lysine (**14**)[24] was diazotized with sodium nitroprusside dihydrate at pH 9.5 to generate alcohol **15**, which was esterified with diazomethane and purified by chromatography before characterization as **16**. Numerous attempts to convert **16** to aldehyde **17** by oxidation with

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pyridinium chlorochromate or dichromate, or by Swern oxidation, led to complex mixtures of **17** plus other products. The low yields of **17** and its apparent instability during chromatography on silica gel led us to abandon this approach. Instead, *p*-bromoaniline (in large excess) was N-alkylated with bromide **18**, obtained by the action of CBr_4/Ph_3P on alcohol **16**, giving **19** in 14% yield from L-lysine. Deprotection of **19** in 6.0 M HCl at 110 °C for 12 h afforded **7** in 92% yield, which also demonstrated that **7** is stable under protein hydrolysis conditions.

Finally, N^{α}-acetyl-N^{ϵ}-(*p*-bromophenyl)-L-lysine (**23**) was prepared from **18** in 20% overall yield by a sequence of deprotection (HBr/HOAc), N-acetylation (AcCl/pyridine) and N-alkylation of *p*-bromoaniline with the resulting bromolysine derivative, and hydrolysis of the methyl ester group.[23]

Experimental Section

Experimental procedures and sources of materials were essentially as described previously.[12] In reporting mass spectral data an asterisk (*) signifies the ^{79}Br peak of a 1:1 isotopic doublet of a bromine-containing fragment ion; relative intensities are given in parentheses.

N^{α}-Acetyl-N^{τ}-(*p*-nitrophenyl)-L-histidine Methyl Ester (10). N-Acetyl-L-histidine methyl ester (**9**, 3.54 g, 16.8 mmol), *p*-fluoronitrobenzene (2.14 mL, 20 mmol), and potassium carbonate (5.1 g, 36.8 mmol) were combined in DMF (8 mL) and heated to 110 °C for 13 h, after which the reaction mixture was cooled and the DMF partially evaporated on a rotary evaporator. The residue was partitioned between brine (50 mL) and ethyl acetate (4 × 70 mL) and the organic phase dried over sodium sulfate. After chromatography (silica gel, 10% methanol in chloroform), compound **10** was obtained as an oil (3.9 g, 68%) which solidified upon standing; recrystallization from ethyl acetate gave colorless crystals (mp 159 °C). ^1H NMR ($CDCl_3$) δ 8.40 (d, 2H), 7.92 (s, 1H), 7.56 (d, 2H), 7.05 (bs, 1H), 7.20 (s, 1H), 4.94 (m, 1H), 3.76 (s, 3H), 3.19 (ABX system, 2H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.9, 169.9, 146.1, 141.8, 140.2, 135.0, 126.0, 120.5, 115.0, 52.4, 52.0, 29.9, 23.2; EIMS, M^+ = 332 (20), 273 (45), 242 (20), 231 (40), 202 (30), 185 (12), 157 (20), 129 (18); CIMS (NH_3) MH^+ = 333 (80). Anal. Calcd for $C_{15}H_{16}N_4O_5$: C, 54.22; H, 4.85; N, 16.86. Found: C, 54.22; H, 5.10; N, 16.90.

Reduction of Compound 10. Compound **10** (2.0 g, 6 mmol) and 5% palladium on charcoal (50% moist, 0.6 g) were combined in ethanol (30 mL) and hydrogenated (40 psi H_2) for 2.5 h at ambient temperature. The catalyst was removed by filtration and the solvent evaporated giving an oily residue (1.6 g) which showed two spots on TLC ($CHCl_3$; R_f 0.44 and 0.22). Flash chromatography on silica gel (50 g), eluting with 5% MeOH in $CHCl_3$, afforded 0.85 g of the high-R_f product (**11a**) and 0.30 g of the low-R_f product. The former was recrystallized from ethyl acetate with addition of pentane (mp 142–143 °C), but the latter was hygroscopic so it was converted to an oxalate salt and recrystallized from ethanol with addition of ether (mp 182–183 °C).

Alternatively, compound **10** (200 mg, 0.6 mmol) was dissolved in 5 mL of absolute ethanol, $SnCl_2 \cdot 2H_2O$ (679 mg, 3 mmol) was added, and the mixture was heated at 70 °C for 1 h and then poured onto ice. The aqueous phase was adjusted to pH 8 by addition of 10% sodium bicarbonate and concentrated to a volume of 3 mL. Extraction of the aqueous phase in 3 × 20 mL chloroform, drying, and concentration gave 135 mg (74%) of compound **11a**.

N^{α}-Acetyl-N^{τ}-(*p*-aminophenyl)-L-histidine methyl ester (11a): ^1H NMR ($CDCl_3$) δ 7.65 (s, 1H, H_c), 7.25 (d,1H), 7.15 (d, 2H, $H_{aa'}$), 6.95 (s, 1H, H_d), 6.75 (d, 2H), 4.90 (m, 1H), 3.85 (bs, 2H), 3.72 (s, 3H), 3.12 (ABX, 2H), 2.1 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.1, 170.2, 146.8, 137.9, 135.5, 128.0, 123.0, 116.5, 115.5, 52.8, 52.0, 29.5, 23; CIMS (NH_3) MH^+ = 303 (85), 243 (90), 201 (25), 173 (60), 108 (15). Anal. Calcd for $C_{15}H_{18}N_4O_3$: C, 59.59; H, 6.00; N, 18.53. Found: C, 59.29; H, 6.00; N, 18.20.

N^{α}-Acetyl-N^{τ}-(*p*-aminophenyl)-L-histidine methyl ester (11b): ^1H NMR ($CDCl_3$) δ 7.67 (s, 1H, H_c), 7.23 (d, 2H, $H_{aa'}$), 7.19 (d, 1H), 7.08 (d, 2H), 6.99 (s, 1H, H_d), 4.88 (m, 1H), 3.73 (s, 3H), 3.16 (ABX, 2H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.2, 170.4, 150.8, 137.9, 135.4, 130.5, 122.0, 116.6, 114.8, 52.6, 52.4, 30.0, 22.8; CIMS (NH_3) MH^+ = 303 (48), 243 (65), 201 (15), 173 (35), 108 (35).

N^{α}-Acetyl-N^{τ}-(*p*-bromophenyl)-L-histidine Methyl Ester (12). *tert*-Butyl nitrite (0.79 mL, 3.90 mmol) and cupric bromide (1.06 g, 4.76 mmol) were combined in acetonitrile (5 mL) and warmed to 65 °C under nitrogen. Compound **11a** (1.20 g, 3.97 mmol) in 5 mL of acetonitrile was introduced as a slurry over 10 min, during which time the evolution of a gas was observed. The resulting dark colored solution was stirred for additional 15 min and the solvent removed under vacuum. The crude residue was chromatographed twice on florosil (60 g), eluting with 0–5% MeOH in $CHCl_3$ to obtain 0.73 g (50%) of compound **12**. Recrystallization from ethyl acetate with addition of pentane gave pale yellow needles (mp 130–131 °C). ^1H NMR ($CDCl_3$) δ 7.68 (s, 1H), 7.50 (d, 2H), 7.21 (d, 1H), 7.16 (d, 2H), 6.99 (s, 1H), 4.78 (m, 1H), 3.63 (s, 3H), 3.05 (ABX, 2H), 1.96 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.0, 170.1, 139.1, 136.0, 135.0, 133.0, 122.6, 120.9, 115.6, 52.32, 52.23, 29.66, 23.16; CIMS (NH_3) MH^+ 366* (90), 306* (50), 264* (20), 235* (30), 155 (10), 129 (23). Anal. Calcd for $C_{15}H_{16}N_3O_3Br$: C, 49.20; H, 4.40; N, 11.47. Found: C, 48.95; H, 4.30; N, 11.26.

N^{α}-Acetyl-N^{τ}-(*p*-bromophenyl)-L-histidine (13). Compound **12** (500 mg, 1.36 mmol) and 1.0 M aqueous NaOH (3 mL) were combined and stirred for 16 h at rt. After careful acidification to pH 5 with 6 M HCl, extraction of the product with chloroform proved ineffective. Therefore the aqueous phase was concentrated to 1 mL volume and applied to a bed of C-18 reverse-phase bonded silica gel (15 g) which had previously been washed with methanol and then water. The column was eluted with water (40 mL) and then methanol (50 mL); on concentration the methanol fraction gave compound **13** as a white crystalline solid (120 mg, 31%): mp 169–170 °C; $[\alpha]^{23}_D$ = +40.6° (*c* 0.5, MeOH); ^1H NMR (CD_3OD) δ 8.20 (s, 1H), 7.65 (d, 2H), 7.56 (d, 2H), 7.43 (s, 1H), 4.72 (m, 1H), 3.29 (ABX, 1H), 3.16 (ABX, 1H), 1.95 (s, 3H); ^{13}C NMR (CD_3OD) δ 174.7, 139.0, 135.7, 135.3, 130.8, 125.4, 123.56, 112.0, 32.5, 24.0; CIMS (NH_3) MH^+ 352* (60), 306* (15), 264* (25), 248* (25), 235* (26), 129 (30); FAB negative, M – H^- 350*. Anal. Calcd for $C_{14}H_{14}N_3O_3Br \cdot H_2O$: C, 45.42; H, 4.36; N, 11.35. Found: C, 45.65; H, 4.20; N, 11.10.

N^{τ}-(*p*-Bromophenyl)-L-histidine Hydrochloride (8·HCl). Compound **12** (10 mg, 0.02 mmol) was heated in 6.0 M HCl (0.5 mL) at 110 °C for 11 h and the excess acid removed under vacuum giving the HCl salt of compound **8** (10 mg, 92%) as a white crystalline but hygroscopic solid: ^1H NMR (CD_3OD) δ 9.5 (s, 1H), 8.12 (s, 1H) , 7.89 (d, 2H), 7.75 (d, 2H), 4.48 (m, 1H), 3.56 (ABX, 2H); CIMS (NH_3) MH^+ 310*; FAB-MS negative M – H^- 308*.

(2*S*)-2-[(Benzyloxycarbonyl)amino]-6-hydroxyhexanoic Acid Methyl Ester (16). N^{α}-Cbz-L-lysine (**14**, 10.5 g, 37.5 mmol) was dissolved in water (150 mL) and the solution warmed to 60 °C and adjusted to pH 9.5 with 4 M NaOH. Sodium nitroprusside dihydrate (20.0 g, 67.1 mmol) was added slowly with vigorous stirring over 45 min. Throughout the addition, the pH was maintained at 9.5 by addition of 4 M NaOH and the temperature at 60 °C. The red brown slurry was stirred at 60 °C and pH 9.5 for a further 6 h. The mixture was filtered through celite, acidified with hydrochloric acid to pH 1 at 0 °C, and extracted with ethyl acetate (3 × 100 mL). The combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated giving 9.5 g of crude **15** as a yellow oil. This oil was dissolved in a 1:1 mixture of ether and ethyl acetate (400 mL total), and diazomethane (3.0 g) in 250 mL ether was added. After standing overnight the solvent was removed and the oily residue thus obtained was chromatographed over silica gel (70 g), eluting with 50% pentane in ethyl acetate and then 10% methanol in ethyl acetate, to obtain **16** (3.79 g, 35%) as a yellowish oil: ^1H-NMR ($CDCl_3$) δ 7.40 (m, 5H), 5.42 (d, 1H), 5.15 (s, 2H), 4.43 (m, 1H), 3.78 (s, 3H), 3.65 (t, 2H), 3.50 (bs, 1H), 1.95–1.2 (m, 6H); ^{13}C-NMR ($CDCl_3$) δ 173.0, 155.8, 136.2, 128.4, 127.5, 67.10, 62.2, 53.5, 52.8, 32.5, 32.0, 21.8; CIMS (NH_3) MH^+ = 296 (2), 252 (11), 236 (3), 220 (3), 192 (6), 156 (4), 128 (8), 108 (5), 91 (9). Anal. Calcd for $C_{15}H_{21}NO_5$: C, 61.0; H, 7.17; N, 4.74. Found: C, 60.58; H, 7.30; N, 4.96.

(2S)-2-[(Benzyloxycarbonyl)amino]-6-bromohexanoic Acid Methyl Ester (18). Alcohol **16** (1.0 g, 3.38 mmol) and CBr_4 (1.40 g, 4.23 mmol) were combined in dichloromethane (5 mL) and cooled to 0 °C with stirring. Triphenylphosphine (1.33 g, 5.08 mmol) was added over 10 min and the mixture stirred for additional 30 min. The solvent was evaporated and the residue chromatographed on silica gel (50 g). Elution with 40% ethyl acetate in pentane gave **18** (1.06 g, 87%) as an oil: ^{1}H-NMR ($CDCl_3$): 7.36 (m, 5H), 5.51 (d, 1H), 5.13 (s, 2H), 4.40 (m, 1H), 3.76 (s, 3H), 3.39 (t, 2H), 1.88–1.82 (m, 2H), 1.71–1.68 (m, 2H), 1.53–1.48 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ 172.8, 155.9, 136.2, 128.5, 128.2, 128.1, 67.0, 53.6, 52.4, 33.2, 32.0, 31.6, 23.7; CIMS (NH_3) MH^+ = 358* (1), 314* (2), 298* (2), 278 (15), 234 (30), 218 (35), 174 (55), 142 (25), 108 (35), 91 (90).

N^{α}-(Benzyloxycarbonyl)-N^{ϵ}-(*p*-bromophenyl)-L-lysine Methyl Ester (19). Compound **18** (1.0 g, 2.79 mmol), *p*-bromoaniline (1.921 g, 11.1 mmol), and potassium carbonate (570 mg, 4.13 mmol) were combined in acetonitrile (5 mL) and refluxed for 10 h. The mixture was filtered, the filtrate concentrated, and the residue thus obtained chromatographed on silica gel (60 g). Eluting with 20–30% ethyl acetate in pentane gave compound **19** (790 mg, 63%) as a white solid which was recrystallized from 40% ethyl acetate in pentane: mp 42–43 °C; $[\alpha]^{23}_D$ = −11.52° (*c* 0.5, MeOH); ^{1}H-NMR ($CDCl_3$) δ 7.38 (m, 5H), 7.25 (d, 2H), 6.45 (d, 2H), 5.38 (d, 1H), 5.12 (s, 2H), 4.41 (m, 1H), 3.78 (s, 3H), 3.70 (bs, 1H), 3.05 (t, 2H), 1.95–1.36 (m, 6H); ^{13}C-NMR ($CDCl_3$) 172.9, 155.9, 147.3, 136.2, 131.9, 128.6, 128.3, 128.2, 114.2, 108.7, 67.1, 53.6, 52.5, 43.6, 32.6, 28.8, 22.8; CIMS (NH_3) δ MH^+ = 449* (20), 341* (55), 184* (75), 142 (15), 108 (90), 91 (88), 77 (87). Anal. Calcd for $C_{21}H_{25}N_2O_4Br$: C, 56.13: H, 5.61; N, 6.23. Found: C, 56.33; H, 5.69; N, 6.19.

N^{ϵ}-(*p*-Bromophenyl)-L-lysine Hydrochloride (7·HCl). Compound **19** (45 mg, 0.010 mmol) and 6.0 M HCl (2 mL) were heated at 110 °C for 11 h, and the reaction mixture was dried under vacuum at 70 °C giving the hydrochloride salt of compound **7** (34 mg, 92%) as a hygroscopic white solid: ^{1}H-NMR (CD_3OD) δ 7.70 (d, 2H), 7.45 (d, 2H), 4.04 (m, 1H), 3.43 (t, 2H), 2.03 (m, 2H), 1.76 (m, 2H), 1.63 (m, 2H); ^{13}C-NMR (CD_3OD) δ 173.2, 139.2, 136.0, 126.3, 124.3, 55.2, 53.4, 32.6, 28.5, 24.8; CIMS (NH_3) MH^+ = 301* (30), 257* (5), 238* (30), 223 (12) 184* (55), 171* (50), 160 (15), 130 (23), 106 (35), 93 (50).

(2S)-2-Amino-6-bromohexanoic Acid Methyl Ester Hydrobromide (20). Compound **18** (2.0 g, 5.6 mmol) and 2 mL of HBr in acetic acid (32–34%) were stirred for 1 h at rt. Ether (50 mL) was added and the resulting cloudy mixture cooled to 5 °C for 1 h. The solvent was decanted and concentrated to ca. 10 mL to obtain more precipitate. The precipitates were combined, washed with ether, and dried in vacuum at 50 °C overnight to give compound **20** (1.54 g, 90%) as a white crystalline solid: mp 117–118 °C; ^{1}H-NMR (CD_3OD) δ 4.10 (t, 1H), 3.85 (s, 3H), 3.49 (t, 2H), 1.96–1.91 (m, 4H), 1.89–1.86 (m, 2H); ^{13}C-NMR (CD_3OD) δ 172.4, 55.4, 55.3, 35.2, 34.7, 32.1, 26.1; CIMS (NH_3) MH^+ = 224* (95), 164* (20), 144 (10). Anal. Calcd for $C_7H_{15}NO_2Br_2$: C, 27.56; H, 4.95; N, 4.59. Found: C, 27.48; H, 4.90; N, 4.38.

(2S)-2-(Acetamido)-6-bromohexanoic Acid Methyl Ester (21). Compound **20** (1.0 g, 3.30 mmol) and pyridine (0.59 mL, 7.24 mmol) were combined in dichloromethane (5 mL) and cooled to 0 °C. Acetyl chloride (0.281 mL, 3.96 mmol) was introduced under nitrogen during 5 min and the resulting mixture stirred at 0 °C for 10 min and at rt for 30 min before pouring over 5 mL of ice-cold water. The organic phase was separated and the aqueous phase extracted with dichloromethane (3 × 5 mL). The combined organic extracts were washed with 1 M HCl (10 mL) and water (10 mL), dried over sodium sulfate, and evaporated yielding **21** (0.830 g, 95%) as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ 6.43 (d, 1H), 4.63 (m, 1H), 3.76 (s, 3H), 3.41 (t, 2H), 2.04 (s, 3H), 1.93–1.45 (m, 6H); ^{13}C-NMR ($CDCl_3$) δ 172.9, 170.0, 52.4, 51.8, 33.2, 31.9, 31.4, 23.7, 23.0; CIMS (NH_3) MH^+ = 266* (80), 164* (10). Anal. Calcd for $C_9H_{16}NO_3Br$: C, 40.62; H, 6.05; N, 5.26. Found: C, 40.70; H, 6.00; N, 5.18.

N^{α}-Acetyl-N^{ϵ}-(*p*-bromophenyl)-L-lysine Methyl Ester (22). Compound **21** (0.921 g, 3.47 mmol), *p*-bromoaniline (2.39 g, 13.90 mmol), and potassium carbonate (0.960 g, 6.95 mmol) were combined in acetonitrile (10 mL) and refluxed for 20 h. The mixture was filtered, the filtrate concentrated, and the residue thus obtained chromatographed on silica gel (50 g). Elution with 10–50% ethyl acetate in pentane gave compound **22** (0.640 g, 52%) as a white solid which was recrystallized from ethyl acetate–pentane: mp 101–102 °C; ^{1}H-NMR ($CDCl_3$) δ 7.24 (d, 2H), 6.47 (d, 2H), 6.07 (d, 1H), 4.66 (m, 1H), 3.76 (s, 3H), 3.08 (t, 2H), 2.04 (s, 3H), 1.91–1.40 (m, 6H); ^{13}C-NMR ($CDCl_3$) δ 173.0, 169.8, 147.3, 131.8, 114.1, 108.6, 52.4, 51.8, 43.5, 32.5, 28.7, 23.2, 22.7; CIMS (NH_3) MH^+ = 357* (90), 269 (10), 246 (20), 184* (20). Anal. Calcd for $C_{15}H_{21}N_2O_3Br$: C, 50.43; H, 5.93, N, 7.84. Found: C, 50.40; H, 6.12; N, 7.69.

N^{α}-Acetyl-N^{ϵ}-(*p*-bromophenyl)-L-lysine (23). Compound **22** (400 mg, 1.12 mmol) and NaOH solution (1.0 M, 4 mL) were stirred for 16 h at rt. The mixture was then brought to pH 5 with 6 M HCl, concentrated to 1 mL, and extraced with chloroform (5 × 10 mL). As the extraction with chloroform proved inefficient, the aqueous phase was evaporated to dryness and the residue chromatographed over silica gel (30 g). Eluting with 10–30% methanol in chloroform gave **23** (175 mg, 45%) as an oil which crystallized upon trituration with chloroform. The product was recrystallized from water: mp 143–144 °C; $[\alpha]^{23}_D$ = +6.8° (*c* 0.5, MeOH); ^{1}H-NMR (CD_3OD) δ 7.89 (s, 1H), 7.19 (d, 2H), 6.51 (d, 2H), 4.42 (m, 1H), 3.07 (ABX, 2H), 2.01 (s, 3H), 1.90–1.51 (m, 6H); ^{13}C-NMR (CD_3OD) δ 184.0, 175.0, 151.0, 134.2, 116.9, 110.4, 46.0, 34.0, 31.2, 26.1, 24.0; CIMS (NH_3) 343* (100), 265 (20), 238* (10), 184* (25). Anal. Calcd for $C_{14}H_{19}N_2O_3Br$: C, 48.99; H, 5.58; N, 8.16. Found: C, 44.86; H, 5.56; N, 8.14.

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