Protein Engineering: Design of Single-Residue-Anchored Metal-Uptake Systems

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Introduction

During the past decade, we have examined the profile of the side chains of coded amino acids from the vantage of protein evolution and protein engineering as well as strategies for augmenting their versatility.[1] None of the coded amino acid side chains has independent metal-uptake potential. In the domain of copper proteins,[2] vital operations involving metal ion participation are accomplished by the binding region, generally involving a span of amino acid residues, needed to align the ligand sites correctly. The present work reports the crafting of metal-uptake sites around a single coded amino acid side chain, by suitable modification. This would amount to a "minimalistic designed approach" to metalloenzyme mimics, wherein the necessary metal ion would be placed on the side chain of a single residue, rather than a combination that would require a span of amino acids.[3] The first syntheses of modified tyrosine analogues, which have independent metal ion uptake potential and which can be incorporated into peptide segments by the usual procedures, are presented.[4]

Experimental Section[5]

Materials and Methods. All amino acids used were of the L configuration. Chiral HPLC analysis was performed on a Shimadzu LC instrument with a Waters variable UV detector at 254 nm. ^{1}H NMR spectra were recorded at 60 MHz (Hitachi R600) or 80 MHz (Bruker WP 80). IR spectra were recorded on PE 580, PE 1600 and FT-IR instruments. Electronic spectra were recorded using a PE Lambda-2 UV−vis spectrophotometer. Optical rotations were measured using an automatic JASCO digital polarimeter. The FAB mass spectra (*m/z* values) were recorded on samples in a matrix of *m*-nitrobenzyl alcohol using a JEOL SX 102/DA 6000 mass spectrometer. EPR spectra were recorded on a Varian E-109 spectrometer operating at the X-band using 2,2-bis(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) as the external standard. Elemental analyses were carried out on an automatic C, H, N analyzer. The organic extracts were invariably dried over anhydrous $MgSO_4/Na_2SO_4$, and solvents were evaporated in vacuo. Reactions were monitored wherever possible by thin-layer chromatography (TLC) using silica gel (Merk).

Acetylacetone−Ethylenediamine Mono(Schiff base) (AEH).[6] Under vigorous stirring, a solution of acetylacetone (10.3 mL), 100 mmol) in $CHCl_3$ (50 mL) was added in drops to a solution of ethylenediamine (6.7 mL, 100 mmol) in $CHCl_3$ (100 mL). The reaction mixture was left to stir for 10 h, separated water was removed, the solvent was evaporated in vacuo, and the crude product (11.9 g, 84%) was used without further purification. IR (neat) (cm^{-1}): 3289, 1734, 1609, 1560, 1437, 1325. ^{1}H NMR ($CDCl_3$): δ 1.68 (m, 2H, NH_2), 2.02 (s, 6H, $CH_3 \times 2$), 2.9 (m, 2H, H_2N-CH_2), 3.38 (m, 2H, $=NCH_2$), 5.03 (s, 1H, CH), 10.97 (s, 1H, OH).

3-Acetyltyrosine Hydrochloride [Tyr(3-Ac)HCl].[7] Yield: 79%. Mp: 220−223 °C dec (lit.[7] mp 220−224 °C). HPLC: analysis using Phenominex G 3020 column, MeOH:H_2O = 75:25, flow rate 1 mL/min, retention time 3.831 min; MeOH, flow rate 1 mL/min, retention time 5.356 min; Phenominex G 3014 column, MeOH:H_2O = 75:25, flow rate 0.6 mL/min, retention time 3.452 min, reported single peaks. IR (KBr) (cm^{-1}): 3000 (br), 1742, 1640, 1582, 1518, 1499. ^{1}H NMR (D_2O): δ 2.3 (s, 3H, $COCH_3$), 3.0 (d, 2H, $C^{\beta}H_2$), 4.12 (t, 1H, $C^{\alpha}H$), 6.71 (d, 1H, Tyr C^5H), 7.26 (d, 1H, Tyr C^6H), 7.54 (s, 1H, Tyr C^2H). FAB MS: 224 $(M + H)^+ - HCl$. $[\alpha]^{25}_D$ −2.33° (*c* 1.0, MeOH)

General Procedures

A. Synthesis of Peptides. 1-Hydroxybenzotriazole (HOBt, 1 mmol) and dicyclohexylcarbodiimide (DCC, 1 mmol) were added sequentially at 0 °C to a stirred solution of N-protected amino acid (1 mmol) in dry CH_2Cl_2 (20 mL) or in dry DMF (5 mL). After a period of 0.25 h, the reaction mixture was admixed with the amino acid methyl ester, prepared at 0 °C from the corresponding ester hydrochloride (1 mmol) and triethylamine (1.2 mmol) in dry CH_2Cl_2 or dry DMF. The combined mixture was left to stir at room temperature for 48 h, the precipitated DC urea was filtered off, the residue was washed with CH_2Cl_2 (2×10 mL), and the combined filtrates were washed with cold 2 N H_2SO_4 (20 mL) and saturated $NaHCO_3$ (20 mL). The organic extract was dried and evaporated in vacuo. The residue was directly crystallized from an EtOAc−hexane mixture or purified on a short column of silica gel using PhH−EtOAc as eluent.

B. 3-Acetyltyrosine−AEH Ligand Precursors. 1. By Reaction with AEH. A solution of 3-acetyltyrosine-containing

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(3) The proteolytic enzyme papain has been successfully transformed to redox-active flavopapain by positioning of the redox-active site close to the substrate binding site (Kaiser, E. T. *Angew. Chem., Int. Ed. Engl*. **1988**, *27*, 913). Therefore, it is likely that when a metal-binding site can be crafted in close proximity to the substrate-binding site of a redox-active metalloprotein—in place of the natural one—the expected metalloenzyme activity would be observed.

(4) A preliminary account peripherally covering portions of the present work has been published.[1c]

(5) The methodology is illustrated with the tripeptide Ala-Tyr(3-Ac)-Ser and with the formation of the ligand−copper complexes. The protocols have been generalized with a number of examples leading to, in addition to copper, nickel and cobalt complexes. These are available in the Supporting Information.

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compound (1 mmol) in MeOH (15 mL) was admixed with acetylacetone−ethylenediamine mono(Schiff base) (AEH) (1 mmol) in MeOH (5 mL). The reaction mixture was left to stir at 25 °C for 10 h, evaporated, and crystallized from MeOH.

2. By Assembly. Under vigorous stirring, a solution of acetylacetone (1 mmol) in $CHCl_3$ (50 mL) was added dropwise to a solution of ethylenediamine (EDA) (1 mmol) in $CHCl_3$ (50 mL). The reaction mixture was left to stir for 0.5 h, and a solution of the 3-acetyltyrosine containing compound (1 mmol) in $CHCl_3$ (50 mL) was added dropwise. The mixture was stirred for 10 h, solvent was evaporated, and the residue was crystallized from methanol.

C. 3-(Oximinoacetyl)tyrosine Ligand Precursors. A stirred mixture of 3-acetyltyrosine containing compound (1 mmol) and hydroxylamine hydrochloride (2 mmol) in MeOH (15 mL) was treated with an aqueous solution of $NaHCO_3$ (2 mmol in 3 mL) at 25 °C for 12 h. Solvent was evaporated, and the residue was extracted with EtOAc (15 mL), washed with water (2 × 5 mL), dried, evaporated, and crystallized from MeOH−H_2O (3:1) to afford the oximino compounds.

D. 3-Acetyltyrosine−EDA Ligand Precursors. A solution of 3-acetyltyrosine-containing compound (2 mmol) in MeOH (20 mL) was admixed with ethylenediamine (EDA) (1 mmol) in MeOH (5 mL). The reaction mixture was held at 50 °C for 2 h and then at 25 °C for 10 h. The generally crystalline solids were filtered off and dried.

E. Copper−Ligand Complexes. A solution of the ligand precursor in MeOH−CH_3CN was mixed with an equivalent amount of $Cu(OAc)_2$ in MeOH, the mixture held at 60 °C for 0.5 h, solvent evaporated, and the metal complex crystallized from MeOH.

***N*-(*tert*-Butyloxycarbonyl)alanyl-3-acetyltyrosylserine Methyl Ester [Boc-Ala-Tyr(3-Ac)-Ser-OMe] (1). Boc-Ala-Tyr(3-Ac)-OMe.** This was prepared from Boc-Ala[8] (1.6 g, 8.5 mmol) and Tyr(3-Ac)-OMe·HCl[7] (2.45 g, 9 mmol) (general procedure A). Yield: 2.31 g (67%). Mp: 106−107 °C. IR (KBr) (cm^{-1}): 3340, 2982, 1750, 1685, 1651, 1525. ^1H NMR ($CDCl_3$): δ 1.28 (d, 3H, Ala CH_3), 1.37 (s, 9H, Boc CH_3), 2.63 (s, 3H, $COCH_3$), 3.10 (d, 2H, Tyr C$^\beta$H$_2$), 3.75 (s, 3H, $COOCH_3$), 4.06 (m, 1H, Ala, C$^\alpha$H), 4.84 (m, 2H, Tyr C$^\alpha$H + Ala NH), 6.63 (d, 1H, Tyr NH), 6.90 (d, 1H, Tyr C^{5}H), 7.22 (d, 1H, Tyr C^{6}H), 7.53 (s, 1H, Tyr C^{2}H), 11.16 (s, 1H, OH). FAB MS: 409 $(M + H)^+$. $[\alpha]^{25}_D$ −5.96° (*c* 1.0, MeOH). Anal. Calcd for $C_{20}H_{28}N_2O_7$: C, 58.80; H, 6.86; N, 6.86. Found: C, 58.89; H, 7.02; N, 7.13.

Boc-Ala-Tyr(3-Ac)-OH. A solution of Boc-Ala-Tyr(3-Ac)-OMe (1.0 g, 2.45 mmol) in MeOH (15 mL) was cooled to 0 °C, treated with aqueous NaOH (2 N, 7 mL) and stirred at room temperature for 4 h. The reaction mixture was concentrated to ∼7 mL (without heating) in vacuo, cooled in ice, acidified to pH 3 with 10% HCl, extracted with EtOAc (3 × 25 mL), dried, and evaporated to give 0.945 g (98%) of the acid, Boc-Ala-Tyr(3-Ac)-OH. IR (KBr) (cm^{-1}): 3349, 2979, 2933, 1716, 1644, 1521, 1488. ^1H NMR ($CDCl_3$−DMSO-d_6): δ 1.35 (d, 3H, Ala CH_3), 1.50 (s, 9H, Boc CH_3), 2.65 (s, 3H, $COCH_3$), 3.10 (d, 2H, Tyr C$^\beta$H$_2$), 4.20 (q, 1H, Ala C$^\alpha$H), 4.80 (q, 1H, Tyr C$^\alpha$H) 5.25 (d, 1H, Ala NH), 6.80 (d, 1H, Tyr NH), 7.00 (d, 1H, Tyr C^{5}H), 7.30 (d, 1H, Tyr C^{6}H), 7.55 (s, 1H, Tyr C^{2}H), 11.60 (s, 1H, phenolic OH). FAB MS: 395 $(M + H)^+$.

Boc-Ala-Tyr(3-Ac)-Ser-OMe (1). This was prepared from Boc-Ala-Tyr(3-Ac)-OH (0.9 g, 2.3 mmol) and Ser-OMe·HCl[9] (0.39 g, 2.5 mmol) by general procedure A. Yield: 0.72 g (64%). Mp: 172−173 °C. IR (KBr) (cm^{-1}): 3299, 2931, 1747, 1687, 1647, 1530. ^1H NMR ($CDCl_3$): δ 1.31 (d, 3H, Ala CH_3), 1.47 (s, 9H, Boc CH_3), 2.65 (s, 3H, $COCH_3$), 3.16 (d, 2H, Tyr C$^\beta$H$_2$), 3.65 (s, 3H, $COOCH_3$), 3.81 (d, 2H, Ser C$^\beta$H$_2$), 4.13 (m, 1H, Ala C$^\alpha$H), 4.69 (m, 2H, Tyr C$^\alpha$H + Ser C$^\alpha$H), 5.03 (d, 1H, Ala NH), 6.78−7.80 (m, 5H, aromatic + Tyr NH + Ser NH), 12.1 (s, 1H, phenolic OH). FAB MS: 496 $(M + H)^+$. $[\alpha]^{25}_D$ −21.0° (*c* 0.5, MeOH). Anal. Calcd for $C_{23}H_{33}N_3O_9$: C, 55.75; H, 6.66; N, 8.48. Found: C, 55.72; H, 6.54; N, 8.62.

***N*-(Benzyloxycarbonyl)alanyl-3-acetyltyrosylserine Methyl Ester [Z-Ala-Tyr(3-Ac)-Ser-OMe] (2).** Was prepared precisely as described for **1** (vide supra). Yield: 54%. Mp: 174−176 °C. IR (KBr) (cm^{-1}): 3319, 2927, 1743, 1687, 1641, 1538. ^1H NMR ($CDCl_3$): δ 1.34 (d, 3H, Ala CH_3), 2.69 (s, 3H, $COCH_3$), 3.06 (d, 2H, Tyr C$^\beta$H$_2$), 3.75 (s, 3H, $COOCH_3$), 3.84 (d, 2H, Ser C$^\beta$H$_2$), 4.13 (m, 1H, Ala C$^\alpha$H), 4.28−4.94 (m, 2H, Tyr C$^\alpha$H + Ser C$^\alpha$H), 5.06 (s, 2H, Z CH_2), 6.53 (d, 1H, Ala NH), 6.88 (d, 1H, Tyr C^{5}H), 7.09−7.94 (m, 9H, Tyr C^{6}H + Tyr C^{2}H + Z aromatic + Tyr NH + Ser NH), 12.16 (s, 1H, phenolic OH). FAB MS: 530 $(M + H)^+$. Anal. Calcd for $C_{26}H_{31}N_3O_9$: C, 58.97; H, 5.86; N, 7.93. Found: C, 58.46; H, 6.13; N, 8.17.

***N*-(*tert*-Butyloxycarbonyl)alanyl-3-acetyltyrosylserine Methyl Ester−AEH Ligand Precursor (3).** This was prepared from Boc-Ala-Tyr(3-Ac)-Ser-OMe (**1**) (0.04 g, 0.08 mmol) by general procedure B1. Yield: 0.036 g (72%). Yield from general procedure B2: 0.040 g (80%). Mp: 164−166 °C. IR (KBr) (cm^{-1}): 3276, 2928, 1743, 1668, 1635, 1537, 1485. ^1H NMR ($CDCl_3$): δ 1.34 (m, 12H, Ala CH_3 + Boc CH_3), 1.93 (s, 6H, AEH CH_3 × 2), 2.62 (s, 3H, N=CCH_3), 3.12 (d, 2H, Tyr C$^\beta$H$_2$), 3.43−4.25 (m, 10H, $COOCH_3$ + $-CH_2CH_2-$ + Ala C$^\alpha$H + Ser C$^\beta$H$_2$), 4.68 (m, 2H, Tyr C$^\alpha$H + Ser C$^\alpha$H), 5.00 (s, 1H, enolic CH), 5.31 (d, 1H, Ala NH), 6.78−7.78 (m, 5H, Tyr NH + Ser NH + aromatic), 10.93 (s, 1H, enolic OH). 12.15 (s, 1H, phenolic OH). FAB MS: 620 $(M + H)^+$. Anal. Calcd for $C_{30}H_{45}N_5O_9$: C, 58.15; H, 7.27; N, 11.31. Found: C, 58.31; H, 7.31; N, 11.70.

Copper−Ligand Complex 4. This was prepared from ligand precursor **3** (0.04 g, 0.064 mmol) and $Cu(OAc)_2·H_2O$ (0.014 g, 0.064 mmol) by general procedure E. Yield: 0.032 g (73%). Mp: 142−144 °C. IR (KBr) (cm^{-1}): 3410, 3290, 1730, 1632, 1580, 1503. EPR ($CHCl_3$, 298 K): $A_0 = 89 \times 10^{-4}$ cm^{-1}, $g_0 = 2.109$. EPR ($CHCl_3$, 77 K): $g_{||} = 2.186$, $g_\perp = 2.051$, $A_{||} = 209 \times 10^{-4}$ cm^{-1}, $A_\perp = 29 \times 10^{-4}$ cm^{-1}. FAB MS: 705 $(M + Na)^+$. Anal. Calcd for $C_{30}H_{43}N_5O_9Cu$: C, 52.90; H, 6.32; N, 10.29. Found: C, 52.51; H, 5.91; N, 9.89.

Ligand Precursor *N*-(*tert*-Butyloxycarbonyl)alanyl-3-oximinoacetyltyrosylserine Methyl Ester [Boc-Ala-Tyr(3-oximinoacetyl)-Ser-OMe] (5). This was prepared from Boc-Ala-Tyr(3-Ac)-Ser-OMe (**1**) (0.1 g, 0.2 mmol) by general procedure C. Yield: 0.086 g (83%). Mp: 104−106 °C. IR (KBr) (cm^{-1}): 3317, 2928, 1743, 1686, 1660, 1521. ^1H NMR ($CDCl_3$−DMSO-d_6): δ 1.28 (d, 3H, Ala CH_3), 1.44 (s, 9H, Boc CH_3), 2.31 (s, 3H, oximino−CH_3), 3.09 (d, 2H, Tyr C$^\beta$H$_2$), 3.78 (m, 5H, $COOCH_3$ + Ser C$^\beta$H$_2$), 4.09 (m, 1H, Ala C$^\alpha$H), 4.63 (m, 2H, Tyr C$^\alpha$H + Ser C$^\alpha$H), 5.75 (d, 1H, Ala NH), 6.75−7.68 (m, 5H, aromatic + Tyr NH + Ser NH), 10.80 (s, 1H, N−OH), 11.70 (s, 1H, phenolic OH). FAB MS: 511 $(M + H)^+$.

Copper−Ligand Complex 7. This was prepared from **5** (0.03 g, 0.058 mmol) and $Cu(OAc)_2·H_2O$ (0.006 g, 0.029 mmol) by general procedure E. Yield: 0.026 g (84%). Mp: 255−257 °C. IR (KBr) (cm^{-1}): 3315, 2977, 2929, 1744, 1652, 1510. EPR ($CHCl_3$, 298 K): $A_0 = 99 \times 10^{-4}$ cm^{-1}, $g_0 = 2.116$. EPR

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(DMSO, 77 K): $g_{||}$ 2.236, $g_{\perp} = 2.053$, $A_{||} = 203 \times 10^{-4}$ cm^{-1}, $A_{\perp} = 47 \times 10^{-4}$ cm^{-1}. UV−vis (DMSO): 263, 342, 631 nm. Anal. Calcd for $C_{46}H_{66}N_8O_{18}Cu$: C, 46.72; H, 5.59; N, 9.48. Found: C, 46.29; H, 5.32; N, 9.02.

Ligand Precursor *N*-(Benzyloxycarbonyl)alanyl-3-oximinoacetyltyrosylserine Methyl Ester [Z-Ala-Tyr(3-oximinoacetyl)-Ser-OMe] (6). This was prepared from **2** (0.15 g, 0.28 mmol) by general procedure C. Yield: 0.128 g (84%). Mp: 204−206 °C. IR (KBr) (cm^{-1}): 3450, 2948, 1720, 1678, 1650. FAB MS: 545 $(M + H)^+$. Anal. Calcd for $C_{26}H_{32}N_4O_9$: C, 57.35; H, 5.88; N, 10.29. Found: C, 57.63; H, 5.29; N, 10.53.

Copper−Ligand Complex 8. This was prepared from **6** (0.05 g, 0.092 mmol) and $Cu(OAc)_2 \cdot H_2O$ (0.009 g, 0.046 mmol) by general procedure E. Yield: 0.034 g (64%). Mp: 245−247 °C. IR (KBr) (cm^{-1}): 3288, 3064, 2928, 1744, 1694, 1646, 1541. EPR ($CHCl_3$ 278 K): $A_0 = 99 \times 10^{-4}$ cm^{-1}, $g_0 = 2.115$. EPR (DMSO, 77 K): $g_{||} = 2.220$, $g_{\perp} = 2.055$, $A_{||} = 202 \times 10^{-4}$ cm^{-1}, $A_{\perp} = 47.5 \times 10^{-4}$ cm^{-1}. UV−vis (DMSO): 260, 358, 423 (sh), 654 nm.

Bis *N*-(Benzyloxycarbonyl)alanyl-3-acetyltyrosylserine Methyl Ester−EDA Ligand Precursor 9. This was prepared from **2** (0.052 g, 0.10 mmol) by general procedure D. Yield: 0.047 g (89%). Mp: 249−251 °C. IR (KBr) (cm^{-1}): 3286, 1740, 1683, 1628, 1528. FAB MS: 1083 $(M + H)^+$. Anal. Calcd for $C_{54}H_{66}N_8O_{16}$: C, 59.88; H, 6.09; N, 10.35. Found: C, 60.32; H, 6.14; N, 10.88.

Copper−Ligand Complex 10. This was prepared from **9** (0.044 g, 0.04 mmol) and $Cu(OAc)_2 \cdot H_2O$ (0.008 g, 0.04 mmol) by general procedure E. Yield: 0.04 g (87%). Mp: 219−220 °C. IR (KBr) (cm^{-1}): 3303, 2926, 1744, 1653, 1588, 1532. EPR ($CHCl_3$, 278 K): $A_0 = 99 \times 10^{-4}$ cm^{-1}, $g_0 = 2.112$. EPR ($CHCl_3$, 77 K): $g_{||} = 2.205$, $g_{\perp} = 2.058$, $A_{||} = 206 \times 10^{-4}$ cm^{-1}, $A_{\perp} = 45.5 \times 10^{-4}$ cm^{-1}. FAB MS: 1167 $(M + Na)^+$, 1145 $(M + H)^+$. UV−vis (DMSO): 262, 368, 559 nm.

Results and Discussion

The ready preparation of compounds **1** and **2** showed that the 3-acetyltyrosine unit is amenable to incorporation into peptides by normal synthetic methodology.[10]

Attempts to prepare metal complexes, taking advantage of the *o*-hydroxy acetyl unit in **1** and **2** failed, thus necessitating modifications. These endeavors (vide infra) resulted in substrates derived from **1** and **2**, having ready metal-uptake[5] potential and having three types of structural profiles (Scheme 1). These are (1) the Schiff bases derived from AEH [acetylacetone−ethylenediamine (EDA) mono(Schiff base)] (**I**), (2) simple oximes (**II**), and (3) bis(Schiff bases) derived from EDA (**III**). It should be noted that whereas the metal complexes derived from AEH involve only a single unit of **1** or **2**, the others need two.

The transformation of 3-acetyltyrosine side chains to ligand precursors is illustrated in Scheme 1.

Of particular interest was the reaction of the 3-acetyltyrosine side chains with the mono(Schiff base) AEH, derived from ethylenediamine (EDA) and acetylacetone. The reaction afforded in uniformly good yields, ligand precursors **I** (Scheme 1). The formation of the mono(Schiff base) of acetylacetone with EDA was possible because strong intramolecular hydrogen bonding (Scheme 1) makes the second Schiff base formation difficult. From a practical vantage it is preferable to assemble **I**, in situ, by addition of the 3-acetyltyrosine unit to EDA to which 1 equiv of acetylacetone has been added. Thus 3-acetyltyrosine-containing peptide segments can be swiftly converted to metal uptake systems by in situ generated AEH.

The ready formation of ligand precursors from the peptide Ala-Tyr(3-Ac)-Ser and AEH is illustrated with the **1** → **3** change (Scheme 1), which can be brought about either by addition of preformed AEH or, invariably in about 5−10% higher yields, by assembly by total in situ operations. The NMR spectrum of **3** was in complete agreement with the structure proposed, and a noteworthy feature was the presence of the phenolic proton signal at 12.15 ppm and the enolic one at 10.93 ppm. The mass spectrum exhibited a sharp peak at *m/z* 620 corresponding to $(M + H)^+$. Compound **3** afforded copper complex **4** in 73% yield, which exhibited excellent EPR profiles at both 298 and 77 K. The appropriate parameters thus derived clearly show the compound is square planar. The FAB mass spectrum exhibited a base peak at *m/z* 705 corresponding to $(M + Na)^+$.

The ready formation of metal complexes from oximes derived from 3-acetyltyrosine side chains was an interesting development, in the light of the inability of the parent carbonyls to form such complexes. This is illustrated with the formation of oximes **5** and **6** from Ala-Tyr(3-Ac)-Ser-OMe (**1** and **2**). Thus tripeptides **1** and **2** gave the ligand precursor oximes **5** and **6** in respectively 83% and 84% yields, which have been fully characterized. These readily afforded copper complexes **7** and **8** in excellent yields. Both **7** and **8** gave the expected EPR profiles for square planar complexes at 77 and 298 K. The EPR at 298 K showed the characteristic four-line pattern and five-line hyperfine splitting of the fourth line, indicating metal coordination in these complexes is through two nitrogens and two oxygens. This profile is common for such complexes. The nature of coordination here places the oxime hydroxyl group anti to the metal center, and this could be taken advantage of to attach additional ligands. Thus, oximination followed by metal complexation of the 3-acetyltyrosine-containing peptides could provide a strategy for folding and dimerization of peptide segments and having avenues for further ligand attachment.

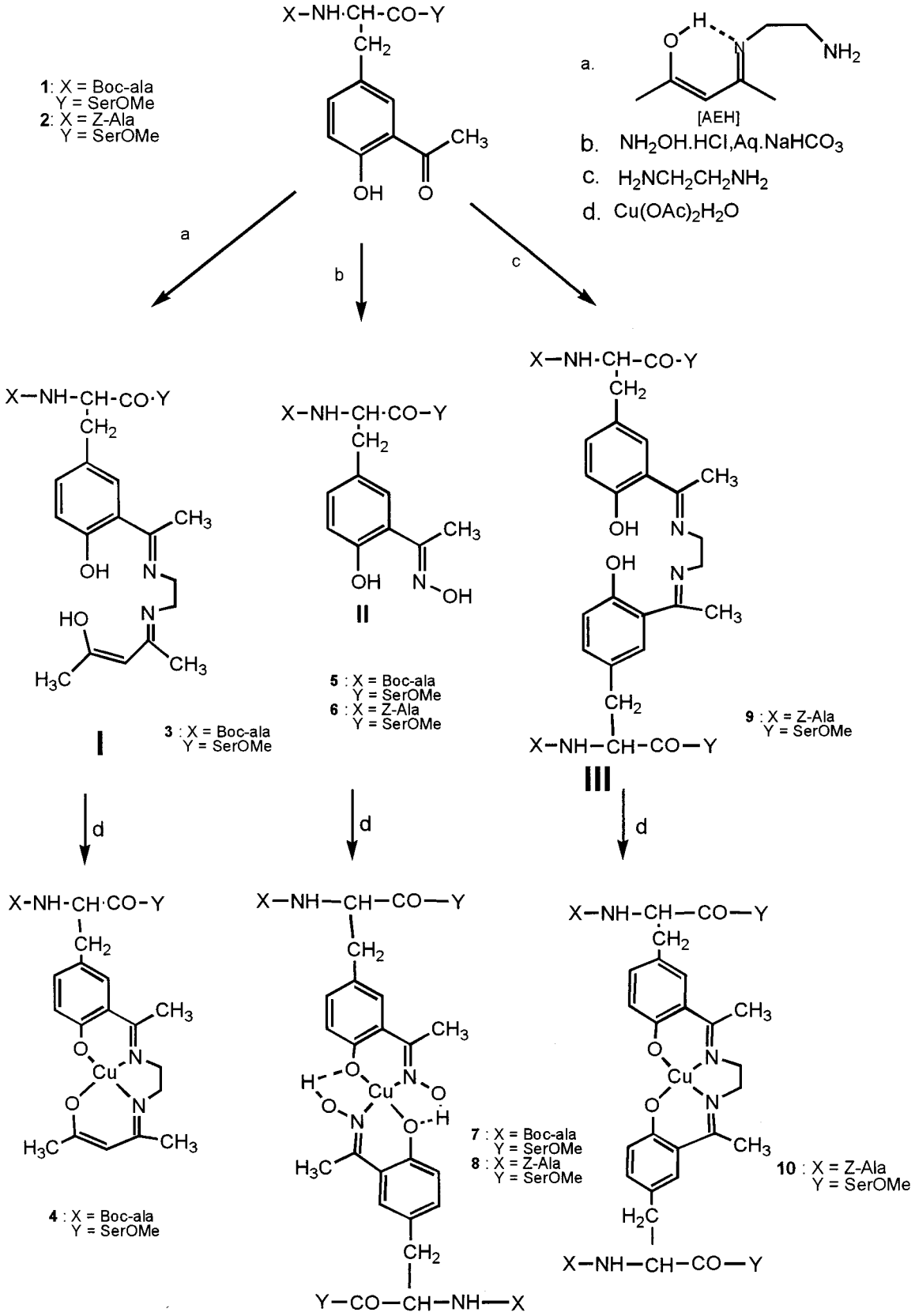
Closely related to the above would be the covalent linking of the 3-acetyltyrosine side chains either in an intramolecular fashion to provide, in peptide segments, cross-linking or in an intermolecular mode to dimers, in both events leading to metal-uptake systems. This aspect has been successfully demonstrated by construction of ligand precursors **III**, arising from linking of 3-acetyltyrosine units with ethylenediamine (EDA) (Scheme 1) as illustrated with the transformation of tripeptide **2** to ligand precursor **9** in 90% yields (Scheme 1). The copper complex **10**, easily prepared from **9**, exhibited an EPR profile typical of square planar complexes. Both **9** and **10** showed $(M + H)^+$ peaks in the FAB MS.

It is interesting to note that once the 3-acetyltyrosine units are in position, condensation with agents described here, particularly AEH, should not pose any problem, since none of the coded amino acid side chains harbor carbonyl functions.

(10) (a) On specific suggestion by a reviewer, the dipeptide alanyl-3-acetyltyrosine was prepared in quantitative yields by the F-MOC solid phase method and fully characterized, thus demonstrating that 3-acetyltyrosine can equally be adapted to solid-phase synthesis. We thank the reviewer for this valuable suggestion. (b) N.T. has prepared a decapeptide with an automated synthesizer, having the residues, in addition to 3-acetyltyrosine, lysine and arginine.

(11) Typical observed EPR parameters at 298 K for copper complexes: $A_0 = 89 \times 10^{-4}$ cm^{-1}, $g_0 = 2.109$. Corresponding values for laccase: $A_0 = 92 \times 10^{-4}$ cm^{-1}, $g_0 = 2.190$. [(a) Vanngard, T. In *Biological Applications of Electron Spin Resonance*; Swartz, H. W., Bolton, J. R., Borg, D. C., Eds.; Wiley: New York, 1972; Chapter 9. (b) Rist, G. H.; Hyde, J. S.; Vanngard, T. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 79. (c) Malmstrom, B. G.; Reinhammar, B.; Vanngard, T. *Biochim. Biophys. Acta* **1970**, *205*, 48. Additional data including spectra are available in the Supporting Information.

Scheme 1



The foregoing account has demonstrated that the placement of Cu(II) can be accomplished by taking advantage of the 3-acetyltyrosine side chain, in place of peptide segments, generally needed for this purpose, in Nature.

The EPR profile of Cu(II) in these minimalistic environments matches well with that prevalent in copper proteins.[11]

We feel that the strategy shown here to craft metal-uptake systems, with 3-acetyltyrosine-containing peptide segments, merits further development. Options would include incorporation in peptide synthesis[10b] as well as in ribosomal protein synthesis using suppressor t-RNA. Endeavors along these paths are underway.

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Supporting Information Available: Experimental procedures, spectral and analytical data, and ^{1}H NMR and EPR spectra. This material is available free of charge on the Internet at http://pubs.acs.org.

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