Pergamon

Tetrahedron Letters, Vol. 35, No. 3, pp. 447-450, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(93)E0243-D

The Crafting of Peptide Segments with Cu^{II} Uptake Potential

Subramania Ranganathan* and Natarajan Tamilarasu

Department of Chemistry, Indian Institute of Technology,
Kanpur 208 016, INDIA.

Abstract: *Ethylenediamine-acetyl acetone mono-Schiff base (AEH) and hydroxylamine hydrochloride readily condense with peptides, prepared by normal procedures, having 3-acetyl tyrosine side chains, to templates having two types of structural profile with AEH having independent Cu^{II} uptake potential and with hydroxylamine hydrochloride requiring two peptide units.*

The interaction of oxygen with copper centered co-ordination spheres nestled in the cradle, crafted from the protein manifold, brings about key biological transformations. Such manifestations are seen, *inter alia*, in, normal copper enzymes associated with dismutation of superoxide radical, oxidation of amines, oxidation of hydroxy functions and hydroxylations, in, blue copper proteins involved in outer sphere long range electron transfer, hemocyanins that reversibly bind oxygen, bi-nuclear copper proteins associated with oxygen transport and aromatic hydroxylations and the multi-copper oxydases that couple four one electron oxidations of substrates to the reduction of oxygen to water. In all these cases the required copper co-ordination is accomplished by careful placement of a handful of coded amino acid side chains, notable amongst which are histidine, tyrosine, aspartic acid, cysteine and methionine[1]. These combinations are necessitated by the fact that none of the 20 coded amino acid side chains has independent copper uptake potential.

Our continued endeavours in the domain of protein engineering[2] highlighted the possible advantages from placement of a redox template at specified locations in peptides and proteins. This communication reports the first synthesis of modified tyrosine analogs which have independent copper uptake potential and which can be incorporated into peptide segments by the usual procedures.

The reaction of L-tyrosine(Tyr) with AcCl-$AlCl_3$ in nitrobenzene afforded optically active 3-acetyl tyrosine hydrochloride [Tyr(3-Ac)HCl, (1), mp 220°-223° , 69%][3,4,5]. Esterification of (1) afforded Tyr(3-Ac)-OMe.HCl (2) which was N-protected to BzNH-Tyr(3-Ac)-OMe(3) on the one hand and coupled with Boc-Ala to give Boc-Ala-Tyr(3-Ac)-OMe(4) on the other. Compound (4) was saponified and coupled with Ser-OMe.HCl to yield Boc-Ala-Tyr(3-Ac)-Ser-OMe(5)(Scheme-1)[6]. These experiments demonstrated that (1) could be conveniently incorporated into peptides.

All efforts to prepare copper complexes of (3), (4) and (5) failed, thus necessitating further modifications. These endeavours resulted in substrates having ready copper uptake potential and having two types of structural profile.

A noteworthy reaction of (3), (4) and (5) is their ready transformation to oximes with $NH_2OH.HCl$ in MeOH, to respectively (6), (7) and (8) (Scheme-2,I)[7]. The oximino compounds (6), (7) and (8) on treatment with $Cu(OAc)_2$ in MeOH-H_2O readily afforded, respectively, the metal templates (12), (13) and (14) (Scheme-2,II)[8].

The UV and EPR profile of the Cu^{II} complexes (12), (13) and (14) show that they are largely square planar and that the oximino group metal coordination takes place through nitrogen. These complexes should therefore provide further avenues for elaboration via the free hydroxyl units.

Thus, copper templated dimers can be prepared by incorporation of (1) into peptides. The utility of this approach would be restricted because in most cases the dimer formation would not be desirable. Therefore it was felt that, in order for the methodology to gain general acceptance, it was necessary to transform the Tyr-3Ac side chain, in a peptide environment, to one having independent potential for copper uptake. The logical approach would be Schiff base formation with construct that can provide the required ligand disposition to form metal templates.

Surprisingly, in spite of extensive literature on Cu^{II} coordinated to $(nitrogen)_2(oxide)_2$ ligands, they invariably arise from symmetric dimers. Amongst the few methodologies available for unsymmetrical templates[9], the readily available acetyl acetone - ethylenediamine mono Schiff base (AEH)[10] proved most satisfactory. AEH readily formed , from (3), (4) and (5), Schiff bases (9), (10) and (11) in MeOH at rt[11] (Scheme-2,III) , which on brief MeOH reflux with $Cu(OAc)_2$ afforded the copper complexes (15), (16) and (17) (Scheme-2,IV)[12]. The UV and EPR profile of the complexes show that they are largely square planar. The Z protected analogs of (13),(14) and (16) have been similarly prepared.

The chemical and redox profile of the metal templates reported here is under study. The ligand environments constructed here can naturally, accommodate other metal ions, thus giving rise to a broader methodology for the crafting of a variety of metal templates in a peptide/protein environment.This aspect has been demonstrated with the preparation and characterisation of Co^{II}, Ni^{II} complexes of (9), (6), (7) and (8).

The methodology delineated, enabling the placement of metal templates at specified sites in peptides, would offer advantages from the vantage of protein design, energy transfer, redox processes and enzyme models.

We are grateful to Dr. Darshan Ranganathan (IITK) for advice and to Dr. R. N. Mukherji and Dr. P. K. Bharadwaj (IITK), Kanpur for useful discussions. Financial assistance from CSIR, DST, New Delhi is gratefully acknowledged.

REFERENCES AND FOOTNOTES

1. Solomon,E.I.; Baldwin,M.J.; Lowery,M.D. Chem.Rev. 1992, *92*, 521.

2. Ranganathan,S.; Ranganathan,D.; Bamezai,S.; Singh,W. P.; Bhattacharyya,D.; Singh,G.P.; Rathi,R.; Jayaraman,N.; Patel,B.K. Pure. Appl. Chem. 1990, *62*, 1437 and references cited therein; Ranganathan,S.; Jayaraman,N. J. Chem. Soc., Chem. Commun., 1991, 934; Ranganathan,S.; Ranganathan,D.; Bhattacharyya,D. Tetrahedron Lett., 1991, *32*, 5615; Ranganathan,S.; Jayaraman,N. Tetrahedron, 1992, *48*, 931; Ranganathan,S.; Jayaraman,N. Tetrahedron Lett., 1992, *33*, 6681; Ranganathan,S.; Patel,B.K. Tetrahedron Lett., 1993, *34*, 2533.

3. Boger,D.L.; Yohannes,D. J. Org. Chem., 1987, *52*, 5283.

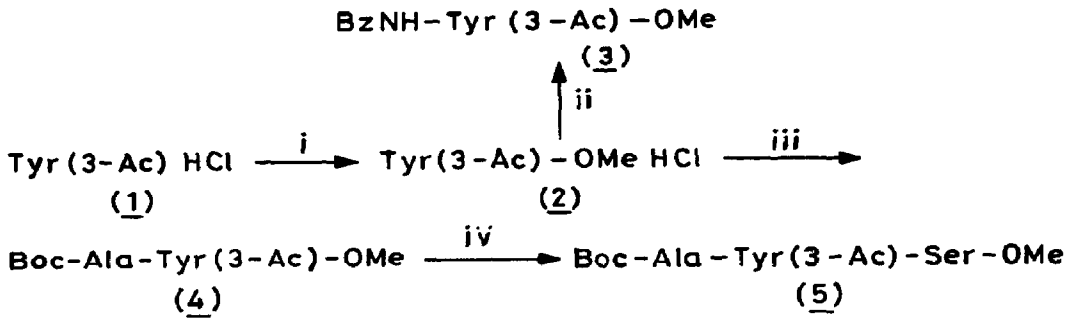
4. Amino acids used in the present study were of L-configuration. All new compounds were fully characterised on the basis of their spectral and analytical data.

5. Preparation of Tyr(3-Ac)HCl (1) : A stirred suspension of L-Tyrosine (10g, 0.055 mol) in nitrobenzene (240 mL) was admixed with $AlCl_3$ (28.8g, 0.216 mol) and freshly distilled acetyl chloride (5.12g,0.065mol). The reaction mixture was held at 100° for 6h, allowed to attain room temperature and poured onto a mixture of con.HCl(55mL) and ice(340g). The aqueous solution was concentrated to ~ 200mL and kept in the refrigerator. The separated solid was recrystallized from 5N(HCl). ir(KBr) ν_{max} cm^{-1}: 2858,1742,1640,1582; nmr(80MHz)(D_2O) δ: 2.3 (s, 3H), 3.0 (d, 2H, J=7.5Hz), 4.12 (t, 1H, J=7.5Hz), 6.71 (d, 1H, J=8.75Hz), 7.26 (d, 1H, J=8.75Hz), 7.54 (s, 1H); FAB MS: 224 $(MH)^+$–HCl; $[\alpha]_D^{25}$ = -2.33° (c, 1.0, MeOH).

   Chiral retention in the reaction has been established via transformation to DOPA and comparison with authentic sample.

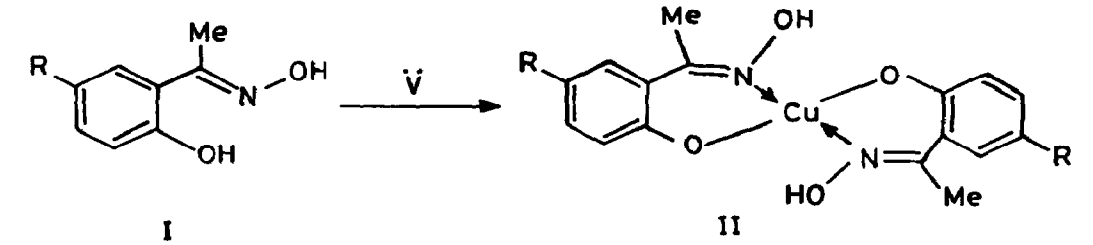
6. Tyr(3-Ac)-OMe.HCl [(2), mp 186°-187° ,99%]; ir (KBr) ν_{max} cm^{-1}: 2923, 1751, 1638, 1490 ; nmr (80 MHz) (D_2O) δ: 2.5 (s, 3H), 3.12 (d, 2H, J=7.5Hz), 3.71 (s, 3H), 6.87 (d, 1H, J=8.75Hz), 7.34 (d, 1H, J=8.75Hz), 7.67 (s, 1H);FAB MS: 238 $(MH)^+$–HCl ; $[\alpha]_D^{25}$ = -3.43° (c, 0.3, MeOH). BzNH-Tyr(3-Ac)-OMe[(3), mp 131°-133°; 82%], ir(KBr) ν_{max} cm^{-1}: 1750, 1637, 1580, 1517; nmr (80 MHz) ($CDCl_3$) δ:2.51 (s, 3H), 3.31 (d, 2H, J=5.0Hz), 3.84 (s, 3H), 5.12 (d,d, 1H, J=7.5Hz), 6.65 (d, 1H, J=8.75Hz), 6.87-7.93 (m, 8H), 11.43 (s, 1H); FAB MS: 342$(MH)^+$; $[\alpha]_D^{25}$ = -55.22° (c, 1.1, MeOH). Boc-Ala-Tyr(3-Ac)-OMe [(4), mp 106°; 67%]; ir(KBr) ν_{max} cm^{-1}: 3340, 2982, 1750, 1686, 1652, 1523 ; nmr (80 MHz) ($CDCl_3$)δ: 1.28 (d, 3H, J=5.0Hz), 1.37 (s, 9H), 2.63 (s, 3H), 3.1 (d, 2H, J=6.25Hz), 3.75 (s, 3H), 4.06 (m, 1H), 4.84 (m, 2H), 6.63 (d, 1H, J=8.75Hz), 6.9 (d, 1H, J=8.75Hz), 7.22 (d, 1H, J=8.75Hz), 7.53(s, 1H), 11.44 (s, 1H); FAB MS: 409$(MH)^+$; $[\alpha]_D^{25}$ = -5.96° (c, 1.0, MeOH). Boc-Ala-Tyr(3-Ac)-Ser-OMe [(5), mp 172° ; 32%]; ir(KBr) ν_{max} cm^{-1}: 3299, 1746, 1687, 1647, 1530; nmr (80 MHz) ($CDCl_3$) δ: 1.44 (m, 12H), 2.65 (s, 3H), 3.16 (d, 2H, J=6.25Hz), 3.65 (s, 3H), 3.81 (d, 2H, J=3.75Hz), 4.13 (m, 1H), 4.69 (m, 2H), 5.03 (d,

Scheme 1

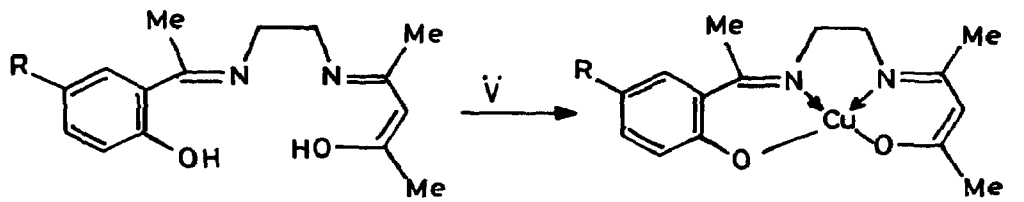


(i) : MeOH/HCl ; (ii) : BzCl / Na_2CO_3 ; (iii): Et_3N, Boc-Ala, DCC, HOBt ; (iV) a. NaOH/H^+, b. Ser-OMe, DCC, HOBt.

Scheme 2



I II



III IV

R	I	II	III	IV
BzNHCH(COOMe)CH_2 -	(6)	(12)	(9)	(15)
Boc Ala CH(COOMe)CH_2 -	(7)	(13)	(10)	(16)
Boc Ala CH(Ser-OMe)CH_2 -	(8)	(14)	(11)	(17)

(V) : $Cu(OAc)_2$, MeOH : H_2O

1H, J=6.25Hz), 6.78-7.50 (m, 4H), 7.7 (s, 1H), 12.1 (s, 1H);FAB MS: 496$(MH)^+$; $[\alpha]_D^{25}$ =-21.0° (*c*, 0.5, MeOH).

7. General procedure for (6),(7),(8): A solution of the substrate (1.2 mmol) and $NH_2OH.HCl$ (2.4 mmol) in MeOH (15 ml) was admixed with a solution of $NaHCO_3$ (2.4 mmol) in H_2O (4 ml). The reaction mixture was left stirred at room temperature for 8 h, evaporated, extracted with EtOAc (15 ml), washed with water (2x5 ml), dried, evaporated and crystallized from MeOH : H_2O :: 3 : 1. BzNHTyr (3-oximino acetyl)-OMe [(6); mp 172° ; 79%]; ir(KBr) ν_{max} cm^{-1}: 1706, 1634, 1580, 1526 ; nmr(80 MHz) ($CDCl_3$-DMSO-d_6) δ : 2.26 (s,3H), 3.2 (d,2H,J=7.0 Hz), 3.8 (s,3H), 5.0(m,1H), 6.73-7.93(m,9H), 11.0(s,1H), 11.8(s,1H); FAB MS: 357$(MH)^+$. Boc-Ala-Tyr(3-oximino acetyl)-OMe [(7); mp 172° ; 71%]; ir(KBr)ν_{max} cm^{-1}: 3426, 3339, 3258, 1742, 1690, 1670, 1623, 1537 ; nmr(80 MHz) ($CDCl_3$) δ: 1.47 (m,12H), 2.28(s,3H), 3.13(d,2H,J=5.0 Hz), 3.84(s,3H), 4.17(m,1H), 4.93(m,1H), 5.37(d,1H, J=6.25 Hz), 6.47-7.34(m,4H), 9.43(s,1H), 11.74(s,1H); FAB MS :424 $(MH)^+$. Boc-Ala-Tyr (3-oximino acetyl)-Ser-OMe [(8); mp 104°; 83%]; ir(KBr) ν_{max} cm^{-1}: 3317, 1743, 1686, 1660, 1521 ; nmr(80 MHz) ($CDCl_3$-DMSO-d_6) δ: 1.34 (m,12H), 2.31(s,3H), 3.09(d,2H, J=6.25Hz), 3.78(m,5H), 4.09 (m,1H), 4.63(m,2H), 5.75(d,1H), 6.75-7.68(m,5H), 10.8(s,1H), 11.7(s,1H); FAB MS: 511 $(MH)^+$.

8. General procedure for (12), (13), (14): A solution of the oxime (2 mmol) in MeOH (15ml) was admixed with a solution of $Cu(OAc)_2$ (1 mmol) in H_2O (3ml). The reaction mixture was left stirred at rt for 0.5h, the copper complex filtered, washed with water and dried.(12): mp 253° ; 78%; UV ($CHCl_3$) λ_{max} nm : 259, 345, 647; ir (KBr) ν_{max} cm^{-1} : 3289, 2950, 1740, 1637, 1579, 1530 , FAB MS: 774$(M)^+$; epr ($CHCl_3$,rt): A_{iso} = 90G, g_{iso} = 2.1137; (13): mp 226° ; 67%; UV ($CHCl_3$) λ nm : 254, 345, 648; ir (KBr) ν_{max} cm^{-1} : 3387, 3329, 1740, 1666, 1520. FAB MS: 908$(M)^+$; epr ($CHCl_3$, rt): A_{iso} = 105G, g_{iso} = 2.1171; (14): mp 255° ; 84%; ir (KBr) ν_{max} cm^{-1} : 3315, 1744, 1651, 1509 . FAB MS: 1082$(M)^+$.

9. Costes,J.P.; Dahan,F.; Laurent,J.P. Inorg. Chem.1985, *24* 1018; Cros,G.; Laurent,J.P.; Dahan,F. Inorg. Chem. 1987, *26*, 596.

10. Preparation of AEH: Under vigorous stirring,a solution of acetyl acetone (0.1 mol) in $CHCl_3$ (50ml) was added, in drops, to a solution of ethylenediamine (EDA) (0.1 mol) in $CHCl_3$ (100ml). The reaction mixture was left stirred for 10h, the separated water was removed, solvents evaporated and the crude product used as such without delay. AEH: liquid; 84 %, nmr (80MHz) ($CDCl_3$) δ : 1.66 (m, 2H), 2.02 (s, s, 6H), 2.9 (m, 2H), 3.38 (m, 2H), 5.03 (s, 1H), 10.97 (s, 1H).

11. General procedure for (9), (10), (11): A solution of the substrate (2mmol) in MeOH (20ml) was admixed with AEH (2 mmol) in MeOH (5ml). The reaction mixture was left stirred at rt. for 10h, evaporated and crystallized from MeOH. (9) mp 190°-191° ; 60%; ir (KBr)ν_{max} cm^{-1}: 3274, 2949,1745, 1642, 1605, 1582, 1558, 1466 ; nmr (80 MHz) ($CDCl_3$) δ: 1.98 (s, s, 6H), 2.22 (s,3H), 3.22 (d, 2H), 3.65-3.97 (m, 7H), 5.0 (m, 2H), 6.66 (d, 1H), 6.75-7.9 (m, 8H), 10.97 (s,1H), 15.37 (s, 1H); FAB MS :466 $(MH)^+$; (10) mp 163° ; 68%; ir (KBr) ν_{max} cm^{-1}: 3326, 3296, 2980, 1744, 1665, 1606, 1560, 1435, 1366 ; nmr (80 MHz) ($CDCl_3$) δ : 1.31 (m, 12H), 1.86 (s,s, 6H), 2.28 (s, 3H), 3.0 (d, 2H), 3.5-4.17 (m, 8H), 4.56-5.16 (m, 3H), 6.44-7.25 (m, 4H), 10.81 (s, 1H), 15.15 (s, 1H); FAB MS :533 $(MH)^+$; (11) mp 164° ; 72%; ir (KBr) ν_{max} cm^{-1}: 3276, 2928, 1743, 1688, 1635, 1537, 1485, 1438, 1213 ; nmr (80 MHz) ($CDCl_3$) δ : 1.34(m, 12H), 1.93 (s,s, 6H), 2.62 (s, 3H), 3.12 (d, 2H), 3.43-4.25 (m, 10H), 4.68 (m, 2H), 5.0 (s, 1H), 5.31(d, 1H), 6.78-7.78(m, 5H), 10.93 (s, 1H), 12.15 (s, 1H); FAB MS: 620 $(MH)^+$.

12. General procedure for (15),(16), (17): A solution of precursor Schiff base (0.2mmol) in MeOH (25ml) was admixed with $Cu(OAc)_2$ (0.2 mmol), held at 60°C for 0.5h, evaporated and crytallized from MeOH. (15) mp 115° -118° ; 74%; UV ($CHCl_3$) λ_{max} nm: 275, 313, 548; ir (KBr) ν_{max} cm^{-1}: 3436, 1734, 1640, 1592, 1514, 1409, 1352 ; epr (rt) A_{iso} = 85G, g_{iso} =2.1119, (-196°C) $A_{\|}$ = 200G, $g_{\|}$ = 2.209, $g_{\perp}$ = 2.0882; FAB MS : 527$(MH)^+$; (16) mp 125° -130° ; 68%; UV ($CHCl_3$): λ_{max} nm : 274, 313, 547; ir ($CHCl_3$) ν_{max} cm^{-1}: 3420, 3300, 1730, 1650, 1580, 1500, 1435, 1355 ; epr (rt) A_{iso} = 90G, g_{iso} = 2.1104, (-196°C) $A_{\|}$ = 190G, $g_{\|}$ = 2.2053, $g_{\perp}$ = 2.0592; FAB MS 594 (M^+); (17) mp 142°-150° ; 73%; ir $(CHCl)_3$ ν_{max} cm^{-1}: 3410, 3290, 1730, 1632, 1580, 1503, 1427, 1360; epr (rt) A_{iso} = 90G, g_{iso} = 2.1119, (-196°C) $A_{\|}$= 210G, $g_{\|}$ = 2.1873, $g_{\perp}$ = 2.0592.

(*Received in UK* 10 *August* 1993; *revised* 18 *November* 1993; *accepted* 19 *November* 1993)