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ENZYMATIC OXIDATIVE COUPLING OF MONOHALOGENATED TYROSINE DERIVATIVES

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Abstract: The regioselectivity of peroxidase-catalyzed oxidative phenolic coupling of monohalogenated tyrosine derivatives could be altered by the halogen substituent and the enzyme-substrate ratio. This ability to shift the coupling pattern (C–C vs. C–O) provides chemists with added versatility for synthetic applications.

A wide variety of naturally occurring amino acids (thyroxine, dityrosine, isodityrosine, trityrosine, isotrityrosine), cyclopeptides (piperazinomycin, K-13, bouvardin, bastadin 6 and OF-4949), and glycopeptide antibiotics (vancomycin, ristocetin and complestatin) contain diaryl ether and/or diphenyl linkages.[1] The molecular complexities of the cross-linked cyclic peptides such as vancomycin and ristocetin are challenging synthetic targets and have attracted the attention of many synthetic chemists. The crucial step in the synthesis of these molecules is the construction of the diphenyl ether linkages and several promising methods are now available for this purpose.[1] However, from these attempts, it is apparent that existing methodologies have limitations [2] and therefore there is a need to develop alternative strategies to supplement current methodologies.

Although little is known about the mechanistic details of vancomycin biosynthesis,[3] it is likely that it occurs via radical oxidative cyclization of aromatic amino acids leading to the formation of the C-C and C-O linkages.[4] Earlier, we have shown that horseradish peroxidase (HRP) efficiently catalyzed the oxidative C-O coupling of 3,5-dibromo and 3,5-dichloro-L-tyrosine derivatives to form the isodityrosine framework, whereas HRP promoted C-C coupling of L-tyrosine and 3,5-diiodo-L-tyrosine derivatives to form the dityrosine skeleton.[5] To investigate further the scope of this enzymatic oxidative coupling reaction, we now report our studies on the monohalogenated tyrosine derivatives.

It is well known that substituents at the ortho position on the aromatic ring could markedly influence the oxidation potential, the pKa and the regioselectivity of oxidative coupling of aromatic compounds. Hence, it was of interest to examine the pattern of oxidative coupling of monohalogenated tyrosine derivatives. For convenience we used several readily available monohalogenated tyrosine derivatives for our study since we have previously shown that no racemization

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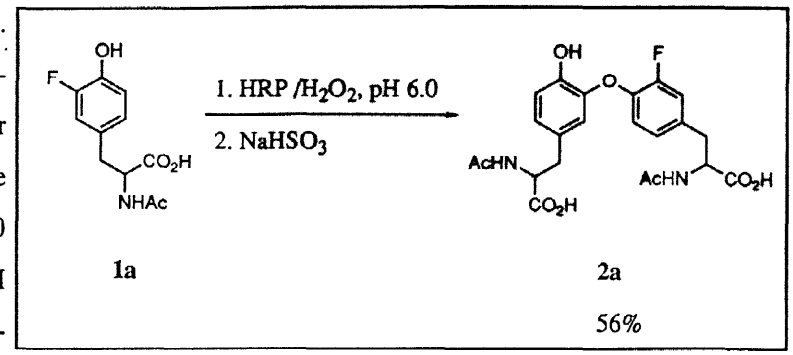
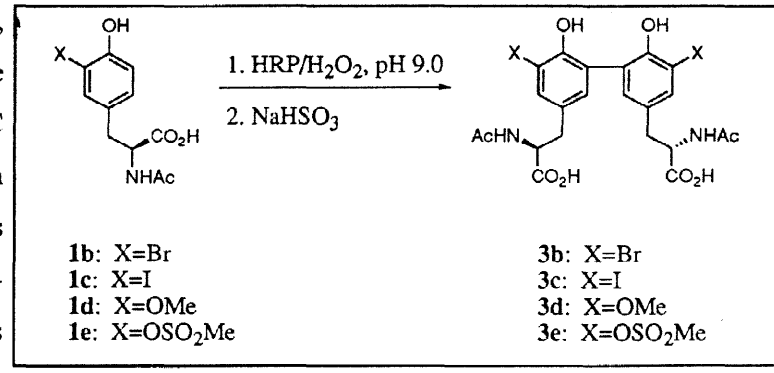
occurred during enzymatic oxidative coupling. Thus N-acetyl-3-fluoro-DL-tyrosine, **1a**, was incubated with HRP (6 units/μmol) in phosphate buffer containing 10% CH_3CN at pH 6.0 in the presence of H_2O_2 (1.2 eq) at 25°C . After stirring for 40 minutes, the reaction was quenched with 1M $NaHSO_3$. Following the work-up and isolation procedure previously described,[5] the product **2a** was isolated in 56% yield,[6] accompanied by some residual **1a**. This result clearly showed that N-acetyl-3-DL-fluoro-tyrosine was converted cleanly by HRP to only **2a**, the C-O coupling product and no C-C coupled product was detected.



1a **2a** 56%

In contrast, the N-acetyl-tyrosine derivatives, **1b-1e** (where X = Br, I, OMe and OMs), were converted by HRP into their respective C-C coupling products, **3b-3e** in yields ranging from 35% to 75% under similar reaction conditions (Table 1).[7] Further, no significant quantities of C-O coupling products were detected under various experimental conditions such as pH, organic solvent, and substrate-enzyme ratio.



1b: X=Br
1c: X=I
1d: X=OMe
1e: X=OSO_2Me

3b: X=Br
3c: X=I
3d: X=OMe
3e: X=OSO_2Me

Table 1. Peroxidase-catalyzed Oxidative Coupling of **1** [a]

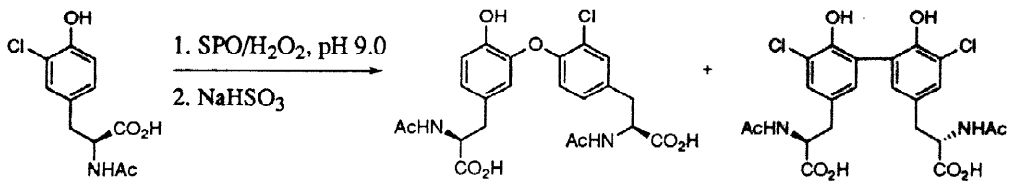
Sub	X	pH	Co-solvent (10%)	Enz/Sub (units/μmol)	Time (min)	Isolated yield (%) **2**	Isolated yield (%) **3**
1a (DL)	F	6.0	CH_3CN	6.0	40	56	
1b (L)	Br	9.0	CH_3CN	6.0	40		40
1c (L)	I	9.0	CH_3CN	6.0	60		75
1d (DL)	OMe	9.0	—	1.0	10		48
1e (L)	OMs	9.0	CH_3CN	6.0	40		35
1f[b] (L)	Cl	9.0	—	1.0	40	43	
1f[b] (L)	Cl	9.0	—	0.1	20		30

[a] A mixture of substrate (1.0 mmol, 2 mM), enzyme and H_2O_2 (1.2 mmol) were incubated in aqueous buffer (10% CH_3CN if needed, total volume 50 mL) at 24°C until complete consumption of substrate (monitored with HPLC); reactions were quenched with 1M $NaHSO_3$.

[b] Soybean peroxidase; horseradish peroxidase was used in the other incubations.

On the other hand, the coupling pattern of N-acetyl-3-chloro-L-tyrosine, **1f**, was found to be dependent on the enzyme-substrate ratio. When the enzyme-substrate ratio was low (0.1unit/μmol), soybean peroxidase (SBP) converted **1f** predominantly into the C-C coupling product, **3f**, whereas at a high enzyme-substrate ratio (1.0 unit/μmol), this

peroxidase catalyzed the transformation of **1f** into the C-O coupling product **2f predominantly.**[8] **Similar coupling pattern was observed with HRP but the** product profile was more complex.



1f **2f** **3f**

SPO (units/μmol)		
0.1	trace	30%
1.0	43%	trace

The importance of enzyme-substrate ratio also has been observed in other peroxidase-catalyzed oxidations of aromatic substrates and this phenomenon may be rationalized similarly. For example, in the oxidation of indole-3-acetate, a high enzyme-substrate ratio resulted in the formation of peroxidase compound II and methyleneoxindole was obtained as the major product.[9] On the other hand at low enzyme-substrate ratio, only ferroperoxidase and compound III were formed and indole-3-aldehyde became the major product of oxidation. As a rule, less enzyme is needed for C-C than for C-O coupling reactions and the oxidative phenolic C-C coupling of unhalogenated or monohalogenated tyrosine derivatives is best achieved in the pH range of 8-10.

In summary, we have shown that the regioselectivity of peroxidase-catalyzed oxidative phenolic coupling of monohalogenated tyrosine derivatives could be altered by the halogen substituent and the enzyme-substrate ratio. This ability to shift the coupling pattern provides chemists with added versatility for synthetic applications. The usefulness of this enzymatic phenolic coupling methodology was demonstrated by the successful synthesis of the Bastadins[10] and was shown to have distinct advantages over the TTN oxidation method.[1a]

Acknowledgment: This investigation was supported in part by a grant from the National Institutes of Health.

References and Notes

1. See recent reviews on the synthesis of isodityrosine-derived cyclic peptides and references cited therein: **a)** Yamamura, S.; Nishiyama, S. In *Studies in Natural Products Chemistry*; Rahman, A. U. (ed.); Elsevier Sci. Publ. B. V. 1992; Vol. 10, pp. 629-669. **b)** Ramarao, A. V.; Gurjar, M. K.; Reddy, K. L.; Rao, A. S. *Chem. Rev.* **1995**, 2135-2167. **c)** Evans, D. A.; Devries, K. M. In *Glycopeptide Antibiotics*, Nagarajan, R. (ed.); Marcel Dekker: New York, 1994; pp. 63-101.
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6. **2a**: ^{1}H NMR (DMSO-d_6) δ7.95 (d, *J*=8.3, 1H), 7.90 (d, *J*=8, 1H), 7.17 (d, *J*=9, 1H), 6.85 (d, *J*=9, 1H), 6.75 (s, 2H), 6.70 (d, *J*=7, 1H), 6.60 (m, 1H), 4.30 (m, 2H), 3.02 (m, 2H), 2.75 (m, 2H), 1.80 (s, 3H), 1.75 (s, 3H); ^{1}H NMR (Methanol-d_4) δ7.12 (d, *J*=11.8, 1H), 6.99 (d, *J*=9, 1H), 6.88 (s, 2H), 6.83 (m, 1H), 6.67 (d, *J*=7, 1H), 4.61 (m, 1H), 4.51 (m, 1H), 3.18 (m, 1H), 3.08 (m, 1H), 2.98 (m, 1H), 2.85 (m, 1H), 1.97 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (DMSO-d_6) δ174.0 (2C), 169.1, 169.0, 153.5, 149.5, 147.0, 143.3, 142.0, 134.5, 129.4, 125.3, 124.5, 121.0, 117.5, 116.8, 54.2 (2C), 35.2 (2C), 22.5 (2C); HR MALDI MS calcd for $C_{22}H_{22}FN_2O_8$ ([M-H]$^-$) 461.136, found 461.135.
7. **3b**: ^{1}H NMR (Acetone-d_6) δ7.54 (d, *J*=8.3, 2H), 7.42 (d, *J*=2.2, 2H), 7.10 (d, *J*=2.2, 2H), 4.78 (m, 2H), 3.15 (dd, *J*=5.4, 2H), 2.95 (dd, *J*=5.4, 2H), 1.93 (s, 6H); ^{1}H NMR (Methanol-d_4) δ7.36 (s, 2H), 7.09 (s, 2H), 4.57 (m, 2H), 3.12 (m, 2H), 3.04 (m, 2H), 1.97 (s, 6H); ^{13}C NMR (Methanol-d_4) δ176.7 (2C), 172.9 (2C), 151.3 (2C), 134.5 (2C), 132.9 (2C), 131.8 (2C), 128.9 (2C), 112.5 (2C), 56.4 (2C), 37.3 (2C), 22.6 (2C); HR MALDI MS calcd for $C_{22}H_{23}Br_2N_2O_8$ ([M+H]$^+$) 600.982, found 600.983, calcd for $C_{22}H_{22}Br_2N_2O_8Na$ ([M+Na]$^+$) 622.964, found 622.965. **3c**: ^{1}H NMR (Acetone-d_6) δ7.63 (dd, *J*=2, 2H), 7.41 (dd, *J*=9, 2H), 7.11 (d, *J*=2, 1H), 7.09 (d, *J*=2, 1H), 4.79 (m, 2H), 3.14 (m, 2H), 2.94 (m, 2H), 1.93 (s, 3H), 1.90 (s, 3H); ^{13}C NMR (Acetone-d_6) δ172.9 (2C), 171.1, 170.9, 153.2, 153.1 140.84, 140.77, 133.7, 133.5, 131.8 (2C), 125.8, 125.4, 86.6, 86.4, 54.07, 53.97, 36.9, 36.7, 22.6 (2C); FAB MS m/z (relative intensity) 695.9 (100, [M+H]$^+$). **3d**: ^{1}H NMR (Acetone-d_6) δ7.58 (bs, 2H), 7.38 (dd, *J*=9, 2H), 6.88 (m, *J*=2.2, 2H), 6.78 (m, *J*=2.2, 2H), 4.80 (m, 2H), 3.82 (s, 6H), 3.15 (m, 2H), 2.95 (m, 2H), 1.93 (s, 6H); ^{1}H NMR (Methanol-d_4) δ6.83 (s, 1H), 6.80 (s, 1H), 6.77 (s, 2H), 4.61 (m, 2H), 3.89 (s, 6H), 3.14 (m, 2H), 3.02 (m, 2H), 1.95 (s, 6H); ^{13}C NMR (Methanol-d_4) δ177.3, 177.1, 173.2 (2C), 149.5, 149.4, 143.3 (2C), 130.3 (2C), 127.3 (2C), 125.5 (2C), 113.1 (2C), 56.8 (2C), 56.6 (2C), 38.4, 38.3, 22.8 (2C); HR MALDI MS calcd for $C_{24}H_{29}N_2O_{10}$ ([M+H]$^+$) 505.182, found 505.182, calcd for $C_{24}H_{28}N_2O_{10}Na$ ([M+Na]$^+$) 527.164, found 527.165. **3e**: ^{1}H NMR (Methanol-d_4) δ7.08 (m, 2H), 7.00 (m, 2H), 4.45 (m, 2H), 3.17 (s, 6H), 3.16 (m, 2H), 3.04 (m, 2H), 1.90 (s, 6H); ^{13}C NMR (Methanol-d_4) δ177.5, 177.3, 172.9 (2C), 147.5 (2C), 139.4 (2C), 132.0 (2C), 130.2, 129.7, 125.8 (2C), 118.3 (2C), 56.6 (2C), 38.1 (2C), 37.2 (2C), 22.8 (2C); HR MALDI MS calcd for $C_{24}H_{29}N_2O_{14}S_2$ ([M+H]$^+$) 633.106, found 633.108, calcd for $C_{24}H_{28}N_2O_{14}S_2Na$ ([M+Na]$^+$) 655.088, found 655.088.
8. **2f**: ^{1}H NMR (Acetone-d_6/D_2O) δ7.25 (s, 1H), 7.15 (d, *J*=9, 1H), 6.94 (s, 2H), 6.76 (d, *J*=9, 2H), 4.55 (m, 2H), 3.15 (m, 2H), 2.85 (m, 2H), 1.98 (s, 3H), 1.94 (s, 3H); ^{1}H NMR (D_2O/NaOH) δ7.40 (s, 1H), 7.06 (d, *J*=9, 1H), 6.96 (d, *J*=9, 1H), 6.68 (d, *J*=9, 2H), 6.62 (s, 1H), 4.20 (m, 1H), 4.15 (m, 1H), 3.08 (m, 1H), 3.00 (m, 1H), 2.82 (m, 1H), 2.74 (m, 1H), 1.95 (s, 3H), 1.90 (s, 3H); ^{13}C NMR (D_2O/NaOH) δ183.0, 182.0, 176.0, 175.5, 160.5, 156.0, 148.5, 136.0, 134.5, 133.0, 129.5, 126.5, 125.5, 125.0, 124.5, 121.0, 60.5, 59.9, 39.9 (2C), 25.5 (2C). **3f**: ^{1}H NMR (Acetone-d_6) δ7.58 (t, *J*=10, 2H), 7.24 (m, 2H), 7.04 (m, 2H), 4.76 (m, 2H), 3.15 (m, 2H), 2.92 (m, 2H), 1.92 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (Acetone-d_6) δ173.6 (2C), 171.7 (2C), 149.6 (2C), 131.8 (2C), 130.8 (2C), 130.6 (2C), 127.8 (2C), 121.9 (2C), 54.2 (2C), 36.9 (2C), 22.5 (2C); FAB MS m/z (relative intensity) 517 (16), 515 (66), 513 (100, [M+H]$^+$).
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