Communications

Enzymatic Oxidative Phenolic Coupling

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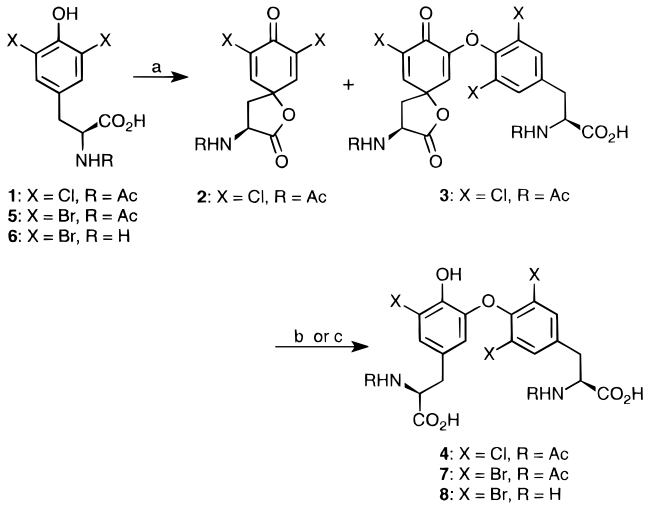
Oxidative phenolic coupling, either by a homolytic or heterolytic mechanism, is of great importance in natural products chemistry.[1] Many heterocyclic products, including alkaloids, cyclic peptides, and glycopeptide antibiotics, are biosynthesized via enzyme-catalyzed C−O or C−C bond formation.[2]

The diaryl ether linkage, formed by phenolic coupling of two tyrosine units is present in a diverse array of biologically-active natural products ranging from K-13,[3] OF4949,[4] and bouvardin[3,5] to the structurally complex glycopeptide antibiotics such as the vancomycin family.[6] Recently, many synthetic efforts have been directed to developing improved methods for the construction of the basic isodityrosine skeleton, which has been achieved either by the Ullmann[7] ether synthesis or by thallium(III)-promoted oxidative coupling[8] of tyrosine derivatives. However, the drastic reaction conditions used in these procedures required extensive protection and deprotection of the sensitive functionalities present in the parent molecule and only modest yields of desired product(s) were obtained. Consequently, the development of an alternative mild method for the synthesis of the diaryl ether linkage in high yields is still very much warranted.

Oxidative enzymes such as peroxidases, laccases, and β-tyrosinases are known to catalyze oxidative phenolic coupling of many aromatic substrates,[1,2] but the yield of the desired products are generally very low. Kametani and co-workers[9] had reported the oxidation of *N*-coclaurine with homogenized potato peels in the presence of hydrogen peroxide to give a mixture of dimer and trimer with C−O−C head to tail coupling but in only 1.6% yield. Similar yields were obtained with isoquinoline derivatives.[10] Although Zenk[11] reported the isolation of a unique cytochrome P-450 enzyme that mediates regio- and stereoselective intermolecular C−O bond formation to furnish natural dimeric alkaloids. The extremely low levels of this plant enzyme and its detergent instability during solubilization of the complex out of the membrane make it unsuitable for synthetic use. Here, we report a novel efficient synthesis of the isodityrosine skeleton using peroxidase to catalyze the C−O coupling of dihalogenated tyrosine derivatives.

Scheme 1



1: X = Cl, R = Ac
5: X = Br, R = Ac
6: X = Br, R = H

2: X = Cl, R = Ac

3: X = Cl, R = Ac

4: X = Cl, R = Ac
7: X = Br, R = Ac
8: X = Br, R = H

Since horseradish peroxidase (HRP) [EC 1.11.1.7] catalyzed the C−C coupling of tyrosine to form dityrosine[12] and nonhomogeneous oxidized polymerized derivatives, we decided to examine the action of this commercially available enzyme on dihalogenated tyrosine derivatives. When *N*-acetyl-3,5-dichloro-L-tyrosine, **1**, was incubated with HRP at pH 6.0 in the presence of H_2O_2 at 24 °C for 20 min, products **2** and **3** were isolated by silica gel chromatography in 7% and 55% yield, respectively. In turn, **3** was further chromatographed to give two diastereomeric lactones, **3a** and **3b**. Reduction of **2** and **3** separately with Zn/TFA at 0 °C afforded **1** and **4**, respectively, each in over 90% yield. Since the product profile and yield were found to depend critically on the experimental conditions used, we list below an improved representative procedure, developed after much experimentation, that furnished **4** directly in 76% isolated yield along with 12% of recovered **1**.

To a clear solution of **1** (292 mg) in 45 mL of 0.2 M phosphate buffer pH 6.0 and 5 mL of acetonitrile at 24 °C was added, under stirring, 2000 units of horseradish peroxidase (HRP, Sigma), followed by 1.2 mL of 1 M H_2O_2. The reaction mixture was stirred for 10 min, quenched with 3 mL of 1 M $NaHSO_3$, and the pH of the mixture was adjusted to 7.5 with 1 M NaOH (6 mL). This mild reduction procedure was found to give higher product yields than Zn/TFA or $CrCl_2$. After stirring for 10 min, the mixture was acidified to pH 3.0 with 2 M $KHSO_4$ (10 mL) and then extracted with ethyl acetate (3 × 60 mL). The combined organic extract was washed with brine (40 mL), dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was chromatographed over a silica gel column, which was eluted with a solvent mixture consisting of EtOAc−acetone−HOAc (1/0/0 to 100/10/1) to give recovered **1** (35 mg, 12%) and the desired product, **4** (208 mg, 76%), as a white solid (Scheme 1).

N-Acetyl-3,5-dibromo-L-tyrosine (**5**) and the unpro-

(1) McDonald, P. D.; Hamilton, G. A. Mechanisms of Phenolic Oxidative Coupling Reactions. In *Oxidation in Organic Chemistry*; Trahanovsky, W. S., Ed.; Academic Press: New York, 1973; Vol. 5b, pp 97−134.

(2) Dhingra, O. M. *Intramolecular Oxidative Coupling of Aromatic Substrates*. In *Oxidation in Organic Chemistry*; Trahanovsky, W. S., Ed; Academic Press: New York, 1982; Vol. 5d, pp 207−278.

(3) Yasuzawa, T.; Shirahata, K.; Sano, H. J. *Antibiot.* **1987**, *40*, 455−458.

(4) (a) Tamai, S.; Kaneda, M.; Nakamura, S. J. *Antibiot.* **1982**, *35*, 1130−1136. (b) Nishiyama, K.; Suzuki, S.; Yamura, S. *Tetrahedron Lett.* **1986**, *27*, 4481−4484.

(5) Kase, H.; Kaneko, M.; Yamada, K. J. *Antibiot.* **1987**, *40*, 450−454.

(6) For a review on vancomycin and related antibiotics, see: Williams, D. H.; Rajananda, V.; Williamson, M. P.; Bojesen, G. In *Topics in Antibiotic Chemistry*; Sammes, P. G., Ed.; John Wiley & Sons Inc.: New York, 1980; Vol. 5, pp 118−158.

(7) (a) Tomita, M.; Fujitani, K.; Aoyagi, Y. *Chem. Pharm. Bull.* **1965**, *13*, 1341−1345. (b) Boger, D. L.; Yohannes, D. *J. Org. Chem.* **1989**, *54*, 2502; **1990**, *54*, 2498−2502; **1990**, *55*, 6000−6017.

(8) (a) Suzuki, Y.; Nishiyama, S.; Yamamura, S. *Tetrahedron Lett.* **1989**, *30*, 6043−6046. (b) Evans, D. A.; Ellman, J. A.; DeVries, K. M. *J. Am. Chem. Soc.* **1989**, *111*, 8912−8914.

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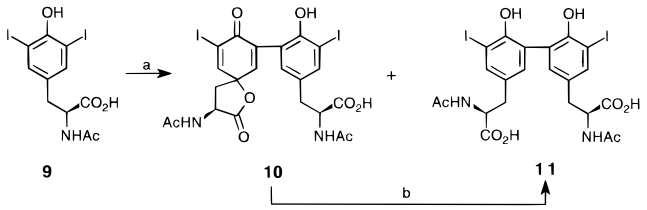
Scheme 2



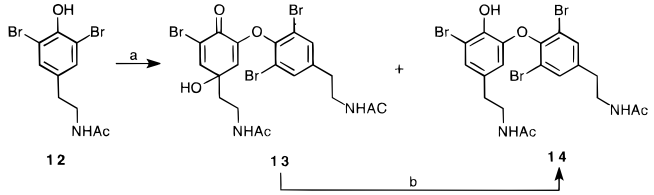
(a) HRP / H_2O_2, pH 6. (b) $NaHSO_3$ / NaOH.

tected amino acid, 3,5-dibromo-L-tyrosine (**6**), were similarly oxidized by HRP at a more rapid rate than **1**, but the yield of the corresponding products were somewhat lower (42% of **7** and 58% of **8**). On the other hand, when *N*-acetyl-3,5-diiodo-L-tyrosine (**9**) was exposed to HRP, a mixture of C−C coupling products, **10** and **11**, was isolated (Scheme 2). The overall yield of **11** after reduction of the mixture was 45%. These results clearly show that the mode of oxidation catalyzed by HRP differs from that promoted by thallium trinitrate, which catalyzed the C−O coupling of diiodotyrosine derivatives[13] in moderate yields.

For substrates lacking a carboxylic acid function such as *N*-acetyl-3,5-dibromotyramine, **12**, it was also oxidized by HRP at pH 4.0 to afford the C−O coupled product, but via the intermediacy of **13**, which upon reduction gave **14** in 65% yield (Scheme 3). Also, *N*-acetyl-3,5-dichloro-L-tyrosine methyl ester, **15**, was converted, presumably via **16**, into **17** in 62% yield under similar reaction conditions (Scheme 4).[14]

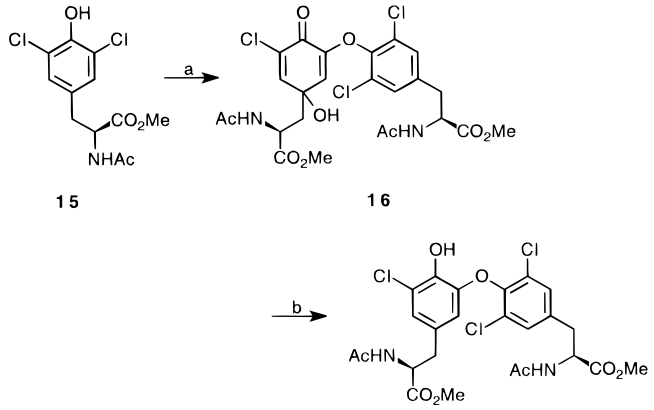
In summary, we have developed a novel efficient enzymatic method to catalyze the C−O coupling of dibromo- and dichlorotyrosine derivatives to yield the isodityrosine framework, the basic building block of many natural products.[3−6,15] In all these enzymatic transformations, no racemization of the chiral centers was observed. The method requires no protection of the functional groups and gives higher yield than previously reported methods. This provides the synthetic chemist with an alternative oxidative coupling technology especially when sensitive functionalities are present in substrates. The application of this methodology for the synthesis of complex natural products will be reported in due course.

Scheme 3



(a) H_2O_2 / HRP, pH 4. (b) $CrCl_2$, pH 3.

Scheme 4



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Supporting Information Available: Experimental procedures and characterization data (5 pages).

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(9) Kametani, T.; Nemoto, H.; Kobari, T.; Takano, S. *Chem. Pharm. Bull.* **1970**, *18*, 181−186.
(10) Kametani, T.; Takano, S.; Kobari, T. *Tetrahedron Lett.* **1968**, 4565−4568.
(11) (a) Zenk, M. H.; Gerardy, R.; Stadler, R. *J. Chem. Soc., Chem. Commun.* **1989**, 1725−1727. (b) Stadler, R.; Zenk, M. H. *J. Biol. Chem.* **1993**, *268*, 823−831.
(12) Gross, A. J.; Sizer, I. W. *J. Biol. Chem.* **1959**, *234*, 1611−1614.
(13) Suzuki, Y.; Nishiyama, S.; Yamamura, S. *Tetrahedron Lett.* **1990**, 4053−4058.
(14) *N*-Acetyl-3,5-dibromo-4-hydroxy-D-phenylglycine was similarly oxidized by HRP to give the corresponding C−O coupled product.
(15) Franklin, M. A.; Penn, S. G.; Lebrilla, C. B.; Lam, T. H.; Pessah, I. N.; Molinski, T. F. *J. Nat. Prod.* **1996**, *59*, 1121−1127.