3.25 (dd, 1 H, $J_1 = J_2 = 8.5$ Hz), 3.10 (d, 1 H, $J = 13.2$ Hz), 3.02 (d, 1 H, $J = 13.2$ Hz), 2.52 (s, 2 H), 2.27 (ddd, 1 H, $J_1 = 12.7$, $J_2 = 4.6$, $J_3 = 1.7$ Hz), 1.53 (ddd, 1 H, $J_1 = J_2 = 11.2$, $J_3 = 12.7$ Hz), 1.33 (d, 3 H, $J = 6.3$ Hz), 1.26 (s, 3 H), 0.93 (s, 9 H), 0.89 (s, 9 H), 0.14 (s, 3 H), 0.13 (s, 3 H), 0.12 (s, 3 H), 0.09 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 182.44, 182.38, 173.3, 158.7, 156.8, 143.0, 138.8, 138.4, 136.1, 132.7, 125.0, 124.7, 123.8, 123.1, 78.3, ca. 77 (concealed in solvent peaks), 74.7, 71.7, 71.4, 62.3, 62.0, 51.7, 44.4, 42.3, 41.1, 27.3, 26.3, 26.2, 19.2, 18.3, 18.1, -2.6, -2.9, -3.8, -4.1; IR (neat) 3530, 2950, 1730, 1675, 1580, 1570, 1470, 1445, 1390, 1325, 1250, 1110, 1010, 835, 780, 760 cm^{-1}; $[\alpha]^{27}_D$ -3° (*c* 1.2, $CHCl_3$); HRMS *m/z* 756.3750 (756.3722 calcd for $C_{40}H_{60}O_{10}Si_2$, M^+).

Vineomycinone B_2 Methyl Ester (7). To a solution of **46** (34.1 mg, 0.0451 mmol) in CH_2Cl_2 (6 mL) was added a solution of BBr_3 (303 mg, 1.21 mmol) in CH_2Cl_2 (1 mL) at -78 °C. After 25 min, saturated $NaHCO_3$ was added. The mixture was stirred for 5 min, acidified with 1 N HCl, and extracted with $CHCl_3$. The extract was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Purification of the product by PTLC (hexane/EtOAc = 1/9) afforded vineomycinone B_2 methyl ester (**7**) (20.3 mg, 90.0%). Recrystallization from $CHCl_3$-hexane gave **7** as orange needles: mp 186-187 °C; $R_f = 0.52$ (EtOAc); ^{1}H NMR ($CDCl_3$) δ 13.2 (s, 1 H), 13.1 (s, 1 H), 7.91 (d, 1 H, $J = 7.9$ Hz), 7.85 (d, 1 H, $J = 7.9$ Hz), 7.80 (d, 1 H, $J = 7.9$ Hz), 7.69 (d, 1 H, $J = 7.9$ Hz), 4.94 (dd, 1 H, $J_1 = 11.2$, $J_2 = 1.8$ Hz), 3.86 (ddd, 1 H, $J_1 = 11.2$, $J_2 = 9.0$, $J_3 = 4.9$ Hz), 3.73 (s, 3 H), 3.53 (dq, 1 H, $J_1 = 9.0$, $J_2 = 6.2$ Hz), 3.22 (dd, 1 H, $J_1 = J_2 = 9.0$ Hz), 3.11 (d, 1 H, $J = 13.6$ Hz), 3.02 (d, 1 H, $J = 13.6$ Hz), 2.59 (d, 1 H, $J = 15.8$ Hz), 2.55 (d, 1 H, $J = 15.8$ Hz), 2.54 (ddd, 1 H, $J_1 = 12.8$, $J_2 = 4.9$, $J_3 = 1.8$ Hz), 1.48 (ddd, 1 H, $J_1 = 12.8$, $J_2 = J_3 = 11.2$ Hz), 1.42 (d, 3 H, $J = 6.2$ Hz), 1.31 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 188.2, 188.1, 173.3, 161.3, 159.0, 139.6, 138.3, 134.7, 133.3, 131.84, 131.78, 119.4, 118.9, 115.6, 115.5, 78.0, 75.9, 73.1, 71.8, 71.3, 51.8, 44.4, 40.5, 39.4, 27.3, 18.1; IR (neat) 3400, 2930, 1725, 1625, 1580, 1430, 1255, 1090, 1070, 790, 760 cm^{-1}; $[\alpha]^{29}_D$ +118° (*c* 1.05, dioxane); HRMS *m/z* 485.1438 (485.1446 calcd for $C_{25}H_{25}O_{10}$, $M^+ - CH_3$). Anal. Calcd for $C_{26}H_{28}O_{10}$: C, 62.39; H, 5.64. Found: C, 62.57; H, 5.54.

12-*epi*-Vineomycinone B_2 Methyl Ester (*epi*-7): orange needles; mp 193-194 °C ($CHCl_3$-hexane); $R_f = 0.52$ (EtOAc); ^{1}H NMR ($CDCl_3$) δ 13.2 (s, 1 H), 13.1 (s, 1 H), 7.92 (d, 1 H, $J = 7.8$ Hz), 7.85 (d, 1 H, $J = 7.8$ Hz), 7.80 (d, 1 H, $J = 7.8$ Hz), 7.69 (d, 1 H, $J = 7.8$ Hz), 4.94 (dd, 1 H, $J_1 = 11.2$, $J_2 = 1.5$ Hz), 3.86 (ddd, 1 H, $J_1 = 11.2$, $J_2 = 9.0$, $J_3 = 4.9$ Hz), 3.73 (s, 3 H), 3.54 (dq, 1 H, $J_1 = 9.0$, $J_2 = 5.9$ Hz), 3.22 (dd, 1 H, $J_1 = J_2 = 9.0$ Hz), 3.09 (d, 1 H, $J = 13.7$ Hz), 3.04 (d, 1 H, $J = 13.7$ Hz), 2.57 (s, 2 H), 2.54 (ddd, 1 H, $J_1 = 12.7$, $J_2 = 4.9$, $J_3 = 1.5$ Hz), 1.48 (ddd, 1 H, $J_1 = 12.7$, $J_2 = J_3 = 11.2$ Hz), 1.43 (d, 3 H, $J = 5.9$ Hz), 1.31 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 188.2, 188.1, 173.3, 161.3, 159.0, 139.6, 138.3, 134.7, 133.3, 131.84, 131.78, 119.4, 118.9, 115.6, 115.5, 78.0, 75.9, 73.1, 71.8, 71.3, 51.8, 44.4, 40.5, 39.4, 27.2, 18.1; IR (neat) 3300, 2930, 1720, 1620, 1580, 1430, 1255, 1090, 1065, 790, 755 cm^{-1}; $[\alpha]^{28}_D$ +111° (*c* 0.45, dioxane); HRMS *m/z* 469.1505 (469.1497 calcd for $C_{25}H_{25}O_9$, $M^+ - OCH_3$). Anal. Calcd for $C_{26}H_{28}O_{10}$: C, 62.39; H, 5.64. Found: C, 62.18; H, 5.51.

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Supplementary Material Available: Physical properties for compounds **10**, **13**, **17**, **18**, **26**, **30**, **31**, **33**, **34**, **35**, *epi*-**40a-42a**, *epi*-**40b-42b**, *epi*-**45**, and *epi*-**46** (8 pages). Ordering information is given on any current masthead page.

Preparation of Chiral Cyanohydrins by an Oxynitrilase-Mediated Transcyanation

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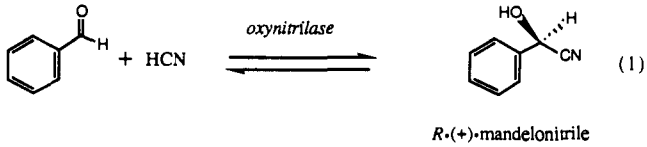
Abstract: The transcyanation of aromatic and aliphatic aldehydes **1** (RCHO) with acetone cyanohydrin is catalyzed by the enzyme D-oxynitrilase to afford (*R*)-cyanohydrins **2** (RCH(OH)CN). The biocatalytic method using acetone cyanohydrin gives products of high enantiomeric purity with better consistency than similar conditions using hydrogen cyanide as the cyanide source. The use of an ether-buffer biphasic solvent system is essential for producing products of optimum optical purity, but the solubility properties of the substrate have a pronounced effect on the enantiomeric purity of the final product. A discussion relating the solubility partition coefficient (log *P*) of a substrate to the enantiomeric purity of the product as a guide for predicting the outcome with new substrates is presented. Application of the method to the preparation of the following cyanohydrins is reported (R, ee): **a**, C_6H_5, 92% ee; **b**, 3,4-$(CH_2O_2)C_6H_3$, 90% ee; **c**, 2-$(CH_3O)C_6H_4$, 96% ee; **d**, $C_6H_5CH_2$, 88% ee; **e**, $CH_3SCH_2CH_2$, 92% ee; **f**, $CH_3(CH_2)_5CH_2$, 92% ee; **g**, $(CH_3)_3C$, 92% ee; **h**, c-C_6H_{11}, 96% ee; **i**, $CH_3O_2C(CH_2)_6CH_2$, 97% ee; **j**, (*E,E*)-$CH_3CH{=}CHCH{=}CH$, 96% ee; **k**, $(CH_3)_2C{=}CHCH_2CH_2C(CH_3){=}CH$, 99% ee.

The flavoprotein D-oxynitrilase (mandelonitrile-benzaldehyde lyase (EC 4.1.2.10)) catalyzes the reversible condensation of benzaldehyde with hydrogen cyanide to form (*R*)-(+)-mandelonitrile[1,2] (eq 1). Since its original isolation and purification, attempts have been made to use this enzyme as a biocatalyst for preparing chiral cyanohydrins.[3,4] Early work on the



(1)

R-(+)-mandelonitrile

oxynitrilase-mediated condensation of unnatural substrates with hydrogen cyanide met with varying degrees of success. For example, achieving high enantioselectivity in aqueous media was often difficult due to a competing nonenzymic reaction of the substrate with cyanide.[5] Also, the enantiomeric purity of the

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(1) (a) Becker, W.; Pfeil, E. *Biochem. Z.* **1966**, *346*, 301-321. (b) Becker, W.; Benthin, U.; Eschenhof, E.; Pfeil, E. *Ibid.* **1963**, *337*, 156-166.

(2) (a) Jorns, M. S. *J. Biol. Chem.* **1979**, *254*, 12145-12152. (b) Jorns, M. S.; Ballenger, C.; Kinney, G.; Pokora, A.; Vargo, D. *Ibid.* **1983**, *258*, 8561-8567.

(3) Becker, W.; Pfeil, E. *J. Am. Chem. Soc.* **1966**, *88*, 4299-4300.

(4) For other methods of preparing chiral cyanohydrins by enzymatic transformations involving hydrolysis of cyanohydrin esters using enzymes such as lipases, see: (a) Wang, Y.-F.; Chen, S.-T.; Liu, K.-C.; Wong, C.-H. *Tetrahedron Lett.* **1989**, *30*, 1917-1920. (b) Ohta, H.; Kimura, Y.; Sugano, Y. *Ibid.* **1988**, *29*, 6957-6960. (c) Matsuo, N.; Ohno, N. *Ibid.* **1985**, *26*, 5533-5536. (d) Ohta, H.; Miyamae, Y.; Tsuchihashi, G.-I. *Agric. Biol. Chem.* **1989**, *53*, 281-283. (e) Ohta, H.; Miyamae, Y.; Tsuchihashi, G.-I. *Ibid.* **1989**, *53*, 215-222. (f) Ohta, H.; Miyamae, Y.; Tsuchihashi, G.-I. *Ibid.* **1989**, *50*, 3181-3184.

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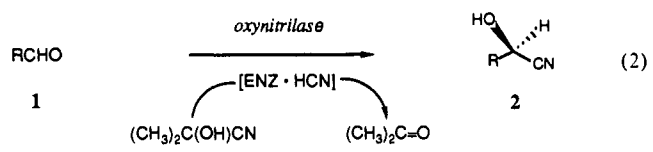
enzymic products could be further compromised by their racemization in the aqueous buffer during the course of the reaction. Some improvement in product enantiomeric purity has been accomplished by conducting the reaction in water-immiscible organic solvents.[6] It was suggested that the organic solvent suppresses the nonenzymic pathway more than the enzyme path, but this method works well primarily for aromatic substrates. The enantiomeric purities of cyanohydrins from aliphatic aldehydes, though improved by organic solvent conditions, show considerable variation as a function of substrate structure. The inconsistent behavior for this category of substrates accords with the broad range of rate and equilibrium constants[7] for the nonenzymic addition of cyanide to aliphatic aldehydes accounting for various degrees of nonenzymic competition and/or racemization. This biocatalytic method might be more broadly applicable by modifying the nature of the nonenzymic process of cyanohydrin formation.

We anticipated that one strategy for improving the scope of the enzymatic method for a broader range of substrates was to introduce a chemical shunt that could divert the flux of reactants away from the nonenzymic direction in favor of the enzymic pathway. This shunt could be in the form of an alternate cyanide carrier capable of generating either an active cyanide-bound form of the lyase or producing sufficiently low levels of HCN that the nonenzyme path is minimal relative to the enzyme-catalyzed process (eq 2). It is highly desirable that such a cyanide carrier



(2)

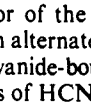
shows minimal reaction with the substrate in the absence of enzyme. Acetone cyanohydrin was attractive as a possible carrier for this reaction since it has been used for the transcyanation of carbonyl compounds under nonenzymic conditions. Furthermore, it reacts slowly with the carbonyl partner in the absence of a strong acid or base mediator.[7] We now report a method of preparing cyanohydrins of consistently high enantiomeric purity by the transcyanation of aldehydes with acetone cyanohydrin and oxynitrilase. Additionally, a tentative working model for predicting the substrate reactivity profile for oxynitrilase is presented as well as the substrate structural parameters that limit application of the method.

Results and Discussion

Oxynitrilase Transcyanation. Model studies involving the conversion of phenylacetaldehyde (**1d**) to the (*R*)-(+)-cyanohydrin **2d** (cf. Table I) revealed that the addition of 500 μL of a solution of oxynitrilase (Sigma Co., 10 mg mL^{-1}; activity[9] = 190 units/mg of protein) in 0.4 M acetate buffer (pH 5.0) to a solution of 1 mmol of **1d** and 1.3 equiv of acetone cyanohydrin in 11 mL of ether at 23 °C gave, after 7 h, an 83% isolated yield of cyanohydrin **2d** with an 88% ee. A control in the absence of enzyme, or using a boiled enzyme solution, gave only an 8% yield of completely racemic **2d**. Consistent with the observations of Effenberger et al.,[6] the use of ethanol–buffer mixtures as the reaction solvent gave a product in high chemical yield but with low enantiomeric purity. Also, we observed that the use of a buffer–organic biphasic media afforded higher yields and faster reaction rates than did the use of "organic solvent conditions"[10] with a low (<1%) water content. However, the use of the ether–buffer biphasic media with HCN as the cyanide source gave a product of only 42% ee. Lactonitrile failed to serve as a suitable cyanide carrier in place of acetone cyanohydrin, whereas (±)-mandelonitrile was equally effective as a transcyanating reagent, affording product ee's comparable to those obtained with acetone cyanohydrin. The principal properties that make acetone cyanohydrin superior to other cyanide sources are its commercial availability, miscibility with the buffer phase, and formation of an easily separable, volatile byproduct (acetone). Other reaction variables also influenced the enantioselectivity of the reaction. For example, the optical purity of the products increased with decreasing pH of the reaction buffer, but we observed that the rate of the enzymatic reaction drops sharply and approaches zero below pH 5.0. At pH 4.8, only a 5% yield of **2d** was obtained and with only a slight increase in ee.

As shown in Table I, oxynitrilase catalyzed the transcyanation of a variety of aromatic and aliphatic substrates with acetone cyanohydrin to afford products of high enantiomeric purity. The enzyme tolerates considerable structural variation within both aromatic and aliphatic substrates, and in most cases the product enantiomeric purity was 90–99% ee. Generally, products with slightly higher ee's, but lower chemical yields, than those in Table I could be obtained by using shorter reaction times. The enantiomeric excess for each product was determined by conversion

Table I. Oxynitrilase-Catalyzed Trancyanation of Aldehydes **1** to Cyanohydrins **2**[a]

	Substrate 1	Product 2	time (h)	yield[b] (%)	% ee[c]	$[\alpha]_D$,[d] confign
a	C_6H_5CHO		10	72	92	+40.6°, *R*
b[e]	3,4-$(CH_2O_2)C_6H_3CHO$		14	35	90	+36°, *R*
c	2-$CH_3OC_6H_4CHO$		16	65	96	+4.6°, *R*
d	$C_6H_5CH_2CHO$		18	83	88 (40)	+10.5°, *R*
e	$CH_3SCH_2CH_2CHO$		7	60	92 (80)	+24.6°, *R*
f	$CH_3(CH_2)_5CHO$		22	65	92	+16.1°, *R*
g	$(CH_3)_3CCHO$		4	58	92 (73)	+14.5°, *R*
h	c-$C_6H_{11}CHO$		10	72	96	+4.8°, *R*
i	$CH_3OC(CH_2)_7CHO$		22	68	97	+6.9°, *R*
j[e]			48	36	96	-24.9°, *R*
k[e]			42	46	99	-52°, *R*

[a] All reactions were performed with 1 mmol of substrate. [b] Yields correspond to isolated, chromatographically purified products. [c] Determined by conversion of the cyanohydrin to the corresponding (+)-MTPA ester followed by HPLC analysis at 256 nm. Values in parentheses are those reported for cyanohydrins prepared by using hydrogen cyanide and oxynitrilase; see ref 6a. [d] All rotations were measured at 23 °C as solutions in chloroform, c = 1 at the sodium D-line. [e] Twice the amount of enzyme solution was used.

(5) (a) Becker, W.; Freund, H.; Pfeil, E. *Angew. Chem.* **1965**, *77*, 1139. (b) Here the term aqueous media includes homogeneous reaction media consisting of a bulk aqueous buffer in combination with water-miscible organic solvents such as ethanol.

(6) (a) Effenberger, F.; Ziegler, T.; Forster, S. *Angew. Chem., Int. Ed. Eng.* **1987**, *26*, 458–459. (b) Ziegler, T.; Horsch, B.; Effenberger, F. *Synthesis* **1990**, 575–578.

(7) (a) Ching, W.-M.; Kallen, R. G. *J. Am. Chem. Soc.* **1978**, *100*, 6119–6124. (b) Okano, V.; doAmaral, L.; Cordes, E. H. *Ibid.* **1976**, *98*, 4201–4203. (c) Schlesinger, G.; Miller, S. L. *Ibid.* **1973**, *95*, 3729–3735. (d) Young, P. R.; McMahon, P. E. *Ibid.* **1979**, *101*, 4678–4681. (e) Finiels, A.; Geneste, P. *J. Org. Chem.* **1979**, *44*, 1577–1578. (f) Baker, J. W.; Barrett, G. F. C. *J. Chem. Soc.* **1952**, 2831–2839.

(8) (a) Kobayashi, Y.; Hayashi, H.; Miyaji, K.; Inoue, S. *Chem. Lett.* **1986**, 931–934. (b) Mori, A.; Kinoshita, K.; Osaka, M.; Inoue, S. *Ibid.* **1990**, 1171–1172 and references therein.

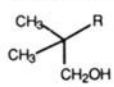
(9) Hochuli, E. *Helv. Chim. Acta* **1983**, *66*, 489–493.

(10) (a) Klibanov, A. M. *Trends Biochem. Sci. (Personal Ed.)* **1989**, *14*, 141–144. (b) *Biocatalysis in Organic Media*; Laane, C., Tramper, J., Lill, M. D., Eds.; Elsevier: Amsterdam, 1987.

to their (+)-MTPA esters[11] followed by normal-phase HPLC analysis and comparison of the enzyme products with the diastereomeric esters prepared from their racemic counterparts. The absolute configurations for **2a**, **2h**, and **2j** were assigned to be *R* by comparison of their rotations with literature values,[12,13,14] while the others were assigned from the signs of the optical rotations of their hydrolysis products that were generated by treating the cyanohydrin with aqueous acid to give a known chiral α-hydroxy acid.[15] The configuration for **2k** is not known with certainty, but is assigned by analogy to the consistent pattern for **2a–i**. Thus, oxynitrilase appears to give the same absolute sense of chirality regardless of the steric encumbrance within the hydrophobic substituent.

In keeping with previous findings,[6] the use of biphasic media with the enzymatic transcyanation process is essential for obtaining products of optimum enantiomeric purity. Both substrates and products are then partitioned between the organic and aqueous phases, and this partitioning has a pronounced influence on the enantiomeric purity of the enzyme-derived product. The optical purity of products is significantly decreased for substrates that display appreciable partitioning into the aqueous phase. For example, the highly water soluble nicotinaldehyde (**3**) gave cyanohydrin **4** with only 2% ee compared to the structurally similar benzaldehyde (**1a**) that affords (*R*)-(+)-mandelonitrile (**2a**) in 92% ee. A similar trend was found for hydroxypivalaldehyde (**5**)[16] and pivalaldehyde (**1g**), which gave, respectively, **6** and **2g** in 20% ee and 92% ee. Conversely, substrates whose water solubilities

3, R = CHO
4, R = CH(OH)CN



5, R = CHO
6, R = CH(OH)CN

are low (e.g., **2k**) give almost enantiomerically pure products. The possibility that the ee's for **4** and **6** were caused by the heterogroup interfering with catalytic functions in the active site of the enzyme could be discounted by observing that the products ee's were increased to 5% and 30%, respectively, when the reaction was terminated after a substantially shorter reaction time (3 h). Consequently, the solubility characteristics of both the substrate and the product strongly influence the overall enantiomeric purity of the product because of the extensive racemization of the latter, which occurs rapidly in water.

The synthetic development of the enzymic reaction would benefit from the ability to predict the solubility properties of a product and avoid the problems associated with the solubility effects as noted above. The solubility behavior of a solute in a biphasic system is expressed by the logarithm of its partition coefficient (log *P*),[17] which is a measure of its distribution in the two phases. While approximate log *P* values can usually be calculated from fragmental hydrophobicity constants for a variety of structural fragments or substituents,[18] these data are not available for the cyanohydrin functionality.[19] However, the log *P* values for the aldehyde substrates can be readily calculated,

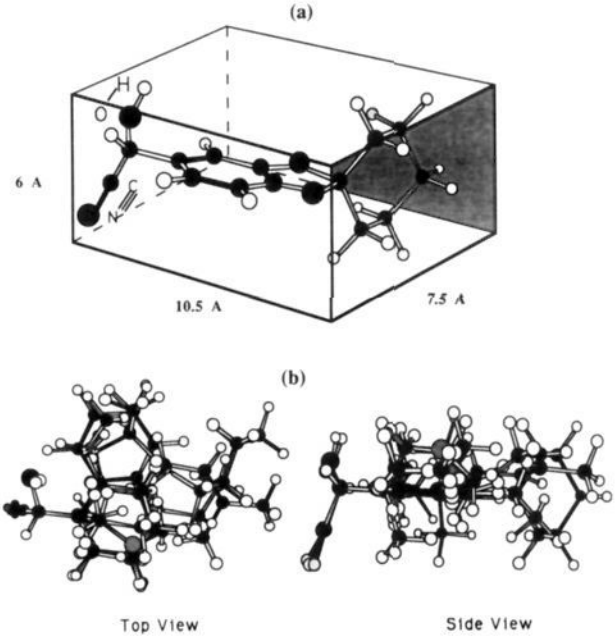


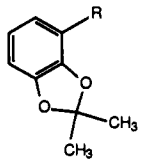
Figure 1. Model for predicting the steric limits on modified substrates for oxynitrilase. (a) Approximate dimensions (angstroms) of the minimum accessible volume near the active site of oxynitrilase. Cyanohydrin **10** is shown in the figure for clarity. The cubic domain in (a) represents the boundaries of the computer-generated superimposed volumes (b) for the cyanohydrin products **2a–i**, **2k**, **8**, and **10** and selected ones of ref 6. Also, the model shows that the right face of the region is not limited (see discussion). (b) Side and top views of the computer-generated superimposed ball-and-stick models of cyanohydrin products. The dimensions of these volumes were used for constructing the accessible region of (a).

and we used these values as an indication of the general solubility trends for their corresponding cyanohydrins. Noteworthy, the log *P* values for substrates **1a–k** are all positive, ranging from +0.01 to +2.11, and the corresponding ee's range from 88 to 99%. By contrast, log *P* for substrates **3** and **5** are −0.65 and −0.717, with ee's of 2% and 30%, respectively. The negative partition coefficients for **3** and **5** indicate a high degree of partitioning in the aqueous phase.[20] These values suggest that the corresponding cyanohydrins also have a high degree of water solubility that can lead to extensive racemization.[21] Because we have observed that substrates with negative log *P* values usually give cyanohydrins of poor optical purity, the enzymatic method appears best suited to substrates whose partition coefficients are strongly positive. Careful consideration should be given to this structural parameter when applying this current method to new substrates. Furthermore, these results presently led us to presume that the beneficial effect of using an organic solvent (i.e., biphasic media) in the enzyme reaction may stem primarily from averting racemization by diminishing the product's residence time in water.

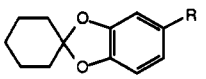
Substrate Model for Oxynitrilase. In order to promote further application of the enzymatic method to organic synthesis, it is also desirable to have a model for predicting the specificity of oxynitrilase. At the present time, no structural data for either the active site or the binding site of oxynitrilase is available. An investigation by Jorns and co-workers[2] has shown that, despite oxynitrilase's dependence on FAD, this cofactor is not directly involved at the catalytic site, nor do its redox properties change during the catalytic cycle. The absence of additional structural data requires an empirical approach toward developing a working model of the binding site and catalytic region. An additional constraint that restricts the extent to which the empirical exploration of the binding topography can be performed is the

(11) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
(12) Tanaka, K.; Mori, A.; Inoue, S. *J. Org. Chem.* **1990**, *55*, 181–185.
(13) Minamikawa, H.; Hayakawa, S.; Yamada, T.; Iwasawa, N.; Narasaka, K. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4379–4383.
(14) Matthews, B. R.; Jackson, W. R.; Jayatilake, G. S.; Wilshire, C.; Jacobs, H. A. *Aust. J. Chem.* **1988**, *41*, 1697–1709.
(15) Hartwig, W.; Schollkopf, U. *Liebigs Ann. Chem.* **1982**, 1952–1970.
(16) Nerdel, F.; Frank, D.; Lengert, H.-J.; Weyerstahl, P. *Chem. Ber.* **1968**, *101*, 1850–1862.
(17) (a) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979. (b) Hansch, C. *Acc. Chem. Res.* **1969**, *2*, 232–239.
(18) Rekker, R. F. *The Hydrophobic Fragmental Constant*; Elsevier: Amsterdam, 1977.
(19) Because the cyanohydrin group displays chemical and electronic properties somewhat different from those of ordinary alcohols, or nitriles, we elected to treat it as a separate functional group, and thus, prefer not to use a combination of fragmental constants for the individual OH and CN units to calculate log *P* values.
(20) Grant, D. J. W.; Higuchi, T. In *Techniques in Chemistry*; Saunders, W. H., Jr., Weissberger, A., Eds.; Wiley: New York, 1990; Vol. 21, pp 12–88.
(21) The optical rotation of (+)-mandelonitrile (**2a**) is unchanged after 24 h in chloroform solution, but decreases by 7.5° after only 2 h in aqueous buffer solution.

solubility characteristics of the substrate as were discussed in the previous section. Notwithstanding, a model representing the molecular volume corresponding to mandelonitrile (127.7 Å^3)[22a] provides a reasonable starting point for constructing this model. All starting molecular geometries were generated by using computer-assisted modeling (MACROMODEL V3.0),[22b] and their energies were minimized to a gradient of 10^{-2} kcal/(Å mol) by using the MM2 force field. By using our experimental data, supplemented by previously reported data, the molecular volumes of substrates possessing acyclic substituents such as methoxy or methyl groups in the ortho, meta, or para positions were superimposed on the mandelonitrile volume, maintaining a fixed position of the CH(OH)CN moieties.[23] This afforded a secondary volume that was expanded principally in the plane occupied by the aromatic ring of mandelonitrile. By including aromatic substrates that possess either branched or spirocyclic substituents fused to adjacent positions such as in compounds **8** and **10**, the model is expanded in a third direction above and below the plane of the aromatic ring. Lastly, introducing the additional volumes of the appendages for selected aliphatic substrates in Table I gives the minimum cavity depicted by the cubic region shown in Figure 1a. The cubic region represents the boundaries of the superimposed molecular volumes shown by Figure 1b.



7, R = CHO
8, R = CH(OH)CN



9, R = CHO
10, R = CH(OH)CN

The allowable space in the binding region may be larger than that shown in Figure 1, but it is not likely to be smaller than boundaries set by this model. One important feature of this model is that one face of the six-sided volume remains unconfined. This is essential to account for the ability of the enzyme to tolerate the lengthy substituent of substrate **1i**. The present data do not allow us to exclude the possibility that other faces of the model may also be unrestricted. Additional work will be necessary to determine the exact limits of the dimensions for the volume shown in Figure 1.

Conclusion. The present investigation demonstrates that oxynitrilase is capable of catalyzing a transcyanation reaction of aldehydes with acetone cyanohydrin. The method described herein provides consistent enantioselectivities for a broader range of aliphatic substrates and avoids the use of the more hazardous agent hydrogen cyanide. Product racemization in the aqueous phase remains a major obstacle of the method, which limits product enantiomeric purity. This is especially problematic for substrates displaying a high degree of water solubility. To the tenuous extent that partition coefficients infer substrate and product solubility trends in biphasic media, we note that the highest level of stereoselectivity is observed for substrates with positive partition coefficient values (log $P \geq +0.1$), and poor ee's result for substrates whose calculated coefficients are negative. The enzyme accomodates substituents of considerable structural variation, and at the present time, we suggest the minimum dimensions of the cavity in the immediate vicinity of the binding region are approximately defined by a 6 × 7.5 × 10.5 Å pocket having at least one open face. The model is based on a total of approximately 25 substrates, all of which apparently afford the same sense of asymmetric induction at the prochiral carbonyl group. Therefore, the model is useful as an approximate guide to predict whether a substrate is too sterically encumbered to bind to oxynitrilase. Refinement of this model will be possible as additional results from this or other laboratories become available. Work is in progress toward this goal, and the results of these studies will appear in subsequent papers.

Experimental Section

^{1}H NMR spectra were obtained at 300 MHz on a Varian Gemini 300, at 400 MHz on a Varian VXR400, or at 500 MHz on a Varian VXR500 spectrometer in $CDCl_3$ solution. ^{13}C NMR spectra were recorded at 75 MHz on a Varian Gemini 300 spectrometer. Infrared spectra were determined on either a Perkin-Elmer 1420 or a Perkin-Elmer 983 spectrometer. Mass spectral data were obtained on a Finnigan 4510 GC/mass spectrometer. The abbreviation TF denotes thin film, KBr denotes a potassium bromide wafer, and HRMS denotes high-resolution mass spectrum. Optical rotation measurements were made as chloroform solutions at the sodium D-line on a Thorn NPL automatic polarimeter. HPLC analyses were performed on a Waters Model 501 system using a 25-cm SiO_2 (5 μm) column monitoring at 256 nm and eluting with 4:96 ethyl acetate–hexane at a flow rate of 2.0–3.6 mL min^{-1}.

Oxynitrilase (mandelonitrile lyase) was obtained from Sigma Co. as a suspension in 3.2 M ammonium sulfate solution, and the enzyme was partially purified prior to use as described below. (±)-Mandelonitrile, acetone cyanohydrin, and all commercial aldehydes were purchased from Aldrich Chemical Co. All aldehydes were purified by distillation prior to use to ensure removal of acid impurities. Acetone cyanohydrin was purified by distillation prior to use.

Preparation of Methyl 9-Oxononanoate (1i). To a solution of 5.12 g (18.5 mmol) of methyl 9,10-oxidooctanoate[24] in 500 mL of 30% aqueous tetrahydrofuran at 23 °C was added 250 mL of 3% aqueous perchloric acid. The mixture was stirred for 3 h at 23 °C followed by neutralization of the solution to pH 7 by the slow portionwise addition of sodium bicarbonate. The mixture was extracted with 3 × 200 mL of ether, and the organic phase was washed successively with 1 × 100 mL of water and 1 × 100 mL of brine and then dried over anhydrous magnesium sulfate. Evaporation of solvent gave a crude colorless diol[25] that was dissolved in 500 mL of tetrahydrofuran. Then was added 30 mL of a saturated aqueous solution of sodium periodate, and the mixture was stirred for 4 h at 23 °C. The mixture was extracted with 3 × 200 mL of ether, and the organic phase was washed successively with 1 × 100 mL of water and 1 × 100 mL of brine and dried over anhydrous magnesium sulfate. Evaporation of solvent gave a colorless oil that was chromatographed on a flash silica gel column in 1:20 ethyl acetate–hexane to afford 2.70 g (50%) of **1i** whose NMR data were consistent with those reported previously:[25] ^{1}H NMR ($CDCl_3$) δ 1.22–1.85 (br m, 10 H), 2.36 (t, J = 7 Hz, 2 H), 2.45 (t, J = 7 Hz, 2 H), 3.85 (s, 3 H), 9.80 (br s, 1 H).

Preparation of (*E*)-3,7-Dimethyl-2,6-octadienal (1k). The procedure of Corey[26] was followed by using 3.08 g (20 mmol) of (*E*)-3,7-dimethyl-2,6-octadienol and 9.7 g (25 mmol) of pyridinium dichromate in 20 mL dimethylformamide at −10 °C to afford, after workup and distillation, 2.64 g (86%) of geranial **1k**. ^{1}H NMR of **1k** was consistent with literature values and showed that **1k** was a 15:1 mixture of *E*/*Z* isomers.

Preparation of Aldehyde 7. To a solution of 1.5 g (10 mmol) of 2,2-dimethyl-1,3-benzodioxole[27] in 15 mL of tetrahydrofuran at 0 °C under a nitrogen atmosphere was added dropwise over a 15-min period 7.97 mL (10.5 mmol) of 1.3 M *n*-butyllithium. The mixture was stirred for 1 h at 0 °C and then 2 h at 23 °C followed by the addition of a solution of 0.76 mL (11 mmol) of dimethylformamide in 5 mL of tetrahydrofuran. The mixture was stirred for 1 h at 23 °C, and the reaction was quenched by the dropwise addition of 50 mL of a saturated aqueous solution of ammonium chloride. The reaction mixture was extracted with 2 × 150-mL portions of ether, and the combined extracts were washed successively with 1 × 50 mL of water and 1 × 50 mL of brine and dried over anhydrous sodium sulfate. Evaporation of solvent followed by flash silica gel chromatography in 1:50 ethyl acetate–hexane afforded 178 mg (10%) of **7**: IR (KBr) 2830, 2740, 1680, 1525, 1465 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.75 (s, 6 H), 6.80–6.95 (m, 2 H), 7.23 (d, J = 5 Hz, 1 H), 10.12 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.74, 113.30, 119.35, 120.22, 120.52, 121.20, 148.93, 149.81, 188.39; HRMS for $C_{10}H_{10}O_3$, calcd 178.0631, obsd *m/e* 178.0658 (M^+), 162, 58.

Preparation of 5-Bromospiro[1,3-benzodioxole-2,1′-cyclohexane]. To a solution of 4.75 g (25 mmol) of spiro[1,3-benzodioxole-2,1′-cyclohexane][27] in 15 mL of chloroform was added 4.67 g (26.25 mmol) of *N*-bromosuccinimide, and the mixture was refluxed for 3 h. The mixture

(22) (a) This volume was determined by using MACROMODEL V3.0. (b) Still, W. C.; Mohamadi, F.; Richards, N. J. G.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T. MACROMODEL V3.0, Department of Chemistry, Columbia University, New York, NY 10027.
(23) The rotational relationship of the cyanohydrin group to the rest of the molecular structure varied for each substrate so as to maximize the overlap of atoms during superimposition of the various structures.

(24) Zie Ken Jie, M. S. F.; Zheng, Y. F. *Chem. Phys. Lipids* **1989**, *49*, 167–179.
(25) Novak, L.; Baan, G.; Marosfalvi, J.; Szantay, C. *Chem. Ber.* **1980**, *113*, 2939–2949.
(26) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399–402.
(27) Slooff, G. *Recl. Trav. Chim. Pays-Bas* **1935**, *54*, 995–1010.

was cooled to 0 °C and filtered, followed by evaporation of the filtrate to afford a pale yellow oil that was distilled in vacuo to afford 4.70 g (70%) of the title compound, which solidified on standing: bp 170–175 °C (18 Torr); IR (KBr) 1477, 1235, 794 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (m, 2 H), 1.78 (m, 4 H), 1.90 (t, J = 7 Hz, 4 H), 6.60 (d, J = 6.5 Hz, 1 H), 6.89 (s, 1 H), 6.90 (d, J = 6.5 Hz, 1 H); HRMS for $C_{12}H_{13}BrO_2$, calcd 270.0075, obsd m/e 270.0066 (M^+), 268, 227, 225, 81.

Preparation of Aldehyde 9. To a suspension of 1.185 g (49 mmol) of magnesium turnings in 20 mL of ether at 23 °C under a nitrogen atmosphere was added dropwise over a 30-min period a mixture of 4.03 g (15 mmol) of 5-bromospiro[1,3-benzodioxole-2,1′-cyclohexane] and 3.27 g (30 mmol) of ethyl bromide in 40 mL of ether during which time the mixture began to reflux. After complete addition, the mixture was refluxed for 1 h and the mixture was cooled to 23 °C. A solution of 3.48 mL (45 mmol) of dimethylformamide in 4 mL of ether was added dropwise over a 5-min period, and the mixture was stirred for 1 h at 23 °C. The reaction was quenched by the dropwise addition of 50 mL of a saturated aqueous solution of ammonium chloride. The reaction mixture was extracted with 2 × 150-mL portions of ether, and the combined extracts were washed successively with 1 × 50 mL of water and 1 × 50 mL of brine and dried over anhydrous sodium sulfate. Evaporation of solvent followed by flash silica gel chromatography in 1:20 ethyl acetate–hexane afforded 1.62 g (49%) of **9**: IR (TF) 2785, 2835, 1676, 1592, 1483 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52 (m, 2 H), 1.75 (m, 4 H), 1.94 (t, J = 7 Hz, 4 H), 6.84 (d, J = 6.5 Hz, 1 H), 7.25 (s, 1 H), 7.35 (d, J = 6.5 Hz, 1 H), 9.76 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 22.78, 22.99, 34.80, 106.51, 108.02, 120.64, 128.22, 131.47, 148.40, 152.50, 190.04; HRMS for $C_{13}H_{14}O_3$, calcd 218.0944, obsd m/e 218.0968 (M^+), 174, 81.

Preparation of Oxynitrilase Buffer Solution. The commercial enzyme was obtained as a suspension in 3.2 M ammonium sulfate solution. Prior to use, the crude enzyme was partially purified to remove the ammonium salts by using the following procedure: 25 g of Sephadex G-25 (80 mesh) was suspended in 200 mL of 0.1 M acetate buffer (pH 5.0) containing 0.15% of NaCl, and the suspension was cooled to 0 °C. After equilibrating for 0.5 h at 0 °C, the suspension was transferred to a 2.5 cm × 500 cm column and the suspension was tightly packed at 0 °C under a positive pressure of nitrogen. The crude, yellow-colored, enzyme suspension (25 mg of protein, activity approximately 200 units/mg of protein) was applied to the top of the column and eluted with 0.1 M acetate buffer (pH 5.0) containing 0.15% NaCl. The protein was eluted under a slight positive pressure of nitrogen at a rate of 5 mL min^{-1}. Sample fractions of approximately 5 mL were collected. The fractions containing the enzyme protein were easily detected visually due to the yellow color of the lyase. The combined fractions (average 10–15 mL) were concentrated in vacuo (0.1 Torr) to a volume of 2.5 mL at a temperature not exceeding 15 °C. The final concentration of the enzyme solution was 10 mg of protein/mL. The activity was determined by the previously described assay method.[9] This solution could be stored at 0–4 °C for approximately 3 weeks without appreciable loss of activity.

General Procedure for the Preparation of Chiral Cyanohydrins Using Oxynitrilase. To a solution of 120 mg (1 mmol) of phenylacetaldehyde (**1d**) and 110 mg (1.3 mmol) of acetone cyanohydrin in 11 mL of ether at 23 °C was added 0.50 mL of the oxynitrilase buffer solution (0.4 M acetate (pH 5.0)) prepared as described above. The mixture was stirred for 18 h at 23 °C and then diluted with 50 mL of ether. The aqueous phase was extracted with 2 × 10 mL of ether, and the combined organic phases were dried over anhydrous magnesium sulfate. Evaporation of solvent gave a pale amber liquid that was chromatographed on a flash silica gel column in 1:30:50 ethyl acetate–benzene–dichloromethane to afford 122 mg (83%) of **2d**: $[\alpha]_D$ = +10.5°; 88% ee; IR (TF) 3420, 2300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.08 (d, J = 5 Hz, 2 H), 3.37 (d, J = 3 Hz, 1 H, OH), 4.59 (dd, J = 3 Hz and 5 Hz, 1 H), 7.31 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 41.15, 62.08, 119.38, 127.67, 128.78, 129.57, 133.88. These data were consistent with those previously reported[28] for racemic **2d**. Additionally, for each substrate, a control incubation in the absence of the enzyme was conducted in parallel during the enzymatic reaction.

Summary of Yields (%), Optical Rotations, and Spectral Data for Cyanohydrins 2a–k, 8, and 10. 2a ((+)-mandelonitrile): 72%; $[\alpha]_D$ = +40.6°; ^1H NMR ($CDCl_3$) δ 3.80 (br s, 1 H, OH), 5.50 (br s, 1 H), 7.28–7.59 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 63.60, 118.92, 126.75, 129.04, 129.82, 135.26. These data were consistent with those reported for (+)-mandelonitrile.[12]

2b: 35%; $[\alpha]_D$ = +36°; IR (TF) 3400, 2250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.35 (s, 1 H, OH), 5.42 (s, 1 H), 6.10 (s, 2 H), 6.83 (d, J = 6.8 Hz, 1 H), 6.98 (s, 1 H), 6.99 (d, J = 6.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 63.08, 101.40, 107.11, 108.55, 119.82, 120.86, 129.02, 148.33, 148.80. These data were consistent with those reported[29] for racemic **2b**.

2c: 65%; $[\alpha]_D$ = +4.6°; IR (TF) 3400, 2250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.48 (d, J = 5 Hz, 1 H, OH), 3.85 (s, 3 H), 5.56 (d, J = 5 Hz, 1 H), 6.94–7.42 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 55.38, 60.14, 110.97, 118.63, 121.28, 123.76, 128.15, 131.02, 156.81. These data were consistent with those reported[30] for racemic **2c**.

2e: 60%; $[\alpha]_D$ = 24.6°; IR (TF) 3420, 2250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.12 (t, J = 6.5 Hz, 2 H), 2.13 (s, 3 H), 2.70 (q, J = 6.5 Hz, 2 H), 3.75 (d, J = 6 Hz, 1 H, OH), 4.70 (q, J = 6.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 15.27, 29.02, 33.63, 59.92, 119.61. These data were consistent with those previously reported[6b] for **2e**.

2f: 65%; $[\alpha]_D$ = +16.1°; IR (TF) 3430, 2250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (t, J = 7 Hz, 3 H), 1.25–1.54 (m, 8 H), 1.83 (q, J = 7 Hz, 2 H), 3.35 (br s, 1 H, OH), 4.46 (t, J = 7 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 13.70, 22.42, 24.31, 28.48, 31.20, 34.55, 61.23, 120.31. These data were consistent with those previously reported[31] for racemic **2f**.

2g: 58%; $[\alpha]_D$ = +14.5°; IR (TF) 3450, 2250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.06 (s, 9 H), 3.10 (br s, 1 H, OH), 4.12 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 24.70, 35.25, 70.57, 119.21. These data were consistent with those previously reported[15] for racemic **2g**.

2h: 72%; $[\alpha]_D$ = +16.1°; IR (TF) 3420, 2250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.04–1.39 (m, 5 H), 1.60–2.20 (m, 6 H), 3.20 (br s, 1 H, OH), 4.26 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.36, 25.39, 25.88, 27.77, 28.08, 42.15, 66.22, 119.33. These data were consistent with those previously reported[13,6b] for **2h**.

2i: 68%; $[\alpha]_D$ = +6.9°; IR (TF) 3400, 2250, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (m, 6 H), 1.48 (m, 2 H), 1.61 (m, 2 H), 1.81 (q, J = 7 Hz, 2 H), 2.30 (t, J = 7 Hz, 2 H), 3.64 (d, J = 7 Hz, 1 H), 3.65 (s, 3 H), 4.45 (q, J = 7 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 24.32, 24.70, 28.52, 28.71, 28.80, 34.08, 35.13, 51.50, 61.11, 120.04, 174.56. HRMS for $C_{11}H_{19}NO_3$, calcd 213.1366, obsd m/e 213.1368 (M^+), 186, 182, 73, 60.

2j: 36%; $[\alpha]_D$ = −24.9°; IR (TF) 3410, 2250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.81 (d, J = 7 Hz, 3 H), 3.05 (br s, 1 H, OH), 4.99 (d, J = 7 Hz, 1 H), 5.62 (dd, J = 15 Hz, 7 Hz, 1 H), 5.91 (dq, J = 15 Hz, 7 Hz, 1 H), 6.10 (dd, J = 15 Hz, 10.5 Hz, 1 H), 6.49 (dd, J = 15 Hz, 10.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 18.10, 61.55; 118.35, 122.61, 129.14, 134.60, 135.63. These data were consistent with those previously reported[14] for **2j**.

2k: 46%; $[\alpha]_D$ = −52°; IR (TF) 3400, 2250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.62 (s, 3 H), 1.70 (s, 3 H), 1.77 (s, 3 H), 2.08–2.20 (m, 5 H), 5.07 (t, J = 7 Hz, 1 H), 5.14 (dd, J = 7 Hz, 6.5 Hz, 1 H), 5.42 (dd, J = 6.5 Hz, 7 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 16.85, 17.60, 25.88, 26.26, 39.15, 57.88, 118.80, 119.15, 122.91, 132.47, 145.10. HRMS for $C_{11}H_{17}NO$, calcd 179.1310, obsd m/e 179.1323 (M^+), 164, 152.

8: 8%; $[\alpha]_{Hg}$ = +30.3° (c = 0.31); IR (TF) 3400, 2250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.68 (s, 6 H), 2.78 (d, J = 6.5 Hz, 1 H, OH), 5.57 (t, J = 6.5 Hz, 1 H), 6.76–7.00 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 25.63, 59.00, 109.96, 116.84, 118.14, 119.18, 119.66, 121.92, 145.14, 148.03. HRMS for $C_{11}H_{11}NO_3$, calcd 205.0736, obsd m/e 205.0749 (M^+), 178, 162, 58.

10: 8%; $[\alpha]_{Hg}$ = +41.3° (c = 0.22); IR (TF) 3400, 2250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (m, 2 H), 1.73 (m, 4 H), 1.90 (t, J = 7 Hz, 4 H), 2.73 (br s, 1 H, OH), 5.42 (br s, 1 H), 6.75 (d, J = 6.5 Hz, 1 H), 6.90–6.97 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 22.88, 24.24, 35.00, 63.63, 107.01, 108.45, 118.91, 120.06, 120.25, 128.61, 148.62, 149.21. HRMS for $C_{14}H_{15}NO_3$, calcd 245.1048, obsd m/e 245.1021 (M^+), 218, 174, 81.

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(28) Dyong, I.; Bertram, H. P. *Chem. Ber.* **1973**, *106*, 2654–2662.

(29) Kihara, M.; Ohnishi, K.; Vangveravong, S.; Kobayashi, S. *Chem. Pharm. Bull.* **1989**, *37*, 870–876.

(30) Ghosh, S.; Datta, I.; Chakraborty, R.; Das, T. K.; Sengupta, J.; Sarkar, D. *Tetrahedron* **1989**, *45*, 1441–1446.

(31) Torii, S.; Inokuchi, T.; Takagishi, S.; Horike, H.; Kuroda, H.; Uneyama, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2173–2188.