pH 6.8, and 10 mM sodium sulfite. The protein concentration was 2-4 mg/mL. The total ligand concentration was between 0.05 and 4 mM. The dialysis cells were allowed to equilibrate 5 h under slow rotation. The protein was precipitated by the addition of trichloroacetic acid to a final concentration of 5%. The ligand concentrations were determined by HPLC analysis. HPLC conditions for the analysis of ligand concentrations are given in Table V. Scatchard plots were evaluated using the program LIGAND (J. P. Munson).

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**Supplementary Material Available:** $^1H$ and $^{19}F$ NMR spectra of compounds **32**, **33**, and **40**, a $^1H$ NMR spectrum of **42**, $^1H$ and $^{19}F$ spectra and reversed-phase HPLC trace of **11**, $^1H$ NMR and $^{19}F$ NMR spectra and HPLC data for compound **13**, and $^1H$ and $^{19}F$ NMR data for compound **53** (41 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and may be ordered from the ACS; see any current masthead page for ordering information.

# One-Pot Synthesis of Optically Active Cyanohydrin Acetates from Aldehydes via Lipase-Catalyzed Kinetic Resolution Coupled with in Situ Formation and Racemization of Cyanohydrins

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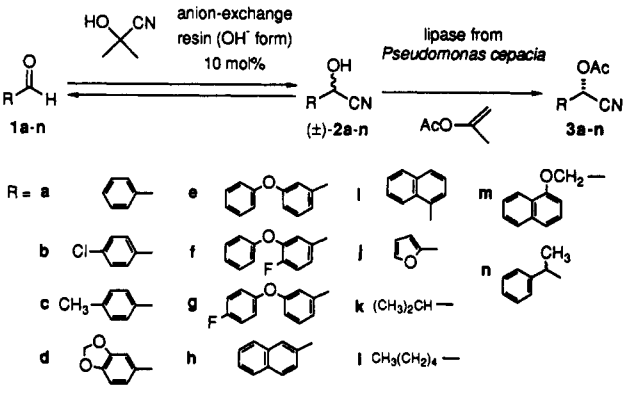
A novel one-pot synthesis of optically active cyanohydrin acetates from aldehydes has been accomplished by lipase-catalyzed kinetic resolution coupled with in situ formation and racemization of cyanohydrins in an organic solvent. Racemic cyanohydrins **2**, generated from aldehydes **1** and acetone cyanohydrin in diisopropyl ether under the catalysis of basic anion-exchange resin ($OH^-$ form), were acetylated stereoselectively by a lipase from *Pseudomonas cepacia* (Amano) with isopropenyl acetate as an acylating reagent. The (*S*)-isomer of **2** was preferentially acetylated by the lipase, while the unreacted (*R*)-isomer was continuously racemized through reversible transhydrocyanation catalyzed by the resin. These processes thus enabled one-stage conversion of various aldehydes **1a-n** into the corresponding (*S*)-cyanohydrin acetates **3a-n** with up to 94% ee in 63-100% conversion yields. The racemization of the optically active cyanohydrin **2e** by Amberlite IRA-904 ($OH^-$ form) was found to be much faster than the enzymatic acetylation, confirming the effective second-order asymmetric transformation. Enzymatic deacetylation of (*S*)-cyanohydrin acetates in an organic solvent and the synthesis of optically active pyrethroids are also described.

Optically active cyanohydrins are important starting materials for the synthesis of a number of chiral pharmaceuticals and agricultural chemicals because cyanohydrins are easily transformed into multifunctional chiral synthons such as β-hydroxy amines,[1] α-hydroxy carboxylic acids,[1c,2] and α-hydroxy ketones.[3] Among several chemical[4,5] and biochemical approaches[1-3,6-8] for the synthesis of optically active cyanohydrins, kinetic resolution by lipases[7] or microorganisms[8] has been extensively studied; optically active cyanohydrin esters were conveniently prepared by stereoselective hydrolysis or transesterification catalyzed by these biocatalysts. However, the recovery of optically active cyanohydrins from the reaction mixture has failed in many cases[7a,b,8b,d,e] because cyanohydrins are unstable and susceptible to decomposition or racemization in aqueous media. The unstable nature of cyanohydrins has thus hampered the enzymatic approach to the kinetic resolution of cyanohydrins. In addition, these approaches were all based on conventional kinetic resolution where the maximum obtainable yield of one enantiomer cannot exceed 50%, and the product ee is dependent on the conversion.[9] It is therefore highly desirable to develop a new method for the enzymatic kinetic resolution of cyanohydrins.

**Scheme I**




Introducing in situ racemization of substrate is a promising approach to this because it would allow for quanti-

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**Table I. One-Pot Synthesis of Optically Active Cyanohydrin Acetates 3a–n from Aldehydes 1a–n Catalyzed by Lipase and Amberlite IRA-904 ($OH^-$ form)[a]**

| | | | conversion[b] | | acetate 3 | | |
|---|---|---|---|---|---|---|---|
| entry | aldehydes | reaction time (days) | 1 → 2 (%) | 2 → 3 (%) | yield[c] (%) | ee[d] (%) | abs config[e] |
| 1 | 1a | 6.3 | 100 | 100 | 96 | 84 | *S* |
| 2 | 1b | 3.8 | 97 | 93 | 83 | 84 | *S* |
| 3 | 1c | 2.5 | 88 | 82 | 64 | 91 | *S* |
| 4 | 1d | 6.5 | 89 | 96 | 81 | 91 | *S* |
| 5 | 1e | 2.9 | 95 | 88 | 80 | 89 | *S* |
| 6[f] | 1e | 2.9 | 92 | 71 | 62 | 91 | *S* |
| 7 | 1f | 3.0 | 96 | 93 | 88 | 91 | *S*[g] |
| 8 | 1g | 3.0 | 98 | 98 | 92 | 87 | *S*[g] |
| 9 | 1h | 6.3 | 98 | 98 | 88 | 85 | *S* |
| 10 | 1i | 6.1 | 91 | 79 | 70 | 70 | *S* |
| 11 | 1j | 6.0 | 96 | 76 | 57 | 47 | *R*[k] |
| 12[h] | 1k | 9.3 | 100 | 63 | 47 | 51 | *S* |
| 13[h] | 1l | 6.0 | 100 | 88 | 83 | 15 | *S* |
| 14 | 1m | 3.0 | 100 | 84 | 68 | 78 | *R*[k] |
| 15 | 1n | 7.6 | 100 | 43[i]/41[j] | 32/20 | 82/85 | *S*[g] |

[a] Typical conditions: benzaldehyde (1a) (265 mg, 2.5 mmol), acetone cyanohydrin (426 mg, 5.0 mmol), isopropenyl acetate (751 mg, 7.5 mmol), immobilized lipase (250 mg), Amberlite IRA-904 ($OH^-$ form, 96 mg, 10 mol % equiv to 1a), 3-Å ground molecular sieves (100 mg), dry diisopropyl ether (20 mL), 40 °C. [b] Determined by $^1H$ NMR. [c] Isolated yield from aldehyde 1. [d] Determined by $^1H$ NMR in the presence of the chiral shift reagent, Eu(hfc)$_3$. [e] Determined by comparing the sign of the optical rotation with that of reported values (see the Experimental Section). [f] The lipase and the resin were recovered from the reaction mixture of entry 5 and reused. [g] The absolute configuration was assigned to be *S* by the comparison of the sign of the optical rotation and $^1H$ NMR spectrum in the presence of Eu(hfc)$_3$ with those of (*S*)-3a–e (see the Experimental Section). [h] Reaction temperature was 25 °C. [i] Conversion yield of the fast eluting diastereomer 3n [TLC, $R_f$ = 0.34, hexane (12)–AcOEt (1); $^1H$ NMR δ (OAc) 2.06]. [j] Conversion yield of the slow eluting diastereomer 3n′ [TLC, $R_f$ = 0.30, hexane (12)–AcOEt (1); $^1H$ NMR δ (OAc) 2.12]. [k] A consequence of the Cahn–Ingold–Prelog sequence rule.

tative conversion of racemic substrate into a single enantiomer of product, thereby maximizing the chemical and optical yields of one enantiomer irrespective of reaction conversion. So far this type of enzymatic second-order asymmetric transformation has only been achieved in a limited number of cases,[10] since several factors have to be considered. First, racemization of substrate must be faster than subsequent enzymatic reaction. Second, the product must be stereochemically stable under the reaction conditions where the substrate is racemized. Third, the enzymatic reaction must be highly stereoselective.

Lipase-catalyzed acylation of cyanohydrins is especially attractive in this regard because cyanohydrins undergo facile racemization by reversible addition of HCN to a carbonyl group under basic conditions, and the racemization is greatly reduced when the hydroxyl group of cyanohydrin is protected by acylation.[11] Moreover, a lipase from *Pseudomonas* sp. showed high stereoselectivity in the acetylation of mandelonitrile in diisopropyl ether.[7c] Considering that the base-catalyzed addition of HCN to a carbonyl compound is reversible, we reasoned that it is possible to generate cyanohydrins in situ, as well as to racemize them, from the corresponding aldehydes through the same transhydrocyanation process using an appropriate hydrogen cyanide source and a base catalyst.

We describe here a novel one-pot synthesis of optically active cyanohydrin acetates **3a–n** from the corresponding aldehydes **1a–n** by lipase-catalyzed kinetic resolution coupled with in situ generation and racemization of cyanohydrins in an organic solvent (Scheme I).[12]

## Results and Discussion

Aldehydes **1a–n** were converted to the corresponding racemic cyanohydrins **2a–n** through transhydrocyanation with acetone cyanohydrin in diisopropyl ether, catalyzed by a strongly basic anion-exchange resin, Amberlite IRA-904 ($OH^-$ form). In another part, the resulting cyanohydrins **2a–n** were acetylated in a stereoselective manner by a lipase from *Pseudomonas cepacia* (Amano) with isopropenyl acetate as an acyl donor[13] to give optically

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**Table II. Effects of Anion-Exchange Resins and Counteranions ($X^-$) on One-Pot Synthesis of (*S*)-3e[a]**

| entry | anion-exchange resin | counteranion ($X^-$) | reaction time (days) | conversion[b] (%) 1e → 2e | (*S*)-3e yield (%)[b] | (*S*)-3e ee (%)[c] |
|---|---|---|---|---|---|---|
| 1 | Amberlyst A-27[d] | $OH^-$ | 2.9 | 90 | 69 | 88 |
| 2 | Duolite A-162[e] | $OH^-$ | 2.1 | 97 | 90 | 80 |
| 3 | Duolite A-162 | $AcO^-$ | 1.0 | 100 | 99 | 90 |
| 4 | Amberlite IRA-400[f] | $OH^-$ | 1.5 | 0 | 0 | –[h] |
| 5 | Amberlyst A-21[g] | | 1.5 | 3 | 0 | –[h] |
| 6 | Amberlite IRA-904[d] | $OH^-$ | 1.0 | 93 | 80 | 91 |
| 7 | Amberlite IRA-904 | $CO_3^{2-}$ | 1.0 | 95 | 90 | 93 |
| 8 | Amberlite IRA-904 | $CN^-$ | 1.0 | 95 | 86 | 87 |
| 9 | Amberlite IRA-904 | $HCO_3^-$ | 1.0 | 95 | 88 | 87 |
| 10 | Amberlite IRA-904 | $AcO^-$ | 1.0 | 92 | 78 | 91 |
| 11 | Amberlite IRA-904 | $TsO^-$ | 3.1 | 0 | 0 | –[h] |
| 12 | Amberlite IRA-904 | $Cl^-$ | 1.0 | 12 | 7 | –[h] |
| 13 | Amberlite IRA-904 | (*R*)-binaphthol[i] | 0.8 | 94 | 84 | 94 |
| 14 | Amberlite IRA-904 | (*S*)-binaphthol[i] | 0.8 | 93 | 84 | 94 |

[a] Conditions: 3-phenoxybenzaldehyde (1e) (198 mg, 1.0 mmol), acetone cyanohydrin (170 mg, 2.0 mmol), isopropenyl acetate (300 mg, 3.0 mmol), immobilized lipase (100 mg), anion-exchange resin ($X^-$ form, 10 mol % equiv to 1e), 3-Å ground molecular sieves (40 mg), dry diisopropyl ether (8 mL), 40 °C. [b] Determined by $^1H$ NMR. [c] Determined by $^1H$ NMR with $Eu(hfc)_3$. [d] Strongly basic and macroporous resin [Type I, $-N^+(CH_3)_3$]. [e] Strongly basic and macroporous resin [Type II, $-N^+(CH_3)_2(CH_2CH_2OH)$]. [f] Strongly basic and gel type resin (Type I). [g] Weakly basic and macroporous resin [tertiary amino group]. [h] Not determined. [i] (*R*)- or (*S*)-binaphthol monoanion.

active cyanohydrin acetates **3a–n**. The reversible nature of the base-catalyzed transhydrocyanation enabled continuous racemization of the unreacted cyanohydrins **2a–n**, thereby effecting the total conversion of the aldehydes **1a–n** into the optically active **3a–n**. Acetone cyanohydrin was chosen as the hydrogen cyanide source because it is easier to handle and less toxic than HCN;[5,6b] besides, it is not subjected to acetylation by the lipase probably due to its steric bulk.[14] Therefore it remains as an effective hydrogen cyanide donor throughout the reaction. In addition, both acetone cyanohydrin and isopropenyl acetate produced acetone as the sole byproduct, which gave no unfavorable effects either on the lipase or on the reaction itself.[15]

The results are summarized in Table I. The proceeding of the reaction was monitored by $^1H$ NMR, and the reaction conversion of each step (the cyanohydrin formation and the enzymatic acetylation) was calculated from the ratio of each component **1**, **2**, and **3** in the reaction mixture. The reaction gave optically active cyanohydrin acetates **3a–i** in one stage from the corresponding aldehydes **1a–i**. For example, benzaldehyde (**1a**) was quantitatively converted into (*S*)-mandelonitrile acetate (**3a**) with 84% ee in 96% isolated yield (entry 1). The lipase tolerates considerable structural variation within substituted benzaldehyde cyanohydrins and gave the corresponding acetates **3b–i** with up to 91% ee in high chemical yields. The absolute configuration of **3a–i** was found to be *S* by comparing $^1H$ NMR and the sign of the optical rotation with those reported (see the Experimental Section). The lipase and the anion-exchange resin were insoluble in the reaction solvent and were recovered by filtration and reused. As shown in entry 6, the recovered enzyme/resin gave the product **3e** with comparable optical yield although the enzymatic acetylation was somewhat slower than that for the first use. The reaction of 2-furaldehyde (**1j**) was considerably slower and gave the acetate **3j** in moderate chemical and optical yields. The poor result can be attributed to the failure in the enzymatic acetylation, since cyanohydrin formation for this substrate proceeded in much the same way as for the other aldehydes.

A preliminary experiment with benzaldehyde as the substrate revealed that the lipase from *P. cepacia* (Amano) was the best enzyme in terms of catalytic activity and stereoselectivity, among several lipase preparations including four different *Pseudomonas* lipases (supplied by Toyobo, Nagase, Toyo Jozo, and Kurita Co., Ltds.) and other microbial lipases from *Chromobacterium viscosum* (Toyo Jozo) or *Candida cylindracea* (Lipase OF, Meito Co., Ltd.). We noticed that the *P. cepacia* lipase immobilized on Hyflo Super-Cel (see the Experimental Section) was much more active than the commercial grade of bulk enzyme (Amano P or PS) containing the same lipase of the same origin. The preliminary experiment also revealed that diisopropyl ether was the best solvent among several other organic solvents tested (benzene, toluene, hexane, cyclohexane, $CCl_4$, $CH_3CCl_3$, $CH_2Cl_2$, THF, *t*-BuOH, and $CH_3CN$) for the cyanohydrin formation and the stereoselectivity in enzymatic acetylation. Therefore the immobilized lipase (*P. cepacia*, Amano) and dry diisopropyl ether were used throughout the study.

In contrast to the reaction with aromatic aldehydes **1a–i**, the lipase did not well discriminate the enantiomers of the cyanohydrins derived from simple aliphatic aldehyde **1k** and **1l**, giving the corresponding acetates **3k** and **3l** in moderate or low optical yields (entries 12 and 13). Introducing a large aromatic ring in the vicinity of the carbonyl group, however, caused a large increase in the product ee (entries 14 and 15). These results are consistent with an increasing number of observations regarding the stereochemical preference of this lipase that the most efficiently resolved secondary alcohols by the lipase are those having substituents which differ significantly in size and, in particular, those having an aromatic ring as one of the substituents.[16]

The reaction of racemic aldehyde **1n** is interesting because an additional chiral center would afford an additional set of diastereoisomers and the chiral center in **1n** is also racemizable.[17] We conducted the reaction of **1n** with the hope that the lipase would discriminate one stereoisomer

(14) A mixture of acetone cyanohydrin and isopropenyl acetate (1.5 equiv) in dry diisopropyl ether was incubated with the immobilized lipase, but no acetylation was observed even after 8.7 days.

(15) When vinyl acetate was used as an acylating reagent, a byproduct, acetaldehyde, participated in the reaction and the corresponding cyanohydrin acetate accumulated. This side reaction consumed both acetone cyanohydrin and vinyl acetate, preventing the main reaction from completion.

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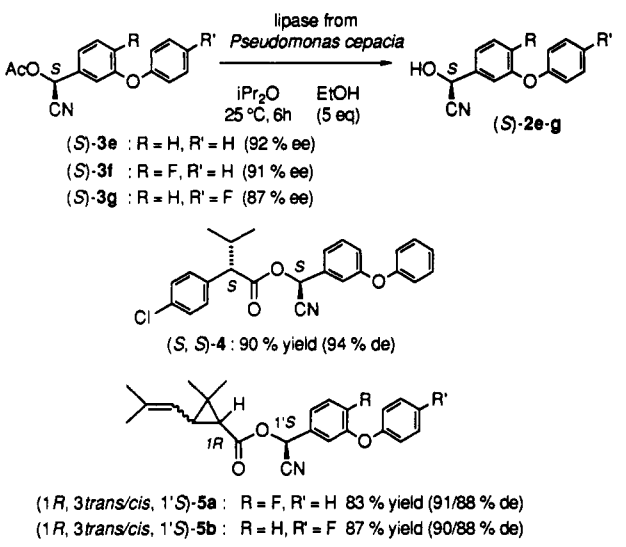
of cyanohydrin **2n** out of four to afford one stereoisomer of **3n** selectively. Contrary to our initial expectation, the reaction yielded a 1:1 mixture of syn and anti diastereoisomers (entry 15), yet the optical purity of each diastereoisomer was 82 and 85% ee.[18] This result indicated that the lipase well discriminated the stereochemistry on the chiral carbon to which the hydroxy group was attached, but not on the adjacent chiral center.

A further interesting observation concerns the catalytic activity of the anion-exchange resin. As shown in Table II, strongly basic and macroporous resins such as Amberlite IRA-904, Amberlyst A-27, and Duolite A-162 were found almost equally effective for the one-pot synthesis of (*S*)-**3e**.[19] A somewhat low ee for Duolite A-162 (80%) may be attributed to nonenzymatic acetylation of racemic **2e** catalyzed by this resin (vide infra).[20] Neither a macroporous but weakly basic resin [Amberlyst A-21 (free base form)] nor a strongly basic but gel-type resin [Amberlite IRA-400 ($OH^-$ form)] catalyzed the transhydrocyanation under the same reaction conditions. This is probably because the tertiary amine function in the weakly basic resin was not basic enough, and the counteranion ($OH^-$) trapped by the gel-type resin was inaccessible to substrates by diffusion limit.

Table II also shows the effects of counteranions of Amberlite IRA-904 on the reaction. Several different anions including $OH^-$, $CO_3^{2-}$, $CN^-$, $HCO_3^-$, $AcO^-$, $Cl^-$, and $TsO^-$ were chosen in descending the order of basicity and tested for their catalytic activity. Surprisingly, no difference was observed for $OH^-$, $CO_3^{2-}$, $CN^-$, $HCO_3^-$, and $AcO^-$, despite the large difference in basicity ($pK_a$'s of the conjugate acids range from 15 to 5!). In most cases the chemical and optical yields of (*S*)-**3e** were 86–90 and 87–93%, respectively. However, the resins with $TsO^-$ or $Cl^-$ as counteranions were inactive, presumably because the basicity was too low. It is worth noting that the nonenzymatic acetylation observed for Duolite A-162 was suppressed by changing the counteranion from $OH^-$ to $AcO^-$, and the product ee was improved accordingly (entries 2 and 3).[21] From the practical point of view, it is advisable to use the anion-exchange resins with weakly basic counteranions such as $AcO^-$ or $HCO_3^-$, because they are easier to make and more stable than the $OH^-$ form.

If the transhydrocyanation is stereoselective and preferentially affords (*S*)-cyanohydrin whch is the fast reacting stereoisomer in the subsequent enzymatic acetylation, the product ee as well as the reaction rate would be further improved by cooperative action of both steps. We attempted the reaction using an anion-exchange resin with a chiral counteranion, (*R*)- or (*S*)-[1,1′-binaphthyl]-2,2′-diol (binaphthol) mono anion (entries 13 and 14), with the hope that these chiral bases catalyzed the transhydrocyanation

**Scheme II**




in a stereoselective manner. However, no difference was observed between these two catalysts with regard to the reaction rate and the product ee. This result can be explained either by the failure of stereoselective transhydrocyanation catalyzed by the chiral anion or by the kinetic consequences that the resin-catalyzed racemization of **2e** was so fast that the stereoselectivity was determined only by the enzymatic acetylation. The latter was actually the case for the reaction with Amberlite IRA-904 ($OH^-$ form). The resin-catalyzed racemization of optically active cyanohydrin (*R*)-**2e** was measured in diisopropyl ether. The racemization obeyed good pseudo-first-order kinetics, and the half-life ($t_{1/2}$) of the optical activity of (*R*)-**2e** was found to be 74 min under the similar conditions as used for the typical one-pot synthesis of **3e**.[22] Since the enzymatic acetylation usually took 1 or 2 days under these conditions, the racemization of cyanohydrins was fast enough to effect the second-order asymmetric transformation. Our initial approach with quinidine or quinine as a catalyst for transhydrocyanation resulted in a long reaction time as well as a low optical yield of the product, although the same lipase was used.[23] Considering that the quinidine-catalyzed racemization of cyanohydrin **2e** was considerably slow ($t_{1/2}$ = 346 min),[23] the speed-up of the racemization step was a key to the effective second-order asymmetric transformation.

The (*S*)-isomer of the cyanohydrins **2e–g** is the desired enantiomer for the synthesis of optically active pyrethroids featuring high insecticidal activity.[24] We synthesized optically active fenvalerate (**4**) and the chrysanthemate esters **5a** and **5b** from (*S*)-**3e–g** while retaining their optical purity (Scheme II). The key step is the enzymatic cleavage of the ester bond of (*S*)-**3e–g** in an organic solvent, since the intermediate cyanohydrins **2e–g** are susceptible to decomposition or racemization under conventional alkaline conditions for ester hydrolysis. The ester bond of (*S*)-**3e–g** was successfully cleaved by ethanolysis using the same lipase as a catalyst in diisopropyl ether. Cyanohydrin ester is an activated ester[25] and the enzymatic ethanolysis

(18) The $^1H$ NMR peak of the acetyl proton was separated for each **3n** and **3n′** in the presence of Eu(hfc)$_3$. The peak of the lower field was larger than that of the higher field for each compound. This observation was common to all the (*S*)-cyanohydrin acetates **3a–m**, and therefore we assigned the major enantiomer of each diastereoisomer **3n** and **3n′** as possesing *S* configuration at cyanohydrin carbon atom.

(19) Amberlite IRA-904 and Amberlyst A-27 are strongly basic and macroporous resins [Type I, $-N^+(CH_3)_3$]. Duolite A-162 is a strongly basic and macroporous, but Type II resin [$-N^+(CH_3)_2(CH_2CH_2OH)$].

(20) The nonenzymatic acetylation of (±)-**2e** catalyzed by the anion-exchange resins ($OH^-$ form) was measured in the absence of the lipase after 2.9 days: Duolite A-162 (39%); Amberlite IRA-904 (8%); Amberlyst A-27 (0%).

(21) The nonenzymatic acetylation of (±)-**2e** was 33% ($OH^-$ form) and 11% ($AcO^-$ form) in 22 h at 40 °C. In a separate experiment, no racemization of (*S*)-**3e** was observed under the reaction conditions used ($AcO^-$ form 40 °C, 3 days), suggesting that still somewhat low ee observed was the result of either nonenzymatic acetylation or the limit of the ability of this enzyme.

(22) Optically active cyanohydrin (*R*)-**2e** was racemized completely in 6.2 h in the presence of 10 mol % of Amberlite IRA-904 ($OH^-$ from) and acetone cyanohydrin (10 equiv) in dry diisopropyl ether at 25 °C. In the absence of acetone cyanohydrin, the decomposition of cyanohydrin **2e** was observed.

(23) Inagaki, M.; Hatanaka, A.; Mimura, M.; Hiratake, J.; Nishioka, T.; Oda J. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 111–120.

(24) Aketa, K.; Ohno, N.; Itaya, N.; Nakayama, I.; Yoshioka, H. *Agric. Biol. Chem.* **1978**, *42*, 895–896.

of **3e–g** went to completion with only a 5 molar excess of ethanol. No racemization or decomposition was observed during the cleavage.[26] The reaction of the resulting cyanohydrin (*S*)-**2e–g** with the appropriate acid chlorides afforded pyrethroids **4**, **5a**, and **5b** with 88–94% de in 83–90% overall chemical yields.

A more smart approach to the synthesis of fenvalerate (**4**) would be the direct coupling of the acid portion with cyanohydrins by the lipase in one-stage. We attempted one-pot synthesis of **4** from 3-phenoxybenzaldehyde (**1e**), acetone cyanohydrin, and vinyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate as an acyl donor. Unfortunately, no fenvalerate was obtained after a prolonged incubation (40 °C, 4 days). The vinyl ester was remained unchanged in the reaction mixture, while the aldehyde **1e** was transformed into the cyanohydrin **2e** (80%). The failure of the reaction was probably because the lipase did not accept such a sterically hindered ester as a substrate.[27] This transformation, however, should be possible by choosing the appropriate lipase which is capable of catalyzing the transformation of sterically hindered carboxylic acids. Cloning and expression of a lipase gene from another *Pseudomonas* sp. are currently going on in our laboratory,[28] and we are actively pursuing the genetic alteration of the lipase protein for the effective transformation of sterically hindered carboxylic acids.

In conclusion, a novel system for the kinetic resolution with in situ racemization has been established. The combination of reversible transhydrocyanation catalyzed by anion-exchange resin and stereoselective acetylation catalyzed by lipase enabled one-stage conversion of various aldehydes into optically active cyanohydrin acetates with up to 94% ee in 63–100% conversion yield. This method affords the (*S*)-isomer of cyanohydrin acetates, which is the opposite enantiomer to that obtained by oxynitrilase-catalyzed addition of HCN to aldehydes.[1,3,6] Considering that the commercially available oxynitrilase are all (*R*)-cyanohydrin producers[29] and that in most cases the enzymatic or microbial hydrolysis of cyanohydrin esters gave (*R*)-cyanohydrin esters as the isolable product,[7b,8d,e] the present method is extremely important to the asymmetric synthesis of chiral compounds whose stereochemistry is correlated to (*S*)-cyanohydrins.

## Experimental Section

**General Methods.** $^1$H NMR (200 or 400 MHz) and $^{13}$C NMR (50 MHz) spectra were determined in $CDCl_3$. Mass spectra were obtained using electron ionization at 70 eV. Melting points are uncorrected. The products were isolated by flash column chromatography on silica gel [silica gel 60, spherical (150–325 mesh), Nacalai Tesque Co. (Kyoto, Japan)] or bulb-to-bulb distillation. Diisopropyl ether was distilled over $CaH_2$ and stored over 4-Å molecular sieves. The aldehydes **1a–e**, **1h–l**, and **1n** were commercially available and were purified by distillation or recrystallization under Ar atmosphere before use. The aldehydes **1f** and **1g** were generous gifts from Sumitomo Chemical Co., Ltd. (Osaka, Japan). Commercial grade of isopropenyl acetate was purified by distillation. The anion-exchange resins, Amberlite IRA-904, IRA-400, IRA-35, Amberlyst A-27, and A-21, were purchased from Organo Co., Ltd. (Tokyo, Japan). Duolite A-162 was purchased from Sumitomo Chemical Co., Ltd. The resin (20 mL) was conditioned by a standard procedure, and the counteranion was changed to $OH^-$ by washing the resin with 1 N NaOH (8 × 50 mL) and deionized water (8 × 60 mL), successively. For the preparation of the resins with various counteranions, the resin was washed with 1 N solution of sodium carbonate (for $CO_3^{2-}$ form), potassium cyanide (for $CN^-$ form), sodium bicarbonate (for $HCO_3^-$ form), sodium acetate (for $AcO^-$ form), hydrochloric acid (for $Cl^-$ form), and *p*-toluenesulfonic acid (for $TsO^-$ form). The resulting resins were washed well with deionized water and acetone for removing most of water, successively, and dried over $CaCl_2$ under reduced pressure in a desiccator for 3 days. (*S*)-2-(4-Chlorophenyl)-3-methylbutanoic acid and (1*R*,*cis*/*trans*)-chrysanthemic acid were kindly provided by Sumitomo Chemical Co., Ltd. The acids were converted to the corresponding acid chlorides with $SOCl_2$ (cat. DMF, $CH_2Cl_2$, reflux 2 h) and used without purification for the synthesis of pyrethroids. Vinyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate was prepared by heating the acid in vinyl acetate (10 mol % *p*-TosOH, reflux 8 h, 70%).

**Immobilization of Lipase.** The lyophilized powder of lipase protein from *P. cepacia* (Amano) (80 mg) was dissolved in 20 mM Tris-HCl buffer (pH 8.0) at 0 °C. Sucrose (240 mg) was added to the solution and stirred for 10 min. Hyflo Super-Cel (8.0 g), which was washed with deionized water and dried in an oven, was added to the solution, and the mixture was stirred for 15 min. The resulting paste was spread on a Petri dish and kept in a refrigerator. After the mixture became visibly dry, it was dried further in a desiccator over $CaCl_2$ under reduced pressure for 2 days.

**One-Pot Synthesis of Optically Active Cyanohydrin Acetates (3) from Aldehydes (1). (*S*)-(+)-1-Cyano-1-phenylmethyl Acetate (3a): Typical Procedure.** Benzaldehyde (**1a**) (265 mg, 2.5 mmol), acetate cyanohydrin (426 mg, 5.0 mmol), and isopropenyl acetate (751 mg, 7.5 mmol) were dissolved in dry diisopropyl ether (20 mL). To this solution were added anion-exchange resin IRA-904 ($OH^-$ form, 96 mg, 0.25 mmol equiv), the immobilized lipase (250 mg), and 3-Å ground molecular sieves (100 mg). The resulting suspension was stirred for 6.3 days at 40 °C under an argon atmosphere. The reaction mixture was filtered, and the filtrate was washed successively with 2 N HCl (10 mL), saturated $NaHCO_3$(aq) (10 mL), and brine (10 mL) and then dried ($Na_2SO_4$). Solvent was removed in vacuo, and the residual oil was analyzed with $^1$H NMR. The reaction was found to be completed (100% conversion) from the ratio of three components [CHO proton for the aldehyde **1a** ($\delta$ 10.00), CH proton of the cyanohydrin **2a** ($\delta$ 5.55), and CH proton of the acetate **3a** ($\delta$ 6.42)]. The crude product was purified by flash column chromatography [hexane (12)–AcOEt (1)] to give **3a** as a colorless oil (416 mg, 96%): $[\alpha]^{25}_D$ = +19.9° (*c* 1.94, benzene) [lit.[7e] $[\alpha]_D$ = −15° (*c* 1.9, benzene) for the (*R*)-isomer with 60% ee]; the optical purity of (+)-**3a** was 84% by $^1$H NMR in the presence of a chiral shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium(III) derivative, Eu(hfc)$_3$ [about 10 mg for 4 mg of **3a** in 800 $\mu$L of $CDCl_3$; $\delta$ (OAc) 2.96 (*R*, minor) and 3.06 (*S*, major)]: IR (neat) 2250 (C≡N) and 1755 (C=O) cm$^{-1}$; $^1$H NMR $\delta$ 2.17 (s, 3 H, OAc), 6.42 (s, 1 H, CH), and 7.42–7.58 (m, 5 H$_{arom}$); $^{13}$C NMR $\delta$ 20.47 ($CH_3CO$), 62.85 (CH), 116.11 (C≡N), 127.88, 129.25, 130.41, 131.74, and 168.93 (C=O); MS *m*/*z* (relative intensity) 175 ($M^+$, 6), 133 (35), 116 (28), 115 (41), 105 (16), 89 (10), and 43 (100). Anal. Calcd for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.34; H, 5.23; N, 7.90.

Compounds **3b–n′** were prepared from the corresponding aldehydes **1b–n** by the same procedure as above. Reaction temperature was 40 °C (**1b–j,n**) or 25 °C (**1l,m**). Only the starting aldehyde, conversion yield, purification method, isolated yield, and the optical purity (determined by $^1$H NMR) are given for each compound **3b–n′**. The physical and spectral data for **3b–n′** are available in supplementary material.

**(*S*)-(+)-1-Cyano-1-(4-chlorophenyl)methyl acetate (3b)** was prepared from 4-chlorobenzaldehyde (**1b**) in 90% conversion yield. Column chromatography [hexane (15)–AcOEt (1)] gave **3b** as a

(25) West, J. B.; Scholten, J.; Stolowich, N. J.; Hogg, J. L.; Scott, A. I.; Wong, C.-H. *J. Am. Chem. Soc.*, **1988**, *110*, 3709–3710. Barbas, C. F., III; Matos, J. R.; West, J. B.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 5162–5166.

(26) The optically active **2e** obtained by the enzymatic cleavage of (*S*)-**3e** (84% ee) was acetylated again ($Ac_2O$, pyridine, room temperature, overnight) to give (*S*)-**3e** with exactly the same optical purity as the starting material (*S*)-**3e**.

(27) This was evidenced by the fact that the lipase did not catalyze the transesterification between this vinyl ester and ethanol (in diisopropyl ether 40 °C, 2 days).

(28) Chihara-Siomi, M.; Yoshikawa, K.; Oshima-Hirayama, N.; Yamamoto, K.; Sogabe, Y.; Nakatani, T.; Nishioka, T.; Oda, J. *Arch. Biochem. Biophys.* **1992**, *296*, 505–513.

(29) The commercially available oxynitrilase from almonds (Sigma) catalyzes the formation of (*R*)-cyanohydrins by asymmetric addition of HCN to aldehydes (refs 1, 3, and 6).

colorless oil (436 mg, 83%): $[\alpha]^{25}_D$ = +31.5° (c 1.17, benzene) [lit.[4f] $[\alpha]^{25}_D$ = +31.2° (c 2.08, benzene) for the (S)-isomer with 81% ee]; 84% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.77 (R, minor) and 2.86 (S, major)].

**(S)-(+)-1-Cyano-1-(4-methylphenyl)methyl acetate (3c)** was prepared from 4-methylbenzaldehyde (**1c**) in 72% conversion yield. Column chromatography [hexane (20)–AcOEt (1)] gave **3c** as a colorless oil (303 mg, 64%): $[\alpha]^{25}_D$ = +30.4° (c 1.41, benzene) [lit.[8e] $[\alpha]_D$ = −29° (c 1.4, benzene) for the (R)-isomer with >95% ee]; 91% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.85 (R, minor) and 2.95 (S, major)].

**(S)-(+)-1-Cyano-1-(3,4-(methylenedioxy)phenyl)methyl acetate (3d)** was prepared from 3,4-(methylenedioxy)benzaldehyde (**1d**) in 85% conversion yield. Column chromatography [hexane (10)–AcOEt (1)] gave **3d** as a colorless oil (443 mg, 81%): $[\alpha]^{25}_D$ = +42.7° (c 1.53, benzene) [lit.[8e] $[\alpha]_D$ = −44° (c 1.7, benzene) for the (R)-isomer with 99.5% ee]; 91% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.77 (R, minor) and 2.86 (S, major)].

**(S)-(+)-1-Cyano-1-(3-phenoxyphenyl)methyl acetate (3e)** was prepared from 3-phenoxybenzaldehyde (**1e**) in 84% conversion yield. Column chromatography [hexane (10)–AcOEt (1)] gave **3e** as a colorless oil (1.07 g, 80%): $[\alpha]^{25}_D$ = +27.9° (c 1.06, benzene) [lit.[30] $[\alpha]^{20}_D$ = +17.1° (10% in benzene) for the enantiomerically pure (S)-isomer]; 89% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 3.07 (R, minor) and 3.20 (S, major)].

**(S)-(+)-1-Cyano-1-(4-fluoro-3-phenoxyphenyl)methyl acetate (3f)** was prepared from 4-fluoro-3-phenoxybenzaldehyde (**1f**) in 89% conversion yield. Chromatography [hexane (8)–AcOEt (1)] gave **3f** as a colorless oil (594 mg, 88%): $[\alpha]^{25}_D$ = +24.8° (c 1.17, benzene); 91% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.69 (minor) and 2.79 (major)]; The absolute configuration of (+)-**3f** was assigned to be S from the sign of the optical rotation and relative intensity of the two $^1$H NMR peaks, compared with those of **3a–e**.

**(S)-(+)-1-Cyano-1-[3-(4-fluorophenoxy)phenyl]methyl acetate (3g)** was prepared from 3-(4-fluorophenoxy)benzaldehyde (**1g**) in 96% conversion yield. Column chromatography [hexane (10)–AcOEt (1)] gave **3g** as a colorless oil (625 mg, 92%): $[\alpha]^{25}_D$ = +26.3° (c 1.13, benzene); 87% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.61 (minor) and 2.69 (major)]; the absolute configuration of (+)-**3g** was assigned to be S on the same basis as (S)-(+)-**3f**.

**(S)-(+)-1-Cyano-1-(2-naphthyl)methyl acetate (3h)** was prepared from 2-naphthaldehyde (**1h**) in 96% conversion yield. Column chromatography [hexane (12)–AcOEt (1)] gave **3h** as a colorless crystalline solid (497 mg, 88%): mp 35 °C; $[\alpha]^{25}_D$ = +20.9° (c 1.13, $CHCl_3$) [lit.[22] $[\alpha]^{25}_D$ = +21.7° (c 1.01, $CHCl_3$) for the (S)-isomer with 85% ee]; 85% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.86 (R, minor) and 2.93 (S, major)].

**(S)-(−)-1-Cyano-1-(1-naphthyl)methyl acetate (3i)** was prepared from 1-naphthaldehyde (**1i**) in 72% conversion yield. Column chromatography [hexane (10)–AcOEt (1)] gave **3i** as a colorless crystalline solid (395 mg, 70%): mp 48 °C; $[\alpha]^{25}_D$ = −25.6° (c 1.04, $CHCl_3$) [lit.[22] $[\alpha]^{25}_D$ = −25.3° (c 1.02, $CHCl_3$) for the (S)-isomer with 69% ee]; 70% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.81 (R, minor) and 2.90 (S, major)].

**(R)-(+)-1-Cyano-1-(2-furyl)methyl acetate (3j)** was prepared from 2-furaldehyde (**1j**) in 73% conversion yield. Column chromatography [hexane (6)–AcOEt (1)] gave **3j** as a colorless oil (472 mg, 57%): $[\alpha]^{25}_D$ = +11.3° (c 1.24, $CHCl_3$) [lit.[22] $[\alpha]^{25}_D$ = +12.8° (c 1.02, $CHCl_3$) for the (R)-isomer with 47% ee]; 47% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.55 (S, minor) and 2.59 (R, major)].

**(S)-(−)-1-Cyano-2-methylpropyl acetate (3k)** was prepared from 2-methylpropanal (**1k**) in 63% conversion yield. Column chromatography [hexane (15)–AcOEt (1)] gave **3k** as a colorless oil (757 mg, 54%): $[\alpha]^{25}_D$ = −42.9° (c 1.02, benzene) [lit.[22] $[\alpha]^{25}_D$ = −60.6° (c 1.19, benzene) for the (S)-isomer with 69% ee]; 51% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.50 (R, minor) and 2.56 (S, major)].

**(S)-(−)-1-Cyano-1-hexyl acetate (3l).** Prepared from hexanal (**1l**) in 88% conversion yield. Column chromatography on silica gel eluting with [hexane (15)–AcOEt (1)] gave **3l** as a colorless oil (1.41 g, 83%): $[\alpha]^{25}_D$ = −8.4° (c 2.126, benzene) [lit.[8c] $[\alpha]_D$ = +74° (c 2, benzene) for the (R)-isomer with 97% ee]; 15% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.32 (R, minor) and 2.36 (S, major)].

**(R)-(−)-1-Cyano-2-(1-naphthyloxy)ethyl acetate (3m)** was prepared from 2-(1-naphthyloxy)acetaldehyde (**1m**)[7a] in 84% conversion yield. Column chromatography [hexane (6)–AcOEt (1)] gave **3m** as a colorless oil (435 mg, 68%): $[\alpha]^{23}_D$ = −28.3° (c 1.02, $CHCl_3$) [lit.[7a] $[\alpha]^{23}_D$ = +36.1° (c 1.19, $CHCl_3$) for the (S)-isomer with 87.4% ee]; 78% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.38 (S, minor) and 2.41 (R, major)].

**Optically active 1-cyano-2-phenylpropyl acetates (3n and 3n′)** were prepared from (±)-2-phenylpropanal in 84% conversion yield for the diastereoisomeric mixture. Each diastereoisomer, **3n** and **3n′**, was separated by column chromatography [hexane (40)–AcOEt (1)]. Diastereoisomer **3n** as a colorless oil (160 mg, 32%): $[\alpha]^{25}_D$ = −40.7° (c 1.04, $CHCl_3$); 82% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.39 (minor) and 2.45 (major)]. Diastereoisomer **3n′** as a colorless oil (100 mg, 20%): $[\alpha]^{25}_D$ = −65.8° (c 1.02, $CHCl_3$); 85% ee [$^1$H NMR with Eu(hfc)$_3$; δ (OAc) 2.57 (minor) and 2.64 (major)].

**Racemization of Optically Active Cyanohydrin (R)-2e by Amberlite IRA-904 ($OH^-$ form).** The optically active cyanohydrin (R)-**2e** [104 mg, 0.459 mmol, 87% ee, prepared by lipase-catalyzed kinetic resolution[7c] of (±)-**2e**] and acetone cyanohydrin (391 mg, 4.59 mmol) were dissolved in dry diisopropyl ether (5 mL). The optical rotation of this solution was $[\alpha]^{25}_D$ = +19°. To this solution were added Amberlite IRA-904 ($OH^-$ form, 17.7 mg, 0.046 mmol equiv) and 3-Å ground molecular sieves (18.4 mg), and the mixture was stirred at 25 °C. A small portion of the mixture (100 μL) was taken after a certain period of time, and its optical rotation was measured. Natural logarithm of the optical rotation was plotted against the incubation time to give a straight line; the half-life ($t_{1/2}$ = 74 min) was calculated from the slope of the line.

**Synthesis of Optically Active Pyrethroids 4, 5a, and 5b. (S,S)-Fenvalerate (4): Typical Procedure.** The optically active (S)-**3e** (500 mg, 1.87 mmol, 92% ee) was dissolved in dry isopropyl ether (60 mL). Ethanol (431 mg, 9.35 mmol) and the lipase (870 mg) were added to the solution, and the mixture was stirred for 6 h at 25 °C. The conversion reached 92%. The lipase powder was removed by filtration, and the filtrate was evaporated to give (S)-**2e** as a colorless oil. The residual oil was dissolved in dry $CH_2Cl_2$ (8 mL) and was added to a solution of (S)-2-(4-chlorophenyl)-3-methylbutyric chloride (475 mg, 2.06 mmol, 100% ee) in dry $CH_2Cl_2$ (15 mL) at 0 °C. After the mixture was stirred for 1 h at 0 °C, pyridine (444 mg, 5.61 mmol) was added and the mixture was stirred overnight at room temperature. The resulting mixture was washed successively with 2 N HCl (10 mL), saturated $NaHCO_3$ (15 mL), and brine (10 mL) and then dried ($Na_2SO_4$). Column chromatography [hexane (15)–AcOEt (1)] afforded (S,S)-fenvalerate (**4**) as a colorless oil (707 mg, 90%): $[\alpha]^{25}_D$ = −9.6° (c 6.91, $CHCl_3$) [lit.[23] $[\alpha]_D$ = −11.2° (c 6.5, $CHCl_3$)]; 94% de [$^1$H NMR δ (CHCN) 6.30 (S,R, minor) and 6.34 (S,S, major)].

**(1R,3-*trans*/*cis*,1′S)-5a.** The acetate (S)-(+)-**3f** (135 mg, 0.47 mmol, 91% ee) was deacetylated by the same procedure as above, and the resulting (S)-**2f** was coupled with (1R,3-*trans*/*cis*)-chrysanthemic acid chloride (93 mg, 0.5 mmol, trans/cis = 8/2) in dry $CH_2Cl_2$ (15 mL) and pyridine (119 mg, 1.5 mmol). Preparative TLC developed twice with [hexane (8)–AcOEt (1)] gave (1R,1′S)-**5a** as a trans/cis mixture (84/16) (154 mg, 83% overall yield from (S)-**3f**): $[\alpha]^{25}_D$ = +3.1° (c 1.02, $CHCl_3$). Two $^1$H NMR peaks (CHCN proton) corresponding to the diastereoisomers were observed for each trans and cis isomer, the de of each trans and cis isomer being calculated from the integration of these peaks. The de of the trans isomer was 91%: $^1$H NMR δ (CHCN) 6.32 [(1R,3-*trans*,1′R)-**5a** (minor)] and 6.34 [(1R,3-*trans*,1′S)-**5a** (major)]. The de of the cis isomer was 88%: $^1$H NMR δ (CHCN) 6.30 [(1R,3-*cis*,1′R)-**5a** (minor)] and 6.32 [(1R,3-*cis*,1′S)-**5a** (major)].

**(1R,3-*trans*/*cis*,1′S)-5b.** By the same procedure as for the preparation of **5a**, the acetate (S)-(+)-**3g** (135 mg, 0.47 mmol, 87% ee) was deacetylated and the resulting **2g** was coupled with (1R,3-*trans*/*cis*)-chrysanthemic acid chloride to afford (1R,1′S)-**5b** as a trans/cis mixture (84/16) (161 mg, 87% overall yield): $[\alpha]^{25}_D$ = +0.9° (c 1.02, $CHCl_3$); The de of the trans isomer was 90%: $^1$H NMR δ (CHCN) 6.38 [(1R,3-*trans*,1′R)-**5b** (minor)] and 6.39 [(1R,3-*trans*,1′S)-**5b** (major)]. The de of the cis isomer was 88%:

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$^1$H NMR δ (CHCN) 6.36 [(1*R*,3-*cis*,1′*R*)-**5b** (minor)] and 6.38 [(1*R*,3-*cis*,1′*S*)-**5b** (major)].

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**Supplementary Material Available:** Physical and spectral data for **3b-n′**, fenvalerate (**4**), and the chrysanthemates **5a,b** and a plot of the time course of the racemization of optically active cyanohydrin **2e** (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

# Design of Pyrimido[4,5-*g*]quinazoline-Based Anthraquinone Mimics. Structure–Activity Relationship for Quinone Methide Formation and the Influence of Internal Hydrogen Bonds on Quinone Methide Fate

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Pyrimido[4,5-*g*]quinazolinequinone derivatives were synthesized as anthraquinone-like reductive alkylating agents. Like many naturally-occurring antibiotics, these quinone derivatives are designed to afford an alkylating quinone methide species upon reduction and leaving-group elimination. Kinetic studies of pyrimido[4,5-*g*]-quinazoline hydroquinones provided evidence of quinone methide intermediates able to trap nucleophiles (alkylation) and protons (ketonization). The rate of quinone methide formation is determined by the hydroquinone free energy. Thus, a linear free energy relationship for quinone methide formation was obtained by plotting rates of quinone methide formation as the log versus the quinone reduction potential. The pyrimido[4,5-*g*]quinazoline quinone methides fall on this free energy plot, showing that these species are formed by the same mechanism as the other structurally-diverse quinone methides previously studied in this research group. Internal hydrogen bonds present in pyrimido[4,5-*g*]quinazoline derivatives influence the fate of the quinone methide species as well as the rate of hydroquinone oxidation in the presence of oxygen. Such hydrogen bonds stabilize the hydroquinone species, thereby resulting in slow rates of hydroquinone oxidation to quinone in alkaline aerobic buffer. Stabilization of the hydroquinone also results in substantial nucleophile trapping by the quinone methide. Without internal hydrogen bonds, hydroquinone oxidations are rapid and the quinone methide traps only electrophiles.

Efforts in this laboratory have been directed toward the design and study of reductive alkylating agents based on heterocyclic ring systems.[2-7] Reductive alkylating agents are quinones functionalized with a leaving group so as to permit quinone methide formation upon quinone reduction. The quinone methide species can trap nucleophiles (alkylation) as well as electrophiles (ketonization). The low reduction potentials exhibited by some tumor cells[8] have generated an interest in reductive alkylating agents as selective antitumor drugs.[9] Indeed, many naturally occurring antitumor drugs may act as reductive alkylating agents.[10]

Chart I

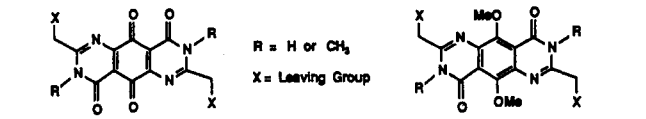
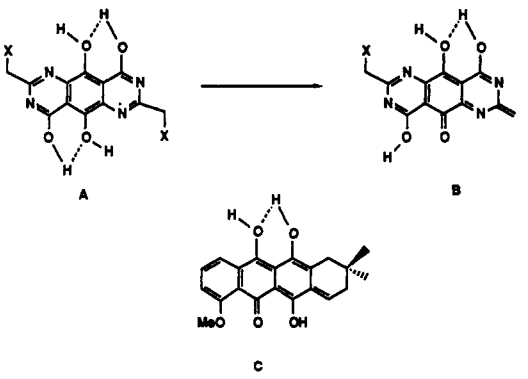



Chart II




The subjects of this paper are the synthesis, physical chemistry, and cytotoxic properties of the pyrimido[4,5-*g*]quinazoline alkylating agents in Chart I. The pyrimido[4,5-*g*]quinazolinetetrone derivatives were designed as reductive alkylating agents while the dione derivatives were

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