

Enzymatic Preparation of Optically Active Cyanohydrin Acetates¹

Andreas van Almsick,^a Joachim Buddrus,^b Petra Hönicke-Schmidt,^a Kurt Laumen,^a and Manfred P. Schneider^{*a}

^a *Fb 9 - Bergische Universität-GH-Wuppertal, D-5600 Wuppertal 1, West Germany*

^b *Institut für Spektrochemie und angewandte Spektroskopie, Bunsen-Kirchhoff-Strasse 11, D-4600 Dortmund 1, West Germany*

A series of cyanohydrin acetates (**1**)–(**47**) of widely varying structures, potential chiral building blocks for numerous synthetic applications, has been prepared in good chemical and often high optical yields by enzymatic hydrolysis of their racemic acetates in the presence of an ester hydrolase from *Pseudomonas* sp.

Molecules with cyanohydrin substructures are useful starting materials in organic synthesis. They can be converted readily into α -hydroxycarboxylic acids,² α -hydroxyaldehydes,³ or ethanolamine derivatives,⁴ including a large number of biologically (physiologically) active compounds. Obviously, enantiomerically pure or enriched cyanohydrins could be of considerable importance for the synthesis of many of these target molecules in optically active form. Although cyanohydrins of limited structural variety are accessible with sometimes high enantiomeric purity *via* oxynitrilase catalysed addition of HCN to aldehydes,⁵ the resulting products are rather unstable with a tendency towards racemization *via* their equilibrium with HCN and the corresponding aldehydes (*cf.* equation 2). In contrast, enantiomerically pure derivatives of cyanohydrins, *e.g.* the acetates (**1**)–(**47**), are chemically quite stable, also towards racemization, storable and seemed therefore attractive synthetic targets.

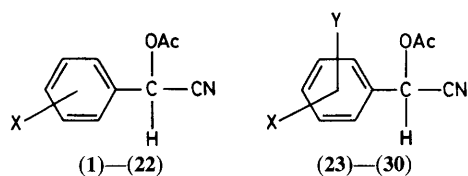
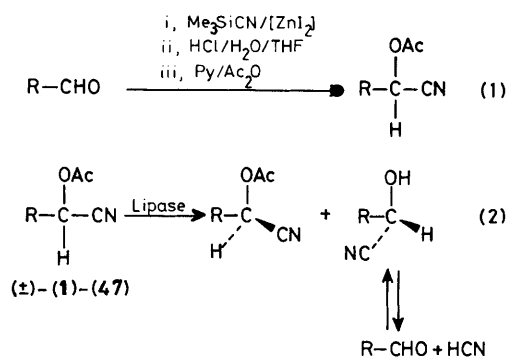
Ester hydrolases (esterases, lipases) are well known for their capability of enantiomer differentiation in racemic esters. In view of our successful preparation of a large number of secondary alcohols and their acetates by esterase catalysed hydrolysis⁶ and reports in other laboratories⁷ we felt that this method would also provide an attractive and simple route to enantiomerically pure cyanohydrin derivatives of widely varying structures.

A simple kinetic evaluation reveals that in such an enzymatic hydrolysis high chemical and optical yields for both enantiomers can usually only be obtained if their rates of hydrolysis are very different, *e.g.* by a factor of at least $E > 100$ (E is a kinetic enantiomeric ratio as defined in *ref.* 8). This further implies that in cases of lower E -values only one enantiomer (the remaining one) can be obtained enantiomerically pure. While we were quite confident in being able to isolate the nonhydrolysed acetates from the reaction

Table 1. Enzymatic hydrolysis of the racemic esters (**1**)–(**47**).^a

Substrate	Method	% Conversion	% Yield ^b	% E.e.	E^c	Configuration
(1)	A	52.7	42	≥ 98	≥ 71	<i>R</i>
(2)	A	53.2	43	93	34	<i>R</i>
(3)	A	53.6	42	≥ 98	≥ 55	<i>R</i>
I	A	49.7–51.6	35–49	≥ 98	> 100	<i>R</i>
(5)	A	56.0	39	82	11	<i>R</i>
(8)	A	52.0	39	70	10	<i>R</i>
(11)	A	51.9	42	72	11	<i>R</i>
(11)	B	51.8	40	80	16	<i>R</i>
(14)	B	52.8	42	95	45	<i>R</i>
(16)	B	53.1	46	≥ 98	≥ 63	<i>R</i>
II	B	49.6–51.4	44–46	≥ 98	> 100	<i>R</i>
(21)	A	44.2	46	5	1.2	<i>R</i>
(24)	A	50.0	47	0	1	(\pm)
(25)	A	—	—	—	—	—
(28)	B	51.8	40	93	47	<i>R</i>
(29)	B	52.9	35	≥ 98	≥ 67	<i>R</i>
(31)	B	55.5	32	86	15	<i>R</i>
(32)	B	52.0	40	90	33	<i>R</i>
(33)	B	52.0	44	≥ 98	≥ 91	<i>R</i>
(34)	A	52.2	38	≥ 98	≥ 84	<i>R</i>
(35)	A	54.3	45	≥ 98	≥ 47	<i>R</i>
(36)	A	50.0	45	30	2.4	<i>R</i>
(37)	A	60.2	30	93	14	<i>R</i>
(38)	A	51.0	35	40	3.3	<i>R</i>
(39)	A	55.7	40	89	17	<i>R</i>
(40)	A	53.8	45	≥ 98	≥ 53	<i>R</i>
(42)	A	53.7	30	95	37	<i>R</i>
(43)	A	55.1	31	92	22	<i>R</i>
(44)	A	52.0	42	80	16	<i>S</i>
(45)	A	58.0	33	≥ 98	≥ 26	<i>S</i>
(46)	A	58.0	33	≥ 98	≥ 26	<i>S</i>
(47)	A	50.7	30	≥ 98	> 100	1 <i>R</i> ,5; 1' <i>R</i>

^a Method A: 10 mmol substrate, 20 g pH 7 phosphate buffer (0.1 M), 20–40 °C, 800 u (100 mg) lipase. Method B: like method A + 5 ml THF. I: substrates (**4**), (**6**), (**7**), (**9**), (**10**), (**12**), (**13**), (**15**), (**19**), (**20**), (**22**), (**23**), (**26**), (**27**), (**41**); II: substrates (**17**), (**18**), (**30**). ^b Based on racemate = 100%. ^c *Ref.* 9.



X	o	m	p
(1)–(22)			
(1)	H	H	H
(2)–(4)	F	F	F
(5)–(7)	Cl	Cl	Cl
(8)–(10)	Br	Br	Br
(11)–(13)	Me	Me	Me
(14)–(16)	OMe	OMe	OMe
(17)–(18)	—	OCH ₂ Ph	OCH ₂ Ph
(19)–(20)	—	OPh	OPh
(21)	NO ₂		
(22)			CH(Me) ₂

(23)	X = 2-OMe; Y = 3-OMe
(24)	X = 2-OMe; Y = 4-OMe
(25)	X = 2-OMe; Y = 5-OMe
(26)	X = 3-OMe; Y = 4-OMe
(27)	X = 3-OMe; Y = 5-OMe
(28)	X = 3-Cl; Y = 4-Cl
(29)	X = 3-OCH ₂ Ph; Y = 4-OCH ₂ Ph
(30)	X, Y = 3,4-O-CH ₂ -O-

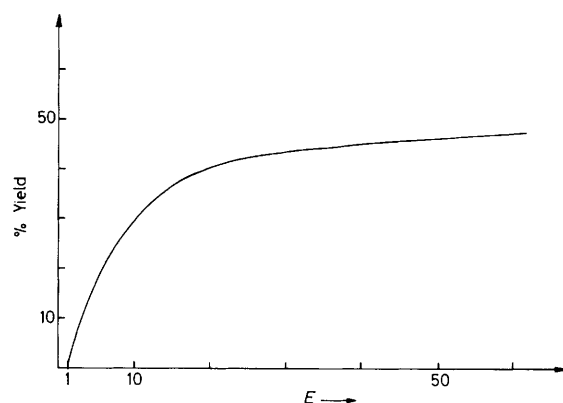
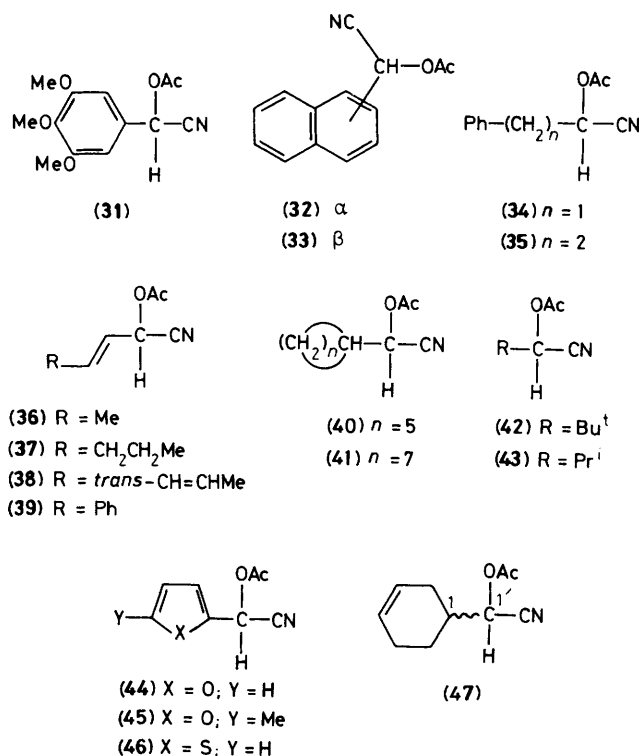


Figure 1. Plot of the theoretical yield of the remaining substrate as a function of the enantiomeric ratio E (e.e. was fixed at 98%).

mixtures without problems, it seemed most likely that the thus produced cyanohydrins would be unstable under the reaction conditions employed, thus preventing their isolation. Consequently, values of $E > 50$ seemed to be sufficient for the production of the desired cyanohydrin acetates in good chemical yields and enantiomeric purities of $\geq 98\%$ enantiomeric excess (e.e.) (Figure 1).

We were very pleased to find that the racemic cyanohydrin acetates (±)-(1)–(47), readily accessible from the corre-



sponding aldehydes *via* their trimethylsilyl ethers (equation 1),⁹ could be hydrolysed with high enantioselectivity in the presence of an ester hydrolase from *Pseudomonas sp.*¹⁰ leading to the optically active acetates (1)–(47) in good chemical and frequently high optical yield (Table 1).†

Again in agreement with our expectations, and in support of the arguments above, the cyanohydrins formed under the employed conditions† (Table 1) were either isolated with lower enantiomeric purity, in racemic form (equation 2), or totally eluded isolation by chromatography on SiO₂. The α-cyanobenzylalcohol derivatives are particularly unstable, while the 1-cyanoalkanols can be isolated with only a small loss of enantiomeric purity.

The enantiomeric purities of (1)–(47) were determined by 400 MHz ¹H n.m.r. using the optical shift reagents Eu(hfc)₃ and Pr(hfc)₃. With base line signal separations and careful calibration using the racemic acetates we are confident that the determinations are accurate to at least ±2%. The absolute configurations of (*R*)-(1), (14)–(16), (30), (36), and (*S*)-(46) have been determined by direct comparison with the products derived by acetylation of the known cyanohydrins obtained

† In a typical experiment, (±)-(1) (1.75 g, 10 mmol) was mixed with 0.1 M phosphate buffer (20 g, pH 7, 20°C) and enzymatically hydrolysed in the presence of lipase SAM-2 (100 mg, 800 u; standard: tributyrin). The rapidly decreasing pH, an indication of the beginning of hydrolysis, was kept constant throughout the reaction by continuous addition of 1 M NaOH solution from an autoburette. The reaction was terminated after the consumption of 5.3 ml of base (11 h) by extraction with ether. After separation of the crude product mixture by flash chromatography on SiO₂ [Et₂O–light petroleum (1:4)] the acetate/aldehyde mixture was dissolved in ethanol (5 ml). This solution was mixed with saturated Na₂S₂O₅ solution (5 ml) and stirred vigorously overnight. The pure acetate was extracted with ether and the organic solution washed with water and dried (MgSO₄). Evaporation of the solvent under reduced pressure yielded the acetate as a yellow oil, which was distilled *in vacuo*.

(+)-(R)-(1): 735 mg, 4.20 mmol, 42% yield, [α]_D²⁰ 8.1° [c 10, CHCl₃ (1% EtOH)], ≥98% enantiomeric excess (e.e.).

via asymmetric, oxynitrilase catalysed addition of HCN to the corresponding aldehydes.⁵ On this basis and the fact that the directions of signal shifts in all n.m.r. spectroscopic experiments using both of the above reagents were identical in all cases, all products (*R*)-(1)—(43), (47), [and (*S*)-(44)—(46), a consequence of the Cahn–Ingold–Prelog rules], were tentatively assigned to be of the same absolute configuration. Studies for further confirmation of these assignments are currently under way.

Under the standard method of enzymatic hydrolysis described,[†] several, especially crystalline or highly viscous liquid substrates [*e.g.* (11), (14), (16)—(18), and (28)—(33)] initially showed only little or no transformation. Both the addition of tetrahydrofuran (THF) as cosolvent (method B, Table 1; leading to the formation of readily converted emulsions) and the application of higher temperatures were successfully employed to overcome these deficiencies.

Although only documented so far in one case (11), and to be confirmed by further examples it seems that for some substrates slightly higher enantiomeric purities can be achieved by the addition of THF to the reaction mixture. There were no indications of substrate or product inhibition in the described reactions; scaled-up experiments into the molar range are currently under way.

We feel that the results presented provide synthetic organic chemists with an extremely useful and facile route to a whole class of potentially valuable chiral auxiliaries; synthetic applications being presently studied in our laboratory.

We thank the Ministerium für Wissenschaft und Forschung - NRW, the Fonds der Chemischen Industrie, and the

Eastman Kodak Co., Rochester, NY (U.S.A.), for financial support.

Received, 10th October 1988; Com. 8/04029J

References

- 1 'Enzymes in Organic Synthesis 15.' Part of the planned Ph.D dissertation of A. van Almsick, Wuppertal.
- 2 B. B. Corson and R. A. Dodge, 'Organic Synthesis,' vol. 1., ed. H. Gilman, Wiley, New York, 1956, p. 336.
- 3 P. Tinapp, *Chem. Ber.*, 1971, **104**, 2266.
- 4 T. Satoh, S. Suzuki, Y. Suzuki, Y. Miyaji, and Z. Imai, *Tetrahedron Lett.*, 1969, 4555.
- 5 W. Becker, H. Freund, and E. Pfeil, *Angew. Chem.*, 1965, **77**, 1139; W. Becker and E. Pfeil, *J. Am. Chem. Soc.*, 1966, **88**, 4299; F. Effenberger, Th. Ziegler, and S. Föster, *Angew. Chem.*, 1987, **99**, 491; J. Oku and S. Inoue, *J. Chem. Soc., Chem. Commun.*, 1981, 229.
- 6 K. Laumen and M. P. Schneider, *J. Chem. Soc., Chem. Commun.*, 1988, 598.
- 7 N. Matsuo and N. Ohno, *Tetrahedron Lett.*, 1985, **26**, 5533; H. Ohta, Y. Miyamae, and G. Tsuchihashi, *Agric. Biol. Chem.*, 1986, **50**, 3181; H. Hirohara, S. Mitsuda, E. Ando, and R. Komaki, in 'Biocatalysts in Organic Syntheses,' 'Studies in Organic Chemistry,' Elsevier, Amsterdam, 1985, vol. 22, p. 119.
- 8 C. S. Chem, Y. Fujimoto, G. Girdaukas, and C. J. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7294.
- 9 P. G. Gassman and J. J. Talley, *Tetrahedron Lett.*, 1978, **40**, 3773.
- 10 Lipase SAM-2 Amano Pharmaceutical Co., supplied by Fluka Chemie AG, CH-9470 Buchs, Switzerland (Cat. No. 62312) and Mitsubishi Int. GmbH, D-4000 Düsseldorf 1, West Germany.