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Preparation of the stereoisomers of 2-cyanocycloalkanols by lipase-catalysed acylation

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Abstract: Enantiopure (1*R*,2*R*)-, (1*S*,2*S*)-, (1*S*,2*R*)- and (1*R*,2*S*)-2-cyanocyclopentanol and -cyclohexanol isomers were prepared through the *Pseudomonas cepacia* lipase-catalysed acetylation of the racemic *cis* and *trans* compounds with vinyl acetate in diisopropyl ether. The quasi-irreversible nature of acylations with 2,2,2-trifluoroethyl esters is evident.
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2-Cyanocycloalkanols with two stereogenic centres are important synthetic targets for the preparation of 1,2-disubstituted 1,3-difunctional cycloalkanes, which can be subjected to further transformation to produce, for example, 1,3-heterocycles.[1–3] Thus, hydrogenation of the cyano group provides a valuable amino group in the formation of alicyclic 1,3-amino alcohols. Chemical or enzymatic hydrolysis of the cyano group, on the other hand, leads to the formation of amides and carboxylic acids.[4,5] Methods have been described for the preparation of racemic *cis*- and *trans*-2-cyanocycloalkanols.[6–10] As targets for biologically active or pharmaceutically important compounds, the enantiopurity of 2-cyanocycloalkanols is crucial.

The enantiomers of *trans*-2-cyanocyclohexanol were previously separated by the lipase-catalysed hydrolysis of the butanoate.[11–13] Separate runs under kinetically controlled conditions were needed for the preparation of the hydrolysed alcohol (>98% ee) and unreacted ester (95% ee) enantiomers, with approximately 40% theoretical yields (according to the racemic butanoate) for both. In the course of our studies on the lipase-catalysed resolution of racemates in anhydrous organic solvents, it has become clear that acylation of the alcohol or amino function attached directly to the ring system is the best way to resolve alicyclic 2-hydroxy- or 2-aminocycloalkane carboxylic acid esters.[14–16] In these cases, lipase PS from *Pseudomonas cepacia* often exclusively acylated the functional groups at the 2*R* centres of various ethyl carboxylates. Encouraged by this, we have now studied the possibility of the preparation of all four stereoisomers of both 2-cyanocyclopentanol **1** and -hexanol **2** through the lipase PS-catalysed acylation in diisopropyl ether in such a way that the two enantiomers of the racemic *cis* or *trans* isomer are always obtained simultaneously in a single run (Scheme 1).

1 (n = 1) **1a** **1b**
2 (n = 2) **2a** **2b**

Scheme 1.

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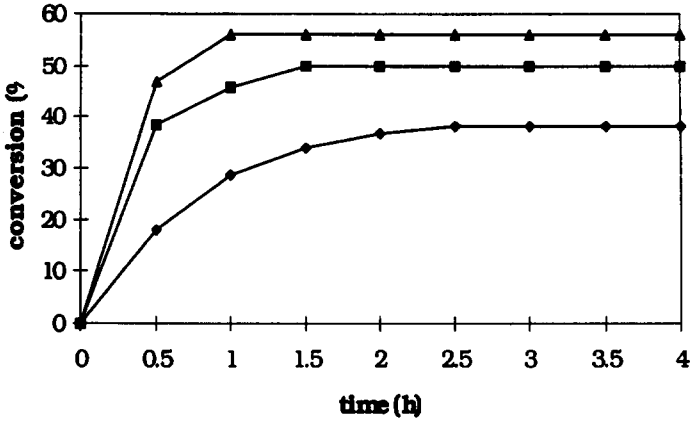

Figure 1. Conversion versus time for the lipase PS-catalysed acetylation of *trans*-2-cyanocyclohexanol with (▲) Ac_2O, (■) $CH_3CO_2CH{=}CH_2$ and (◆) $CH_3CO_2CH_2CF_3$ in diisopropyl ether.

Results and discussion

For an irreversible acyl transfer, modern chromatographic techniques often allow determination of the enantioselectivity ratio[17] (*E*) at an early stage of an enzymatic resolution. This ratio, in turn, can be used to determine how the enantiomeric excess (ee) for the product and unreacted substrate enantiomers changes as a function of conversion.[14] Accordingly, the best termination conversion for kinetic resolution can be estimated. This theoretical consideration clearly reveals that, for an enantioselective reaction, the less reactive enantiomer is always obtained in higher enantiopurity (when the reaction exceeds 50% conversion) than that of the new enzymatically produced product counterpart (when the reaction is terminated before 50% conversion). The enantiopurity difference between the two enantiomers diminishes with increasing *E*, enantiospecific reactions finally producing two enantiopure compounds simultaneously. In accordance with the highly enantioselective acyl transfers, $E \gg 100$ was always obtained for the lipase PS-catalysed acylations in the present study, with the promise of fulfilling the aim of the work.

In order to achieve irreversible acyl transfer, acid anhydrides and vinyl or 2,2,2-trifluoroethyl esters are the most common acyl donors for lipase-catalysed acylation in organic solvents.[14] However, due to a nonenzymatic or quasi-irreversible reaction or to a Schiff base formation, the ee values of the resolved products may be lower than expected on the basis of *E*. That is why various acetic acid derivatives as acyl donors were first studied for the lipase PS-catalysed acetylation of *trans*-2-cyanocyclohexanol in diisopropyl ether (Figure 1). The time needed to reach the theoretical 50% conversion obviously depends on the nature of the acyl donor. Acetylation of *trans*-2-cyanocyclohexanol with acetic anhydride leads to chemical esterification. Accordingly, the *E* value is not reliable, the conversion exceeds the 50% limit and the ester enantiomer produced exhibits low enantiopurity [(▲) Figure 1, Table 1]. On the other hand, the less reactive (1*S*,2*R*) alcohol is enantiopure. The problem of chemical acylation is the same when propionic anhydride is used as acyl donor (Table 1). The absolute configuration (1*S*,2*R*) of **2a** was recently proved adequately by means of X-ray diffraction.[13]

In accordance with the excellent enantioselectivity ($E \gg 100$), 2,2,2-trifluoroethyl acetate produces the acetylated *trans*-2-cyanocyclohexanol with ee >99% (Table 1, entry 1). However, independently of the acyl group of the 2,2,2-trifluoroethyl ester and contrary to expectation, the enantiopurity of the less reactive alcohol enantiomer of repeated experiments attains the maximum value ≤95% as the reaction slowly approaches 49% conversion [(◆) Figure 1]. Evidently, the reaction stops on product inhibition or at equilibrium with respect to the reactive (1*R*,2*S*) enantiomer. For the acylation of *cis*-2-cyanocyclopentanol with 2,2,2-trifluoroethyl esters, on the other hand, the reactions smoothly proceed to 50% conversion (Table 2). This observation, together with the excellent results obtained with the

Table 1. Lipase PS[a]-catalysed acylation of *trans*-2-cyanocyclohexanol (0.1 M) with RCO_2R^1 (0.1–0.2 M) in diisopropyl ether

R	R^1	Time/h	Conversion/%	$ee_{alcohol}$/%	ee_{ester}/%
CH_3	CH_2CF_3	4	38	52	>99
CH_3CH_2	CH_2CF_3	5	48	88	96
$CH_3(CH_2)_2$	CH_2CF_3	24	48	94	>99
$CH_3(CH_2)_4$	CH_2CF_3	24	49	95	>99
$ClCH_2$	CH_2CF_3	8	49	94	>99
CH_3	$CH{=}CH_2$	1.5	50	>99	>99
$CH_3(CH_2)_2$	$CH{=}CH_2$	5	51	>99	96
CH_3	$CH_3C{=}O$	1	56		
CH_3CH_2	$CH_3CH_2C{=}O$	2.5	58	>99	54

[a]Enzyme preparation (50 mg ml^{-1}) containing lipase [20% (w/w)].

Table 2. Lipase PS[a]-catalysed acylation of *cis*-2-cyanocyclopentanol (0.1 M) with RCO_2R^1 (0.1–0.2 M) in diisopropyl ether

R	R^1	Time/h	Conversion/%	$ee_{alcohol}$/%	ee_{ester}/%
CH_3CH_2	CH_2CF_3	0.5	50	96	95
$CH_3(CH_2)_4$	CH_2CF_3	0.5	50	96	98
CH_3	$CH{=}CH_2$	0.3	50	99	99

[a]Enzyme preparation (50 mg ml^{-1}) containing lipase [20% (w/w)].

use of vinyl esters (Tables 1 and 2), favours the equilibrium proposal for the lipase PS-catalysed acylation of the *trans* compound.

Vinyl acetate is clearly the best acyl donor for the lipase PS-catalysed acylation of *trans*-2-cyanocyclohexanol (Table 1, (■) Figure 1). Thus, 50% conversion is reached within 1–1.5 h. At this point the reaction stops, leading to the enantiopure acetate and unreacted alcohol enantiomers (Table 1).

Under the resolution conditions, the lipase PS-catalysed acylation of *cis*-2-cyanocyclopentanol stops at 50% conversion within less than an hour, the (1*R*,2*R*) enantiomer now being the reactive counterpart (Table 2). Accordingly, vinyl acetate as an easily available and economical acyl donor was chosen for the preparative-scale resolution of racemic *cis*- and *trans*-2-cyanoalkanols **1** and **2** (n=1 and 2, respectively) in the presence of lipase PS. The results described in the Experimental section reveal excellent enantiodiscrimination for the resolution of the four racemic mixtures, allowing the simultaneous preparation of the unreacted (1*S*,2*S*) alcohol and (1*R*,2*R*) ester enantiomers in the case of the *cis* isomers, and the (1*S*,2*R*) alcohol and (1*R*,2*S*) ester enantiomers in the case of the *trans* isomers at 50% conversion. The resolved products were further separated by column chromatography.

Experimental

Materials and methods

Racemic *trans*-cyanoalcohols **1** (n=1) and **2** (n=2) were obtained from 1,2-epoxycyclopentane and hexane, respectively, with hydrogen cyanide.[1,11] The corresponding racemic *cis*-cyanoalcohols were prepared by the decarboxylative ring opening of 3-carboxyisoxazolines, using the (3+2) cycloaddition of carbethoxyformonitrile oxide and the corresponding alkene.[8] Racemic cyanoalcohol esters for the ee determination and 2,2,2-trifluoroethyl esters as acyl donors were prepared from the corresponding alcohol and acid chloride or anhydride. Vinyl acetate was from Aldrich Chemical Corporation and vinyl butyrate from Tokyo Kasei Kogyo Corporation. Lipase PS from *Pseudomonas cepacia* was

purchased from Amano Pharmaceuticals. For preparation of the catalyst, the enzyme was dissolved in Tris-HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by absorption on Celite (17 g) (Sigma). The lipase preparation thus contained 20% (w/w) of the lipase. The solvents were of the best analytical grade and were dried over molecular sieves (3 Å). For gram-scale resolution, diisopropyl ether was distilled just before use.

In a typical small-scale experiment, a solution (3 ml) of cyanoalcohol **1** or **2** (0.1 M) in diisopropyl ether was added to the lipase preparation (50 mg ml^{-1}), and vinyl acetate (0.2 M in the reaction mixture) was added. The mixture was shaken at room temperature. The progress of the reaction was followed by taking samples (0.1 ml) from the reaction mixture at intervals. The unreacted alcohol in the sample was derivatized with pentanoic or propionic anhydride in the presence of 4-dimethylaminopyridine and pyridine before the gas chromatographic analysis. For the resolutions of *cis*-**1** and -**2** and *trans*-**1**, the ee's of the unreacted alcohol **1a** and **2a** and the ester **1b** and **2b** enantiomers produced were determined by using GLC on a 25 m Chrompack CP-Cyclodextrine-β-2,3,6-M-9 column. The ee values for *trans*-**2a** and -**2b** were determined using a Chirasil-*L*-Val column in the presence of hexadecane as internal standard.

Gram-scale resolution of cis-*2-cyanocyclopentanol*

Racemic *cis*-**1** (1.00 g; 9.00 mmol) and vinyl acetate (1.69 ml; 18 mmol) in diisopropyl ether (90 ml) were added to the lipase PS preparation (4.50 g). The mixture was stirred at room temperature for 20 minutes. The reaction stopped at 50% conversion with 99% ee for both unreacted (1*S*,2*S*)-**1a** and produced (1*R*,2*R*)-**1b** (0.63 g, 4.1 mmol; $[\alpha]_D^{20}$ +36.6 (c=2.5, CH_2Cl_2); ee 99%). The enzyme was filtered off, the solvent was evaporated off and the resolved alcohol and ester enantiomers were separated by column chromatography on silica gel, with elution with EtOAc/hexane (1/2). The fraction containing **1a** (0.45 g, 4.0 mmol; $[\alpha]_D^{20}$ +4.9 (c=2.5, CH_2Cl_2); ee 99%) had an impurity affecting the value of the optical rotation. For this reason, the cyanoalcohol **1a** was acetylated to form the ester **1b′** which was then purified by column chromatography (0.38 g, 2.4 mmol; $[\alpha]_D^{20}$ −36.4 (c=2.5, CH_2Cl_2); ee 99%).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **1b** and **1b′**: 1.7–2.2 (6H, m, $3CH_2$) 2.0 (3H, s, CH_3) 3.0 (1H, m, C*H*CN) 5.2 (1H, m, C*H*OCOCH_3). Analysis: calculated for $C_8H_{11}NO_2$: C, 62.75; H, 7.19; N, 9.15; found for **1b**: C, 62.13; H, 7.26; N, 9.02; and for **1b′**: C, 61.88; H, 6.99; N, 9.02.

Gram-scale resolution of trans-*2-cyanocyclopentanol*

With the procedure described above, racemic *trans*-**1** (1.00 g; 9 mmol) afforded the unreacted (1*S*,2*R*)-**1a** and the produced ester (1*R*,2*S*)-**1b** in 1 h.

1a (0.38 g, 3.36 mmol; $[\alpha]_D^{20}$ +78.9 (c=2.5, CH_2Cl_2); ee 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.6–2.3 (6H, m, $3CH_2$) 2.7 (1H, m, C*H*CN) 4.4 (1H, m, C*H*OH). Analysis: calculated for C_6H_9NO: C, 64.86; H, 8.11; N, 12.61; found: C, 63.50; H, 7.97; N, 12.13).

1b (0.58 g, 3.83 mmol; $[\alpha]_D^{20}$ −71.4 (c=2.5, CH_2Cl_2); ee 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.7–2.2 (6H, m, $3CH_2$) 2.3 (3H, s, CH_3) 2.8 (1H, m, C*H*CN) 5.2 (1H, m, C*H*OCOCH_3). Analysis: calculated for $C_8H_{11}NO_2$: C, 62.75; H, 7.19; N, 9.15; found: C, 62.21; H, 7.19; N, 9.11.

Gram-scale resolution of cis-*2-cyanocyclohexanol*

Racemic *cis*-**2** (0.36 g; 2.88 mmol) and vinyl acetate (0.54 ml; 5.76 mmol) in diisopropyl ether (30 ml) were added to the lipase PS preparation (1.50 g). The mixture was stirred at room temperature for 18 minutes. The reaction stopped at 50% conversion with 95% ee for the unreacted (1*S*,2*S*)-**2a** and 98% ee for the produced (1*R*,2*R*)-**2b** (0.23 g, 1.38 mmol; $[\alpha]_D^{20}$ +86.8 (c=2.5, CH_2Cl_2); ee 96%). The normal work-up led to the unreacted alcohol **2a** (0.17 g, 1.36 mmol; $[\alpha]_D^{20}$ −28.8 (c=2.5, CH_2Cl_2); ee 95%), containing an impurity which was removed as described above in the case of *cis*-**1a**. The acetylated derivative **2b′** (0.16 g, 0.98 mmol: $[\alpha]_D^{20}$ −83.5 (c=2.5, CH_2Cl_2); ee 98%) was obtained.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **2b** and **2b′**: 1.3–2.2 (6H, m, 3×CH_2) 2.1 (3H, s, CH_3) 3.1 (1H, m, C*H*CN) 4.7 (1H, m, C*H*OCOCH$_3$). Analysis: calculated for $C_9H_{13}NO_2$: C, 64.67; H, 7.78; N, 8.38. Found for **2b′**: C, 64.07; H, 7.78; N, 8.38; and for **2b**: C, 63.31; H, 7.94; N, 8.21.

Gram-scale resolution of trans-*2-cyanocyclohexanol*

With the procedure described above, racemic *trans*-**2** (0.36 g; 2.88 mmol) afforded the unreacted (1*S*,2*R*)-**2a** and the produced ester (1*R*,2*S*)-**2b** in 2.5 h.

2a (0.13 g, 1.04 mmol; $[\alpha]_D^{20}$ +52.0 (c=2.5, CH_2Cl_2); lit.[11,13] $[\alpha]_D^{20}$ +53.9 (c=1, CH_2Cl_2); ee 9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.0–2.2 (8H, m, 4×CH_2) 2.4 (1H, m, C*H*CN) 3.7 (1H, m, C*H*OH). Analysis: calculated for $C_7H_{11}NO$: C, 67.20; H, 8.80; N, 11.20; found: C, 66.35; H, 8.69; N, 10.91.

2b (0.21 g, 1.25 mmol; $[\alpha]_D^{20}$ −52.0 (c=2.5, CH_2Cl_2); ee 99%). ^{1}H NMR (400 MHz, CDC_3) δ (ppm): 1.2–2.2 (8H, m, 4×CH_2) 2.1 (3H, s, CH_3) 2.6 (1H, m, C*H*CN) 4.8 (1H, m, C*H*OCOCH$_3$). Analysis: calculated for $C_9H_{13}NO_2$: C, 64.67; H, 7.78; N, 8.38; found: C, 63.96; H, 7.79; N, 8.29.

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