ENANTIOSELECTIVE PREPARATION OF THE STEREOISOMERS OF 4-METHYLHEPTAN-3-OL USING *Candida antarctica* LIPASE B

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The four stereoisomers of 4-methylheptan-3-ol were prepared through fractional crystallization of a mixture of their racemic 4-methylhept-3-yl 3,5-dinitrobenzoates, followed by hydrolysis of each crystalline racemate and enantioselective, lipase-mediated transesterification of each pair of enantiomeric alcohols liberated, using the *Candida antarctica* lipase B as catalyst and *S*-ethyl octanethioate as the acyl donor. An analytical method of determining the enantiomeric composition of mixtures of 4-methylheptan-3-ols was developed. The optical purity of the separated isomers was in the range of 29–97%.
Key words: 4-Methylheptan-3-ol; *Candida antarctica* lipase B; Enzymatic resolution.

4-Methylheptan-3-ol (**1**) is a secondary alcohol, whose stereoisomers are ecologically active in several kinds of organisms. (3*R*,4*S*)-4-Methylheptan-3-ol is the trail pheromone of the ant *Leptogenus diminuta*[1]. Stereoisomers of 4-methylheptan-3-ol (**1**) are components of the aggregation pheromone of elm bark beetles and other bark beetles in the Scolytidae family[2].

Elm bark beetles are the vectors of the Dutch elm disease (*Ceratocystis ulmi*), a fungus disease which kills elm trees[3]. The efforts to control the beetles and thereby the disease are severely hampered by the lack of knowledge regarding the chemical communication of some *Scolytus* species. This is partly due to the lack of enantiomerically pure samples of this secondary alcohol.

A number of syntheses of one or two isomers of 4-methylheptan-3-ol (**1**) were reported in the literature[4], but only two (rather tedious) syntheses of all four isomers[5]. We chose an enzymatic method, lipase-mediated transesterification, which had been shown to be a fast and facile way of resolving secondary alkan-3-ols, *e.g.* nonan-3-ol and

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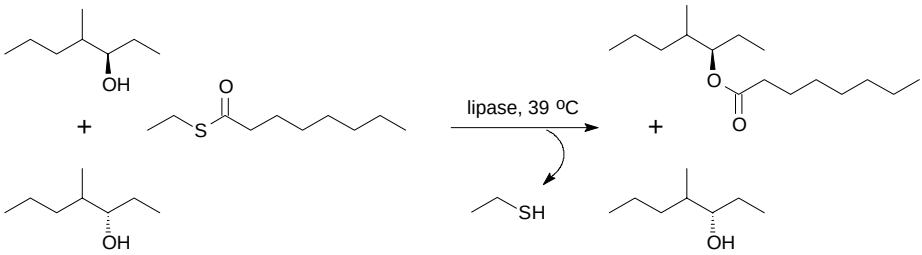
undec-1-yn-3-ol[6]. This reaction, mediated by *Candida antarctica* lipase B (CALB) as catalyst and with *S*-ethyl octanethioate as an acyl donor, was carried out at 39 °C and atmospheric pressure (Scheme 1). Under these conditions the ethanethiol produced (b.p. 35 °C) evaporated and the reaction equilibrium was shifted in the desired direction[7].



SCHEME 1

Other types of acyl donors, methyl octanoate[8], ethyl octanoate[9] and vinyl octanoate[10], have been employed. Problems with the water activity may arise when methyl octanoate is used, since the methanol formed is removed by 3Å molecular sieves, which also remove water[10,11]. When employing ethyl octanoate, it is not possible to evaporate the ethanol at a temperature that will not coagulate the enzyme, without using an inconvenient reduced pressure technique[9]. The disadvantage of using vinyl octanoate as acyl donor is that the acetaldehyde formed after tautomerization of the leaving group may react with basic moieties in the enzyme, thereby impeding or blocking the catalyzing capacity[12].

This paper describes the diastereomeric separation of the *erythro* and *threo* forms of 4-methylheptan-3-ol using fractional crystallization (Scheme 2) and the lipase-mediated separation of the enantiomers of the *erythro* and *threo* forms. Schematic separation of all stereoisomers of 4-methylheptan-3-ol is shown in Scheme 3.

EXPERIMENTAL

The chemicals were purchased from Aldrich. *S*-Ethyl octanethioate was prepared from octanoyl chloride and ethanethiol according to Orrenius *et al.*[6]. The lipases were kindly supplied Novo Nordisk A/S, Denmark. The lipase (component B) derived from *Candida antarctica* was a product from Novo Nordisk A/S (Novozym™ 435). The enzyme was used as an immobilized preparation on a macroporous resin, containing approximately 1% enzyme (w/w). The catalytic activity was approximately 40 000 lipase units per gram of enzyme preparation. Melting points were taken with a Gallenkamp apparatus and were uncorrected.

Spectroscopy. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 ACF spectrometer. Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. The optical rotations were obtained using a Perkin–Elmer 241 polarimeter. The gas chromatograms were recorded on a Varian 3500 instrument supplied with a 30 m DB-FFAP fused silica capillary column.

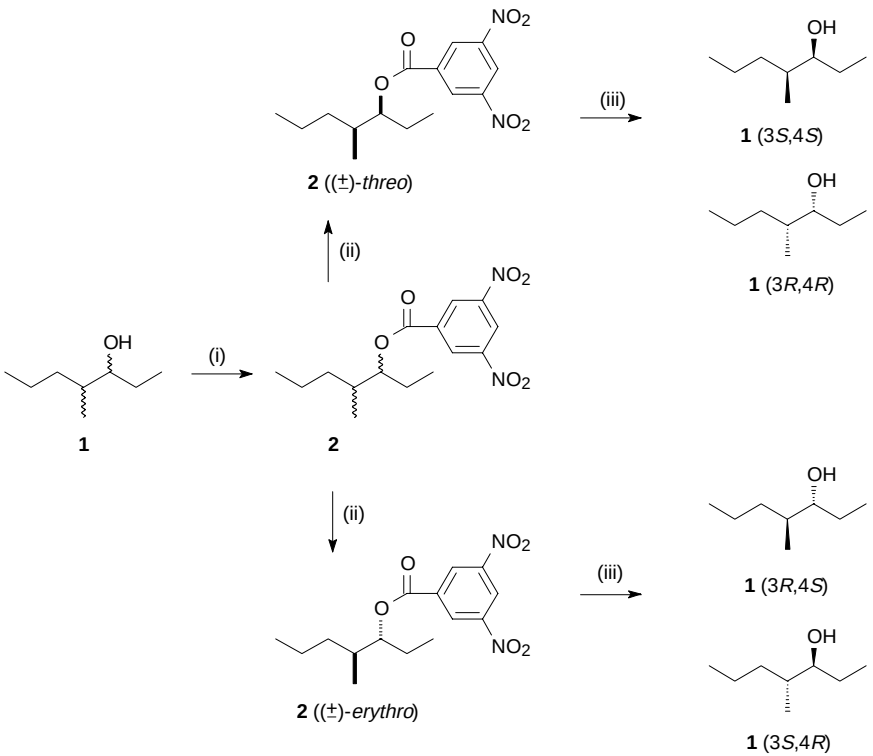
Carbamate derivatization. For the stereochemical analysis by chiral gas chromatography (GC), the 4-methylheptan-3-ols were derivatized with methyl isocyanate, yielding the corresponding carba-

mates. A few drops of neat methyl isocyanate were added to the sample to be analyzed and the mixture was left to react during 3–5 h at room temperature. The analysis was then performed on a Varian 3300 instrument supplied with a 50 m permethylated β-cyclodextrin column (isothermal 115 °C, 24 kPa). The elution order of the carbamate derivatives was *S*,*S*; *S*,*R*; *R*,*R*; *R*,*S*.

4-Methylheptan-3-ol (**1**). Two standard Grignard reactions were used for synthesizing mixtures of stereoisomeric 4-methylheptan-3-ols[13]. The reaction of 2-pentylmagnesium bromide with propanal resulted in equal amounts of the four isomers, while the reaction of ethylmagnesium bromide with 2-methylpentanal resulted in a mixture with a 2 : 1 *threo:erythro* diastereomeric ratio.

4-Methylhept-3-yl 3,5-Dinitrobenzoate (**2**)

A stirred solution of 4-methylheptan-3-ol (5.00 g, 38.4 mmol), dicyclohexylcarbodiimide (11.9 g, 57.7 mmol) and 4-dimethylaminopyridine (0.38 g, 3.1 mmol) in dry CH_2Cl_2 (85 ml) was ice-cooled and 3,5-dinitrobenzoic acid (12.2 g, 57.7 mmol) was added[14]. The mixture was stirred at room temperature for 12 h, and then diluted with pentane (20 ml). The mixture was filtered and the solid material was washed with CH_2Cl_2. The combined filtrate and washings were concentrated *in vacuo* and



(i) 3,5-dinitrobenzoic acid, 4-dimethylaminopyridine, dicyclohexylcarbodiimide, CH_2Cl_2; (ii) fractional crystallization; (iii) KOH/THF/methanol

SCHEME 2

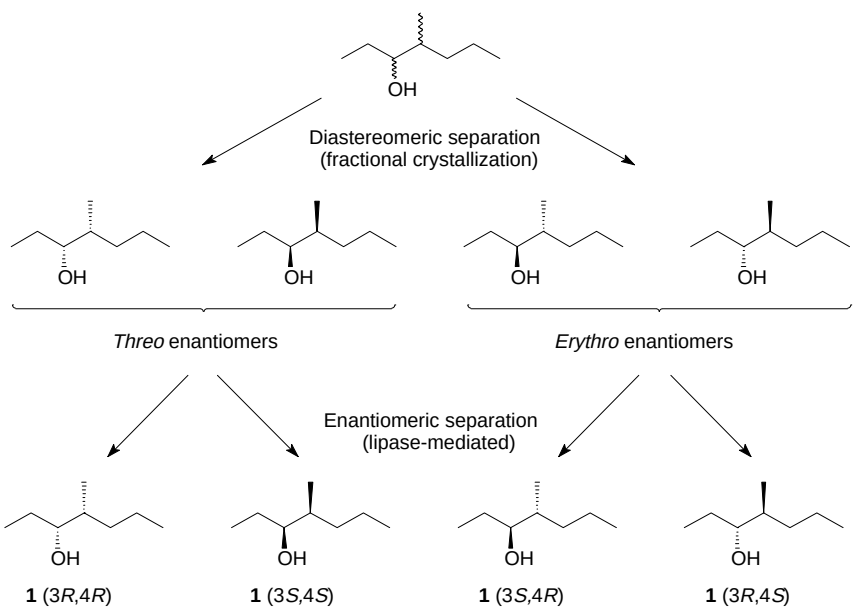
then subjected to silica gel chromatography. The yield of dinitrobenzoate **2** was 11.85 g (95%). The *erythro* isomers of **2** were found to crystallize more readily than their *threo* counterparts of **2** and the diastereomers could be separated by repeated crystallization at low temperature (–50 °C) from hexane. A problem that appeared was that when the mixture consisted of 67% *erythro*- and 33% *threo*-benzoate **2**, no further fractional crystallization could be achieved. Apparently fairly stable mixed crystals were formed.

The erythro diastereoisomers of **2**: White crystals. The yield was 880 mg (18%), m.p. 59–61 °C. ^{1}H NMR spectrum ($CDCl_3$): 9.22 t, 1 H, J = 2 (ArH-4); 9.14 d, 2 H, J = 2 (ArH-2,6); 5.09 app. q, 1 H, J = 5.6, J' = 6.9 (C**H**OCOAr); 1.17–1.92 m, 7 H (3 × CH_2, CH); 0.97 d, 3 H, J = 6.8 (CH–C**H**$_3$); 0.95 t, 3 H, J = 7.4 (CH_3); 0.91 t, 3 H, J = 6.9 (CH_3). ^{13}C NMR spectrum ($CDCl_3$): 10.1 (CH_3), 14.2 (CH_3), 15.2 (CH_3), 20.1 (CH_2), 23.3 (CH_2), 34.3 (CH_2), 35.8 (CH–Me), 82.9 (CH–O), 122.2 (Ar), 129.3 (Ar), 134.5 (Ar), 148.7 (Ar), 162.3 (C=O).

The threo diastereomers of **2**: Yellow crystals. The yield was 460 mg (7%), m.p. 19–21 °C. ^{1}H NMR spectrum ($CDCl_3$): 9.21 t, 1 H, J = 2 (ArH-4); 9.12 t, 2 H, J = 2 (ArH-2,6); 5.13 ddd, 1 H, J = 8.0, J' = 4.7, J'' = 4.5 (CHOCOAr); 1.12–1.92 m, 7 H (3 × CH_2, CH); 1.00 d, 3 H, J = 6.9 (CH–C**H**$_3$); 0.94 t, 3 H, J = 6.4 (CH_3); 0.88 t, 3 H, J = 7.0 (CH_3). ^{13}C NMR spectrum ($CDCl_3$): 10.2 (CH_3), 14.2 (CH_3), 14.4 (CH_3), 20.2 (CH_2), 24.4 (CH_2), 35.3 (CH_2), 35.7 (CH–Me), 82.2 (CH–O), 122.2 (Ar), 129.3 (Ar), 134.4 (Ar), 148.7 (Ar), 162.3 (C=O).

erythro-4-Methylheptan-3-ol (**1**)

Aqueous 1 M KOH (2.85 ml, 2.85 mmol) was added dropwise to a stirred and ice-cooled solution of (±)-*erythro*-4-methylhept-3-yl 3,5-dinitrobenzoate (**2**) (880 mg, 2.71 mmol) in THF–MeOH (1 : 1, 14 ml). After the addition, the mixture was stirred for 2 h at 0 °C before being diluted with aqueous $NaHCO_3$ and extracted with ether. The ether solution was washed with brine, dried ($MgSO_4$) and concentrated



SCHEME 3

in vacuo. The residue was subjected to silica gel chromatography. The diastereomeric purity was 96% and the yield was 151 mg (43%). ^{1}H NMR spectrum ($CDCl_3$): 3.34 m, 1 H (C**H**OH); 1.56 s, 1 H (CHO**H**); 1.05–1.55 m, 7 H (3 × CH_2, CH); 0.96 t, 3 H, J = 7.4 (CH_3); 0.90 t, 3 H, J = 7.0 (CH_3); 0.88 d, 3 H, J = 6.9 (CH–C**H**$_3$).^{13}C NMR spectrum ($CDCl_3$): 10.3 (CH_3), 14.3 (CH_3), 15.2 (CH_3), 20.3 (CH_2), 26.1 (CH_2), 34.1 (CH_2), 38.2 (CH–Me), 77.5 (CH–O). The NMR data were in accord with those reported in the literature[15].

threo-4-Methylheptan-3-ol (**1**)

Similarly, (±)-*threo*-4-methylhept-3-yl 3,5-dinitrobenzoate (**2**) (710 mg, 2.19 mmol) was hydrolyzed in THF–MeOH (1 : 1, 13 ml; 1 M KOH (2.29 ml, 2.29 mmol)). The diastereomeric purity was 96% and the yield was 77 mg (27%). ^{1}H NMR spectrum ($CDCl_3$): 3.41 app. quintet, 1 H, J = 7.9, J' = 8.3 (CHOH); 1.12–1.53 m, 8 H (3 × CH_2, CH, CHO**H**); 0.95 t, 3 H, J = 7.4 (CH_3); 0.90 t, 3 H, J = 7.0 (CH_3); 0.86 d, 3 H, J = 6.9 (CH–C**H**$_3$). ^{13}C NMR spectrum ($CDCl_3$): 10.5 (CH_3), 13.4 (CH_3), 14.3 (CH_3), 20.4 (CH_2), 27.2 (CH_2), 35.6 (C5), 37.4 (CH–Me), 76.6 (CH–O). The NMR data were in accord with those reported in the literature[15].

Lipase-Mediated Enantiomeric Resolution

Lipase screening procedure. A mixture of all four 4-methylheptan-3-ols (65 mg, 0.5 mmol) was dissolved in *S*-ethyl octanethioate (284 mg, 1.5 mmol) and hexadecane (30 μl) was added as an internal standard. The reaction was started by the addition of the lipase (20 mg). The reaction was carried out at 39 °C with magnetic stirring. The conversion was measured by following the consumption of the alcohol in relation to the internal standard and to the amount of ester produced (Table I).

(3R,4S)-(–)-4-Methylheptan-3-ol (**1**): The *erythro* forms of compound **1** (151 mg, 1.16 mmol) were dissolved in *S*-ethyl octanethioate (437 mg, 2.32 mmol) and hexadecane (115 μl) was added as an internal standard. The reaction was started by the addition of immobilized *Candida antarctica* lipase B (46 mg) and was carried out at 39 °C with stirring. The reaction reached a conversion of 43% (according to GC). The amount of 68% of the acyl donor was transformed into octanoic acid. The reaction mixture was subjected to silica gel chromatography. The amount of unreacted (3*R*,4*S*)-(–)-4-methylheptan-3-ol recovered was 16 mg (11%), 93% isomeric purity, 96% ee.

(3S,4R)-(+)-4-Methylheptan-3-ol (**1**): The fraction containing the ester formed was treated with KOH/MeOH. The yield of the (3*S*,4*R*)-(+)-4-methylheptan-3-ol was 41 mg (27%), 65% isomeric purity, 35% ee.

(3S,4S)-(+)-4-Methylheptan-3-ol (**1**): The *threo* forms of compound **1** were resolved by the procedure described above for the *erythro* analogue: Compound **1** (*threo*) (77 mg, 0.59 mmol), *S*-ethyl octanethioate (230 mg, 1.18 mmol), hexadecane (60 μl). A conversion of 42% was reached and 77% of the acyl donor was transformed to octanoic acid. The amount of unreacted alcohol recovered was 12 mg (16%), 61% isomeric purity, 29% ee.

(3R,4R)-(–)-4-Methylheptan-3-ol (**1**): The ester was hydrolyzed with KOH in methanol. The yield of alcohol was 6 mg (8%), 93% isomeric purity, 95% ee.

RESULTS AND DISCUSSION

Diastereomeric Separation of 4-Methylheptan-3-ol

A racemic mixture of the four isomers of 4-methylheptan-3-ol was converted into a corresponding mixture of their 3,5-dinitrobenzoates[14]. The *erythro* racemate was found

to crystallize more readily than its *threo* counterpart. The highest diastereomeric purity of the *erythro* racemate, which was obtained after repeated crystallization in hexane at low temperature, was >98%. The *threo* dinitrobenzoate racemate could be obtained in 99% diastereomeric purity by fractional crystallization.

Lipase Screening

Five lipases were tested for their ability of catalyze the esterification reaction of all four 4-methylheptan-3-ols with octanethioate as acyl donor (Table I). The degree of conversion was monitored by GC, following the consumption of the alcohol in relation to an internal standard (hexadecane) and to the amount of ester produced. The rate of the reaction brought about by *Candida antarctica* A was too high and the selectivity was comparatively low. The reaction rates obtained with *Candida antarctica* B (CALB) and *Candida rugosa* were both suitable but CALB was chosen since it is generally the most selective of the two ones.

Enantioselective Lipase-Mediated Acylation of 4-Methylheptan-3-ols

The enantioselective, enzyme-mediated transesterification method described above[7] was applied to the enantiomeric resolution of the *threo* racemate of 4-methylheptan-3-ol obtained by hydrolysis of the dinitrobenzoates.

The reaction was started by addition of the immobilized *Candida antarctica* lipase B to the mixture of acyl donor and (±)-*threo*-4-methylheptan-3-ol. The reaction was monitored by GC. The enzyme was removed by filtration and the reaction mixture was subjected to silica gel chromatography, whereby the esters produced and the unreacted alcohols were separated. The fraction containing the esters was hydrolyzed (KOH/MeOH).

The *erythro*-4-methylheptan-3-ols were resolved by means of the same procedure (see Experimental).

TABLE I
Screening of lipases for resolution of racemic 4-methylheptan-3-ols (**1**)

Lipase	Synonym	Reaction time, h	Conversion, %
Mucor miehei	Lipozyme	60	0
Humicola lanuginosa	SP 400	24	0
Candida cylindracea	*Candida rugosa*	60	≈20
Candida antarctica A	SP 429	24	≈80
Candida antarctica B	Novozym™ 435	60	≈25

Chiral Analysis and Determination of the Absolute Configuration

The alcohols liberated on hydrolysis were derivatized with methyl isocyanate. The carbamates formed were then separated by means of GC on a permethylated β-cyclodextrin column. The enantiomeric excess values (see Table II) were then calculated assuming that the stereochemistry of the carbamates was identical with that of the 4-methylheptan-3-ols (*i.e.* that the derivatization procedure did not affect the stereocentres at carbon atoms 3 and 4).

The correlation of a gas chromatography peak to an absolute configuration of a stereoisomer of 4-methylheptan-3-ol was made by comparison of the sign of the optical rotation with those in the literature[16]. The assignment was consistent with the fact that the enzyme used, *Candida antarctica* lipase B, reacts selectively with *R*-alcohols[17]. It was also in accordance with Cram's rule[18], that the 4-methylheptan-3-ol formed in a Grignard reaction of 2-methylpentanal with ethylmagnesium bromide consists of *ca* 2 : 1 diastereomeric mixture, in which the *threo* isomer is the major component. The absolute configurations of the four stereoisomers (see Scheme 2) of 4-methylheptan-3-ol are correlated to their optical rotations as follows: 3*R*,4*R*-(+)-*threo*, 3*S*,4*S*-(–)-*threo*, 3*S*,4*R*-(+)-*erythro* and 3*R*,4*S*-(–)-*erythro*.

Enantiomeric Excess and E-Values

In 1982 Chen *et al*. introduced the notion of enantiomeric ratio, *E*, as a parameter to quantify the kinetic resolution of enantiomers and to characterize the enantioselective properties of an enzyme[19]. Parameter *E* describes to what extent the enzyme discriminates between two competing enantiomers. In the case of an irreversible reaction ($K_{eq} \to \infty$) and when enantiomeric excess values of both the substrate (ee_s) and the product (ee_p) are known, the enantiomeric ratio, *E*, can be calculated directly from Eq. (*1*) (ref.[20]).

$$E = \frac{\ln [(1 - ee_s)/(1 + ee_s/ee_p)]}{\ln [(1 + ee_s)/(1 + ee_s/ee_p)]} \qquad (1)$$

TABLE II
Compositions of the enantiomerically enriched carbamate fractions (by chiral GC)

Sample	(3*R*,4*S*)	(3*S*,4*R*)	(3*S*,4*S*)	(3*R*,4*R*)	ee, %
(3*R*,4*S*)-*erythro*	**93**	2	–	5	96
(3*S*,4*R*)-*erythro*	32	**65**	3	1	35
(3*S*,4*S*)-*threo*	3	3	**61**	33	29
(3*R*,4*R*)-*threo*	3	–	5	**93**	95

Using the figures from Table I the *E* values can be calculated:

$$E\ (erythro) = 69 \qquad E\ (threo) = 52.$$

The enantiomeric excess at 50% conversion can be estimated from these values (ee_s) = (ee_p):

$$ee\ (erythro) = 91 \qquad ee\ (threo) = 89.$$

β *Selectivity*. It was observed in a transesterification experiment with all four stereoisomers that the lipase catalyzed the reaction in a slightly diastereoselective manner. The (3*R*,4*R*)-*threo* isomer was acylated faster than the (3*R*,4*S*)-*erythro* isomer and the (3*S*,4*S*)-*threo* isomer reacted faster than its (3*S*,4*R*)-*erythro* isomer (see Table III).

TABLE III
Transesterification of all four stereoisomers of 4-methylheptan-3-ol (**1**)

Compound	Carbamate retention time order	Unreacted alcohol, %	Hydrolyzed ester, %
(3*S*,4*S*)-*threo*	1	32	<1
(3*S*,4*R*)-*erythro*	2	42	<1
(3*R*,4*R*)-*threo*	3	7	52
(3*R*,4*S*)-*erythro*	4	18	46

CONCLUSION

Our results showed that the *Candida antarctica* lipase B enantioselectively catalyzed the acylation of 4-methylheptan-3-ol. The diastereomers (*threo* and *erythro*) of 4-methylheptan-3-ol were separated through fractional crystallization of the corresponding 3,5-dinitrobenzoates. The ee values of the separated isomers were in the range of 29–96%. The enantiomeric ratio *E* was 45–70, indicating a theoretical ee of 90% for both the 3*R* and the 3*S* isomers at 50% conversion and that much purer 3*S* isomers can be obtained by running the reaction much closer to 50% conversion.

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