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Resolution of 2-Methylalkanoic esters: Enantioselective Aminolysis by (*R*)-1-Phenylethylamine of Ethyl 2-Methyloctanoate Catalysed by Lipase B from *Candida antarctica*

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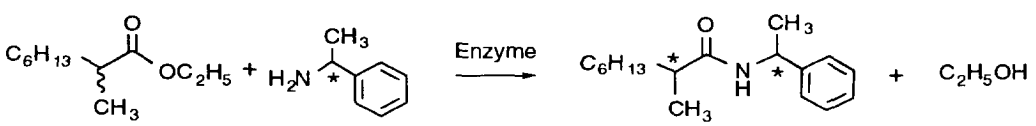
Abstract: Enantiomerically pure (*R*)-1-phenylethylamine and ethyl *rac*-2-methyloctanoate in the presence of lipase from *Candidada antarctica* (Novozym 435) reacted to give (*R*)-2-methyloctanoic (*R*)-1-phenylethylamide as the predominant diastereomer of 30 – 40% *de* at ≈ 100% conversion. Ethyl *rac*-2-methyldecanoate and ethyl *rac*-2-methylhexanoate gave similar results. The diastereomeric excess was almost constant from 0% – 100% conversion probably due to epimerisation of the α-carbon of the acyl moiety under the reaction conditions. The diastereomeric amides were obtained pure after liquid chromatography. Copyright © 1996 Elsevier Science Ltd

Resolutions of racemic amines *via* enzymatic aminolysis of achiral esters have recently been described.[1] The simultaneous resolution of ethyl 2-chloropropionate and some 1-amino-1-ethyl derivatives has also been described.[2a] Enzymatic ester ammoniolysis has been shown to provide a mild procedure for enantioselective synthesis of amides.[2b] The aminolysis of ethyl 2-methylbutanoate with benzylamine catalysed by lipase B from *Candida antarctica* [*CAL*, Novozym 435 (immobilised on polypropene)] in hexane yields the amide in 78% enantiomeric excess (*ee*) at 25% conversion.[3] Whereas chiral amines are resolved with high enantiomeric ratios ($E > 100$)[1], chiral esters give much more modest results with E between 1–10[2,3]. We have recently been studying the enzyme catalysed resolution of 2-methylalkanoic acids and 2-methyl-1-alcohols by esterification[4] and transesterification[4f,5] respectively. We routinely determine the enantiomeric purities of the products by reduction of the esters to the alcohols and then oxidation of these to the acids followed by conversion of the latter to the 2-methylacyl *N*-(1-phenylethyl)amides using enantiomerically pure (*S*)- or (*R*)-1-phenylethylamine.[4c,5] The diastereomeric ratios of the amides formed are easily determined by GC.[4c–f,5] If one of the readily available pure enantiomers of 1-phenylethylamine could act as the nucleophile for a 2-methylacyl enzyme, an easily analysed and separable diastereomeric mixture of two amides would be obtained and both conversion and stereoselectivity could be measured at the same time.



1 **2** **3**

We now wish to report our study of the aminolysis reactions of racemic ethyl 2-methyloctanoate **1** with enantiomerically pure (*S*)- or (*R*)-1-phenylethylamine **2** catalysed by various lipases giving a mixture of the diastereomeric amides **3**.

Among the enzymes tested [CRL, Amano PS, Subtilisin, Novozym 435, SP 523, SP 524, SP 525, SP 526 and SP 539] only SP 526 and lipase from *Candida antarctica* (Novozym 435, Table 1, entries 1–7, 9–13)

displayed any significant activity and the latter was the most effective. In the latter case (*R*)-1-phenylethylamine (*R*-**2**) was the reactive enantiomer.

Usually reactions of this type are run in an organic solvent. In hexane at room temperature or 30 °C the reaction was extremely slow (entries 1, 2) and only slightly faster at 50 °C. This might be due to a reversible reaction with the ethanol formed which should compete favourably[1c] as a nucleophile for the acyl enzyme under the reaction conditions. Using some more polar solvents like anisol, acetone, dioxane, pyridine or sulpholane did not significantly increase the reaction rates at 30 °C. The diastereomeric excesses (*de*) of the products in these reactions were around 35–50%. However, the conversions reached within reasonable time were far too low to be preparatively useful. The results obtained with the various solvents tried so far were used in a simplex strategy[6] to find a better one from the principal properties of solvents. This led us to test diphenyl ether (DPE,Table 1, entries 5–8).

Table 1 Aminolysis of ester *rac*-**1** with amines *R*- and *S*-**2** with Lipase from *Candida antarctica* (unless otherwise stated). *c* = Conversion, TMSU = Tetramethylenesulphone (= Sulpholane), DPE = Diphenylether.

Entry	Solvent	Conf (**2**)	Temp (°C)	Time (hrs)	c (%)	*de*[g] (%)
1	Hexane	R	23	384	6	48
2	Hexane	S	23	144	0	-
3	Dioxane	R	30	600	3	54
4	TMSU	R	30	600	4	38
5	DPE	R	30	2160	16	49
6	DPE	R	70	31	45	30
7	DPE	S	70	408	16	16[h]
8[a]	DPE	R	70	408	0	-

Entry	Solvent	Conf (**2**)	Temp (°C)	Time (h)	c (%)	*de* (%)
9[b]	(neat)	R	23	10	8	35
				34	30	31
				58	42	27
10[c]	(neat)	R	70	5	8	33
				13	20	27
				26	35	25
				70	87	47
				115	99	45
11[d]	(neat)	R	70	960	58	25
12[e]	(neat)	R	70	48	26	25
13[f]	(neat)	R	70	95	76	25

[a] no enzyme. [b] reduced pressure 100 mmHg; twice normal scale. [c] Scale: 24 times normal (Fig. 1). [d] fivefold molar excess of amine. [e] substrate: ethyl 2-methyldecanoate. [f] substrate: ethyl 2-methylhexanoate. [g] % *de* of *RR*-**3** = [% *RR*-**3** – % *SR*-**3**] / [% *RR*-**3** + % *SR*-**3**]; First letter in *RR*-**3** and *SR*-**3** defines stereochemistry in the acyl and second in the amine moiety. [h] % de of *RS*-**3**.

Novozym 435 is a very heat tolerant product with a maximum activity in the range 70–80 °C. Therefore, in order to increase the rate and simultaneously remove the ethanol formed, the reaction temperature was increased and an open vessel was used. Thus at 70 °C this reaction in DPE gave after 31 h a 45% conversion to a product of 30% *de*. (Table 1, entry 6).

Due to its very high boiling point diphenylether is an unsuitable solvent for practical purposes. Therefore we tried the reaction without a solvent (*cf* ref 1c). Thus, when run neat at 23 °C under reduced pressure in order to remove the ethanol formed and prevent water absorption, which could lead to acid formation,[1c] the reaction proceeded to 42% conversion to give a product of 27% *de* after 58 h (Table 1, entry 9). Similar or slightly better *de*:s and increased rates were observed at 70 °C: 115 h, 99% conversion, 45% *de* (open vessel to permit ethanol to escape, Table 1, entry 10). The predominating diastereomeric amide **3** formed always had the (*R*)-configuration at the α-carbon of the acyl moiety regardless of the configuration of the amine (Table 1, entry 7). The (*S*)-amine reacted very slowly even at 70 °C (Table 1, entry 7). The rate ratio between the fast reacting pure enantiomer of amine *R*-**2** and the slow one, *S*-**2**, gave an approximate[8] enantiomeric ratio for the resolution of the racemic amine of $E \approx 75$. This is similar to the *E*-values that are

obtained with *rac*-**2** and ethyl esters.[1c]

The amides were not formed spontaneously under these conditions as an experiment without enzyme clearly demonstrated (Table 1, entry 8).

The ethyl esters of racemic 2-methyldecanoic and 2-methylhexanoic acids (Table 1, entries 12 and 13) reacted with the amine *R*-**2** in a similar way and both amide products were obtained in 25% *de*.

Vinyl esters are sometimes used as efficient acyl donors.[7] In an attempted aminolysis with **2**, *racemic* vinyl 2-methyloctanoate reacted much more slowly than the ethyl ester **1** in *n*-hexane. We have also examined some alternative substrates such as ethyl β-methyl-2-thiophenepropionate, 3,4-dimethyl-γ-butyrolactone but neither of them gave useful rates.

It is interesting to note that the *de* of the product was constant or even increased slightly at conversions higher than 50% (Table 1, entry 10 and Fig 1.). This is not possible unless the stereogenic α-carbon of the acyl moiety is continually epimerised during the reaction.

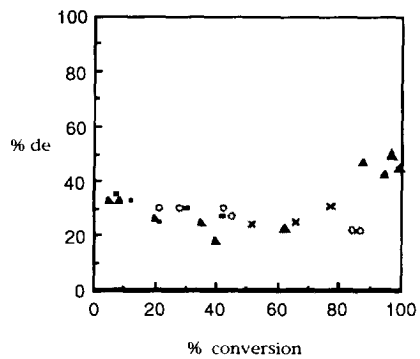


Fig. 1. Variation of product *de* with conversion in the Novozym 435 catalysed (*R*)-1-phenylethyl aminolysis of ethyl *rac*-2 methyloctanoate. x: 0.4 g scale, 70 °C, neat. ▲4 g scale, 70 °C, (entry 10). o: 0.15 g scale, 70 °C, solvent diphenylether, (entry 6). ■ :0.3 g scale, reduced pressure (entry 9).

First the configurational stability of the product was investigated. The possibility that the *de* of the enzymatically produced major amide *RR*-**3** reflects the thermodynamic equilibrium position *via* base catalysed epimerisation of the initially formed product was first tested. None of the pure amides *SR*- and *RR*-**3** were appreciably racemised (< 2% loss in *de*) in the presence of phenylethylamine neat at 70 °C for 75 h. In addition, as mentioned above the extremely slow enzyme catalysed reaction of the unreactive amine enantiomer, *S*-**2**, gave the same sense of chirality in the acyl moiety i.e predominantly the amide *RS*-**3** (16% *de* at 16% conv., Table 1, entry 7). Since different diastereomers of the amides **3** were obtained from each of the enantiomeric amines the amides must have formed under kinetic rather than thermodynamic control.

When recovering the remaining ester from an experiment (Fig. 1: x, neat, 70 °C) yielding the amide product (31% *de* at 76% conversion) we found that this eester was virtually racemic (≈5% *ee*).. Assuming irreversibility in the amide forming step, the *ee* of a configurationally stable substrate at this conversion should be 98%.[9] Thus, continous epimerisation of the α-carbon of the acyl moiety must have taken place before product formation during the reaction.

Substrate racemisation in enzymatic resolutions are known in some cases.[10] In the case at hand, base catalysed enolisation caused by phenylethylamine might be responsible for our results. In order to test this, a mixture of 1-phenylethylamine (*R*-**2**) and enantiomerically enriched ethyl (*S*)-2-methyloctanoate was allowed to react neat at 70 °C without enzyme for 80 h. However, no appreciable racemisation was observed in this experiment.

Since neither the substrate nor the product was epimerised at the α- carbon of the acyl moiety in the absence of the enzyme, the latter in conjunction with the amine is probably responsible for the observed epimerisation. An hypothetical explanation is that the basicities of some groups in the protein is locally enhanced in the basic reaction environment, creating catalytic sites which can racemise the ester more efficiently than the free amine is able to do. Alternatively, the acyl enzyme might be the species that is epimerised. We have at present no explanation for the intriguing increase in *de* observed at high conversions in the aminolysis experiment performed on a 4 g scale (entry 10 and Fig 1.).

On a 4 g scale the diastereomeric amide mixture was easily separated by chromatography affording, after recrystallisation, the enantiomerically pure diastereomeric amides *RR*-**3** (major product 47% yield) and the *SR*-**3** (minor product 33% yield).

2-Methylalkyl derivatives have previously been resolved by classical diastereomer formation using enantiomerically pure 1-phenylethylamine.[11] The amides were formed from the acid chlorides by conventional chemical reactions and the amides were separated by chromatography and transformed into useful enantiomerically pure 2-methylalkyl derivatives.[11] If (*R*)-2-methylalkyl derivatives are needed, the present methodology offers a superior procedure since the complete conversion to a product of moderate but significant diastereomeric excess constitutes an advantage over the previous resolution procedure.

Further studies of the origin of the observed racemisation and the increase in *de* at the end of the reaction are under way.

Experimental

^1H and ^{13}C NMR were measured on a Jeol EX 270 instrument using $CDCl_3$ as solvent and TMS as an internal reference. Refractive indices were obtained with a IA Pleuger 2WA. Optical rotations were measured on a Perkin Elmer 241 polarimeter. FT-IR spectra were recorded (neat between NaCl plates for liquid sample or as a tablet of KBr for a solid sample) using a Nicolet 5SXC spectrometer. Mass spectra were recorded using GC-MS (Varian 3300 and an ion trap detector, Finnigan ITD800). Preparative liquid chromatography (MPLC) was performed on straight phase silica gel (Merck 60, 230–400 mesh, 0.040–0.063 mm) employing a gradient technique[12] using an increasing concentration (0–100%) of distilled ethyl acetate in distilled cyclohexane, as eluent. Boiling and melting points are uncorrected. Elemental analyse was carried out by Mikrokemi, Uppsala, Sweden.

Determination of conversion and diastereomeric excess. The conversions in the aminolysis reactions were determined using a Shimadzu 7AG gas chromatograph equipped with a 30 m x 0.25 mm I.D. capillary column with liquid phase DB-5, film thickness = 0.4 μm (N_2 30 mL/min.; temp 180 °C isothermal). The conversions were calculated from the areas of the amide peaks relative to the peak of the ester. The *ee:s* of the products were determined using the same chromatograms. Retention times: **1**: 3.2 min.; *RR*-**3** or *SS*-**3**: 24.7 min. and *RS*-**3** or *SR*-**3**: 26.5 min. The *ee* of the remaining ester substrate was analysed by GC after reduction to the alcohol, oxidition to the acid and conversion to the appropriate phenylethylamide.[4c]

Racemic 2-methyloctanoic acid was prepared as described in the literature.[4c]

Ethyl 2-methyloctanoate[15] (**1**) To ethanol (70 mL) and 2-methyloctanoic acid (5.34 g, 28.7 mmol) was cautiously added conc. H_2SO_4 (0.2 mL). The mixture was refluxed for 40 h. After cooling, the mixture was poured into water (50 mL) and extracted with pentane (3x 100 mL). The organic phase was shaken twice with a 15% aqueous solution of sodium carbonate (100 mL) and then with water (100 mL). The organic phase was dried ($MgSO_4$) filtered and concentrated *in vacuo*. Distillation (b.p. 40-43 °C / 0.04 mmHg followed by liquid chromatography gave 4.7 g (77%) of pure ester (>99% by GC). ^1H NMR (270 MHz): δ

0.87 (3H, t, J = 6.9 Hz), 1.13 (3H, d, J = 6.1 Hz), 1.22–1.67 (13H, m), 2.20 (1H, q, J = 6.8 Hz), 4.12 (2H, q, J = 7.1 Hz). ^{13}C NMR (67.8 MHz): δ 176.93, 60.00, 39.55, 33.80, 31.68, 29.15, 27.15, 22.55, 17.04, 14.23, 14.00 ppm. FT-IR: 2930, 2859, 1739, 1482, 1377, 1252, 1175, 1095, 1033, 860 cm^{-1}. Mass spectrum, m/z (relative intensity): 188 ($M+2^+$, 100%), 139 (4), 129 (5), 115 (14), 102 (95), 87 (8), 73 (48), 57 (40), 41 (88). n^{20}_D = 1.4188. The compound is known, but no spectral or physical data is given. [15]

Ethyl 2-methyldecanoate was prepared as described above but starting from 2-methyldecanoic acid. ^{1}H NMR (270 MHz): δ 4.12 (2H, q, J = 6.7 Hz), 2.40 (2H, q, J = 6.9 Hz), 1.62 (1H, m), 1.22–1.29 (15H, m), 1.13 (3H, d, J = 6.9 Hz), 0.88 (3H, m). ^{13}C NMR (67.8 MHz): δ 176.94, 60.02, 39.55, 33.82, 31.84, 29.51, 29.41, 29.24, 27.2, 22.6, 17.1, 14.25, 14.05 ppm. FT-IR: 2927, 2858, 1736, 1484, 1376, 1257, 1180, 860, 761, 722 cm^{-1}. Mass spectrum, m/z (relative intensity): 216 ($M+2^+$, 100%), 171 (3), 157 (4), 129 (3), 115 (13), 102 (84), 83 (12), 73 (35), 55 (34), 41 (66). n^{20}_D = 1.429. Anal. calc for $C_{13}H_{26}O_2$: C 72.8%, H 12.2%. Found: C 72.3%, H 12.4%.

Ethyl 2-methylhexanoate was prepared as described above but starting from 2-methylhexanoic acid. ^{1}H NMR (270 MHz): δ 0.89 (3H, t, J = 6.8 Hz), 1.13 (3H, d, J = 6.9 Hz), 1.22–1.71 (9H, m), 2.40 (1H, q, J = 6.9 Hz), 4.12 (2H, q, J = 7.15 Hz). ^{13}C NMR (67.8 MHz): δ 176.90, 60.03, 39.55, 33.53, 29.43, 22.60, 17.07, 14.27, 13.93 ppm. FT-IR: 2933, 2666, 1736, 1482, 1376, 1257, 1181, 1039, 918, 861, 732 cm^{-1}. Mass spectrum, m/z (relative intensity): 159 ($M+1^+$, 50%), 131 (4), 115 (8), 102 (85), 85 (27), 74 (52), 56 (28), 43 (100). n^{20}_D = 1.4295. The compound is known, but no spectral or physical data is given. [16]

Vinyl 2-methyloctanoate. was prepared by the method used in ref. [14] for vinyl octanoate) Vinyl acetate (20 mL, 216 mmol) and 2-methyloctanoic acid (6.4 g, 35 mmol) was stirred for a few minutes and then mercuric acetate (0.16 g) was added. After stirring for about 0.5 h 100% H_2SO_4 (0.2 mL) was added dropwise. The mixture was refluxed for 24 h. After cooling the mixture was poured into pentane (200 mL) and stirred for 15 h. The solution was washed with saturated sodium carbonate (3x150 mL). After drying, the pentane was evaporated off and the vinyl ester was distilled twice in a bulb to bulb apparatus to give 1.7 g (23%); bath temp. 70 °C / 0.1 mmHg. ^{1}H NMR (270 MHz): δ 0.85 (3H, t, J = 5.4 Hz), 1.12 (3H, d, J = 8.1 Hz) 1.2-1.8 (16H, m), 2.51 (1H, sextet, J = 8.1 Hz), 4.55 (1H, d, J = 8.1 Hz) , 4.95 (1H, d, J = 16.2 Hz), 7.32 (1H, dd, J = 8.1 Hz) ppm. The compound was not stable when stored at +8 °C.

Aminolysis: General procedure. Novozym 435 (30 mg) was added to a solution of ethyl 2-methyloctanoate (**1**, 184 mg, 1.0 mmol) and (*R*)-1-phenylethylamine (*R*-**2**, 122 mg, 1.0 mmol) either neat or in 8 mL of solvent (unless otherwise stated, see Table 1). The mixture was stirred at 500 rpm at temperatures between 23-70 °C for various periods of time (see Table 1).

Semipreparative aminolysis. Ethyl *rac*-2-methyloctanoate (**1**, 4.5 g, 24 mmol), (*R*)-1-phenylethylamine (*R*-**2**, 3.5 g, 29 mmol) and Novozym 435 (720 mg) was stirred at 70 °C for 115 h when, according to GC, no starting material remained. After cooling, dichloromethane was added to the product mixture and the immobilised enzyme particles were filtered off and washed with dichloromethane. The resulting solution (15 mL) was mixed with silica gel (14 g) and the solvent evaporated off. The residue was chromatographed (silica gel 230 g), elution with 0% –100% ethyl acetate in cyclohexane followed by 1.25–5% ethanol in ethyl acetate gave first *SR*-**3** (1.5 g) followed by mixed fractions (1.2 g) and finally *RR*-**3** (2.5 g) (Total yield 83%).

(S)-2-Methyloctanoic (R)-1-phenylethylamide SR-**3**. The early fractions from above were recrystallised twice from cyclohexane to give 1 g of colourless crystals; m.p. 95–97 °C; $[\alpha]^{20}_D$ +92.0 [(c 1, $CHCl_3$) Lit.[13] $[\alpha]^{20}_D$ –91.7 for *RS*-**3**]. ^{1}H NMR (270 MHz): δ 0.88 (3H, t, J = 7.3 Hz), 1.11 (3H, d, J = 6.9 Hz), 1.2–1.4

(10H, m), 1.49 (3H, d, J = 6.9 Hz), 2.1–2.2 (1H, m), 5.1-5.2 (1H, quintet, J = 7.1 Hz), 5.6 (1H, bd, J = 6.6 Hz), 7.3 (5H, m) ppm. Essentially the same as the published 400 MHz NMR-data except that in these the peaks at δ 5.1–5.2 and 5.6 ppm were omitted[13]. ^{13}C (67.8 MHz): δ 175.6, 143.4, 128.7 (overlap), 127.3, 126.2 (overlap), 48.4, 41.7, 34.4, 31.8, 29.3, 27.5, 22.6, 21.7, 17.9, 14.1 ppm. FT-IR: 3299, 2988, 2922, 2851, 1638, 1547, 1231, 1127, 696 cm^{-1}. Mass spectrum, m/z (relative intensity): 261 (M^+ 9%), 190 (7), 177 (57), 162 (11), 120 (15), 105 (100), 86 (12), 73 (26), 57 (23), 41 (45).

*(R)-2-Methyloctanoic (R)-1-phenylethylamide RR-***3**. The late fractions from above were recrystallised twice from cyclohexane to give 2 g of colourless crystals; m.p. 53–54 °C; $[\alpha]^{20}_D$ +63.8 (c 1, $CHCl_3$). ^{1}H NMR (270 MHz): δ 0.85 (3H, t), 1.14 (3H, d, J = 6.6 Hz), 1.2–1.3 (10H, m), 1.48 (3H, d, J = 6.6 Hz), 2.1–2.2 (1H, m), 5.1-5.2 (1H, quintet, J = 6.9 Hz), 5.6 (1H, bd, J = 6.9 Hz), 7.3 (5H, m) ppm. ^{13}C (67.8 MHz, $CDCl_3$): δ 175.7, 143.5, 128.6 (overlap), 127.3, 126.2 (overlap), 48.4, 41.7, 34.5, 31.7, 29.3, 27.4, 22.6, 21.7, 17.9, 14.1 ppm. FT-IR: 3310, 2927, 2849, 1640, 1537, 1448, 1368, 1241, 1131, 758, 700 cm^{-1} Mass spectrum, m/z (relative intensity): 261 (M^+ 9%), 190 (7), 177 (65), 162 (12), 120 (16), 105 (100), 86 (13), 73 (25), 57 (23), 41 (49).

*Ethyl (S)-2-methyloctanoate. (S-***1***)* (*S*)-2-Methyloctanoic acid (949 mg, 6 mmol, 86% ee) was esterified with ethanol in cyclohexane using immobilized[4e] *Candida rugosa* lipase (water activity = 0.8) using the method described for long chain alcohols.[4c-e] After 30% conversion ethyl (*S*)-2-methyloctanoate (200 mg, 99%, $[\alpha]^{20}_D$ +21 ° (c 1, n-hexane) was obtained pure by MPLC.

*Attempted epimerisation of (S)-2-Methyloctanoic (R)-1-phenylethylamide SR-***3** *and (R)-2-Methyloctanoic (R)-1-phenylethylamide RR-***3**. The amide *SR*-**3** (392 mg, 1.5 mmol) and amide *RR*-**3** (392 mg, 1.5 mmol) from above were stirred in separate vessels, each with (*R*)-1-phenylethylamine (*R*-**2**, 303 mg, 2.5 mmol) without solvent at 500 rpm at 70 °C for 84 h. GC-analysis showed that the diastereomeric ratio of the both amides had decreased less than 2%.

Attempted base catalyzed racemization of ethyl (S)-2-methyloctanoate. Ethyl (*S*)-2-methyloctanoate (*S*-**1**, 551 mg, 3 mmol, $[\alpha]^{20}_D$ +17.8 ± 0.3 (c 1, hexane) and (*R*)-1-phenylethylamine (367 mg, 3 mmol) was mixed under argon and kept in a closed vessel at 70 °C for 80 h after which the mixture was diluted with ether and exhaustively shaken first with 0.2 M HCl (aq) followed by drying ($MgSO_4$), evaporation of the solvent and purification as above. The optical rotation of the recovered ethyl (*S*)-2-methyloctanoate product had decreased to $[\alpha]^{20}_D$ +17.3 ± 0.3 (c 1, hexane), i. e. no significant racermisation had occurred.

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