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Asymmetric Synthesis of Aliphatic 2-Hydroxy Ketones by Enzymatic Carboligation of Aldehydes

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Dedicated to Professor Maria-Regina Kula on the occasion of her 70th birthday

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Benzaldehyde lyase (BAL) and benzoylformate decarboxylase (BFD) catalyse the asymmetric ligation of aliphatic aldehydes to afford enantiomerically enriched 2-hydroxy ketones. Carboligation of linear aldehydes with both enzymes results in high levels of conversion and in enantioselectivities of up to 80 % *ee*. In cases involving branched aliphatic aldehydes, BAL enables the carboligation of 3-methylbutanal with a high level of conversion and an enantioselectivity of 89 % *ee*.

Introduction

Thiamindiphosphate-dependent (ThDP-dependent) enzymes catalyse different types of reactions in biosynthesis.[1] It is well established that the asymmetric carboligation of two aromatic aldehydes (benzoin condensation) or cross-carboligation between aromatic and aliphatic aldehydes can be performed with high regio- and stereocontrol with the aid of ThDP-dependent enzymes.[2] However, the asymmetric benzoin-type condensation of aliphatic aldehydes by either enzymatic or nonenzymatic methods has only rarely been described. In this field, Schmauder and Gröger have described the whole-cell (*Saccharomyces cerevisiae*) catalysed formation of acetoin and mixed aliphatic acyloins,[3] while Chen and Jordan have demonstrated that pyruvate decarboxylase (PDC) is an active catalyst in the formation of acetoin.[4] Later on, Bringer-Meyer and Sahm[5] and Crout et al.[6] described acyloin formation through the use of PDC from *Zymomonas mobilis*, while Berger et al. reported similar transformations with different yeast strains or with isolated PDC.[7] In most of these transformations, acetaldehyde, used either directly or through decarboxylation of pyruvate, reacts predominantly as the donor substrate.

Non-enzymatic thiazolium- and triazolium-catalysed asymmetric acyloin formation has been described (e.g., by Knight and Leeper[8] and by Enders and Breuer[9]). In both cases, however, the enantiomeric excesses of the products were below 33 %. Other non-enzymatic methods for the enantioselective synthesis of aliphatic acyloins, such as Sharpless asymmetric dihydroxylation[10] or selective oxidation of vicinal diols, have also been developed.[11]

Lipase-catalysed racemic resolution of 2-hydroxy ketones[12] has also been applied to aliphatic acyloins,[13a] together with other enzymatic approaches such as diketone reductions.[13b–13d] The use of aliphatic aldehydes as substrates in benzoin-type condensations provides access to enantiomerically pure acyloins in 100 % theoretical yield.

Since coupling with aromatic aldehydes has emerged in the last years as a powerful tool for the synthesis of 2-hydroxy ketones, we envisaged a similar transformation with aliphatic aldehydes. As enzymes we chose the ThDP-dependent benzaldehyde lyase (BAL) from *Pseudomonas fluorescens* biovar I (EC 4.1.2.38)[14] and benzoylformate decarboxylase (BFD) from *Pseudomonas putida* (EC 4.1.17)[15] which have proven to be very efficient catalysts for the production of benzoins and 2-hydroxy propiophenones.[16,17] In the case of BFD the carboligation of two molecules of aliphatic aldehydes to yield acetoin (13 % *ee*) or 1-cyclohexyl-

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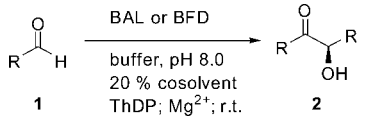
2-hydroxypropanone (61% *ee*) has been reported by some of us,[18] while the BAL-catalysed formation of 4-hydroxyoctan-3-one has been published recently.[19]

Here we describe the enzymatic carboligation of linear and branched aliphatic aldehydes to yield aliphatic 2-hydroxy ketones in high yields and with moderate to high enantiomeric excesses.

Results and Discussion

The biocatalytic carboligation of aliphatic aldehydes was performed by use of reaction conditions employed for the synthesis of acetoin by the coupling of acetaldehyde (see Scheme 1).[15]



Scheme 1. Enzymatic carboligation of linear aliphatic aldehydes.

The reaction was performed in aqueous buffer with isolated enzyme (BAL or BFD) in the presence of the cofactors ThDP and Mg^{2+}. Since cosolvents are assumed to influence the activity and selectivity, we included the investigation of DMSO and propan-2-ol as cosolvents in our studies. The results for a range of linear and branched aldehydes with both enzymes are summarized in Table 1.

The first experiment we conducted involved the carboligation of acetaldehyde (**1a**; Entries 1, 2). Both BAL and BFD gave (*R*)-acetoin (**2a**) with high conversions of more than 90%, but in both cases the enantioselectivities were low (≤40% *ee*), which is in agreement with the literature.[15] Enantiomeric excesses were determined by chiral GC, and showed that BAL and BFD produce the same favoured enantiomer as PDC from *Saccharomyces cerevisiae*, allowing the assignment of the absolute configuration of the acetoin (**2a**).[6] The tested cosolvents do not significantly influence the enantioselectivity of BAL and BFD in this reaction.

With propanal (**1b**), the desired product 4-hydroxyhexan-3-one (**2b**) was formed with levels of conversion of more than 90% (Entries 3–6) with both enzymes BAL and BFD. BAL gave the (*S*)-acyloin **2b** as the major enantiomer, the absolute configuration being assigned from the positive Cotton effect at 278 nm in its circular dichroism (CD) spectrum. It has been shown for several aliphatic hydroxy ketones that such positive and negative CD bands correspond to the (*S*) and (*R*) enantiomers, respectively.[20] While the (*S*) enantiomer was formed preferentially with BAL (≤60% *ee*), BFD favoured the opposite (*R*) enantiomer with an *ee* of 63% (shown by chiral GC). The presence of cosolvents resulted in reduced enantioselectivity when BAL was used, showing that this transformation is influenced considerably by external parameters.

n-Butanal (**1c**) was the most suitable substrate in terms of enantioselectivity in this series of linear aliphatic aldehydes (Entries 7–10). Without cosolvent the enantioselectivity in

Table 1. Carboligation of linear aldehydes catalysed by BAL and BFD. Enantiomeric excesses were determined by GC separation of compounds **2** on chiral phases.

Entry	Aldehyde (**1**), R	Product	Enzyme (20% cosolvent)	Conversion [%]	*ee* of **2** [%]
1	acetaldehyde (**1a**), CH_3	**2a**	BAL	> 90	40 (*R*)
2		**2a**	BFD	> 90	34 (*R*)
3	propanal (**1b**), CH_3CH_2	**2b**	BAL	> 90	60 (*S*)
4		**2b**	BAL (DMSO)	n.d.[a]	53 (*S*)
5		**2b**	BAL (propan-2-ol)	> 90	20 (*S*)
6		**2b**	BFD	> 90	63 (*R*)
7	*n*-butanal (**1c**), $CH_3(CH_2)_2$	**2c**	BAL	> 90	50 (*R*)
8		**2c**	BAL (DMSO)	> 90	55 (*R*)
9		**2c**	BAL (propan-2-ol)	> 90	80 (*R*)
10		**2c**	BFD	> 90	80 (*R*)
11	*n*-pentanal (**1d**), $CH_3(CH_2)_3$	**2d**	BAL	> 90	30 (*R*)
12		**2d**	BAL (propan-2-ol)	> 90	60 (*R*)
13		**2d**	BFD	> 90	65 (*R*)
14	isobutyraldehyde (**1e**), $(CH_3)_2CH$	**2e**	BAL	< 1	–
15		**2e**	BFD	< 1	–
16	pivalaldehyde (**1f**), $(CH_3)_3C$	**2f**	BAL	< 1	–
17		**2f**	BFD	< 1	–
18	isovaleraldehyde (**1g**), $(CH_3)_2CHCH_2$	**2g**	BAL	> 90	89 (*R*)
19		**2g**	BAL (DMSO)	> 90	86 (*R*)
20		**2g**	BFD	60	85 (*R*)
21		**2g**	BFD (DMSO)	n.d.[a]	86 (*R*)
22	*tert*-butylacetaldehyde (**1h**), $(CH_3)_3CCH_2$	**2h**	BAL	< 1	–
23		**2h**	BFD	< 1	–

[a] n.d.: not determined.

the BAL-catalysed reaction forming (*R*)-5-hydroxyoctan-4-one (**2c**) was 50% *ee*, while addition of DMSO slightly enhanced the enantioselectivity to 55% *ee*. Remarkably, on addition of propan-2-ol the enantioselectivity was raised to 80% *ee*, indicating that reaction engineering can be used successfully for enhancing enantioselectivity in such transformations. Both BAL and BFD produced the same enantiomer of **2c** (chiral GC), as was also the case for *n*-pentanal (**1d**) as a substrate. Absolute (*R*) configurations were assigned to **2c** and **2d** by circular dichroism (negative band at 278 nm).

In all BFD-catalysed reactions there was no significant influence of the cosolvent (data not shown) on the selectivity of the BFD, while the positive influence of propan-2-ol on BAL was also confirmed when *n*-pentanal (**1d**) was used as a substrate (Entries 11–13). The enantioselectivity was enhanced from 30% *ee* (no cosolvent or DMSO) to 60% *ee* with a conversion of more than 90% for (*R*)-6-hydroxydecan-5-one (**2d**). BFD furnished enantioselectivities of about 65% *ee* with high levels of conversion of >90%.

We had thus been able to show that linear aliphatic 2-hydroxy ketones are accessible by enzymatic carboligation. Subsequently, we studied the applicability of BAL- and BFD-catalysed carboligation with branched aliphatic aldehydes. The existence of additional methyl groups close to the aldehyde group is regarded as representing higher steric hindrance for the enzymes, which should reduce the reaction rate, but might result in better enantioselectivity. We varied the degree of steric hindrance by using isopropyl or *tert*-butyl groups, either adjacent to the carbonyl group or separated from the carbonyl group by a methylene spacer.

No 2-hydroxy ketone could be observed on attempted BFD-catalysed carboligation with the branched aliphatic aldehydes isobutyraldehyde (**1e**), pivalaldehyde (**1f**) and *tert*-butylacetaldehyde (**1h**). Wild-type BFD seems to be very sensitive to the steric demand of the aldehyde, as is also known from aromatic aldehydes.[21] Only in the case of isovaleraldehyde (**1g**; Entries 20, 21) was product formation observed, resulting in the same enantiomer as obtained with use of BAL. Thus, with isovaleraldehyde (**1g**) as a substrate, the formation of (*R*)-5-hydroxy-2,7-dimethyloctan-4-one (**2g**) with an enantioselectivity of up to 89% *ee* was observed. The absolute configuration of **2g** was established as (*R*) from the CD spectrum (negative band at 278 nm) of the crude product. Additionally, the *ee* and absolute configuration of **2g** were confirmed by Mosher ester methodology (NMR spectroscopy and separation of diastereomers by GCMS). The high enantioselectivity might be caused by the sterically demanding isopropyl group. The other tested branched aliphatic substrates with isobutyraldehyde (**1e**), pivalaldehyde (**1f**) and *tert*-butylacetaldehyde (**1h**) did not react in the presence of BAL. Here the steric demand is even higher than in **1g** as there is no methylene spacer present, or in one case there is even a *tert*-butyl group. Monitoring the course of reactions of BAL and BFD in detail demonstrated the differences in activities of the two enzymes. Consumption of **1g** (63 mM) in the BAL-catalysed reaction was complete after 5.5 h (Figure 1), whereas the BFD-catalysed reaction was significantly slower. The calculated space-time yields with 0.3 mg mL^{-1} enzyme are 0.26 g L^{-1} d^{-1} (BFD) and 13.15 g L^{-1} d^{-1} (BAL).

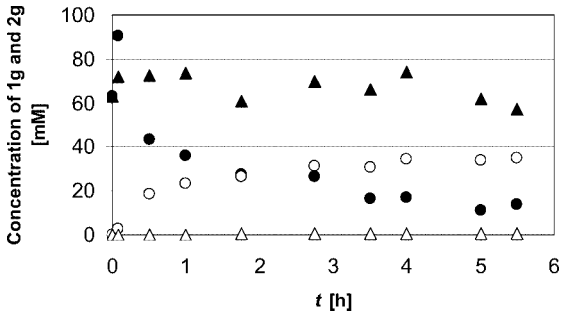


Figure 1. Time course for the production of 5-hydroxy-2,7-dimethyloctan-4-one (**2g**, open symbols) from isovaleraldehyde (**1g**, closed symbols) catalysed by purified BFD (triangles) and by BAL (circles).

In order to confirm the racemic resolution of the enzymatically produced 2-hydroxy ketones (chiral GC), we also synthesized compounds **2e** and **2g** by non-enzymatic acyloin condensation as described by Fleming et al.[22] Analytical data (NMR, GCMS) of the enzymatic products and racemic material are identical in all respects. In the case of **2b**, the NMR spectroscopic data (enzymatic products) are not in accordance with literature values;[23] the correctness of the structure of **2b** was confirmed by COSY and HSQC experiments.

Conclusions

In terms of the recently introduced donor–acceptor concept of Müller et al.,[24] it has been shown that aliphatic aldehydes are both suitable donors and acceptors as long as the steric hindrance is not too high. These examples also show that the substrate spectra of these C–C-ligating ThDP-dependent enzymes are broader than described in the literature so far, so further interesting substrates could be expected to be applied in this attractive carboligating reaction. By a combination of reaction engineering and random and/or site-directed mutagenesis enantioselectivity might even be increased further.[25]

Experimental Section

Chemicals and Biocatalysts: All reagents were commercially available from Sigma–Aldrich and were used without further purification. Benzaldehyde lyase from *Pseudomonas fluorescens* Biovar I and benzoylformate decarboxylase from *Pseudomonas putida* were produced in *E. coli* cells.[26] The preparation of BAL and BFD was carried out by sonication (ca. 3–5 min) of the corresponding *E. coli* cells (2 g), which were dissolved in phosphate buffer (50 mmol L^{-1}, 20 mL) together with $MgSO_4$ (2.5 mmol L^{-1}) and ThDP (0.3 mmol L^{-1}). The disrupted cells were centrifuged at 4000 rpm at 4 °C for 20 min, the pellet was removed, and the supernatant was applied for catalysis. The biocatalytic characterization was carried out as described in the literature.[19,27] The volumetric activity of this solution was determined to 100 U mL^{-1}. One unit (U) of BAL

activity is defined as the amount of enzyme that catalyses the cleavage of 1 μmol benzoin (1.5 mmol L^{-1}) into benzaldehyde in potassium phosphate buffer (20 mmol L^{-1}, pH = 7.0) containing $MgSO_4$ (2.5 mmol L^{-1}), ThDP (0.15 mmol L^{-1}) and PEG 400 (15 vol-%) at 30 °C. One unit of BFD is defined as the amount of enzyme that catalyses the cleavage of 1 μmol of benzoyl formate (30 mmol L^{-1}) into benzaldehyde and CO_2 in potassium phosphate buffer (50 mmol L^{-1}, pH = 6.5) containing $MgSO_4$ (2.5 mmol L^{-1}) and ThDP (0.1 mmol L^{-1}) per minute at 30 °C.

Typical Carboligation Procedure: Variable amounts of BAL or BFD (500 U) were added to a mixture (50 mL) of potassium phosphate buffer (50 mmol L^{-1}, with 2.5 mmol L^{-1} $MgSO_4$ and 0.3 mmol L^{-1} ThDP, pH = 8.0) both in the absence and in the presence of cosolvent (DMSO, propan-2-ol, 20 vol-%) and the substrate (50 mmol L^{-1}) The reaction mixture was stirred at room temperature for 24 h. For the workup, the reaction medium was extracted with dichloromethane (4 × 50 mL) and the organic phase was washed several times with water. After drying of the organic layer with $MgSO_4$, the solvent was removed under reduced pressure. The products were obtained practically pure without need of further purification. In the case of 5-hydroxy-2,7-dimethyloctan-4-one (**2g**), the course of the reaction was monitored in order to determine space-time yields for BAL and BFD. The reaction was performed with isovaleraldehyde (**1g**, 63 mmol L^{-1}) in potassium phosphate buffer (50 mmol L^{-1}) with $MgSO_4$ (2.5 mmol L^{-1}) and ThDP (0.1 mmol L^{-1} ThDP) at pH = 8.0 and 30 °C, together with DMSO (20%), and purified BAL and BFD (0.3 mg each per mL reaction volume). Samples taken every 0.5–1 h were analysed by GC.

Analytical Performance: The levels of conversion and the enantiomeric excesses were monitored by chiral GC, with a Chirasil-DEX CB (Varian, 25 m × 0.32 mm), or an FS Lipodex D (50 m × 0.25 mm), with an FID detector. NMR spectra were recorded with a Bruker DPX 400. Chemical shifts are reported in ppm relative to $CHCl_3$ (^{1}H NMR: δ = 7.27 ppm) or $CDCl_3$ (^{13}C NMR: δ = 77.0 ppm) as internal standards. GCMS spectra were determined with an HP 6890 series GC system fitted with an HP 5973 mass-selective detector [Hewlett Packard; column HP-5MS, 30 m × 250 μm; T_{GC}(injector) = 250 °C, T_{MS}(ion source) = 200 °C, time program (oven): $T_{0\ min}$ = 60 °C, $T_{3\ min}$ = 60 °C, $T_{14\ min}$ = 280 °C (heating rate 20 °C min^{-1}), T_{19min} = 280 °C]. CD spectra were recorded with a JASCO J-810 Spectropolarimeter in acetonitrile as solvent.

3-Hydroxybutan-2-one (2a): Colourless liquid. Conversion: > 90%. GC (Lipodex D, $T_{injector}$ = 190 °C, T_{column} 70 °C): $t_R(R)$ = 13.4 min, $t_R(S)$ = 18.3 min [*ee* 40% (*R*) BAL; 34% (*R*) BFD]. ^{1}H NMR: δ = 1.40 (d, *J* = 7.2 Hz, 3 H), 2.21 (s, 3 H), 3.52 (s, 1 H, OH), 4.25 (q, *J* = 7.2 Hz, 1 H) ppm.

4-Hydroxyhexan-3-one (2b): Colourless liquid. Conversion: > 90%. 54% isolated yield (BAL, without cosolvent). GC (Lipodex D, $T_{injector}$ = 190 °C, T_{column} 60 °C): $t_R(R)$ = 30.6 min, $t_R(S)$ = 31.8 min [*ee* 20–60% (*S*) BAL; 63% (*R*) BFD]. ^{1}H NMR (400 MHz, CD_2Cl_2): δ = 0.95 (t, *J* = 7.3 Hz, 3 H), 1.12 (t, *J* = 7.3 Hz, 3 H), 1.61 (m, *J* = 14.4, 7.3, 6.9 Hz, 1 H), 1.91 (dqd, *J* = 14.4, 7.3, 4.0 Hz, 1 H), 2.47 (dq, *J* = 17.9, 7.3 Hz, 1 H), 2.55 (dq, *J* = 17.9, 7.3 Hz, 1 H), 3.41 (br., 1 H), 4.17 (dd, *J* = 6.9, 4.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, CD_2Cl_2): δ = 7.2, 8.6 (CH_3), 26.8, 30.9 (CH_2), 76.9 (CHOH), 212.8 (C=O) ppm. GCMS t_R = 4.13 min. MS (EI, 70 eV): *m*/*z* (%) = 116 (3) $[M]^+$, 87 (2), 73 (2), 59 (100).

5-Hydroxyoctan-4-one (2c): Yellow liquid. Conversion: > 90%. 71% isolated yield (BAL, DMSO as cosolvent). GC (Lipodex D, $T_{injector}$ = 190 °C, T_{column} 70 °C): $t_R(R)$ = 46.2 min, $t_R(S)$ = 49.5 min [*ee* 50–80% (*R*) BAL; 80% (*R*) BFD]. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.93 (t, *J* = 7.4 Hz, 3 H), 0.94 (t, *J* = 7.2 Hz, 3 H), 1.34 (m, 3 H), 1.65 ("sext", *J* = 7.3 Hz, 2 H), 1.72–1.82 (m, 1 H), 2.36–2.51 (m, 2 H), 4.16 (t, *J* = 3.5 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.7, 13.8 (CH_3), 17.0, 18.1, 35.7, 39.7 (CH_2), 76.2 (CHOH), 212.2 (C=O) ppm. GCMS: t_R = 6.27 min. MS (EI, 70 eV): *m*/*z* (%) = 144 (1) $[M]^+$, 102 (12), 73 (66), 55 (100).

6-Hydroxydecan-5-one (2d): Colourless liquid. Conversion: > 90%. 91% isolated yield (BAL, DMSO as cosolvent). GC (Lipodex D, $T_{injector}$ = 190 °C, T_{column} 70 °C): $t_R(R)$ = 99.5 min, $t_R(S)$ = 99.7 min [*ee* 30–60% (*R*) BAL; 65% (*R*) BFD]. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.928 (t, *J* = 7.1 Hz, 3 H), 0.933 (t, *J* = 7.3 Hz, 3 H), 1.28–1.67 (m, 9 H), 1.8–1.9 (m, 1 H), 2.4–2.55 (m, 2 H), 4.18 (dd, *J* = 7.4, 3.7 Hz, 1 H, C*H*OH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.7, 13.8 (CH_3), 22.3, 22.5, 25.7, 26.9, 33.4, 37.5 (CH_2), 76.3 (CHOH), 212.5 (C=O) ppm. GCMS: t_R = 7.99 min. MS (EI, 70 eV): *m*/*z* (%) = 172 (0.1) $[M]^+$, 116 (5), 87 (35), 69 (100), 57 (31).

2,5-Dimethyl-4-hydroxyhexan-3-one (2e) (Nonenzymatic Synthesis): Colourless liquid. ^{1}H NMR (400 MHz, CDl_3): δ = 0.70 (d, *J* = 6.7 Hz, 3 H), 1.11 (d, *J* = 6.8 Hz, 3 H), 1.12 (d, *J* = 6.8 Hz, 3 H), 1.13 (d, *J* = 6.8 Hz, 3 H), 2.18 ("sept", *J* = 6.8 Hz, 1 H), 2.80 ("dsept", *J* = 6.7, 2.5 Hz, 1 H), 3.47 (br., 1 H), 4.20 (br., 1 H) ppm. ^{13}C NMR (100 MHz, CDl_3): δ = 14.9, 17.3, 19.7, 20.2 (CH_3), 31.1, 36.1 (CH), 79.1 (*C*HOH), 216.2 (C=O) ppm. GCMS: t_R = 5.7 min. MS (EI, 70 eV): *m*/*z* (%) = 144 (3) $[M]^+$, 102 (20), 87 (25), 73 (100), 55 (60).

2,7-Dimethyl-5-hydroxyoctan-4-one (2g): Yellow liquid. Conversion: > 90%. 65% isolated yield (BAL, *tert*-butyl methyl ether as cosolvent[28]). GC (Lipodex D, $T_{injector}$ = 190 °C, T_{column} 70 °C): $t_R(R)$ = 82.0 min, $t_R(S)$ = 86.1 min [*ee* 86–89% (*R*) BAL; 85% (*R*) BFD]. ^{1}H NMR (400 MHz, CDl_3): δ = 0.94 (d, *J* = 6.7 Hz, 3 H), 0.95 (d, *J* = 6.7 Hz, 3 H), 0.97 (d, *J* = 6.6 Hz, 3 H), 1.0 (d, *J* = 6.6 Hz, 3 H), 1.39 (ddd, *J* = 13.7, 10.3, 4.2 Hz, 1 H), 1.51 (ddd, *J* = 13.7, 9.6, 3.0 Hz, 1 H), 1.96 (m, 1 H), 2.23 (m, 1 H), 2.36 (m, 2 H), 3.51 (br., 1 H), 4.15 (dd, *J* = 10.3, 3.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, CDl_3): δ = 21.2, 22.5, 22.6, 23.6 (CH_3), 24.6, 24.8 (CH), 42.6, 46.7 (CH_2), 75.3 (CHOH), 212.5 (C=O) ppm. GCMS: t_R = 7.51 min. MS (EI, 70 eV): *m*/*z* (%) = 170 (10) $[M - 2H]^+$, 116 (30), 85 (70), 69 (100), 57 (70).

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