Efficient Enzymatic Amine Resolution at High Substrate Input Using Diethyl Malonate as an Acyl Donor of Low Hazard Potential

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Z. Naturforsch. **2012**, *67b*, 1123 – 1126
DOI: 10.5560/ZNB.2012-0127
Received May 17, 2012

Dedicated to Professor Dr. Dr. Heribert Offermanns on the occasion of his 75th birthday

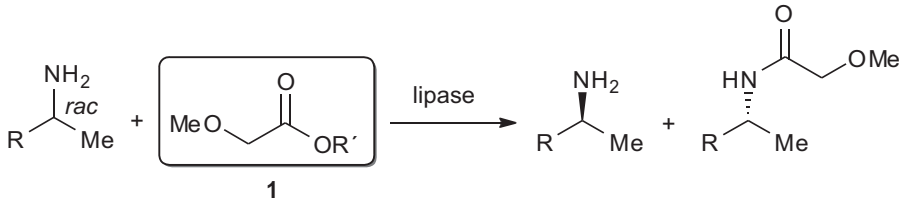
Diethyl malonate turned out to be both a "green" and highly efficient acyl donor in the lipase-catalyzed resolution of amines, thus representing an attractive alternative to currently applied acyl donors. By means of this acyl donor a highly efficient enzymatic process for the resolution of amines, running at high substrate input of up to 200 g/L in an organic solvent classified as "usable" according to the Pfizer Solvent Selection Guide, is presented.

Key words: Amines, Amides, Enantioselective Synthesis, Biocatalysis, Enzymatic Resolution

Introduction

A large percentage of today's drugs contain chiral amine frameworks, and their synthesis often requires the use of enantiomerically pure amines as building blocks [1]. In order to provide enantiomerically pure amines as drug intermediates in an economically attractive fashion, the development of efficient methodologies for their enantioselective synthesis is a key task for organic chemists. Among existing synthetic approaches, comprising classic chemical resolution *via* diastereomeric salts as well as asymmetric chemo- and biocatalysis, lipase-catalyzed resolution of amines developed at BASF certainly ranks among the top processes for chiral amine synthesis in terms of both synthetic efficiency and technical attractiveness [2 – 4]. This is underlined by the impressive production volume of this technology at BASF being in the multi-thousand tons range [4]. Key feature of this outstanding BASF process technology is the use of an α-heteroatom-substituted acetate such as a methoxyacetate (**1**) as an acyl donor for the enantioselective acylation of (racemic) amines as substrates (Scheme 1) [2 – 7].

What makes this process additionally attractive from the perspective of sustainability is the opportunity to recycle the unwanted enantiomer through racemization. To further improve the sustainability of the resolution process, the search for alternative acyl donors showing a low hazard potential (which ideally means being listed as "no hazardous product") and at the same time excellent reactivity is desirable. For comparison, ethyl methoxyacetate is classified as flammable, and the corresponding methoxyacetic acid is listed as a toxic compound [8, 9]. On the other hand, ethyl acetate [10] (and related non-activated aliphatic esters) show a tremendous decrease of reactivity compared to methoxyacetate [2 – 6]. Thus, identifying acyl donors, which are highly reactive acylating agents and attractive from both a hazard potential and economical perspective, still represents a challenging task. In our attempts addressing this issue we became interested in



Scheme 1. Industrial process technology at BASF for amine resolution.

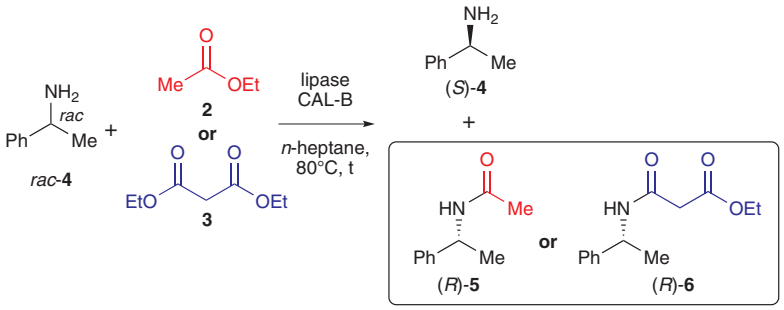
an evaluation of diethyl malonate as an acyl donor due to the following features which appear to make this compound promising: (i) cheap and readily available chemical produced on bulk scale, (ii) (relatively) low hazard potential (classification as no dangerous material according to the MSDS of Merck Chemicals; however, in the MSDS of Sigma-Aldrich this compound is classified as irritating to eyes) [11], and (iii) an activating moiety as a substituent for the carbon atom in α-position, namely a carboxylate ester moiety, which could result in an increased reactivity.

So far malonates are rarely known as acyl donors in enzymatic amine resolution, and only dimethyl malonate was used in a few earlier experimental studies with bifunctionalized compounds as substrates such as diamines and amino alcohols [12 – 14]. In the following, we report our results demonstrating that diethyl malonate is a "green" and highly efficient acyl donor in the lipase-catalyzed resolution of amines, thus representing an attractive alternative to currently applied acyl donors. In addition, based on this sustainable donor we report a highly efficient process running at high substrate concentration of 1 M in an organic solvent classified as usable according to the Pfizer Solvent Selection Guide [15].

Results and Discussion

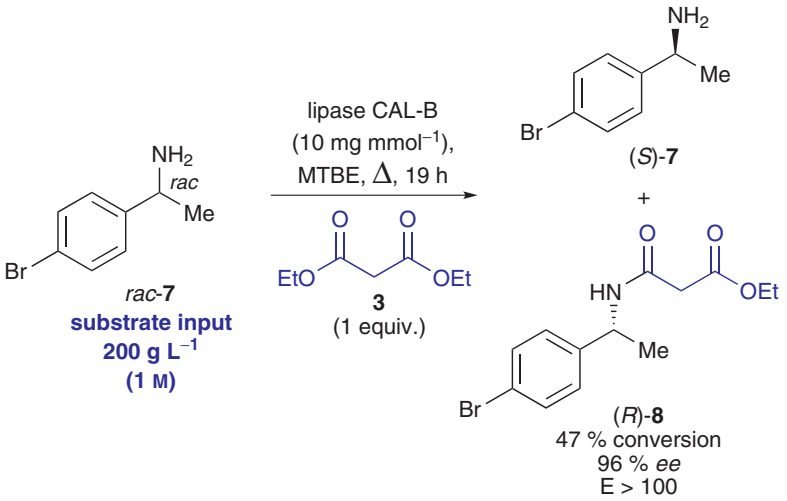
At first we chose the lipase-catalyzed acylation of racemic (1-phenylethyl)amine (*rac*-**4**) as a model reaction for our work. Initial reaction conditions were chosen according to a recent process by Gastaldi and Gil *et al.* for enzymatic resolution of various amines [6], which proceeds efficiently with even non-activated

Table 1. Resolution of (1-phenylethyl)amine (*rac*-**4**) with ethyl acetate (**2**) and diethyl malonate (**3**) as acyl donors.



Entry[a]	Donor	*c* (M)[b]	CAL-B loading[c]	t (h)	conv. (%)[d]	*ee* **5** / **6** (%)[e]	E[f]
1	**2**	0.1	200	4.5	50	98 (*R*)	>100
2	**2**	0.1	200	2	32	98 (*R*)	>100
3	**2**	0.1	50	4.5	33	99 (*R*)	>100
4	**3**	0.1	200	4.5	50	97 (*R*)	>100
5	**3**	0.1	200	2	47	96 (*R*)	>100
6	**3**	0.1	40	4.5	45	96 (*R*)	>100
7	**3**	1.0	10	19	46	97 (*R*)	>100

[a] General biotransformation protocol: After dissolving *rac*-(1-phenylethyl)amine (*rac*-**4**, 0.1 – 1.0 M) in *n*-heptane, acyl donor **2** or **3** (1 equiv.) and lipase CAL-B (Novozym 435; 10 – 200 mg mmol^{-1}) are added, and the mixture is stirred at 80 °C for a reaction time of 2 – 19 h. Then, the mixture is cooled, and the enzyme immobilisate is filtered and washed with methylene chloride. After removing the volatile components *in vacuo* the conversion is determined from the resulting crude product. After work-up (consisting of acidification with HCl and subsequent extraction with methylene chloride for separation of the amide from the amine) the enantiomeric excess of amide (*R*)-**5** or (*R*)-**6** is determined; [b] concentration of substrate *rac*-**4**; [c] the catalyst loading is given in mg of lipase CAL-B per mmol of substrate *rac*-**4**; [d] the conversion is determined *via* ^{1}H NMR spectroscopy; [e] the enantiomeric excess is determined *via* chiral HPLC (column: Daicel Chiralpak AD-H; eluent: isohexane : isopropanol (95:5 (v/v)); 210 nm; flow: 0.8 mL min^{-1}); [f] E-value determined from conversion and *ee*-value of amide (*R*)-**5** or (*R*)-**6**.



Scheme 2. Enzymatic amine resolution with diethyl malonate in MTBE.

acyl donors such as ethyl acetate (**2**) in heptane at elevated temperature (80 °C). When we used – in analogy to those reaction conditions – 12 g L^{-1} (100 mM) of racemic (1-phenylethyl)amine (*rac*-**4**) in combination with ethyl acetate (**2**) as an acyl donor in *n*-heptane at 80 °C, a conversion of 50 % and 98 % *ee* of the formed amide (*R*)-**5** was obtained after 4.5 h (Table 1, entry 1). At a decreased reaction time of 2 h, however, the biotransformation was incomplete with 32 % (entry 2). In addition, lowering the catalyst loading by a factor of 4 led to a decrease of the conversion to 33 % after 4.5 h, indicating that ethyl acetate (**2**) is a less efficient acyl donor (entry 3).

Taking these biotransformations as a "benchmark" we next studied the acylation with diethyl malonate (**3**) as an acyl donor, and we were pleased to find a significantly increased reactivity while maintaining excellent enantioselectivity (Table 1, entries 4 – 6). After having demonstrated as a proof of concept the suitability of diethyl malonate (**3**) in an initial "model reaction" (entry 4), in further experiments at shorter reaction time or lower catalyst loading significantly improved reaction rates compared to the analogous reactions with ethyl acetate (**2**) were found. After a reaction time of 2 h high conversion (47 %) and enantiomeric excess (96 % *ee*) were obtained (entry 5), compared to only 32 % when using ethyl acetate (entry 2). Furthermore, and in contrast to ethyl acetate, a lower catalyst loading led to still a high conversion (entries 3, 6). After obtaining these encouraging results this resolution process has been subject to optimization with respect to an increased volumetric productivity at decreased catalyst demand. After a reaction time of 19 h even at an elevated substrate loading of 121 g L^{-1} of *rac*-**4** the desired amide (*R*)-**6** was formed with 46 % conversion and 97 % *ee*, corresponding to a high enantioselectivity of the process with an E-value [16] of > 100 (entry 7). Thus, compared to ethyl acetate (**2**), a dramatic improvement of reactivity has been observed.

Next, this type of enzymatic resolution process was further studied, varying both substrate and solvent component. As a substrate racemic (1-(4'-bromophenyl)ethyl)amine (*rac*-**7**) was used, and as a solvent we evaluated MTBE, which appeared to be interesting in terms of both amine substrate and amide product solubility. When running the resolution with diethyl malonate (**3**) as acyl donor under reflux conditions (55 °C), with a lower catalyst loading of only 10 mg $mmol^{-1}$ and at a high substrate input of 200 g L^{-1} (1 M), the desired amide (*R*)-**8** was formed with high conversion of 47 % and 96 % *ee* (Scheme 2). This corresponds to a high enantioselectivity with an E-value of > 100, and taken together with the high reaction rate it demonstrates that diethyl malonate is an excellent acyl donor suitable for use also in a solvent acceptable from the perspective of sustainability.

Conclusion

In summary, diethyl malonate (**3**) has been demonstrated to be both a "green" and highly efficient acyl

donor in the lipase-catalyzed resolution of amines, thus representing an attractive sustainable alternative to currently applied acyl donors. By means of this bulk chemical of low hazard potential as an acyl donor a highly efficient process for enzymatic amine resolution running at high substrate input of up to 200 g L^{-1} in an organic solvent classified as "usable" according to the Pfizer Solvent Selection Guide has been developed. Future work will also address the expansion of this protocol to the enantioselective synthesis of other amines in highly enantiomerically enriched form as well as further process development.

[1] Overview: A. Kleemann, J. Engel, B. Kutscher, D. Reichert, *Pharmaceutical Substances*, 5th ed., Thieme, Stuttgart, **2009**.

[2] F. Balkenhohl, K. Ditrich, B. Hauer, W. Ladner, *J. Prakt. Chem.* **1997**, *339*, 381 – 384.

[3] K. Ditrich, *Synthesis* **2008**, 2283 – 2287.

[4] Review: M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßeler, R. Stürmer, T. Zelinski, *Angew. Chem. Int. Ed.* **2004**, *43*, 788 – 824.

[5] Work of other authors with CALB and ethyl methoxyacetate as an acyl donor in MTBE: I. I. Gill, J. Das, R. N. Patel, *Tetrahedron: Asymmetry* **2007**, *18*, 1330 – 1337.

[6] Work of other authors with CALB and ethyl methoxyacetate as well as other acyl donors at elevated temperature: M. Nechab, N. Azzi, N. Vanthuyne, M. Bertrand, S. Gastaldi, G. Gil, *J. Org. Chem.* **2007**, *72*, 6918 – 6923.

[7] Overview of the different enzymatic approaches towards chiral amines, see: S. Buchholz, H. Gröger in *Biocatalysis in the Pharmaceutical and Biotechnology Industries* (Ed.: R. N. Patel), CRC Press, New York, **2006**, chapter 34, pp. 829 – 847.

[8] The risk phrases and hazard symbols of ethyl methoxyacetate are as follows: R10 and none hazard symbol according to the MSDS data sheet of Sigma-Aldrich, Germany (as stated on March 8, 2012).

[9] The risk phrases and hazard symbols of methoxyacetic acid are as follows: R60 – 61-22 – 34 and symbol "T" according to the MSDS data sheets of Sigma-Aldrich, Germany, and Merck Chemicals, Germany (as stated on March 8, 2012).

[10] The risk phrases and hazard symbols of ethyl acetate are as follows: R11 – 36-66 – 67 and hazard symbols "F" and "Xi" according to the MSDS data sheet of Sigma-Aldrich, Germany, and R11 – 36 and hazard symbols "F" and "Xi" according to the MSDS data sheet of Merck Chemicals, Germany (as stated on March 8, 2012).

[11] The risk phrases and hazard symbols of diethyl malonate are as follows: R36 and hazard symbol "Xi" according to the MSDS data sheet of Sigma-Aldrich, Germany (as stated on March 8, 2012), and no risk phrase, none hazard symbol and listed as "no hazardous product" (as specified in the Directive 67/548/EEC) according to the MSDS data sheet of Merck Chemicals, Germany (as stated on March 8, 2012).

[12] V. Gotor-Fernández, R. Brieva, V. Gotor, *J. Mol. Catal. B: Enzym.* **2006**, *40*, 111 – 120.

[13] A. Luna, I. Alfonso, V. Gotor, *Org. Lett.* **2002**, *4*, 3627 – 3629.

[14] I. Alfonso, C. Astorga, F. Rebolledo, V. Gotor, *Chem. Commun.* **1996**, 2471 – 2472.

[15] Pfizer Solvent Selection Guide: K. Alfonsi, J. Colberg, P. J. Dunn, T. Fevig, S. Jennings, T. A. Johnson, H. P. Kleine, C. Knight, M. A. Nagy, D. A. Perry, M. Stefaniak, *Green Chem.* **2008**, *10*, 31 – 36.

[16] The E-value describes the preference (selectivity) of a resolution process for one enantiomer over the other, thus indicating the enantioselectivity of this process; for details and formula for its calculation, see: K. Faber, *Biotransformation in Organic Chemistry*, 5th ed., Springer, Heidelberg, **2004**.