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Chemoenzymatic route to Tyrphostins involving lipase-catalyzed kinetic resolution of 1-phenylethanamine with alkyl cyanoacetates as novel acylating agents

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ABSTRACT

Ethyl and isopropyl cyanoacetates were tested as acylating agents in the kinetic resolution of racemic 1-phenylethanamine *rac*-**1** catalyzed by lipase B from *Candida antarctica*. The best conversion combined with high enantioselectivity was achieved with ethyl cyanoacetate **2a** as the acylating agent and immobilized lipase B from *Candida antarctica* (*Ca*LB N435) as the biocatalyst. Enantiomers of the amides (*R*)-**3** and (*S*)-**3** were obtained with high enantiopurity (ee >98%) by lipase-catalyzed kinetic resolution and by chemical conversion of the residual (*S*)-**1**, respectively. The amides were reacted with various aromatic aldehydes **4a–c,e** in Knoevenagel condensation to yield Tyrphostins *rac*-**5a–c,e**, (*R*)-**5a–c,e** and (*S*)-**5a–c,e**, which were tested as protein tyrosine kinase inhibitors on human cancer cell lines HCT 116, A549, PC9, PC9ER, Jurkat, and MV4-11. Although some of the novel Tyrphostins exhibited weak biological activities (EC_{50} ~6–60 μM), none of them proved to have a significant effect on the growth of the investigated cell lines.

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1. Introduction

Chiral amines and their derivatives, especially in enantiopure form are important building blocks of several drugs.[1–3] Environmentally friendly biocatalysts, notably hydrolases, mostly lipases, are frequently used to produce enantiopure amides.[4] Since lipases are relatively thermostable and are often highly selective in their reactions with a wide range of substrates, they are broadly used in enantio- and regioselective reactions such as hydrolysis, esterification, transesterification, aminolysis, and ammoniolysis.[4] One of the most often used lipases in the industry is lipase B from *Candida antarctica* (*Ca*LB) in its immobilized form. Most of the kinetic resolutions of racemic amines with *Ca*LB were run in organic solvents[3–6] or even under neat, solvent free conditions.[7] Recently, dynamic-kinetic resolution methods using *Ca*LB have been reported,[8] to give much better conversion than simple kinetic resolutions. *Ca*LB biocatalyst is suitable for the *N*-acylation of a wide range of aromatic and aliphatic amines even under continuous-flow conditions.[6]

Several acylating agents can be used for lipase-catalyzed stereoselective biotransformations. For example esters, such as ethyl acetate,[6] isopropyl acetate,[7] isopropyl butanoate, isopropyl methoxyacetate[7,9] or acids, such as myristic acid[10] may be useful. The kinetic resolution of 1-phenylethanamine and other amines with lipases and ethyl methoxyacetate[11,12] has been applied on an industrial scale by BASF since 1993. This industrial biotransformation on a multiton scale was enabled by the use of ethyl methoxyacetate as an activated acylating agent, which enhanced the rate of resolution more than 100 times compared to butyl acetate due to the presence of an electron withdrawing group at the α-position.[11] The excellent reactivity of the activated methoxyacetates with *Ca*LB could be rationalized by the electronegativity of the methoxy oxygen of the acyl donor and the existence of a weak hydrogen bond to the β-oxygen atom of the methoxyacetate moiety in the so-called acyl-enzyme intermediate.[13] In enzymatic *N*-acylations with activated acylating agents, chemical (non-enzymatic) *N*-acylation may pose problems, which require careful optimization of the process. The pK_a of an acid[14] correlates with the acylation ability of its ester. In the case of an ester from a strong acid (pK_a <2.0), chemical acylation is fast. Consequently, poor enantioselectivity can be anticipated under enzymatic

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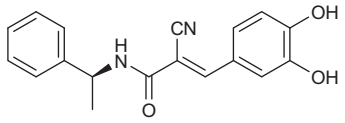
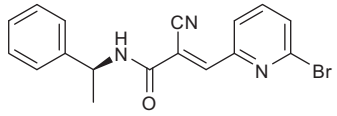
conditions due the concurrent non-selective chemical reaction. When the pK_a >4 acylation is slower under enzymatic conditions as well. The acidity of cyanoacetic acid (pK_a = 2.47,[14] or pK_a = 3.8, in CH_3CN/H_2O 1:1[15]) allowed smooth chemical *N*-acylation with its methyl ester.[16] Although this acidity presumably allows the optimization of the enzyme-catalyzed reaction with its esters, to the best of our knowledge, no enzymatic *N*-acylation with cyanoacetate esters has been studied so far.

Cyanoacetate esters are synthetically important compounds due to their role in a variety of chemical transformations such as *N*-acylation with amines[17] and Knoevenagel condensation with aldehydes.[18] Hydrolysis of the nitrile group offers possibilities for further functionalization. The Knoevenagel condensation is an important tool for constructing an α,β-unsaturated structural unit from a carbonyl compound and an active methylene compound; several new catalysts have been introduced and many reaction conditions have been described.[19] From the enantiopure amide of (*S*)-1-phenylethanamine and cyanoacetic acid, via Knoevenagel condensation Tyrphostins such as Tyrphostin B50 or WP 1066[20,21] can be obtained (Fig. 1).



Tyrphostin B50



WP-1066

Figure 1. Tyrphostins with an α-amide group as protein tyrosine kinase inhibitors.

Tyrphostins are inhibitors of protein tyrosine kinases.[21,22] The function of protein tyrosine kinases is at the beginning of the signal transfer chain in cells. Protein tyrosine kinases are also significant in the transduction of growth factors. The development of tyrosine phosphorylation inhibitors has transformed the approach to cancer therapy and is likely to affect other fields of medicine. In spite of the conservation among protein tyrosine kinases, one can develop small molecules that block the activity of a narrow spectrum of protein tyrosine kinases, which in turn exhibit much less toxicity than the currently used chemotherapeutic agents.[22] Several types of the Tyrphostins have been described including conformationally constrained aromatic and heteroaromatic compounds, and compounds including α-keto or α-amide groups.[21,22] Compounds including an α-keto or α-amide group are at least one order of magnitude stronger inhibitors rate than the others.[21]

Herein we report the preparation and biological evaluation of both enantiomeric forms of several Tyrphostins containing an α-amide function by a chemoenzymatic route, which applies alkyl cyanoacetates as novel acylating agents in lipase-catalyzed kinetic resolutions of 1-phenylethanamine.

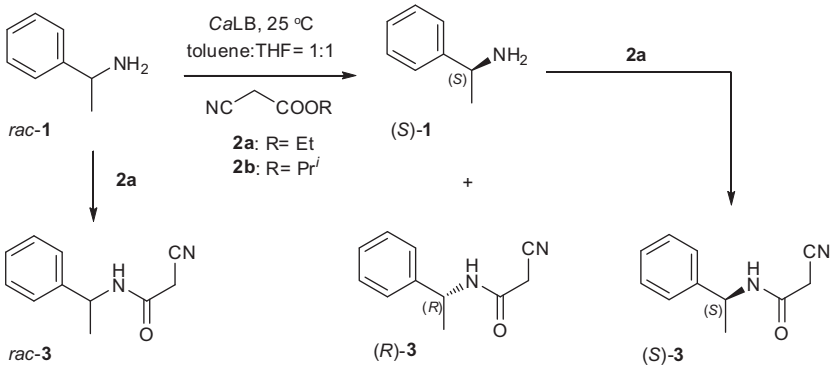
2. Results and discussion

Herein our aim was to investigate the enzymatic *N*-acylation of racemic 1-phenylethanamine *rac*-**1** with cyanoacetate esters **2a**,**b**, to optimize the reaction conditions of the kinetic resolution and to study the correlation of conversion and enantiopurity. The products from the enzymatic reaction were converted via Knoevenagel condensation to potential protein tyrosine kinase inhibitors. The acylation reaction (Scheme 1) was investigated using three different immobilized forms of the lipase B from *Candida antarctica* (*Ca*LB) as biocatalysts (Immozyme *Ca*LB T2-150, *Ca*LB G250P, *Ca*LB N435) (Table 1). First, the biocatalysts were screened for the best conversion and high enantiomeric excess in the kinetic resolution resulting in a mixture of amide (*R*)-**3** of high purity and the residual amine (*S*)-**1** (Table 1). Although the *Ca*LB catalyzed kinetic resolution of **1** with ethyl acetate proceeded smoothly in toluene,[6] in case of acylation with ethyl 2-cyanoacetate **2a** the amide (*R*)-**3** precipitation was observed from pure toluene. The solubility of (*R*)-**3** was better in THF, which was used as a cosolvent in *Ca*LB catalyzed kinetic resolutions.[23] Therefore, 0.5 equiv of ethyl 2-cyanoacetate **2a** (related to the amount of racemic **1**) was used in THF/toluene 1:1 as the solvent. This solvent system did not deplete the biocatalyst and secured the solubility of the resulting

Table 1
Comparison of the *Ca*LB biocatalyst in the kinetic resolution of 1-phenylethanamine *rac*-**1** with ethyl 2-cyanoacetate **2a** (at room temperature, in toluene–THF 1:1, for 24 h)

Entry	Form of *Ca*LB	Mode of immobilization	*c* (%)	$ee_{(R)\text{-}3}$ (%)	$ee_{(S)\text{-}1}$ (%)
1	Immozyme *Ca*LB T2-150	Covalent[a]	20.0	91.0	22.7
2	*Ca*LB G250P	Adsorption[b]	17.1	99.3	20.7
3	*Ca*LB N435	Adsorption[c]	32.2	99.9	47.4

[a] On mezoporous beads, particle size: 150–300 μm.
[b] Phenyl functionalized silica-gel, pore diameter: 25 nm.
[c] On macroporous acrylic resin.



Scheme 1. Chemoenzymatic synthesis of amides *rac*-**3**, (*R*)-**3** and (*S*)-**3** from *rac*-**1**-phenylethanamine *rac*-**1** involving *Ca*LB-catalyzed kinetic resolution with alkyl 2-cyanoacetates **2a**,**b**.

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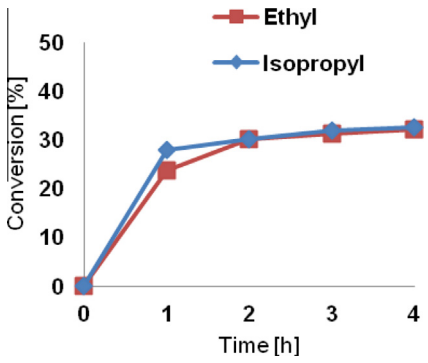


Figure 2. The progress of *Ca*LB-catalyzed kinetic resolution of 1-phenylethanamine *rac*-**1** using ethyl 2-cyanoacetate **2a** and isopropyl 2-cyanoacetate **2b** as acylating agents.

Table 2
Various modes of addition of ethyl 2-cyanoacetate **2a** in the *Ca*LB N435-catalyzed kinetic resolution of *rac*-**1**

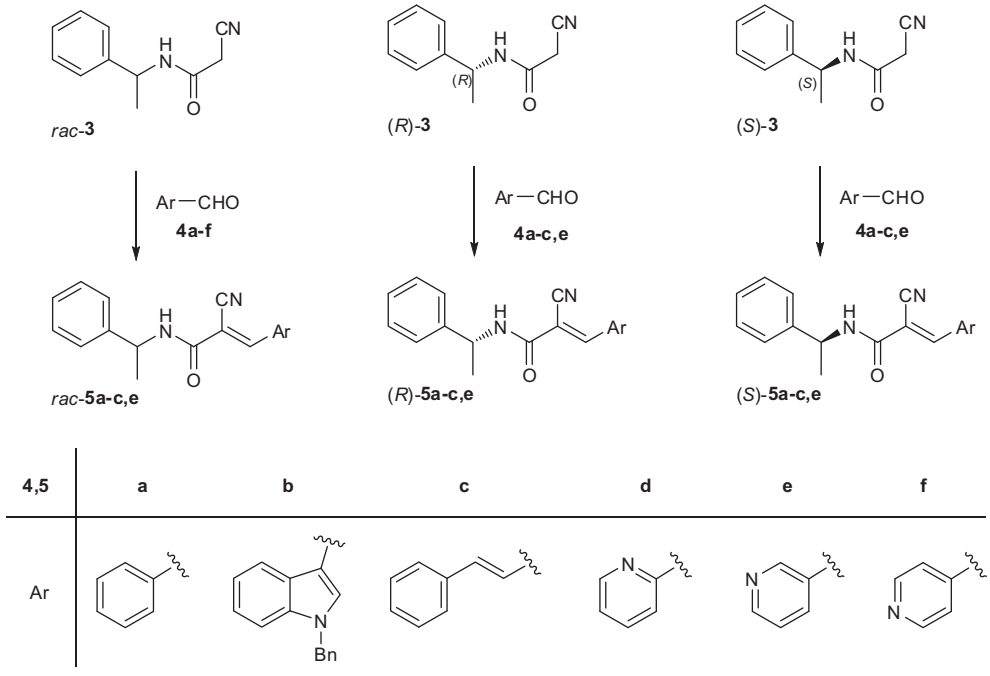
Reaction time (h)	c (%)	$ee_{(R)\text{-}3}$ (%)	$ee_{(S)\text{-}1}$ (%)	U_B (μmol min^{-1} g^{-1})
Method A[a]				
2	26.4	99.9	35.9	9.10
4	28.8	99.9	40.4	4.96
6	29.5	99.9	41.8	3.38
24	31.7	99.9	46.4	0.91
Method B[b]				
2	44.1	99.9	78.8	15.18
4	47.6	99.3	90.1	8.19
6	48.9	99.0	94.8	5.61
24	50.1	98.2	98.5	1.44
Method C[c]				
2	27.3	99.9	37.5	9.40
4	41.2	99.5	69.9	7.10
6	47.4	99.1	89.1	5.44
24	50.1	98.9	99.3	1.44

[a] Method A: 0.5 equiv of **2a**.
[b] Method B: 1.0 equiv of **2a**.
[c] Method C: 0.5 equiv of **2a**, 0.25 equiv of **2a** at 2 h and further 0.25 equiv of **2a** at 4 h.

amide (*R*)-**3** and the starting amine **1**. Since the best conversions and the highest enantiopurity [$ee_{(R)\text{-}3}$ >99.9%] for amide (*R*)-**3** were obtained with the *Ca*LB N435 form, this biocatalyst was used in further studies.

Our hypothesis was that the non-enzymatic (i.e., non-stereoselective) *N*-acylation with isopropyl ester **2b** would be slower than with the ethyl ester **2a**, resulting in a higher proportion of enzyme-catalyzed acylation and thus resulting in higher enantiopurity. A test reaction between racemic **1** and alkyl cyanoacetates **2a**,**b** in the absence of enzyme under the planned conditions resulted in only negligible conversions (0.2% with **2a**, 0.3% with **2b**, after 4 h at room temperature). Next, the ethyl and isopropyl 2-cyanoacetates **2a** and **2b** (0.5 equiv) were compared as acylating agents in the enzymatic *N*-acylation of *rac*-**1** (Fig. 2). A paired T-statistic for the conversion values showed that the conversion values did not differ significantly ($t = -1.3674$; $n = 3$; $p = 0.2649$), while the enantiomeric excess of the product (*R*)-**3** was over 99% at all conversions. Since the nature of the leaving group did not significantly affect the outcome of the kinetic resolution, ethyl-2-cyanoacetate **2a** was used as acylating agent in all further experiments.

Next, optimization of the mode of addition of the acylating agent **2a** was performed to approximate the theoretically possible maximum conversion (50%) in the kinetic resolution of *rac*-**1**. It is known that increasing the amount of the acylating agent is usually helpful to achieve higher conversions. Therefore, in addition to our first approach using only the theoretically calculated 0.5 equiv acylating agent **2a** related to *rac*-**1** (Method A), two other methods were investigated using increased amounts of **2a** (Table 2). In one case, 1 equiv of **2a** related to *rac*-**1** was applied (Method B), while in the other case 0.5 equiv of **2a** was added at the beginning of the reaction and a further 0.25–0.25 equiv was added after 2 and 4 h (Method C). When increased amounts of acylating agent **2a** were applied, the conversion approached the theoretical limit (50%, from *rac*-**1**). Although the increased amount of **2a** promoted the chemical acylation, it resulted in somewhat lower $ee_{(R)\text{-}3}$ values than with only 0.5 equiv of acylating agent [e.g. at 24 h $ee_{(R)\text{-}3}$ was 98.2 and 98.9% for Method B and C, respectively, compared to $ee_{(R)\text{-}3}$ = 99.9% for Method A], the conversion reached the theoretical limit (50%). Application of Method C combining the good



Scheme 2. Knoevenagel condensation of amides *rac*-**3**, (*R*)-**3** and (*S*)-**3** with aldehydes **4a**–**f** leading to the Tyrphostins *rac*-**5a**–**c**,**e**, (*R*)-**5a**–**c**,**e** and (*S*)-**5a**–**c**,**e**.

Table 3
Knoevenagel condensation of amide *rac*-**3** with aldehydes **4a–f** leading to racemic Tyrphostins **5a–c,e**

Entry	Aldehyde	Product	Yield (%)
1	Benzaldehyde **4a**	*rac*-**5a**	70[a]
2	1-Benzyl-1*H*-indole-3-carbaldehyde **4b**	*rac*-**5b**	95[b]
3	Cinnamaldehyde **4c**	*rac*-**5c**	44[a]
4	Pyridine-2-carbaldehyde **4d**	—	0
5	Pyridine-3-carbaldehyde **4e**	*rac*-**5e**	88[b]
6	Pyridine-4-carbaldehyde **4f**	—	0

[a] After silica gel chromatography.
[b] After precipitation and washing with Et_2O.

Table 4
Antiproliferative effect of racemic and enantiopure Tyrphostins **5a–c,e** on various human cancer cell lines (average statistical error: 10%; >60: no significant effect)

Compound	HCT 116 EC_{50} (μM)	A549 EC_{50} (μM)	PC9 EC_{50} (μM)	PC9ER EC_{50} (μM)	Jurkat EC_{50} (μM)	MV4-11 EC_{50} (μM)
rac-**5a**	33.3	11.0	47.6	34.2	23.7	23.2
(*R*)-**5a**	30.6	16.0	31.3	31.5	19.6	21.0
(*S*)-**5a**	31.6	14.1	40.4	33.5	22.1	22.0
rac-**5b**	>60	14.0	31.9	>60	>60	>60
(*R*)-**5b**	>60	16.9	57.3	>60	>60	>60
(*S*)-**5b**	>60	24.1	31.6	>60	30.2	39.2
rac-**5c**	14.2	15.7	14.1	13.8	8.0	6.0
(*R*)-**5c**	23.3	20.1	25.3	23.3	12.3	8.6
(*S*)-**5c**	13.5	13.2	11.4	11.9	8.5	6.9
rac-**5e**	37.5	23.1	38.9	40.2	23.4	25.0
(*R*)-**5e**	28.4	15.6	32.3	32.5	13.5	13.0
(*S*)-**5e**	31.3	17.4	34.7	34.5	22.1	22.0

conversion with high selectivity enabled the preparation of the residual amine (*S*)-**3** with excellent enantiopurity [$ee_{(S)\text{-}\mathbf{1}}$ = 99.3%] as well. After separation, (*S*)-**1** could be readily converted by chemical acylation into (*S*)-**3** (Scheme 1).

First, the Knoevenagel condensation was tested between the racemic amine *rac*-**1** and a series of aldehydes **4a–f** including benzaldehyde **4a**, *N*-benzyl indole-3-carbaldehyde **4b**, cinnamaldehyde **4c**, and three different pyridinecarbaldehydes **4d–f** (Scheme 2, Table 3). The best yields were achieved in condensations with the *N*-protected indole-3-carbaldehyde **4b** and pyridine-3-carbaldehyde **4e** (Table 3, entries 2 and 5). The yield was only moderate with the benzaldehyde **4a** (Table 3, entry 1), and poor with cinnamaldehyde **4c** (Table 3, entry 3). Under these condensation conditions, no identifiable products were formed with pyridine-2- and 4-carbaldehydes **4d,f**.

After this test with the racemic amide *rac*-**3**, the Knoevenagel condensation was performed with aldehydes **4a–c,e** and the enantiopure amides (*R*)-**3** and (*S*)-**3** to give the Tyrphostin enantiomers (*R*)-**5a–c,e** and (*S*)-**5a–c,e**.

The biological activity of these compounds was tested on different carcinoma and leukemia cell lines. The results in Table 4 indicate that the Tyrphostin-like compound *rac*-**5a–c,e**, (*R*)-**5a–c,e**, and (*S*)-**5a–c,e** had no or very weak inhibitory activities and none of them fall in the range of good inhibitors characterized by EC_{50} <1 μM.

3. Conclusion

Variously immobilized forms of *Ca*LB (Immozyme *Ca*LB T2-150, *Ca*LB G250P, *Ca*LB N435) were tested in the kinetic resolution of racemic 1-phenylethanamine *rac*-**1** with ethyl and isopropyl 2-cyanoacetates **2a,b** as novel acyl activated acylating agents. The ethyl and isopropyl 2-cyanoacetates **2a,b** resulted in similar conversions and enantioselectivity in the enzymatic *N*-acylation of 1-phenylethanamine *rac*-**1**, indicating that the leaving group did not significantly affect the reaction. The most efficient biocatalyst Novozyme® N435 (*Ca*LB N435) enabled us to reach high enantiopurity [$ee_{(R)\text{-}\mathbf{3}}$ = 99.9%] and the theoretical 50% conversion limit in the kinetic resolution of 1-phenylethanamine *rac*-**1** with ethyl 2-cyanoacetate **2a**. Under the optimal reaction conditions using 1 equiv of the acylating agent, excellent enantiopurity of the product (*R*)-**3** was observed [$ee_{(R)\text{-}\mathbf{3}}$ = 99%] at high conversion (c = 47.6%) after 4 h reaction time. A chemoenzymatic process involving chemical conversion of the residual amine (*S*)-**1** into amide (*S*)-**3**, and Knoevenagel condensation of the produced amides *rac*-**3**, (*R*)-**3** and (*S*)-**3** with aromatic aldehydes **4a–f** resulted in some novel Tyrphostins *rac*-**5a–c,e**, (*R*)-**5a–c,e**, and (*S*)-**5 a–c,e**. Unfortunately, none of the novel Tyrphostins exhibited strong inhibition (EC_{50} <1 μM) on different carcinoma and leukemia cell lines: HCT 116, A549, PC9, PC9ER, Jurkat, and MV4-11

4. Experimental

4.1. Materials and methods

4.1.1. Materials, enzymes and cell lines

rac-**1**-Phenylethanamine, ethyl cyanoacetate, isopropyl cyanoacetate, and the aldehydes were purchased from Sigma–Aldrich (Saint Louis MO, USA).

*Ca*LB G250P (lipase B from *Candida antarctica*, adsorbed on phenyl-functionalized silica gel) was the product of SynBiocat Ltd (Budapest, Hungary). Immozyme *Ca*LB T2-150 (lipase B from *Candida antarctica*, covalently attached to dry acrylic beads of 150–300 μm particle size) was the product of ChiralVision BV (Leiden, The Netherlands). *Ca*LB N435 (Novozym®, lipase B from *Candida antarctica*, recombinant, expressed in *Aspergillus niger*, adsorbed on acrylic resin) was obtained from Sigma–Aldrich (Table 1).

Solvents (toluene, tetrahydrofuran, ethanol, hexane, acetone) from Merck (Budapest, Hungary) were dried and/or freshly distilled prior to use.

A549 lung, HCT116 colorectal carcinoma and Jurkat acute T cell leukemia cell lines were purchased from ATCC (American Type Culture Collection, Manassas, VA). The PC9 and PC9-ER lung adenocarcinoma cell lines were kindly provided by the Cancer Research UK (London Research Institute, Signal Transduction laboratory, London, UK). PC9-ER cells were made erlotinib-insensitive by prolonged incubation with erlotinib. MV4-11 biphenotypic B myelomonocytic leukemia cell line was purchased from CLS (Cell Lines Service, Eppelheim, Germany). IMDM and RPMI-1640 medium, fetal bovine serum and antibiotic-antimycotic solution were purchased from Sigma–Aldrich.

4.1.2. Methods

Thin layer chromatography was carried out using Kieselgel 60 F_{254} (Merck) sheets. Spots were visualized under UV light (Vilber Lourmat VL-6.LC, 254 nm and 365 nm) or by treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates.

The enantiomeric excess of the residual amine (*S*)-**1** [$ee_{(S)\text{-}\mathbf{1}}$] was analyzed by GC on an Agilent 5890 equipment using Hydrodex β-TBDAc column (Machery-Nagel; 25 m × 0.25 mm × 0.25 μm, heptakis-(2,3-di-*O*-acetyl-6-*O*-*t*-butyl-dimethylsilyl)-β-cyclodextrin), FID (250 °C), injector (250 °C), H_2 (12 psi, split ratio: 1:50). Oven program: 100–180 °C with 8 °C min^{-1}, 15 min at 180 °C. Retention times, t_r (min): 2.9 [(*S*)-**1**], 3.1 [(*R*)-**1**], 22.1 [*rac*-**3**].

Enantiomeric excess of the produced amide (*R*)-**3** [$ee_{(R)\text{-}\mathbf{3}}$] was analyzed by HPLC on a HP1090 equipment using Chiralpak IB column [Daicel; 2.1 mm × 150 mm × 5 μm, cellulose tris-(3,5-dimethylphenylcarbamate) immobilized on 5 μm silica gel], eluent/hexane/propan-2-ol 95/5 v/v 0.10 ml min^{-1}, column

temperature: 25 °C, λ = 220 nm. Retention times, t_r (min): 4.6 [*rac*-**1**], 42.9 [(*R*)-**3**], 48.8 [(*S*)-**3**].

Conversion of reactions (c) were calculated by the equation $c = (ee_{(S)\text{-}\mathbf{1}})/[ee_{(S)\text{-}\mathbf{1}} + ee_{(R)\text{-}\mathbf{3}}]$ using the enantiomeric excess values of the residual substrate (*S*)-**1** and the produced (*R*)-**3**.

The NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-300 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C, and signals are given in ppm on the δ scale.

Infrared spectra were recorded on a Bruker ALPHA FT-IR spectrometer and wavenumbers of bands are listed in cm^{-1}.

Optical rotation was measured on Perkin–Elmer 241 polarimeter at the D-line of sodium. The polarimeter was calibrated with measurements of both enantiomers of menthol.

The proliferation assay was made on 384 well plates (Perkin Elmer, Boston, MA). The antiproliferative activity of the compounds was determined by CellTiter-Glo®Luminescent Cell Viability Assay (Promega, Madison, WI) according to the manufacturer's instructions. The luminescent signal was measured by Analyst®GT Multimode Reader (Molecular Devices, Sunnyvale, CA). XLfit (IDBS) software was used to generate the dose-effect curves for EC_{50} determination.

4.2. Chemical acylation of 1-phenylethanamine 1, (*S*)-1 by ethyl 2-cyanoacetate 2a

Amides *rac*-**3** or (*S*)-**3** were prepared from the corresponding amine [racemic amine **1** or (*S*)-**1** obtained from the lipase-catalyzed kinetic resolution, see Section 4.3] by a modified method of Kumar et al.[16] The reaction mixture of ethyl 2-cyanoacetate **2a** (113 mg, 0.01 mol) and 1-phenylethanamine *rac*-**3** or (*S*)-**3** (121 mg, 0.01 mol) was added to toluene (2 mL) by stirring at room temperature for 72 h. The solvent was removed under reduced pressure. The product was washed with cooled diethyl ether (2 × 3 mL).

rac-***3***: 61% (precipitated from the reaction mixture and washed by Et_2O); colorless crystals; mp: 113 °C, lit.[24] mp 100–101 °C, ^1H NMR ($CDCl_3$): 1.54 (3H, d, J = 7.2 Hz, CH_3), 3.31 (2H, s, CH_2), 5.10 (1H, dq, J = 7.2 Hz and J = 7.3 Hz, CH), 6.53 (1H, br, NH), 7.28–7.40 (5H, m, ArH); ^{13}C NMR ($CDCl_3$): 21.7 (CH_3), 26.1 (CH_2), 50.2 (CH), 114.9 (CN), 126.3 (2xCH), 128.0 (CH), 129.1 (2xCH), 142.2 (C) 160.3 (C=O); IR (KBr): 3279, 3068, 2975, 2921, 2258, 1648, 1554, 669. The IR and NMR spectra were in agreement with the data in literature.[24]

(*S*)-***3***: 59% (precipitated from the reaction mixture, washed by Et_2O); colorless crystals; mp: 141 °C, $[\alpha]_D^{28}$ = −78.6 (c 1.0, CH_2Cl_2), ee = 99.3% (by HPLC). The IR and NMR spectra were in agreement with those obtained for the racemic amide *rac*-**3**.

4.3. *Ca*LB-catalyzed kinetic resolution of 1-phenylethanamine 1 with alkyl 2-cyanoacetates 2a, b

4.3.1. Selection of the biocatalyst

1-Phenylethanamine *rac*-**1** (100 mg, 0.825 mmol), ethyl 2-cyanoacetate **2a** (46 mg, 0.5 equiv), and 200 mg of the biocatalyst (*Ca*LB G250P, *Ca*LB T2-150 or *Ca*LB N435: see Table 1) were added to a mixture of toluene–THF 1:1 (10 mL) and the resulting mixture was shaken (400 rpm) at room temperature for 24 h. At the end of the reaction, the biocatalyst was filtered off and the filtrate was evaporated in vacuum. The residue was dissolved in 5% HCl (7.5 mL) and the solution extracted with dichloromethane (3 × 12 mL). The organic phase was dried and evaporated to give pure (*R*)-**3**. To the aqueous phase was added 40% NH_3 solution (5 mL) and then extracted with dichloromethane (3 × 12 mL). The organic phase was dried and evaporated to give unreacted (*S*)-1-phenylethanamine (*S*)-**1**.

(*R*)-***3***: 29% (entry 3 in Table 1); colorless crystals; mp: 142 °C, lit.[16] mp: 120–122 °C, $[\alpha]_D^{28}$ = +80.1 (c 1.0, CH_2Cl_2), ee = 99.9% (by HPLC). The IR and NMR spectra were in agreement with those obtained for the racemic amide *rac*-**3**.

4.3.2. Comparison of ethyl and isopropyl 2-cyanoacetates 2a and 2b as acylating agents

1-Phenylethanamine *rac*-**1** (50 mg, 0.41 mmol), *Ca*LB N435 (100 mg), and alkyl 2-cyanoacetate (ethyl 2-cyanoacetate **2a**, 23 mg, 0.5 equiv; or isopropyl 2-cyanoacetate **2b**, 26 mg, 0.5 equiv) were added to a mixture of toluene–THF 1:1 (2 mL) and the resulting mixture was shaken (400 rpm) at room temperature for 4 h (sampling at 1, 2, 3, and 4 h was performed: see Fig. 2). The reaction mixtures were then processed as described in Section 4.3.1.

A blank test was performed with *rac*-**1** (50 mg, 0.41 mmol) and alkyl 2-cyanoacetate **2a** (23 mg, 0.5 equiv) or **2b** (26 mg, 0.5 equiv) in a mixture of toluene–THF 1:1 (2 mL) shaken (400 rpm) at room temperature for 4 h. The conversion was checked by GC (0.2% with **2a**, 0.3% with **2b**).

4.3.3. Mode of ethyl 2-cyanoacetate 2a addition

To a mixture of toluene–THF 1:1 (2 mL) were added 1-phenylethanamine *rac*-**1** (50 mg, 0.41 mmol), *Ca*LB N435 (100 mg), and ethyl 2-cyanoacetate **2a** (Method A: 23 mg, 0.5 equiv; Method B: 46 mg, 1 equiv; Method C: 23 mg, 0.5 equiv, further 11.5 mg, 0.25 equiv after 2 h and further 11.5 mg, 0.25 equiv after 4 h) and the resulting mixture was shaken (400 rpm) at room temperature for 24 h (sampling at 2, 4 6, and 24 h was performed: see Table 2). The reaction mixtures were then processed as described in Section 4.3.1. (*S*)-**1**: 41% (Method C in Table 2); pale yellow liquid; $[\alpha]_D^{28}$ = −31.1 (c 10, ethanol), {lit.: $[\alpha]_D^{28}$ = −40.3 (neat)[25]}; ee = 99.3% (by GC).

4.4. Preparation of Tyrphostins

An aldehyde **4a–f** (1 mmol), and an amide *rac*-**3**, (*S*)-**3** or (*R*)-**3** (1 mmol) were heated at reflux in ethanol (8 mL) containing piperidine (1 drop) for 4 h. The mixture was cooled and stored at 4 °C for 24 h. The precipitated crystalline product was filtered off and washed with cold diethyl ether. In the case of no precipitate forming, the product was isolated by column chromatography (silica gel, with a mixture of hexane–acetone 10:4) from the residue after evaporation of the solvent. The results with *rac*-**3** are compiled in Table 3.

rac-***5a***: 73% (after silica gel chromatography), colorless crystals; mp: 123 °C; ^1H NMR ($CDCl_3$): 1.62 (3H, d, J = 7.2 Hz, CH_3), 5.27 (1H, m, CH), 6.56 (1H, d, J = 5.7 Hz, NH), 7.27–7.55 (8H, m, ArH), 7.93 (2H, d, J = 6.6 Hz, ArH), 8.35 (1H, s, CH=); ^{13}C NMR ($CDCl_3$): 22.0 (CH_3), 50.4 (CH), 106.0 (C=), 117.0 (CN), 126.4 (2 × CH), 128.0 (CH), 129.1 (2 × CH), 129.5 (2 × CH), 130.9 (2 × CH), 132.0 (C), 133.0 (CH), 143.0 (C), 153.5 (CH=), 159.0 (C=O); IR (KBr): 3295, 3062, 3032, 29702, 2939, 2215, 1666, 1654, 1597, 1541, 1523, 701, 687.

(*R*)-***5a***: 71% (after silica gel chromatography), colorless crystals; mp: 73 °C; $[\alpha]_D^{28}$ = −4.1 (c 1.0, CH_2Cl_2), ee = 99.9% [by HPLC of the starting amide (*R*)-**3**].

(*S*)-***5a***: 69%; (after silica gel chromatography), colorless crystals; mp: 72 °C, $[\alpha]_D^{28}$ = +4.0 (c 1.0, CH_2Cl_2), ee = 99.3% [by HPLC of the starting amide (*S*)-**3**].

rac-***5b***: 95% (precipitated from the reaction mixture, washed by Et_2O), yellow crystals; mp: 228 °C, ^1H NMR ($CDCl_3$): 1.62 (3H, d, J = 6.6 Hz, CH_3), 5.30 (1H, m, CH), 5.43 (2H, s, CH_2), 6.39 (1H, d, J = 7.5 Hz, NH), 7.16–7.19 (2H, m, ArH), 7.28–7.41 (11H, m, ArH), 7.86–7.88 (1H, m, ArH), 8.45 (1H, s, CH=), 8.70 (1H, s, ArH); ^{13}C NMR ($CDCl_3$): 22.1 (CH_3), 50.0 (CH), 51.5 (CH_2), 95.9 (C=), 110.8 (C), 111.1 (CN), 119.1 (CH), 119.5 (CH), 122.7 (CH), 124.1 (CH),

126.4 (2 × CH), 127.1 (2 × CH), 127.8 (CH), 128.5 (CH), 128.8 (C), 129.0 (2 × CH), 129.3 (2 × CH), 133.1 (CH), 135.6 (C), 136. 6 (C), 142.9 (C), 144.4 (CH═), 161.1 (C═O); IR (KBr): 3331, 3054, 3034, 2979, 2932, 2211, 1655, 1568, 1507, 1257, 1177, 738, 702.

(R)-***5b***: 91% (precipitated from the reaction mixture, washed by Et_2O), yellow crystals; mp: 190 °C, $[\alpha]_D^{28} = -15.0$ (*c* 1.0, CH_2Cl_2), ee = 99.9% [by HPLC of the starting amide (*R*)-**3**].

(S)-***5b***: 93% (precipitated from the reaction mixture, washed by Et_2O), yellow crystals; mp: 191 °C, $[\alpha]_D^{28} = +15.5$ (*c* 1.0, CH_2Cl_2), ee = 99.3% [by HPLC of the starting amide (*S*)-**3**].

rac-***5c***: 46% (after silica gel chromatography), yellow crystals; mp: 127 °C, ^{1}H NMR ($CDCl_3$): 1.61 (3H, d, *J* = 7.0 Hz, CH_3), 5.25 (1H, m, CH), 6.41 (1H, d, *J* = 7.8 Hz, NH), 7.25 (1H, d, *J* = 5.6 Hz, CH═), 7.30–7.59 (10H, m, ArH), 7.59 (1H, d, *J* = 5.7 Hz, CH═), 8.08 (1H, dd, *J* = 5.7 and 5.7 Hz, CH═); ^{13}C NMR ($CDCl_3$): 21.9 (CH_3), 50.1 (CH), 105.8 (C═), 116.1 (CN), 123.3 (CH), 126.4 (2 × CH), 127.9 (CH), 128.5 (2 × CH), 129.1 (2 × CH), 129.1 (2 × CH), 131.1 (C═), 135.1 (C), 142.5 (C), 148.1 (C═), 153.7 (C═), 159.6 (C═O); IR (KBr): 3351, 3059, 3031, 2976, 2933, 2217, 1660, 1612, 1584, 1521, 1254, 749, 700.

(R)-***5c***: 43% (after silica gel chromatography), yellow crystals; mp: 107 °C, $[\alpha]_D^{28} = +28.7$ (*c* 1.0, CH_2Cl_2), ee = 99.9% [by HPLC of the starting amide (*R*)-**3**].

(S)-***5c***: 44% (after silica gel chromatography), yellow crystals; mp: 106 °C, $[\alpha]_D^{28} = -25.8$ (*c* 1.0, CH_2Cl_2), ee = 99.3% [by HPLC of the starting amide (*S*)-**3**].

rac-***5e***: 88% (precipitated from the reaction mixture, washed by Et_2O), colorless crystals; mp: 119 °C; ^{1}H NMR ($CDCl_3$): 1.62 (3H, d, *J* = 6.9 Hz, CH_3), 5.25 (1H, m, CH), 7.16–7.44 (5H, m, ArH), 7.49 (1H, dd, *J* = 8.2 and 4.9 Hz, ArH), 8.35 (1H, s, NH), 8.45 (1H, d, *J* = 8.2 Hz, ArH), 8.74 (1H, d, *J* = 4.9 Hz, ArH), 8.94 (1H, s, ArH); ^{13}C NMR ($CDCl_3$): 21.8 (CH_3), 50.5 (CH), 107.2 (C═), 116.4 (CN), 124.4 (CH), 126.4 (2 × CH), 128.1 (CH), 128.8 (CH), 129.1 (2 × CH), 136.5 (C), 142.1 (C), 149.4 (CH), 151.9 (C═), 152.5 (CH), 158.6 (C═O); IR (KBr): 3324, 3286, 3054, 3032, 2991, 2943, 2222, 1645, 1540, 701.

(R)-***5e***: 91% (precipitated from the reaction mixture, washed by Et_2O), colorless crystals; mp: 90 °C, $[\alpha]_D^{28} = +5.8$ (*c* 1.0, CH_2Cl_2), ee = 99.9% [by HPLC of the starting amide (*R*)-**3**].

(S)-***5e***: 87% (precipitated from the reaction mixture, washed by Et_2O), colorless crystals; mp: 90 °C, $[\alpha]_D^{28} = -5.9$ (*c* 1.0, CH_2Cl_2), ee = 99.3% [by HPLC of the starting amide (*S*)-**3**].

4.5. Protocols for bioassays

From each of human cancer cell lines (A549, PC9, PC9ER, HCT116, Jurkat, and MV4-11), 1000 cells were plated in each well. The MV4-11 cells were maintained in IMDM medium, all the other cells in RPMI-1640 medium in the presence of 10% fetal bovine serum and antibiotic-antimycotic solution in a humidified atmosphere with 5% CO_2 at 37 °C. Following adherence, the cells were incubated with **5a**–**c**,**e** at 10 different concentrations in the range of 3.05 nM–60 μM (3-fold serial dilution) for 72 h.

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