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Enantioselective synthesis of a chiral pyridazinone derivative by lipase-catalyzed hydrolysis

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Abstract—The lipase-catalyzed resolution of 2-(acyloxymethyl)-4,5-dihydro-5-methylpyridazin-3(2*H*)-one derivatives in organic solvents containing water was demonstrated to be a practical method for the synthesis of a chiral pyridazinone bearing a pyrazolopyridine ring, which is a potent phosphodiesterase inhibitor.

1. Introduction

Pyridazinone derivatives exhibit a variety of pharmaceutically useful biological activities.[1–3] Previously, we reported that a chiral pyridazin-3(2*H*)-one bearing a pyrazolopyridine ring was an effective bronchodilatory agent due to its phosphodiesterase inhibitory activity.[4] However, resolution of the racemate using chiral columns was impractical and a synthetic route toward the chiral derivative was unsatisfactory due to the requirement for an expensive starting material as well as the loss of enantiomeric purity in the course of the synthesis.[4,5] Recently, many efforts have been made toward the exploitation of synthetic methods for biologically active chiral pyridazinone derivatives: the resolution of the diastereoisomeric salts with tartaric acid[6] or chiral amines,[7] syntheses starting from chiral substrates (chiral-pool methods),[8,9] asymmetric hydrogenation[10,11] and enzymatic resolution[12] of various intermediates were reported. However, these methods also seem to be impractical due to the low enantiomeric purities they afford or the need for multiple synthetic steps.

Previously Achiwa et al. reported that the enzymatic hydrolysis of acyloxymethyl groups on some heterocyclic rings was an effective strategy for enantioselective synthesis.[13–16] Thus, we considered the application of these methods for the resolution of racemic pyridazinone derivatives. We wish to describe herein the enantioselective and practical synthesis of a chiral pyridazinone by the lipase-catalyzed resolution of 2-(acyloxymethyl)pyridazinone derivatives in organic solvents containing water.

2. Results and discussion

We synthesized 6-(2-isopropylpyrazolo[1,5-*a*]pyridin-3-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one **6** as a racemic mixture in four steps starting with Friedel–Crafts acylation (77%) of 2-isopropylpyrazolo[1,5-*a*]-pyridine[17] **1** with 2-bromopropionyl bromide **2**. Extension of the carbon chain was performed by nucleophilic substitution with dimethyl malonate to provide **4** in 86% yield. Hydrolysis of **4** and subsequent decarboxylation in hydrobromic acid gave **5** in 90% yield. Treatment of **5** with hydrazine monohydrate then afforded the pyridazinone (±)-**6** in 92% yield (Scheme 1).

Propionyloxymethyl and pivaloyloxymethyl groups were introduced on the nitrogen atom at the 2-position of (±)-**6** by the reactions of chloromethyl propionate and chloromethyl pivalate in the presence of potassium carbonate, and the acyloxymethyl derivatives (±)-**7** and (±)-**8** were obtained in 89 and 88% yields, respectively. In the previous report, acyloxymethyl groups were introduced in moderate yields with sodium hydride as the base,[15] but we found that the use of potassium carbonate was more effective for the present transformation.

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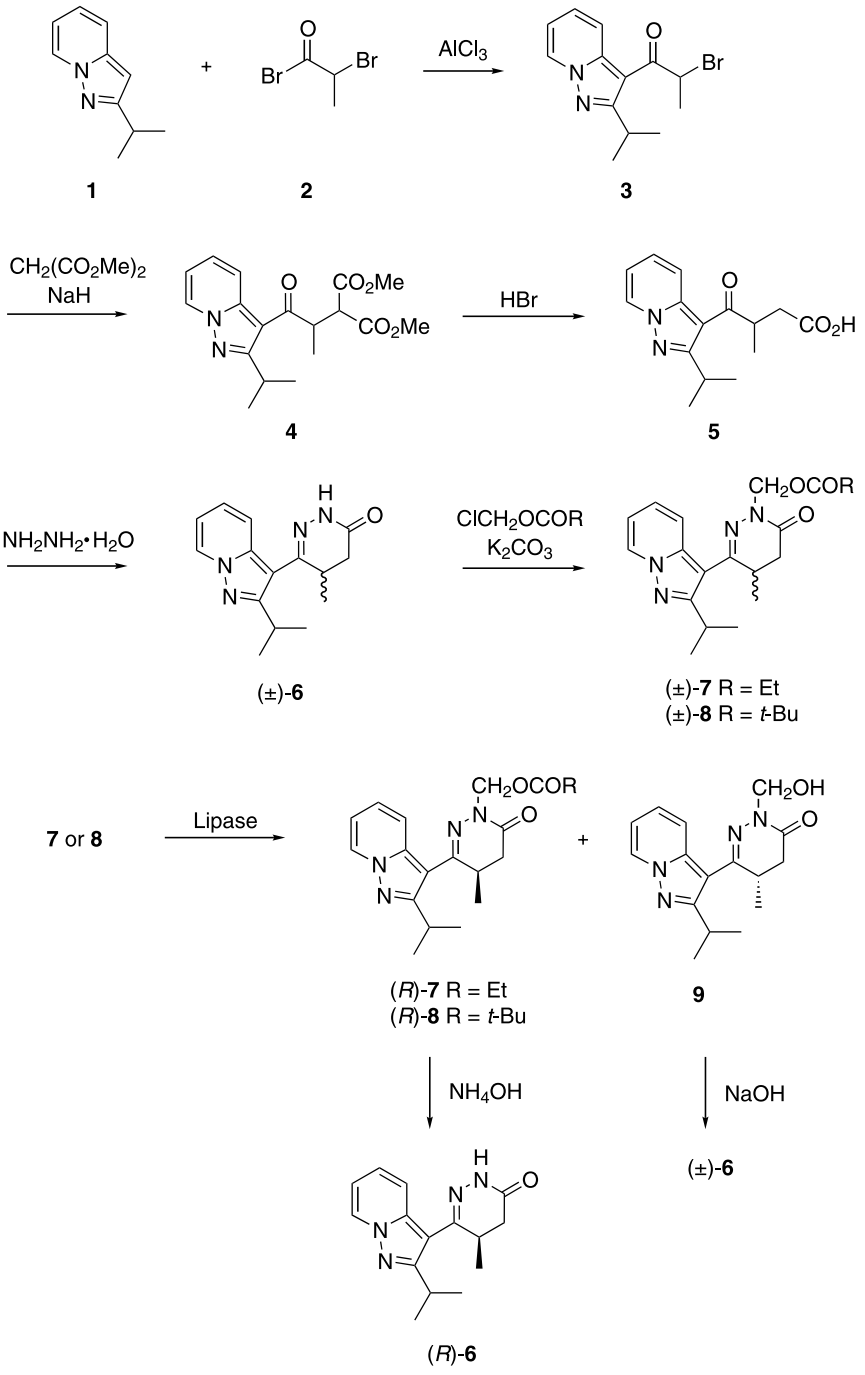

Scheme 1.

We next investigated the activities of enzymes in hydrolyses of (±)-**7** and (±)-**8**. We attempted the hydrolysis reactions with more than 20 commercially available enzymes. A number of the results, where activity was observed, are listed in Table 1. (*R*)-4,5-Dihydro-6-(2-isopropylpyrazolo[1,5-*a*]pyridin-3-yl)-5-methylpyridazine-3(2*H*)-one (*R*)-**6** has been found to exhibit phosphodiesterase inhibitory activity,[4] and the absolute configuration of (*R*)-**6** has been determined by an alternative synthesis starting from (*S*)-2-chloropropionic acid according to a similar procedure described in the literature.[5,8,9] We therefore sought the most efficient conditions to obtain higher chemical and enantiomeric purity of the recovered (*R*)-**7** or (*R*)-**8** (which have *R*-configuration at the 5-position). The efficiency of the hydrolysis was estimated from the *E*-values, which are calculated by the following equation.[18]

$$E = \ln[(1-c)(1-\mathrm{ee}(S))]/\ln[(1-c)(1+\mathrm{ee}(S))] = \ln[1-c(1+\mathrm{ee}(P))]/\ln[1-c(1-\mathrm{ee}(P))]$$

Where *E*: *E*-value, *S*: substrate, *P*: product, *c*: conversion.

Table 1. Screening results of the lipase-catalyzed hydrolysis of (±)-**7** and (±)-**8**

Entry	Substrate	Solv[a].	Enzyme	Time	Conv.	Hydrolysis (**9**)		Recovered **7** or **8**		*E*
						Yield[b] (%)	Ee[b] (%)	Yield[b] (%)	Ee[b] (%)	
1	(±)-**7**	A	Lipase Type XI	24	41.4	37.5	30.7 (*S*)	58.6	20.7 (*R*)	2.2
2	(±)-**7**	A	Lipase AK	24	47.3	46.7	73.0 (*S*)	52.7	66.8 (*R*)	13.5
3	(±)-**7**	A	Lipase F-AP15	24	20.8	20.8	42.8 (*S*)	79.2	15.6 (*R*)	4.6
4	(±)-**7**	A	Lipase M	24	45.3	45.3	31.6 (*S*)	54.7	32.4 (*R*)	3.1
5	(±)-**7**	A	Lipase PS	24	13.9	12.0	50.4 (*S*)	86.1	10.3 (*R*)	5.0
6	(±)-**7**	B	Lipase Type I	0.08	57.5	57.5	41.7 (*S*)	42.5	58.6 (*R*)	4.4
7	(±)-**7**	B	Lipase Type IA	2	53.3	53.3	42.6 (*S*)	46.7	53.2 (*R*)	4.5
8	(±)-**7**	B	Lipase A	0.5	71.7	71.7	16.3 (*S*)	28.3	45.5 (*R*)	2.1
9	(±)-**7**	B	Lipase AK	0.5	62.6	62.4	52.3 (*S*)	37.4	79.7 (*R*)	6.5
10	(±)-**7**	B	Lipase M	0.5	59.5	59.5	33.5 (*S*)	40.5	47.0 (*R*)	3.0
11	(±)-**7**	B	Lipase PS	1.5	48.1	47.7	73.0 (*S*)	51.9	68.2 (*R*)	13.2
12	(±)-**7**	B	Protease A	0.03	63.1	61.9	22.2 (*S*)	36.9	42.5 (*R*)	2.4
13	(±)-**7**	B	Protease M	0.03	80.4	79.9	12.8 (*S*)	19.6	56.9 (*R*)	2.1
14	(±)-**7**	B	Promerain F	2	71.5	71.4	26.7 (*S*)	28.5	69.5 (*R*)	3.4
15	(±)-**7**	B	Papain W-40	2	52.7	52.1	38.1 (*S*)	47.3	43.9 (*R*)	3.4
16	(±)-**8**	B	Lipase Type II	24	91.0	91.0	52.6 (*S*)	9.0	54.0 (*R*)	1.6
17	(±)-**8**	B	Lipase	6	40.2	40.2	39.9 (*S*)	59.8	13.7 (*R*)	1.7
18	(±)-**8**	B	Lipase AK	24	0	0	–	100	–	–
19	(±)-**8**	B	Lipase PS	24	5.8	5.8	67.6 (*S*)	94.2	2.7 (*R*)	2.6
20	(±)-**8**	B	Protease A	24	95.0	95.0	13.8 (*S*)	5.0	32.8 (*R*)	1.3
21	(±)-**8**	B	Pancreatin F	24	41.3	41.3	47.3 (*S*)	58.7	19.9 (*R*)	2.2

[a] Solvent system A: diisopropyl ether containing water; B: acetone–phosphate buffer solution (pH 6.8).
[b] Chemical yields and enantiomeric purities were determined by HPLC analyses.

Table 1 summarizes the results from the lipase-catalyzed hydrolysis of (±)-**7** and (±)-**8** in diisopropyl ether saturated with water or an acetone-buffer solution. The outcome of the screening test indicates that the use of lipase AK with the highest *E*-value (13.5) seems to be efficacious (Table 1, entry 2). When the lipase-catalyzed hydrolysis of (±)-**7** was carried out in acetone-buffer system, which is widely employed for the reactions with enzymes, the rates of the reactions were generally faster than those in diisopropyl ether solution, and under these conditions lipase PS shows the highest *E*-value (13.2) (Table 1, entry 11). On the other hand, the lipase-catalyzed hydrolysis with (±)-**8** in the acetone-buffer system showed rather low *E*-values, and the use of lipase AK unexpectedly resulted in the recovery of the substrate (Table 1, entry 18). From these results, the conditions with lipase AK in organic solvents containing water as well as those with lipase PS in the acetone-buffer system seemed to be available for the enzymatic resolution of (±)-**7**. Examination of the relationship between the conversion of the reaction and the enantiomeric excess of (*R*)-**7** showed that the formation of (*R*)-**7** with more than 90% ee was attained when the conversion reached ca. 60% for both enzymes. Although the reaction with lipase AK in diisopropyl ether was slower than that with lipase PS in the acetone-buffer system, the use of the organic solvent is advantageous from the viewpoints of enzyme recovery and enhanced substrate concentration. We therefore decided to employ these conditions with lipase AK for a large-scale synthesis.

In order to further optimize the reaction conditions, we examined the effect of the solvent systems, concentration of the substrate, and the ratio of the substrate versus the enzyme, the results of which are summarized in Tables 2–4, respectively. In the screening test for organic solvents containing 0.4% water, acetonitrile was found to be much more effective than diisopropyl ether, and the highest *E*-value (55.7) was observed using acetonitrile–water (Table 2). Generally, the reactions with *E*-values of over 20 are considered synthetically useful.[18]

The optimum concentrations of (±)-**7** were determined to be 10 mg/cm^3 in diisopropyl ether and 30 mg/cm^3 in acetonitrile (Table 3). Furthermore, when a three- or four-fold excess of lipase AK was used (based on the substrate (±)-**7**), the conversion and the enantiomeric purity were satisfactory (Table 4). Consequently, the

Table 2. Hydrolysis of (±)-**7** using lipase AK in various solvents[a]

Solvent	Conversion[b] (%)	Recovered (*R*)-**7** ee[b] (%)	*E*-value
Diisopropyl ether	55.2	86.1	15.2
Tetrahydrofuran	10.9	8.5	6.0
1,4-Dioxane	19.3	19.1	10.7
Ethyl methyl ketone	8.4	6.3	5.8
Acetonitrile	45.7	77.4	55.7

[a] Reaction conditions: Lipase AK (20 mg) was added to a solution of (±)-**7** (10 mg) in a solvent (containing 0.4% H_2O, 1 cm^3). The mixture was stirred for 24 h at room temperature.
[b] Conversions and enantiomeric purities were determined by HPLC analyses.

Table 3. Hydrolysis of (±)-**7** in various concentrations[a]

Concentration (mg/cm^3)	Conversion (%)	
	Diisopropyl ether	Acetonitrile
100	23.2	11.3
50	38.0	28.4
30	–	42.2
20	53.0	41.6
10	71.9	42.0
5	75.3	–
2	70.9	–
1	73.9	–

[a] Reaction conditions: Lipase AK (20 mg) was added to a solution (1 cm^3) of (±)-**7** in the solvent (diisopropyl ether saturated with H_2O, or acetonitrile containing 10 equiv. of H_2O for the substrate). The mixture was stirred for 24 h at room temperature.

large-scale enzymatic hydrolysis of (±)-**7** was performed with a four-fold excess of lipase AK in acetonitrile containing water, and the pyridazinone derivative (*R*)-**7** bearing an (*R*)-5-methyl group was recovered in 50% yield (91% ee) along with the formation of **9** in 49% yield. Compound **9** bears a hydroxymethyl group on the nitrogen atom at the 2-position, which is usually eliminated at the same time as the ester hydrolysis.[13–16] The reason why the hydroxymethyl group was retained in this case is unclear, but the formation of a hydrogen bond with the neighboring carbonyl group may serve to stabilize this structure.

Finally, the pyridazinone (*R*)-**7** was hydrolyzed with aqueous ammonium hydroxide in methanol at room temperature to afford (*R*)-**6** in 86% yield (98% ee) after recrystallization. On the other hand, treatment of **9** with aqueous sodium hydroxide in ethanol afforded (±)-**6** (90%) as a racemic mixture, which can be recycled for the enzymatic hydrolysis.

In summary, we have demonstrated that the lipase-catalyzed hydrolysis of the acyloxymethylpyridazinone (±)-**7** was an efficient and practical pathway toward the chiral pyridazinone derivative (*R*)-**6** with sufficient enantiomeric purity. It is also noteworthy that the lipase-catalyzed hydrolysis of **7** exhibited high enantioselectivity despite the fact that the stereogenic center is remote (six-bonds apart) from the reactive center.

3. Experimental

3.1. General

Melting points were determined on a Yanagimoto micro melting point apparatus MP-500D. Elemental analyses were performed with a Yanagimoto CHN CORDER MT-5. ^{1}H NMR spectra were recorded on a JEOL JNM-EX400 or JEOL JNM-EX90 spectrometer using tetramethylsilane as an internal standard. ^{13}C NMR spectra were recorded on a JEOL JNM-LA400 spectrometer using tetramethylsilane as an internal standard. Infrared spectra (IR) were taken on a Hitachi infrared spectrophotometer 270-30. Mass spectra and high-resolution mass spectra (HR-MS) were recorded on a JEOL JMS-SX102A. Column chromatography was carried out on a silica gel (Kieselgel 60H, Merck). Enantiomeric purities were determined by HPLC analyses with a Hitachi L-6200 HPLC system equipped with a column packed with Chiralcel OD (Daicel Chemical Industries, Ltd.), eluent: hexane–2-propanol 9/1, flow rate: 1.0 cm^3/min, column temperature: room temperature, and wave length: 230 nm. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. The following enzymes were used: lipase A, lipase AK, lipase F-AP15, lipase M, lipase PS, protease A, protease M, promerain F, papain W-40, and pancreatin F (from Amano Seiyaku Co., Ltd.); lipase (from Wako Pure Chemical Industries, Ltd.); lipase type I, lipase type IA, lipase type II, and lipase type XI (from Sigma-Aldrich Co.).

3.1.1. 2-Bromo-1-(2-isopropylpyrazolo[1,5-*a*]pyridin-3-yl)propan-1-one, 3. Anhydrous aluminum(III) chloride (185 g, 1.39 mol) was added to an ice-cooled solution of 2-isopropylpyrazolo[1,5-*a*]pyridine[17] **1** (110 g, 0.69 mol) in dichloromethane (600 cm^3). The mixture was refluxed, and 2-bromopropionyl bromide **2** (150 g, 0.70 mol) was slowly added over 1.5 h. After heating the

Table 4. Hydrolysis of (±)-**7** using lipase AK with various amounts of enzyme

Solvent	Proportion of enzyme[c]	24 h		48 h	
		Conversion[d] (%)	Ee[d] (%)	Conversion[d] (%)	Ee[d] (%)
	1/100	3.6	–	–	–
	1/10	4.2	–	–	–
Diisopropyl ether[a]	1	11.4	–	–	–
	2	55.2	–	–	–
	10	90.0	–	–	–
	2	45.7	77.4	50.5	90.2
Acetonitrile[b]	3	49.3	93.6	54.7	98.9
	4	55.1	99.1	57.9	99.5

[a] Saturated with H_2O (water content, ca. 0.4%). The substrate concentration was 10 mg/cm^3.
[b] Containing 10 equiv. of H_2O. The concentration of the substrate was 30 mg/cm^3.
[c] Weight proportion of enzyme to the substrate.
[d] Conversions and enantiomeric purities were determined by HPLC analyses.

mixture under reflux for 2 h, the reaction mixture was cooled, poured into ice-water (2000 cm^3), and extracted with dichloromethane. The combined extracts were washed with water, and dried over sodium sulfate. The solution was concentrated in vacuo to give **3** (156 g, 77%) as a pale brown solid: mp 92–93°C (from hexane); IR (KBr) 1640, 1508 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) $\delta=1.41$ [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 1.44 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 1.94 (3H, d, $J=6.8$ Hz, $COCHBrCH_3$), 3.79 (1H, septet, $J=6.8$ Hz, $CHMe_2$), 5.06 (1H, q, $J=6.8$ Hz, $COCHMe$), 6.96 (1H, td, $J=6.8$ and 1.3 Hz, 6′-H), 7.47 (1H, ddd, $J=9.0$, 6.8, and 1.3 Hz, 5′-H), 8.12 (1H, dd, $J=9.0$ and 1.3 Hz, 4′-H), 8.51 (1H, dd, $J=6.8$ and 1.3 Hz, 7′-H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=20.4$ (C-3), 21.8 [$CH(CH_3)_2$], 22.7 [$CH(CH_3)_2$], 28.0 ($CHMe_2$), 45.8 ($COCHBrMe$), 106.8 (C-3′), 113.8 (C-4′), 119.5 (C-6′), 128.5 (C-5′), 129.5 (C-7′), 141.6 (C-3a′), 165.0 (C-2′), 187.9 (C=O); MS m/z (rel. intensity) 296/294 (M^+, 7/7), 187 (M–CH_3CHBr, 100). Found: C, 52.83; H, 5.09; N, 9.66%. Calcd for $C_{13}H_{15}BrN_2O$: C, 52.90; H, 5.12; N, 9.49%.

3.1.2. Dimethyl 2-[2-(2-isopropylpyrazolo[1,5-*a*]pyridin-3-yl)-1-methyl-2-oxoethyl]malonate, 4. Dimethyl malonate (239 g, 1.82 mol) was added to an ice-cooled suspension of sodium hydride (60%, 73.6 g, 1.84 mol) in DMF (1200 cm^3). After stirring for 1 h, a solution of **3** (267 g, 0.91 mol) in DMF (750 cm^3) was slowly added over 3 h. The mixture was stirred for additional 5 h at room temperature. The reaction mixture was added into ice-water (4000 cm^3) and extracted with ethyl acetate. The combined extracts were washed with water and brine, prior to drying over sodium sulfate. The solvent was removed in vacuo, and the residue was crystallized from hexane–diisopropyl ether (1/1) to give **4** (269 g, 86%) as a white powder: mp 101–102°C (from hexane–diisopropyl ether, 1/1); IR (KBr) 1764, 1744, 1642, 1506 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) $\delta=1.25$ (3H, d, $J=6.8$ Hz, $COCHCH_3$), 1.41 [6H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 3.60–4.18 [3H, m, $CHMe_2$ and $COCHCH(CO_2Me)_2$], 3.68 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 6.92 (1H, td, $J=6.8$ and 1.3 Hz, 6′-H), 7.42 (1H, ddd, $J=9.0$, 6.8, and 1.3 Hz, 5′-H), 8.10 (1H, dd, $J=9.0$ and 1.3 Hz, 4′-H), 8.49 (1H, dd, $J=6.8$ and 1.3 Hz, 7′-H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=16.2$ ($COCHCH_3$), 21.7 [$CH(CH_3)_2$], 22.7 [$CH(CH_3)_2$], 27.8 ($CHMe_2$), 43.9 ($COCHMe$), 52.71 (OCH_3), 52.73 (OCH_3), 54.3 [$CH(CO_2Me)_2$], 108.0 (C-3′), 113.4 (C-4′), 119.0 (C-6′), 128.1 (C-5′), 129.3 (C-7′), 141.7 (C-3a′), 164.5 (C-2′), 169.1 (CO_2Me), 169.5 (CO_2Me), 195.6 [$COCHCH(CO_2Me)_2$]; MS m/z (rel. intensity) 346 (M^+, 20), 187 [M–$CHMeCH(CO_2Me)_2$, 100]. Found: C, 62.43; H, 6.42; N, 8.30. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.42; H, 6.40; N, 8.09%.

3.1.3. 4-(2-Isopropylpyrazolo[1,5-*a*]pyridin-3-yl)-3-methyl-4-oxobutyric acid, 5. The dimethyl ester **4** (268 g, 0.77 mol) was added to 47% hydrobromic acid (800 cm^3) and stirred at 80–90°C for 5 h. The reaction mixture was poured into ice-water (3000 cm^3) and extracted with dichloromethane. The combined extracts were washed with water and dried over sodium sulfate. The solution was concentrated in vacuo, and the residue was triturated with diisopropyl ether. The resulting solid was collected by suction to give **5** (191 g, 90%) as a white solid: mp 134–136°C (from diisopropyl ether); IR (KBr) 3032, 1706, 1642, 1508 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) $\delta=1.29$ (3H, d, $J=6.8$ Hz, $COCHCH_3$), 1.37 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 1.40 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 2.49 (1H, dd, $J=16.7$ and 5.7 Hz, CH_2CO_2H), 3.05 (1H, dd, $J=16.7$ and 7.9 Hz, CH_2CO_2H), 3.60–3.93 (2H, m, $CHMe_2$ and $COCHMe$), 6.91 (1H, td, $J=6.8$ and 1.3 Hz, 6′-H), 7.41 (1H, ddd, $J=9.0$, 6.8, and 1.3 Hz, 5′-H), 8.06 (1H, dd, $J=9.0$ and 1.3 Hz, 4′-H), 8.53 (1H, dd, $J=6.8$ and 1.3 Hz, 7′-H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=17.7$ ($COCHCH_3$), 21.8 [$CH(CH_3)_2$], 22.6 [$CH(CH_3)_2$], 27.8 ($CHMe_2$), 37.1 ($COCHMe$), 40.2 (CH_2COOH) 108.3 (C-3′), 113.5 (C-4′), 118.9 (C-6′), 128.2 (C-5′), 129.4 (C-7′), 141.6 (C-3a′), 164.6 (C-2′), 177.6 (CO_2H), 197.2 (C=O); MS m/z (rel. intensity) 274 (M^+, 13), 187 (M–$CHMeCH_2CO_2H$, 100). Found: C, 65.47; H, 6.66; N, 10.18. Calcd for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21%.

3.1.4. (*RS*)-6-(2-Isopropylpyrazolo[1,5-*a*]pyridin-3-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one, (±)-6. Hydrazine monohydrate (80.0 g, 1.60 mol) was added to a suspension of **5** (190 g, 0.69 mol) in ethanol (800 cm^3). After heating the mixture under reflux for 10 h, water (4000 cm^3) was added and the mixture was stirred for 1 h. The resulting precipitates were collected by suction filtration to give (±)-**6** (173 g, 92%) as a white powder: mp 122–123°C (from ethanol); IR (KBr) 3236, 2964, 1678, 1636 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) $\delta=1.24$ (3H, d, $J=7.0$ Hz, 5-CH_3), 1.37 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 1.44 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 2.49 (1H, dd, $J=16.7$ and 7.0 Hz, 4-H), 2.83 (1H, dd, $J=16.7$ and 6.2 Hz, 4-H), 3.12–3.61 (2H, m, $CHMe_2$ and 5-H), 6.78 (1H, td, $J=6.8$ and 1.3 Hz, 6′-H), 7.19 (1H, ddd, $J=9.0$, 6.8, and 1.3 Hz, 5′-H), 7.71 (1H, dd, $J=9.0$ and 1.3 Hz, 4′-H), 8.44 (1H, dd, $J=6.8$ and 1.3 Hz, 7′-H), 8.87 (1H, br s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=16.0$ (5-CH_3), 22.4 [$CH(CH_3)_2$], 23.2 [$CH(CH_3)_2$], 27.4 ($CHMe_2$), 30.9 (C-5), 34.1 (C-4), 104.1 (C-3′), 112.1 (C-4′), 117.8 (C-6′), 125.2 (C-5′), 128.9 (C-7′), 139.4 (C-3a′), 152.3 (C-6), 160.3 (C-3), 166.8 (C-2′); MS m/z (rel. intensity) 270 (M^+, 100). HR-MS found: 270.1457. Calcd for $C_{15}H_{18}N_4O$: 270.1481.

3.1.5. (*RS*)-4,5-Dihydro-6-(2-isopropylpyrazolo[1,5-*a*]pyridin-3-yl)-5-methyl-2-(propionyloxymethyl)-pyridazin-3(2*H*)-one, (±)-7. A mixture of the pyridazinone (±)-**6** (71.9 g, 0.27 mol), potassium carbonate (217 g, 1.57 mol), and DMF (800 cm^3) was stirred at room temperature. Chloromethyl propionate (71.8 g, 0.59 mol) was added over 2 h, and the mixture was stirred for additional 19 h at room temperature. Insoluble materials were removed by filtration. Water (3000 cm^3) was added into the filtrate and the mixture was extracted with ethyl acetate. The combined extracts were washed with water and brine, prior to drying over sodium sulfate. The solvent was removed in vacuo, and

the residue was purified by column chromatography (silica gel, hexane–ethyl acetate, 1/1) to give (±)-**7** (84.8 g, 89%) as an yellowish oil: IR (neat) 2972, 1738, 1694 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) $\delta=1.16$ (3H, t, $J=7.5$ Hz, CH_2CH_3), 1.22 (3H, d, $J=6.5$ Hz, 5-CH_3), 1.37 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 1.44 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 2.39 (2H, q, $J=7.5$ Hz, CH_2Me), 2.54 (1H, dd, $J=16.5$ and 2.6 Hz, 4-H), 2.86 (1H, dd, $J=16.5$ and 6.5 Hz, 4-H), 3.20–3.62 (2H, m, $CHMe_2$ and 5-H), 5.80 (1H, d, $J=9.7$ Hz, NCH_2O), 5.94 (1H, d, $J=9.7$ Hz, NCH_2O), 6.80 (1H, td, $J=6.8$ and 1.5 Hz, 6′-H), 7.23 (1H, ddd, $J=9.0$, 6.8, and 1.1 Hz, 5′-H), 7.78 (1H, dd, $J=9.0$ and 1.1 Hz, 4′-H), 8.44 (1H, dd, $J=6.8$ and 1.1 Hz, 7′-H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=9.0$ (CH_2CH_3), 16.0 (5-CH_3), 22.3 [$CH(CH_3)_2$], 23.1 [$CH(CH_3)_2$], 27.5 ($CHMe_2$), 27.6 (CH_2Me), 31.1 (C-5), 34.7 (C-4), 71.1 (NCH_2O), 103.5 (C-3′), 112.3 (C-4′), 118.0 (C-6′), 125.6 (C-5′), 128.9 (C-7′), 139.6 (C-3a′), 153.7 (C-6), 160.6 (C-3), 165.7 (C-2′), 173.9 ($OCOCH_2CH_3$); MS m/z (rel. intensity) 356 (M^+, 53), 226 (M–CH_2OCOEt–*i*-Pr, 100). HR-MS found: 356.1832. Calcd for $C_{19}H_{24}N_4O_3$: 356.1848.

3.1.6. (*RS*)-4,5-Dihydro-6-(2-isopropylpyrazolo[1,5-*a*]-pyridin-3-yl)-2-(2,2-dimethylpropionyloxymethyl)-5-methylpyridazin-3(2*H*)-one, (±)-8. A mixture of the pyridazinone (±)-**6** (1.0 g, 3.7 mmol), potassium carbonate (3.02 g, 21.9 mmol), and DMF (11 cm^3) was stirred at room temperature. Chloromethyl pivarate (1.23 g, 8.2 mmol) was added over 3 min, and the mixture was stirred for 24 h at room temperature. Insoluble materials were removed by filtration. Water (42 cm^3) was added into the filtrate and the mixture was extracted with ethyl acetate. The combined extracts were washed with water and brine, prior to drying over sodium sulfate. The solvent was removed in vacuo, and the residue was separated by column chromatography (silica gel, hexane–ethyl acetate, 1/1) to give (±)-**8** (1.24 g, 88%) as a yellowish oil: IR (neat) 2972, 1736, 1698 cm^{-1}: ^1H NMR (400 MHz, $CDCl_3$) $\delta=1.218$ (3H, d, $J=6.8$ Hz, 5-CH_3), 1.222 [9H, s, $C(CH_3)_3$], 1.38 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 1.44 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 2.58 (1H, dd, $J=16.6$ and 2.0 Hz, 4-H), 2.83 (1H, dd, $J=16.6$ and 6.8 Hz, 4-H), 3.34 (1H, quintet of d, $J=6.8$ and 2.0 Hz, 5-H), 3.47 (1H, septet, $J=6.8$ Hz, $CHMe_2$), 5.72 (1H, d, $J=9.8$ Hz, NCH_2O), 6.02 (1H, d, $J=9.8$ Hz, NCH_2O), 6.81 (1H, td, $J=6.8$ and 1.0 Hz, 6′-H), 7.23 (1H, ddd, $J=8.8$, 6.8, and 1.0 Hz, 5′-H), 7.79 (1H, dd, $J=8.8$ and 1.0 Hz, 4′-H), 8.44 (1H, dd, $J=6.8$ and 1.0 Hz, 7′-H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=15.9$ (5-CH_3), 22.3 [$CH(CH_3)_2$], 23.1 [$CH(CH_3)_2$], 27.0 [$C(CH_3)_3$], 27.1 [$C(CH_3)_3$], 27.6 ($CHMe_2$), 31.1 (C-5), 34.7 (C-4), 39.0 (CMe_3), 71.1 (NCH_2O), 103.5 (C-3′), 112.3 (C-4′), 118.1 (C-6′), 125.5 (C-5′), 128.9 (C-7′), 139.6 (C-3a′), 153.7 (C-6), 160.6 (C-3), 165.4 (C-2′), 177.8 ($OCOCMe_3$), 1C missing; MS m/z (rel. intensity) 384 (M^+, 83), 226 (M–CH_2OCOEt–*i*-Pr, 100). HR-MS found: 384.2183. Calcd for $C_{21}H_{28}N_4O_3$: 384.2161.

3.1.7. General screening procedure for lipase-catalyzed hydrolysis of the acyloxymethyl derivatives. Method 1. A mixture of the solution of acyloxymethyl derivatives (0.2 cm^3, 1 mg/cm^3) and a lipase (ca. 2–3 mg) in diisopropyl ether saturated with water (water content: ca. 0.5%) was stirred for 24 h at room temperature. The reaction mixture was diluted with acetic acid (0.5 cm^3) and separated by centrifugation. The supernatant solution was concentrated in vacuo. Enantiomeric purities were determined by HPLC analyses.

Method 2. A mixture of the solution of acyloxymethyl derivatives (0.02 cm^3, 10 mg/cm^3) and a lipase (ca. 2–3 mg) in an acetone–phosphate buffer solution (0.18 cm^3, pH 6.8) was stirred for 24 h at room temperature. The reaction mixture was extracted with ethyl acetate (0.5 cm^3), and the solvent was removed in vacuo. The residue was diluted with ethanol (50 mm^3). Enantiomeric purities were determined by HPLC analyses.

Retention times of the substrates and products are as follows: (*R*)-**7**: 23 min, (*S*)-**7**: 33 min, (*R*)-**8**: 34 min, (*S*)-**8**: 63 min, (*R*)-**9**: 45 min, and (*S*)-**9**: 53 min.

3.1.8. (*R*)-4,5-Dihydro-6-(2-isopropylpyrazolo[1,5-*a*]-pyridin-3-yl)-5-methyl-2-(propionyloxymethyl)pyridazin-3(2*H*)-one, (*R*)-7 and (*S*)-4,5-dihydro-2-(hydroxymethyl)-6-(2-isopropylpyrazolo[1,5-*a*]pyridin-3-yl)-5-methylpyridazin-3(2*H*)-one, (*S*)-9. A solution of ester (±)-**7** (27.1 g, 0.076 mol) in acetonitrile (905 cm^3) was added water (13.6 g) and lipase AK (106 g). The mixture was stirred for 42 h at room temperature. Insoluble materials were removed by filtration. The filtrate was concentrated in vacuo, and the residue was dissolved in ethyl acetate (800 cm^3). The solution was washed with aqueous sodium hydrogencarbonate and brine, prior to drying over sodium sulfate. The solvent was removed in vacuo, and the residue was separated by column chromatography (silica gel, hexane–ethyl acetate, 1/1) to give (*R*)-**7** (13.5 g, 50%, 91% ee) and **9** (11.3 g, 49%).

3.1.8.1. (*R*)-4,5-Dihydro-6-(2-isopropylpyrazolo[1,5-*a*]-pyridin-3-yl)-5-methyl-2-(propionyloxymethyl)pyridazin-3(2*H*)-one, (*R*)-7. Yellow liquid; IR (neat) 1738, 1686, 1634 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) $\delta=1.16$ (3H, t, $J=7.0$ Hz, CH_2CH_3), 1.22 (3H, d, $J=7.3$ Hz, 5-CH_3), 1.37 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 1.44 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 2.39 (2H, q, $J=7.6$ Hz, CH_2Me), 2.58 (1H, dd, $J=16.6$ and 2.0 Hz, 4-H), 2.83 (1H, dd, $J=16.6$ and 7.0 Hz, 4-H), 3.31–3.50 (2H, m, $CHMe_2$ and 5-H), 5.83 (1H, d, $J=9.8$ Hz, NCH_2O), 5.91 (1H, d, $J=9.8$ Hz, NCH_2O), 6.81 (1H, t, $J=6.8$ Hz, 6′-H), 7.24 (1H, dd, $J=9.0$ and 6.8 Hz, 5′-H), 7.78 (1H, d, $J=9.0$ Hz, 4′-H), 8.44 (1H, d, $J=6.8$ Hz, 7′-H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=9.0$ (CH_2CH_3), 16.0 (5-CH_3), 22.3 [$CH(CH_3)_2$], 23.1 [$CH(CH_3)_2$], 27.5 ($CHMe_2$), 27.6 (CH_2Me), 31.1 (C-5), 34.7 (C-4), 71.1 (NCH_2O), 103.5 (C-3′), 112.3 (C-4′), 118.0 (C-6′), 125.6 (C-5′), 128.9 (C-7′), 139.6 (C-3a′), 153.7 (C-6), 160.6 (C-3), 165.7 (C-2′), 173.9 ($OCOCH_2CH_3$). MS m/z (rel. intensity) 356 (M^+, 54), 226 (M–CH_2OCOEt–*i*-Pr, 100). HR-MS found: 356.1841. Calcd for $C_{19}H_{24}N_4O_3$: 356.1848.

3.1.8.2. (*S*)-4,5-Dihydro-2-(hydroxymethyl)-6-(2-isopropylpyrazolo[1,5-*a*]pyridin-3-yl)-5-methylpyridazin-3(2*H*)-one, 9. Yellow liquid; IR (neat) 3424, 2972, 1634 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) $\delta=1.21$ (3H, d, $J=6.6$ Hz, 5-CH_3), 1.38 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 1.44 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 2.50 (1H, dd, $J=16.5$ and 2.6 Hz, 4-H), 2.83 (1H, dd, $J=16.5$ and 6.6 Hz, 4-H), 3.12–3.62 (2H, m, $CHMe_2$ and 5-H), 3.95 (1H, br, OH), 5.32 (2H, br s, NCH_2O), 6.77 (1H, td, $J=6.8$ and 1.3 Hz, 6′-H), 7.20 (1H, ddd, $J=9.0$, 6.8, and 1.3 Hz, 5′-H), 7.76 (1H, dd, $J=9.0$ and 1.3 Hz, 4′-H), 8.44 (1H, dd, $J=6.8$ and 1.3 Hz, 7′-H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=16.0$ (5-CH_3), 22.4 [$CH(CH_3)_2$], 23.1 [$CH(CH_3)_2$], 27.6 ($CHMe_2$), 31.1 (C-5), 34.7 (C-4), 72.7 (NCH_2OH), 103.8 (C-3′), 112.2 (C-4′), 117.9 (C-6′), 125.4 (C-5′), 128.9 (C-7′), 139.5 (C-3a′), 152.9 (C-6), 160.4 (C-3), 166.0 (C-2′); MS m/z (rel. intensity) 300 (M^+, 21) 270 (M–CH_2O, 100). HR-MS found: 300.1605. Calcd for $C_{16}H_{20}N_4O_2$: 300.1586.

3.1.9. (*R*)-4,5-Dihydro-6-(2-isopropylpyrazolo[1,5-*a*]pyridin-3-yl)-5-methylpyridazin-3(2*H*)-one, (*R*)-6. To a solution of the (*R*)-**7** (13.5 g, 0.038 mol) in methanol (300 cm^3) was added aqueous ammonium hydroxide (25%, 30 cm^3). The mixture was stirred for 16 h at room temperature. The reaction mixture was concentrated in vacuo, and the residue was added to aqueous sodium hydrogencarbonate. The mixture was extracted with dichloromethane, and the organic phase was dried over sodium sulfate. The solvent was removed in vacuo, and the residue was separated by column chromatography (silica gel, dichloromethane–methanol, 40/1). The resulting solid was recrystallized from dichloromethane–2-propanol–diisopropyl ether to give (*R*)-**6** (8.83 g, 86%) as a white solid: mp 158–159°C (from); IR (neat) 3229, 2959, 1674, 1636 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) $\delta=1.24$ (3H, d, $J=6.8$ Hz, 5-CH_3), 1.37 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 1.43 [3H, d, $J=6.8$ Hz, $CH(CH_3)_2$], 2.51 (1H, dd, $J=16.6$ and 2.4 Hz, 4-H), 2.81 (1H, dd, $J=16.6$ and 6.8 Hz, 4-H), 3.29 (1H, quintet of d, $J=6.8$ and 2.4 Hz, 5-H), 3.45 (1H, septet, $J=6.8$ Hz, $CHMe_2$), 6.79 (1H, td, $J=6.8$ and 1.0 Hz, 6′-H), 7.20 (1H, ddd, $J=8.8$, 6.8, and 1.0 Hz, 5′-H), 7.71 (1H, dd, $J=8.8$ and 1.0 Hz, 4′-H), 8.44 (1H, dd, $J=6.8$ and 1.0 Hz, 7′-H), 8.62 (1H, br s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=16.0$ (5-CH_3), 22.4 [$CH(CH_3)_2$], 23.2 [$CH(CH_3)_2$], 27.4 ($CHMe_2$), 30.9 (C-5), 34.1 (C-4), 104.0 (C-3′), 112.1 (C-4′), 117.8 (C-6′), 125.2 (C-5′), 128.9 (C-7′), 139.4 (C-3a′), 152.3 (C-6), 160.3 (C-3), 166.7 (C-2′); MS m/z (rel. intensity) 270 (M^+, 100). Found: C, 66.28; H, 6.85; N, 20.63. Calcd for $C_{15}H_{18}N_4O$: C, 66.65; H, 6.71; N, 20.73%. $[\alpha]_D^{24}=-185$ (c 1.07, $CHCl_3$), 98% ee.

3.1.10. Elimination of the hydroxymethyl group and racemization of 9. Aqueous sodium hydroxide solution (20%, 80 cm^3) was added to a solution of the hydroxymethyl derivative **9** (2.01 g, 6.69 mmol) in ethanol (80 cm^3). The mixture was stirred for 18 h at room temperature. The reaction mixture was poured into water (200 cm^3) and the product was extracted with ethyl acetate. The combined extracts were washed with water and brine, prior to drying over sodium sulfate. The solution was concentrated in vacuo to give (±)-**6** (1.63 g, 90%) as a white powder.

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