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Arthrobacter sp.: a lipase of choice for the kinetic resolution of racemic arylazetidinone precursors of taxanoid side chains

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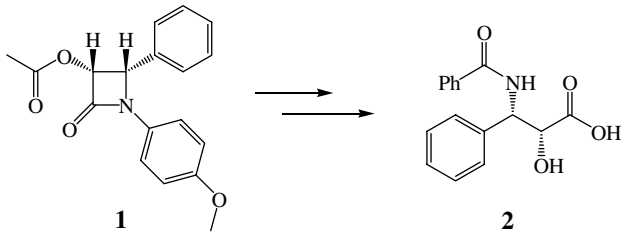
Abstract—The native strain of *Arthrobacter* sp. (MTCC 5125) bearing a lipase has been found to be the most effective in the kinetic resolution of racemic arylazetidinones for producing *cis*-(3*R*,4*S*)-3-acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone, *cis*-(3*R*,4*R*)-3-acetoxy-1-(4-methoxyphenyl)-4-(2-furanyl)-2-azetidinone, *cis*-(3*R*,4*R*)-3-acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone, and *cis*-3-acetoxy-4-(*t*-butyl)-2-azetidinone products. The resolved compounds, which were obtained in high enantiopurity are important intermediates of amino acid side chains of paclitaxel as well as a new generation of taxanoids. The use of co-solvents dramatically improved the resolution efficacy of the lipase.

1. Introduction

Paclitaxel, a complex polyoxygenated diterpenoid isolated from the bark of *Taxus brevifolia*,[1] has been extensively used in anti-cancer therapy.[2] Continuing efforts are being made to meet its high world-wide demand by semi-synthetic methods. The discovery of 10-deacetyl baccatin III (10-DAB) in 1981 opened the gateway to semi-synthetic paclitaxel, wherein 10-DAB was obtained from *Taxus baccata* and the C-13 side chain was prepared synthetically.[3,4] M/s Bristol-Myers Squibb, regarded as the major producer of paclitaxel, reportedly exploited the semi-synthetic route.[5] The basic requirement in this method is to prepare the *N*-benzoyl-β-phenylisoserine side chain **2** with a protected 2′-OH group and 10-DAB protected at the 7-OH. Ever since the initial work of Potier,[6] a number of attempts have been made to convert 10-DAB to paclitaxel, all of these methods generally relied on the coupling of a 7-protected baccatin derivative to a protected phenylisoserinate, which in turn can be prepared from either glycidates[3] or azetidinones, for example, **1** (Scheme 1).[4]



Scheme 1.

2-Azetidinones are well known due to their potent bioactivity and importance as a part of the family of β-lactam antibiotics.[7] Furthermore, asymmetric syntheses,[8] and the preparation of their chiral intermediates either by chemical[9] or chemoenzymatic[10] methods have also been described. However, biocatalytic methods are now increasingly being employed due to the efficacy of enzymatic resolution and simple reaction conditions. Thus, the use of the lipase from *Pseudomonas* sp. has been more frequent,[4,11] whereas PPL and PLE have also been used for the stereoselective hydrolysis of various 2-azetidinone derivatives.[12]

Studies on the structure modifications of paclitaxel have been carried out to design newer analogs, which display improved bioactivity and low toxicity. The phenyl group at the 3′-position on the C-13 side chain has been an important and easy target for the synthesis of new compounds. A number of such new generation taxanoids have been

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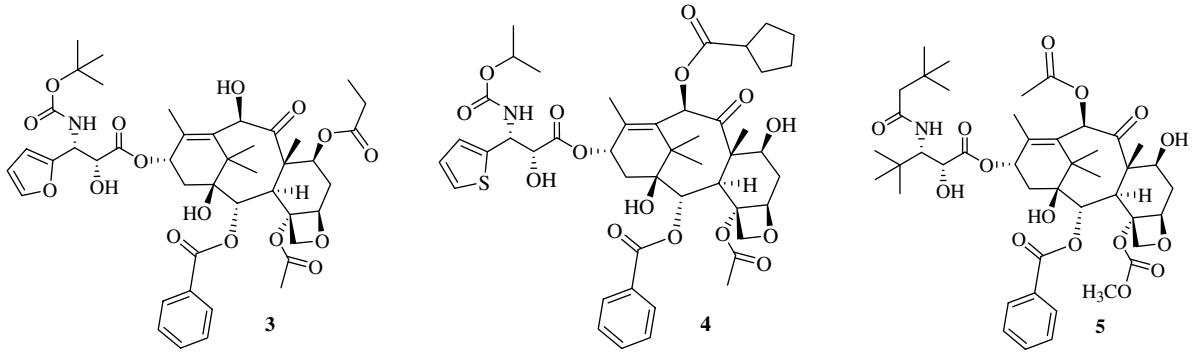
synthesized and are currently under various stages of clinical trials, for example, MAC-321 (TL-00139)[13] **3**; MST-997 (TL-909)[14] **4**; BMS-275183 **5**.[15]



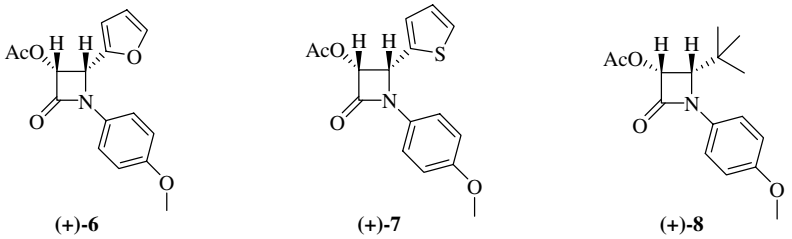
The azetidinone precursors of side chain of new generation of semi-synthetic paclitaxel analogues **3**–**5** are represented by structures **6**–**8**, respectively. A process of resolution of β-lactam (±)-**6** via enantioselective hydrolysis using homogenized beef liver to give a product in >95% enantiopurity in 40% yield has been disclosed in a patent by Holtan.[11a] The enzymatic resolution of a side chain precursor of **4** has also been described by Bisht et al.[4a] using PS-30 to obtain both enantiomers in >97% enantiopurity. The side chain precursor of Taxotere, that is, (±)-*cis*-3-acetoxy-4-(1,1-dimethylethyl)-2-azetidinone **8** with lipase PS-30 or crude BMS lipase from *Pseudomonas*, has also been reported by Patel et al.[11b,c] However, an improved and highly efficient kinetic resolution process of the above racemic precursors with high enantiopurity of the products (∼99%) will remain a prerequisite for industry due to their biological importance as well as future demand. In our ongoing program of the development of efficient kinetic resolutions methods, we have reported the preparation of various chiral drugs/drug intermediates and chiral auxiliaries using indigenous and commercial sources of biocatalysts, whole cell preparations as well as immobilized enzymes.[16] Herein, we report a highly efficient kinetic resolution protocol for the preparation of **1** and **6**–**8** wherein the whole cells preparation of ABL was successfully used for the kinetic resolution of all the racemates.

phenyl)-4-*t*-butyl-2-azetidinone **8**, *cis*-3-propyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone **10**, *cis*-3-butyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone **11**, and *cis*-3-hexyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone **12**, were first carried out via a Staudinger reaction. The Schiff's base from the benzaldehyde and *p*-anisidine was prepared using microwaves and the rest by acid catalyzed (*p*-toluenesulfonic acid) reaction between the corresponding aldehydes and *p*-anisidine using a Dean–Stark apparatus.

The Schiff's bases thus prepared were reacted with acetoxyacetyl chloride (AAC) in the presence of triethylamine (TEA) in dry dichloromethane to yield β-lactams (72–90%) via a [2+2] cycloaddition reaction (Scheme 2). In all of the reactions, only one diastereomer with a *cis*-configuration was obtained as confirmed by ^{1}H NMR.

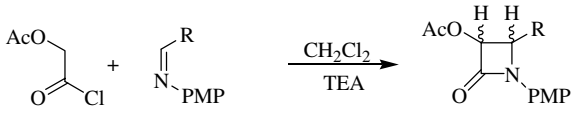
After the formation of racemic 2-azetidinones, a preliminary screen was undertaken to select the most suitable lipase for enantioselective hydrolysis. A set of 14 lipases and whole cell preparations, both from commercial sources, as well as the institutes microbial repository were used to affect the hydrolysis. The results of the preliminary screening are summarized in Table 1.

It is apparent from Table 1 that four lipases, that is, PS, PS-C, AS, and *Arthrobacter* sp. (MTCC 5125, ABL) were able to hydrolyze the substrates and ABL was the only one which hydrolyzed all four substrates (shown with a positive sign). Detailed studies of the kinetic resolution of racemic β-lactams were undertaken via stereoselective hydrolysis of the corresponding racemic esters, which were incubated with selected lipases, that is, *Aspergillus niger* (Amano AS), *Pseudomonas* sp. now known as *Burkholderia cepacia* (Amano PS), *B. cepacia* on ceramics (Amano PS-C), and whole cells of *Arthrobacter* sp. (ABL).



2. Results and discussion

For the proposed kinetic resolution studies, the syntheses of racemic *cis*-3-acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone **1**, *cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone **6**, *cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone **7**, *cis*-3-acetoxy-1-(4-methoxy-



1, R = phenyl
7, R = 2-thienyl

6, R = 2-furyl
8, R = *t*-butyl

Scheme 2.

Table 1. Screening of lipases on substrates **1**, **6**, **7**, and **8**

Enzyme	Substrates			
	1	**6**	**7**	**8**
PS	+	+	+	–
PS-C	+	+	+	–
PF	–	–	–	nd
AS	+	+	+	–
ABL	+	+	+	+
CRL	–	–	–	–
PLAP	–	–	–	–
MJ	–	–	–	nd
RRL–1789	–	–	–	–
MM	–	–	–	nd
Lipase G	–	–	–	nd
RRLBB-1	–	–	–	–
PPL	–	–	–	nd
CCL	–	–	–	nd

(+) = Hydrolysis; (–) = no reaction; (nd) = not determined; PS (*Burkholderia cepacia*, Amano PS), PS-C (*Burkholderia cepacia* on ceramics, Amano PS-C), PF (*Pseudomonas florescence*), MJ (*Mucor javanicus*), ABL (*Arthrobacter* sp.), CRL (*Candida rugosa* lipase), PPL (Porcine pancreatic lipase), RRLBB1 (*Bacillus subtilis*), RRL-1789 (*Bacillus* sp.), Acylase (Amano Acylase), MM (*Mucor miehei*), AS (*Aspergillus niger*, Amano AS), Lipase G, CCL (*Candida cylindracea*), PLAP (Pig Liver Acetone Powder).

2.1. Resolution of (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone 1

The stereoselective hydrolysis was carried out in an aqueous 0.1 M-phosphate buffer at pH 7.0 in the temperature range 25–26 °C. Due to the slow rate of hydrolysis (15% conversion in 5 h, ee = 99%) the use of co-solvents was envisaged (Scheme 3). The addition of organic co-solvents during enzymatic hydrolysis is known to influence the rates of hydrolysis as well as enatioselectivity.[17]

The addition of organic co-solvents in ratios ranging from 10% to 50% in aqueous pH 7.0 phosphate buffer medium was studied and their effect on the rates of hydrolysis and enantioselectivity are shown in Tables 2 and 3. It was

Table 2. Enzymatic hydrolysis of (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (±)-**1** at 26 °C at 20 g/L

Enzyme	Co-solvent	Conversion	*T* (h)	ee_s	ee_p	*E*
Amano AS	CH_3CN	46	78	84	96	125
	DMF	49	78	82	85	31
	DMSO	49	78	83	87	38
Amano PS	CH_3CN	49	24	96	99	752
	DMF	48	24	93	98.5	425
	DMSO	49	24	97	98	357
Amano PSC	CH_3CN	50	10	99	99	>1000
	DMF	50	6	99	99	>1000
	DMSO	49	9	99	99	752
ABL	CH_3CN	**50**	**6**	**99**	**99**	**>1000**
	DMF	**50**	4.5	**>99**	**>99**	**>1000**
	DMSO	**50**	**5**	**>99**	**>99**	**>1000**

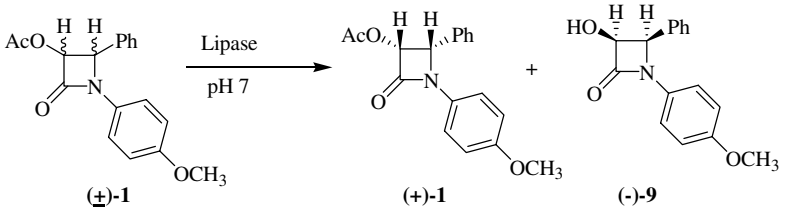
Table 3. ABL catalyzed hydrolysis of (±)-**1** after 5 h at 20 g/L concentration at 26 °C[a]

Co-solvent	10%	20%	30%	40%	50%
DMF	50	47	<1	Nil	Nil
Acetone	45	10	<1	Nil	Nil
CH_3CN	41	<5	Nil	Nil	Nil
DMSO	37	45	50	42	<1
Toluene	20	Nd	Nd	Nd	Nd
DCM	17	Nd	Nd	Nd	Nd

Nd—not determined.
ABL concentration 2% (wet weight/v).
[a] 8.2% conversion in 5 h in buffer only (in the absence of any co-solvent).

observed that when compared to commercial lipases, ABL was more effective in stereoselective hydrolysis, furnishing only one enantiomer (ee >99%) with high space–time yields. ABL also displayed high activity in almost all the co-solvents used and remained active even in the presence of 30–40% DMSO, whereas other enzymes became inactive at those concentrations (Table 3). Only PSC which is a ceramics immobilized lipase could match the efficacy of ABL for the resolution of (±)-**1**.

In order to further optimize the reaction conditions, stereoselective hydrolysis at varied concentrations ranging from 20 to 100 g/L was attempted. It was observed that ABL was quite active at 40–50 g/L (Fig. 1). At a concentration of 20 g/L, 50% conversion was obtained in 5 h, whereas at 40 g/L, 50% conversion was achieved in 10 h and 24 h at 60 g/L. Space–time yield calculations show that a concentration of 40 g/L is the optimum (6.43 mmol/h). On



(±)-1 (+)-1 (-)-9

Scheme 3.

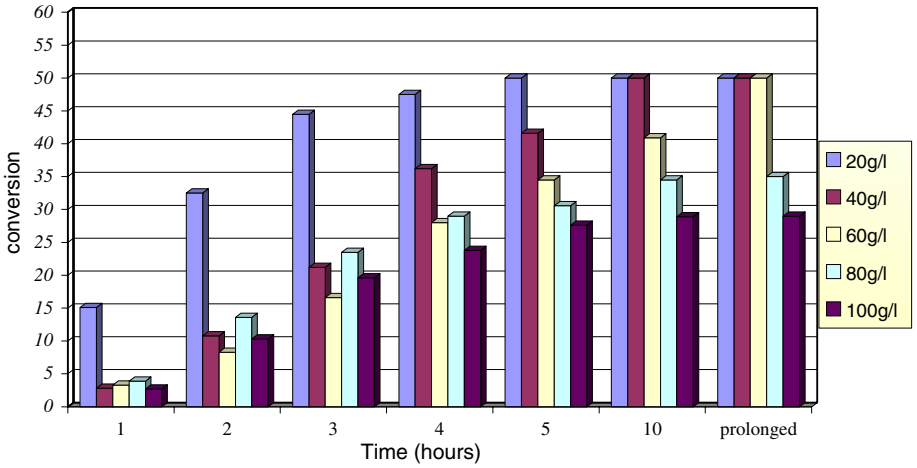


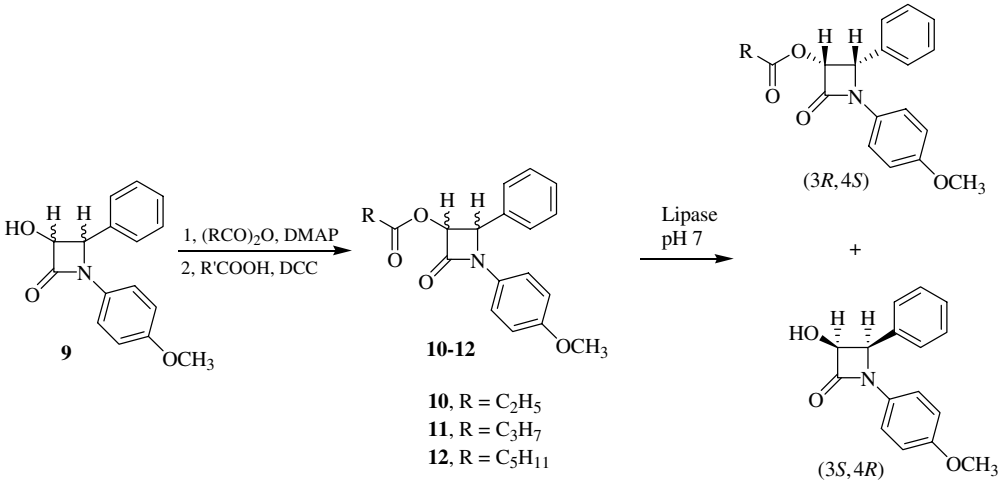
Figure 1. Concentration v/s conversion in DMF using ABL at 26 °C.

increasing the concentrations to 80–100 g/L, no conversion beyond 35% was observed.

In addition to the above studies, the effect of the size of acyl groups at C-3 were also investigated for the resolution of racemic *cis*-3-acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone **1** with respect to different lipases. Therefore, the alkyl acylates, that is, *cis*-3-propyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone **10**, *cis*-3-butyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone **11**, and *cis*-3-hexyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone **12** were prepared and subjected to kinetic resolution using all four lipases (Scheme 4, Table 4).

During the hydrolysis of esters **10**–**12**, ABL behaved more or less in a similar manner as in **1**, whereas both PS and PS-C showed a marked improvement of enantioselectivity as well as rates of hydrolysis of **11** and **12** in the presence of 10% DMF. The commercial lipases PS and PS-C hydrolyzed the racemic propionate ester **10** in only 8 h (~50% conversion, ee 99%) whereas it took more than 24 h to hydrolyze the racemic acetoxy derivative **1**. A significant improvement in the rates of hydrolysis with higher esters for both PS and PS-C was probably missed by other workers.

In order to fully explore the ability of the native strain bearing lipase from *Arthrobacter* sp., a unit wise comparative



Scheme 4.

Table 4. Effect of alky acyloxy groups with 10% DMF at 26 °C

Enzyme	Propyloxy (**10**)				Butyloxy (**11**)				Hexyloxy (**12**)			
	T (h)	*C* (%)	ee_s	ee_p	*T* (h)	*C* (%)	ee_s	ee_p	*T* (h)	*C* (%)	ee_s	ee_p
AS	8	34	24	78	48	0	—	—	24	0	—	—
PS	8	44	89	99	48	47	92	99	24	49	99	99
PSC	8	48	96	99	48	49	99	99	24	46	87	99
ABL	8	49	99	99	48	21	27	92	24	48	<5	<5

Table 5. Unit wise comparative study of ABL on (±)-**1** at 26 °C

Enzyme	Conversion percentage					
	1 h	2 h	3 h	4 h	5 h	6 h
ABL (wet pellet)	9	18	33	41	49	50
ABL (lyophilized whole cells)	26	42	49	50	50	50
ABL (cell free)	19	21	31	34	39	42
ABL (cell free, lyophilized)	8	13	20	26	34	38

Reactions were carried out using ~40,000 units in each experiment.

study was carried out by using (a) whole cells wet pellet; (b) whole cells lyophilized; (c) cell free extract; and (d) cell free lyophilized powder.

The results of these studies clearly show that ABL prepared in the form of whole cells lyophilized powder was the most suitable to affect the enantioselective hydrolysis as shown in Table 5.

As already discussed, a new generation of taxanoids such as **3**–**5** are undergoing clinical trials, therefore the synthesis and resolution to obtain the optically active amino acid side chain of these analogues is a challenging task. Moreover, it was also our endeavor to explore the stereoselective hydrolytic capability of ABL as well as other commercial lipases with respect to other important azetidinones. The racemic azetidinones, that is, *cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone **6**, *cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone **7**, and *cis*-3-acetoxy-1-(4-methoxyphenyl)-4-*t*-butyl-2-azetidinone **8** were synthesized for kinetic resolution studies and development of amino acid side chains having 2-furyl, 2-thienyl and *t*-butyl groups, respectively, at C-4.

2.2. Resolution of (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone 6

Enantiomerically pure *cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone **6** is the precursor of the amino acid side chain of paclitaxel analogue **3** [MAC-321(TL-00139)]. Racemate **6** was subjected to kinetic resolution studies using the panel of lipases in the presence of organic co-solvents (Scheme 5) and the results are summarized in Table 6. It is apparent from these results that ABL as well as PS-C were the most suitable lipases for producing the desired ester with high enantiopurity ($E = 1057$).

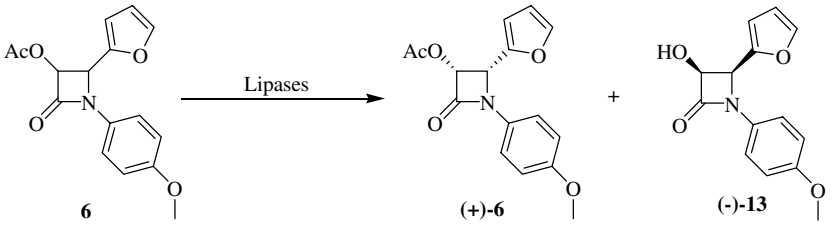
Table 6. Kinetic resolution of (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone (±)-**6** (30 °C)

Enzyme	Co-solvent	T (h)	C (%)	ee_s	ee_p	E
AS	Nil	24	23	5	27	<5
	DMF	24	44	45	58	6
	DMSO	24	38	24	41	<5
PS	Nil	24	27	41	99	285
	DMF	24	50	99	99	>1000
	DMSO	24	43	85	99	449
PS-C	Nil	15	41	69	99	412
	DMF	15	50	99	99	>1000
	DMSO	15	50	99	99	>1000
ABL	Nil	24	30	45	99	302
	DMF	24	50	98.5	98.5	650
	DMSO	20	50	99	99	>1000

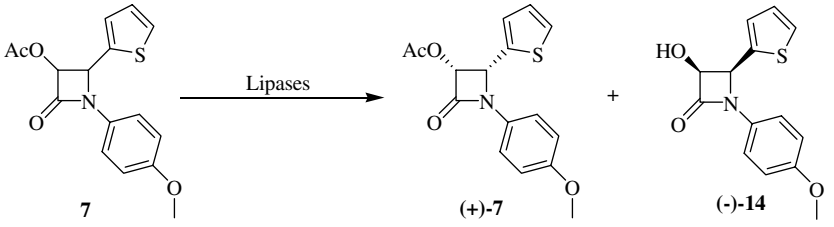
On the basis of the reported specific rotation values, the absolute configuration at C-3 was also established as (*R*) in case of unhydrolyzed esters and (*S*) in case of the alcohols.

2.3. Resolution of (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone 7

Racemic *cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone **7** is another important precursor of paclitaxel analogue **4** [MST-997 (TL-909)]. Its racemate was also successfully resolved using ABL and other lipases in the presence of organic co-solvents (Scheme 6) to produce (+)-**7** and (−)-**14**, respectively. The results of these experiments are summarized in Table 7. Here again ABL clearly



Scheme 5.



Scheme 6.

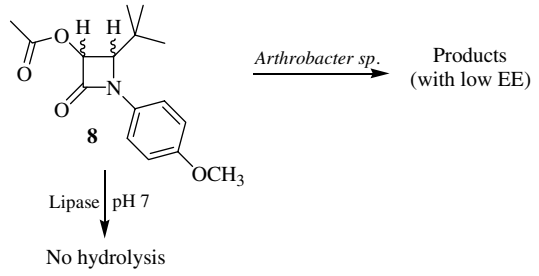
Table 7. Kinetic resolution of (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone **7** at 30 °C

Enzyme	Co-solvent	*T* (h)	*C* (%)	ee_s	ee_p	*E*
AS	Nil	8	30	42	80	13
	CH_3CN	8	48	83	86	32
	DMF	8	48	76	83	25
	DMSO	8	49	84	87	38
PS	Nil	8	36	58	98	173
	CH_3CN	8	44	79	98	232
	DMF	8	47	89	99	581
	DMSO	8	50	92	93	94
PS-C	Nil	8	36	55	98	173
	CH_3CN	8	49	95	98	357
	DMF	8	48	87	96	146
	DMSO	8	47	86	97	183
ABL	Nil	8	32	47	99	316
	CH_3CN	8	46	85	99	536
	DMF	8	48	92	99	645
	DMSO	8	47	89	99	581

scored over other lipases, thus affecting 48% conversion in only 8 h ($E = 645$).

2.4. Resolution of (±)-*cis*-3-acetoxy-4-*t*-butyl-2-azetidinone 15

Racemic *cis*-3-acetoxy-1-(4-methoxyphenyl)-4-*t*-butyl-2-azetidinone **8**, which is a side chain precursor of an oral taxane known as Taxotere,[18] was also subjected to lipase catalyzed kinetic resolution. It was observed that all the lipases used were unable to hydrolyze it, including ABL which could hydrolyze up to 10% in 16 h with very low ee (<5%). The low acceptance of **8** by lipases is probably due to the steric effect of the *t*-butyl group (Scheme 7).



Scheme 7.

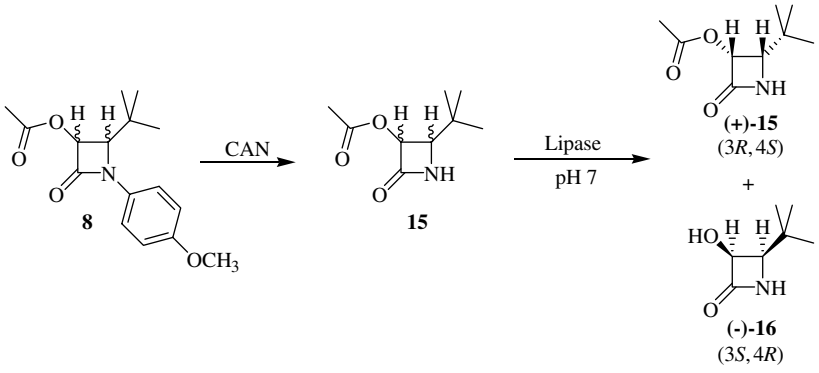
Table 8. Kinetic resolution of (±)-*cis*-3-acetoxy-4-*t*-butyl-2-azetidinone **15** (22 °C)

Enzyme	Co-solvent	*T* (h)	*C* (%)	ee_s	ee_p	*E*
AS	Nil	4	41	68	99	8
	CH_3CN	4	22	12	99	<5
	DMF	4	26	4	99	<5
PS	Nil	4	48	98	98	311
	CH_3CN	4	50	99	99	>1000
	DMF	4	50	99	99	>1000
PS-C	Nil	4	50	99	99	>1000
	CH_3CN	4	50	99	99	>1000
	DMF	4	50	99	99	>1000
ABL	Nil	4	50	99	99	>1000
	CH_3CN	4	50	99	99	>1000
	DMF	4	50	99	99	>1000

The problem of hydrolyzing azetidinone derivative **8** was, however, resolved by removing the *p*-methoxyphenyl group on reacting with ammonium ceric nitrate (CAN) **15**. The resulting *cis*-3-acetoxy-4-*t*-butyl-2-azetidinone **15** was now easily amenable to ABL, PS, and PS-C. Thus racemic **15** was hydrolyzed stereoselectively to produce enantiomers (+)-**15** and (−)-**16** in 99% ee after 4 h (Scheme 8, Table 8).

3. Conclusions

In conclusion the hydrolytic activity of a series of lipases toward 2-azetidinone derivatives which are important precursors of amino acid side chain of paclitaxel. 2-Azetidinone derivatives with varying substitutions at C-3 were synthesized and kinetically resolved using lipases. The kinetic resolutions of (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone, (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone, and (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone were achieved using ABL with ee ~99%. The use of co-solvents, such as CH_3CN, DMF, and DMSO, dramatically improved the enantioselectivity and reduced reaction timings. Taxotere side chain intermediate (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-*t*-butyl-2-azetidinone, which could not be directly hydrolyzed by any one of the lipases used, was effectively resolved (ee 99%) after its conversion to (±)-*cis*-3-acetoxy-4-*t*-butyl-2-azetidinone. In conclusion *Arthrobacter* sp. (ABL), the native strain bearing lipase



Scheme 8.

was found to be the most effective that could kinetically resolve all the substrates used in the present study with high ee.

4. Experimental

4.1. General

NMR spectra were recorded on Bruker 200 MHz and 500 MHz spectrometers in the indicated solvents with tetramethylsilane as the internal standard. IR was recorded on FT-IR Bruker 270-30 spectrophotometer. Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter in the indicated solvents. MS were recorded on Bruker esquire 3000 and Thermo-Finnigan TSQ 5000. GC–MS was carried out on Shimadzu GC–MS *QP*2000. Elemental analysis was performed on an Elementar CHNS analyzer. Melting points were determined by open capillary method on Buchi B-542 apparatus and are uncorrected. TLC was performed on Silica gel 60 F_{254} (Merck), using ethyl acetate–hexane (20:80, 30:70, or 40:60) as mobile phase. Chiral HPLC was performed on Shimadzu and Thermo-Finnigan equipments using (*R*,*R*) Whelk-O1, Chiralcel OJ-H and OD-H chiral columns, and hexane–isopropyl alcohol–acetic acid (97:3:0.1), hexane–ethyl alcohol (98.1:1.9) and hexane–isopropyl alcohol–acetic acid (95:5:0.1) as mobile phases. The UV detection was done at 210, 254, and 260 nm.

4.2. Preparation of the cell biomass of ABL

The microorganism *Arthrobacter* sp. (ABL) was isolated from the fresh water samples collected from Jammu hills and has been deposited in MTCC culture collection at IMT, Chandigarh (MTCC No. 5125) under Budapest Treaty (2004).

ABL cell biomass was prepared in 10 L fermentor containing medium (1% peptone, and 0.5% NaCl and 0.5% beef extract, pH 7.0). The medium was inoculated with an overnight preculture prepared in the same broth. The culture was grown at 30 °C for 16–18 h at 200 rpm. The cell pellet was separated from the broth by centrifugation at 10,000*g* for 15 min at 4 °C. The cell pellet was preserved at −20 °C till further use. The moisture contents in the wet cell pallet was estimated as 75–80%.

4.3. ABL enzyme isolation

Lipase from *Arthrobacter* sp. was obtained by ultrasonication of cells (1 U/mg wet biomass) in phosphate buffer, pH 7.0 using MSE Manor Roya Crawley RH 10 2QQ cell disrupter at 16 kHz. Cell free extract (CFE) as crude enzyme preparation was used directly. The CFE obtained from the above method was partially purified by 60% ammonium sulphate precipitation. The precipitates were then dissolved in phosphate buffer (0.1 M, pH 7.0) and dialyzed against the phosphate buffer (10 mM, pH 7.0). The partially purified enzyme was lyophilized (specific activity 40 units per mg protein) and stored at −20 °C till use.

4.4. General method of preparation of the imines

An imine from the benzaldehyde and *p*-anisidine was prepared by irradiating for 3 min an equimolar solution (50 mmol) in ethanol (50 mL) in a commercial microwave oven at 150 W, and the rest were prepared by refluxing in toluene (100 mL). The water formed during the reaction was removed azeotropically using Dean–Stark apparatus. Excess of the solvent was removed and the imines were stored under moisture free conditions and used as such without further purification.

4.5. (±)-*cis*-3-Acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone 1

Triethylamine (1.51 g, 15.0 mmol) was slowly added to a stirred mixture of imine (from benzaldehyde and *p*-anisidine) (2.11 g, 10.0 mmol) in dry dichloromethane (75 mL) at 0–5 °C. Acetoxyacetyl chloride (1.71 g, 10.0 mmol) dissolved in 50 mL dichloromethane was added over a period of 30 min while maintaining the temperature between 0 and 5 °C and stirring continued for 8 h at room temp. After the reaction completed (TLC), the mixture was washed with 5% aq $NaHCO_3$ (50 mL), 5% HCl (50 mL), and water (3×50 mL). The organic layer was dried and evaporated under reduced pressure. The product was purified using column chromatography (230–400 mesh silica gel and dichloromethane and hexane). Yield: 2.7 g (87%), mp 160–162 °C. IR (KBr): 3476, 2953, 1744, 1515, 1399, 1371, 1223, 1107, 1025 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.69 (s, 3H, –OCOCH_3), 3.75 (s, 3H, –OCH_3), 5.35 (d, 1H, $J = 4.82$ Hz, C_4–*H*), 5.94 (d, 1H, $J = 4.83$ Hz, C_3–*H*), 6.81 (d, 2H, $J = 8.91$ Hz, Ar–*H*), 7.29 (d, 2H, $J = 8.92$ Hz, Ar–*H*), 7.34 (s, 5H, Ar–*H*). ^{13}C NMR (125 MHz): δ 19.8, 55.5, 61.5, 76.4, 114.4, 118.8, 127.9, 128.5, 128.8, 130.1, 132.3, 156.0, 161.3 172.0. MS (*m*/*z*): 311, 212, 167, 162, 149, 120. Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.30; H, 5.47; N, 4.51.

4.6. (±)-*cis*-3-Acetoxy-4-(2-furyl)-1-(4-methoxyphenyl)-2-azetidinone 6

Compound **6** was prepared from triethylamine (3.0 g, 30.0 mmol), imine (from furfural and *p*-anisidine) (2.01 g, 10.0 mmol), and acetoxyacetyl chloride (1.71 g, 10.0 mmol) following the above mentioned procedure for **1** and maintaining the temperature at −5 to 0 °C. Yield: 2.7 g (90%), mp 159–161 °C. IR (KBr): 3476, 2959, 1743, 1516, 1378 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.93 (s, 3H, –OCOCH_3), 3.76 (s, 3H, –OCH_3), 5.39 (d, 1H, $J = 4.72$ Hz, C_4–*H*), 5.95 (d, 1H, $J = 4.73$ Hz, C_3–*H*), 6.39 (dd, 1H, $J = 3.19$ and 1.36 Hz, furyl C_4–*H*), 6.43 (d, 1H, $J = 3.26$ Hz, furyl C_3–*H*), 6.83 (d, 2H, $J = 9.00$ Hz, Ar–*H*), 7.29 (d, 2H, $J = 9.00$ Hz, Ar–*H*), 7.45 (s, 1H, $J = 0.97$ Hz, furyl C_5–*H*). ^{13}C NMR (125 MHz): δ 20.0, 55.3, 55.4, 76.1, 110.8, 110.8, 114.4, 118.6, 130.3, 143.7, 146.8, 156.7, 161.0, 169.6. MS (*m*/*z*): 301, 260, 242, 232, 230, 214, 202, 186, 153, 111. Anal. Calcd for $C_{16}H_{15}NO_5$: C, 63.78; H, 5.02; N, 4.65. Found: C, 63.72; H, 5.00; N, 4.61.

4.7. (±)-*cis*-3-Acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone 7

Compound **7** was prepared from triethylamine (1.51 g, 15.0 mmol), imine (from thienaldehyde and *p*-anisidine) (2.17 g, 10.0 mmol), and acetoxyacetyl chloride (1.71 g, 10.0 mmol), following the method of the preparation of **1** while maintaining the temperature from −5 to 0 °C. Yield: 2.65 g (84%), mp 148–149 °C. IR (KBr): 3479, 2985, 1748, 1514, 1375 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.85 (s, 3H, –OCOCH_3), 3.74 (s, 3H, –OCH_3), 5.60 (d, 1H, $J = 4.7$ Hz, C_4–*H*), 5.94 (d, 1H, $J = 4.7$ Hz, C_3–*H*), 6.80 (d, 2H, $J = 9.0$ Hz, Ar–*H*), 7.0 (dd, 1H, $J = 3.59$ and 3.60 Hz, thienyl C_4–*H*), 7.09 (d, 1H, $J = 2.81$ Hz, thienyl C_4–*H*), 7.30 (d, 2H, $J = 9.10$ Hz, Ar–*H*), 7.31 (s, 1H, thienyl C_5–*H*). ^{13}C NMR (125 MHz), 20.0, 55.4, 57.6, 76.5, 114.4, 118.8, 126.8, 127.1, 128.1, 130.1, 135.6, 156.7, 161.0, 169.3. MS (*m*/*z*): 317, 218, 217, 202, 173, 168, 149, 126, 97. Anal. Calcd for $C_{16}H_{15}NO_4S$: C, 60.55; H, 4.76; N, 4.41; S, 10.10. Found: C, 60.56; H, 4.73; N, 4.42; S, 10.08.

4.8. (±)-*cis*-3-Acetoxy-4-(*t*-butyl)-1-(4-methoxyphenyl)-2-azetidinone 8

Compound **8** was prepared from triethylamine (3.02 g, 30.0 mmol), imine (pivaldehyde and *p*-anisidine) (1.91 g, 10.0 mmol), and acetoxyacetyl chloride (1.71 g, 10.0 mmol), following the above mentioned procedure for **1** and maintaining temperature at –78 °C. Yield: 2.1 g (72%), mp 187–189 °C. IR (KBr): 2964, 1758, 1740, 1516, 1371, 1228, 1181, 1121, 1031, 824 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.97 (s, 9H, *t*-butyl), 2.14 (s, 3H, –OCOCH_3), 3.75 (s, 3H, –OCH_3), 4.22 (d, 1H, $J = 5.36$ Hz, C_4–*H*), 6.12 (d, 1H, $J = 5.36$ Hz, C_3–*H*), 6.85 (d, 2H, $J = 8.95$ Hz, Ar–H), 7.27 (d, 2H, $J = 8.95$ Hz, Ar–*H*). ^{13}C NMR (125 MHz): δ 20.9, 27.0, 34.6, 55.5, 66.9, 73.5, 114.3, 122.3, 130.0, 157.2, 164.0, 169.4. MS (*m*/*z*): 292, 291, 192, 165, 164, 150, 149, 134. Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81. Found: C, 65.93; H, 7.22; N, 4.78.

4.9. (±)-*cis*-3-Hydroxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone 9

cis-3-Acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (4 g, 12.8 mmol) dissolved in 250 mL of THF was added dropwise over a period of 1 h to a stirring mixture of 1 M KOH (240 mL) and THF (160 mL) at 0 °C. The reaction mixture was stirred further for another hour. After completion of the reaction, it was diluted with 200 mL of THF and saturated solution of $NaHCO_3$, extracted with ethyl acetate (3 × 200 mL), washed, dried, and evaporated under reduced pressure to give **9**. Yield: 3.36 g (97%), mp 210–212 °C. IR (KBr): 3309, 1719, 1514, 1405, 1251, 1181, 1116, 1029 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$+DMSO-d^6) δ 3.74 (s, 3H, –OCH_3), 5.16 (d, 1H, $J = 4.7$ Hz, C_4–*H*), 5.19 (d, 1H, $J = 4.7$ Hz, C_3–*H*), 6.78 (d, 2H, $J = 9.01$ Hz, Ar–*H*), 7.28 (d, 2H, $J = 9.01$ Hz, Ar–*H*), 7.35 (m, 5H, Ar–*H*), ^{13}C NMR (125 MHz), 55.3, 62.6, 77.0, 114.2, 118.6, 127.8, 128.1, 128.4, 130.8, 134.1, 156.1, 166.3. MS (*m*/*z*): 269, 212, 196, 167, 149, 120. Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.29; H, 5.60; N, 5.18.

4.10. (±)-*cis*-3-Propyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone 10

A solution of (±)-*cis*-3-hydroxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (1.0 g, 3.72 mmol), propionic anhydride (520 mg, 4.0 mmol), and DMAP (10 mg) in dichloromethane (20 mL) was kept overnight at room temperature. After the completion of the reaction, the contents were poured into ice-cold water and extracted with dichloromethane (3 × 50 mL). The organic layer was washed, dried, and evaporated to furnish the crude product which was further purified by crystallization to give **10**. Yield: 1.16 g (96%), mp 127 °C. IR (KBr): 3473, 1743, 1515, 1401 1243, 1110, 1032, 981 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.71 (t, 3H, $J = 7.56$ Hz, –OCOCH_2CH_3), 1.77–2.10 (m, 2H, –OCOCH_2CH_3), 3.73 (s, 3H, –OCH_3), 5.34 (d, 1H, $J = 4.83$ Hz, C_4–*H*), 5.94 (d, 1H, $J = 4.83$ Hz, C_3–*H*), 6.79 (d, 2H, $J = 8.93$ Hz, Ar–*H*), 7.27 (d, 2H, $J = 8.92$ Hz, Ar–*H*), 7.28 (s, 5H, phenyl). ^{13}C NMR (50 MHz, $CDCl_3$): δ 8.6, 26.8, 55.4, 61.5, 76.1, 114.4, 118.8, 127.9, 128.5, 128.7, 130.3, 132.4, 156.6, 161.5, 172.7. MS (*m*/*z*): 325, 212, 196, 176, 149, 134, 120, 91. Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 70.16; H, 5.87; N, 4.26.

4.11. (±)-*cis*-3-Butyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone 11

It was prepared from (±)-*cis*-3-hydroxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (1.0 g, 3.72 mmol), butyric anhydride (630 mg, 4.0 mmol), and DMAP (10 mg) in dichloromethane (20 mL) following the procedure for **10**. Yield: 1.2 g (95%), mp 129 °C. IR (KBr): 3477, 1747, 1519, 1456, 1402 1245, 1111, 1029, 842, 805 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.61 (t, 3H, $J = 7.34$ Hz, –OCOCH_2CH_2CH_3), 1.11–1.25 (h, 2H, –OCOCH_2-CH_2CH_3), 1.75–2.05 (m, 2H, –OCOCH_2CH_2CH_3), 3.73 (s, 3H, –OCH_3), 5.33 (d, 1H, $J = 4.85$ Hz, C_4–*H*), 5.94 (d, 1H, $J = 4.85$ Hz, C_3–*H*), 6.80 (d, 2H, $J = 8.98$ Hz, Ar–*H*), 7.28 (d, 2H, $J = 8.96$ Hz, Ar–*H*), 7.30 (s, 5H, phenyl). ^{13}C NMR (50 MHz): δ 13.3, 17.8, 35.2, 55.4, 61.6, 76.2, 114.4, 118.8, 127.9, 128.5, 128.7, 130.3, 132.4, 156.6, 161.5, 171.9. MS (*m*/*z*): (M+1) 340, 213, 197, 149, 134, 120, 91, 71. Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.71; H, 6.20; N, 4.11.

4.12. (±)-*cis*-3-Hexyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone 12

A solution of (±)-*cis*-3-hydroxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (1.0 g, 3.72 mmol), caproic acid (500 mg, 4.3 mmol), and DCC (in excess) in dichloromethane (100 mL) was stirred at room temperature for 24 h. After completion of the acylation reaction, the contents were passed through a pad of silica (230–400 mesh). The solution upon evaporation furnished the crude product which on purification by crystallization gave **12**. Yield: 1.3 g (95%), mp 110 °C. IR (KBr): 3427, 1747, 1517, 1455, 1400, 1244, 1157, 1111, 1065, 986, 835, 698 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.77 (t, 3H, $J = 7.3$ Hz, –OCO$(CH_2)_4CH_3$), 0.90–0.96 (m, 2H, –OCO$(CH_2)_3$-CH_2CH_3), 1.07–1.16 (m, 2H, –OCO$(CH_2)_2CH_2CH_2CH_3$), 1.81–1.87 (m, 2H, –OCOCH$_2CH_2(CH_2)_2CH_3$), 1.97–2.03 (m, 2H, –OCOC$H_2(CH_2)_3CH_3$), 3.72 (s, 3H, –OCH_3), 5.31 (d, 1H, $J = 4.77$ Hz, C_4–H), 5.92 (d, 1H, $J = 4.79$ Hz, C_3–H), 6.78 (d, 2H, $J = 8.71$ Hz, Ar–H), 7.25 (d, 2H, $J = 8.62$ Hz, Ar–H), 7.30 (s, 5H, phenyl). ^{13}C NMR (125 MHz): δ 13.7, 22.1, 23.9, 30.8, 33.3, 55.4, 61.5, 76.1, 114.4, 118.8, 127.9, 128.3, 128.8, 130.3, 132.4, 156.6, 161.5, 172.1. MS (*m*/*z*): 367, 218, 213, 196, 149, 134, 120, 99, 91. Anal. Calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81. Found: C, 71.86; H, 6.82; N, 3.80.

4.13. (±)-*cis*-3-Acetoxy-4-(1,1-dimethylethyl)-2-azetidinone 15

A solution of (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(1,1-dimethylethyl)-2-azetidinone (2.91 g, 10.0 mmol) dissolved in 200 mL of acetonitrile was added slowly to a solution of ceric ammonium nitrate (16.0 g, 30 mmol) in 100 mL of water at 0 °C. The mixture was stirred for 2 h, diluted with 200 mL water, extracted with ethylacetate (3×100 mL), and neutralized with 5% $NaHCO_3$ solution. The organic layer was combined, washed with 10% Na_2SO_3, 5% $NaHCO_3$, and brine successively, dried over sodium sulfate, and concentrated under reduced pressure. The crude oil obtained was chromatographed over a silica gel column (60–120 mesh using ethylacetate and hexane as eluent) to give **15**. Yield: 1.65 g (89%), mp 68–69°C. IR (KBr): 3265, 2919, 1792, 1740, 1375, 1228, 1128 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.98 (s, 9H, *t*-butyl), 2.16 (s, 3H, –OCOCH_3), 3.61 (d, 1H, $J = 5.09$ Hz, C_4–H), 5.99 (d, 1H, $J = 5.07$ Hz, C_3–H). ^{13}C NMR (50 MHz): δ 20.8, 26.0, 32.8, 63.2, 74.8, 166.6, 169.3. MS (*m*/*z*): 186, 142, 127, 100, 85. Anal. Calcd for $C_9H_{15}NO_3$: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.33; H, 8.11; N, 7.57.

5. General method for enzyme catalyzed resolution of 2-azetidinones

5.1. (+)-*cis*-(3*R*,4*S*)-3-Acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (+)-1

Racemic (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (400 mg) was added to a solution of aqueous phosphate buffer (18 mL, 0.1 M, pH 7.0) and dimethylformamide (2.0 mL). A wet pallet of whole cell preparation of ABL (400 mg, 1100 units/mg) was added to the above solution with continuous stirring while maintaining temperature at 26 ± 1 °C. Thin-layer chromatography (TLC) and chiral high performance liquid chromatography (HPLC) was carried out to monitor the progress of the reaction after every hour. The completed reaction (5–6 h approx., 50% conversion) was terminated by adding ethyl acetate and centrifuged to remove cells and the suspended particles. The decanted clear solution and the centrifuged mass were extracted separately with ethyl acetate (3×40 mL). The organic layers combined and washed with water and dried. The solvent removed under reduced pressure to furnish a mixture comprising hydrolyzed alcohol and unhydrolyzed ester, which was separated by column chromatography (230–400 mesh silica gel using dichloromethane and hexane as eluent) giving (+)-*cis*-(3*R*,4*S*)-3-acetoxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (+)-**1** (180 mg, 90%) having ee >99.5%, $[\alpha]_D^{25} = +8.1$ (*c* 1.0, $CHCl_3$) and hydrolyzed alcohol (−)-*cis*-(3*S*,4*R*)-3-hydroxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (−)-**9** (160 mg, 92%), ee >99.5% (chiral HPLC), $[\alpha]_D^{25} = -179.0$ (*c* 1.0, $CHCl_3$).

5.2. (+)-*cis*-(3*R*,4*R*)-3-Acetoxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone (+)-6

A mixture of racemic (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone (200 mg), aqueous phosphate buffer (9 mL, 0.1 M, pH 7.0), dimethylsulfoxide (1 mL), and whole cells of ABL (200 mg) was stirred continuously at 30 ± 1 °C. After a certain degree of conversion, the reaction was stopped following the above general procedure to give unhydrolyzed ester (+)-*cis*-(3*R*,4*R*)-3-acetoxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone (+)-**6** (89 mg, 89%), ee >99.5%, $[\alpha]_D^{25} = +13.6$ (*c* 1.0, $CHCl_3$) and hydrolyzed alcohol (−)-*cis*-(3*S*,4*S*)-3-hydroxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone (−)-**13** (81 mg, 92%), ee >99.5% (chiral HPLC), $[\alpha]_D^{25} = -222.0$ (*c* 1.0, $CHCl_3$).

5.3. (+)-*cis*-(3*R*,4*R*)-3-Acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone (+)-7

A mixture of racemic (±)-*cis*-3-acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone (200 mg), aqueous phosphate buffer (9 mL, 0.1 M, pH 7.0), dimethylformamide (1 mL), and whole cells of ABL (200 mg) was stirred continuously at 30 ± 1 °C. After a certain degree of conversion the reaction was stopped following the above general procedure to give unhydrolyzed ester (+)-*cis*-(3*R*,4*R*)-3-acetoxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone (+)-**7** (91 mg, 90%) having ee >99%, $[\alpha]_D^{25} = +4.5$ (*c* 0.94, $CHCl_3$) and hydrolyzed alcohol (−)-*cis*-(3*S*,4*S*)-3-hydroxy-1-(4-methoxyphenyl)-4-(2-thienyl)-2-azetidinone (−)-**14** (81 mg, 91%), ee 99% (chiral HPLC), $[\alpha]_D^{25} = -162.8$ (*c* 0.42, $CHCl_3$).

5.4. (+)-*cis*-(3*R*,4*S*)-3-Propyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (+)-10

A mixture of racemic (±)-*cis*-3-propyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (400 mg), aqueous phosphate buffer (18 mL, 0.1 M, pH 7.0), dimethylformamide (2 mL), and whole cells of ABL (400 mg) was stirred continuously at 25 ± 1 °C. After a certain degree of conversion the reaction was stopped following the above general procedure to get unhydrolyzed ester (+)-*cis*-(3*R*,4*S*)-3-propyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (+)-**10** (175 mg, 87%), ee >99.5%, $[\alpha]_D^{25} = +3.5$ (*c* 0.6, $CHCl_3$) and hydrolyzed alcohol (−)-*cis*-(3*S*,4*R*)-3-hydroxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (−)-**9** (160 mg, 92%), ee >99.5% (chiral HPLC), $[\alpha]_D^{25} = -190$ (*c* 0.4, $CHCl_3$).

5.5. (+)-*cis*-(3*R*,4*S*)-3-Butyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (+)-11

A mixture of racemic (±)-*cis*-3-butyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (400 mg), aqueous phosphate buffer (18 mL, 0.1 M, pH 7.0), dimethylformamide (2 mL), and lipase PS-C (400 mg) was stirred continuously at 25 ± 1 °C. After a certain degree of conversion, the reaction was stripped following the above general procedure to obtain unhydrolyzed ester (+)-*cis*-(3*R*,4*S*)-3-butyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (+)-**11** (180 mg, 90%) having ee >99.5%, $[\alpha]_D^{25} = +7.6$ (*c* 0.54, $CHCl_3$) and hydrolyzed alcohol (−)-*cis*-(3*S*,4*R*)-3-hydroxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (−)-**9** (159 mg, 92%), ee >99.5% (chiral HPLC), $[\alpha]_D^{25} = -174.0$ (*c* 1.0, $CHCl_3$).

5.6. (+)-*cis*-(3*R*,4*S*)-3-Hexyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (+)-12

A mixture of racemic (±)-*cis*-3-hexyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (400 mg), aqueous phosphate buffer (18 mL, 0.1 M, pH 7.0), dimethylformamide (2 mL), and lipase PS (400 mg) was stirred continuously at 25 ± 1 °C. After a certain degree of conversion, the reaction was stopped following the above general procedure to obtain unhydrolyzed ester (+)-*cis*-(3*R*,4*S*)-3-hexyloxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (+)-**12** (173 mg, 88%), ee >99.5%, $[\alpha]_D^{25} = +11.6$ (*c* 1.0, $CHCl_3$) and hydrolyzed alcohol (−)-*cis*-(3*S*,4*R*)-3-hydroxy-1-(4-methoxyphenyl)-4-phenyl-2-azetidinone (−)-**9** (161 mg, 92%) having ee >99.5% (chiral HPLC), $[\alpha]_D^{25} = -174.0$ (*c* 1.0, $CHCl_3$).

5.7. Kinetic resolution of (±)-*cis*-3-acetoxy-4-(1,1-dimethylethyl)-2-azetidinone 15

A mixture of racemic (±)-*cis*-3-acetoxy-4-(1,1-dimethylethyl)-2-azetidinone (130 mg, 0.72 mmol) and whole cells of ABL (150 mg) in aqueous phosphate buffer (4 mL, 0.1 M, pH 7.0) with the continuous stirring at 20 ± 1 °C. After a certain degree of conversion, the reaction was terminated and contents extracted with ethyl acetate (3 × 20 mL). The organic phase was washed with water, dried over sodium sulfate and concentrated in vacuo to give the crude product, which was heated in a mixture of toluene–heptane (55:45) for 3 h. The reaction mixture on cooling gave (+)-*cis*-3-acetoxy-4-(1,1-dimethylethyl)-2-azetidinone (+)-**15** (56 mg, 86%), ee 98.5%, $[\alpha]_D^{25} = +67.4$ (*c* 1.1, $CHCl_3$) recovered from the organic layer and (−)-*cis*-3-hydroxy-4-(1,1-dimethylethyl)-2-azetidinone (−)-**16** (42 mg, 82%), ee 99%, $[\alpha]_D^{25} = -91.2$ (*c* 0.6, CH_3OH) as a white solid.

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