Candida cylindracea Lipase-catalysed Hydrolysis of Methyl Aziridine-2-carboxylates and -2,3-dicarboxylates

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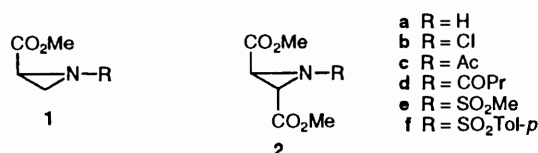
N-Substituted aziridine-2-carboxylates and -2,3-dicarboxylates have been resolved with good to excellent stereochemical purity by enzymatic hydrolysis catalysed by lipase from *Candida cylindracea*.

Biocatalytic synthesis of optically active compounds is well established [1] and, recently, we successfully used ester hydrolases to obtain optically active aziridinecarboxylates,[2] intermediates for the synthesis of, in particular, amino acids [3] and β-lactams.[4] Here we report the results of the enzymatic hydrolysis of *N*-unsubstituted and *N*-substituted aziridinecarboxylates, catalysed by *Candida cylindracea* lipase (CCL). Racemic methyl aziridine-2-carboxylate **1a** and -2,3-dicarboxylate **2a** were used as substrates both for enzymatic hydrolysis and for the synthesis of *N*-chloro-, *N*-acyl- and *N*-sulfonyl derivatives **1b–f** and **2b–e**.

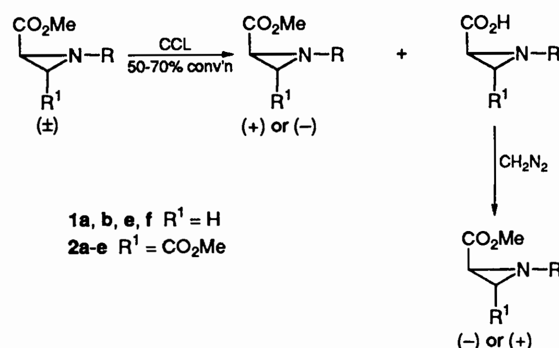


N-Activation by acylation or sulfonylation, in particular, is known to be a prerequisite for successful nucleophilic ring-opening reactions.[3]

Results and Discussion

Synthesis of Racemic Aziridines **1a–f** *and* **2a–e**.—The substrates used for the lipase-catalysed reactions were easily prepared in high overall yields as follows: racemic aziridines **1a** [5] and **2a** [6,7] were synthesized by reaction with ammonia from the commercially available methyl α,β-dibromopropionate and from dimethyl α,β-dibromosuccinate, respectively. The selected *N*-functional groups, *viz.*, chlorine, acyl and sulfonyl, were introduced using standard procedures.[3] Chlorination of aziridines **1a** and **2a** into **1b** [8] (80%) and **2b** [6] (98%) derivatives was carried out with *tert*-butyl hypochlorite, as described elsewhere. Acetylation of **1a** and **2a** into **1c** [9] (70%) and **2c** (65%) was carried out with acetyl chloride and triethylamine; however, the yield of **2c** was higher (80%) when pyridine and acetic anhydride were used. For introduction of the butyryl group, butyric acid in the presence of dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine gave the best results, yielding **1d** (86%) and **2d** (80%). The reaction of **1a** and **2a** with methanesulfonyl chloride or toluene-*p*-sulfonyl chloride and triethylamine (or pyridine) afforded compounds **1e**, **f** (70%) and **2e** (80%), whereas no reaction was observed for the tosylation of **2a**. Analytical and spectroscopic data of new compounds **1d–f** and **2c–e** were in close agreement with the structures.

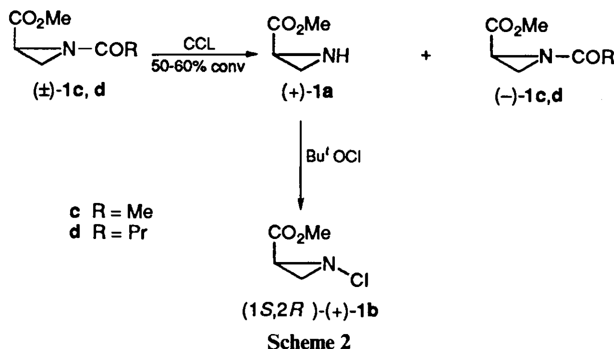
Enzymatic Resolution.—CCL-catalysed hydrolysis of the aziridines **1a–f** and **2a–e** were carried out in phosphate buffer (0.1 mol dm^{-3} and NaCl 0.1 mol dm^{-3}; pH 7.5) at room temperature. The suspensions were vigorously shaken and the reactions stopped when conversion reached 60–80%. Under these conditions, CCL catalysed the hydrolyses of aziridines **1a–f** and **2a–e** with low to high enantioselectivity and with high chemoselectivity. The enantioselectivity, expressed as enantiomer ratio *E*, and the conversions were calculated from the values of the enantiomeric excesses (ee's) of the unhydrolysed (ee_s) and hydrolysed products (ee_p).[10] CCL showed a very high esterolytic activity towards compounds **1a**, **b**, **e**, **f** and **2a–e**. So, for racemic aziridines **1a, b, e, f** and **2a–e**, up to 50–80% conversion, the enzymatic hydrolysis afforded the unhydrolysed compounds and the corresponding acids as hydrolysis products (Scheme 1).



Scheme 1

The optically active unchanged esters **1a, b, e, f** and **2a–e** were isolated from the reaction mixture by extraction with dichloromethane followed by column chromatography on silica gel (dichloromethane–diethyl ether as eluent). The optically active enzymatic hydrolysis products, aziridinecarboxylic acids, were recovered from the aqueous phase by acidification with HCl 10% and extraction with ethyl ether. Their optical purities were estimated through conversion into the corresponding esters by treatment with diazomethane. Owing to its high volatility, *N*-unsubstituted aziridine **1a** was recovered by conversion into the more stable derivatives **1b** or **1c**, whose absolute configurations and optical purities are known from the literature.[8,9] The results, reported in Table 1, show that the CCL-catalysed hydrolyses proceeded with low enantioselectivity ($E \leqslant 2$) towards aziridines **1a**, **b**, **e** and **2a**, **b**, **e**, thus affording products with 5–30% enantiomeric excesses. A considerable enhancement of the optical purities was observed for the *N*-sulfonyl derivative **1f** (ee 72%) and for the *N*-acyl derivatives **2c**, **d**, which are obtained in nearly optically pure forms (ee ⩾ 95%). Surprisingly, for aziridines **1c**, **d**, CCL catalysed the chemo- and enantio-selective hydrolysis of the amide bond. The enzymatic reaction afforded, up to 50% conversion, the unhydrolysed esters **1c**, **d** and the aziridine **1a** as enzymatic hydrolysis products (Scheme 2).

Both optically active unchanged esters **1c**, **d** and the aziridine **1a** were extracted from the aqueous phase with dichloromethane. After evaluation of the ratio between compounds **1c**, **d** and **1a** by gas chromatography, the organic phase was treated with the required amount of *tert*-butyl hypochlorite at −10 °C, to convert the volatile aziridine **1a** into **1b**. The reaction mixture

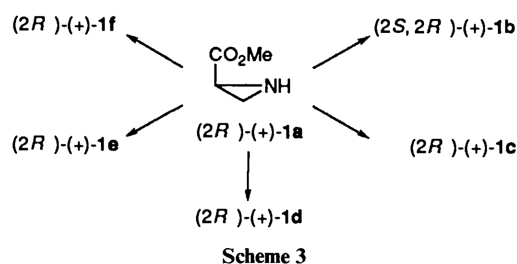


Scheme 2

was concentrated and chromatographed on silica gel (dichloromethane–diethyl ether as eluent) to afford the unchanged esters and the aziridine **1b**, which were both optically active. Once more, as observed for derivatives **2c**, **d**, the CCL-catalysed hydrolysis of *N*-acylaziridines **1c**, **d** proceeded with high enantioselectivity to afford the unchanged derivatives with 60–90% enantiomeric excesses. All optical purities of aziridines **1a–f** and **2a–e** were determined by analysis of the ^{1}H NMR spectra recorded in the presence of the chiral shift reagent, Eu(hfc)$_3$, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III).

The results in Table 1 show that *N*-acylaziridines were hydrolysed with high enantio- and chemo-selectivity within shorter reaction times. *N*-Butyrylaziridines **1d** and **2d** were hydrolysed faster than the other *N*-substituted compounds; changing the *N*-substituent from acetyl to butyryl in aziridine-2-carboxylates brought about a significant increase in enantioselectivity. Moreover, we observed that, while higher conversion did not improve the optical purity of **1c**, when the reaction was stopped at 20% of conversion, the aziridine **1a** was obtained in ee 80%.

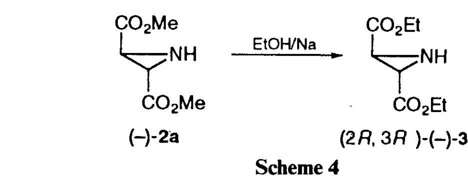
Absolute Configurations of the Aziridines **1a–f** *and* **2a–e**.—Knowing the absolute configuration of (+)-**1b**[8] and (−)-**1c**[9] enabled us to assign the absolute configurations to aziridines (+)-**1a**, **d**, **e**, **f** through the chemical correlations of Scheme 3.



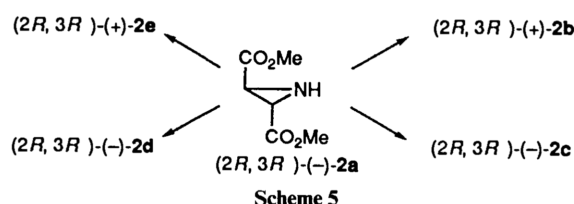
Scheme 3

In particular, by chlorination, acylation and sulfonylation of (+)-**1a**, we obtained the aziridines (+)-**1b–f**. Since these reactions do not involve the carbon chiral centre, compounds **1a–f** must have the same configuration at the chiral carbon atom. The literature[8,9] assigns the 2*R* configuration to (+)-**1b** and (+)-**1c**, so we were able to infer the 2*R* configuration for (+)-**1a**, **d**, **e**, **f** as well.*

We proceeded likewise for the aziridines **2a–e**. The aziridine



Scheme 4



Scheme 5

(−)-**2a** was correlated by transesterification with the levorotatory diethyl aziridine-2,3-dicarboxylate **3** of known 2*R*,3*R* configuration at the chiral ring carbon atoms.[12] We were able to deduce the same 2*R*,3*R* configuration for (−)-**2a** (Scheme 4).

Starting from the aziridine (2*R*,3*R*)-(−)-**2a** we obtained: the aziridine (+)-**2b** by chlorination; the aziridines (−)-**2c** and (−)-**2d** by acylation and the aziridine (+)-**2e** by sulfonylation (Scheme 5). All these compounds must have the same 2*R*,3*R* configuration at the chiral carbon atom.†

It is well known[14] that the CLRS-NMR technique can be used as a tool for assigning absolute configurations from the observed sense of non-equivalence of partially enriched enantiomers. ^{1}H NMR spectra of the optically active aziridines **1b–f** and **2a–e**, obtained from enzymatic hydrolysis, were recorded in the presence of Eu(hfc)$_3$. They showed a chemical shift doubling for the ring-carbon substituents as well as for the nitrogen substituents: the magnitudes of the observed non-equivalences were quite high so as to allow the enantiomeric purities and the senses of non-equivalence to be determined (Table 2).

It is worth noting that all aziridines showed senses of non-equivalence that can be correlated with their absolute configuration; moreover, a reverse sense of non-equivalence for the carbon substituents with respect to the nitrogen substituents was observed. All aziridines having *R* configuration at the chiral carbon atoms exhibited a highfield sense of non-equivalence (H) relative to the carbon substituents and (where observable) a low sense of non-equivalence (L) for the nitrogen substituents. On the other hand, we observed a low sense of non-equivalence for the carbon substituents in aziridines having *S* configuration.

Interestingly, from configurational correlations we can observe that the CCL-catalysed ester-bond hydrolysis occurs preferentially at the *S* enantiomer, while the amide-bond hydrolysis occurs preferentially at the *R* enantiomer. These results further demonstrate that CCL hydrolysis of *N*-acylaziridine-2-carboxylates or -2,3-dicarboxylates, affords easy access to enantiomerically pure aziridine derivatives in good yields.

Experimental

^{1}H NMR spectra were recorded in CDCl$_3$ on a VARIAN XL-200 spectrometer. Chemical shifts are reported in δ values from TMS as internal standard. Coupling constant values are given in Hz. Optical rotations were measured on a Perkin-Elmer 241 polarimeter in CHCl$_3$ solution and are in 10^{-1} deg cm^2 g^{-1}. Enantiomeric purities (ee %) and non-equivalence senses were determined by ^{1}H NMR spectra recorded in the presence of the

* It is reported[9,11] that aziridines *N*-acylation or sulfonylation decrease significantly the barrier to inversion at the nitrogen atom. Therefore, we think that the nitrogen atom does not have configurational stability at room temperature in derivatives **1c–f**; for this reason we did not report the nitrogen configuration. Measurements for determining the barriers to pyramidal inversion and the relative configuration of derivatives of type **1** are now in course.

† In all these derivatives nitrogen is an achiral centre independently from its configurational stability: in these compounds nitrogen is chirotopic but nonstereogenic.[13]

Table 1 Enzymatic hydrolysis of aziridines **1a–f** and **2a–e** catalysed by lipase from *Candida cylindracea*

	Reaction conditions[a]			Unchanged aziridine					Hydrolysed aziridine		
Substrate	t/h	E:S	Conv. (%)[b]	Yield (%)[c]	$[\alpha]_D$[d]	ee (%)[e]	Abs. conf.[f]	E[b]	Yield (%)[c]	ee (%)[e]	Abs. conf.[f]
1a	24	1:2	50	30	+3.8	5	(2*R*)	2	—	—	—
1b	3	1:1	80	15	+57.1	30	(1*S*,2*R*)	2	40	8	(1*R*,2*S*)
1c	4	1:4	55	20	−46.1	63	(2*S*)	6	35	40[g]	(2*R*)
1d	1	1:20	60	35	−74.3	90	(2*S*)	12	40	60	(2*R*)
1e	24	1:2	60	37	+13.0	14	(2*R*)	2	—	—	—
1f	30	1:1	52	40	+33.6	72	(2*R*)	10	40	64	(2*S*)
2a	24	1:4	55	35	−51.5	30	(2*R*,3*R*)	2	40	4	(2*S*,3*S*)
2b	2	1:2	38	52	+25.7	26	(2*R*,3*R*)	5	24	42	(2*S*,3*S*)
2c	24	1:1	60	30	−56.4	⩾95	(2*R*,3*R*)	16	45	60	(2*S*,3*S*)
2d	3	1:1	70	25	−34.8	⩾95	(2*R*,3*R*)	8	58	30	(2*S*,3*S*)
2e	24	1:2	56	40	+4.9	13	(2*R*,3*R*)	2	45	10	(2*S*,3*S*)

[a] All hydrolyses were performed in phosphate buffer (pH 7.5; 0.1 mol dm^{-3}) at room temperature with an enzyme/aziridine ratio (E:S) as reported. [b] Enantiomer ratio E and conversions were calculated from enantiomeric excesses of unchanged aziridine (ee_s) and of hydrolysed products (ee_p), as reported in ref. 9. [c] Isolated yield. [d] Data for chloroform solution. [e] ±2%; optical yield (ee) determined by ^1H NMR on methyl ester signal after addition of chiral $Eu(hfc)_3$. [f] Absolute configurations of aziridines **1b** and **1c** reported in refs. 8 and 10, respectively, and determined by chemical correlation for aziridines **1c–f** and **2a–e**. [g] ee 80% was obtained at 20% conversion.

Table 2 Proton chemical shift non-equivalences of aziridines **1b–f** and **2a, c–e** in the presence of $Eu(hfc)_3$

	Aziridine		^1H NMR			
			δ(ppm)[c]		Non-equival. sense[d]	(Δν, Hz)[e]
	$[\alpha]_D$[a]	Abs. conf.[b]	$COOCH_3$	*R*	$COOCH_3$	*R*
1b	+107.0	(1*S*,2*R*)	3.8	—	H (14.7)	—
1c	−20.7	(2*S*)	3.8	2.16	L (8.3)	H (24.1)
1d	−20.7	(2*S*)	3.8	—	L (19.0)	—
1e	+13.2	(2*R*)	3.8	3.1	H (4.7)	L (11.2)
1f	+33.6	(2*R*)	3.8	2.16	H (15.4)	—
2a	−49.2	(2*R*,3*R*)	3.8	—	H (11.4)	—
2b	−42.1	(2*S*,3*S*)	3.9	—	L (64.8)	—
2c	−33.4	(2*R*,3*R*)	3.8	2.1	H (16.6)	L (50.0)
2d	−23.0	(2*R*,3*R*)	3.8	—	H (14.2)	—
2e	+4.9	(2*R*,3*R*)	3.8	3.2	H (6.0)	L (7.8)

[a] Measured in $CHCl_3$. [b] By chemical correlations. [c] Chemical shifts in $CDCl_3$ reported in δ values from TMS as internal standard. [d] H refers to the highfield position of the predominant enantiomer with respect to the other enantiomer; L (lowfield position) indicates the converse. [e] Non-equivalences recorded from the spectra in $CDCl_3$ after addition of $Eu(hfc)_3$ in a CLSR/aziridine molar ratio of 0.5.

chiral lanthanide shift reagent (CLSR), $Eu(hfc)_3$, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), in the CLSR/aziridine molar ratio 0.5. Accuracy was within ±2%. Mass spectra were determined on a Hewlett-Packard 5970 mass selective detector. GLC analyses were performed on a Hewlett-Packard 5890 A gas chromatograph (capillary column DB-1, 5 μm, 30 m × 0.53 mm I.D.). Elemental analyses were performed with a Carlo Erba Elemental Analyzer Mod. 1106. $Eu(hfc)_3$ was purchased from Ega-Chemie and used without additional purification. Methyl α,β-dibromopropionate was purchased from Fluka. *Candida cylindracea* lipase (CCL) was purchased from Sigma and used without additional purification. Dimethyl α,β-dibromosuccinate (yield 90%) was obtained by bromination of the methyl fumarate following the procedure described in the literature for α,β-dibromosuccinic acid.[15] Dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) were purchased from Fluka. Racemic aziridines **1a–f** and **2a–e** were synthesized as follows.

Methyl Aziridine-2-carboxylate **1a**.[5]—Gaseous ammonia was bubbled through a solution of methyl α,β-dibromopropionate (13.5 g, 55 mmol) in acetonitrile (250 cm^3), cooled at −20 °C, for 2 h after which the reaction mixture was stirred for 3 h. After removal of the cooling bath, excess of ammonia was eliminated from the mixture with a nitrogen stream and the NH_4Br was filtered off. The filtrate was then evaporated and the residue chromatographed on silica gel (dichloromethane–diethyl ether, 80:20) to yield pure **1a** (2.5 g, 45%) as a volatile oil, b.p. 50 °C at 18 mmHg; δ_H 1.42 (1 H, m), 1.88 (1 H, dd, J 1.5, 5.5), 2.02 (1 H, dd, J 1.5, 3), 2.55 (1 H, dd, J 5.2, 2.8) and 3.78 (3 H, s); m/z 102 ($M + 1^+$).

Methyl 1-Chloroaziridine-2-carboxylate **1b**.[8]—*tert*-Butyl hypochlorite in dichloromethane (0.7 mol dm^{-3}; 14 cm^3) was added dropwise to a stirred solution of **1a** (1 g, 10 mmol) in dichloromethane (15 cm^3), cooled at 0 °C. After 30 min the solvent was evaporated and the residue chromatographed on silica gel (dichloromethane–diethyl ether, 80:20) to give pure **1b** as an oil (1.07 g, 80%); δ_H 2.54 (1 H, dd, J 2.6, 8), 2.7 (1 H, dd, J 2.7, 5.3), 2.99 (1 H, dd, J 5.4, 8) and 3.77 (3 H, s); m/z 135 (M^+).

Methyl 1-Acetylaziridine-2-carboxylate **1c**.[9]—A solution of acetyl chloride (1.06 g, 14 mmol) in chloroform (30 cm^3) was added dropwise to a chloroform solution (80 cm^3) of **1a** (1.36 g, 13 mmol) and triethylamine (1.35 g, 13 mmol), cooled at −5 °C. The mixture was stirred for 2 h at room temperature and then washed with water, dried (Na_2SO_4) and concentrated. The crude product was chromatographed on silica gel (dichloromethane–diethyl ether, 80:20) to give pure **1c** (1.4 g, 70%) as an oil; δ_H 2.16 (3 H, s), 2.5 (1 H, dd, J 5.5, 1.7), 2.58 (1 H, dd, J 3, 1.7), 3.16 (1 H, dd, J 3, 5.5), 3.8 (3 H, s); m/z 144 ($M + 1^+$).

Methyl 1-Butyrylaziridine-2-carboxylate **1d**.—A few crystals of DMAP were added to an anhydrous diethyl ether solution (25 cm^3) of aziridine **1a** (0.4 g, 4 mmol), butyric acid (0.41 g, 4.65 mmol) and DCC (0.98 g, 4.75 mmol), cooled to 0 °C. After 24 h at room temperature, dicyclohexylurea was filtered off and washed with anhydrous diethyl ether and the organic phase was dried ($MgSO_4$) and concentrated. The crude product was chromatographed on silica gel (dichloromethane–diethyl ether, 80:20) to yield pure **1d** (0.58 g, 86%) as an oil (Found: C, 56.2; H, 7.7; N, 8.1; M^+, 171. $C_8H_{13}NO_3$ requires C, 56.1; H, 7.65; N, 8.2%; *M*, 171); δ_H 0.91 (3 H, t), 1.70 (2 H, m), 2.39 (2 H, m), 2.50 (1 H, dd, *J* 1.7, 5.5), 2.57 (1 H, dd, *J* 1.7, 3), 3.12 (1 H, dd, *J* 3, 5.5) and 3.80 (3 H, s).

Methyl 1-Methylsulfonylaziridine-2-carboxylate **1e**.—Triethylamine (1.0 g, 9.89 mmol), a few crystals of DMAP and methanesulfonyl chloride (0.85 g, 7.42 mmol) were added sequentially to a solution of **1a** (0.5 g, 4.94 mmol) in dichloromethane (12 cm^3) cooled to −10 °C. After 2 h at room temperature, the mixture was filtered, washed with a saturated aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4) and concentrated. The crude product was chromatographed on silica gel (dichloromethane–diethyl ether, 80:20) to give pure **1e** (0.62 g, 70%) as an oil, b.p. 100 °C at 8 mmHg (Found: C, 33.1; H, 5.5; N, 7.5; $M + 1^+$, 180. $C_5H_9NO_4S$ requires C, 33.5; H, 5.1; N, 7.8%; *M*, 179); δ_H 2.63 (1 H, d, *J* 4.2), 2.77 (1 H, d, *J* 7.2), 3.15 (3 H, s), 3.32 (1 H, dd, *J* 4.2, 7.2) and 3.83 (3 H, s).

Methyl 1-(p-Tolylsulfonyl)aziridine-2-carboxylate **1f**.—Following the procedure described for **1e**, pure **1f** (87 mg, 70%) was obtained after chromatography from aziridine **1a** (50 mg, 0.5 mmol), triethylamine (80 mg, 0.8 mmol) and toluene-*p*-sulfonyl chloride (100 mg, 0.5 mmol) in chloroform solution (reaction time 6 h) (Found: C, 51.5; H, 5.5; N, 5.4; M^+, 255. $C_{11}H_{13}NO_4S$ requires C, 51.75; H, 5.1; N, 5.5%; *M*, 255); δ_H 2.46 (3 H, s), 2.56 (1 H, d, *J* 4.1), 2.77 (1 H, d, *J* 7), 3.35 (1 H, dd, *J* 4, 7), 3.75 (3 H, s), 7.3–7.9 (4 H, m).

Dimethyl Aziridine-2,3-dicarboxylate **2a**.[6]—Dimethyl α,β-dibromosuccinate (9 g, 0.03 mol) in acetonitrile (60 cm^3) was added dropwise to a solution of gaseous ammonia (2.0 g, 0.12 mol) in acetonitrile (10 cm^3) cooled to −5 °C. The reaction mixture was stirred for 16 h at room temperature and then filtered and evaporated. The residue was chromatographed on silica gel (diethyl ether–light petroleum, 90:10) to give pure **2a** (1.9 g, 40%) as an oil; δ_H 1.83 (1 H, t), 2.88 (1 H, d), 2.92 (1 H, d), 3.77 (3 H, s) and 3.82 (3 H, s); *m/z* 160 ($M + 1^+$).

Dimethyl 1-Chloroaziridine-2,3-dicarboxylate **2b**.[6]—Following the procedure described for **1b**, pure **2b** (0.65 g, 98%) was obtained by chlorination of the aziridine **2a** (0.54 g, 3.4 mmol) with *tert*-butyl hypochlorite in dichloromethane solution at 0 °C (reaction time 30 min); δ_H 3.43 (1 H, d), 3.45 (1 H, d), 3.79 (3 H, s) and 3.87 (3 H, s); *m/z* 193 (M^+).

Dimethyl 1-Acetylaziridine-2,3-dicarboxylate **2c**.—Following the procedure described for **1c**, pure **2c** (0.4 g, 65%) was obtained after column chromatography (silica gel, dichloromethane–diethyl ether, 80:20) from the aziridine **2a** (0.5 g, 3.14 mmol), triethylamine (0.5 g, 5 mmol) and acetyl chloride (0.38 g, 4.84 mmol) in chloroform solution (reaction time 7 h). A higher yield (83%) was obtained from the aziridine **2a** (0.42 g, 2.1 mmol) and acetic anhydride (1.08 g, 10 mmol) in pyridine at 60 °C for 1 h. Compound **2c** was a white solid, m.p. 42–44 °C (Found: C, 47.4; H, 7.7; N, 6.8%; M^+, 201. $C_8H_{11}NO_5$ requires C, 47.8; H, 7.5; N, 7.0%; *M*, 201); δ_H 2.14 (3 H, s), 3.46 (2 H, s) and 3.82 (6 H, s).

Dimethyl 1-Butyrylaziridine-2,3-dicarboxylate **2d**.—Following the procedure described for **1d**, pure **2d** (0.57 g, 80%), as a white solid, was obtained after chromatography (silica gel, dichloromethane–diethyl ether, 80:20) from **2a** (0.5 g, 3.14 mmol), butyric acid (0.33 g, 3.75 mmol), DCC (0.78 g, 3.78 mmol) and a few crystals of DMAP in anhydrous diethyl ether (reaction time 24 h); m.p. 34–36 °C (Found: C, 52.2; H, 6.8; N, 6.0%; M^+, 229. $C_{10}H_{15}NO_5$ requires C, 52.4; H, 6.6; N, 6.1%; *M*, 229); δ_H 0.96 (3 H, t), 1.68 (2 H, m), 2.34 (2 H, m), 3.45 (2 H, s) and 3.81 (6 H, s).

Dimethyl 1-Methylsulfonylaziridine-2,3-dicarboxylate **2e**.—Following the procedure described for **1e**, pure **2e** (0.2 g, 80%) was obtained after chromatography (silica gel, dichloromethane–diethyl ether, 80:20) from **2a** (0.16 g, 1 mmol), triethylamine (0.2 g, 1.98 mmol), methanesulfonyl chloride (0.17 g, 1.5 mmol) and a few crystals of DMAP (reaction time 1 h). Compound **2e** was a white solid, m.p. 87–90 °C (Found: C, 35.1; H, 4.5; N, 5.8%; $M + 1^+$, 238. $C_7H_{11}NO_6S$ requires C, 35.45; H, 4.7; N, 5.9%; *M*, 237); δ_H 3.24 (3 H, s), 3.77 (2 H, s) and 3.83 (6 H, s).

General Procedure for CCL-catalysed Hydrolysis of the Aziridines **1a–f** *and* **2a–e**.—Racemic aziridines (150 mg) were added to 0.1 mol dm^{-3} potassium phosphate buffer [5 cm^3; containing NaCl (0.1 mol dm^{-3})] pH 7.5 and treated with *Candida cylindracea* lipase with vigorous stirring at room temperature. The reaction times and the employed enzyme/aziridine (E/S) ratios (w/w) are reported in Table 1.

Hydrolyses were followed by GLC and TLC and stopped at 60–80% conversion. When hydrolysis occurred at the ester group, as for aziridines **1a**, **b**, **e**, **f** and **2a–e**, the reaction mixture was extracted with dichloromethane and the organic extracts were washed with water, dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (silica gel, dichloromethane–diethyl ether, 80:20 eluent) to obtain the unchanged esters. The enzymatic hydrolysis products, aziridinecarboxylic acids, were recovered from the aqueous phase of the reaction mixture by acidification with 10% HCl followed by extraction with diethyl ether. The carboxylic acid enantiomeric excesses were estimated through their conversions into the corresponding esters by washing the combined organic layers with water, drying them ($MgSO_4$) and treating them with diazomethane. After evaporation of the solvent, the residue was purified by column chromatography (silica gel, dichloromethane–diethyl ether, 80:20 as eluent) to obtain the hydrolysed esters.

When hydrolysis occurred at the amide bond, as for aziridines **1c**, **d**, both unchanged aziridines and the product of hydrolysis (aziridine **1a**) were extracted from the reaction mixture with dichloromethane. The ratio between the unchanged compound and the hydrolysis product was evaluated by gas chromatography and the organic extracts were treated with *tert*-butyl hypochlorite, at −10 °C, in sufficient quantity to convert the volatile aziridine **1a** into the more stable *N*-chloro derivative **1b**. Unchanged aziridines and aziridine **1b** were isolated by concentration of the reaction mixture followed by the chromatography of the residue (silica gel, dichloromethane–diethyl ether, 80:20 as eluent).

All recovered compounds showed the expected spectroscopic properties. The enantiomer ratio *E* and the conversions (*c*), reported in Table 1, were calculated from the relationships (1) and (2),[10] respectively.

$$E = \frac{\ln[(1 - c)(1 - ee_s)]}{\ln[(1 - c)(1 + ee_s)]} \quad (1) \qquad c = \frac{ee_s}{ee_s + ee_p} \quad (2)$$

The value of ee_s refers to the enantiomeric excess (ee) of the

unhydrolysed products and ee_p to the ee of the hydrolysed ones.

Methyl (2R)-(+)-*aziridine*-2-*carboxylate* **1a**. Owing to its volatility, the unchanged aziridine **1a** was recovered through conversion into the corresponding *N*-acetyl derivative **1c** following the procedure described for the synthesis of racemic **1c** from **1a**; yield 30%; (2*R*)-(+)-**1c**,[9] $[\alpha]_D$ +3.8 (*c* 1), ee 5%.

Methyl (1S,2R)-(+)-1-*chloroaziridine*-2-*carboxylate* **1b**.[8] Yield 15%; (1*S*,2*R*)-(+)-**1b**, $[\alpha]_D$ +57.1 (*c* 1.3), ee 30%. The hydrolysis product was isolated by conversion into the ester by esterification with diazomethane: yield 40%; (1*R*,2*S*)-(−)-**1b**, $[\alpha]_D$ −14.9 (*c* 1.3), ee 8%.

Methyl (2S)-(−)-1-*acetylaziridine*-2-*carboxylate* **1c**.[9] Yield 20%; (2*S*)-(−)-**1c**, $[\alpha]_D$ −46.1 (*c* 0.9), ee 63%. The hydrolysis product, the aziridine **1a**, was isolated as **1b** by chlorination, following the procedure described for the synthesis of racemic **1b** from **1a**: yield 35%; (1*S*,2*R*)-(+)-**1b**, $[\alpha]_D$ +76.1 (*c* 0.5), ee 40%. At 20% conversion, after 1 h, the unchanged aziridine (2*R*)-(−)-**1c**, $[\alpha]_D$ −19.5 (*c* 1.5), ee 27% and the hydrolysis product (1*S*,2*R*)-(+)-**1b**, $[\alpha]_D$ +154.6 (*c* 0.6), ee 80%, were isolated. Surprisingly, higher conversion (>60%) did not improve the optical purity of **1c**.

Methyl (2S)-(−)-1-*butyrylaziridine*-2-*carboxylate* **1d**. Yield 35%; (2*S*)-(−)-**1d**, $[\alpha]_D$ −74.3 (*c* 1.2), ee 90%. The hydrolysis product, the aziridine **1a**, was isolated as **1c** by acylation, following the procedure described for the synthesis of racemic **1c** from **1a**: yield 40%; (2*R*)-(+)-**1c**, $[\alpha]_D$ +45.4 (*c* 0.7), ee 60%.

Methyl (2R)-(+)-1-*methylsulfonylaziridine*-2-*carboxylate* **1e**. Unchanged aziridine (2*R*)-(+)-**1e** (37%) was obtained with $[\alpha]_D$ +13.0 (*c* 3.6), ee 14%. Absolute configuration: aziridine (+)-**1e**, which must have the same 2*R* configuration at the chiral carbon atom, was obtained by sulfonylation of (2*R*)-(+)-**1a** with methanesulfonyl chloride, following the procedure described for the synthesis of racemic **1e** from **1a**.

Methyl (2R)-(+)-1-(p-*tolylsulfonyl*)*aziridine*-2-*carboxylate* **1f**. Unchanged aziridine (2*R*)-(+)-**1f** (40%) was obtained with $[\alpha]_D$ +33.6 (*c* 1.8), ee 72%. The hydrolysis product was esterified with diazomethane to yield (2*S*)-(−)-**1f** (40%), $[\alpha]_D$ −30.2 (*c* 1.4), ee 64%. Absolute configuration: (+)-**1f**, which must have the same 2*R* configuration at the chiral carbon atom, was obtained by sulfonylation of (2*R*)-(+)-**1a** with toluene-*p*-sulfonyl chloride, as reported for the synthesis of racemic **1f** from **1a**.

Dimethyl (2R,3R)-(−)-*aziridine*-2,3-*dicarboxylate* **2a**. Unchanged aziridine (2*R*,3*R*)-(−)-**2a** (35%) was obtained with $[\alpha]_D$ −51.5 (*c* 0.5), ee 30%. The hydrolysis product was esterified with diazomethane affording (2*S*,3*S*)-(+)-**2a** (40%), $[\alpha]_D$ +6.2 (*c* 2), ee 4%. The absolute configuration of (−)-**2a** was assigned by conversion of the aziridine (−)-**2a** (30 mg, 0.2 mmol) into diethyl (2*R*,3*R*)-(−)-aziridine-2,3-dicarboxylate (90%)[12] by transesterification (5 min) in ethyl alcohol and in the presence of a trace of sodium.

Dimethyl (2R,3R)-(+)-1-*chloroaziridine*-2,3-*dicarboxylate* **2b**. Unchanged aziridine (2*R*,3*R*)-(+)-**2b** (52%) was obtained with $[\alpha]_D$ +25.7 (*c* 1), ee 26%. The hydrolysis product was esterified with diazomethane to yield (2*S*,3*S*)-(−)- **2b** (24%), $[\alpha]_D$ −42.1 (*c* 0.5), ee 42%. Absolute configuration: (+)-**2b** was obtained by chlorinating a sample of (2*R*,3*R*)-(−)-**2a** with *tert*-butyl hypochlorite.

Dimethyl (2R,3R)-(−)-1-*acetylaziridine*-2,3-*dicarboxylate* **2c**. Unchanged aziridine (2*R*,3*R*)-(−)-**2c** (30%) was obtained with $[\alpha]_D$ −56.4 (*c* 1.4), ee ⩾ 95%. The hydrolysis product, the monoester derivative, was esterified with diazomethane to afford (2*S*,3*S*)-(+)-**2c** (45%), $[\alpha]_D$ +33.4 (*c* 0.6), ee 60%. Absolute configuration: (−)-**2c** was obtained by acetylation of (2*R*,3*R*)-(−)-**2a** with acetyl chloride, following the procedure described for the synthesis of racemic **2c** from **2a**.

Dimethyl (2R,3R)-(−)-1-*butyrylaziridine*-2,3-*dicarboxylate* **2d**. Unchanged aziridine (2*R*,3*R*)-(−)-**2d** (25%) was obtained with $[\alpha]_D$ −34.8 (*c* 0.8), ee ⩾ 95%. The hydrolysis product, the monoester derivative, was esterified with diazomethane to yield (2*S*,3*S*)-(+)-**2d** (58%), $[\alpha]_D$ +9.7 (*c* 0.5), ee 30%. Absolute configuration: (−)-**2d** was obtained by acylation of (2*R*,3*R*)-(−)-**2a** with butyric acid, DCC and DMAP, following the procedure described for the synthesis of racemic **2d** from **2a**.

Dimethyl (2R,3R)-(+)-1-*methylsulfonylaziridine*-2,3-*dicarboxylate* **2e**. Unchanged aziridine (2*R*,3*R*)-(+)-**2e** (40%) was obtained with $[\alpha]_D$ +4.9 (*c* 1.2), ee 13%. The hydrolysis product, the monoester derivative, was esterified with diazomethane to afford (2*S*,3*S*)-(−)-**2e** (45%), $[\alpha]_D$ −3.8 (*c* 0.6), ee 10%. Absolute configuration: (+)-**2e** was obtained by treating (2*R*,3*R*)-(−)-**2a** with methanesulfonyl chloride, following the procedure described for the synthesis of racemic **2e** from **2a**.

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