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Lipase-Catalyzed Resolution of 2-Azabicyclo[2.2.1]hept-5-en-3-ones[1]

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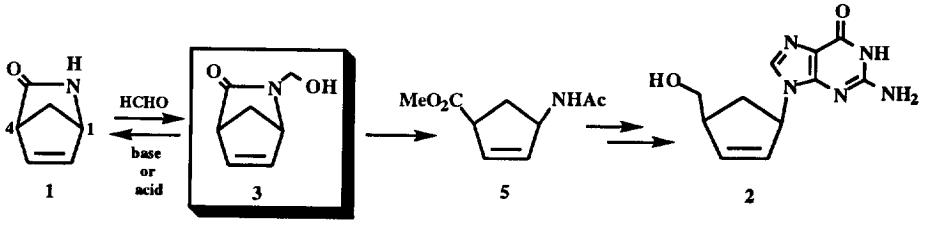
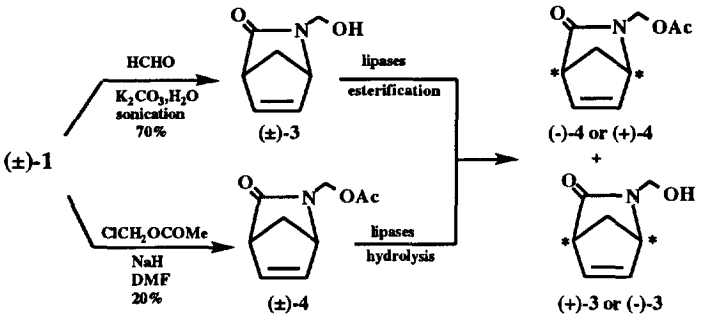
Abstract: The lipase-catalyzed asymmetric resolution of 2-azabicyclo[2.2.1]hept-5-en-3-ones is reported; non-racemic chiral 2-azabicyclo[2.2.1]hept-5-en-3-ones were obtained conveniently by lipase-catalyzed enantioselective transesterification or hydrolysis of *N*-hydroxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one or *N*-acyloxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one.

Bicyclic lactam, 2-azabicyclo[2.2.1]hept-5-en-3-one **1** has great potential as a synthetic intermediate. Thus, the lactam **1** may become a synthon of carbocyclic sugar amines,[2] carbonucleosides,[3] and carbocyclic dinucleotide analogues.[3] Particularly , the synthetic potential of (-)-**1** proved as an useful synthon for the synthesis of (-)-carbovir **2**,[3] which was shown to have similar activity to AZT(zidovudine) against HIV.[3d,e,g] Therefore an effecient synthetic method for the preparation of the appropriate one enantiomer of the bicyclic lactam synthon is required. A lipase is a typical enzyme to be accepted for routine use in organic synthesis, because it requires no coenzymes and is commercially available and inexpensive. We wish to report the facile synthesis of non-racemic chiral 2-azabicyclo[2.2.1]hept-5-en-3-ones by a lipase-catalyzed enantioselective transesterification and hydrolysis of (±)-**1** having a hydroxymethyl group or acyloxymethyl group on the nitrogen, in which the stereogenic centers are remote from the reactions site.[4] Generally, most substrates of the lipase-catalyzed asymmetric resolution have been compounds in which the stereogenic carbon atoms were adjacent to the reaction site.[5] Furthermore, the chiral bicyclic lactam **3** obtained was converted conveniently into the chiral carbosugar intermediate **5** of **2**.



Scheme 1

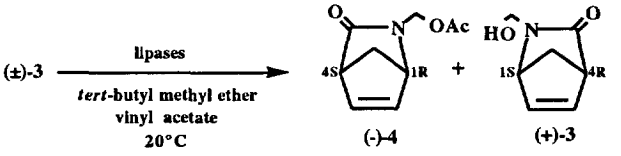
N-Hydroxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one **3** was prepared easily by treatment of commercially available racemate **1** with paraformaldehyde in the presence of potassium carbonate and water under sonication for 6h in good yield (Scheme 2). *N*-Acetoxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one **4** was prepared by reaction of sodium salt of racemate **1** with acetoxymethylchloride (Scheme 2). The structures of **3** or **4** were characterized by IR, ^{1}H-NMR spectroscopy, mass and high-resolution mass spectrometry.



Scheme 2

First, we examined the transesterification of (±)-**3** with vinyl acetate by the three Amano lipases [PS (*Pseudomonas cepacia*), AK (*Pseudomonas fluorescens*), AY (*Candida rugosa*)] and two new Amano immobilized lipase PS (imm-PS on diatomite and imm-PS on Toyonite-200-P) in *tert*-butyl methyl ether. The results are summarized in Table 1. Both lipase PS and AK catalyzed the transesterification of the hydroxyl group on (±)-**3** rapidly with a high degree of enantioselectivity, but the rate of the reaction by lipase AY was slow, and satisfactory result in terms of enantioselectivity were not obtained. While, the transesterification using the two new immobilized lipase PS proceeded smoothly and high enantioselectively. In particular, the immobilized lipase PS on diatomite proved to have efficient catalytic ability [reaction time (0.5 hour), acetate (-)-**4** (43%, 93%ee), recovery (+)-**3** (48%, 98%ee)].

Table 1: Lipase-catalyzed transesterification[a] of (±)-3



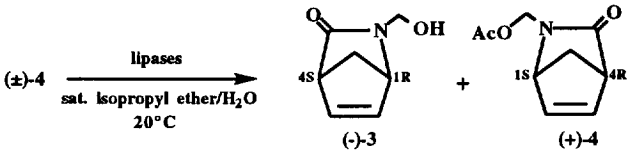
			Product (-)-4		Recovery (+)-3	
Lipase	Time(h)	Convn(%)[b]	C.Y.(%)[c]	Ee(%)[d]	C.Y.(%)	Ee(%)
PS	2	49	38	94	40	89
AK	5	50	31	89	40	91
AY	10	28	5	26	40	10
Imm-PS on diatomite	0.5	51	43	93	48	98
Imm-PS on Toyonite	1	51	39	86	42	91

a. Conditions: (±)-**3** (0.2g, 1.44mmol), five lipases (0.2g), vinyl acetate (0.6g, 7.2 mmol), *tert*-butyl methyl ether (100ml) b. Conversion: ref.6. c. Isolated yield. d. The enantiomeric excesses were determined by HPLC on Chiralcel OD (Daicel, Japan) column (hexane/isopropyl ether).

Having obtained the results in the resolution of racemic alcohol (±)-**3** under transesterification conditions, we next examined the resolution of racemic acetate (±)-**4** under hydrolytic conditions using isopropyl ether saturated with water in the presence of three good lipases (lipase PS, immobilized lipase PS on diatomite, and immobilized lipase PS on Toyonite-200-P). The results are summarized in Table 2. The best result was obtained

by using lipase PS [alcohol (-)-**3** (41%, 95%ee), recovery (+)-**4** (41%, 96%ee)]. Interestingly, the catalytic ability of two immobilized lipases reversed in hydrolysis as compared to transesterification. Namely, good reaction rate and enantioselectivity [reaction time (1 hour), alcohol (-)-**3** (41% 75%ee), recovery(+)-**4** (35%, >99%ee)] were achieved by using immobilized lipase PS on Toyonite-200-P.

Table 2: Lipase-catalyzed hydrolysis[a] of (±)-4



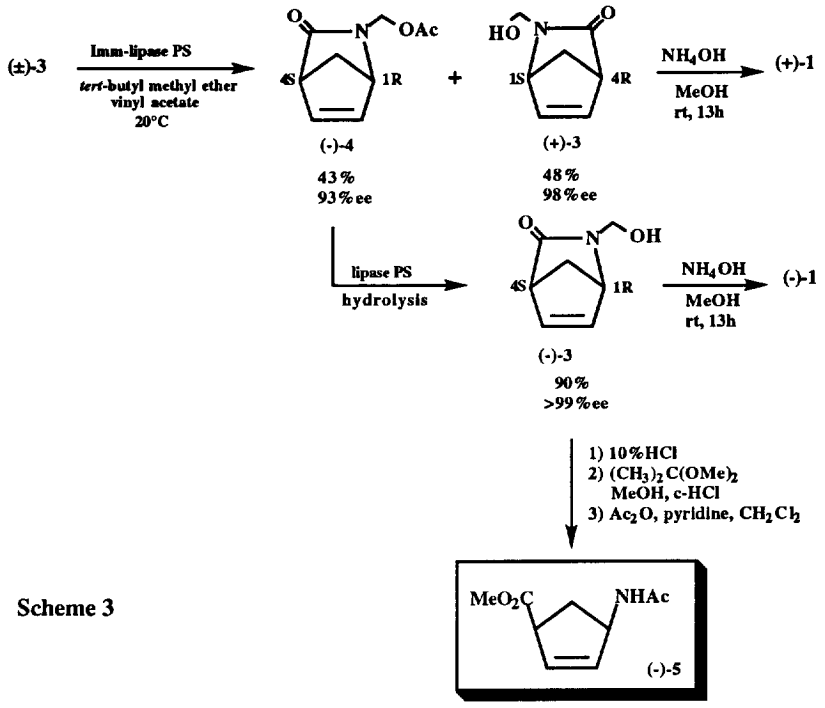
Lipase	Time(h)	Convn(%)[b]	Product (-)-3 C.Y.(%)[c]	Product (-)-3 Ee(%)[d]	Recovery (+)-4 C.Y.(%)	Recovery (+)-4 Ee(%)
PS	3	50	41	95	41	96
Imm-PS on diatomite	3	52	43	81	33	88
Imm-PS on Toyonite	1	58	41	75	35	>99

a. Conditions: (±)-**4** (0.2g, 1.11mmol), three lipases (0.2g), sat. isopropyl ether-H_2O (100 ml). b. Conversion: ref.6. c. Isolated yield. d. The enantiomeric excesses were determined by HPLC on Chiralcel OD (Daicel, Japan) column (hexane/isopropyl ether).

The chiral acetate (-)-**4** and alcohol (+)-**3**, obtained by catalytic transesterification using immobilized lipase PS on diatomite, were converted efficiently into the chiral precursors (-)-**5** or (-)-**1** of carbovir **2** as follows (Scheme 3). The (+)-**3** (48%, 98%ee) was purified by recrystallization (isopropyl ether) to extremely high enantiomeric purity (>99%ee), mp 60-62°C, $[\alpha]_D$ +344 (c2.1, $CHCl_3$). Furthermore, The hydrolysis of acetate (-)-**4**, viscous oil, 93%ee, $[\alpha]_D$ -104 (c2.5, $CHCl_3$) gave the *N*-hydroxymethyl form (-)-**3**, mp 58-59°C, $[\alpha]_D$ -343 (c2.1, $CHCl_3$) using pase PS under mild conditions in excellent chemical (90%) and enantiomeric (>99%ee) yields. The enantiomerically pure (-)-**3** obtained was converted conveniently into the carbocyclic sugar intermediate (-)-**5**, 73%, >99%ee, $[\alpha]_D$ -85.0 (c1.0., MeOH), lit.[3f] : $[\alpha]_D$ -84.4 (c1.0., MeOH) according to the method of Roberts *et. al.* [3f] in one step. And (+)-**3** or (-)-**3** obtained were also converted conveniently into (+)-**1**, 38%, >99%ee, mp 89-90°C, $[\alpha]_D$ +554 (c1.2, CHCl3), lit.[3d] : $[\alpha]_D$ +555±15 or (-)-**1**, 51%, >99%ee, mp 86-88°C, $[\alpha]_D$ -560 (c1.1, CHCl3), lit.[3d] : $[\alpha]_D$ -568±15, respectively, by treatment with ammonium hydroxide in methanol.

The absolute configurations of bicyclic lactams (+)-**3** (1*S*,4*R*), (-)-**4** (1*R*,4*S*) and (-)-**3** (1*R*,4*S*) were determined by their conversion into (+)-**1** (1*S*,4*R*) or (-)-**1** (1*R*,4*S*) as mentioned above,[3f] respectively. The enantiomeric purities of the chiral compounds **1**, **3** and **4** were determined by HPLC analysis using a chiral column [Chiralpack AS(Daicel,Japan)] and these structures were characterized by IR, ^{1}H-NMR spectroscopy, mass and high-resolution mass spectrometry.

In conclusion, we have established an efficient synthetic method of both enantiomeric bicyclic lactams (-)-**1** or (+)-**1**, and the *N*-hydroxymethyl derivative (-)-**3** was converted easily into the corresponding carbocyclic sugar intermediate (-)-**5** of non-racemic carbovir.



Scheme 3

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with a PERKIN ELMER 1725X spectrophotometer. ^{1}H-NMR spectra were recorded on a JEOL JNM-PMX 60 and a JEOL JNM-EX 270 spectrometers with TMS as an internal standard. The coupling patterns are indicated as follows: singlet=s, doublet=d, triplet=t, multiplet=m, and broad=br. MS were taken on a Hitachi RMG-6MG and a JEOL-JNM-DX 303 spectrometers. Optical rotations were measured with a JASCO-DIP-360 digital polarimater. The e e values of the *N*-hydroxymethyl and *N*-acetoxymethyl bicyclic lactams were determined by HPLC analysis using chiralcel OD.

Synthesis of *N*-hydroxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (±)-3: A mixture of (±)-**1** (1.09g, 10mmol), paraformaldehyde (1g) and potassium carbonate (0.2g, 1.5mmol) in H_2O (20ml) was treated under sonication for 6h at room temperature. Extraction with $CHCl_3$, dring ($MgSO_4$), and removal of the solvent *in vacuo* provided the crude *N*-hydroxymethyl form which was purified by silica gel TLC (ether). (±)-**3**: 0.97g, 70%, colorless prisms (ether), mp 58-59°C. High resolution MS m/z : Calcd $C_7H_9NO_2$ (M^+) : 139.0633. Found : 139.0645. IR (NaCl)cm^{-1} : 3376, 1696. ^{1}H-NMR ($CDCl_3$) δ: 1.93-2.50 (2H, m), 3,20-3.43 (1H, m), 4.23-4.50 (1H, m), 4.50 (1H, d, J=10Hz), 4.83 (1H, d, J=10Hz), 6.40-6.73 (1H, m), 6.83-7.13 (1H, m).

Synthesis of *N*-acetoxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (±)-4: The bicyclic lactam (±)-**1** (1.09g, 10mmol) was added to the suspension of sodium hydride (60% in oil) (0.4g, 10mmol) in dry DMF (20ml) at 0°C. Stirring was continued for 1h, chloromethyl acetate (1.09g, 10mmol) was added and the

mixture was stirred for 1h at room temperature. The reaction mixture was poured into ice-cooled water and extracted with ether. The extract was washed with brine and dried ($MgSO_4$). The solvent was removed and residual oil was chromatographed on silica gel column eluted with ether to give a colorless oil.
(±)-**4**: 0.36g, 20%, colorless oil, IR (NaCl)cm^{-1} : 1723. ^{1}H-NMR ($CDCl_3$) δ : 2.00 (3H, s), 2.10-2.53 (2H, m), 3.20-3.50 (1H, m), 4.30-4.53 (1H, m), 5.20 (2H, s), 6.47-6.70 (1H, m), 6.70-6.93 (1H, m).

General procedure for lipase-catalyzed transesterification of *N*-hydroxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (±)-3: A mixture of (±)-**3** (0.2g, 1.44mmol), lipases (PS, AK, AY, Immobilized lipase PS on diatomite, and Immobilized lipase PS on Toyonite-200-P) (0.2g) and vinyl acetate (0.6g, 7.2mmol) in *tert*-butyl methyl ether (100ml) was stirred at room temperature, respectively. The lipase was removed by filtration and washed with ether.The combined organic layer was concentrated, and the residue was chromatographed on a silica gel column eluted with ether to give the corresponding (1*R*, 4*S*)-*N*-acetoxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (-)-**4** and (1*S*, 4*R*)-*N*-hydroxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (+)-**3**. Enantiomeric excesses of (-)-**4** or (+)-**3** were determined by HPLC analysis using chiral column [Chiralcel OD, hexane-isopropyl ether (9:1), flow rate: 0.5ml/1min, Rt (min): 25min for (+)-**3**, 35min for (-)-**4**]. The reaction times and yields are listed in Table 1. The spectral data of the products (-)-**4** or (+)-**3** were identical with those of the corresponding racemic compounds (±)-**3** or (±)-**4**.

General procedure for lipase-catalyzed hydrolysis of *N*-acetoxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (±)-4: A mixture of (±)-**4** (0.2g, 1.11mmol) and lipases (PS, Immobilized lipase PS on diatomite, and Immobilized lipase PS on Toyonite-200-P) (0.2g) in isopropyl ether saturated with water (100ml) was stirred at room temperature, respectively. The lipase was removed by filtration.The filtrate was concentrated, and the residue was chromatographed on a silica gel column eluted with ether to give the corresponding (1*R*, 4*S*)-*N*-hydroxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (-)-**3** and (1*S*, 4*R*)-*N*-acetoxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (+)-**4**. Enantiomeric excesses of (-)-**3** or (+)-**4** were determined by HPLC analysis using chiral column [Chiralcel OD, hexane-isopropyl ether (9:1), flow rate: 0.5ml/1min, Rt (min): 22min for (-)-**3**, 40min for (+)-**4**]. The reaction times and yields are listed in Table 2. The spectral data of the products (-)-**3** or (+)-**4** were identical with those of the corresponding racemic compounds (±)-**3** or (±)-**4**.

Lipase-catalyzed hydrolysis of (1*R*, 4*S*)-*N*-acetoxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (-)-4: A mixture of (-)-**4** (93%ee, 0.3g, 1.66mmol) and lipase PS (0.3g) in isopropyl ether saturated with water (100ml) was stirred for 3h at room temperature. The lipase was removed by filtration.The filtrate was concentrated, and the residue was chromatographed on a silica gel column eluted with ether to give the corresponding (1*R*, 4*S*)-2-hydroxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (-)-**3** (0.22g, 90%, >99%ee). Enantiomeric excess of (-)-**3** was determined by HPLC analysis using chiral column (Chiralpak AS).

Conversion of (+)-3 or (-)-3 to (+)-1 or (-)-1: A solution of (+)-**3** (>99%ee, 0.1g, 0.72mmol) or (-)-**3** (>99%ee, 0.1g, 0.72mmol) and 28% NH_4OH (1ml) in MeOH (10ml) was stirred at room temperature for 12h, respectively. The solvent was removed under reduced pressure and the residue was purified by silica gel TLC (ether) to give (+)-**1** or (-)-**1**, respectively. The spectral data of the products were identical with those of

(±)-**1**. (+)-**1**: 0.035g, 45%, >99%ee, mp 89-90°C, $[\alpha]_D$ +554 (c1.2, $CHCl_3$), lit.[3d] : $[\alpha]_D$ +555±15. (-)-**1**: 0.04g, 51%, >99%ee, mp 86-88°C, $[\alpha]_D$ -560 (c1.1, $CHCl_3$), lit.[3d] : $[\alpha]_D$ -568±15.

Conversion of (-)-3 to (-)-5: A mixture of *N*-hydroxymethyl form (-)-**3** (>99%ee, 0.15g, 1.4mmol) in 10%HCl (10ml) was refluxed for 2h under N_2. The mixture was concentrated under reduced pressure and the residue was stirred in a mixture of MeOH (2ml), conc. HCl (0.6ml), and dimethoxypropane (10ml) at room temperature for 24h. The mixture was concentrated under reduced pressure. To the resulting solid, dichloromethane (10ml), acetic anhydride (0.28g, 2.7mmol), and pyridine (0.3g, 3.7mmol) were added. And the reaction mixture was stirred for 24h at room temperature under nitrogen, then was diluted with chloroform (50ml) and washed with water (20ml). The combined extract was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by silica gel TLC (ether) to give (-)-**5**. (-)-**5**: 0.19g, 73%, >99%ee, $[\alpha]_D$ -85.0 (c1.0, MeOH), lit.[3f] : $[\alpha]_D$ -84.4.

Acknowledgement

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