Synthesis of novel optically pure β-lactams

Yan Yang, Fengyun Wang, Fernande D. Rochon, and Margaret M. Kayser

Abstract: Several new β-lactams were synthesized as racemates via a Staudinger reaction. The corresponding optically pure compounds were obtained in subsequent biotransformation steps either through baker's yeast reduction or lipase resolution. Their absolute configurations were established. The X-ray crystal structures of three new substituted β-lactams are reported here. These compounds represent key building blocks for a variety of medicinally important molecules, including inhibitors of aspartyl proteases and Taxol® analogues.

Key words: optically pure β-lactams, lipase resolutions, baker's yeast reductions, Staudinger reaction.

Résumé : Faisant appel à la réaction de Staudinger, on a réalisé la synthèse de plusieurs nouvelles β-lactames racémiques. On a obtenu les composés correspondants optiquement purs par le biais de biotransformations subséquentes impliquant une réduction par la levure de boulanger ou par résolution avec une lipase. On en a déterminé les configurations absolues. On a déterminé les structures cristallines de trois β-lactames substituées par diffraction des rayons X. Ces composés sont des unités pouvant servir à la synthèse de plusieurs molécules médicinalement importantes, y compris des inhibiteurs de protéases de l'aspartyle et des analogues du Taxol®.

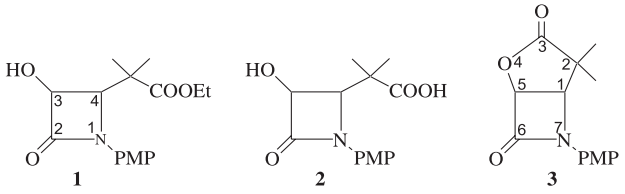
Mots clés : β-lactames optiquement pures, résolutions à la lipase, réductions avec de la levure de boulanger, réaction de Staudinger.

[Traduit par la Rédaction]

Introduction

The β-lactam skeleton represents a core structure of numerous natural and synthetic antibiotics (1). The 3-hydroxy-substituted β-lactams are convenient precursors of β-amino-α-hydroxy esters that constitute key fragments of many biologically important natural products such as aminosugars, α-hydroxy-β-amino acids, glycosphingolipids, peptidic enzyme inhibitors, and alkaloids (2). Furthermore, 3-hydroxy-β-lactams are used for the construction of analogues of the anticancer drug Taxol® (3). It is not surprising, therefore, that the development of enantioselective syntheses of such β-lactams has been pursued by numerous laboratories around the world.

The extensive structure–activity relationship (SAR) studies have shown that the phenylisoserine C-13 side chain of paclitaxel (Taxol®, Fig. 1), essential for anticancer activity, tolerates a variety of modifications (4), and even small changes significantly influence the bioactive properties of the drug (3, 5). The therapeutic potential of paclitaxel is limited by its high hydrophobicity and development of resistance. Search for analogues with better water solubility, easier to administer, fewer side effects, and active on multidrug-resistant (MDR) cells is an on-going process (6). We have been interested in developing the synthesis of optically pure C-13 side chains in which 3′-phenyl is replaced with bulky, polar groups. The preparation of side chains in the form of 3-hydroxy-β-lactams is particularly appealing since it is in this form that the prospective side chains are generally coupled with baccatin III. Here, we report the syntheses and characterizations of several isomers of 4-(1-ethoxycarbonyl-1-methylethyl)-3-hydroxy-β-lactam (**1**), the 4-(1-carboxy-1-methylethyl)-3-hydroxy-β-lactam (**2**), and the corresponding lactone (**3**).



A number of methods for the preparation of β-lactams, such as ketene–imine cycloaddition (1*a*, 7), metalloester enolate–imine condensation (8), isocyanate–alkene cycloaddition (9), and alkyne–nitrone cycloaddition (10), have been developed over the years. Among these, the most widely used is the Staudinger reaction, in which ketene–imine addition leads to a β-lactam ring system. Optically pure β-lactams prepared via asymmetric synthesis employed either a combination of chiral imine and achiral ketenes, achiral imines and chiral ketenes (1*a*, 11), or a direct cyclization of ketene–imine in the presence of a chiral catalyst (12). Only a few studies resorted to biocatalytic approaches such as lipase-catalyzed resolution (13) or baker's yeast reduction (14). Since our preliminary goal was to syn-

Received 22 March 2004. Published on the NRC Research Press Web site at http://canjchem.nrc.ca on 5 February 2005.

Dedicated to Professor Peter Stanetty on the occasion of his 60th birthday.

Y. Yang, F. Wang, and M.M. Kayser.[1] Department of Physical Sciences, University of New Brunswick, Saint John, NB E2L 4L5, Canada.
F.D. Rochon. Département de chimie, Université du Québec à Montréal, C. P. 8888, Succ. Centre-Ville Montréal, QC H3C 3P8, Canada.

[1]Corresponding author (e-mail: kayser@unbsj.ca).

 doi: 10.1139/V04-159

Scheme 1.

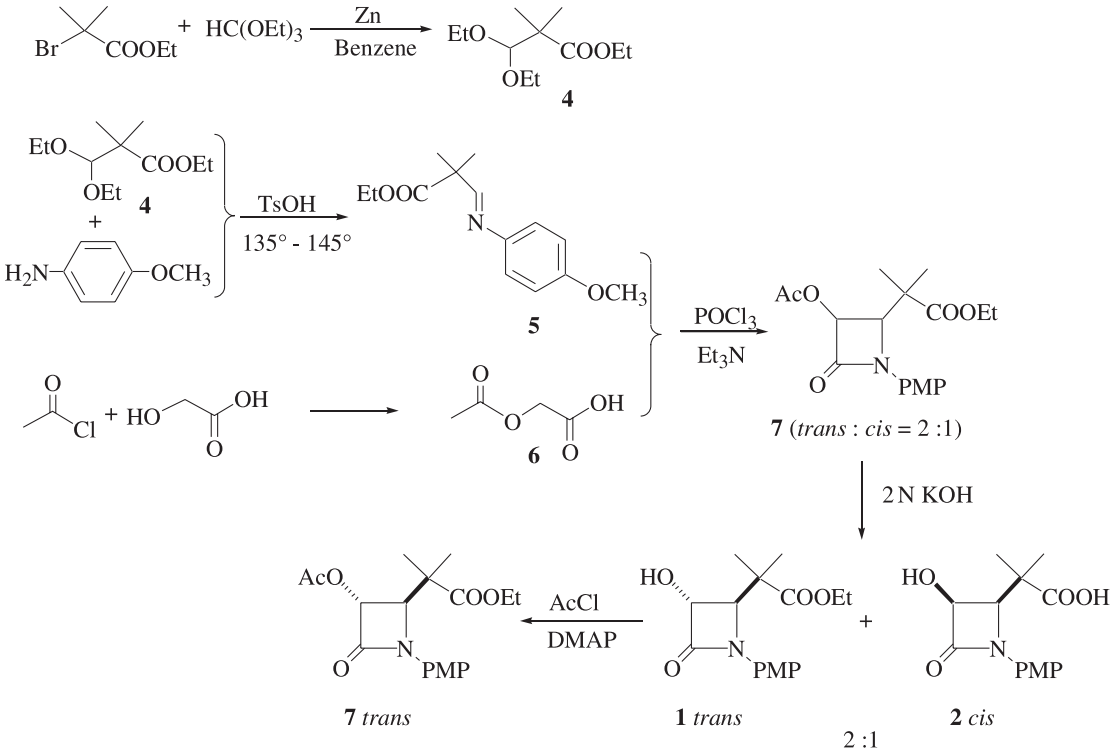
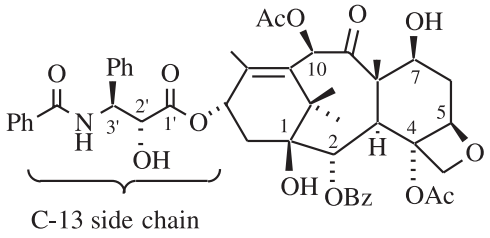


Fig. 1. Structure of paclitaxel (Taxol®).



thesize and characterize all the diastereomers and enantiomers, we decided to prepare the racemic β-lactams through Staudinger cyclization (Scheme 1) and to obtain enantiopure β-lactams in the subsequent biotransformation steps.

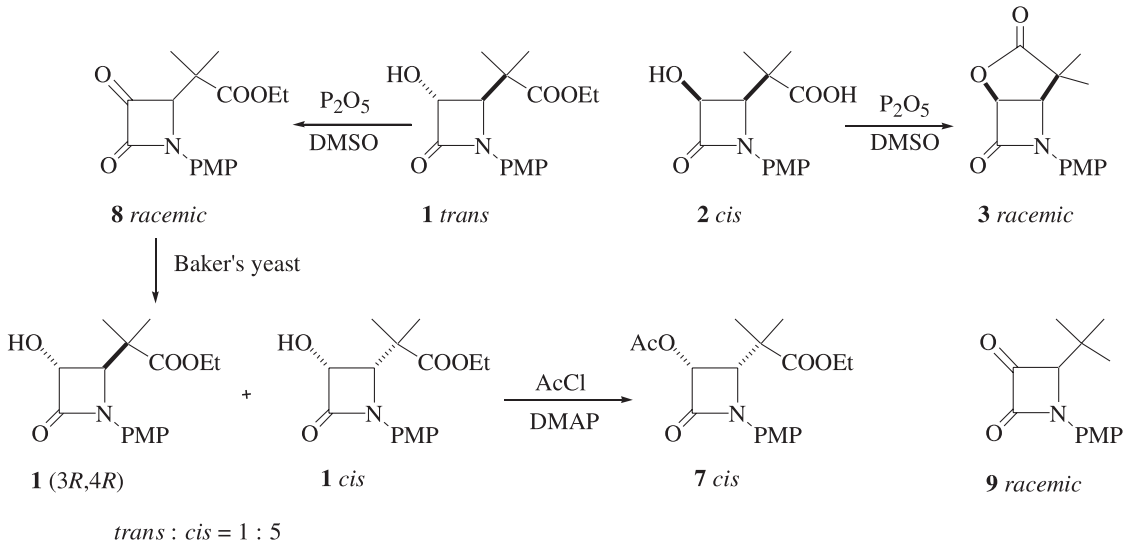
Results and discussion

Condensation of 3,3-diethoxy-2,2-dimethylpropionic acid ethyl ester (**4**) (15) with *p*-anisidine gave 3-(4-methoxyphenylimino)-2,2-dimethylpropionic acid ethyl ester (**5**), which was used in the following step without purification. Cycloaddition of **5** with the in-situ-generated acetoxyacetyl chloride in the presence of anhydrous triethylamine gave acetyl β-lactam **7** (PMP = *p*-methoxyphenyl) in 53% yield after two steps. The β-lactam product **7** consisted of trans and cis diastereomers in the ratio of 2:1 based on the ^{1}H NMR spectrum of the product mixture. Hydrolysis of **7** (2 N KOH) gave two compounds as shown in Scheme 1. It was interesting to note that while the *trans*-acetyl β-lactam was selectively hydrolyzed to the corresponding *trans*-3-hydroxy β-lactam (**1**) as expected, its cis diastereomer was hydrolyzed at both acetyl and acetate groups, yielding *cis*-3-hydroxy β-lactam (**2**). Apparently in the cis isomer, the hindered ester function undergoes hydrolysis aided by the strategically located 3-OH group. The two compounds were easily separated during the extraction process (see Experimental). This turned out to be very useful in the characterization of **7**-*trans* and **7**-*cis*, since the original mixture from the Staudinger reaction was inseparable by chromatography. The pure sample of **7**-*trans* was prepared by acylation of **1**-*trans* (Scheme 1), while **7**-*cis* was obtained from baker's yeast reduction of the α-keto-β-lactam (**8**, Scheme 2). Their stereochemistry was assigned on the basis of coupling constants for the *cis*- and t*rans*-β-lactams (16). Oxidation of compound **1** with DMSO/phosphorus pentoxide provided the α-keto-β-lactam (**8**). Under the same conditions, **2**-*cis* was dehydrated to a racemic mixture of fused lactone–lactams **3** as shown in Scheme 2.

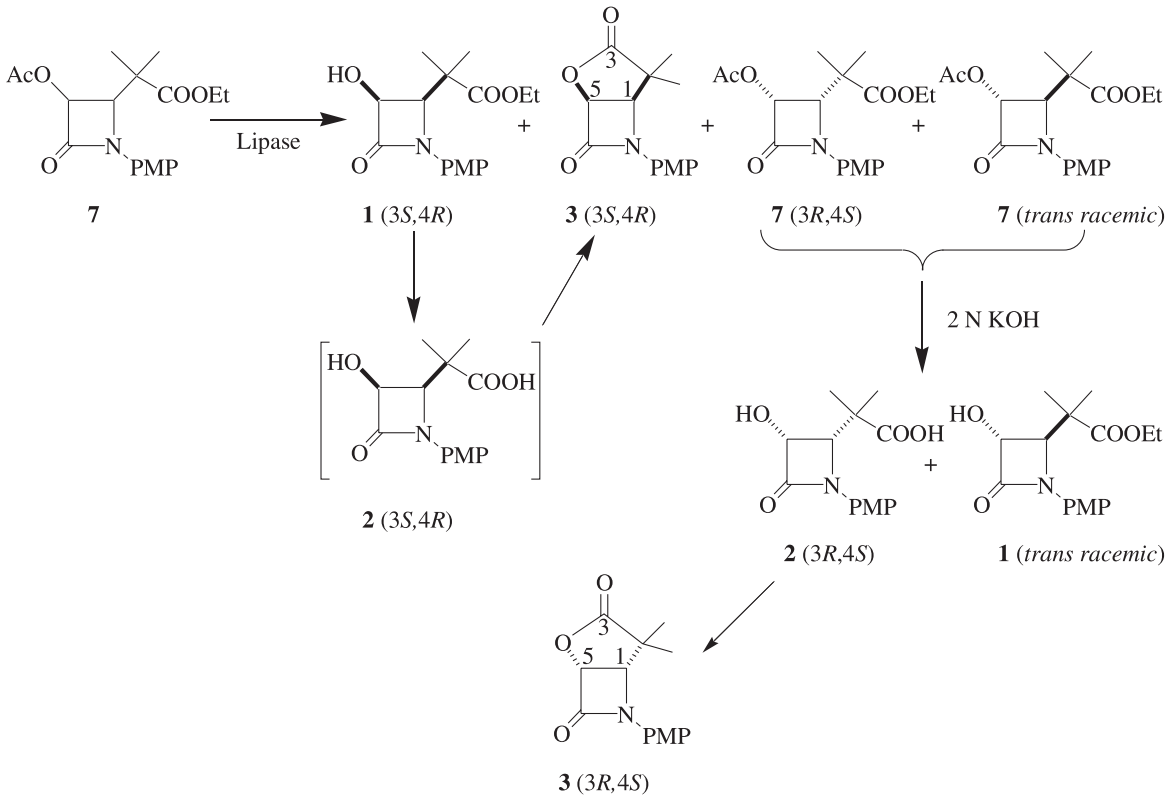
In an initial attempt to obtain optically pure 3-hydroxy-β-lactams (**1**), we performed baker's yeast reduction of the racemic 3-oxo-β-lactam (**8**). This strategy was successful for the *tert*-butyl analogue **9**, where it yielded easily separable 3-hydroxy diastereomers 3*R*,4*S*-*cis* and 3*R*,4*R*-*trans* (14). Unfortunately, in the case of ketone **8**, the reduction product consisted of an inseparable mixture of essentially racemic **1**-*cis* and an optically pure **1**-*trans* isomer, tentatively identified as 3*R*,4*R* (Scheme 2). The ratio of cis to trans (5:1) was measured from the ^{1}H NMR spectrum of the product mixture.

Since a racemic mixture of *cis*-3-acetoxy-4-phenyl-β-lactams was reported to be enantioselectively hydrolyzed by Amano lipase (13*a*), we decided to test the ability of this enzyme to resolve the mixture of lactams **7**. It turned out that the mixture of the isomers of **7** could be resolved efficiently, particularly since the **7**-*trans* is not a suitable substrate for the lipase PS "Amano" (Scheme 3).

Scheme 2.



Scheme 3.



Of the four products formed in the course of the lipase resolution, compounds **1** and **3** were easily separated by chromatography and the absolute configuration of **1** (3*S*,4*R*) was established with certainty by X-ray diffraction methods. The whole diffraction sphere was measured with Cu-Kα radiation. The absolute structure parameter (Flack) determined using the option TWIN/BASF 0.5 indicated the right configuration (Table 1).

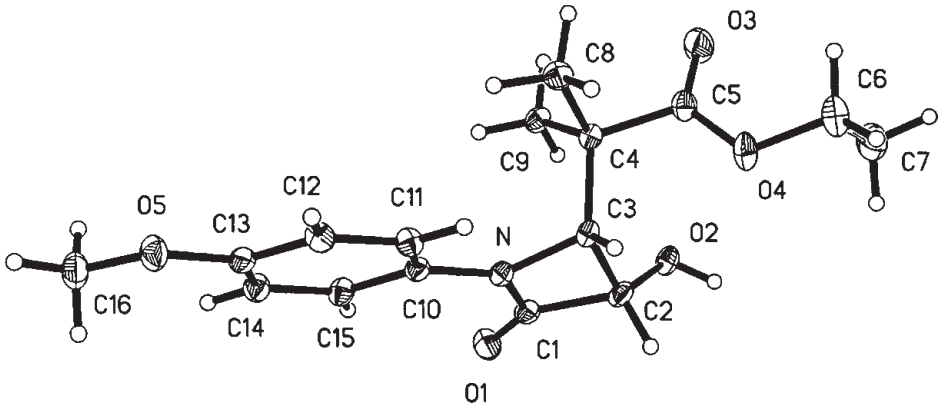
The molecular structure of **1** (3*S*,4*R*) is shown in Fig. 2. The internal angles in the four-membered ring vary between 85.4(1)° and 95.1(2)°. The N—C and O—C bond distances close to the carbonyl groups are shorter than the others. For example, the distances N—C1 = 1.360(3) Å and O4—C5 = 1.335(3) Å, while N—C3 = 1.488(3) Å and O4—C6 = 1.461(3) Å.

The lactone–lactam **3** (1*R*,5*S*), apparently resulting from hydrolysis of the ethoxy carbonyl group followed by cyclization, was isolated and its absolute configuration was initially established by chiral-phase HPLC analysis. This was possible because the four isomers of **7** are clearly re-

Table 1. Crystal data for compounds **1**, **3**, and **11**.

Compound	**1**	**3**	**11**
Empirical formula	$C_{16}H_{21}NO_5$	$C_{14}H_{15}NO_4$	$C_{23}H_{18}BrNO_4$
Formula weight	307.34	261.27	452.29
Temperature (K)	223(2)	223(2)	293
Wavelength (Å)	1.541 78	1.541 78	0.710 73
Crystal system	Monoclinic	Monoclinic	Orthorhombic
Space group	$P2_1$	$P2_1$	$P2_12_12_1$
a (Å)	11.383 2(2)	5.896 2(1)	8.901(3)
b (Å)	5.895 9(1)	18.571 0(2)	10.392(4)
c (Å)	12.270 9(2)	11.529 2(1)	21.842(8)
β (°)	110.431(1)	90.762(1)	90.
Volume (Å^3)	771.74(2)	1 262.32(3)	2020.4(13)
Z	2	4	4
D_{calcd} (Mg m^{-3})	1.323	1.375	1.487
Absorption coefficient (mm^{-1})	0.815	0.842	2.064
F(000)	328	552	920
θ range for data collection (°)	3.84–72.9	3.83–72.77	1.86–25.0
Reflections collected	9298	15 210	2052
Independent reflections (R_{int})	2711 (0.051 1)	4 802 (0.029 8)	2052
Observed reflections ($I > 2\sigma(I)$)	2656	4 570	899
Goodness-of-fit on F^2	1.041	1.045	1.048
Final R_1 [$I > 2\sigma(I)$]	0.051 5	0.035 0	0.063 6
wR_2 (all data)	0.139 6	0.073 0	0.108 2
Absolute structure parameter	0.1(2)	0.00(17)	0.00(3)

Fig. 2. Crystal structure of *cis*-3-hydroxy-β-lactam (**1**, 3*S*,4*R*).



solved on the chiral-phase HPLC. Furthermore, in the course of the HPLC-monitored lipase resolution we were able to observe that only *one* of the cis enantiomers was hydrolyzed; its antipode and the two trans diastereomers were unaffected. Additional evidence for the configuration of **3** (1*R*,5*S*) was obtained when a mixture of **1** (3*S*,4*R*) and **3** (1*R*,5*S*) under extended treatment with an excess of the lipase was transformed entirely into the lactone **3** (1*R*,5*S*). Finally, we were able to obtain a suitable crystal, and the proposed absolute configuration of **3** (1*R*,5*S*) was confirmed by X-ray diffraction. Again, the absolute structure parameter (Flack) confirmed the correct enantiomer (Table 1). The compound crystallized with two independent molecules in the unit cell. The two molecules are very similar as shown by identical equivalent bond distances and angles. The molecular structure of one of the two molecules is shown in Fig. 3.

The conformations of the two molecules are slightly different, especially in the region of the five-membered ring. For example, the torsion angles in the first molecule N1-C3-C4-C5 = –91.9(2)°, N1-C3-C4-C7 = 27.5(3)°, and N1-C3-C4-C6 = 153.8(2)°, while the corresponding angles in the second molecule are N2-C17-C19-C18 = –82.4(2)°, N2-C17-C19-C20 = 38.7(3)°, and N2-C17-C19-C27 = 165.3(2)°. Again, the N—C and O—C bond distances close to the carbonyl bonds are shorter than the others.

The remaining products from the lipase resolution, **7** (3*R*,4*S*) and **7**-*trans* (racemic), were hydrolyzed with 2 N

Fig. 3. Crystal structure of the fused lactone–lactam **3** (1*R*,5*S*).

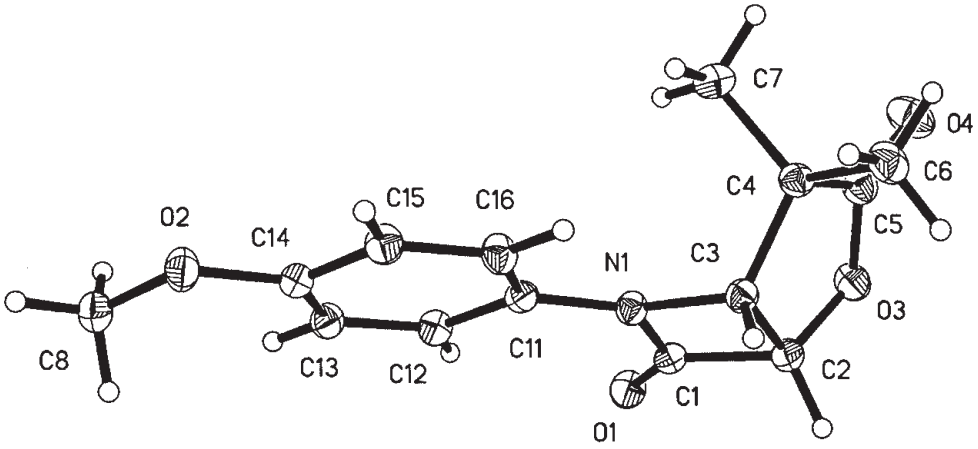
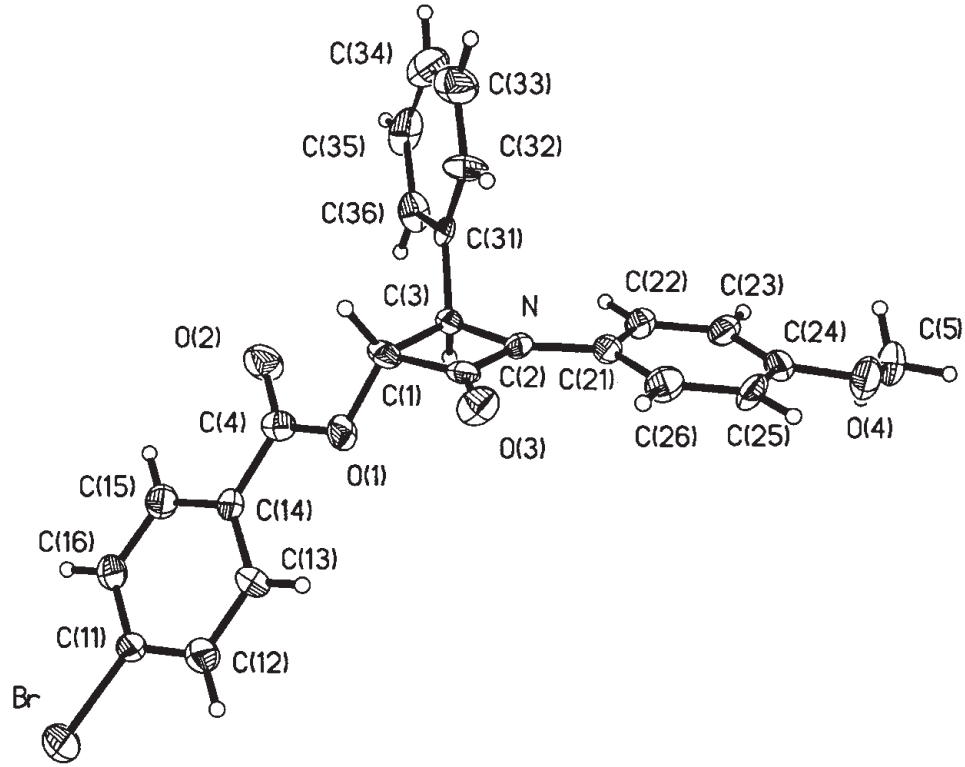


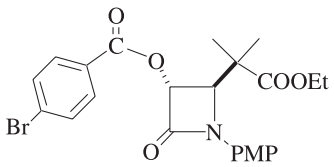
Fig. 4. Crystal structure of *trans*-β-lactam (**11**, 3*R*,4*R*).



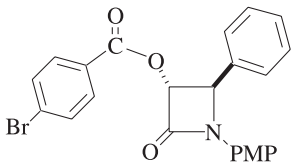
KOH to give **2** (3*R*,4*S*) and **1**-*trans* (racemic), which were separated during the work-up. The subsequent treatment of **2** (3*R*,4*S*) with DMSO/phosphorous pentoxide gave lactone **3** (1*S*,5*R*), which is clearly distinguishable from its antipode on the chiral-phase HPLC and has opposite specific rotation (Scheme 3).

The attempts to resolve racemic **1**-*trans* using lipases PS "Amano", PS-C "Amano" II, AY "Amano", and AK "Amano" were not successful. To characterize fully and to establish unambiguously the absolute configurations of the trans products, we decided to separate and identify the **1**-*trans* enantiomer from the baker's yeast reduction (Scheme 2). We were unable to separate the overlapping cis and trans isomers, however, on treatment with 2 N KOH, only the racemic **1**-*cis* was hydrolysed to the corresponding acid and the enantiopure **1**-*trans* was separated during the work-up. Unfortunately, neither **1**-*trans* nor its *p*-bromobenzoyl derivative **10** could produce crystals suitable for X-ray crystallographic analysis. Thus, the (3*R*,4*R*) absolute configuration was tentatively assigned by analogy to other related *trans*-β-lactams obtained from yeast-catalyzed reductions: the phenyl-substituted **11** (3*R*,4*R*) (17) whose crystal structure was determined and is shown in Fig. 4 (Table 1), and *tert*-butyl-substituted lactam **12** (3*R*,4*R*) reported earlier (14).

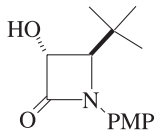
In summary, we have synthesized and fully characterized several new β-lactams. The absolute configurations were deduced from chiral-phase HPLC and confirmed through X-ray crystal structure determination. We are currently developing and optimizing more efficient and better-yielding



10 (3*R*,4*R*)



11 (3*R*,4*R*)



12 (3*R*,4*R*)

protocols for the preparations of these and several other related compounds.

Experimental

The X-ray crystallographic data for compounds **1** and **3** were collected using a Bruker Platform diffractometer, equipped with a Bruker SMART 2K charged-coupled device (CCD) area detector using the program SMART and normal focus sealed tube source graphite-monochromated Cu-Kα radiation. The crystal-to-detector distance was 4.908 cm, and the data collection was carried out in 512 pixel × 512 pixel mode, utilizing 4 pixel × 4 pixel binning. The initial unit cell parameters were determined by a least-squares fit of the angular setting of strong reflections, collected by a 9.0° scan in 30 frames over four different parts of the reciprocal space (120 frames total). One complete sphere of data was collected, to better than 0.8\Å resolution. Upon completion of the data collection, the first 101 frames were recollected to improve the decay correction analysis. The crystal structure of the bromo compound (**11**) was determined on a Siemens P4 instrument using graphite-monochromated Mo-Kα radiation and the XSCANS program (18). The intensity data were corrected for the effects of Lorentz and polarization. An absorption correction based on the equations of the crystal faces was applied for crystal **11**. The coordinates of the first set of atoms (Br for **11**) were determined by direct methods and the positions of all the other atoms were found by the standard Fourier methods. The structures were refined on F_0^2 using all reflections. The H atoms were fixed in idealized positions using a riding model with displacement parameters = $1.2U_{eq}$ (1.5 for methyl groups) of the parent atom. The SHELXTL system (19) was used for all calculations and drawings. The experimental details and crystal data are listed in Table 1. The absolute structure of the three compounds was established with certainty. For crystals **1** and **3**, the data were measured with Cu-Kα radiation on the entire sphere. Crystal **11** was measured with Mo-Kα, but it contains a heavy atom, which makes it very easy to determine the absolute configuration. The absolute Flack parameter was obtained using the option TWIN/BASF 0.5 in the refinement program. The crystal of compound **3** contains two independent molecules in the unit cell.

All chemicals were purchased from commercial suppliers and were used as received except where noted. Dry methylene chloride was freshly prepared by distillation from calcium hydride. Triethylamine was freshly distilled from sodium hydride. Commercially available DMSO was treated with molecular sieves (4 Å). Thin layer chromatography was performed on Sigma-Aldrich 0.2 mm aluminum-backed silica gel plates with UV indicator and was visualized under a UV lamp. Flash chromatography was performed on 230–400 mesh silica gel (Silicycle). Chiral-phase HPLC analyses were performed on a Chiracel OD-H column (4.6 mm × 150 mm) using hexane:iso-propanol (90:10) as the mobile phase. A UV detector set at 254 nm was used to monitor the reactions. Capillary gas chromatography was performed on a DB-1301 (15 m × 0.53 mm × 1.0 μm) column from J & W Scientific. Lipases were generous gifts from Amano Enzyme USA Co., Ltd. Commercial baker's yeast was obtained from a local grocery chain.

Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Specific rotation ($[\alpha]_D$) was measured on a PerkinElmer 241 polarimeter at room temperature with energy source Na 589 and is given in $10^{-1\circ}$ cm^2 g^{-1}. IR spectra were recorded as thin films on a Mattson Satellite FT IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution, except where otherwise specified, at room temperature either on a Bruker AC-250 FT-NMR spectrometer or a Varian Unity 400 FT-NMR spectrometer; chemical shifts (δ) are reported in ppm using Me_4Si as the internal standard. *J* values are expressed in Hz. The high-resolution mass spectra were obtained on a Kratos MS50TC mass spectrometer.

(±)-3-Acetoxy-4-(1-ethoxycarbonyl-1-methylethyl)-1-(4-methoxyphenyl)-2-azetidinone (7)

A mixture containing *p*-anisidine (15.4 g, 0.125 mol), ethyl 3,3-diethoxy-2,2-dimethylpropanoate (**4**) (30.0 g, 0.137 mol), and *p*-toluenesulfonic acid monohydrate (0.100 g, 0.526 mmol) was heated at reflux for 5 h. The reaction mixture was cooled and evaporated under vacuum to remove the resulting ethanol. The imine **5** thus obtained was used in the next step without purification. Phosphorus oxychloride (28.0 g, 0.182 mol) in dry methylene chloride (100 mL) was added dropwise to a solution of imine **5**, acetoxyacetic acid **6** (23.4 g, 0.198 mol), and triethylamine (69.9 mL, 0.502 mol) in methylene chloride (500 mL) maintained at 5 °C. The stirred reaction was warmed to room temperature and monitored by TLC until complete disappearance of the imine (48 h). The reaction was quenched with 2 N HCl (400 mL) solution, and extracted with methylene chloride (3 × 400 mL). The combined organic layers were washed sequentially with a saturated solution of $NaHCO_3$ and brine, dried over magnesium sulfate, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel to give compound **7** as a mixture of diastereomers (trans:cis = 2:1) with a yield of 23.0 g (53%) over two steps from *p*-anisidine. **7**-*trans*: IR ($CHCl_3$, cm^{-1}): 2981, 2940, 1765, 1731, 1513, 1372, 1221, 1149. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.17 (3H, t, *J* = 7.2, CH_2CH_3), 1.21 (3H, s, CCH_3), 1.29 (3H, s, CCH_3), 2.17 (3H, s, $COCH_3$), 3.78 (3H, s, OCH_3), 4.03 (2H, q, *J* = 7.0, CH_2CH_3), 4.49 (1H, d, *J* = 1.8, NC*H*), 5.74 (1H, d, *J* = 1.8, OC*H*), 6.86 (2H, d, *J* = 9.1, Ar*H*), 7.21 (2H, d, *J* = 8.9, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.9 (CH_2CH_3), 20.0 (CCH_3), 20.7

(CCH_3), 22.9 ($COCH_3$), 43.5 (CCH_3), 55.5 (OCH_3), 61.3 (OCH_2), 66.7 (NCH), 75.4 (OCH), 114.4, 123.1, 128.7, 157.8, 162.5 (*C*O), 169.2 (*C*O), 175.3 (*C*O). **7**-*cis*: ^{1}H NMR (400 MHz; $CDCl_3$) δ: 1.26 (3H, t, *J* = 7.3, CH_2CH_3), 1.29 (3H, s, CCH_3), 1.32 (3H, s, CCH_3), 2.10 (3H, s, $COCH_3$), 3.80 (3H, s, OCH_3), 4.11 (2H, q, *J* = 7.0, CH_2CH_3), 4.76 (1H, d, *J* = 5.3, NCH), 5.74 (1H, d, *J* = 5.3, OCH), 6.89 (2H, d, *J* = 9.0, ArH), 7.33 (2H, d, *J* = 9.2, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.9 (CH_2CH_3), 20.0 (CCH_3), 20.7 (CCH_3), 22.9 ($COCH_3$), 44.5 (CCH_3), 54.2 (OCH_3), 61.0 (OCH_2), 63.2 (NCH), 73.3 (OCH), 114.4, 121.5, 129.7, 157.3, 163.2 (*C*O), 168.8 (*C*O), 175.0 (*C*O). HR-MS calcd. for $C_{18}H_{23}NO_6$ (M^+): 349.1525; found: 349.1511.

3-Hydroxy-1-(4-methoxyphenyl)-2-azetidinone (1)

A solution of **7** (mixture) (5.0 g, 0.014 mol) in THF (100 mL) was slowly treated with 2 N KOH (50 mL) at 0 °C. The reaction was stirred at 0 °C until TLC indicated complete conversion (2 h). The solution was extracted with ethyl acetate (3 × 80 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and evaporated to dryness to afford racemic **1**-*trans* (2.8 g) as a colorless solid. The water layer was acidified with 2 N HCl to pH 2 ~ 3, and then extracted with ethyl acetate. The organic layers were combined and washed with brine, dried over magnesium sulfate, filtered, and evaporated to dryness to afford racemic **2**-*cis* (1.2 g) as a colorless solid. (±)-*trans*-4-(1-Ethoxycarbonyl-1-methylethyl)-3-hydroxy-1-(4-methoxyphenyl)-2-azetidinone (**1**): mp 78 to 79 °C. IR ($CHCl_3$, cm^{-1}): 3387, 2939, 1730, 1513, 1249, 1150. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.16 (3H, s, CCH_3), 1.18 (3H, t, *J* = 7.2, CH_2CH_3), 1.27 (3H, s, CCH_3), 3.78 (3H, s, OCH_3), 4.05 (2H, q, *J* = 7.2, CH_2), 4.38 (1H, d, *J* = 1.7, NCH), 4.73 (1H, d, *J* = 1.7, HOCH), 6.84 (2H, d, *J* = 9.0, ArH), 7.16 (2H, d, *J* = 8.9, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (CH_2CH_3), 20.4 (CCH_3), 22.8 (CCH_3), 43.6 (CCH_3), 55.5 (OCH_3), 61.2 (OCH_2), 68.6 (NCH), 76.7 (OHCH), 114.4, 123.0, 129.0, 157.7, 167.2 (*C*O), 175.6 (*C*O). HR-MS calcd. for $C_{16}H_{21}NO_5$ (M^+): 307.1420; found: 307.1428. (±)-*cis*-4-(1-Carboxy-1-methylethyl)-3-hydroxy-1-(4-methoxyphenyl)-2-azetidinone (**2**): mp 168 to 169 °C. IR ($CHCl_3$, cm^{-1}): 3373, 2979, 2839, 1724, 1513, 1248, 1170. ^{1}H NMR (400 MHz, d_6-acetone) δ: 1.32 (3H, s, CCH_3), 1.35 (3H, s, CCH_3), 3.79 (3H, s, OCH_3), 4.74 (1H, d, *J* = 5.3, NCH), 5.16 (1H, d, *J* = 5.3, OHCH), 6.92 (2H, d, *J* = 9.0), 7.40 (2H, d, *J* = 9.0). ^{13}C NMR (100 MHz, d_6-acetone) δ: 22.1 (CCH_3), 26.8 (CCH_3), 45.6 (CCH_3), 56.5 (OCH_3), 65.7 (NCH), 77.7 (OCH), 115.7, 122.7, 132.8, 158.4, 168.8 (*C*O), 178.6 (*C*O). HR-MS calcd. for $C_{14}H_{17}NO_5$ (M^+): 279.1107; found: 279.1113.

(±)-4-(1-Ethoxycarbonyl-1-methylethyl)-1-(4-methoxyphenyl)-3-oxo-2-azetidinone (8)

Phosphorus pentoxide (6.0 g, 0.042 mol) was added to dry DMSO (90 mL) and stirred at room temperature for 30 min. Racemic **1**-*trans* (7.5 g, 0.025 mol) in DMSO (45 mL) was added over an 1 h period. The resulting mixture was stirred at room temperature until TLC showed complete conversion (48 h). The reaction was quenched with a cold saturated aqueous solution of sodium bicarbonate (100 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic phases were washed with water (5 × 150 mL), followed by brine and dried over sodium sulfate. The solvent was removed under vacuum. The residue was purified by flash chromatography on silica gel and crystallized from ethyl acetate – hexane to give 5.3 g (71%) of **8** as yellow crystals; mp 64 to 65 °C. IR ($CHCl_3$, cm^{-1}): 2981, 1815, 1764, 1513, 1253, 1149. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.18 (3H, t, *J* = 7.0, CH_2CH_3), 1.27 (3H, s, CCH_3), 1.37 (3H, s, CCH_3), 3.82 (3H, s, OCH_3), 4.07 (2H, q, *J* = 7.2, CH_2), 4.95 (1H, s, NCH), 6.92 (2H, d, *J* = 9.0), 7.38 (2H, d, *J* = 9.2). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.8 (CH_2CH_3), 21.0 (CCH_3), 23.6 (CCH_3), 44.9 (CCH_3), 55.5 (OCH_3), 61.7 (OCH_2), 76.7 (NCH), 114.6, 121.5, 128.9, 158.4, 161.0 (*C*O), 174.4 (*C*O), 193.3 (*C*O). HR-MS calcd. for $C_{16}H_{19}NO_5$ (M^+): 305.1263; found: 305.1274.

(±)-*cis*-7-(4-Methoxyphenyl)-2,2-dimethyl-4-oxa-7-aza-bicyclo[3.2.0]heptane-3,6-dione (3)

Phosphorus pentoxide 1.8 g (0.013 mmol) was added to dry DMSO (35 mL) and stirred at room temperature for 30 min. Racemic **2**-*cis* (2.4 g, 0.0090 mol) in DMSO (15 mL) was added over 30 min. The resulting mixture was stirred at room temperature until TLC indicated complete conversion (48 h). The reaction was quenched with a cold saturated aqueous solution of sodium bicarbonate (40 mL) and extracted with ethyl acetate (3 × 40 mL). The combined organic phases were washed with water (5 × 80 mL) and brine, dried over sodium sulfate, and concentrated under reduced pressure. Crystallization of the crude residue from ethyl acetate – hexane afforded 1.9 g (85%) of **3** as a colorless crystalline solid; mp 169–169.5 °C. IR ($CHCl_3$, cm^{-1}): 2976, 2936, 2838, 1781, 1513, 1385, 1247, 1149. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.28 (3H, s, CCH_3), 1.39 (3H, s, CCH_3), 3.80 (3H, s, OCH_3), 4.49 (1H, d, *J* = 4.6, NCH), 5.44 (1H, d, *J* = 4.6, OCH), 6.90 (2H, d, *J* = 8.9), 7.31 (2H, d, *J* = 8.9). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ: 20.4 (CCH_3), 25.1 (CCH_3), 42.8 (CCH_3), 55.5 (OCH_3), 61.2 (NCH), 79.8 (OCH), 114.6, 118.7, 130.3, 157.1, 160.7 (*C*O), 180.1 (*C*O). HR-MS calcd. for $C_{14}H_{15}NO_4$ (M^+): 261.1001; found: 261.1006.

Lipase-catalyzed hydrolysis of 7

The reaction was carried out in a 0.2 mol L^{-1} potassium phosphate buffer (pH 7.5, 50 mL) containing a mixture of cis and trans diastereomers of **7** (2.1 g, 0.006 mol) and lipase PS "Amano" (2.1 g). The reaction mixture was stirred at room temperature. The kinetic resolution of **7**-*cis* was monitored by chiral HPLC (the **7**-*trans* diastereomers were not hydrolysed by the lipase). After 26 h, 50% conversion of **7**-*cis* was observed. The mixture was extracted with ethyl acetate, and the combined ethyl acetate layers were washed with brine and dried over magnesium sulfate. Removal of the solvent afforded a mixture of unreacted **7** (3*R*,4*S*) and racemic **7**-*trans* as well as the compounds **1** (3S,4*R*) and **3** (1*R*,5*S*). Separation on a silica gel column gave unreacted material **7** (1.2 g), optically pure 3-hydroxy-β-lactam (**1**) (3S,4*R*) as a colorless crystal (0.080 g), and lactone **3** (1*R*,5*S*) as a colorless crystal (0.18 g). The recovered mixture **7** (7.5 g, 0.022 mol) was hydrolyzed with 2 N KOH, as described above, to yield optically pure **2** (3*R*,4*S*) as a white solid (1.1 g) and racemic **7**-*trans* as a colorless solid (5.0 g).

1 (3*S*,4*R*): mp 139–139.8 °C. $[\alpha]_D$ = –170 (*c* 1.00 in CH_2Cl_2). **3** (1*R*, 5*S*): mp 140 to 141 °C. $[\alpha]_D$ = –110 (*c* 1.00 in CH_2Cl_2). **3** (1*S*, 5*R*) prepared from **2** (3*R*,4*S*) in 91% yield: mp 139 to 140 °C. $[\alpha]_D$ = +108 (*c* 1.00 in CH_2Cl_2). **2** (3*R*, 4*S*): mp 158 to 159 °C. $[\alpha]_D$ = +13.4 (*c* 1.00 in CH_3COCH_3).

Procedure for baker's yeast reduction of 8

A suspension of baker's yeast (10 g) and dextrose (40 g) in sterilized water (500 mL) was stirred at 30 °C in a fermentor with aeration for 15 min. Compound **8** (1.00 g) was added and stirring continued for 26 h. The reaction was monitored by chiral HPLC. After complete conversion, the reaction mixture was saturated with salt and centrifuged at 3000× *g* for 10 min to remove yeast cells. The supernatant was extracted with ethyl acetate. The cell pellet was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered. Vacuum evaporation afforded the crude mixture (0.82 g) of optically pure **1**-*trans*, and mixtures of **1**-*cis* and **3**. The crude reaction product (0.29 g) was hydrolyzed with 2 N KOH as described before. Optically pure **1**-*trans* (3*R*,4*R*) was isolated as a colorless oil (0.029 g); **1**-*cis* and **3** were hydrolyzed to the racemic acid **2** and were isolated from the aqueous layer as a colorless solid (0.17 g). **1** (3*R*,4*R*): $[\alpha]_D$ = +84.9 (*c* 1.65 in CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.17 (3H, s, CCH_3), 1.18 (3H, t, *J* = 7.10, CH_2CH_3), 1.28 (3H, s, CCH_3), 3.78 (3H, s, OCH_3), 4.05 (2H, q, *J* = 7.05, CH_2CH_3), 4.35 (H, d, *J* = 1.70, NC*H*), 4.75 (1H, d, *J* = 1.6, OHC*H*), 6.82 (2H, d, *J* = 9.2, Ar*H*), 7.14 (2H, d, *J* = 9.2).[2]

Acknowledgements

Financial support by the Natural Sciences and Engineering Research Council of Canada (NSERC) (FDR and MMK) and Medical Research Fund of New Brunswick (MMK) are gratefully acknowledged. The NMR spectra were recorded at the Atlantic Regional Magnetic Resonance Centre at Dalhousie University, Halifax, Nova Scotia, Canada. The authors thank Dr. D.L. Hooper for the spectra with chiral solvating agents. The help of Stéphane M. Foulem, an undergraduate participant, is gratefully acknowledged.

References

1. For reviews on β-lactam antibiotics see: (*a*) G.I. Georg and V.T. Ravikumar. *In* The organic chemistry of β-lactams. *Edited by.* G.I. Georg. VCH, New York. 1993; (*b*) R. Southgate, C. Branch, S. Coulton, and E. Hunt. *In* Recent progress in the chemical synthesis of antibiotics and related microbial products. Vol. 2. *Edited by* G. Lukacs. Springer-Verlag, Berlin. 1993. p. 621; (*c*) F.H. Van der Steen and G. van Koten. Tetrahedron, **47**, 7503 (1991).
2. C. Palomo, J.M. Aizpurua, I. Ganboa, and M. Oiarbide. Synlett, 1813 (2001); C. Palomo, J.M. Aizpurua, and I. Ganboa. *In* Enantioselective synthesis of β-amino acids. *Edited by* E. Juaristi. Wiley-VCH, New York. 1997; C. Palomo, J.M. Aizpurua, I. Ganboa, and M. Oiarbide. Pure Appl. Chem. **72**, 1763 (2000); R. Herranz, J. Castro-Pichel, S. Vinuesa, and M.T. Garcia-Lopez. J. Org. Chem. **55**, 2232 (1990), and refs. cited therein; Y. Kobayashi, Y. Takemoto, Y. Ito, and S. Terashima. Tetrahedron Lett. **31**, 3031(1990); K. Iizuka, T. Kamijo, T. Kubota, K. Akahane, H. Umeyama, and Y. Kiso. J. Med. Chem. **31**, 701 (1988); T. Aoyagi, H. Tobe, F. Kojima, M. Hamada, T. Takeuchi, and H. Umezawa. J. Antibiot. **31**, 636 (1978).
3. R.A. Holton, R.J. Biedinger, and P.D. Boatman. Semisynthesis of Taxol® and Taxotere. *In* Taxol® science and applications. *Edited by* M. Suffness. Boca Raton, CRC Press, Florida. 1995.
4. S. Lin, X. Geng, C. Qu, R. Tynebor, D.J. Gallagher, E. Pollina, J. Rutter, and I. Ojima. Chirality, **12**, 431 (2000); I. Ojima, S. Lin, J.C. Slater, T. Wang, P. Pera, R.J. Bernacki, C. Ferlini, and G. Scambia. Bioorg. Med. Chem. **8**, 1619 (2000); T. Yamaguchi, N. Harada, K. Ozaki, H. Arakawa, K. Oda, N. Nakanishi, K. Tsujihara, and T. Hashiyama. Bioorg. Med. Chem. Lett. **9**, 1639 (1999); S.M. Ali, M.Z. Hoemann, J. Aube, G.I. Georg, and L.A. Mitscher. J. Med. Chem. **40**, 236 (1997); I. Ojima, J.C. Slater, E. Michaud, S.D. Kuduk, P.-Y. Bounaud, P. Vrignaud, M.-C. Bissery, J.M. Veith, P. Pera, and R.J. Bernacki. J. Med. Chem. **39**, 3889 (1996); G.I. Georg, T.C. Boge, Z.S. Cheruvallath, J.S. Clowers, G.C.B. Harriman, M. Hepperle, and H. Park. The medicinal chemistry of Taxol®. *In* Taxol® science and applications. *Edited by* M. Suffness. Boca Raton, CRC Press, Florida. 1995; S.M. Ali, M.Z. Hoemann, J. Aubé, L.A. Mitscher, and G.I. Georg. J. Med. Chem. **38**, 3821 (1995); G.I. Georg, G.C.B. Harriman, M. Hepperle, and R.H. Himes. Bioorg. Med. Chem. Lett. **4**, 1381 (1994).
5. For recent reviews see: X. Wang, H. Itokawa, and K.-H. Lee. *In* Taxus: The genus taxus. Medical and aromatic plants — Industrial profiles. Vol. 32. *Edited by* H. Itokawa and K.H. Lee. Taylor and Francis, London. 2003; D.G.I. Kingston. Chem. Commun. 867 (2001); D.G.I. Kingston. J. Nat. Prod. **63**, 726 (2000).
6. T. Konno, J. Watanabe, and K. Ishihara. J. Biomed. Mater. Res. **65A**, 210 (2003); A.L. Seligson, R.C. Terry, J.C. Bressi, J.G. Douglass III, and M. Sovak. Anti-Cancer Drugs, **12**, 305 (2001), and refs. cited therein; M.I. Nicoletti, T. Colombo, C. Rossi, C. Monardo, S. Stura, M. Zucchetti, A. Riva, P. Morazzoni, M.B. Donati, E. Bombardelli, M. D'Incalci, and R. Giavazzi. Cancer Res. **60**, 842 (2000); D. Sampath, C.M. Discafani, F. Loganzo, C. Beyer, H. Liu, X. Tan, S. Musto, T. Annable, P. Gallagher, C. Rios, and L.M. Greenberger. Mol. Cancer Ther. **2**, 873 (2003). For a review see: I. Ojima, S. Lin, and W. Tao. Curr. Med. Chem. **6**, 927 (1999).
7. For reviews see: C. Palomo, J.M. Aizpurua, I. Ganboa, and M. Oiarbide. Eur. J. Org. Chem. 3223 (1999). Some recent examples: M.D. Mihovilovic, A. Feicht, and M.M. Kayser. J. Prakt. Chem. (Weinheim, Ger.), **342**, 585 (2000); B.K. Banik and F.F. Becker. Tetrahedron Lett. **41**, 6551 (2000).
8. For reviews see: M. Benaglia, M. Cinquini, and F. Cozzi. Eur. J. Org. Chem. 563 (2000); D.J. Hart and D.-C. Ha. Chem. Rev. **89**, 1447 (1989). A recent example: S. Schunk and D. Enders. Org. Lett. **2**, 907 (2000).

[2] Supplementary data may be purchased from the Directory of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada (http://www.nrc.ca/cisti/irm/unpub_e.shtml for information on ordering electronically). CCDC 220029, 220030, and 225739 contain the crystallographic data for this manuscript. These data can be obtained, free of charge, via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033; or deposit@ccdc.cam.ac.uk).

9. J.R. Malpass. *In* Comprehensive organic chemistry: The synthesis and reactions of organic compounds. Vol. 2. *Edited by* D. Barton and W.D. Ollis. Pergamon Press, Oxford. 1979. p. 22; M. Chmielewski, Z. Kaluza, and B. Furman. Chem. Commun. 2689 (1996).
10. A. Basak, S.C. Ghosh, T. Bhowmick, A.K. Das, and V. Bertolasi. Tetrahedron Lett. **43**, 5499 (2002); M.M.-C. Lo and G.C. Fu. J. Am. Chem. Soc. **124**, 4572 (2002); M. Miura, M. Enna, K. Okuro, and M. Nomura. J. Org. Chem. **60**, 4999 (1995); M. Kinugasa and S. Hashimoto. J. Chem. Soc. Chem. Commun. 466 (1972).
11. M. Arun, S.N. Joshi, V.G. Puranik, B.M. Bhawal, and A.R.A.S. Deshmukh. Tetrahedron, **59**, 2309 (2003); R. Fernández, A. Ferrete, J.M. Lassaletta, J.M. Llera, and E. Martin-Zamora. Angew. Chem. Int. Ed. **41**, 831(2002); S.N. Joshi, A.R.A.S. Deshmukh, and B.M. Bhawal. Tetrahedron: Asymmetry, **11**, 1477 (2000); S. Brown, A.M. Jordan, N.J. Lawrence, R.G. Pritchard, and A.T. McGown. Tetrahedron Lett. **39**, 3559 (1998); I. Ojima and F. Delaloge. Chem. Soc. Rev. **26**, 377 (1997); S. Matsui, Y. Hashimoto, and K. Saigo. Synthesis, 1161 (1998); Y. Hashimoto, A. Kai, and K. Saigo. Tetrahedron Lett. **36**, 8821 (1995); T. Fujisawa, A. Shibuya, D. Sato, and M. Shimizu. Synlett, 1067 (1995).
12. P.A. Magriotis. Angew. Chem. Int. Ed. **40**, 4377 (2001); B.L. Hodous and G.C. Fu. J. Am. Chem. Soc. **124**, 1578 (2002); A.E. Taggi, A.M. Hafez, H. Wack, B. Young, W.J. Drury III, and T. Lectka. J. Am. Chem. Soc. **122**, 7831 (2000); H. Wack, W.J. Drury III, A.E. Taggi, D. Ferraris, and T. Lectka. Org. Lett. **1**, 1985 (1999).
13. (*a*) R. Brieva, J.Z. Crich, and C.J. Sih. J. Org. Chem. **58**, 1068 (1993); (*b*) E. Forró and F. Fülöp. Tetrahedron: Asymmetry, **12**, 2351 (2001); (*c*) W. Adam, P. Groer, H.-U. Humpf, and C.R. Saha-Möller. J. Org. Chem. **65**, 4919 (2000); (*d*) A. Basak, T. Mahato, G. Bhattacharya, and B. Mukherjee. Tetrahedron Lett. **38**, 643 (1997); (*e*) J.A. Carr, T.F. Al-Azemi, T.E. Long, J.-Y. Shim, C.M. Coates, E. Turos, and K.S. Bisht. Tetrahedron, **59**, 9147 (2003).
14. M.M. Kayser, Y. Yang, M.D. Mihovilovic, A. Feicht, and F.D. Rochon. Can. J. Chem. **80**, 796 (2002).
15. N.C. Deno. J. Am. Chem. Soc. **69**, 2233 (1947).
16. It is generally recognized that *cis*- β-lactams have larger coupling constants than the corresponding trans diastereomers. C. Palomo, J.M. Aizpurua, F. Cabré, J.M. Garcia, and J.M. Odriozola. Tetrahedron Lett. **35**, 2721 (1994). Also see B.K. Banik and F.F. Becker, in ref. 7. In the case of compound **7**, the *J* coupling constant for cis isomers is 5.3 Hz, whereas that for the trans is 1.8 Hz.
17. M.M. Kayser, M.D. Mihovilovic, J. Kearns, A. Feicht, and J.D. Stewart. J. Org. Chem. **64**, 6603 (1999).
18. XSCANS. PC Version 5 [computer program]. Bruker AXS Inc., Madison, Wisconsin. 1995.
19. SHELXTL. Release 5.10 [computer program]. Bruker AXS Inc., Madison, Wisconsin. 1997.