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Synthesis of 3-Substituted-clavams: Antifungal Properties, DD-Peptidase and β-Lactamase Inhibition

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Abstract The [2+2]cycloaddition of chlorosulfonyl isocyanate to vinyl and (*Z*)-propenyl ethers derived from the 2-*O*-sulfonylated (*R*)- and (*S*)-1-(furyl-2′)-1,2-ethanediols furnished the 4-alkoxy-azetidin-2-ones with a good to moderate stereoselectivity. The intramolecular alkylation of the β-lactam nitrogen atom led to the corresponding 3-(furyl-2′)- and 6-methyl-3-(furyl-2′)-clavams. The transformation of the furyl residue into an alkoxycarbonyl group led to clavams related to the natural compounds. The synthesized clavams exhibited moderate inhibitory activities against DD-peptidase 64-575 and β-lactamase (penase) as well as antifungal activities.

Keywords furyloglycol, [2+2]cycloaddition, β-lactams, clavams

Introduction

The natural clavams (4-dethia-4-oxa-penams)† represent an interesting and unique group of β-lactam antibiotics, which exist in nature as both (5*R*) and (5*S*) bridgehead carbon atom stereoisomers. While a fair number of different clavams were isolated [1∼5], only the clavulanic acid (**1**) [1] and its simple *O*-acyl derivatives with the (5*R*)-configuration at the ring junction showed strong β-lactamase inhibition and weak antibacterial activity. Other clavams, represented by the family (**2**∼**6**), with (*S*)-configuration at C-5, exhibit inhibitory activities against a number of fungal species (Fig. 1) [2∼5]. It should be stressed that a variety of compounds having β-lactam fragments have been found to display very interesting, but not antibacterial activities [6].

The synthesis of **1**∼**6** typically begins with the condensation of commercially available 4-acetoxy-azetidinone (**7**) with a separately prepared chiral alcohol, followed by the intramolecular alkylation of the β-lactam nitrogen atom [7∼10]. The drawback of such a strategy appears to be related to the usually low asymmetric

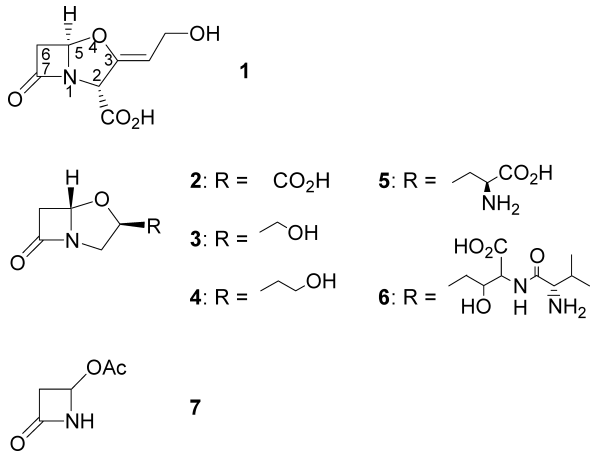

Fig. 1 Structures of clavams (**1**∼**6**) and 4-acetoxy-azetidinone (**7**).

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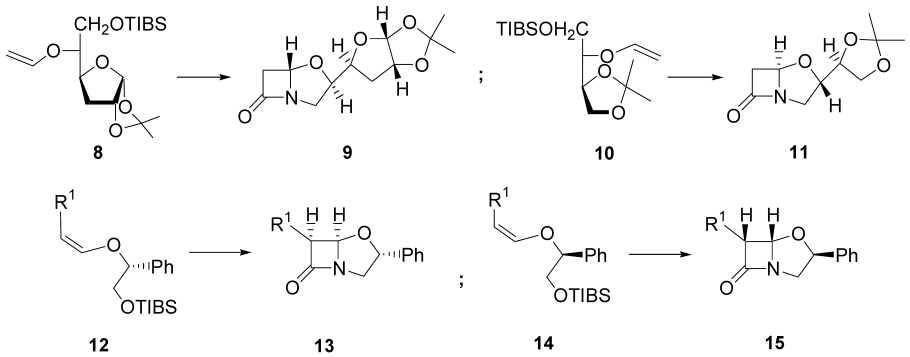


Scheme 1

induction at C-4 of the azetidin-2-one ring [7～11].

We have shown that the [2+2]cycloaddition of chlorosulfonyl isocyanate (CSI) to the chiral vinyl ethers with a stereogenic center next to the oxygen atom, is an attractive alternative to the approach based on the condensation of **7** with chiral alcohols [11～14]. It has been demonstrated that the [2+2]cycloaddition method is remarkably stereospecific and leads to the *cis*-substituted azetidinones from (*Z*)-olefins while the *trans*-substituted products are obtained from the respective (*E*)-olefins. These reactions usually proceed with an excellent stereoselectivity even in the case of open-chain vinyl ethers [15～17].

We have previously reported that starting from 1,2-*O*-isopropylidene-D-glucofuranose we have synthesized clavam (**9**, Scheme 1) [16] which shares its basic skeleton with several natural antifungal compounds, illustrated by **2**～**6**. Similarly, starting from the L-tartaric acid clavam which is structurally related to **1** but lacks the C-2 carboxylic function, **11** [17], was also prepared (Scheme 1). Interestingly, **11** displays a marked anti-β-lactamase activity [17]

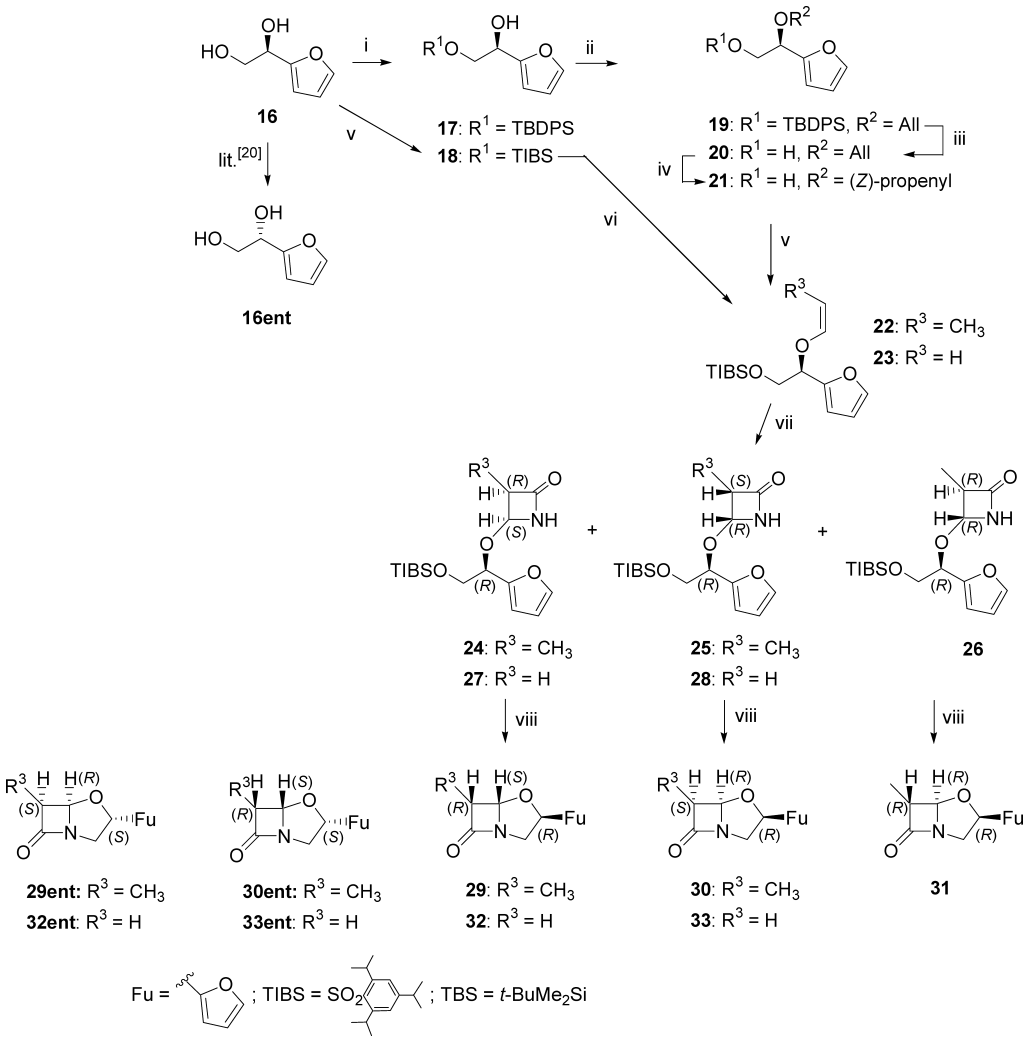
Very recently we have shown that starting from the commercial 1-phenyl-1,2-ethanediol (both enantiomers are available) and using the same standard methodology, it is possible to obtain the corresponding 3-phenyl-clavams (**13**, **15**) with a relatively good asymmetric induction (Scheme 1) [18].

Since the structurally related 1-(furyl-2′)-1,2-ethanediol (**16**) is readily available from D-glucal [19] and its enantiomeric form **16ent** can be accessed by the simple inversion of the configuration using the Mitsunobu procedure (Scheme 2) [20], it was of interest to investigate the course of [2+2]cycloaddition to the 1-*O*-vinyl and (*Z*) 1-*O*-propenyl ethers of **16** and **16ent** (Scheme 2). In addition we wished to synthesize the corresponding 3-furyl-clavams *via* intramolecular alkylation of the nitrogen atom in the intermediary adducts. It is worthwhile to point out that the furyl residue can be easily transformed under mild conditions into a number of other functional groups, including the carboxylic function present in natural clavam **2**. In contrast, the conversion of the phenyl group, which was present in the previously synthesized clavams [18] into other useful substituents does not appear to be feasible without simultaneous decomposition of the clavam skeleton. Considering the broad spectrum of bioactivity of simple clavams, the synthesis and examination of properties of related 3-(furyl-2′) clavams appears promising.

Results and Discussion

Synthesis

Our previous studies carried out with the vinyl ethers **8** [16] and **10** [17], substituted at C-1 with five-membered rings and protected at the primary hydroxyl group with the 2,4,6-triisopropyl-benzenesulfonyl (TIBS) group, demonstrated the high degree of stereoselectivity of the [2+2]cycloaddition. We observed that the configuration at the carbon atom bearing a vinyloxy group in **8** and **10** effectively controlled the configuration of intermediary cycloadducts as well as the configuration at C-5 in the resulting clavams **9** and **11**, respectively. Considering that the absolute configuration of the five-membered ring stereogenic centers in **8** and **10** did not substantially change the observed diastereoselectivity of the cycloaddition, we could expect that the replacement of a five-membered ring (furanose or dioxolane) with the (furyl-2′) ring (**16**/**16ent**) should not affect the high degree of asymmetric induction. The primary hydroxyl group in **16** was protected by a *tert*-butyldiphenylsilyl (TBDPS) group to give **17**, or by a TIBS group to give **18**. The TBDPS-protected (furyl-2′)-glycol



Scheme 2

(**17**) was alkylated with allyl bromide and then the ether **19** was desilylated to give the alcohol **20**. Subsequently, the (*Z*)-propenyl ether **21** was obtained from **20** by the rearrangement in the presence of *t*-BuOK in DMSO [21]. The free hydroxyl group in **21** was sulfonylated with TIBSCl to afford **22**, using the same methodology as used for the preparation of **18** (Scheme 2).

The simple vinyl ether **23** was obtained directly from **16** (Scheme 2) using the standard *trans*-etherification procedure with vinyl *n*-butyl ether in the presence of mercury acetate [22], or by the alternative method, involving reaction of the alcohol with vinyl acetate in the presence of $[IrCl(cod)]_2$ [23]. It should be stressed that both TIBS-protected vinyl and (*Z*)-propenyl ethers derived from the phenyl-1,2-ethanediol displayed the same direction and a similar relatively high degree of asymmetric induction [18]. Bearing this fact in mind, we decided to make the cycloaddition reactions using both the TIBS-protected (*Z*)-propenyl **22** and vinyl **23** ethers (Scheme 2). The corresponding enantiomeric forms **17ent**～**23ent** were obtained from **16ent** following the same procedures as that used for the preparation of enantiomers **17**～**23**.

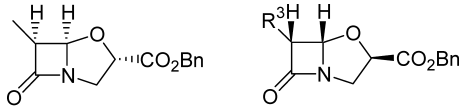
The [2+2]cycloaddition of CSI to the (*Z*)-propenyl ether **22** [24] under standard conditions at −70°C [11～14] proceeded with a good diastereoselectivity to give the adducts **24** and **25** in a ratio of about 9 : 1. At a higher reaction temperature (−50°C) the formation of an additional compound, adduct **26** was observed (the ratio of **24** : **25** : **26** amounts to 6 : 1 : 3). The configuration of the cycloadducts **24**～**26** was established after their cyclization to the corresponding clavams **29**～**31** (Scheme 2). The (1*R*,3′*R*,4′*R*) configuration assigned for **26** and the ratio of **24** : **25** : **26** at −50°C indicated that the adduct **24** underwent epimerization at the stage of the *N*-

chlorosulfonylated [2+2]cycloadduct. Similar epimerization has also been noticed in the past [25].

4-Alkoxy-azetidinones (**24**~**26**) were subjected independently to intramolecular *N*-alkylation under standard PTC conditions to provide the corresponding β-lactams **29**~**31** (Scheme 2). The relative configurations of clavams **29** and **30** was established as a result of the analysis of their NOE's in ^{1}H-NMR [24] (protons H-3 and H-5 displayed spin–spin interaction in the case of diastereomer **30** whereas in the case of **29** such interaction was not observed) as well as values of their respective $J_{5,6}$ coupling constants which were found to be 3.1 Hz for **29** and 3.0 Hz for **30**. On the basis of the above we assigned the *syn*-orientation of these protons for both compounds. Since the NOE results were uncertain in the case of clavam **31**, the (5*R*)-configuration of this compound was independently established by its CD-spectrum which showed a positive CD band attributed to the n–π^* β-lactam amide transition [18] while the value of the $J_{5,6}$ coupling constant equal to 1 Hz indicated the *anti*-orientation of both protons. The structure and configuration of **29** was also independently corroborated by X-ray crystallography [18b].

In comparison with the [2+2]cycloaddition to (*Z*)-propenyl ether **22**, the analogous reaction of CSI with ether **23** led to the corresponding adducts **27** and **28** with a lower stereoselectivity, 6 : 1 respectively. The same tendency has been noticed for the cycloaddition to phenyl-1,2-ethanediol congeners [18a]. The intramolecular alkylation of the nitrogen atom in **27** and **28** afforded clavams **32** and **33**. Their relative configuration was assigned as discussed above. In all cases the direction of the asymmetric induction followed exactly the direction of the induction noticed for the corresponding phenyl congeners **12** and **14**, thus corroborating the stereochemical model of the transition state for the [2+2]cycloaddition of CSI and vinyl ethers as proposed by us recently [18a]. Starting from the diol **16ent** and following the same procedures, the enantiomers **24ent**, **25ent**, **27ent**~**30ent**, **32ent** and **33ent** were synthesized and characterized; the enantiomeric forms of **26** and **31** (*i.e.* **26ent** and **31ent**) were not obtained.

In order to demonstrate the usefulness of the (furyl-2′) group, **29**, **29ent** and **32** were subsequently transformed into the benzyl esters **34**, **34ent**, and **35**, respectively (Fig. 2). **35** is a benzyl ester of natural clavam **2** [2, 4]. Starting from the commercially available 4-acetoxy-azetidinone, the racemic **35** has been synthesized by a Spanish group [26]. The same group reported the transformation of **35** into the sodium salt of the racemic acid **2** [26].



34ent **34**: $R^3 = CH_3$ **35**: $R^3 = H$

Fig. 2 Structures of benzyl esters **34**, **34ent** and **35**.

Inhibition of DD-peptidase 64-575 and β-Lactamase

29~**34**, **29ent**, **30ent**, **32ent**~**34ent** obtained as described above were tested for biological activity. In order to have fully reliable results, all tests were repeated using independently synthesized compounds. The results of the inhibition of enzymes by synthetic clavams are presented in Table 1.

DD-Carboxypeptidases/transpeptidases (DD-peptidases) inhibition is the mode of action of β-lactam antibiotics. The relation between the chemical structure of β-lactams and their antibacterial activity is relatively well known. All β-lactam antibiotics possess the (*R*) configuration at C-5 (penicillins) or C-6 (cephalosporins). The monobactams and nocardicins with either the (4*S*) or (4*R*) configuration or without the stereogenic center at C-4 of the azetidin-2-one ring usually show a low antibacterial activity [27].

Clavams **29** [(*S*) configuration at C-5] and **32ent** [(*R*) configuration at C-5] showed inhibition of the DD-carboxypeptidase/DD-transpeptidase 64-575 (DD-peptidase 64-575), expressed by $IC_{50}(M) = 3.4\times10^{-3}$ and 1.3×10^{-3}, respectively (Table 1). In comparison, the 50%-inhibition of the enzyme 64-575 by cephamandole, penicillin G and clavulanic acid was achieved at much lower concentrations, according to the published data: $IC_{50}(M) = 1.5\times10^{-8}$, 6.1×10^{-7} and 2.0×10^{-6}, respectively [29, 30]. The clavam **32ent** showed the β-lactamase inhibition, expressed by the value of $IC_{50}(M) = 1.9\times10^{-3}$. Other tested clavams did not show measureable β-lactamase inhibition.

Antifungal and Antibacterial Activity

29~**34**, **29ent**, **30ent**, **32ent**~**34ent** were tested for antifungal activity using the disk diffusion method (Table 2, Fig. 3). The clavams **29**, **29ent**, **32**, **32ent**, **34** showed antifungal activity against *Candida albicans* ATTC 90028. **30**, **30ent**, **31**, **33**, **33ent**, **34ent** did not exhibit measureable antifungal activity.

The clavams **29** and **32ent** were also tested for antibacterial activity. Neither of them showed a measureable antibacterial activity against *Escherichia coli* ATCC 25922.

In respect to the configuration at C-5, some natural clavams were described as either possessing antifungal and antibacterial activity or to be inhibitors of β-lactamases

Table 1 Inhibition of DD-peptidase 64-575 by clavams

Clavams	DD-Peptidase inhibition IC_{50} (M)*
29	3.4×10^{-3}
30	2.5×10^{-2}
31	1.0×10^{-2}
32	6.6×10^{-3}
33	2.8×10^{-2}
29ent	5.4×10^{-3}
30ent	3.0×10^{-2}
32ent	1.3×10^{-3}
33ent	Nonmeasureable
34	4.5×10^{-3}
34ent	1.6×10^{-2}

* IC_{50} (M): molar concentration of clavams inhibiting the DD-peptidase 64-575 in 50%.

Table 2 Antifungal activities against *Candida albicans* ATTC 90028

Clavams	Inhibition zones* (mm)		
	50 μg	100 μg	200 μg
29	0	6	14
30	0	0	0
31	0	0	0
32	15	30	35
33	0	0	0
29ent	0	0	15
30ent	0	0	0
32ent	15	25	30
33ent	0	0	0
34	12	22	30
34ent	0	0	0

* Zones of fungal growth inhibiton.

[1~5, 30]. The clavams with the (*S*)-configuration at C-5 are usually active against bacteria and fungi. The antifungal mode of action of these compounds is related to their interference with eukaryotic RNA synthesis [30]. Instead of β-lactam murein cross-linking disturbance, some of the (*S*)-clavams inhibit the bacterial methionine biosynthesis. Clavulanic acid with the (*R*)-configuration at C-5 possesses poor activity against bacteria, but it is known to be a strong inhibitor of β-lactamases [1, 30].

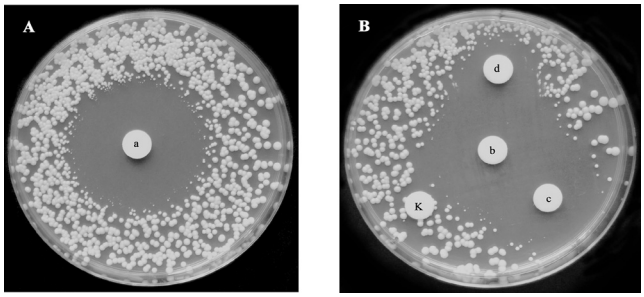


Fig. 3 Inhibition zones of the growth of *Candida albicans* ATTC 90028 by the clavam **32** at the following concentrations. (A) a: 200 μg, (B) b: 100 μg, c: 50 μg, d: 25 μg, K: EtOH control disk without the clavam **32**.

Conclusion

It was shown that the [2+2]cycloaddition of CSI to the (*Z*)-propenyl ether **22** at −70°C proceeds with a relatively high stereoselectivity. At a higher temperature (−50°C) a partial epimerization of the *cis*-adduct leading to the respective *trans*-adduct was observed. The cycloaddition to the simple vinyl ether **23** proceeds with a lower stereoselectivity. The direction and magnitude of the stereocontrol of the cycloaddition follows the general trend observed for respective phenylglycol congeners [18a].

The clavams **29**~**34**, **29ent**, **30ent**, **32ent**, **34ent** showed modest inhibition of the DD-peptidase 64-575. In addition, clavam **32ent** exhibited also a modest inhibition of β-lactamase. The clavams **29**, **29ent**, **32**, **32ent**, **34** showed antifungal activity against *C. albicans* ATTC 90028. Further experimentation aimed at the rational structural modifications of the tested clavams may lead to compounds with a high antibacterial and/or antifungal activity.

Experimental

General Remarks

Melting points were determined on a Koefler hot-stage apparatus. NMR spectra were recorded using Bruker Avance 500 and Varian Mercury 400 instruments. IR spectra were recorded on a Perkin-Elmer FTIR Spectrum 200 spectrophotometer. Optical rotations were measured using a JASCO P 3010 polarimeter at 22±3°C. Mass spectra were recorded using AMD-604 Inectra GmbH and HPLC-MS with Mariner and API 356 detectors. Column chromatography was performed using E. Merck Kiesel Gel (230~400 mesh).

(1*R*) **16** [19] and its enantiomeric form (1*S*) **16ent** [20]

were prepared by literature procedures from D-glucal.

Since cycloaddition to both enantiomeric forms of vinyl ethers and (*Z*)-propenyl ethers derived from **16** and **16ent** diols provides the same information on stereoselectivity of the reaction, the representative procedures and full characterization of ethers **20**～**25ent**, **27ent**～**ent**, **32ent**～**34ent** were provided for enantiomers derived from (*R*) 1-furyl-1,2-ethanediol (**16**).

(1*R*) 2-*t*-Butyldiphenylsiloxy-1-(furyl-2′)-1-hydroxy-ethane (**17**)

To a solution of ethanediol **16** (1.28 g, 10 mmol) in CH_2Cl_2 (25 ml) *t*-butyldiphenylchlorosilane (2.75 g, 10 mmol) and DMAP (1.22 g, 10 mmol) were added. After 1 hour the reaction mixture was evaporated and purified by chromatography using hexane/EtOAc 8 : 2 (v/v) as an eluent to afford **17** (2.9 g, 81%); $[\alpha]_D^{22}$ +3.7 (*c* 1.0, CH_2Cl_2); IR (film): ν 3568 cm^{-1} (OH); ^{1}H-NMR ($CDCl_3$): δ 1.06 (9H, s, *t*-Bu), 3.90 (1H, dd, *J*=4.5, 10.2 Hz, H-2), 3.92 (1H, dd, *J*=6.6, 10.2 Hz, H-2a), 4.83 (1H, dd, *J*=4.5, 6.6 Hz, H-1), 6.29 (1H, m, H-2′), 6.32 (1H, dd, *J*=1.9, 3.3 Hz, H-2′, H-3′), 7.34～7.72 (11H, m, H-4′, phenyls); ESIHR-MS *m*/*z* $(M+Na)^+$ Calcd for $C_{22}H_{26}O_3SiNa$: 389.1543. Found: 389.1530.

(1*R*) 1-Allyloxy-2-*t*-butyldiphenylsiloxy-1-(furyl-2′)-ethane (**19**)

To a cold solution (at 5°C) of **17** (3.30 g, 9.9 mmol) in dry DMF (25 ml) the NaH suspension in mineral oil (60%; 0.45 g, 11.4 mmol) was added. The solution was stirred for 5 minutes and allyl bromide (1.50 g, 12.4 mmol) was added dropwise. Cooling bath was removed and reaction mixture was stirred for 1.5 hours (TLC monitoring) at room temperature. The excess of NaH was decomposed with MeOH (3.0 ml). The reaction mixture was diluted with brine (20 ml) and extracted with *t*-butyl-methyl ether. The combined organic extracts were dried with magnesium sulfate, filtered and evaporated to dryness. The residue was purified by a column chromatography on silica gel using hexane - EtOAc 9.5 : 0.5 (v/v) as eluent and gave **19** (2.6 g, 76%); $[\alpha]_D^{22}$ +31.7 (*c* 1.0, CH_2Cl_2); IR (film): absence of hydroxyl group; ^{1}H-NMR ($CDCl_3$): δ 1.01 (9H, s, *t*-Bu), 3.91 (1H, dd, *J*=5.9, 10.4 Hz, H-2), 4.00 (1H, dd, *J*=6.7, 10.4 Hz, H-2a), 3.91～4.07 (2H, m, allyl), 4.51 (dd, *J*=5.9, 6.7 Hz, H-1), 5.15 and 5.26 (2H, 2m, allyl), 5.87 (1H, m, allyl), 6.28 (1H, m, furyl), 6.33 (1H, dd, *J*=1.8, 3.2 Hz, furyl), 7.34～7.72 (11H, m, H-4′, phenyls); ESIHR-MS *m*/*z* $(M+Na)^+$ Calcd for $C_{25}H_{30}O_3SiNa$: 429.1856. Found: 429.1852.

(1*R*) 1-Allyloxy-2-hydroxy-1-(furyl-2′)-ethane (**20**)

19 (3.6 g, 9.0 mmol) was dissolved in THF (30 ml) and treated with tetrabutylammonium fluoride hydrate (2.9 g, 9.0 mmol). The reaction was complete after 1 hour (TLC monitoring). Subsequently the solvent was evaporated and the residue was purified by a column chromatography on silica gel using hexane/EtOAc 8 : 2 (v/v) as eluent, to give **20** (1.0 g, 71%); $[\alpha]_D^{22}$ +27.5 (*c* 1.0, CH_2Cl_2); IR (film): ν 3593 cm^{-1} (OH); ^{1}H-NMR ($CDCl_3$): δ 3.76 (1H, dd, *J*=4.1, 11.6 Hz, H-2a), 3.91 (1H, dd, *J*=8.0, 11.6 Hz, H-2b), 3.92 (1H, m, allyl), 4.05 (1H, m, allyl), 4.51 (1H, dd, *J*=4.1, 8.0 Hz, H-1), 5.18 (1H, m, allyl), 5.26 (1H, m, allyl), 5.88 (1H, m, allyl), 6.33 (1H, m, furyl), 6.35 (1H, dd, *J*=1.8, 3.2 Hz, furyl), 7.40 (1H, m, furyl); EIHR-MS *m*/*z* $(M)^+$ Calcd for $C_9H_{12}O_3$: 168.0786. Found: 168.0781.

(1*S*) 1-Allyloxy-2-hydroxy-1-(furyl-2′)-ethane (**20ent**)

$[\alpha]_D^{22}$ −28.5 (*c* 1.0, CH_2Cl_2).

(1*R*) 2-Hydroxy-1-(*Z*-prop-1′-enyloxy)-1-(furyl-2′)-ethane (**21**)

A solution of allyl ether **20** (1.0 g, 6.0 mmol) and freshly sublimed *t*-BuOK (2.0 g) in DMSO (15 ml), was stirred at 50°C for 1 hour. The reaction mixture was diluted with brine (20 ml) then extracted with *t*-butyl - methyl ether. The combined organic extracts were dried with magnesium sulfate, filtered and evaporated to dryness. The residue was purified by a column chromatography on silica gel using hexane - EtOAc 8 : 2 (v/v) as eluent, to give **21** (0.9 g, 90%); mp 48～49.5°C; $[\alpha]_D^{22}$ +29.0 (*c* 0.8, CH_2Cl_2); IR (film): ν 1669 cm^{-1} (C=C), 3597 cm^{-1} (OH); ^{1}H-NMR ($CDCl_3$): δ 1.62 (3H, dd, *J*=1.7, 6.8 Hz, CH_3), 3.89 (dd, *J*=4.0, 11.8 Hz, H-2a), 4.03 (1H, dd, *J*=7.8, 11.8 Hz, H-2b), 4.52 (1H, q, *J*=6.8 Hz, H-2′), 4.77 (1H, dd, *J*=4.0, 7.8 Hz, H-1), 6.08 (1H, dq, *J*=6.8, 1.7 Hz, H-1′), 6.38 (2H, m, H-2″, H-3″), 7.43 (1H, m, H-4″); EIHR-MS *m*/*z* $(M)^+$ Calcd for $C_9H_{12}O_3$: 168.0786. Found: 168.0788.

(1*S*) 2-Hydroxy-1-(*Z*-prop-1′-enyloxy)-1-(furyl-2′)-ethane (**21ent**)

$[\alpha]_D^{22}$ −27.0 (*c* 0.5, CH_2Cl_2).

(1*R*) 2-(2,4,6-Triisopropylbenzenesulfonyloxy)-1-(*Z*-prop-1′-enyloxy)-1-(furyl-2′)-ethane (**22**)

To a solution of **21** (1.68 g, 10 mmol) and Et_3N (1.2 g, 11 mmol) in CH_2Cl_2 (30 ml) 2,4,6-triisopropylbenzene sulfonyl chloride (3.3 g, 11 mmol) was added. After 2 hours brine (50 ml) was added to the reaction mixture. The mixture was extracted with *t*-butyl methyl ether (3×40 ml). The combined organic extracts were dried with magnesium sulfate, filtered and evaporated to dryness. The residue was

purified by a column chromatography on silica gel using hexane - EtOAc 9 : 1 (v/v) as eluent, to give corresponding sulfonate **22** in 73% yield; mp 56∼57°C; $[\alpha]_D^{22}$ +0.9 (*c* 1.6, CH_2Cl_2); IR (film): ν 1670 cm^{-1} (C=C); ^{1}H-NMR ($CDCl_3$): δ 1.24, 1.25 and 1.26 (18H, 3d, TIBS), 1.51 (3H, dd, *J*=1.7, 6.8 Hz, CH_3), 2.91 (1H, sept., *J*=6.9 Hz, TIBS), 4.13 (2H, sept., *J*=6.7 Hz, TIBS), 4.39 (2H, d, H-2, 2′), 4.45 (1H, dq, *J*=6.2, 6.8 Hz, H-2′), 4.91 (1H, t, H-1), 5.94 (1H, dq, *J*=6.2, 1.7 Hz, H-1′), 6.33 (1H, dd, *J*=1.8, 3.3 Hz, furyl), 6.35 (1H, bd, furyl), 7.18 (2H, s, TIBS), 7.37 (1H, dd, *J*=0.8, 1.7 Hz, furyl); ESIHR-MS *m/z* $(M+Na)^+$ Calcd for $C_{24}H_{34}O_5SNa$: 457.2019. Found: 457.2008.

(1*S*) 2-(2,4,6-Triisopropylbenzenesulfonyloxy)-1-(*Z*-prop-1′-enyloxy)-1-(furyl-2′)-ethane (**22ent**)
$[\alpha]_D^{22}$ −1.0 (*c* 1.0, CH_2Cl_2).

(1*R*,3′*R*,4′*S*) and (1*R*,3′*S*,4′*R*) 1-(3′-Methyl-azetidin-2′-on-4′-yloxy)-2-(2,4,6-triisopropylbenzenesulfonyl)-1-(furyl-2′)-ethane (**24** and **25**)
To a well stirred suspension of anhydrous potassium carbonate (0.8 g) in solution of (*Z*)-propenyl ether **22** (1.1 g, 4.0 mmol) in dry toluene (20 ml), chlorosulfonyl isocyanate (600 μl, 7.0 mmol) was added during 5 minutes, under an argon atmosphere at −70°C. The mixture was stirred at the same temperature for another 30 minutes and then it was diluted with toluene cooled to −70°C (20 ml). Subsequently, the Red-Al solution in toluene (1 M, 4.6 ml) was slowly added and the reaction mixture was stirred for 15 minutes. The cooling bath was removed and water (0.8 ml) was added at 0°C. Stirring was continued for additional 10 minutes. The suspension was filtered through Celite and the solvent was evaporated. The residue was separated by a column chromatography on silica gel using hexane - EtOAc 7 : 3 (v/v) as eluent, to give **24** (1.08 g, 56%) and **25** (0.12 g, 6%).

24: $[\alpha]_D^{22}$ +6.7 (*c* 1.25, CH_2Cl_2); IR (film): ν 1773 cm^{-1} (C=O), 3410 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$): δ 1.22 (3H, d, *J*=7.5 Hz, CH_3), 1.24, 1.25, 1.26 (18H, 3d, TIBS), 2.92 (1H, sept, TIBS), 3.32 (1H, qdd, *J*=7.5, 2.0, 4.2 Hz, H-3′), 4.10 (2H, sept, TIBS), 4.32 (1H, dd, *J*=4.9, 11.0 Hz, H-2a), 4.41 (1H, dd, *J*=7.7, 11.0 Hz, H-2b), 4.84 (1H, dd, *J*=4.9, 7.7 Hz, H-1), 5.11 (1H, d, *J*=4.4 Hz, H-4′), 6.38 (1H, dd, *J*=1.8, 3.3 Hz, furyl), 6.39 (1H, bd, furyl), 7.19 (2H, s, TIBS), 7.42 (1H, m, furyl); ESIHR-MS *m/z* $(M+Na)^+$ Calcd for $C_{25}H_{35}NO_6SNa$: 500.2077. Found: 500.2097.

25: $[\alpha]_D^{22}$ +10.7 (*c* 1.12, CH_2Cl_2); IR (film): ν 1774 cm^{-1} (C=O), 3410 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$): δ 1.09 (3H, d, *J*=7.5 Hz, CH_3), 1.25, 1.26, 1.26 (18H, 3d, TIBS), 2.92 (1H, sept, TIBS), 3.23 (1H, qdd, *J*=7.5, 2.2, 4.2 Hz, H-3′), 4.10 (2H, sept, TIBS), 4.36 (1H, dd, *J*=5.0, 11.0 Hz, H-2a), 4.38 (1H, dd, *J*=7.2, 11.0 Hz, H-2b), 4.88 (1H, dd, *J*=5.0, 7.2 Hz, H-1), 5.10 (1H, d, *J*=4.2 Hz, H-4′), 6.37 (2H, m, furyl), 7.19 (2H, s, TIBS), 7.39 (1H, bd, furyl); ESIHR-MS *m/z* $(M+Na)^+$ Calcd for $C_{25}H_{35}NO_6SNa$: 500.2077. Found: 500.2085.

(1*S*,3′*S*,4′*R*) 1-(3′-Methyl-azetidin-2′-on-4′-yloxy)-2-(2,4,6-triisopropylbenzenesulfonyl)-1-(furyl-2′)-ethane (**24ent**)
$[\alpha]_D^{22}$ −8.7 (*c* 0.3, CH_2Cl_2).

(1*S*,3′*R*,4′*S*) 1-(3′-Methyl-azetidin-2′-on-4′-yloxy)-2-(2,4,6-triisopropylbenzenesulfonyl)-1-(furyl-2′)-ethane (**25ent**)
$[\alpha]_D^{22}$ −9.7 (*c* 0.5, CH_2Cl_2).

(1*R*,3′*R*,4′*S*) 1-(3′-Methyl-azetidin-2′-on-4′-yloxy)-2-(2,4,6-triisopropylbenzenesulfonyl)-1-(furyl-2′)-ethane (**26**)
$[\alpha]_D^{22}$ +58.6 (*c* 1.0, CH_2Cl_2); IR (film): ν 1775 cm^{-1} (C=O), 3411 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$): δ 1.11 (3H, d, *J*=7.5 Hz, CH_3), 1.25, 1.26, 1.27 (18H, 3d, TIBS), 2.87 (1H, dq, *J*=1.1, 7.5 Hz, H-3′), 2.92 (1H, sept, TIBS), 4.10 (2H, sept, TIBS), 4.31 (1H, dd, *J*=4.6, 11.0 Hz, H-2a), 4.35 (1H, dd, *J*=7.9, 11.0 Hz, H-2b), 4.68 (1H, d, *J*=1.1 Hz, H-4′), 4.86 (1H, dd, *J*=4.6, 7.9 Hz, H-1), 6.38 (1H, dd, *J*=1.8, 3.3 Hz, furyl), 6.39 (1H, bdd, *J*=0.9, 3.3 Hz, furyl), 7.19 (2H, s, TIBS), 7.40 (1H, dd, *J*=0.9, 1.8 Hz, furyl); ESIHR-MS *m/z* $(M+Na)^+$ Calcd for $C_{25}H_{35}NO_6SNa$: 500.2077. Found: 500.2067.

(3*R*,5*S*,6*R*) 6-Methyl-3-(furyl-2′)-4-dethia-4-oxa-penam (**29**)
To a solution of 4-alkoxyazetidinone **24** (0.24 g, 2.5 mmol) in acetonitrile (15 ml) were added tetrabutylammonium bromide (0.32 g, 1.0 mmol) and potassium carbonate (0.8 g). The mixture was heated under reflux for 40 minutes, cooled, diluted with toluene (10 ml) and filtered. The colorless solution was washed with water (5.0 ml), dried with magnesium sulfate, filtered and evaporated to dryness. The residue was purified on a silica gel using hexane - EtOAc 1 : 1 (v/v) as an eluent, to give corresponding clavam (0.076 g, 83%); mp 40∼41°C; $[\alpha]_D^{22}$ −140.6 (*c* 1.1 CH_2Cl_2); IR (film): ν 1782 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$): δ 1.21 (3H, d, *J*=7.7 Hz, CH_3), 3.17 (1H, ddd, *J*=0.8, 7.0, 11.8 Hz, H-2), 3.48 (1H, ddq, *J*=0.8, 3.2, 7.7 Hz, H-6), 4.07 (1H, dd, *J*=6.8, 11.8 Hz, H-2í), 5.17 (1H, t, *J*=6.8, 7.0 Hz, H-3), 5.43 (1H, d, *J*=3.2 Hz, H-5), 6.36 (1H, dd, *J*=1.8, 3.2 Hz, furyl), 6.37 (1H, bd, furyl), 7.44 (1H, dd, *J*=0.9, 1.8 Hz, furyl); EIHR-MS *m/z* $(M)^+$ Calcd for $C_{10}H_{11}NO_3$: 193.0730. Found: 193.0739.

(3*S*,5*R*,6*S*) 6-Methyl-3-(furyl-2′)-4-dethia-4-oxa-penam (**29ent**)
$[\alpha]_D^{22}$ +142.2 (*c* 1.1 CH_2Cl_2).

(3*R*,5*R*,6*S*) 6-Methyl-3-(furyl-2′)-4-dethia-4-oxa-penam (**30**)
$[\alpha]_D^{22}$ +91.6 (*c* 0.6, CH_2Cl_2); IR (film): ν 1779 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$): δ 1.14 (3H, d, *J*=7.6 Hz, CH_3), 3.32 (1H, dd, *J*=7.5, 11.0 Hz, H-2), 3.45 (1H, dq, *J*=3.0, 7.6 Hz, H-6), 3.93 (1H, dd, *J*=6.3, 11.0 Hz, H-2′), 5.29 (1H, d, *J*=3.0 Hz, H-5), 5.33 (1H, dd, *J*=6.3, 7.5 Hz, H-3), 6.34 (1H, dd, *J*=1.8, 3.3 Hz, furyl), 6.38 (1H, bd, *J*=3.3 Hz, furyl), 7.41 (1H, bd, *J*=1.8 Hz, furyl); EIHR-MS *m*/*z* $(M)^+$ Calcd for $C_{10}H_{11}NO_3$: 193.0730. Found: 193.0739.

(3*S*,5*S*,6*R*) 6-Methyl-3-(furyl-2′)-4-dethia-4-oxa-penam (**30ent**)
$[\alpha]_D^{22}$ −89.0 (*c* 0.5, CH_2Cl_2).

(3*R*,5*R*,6*R*) 6-Methyl-3-(furyl-2′)-4-dethia-4-oxa-penam (**31**)
$[\alpha]_D^{22}$ +142.9 (*c* 0.5, CH_2Cl_2); IR (film): ν 1778 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$): δ 1.36 (3H, d, *J*=7.7 Hz, CH_3), 3.14 (1H, q, *J*=7.7 Hz, H-6), 3.31 (1H, dd, *J*=7.3, 11.3 Hz, H-2), 4.00 (1H, dd, *J*=5.2, 11.3 Hz, H-2′), 5.03 (1H, s, H-5), 5.30 (1H, dd, *J*=5.2, 7.3 Hz, H-3), 6.33 (1H, dd, *J*=1.8, 3.3 Hz, furyl), 6.33 (1H, bd, *J*=3.3 Hz, furyl), 7.41 (1H, dd, *J*=0.9, 1.8 Hz, furyl); EIHR-MS *m*/*z* $(M)^+$ Calcd for $C_{10}H_{11}NO_3$: 193.0730. Found: 193.0730.

(1*R*) 2-(2,4,6-Triisopropylbenzenesulfonyloxy)-1-vinyloxy-1-(furyl-2′)-ethane (**23**)
16 (0.25 g, 2.0 mmol) dissolved in CH_2Cl_2 (5.0 ml) was treated with Et_3N (0.4 g, 4.0 mmol) and 2,4,6-triisopropylbenzene sulfonyl chloride (0.6 g, 2.0 mmol) and the solution was left for 1 hour. Subsequently the solvent was evaporated and the residue was purified on silica gel using hexane - EtOAc 9 : 1 (v/v) as an eluent. The partially purified (1*R*/1*S*) 2-(2,4,6-triisopropylbenzenesulfonyloxy)-1-furyl-1-hydroxy-ethane (**18**) was dissolved in butyl-vinyl ether (5.0 ml) treated with $(AcO)_2Hg$ (0.02 g) and refluxed for 1 hour. The mixture was then evaporated and the residue was purified on silica gel column using hexane - EtOAc 9 : 1 (v/v) as an eluent to afford **23** (0.49 g, 60%); $[\alpha]_D^{22}$ +30.3 (*c* 0.5, CH_2Cl_2); IR (film): ν 1599 cm^{-1} (C=C); ^{1}H-NMR ($CDCl_3$): δ 1.27, 1.28 and 1.29 (18H, 3d, TIBS), 2.94 (1H, sept., *J*=6.9 Hz, TIBS), 4.08 (1H, dd, *J*=2.1, 6.6 Hz, H-2′a), 4.16 (2H, sept., *J*=6.7 Hz, TIBS), 4.35 (1H, dd, *J*=2.1, 14.1 Hz, H-2′b), 4.40 (1H, dd, *J*=5.5, 11.0 Hz, H-2a), 4.41 (1H, dd, *J*=6.6, 11.0 Hz, H-2b), 5.12 (1H, t, *J*=5.5, 6.6 Hz, H-1) 6.29 (1H, dd, *J*=6.6, 14.1 Hz, H-1′), 6.35 (1H, dd, *J*=1.8, 3.3 Hz, furyl), 6.35 (1H, bd, *J*=3.3 Hz, furyl), 7.21 (2H, s, TIBS), 7.41 (1H, m, furyl); ESIHR-MS *m*/*z* $(M+Na)^+$ Calcd for $C_{23}H_{32}O_5SNa$: 443.1863. Found: 443.1844.

(1*S*) 2-(2,4,6-Triisopropylbenzenesulfonyloxy)-1-vinyloxy-1-(furyl-2′)-ethane (**23ent**)
$[\alpha]_D^{22}$ −30.0 (*c* 0.5, CH_2Cl_2).

(1*R*,4′*S*) and (1*R*,4′*R*) 1-(Azetidin-2′-on-4′-yloxy)-2-(2,4,6-triisopropylbenzenesulfonyloxy)-1-(furyl-2′)-ethane (**27** and **28**)
Cycloaddition to **23** was performed following the procedure described for **24**; 58% yield. A sample of a mixture **27** and **28** in a ratio of about 6 : 1 was separated into pure components. **27**: $[\alpha]_D^{22}$ +17.7 (*c* 0.26, CH_2Cl_2); IR (film): ν 1776 cm^{-1} (C=O), 3414 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$): δ 1.24, 1.25, 1.26 (18H, 3d, TIBS), 2.83 (1H, bd, *J*=15.2 Hz, H-3′a), 2.92 (1H, sept, TIBS), 3.07 (1H, ddd, *J*=2.7, 4.0, 15.2 Hz, H-3′b), 4.11 (2H, sept, TIBS), 4.32 (1H, dd, *J*=4.5, 11.0 Hz, H-2a), 4.37 (1H, dd, *J*=7.9, 11.0 Hz, H-2b), 4.86 (1H, dd, *J*=4.5, 7.9 Hz, H-1), 5.11 (1H, d, *J*=1.2, 4.0 Hz, H-4′), 6.38 (1H, dd, *J*=1.8, 3.3 Hz, furyl), 6.39 (1H, bd, furyl), 7.19 (2H, s, TIBS), 7.42 (1H, m, furyl); ESIHR-MS *m*/*z* $(M+Na)^+$ Calcd for $C_{24}H_{33}NO_6SNa$: 486.1921. Found: 486.1900.

(1*S*,4′*R*) 1-(Azetidin-2′-on-4′-yloxy)-2-(2,4,6-triisopropyl benzenesulfonyloxy)-1-(furyl-2′)-ethane (**27ent**)
$[\alpha]_D$ −19.0 (*c* 0.5, CH_2Cl_2).

28: $[\alpha]_D^{22}$ +15.6 (*c* 0. 3, CH_2Cl_2); IR (film): ν 1778 cm^{-1} (C=O), 3415 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$): δ 1.25, 1.26, 1.26 (18H, 3d, TIBS), 2.73 (1H, bd, *J*=15.1 Hz, H-3′a), 2.92 (1H, sept, TIBS), 3.00 (1H, ddd, *J*=2.8, 3.9, 15.1 Hz, H-3′b), 4.10 (2H, sept, TIBS), 4.33 (1H, dd, *J*=4.8, 11.0 Hz, H-2a), 4.36 (1H, dd, *J*=7.4, 11.0 Hz, H-2b), 4.86 (1H, dd, *J*=4.8, 7.4 Hz, H-1), 5.09 (1H, d, *J*=1.3, 3.9 Hz, H-4′), 6.37 (2H, m, furyl), 7.19 (2H, s, TIBS), 7.41 (1H, m, furyl); ESIHR-MS *m*/*z* $(M+Na)^+$ Calcd for $C_{24}H_{33}NO_6SNa$: 486.1921. Found: 486.1914.

(1*S*,4′*S*) 1-(Azetidin-2′-on-4′-yloxy)-2-(2,4,6-triisopropyl benzenesulfonyloxy)-1-(furyl-2′)-ethane (**28ent**)
$[\alpha]_D^{22}$ −15.8 (*c* 1.1, CH_2Cl_2).

(3*R*,5*S*) and (3*R*,5*R*) 3-(Furyl-2′)-4-dethia-4-oxa-penam (**32** and **33**)
A 6 : 1 mixture of **27** and **28** was subjected to intramolecular alkylation following procedure described for **29**. The mixture of **32** and **33** was separated into pure

components on a silica gel column using hexane - EtOAc 7 : 3 (v/v) as an eluent in 80% overall yield.

32: $[\alpha]_D^{22}$ −65.3 (*c* 1.1, CH_2Cl_2); IR (film): ν 1782 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$): δ 2.92 (1H, bd, *J*=16.3 Hz, H-6), 3.32 (1H, ddd, *J*=0.8, 6.4, 11.6 Hz, H-2), 3.34 (1H, ddd, *J*=0.8, 2.8, 16.3 Hz, H-6′), 4.13 (1H, dd, *J*=6.9, 11.6 Hz, H-3), 5.30 (1H, t, *J*=6.4, 6.9 Hz, H-3), 5.44 (1H, d, *J*=2.8 Hz, H-5), 6.36 (2H, m, furyl), 7.43 (1H, m, furyl); EIHR-MS *m/z* $(M)^+$ Calcd for $C_9H_9NO_3$: 179.0582. Found: 179.0591.

(3*S*,5*R*) 3-(Furyl-2′)-4-dethia-4-oxa-penam (**32ent**)
$[\alpha]_D$ +66.0 (*c* 0.3 CH_2Cl_2).

33: $[\alpha]_D^{22}$ +46.3 (*c* 0.2, CH_2Cl_2); IR (film): ν 1782 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$): δ 2.97 (1H, d, *J*=16.2 Hz, H-6), 3.30 (1H, bdd, *J*=2.9, 16.2 Hz, H-6′), 3.34 (1H, dd, *J*=7.9, 11.1 Hz, H-2), 4.00 (1H, dd, *J*=5.8, 11.1 Hz, H-2′), 5.32 (1H, dd, *J*=5.8, 7.9 Hz, H-3), 5.33 (1H, d, *J*=2.9 Hz, H-5), 6.34 (1H, dd, *J*=1.8, 3.3 Hz, furyl), 6.37 (1H, bd, *J*=3.3 Hz, furyl), 7.42 (1H, dd, *J*=0.8, 1.8 Hz, furyl); EIHR-MS *m/z* $(M)^+$ Calcd for $C_9H_9NO_3$: 179.0582. Found: 179.0578.

(3*S*,5*S*) 3-(Furyl-2′)-4-dethia-4-oxa-penam (**33ent**)
$[\alpha]_D^{22}$ −47.0 (*c* 0.3, CH_2Cl_2).

(3*R*,5*S*,6*R*) 3-Benzyloxycarbonyl-6-methyl-4-dethia-4-oxa-penam (**34**)

To the solution of **29** (0.10 g, 0.5 mmol) in the mixture of CCl_4 (0.7 ml), acetonitrile (0.7 ml) and water (1.1 ml) sodium metaperiodate (1.5 g, 7.0 mmol) and $RuCl_2$ (0.02 g) were added. The mixture was stirred at room temperature for 40 minutes. Subsequently K_2CO_3 (0.9 g), Bu_4NBr (0.9 g), and BnBr (0.34 g, 2.0 mmol) were added and the mixture was heated to 50°C for 5 minutes. Then it was diluted with water (5.0 ml) and extracted with EtOAc. The extract was dried and evaporated to dryness. The residue was purified on a silica gel using hexane - EtOAc 8 : 2 (v/v) as an eluent, to give corresponding clavam (0.075 g, 59%). $[\alpha]_D^{22}$ −39.6 (*c* 0.5, CH_2Cl_2); IR (film): ν 1749 (C=O), 1785 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$): δ 1.21 (3H, d, *J*=7.7 Hz, CH_3), 3.17 (1H, ddd, *J*=0.8, 7.0, 11.8 Hz, H-2), 3.48 (1H, ddq, *J*=0.8, 3.2, 7.7 Hz, H-6), 4.07 (1H, dd, *J*=6.8, 11.8 Hz, H-2′), 5.17 (1H, t, *J*=6.8, 7.0 Hz, H-3), 5.43 (1H, d, *J*=3.2 Hz, H-5), 6.36 (1H, dd, *J*=1.8, 3.2 Hz, furyl), 6.37 (1H, bd, furyl), 7.44 (1H, dd, *J*=0.9, 1.8 Hz, furyl); ESIHR-MS *m/z* $(M+Na)^+$ Calcd for $C_{14}H_{15}NO_4Na$: 284.0893. Found: 284.0895.

(3*S*,5*R*,6*S*) 3-Benzyloxycarbonyl-6-methyl-4-dethia-4-oxa-penam (**34ent**)
$[\alpha]_D^{22}$ +40.0 (*c* 0.5, CH_2Cl_2).

(3*R*,5*S*) 3-Benzyloxycarbonyl-4-dethia-4-oxa-penam (**35**)

35 was obtained according to the procedure described for **34**; $[\alpha]_D^{22}$ −20.3 (*c* 0.3, CH_2Cl_2); IR (film): ν 1750 (C=O), 1787 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$): δ 2.90 (1H, d, *J*=16.2 Hz, H-6), 3.10 (1H, ddd, *J*=1.0, 4.8, 11.6 Hz, H-2), 3.32 (1H, ddd, *J*=1.0, 2.7, 16.2 Hz, H-6′), 4.15 (1H, dd, *J*=8.1, 11.6 Hz, H-2′), 4.87 (1H, dd, *J*=4.8, 8.1 Hz, H-3), 5.21 (2H, s, benzyl), 5.49 (1H, d, *J*=2.7 Hz, H-5); ESIHR-MS *m/z* $(M+Na)^+$ Calcd for $C_{13}H_{13}NO_4Na$: 270.0737. Found: 270.0726.

Assay of DD-carboxypeptidase Activity (Table 1)

The enzyme activity was measured as described previously [28, 29, 31]. Samples for assay of the DD-carboxypeptidase activity consisted of 10 μl of DD-carboxypeptidase from *Saccharopolyspora erythraea* PZH TZ 64-575 (40 units/mg), 20 μl of substrate solution containing 4.52 mg/ml N^{α}, N^{ε}-diacetyl-L-lysyl-D-alanyl-D-alanine in 0.1 M phosphate buffer, pH 8.0 and 10 μl of 0.1 M phosphate buffer, pH 8.0. A standard sample contained 20 μl of D-alanine in distilled water. The reaction mixture for assay of the DD-carboxypeptidase activity consisted of 60 μl of 0.05 mg/ml flavin adenine dinucleotide in 0.1 M phosphate buffer, pH 8.0, 10 μl of 0.05 mg/ml horseradish peroxidase (1230 units/mg) in distilled water, 5.0 μl of 5.0 mg/ml *o*-dianisidine in methanol, and 2.0 μl of 11.77 mg/ml D-amino acid oxidase from porcine kidney (6.7 units/mg) in 0.1 M phosphate buffer, pH 8.0. Samples were incubated for 30 minutes at 37°C and then boiled for 2 minutes. After cooling, 77 μl of the reaction mixture was added, and all samples were incubated for 10 minutes at 37°C. Next, was added 350 μl to each sample of a mixture consisting of MeOH, distilled water and sulfuric acid (5 : 5 : 6 by volume). Extinction of the resulting solution was measured at 540 nm.

The inhibition of DD-peptidase 64-575 by the discussed above clavams was evaluated [32]. Mixtures of 10 μl of DD-peptidase 64-575 (40 units/mg), 5.0 μl solution of a clavam in methanol and 5.0 μl of 0.1 M phosphate buffer, pH 8.0 were incubated for 45 minutes at 37°C. The concentration of the clavams in the mixture was from 0.04 to 0.0001 M. Following the incubation, 20 μl of substrate solution was added to 20 μl of each sample and the resulting mixtures were incubated again.

Assay of β-Lactamase Activity

The inhibition of penicillinase was evaluated following a

literature method [33]. The samples for the assay of inhibition of β-lactamase consisted of 10 μl of penicillinase (Penase, 5×10^6 IU/ml, Bacto), 20 μl 0.1 M phosphate buffer, pH 7.0, 10 μl solution of clavams in methanol. The samples were incubated for 30 minutes at 37°C, then 30 μl of nitrocephin, 430 μl 0.1 M phosphate buffer pH 7.0 were added and all the samples were incubated for 10 minutes at 37°C. Absorption was measured at 482 nm.

The inhibition of β-lactamase was expressed as a IC_{50} (M), molar concentration of inhibitor decreasing β-lactamase activity in 50%.

Antifungal Susceptibility Testing (Table 2) [34, 35]
All studies were conducted with *Candida albicans* ATTC 90028. The strain was grown 48 hours onto solid Sabourand's medium (Difco) at 35°C. The inoculum with yeast cells was standardized to 0.1 McFarland (McF) (Densitometr, Bio Merieux).

Antifungal Assay (Table 2, Fig 3)
Antifungal activity was measured by the paper-disk method. Disks with absorbed tested compounds were placed onto the surface of solid Sabourand's medium inoculated with *Candida albicans* ATCC 90028 (0.1 McF). Sizes of the zones of inhibition (mm diameter) were observed after 48 hours incubation at 35°C.

Antibacterial Susceptibility Testing
The studies were conducted with *Escherichia coli* ATCC 25922. The strain was grown for 24 hours onto a solid Mueller-Hinton medium (Difco) at 37°C. The inoculum with bacterial cells was standardized to 0.5 McF (Densitometer, Bio Merieux).

Antibacterial Assay
Antibacterial activity was measured by the paper-disk method. Disks with absorbed tested compounds were placed onto the surface of a solid Mueller-Hinton medium inoculated with *Escherichia coli* ATCC 25922 (0.5 McF). Sizes of the zones of inhibition (mm diameter) were observed after 18 hours incubation in 37°C.

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