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An entry to 7-amino- and to 2-ethoxycarbonyl-5-dethia-5-oxa-cephams from 1,3-alkylidene-L-erythritol

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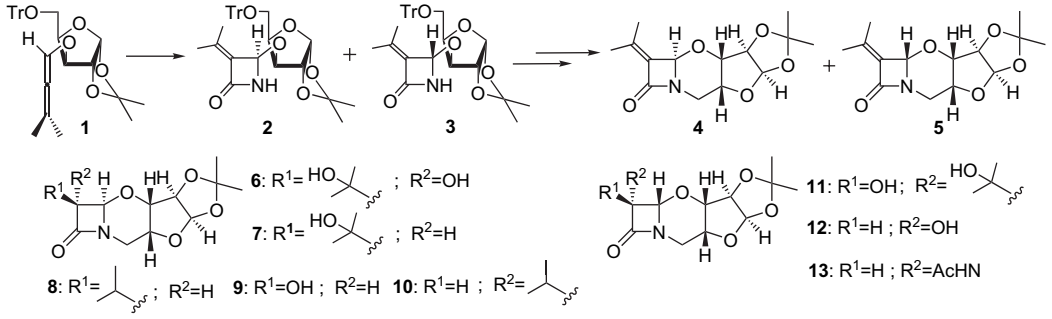
Abstract—The alkoxyallene derived from 1,3-benzylidene-L-erythritol when treated with chlorosulfonyl isocyanate provided diastereomeric β-lactams with moderate stereoselectivity. After the intramolecular alkylation of the nitrogen atom, these afforded compounds having oxa-cepham skeletons. The *exo*-isopropylidene group enabled the introduction of a variety of substituents to the C-7 carbon atom of the cepham, whereas removal of the benzylidene protection followed by the oxidation of 3-OH to the ketone allowed carboxylation of the C-2 carbon atom.

1. Introduction

Recently, we have demonstrated that the [2+2]cycloaddition of chlorosulfonyl isocyanate (CSI) to alkoxyallene **1** derived from the 1,2-*O*-isopropylidene-D-xylofuranose provided β-lactams **2** and **3** with a moderate stereoselectivity.[1] The intramolecular alkylation of the nitrogen atom in **2** and **3** afforded cephams **4** and **5** having an *exo*-isopropylidene group (Scheme 1). Compounds **4** and **5** were used as substrates for a variety of transformations leading to the introduction of isopropyl, hydroxyisopropyl, oxygen, and nitrogen functions at the α position to the β-lactam carbonyl group (**6**–**13**).[2] These transformations followed, in part, Buynak and co-workers[3] study on the functionalization of 3-alkylidene-azetidin-2-ones. Reactions described by us proceeded in high stereoselectivity, with control of the configuration of the cephams thus formed. The introduction of the amino function (**13**) was successfully performed for the cepham **5** only, having the (*S*) configuration at the bridgehead carbon atom.[2]

Due to the specific multifunctional character of the 1,2-*O*-isopropylidene-D-xylofuranose scaffold, however, the cephams obtained are of limited value, since the acid catalyzed hydrolysis of the acetal center derived from the sugar precursor could not be made without the opening of the azetidinone ring. The successful opening of the furanoid fragment was performed as a base induced β-elimination process.[4]

The [2+2]cycloaddition of CSI to alkoxyallene **14** derived from benzylidene erythritol provided azetidinones **15** and



Scheme 1.

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16, which were subjected to intramolecular alkylation affording corresponding tricyclic cephams.[5] Compounds **17** and **18** were synthesized with the intention to introduce a carboxylic function to the C-2 atom and a variety of substituents to the C-7 carbon atom of the 3,4-disubstituted 5-oxacepham skeleton (Chart 1).

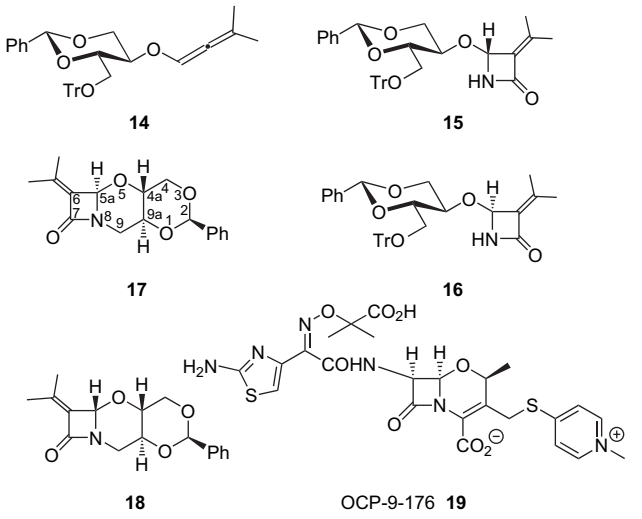
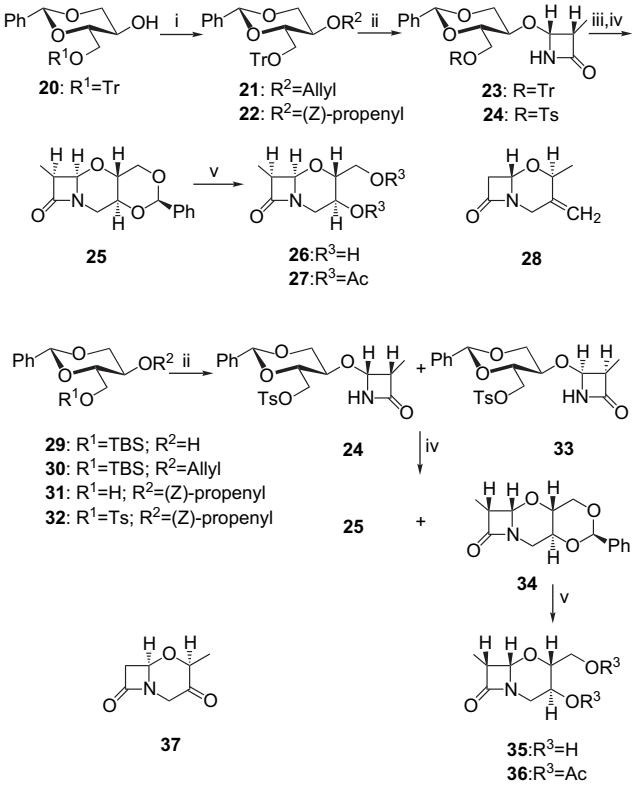


Chart 1.



Scheme 2. (i) (a) NaH/DMF, CH_2=$CHCH_2Br$; (b) *t*-BuOK/DMSO; (ii) CSI, Na_2CO_3/Red-Al; (iii) (a) TsOH/MeOH; (b) TsCl/Py; (iv) K_2CO_3, Bu_4NBr/CH_3CN; and (v) (a) Pd/C, H_2; (b) Ac_2O/Py.

2. Results and discussion

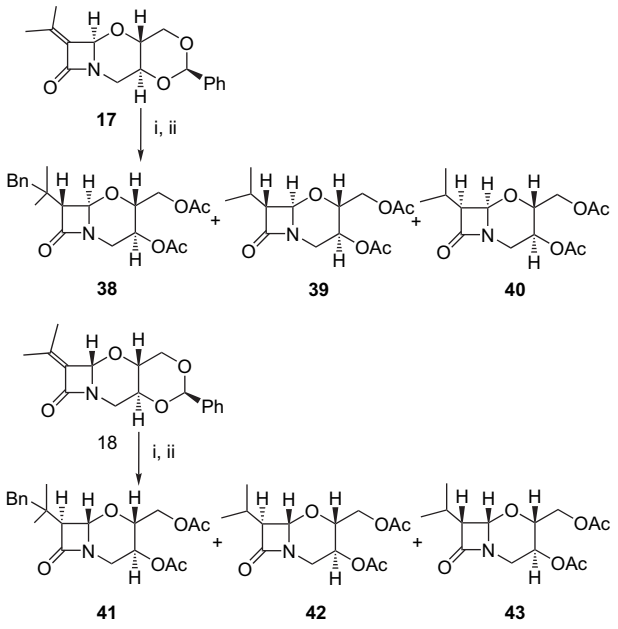
Introduction of the benzylidene grouping to the L-erythritol is a crucial step for successful functionalization of the 5-oxacepham skeleton, since the reductive removal of the protection should be performed easily without any decomposition of the β-lactam ring. The debenzylidation leaves a free OH group at C-3 of the cepham, which after oxidation to the ketone should allow us to introduce an alkoxycarbonyl function to the C-2 carbon atom.[6] Moreover, the cepham obtained contains the hydroxymethyl group at C-4, which may increase its biological activity. At the end of the eighties the Merck and Meiji groups[7] reported a new 4-methyl-cephalosporin **19**, which offers the stability toward β-lactamases together with a significant antibacterial activity.

The usefulness of the benzylidene erythritol scaffold[8] was demonstrated using simple diastereomeric models **25** and **34** obtained by the standard reaction sequence developed for the ethylidene congeners (Scheme 2).[9,20] [2+2]Cycloaddition of chlorosulfonyl isocyanate to **20** proceeded with excellent stereoselectivity, and provided, after intramolecular alkylation of the β-lactam nitrogen atom, only one oxacepham **25**. Its diastereomer **34**, having the (*S*) configuration at the bridgehead carbon atom, was obtained by the same reaction sequence, starting from the tosyloxymethyl propenyl ether **32**, which gave lower stereoselectivity in the cycloaddition. The minor product of the cycloaddition (**33**) after the intramolecular alkylation provided the cepham **34**. All these reactions followed our earlier observations made for ethylidene analogs.[9]

As it was expected, hydrogenation of **25** over palladium gave **26** in a very good yield. Removal of the benzylidene fragment caused the inversion in the conformation of the six-membered oxazine ring. Consequently hydroxymethyl and hydroxy groups switched from diequatorial geometry coerced by the rigid trans decalin system to the distorted diaxial geometry of the oxazine ring. This was demonstrated by the change of coupling constants $J_{3,4}$ from about 9.5 Hz to 2.2 Hz. Similar conformation of the six-membered oxazine ring has been found by us recently for cepham **28**.[10] Such a conformational switch demonstrates the angular strain existing in **25**. Contrary to that, the hydrogenation of the alternative diastereomer **34**, having *syn* protons at C-5a and C-6a carbon atoms, provided cepham **36**, which did not show an inversion in the conformation of the six-membered oxazine ring in comparison with the decalin precursor **34**. The coupling constant $J_{3,4}$=9.8 Hz remains large, proving the diaxial position of both protons. This shows that the conformation of the oxacepham having the β-lactam ring fused to the six-membered oxazine is well defined. The bridgehead proton H-6 of the molecule must be located in the pseudoaxial position. One can compare X-ray structures of **28** and **37** reported by us recently.[10] The reverse conformational arrangement can occur only if the cepham fragment is a part of the bigger rigid molecule, for example, a trans decalin system.

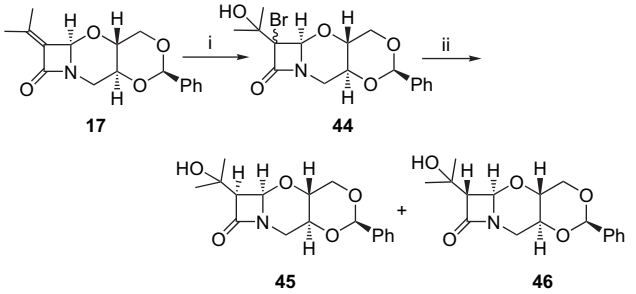
Readily available oxacepham **17** and its C-6 epimer **18**[5] were selected to demonstrate both functionalizations, i.e., introduction of substituents at the C-7 carbon atom and alkoxycarbonylation of the C-2. The sodium in liquid ammonia reduction of compound **17**, which should remove the benzylidene protection and reduce the double bond, led to the

mixture of three products **38**, **39**, and **40** in a ratio of about 1.3:1.2:1, respectively, whereas compound **18** under the same conditions provided a corresponding mixture of **41**, **42**, and **43** in the ratio of about 2:2:1 (Scheme 3). The transfer of the benzyl radical or anion to the α,β-unsaturated amide is worth mentioning. Addition of acrylamide to the reaction mixture in order to trap the reactive intermediate did not change significantly the proportion of the reaction products. Low stereoselectivity of reduction of the double bond was another feature that differentiated reduction of **17** or **18** from that of **4**, which proceeded exclusively to the trans arrangement of protons at C-6 and C-7 of the cepham skeleton.[2]



Scheme 3. (i) Na/NH_3 and (ii) Ac_2O/Py.

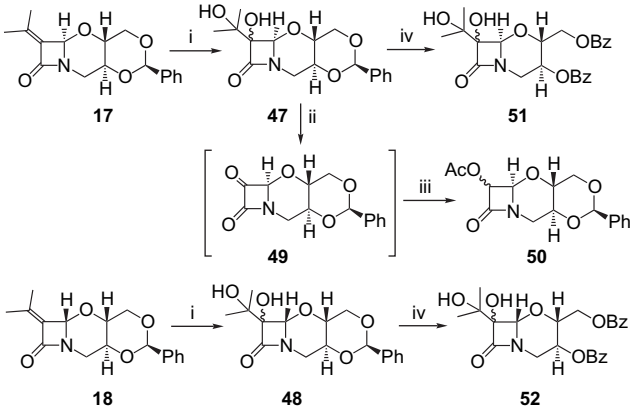
The isopropylidene group in compound **17** can be easily transformed into hydroxyisopropyl by the sequence of reactions involving the bromohydrin formation followed by the reductive removal of the bromine atom (Scheme 4).



Scheme 4. (i) NBS/DMSO–H_2O and (ii) Bu_3SnH/AIBN/toluene.

The treatment of **17** with NBS in wet DMSO,[11] according to the known procedure,[3f] provided a mixture of bromohydrins **44**, which upon treatment with tributyltin hydride gave two diastereomers **45** and **46** in the ratio of 4.4:1, respectively (Scheme 4). The relatively high stereoselectivity of debromination, which did not depend upon the proportion of bromohydrins, was in agreement with the previous observations.[2]

Since ozonolysis of the *exo* double bond in β-lactams led to the decomposition of the azitidin-2-one ring,[12] we used a cis hydroxylation–glycolic cleavage sequence to split the double bonds in **17** and **18**. Introduction of substituents to the C-3 carbon atom of the azetidin-2-one ring via 2,3-dione stage has been reported recently.[13] Oxidation of the cephams **17** and **18** independently with $RuCl_3/NaIO_4$ in $H_2O/CH_3CN/CHCl_3$ mixture, for 30 min,[14] afforded corresponding mixtures of diols **47** and **48** in a good yields (Scheme 5). Glycolic cleavage of **47**[15], followed by reduction of unstable ketone **49** with sodium borohydride provided a mixture of alcohols in a very low yield, which were characterized as acetates **50**. This low yield could be explained by the mentioned above strain, which exists in (6*R*) β-lactam ring fused to the trans decalin system and is manifested by the easy opening of the four-membered β-lactam ring.

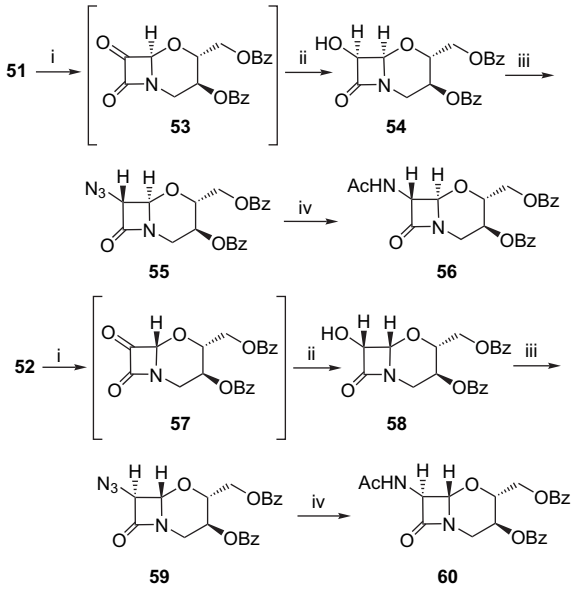


Scheme 5. (i) $RuCl_3/NaIO_4$, $H_2O/CH_3CN/CHCl_3$; (ii) H_5IO_6/AcOEt; (iii) (a) $NaBH_4$; (b) Ac_2O/Py; and (iv) (a) H_2, Pd/C; (b) BzCl/Py.

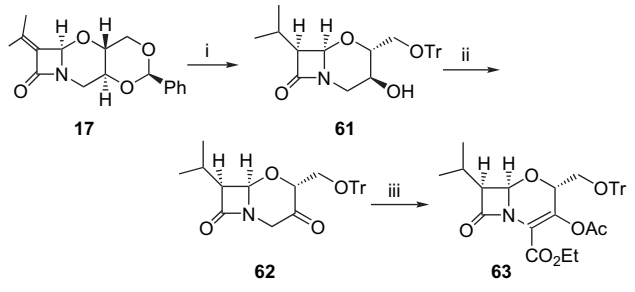
The hydrogenolysis of the benzylidene fragment in diol **47** released the conformational strain providing a tetraol, which was protected at the primary and secondary hydroxyl groups and gave compound **51**. Glycolic cleavage performed on diol **51** proceeded in much better yield. Unstable 7-keto compound **53** was thus obtained, which without purification was immediately reduced to the corresponding 7-ol **54**. Subsequent triflation of the hydroxyl group, followed by nucleophilic substitution with azide, provided **55** with the inversion in the configuration at C-7 carbon atom. Hydrogenation of the azide **55** and acetylation of the resulting amino group ended the reaction sequence affording **56**. The same reaction sequence was performed on **48**, yielding the corresponding acetamide **60**. All transformations proceeded in good yield (Scheme 6).

Hydrogenation of compound **17** over palladium followed by tritylation of the primary hydroxyl group provided only one diastereomer **61** having cis located H-6 and H-7 protons. Subsequent oxidation of the secondary hydroxyl group afforded the ketone **62**. Reaction of **62** with 1.1 equiv of KHMDS at −78 °C in toluene followed by the addition of ethyl cyanoformate provided, after acetylation, the cepham **63** in 60% yield (Scheme 7). This relatively high yield of ethoxycarbonylation was in contrast to our previous observations.[6]

Oxacephams **17**, **18**, **56**, and **63** were tested for their biological activity. An inhibition of the DD-carboxypeptidase activity and,



Scheme 6. (i) H_5IO_6/AcOEt; (ii) $NaBH_4/H_2O$; (iii) (a) Tf_2O/Py; (b) NaN_3/DMF; and (iv) (a) Pd/C, H_2; (b) Ac_2O, Py.



Scheme 7. (i) (a) Pd/C, H_2; (b) TrCl, Py; (ii) PCC, MS 4 Å, CH_2Cl_2, reflux; and (iii) (a) KHMDS, $NCCO_2Et$, toluene; (b) Ac_2O, Py.

separately, an inhibition of β-lactamase was measured.[16–19] Within studied series, all tested oxacephams showed low activity of DD-peptidase. All tested compounds did not show any significant activity as inhibitors of the β-lactamase either.

3. Conclusions

In summary, we have demonstrated that the [2+2]cycloadducts of CSI to 2-*O*-allenyl-1,3-benzylidene-L-erythritol are versatile intermediates for the preparation of a wide range of 7-substituted-5-oxacephams and for the introduction of carboxylic function to the C-2 carbon atom. Except the cycloaddition reaction that proceeded with a moderate stereoselectivity, the other transformations offer high stereoselectivities, and therefore may provide substituents at C-7, existing in many active β-lactam antibiotics.

4. Experimental

4.1. 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. Mass spectra were recorded using AMD-604 Inectra GmbH and HPLC–MS with Mariner and API 356 detectors. Optical rotations were measured using JASCO P 3010 polarimeter at 22±3 °C. Column chromatography was performed using E. Merck Kiesel Gel (230–400 mesh).

Compounds **21**–**25** and **30**–**34** were obtained according to the procedures reported previously for ethylidene analogs.[9] Detailed procedures, spectral and analytical data are provided in Supplementary data.[20]

4.1.1. (3*S*,4*R*,6*R*,7*S*)-3-Hydroxy-4-hydroxymethyl-7-methyl-5-oxa-cepham (26). Compound **25** (0.07 g, 0.25 mmol) dissolved in MeOH (10 mL) was hydrogenated in the presence of 5% Pd/C (0.007 g) for 1.5 h. Subsequently the mixture was filtered through Celite and evaporated. The crude product was purified by chromatography using AcOEt/MeOH, 4:1 v/v as an eluent to afford **26** (0.04 g, 83%). $[\alpha]_D^{22}$ +21.7 (*c* 0.1, CH_2Cl_2). IR (film): 1740, 3367 cm^{-1}. HRMS (ESI), *m*/*z* $(M+H)^+$, calcd for $C_8H_{15}O_4N$: 188.0917, found: 188.0922. ^1H NMR (400 MHz, D_2O) *δ*: 1.24 (d, *J*=7.5 Hz, 3H, CH_3), 3.38 (ddd, *J*=1.8, 2.9, 14.7 Hz, 1H, H-2), 3.50 (ddq, *J*=1.8, 3.7, 7.5 Hz, 1H, H-7), 3.64 (ddd, *J*=1.1, 2.0, 14.7 Hz, 1H, H-2′), 3.77–3.85 (m, 2H, H-3, CH_AH_BOH), 4.06–4.14 (m, 2H, H-4, CH_AH_BOH), 5.29 (d, 1H, *J*=3.7 Hz, H-6). Acetate **27**. $[\alpha]_D^{22}$ +83.3 (*c* 0.5, CH_2Cl_2). IR (film): 1745, 1770 cm^{-1}. HRMS (ESI), *m*/*z* $(M+Na)^+$, calcd for $C_{12}H_{17}O_6NNa$: 294.0948, found: 294.0953. ^1H NMR (500 MHz, C_6D_6) *δ*: 1.19 (d, *J*=7.5 Hz, 3H, CH_3), 1.59, 1.67 (2s, 6H, 2Ac), 2.51 (ddd, *J*=1.4, 3.0, 15.1 Hz, 1H, H-2), 2.86 (ddq, *J*=1.4, 3.6, 7.5 Hz, 1H, H-7), 3.66 (dd, *J*=4.9, 12.0 Hz, 1H, CH_AH_BOAc), 3.67 (dt, *J*=1.3, 1.8, 15.1 Hz, 1H, H-2′), 4.00 (m, 1H, H-4), 4.13 (dd, *J*=7.7, 12.0 Hz, 1H, CH_AH_BOAc), 4.25 (ddd, *J*=1.8, 2.2, 3.0 Hz, 1H, H-3), 4.58 (d, *J*=3.6 Hz, 1H, H-6).

4.1.2. (3*S*,4*R*,6*S*,7*R*)-3-Hydroxy-4-hydroxymethyl-7-methyl-5-oxa-cepham (35). Compound **34** was hydrogenated according to the procedure described above to afford **35** (85%). $[\alpha]_D^{22}$ −10.8 (*c* 0.5, CH_2Cl_2). IR (film): 1743, 3378 cm^{-1}. HRMS (ESI), *m*/*z* $(M+H)^+$, calcd for $C_8H_{15}O_4N$: 188.0917, found: 188.0906. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 1.20 (d, *J*=7.5 Hz, 3H, CH_3), 2.77 (ddd, *J*=2.6, 9.7, 13.1 Hz, 1H, H-2), 3.36 (ddq, *J*=1.7, 3.7, 7.5 Hz, 1H, H-7), 3.49 (m, 1H, CH_AH_BOH), 3.78 (m, 1H, H-3), 3.91 (m, 2H, H-4, CH_AH_BOH), 4.11 (dd, *J*=6.2, 13.1 Hz, H-2′), 4.97 (d, *J*=3.7 Hz, 1H, H-6). Acetate **36**: $[\alpha]_D^{22}$ −15.1 (*c* 0.1, CH_2Cl_2). IR (film): 1746, 1773 cm^{-1}. HRMS (ESI), *m*/*z* $(M+Na)^+$, calcd for $C_{12}H_{17}O_6NNa$: 294.0948, found: 294.0925. ^1H NMR (500 MHz, C_6D_6) *δ*: 1.08 (d, *J*=7.5 Hz, 3H, CH_3), 1.53, 1.61 (2s, 6H, 2Ac), 2.25 (ddd, *J*=1.6, 9.5, 13.0 Hz, 1H, H-2), 2.79 (ddq, *J*=1.6, 3.7, 7.5 Hz, 1H, H-7), 3.22 (ddd, *J*=2.9, 4.6, 9.8 Hz, 1H, H-4), 4.09 (dd, *J*=2.9, 12.1 Hz, 1H, CH_AH_BOAc), 4.12 (dd, *J*=4.6, 12.1 Hz, 1H, CH_AH_BOAc), 4.17 (dd, *J*=6.3, 13.0 Hz, 1H, H-2′), 4.19 (d, *J*=3.7 Hz, 1H, H-6), 4.66 (dt, *J*=6.4, 9.5, 9.8 Hz, 1H, H-3).

4.1.3. (3*S*,4*R*,6*R*,7*R*)-3-Acetoxy-4-(acetoxymethyl)-7-(1′-benzyl-1′-methyl-ethyl)-5-oxa-cepham (38), (3*S*,4*R*,6*R*,7*R*) and (3*S*,4*R*,6*R*,7*S*)-7-isopropyl-3-acetoxy-4-(1′-acetoxymethyl)-5-oxa-cepham (39 and 40). To a stirring solution

of sodium (0.040 g, 1.7 mmol) in liquid ammonia (40 mL) at 60 °C, compound **17** (0.050 g, 0.166 mmol) in dry THF (4 mL) was added dropwise. The temperature was maintained for 40 min. Subsequently NH_4Cl (0.5 g) was added and the mixture was left until evaporation of ammonia. To the residue, 10 mL of water was added and the mixture was extracted with AcOEt (3×20 mL). The extract was dried and evaporated. The crude products were acetylated with Ac_2O/pyridine mixture. Standard workup and chromatographical separation using hexane/AcOEt, 7:3 v/v as an eluent provided compound **38** (0.013 g, 20%) and a mixture **39/40** (0.017 g, 34%) in a ratio of about 1.2:1, respectively.

Compound **38**: $[\alpha]_D^{22}$ +44.2 (*c* 0.4, CH_2Cl_2). IR (film): 1745, 1769 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{21}H_{27}NO_6Na$: 412.1731, found: 412.1756. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 0.97 and 1.03 (2s, 6H, $2CH_3$), 2.11 (s, 6H, 2Ac), 2.63 and 2.68 (2d, *J*=13.2 Hz, 2H, CH_2Ph), 3.06 (s, 1H, H-7), 3.32 (dd, *J*=3.51, 15.03 Hz, 1H, H-2), 3.87 (m, 1H, H-2′), 4.20 (m, 1H, H-4), 4.25 (m, 1H, CH_AH_BOAc), 4.53 (dd, *J*=6.12, 11.13 Hz, 1H, CH_AH_BOAc), 4.70 (m, 1H, H-3), 4.95 (s, 1H, H-6), 7.18–7.28 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$) *δ*: 20.76, 21.02, 24.35, 25.22, 33.95, 39.60, 47.14, 60.88, 64.39, 68.97, 72.96, 75.57, 126.25, 127.87, 128.32, 130.88, 137.43, 169.99, 170.36.

Compounds **39** and **40**, taken for the mixture. IR (film): 1745, 1769 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{14}H_{21}NO_6Na$: 322.1261, found: 322.1275. ^1H NMR (500 MHz, $CDCl_3$) *δ*: **39**: 1.01 (d, *J*=6.7 Hz, 3H, CH_3), 1.06 (d, *J*=6.7 Hz, 3H, CH_3), 1.98 (m, 1H, C*H*–$(CH_3)_2$), 2.10 and 2.12 (2s, 6H, 2Ac), 2.98 (d, *J*=6.7 Hz, 1H, H-7), 3.30 (dd, *J*=3.4, 15.1 Hz, 1H, H-2), 3.86 (m, 1H, H-2′), 4.22 (m, 1H, H-4), 4.30 (m, 1H, CH_AH_BOAc), 4.56 (dd, *J*=6.5, 11.2 Hz, 1H, CH_AH_BOAc), 4.70 (m, 1H, H-3), 4.95 (s, 1H, H-6). Compound **40**: 0.96 (d, *J*=6.6 Hz, 3H, CH_3), 1.16 (d, *J*=6.7 Hz, 3H, CH_3), 2.10 and 2.11 (2s, 6H, 2Ac), 2.24 (m, 1H, C*H*–$(CH_3)_2$), 2.95 (ddd, *J*=1.4, 3.5, 10.9 Hz, 1H, H-7), 3.30 (ddd, *J*=1.4, 3.3, 15.0 Hz, 1H, H-2), 3.84 (m, 1H, H-2′), 4.24 (m, 1H, H-4), 4.28 (dd, *J*=5.5, 11.8 Hz, 1H, CH_AH_BOAc), 4.54 (dd, *J*=6.7, 11.8 Hz, 1H, CH_AH_BOAc), 4.70 (m, 1H, H-3), 5.18 (d, *J*=3.5 Hz, 1H, H-6).

4.1.4. (3*S*,4*R*,6*S*,7*S*)-3-Acetoxy-4-(acetoxymethyl)-7-(1′-benzyl-1′-methyl-ethyl)-5-oxa-cepham (41), (3*S*,4*R*,6*S*,7*S*) and (3*R*,4*R*,6*S*,7*R*)-7-isopropyl-3-acetoxy-4-(1′-acetoxymethyl)-5-oxa-cepham (42 and 43). Compound **41** (23%) and a mixture **42/43** (32%) were obtained from compound **18** according to the procedure described for compounds **38**–**40**.

Compound **41**: $[\alpha]_D^{22}$ +36.4 (*c* 0.1, CH_2Cl_2). IR (film): 1746, 1765 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{21}H_{27}NO_6Na$: 412.1731, found: 412.1755. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 0.98, 1.04 (2s, 6H, $2CH_3$), 2.17 (s, 6H, 2Ac), 2.61 and 2.73 (2d, *J*=13.3 Hz, 2H, CH_2Ph), 2.78 (dd, *J*=9.5, 13.0 Hz, 1H, H-2a), 3.03 (s, 1H, H-7), 3.72 (ddd, *J*=2.4, 5.1, 9.9 Hz, 1H, H-4), 4.19 (dd, *J*=2.4, 12.2 Hz, 1H, CH_AH_BOAc), 4.25 (dd, *J*=5.1, 12.2 Hz, 1H, CH_AH_BOAc), 4.33 (dd, *J*=6.3, 13.0 Hz, 1H, H-2b), 4.73 (dt, *J*=6.5, 9.5, 9.9 Hz, 1H, H-3), 4.78 (s, 1H, H-6), 7.19–7.29 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$) *δ*: 20.75, 21.00, 24.36, 25.23, 33.95, 39.62, 47.17, 60.92, 64.42, 69.01, 72.98, 75.61, 126.26, 127.87, 130.88, 137.45, 169.98, 170.34, 170.36.

Compounds **42** and **43**, taken for the mixture. IR (film): 1732, 1772 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{14}H_{21}NO_6Na$: 322.1261, found: 322.1279. ^1H NMR (500 MHz, $CDCl_3$) *δ*: **42**: 1.01 (d, *J*=6.8, 3H, CH_3), 1.06 (d, *J*=6.7 Hz, 3H, CH_3), 2.00 (m, 1H, C*H*–$(CH_3)_2$), 2.06 and 2.09 (2s, 6H, 2Ac), 2.77 (dd, *J*=9.5, 13.0 Hz, 1H, H-2), 2.96 (d, *J*=6.7 Hz, 1H, H-7), 3.7 (m, 1H, H-4), 4.20 (dd, *J*=2.4, 12.2 Hz, 1H, CH_AH_BOAc), 4.24 (m, 1H, CH_AH_BOAc), 4.40 (dd, *J*=6.4, 13.0 Hz, 1H, H-2′), 4.72 (dt, *J*=6.4, 9.5 Hz, 1H, H-3), 4.80 (s, 1H, H-6). **43**: 0.95 (d, *J*=6.5 Hz, 3H, CH_3), 1.13 (d, *J*=6.7 Hz, 3H, CH_3), 2.06 and 2.08 (2s, 6H, 2 Ac), 2.15 (m, 1H, C*H*–$(CH_3)_2$), 2.75 (ddd, *J*=1.6, 9.5, 13.0 Hz, 1H, H-2a), 2.94 (ddd, *J*=1.6, 3.6, 10.9 Hz, 1H, H-7), 3.75 (m, 1H, H-4), 4.23 (m, 2H, CH_2OAc), 4.29 (dd, *J*=6.4, 13.0 Hz, 1H, H-2b), 4.71 (m, 1H, H-3), 4.98 (d, *J*=3.6 Hz, 1H, H-6).

4.1.5. (2*R*,4a*R*,5a*R*,6*R*,9a*S*) and (2*R*,4a*R*,5a*R*,6*S*,9a*S*)-6-Bromo-6-(1′-hydroxy-1′-methyl-ethyl)-2-phenyl-1,3,5-trioxa-8-aza-cyclobuta[*b*]decalin-7-on (44). To a stirring solution of **17** (0.040 g, 0.13 mmol) in water (0.01 mL, 0.55 mmol) and DMSO (5 mL) NBS (0.034 g, 0.19 mmol) was added. Stirring was continued at room temperature for 12 h. Subsequently the mixture was poured into water (10 mL) and extracted with Et_2O (2×20 mL). Organic layer was dried and evaporated. The residue was purified by chromatography using hexane/AcOEt, 1:1 v/v as an eluent to afford **44** as a mixture of two diastereomers in a ratio of about 10:1 (0.032 g, 62%). HRMS (ESI) taken for the mixture, *m/z* $(M+Na)^+$, calcd for $C_{17}H_{20}BrNO_5Na$: 420.0417, found: 420.0439. ^1H NMR (500 MHz, $CDCl_3$) *δ*: the major isomer: 1.42 and 1.54 (2s, 6H, $2CH_3$), 3.66 (dd, *J*=7.2, 12.7 Hz, 1H, H-9), 3.78 (m, 2H, H-4, H-9a), 4.12 (m, 2H, H-4a, H-9′), 4.44 (dd, *J*=2.1, 10.7 Hz, 1H, H-4′), 5.31 (s, 1H, H-2), 5.55 (s, 1H, H-5a), 7.36–7.46 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$) *δ*: 26.27, 26.41, 41.82, 65.40, 68.74, 72.11, 73.97, 79.99, 80.26, 102.05, 126.14, 128.33, 128.39, 129.39, 136.64, 165.68.

4.1.6. (2*R*,4a*R*,5a*R*,6*S*,9a*S*) and (2*R*,4a*R*,5a*R*,6*R*,9a*S*)-6-(1′-Hydroxy-1′-methyl-ethyl)-2-phenyl-1,3,5-trioxa-8-aza-cyclobuta[*b*]decalin-7-on (45 and 46). A solution of tri-*n*-butyltin hydride (0.37 mL, 0.14 mmol) and AIBN (0.021 g, 0.16 mmol) in toluene (2 mL) was added to a hot solution (95 °C) of bromohydrins **44** (0.027 g, 0.07 mmol) in toluene (3 mL). The stirring and temperature was maintained for additional 40 min. Subsequently the temperature of the mixture was cooled to room temperature and the solvent was evaporated. The residue was purified by chromatography using hexane/AcOEt, 1:1 v/v as an eluent to afford a mixture of **45/46** in a ratio of about 4.4:1 (0.019 g, 85%). IR (film): 1755, 3457 cm^{-1}. HRMS (ESI) taken for the mixture, *m/z* $(M+Na)^+$, calcd for $C_{17}H_{21}NO_5Na$: 342.1312, found: 342.1317. ^1H NMR (500 MHz, $CDCl_3$) *δ*: major component **45**: 1.38 and 1.46 (2s, 6H, $2CH_3$), 3.23 (dd, *J*=1.9, 3.4 Hz, 1H, H-6), 3.59 (ddd, *J*=1.9, 7.1, 12.0 Hz, 1H, H-9), 3.74 (dd, *J*=8.7, 10.9 Hz, 1H, H-4), 3.76 (dd, *J*=9.5, 12.0 Hz, 1H, H-9′), 4.05 (m, *J*=4.9, 8.7, 9.5 Hz,

1H, H-4a), 4.12 (dt, *J*=7.1, 9.5, 9.5 Hz, 1H, H-9a), 4.34 (dd, *J*=4.9, 10.9 Hz, 1H, H-4′), 5.37 (d, *J*=3.4 Hz, 1H, H-5a), 5.55 (s, 1H, H-2), 7.36–7.45 (m, 5H, Ph). Compound **46** inter alia: 1.34 and 1.40 (2s, 6H, $2CH_3$), 3.20 (s, 1H, H-6), 4.4 (dd, *J*=5.0, 10.7 Hz, 1H, H-4′), 5.24 (s, 1H, H-5a), 5.54 (s, 1H, H-2).

4.1.7. (2*R*,4a*R*,5a*R*,6*S*,9a*S*) and (2*R*,4a*R*,5a*R*,6*R*,9a*S*)-6-Hydroxy-6-(1′-hydroxy-1′-methyl-ethyl)-2-phenyl-1,3,5-trioxa-8-aza-cyclobuta[*b*]decalin-7-on (47). A solution of **17** (0.070 g, 0.23 mmol) in CH_3CN (10 mL), $CHCl_3$ (2 mL), and water (5 mL) was treated with $NaIO_4$ (0.245 g, 1.15 mmol) and $RuCl_3 \cdot H_2O$ (0.001 g). The stirring was continued until disappearance of the substrate (TLC), about 30 min. The mixture was treated with water (10 mL) and extracted with AcOEt (3×20 mL). Organic layer was dried and evaporated. The residue was purified by chromatography using hexane/AcOEt, 7:3 v/v as an eluent to afford **47** as a mixture of diastereoisomers in a ratio of about 2.8:1 (0.067 g, 87%). IR (film): 3293, 3222, 1755 cm^{-1}. HRMS (ESI) taken for the mixture, *m*/*z* $(M+Na)^+$, calcd for $C_{17}H_{21}NO_6Na$: 358.1261, found: 358.1271. ^1H NMR (500 MHz, $CDCl_3$) *δ*: major component: 3.63–3.82 (m, 3H, H-4, H-9, H-9′), 3.92 (m, 1H, H-9a), 4.16 (m, 1H, H-4a), 4.33 (dd, *J*=5.0, 10.9 Hz, 1H, H-4′), 5.15 (s, 1H, H-2), 5.54 (s, 1H, H-5a), 7.34–7.46 (m, 5H, Ph). Minor component inter alia: 4.43 (dd, *J*=5.0, 10.9 Hz, 1H, H-4), 5.22 (s, 1H, H-2), 5.55 (s, 1H, H-5a).

4.1.8. (2*R*,4a*R*,5a*S*,6*S*,9a*S*) and (2*R*,4a*R*,5a*S*,6*R*,9a*S*)-6-Hydroxy-6-(1′-hydroxy-1′-methyl-ethyl)-2-phenyl-1,3,5-trioxa-8-aza-cyclobuta[*b*]decalin-7-on (48). Compound **48** (90%) in a ratio of about 4.5:1 was obtained from **18** following procedure described for **47**. IR (film): 1756, 3220, 3292 cm^{-1}. HRMS (ESI), *m*/*z*, $(M+Na)^+$, calcd for $C_{17}H_{21}NO_6Na$: 358.1261, found: 358.1271. ^1H NMR (500 MHz, $CDCl_3$) major product *δ*: 1.28 and 1.39 (2s, 6H, $2CH_3$), 3.04 (dd, *J*=10.0, 12.6 Hz, 1H, H-9), 3.62 (ddd, *J*=4.8, 9.1, 9.9 Hz, 1H, H-4a), 3.68–3.74 (m, 2H, H-4, H-9a), 4.17 (dd, *J*=5.7, 12.6 Hz, 1H, H-9′), 4.30 (dd, *J*=4.8, 10.7 Hz, 1H, H-4′), 5.00 (s, 1H, H-2), 5.52 (s, 1H, H-5a), 7.18–7.38 (m, 5H, Ph).

4.1.9. (2*R*,4a*R*,5a*R*,6*S*,9a*S*) and (2*R*,4a*R*,5a*R*,6*R*,9a*S*)-6-Acetoxy-2-phenyl-1,3,5-trioxa-8-aza-cyclobuta[*b*]decalin-7-on (50). A solution of **47** (0.065 g, 0.19 mmol) in AcOEt (5 mL) was treated with H_5IO_6 (0.043 g, 0.19 mmol). Upon stirring at temperature 0–5 °C, $NaBH_4$ (0.01 g, 0.026 mmol) in water (2 mL) was added. After 20 min, 10 mL of water was added and the mixture was extracted with AcOEt (3×20 mL). The extract was dried and evaporated. The crude product was acetylated with Ac_2O/Py mixture to afford, after standard workup, compound **50** in a ratio of about 5.5:1 (0.003 g, 5%). IR (film): 1755, 1782 cm^{-1}. HRMS (ESI), *m*/*z* $(M+Na)^+$, calcd for $C_{16}H_{17}NO_6Na$: 342.0948, found: 342.0953. ^1H NMR (500 MHz, C_6D_6) major isomer (6*S*) *δ*: 3.00 (ddd, *J*=1.2, 7.6, 12.4 Hz, 1H, H-9), 3.32 (m, 1H, H-9′), 3.44 (dd, *J*=9.9, 10.3 Hz, 1H, H-4), 3.80 (m, 2H, H-9a, H-4a), 4.19 (dd, *J*=5.1, 10.3 Hz, 1H, H-4′), 4.79 (d, *J*=2.5 Hz, 1H, H-5a), 5.25 (s, 1H, H-2), 5.31 (dd, *J*=1.7, 2.5 Hz, 1H, H-6), 7.27–7.62 (m, 5H, Ph). Minor isomer (6*S*) inter alia: 4.86 (s, 1H, H-5a), 5.19 (s, 1H, H-2), 5.54 (s, 1H, H-6).

4.1.10. (3*S*,4*R*,6*R*,7*R*) and (3*S*,4*R*,6*R*,7*S*)-3-Benzoyloxy-4-benzoyloxymethyl-7-hydroxy-7-(1′-hydroxy-1′-methyl-ethyl)-5-oxa-cepham (51). Compound **47** (0.060 g, 0.18 mmol) in MeOH (10 mL) was treated with 10% Pd/C (3 mg) and stirred under a hydrogen atmosphere for 2 h. Subsequently the mixture was filtered and evaporated. The residue was treated with BzCl/Py mixture. After standard workup, compound **51** was obtained (0.063 g, 77%) in a ratio 6:1. IR (film): 3331, 1787, 1747, 1724 cm^{-1}. HRMS (ESI), *m*/*z* $(M+Na)^+$, calcd for $C_{24}H_{25}NO_8Na$: 478.1472, found: 478.1487. ^1H NMR (500 MHz, $CDCl_3$) major isomer *δ*: 1.33 and 1.45 (2s, 6H, $2CH_3$), 3.62 (dd, *J*=3.3, 15.1 Hz, 1H, H-2), 4.00 (m, 1H, H-2′), 4.60 (m, 2H, H-4, CH_AH_BOBz), 4.80 (dd, *J*=6.3, 12.0 Hz, 1H, CH_AH_BOBz), 5.08 (m, 1H, H-3), 5.28 (s, 1H, H-6), 7.19–8.02 (m, 10H, 2×Ph).

4.1.11. (3*S*,4*R*,6*S*,7*R*) and (3*S*,4*R*,6*S*,7*S*)-3-Benzoyloxy-4-benzoyloxymethyl-7-hydroxy-7-(1′-hydroxy-1′-methyl-ethyl)-5-oxa-cepham (52). Compounds **52** (75%) in a ratio 2.1:1 were obtained from **48** according to the procedure described for **51**. IR (film): 1721, 1761, 3423, 3519 cm^{-1}. HRMS (ESI), *m*/*z* $(M+Na)^+$, calcd for $C_{24}H_{25}NO_8Na$: 478.1472, found: 478.1498. ^1H NMR (500 MHz, $CDCl_3$) major isomer *δ*: 1.35, 1.46 (2s, 6H, $2CH_3$), 3.08 (dd, *J*=9.5, 12.9 Hz, 1H, H-2), 4.26–4.34 (m, 1H, H-4), 4.53 (dd, *J*=4.2, 12.3 Hz, 1H, CH_AH_BOBz), 4.56 (dd, *J*=6.3, 12.3 Hz, 1H, CH_AH_BOBz), 4.67 (dd, *J*=2.6, 12.9 Hz, 1H, H-2′), 5.10 (s, 1H, H-6), 5.18 (dt, *J*=6.4, 9.5, 9.5 Hz, 1H, H-3), 7.43–8.00 (m, 10H, 2×Ph).

4.1.12. (3*S*,4*R*,6*R*,7*S*)-3-Benzoyloxy-4-benzoyloxymethyl-7-hydroxy-5-oxa-cepham (54). A solution of compound **51** (0.055 g, 0.12 mmol) in AcOEt (5 mL) was treated with H_5IO_6 (0.027 g, 0.12 mmol) and stirred at room temperature for 45 min. Subsequently the mixture was cooled to −5 °C and a solution of $NaBH_4$ (0.01 g, 0.026 mmol) in water (2 mL) was added. Stirring was continued for 30 min and then water (10 mL) was added and the mixture was extracted with AcOEt (3×20 mL). Organic layer was dried and evaporated. The residue was purified by chromatography using hexane/AcOEt, 3:2 v/v as an eluent to afford **54** (0.031 g, 65%). $[\alpha]_D^{22}$ +10.4 (*c* 0.2, CH_2Cl_2). IR (film): 1721, 1757, 3394 cm^{-1}. HRMS (ESI), *m*/*z* $(M+Na)^+$, calcd for $C_{21}H_{19}NO_7Na$: 420.1054, found: 420.1072, ^1H NMR (500 MHz, $CDCl_3$) *δ*: 3.58 (m, 1H, H-2), 4.07 (m, 1H, H-2′), 4.63–4.70 (m, 2H, H-4, CH_AH_BOBz), 4.93–4.99 (m, 2H, H-7, CH_AH_BOBz), 5.13 (m, 1H, H-3), 5.44 (dd, *J*=3.2 Hz, 1H, H-6), 7.48–8.07 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$) *δ*: 39.55, 61.78, 65.59, 73.47, 74.80, 78.86, 128.56, 2×128.67, 128.97, 129.72, 129.92, 2×133.66, 165.57, 165.91, 172,39.

4.1.13. (3*S*,4*R*,6*R*,7*R*)-7-Azido-3-benzoyloxy-4-benzoyloxymethyl-5-oxa-cepham (55). Tf_2O (0.047 g, 0.17 mmol) was added to pyridine (2 mL) at −20 °C under argon. After 5 min a solution of **54** (0.056 g, 0.14 mmol) in pyridine (2 mL) was added and the mixture was left for 30 min. Subsequently the solvent was removed under diminished pressure. The residue was dissolved in DMF (10 mL), treated with NaN_3 (0.065 g, 1.0 mmol) and heated to 70 °C for 30 min until disappearance of the triflate (TLC). Subsequently, the mixture was cooled to room temperature, treated

with water (20 mL) and extracted with AcOEt (3×20 mL). Organic layer was dried and evaporated. The crude product was purified by chromatography using hexane/AcOEt, 9:1 v/v as an eluent to afford **55** (44 mg, 75%). $[\alpha]_D^{22}$ +69.4 (*c* 1.8, CH_2Cl_2). IR (film): 1722, 1782, 2114 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{21}H_{18}N_4O_6Na$: 445.1119, found: 445.1133. ^1H NMR (500 MHz, $CDCl_3$) δ: 3.65 (dd, *J*=3.1, 15.0 Hz, 1H, H-2), 4.08 (m, 1H, H-2′), 4.58 (s, 1H, H-7), 4.61 (m, 2H, H-4, CH_AH_BOBz), 4.86 (m, 1H, CH_AH_BOBz), 5.10 (m, 1H, H-3), 5.29 (s, 1H, H-6), 7.47–8.06 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$) δ: 40.70, 60.02, 64.74, 72.39, 73.62, 79.65, 128.65, 128.73, 128.98, 128.99, 129.73, 129.82, 133.72, 133.74, 163.80, 165.48, 165.89.

4.1.14. (3*S*,4*R*,6*R*,7*R*)-7-Acetamino-3-benzoyloxy-4-benzoyloxymethyl-5-oxa-cepham (56). Compound **55** (0.030 g, 0.07 mmol) and 5% Pd/C in AcOEt (10 mL) were hydrogenated for 2 h. Subsequently the mixture was filtered through Celite and evaporated. The residue was acetylated with Ac_2O/Py mixture. Subsequently the mixture was evaporated and purified by chromatography using AcOEt to afford **56** (0.028 g, 90%). $[\alpha]_D^{22}$ +38.6 (*c* 0.4, CH_2Cl_2). IR (film): 1722, 1775, 3300 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{23}H_{22}N_2O_7Na$: 461.1319, found: 461.1341. ^1H NMR (500 MHz, $CDCl_3$) δ: 2.04 (s, 3H, Ac), 3.68 (dd, *J*=3.2, 14.9 Hz, 1H, H-2), 4.08 (m, 1H, H-2′), 4.60 (m, 1H, H-4), 4.64 (m, 1H, CH_AH_BOBz), 4.82 (dd, *J*=5.2, 11.0 Hz, 1H, CH_AH_BOBz), 4.86 (d, *J*=7.6 Hz, 1H, H-7), 5.10 (m, 1H, H-3), 5.38 (s, 1H, H-6), 6.01 (d, *J*=7.6 Hz, 1H, NH), 7.46–8.05 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$) δ: 22.86, 40.62, 62.10, 64.99, 65.68, 73.40, 80.72, 128.60, 128.67, 2×129.08, 129.76, 129.85, 133.60, 133.65, 165.52, 165.64, 165.95, 170.20.

4.1.15. (3*S*,4*R*,6*S*,7*R*)-3-Benzoyloxy-4-benzoyloxymethyl-7-hydroxy-5-oxa-cepham (58). Compound **58** was obtained from the mixture **52** (74%) according to the procedure described for **54**. $[\alpha]_D^{22}$ +46.7 (*c* 3, CH_2Cl_2). IR (film): 1722, 1759, 3424 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 3.03 (m, 1H, H-2), 4.27 (ddd, *J*=2.7, 5.6, 9.8 Hz, 1H, H-4), 4.50 (m, 2H, CH_AH_BOBz, H-7), 4.69 (dd, *J*=2.7, 12.2 Hz, 1H, CH_AH_BOBz), 4.93 (ddd, *J*=1.2, 3.2, 11.8 Hz, 1H, H-2′), 5.12 (dt, *J*=6.3, 9.5, 9.8 Hz, 1H, H-3), 5.14 (d, *J*=3.2 Hz, 1H, H-6), 7.42–8.02 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$) δ: 41.59, 63.37, 63.99, 75.17, 78.19, 79.48, 128.47, 128.59, 128.74, 129.4, 129.75, 129.81, 133.33, 133.74, 164.84, 166.22, 171.12.

4.1.16. (3*S*,4*R*,6*S*,7*R*)-7-Azido-3-benzoyloxy-4-benzoyloxymethyl-5-oxa-cepham (59). Compound **59** was obtained from **58** (85%) according to the procedure described for compound **55**. $[\alpha]_D^{22}$ −15.43 (*c* 0.3, CH_2Cl_2). IR (film): 1732, 1783, 2113 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{21}H_{18}N_4O_6Na$: 445.1119, found: 445.1130. ^1H NMR (500 MHz, $CDCl_3$) δ: 3.06 (dd, *J*=9.4, 13.1 Hz, 1H, H-2), 4.16 (ddd, *J*=2.6, 5.5, 9.8 Hz, 1H, H-4), 4.41 (dd, *J*=5.5, 12.3 Hz, 1H, CH_AH_BOBz), 4.53 (dd, *J*=6.4, 13.1 Hz, 1H, H-2′), 4.56 (s, 1H, H-7), 4.70 (dd, *J*=2.6, 12.3 Hz, 1H, CH_AH_BOBz), 5.02 (s, 1H, H-6), 5.08 (dt, *J*=6.4, 9.8, 1H, H-3), 7.42–8.02 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$) δ: 42.37, 63.13, 63.70, 70.92, 75.62, 83.14, 128.40, 128.50, 128.56, 129.26, 129.68, 129.73, 133.284, 133.77, 161.94, 164.73, 166.00.

4.1.17. (3*S*,4*R*,6*S*,7*R*)-7-Acetamino-3-benzoyloxy-4-benzoyloxymethyl-5-oxa-cepham (60). Compound **60** was obtained from **59** (92%) according to the procedure described for **56**. $[\alpha]_D^{22}$ +17.9 (*c* 0.4, CH_2Cl_2). IR (film): 1722, 1777, 3313 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{23}H_{22}N_2O_7Na$: 461.1319, found: 461.1338. ^1H NMR (500 MHz, $CDCl_3$) δ: 2.02 (s, 3H, Ac), 3.08 (dd, *J*=9.4, 13.0 Hz, 1H, H-2), 4.14 (ddd, *J*=2.7, 5.4, 9.7 Hz, 1H, H-4), 4.42 (dd, *J*=5.4, 12.2 Hz, 1H, CH_AH_BOBz), 4.52 (dd, *J*=6.4, 13.0 Hz, 1H, H-2′), 4.62 (d, *J*=7.35 Hz, 1H, H-7), 4.66 (dd, *J*=2.7, 12.2 Hz, 1H, CH_AH_BOBz), 5.07 (dt, *J*=6.4, 9.7, 1H, H-3), 5.24 (s, 1H, H-6), 5.20 (d, *J*=7.4 Hz, 1H, NH), 7.34–8.05 (10H, m, 2×Ph). ^{13}C NMR ($CDCl_3$) δ: 22.78, 42.28, 63.33, 64.07, 64.32, 75.49, 84.04, 128.41, 128.58, 128.74, 129.47, 129.76, 129.79, 133.24, 133.72, 164.06, 164.93, 166.20, 170.64.

4.1.18. (3*S*,4*R*,6*R*,7*S*)-7-Isopropyl-3-hydroxy-4-trityloxymethyl-5-oxa-cepham (61). Compound **17** (0.13 g, 0.43 mmol) in MeOH (10 mL) was hydrogenated in the presence of a catalytic amount of 10% Pd/C for 4 h. Subsequently the mixture was filtered and evaporated. The crude product was treated with TrCl in pyridine at 70 °C for 2 h. After standard workup and chromatographical purification, compound **61** was obtained (0.176 g, 90%). $[\alpha]_D^{22}$ +44.1 (*c* 0.4, CH_2Cl_2). IR (film): 1748, 3431 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{29}H_{31}NO_4Na$: 480.2145, found: 480.2154. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.97 (d, *J*=6.5 Hz, 3H, CH_3), 1.15 (d, *J*=6.7 Hz, 3H, CH_3), 2.20 (m, 1H, $CH(CH_3)_2$), 2.86 (ddd, *J*=1.4, 3.5, 11.3 Hz, 1H, H-7), 3.04 (dd, *J*=1.4, 3.1, 14.1 Hz, 1H, H-2), 3.41 (dd, *J*=6.4, 10.1 Hz, 1H, CH_AH_BOTr), 3.51 (dd, *J*=5.6, 10.1 Hz, 1H, CH_AH_BOTr), 3.64 (dt, *J*=1.3, 14.1 Hz, 1H, H-2′), 3.74 (m, 1H, H-3), 4.15 (m, 1H, H-4), 5.00 (d, *J*=3.5 Hz, 1H, H-6), 7.27–7.44 (m, 15H, OTr).

4.1.19. (3*S*,4*R*,6*R*,7*S*)-7-Isopropyl-3-oxo-4-trityloxymethyl-5-oxa-cepham (62). Compound **61** (0.1 g, 0.22 mmol) in CH_2Cl_2 (10 mL) was treated with PCC (0.056 g, 0.26 mmol) and molecular sieves MS 4 Å (0.02 g). The reaction mixture was stirred under reflux until disappearance of the substrate (4 h, TLC). Subsequently it was filtered by Celite and concentrated. The residue was filtered by chromatography using hexane/AcOEt, 7:3 v/v as an eluent to afford **62** (0.09 g, 90%). $[\alpha]_D^{22}$ +77.13 (*c* 0.3, CH_2Cl_2). IR (film): 1763, 1778 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{29}H_{29}NO_4Na$: 478.1989, found: 478.2009. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.99 (d, *J*=6.4 Hz, 3H, CH_3), 1.18 (dd, *J*=6.7 Hz, 3H, CH_3), 2.55 (m, 1H, $CH(CH_3)_2$), 3.06 (ddd, *J*=1.6, 3.5, 11.6 Hz, 1H, H-7), 3.56 (dd, *J*=2.3, 10.4 Hz, 1H, CH_AH_BOTr), 3.69 (dd, *J*=4.7, 10.4 Hz, 1H, CH_AH_BOTr), 3.84 (dt, *J*=1.6, 19.5 Hz, 1H, H-2), 4.41 (m, 1H, H-4), 4.47 (d, *J*=19.5 Hz, 1H, H-2′), 5.74 (d, *J*=3.5 Hz, 1H, H-6), 7.27–7.38 (m, 15H, OTr). ^{13}C NMR ($CDCl_3$) δ: 20.49, 21.48, 24.51, 60.34, 62.33, 65.66, 77.52, 79.80, 87.78, 127.36, 128.00, 128.52, 143.08, 172.51, 201.37.

4.1.20. (4*R*,6*R*,7*S*)-3-Acetoxy-7-isopropyl-2-ethoxycarbonyl-4-trityloxymethyl-5-oxa-2-cephem (63). Compound **62** (0.017 g, 0.038 mmol) in toluene (5 mL) at −45 °C under argon was treated with KHMDS (0.045 mmol, 0.091 mL, 0.5 M in toluene). After 30 min

ethyl cyanoformate (0.045 mmol, 0.004 mL) was added. Stirring was continued for 30 min and then the solution was treated with Ac_2O (0.5 mL) in pyridine (5 mL) with catalytic amount of DMAP. The mixture was stirred for 4 h at room temperature and then brine (10 mL) was added. The mixture was extracted with AcOEt (3×20 mL). The extract was dried and evaporated. The residue was purified by chromatography using hexane/AcOEt, 95:5 v/v as an eluent to afford **63** (0.012 g, 60%). $[\alpha]_D^{22}$ −54.3 (*c* 0.1, CH_2Cl_2). IR (film): 1727, 1776 cm^{-1}. HRMS (ESI), *m/z* $(M+Na)^+$, calcd for $C_{34}H_{35}NO_7Na$: 592.2306, found: 592.2329. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 1.02 (d, *J*=6.4, 3H, CH_3), 1.14 (d, *J*=6.7 Hz, 3H, CH_3), 1.32 (t, *J*=7.1 Hz, 3H, OCH_2CH_3), 2.04 (s, 3H, OAc), 2.25 (m, 1H, $CH(CH_3)_2$), 3.10 (dd, *J*=3.9, 11.8 Hz, 1H, H-7), 3.31 (dd, *J*=2.6, 10.6 Hz, 1H, CH_AH_BOTr), 3.39 (dd, *J*=4.7, 10.6 Hz, 1H, CH_AH_BOTr), 4.24 (dq, *J*=10.7, 7.1 Hz, 1H, $OCH_AH_BCH_3$), 4.34 (dq, *J*=10.8, 7.1 Hz, 1H, $OCH_AH_BCH_3$), 4.59 (m, 1H, H-4), 5.44 (d, *J*=3.9 Hz, 1H, H-6), 7.25–7.43 (15H, m, OTr). ^{13}C NMR ($CDCl_3$) *δ*: 14.07, 20.36, 20.46, 21.64, 24.20, 60.37, 61.43, 61.63, 63.57, 72.89, 76.19, 87.15, 119.49, 127.26, 127.93, 128.65, 143.26, 159.74, 167.32, 169.18.

4.1.21. Assay of DD-carboxypeptidase activity. The enzyme activity was measured as described previously.[16,17] Samples for the 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. Standard sample contained 20 μL of D-alanine in distilled water.

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 μL of 5 mg/mL *o*-dianisidine in methanol, and 2 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 min at 37 °C and then boiled for 2 min. After cooling, 77 μL of the reaction mixture was added, and all samples were incubated for 10 min at 37 °C. Next, to each sample 350 μL of mixture consisting of methanol, distilled water, and sulfuric acid (5:5:6 by volume) were added. Extinction of the resulting solution was measured at 540 nm.

The inhibition of DD-peptidase 64-575 by the oxacephams discussed above was evaluated.[17,18] Mixtures of 10 μL of DD-peptidase 64-575 (40 units/mg), 5 μL solution of an oxacepham in methanol, and 5 μL of 0.1 M phosphate buffer, pH 8.0 were incubated for 45 min at 37 °C. The concentration of a cepham in the mixture was from 2.3×10^{-2} to 1.3×10^{-5} M. Following the incubation, 20 μL of substrate solution was added to 20 μL of each sample and resulted mixtures were incubated again.

The inhibition of penicillinase was evaluated following the literature method.[19] 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 and 10 μL solution of oxacephams in methanol. The samples were incubated for 30 min at 37 °C. Then 30 mL of nitrocephin and 430 μL, 0.1 M phosphate buffer pH 7.0 were added, and all the samples were incubated for 10 min at 37 °C. Absorption was measured at 482 nm.

The following oxacephams were tested for both activities: **17**, **18**, **56**, and **63**.

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Supplementary data

Supplementary data including spectral and analytical data for compounds **21**–**25** and **31**–**34** are available on the www under http://www.sciencedirect.com or from the authors. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.08.074.

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