JOC Notes

Oppolzer Sultam Directed Aldol as a Key Step for the Stereoselective Syntheses of Antitumor Antibiotic Belactosin C and Its Synthetic Congeners†,‡

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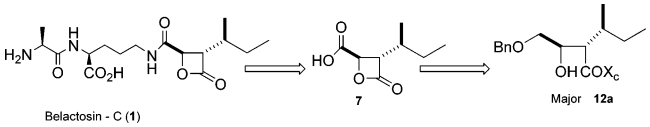


An efficient protocol has been developed using D-(2*R*)-Oppolzer sultam as a chiral auxiliary for generating anti/syn diastereomers with high enantiopurity and utilized in the efficient synthesis of natural product belactosin C and their synthetic congeners. It has been observed that a variation in the stoichiometry of the Lewis acid led to a difference in anti/syn selectivity.

Small molecules that target the 20S proteosome inhibition appear to be an apt choice for cancer chemotherapy.[1] Lactacystin, a *Steptomyces* metabolite isolated by Omura et al., is an irreversible, covalent inhibitor of the chymotrypsin-like and trypsin-like activity and a weak, reversible inhibitor of the PGPH activity of the 20S proteasome.[2] A related compound of class PS-519 is currently in preclinical development for the treatment of ischemia−reperfusion injury in stroke and myocardial infarction.[3] Recently, Asai et al. identified belactosin A and C molecules that inhibits the 20S proteasome in vitro (IC = 0.4 μM, chymotrypsin-like activity)[4] in a yeast-based assay of *Steptomyces* metabolite (Figure 1). Initial studies of the belactosins revealed that this could be a good lead compound for

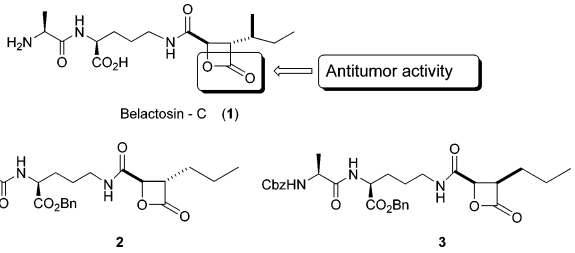
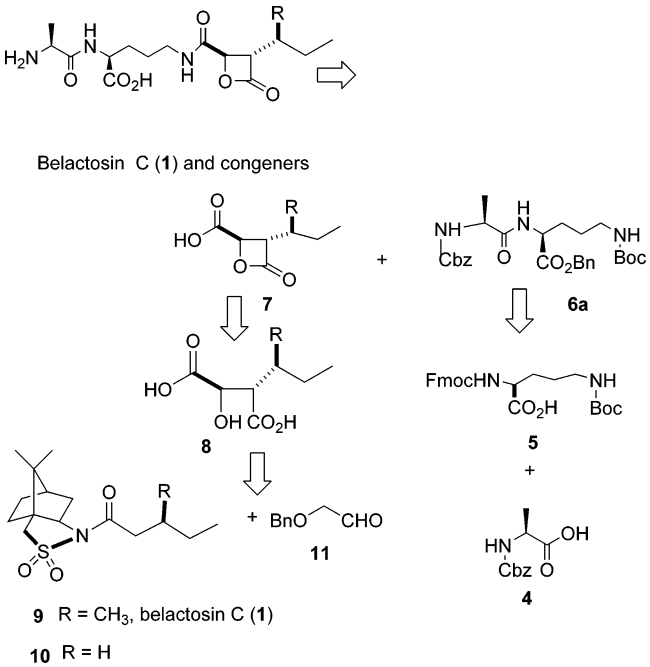


FIGURE 1. Belactosin C (**1**) and its congeners **2** and **3**.

SCHEME 1. Retrosynthesis of Belactosin C and Congeners



cancer by regulating the ubiquitin−proteosome pathways.[5] Interestingly, both belactosins A and C (**1**) exhibit inhibitory activity comparable to that of lactacystin. In addition, the degradation studies suggested the β-lactone moiety to be responsible for antiproliferative activity. Two impressive total syntheses of belactosins A and C (**1**) and their homoanalogues have since been reported.[6]

To broaden the therapeutical value and also to understand the mode of action, we initiated a synthetic program to prepare new variants of this unique natural product, belactosin C (**1**). Since the β-lactone moiety is pivotal for bioactivity, we set out to investigate the influence of the relative stereochemistry of β-lactone ring and stereochemistry adjacent to the β-lactone on the anti cancer activity.

In this paper, we describe the synthesis of belactosin C (**1**) and their variants **2** and **3** (Scheme 1) based on Oppolzer sultam-

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‡ Dedicated to Dr. Ganesh Pandey on occasion of his 51st birthday.

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⊥ NMR Division.

(1) Garcia-Echeverria, *C Mini Rev. Med. Chem.* **2002**, 247.

(2) (a) Omura, S.; Fujimoto, T.; Otoguru, K.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H. *J. Antibiot.* **1991**, *44*, 113. (b) Omura, S.; Matsuzaki, K.; Fujimoto, T.; Kosuge, K.; Furuya, T.; Fujita, S.; Nakagawa, A. *J. Antibiot.* **1991**, *44*, 117.

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(6) (a) Armstrong, A.; Scutt, J. N.; *Chem. Commun.* **2004**, 510−511. (b) Larionov, O. V.; de Meijere, A. *Org. Lett.* **2004**, *6*, 2153−2156.

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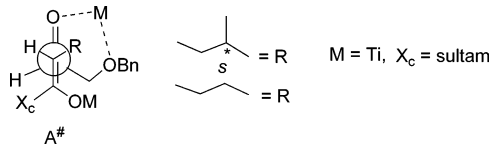
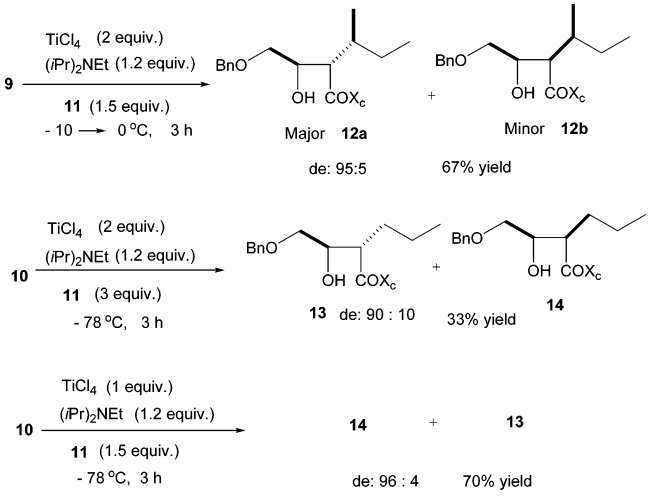


FIGURE 2. Proposed transition state for aldolization.

SCHEME 2. Synthesis of Aldol Products 12a, 13, and 14



directed asymmetric aldol reaction as a key step to obtain three diastereo pure β-lactones **16**, **18**, and **21**. The study was initiated by the treatment of a cold (−78 °C) solution of acylsultam **9**[7] with 1 equiv of $TiCl_4$ and 1.2 equiv of diisopropylethylamine followed by addition of aldehyde **11**.

Surprisingly, after workup, only starting material was recovered. Also, the same reaction conducted at an elevated temperature (−45 °C) did not yield any trace of aldol product, while the reaction carried out between −10 to 0 °C for 3 h afforded a product in moderate yield and 70:30 diastereoselectivity in favor of **12a** (Scheme 2). The major and minor aldol products were readily separated by flash column chromatography on silica gel. Using 2 equiv of $TiCl_4$ under otherwise identical conditions, the anti isomer **12a** was obtained exclusively (anti/syn >95:<5, 67% yield). The stereochemical assignments to major and minor products were unambiguously confirmed by X-ray crystallography[8] (Figure 3). Interestingly, contrary to the expected syn selectivity,[9] the major isomer with absolute configuration of 1′*R*,2*S* was found to result from an anti-selective aldol reaction.

To check the influence of the resident stereogenic center in **9** (i.e., Me) on the observed sense of stereoselectivity, we treated **10** (lacking the methyl group) with 2 equiv of $TiCl_4$ and 1.2 equiv of diisopropylethylamine between −10 and 0 °C for 3 h followed by addition of aldehyde **11** to yield product as a 60:40 mixture of *anti*-**13**/*syn*-**14** isomers, respectively. The diasteroeoselectivity markedly improved when the same reaction was conducted at −78 °C, where the *anti*/*syn* products were formed in 90:10 ratio.[10] Treatment of the compound **10** with 1 equiv of $TiCl_4$, 1.2 equiv of Hünig's base at −78 °C followed by the aldehyde yielded **14** as sole product which was isolated by column chromatography as a crystalline solid (Table 1). The absolute stereochemistry of **14** was confirmed by X-ray crystallography (Figure 3) and was found to be in agreement with the expected *syn* selectivity (*syn*/*anti* >96:<4, 70% yield).[9b]

The observed *anti* selectivity in the reaction of **9** or **10** with aldehyde **11** can be explained by invoking open transition state A# as proposed by Oppolzer et. al (Figure 2).[11−13]

Proceeding ahead toward the synthesis of belactosin C (**1**), attempted hydrolysis of **12a** under basic hydroperoxide conditions led to the recovery of starting material. Consequently, a

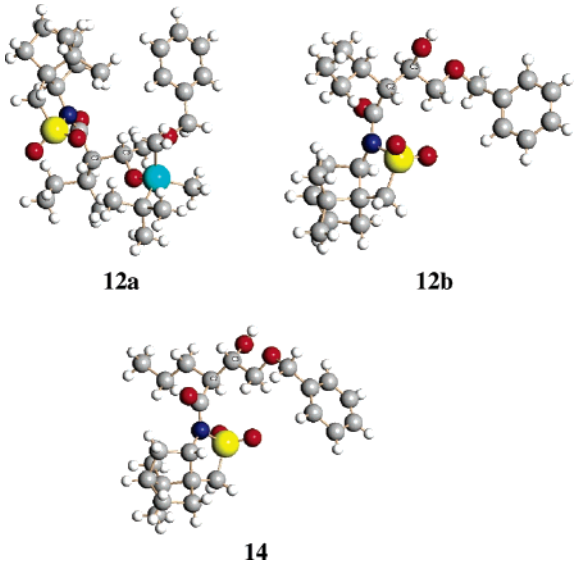


FIGURE 3. X-ray crystal structure of **12a**, **12b**, and **14**.

TABLE 1. Dependence of Diasteroselectivity on the Stoichiometry of Lewis Acid

entry	substrate	*T* (°C)	$TiCl_4$ (equiv)	aldehyde (equiv)	de (anti/syn)	% yield
1	**9**	−10	1	1.5	70:30	55
2	**9**	−10	2	1.5	95:5	67
3	**10**	−10	2	1.5	60:40	15
4	**10**	−78	2	3[a]	90:10	33
5	**10**	−78	1	1.5	4:96	70

[a] Use of 3 equiv of aldehyde increased the product yield.

(7) The acyl-sultam prepared in two steps in overall yield 80% (a) Michael, R.; Ofer, S.; Volker, S.; Meir, B.; Boris, Y. *Tetrahedron: Asymmetry* **1999**, *10*, 841−853. (b) Ashot, K.; Armenak, Kh. M.; Walter, F. S. *Tetrahedron* **2003**, *59*, 5475−5480.

(8) The major diastereomer **12a** was crystallized as silyl ether. The minor distereomer **12b** initially isolated as liquid but slowly it was crystallized out after a period of one month. The absolute stereochemistry 1′*R*,2*R* was assigned.

(9) (a) Oppolzer, W.; Blagg, J.; Rodriguez.; Walther, E. *J. Am. Chem. Soc.* **1990**, *112*, 2167−2172. (b) Evans, D. A.; Rieger, D. L.; Bilodeau, Bilodeau, M. T.; Urpi, F. *J. Am. Chem. Soc.* **1991**, *113*, 1047−1049. (c) Evans, D. A.; Tedrow, J. S.; Shaw, J. T.; Downey, C. W. *J. Am. Chem. Soc.* **2002**, *124*, 392−393. (d) Crimmins, M. T.; King, B. W.; Tabet, E. A. *J. Am. Chem. Soc.* **1997**, *119*, 7883. (e) Ghosh, A. K.; Liu, C. *J. Am. Chem. Soc.* **2003**, *125*, 2374−2375.

(10) The anti/syn ratio was estimated by ^{1}H NMR spectrum of crude product. Our attempt to obtain a crystal of **13** was not successful. The absolute stereochemistry was assigned by analogy of **12a**.

(11) Oppolzer, W.; Lienard, P. *Tetrahedron Lett.* **1993**, *34*, 4321−4324.

(12) When compared to the chiral amide **9**, the achiral amide **10** undergoes aldolization at lower temperature (−78 °C), probably due to its greater reactivity. The *N*, *O*-Ketone acetal derived from silylation of the enolate of **9** with TBSCl failed to yield the aldol product upon reaction with a mixture of aldehyde **11** and $TiCl_4$.[13]

(13) (a) Walker, M. A.; Heathcock. C. H. *J. Org. Chem.* **1991**, *56*, 5747. (b) Oppolzer, W.; Christian, S.; Ines, R.; Bernardinelli, G. *Tetrahedron Lett.* **1991**, *32*, 61−64. (c). Ghosh. A. K.; Onishi, M. *J. Am. Chem. Soc.* **1996**, *118*, 2527−2528.

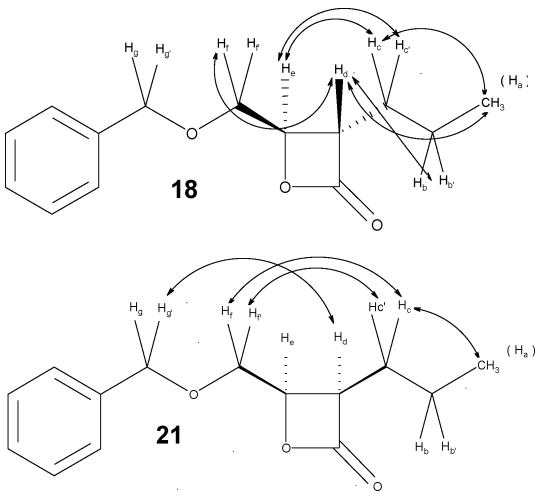


FIGURE 4. NOE studies of **18** and **21**.

reduction/oxidation strategy was adopted. Reduction of **12a** with LAH to alcohol **15** followed by a two-step oxidation afforded **8** in 40% yield. On the other hand, compounds **13** and **14** were smoothly hydrolyzed to the corresponding β-hydroxy acids **17** and **20**. Despite a plethora of methods available for lactonization,[14] we met success only with BOPCl/Et_3N. Thus, lactonization of **8**, **17**, and **20** afforded **16**, **18** and **21** respectively. The presence of strong NOE between H_f and H_f' and H_c and H_c' for compound **21** confirmed syn stereochemistry, whereas the absence of the NOE between the same protons in the compound **18** and between protons H_d and H_e (Figure 4) supports anti stereochemistry (see the Suporting Information).

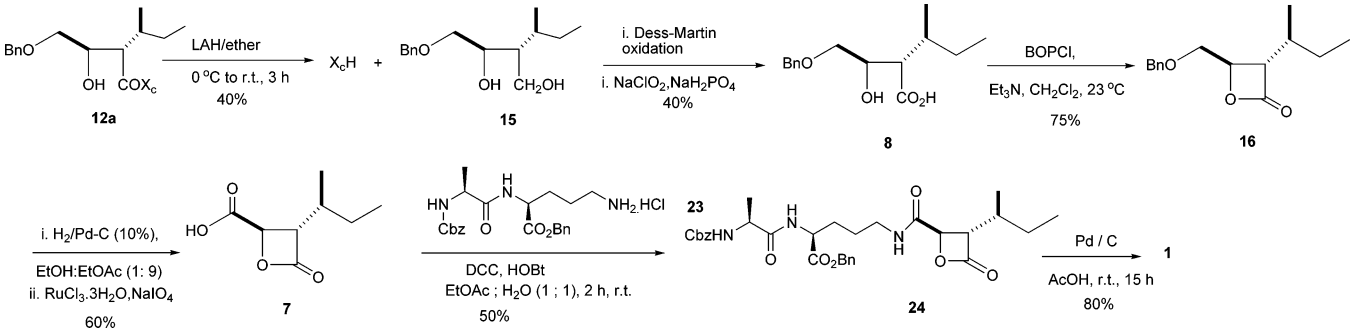
The lactones **16**, **18**, and **21** were subjected to hydrogenolysis followed by a one-step oxidation to afford β-lactone carboxylic acids **7**, **19**, and **22,** respectively. Attempted coupling of **7**, **19**, and **22** with dipeptide **23**[15] using the reported conditions[6a] suffered from low yields. After considerable experimentation, we developed a procedure that furnished protected belactosin derivatives **24**, **2**, and **3** in moderate isolated yields (Schemes 3 and 4).[16] The analytical data of compound **24** were in full agreement with the reported data.[6b] Finally, the compound **24** was subjected to hydrogenolysis followed by filtration and removal of water with lyophilizer to give the product **1** in 20% overall yield from β-lactone **16**. The analytical data of **1** were in full agreement with reported data of natural product.[4]

In conclusion, an efficient method was developed using D-(2*R*)-sultam as a chiral auxiliary for generating *anti* and *syn* diastereomers with high enantiopurity, which facilitated the synthesis of natural product belactosin C (**1**) and synthetic congeners **2** and **3**. This process is significant in that the Lewis acid here induces different chirogenicity with variation of stoichiometry. Further work is in progress to prepare various analogues and evaluate for the anti cancer activity.

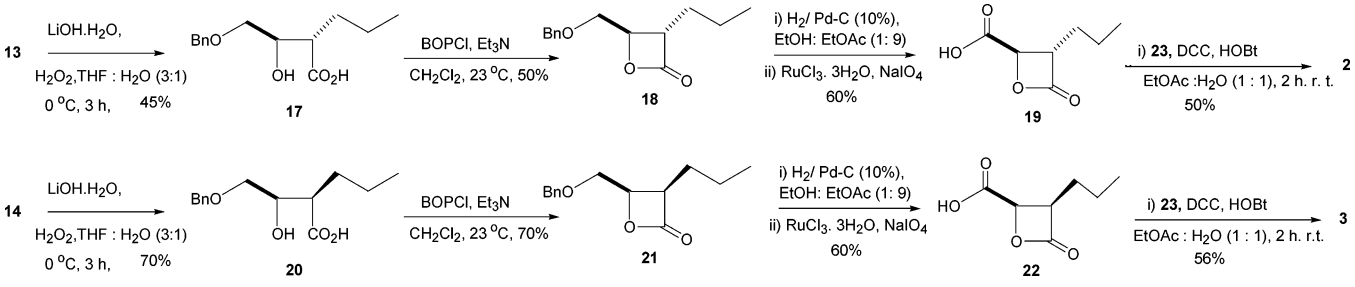
Experimental Section

***N*-[(2*S*,3*S*-Methyl)-2-[(2′-benzyloxy-1′*R*-hydroxy)ethyl]pentanoyl]borane-10,2-sultam (12a).** $TiCl_4$ (24.3 g or 14.0 mL, 128 mmol) was added dropwise to a 0.2 M solution of **9** (20.0 g, 63.9 mmol) in CH_2Cl_2 at −10 °C under nitrogen atmosphere resulting in a yellow slurry. To this was added diisopropylethylamine (13.4 mL, 76.7 mmol) dropwise, and the resulting deep red solution was stirred maintaining the temperature between −10 and 0 °C. After 45 min, aldehyde **11** (13.4 mL, 95.8 mmol) was added dropwise, and stirring was continued for a further 3 h at 0 °C. The reaction was terminated by addition of 1:1 (v/v) saturated aq NH_4Cl solution, and the reaction mixture was warmed to ambient temperature. The contents were diluted with water and extracted with organic solvent. The combined organic layers were evaporated and dried. The residue was purified by column chromatography to give the aldol product **12a** as white solid. Further, the product was crystallized from EtOH (19.8 g, 67%,): $[\alpha]^{25}_D$ −61.5 (*c* 1, $CHCl_3$); ^{1}H NMR (200 MHz) δ 0.88 (3H, t, J = 7.4 Hz), 0.90 (3H, s), 1.00 (3H, d, J = 6.7 Hz), 1.08 (3H, s), 1.20−1.41 (4H, m), 1.72−2.11(6H, m), 2.90−3.02 (1H, m), 3.40−3.50 (3H, m), 3.57 (1H, m), 3.85 (1H, t, J = 7.4 Hz), 4.05 (1H, m), 4.49 (1H, d, J = 11.8), 4.58 (1H, d, J = 11.8), 7.20−7.41 (5H, m); ^{13}C NMR (75 MHz) δ 12.4, 16.0,

SCHEME 3. Synthesis of Belactosin C



SCHEME 4. Synthesis of Belactosin Congeners 2 and 3



19.9, 19.9, 20.7, 26.4, 27.4, 33.0, 35.1, 38.6, 44.6, 47.9, 52.8, 53.3, 65.6, 70.7, 73.3, 127.6, 138.0, 173.9; IR (KBr) 3521, 3091, 2910, 2961, 1673, 1453, 1394, 1317, 1220, 1131, 1065, 736, 542, 467 cm^{-1}; LSIMS (FAB) *m*/*z* 464 ($M + H^+$), 306 (71), 249 (100), 216 (40), 154 (92), 136 (90), 107 (52); HRMS ($M + Na^+$) calcd for $C_{25}H_{37}NO_5NaS$ 486.2290, found 486.2286. (For isomers **13** and **14**, see the Supporting Information.)

Typical Procedure for the Peptide Coupling 5: (2*S*)-[(2*S*)-Benzyloxycarbonylaminopropionylamino]-5-{[(3*S*)-((1*S*)-methylpropyl)-4-oxo-oxetane-(2*R*)-carbonyl]amino}pentanoic Acid Benzyl Ester (24). To a solution of **23** (1.16 g, 2.50 mmol) in H_2O/EtOAc (1:1; 7.3 mL) were added sequentially DCC (1.24 g, 6.01 mmol), HOBT (810 mg, 6.00 mmol), and acid **7** (500 mg, 2.90 mmol) under nitrogen atmosphere at rt. After 2 h, the reaction mixture was filtered and washed with EtOAc (5 mL). The aqueous layer was separated, and the organic layer was washed with 1 M aq NaOH solution (2 × 20 mL) followed by saturated aq $NaHCO_3$ solution (2 × 20 mL). The organic layer was concentrated under reduced pressure. The residue was purified by column chromatography to give **24** as a solid (840 mg, 50%): $[\alpha]^{25}_D$ +3.0 (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz) δ 0.94 (3H, t, J = 7.5 Hz), 1.06 (3H, d, J = 7.5 Hz), 1.18−1.35 (1H, m), 1.37 (3H, d, J = 7.5 Hz), 1.4−1.5 (2H, m), 1.5−1.7 (2H, m), 1.8−2.0 (2H, m), 3.07−3.35 (2H, m), 3.56 (1H, dd, J = 4.5, 7.5 Hz), 4.21−4.39 (1H, m), 4.53 (1H, d, J = 4.5 Hz), 4.54−4.64 (1H, m), 5.08 (2H, s), 5.15 (2H, d, J = 8.3 Hz), 5.60 (1H, d, J = 7.5 Hz), 6.73 (1H, brs), 6.95 (1H, d, J = 7.5 Hz), 7.26−7.38 (10H, m); ^{13}C NMR (75 MHz) δ 10.9, 16.3, 18.4, 25.2, 26.6, 29.3, 33.8, 38.4, 50.5, 51.8, 62.9, 67.1, 67.4, 70.8, 128.0, 128.2, 128.4, 128.6, 128.5, 128.6, 135.1, 136.2, 156.2, 168.2, 169.1, 171.6, 172.4; IR (KBr) 3317, 3066, 2963, 2931, 1835, 1727, 1666, 1536, 1455, 1250, 1101, 908, 746, 698 cm^{-1}; LSIMS (FAB) *m*/*z* 582 ($M + H^+$), 181(10), 154 (15), 136 (18), 109 (21), 91 (100), 81(40), 69 (61), 55 (80); HRMS [$M + H^+$] calcd for $C_{31}H_{40}N_3O_8$ 582.2815, found 582.2818.

(2*S*)-[(2*S*)-Benzyloxycarbonylaminopropionylamino]-5-{[(3*S*)-propyl)-4-oxo-oxetane-(2*R*)-carbonyl]amino}pentanoic Acid Benzyl Ester (2). This compound was prepared using the above typical procedure **5**: yield (900 mg, 50%); $[\alpha]^{25}_D$ −3.88 (*c* 1, $CHCl_3$); ^{1}H NMR (200 MHz) δ 0.92 (3H, t, J = 7.2 Hz), 1.35 (3H, d, J = 7.2 Hz), 1.42−1.7 (4H, m), 1.7−1.9 (4H, m), 3.1−3.35 (2H, m), 3.55−3.65 (1H, m), 4.25−4.35 (1H, m), 4.45 (1H, d, J = 4.0 Hz), 4.52−4.65 (1H, m), 5.09 (2H, s), 5.2 (2H, d, J = 4.8 Hz), 5.51(1H, d, J = 7.2 Hz), 6.75(1H, brs), 6.95 (1H, d, J = 7.2 Hz), 7.26−7.4 (10H, m); ^{13}C NMR (75 MHz) δ 13.5, 18.4, 19.9, 25.2, 29.3, 30.0, 38.4, 50.5, 51.8, 57.5, 67.0, 67.4, 72.9, 128.0, 128.2, 128.4, 128.5, 128.6, 128.7, 135.1, 136.2, 156.1, 167.9, 169.5, 171.6, 172.4; IR (KBr) 3317, 2965, 1837, 1736, 1654, 1531, 1453, 1248, 1188, 11189, 746, 698 cm^{-1}; HRMS [$M + H^+$] cacld for $C_{30}H_{38}N_3O_8$ 568.2658, found 568.2660.

(2*S*)-[(2*S*)-Benzyloxycarbonylaminopropionylamino]-5-{[(3*R*)-propyl-4-oxo-oxetane-(2*R*)-carbonyl]amino}pentanoic Acid Benzyl Ester (3). This compound was prepared using the above typical procedure **5**: yield (1.0 g, 56%); $[\alpha]^{25}_D$ +21 (*c* 1,$CHCl_3$); ^{1}H NMR (200 MHz) δ 0.9 (3H, t, J = 6.95 Hz), 1.35 (3H, d, J = 6.95 Hz), 1.42−1.7 (6H, m), 1.70−1.91 (2H, m), 3.11−3.40 (2H, m), 3.85−3.88 (1H, m), 4.28−4.29 (1H, m), 4.60 (1H, m), 4.80 (1H, d, J = 6.95 Hz), 5.1 (2H, s), 5.15 (2H, d, J = 4.6 Hz), 5.51 (1H, d, J = 6.95 Hz), 6.80 (1H, brs), 6.90 (1H, d, J = 8.5 Hz), 7.26−7.41 (10H, m); ^{13}C NMR (50 MHz) δ 13.5, 18.4, 19.9, 25.2, 27.1, 29.3, 38.4, 50.5, 51.8, 57.5, 67.0, 67.4, 72.9, 128.0, 128.2, 128.3, 128.5, 128.6, 128.7, 135.1, 136.2, 156.1, 167.9, 169.5, 171.6, 172.0; IR (KBr) 3311, 2965, 1837, 1736, 1654, 1531, 1453, 1248, 1188, 11189 746, 698 cm^{-1}; LSIMS (FAB) *m*/*z* 568 ($M + H^+$), 154 (30), 91(95), 69 (69), 55 (100); HRMS [$M + H^+$] calcd for $C_{30}H_{38}N_3O_8$ 568.2658, found 568.2682.

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Supporting Information Available: Experimental details and analytical data of all compounds as well as crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0516887

(14) (a) Corey, E. J.; Li, W.; Reichard. G. A. *J. Am. Chem. Soc*. **1998**, *120*, 2330−2336. (b) Among surveyed reagents used for this transformation, only $PhSO_2Cl$/pyridine gave the product but in poor yields.

(15) For preparation of dipeptide **23**, see the Supporting Information.

(16) Synthetic analogues **3**, **4**, and **24**, which were intended to be applied for testing against cancer cell lines, were not deprotected; belactosin C itself has greater activity in protected form.[5]