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The Structural Requirements for Inhibition of Proteasome Function by the Lactacystin-Derived β-Lactone and Synthetic Analogs

E. J. Corey,* Wei-Dong Z. Li, Tohru Nagamitsu, and Gabriel Fenteany

Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138

Dedicated to the memory of the late Professor Derek H. R. Barton.

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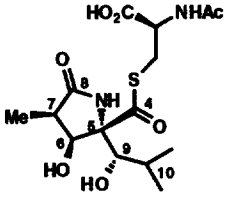
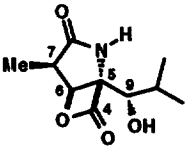
Abstract: *The synthesis of analogs of the lactacystin-derived β-lactone (2) in which the substituents at C(5), C(7) and C(9) were systematically varied has led to a well defined structure-activity correlation for the highly selective inhibition of the mammalian 20 S proteasome.*

Lactacystin (**1**), a microbial product, first isolated by Ōmura et al.,[1,2] was originally obtained from a screening effort based on a nerve growth factor (NGF) -like capacity to induce neurite sprouting in a mouse neuroblastoma cell line (Neuro-2A) and considered to be a mimic of NGF.[1,2] However, more recent work has shown this not to be the case.[3,4] Instead, lactacystin has been found to act with exquisite specificity and in a unique fashion to inhibit irreversibly the proteolytic activity of the 20 S proteasome, a cylindrical complex of 28 protein subunits which is responsible for the hydrolytic fragmentation of ubiquitinated proteins. The thiol ester function of lactacystin is sufficiently reactive to allow spontaneous conversion to the β-lactone **2**[5] which similarly deactivates the 20 S proteasome, *but at a much faster rate.*[4,5] The major source of inactivation of the 20 S proteasome appears to be the acylation of the *N*-terminal threonine subunit, a key participant in proteolytic catalysis,[3,4] to form inactivated proteasome. This result was confirmed by X-ray crystallographic studies of the lactacystin inactivated 20 S proteasome at 2.4 Å resolution.[6,7] Because the proteasome machinery is involved in the degradation of many proteins, including not only misfolded and denatured molecules[8] but also proteins involved in cell cycle progression[9] and regulation of gene transcription,[10] lactacystin has emerged as a very important new tool for the study of protein biochemistry and cell biology.[11] Lactacystin prepared by the route of the first total synthesis[5,12,13] has been used in several hundred biological laboratories. The structural and biological uniqueness of **1** has stimulated several additional syntheses by a variety of multistep processes.[14-16]

Lactacystin and β-lactone **2** are remarkable because they exemplify dramatically the ability of a small molecule to shut down the functioning of a very large poly-macromolecular machine and to exert this inhibition with great selectivity on the 20 S proteasome in the presence of countless other proteins as potential targets. This paper reports studies on the molecular basis of the very high selectivity and potency of **2** in this regard.



***Lactacystin* (1)**



2

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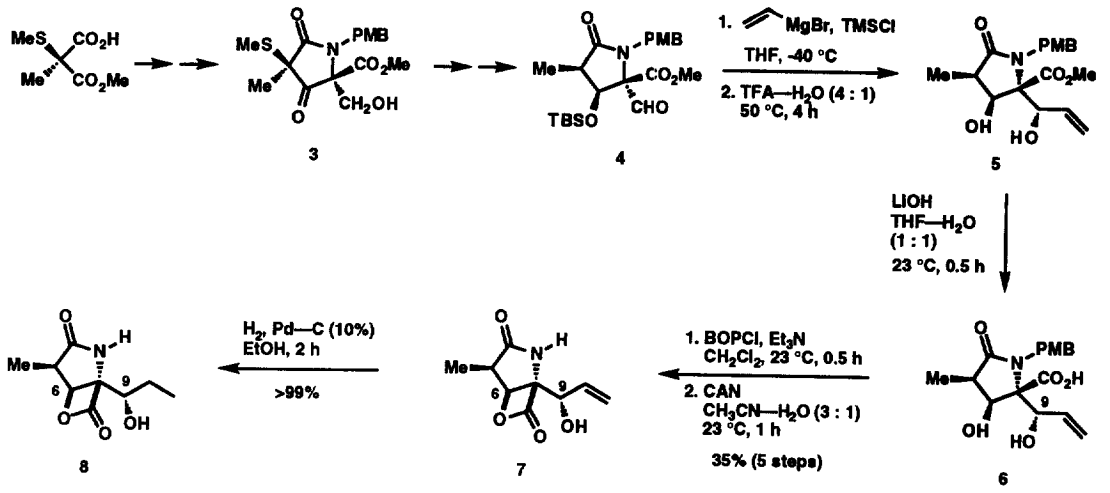
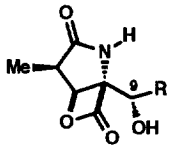
Scheme 1



Knowledge of the structural requirements for inhibition by **2** or its structural analogs might also be of value in the design of proteasome inhibitors which are species specific, e.g. bacterial or protozoal vs mammalian. As a first step toward these objectives we recently developed a new enantioselective synthesis of **1** and **2** designed to allow the introduction of the C(9) substituent at the end of the synthesis and thereby to facilitate the preparation of analogs of **1** and **2** in which the isopropyl subunit at C(9) is replaced by other lipophilic groups.[17] It is apparent from the X-ray crystal structure of the lactacystin inactivated 20 S proteasome that the isopropyl group of **1** and **2** fits into a lipophilic pocket,[7] the exact dimensions of which are not clear. The key late intermediate **3** in the recently developed synthesis of **2**,[17] was used for the synthesis of the C(9)-vinyl and C(9)-ethyl analogs of β-lactone **2** for in vitro studies of 20 S proteasome inactivation as shown in Scheme 1. Addition of vinylmagnesium bromide to a solution of **4** and trimethylchlorosilane in THF at -40 °C afforded diastereoselectively after desilylation the allylic alcohol **5**. (For proof of stereochemistry at C(9), see experimental section.) The presence of trimethylchlorosilane during the reaction of aldehyde **4** with the Grignard reagent was essential to prevent retroaldol cleavage of the alkoxide of **5** in the reaction mixture.[17] Dihydroxy ester **5** was converted to the vinyl substituted β-lactone **7** by the sequence: (1) alkaline ester hydrolysis to form the dihydroxy acid **6**, (2) β-lactonization and (3) *N*-deprotection.[18] Hydrogenation of **7** afforded the corresponding 9-ethyl analog (**8**) of β-lactone **2**. The C(9) primary alcohol-β-lactone (**9**, Table 1) was synthesized directly from the primary alcohol corresponding to aldehyde **4**[17] by ester hydrolysis, β-lactonization and *N*-deprotection. The 9-phenyl analog (**10**) was synthesized by a route analogous to that used for **7**. 9-Allyl and 9-methallyl analogs (**11** and **12**, respectively) were prepared by a chromium-catalyzed[19] addition of allyl or methallyl bromide to aldehyde **4a** as shown in Scheme 2. Catalytic hydrogenation of **11** and **12** afforded the C(9)-propyl (**13**) and C(9)-isobutyl (**14**) analogs of β-lactone **2** (formulas shown in Table 1).

The effectiveness of the above described analogs of β-lactone **2**, β-lactones **7** - **14**, as irreversible inhibitors of the 20 S proteasome was determined by measuring the rates of inhibition of chymotrypsin-like peptidase activity (as previously described[3]), with the results summarized in Table 1. Clearly the most active compound is β-lactone **2**, corresponding to natural lactacystin. Replacement of the C(9)-isopropyl group of **2** by hydrogen (as in **9**) greatly reduces activity and substitution by phenyl (as in **10**) abolishes proteasome inhibition.

Table 1. Kinetics of inhibition of the chymotrypsin-like peptidase activity of purified 20 S proteasome (from bovine brain[3]) by β-lactone analogs of lactacystin: $k_{assoc} = k_{obs}/[I]$ ($M^{-1}s^{-1}$). (triplicate runs)

Analog Structure	k_{assoc} ($M^{-1}s^{-1}$)
	
R = $CH(CH_3)_2$ (**2**)	3059 ± 478
R = H (**9**)	9.7 ± 6.2
R = C_6H_5 (**10**)	no inhibition
R = C_2H_5 (**8**)	290 ± 12
R = $CH=CH_2$ (**7**)	188 ± 11
R = $CH_2CH_2CH_3$ (**13**)	192 ± 35
R = $CH_2CH=CH_2$ (**11**)	255 ± 40
R = $CH_2CH(CH_3)_2$ (**14**)	17.4 ± 2.4
R = $CH_2C(CH_3)=CH_2$ (**12**)	64.7 ± 2.2

Similarly, activity relative to **2** is greatly diminished with C(9) substituents which are either slightly smaller than isopropyl (e.g. ethyl (**8**) or vinyl (**7**)) or larger than isopropyl (e.g. allyl (**11**), *n*-propyl (**13**), methallyl (**12**) or isobutyl (**14**)). Thus, it seems clear that the C(9)-isopropyl substituent of the natural products **1** and **2** is optimal for mammalian proteasome inhibition, implying a fairly snug fit for isopropyl in the complementary binding pocket of the proteasome.

Previous biological studies in this Laboratory established the C(5)-α-hydroxyisobutyl sidechain for bioactivity.[4a] In addition, it was shown that there must be a hydroxyl group at C(6) cis to the C(4)-carboxylic group (i.e. suitably placed for β-lactone formation); 6-desoxy- and 6(*R*)-hydroxy analogs of lactacystin were completely inactive in proteasome inhibition. Similar studies were carried out with regard to the C(9)-hydroxyl

Scheme 2

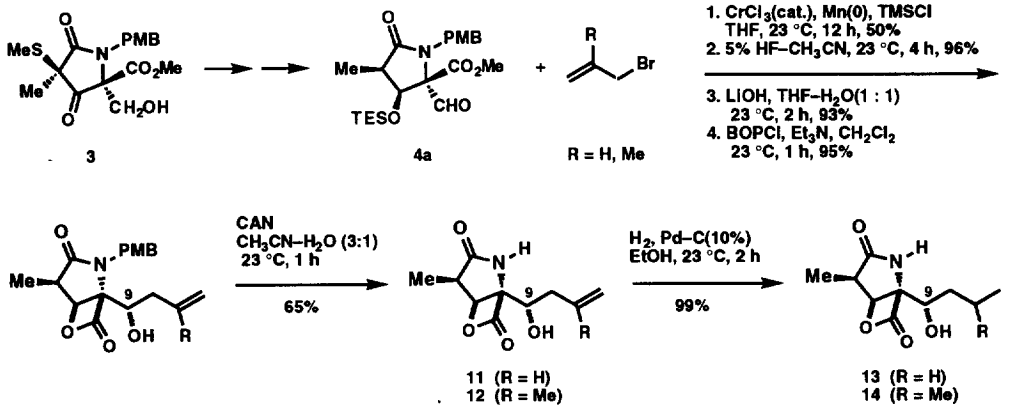
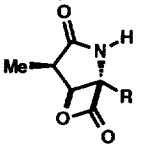
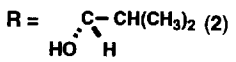
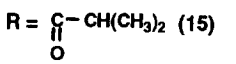
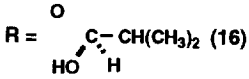
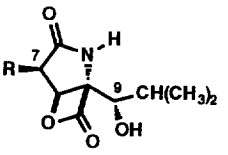


Table 2. Kinetics of inhibition of the chymotrypsin-like peptidase activity of purified 20 S proteasome (from bovine brain) by β-lactone analogs of lactacystin: $k_{assoc} = k_{obs}/[I]$ ($M^{-1}s^{-1}$). (triplicate runs)

Analog Structure	k_{assoc} ($M^{-1}s^{-1}$)
	
R =  (2)	3059 ± 478
R =  (15)	no inhibition
R =  (16)	65 ± 9.6
R = $CH_2-CH(CH_3)_2$ (17)	235 ± 16
R = $CH=C(CH_3)_2$ (18)	98 ± 5

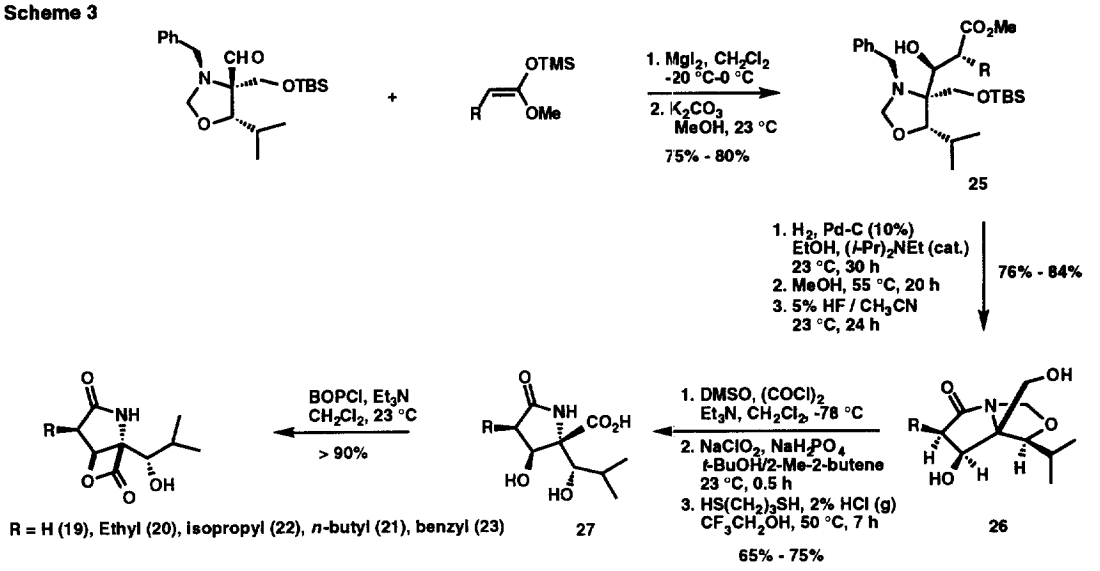
function as summarized in Table 2. Analogs **15** and **16** were prepared as previously described.[17] Analog **17** was prepared by catalytic hydrogenation of **18** (H_2, Pd–C) which was synthesized from aldehyde **4** by the sequence (1) Wittig reaction with $(CH_3)_2C=PPh_3$, (2) ester hydrolysis, (3) β-lactonization, and (4) *N*-deprotection, essentially using the methodology outlined in Scheme 1. Comparison of the data in Table 2 reveals that the functionality and configuration at C(9) of **2** are critical to proteasome inhibition and that the lactacystin derived β-lactone **2** is optimal.

Table 3. Kinetics of inhibition of the chymotrypsin-like peptidase activity of purified 20 S proteasome (from bovine brain) by β-lactone analogs of lactacystin: $k_{assoc} = k_{obs}/[I]$ ($M^{-1}s^{-1}$). (triplicate runs)

Analog Structure	k_{assoc} ($M^{-1}s^{-1}$)
	
R = CH_3 (2)	3059 ± 478
R = H (19)	450 ± 77
R = CH_3CH_2 (20)	6679 ± 553
R = $CH_3(CH_2)_3$ (21)	7275 ± 466
R = $(CH_3)_2CH$ (22)	8465 ± 1572
R = $C_6H_5CH_2$ (23)	2227 ± 180
7-*epi*-**2** (24)	1250 ± 66

We have also investigated the relationship between the nature of the substitution at C(7) in analogs of β-lactone **2** and ability to inactivate the mammalian 20 S proteasome. The analogs which were prepared and tested (**19** - **24**) are shown in Table 3. The synthesis of these compounds was accomplished by the recently described modification[13] of our original synthetic route[12] to **1** and **2**, as summarized in Scheme 3. The data in Table 3 indicate that whereas the replacement of the C(7)-methyl group of the β-lactone **2** derived from lactacystin by the smaller hydrogen leads to reduced activity (**19**), replacement of the C(7) methyl group by the larger substituents ethyl (**20**), *n*-butyl (**21**), or isopropyl (**22**) results in an approximate doubling of the proteasome inhibitory activity relative to **2**. The C(7)-diastereomer of **2** (**24**)[20] and the C(7)-benzyl analog (**23**) are somewhat less active, but still potent. Thus, in the case of the C(7)-substituent the activity of the natural lactacystin-derived β-lactone **2**, while high, is not quite optimum.

Scheme 3



Discussion of Results

In vitro experiments with radiolabelled lactacystin in whole cells have shown that almost all the radioactivity becomes associated with the proteasome, demonstrating a remarkable specificity for that entity over a very large number of other proteins. In view of this selectivity, it is perhaps less surprising that the structure of lactacystin **1** and the corresponding β-lactone are nearly optimal for proteasome inhibition. The data in Table 1 emphasize that the isopropyl group at C(9) cannot be improved upon. In fact, the whole (*S*)-1-hydroxy isobutyl substituent at C(5) which contains that isopropyl group is also clearly optimal. The only suboptimal part of the lactacystin structure appears to be the substituent at C(7). The replacement of the 7-methyl substituent of lactacystin by isopropyl improves activity by a factor of *ca* 2.8. Taken together, the results on biological activity presented herein make it appear that further enhancements of the bioactivity of lactacystin derivatives are unlikely. If so, it is clear that Nature has nearly optimized the design of this small but potent and selective molecule, β-lactone **2**.

Experimental

General. All anhydrous solvents (THF, CH_2Cl_2, Et_2O, CH_3CN, Benzene) were dried by standard techniques and freshly distilled before use. All reactions were carried out under N_2 and monitored by TLC. All organic extracts were washed with brine before being dried over $MgSO_4$. All oily products were purified by flash silica gel chromatography (SGC) eluting with a solvent mixture of hexane and ethyl acetate. Melting points (mp) were determined using a Fisher-Johns hot stage apparatus and are uncorrected. Specific optical rotations were measured using a Perkin-Elmer 241 polarimeter at 23 °C with a sodium lamp (D line, 589 nm). FTIR spectra were recorded on a Nicolet 5 ZDX FTIR spectrometer. ^1H and ^{13}C NMR spectra were recorded on a *Brucker* AM series instrument. Mass spectral analyses and high resolution mass spectral analyses (HRMS) were measured on a JEOL model AX-505 or SX-102 spectrometer by either means of EI, CI or FAB. X-ray crystallographic data was collected using a Siemens SMART CCD (charge coupled device) based diffractometer equipped with an LT-2-low-temperature apparatus at 213 K. A suitable crystal (crystallized from ethyl acetate or a solvent mixture of EtOAc–Hexane) was chosen and mounted on a glass fiber using grease. Data was measured using omega scans of 0.3 ° per frame for 30 seconds, such that a hemisphere was collected. Cell parameters were retrieved using SMART software and refined using SAINT on all observed reflections. Data reduction was performed using SAINT software which corrects for Lp and decay. The structure was solved by direct methods using the SHELXS-90 program and refined by least squares method on F^2, SHELXL-93, incorporated in SHELXTL-IRIX V 5.03.

General procedure for the preparation of β-lactone analogs 7, 8 and 10 by diastereoselective Grignard addition of aldehyde 4.

To a stirred solution (0.1M) of aldehyde **4** and freshly distilled trimethylsilyl chloride (TMSCl, 5 eq) was added dropwise a solution of vinylmagnesium bromide or phenylmagnesium bromide (2.0 eq) at -50 °C. The resulting mixture was stirred at -50 °C to -40 °C for 0.5 h and quenched with sat. aqueous NH_4Cl solution. After extractive workup with ether (3 x), the combined extracts were washed with water, brine and dried, concentrated in vacuo. The resulting oil residue was purified by flash SGC to give corresponding Grignard adduct. **Vinyl Grignard adduct of aldehyde 4:** 93% yield, colorless oil; $[\alpha]_D^{23}$ -12 (*c* 0.05, $CHCl_3$); FTIR (film) ν_{max}: 3395, 1698, 1683, 1514, 1246 cm^{-1}; ^1H-NMR (500 MHz, $CDCl_3$) δ 0.05 [s, 3 H, $(CH_3)_3CSi(C\underline{H}_3)CH_3$], 0.08 [s, 3 H, $(CH_3)_3CSi(C\underline{H}_3)_2$], 0.84 [s,9 H, $(C\underline{H}_3)_3CSi(CH_3)CH_3$], 1.19 (d, *J* = 7.7 Hz, 3 H, 7-$C\underline{H}_3$), 2.59 (m, 1 H, 7-H), 3.43 (s, 3 H, CO_2CH_3), 3.78 (s, 3 H, $CH_2C_6H_4OC\underline{H}_3$), 4.32 (d, *J* = 15.0 Hz, 1 H, $C\underline{H}_2C_6H_4OCH_3$), 4.37 (d, *J* = 15.0 Hz, 1 H, $C\underline{H}_2C_6H_4OCH_3$), 4.60 (d, *J* = 9.2 Hz, 1 H, 6-H), 4.86 (bs, 1 H, C<u>H</u>OH), 5.23 (m, 1 H, C<u>H</u>=CH_2), 5.46 (m, 2 H, CH=C<u>H</u>$_2$), 6.81 (d, *J* = 8.6 Hz, 2 H, $CH_2C_6\underline{H}_4OCH_3$), 7.22 (d, *J* = 8.6 Hz, 2 H, $CH_2C_6\underline{H}_4OCH_3$) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 178.1, 173.0, 159.1, 133.0, 130.5, 128.7, 118.7, 113.8, 75.4, 70.8, 68.7, 55.3, 52.0, 45.1, 40.7, 25.7, 17.9, 11.4, -4.2, -4.9 *ppm*; HRMS (FAB, NBA+NaI) *m/z* calcd for $C_{24}H_{37}NO_6SiNa$ 486.2288, found for $[M+Na]^+$ 486.2292; **Phenyl Grignard adduct of 4:** 90% yield, colorless crystals; m.p. 173 °–174 °C; $[\alpha]_D^{23}$ +32.4 (*c* 0.25, $CHCl_3$); FTIR (film) ν_{max}: 3395, 1679, 1674, 1514, 1246 cm^{-1}; ^1H-NMR (400 MHz, $CDCl_3$) δ 0.06 [s, 3 H, $(CH_3)_3CSi(C\underline{H}_3)CH_3$], 1.12 [s, 3 H, $(CH_3)_3CSi(C\underline{H}_3)_2$], 0.84 [s,9 H, $(C\underline{H}_3)_3CSi(CH_3)CH_3$], 1.04 (d, *J* = 7.6 Hz, 3 H, 7-$C\underline{H}_3$), 1.58 (bs, 1 H, O<u>H</u>), 1.79 (m, 1 H, 7-H), 3.24 (s, 3 H, CO_2CH_3), 3.79 (s, 3 H, $CH_2C_6H_4OC\underline{H}_3$), 4.11 (d, *J* = 15.0 Hz, 1 H, $C\underline{H}_2C_6H_4OCH_3$), 4.70 (bs, 1 H, C<u>H</u>OH), 4.71 (d, *J* = 8.5 Hz, 1 H, 6-H), 4.84 (d, *J* = 15.0 Hz, 1 H, $C\underline{H}_2C_6H_4OCH_3$), 6.84 (d, *J* = 8.7 Hz, 2 H, $CH_2C_6\underline{H}_4OCH_3$), 7.26 (m, 7 H, $CH_2C_6\underline{H}_4OCH_3$,$C_6\underline{H}_5$) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 178.2, 173.3, 158.9, 137.5, 130.2, 129.4, 128.4, 127.0, 113.7, 76.6, 72.5, 69.3, 55.6, 51.9, 46.5, 40.4, 25.8, 18.0, 11.2, -4.3, -4.4 *ppm*; MS (FAB, NBA+NaI) HRMS *m/z* calcd for $C_{28}H_{39}NO_6SiNa$ 536.2444, found for $[M+Na]^+$ 536.2452; **Summary of X-ray crystal data**: $C_{28}H_{39}NO_6Si$; FW = 513.69; a = 9.3357(7) Å; b = 14.3713(11) Å; c = 21.781(2) Å; α = 90°; β = 90°; γ = 90°; Vol = 2922.3(4) $Å^3$; orthorhombic; $P2_12_12_1$; Z = 4; crystal size = 0.1 x 0.2 x 0.4 mm; GOF = 1.146; final R indices [$I>2\sigma(I)$], R_1 = 5.73%, wR_2 = 9.88%; R indices (all data), R_1 = 10.41%, wR_2 = 12.46%.[21]

β-Lactone 7. The vinyl Grignard adduct of aldehyde **4** was taken up into a mixture of trifluoroacetic acid and water (*v/v*, 4 : 1, 0.1 *M*) and heated to 50 ° to 60 °C for 4 h. The resulting reaction mixture was cooled to

0 °C and quenched with sat. aqueous $NaHCO_3$ solution and extracted with ethyl acetate (3 x). The combined organic layers were washed with brine, dried and concentrated in vacuo, without further purification, the resulting diol ester **5** was dissolved in a mixture of THF–H_2O containing LiOH (*v/v*, 1 : 1, 0.1 *M*) and was stirred for 1 h at 23 °C. The reaction mixture was neutralized and acidified with 2*N* HCl to pH 2–3 and extracted with ethyl acetate (4 x). The combined extracts were washed with brine, dried and concentrated in vacuo to give the dihydroxy acid **6** as a colorless oil, which was taken up into CH_2Cl_2 (0.1 *M*) and treated with triethylamine (3.0 eq) and followed by BOPCl(1.5 eq) at 23 °C. The resulting reaction mixture was stirred for 0.5 h and quenched with sat. aqueous $NaHCO_3$ solution and extracted with ethyl acetate (3 x). The combined extracts were washed with brine, dried and concentrated in vacuo. The residue was purified by flash SGC to give the *N-p*-methoxybenzyl (PNB) derivative of **7** (49% yield over 4 steps), as a colorless oil; $[\alpha]_D^{23}$ -83.3 (*c* 0.03, $CHCl_3$); FTIR (film) ν_{max}: 3412, 1836, 1692, 1514, 1248 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.34 (d, *J* = 7.5 Hz, 1 H, 7-CH_3), 2.67 (d, *J* = 4.3 Hz, 1 H, O<u>H</u>), 2.63 (m, 1 H, 7-H), 3.80 (s, 3 H, $CH_2C_6H_4OC\underline{H}_3$), 4.27 (d, *J* = 15.0 Hz, 1 H, $C\underline{H}_2C_6H_4OCH_3$), 4.66 (bs, 1 H, C<u>H</u>OH), 4.89 (d, *J* = 15.0 Hz, 1 H, $C\underline{H}_2C_6H_4OCH_3$), 5.03 (d, *J* = 6.0 Hz, 1 H, 6-H), 5.13 (dd, *J* = 10.2, 1.5 Hz, 1H, CH=C<u>H</u>$_2$), 5.18 (dd, *J* = 17.1, 1.5 Hz, 1 H, CH=C<u>H</u>$_2$), 5.29 (ddd, *J* = 17.1, 10.2, 4.5 Hz, 1 H, C<u>H</u>=CH$_2$), 6.84 (d, *J* = 8.7 Hz, 2 H, $CH_2C_6\underline{H}_4OCH_3$), 7.26 (d, *J* = 8.7 Hz, 2 H, $CH_2C_6\underline{H}_4OCH_3$) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.5, 167.8, 159.4, 132.7, 130.0, 128.6, 118.9, 114.1, 83.2, 72.9, 66.0, 55.3, 45.1, 38.5, 8.3 *ppm*; MS (CI, NH_3) HRMS *m/z* calcd for $C_{17}H_{23}N_2O_5$ 335.1607, found for $(M+NH_4)^+$ 335.1601; **9(*S*)** diastereomer of ***N*-PMB** derivative of **7:** 5% (4 steps), colorless crystals; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.26 (d, *J* = 7.5 Hz, 1 H, 7-CH_3), 2.26 (m, 1 H, 7-H), 3.82 (s, 3 H, $CH_2C_6H_4OC\underline{H}_3$), 4.53 (d, *J* = 15.0 Hz, 1 H, $C\underline{H}_2C_6H_4OCH_3$), 4.73 (d, *J* = 15.0 Hz, 1 , $C\underline{H}_2C_6H_4OCH_3$), 5.19 (d, *J* = 5.9 Hz, 1 H, 6-H), 6.28 (br s, 1 H, C<u>H</u>OH), 6.86 (d, *J* = 8.6 Hz, 2 H, $CH_2C_6\underline{H}_4OCH_3$), 7.27 (m, 7 H, $CH_2C_6\underline{H}_4OCH_3$, $C_6\underline{H}_5$) *ppm*.

The *N*-PMB derivative of **7** was dissolved in a mixture of CH_3CN and water (*v/v*, 3 : 1, 0.1 *M*) and treated with CAN (5-6 eq) at 23 °C. The resulting mixture was stirred for 2 h and quenched with sat. aqueous $NaHCO_3$. After extractive workup with ethyl acetate (3 x), the combined extracts were washed with brine, dried and concentrated *in vacuo*. The residue was purified by flash SGC to afford the β-lactone **7** as colorless crystals (from ethyl acetate), m.p. 128 °–129 °C; $[\alpha]_D^{23}$ -148 (*c* 0.025, $CHCl_3$); FTIR (film) ν_{max}: 3387, 1828, 1698 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.32 (d, *J* = 7.5 Hz, 3 H, 7-CH_3), 1.60 (bs, 1 H, O<u>H</u>), 2.68 (m, 1 H, 7-H), 4.63 (d, *J* = 5.3 Hz, 1 H, C<u>H</u>OH), 5.08 (d, *J* = 6.3 Hz, 1 H, 6-H), 5.44 (d, *J* = 10.6, 1 H, CH=C<u>H</u>$_2$), 5.55 (d, *J* = 17.1 Hz, 1 H, CH=C<u>H</u>$_2$), 5.92 (ddd, *J* = 17.1, 10.6, 5.3 Hz, 1 H, CH=C<u>H</u>$_2$) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 176.7, 168.1, 133.2, 120.0, 77.3, 75.4, 68.9, 38.7, 8.1 *ppm*; MS (CI, NH_3) HRMS *m/z* calcd for $C_9H_{15}N_2O_4$ 215.1032, found for $(M+NH_4)^+$ 215.1033; **Summary of X-ray crystal data:** $C_9H_{10}NO_4$; FW = 196.18; a = 15.024(2) Å; b = 6.6266(9) Å; c = 9.9474(14) Å; α = 90°; β = 90°; γ = 90.911(3)°; Vol = 990.2(2) $Å^3$; Monoclinic; C_2; Z = 4; crystal size = 0.1 x 0.05 x 0.05 mm; GOF = 1.085; final R indices [I>2σ(I)], R_1 = 4.44%, wR_2 = 9.77%; R indices (all data), R_1 = 5.83%, wR_2 =10.85%.[21]

β-Lactone 10, colorless crystals; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.26 (d, *J* = 10.0 Hz, 1 H, CH_3), 1.57 (bs, 1 H, O<u>H</u>), 2.43 (m, 1 H, 7-H), 5.09 (d, *J* = 8.1 Hz, 1 H, CHOCO), 6.11 (bs, 1 H, C<u>H</u>OH), 6.15 (bs, 1 H, N<u>H</u>), 7.39 (m, 5 H, $C_6\underline{H}_5$) *ppm*.

β-Lactone 8. A solution of β-lactone **7** (5.0 mg, 0.03 mmol) in ethanol (0.5 mL) was treated with 10% palladium on carbon (1 mg, 20% *wt.*) and hydrogen (balloon, 1 atm.). The resulting mixture was stirred for 2 h at 23 °C and filtered through a pad of Celite. The filtrate was concentrated *in vacuo* and the resulting residue was purified by flash SGC with ethyl acetate as eluent to give 9-ethyl β-lactone **8** (5.0 mg, >99%) as colorless crystals; m.p. 108 °-109 °C; $[\alpha]_D^{23}$ -127 (*c* 0.015, $CHCl_3$); FTIR (film) ν_{max} 3387, 1827, 1698 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.07 (t, *J* = 7.4 Hz, 1 H, $CH_2C\underline{H}_3$), 1.31 (d, *J* = 7.5 Hz, 1 H, 7-CH_3), 1.43 (m, 1 H, C<u>H</u>$_2$CH$_3$), 1.75 (m, 1 H, C<u>H</u>$_2$CH$_3$), 2.04 (bs, 1 H, O<u>H</u>), 2.71 (m, 1 H, 7-H), 3.95 (dd, *J* = 10.2, 2.7 Hz, 1 H, C<u>H</u>OH), 5.10 (d, *J* = 6.2 Hz, 1 H, 6-H), 7.26 (br s, NH) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.6, 169.1, 79.4, 76.1, 69.5, 38.8, 25.0, 9.9, 8.1 *ppm*; MS (CI, NH_3) HRMS *m/z* calcd for $C_9H_{17}N_2O_4$ 217.1188, found for $(M+NH_4)^+$ 217.1177.

β-Lactone 11 or 12. To a stirred suspension of $CrCl_3$ (6 mg, 0.04 mmol) and manganese powder (200 mesh, 33 mg, 0.61 mmol) in THF (0.8 mL) was added dropwise a solution of aldehyde **4a** (88 mg, 0.21 mol) in THF (1.0 mL), allyl bromide (61 μL, 0.71 mmol) or methallyl bromide (71 μL, 0.71 mmol) and TMSCl (116 μL, 0.91 mmol) at 23 °C. After stirring for 12 h, water (2 mL) was added and the resulting mixture was stirred for an additional 1 h and extracted with ethyl acetate (3 x 5 mL). The combined organic layers were washed with brine, dried, and concentrated *in vacuo*. The resulting residue was filtered through a pad of silica gel eluting with 1 : 4–EtOAc–hexane and the filtrate was concentrated *in vacuo* to give the crude allylation product (mixture of free hydroxyl adduct and O-TMS silylated adduct) as a colorless oil, which without further purification, was dissolved in a 5% HF-acetonitrile solution (1 mL), stirred for 4 h at 23 °C, and placed directly on a pad of silica gel packed with ethyl acetate. The pad was rinsed with ethyl acetate (10 mL), the filtrate was concentrated to give the crude diol ester as a colorless oil, which was taken up in a mixture of THF (0.6 mL) and H_2O (0.6 mL) and was treated with LiOH (24 mg, 1.0 mmol) in one portion. After stirring for 1 h at 23 °C, the reaction mixture was acidified to pH 2–3 with 2*N* HCl, extracted with ethyl acetate (5 x 10 mL), the combined organic layers were washed with brine, dried and concentrated *in vacuo* to afford crude diol acid as a colorless oil, which without further purification, was suspended directly in CH_2Cl_2 (1 mL) and treated with Et_3N (56 μL, 0.40 mmol) and BOPCl (50 mg, 0.20 mmol). The resulting mixture was stirred for 2 h at 23 °C, treated with sat. aqueous $NaHCO_3$ and extracted with ethyl acetate (3 x 5 mL). The combined organic layers were washed with brine, dried and concentrated *in vacuo*. The crude residue was purified by flash SGC with hexane/ethyl acetate (1 : 1 *v/v*) as eluent to give *N*-PMB-protected β-lactone **11** (28 mg, 42%) or the *N*-PMB-protected β-lactone **12** (31 mg, 45%). ***N*-PMB-derivative of 11**, colorless oil; $[\alpha]_D^{23}$ -77 (*c* 0.1, $CHCl_3$); FTIR (film) ν_{max}: 3397, 1836, 1695, 1516, 1249 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.36 (d, *J* = 7.5 Hz, 3 H, 7-C$\underline{H}_3$), 1.59 (m, 1 H, C$\underline{H}_2$CH=CH$_2$), 1.83 (dd, 1 H, J = 4.7; 14.1 Hz, C$\underline{H}_2$CH=CH$_2$), 2.20 (bs, 1 H, O$\underline{H}$), 2.71 (m, 1 H, 7-H), 3.80 (s, 3 H, CH$_2$C$_6$H$_4$OC$\underline{H}_3$), 4.12 (br s, 1 H, C$\underline{H}$OH), 4.14 (d, *J* = 15.0 Hz, 1 H, C$\underline{H}_2$C$_6$H$_4$OCH$_3$), 4.87 (d, *J* = 17.0 Hz, 1 H, CH$_2$CH=C$\underline{H}_2$), 4.97 (d, *J* = 15.0 Hz, 1 H, C$\underline{H}_2$C$_6$H$_4$OCH$_3$), 5.04 (d, *J* = 10.2 Hz, 1 H, CH$_2$CH=C$\underline{H}_2$), 5.11 (d, *J* = 6.0 Hz, 1 H, 6-H), 5.38 (m, 1 H, CH$_2$C$\underline{H}$=CH$_2$), 6.85 (d, *J* = 8.6 Hz, 2 H, CH$_2$C$_6$H$_4$OCH$_3$), 7.28 (d, *J* = 8.6 Hz, 2 H, CH$_2$C$_6$H$_4$OCH$_3$) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.1, 168.1, 159.6, 132.3, 130.1, 130.0, 119.7, 114.3, 77.3, 73.1, 65.5, 55.4, 45.2, 38.5, 35.5, 8.5 *ppm*; HRMS (FAB, NBA+NaI) *m/z* calcd for $C_{18}H_{21}NO_5Na$ 354.1317, found for $[M+Na]^+$ 354.1315; ***N*-PMB-derivative of 12,** colorless oil; $[\alpha]_D^{23}$ -172 (*c* 0.05, $CHCl_3$); FTIR (film) ν_{max}: 3395, 1833, 1701, 1520, 1248 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.25 [s, 3 H, CH$_2$C(C$\underline{H}_3$)=CH$_2$], 1.37 (d, *J* = 7.5 Hz, 3 H, 7-C$\underline{H}_3$), 1.58 [d, *J* = 13.1 Hz, 1 H, C$\underline{H}_2$C(CH$_3$)=CH$_2$], 1.73 [d, *J* = 13.1 Hz, 1 H, C$\underline{H}_2$C(CH$_3$)=CH$_2$], 2.22 (bs, 1 H, O$\underline{H}$), 2.72 (m, 1 H, 7-H), 3.78 (s, 3H, CH$_2$C$_6$H$_4$OC$\underline{H}_3$), 4.13 (d, *J* = 15.1 Hz, 1 H, C$\underline{H}_2$C$_6$H$_4$OCH$_3$), 4.14 (d, *J* = 13.1 Hz, 1 H, C$\underline{H}$OH), 4.63 [br s, 1 H, CH$_2$C(CH$_3$)=C$\underline{H}_2$], 4.82 [br s, 1 H, CH$_2$C(CH$_3$)=C$\underline{H}_2$], 5.03 (d, *J* = 15.1 Hz, 1 H, C$\underline{H}_2$C$_6$H$_4$OCH$_3$), 5.12 (d, *J* = 6.0 Hz, 1 H, 6-H), 6.83 (d, *J* = 8.7 Hz, 2 H, CH$_2$C$_6$H$_4$OCH$_3$), 7.26 (d, *J* = 8.7 Hz, 2 H, CH$_2$C$_6$H$_4$OCH$_3$) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.2, 168.5, 159.6, 140.1, 130.0, 129.1, 115.4, 114.4, 77.3, 73.0, 63.5, 55.4, 45.3, 39.3, 38.7, 21.4, 8.4; MS (FAB) HRMS (FAB, NBA+NaI) *m/z* calcd for $C_{19}H_{23}NO_5Na$ 368.1474, found for $[M+Na]^+$ 368.1458.

A stirred mixture of the *N*-PMB-derivative of β-lactone **11** or **12** (0.08 mmol) in acetonitrile (0.63 mL) and distilled water (0.21 mL) was treated with ceric ammonium nitrate (CAN, 186 mg, 0.34 mmol). After stirring for 1 h at 23 °C, the reaction was treated with sat. aqueous $NaHCO_3$ (2 mL) and extracted with ethyl acetate (5 x10 mL). The combined organic layers were washed with brine, dried and concentrated *in vacuo*. The resulting residue was purified by flash SGC with ethyl acetate as eluent to afford the corresponding β-lactones **11** or **12**. **β-Lactone 11**, 65%, colorless oil; $[\alpha]_D^{23}$ -60 (*c* 0.15, $CHCl_3$); FTIR (film) ν_{max}: 3300, 1828, 1709 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.31 (d, *J* = 7.5 Hz, 3 H, 7-C$\underline{H}_3$), 2.20 (m, 1 H, C$\underline{H}_2$CH=CH$_2$), 2.52 (m, 1 H, C$\underline{H}_2$CH=CH$_2$), 2.71 (m, 1 H, 7-H), 3.18 (bs, 1 H, O$\underline{H}$), 4.17 (dd, *J* = 8.5, 3.4 Hz, 1 H, C$\underline{H}$OH), 5.15 (d, *J* = 6.2 Hz, 1 H, 6-H), 5.18 (dd, *J* = 3.5, 1.2 Hz, 1 H, CH$_2$C$\underline{H}$=C$\underline{H}_2$), 5.22 (d, *J* = 1.2 Hz, 1 H, CH$_2$CH=C$\underline{H}_2$),5.86 (m, 1 H, CH$_2$C$\underline{H}$=CH$_2$), 7.34 (bs, 1 H, N$\underline{H}$).*ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) d 177.6, 169.2, 132.4, 119.9, 79.1, 76.0, 66.9, 38.6, 36.5, 8.1 *ppm*; HRMS (CI, NH_3) *m/z* calcd for $C_{10}H_{17}N_2O_4$

229.1188, found for $[M+NH_4]^+$ 229.1197; **β-Lactone 12**, 65%, colorless crystal; m.p. 141 °–142 °C; $[\alpha]_D^{23}$ -115 (*c* 0.02, $CHCl_3$); FTIR (film) ν_{max}: 3320, 1829, 1710 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.33 (d, *J* = 7.6 Hz, 3 H, 7-CH_3), 1.59 (bs, 1H, O<u>H</u>), 1.80 [s, 3 H, $CH_2C(CH_3)=CH_2$], 2.08 (dd, *J* = 13.8, 10.5 Hz, 1 H, $CH_2C(CH_3)=CH_2$], 2.42 (d, 1 H, *J* = 13.8 Hz, CH_2C=)2.71 (m, 1 H, 7-H), 4.20 (dd, *J* = 10.5, 2.5 Hz, 1 H, CHOH), 4.89 [br s, 1 H, $CH_2C(CH_3)=CH_2$], 5.00 [br s, 1 H, $CH_2C(CH_3)=CH_2$], 5.12 (d, *J* = 6.1 Hz, 1 H, 6-H), 6.55 (bs, 1 H, N<u>H</u>) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.0, 168.5, 140.1, 115.4, 78.8, 75.7, 65.3, 40.3, 38.7, 22.2, 8.1 *ppm*; HRMS (CI, NH_3) *m/z* calcd for $C_{11}H_{19}N_2O_4$ 243.1345, found for $[M+NH_4]^+$ 243.1340; **Summary of X-ray crystal data:** $C_{11}H_{15}NO_4$; FW = 225.24; a = 13.1456(3) Å; b = 9.7557(1) Å; c = 15.3696(4) Å; α = 90°; β = 114.693(1)°; γ = 90°; Vol = 1790.83(6) $Å^3$; Monoclinic; $P2_1$; Z = 6; crystal size = 1 x 1 x 0.5 mm; GOF = 1.136; final R indices [I>2σ(I)], R_1 = 4.64%, wR_2 = 9.39%; R indices (all data), R_1 = 6.64%, wR_2 = 10.77%.[21]

β-Lactone 13 or 14. A stirred solution of β-lactone **11** or **12** (5.0 mg each, 0.02 mmol) in ethanol (0.5 mL) was treated with 10% palladium on carbon (1 mg, 20% *wt.*) and hydrogen (balloon, 1 atm.). The resulting mixture was stirred for 2 h at 23 °C and filtered through a pad of Celite. The filtrate was concentrated *in vacuo* and the residue was purified by flash SGC with ethyl acetate as eluent to give β-lactone **13** or **14** quantitatively. **β-Lactone 13**, colorless crystals; m.p. 124 °-125 °C; $[\alpha]_D^{23}$ -87 (*c* 0.03, $CHCl_3$); FTIR (film) ν_{max}: 3420, 1828, 1705 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.97 (t, *J* = 6.9 Hz, 3 H, $CH_2CH_2CH_3$), 1.32 (d, *J* = 7.5 Hz, 3 , 7-CH_3), 1.43 (m, 2 H, $CH_2CH_2CH_3$), 1.62 (m, 2 H, $CH_2CH_2CH_3$), 2.54 (bs, 1 H, O<u>H</u>), 2.71 (m, 1 H, 7-H), 4.04 (bd, *J* = 8.3 Hz, 1 H, CHOH), 5.09 (d, *J* = 6.1 Hz, 1 H, 6-H), 6.81 (bs, 1 H, N<u>H</u>) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.2, 169.0, 79.3, 76.0, 67.9, 38.8, 33.8, 18.7, 13.8, 8.1 *ppm*; HRMS (CI, NH_3) *m/z* calcd for $C_{10}H_{19}N_2O_4$ 231.1345, found for $[M+NH_4]^+$ 231.1336; **β-Lactone 14**, colorless crystals; m.p. 127 °–128 °C; $[\alpha]_D^{23}$ -170 (*c* 0.01, $CHCl_3$); FTIR (film) ν_{max}: 3360, 1829, 1709 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.94 [d, *J* = 6.7 Hz, 3 H, $CH_2CH(CH_3)CH_3$], 0.96 [d, *J* = 6.7 Hz, 3 H, $CH_2CH(CH_3)CH_3$], 1.32 (d, *J* = 7.4 Hz, 3 H, 7-CH_3), 1.38 [m, 2H, $CH_2CH(CH_3)CH_3$], 1.89 [m, 1 H, $CH_2CH(CH_3)CH_3$], 2.02 (br s, 1 H, OH), 2.70 (m, 1 H, 7-H), 4.11 (bd *J* = 10.0 Hz, 1 H, CHOH), 5.09 (d, *J* = 6.0 Hz, 1 H, 6-H), 6.22 (bs, 1 H, N<u>H</u>) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.0, 168.8, 79.0, 75.8, 66.5, 40.6, 38.7, 24.3, 23.6, 21.3, 8.1 *ppm*; HRMS (CI, NH_3) *m/z* calcd for $C_{11}H_{21}N_2O_4$ 245.1501, found for $[M+NH_4]^+$ 245.1504.

β-Lactone 9. β-Lactone **9** was prepared from intermediate **3** by the sequence (1) ketone reduction, (2) desulfurization, (3) β-lactonization and (4) *N*-deprotection. *N*-PMB-derivative of **9**, ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.16 (dd, 1 H, *J* = 4.7; 8.3 Hz, <u>H</u>OCH$_2$), 1.38 (d, 3 H, *J* = 7.4 Hz, CH_3CH), 2.81 (m, 1 H, CHCH$_3$), 3.72 (dd, 1 H, *J* = 4.7; 12.6 Hz, CH_2OH), 3.80 (s, 3 H, CH_3O), 3.93 (dd, 1 H, *J* = 8.3; 12.6 Hz, CH_2OH), 4.10 and 5.09 (each d, 1 H, *J* = 15.1 Hz, CH_2Ph), 5.05 (d, 1 H, *J* = 6.2 Hz, CHOCO), 6.87 and 7.27 (each d, 2 H, *J* = 8.6 Hz, ArH) *ppm*; HRMS (FAB, NBA+NaI) *m/z* calcd for $[C_{15}H_{17}NO_5Na]^+$ 314.1004; found for $[M+Na]^+$ 314.1002; **β-Lactone 9**, white solids, $[\alpha]_D^{23}$ -92.1 (*c* 0.19, EtOAc); FTIR (film) ν_{max}: 3350 (br s, OH), 1834, 1829, 1708, 1704, 1698 cm^{-1}; ^{1}H-NMR (400 MHz, Py-d_5) δ 1.40 (d, 3 H, *J* = 7.3 Hz, CH_3CH), 2.99 (m, 1 H, CHCH$_3$), 4.13 (d, 1 H, *J* = 11.9 Hz, CH_2OH), 4.44 (d, 1 H, *J* = 11.9 Hz, CH_2OH), 5.10 (br, OH), 5.59 (d, 1 H, *J* = 6.2 Hz, CHOCO), 10.65 (br s, NH) *ppm*; ^{13}C-NMR (100 MHz, Acetone-d_6) δ 8.4, 38.9, 58.6, 76.8, 100.1, 170.5, 176.8 *ppm*; HRMS (CI, NH_3) *m/z* calcd for $[C_7H_{13}N_2O_4]^+$ 189.0875; found for $[M+NH_4]^+$ 189.0883.

Preparation of β-Lactone 15. To a stirred solution of *clasto*-lactacystin β-lactone **2** (4.0 mg, 0.018 mmol) was added Dess-Martin periodinane (12 mg, 0.0283 mmol) in one portion. After stirring for 1 h at room temperature, the reaction mixture was treated with a mixture of sat. aqueous $NaHCO_3$ / $Na_2S_2O_3$ (*v/v* 1 : 1, 1 mL) and stirred for another 15 min. The aqueous phase was extracted with EtOAc (2 x 5 mL) and the combined organic phases were washed with brine, dried and concentrated *in vacuo*. The residue was put upon a pad of silica gel and eluted with a solvent mixture of EtOAc–hexane (*v/v* 1 : 1). The filtrate was concentrated *in vacuo* to give the 9-oxo *clasto*-lactacystin β-lactone **15** (3.6 mg, 90%) as colorless crystals. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.19 and 1.21 (each d, 3 H, *J* = 7. 0 Hz, $(CH_3)_2$CH), 1.37 (d, 3 H, *J* = 7.4 Hz, CH_3CH), 2.77 (m, 1 H,

CH(CH$_3$)$_2$), 3.00 (m, 1 H, CHCH$_3$), 5.24 (d, 1 H, J = 6.3 Hz, CHOCO), 6.75 (br s, NH) *ppm*; ^{13}C-NMR (125 MHz, CDCl$_3$) δ 8.1, 17.8, 18.1, 37.5, 38.6, 76.2, 164.8, 176.9, 203.0 *ppm*.

β-Lactone 16. A stirred solution of 9-oxo *clasto*-lactacystin β-lactone **15** (3.0 mg, 0.0142 mmol) in THF (1.5 mL) was treated with NaBH$_4$ (1.5 mg, 0.040 mmol) in one portion at -78 °C. After stirring for 0.5 h at that temperature, the reaction was quenched with water (1 mL) at -78 °C and extracted with EtOAc (3 x 3 mL). The combined extracts were washed with brine, dried and concentrated *in vacuo*. The crude residue was purified by flash SGC to afford *clasto*-lactacystin β-lactone (**2**) as major product (2.1 mg) and the 9*R*-hydroxy *clasto*-lactacystin β-lactone **16** (0.9 mg, 30%) as minor product. ^{1}H-NMR (500 MHz, CDCl$_3$) δ 0.94 and 1.09 (each d, 3 H, J = 6.8 Hz, (CH$_3$)$_2$CH), 1.35 (d, 3 H, J = 7.5 Hz, CH$_3$CH), 1.90 (dd, J = 5.4; 6.8 Hz, 1 H, CH(CH$_3$)$_2$), 2.28 (d, 1 H, J = 2.0 Hz, OH), 2.76 (dq, 1 H, J = 6.1; 7.5 Hz, CHCH$_3$), 3.80 (dd, 1 H, J = 2.0; 5.4 Hz, CHOH), 5.10 (d, 1 H, J = 6.1 Hz, CHOCO), 6.18 (br s, NH) *ppm*.

β-lactones 17 and 18. To a stirred suspension of isopropyltriphosphonium iodide (420 mg, 0.972 mmol) in THF (8.0 mL) was added *n*-BuLi (1.6 *M* in hexane, 0.60 mL, 0.96 mmol) dropwise within 5 min at 0 °C. The resulting mixture was stirred for 15 min at that temperature to give a dark-red solution of corresponding ylide, whereupon a solution of aldehyde **4a** (170 mg, 0.39 mmol) in THF (2.0 mL) was added dropwise at 0 °C. After stirring for 5 min, the reaction mixture was treated with sat. aqueous NH$_4$Cl and extracted with ether (2 x 30 mL). The combined extracts were washed with brine, dried and concentrated *in vacuo*. The crude oil was purified by flash SGC eluting with a solvent mixture of EtOAc–hexane (1 : 2 *v/v*) to give the corresponding olefination product (116.5 mg, 60%) as a colorless oil. ^{1}H-NMR (400 MHz, CDCl$_3$) δ 0.52 (q, 6 H, J = 8.0 Hz, CH$_2$Si), 0.89 (t, 9 H, J = 8.0 Hz, CH$_3$CH$_2$Si), 1.18 (d, 3 H, J = 7.7 Hz, CH$_3$CH), 1.44 and 1.81 (each s, 3 H, (CH$_3$)$_2$C=), 2.72 (dq, 1 H, J = 7.7; 9.7 Hz, CHCH$_3$), 3.22 (s, 3 H, CH$_3$O), 3.76 (s, 3 H, CH$_3$O), 3.88 and 4.62 (each d, 1 H, J = 14.2 Hz, CH$_2$Ph), 4.52 (d, 1 H, J = 9.7 Hz, O-CH), 6.11 (br s, 1 H, CH=), 6.75 and 7.17 (each d, 2 H, J = 8.6 Hz, ArH) *ppm*; HRMS (FAB, NBA+NaI) *m/z* calcd for [C$_{25}$H$_{39}$NO$_5$SiNa]$^+$ 484.2495; found for [M+Na]$^+$ 484.2493; calcd for [C$_{25}$H$_{40}$NO$_5$Si]$^+$ 462.2676; found for [M+H]$^+$ 462.2679.

The above olefination product (110.0 mg, 0.239 mmol) was hydrogenated in EtOH (2 mL) at 1 atm (balloon) and room temperature in the presence of 10% Pd–C (10 mg) as catalyst. After stirring for 12 h, the reaction mixture was filtered through a pad of Celite and concentrated *in vacuo* to give the desired hydrogenation product (109 mg, 98%) as a colorless oil. ^{1}H-NMR (300 MHz, CDCl$_3$) δ 0.58 (q, 6 H, J = 8.0 Hz, CH$_2$Si), 0.82 (d, 3 H, J = 7.2 Hz, CH$_3$), 0.92 (t, 9 H, J = 8.0 Hz, CH$_3$CH$_2$Si), 0.98 (d, 3 H, J = 7.2 Hz, CH$_3$), 1.20 (d, 3 H, J = 7.7 Hz, CH$_3$CH), 1.58 (m, 1 H, CHCH$_2$), 1.98 (dd, 2 H, J = 6.0; 11.1 Hz, CH$_2$CH), 2.69 (m, 1 H, CHCH$_3$), 3.23 (s, 3 H, CH$_3$O), 3.77 (s, 3 H, CH$_3$O), 4.06 and 4.68 (each d, 1 H, J = 14.3 Hz, CH$_2$Ph), 4.53 (d, 1 H, J = 7.8 Hz, CHOTES), 6.79 and 7.21 (each d, 2 H, J = 8.6 Hz, ArH) *ppm*; HRMS (FAB, NBA+NaI) *m/z* calcd for [C$_{25}$H$_{41}$NO$_5$SiNa]$^+$ 486.2652; found for [M+Na]$^+$ 486.2632.

The above olefination product and its dihydro derivative (42 mg, 0.091 mmol) were separately desilylated by treatment with 8% HF-CH$_3$CN (2 mL) at 23 °C for 10 h and followed by filtration through a pad of silica gel eluting with EtOAc. The filtrate was concentrated *in vacuo* to give the crude desilylated product, which without purification, was taken up in a mixture of THF-H$_2$O (1 : 1 *v/v* 1.5 mL) and treated with LiOH (5-8 eq) at 23 °C. After stirring for 1 h, the reaction mixture was acidified with 2*N* HCl to pH 2–3 and followed by extractive workup with EtOAc (3x5 mL). The combined extracts were washed with brine, dried and concentrated *in vacuo* to afford the crude acid as an oil, which was suspended in CH$_2$Cl$_2$ (2.5 mL) and treated with Et$_3$N (38 μL, 0.27 mmol, 3 eq), followed by BOPCl (35 mg, 0.136 mmol, 1.5 eq) at room temperature. After stirring for 1 h, the reaction was quenched with sat. aqueous NaHCO$_3$ and extracted with EtOAc (3 x 5 mL). The combined EtOAc extracts were washed with brine, dried and concentrated *in vacuo* to give a residue which was purified by flash SGC to afford the corresponding *N*-PMB β-lactones. Cleavage of the *N*-PMB protecting group by treatment with CAN in CH$_3$CN–H$_2$O (*v/v*, 3 : 1, 23 °C, 2 h) followed by extractive workup and SGC purification gave the desired β-lactone **17** or **18** in an overall yield of 45% and 60% respectively. **β-Lactone 17**, [α]$_D^{23}$ -84 (*c* 0.10, CHCl$_3$); ^{1}H-NMR (500 MHz, CDCl$_3$) δ 0.94 and 1.03 (each d, 3 H, J = 6.4 Hz, (CH$_3$)$_2$CH), 1.34 (d, 3

H, J = 7.5 Hz, CH_3CH), 1.79 (dd, 1 H, J = 8.2; 14.2 Hz, CH_2CH), 1.86 (m, 1 H, $CH(CH_3)_2$), 1.98 (dd, 1 H, J = 5.1; 14.2 Hz, CH_2CH), 2.75 (m, 1 H, $CHCH_3$), 4.94 (d, 1 H, J = 6.2 Hz, CHOCO), 6.13 (brs, NH) *ppm*; HRMS (CI, NH_3) *m/z* calcd for $[C_{10}H_{19}N_2O_3]^+$ 215.1396; found for $[M+NH_4]^+$ 215.1397; **β-lactone 18**, ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.35 (d, 1 H, J = 7.4 Hz, CH_3CH), 1.77 and 1.84 (each s, 3 H, $(CH_3)_2C$=), 2.79 (m, 1 H, $CHCH_3$), 4.99 (d, 1 H, J = 6.0 Hz, CHOCO), 5.35 (br s, 1 H, CH=), 6.03 (br s, NH) *ppm*; HRMS (CI, NH_3) *m/z* calcd for $[C_{10}H_{17}N_2O_3]^+$ 213.1239; found for $[M+NH_4]^+$ 213.1245.

β-lactone analogs 19, 20, 21, 22, 23 and **24** were prepared by the method previously reported[13] as outlined in Scheme 3. The chemical yields and spectral data of intermediates for the preparation of β-lactone **19** and β-lactone **24** are listed below.

Mukaiyama aldol product (Scheme 3, **25**, R = H); 90% (total yield along with corresponding TBS ether), colorless oil, $[\alpha]_D^{23}$ -9.0 (*c* 0.50, EtOAc); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.12 (s, 6 H, CH_3Si), 0.92 (s, 9 H, t-BuSi), 1.00 (d, 3 H, J = 6.7 Hz, CH_3), 1.03 (d, 3 H, J = 6.7 Hz, CH_3), 2.04 (m, 1 H, $CH(CH_3)_2$), 2.57 (dd, 1 H, J = 10.5; 15.4 Hz, CH_2CO), 2.68 (dd, 1 H, J = 2.0; 15.4 Hz, CH_2CO), 3.58 (br s, OH), 3.74 (s, 3 H, OCH_3), 3.75 (d, 1 H, J = 5.6 Hz, CHOH), 3.79 (d, 1 H, J = 13.7 Hz, CH_2Ph), 3.92 (d, 1 H, J = 10.8 Hz, CH_2OSi), 4.04 (d, 1 H, J = 10.8 Hz, CH_2OSi), 4.24 (d, 1 H, J = 1.9 Hz, NCH_2O), 4.31 (d, 1 H, J = 13.7 Hz, CH_2Ph), 4.35 (d, 1 H, J = 1.9 Hz, NCH_2O), 4.51 (br d, 1 H, J = 10.0 Hz, HOCH), 7.16 - 7.35 (m, 5 H, ArH) *ppm*; ^{13}C-NMR (125 MHz, $CDCl_3$) δ -5.8, -5.7, 18.1, 19.1, 22.11, 25.7, 25.8, 28.9, 37.4, 40.1, 51.1, 51.9, 57.5, 61.0, 68.6, 68.8, 84.8, 127.1, 128.0, 139.5, 173.2 *ppm*; HRMS (FAB, NBA+NaI) *m/z* calcd for $[C_{24}H_{41}NO_5SiNa]^+$ 474.2652; found for $[M+Na]^+$ 474.2656.

Bicyclic diol (Scheme 3, **26**, R = H) of corresponding β-lactone **19**: 70%(3 steps), colorless crystals (EtOAc), $[\alpha]_D^{23}$ +45.0 (*c* 0.01, EtOAc); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.03 (dd, 1 H, J = 1.3; 6.8 Hz, $(CH_3)_2CH$), 1.67 (m, 1 H, $CH(CH_3)_2$), 2.24 (br s, OH), 2.88 (dd, 1 H, J = 9.0; 16.8 Hz, CH_2CO), 2.99 (dd, 1 H, J = 8.9; 16.8 Hz, CH_2CO), 3.05 (d, 1 H, J =10.5 Hz, OCH), 3.80 (d, 1 H, J = 11.7 Hz, CH_2OH), 4.09 (d, 1 H, J = 1.7 Hz, CH_2OH), 4.38 (br t, 1 H, J = 8.6 Hz, CHOH), 4.70 (dd, 1 H, J = 1.2; 4.9 Hz, NCH_2O), 5.13 (d, 1 H, J = 4.9 Hz, NCH_2O) *ppm*; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 18.2, 20.6, 27.7, 46.1, 60.2, 72.2, 73.7, 75.6, 90.4, 174.1 *ppm*; HRMS (CI, NH_3) *m/z* calcd for $[C_{10}H_{18}NO_4]^+$ 216.1236; found for $[M+H]^+$ 216.1238; **Summary of X-ray crystal data**: $C_{10}H_{17}NO_4$; FW = 215.25; a = 6.6289(2) Å; b = 8.5550(2) Å; c = 19.5992(3) Å; α = 90°; β = 90°; γ = 90°; Vol = 1111.32(5) Å^3; orthorhombic; $P2_12_12_1$; Z = 4; crystal size = 0.4 x 0.3 x 0.25 mm; GOF = 1.098; final R indices [I>2σ(I)], R_1 = 4.49%, wR_2 = 10.46%; R indices (all data), R_1 = 5.08%, wR_2 = 11.50%.[21]

Diol acid (Scheme 3, **27**, R = H) of corresponding β-lactone **19**: 56% (3 steps), white solids, $[\alpha]_D^{23}$ +20 (*c* 0.01, MeOH); ^{1}H-NMR (500 MHz, Py-d_5) δ 1.31 (d, 3 H, J = 6.7 Hz, CH_3), 1.35 (d, 3 H, J = 6.7 Hz, CH_3), 2.36 (m, 1 H, $CHCH_3$), 2.78 (d, 1 H, J = 16.4 Hz, CH_2CO), 3.58 (dd, 1 H, J = 6.2; 16.4 Hz, CH_2CO), 4.70 (d, 1 H, J = 6.6 Hz, CHOH), 5.34 (d, 1 H, CHOH), 9.39 (s, 1 H, NH) *ppm*; ^{13}C-NMR (125 MHz, Py-d_5) δ 14.2, 19.5, 20.9, 22.8, 31.7, 32.2, 42.5, 47.9, 74.3, 78.0, 79.6, 174.6, 177.5 *ppm*; HRMS (FAB, NBA+NaI) *m/z* calcd for $[C_9H_{16}NO_5]^+$ 218.1028; found for $[M+H]^+$ 218.1027.

β-Lactone 19: 30% (along with *ca.* 60% of decarboxylated by-product), white solids, $[\alpha]_D^{23}$ -123 (c 0.02, EtOAc); ^{1}H-NMR (400 MHz, Py-d_5) δ 0.94 (d, 3 H, J = 6.7 Hz, CH_3), 1.08 (d, 3 H, J = 6.7 Hz, CH_3), 2.10 (m, 1 H, $CH(CH_3)_2$), 3.00 (d, 1 H, J = 18.3 Hz, CH_2CO), 3.10 (dd, 1 H, J = 5.9; 18.3 Hz, CH_2CO), 4.35 (dd, 1 H, J = 6.8; 3.6 Hz, CHOH), 5.66 (d, 1 H, J =5.9 Hz, CH), 7.86 (d, 1 H, J = 6.8 Hz, CHOH), 10.50 (s, 1 H, NH) *ppm*; ^{13}C-NMR (125 MHz, Py-d_5) δ 16.4, 20.2, 29.8, 36.0, 70.5, 74.3, 82.6, 172.3, 175.1 *ppm*; HRMS (CI, NH_3) *m/z* calcd for $[C_9H_{17}N_2O_4]^+$ 217.1188; found for $[M+NH_4]^+$ 217.1187.

β-Lactone 24: ^{1}H-NMR(300 MHz, $CDCl_3$) δ 1.03 (d, 3 H, J = 6.7 Hz), 1.09 (d, 3 H, J = 6.8 Hz), 1.36 (d, 3 H, J = 7.9 Hz), 1.85 (m, 1 H), 2.86 (q, 1 H, J = 7.9 Hz), 3.83 (m, 1 H, CH-OH), 4.84 (s, 1 H, CH-OCO), 6.03 (br s, 1 H, NH) *ppm*.

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18. In this β-lactonization and in each of the others reported herein it was not necessary to protect the C(9)-hydroxyl since β-lactone formation occurred exclusively at the C(6)-hydroxyl. This highly selective β-lactonization to form the 5/4 fused β-lactone is the result of favorable entropic and steric factors.
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20. Analog **24** (7*S*-epimer of **2**) was synthesized by a method parallel to that used for the modified preparation **1** and **2**[13] by employing the minor diastereomer[13] of the magnesium-catalyzed Mukaiyama aldol reaction.
21. Detailed X-ray crystallographic data are available from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.