Synthesis and MMP-Inhibitory Activity of Gelastatin Analogues

by **Ju-Hee Cho** and **Soo Y. Ko***

Department of Chemistry and Division of Molecular Life Sciences, Ewha Womans University, Seoul 120-750, Korea

and

Eugene Oh, **Jae C. Park**, and **Ji U. Yoo**

Samsung Advanced Institute of Technology, Taejon 305-380, Korea

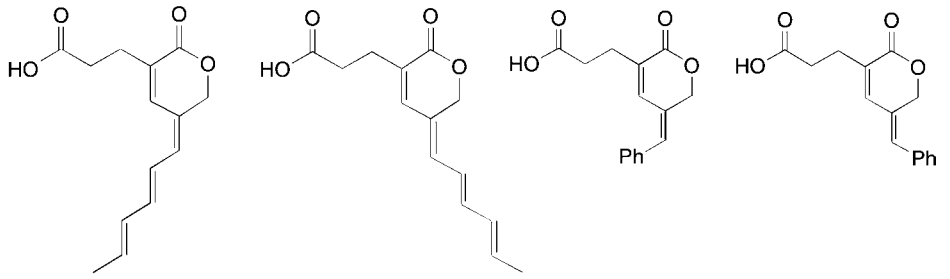
Dedicated to Professor *Dieter Seebach* on the occasion of his 65th birthday

Gelastatin A and B, isolated from culture broth of *Westerdykella multispora* F 50733, have been reported to exhibit MMP-inhibitory activities at a sub-micromolar level. In an effort to exploit this lead, we synthesized gelastatin analogues in which the conjugated triene unit of natural gelastatins was replaced by the benzylidene group. The (*Z*)-isomeric synthetic benzylidene-gelastatin exhibited MMP-inhibitory activities comparable to those reported for the natural products. Therefore, this compound appears to be a viable lead in searching for therapeutically useful MMP inhibitors.

Introduction. – Matrix metalloproteinases (MMPs) are a family of Zn-containing endopeptidases that mediate the breakdown of connective tissue [1]. About 20 different members of MMPs are known so far, which are believed to be involved in various disease states such as cancer, inflammation, and degenerative diseases. The development of MMP inhibitors are, therefore, of therapeutic interest. In particular, MMP-2 and MMP-9, also known as gelatinase A and B, respectively, are two type-IV collagenases and are reported to be closely associated with invasion and metastasis in several cancers [2]. Consequently, inhibitors of these two enzymes are sought as possible anti-cancer agents.

Gelastatin A (**1**) and B (**2**) have been isolated from culture broth of *Westerdykella multispora* F 50733 and, have been reported to exhibit, as a 2:1 mixture, MMP-inhibitory activities at the sub-micromolar level [3]. As this initial report was made with the unseparable mixture of the geometric isomers, the identity of the more bioactive component was not yet known. In addition, the conjugated triene chain of the natural products was thought to be conformationally flexible, not easily amenable to derivatization, and possibly synthetically cumbersome as well. In an effort aimed identify a viable lead based on the natural gelastatin structure, we set out to synthesize gelastatin analogues **3** in which the benzylidene group replaced the triene unit.

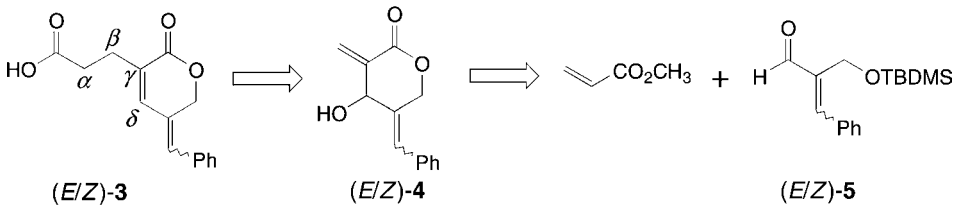
Results and Discussion. – *Synthesis.* To establish the bioactive geometry of the lead structure, we needed to obtain the (*E*/*Z*)-isomers of the benzylidene-substituted gelastatins (*E*)-**3** and (*Z*)-**3** separately. As it was not guaranteed at the outset that the two geometric isomeric products would be separable at the final stage, we devised a



synthetic plan in which each route leading to the respective (*E*/*Z*)-isomeric products would be set apart from each other at an early stage of the synthesis.

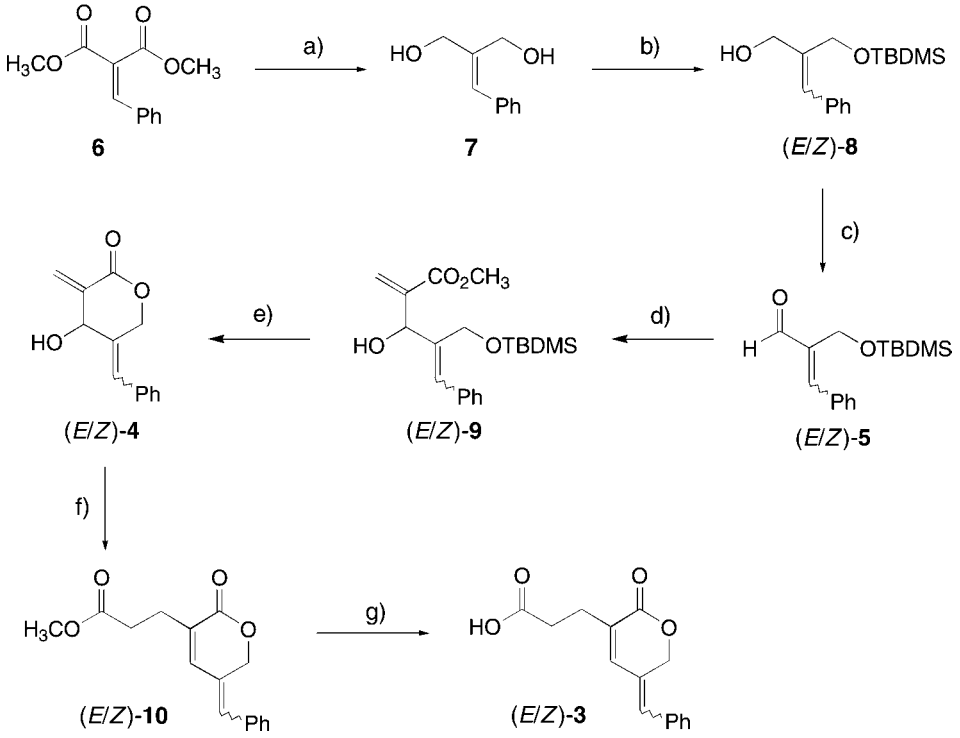
Recognizing the γ,δ-unsaturated carboxy unit in the target molecule, we planned to arrive at the final product *via Claisen* rearrangement of the allylic alcohols **4**, which would be constructed *via Baylis-Hillman* reaction (*Scheme 1*). The required (*E*/*Z*)-aldehydes **5**, it was hoped, would either be prepared individually or be separable chromatographically. It turned out that the two isomeric aldehydes could be synthesized individually, but they were also separable on a silica-gel column and might be more conveniently prepared as a mixture, and then separated. The synthetic sequence is shown in *Scheme 2*.

Scheme 1



Knoevenagel-condensation product **6** was reduced to diol **7** [4]. Monosilyl protection yielded two (*E*/*Z*)-isomeric products **8**, which were separable on a silica-gel column. The geometric assignment based on NMR spectroscopy was inconclusive at this stage, but the issue was settled after the next step. Upon oxidation with pyridinium chlorochromate (PCC), each of the two (*E*/*Z*)-isomeric allylic alcohols, (*E*)-**8** and (*Z*)-**8**, yielded the corresponding aldehyde, (*E*)-**5** and (*Z*)-**5**. Based on the NMR-spectroscopic data, the (*E*)- and (*Z*)-aldehydes were identified. When the oxidation reaction mixture was allowed to stirr for a long time, a mixture of the (*E*/*Z*)-aldehydes was obtained starting from either of the pure allylic alcohols. Clearly, (*E*/*Z*)-isomerization took place under the reaction conditions. As the isomeric aldehydes turned out to be distinguishable on a silica-gel column, the oxidation with PCC might be performed on the (*E*/*Z*)-isomeric mixture of the silyl-protected allylic alcohols, during which (*E*/*Z*)-isomerization might also take place. The (*E*/*Z*)-isomeric aldehydes could then be separated and identified.

Scheme 2



a) $(i\text{-Bu})_2AlCl$ (DIBAL), 67%. b) $(t\text{-Bu})Me_2SiCl$ (TBDMS-Cl), NaH; 37% (*E*)-**8** and 36% (*Z*)-**8**. c) Pyridinium chlorochromate (PCC); 67% (from (*Z*)-**8** to (*E*)-**5**); from (*E*)-**8**, 9% (*Z*)-**5** and 49% (*E*)-**5**. d) 1,4-Diazabicyclo[2.2.2]octane (DABCO)/triethanolamine/$[La(OTf)_3]$; 29% (from (*E*)-**5** to (*E*)-**9**); Bu_3P, 10% (from (*Z*)-**5** to (*Z*)-**9**). e) Bu_4NF (TBAF); 66% (from (*E*)-**9** to (*E*)-**4**); 68% (from (*Z*)-**9** to (*Z*)-**4**). f) MeC(OMe)3, propanoic acid; 47% (from (*E*)-**4** to (*Z*)-**10**); 31% (from (*Z*)-**4** to (*E*)-**10**). g) LiOH; 72% (from (*Z*)-**10** to (*Z*)-**3**); 73% (from (*E*)-**10** to (*E*)-**3**).

With each of the pure aldehydes (*E*)-**5** and (*Z*)-**5**, *Baylis-Hillman* reaction was attempted. A notoriously slow process, this otherwise useful C,C bond-forming protocol has been a focus of much research activity recently, and some improvements have been reported to accelerate the reaction rate [5]. Still, the *Baylis-Hillman* reaction between the aldehyde (*E*)-**5** and methyl acrylate, when performed in the presence of DABCO/triethanolamine/$[La(OTf)_3]$ [6], required 14 d to give the desired adduct (*E*)-**9** in 29% yield. In contrast, the aldehyde (*Z*)-**5**, under the same reaction conditions, underwent a (*Z*/*E*)-isomerization first, for the isomerized aldehyde (*E*)-**5** to react later to give the same *Baylis-Hillman* adduct (*E*)-**9**. While this observation could perhaps be exploited to devise an efficient synthetic plan for the benzylidene derivative (*Z*)-**3**[1]), our task at present was to obtain both the (*E*)- and (*Z*)-isomers of the benzylidene-substituted gelastatins (*E*/*Z*)-**3** separately. Therefore, we needed to find a route to give

[1]) The *Baylis-Hillman* product (*E*)-**9** leads to the final (*Z*)-**3**, and (*Z*)-**9** to (*E*)-**3**. This is due to the change in the substituents' priorities following the *Claisen*-rearrangement step; the C=C bond geometry is retained.

the *Baylis-Hillman* (*Z*)-adduct. Of various sets of the *Baylis-Hillman*-reaction conditions tried, Bu_3P-mediated reaction protocol produced the desired *Baylis-Hillman* (*Z*)-adduct (*Z*)-**9** without a (*Z*/*E*)-isomerization, albeit in very low yield (10%) [7].

Each of the (*E*)- and (*Z*)-*Baylis-Hillman* adducts **9** were desilylated (Bu_4NF) that resulted in a concurrent lactonization to give **4**. Ortho-ester *Claisen* rearrangement produced the desired skeleton **10**. Hydrolysis of each of the (*E*/*Z*)-isomers yielded the desired benzylidene-substituted gelastatin **3**. It turned out that the two geometric isomers, (*E*)-**3** and (*Z*)-**3** were distinguishable on a silica-gel TLC plate.

The NMR data of the synthetic benzylidene analogues compared well with those reported for the natural products, reinforcing the validity of our original (*E*/*Z*)-geometric assignment early in the synthesis (*Figure*).

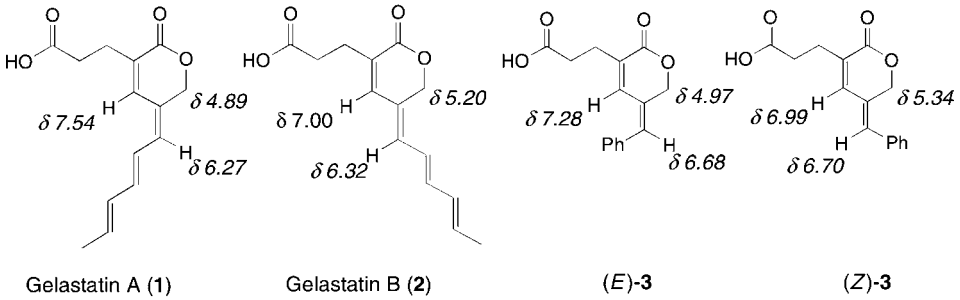


Figure. *NMR Data of natural products* **1** *and* **2** *and of synthetic benzylidene analogues* (E)-**3** *and* (Z)-**3**

MMP-Inhibitory Activity. The synthetic benzylidene-substituted gelastatins (*E*)-**3** and (*Z*)-**3** were bioassayed against purified MMP-2 and MMP-9 according to a protocol based on the cleavage of the fluorogenic peptide MCA-Pro-Leu-Gly-Dpa-Ala-Arg-NH_2 [8]. Compound (*Z*)-**3** was observed to exhibit an inhibitory activity of IC_{50} 1.98 μM against MMP-2 and IC_{50} 17.6 μM against MMP-9; for (*E*)-**3**, it was IC_{50} 21.5 μM against MMP-2 and IC_{50} 279 μM against MMP-9.

Conclusions. – We synthesized gelastatin analogues in which the conjugated triene unit of natural gelastatins was replaced by the benzylidene group. The isomer (*Z*)-**3** exhibited an inhibitory activity of IC_{50} 1.98 μM against MMP-2. Therefore, this compound appears to be a viable lead in searching for therapeutically useful MMP inhibitors. While the present synthesis had been designed to produce both the (*E*)- and (*Z*)-isomers of the benzylidene-substituted gelastatins separately, future work may be focused on the production of more bioactive (*Z*)-isomeric derivatives, which would also include various substitution patterns on the aromatic ring covering wide ranges of electronic and steric effects [9]. The synthetic scheme described herein may not be ideal for this kind of structural optimization effort, as it would be served better with a more divergent synthetic strategy. The results with (*Z*)-**3** are encouraging enough to warrant further studies along these lines.

Experimental Part

Reduction of Dimethyl 2-Benzylidenepropanedioate (**6**) *to 2-Benzylidenepropane-1,3-diol* (**7**). Compound **6** (2.243 g, 10.2 mmol) was dissolved in toluene (50 ml), and the soln. was cooled in an ice bath. DIBAL (1.0M soln. in toluene, 50 ml, 50 mmol) was added slowly. The mixture was stirred at 0° for 5 h. The reaction was quenched by adding 1.0N HCl. The mixture was extracted with AcOEt and the org. phase washed with brine. Following drying (Na_2SO_4) and concentration, the crude product was purified by CC (silica-gel; hexane/AcOEt 1:3) to afford **7** (1.125 g, 67%). ^{1}H-NMR ($CDCl_3$): 3.46 (br., 2 H); 4.34 (*d*, *J* = 11.0, 4 H); 6.58 (*s*, 1 H); 7.25 (*m*, 5 H).

Silylation of **7** *to Give 2-[(*E/Z*)-Benzylidene]-3-[(*tert*-butyl)dimethylsilyloxy]propan-1-ol* ((*E*/*Z*)-**8**). Compound **7** (1.00 g, 6.1 mmol) was dissolved in THF (12 ml), and NaH (55%, 265 mg, 6.1 mmol) was added. The mixture was stirred at r.t. for 45 min. TBDMS-Cl (916 mg, 6.1 mmol) was added, and the entire mixture was stirred at r.t. for further 2 h. It was diluted with AcOEt and washed with 10% K_2CO_3 soln., then with brine. Following drying (Na_2SO_4) and concentration, the crude product was purified by CC (silica-gel; hexane/AcOEt 5:1) to afford (*E*)-**8** and (*Z*)-**8**[2]).

*Data of (*E*)-***8***:* 632 mg (37%). R_f (hexane/AcOEt 4:1) 0.54. ^{1}H-NMR ($CDCl_3$): 0.14 (*s*, 6 H); 0.95 (*s*, 9 H); 2.44 (br., 1 H); 4.35 (*s*, 2 H); 4.41 (*d*, *J* = 1.3, 2 H); 6.61 (*s*, 1 H); 7.31 (*m*, 5 H).

*Data of (*Z*)-***8***:* 605 mg (36%). R_f (hexane/AcOEt 4:1) 0.42. IR: 3250, 3056, 2859, 1712, 1458, 1412, 1069. ^{1}H-NMR ($CDCl_3$): 0.06 (*s*, 6 H); 0.90 (*s*, 9 H); 2.50 (br., 1 H,); 4.35 (*s*, 2 H); 4.49 (*s*, 2 H); 6.62 (*s*, 1 H); 7.31 (*m*, 5 H).

*Oxidation of (*E/Z*)-***8*** to Give 2-[(*E/Z*)-Benzylidene]-3-[(*tert*-butyl)dimethylsilyloxy]propanal* ((*E*/*Z*)-**5**). Compound (*Z*)-**8** (5.37 g, 19.3 mmol) was dissolved in CH_2Cl_2 (220 ml). PCC (8.35 g, 38.66 mmol) and *Celite* (8.39 g) were added. The mixture was stirred for 4 h, then filtered through a pad of silica-gel, which was then washed with CH_2Cl_2. The filtrate was concentrated and the crude product was purified by CC (silica-gel; hexane/AcOEt 9:1) to afford the corresponding aldehyde (*E*)-**5** (3.60 g, 67%). The isomeric alcohol (*E*)-**8** was oxidized likewise to afford the corresponding aldehyde (*Z*)-**5** (9%) together with the (*Z*/*E*)-isomerized product (*E*)-**5** (49%), which were separated by CC (silica-gel; hexane/AcOEt 12:1).

*Data of (*E*)-***5***:* R_f (hexane/AcOEt 5:1) 0.65. IR: 3068, 2956, 2931, 2858, 1712, 1684, 1469, 1362, 1264, 1081. ^{1}H-NMR ($CDCl_3$): 0.13 (*s*, 6 H); 0.91 (*s*, 9 H); 4.48 (*s*, 2 H); 7.41 (*s*, 1 H); 7.44 (*m*, 3 H); 7.77 (*m*, 2 H); 9.60 (*s*, 1 H).

*Data of (*Z*)-***5***:* R_f (hexane/AcOEt 5:1) 0.79. ^{1}H-NMR ($CDCl_3$): 0.13 (*s*, 6 H); 0.96 (*s*, 9 H); 4.52 (*d*, *J* = 2.0, 2 H); 7.40 (*m*, 5 H); 7.81 (*s*, 1 H); 9.89 (*s*, 1 H).

Baylis-Hillman *Reaction of (*E*)-***5*** to Give Methyl (*E*)-4-{[(*tert*-Butyl)dimethylsilyloxy]methyl}-3-hydroxy-2-methylene-5-phenylpent-4-enoate* ((*E*)-**9**). Compound (*E*)-**5** (1.45 g, 5.26 mmol) was placed in a flask. Methyl acrylate (0.47 ml, 5.26 mmol), DABCO (590 mg, 5.26 mmol), [$La(OTf)_3$] (154 mg, 0.263 mmol), and triethanolamine (0.35 ml, 2.63 mmol) were added successively. A minimum amount of MeCN (0.4 ml) was added to keep the soln. homogeneous. An equiv. of methyl acrylate (5.26 mmol) was added at 48-h interval for 14 d. The mixture was diluted with AcOEt and washed with 10% citric acid, then with brine. Following drying (Na_2SO_4) and concentration, the crude product was purified by CC (silica-gel; hexane/AcOEt 10:1, then 4:1) to recover unreacted (*E*)-**5** (47%), and to afford (*E*)-**9** (536 mg, 29%). R_f (hexane/AcOEt 5:1) 0.32. IR: 3481, 2953, 2931, 2858, 1724, 1630, 1493, 1440, 1391, 1259, 1194, 1151, 1052. ^{1}H-NMR ($CDCl_3$): 0.05 (*s*, 6 H); 0.90 (*s*, 9 H); 3.75 (*s*, 3 H); 3.59 (*s*, 1 H); 4.35 (*s*, 2 H); 5.29 (*d*, *J* = 4.4, 1 H); 6.10 (*t*, *J* = 1.4, 1 H); 6.42 (*s*, 1 H); 6.76 (*s*, 1 H); 7.30 (*m*, 5 H). MS: 363 ($[M+H]^+$).

Baylis-Hillman *Reaction of (*Z*)-***5*** to Give (*Z*)-***9***.* Aldehyde (*Z*)-**5** (501 mg, 1.81 mmol) was dissolved in toluene (20 ml). Methyl acrylate (0.49 ml, 5.43 mmol) was added, followed by Bu_3P (0.135 ml, 0.543 mmol). The mixture was stirred at r.t. for 48 h, then treated with another portion of methyl acrylate (0.49 ml, 5.43 mmol), and stirred for further 48 h. It was diluted with AcOEt and washed with 10% citric acid, then with brine. Following drying (Na_2SO_4) and concentration, the crude product was purified by CC (silica-gel; hexane/AcOEt 5:1) to isolate unreacted and isomerized aldehyde (*E*/*Z*)-**5** (51%) and to afford (*Z*)-**9** (64 mg, 10%). R_f (hexane/AcOEt 5:1) 0.40. ^{1}H-NMR ($CDCl_3$): 0.10 (*s*, 6 H); 0.92 (*s*, 9 H); 3.62 (*s*, 3 H); 3.75 (*d*, *J* = 7.2, 1 H); 4.12 (*d*, *J* = 12.4, 1 H); 4.43 (*dd*, *J* = 12.2, 1.4, 1 H); 5.54 (*d*, *J* = 7.1, 1 H); 6.03 (*t*, *J* = 1.4, 1 H); 6.38 (*t*, *J* = 1.2, 1 H); 6.73 (*s*, 1 H); 7.36 (*m*, 5 H). MS: 363 ($[M+H]^+$).

*Desilylation/Lactonization of (*E/Z*)-***9*** to Give 5-[(*E/Z*)-Benzylidene]-3,4,5,6-tetrahydro-4-hydroxy-3-methylene-2*H*-pyran-2-one* ((*E*/*Z*)-**4**). Compound (*E*)-**9** (1.34 g, 3.7 mmol) was dissolved in THF (40 ml), and the soln. was cooled in an ice bath. Bu_4NF (1.94 g, 7.4 mmol) was added, and the mixture was stirred at 0° for

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[2]) The (*E*/*Z*)-assignment was made after the subsequent step. See *Results and Discussion*.

20 min. The mixture was diluted with AcOEt and washed with 10% citric acid, then with brine. Following drying (Na_2SO_4) and concentration, the crude product was purified by CC (silica-gel; CH_2Cl_2/acetone 9:1) to afford (*E*)-**4** (530 mg, 66%). The isomeric (*Z*)-**9** was converted likewise to the corresponding lactone (*Z*)-**4** (68%).

*Data of (*E*)-***4***:* R_f (hexane/AcOEt 1:1) 0.33. IR: 3180, 3068, 2987, 1711, 1416, 1350, 1266, 1178, 1027. ^{1}H-NMR ($CDCl_3$): 2.63 (br., 1 H); 5.04 (*d*, *J* = 14.7, 1 H); 5.09 (*s*, 1 H); 5.25 (*d*, *J* = 14.7, 1 H); 6.00 (*t*, *J* = 1.2, 1 H); 6.47 (*t*, *J* = 1.1, 1 H); 6.90 (*s*, 1 H); 7.14 (*m*, 2 H); 7.35 (*m*, 3 H).

*Data of (*Z*)-***4***:* R_f (hexane/AcOEt 1:1) 0.33. ^{1}H-NMR ($CDCl_3$): 2.24 (br., 1 H); 4.72 (*d*, *J* = 12.7, 1 H); 5.32 (*m*, 2 H); 5.58 (*s*, 1 H); 6.48 (*s*, 1 H); 6.79 (*s*, 1 H); 7.38 (*m*, 5 H).

Claisen *Rearrangement of (*E/Z*)-***4*** to Give Methyl 3-[5-[(*E/Z*)-Benzylidene]-5,6-dihydro-2-oxo-2*H*-pyran-3-yl]propanoate* ((*Z*/*E*)-**10**). Compound (*E*)-**4** (45 mg, 0.21 mmol) was dissolved in $MeC(OMe)_3$ (5 ml) and propanoic acid (0.015 ml) was added. The mixture was heated to reflux for 15 h. The mixture was diluted with AcOEt and washed with 10% $NaHCO_3$, then with brine. Following drying (Na_2SO_4) and concentration, the crude product was purified by CC (silica-gel; hexane/AcOEt 3:2) to afford (*Z*)-**10** (27 mg, 47%). The isomer (*Z*)-**4** was converted likewise to the corresponding product (*E*)-**10** (31%).

*Data of (*Z*)-***10***:* R_f (hexane/AcOEt 3:2) 0.39. IR: 3056, 2948, 2848, 2658, 1729, 1697, 1443, 1166, 1124, 1017. ^{1}H-NMR ($CDCl_3$): 2.62 (*m*, 2 H); 2.73 (*m*, 2 H); 3.67 (*s*, 3 H); 5.36 (*d*, *J* = 2.1, 2 H); 6.70 (*s*, 1 H); 7.01 (*s*, 1 H); 7.17 (*d*, *J* = 6.9, 2 H); 7.42 (*m*, 3 H). MS: 272 (M^+).

*Data of (*E*)-***10***:* R_f (hexane/AcOEt 5:4) 0.55. ^{1}H-NMR ($CDCl_3$): 2.61 (*m*, 2 H); 2.72 (*m*, 2 H); 3.67 (*s*, 3 H); 4.97 (*d*, *J* = 1.4, 2 H); 6.68 (*s*, 1 H); 7.35 (*m*, 6 H).

*Hydrolysis of (*Z/E*)-***10*** to Give 3-[5-[(Z/E)-Benzylidene]-5,6-dihydro-2-oxo-2*H*-pyran-3-yl]propanoic Acid* ((*Z*/*E*)-**3**). Compound (*Z*)-**10** (9.8 mg, 0.036 mmol) was dissolved in MeOH/H_2O (3:1 (*v*/*v*), 4 ml) and LiOH monohydrate (1.9 mg, 0.043 mmol) was added. The mixture was stirred at r.t. for 14 h, and the reaction was quenched by adding 1.0N HCl. The mixture was diluted with AcOEt and washed with brine. Following drying (Na_2SO_4) and concentration, the crude product was purified by CC (silica-gel; AcOEt then AcOEt/MeOH 9:1) to afford (*Z*)-**3** (6.7 mg, 72%). The isomer (*E*)-**10** was converted likewise to the corresponding product (*E*)-**3** (73%).

*Data of (*Z*)-***3***:* R_f (AcOEt) 0.21. M.p. 127–128°. IR: 3114, 3060, 2632, 1707, 1443, 1231, 1120, 1067. ^{1}H-NMR ($CDCl_3$): 2.69 (*m*, 4 H); 5.34 (*dd*, *J* = 7.4, 2.1, 2 H); 6.70 (*s*, 1 H); 6.99 (*d*, *J* = 6.9, 1 H); 7.17 (*m*, 2 H); 7.42 (*m*, 3 H). MS: 258 (M^+).

*Data of (*E*)-***3***:* R_f (AcOEt) 0.32. ^{1}H-NMR ($CDCl_3$): 2.69 (*m*, 4 H); 4.97 (*s*, 2 H); 6.68 (*s*, 1 H); 7.34 (*m*, 6 H).

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