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PAPER

Synthesis and biological activity of simplified belactosin C analogues†‡

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Successful biochemical studies of the natural products belactosin A and C and their acylated congeners have shown a β-lactonecarboxamide moiety to be a possible core structure of powerful proteasome inhibitors. As a part of further investigations, variously decorated simplified β-lactonecarboxamides have been synthesized in order to understand structure–biological activity relations in detail, to find ways of improving their biological activity and stability and to reduce the complexity of their preparation. Biological tests showed that the best compounds possess a high potential against phytopathogenic fungi in the greenhouse.

Introduction

Since their isolation, members of the belactosin family of proteasome inhibitors have attracted interest from both biological and synthetic points of view. In order to better understand their proteasome inhibitory power and the important structure–activity relationships, the prevailing interactions between such molecules and the proteasome should be known. Towards this end, a protected homobelactosin C was initially cocrystallized with the 20S proteasome from *Saccharomyces cerevisiae*, and the structure of this crystal was elucidated by X-ray diffraction.[1] On the basis of this analysis, it was decided which groups in the inhibitor should be modified in order to improve potentially favorable interactions with the proteasome. With this in mind, several belactosin C congeners bearing various protective groups have meanwhile been synthesized, some of them cocrystallized with the 20S proteasome, and their structures investigated.[2] As those compounds showed interesting biological activities against HeLa cells, further modifications on the β-lactone nucleus and the side chain were initiated in order to achieve two purposes: to increase the biological activity of the molecules and simultaneously decrease the complexity of their preparation. Those parts of the belactosin structure that were considered for modification are marked in Fig. 1.

The most drastic modification would be to replace the whole dipeptide residue on the β-lactonecarboxamide by a relatively small and easily accessible amide moiety which, at the same time, would considerably decrease the cost of the synthesis. Of course, any such modification should not go along without a loss, but with an increase of biological activity. Looking at the structure of the first cocrystallisate, a 3,5-dimethoxybenzylamido group should be a good mimic of the dipeptide residue. Therefore, the [2*R*,3*S*(1*S*)]-*N*-(3,5-dimethoxybenzyl)-3-(1-*sec*-butyl)-4-oxooxetane-2-carboxamide (**2**) was targeted, and in order to determine the influence of the *sec*-butyl group on the biological activity, the analogue **3** bearing an isopropyl residue at the C-4 position of the β-lactone moiety was considered as well (Fig. 2).

As detailed investigations of the biological activities of salinosporamide A had shown, an additional substituent at the C-2 position of the β-lactone unit can be favorable.[3] Accordingly, simplified belactosin analogues bearing an additional methyl group at C-2 were targeted as well. For a better understanding of the structure–activity relationship, the β-lactonecarboxamides both with the (2*R*,3*S*)-configuration as in the natural product as well as the derivatives (2*S*,3*R*)-**4** and **5**–**7** with the opposite configuration (Fig. 2) were approached.

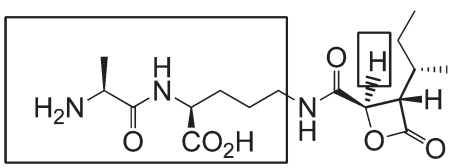

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Fig. 1 Structure of belactosin C with possible modifications.

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† Dedicated to Professor Francois Diederich on the occasion of his 60th birthday.

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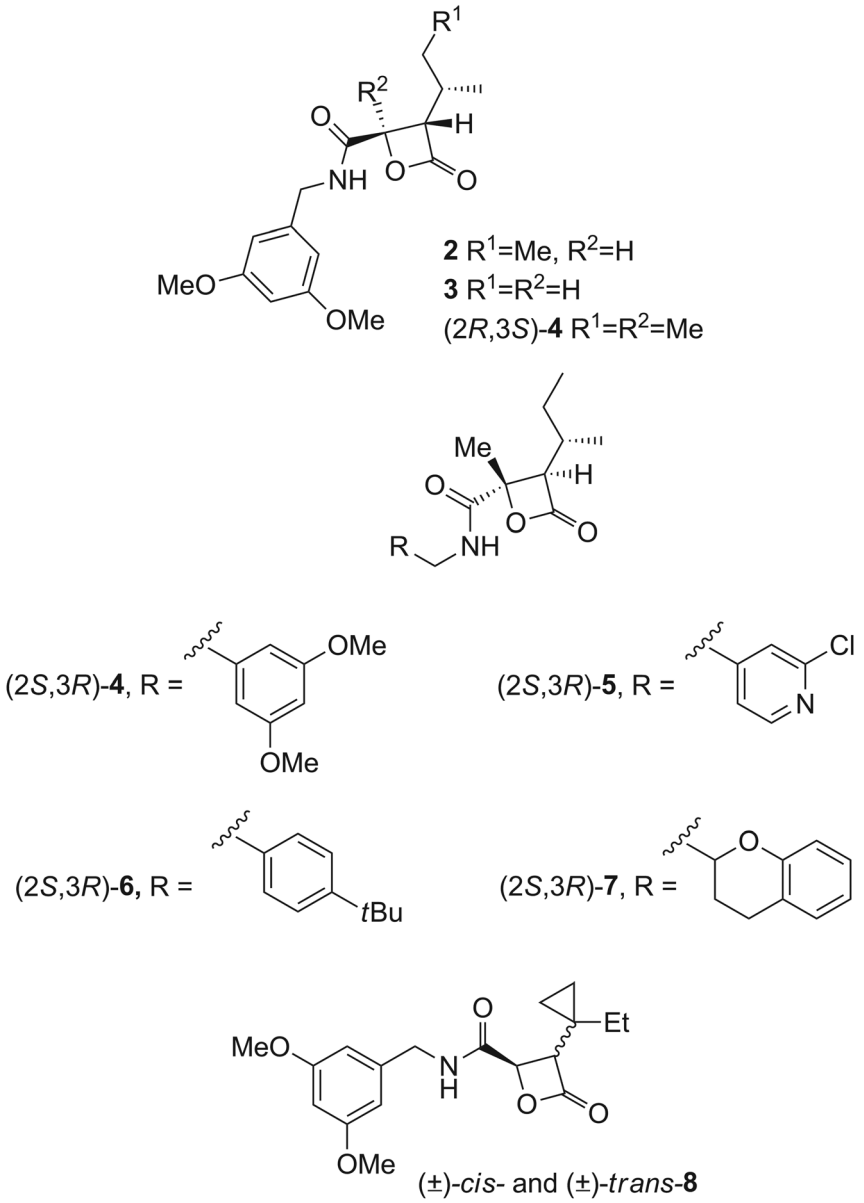


Fig. 2 Targeted belactosin analogues.

Another worthwhile simplification of the belactosin molecule would be the removal of the stereogenic center in the side chain of the β-lactone moiety, and this would be achieved by introducing a 1-ethylcyclopropyl residue instead of the *sec*-butyl group. This looked particularly promising as the structure of the cocrystallized homobelactosin C[1] revealed enough space for a sterically more demanding side chain.

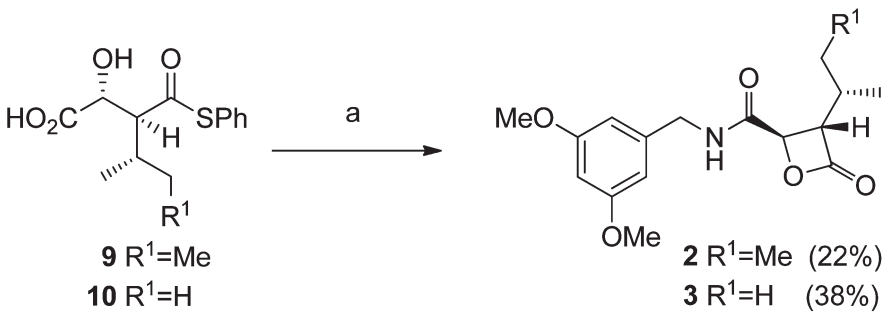
Results and discussion

Synthesis of simplified belactosin analogues

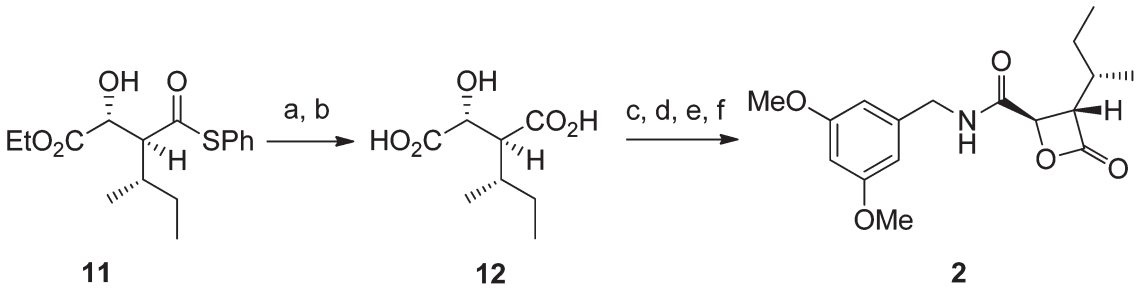
The β-lactonecarboxamides **2** and **3** were prepared adopting the established protocol for the condensation of the malic acid derivatives **9** and **10** with the belactosin dipeptide fragment[1] to the condensation with 3,5-dimethoxybenzylamine, which also occurred with immediately ensuing β-lactonization (Scheme 1).

Since the yields of 22 and 38%, respectively, were unsatisfactory, another approach to **2** was developed. Towards that, the free malic acid **12** was prepared by a two-step hydrolysis of the phenylthio ethyl ester **11**, and the resulting **12** was regioselectively converted to its 3,5-dimethoxybenzylamide by treatment with trichloroacetic acid anhydride, then 3,5-dimethoxybenzylamine and finally sodium hydroxide. The monoamide of the β-hydroxy acid was eventually lactonized by treatment with benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) in the presence of triethylamine. The overall yield of **2** in this sequence was 41% (Scheme 2).

The belactosin analogues **4**–**7** bearing a methyl group in the second position of the β-lactone ring were synthesized from the readily accessible (*S*)-3-methylpentanethioic acid *S*-phenyl ester (**13**). After an aldol reaction with *tert*-butyl pyruvate with immediately ensuing lactone ring closure of the intermediate **14**, a mixture of all four possible diastereomeric *tert*-butyl



Scheme 1 Synthesis of 4-oxooxetane-2-carboxamides **2** and **3**. Reagents and conditions: (a) 3,5-Dimethoxybenzylamine, EDC, HOAt, TMP, −30 to 0 °C.



Scheme 2 An alternative preparation of the oxooxetanecarboxamide **2**. Reagents and conditions: (a) CF_3CO_2Ag, THF–H_2O (4 : 1), 55 °C, 16 h, 89%. (b) 10% aq. HCl, dioxane (1 : 6), 60 °C, 51 h, 90%. (c) $(CCl_3CO)_2O$, dioxane, 75 °C, 3 h. (d) 3,5-Dimethoxybenzylamine, THF, 0 °C, 1 h. (e) aq. NaOH, 16 h, r.t. (f) Benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), Et_3N, CH_2Cl_2, 0 °C to r.t., 3 h, 51% over 3 steps.

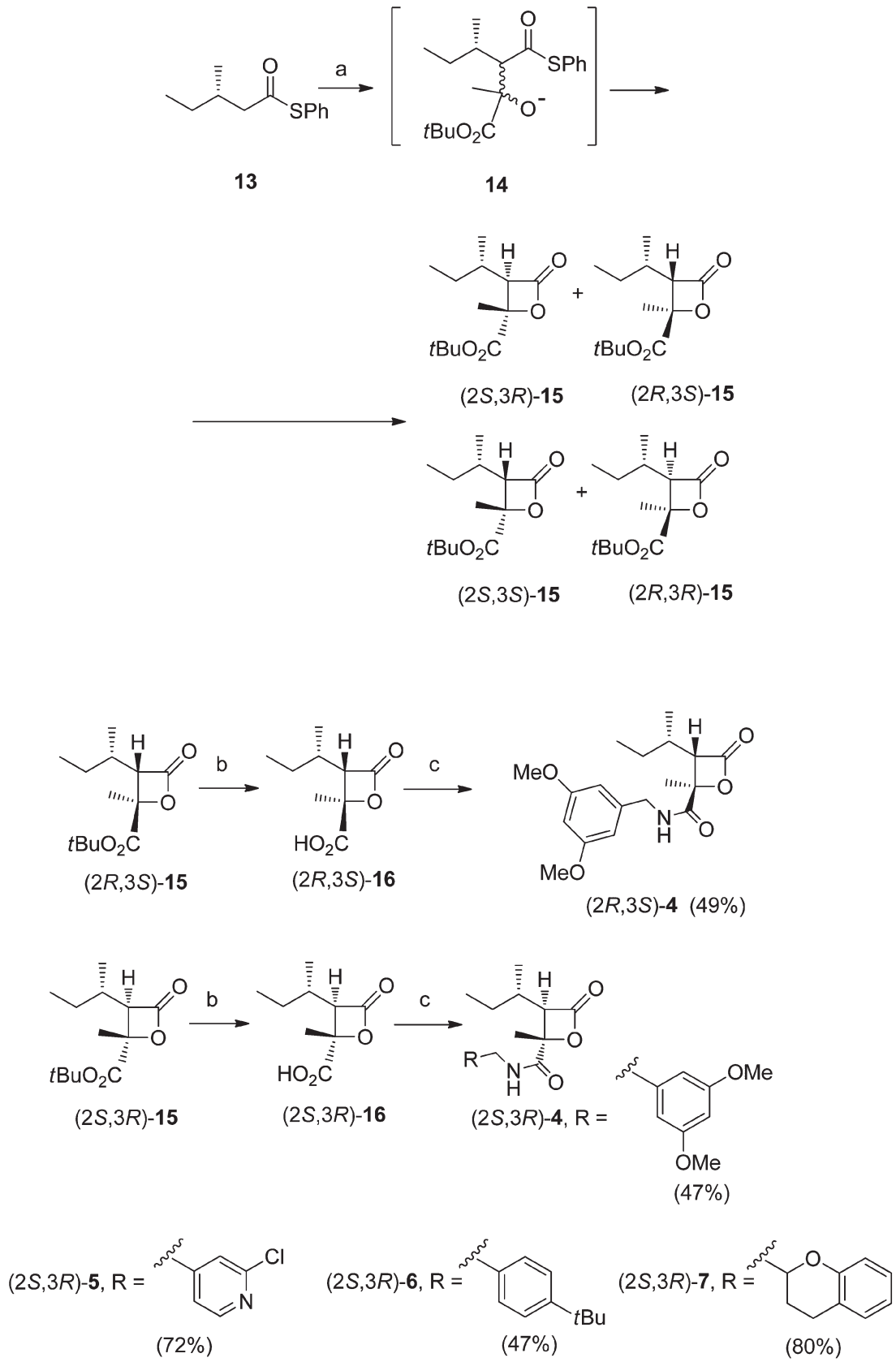
2-oxooxetane-4-carboxylates **15** was obtained with the diastereomer (2*S*,3*R*)-**15** predominating. The pure (2*S*,3*R*)-**15** could be obtained by crystallization of the mixture from hexane–diethyl ether. The second diastereomer, (2*R*,3*S*)-**15** with a *trans*-orientation of the *tert*-butoxycarbonyl and the *sec*-butyl substituent on the β-lactone ring, was isolated in pure form from the mixture by repeated column chromatography and recrystallizations. Subsequent cleavage of the *tert*-butyl ester groups in both *trans*-isomers led to the diastereomeric acids (2*S*,3*R*)-**16** and (2*R*,3*S*)-**16**, which were condensed with the corresponding amines to give the oxooxetanecarboxamides (2*S*,3*R*)-**4**, (2*R*,3*S*)-**4** and **5**–**7** (Scheme 3).

Both the *cis*- and *trans*-3-(1-ethylcyclopropyl)-2-oxooxetanecarboxamides *cis*-**8** and *trans*-**8** were synthesized as racemates from the well-established versatile building block *tert*-butyl 2-chloro-2-cyclopropylideneacetate (**17**).[4] The sequence started with a one-pot transformation involving Michael addition of ethylmagnesium bromide and ensuing aldol reactions with ethyl glyoxylate that led to an inseparable mixture of two diastereomers of **18** (ratio 4 : 1). Then the chlorine substituent was reductively removed by treatment of **18** with ammonium formate in the presence of palladium on charcoal to give **19** (a 4 : 1 mixture of diastereomers). The ethyl ester was selectively hydrolyzed with lithium hydroxide in aqueous tetrahydrofuran, and the free carboxyl group was condensed with 3,5-dimethoxybenzylamine. The diastereomeric carboxamides (*R**,*R**)- and (*R**,*S**)-**20** could be separated by column chromatography, and the individual diastereomers were converted to the oxooxetanecarboxamides *cis*-**8** and *trans*-**8** by acid-catalyzed cleavage of the *tert*-butoxycarbonyl groups and subsequent HATU-mediated cyclization of the β-hydroxy acids (Scheme 4).

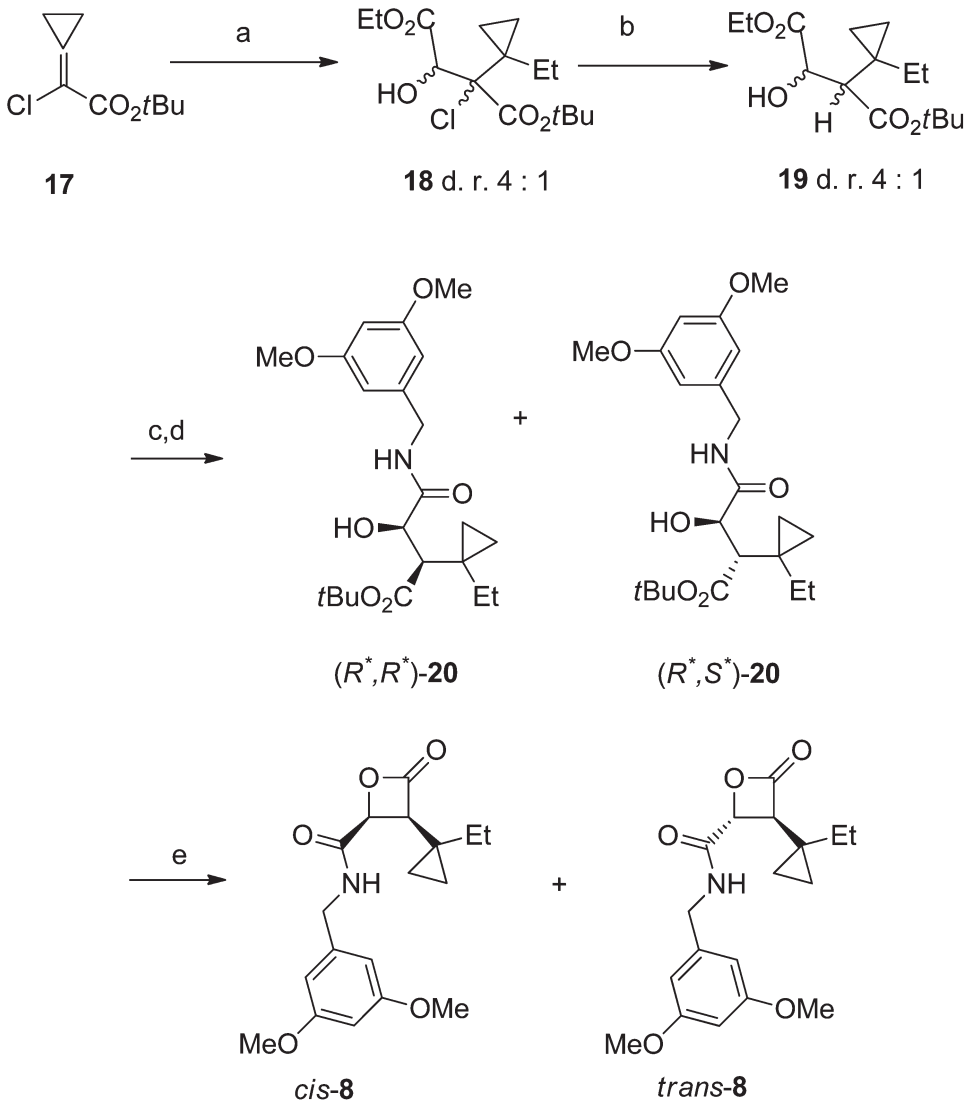
Biological activities of simplified belactosin analogues

Since the *in vivo* biological activity of a given substance in the greenhouse depends not only on the *in vitro* biological activity at the target, but also on such parameters as solubility, cell permeability, and others that cannot be easily taken into account, it was decided to synthesize a library of compounds to be able to choose the most potent ones. Following the previously expressed hypothesis that the binding site in the proteasome is structurally rather flexible, 200 diverse amines were condensed with the hydroxy acid **9**,[5] and the activities of the corresponding amides were determined against phytopathogenic fungi in the greenhouse (Table 1). Many of these amides showed activities comparable to that of the protected belactosine C at the target, but significantly better activity in the greenhouse. This effect may be related to beneficial physicochemical properties and is more strongly affected by the amines used.[6] For example, the target activity appears to be independent of the substitution pattern on the phenyl ring and the spacer between the phenyl ring and the amide nitrogen. Compound **23** turned out to be by far the best compound in the greenhouse.

The α-branched side chain in the 3-position of the lactone appears to be important for good activity, compare *e.g.* the 1-methylpropyl analogue **2** with the 2-methylpropyl derivative (not shown) (Table 2). The isopropyl group in the same position



Scheme 3 Preparation of the 2-oxooxetanecarboxamides (2*R*,3*S*)-**4**, (2*S*,3*R*)-**4** and **5**–**7**. Reagents and conditions: (a) (1) $LiNiPr_2$, THF, −78 °C, then *tert*-butyl pyruvate −78 to 0 °C, 1.5 h, 36%; (2) crystallization from Et_2O–hexane, 22%. (b) CF_3CO_2H, CH_2Cl_2, 16 h, 96%. (c) RCH_2NH_2, EDC.



Scheme 4 Preparation of the 2-oxooxetanecarboxamides **8**. Reagents and conditions: (a) EtMgBr, Et_2O–THF, 0 °C to r.t., then ethyl glyoxylate (50% in toluene), 0 °C to r.t., 15 min, 58%. (b) HCO_2NH_4, Pd/C, MeOH, 0 °C, 2 h, 88%. (c) LiOH, H_2O–THF, r.t., 45 min, 99%. (d) 3,5-Dimethoxybenzylamine, EDC, $EtNiPr_2$, DMF, CH_2Cl_2, −30 °C, 1 h, then r.t., 28 h. (e) (1) CF_3CO_2H, Et_3SiH, CH_2Cl_2, r.t., 7 h; (2) HATU (2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), $EtNiPr_2$, CH_2Cl_2, 0 °C to r.t., 1 h, 67%.

affects the target activity slightly, but resulted in very weak greenhouse efficacy. The *gem*-disubstituted spirocyclopropanated analogues *trans*-**8** and *cis*-**8** are much weaker even at the target, with only little activity for the *trans*-configured lactone *trans*-**8**. Surprisingly weak were also all analogues bearing a methyl group in the 2-position of the lactone.

Conclusion

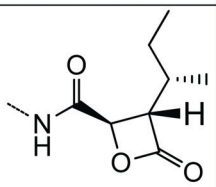
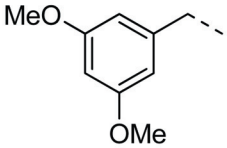
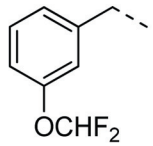
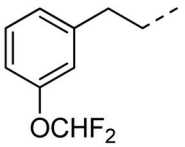
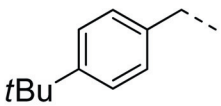
Simplified analogues of the natural dipeptide belactosin show very good activities against the 20S proteasome,[8] which are translated into promising fungicidal activities against some phytopathogenic fungi in the greenhouse. This opens up possible applications in crop protection and underlines once more the potential of natural products[9] for the identification of novel chemical classes with a novel mode of action. Based on various developed methodologies, a first structure–activity relationship has been established. This includes broad variation of the amine part as well as the side chain in the 3-position and modulation of the 2-position of the lactone ring. The target activity reacts in a very flexible way on the variation of the amine part, but has a strong effect on the greenhouse activity. Smaller residues likely help to optimize the physico-chemical properties and thereby improve the transfer of target activity to the greenhouse. Substituents in the 2-position of the lactone are not allowed, whereas the side chain in the 3-position requires an α-branching methyl group.

Experimental part

General remarks

^1H and ^{13}C NMR spectra were recorded at 250, 300, 600 (^1H), and 75.5, 151 MHz (^{13}C), additional APT (attached proton test)

Table 1 Activities of selected belactosin analogues against the 20S proteasome and phytopathogenic fungi in the greenhouse

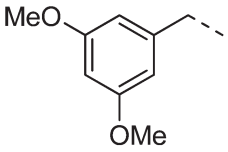
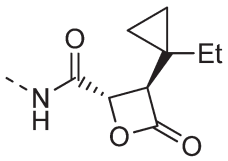
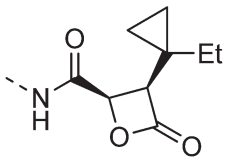
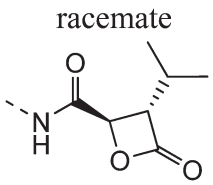
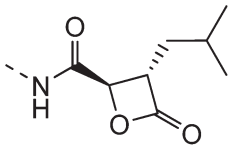
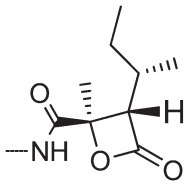
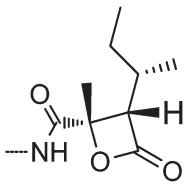
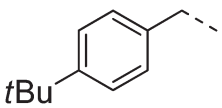
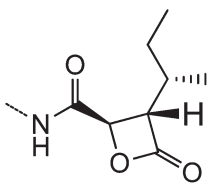
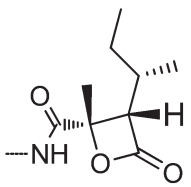
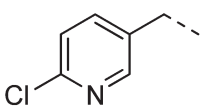
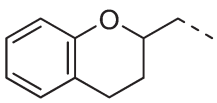
Compound	Side Chain	Pharmacophore	pI_{50} (proteasome)	Oomycetes	Powdery Mildew	Leaf Spots	Basidomycetes	Botrytis
Homobelactosin C	Cbz-Ala-Lys(OBn)		7.3					
2			6.9					
21*			6.6					
22*			6.0					
23*			7.1					
green	>95% at 500 ppm							
yellow	60 – 90% at 500 ppm							
red	<60% at 500 ppm							

[a]Compounds **21**, **22** and **23** were synthesized according to the published procedure.[5,7]

on Bruker AM 250, Varian AMX 300 and Inova 600 instruments for $CDCl_3$ solutions if not otherwise specified, chemical shifts δ in ppm, coupling constants J in Hz. IR: Bruker IFS 66 (FT-IR) spectrometer, measured as KBr pellets or as films between KBr plates. MS (ESI): Finnigan MAT 95 spectrometer. Optical rotations: Perkin-Elmer 241 digital polarimeter, 1 dm cell. Starting materials: CH_2Cl_2 was distilled from P_4O_{10}. (2*R*,3*S*,4*S*)-2-Hydroxy-4-methyl-3-phenylsulfanylcarbonylhexanoic acid ethyl ester (**11**) and (*S*)-3-methylpentanethioic acid *S*-phenyl ester (**13**) were prepared according to de Meijere and Larionov.[5] (2*R*,3*S*)-2-Hydroxy-4-methyl-3-phenylsulfanylcarbonylpentanoic acid ethyl ester (**10**) was prepared according to Evans *et al.*[10] *tert*-Butyl pyruvate was obtained according to Carpino.[11] All other chemicals were used as commercially available. All reactions were conducted under an atmosphere of dry nitrogen. If not stated otherwise, organic extracts were dried over Na_2SO_4. Abbreviations used: BOP: benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; HOAt: 1-hydroxy-7-azabenzotriazole; EDC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; TMP: 2,4,6-trimethylpyridine; HOBt: 1-hydroxybenzotriazole; HATU: *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate.

Ethyl (2*R*,3*S*,4*S*)-2-hydroxy-4-methyl-3-carboxylhexanoate. Silver trifluoroacetate (7.7 g, 35 mmol) was added to a solution of the malic acid derivative **9** (5 g, 17.5 mmol) in THF (20 mL) and H_2O (5 mL). The mixture was stirred at 55 °C for 16 h. The precipitate was filtered off and washed with THF. Then the volatiles were removed from the filtrate under reduced pressure. Water (20 mL) was added to the residue, and this mixture was extracted with CH_2Cl_2. The solution was dried (Na_2SO_4), and the solvent was distilled off to yield 3.0 g (89%) of the product.

Table 2 SAR for analogues of belactosin C with modifications in the side chain in the 3-position and substituents in the 2-position of the lactone

Compound	Side chain	Pharmacophore	pI_{50} (proteasome)
trans-**8**			5.3
cis-**8**		racemate 	4.5
3		racemate 	6.1
Not numbered			4.7
(2*R*,3*S*)-**4**			<4.0
(2*S*,3*R*)-**4**			<4.0
23			7.1
6			<4.0
5			4.4
7			4.7

^{1}H NMR (300 MHz): 0.92 (3 H, t, J = 7 Hz), 1.03 (3 H, d, J = 7 Hz), 1.20–1.30 (1 H, m), 1.27 (3 H, t, J = 7 Hz), 1.44–1.56 (1 H, m), 1.98–2.09 (1 H, m), 2.74 (1 H, dd, J = 8, 3 Hz), 4.17–4.29 (2 H, m), 4.38 (1 H, d, J = 3 Hz) ppm. ^{13}C NMR (125.7 MHz): 11.2 (CH_3), 14.0 (CH_3), 16.1 (CH_3), 27.3 (CH_2), 33.5 (CH), 53.1 (CH), 62.0 (CH_2), 69.3 (CH), 173.8 (C), 178.2 (C) ppm. IR (KBr): $\tilde{\nu}$ = 2967 cm^{-1}, 2937, 1734, 1465, 1386, 1202, 1141, 1096, 1027. $[\alpha]_D^{20}$ = +0.0° (c 1.0, $CHCl_3$). MS (ESI): positive ion mode: m/z = 241 ([M + Na^+], 100). Negative ion mode: m/z = 217 ([M − H^+], 100). HRMS (ESI) [M + H^+] calcd for $C_{10}H_{19}O_5^+$: 219.1233; found: 219.1227.

[2*R*,3*S*(1*S*)]-3-*sec*-Butyl-2-hydroxybutanedioic acid (12). A solution of ethyl (2*R*,3*S*,4*S*)-2-hydroxy-4-methyl-3-carboxylhexanoate (1.6 g, 7.3 mmol) in a mixture of dioxane (95 mL) and 10% aq. HCl (15 mL) was stirred at 60 °C for 50 h. Then the volatiles were removed under reduced pressure, and the solid residue was recrystallized from hexane–EtOAc 1 : 2 to yield 1.2 g (86%) of **12**, m.p. 140–1 °C. ^{1}H NMR (300 MHz, [d_6] DMSO): δ = 0.84 (3 H, t, J = 7 Hz), 0.93 (3 H, d, J = 7 Hz), 1.02–1.21 (1 H, m), 1.39–1.53 (1 H, m), 1.66–1.80 (1 H, m), 2.48–2.55 (1 H, m), 4.16 (1 H, d, J = 6 Hz), 12.20–12.40 (2 H, br m) ppm. ^{13}C NMR (125.7 MHz, [d_6] DMSO): δ = 11.2 (CH_3), 116.3 (CH_3), 26.7 (CH_2), 33.8 (CH), 43.4 (CH_2), 55.4 (CH_3), 63.0 (CH), 70.7 (CH), 99.6 (CH), 105.8 (CH), 139.3 (C), 161.1 (C), 167.8 (C), 169.1 (C) ppm. IR (KBr): $\tilde{\nu}$ = 2964 cm^{-1}, 1702, 1289, 1090, 914. $[\alpha]_D^{20}$ = −11.5° (c 1.0, $CHCl_3$). MS (ESI): positive ion mode: m/z = 235 ([M − H^+ + 2Na^+], 100), 213 ([M + Na^+], 40). Negative ion mode: m/z = 189 ([M − H^+], 100). Calcd for $C_8H_{14}O_5$: C 50.52%, H 7.42%; found: C 50.35%, H 7.55%.

[2*R*,3*S*(1*S*)]-3-*sec*-Butyl-*N*-(3,5-dimethoxybenzyl)-4-oxooxetane-2-carboxamide (2)

Method A. Compound **2** was prepared from 3,5-dimethoxybenzylamine (0.84 g, 5.00 mmol), (2*R*,3*S*,4*S*)-2-hydroxy-4-methyl-3-phenylsulfanylcarbonylhexanoic acid (**9**) (1.40 g, 4.96 mmol), TMP (2.40 g, 19.83 mmol), EDC·HCl (1.90 g, 9.92 mmol) and HOAt (0.70 g, 5.15 mmol) in a mixture of DMF (17 mL) and CH_2Cl_2 (66 mL) adopting the established procedure for belactosin C.[4] Yield: 366 mg (22%).

Method B. A mixture of [2*R*,3*S*(1*S*)]-3-*sec*-butyl-2-hydroxybutanedioic acid (**11**) (0.7 g, 3.7 mmol), trichloroacetic acid anhydride (2.3 g, 7.4 mmol) and dioxane (2 mL) was stirred at 75 °C for 3 h. Then the volatiles were removed under reduced pressure at r.t., the residue was dissolved in THF (10 mL) and the solution cooled to 0 °C. 3,5-Dimethoxybenzylamine (1.4 g, 8.4 mmol) was added, and the reaction mixture was stirred first at 0 °C for 1 h, then at r.t. for 16 h. The volatiles were removed *in vacuo*, the residue was taken up in an aq. solution of NaOH (1.5 mL, 6 N), and the mixture was stirred at r.t. for 16 h. Then the solution was neutralized with conc. HCl, dichloromethane (20 mL) was added, and the mixture was washed with 1 N HCl (2 × 5 mL). The organic phase was dried (Na_2SO_4). The solvent was distilled off, the residue was purified by flash chromatography on silica; by-products were removed by eluting with pentane–diethyl ether (1 : 4), and the desired product was eluted with MeOH. The solvent was distilled off *in vacuo*, and the crude malic acid monoamide was directly used for the next step.

To a solution of the crude malic acid monoamide (0.5 g, 1.5 mmol) in CH_2Cl_2 (40 mL) was added at 0 °C first Et_3N (0.45 g, 4.5 mmol), then BOP (0.92 g, 2.1 mmol). The cooling bath was removed, and the reaction mixture was stirred at r.t. for 3 h. Then the solvent was distilled off *in vacuo* and the residue subjected to column chromatography (eluent pentane–diethyl ether 1 : 1) to give **2** as a colorless solid. Yield 240 mg (51%), R_f = 0.16 [pentane–diethyl ether (1 : 1)], m.p. 104–5 °C. ^{1}H NMR (300 MHz): δ = 0.94 (3 H, t, J = 7 Hz), 1.07 (3 H, d, J = 7 Hz), 1.22–1.40 (1 H, m), 1.57–1.72 (1 H, m), 1.91–2.06 (1 H, m), 3.61 (1 H, dd, J = 4, 8 Hz), 3.77 (6 H, s), 4.29–4.50 (2 H, m), 4.63 (1 H, d, J = 4 Hz), 6.35–6.43 (3 H, m), 6.70–6.78 (1 H, m) ppm. ^{13}C NMR (125.7 MHz): δ = 11.0 (CH_3), 16.3 (CH_3), 26.7 (CH_2), 33.8 (CH), 43.4 (CH_2), 55.4 (CH_3), 63.0 (CH), 70.7 (CH), 99.6 (CH), 105.8 (CH), 139.3 (C), 161.1 (C), 167.8 (C), 169.1 (C) ppm. IR (KBr): $\tilde{\nu}$ = 3302 cm^{-1}, 2958, 1828, 1651, 1601, 1473, 1206, 1151, 915, 838. $[\alpha]_D^{20}$ = +2.2° (c 1.0, $CHCl_3$). MS (ESI) m/z = 366 ([M + $HCOO^-$], 68), 320 ([M − H^+], 100). Calcd for $C_{17}H_{23}NO_5$: C 63.54%, H 7.21%, N 4.36%; found: C 63.34%, H 7.05%, N 4.24%.

[2*R*,3*S*(1*S*)]-3-*sec*-Butyl-*N*-(4-*tert*-butylbenzyl)-4-oxooxetane-2-carboxamide (23). Compound **23** was prepared from 4-*tert*-butylbenzylamine, (2*R*,3*S*,4*S*)-2-hydroxy-4-methyl-3-phenylsulfanylcarbonylhexanoic acid (**9**), TMP, EDC·HCl and HOAt adopting the established procedure for belactosin C.[5] Yield: 14%. ^{1}H NMR (300 MHz, CD_3CN): δ = 0.91 (3 H, t, J = 7 Hz), 1.01 (3 H, d, J = 7 Hz), 1.29 (9 H, s), 1.50–1.65 (1 H, m), 1.84–1.97 (3 H, m), 3.66 (1 H, dd, J = 4, 8 Hz), 4.25–4.47 (2 H, m), 4.69 (1 H, d, J = 4 Hz), 7.21 (2 H, d, J = 8 Hz), 7.38 (2 H, d, J = 8 Hz) ppm. MS (ESI) m/z = 318 ([M + H], 100).

(2*R*,3*S*)-2-Hydroxy-4-methyl-3-phenylsulfanylcarbonylpentanoic acid (10). To a solution of ethyl (2*R*,3*S*)-2-hydroxy-4-methyl-3-phenylsulfanylcarbonylpentanoate[5] (2.90 g, 9.8 mmol) in dioxane (70 mL) was added 10% aq. HCl (11.6 mL), and the mixture was stirred at 55–60 °C for 50 h. It was then concentrated to dryness under reduced pressure at 45 °C, and the solid residue was recrystallized from EtOAc (10 mL) and hexane (30 mL) to give the acid **10** (2.05 g, 78%) as a colorless solid.

$[\alpha]_D^{20}$ = +11.9° (c = 0.62, $CHCl_3$). ^{1}H NMR (300 MHz): δ = 1.10 (3 H, d, J = 6.5 Hz), 1.12 (3 H, d, J = 6.6 Hz), 2.25–2.41 (1 H, m), 2.88 (1 H, dd, J = 9.4, 3.0 Hz), 4.50 (1 H, d, J = 3.0 Hz), 7.27–7.30 (2 H, m), 7.35–7.39 (3 H, m), 10.7 (1 H, br s) ppm. ^{13}C NMR (75.5 MHz): δ = 20.2 (CH_3), 21.1 (CH_3), 28.3 (CH), 61.9 (CH), 70.2 (CH), 126.5 (C), 129.3 (CH), 129.8 (CH), 134.3 (CH), 177.5 (C), 200.5 (C) ppm. IR (KBr): $\tilde{\nu}$ = 3437 cm^{-1}, 3020, 2085, 1676, 1215, 756. MS (ESI): m/z = 842.8 (38) [M + K^+], 574.9 (100) [2M + K^+], 290.9 (16) [M + Na^+]; negative ion mode: 841.0 (57) [3M + K^+ − 2H^+], 534.8 (100), 266.8 (9) [M − H^+]. $C_{13}H_{16}O_4S$ (268.33) calcd C 58.19, H 6.01; found C 57.99, H 5.79.

(2*R*,3*S*)-*N*-(3,5-dimethoxybenzyl)-3-isopropyl-4-oxooxetane-2-carboxamide (3). A solution of the acid **10** (161 mg, 0.6 mmol) in CH_2Cl_2 (8 mL) was added to a solution of 3,5-dimethoxybenzylamine (100.2 mg, 0.6 mmol) in CH_2Cl_2 (2 mL) at −30 °C. HOAt (0.085 g, 0.603 mmol), TMP (0.146 g, 1.2 mmol), and EDC (186 mg, 1.2 mmol) were then added, the reaction mixture was stirred at −30 °C for 6 h, then at r.t. for 30 h. It was then

concentrated under reduced pressure. The oily residue was purified by column chromatography [hexane–EtOAc (2 : 1)] to give the desired product **3** (70 mg, 38%) as a colorless solid.

R_f = 0.30 (hexane–EtOAc 2 : 1). $[\alpha]_D^{20}$ = +0.4° (*c* = 0.8, $CHCl_3$). ^1H NMR (300 MHz): δ = 1.11 (3 H, d, *J* = 6.7 Hz), 1.13 (3 H, d, *J* = 6.7 Hz), 2.15–2.26 (1 H, m), 3.50 (1 H, dd, *J* = 8.3, 4.5 Hz), 3.78 (6 H, s), 4.34 (1 H, dd, *J* = 14.6, 5.6 Hz), 4.47 (1 H, dd, *J* = 14.6, 6.2 Hz), 4.63 (1 H, d, *J* = 4.5 Hz), 6.38 (1 H, m), 6.42 (2 H, m), 6.75 (1 H, br s) ppm. ^{13}C NMR (75.5 MHz): δ = 19.4 (CH_3), 20.0 (CH_3), 27.9 (CH), 43.3 (CH_2), 55.3 (CH_3), 64.4 (CH), 71.2 (CH), 99.5 (CH), 105.8 (CH), 139.3 (C), 161.1 (C), 167.7 (C), 168.7 (C) ppm. IR (film): $\tilde{\nu}$ = 3056 cm^{-1}, 2963, 2882, 1838, 1611, 1521, 1465, 1386, 1206, 1153, 1066. MS (ESI): *m*/*z* = 637.2 (85) [2M + Na^+], 330.1 (100) [M + Na^+]. HRMS (ESI) [M + Na^+] calcd for $C_{16}H_{21}NO_5Na$: 330.1312; found: 330.1319.

***tert*-Butyl [2*S*,3*R*(1*S*)]-3-*sec*-Butyl-2-methyl-4-oxooxetane-2-carboxylate ((2*S*,3*R*)-15) and *tert*-butyl [2*R*,3*S*(1*S*)]-3-*sec*-butyl-2-methyl-4-oxooxetane-2-carboxylate ((2*R*,3*S*)-15).** *n*-Butyllithium (2.5 M solution in hexane, 4.2 mL, 10.5 mmol) was added dropwise at 0 °C over 5 min to a solution of diisopropylamine (1.55 mL, 11.0 mmol) in THF (50 mL). After 15 min, the ice bath was replaced with a dry ice–acetone bath (−78 °C), and a solution of phenyl (*S*)-3-methylpentanethioate (**13**) (2.08 g, 10.0 mmol) in THF (2 mL) was added dropwise at −78 °C over 15 min. After 30 min, a pre-cooled (−78 °C) solution of *tert*-butyl pyruvate (1.44 g, 10.0 mmol) in THF (15 mL) was added dropwise within 20 min. The reaction mixture was stirred at −78 °C for 40 min and then allowed to warm to 0 °C over 1.5 h. A half-saturated solution of NH_4Cl (50 mL) was then added, and the resulting mixture was partitioned between water (100 mL) and diethyl ether (100 mL). The organic phase was extracted with a 10% solution of K_2CO_3 (2 × 150 mL) and brine (100 mL), dried, filtered and concentrated to afford a yellow oil. Column chromatography on silica gel (pentane–diethyl ether 7 : 1) gave 1.38 g (57%) of a mixture of four diastereomers as a colorless oil (TLC – hexane–diethyl ether 7 : 1, R_f = 0.57). After crystallization from pentane at −24 °C, 560 mg of a colorless solid consisting predominantly of two diastereomers (according to a ^1H NMR spectrum of this mixture) was collected. Repeated column chromatography (pentane–diethyl ether 7 : 1) and crystallization of two fractions from pentane at −24 °C afforded two diastereomers in a pure form.

Diastereomer (2*S*,3*R*)-**15**. m.p. 46 °C, $[\alpha]_D^{20}$ = −11.6° (*c* = 0.25, $CHCl_3$). ^1H ($CDCl_3$, 300 MHz): δ = 0.95 (3 H, t, *J* = 7.3 Hz), 0.96 (3 H, d, *J* = 6.6 Hz), 1.26 (1 H, m), 1.52 (9 H, s), 1.71 (3 H, s), 1.96 (2 H, m), 3.41 (1 H, d, *J* = 11.7 Hz) ppm. ^{13}C ($CDCl_3$, 75.5 MHz): δ = 10.4 (CH_3), 16.9 (CH_3), 17.8 (CH_3), 26.1 (CH), 27.8 (CH_3), 31.0 (CH_2), 62.8 (CH), 77.9 (C), 83.2 (C), 169.1 (C), 169.7 (C) ppm. IR (KBr): 2979 cm^{-1}, 1831, 1741, 1371, 1312, 1151, 1047, 880. MS (ESI) *m*/*z* = 506.9 (90) [2M + Na^+], 264.9 (100) [M + Na^+]. HRMS (ESI) [M + Na^+] calcd for $C_{13}H_{22}O_4Na^+$: 265.14103; found: 265.14113.

Diastereomer (2*R*,3*S*)-**15**. m.p. 51–52 °C, $[\alpha]_D^{20}$ = −63.0° (*c* = 1.07, $CHCl_3$). ^1H ($CDCl_3$, 300 MHz): δ = 0.92 (3 H, t, *J* = 7.3 Hz), 0.92 (3 H, d, *J* = 6.6 Hz), 1.23–1.27 (1 H, m), 1.53 (9 H, s), 1.76 (3 H, s), 1.91 (2 H, m), 3.21 (1 H, d, *J* = 11.0 Hz) ppm. ^{13}C ($CDCl_3$, 75.5 MHz): δ = 10.4 (CH_3), 17.0 (CH_3), 23.2 (CH_3), 26.3 (CH), 32.3 (CH_2), 67.3 (CH), 78.9 (C), 83.6 (C), 168.5 (C), 168.9 (C) ppm. IR (KBr): 2982 cm^{-1}, 1831, 1743, 1375, 1310, 1153, 1050, 880. MS (ESI) *m*/*z* = 506.9 (90) [2M + Na^+], 264.9 (100) [M + Na^+]. HRMS (ESI) [M + Na^+] calcd for $C_{13}H_{22}O_4Na^+$: 265.14103; found: 265.14110.

[2*S*,3*R*(1*S*)]-3-*sec*-Butyl-2-methyl-4-oxooxetane-2-carboxylic acid ((2*S*,3*R*)-16). To a cooled solution of the β-lactonecarboxylate (2*S*,3*R*)-**15** (667 mg, 2.76 mmol) in dichloromethane (18.7 mL) was added dropwise at 0 °C trifluoroacetic acid (18.7 mL). The mixture was stirred at this temperature for 16 h, then the solvents were removed *in vacuo*, and the residue azeotroped from anhydrous toluene (20 mL). After further concentration, the mixture became a colorless solid, which was used without further purification.

(2*S*,3*R*)-**16**. m.p. 69–70 °C, $[\alpha]_D^{20}$ = −5.6° (*c* = 0.7, $CHCl_3$). ^1H ($CDCl_3$, 300 MHz): δ = 0.96 (3 H, t, *J* = 7.3 Hz), 0.98 (3 H, d, *J* = 6.6 Hz), 1.29 (1 H, m), 1.80 (3 H, s), 1.94 (1 H, m), 2.05 (m, 1 H), 3.56 (d, *J* = 11.7 Hz, 1 H), 10.17 (br s, 1 H) ppm. ^{13}C ($CDCl_3$, 125 MHz): δ = 10.4 (CH_3), 16.9 (CH_3), 17.6 (CH_3), 26.2 (CH), 31.1 (CH_2), 63.5 (CH), 77.1 (C), 168.1 (C), 176.2 (C) ppm. IR (KBr): 2975 cm^{-1}, 1828, 1734, 1467, 1381, 1309, 1194, 1101, 1042, 1016, 933, 881, 819, 733. MS (ESI) *m*/*z* = 187.1 (6) [M + H^+], 393.1 (100) [2M − 2H + Na^+], 371.0(50) [2M − H^+], 185.0 (21) [M − H^+]. HRMS (ESI) [M + Na^+] calcd for $C_9H_{14}O_4Na^+$: 209.07843; found: 209.07851.

[2*R*,3*S*(1*S*)]-3-*sec*-Butyl-2-methyl-4-oxooxetane-2-carboxylic acid ((2*S*,3*R*)-16). [2*R*,3*S*(1*S*)]-3-*sec*-Butyl-2-methyl-4-oxooxetane-2-carboxylic acid was synthesized according to the same procedure as (2*S*,3*R*)-**16**.

(2*R*,3*S*)-**16**. m.p. 92–93 °C, $[\alpha]_D^{20}$ = −94.3° (*c* = 0.28, $CHCl_3$). ^1H ($CDCl_3$, 300 MHz): δ = 0.93 (3 H, t, *J* = 7.3 Hz), 0.95 (3 H, d, *J* = 6.6 Hz), 1.28 (1 H, m), 1.86 (3 H, s), 1.89 (1 H, m), 1.99 (1 H, m), 3.36 (1 H, d, *J* = 11.0 Hz), 9.58 (1 H, br s) ppm. ^{13}C ($CDCl_3$, 125 MHz): δ = 10.4 (CH_3), 16.9 (CH_3), 22.9 (CH_3), 26.4 (CH), 32.6 (CH_2), 68.0 (CH), 78.5 (C), 167.9 (C), 175.0 (C) ppm. IR (KBr): 2970 cm^{-1}, 1828, 1732, 1468, 1384, 1311, 1195, 1101, 1043, 1016, 936, 880, 821, 734. MS (ESI) *m*/*z* = 187.1 (16) [M + H^+], 393.1 (100) [2M − 2H + Na^-], 371.0 (55) [2M − H^-], 185.0 (14) [M − H^-]. HRMS (ESI) [M + Na^+] calcd for $C_9H_{14}O_4Na^+$: 209.07843; found: 209.07849.

[2*S*,3*R*(1*S*)]-3-*sec*-Butyl-*N*-(3,5-dimethoxybenzyl)-2-methyl-4-oxooxetane-2-carboxamide ((2*S*,3*R*)-4). To a cooled solution of the acid (2*S*,3*R*)-**16** (89 mg, 0.48 mmol) in CH_2Cl_2 (4 mL), kept at 0 °C, was added distilled water (4 mL), then HOBt × $2H_2O$ (328 mg, 1.92 mmol) and EDC (183 mg, 0.96 mmol). The biphasic mixture was then stirred rapidly at 0 °C for 10 min, and the organic phase was transferred to a cooled solution (0 °C) of 3,5-dimethoxybenzylamine (80 mg, 0.48 mmol) and TMP (203 mg, 1.44 mmol) in CH_2Cl_2 (4 mL). The water phase was extracted with CH_2Cl_2 (2 × 2 mL), and the combined extracts were added to the same solution with 3,5-dimethoxybenzylamine. The mixture was then further stirred at 0 °C for 2 h, and the solvents were removed *in vacuo*. The product was isolated by column chromatography (pentane–diethyl ether 5 : 1. TLC: pentane–diethyl ether 1 : 1, R_f = 0.59) to give 75 mg (47%) of (2*S*,3*R*)-**4** as a colorless oil. $[\alpha]_D^{20}$ = −0.8° (*c* = 0.78, $CHCl_3$).

^{1}H ($CDCl_3$, 300 MHz): δ = 0.94 (3 H, t, J = 7.3 Hz), 1.04 (3 H, d, J = 6.2 Hz), 1.26 (1 H, m), 1.75 (3 H, s), 1.92 (1 H, m), 2.04 (1 H, m), 3.35 (1 H, d, J = 11.7 Hz), 3.78 (6 H, s), 4.32 (dd, 1 H, J = 14.6, 5.6 Hz), 4.45 (dd, 1 H, J = 14.6, 6.1 Hz), 6.40 (3 H, m), 6.72 (1 H, br s) ppm. ^{13}C ($CDCl_3$, 75 MHz): δ = 10.4 (CH_3), 16.9 (CH_3), 18.2 (CH_3), 26.1 (CH), 31.1 (CH_2), 43.4 (CH), 55.3 (CH_3), 63.3 (CH_2), 79.5 (C), 99.6 (CH), 105.7 (CH), 139.5 (C), 161.1 (C), 168.9 (C), 170.9 (C) ppm. IR (film): 3082 cm^{-1}, 2968, 2867, 1835, 1615, 1528, 1469, 1388, 1210, 1158, 1066, 734. MS (ESI) m/z = 693.3 (46) [2M + Na^+], 432.2 (64), 358.2 (100) [M + Na^+]. HRMS (ESI) [M + Na]$^+$ calcd for $C_{18}H_{25}O_5NNa^+$: 358.1625; found: 358.1629.

[2*R*,3*S*(1*S*)]-3-*sec*-Butyl-*N*-(3,5-dimethoxybenzyl)-2-methyl-4-oxooxetane-2-carboxamide ((2*R*,3*S*)-4). The compound (2*R*,3*S*)-**4** was prepared according to the same procedure as (2*S*,3*R*)-**4** starting from (2*R*,3*S*)-**16** (89 mg, 0.48 mmol). Yield: 78 mg (49%). $[\alpha]_D^{20}$ = −7.1° (c = 0.99, $CHCl_3$). ^{1}H ($CDCl_3$, 300 MHz): δ = 0.86 (3 H, t, J = 7.3 Hz), 1.04 (3 H, d, J = 6.2 Hz), 1.22 (1 H, m), 1.77 (2 H, m), 1.82 (3 H, s), 3.29 (1 H, d, J = 9.9 Hz), 3.78 (6 H, s), 4.36 (1 H, dd, J = 14.5, 5.9 Hz), 4.44 (1 H, dd, J = 14.6, 6.0 Hz), 6.41 (3 H, m), 6.86 (1 H, br s) ppm. ^{13}C ($CDCl_3$, 75 MHz): δ = 10.4 (CH_3), 17.4 (CH_3), 23.9 (CH_3), 25.9 (CH), 32.9 (CH_2), 43.5 (CH), 55.3 (CH_3), 66.9 (CH_2), 80.9 (C), 99.6 (CH), 105.8 (CH), 139.6 (C), 161.1 (C), 168.7 (C), 168.8 (C) ppm. IR (film): 3084 cm^{-1}, 2960, 2861, 1834, 1614, 1530, 1472, 1388, 1211, 1159, 1063, 733. MS (ESI) m/z = 693.3 (46) [2M + Na^+], 432.2 (64), 358.2 (100) [M + Na^+]. HRMS (ESI) [M + Na^+] calcd for $C_{18}H_{25}O_5NNa^+$: 358.1625; found: 358.1628.

[2*S*,3*R*(1*S*)]-3-*sec*-Butyl-*N*-(2-chloropyrid-4-ylmethyl)-2-methyl-4-oxooxetane-2-carboxamide (5). To a cooled solution of the acid (2*S*,3*R*)-**16** (100 mg, 0.54 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added distilled water (5 mL), then HOBt·$2H_2O$ (368 mg, 2.15 mmol) and EDC (206 mg, 1.08 mmol). The biphasic mixture was stirred rapidly at 0 °C for 10 min, followed by dropwise transfer of the organic phase to a cooled solution (0 °C) of (2-chloropyridylmethyl)amine (76 mg, 0.54 mmol) and TMP (228 mg, 1.61 mmol) in CH_2Cl_2 (5 mL). The mixture was then further stirred at this temperature for 2 h, followed by removal of all solvents *in vacuo*. The crude product was directly purified by column chromatography (pentane–diethyl ether 2 : 1) to give 120 mg (72%) of the product **5** as a colorless solid. R_f = 0.60 (diethyl ether). $[\alpha]_D^{20}$ = +8.6° (c = 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.91 (3 H, t, J = 7.5 Hz), 0.98 (d, J = 6.5 Hz, 3 H), 1.30–1.13 (1 H, m), 1.69 (3 H, s), 1.90–1.80 (1 H, m), 1.93–2.05 (1 H, m), 3.28 (1 H, d, J = 11.8 Hz), 4.34 (1 H, dd, J = 15.0, 5.9 Hz), 4.44 (1 H, dd, J = 15.0, 6.0 Hz), 7.08 (1 H, br s), 7.28 (1 H, d, J = 8.1 Hz), 7.59 (1 H, dd, J = 8.4 Hz, J = 2.5 Hz), 8.30 (1 H, d, J = 2.4 Hz) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 10.33 (CH_3), 16.78 (CH_3), 18.12 (CH_3), 26.04 (CH), 31.04 (CH_3), 40.02 (CH_2), 63.33 (CH), 79.45 (C), 124.33 (CH), 132.20 (C), 138.49 (CH), 149.07 (CH), 150.80 (C), 168.81 (C), 171.31 (C) ppm. IR (film): 3262 cm^{-1}, 2971, 1824, 1670, 1541, 1463, 1387, 1200, 1108, 1036, 862, 811, 739. MS (ESI): positive ion mode: m/z = 311.1 (100) [M + H^+]. Negative ion mode: m/z = 309.1 ([M − H], 100), 355.0 ([M + $HCOO^-$], 64). HRMS (ESI) [M + H]$^+$ calcd for $C_{15}H_{20}ClN_2O_3^+$: 311.11570; found: 311.11559.

[2*S*,3*R*(1*S*)]-3-*sec*-Butyl-*N*-(4-*tert*-butylbenzyl)-2-methyl-4-oxooxetane-2-carboxamide (6). To a cooled solution of the acid (2*S*,3*R*)-**16** (82 mg, 0.44 mmol) in CH_2Cl_2 (4 mL), kept at 0 °C, was added distilled water (4 mL), then HOBt·$2H_2O$ (302 mg, 1.76 mmol) and EDC (169 mg, 0.88 mmol). The biphasic mixture was stirred rapidly at 0 °C for 10 min, followed by dropwise transfer of the organic phase to a cooled solution (0 °C) of *tert*-butylbenzylamine (72 mg, 0.44 mmol) and TMP (187 mg, 1.32 mmol) in CH_2Cl_2 (4 mL). The mixture was then further stirred at this temperature for 2 h, followed by removal of all solvents *in vacuo*. The crude material was directly purified by column chromatography (eluent pentane–diethyl ether 5 : 1) to give 69 mg (47%) of the product **6** as a colorless solid. R_f = 0.59 (pentane–diethyl ether 1 : 1). $[\alpha]_D^{20}$ = −12.8° (c = 0.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.94 (3 H, t, J = 7.5 Hz), 1.03 (3 H, d, J = 6.5 Hz), 1.18–1.28 (1 H, m), 1.31 (s, 9 H), 1.73 (s, 3 H), 1.95–1.87 (m, 1 H), 2.05–1.95 (m, 1 H), 3.34 (d, J = 11.8 Hz, 1 H), 4.31 (1 H, dd, J = 14.5, 5.6 Hz), 4.40 (1 H, dd, J = 14.5, 5.9 Hz), 6.69 (br s, 1 H), 7.19 (d, J = 8.1 Hz, 2 H), 7.36 (d, J = 8.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 10.35 (CH_3), 16.82 (CH_3), 18.20 (CH_3), 26.08 (CH), 31.09 (CH_2), 31.23 (CH_3), 34.48 (C), 43.01 (CH_2), 63.24 (CH), 79.53 (C), 125.74 (CH), 127.55 (CH), 134.05 (C), 150.84 (C), 168.97 (C), 170.80 (C) ppm. IR (film): 3339 cm^{-1}, 2965, 1831, 1671, 1534, 1464, 1192, 1016, 865, 823. MS (DCI) m/z = 680.8 ([2M + NH_4^+], 28), 663.7 ([2M + H^+], 2), 349.5 ([M + NH_4^+], 100), 332.4 ([M + H^+], 10). HRMS (ESI) [M + H]$^+$ calcd for $C_{20}H_{30}O_3N$: 332.22192; found: 332.22192.

[2*S*,3*R*(1*S*)]-3-*sec*-Butyl-*N*-(chroman-2-ylmethyl)-2-methyl-4-oxooxetane-2-carboxamide (7). To a cooled solution of the acid (2*S*,3*R*)-**16** (100 mg, 0.54 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added distilled water (5 mL), then HOBt·$2H_2O$ (368 mg, 2.15 mmol) and EDC (206 mg, 1.08 mmol). The biphasic mixture was stirred rapidly at 0 °C for 10 min, followed by dropwise transfer of the organic phase to a cooled solution (0 °C) of (chroman-2-ylmethyl)amine (88 mg, 0.54 mmol) and TMP (228 mg, 1.61 mmol) in CH_2Cl_2 (5 mL). The mixture was then further stirred at this temperature for 2 h, followed by removal of all solvents *in vacuo*. The crude material was directly purified by column chromatography (pentane–diethyl ether 2 : 1. TLC: diethyl ether, R_f = 0.78) to give 144 mg (80%) of the product **7** as a colorless oil. $[\alpha]_D^{20}$ = −15.3° (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.94 (3 H, t, J = 7.5 Hz), 1.02 (3 H, d, J = 6.4 Hz), 1.30–1.13 (2 H, m), 1.73 (3 H, s), 1.85–2.05 (3 H, m), 2.94–2.72 (2 H, m), 3.31–3.51 (1 H, m), 3.34 (1 H, d, J = 11.7 Hz), 3.83–3.67 (1 H, m), 4.05–4.15 (1 H, m), 6.81 (1 H, d, J = 7.9 Hz), 6.87 (1 H, d, J = 7.2 Hz), 6.89 (1 H, br s), 7.04 (1 H, d, J = 7.2 Hz), 7.09 (1 H, t, J = 7.2 Hz) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 10.35 (2 CH_3), 16.83 (CH_3), 16.84 (CH_3), 18.22 (CH_3), 18.26 (CH_3), 24.27 (CH_2), 24.31 (CH_2), 24.84 (CH_2), 25.04 (CH_2), 26.10 (CH), 26.11 (CH), 31.08 (CH_2), 31.10 (CH_2), 43.25 (CH_2), 43.39 (CH_2), 63.32 (CH), 63.34 (CH), 74.23 (CH), 74.36 (CH), 79.49 (C), 79.52 (C), 116.71 (CH), 116.76 (CH), 120.61 (CH), 120.63 (CH), 121.48 (C), 121.51 (C), 127.35 (2 CH), 129.48 (CH), 129.50 (CH),

154.02 (C), 154.08 (C), 168.92 (C), 168.93 (C), 171.27 (C), 171.36 (C) ppm. IR (film): 3351 cm^{-1}, 2965, 1830, 1677, 1583, 1534, 1489, 1458, 1384, 1234, 1108, 1033, 817, 756. MS (ESI): positive ion mode: m/z = 386.3 ([M − H + 2Na$^+$], 100), 354.2 ([M + Na$^+$], 74). Negative ion mode: m/z = 330.3 ([M − H], 100). HRMS (ESI) [M + Na]$^+$ calcd for $C_{19}H_{25}O_4NNa^+$: 354.16758; found: 354.16750.

Ethyl 3-(*tert*-butoxycarbonyl)-3-chloro-3-(1-ethylcyclopropyl)-2-hydroxypropionate (18). Ethylmagnesium bromide (2.8 mL, 6.9 mmol, 2.45 M solution in diethyl ether) was added at 0 °C over 10 min to a solution of *tert*-butyl 2-chlorocyclopropylideneacetate[4c] (**17**) (1.44 g, 6.9 mmol) in THF. This mixture was stirred at 0 °C for 5 min and then allowed to warm up to r.t. It was cooled to 0 °C again, and a solution of ethyl glyoxylate (1.41 g, 50% in toluene, 6.9 mmol) in THF (10 mL) was added. The reaction mixture was stirred at 0 °C for 5 min and then at r.t. for 10 min. It was cooled to 0 °C again, and the reaction was quenched by addition of saturated NH_4Cl. The precipitate was filtered off, the phases were separated, and the aqueous phase was extracted with diethyl ether. The combined organic phases were dried over $MgSO_4$. The volatiles were removed under reduced pressure, and the residue was purified by column chromatography (diethyl ether–hexane 1 : 3) to give the product **18** as a colorless oil. According to its NMR spectrum, it was a 4 : 1 mixture of two diastereomers. Yield (1.29 g, 58%), R_f = 0.27 (diethyl ether–hexane 1 : 3). ^{1}H NMR (250 MHz): 0.35–0.45 (1 H, m), 0.52–0.57 (1 H, m), 0.74 (3 H, t, J = 7 Hz), 0.80–0.84 (1 H, m), 1.03–1.06 (1 H, m), 1.26 (3 H, d, J = 7 Hz), 1.51 (9 H, s), 1.79–1.84 (2 H, m), 3.22 (0.8 H, d, J = 7 Hz), 3.67 (0.2 H, d, J = 10 Hz), 4.21–4.29 (2 H, m), 4.87 (0.2 H, d, J = 10 Hz), 4.98 (0.8 H, d, J = 7 Hz) ppm. ^{13}C NMR (62.5 MHz): 5.86 (CH_2), 7.69 (CH_2), 9.90 (CH_2), 9.99 (CH_3), 14.04 (CH_3), 23.95 (CH_2), 25.81 (C), 27.78 (CH_3), 62.53 (CH_2), 74.23 (CH), 80.27 (C), 83.13 (C), 166.70 (C), 171.43 (C) ppm. MS (DCI) m/z = 338 ([M + NH$_4^+$], 100)/340 ([M + NH$_4^+$], **35**).

Ethyl 3-(*tert*-butoxycarbonyl)-3-(1-ethylcyclopropyl)-2-hydroxypropionate (19). Palladium on carbon (170 mg, 5%) was added to a solution of **18** (435 mg, 1.36 mmol) and ammonium formate (427 mg, 6.78 mmol) in methanol (20 mL) at 0 °C. The mixture was stirred at r.t. for 2 h and after that filtered through a pad of Celite. The Celite was washed with diethyl ether and water. The filtrate was washed with saturated $NaHCO_3$, and the volatiles were removed under reduced pressure. The residue was dissolved in Et_2O, the solution washed with saturated $NaHCO_3$ and NaCl solutions. The ethereal solution was dried over $MgSO_4$, and the volatiles were removed under reduced pressure to give, after column chromatography (diethyl ether–hexane 1 : 3), 340 mg (88%) of the product **19** as a colorless solid. R_f = 0.15 (diethyl ether–hexane 1 : 3). ^{1}H NMR (250 MHz): δ = 0.38–0.42 (3 H, m), 0.58–0.62 (1 H, m), 0.80 (3 H, t, J = 7 Hz), 1.26–1.29 (3 H, m), 1.42–1.44 (9 H, m), 1.44–1.70 (2 H, m), 2.30–2.39 (2 H, m), 3.16 (0.8 H, m), 3.41 (0.2 H, m), 4.18–4.30 (2 H, m), 4.39 (0.2 H, m), 4.58 (0.8 H, m) ppm. ^{13}C NMR (62.5 MHz): δ = 9.25 (CH_2), 10.15 (CH_3), 11.23 (CH_2), 14.07 (CH_3), 20.01 (C), 21.04 (C), 26.61 (CH_2), 26.81 (CH_2), 28.00 (CH_3), 54.54 (CH), 54.88 (CH), 61.52 (CH_2), 61.63 (CH_2), 70.62 (CH), 71.43 (CH), 81.17 (C), 81.56 (C), 171.67 (C), 171.75 (C), 173.28 (C), 178.58 (C) ppm. MS (DCI) m/z = 304 ([M + NH$_4^+$], 100).

3-(*tert*-Butoxycarbonyl)-3-(1-ethylcyclopropyl)-2-hydroxypropionic acid. A solution of LiOH (154 mg, 6.4 mmol) in H_2O (10 mL) was added to a solution of **19** (920 mg, 3.21 mmol) in THF (15 mL) at r.t. Within 45 min the reaction was complete. Then H_2O (20 mL) was added, and the THF was removed from the reaction mixture under reduced pressure. The residue was washed with Et_2O, acidified with 1 N $KHSO_4$, extracted with diethyl ether, washed with saturated NaCl solution and dried over $MgSO_4$. The volatiles were removed under reduced pressure to give 820 mg (99%) of the title compound as a colorless oil. ^{1}H NMR (250 MHz): 0.41–0.50 (3 H, m), 0.60–0.68 (1 H, m), 0.75–0.85 (3 H, m), 1.41–1.42 (9 H, m), 1.40–1.50 (1 H, m), 1.60–1.67 (1 H, m), 2.38–2.53 (1 H, m), 4.41–4.63 (1 H, m), 4.18–4.30 (2 H, m), 4.39 (0.2 H), 4.58 (0.8 H) ppm. MS (DCI) m/z = 276 ([M + NH$_4^+$], 100).

3-(*tert*-Butoxycarbonyl)-*N*-(3,5-dimethoxybenzyl)-3-(1-ethylcyclopropyl)-2-hydroxypropionamide (20). HOAt (380 mg, 2.79 mmol) was added at −30 °C to a solution of 3-(*tert*-butyloxycarbonyl)-3-(1-ethylcyclopropyl)-2-hydroxypropionic acid (720 mg, 2.79 mmol) in CH_2Cl_2 (30 mL) followed by DMF (5 mL), EDC (660 mg, 4.25 mmol) and a solution of 3,5-dimethoxybenzylamine (500 mg, 2.99 mmol) as well as ethyldiisopropylamine (550 mg, 4.25 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at a temperature from −30 to −20 °C for 5 h, and then at r.t. for 28 h. CH_2Cl_2 (60 mL) was added to this mixture, and it was subsequently washed with 1 M aq. $KHSO_4$, H_2O, aq. $NaHCO_3$, aq. NaCl solution and dried over $MgSO_4$. The volatiles were removed under reduced pressure, and the residue was purified by column chromatography (eluent diethyl ether–hexane 4 : 1) to give 1.13 g (99%) of a mixture of two diastereomers that were separated by repeated column chromatography (eluent diethyl ether–hexane 4 : 1).

Major isomer (*R**,*R**)-**16**: R_f = 0.24 (diethyl ether–hexane 4 : 1). ^{1}H NMR (250 MHz): δ = 0.32–0.38 (2 H, m), 0.48–0.52 (1 H, m), 0.61–0.65 (1 H, m), 0.76 (3 H, t, J = 7 Hz), 1.30–1.40 (1 H, m), 1.43 (9 H, s), 1.67–1.80 (1 H, m), 2.39 (1 H, d, J = 5 Hz), 3.76 (6 H, s), 3.96 (1 H, d, J = 3 Hz), 4.33 (1 H, dd, J = 15, 6 Hz), 4.45 (1 H, dd, J = 15, 7 Hz), 4.56 (1 H, dd, J = 5, 3 Hz), 6.33 (1 H, t, J = 2 Hz), 6.42 (1 H, d, J = 2 Hz), 7.05 (1 H, br s) ppm. ^{13}C NMR (62.5 MHz): 9.56 (CH_2), 10.20 (CH_3), 11.22 (CH_2), 20.46 (C), 27.52 (CH_2), 27.92 (CH_3), 43.18 (CH_2), 52.79 (CH), 55.21 (CH_3), 72.16 (CH), 81.57 (C), 99.26 (CH), 105.66 (CH), 140.15 (C), 160.90 (C), 171.36 (C), 174.55 (C) ppm. MS (DCI) m/z = 408 ([M + H$^+$], 100).

Minor isomer (*R**,*S**)-**16**: R_f = 0.28 (diethyl ether–hexane 4 : 1). ^{1}H NMR (250 MHz): δ = 0.35–0.55 (3 H, m), 0.75–0.78 (1 H, m), 0.84 (3 H, t, J = 7 Hz), 1.41 (9 H, s), 1.42–1.64 (2 H, m), 2.91 (1 H, d, J = 2 Hz), 3.77 (6 H, s), 4.23 (1 H, dd, J = 10, 2 Hz), 4.28 (1 H, dd, J = 15, 6 Hz), 4.45 (1 H, dd, J = 15, 7 Hz), 4.75 (1 H, dd, J = 10 Hz), 6.33 (1 H, t, J = 2 Hz), 6.42 (1 H, d, J = 2 Hz), 7.24 (1 H, br s) ppm. ^{13}C NMR (62.5 MHz): 10.06 (CH_2), 10.33 (CH_3), 11.16 (CH_2), 22.21 (C), 27.88 (CH_2), 27.89 (CH_3), 43.14 (CH_2), 50.08 (CH), 55.23 (CH_3), 73.62 (CH), 82.22 (C), 99.21 (CH), 105.35 (CH), 140.45 (C), 160.92 (C),

173.07 (C), 174.93 (C) ppm. MS (DCI) m/z = 408 ([M + H^+], 100).

***trans*-N-(3,5-Dimethoxybenzyl)-3-(1-ethylcyclopropyl)-4-oxo-oxetane-2-carboxamide (*trans*-8).** Trifluoroacetic acid (380 μL, 5.1 mmol) was added to a solution of (*R**,*S**)-**16** (147 mg, 0.361 mmol) and triethylsilane (150 μL, 0.9 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred at r.t. for 7 h. Then the solvent was removed under reduced pressure, toluene was added and removed again under reduced pressure. This operation was repeated two more times. The residue was dissolved in CH_2Cl_2 (5 mL), the solution cooled to 0 °C, ethyldiisopropylamine (170 μL, 1.01 mmol) and HATU (165 mg, 0.99 mmol) were added and the mixture stirred at r.t. for 1.5 h. Then the mixture was washed successively with aq. $NaHCO_3$, H_2O, aq. $KHSO_4$, H_2O, aq. NaCl, and dried over $MgSO_4$. The solvent was removed, and the residue was purified by column chromatography (diethyl ether–hexane 4 : 1) to give 81 mg (67%) of the product *trans*-**8**. R_f = 0.44 (diethyl ether–hexane 1 : 4). ^{1}H NMR (250 MHz): δ = 0.45–0.60 (3 H, m), 0.80–0.84 (1 H, m), 0.92 (3 H, t, J = 7 Hz), 1.33–1.37 (1 H, m), 1.62–1.68 (1 H, m), 3.77 (6 H, s), 3.90 (1 H, d, J = 4.6 Hz), 4.02(1 H, d, J = 4.6 Hz), 4.32 (1 H, dd, J = 5.6, 14.6 Hz), 4.44 (1 H, dd, J = 5.6, 14.6 Hz), 6.35–6.40 (3 H, m), 6.78 (1 H, br s) ppm. ^{13}C NMR (62.5 MHz): δ = 8.83 (CH_2), 9.15 (CH_3), 10.12 (CH_2), 19.37 (C), 28.71 (CH_2), 43.24 (CH_2), 55.23 (2 CH_3), 61.55 (CH), 72.18 (CH), 99.45 (CH), 105.70 (2 CH), 139.30 (C), 161.01 (C), 167.47 (C), 167.92 (C) ppm. IR (KBr): $\tilde{\nu}$ = 2967 cm^{-1}, 1837, 1708, 1669, 1598, 1155. MS (EI) m/z = 333 ([M^+], 25), 261 (15), 166 (45), 152 (34), 151 (100).

***cis*-N-(3,5-Dimethoxybenzyl)-3-(1-ethylcyclopropyl)-4-oxo-oxetane-2-carboxamide (*cis*-8).** Trifluoroacetic acid (870 μL, 11.6 mmol) was added to a solution of (*R**,*R**)-**16** (337 mg, 0.827 mmol) and triethylsilane (340 μL, 2.1 mmol) in CH_2Cl_2 (4 mL). The mixture was stirred at r.t. for 7 h. Then the solvent was removed under reduced pressure, toluene was added and removed again under reduced pressure. This operation was repeated two more times. The residue was dissolved in CH_2Cl_2 (10 mL), the solution cooled to 0 °C, ethyldiisopropylamine (385 μL, 2.32 mmol) and HATU (377 mg, 0.99 mmol) were added, and the mixture was stirred at rt for 1.5 h. Then the mixture was washed successively with aq. $NaHCO_3$, H_2O, aq. $KHSO_4$, H_2O, aq. NaCl and dried over $MgSO_4$. The solvent was removed, and the residue was purified by column chromatography (diethyl ether–hexane 4 : 1) to give 92 mg (33%) of the product *cis*-**8**. R_f = 0.29 (diethyl ether–hexane 1 : 4). ^{1}H NMR (250 MHz): 0.22–0.38 (2 H, m), 0.42–0.46 (1 H, m), 0.80–0.90 (5 H, m), 2.05–2.07 (1 H), 3.77 (6 H, s), 4.40–4.42 (2 H, m), 4.57 (1 H, d, J = 7 Hz), 4.95 (1 H, d, J = 7 Hz), 6.37 (1 H, t, J = 2 Hz), 6.47 (2 H, d, J = 2 Hz), 6.88 (1 H, br s) ppm. ^{13}C NMR (62.5 MHz): 7.55 (CH_2), 10.06 (CH_3), 10.22 (CH_2), 17.89 (C), 27.55 (CH_2), 43.43 (CH_2), 55.23 (2 CH_3), 58.13 (CH), 72.66 (CH), 99.46 (CH), 106.17 (2 CH), 139.25 (C), 160.93 (2 C), 166.25 (C), 168.33 (C), 171.43 (C) ppm. IR (KBr): $\tilde{\nu}$ = 2962 cm^{-1}, 2840, 1829, 1668, 1596, 1533, 1471. MS (EI) m/z = 333 ([M^+], 25), 261 (15), 166 (45), 152 (34), 151 (100).

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