Total Synthesis, Absolute Configuration, and Biological Activity of Xyloallenoide A

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The novel natural product xyloallenoide A, isolated from the marine mangrove endophytic fungus from the South China Sea, and its diastereoisomer xyloallenoide A1, which contain *N*-methyl-substituted amino acids, were synthesized. The absolute configurations of the amino acid units of xyloallenoide A were finally confirmed to be L-Lys, Me-D-Val, and Me-L-Ala. This report represents a practical and attractive alternative for the synthesis of *N*-methyl-substituted cyclotripeptides. In the preliminary bioassay, synthetic xyloallenoide A showed marginal activities against KB ($IC_{50} = 9.6$ μM) and KBv200 cells ($IC_{50} = 10.3$ μM), and xyloallenoide A1 was inactive against KB and KBv200 cells.

Introduction. – The marine microorganisms are a rich source of novel and unusual secondary metabolites [1], many of which have already shown considerable promise for the development as therapeutic agents [2], including agents showing antihypertensive, antioxidative, antithrombotic, and anticancer activities [3].

The cyclopeptide moiety is ubiquitous in many natural and biologically active compounds as well as in advanced organic materials [4]. In the past, the liquid phase synthesis was a successful method to construct these motifs [5]. Meanwhile, the solid-phase synthesis is an important alternative to liquid-phase peptide synthesis [6]. However, to the best of our knowledge, few successful examples have been reported to date on the cyclization of *N*-methyl-subtituted amino acids to peptides, especially for the synthesis of *N*-methyl-substituted cyclotripeptides.

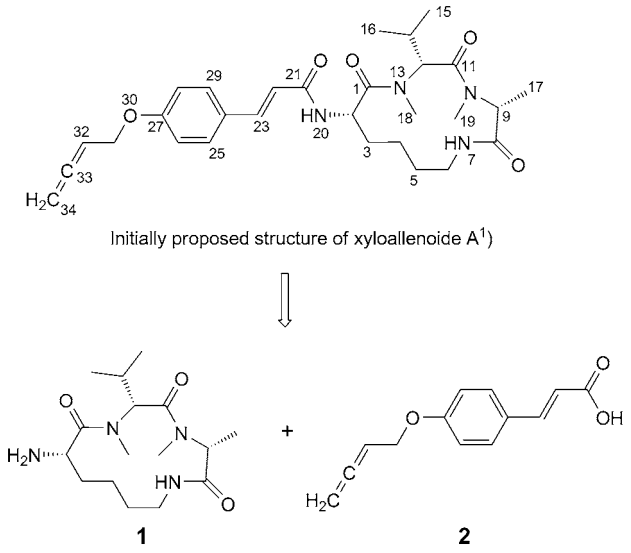
Xyloallenoide A is a structurally unique compound, containing a cyclic tripeptide and an allenic ether linkage, which was isolated from the mangrove fungus *Xylaria* sp. 2508 in the South China Sea [7]. Herein, we report the first total synthesis of this compound. Cytotoxicity assays showed that synthetic xyloallenoide A showed marginal activities against KB ($IC_{50} = 9.6$ μM) and KBv200 cells ($IC_{50} = 10.3$ μM).

Results and Discussion. – The retrosynthetic analysis, shown in *Scheme 1*[1]), leads to only two fragments: the cyclic tripeptide **1** and (2*E*)-3-[4-(buta-2,3-dienyl-1-oxy)-

[1]) Arbitrary atom numbering of xyloallenoide A und A1 (cf. *Scheme 1*); for systematic names, see *Exper. Part*.

phenyl]prop-2-enoic acid (**2**). The two fragments may be combined by formation of an amide bond [8].

Scheme 1. *Retrosynthetic Analysis of the Initially Proposed Structure of Xyloallenoide A*



The cyclic tripeptide **1** contains three amino acids (Lys, Me-D-Val, and Me-D-Ala). Thus, there are three possible sites (A, B, and C) for the final cyclization. Considering the steric hindrance of the *N*-methyl groups, we chose A as the cyclization site (*Scheme 2*). The preparation of tripeptide **3** can be executed in two ways, by *Route 1* and *2*.

Based on *Route 1* in *Scheme 2*, the required linear tripeptide **3** was first synthesized as shown in *Scheme 3*, starting from Boc-D-Val-OH (**8**) and Boc-D-Ala-OH (**9**) (Boc = ^{t}BuOC(=O)). The *N*-methylations were performed with MeI/NaH in THF at room temperature (→ **10** and **7** (after esterification), resp.). Dipeptide **11** was obtained by coupling of **7** and **10**, promoted by BOP and ^{i}Pr$_2$NEt, in a gratifying 92% yield, which was further subjected to Boc-deprotection to provide **5** in 95% yield (BOP = [(1*H*-benzotriazol-1-yl)oxy][tris(dimethylamino)]phosphonium hexafluorophosphate). Unfortunately, the coupling of **5** and Boc-L-Lys(Cbz)-OH (Cbz = PhCH$_2$OC(=O); **4**) afforded tripeptide **3** in a very low yield (7%); instead, a larger amount of a by-product, cyclodipeptide **12**, was produced (79%). A variety of coupling reagents (DCC, BOP-Cl, EDCI/HOAt, *etc.*[2])) were examined for this coupling, but none led to satisfactory results. It was apparent that cyclization of dipeptide **5** occured very easily. In the competition with the coupling to give tripeptide **3**, the cyclization was the dominant reaction.

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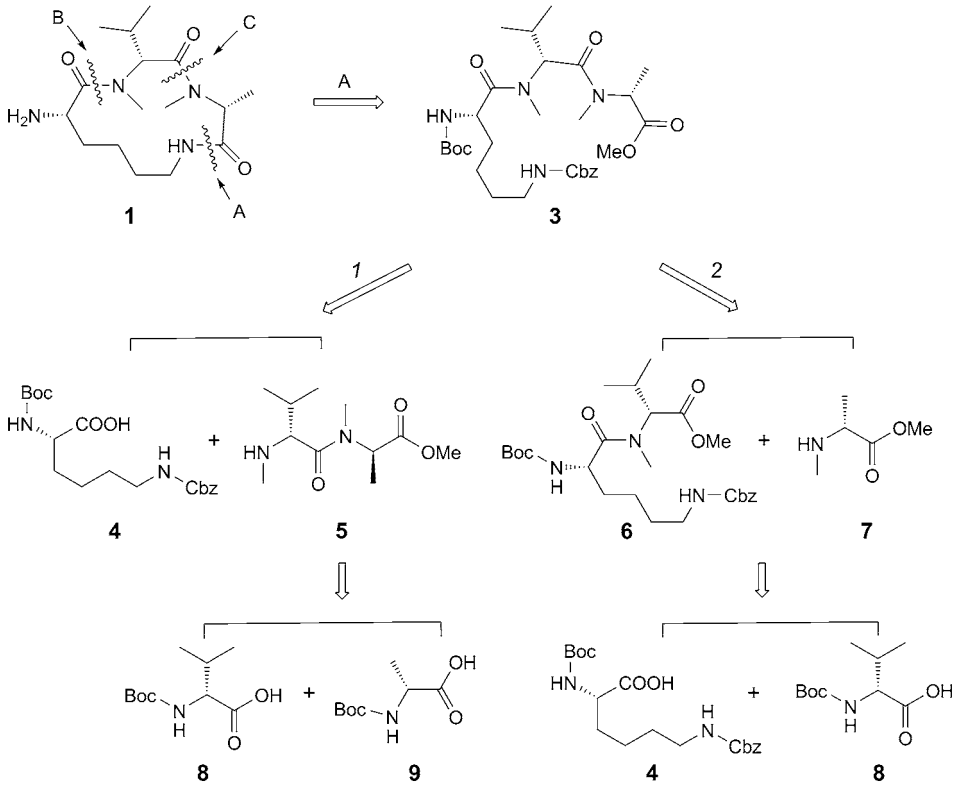
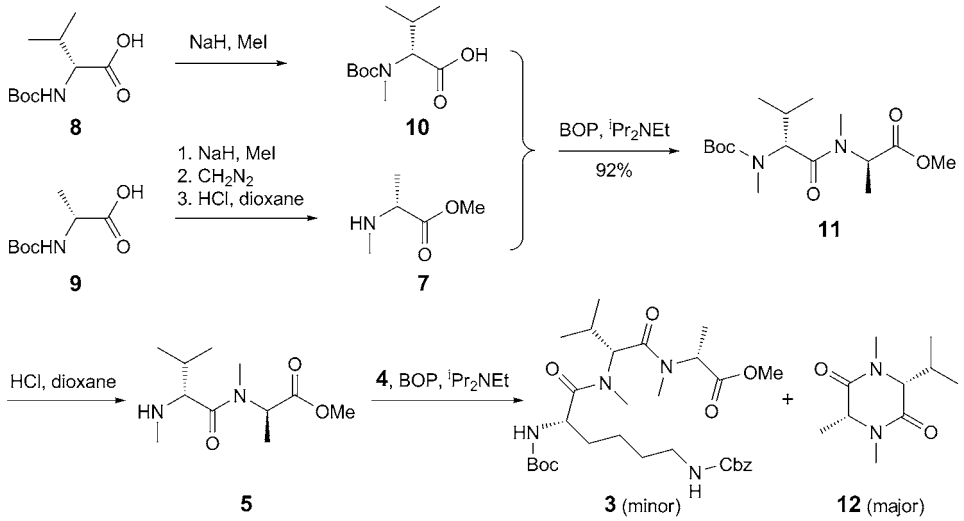
[2]) DCC = Dicyclohexylcarbodiimide; BOP-Cl = *P*,*P*-bis(2-oxooxazolidin-3-yl)phosphonic chloride; EDCI = 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; HOAt = 1-hydroxy-7-aza-1*H*-benzotriazole.

Scheme 2. *Retrosynthetic Analysis of* **1**

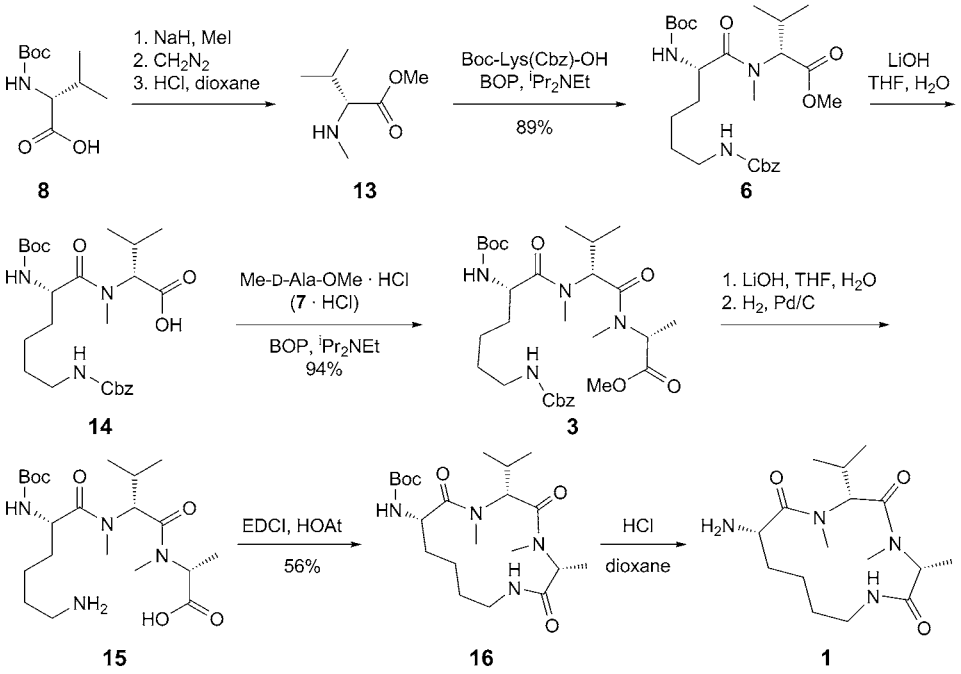


Scheme 3. *Preparation of Compound* **3**



Thus, we attempted *Route 2* (*Scheme 2*). Dipeptide **6** was prepared by coupling BocLys(Cbz)-OH (**4**) and Me-D-Val-OMe (**13**) in 89% yield, and subsequently hydrolyzed to provide acid **14** in 94% yield (*Scheme 4*). Coupling of **14** and Me-D-Ala-OMe (**7**) afforded tripeptide **3**, again in high yield (94%). It was interesting that the subtle change in the synthetic route gave an excellent result (*cf. Schemes 3* and *4*). Saponification of tripeptide **3** with LiOH in THF/H_2O, followed by removal of the Cbz group, provided the cyclization precursor **15** in two steps with 93% overall yield. The cyclization was attempted under various conditions. With BOP as a catalyst under high dilution, the cyclization of **15** gave the product in so low yield that **16** could not be isolated, and was detected only by ESI mass spectrometry. But alternatively, with BOP-Cl or EDCI/HOAt as reagent, **16** was produced in modest 42 and 56% yield, respectively. Final deprotection of the Boc group in **16** gave **1** in 96% yield.

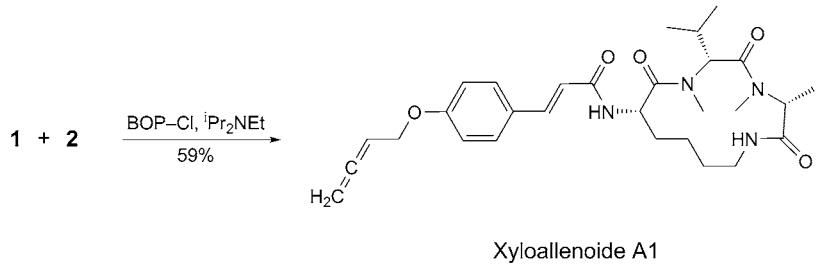
Scheme 4. *Preparation of Compound* **1**



Fragment **2** was prepared from prop-2-yn-1-ol and 4-hydroxycinnamic acid (=(2*E*)-3-(4-hydroxyphenyl)prop-2-enoic acid) according to [9–11]. The final coupling of fragments **1** and **2** by BOP-Cl gave xyloallenoide A1 in an acceptable 59% yield (*Scheme 5*).

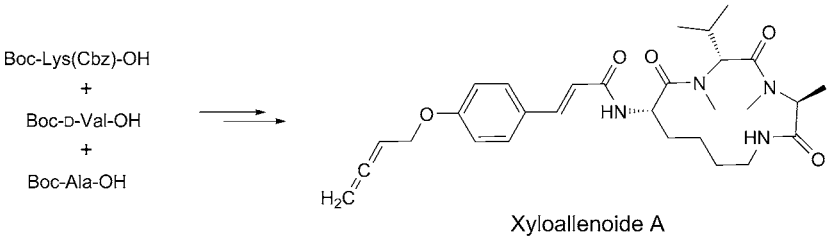
Comparing the characteristics of the synthetic compound relative to those of the natural xyloallenoide A, their melting points, IR spectra, and HR-MS were identical, but the NMR data showed some inconsistencies, mainly concerning the signals of H–C(9) ($\Delta\delta$ (H) = 0.42), Me(17) ($\Delta\delta$ (H) = 0.23), C(9) ($\Delta\delta$ (C) = 4.3), and C(17) ($\Delta\delta$ (C) = 3.2), which were attributed to the methyl alaninmoiety. It is important to

Scheme 5. *Preparation of Xyloallenoide A1*



mention that the value of the optical rotation of the synthetic compound ($[\alpha]_D^{25} = +39.4$ ($c = 0.031$, $CHCl_3$)) was nearly equal to that of the natural product ($[\alpha]_D^{25} = -34.6$ ($c = 0.058$, $CHCl_3$)), but with the opposite sign. These differences led us to presume that the configuration of the methyl-alanine residue in the natural xyloallenoide A was probably L, and not D. To confirm this, we repeated the synthesis shown in *Schemes 3–5* with Boc-Ala (*ent*-**9**) *via* Me-Ala-OMe (*ent*-**7**) instead of Boc-D-Ala (**9**). This gave indeed xyloallenoide A (overall yield from Boc-D-Val-OH (**8**), 18.4%; *Scheme 6*) since all its characteristic data were consistent with those of the natural product. Thus, the absolute configurations of the amino acid units of xyloallenoide A were finally confirmed to be L-Lys, D-Me-Val, and Me-L-Ala.

Scheme 6. *Preparation of Xyloallenoide A*



In the preliminary bioassay, synthetic xyloallenoide A showed marginal activities against KB ($IC_{50} = 9.6$ μM) and KBv200 cells ($IC_{50} = 10.3$ μM) and was, inactive against SW620 ($IC_{50} = 33$ μM), the diastereoisomer xyloallenoide A1 was inactive against KB ($IC_{50} = 36$ μM) and KBv200 cells ($IC_{50} = 43$ μM).

This research was supported by the *863-Foundation of China* (2006A092422 and 2006A092419), the *National Natural Science Foundation of China* (20572136), and the Guangdong Provincial Public Laboratory of Food Industry, China.

Experimental Part

General. Starting materials, Boc- or Cbz-protected amino acids, reagents, and solvents were purchased from commercial suppliers and were used without further purification. M.p.: *Fisher–Johns* hot-stage apparatus; uncorrected. IR Spectra: *Bruker-Equinox-55* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-Avance-400* NMR spectrometer, in (D_6)DMSO or $CDCl_3$; δ in ppm rel. to

Me_4Si as internal standard, *J* in Hz. EI-MS: *DSQ* EI-mass spectrometer; in *m*/*z*. HR-EI-MS: *MAT95XP* high-resolution mass spectrometer; in *m*/*z*.

Methyl N-*Methyl*-D-*alaninate* (Me-D-Ala-OMe; **7**) [12]. To a stirred soln. of Boc-D-Ala-OH (**9**; 2.0 g, 10.6 mmol) and MeI (6.59 ml, 106 mmol) in THF (34 ml), a 60% NaH dispersion in mineral oil (1.27 g, 106 mmol) was added in several portions. After 24 h stirring at r.t., the reaction was quenched by adding AcOEt (5 ml) and H_2O (5 ml). The mixture was concentrated and the residue partitioned between Et_2O (50 ml) and H_2O (100 ml). The aq. layers were combined and acidified with 5% citric acid to pH 3. This soln. was then extracted with AcOEt (3 × 200 ml). The combined extract was washed with H_2O (1 × 100 ml), 5% $Na_2S_2O_4$ soln. (1 × 100 ml), and brine (1 × 100 ml), dried ($MgSO_4$), and concentrated; 1.96 g (91.0%) of pure Boc,Me-D-Ala-OH as a white solid. M.p. 97.6–98.2° (AcOEt/hexane). ^{1}H-NMR ($CDCl_3$, 300 MHz): 10.54 (*s*, 1 H); 4.71 (*dd*, *J* = 7.43, 10.6, 1 H); 2.89 (*s*, 3 H); 1.49 (*s*, 9 H); 1.47 (*d*, *J* = 8.06, 3 H).

The acid Boc,Me-D-Ala-OH was converted to the corresponding methyl ester by the following procedure: KOH (2.15 g, 38.3 mmol) was dissolved in EtOH/Et_2O 2:1 (60 ml). This soln. was treated with *Diazald*® (1.64 g, 7.65 mmol). The CH_2N_2 generated at r.t. was blown *via* a stream of N_2 into a flask containing Boc,Me-D-Ala-OH (1.04 g, 5.1 mmol) in Et_2O at 0° until the yellow color in both flasks disappeared. The Et_2O soln. was concentrated to give 1.09 g (98%) of pure ester Boc,Me-D-AlaOMe as a colorless oil. ^{1}H-NMR (D_2O, 300 MHz): 4.54–4.67 (*m*, 1 H); 3.69 (*s*, 3 H); 2.81 (*d*, *J* = 19.6, 3 H); 1.44 (*s*, 9 H); 1.38 (*d*, *J* = 7.2, 3 H).

The ester Boc,Me-D-Ala-OMe (1.06 g, 4.9 mmol) was dissolved in 4N HCl in dioxane (50 ml, 200 mmol) and stirred at r.t. for 1.5 h. The mixture was concentrated: 0.71 g (92%) of **7** · HCl. ^{1}H-NMR (D_2O, 300 MHz): 4.03–4.15 (*m*, 1 H); 3.81 (*s*, 3 H); 2.71 (*d*, *J* = 5.76, 3 H); 0.52 (*d*, *J* = 2.48, 3 H).

N-*[(*tert-*Butoxy)carbonyl]*-N-*methyl*-D-*valine* (Boc,Me-D-Val-OH; **10**) [12]. As described above for Boc,Me-D-Ala-OH (*cf.* **7**), with Boc-D-Val-OH (**8**; 4.0 g, 18.4 mmol), MeI (10.46 ml, 184 mmol), THF (68 ml), and 60% NaH dispersion (2.2 g, 184 mmol). Quenching with AcOEt (10 ml) and H_2O (10 ml), partitioning with Et_2O (100 ml) and H_2O (200 ml), and workup as described: 3.71 g (87.2%) of pure **10**. White solid. M.p. 53.5–54.8°. ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.47 (*s*, 1 H); 4.06–4.26 (*dd*, *J* = 6.32, 7.85, 1 H); 2.86 (*s*, 3 H); 1.99–2.1 (*s*, 1 H); 1.45 (*s*, 9 H); 1.03 (*d*, *J* = 6.56, 3 H); 0.91 (*d*, *J* = 6.7, 3 H).

Methyl N-*[(*tert-*Butoxy)carbonyl]*-N-*methyl*-D-*valyl*-N-*methyl*-D-*alaninate* (Boc-Me-D-Val-Me-D-Ala-OMe; **11**). A soln of **7** · HCl (0.576 g, 2.49 mmol) and **10** (0.431 g, 2.74 mmol) in DMF (7 ml) was treated with BOP (1.21 g, 2.74 mmol) and ^{i}Pr$_2$NEt (1.12 ml, 6.23 mmol). After 3 h stirring at r.t., the mixture was concentrated and the residue partitioned between 10% citric acid (50 ml) and AcOEt (70 ml). The aq. layer was extracted with AcOEt (2 × 70 ml), the combined org. layer washed with sat. aq. $NaHCO_3$ soln. (70 ml) and brine (70 ml), dried ($MgSO_4$), and concentrated, and the residue purified by flash chromatography (SiO_2, AcOEt/hexane 1:9): 0.757 g (92%) of pure **11**. Colorless oil. $[\alpha]_D^{25} = +136.92$ (*c* = 0.305, $CHCl_3$). IR (KBr): 3530, 2967, 2934, 2874, 1748, 1701, 1680, 1657, 1645, 1479, 1456, 1393, 1306, 1159, 883, 769. ^{1}H-NMR ($CDCl_3$, 400 MHz): 4.82 (*q*, *J* = 6.8, 1 H); 4.40 (*d*, *J* = 12.8, 1 H); 3.41 (*s*, 3 H); 2.76 (*s*, 3 H); 2.47 (*s*, 3 H); 2.05–2.07 (*m*, 1 H); 1.18 (*s*, 9 H); 1.13 (*d*, *J* = 5.6, 3 H); 0.59 (*d*, *J* = 6.4, 3 H); 0.56 (*d*, *J* = 6.4, 3 H). ^{13}C-NMR ($CDCl_3$): 171.3; 170.3; 155.5; 79.2; 59.1; 53.8; 51.4; 31.5; 28.7; 27.8; 27.6; 18.9; 17.6; 13.7. EI-MS: 330 (M^+).

Methyl N-*Methyl*-D-*valyl*-N-*methyl*-D-*alaninate* (Me-D-Val-Me-D-Ala-OMe; **5**). A soln. of **11** (1.62 g, 4.9 mmol) in 4N HCl in dioxane (50 ml, 200 mmol) was stirred at r.t. for 1.5 h. The mixture was concentrated: 1.24 g (95%) of **5** · HCl. Solid. M.p. 179–180°. $[\alpha]_D^{25} = +61.02$ (*c* = 0.031, $CHCl_3$). IR (KBr): 3580, 3397, 2961, 2940, 2872, 1688, 1622, 1520, 1398, 1296, 1086, 561. ^{1}H-NMR ($CDCl_3$, 400 MHz): 4.35 (*q*, *J* = 9.6, 1 H); 4.33–4.37 (*m*, 1 H); 3.59 (*s*, 3 H); 3.03 (*s*, 3 H); 2.39 (*s*, 3 H); 2.31–2.34 (*m*, 1 H); 1.32 (*d*, *J* = 6.8, 3 H); 0.98 (*d*, *J* = 9.2, 6 H). ^{13}C-NMR ($CDCl_3$): 171.4; 169.9; 60.2; 58.5; 50.8; 37.9; 30.8; 30.5; 18.9; 18.7; 14.1. FAB-MS: 231 ($[M+1]^+$).

Methyl N^2-*[(*tert-*Butoxy)carbonyl]*-N^6-*[(phenylmethoxy)carbonyl]*-L-*lysyl*-N-*methyl*-D-*valyl*-N-*methyl*-D-*alaninate* (Boc-Lys(Cbz)-Me-D-Val-Me-D-Ala-OMe; **3**). As described for **11**, with **5** · HCl (1.74 g, 6.53 mmol) and Boc-Lys(Cbz)-OH (**4**; 2.74 g, 7.18 mmol), DMF (25 ml), BOP (3.17 g, 7.18 mmol), and ^{i}Pr$_2$NEt (5.60 ml, 31.2 mmol) (12 h stirring at r.t.). Partitioning with 10% citric acid (100 ml) and AcOEt (100 ml), extraction with AcOEt (2 × 100 ml), and workup as described (eluent AcOEt/hexane 2:3): 0.27 g (7%) of pure **3** and *(3*R*,6*R*)-1,3,4-trimethyl-6-(1-methylethyl)piperazine-2,5-*

dione (**12**; 79%). **3**: colorless oil. $[\alpha]_D^{25} = +52.24$ ($c = 0.495$, $CHCl_3$). IR (KBr): 3339, 3327, 2965, 2940, 2872, 1744, 1709, 1632, 1524, 1456, 1250, 1171, 754, 698. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.24 (*s*, 5 H); 5.52 (*s*, 2 H); 4.46 (*d*, $J = 8.0$, 1 H); 4.99 (*s*, 2 H); 4.89 (*d*, $J = 5.6$, 1 H); 4.48 (*s*, 1 H); 4.02 (*q*, $J = 7.2$, 1 H); 3.60 (*s*, 3 H); 3.02–3.14 (*m*, 2 H); 2.91 (*s*, 3 H); 2.82 (*s*, 3 H); 2.11–2.28 (*m*, 1 H); 1.51–1.67 (*m*, 2 H); 1.37–1.52 (*m*, 2 H); 1.33 (*s*, 9 H); 1.29 (*d*, $J = 9.6$, 3 H); 1.06–1.19 (*m*, 2 H); 0.83 (*d*, $J = 6.4$, 3 H); 0.71 (*d*, $J = 6.4$, 3 H). ^{13}C-NMR ($CDCl_3$): 172.6; 171.4; 169.7; 168.8; 156.1; 136.3; 127.9; 127.6; 127.5; 79.1; 65.9; 57.9; 52.8; 51.6; 40.2; 31.6; 29.7; 28.9; 28.8; 27.8; 26.9; 21.9; 19.1; 17.8; 17.4; 13.8. EI-MS: 592 ($[M + H]^+$).

Methyl N-*Methyl*-D-*valinate* (Me-D-Val-OMe; **13**) [12]. As described for Me-D-Ala-OMe (**7**), from Boc-D-Val-OH. ^{1}H-NMR (D_2O, 300 MHz): 4.02–4.18 (*m*, 1 H); 3.81 (*s*, 3 H); 2.71 (*d*, $J = 5.76$, 3 H); 0.52 (*d*, $J = 2.48$, 3 H).

Methyl N^2-*[(*tert-*Butoxy)carbonyl]*-N^6-*[(phenylmethoxy)carbonyl]*-L-*lysyl*-N-*methyl*-D-*valinate* (Boc-Lys(Cbz)-Me-D-Val-OMe; **6**). As described for **11**, with **13**·HCl (0.736 g, 2.49 mmol), Boc-Lys(Cbz)-OH (**4**; 1.044 g, 2.74 mmol), DMF (7 ml), BOP (1.21 g, 2.74 mmol), and ^{i}Pr$_2$NEt (1.12 ml, 6.23 mmol) (24 h stirring at r.t.). Partitioning, extraction, and workup as described (eluent AcOEt/hexane 1:5): 1.124 g (89%) of pure **6**. $[\alpha]_D^{25} = +50.32$ ($c = 0.186$, $CHCl_3$). IR (KBr): 3323, 2968, 2936, 2872, 1717, 1701, 1647, 1526, 1250, 1169, 1015, 739, 698. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.24 (*s*, 5 H); 4.98 (*s*, 2 H); 4.69 (*d*, $J = 10.8$, 1 H); 4.01 (*q*, $J = 7.2$, 1 H); 3.59 (*s*, 3 H); 3.03–3.17 (*m*, 2 H); 2.94 (*s*, 3 H); 2.06–2.19 (*m*, 1 H); 1.49–1.62 (*m*, 2 H); 1.35–1.49 (*m*, 2 H); 1.32 (*s*, 9 H); 1.23–1.38 (*m*, 2 H); 0.89 (*d*, $J = 6.4$, 3 H); 0.73 (*d*, $J = 6.4$, 3 H). ^{13}C-NMR ($CDCl_3$): 172.9; 170.8; 156.3; 155.4; 136.4; 128.2; 127.8; 127.7; 79.3; 66.2; 61.7; 51.8; 50.0; 40.5; 32.7; 31.1; 28.9; 28.1; 27.1; 21.9; 19.5; 18.5. EI-MS: 507 (M^+).

N^2-*[(*tert-*Butoxy)carbonyl]*-N^6-*[(phenylmethoxy)carbonyl]*-L-*lysyl*-N-*methyl*-D-*valine* (Boc-Lys(Cbz)-Me-D-Val-OH; **14**). To a stirred and cooled (0°) soln. of **6** (0.72 g, 1.42 mmol) in THF/H_2O 2:1 (30 ml), LiOH (0.70 g, 17.07 mmol) was added. After 12 h stirring, the mixture was quenched with aq. NH_4Cl soln. (50 ml) and extracted with AcOEt (3 × 80 ml). The extracts were washed with H_2O (50 ml) and brine (50 ml), dried ($MgSO_4$), and concentrated: 0.66 g (94%) of **14**. Colorless oil. $[\alpha]_D^{25} = +80.59$ ($c = 0.1685$, $CHCl_3$). IR (KBr): 3331, 3325, 2967, 2936, 2872, 1701, 1636, 1524, 1368, 1254, 1169, 1024, 698. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.32 (*s*, 5 H); 5.07 (*s*, 2 H); 4.71 (*d*, $J = 10.4$, 1 H); 4.11 (*q*, $J = 7.2$, 1 H); 3.08–3.22 (*m*, 2 H); 3.06 (*s*, 3 H); 2.06–2.25 (*m*, 1 H); 1.59–1.73 (*m*, 2 H); 1.47–1.61 (*m*, 2 H); 1.41 (*s*, 9 H); 1.07–1.25 (*m*, 2 H); 0.87 (*d*, $J = 6.4$, 3 H); 0.83 (*d*, $J = 6.4$, 3 H). ^{13}C-NMR ($CDCl_3$): 173.6; 172.4; 156.4; 155.5; 136.3; 128.1; 127.7; 127.6; 79.4; 66.3; 60.1; 50.1; 40.3; 32.2; 31.7; 29.1; 27.9; 26.9; 21.9; 19.6; 18.6. EI-MS: 493 ($[M + H]^+$).

Methyl N^2-*[(*tert-*Butoxy)carbonyl]*-N^6-*[(phenylmethoxy)carbonyl)*-L-*lysyl*-N-*methyl*-D-*valyl*-N-*methyl*-D-*alaninate* (Boc-Lys(Cbz)-Me-D-Val-Me-D-Ala-OMe; **3**). As described for **11**, with **14** (0.96 g, 1.95 mmol), **7**·HCl (0.40 g, 2.54 mmol), DMF (15 ml), BOP (1.12 g, 2.54 mmol), and ^{i}Pr$_2$NEt (2.24 ml, 12.46 mmol). (24 h stirring at r.t.). Partitioning with 10% citric acid (100 ml) and AcOEt (100 ml), extraction with AcOEt (2 × 100 ml), and workup as described with sat. $NaHCO_3$ soln. (100 ml) and brine (100 ml) (eluent AcOEt/hexane 2:3): to give 1.15 g (94%) of pure **3**. Colorless oil. $[\alpha]_D^{25} = +52.24$ ($c = 0.495$, $CHCl_3$). IR (KBr): 3339, 3327, 2965, 2940, 2872, 1744, 1709, 1632, 1524, 1456, 1250, 1171, 754, 698. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.24 (*s*, 5 H); 5.52 (*s*, 2 H); 4.46 (*d*, $J = 8.0$, 1 H); 4.99 (*s*, 2 H); 4.89 (*d*, $J = 5.6$, 1 H); 4.48 (*s*, 1 H); 4.02 (*q*, $J = 7.2$, 1 H); 3.60 (*s*, 3 H); 3.01–3.14 (*m*, 2 H); 2.91 (*s*, 3 H); 2.82 (*s*, 3 H); 2.16–2.31 (*m*, 1 H); 1.53–1.67 (*m*, 2 H); 1.39–1.54 (*m*, 2 H); 1.33 (*s*, 9 H); 1.29 (*d*, $J = 9.6$, 3 H); 1.10–1.24 (*m*, 2 H); 0.83 (*d*, $J = 6.4$, 3 H); 0.71 (*d*, $J = 6.4$, 3 H). ^{13}C-NMR ($CDCl_3$): 172.6; 171.4; 169.7; 168.8; 156.1; 136.3; 127.9; 127.6; 127.5; 79.1; 65.9; 57.9; 52.8; 51.6; 40.2; 31.6; 29.7; 28.9; 28.8; 27.8; 26.9; 21.9; 19.1; 17.8; 17.4; 13.8. HR-EI-MS: 592.3444 (M^+, $C_{30}H_{48}N_4O_8^+$; calc. 592.3467).

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*lysyl*-N-*methyl*-D-*valyl*-N-*methyl*-D-*alanine* (Boc-Lys-Me-D-Val-Me-D-Ala-OH; **15**). As described for **14**, with **3** (1.15 g, 1.94 mmol), THF/H_2O 2:1 (30 ml), and LiOH (0.80 g, 19.40 mmol). Workup with AcOEt (3 × 100 ml), H_2O (80 ml), and brine (80 ml): 1.12 g (100%) of Boc-Lys(Cbz)-Me-D-Val-Me-D-Ala-OH. Colorless oil. $[\alpha]_D^{25} = +27.30$ ($c = 0.2667$, $CHCl_3$). IR (KBr): 3568, 3321, 2967, 2938, 1705, 1697, 1632, 1524, 1456, 1250, 1169, 1022, 754. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.07 (*s*, 1 H); 7.22 (*s*, 5 H); 5.54 (*d*, $J = 7.6$, 1 H); 5.00 (*d*, $J = 5.2$, 1 H); 4.96 (*s*, 2 H); 4.48 (*s*, 1 H); 3.99 (*q*, $J = 7.2$, 1 H); 2.91–3.09 (*m*, 2 H); 2.90 (*s*, 3 H); 2.87 (*s*, 3 H); 2.16–2.27 (*m*, 1 H); 1.43–1.56 (*m*, 2 H); 1.32–1.47 (*m*, 2 H); 1.26–1.39 (*m*, 2 H); 1.29 (*s*, 9 H); 1.25 (*d*, $J = 11.2$, 3 H); 0.82 (*d*, $J = 6.4$, 3 H); 0.80

(d, J = 6.4, 3 H). ^{13}C-NMR ($CDCl_3$): 173.9; 173.1; 171.1; 169.9; 156.4; 136.3; 128.2; 127.8; 127.7; 79.5; 66.3; 60.2; 50.4; 40.4; 31.6; 30.0; 29.0; 28.1; 27.2; 26.4; 22.2; 19.2; 18.1; 17.6; 13.9. EI-MS: 578 ($[M+H]^+$).

A soln. of Boc-Lys(Cbz)-Me-D-Val-Me-D-Ala-OH (1.12 g, 1.94 mmol) in EtOH (60 ml) was hydrogenated at atmospheric pressure on 10% Pd/C (0.6 g). Filtering through *Celite* and concentration gave 0.86 g (93%) of **15**. White foamy residue, which was used without subsequent purification. M.p. 176–177°. $[\alpha]_D^{25} = +19.64$ (c = 0.389, $CHCl_3$,). IR (KBr): 3505, 2968, 2938, 1701, 1632, 1395, 1171, 754. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.01 (s, 1 H); 4.91 (d, J = 6.0, 1 H); 4.88 (d, J = 6.0, 1 H); 4.60 (q, J = 7.2, 1 H); 3.33 (s, 2 H); 2.85–2.99 (m, 2 H); 2.81 (s, 3 H); 2.68 (s, 3 H); 2.20–2.33 (m, 1 H); 1.46–1.61 (m, 2 H); 1.36 (s, 9 H); 1.27 (d, J = 7.2, 3 H); 1.18 (d, J = 6.8, 2 H); 0.84 (d, J = 6.4, 3 H); 0.79 (d, J = 6.4, 3 H). ^{13}C-NMR ($CDCl_3$): 176.9; 172.9; 170.1; 155.3; 79.5; 57.9; 50.1; 39.2; 31.1; 30.4; 29.4; 28.2; 27.4; 26.8; 21.8; 19.2; 18.1; 16.7; 15.1. EI-MS: 444 ($[M+H]^+$).

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*lysyl*-N-*methyl*-D-*valyl*-N-*methyl*-D-*alanine* (*3*^{1} → *1*^{6})-*Lactam* (= (*3*R,*6*R,*9*R)-*9*-{[(tert-*Butoxy)carbonyl]amino}-*3,4,7*-*trimethyl*-*6*-(*1*-*methylethyl*)-*1,4,7*-*triazacyclotridecane*-*2,5,8*-*trone*; cyclo(Me-D-Ala-Boc-Lys-Me-D-Val); **16**): *Method I:* As described for **11**, with **15** (0.50 g, 1.13 mmol), THF/DMF 9:1 (1000 ml; 0.001M), BOP (1.00 g, 2.26 mmol, 2 equiv.), and ^{i}Pr$_2$NEt (1.12 ml, 6.23 mmol) (2 d stirring at r.t.). Partitioning, extraction, and workup as described (eluent AcOEt/hexane 1:1): 34 mg (7%) of pure **16**.

Method II: A soln. of **15** (0.50 g, 1.13 mmol) and HOAt (0.77 g, 5.63 mmol, 5.0 equiv.) in CH_2Cl_2/DMF 9:1 (1000 ml, 0.001M) was treated dropwise with a soln. of EDC·HCl (1.08 g, 5.63 mmol, 5.0 equiv.) in CH_2Cl_2/DMF 5:1 (3 ml) at 0°. The resulting soln. was stirred for 3 h at 0° and then for 2 d at r.t. H_2O (80 ml) was added to quench the reaction. After concentration, the aq. phase was extracted with AcOEt (4 × 100 ml), the combined org. layer washed with 5% citric acid (50 ml), sat. $NaHCO_3$ soln. (50 ml), and brine (50 ml), dried ($MgSO_4$), and concentrated, and the crude product purified by flash chromatography (AcOEt/hexane 1:1): 0.27 g (56%) of **16**.

Method III: A soln. of **15** (0.50 g, 1.13 mmol) in THF/DMF 9:1 (1000 ml, 0.001M) was cooled in an ice bath with stirring under N_2. The cold mixture was treated with ^{i}Pr$_2$NEt (1.12 ml, 6.23 mmol), followed, in one portion, by BOP-Cl (0.58 g, 2.26 mmol, 2 equiv.). The mixture was stirred in the cold until TLC analysis indicated that the amino component was completely consumed or no longer progressing. Then, H_2O (80 ml) was added to quench the reaction. After concentration, the aq. phase was extracted with AcOEt (4 × 100 ml), the combined org. layer washed with 10% aq. $KHSO_4$ soln., H_2O, 1N $NaHCO_3$, 50% brine, and brine, then dried ($MgSO_4$), and concentrated, and the crude product purified by flash chromatography (AcOEt/hexane 1:1): 0.21 g (42%) of pure **16**. M.p. 251–252°. $[\alpha]_D^{25} = +42.60$ (c = 0.100, $CHCl_3$). IR (KBr): 3354, 2936, 2874, 1709, 1668, 1641, 1628, 1516, 1366, 1254, 1167, 999. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.06 (s, 1 H); 5.42 (d, J = 8.4, 1 H); 5.20 (d, J = 10.8, 1 H); 5.15 (d, J = 6.8, 1 H); 3.21–3.43 (m, 2 H); 3.13 (s, 3 H); 3.08 (s, 3 H); 2.26–2.39 (m, 1 H); 1.81–1.92 (m, 2 H); 1.60–1.72 (m, 2 H); 1.44–1.54 (m, 2 H); 1.35 (s, 9 H); 1.25 (d, J = 7.2, 3 H); 0.88 (d, J = 4.4, 3 H); 0.86 (d, J = 4.4, 3 H). ^{13}C-NMR ($CDCl_3$): 174.1; 172.2; 170.4; 155.4; 58.5; 51.1; 50.7; 38.0; 36.8; 31.1; 30.7; 28.5; 28.1; 26.9; 24.9; 20.0; 19.0; 18.9; 12.9. HR-EI-MS: 426.2841 (M^+, $C_{21}H_{38}N_4O_5^+$; calc. 426.2837).

L-*Lysyl*-N-*methyl*-D-*valyl*-*N*-*methyl*-D-*alanine* (*3*^{1} → *1*^{6})-*Lactam* (= (*3*R,*6*R,*9*R)-*9*-*Amino*-*3,4,7*-*trimethyl*-*6*-(*1*-*methylethyl*)-*1,4,7*-*triazacyclotridecane*-*2,5,8*-*trione*; cyclo(Me-D-Ala-Lys-Me-D-Val); **1**). Compound **16** (0.20 g, 0.47 mmol) was dissolved in 4N HCl/dioxane (20 ml, 80 mmol) and stirred at r.t. for 1.5 h. The mixture was concentrated: 0.16 g (96%) of **1**·HCl. White solid. M.p. 258–260°. $[\alpha]_D^{25} = +59.44$ (c = 0.018, $CHCl_3$). IR (KBr): 3397, 2961, 2940, 2642, 1688, 1622, 1520, 1399, 1398, 1296, 1101, 1016, 561. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.42 (s, 2 H); 6.17 (s, 1 H); 5.25 (d, J = 10.8, 1 H); 5.16 (q, J = 6.8, 1 H); 4.11 (q, J = 7.2, 1 H); 3.61–3.75 (m, 2 H); 3.20 (s, 3 H); 3.11 (s, 3 H); 2.24–2.42 (m, 1 H); 1.83–1.96 (m, 2 H); 1.58–1.71 (m, 2 H); 1.30 (d, J = 6.8, 3 H); 1.11–1.26 (m, 2 H); 0.97 (d, J = 6.0, 3 H); 0.94 (d, J = 6.0, 3 H). ^{13}C-NMR ($CDCl_3$): 171.5; 170.5; 170.1; 58.6; 51.1; 50.9; 37.9; 31.1; 30.6; 28.1; 26.8; 19.4; 18.9; 18.7; 14.1; 12.7. HR-FAB-MS: 327.2367 (M^+, $C_{16}H_{31}O_3N_4^+$; calc. 327.2391).

Xyloallenoide A1 = N^2-*{(*2E*)*-*3*-*[4*-(*Buta*-*2,3*-*dien*-*1*-*yloxy)phenyl]*-*1*-*oxoprop*-*2*-*en*-*1*-*yl}*-L-*lysyl*-N-*methyl*-D-*valyl*-N-*methyl*-D-*alanine* (*3*^{1} → *1*^{6})-*Lactam* (= (*2*E)-*3*-[*4*-(*Buta*-*2,3*-*dien*-*1*-*yloxy)phenyl]*-N-*[(3*R,*6*R,*9*S)-*3,4,7*-*trimethyl*-*6*-(*1*-*methylethyl*)-*2,5,8*-*trioxo*-*1,4,7*-*triazacyclotridec*-*9*-*yl]prop*-*2*-*enamide*). A soln. of **1** (120 mg, 0.331 mmol) and **2** (136 mg, 0.662 mmol) in DMF (15 ml) was treated with ^{i}Pr$_2$NEt (1.12 ml, 6.23 mmol), followed, in one portion, by BOP-Cl (0.876 g, 1.99 mmol). Further treatment and

workup as described for **16**, *Method III* (eluent AcOEt), a 0.103 g (56%) of pure xyloallenoide A1. Overall yield from Boc-D-Val-OH (**8**), 17.2%. Colorless crystals. M.p. 82–83°. $[\alpha]_D^{25} = +39.355$ ($c = 0.031$, $CHCl_3$). IR (KBr): 33.4, 2960, 2937, 2870, 1958, 1670, 1618, 1603, 1508, 1465, 1404, 1246, 1221, 1173, 1089, 1007, 827. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.42 (*d*, *J* = 15.6, 1 H); 7.29 (*d*, *J* = 6.8, 2 H); 7.03 (*d*, *J* = 7.2, 1 H); 6.79 (*d*, *J* = 6.8, 2 H); 6.23 (*d*, *J* = 15.6, 1 H); 6.06 (*s*, 1 H); 5.31 (*tt*, *J* = 6.8, 1 H); 5.22 (*d*, *J* = 10.8, 1 H); 5.15 (*q*, *J* = 6.8, 1 H); 5.06 (*dt*, *J* = 6.0, 6.8, 1 H); 4.80 (*dt*, *J* = 2.4, 6.8, 2 H); 4.50 (*dt*, *J* = 2.4, 6.8, 2 H); 3.49–3.68 (*m*, 2 H); 3.20 (*s*, 3 H); 3.09 (*s*, 3 H); 2.17–2.41 (*m*, 1 H); 1.71 (*d*, *J* = 5.6, 2 H); 1.43–1.64 (*m*, 2 H); 1.25 (*d*, *J* = 5.2, 3 H); 1.02–1.18 (*m*, 2 H); 0.89 (*d*, *J* = 6.0, 3 H); 0.87 (*d*, *J* = 6.0, 3 H). ^{13}C-NMR ($CDCl_3$): 209.7; 174.1; 172.1; 170.4; 165.8; 159.8; 140.9; 129.5; 129.9; 118.2; 115.2; 86.9; 77.1; 66.0; 58.6; 51.1; 50.1; 38.1; 32.2; 31.2; 30.7; 28.3; 27.1; 20.1; 19.2; 19.0; 12.9. HR-EI-MS: 524.2988 (M^+, $C_{29}H_{40}N_4O_5^+$; calc. 524.2993).

Xyloallenoide A = N^2-*{(2*E*)-3-[4-(Buta-2,3-dien-1-yloxy)phenyl]-1-oxoprop-2-en-1-yl}*-L-*lysyl*-N-*methyl*-D-*valyl*-N-*methyl*-L-*alanine* (*3^1* → *1^6*)-*Lactam* (= *(2*E*)-3-[4-(Buta-2,3-dien-1-yloxy)phenyl]*-N-*[(3*S*,6*R*,9*S*)-3,4,7-trimethyl-6-(1-methylethyl)-2,5,8-trioxo-1,4,7-triazacyclotridec-9-yl]prop-2-enamide*). The isomer xyloallenoide A was synthesized according to the above described procedures from Boc-Ala (*ent*-**9**); *cf.* **7** *et seq.* Overall yield from Boc-D-Val-OH (**8**), 18.4%. M.p. 82–83°. $[\alpha]_D^{25} = -36.6$ ($c = 0.046$, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.54 (*d*, *J* = 15.6, 1 H); 7.42 (*d*, *J* = 8.8, 2 H); 6.88 (*d*, *J* = 8.8, 2 H); 6.54 (*d*, *J* = 7.6, 1 H); 6.28 (*d*, *J* = 15.6, 1 H); 5.86 (*s*, 1 H); 5.37 (*tt*, *J* = 6.8, 1 H); 5.18 (*d*, *J* = 10.4, 1 H); 5.10 (*dt*, *J* = 2.8, 8.0, 1 H); 4.86 (*dt*, *J* = 2.4, 6.8, 2 H); 4.72 (*q*, *J* = 6.8, 1 H); 4.57 (*dt*, *J* = 2.4, 6.8, 2 H); 3.60 (*m*, 2 H); 3.09 (*s*, 3 H); 2.92 (*s*, 3 H); 2.43 (*m*, 1 H); 1.90 (*m*, 2 H); 1.61 (*m*, 2 H); 1.58 (*m*, 2 H); 1.47 (*d*, *J* = 7.2, 3 H); 0.89 (*d*, *J* = 6.4, 3 H); 0.78 (*d*, *J* = 6.4, 3 H). ^{13}C-NMR ($CDCl_3$): 209.5; 173.1; 170.2; 169.5; 165.4; 159.8; 141.2; 129.4; 127.6; 117.8; 115.2; 86.8; 76.8; 65.9; 57.9; 55.2; 49.1; 36.2; 30.6; 29.5; 27.9; 27.3; 24.8; 19.6; 18.0; 17.9; 15.9. HR-EI-MS: 524.2984 (M^+, $C_{29}H_{40}N_4O_5^+$; calc. 524.2985).

Cytotoxicity Assay [13]. Human oral squamous carcinoma cell line KB and KBv200, human colon adenocarcinoma SW620 cells were grown as adherent monolayers in flasks in RPMI 1640 with 10% fetal bovine serum (FBS), and cultured at 37° in an atmosphere of 5% CO_2 in air (100% humidity). The cells were collected with trypsin and re-suspended in a final concentration of 4×10^5 cells/ml; 0.18 ml aliquots were seeded in 96-well multiplates. After 24 h of incubation, 10 µl of modulator and 10 µl of anticancer drug were added. After 68 h, 10 µl of a 10 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) soln. were added to each well, and the plate was incubated for 4 h, allowing the viable cells to reduce the yellow MTT into dark-blue formazan crystals, which were dissolved in 150 µl of DMSO. The cell growth inhibition was evaluated by the MTT method by using triplicate assays [14]. The concentrations required to inhibit growth by 50% (IC_{50} values) were calculated from the cytotoxicity curves (*Bliss*'s software).

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