Isolation, Structural Assignment and Total Synthesis of Barmumycin

Adriana Lorente,[a] Daniel Pla, [a, †] Librada M. Cañedo,[b] Fernando Albericio[a,c,d,*] and Mercedes Álvarez[a,c,e]*

[a]*Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain.* [b] *Instituto Biomar, S.A. Parque Tecnológico de León -M10.4, E-24009 Armunia, León, Spain,* [c]*CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park, Baldiri Reixac 10, 08028 Barcelona, Spain;* [d]*Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain;* [e]*Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.*

fernando.albericio@irbbarcelona.org ; mercedes.alvarez@irbbarcelona.org

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Abstract

Barmumycin was isolated from an extract of the marine actinomycete *Streptomyces* sp. BOSC-022A and found to be cytotoxic against various human tumor cell lines. Based on preliminary one- and two-dimensional ^{1}H- and ^{13}C-NMR spectra, the natural compound was initially assigned the structure of macrolactone-type compound **1**, which was later prepared by two different routes. However, major spectroscopic differences between isolated **barmumycin** and **1** led to revision of the proposed structure as *E*-**16**. Based on synthesis of

† Current address: Memorial Sloan-Kettering Cancer Center, NY-10065, USA, PlaQuerD@mskcc.org

this new compound, and subsequent spectroscopic comparison of it to an authentic sample of **barmumycin**, the structure of the natural compound was indeed confirmed as that of *E*-**16**.

Introduction

Natural products from terrestrial plants and microorganisms have long been a traditional source of drugs; however, over the past few years, marine organisms have garnered ever-increasing attention as a rich bank of new bioactive compounds.[1] Marine actinomycetes have also proven to be an important source of biologically active compounds.[2]

Among the marine actinomycetes that our group has studied, those of the genus *Streptomyces* have clearly shown most pharmacological potential; however, in many bioactive cultures they have yielded only compounds that are already known. During ongoing research efforts to explore the biosynthetic potential of rare marine microorganisms, we isolated two known compounds pretomaymycin[3] and oxotomaymycin[4] (Figure 1) plus the previously unknown compound **barmumycin** from the culture broth of the marine actinomycete *Streptomyces* sp. BOSC-022A, isolated from a tunicate collected off the Scottish coast. **Barmumycin** and its diacetate show antitumor activity at micromolar concentrations in all 12 cancer cell lines tested (see Table 1 in the Supporting Information). Herein we report the isolation, total synthesis and structure elucidation of **barmumycin**.

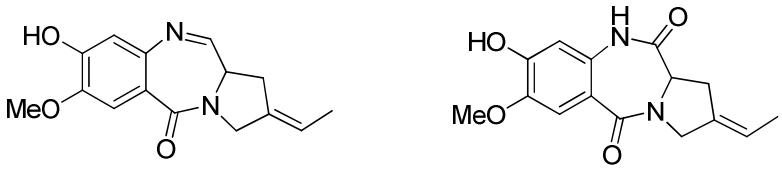


Figure 1. The two known compounds isolated from *Streptomyces* sp. BOSC-022A

Results and Discussion

The molecular formula of **barmumycin** was determined to be $C_{15}H_{19}NO_4$, by HRMS MALDI-TOF, it gave an $(M+H)^+$ ion at *m/z* 278.13840 (calcd. *m/z* 278.13869 for $C_{15}H_{20}NO_4$).

Reaction of **barmumycin** with acetic anhydride and pyridine gave a diacetyl derivative, confirmed by MS, pointing the presence of two OH and/or NH protons (see Figure 2).

^{1}H-NMR shows three groups of protons (see Table 2 in the Supporting Information). The first group is in the aromatic region and contains three protons, at 6.90 (d), 7.03 (d) and 7.08 (s) ppm; the chemical shifts indicated a 1,2,4-substituted electron-rich benzene ring. The second group corresponds to a single vinylic proton at 5.34 ppm (m). The third region comprises an upfield includes a CH shift at 4.67 ppm (m); two methyl groups, seen at 3.90 ppm (s) and 1.62 ppm (d); and the signals of three CH_2 at 4.07 (bt), 4.18 (bd), 3.75 (m), 2.29 (m) and 2.71 (m) ppm. Based on these data, the MS findings, and further data from one-dimensional and two-dimensional (COSY, HMBC and NOESY) ^{1}H- and ^{13}C-NMR experiments, we initially proposed that the structure of the isolated natural product was that of benzomacrolactone **1**, derived from 5-methoxy-2-aminobenzoic acid with an exocyclic *E*-ethylidene and one alcohol function (Figure 2).

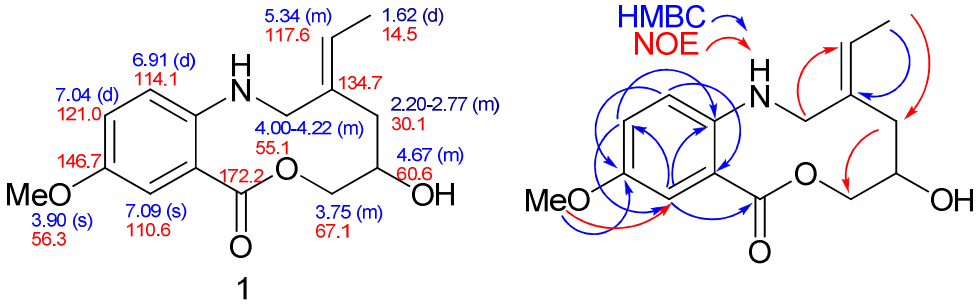
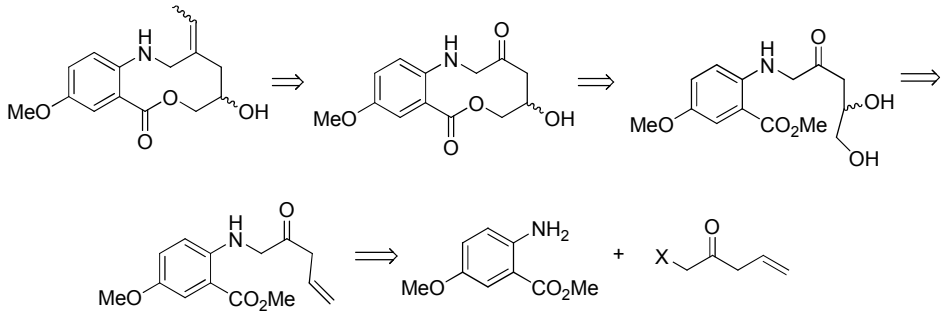


Figure 2. Structure of **1**, showing ^{1}H- NMR (blue) and ^{13}C-NMR (red) chemical shifts (left); and its HMBC and NOE correlations (right).

Decanolides are chemical entities abundant in terrestrial organisms, though only a few (*e.g.* modiolides A and B,[5] and xestodecalactones A-C[6]) have been isolated from marine sources. To the best of our knowledge, the aniline moiety within its ten-membered lactone had never been reported in naturally occurring macrocycles.

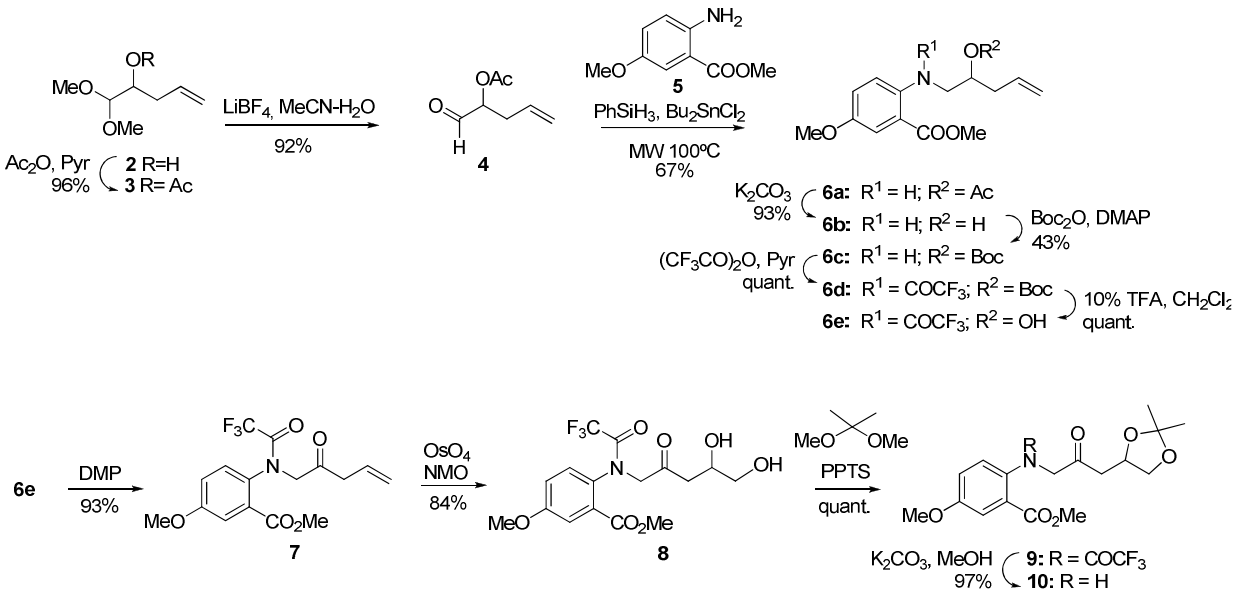
We sought to synthesize **1** to compare it against an authentic sample of **barmumycin** in order to assess its structural assignment. Our retrosynthetic analysis of **1** entailed formation of the exocyclic double bond *via* Wittig reaction; dihydroxylation of a double bond to give the alcohol required for lactonization; and finally, introduction of a functionalized five-carbon chain onto the nitrogen of methyl 2-amino-5-methoxybenzoate (Scheme 1).



Scheme 1. Retrosynthetic analysis of compound **1**.

The functionalized five-carbon chain on the aniline nitrogen was introduced by two different ways: *via* reductive amination (Scheme 2) and *via N*-alkylation (Scheme 3).

Homoallylic alcohol **2** was obtained by Barbier reaction of 2,2-dimethoxyacetaldehyde with allyl bromide and indium powder in water (95% yield).[7]

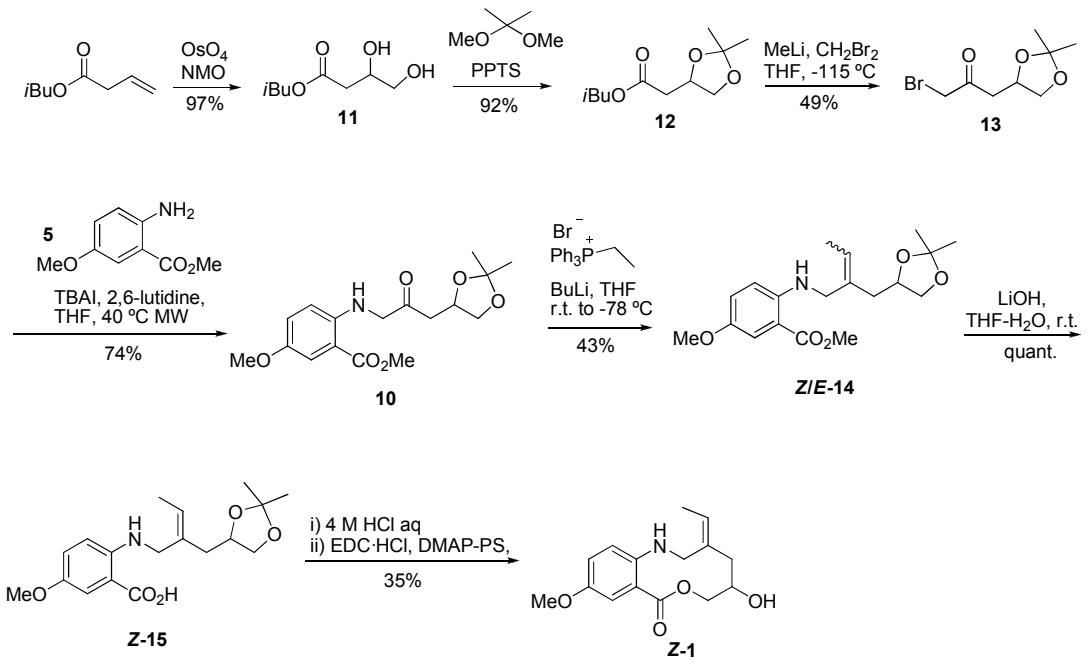


Scheme 2. Route A for the synthesis of **10**.

Attempts at direct deprotection of the dimethylacetal under acidic conditions led to polymerization of **2**;[8] therefore, the alcohol had to be protected. Acetylation of the alcohol to give compound **3**,[7] followed by dimethyl acetal deprotection using $LiBF_4$ in MeCN-H_2O,[9] gave the aldehyde **4** in excellent yield. Reductive amination of **4** with aniline **5**[10] required special conditions due to the poor nucleophilicity of the aniline (which is deactivated by the methyl ester group in *ortho* position): thus, reaction of **4**, **5**, phenylsilane, and dibutyltin dichloride under microwave irradiation for short reaction times gave the aminoalkene **6a** in 67% yield.[11,12] Attempts at protecting the aniline NH in **6a** as a ^{t}Bu carbamate failed due to its poor reactivity; therefore, **6a** was treated with K_2CO_3 in MeOH to give the deacetylated derivative **6b**. However, all attempts at oxidizing **6b** to its ketone derivative resulted in decomposition of the starting material.[13] Thus, the aniline had to be protected, but this was not possible in the presence of the unprotected alcohol. Exploiting the lack of reactivity of the aromatic amine towards Boc protection, and using standard conditions, **6b** was converted into its ^{t}Bu carbonate derivative **6c** in 43% yield.[14] The aniline group of **6c** was then orthogonally

protected using $(CF_3CO)_2O$ in pyridine to afford the trifluoroacetamide derivative **6d** in quantitative yield. Treatment of **6d** with 10% TFA in CH_2Cl_2 to remove the carbonate gave the free alcohol **6e** in quantitative yield. Compound **6e** was then oxidized with Dess Martin periodinate (DMP)[15] to yield the ketone **7** in 93% yield. Slow addition of **7** to *N*-methylmorpholine oxide (NMO) and a catalytic amount of OsO_4 in acetone-H_2O, to generate the corresponding diol **8** while preventing double bond isomerization gave **8** in good yield. Diol **8** was further protected by conversion into its 2,2-dimethyl-1,3-dioxolane derivative **9**, using 2,2-dimethoxypropane plus pyridinium *p*-toluenesulfonate (PPTS) as catalyst (quantitative yield). Deprotection of the amine in **9** *via* mild basic hydrolysis gave the free amine **10** in 97% yield.

A faster and better-yielding synthesis of **10** (Scheme 3) was done in parallel to the route described above. The first step was dihydroxylation of *iso*-butyl but-3-enoate. The introduction of the bromomethyl residue was planned for a later step. The oxidation conditions described above afforded *iso*-butyl 3,4-dihydroxybutanoate (**11**), which was then further protected as the 2,2-dimethyl-1,3-dioxolane derivative **12**, in excellent overall yield for both steps. The key step, transformation of **12** into the bromoketone **13** using bromomethyllithium, gave **13** in 49% yield. *N*-Alkylation of **5** with **13** under microwave irradiation gave **10**.



Scheme 3. Route B for the synthesis of **10**.

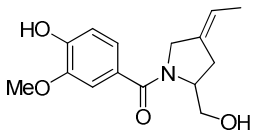
Wittig chemistry was employed to introduce the ethylidene chain. Reaction of **10** with the Wittig ylide derived from ethyltriphenylphosphonium bromide yielded **14** (43%) as a mixture of *Z*/*E*-diastereomers.[16]

Z/E-**14** was transformed into **1** in three successive reactions: hydrolysis of the methyl ester; acetonide deprotection under acidic conditions; and macrocyclization. The acid *Z*-**15** was obtained by purification of the Z/E mixture of acids by semipreparative HPLC.[17]

Racemic *Z*-**1** was obtained in 35% yield by acetal deprotection followed by macrocyclisation using EDC·HCl and solid-supported DMAP in a 5 mM CH_2Cl_2 solution. The ^{1}H-NMR spectrum of *Z*-**1** showed two doublets for the CH_3 linked to the double bond (1.42 ppm and 1.45 ppm) and two quadruplets for the vinylic proton (5.51 and 5.53 ppm).[18] These data could be explained by the presence of two highly populated conformations of *Z*-**1** at room temperature. Therefore, we studied peak coalescence by ^{1}H-NMR run at different

temperatures. Spectra from the initial experiments, run up to 55 ºC in $CDCl_3$ as solvent, exhibited this trend, but coalescence was not reached at this temperature limit. Finally, coalescence was almost reached in DMSO-d_6 as solvent at 145 ºC (see Table 4 in the Supporting Information). Moreover, comparison of spectroscopic data for **barmumycin** with those for *Z*-**1** revealed dramatic differences in the chemical shifts (see Tables 2 and 3 in the Supporting Information). This discrepancy, although the conflicting stereochemistry of the two compounds (*Z*-**1** and *E*- **barmumycin**), led us to pursue a new structural assignment.

Re-evaluation of all possible alternative structures led us to systematic elucidation of *E*-**16** as a novel structure for **barmumycin** (Figure 3). Interestingly, the very close structural resemblance of **16** to the pretomaymycin and oxotomaymycin isolated from the extract (Figure 1) suggests that all three molecules could derive from the same biogenetic pathway.



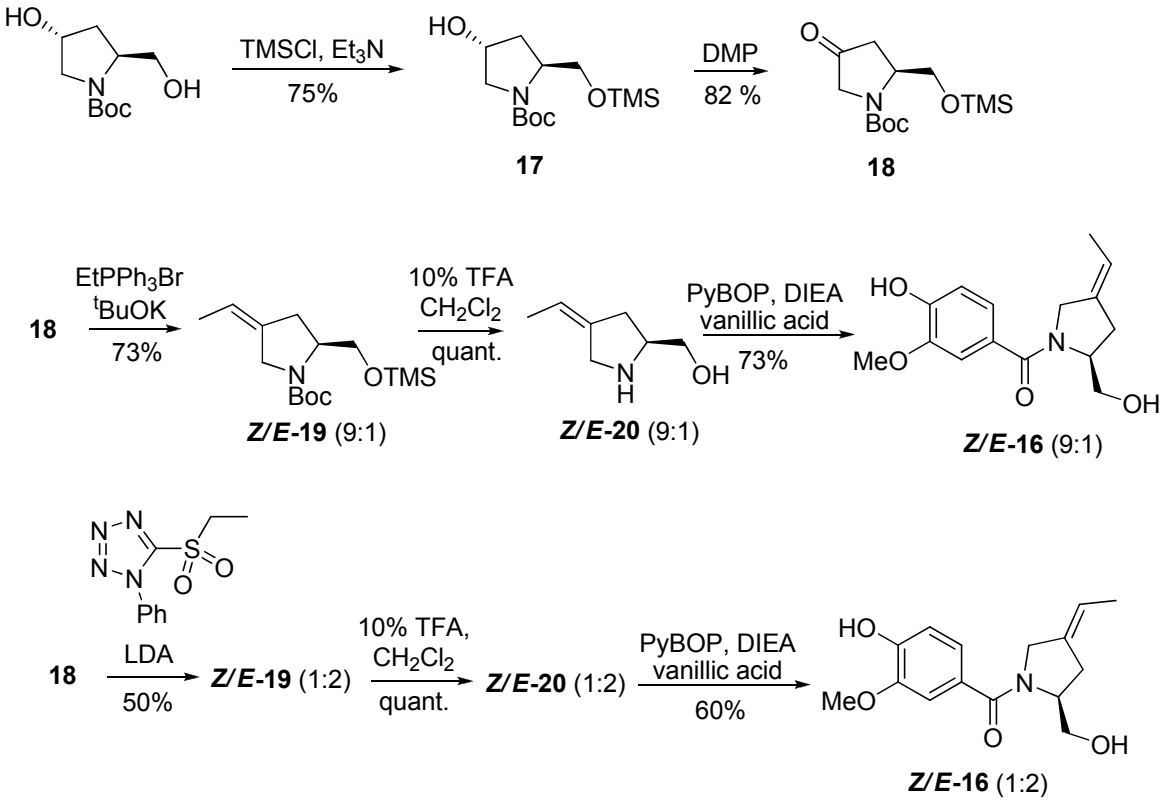
16

Figure 3. Structure proposed for **barmumycin** upon re-evaluation of NMR data.

In order to confirm that the structure of **barmumycin** is actually that of *E*-**16**, we synthesized the latter and subsequently compared it to an authentic sample of the former. This began with selective silyl protection of the primary alcohol in the commercially available *N*-Boc-*trans*-4-hydroxy-L-prolinol followed by oxidation of the secondary alcohol in the derivative **17**, which afforded ketone **18** in 62% yield over two steps (Scheme 4). Wittig chemistry was again employed to introduce the ethylidene chain: reaction of **18** with the Wittig ylide derived from ethyltriphenylphosphonium bromide yielded **19** as a 9:1 mixture of *Z/E*-diastereomers. *Z/E*-**19** was used directly without separation, as a single purification was planned for the final step of the synthesis. The TMS ether and the *tert*-butyl carbamate of *Z/E*-

19 were deprotected with 10% TFA in CH_2Cl_2 to give the pyrrolidine derivative *Z/E*-**20**. Coupling of *Z/E*-**20** to vanillic acid using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and *N*,*N*-diisopropylethylamine (DIEA) gave a 9:1 mixture of *Z/E*-**16** diastereomers. The configuration of the double bond was established by the NOESY correlations in *Z/E*-**16** between $^5CH_2$ (4.00–4.20 ppm) and the CH_3 (1.60 ppm) (see NOESY correlations in the Supporting Information).

None of the Horner-Wadsworth-Emmons reactions tested (using the appropriate phosphonate and different bases[19]) gave the desired *E*-**19**. However, the Kocienski variant of the Julia–Lythgoe olefination[20] afforded a 2:1 mixture of *E/Z*-**19**. This process entails nucleophilic addition of 5-(ethylsulfonyl)-1-phenyl-1*H*-tetrazole anion to the ketone followed by transposition and elimination to give the double bond. Again, deprotection of the hydroxyl group and the amine group was obtained using 10% TFA in CH_2Cl_2, and coupling of *E/Z*-**20** to vanillic acid using PyBOP and DIEA gave a 2:1 mixture of *E/Z*-**16**. This mixture was purified by semipreparative HPLC to obtain *E*-**16** as a single diastereomer. The configuration of the double bond was established by the NOESY correlations in *E*-**16** between $^5CH_2$ (4.00–4.22 ppm) and the vinyl proton (5.30-5.38 ppm) (see NOESY correlations in the Supporting Information).



Scheme 4. Preparation of *Z/E*-**16** staring from *N*-Boc-*trans*-4-hydroxy-L-prolinol.

Comparison of spectroscopic data obtained for *E*-**16** and **barmumycin** confirmed that the revised structure is indeed the structure of the natural product.

In summary, the previously unreported marine compound **barmumycin** was isolated, and its chemical formula was determined *via* mass spectrometry. Based on preliminary NMR data, **barmumycin** was initially assigned the structure of compound **1**. To confirm this assignment, compound **1** was synthesized following two different strategies starting from an ortho-aminobenzoic ester: one based on reductive amination, and one based on *N*-alkylation, which was shorter and higher-yielding. However, comparison of the NMR spectra for **1** with

those for isolated **barmumycin** showed dramatic differences. The structure of **barmumycin** was reassessed, and most probable option conceived was compound *E*-**16**, which was subsequently prepared (in five steps and 18% overall yield) for comparison with the natural compound. The spectroscopic data for *E*-**16** fully coincided with that for **barmumycin**, thereby confirming that the two structures are equivalent. This work is a new example of the importance of total synthesis for structural characterization and confirmation of natural products[21].

Experimental section

See Supporting Information for General Procedures.

Extraction and isolation of barmumycin

The culture broth (10 L) was separated by filtration into a mycelial cake and cultured filtrate (9 L). A 500 mL aliquot of the absorber resin XAD-1180 was added to the filtrate. Compound **barmumycin** was eluted from the resin by double extraction with a 3:1:1 mixture of EtOAc-MeOH-H_2O (1.8 L). The active fractions were concentrated in the organic phase, which was concentrated to dryness *in vacuo* to yield 950 mg of crude extract. This extract purified by vacuum flash chromatography using a mixture of *n*-hexane-EtOAc and EtOAc-MeOH, whereby the fractions containing **barmumycin** (220 mg) were eluted with 9:1 EtOAc-MeOH. The active fractions were purified by silica gel chromatography using $CHCl_3$-MeOH mixtures. Cytotoxicity was detected in the fractions eluted with 96:4 $CHCl_3$-MeOH (20 mg). Further purification with a C18 column by HPLC afforded 6 mg of pure **barmumycin** (elution with 54:46 H_2O/MeOH). This quantity of **barmumycin** was treated with 0.5 mL of pyridine and 0.5 ml of Ac_2O to afford 7 mg of the corresponding diacetate. The molecular formula of **barmumycin** was determined to be $C_{15}H_{19}NO_4$ by HPLC-APESI MS, in which it gave an $(M+Na)^+$ peak at 300 and $(M-H)^-$

276. **Barmumycin** gave an $(M+H)^+$ peak at 278 and $(M-H)^-$ 276 in HPLC-APCI MS and it gave an $(M+H)^+$ ion at *m/z* 278.13840 (calcd. *m/z* 278.13869 for $C_{15}H_{20}NO_4$) in HRMALDI-TOF MS.

The diacetyl derivative of **barmumycin** gave an $(M+H)^+$ peak at 362 and an $(M+Na)^+$ peak at 384, by HPLC-APCI MS and HPLC-ESI MS.

2-Acetoxypent-4-enal (4). $LiBF_4$ (6.05 g, 64.5 mmol) was added to a solution of **3** (4.05 g, 21.5 mmol) in 98:2 MeCN/H_2O (110 mL) and the mixture was stirred for 72 h at room temperature. The solvents were removed *in vacuo*. The crude was dissolved in CH_2Cl_2, washed with water and brine, and then concentrated *in vacuo* to yield **4** (2.82 g, 92%) as a yellowish oil. IR (KBr film) ν 3080, 2932, 1744, 1373, 1237, 1048 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.18 (s, 3H); 2.32–2.65 (m, 2H); 5.06–5.30 (m, 3H); 5.65–5.87 (m, 1H); 9.54 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 20.4 (q); 33.1 (t); 77.2 (d); 119.0 (t); 131.4 (d); 170.4 (s); 197.9 (d). MS (ESI-TOF) 143 (M+1, 100).

Methyl 2-(2-acetoxypent-4-enylamino)-5-methoxybenzoate (6a). $PhSiH_3$ (2.01 g, 18.6 mmol) was added to a THF solution (12 mL) of **5** (2.02 g, 9.3 mmol), **4** (1.32 g, 9.3 mmol) and Bu_2SnCl_2 (282.3 mg, 0.9 mmol) in a sealed tube. The mixture was heated to 100 ºC under MW irradiation for 15 min. The solvents were removed *in vacuo*. Purification by silica gel column chromatography (100:0 to 95:5 hexane-Et_2O) yielded **6a** (1.93 g, 67%) as a yellowish oil. IR (KBr film) ν 3479, 3370, 2951, 1739, 1691, 1520, 1223, 1042 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.06 (s, 3H, Me); 2.41–2.46 (m, 2H); 3.35–3.37 (m, 2H); 3.76 (s, 3H, OMe); 3.86 (s, 3H, OMe); 5.09-5.17 (m, 3H); 5.78 (ddt, *J* = 17.2, 10.1 and 7.1 Hz, 1H); 6.74 (d, *J* = 9.2 Hz, 1H); 7.04 (dd, *J* = 9.2, 3.1 Hz, 1H); 7.42 (d, *J* = 3.1 Hz, 1H); 7.55 (bs, 1H, NH). ^{13}C

NMR (100.6 MHz, $CDCl_3$) δ 21.0 (q); 36.3 (t); 45.6 (t); 51.5 (q); 55.9 (q); 71.6 (d); 110.2 (s); 112.9 (d); 114.3 (d); 118.3 (t); 123.3 (d); 133.0 (d); 146.0 (s); 149.5 (s); 168.6 (s); 170.6 (s). MS (ESI-TOF) 308 (M+1, 82). HRMS *m/z* calcd. for $C_{16}H_{22}NO_5$ 308.1498, found 308.1504.

Methyl 2-(2-hydroxypent-4-enylamino)-5-methoxybenzoate (6b). K_2CO_3 (953.5 mg, 6.9 mmol) was added to a solution of **6a** (1.92 g, 6.27 mmol) in MeOH (75 mL). The mixture was stirred for 1 h at room temperature. The solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2, and then washed with water and brine. The organic extracts were dried over $MgSO_4$, and then concentrated *in vacuo*. Purification by silica gel column chromatography (85:15 to 75:25 hexane/EtOAc) yielded **6b** (1.55 g, 93%) as a yellow oil. IR (KBr film) ν 3370, 1688, 1518, 1437, 1222, 1073 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.27-2.43 (m, 2H); 3.20 (dd, *J* = 13.0 and 7.7 Hz, 1H); 3.33 (dd, *J* = 13.0 and 4.5 Hz, 1H); 3.77 (s, 3H, OMe); 3.87 (s, 3H, OMe); 3.96 (m, 1H); 5.17 (m, 1H); 5.19 (m, 1H); 5.87 (m, 1H); 6.75 (d, *J* = 9.1 Hz, 1H); 7.04 (dd, *J* = 9.1, 3.1 Hz, 1H); 7.44 (d, *J* = 3.1 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 39.5 (t); 49.3 (t); 51.7 (q); 56.0 (q); 69.3 (d); 110.8 (s); 113.6 (d); 114.5 (d); 118.5 (t); 123.2 (d); 133.9 (d); 145.8 (s); 149.9 (s); 168.6 (s). MS (ESI-TOF) 266 (M+1, 100). HRMS *m/z* calcd. for $C_{14}H_{20}NO_4$ 266.1392, found 266.1398.

Methyl 2-(2-(*tert*-butoxycarbonyloxy)pent-4-enylamino)-5-methoxybenzoate (6c). Boc_2O (823 mg, 3.77 mmol) was added to a solution of **6b** (909.4 mg, 3.43 mmol) and DMAP (125.6 mg, 1.03 mmol) in dry CH_2Cl_2 (50 mL). The reaction mixture was stirred for 40 h at room temperature and then concentrated *in vacuo*. Purification by silica gel column chromatography (100:0 to 95:5 hexane-EtOAc) yielded **6c** (543 mg, 43%) as a yellowish oil. IR (KBr film) ν 3369, 3078, 1730, 1709, 1500, 1368 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9H); 2.41–2.48 (m, 2H); 3.39 (d, *J* = 6.1 Hz, 2H); 3.76 (s, 3H, OMe); 3.85 (s, 3H, OMe);

4.91 (p, *J* = 6.1 Hz, 1H); 5.12 (m, 1H); 5.17 (m, 1H); 5.82 (m, 1H); 6.74 (d, *J* = 9.2 Hz, 1H); 7.04 (dd, *J* = 9.2, 3.1 Hz, 1H); 7.42 (d, *J* = 3.1 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 27.7 (q); 36.5 (t); 45.9 (t); 51.6 (q); 56.0 (q); 74.4 (d); 82.2 (s); 110.4 (s); 112.9 (d); 114.4 (d); 118.4 (t); 123.3 (d); 132.9 (d); 145.8 (s); 149.6 (s); 153.2 (s); 168.5 (s). MS (ESI-TOF) 366 (M+1, 100). HRMS *m/z* calcd. for $C_{19}H_{28}NO_6$ 366.1917, found 366.1917.

Methyl 2-[*N*-(2-(*tert*-butoxycarbonyloxy)pent-4-enyl)-trifluoroacetamido]-5-methoxybenzoate (6d). TFAA (0.3 mL, 2.2 mmol) was added to a cooled (0 ºC) solution of **6c** (542.9 mg, 1.49 mmol) in pyridine (20 mL) and the mixture was stirred at 0 ºC for 90 min. The crude was concentrated *in vacuo*, dissolved in CH_2Cl_2, and then washed with NH_4Cl, water and brine. The organic extracts were dried over $MgSO_4$, and then concentrated *in vacuo* to yield **6d**, as a mixture of rotamers, (685 mg, quant.), as a yellowish oil. IR (KBr film) ν 1736, 1707, 1502, 1282 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 and 1.47 (2s, 9H); 2.32–2.40 (m, 2H, CH_2); 3.04 and 3.31 (2dd, *J* = 14.4, 9.3 and 14.8, 2.2 Hz, 1H, CH_2); 3.86 (s, 3H, OMe); 3.87 (s, 3H, OMe); 4.41 and 4.51 (2dd, *J* = 14.4, 2.9 and 14.8, 8.9 Hz, 1H, CH_2); 4.82–4.87 and 5.17–5.23 (2m, 1H, CH); 5.05–5.17 (m, 2H); 5.65–5.81 (m, 1H); 7.04 and 7.10 (2dd, *J* = 8.8, 3.0 Hz, 1H); 7.24 and 7.51 (2d, *J* = 8.8 Hz, 1H); 7.55 and 7.57 (2d, *J* = 3.0 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 27.6 and 27.7 (3q); 36.7 and 37.1 (t); 52.7 (q); 54.0 and 55.8 (t); 55.7 (q); 73.3 and 73.7 (d); 82.3 (s); 116.4 and 116.8 (d); 118.5 and 118.7 (d); 118.6 (t); 132.2 and 132.3 (d); 132.7 (d); 152.7 (s); 153.1 (s); 159.6 and 159.7 (s); 164.8 and 164.9 (s); 171.1 (s). ^{19}F NMR (376 MHz, $CDCl_3$) δ -68.8 and -69.0 (2s). MS (ESI-TOF) 945 (2M+Na, 15). HRMS *m/z* calcd. for $C_{42}H_{52}N_2O_{14}F_6Na$ 945.3220, found 945.3208.

Methyl 5-methoxy-2-[*N*-(2-hydroxypent-4-enyl)trifluoroacetamido]benzoate (6e). A 10% solution of TFA in CH_2Cl_2 (50 mL) was added to **6d** (494.9 mg, 1.07 mmol) and the mixture

was stirred at room temperature for 25 min. Elimination of the solvent gave **6e** (387 mg, quant.) as a yellow oil. IR (KBr film) ν 3370, 2950, 1688, 1519, 1437, 1222, 1074 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20–2.30 (m, 2H, CH_2); 3.46–3.49 and 3.54–3.61 (m, 1H); 3.88 (s, 3H, OMe); 3.90 (s, 3H, OMe); 4.02–4.15 (m, 2H); 4.89 (bs, 1H, OH); 5.10–5.14 (m, 2H); 5.72–5.81 (m, 1H); 7.09–7.14 (m, 1H); 7.31 and 7.39 (2d, *J* = 8.8 Hz, 1H); 7.55 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 39.3 and 39.4 (t); 52.9 (q); 55.8 (q); 58.4 and 59.0 (t); 68.2 and 68.8 (d); 114.9 (s); 116.2 and 116.7 (d); 118.8 (t); 118.9 and 119.0 (d); 120.1 (s); 120.9 (s); 122.8 (s); 131.1 (s); 131.9 and 132.1 (d); 133.1 and 133.4 (d); 159.8 (s). ^{19}F NMR (376 MHz, $CDCl_3$) δ −68.7 (s). MS (ESI-TOF) 362 (M+1, 37). HRMS *m/z* calcd. for $C_{16}H_{19}NO_5F_3$ 362.1215, found 362.1211.

Methyl 5-methoxy-2-[*N*-(2-oxopent-4-enyl)trifluoroacetamido]benzoate (7). Dess-Martin periodinane (606.4 mg, 1.43 mmol) was added to a solution of **6e** (469.6 mg, 1.30 mmol) in anhydrous CH_2Cl_2 (35 mL). The mixture was stirred for 1 h, and then diluted with Et_2O and hexane to a final concentration of 30:20:50 CH_2Cl_2/Et_2O/hexane. The solution was filtered through a silica gel pad. The solvents were removed *in vacuo* to yield **7** (433 mg, 93%) as a yellowish oil. IR (KBr film) ν 1707, 1704, 1502, 1291, 1204, 1153 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.18 (dd, *J* = 16.8 and 6.7 Hz, 1H); 3.27 (dd, *J* = 16.8 and 7.2 Hz, 1H); 3.85 (m, 1H); 3.86 (s, 3H, OMe); 3.89 (s, 3H, OMe); 5.13–5.23 (m, 3H); 5.95 (m, 1H); 7.06 (dd, *J* = 8.8 and 3.0 Hz, 1H); 7.51 (d, *J* = 3.0 Hz, 1H); 7.59 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 45.0 (t); 52.7 (q); 55.7 (q); 60.4 (t); 114.7 (s); 116.5 (d); 117.6 (s); 118.4 (d); 119.9 (t); 129.0 (s); 129.1 (d); 131.6 (s); 132.6 (d); 159.8 (s); 165.0 (s); 200.8 (s). ^{19}F NMR (376 MHz, $CDCl_3$) δ -68.8 (s). MS (ESI-TOF) 360 (M+1, 100). HRMS *m/z* calcd. for $C_{16}H_{17}NO_5F_3$ 360.1059, found 360.1072.

Methyl 5-methoxy-2-[*N*-(2,4,5-trihydroxypent-2-enyl)trifluoroacetamido]benzoate (8). A solution of **7** (775 mg, 2.16 mmol) in acetone (20 mL) was added dropwise over 10 h to a stirring solution of *N*-methylmorpholine oxide (337 mg, 2.37 mmol) and OsO_4 (catalytic amount) in 60:40 acetone/H_2O (64 mL). The mixture was stirred for 20 h at room temperature then quenched with 40% aq. $NaHSO_3$ (3 mL) and subsequently concentrated *in vacuo*. The residue was dissolved in EtOAc, dried over $MgSO_4$, filtered, and then re-concentrated *in vacuo*. Purification by silica gel column chromatography (100:0 to 90:10 CH_2Cl_2-MeOH) yielded **8** (628 mg, 84%) as a brownish oil. IR (KBr film) ν 1725, 1605, 1503, 1293, 1204 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 3.25–3.30 (m, 1H); 3.36–3.43 (m, 1H); 3.63–3.71 (m, 1H, CH); 3.84 (s, 6H, 2OMe); 4.12–4.13 (m, 1H); 4.16 and 4.33 (2d, *J* = 18.5 Hz, 1H); 5.14 and 5.33 (2d, *J* = 18.5 Hz, 1H); 7.27 and 7.29 (2dd, *J* = 3.0 Hz, 1H); 7.41–7.48 (m, 2H). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 52.6 (q); 55.7 (q); 59.4 and 60.2 (t); 61.3 (t); 72.2 and 72.9 (d); 76.0 and 76.1 (d); 116.0 (d); 118.5 (d); 128.6 (s); 128.7 (s); 131.2 (s); 131.9 (d); 159.2 (s); 164.4 (s); 206.2 (s); 207.5 (s). ^{19}F NMR (376 MHz, $CDCl_3$) δ -68.8 (s). MS (ESI-TOF) 392 (M-1, 100); 394 (M+1, 40). HRMS *m/z* calcd. for $C_{16}H_{17}NO_7F_3$ 392.0957, found 392.0946.

Methyl 2-[*N*-(3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxopropyl)trifluoroacetamido]-5-methoxybenzoate (9). Pyridinium *p*-toluenesulfonate (18.7 mg, 74 mmol) was added to a solution of **8** (584 mg, 1.49 mol) and 2,2-dimethoxypropane (1.82 mL, 14.8 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred at 40 ºC for 16 h and then concentrated *in vacuo*. Purification by silica gel column chromatography (90:10 to 40:60 hexane-EtOAc) yielded **9** (642 mg, quant.) as a yellowish oil. IR (KBr film) ν 1775, 1730, 1381, 1172, 1087, 1047 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.30 and 1.36 (2s, 6H); 2.59 and 2.69 (2dd, *J* = 16.3, 6.8 and 16.7, 6.0 Hz, 1H, CH_2); 2.81 and 2.90 (2dd, *J* = 16.7, 6.8 and 16.3, 6.0 Hz, 1H, CH_2); 3.54

and 3.60 (2dd, *J* = 8.4, 6.6 Hz, 1H); 3.77–3.83 (m, 1H); 3.85 (s, 3H, OMe); 3.87 (s, 3H, OMe); 4.12–4.18 (m, 1H); 4.40–4.48 (m, 1H); 5.08 (d, *J* = 17.9 Hz, 1H); 5.13 (d, *J* = 17.9 Hz, 1H); 7.048 and 7.051 (2dd, *J* = 8.8 and 3.0 Hz, 1H); 7.50 (d, *J* = 3.0 Hz, 1H); 7.55 and 7.57 (2d, *J* = 8.8 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 25.4 (q); 26.8 (q); 44.3 and 44.4 (t); 52.6 (q); 55.7 (q); 61.2 and 61.4 (t); 69.0 and 69.2 (t); 71.3 (d); 109.1 (s); 114.6 (s); 116.4 and 116.5 (d); 117.5 (s); 118.4 (d); 129.0 (s); 131.6 (s); 132.5 and 132.6 (d); 159.8 (s); 164.9 (s); 200.8 (s). ^{19}F NMR (376 MHz, $CDCl_3$) δ -68.8 (s). MS (ESI-TOF) 434 (M+1, 100).

Isobutyl 3,4-dihydroxybutanoate (11). A solution of isobutyl but-3-enoate (10.5 g, 73.6 mmol) in acetone (50 mL) was added dropwise over 20 h to a solution of *N*-methylmorpholine oxide (10.9 g, 80.9 mmol) and a catalytic amount OsO_4 in 60:40 acetone/H_2O (250 mL). The reaction was quenched with $NaHSO_3$ 40% aq. sol. (3 mL) and concentrated *in vacuo*. The crude was dissolved in EtOAc, filtered through silica gel and the eluent was concentrated *in vacuo* to yield **11** (12.6 g, 97%) as a yellow oil. IR (KBr film) ν 3402, 2962, 1729, 1470, 1381, 1170, 1043 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (d, *J* = 6.8 Hz, 6H); 2.00–1.89 (m, 1H); 2.51 (dd, *J* = 16.4 and 4.0 Hz, 1H, CH_2); 2.58 (dd, *J* = 16.4 and 8.4 Hz, 1H, CH_2); 3.58 (dd, *J* = 11.2 and 6.4 Hz, 1H, CH_2); 3.69 (dd, *J* = 11.2 and 3.6 Hz, 1H, CH_2); 3.91 (d, *J* = 6.8 Hz, 2H); 4.10–4.18 (m, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.2 (2q); 27.8 (d); 37.9 (t); 65.9 (t); 68.8 (d); 71.2 (t); 172.8 (s). MS (ESI-TOF) 177 (M+1, 45); 199 (M+Na, 100). HRMS (+ESI): *m/z* calcd. for $C_8H_{17}O_4$ (M+1) 177.1127, found 177.1127. calcd. for $C_8H_{16}O_4Na$ (M+Na) 199.0946, found 199.0945.

Isobutyl 2,2-dimethyl-1,3-dioxolan-4-yl acetate (12). Pyridinium *p*-toluenesulfonate (120 mg, 0.48 mmol) was added to a solution of **11** (15.4 g, 87.26 mmol) in 50:50 2,2-dimethoxypropane/CH_2Cl_2 (300 mL). The reaction mixture was stirred at room temperature

for 16 h and then the solvent was removed *in vacuo*. Purification by silica gel column chromatography (50:50 hexane-EtOAc) yielded **12** (17.4 g, 92%) as a yellowish oil. IR (KBr film) ν 1736, 1380, 1370 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (d, *J* = 6.8 Hz, 6H); 1.35 (s, 3H); 1.41 (s, 3H); 1.98–1.87 (m, 1H); 2.52 (dd, *J* = 15.7 and 7.6 Hz, 1H, CH_2); 2.72 (dd, *J* = 15.7 and 6.4 Hz, 1H, CH_2); 3.65 (dd, *J* = 8.4 and 6.4 Hz, 1H, CH_2); 3.88 (d, *J* = 6.4 Hz, 2H); 4.16 (dd, *J* = 8.4 and 6.0 Hz, 1H, CH_2); 4.40–4.51 (m, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.0 (2q); 25.5 (q); 26.9 (q); 27.6 (d); 39.0 (t); 69.2 (t); 70.8 (t); 72.1 (d); 109.1 (s); 170.6 (s).

1-Bromo-3-(2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-one (13). A 1.6 M solution of MeLi in Et_2O (5 mL, 8 mmol) was added to a solution of **12** (865 mg, 4 mmol) and dibromomethane (557 µL, 8 mmol) in THF (20 mL) at -116 ºC. The solution was stirred for 3 h and then quenched with sat. NH_4Cl (60 ml). The residue was immediately extracted with CH_2Cl_2. The organic layers were dried over $MgSO_4$, filtered, and then concentrated *in vacuo*. Purification by silica gel column chromatography (80:20 to 70:30 hexane-EtOAc) yielded **13** (460 mg, 49%) as a yellow oil. IR (KBr film) ν 1719, 1370, 840 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (s, 3H); 1.42 (s, 3H); 2.82 (dd, *J* = 16.6 and 6.0 Hz, 1H, CH_2); 3.07 (dd, *J* = 16.6 and 6.8 Hz, 1H, CH_2); 3.60 (dd, *J* = 8.4 and 6.4 Hz, 1H, CH_2); 3.94 (s, 2H); 4.18 (dd, *J* = 8.4 and 6.0 Hz, 1H, CH_2); 4.44–4.51 (m, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 25.4 (q); 26.8 (q); 34.6 (t); 44.16 (t); 69.2 (t); 71.8 (d); 109.3 (s); 199.7 (s). MS (ESI-TOF) 259 (MBr^{79}+Na, 100); 261 (MBr^{81}+Na, 98). HRMS (+ESI): *m/z* calcd. for $C_8H_{13}O_3NaBr$ (M+Na) 258.9944, found 258.9946.

Methyl 2-[3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxopropylamino]-5-methoxybenzoate (10): *Route A*. K_2CO_3 (68 mg, 0.5 mmol) was added to a solution of **9** (50 mg, 0.11 mmol) in

MeOH and the mixture was stirred for 1 h. The solvent was removed *in vacuo* and the resulting residue was purified by silica gel column chromatography (95:5 to 80:20 hexane/EtOAc) to yield **10** (38 mg, 97%) as a yellowish oil.

Route B. 2,6-Lutidine (1.6 mL, 13.74 mmol) and tetrabutylammonium iodide (3.08 g, 8.33 mmol) were added to a solution of **5** (1.21 g, 6.66 mmol) and **13** (1.37 g, 5.76 mmol) in 1,4-dioxane (12 mL). The reaction mixture was stirred at 40 ºC for 15 min under microwave irradiation. Purification by silica gel column chromatography (95:5 to 80:20 hexane-EtOAc) yielded **10** (1.44 g, 74%) as a yellow oil. IR (KBr film) ν 3350, 1705, 1692, 1521, 1286, 1225, 1045 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 3H); 1.40 (s, 3H); 2.64 (dd, *J* = 16.2, 6.5 Hz, 1H, CH_2); 2.94 (dd, *J* = 16.2, 6.5 Hz, 1H, CH_2); 3.58 (dd, *J* = 8.4, 6.7 Hz, 1H); 3.76 (s, 3H, OMe); 3.89 (s, 3H, OMe); 4.08 (bd, 2H, CH_2); 4.18 (dd, *J* = 8.4 and 6.0 Hz, 1H); 4.46-4.52 (m, 1H); 6.46 (d, *J* = 9.1 Hz, 1H); 7.02 (dd, *J* = 9.1 and 3.1 Hz, 1H); 7.45 (d, *J* = 3.1 Hz, 1H); 7.99 (bt, 1H, NH). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 25.4 (q); 26.8 (q); 44.2 (t); 51.7 (q); 54.2 (t); 55.9 (q); 69.3 (t); 71.8 (d); 109.1 (s); 110.8 (s); 112.7 (d); 114.8 (d); 123.1 (d); 144.7 (s); 149.9 (s); 168.4 (s); 204.7 (s). MS (ESI-TOF) 338 (M+1, 47); 675 (2M+1, 100). HRMS *m/z* calcd. for $C_{17}H_{24}NO_6$ 338.1598, found 338.1603.

(*Z*/*E*)-Methyl 2-[2-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)but-2-enylamino]-5-methoxybenzoate (*Z*/*E*-14). A 2.5 M solution of BuLi in hexane (1.27 mL, 3.17 mmol) was added to a mixture of ethyltriphenylphosphonium bromide (1.18 g, 3.17 mmol) in anhydrous THF (13 mL). The reaction mixture was stirred at room temperature for 1 h and then cooled to -78 ºC. A solution of **10** (337 mg, 1.58 mmol) in anhydrous THF (3 mL) was added dropwise to the reaction mixture. The reaction mixture was stirred at -78 ºC for 30 min and subsequently allowed to warm to room temperature for an additional 30 min. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography with

hexane-EtOAc (95:5 to 80:20) to yield *Z*-**14** and *E*-**14** as a yellowish oil (239 mg, 43%; 73:27 *Z*/*E*, as determined by ^{1}H NMR). IR (KBr film) ν 3373, 1689, 1517, 1222, 1065 cm^{-1}. *Z*-**14:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 3H); 1.40 (s, 3H); 1.74 (d, *J* = 6.9 Hz, 3H); 2.23–2.44 (m, 2H, CH_2); 3.53 (dd, *J* = 7.8 and 7.4 Hz, 1H); 3.76 (s, 3H, OMe); 3.82 (bs, 2H, CH_2); 3.85 (s, 3H, OMe); 4.00 (dd, *J* = 7.8 and 6.0 Hz, 1H); 4.19–4.26 (m, 1H); 5.56 (q, *J* = 6.9 Hz, 1H); 6.63 (d, *J* = 9.2 Hz, 1H); 7.03 (dd, *J* = 9.2 and 3.2 Hz, 1H); 7.42 (d, *J* = 3.2 Hz, 1H). *E*-**14:** ^{1}H NMR (100.6 MHz, $CDCl_3$) δ 1.35 (s, 3H); 1.43 (s, 3H); 1.65 (d, *J* = 6.9 Hz, 3H); 2.23–2.44 (m, 2H, CH_2); 3.56 (dd, *J* = 7.6 and 7.6 Hz, 1H); 3.75 (s, 3H, OMe); 3.82 (bs, 2H, CH_2); 3.86 (s, 3H, OMe); 4.02–4.05 (m, 1H); 4.19–4.26 (m, 1H); 5.62 (q, *J* = 6.9 Hz, 1H); 6.63 (d, *J* = 9.2 Hz, 1H); 7.00 (dd, *J* = 9.2 and 3.1 Hz, 1H); 7.41 (d, *J* = 3.1 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 13.4 (q); 25.7 (q); 27.0 (q); 32.6 and 42.8 (t); 40.0 (t); 51.5 (q); 56.0 (q); 69.3 (t); 75.0 (d); 108.9 (s); 112.8 and 114.1 (d); 114.4 (d); 123.3 (d); 125.4 (d); 133.0 (s); 133.7 (s); 146.4 (s); 149.4 (s); 168.6 (s). MS (ESI-TOF) 350 (M+1, 35); 372 (M+Na, 45); 721 (2M+Na, 100). HRMS *m/z* calcd. for $C_{19}H_{28}NO_5$ 350.1962, found 350.1961.

(*Z*)-2-[2-((2,2-Dimethyl-1,3-dioxolan-4-yl)methyl)but-2-enylamino]-5-methoxybenzoic acid (*Z*-15). LiOH (310 mg, 7.39 mmol) was added to a solution of *Z*/*E*-**14** (258 mg, 0.74 mmol) in 75:25 H_2O/THF (20 mL). The reaction mixture was sonicated at room temperature for 1 h and then stirred at 50 ºC for 24 h. The crude mixture was washed with Et_2O. The aqueous phase was cooled to 0 ºC, acidified to pH 7 with 4 M HCl (1.7 mL) and extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, and then concentrated *in vacuo* to yield *Z*/*E*-**15** (250 mg, quant.; 73:27 *Z*/*E*, as determined by ^{1}H NMR) as a yellowish oil. Purification by semipreparative HPLC performed on a 15.5 g Redisep Gold C_{18} (20-40 μm) column, with UV detection at 254 nm, a flow rate of 18 mL/min, and H_2O-

CH_3CN as eluents (gradient: 80:20 to 60:40 in 50 min), yielded *Z*-**15** (80 mg, 72% recovery). IR (KBr film) ν 3375, 2985, 1668, 1577, 1516, 1371, 1216, 1041 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 3H); 1.42 (s, 3H); 1.74 (d, *J* = 6.9 Hz, 3H); 2.27 (dd, *J* = 14.3 and 5.7 Hz, 1H, CH_2); 2.41 (dd, *J* = 14.3 and 7.1 Hz, 1H, CH_2); 3.55 (t, *J* = 8.0 Hz, 1H); 3.78 (s, 3H, OMe); 3.86 (bs, 2H); 4.02 (dd, *J* = 8.0 and 6.0 Hz, 1H); 4.20–4.27 (m, 1H); 5.58 (q, *J* = 6.9 Hz, 1H); 6.69 (d, *J* = 9.1 Hz, 1H); 7.08 (dd, *J* = 9.1 and 3.1 Hz, 1H); 7.49 (d, *J* = 3.1 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.5 (q); 25.7 (q); 27.0 (q); 39.8 (t); 43.3 (t); 56.0 (q); 69.2 (t); 75.1 (d); 109.1 (s); 109.8 (s); 113.9 (d); 114.6 (d); 124.5 (d); 125.8 (d); 133.3 (s); 146.3 (s); 150.0 (s); 172.5 (s). MS (ESI-TOF) 336 (M+1, 100); 337 (M+2, 23).

(*Z*)-3-Ethylidene-5-hydroxy-10-methoxy-1,2,3,4,5,6-hexahydrobenzo[*c*][1,5]oxazecin-8-one (1). *Z*-**15** (30 mg, 0.09 mmol) was stirred with 4 M HCl (4 mL) for 30 min and then concentrated *in vacuo* to yield (*Z*)-2-(2-ethylidene-4,5-dihydroxypentylamino)-5-methoxybenzoic acid hydrochloride. The resulting residue was used in the following step without further purification. A mixture of EDC·HCl (69 mg, 0.36 mmol), (*Z*)-2-[2-ethylidene-4,5-dihydroxypentylamino]-5-methoxybenzoic acid hydrochloride, solid supported DMAP (18 mg, 0.09 mmol), and molecular sieves 4 Å (254 mg) in dry CH_2Cl_2 (20 mL) was stirred at room temperature for 3 h. The crude mixture was filtered, treated with sat. NH_4Cl, and then extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and then concentrated *in vacuo*. Purification by silica gel column chromatography (100:0 to 80:20 CH_2Cl_2/MeOH) yielded *Z*-**1** as a brownish oil (9 mg, 35%). IR (KBr film) ν 3397, 1640, 1498, 1436, 1292, 1228, 1039 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 and 1.45 (2d, *J* = 6.9 Hz, 3H); 2.09–2.20 (m, 2H, CH_2); 3.44–3.51 and 3.59–3.69 (2m, 2H, CH_2); 3.73 (s, 3H, OMe); 3.79–3.90 (m, 1H); 4.35 (dd, *J* = 14.3 and 6.1 Hz, 1H, CH_2); 4.82 (dd, *J* = 14.3, 3.1

Hz, 1H, CH_2); 5.51 and 5.53 (2q, *J* = 6.9 Hz, 1H); 6.72–6.76 (m, 2H); 6.92 (2d, *J* = 8.5, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.4 (q); 38.2 and 38.9 (t); 46.9 and 47.1 (t); 55.5 (q); 66.2 and 66.8 (t); 70.4 and 70.9 (d); 111.5 and 111.6 (d); 116.5 (d); 127.0 and 127.1 (d); 127.9 and 128.4 (d); 130.2 and 130.5 (s); 130.7 (s); 136.1 and 136.2 (s); 158.9 (s); 169.1 and 169.2 (s). MS (ESI-TOF) 299 (M+Na, 13); 555 (2M+1, 100); 577 (2M+Na, 100). HRMS *m/z* calcd. for $C_{30}H_{38}N_2O_8Na$ 577.2520, found 577.2521.

(2*S*,4*R*)-1-*tert*-Butoxycarbonyl-4-hydroxy-2-(trimethylsilyloxymethyl)pyrrolidine (17).. TMSCl (0.65 mL, 4.85 mmol) was added to a solution of *N*-Boc-trans-4-hydroxy-L-prolinol (1.054 g, 4.85 mmol), Et_3N (0.67 mL, 4.85 mmol), and DMAP (50 mg, 0.4 mmol) in CH_2Cl_2 (25 mL). The reaction mixture was stirred at 0 ºC for 16 h. After this time the reaction mixture was washed with water, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. Purification by silica gel column chromatography with hexane-EtOAc (70:30 to 50:50) yielded **17** (444 mg, 32%) as a colorless oil. IR (KBr film) ν 3434, 1692, 1678, 1408, 1119 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 0.12 and 0.13 (2s, 9H); 1.50 and 1.51 (2s, 9H); 1.90–2.04 (m, 1H, CH_2); 2.18 (dt, *J* = 17.2 and 5.8 Hz, 1H, CH_2); 3.35–3.48 (m, 2H, CH_2); 3.63 and 3.65 (2d, *J* = 10.2 Hz, 1H, CH_2); 3.78 and 3.90 (2dd, *J* = 10.2 and 4.6 Hz, 1H, CH_2); 3.94–4.02 (m, 1H, CH); 4.38–4.44 (m, 1H, CH). ^{13}C NMR (100.6 MHz, CD_3OD) δ –0.5 (q); 28.8 (q); 37.3 and 38.2 (t); 55.9 and 56.4 (t); 58.8 and 58.9 (d); 63.6 and 64.6 (t); 70.1 and 70.6 (d) 80.8 and 81.1 (s); 155.3 (s). HRMS *m/z* calcd. for $C_{13}H_{28}NO_4Si$ 290.1782, found 290.1784.

(*S*)-1-*tert*-Butoxycarbonyl-2-(trimethylsilyloxymethyl)pyrrolidin-4-one (18). DMP (369 mg, 0.87 mmol) was added to a solution of **17** (228 mg, 0.78 mmol) in CH_2Cl_2 (10 mL) and the reaction mixture was stirred at room temperature for 15 min. After this time sat. $NaHCO_3$

and sat. $Na_2S_2O_3$ were added and the reaction mixture was stirred for additional 10 minutes. The residue was extracted with CH_2Cl_2, the combined organic extracts were dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. Purification by silica gel column chromatography with hexane-EtOAc (80:20) yielded **18** (182 mg, 82%) as a colorless oil. IR (KBr film) ν 1765, 1701, 1401, 1106 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 0.12 (s, 9H); 1.54 (s, 9H); 2.41 (d, *J* = 18.0 Hz, 1H, CH_2); 2.77–2.93 (m, 1H, CH_2); 3.57–3.68 (m, 2H, CH_2); 3.86 (d, *J* = 18.0 Hz, 1H, CH_2); 3.99 (dd, *J* = 22.1, 10.0 Hz, 1H, CH_2); 4.38 and 4.40 (2bs, 1H, CH). ^{13}C NMR (100.6 MHz, CD_3OD) δ –0.9 (q); 28.7 (q); 41.1 and 41.7 (t); 54.4 and 54.9 (t); 56.8 and 57.4 (d); 65.3 and 66.1 (t), 81.6 (s). HRMS *m/z* calcd. for $C_{13}H_{26}NO_4Si$ 288.1626, found 288.1628.

(*S,Z*)-1-*tert*-Butoxycarbonyl-4-ethylidene-2-((trimethylsilyloxy)methyl)pyrrolidine (*Z*-19). ^{t}BuOK (213 mg, 1.9 mmol) was added to a solution of ethyltriphenylphosphonium bromide (705 mg, 1.9 mmol) in THF (5 ml), the mixture was stirred for 1 h. After this time **18** (180 mg, 0.62 mmol) was added and the mixture was stirred for additional 30 min. Water was added and the residue was extracted with Et_2O, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. Purification by silica gel column chromatography with hexane-EtOAc (95:5) yielded *Z*/*E*-**19** (136 mg, 73 %) in portion 9:1 as a colorless oil. IR (KBr film) ν 1702, 1397, 1251, 1109 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.09 (s, 9H,); 1.48 (s, 9H); 1.58 (d, *J* = 6.8 Hz, CH_3); 2.45–2.54 (m, 1H, CH_2); 2.56–2.67 (m, 1H, CH_2); 3.20–3.45 (m, 1H, CH_2); 3.55–3.67 (m, 1H, CH_2); 3.78–4.05 (m, 3H, CH + CH_2); 5.32–5.40 (m, 1H, CH). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ –0.5 (q); 14.5 (q); 28.5 (q); 34.0 and 34.5 (t); 47.5 (t) 57.5 and 57.7 (d); 62.7 and 63.1 (t); 79.4 (s); 115.9 and 116.2 (d); 136.7 (s); 154.2 (s). HRMS *m/z* calcd. for $C_{15}H_{30}NO_3Si$ 300.1989, found 300.1990.

(*S,Z*)-4-Ethylidene-2-(hydroxymethyl)pyrrolidine (*Z*-20). A solution of *Z*/*E*-**19** in portion 9:1 (136 mg, 0.45 mmol) in 10% TFA in CH_2Cl_2 (5 mL) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography with CH_2Cl_2-MeOH (98:2 to 90:10) to obtain *Z*/*E*-**20** (105 mg) in portion 9:1 quantitative yield. IR (KBr film) ν 3380, 1677, 1435, 1135 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (d, *J* = 8.0 Hz, 3H, CH_3); 2.34–2.45 (m, 1H, CH_2); 2.58–2.68 (m, 1H, CH_2); 3.65–3.95 (m, 5H); 5.50–5.58 (m, 1H, =CH). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.7 (q); 32.2 (t); 45.8 (t); 60.5 (t); 61.4 (d); 120.6 (d); 130.9 (s). HRMS *m/z* calcd. for $C_7H_{14}NO$ 128.1069, found 128.1070.

(*S,Z*)-*N*-(4-Hydroxy-3-methoxybenzoyl)-4-ethylidene-2-(hydroxymethyl)pyrrolidine (*Z*-16). PyBOP (135 mg, 0.26 mmol) was added to a solution of DIEA (0.1 mL, 0.55 mmol) and vanillic acid (44 mg, 0.26 mmol) in THF (5 mL), and the mixture was stirred for 10 min. Then, a solution of *Z*/*E* **-20** in portion 9:1 (26 mg, 0.21 mmol) in THF was added and the mixture was stirred for 1 h. The solvent was removed under reduced pressure and the residue was disolved in EtOAc, washed with sat. $NaHCO_3$, and sat. NH_4Cl. Purification by silica gel column chromatography with EtOAc yielded *Z*-**16** (45 mg, 73%) in portion 9:1 as a colorless oil. $[\alpha]_D = -21.5$ (*c* 0.75, CH_2Cl_2). IR (KBr film) ν 3288, 1600, 1585, 1431, 1277, 1207 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (bs, 3H, CH_3); 2.27–2.40 (m, 1H, CH_2); 2.66–2.78 (m, 1H, CH_2); 3.65–3.75 (m, 2H, CH_2); 3.90 (s, 3H, OMe); 4.00–4.20 (m, 2H, CH_2); 4.53–4.69 (m, 1H, CH); 5.30–5.45 (m, 1H); 6.91 (d, *J* = 8.1 Hz, 1H); 7.05 (dd, *J* = 8.1 and 1.8 Hz, 1H); 7.09 (d, *J* = 1.8 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.5 (q); 34.1 (t); 51.5 (t); 56.1 (q); 59.9 (d); 66.4 (t); 110.4 (d); 114.0 (d); 117.7 (d); 120.7 (d); 128.0 (s); 134.4 (s); 146.6 (s); 147.6 (s); 172.0 (s). HRMS *m/z* calcd. for $C_{15}H_{20}NO_4$ 278.1387, found 278.1387.

(*S,E*)-1-*tert*-Butoxycarbonyl-4-ethylidene-2-(trimethylsilyloxymethyl)pyrrolidine (*E*-19). A 2M solution of LDA in THF (0.25 mL, 0.5 mmol) was added to a solution of 5-(ethylsulfonyl)-1-phenyl-*1H*-tetrazole (119 mg, 0.5 mmol) in THF (4 ml) at −78 ºC and the mixture was stirred for 10 min. After this time, **18** (116 mg, 0.4 mmol) was added and the mixture was stirred for additional 30 min. The reaction mixture was quenched with sat. NH_4Cl and was extracted with CH_2Cl_2. The organic fractions were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. Purification by silica gel column chromatography with hexane-EtOAc (97:3) yielded *Z*/*E*-**19** (58 mg, 50 %) in portion 1:2 as a colorless oil. IR (KBr film) ν 1702, 1397, 1251, 1109 cm^{-1}. *E* diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.09 (s, 9H); 1.46 (s, 9H); 1.62 (d, *J* = 6.7 Hz, CH_3); 2.41–2.52 (m, 1H, CH_2); 2.54–2.66 (m, 1H, CH_2); 3.20–3.45 (m, 1H, CH_2); 3.55–3.65 (m, 1H, CH_2); 3.72–3.80 (m, 1H, CH_2); 3.87–4.05 (m, 2H); 5.32–5.42 (m, 1H, CH). *E* diastereomer: ^{13}C NMR (100.6 MHz, $CDCl_3$) δ –0.5 (q); 14.4 (q); 28.5 (q); 29.8 and 30.4 (t); 50.8 and 51.4 (t); 57.5 and 57.7 (d); 62.7 and 63.1 (t); 79.4 (s); 115.5 and 116.1 (d); 136.7 (s); 154.2 (s). HRMS *m/z* calcd. for $C_{15}H_{30}NO_3Si$ 300.1989, found 300.1990.

(*S,E*)-4-Ethylidene-2-(hydroxymethyl)pyrrolidine (*E*-20). A solution of *Z*/*E*-**19** in portion 1:2 (95 mg, 0.31 mmol) in 10% TFA in CH_2Cl_2 (5 mL) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography with CH_2Cl_2-MeOH (98:2 to 90:10) to obtain *Z/E*-**20** in portion 1:2 in quantitative yield. IR (KBr film) ν 3380, 1677, 1435, 1135 cm^{-1}. *E* diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (d, *J* = 6.7 Hz, 3H, CH_3); 2.21–2.30 (m, 1H, CH_2); 2.58–2.68 (m, 1H, CH_2); 3.65–3.95 (m, 5H); 5.50–5.58 (m, 1H, CH). *E* diastereomer: ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.7 (q); 28.2 (t); 48.7 (t); 60.8 (t); 61.6 (d); 120.6 (d); 130.9 (s). *E*

diastereomer: ^{1}H NMR (400 MHz, CD_3OD) δ 1.68 (d, *J* = 6.9 Hz, 3H, CH_3); 2.40 (dd, *J* = 16.4 and 8.4 Hz, 1H, CH_2); 2.75 (dd, *J* = 16.4 and 7.0 Hz, 1H, CH_2); 3.60–3.98 (m, 5H); 5.55–5.67 (m, 1H, CH). *E* diastereomer: ^{13}C NMR (100.6 MHz, CD_3OD) δ 14.8 (q); 29.1 (t); 50.1 (t); 61.2 (t); 62.7 (d); 121.0 (d); 133.1 (s). HRMS *m/z* calcd. for $C_7H_{14}NO$ 128.1069, found 128.1070.

(*S*,*E*)-*N*-(4-Hydroxy-3-methoxybenzoyl)-4-ethylidene-2-(hydroxymethyl)pyrrolidine (*E*-16). PyBOP (93 mg, 0.18 mmol) was added to a solution of DIEA (64 µL, 0.37 mmol) and vanillic acid (30 mg, 0.18 mmol) in THF (5 mL), and the mixture was stirred for 10 min. After this time a solution of *Z*/*E*-**20** in portion 1:2 (19 mg, 0.15 mmol) in THF was added and the mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was disolved in EtOAc, washed with sat. $NaHCO_3$, and sat. NH_4Cl. Purification by silica gel column chromatography with EtOAc yielded *Z*/*E*-**16** (25 mg, 60%) in portion 1:2. Purification by semipreparative HPLC using a Waters XBridge C18 column (10×100 mm, 5µm), UV detection at 254 nm, with a flow of 3 mL/min, and using H_2O-CH_3CN 18:82 as solvent system in isocratic conditions, yielded *E*-**16** (5.7 mg). The ^{1}H-NMR and ^{13}C-NMR are identical as described for the natural product in Table 2 in the Supporting information. $[\alpha]_D$ = − 51.2 (*c* 0.25, CH_2Cl_2). IR (KBr film) ν 3288, 1600, 1585, 1431, 1277, 1207 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (d, *J* = 6.8, 3H, CH_3); 2.20–2.35 (m, 1H, CH_2); 2.67–2.77 (m, 1H, CH_2); 3.75 (bs, 2H, CH_2); 3.90 (s, 3H, OMe); 4.00–4.22 (m, 2H, CH_2); 4.67 (bs, 1H, CH); 5.34 (bs, 1H, CH); 6.91 (d, *J* = 7.8 Hz, 1H, Ar); 7.04 (d, *J* = 7.8, 1H, Ar); 7.09 (s, 1H, Ar). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.5 (q); 30.1 (t); 55.1 (t); 56.3 (q); 60.6 (d); 67.1 (t); 110.6 (d); 114.1 (d); 117.6 (d); 121.0 (d); 128.3 (s) 134.7 (s); 146.7 (s); 147.8 (s); 172.0 (s). HRMS *m/z* calcd. for $C_{15}H_{20}NO_4$ 278.1387, found 278.1387.

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Supporting Information Available: General procedures, tables of the bioactivity of isolated **barmumycin** and of its diacetyl derivative; NMR data Tables 2 and 3; ^{1}H- and ^{13}C-NMR spectra of compounds **6a-e**, **7**-**14**, **17**, **18**, *Z/E*-**19** and *Z/E*-**20**; and the ^{1}H- and ^{13}C-NMR spectra with two-dimensional NMR experiments for compounds *Z*-**15**, *Z*-**1**, *Z*-**16** and *E*-**16**. This information is available free of charge via the Internet at http://pubs.acs.org.

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12. Other reduction conditions proved unsuccessful. These included $NaBH(OAc)_3$ in THF at room temperature for 16 h; $NaBH(OAc)_3$ in CH_2Cl_2/AcOH at room temperature for 5 h; and $NaBH(OAc)_3$ in toluene at 110 ºC for 2 h.

13. The aniline **5** was isolated from the oxidation degradation mixture. Its formation could be rationalized through hydrolysis of the enamine resulting from enolization of the keto-compound.

14. Under these conditions methyl 2-(5-allyl-2-oxooxazolidin-3-yl)-5-methoxybenzoate was isolated as a by-product in 27% yield.

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16. The *Z/E* stereoisomers were in a ratio of 73:27 (based on NMR signal areas).

17. The NOESY correlations between $^4CH_2$ (2.27 and 2.41 ppm) and the vinyl proton (5.58 ppm), confirmed the stereochemistry of ***Z*-15** (see NOESY interactions in the Supporting Information).

18. The NOESY correlations between $^2CH_2$ (4.35 and 4.82 ppm) and CH_3 (1.42 and 1.45 ppm), and between $^4CH_2$ (2.09-2.20 ppm) and the vinyl proton (5.51 and 5.53 ppm), confirmed the stereochemistry of ***Z*-1** (see NOESY interactions in the Supporting Information).

19. The reaction was performed with $(EtO)_2P(O)CH_2CH_3$ and either LDA, K^{t}BuO or NaHMDS as base.

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