

# Synthesis of sugar-modified analogs of bredinin (mizoribine), a clinically useful immunosuppressant, by a novel photochemical imidazole ring-cleavage reaction as the key step †<sup>1</sup>

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The imidazole nucleoside bredinin (mizoribine) is a clinically useful immunosuppressant. Derivatization of bredinin by the usual nucleoside chemistry is often troublesome due to the unusual zwitterionic structure of the base moiety. We achieve the synthesis of 5'-modified analogs of bredinin *via* a novel photochemical imidazole ring-cleavage reaction as the key step. When a solution of 2',3'-*O*-isopropylidenebredinin **5** in 0.1 M AcOH is irradiated with a high-pressure mercury lamp, an imidazole ring-cleavage reaction occurs to give the 2-aminomalonomamide riboside derivative **16** in 71% yield. Appropriate modifications of the 5'-position of **16** and subsequent condensation with (EtO)<sub>3</sub>CH to reconstruct the imidazole base moiety follow. Using this imidazole ring-cleavage–reconstruction strategy, some biologically important 5'-modified bredinin analogs, *i.e.*, the 5'-phosphate **2**, the 5'-deoxy derivative **3**, and the 5'-*O*-aminopropylcarbamate **4** are efficiently synthesized.

## Introduction

Bredinin (mizoribine, **1**) is an imidazole nucleoside antibiotic isolated from *Eupenicillium brefeldianum*<sup>2</sup> and now clinically used as an immunosuppressant,<sup>3,4</sup> especially for the transplantation of viscera<sup>3a,b</sup> and for autoimmune diseases such as rheumatism.<sup>3c</sup> Bredinin as well as its analogs also have anti-tumor effects in experimental tumor models,<sup>5</sup> and recently its significant antiviral effect was also reported.<sup>6</sup> Therefore, chemical modification studies of bredinin may result in the development of useful compounds with efficient pharmacological effects. However, derivatization of bredinin by the conventional methods used in nucleoside chemistry is often troublesome,<sup>5a,7</sup> probably because of the unusual zwitterionic structure of the base moiety. Thus, despite its biological interest, only a few studies on the synthesis of derivatives of bredinin have been reported.<sup>5</sup>

We planned to synthesize the currently biologically important 5'-modified derivatives of bredinin, the 5'-phosphate **2**, the 5'-deoxy derivative **3**, and the 5'-*O*-(3-aminopropyl)carbamate **4** (Fig. 1). However, our first attempt at synthesizing the target compounds from 2',3'-*O*-isopropylidenebredinin **5**, which was readily obtained by treating bredinin with TsOH–acetone, was unsuccessful. When an electron-withdrawing group was introduced at the 5'-position, intramolecular attack by the 2-oxygen of the base moiety quickly occurred. For example, treatment of **5** under the usual mesylation conditions or phosphotriester method produced none of the desired 5'-*O*-mesylester **6** or 5'-phosphate **7**. Both reactions instead gave the 5,5'-anhydro derivative **8** as the major product (Scheme 1). We also tried protecting the phenolic hydroxy group of the base moiety of bredinin with acyl and silyl groups, but this also proved unsuccessful.

Previously, we reported the synthesis of bredinin from 5-

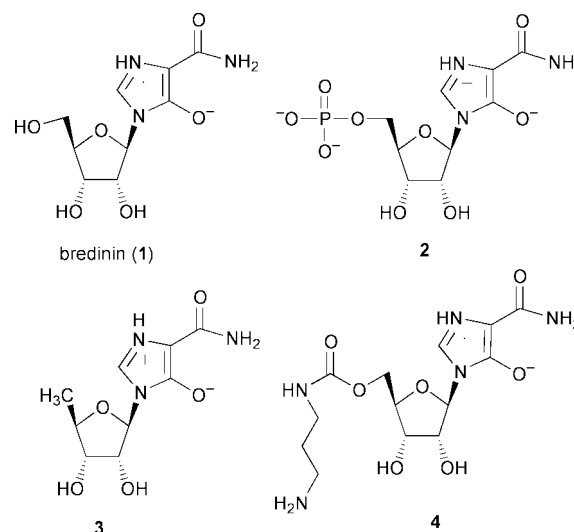
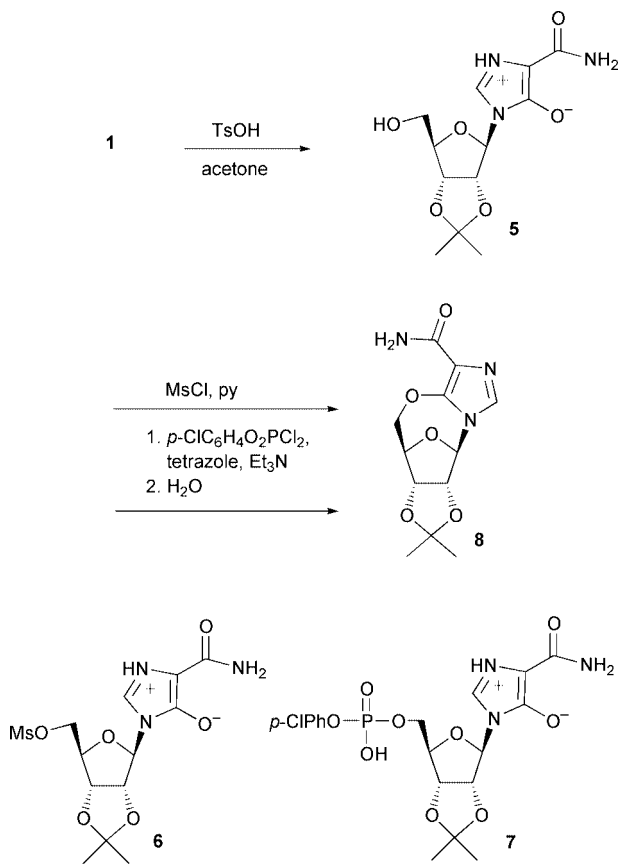


Fig. 1

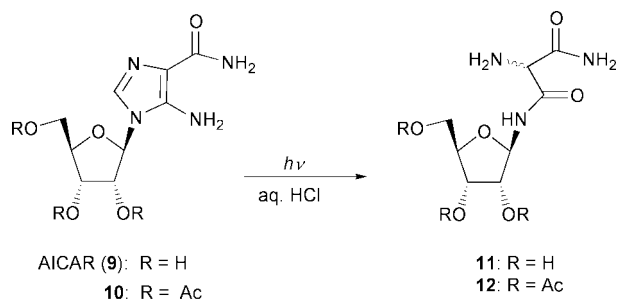
amino-4-carbamoylimidazol-1-yl-β-D-ribofuranoside (AICAR, **9**) using a novel photoreaction.<sup>7</sup> When an acidic aqueous solution of **9** or its triacetate **10** was irradiated with a UV lamp, an imidazole ring-cleavage reaction occurred to give the 2-aminomalonomamide derivative **11** or **12**, respectively as the major product. We found that upon heating with (EtO)<sub>3</sub>CH in DMF, compounds **11** and **12** were converted into bredinin **1** and its triacetate **13**, respectively, as shown in Scheme 2.<sup>7</sup> Accordingly, 2-aminomalonomamide ribosides, such as **11** and **12**, are considered synthetic equivalents of bredinin. Although **11** or its tri-*O*-acetate **12** might also be useful intermediates for preparing various bredinin derivatives,<sup>5a</sup> the yields of **11** and **12** from AICAR or tri-*O*-acetyl-AICAR were low (about 30%) and were obtained on only a 100 mg scale once.<sup>5a,7</sup>

In this paper, we describe an efficient preparation of **12** and the corresponding 2',3'-*O*-acetone **16** (see Scheme 5, below) from bredinin by a photochemical reaction, and conversion of **16** into the biologically important 5'-modified bredinin

† <sup>1</sup>H NMR spectral charts of **15**, **19**, **20**, **22** and **4**, and H–H COSY spectral charts of **15**, **17**, **18**, **24** and **4** are available as supplementary data from BLDSC (SUPPL. NO. 57713, 11 pp.) or the RSC Library. See Instructions for Authors available *via* the RSC web page (<http://www.rsc.org/authors>).



Scheme 1



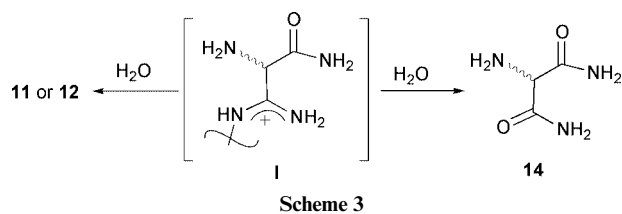
Scheme 2

derivatives, *i.e.*, the 5'-phosphate **2**, the 5'-deoxy derivative **3**, and the 5'-*O*-(3-aminopropyl)carbamate **4** (Fig. 1), *via* reconstruction of the imidazole base moiety.<sup>8</sup>

## Results and discussion

### Photoreaction of bredinin

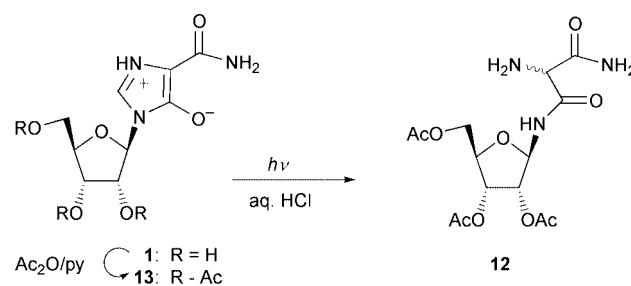
Although the reaction mechanism of the imidazole ring-cleavage reaction of AICAR is unclear, it is likely that the 2-aminomalonyl-ribose **11** and **12** are produced *via* acidic hydrolysis of the amidinium intermediate **I** (Scheme 3). In the acidic hydrolysis of **I**, two pathways, producing the desired 2-aminomalonyl-ribose **11** or **12** and the undesired



Scheme 3

2-aminomalonyl-ribose **14**, respectively, are possible. We expected that the desired 2-aminomalonyl-ribose **11** or **12** might be obtained efficiently if the photoreaction was carried out with bredinin **1**, or its sugar-protected derivatives, as a substrate, since the acidic hydrolysis process of the amidinium moiety would not be needed in this case. Thus, we examined the reaction with bredinin.

We first investigated the photoreaction with bredinin 2',3',5'-tri-*O*-acetate **13** (Scheme 4), which was readily prepared from

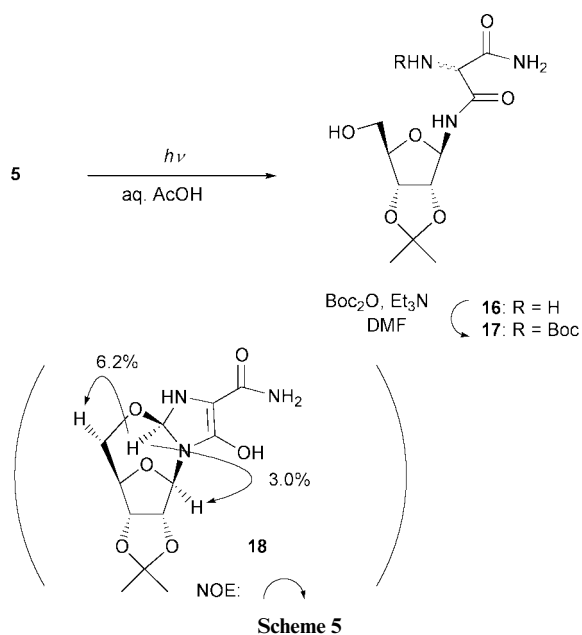


Scheme 4

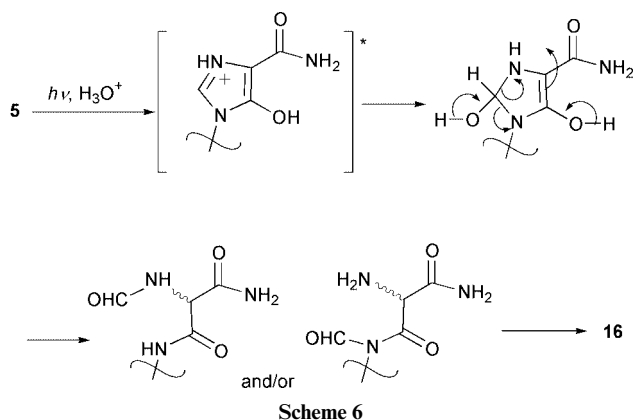
bredinin by the usual method with  $\text{Ac}_2\text{O}$ -pyridine, in order to make the detection and purification of the reaction products easy. When a solution of **13** in 0.1 M HCl was irradiated with a high-pressure mercury lamp (100 W) under bubbling of argon, the UV-absorption of the solution rapidly faded, and work-up gave **12** in 79% yield as a diastereomeric mixture at the 2-position, which was identical with the photoreaction product of tri-*O*-acetyl-AICAR **10** previously reported.<sup>7</sup>

Next, the photoreaction of 2',3'-*O*-isopropylidenebredinin **5** (Scheme 5) was carried out in  $\text{aq. AcOH}$  to avoid hydrolysis of the acetonide moiety. Thus, a solution of **5** in 0.1 M AcOH was irradiated with a high-pressure mercury lamp to give the desired imidazole ring-cleavage product **16** in 71% yield as a diastereomeric mixture at the 2-position, as in the above reaction with the triacetate **13**. In this reaction, the 2,5'-cyclized product **18** was also obtained, in 10% yield, as a by-product, the structure of which was confirmed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR, HRMS and elemental analytical data. The configuration at the 2-position was assigned as *S* by an NOE experiment (Scheme 5). When the photoreaction was carried out with a low-pressure instead of a high-pressure mercury lamp (60 W), the imidazole ring-cleavage product **16** was formed in 68% yield, with none of the cyclized product **18** produced. The 2-amino group of the photoproduct **16** was protected with a Boc group by the usual method for further derivatization.

There is no precedent for such a photochemical ring-cleavage reaction of imidazole derivatives except for our previous results with AICAR. Although the mechanism of the photoreaction is not clear, protonation of the base moiety would significantly facilitate the reaction (the  $\text{p}K_a$  of bredinin is 6.75<sup>2a</sup>), because



when performed under neutral conditions the photoreaction proceeded only slowly. Formation of the cyclized product **18** as a by-product, which is the result of an addition of a 5'-hydroxy group at the 2-position, suggested a possible reaction pathway giving **16** via hydration at the 2-position followed by deformylation (Scheme 6). However this mechanism may not be plausible,



since, when the *N*<sup>2</sup>-formyl derivative **15**, prepared from **12** by treatment with formic acid and DCC,<sup>9</sup> was subjected to the above acidic photoreaction conditions with a high-pressure mercury lamp, deformylation did not occur and none of the product **12** was obtained (Scheme 4).

This photoreaction of bredinin derivatives is very useful, since more than 5 g of the photoproduct can be readily obtained at once, while only 100 mg of the product was obtained by the reaction with AICAR derivatives. Therefore, we planned to convert the photoproduct into bredinin derivatives of biological importance.

#### Synthesis of bredinin 5'-phosphate

The mechanism of action of bredinin as an immunosuppressant has been studied.<sup>3,4,10</sup> In cells, bredinin is metabolized into the 5'-phosphate **2** (Fig. 1) by adenosine kinase,<sup>10</sup> which inhibits cellular inosine monophosphate (IMP) dehydrogenase, an essential enzyme in the *de novo* synthesis of guanine ribonucleotides. Bredinin inhibits T lymphocyte proliferation due to this antimetabolic effect. Accordingly, the 5'-phosphate **2**, the active form of bredinin in cells, should be very useful as a tool in pharmacological studies. However, an efficient method for preparing **2** has not been developed, probably because

of bredinin's unusual chemical features; for instance, when bredinin was treated under Yoshikawa's phosphorylation conditions,<sup>11</sup> which is the most useful method for preparing nucleoside 5'-phosphates, the desired product **2** was obtained in only 3% yield.<sup>12</sup> Attempted phosphorylations at the 5'-hydroxy position of 2',3'-*O*-isopropylidenebredinin **5** by the phosphotriester method described above, as well as by the phosphoramidite method, by our hand, were also unsuccessful.

We sought to develop a practical method for preparing **2** via the above photoreaction of bredinin. Therefore, we investigated introducing a phosphate unit at the 5'-position of *N*<sup>2</sup>-protected photoproduct **17**, and found that a phosphoramidite method with *o*-xylylene *N,N*-diethylphosphoramidite (XEPA)<sup>13</sup> was effective in this system (Scheme 7). Treatment of **17** with XEPA and tetrazole in CH<sub>2</sub>Cl<sub>2</sub>, followed by oxidation with aq. I<sub>2</sub>, gave the corresponding 5'-phosphotriester **19** in 70% yield. The isopropylidene and Boc groups of **19** were removed simultaneously with 90% aq. TFA, and the resulting product, without purification, was heated with (EtO)<sub>3</sub>CH in DMF at 90 °C to give the bredinin 5'-phosphate derivative **20** in 47% yield from **19**. Hydrogenation of **20** with Pd-carbon in MeOH furnished bredinin 5'-phosphate **2**, which was isolated as a disodium salt in 89% yield, after successive treatment with Dowex 50 (H<sup>+</sup>) and Diaion WK-20 (Na<sup>+</sup>) resins.

#### Synthesis of 5'-deoxybredinin

4-Carbamoylimidazolium-5-olate **25**, the aglycone of bredinin, is known to show potent antitumor effects *in vivo* stronger than those of bredinin itself.<sup>5c</sup> We designed 5'-deoxybredinin **3** as a potential antitumor agent. This compound was expected to release the aglycone **25** efficiently in tumor cells by the action of nucleoside phosphorylase (NP) (Scheme 8), since the activity of this enzyme is significantly increased in tumor cells compared with normal cells.<sup>14,15</sup>

The synthesis of **3** is shown in Scheme 7. The *N*<sup>2</sup>-(Boc-amino)malonamide riboside derivative **17** was successively treated with MsCl-py and NaI-methyl ethyl ketone (MEK) to give the corresponding 5'-deoxy-5'-iodo derivative **21** in 90% yield. The 5'-iodide **21** was then subjected to radical reduction with Bu<sub>3</sub>SnH-azoisobutyronitrile (AIBN) in benzene to give the 5'-deoxy derivative **22** quantitatively. Removal of the protecting groups of **22** with 90% TFA and subsequent reconstruction of the imidazole ring with (EtO)<sub>3</sub>CH afforded the target 5'-deoxybredinin **3** in 77% yield.

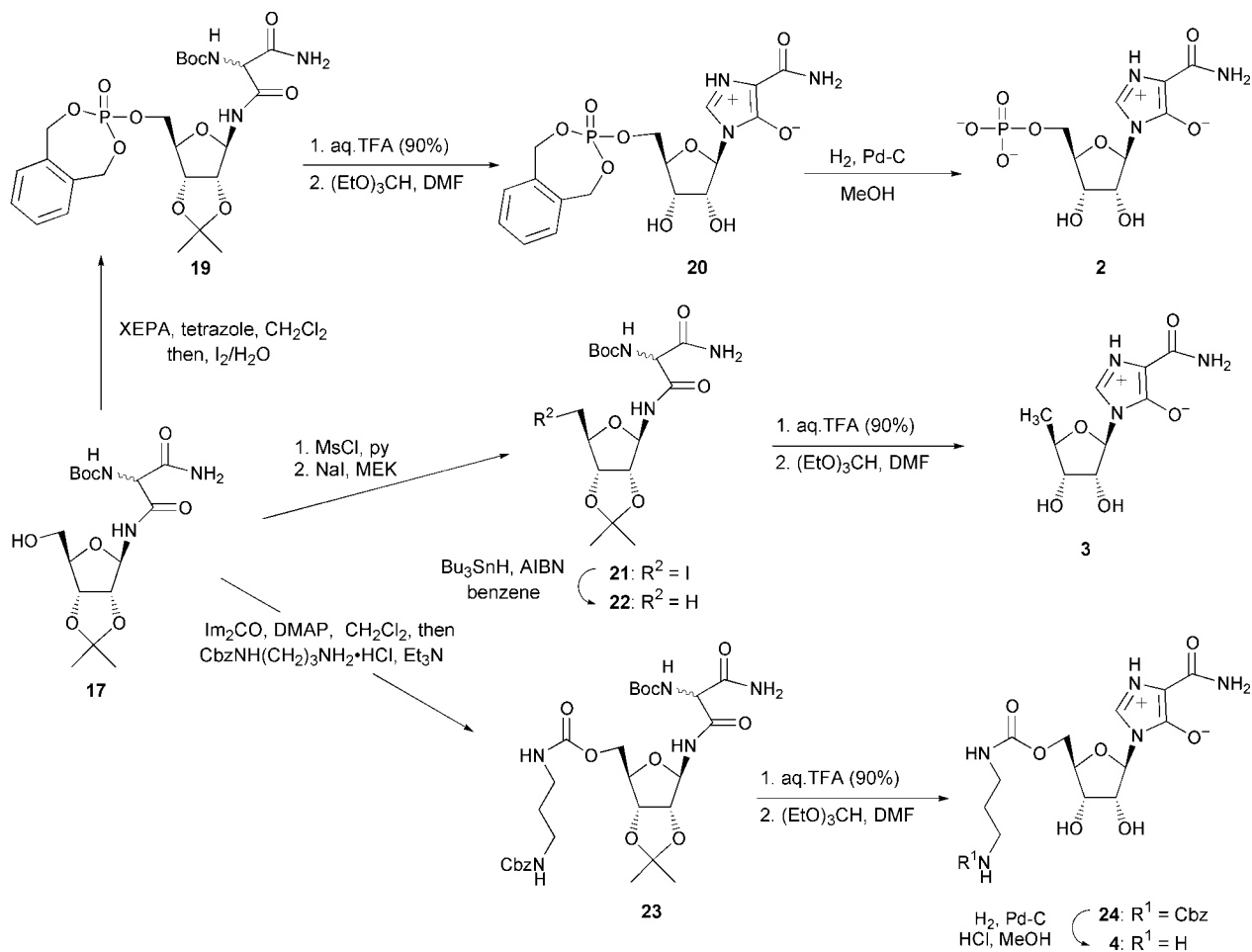
#### Synthesis of bredinin 5'-*O*-(3-aminopropyl)carbamate

The 5'-*O*-(3-aminopropyl)carbamate **4** was designed as a bredinin derivative having an aminoalkyl tether, which can bond covalently to other molecules. Such a bredinin derivative would be very useful for biological studies, which include synthesis of haptens for preparing antibodies to bredinin and also preparation of bredinin-attached resins for affinity chromatography.

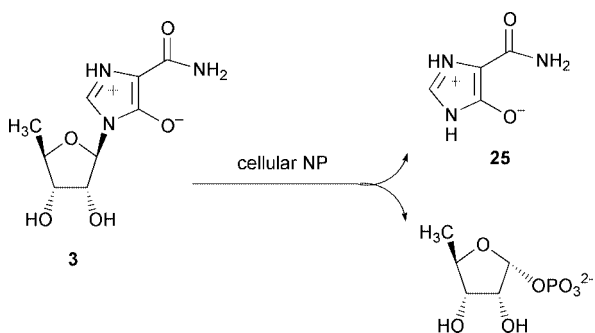
Compound **17** was successively treated with carbonyldiimidazole-4-(dimethylamino)pyridine (DMAP) and *N*-Cbz-propanediamine-Et<sub>3</sub>N in CH<sub>2</sub>Cl<sub>2</sub> to give the 5'-*O*-carbamate derivative **23** in 91% yield. Deprotection and reconstruction of the base by the above method gave the bredinin 5'-*O*-aminopropylcarbamate **24**, which was hydrogenated with Pd-C in the presence of HCl in MeOH to afford the target compound **4**, as shown in Scheme 7.

#### Conclusions

We have found that the imidazole base moiety of bredinin is cleaved easily by irradiation under aqueous acidic conditions to give 2-aminomalonyl riboside derivatives and that the base moiety of bredinin can be reconstructed when the photoproduct is heated with (EtO)<sub>3</sub>CH. Using this imidazole ring-



Scheme 7



Scheme 8

cleavage–reconstruction strategy, several biologically important 5'-modified brevidin analogs were efficiently synthesized.

## Experimental

Mps were measured on a Yanagimoto MP-3 micro-melting point apparatus and are uncorrected.  $^1\text{H}$  NMR spectra were recorded at 100, 400, and 500 MHz ( $^1\text{H}$ ) and at 100 MHz ( $^{13}\text{C}$ ). Chemical shifts ( $\delta$ ) and coupling constants ( $J$ ) are reported in ppm downfield from TMS and in Hz, respectively. Assignments of  $^1\text{H}$  and  $^{13}\text{C}$  NMR described are based on COSY and/or DEPT spectra. Mass spectra were obtained by fast-atom bombardment (FAB) or chemical ionization (CI). Thin-layer chromatography (TLC) was done on Merck 60F<sub>254</sub> coated plates. Silica gel chromatography was done with Merck silica gel 5715 or 9385. Reactions were carried out under an argon atmosphere.

### 2',3'-*O*-Isopropylidenebrevidinin 5

A suspension of brevidinin 1 (10.4 g, 40 mmol) and  $\text{TsOH}\cdot\text{H}_2\text{O}$

(16.0 g, 84.0 mmol) in acetone (800 mL) was stirred at room temperature for 2 h, and then the resulting solution was neutralized with  $\text{NH}_4\text{OH}$  (28% aq.). The resulting precipitate was filtered off and washed well with EtOH, and the filtrate was evaporated. The residue was purified by column chromatography ( $\text{SiO}_2$ ;  $\text{CHCl}_3$ –MeOH, 10:1) to give 5 (9.63 g, 80%) as a solid; mp 209–212 °C (from MeOH) (Found: C, 48.14; H, 5.82; N, 13.86.  $\text{C}_{12}\text{H}_{17}\text{N}_3\text{O}_6$  requires C, 48.16; H, 5.73; N, 14.04%);  $^1\text{H}$  NMR (400 MHz;  $\text{DMSO}-d_6 + \text{D}_2\text{O}$ )  $\delta$  8.27 (s, 1 H), 5.73 (d, 1 H,  $J = 2.6$ ), 5.16 (dd, 1 H,  $J = 5.9, 2.6$ ), 4.85 (dd, 1 H,  $J = 5.9, 2.6$ ), 4.15 (m, 1 H), 3.57 (m, 2 H), 1.49 and 1.30 (each s, each 3 H); MS (FAB, positive)  $m/z$  300 ( $\text{MH}^+$ ).

### 5,5'-Anhydrobrevidinin 8

A mixture of 5 (299 mg, 1.0 mmol) and  $\text{MsCl}$  (88  $\mu\text{L}$ , 1.3 mmol) in pyridine (8 mL) was stirred at 0 °C for 1 h. After addition of water (1.0 mL), the resulting mixture was evaporated, and the residue was partitioned between EtOAc and brine. The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), evaporated, and purified by column chromatography ( $\text{SiO}_2$ ;  $\text{CHCl}_3$ –MeOH, 100:1, then 20:1) to give 8 (138 mg, 36%) as a foam;  $^1\text{H}$  NMR (100 MHz;  $\text{CDCl}_3 + \text{D}_2\text{O}$ )  $\delta$  7.19 (s, 1 H), 5.76 (s, 1H), 5.07 (d, 1 H,  $J = 5.6$ ), 4.77 (d, 1 H,  $J = 5.6$ ), 4.69 (d, 1 H,  $J = 2.2$ ), 4.56 (dd, 1 H,  $J = 12.9, 2.2$ ), 4.06 (d, 1 H,  $J = 12.9$ ), 1.54 and 1.53 (each s, each 3 H); MS (CI)  $m/z$  282 ( $\text{MH}^+$ ); UV (MeOH)  $\lambda_{\text{max}}$  280 nm.

### 2',3',5'-Tri-*O*-acetylbrevidinin 13

A mixture of brevidinin 1 (5.18 g, 20 mmol) and  $\text{Ac}_2\text{O}$  (9.5 mL, 100 mmol) in pyridine (50 mL) was stirred at room temperature for 3 h. After MeOH was added, the solvent was evaporated, and the residue was treated with hot  $\text{CHCl}_3$ –MeOH to give crystalline 13 (5.13 g, 67%); mp 188–190 °C (Found: C, 47.05;

H, 5.09; N, 10.90. C<sub>15</sub>H<sub>19</sub>N<sub>3</sub>O<sub>9</sub> requires C, 46.76; H, 4.97; N, 10.90%; <sup>1</sup>H NMR (100 MHz; DMSO-*d*<sub>6</sub>) δ 8.35 (s, 1 H), 6.88 (br s, 2 H), 5.80 (m, 2 H), 5.52 (m, 1 H), 4.42–4.11 (m, 3 H), 2.08, 2.07 and 2.03 (each s, each 3 H); MS (CI) *m/z* 385 (M<sup>+</sup>); UV (MeOH) λ<sub>max</sub> 284 nm.

### 2-Amino-*N*-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)malonamide 12

A solution of **13** (4.10 g, 10.6 mmol) in aq. HCl (0.1 M; 500 mL) was irradiated with a high-pressure mercury lamp (100 W, Pyrex filter) at room temperature under bubbling of argon for 3.5 h. After being neutralized with aq. NaHCO<sub>3</sub> (0.8 M), the resulting mixture was concentrated to about 50 mL, and then NaCl was added. The resulting solution was extracted with CHCl<sub>3</sub> (3 times), and the combined organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated. The residue was purified by flash column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>–MeOH, 17:1) to give **12** (3.14 g, 79%) as a foam, the <sup>1</sup>H NMR spectral data of which were identical with those reported previously.<sup>7a</sup>

### 2-Formamido-*N*-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)malonamide 15

A mixture of DCC (413 mg, 2.0 mmol) and formic acid (151 μL, 4.0 mmol) in CHCl<sub>3</sub> (5 mL) was stirred at 0 °C for 10 min. A solution of **12** (357 mg, 1.0 mmol) in pyridine (3 mL) was added, and the resulting mixture was stirred at 0 °C for 15 min and then at room temperature for 1 h. The resulting white precipitate was filtered off, and the filtrate was evaporated. The residue was partitioned between CHCl<sub>3</sub> and brine, and the aqueous layer was extracted with CHCl<sub>3</sub> (5 times). The combined organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated, and purified by flash column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>–MeOH, 30:1, then 25:1) to give **15** (187 mg, 46%) as a foam: <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 8.32 and 8.30 (each s, each 0.5 H, CHO), 8.18 and 8.10 (each d, each 0.5 H, 1-NH, *J* = 8.5 and 8.8, respectively), 7.65 and 7.61 (each d, each 0.5 H, NHCHO, *J* = 6.4 and 6.4, respectively), 7.00 (br s, 1H, CONH<sub>2</sub>), 6.40 and 6.31 (each br s, each 0.5 H, CONH<sub>2</sub>), 5.68–5.61 (m, 1 H, H-1'), 5.32–5.26 (m, 2 H, H-2' and -3'), 5.10 and 5.07 (each d, each 0.5 H, H-3, *J* = 6.4 and 6.4, respectively), 4.30–4.15 (m, 3 H, H-4' and H<sub>2</sub>-5'), 2.11, 2.10, 2.09 and 2.08 (each s, total 9 H, Ac); <sup>13</sup>C NMR (100 MHz; CDCl<sub>3</sub>) δ 171.01, 170.92, 170.17, 170.01, 169.97, 169.93, 168.40, 167.72, 166.93 and 166.51 (COCH<sub>3</sub> and CONH<sub>2</sub>), 166.93 and 166.51 (CHO), 82.99 and 82.66 (C-1'), 78.80 and 78.75 (C-4'), 73.59, 73.23, 70.81 and 70.70 (C-2' and -3'), 63.46 (C-5'), 56.16 and 55.93 (C-3), 20.76 and 20.52 (acetyl Me); HRMS (FAB, positive) 404.1292 (MH<sup>+</sup>). C<sub>15</sub>H<sub>22</sub>N<sub>3</sub>O<sub>10</sub> requires *m/z*, 404.1305.

### 2-Amino-*N*-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)malonamide 16

**Reaction using a low-pressure mercury lamp.** A solution of **5** (5.96 g, 20.0 mmol) in aq. AcOH (0.1 M; 600 mL) was irradiated with a low-pressure mercury lamp (60 W, quartz filter) at room temperature under bubbling of argon for 12 h. After being neutralized with aq. NaHCO<sub>3</sub> (0.8 M), the resulting mixture was evaporated. The residue was purified by column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>–MeOH, 10:1, then 5:1) to give **16** (3.98 g, 68%) as a foam: <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub> + D<sub>2</sub>O) δ 5.44 (d, 1 H, *J* = 2.0), 4.71 (d, 1 H, *J* = 6.4), 4.51 (dd, 1 H, *J* = 6.4, 2.0), 4.04 (m, 1 H), 3.81 and 3.79 (each s, each 0.5 H, disappeared after 1 h from D<sub>2</sub>O-addition), 3.53–3.43 (m, 2 H), 1.42 and 1.26 (each s, each 3 H); MS (FAB, positive) *m/z* 290 (MH<sup>+</sup>). This compound was rather unstable and therefore was immediately used for the next reaction.

**Reaction with high-pressure mercury lamp.** A solution of **5** (1.49 g, 5.0 mmol) in aq. AcOH (0.1 M; 500 mL) was irradiated

with a high-pressure mercury lamp (100 W, Pyrex filter) at room temperature under bubbling of argon for 6 h. The resulting white precipitate was filtered off and washed with water to give 2,5'-cyclized product **18** (145 mg, 10%) as crystals, mp 241–242 °C (Found: C, 46.81; H, 5.70; N, 13.80. C<sub>12</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>·1/2H<sub>2</sub>O requires C, 46.75; H, 5.89; N, 13.63%); <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>/D<sub>2</sub>O) δ 5.37 (s, 1 H, H-1'), 5.04 (s, 1 H, H-2), 4.83 (d, 1 H, H-3', *J* = 5.9), 4.75 (d, 1 H, H-2', *J* = 5.9), 4.48 (m, 1 H, H-4'), 4.11 (dd, 1 H, H-5'a, *J* = 13.2, 1.0), 3.74 (dd, 1 H, H-5'b, *J* = 13.2, 2.0), 1.40 and 1.28 (each s, each 3 H, CHMe<sub>2</sub>); NOE irradiated H-1', observed H-2 (1.1%), H-2' (5.8%); irradiated H-2, observed H-1' (3.0%), H-5'b (6.2%); <sup>13</sup>C NMR (100 MHz; DMSO-*d*<sub>6</sub>) δ 169.68 (C), 165.46 (C), 111.89 (C), 88.63 (CH), 87.76 (CH), 85.92 (CH), 84.55 (CH), 80.96 (CH), 72.33 (C), 71.94 (CH<sub>2</sub>), 25.95 (CH<sub>3</sub>), 24.43 (CH<sub>3</sub>); MS (CI) *m/z* 300 (MH<sup>+</sup>).

The filtrate was neutralized with aq. NaHCO<sub>3</sub> (0.8 M) and evaporated. The residue was purified by column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>–MeOH, 10:1 then 5:1) to give **16** (1.02 g, 71%) as a foam.

### 2-(*tert*-Butoxycarbonylamino)-*N*-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)malonamide 17

A mixture of **16** (867 mg, 3.0 mmol), Boc<sub>2</sub>O (981 mg, 4.5 mmol), and Et<sub>3</sub>N (0.63 mL, 4.5 mmol) in 1,4-dioxane (15 mL) was stirred at room temperature for 2.5 h. The resulting mixture was evaporated, and the residue was partitioned between EtOAc and brine. The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated, and purified by column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>–MeOH, 20:1) to give **17** (866 mg, 74%) as a foam (Found: C, 48.68; H, 6.85; N, 10.27. C<sub>16</sub>H<sub>27</sub>N<sub>3</sub>O<sub>8</sub>·1/3H<sub>2</sub>O requires C, 48.60; H, 7.05; N, 10.63%); <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>) δ 8.61 (br d, 1 H, 1-NH, *J* = 8.3), 7.47, 7.45 and 7.40 (each br s, total 2 H, CONH<sub>2</sub>), 6.63 (br d, 0.5 H, 2-NH, *J* = 8.3), 6.61 (br d, 0.5 H, 2-NH, *J* = 8.8), 5.41 (dd, 0.5 H, H-1', *J* = 1.5, 8.3), 5.38 (dd, 0.5 H, H-1', *J* = 1.9, 8.3), 5.19 (t, 0.5 H, 5'-OH, *J* = 4.9), 5.12 (t, 0.5 H, 5'-OH, *J* = 5.4), 4.70 (m, 1 H, H-3'), 4.58–4.48 (m, 2 H, H-2 and -2'), 4.01 (m, 1 H, H-4'), 3.52–3.37 (m, 2 H, H-5'), 1.42–1.26 (m, 15 H, *t*-Bu and Pr<sup>t</sup>); MS (FAB, positive) *m/z* 390 (MH<sup>+</sup>).

### 2-(*tert*-Butoxycarbonylamino)-*N*-[3-oxo-5-*O*-(2,4,3-benzodioxaphosphhepan-3-yl)-2,3-*O*-isopropylidene-β-D-ribofuranosyl]malonamide 19

A mixture of **17** (78 mg, 0.20 mmol), tetrazole (32 mg, 0.46 mmol), and 3-diethylamino-2,4,3-benzodioxaphosphhepane XEPA (72 mg, 0.30 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was stirred at room temperature for 1 h. To the mixture was added a solution of I<sub>2</sub> (120 mg, 0.47 mmol) in aq. THF (95%; 4 mL), and the whole was stirred at room temperature for 10 min and then quenched with saturated aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The resulting mixture was partitioned between CHCl<sub>3</sub> and brine. The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated, and purified by column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>–EtOAc, 1:1, then CHCl<sub>3</sub>–MeOH, 50:1) to give **19** (81 mg, 70%) as a foam; <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 8.15–8.04 (m, 1 H), 7.40–7.28 (m, 4 H), 7.03 (br s, 0.5 H), 6.95 (br s, 0.5 H), 6.03–5.90 (m, 2 H), 5.64 (m, 1 H), 5.31–5.16 (m, 4 H), 4.80–4.70 (m, 3 H), 4.36 (m, 1 H), 4.31–4.21 (m, 2 H), 1.52–1.32 (m, 15 H); MS (FAB, positive) *m/z* 572 (MH<sup>+</sup>).

### 5'-*O*-(3-Oxo-2,4,3-benzodioxaphosphhepan-3-yl)breedinin 20

A solution of **19** (1.27 g, 3.4 mmol) in 90% aq. TFA was stirred at room temperature for 15 min. After water was added, the solvent was evaporated, and the residue was coevaporated with water. The residue and Et<sub>3</sub>N (1.0 mL) were dissolved in MeOH (15 mL), and then the solvent was evaporated. The residue was purified by column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>–MeOH, 10:1, then 5:1) to give a foam (1.01 g). A mixture of the foam

and (EtO)<sub>3</sub>CH (734  $\mu$ L, 4.4 mmol) in DMF (25 mL) was heated at 90 °C for 15 min. The resulting mixture was evaporated, and the residue was purified by column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>-MeOH, 10:1, then 5:1) to give **20** (487 mg, 47%) as a solid; <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub> + D<sub>2</sub>O)  $\delta$  8.28 (s, 1 H), 7.46–7.41 (m, 4 H), 5.57 (d, 1 H, *J* = 4.9), 5.37–5.29 (m, 2 H), 5.11–5.01 (m, 2 H), 4.39 (dd, 1 H, *J* = 4.9, 4.9), 4.29 (m, 1 H), 4.24–4.19 (m, 2 H), 4.09 (m, 1 H); HRMS (FAB, positive) 442.1036 (MH<sup>+</sup>. C<sub>17</sub>H<sub>21</sub>N<sub>3</sub>O<sub>9</sub>P requires *m/z*, 442.1015); UV (H<sub>2</sub>O)  $\lambda_{\text{max}}$  280 nm.

#### Bredinin 5'-phosphate sodium salt 2

A mixture of **20** (297 mg, 0.67 mmol) and Pd-C (10%, 100 mg) in MeOH (10 mL) was stirred at room temperature under atmospheric pressure of H<sub>2</sub> for 30 min. The catalyst was filtered off, and the filtrate was evaporated. The residue was partitioned between CHCl<sub>3</sub> and water, and the aqueous layer was concentrated *in vacuo*, and then applied to a column of Dowex 50 resin (H<sup>+</sup>-form, 1.8  $\times$  8 cm, packed with water). The column was eluted with water (300 mL), and the appropriate fractions containing **2** were concentrated to about 3 mL *in vacuo*. The resulting solution was applied to a column of Diaion WK-20 resin (Na<sup>+</sup>-form, 1.8  $\times$  10 cm, packed with water). The column was eluted with water. The appropriate fractions containing **2** were evaporated, and the residue was freeze-dried to give pure **2** (230 mg, sodium salt, 89%) as a solid (Found: C, 27.15; H, 4.17; N, 10.04. C<sub>9</sub>H<sub>13</sub>N<sub>3</sub>NaO<sub>9</sub>P·7/3H<sub>2</sub>O requires C, 26.81; H, 4.42; N, 10.42%); <sup>1</sup>H NMR (400 MHz; D<sub>2</sub>O)  $\delta$  8.38 (s, 1 H), 5.81 (d, 1 H, *J* = 4.4), 4.49 (dd, 1 H, *J* = 4.4, 4.9), 4.41 (dd, 1 H, *J* = 2.4, 4.9), 4.28 (m, 1 H), 4.14–4.04 (m, 2 H); <sup>31</sup>P NMR (D<sub>2</sub>O; 125 MHz, decoupled with <sup>1</sup>H)  $\delta$  1.85 (s); <sup>13</sup>C NMR (125 MHz; D<sub>2</sub>O)  $\delta$  165.03, 156.36, 127.03, 101.32, 87.43 and 84.41 (d, *J* = 7.5), 75.61, 70.58 and 64.45; MS (FAB, positive) *m/z* 362 (MNa<sup>+</sup>); MS (FAB, negative) *m/z* 338 [(M - H)<sup>-</sup>]; UV (H<sub>2</sub>O)  $\lambda_{\text{max}}$  279 nm.

#### 2-(*tert*-Butoxycarbonylamino)-*N*-(5-deoxy-5-iodo-2,3-*O*-isopropylidene- $\beta$ -D-ribofuranosyl)malonamide 21

A mixture of **17** (1.17 g, 3.0 mmol) and MsCl (302  $\mu$ L, 3.9 mmol) in pyridine (30 mL) was stirred at 0 °C for 1 h. After addition of water, the solvent was evaporated, and the residue was partitioned between CHCl<sub>3</sub> and brine. The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated. A mixture of the residue and NaI (1.12 g, 7.5 mmol) in MEK (30 mL) was heated under reflux for 1 h. The resulting precipitate was filtered off, and the filtrate was evaporated. The residue was partitioned between CHCl<sub>3</sub> and brine, and the organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated. The residue was purified by column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>-EtOAc, 4:1) to give **21** (1.35 g, 90%) as a solid (Found: C, 38.55; H, 5.28; N, 8.22. C<sub>16</sub>H<sub>26</sub>IN<sub>3</sub>O<sub>7</sub> requires C, 38.49; H, 5.25; N, 8.42%); <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>)  $\delta$  7.81 (d, 0.5 H, *J* = 8.3), 7.76 (d, 0.5 H, *J* = 6.8), 6.95 and 6.91 (each br s, each 0.5 H), 6.01 (dd, 1 H, *J* = 4.9, 4.9), 5.95 (br s, 1 H), 5.59 (m, 1 H), 4.76–4.67 (m, 3 H), 4.23 (m, 1 H), 3.33–3.22 (m, 2 H), 1.53–1.34 (m, 15 H); MS (FAB, positive) *m/z* 500 (MH<sup>+</sup>).

#### 2-(*tert*-Butoxycarbonylamino)-*N*-(5-deoxy-2,3-*O*-isopropylidene- $\beta$ -D-ribofuranosyl)malonamide 22

A mixture of **21** (998 mg, 2.0 mmol), Bu<sub>3</sub>SnH (810  $\mu$ L, 3.0 mmol), and AIBN (100 mg, 0.61 mmol) in benzene (30 mL) was heated under reflux for 2 h. The solvent was evaporated, and the residue was purified by column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>-MeOH, 30:1) to give **22** (736 mg, 98%) as a foam; <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  8.77–8.68 (m, 1 H), 7.41 (br s, 2 H), 6.63–6.58 (m, 1 H), 5.30–5.21 (m, 1 H), 4.70–4.42 (m, 4 H), 4.02–3.98 (m, 1 H), 1.46–1.16 (m, 15 H), 0.88 (m, 3 H); MS (FAB, positive) *m/z* 374 (MH<sup>+</sup>).

#### 5'-Deoxybredinin 3

Compound **3** (385 mg, 77%) as a solid was obtained from **22** (495 mg, 2.0 mmol) as described above for the synthesis of **20**, after purification by column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>-MeOH, 10:1 then 5:1); mp 217–219 °C (from MeOH, decomp.) (Found: C, 44.32; H, 5.52; N, 16.91. C<sub>9</sub>H<sub>13</sub>N<sub>3</sub>O<sub>5</sub>·1/7MeOH requires C, 44.62; H, 5.51; N, 16.71%); <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub> + D<sub>2</sub>O)  $\delta$  8.29 (s, 1 H), 6.87 (br s, 2 H), 5.48 (d, 1 H, *J* = 4.8), 4.34 (dd, 1 H, *J* = 4.8, 5.1), 3.91–3.79 (m, 2 H), 1.24 (d, 3 H, *J* = 6.2); <sup>13</sup>C NMR (100 MHz; D<sub>2</sub>O)  $\delta$  161.71, 155.21, 124.43, 98.52, 86.05, 79.34, 74.43, 72.98, 18.74; MS (FAB, positive) *m/z* 244 (MH<sup>+</sup>); UV  $\lambda_{\text{max}}$  278 nm (H<sub>2</sub>O).

#### *N*-(5-*O*-[3-(Benzyloxycarbonylamino)propylcarbamoyl]-2,3-*O*-isopropylidene- $\beta$ -D-ribofuranosyl]-3-(*tert*-butoxycarbonylamino)malonamide 23

A mixture of **17** (97 mg, 0.25 mmol), carbonyldiimidazole (61 mg, 0.50 mmol) and DMAP (67 mg, 0.55 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was stirred at room temperature for 6 h. To the mixture was added a mixture of 3-(benzyloxycarbonylamino)propylamine hydrochloride<sup>16</sup> (257 mg, 1.0 mmol) and Et<sub>3</sub>N (139  $\mu$ L, 1.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL), and the whole was stirred at room temperature for 16 h. After water was added, the resulting mixture was partitioned, and the organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. The residue was purified by column chromatography (SiO<sub>2</sub>; CHCl<sub>3</sub>-MeOH, 80:1) to give **23** (138 mg, 91%) as a foam (Found: C, 53.79; H, 6.51; N, 11.00. C<sub>28</sub>H<sub>41</sub>N<sub>5</sub>O<sub>11</sub> requires C, 53.92; H, 6.63; N, 11.23%); <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub> + D<sub>2</sub>O)  $\delta$  7.37–7.32 (m, 5 H), 5.87 (s, 0.7 H), 5.81 (s, 0.3 H), 5.08 (s, 2 H), 4.72–4.41 (m, 5 H), 3.91 (d, 0.7 H, *J* = 12.4), 3.83 (d, 0.3 H, *J* = 11.9), 3.39–3.18 (m, 4 H), 1.73–1.31 (m, 17 H); HRMS (FAB, positive) 624.2888 (MH<sup>+</sup>. C<sub>28</sub>H<sub>42</sub>N<sub>5</sub>O<sub>11</sub> requires *m/z* 624.2881).

#### 5'-*O*-[3-(Benzyloxycarbonylamino)propylcarbamoyl]bredinin 24

A solution of **23** (249 mg, 0.40 mmol) in 90% TFA (3 mL) was stirred at room temperature for 10 min, and then the solution was evaporated. After the residue was coevaporated with water and then with EtOH (twice), the residue was dissolved in EtOH (2 mL) and Et<sub>3</sub>N (1 mL), and the solution was evaporated. The residue was coevaporated with EtOH (3 times), and then heated at 40 °C *in vacuo* for 3 h. The resulting syrup was heated at 90 °C with (EtO)<sub>3</sub>CH (67  $\mu$ L, 0.52 mmol) in DMF (5 mL) for 20 min, and then the solvent was evaporated. The residue was dissolved in MeOH (1 mL) and applied to a column of Dowex 50 resin (H<sup>+</sup>-form, 2  $\times$  15 cm, packed with MeOH). The column was washed with MeOH (200 mL), and then developed with 50% aq. MeOH. Appropriate fractions were evaporated to give **24** (132 mg, 67%) as a foam (Found: C, 50.26; H, 5.61; N, 13.84. C<sub>21</sub>H<sub>27</sub>N<sub>5</sub>O<sub>9</sub>·0.5H<sub>2</sub>O requires C, 50.1; H, 5.81; N, 13.91%); <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub> + D<sub>2</sub>O)  $\delta$  8.24 (s, 1 H, H-2), 5.55 (d, 1 H, *J* = 5.9), 5.00 (s, 2 H, benzyl CH<sub>2</sub>), 4.34 (dd, 1 H, H-2', *J* = 5.9, 4.9), 4.16 (dd, 1 H, H-5'a, *J* = 11.7, 2.4), 4.05–3.99 (m, 3 H, H-3', -4', -5'b), 3.00–2.95 (m, 4 H, NCH<sub>2</sub>  $\times$  2), 2.98 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); HRMS (FAB, positive) 494.1884 (MH<sup>+</sup>. C<sub>21</sub>H<sub>28</sub>N<sub>5</sub>O<sub>9</sub> requires *m/z* 494.1887); UV (MeOH)  $\lambda_{\text{max}}$  283 nm.

#### 5'-*O*-(3-Aminopropylcarbamoyl)bredinin hydrochloride 4

A mixture of **24** (49 mg, 0.10 mmol), Pd-C (10%; 50 mg), and 12 M HCl (8.8  $\mu$ L, 0.105 mmol) in MeOH (5 mL) was stirred at room temperature under atmospheric pressure of H<sub>2</sub> for 30 min. The catalyst was filtered off, and the filtrate was evaporated. The residue was coevaporated with EtOH (3 times), and then the residue was treated with EtOH to give **4**·HCl (34 mg, 86%) as a solid; <sup>1</sup>H NMR (400 MHz; D<sub>2</sub>O)  $\delta$  8.08 (s, 1 H, H-2), 5.78 (d, 1 H, H-1', *J* = 3.4), 4.62 (dd, 1 H, H-2', *J* = 5.4, 3.4),

4.50 (dd, 1 H, H-5'a,  $J = 12.2, 1.8$ ), 4.41 (dd, 1 H, H-3',  $J = 5.4, 5.4$ ), 4.31–4.25 (m, 2 H, H-4', -5'b), 3.23 (t, 1 H, NCH<sub>2</sub>,  $J = 6.8$ ), 3.02 (t, 2 H, NCH<sub>2</sub>,  $J = 7.8$ ), 1.86 (m, 2 H, NCH<sub>2</sub>-CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR (125 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  164.62, 158.30, 155.97, 126.24, 101.46, 87.41, 82.00, 73.64, 70.00, 63.80, 37.56, 37.23, 27.32; HRMS (FAB, positive) 360.1521 (NH<sup>+</sup>. C<sub>13</sub>H<sub>22</sub>N<sub>5</sub>O<sub>7</sub> requires *m/z* 360.1519); UV  $\lambda_{\text{max}}$  279 nm (H<sub>2</sub>O).

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## Notes and references

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