

# Synthesis and Biological Evaluation of Quinaldopeptin

Katsushi Katayama,<sup>†</sup> Takuya Okamura,<sup>†</sup> Takuya Sunadome,<sup>†</sup> Koji Nakagawa,<sup>†</sup> Hiroshi Takeda,<sup>†</sup> Motoo Shiro,<sup>§</sup> Akira Matsuda,<sup>†</sup> and Satoshi Ichikawa<sup>†,‡,\*</sup>

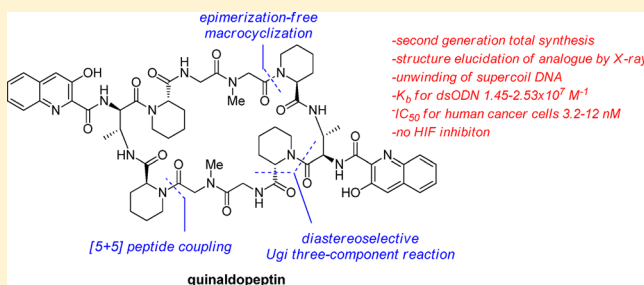
<sup>†</sup>Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

<sup>‡</sup>Center for Research and Education on Drug Discovery, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

<sup>§</sup>Rigaku Corporation, 3-9-12 Matsubara, Akishima, Tokyo 196-0003, Japan

## S Supporting Information

**ABSTRACT:** The second-generation total synthesis of quinaldopeptin (**1**) was established via a Staudinger/aza-Wittig/diastereoselective Ugi three-component reaction sequence and a racemization-free [5 + 5] coupling and macrolactamization. A single-crystal X-ray structure of the chromophore analogue **26** confirmed the structural and stereochemical assignments of the macrocycle. Synthetic **1** successfully unwound supercoiled DNA to form a relaxed DNA in a dose-dependent manner, the binding affinity of **1** to four dsODNs was within a similar range ( $K_b = 1.45\text{--}2.53 \times 10^7 \text{ M}^{-1}$ ), and the sequence selectivity was subtle. It was suggested that **1** possesses biological behaviors similar to those of sandramycin (**2**) in terms of cytotoxic activity against human cancer cell lines ( $\text{IC}_{50} = 3.2\text{--}12 \text{ nM}$ ) and HIF-1 inhibitory activity.



## INTRODUCTION

Quinaldopeptin (**1**) was first isolated from the culture broth of *Streptovorticillium album* (actinomycetes strain Q132-6) in 1990<sup>1</sup> and constitutes one of the members in a C<sub>2</sub>-symmetric cyclic decapeptide class that includes sandramycin (**2**),<sup>2</sup> luzopeptins,<sup>3</sup> and quinoxapeptins (Figure 1).<sup>4</sup> This class of natural products possesses high-affinity double-strand DNA binding through a phenomenon called bisintercalation.<sup>5</sup> Quinaldopeptin (**1**) has a strong activity against B16 (murine melanoma) and Moser (human colorectal carcinoma) cells in vitro with  $\text{IC}_{50}$  values of 0.6 and 32 nM, respectively. In vivo antitumor activity was also tested against lymphocytic leukemia P388 in mice, and **1** exhibits greater potency than mitomycin C, one of the classical cancer chemotherapeutic agents. Accordingly, **1** or **2** could be a potential candidate for therapeutic use in cancer chemotherapy. Although extensive efforts have been devoted to synthesize and elucidate the DNA binding properties of **2**,<sup>6,7</sup> luzopeptins,<sup>8,9</sup> and quinoxapeptins,<sup>10</sup> there has been no similar effort for **1**. Recently, we have accomplished the first total synthesis of **1** by solid-phase peptide synthesis.<sup>11</sup> Our first-generation synthesis featuring a macrocyclization site was conducted at the less sterically hindered glycine (Gly) residue as the N-terminus and L-pipecolic acid (L-Pip) residue as the C-terminus in the linear decapeptide **3** (Scheme 1). Unfortunately, the cyclization did not proceed smoothly and severe racemization at the L-Pip residue occurred. As a result, the undesired epimer was the major product (desired/epimer = 1/1.5). In this study, we describe a substantially improved second-generation total synthesis of **1**. DNA binding properties and cytotoxic activity against a range of human cancer cell lines are also reported. Echinomycin is a C<sub>2</sub>-

symmetric cyclic octadecadepsipeptide, which similarly acts as a double-strand DNA bisintercalator. It inhibits the binding of hypoxia-inducible factor (HIF) proteins to a hypoxia-responsive element (HRE) sequence. Considering the similarities of the structures and the modes of binding to double-stranded DNA, the ability of **1** and **2** to inhibit the HIF pathway was also evaluated.

## RESULTS AND DISCUSSION

We have recently established the total synthesis of **2** as well as analogues of the macrocyclic moiety.<sup>12</sup> Our approach to the synthesis includes a preparation of a pentadepsipeptide by a Staudinger/aza-Wittig/diastereoselective Ugi three-component reaction (U3CR) sequence and a racemization-free [5 + 5] coupling and macrolactamization, which can be applicable to the total synthesis of **1**. Our revised retrosynthetic analysis of **1** is illustrated in Scheme 2. The quinaldine chromophores **5** were installed on macrocycle **4** in the late stages of the synthesis. The macrocycle **4** was disconnected at the amide moiety linking sarcosine (Sar) and L-pipecolic acid (L-Pip) residues. Since the Sar residue has no substituent at the  $\alpha$  position, the peptide coupling is free from racemization during both [5 + 5] coupling and macrolactamization. The sequential Staudinger/aza-Wittig/diastereoselective U3CR<sup>13,14</sup> of the azidoaldehyde **10** with the isonitrile **7** and the carboxylic acid **8** to provide the pentapeptide **6** forms a nonproteinogenic amino acid, L-Pip residue, with simultaneous linking to the two dipeptides **7** and **8** at the C- and

Received: January 8, 2014

Published: February 20, 2014

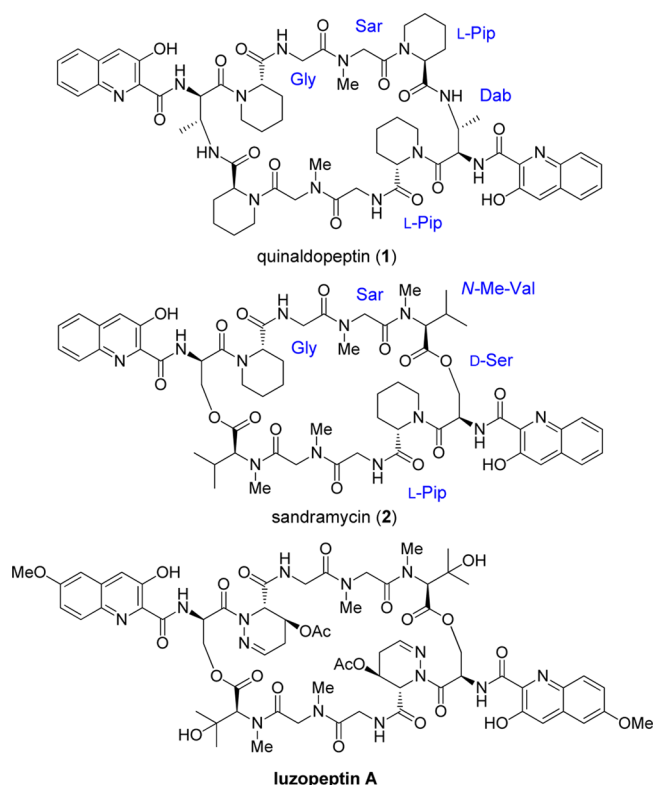
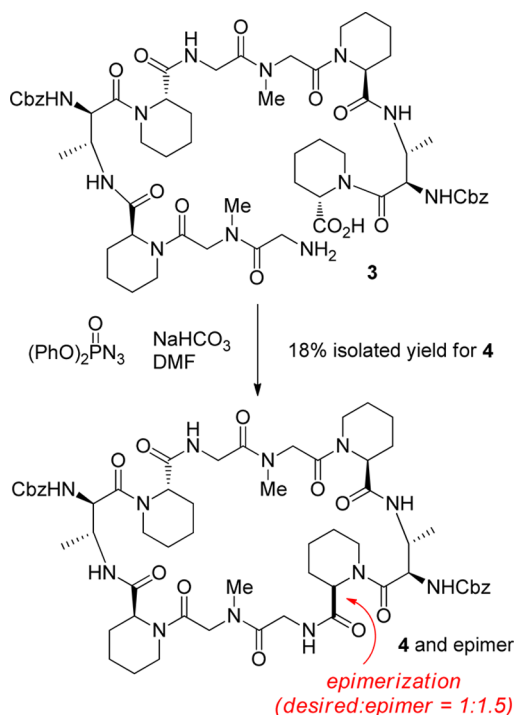


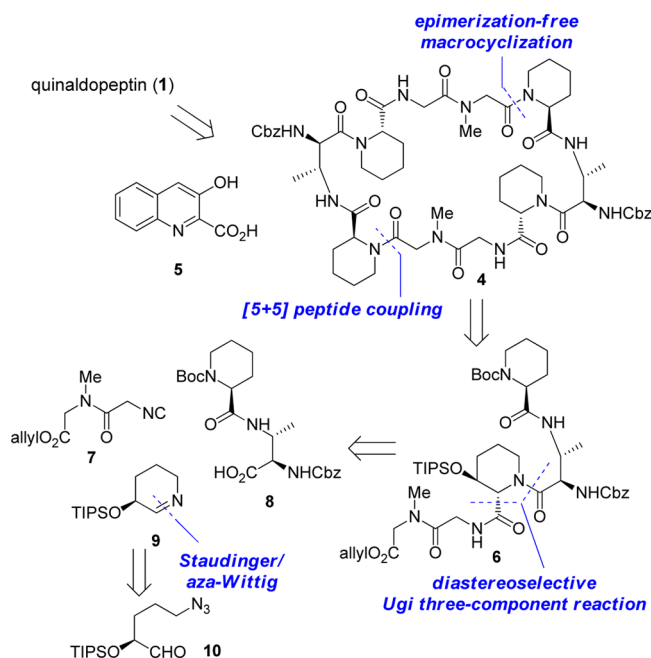
Figure 1. Structures of quinaldopeptin, sandramycin, and luzopeptin A.

### Scheme 1. Previous Total Synthesis of Quinaldopeptin



N-termini. It is a key issue to control the stereoselectivity of the newly formed stereogenic center in the U3CR using a cyclic imine, and several approaches were investigated by introducing a substituent to the cyclic imine.<sup>15–26</sup> Most of the successful examples of achieving good diastereoselectivity involve the introduction of two substituents in a *cis* relationship at the 2,3-positions of five-membered cyclic imines and at the 3,4- or 2,5-

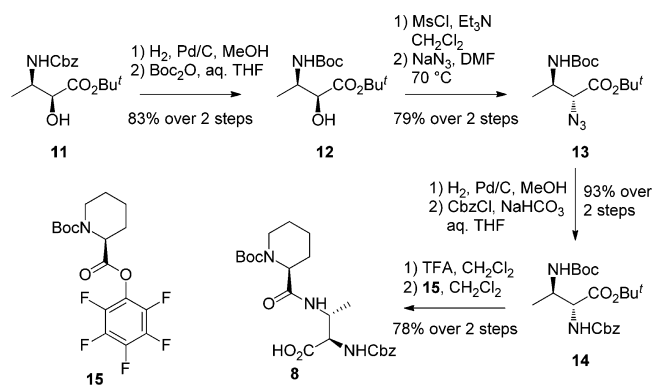
### Scheme 2. Retrosynthetic Analysis of Quinaldopeptin



positions of six-membered cyclic imines.<sup>15,16</sup> On the other hand, the introduction of one substituent gave unsatisfactory diastereoselectivity.<sup>14</sup> In our previous study of the total synthesis of 2,<sup>12</sup> a bulky silyloxy substituent introduced at the 3-position of 9 was effective in controlling the diastereoselectivity up to *S/R* = 85/15; therefore, this strategy was used in the present study.

The dipeptide 8, the carboxylic component of the U3CR, was prepared as shown in Scheme 3. The Cbz group of the known

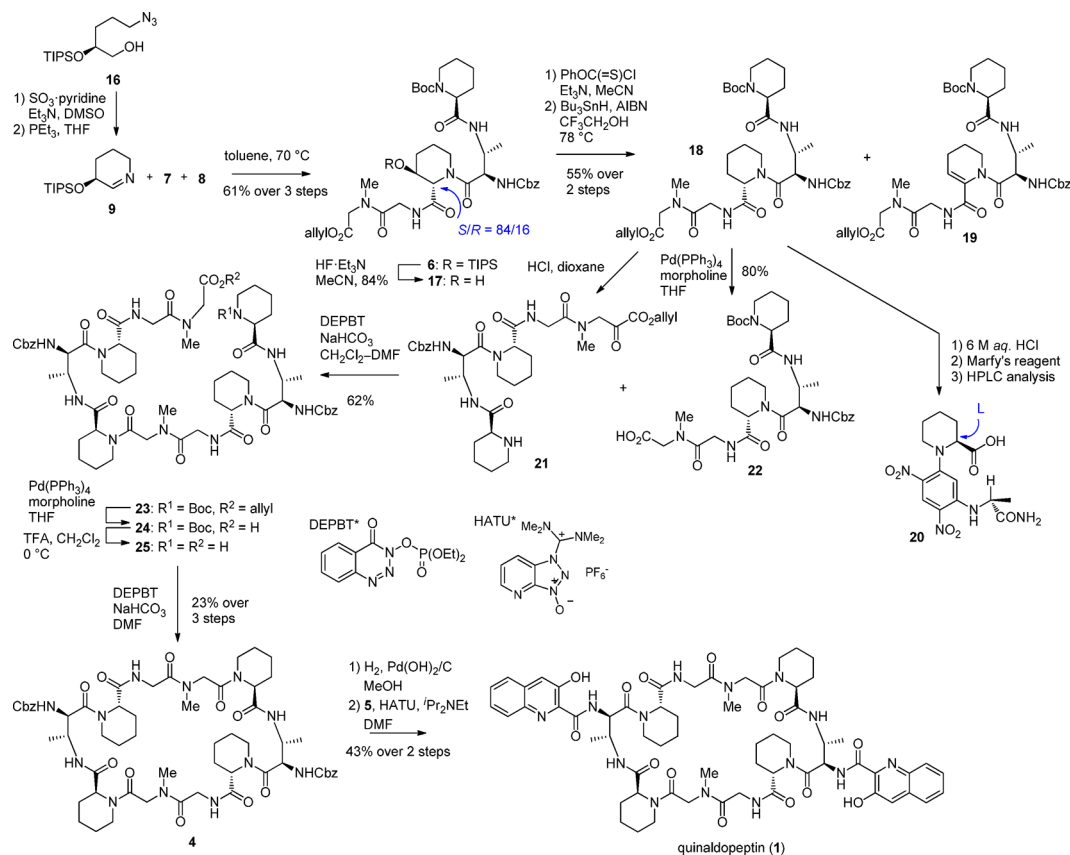
### Scheme 3. Preparation of Dipeptide 8



alcohol 11<sup>27</sup> was removed, and the liberated amine was reprotected with a Boc group to give 12. The hydroxyl group of 12 was mesylated, and *S<sub>N</sub>2* displacement with azide ion provided the azide 13 in 79% over two steps. Reduction of the azide group of 13 by catalytic hydrogenation was followed by protection of the resulting amine with a Cbz group to provide the fully protected (2*R*,3*R*)-2,3-diaminobutanoic acid (Dab) derivative 14. The Boc and the *tert*-butyl groups were both removed with TFA in  $\text{CH}_2\text{Cl}_2$ , and the resulting amino acid was coupled with Boc-L-Pip pentafluorophenyl ester (15) to afford the dipeptide 8 in 78% yield over two steps.

With the dipeptide carboxylic acid component 8 in hand, the total synthesis of 1 was investigated as shown in Scheme 4. The azido alcohol 16<sup>12</sup> was oxidized by  $\text{SO}_3$ -pyridine and  $\text{Et}_3\text{N}$  in

Scheme 4. Second-Generation Total Synthesis of Quinaldopeptin



DMSO to afford the aldehyde **10** (Scheme 2), which was directly used for the following Staudinger/aza-Wittig/U3CR sequence. The azido aldehyde **10** was treated with  $\text{PEt}_3$  in THF, which resulted in clean conversion to the corresponding cyclic imine **9**.<sup>12</sup> The imine **9** was subsequently reacted with the isonitrile **7**<sup>12</sup> and the carboxylic acid **8** in toluene at 70 °C to afford the desired pentapeptide **6** with 84/16 diastereoselectivity at the newly formed stereogenic center of the Pip residue. The diastereomers were easily separated by silica gel column chromatography, and the major diastereomer **6** was obtained in 61% yield over three steps from **16**. The stereochemistry of the  $\alpha$  position of the newly constructed Pip residue of **6** was determined to be *S* by conventional amino acid analysis, as described later. The impact of the bulky silyloxy substituent introduced at the 3-position of **9** on the stereoselectivity was great, providing good control of the diastereoselectivity up to 84/16, which was acceptable in pursuing the total synthesis of **1** as well as its analogues. Deprotection of the TIPS group of **6** by  $\text{HF}\cdot\text{Et}_3\text{N}$  in MeCN gave **17** in 84% yield. Then the deoxygenation of the resulting hydroxyl group of **17** was examined. The resulting secondary hydroxyl group was converted to the corresponding phenylthionocarbonate ( $\text{PhOC}(=\text{S})\text{Cl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ ). First, the phenylthionocarbonate was heated under reflux in the presence of 2,2'-azobis(isobutyronitrile) (AIBN) as a radical initiator.<sup>28</sup> However, the desired deoxygenated product **18** was not obtained at all, and  $\beta$  elimination was predominant under these conditions to give the enamide **19** as a major product. The reaction at low temperature by using  $\text{Et}_3\text{B}$  or 2,2'-azobis(4-methoxy-2,4-dimethylvaleronitrile) (V-70)<sup>29</sup> as an initiator also gave **19**, and the desired **18** was not obtained at all. Extensive efforts to circumvent the  $\beta$  elimination were conducted, and the use of

trifluoroethanol as a solvent was found to give a good result. That is, the phenylthionocarbonate and AIBN were heated in trifluoroethanol at 78 °C for 1 h to provide **18** in 55% yield over two steps from **17**. As described later, the  $\alpha$  position of the newly constructed Pip residue had the *S* configuration, so that the acidic  $\alpha$ -hydrogen and the phenoxylthiocarbonyloxy group had a *syn*-periplanar relationship. Facile *syn* elimination initiated by intramolecular deprotonation by the sulfur atom in the phenoxylthiocarbonyloxy group results. The defeat of the *syn* elimination by the choice of the solvent could be attributed to the difference in conformation of the Pip residue. Trifluoroethanol has a relatively acidic proton ( $\text{pK}_\text{a} = 12.4$ ), which can form a hydrogen bond with the carboxamide oxygens of the peptide frame.<sup>30</sup> This in turn could cause a conformational change including a distortion of the piperidine ring, which would suppress the *syn* elimination, thereby favoring the desired radical deoxygenation. In order to determine the absolute stereochemistry at the newly formed stereogenic center of the Pip residue, the deoxygenated pentapeptide **18** was heated under reflux in 6 M aqueous HCl for 24 h, and the resulting mixture was treated with Marfey's reagent.<sup>31</sup> The reaction mixture was analyzed by reverse-phase HPLC (ODS, 10–60% MeCN/ $\text{H}_2\text{O}$  linear gradient containing 0.1% TFA). The peak corresponding to the Pip derivative matched that of authentic **20** derived from L-Pip (Supporting Information).

Deprotection of either the Boc group (HCl, dioxane) or the allyl group ( $\text{Pd}(\text{PPh}_3)_4$ , morpholine, THF, 80%) of **18** gave the amine **21** or the carboxylic acid **22**, respectively. The [5 + 5] assembly of **21** and **22** was conducted by the peptide coupling under the conditions using 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT)<sup>32</sup> and  $\text{NaHCO}_3$  in  $\text{CH}_2\text{Cl}_2$ –

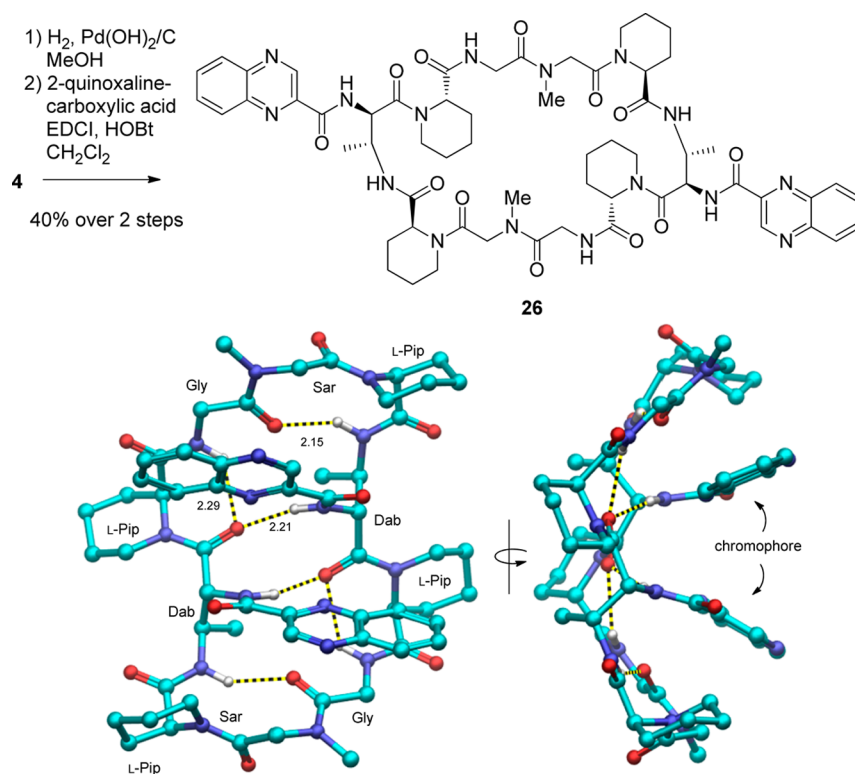


Figure 2. X-ray crystal structure of analogue 26.

DMF to afford the decapeptide **23** in 62% yield. Deprotection of the Boc and the allyl groups of **23** gave the free linear decapeptide **25**, which was then cyclized by treatment with DEPBT in DMF to afford the cyclic decapeptide **4** in 23% yield over three steps from **23**. The cyclic decapeptide **4** obtained in this study is identical with material synthesized in our previous study.<sup>11</sup> The cyclization of **25** still gave a low chemical yield of **4** and required a prolonged reaction time and higher reaction temperature in spite of the alteration of the cyclization site from our previous approach with **3** as a precursor (Scheme 1). It was observed that quinaldopeptin (**1**) and its synthetic precursor **4** adopt multiple conformers in the solvents used to acquire their  $^1\text{H}$  NMR spectra.<sup>11</sup> This observation is in contrast to that of **2** and its cyclic intermediate, which in solution adopts a single conformation.<sup>6,12</sup> The cyclization of the corresponding linear decapeptide in the synthesis of **2** proceeded smoothly. Presumably, the macrocyclic framework of **4** would be inherently strained and, thus, it would be difficult for the cyclization precursors such as **3** and **25** to adopt conformations suitable for successful cyclization. However, in this strategy, no epimers were detected in the macrocyclization of **25** or the [5 + 5] assemblage of **21** and **22**. This allowed us to subject the reaction mixture to harsher reaction conditions and isolate the desired products easily. Ciufolini et al. directly synthesized a macrocycle of luzopeptin E2 in 26% yield by a dimerization/cyclization strategy with the free pentadepsipeptide.<sup>9</sup> Accordingly, the dimerization/cyclization route was also investigated in this study with the free peptapeptide prepared by deprotection of both *N*- and *C*-terminal protecting groups of the pentapeptide **18** in order to directly obtain the macrocycle **4**. However, none of these efforts were successful. This was also true for our recent total synthesis of **2**. Finally, the Cbz groups of **4** were removed by hydrogenolysis, and the liberated amines were coupled with  $\text{S}^{33}$  using 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluro-

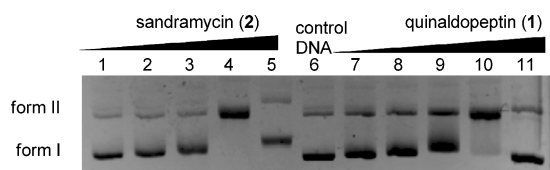
nium hexafluorophosphate (HATU) and  $\text{Pr}_2\text{NEt}$  in DMF to afford **1** in 43% yield over two steps, establishing a second-generation total synthesis of **1**.

In a manner similar to the synthesis of **1**, the chromophore analogue **26** was also prepared, as shown in Figure 2. A single-crystal X-ray structure of **26** was obtained and confirmed the structural and stereochemical assignments of the macrocycle and further revealed a rigid cyclic decapeptide conformation. The overall shape of the crystal structure of **26** is rectangular with a 2-fold axis of symmetry and is folded with six intramolecular hydrogen bonds. The Gly amide carbonyl oxygen atom is engaged in a transannular hydrogen bond (2.15 Å) to the  $\beta$ -NH of the Dab residue across the ring, and the Dab amide carbonyl oxygen atom has two hydrogen bond networks with both the adjacent secondary amide NH of Gly (2.29 Å) and the  $\alpha$ -NH of the Dab (2.21 Å) residues across the ring. These conformational features resulted in a kink at the L-Pip residue between the Gly and the Dab residues, and two chromophores embedded inside the kink, in which the interchromophore distance is 4–6.5 Å. This structure is quite different from that found in the X-ray structure of luzopeptin A (Figure 1).<sup>34</sup> The macrocyclic framework of luzopeptin A is rather flat and two chromophores are oriented toward the perimeter of the macrocycle with an interchromophore distance of 17–19.5 Å. It was reported that natural **1** did not give clear signals in  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra.<sup>1</sup> Those of **26** also exhibited broadened NMR spectra. This made it difficult to elucidate their structures. The X-ray crystal structure of **26** directly and unambiguously determined the relative and absolute stereochemistry of the cyclic peptide framework of **1** as well as **26**, as shown in Schemes 1 and 2.

The DNA binding properties of **1** were totally unknown. These were evaluated in comparison with **2**, the DNA binding properties of which were elucidated by Boger's group.<sup>6–8,10</sup> Treatment of negatively supercoiled  $\Phi\text{X174}$  DNA (form I,



Figure 3, lane 6) with synthetic **1** resulted in successful unwinding to form a relaxed DNA (form II) in a dose-dependent



**Figure 3.** DNA unwinding experiment of **1** and **2**. Lanes 1–5, sandramycin-treated  $\Phi$ X174 DNA; lane 6, untreated supercoiled  $\Phi$ X174 DNA, 95% supercoiled form and 5% relaxed DNA; lanes 7–11, quinaldopeptin-treated  $\Phi$ X174 DNA. The [agent]-to-[base pair] ratios were 0.022 (lanes 1 and 7), 0.033 (lanes 2 and 8), 0.044 (lanes 3 and 9), 0.11 (lanes 4 and 10), 0.22 (lanes 5 and 11).

manner (lanes 7–10), and further treatment resulted in rewinding (lane 11). This was in good accordance with the results of **2** (lanes 1–5) and is indicative that **1** acts as a DNA bisintercalator. The DNA binding affinity and sequence selectivity of **1** were then evaluated by a fluorescence quenching experiment, where a decrease of the fluorescence intensity of the chromophores was measured during titrations with four self-complementary hexamer double-stranded oligodeoxynucleotides (dsODNs) (Figure 4b). The binding affinities to these dsODNs were in a similar range ( $K_b = 1.45$ – $2.53 \times 10^7 \text{ M}^{-1}$ ), and **1** binds preferentially to a 5'-d(TA)-3' sequence (Table 1). The overall DNA binding ability of **1** was slightly weaker than that of **2** ( $K_b = 8.0$ – $23.0 \times 10^7 \text{ M}^{-1}$ ) but still of the same order of magnitude under our experimental conditions. In the previous

**Table 1.** DNA Binding Properties

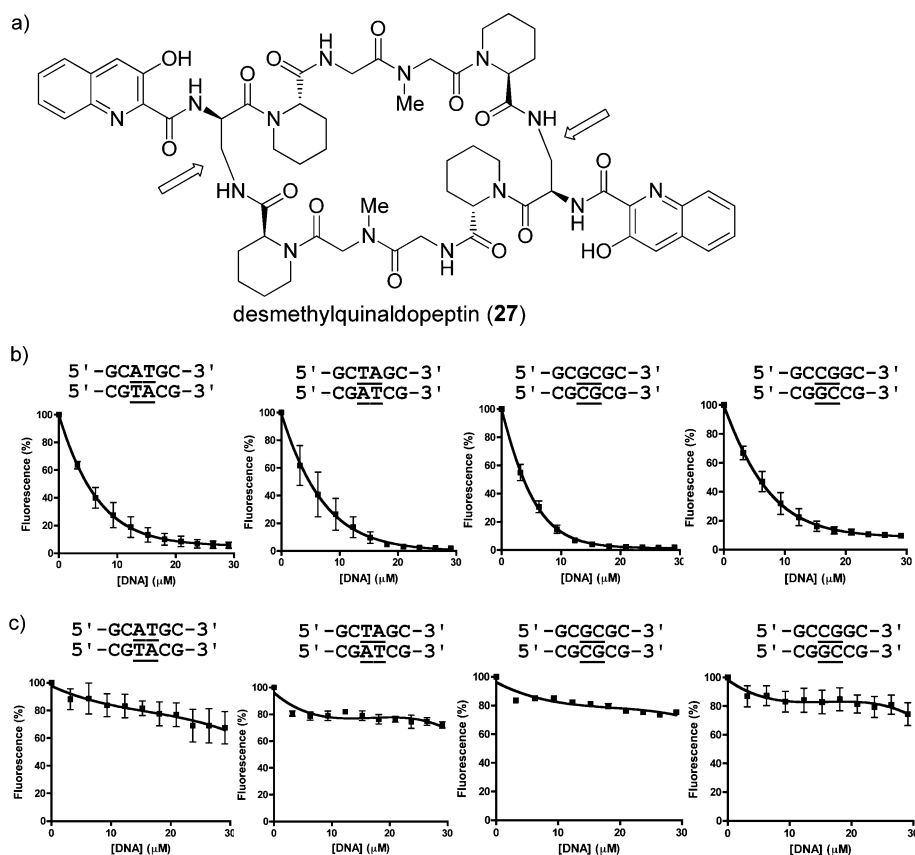
		sequence			
		5'-GCATGC-3' 3'-CGTACG-5'	5'-GCGCGC-3' 3'-CGCGCG-5'	5'-GCTAGC-3' 3'-CGATCG-5'	5'-GCCGGC-3' 3'-CGGCCG-5'
<b>1</b>	$K_b (10^7 \text{ M}^{-1})$	1.48	2.53	1.45	1.52
	$\Delta G^0 (\text{kcal/mol})$	-9.8	-10.1	-9.7	-9.8
<b>2</b>	$K_b (10^7 \text{ M}^{-1})$	23.0	14.5	8.5	8.0
	$\Delta G^0 (\text{kcal/mol})$	-11.4	-11.0	-10.8	-10.8

study, we synthesized a simple analogue of **1**, desmethylquinaldopeptin (**27**, Figure 4a), with two methyl groups at the Dab residues (indicated by the arrows) missing, by solid-phase peptide synthesis. The cytotoxic activity of **27** was reduced approximately by 2 orders of magnitude (171–457 nM; Table 2)

**Table 2.** Cytotoxic Activity against Cancer Cell Lines<sup>a</sup>

	IC <sub>50</sub> (nM)					
	HCT-118	RPMI8226	A431	RKO	SU-DHL6	SU-DHL10
<b>1</b>	3.2	11	12	5	11	12
<b>2</b>	0.8	3.8	3.1	1.3	5.9	3.3
<b>26</b>	77	26	64	86	350	510
<b>27</b>	279	171	457	264	213	307
<b>28</b>	0.7	1.0	1.4	0.8	0.8	0.8

<sup>a</sup>Definitions: HCT-118 and RKO, human colon cancer cells; A431, human epidermal cancer cells; RPMI8226, human myeloma cells; SU-DHL6 and SU-DHL10, human diffuse large B-cell lymphoma cells.



**Figure 4.** DNA binding properties of **1** and its desmethyl analogue **27**: (a) structure of desmethylquinaldopeptin (**27**); (b) fluorescence quenching experiment of **1** with four hexamer double-strand ODNs; (c) fluorescence quenching experiment of **27** with four hexamer double-strand ODNs.

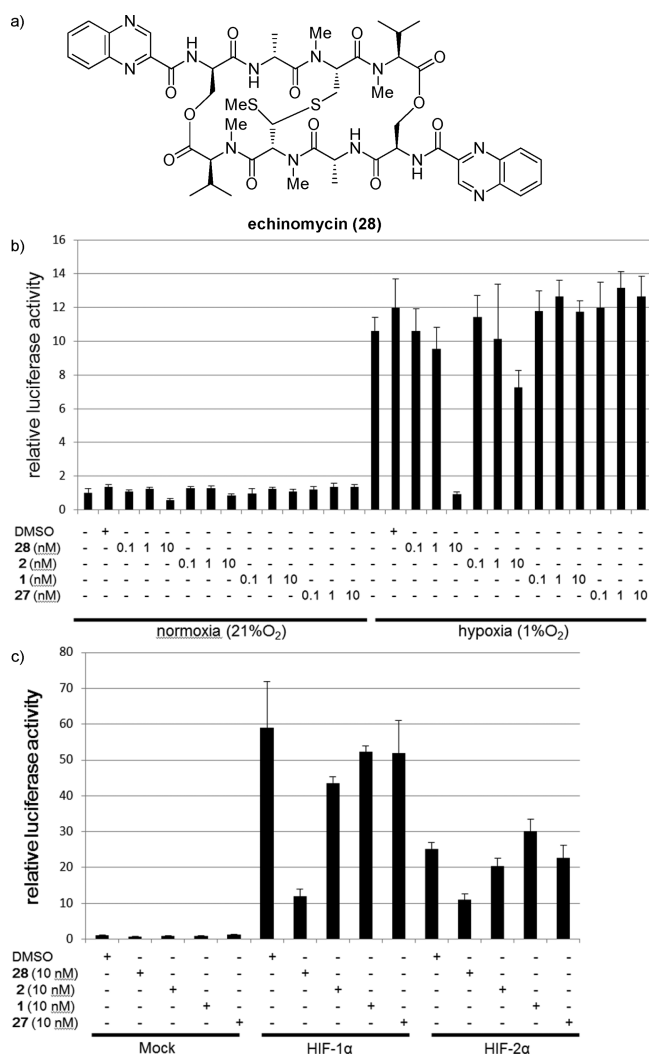
in comparison to synthetic **1** (3.2–12 nM). Presumably, truncation of the methyl groups would cause a conformational change of the macrocycle, which would not be preferred in DNA binding. In order to support this hypothesis, the DNA binding affinity of **27** was also evaluated (Figure 4c). Only ca. 30% fluorescence quenching was observed even at higher concentrations of dsODNs in every experiment with four dsODNs, and the DNA binding constants could not be measured. Thus, **27** is a weak DNA binder in comparison to **1**. The cytotoxic activities of **1**, **2**, and **26** against a range of human cancer cell lines were then compared side by side (Table 2). The activity correlated well to the DNA binding affinity, and **2** is more active than **1** against all cell lines tested. The chromophore analogue **26** was 5–40-fold less cytotoxic.

Echinomycin<sup>35</sup> (**28**, Figure 5a), which is another class of C2-symmetric cyclic octadecadepsipeptide bisintercalator,<sup>36</sup> exhibits a strong cytotoxicity against human cancer cells, and phase I and II clinical trials have been pursued.<sup>37</sup> Hypoxia-inducible factor-1 (HIF-1) is a transcription factor that controls genes involved in

angiogenesis, migration, and invasion, all of which are important for tumor progression and metastasis.<sup>38,39</sup> HIF-1 consists of the constitutively expressed subunit HIF-1 $\beta$  and the oxygen-regulated subunit HIF-1 $\alpha$ , HIF-2 $\alpha$ , or HIF-3 $\alpha$ . HIF-1 $\alpha$  is ubiquitous, and its paralog HIF-2 $\alpha$  is more cell-specific. Both 1 $\alpha$  and 2 $\alpha$  subunits are highly conservative and bind to the same hypoxia-response element (HRE), although their effect on the expression of some genes may vary. Echinomycin is known to inhibit the binding of transcription factor HIF-1 $\alpha$  to HRE, which contains 5'-ACGT-3' in its core sequence.<sup>38</sup> In this study, **28** also exhibited a strong cytotoxic activity similar to that of **1** and **2** (Table 2). Considering the similarities of its structure and the mode of action, the ability of **1** or **2** to inhibit the HIF-1 pathway was the subject of a preliminary investigation by a reporter gene assay in human embryonic kidney 293 (HEK293) cells (Figure 5b). First, the effect of **1**, **2**, and **27** or **28** as a positive control on a HRE-dependent transcriptional activity was evaluated. That is, HEK293 cells were transiently transfected with the pGL3-5xHRE-Luc reporter plasmid and the internal control pGL4.75 (hRLuc/CMV) plasmid. After 48 h, the cells were incubated under normoxia (21% O<sub>2</sub>) or hypoxia (1% O<sub>2</sub>) for 16 h in the presence or absence of the **1**, **2**, and **27** or **28**, and the luciferase activities were measured. Echinomycin (**28**) completely inhibited the HIF-dependent transcriptional activity in HEK293 cells under hypoxia at 10 nM. In contrast to the case for **28**, **2** exhibited a very weak inhibitory activity of the transcription activity at the same concentration<sup>12</sup> and no inhibitory activity was observed at all for **1** and **27**. Next, the selectivity between HIF-1 $\alpha$  and HIF-2 $\alpha$  inhibition at 10 nM of agents was also investigated by transfecting HIF-1 $\alpha$  or HIF-2 $\alpha$  expression vector together with the pGL3-5xHRE-Luc reporter plasmid and the internal control pGL4.75 (hRLuc/CMV) plasmid (Figure 5c). After transfection, the cells were exposed to 10 nM of agents for 16 h under normoxic conditions, and the luciferase activities were measured. A trend was observed similar to that in the HRE-dependent transcriptional assay. That is, **2** showed a weak inhibition on both HIF-1 $\alpha$  and HIF-2 $\alpha$  dependent transcriptional activities, which were strongly inhibited by treatment with **28**, and quinaldopeptin and its analogue **27** did not affect the activities. In conjunction with the fact that **1** and **2** exhibit cytotoxic activity with potency similar to that of **28**, these data suggest that the primary target of **1** and **2** is not the binding of transcription factor HIF-1 to the HRE, and the mode of action of **1** and **2** is different from that of **28**. Transcriptional pathways other than HIF could be a target of cyclic decapeptides **1** and **2**, and detailed studies will be necessary to elucidate the mode of action.

## CONCLUSIONS

In summary, the second-generation total synthesis of **1** was established via the diastereoselective U3CR. In conjunction with our total synthesis of **2**, this strategy is applicable to the synthesis of this class of natural products as well as their analogues. A single-crystal X-ray structure of the chromophore analogue **26** confirmed the structural and stereochemical assignments of the macrocycle of **1**. The DNA binding properties of **1** were evaluated in comparison with those of **2**. Synthetic **1** successfully unwound supercoiled DNA to form a relaxed DNA in a dose-dependent manner. The binding affinity of **1** to four dsODNs was within a similar range, and the sequence selectivity was subtle. The overall DNA binding ability of **1** was slightly weaker than that of **2** but still the same order of magnitude. In addition, it was suggested that **1** possesses biological behaviors similar to that



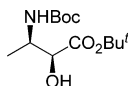
**Figure 5.** Effect of **1** and **2** on HIF-dependent transcriptional activity: (a) structure of echinomycin (**28**); (b) HEK293 cells seeded transiently transfected with the pGL3-5xHRE-Luc reporter plasmid; (c) HEK293 cells seeded in a 24-well plate transiently transfected with HIF-1 $\alpha$  or HIF-2 $\beta$  expression vector together with the pGL3-5xHRE-Luc reporter plasmid. Echinomycin (**28**) was tested as a positive control.

of **2** in terms of cytotoxic activity against human cancer cell lines and HIF-1 inhibitory activity.

## EXPERIMENTAL SECTION

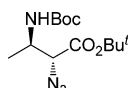
**General Experimental Methods.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts are reported in parts per million ( $\delta$ ) relative to tetramethylsilane (0.00 ppm) as internal standard unless otherwise noted. Coupling constants ( $J$ ) are reported in hertz (Hz). Multiplicities are given as follows; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Data are presented as follows: chemical shift (multiplicity, integration, coupling constant). Assignments are based on  $^1\text{H}$ – $^1\text{H}$  COSY, HMBC, and HMQC NMR spectra. The mass analyzer type used for the HRMS measurements was TOF. All reactions except those carried out under aqueous conditions were performed under an atmosphere of argon, unless otherwise noted.

**(2S,3R)-tert-Butyl 2-Hydroxy-3-tert-butoxycarbonylamino-butanoate (**12**).**



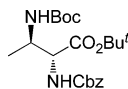
A mixture of **11** (5.0 g, 16 mmol) and Pd/C (1.0 g, 20% w/w) in MeOH (80 mL) was vigorously stirred under a  $\text{H}_2$  atmosphere (balloon pressure) at room temperature for 3 h. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated in vacuo to give the amine. A mixture of the amine and  $\text{NaHCO}_3$  (14 g, 160 mmol) in THF (60 mL) and  $\text{H}_2\text{O}$  (20 mL) was treated with  $\text{Boc}_2\text{O}$  (5.6 mL, 24 mmol) at  $0^\circ\text{C}$  and stirred at room temperature for 11 h. The whole mixture was partitioned between AcOEt (300 mL) and 1 M aqueous HCl (100 mL), and the organic phase was washed with saturated aqueous  $\text{NaHCO}_3$  (100 mL) and saturated aqueous NaCl, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ( $\phi$  3.0  $\times$  18 cm, 25% AcOEt–75% hexane) to afford **12** (3.7 g, 83%) as a colorless oil:  $[\alpha]_D^{20} = +10.6^\circ$  ( $c$  0.89,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  4.72 (d, 1H, NH,  $J = 10.3$  Hz), 4.17 (d, 1H, H- $\alpha$ ,  $J = 8.1$  Hz), 3.97 (dd, 1H, H- $\beta$ ,  $J = 2.3$ ,  $J = 5.5$  Hz), 3.10 (m, 1H, OH), 1.48 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ), 1.40 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ), 1.23 (d, 3H,  $\text{CH}_3$ ,  $J = 5.5$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  172.8, 155.0, 83.5, 79.3, 73.6, 48.5, 28.5, 28.0, 18.7; ESIMS-LR  $m/z$  298 [(M + Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{13}\text{H}_{25}\text{NNaO}_5$  298.1630, found 298.1621.

**(2R,3R)-tert-Butyl 2-Azido-3-tert-butoxycarbonylaminobutanoate (**13**).**



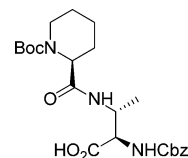
A solution of **12** (3.4 g, 13 mmol) in  $\text{CH}_2\text{Cl}_2$  (130 mL) was treated with  $\text{Et}_3\text{N}$  (2.6 mL, 19 mmol) and  $\text{MsCl}$  (1.5 mL, 19 mmol) at  $0^\circ\text{C}$ , and the mixture was stirred at room temperature for 1 h. The whole mixture was partitioned between AcOEt (500 mL) and  $\text{H}_2\text{O}$  (200 mL), and the organic phase was washed with saturated aqueous NaCl, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. A solution of the residue in DMF (130 mL) was treated with  $\text{NaN}_3$  (1.2 g, 19 mmol) at room temperature, and the mixture was stirred at  $70^\circ\text{C}$  for 16 h. The mixture was concentrated in vacuo, and the residue was partitioned between AcOEt (300 mL) and  $\text{H}_2\text{O}$  (100 mL  $\times$  3). The organic phase was washed with saturated aqueous NaCl, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ( $\phi$  3.0  $\times$  18 cm, 20% AcOEt–80% hexane) to afford **13** (3.0 g, 79%) as a colorless oil:  $[\alpha]_D^{20} = +27.5^\circ$  ( $c$  1.62,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  4.73 (d, 1H, NH,  $J = 10.3$  Hz), 4.19 (s, 2H, H- $\alpha$ ,  $\beta$ ), 1.50 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ), 1.44 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ), 1.08 (d, 3H,  $\text{CH}_3$ ,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  167.5, 155.0, 83.6, 80.0, 65.8, 47.6, 28.4, 28.1, 15.8; ESIMS-LR  $m/z$  323 [(M + Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{13}\text{H}_{24}\text{N}_4\text{NaO}_4$  323.1695, found 323.1686.

**(2R,3R)-tert-Butyl 2-Benzoyloxycarbonylamino-3-tert-butoxycarbonylaminobutanoate (**14**).**



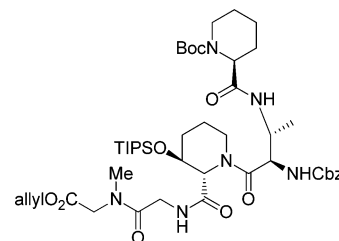
A mixture of **13** (3.0 g, 9.8 mmol) and Pd/C (590 mg, 20% w/w) in MeOH (100 mL) was vigorously stirred under a  $\text{H}_2$  atmosphere at room temperature for 2 h. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated in vacuo to give the amine. A mixture of the amine and  $\text{NaHCO}_3$  (820 mg, 98 mmol) in THF (74 mL) and  $\text{H}_2\text{O}$  (24 mL) was treated with  $\text{CbzCl}$  (2.1 mL, 15 mmol) at  $0^\circ\text{C}$  and stirred at room temperature for 11 h. The whole mixture was partitioned between AcOEt (400 mL) and 1 M aqueous HCl (150 mL), and the organic phase was washed with saturated aqueous  $\text{NaHCO}_3$  (150 mL) and saturated aqueous NaCl, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ( $\phi$  3.0  $\times$  18 cm, 14% AcOEt–86% hexane) to afford **14** (3.7 g, 93%) as a colorless oil:  $[\alpha]_D^{20} = +1.05^\circ$  ( $c$  1.37,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.37–7.27 (m, 5H, Ph), 5.64 (br s, 1H, Boc-NH), 5.12 (q, 2H,  $\text{CH}_2\text{Ph}$ ,  $J = 12.0$  Hz), 5.01 (br s, 1H, Cbz-NH), 4.44 (m, 1H, H- $\alpha$ ), 4.11 (m, 1H, H- $\beta$ ), 1.46 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ), 1.44 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ), 1.09 (d, 3H,  $\text{CH}_3$ ,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  169.6, 156.6, 155.5, 136.3, 128.7, 128.3, 127.1, 83.0, 79.8, 67.3, 65.5, 58.3, 48.8, 28.5, 28.1, 16.4; ESIMS-LR  $m/z$  431 [(M + Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{21}\text{H}_{32}\text{N}_2\text{NaO}_6$  431.2158, found 431.2148.

**(2R,3R)-3-(N-tert-Butoxycarbonyl-L-pipecolylamino)-2-benzoyloxycarbonylaminobutanoate (**8**).**



A solution of **14** (160 mg, 0.40 mmol) in 80% TFA/20%  $\text{CH}_2\text{Cl}_2$  (4 mL) was stirred at  $0^\circ\text{C}$  for 12 h. The mixture was concentrated in vacuo. A mixture of the residue and  $\text{NaHCO}_3$  (130 mg, 1.6 mmol) in DMF (4 mL) was treated with Boc-L-Pip pentafluorophenyl ester **15** (160 mg, 0.4 mmol) at  $0^\circ\text{C}$ , and the mixture was stirred at room temperature for 24 h. The mixture was partitioned between AcOEt (50 mL) and 1 M aqueous HCl (30 mL  $\times$  3), and the organic phase was washed with saturated aqueous NaCl, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ( $\phi$  1.6  $\times$  15 cm, 5% MeOH– $\text{CHCl}_3$ ) to afford **8** (150 mg, 78%) as a colorless amorphous solid:  $[\alpha]_D^{23} = -28.6^\circ$  ( $c$  1.03, MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz, a mixture of several rotamers at  $20^\circ\text{C}$ ; selected data for the major rotamer)  $\delta$  7.37–7.30 (m, 5H, Ph), 5.12 (s, 2H,  $\text{CH}_2\text{Ph}$ ), 4.64 (s, 1H, Dab-NH), 4.47 (m, 1H, Dab- $\alpha$ -CH), 4.36 (m, 1H, Pip- $\alpha$ -CH), 3.91 (m, 1H, Pip- $\epsilon$ -CH), 2.99 (dd, 1H, Pip- $\epsilon$ -CH,  $J = 10.3$ ,  $J = 13.2$  Hz), 2.18 (d, 1H, Pip- $\beta$ -CH,  $J = 11.5$  Hz), 1.58–1.31 (m, 5H, Pip- $\beta$ -CH, Pip- $\gamma$ -CH $_2$  and Pip- $\delta$ -CH $_2$ ), 1.45 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ), 1.11 (d, 3H, Dab- $\gamma$ -CH $_3$ ,  $J = 6.3$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  174.2, 171.7, 157.5, 155.7, 136.8, 128.2, 127.8, 127.7, 80.3, 66.5, 58.6, 42.3, 40.9, 27.4, 26.6, 24.5, 20.0, 14.4; ESIMS-LR  $m/z$  486 [(M + Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{23}\text{H}_{33}\text{N}_3\text{NaO}_7$  486.2216, found 486.2208.

### Pentapeptide **6**.

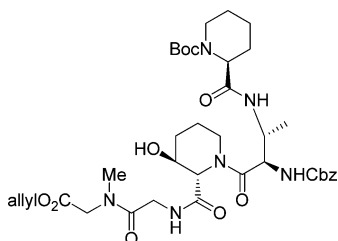


A solution of **16** (2.4 g, 8.0 mmol) and  $\text{Et}_3\text{N}$  (3.3 mL, 24.0 mmol) in DMSO (53 mL) was treated with sulfur trioxide pyridine complex (3.9 g, 24 mmol) at room temperature for 30 min. The reaction mixture was neutralized by 1 M aqueous HCl at  $0^\circ\text{C}$ , and the mixture was extracted with AcOEt (300 mL). The organic phase was washed with saturated aqueous  $\text{NaHCO}_3$  (100 mL),  $\text{H}_2\text{O}$  (100 mL), and saturated aqueous NaCl, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo to afford an aldehyde (2.4 g) as a yellow oil. A solution of the aldehyde in THF (80 mL) was treated with  $\text{PET}_3$  (20% toluene solution, 6.4 mL, 9.6 mmol) at  $0^\circ\text{C}$ , and the mixture was stirred at room temperature for 30 h. The



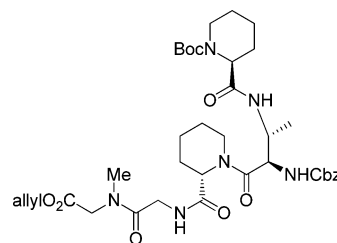
resulting mixture was concentrated in vacuo to afford **9** as a yellow oil. This material was used in the next reaction without further purification. A solution of carboxylic acid **8** (230 mg, 0.5 mmol), imine **9** (390 mg, 1.5 mmol), and isonitrile **7** (330 mg, 1.5 mmol) in toluene (1 mL) was stirred at 70 °C for 48 h. The mixture was partitioned between AcOEt (50 mL) and 1 M aqueous HCl (30 mL), and the organic phase was washed with saturated aqueous NaHCO<sub>3</sub> (30 mL), H<sub>2</sub>O (30 mL), and saturated aqueous NaCl, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ( $\phi$  1.6 × 19 cm, 33% AcOEt–67% hexane) to afford **6** (280 mg, 61%) as a colorless amorphous solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C; selected data for the major rotamer)  $\delta$  8.29 (br s, 1H, Gly-NH), 7.35–7.26 (m, 5H, Ph), 5.87 (ddd, 1H, CH<sub>2</sub>=CHCH<sub>2</sub>O,  $J$  = 5.2,  $J$  = 6.9,  $J$  = 14.2 Hz), 5.85–5.83 (m, 1H, Dab- $\alpha$ -NH), 5.43 (d, 1H, Sar- $\alpha$ -CH,  $J$  = 4.6 Hz), 5.24 (ddd, 2H, CH<sub>2</sub>=CHCH<sub>2</sub>O,  $J$  = 1.2,  $J$  = 9.1,  $J$  = 17.2 Hz), 5.15 (m, 1H, SiOPip- $\alpha$ -CH,  $J$  = 5.7 Hz), 5.07 (m, 1H, Pip- $\alpha$ -CH,  $J$  = 8.6 Hz), 5.02 (s, 2H, CH<sub>2</sub>Ph), 4.60 (d, 2H, CH<sub>2</sub>=CHCH<sub>2</sub>O,  $J$  = 5.2 Hz), 4.36 (dd, 1H, Dab- $\alpha$ -CH,  $J$  = 5.7,  $J$  = 17.2 Hz), 4.32–4.23 (m, 4H, SiOPip- $\epsilon$ -CH, Pip- $\epsilon$ -CH), 4.22 (d, 2H, Gly- $\alpha$ -CH,  $J$  = 17.2 Hz), 4.08 (d, 1H, Sar- $\alpha$ -CH,  $J$  = 15.8 Hz), 3.92 (dd, 1H, Dab- $\beta$ -CH,  $J$  = 4.0,  $J$  = 17.8 Hz), 3.10–3.03 (m, 1H, SiOPip- $\beta$ -CH), 3.02 (s, 3H, Sar-NCH<sub>3</sub>), 2.29 (s, 1H, Pip- $\beta$ -CH), 1.95–1.40 (m, 8H, Pip-(CH<sub>2</sub>)<sub>2</sub>), 1.43 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.18–1.13 (m, 3H, SiCH), 1.09–0.96 (m, 21H, SiCH(CH<sub>3</sub>)<sub>2</sub>, Dab- $\gamma$ -CH); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  171.2, 169.5, 168.8, 168.5, 168.3, 168.0, 156.6, 136.4, 131.6, 128.6, 128.2, 128.1, 118.8, 80.5, 70.3, 67.0, 66.4, 65.9, 56.9, 53.4, 50.6, 49.5, 42.6, 41.3, 35.4, 30.6, 28.4, 25.8, 25.7, 24.7, 20.9, 20.5, 18.1, 14.1, 12.1; ESIMS-LR  $m/z$  937 [(M + Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>46</sub>H<sub>74</sub>N<sub>6</sub>NaO<sub>11</sub>Si 937.5083, found 937.5073.

#### Pentapeptide 17.



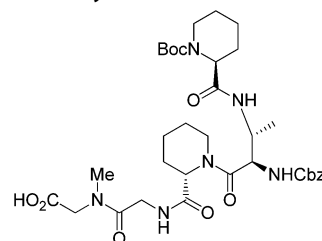
A solution of **6** (300 mg, 0.33 mmol) in MeCN (3 mL) was treated with Et<sub>3</sub>N·3HF (540  $\mu$ L, 3.3 mmol) at room temperature, and the mixture was stirred at 50 °C for 24 h. The resulting mixture was partitioned between AcOEt (30 mL) and saturated aqueous NaHCO<sub>3</sub> (50 mL × 3), and the organic phase was washed with H<sub>2</sub>O (50 mL) and saturated aqueous NaCl, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ( $\phi$  2.2 × 20 cm, 90% AcOEt–10% hexane) to afford **17** (210 mg, 84%) as a yellow oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C; selected data for the major rotamer)  $\delta$  7.30 (br s, 5H, Ph), 6.93 (br s, 1H, Gly-NH), 6.11 (d, 1H, Dab- $\alpha$ -NH,  $J$  = 6.3 Hz), 5.85 (ddd, 1H, CH<sub>2</sub>=CHCH<sub>2</sub>O,  $J$  = 5.8,  $J$  = 6.3,  $J$  = 10.3 Hz), 5.40 (d, 1H, Sar- $\alpha$ -CH,  $J$  = 5.8 Hz), 5.26 (dd, 2H, CH<sub>2</sub>=CHCH<sub>2</sub>O,  $J$  = 10.3 Hz,  $J$  = 16.1 Hz), 5.02 (s, 2H, CH<sub>2</sub>Ph), 4.72–4.61 (m, 1H, Pip- $\alpha$ -CH), 4.57 (d, 2H, CH<sub>2</sub>=CHCH<sub>2</sub>O,  $J$  = 10.9 Hz), 4.23–3.90 (m, 5H, Dab- $\alpha$ -CH, Gly- $\alpha$ -CH, Sar- $\alpha$ -CH, Dab- $\beta$ -CH), 3.78 (s, 1H, Pip- $\beta$ -CH), 3.30 (t, 2H, Pip- $\epsilon$ -CH,  $J$  = 12.6 Hz), 3.03–2.94 (m, 3H, Sar-NCH<sub>3</sub>), 2.26 (br s, 2H, Pip- $\beta$ -CH), 1.98–1.43 (m, 12H, Pip-(CH<sub>2</sub>)<sub>3</sub>), 1.42 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.06 (d, 3H, Dab- $\gamma$ -CH,  $J$  = 6.3 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  171.3, 169.9, 169.1, 168.8, 168.5, 168.0, 156.5, 136.3, 131.5, 128.5, 128.2, 128.1, 119.0, 68.6, 67.0, 66.5, 66.0, 56.1, 53.5, 49.6, 42.9, 41.3, 35.4, 35.2, 29.8, 28.4, 25.8, 25.7, 25.0, 24.4, 24.3, 20.4, 14.3; ESIMS-LR  $m/z$  781 [(M + Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>37</sub>H<sub>54</sub>N<sub>6</sub>NaO<sub>11</sub> 781.3748, found 781.3741.

#### Pentapeptide 18.



A solution of **17** (100 mg, 0.13 mmol), DMAP (24 mg, 0.20 mmol), and Et<sub>3</sub>N (55  $\mu$ L, 0.40 mmol) in MeCN (1.5 mL) was treated with phenyl chlorothioformate (54  $\mu$ L, 0.40 mmol) at room temperature for 12 h. The resulting mixture was partitioned between AcOEt (50 mL) and 1 M aqueous HCl (30 mL), and the organic phase was washed with saturated aqueous NaHCO<sub>3</sub> (30 mL), H<sub>2</sub>O (30 mL), and saturated aqueous NaCl, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue in trifluoroethanol (1.3 mL) was treated with Bu<sub>3</sub>SnH (160  $\mu$ L, 0.53 mmol) and AIBN (11 mg, 0.066 mmol), and the mixture was stirred at 78 °C for 1 h. The resulting mixture was partitioned between AcOEt (30 mL) and saturated aqueous KF (10 mL × 2), and the organic phase was washed with H<sub>2</sub>O (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography ( $\phi$  1.2 × 17 cm, 90% AcOEt–10% hexane) to afford **18** (54 mg, 55%) as a colorless amorphous solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C; selected data for the major rotamer)  $\delta$  7.33–7.26 (m, 5H, Ph), 6.89 (br s, 1H, Gly-NH), 6.07 (br s, 1H, Dab- $\alpha$ -NH), 5.89 (ddd, 1H, CH<sub>2</sub>=CHCH<sub>2</sub>O,  $J$  = 5.7,  $J$  = 6.9,  $J$  = 10.3 Hz), 5.33–5.25 (m, 1H, Sar- $\alpha$ -CH), 5.27 (dd, 2H, CH<sub>2</sub>=CHCH<sub>2</sub>O,  $J$  = 10.3,  $J$  = 16.4 Hz), 5.11–5.08 (m, 1H, Pip- $\alpha$ -CH), 5.06 (s, 2H, CH<sub>2</sub>Ph), 4.94 (m, 1H, Pip- $\alpha$ -CH), 4.60 (d, 2H, CH<sub>2</sub>=CHCH<sub>2</sub>O,  $J$  = 6.3 Hz), 4.25–3.90 (m, 5H, Dab- $\alpha$ -CH, Gly- $\alpha$ -CH, Sar- $\alpha$ -CH, Dab- $\beta$ -CH), 3.30 (t, 2H, Pip- $\epsilon$ -CH,  $J$  = 12.6 Hz), 3.10–3.04 (m, 3H, Sar-NCH<sub>3</sub>), 2.31–2.23 (br s, 2H, Pip- $\beta$ -CH), 1.80–1.46 (m, 12H, Pip-(CH<sub>2</sub>)<sub>3</sub>), 1.42 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.10 (d, 3H, Dab- $\gamma$ -CH,  $J$  = 6.9 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  171.4, 170.2, 169.8, 168.9, 168.6, 168.1, 156.7, 136.4, 131.6, 131.2, 128.6, 128.2, 128.1, 119.1, 80.5, 67.2, 66.0, 53.8, 53.0, 44.1, 41.5, 41.1, 35.6, 35.4, 29.9, 28.5, 26.2, 25.8, 25.7, 25.0, 20.6, 20.5, 15.3; ESIMS-LR  $m/z$  765 [(M + Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>37</sub>H<sub>54</sub>N<sub>6</sub>NaO<sub>10</sub> 765.3799, found 765.3794.

#### Pentapeptide Carboxylic Acid 22.

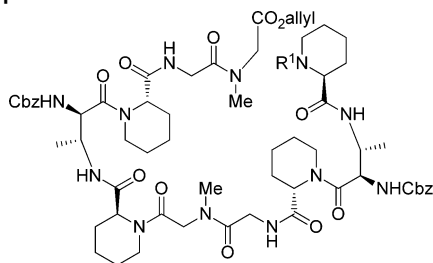


A solution of compound **18** (420 mg, 0.57 mmol) and morpholine (150  $\mu$ L, 0.17 mmol) in THF (6 mL) was treated with Pd(PPh<sub>3</sub>)<sub>4</sub> (200 mg, 0.17 mmol) at room temperature for 30 min. The mixture was partitioned between AcOEt (150 mL × 3) and 1 M aqueous HCl (50 mL), and the organic phase was washed with saturated aqueous NaCl, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ( $\phi$  2.2 × 17 cm, 7% MeOH–93% CHCl<sub>3</sub>) to afford **22** (320 mg, 80%) as a white amorphous solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz, a mixture of several rotamers at 20 °C; selected data for the major rotamer)  $\delta$  7.40–7.23 (m, 5H, Ph), 5.21 (m, 1H, Sar- $\alpha$ -CH), 5.08–5.02 (m, 2H, CH<sub>2</sub>Ph,  $J$  = 8.6 Hz), 5.00 (s, 1H, Pip- $\alpha$ -CH), 4.67 (s, 1H, Pip- $\alpha$ -CH), 4.30–3.82 (m, 5H, Dab- $\alpha$ -CH, Gly- $\alpha$ -CH, Sar- $\alpha$ -CH, Dab- $\beta$ -CH), 3.60 (d, 1H, Pip- $\epsilon$ -CH,  $J$  = 12.6 Hz), 3.06–2.91 (m, 4H, Pip- $\epsilon$ -CH, Sar-NCH<sub>3</sub>), 2.36 (m, 1H, Pip- $\beta$ -CH), 2.19 (m, 1H, Pip- $\beta$ -CH), 1.80–1.46 (m, 12H, Pip-(CH<sub>2</sub>)<sub>3</sub>), 1.45 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.14 (s, 3H, Dab- $\gamma$ -CH); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz)  $\delta$  176.4, 173.2, 172.7, 171.7, 170.9, 170.6, 158.4, 137.9, 129.4, 129.0, 81.4, 67.8, 58.2, 55.3, 54.7, 54.7, 53.8, 52.6, 48.1, 44.9, 43.4, 42.1, 36.2, 35.8, 28.6, 27.7, 27.4, 26.7, 26.5, 25.9, 25.7, 22.1, 21.4, 18.4, 15.3; ESIMS-LR



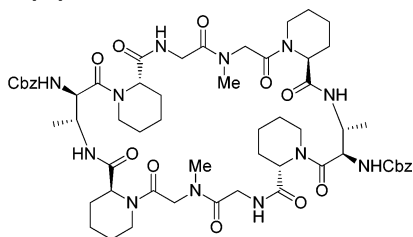
$m/z$  701  $[(M + Na)^+]$ ; ESIMS-HR calcd for  $C_{34}H_{49}N_6O_{10}$  701.3510, found 701.3532.

#### Decapeptide 23.



Compound **18** (340 mg, 0.46 mmol) was treated with 4 M HCl in dioxane (4.6 mL) at room temperature for 30 min. The mixture was concentrated in vacuo to afford the crude amine hydrochloride **21** (300 mg, quantitative) as a white solid. The amine was added to a mixture of **22** (320 mg, 0.46 mmol),  $NaHCO_3$  (150 mg, 1.8 mmol), and (3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT; 550 mg, 1.8 mmol) in  $CH_2Cl_2$  (4 mL) and DMF (0.5 mL) at 0 °C, and the whole mixture was stirred at room temperature for 24 h. The resulting mixture was partitioned between AcOEt (50 mL) and 1 M aqueous HCl (10 mL), and the organic phase was washed with saturated aqueous  $NaHCO_3$  (30 mL),  $H_2O$  (30 mL), and saturated aqueous NaCl, dried ( $Na_2SO_4$ ), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ( $\phi$  2.2 × 18 cm, 90% AcOEt–10% hexane) to afford **23** (380 mg, 62%) as a colorless amorphous solid:  $^1H$  NMR ( $CDCl_3$ , 500 MHz, a mixture of several rotamers at 20 °C; selected data for the major rotamer)  $\delta$  7.38–7.23 (m, 10H, Ph), 6.93 (br s, Dab- $\alpha$ -NH), 5.91 (m, 1H,  $CH_2=CHCH_2O$ ), 5.25 (m, 2H,  $CH_2=CHCH_2O$ ), 5.23–5.15 (m, 2H, Pip- $\alpha$ -CH), 5.11–5.00 (m, 4H,  $CH_2Ph$ ), 4.66 (m, 2H, Dab- $\alpha$ -CH), 4.65–4.62 (m, 2H,  $CH_2=CHCH_2O$ ), 4.30–3.91 (m, 10H, Gly- $\alpha$ -CH, Dab- $\beta$ -CH, Sar- $\alpha$ -CH), 3.75–3.60 (m, 4H, Pip- $\epsilon$ -CH), 3.33 (m, 4H, Pip- $\epsilon$ -CH), 3.07–2.92 (m, 6H, Sar- $NCH_3$ ), 2.30–2.15 (m, 4H, Pip- $\beta$ -CH), 1.84–1.30 (m, 20H, Pip- $(CH_2)_2$ , Pip- $\beta$ -CH), 1.44 (s, 9H,  $C(CH_3)_3$ ), 1.24 (m, 3H, Dab- $\gamma$ -CH), 1.09 (m, 3H, Val- $\gamma$ -CH);  $^{13}C$  NMR ( $CDCl_3$ , 125 MHz)  $\delta$  171.3, 171.0, 170.8, 170.3, 170.0, 169.7, 168.8, 168.7, 168.5, 168.4, 168.2, 156.6, 156.0, 136.4, 136.3, 131.5, 128.49, 128.45, 128.0, 127.9, 127.8, 127.3, 118.9, 80.3, 67.6, 67.0, 65.9, 53.7, 52.9, 49.5, 43.9, 41.3, 41.2, 40.9, 35.6, 35.3, 35.1, 28.4, 25.8, 25.6, 25.5, 24.9, 21.0, 20.5, 20.4, 14.2; ESIMS-LR  $m/z$  1349  $[(M + Na)^+]$ ; ESIMS-HR calcd for  $C_{66}H_{94}N_{12}NaO_{17}$  1349.6758, found 1349.6741.

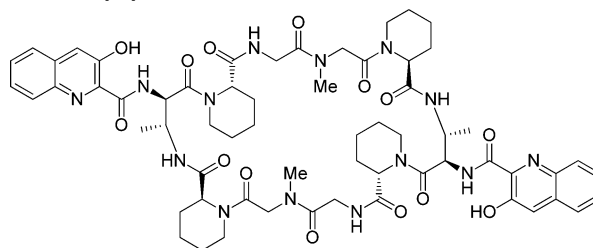
#### Cyclic Decapeptide 4.



A solution of **23** (62 mg, 0.047 mmol) and morpholine (12  $\mu$ L, 0.14 mmol) in THF (1 mL) was treated with  $Pd(PPh_3)_4$  (16 mg, 14  $\mu$ mol) at room temperature for 30 min. The mixture was partitioned between AcOEt (30 mL × 3) and 1 M aqueous HCl (10 mL), and the organic phase was washed with saturated aqueous NaCl, dried ( $Na_2SO_4$ ), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ( $\phi$  1.2 × 14 cm, 10% MeOH–90%  $CHCl_3$ ) to afford **24** (46 mg, 0.036 mmol) as a white solid. The carboxylic acid **24** (46 mg, 0.036 mmol) was treated with 4 M HCl in dioxane (1 mL) for 30 min. The mixture was concentrated in vacuo to afford the crude amino acid **25** (43 mg, 0.036 mmol, theoretical quantitative) as a white solid. A solution of the residue and  $NaHCO_3$  (12 mg, 0.14 mmol) in DMF (7 mL) was treated with DEPBT (42 mg, 0.14 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 2 days and 40 °C for 2 days. The resulting mixture was partitioned between AcOEt (30 mL) and 1 M aqueous HCl (10 mL), and the organic phase was washed

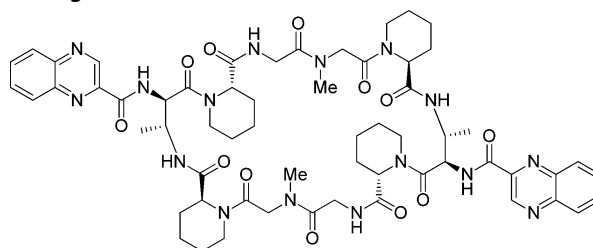
with saturated aqueous  $NaHCO_3$  (10 mL),  $H_2O$  (10 mL), and saturated aqueous NaCl, dried ( $Na_2SO_4$ ), filtered, and concentrated in vacuo. The residue was purified by flash silica gel column chromatography ( $\phi$  0.9 × 18 cm, 3% MeOH– $CHCl_3$ ) to afford **4** (12 mg, 23%) as a colorless amorphous solid:  $[\alpha]_D^{20} = -37.0^\circ$  (c 0.31, MeOH);  $^1H$  NMR ( $CD_3OD$ , 500 MHz, a mixture of several rotamers at 20 °C; selected data for the major rotamer)  $\delta$  7.45–7.21 (m, 10H, Ph), 5.35–4.90 (m, 6H,  $CH_2Ph$ , Gly- $\alpha$ -CH), 4.84–3.99 (m, 6H, Gly- $\alpha$ -CH, Pip- $\alpha$ -CH, Dab- $\alpha$ -CH), 3.82–3.35 (m, 4H, Sar- $\alpha$ -CH), 3.19–3.03 (m, 6H, Sar- $NCH_3$ ), 2.94–2.89 (m, 4H, Pip- $\epsilon$ -CH), 2.26–2.10 (m, 2H, Dab- $\beta$ -CH), 1.62–1.23 (m, 20H, Pip- $(CH_2)_3$ ), 1.30–1.11 (m, 6H, Dab- $\gamma$ -CH);  $^{13}C$  NMR ( $CD_3OD$ , 125 MHz)  $\delta$  173.4, 172.5, 172.3, 172.2, 171.5, 171.0, 170.7, 170.2, 158.5, 158.4, 138.2, 129.5, 129.1, 79.5, 71.6, 67.8, 67.6, 67.5, 54.6, 45.1, 42.0, 41.7, 36.2, 35.9, 33.1, 30.8, 30.5, 28.7, 28.5, 27.6, 26.8, 26.4, 26.1, 25.6, 23.7, 21.8, 21.5, 21.4, 18.5, 14.4, 13.2; ESIMS-LR  $m/z$  1191  $[(M + Na)^+]$ ; ESIMS-HR calcd for  $C_{58}H_{80}N_{12}NaO_{14}$  (M + Na) $^+$  1191.5814, found 1191.5798. These data are identical with those obtained previously.<sup>11</sup>

#### Quinaldopeptin 1.



A mixture of **4** (5.8 mg, 5.0  $\mu$ mol) in MeOH (1 mL) and 10%  $Pd(OH)_2/C$  (1 mg) was vigorously stirred under a  $H_2$  atmosphere at room temperature for 1 h. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated in vacuo to give the diamine. The residue in DMF (1 mL) was treated with HATU (7.6 mg, 20  $\mu$ mol),  $Pr_2NEt$  (3.5  $\mu$ L, 20  $\mu$ mol), and chromophore **5** (3.8 mg, 20  $\mu$ mol) at 0 °C, and the mixture was stirred at room temperature for 17 h. The resulting mixture was partitioned between AcOEt (30 mL) and 1 M aqueous HCl (10 mL), and the organic phase was washed with saturated aqueous  $NaHCO_3$  (10 mL),  $H_2O$  (10 mL), and saturated aqueous NaCl, dried ( $Na_2SO_4$ ), filtered, and concentrated in vacuo. The residue was purified by flash silica gel column chromatography (3% MeOH–97%  $CHCl_3$ ) to afford quinaldopeptin (**1**; 2.7 mg, 43%) as a yellow solid:  $^1H$  NMR ( $CD_3OD$ , 500 MHz; selected peaks are given because of multiple conformers)  $\delta$  7.90 (br d, 1H,  $J = 5.8$  Hz), 7.81–7.26 (m, 4H), 5.86 (br s, 1H), 5.67 (br s, 1H), 5.30 (d, 1H,  $J = 4.1$  Hz), 5.12 (d, 1H,  $J = 4.0$  Hz), 4.70 (d, 1H,  $J = 18.3$  Hz), 4.60 (d, 1H,  $J = 10.3$  Hz), 4.47 (d, 1H,  $J = 13.2$  Hz), 4.30 (br s, 1H), 4.23 (m, 2H), 4.07 (d, 1H,  $J = 18.9$  Hz), 3.95 (d, 1H,  $J = 17.2$  Hz), 3.65 (m, 1H), 3.43 (m, 1H), 2.87 (m, 1H), 3.16 (br s, 1H), 3.22–2.99 (m), 2.96 (s, 3H), 2.89–2.81 (m), 2.30 (br d, 1H,  $J = 12.1$  Hz), 2.20 (br d, 1H,  $J = 13.7$  Hz), 1.87–1.37 (m), 1.21 (d, 3H,  $J = 7.5$  Hz);  $^{13}C$  NMR ( $CD_3OD$ , 125 MHz; selected peaks are given because of multiple conformers)  $\delta$  172.7, 172.6, 172.4, 171.9, 170.1, 169.7, 154.4, 135.8, 133.2, 130.5, 129.9, 129.8, 129.5, 128.5, 128.2, 127.4, 121.0, 57.1, 54.5, 54.4, 53.7, 52.6, 45.2, 42.0, 41.9, 36.2, 33.1, 30.7, 30.5, 30.3, 28.1, 27.9, 27.0, 26.4, 25.2, 23.7, 21.6, 21.4, 21.3, 14.4, 13.2; ESIMS-LR  $m/z$  1265  $[(M + Na)^+]$ ; ESIMS-HR calcd for  $C_{62}H_{78}N_{14}NaO_{14}$  1265.5720, found 1265.5728. These data are identical with those obtained previously.<sup>11</sup>

#### Analogue 26.



A solution of the 2-quinoxalinecarboxylic acid (13.9 mg, 0.08 mmol) and HOBt (16.2 mg, 0.12 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was treated with EDCI (15.3 mg, 0.08 mmol), and the reaction mixture was stirred at room temperature for 30 min. The mixture was added to the diamine obtained from **4** by hydrogenolysis (18.1 mg, 0.02 mmol), and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was poured onto 1 M aqueous HCl and extracted with AcOEt. The combined organic layer was washed with saturated aqueous  $\text{NaHCO}_3$  and saturated aqueous NaCl, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ( $\text{SiO}_2$ , 1% MeOH–99%  $\text{CHCl}_3$ ) to afford **26** (9.7 mg, 40%) as a colorless glass. Part of the material was crystallized from MeOH to give a colorless prism, which was used for the X-ray crystal structure analysis: mp >300 °C;  $[\alpha]_D^{20} = -409.6^\circ$  (c 0.64,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of rotamers; selected data for the major rotamer)  $\delta$  10.34 (br s, 2H, NH), 8.88 (br s, 2H, NH), 8.01 (br s, 2H), 7.93 (br s, 2H), 7.80 (m, 6H), 7.63 (br s, 2H), 7.01 (d, 2H,  $J = 9.2$  Hz), 5.90 (br s, 2H), 5.21 (s, 2H), 5.04 (br s, 2H), 4.73 (br s, 2H), 4.48 (m, 2H), 3.99 (br s, 2H), 3.61 (br s, 2H), 3.20 (br s, 2H), 2.66 (br s, 6H), 2.46 (d, 2H,  $J = 12.6$  Hz), 2.20–2.10 (m, 6H), 1.82 (m, 4H), 1.70–1.61 (m, 16H), 1.43 (d, 6H, Dab- $\text{CH}_3$ ,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 125 MHz, a mixture of rotamers; selected data for the major rotamer)  $\delta$  172.7, 172.5, 172.2, 172.0, 171.9, 165.1, 145.1, 144.2, 133.1, 132.6, 132.3, 131.0, 130.9, 130.4, 129.9, 129.6, 57.1, 54.5, 52.7, 42.2, 41.8, 41.5, 36.3, 28.0, 26.7, 25.2, 23.8, 21.6, 21.3, 13.1; ESIMS-LR  $m/z$  1235  $[(M + \text{Na})^+]$ ; ESIMS-HR calcd for  $\text{C}_{60}\text{H}_{76}\text{N}_{16}\text{NaO}_{12}$  1235.57208, found 1235.57142.

**DNA Binding Constant Measurements.** The temperature was maintained at 24 °C throughout the experimental work. A 300  $\mu\text{L}$  quartz cuvette was used in all experiments. Double-stranded oligodeoxynucleotide (dsODN) was dissolved in 10 mM Tris-HCl buffer solution containing 75 mM NaCl. The excitation and emission spectra were recorded with a sample (200  $\mu\text{L}$ ) containing 10 mM Tris-HCl, 75 mM NaCl buffer, and 2  $\mu\text{L}$  of a DMSO stock solution of the agent. The final concentration for the agents was 10  $\mu\text{M}$ . For the determination of the dsODN binding constant, a 200  $\mu\text{L}$  sample containing 10  $\mu\text{M}$  **1** or **2** was titrated with 2  $\mu\text{L}$  of dsODN (320  $\mu\text{M}$ ) solution. The quenching of fluorescence was measured by spectrofluorimeter 5 min after each addition of dsODN to allow binding equilibration with 360 nm excitation and 530 nm fluorescence, and the results are graphically represented in Figure 3b,d.

**General Procedure for Agarose Gel Electrophoresis.** All agents were dissolved in DMSO as stock solutions, stored at –30 °C in the dark, and diluted to the working concentrations in DMSO prior to addition to the DNA solution. A buffered DNA solution containing 0.25  $\mu\text{g}$  of supercoiled  $\Phi\text{X}$  174 RF I DNA ( $1.0 \times 10^{-8}$ ) in 9  $\mu\text{L}$  of 50 mM Tris-HCl buffer solution was treated with 1  $\mu\text{L}$  of agent in DMSO (the control DNA was treated with 1  $\mu\text{L}$  of DMSO). The [agent] to [DNA] base pair ratios were as follows: 0.022 (lane 1), 0.033 (lane 2), 0.044 (lane 3), 0.11 (lane 4), 0.22 (lane 5) for **2**; 0 (lane 6 control DNA), 0.022 (lane 7), 0.033 (lane 8), 0.044 (lane 9), 0.11 (lane 10), 0.22 (lane 11) for **1**. The reaction mixtures were incubated at 37 °C for 1 h and quenched with 5  $\mu\text{L}$  of loading buffer. Electrophoresis was conducted on a 0.9% agarose gel at 90 V for 1 h. The gel was stained with 0.1  $\mu\text{g}/\text{mL}$  ethidium bromide, visualized on a lumino imaging analyzer.

**Cytotoxicity Assay.** Antiproliferative activities of the compounds against HCT-116, HT-29, and CCRF-CME cells were measured using the CellTiter-Glo Luminescent Cell Viability Assay according to the manufacturer's protocol. Briefly, cells ( $1.5\text{--}3 \times 10^3$  cells/well) in a 96-well plate were cultured in RPMI medium containing 10% fetal bovine serum in the presence of test compounds at 37 °C for 72 h under a 5%  $\text{CO}_2$  atmosphere. Then, CellTiter-Glo reagent was added, and plates were shaken and the luminescence was monitored 30 min after reagent addition with the plates held at room temperature.

**Cell Culture and Transfection.** Human embryonic kidney (HEK) 293 cells were cultured in Dulbecco's modified Eagle's medium (Wako, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA) at 37 °C under a 5%  $\text{CO}_2$  atmosphere. Transfection was performed by using FuGENE HD (Promega, Madison, WI) according to the manufacturer's protocol.

**Reporter Gene Assay.** HEK293 cells seeded in 48-well plates were transfected with pGL3-5xHRE-Luc and pGL4.75 hRluc-CMV (Promega), together with HIF-1 $\alpha$  or HIF-2 $\alpha$  expression vectors as indicated. Twenty-four hours after transfection, the cells were exposed to echinomycin, sandramycin, quinaldpeptin, or desmethylquinaldpeptin for 16 h under normoxic (21%  $\text{O}_2$ ) or hypoxic (1%  $\text{O}_2$ ) conditions. Luciferase activities were measured using Dual-Luciferase Reporter Assay System (Promega) and a luminescencer.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Text, figures, tables, and a CIF file giving amino acid analysis of **18**, NMR spectra of new compounds, and an X-ray crystal structure analysis of **26**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*S.I.: tel, (+81) 11-706-3229; fax, (+81) 11-706-4980; e-mail, [ichikawa@pharm.hokudai.ac.jp](mailto:ichikawa@pharm.hokudai.ac.jp).

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This research was supported by JSPS Grants-in-Aid for Challenging Exploratory Research (SI, Grant No. 22659020), Scientific Research on Innovative Areas "Chemical Biology of Natural Products" (SI, Grant No. 24102502), and Scientific Research (B) (SI, Grant No. 25293026). We thank Ms. S. Oka and Ms. A. Tokumitsu (Center for Instrumental Analysis, Hokkaido University) for measurement of the mass spectra.

## ■ REFERENCES

- (1) Toda, S.; Sugawara, K.; Nishiyama, Y.; Ohbayashi, M.; Ohkusa, N.; Yamamoto, H.; Konishi, M.; Oki, T. *J. Antibiot.* **1990**, *43*, 796–808.
- (2) (a) Matson, J. A.; Bush, J. A. *J. Antibiot.* **1989**, *42*, 1763–1767. (b) Matson, J. A.; Colson, K. L.; Belofsky, G. N.; Bleiberg, B. B. *J. Antibiot.* **1993**, *46*, 162–166.
- (3) (a) Ohkuma, H.; Sakai, F.; Nishiyama, Y.; Ohbayashi, M.; Imanishi, H.; Konishi, M.; Miyaki, T.; Koshiyama, H.; Kawaguchi, H. *J. Antibiot.* **1980**, *33*, 1087–1097. (b) Tomita, K.; Hoshino, Y.; Sasahira, T.; Kawaguchi, H. *J. Antibiot.* **1980**, *33*, 1098–1102. (c) Konishi, M.; Ohkuma, H.; Sakai, F.; Tsuno, T.; Koshiyama, H.; Naito, T.; Kawaguchi, H. *J. Antibiot.* **1981**, *34*, 148–159.
- (4) Lingham, R. B.; Hsu, A. H. M.; O'Brien, J. A.; Sigmund, J. M.; Sanchez, M.; Gagliardi, M. M.; Heimbuch, B. K.; Genilloud, O.; Martin, I.; Diez, M. T.; Hirsch, C. F.; Zink, C. D. L.; Liesch, J. M.; Koch, G. E.; Gartner, S. E.; Garrity, G. M.; Tsou, N. N.; Salituro, G. M. *J. Antibiot.* **1996**, *49*, 253–259.
- (5) For reviews, see: (a) Dawson, S.; Malkinson, J. P.; Paumier, D.; Searcey, M. *Nat. Prod. Rep.* **2007**, *24*, 109–126. (b) Zolova, O. E.; Mady, A. S. A.; Garneau-Tsodikova, S. *Biopolymers* **2010**, *93*, 777–790.
- (6) Boger, D. L.; Chen, J.-H.; Saitonz, K. W. *J. Am. Chem. Soc.* **1996**, *118*, 1629–1644.
- (7) (a) Boger, D. L.; Chen, J.-H.; Saionz, K. W.; Jin, Q. *Bioorg. Med. Chem.* **1998**, *6*, 85–102. (b) Boger, D. L.; Chen, J.-H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 919–922. (c) Boger, D. L.; Saionz, K. W. *Bioorg. Med. Chem.* **1999**, *7*, 315–321.
- (8) (a) Boger, D. L.; Schüle, G. *J. Org. Chem.* **1998**, *63*, 6421–6424. (b) Boger, D. L.; Ledebauer, M. W.; Kume, M. *J. Am. Chem. Soc.* **1999**, *121*, 1098–1099.
- (9) (a) Ciufolini, M. A.; Xi, N. *J. Org. Chem.* **1997**, *62*, 2320–2321. (b) Ciufolini, M. A.; Valognes, D.; Xi, N. *J. Heterocycl. Chem.* **1999**, *36*, 1409–1419. (c) Valognes, D.; Belmont, P.; Xi, N.; Ciufolini, M. A. *Tetrahedron Lett.* **2001**, *42*, 1907–1909. (d) Ciufolini, M. A.; Swaminathan, S. *Tetrahedron Lett.* **1989**, *23*, 3027–3028. (e) Ciufolini,

- M. A.; Valognes, D.; Xi, N. *Tetrahedron Lett.* **1999**, *40*, 3693–3696.
- (f) Ciufolini, M. A.; Valognes, D.; Xi, N. *Angew. Chem., Int. Ed.* **2000**, *39*, 2493–2495.
- (10) Boger, D. L.; Ledebor, M. W.; Kume, M.; Searcey, M.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 11375–11383.
- (11) Ichikawa, S.; Okamura, T.; Matsuda, A. *J. Org. Chem.* **2013**, *78*, 12662–12670.
- (12) Katayama, K.; Nakagawa, K.; Takeda, H.; Matsuda, A.; Ichikawa, S. *Org. Lett.* **2014**, *16*, 428–431.
- (13) Review: Domling, A.; Ugi, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168–3210.
- (14) (a) Nutt, R. F.; Joullie, M. M. *J. Am. Chem. Soc.* **1982**, *104*, 5852–5853. (b) Bowers, M. M.; Carroll, P.; Joullie, M. M. *J. Chem. Soc., Perkin Trans. 1* **1989**, 857–865.
- (15) Znabet, A.; Ruijter, E.; de Kanter, F. J. J.; Köhler, V.; Helliwell, M.; Turner, N. J.; Orru, R. V. A. *Angew. Chem., Int. Ed.* **2010**, *49*, 5289–5292.
- (16) Chapman, T. M.; Davies, I. G.; Gu, B.; Block, T. M.; Scopes, D. I. C.; Hay, P. A.; Courtney, S. M.; McNeill, L. A.; Schofield, C. J.; Davis, B. G. *J. Am. Chem. Soc.* **2005**, *127*, 506–507.
- (17) Timmer, M. S. M.; Risseuw, M. D. P.; Verdoes, M.; Filippov, D. V.; Plaisier, J. R.; van der Marel, G. A.; Overkleeft, H. S.; van Boom, J. H. *Tetrahedron: Asymmetry* **2005**, *6*, 177–185.
- (18) Maison, W.; Lützen, A.; Kosten, M.; Schlemminger, I.; Westerhoff, O.; Saak, W.; Martens, J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1867–1871.
- (19) Sperger, C. A.; Mayer, P.; Wanner, K. T. *Tetrahedron* **2009**, *65*, 10463–10469.
- (20) (a) Zhu, D.; Chen, R.; Liang, H.; Li, S.; Pan, L.; Chen, X. *Synlett* **2010**, 897–900. (b) Zhu, D.; Xia, L.; Pan, L.; Li, S.; Chen, R.; Mou, Y.; Chen, X. *J. Org. Chem.* **2012**, *77*, 1386–1395. (c) Xia, L.; Li, S.; Chen, R.; Liu, K.; Chen, X. *J. Org. Chem.* **2013**, *78*, 3120–3131.
- (21) El Kaïm, L.; Grimaud, L.; Oble, J.; Wagschal, S. *Tetrahedron Lett.* **2009**, *50*, 1741–1743.
- (22) Gröger, H.; Hatam, M.; Martens, J. *Tetrahedron* **1995**, *51*, 7173–7180.
- (23) Banfi, L.; Basso, A.; Guanti, G.; Merlo, S.; Repetto, C.; Riva, R. *Tetrahedron* **2008**, *64*, 1114–1134.
- (24) Iizuka, T.; Takiguchi, S.; Kumakura, Y.; Tsukioka, N.; Higuchi, K.; Kawasaki, T. *Tetrahedron Lett.* **2010**, *51*, 6003–6005.
- (25) Gulevich, A. V.; Shevchenko, N. F.; Balenkova, E. S.; Roeschenthaler, G. V.; Nenajdenko, V. G. *Synlett* **2009**, 403–406.
- (26) Mehta, V. P.; Modha, S. G.; Ruijter, E.; van Hecke, K.; Van Meervelt, L.; Pannecouque, C.; Balzarini, J.; Orru, R. V. A.; van der Eycken, E. *J. Org. Chem.* **2011**, *76*, 2828–2839.
- (27) Han, H.; Yoon, J.; Janda, K. D. *J. Org. Chem.* **1998**, *63*, 2045–2048.
- (28) (a) Robins, M. J.; Wilson, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 932–933. (b) Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059–4065.
- (29) (a) Kita, Y.; Sano, A.; Yamaguchi, T.; Oka, M.; Gotanda, K.; Matsugi, M. *Tetrahedron Lett.* **1997**, *38*, 3549–3552. (b) Kita, Y.; Gotanda, K.; Murata, K.; Suemura, M.; Sano, A.; Yamaguchi, T.; Oka, M.; Matsugi, M. *Org. Process Res. Dev.* **1998**, *2*, 250–254.
- (30) Hinou, H.; Hyugaji, K.; Garcia-Martin, F.; Nishimura, S.; Albericio, F. *RSC Adv.* **2012**, *2*, 2729–2731.
- (31) For a review, see: Bhushan, R.; Brückner, H. *Amino Acids* **2004**, *27*, 231–247.
- (32) Jiang, H.; Li, X.; Fan, Y.; Ye, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91–93.
- (33) Boger, D. L.; Chen, J.-H. *J. Org. Chem.* **1995**, *60*, 7369–7371.
- (34) Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 1243–1244.
- (35) Formica, J. V.; Waring, M. J. *Antimicrob. Agents Chemother.* **1983**, *24*, 735–741.
- (36) For other representative cyclic octadepsipeptides, see the following. Triostin A, isolation: (a) Shoji, J.; Katagiri, K. *J. Antibiot., Sect. A* **1961**, *14*, 335–339. Triostin A, total synthesis: (b) Chakravarty, P. K.; Olsen, R. K. *Tetrahedron Lett.* **1978**, *19*, 1613–1616. (c) Shin, M.; Inouye, K.; Otsuka, H. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2203–2210. Thiocoraline, isolation: (d) Romero, F.; Espliego, F.; Pérez, B. J.; García, de Q. T.; Grávalos, D.; de la Calle, F.; Fernández-Puentes, J. L. *J. Antibiot.* **1997**, *50*, 743–747. Thiocoraline, total synthesis: (e) Boger, D. L.; Ichikawa, S. *J. Am. Chem. Soc.* **2000**, *122*, 2956–2957. (f) Boger, D. L.; Ichikawa, S.; Tse, W. C.; Hedrick, M. P.; Jin, Q. *J. Am. Chem. Soc.* **2001**, *123*, 561–568. It is unknown whether these natural products inhibit HIF-1.
- (37) (a) Muss, H. B.; Blessing, J. A.; DuBeshter, B. *Am. J. Clin. Oncol.* **1993**, *16*, 492–493. (b) Wadler, S.; Tenteromano, L.; Cazenave, L.; Sparano, J. A.; Greenwald, E. S.; Rozenblit, A.; Kaleya, R.; Wiernik, P. H. *Cancer Chemother. Pharmacol.* **1994**, *34*, 266–269. (c) Chang, A. Y.; Kim, K.; Boucher, H.; Bonomi, P.; Stewart, J. A.; Karp, D. D.; Blum, R. H. *Cancer* **1998**, *82*, 292–300.
- (38) (a) Semenza, G. L. *Trends Mol. Med.* **2001**, *7*, 345–50. (b) Semenza, G. L. *Nat. Rev. Cancer* **2003**, *3*, 721–732.
- (39) Kong, D.; Park, E. J.; Stephen, A. G.; Calvani, M.; Cardellina, J. H.; Monks, A.; Fisher, R. J.; Shoemaker, R. H.; Melillo, G. *Cancer Res.* **2005**, *65*, 9047–9055.