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Total Synthesis of Quinaldopeptin and Its Analogues

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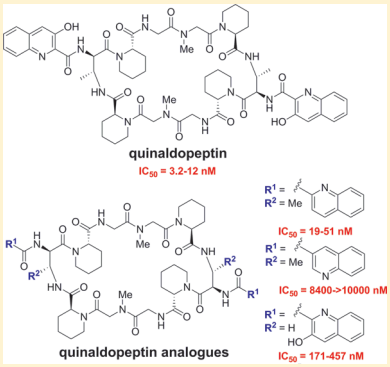
Supporting Information

ABSTRACT: The first total synthesis of quinaldopeptin (**1**) was accomplished. Our approach to the synthesis of **1** includes the solid-phase peptide synthesis of the linear decapeptide **4** followed by macrocyclization and introduction of the quinoline chromophores **2** at a late stage of the synthesis. As for the preparation of **4**, a fragment coupling approach was applied considering the *C*2 symmetrical structure of **1**. Chromophore analogues **22** and **23** and desmethyl analogue **27** were also prepared in a manner similar to the synthesis of **1**. Synthetic **1** exhibits a strong cytotoxicity with the IC_{50} value of 3.2 nM. On the other hand, the activity of **23** and **27** was largely reduced.



INTRODUCTION

A class of *C*2-symmetric cyclic decadepsipeptides such as sandramycin,[1] luzopeptines,[2] and quinoxapeptines[3] are known to exhibit a variety of promising biological properties including antitumor, antibacterial, and antiviral activities (Figure 1). They can bind to double-stranded DNA by the phenomenon called bisintercalation.[4] It is presumed that the binding of this class of natural products to a gene suppresses the aberrant activation and expression of the corresponding *cis*-element by inhibiting the binding of transcription factors to the *cis*-element. Quinaldopeptin (**1**),[5] which was first isolated from the culture broth of *Streptoverticillium album* (actinomycetes strain Q132-6), constitutes one of the members of this class of natural products. Quinaldopeptin has a strong activity against melanoma B16 and Moser cells *in vitro* with IC_{50} values of 0.6 and 32 nM, respectively. Furthermore, *in vivo* antitumor activity against lymphocytic leukemia P388 in mice is promising, indicating that **1** can exhibit greater potency than the chemotherapeutic agent, mitomycin C. Therefore, **1** could be a potential candidate for therapeutic use in anticancer chemotherapies. Although DNA binding properties as well as the structure−activity relationship has been extensively elucidated for sandramycin, luzopeptines, and quinoxapeptines by Boger's group,[6,7a,b,8,9] those for **1** are completely unknown. In order to investigate the structure−activity relationship of **1**, it is necessary to establish its synthetic strategy first. Herein, we describe the first total synthesis of **1**.

RESULTS AND DISCUSSION

Quinaldopeptin consists of glycin (Gly), sarcosin (Sar), and two nonproteinogenic amino acids, L-pipecolic acid (L-Pip)[10] and (2*R*,3*R*)-2,3-diaminobutanoic acid (Dab) residues, and all of the amino acid components of **1** are linked with amide bonds, which is one of the major structural differences between **1** and other *C*2-symmetric cyclic decapeptide natural products. Total synthesis of sandramycin,[6] luzopeptines,[7] and quinoxapeptines[8] as well as its analogues[9] has been accomplished via a sequential peptide coupling approach in a solution phase. For the synthesis of **1**, we planned to apply solid-phase peptide synthesis (SPPS).[11] The strategy is applicable to parallel synthesis which would enable access to a range of analogues. A proposed retrosynthetic analysis of **1** is shown in Scheme 1. Our approach to the synthesis of **1** includes the SPPS of the linear decapeptide **4** followed by macrocyclization and the introduction of the quinoline chromophores **2** at a late stage. The macrocyclization site of **3** was planned at the less sterically hindered Gly residue (as the *N*-terminus) and L-Pip (as the *C*-terminus) that are derived from the linear decapeptide precursor **4**. A fragment coupling approach[11d−f] was applied for the synthesis of **4**, considering the *C*2 symmetrical structure of **1**. As a synthetic strategy, the pentapeptide **5** was assembled on a solid support. 2-Chlorotrityl (2CT)[12] was used as the solid support. A part of the resin-bound pentapeptide **5** was cleaved from the resin to give the pentapeptide carboxylic acid **7**, and the remainder was deprotected to give the amine **6**. A coupling reaction between **7** and the resin-bound amine **6** followed by cleavage from the resin would yield the linear decapeptide **4**. Generally, the SPPS requires an excess amount (4−5 equiv) of *N*-protected amino acid derivatives in coupling steps. In addition, it is difficult to recover the excess or unused amino acid, because the amino acid would convert to the active ester in the reaction mixture. This is a severe drawback especially in the case when the target peptide contains precious non-proteinogenic amino acids that would require a number of steps for the preparation. However, the unreacted amino acids from

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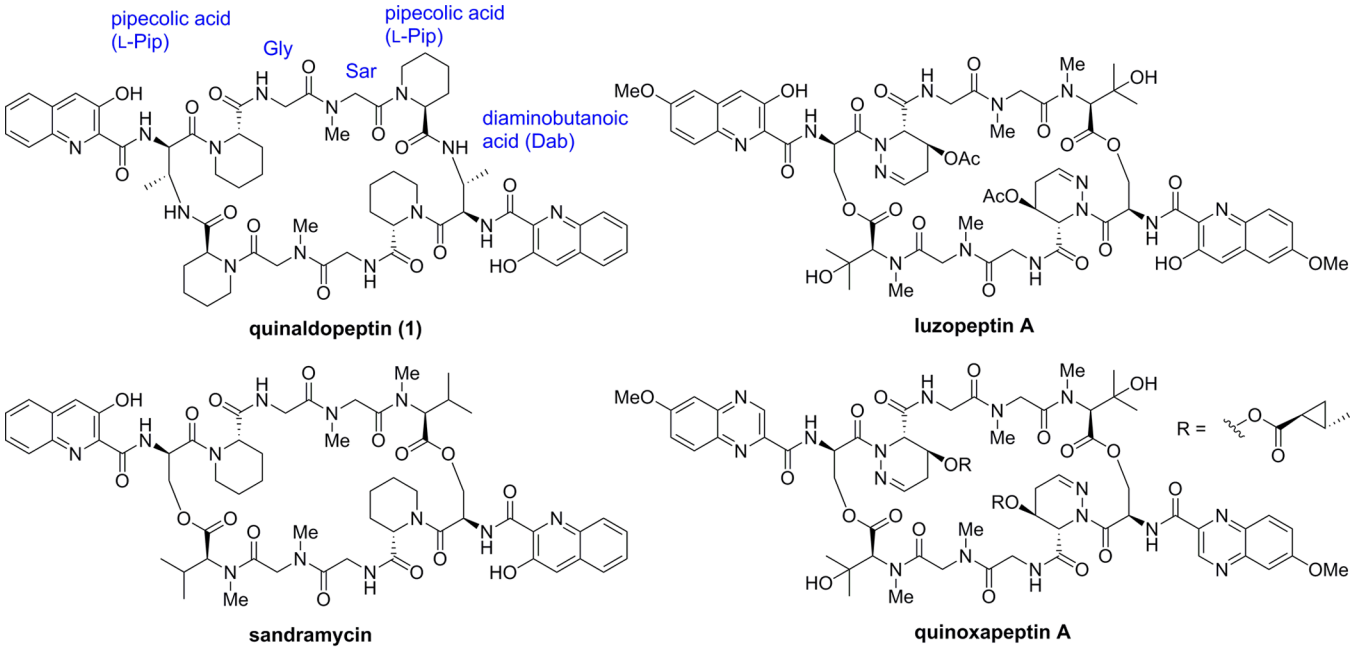
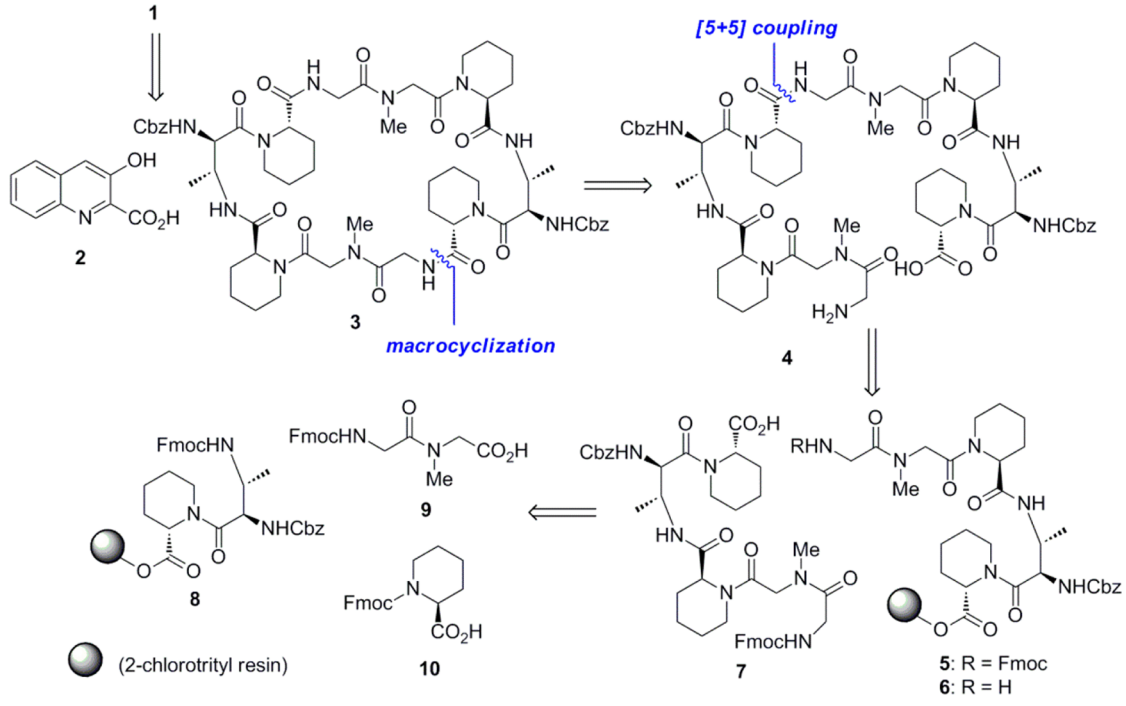


Figure 1. Structure of cyclic decapeptide bisintercalator natural products.

Scheme 1. Retrosynthetic Analysis of Quinaldopeptin (1)



the first step of the SPPS, which is the immobilization of the substrate to the 2CT chloride resin, could be recovered because the reaction is simply an alkylation of the carboxylate ion. Therefore, we strategically immobilize the dipeptide containing the Dab to yield **8**, followed by sequential coupling of Fmoc protected amino acids **9**[13] and **10**. Based on this strategy, we synthesized a suitably protected Dab residue **18** as the starting unit for the synthesis of **1** by the SPPS approach (Scheme 2). To synthesize **18**, *tert*-butyl crotonate (**11**) was converted to the known *α*-azido-*β*-aminobutanoate derivative **12**.[14] The azide group of **12** was reduced by a Staudinger reaction, and the liberated amine was protected with a Boc group to give **13**. After the exchange of the Cbz group of **13** to the Fmoc, deprotection of both the Boc and *tert*-butyl group of **14** with 80% *aq.* TFA followed by the protection of the *α*-amino group by the Cbz group yielded the suitably protected *α*,*β*-diaminobutylic acid **15**. Compound **15** was then coupled with the allyl ester of L-Pip **16**[15] by *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and *i*-Pr_2NEt to afford the dipeptide **17** in 73% yield. Deprotection of the allyl group afforded the desired starting unit dipeptide **18**. With the dipeptide **18** in hand, the total synthesis of **1** was

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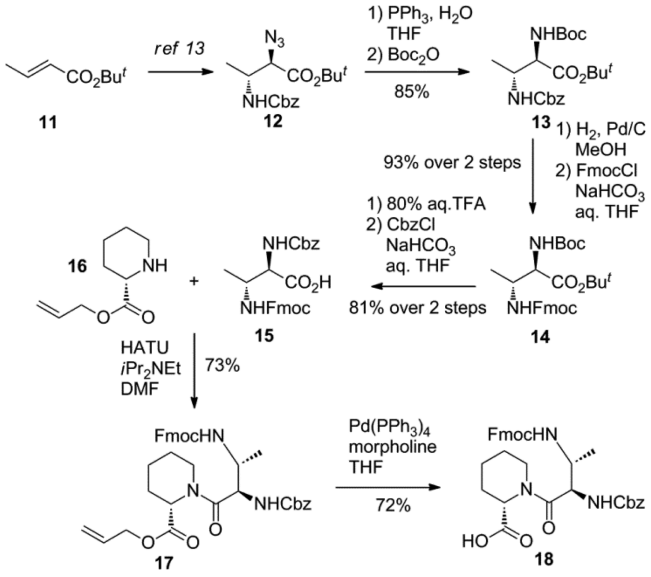
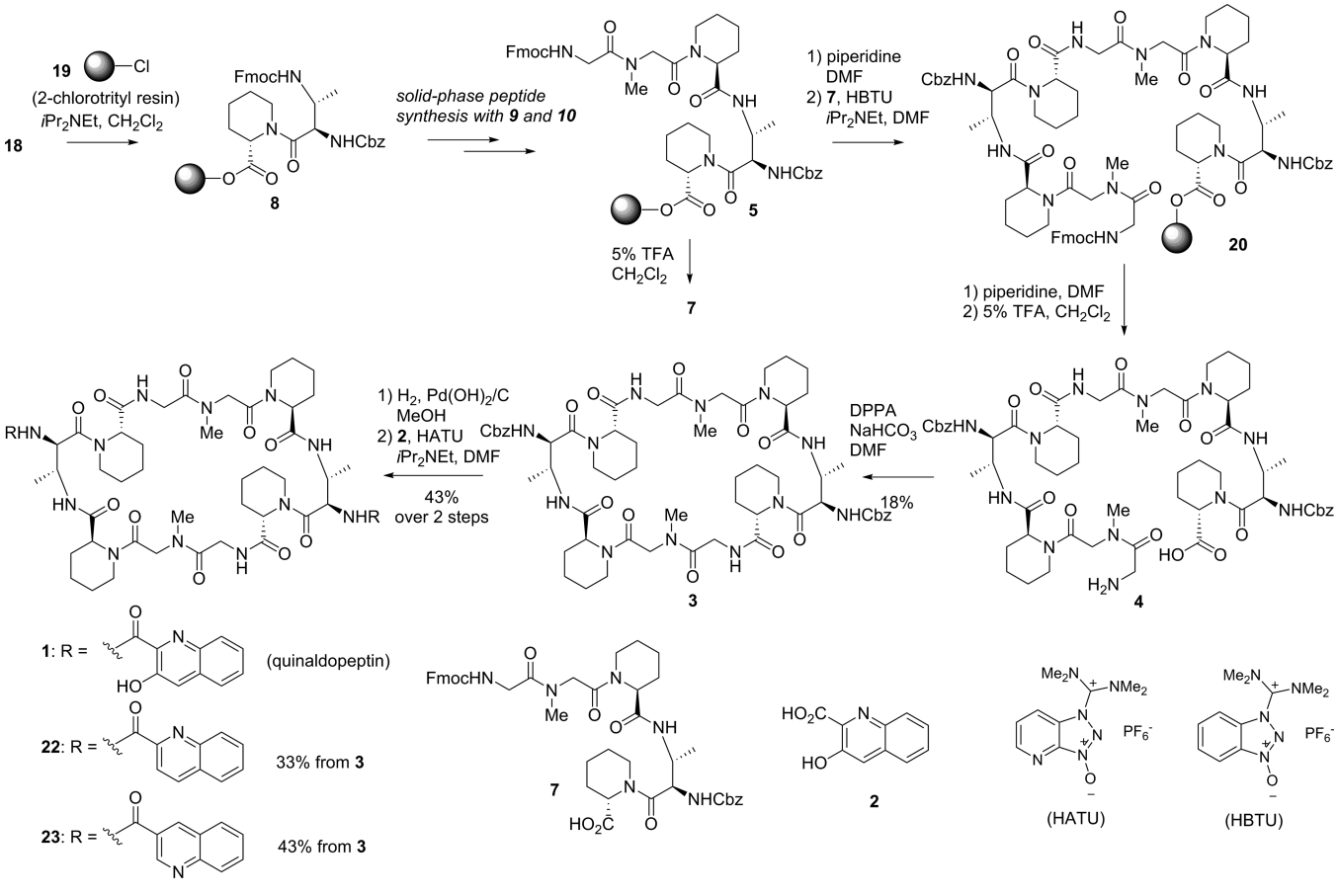
Scheme 2. Preparation of Dipeptide 18



pursued (Scheme 3). Compound **18** was immobilized to the 2CT chloride resin (**19**, 1.57 mmol/g resin). Elongation of the L-Pip and Gly-Sar residues was achieved by a conventional Fmoc-SPPS with **9** and **10** to give solid-supported pentapeptide **5**. Five-sevenths of **5** was treated with 5% TFA/CH_2Cl_2 to give the crude carboxylic acid **7**. The Fmoc group of the remaining two-sevenths of **5** was deprotected to give the amine **6**, which was coupled with **7** by HBTU to yield the decapeptide on the resin **20**. Deprotection of the Fmoc group by piperidine and the release from the resin by 5% TFA/CH_2Cl_2 afforded the crude decapeptide **4**, a precursor to the cyclization. The cyclization of **4** was conducted with 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxy-7-azabenzotriazole (HOAt), and $NaHCO_3$ in CH_2Cl_2 (5 mM) at rt. However, only a trace amount of the desired cyclic peptide **3** was obtained even after a prolonged reaction time (a week). Despite extensive efforts to obtain **3** with a variety of conditions using various coupling reagents, additives, solvents, or temperature, it was difficult to increase the reaction rate. A prolonged reaction time caused a severe racemization at the *C*-terminal Pip residue. The conditions using diphenylphosphoryl azide (DPPA)[16] and $NaHCO_3$ in DMF (5 mM) for 6 days gave the desired cyclic peptide **3** (18%) as well as its epimer (ca. 27%). The stereogenic center at the Pip residue of **3** was confirmed by conventional amino acid analysis[17] as shown in Scheme 4. For the analysis, **3** was heated under reflux in 6 M *aq*. HCl for 24 h, and the resulting mixture was treated with Marfey's reagent. The reaction mixture was analyzed by reversed phase HPLC (ODS, 10−60% MeCN−H_2O linear gradient containing 0.1% TFA) and compared with a reference sample **21** derived from L- or D-Pip[18] (Figure S1 in Supporting Information). The arylamine **21** obtained from the hydrolysis products of **3** was identical to the reference L-**21**, whereas the D-**21** analogue was not detected. This analysis unambiguously determined the stereochemistry at the stereogenic center of four L-Pip residues. Finally, the Cbz groups of **3** were removed,

Scheme 3. Total Synthesis of Quinaldopeptin (1) and Its Analogues



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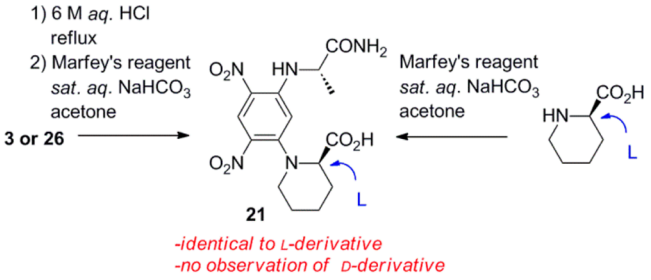
Scheme 4. Amino Acid Analysis of 3 and 26



and quinaldic acid derivative **2**[19] was attached to the liberated amines to successfully afford the quinaldopeptin (**1**) in 43% over 2 steps. It was reported that natural **1** did not give clear signals in ^{1}H and ^{13}C NMR spectra.[5] Those of synthetic **1** as well as **3** also exhibited complex and broadened NMR spectra despite our efforts to obtain clear spectra by changing the solvents and temperature. However importantly, the ^{1}H NMR spectrum of synthetic **1** as well as optical rotation [synthetic $[\alpha]_D^{22}$ −120.8 (*c* 0.27, MeOH); natural $[\alpha]_D^{20}$ −129.3 (*c* 0.19, MeOH)] and HPLC analysis (SiO_2, MeOH/$CHCl_3$ 0−10% linear gradient, 14.34 min for natural and synthetic **1**, respectively) as well as coinjection in HPLC of synthetic material with natural **1** proved that synthetic **1** was identical to natural **1** (Figures S2 and S3 in Supporting Information). Similar to the synthesis of **1**, two chromophore analogues **22** and **23** were also prepared. In contrast to **1** and **22**, **23** gave clear signals in ^{1}H and ^{13}C NMR spectra, which indicate that it adopts a single and rigid solution conformation. The *C*2-symmetrical cyclic structure was confirmed by the fact that only a half of the peaks could be observed in both ^{1}H and ^{13}C NMR spectra. In conjunction with amino acid and HPLC analyses as well as the biological data (described later), the structure of **1** and that of the key intermediate **3** also were unambiguously confirmed as shown in Scheme 3.

As a preliminary study, the synthesis of desmethyl analogue **27** was planned using a readily available diaminopropanoic acid derivative **25** by following a strategy similar to that for the synthesis of **1** in order to see the impact of the methyl groups at the Dab residues on the biological activity. As for the cyclization step to prepare **26**, the conditions using EDCI, HOBt, and $NaHCO_3$ and CH_2Cl_2 (5 mM) gave a better result (30%) than in the case of the synthesis of **3**. However, the corresponding epimer was also obtained (ca. 21%) at the L-Pip residue. The ^{1}H NMR spectra of **27** also exhibited more complex and broadened NMR spectra than those of **1**, and efforts to obtain clear spectra were unsuccessful similar to the case of **1** and **3**. Due to the presence of multiple conformers, we were unable to obtain the ^{13}C NMR spectrum of the compound, and an attempt to obtain the spectrum with a concentrated solution only caused the precipitation of the excess compound. It was confirmed by the amino acid analysis that **26** contained only L-Pip residues and no epimers were contaminated (Scheme 4, Figure S1g). This indicated that **27** existed as a mixture of multiple conformers including asymmetric ones in the solution.

The cytotoxicity of **1**, **22**, **23**, and **27** was tested against a range of human cancer cell lines (Table 1). Synthetic **1** exhibited strong cytotoxicity with IC_{50} values ranging from 3.2 to 12 nM. The observed strong cytotoxicity also confirmed the accomplishment of the total synthesis of **1**. 2-Quinoline derivative **22**, which is an analogue of **1** with no hydroxyl

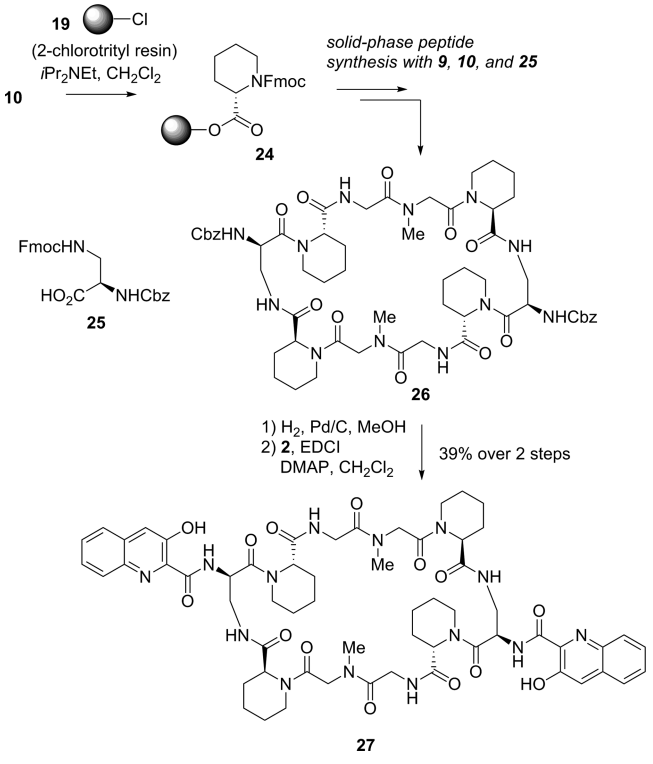
Table 1. Cytotoxic Activity of 1 and Its Analogues against Human Cancer Cell Lines[a]

	IC_{50} (nM)					
	HCT-116	RPMI8226	A431	RKO	SU-DHL6	SU-DHL10
1	3.2	11	12	5	11	12
22	41	19	42	42	51	91
23	8400	>10 000	>10 000	9683	>10 000	>10 000
27	279	171	457	264	213	307

[a]HCT-116 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.

group at the chromophore moiety, retained cytotoxicity although it reduced the activity by a factor of 1.7−13. On the other hand, 3-quinoline analogue **23**, which is an isomer of **22** at the chromophore moiety, almost lost the cytotoxicity. The solution conformation of **23** was single and rigid in CD_3OD and was clearly different from that of **1** and **22**. Presumably the solution conformation of **23** would be disfavored in DNA binding to nearly result in loss of function. The activity of **27** was also reduced by approximately 2 orders of magnitude (171−457 nM). Although data are not shown, the analogues of **27**, where two L-Pip residues on either the *N*- or *C*-terminal of the diaminopropanoic acid residues or all the L-Pip residues were replaced by L-Pro residues, were also synthesized as in Scheme 5. The cytotoxicity of these analogues were largely reduced (IC_{50} 2000−20 000 nM). Truncation of the methyl groups or ring contraction caused a conformational change of the macrocycle, which would not be preferred in DNA binding. The DNA binding property and structural analysis of **1** will be reported elsewhere soon.

Scheme 5. Synthesis of Desmethyl Analogue 27



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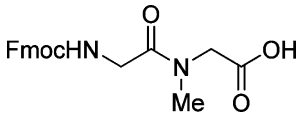
CONCLUSION

The first total synthesis of quinaldopeptin (**1**) as well as its analogues was accomplished employing the SPPS approach. Synthetic **1** exhibited strong cytotoxicity against a range of human cancer cell lines. On the other hand, the activity of **23** and **27** was largely reduced. Further studies on its structure−activity relationship will be pursued and discussed soon.

EXPERIMENTAL SECTION

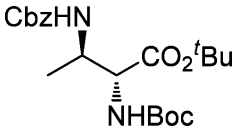
General Experimental Methods. ^{1}H and ^{13}C NMR chemical shifts were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as an internal standard unless otherwise noted. Coupling constants (J) were reported in herz (Hz). Abbreviations of multiplicity were as follows; s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad. Data were presented as follows: chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^{1}H−^{1}H COSY, HMBC, and HMQC NMR spectra. The mass analyzer type used for the HRMS measurements was TOF. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F254 plates. Normal-phase column chromatography was performed on Merck silica gel 5715 or Kanto Chemical silica gel 60N (neutral). Flash column chromatography was performed on Merck silica gel 60. All reactions except that carried out in aqueous phase were carried out under an argon atmosphere, unless otherwise noted. Isolated yields were calculated by weighing products. The weight of the starting materials and the products were not calibrated.[20]

6-[(9-Fluorenylmethoxycarbonyl)amino]glycylsarcocine (9). A solution of glycylsarcosine (2.48 g, 17.0 mmol) and $NaHCO_3$ (3.14



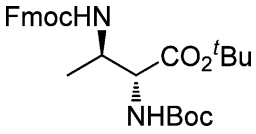
g, 37.4 mmol) in dioxane/H_2O (125/125 mL) was treated with FmocCl (4.84 g, 18.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The mixture was diluted with AcOEt, and the organic phase was washed with 1 M *aq.* HCl, and saturated *aq.* NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was triturated from hexane to afford **9** (5.87 g, 94%) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz, 2:1 mixture of rotamers, data for the major rotamer) δ 7.25 (m, 9H, fluorenyl and N*H*), 4.27−4.20 (m, 3H, OCH_2-fluorenyl and *H*-fluorenyl), 3.98 (s, 2H, Gly-α-CH_2), 3.89 (d, 2H, Sar-α-CH_2, J = 6.3 Hz), 2.98 (s, 3H, NCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz, 2:1 mixture of rotamers, data for the major rotamer) δ 170.7, 169.2, 156.5, 143.9, 140.7, 127.7, 127.1, 125.3, 120.1, 99.5, 65.7, 49.2, 46.6, 34.9; ESIMS-LR m/z 391 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{20}H_{20}N_2NaO_5$ 391.1270, found 391.1261.

(2*R*,3*R*)-*tert*-Butyl 3-[(benzyloxycarbonyl)amino]-2-[(*tert*-butoxycarbonyl)amino] Butanoate (13). A solution of **12**[13]



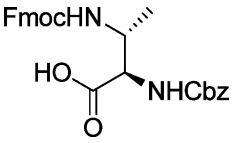
(2.49 g, 7.47 mmol) in THF/H_2O (50/20 mL) was treated with PPh_3 (5.88 g, 22.4 mmol) at room temperature, and the mixture was stirred at 40 °C for 2 h. The resulting mixture was treated with $(Boc)_2O$ (1.81 mL, 7.84 mmol) and stirred at room temperature for 24 h. The mixture was diluted with AcOEt, which was washed with H_2O and saturated *aq.* NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, 14% AcOEt−hexane) to afford **13** (2.42 g, 80%) as a colorless oil. $[\alpha]^{23}_D$ −1.5 (c 0.59, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.36−7.25 (m, 6H, aromatic and N*H*), 7.05 (d, 1H, N*H*, J = 10.9 Hz), 4.98 (q, 2H, CH_2Ph, J = 8.6, 16.0 Hz), 4.00 (t, 1H, α-C*H*, J = 9.7 Hz), 3.88 (m, 1H, β-C*H*), 1.36 (s × 2, 18H, ^{t}Bu × 2), 1.01 (d, 3H, γ-CH_3, J = 8.6 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.0, 155.6, 155.3, 137.1, 128.3, 127.8, 127.7, 80.5, 78.3, 65.2, 58.3, 47.3, 28.2, 27.6, 16.8; ESIMS-LR m/z 431 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{21}H_{32}N_2NaO_6$ 431.2153, found 431.2154.

(2*R*,3*R*)-*tert*-Butyl 3-[(fluorenylmethoxycarbonyl)amino]-2-[(*tert*-butoxycarbonyl)amino] Butanoate (14). A mixture of **13**



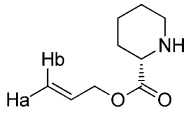
(2.42 g, 5.92 mmol) and 10% Pd/C (242 mg) in MeOH (30 mL) was vigorously stirred under a hydrogen atmosphere at room temperature for 30 min. The insoluble portion was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to give a crude amine (1.62 g), which was used in the next reaction without further purification. To a stirred solution of the resulting amine in THF/H_2O (30/10 mL) was added $NaHCO_3$ (1.09 g, 13.0 mmol) and FmocCl (1.68 g, 6.51 mmol). The mixture was stirred at room temperature for 1 h. The reaction mixture was poured into H_2O (200 mL) and extracted with AcOEt. The combined organic phase was washed with H_2O and saturated *aq.* NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, 29% AcOEt−hexane) to afford **14** (2.73 g, 93% over 2 steps) as a white foam. $[\alpha]^{23}_D$ −15.9 (c 0.89, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.89−7.29 (m, 9H, fluorenyl and N*H*), 7.06 (d, 1H, N*H*, J = 10.3 Hz), 4.29−4.17 (m, 3H, OCH_2-fluorenyl and *H*-fluorenyl), 3.99 (t, 1H, α-C*H*, J = 9.8 Hz), 3.88 (m, 1H, β-C*H*), 1.35 (s × 2, 18H, ^{t}Bu × 2), 1.02 (d, 3H, γ-CH_3, J = 8.6 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.0, 155.7, 155.3, 143.8, 140.7, 127.6, 127.1, 125.2, 120.1, 80.5, 78.3, 65.5, 58.4, 47.2, 46.7, 28.2, 27.6, 16.7; ESIMS-LR m/z 418 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{28}H_{36}N_2NaO_6$ 519.2466, found 519.2466.

(2*R*,3*R*)-3-[(9-Fluorenylmethoxycarbonyl)amino]-2-[(benzyloxycarbonyl)amino] Butanoic Acid (15). Compound **14** (2.73 g,



5.51 mmol) was treated with 80% *aq.* TFA (40 mL), and the resulting mixture was stirred at room temperature for 4 h. The mixture was concentrated *in vacuo*. A mixture of the residue and $NaHCO_3$ (2.31 g, 27.5 mmol) in THF/H_2O (40/10 mL) was treated with benzyl chloroformate (1.58 mL, 11.2 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 12 h. The mixture was diluted with AcOEt, and the organic phase was washed with H_2O and saturated *aq.* NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, 5% MeOH−$CHCl_3$) to afford **15** (2.12 g, 81% over 2 steps) as a white solid. $[\alpha]^{21}_D$ −7.9 (c 0.91, DMSO); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.86−7.26 (m, 13H, aromatic), 7.19 (s, 1H, N*H*), 6.85 (s, 1H, N*H*), 4.98 (q, 2H, CH_2Ph, J = 12.1, 13.2 Hz), 4.28−3.99 (m, 5H, OCH_2-fluorenyl, *H*-fluorenyl, α-C*H* and β-C*H*), 0.96 (d, 3H, γ-CH_3, J = 6.9 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 156.4, 155.3, 144.1, 143.8, 140.7, 137.0, 128.4, 127.8, 127.7, 127.6, 127.1, 127.0, 125.5, 125.3, 120.1, 65.5, 58.7, 48.6, 46.7, 15.9; ESIMS-LR m/z 497 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{27}H_{26}N_2NaO_6$ 497.1689, found 497.1678.

Allyl L-Pipecolate Hydrochloride (16). A solution of Boc-L-Pip-OH (2.29 g, 10.0 mmol) and $CsCO_3$ (3.58 g, 11.0 mmol) in DMF (50



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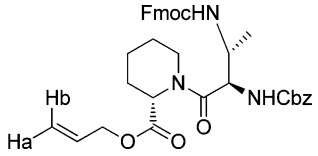
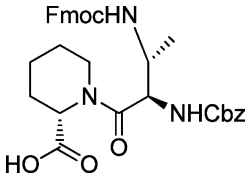
mL) was treated with allyl bromide (930 μL, 11.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The mixture was diluted with AcOEt, and the organic phase was washed with H_2O (5 times) and saturated *aq.* NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The compound was used in the next reaction without further purification. The Boc-L-Pip-OAll was treated with 4 M HCl in AcOEt for 30 min. The resulting solution was concentrated *in vacuo* to afford **16** (2.01 g, 98% over 2 steps) as a white solid. $[\alpha]^{21}_D$ −12.1 (*c* 1.06, MeOH); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 5.90 (m, 1H, CH_2=C*H*CH_2O), 5.37 (dd, 1H, H_a, J_{gem} = 1.7 Hz, J_{cis} = 17.2 Hz), 5.23 (dd, 1H, H_b, J_{gem} = 1.7 Hz, J_{trans} = 10.9 Hz), 4.66 (m, 2H, CH_2=CHCH_2O), 4.09 (d, 1H, α-C*H*, *J* = 8.0 Hz), 3.18 (d, 1H, ε-CH_{ex}, *J* = 12.6 Hz), 2.87 (m, 1H, ε-CH_{ax}), 2.05 (d, 1H, β-CH_{ex}, *J* = 10.3 Hz), 1.19 (m, 4H, γ-CH_2 and δ-CH_2), 1.56 (m, 1H, β-CH_{ax}); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 168.4, 131.8, 118.4, 65.8, 55.4, 43.3, 25.6, 21.2, 21.0; ESIMS-LR *m*/*z* 170 [(M + H)$^+$]; ESIMS-HR calcd for $C_9H_{16}NO_2$ 170.1181, found 170.1176.

Fmoc-Dab(Cbz)-L-Pip-OAll (17). A solution of **15** (20.0 mg, 0.042 mmol) and ^{i}Pr$_2$NEt (44 μL, 0.25 mmol) in DMF (1 mL) was



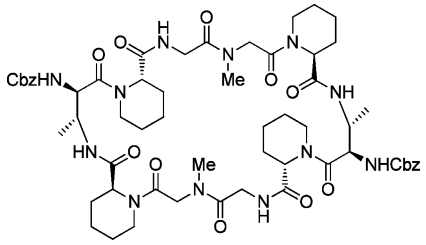
treated with HATU (32 mg, 0.084 mmol) at 0 °C, and the mixture was stirred at the same temperature for 30 min. The amine **16** (13 mg, 0.063 mmol) was then added to the mixture, which was stirred at room temperature for 2 h. The mixture was diluted with AcOEt, which was washed with 1 M *aq.* HCl, saturated *aq.* $NaHCO_3$, and saturated *aq.* NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, 20% AcOEt−hexane) to afford **17** (19.1 mg, 73%) as a white foam. $[\alpha]^{21}_D$ −40.7 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 500 MHz, a mixture of rotamers, data for the major rotamer) δ 7.77−7.15 (m, 13H, fluorenyl and Ph), 5.99 (m, 1H, CH_2=C*H*CH_2O), 5.28 (dd, 1H, H_a, J_{gem} = 1.7 Hz, J_{cis} = 17.2 Hz), 5.18 (dd, 1H, H_b, J_{gem} = 1.7 Hz, J_{trans} = 10.9 Hz), 5.16 (br s, 1H, Pip-α-C*H*), 5.05 (s, 2H, CH_2Ph), 4.59 (m, 2H, CH_2=CHCH_2O), 4.45 (dd, 1H, OC*H*-fluorenyl, *J* = 6.3, 10.3 Hz), 4.33 (m, 1H, Pip-ε-C*H*), 4.21 (m, 2H, OC*H*-fluorenyl, Pip-ε-C*H*), 3.89 (m, 1H, Pip-β-C*H*), 2.24 (d, 1H, C*H*-fluorenyl, *J* = 13.1 Hz), 1.74−1.27 (m, 6H, Pip-(CH_2)$_3$), 1.09 (d, 3H, Dab-γ-CH_3, *J* = 6.9 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 172.2, 171.7, 158.4, 158.2, 145.7, 145.1, 142.6, 138.0, 133.2, 129.4, 130.0, 128.9, 128.7, 128.1, 128.0, 126.5, 126.2, 121.0, 118.6, 67.8, 66.8, 55.0, 54.1, 50.0, 44.8, 27.7, 26.4, 21.8, 14.7; ESIMS-LR *m*/*z* 648 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{36}H_{39}N_3NaO_7$ 648.2686, found 648.2676.

Fmoc-Dab(Cbz)-L-Pip-OH (18). A solution of **17** (100 mg, 0.16 mmol) and morpholine (41.5 μL, 0.48 mmol) in THF (2 mL) was



treated with $Pd(PPh_3)_4$ (18.5 mg, 0.016 mmol) at room temperature for 1 h. The mixture was diluted with AcOEt, which was washed with 1 M *aq.* HCl and saturated *aq.* NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2 and SH silica, 50% AcOEt−hexane) to afford **9** (67 mg, 72%) as a yellow foam. $[\alpha]^{21}_D$ −37.1 (*c* 0.78, MeOH); ^{1}H NMR (CD_3OD, 500 MHz, a mixture of rotamers, data for the major rotamer) δ 7.77−7.15 (m, 13H, fluorenyl and Ph), 5.16 (br s, 1H, Pip-α-C*H*), 5.05 (s, 2H, CH_2Ph), 4.84 (m, 1H, Dab-α-C*H*), 4.47 (dd, 1H, OC*H*-fluorenyl, *J* = 6.3, 10.3 Hz), 4.33 (m, 1H, Pip-ε-C*H*), 4.21 (m, 2H, OC*H*-fluorenyl, Pip-ε-C*H*), 3.88 (m, 1H, Pip-β-C*H*), 2.27 (d, 1H, C*H*-fluorenyl, *J* = 13.3 Hz), 1.77−1.28 (m, 6H, Pip-(CH_2)$_3$), 1.09 (d, 3H, Dab-γ-CH_3, *J* = 6.9 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 173.8, 171.9, 158.4, 158.3, 145.7, 145.1, 142.6, 142.5, 138.0, 129.4, 129.0, 128.9, 128.8, 128.7, 128.1, 128.0, 126.5, 126.2, 120.9, 67.9, 67.8, 55.0, 53.9, 50.1, 44.7, 27.9, 26.6, 22.0, 14.6; ESIMS-LR *m*/*z* 608 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{33}H_{35}N_3NaO_7$ 608.2373, found 608.2365.

Cyclo[Gly-Sar-L-Pip-Dab(Cbz)-L-Pip]$_2$ (3). Solid-phase peptide syntheses were performed in polypropylene syringes (10 mL) fitted



with polyethylene porous discs. Solvents and soluble reagents were removed by suction. The Fmoc group was removed with piperidine/DMF (1:4, 3 min, then 1:8, 12 min). Washings between deprotection, couplings, and final deprotection steps were performed with DMF (7 mL × 3) and CH_2Cl_2 (7 mL × 3). Peptide synthesis transformations and washes were performed at 25 °C. The 2CT chloride resin (**19**, 500 mg, 1.57 mmol/g) was placed in a 10 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was then washed with CH_2Cl_2 (7 mL × 3 min), and a solution of Fmoc-Dab(Cbz)-L-Pip-OH (**18**, 552 mg, 0.94 mmol) and ^{i}Pr$_2$NEt (575 μL, 4.8 equiv) in CH_2Cl_2 (5 mL) was then added. The mixture was stirred at 25 °C for 1 h. The Fmoc-Dab(Cbz)-L-Pip-O-2CT resin **8** was subjected to the following washings/treatments: filtration, CH_2Cl_2−MeOH−^{i}Pr$_2$NEt (17:2:1, 7 mL × 3), DMF (7 mL × 3), and CH_2Cl_2 (7 mL × 3). The resin **8** was dried over P_2O_5 overnight. The loading, calculated by measuring absorbance at 290 nm, was 0.834 mmol/g.

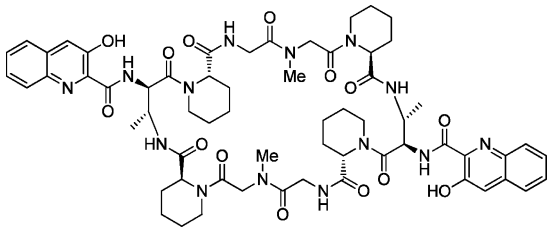
The elongation of the peptide was achieved by sequential addition of the corresponding Fmoc-protected amino acid **9** and **10** with either HBTU (for the primary amine, 775 mg, 4.9 equiv) or HATU (for the secondary amine, 777 mg, 4.9 equiv) and ^{i}Pr$_2$NEt (436 μL, 6.0 equiv) as coupling reagents in DMF (3 mL) in preactivation mode. The mixture was stirred for 1 h, and after filtration the corresponding colorimetric test (Kaiser test for the primary amine and chloranil test for the secondary amine) indicated the completion of the coupling. Next, the peptide resin was washed with DMF (7 mL × 3) and CH_2Cl_2 (7 mL × 3) and treated with a solution of piperidine/DMF (1:4, 3 min, then 1:8, 12 min) to remove the Fmoc group, and the coupling plus Fmoc removal was then measured. The peptide resin was split into fractions (5/7 and 2/7). The former was cleaved from the resin with 5% TFA in CH_2Cl_2 solution (5 mL × 3), and the combined filtrates were concentrated *in vacuo*. The residue was precipitated in ether to afford the crude pentapeptide **7** (867 mg) as a pale yellow solid (ESIMS-LR (negative mode) *m*/*z* 823 [(M − H)$^-$]; ESIMS-HR (negative mode) calcd for $C_{44}H_{51}N_6O_{10}$ 823.3672, found 823.3685).

The remaining 2/7 fraction of **5** was treated with a piperidine/DMF (1:4, 3 min, 1:8, 12 min) solution as described above to give the free amine **6**. The pentapeptide **7** was added to the free amine peptide resin fraction, with HBTU (193 mg, 2.8 equiv) and ^{i}Pr$_2$NEt (554 μL, 6.0 equiv) as coupling reagents in DMF (3 mL) in preactivation mode. The mixture was stirred at room temperature for 2 h. Then, the peptide resin was washed with DMF (7 mL × 3) and CH_2Cl_2 (7 mL × 3) and treated with a solution of piperidine/DMF (1:4, 3 min, then 1:8, 12 min). The resin **20** was treated with 5% TFA in CH_2Cl_2 solution (5 mL × 3), and the combined filtrates were concentrated *in vacuo* to afford the crude decapeptide **4** (582 mg) as a pale yellow solid (ESIMS-LR *m*/*z* 1187 [(M + H)$^+$]; ESIMS-HR calcd for $C_{58}H_{83}N_{12}O_{15}$ 1187.6101, found 1187.6101).

A sample of linear decapeptide **4** (20 mg, 0.017 mmol) and $NaHCO_3$ (5.6 mg, 0.067 mmol) in DMF (3.4 mL) was treated with DPPA (15 μL, 0.067 mmol), and the reaction mixture was stirred at

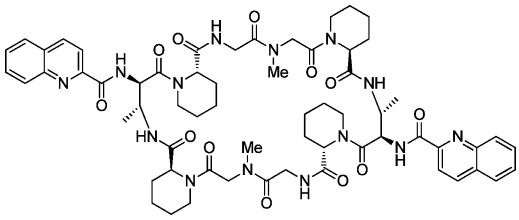
room temperature for 6 days. The reaction mixture was poured onto 1 M *aq*. HCl (ca. 20 mL) and extracted with AcOEt. The combined organic phases were washed with saturated *aq*. $NaHCO_3$ and saturated *aq*. NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (SiO_2, 1−2% MeOH−$CHCl_3$) to afford **3** (3.6 mg, 18%) as a colorless glass. Data for **3**: $[\alpha]^{20}_D$ −37.0 (*c* 0.31, MeOH); ^{1}H NMR (CD_3OD, 500 MHz, selected peaks are listed because of multiple conformers) δ 7.45−7.21 (m, 10H), 5.35−4.90 (m, 6H), 4.84−3.99 (m, 6H), 3.82−3.35 (m, 4H), 3.19−3.03 (m, 6H), 2.94−2.89 (m, 4H), 2.26−2.10 (m, 2H), 1.62−1.23 (m, 20H), 1.30−1.11 (m, 6H); ^{13}C NMR (CD_3OD, 125 MHz, selected peaks are listed because of multiple conformers) δ 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)$^+$ 1191.5815, found 1191.5798.

Quinaldopeptin (1). A mixture of **3** (5.8 mg, 5.0 μmol) in MeOH (1 mL) and 10% $Pd(OH)_2$/C (1 mg) was vigorously stirred under a



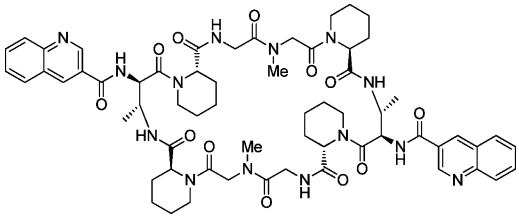
H_2 atmosphere at room temperature for 1 h. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to give the diamine. The residue in DMF (1 mL) was treated with HATU (7.6 mg, 20 μmol), ^{i}Pr$_2$NEt (3.5 μL, 20 μmol), and chromophore **2** (3.8 mg, 20 μmol) 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−$CHCl_3$) to afford quinaldopeptin (**1**, 2.7 mg, 43%) as a yellow solid. Mp >300 °C (lit. >300 °C); $[\alpha]^{22}_D$ −120.8 (*c* 0.27, MeOH); natural $[\alpha]^{20}_D$ −129.3 (*c* 0.19, MeOH); ^{1}H NMR (CD_3OD, 500 MHz, selected peaks are listed because of multiple conformers) δ 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, 2H), 2.96 (s, 3H), 2.89−2.69 (m, 4H), 2.30 (br d, 1H, *J* = 12.1 Hz), 2.20 (br d, 1H, *J* = 13.7 Hz), 1.87−1.37 (m, 16H), 1.21 (d, 3H, *J* = 7.5 Hz); ^{13}C NMR (CD_3OD, 125 MHz, selected peaks are listed because of multiple conformers) δ 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 $C_{62}H_{78}N_{14}NaO_{14}$ (M + Na)$^+$ 1265.5720, found 1265.5728.

2-Quinoline Analogue (22). In a manner similar to the synthesis of **1**, **22** (colorless glass, 9.2 mg, 33%) was prepared from **3** using 2-quinolinecarboxylic acid (15.9 mg, 0.092 mmol), HOBt (18.6 mg, 0.14 mmol), and EDCI (17.6 mg, 0.092 mmol) after purification by silica gel column chromatography (SiO_2, 1% MeOH−$CHCl_3$). $[\alpha]^{21}_D$ −116.3 (*c* 0.67, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz, a mixture of rotamers, data for the major rotamer) δ 8.23 (d, 2H, *J* = 8.0 Hz), 8.08 (d, 2H, *J* = 8.6 Hz), 8.00 (d, 2H, *J* = 8.6 Hz), 7.83 (d, 2H, *J* = 7.5 Hz), 7.56 (t, 2H, *J* = 6.9 Hz), 7.60 (t, 2H, *J* = 6.9 Hz), 5.72 (br s, 2H, Dab-α-C*H*), 5.30 (d, 2H, Pip-α-CH_{ex}, *J* = 5.2 Hz), 4.62 (m, 2H, Pip-α-C*H*), 4.50−3.97 (m, 12H, Dab-β-C*H*, Gly-α-CH_2, Pip-ε-C*H*, Sar-α-



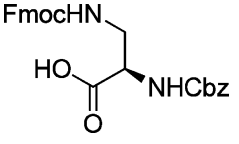
CH_2), 3.22−2.80 (m, 4H, Pip-ε-CH_2), 2.32 (d, 2H, Pip-β-CH_2, *J* = 12.6 Hz), 2.23 (d, 2H, Pip-β-CH_2, *J* = 12.6 Hz), 1.90−1.22 (m, 16H, Pip-γ-CH_2, Pip-δ-CH_2, Pip-γ-CH_2 and Pip-δ-CH_2), 1.17 (d, 6H, Dab-CH_3, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.7, 172.6, 172.3, 172.0, 171.3, 166.4, 150.7, 147.8, 138.6, 131.4, 130.8, 130.6, 129.2, 128.9, 119.6, 79.5, 57.2, 54.6, 52.7, 52.1, 45.1, 42.2, 41.8, 36.2, 27.9, 26.9, 26.3, 25.3, 21.7, 21.6, 13.2; ESIMS-LR *m*/*z* 1233 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{62}H_{78}N_{14}NaO_{12}$ 1233.5816, found 1233.5810.

3-Quinoline Analogue (23). In a manner similar to the synthesis of **1**, **23** (colorless glass, 11.9 mg, 43%) was prepared from **3** using 3-



quinolinecarboxylic acid (15.9 mg, 0.092 mmol), HOBt (18.6 mg, 0.14 mmol), and EDCI (17.6 mg, 0.092 mmol) after purification by silica gel column chromatography (SiO_2, 1% MeOH−$CHCl_3$). $[\alpha]^{20}_D$ −135.7 (*c* 0.32, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 9.33 (d, 2H, N*H*, *J* = 9.7 Hz), 8.34 (d, 2H, *J* = 1.7 Hz), 8.22 (d, 2H, *J* = 8.0 Hz), 8.06 (s, 2H), 7.99 (d, 2H, *J* = 8.6 Hz), 7.88 (dt, 2H, *J* = 1.7, 8.6 Hz), 7.68 (t, 2H, *J* = 8.0 Hz), 7.21 (d, 2H, Dab-N*H*-Pip, *J* = 8.0 Hz), 5.70 (m, 2H, Dab-α-C*H*), 5.13 (d, 2H, Pip-α-CH_{ex}, *J* = 4.6 Hz), 4.68 (dd, 2H, Pip-α-C*H*), 4.53 (m, 2H, Dab-β-C*H*), 4.37 (d, 2H, Gly-α-CH_2, *J* = 17.2 Hz), 3.91 (t, 2H, Pip-ε-C*H*), 3.74 (d, 2H, Gly-α-CH_2, *J* = 17.2 Hz), 3.53 (d, 2H, Sar-α-CH_2, *J* = 15.5 Hz), 3.07 (d, 2H, Sar-α-CH_2, *J* = 15.5 Hz), 3.01 (d, 2H, Pip-ε-CH_2), 2.71 (t, 2H, Pip-ε-CH_2), 2.37 (d, 2H, Pip-β-CH_2), 2.17 (d, 2H, Pip-β-CH_2), 1.97 (m, 2H, Pip-γ-CH_2), 1.81 (m, 2H, Pip-δ-CH_2), 1.55−1.15 (m, 12H, Pip-γ-CH_2 and Pip-δ-CH_2), 1.49 (d, 6H, Dab-CH_3, *J* = 6.9 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.7, 173.4, 171.0, 170.8, 170.5, 166.2, 149.8, 149.4, 137.8, 133.0, 131.4, 128.7, 128.6, 128.1, 126.0, 79.5, 56.8, 54.7, 53.5, 51.6, 45.2, 43.9, 41.4, 37.1, 28.3, 26.2, 25.8, 25.5, 21.2, 20.7, 15.1; ESIMS-LR *m*/*z* 1233 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{62}H_{78}N_{14}NaO_{12}$ 1233.5816, found 1233.5816.

(*R*)-3-(9-Fluorenylmethoxycarbonylamino)-2-(benzyloxycarbonylamino)propanoic Acid (25). A solution of (2*R*)-2-*N*-

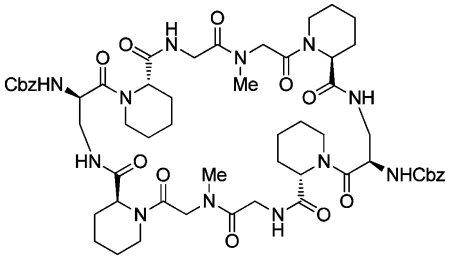


benzyloxycarbonyl-2,3-diaminopropanoic acid (1.00 g, 4.20 mmol) and $NaHCO_3$ (776 mg, 9.24 mmol) in dioxane/H_2O (38/12 mL) was treated with FmocCl (1.20 g, 4.62 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The mixture was diluted with AcOEt, and the organic phase was washed with 1 M *aq*. HCl and saturated *aq*. NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was triturated from hexane to afford **25** (1.74 g, 90%) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.88−7.29 (m, 14H, aromatic and N*H*), 7.09 (d, 1H, N*H*, *J* = 6.3 Hz), 4.98 (s, 2H, CH_2Ph), 4.26−3.97 (m, 3H, OCH_2fluorenyl, *H*-fluorenyl), 3.33 (m, 2H, α-C*H* and β-C*H*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 172.4, 156.2, 155.9, 143.9, 140.7, 137.0, 128.4, 127.8, 127.6, 125.3, 127.1,

dx.doi.org/10.1021/jo402267r | *J. Org. Chem.* 2013, 78, 12662−12670

120.1, 65.6, 65.4, 54.7, 46.7, 42.4; ESIMS-LR m/z 483 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{26}H_{24}N_2NaO_6$ 483.15266, found 483.15215.

Cyclo(Gly-Sar-L-Pip-Dap(Cbz)-L-Pip)$_2$ (26). Solid-phase peptide synthesis of **26** was performed in a manner similar to the synthesis of



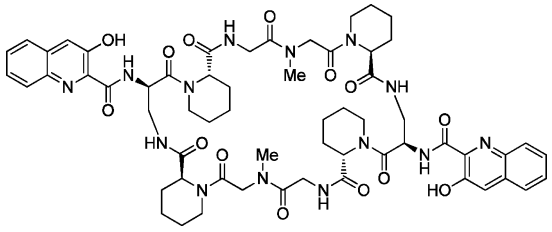
3. A solution of Fmoc-L-Pip-OH (**10**, 551 mg, 0.79 mmol) and ^{i}Pr$_2$NEt (1.09 mL, 4.0 equiv) in CH_2Cl_2 (5 mL) was then added. The mixture was stirred for 1 h at 25 °C. The Fmoc-L-Pip-O-2CT resin was subjected to the following washings/treatments: filteration, CH_2Cl_2−MeOH−^{i}Pr$_2$NEt (17:2:1, 7 mL × 3), DMF (7 mL × 3), and CH_2Cl_2 (7 mL × 3). The resin was dried over P_2O_5 overnight. The loading, calculated by measuring absorbance at 290 nm, was 0.998 mmol/g.

The elongation of the peptide was achieved by sequential addition of the Fmoc-protected amino acid with either HBTU (for the primary amine, 548 mg, 2.9 equiv) or HATU (for the secondary amine, 550 mg, 2.9 equiv) and ^{i}Pr$_2$NEt (521 μL, 6.0 equiv) as coupling reagents in DMF (4 mL) in preactivation mode. The mixture was stirred for 1 h, and after filtration the corresponding colorimetric test (Kaiser test for the primary amine and cloranil test for the secondary amine) indicated the completion of the coupling. Next, the peptide resin was washed with DMF (7 mL × 3) and CH_2Cl_2 (7 mL × 3) and treated with a solution of piperidine/DMF (1:4, 3 min, then 1:8, 12 min) to remove the Fmoc group, and the coupling plus Fmoc removal was then measured. The peptide resin was split into fractions (1/3 and 2/3). The former was treated with a piperidine/DMF (1:4, 3 min, 1:8, 12 min) solution as described above, while the latter was cleaved from the resin with 5% TFA in CH_2Cl_2 solution (5 mL × 3), and the combined filtrates were concentrated *in vacuo*. The residue was precipitated in ether to afford the crude pentapeptide (278 mg) as a pale yellow solid (ESIMS-LR (negative mode) m/z 833 [(M − Na)$^-$]; ESIMS-HR (negative mode) calcd for $C_{43}H_{50}N_6NaO_{10}$ 833.34806, found 833.34782).

The crude pentapeptide was added to the unprotected peptide resin fraction with HBTU (104 mg, 1.7 equiv) and ^{i}Pr$_2$NEt (102 μL, 3.6 equiv) as coupling reagents in DMF (2 mL) in preactivation mode. The mixture was stirred at room temperature for 2 h. Then, the peptide resin was washed with DMF (7 mL × 3) and CH_2Cl_2 (7 mL × 3) and treated with a solution of piperidine/DMF (1:4, 3 min, then 1:8, 12 min). The resin was treated with 5% TFA in CH_2Cl_2 solution (5 mL × 3), and the combined filtrates were concentrated to dryness under reduced pressure. The residue was precipitated in ether to afford the crude decapeptide (121 mg, 64%) as a pale yellow solid (ESIMS-LR m/z 1159 [(M + H)$^+$]; ESIMS-HR calcd for $C_{56}H_{79}N_{12}O_{15}$ 1159.57824, found 1159.57738).

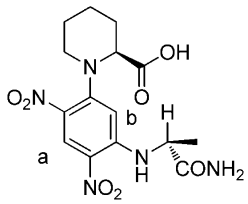
A sample of linear decapeptide (14.8 mg, 0.013 mmol) in CH_2Cl_2 (2.6 mL) was treated sequentially with EDCI (7.5 mg, 0.039 mmol), HOBt (5.3 mg, 0.039 mmol), and $NaHCO_3$ (6.5 mg, 0.077 mmol), and the reaction mixture was stirred at room temperature for 5 days. The reaction mixture was poured onto 1 M *aq*. HCl (20 mL) and extracted with AcOEt (20 mL). The combined organic phases were washed with saturated *aq*. $NaHCO_3$ (20 mL) and saturated *aq*. NaCl (10 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Preparative TLC (thin SiO_2, 20 × 20 cm, 10% MeOH−$CHCl_3$ eluent) afforded **26** (4.7 mg, 31%) as a colorless glass. ^{1}H NMR ($CDCl_3$/CD_3OD = 9/1, 500 MHz, selected peaks are listed because of multiple conformers) δ 7.25−7.04 (m, 10H), 5.12−4.88 (m, 8H), 4.58−4.51 (m, 6H), 4.13−4.04 (m, 4H), 3.23−2.81 (m, 10H), 2.06−1.83 (m, 4H), 1.65−1.12 (m, 20H); ^{13}C NMR ($CDCl_3$/CD_3OD = 9/1, 125 MHz, selected peaks are listed because of multiple conformers) δ 172.1, 170.5, 170.3, 168.2, 167.8, 156.3, 136.7, 128.4, 128.1, 127.9, 127.7, 66.0, 53.1, 52.2, 50.6, 44.2, 43.4, 40.9, 36.7, 29.6, 25.5, 25.1, 24.9, 24.5, 19.6; ESIMS-LR m/z 1163 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{56}H_{76}N_{12}NaO_{14}$ (M + Na)$^+$ 1163.5502, found 1163.5500.

Desmethylquinaldopeptin (27). A mixture of **26** (20 mg, 0.017 mmol) and Pd/C (10 mg) in MeOH (5 mL) was vigorously stirred for



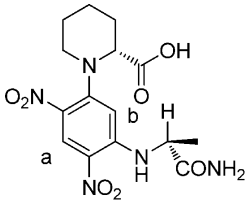
3 h under hydrogen atmosphere. The Pd/C was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to give the crude diamine. A solution of **2** (12.7 mg, 0.068 mmol) and DMAP (8.3 mg, 0.068 mmol) in CH_2Cl_2 (1 mL) was treated with EDCI (13 mg, 0.068 mmol), and the reaction mixture was stirred at room temperature for 30 min. The mixture was added to the diamine, and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was poured onto 1 M *aq*. HCl (5 mL) and extracted with AcOEt. The combined organic phases were washed with saturated *aq*. $NaHCO_3$ and saturated *aq*. NaCl (5 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Preparative TLC (thin SiO_2, 20 × 20 cm, 10% MeOH−$CHCl_3$ eluent) afforded **27** (8.0 mg, 39%) as a white solid colorless glass. $[\alpha]^{21}_D$ −60.6 (*c* 0.49, MeOH); ^{1}H NMR ($CDCl_3$/CD_3OD = 9/1, 500 MHz, selected peaks are listed because of multiple conformers) δ 7.46−7.35 (m, 10H), 5.74−4.93 (m, 6H), 4.56−3.50 (m, 14H), 3.05−2.89 (m, 4H), 2.45−2.22 (m, 4H), 1.87−1.12 (m, 16H); the ^{13}C NMR spectrum is not able to be obtained because of multiple conformers. Increasing the amount of the compound also caused precipitation. ESIMS-LR m/z 1237 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{60}H_{74}N_{14}NaO_{14}$ 1237.5407, found 1237.5383.

L-Ala-NH$_2$-DNP-L-Pip (L-21). A solution of L-pipecolic acid (5 mg, 0.038 mmol) in acetone (1 mL) and saturated *aq*. $NaHCO_3$ (1 mL)



was treated with 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (12.5 mg, 0.046 mmol) at 40 °C for 6 h. The reaction was quenched by 1 M *aq*. HCl at 0 °C. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (SiO_2, 20% MeOH−CH_2Cl_2) to afford L-**21** (10 mg, 69%) as a yellow powder. ^{1}H NMR (CD_3OD, 500 MHz) δ 8.82 (s, 1H, Ha), 6.13 (s, 1H, Hb), 4.27 (q, 1H, Ala-α-C*H*, J = 6.9 Hz, J = 13.8 Hz), 4.09 (br s, 1H, Pip-α-C*H*), 3.45 (m, 1H, Pip-ε-CH_{ax}), 3.22 (d, 1H, Pip-ε-CH_{eq}, J = 12.6 Hz), 2.22 (d, 1H, Pip-β-CH_{eq}, J = 10.9 Hz), 1.78−1.28 (m, 4H, Pip-γ-CH_2 and Pip-δ-CH_2), 1.57 (d, 3H, Ala-β-CH_3, J = 6.9 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.8, 174.5, 153.3, 147.7, 131.5, 129.4, 126.0, 103.0, 62.3, 53.5, 28.5, 25.9, 21.7, 19.2; ESIMS-LR m/z 380 [(M + H)$^+$]; ESIMS-HR calcd for $C_{15}H_{18}N_5O_7$ 380.12117, found 380.12135.

L-Ala-NH$_2$-DNP-D-Pip (D-21). A solution of D-pipecolic acid (5 mg, 0.038 mmol) in acetone (1 mL) and saturated *aq*. $NaHCO_3$ (1 mL)



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was treated with 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (12.5 mg, 0.046 mmol) at 40 °C for 6 h. The reaction was quenched by 1 M *aq.* HCl at 0 °C. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (SiO_2, 20% MeOH–CH_2Cl_2) to afford D-**21** (7.2 mg, 50%) as a yellow powder. $[\alpha]^{20}_{D}$ −46.9 (*c* 0.23, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 8.80 (s, 1H, Ha), 6.10 (s, 1H, Hb), 4.22 (q, 1H, Ala-α-C*H*, *J* = 6.9 Hz, *J* = 13.7 Hz), 4.00 (br s, 1H, Pip-α-C*H*), 3.59 (m, 1H, Pip-ε-CH_{ax}), 3.17 (d, 1H, Pip-ε-CH_{eq}, *J* = 12.6 Hz), 2.25 (d, 1H, Pip-β-CH_{ax}, *J* = 13.2 Hz), 1.75–1.29 (m, 4H, Pip-γ-CH_2 and Pip-δ-CH_2), 1.60 (d, 3H, Ala-β-CH_3, *J* = 6.9 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 177.7, 153.4, 147.8, 131.7, 129.3, 125.7, 102.9, 63.3, 54.1, 28.8, 26.0, 21.9, 19.1; ESIMS-LR *m*/*z* 380 [(M + H)$^+$]; ESIMS-HR calcd for $C_{15}H_{18}N_5O_7$ 380.12117, found 380.12142.

Amino Acid Analysis of 3 and 26. Compound **3** or **26** (1.0 mg, 0.88 μmol) was treated with 6 M *aq.* HCl (1.0 mL) at 100 °C for 24 h. The resulting mixture was concentrated *in vacuo*. The residue in acetone (1 mL) and saturated *aq.* $NaHCO_3$ (1 mL) was treated with 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (4.90 mg, 0.018 mmol) at 40 °C for 6 h. The crude mixture was dissolved in DMSO (1.0 mL) and analyzed by HPLC (YMC J'sphere ODS M80, 4.6 × 150 mm, 0.1% TFA 25–50% MeCN–H_2O for 40 min, 9.2 min).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of all the new compounds except ^{13}C NMR spectra of **27**, and amino acid analysis of **3** and **26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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