Total Syntheses of (+)-P-3A, *epi*-(−)-P-3A, and (−)-Desacetamido P-3A

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*Received July 21, 1993**

Abstract: Full details of the first total syntheses of (+)-P-3A (**1**), *epi*-(−)-P-3A (**2**), and (−)-desacetamido P-3A (**3**) are disclosed. Key strategic elements of the approach include the implementation of an inverse electron demand [4 + 2] cycloaddition reaction of 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine with in situ generated 1,1-diaminoethene for one-step preparation of an appropriately functionalized pyrimdine core and the subsequent use of a diastereoselective *N*-acyloxazolidinone enolate–imine addition reaction for stereocontrolled introduction of the pyrimidine C2-acetamido side chain. The demonstration and comparison of the functional cleavage of duplex DNA by Fe(II)–**1**–**3** are described. The Fe(II) complex of (+)-P-3A proved to be only 0.8–0.5 times less effective than Fe(II)–deglycobleomycin A_2 at producing cleavage of duplex DNA. Like Fe(II)–bleomycin A_2 or deglycobleomycin A_2, Fe(II)–**1**–**3** produced both single- and double-strand cleavage, although with a decreased propensity for double-stranded cleavage. Unlike bleomycin A_2 or deglycobleomycin A_2, Fe(II)–**1**–**3** or Fe(III)–**1**–**3** were found to cleave duplex DNA with no discernable sequence selectivity, indicating that the metal chelation subunit alone may be insufficient for observation of the characteristic bleomycin A_2 DNA cleavage selectivity. In addition, Fe(II)–**1** proved to be 3–5 times more efficient than Fe(II)–**2** and Fe(II)–**3** at producing DNA cleavage, indicating that the pyrimidine C2-acetamido side chain significantly affects cleavage efficiency although it is not intimately involved in the metal chelation.

P-3A (**1**)[1] is a peptide-derived natural product, isolated in the conduct of biosynthetic studies of the bleomycins, whose structure was unambiguously established in a single-crystal X-ray structure determination of its copper(II) complex. It represents the simplest member of the class of agents related to the clinically important bleomycin antitumor antibiotics, whose biological properties are thought to be derived through their metal-dependent oxidative cleavage of duplex DNA.[2] The timely identification of P-3A and the structural characterization of its copper(II) complex established the functionality responsible for metal chelation and provided the initial observation which suggested that the C2-acetamido side chain of P-3A and the related bleomycins may not be intimately involved in the metal coordination (Figure 1). Herein, we provide full details of the first total syntheses of (−)-P-3A (**1**), *epi*-(−)-P-3A (**2**), and (−)-desacetamido P-3A (**3**),[3] based on the inverse electron-demand Diels–Alder reaction[4] of 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine (**4**)[5] in studies which first established the viability of this concise approach to the pyrimidine nucleus central to the structure of P-3A (**1**), pyrimidoblamic acid,[6] bleomycin A_2,[7] and structurally related agents.[8,9] Key to the completion of the synthesis of P-3A (**1**) and central to the extension of the strategy to the bleomycins was the implementation of a diastereoselective *N*-acyloxazolidinone enolate–imine addition reaction for stereocontrolled introduction of the pyrimidine C2-acetamido side chain.

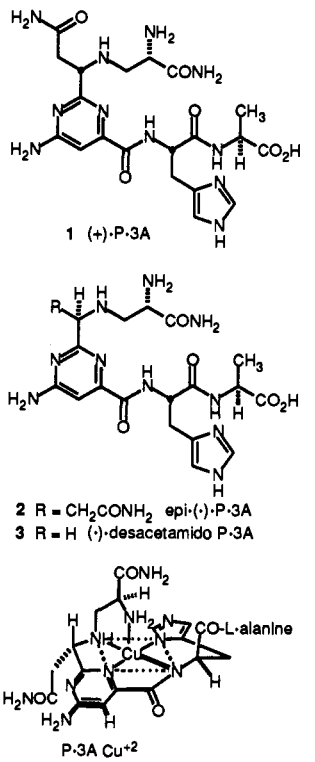

Figure 1.

The pyrimidine nucleus constitutes the core of the iron(II) chelation subunit required for O_2 activation and the subsequent double-stranded DNA cleavage thought to be responsible for the

* Abstract published in *Advance ACS Abstracts*, November 15, 1993.

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therapeutic action of the bleomycins. Consistent with this functional property of the bleomycins, the demonstration and comparative examination of the DNA cleavage properties of the Fe(II) complexes of **1–3** are detailed, and two significant observations are disclosed. The Fe(II) complexes of (+)-P-3A (**1**) as well as **2** and **3** were found to cleave duplex DNA above background Fe(II) with a relative efficiency that approaches that of deglycobleomycin A_2 but with no discernible sequence selectivity. This represents the first demonstration that a bleomycin-related metal chelation subunit alone is insufficient for observation of the characteristic DNA cleavage selectivity. In addition, the Fe(II) complex of P-3A (**1**) proved to be 3–5 times more efficient than Fe(II)–**2** and Fe(II)–**3** at cleaving DNA, indicating that the C2-acetamido side chain significantly affects DNA cleavage efficiency, although it is apparently not intimately involved in the metal chelation.

1,3,5-Triazine → Pyrimidine Heteroaromatic Azadiene Diels–Alder Reaction: Synthesis of the Pyrimidine Core. Key to the effective synthesis of **1–3** is the concise preparation of an appropriately functionalized and fully substituted pyrimidine at the core of the structure. A particularly effective one-step approach to this pyrimidine core was devised based on the development of an inverse electron demand Diels–Alder reaction of 1,3,5-triazines[3,5] with in situ generated amino enamines. Treatment of **4**,[5,10] prepared by acid-catalyzed trimerization of ethyl cyanoformate (95–100%), with acetamidine hydrochloride (**5**) provided the pyrimidine **9** in a reaction that proceeds through reversible, in situ tautomerization of **5** to 1,1-diaminoethene and its participation in an effective [4 + 2] cycloaddition reaction with **4** (Scheme I). The subsequent elimination of ammonia, imine tautomerization to enamine **8**, and retro-Diels–Alder loss of ethyl cyanoformate under the reaction conditions provided **9** in a reaction cascade for which the conversions proved sensitive to the reaction conditions (Table I). The use of a polar, aprotic solvent and thermal conditions (>80 °C) promote amidine tautomerization and are required for affecting the retro-Diels–Alder reaction of the initial [4 + 2] cycloadduct. Consistent with past observations made in studies of related heteroaromatic azadiene Diels–Alder reactions[4,5] in which acid catalysts proved to accelerate both the retro-Diels–Alder reaction and the subsequent aromatization reaction, the retro-Diels–Alder reaction of **8** and the tautomerization reaction of **7** in the conversion to **9** are facilitated by the presence of HCl derived from the use of the amidine hydrochloride. Efforts to employ the amidine free base of **5** as well as the corresponding methyl imidate or imidate hydrochloride proved significantly less successful.

Scheme I

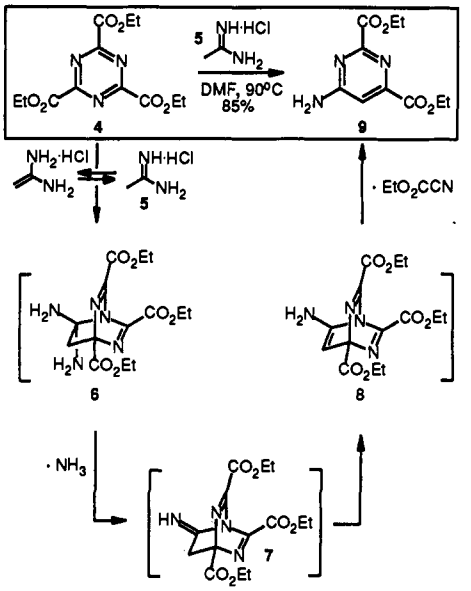

Table I. Representative Results of a Study of the [4 + 2] Cycloaddition Reaction of **4** with **5** and **17**

entry	conditions	product	yield (%)
1	pyridine 60 °C, 28 h	**9**	20
2	pyridine 115 °C, 30 h	9	55
3	DMF 80 °C, 22 h	**9**	70
4	DMF 90 °C, 22 h	**9**	85
5	DMF 100 °C, 22 h	**9**	81
6	DMF 110 °C, 22 h	9	73
7	DMF 120 °C, 26 h	**9**	50
8	DMF 130 °C, 26 h	**9**	35
9	DMF, K_2CO_3 100 °C, 48 h	9	42
10	dioxane 100 °C, 48 h	**9**	0
11	CH_3CN 84 °C, 48 h	**9**	0
12	DMF 90 °C, 20 h	**19**	78
13	DMF 90 °C, 48 h	**19**	90

Synthesis of (–)-Desacetamido P-3A (3). The additional key to the use of **9** in the preparation of the heterocyclic core of **1–3** was the selective differentiation of the pyrimidine C2- and C4-esters. This differentiation was accomplished through selective reduction of the more electrophilic C2-ethoxycarbonyl group to provide **10**.[11] Characteristic of the enhanced electrophilic nature

(6) Structure determination: (a) Yoshioka, T.; Muraoka, Y.; Takita, T.; Maeda, K.; Umezawa, H. *J. Antiobiot.* **1972**, *25*, 625. Synthesis: (b) Umezawa, Y.; Morishima, H.; Saito, S.; Takita, T.; Umezawa, H.; Kobayashi, S.; Otsuka, M.; Narita, M.; Ohno, M. *J. Am. Chem. Soc.* **1980**, *102*, 6630. (c) Otsuka, M.; Narita, M.; Yoshida, M.; Kobayashi, S.; Ohno, M.; Umezawa, Y.; Morishima, H.; Saito, S.; Takita, T.; Umezawa, H. *Chem. Pharm. Bull.* **1985**, *33*, 520. (d) Arai, H.; Hagmann, W. K.; Suguna, H.; Hecht, S. M. *J. Am. Chem. Soc.* **1980**, *102*, 6631. (e) Aoyagi, Y.; Chorghade, M. S.; Padmapriya, A. A.; Suguna, H.; Hecht, S. M. *J. Org. Chem.* **1990**, *55*, 6291. (f) Boger, D. L.; Menezes, R. F.; Honda, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 273.

(7) Synthesis: Takita, T.; Umezawa, Y.; Saito, S.; Morishima, H.; Umezawa, H.; Muraoka, Y.; Suzuki, M.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1981**, *22*, 671. Takita, T.; Umezawa, Y.; Saito, S.; Morishima, H.; Naganawa, H.; Umezawa, H.; Tsuchiya, T.; Miyake, T.; Kageyama, S.; Umezawa, S.; Muraoka, Y.; Suzuki, M.; Otsuka, M.; Narita, M.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1982**, *23*, 521. Saito, S.; Umezawa, Y.; Morishima, H.; Takita, T.; Umezawa, H.; Narita, M.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1982**, *23*, 529. Aoyagi, Y.; Suguna, H.; Murugesan, N.; Ehrenfeld, G. M.; Chang, L.-H.; Ohgi, T.; Shekhani, M. S.; Kirkup, M. P.; Hecht, S. M. *J. Am. Chem. Soc.* **1982**, *104*, 5237. Aoyagi, Y.; Katano, K.; Suguna, H.; Primeau, J.; Chang, L.-H.; Hecht, S. M. *J. Am. Chem. Soc.* **1982**, *104*, 5537. Saito, S.; Umezawa, Y.; Yoshioka, T.; Takita, T.; Umezawa, H.; Muraoka, Y. *J. Antibiot.* **1983**, *36*, 92. Boger, D. L.; Menezes, R. F.; Honda, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 273.

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(11) Selective hydrolysis, transesterification, or aminolysis under a variety of reaction conditions provided unsatisfactory product mixtures derived from reaction of the C2-ethoxycarbonyl group and of both the C2- and the C4-ethoxycarbonyl groups, along with recovered starting material.

Scheme II

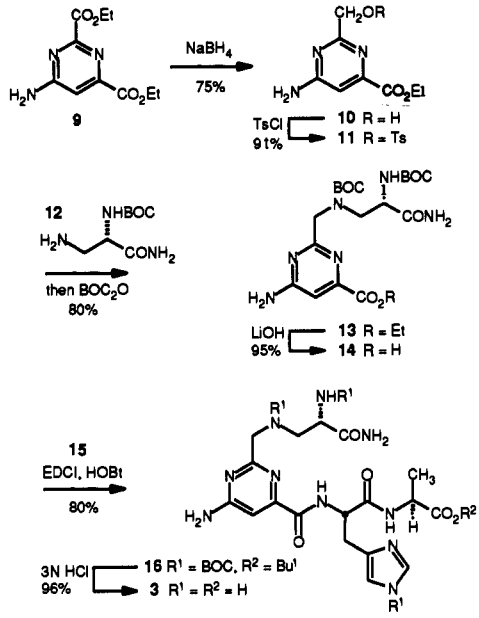


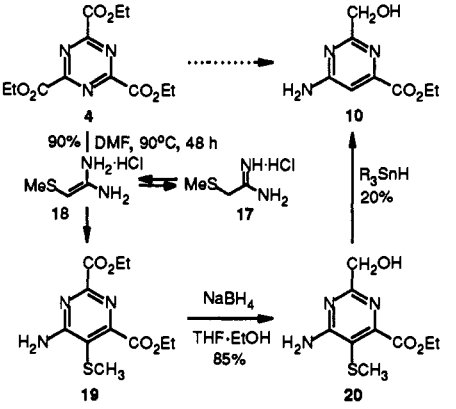
Table II. Representative Results of the Selective Reduction of **9**

entry	conditions[a]	2-CH_2OH:4-CH_2OH	yield (%)
1	MeOH −25 °C, 72 h	no reaction	0[b]
2	EtOH −40 °C, 72 h	2:1	38[b]
3	EtOH −40 to −15 °C, 131 h	2:1	78
4	iPrOH −30 °C, 72 h	6:1	65[c]
5	tBuOH–THF (1:2) −20 °C, 18 h	7:1	70
6	tBuOH–THF (1:2) −30 °C, 24 h	6–7:1	87
7	tBuOH–THF (1:5) −20 °C, 18 h	7:1	58
8	tBuOH–EtOH (3:1) −25 °C, 28 h	6:1	55
9	EtOH–$CHCl_3$ (1:1) −30 °C, 102 h	3:1	67
10	EtOH–$CHCl_3$ (1:1) $Mg(ClO_4)_2$ (2 equiv) −20 °C, 2 h	2-CH_2OH only	25[d]

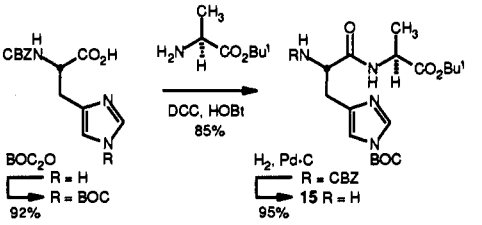
[a] 1.0 equiv of $NaBH_4$. [b] Starting material recovered. [c] The isopropyl ester was isolated due to transesterification. [d] No 4-CH_2OH detected by ^1H NMR.

of the C2-ethoxycarbonyl group, the selective reduction of **9** may be conducted effectively with $NaBH_4$ at low temperature (−30 to −20 °C, Scheme II). Although the reduction was found to proceed in a satisfactory manner in EtOH, the reaction proved faster, cleaner, and more selective (6–7:1 vs 2–4:1 C2-CH_2OH:C4-CH_2OH) when conducted in 2-PrOH (3 days) or 1:2 *t*-BuOH–THF (7:1 C2-CH_2OH: C4-CH_2OH, 87%, 24 h), presumably due to the enhanced stability of the reagent to the reaction conditions (Table II). Notably, the use of THF as a cosolvent with *t*-BuOH permitted the use of this unreactive alcohol as solvent at reaction temperatures below its freezing point, providing reaction conditions under which the reagent proved stable and competitive transesterification of the substrate was not observed. On large scales, simple recrystallization of the reduction product proved sufficient to provide pure **10**. Consequently, the preliminary finding that reduction of **9** in the presence of $Mg(ClO_4)_2$ provided **10** cleanly, albeit in low yield, presumably through selective chelation with the substrate, was not further pursued. 1D ^1H NOE NMR of the isomeric alcohols and 2D ^1H–^1H NOESY NMR of the corresponding tosylates derived from the

Scheme III



major and minor reduction products unambiguously confirmed the isomer assignments through observation of a diagnostic -CH_2-OR/C5-H NOE crosspeak for the minor isomer. The completion of the synthesis of **3** was accomplished by conversion of **10** to the tosylate **11** (91%), followed by clean displacement with amine **12**[12] and subsequent protection of the secondary amine to provide **13** (80%, two steps).[13] Notably, the pyrimidine C6-amine proved to be sufficiently unreactive that its participation in competitive reactions was not observed and its deliberate protection proved unwarranted. Ethyl ester hydrolysis (95%) and EDCI-promoted coupling of **14** with N^{im}-Boc-L-His-L-Ala-OBu^t (**15**, eq 1) provided desacetamido P-3A in its fully protected form **16** (80%). Initial



(1)

attempts to use the methyl ester of **15** in the coupling with **14** suffered from competitive lactamization with piperazinedione formation. Acid-catalyzed deprotection of **16** provided (−)-desacetamido P-3A [**3**, 96%, $[\alpha]^{22}_D$ −13.3 (*c* 0.15, CH_3OH), −18 (*c* 0.15, 0.1 N HCl)].

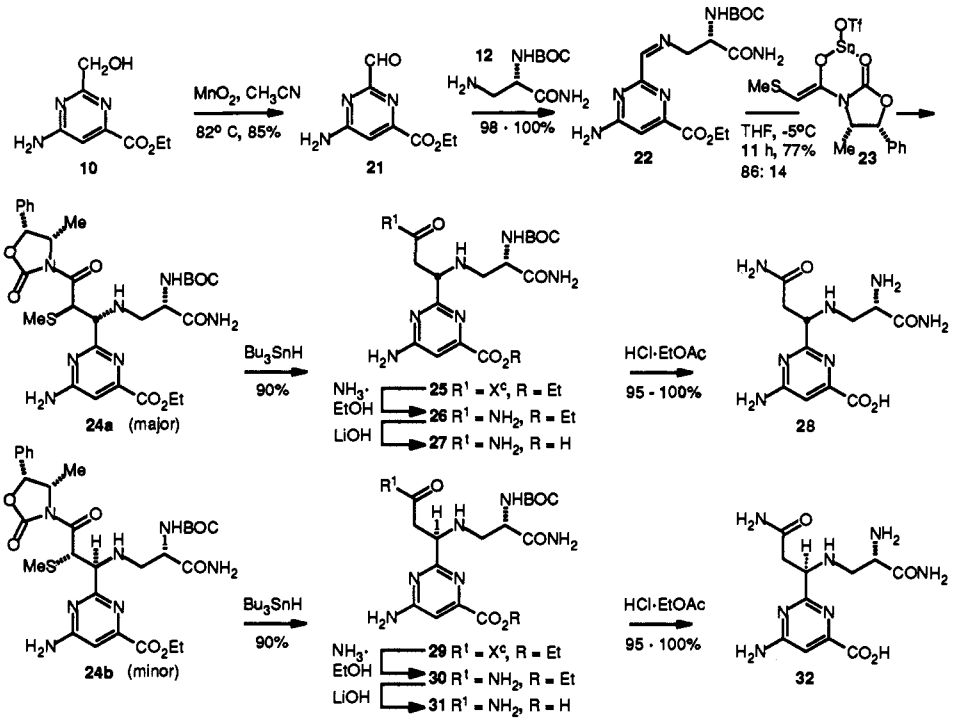
In an alternative approach to the preparation of **10**, the key C2- and C4-ethoxycarbonyl differentiation was anticipated to be simplified with the selective reduction of the electronically more reactive *and* sterically less hindered C2-ethoxycarbonyl group of **19**. Treatment of **4** with (methylthio)acetamidine hydrochloride[14] (**17**, DMF, 90 °C, 48 h) provided **19** directly in excellent conversion (90%) in a reaction cascade that proceeds by reversible tautomerization of **17** to **18**, [4 + 2] cycloaddition of **18** with **4**, elimination of ammonia from the initial [4 + 2] cycloadduct, imine to enamine tautomerization, and retro-Diels–Alder loss of ethyl cyanoformate (Scheme III). Consistent with expectations, selective reduction of **19** with $NaBH_4$ proved straightforward and provided **20** exclusively under unexceptional reaction con-

(12) For the preparation and use of *N*-Boc-L-β-aminoalanine methyl ester, see: Otsuka, M.; Kittaka, A.; Iimori, T.; Yamashita, H.; Kobayashi, S.; Ohno, M. *Chem. Pharm. Bull.* **1985**, *33*, 509. For alternative preparations of **12**, see ref 6b,d.

(13) Similarly, amine displacement of the 2-(chloromethyl)pyrimidine provided **13** in a comparable conversion. Alternatively, **13** may be prepared from imine **22** through catalytic hydrogenation (0.1 wt equiv PtO_2, H_2, 25 °C, 28 h) and subsequent protection of the secondary amine (Boc_2O), but the sequence detailed in Scheme II proved superior, shorter, and technically more convenient. Attempts to conduct the catalytic hydrogenation of **22** with 5–10% Pd–C proved much less successful, presumably due to catalyst poisoning by the substrate or product.

(14) Amidine **17** was prepared from (methylthio)acetonitrile by the following reactions: (i) MeOH, HCl, Et_2O, −20 °C, 12 h; (ii) NH_3, EtOH, 25 °C, 3 h, 72% for the two steps.

Scheme IV



ditions (THF–EtOH 1:1, 25 °C, 2 h, 85%). In the examination of the reduction of **19** to **20**, the use of EtOH without THF as a cosolvent and shorter (0.5 h, 56%) or longer (12 h, overreduction) reaction times led to lower conversions. Subsequent desulfurization of **20** to provide **10** has not proven straightforward with common reagents, although this has not been exhaustively investigated. Reduction of **20** with Bu_3SnH (2–4 equiv) or Ph_3SnH (2.2–6 equiv) under a range of conditions provided **10** in approximately 20% yield under the best conditions examined. Alternative reagents including Raney Ni, $NiCl_2$–$NaBH_4$, and Zn/THF–NH_4Cl under a range of conventional reaction conditions failed to improve on these observations. Consequently, the use of **20** was not further investigated given the ease with which the direct reduction of **9** may be conducted. However, the clean generation of **19** and **20** has served to establish the generality of the approach to the preparation of modified P-3A and pyrimidoblamic acid pyrimidine cores bearing a range of C5 substituents.

Diastereoselective *N*-Acyloxazolidinone Enolate–Imine Addition Reaction: Total Synthesis of (+)-P-3A and *epi*-(−)-P-3A. The final strategic element required for extension of the studies to the total synthesis of P-3A (**1**) was the stereocontrolled introduction of the C2-acetamido side chain. Prior studies on the synthesis of the pyrimidoblamic acid subunit of the bleomycins[6] by Hecht and Umezawa-Ohno have employed nonselective approaches to the introduction of the acetamido side chain requiring a separation of the resulting 1:1 mixture of diastereomers. In parallel with synthetic studies on pyrimidoblamic acid and bleomycin A_2,[6,7] we have examined the diastereoselective addition of optically active enolates with imines as a potential solution to this problem.[15,16] In these studies, the imine addition reactions of a range of optically active enolates were examined, and the Evans' optically active *N*-acyloxazolidinones[17] were found to provide a diastereoselective imine addition reaction suitable for C2-acetamido side-chain introduction.[18] With this methodology in hand, its application in the total synthesis of (+)-P-3A was pursued.

Oxidation of **10** to provide aldehyde **21** proved technically more challenging than its structure might suggest. After considerable experimentation, the modest conversion observed under standard MnO_2 oxidation conditions (10 equiv, CH_2Cl_2, 25 °C, 20–45%) that may be attributed principally to the limited solubility of **10** and **21** in CH_2Cl_2 was significantly improved by conducting the reaction in refluxing CH_3CN. Further use of dilute reaction conditions (0.05 M), which eliminated a competitive self-condensation reaction of **21** resulting in imine formation, provided excellent conversion of **10** to **21** (10 equiv of MnO_2, 0.05 M CH_3CN, 82 °C, 3 h, 85%, Scheme IV). A range of alternative oxidants[19] were not successful at providing **21**, and their failure most likely may be attributed to the solubility properties of **10** under the conventional reaction conditions. Condensation of **21** with **12** provided the imine **22**.

Treatment of imine **22** with the stannous (*Z*)-enolate **23**[20-28] (2.0 equiv) in the presence of additional $Sn(OTf)_2$ (2.0 equiv)

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(19) This included the examination of $Ba(MnO_4)_2$, PCC, PDC, $(COCl)_2$–DMSO, TFAA–DMSO, and DDQ.

(20) The stannous enolate **23**[24] proved more effective than the titanium enolate[22,23] ($TiCl_3$, 20-30% yield, 9:1 **24a**:**24b**), which provided the same products with a comparable level of diastereoselectivity. The di-*n*-butylboronyl enolate[21] proved ineffective.

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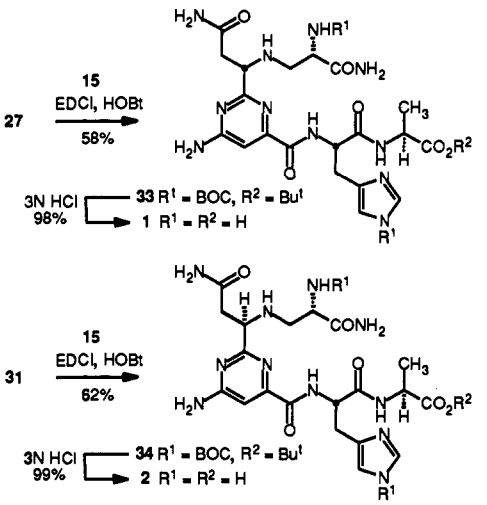
Scheme V

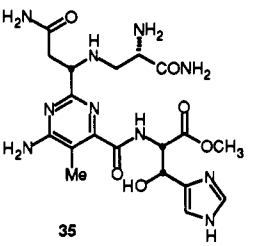


provided a separable 86:14 (6:1) mixture of two diastereomeric *anti* imine addition products **24a** and **24b**, respectively, in good yield (77%). In agreement with observations made in related studies,[18] the reaction provides predominantly the *anti* imine adduct **24a** and requires a minimal number of protecting groups for the reactive functionality in imine **22**. Prolonged exposure of *anti*-**24a** to the reaction conditions resulted in epimerization to provide a third diastereomer, *syn*-**24a** (−5 °C, 11 h, >20:1 *anti*:*syn*-**24a** versus 0 °C, 29 h, 3:2 *anti*:*syn*-**24a**). Reductive desulfurization (Bu_3SnH, 90–95%) of the major diastereomer **24a** and subsequent careful aminolysis of **25** (0 °C, 1 h) afforded **26**. Ethyl ester hydrolysis provided the N^{α}-*tert*-butyloxycarbonyl derivative **27**, and subsequent acid-catalyzed deprotection provided **28**, $[\alpha]^{25}_D$ −23 (*c* 0.065, H_2O). Subjection of the minor diastereomer **24b** to the same sequence provided **29–31** and **32**, $[\alpha]^{25}_D$ +20 (*c* 0.10, H_2O). The diastereomeric relationship of **26–28** and **30–32** confirmed that **24a** and **24b** constitute isomers at the newly introduced amine center.

The absolute configuration of the adducts was determined through the incorporation of **24a**, the major imine addition product of **23** with imine **22**, into natural (+)-P-3A (**1**) via **25–27** and the conversion of the minor adduct into the diastereomeric series **29–32**. The relative stereochemical assignments of the major and minor adducts **24a** and **24b**, as well as that of *syn*-**24a** derived from epimerization of *anti*-**24a**, were made on the basis of characteristic ^1H NMR chemical shifts, coupling constants, and $w_{1/2}$ values in conjunction with 2D ^1H–^1H NMR.[29]

Direct coupling of **27** with **15** provided **33** and was found to be conveniently conducted without protection of the unreactive arylamine or hindered secondary amine of **27** (Scheme V). Final acid-catalyzed deprotection of **33** provided (+)-P-3A (**1**), $[\alpha]^{25}_D$ +80 (*c* 0.015, H_2O), identical in all compared respects with authentic material[30] (Table III–IV). In an analogous fashion, coupling of **31** with **15** provided **34**, and subsequent acid-catalyzed deprotection provided *epi*-(−)-P-3A (**2**), $[\alpha]^{25}_D$ −34 (*c* 0.02, H_2O).

Functional DNA Cleavage. A study of the comparative abilities of the Fe(II) complexes of P-3A (**1**), *epi*-P-3A (**2**), and desacetamido P-3A (**3**)[31,32] to cleave duplex DNA in the presence of oxygen was conducted through examination of single-strand and double-strand cleavage of supercoiled *φX174* RFI DNA (Form I) to produce relaxed (Form II) and linear (Form III) DNA, respectively. The Fe(II) complexes of **1–3** were found to effectively produce both single- and double-strand cleavage of *φX174* DNA (Table V). Surprisingly, Fe(II)–**1** proved to be only slightly less efficient than Fe(II)–deglycobleomycin A_2 (relative efficiency, 1:0.8–0.5 deglycobleomycin A_2:**1**) and substantially more effective than Fe(II)–**35**, which proved indistinguishable from Fe(II) itself. In addition, the comparison of the efficiency of DNA cleavage by Fe(II)–**1**, Fe(II)–**2**, and Fe(II)–**3** permitted the assessment of the relative importance and functional role of the C2-acetamido side chain. Although the side chain has been shown not to be intimately involved in the metal chelation (*cf.* Figure 1), it may contribute to the efficiency of DNA cleavage by enhancing binding affinity or orientation with duplex DNA or by constituting one side or component of the oxygen binding pocket sterically protecting the reactive iron–oxygen intermediate. Consistent with such suggestions, Fe(II)–**1** proved to be 3–5 times more efficient than both Fe(II)–**2** and Fe(II)–**3** in cleaving supercoiled *φX174* DNA. These results proved to be analogous to observations made in our recent comparison of Fe(II)–deglycobleomycin A_2 and Fe(II)–desacetamidodeglycobleomycin A_2, in which the agent lacking the pyrimidoblamic acid C2-acetamido side chain proved to be 3–5 times less effective in cleaving *φX174* RFI DNA.[9] The lack of comparable DNA cleavage in control studies in which the agents alone in the absence of Fe(II) or Fe(II) alone in the absence of agent at identical concentrations assures that the DNA cleavage reactions are derived from the Fe(II) complexes of **1–3**.



35

In addition, Fe(II)–**1–3** proved effective in producing linear DNA resulting from double-strand DNA cleavage but less efficient at doing so than deglycobleomycin A_2. A statistical treatment of the time dependence of the relative amount of circular versus linear DNA generated in the DNA cleavage reaction suggests that the linear DNA generated with Fe(II)–**1–3** is not simply the consequence of random, unrelated single-strand DNA

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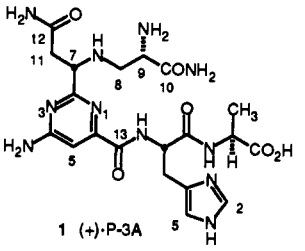
(29) The assignment of the absolute configuration of *anti*-**24a** and *anti*-**24b** was based on the conversion of **24a** to (+)-P-3A (**1**), for which an X-ray crystal structure determination unambiguously established relative and absolute configuration.[1] Adduct *anti*-**24b** provided the diastereomer *epi*-(−)-P-3A (**2**), isomeric at the C7 center. Deliberate epimerization of the C11 center of *anti*-**24a** provided *syn*-**24a**, spectroscopically distinguishable from *anti*-**24a** and **-24b**. The assignment of the relative stereochemistry of *anti*-**24a** and *anti*-**24b** was based on characteristic C7-H/C11-H ^1H NMR coupling constants and proved analogous with prior stereochemical studies of the *N*-acyloxazolidinone enolate–imine addition reactions,[6f,18] for which unambiguous stereochemical assignments are available by X-ray.[18]
(30) The ^1H NMR of **1** versus **2** proved especially diagnostic, with the natural product and synthetic **1** exhibiting a characteristic set of two doublets of doublets at 2.92 (*J* = 7.0, 14.0 Hz) and 2.96 (*J* = 5.5, 14.0 Hz) appearing as a clear doublet split AB quartet for C11-H_2. The *epi*-P-3A (**2**) C11-H_2 AB quartet collapses to an apparent doublet.
(31) Boger, D. L.; Yang, W. *BioMed. Chem. Lett.* **1992**, *2*, 1649.
(32) For representative recent studies of the metal dependent bleomycin oxidative cleavage of duplex DNA: Guan, L. L.; Kuwahara, J.; Sugiura, Y. *Biochemistry* **1993**, *32*, 6141. Holmes, C. E.; Carter, B. J.; Hecht, S. M. *Biochemistry* **1993**, *32*, 4293. Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Absalon, M. J.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1993**, *32*, 2601. McGall, G. H.; Rabow, L. E.; Ashley, G. W.; Wu, S. H.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1992**, *114*, 4958. Tan, J. D.; Hudson, S. E.; Brown, S. J.; Olmstead, M. M.; Mascharak, P. K. *J. Am. Chem. Soc.* **1992**, *114*, 3841. Boger, D. L.; Menezes, R. F.; Yang, W. *BioMed. Chem. Lett.* **1992**, *2*, 959.

Table III. ^{1}H NMR of **1–3** (400 MHz)

assignment	**1** (D_2O)	**2** (D_2O)	**3** (CD_3OD)
C2-H (His)	8.46 (s)	8.46 (s)	8.79 (s)
C5-H (His)	7.18 (s)	7.16 (s)	7.46 (s)
C5-H	6.93 (s)	6.93 (s)	7.03 (s)
α-CH (His)	4.76 (dd, J = 5.2, 5.8 Hz)	4.75 (dd, J = 5.8, 5.9 Hz)	4.75 (dd, J = 5.4, 5.8 Hz)
C9-H	4.50 (dd, J = 6.8, 7.6 Hz)	4.54 (dd, J = 6.9, 7.5 Hz)	4.64 (dd, J = 6.8, 7.5 Hz)
C7-H	4.34 (dd, J = 5.5, 7.0 Hz)	4.34 (dd, J = 5.8, 6.5 Hz)	4.42 (d, J = 14 Hz)
			4.28 (d, J = 14 Hz)
α-CH (Ala)	4.23 (q, J = 7.4 Hz)	4.23 (q, J = 7.4 Hz)	4.35 (q, J = 7.4 Hz)
β-CH_2 (His)	3.57 (dd, J = 5.2, 14.0 Hz)	3.56 (dd, J = 5.9, 14.0 Hz)	3.72 (dd, J = 6.0, 14.0 Hz)
	3.44 (dd, J = 5.8, 14.0 Hz)	3.47 (dd, J = 5.8, 14.0 Hz)	3.65 (dd, J = 6.0, 14.0 Hz)
C8-H_2	3.27 (dd, J = 6.8, 14.0 Hz)	3.27 (dd, J = 6.9, 14.0 Hz)	3.45 (dd, J = 6.8, 14.0 Hz)
	3.18 (dd, J = 7.6, 14.0 Hz)	3.17 (dd, J = 7.5, 14.0 Hz)	3.38 (dd, J = 7.5, 14.0 Hz)
C11-H_2	2.96 (dd, J = 5.5, 14.0 Hz)	2.97 (app d, J = 8.7 Hz)	
	2.92 (dd, J = 7.0, 14.0 Hz)		
CH_3	1.27 (d, J = 7.2 Hz)	1.27 (d, J = 7.2 Hz)	1.44 (d, J = 7.5 Hz)



1 (+)-P-3A

Table IV. ^{13}C NMR of **1** and **2** (D_2O, 100 MHz)

assignment	**1** (D_2O)	**2** (D_2O)	bleomycin A_2[a]
CO_2H	178.8	178.7	
C12	176.3	176.3	177.0
CO (His)	173.4	173.3	169.8
C10	170.9	170.7	171.9
C13	167.5	167.4	168.5
C2	166.8	166.9	166.1
C4	164.8	164.8	165.5
C6	155.6	155.8	153.0
C2 (His)	136.0	136.0	137.7
C4 (His)	130.5	130.5	135.3
C5 (His)	120.0	120.0	118.6
C5	106.2	106.1	111.3
C9	62.1	62.0	60.5
α-CH (His)	55.1	55.1	57.8
C7	52.7	52.6	53.3
α-CH (Ala)	51.6	51.4	
C8	48.8	48.4	47.8
C11	38.4	37.9	41.0
β-CH_2 (His)	29.3	29.2	
CH_3	18.5	18.5	

[a] Taken from Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Umezawa, Y.; Naganawa, H.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 801.

cleavage events.[33,34] The reactions exhibit fast kinetics in the first 5–10 min, and the decreasing rate of DNA cleavage may reflect metal complex reactivation kinetics or the conversion to a less reactive or inactive agent in the time course of the assay. Assuming a Poisson distribution for the formation of single-stranded and double-stranded breaks, the Freifelder–Trumbo equation[33] was used to calculate the average number of single- to double-stranded DNA cleavages. The data from the first 5–10 min could be fitted to a linear equation with a ratio of 1:30, 1:38, and 1:40 double- to single-stranded cuts observed for Fe(II)–**1**, Fe(II)–**2**, and Fe(II)–**3**, respectively. A theoretical ratio of approximately 1:100 is required in order for the linear DNA to be the result of the random accumulation of single-strand breaks within the 5586 base-pair size of *ϕX174* DNA, assuming that sequential cleavage on the complementary strands within 15 base pairs is required to permit linearization of the hybridized DNA.

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(34) Dedon, P. C.; Goldberg, I. H. *Chem. Res. Toxicol.* **1992**, *5*, 311.

Table V. Summary of DNA Cleavage Properties of **1–3**

agent	relative efficiency of DNA cleavage[a]	ratio of double- to single-stranded DNA cleavage[b]	DNA cleavage selectivity[c]
bleomycin	4–10	1:6	5′-GC, 5′-GT > 5′-GA
deglycobleomycin A_2	1.25–2	1:12	5′-GC, 5′-GT > 5′-GA
(+)-P-3A (**1**)	1	1:30	none
(–)-*epi*-P-3A (**2**)	0.33–0.2	1:38	none
(–)-desacetamido P-3A (**3**)	0.33–0.2	1:40	none
Fe(II)	0.1–0.05	1:98	none

[a] Relative efficiency of supercoiled ϕX174 DNA cleavage. [b] Ratio of double- to single-stranded supercoiled ϕX174 DNA cleavage calculated as $F_{III} = n_2 \exp(-n_2)$, $F_I = \exp -(n_1 + n_2)$. [c] Examined within singly 5′-end-labeled w794 DNA.

Experimentally, it was determined that Fe(II) alone produced a ratio of 1:98 double:single-strand breaks under these conditions, fully consistent with the theoretical ratio. In contrast, Fe(II)–bleomycin A_2 and Fe(II)–deglycobleomycin A_2 produce the double-strand DNA cleavage with a greater frequency of 1:6 and 1:12, respectively, under our conditions, suggesting that complementary strand cleavage within 15 base pairs occurs more often and, as previously detailed,[33] may be related by a single binding event.

The selectivity of DNA cleavage was examined within duplex w794 DNA[35,36] by monitoring strand cleavage of singly ^{32}P 5′-end-labeled double-stranded DNA after exposure to the Fe(II) complexes of **1–3** in the presence of O_2. Thus, incubation of the labeled duplex DNA with the agents in the presence of equimolar $Fe(NH_4)_2(SO_4)_2$ and O_2 or equimolar $FeCl_3$ and H_2O_2 led to DNA cleavage. Removal of the agent by EtOH precipitation of the DNA, resuspension of the treated DNA in aqueous buffer, and gel electrophoresis of the resultant DNA under denaturing conditions adjacent to Sanger sequencing standards permitted the identification of the sites of DNA cleavage. Identical to trends observed with supercoiled *ϕX174* DNA, the relative efficiency of w794 DNA cleavage was found to be bleomycin A_2 > deglycobleomycin A_2 > (+)-P-3A > *epi*-(–)-P-3A, (–)-desacetamido P-3A. In contrast to bleomycin A_2 or deglycobleomycin A_2, which exhibited the characteristic 5′-GC and 5′-GT > 5′-GA DNA cleavage selectivity,[37] Fe(II)– or Fe(III)-**1–3** exhibited DNA cleavage with little or no discernible selectivity. Notably, the DNA cleavage by Fe(II)–**1** or Fe(III)-**1** occurs under

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(36) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.

(37) D'Andrea, A. D.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3608. Takeshita, M.; Grollman, A. P.; Ohtsubo, E.; Ohtsubo, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5983. Mirabelli, C. K.; Ting, A.; Huang, C.-H.; Mong, S.; Crooke, S. T. *Cancer Res.* **1982**, *42*, 2779. Kross, J.; Henner, W. D.; Hecht, S. M.; Haseltine, W. A. *Biochemistry* **1982**, *21*, 4310.

conditions and concentrations for which Fe(II) alone in the absence of agent fails to significantly cleave DNA.

Thus, the observations represent an important demonstration that agents related to the iron chelation subunit of bleomycin A_2 may effectively cleave DNA above a control Fe(II) background and that they may do so with no discernible sequence selectivity.[38] This illustrates that a bleomycin-related metal chelation subunit may be insufficient for sequence-selective DNA cleavage, and the observations are consistent with studies which suggest that the C-terminus of bleomycin A_2 including the bithiazole and tri- or tetrapeptide subunits may be necessary for observation of the bleomycin A_2 duplex DNA cleavage selectivity.[8f,39,40] In addition, comparison of the DNA cleavage properties of Fe(II)–**1** with those of Fe(II)–**2** and Fe(II)–**3** suggests a prominent role for the C2-acetamido side chain of **1**. Like observations made in the comparison of deglycobleomycin A_2 and desacetamidodeglycobleomycin A_2,[9] both the efficiency of DNA cleavage and the ratio of double- to single-strand DNA cleavage events are reduced significantly by the removal or epimerization of the C2-acetamido side chain.

Experimental Section[41]

6-Amino-2,4-bis(ethoxycarbonyl)pyrimidine (9). A solution of 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine[10] (**4**, 301 mg, 1.0 mmol) in anhydrous DMF (1.0 mL) under Ar was treated with acetamidine hydrochloride (**5**, 149 mg, 1.5 mmol, 1.5 equiv) at 25 °C, and the reaction mixture was warmed at 90 °C (22 h). Removal of the solvent in vacuo and recrystallization (EtOAc–hexane) afforded pure **9** (192 mg, 241 mg theoretical, 80%; 80–87%) as a white solid: mp 168–169 °C (white needles, EtOAc–hexane); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.68 (2H, br s, NH_2), 7.13 (1H, s, C5-H), 4.31 (2H, q, J = 7.2 Hz), 4.29 (2H, q, J = 7.2 Hz), 1.28 (3H, t, J = 7.2 Hz), 1.27 (3H, t, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 165.8 (e, C-6), 164.6 (e, C_4-CO_2Et), 164.3 (e, C_2-CO_2Et), 158.1 (e, C-2), 153.9 (e, C-4), 107.6 (o, C-5), 62.0 (e), 61.8 (e), 14.2 (o, two carbons); ^{13}C–^{1}H long-range coupling NMR (DMSO-d_6, 125 MHz, diagnostic coupling constants) δ 164.6 (dt, J(C/C5-H) = 3 Hz, J(C/OCH_2CH$_3$) = 4 Hz, C_4-CO_2Et), 164.3 (t, J(C/OCH_2CH$_3$) = 4 Hz, C_2-CO_2Et), 107.6 (dt, J(C/NH_2) = 5 Hz, J(C/C5-H) = 170 Hz, C-5); IR (KBr) ν_{max} 3442, 3306, 3192, 2980, 1739, 1719, 1650, 1596, 1536, 1435, 1260, 1226, 1026, 972, 866, 746 cm^{-1}; UV (CH_3OH) λ_{max} 209 nm (ϵ 17 000); CIMS (2-methylpropane) m/e (M^+ + H, base); CIHRMS (2-methylpropane) m/e 240.0987 (M^+ + H, $C_{10}H_{13}N_3O_4$ requires 240.0984).

Anal. Calcd for $C_{10}H_{13}N_3O_4$: C, 50.21; H, 5.44; N, 17.57. Found: C, 50.10; H, 5.82; N, 17.65.

6-Amino-4-(ethoxycarbonyl)-2-(hydroxymethyl)pyrimidine (10). A solution of **9** (186 mg, 0.78 mmol) in anhydrous t-BuOH–THF (1:2, 4 mL) was cooled to –25 °C and treated with $NaBH_4$ (29.4 mg, 0.78 mmol, 1.0 equiv) under Ar. After being stirred at –25 °C for 18 h, the reaction mixture was treated with saturated aqueous NH_4Cl (10 mL) and stirred at 25 °C for 1 h. The mixture was extracted with 20% 2-PrOH–$CHCl_3$ (5 × 20 mL), and the combined organic extracts were dried ($MgSO_4$). Removal of solvent in vacuo afforded a mixture of the 2- and 4-(hydroxymethyl)pyrimidines (2-CH_2OH:4-CH_2OH = 7:1, 122 mg, 153 mg theoretical, 80%) as a white solid. The two isomeric alcohols[42] were separated by preparative centrifugal thin-layer chromatography (SiO_2, 5% DMF–EtOAc), and the selectivity of the reduction varied from 4:1 to 2:1 (C2-CH_2OH:C4-CH_2OH) in EtOH, 6:1 in 2-PrOH (75–88% yield, –20 °C, 3 days), and 6-7:1 in t-BuOH–THF (1:2, 80–87%, –30 to –25 °C, 24 h). For the reaction detailed above, pure **10** was isolated in 70% yield (107 mg): mp 154 °C sharp (white needles, EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.06 (1H, s, C5-H), 5.24 (2H, br s, NH_2), 4.70 (2H, s, CH_2OH), 4.45 (2H, q, J = 7.1 Hz), 3.62 (1H, br s, OH), 1.42 (3H, t, J = 7.1 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.9 (e, C-6), 165.3 (e, CO_2Et), 165.0 (e, C-2), 153.3 (e, C-4), 104.3 (o, C-5), 64.9 (e, CH_2OH), 61.9 (e, CH_2CH$_3$), 14.6 (o, CH_2CH_3); irradiation of either CH_2OH or C5-H in **10** did not result in a difference NOE, while irradiation of CH_2OH in the minor isomer, 6-amino-2-(ethoxycarbonyl)-4-(hydroxymethyl)pyrimidine, gave a 5% NOE enhancement of C5-H; IR (KBr) ν_{max} 3355, 3162, 2996, 1736, 1652, 1597, 1547, 1492, 1470, 1397, 1338, 1250, 1138, 1068, 1022, 974 cm^{-1}; EIMS m/e (relative intensity) 197 (M^+, 3), 125 (100), 94 (10), 67 (90); CIMS (2-methylpropane) m/e 198 (M^+ + H, base).

Anal. Calcd for $C_8H_{11}N_3O_3$: C, 48.73; H, 5.58; N, 21.32. Found: C, 48.50; H, 5.80; N, 21.39.

6-Amino-4-(ethoxycarbonyl)-2-[((*p*-toluenesulfonyl)oxy)methyl]pyrimidine (11). A suspension of **10** (169 mg, 0.86 mmol) in THF–CH_2Cl_2 (1:1, 3 mL) was treated with K_2CO_3 (237 mg, 1.72 mmol, 2.0 equiv) and TsCl (246 mg, 1.29 mmol, 1.5 equiv) at 25 °C under Ar, and the reaction mixture was stirred at 25 °C (17 h). The crude reaction mixture was filtered through Celite (CH_2Cl_2, 3 × 5 mL), and the solvent was removed in vacuo. Chromatography (SiO_2, 1.5 × 5 cm, 50% EtOAc–hexane) afforded **11** (275 mg, 301 mg theoretical, 91%) as a white solid: mp 151 °C sharp (white needles, EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.83 (2H, d, J = 8.1 Hz), 7.35 (2H, d, J = 8.0 Hz), 7.07 (1H, s, C5-H), 6.51 (2H, br s, NH_2), 5.05 (2H, s, CH_2OTs), 4.42 (2H, q, J = 7.1 Hz), 2.45 (3H, s), 1.40 (3H, t, J = 7.1 Hz); 2D ^{1}H–^{1}H NOESY NMR (DMSO-d_6, 200 MHz) did not reveal a NOE crosspeak between –CH_2OTs and C5-H for **11** but did so for the minor isomer derived from the $NaBH_4$ reduction of **9**; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.0 (e, CO_2Et), 164.1 (e, C-2), 163.0 (e, C-6), 154.0 (e, C-4), 145.5 (e), 131.9 (e) 130.0 (o) 128.3 (o), 105.6 (o, C-5), 70.8 (e, CH_2OTs), 62.4 (e), 21.7 (o, CH_3), 14.2 (o); IR (KBr) ν_{max} 3324, 3166, 2988, 2366, 1734, 1700, 1684, 1654, 1636, 1542, 1430, 1364, 1134, 990 cm^{-1}; CIMS (2-methylpropane) m/e (relative intensity) 352 (M^+ + H, 5), 201 (100).

Anal. Calcd for $C_{15}H_{17}N_3O_5S$: C, 51.28; H, 4.84; N, 11.97; S, 9.14. Found: C, 51.44; H, 4.84; N, 11.76; S, 8.91.

(*S*)-3-Amino-2-[(*tert*-butyloxycarbonyl)amino]propionamide (12). A solution of Ph_3P (4.85 g, 18.5 mmol, 1.5 equiv) in THF (50 mL) was cooled to –78 °C and treated with DEAD (3.39 g, 18.5 mmol, 1.5 equiv), HN_3–benzene[12,43] (18 mL, 1.03 M, 18.5 mmol, 1.5 equiv), and a solution of *N*-Boc-L-serine methyl ester (2.7 g, 12.3 mmol) in THF (10 mL). The mixture was stirred at 25 °C for 24 h before the solvent was removed in vacuo. Chromatography (SiO_2, 5 × 18 cm, 20% Et_2O–hexane) afforded methyl (*S*)-3-azido-2-[(*tert*-butyloxycarbonyl)amino]propionate[12] (2.73 g, 3.0 g theoretical, 91%) as a colorless oil.

A solution of methyl (*S*)-3-azido-2-[(*tert*-butyloxycarbonyl)amino]-propionate (2.86 g, 11.7 mmol) in CH_3OH (30 mL) was cooled to –20 °C and treated with 10% NH_3 in CH_3OH (20 mL). After 1 h at –20 °C, the reaction mixture was stirred at 24 °C (6 h). Removal of solvent in vacuo followed by chromatography (SiO_2, 2 × 15 cm, 70% Et_2O–hexane) afforded (*S*)-3-azido-2-[(*tert*-butyloxycarbonyl)amino]propionamide[44] (2.08 g, 2.45 g theoretical, 85%) as a white solid: mp 83–84 °C (white needles, Et_2O–hexane); $[\alpha]^{22}_D$ +61 (*c* 0.65, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.29 (1H, br s, CONH*H*), 5.59 (1H, br s, CONH*H*), 5.30 (1H, br d, J = 6.3 Hz, NHBOC), 4.31 (1H, m, C*H*NH BOC), 3.89 (1H, dd, J = 12.3, 4.4 Hz, C*H*HCH), 3.55 (1H, dd, J = 12.3, 5.8 Hz, CH*H*CH), 1.47 (9H, s, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 177.4 (e), 155.6 (e) 80.9 (e), 53.3 (o), 52.0 (e), 28.1 (o); IR (KBr) ν_{max} 3332, 2980, 2934, 2114, 1702, 1508, 1452, 1394, 1368, 1252, 1166, 1050, 1026, 866, 782, 748 cm^{-1}; ClMS (2-methylpropane) m/e 230 (M^+ + H, base).

Anal. Calcd for $C_8H_{15}N_5O_3$; C, 41.92; H, 6.60; N, 30.55. Found: C, 41.78; H, 6.79; N, 30.23.

A solution of (*S*)-3-azido-2-[(*tert*-butyloxycarbonyl)amino]propionamide (295 mg, 1.29 mmol) in CH_3OH (10 mL) was treated with 10% Pd–C (30 mg, 0.1 wt equiv) under H_2 for 5 h. The reaction mixture was filtered through Celite (CH_3OH, 3 × 10 mL), and solvent was removed

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(41) ^{13}C NMR (e) and (o) refer to even and odd number of attached protons determined through DEPT ^{13}C NMR.

(42) For 6-amino-2-(ethoxycarbonyl)-4-(hydroxymethyl)pyrimidine: ^{1}H NMR (CD_3OD, 400 MHz) δ 6.80 (1H, s, C5-H), 4.52 (2H, s, CH_2OH), 4.39 (2H, q, J = 7.0 Hz), 1.39 (3H, t, J = 7.0 Hz); IR (neat film) ν_{max} 3311, 3172, 1732, 1654, 1601, 1480, 1277, 1233, 1209, 1165, 1020, 976, 860, 754 cm^{-1}. For 6-amino-2,4-bis(hydroxymethyl)pyrimidine: ^{1}H NMR (CD_3OD, 400 MHz) δ 6.55 (1H, s, C5-H), 4.46 (4H, s, CH_2OH); IR (neat film) ν_{max} 3341, 3202, 1662, 1607, 1429, 1394, 1339, 1081, 991, 847 cm^{-1}.

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(44) Alternatively, treatment of *N*-Boc-L-serinamide with HN_3–benzene, Ph_3P–DEAD (THF, –70 °C to 24 °C) provided the azide in good yield (>70%), but purification from the Mitsunobu reaction byproducts proved tedious. Similarly, NaN_3 displacement (DMF, 50 °C, 55–84%) of the mesylate derived from *N*-Boc-L-serinamide provided the azide, but competitive β-elimination proved problematic on larger scales.

in vacuo to afford pure **12**[12] as a white foam (258 mg, 266 mg theoretical, 97%) which was used directly in the next reaction.

(*S*)-N^{α},N^{β}-Bis(*tert*-butyloxycarbonyl)-N^{β}-[(4-(ethoxycarbonyl)-6-aminopyrimidin-2-yl)methyl]-β-aminoalaninamide (13). A solution of **11** (20 mg, 0.057 mmol) and **12** (41.3 mg, 0.20 mmol, 3.5 equiv) in CH_3CN (0.2 mL) was treated with $NaHCO_3$ (19.2 mg, 0.23 mmol, 4 equiv), and the mixture was stirred under Ar at 25 °C (24 h). Removal of solvent in vacuo afforded the crude product, which was dissolved in THF–H_2O (1:1, 1 mL) and treated with di-*tert*-butyldicarbonate (75 mg, 0.34 mmol, 6 equiv) and K_2CO_3 (15.8 mg, 0.11 mmol, 2 equiv). The resulting mixture was stirred at 25 °C (24 h). Saturated aqueous $NaHCO_3$ (5 mL) was added, and the mixture was extracted with 20% 2-PrOH–$CHCl_3$ (5 × 10 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. Chromatography (SiO_2, 0.5 × 10 cm, 50–100% EtOAc–hexane gradient elution) afforded **13** (22 mg, 27.4 mg theoretical, 80% for two steps) as a white solid: mp 159 °C sharp (white needles, EtOAc–hexane); $[\alpha]^{22}_D$ +42 (*c* 2.4, $CHCl_3$); ^{1}H NMR (CD_3OD, 300 MHz) δ 7.08 (1H, s, C5-H), 4.60–4.30 (5H, m), 3.82–3.55 (2H, m, CH_2CH), 1.43–1.27 (18H, m, two $C(CH_3)_3$); ^{13}C NMR (CD_3OD, 75 MHz) δ 176.2 (e), 173.3 (e), 169.6 (e), 166.6 (e), 158.0 (e, C-2), 157.7 (e, C-6), 154.8 (e, C-4), 105.5 (o, C-5), 105.1 (e, CH_2NBOC), 82.3 (e), 81.5 (e), 80.8 (e, CH_2CH), 63.2 (e, OCH_2CH_3), 55.6 (o, CHNHBOC), 28.6 (o), 28.4 (o), 14.4 (o); IR (KBr) ν_{max} 3358, 2978, 1686, 1650, 1582, 1460, 1408, 1368, 1334, 1250, 1166, 1074, 1020, 874, 764 cm^{-1}; CIMS (2-methylpropane) *m/e* 483 (M^+ + H, base); CIHRMS (2-methylpropane) *m/e* 483.2567 (M^+ + H, $C_{21}H_{34}N_6O_7$ requires 483.2567).

Anal. Calcd for $C_{21}H_{34}N_6O_7$: C, 52.28; H, 7.05; N, 17.43. Found: C, 51.91; H, 7.34; N, 17.07.

(*S*)-N^{α},N^{β}(*tert*-butyloxycarbonyl)-N^{β}-[(4-carboxy-6-aminopyrimidin-2-yl)methyl]-β-aminoalaninamide (14). A solution of **13** (68 mg, 0.14 mmol) in THF–EtOH–H_2O (3:2:1, 2 mL) at 25 °C was treated with aqueous 1 N LiOH (0.43 mL, 0.43 mmol, 3 equiv), and the mixture was stirred for 3 h. The reaction mixture was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (2 × 15 mL). Aqueous 1 N HCl (0.43 mL, 0.43 mmol, 3 equiv) was added to the aqueous phase, which was extracted with 15% 2-PrOH–$CHCl_3$ (5 × 15 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo to afford **14** (61 mg, 64 mg theoretical, 95%) as a white foam: mp 129–130 °C; $[\alpha]^{22}_D$ +3.1 (*c* 0.9, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.15 (1H, s, C5-H), 4.80–4.40 (3H, m), 3.90–3.60 (2H, m, CH_2CH), 1.50, 1.41 and 1.29 (18H, $C(CH_3)_3$); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.7 (e), 167.7 (e), 165.0 (e), 163.8 (e), 157.7 (e, C-2), 157.3 (e, C- 6), 152.0 (e, C-4), 103.9 (o, C-5), 82.8 (e, $C(CH_3)_3$), 82.2 (e, $C(CH_3)_3$), 80.8 (e, CH_2NBOC), 64.7 (e, CH_2CH), 55.4 (o, CHNHBOC), 28.7 (o, $C(CH_3)_3$), 28.4 (o, $C(CH_3)_3$); IR (KBr) ν_{max} 3510, 3390, 2970, 2890, 1685, 1470, 1450, 1390, 1250, 1160, 1005 cm^{-1}; FABHRMS (NBA) *m/e* 455.2254 (M^+ + H, $C_{19}H_{30}N_6O_7$ requires 455.2254).

Anal. Calcd for $C_{19}H_{30}N_6O$: C, 50.21; H, 6.65; N, 18.49. Found: C, 50.30; H, 6.51; N, 18.56.

[N^{im}-(*tert*-Butyloxycarbonyl)-L-histidyl]-L-alanine *tert*-Butyl Ester (15). A solution of N^{α}-CBZ-L-histidine (858 mg, 2.97 mmol) in THF–H_2O (1:1, 10 mL) was cooled to 0 °C and treated with aqueous 2 N NaOH (3 mL, 2 equiv) and di-*tert*-butyldicarbonate (681 mg, 3.12 mmol, 1.05 equiv). The reaction mixture was stirred at 25 °C under Ar for 8 h. Aqueous 2 N HCl (3 mL, 2 equiv) was added, and the resulting mixture was extracted with EtOAc (3 × 15 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo to afford N^{α}-CBZ-N^{im}-Boc-L-histidine as a white solid (1.06 g, 1.16 g theoretical, 92%).[45]

A solution of N^{α}-CBZ-N^{im}-Boc-L-histidine (627 mg, 1.6 mmol) in CH_2Cl_2 (10 mL) was treated with HOBt–H_2O (261 mg, 1.9 mmol, 1.2 equiv) and DCC (365 mg, 1.77 mmol, 1.1 equiv) at 0 °C under Ar for 5 min. L-Alanine *tert*-butyl ester hydrochloride (380 mg, 2.1 mmol, 1.3 equiv) and $NaHCO_3$ (189 mg, 2.25 mmol, 1.4 equiv) were added, and the mixture was stirred at 25 °C (24 h). The mixture was filtered through Celite (CH_2Cl_2, 5 × 10 mL), and the solvent was removed in vacuo. Chromatography (SiO_2, 2 × 10 cm, 40% EtOAc–hexane) afforded N^{α}-CBZ-**15** as a white solid (706 mg, 831 mg theoretical, 85%): mp 50–51 °C (white needles, EtOAc–hexane); $[\alpha]^{22}_D$ −11 (*c* 0.3, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92 (1H, br s, C2-H(His)), 7.35 (1H, s, C5-H(His)), 7.20 (1H, br s, CONH), 6.31 (1H, br d, *J* = 7.3 Hz, NHCBZ), 5.06 (1H, d, *J* = 12.3 Hz, CHHPh), 5.01 (1H, d, *J* = 12.3 Hz, CHHPh), 4.47 (1H, m, H_{α}-His), 4.30 (1H, dq, *J* = 7.1, 7.0 Hz, H_{α}-Ala), 3.03 (1H, dd, *J* = 14.7, 4.8 Hz, CHHCH(His)), 2.90 (1H, dd, *J* = 14.7, 5.6 Hz, CHHCH(His)), 1.52 (9H, s, $C(CH_3)_3$ of N^{im}-Boc), 1.36 (9H, s, $C(CH_3)_3$), 1.19 (3H, d, *J* = 7.0 Hz, $CHCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.6 (e), 170.4 (e), 156.1 (e), 146.7 (e), 138.7 (e), 136.7 (o), 136.2 (o), 128.4 (o), 128.0 (o), 126.3 (e), 114.9 (o), 85.7 (e), 81.7 (e), 66.9 (e), 54.5 (o), 48.6 (o), 30.3 (e), 27.8 (o), 27.7 (o), 18.2 (o); IR (KBr) ν_{max} 3300, 2977, 2922, 1756, 1728, 1667, 1533, 1456, 1389, 1289, 1256, 1153, 1048, 1011, 843, 774, 744, 697 cm^{-1}; FABMS (NBA) *m/e* 517 (M^+ + H, base); FABHRMS (NBA) *m/e* 517.2689 (M^+ + H, $C_{26}H_{36}N_4O_7$ requires 517.2662).

Anal. Calcd for $C_{26}H_{36}N_4O_7$: C, 60.45; H, 7.02; N, 10.85. Found: C, 60.45; H, 7.02; N, 10.90.

A solution of N^{α}-CBZ-N^{im}-Boc-L-His-L-Ala-OtBu (134 mg, 0.26 mmol) in CH_3OH (10 mL) was treated with 10% Pd–C (13.4 mg, 0.1 wt equiv) under H_2 for 8 h. The reaction mixture was filtered through Celite (CH_3OH, 3 × 10 mL), and solvent was removed in vacuo to afford pure **15** as a white foam (94 mg, 99 mg theoretical, 95%), which was used directly in the next reaction. For **15**: $[\alpha]^{22}_D$ +65 (*c* 0.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (1H, d, *J* = 1.0 Hz, C2-H(His)), 7.83 (1H, br d, *J* = 7.5 Hz, NH), 7.11 (1H, s, C5-H(His)), 4.38 (1H, dq, *J* = 7.5, 7.2 Hz, H_{α}-Ala), 3.61 (1H, m, H_{α}-His), 3.00 (1H, dd, *J* = 14.5, 4.0 Hz, CHHCH), 2.75 (1H, dd, *J* = 14.5, 8.2 Hz, CHHCH), 1.54 (9H, s, $C(CH_3)_3$ of N^{im}-Boc), 1.40 (9H, s, $C(CH_3)_3$), 1.27 (3H, d, *J* = 7.2 Hz, $CHCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7 (e), 172.1 (e), 146.8 (e), 140.0 (o, C-2(His)), 136.6 (e, C-4(His)), 114.4 (o, C-5(His)), 85.4 (e, $C(CH_3)_3$), 81.6 (e, $C(CH_3)_3$), 54.9 (o, C_{α}-Ala), 48.1 (o, C_{α}-His), 32.8 (e, CH_2CH), 27.8 (o, $C(CH_3)_3$), 27.7 (o, $C(CH_3)_3$), 18.5 (o, $CHCH_3$); IR (KBr) ν_{max} 3368, 3318, 2987, 2926, 1760, 1725, 1674, 1454, 1243, 1012, 836 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 515.1271 (M^+ + Cs, $C_{18}H_{30}N_4O_5$ requires 515.1267).

N^{α}-[2-[[*N*-*tert*-Butyloxycarbonyl-*N*-[(*S*)-2-[*N*-(*tert*-butyloxycarbonyl)-amino]-2-(carbamoyl)ethyl]amino]methyl]-6-aminopyrimidine-4-carbonyl][N^{im}-(*tert*-butyloxycarbonyl)-L-histidyl]-L-alanine *tert*-Butyl Ester (16). A solution of **14** (61 mg, 0.13 mmol) in DMF (0.7 mL) was cooled to 0 °C under Ar and treated with EDCI (33 mg, 0.17 mmol, 1.3 equiv) and HOBt–H_2O (25 mg, 0.18 mmol, 1.4 equiv). After 5 min, **15** (80 mg, 0.21 mmol, 1.6 equiv) was added, and the reaction mixture was stirred under Ar at 22 °C (3 days). A solution of 10% 2-PrOH–$CHCl_3$ (20 mL) was added, and the mixture was washed with H_2O (5 × 15 mL). The organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. Chromatography (SiO_2, 0.8 × 15 cm, 50–100% EtOAc–hexane and 5% CH_3OH–CH_2Cl gradient elution) afforded **16** (87 mg, 109 mg theoretical, 80%) as a white foam: $[\alpha]^{22}_D$ +65 (*c* 0.02, $CHCl_3$); ^{1}H NMR (CD_3OD, 300 MHz) δ 8.12 (1H, s, C2-H(His)), 7.33 (1H, s, C5-H(His)), 6.93 (1H, s, C5-H), 4.80–4.20 (5H, m), 3.08 (2H, m, CH_2CH(His)), 1.58 (9H, s, $C(CH_3)_3$ of N^{im}-Boc), 1.42–1.21 (27H, m) 1.32 (3H, d, *J* = 7.3 Hz, $CHCH_3$); ^{13}C NMR (CD_3OD, 75 MHz) δ 173.6 (e), 172.8 (e), 168.8 (e), 167.3 (e), 166.0 (e), 158.9 (e, C-2), 156.5 (e, C-6), 148.5 (e, C-4), 139.7 (o, C-2(His)), 138.9 (e, C-4(His)), 116.8 (o, C-5(His)), 102.0 (o, C-5), 87.2 (e, $C(CH_3)_3$), 82.8 (e, $C(CH_3)_3$), 81.6 (e, $C(CH_3)_3$), 81.0 (e, $C(CH_3)_3$), 64.4 (e, CH_2NBoc), 62.1 (o, C_{α}-His), 54.1 (o, CHNHBoc), 51.9 (e, CH_2NHBoc), 47.2 (o, C_{α}-Ala), 31.7 (e, CH_2CH(His)), 28.7 (o, $C(CH_3)_3$, 28.6 (o, $C(CH_3)_3$), 28.3 (o, $C(CH_3)_3$), 28.1 (o, $C(CH_3)_3$), 17.5 (o, $CHCH_3$); IR (KBr) ν_{max} 3334, 2928, 1750, 1700, 1684, 1654, 1636, 1558, 1542, 1522, 1508, 1458, 1396, 1158, 1010, 842 cm^{-1}; FABMS (NBA) *m/e* (relative intensity) 819 (M^+ + H, 20), 719 (base); FABHRMS (NBA) *m/e* 819.4381 (M^+ + H, $C_{37}H_{58}N_{10}O_{11}$ requires 819.4365).

(–)-Desacetamido P-3A (3). A solution of **16** (4 mg, 4.9 μmol) in EtOAc (0.1 mL) was cooled to 0 °C and treated with 3 N HCl in EtOAc (3 mL). The reaction mixture was stirred at 22 °C (1 h). Removal of solvent in vacuo afforded **3** as a white solid. Pure **3** (2.7 mg, 2.8 mg theoretical, 96%) was obtained by trituration with anhydrous CH_2Cl_2 (3 × 5 mL): mp 188 °C dec; $[\alpha]^{22}_D$ −13.3 (*c* 0.15, CH_3OH), −18.0 (*c* 0.15, 0.1 N aqueous HCl); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.79 (1H, s, C2-H(His)), 7.46 (1H, s, C5-H(His)), 7.03 (1H, s, C5-H), 4.75 (1H, dd, *J* = 5.4, 5.8 Hz), 4.64 (1H, dd, *J* = 6.8, 7.5 Hz), 4.42 (1H, d, *J* = 14 Hz), 4.32 (1H, q, *J* = 7.4 Hz), 4.28 (1H, d, *J* = 14 Hz), 3. 72 (1H, dd, *J* = 6.0, 14.0 Hz), 3.65 (1H, dd, *J* = 6.0, 14.0 Hz), 3.45 (1H, dd, *J* = 6.8, 14.0 Hz), 3.38 (1H, dd, *J* = 7.5, 14.0 Hz), 1.44 (3H, d, *J* = 7.5 Hz, $CHCH_3$); IR (KBr) ν_{max} 3470, 3410, 3342, 2990, 2930, 1720, 1666, 1637, 1548, 1455, 1431, 1396, 1370, 1255, 1237, 1155, 1091, 1044, 1008, 979, 932, 897, 808, 779, 615 cm^{-1}; FABMS (NBA) *m/e* (relative intensity) 463 (M^+ + H, 10), 176 (100); FABHRMS (NBA) *m/e* 463.2160 (M^+ + H, $C_{18}H_{26}N_{10}O_5$ requires 463.2166).

6-Amino-2,4-bis(ethoxycarbonyl)-5-(methylthio)pyrimidine (19). A solution of **4**[10] (1.19 g, 4 mmol) in anhydrous DMF (10 mL) under Ar

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was treated with 2-(methylthio)acetamidine hydrochloride (**17**,[14] 1.12 g, 8 mmol, 2.0 equiv) at 25 °C, and the reaction mixture was warmed at 90 °C (48 h). Removal of the solvent in vacuo and chromatography (SiO_2, 3 × 15 cm, 50% EtOAc–hexane) afforded pure **19** (1.03 g, 1.14 g theoretical, 90%) as a white solid: mp 148 °C sharp (white needles, EtOAc–hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.48 (2H, q, J = 7.2 Hz), 4.47 (2H, q, J = 7.2 Hz), 2.35 (3H, s), 1.43 (3H, t, J = 7.2 Hz), 1.42 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.1 (e), 164.9 (e), 163.3 (e, C-2), 162.6 (e, C-6) , 155.7 (e, C-4), 111.3 (e, C-5), 62.8 (e), 62.3 (e), 17.9 (o, SCH_3), 14.1 (o), 14.0 (o); IR ($CHCl_3$) ν_{max} 3500, 3392, 2992, 1739, 1599, 1544, 1448, 1383, 1231, 1199, 1026 cm^{-1}; FABHRMS (NBA) m/e 286.0878 (M^+ + H, $C_{11}H_{15}N_3O_4S$ requires 286.0861).

Anal. Calcd for $C_{11}H_{15}N_3O_4S$: C, 46.31; H, 5.30; N, 14.73; S, 11.24. Found: C, 46.38; H, 5.30; N, 14.70; S, 11.30.

6-Amino-4-(ethoxycarbonyl)-2-(hydroxymethyl)-5-(methylthio)pyrimidine (20). A solution of **19** (782 mg, 2.74 mmol) in EtOH–THF (1:1, 14 mL) was cooled to 0 °C and treated with $NaBH_4$ (208 mg, 5.5 mmol, 2.0 equiv) under Ar. The reaction mixture was stirred at 25 °C (2 h) and treated with saturated aqueous NH_4Cl (15 mL). The mixture was extracted with 10% 2-PrOH–$CHCl_3$ (3 × 15 mL), and the combined organic extracts were dried (Na_2SO_4). Removal of solvent followed by chromatography (SiO_2, 2 × 15 cm, 70% EtOAc–hexane) afforded pure **20** (567 mg, 667 mg theoretical, 85%) as a white solid: mp 94 °C sharp (white needles, EtOAc–hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.39 (2H, br s, NH_2), 4.63 (2H, s, CH_2OH), 4.46 (2H, q, J = 7.2 Hz), 2.30 (3H, s, SCH_3), 1.42 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.6 (e), 165.4 (e, C-2), 164.6 (e, C-6), 162.3 (e, C-4), 106.8 (e, C-5), 64.0 (e, CH_2OH), 62.2 (e, CH_2CH_3), 18.0 (o, SCH_3), 14.0 (o, CH_2CH_3); IR ($CHCl_3$) ν_{max} 3510, 3392, 2993, 2928, 1738, 1600, 1541, 1453, 1366, 1227, 1091, 1025 cm^{-1}; FABHRMS (NBA–CsI) m/e 375.9736 (M^+ + Cs, $C_9H_{13}N_3O_3S$ requires 375.9729).

Anal. Calcd for $C_9H_{13}N_3O_3S$: C, 44.43; H, 5.39; N, 17.27; S, 13.18. Found: C, 44.41; H, 5.19; N, 17.25; S, 13.10.

6-Amino-4-(ethoxycarbonyl)pyrimidine-2-carboxaldehyde (21). A solution of **10** (190 mg, 0.96 mmol) in anhydrous CH_3CN (20 mL) was treated with activated MnO_2 (839 mg, 9.6 mmol, 10 equiv) at 25 °C, and the resulting suspension was warmed at 82 °C (3 h). The cooled reaction mixture was filtered through Celite (CH_3CN, 5 × 10 mL), and the solvent was removed in vacuo. Flash chromatography (SiO_2, 1 × 3 cm, 80% EtOAc–hexane) afforded pure **21** (160 mg, 188 mg theoretical, 85%) as a white foam: mp 87 °C dec; ^1H NMR ($CDCl_3$, 300 MHz) δ 10.0 (1H, s, CHO), 7.31 (1H, s, C5-H), 5.80 (2H, br s, NH_2), 4.50 (2H, q, J = 7.1 Hz), 1.45 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 191.9 (o, CHO), 165.9 (e, CO_2Et), 164.4 (e, C-2), 160.2 (e, C-6), 153.8 (e, C-4), 108.8 (o, C-5), 61.8 (e), 14.1 (o); IR (K Br) ν_{max} 3350, 2973, 2902, 2863, 1731, 1696, 1656, 1621, 1328, 1248, 1144, 1054, 816 cm^{-1}; FABHRMS (NBA) m/e 196.0702 (M^+ + H, $C_8H_9N_3O_3$ requires 196.0722).

Anal. Calcd for $C_8H_9N_3O_3$: C, 49.23; H, 4.65; N, 21.53. Found: C, 49.38; H, 4.43; N, 21.66.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{β}-[[6-amino-4-(ethoxycarbonyl)pyrimidin-2-yl]methylene]amino]-(*S*)-β-aminoalaninamide (22). A solution of **21** (116 mg, 0.595 mmol) and **12** (121 mg, 0.595 mmol, 1.0 equiv) in anhydrous CH_3CN (8 mL) was treated with 4-Å molecular sieves (2 g) at 25 °C, and the suspension was stirred at 25 °C (24 h). The reaction mixture was filtered through Celite (CH_3CN, 5 × 15 mL). Removal of solvent in vacuo afforded **22** (226 mg, 226 mg theoretical, quantitative) as a white foam: $[\alpha]^{22}_D$ +15.5 (*c* 0.65, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.30 (1H, s, CH=N), 7.01 (1H, s, C5-H), 6.02 (2H, s, NH_2), 4.80-4.50 (3H, m, CH_2CH_3, CH_2CH) 4.10–3.90 (2H, m, CH_2CH), 1.40–1.20 (12H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.3 (e, C=N), 168.5 (e), 165.0 (e), 164.3 (e), 164.2 (e, C-6), 161.1 (e), 153.4 (e, C-4), 107.3 (e, C-5), 80.1 (e, $C(CH_3)_3$), 62.6 (e, CH_2CH_3), 62.2 (o, CH_2CH), 61.5 (e, CH_2CH), 28.1 (o, $C(CH_3)_3$), 14.0 (o, CH_2CH_3); IR ($CHCl_3$) ν_{max} 3550, 3350, 2990, 1750, 1716, 1652, 1502, 1470, 1208, 1056, 916 cm^{-1}; FABHRMS (NBA–CsI) m/e 513.0863 (M^+ + Cs, $C_{16}H_{24}N_6O_5$ requires 513.0859).

Diastereoselective Reaction of the Stannous (*Z*)-Enolate of (4*S*,5*R*)-3-(Methylthioacetyl)-4-methyl-5-phenyl-2-oxazolidinone with N^{α}-(*tert*-Butyloxycarbonyl)-N^{β}-[(6-amino-4-(ethoxycarbonyl)pyrimidin-2-yl)-methyl]-(*S*)-β-aminoalaninamide. A solution of $Sn(OTf)_2$ (409 mg, 0.98 mmol, 4.0 equiv) in dry THF (1 mL) under Ar cooled to –78 °C was treated sequentially with (4*S*,5*R*)-3-(methylthioacetyl)–4–methyl–5–phenyl–2–oxazolidinone (131 mg, 0.49 mmol, 2.0 equiv) in dry THF (1 mL) and *i*-Pr_2NEt (140 mg, 1.08 mmol, 4.4 equiv). The mixture was stirred for 1 h at –20 °C and then recooled to –78 °C. A solution of **22** (93.5 mg, 0.25 mmol) in dry THF (2 mL) was added, and the reaction mixture was allowed to warm to –5 °C, where it was stirred for 11 h. The reaction mixture was poured into a two-phase solution of CH_2Cl_2 (15 mL) and saturated aqueous $NaHCO_3$ (15 mL) with vigorous stirring. The mixture was filtered through Celite (CH_2Cl_2, 3 × 10 mL), and the organic layer was washed with saturated aqueous NaCl (2 × 10 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 1.5 × 3 cm, 5% CH_3OH–CH_2Cl_2) afforded 123 mg (159 mg theoretical, 77%) of a 86:14 mixture of **24a**:**24b**. Preparative centrifugal thin-layer chromatography (SiO_2, 60–100% EtOAc–hexane graduent elution) afforded **24a** (94.4 mg) and **24b** (16.6 mg) as white foams.

Ethyl (*R*)-2-[1-[[(*S*)-2-[(*tert*-butyloxycarbonyl)amino]-2-carbamoylethyl]amino]-2-[((4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinyl)carbonyl]-2-(*S*)-(methylthio)-1-ethyl]-6-aminopyrimidine-4-carboxylate (24a): $[\alpha]^{25}_D$ –26 (*c* 4.8, $CHCl_3$); ^1H NMR (CD_3OD, 400 MH) δ 7.32–7.41 (5H, m), 7.02 (1H, s, C5-H), 5.78 (1H, d, J = 7.3 Hz), 5.10 (1H, d, J = 10.9 Hz), 4.82 (1H, m), 4.36 (2H, q, J = 7.1 Hz), 4.15 (1H, d, J = 10.9 Hz), 3.98 (2H, m), 2.70 (2H, m), 2.04 (3H, s, SCH_3), 1.40–1.20 (12H, m), 0.93 (3H, d, J = 6.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.4 (e), 171.4 (e), 171.0 (e), 166.9 (e), 166.0 (e), 158.9 (e, C-2), 154.4 (e, C-6), 154.1 (e, C-4), 135.1 (e), 129.7 (o), 129.6 (o), 127.0 (o), 105.8 (o, C-5), 80.2 (e, $C(CH_3)_3$), 65.5 (o, CHAr), 63.5 (o, $CHSCH_3$), 63.1 (e, CH_2CH_3) 56.4 (o, CHPh), 53.6 (o, $CHCH_3$), 50.2 (o, $CHCH_2$), 47.9 (e, CH_2CH), 28.7 (o, $C(CH_3)_3$), 15.4 (o, SCH_3), 14.5 (o, CH_2CH_3), 12.2 (o, $CHCH_3$); IR ($CHCl_3$) ν_{max} 3420, 2995, 2980, 1770, 1715, 1650, 1565, 1358, 1210, 1130, 950 cm^{-1}; FABHRMS (NBA) m/e 646.2672 (M + H^+, $C_{29}H_{39}N_7O_8S$ requires 646.2659).

Ethyl (*S*)-2-[1-[[(*S*)-2-[(*tert*-butyloxycarbonyl)amino]-2-carbamoylethyl]amino]-2-[((4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinyl)carbonyl]-2-(*S*)-(methylthio)-1-ethyl]]-6-aminopyrimidine-4-carboxylate (24b): $[\alpha]^{25}_D$ +29 (*c* 2.6, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.45–7.20 (5H, m), 7.02 (1H, s, C-5), 5.78 (1H, d, J = 7.4 Hz), 4.95 (1H, d, J = 9.5 Hz), 4.85 (1H, m), 4.42 (2H, q, J = 7.2 Hz), 4.22 (1H, d, J = 9.5 Hz), 4.14 (1H, m), 2.90–2.70 (2H, m), 2.10 (3H, s, SCH_3), 1.45–1.20 (12H, m), 0.86 (3H, d, J = 6.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.0 (e), 169.8 (e), 169.2 (e), 164.9 (e), 164.6 (e), 155.9 (e, C-2) 153.7 (e, C-6), 152.7 (e, C-4), 133.0 (e), 128.8 (o), 128.7 (o), 125.6 (o), 105.4 (o, C-5), 78.9 (e, $C(CH_3)_3$), 64.4 (o, CHAr), 62.0 (o, $CHSMe$), 60.3 (o, CH_2CH_3), 55.2 (o, CHPh), 53.5 (o, $CHCH_3$), 49.2 (o, $CHCH_2$), 46.6 (e, CH_2CH), 28.2 (o, $C(CH_3)_3$), 14.7 (o, SCH_3), 14.1 (o, CH_2CH_3), 11.8 (o, $CHCH_3$); IR ($CHCl_3$) ν_{max} 3422, 2995, 1779, 1725, 1689, 1612, 1575, 1450, 1362, 1215, 1125, 960 cm^{-1}; FABHRMS (NBA–CsI) m/e 778.1635 (M^+ + Cs, $C_{29}H_{39}N_7O_8S$ requires 778.1632).

Ethyl (*S*)-2-[1-[[(*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-2-carbamoylethyl]amino]-2-[((4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinyl)carbonyl]ethyl]-6-aminopyrimidine-4-carboxylate (25). A solution of **24a** (52.4 mg, 0.08 mmol) in C_6H_6 (1 mL) was treated with Bu_3SnH (94 mg, 0.32 mmol, 4.0 equiv) and AIBN (1.3 mg, 0.008 mmol, 0.1 equiv) and was warmed at 80°C (45 min) under Ar. The mixture was allowed to cool to 23 °C, and the solvent was evaporated in vacuo. Flash chromatography (SiO_2, 1 × 5 cm, 5% CH_3OH–CH_2Cl_2) afforded **25** as a white foam (43.1 mg, 47.9 mg theoretical, 90%): mp 125–127 °C (EtOAc–hexane); $[\alpha]^{25}_D$ –10.2 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50 (5H, m, Ph), 7.00 (1H, s, C5-H), 5.69 (1H, d, J = 7.2 Hz), 4.75 (1H, m), 4.62 (1H, m, CHAr), 4.40 (2H, q, J = 7.1 Hz), 4.30 (1H, m, CH_2CH), 3.80–3.45 (3H, m, CH_2CHAr, $CHHCH$), 2.85 (1H, m, $CHHCH$), 1.43–1.40 (12H, m), 0.90 (3H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.0 (e), 171.2 (e), 170.6 (e), 168.5 (e), 164.6 (e), 156.2 (e, C-2), 154.2 (e, C-6), 153.2 (e, C-4), 133.5 (e), 128.5 (o), 125.7 (o), 104.5 (o, C-5), 79.2 (e, $C(CH_3)_3$), 67.1 (o, CHAr), 62.1 (e, CH_2CH_3), 60.2 (o, CHPh), 54.7 (o, $CHCH_3$), 52.2 (o, $CHCH_2$), 31.2 (e, CH_2CH), 29.4 (e, CH_2CH), 28.3 (o, $C(CH_3)_3$), 14.2 (o, CH_2CH_3), 13.4 (o, $CHCH_3$); IR ($CHCl_3$) ν_{max} 3410, 3010, 2990, 1778, 1695, 1628, 1560, 1450, 1365, 1210, 1155, 1078 cm^{-1}; FABHRMS (NBA) m/e 600.2758 (M + H^+, $C_{28}H_{37}N_7O_8$ requires 600.2782).

N^{α}-(*tert*-Butyloxycarbonyl)-N^{β}-[1-amino-3(*S*)-(4-amino-6-(ethoxycarbonyl)pyrimidin-2-yl)propan-3-yl]-(*S*)-β-aminoalaninamide (26). Solid **25** (13.8 mg, 0.023 mmol) was treated with an EtOH solution of NH_3 (14%, 10 mL), and the solution was stirred for 1.5 h at 0°C. The solvent was evaporated in vacuo, and flash chromatography (SiO_2, 0.5 × 5 cm, 10:1 CH_2Cl_2–CH_3OH) afforded **26** as a white solid (7.1 mg, 10.1 mg theoretical, 70%): mp 146–148 °C (2-PrOH–hexane); $[\alpha]^{22}_D$ –8.7 (*c* 0.25, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.01 (1H, s, C5-H), 4.41 (2H, q, J = 7.1 Hz, CH_2CH_3), 4.21 (1H, m), 4.12 (1H, m), 2.95 (2H, m), 2.67 (2H, m), 1.41 (12H, m); ^{13}C NMR (CD_3OD, 100 MHz)

δ 176.5, 176.4, 167.0, 154.6, 154.5, 105.4 (o, C-5), 80.7 (e, $C(CH_3)_3$), 64.7, 63.2, 55.7, 49.7, 47.9, 28.7, 14.5; IR (CH_3OH) ν_{max} 3400, 3009, 2980, 1729, 1680, 1550, 1410, 1250, 1050, 940 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 572.1234 (M^+ + Cs, $C_{18}H_{29}N_7O_6$ requires 572.1234).

N^{α}-(*tert*-Butyloxycarbonyl)-N^{β}-[1-amino-3(*S*)-(4-amino-6-carboxypyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalaninamide (27). A solution of **25** (11 mg, 0.025 mmol) in THF–CH_3OH–H_2O (3:1:1, 0.3 mL) at 0°C was treated with aqueous 1 N LiOH (37 μL, 0.037 mmol, 1.5 equiv), and the mixture was stirred at 0°C for 1.5 h. After evaporation of most of the THF–CH_3OH, the aqueous phase was acidified to pH 4–5 with the addition of aqueous 1.2 N HCl, and the solvent was evaporated in vacuo. The residue was charged onto a column of Dowex (1 × 8 cm, acetate form, 50–100 mesh). The column was washed with H_2O, and subsequent elution with 6% HOAc–H_2O afforded **27** as a white powder (8.9 mg, 10.3 mg theoretical, 88%): mp 205–207 °C (EtOH–hexane); $[\alpha]^{25}_D$ –26 (*c* 0.15, H_2O); ^1H NMR (D_2O, 400 MHz) δ 6.91 (1H, s), 4.35 (2H, m), 3.31 (1H, m), 3.06 (1H, m), 2.91 (2H, m), 1.38 (9H, s); IR (neat) ν_{max} 3428, 3157, 1718, 1686, 1578, 1467, 1256, 1108, 868 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 544.0920 (M^+ + Cs, $C_{16}H_{25}N_7O_6$ requires 544.0921).

In an alternative purification of **27**, crude acid (0.023 mmol theoretical) was chromatographed on C-18 (2 × 0.5 cm, H_2O eluant) to afford **27** (9.3 mg, 9.36 mg theoretical, 99%).

N^{β}-[1-Amino-3(*S*)-(4-amino-6-carboxypyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalaninamide (28). The solid **27** (1.2 mg, 0.003 mmol) was treated with 3 N HCl–EtOAc (0.4 mL), and the mixture was stirred at 25 °C for 1 h. The solvent was evaporated in vacuo to give pure **28**–hydrochloride (1.3 mg, 1.3 mg theoretical, 100%) as a clear hygroscopic solid: $[\alpha]^{25}_D$ –23 (*c* 0.065, H_2O); ^1H NMR (D_2O, 400 MHz) δ 7.05 (1H, s), 4.29 (1H, dd, *J* = 5.6, 7.6 Hz), 4.14 (1H, dd, *J* = 5.6, 4.8 Hz), 3.21 (1H, dd, *J* = 4.8, 13.6 Hz), 3.11 (1H, dd, *J* = 5.6, 13.6 Hz), 2.91 (1H, dd, *J* = 5.6, 16.0 Hz), 2.85 (1H, dd, *J* = 7.6, 16.0 Hz); IR (neat) ν_{max} 3442, 3236, 1 700, 1678, 1498, 1152, 1062, 819 cm^{-1}; FABHRMS (NBA–NaI) *m/e* 334.1244 (M^+ + Na, $C_{11}H_{17}N_7O_4$ requires 334.1240).

Ethyl (*R*)-2-[1-[[(*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-2-carbamoylethyl]amino]-2-[[(4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinyl]carbonyl]ethyl]-6-aminopyrimidine-4-carboxylate (29). A solution of **24h** (16.4 mg, 0.03 mmol) in C_6H_6 (1 mL) was treated with Bu_3SnH (30 mg, 0.10 mmol, 4 equiv) and AIBN (1 mg, 0.006 mmol, 0.2 equiv), and the solution was warmed at 80 °C (45 min) under N_2. The mixture was allowed to cool to 23 °C, and the solvent was evaporated in vacuo. Flash chromatography (SiO_2, 1 × 3 cm, 5% CH_3OH–CH_2Cl_2) afforded **29** (14 mg, 15 mg theoretical, 93%) as a white solid: mp 135°C sharp (EtOAc–hexane); $[\alpha]^{25}_D$ +6.0 (*c* 0.75, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.50–7.25 (5H, m), 7.02 (1H, s, C5-H), 5.82 (1H, d, *J* = 7.2 Hz), 4.79 (1H, m), 4.39 (2H, q, *J* = 7.1 Hz), 4.22 (1H, m), 4.09 (1H, m), 3.50 (2H, m), 3.26 (1H, m), 2.90 (2H, m), 1.50–1.30 (12H, m), 0.81 (3H, d, *J* = 6.6 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.3, 172.3, 171.5, 166.9, 166.0, 157.7, 154.8, 154.5, 135.3, 129.7, 129.6, 127.0, 105.4, 80.4, 63.1, 56.0, 50.0, 49.6, 41.8, 29.1, 28.7, 28.1, 14.8, 14.5; IR ($CHCl_3$) ν_{max} 3420, 3019, 2998, 2960, 1735, 1689, 1610, 1570, 1370, 1220, 1120, 1080 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 732.1758 (M^+ + Cs, $C_{28}H_{37}N_7O_8$ requires 732.1755).

N^{α}-(*tert*-Butyloxycarbonyl)-N^{β}-[1-amino-3(*R*)-4-amino-6-(ethoxycarbonyl)pyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalaninamide (30). Compound **29** (39.4 mg, 0.066 mmol) was subjected to aminolysis as described for **26** to provide **30** (19.1 mg, 28.9 mg theoretical, 66%, 66–77%) as a white solid: mp 79–81 °C (2-PrOH–hexane): $[\alpha]^{25}_D$ +24 (*c* 0.75, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.02 (1H, s, C5-H), 4.37 (2H, q, *J* = 7.1 Hz), 4.20–4.01 (2H, m), 3.00–2.50 (4H, m), 1.50–1.20 (12H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.7, 176.6, 167.0, 166.1, 154.8, 154.7, 105.2, 7 8.5, 63.0, 62.1, 55.9, 50.0, 47.9, 28.9, 14.4; IR (neat) ν_{max} 3400, 3010, 2990, 1730, 1620, 1560, 1415, 1230, 1050, 950 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 572.1234 (M^+ + Cs, $C_{18}H_{29}N_7O_6$ requires 572.1230).

N^{α}-(*tert*-Butyloxycarbonyl)-N^{β}-[1-amino-3(*R*)-4-amino-6-carboxypyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalaninamide (31). Compound **30** (14.2 mg, 0.032 mmol) was subjected to hydrolysis and purification as described for **27** to provide **31** (13.2 mg, 13.3 mg theoretical, 99%): mp 210–212 °C (EtOH–hexane); $[\alpha]^{25}_D$ + 14 (*c* 0.16, H_2O); ^1H NMR (D_2O, 400 MHz) δ 7.11 (1H, s), 3.56 (1H, m), 3.44 (1H, m), 3.15 (4H, m); IR (neat) ν_{max} 3425, 3120, 1708, 1692, 1583, 1452, 1258, 1162, 890 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 544.0921 (M^+ + Cs, $C_{16}H_{25}N_7O_6$ requires 544.0917).

N^{β}-[1-Amino-3(*R*)-(4-amino-6-carboxypyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalaninamide (32). The solid **31** (2.0 mg, 0.005 mmol) was subjected to acid-catalyzed deprotection in the same fashion as **28** to give **32**–hydrochloride (2.2 mg, 2.2 mg theoretical, 100%) as a clear, hygroscopic solid: $[\alpha]^{25}_D$ + 20 (*c* 0.10, H_2O); ^1H NMR (D_2O, 400 MHz) δ 7.06 (1H, s), 4.36 (1H, dd, *J* = 6.6, 7.6 Hz), 4.13 (1H, dd, *J* = 5.5, 6.2 Hz), 3.21 (1H, dd, *J* = 8.7, 13.5 Hz), 3.14 (1H, dd, *J* = 6.2, 13.5 Hz), 2.91 (1H, dd, *J* = 5.5, 14.1 Hz) , 3.85 (1H, dd, *J* = 7.5, 14.1 Hz); IR (neat) ν_{max} 3438, 3226, 1700, 1681, 1510, 1062, 825 cm^{-1}; FABHRMS (NBA–NaI) *m/e* 334.1250 (M^+ + Na, $C_{11}H_{17}N_7O_4$ requires 334.1240).

N^{α}-(*tert*-Butyloxycarbonyl)-N^{β}-[1-amino-3(*S*)-(4-amino-6-(((*tert*-butyloxy)-L-alanyl-N^{im}-(*tert*-butyloxycarbonyl)-L-histidyl)carbonyl)pyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalaninamide (33). A solution of **27** (3.7 mg, 0.009 mmol) in DMF–THF (1:1, 0.2 mL) was treated with **15** (5.9 mg, 0.015 mmol, 1.7 equiv), HOBt (1.3 mg, 0.009 mmol, 1.0 equiv), and EDCl (1.83 mg, 0.009 mmol, 1.05 equiv), and the mixture was stirred at 25 °C (70 h). The reaction mixture was concentrated in vacuo to give an oily solid. Chromatography (SiO_2, 1 × 2 cm, 15–20% CH_3OH–$CHCl_3$ gradient elution) afforded **33** (4.0 mg, 6.97 mg theoretical, 58%) as a thin film: R_f 0.35 (SiO_2, 25% CH_3OH–$CHCl_3$); $[\alpha]^{25}_D$ –40 (*c* 0.15, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.18 (1H, s), 7.50 (1H, s), 6.96 (1H, s), 4.81 (1H, t, *J* = 6.0 Hz), 4.31 (1H, q, *J* = 7.2 Hz), 4.22 (1H, br t), 4.05 (1H, m), 3.22 (2H, m), 2.92 (2H, m), 2.71 (1H, dd, *J* = 5.1, 10.0 Hz), 2.60 (1H, m), 1.64 (9H, s), 1.49 (18H, s), 1.40 (3H, d, *J* = 7.3 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.6, 173.2, 172.9, 170.6, 167.0, 165.6, 155.9, 148.2, 139.5, 138.5, 116.9, 102.1, 87.1, 82.6, 63.5, 61.9, 55.4, 54.4, 50.3, 41.7, 31.4, 29.2, 28.7, 28.2, 28.0, 17.4; IR (neat) ν_{max} 3319, 3243, 2918, 2852, 1680, 1650, 1579, 1374, 1333, 1215, 1162, 1069, 1018 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 908.3039 (M^+ + Cs, $C_{34}H_{53}N_{11}O_{10}$ requires 908.3028).

(+)-P-3A (1). The solid **33** (8.2 mg, 0.011 mmol) was treated with 3.5 N HCl–EtOAc (5 mL), and the mixture was stirred at 25 °C (2 h). The solvent was evaporated in vacuo, and the oily solid was triturated with $CHCl_3$ (1 × 1 mL) to give **1** (5.4 mg, 5.5 mg theoretical, 98%) as a hygroscopic solid: R_f 0.34 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ +80 (*c* 0.015, H_2O); ^1H NMR (D_2O, 400 MHz) δ 8.46 (1H, s), 7.18 (1H, s), 6.93 (1H, s), 4.76 (1H, dd, *J* = 5.2, 5.8 Hz), 4.50 (1H, dd, *J* = 6.8, 7.6 Hz), 4.34 (1H, dd, *J* = 5.5, 7.0 H z), 4.23 (1H, q, *J* = 7.4 Hz), 3.57 (1H, dd, *J* = 5.2, 14.0 Hz), 3.44 (1H, dd, *J* = 5.8, 14.0 Hz), 3.27 (1H, dd, *J* = 6.8, 14.0 Hz), 3.18 (1H, dd, *J* = 7.6, 14.0 Hz), 2.92 (1H, dd, *J* = 5.5, 14.0 Hz), 2.96 (1H, dd, *J* = 7.0, 14.0 Hz), 1.27 (3H, d, *J* = 7.2 Hz); ^{13}C NMR (D_2O, 100 MHz) δ 178.8, 176.3, 173.4, 170.9, 167.6, 166.8, 164.8 155.6, 136.0, 130.5, 120.0, 106.2, 62.1, 55.1, 52.7, 51.6, 48.8, 38.4, 29.3, 18.5; IR (neat) ν_{max} 3307, 3188, 1684, 1654, 1634, 1559, 1538, 1517, 1457, 1414, 1359, 1265, 1162, 1098 cm^{-1}; FABHRMS (NBA) *m/e* 520.2389 (M^+ + H, $C_{20}H_{29}N_{11}O_6$ requires 520.2380).

N^{α}-(*tert*-Butyloxycarbonyl)-N^{β}-[1-amino-3(*R*)-(4-amino-6-(((*tert*-butyloxy)-L-alanyl-N^{im}-(*tert*-Butyloxycarbonyl)-L-histidyl)carbonyl)pyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalaninamide (34). A solution of **31** (13.2 mg, 0.032 mmol) in DMF–TMF (1:1, 0.2 mL) was treated with **15** (18.4 mg, 0.048 mmol, 1.5 equiv), HOBt (4.34 mg, 0.032 mmol, 1.0 equiv), and EDCl (6.4 mg, 0.034 mmol, 1.05 equiv), and the mixture was stirred at 25 °C (70 h). The reaction mixture was concentrated in vacuo to give an oily solid. Chromatography (SiO_2, 1 × 3 cm, 15–20% CH_3OH–$CHCl_3$ gradient elution) afforded **34** (15.4 mg, 24.9 mg theoretical, 62%) as a thin film: R_f 0.32 (SiO_2, 25% CH_3OH–$CHCl_3$); $[\alpha]^{25}_D$ +43 (*c* 0.025, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.19 (1H, s), 7.43 (1H, s), 6.97 (1H, s), 4.86 (1H, t, *J* = 5.6 Hz), 4.31 (1H, q, *J* = 7.2 Hz), 4.25 (1H, br t), 4.09 (1H, m), 3.21 (2H, m), 3.03 (1H, dd, *J* = 5.2, 10.0 Hz), 2.90 (1H, m), 2.70 (1H, m), 2.62 (1H, dd, *J* = 6.0, 10.0 Hz), 1.64 (9H, s), 1.49 (18H, s), 1.40 (3H, d, *J* = 7.2 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.6, 173.2, 172.6, 170.6, 167.0, 165.5, 155.9, 148.2, 139.4, 138.5, 116.9, 102.1, 87.1, 82.6, 63.5, 61.9, 55.5, 54.2, 50.3, 41.7, 31.5, 29.2, 28.7, 28.3, 28.0, 17.4; IR (neat) ν_{max} 3320, 3222, 2920, 1677, 1661 1648, 1581, 1398, 1368, 1331, 1214, 1163, 1090, 1015 cm^{-1}; FABHRMS (NBA) *m/e* 776.4083 (M^+ + H, $C_{34}H_{53}N_{11}O_{10}$ requires 776.4055).

(–)-*epi*-P-3A (2). The solid **34** (13.2 mg, 0.017 mg, 0.017 mmol) was treated with 3.5 N HCl–EtOAc (5 mL), and the mixture was stirred at 25 °C (2 h). The solvent was evaporated in vacuo, and the oily solid was triturated with $CHCl_3$ (3 × 1 mL) to give **2** (8.75 mg, 8.8 mg theoretical, 99%) as a hygroscopic solid: R_f 0.36 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ –34 (*c* 0.02, H_2O); ^1H NMR (D_2O, 400 MHz) δ 8.46 (1H, s), 7.16 (1H, s), 6.93 (1H, s), 4.75 (1H, dd, *J* = 5.8, 5.9 Hz), 4.54 (1H, dd, *J* = 6.9, 7.5 Hz), 4.34 (1H, dd, *J* = 5.8, 6.5 H z), 4.23 (1H, q, *J* = 7.4 Hz), 3.56 (1H, dd, *J* = 5.9, 14.0 Hz), 3.47 (1H, dd, *J* = 5.8, 14.0 Hz), 3.27 (1H, dd, *J* = 6.9, 14.0 Hz), 3.17 (1H, dd, *J* = 7.5, 13.8 Hz). 2.97 (2H, apparent d, *J* = 8.7 Hz), 1.27 (3H, d, *J* = 7.2 Hz); ^{13}C NMR (D_2O, 100 MHz) δ 178.7, 176.3, 173.3,

170.7, 167.4, 166.9, 164.8, 155.8, 136.0, 130.5, 120.0, 106.1, 62.0, 55.1, 52.6, 51.4, 48.4, 37.9, 29.2, 18.5; IR (neat) ν_{max} 3323, 3066, 1697, 1664, 1633, 1625, 1528, 1515, 1441, 1364, 1164, 995 cm^{-1}; FABHRMS (NBA) m/e 520.2380 ($M^+ + H$, $C_{20}H_{29}N_{11}O_6$ requires 520.2380).

General Procedure for the DNA Cleavage Reactions: Relative Efficiency Study. All reactions were run with freshly prepared agent–Fe(II) complexes. The agent–Fe(II) complexes were prepared by combining 1 μL of a water solution of agent at 10 times the specified concentration with 1 μL of a freshly prepared equimolar aqueous ferrous ammonium sulfate solution followed by vortex mixing. Each of the agent–Fe(II) complex solutions was treated with 7 μL of a buffered DNA solution containing 0.25 μg of supercoiled *φX174* RFI DNA (1.4×10^{-8} M) in 50 mM Tris–HCl buffer solution (pH 8). The DNA cleavage reactions were initiated by adding 1 μL of aqueous 10 mM 2-mercaptoethanol. The final concentrations of the agents employed in the study were 0.2–5 μM bleomycin A_2, 0.2–5 μM deglycobleomycin A_2, and 0.2–20 μM each (+)-P-3A (**1**), (–)-*epi*-P-3A (**2**), and (–)-desacetamido P-3A (**3**). The DNA reaction solution was incubated at 25 °C for 1 h. The reactions were quenched with the addition of 5 μL of loading buffer formed by mixing Keller buffer (0.4 M Tris–HCl, 0.05 M NaOAc, 0.0125 M EDTA, pH 7.9) with glycerol (40%), sodium dodecyl sulfate (0.4%), and Bromophenol Blue (0.3%). Electrophoresis was conducted on a 1% agarose gel containing 0.1 μg/mL ethidium bromide at 50 V for 2.5 h, and the gel was immediately visualized on a UV transilluminator and photographed using Polaroid T667 black and white instant film. Direct fluorescence quantitation of DNA in the presence of ethidium bromide was conducted using a Millipore Bio Image 60S RFLP system visualized on a UV (312 nm) transilluminator, taking into account the relative fluorescence intensities of Forms I–III *φX174* DNA (Forms II and III fluorescence is 0.7 times that of Form I).

General Procedure for Quantitation of Double-Stranded and Single-Stranded DNA Cleavage. The agent–Fe(II) complexes were formed by mixing 1 μL of a selected concentration of a water solution of agent with 1 μL of a freshly prepared equimolar aqueous ferrous ammonium sulfate solution. Seven microliters of a buffered DNA solution containing 0.25 μg of supercoiled *φX174* RFI DNA (1.4×10^{-8} M) in 50 mM Tris–HCl buffer solution (pH 8) was added to each of the agent–Fe(II) complex solutions. The final concentrations of the agents employed in the study were 1 μM bleomycin A_2, 2.5 μM deglycobleomycin A_2, 10 μM **1**, and 20 μM **2** or **3**. The DNA cleavage reactions were initiated by adding 1 μL of aqueous 10 mM 2-mercaptoethanol to each of the reaction mixtures. The solutions were thoroughly mixed and incubated at 25 °C for 40, 30, 20, 15, 10, 8, 6, 4, 2, and 1 min, respectively. The reactions were quenched with the addition of 5 μL of loading buffer, and electrophoresis was run on a 1% agarose gel containing 0.1 μg/mL ethidium bromide at 50 V for 2.5 h. Direct fluorescence quantitation of the DNA in the presence of ethidium bromide was conducted using a Millipore Bio Image 60S RFLP system, taking into account the relative fluorescence intensities of Forms I–III1 *φX174* DNA (Forms II and III fluorescence is 0.7 times that of Form I). The ratio of the double-strand to single-strand cleavage was calculated with use of the Freifelder–Trumbo equation[33] assuming a Possion distribution.

General Procedure for Cleavage of 5′-End-Labeled w794 DNA: Selectivity. All reactions were run with freshly prepared agent–Fe(II) complexes. The agent–Fe(II) complexes were prepared by combining 1 μL of a water solution of agent at 10 times the specified concentration with 1 μL of a freshly prepared equimolar aqueous ferrous ammonium sulfate solution. Each of the agent–Fe(II) complex solutions was treated with 7 μL of a buffered DNA solution containing the ^{32}P 5′-end-labeled w794 DNA[36] in 50 mM Tris–HCl buffer solution (pH 8). The final concentrations of the agents employed in the study were 1–10 μM bleomycin A_2, 1–50 μM deglycobleomycin A_2, and 50–1000 μM each **1**–**3**. The DNA cleavage reactions were initiated by adding 1 μL of aqueous 10 mM 2-mercaptoethanol. The DNA reaction solutions were incubated at 37 °C for 30 min. The reactions were quenched with the addition of 2 μL of glycerol, followed by ethanol precipitation and isolation of the DNA. The DNA was resuspended in 10 μL of TE buffer, and formamide dye was added (10 μL) to the supernatant. Prior to electrophoresis, the samples were warmed at 100 °C for 5 min, placed in an ice bath, and centrifuged, and the supernatant was loaded onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the agent-treated DNA. Gel electrophoresis was carried out using a denaturing 8% sequencing gel (19:1 acrylamide:*N*,*N*-methylenebisacrylamide, 8 M urea). Formamide dye contained xylene cyanol FF (0.03%), Bromophenol Blue (0.03%), and aqueous Na_2EDTA (8.7%, 250 mM). Electrophoresis running buffer (TBE) contained Tris base (100 mM), boric acid (100 mM), and $Na_2EDTA–H_2O$ (0.2 mM). Gels were prerun for 30 min with formamide dye prior to loading of the samples. Autoradiography of dried gel was carried out at –78 °C using Kodak X-Omat AR film and a Picker Spectra intensifying screen.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (D.L.B.; CA42056; S.L.C.; GM15302) and a Glaxo fellowship (T.H.), and we thank Dr. T. Nakatani for copies of the IR, ^1H NMR, and ^{13}C NMR spectra of natural P-3A.