

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

Dale L. Boger,* Takeshi Honda, Royce F. Menezes, Steven L. Colletti, Qun Dang, and Wenjin Yang

Contribution from the Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037

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 pyrimidine 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 A₂ at producing cleavage of duplex DNA. Like Fe(II)-bleomycin A₂ or deglycobleomycin A₂, Fe(II)-1-3 produced both single- and double-strand cleavage, although with a decreased propensity for double-stranded cleavage. Unlike bleomycin A₂ or deglycobleomycin A₂, 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 A₂ 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 reaction⁴ 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), pyrimidoblastic

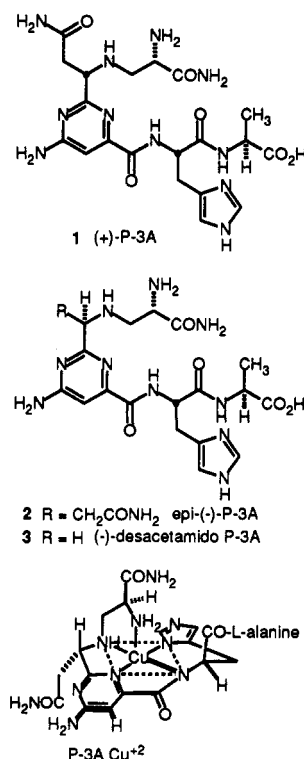


Figure 1.

acid,⁶ bleomycin A₂,⁷ 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.

The pyrimidine nucleus constitutes the core of the iron(II) chelation subunit required for O₂ activation and the subsequent double-stranded DNA cleavage thought to be responsible for the

* Abstract published in *Advance ACS Abstracts*, November 15, 1993.
 (1) Iitaka, Y.; Nakamura, H.; Nakatani, T.; Muraoka, Y.; Fujii, A.; Takita, T.; Umezawa, H. *J. Antibiot.* 1978, 31, 1070.
 (2) Ohno, M.; Otsuka, M. In *Recent Progress in the Chemical Synthesis of Antibiotics*; Lukacs, G., Ohno, M., Eds.; Springer-Verlag: New York, 1990; p 387. Hecht, S. M. *Acc. Chem. Res.* 1986, 19, 383. Stubbe, J.; Kozarich, J. W. *Chem. Rev.* 1987, 87, 1107. Sugiura, Y.; Takita, T.; Umezawa, H. *Metal Ions Biol. Syst.* 1985, 19, 81. Povirk, L. F. In *Molecular Aspects of Anti-Cancer Drug Action*; Neidle, S., Waring, M. J., Eds.; MacMillan: London, 1983. Hecht, S. M. In *Bleomycin: Chemical, Biochemical and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979. Umezawa, H. In *Bleomycin: Current Status and New Developments*; Carter, S. K., Crooke, S. T., Umezawa, H., Eds.; Academic Press: New York, 1978. Umezawa, H. *Pure Appl. Chem.* 1971, 28, 665.
 (3) Boger, D. L.; Dang, Q. *J. Org. Chem.* 1992, 57, 1631.
 (4) Boger, D. L.; Weinreb, S. M. *Hetero Diels-Alder Methodology in Organic Synthesis*; Academic Press: San Diego, CA, 1987. Boger, D. L. *Tetrahedron* 1983, 39, 2869. Boger, D. L. *Chem. Rev.* 1986, 86, 781. Boger, D. L.; Patel, M. *Prog. Heterocycl. Chem.* 1989, 1, 30. Boger, D. L. *Bull. Soc. Chim. Belg.* 1990, 99, 599.
 (5) Boger, D. L.; Dang, Q. *Tetrahedron* 1988, 44, 3379.

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 A₂ 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 (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

Scheme I

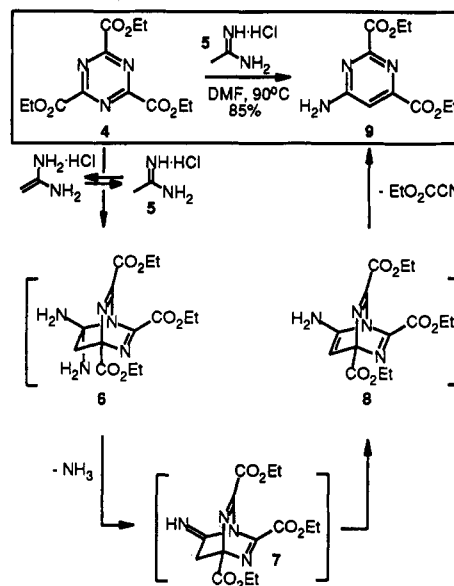


Table I. Representative Results of a Study of the [4 + 2] Cycloaddition Reaction of 4 with 5 and 17

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 ₂ CO ₃ 100 °C, 48 h	9	42
10	dioxane 100 °C, 48 h	9	0
11	CH ₃ CN 84 °C, 48 h	9	0
12	DMF 90 °C, 20 h	19	78
13	DMF 90 °C, 48 h	19	90

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.

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. Antibiot.* **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.

(8) (a) Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M. *Tetrahedron* **1988**, *44*, 2811, 2821. (b) Owa, T.; Haupt, A.; Otsuka, M.; Kobayashi, S.; Tomioka, N.; Itai, A.; Ohno, M.; Shiraki, T.; Uesugi, M.; Sugiura, Y.; Maeda, K. *Tetrahedron* **1992**, *48*, 1193. (c) Shipley, J. B.; Hecht, S. M. *Chem. Res. Toxicol.* **1988**, *1*, 25. (d) Carter, B. J.; Murty, V. S.; Reddy, K. S.; Wang, S. N.; Hecht, S. M. *J. Biol. Chem.* **1990**, *265*, 4193. (e) Carter, B. J.; Reddy, K. S.; Hecht, S. M. *Tetrahedron* **1991**, *47*, 2463. (f) Hamamichi, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278. (g) Tan, J. D.; Hudson, S. E.; Brown, S. J.; Olmstead, M. M.; Mascharak, P. K. *J. Am. Chem. Soc.* **1992**, *114*, 3841.

(9) Boger, D. L.; Menezes, R. F.; Dang, Q. *J. Org. Chem.* **1992**, *57*, 4333. Boger, D. L.; Menezes, R. F. *J. Org. Chem.* **1992**, *57*, 4331. Boger, D. L.; Menezes, R. F.; Dang, Q.; Yang, W. *BioMed. Chem. Lett.* **1992**, *2*, 261.

(10) Ott, E. *Chem. Ber.* **1919**, *52*, 656. Grundmann, C.; Weisse, G.; Seide, S. *Liebigs Ann. Chem.* **1952**, *577*, 77.

(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

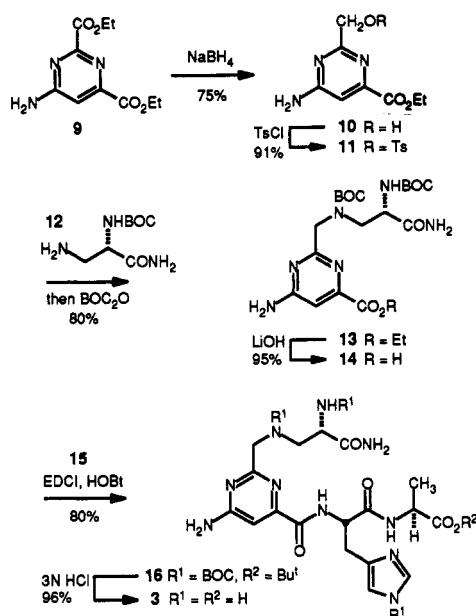


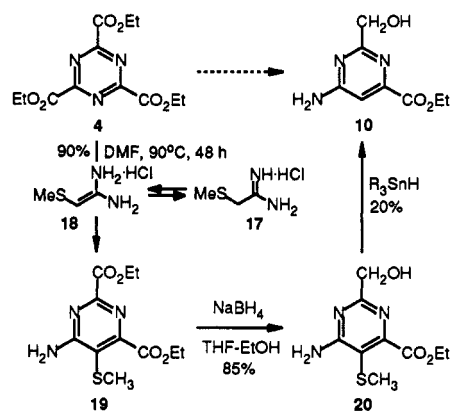
Table II. Representative Results of the Selective Reduction of 9

entry	conditions ^a	2-CH ₂ OH:4-CH ₂ OH	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	<i>t</i> BuOH-THF (1:2) -20 °C, 18 h	7:1	70
6	<i>t</i> BuOH-THF (1:2) -30 °C, 24 h	6-7:1	87
7	<i>t</i> BuOH-THF (1:5) -20 °C, 18 h	7:1	58
8	<i>t</i> BuOH-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 ₂ OH only	25 ^d

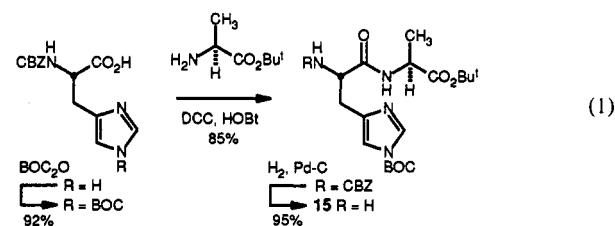
^a 1.0 equiv of NaBH₄. ^b Starting material recovered. ^c The isopropyl ester was isolated due to transesterification. ^d No 4-CH₂OH detected by ¹H NMR.

of the C2-ethoxycarbonyl group, the selective reduction of 9 may be conducted effectively with NaBH₄ 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-CH₂OH: C4-CH₂OH) when conducted in 2-PrOH (3 days) or 1:2 *t*-BuOH-THF (7:1 C2-CH₂OH: C4-CH₂OH, 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(ClO₄)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 -CH₂-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 12¹² and subsequent protection of the secondary amine to provide 13 (80%, two steps).¹³ Notably, the pyrimidine C6-amino 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-OBu¹ (15, eq 1) provided desacetamido P-3A in its fully protected form 16 (80%). Initial



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%, [α]_D²⁰ -13.3 (*c* 0.15, CH₃OH), -18 (*c* 0.15, 0.1 N HCl)].

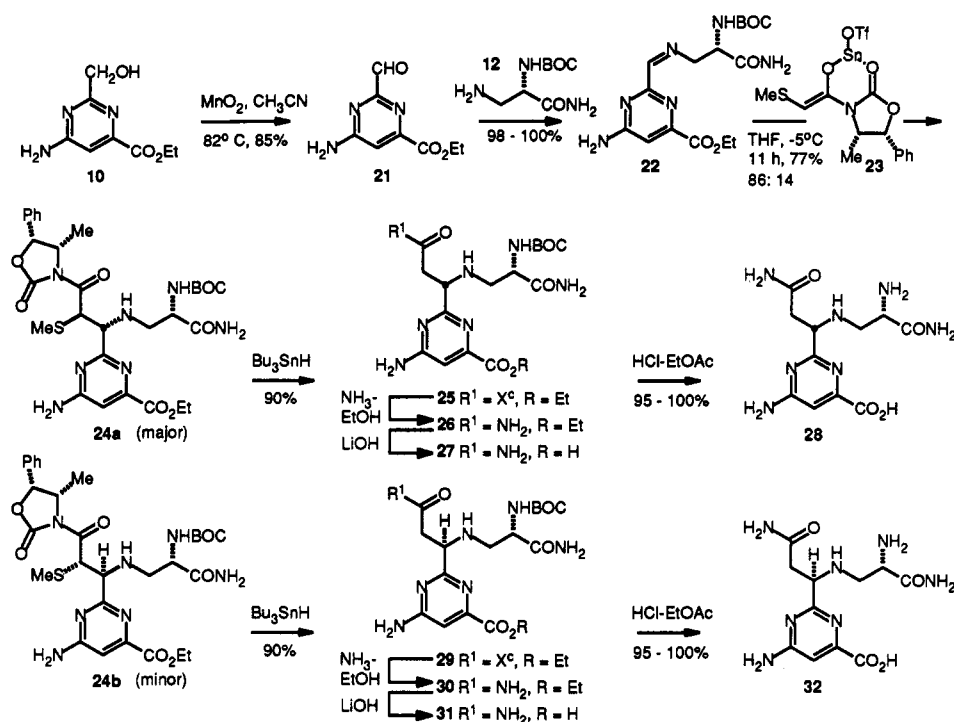
In an alternative approach to the preparation of 10, the key C2- and C4-ethoxycarbonyl differentiation was anticipated to be simplified with the selective reduction of the electronically more reactive and sterically less hindered C2-ethoxycarbonyl group of 19. Treatment of 4 with (methylthio)acetamidine hydrochloride¹⁴ (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 NaBH₄ 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 PtO₂, H₂, 25 °C, 28 h) and subsequent protection of the secondary amine (Boc₂O), 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)acetamidine by the following reactions: (i) MeOH, HCl, Et₂O, -20 °C, 12 h; (ii) NH₃, 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, $\text{NiCl}_2\text{-NaBH}_4$, and $\text{Zn/THF-NH}_4\text{Cl}$ 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 pyrimidoblastic acid pyrimidine cores bearing a range of C5 substituents.

Diastereoselective *N*-Acylloxazolidinone 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 pyrimidoblastic acid subunit of the bleomycins⁶ 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 pyrimidoblastic 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*-acyloxazolidinones¹⁷ 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 oxidants¹⁹ 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 $\text{Sn}(\text{OTf})_2$ (2.0 equiv)

(17) Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem.* **1982**, *13*, 1.

(18) Boger, D. L.; Honda, T. *Tetrahedron Lett.* **1993**, *34*, 1567.

(19) This included the examination of $\text{Ba}(\text{MnO}_4)_2$, PCC, PDC, $(\text{COCl})_2\text{-DMSO}$, 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*-butylboron enolate²¹ proved ineffective.

(21) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127.

(22) Nerz-Stormes, M.; Thornton, E. R. *J. Org. Chem.* **1991**, *56*, 2489.

(23) Evans, D. A.; Clark, J. S.; Metternich, R.; Novack, V. J.; Sheppard, G. S. *J. Am. Chem. Soc.* **1990**, *112*, 866.

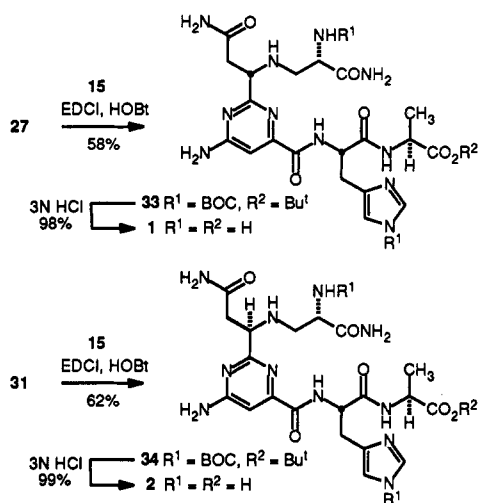
(24) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1986**, *108*, 6757. Evans, D. A.; Gage, J. R.; Leighton, J. L.; Kim, A. S. *J. Org. Chem.* **1992**, *57*, 1961.

(25) Nagao, Y.; Kumagai, T.; Nagase, Y.; Tamai, S.; Inoue, Y.; Shiro, M. *J. Org. Chem.* **1992**, *57*, 4232. Nagao, Y.; Nagase, Y.; Kumagai, T.; Kuramoto, Y.; Kobayashi, S.; Inoue, Y.; Taga, T.; Ikeda, H. *J. Org. Chem.* **1992**, *57*, 4238. Nagao, Y.; Nagase, Y.; Kumagai, T.; Matsunaga, H.; Abe, T.; Shimada, O.; Hayashi, T.; Inoue, Y. *J. Org. Chem.* **1992**, *57*, 4243. Nagao, Y.; Dai, W.-M.; Ochiai, M.; Tsukagoshi, S.; Fujita, E. *J. Org. Chem.* **1990**, *55*, 1148. Nagao, Y.; Dai, W.-M.; Ochiai, M. *Tetrahedron Lett.* **1988**, *29*, 6133.

(15) Hart, D. J.; Ha, D.-C. *Chem. Rev.* **1989**, *89*, 1447. Brown, M. J. *Heterocycles* **1989**, *29*, 2225.

(16) Corey, E. J.; Decicco, C. P.; Newbold, R. C. *Tetrahedron Lett.* **1991**, *32*, 5287. Ojima, I.; Habus, I. *Tetrahedron Lett.* **1990**, *31*, 4289. Hart, D. J.; Lee, C.-S.; Pirkle, W. H.; Hyon, M. H.; Tsiouras, A. *J. Am. Chem. Soc.* **1986**, *108*, 6054. Furukawa, M.; Okawara, T.; Noguchi, Y.; Terawaki, Y. *Chem. Pharm. Bull.* **1978**, *26*, 260. Shibasaki, M.; Ishida, Y.; Iwasaki, G.; Iimori, T. *J. Org. Chem.* **1987**, *52*, 3488. Georg, G. I.; Kant, J.; Gill, H. S. *J. Am. Chem. Soc.* **1987**, *109*, 1129.

Scheme V



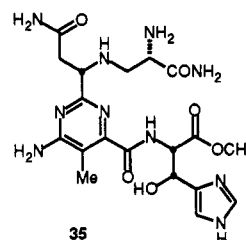
provided a separable 86:14 (6:1) mixture of two diastereomeric *anti* imine addition products **24a** and **24b**, respectively, in good yield (77%). In agreement with observations made in related studies,¹⁸ the reaction provides predominantly the *anti* imine adduct **24a** and requires a minimal number 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** ($-5\text{ }^{\circ}\text{C}$, 11 h, >20:1 *anti*:*syn*-**24a** versus $0\text{ }^{\circ}\text{C}$, 29 h, 3:2 *anti*:*syn*-**24a**). Reductive desulfurization (Bu_3SnH , 90–95%) of the major diastereomer **24a** and subsequent careful aminolysis of **25** ($0\text{ }^{\circ}\text{C}$, 1 h) afforded **26**. Ethyl ester hydrolysis provided the *N* α -*tert*-butyloxycarbonyl derivative **27**, and subsequent acid-catalyzed deprotection provided **28**, $[\alpha]^{25}_{\text{D}} -23$ (c 0.065, H_2O). Subjection of the minor diastereomer **24b** to the same sequence provided **29–31** and **32**, $[\alpha]^{25}_{\text{D}} +20$ (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**), $[\alpha]^{25}_{\text{D}} +80$ (c 0.015, H_2O), identical in all compared respects with

authentic material³⁰ (Table III–IV). In an analogous fashion, coupling of **31** with **15** provided **34**, and subsequent acid-catalyzed deprotection provided *epi*-(–)-P-3A (**2**), $[\alpha]^{25}_{\text{D}} -34$ (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 pyrimidoblastic 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**.



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

(26) Yamada, T.; Suzuki, H.; Mukaiyama, T. *Chem. Lett.* **1987**, 293, 1986, 915. Yamasaki, N.; Murakami, M.; Mukaiyama, T. *Chem. Lett.* **1986**, 1103.

(27) Kobayashi, S.; Hachiya, I. *J. Org. Chem.* **1992**, 57, 1324.

(28) Walker, M. A.; Heathcock, C. H. *J. Org. Chem.* **1991**, 56, 5747.

(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 *anti*-**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

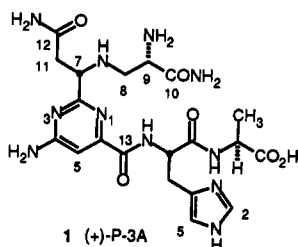
(30) The ^1H NMR of **1** versus **2** proved especially diagnostic, with the natural product and synthetic **1** exhibiting a characteristic set of two doublets of doublets at 2.92 ($J = 7.0, 14.0\text{ Hz}$) and 2.96 ($J = 5.5, 14.0\text{ Hz}$) appearing as a clear doublet split AB quartet for C11-H₂. The *epi*-P-3A (**2**) C11-H₂ 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. ¹H NMR of 1–3 (400 MHz)

assignment	1 (D ₂ O)	2 (D ₂ O)	3 (CD ₃ OD)
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, <i>J</i> = 5.2, 5.8 Hz)	4.75 (dd, <i>J</i> = 5.8, 5.9 Hz)	4.75 (dd, <i>J</i> = 5.4, 5.8 Hz)
C9-H	4.50 (dd, <i>J</i> = 6.8, 7.6 Hz)	4.54 (dd, <i>J</i> = 6.9, 7.5 Hz)	4.64 (dd, <i>J</i> = 6.8, 7.5 Hz)
C7-H	4.34 (dd, <i>J</i> = 5.5, 7.0 Hz)	4.34 (dd, <i>J</i> = 5.8, 6.5 Hz)	4.42 (d, <i>J</i> = 14 Hz)
			4.28 (d, <i>J</i> = 14 Hz)
α-CH (Ala)	4.23 (q, <i>J</i> = 7.4 Hz)	4.23 (q, <i>J</i> = 7.4 Hz)	4.35 (q, <i>J</i> = 7.4 Hz)
β-CH ₂ (His)	3.57 (dd, <i>J</i> = 5.2, 14.0 Hz)	3.56 (dd, <i>J</i> = 5.9, 14.0 Hz)	3.72 (dd, <i>J</i> = 6.0, 14.0 Hz)
	3.44 (dd, <i>J</i> = 5.8, 14.0 Hz)	3.47 (dd, <i>J</i> = 5.8, 14.0 Hz)	3.65 (dd, <i>J</i> = 6.0, 14.0 Hz)
C8-H ₂	3.27 (dd, <i>J</i> = 6.8, 14.0 Hz)	3.27 (dd, <i>J</i> = 6.9, 14.0 Hz)	3.45 (dd, <i>J</i> = 6.8, 14.0 Hz)
	3.18 (dd, <i>J</i> = 7.6, 14.0 Hz)	3.17 (dd, <i>J</i> = 7.5, 14.0 Hz)	3.38 (dd, <i>J</i> = 7.5, 14.0 Hz)
C11-H ₂	2.96 (dd, <i>J</i> = 5.5, 14.0 Hz)	2.97 (app d, <i>J</i> = 8.7 Hz)	
	2.92 (dd, <i>J</i> = 7.0, 14.0 Hz)		
CH ₃	1.27 (d, <i>J</i> = 7.2 Hz)	1.27 (d, <i>J</i> = 7.2 Hz)	1.44 (d, <i>J</i> = 7.5 Hz)

Table IV. ¹³C NMR of 1 and 2 (D₂O, 100 MHz)

assignment	1 (D ₂ O)	2 (D ₂ O)	bleomycin A ₂ ^a
CO ₂ H	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 equation³³ 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 ϕ X174 DNA, assuming that sequential cleavage on the complementary strands within 15 base pairs is required to permit linearization of the hybridized DNA.

(33) Povirk, L. F.; Wubker, W.; Kohnlein, W.; Hutchinson, F. *Nucleic Acids Res.* 1977, 4, 3573. Freifelder, D.; Trumbo, B. *Biopolymers* 1969, 7, 681.

(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 ϕ X174 DNA cleavage. ^b Ratio of double- to single-stranded supercoiled ϕ X174 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 A₂ and Fe(II)–deglycobleomycin A₂ 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 ³²P 5'-end-labeled double-stranded DNA after exposure to the Fe(II) complexes of 1–3 in the presence of O₂. Thus, incubation of the labeled duplex DNA with the agents in the presence of equimolar Fe(NH₄)₂(SO₄)₂ and O₂ or equimolar FeCl₃ and H₂O₂ 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 ϕ X174 DNA, the relative efficiency of w794 DNA cleavage was found to be bleomycin A₂ > deglycobleomycin A₂ > (+)-P-3A > epi-(−)-P-3A, (−)-desacetamido P-3A. In contrast to bleomycin A₂ or deglycobleomycin A₂, 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

(35) Ambrose, C.; Rajadhyaksha, A.; Lowman, H.; Bina, M. *J. Mol. Biol.* 1989, 209, 255.

(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 A₂ 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 A₂ including the bithiazole and tri- or tetrapeptide subunits may be necessary for observation of the bleomycin A₂ duplex DNA cleavage selectivity.^{38,39,40} 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 A₂ and desacetamidodeglycobleomycin A₂,⁹ 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 Section⁴¹

6-Amino-2,4-bis(ethoxycarbonyl)pyrimidine (9). A solution of 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine¹⁰ (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); ¹H NMR (DMSO-*d*₆, 200 MHz) δ 7.68 (2H, br s, NH₂), 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); ¹³C NMR (DMSO-*d*₆, 50 MHz) δ 165.8 (e, C-6), 164.6 (e, C4-CO₂Et), 164.3 (e, C2-CO₂Et), 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); ¹³C-¹H long-range coupling NMR (DMSO-*d*₆, 125 MHz, diagnostic coupling constants) δ 164.6 (dt, *J*(C/C5-H) = 3 Hz, *J*(C/OCH₂CH₃) = 4 Hz, C4-CO₂Et), 164.3 (t, *J*(C/OCH₂CH₃) = 4 Hz, C2-CO₂Et), 107.6 (dt, *J*(C/NH₂) = 5 Hz, *J*(C/C5-H) = 170 Hz, C-5); IR (KBr) *v*_{max} 3442, 3306, 3192, 2980, 1739, 1719, 1650, 1596, 1536, 1435, 1260, 1226, 1026, 972, 866, 746 cm⁻¹; UV (CH₃OH) *λ*_{max} 209 nm (ε 17 000); CIMS (2-methylpropane) *m/e* (M⁺ + H, base); CIHRMS (2-methylpropane) *m/e* 240.0987 (M⁺ + H, C₁₀H₁₃N₃O₄ requires 240.0984).

Anal. Calcd for C₁₀H₁₃N₃O₄: 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 NaBH₄ (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 NH₄Cl (10 mL) and stirred at 25 °C for 1 h. The mixture was extracted with 20% *n*-PrOH-CHCl₃ (5 × 20 mL), and the combined organic extracts were dried (MgSO₄). Removal of solvent in vacuo afforded a mixture of the 2- and 4-(hydroxymethyl)pyrimidines (2-CH₂OH:4-CH₂OH = 7:1, 122 mg, 153 mg theoretical, 80%) as a white solid. The two isomeric alcohols⁴² were separated by preparative centrifugal thin-layer chromatography (SiO₂, 5% DMF-EtOAc), and the selectivity of the reduction varied from 4:1 to 2:1 (C2-CH₂OH:C4-CH₂OH) in EtOH, 6:1 in *n*-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); ¹H NMR (CDCl₃, 300 MHz) δ 7.06 (1H, s, C5-H), 5.24 (2H, br s, NH₂), 4.70 (2H, s, CH₂OH), 4.45 (2H, q, *J* = 7.1 Hz), 3.62 (1H, br s, OH), 1.42 (3H, t, *J* = 7.1 Hz); ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 169.9 (e, C-6), 165.3 (e, CO₂Et), 165.0 (e, C-2), 153.3 (e, C-4), 104.3 (o, C-5), 64.9 (e, CH₂OH), 61.9 (e, CH₂CH₃), 14.6 (o, CH₂CH₃); irradiation of either CH₂OH or C5-H in 10 did not result in a difference NOE, while irradiation of CH₂OH in the minor isomer, 6-amino-2-(ethoxycarbonyl)-4-(hydroxymethyl)pyrimidine, gave a 5% NOE enhancement of C5-H; IR (KBr) *v*_{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 C₈H₁₁N₃O₃: 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)methylpyrimidine (11). A suspension of 10 (169 mg, 0.86 mmol) in THF-CH₂Cl₂ (1:1, 3 mL) was treated with K₂CO₃ (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 (CH₂Cl₂, 3 × 5 mL), and the solvent was removed in vacuo. Chromatography (SiO₂, 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); ¹H NMR (CDCl₃, 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, NH₂), 5.05 (2H, s, CH₂OTs), 4.42 (2H, q, *J* = 7.1 Hz), 2.45 (3H, s), 1.40 (3H, t, *J* = 7.1 Hz); 2D ¹H-¹H NOESY NMR (DMSO-*d*₆, 200 MHz) did not reveal a NOE crosspeak between -CH₂OTs and C5-H for 11 but did so for the minor isomer derived from the NaBH₄ reduction of 9; ¹³C NMR (CDCl₃, 75 MHz) δ 165.0 (e, CO₂Et), 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, CH₂OTs), 62.4 (e), 21.7 (o, CH₃), 14.2 (o); IR (KBr) *v*_{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 C₁₅H₁₇N₃O₅S: 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*-butoxycarbonyl)amino]propionamide (12). A solution of Ph₃P (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), HN₃-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 (SiO₂, 5 × 18 cm, 20% Et₂O-hexane) afforded methyl (S)-3-azido-2-[(*tert*-butoxycarbonyl)amino]propionate¹² (2.73 g, 3.0 g theoretical, 91%) as a colorless oil.

A solution of methyl (S)-3-azido-2-[(*tert*-butoxycarbonyl)amino]propionate (2.86 g, 11.7 mmol) in CH₃OH (30 mL) was cooled to -20 °C and treated with 10% NH₃ in CH₃OH (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 (SiO₂, 2 × 15 cm, 70% Et₂O-hexane) afforded (S)-3-azido-2-[(*tert*-butoxycarbonyl)amino]propionamide⁴⁴ (2.08 g, 2.45 g theoretical, 85%) as a white solid: mp 83–84 °C (white needles, Et₂O-hexane); [α]_D²⁵ +61 (c 0.65, CHCl₃); ¹H NMR (CDCl₃, 200 MHz) δ 6.29 (1H, br s, CONHH), 5.59 (1H, br s, CONHH), 5.30 (1H, br d, *J* = 6.3 Hz, NHBOC), 4.31 (1H, m, CHNH BOC), 3.89 (1H, dd, *J* = 12.3, 4.4 Hz, CHHCH), 3.55 (1H, dd, *J* = 12.3, 5.8 Hz, CHHCH), 1.47 (9H, s, C(CH₃)₃); ¹³C NMR (CDCl₃, 50 MHz) δ 177.4 (e), 155.6 (e) 80.9 (e), 53.3 (o), 52.0 (e), 28.1 (o); IR (KBr) *v*_{max} 3332, 2980, 2934, 2114, 1702, 1508, 1452, 1394, 1368, 1252, 1166, 1050, 1026, 866, 782, 748 cm⁻¹; CIMS (2-methylpropane) *m/e* 230 (M⁺ + H, base).

Anal. Calcd for C₈H₁₅N₃O₃: 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*-butoxycarbonyl)amino]propionamide (295 mg, 1.29 mmol) in CH₃OH (10 mL) was treated with 10% Pd-C (30 mg, 0.1 wt equiv) under H₂ for 5 h. The reaction mixture was filtered through Celite (CH₃OH, 3 × 10 mL), and solvent was removed

(38) Kilkuskie, R. E.; Suguna, H.; Yellin, B.; Murugesan, N.; Hecht, S. *M. J. Am. Chem. Soc.* **1985**, *107*, 260.

(39) Kuwahara, J.; Sugiura, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 2459.

(40) Umezawa, H.; Takita, T.; Sugiura, Y.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron* **1984**, *40*, 501.

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

(42) For 6-amino-2-(ethoxycarbonyl)-4-(hydroxymethyl)pyrimidine: ¹H NMR (CD₃OD, 400 MHz) δ 6.80 (1H, s, C5-H), 4.52 (2H, s, CH₂OH), 4.39 (2H, q, *J* = 7.0 Hz), 1.39 (3H, t, *J* = 7.0 Hz); IR (neat film) *v*_{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: ¹H NMR (CD₃OD, 400 MHz) δ 6.55 (1H, s, C5-H), 4.46 (4H, s, CH₂OH); IR (neat film) *v*_{max} 3341, 3202, 1662, 1607, 1429, 1394, 1339, 1081, 991, 847 cm⁻¹.

(43) Loibner, H.; Zbiral, E. *J. Liebigs Ann. Chem.* **1978**, *78*, Brandstetter, H. H.; Zbiral, E. *Helv. Chim. Acta* **1980**, *63*, 327. Mark, E.; Zbiral, E. *Monaish. Chem.* **1981**, *112*, 215. Wolff, H. *Org. React.* **1967**, *3*, 327.

(44) Alternatively, treatment of *N*-Boc-L-serinamide with HN₃-benzene, Ph₃P-DEAD (THF, -70 °C to 24 °C) provided the azide in good yield (>70%), but purification from the Mitsunobu reaction byproducts proved tedious. Similarly, NaN₃ 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*³,*N*⁶-Bis(*tert*-butoxycarbonyl)-*N*⁶-[(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 CH₃CN (0.2 mL) was treated with NaHCO₃ (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-H₂O (1:1, 1 mL) and treated with di-*tert*-butyldicarbonate (75 mg, 0.34 mmol, 6 equiv) and K₂CO₃ (15.8 mg, 0.11 mmol, 2 equiv). The resulting mixture was stirred at 25 °C (24 h). Saturated aqueous NaHCO₃ (5 mL) was added, and the mixture was extracted with 20% 2-PrOH-CHCl₃ (5 \times 10 mL). The combined organic extracts were dried (Na₂SO₄), and the solvent was removed in vacuo. Chromatography (SiO₂, 0.5 \times 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); [α]_D²⁵ +42 (c 2.4, CHCl₃); ¹H NMR (CD₃OD, 300 MHz) δ 7.08 (1H, s, C5-H), 4.60–4.30 (5H, m), 3.82–3.55 (2H, m, CH₂CH), 1.43–1.27 (18H, m, two C(CH₃)₃); ¹³C NMR (CD₃OD, 75 MHz) δ 176.2 (e), 173.2 (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, CH₂NBOC), 82.3 (e), 81.5 (e), 80.8 (e, CH₂CH), 63.2 (e, OCH₂CH₃), 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, C₂₁H₃₄N₆O₇ requires 483.2567).

Anal. Calcd for C₂₁H₃₄N₆O₇: C, 52.28; H, 7.05; N, 17.43. Found: C, 51.91; H, 7.34; N, 17.07.

(*S*)-*N*³,*N*⁶-(*tert*-butoxycarbonyl)-*N*⁶-[(4-carboxy-6-aminopyrimidin-2-yl)methyl]- β -aminoalaninamide (**14**). A solution of **13** (68 mg, 0.14 mmol) in THF-EtOH-H₂O (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 H₂O (20 mL) and extracted with CH₂Cl₂ (2 \times 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-CHCl₃ (5 \times 15 mL). The combined organic extracts were dried (Na₂SO₄), and the solvent was removed in vacuo to afford **14** (61 mg, 64 mg theoretical, 95%) as a white foam: mp 129–130 °C; [α]_D²⁵ +3.1 (c 0.9, CH₃OH); ¹H NMR (CD₃OD, 400 MHz) δ 7.15 (1H, s, C5-H), 4.80–4.40 (3H, m), 3.90–3.60 (2H, m, CH₂CH), 1.50, 1.41 and 1.29 (18H, C(CH₃)₃); ¹³C NMR (CD₃OD, 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(CH₃)₃), 82.2 (e, C(CH₃)₃), 80.8 (e, CH₂NBOC), 64.7 (e, CH₂CH), 55.4 (o, CHNHBoc), 28.7 (o, C(CH₃)₃), 28.4 (o, C(CH₃)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, C₁₉H₃₀N₆O₇ requires 455.2254).

Anal. Calcd for C₁₉H₃₀N₆O₇: C, 50.21; H, 6.65; N, 18.49. Found: C, 50.30; H, 6.51; N, 18.56.

[*N*⁶-(*tert*-butoxycarbonyl)-L-histidyl]-L-alanine *tert*-Butyl Ester (**15**). A solution of *N*⁶-CBZ-L-histidine (858 mg, 2.97 mmol) in THF-H₂O (1:1, 10 mL) was cooled at 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 \times 15 mL). The combined organic extracts were dried (Na₂SO₄), and the solvent was removed in vacuo to afford *N*⁶-CBZ-*N*⁶-Boc-L-histidine as a white solid (1.06 g, 1.16 g theoretical, 92%).⁴⁵

A solution of *N*⁶-CBZ-*N*⁶-Boc-L-histidine (627 mg, 1.6 mmol) in CH₂Cl₂ (10 mL) was treated with HOBt-H₂O (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 NaHCO₃ (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 (CH₂Cl₂, 5 \times 10 mL), and the solvent was removed in vacuo. Chromatography (SiO₂, 2 \times 10 cm, 40% EtOAc-hexane) afforded *N*⁶-CBZ-**15** as a white solid (706 mg, 831 mg theoretical, 85%): mp 50–51 °C (white needles, EtOAc-hexane); [α]_D²⁵ -11 (c 0.3, CH₃OH); ¹H NMR (CDCl₃, 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, H₂-Ala), 4.30 (1H, dq, *J* = 7.1, 7.0 Hz, H₂-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(CH₃)₃ of *N*⁶-Boc), 1.36 (9H, s, C(CH₃)₃), 1.19 (3H, d, *J* = 7.0 Hz, CHCH₃); ¹³C NMR (CDCl₃, 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, C₂₆H₃₆N₄O₇ requires 517.2662).

Anal. Calcd for C₂₆H₃₆N₄O₇: C, 60.45; H, 7.02; N, 10.85. Found: C, 60.45; H, 7.02; N, 10.90.

A solution of *N*⁶-CBZ-*N*⁶-Boc-L-His-L-Ala-OtBu (134 mg, 0.26 mmol) in CH₃OH (10 mL) was treated with 10% Pd-C (13.4 mg, 0.1 wt equiv) under H₂ for 8 h. The reaction mixture was filtered through Celite (CH₃OH, 3 \times 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**: [α]_D²⁵ +65 (c 0.02, CHCl₃); ¹H NMR (CDCl₃, 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, H₂-Ala), 3.61 (1H, m, H₂-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(CH₃)₃ of *N*⁶-Boc), 1.40 (9H, s, C(CH₃)₃), 1.27 (3H, d, *J* = 7.2 Hz, CHCH₃); ¹³C NMR (CDCl₃, 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(CH₃)₃), 81.6 (e, C(CH₃)₃), 54.9 (o, C α -Ala), 48.1 (o, C α -His), 32.8 (e, CH₂CH), 27.8 (o, C(CH₃)₃), 27.7 (o, C(CH₃)₃), 18.5 (o, CHCH₃); 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, C₁₈H₃₀N₄O₅ requires 515.1267).

*N*⁶-[2-[(*N*⁶-*tert*-butoxycarbonyl)-*N*-(*S*)-2-[(*tert*-butoxycarbonyl)-amino]-2-(carbamoyl)ethyl]amino]methyl]-6-aminopyrimidine-4-carboxyl[*N*⁶-(*tert*-butoxycarbonyl)-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-H₂O (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-CHCl₃ (20 mL) was added, and the mixture was washed with H₂O (5 \times 15 mL). The organic phase was dried (Na₂SO₄), and the solvent was removed in vacuo. Chromatography (SiO₂, 0.8 \times 15 cm, 50–100% EtOAc-hexane and 5% CH₃OH-CH₂Cl gradient elution) afforded **16** (87 mg, 109 mg theoretical, 80%) as a white foam: [α]_D²⁵ +65 (c 0.02, CHCl₃); ¹H NMR (CD₃OD, 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, CH₂CH(His)), 1.58 (9H, s, C(CH₃)₃ of *N*⁶-Boc), 1.42–1.21 (27H, m), 1.32 (3H, d, *J* = 7.3 Hz, CHCH₃); ¹³C NMR (CD₃OD, 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(CH₃)₃), 82.8 (e, C(CH₃)₃), 81.6 (e, C(CH₃)₃), 81.0 (e, C(CH₃)₃), 64.4 (e, CH₂NBOC), 62.1 (o, C α -His), 54.1 (o, CHNHBoc), 51.9 (e, CH₂NHBoc), 47.2 (o, C α -Ala), 31.7 (e, CH₂CH(His)), 28.7 (o, C(CH₃)₃), 28.6 (o, C(CH₃)₃), 28.3 (o, C(CH₃)₃), 28.1 (o, C(CH₃)₃), 17.5 (o, CHCH₃); 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, C₃₇H₅₈N₁₀O₁₁ requires 819.4365).

(-)-Desacetamido P-3A (**3**). A solution of **16** (4 mg, 4.9 μ mol) 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 CH₂Cl₂ (3 \times 5 mL): mp 188 °C dec; [α]_D²⁵ -13.3 (c 0.15, CH₃OH), -18.0 (c 0.15, 0.1 N aqueous HCl); ¹H NMR (CD₃OD, 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, CHCH₃); 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, C₁₈H₂₆N₁₀O₅ 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

(45) Fridkin, M.; Goren, H. *J. Can. J. Chem.* 1971, 49, 1578.

was treated with 2-(methylthio)acetamide 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 (SiO₂, 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 (CDCl₃, 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); ¹³C NMR (CDCl₃, 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, SCH₃), 14.1 (o), 14.0 (o); IR (CHCl₃) *v*_{max} 3500, 3392, 2992, 1739, 1599, 1544, 1448, 1383, 1231, 1199, 1026 cm⁻¹; FABHRMS (NBA) *m/e* 286.0878 (M⁺ + H, C₁₁H₁₃N₃O₄S requires 286.0861).

Anal. Calcd for C₁₁H₁₃N₃O₄S: 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 NaBH₄ (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 NH₄Cl (15 mL). The mixture was extracted with 10% 2-PrOH–CHCl₃ (3 × 15 mL), and the combined organic extracts were dried (Na₂SO₄). Removal of solvent followed by chromatography (SiO₂, 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 (CDCl₃, 400 MHz) δ 6.39 (2H, br s, NH₂), 4.63 (2H, s, CH₂OH), 4.46 (2H, q, *J* = 7.2 Hz), 2.30 (3H, s, SCH₃), 1.42 (3H, t, *J* = 7.2 Hz); ¹³C NMR (CDCl₃, 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, CH₂OH), 62.2 (e, CH₂CH₃), 18.0 (o, SCH₃), 14.0 (o, CH₂CH₃); IR (CHCl₃) *v*_{max} 3510, 3392, 2993, 2928, 1738, 1600, 1541, 1453, 1366, 1227, 1091, 1025 cm⁻¹; FABHRMS (NBA–CsI) *m/e* 375.9736 (M⁺ + Cs, C₉H₁₃N₃O₃S requires 375.9729).

Anal. Calcd for C₉H₁₃N₃O₃S: 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 CH₃CN (20 mL) was treated with activated MnO₂ (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 (CH₃CN, 5 × 10 mL), and the solvent was removed in vacuo. Flash chromatography (SiO₂, 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 (CDCl₃, 300 MHz) δ 10.0 (1H, s, CHO), 7.31 (1H, s, C5-H), 5.80 (2H, br s, NH₂), 4.50 (2H, q, *J* = 7.1 Hz), 1.45 (3H, t, *J* = 7.1 Hz); ¹³C NMR (CDCl₃, 50 MHz) δ 191.9 (o, CHO), 165.9 (e, CO₂Et), 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 (KBr) *v*_{max} 3350, 2973, 2902, 2863, 1731, 1696, 1656, 1621, 1328, 1248, 1144, 1054, 816 cm⁻¹; FABHRMS (NBA) *m/e* 196.0702 (M⁺ + H, C₈H₉N₃O₃ requires 196.0722).

Anal. Calcd for C₈H₉N₃O₃: C, 49.23; H, 4.65; N, 21.53. Found: C, 49.38; H, 4.43; N, 21.66.

N⁶-(tert-Butyloxycarbonyl)-N⁸-[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 CH₃CN (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 (CH₃CN, 5 × 15 mL). Removal of solvent in vacuo afforded **22** (226 mg, 226 mg theoretical, quantitative) as a white foam: [α]_D²⁵ +15.5 (c 0.65, CH₃OH); ¹H NMR (CDCl₃, 400 MHz) δ 8.30 (1H, s, CH=N), 7.01 (1H, s, C5-H), 6.02 (2H, s, NH₂), 4.80–4.50 (3H, m, CH₂CH₃, CH₂CH), 4.10–3.90 (2H, m, CH₂CH), 1.40–1.20 (12H, m); ¹³C NMR (CDCl₃, 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(CH₃)₃), 62.6 (e, CH₂CH₃), 62.2 (o, CH₂CH), 61.5 (e, CH₂CH), 28.1 (o, C(CH₃)₃), 14.0 (o, CH₂CH₃); IR (CHCl₃) *v*_{max} 3550, 3350, 2990, 1750, 1716, 1652, 1502, 1470, 1208, 1056, 916 cm⁻¹; FABHRMS (NBA–CsI) *m/e* 513.0863 (M⁺ + Cs, C₁₆H₂₄N₆O₅ requires 513.0859).

Diastereoselective Reaction of the Stannous (Z)-Enolate of (4S,5R)-3-(Methylthioacetyl)-4-methyl-5-phenyl-2-oxazolidinone with N⁶-(tert-Butyloxycarbonyl)-N⁸-[6-amino-4-(ethoxycarbonyl)pyrimidin-2-yl)methyl]amino-(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 (4S,5R)-3-(methylthioacetyl)-4-methyl-5-phenyl-2-oxazolidinone (131 mg, 0.49 mmol, 2.0 equiv) in dry THF (1 mL) and *i*-Pr₂NEt (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 CH₂Cl₂ (15 mL) and saturated aqueous NaHCO₃ (15 mL) with vigorous stirring. The mixture was filtered through Celite (CH₂Cl₂, 3 × 10 mL), and the organic layer was washed with saturated aqueous NaCl (2 × 10 mL), dried (Na₂SO₄), and concentrated in vacuo. Chromatography (SiO₂, 1.5 × 3 cm, 5% CH₃OH–CH₂Cl₂) afforded 123 mg (159 mg theoretical, 77%) of a 86:14 mixture of **24a**:**24b**. Preparative centrifugal thin-layer chromatography (SiO₂, 60–100% EtOAc–hexane gradient elution) afforded **24a** (94.4 mg) and **24b** (16.6 mg) as white foams.

Ethyl (S)-2-[1-[(S)-2-[(tert-butyloxycarbonyl)amino]-2-carbamoyl-ethyl]amino]-2-[(4S,5R)-4-methyl-5-phenyl-2-oxazolidinyl]carbonyl]-2-(S)-(methylthio)-1-ethyl)-6-aminopyrimidine-4-carboxylate (24a): [α]_D²⁵ –26 (c 4.8, CHCl₃); ¹H NMR (CD₃OD, 400 MHz) 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, SCH₃), 1.40–1.20 (12H, m), 0.93 (3H, d, *J* = 6.5 Hz); ¹³C NMR (CD₃OD, 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(CH₃)₃), 65.5 (o, CHAr), 63.5 (o, CHSCH₃), 63.1 (e, CH₂CH₃), 56.4 (o, CHPh), 53.6 (o, CHCH₃), 50.2 (o, CHCH₂), 47.9 (e, CH₂CH), 28.7 (o, C(CH₃)₃), 15.4 (o, SCH₃), 14.5 (o, CH₂CH₃), 12.2 (o, CHCH₃); IR (CHCl₃) *v*_{max} 3420, 2995, 2980, 1770, 1715, 1650, 1565, 1358, 1210, 1130, 950 cm⁻¹; FABHRMS (NBA) *m/e* 646.2672 (M + H⁺, C₂₉H₃₉N₇O₈S requires 646.2659).

Ethyl (S)-2-[1-[(S)-2-[(tert-butyloxycarbonyl)amino]-2-carbamoyl-ethyl]amino]-2-[(4S,5R)-4-methyl-5-phenyl-2-oxazolidinyl]carbonyl]-2-(S)-(methylthio)-1-ethyl)-6-aminopyrimidine-4-carboxylate (24b): [α]_D²⁵ +29 (c 2.6, CHCl₃); ¹H NMR (CDCl₃, 400 MHz) δ 7.45–7.20 (5H, m), 7.02 (1H, s, C5-H), 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, SCH₃), 1.45–1.20 (12H, m), 0.86 (3H, d, *J* = 6.5 Hz); ¹³C NMR (CD₃OD, 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(CH₃)₃), 64.4 (o, CHAr), 62.0 (o, CHSMe), 60.3 (o, CH₂CH₃), 55.2 (o, CHPh), 53.5 (o, CHCH₃), 49.2 (o, CHCH₂), 46.6 (e, CH₂CH), 28.2 (o, C(CH₃)₃), 14.7 (o, SCH₃), 14.1 (o, CH₂CH₃), 11.8 (o, CHCH₃); IR (CHCl₃) *v*_{max} 3422, 2995, 1779, 1725, 1689, 1612, 1575, 1450, 1362, 1215, 1125, 960 cm⁻¹; FABHRMS (NBA–CsI) *m/e* 778.1635 (M⁺ + Cs, C₂₉H₃₉N₇O₈S requires 778.1632).

Ethyl (S)-2-[1-[(S)-2-[(tert-butyloxycarbonyl)amino]-2-carbamoyl-ethyl]amino]-2-[(4S,5R)-4-methyl-5-phenyl-2-oxazolidinyl]carbonyl]ethyl)-6-aminopyrimidine-4-carboxylate (25). A solution of **24a** (52.4 mg, 0.08 mmol) in C₆H₆ (1 mL) was treated with Bu₃SnH (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 (SiO₂, 1 × 5 cm, 5% CH₃OH–CH₂Cl₂) afforded **25** as a white foam (43.1 mg, 47.9 mg theoretical, 90%): mp 125–127 °C (EtOAc–hexane); [α]_D²⁵ –10.2 (c 0.7, CHCl₃); ¹H NMR (CDCl₃, 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, CH₂CH), 3.80–3.45 (3H, m, CH₂CHAr, CHHCH), 2.85 (1H, m, CHHCH), 1.43–1.40 (12H, m), 0.90 (3H, d, *J* = 6.6 Hz); ¹³C NMR (CDCl₃, 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(CH₃)₃), 67.1 (o, CHAr), 62.1 (e, CH₂CH₃), 60.2 (o, CHPh), 54.7 (o, CHCH₃), 52.2 (o, CHCH₂), 31.2 (e, CH₂CH), 29.4 (e, CH₂CH), 28.3 (o, C(CH₃)₃), 14.2 (o, CH₂CH₃), 13.4 (o, CHCH₃); IR (CHCl₃) *v*_{max} 3410, 3010, 2990, 1778, 1695, 1628, 1560, 1450, 1365, 1210, 1155, 1078 cm⁻¹; FABHRMS (NBA) *m/e* 600.2758 (M + H⁺, C₂₈H₃₇N₇O₈ requires 600.2782).

N⁶-(tert-Butyloxycarbonyl)-N⁸-[1-amino-3-(S)-(4-amino-6-(ethoxycarbonyl)pyrimidin-2-yl)propion-3-yl]-(S)-β-aminoalaninamide (26). Solid **25** (13.8 mg, 0.023 mmol) was treated with an EtOH solution of NH₃ (14%, 10 mL), and the solution was stirred for 1.5 h at 0 °C. The solvent was evaporated in vacuo, and flash chromatography (SiO₂, 0.5 × 5 cm, 10:1 CH₂Cl₂–CH₃OH) afforded **26** as a white solid (7.1 mg, 10.1 mg theoretical, 70%): mp 146–148 °C (2-PrOH–hexane); [α]_D²⁵ –8.7 (c 0.25, CH₃OH); ¹H NMR (CD₃OD, 400 MHz) δ 7.01 (1H, s, C5-H), 4.41 (2H, q, *J* = 7.1 Hz, CH₂CH₃), 4.21 (1H, m), 4.12 (1H, m), 2.95 (2H, m), 2.67 (2H, m), 1.41 (12H, m); ¹³C NMR (CD₃OD, 100 MHz)

δ 176.5, 176.4, 167.0, 154.6, 154.5, 105.4 (o, C-5), 80.7 (e, C(CH₃)₃), 64.7, 63.2, 55.7, 49.7, 47.9, 28.7, 14.5; IR (CH₃OH) ν_{\max} 3400, 3009, 2980, 1729, 1680, 1550, 1410, 1250, 1050, 940 cm⁻¹; FABHRMS (NBA-CsI) m/e 572.1234 (M⁺ + Cs, C₁₈H₂₉N₇O₆ requires 572.1234).

N⁺-(*tert*-Butyloxycarbonyl)-N⁺-[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-CH₃OH-H₂O (3:1:1, 0.3 mL) at 0°C was treated with aqueous 1 N LiOH (37 μ L, 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-CH₃OH, 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 \times 8 cm, acetate form, 50–100 mesh). The column was washed with H₂O, and subsequent elution with 6% HOAc-H₂O afforded **27** as a white powder (8.9 mg, 10.3 mg theoretical, 88%); mp 205–207 °C (EtOH-hexane); $[\alpha]_D^{25}$ -26 (c 0.15, H₂O); ¹H NMR (D₂O, 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, C₁₆H₂₃N₇O₆ requires 544.0921).

In an alternative purification of **27**, crude acid (0.023 mmol theoretical) was chromatographed on C-18 (2 \times 0.5 cm, H₂O eluant) to afford **27** (9.3 mg, 9.36 mg theoretical, 99%).

N⁺-[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: $[\alpha]_D^{25}$ -23 (c 0.065, H₂O); ¹H NMR (D₂O, 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, 1700, 1678, 1498, 1152, 1062, 819 cm⁻¹; FABHRMS (NBA-NaI) m/e 334.1244 (M⁺ + Na, C₁₁H₁₇N₇O₄ requires 334.1240).

Ethyl (R)-2-[(*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-2-carbamoyl-ethylamino]-2-[(*4S,5R*)-4-methyl-5-phenyl-2-oxazolidinyl]carbonyl]ethyl-6-aminopyrimidine-4-carboxylate (29). A solution of **24h** (16.4 mg, 0.03 mmol) in C₆H₆ (1 mL) was treated with Bu₃SnH (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 N₂. The mixture was allowed to cool to 23 °C, and the solvent was evaporated in vacuo. Flash chromatography (SiO₂, 1 \times 3 cm, 5% CH₃OH-CH₂Cl₂) afforded **29** (14 mg, 15 mg theoretical, 93%) as a white solid: mp 135°C sharp (EtOAc-hexane); $[\alpha]_D^{25}$ +6.0 (c 0.75, CH₃OH); ¹H NMR (CD₃OD, 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); ¹³C NMR (CD₃OD, 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 (CHCl₃) 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, C₂₈H₃₇N₇O₈ requires 732.1755).

N⁺-(*tert*-Butyloxycarbonyl)-N⁺-[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); $[\alpha]_D^{25}$ +24 (c 0.75, CH₃OH); ¹H NMR (CD₃OD, 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); ¹³C NMR (CD₃OD, 100 MHz) δ 176.7, 176.6, 167.0, 166.1, 154.8, 154.7, 105.2, 78.5, 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, C₁₈H₂₉N₇O₆ requires 572.1230).

N⁺-(*tert*-Butyloxycarbonyl)-N⁺-[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); $[\alpha]_D^{25}$ +14 (c 0.16, H₂O); ¹H NMR (D₂O, 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, C₁₆H₂₃N₇O₆ requires 544.0917).

N⁺-[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: $[\alpha]_D^{25}$ +20 (c 0.10, H₂O); ¹H NMR (D₂O, 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, C₁₁H₁₇N₇O₄ requires 334.1240).

N⁺-(*tert*-Butyloxycarbonyl)-N⁺-[1-amino-3(*S*)-(4-amino-6-(((*tert*-butyloxy)-L-alanyl)-N⁺-(*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 (SiO₂, 1 \times 2 cm, 15–20% CH₃OH-CHCl₃ gradient elution) afforded **33** (4.0 mg, 6.97 mg theoretical, 58%) as a thin film: R_f 0.35 (SiO₂, 25% CH₃OH-CHCl₃); $[\alpha]_D^{25}$ -40 (c 0.15, CH₃OH); ¹H NMR (CD₃OD, 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); ¹³C NMR (CD₃OD, 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, C₃₄H₅₃N₁₁O₁₀ 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 CHCl₃ (1 \times 1 mL) to give **1** (5.4 mg, 5.5 mg theoretical, 98%) as a hygroscopic solid: R_f 0.34 (SiO₂, 10:9:1 CH₃OH-10% aqueous CH₃-CO₂NH₄-10% aqueous NH₄OH); $[\alpha]_D^{25}$ +80 (c 0.015, H₂O); ¹H NMR (D₂O, 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 Hz), 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); ¹³C NMR (D₂O, 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, C₂₀H₂₉N₁₁O₈ requires 520.2380).

N⁺-(*tert*-Butyloxycarbonyl)-N⁺-[1-amino-3(*R*)-(4-amino-6-(((*tert*-butyloxy)-L-alanyl)-N⁺-(*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 (SiO₂, 1 \times 3 cm, 15–20% CH₃OH-CHCl₃ gradient elution) afforded **34** (15.4 mg, 24.9 mg theoretical, 62%) as a thin film: R_f 0.32 (SiO₂, 25% CH₃OH-CHCl₃); $[\alpha]_D^{25}$ +43 (c 0.025, CH₃OH); ¹H NMR (CD₃OD, 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); ¹³C NMR (CD₃OD, 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, C₃₄H₅₃N₁₁O₁₀ requires 776.4055).

(-)-epi-P-3A (2). The solid **34** (13.2 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 CHCl₃ (3 \times 1 mL) to give **2** (8.75 mg, 8.8 mg theoretical, 99%) as a hygroscopic solid: R_f 0.36 (SiO₂, 10:9:1 CH₃OH-10% aqueous CH₃-CO₂NH₄-10% aqueous NH₄OH); $[\alpha]_D^{25}$ -34 (c 0.02, H₂O); ¹H NMR (D₂O, 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 Hz), 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); ¹³C NMR (D₂O, 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 ($\text{M}^+ + \text{H}$, $\text{C}_{20}\text{H}_{29}\text{N}_{11}\text{O}_6$ 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 A₂, 0.2–5 μM deglycobleomycin A₂, 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 $\mu\text{g}/\text{mL}$ 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 A₂, 2.5 μM deglycobleomycin A₂, 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 $\mu\text{g}/\text{mL}$ 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–III 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 equation³³ assuming a Poisson 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 ³²P 5'-end-labeled w794 DNA³⁶ in 50 mM Tris-HCl buffer solution (pH 8). The final concentrations of the agents employed in the study were 1–10 μM bleomycin A₂, 1–50 μM deglycobleomycin A₂, 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 Na₂EDTA (8.7%, 250 mM). Electrophoresis running buffer (TBE) contained Tris base (100 mM), boric acid (100 mM), and Na₂EDTA-H₂O (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 ¹³C NMR spectra of natural P-3A.