Acknowledgment. We thank members of the Physical Chemistry Department, Hoffmann-La Roche, Inc., for determination of the spectral and microanalytical data for the compounds reported herein. We thank Mr. Patrick P. Riggio for the enantiomeric purity determinations. We also thank Dr. R. W. Kierstead for his support and advice during the course of these studies.

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Synthesis of Pyrimidoblamic Acid and Epipyrimidoblamic Acid

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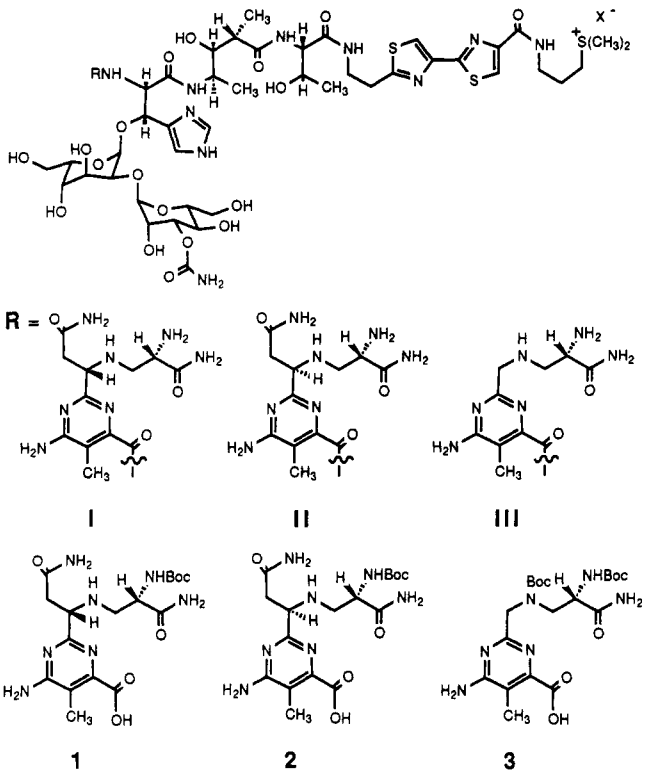
The N-protected pyrimidine moieties of bleomycin (pyrimidoblamic acid, 1) and epibleomycin are reported. In addition to a complete description of a synthetic route outlined earlier (Arai, H.; Hagmann, W. K.; Suguna, H.; Hecht, S. M. *J. Am. Chem. Soc* **1980**, *102*, 6631), a new route is also presented that provides access to multigram quantities of pyrimidoblamic acid. Because bleomycin and epibleomycin, which differ only in the orientation of the propionamide substituent, differ significantly in their Cu(II) chelation and DNA cleavage properties, we also prepared an analogue of pyrimidoblamic acid lacking the propionamide moiety.

The bleomycins are a family of antitumor agents believed to exert their therapeutic effects at the level of DNA strand scission.[1] Much attention has been focused on the bleomycins due to their clinical utility, as well as their interesting structures and mechanism of action.[1,2] Central to the investigation of this class of compounds is the ongoing investigation of synthetic methodology for the elaboration of bleomycin group antibiotics, as well as the actual synthesis of novel bleomycin analogues as mechanistic probes and potential antitumor agents.[3]

In the development of an understanding of the structure and chemical nature of bleomycin (I; bleomycin A_2), the synthesis of the pyrimidine moiety was an exceptionally important achievement.[4] The synthesis provided verification of the revised structure that had been proposed for bleomycin;[5] the synthetic studies leading to the successful syntheses provided key insights into the chemical behavior of bleomycin, particularly the extent to which the pyrimidine ring influenced the functional group chemistry of the propionamide substituent.

Described fully herein is the chemistry that provided initial synthetic access to the pyrimidine moieties of bleomycin (I) and epibleomycin (II)[4b] and a new route that can provide multigram quantities of pyrimidoblamic acid (1). Because of the remarkable differences in behavior of metal chelates of bleomycin and epibleomycin,[6] we also prepared the pyrimidine moiety corresponding to despropionamidobleomycin (III).[7]



Results and Discussion

While inspection of the structure of bleomycin suggests the presence of at least three potentially nucleophilic N atoms within the pyrimidine moiety (I), early chemical

(1) (a) Umezawa, H. *Biomedicine* **1973**, *18*, 459. (b) Umezawa, H. In *Bleomycin: Current Status and New Developments*; Carter, S. K., Crooke, S. T., Umezawa, H., Eds.; Academic Press: New York, 1978; p 15ff. (c) Hecht, S. M. In *Bleomycin: Chemical, Biochemical and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; p 1ff.

(2) (a) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 383. (b) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107.

(3) (a) 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. (b) Aoyagi, Y.; Katano, K.; Suguna, H.; Primeau, J. L.; Chang, L.-H.; Hecht, S. M. *J. Am. Chem. Soc.* **1982**, *104*, 5537.

(4) (a) 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. (b) Arai, H.; Hagmann, W. K.; Suguna, H.; Hecht, S. M. *J. Am. Chem. Soc.* **1980**, *102*, 6631. (c) Otsuka, M.; Narita, M.; Yoshida, M.; Kobayashi, S.; Ohno, M.; Umezawa, Y.; Morishima, H.; Saito, S.; Takita, T.; Umezawa, H. *Chem. Pharm. Bull. Jpn.* **1985**, *33*, 520.

(5) Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Umezawa, Y.; Naganawa, H.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 801.

(6) Muraoka, Y.; Kobayashi, H.; Fujii, A.; Kunishima, M.; Fujii, T.; Nakayama, Y.; Takita, T.; Umezawa, H. *J. Antibiot.* **1976**, *29*, 853.

(7) For previous syntheses of analogs of pyrimidoblamic acid, see: (a) Hagmann, W. K.; Basha, F. Z.; Hashimoto, M.; Frye, R. B.; Kojo, S.; Hecht, S. M. *J. Org. Chem.* **1981**, *46*, 1413. (b) Hénichart, J.-P.; Houssin, R.; Bernier, J.-L.; Catteau, J.-P. *J. Chem. Soc., Chem. Commun.* **1982**, 1295. (c) Sugano, Y.; Kittaka, A.; Otsuka, M.; Ohno, M.; Sugiura, Y.; Umezawa, H. *Tetrahedron Lett.* **1986**, *27*, 3635. (d) Tao, X.; Stephan, D. W.; Mascharak, P. K. *Inorg. Chem.* **1987**, *26*, 754. (e) Suga, A.; Sugiyama, T.; Sugano, Y.; Otsuka, M.; Ohno, M.; Sugiura, Y.; Maeda, K. *Synlett* **1989**, 70.

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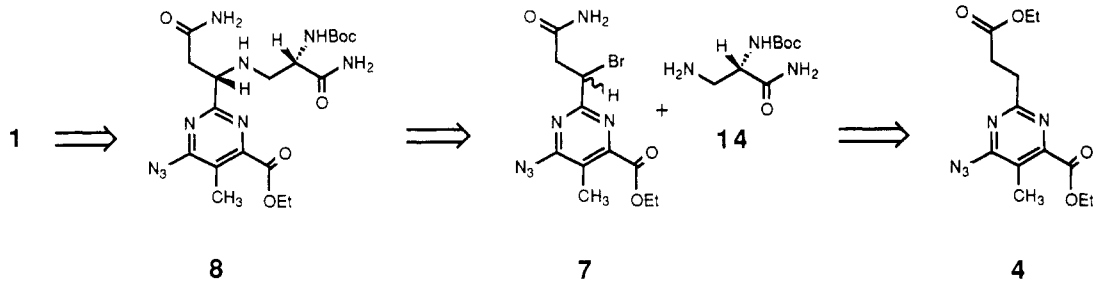
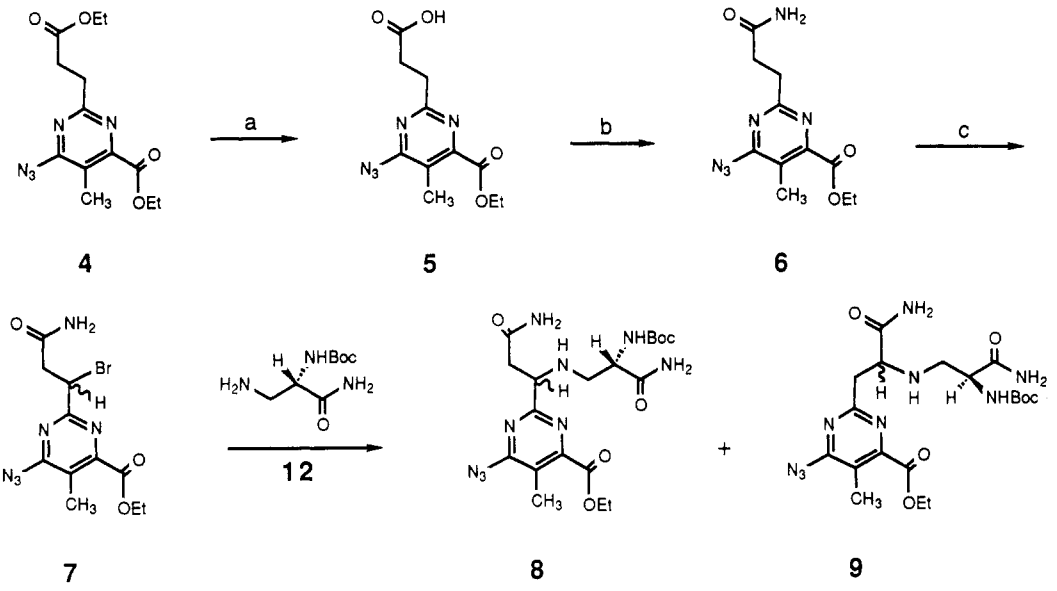
Scheme I. Retrosynthetic Analysis of the Pyrimidine Moiety of Bleomycin



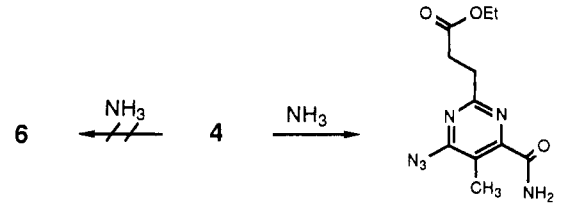
Scheme II[a]



[a] (a) 3:2 THF–2 N HCl, 25 °C, 5 days; (d) $SOCl_2$, CH_2Cl_2, 0 °C; then NH_3, CH_2Cl_2; (c) Br_2, dioxane, 75–80 °C, 15 min.

studies carried out with bleomycin indicated that acylation occurred with facility only on N^{α} of the β-aminoalanineamide group.[8] In view of this finding, it seemed logical to prepare a derivative of pyrimidine I containing a protecting group solely on N^{α} of the β-aminoalanineamide. The Boc group was chosen based on its anticipated ease of removal from the final product,[3b] as well as the favorable physicochemical properties that it would likely impart to otherwise polar intermediates. Accordingly, pyrimidoblamic acid (1) was chosen as a synthetic intermediate of probable utility for the elaboration of bleomycin.

Retrosynthetic analysis (Scheme I) suggested a logical disconnection between the (pyrimidin-2-yl)propionamide and β-aminoalanineamide moieties; based on model studies,[7a] we planned to obtain bromopyrimidine 7 from the known ethyl 3-(4-azido-6-carbethoxy-5-methylpyrimidin-2-yl)propionate (4).[3b,7a] Differentiation of the two ethyl carboxylates in 4 was attempted initially via ammonolysis, but afforded mainly the respective C-6 carboxamide rather than the desired pyrimidinylpropionamide 6. Based on



published data[9] which suggested that selective hydrolysis of the propionate ester might be achieved under acidic conditions by direct participation of a pyrimidine ring nitrogen atom, we studied the solvolysis of 4 in various mixtures of THF and hydrochloric acid. As shown in Table I, conversion of 4 to pyrimidinylpropionic acid 5 was observed under a range of conditions; the best yield (90%) and product purity (mp 144° C) was achieved using a 3:2 mixture of THF and 2 N HCl at 25 °C for 5 days. Compound 5 was obtained as colorless microcrystals from ether after simple extractive workup. That the hydrolysis had involved the propionate, rather than pyrimidine, ester was confirmed via ^1H NMR spectroscopy in D_2O at several pD values. Variation in the pD from 2.30 to 8.70 resulted in the expected upfield shifts of the hydrogens attached to C^{α} (0.23 ppm) and C^{β} (0.07 ppm) of the putative propionate moiety, while the chemical shifts of the remaining hydrogens in 5 were unaffected. Analogous titration of 4 gave no significant change in the chemical shift of any hydrogen.

Table I. Hydrolysis of Pyrimidinylpropionate Ester 4 in Mineral Acid at 25 °C

conditions	time, h	% yield
3:1 THF–5 N HCl	72	70
5:3 THF–3 N HCl	100	76
3:2 THF–2 N HCl	120	90
3:2 THF–1 N HCl	140	80

(8) (a) Huang, C.-H.; Galvan, L.; Crooke, S. T. *Biochemistry* **1979**, *18*, 2880. (b) Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Biochemistry* **1979**, *18*, 3439.

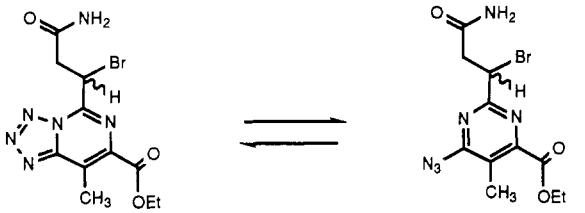
(9) (a) Kröger, M.; Seela, F.; Cramer, F. *Chem. Ber.* **1976**, *109*, 3615. (b) Kirby, A. J.; Mujahid, T. G. *Tetrahedron Lett.* **1978**, 4081.

Table II. Assignment of Azide:Tetrazole Ratio for Azidopyrimidines 4–8[a]

	chemical shift (δ)[b]		equilibrium ratio[c]	
compd	azide	tetrazole	azide	tetrazole
4[d]	e	2.93	0	100
5	2.16	e	100	0
6	e	2.93	0	100
7	2.22	2.93	42	58
8[f]	e	2.90	0	100

[a] Determined in $CDCl_3$–DMSO-d_6, except as noted. [b] Pyrimidine 5-CH_3 group. [c] ±5%. [d] Determined in $CDCl_3$. [e] No resonance apparent. [f] Determined in D_2O.

Pyrimidinylpropionic acid **5** could be converted to the respective propionamide derivative **6** by ammonolysis of an initially formed acid chloride. In a typical experiment, the desired propionamide was isolated in 49% yield by crystallization after extractive workup of the reaction mixture. Bromination of model pyrimidinylpropionates structurally related to **6** afforded mixtures of monobromides (analogous to **7**), geminal dibromides, and vinyl bromides;[7a] small changes in reaction conditions often had a dramatic effect on the ratios and absolute yields of formation of these products. Accordingly, conditions appropriate for the routine preparation of bromide **7** were investigated in detail. It was found that the desired transformation proceeded readily at 75–80 °C in dry dioxane when a modest (20–25%) excess of bromine was employed, relative to pyrimidinylpropionamide **6**. Racemic bromopyrimidine **7** crystallized from ethyl acetate in 67% yield and was characterized fully; interestingly, the ^1H NMR spectrum indicated that this compound existed as an equilibrium mixture of azide (**7**) and tetrazole (**7a**).[10]

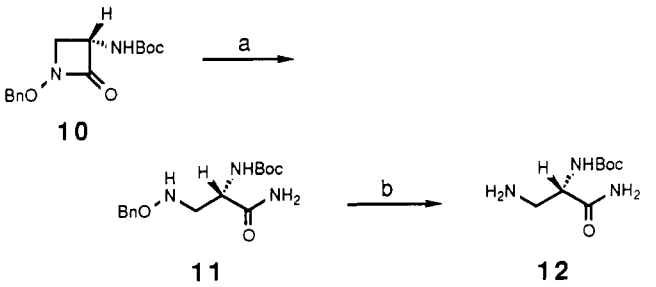


7a **7**

As illustrated in Table II, the pyrimidine 5-CH_3 substituent was diagnostic for each of the forms and permitted assignment of the azide:tetrazole ratio for each of the azidopyrimidine intermediates. Although the factors that determine the equilibrium ratio were not studied in detail, the results can be rationalized if it is assumed that tetrazole formation is disfavored for **7** by the steric effect of the bromine atom, and for **5** by competitive intramolecular H-bonding of the carboxylic acid moiety to the same pyrimidine ring N atom required for tetrazole formation.

(2*S*)-3-Amino-2-[(*tert*-butoxycarbonyl)amino]propionamide (**12**) was prepared starting from (2*S*)-*N*-(benzyloxy)-2-azetidinone (**10**).[11] Treatment of **10** with methanolic ammonia at 0 °C provided (2*S*)-3-[(benzyloxy)amino]-2-[(*tert*-butoxycarbonyl)amino[propionamide (**11**) in good yield. Hydrogenolysis over 10% palladium-on-charcoal gave **12** essentially in quantitative yield as a viscous oil that solidified on standing. Characterization of **12** included verification of absolute configuration, as well as optical purity, by acid-catalyzed conversion of a sample to putative (2*S*)-2,3-diaminopropionic acid. The hydrolyzed sample of **12** had the same spectral and physical

Scheme III[a]

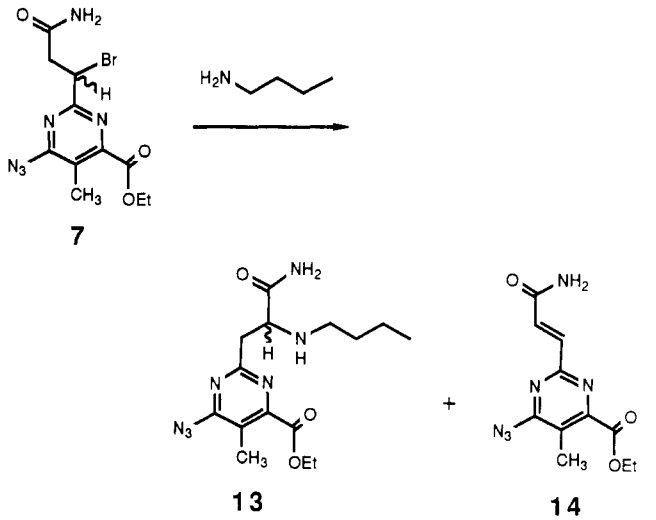


10 **11** **12**

[a] (a) NH_3, MeOH, 0 °C; (b) H_2, Pd/C.

properties as an authentic sample of (2*S*)-2,3-diaminopropionic acid.[12]

Treatment of bromopyrimidine **7** with diaminopropionamide **12** at 25 °C in ethanol containing 1.5 equiv of $NaHCO_3$ provided several products, including putative diastereomers **8**. Fractionation of the reaction mixture and analysis of products by ^1H NMR indicated that the most abundant products from this reaction (~30% yield) were actually diastereomers **9**. This assignment of structure, as well as mechanistic insight into the probable mode of formation of **9**, was provided by treatment of bromopyrimidine **7** with 2.3 equiv of *n*-butylamine in DMF. The products of the reaction included (*n*-butylamino)pyrimidine derivative **13** (25%) and pyrimidinylpropenamide **14** (46%), the latter undoubtedly resulting from elimination of HBr under the reaction conditions. The probable in-



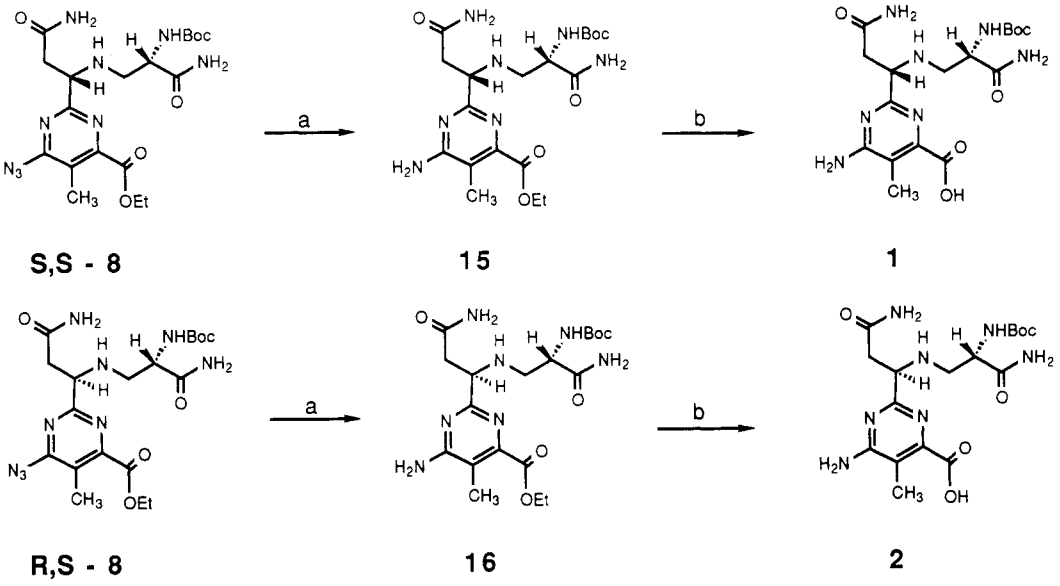
7 **13** **14**

termediacy of **14** in the formation of **13** (and by inference **9**, vide supra) was established by demonstrating that admixture of isolated **14** with 1.2 equiv of *n*-butylamine at 0 °C also provided (*n*-butylamino)pyrimidine **13**.[13] Clarification of the nature of the products obtained upon admixture of **7** and **12** prompted a change in reaction conditions; the use of a less polar solvent (1:1 dioxane–THF) facilitated the desired nucleophilic displacement reaction, and provided a mixture of (*S,S*)-**8** and (*R,S*)-**8** in high yield. Separation was accomplished readily by preparative silica gel TLC and provided each of the diastereomers in 48% yield.

Azidopyrimidines (*S,S*)-**8** and (*R,S*)-**8** were converterd separately to the respective final products **1** and **2**. Thus

(10) See, e.g.: Temple, C., Jr.; McKee, R. L.; Montgomery, J. A. *J. Org. Chem.* **1965**, *30*, 829.
(11) Mattingly, P. G.; Miller, M. J. *J. Org. Chem.* **1980**, *45*, 410.
(12) Obtained from Calbiochem-Behring Corp.
(13) It is interesting to note that two similar ethyl pyrimidinylacrylates failed to undergo conjugate addition by any of several alkylamines tested. See ref 7a.

Scheme IV[a]



[a] (a) H_2, Pd/C; (b) NaOH, aqueous EtOH, 25 °C, 7 days.

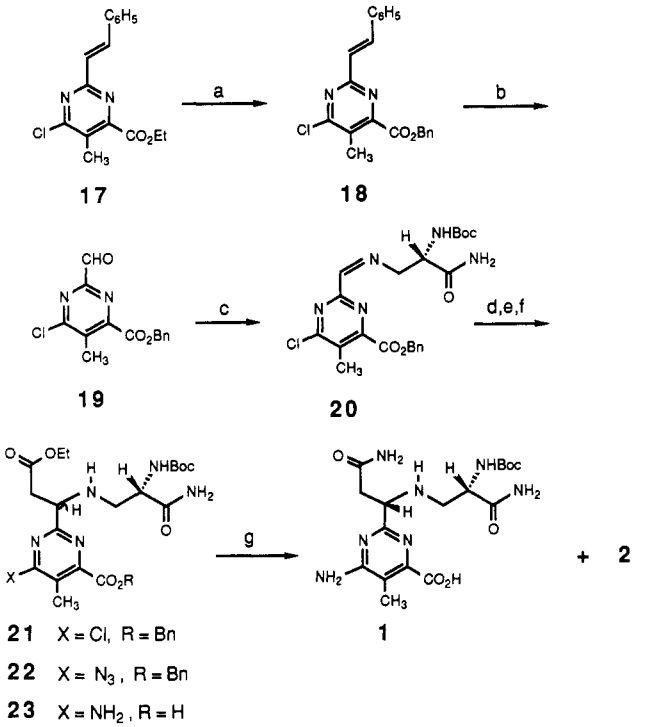
hydrogenolysis of (*S*,*S*)-8 over 10% palladium-on-charcoal afforded 4-aminopyrimidine **15** as colorless needles in 85% yield. Saponification of **15** in aqueous ethanol at 0 °C effected quantitative conversion to pyrimidoblamic acid (**1**), which was isolated as colorless microcrystals, mp 223–225 °C, $[\alpha]^{28}_D$ −33.6° (*c* 0.75, H_2O). The physical and spectral properties of this compound were the same as those reported for pyrimidoblamic acid prepared by a different route;[4a] identical material was also obtained by a third synthetic route (vide infra). Analogous treatment of (*R*,*S*)-8 provided epipyrimidoblamic acid (**2**) in 87% overall yield.

One facet of the foregoing synthesis that is worthy of note was the favorable physical properties (e.g., solubility) of the azidopyrimidine intermediates, which provided significant operational advantages for some of the transformations reported. It seemed of interest to employ this functional group in a synthetic scheme analogous to the one devised in the Umezawa and Ohno laboratories for the synthesis of pyrimidoblamic acid.[4a,c]

Accordingly, (*E*)-1-(6-carbobenzoxy-4-chloro-5-methylpyrimidin-2-yl)-2-phenylethylene (**18**) was prepared by transesterification of the respective ethyl ester,[14] ozonolysis and reductive workup of the formed ozonide with dimethyl sulfide then provided the key intermediate benzyl 4-chloro-2-formyl-5-methylpyrimidine-6-carboxylate (**19**). Condensation of aldehyde **19** with (2*S*)-3-amino-2-[(*tert*-butoxycarbonyl)amino]propionamide (**12**) was carried out in dry CH_3CN over powdered 3-Å molecular sieves. Imine **20** was obtained as a relatively unstable light yellow solid that could not be induced to crystallize; the crude material was used directly for condensation with monoethyl malonate in benzene. The desired product **21** was obtained after extractive and chromatographic workup, albeit only in low yield as also reported for the condensation involving the corresponding ethyl pyrimidine-6-carboxylate.[4a,c]

Diastereomeric **21** was treated with NaN_3 in DMF, which afforded the respective 4-azidopyrimidine (**22**) in quantitive yield. Hydrogenolysis of **22** over 10% palladium-on-charcoal provided 4-aminopyrimidine-6-carboxylate **23** as a light yellow solid in 97% yield after

Scheme V[a]



[a] (a) $C_6H_5CH_2ONa$, $C_6H_5CH_2OH$, 80 °C; (b) O_3, $CHCl_3$–CH_2Cl_2, then $(CH_3)_2S$; (c) **12**, dry CH_3CN, 3-Å molecular sieves, 25 °C; (d) monoethyl malonate, C_6H_6, 42 °C; (e) NaN_3, DMF, 0 °C; (f) H_2, Pd/C; (g) EtOH, NH_3.

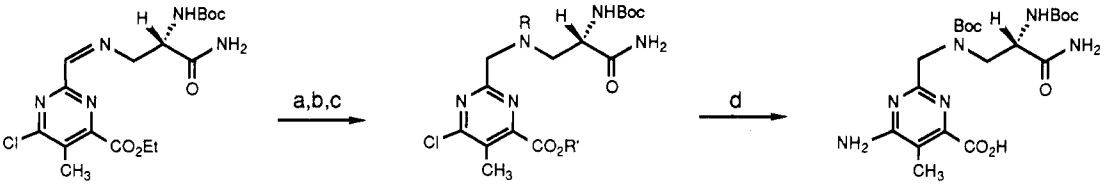
simple extractive workup. Ammonolysis of diastereomeric **23** provided a product mixture (presumably containing **1** and **2**) which deposited pyrimidoblamic acid (**1**) as colorless microcrystals.

Epibleomycin (II) has been noted to bind Cu(II) with exceptional affinity[15] and to mediate DNA damage as an Fe(II) chelate with sequence selectivity that differs from that of bleomycin in certain respects.[16] Because these two

(14) Otsuka, M.; Kobayashi, S.; Ohno, M.; Umezawa, Y.; Morishima, H.; Umezawa, H. *Chem. Pharm. Bull. Jpn.* **1985**, *33*, 515.

(15) (a) Takita, T. In *Bleomycin: Chemical, Biochemical and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; p 156 ff. (b) Shipley, J.; Hecht, S. M., unpublished data.

Scheme VI[a]



24 25 R = H, R' = Et 26 R = Boc, R' = Et 27 R = Boc, R' = H 3

[a] (a) $NaCNBH_3$, MeOH, 0 °C; (b) $(Boc)_2O$, C_6H_5N, 25 °C; (c) NaOH, aqueous EtOH; (d) EtOH, NH_3, 43–45 °C, 10 days.

analogues from each other only in the configuration of the propionamide substituent attached to the pyrimidine moiety of bleomycin, it seemed of interest to prepare the bleomycin analogue lacking the propionamide (III) to permit an assessment of the possible role of this substituent in metal binding and DNA strand scission by bleomycin. Accordingly, we prepared pyrimidine **3** to permit the synthesis of despropionamidobleomycin A_2 (III). This was accomplished starting from N^{α}-(*tert*-butoxycarbonyl)-N^{β}-[[(6-carbethoxy-4-chloro-5-methylpyrimidin-2-yl)methylene]amino]-(*S*)-β-aminoalanineamide (**24**),[4a,c] which was reduced with $Na(CN)BH_3$ in CH_3OH to afford the corresponding (aminomethyl)pyrimidine **25**; the latter was isolated as an oil in 68% yield following extractive workup. Following protection of the (potentially reactive) N^{β} atom via the agency of di-*tert*-butyl pyrocarbonate, saponification of **26** afforded di-*t*-Boc 4-chloropyrimidine **27** as a yellow foam. Ammonolysis at −78 °C in ethanol then gave di-*t*-Boc desamidopyrimidoblamic acid (**3**); after removal of excess NH_3 and extractive workup, compound **3** was obtained as off-white microcrystals from 2-propanol–ethanol. The characterization of **3** included acid-catalyzed removal of the Boc protecting groups; the derived desamidopyrimidoblamic acid was isolated as colorless microcrystals and characterized by ^1H NMR ((D_2O) δ 2.15 (s, 3), 3.66 (d, 2), 4.39 (t, 1), and 4.48 (s, 2)).

The preparation of pyrimidine derivatives described herein permitted completion of the total synthesis of bleomycin[3b] and facilitated verification of the revised structure proposed for bleomycin,[5] as well as the synthesis of a number of structural analogues of bleomycin[17] of utility for dissecting the molecular mechanism by which this antitumor agent mediates destruction of DNA.[2]

Experimental Section

Elemental analyses were carried out by Chemalytics, Inc., or by Atlantic Microlab, Inc. Melting points were taken on a Thomas-Hoover apparatus and are not corrected. Infrared spectra were recorded on a Perkin-Elmer 257 spectrophotometer. Mass spectra were recorded on a Perkin-Elmer Hitachi RMU-6, Varian MAT-44, or Finnigan MAT 4500 Series GC/MS mass spectrometer. High-resolution peak matching was carried out on a Finnigan MAT 8230. NMR spectra were determined on Varian EM-390, Nicolet NT-360, or GE QE-300 NMR spectrometers. Chemical shift values are expressed relative to added $(CH_3)_4Si$, or else using $CDCl_3$ as the solvent and internal standard. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. All reactions were carried out under an argon atmosphere.

Ammonolysis of Ethyl 3-(4-Azido-6-carbethoxy-5-methylpyrimidin-2-yl)propionate (4). A solution of **4** (515 mg, 1.68 mmol) was dissolved in 8.5 mL of methanol that had been saturated with ammonia at 0 °C; the solution was stirred at 25 °C for 1.5 h. The solution was concentrated under diminished pressure to afford ethyl 3-(4-azido-6-carboxamido-5-methylpyrimidin-2-yl)propionate as colorless needles from ethanol, yield 400 mg (85%): mp 197–199 °C dec; IR (Nujol, cm^{-1}) 3175 (br), 1730, 1700, 1645, and 1585; ^1H NMR (DMSO-d_6) δ 1.23 (t, 3, J = 7.0 Hz), 2.73 (s, 3), 2.86 (m, 4), and 4.10 (q, 2, J = 7.0 Hz); mass spectrum (chemical ionization, isobutane) m/z 279.120 ($C_{11}H_{15}N_6O_3$ requires 279.120) $(M + 1)^+$, 265, 251, and 185.

3-(4-Azido-6-carbethoxy-5-methylpyrimidin-2-yl)propionic Acid (5). A solution containing 5.00 g (16.3 mmol) of ethyl 3-(4-azido-6-carbethoxy-5-methylpyrimidin-2-yl)propionate[4b,7a] (**4**) in 100 mL of 3:2 tetrahydrofuran–2 N hydrochloric acid was stirred at 25 °C for 5 days. The solution was extracted with ethyl acetate, and the organic extract was washed with water, dried ($MgSO_4$), and concentrated to dryness under diminished pressure to afford 3-(4-azido-6-carbethoxy-5-methylpyrimidin-2-yl)-propionic acid (**5**), which crystallized from ether as colorless microcrystals, yield 4.10 g (90%): mp 144 °C; ^1H NMR ($CDCl_3$–DMSO-d_6) δ 1.33 (t, 3 J = 7.0 Hz), 2.16 (s, 3), 2.73 (m, 4), 4.30 (q, 2, J = 7.0 Hz) and 10.86 (s, 1); mass spectrum, m/z 279 (M^+) 234, 206, 160, and 55.

Anal. Calcd for $C_{11}H_{13}N_5O_4 \cdot H_2O$: C, 44.44; H, 5.09. Found: C, 44.46; H, 5.10.

3-(4-Azido-6-carbethoxy-5-methylpyrimidin-2-yl)-propionamide (6). Pyrimidinylpropionic acid derivative **5** (1.15 g, 4.12 mmol) was suspended in 40 mL of dry CH_2Cl_2, cooled to 0 °C, and treated with 0.7 mL (1.15 g, 9.63 mmol) of thionyl chloride. The reaction mixture was stirred at 25 °C for 1 h, cooled in an ice bath, and treated dropwise with 25 mL of CH_2Cl_2 that had been saturated with ammonia. Stirring was continued for 10 min, and then the reaction mixture was diluted with 200 mL of ethyl acetate and washed successively with 1 N aqueous $NaHCO_3$, water, and brine. The organic phase was dried ($MgSO_4$) and concentrated under diminished pressure. Crude 3-(azido-6-carbethoxy-5-methylpyrimidin-2-yl)propionamide (**6**) crystallized from ethyl acetate–ether as colorless microcrystals, yield 565 mg (49%): mp 149.5–150 °C; ^1H NMR ($CDCl_3$–DMSO-d_6) δ 1.43 (t, 3, J = 7.0 Hz), 2.89 (s, 3), 2.97 (t, 2, J = 6.0 Hz), 3.71 (t, 2, J = 6.0 Hz), 4.43 (q, 2, J = 7.0 Hz), 6.28 (br s, 1), and 7.14 (br s, 1); mass spectrum, m/z 278 (M^+), 234, 206, 160, 81 and 55.

Anal. Calcd for $C_{11}H_{14}N_6O_3$: C, 47.48; H, 5.07. Found: C, 47.42; H, 5.11.

3-(4-Azido-6-carbethoxy-5-methylpyrimidin-2-yl)-3-bromopropionamide (7). A solution containing 150 mg (0.54 mmol) of 3-(4-azido-6-carbethoxy-5-methylpyrimidin-2-yl)-propionamide (**6**) and 34 μL (105 mg, 0.66 mmol) of bromine in 4 mL of dry dioxane was heated at 75–80 °C for 15 min. The cooled reaction mixture was diluted with 75 mL of ethyl acetate and then washed successively with 15-mL portions of water and brine. The organic phase was dried ($MgSO_4$) and concentrated under diminished pressure (bath temperature <20 °C) to afford

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195 mg of a yellow solid. Crystallization from ethyl acetate afford bromopyrimidine drivative **7** as colorless microcrystals, yield 130 mg (67%): mp 145 °C dec; ^{1}H NMR ($CDCl_3$, DMSO-d_6) (azide) δ 1.40 (t, 3, J = 7.5 Hz), 2.22 (s, 3), 3.10–3.90 (m, 2), 4.41 (q, 2, J = 7.5 Hz), 5.47 (dd, 1, J = 7.5, 7.5 Hz), and 6.13 (br s, 2); (tetrazole) δ 1.44 (t, 3, J = 7.5 Hz), 2.93 (s, 3), 3.1–3.9 (m, 2), 4.46 (q, 2, J = 7.5 Hz), 6.13 (dd, 1, J = 7.5, 9.0 Hz), and 7.20 (br s, 2); mass spectrum (electron impact), m/z 358 and 356 (M^+), 314 and 312, 286 and 284, 240 and 238, 234, 160, 124, 122, 108, 89, and 74.

Anal. Calcd for $C_{11}H_{13}N_6O_3Br$: C, 36.99; H, 3.67. Found: C, 37.00; H, 3.70.

N^α-(*tert*-Butoxycarbonyl)-N^β-[1-amino-3-(4-azido-6-carbethoxy-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanineamide (8). A solution containing 35.7 mg (0.1 mmol) of bromopyridine derivative **7** and 50 mg (0.6 mmol) of $NaHCO_3$ in 10 mL of dioxane and 10 mL of tetrahydrofuran was stirred at 0 °C and treated with 26.5 mg (0.13 mmol) of (2*S*)-3-amino-2-[(*tert*-butoxycarbonyl)amino]propionamide (**12**).[4b] The reaction mixture was stirred at 0 °C for 24 h, filtered through Celite, and concentrated to dryness under diminished pressure. The residual oil was purified by preparative silica gel TLC (development with 10% CH_3OH in $CHCl_3$) to provide the individual diastereomers of **8**. (*S*,*S*)-**8** was isolated as a light yellow oil (R_f 0.43), yield 23 mg (48%): IR (film, cm^{-1}) 3380–3320 (br), 2950, 2850, 1750, 1690, 1585, 1150, 1100, and 1020; ^{1}H NMR (D_2O, DSS) δ 1.35 (t, 3, J = 7.0 Hz), 1.35 (s, 9), 2.90 (s, 3), 2.50–3.25 (m, 4), 3.50–3.65 (m, 1), 4.00–4.17 (m, 1), and 4.48 (q, 2, J = 7.0 Hz); mass spectrum (chemical ionization, isobutane), m/z 480.229 ($C_{19}H_{30}N_9O_6$ requires 480.232) $(M + 1)^+$, 407, 277, 204, and 148. (*R*,*S*)-**8** was also isolated as a light yellow oil (R_f 0.34), yield 23 mg (48%): IR (film, cm^{-1}) 3420–3360 (br), 2960, 2880, 1735, 1670, 1570, 1170, and 1030; ^{1}H NMR (D_2O, DSS) δ 1.37 (t, 3, J = 7.0 Hz), 1.40 (s, 9), 2.81 (s, 3), 2.60–3.20 (m, 4), 3.70 (dd, 1, J = 4.5, 9.0 Hz), 4.11 (m, 1), and 4.42 (q, 2, J = 7.0 Hz); mass spectrum (chemical ionization, isobutane), m/z 480.229 ($C_{19}H_{30}N_9O_6$ requires 480.232) $(M + 1)^+$, 407, 277, 251, 204, and 148.

N^α-(*tert*-Butoxycarbonyl)-N^β-[1-amino-2-(4-azido-6-carbethoxy-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanineamide (9). A reaction mixture containing 250 mg (0.7 mmol) of bromopyrimidine derivative **7**, 230 mg (1.13 mmol) of (2*S*)-3-amino-2-[(*tert*-butoxycarbonyl)amino]propionamide (**12**), and 88 mg (1.0 mmol) of $NaHCO_3$ in 22 mL of 10:1 EtOH–$CHCl_3$ was stirred overnight at 25 °C. The reaction mixture was concentrated under diminished pressure, affording a mixture of several products, including both diastereomers of **9**. One of these diastereomers was isolated in ~30% yield by flash column chromatography (7% MeOH in $CHCl_3$) and precipitated from $CHCl_3$–ether as an amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3, J = 7.5 Hz), 1.40 (s, 9) 2.27 (br s, 2), 2.93 (s, 3) 2.93 (m, 2), 3.83 (m, 2), 4.17 (m, 2), 4.43 (q, 2, J = 7.5 Hz), 5.60 (br s, 2), 5.87 (br s, 1), and 6.80 (br s, 1); mass spectrum (chemical ionization, isobutane), m/z 480 $(M + 1)^+$, 454, 408, 407, 401, 300, 277, 268, 250, 232, and 204.

N^α-(*tert*-Butoxycarbonyl)-N^β-[1-amino-3-(4-amino-6-carbethoxy-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanineamide (15). A solution containing 5 mg (10 μmol) of (*S*,*S*)-**8** in 15 mL of ethanol was treated with 1 drop of 10% aqueous hydrochloric acid and 15 mg of 10% palladium-on-charcoal and stirred under a H_2 atmosphere at 25 °C for 12 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated under diminished pressure. The residue was purified by preparative silica gel TLC (development with 3:1 $CHCl_3$–ethanol; R_f 0.53). Compound **15** crystallized from 2-propanol as colorless needles, yield 4 mg (85%): mp 159–162 °C; $[\alpha]^{25}_D$ – 7.5° (*c* 1.0, C_2H_5OH); IR (KBr, cm^{-1}) 3460–3210 (br), 2960, 2920, 1710, 1670, 1620, 1250, 1160, 1040, 1020, and 750; ^{1}H NMR (D_2O) δ 1.28 (t, 3, J = 7.2 Hz), 1.30 (s, 9), 2.05 (s, 3), 2.60–2.80 (m, 3), 2.90 (m, 1), 3.60 (m, 1), 3.98 (m, 1), and 4.34 (q, 2, J = 7.2 Hz); mass spectrum (chemical ionization, isobutane), m/z 454 $(M + 1)^+$ and 211; high-resolution mass spectrum, m/z 453.233 ($C_{19}H_{31}N_7O_6$ requires 453.233).

A 5-mg sample of (*R*,*S*)-**8** was reduced in the same fashion; isolation by HPTLC (R_f 0.45, 3:1 $CHCl_3$–ethanol) provided 4.3 mg (91%) of **16** as colorless needles from 2-propanol: mp 64–66 °C; $[\alpha]^{25}_D$ +14.8° (*c* 1.0, C_2H_5OH); IR (KBr, cm^{-1}) 3420–3300, 2900, 2820, 1745, 1650, 1580, 1160, 1040, and 1010; ^{1}H NMR (D_2O) δ 1.28 (t, 3, J = 7.2 Hz), 2.05 (s, 3), 2.65–2.80 (m, 3), 2.85–3.00 (m, 1), 3.55 (m, 1), 3.95 (br s, 1), and 4.35 (q, 2, J = 7.2 Hz); mass spectrum (chemical ionization, isobutane), m/z 454.245 ($C_{19}H_{32}N_7O_6$ requires 454.241) $(M + 1)^+$, 354, 337, 204, and 102.

N^α-(*tert*-Butoxycarbonyl)-N^β-[1-amino-3-(4-amino-6-carboxy-5-methylpyrimdin-2-yl)propion-3-yl]-(*S*)-β-aminoalanineamide (1). A solution containing 9 mg (20 μmol) of **15** in 3 mL of ethanol was stirred, cooled to 0 °C, and then treated with 5 mL of 0.1 M aqueous NaOH. The reaction mixture was stirred at 0 °C for 1 h, acidified to pH 5 with 1 M citric acid, and diluted with 5 mL of water. The aqueous solution was washed with water and desalted on an XAD-2 column (applied as a standard NaCl solution, followed by washing with water and elution of the desired product with a gradient of CH_3OH in H_2O (0 → 50%)). The crude product was purified by preparative silica gel TLC (development with 4:1:1 1-butanol–HOAc–H_2O; R_f 0.20). Pyrimidoblamic acid (**1**) was obtained as colorless microcrystals, yield 8.5 mg (100%): mp 185 °C (softens), 220–222° C dec [lit.[4a] mp 223–225 °C dec]; silica gel TLC R_f 0.25 (5:2:3 1-butanol–HOAc–H_2O), R_f 0.10 (DMF), 0.14 (1:1 DMF–CH_3OH); $[\alpha]^{28}_D$ –33.6° (*c* 0.75, H_2O) [lit.[4a] $[\alpha]^{28}_D$ –32.8° (*c* 0.75, H_2O)]; ^{1}H NMR (D_2O) δ 1.30 (s, 9), 1.98 (s, 3), 2.87 (m, 2), 3.15 (m, 1), 3.38 (m, 1), and 4.37 (m, 2); mass spectrum (chemical ionization, isobutane), m/z 425 (M^+), 351, 309, 281, 267, 251, 235, 207, 191, 177, 176, 147, 135, 131, 115, 103, 96, 73, and 69.

A solution containing 5 mg (11 μmol) of **16** in 3 mL of ethanol was stirred, cooled to 0 °C, and then treated dropwise with 5 mL of 0.1 M aqueous NaOH. The reaction mixture was stirred at 0 °C for 1 h, acidified to pH 5 with 1 M citric acid, and worked up as described above for **1**. Epipyrimidoblamic acid (**2**) was obtained as a colorless, amorphous solid, yield 4.5 mg (96%): mp 174 °C (softens), 221 °C dec; silica gel TLC R_f 0.18 (4:1:1 1-butanol–HOAc–H_2O), R_f 0.23 (5:2:3 1-butanol–HOAc–H_2O), R_f 0.10 (DMF), R_f 0.14 (1:1 DMF–CH_3OH); $[\alpha]^{25}_D$ + 20.8° (*c* 0.65, H_2O); ^{1}H NMR (D_2O) δ 1.30 (s, 9), 1.95 (s, 3), 2.80 (d, 2), 2.95 (m, 1), 3.60 (m, 1), and 4.35 (m, 2); mass spectrum (chemical ionization, isobutane), m/z 207, 177, 135, 133, 73, and 56.

(2*S*)-3-[(Benzyloxy)amino]-2-[(*tert*-butoxycarbonyl)-amino]propionamide (11). A solution of 2.00 g (6.85 mmol) of (2*S*)-*N*-(benzyloxy)-2-azetidinone[11] (**10**) in 8 mL of methanol was cooled to 0 °C and saturated with NH_3 for 10 min. The reaction mixture was stirred at 0 °C for 3 h and then concentrated under diminished pressure. The residue crystallized from ether–hexane, providing propionamide **11** as colorless needles, yield 1.90 g (90%): mp 86–89 °C; silica gel TLC R_f 0.18 (EtOAc); $[\alpha]^{25}_D$ –8.9° (*c* 1.25, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9), 3.07 (dd, 1, J = 14, 6 Hz), 3.33 (dd, 1, J = 14, 6 Hz), 4.23 (m, 1), 4.66 (s, 2), 5.50 (m, 2), 5.90 (br s, 1), 6.48 (br s, 1), and 7.30 (s, 5); mass spectrum (chemical ionization, isobutane), m/z 310.178 ($C_{15}H_{24}N_3O_4$ requires 310.176) $(M + 1)^+$ and 254.

(2*S*)-3-Amino-2-[(*tert*-butoxycarbonyl)amino]propion-amide (12). A solution of 9.01 g (29.2 mmol) of (2*S*)-3-[(benzyloxy)amino]-2-[(*tert*-butoxycarbonyl)amino]propionamide (**11**) in 100 mL methanol was treated with 900 mg of 10% palladium-on-charcoal and maintained under a H_2 atmosphere at 25 °C for 4 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel (3.8 × 7.6 cm) column; washing with ethyl acetate effected elution of less polar impurities, after which the desired product was recovered by washing with 3:2 CH_3OH–ethyl acetate. Concentration of the appropriate fractions afforded (2*S*)-3-amino-2-[(*tert*-butoxycarbonyl)amino]propionamide (**12**) as a colorless, viscous oil that crystallized on standing, yield 5.90 g (100%): mp 95–97 °C; $[\alpha]^{25}_D$ +4.6° (*c* 1, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9), 1.63 (s, 2), 2.83 (dd, 1, J = 13, 7.5 Hz), 2.82 (dd, 1, J = 13, 7.5 Hz), 4.08 (m, 1), 5.88 (br d, 1, J = 7.5 Hz), 6.27 (br s, 1), and 7.20 (br s, 1); mass spectrum (chemical ionization, isobutane), m/z 204.134 ($C_8H_{18}N_3O_3$ requires 204.134) $(M + 1)^+$ and 149.

Diaminopropionamide **12** was stored as its hydrochloride salt. A solution of **12** (775 mg, 3.8 mmol) in 17 mL of dry ether was cooled to 0 °C, treated with 8 mL of dry methanol saturated with hydrogen chloride, and diluted with 120 mL of dry ether. The hydrochloride salt of **12** precipitated as colorless microcrystals, which were isolated by filtration, washed with dry ether, and dried

under vacuum, yield 731 mg (80%): mp 179–180 °C; $[\alpha]^{25}_D$ −20.8° (c 1.2, H_2O); ^1H NMR (D_2O) δ 1.41 (s, 9), 3.21 (dd, 1, J = 13, 7.5 Hz), 3.47 (dd, 1, J = 13, 6 Hz), and 4.37 (dd, 1, J = 7.5, 6 Hz).

Free amine **12** were regenerated prior to use by passing a methanolic solution of **12**·HCl through a column of Dowex 1 × 2 resin (HCO_3^-) form; concentration of the ninhydrin-positive fractions afforded **12** as a colorless solid: mp 95–97 °C.

Hydrolysis of (2*S*)-3-Amino-2-[(*tert*-butoxycarbonyl)-amino]propionamide (12). A solution containing 80 mg (0.39 mmol) of diaminopropionamide **12** in 3.5 mL of 3 N hydrochloride acid was heated at reflux for 4 h. The solution was concentrated to dryness, and the residue was dissolved in a small amount of water and treated with 2 mL of ethanol. Colorless microcrystals of (2*S*)-2,3-diaminopropionic acid monohydrochloride were obtained by filtration, yield 55 mg (quantitative): mp 242 °C; $[\alpha]^{25}_D$ +26.3° (c 1.5, 1 N HCl). An authentic sample (Calbiochem-Behring Corporation) of (2*S*)-2,3-diaminopropionic acid hydrochloride gave mp 233 °C dec, no mp depression on admixture with the hydrolysis product; $[\alpha]^{25}_D$ +25.7° (c 1.58, 1 N HCl).

Treatment of 3-(4-Azido-6-carbethoxy-5-methylpyrimidin-2-yl)-3-bromopropionamide (7) with *n*-Butylamine. A solution containing 105 mg (0.29 mmol) of bromopropionamide **7** in 1 mL of DMF was cooled to 0 °C, treated dropwise with 68 μL (50 mg, 0.68 mmol) of *n*-butylamine dissolved in a small volume of DMF, and then stirred at 0 °C for 5 h. The solvent was removed under diminished pressure, and the residue was dissolved in ethyl acetate, washed with saturated $NaHCO_3$, and dried ($MgSO_4$). Concentration afforded a residue that was applied to a silica gel column. The column was washed with 5% ethanol in $CHCl_3$, which effected elution of two products. The first product to elute was 3-(4-azido-6-carbethoxy-5-methylpyrimidin-2-yl)-2-(*n*-butylamino)propionamide (**13**), which was isolated as a colorless oil, yield 26 mg (25%): silica gel TLC R_f 0.69 (10% CH_3OH in $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.98 (t, 3, J = 7.0 Hz), 1.48 (m, 7), 2.61 (t, 2, J = 7.5 Hz), 2.90 (s, 3), 3.85 (m, 3), 4.40 (q, 2, J = 7.0 Hz), 5.90 (br s, 1) and 7.29 (br s, 2); mass spectrum (chemical ionization, isobutane), *m/z* 350.193 ($C_{15}H_{24}N_7O_3$ requires 350.194) $(M + 1)^+$, 147, and 74.

The second product to elute from the silica gel column was (*E*)-3-(4-azido-6-carbethoxy-5-methylpyrimidin-2-yl)propenamide (**14**), which was isolated as a yellow solid, yield 37 mg (46%): silica gel TLC R_f 0.36 (10% CH_3OH in $CHCl_3$); ^1H NMR ($CDCl_3$) (tetrazole) δ 1.47 (t, 3, J = 7.5 Hz), 3.02 (s, 3), 4.52 (q, 2, J = 7.5 Hz), 5.74 (br s, 2), 7.84 (d, 1, J = 15 Hz), and 8.22 (d, 1, J = 15 Hz); (azide) 1.43 (t, 3, J = 7.5 Hz), 2.28 (s, 3), 4.46 (q, 2, J = 7.5 Hz), 5.09 (br s, 2), 7.23 (d, 1, J = 15 Hz), and 7.55 (d, 1, J = 15 Hz).

A sample of (*E*)-3-(4-azido-6-carbethoxy-5-methylpyrimidin-2-yl)propenamide (**14**) (16 mg, 58 μmol) and 7 μL (5 mg, 68 μmol) of *n*-butylamine were dissolved in 0.5 mL of cold DMF. The reaction mixture was stirred 0 °C overnight and then concentrated under diminished pressure. Chromatography of the residue (elution with 5% CH_3OH in $CHCl_3$) afforded 5 mg (25%) of a colorless oil that proved to be identical with **13**. Further washing of the column afforded 3 mg of recovered **14**.

(*E*)-1-(6-Carbobenzoxy-4-chloro-5-methylpyrimidin-2-yl)-2-phenylethylene (18). A solution containing 35.0 g (116 mmol) of (*E*)-1-(6-carbethoxy-4-chloro-5-methylpyrimidin-2-yl)-2-phenylethylene (**17**) and 3.52 g (27 mmol) of sodium benzoxide in 300 mL of dry benzyl alcohol was stirred at 80 °C for 4 h under argon. Excess benzyl alcohol was removed under diminished pressure; the residue crystallized from ethyl acetate as colorless microcrystals, yield 25.8 g (61%): mp 135–137 °C; ^1H NMR ($CDCl_3$) δ 2.38 (s, 3), 5.40 (s, 2), 7.12 (d, 1, J = 15 Hz), 7.38 (m, 10), and 7.97 (d, 1, J = 15 Hz); mass spectrum (chemical ionization, isobutane), *m/z* 365.104 ($C_{21}H_{18}N_2O_2Cl$ requires 365.105) $(M + 1)^+$, 329, and 91.

Benzyl 6-Chloro-2-formyl-5-methylpyrimidine-6-carboxylate (19). A solution of 12.5 g (34 mmol) of pyrimidine **18** in 96 mL of 1:1 $CHCl_3$–CH_2Cl_2 was cooled to −78 °C and saturated with ozone. The reaction mixture was then treated with 48 mL of dimethyl sulfide and maintained under argon at 25 °C for 14 h. The solvent was concentrated under diminished pressure, and the residue was purified by chromatography on a silica gel column (6 × 22 cm); elution was carried out using 5% ether in hexane. Benzyl 6-chloro-2-formyl-5-methylpyrimidine-6-carboxylate (**19**) was obtained as a colorless oil, yield 7.94 g (80%): ^1H NMR ($CDCl_3$) δ 2.47 (s, 3), 5.37 (s, 2), 7.40 (m, 5), and 9.93 (s, 1); mass spectrum (chemical ionization, isobutane) *m/z* 291.052 ($C_{14}H_{12}N_2O_3Cl$ requires 291.054) $(M + 1)^+$, 184, 156, 119, and 91.

N^α-(*tert*-Butoxycarbonyl)-N^β-[[(6-carbobenzoxy-4-chloro-5-methylpyrimidin-2-yl)methylene]amino]-(*S*)-β-aminoalanineamide (20). A solution containing 7.94 g (27 mmol) of benzyl 6-chloro-2-formyl-5-methylpyrimidine-6-carboxylate (**19**) and 5.55 g (27 mmol) of (2*S*)-3-amino-2-[(*tert*-butoxycarbonyl)-amino]propionamide (**12**) in 150 mL of dry CH_3CN was stirred with 200 g of powdered 3-Å molecular sieves at 25 °C for 24 h. The suspension was filtered, and the solid material was washed with dry CH_3CN. The combined CH_3CN filtrate was concentrated under diminished pressure to afford imine **20** as a light yellow solid, yield 10.5 g (81%): ^1H NMR ($CDCl_3$) δ 1.45 (s, 9), 2.50 (s, 3), 3.98 (dd, 1, J = 7.5, 12.5 Hz), 4.13 (dd, 1, J = 6, 12.5 Hz), 4.46 (dd, 1, J = 6, 7.5 Hz), 5.40 (s, 2), 5.67 (br s, 2), 6.82 (br s, 1), 7.35–7.46 (m, 5), and 8.42 (s, 1); mass spectrum (chemical ionization, isobutane), *m/z* 476.173 ($C_{22}H_{27}N_5O_5Cl$ requires 476.170) $(M + 1)^+$, 342, 235, 204, and 148.

N^α-(*tert*-Butoxycarbonyl)-N^β-[1-ethoxy-3-(6-carbobenzoxy-4-chloro-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanineamide (21). A solution containing 41 g (86 mmol) of imine **20** and 101 g (765 mmol) of monoethyl malonate in 650 mL of benzene was stirred at 42 °C for 24 h under argon. The cooled reaction mixture was diluted with 500 mL of ethyl acetate, and the combined solution was washed with 10% aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated under diminished pressure. The crude product was purified by flash chromatography[18] on silica gel; elution was carrier out using 20% ethyl acetate in hexane. N^α-(*tert*-Butoxycarbonyl)-N^β-[1-ethoxy-3-(6-carbobenzoxy-4-chloro-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanineamide (**21**) was isolated as a colorless oil, yield 6.9 g (14%): silica gel TLC R_f 0.35 (4:1 hexane–ethyl acetate); ^1H NMR ($CDCl_3$) δ 1.24 (t, 3, J = 7.2 Hz), 1.45 (s, 9), 2.42 (s, 3), 2.45 (m, 2), 2.75 (dd, 1, J = 9.0, 16.2 Hz), 2.87 (dd, 1, J = 5.4, 16.2 Hz), 3.05 (br s, 1), 3.17 (dd, 1, J = 9.0, 5.4 Hz), 4.52 (q, 2, J = 7.2 Hz), 4.30 (m, 1), 5.37 (br s, 2), 5.42 (s, 2), 5.80 (br s, 1), and 7.40 (m, 5): mass spectrum (chemical ionization, isobutane), *m/z* 564.218 ($C_{26}H_{35}N_5O_7Cl$ requires 564.222) $(M + 1)^+$, 502, 464, 390, 378, 302, 179, 177, 91 and 89.

N^α-(*tert*-Butoxycarbonyl)-N^β-[1-ethoxy-3-(4-azido-6-carbobenzoxy-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanineamide (22). A solution containing 1.10 g (1.95 mmol) of chloropyrimidine **21** and 550 mg (8.46 mmol) of sodium azide in 55 mL of DMF was stirred at 0 °C under argon for 4 h. The cooled reaction mixture was poured into water and extracted with ethyl acetate. The organic extract was dried ($MgSO_4$) and concentrated under diminished pressure to afford azidopyrimidine **22** as a colorless oil, yield 1.11 g (100%): silica gel TLC R_f 0.32 (5% EtOH in ether); NMR ($CDCl_3$) (mixture of tautomers and enantiomers) δ 1.15 (m, 3), 1.40, 1.41, and 1.42 (3 s, 9), 1.65 (br s, 2), 2.20 and 2.96 (2 s, 3), 2.72–2.88 (m, 2), 3.00–3.25 (m, 3), 4.05–4.18 (m, 2), 5.06–5.18 (m, 1), 5.27 (br s, 1), 5.40, 5.50, and 5.52 (3 s, 2), 5.75 (br s, 1), 7.35–7.50 (m, 5); IR (film, cm^{-1}) 3310 (br), 2960, 2900, 2120, 1740, 1700, 1680, 1670, 1480, 1460, and 1165; chemical ionization mass spectrum (positive ion), *m/z* 571.264 ($C_{26}H_{35}N_8O_7$ requires 571.263) $(M + 1)^+$, 557, 545, 385, 359, 302, 242, 204, and 91.

N^α-(*tert*-Butoxycarbonyl)-N^β-[1-ethoxy-3-(4-amino-6-carboxy-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanineamide (23). Azidopyrimidine **22** (1.11 g, 1.95 mmol) was dissolved in 130 mL of CH_3OH, and the solution was treated with 130 mg of 10% palladium-on-charcoal. The reaction mixture was stirred under a H_2 atmosphere at 25 °C for 5 h and then filtered through Celite to remove the catalyst. Concentration of the filtrate under diminished pressure afforded N^α-(*tert*-butoxycarbonyl)-N^β-[1-ethoxy-3-(4-amino-6-carboxy-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanineamide (**23**) as a light yellow solid, yield 855 mg (97%): silica gel TLC R_f 0.23 (1:1 $CHCl_3$–CH_3OH); ^1H NMR (CD_3OH) δ 1.20 (t, 3, J = 7.2 Hz), 1.44 (s, 9), 2.28 (s, 3), 2.89–3.20 (m, 4), 4.12 (d, 2, J = 7.5 Hz), and 4.27 (m, 2); IR (film, cm^{-1}) 3320 (br), 2960, 2920, 1635 (br), 1370, 1240,

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and 1020; chemical ionization mass spectrum (positive ion, methane) m/z 455.221 ($C_{19}H_{31}N_6O_7$ requires 455.225) $(M + 1)^+$, 437, 411, 383, 355, 337, 327, 311, 225, 148, and 131.

N^α-(*tert*-Butoxycarbonyl)-N^β-[1-amino-3-(4-amino-6-carboxy-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanineamide (1). A solution containing 488 mg (1.07 mmol) of pyrimidine **23** in 15 mL of absolute ethanol was cooled to −78 °C and treated with liquid NH_3 to a total volume of 25 mL. The cooled, combined solution was sealed in a glass tube and maintained at 25 °C for 7 days. The sealed tube was opened at −78 °C and then permitted to warm to 25 °C for removal of excess NH_3. The resulting solution was concentrated on a rotary evaporator, and the solid residue was dissolved in water and applied to a (30 × 1.5 cm) XAD-2 column. Elution with a linear gradient of methanol in water (0 → 100%) provided 266 mg of recovered pyrimidine **23** (~30% CH_3OH in H_2O) and then 126 mg of crude **1**. The latter was dissolved in 5 mL of absolute ethanol and maintained at 0 °C for 4 h. Pyrimidoblamic acid (**1**) was deposited as colorless microcrystals, yield 34 mg (15%).

N^α-(*tert*-Butoxycarbonyl)-N^β-[(6-carbethoxy-4-chloro-5-methylpyrimidin-2-yl)methyl]-(*S*)-β-aminoalanineamide (25). A solution containing 1.33 g (3.2 mmol) of N^α-(*tert*-butoxycarbonyl-N^β-[[(6-carbethoxy-4-chloro-5-methylpyrimidin-2-yl)methylene]amino]-(*S*)-β-aminoalanineamide (**24**) in 25 mL of CH_3OH was cooled to 0 °C and treated with 400 mg (6.4 mmol) of sodium cyanoborohydride. The reaction mixture was stirred at 0 °C for 30 min, treated with 1 mL of 1 M citric acid, and concentrated under diminished pressure. The residue was dissolved in 0.1 M citric acid and extracted with ethyl acetate. The aqueous phase was treated with $NaHCO_3$ until it became alkaline and was again extracted with ethyl acetate. This ethyl acetate extract was dried ($MgSO_4$) and concentrated under diminished pressure to afford chloropyrimidine **25** as a yellow oil, yield 915 mg (68%): $[\alpha]^{25}_D$ +12.2° (*c* 2.0, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.40 (t, 3, *J* = 7.5 Hz), 1.41 (s, 9), 2.43 (s, 3), 2.63 (br s, 1), 2.78 (dd, 1, *J* = 13.5, 7.5 Hz), 3.20 (dd, 1, *J* = 13.5, 4.5 Hz), 4.06 (s, 2), 4.12 (dd, 1, *J* = 7.5, 4.5 Hz), 4.45 (q, 2, *J* = 7.5 Hz), 5.87 (br s, 1), and 7.40 (br s, 1); mass spectrum (chemical ionization, isobutane), m/z 416.167 ($C_{17}H_{27}N_5O_5Cl$ requires 416.170) $(M + 1)^+$, 182, 89.

N^α,N^β-Bis(*tert*-butoxycarbonyl)-N^β-[(6-carbethoxy-4-chloro-5-methylpyrimidin-2-yl)methyl]-(*S*)-β-aminoalanineamide (26). A solution containing 900 mg (2.17 mmol) of pyrimidine **25** in 20 mL of dry pyridine was treated with 700 mg (3.21 mmol) of di-*tert*-butyl pyrocarbonate and stirred at 25 °C for 24 h under argon. The reaction mixture was concentrated under diminished pressure, and the residue was dissolved in ethyl acetate, washed successively with 0.1 M aqueous citric acid and brine, and then dried ($MgSO_4$). Concentration of the organic phase afforded a brown oil that was purified by chromatography on a silica gel column. Elution with ethyl acetate provided bis-*t*-Boc-pyrimidine **26** as a colorless oil, yield 562 mg (50%): silica gel TLC R_f 0.44 (EtOAc), R_f 0.31 (4:1 EtOAc–hexanes); ^1H NMR ($CDCl_3$) δ 1.20 (s, 3), 1.40 (t, 3, *J* = 7.5 Hz), 1.41 (s, 15), 2.45 (s, 3), 3.40 (m, 1), 3.95 (m, 1), 4.52 (q, 2, *J* = 7.5 Hz), 4.07–5.10 (m, 4), 5.60 (br s, 1), and 6.00 (br s, 1); mass spectrum (chemical ionization, isobutane), m/z 516.222 ($C_{22}H_{35}N_5O_7Cl$ requires 516.222) $(M + 1)^+$, 416, 161, 129, 117, and 105.

N^α,N^β-Bis(*tert*-butoxycarbonyl)-N^β-[(6-carboxy-4-chloro-5-methylpyrimidin-2-yl)methyl]-(*S*)-β-aminoalanineamide (27). A solution of 550 mg (1.07 mmol) of pyrimidine **26** in 24 mL of ethanol and 12 mL of 0.1 N NaOH was stirred at 0 °C for 1 h, neutralized with 1 N citric acid, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with brine, dried ($MgSO_4$), and concentrated under diminished pressure to afford pyrimidine carboxylic acid **27** as a yellow foam, yield 407 mg (78%): $[\alpha]^{24}_D$ +9.3° (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.18 (s, 6), 1.40 (s, 12), 2.60 (s, 3), 3.20 (m, 1), 4.15 (m, 2), 4.43 (dd, 1, *J* = 18, 9 Hz), 5.00 (dd, 1, *J* = 18, 9 Hz), 5.65 (m, 1), 8.07 (br s, 1), and 9.40 (br s, 1); mass spectrum (chemical ionization, isobutane), m/z 488 $(M + 1)^+$, 390, 388, 346, 344, 161, 142, 118, 105, and 62; high-resolution mass spectrum m/z 487.181 ($C_{20}H_{30}N_5O_7Cl$ requires 487.183).

N^α,N^β-Bis(*tert*-butoxycarbonyl)-N^β-[(4-amino-6-carboxy-5-methylpyrimidin-2-yl)methyl]-(*S*)-β-aminoalanineamide (3). A solution containing 400 mg (0.82 mmol) of pyrimidine **27** in 10 mL of ethanol was cooled to −78 °C and treated with 7 mL of liquid NH_3. The cooled, combined solution was sealed in a glass tube and heated at 43–45 °C for 10 days. The sealed tube was opened at −78 °C and then permitted to warm to 25 °C for removal of excess NH_3. The resulting solution was concentrated on a rotary evaporator, and the solid residue was dissolved in 20 mL of water, acidified with 1 M citric acid, and washed with $CHCl_3$. The aqueous layer was neutralized with $NaHCO_3$, saturated with NaCl, and extracted with 1-butanol. The butanol extract was concentrated under diminished pressure; crystallization of the residue from 2-propanol–ethanol afforded bis-*t*-Boc desamidopyrimidoblamic acid (**3**) as yellowish microcrystals, yield 145 mg (38%): silica gel TLC R_f 0.15 (7:3 $CHCl_3$–CH_3OH); $[\alpha]^{25}_D$ −8.9° (*c* 1.25, CH_3OH); ^1H NMR (D_2O) 1.20 (s, 6), 1.42 (s, 12), 2.02 (s, 3), 3.87 (m, 2), and 4.23–4.50 (m, 3); FAB mass spectrum, m/z 491 $(M + Na)^+$.

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Registry No. 1, 75452-30-1; **2**, 96922-00-8; **3**, 129812-61-9; **4**, 129812-62-0; **5**, 75624-21-4; **6**, 129812-63-1; **7**, 129812-64-2; **7a**, 129812-82-4; (*S*,*S*)-**8**, 129812-65-3; (*R*,*S*)-**8**, 129812-66-4; (*S*,*S*)-**9**, 129833-08-5; (*R*,*S*)-**9**, 129812-81-3; **10**, 71405-00-0; **11**, 75624-25-8; **12** (free base), 75452-33-4; **12**·HCl, 75624-26-9; **13**, 129833-06-3; **14** (azide form), 129812-67-5; **14** (tetrazole form), 129812-83-5; **15**, 75624-28-1; **16**, 75624-27-0; **17**, 129812-68-6; **18**, 129812-69-7; **19**, 129812-70-0; **20**, 129812-71-1; (*S*,*S*)-**21**, 129812-72-2; (*R*,*S*)-**21**, 129833-07-4; (*S*,*S*)-**22** (azide form), 129812-76-6; (*S*,*S*)-**22** (tetrazole form), 129812-84-6; (*R*,*S*)-**22** (azide form), 129812-77-7; (*R*,*S*)-**22** (tetrazole form), 129812-85-7; (*S*,*S*)-**23**, 129812-78-8; (*R*,*S*)-**23**, 129812-79-9; **24**, 75460-34-3; **25**, 129812-73-3; **26**, 129812-74-4; **27**, 129812-75-5; I, 11056-06-7; II, 69913-05-9; $HOOCCH_2COOEt$, 1071-46-1; (2*S*)-$H_2NCH_2CH(NH_2)COOH$·HCl, 1482-97-9; ethyl 3-(4-azido-6-carboxamido-5-methylpyrimidin-2-yl)propionate, 129812-80-2.