cophytonate (3; 5 mg) was obtained from the CH_2Cl_2 extract (7.4 g) as a substance slightly more polar than 1. R_f values in TLC [Merck Kieselgel 60 GF_{254}; CH_2Cl_2–MeOH (24:1)] of 1 and 3 are as follows: 1, 0.46; 3, 0.40.

$NaBH_4$ Reduction of 1. Methyl sarcophytoate (1; 4.9 mg) was treated with $NaBH_4$ (24.5 mg) in MeOH (1 mL) at room temperature for 1 h. After workup the crude product was subjected to HPLC [Inertsil ODS; MeOH–H_2O (17:3)] to give 2 (1.8 mg).

Registry No. 1, 129239-13-0; 3, 129239-14-1.

Supplementary Material Available: NMR data of 1–3, NOESY spectra of 1 and 2, and NMR spectra of 1 and 3 (14 pages). Ordering information is given on any current masthead page.

Preparation of a Protected (2*S*,3*S*)-β-Hydroxyaspartic Acid Suitable for Solid-Phase Peptide Synthesis

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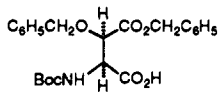
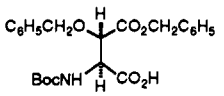
(2*S*,3*S*)-*N*-Boc-3-(benzyloxy)aspartic acid β-benzyl ester (2), a β-hydroxyaspartic acid derivative suitably protected for incorporation into peptides by solid-phase synthesis, was synthesized from *N*-Boc-(*R*)-serine via the intermediate, [4*R*-(*R**,*R**)]-4-[hydroxy[2-(trimethylsilyl)ethynyl]methyl]-2,2,-dimethyl-3-oxazolidinecarboxylic acid 1,1-dimethylethyl ester (5). Key transformations involved the ruthenium tetraoxide mediated oxidation of the ethynyl moiety to form the β-carboxylic acid and, after esterification and oxazolidine ring hydrolysis, dichromate oxidation of the resulting primary alcohol to the α-carboxylic acid. The method is suitable for the preparation of gram quantities of 2.

We recently required access to suitably protected β-hydroxyaspartic acids for incorporation into peptide analogues using solid-phase synthesis. Previously, (2*S*,3*R*)-*N*-Boc-β-(benzyloxy)aspartic acid β-benzyl ester (1) was prepared bearing appropriate protecting groups for peptide synthesis.[1,2] In the present report, we describe a complimentary route leading to (2*S*,3*S*)-*N*-Boc-β-(benzyloxy)aspartic acid β-benzyl ester (2) starting from (*R*)-serine.



1

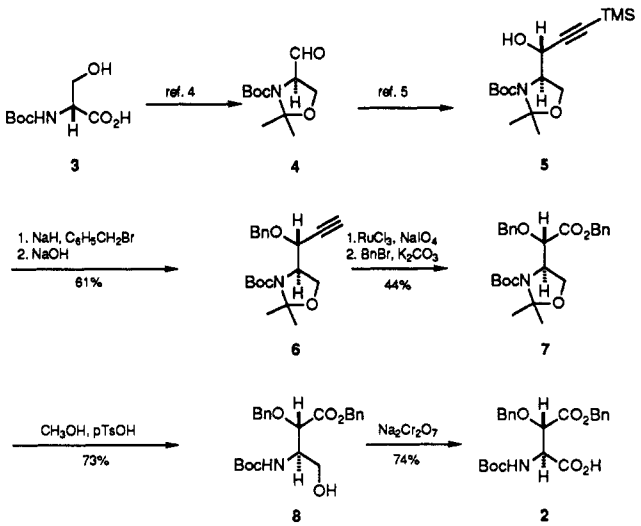


2

The three-step conversion of *N*-Boc-(*R*)-serine to the configurationally stable aldehyde 4[3] and the copper-mediated, diastereoselective addition of (trimethylsilyl)-acetylide to provide the acetylenic alcohol 5 proceeded without the incident as described.[4] Conversion of the alcohol 5 to the corresponding benzyl ether was effected by treatment with sodium hydride and benzyl bromide in DMF at room temperature for 2 h. When the reaction was quenched with water and then stirred for 45 min, complete desilylation occurred to give 6 directly. Oxidation of the acetylenic moiety of 6 in the presence of ruthenium tetraoxide and sodium periodate[5] provided an intermediate carboxylic acid as an oil, which was immediately treated with potassium carbonate and benzyl bromide in DMF to afford the benzyl ester 7 in 44% yield for the two steps.

Attempted selective hydrolysis of the acetonide contained in 7 with methanol in the presence of Amberlyst 15 as generally described by Herold[4] gave the desired alcohol 8 in low yield accompanied by large amounts of very polar products which were not characterized. After considerable experimentation, it was found that a procedure

Scheme I



3 4 5 6 7 8 2

reported by Beaulieu and Schiller was preferable.[6] Thus the acetonide 7 was treated with a catalytic amount of *p*-toluenesulfonic acid in refluxing wet methanol to provided the primary alcohol 8 as a 1:1.5 mixture with recovered starting material. Recycling afforded an overall 73% yield of 8. Finally, oxidation of 8 to the required product 2 was accomplished efficiently using chromic acid in an ether–water two-phase system.[7]

While analysis of the NMR spectra of the intermediates possessing oxazolidine rings was complicated by the

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presence of long-lived rotamers,[3] spectra of the alcohol 8 and the β-benzyloxyaspartic acid 2 could be readily assigned. Since the same alternate (threo) diastereomer could have arisen either from a lack of stereoselectivity in the acetylide addition to the aldehyde 4 or from racemization during the final oxidation of 8, the ^1H and ^{13}C NMR spectra of 2 were compared with the corresponding spectra of the 2S,3R-diastereometer 1. For this purpose the region of the ^1H NMR spectra of 1 and 2 between 4.5 and 4.8 ppm proved most useful. Compound 2 displayed a pair of geminally coupled benzyl ether methylene protons at 4.50 and 4.79 ppm (J = 11.3 Hz) and a doublet at 4.75 ppm (J = 2.5 Hz) representing the hydrogen atom on the β-carbon. The corresponding peaks for 1 appeared at 4.60 and 4.83 ppm (J = 11 Hz) and 4.46 ppm (J = 3.6 Hz), respectively. Comparison of the two indicate that 2 consisted of >95% of a single diastereomer. Thus we conclude that the present method provides a highly diastereoselective and practical route to protected (S,S)-β-hydroxyaspartic acids which with suitable modification should be applicable to various peptide synthesis strategies.

Experimental Section

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Proton and carbon magnetic resonance spectra were recorded on a Varian XL-400 spectrometer, and shifts are reported in ppm downfield from tetramethylsilane used as an internal reference. Electron impact (EI, 70 eV) mass spectra were taken on a VG ZAB-1F mass spectrometer. Preparative high-pressure liquid chromatography (HPLC) was performed on silica gel Prep-Pak 500 cartridges using a Waters Associates Prep LC 500A. Flash silica gel chromatography employed Kiesel gel 60, 70–230 mesh, as supplied by E. Merck, Darmstadt, under a nitrogen pressure of 3–5 psi. DMF was dried over Linde 4A sieves; acetonitrile, carbon tetrachloride, methanol, and ether were supplied by Fisher and used without further purification. Concentration refers to removal of solvent under reduced pressure using a Buchi rotary evaporator.

[4*R*-(*R,*R**)]-4-[Ethynyl(phenylmethoxy)methyl]-2,2-dimethyl-3-oxazolidinecarboxylic Acid 1,1-Dimethylethyl Ester (6).** To a solution of alcohol 5[4] (24.0 g, 73 mmol) and benzyl bromide (8.7 mL, 73 mmol) in DMF (350 mL) at 0 °C was added a 50% dispersion of NaH in mineral oil (3.0 g, 81 mmol). The ice bath was removed, and the reaction mixture was stirred at ambient temperature (2 h). The reaction was quenched by careful dropwise addition of H_2O (2 mL). After stirring at ambient temperature (45 min), aqueous saturated NH_4Cl (5 mL) was added and the mixture was concentrated. The residue was partitioned between H_2O (1 L) and EtOAc (700 mL), and the organic layer was washed with additional H_2O (1 L) and brine (500 mL). The aqueous portions were extracted with EtOAc (500 mL), and the organic layers were combined, dried ($MgSO_4$), filtered, and concentrated to yield a crude oil (27 g). Purification by HPLC (hexanes/EtOAc, 12/1) provided 6 (15.4 g, 44.6 mmol) in 61% yield as a pale yellow oil, which crystallized on standing: mp 41–43 °C; $[\alpha]^{20}_D$ −26.7° (*c* 0.45, EtOH); IR ($CHCl_3$) ν 3305, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) (rotamers 1:1) δ 7.15 (m, 5 H, Ar*H*), 4.82 (2 dd, 1 H, J = 12.0 and 16.5 Hz, ArC*H*HO), 4.73 (br s, 0.5 H, ≡CC*H*O rotamer 1), 4.52 (2 dd, 1 H, J = 12.0 and 16.5 Hz, ArCH*H*O), 4.46 (br s, 0.5 H, ≡CCHO, rotamer 2), 4.23 and 4.02 (m, 1.5 H each C-5 CH_2 and NC*H* (rotamers)), 2.47 and 2.49 (0.5 each H, ≡C*H*), 1.35–1.75 (m, 15 H, C(CH_3)$_2$, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) (rotamers 1:1) δ 189.0, 137.8, 137.3, 130.8, 130.4, 130.3, 128.4, 128.3, 127.9, 94.8, 94.3, 80.6, 80.2, 75.9, 75.5, 71.4, 70.9, 69.2, 68.5, 64.8, 64.6, 59.6, 59.4, 28.4, 26.7, 26.0, 24.9, 23.5; mass spectrum, m/z 330 (M − 15), 230, 200, 198, 144, 100, 91, 57 (base), 41. Anal. Calcd for $C_{20}H_{27}NO_4$: C, 69.54; H, 7.88; N, 4.05. Found: C, 69.53; H, 8.02; N, 4.09.

[4*R*-(*R,*S**)]-2,2-Dimethyl-4-[(phenylmethoxy)[(phenylmethoxy)carbonyl]methyl]-3-oxazolidinecarboxylic Acid 1,1-Dimethylethyl Ester (7).** At room temperature a suspension of $NaIO_4$ (29.6 g, 144 mmol) in H_2O (135 mL) was added to a vigorously stirring solution of the acetylene 6 (15.4 g, 44.6 mmol) in CH_3CN (93 mL) and CCl_4 (93 mL), whereupon $RuCl_3 \cdot H_2O$ (0.2 g) was added in one portion. After 1 h the mixture was filtered through a pad of Celite, and the pad was washed with CH_3CN (4 × 100 mL). The filtrate was concentrated to dryness, and the residue was triturated with hexanes (8 × 200 mL) with the aid of ultrasound. The extracts were passed through Celite, combined with the previous filtrate, and concentrated to produce a tan oil (10.0 g). This residue was dissolved in DMF (300 mL) and stirred in the presence of K_2CO_3 (3.8 g, 27 mmol) and benzyl bromide (3.3 mL, 27 mmol) (16 h). The mixture was partitioned between H_2O (1.7 L) and EtOAc (1.5 L). The organic layer was washed with H_2O (2 × 1.5 L) and brine (1 L), and the combined aqueous layers were reextracted with EtOAc (1.5 L). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to provide 12 g of crude product. Purification by HPLC (hexanes/EtOAc, 12/1) provided 7 (8.90 g, 19.6 mmol) as a pale yellow oil in 44% yield. ^1H NMR ($CDCl_3$) (rotamers) δ 7.24–7.40 (m, 10 H, Ar*H*), 4.98–5.30 (m, 2 H, ArCH_2OC(O)), 4.66 (d, 0.5 H, J = 11.7 Hz, ArC*H*HO (rotamer 1)), 4.60 (d, 0.5 H, J = 11.1 Hz, ArC*H*HO (rotamer 2)), 4.26–4.55 (m, 2 H, ArCH*H*O, O_2CC*H*O), 4.13 (m, 1 H, NC*H*), 4.03 (m, 1 H, α-C-5 C*H*), 3.93 (dd, 1 H, J = 9.5, 6.5 Hz, β-C-5 C*H*), 1.35–1.70 (m, 15 H, C(CH_3)$_2$, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) (rotamers) δ 171.0, 137.5, 137.0, 135.5, 135.0, 128.5, 128.3, 128.1, 127.8, 94.4, 94.0, 80.5, 80.4, 77.0, 76.4, 72.6, 72.3, 67.1, 66.9, 66.3, 63.6, 59.4, 59.1, 58.9, 28.3, 26.5, 26.0, 25.8, 25.1, 25.0, 24.29, 24.1, 22.7; mass spectrum, m/z 455 (M^+), 440, 320, 220, 144, 100, 91, 57 (base), 41.[8]

(2*S*,3*R*)-4-Hydroxy-3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-(phenylmethoxy)butanoic Acid Phenylmethyl Ester (8). Acetonide 7 (2.3 g, 5.1 mmol) was dissolved in 95/5 CH_3OH/H_2O (120 mL) containing *p*-toluenesulfonic acid monohydrate (49 mg, 0.25 mmol), and the mixture was refluxed for 18 h. The mixture was concentrated, and the residue was purified by flash chromatography on silica gel using gradient elution (hexanes/EtOAc, 9:1, 4:1, 1:1) to give pure recovered 7 (1.15 g, 2.53 mmol) and pure 8 (0.77 g, 1.9 mmol). Based on recovered acetonide 7, primary alcohol 8 was obtained as a colorless oil in 73% yield. $[\alpha]^{20}_D$ +9.12° (*c* 1.06, EtOH); ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H, C(CH_3)$_3$), 2.67 (br s, 1 H, O*H*), 3.57 (m, 1 H, C*H*HOH), 3.71 (m, 1 H, CH*H*OH), 4.16 (m, 1 H, NC*H*CH$_2$), 4.38 (m, 1 H, O_2CC*H*O), 4.40 (d, 1 H, J = 11 Hz, ArC*H*HO), 4.78 (d, 1 H, J = 11 Hz, ArCH*H*O), 5.12 (s, 1 H, NH), 5.16 (s, 2 H, ArCH_2OCO), 7.33 (m, 10 H, ArH); ^{13}C NMR ($CDCl_3$) δ 28.3, 53.9, 62.3, 67.1, 72.9, 77.1, 79.8, 128.3, 135.4, 136.9, 155.7, 170.8; IR ($CHCl_3$) ν 3610, 3425, 1742, 1705 cm^{-1}; mass spectrum, m/z 415 (M^+), 160, 144, 107, 91 (base), 57. Anal. Calcd for $C_{23}H_{29}N_1O_6$: C, 66.49; H, 7.04; N, 3.37. Found: C, 66.42; H, 7.07; N, 3.33.

(2*S*,3*S*)-2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3,4-bis(phenylmethoxy)-4-oxobutanoic Acid (2). A solution of chromic acid (3.9 mL, 4.0 equiv), prepared according to Brown,[7] was added dropwise to a vigorously stirring mixture of alcohol 8 (0.77 g, 1.9 mmol) in Et_2O (15 mL). After 3 h the reaction was partitioned between H_2O (40 mL) and Et_2O (40 mL), and the organic layer was washed with brine (40 mL). The aqueous layers were extracted with Et_2O (2 × 40 mL), and the organics were combined, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography on silica gel using gradient elution (hexanes/EtOAc, 4:1, 1:1, 0.5% AcOH in EtOAc). The fractions containing product were pooled and concentrated to dryness, giving pure 2 (0.59 g, 1.4 mmol) in 74% yield. The oil was lyophilized from benzene providing a solid foam that collapsed to exposure to air: $[\alpha]^{20}_D$ −2.23° (*c* 1.07, EtOAc); IR ($CHCl_3$) ν 3435, 1750, 1718 cm^{-1}; ^1H NMR (acetone-d_6) δ 7.20–7.55 (m, 10 H, Ar*H*), 5.88 (d, 1 H, J = 9.0 Hz, N*H*), 5.22 (s, 2 H, ArCH_2OC(O)), 4.82 (m, 1 H, NC*H*), 4.79 (d, 1 H, J = 11.3 Hz, ArC*H*HO), 4.75 (d, 1 H, J = 2.5 Hz, OC*H*), 4.50 (d, 1 H, J = 11.3 Hz, ArCH*H*O), 1.43 (s, 9 H, C(CH_3)$_3$); ^{13}C NMR (acetone-d_6) δ 171.01, 169.83, 156.20, 138.31, 136.73, 129.22, 128.94, 128.57, 79.81, 78.74, 73.41, 67.44, 56.63, 28.43; mass spectrum, m/z 429 (M^+), 440, 328, 284, 264, 107, 91 (base), 79, 57. Anal. Calcd for $C_{23}H_{27}N_1O_7$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.28; H, 6.24; N, 3.15.

(8) Even after extensive recycling on the HPLC, 7 could not be obtained in an analytically pure state. It was sufficiently pure, however, to proceed with the next step.

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Registry No. 2, 129786-69-2; 5, 129786-70-5; 6, 129786-71-6; 7, 129786-72-7; 8, 129786-73-8.

Synthesis of Pyrimidoblamic Acid and Epipyrimidoblamic Acid

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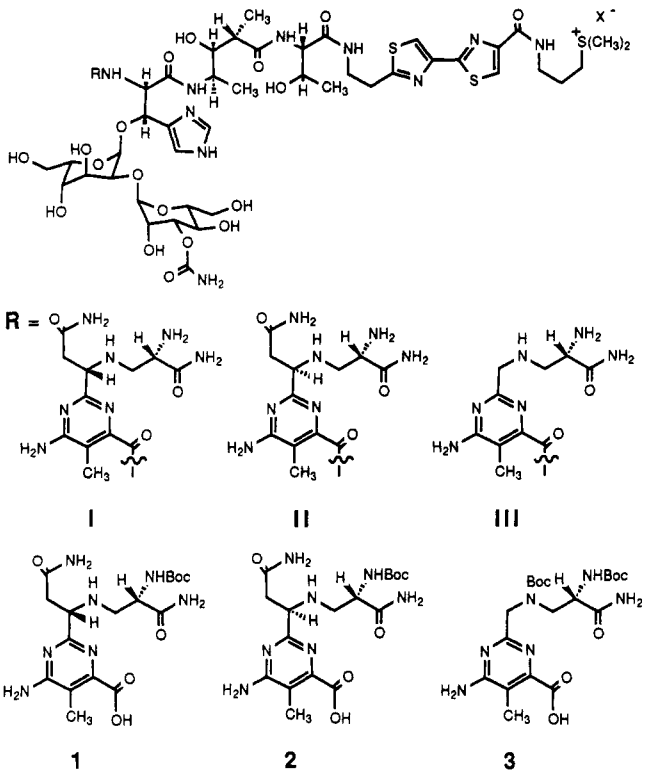
The N-protected pyrimidine moieties of bleomycin (pyrimidoblamic acid, 1) and epibleomycin are reported. In addition to a complete description of a synthetic route outlined earlier (Arai, H.; Hagmann, W. K.; Suguna, H.; Hecht, S. M. *J. Am. Chem. Soc* **1980**, *102*, 6631), a new route is also presented 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

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