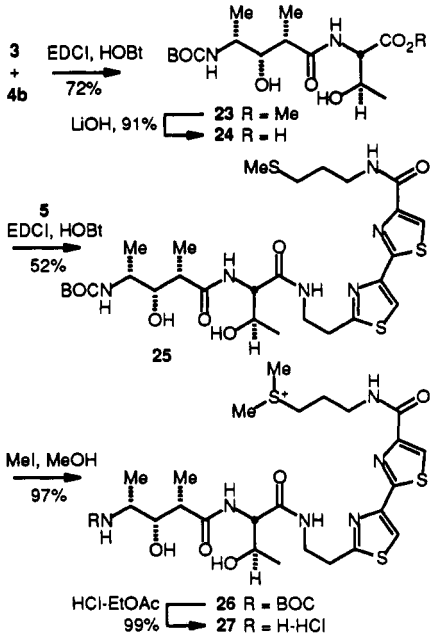
Scheme III



Sakai and co-workers provided **20**[18] which proved to be a convenient, stable storage intermediate (63%, 1.5 equiv of DCC, 1.2 equiv of NHS; 2.0 equiv of $NaHCO_3$, DME, 25 °C, 24 h), Scheme II. S-Methylation of **20** (96%, 50 equiv of CH_3I, CH_3OH, 25 °C, 72 h) provided the *N*-BOC derivative of tripeptide S (**21**),[18] and subsequent acid-catalyzed deprotection afforded tripeptide S[18] (**22**, 95%, 3 N HCl–EtOAc, 25 °C, 1.5 h) identical in all respects to authentic material. Although a linear synthesis of tetrapeptide S based on the coupling of tripeptide S and **3** has been detailed in the independent efforts of Umezawa and Hecht,[8] an alternative and more convergent preparation was employed for the work detailed herein. Coupling of **3** with **4b** (71%, 1.05 equiv of EDCI, 1.0 equiv of HOBt, 4 equiv of $NaHCO_3$, DMF, 25 °C, 24 h) followed by hydrolysis of the methyl ester **23** (91%, 4 equiv of LiOH, THF–CH_3OH–H_2O (3:1:1), 25 °C, 3 h) provided **24**, Scheme III. Coupling of **24** with **5** (52%, 1.05 equiv of EDCI, 1.0 equiv of HOBt, 4 equiv of $NaHCO_3$, DMF, 25 °C, 72 h) afforded **25**[19] which has proven to be a stable storage intermediate in our synthetic efforts. Subsequent S-methylation (97%, 50 equiv of CH_3I, CH_3OH, 25 °C, 80 h) provided the *N*-BOC derivative of tetrapeptide S (**26**)[19] and acid-catalyzed deprotection of **26** (99%, 3 N HCl–EtOAc, 25 °C, 1.5 h) provided tetrapeptide S (**27**).[19] Because of the sensitivity of **21–22** and **26–27** to prolonged storage, they are prepared from **20** and **25** immediately prior to use.

The incorporation of tetrapeptide S (**27**) and subunit **2** in the synthesis of deglyco desacetamidobleomycin A_2 is detailed in the accompanying paper,[20] and the approach detailed herein has been employed in the preparation of structural analogs of **22** and **27**. The incorporation of **22**, **27**, and such agents into structural analogs of bleomycin A_2 will be reported in due course.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (CA42056). We wish to thank Dr. Mona Patel for the initial preparation of **20**, Professor M. Ohno and M. Otsuka for a generous supply of authentic, comparison tripeptide S, and Professor S. M. Hecht for copies of the ^1H NMR spectra of tripeptide S and **25**.

Supplementary Material Available: Full experimental details and characterization for **2–5**, **8**, **11**, **13**, and **20–27** (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(18) For **20**: mp 105–106 °C (EtOAc–hexane); $[\alpha]^{23}_D$ −19.2 (*c* 0.66, CH_3OH) [lit.[8c] mp 106–107 °C (EtOAc–iPr_2O), $[\alpha]^{24}_D$ −20 (*c* 1, CH_3OH)]; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.12 (s, 1 H), 7.85 (s, 1 H), 7.65 (br t, 1 H), 7.25 (br t, 1 H), 5.60 (d, 1 H, *J* = 8 Hz), 4.40 (m, 1 H), 4.15 (m, 1 H), 3.75 (m, 2 H), 3.59 (q, 2 H, *J* = 7 Hz), 3.30 (t, 2 H, *J* = 6 Hz), 2.61 (t, 2 H, *J* = 7 Hz), 2.13 (s, 3 H), 1.98 (p, 2 H, *J* = 7 Hz), 1.40 (s, 9 H), 1.17 (d, 3 H, *J* = 8 Hz). For **21**: $[\alpha]^{23}_D$ −17.4 (*c* 0.095, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.20 (s, 1 H), 8.17 (s, 1 H), 4.20 (m, 1 H), 3.92 (m, 1 H), 3.75 (m, 1 H), 3.61 (t, 3 H, *J* = 6.5 Hz), 3.41 (t, 2 H, *J* = 7.5 Hz), 3.28 (t, 2 H, *J* = 6.5 Hz), 2.96 (s, 6 H), 2.16 (p, 2 H, *J* = 7.0 Hz), 1.42 (s, 9 H), 1.14 (d, 3 H, *J* = 6.5 Hz). For **22**: $[\alpha]^{23}_D$ −16.5 (*c* 0.04, 0.1 N HCl) [lit.[8c] $[\alpha]^{23}_D$ −15 (*c* 0.75, 0.1 N HCl), authentic sample[8c] $[\alpha]^{23}_D$ −16.2 (*c* 0.04, 0.1 N HCl)]; ^1H NMR (CD_3OD, 400 MHz) δ 8.27 (s, 1 H), 8.26 (s, 1 H), 4.05 (m, 1 H), 3.82 (m, 1 H), 3.72 (m, 1 H), 3.66 (m, 3 H), 3.49 (t, 2 H, *J* = 7 Hz), 3.38 (m, 2 H), 3.00 (s, 6 H), 2.18 (p, 2 H, *J* = 7 Hz), 1.25 (d, 3 H, *J* = 6.5 Hz).

(19) For **25**: $[\alpha]^{23}_D$ +14.4 (*c* 0.075, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz) δ 8.12 (s, 1 H), 7.85 (s, 1 H), 7.65 (br t, 1 H), 7.45 (br t, 1 H), 7.02 (br d, 1 H), 4.90 (br d, 1 H), 4.35 (br d, 1 H), 4.30–4.10 (m, 2 H), 3.70 (m, 2 H), 3.65–3.50 (m, 5 H), 3.25 (t, 2 H, *J* = 6.5 Hz), 2.60 (t, 2 H, *J* = 7.0 Hz), 2.59 (br s, 1 H), 2.12 (s, 3 H), 1.95 (p, 2 H, *J* = 7.0 Hz), 1.43 (s, 9 H), 1.22 (d, 3 H, *J* = 7.0 Hz), 1.11 (d, 6 H, *J* = 6.0 Hz). For **26**: $[\alpha]^{25}_D$ +22.2 (*c* 0.055, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.26 (s, 1 H), 8.20 (s, 1 H), 4.38 (d, 1 H, *J* = 4.5 Hz), 4.15 (m, 1 H), 3.70 (m, 3 H), 3.62 (br t, 3 H), 3.45 (t, 2 H, *J* = 7.5 Hz), 3.34 (m, 2 H), 2.99 (s, 6 H), 2.62 (m, 1 H), 2.16 (p, 2 H, *J* = 7.0 Hz), 1.46 (s, 9 H), 1.24 (d, 3 H, *J* = 7.0 Hz), 1.18 (d, 3 H, *J* = 6.5 Hz), 1.17 (d, 3 H, *J* = 6.5 Hz). For **27**: [lit.[8d] $[\alpha]^{20}_{365}$ −52 (*c* 0.5, 0.1 N HCl)]; ^1H NMR (CD_3OD, 400 MHz) δ 8.27 (s, 1 H), 8.25 (s, 1 H), 4.26 (d, 1 H, *J* = 4.5 Hz), 4.12 (m, 1 H), 3.80 (m, 3 H), 3.66 (br t, 3 H), 3.44 (t, 2 H, *J* = 7.5 Hz), 3.34 (m, 2 H), 3.00 (s, 6 H), 2.65 (m, 1 H), 2.20 (p, 2 H, *J* = 7.0 Hz), 1.34 (d, 3 H, *J* = 6.0 Hz), 1.33 (d, 3 H, *J* = 6.0 Hz), 1.19 (d, 3 H, *J* = 6.5 Hz).

(20) Boger, D. L.; Menezes, R. F.; Dang, Q. Following paper in this issue.

Synthesis of Desacetamidopyrimidoblamic Acid and Deglyco Desacetamidobleomycin A_2

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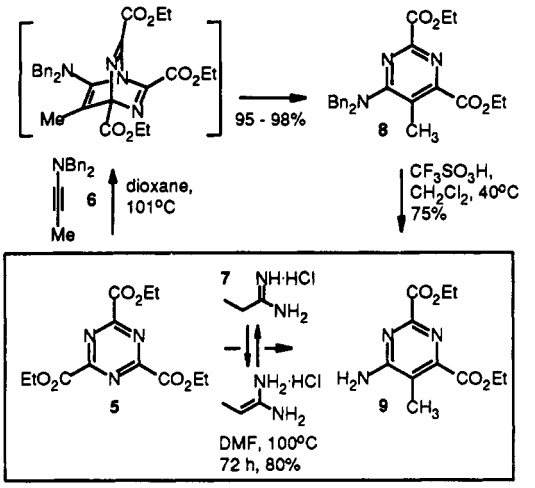
Received February 24, 1992

Summary: A concise synthesis of desacetamidopyrimidoblamic acid (**3**) is detailed based on the inverse electron demand [4 + 2] cycloaddition reaction of 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine (**5**) with 1-bis(benzylamino)-1-propyne or in situ generated 1,1-diaminopropene for the one-step preparation of an appropriately functionalized pyrimidine nucleus. The incorporation of **3** into synthetic deglyco desacetamidobleomycin A_2 (**4**) and the preliminary comparison of the functional cleavage of duplex DNA by Fe(II)-**4** are described. Fe(II)-**4** proved to be 0.3–0.2× as effective as Fe(II)-deglycobleomycin A_2 in its efficiency of cleavage of supercoiled φX174 RFI DNA.

0022-3263/92/1957-4333$03.00/0

The bleomycins are a family of glycopeptide antitumor antibiotics possessing clinically useful activity thought to be mediated through their metal-dependent oxidative cleavage of duplex DNA.[1] Consequently, bleomycin A_2 (1),[2] its naturally occurring congeners,[3] its semisynthetic derivatives and degradation products,[4] and synthetic analogs[5] have been the subject of extensive investigations in efforts to define the fundamental functional roles of their structural subunits. In the preceding article, we detailed a concise synthesis of tetrapeptide S. Herein we detail the synthesis of desacetamidopyrimidoblamic acid (3)[6,7] based on the inverse electron demand Diels–Alder reaction[8] of 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine (5)[9,10] and the incorporation of 3 and tetrapeptide S into deglyco desacetamidobleomycin A_2 (4). Pertinent to the studies detailed herein, the C-2 acetamido side chain of the bleomycins has been shown not to be intimately involved in the key metal chelation and oxygen activation event required for DNA cleavage.[1] Thus, 4 additionally constitutes an important substructure of the natural product which has not yet been evaluated[1-5] and that is accessible only through total synthesis.

Scheme I



Two concise approaches to the preparation of 9 based on the [4 + 2] cycloaddition of 5 have been developed, Scheme I. Treatment of 5 with 1-bis(benzylamino)-1-propyne (6, 2 equiv)[11] provided 8 in excellent yield (95–98%) under thermal reaction conditions (101 °C, dioxane, 21 h). The room temperature [4 + 2] cycloaddition reaction of 5 with 6 is followed by a subsequent retro Diels–Alder reaction with loss of ethyl cyanoformate (40–100 °C), and it is the rate of the latter cycloreversion reaction that dictates the required thermal reaction conditions. Acid-catalyzed debenzylation of 8 under vigorous conditions (CF_3SO_3H, CH_2Cl_2, 40 °C, 12 h, 75%) provided 9, and initial efforts to effect the conversion of 8 to 9 through catalytic hydrogenolysis proved less successful.[12] Alternatively, 9 was derived directly and conveniently from the treatment of 5 with amidine hydrochloride 7 (100 °C, DMF, 72 h, 80%) in a reaction cascade that proceeds with thermal tautomerization of 7 to 1,1-diaminopropene and its [4 + 2] cycloaddition reaction with 5. The sequential elimination of ammonia, imine to enamine tautomeriza-

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(12) The deprotection of 8 under a range of alternative conditions gave the monodebenzylation product as the major or only reaction product in addition to recovered starting material.

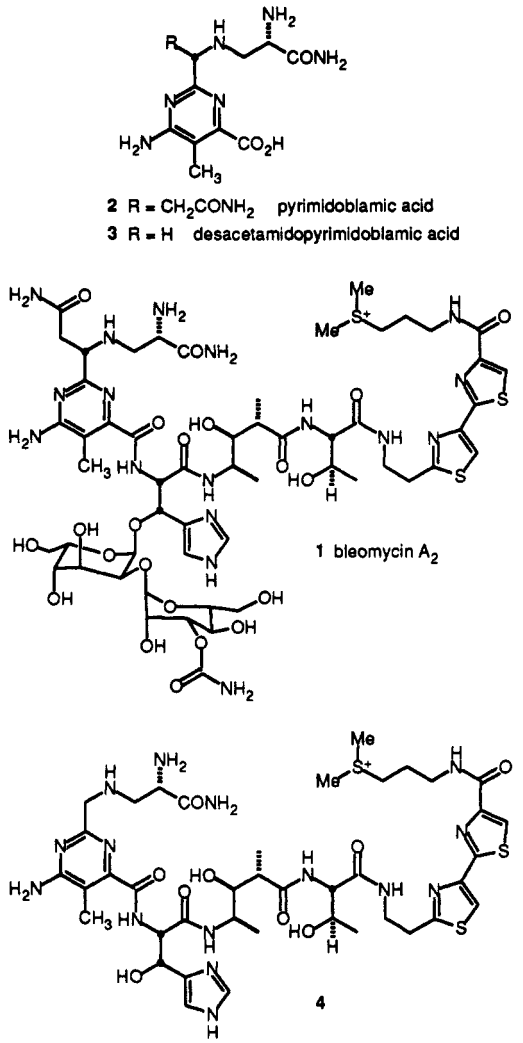
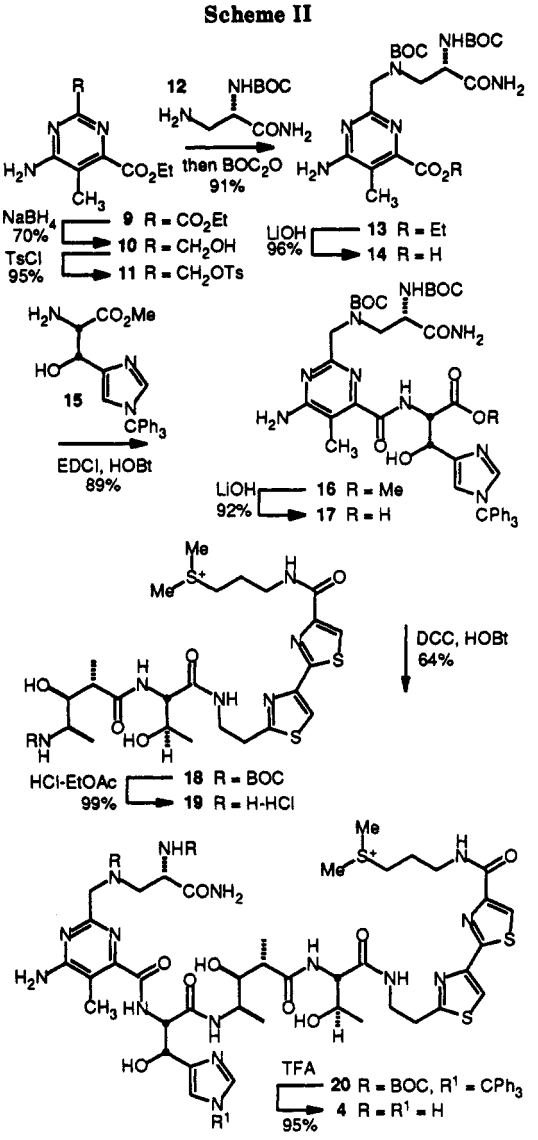


Figure 1.

tion, and subsequent retro Diels–Alder loss of ethyl cyanoformate under the reaction conditions provided **9** directly in excellent yield. The thermal conditions detailed and the deliberate use of the hydrochloride salt of **7** facilitated the amidine tautomerization and proved it necessary to effect the aromatization of the initial cycloadduct.[13]

Selective reduction of sterically and electronically more accessible C2 ethoxycarbonyl group of **9** provided **10** and was effectively conducted with sodium borohydride at low temperature (1.0 equiv, EtOH, 5 °C, 150 h, 70%), Scheme II.[14] Conversion of **10** to the tosylate **11** (1 equiv of TsCl, 2 equiv of K_2CO_3, CH_2Cl_2, 25 °C, 17 h, 95%) followed by clean displacement with **12**[10b] (4 equiv, 2 equiv of $NaHCO_3$, CH_3CN, 25 °C, 26 h) and subsequent protection of the secondary amine provided **13** (8 equiv of BOC_2O, THF–saturated aqueous $NaHCO_3$ (1:1), 25 °C, 15 h, 91% for the two steps). Hydrolysis of the ethyl ester (2 equiv of LiOH, THF–H_2O–CH_3OH (3:1:1), 25 °C, 4 h, 96%) provided the N^{α},N^{β}-bis(*tert*-butyloxycarbonyl) derivative of desacetamidopyrimidoblamic acid (**14**),[6] and subsequent acid-catalyzed deprotection of **14** provided desacetamidopyrimidoblamic acid **3**[6] (3 N HCl–EtOAc, 25 °C, 1 h, 90%). Direct coupling of **14** with erythro N^{π}-(triphenylmethyl)-β-hydroxy-L-histidine methyl ester (**15**)[15] provided **16** (1.05 equiv of EDCl, 1.0 equiv of HOBt, THF–DMF (2:1), 25 °C, 72 h, 89%). Methyl ester hydrolysis (2 equiv of LiOH, THF–CH_3OH–H_2O (3:1:1), 25 °C, 3 h, 92%) followed by *direct* coupling of **17** with synthetic tetrapeptide S (**19**)[15] provided **20**[16] and was found to be conveniently conducted without additional protection of the adorning functionality (3.0 equiv of DCC, 1.0 equiv of HOBt, 2.5 equiv of $NaHCO_3$, DMF, 25 °C, 72 h, 64%). Final exhaustive deprotection of **20** (TFA, 25 °C, 1.5 h, 95%) afforded deglyco desacetamidobleomycin A_2 (**4**).[16]

Scheme II



(13) The use of the propionamidine free base resulted in lower yields of **9** (45%, DMF, 100 °C, 48 h). A summary of representative results of the study of the [4 + 2] cycloaddition reaction is provided in tabular form in supplementary material.

(14) 2D ^1H–^1H NOESY NMR ($CDCl_3$, 200 MHz) did not reveal a NOE crosspeak between $-CH_2OH$ and C5-CH_3 for **10** but did so for the minor isomer derived from the $NaBH_4$ reduction of **9**. A summary of representative results of the study of the reduction of **9** is provided in tabular form in the supplementary material.

(15) Boger, D. L.; Menezes, R. F., preceding paper in this issue.

(16) For **20**: R_f 0.45 (SiO_2, 10:9:1 CH_3OH–10% $CH_3CO_2NH_4$–10% NH_4OH); $[\alpha]^{23}_D$ +51.4 (c 0.035, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.22 (br s, 1 H), 8.12 (br s, 1 H), 7.45 (br s, 1 H), 7.40–7.25 (m, 9 H), 7.15–7.02 (m, 6 H), 6.92 (br s, 1 H), 5.35 (m, 1 H), 5.08 (m, 1 H), 4.80 (m, 1 H), 4.70 (br s, 2 H), 4.44 (m, 2 H), 4.35–4.10 (m, 1 H), 3.78 (m, 2 H), 3.70–3.55 (m, 4 H), 3.28 (m, 2 H), 2.97 (s, 6 H), 2.62 (m, 1 H), 2.27 (s, 3 H), 2.18 (m, 2 H), 1.47 (br s, 12 H), 1.21 (br s, 6 H), 1.16 (m, 9 H). For **4**: R_f 0.2 (SiO_2, 10:9:1 CH_3OH–10% $CH_3CO_2NH_4$–10% NH_4OH); $[\alpha]^{23}_D$ +83 (c 0.03, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.89 (br s, 1 H), 8.26 (br s, 1 H), 8.15 (br s, 1 H), 7.54 (br s, 1 H), 5.36 (m, 1 H), 5.15 (m, 1 H), 4.52–3.95 (m, 8 H), 3.90–3.70 (m, 4 H), 3.65 (br t, 2 H), 3.42 (br t, 2 H), 2.98 (s, 6 H), 2.65 (m, 1 H), 2.31 (s, 3 H), 2.20 (m, 3 H), 1.20 (m, 9 H).

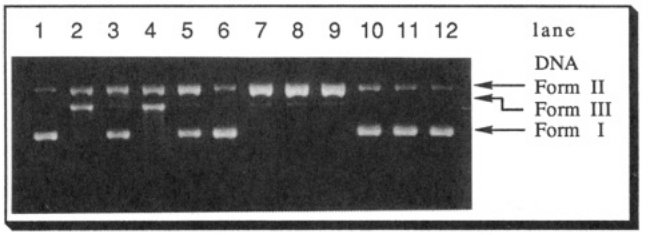


Figure 2. Cleavage of ΦX174 supercoiled DNA by Fe(II)-**4**, Fe(II)-bleomycin A_2, and Fe(II)-deglycobleomycin A_2. Solutions contained 0.25 μg of ΦX174 supercoiled DNA (1.4×10^{-8} M) in 50 mM Tris-HCl, pH 8 containing 10 mM 2-mercaptoethanol. The DNA cleavage reactions were run for 60 min at 25 °C, and electrophoresis was conducted at 50 V (2.5 h) on a 1.0% agarose gel. Lane 1, control ΦX174 DNA 95% Form I (supercoiled), 5% Form II (relaxed); lanes 2–3, 1 and 0.2 μM Fe(II)-bleomycin A_2; lanes 4–6, 5, 1, and 0.2 μM Fe(II)-deglycobleomycin A_2; lanes 7–10, 50, 10, 5, and 1 μM Fe(II)-**4**; lanes 11–12, 5 and 1 μM Fe(II), control. Form I = supercoiled DNA, Form II = relaxed DNA (single-strand cleavage). Form III = linear DNA (double-strand cleavage).

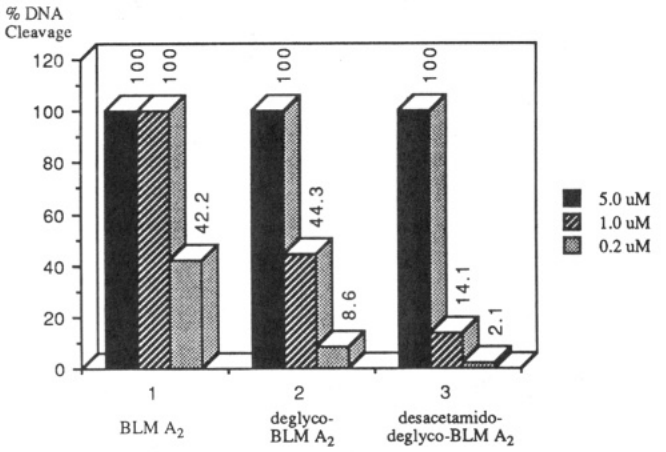


Figure 3. Comparison of the relative efficiency of cleavage of supercoiled ΦX174 RFI DNA by Fe(II)-bleomycin A_2, Fe(II)-deglycobleomycin A_2, and Fe(II)-**4**.

A preliminary study of the ability of the Fe(II) complex of **4** to cleave duplex DNA 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. Like Fe(II)-bleomycin A_2[17] and deglycobleomycin A_2[17], Fe(II)-**4** produced both single- and double-strand cleavage of φX174 RFI DNA, Figure 2. The direct comparison of the efficiency of DNA cleavage by Fe(II)-**4** and Fe(II)-deglycobleomycin A_2 permits the assessment of the relative importance and functional role of the pyrimidoblamic acid C2 acetamido side chain. Although the side chain has been shown not to be intimately involved in the metal chelation, it has been suggested to contribute to the efficiency of DNA cleavage by constituting one side or component of the oxygen binding pocket thereby sterically shielding or protecting the activated and reactive iron-oxo intermediate.[1] Consistent with this latter suggestion, Fe(II)-deglycobleomycin A_2 proved to be 3–5× more effective than Fe(II)-**4** in its efficiency for producing the cleavage of supercoiled φX174 RFI DNA, Figure 3 [relative efficiency: bleomycin A_2 (1), deglycobleomycin A_2 (0.5–0.2), **4** (0.2–0.05)]. Under the conditions of the assay, both Fe(II)-deglycobleomycin A_2 and Fe(II)-**4** produced little or no cleavage at 0.2 μM, significant cleavage at 1 μM, and complete cleavage at 5 μM. Both agents proved to be slightly less efficient that Fe(II)-bleomycin A_2 which produced significant cleavage of the supercoiled DNA at concentrations as low as 0.2 μM with complete cleavage of the DNA at 1 μM. Consistent in each of multiple assays, Fe(II)-deglycobleomycin A_2 proved at least as effective in producing linear DNA resulting from double-stranded DNA cleavage as Fe(II)-bleomycin A_2 itself which in turn generally proved more effective than Fe(II)-**4**. Detailed studies of the DNA cleavage properties of Fe(II)-**4** including additional comparison of its duplex DNA cleavage efficiency and selectivity with that of bleomycin A_2, deglyco bleomycin A_2, and structurally related analogs are in progress and will be reported in due course.

(17) Boger, D. L.; Menezes, R. F.; Yang, W. *Biomed. Chem. Lett.*, in press.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (CA42056). We wish to thank Dr. W. Yang for conducting the DNA cleavage studies of Fe(II)-**4** and Professor S. M. Hecth for copies of the ^{1}H NMR spectra of **17** and desacetamidopyrimidoblamic acid (**3**).

Supplementary Material Available: Experimental details, full physical and spectroscopic characterization for **8–14**, **16**, **17**, **20**, and **3–4**, and two tables detailing studies of the [4 + 2] cycloaddition reactions of **5** and the reduction of **9** (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

A Practical Preparation of α-Alkoxylithium Reagents: Synthesis of Syn or Anti 1,3-Diols

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Received May 19, 1992

Summary: Phenylthio acetals **3** are easily prepared from β-hydroxy aldehydes and can be reduced to anti alkyllithiums **7** and subsequently equilibrated to syn alkyllithiums **9** with excellent stereoselectivity and in good overall yield. A practical preparation of **3** and a reductive lithiation procedure using catalytic naphthalene makes these alkyllithium reagents conveniently available on a multigram scale.

0022-3263/92/1957-4336$03.00/0