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# DEGLYCO GABA,GLY-DESACETAMIDOBLEOMYCIN $A_2$: A SIMPLIFIED SYNTHETIC MODEL FOR BLEOMYCIN $A_2$

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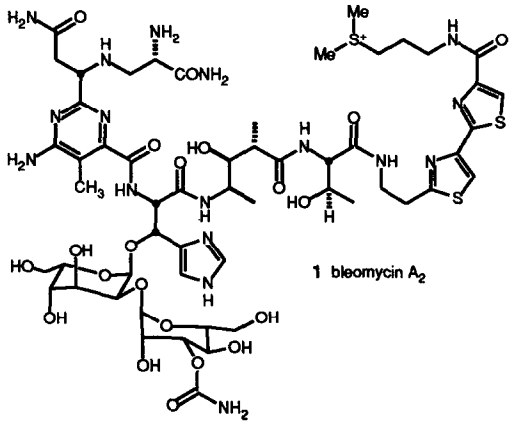
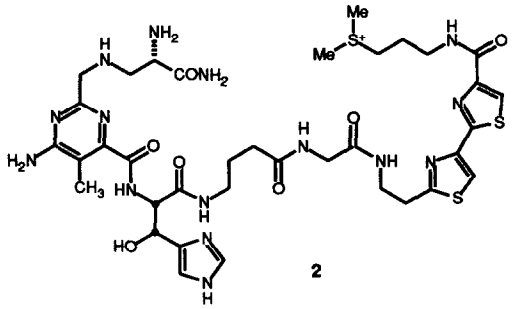
**Abstract.** The synthesis of **2**, a simplified synthetic model for bleomycin $A_2$, and the premliminary demonstration of its functional cleavage of duplex DNA are detailed.

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,[1,3] its semisynthetic derivatives and degradation products,[4] and synthetic analogs[5] have been the subject of extensive study in efforts to define the fundamental functional roles of their structural subunits. The bleomycin substructure responsible for DNA cleavage including the pyrimidoblamic acid subunit in conjunction with the adjacent β-hydroxy-L-histidine provides the necessary functionality and appropriate environment for metal chelation and oxygen activation[1] and the potential contribution that this segment may make in polynucleotide recognition has been recently addressed.[6] Pertinent to the studies detailed herein, the C-2 acetamido side chain of pyrimidoblamic acid has been shown not to be intimately involved in the key metal chelation[7] and oxygen activation event although its potential beneficial effect on the stability of metal complexes has been inferred from simple models.[8] The tri- and tetrapeptide S[9] segment of the agents including the bithiazole and terminal sulfonium cation provides a majority of the bleomycin DNA binding affinity[10] and contributes to[1,11] or potentially dominates[11a] the DNA binding selectivity and polynucleotide cleavage specificity. The relative importance and role of bithiazole intercalation[12] versus minor groove binding,[11] the fundamental recognition elements important to binding affinity and selectivity, and the extent of the tri- and tetrapeptide S contribution to the DNA cleavage selectivity remain to be fully defined.

Figure I



1 bleomycin $A_2$

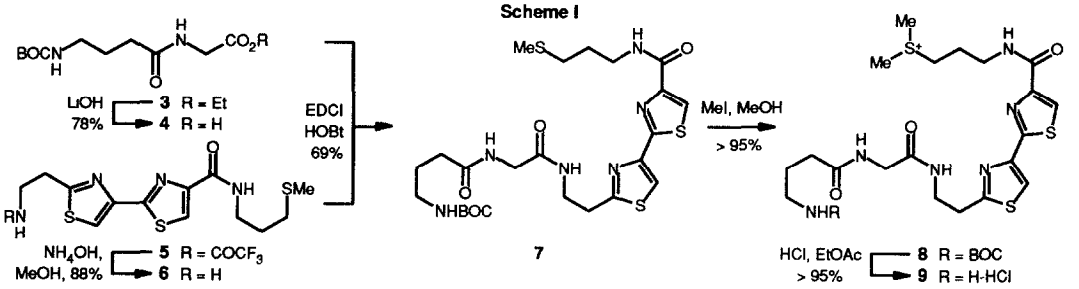


2

In the course of the ongoing development of a total synthesis of bleomycin $A_2$[13] complementary to those disclosed by Umezawa, Ohno and Hecht and in anticipation of studies that address the role of the peripheral substituents adorning the bleomycin skeleton, we have completed and herein detail the synthesis of the simplified analog **2** of bleomycin $A_2$. In addition to establishing the viability of an alternative order of incorporation of the individual subunits including the timing of the requisite *S*-methylation and the anticipated chemistry to be employed in the total synthesis of the natural product,[13] the preparation of **2** provides a key substructure of the natural bleomycins. The agent **2** incorporates the full carbon skeleton of tetrapeptide S[14] lacking each of the peripheral substituents on the linking peptide chain as well as the fully functionalized metal chelation and oxygen activation segment lacking only the nonessential pyrimidoblamic acid[15] C-2 acetamido side chain. Thus, the proposed five major metal coordination centers of bleomycin $A_2$ have been incorporated into **2** and γ-aminobutyric acid (GABA) and glycine (gly) have been substituted for the (2*S*, 3*S*, 4*R*)-4-amino-3-hydroxy-2-methylpentanoic acid and L-threonine subunits of bleomycin $A_2$, respectively.

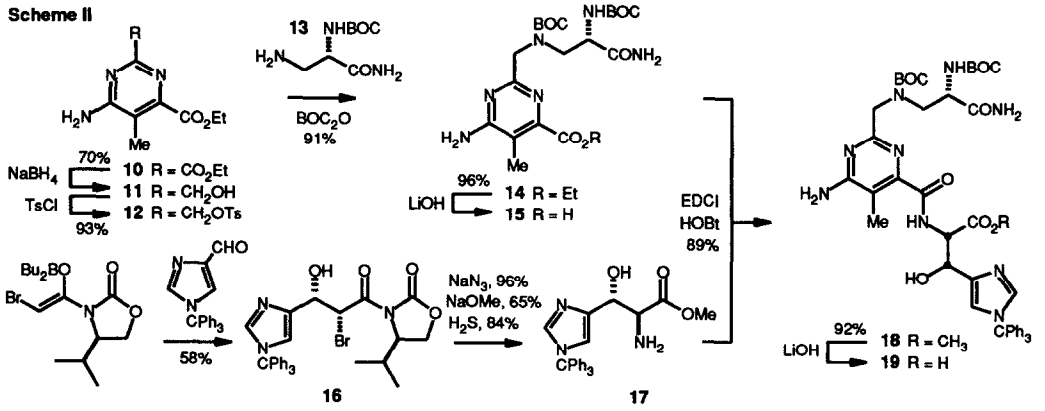
**Synthesis of 2.** Hydrolysis of *N*-BOC-GABA-gly-OEt (**3**,[5f] 3 equiv LiOH, 3:1 THF-$H_2O$, 25°C, 3h, 78%) followed by coupling of **4** with the free base of bithiazole **6**[16] (1.05 equiv EDCI, 1.0 equiv HOBt, DMF, 25°C, 24 h, 69%) provided **7**,[17] Scheme I. *S*-Methylation of **7** (50 equiv $CH_3I$, $CH_3OH$, 25°C, 72 h) provided the *N*-*t*-butyloxycarbonyl derivative **8**[17] of the simplified tetrapeptide S in quantitative yield. Deprotection of **8** (3N HCl-EtOAc, 25°C, 30 min, >95%) provided **9**[17] which was used directly in the coupling with **19**.

**Scheme I**

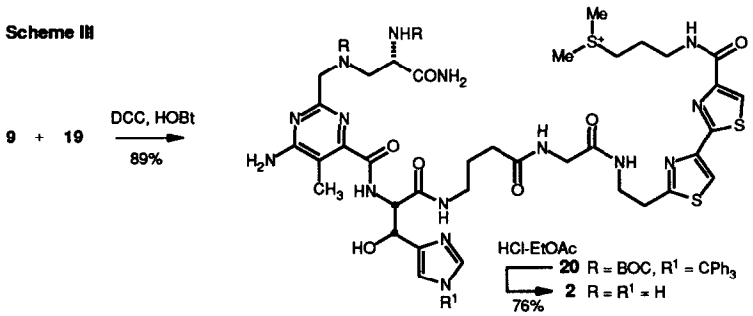



Selective reduction of the electronically more reactive and sterically more accessible C-2 ethoxycarbonyl group of pyrimidine **10**[18] (1.0 equiv $NaBH_4$, EtOH, 5°C, 150 h, 70%) derived from the inverse electron demand Diels-Alder reaction of 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine[19,20] provided **11**, Scheme II. Conversion of **11** to the tosylate **12** (1 equiv TsCl, 2 equiv $K_2CO_3$, $CH_2Cl_2$, 25°C, 17 h, 93%) followed by clean tosylate displacement with **13**[20] (4 equiv, 2 equiv $NaHCO_3$, $CH_3CN$, 25°C, 26 h) and subsequent BOC protection of the secondary amine (8 equiv $BOC_2O$, 2 equiv $NaHCO_3$, THF-$H_2O$ 1:1, 25°C, 15 h, 91% for two steps) afforded **14**. Hydrolysis of the ethyl ester of **14** (2 equiv LiOH, THF-$CH_3OH$-$H_2O$ 3:1:1, 25°C, 4 h, 96%) provided **15**. Coupling of **15** with the erythro β-hydroxy-L-histidine derivative **17** (1.05 equiv EDCI, 1.0 equiv HOBt, THF-

DMF 2:1, 25°C, 72 h, 89%) provided **18**.[18] Notably, **17** was prepared from **16**[21] through adaptation of the approach detailed by Ohno and coworkers[21] with modifications in which the competitive retro aldol reaction was suppressed during the azide displacement reaction (5 equiv $NaN_3$, DMF, 45°C, 1.5 h, 96%) and which provide an appropriately protected derivative of erythro β-hydroxy-L-histidine suitable for direct coupling with **15** without further functionalization ($NaOCH_3$, $CH_3OH$, 0°C, 5 min, 65%; $H_2S$, $H_2O$-$CH_3OH$, 25°C, 48 h, 84%).




Conversion of **18** and **19** (2 equiv LiOH, THF-$CH_3OH$-$H_2O$ 3:1:1, 25°C, 3 h, 92%) followed by penultimate coupling with **9** (3 equiv DCC, 1.0 equiv HOBt, 2.5 equiv $NaHCO_3$, DMF, 25°C, 51 h, 89%) provided **20**[22] in excellent yield, Scheme III. Final deprotection of **20** (3N HCl-EtOAc, 25°C, 2 h, 76%) provided **2**.[22]




**DNA Cleavage Studies.** A preliminary study of the ability of the Fe(II) complex of **2** 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 $A_2$ but unlike simple models,[5h] Fe(II)-**2** produced both single- and double-strand cleavage of ΦX174 RFI DNA. The detailed study of the DNA cleavage properties of Fe(II)-**2**, including the comparision of its duplex DNA cleavage efficiency and selectivity with that of bleomycin $A_2$ and structurally related analogs as well as the extension of the studies detailed herein to more advanced synthetic analogs of bleomycin $A_2$ are in progress and will be reported in due course.

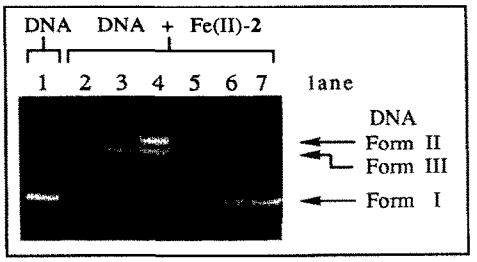



Figure 2. Cleavage of ΦX174 RFI supercoiled DNA by **2**. Solutions contained 0.5 μg ΦX174 RFI supercoiled DNA ($1.4 \times 10^{-8}$ M) in 50 mM Tris-HCl, pH = 8.0 containing 10 mM 2-mercaptoethanol. The DNA cleavage reactions were run for 1.0 h at 25°C and electrophoresis was conduted at 50 V (2.5 h) on a 0.7% agarose gel. Lane 1, control ΦX174 RFI DNA 90% Form I (supercoiled), 10% Form II (relaxed); lane 2-7, $5 \times 10^{-4}$ M, $1 \times 10^{-4}$ M, $5 \times 10^{-5}$ M, $1 \times 10^{-5}$ M, $5 \times 10^{-6}$ M, and $1 \times 10^{-6}$ M Fe(II)-**2**, respectively. Form I = supercoiled DNA, Form II = relaxed DNA (single-strand cleavage), Form III = linear DNA (double-strand cleavage).

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17. For **7**: $^1$H NMR ($CDCl_3$, 400 MHz) δ 8.09 (s, 1H), 7.85 (s, 1H), 7.55 (br t, 1H), 7.19 (br t, 1H), 6.58 (br t, 1H), 4.72 (br t, 1H), 3.94 (d, 2H, *J* = 5.6 Hz), 3.74 (q, 2H, *J* = 6.2 Hz), 3.57 (q, 2H, *J* = 6.7 Hz), 3.25 (t, 2H, *J* = 6.5 Hz), 3.09 (q, 2H, *J* = 6.3 Hz), 2.59 (t, 2H, *J* = 7.1 Hz), 2.23 (t, 2H, *J* = 6.6 Hz), 2.12 (s, 3H), 1.95 (p, 2H, *J* = 7.0 Hz), 1.74 (p, 2H, *J* = 6.3 Hz), 1.42 (s, 9H); $^{13}$C NMR ($CDCl_3$, 400 MHz) δ 181.2, 172.1, 169.8, 169.0, 160.9, 156.2, 150.5, 148.2, 123.5, 116.4, 43.4, 39.1, 38.6, 38.4, 32.8, 31.6, 28.9, 28.4, 26.8, 15.5; IR (neat) $\nu_{max}$ 3314, 3106, 2978, 1654, 1544, 1438, 1394, 1252, 1176, 1138, 1062, 838, 722 cm$^{-1}$; DCIMS *m/e* 585 ($M^+$ + H, base). For **8**: $^1$H NMR ($CD_3OD$, 400 MHz) δ 8.25 (s, 1H), 8.21 (s, 1H), 3.87 (s, 2H), 3.72 (t, 2H, *J* = 6.5 Hz), 3.65 (t, 2H, *J* = 6.4 Hz), 3.46 (t, 2H, *J* = 6.8 Hz), 3.33 (t, 2H, *J* = 6.8 Hz), 3.08 (t, 2H, *J* = 6.6 Hz), 3.01 (s, 6H), 2.31 (t, 2H, *J* = 7.2 Hz), 2.21 (p, 2H, *J* = 7.2 Hz), 1.78 (p, 2H, *J* = 6.7 Hz), 1.47 (s, 9H); $^{13}$C NMR ($CD_3OD$, 400 MHz) δ 176.0, 172.1, 170.7, 167.0, 164.9, 164.1, 151.3, 149.4, 125.4, 118.7, 43.6, 42.6, 40.4, 40.0, 38.6, 33.7, 33.6, 28.8, 26.9, 25.6, 25.4; IR (neat) $\nu_{max}$ 3419, 3288, 3047, 2974, 1704, 1659, 1538, 1326, 1295, 1177, 1067, 1015, 982 cm$^{-1}$; FABHRMS (NBA) *m/e* 599.2149 ($M^+$, $C_{25}H_{39}O_5N_6S_3$ requires 599.2144). For **9**: purification by reverse phase chromatography on $C_{18}$ ($H_2O$ eluant); $^1$H NMR ($CD_3OD$, 400 MHz) δ 3.84 (s, 2H), 3.66 (t, 2H, *J* = 6.8 Hz), 3.61 (t, 2H, *J* = 6.5 Hz), 3.41 (t, 2H, *J* = 7.0 Hz), 3.29 (t, 2H, *J* = 7.5 Hz), 2.99 (t, 2H, *J* = 7 Hz), 2.97 (s, 6H), 2.42 (t, 2H, *J* = 6.9 Hz), 2.16 (p, 2H, *J* = 6.8 Hz), 1.94 (p, 2H, *J* = 7.0 Hz); IR (neat) $\nu_{max}$ 3386, 2968, 1653, 1537, 1356, 1167, 1058 cm$^{-1}$; FABHRMS (DTT/DTE) *m/e* 499.1613 ($M^+$, $C_{20}H_{32}N_6O_3S_3$ requires 499.1620).

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22. For **20**: purification by trituration with $CHCl_3$ followed by reverse phase chromatography on $C_{18}$ (5% - 25% MeOH - $H_2O$ eluant); TLC $R_f$ = 0.28 ($SiO_2$, $CH_3OH$(10) - 10% $CH_3CO_2NH_4$(9) - 10% $NH_4OH$(1); $[\alpha]^{24}_D$ -24.0 (c 0.03, $CH_3OH$); $^1$H NMR ($CD_3OD$, 400 MHz) δ 8.22 (s, 1H), 8.15 (s, 1H), 7.50 (br s, 1H), 7.40 - 7.26 (m, 9H), 7.20 - 7.12 (m, 6H), 7.05 (br s, 1H), 5.40 (br s, 1H), 5.35 (br s, 1H), 4.50 - 4.15 (m, 4H), 3.85 (br s, 2H), 3.75 - 3.55 (m, 5H), 3.45 (t, 2H, *J* = 7 Hz), 3.28 - 3.15 (m, 4H), 2.99 (s, 6H), 2.35 - 2.25 (m, 2H), 2.18 (br s, 3H), 1.90 - 1.60 (m, 4H), 1.47 (br s, 12H), 1.27 (br s, 6H); IR (neat) $\nu_{max}$ 3373, 2978, 2922, 1636, 1550, 1433, 1367, 1250, 1167, 1062 cm$^{-1}$; FABMS (NBA) *m/e* 1345 ($M^+$). For **2**: purification by trituation with $CHCl_3$ and recrystallization from $CH_3OH$-$Et_2O$; $[\alpha]^{24}_D$ +88.8 (c 0.095, $CH_3OH$); $^1$H NMR ($D_2O$, 400 MHz) δ 8.75 (s, 1H), 8.21 (s, 1H), 8.08 (s, 1H), 7.42 (s, 1H), 5.50 (d, 1H), 5.25 (br s, 1H), 4.40 (br s, 1H), 4.25 - 4.12 (m, 3H), 3.82 (s, 2H), 3.65 - 3.55 (m, 5H), 3.39 (t, 2H, *J* = 6.5 Hz), 3.25 (m, 2H), 3.18 (m, 2H), 2.92 (s, 6H), 2.35 - 2.25 (m, 2H), 2.20 - 2.12 (br t, 2H), 1.95 (s, 3H), 1.80 -1.70 (m, 2H); IR (neat) $\nu_{max}$ 3383, 2923, 2851, 1678, 1441, 1200, 1138, 800 cm$^{-1}$; FABHRMS (NBA) *m/e* 902.3381 ($M^+$, $C_{36}H_{52}N_{15}O_7S_3$ requires 902.3336).