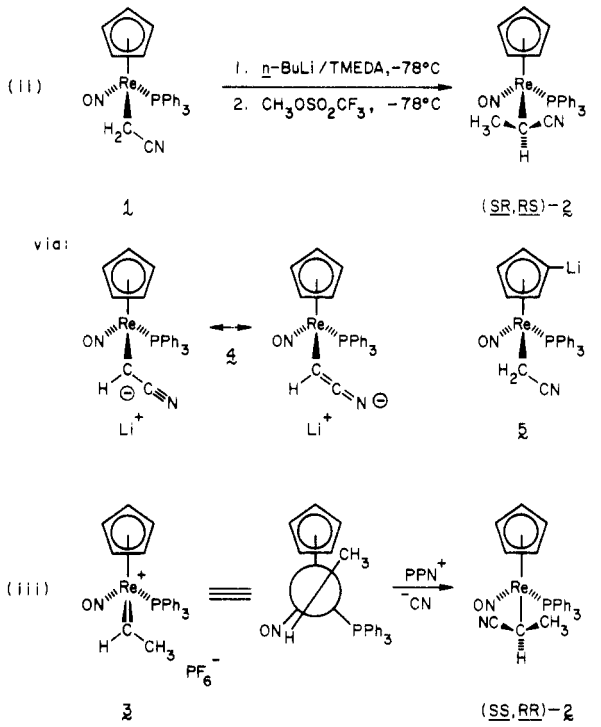

a rigorous assignment of product stereochemistry. Complex (*SS,RR*)-**2** was not formed (detection limit, <1%) in eq ii, as determined by ^{1}H NMR, ^{31}P NMR, and HPLC analyses of the crude reaction mixture.

Evidence was sought for the apparent precursor to (*SR,RS*)-**2**, deprotonated complex $Li^+[(\eta^5\text{-}C_5H_5)Re(NO)(PPh_3)(\bar{C}HCN)]^-$ (**4**).[3a] The reaction of **1** with *n*-BuLi/TMEDA was monitored by ^{31}P NMR at −98 °C. Two resonances (32.15 ppm, br; 25.71 ppm, sh) appeared immediately. The relative areas of these resonances (ca. 2:1) did not change over the course of 3 h. Upon warming (−78 °C, 2.5 h, or −25 °C, 0.5 h), the 25.71 ppm resonance disappeared and the 32.15 ppm resonance sharpened. The spectrum was unchanged by subsequent cooling (−98 °C, 3 h). Addition of $CH_3OSO_2CF_3$ to any of these solutions (−98, −78, −25 °C) gave exclusively (*SR,RS*)-**2**, as observed by ^{31}P NMR monitoring.

Deuterium labeling experiments were conducted to provide additional information on the intermediates described above. Reaction of $(\eta^5\text{-}C_5H_5)Re(NO)(PPh_3)(CD_2CN)$ (**1**-d_2; 91:9 d_2/d_1)[5] with *n*-BuLi/TMEDA and $CH_3OSO_2CF_3$ as in eq ii gave a 31:69 mixture of (*SR,RS*)-**2**-d_2/(*SR,RS*)-**2**-d_1, as determined by mass spectral analysis. An identical reaction of $(\eta^5\text{-}C_5D_5)Re(NO)(PPh_3)(CH_2CN)$ (**1**-d_5; 86:14 d_5/d_4) gave a 62:38 mixture of (*SR,RS*)-**2**-d_5/(*SR,RS*)-**2**-d_4. These data indicate that **1** can be deprotonated either on the CH_2CN ligand (major) to give **4** (32.15 ppm) or the $\eta^5\text{-}C_5H_5$ ligand (minor) to give $(\eta^5\text{-}C_5H_4Li)Re(NO)(PPh_3)(CH_2CN)$ (**5**, 25.71 ppm). Interestingly, only (*SR,RS*)-**2** is obtained when $CH_3OSO_2CF_3$ is added to mixtures of **4** and **5** at temperatures where **4** and **5** do not (or are slow to) equilibrate. One possible explanation is that initially formed (*SR,SR*)-**2** might equilibrate **4** and **5**. Such equilibrations have abundant precedent in organic enolate alkylations.[9]

We sought to determine whether the ion pair acidity[3] of **1** was greater or less than that of CH_3CN ($pK_a(H_2O)$ = 31.5).[10] Hence, in a ^{31}P NMR monitored experiment, **4** (−78 °C) was treated with 3 equiv of CD_3CN. Immediate conversion to **1**-d_x occurred. The solution was kept at 25 °C for 8 h. The **1**-d_x was isolated and shown to be extensively deuterated (d_0:d_1:d_2:d_3:d_4:d_5:d_6:d_7 = <1:6:12:20:31:21:9:1). This established that **4** was not quenched by adventitious proton sources, and that additional H/D exchange between **1** and the resulting $^-CD_2CN$ occurred. Hence, **1** is less acidic than CH_3CN, and the $(\eta^5\text{-}C_5H_5)Re(NO)(PPh_3)$ moiety can be considered a carbanion destabilizing substituent.

Extensions of the above chemistry were explored. First, reaction of **1** with *n*-BuLi/TMEDA and then *n*-C_4H_9I as in eq ii gave (*SR,RS*)-$(\eta^5\text{-}C_5H_5)Re(NO)(PPh_3)(CH(n\text{-}C_4H_9)CN)$ ((*SR,RS*)-**6**)[5] in 53% yield after workup. The product stereochemistry and the reaction stereospecificity were established exactly as was done for (*SR,RS*)-**2** in eq ii and iii. Second, reaction of (*SR,RS*)-**2** with *n*-BuLi/TMEDA and then $CH_3OSO_2CF_3$ as in eq ii gave methylcyclopentadienyl complex (*SR,RS*)-$(\eta^5\text{-}C_5H_4CH_3)Re(NO)(PPh_3)(CH(CH_3)CN)$ ((*SR,RS*)-**7**)[5] in 84% yield upon workup. This reaction proceeded cleanly via an intermediate with a ^{31}P NMR resonance (25.15 ppm) very close to that of **5**. Accordingly, this species is assigned the structure $(\eta^5\text{-}C_5H_4Li)Re(NO)(PPh_3)(CH(CH_3)CN)$ (**8**).

In conclusion, we have established that transition-metal alkyls can be deprotonated as in eq i and that the resulting conjugate base can, when appended to the chiral $(\eta^5\text{-}C_5H_5)Re(NO)(PPh_3)$ moiety, be stereospecifically alkylated. Since the rhenium–carbon σ bond in $(\eta^5\text{-}C_5H_5)Re(NO)(PPh_3)(R)$ complexes can be cleaved with high stereoselectivity both at rhenium and carbon,[11] these transformations should have utility in asymmetric organic synthesis. Efforts to understand the basis for the stereospecificity of eq ii, and to synthesize other transition-metal substituted carbanions, are in progress.

Acknowledgment. We thank the NIH for support of this research. Mass spectrometers utilized were obtained via National Science Foundation and University of Utah Institutional Funds Committee Grants.

Supplementary Material Available: Table of microanalytical, mass spectral, IR, and NMR (^{1}H, ^{13}C, ^{31}P) data for new compounds (4 pages).[5] Ordering information is given on any current masthead page.

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Structure of the Alkali-Labile Product Formed during Iron(II)–Bleomycin-Mediated DNA Strand Scission

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The bleomycins are a group of glycopeptide-derived antibiotics employed clinically for the treatment of certain malignancies including squamous cell carcinomas and Hodgkin's disease.[1] The bleomycins appear to mediate their therapeutic effects primarily at the level of DNA strand scission,[2] a transformation that can be effected by any of four metallobleomycins.[3] The O_2-dependent

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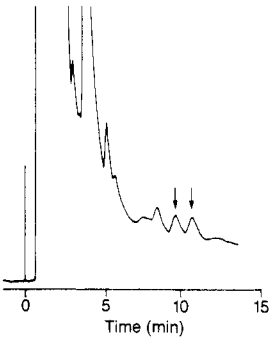
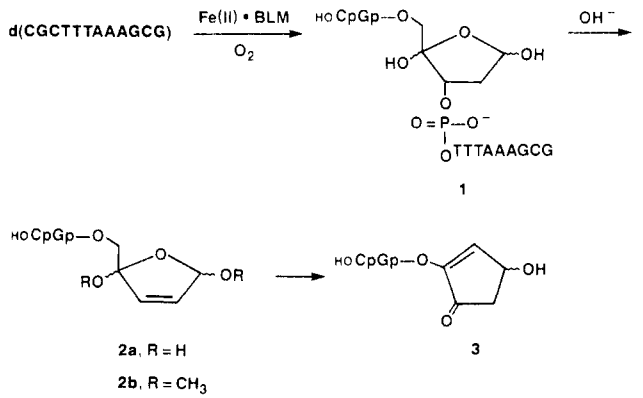


Figure 1. HPLC analysis of Fe(II)–BLM-treated dodecanucleotide, following additional alkali treatment. Separation was achieved on a Rainin Microsorb C_{18} column (3 μm), elution with 0.1 M ammonium formate containing 2.8% CH_3CN at a flow rate of 1.5 mL/min. The eluate was monitored at 254 nm; the authentic diastereomers of **3** eluted at the times (9.7 and 10.8 min) indicated by the arrows.

DNA strand scission mediated by Fe(II)–bleomycin has been studied in detail and shown to be accompanied by the formation of base propenals and oligomers having deoxynucleoside 3′-(phosphoro-2″-*O*-glycolates) at their 3′-termini.[4] It is believed that these products derive from a C-4′ hydroperoxide that results from capture of an initially formed C-4′ deoxyribose radical.[4,5] Also formed in comparable amounts under ambient conditions, and as the predominant products when O_2 is limiting, are free bases and DNA lesions that result in strand scission upon subsequent treatment with alkali.[1a,6] These alkali-labile lesions, which have been proposed to form via C-4′ hydroxyl derivatives of DNA,[5a] have thus far eluded efforts at structural characterization. Herein we describe the structure and chemistry of this alkali-labile lesion.

A recent study in this laboratory has demonstrated the formation of 2′-deoxycytidylyl(3′ → 5′)(2′-deoxyguanosine 3′-(phosphoro-2″-*O*-glycolate)) upon treatment of the dodecamer d(CGCTTTAAAGCG) with Fe(II)–bleomycin + O_2.[7] The structure of this product was verified by comparison with the authentic synthetic dinucleotide; its formation was consistent with the known[8] sequence selectivity of DNA cleavage by bleomycin. To test the hypothesis that an alkali-labile product of structure **1** might also form at the same position,[9] we synthesized two dinucleotides (**2a** and **3**) whose formation from **1** could be envisioned under alkaline conditions.[10]



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Following treatment of d(CGCTTTAAAGCG) with Fe(II)–BLM A_2 + O_2 at neutral pH,[12] the product mixture was analyzed directly by HPLC. As anticipated, HPLC analysis confirmed substantial degradation of the starting dodecanucleotide and formation of cytosine, but neither **2a** nor **3** was present. Further treatment of the dodecamer under conditions (0.1 N NaOH, 60 °C, 2 min) shown previously to effect strand scission of DNA containing alkali-labile lesions resulted in further degradation of the oligomeric products and the accumulation of **3** (albeit not **2a**) as a reaction product. The formation of **3** was verified by comparison with an authentic sample on reversed phase (Figure 1) and anion exchange HPLC columns.[13]

These results strongly suggest the bleomycin-mediated formation of alkali-labile structure **1** from d(CGCTTTAAAGCG) and indicate that subsequent alkali treatment results in oligomer strand scission, as observed for Fe–BLM-treated DNA. Thus, we believe that the alkali-labile product formed from DNA by Fe(II)–BLM + O_2 has structure **1**. Moreover, these results indicate that in addition to participating in the anticipated elimination reaction (i.e., **1** → **2a**), the atoms corresponding to the deoxyribose moiety of cytidine-3 in the original dodecamer undergo a further alkali-mediated rearrangement (to form **3**), analogous to chemical transformations observed previously.[14]

Acknowledgment. We thank Dr. Li-Ho Chang for the synthesis of the dodecanucleotide employed in this study. This work was supported by Research Grants CA-29235 and CA-38544, awarded by the National Cancer Institute, Department of Health and Human Services.

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(9) Alkali-labile product **1** would presumably result from C-4′ hydroxylation of cytidine-3 in the dodecamer, followed by elimination of cytosine.

(10) Dinucleotide **2b** was synthesized in analogy with 2′-deoxycytidylyl(3′ → 5′)(2′-deoxyguanosine 3′-(phosphoro-2″-*O*-glycolate))[7] via the phosphite-mediated coupling of a protected guanosine derivative with 2,5-dihydro-2,5-dimethoxyfurfuryl alcohol and subsequent coupling with (protected) cytidine.[11] Compound **2b**: ^{1}H NMR (D_2O) δ 1.47 (m, 1), 2.25 (m, 1), 2.55 (m, 1), 2.78 (m, 1), 2.95–3.12 (m, 3), 3.25–3.43 (m, 3), 3.59 (m, 2), 3.65–4.10 (m, 5), 4.32 (s, 1), 4.50 (m, 1), 4.90 (s, 1), 5.48 (d, 1, J = 9 Hz), 5.89 (d, 1, J = 7 Hz), 5.95–6.25 (m, 4), 7.53 (d, 1, J = 7 Hz) and 7.99 (s, 1); mass spectrum (chemical ionization), m/z 779 (M + 1), 777 (M − 1). Hydrolysis (0.1 N HCl, 25 °C, 30 min) afforded unstable **2a**, which was characterized by HPLC. Alkali treatment of **2a** (0.1 N NaOH, 60 °C, 2 min) afforded **3**: ^{1}H NMR (D_2O) δ 1.55 (m, 1), 2.19 (m, 2), 2.52 (m, 1), 2.87 (m, 2), 3.48 (m, 2), 3.91 (m, 3), 4.23 (m, 1), 4.44 (m, 1), 4.86 (m, 1), 4.96 (m, 1), 5.84 (br d, 1, J = 7.6 Hz), 5.93 (br t, 1, J = 7 Hz), 6.09 (m, 1), 6.97 (d, 1, J = 2.6 Hz), 7.48 (d, 1, J = 7.6 Hz), 7.91 (br s, 1). Also characterized in detail by ^{1}H NMR and mass spectrometry were the analogous rearrangements of other 1-substituted 2,5-dihydro-2,5-dihydroxyfurfuryl alcohols including the tosylate and guanosine 3′-phosphate derivatives.

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(12) Reaction mixtures (total volume 50 μL) contained d(CGCTTTAAAGCG)[7] (2 mM final nucleotide concentration), 1 mM BLM A_2, and 1 mM $Fe^{II}(NH_4)_2(SO_4)_2$ in 50 mM sodium cacodylate, pH 7.0. Reactions were initiated by addition of Fe(II) and incubated at 25 °C for 15 min prior to HPLC analysis.

(13) Identity on reversed phase HPLC was verified using two different solvent systems. Anion exchange HPLC was carried out on a Dupont 25-cm Zorbax Sax anion exchange column, elution with 0.05 M ammonium phosphate (pH 4.5) at a flow rate of 2 mL/min; although the isomers of **3** could not be resolved, the same elution profile was obtained for authentic **3** and for the dodecamer that had been treated successively with Fe(II)–BLM and alkali. Also employed for study was [5′-^{32}P]d(CGCTTTAAAGCG); the resulting [5′-^{32}P]-**3** was shown to have the same properties as an authentic synthetic sample when analyzed by anion exchange HLPC. In addition, digestion of poly(dG-dC)·poly(dG-dC) with Fe(II)–BLM A_2, followed by alkali treatment, afforded the 2,4-dihydroxycyclopentenone derivative of pGp; the identity of this species was also verified by comparison with an authentic synthetic sample.

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