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Formation and Structure of a Novel Enediyne−RNA Base Covalent Adduct

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Enediyne antitumor antibiotics undergo activation to radical species (Scheme 1) that selectively abstract hydrogen atoms from nucleic acid sugar residues, leading to oxidative strand scission. In the absence of dioxygen, the enediynes neocarzinostatin (NCS) and C-1027 chromophores, instead, form covalent adducts on the deoxyribose of DNA.[1] Recently, in the study of NCS-induced cleavage of RNA−DNA hybrid 5′-r (CACAGAAUUCG)/5′-d (TTCTGTG) molecules (Figure 1), containing an overhang of four unpaired residues at the 3′-end of the RNA strand, we found that a covalent adduct is formed between the drug and U9 of the 11-mer RNA.[2] This reaction was unexpected since it occurred in the presence of dioxygen and involved an unpaired RNA residue. We describe herein the structure elucidation of this unusual adduct and show that unlike enediyne−DNA adducts characterized before,[3] this one involves the base of an RNA residue.

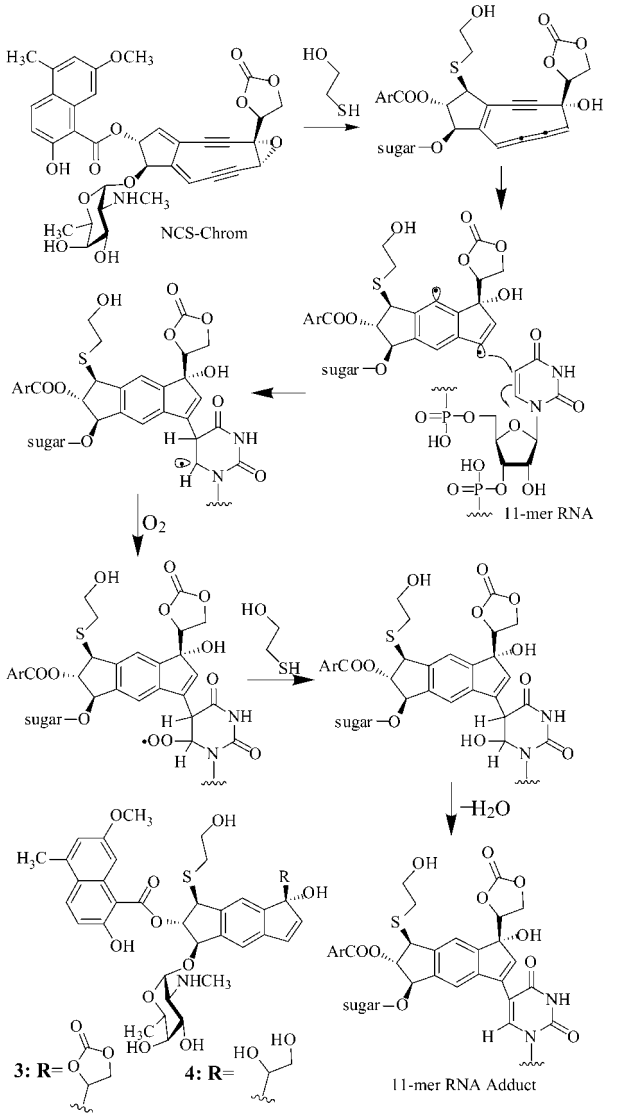
The RNA adduct of 2-mercaptoethanol (BME)-activated NCS-chrom was synthesized and purified by HPLC as described previously.[2] It is confirmed to be an 11-mer RNA adduct of NCS-chrom on the basis of the observation of $[M - 4H]^{4-}$ ion peak at *m*/*e* 1051.11 in its ESI mass spectrum. ^{1}H NMR, DQF-COSY, and ROESY spectra of the 11-mer RNA adduct were obtained, but because the signals seriously overlapped, the complete structure could not be elucidated. To obtain smaller adduct fragments, digestions of the 11-mer RNA adduct with several nucleases[4] were attempted; unfortunately, this did not afford a mononucleotide adduct, presumably due to steric interference by the bulky adducted NCS chromophore.[5] We finally found that hydrolysis[6] of the 11-mer RNA adduct by 2 M trifluoroacetic acid for 2 h at 55 °C yielded two mononucleotide adducts **1** (high-resolution ESI-MS: calcd for $[M - H]^-$, $C_{45}H_{54}N_3O_{21}PS$: 1034.2630; found: 1034.2653) and **2** (high-resolution ESI-MS: calcd for $[M - H]^-$, $C_{46}H_{52}N_3O_{22}PS$: 1060.2423; found: 1060.2438). When a mixture of **1** and **2** was treated with 200 mM Tris-HCl buffer (pH 8.5) for 12 h at room temperature, **2** was quantitatively converted to **1** (Scheme 2), suggesting **1** is a debicarbonate product of **2**.[2a, 7]

About 300 μg of **1** was accumulated by acid hydrolysis and subsequent alkaline (pH 8.5) hydrolysis from the 11-mer RNA adduct, and ^{1}H NMR, DQF−COSY, TOCSY and NOESY spectra[8] were obtained. High-resolution ESI-MS of **2** confirmed its formulation to be **3**+ uridine phosphate-2H, in which **3** is a postactivated product of NCS-chrom activated by BME, and the formulation of **1** was determined to be **4** + uridine phosphate-2H, in which **4** is a debicarbonate product of **3**. ^{1}H NMR spectrum of **1** showed two sets of signals, indicating that **1** is a mixture of two components with a ratio of 6:1. Taking the acid hydrolysis of RNA into account,

5′-**r(CACAGAAUUCG)**-3′
3′-**d(GTGTCTT)**-5′

Figure 1. Sequence of RNA−DNA hybrid substrate. Drug attachment site is underlined.

Scheme 1



1 was suggested to be a mixture of 2′-phosphate and 3′-phosphate. In the ^{1}H NMR spectrum of **1**, all of the signals derived both from **4** and uridine phosphate were observed except for two doublets attributable to H-5 and H-6[8] of **4**, and two doublets attributable to H-5 and H-6 of uridine phosphate. Instead, two singlets (δ 6.54, 8.10 for major component; δ 6.54, 8.08 for minor one) were found

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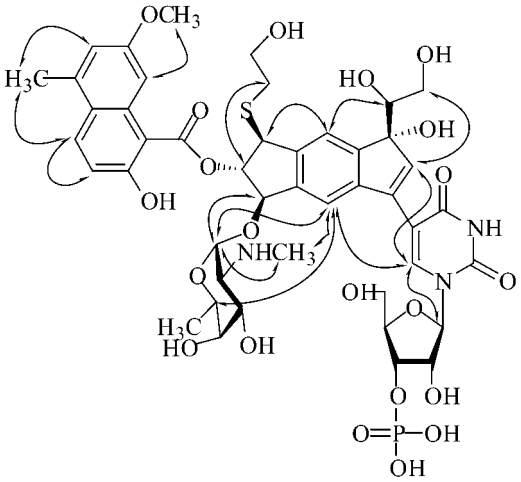
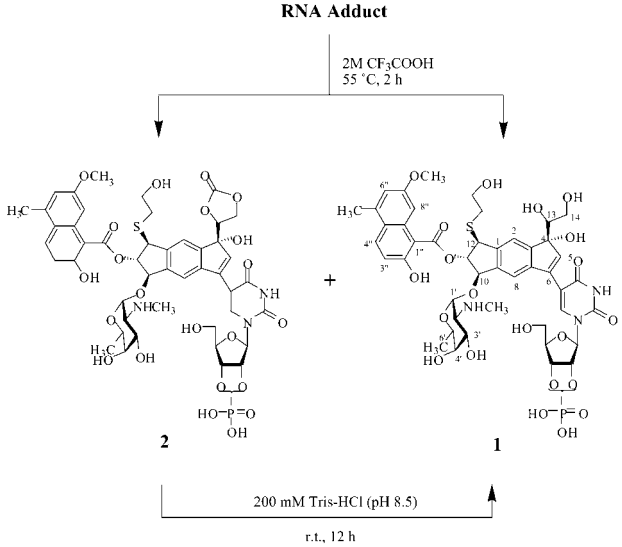


Figure 2. Important NOE correlations observed for compound **1**.

Scheme 2



to be present. Further, the changes in chemical shift in the adduct compared to uridine monophosphate and compounds **3** and **4** show that the double bonds of C5−C6 in the drug and uracil residues are conjugated (see Supporting Information). Considering this and the above mass spectral result of **1**, the linkage of **4** and uridine phosphate in **1** appears to be located between the two double bonds. On the other hand, only four cross-peaks between H-5 and H-6 of the pyrimidine base of RNA were found in the DQF-COSY spectrum of the 11-mer RNA adduct, providing further evidence that the double bond of one of the pyrimidine bases of the RNA adduct was substituted. With the aid of DQF-COSY, TOCSY, and NOESY (Figure 2) spectral evidence, all of the carbon-bound proton signals of the major component of **1** were assigned. Two singlets of the major component (δ 6.54, 8.10) were attributable to H-5 of the postactivated NCS-chrom moiety and H-6 of uridine phosphate, respectively. On this basis, a C−C linkage between C-6 of **4** and C-5 of uridine in **1** was established. Further, by comparing the chemical shifts of the ribose protons of two components of **1** with those of uridine 2′- and 3′- monophosphate, the major component of **1** was judged to be 3′-phosphate. Since the drug attachment site on RNA has been identified to be at the U9 position, the whole structure of 11-mer RNA adduct was elucidated.

RNA−DNA hybrids (including those with "overhangs") exist in biology as intermediates in transcription or reverse transcription. Enediyne adducts on RNA have not been described before.[1] This may be, in part, because duplex RNA structures are poor cleavage targets for enediyne antibiotics.[1a,9,10] RNA−DNA hybrids, however, are good cleavage targets, and hybrids formed between the RNA 11-mer used here and its 11-mer DNA complement show strong cleavage at U9 due to C-1′ attack.[2b] The present report is particularly unusual in that targeting of U9 in the RNA overhang does not involve radical attack on its sugar and, instead, leads to formation of a covalent adduct on its base. Elucidation of its structure also makes it the first nucleic acid adduct of an enediyne antibiotic to be structurally determined.

The proposed mechanism of its formation (Scheme 1) involves adduction of NCS-chrom C-6 radical to the C-5 of uracil of the RNA, oxidation by dioxygen, reduction by BME, and subsequent dehydration.

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Supporting Information Available: ^{1}H NMR chemical shifts and assignment, HPLC charts, and mass spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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