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Internal Hydrogen Abstraction by Activated Neocarzinostatin: Quenching of the Radical at C2 by Hydrogen Atom Transfer from the α Carbon of the Adducted Thiol

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Neocarzinostatin chromophore (NCS-Chrom) (**1**) (Scheme I) is the first of the enediyne-containing antitumor antibiotics shown to cleave DNA by abstracting hydrogen atoms from minor groove accessible sites on the deoxyribose backbone.[1] Thiol adduction at C12 of the bicyclo enediyne ring generates a diradical species of the drug, with radical centers at C2 and C6 (**3**), that eventuates in a stable reduced form of the drug (**4**) following hydrogen atom abstraction from DNA or some other available source.[2-5] Although the sulfhydryl hydrogen can quench the radicals of the activated chromophore at high ratios of thiol to drug,[6] under the experimental conditions used in DNA damage reactions involving relatively low ratios of thiol to drug, hydrogen from the exchangeable sulfhydryl, as well as that from the nonexchangeable DNA source, cannot account for the total hydrogen abstracted into the post-activated thiol–drug adduct.[3-5] Hence, the acidic hydrogens on the carbon α to the sulfur in the thiol have been suggested as a possible source of hydrogen abstracted by the diradical form of NCS-Chrom.[4] We present herein direct evidence of hydrogen atom abstraction from the carbon α to the sulfur of a drug-bound thiol into the C2 position of the drug. Intramolecular quenching of the radical at C2 helps to explain the finding that single-stranded DNA breaks exceed double-stranded lesions.[1]

Since glutathione activates NCS-Chrom in cells,[7] it was the thiol chosen in this study to investigate the hydrogen abstraction reaction. A racemic mixture of γ-L-glutamyl-DL-cysteinylglycine (DL-**2**), with or without deuterium replacing both hydrogens on the α carbon, was prepared by solid-phase peptide synthesis. DL-Cysteine was converted[8-10] into *N*-Fmoc-*S*-trityl-DL-cysteine for peptide synthesis. ^{1}H NMR analysis showed that the deuterium enrichment of the α carbon was more than 90%. The ability of DL-**2** to activate NCS-Chrom was verified by measuring the incorporation of tritium into the drug from [5′-^{3}H]thymidine-labeled λ DNA[4] at a thiol to drug ratio of 5. DL-**2** affords the same amount of tritium abstraction from DNA into the drug as glutathione. The thiol–drug adducts (**4**) were produced by mixing the thiol with NCS-Chrom[4] at a ratio of 8, using sodium citrate and Tris-HCl as buffers. In the absence of DNA, reactions were conducted at a temperature range from 0 to 20 °C, a pH range from 4 to 8.5, and with methanol at 1 to nearly 100%. In reactions involving calf thymus DNA, the ratio of DNA phosphorus to drug was 10.

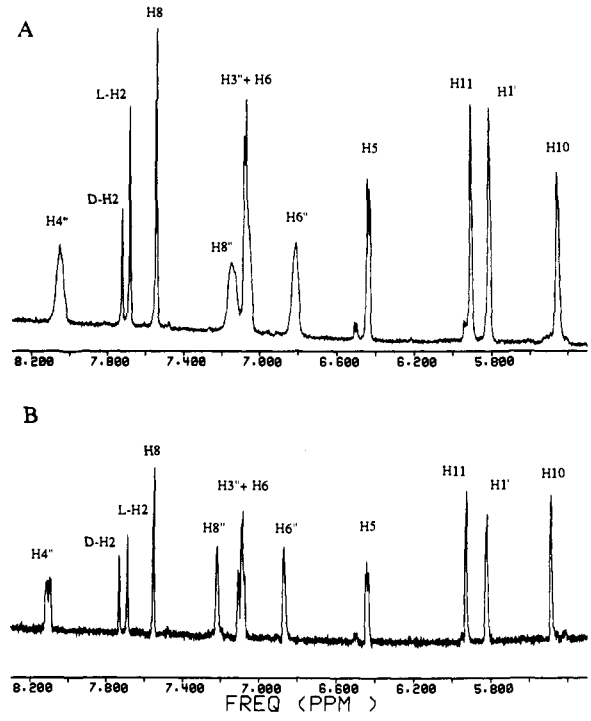
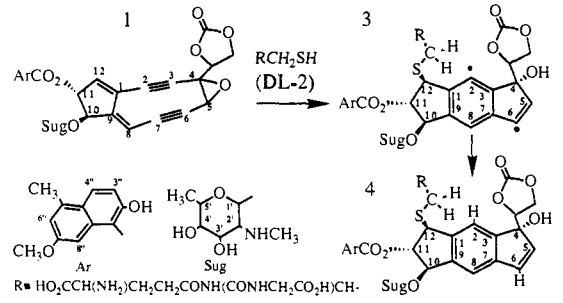


Figure 1. ^{1}H NMR spectra (500 MHz) (δ 5.3–8.3) of purified thiol–drug adducts produced by mixing thiol and NCS-Chrom (8:1) at pH 7.6 in a >99% methanolic solution at 20 °C: DL-**2**–NCS-Chrom adduct (A); [α,α-$^2H_2$]-DL-**2**–NCS-Chrom adduct (B). The NMR solvent was 2 mM ^{2}HCl in ^2H_2O.

Scheme I



Analysis of the 500-MHz ^{1}H NMR spectra of the DL-**2**–drug adduct (Figure 1) revealed that the majority of the resonance signals from the rearranged central core of the post-activated drug exhibited close, if not identical, chemical shifts for the D and L forms of the compound. The signal for the D form of H2, however, was well-separated from that of the L form by 0.05 ppm downfield. Analysis of the spectra of the DL-**2**–drug and [α,α-$^2H_2$]-DL-**2**–drug adducts from paired experiments showed that 20–30% of deuterium was consistently incorporated into C2 of the L-form adduct (seven sets of experiments, two with DNA) under the various conditions. There was no obvious reduction of the signal at H2 of the D-form adduct or at H6 in the spectra of the [α,α-$^2H_2$]-DL-**2**–drug adduct. In fact, the H2 peak of the D-form adduct is slightly greater than that of the D form of the nonlabeled adduct. The amount of deuterium transfer to C2 is not very sensitive to changes in temperature, percent of methanol, pH, or the presence of DNA. However, the yield of the two isomeric adducts, as judged from the separate ^{1}H NMR signals at H2, varies substantially with the content of methanol and the presence of DNA.

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The ratio of the L to the D form of the adduct in the absence of DNA increases with increasing methanol and decreases with the presence of DNA. The finding that deuterium is incorporated selectively into the C2 position of the L form of the thiol–drug adduct suggests that the steric geometry of the thiol which donates the hydrogen is relatively fixed in its relationship to the drug. Accordingly, the mechanism of hydrogen abstraction favors an internal hydrogen transfer into C2 of the drug from the attached L-form thiol at C12.

Although NCS-Chrom is converted into a diradical intermediate upon thiol activation that is capable of bistranded interaction with DNA, it has been puzzling that single-stranded lesions are much more prevalent than double-stranded lesions.[11] The finding of significant intramolecular hydrogen abstraction from the adducted thiol by the radical center at C2, resulting in the conversion of a bifunctional agent into a monofunctional one, may provide an explanation.[12] This result is consistent with recent evidence that the C6 radical is specifically responsible for hydrogen abstraction from the C-5′ position of deoxyribose of T residues,[5] the lesion primarily responsible for single-strand breaks.

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(12) Whether or not the amount of deuterium incorporated from the α carbon of the thiol into C2 is sufficient to explain entirely the difference between the double- and single-stranded lesions of DNA depends on the magnitude of the deuterium isotope selection effect. Internal hydrogen transfer, such as from C12 of the drug into C2[6c] or from the β carbon into C2,[4] may also contribute to the quenching of the C2 radical. These possibilities are under investigation.

Tri-3-(2-butyl)-6-methylsalicylide. A Novel, Versatile Tri-*o*-thymotide-Based Clathrate Host Having Chiral Centers

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Activity in supramolecular chemistry continues to develop with many impressive achievements being recorded. Clathrate inclusion complexes allow a broad category of host–guest phenomena to be readily studied, and they have also been the subject of increasing recent interest.[1] The unique properties of tri-*o*-thymotide[2] (TOT, **4**) provide an especially suitable system for study because TOT affords a host lattice for inclusion compounds with guest molecules having a wide variety of sizes, shapes, and functionalities[3] and

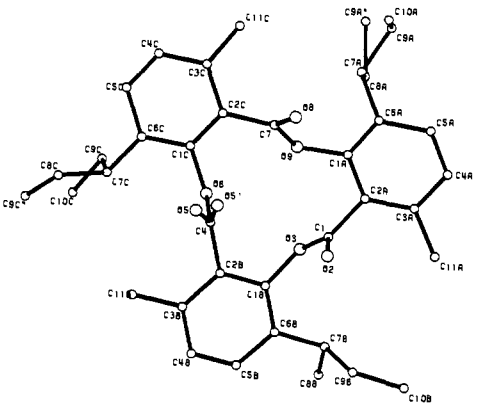
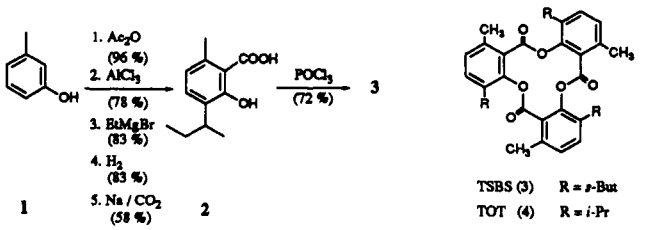


Figure 1. Molecular structure of TSBS (**3**) in the (*S*)-(+)-2-butanol clathrate.[11] The hydrogen atoms have been eliminated for clarity. The *sec*-butyl group disorder in subunits A and C is indicated by the extra atoms $C_{9A''}$ and $C_{9C''}$, respectively; this is effectively methyl–ethyl substitutional disorder. In addition, one of the three carbonyl oxygens ($O_5/O_{5'}$) displays positional disorder with 0.5:0.5 occupancy values.

Scheme I



because of its tendency to undergo spontaneous optical resolution on crystallization.[4] While modification of its chemical structure has been carried out over the past 40 years, none of the analogues retained the versatile properties of TOT itself.[5] In order to better understand the physical properties which make TOT such a unique host, we report the preparation of tri-3-(2-butyl)-6-methylsalicylide (TSBS), a TOT analogue which is unusual because of the presence of chiral *sec*-butyl units and its broad clathrate-forming properties.

TSBS (**3**) was prepared by the cyclodehydration[6] of racemic 3-(2-butyl)-6-methylsalicylic acid (**2**),[7] starting from *m*-cresol (**1**) (Scheme I), and isolated as the TSBS/*n*-hexane complex (mp 132 °C).[8] Crystallization of TSBS from all solvents used thus far has afforded complexes, and more than 30 clathrates (both cage- and channel-type) have been characterized. The included guests have alcohol, carboxylic acid, ester, ketone, haloalkane, and alkane functionalities and represent sizes and shapes which are common to TOT clathrates.[9] A few modified TOT analogues form

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(7) Data for **2**: mp 85–7 °C; ^{1}H NMR δ 0.86 (3 H, t, J = 7 Hz), 1.20 (3 H, d, J = 7 Hz), 1.61 (2 H, q, J = 8 Hz), 2.59 (3 H, s), 3.15 (1 H, m), 6.72 (1 H, d, J = 8 Hz), 7.23 (1 H, d, J = 8 Hz), 9.06 (2 H, br s).

(8) Guest-free host **3** was obtained by heating the clathrate crystals under vacuum at 80 °C (30 mmHg) for 7 days or at 100 °C (0.1 mmHg) for 4 h; the microcrystalline powder obtained had mp 178–9 °C: ^{1}H NMR δ 0.78, 0.92 (9 H, two triplets, J = 8 Hz), 1.17, 1.26 (9 H, two doublets, J = 8 Hz), 1.60 (6 H, quintet, J = 7 Hz), 2.47 (9 H, s), 2.84 (3 H, sextet, J = 8 Hz), 7.22 (3 H, d, J = 7 Hz), 7.38 (3 H, d, J = 7 Hz). Anal. Calcd C, 75.79; H, 7.37. Found: C, 75.95; H, 7.45.

(9) For example, TSBS forms cage-type, 2:1 (space group $P3_121$) clathrates with nitromethane, ethyl acetate, 2-butanol, trifluoroacetic acid, and 2-chlorobutane; a channel-type, 2:1 (space group $P6_1$), clathrate forms with 2-octanone. The 2:1 clathrates with chloroform, tetrachloromethane, 1-bromobutane, and diiodomethane are believed to be cage-type, but X-ray data has not been collected.

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