Model Systems for Hydrogen Atom Abstraction from DNA

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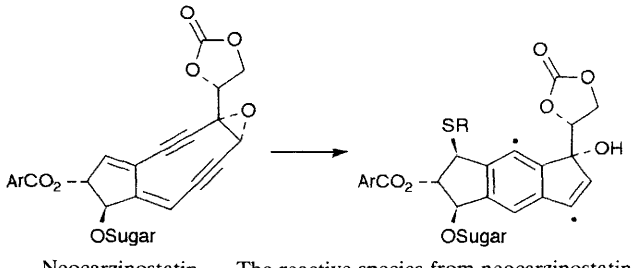
Addition of thiyl radicals to alkynes produces vinyl radicals that abstract hydrogen atoms from tetrahydrofurans and a tetrahydropyran mimicking the crucial stages of the action of the anti-tumour agents, neocarzinostatin, esperamicin and calicheamicin.

Many anti-tumour agents work by interaction with DNA, and the chemical mechanisms of these compounds fall principally into two categories: alkylating agents (nitrogen mustards, mitomycin, CC-1065)[1] and radical generators (bleomycin,[2] neocarzinostatin, esperamicin and calicheamicin[3]).

The latter set can cleave DNA by hydrogen atom abstraction from the deoxyribose sugars. Reaction of the resulting radicals with molecular oxygen leads to destruction of DNA. (See Scheme 1 for one destructive path for DNA resulting from initial hydrogen atom abstraction[4] at the 4′ position). Great efforts are being expended to design molecules that mimic these compounds.[5] To date, the efforts have strived towards compounds as structurally similar as possible to the original natural products. These drugs with a radical-based mechanism have one common starting point. They abstract a hydrogen atom from the 1′, 4′ or 5′ positions on the deoxyribose moieties. The subsequent decomposition of the DNA is independent of the drug.

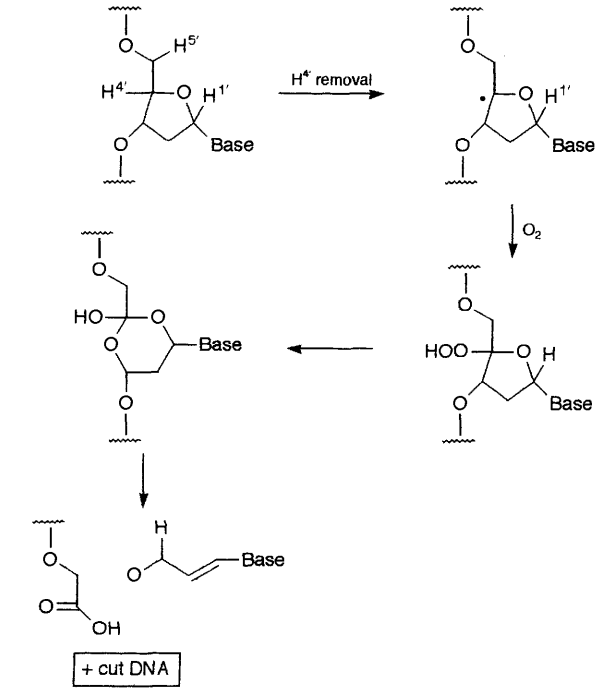
Glutathione, GSH, occurs in every mammalian cell,[6] and is responsible for a host of redox processes. Many of these occur *via* the stabilised glutathionyl radical,[7] which cannot readily abstract hydrogen atoms from deoxyribose,[8] but can add[9] to an alkyne. The resulting vinyl radical should be able to abstract a hydrogen atom from the positions of importance on the deoxyribose, *i.e.* the 1′, 4′ and 5′ sites.

Scheme 2 shows the general reactivity planned for the model reactions described here. A thiyl radical is generated to react intermolecularly with an alkyne giving radical **A**, so placed to be able to remove a hydrogen atom intramolecularly from a carbon with a neighbouring oxygen, thus leaving carbon-centred radical, **B**. The formation of **B** is reported by

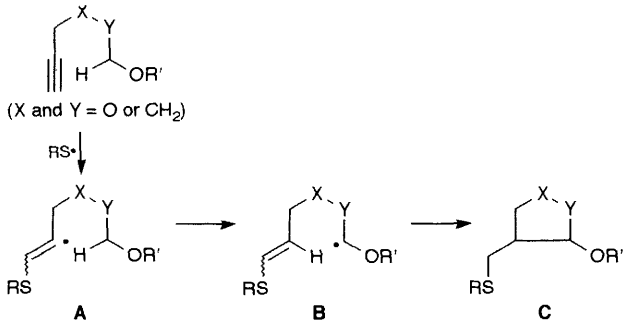


Neocarzinostatin chromophore

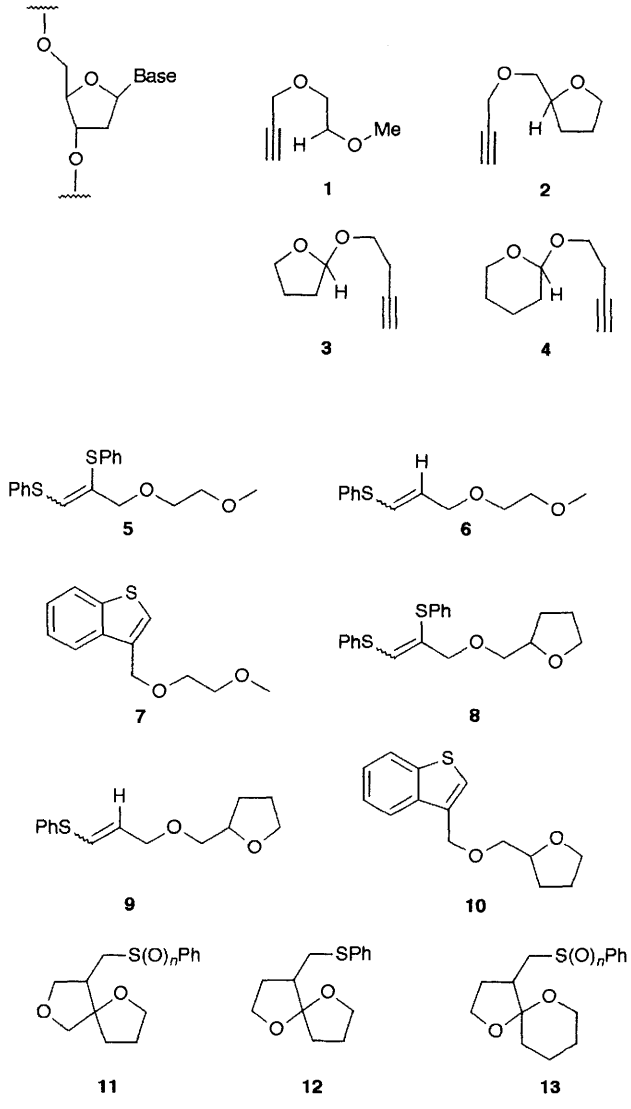
The reactive species from neocarzinostatin, which is responsible for hydrogen atom abstraction from DNA



Scheme 1



Scheme 2



cyclisation followed by hydrogen atom abstraction from an unspecified source to give product, **C**.

This model is a multi-step radical scheme crucially dependent on relative rates and concentrations of different reactions. Accordingly, high yields were not predicted for the process. The thiyl radicals were generated by photolysis of diphenyl disulphide with a sun-lamp.

Our simple models were compounds **1**, **2**, **3** and **4**. In the reaction of the simplest model, **1** no cyclic product was observed. This reaction and others in the series were very complex, and our product isolation was guided by NMR of the overall reaction mixture and of samples resulting from exhaustive chromatographic fractionation. The isolated products were **5**, **6** and **7**.

These all result from initial addition of the thiyl radical to the alkyne. Product **5** arises by the intermediate vinyl radical attacking diphenyl disulphide rather than abstracting a hydrogen atom. The thioether **6** results from hydrogen atom abstraction by the vinyl radical either intra- or inter-molecularly, while the formation of the benzothiophene **7** occurs by addition of the vinyl radical to the aromatic ring, followed by oxidative rearomatisation. Such a process has precedent[10] albeit at a much higher temperature.

The cyclic ether **2** models reaction at the 4′ position. Products **8**, **9** and **10** analogous to the above products were isolated, but now the spiroether **11** (n = 0) (0.2%)† was also seen. Purification of this could not be completely accomplished at this stage, so, by treatment with oxone a single diastereoisomer of the corresponding sulphone **11** (n = 2) {δ[$(CD_3)_2CO$] 90.24, spiro carbon} was completely purified.

To represent models for 1′ hydrogen abstraction, ethers **3** and **4** were examined. A single diastereoisomer of the spiroketal **12** (1.2%) was isolated from reaction of **3** {δ[$(CD_3)_2CO$] 116.58, spiro carbon}. The spiroketal sulphide **13** (n = 0) from reaction of **4** was purified by conversion to the sulphone **13** (n = 2) by treatment with oxone {δ($CDCl_3$) 104.86, spiro carbon}.

These reactions demonstrate that very simple systems–here non-aggressive thiyl radical plus alkynes–can generate reactive vinyl radicals capable of hydrogen atom abstraction from deoxyribose-like structures, and thus model the initial stages of the radical-induced DNA damage seen in the anti-tumour agents neocarzinostatin, esperamicin and calicheamicin. Our challenge now is to see if a simple system such as that used here can be made more efficient and can be effectively targeted, by attachment to appropriate carriers, to show sequence-specific reaction with DNA, and hence behave as a rationally designed biocompatible anti-tumour agent.

† The reported yields are based on isolated mass of pure compounds. All products had ^1H, ^{13}C NMR, IR and mass spectra in accord with the assigned structures.

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