# Modelling Radical-initiated DNA Cleavage by Vinyl Epoxides

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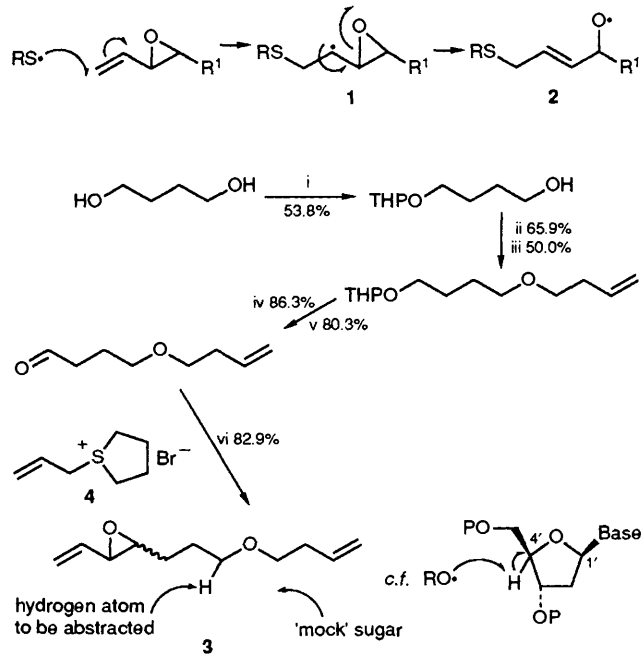
Allyloxy radicals, formed by addition of thio radicals to vinyl epoxides, are shown to abstract hydrogen atoms and to add to alkene π-bonds in reactions which model the chemistry that would be required to induce DNA damage; this suggests that vinyl epoxides could have uses as radiosensitizers for treatment of resistant tumours.

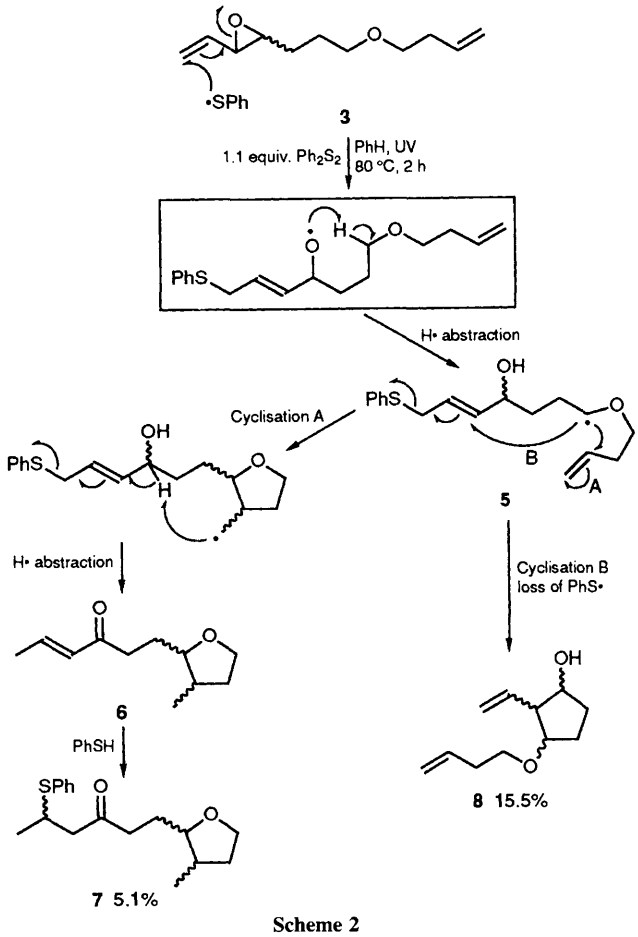
Glutathionyl radicals (GS•) are easily formed *in vivo* from glutathione (GSH) on reaction with harmful hydroxyl or hydroperoxyl radicals.[1] Glutathione thus exerts a protective effect on cells when challenged with these reactive radicals. Certain radiation-resistant tumour cell lines produce vastly increased levels of glutathione,[2] and this has been implicated as a major mechanism of resistance to radiotherapy (radiolysis of water produces hydroxyl radicals). We have recently proposed[3] a method for reversing this protection by allowing the glutathionyl radicals, which are not capable of damaging DNA, to add to alkynes so producing vinyl radicals which can react with DNA (as seen for example in the chemistry of neocarzinostatin, esperamicin and calicheamicin[4]). An alternative approach is proposed here and features a vinyl epoxide. Thio radical addition to a vinyl epoxide gives rise to a carbon-centred radical **1**. The epoxide can then fragment *via* C–O bond cleavage[5] to give an allyloxyl radical **2**. By analogy with the hydroxyl radical, such an oxygen-centred radical might be very reactive and capable of DNA damage. If the vinyl epoxide were attached to a DNA intercalator, then activation by GS• would produce the reactive oxyl-radical in the vicinity of DNA, enhancing the possibility of DNA damage.

Hydroxyl radicals are known to attack DNA in two ways:[6] (*a*) *via* hydrogen atom abstraction from the 1′, 4′, 5′ sites of the deoxyribose sugar ring, eventually leading to cleavage of the DNA backbone,[7] and (*b*) *via* addition to the π-bonds of the DNA bases.[8] Allyloxyl radicals[9] have alternative reactions available to them not available to hydroxyl radicals, and so studies were undertaken to probe the ability of allyloxyl radicals to perform the types of rections which lead to DNA cleavage. This paper describes these reactions.

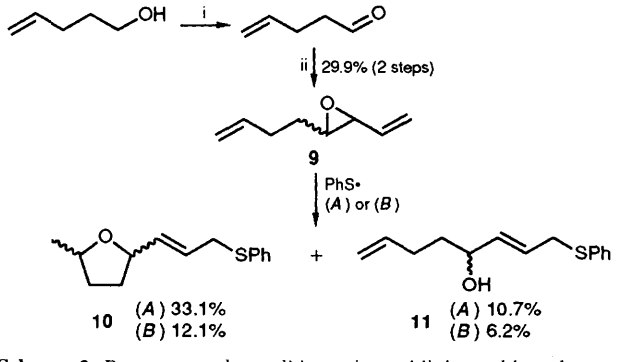
Modelling hydrogen atom abstraction from DNA sugars was accomplished by synthesising[10] a non-cyclic ether analogue of a DNA deoxyribose sugar **3**, in six steps from



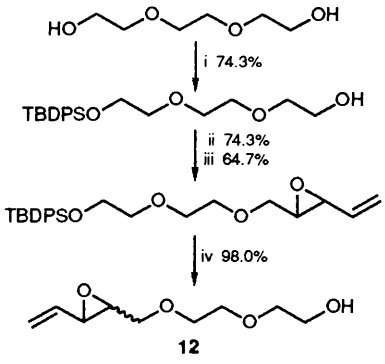

**Scheme 1** *Reagents and conditions*: i, diol (10 equiv.), dihydropyran, pyridine toluene-*p*-sulfonate (PPTS), $CH_2Cl_2$, tetrahydrofuran (THF), 5 h; ii, TsCl (1.5 equiv.), pyridine (2 equiv.), $CHCl_3$, 0 °C, 4 h; iii, but-3-en-1-ol (2 equiv.), $Bu^t_4NHSO_4$, PhH, 12.5 mol $dm^{-3}$ NaOH, 60 °C, 20 h; iv, Dowex ($H^+$), MeOH, 60–65 °C, 3 h; v, $(COCl)_2$, dimethyl sulfoxide (DMSO), $NEt_3$, $CH_2Cl_2$, −65 to 25 °C, 1 h; vi, $BnEt_3NCl$, 10 mol $dm^{-3}$ NaOH, $CH_2Cl_2$, −20 to 0 °C, 30 min (THP = tetrahydropyranyl, Bn = benzyl)




**Scheme 2**




**Scheme 3** *Reagents and conditions*: i, pyridinium chlorochromate (PCC), NaOAc, $SiO_2$, $CH_2Cl_2$, 2 h; ii, **4**, $BnEt_3NCl$, $CH_2Cl_2$, 10 mol $dm^{-3}$ NaOH, −25 to 0 °C, 1.5 h; (*A*) PhSH, AIBN, THF, 67 °C, 18 h; (*B*) PhSH, $O_2$, hexane, 25 °C, 3 days




**Scheme 4** *Reagents and conditions*: i, diol (10 equiv.), TBDPSCl, imidazole, dimethylformamide (DMF), 18 h; ii, $(COCl)_2$, DMSO, $NEt_3$, $CH_2Cl_2$, −70 to 25 °C, 1 h; iii, **4**, $BnEt_3NCl$, 10 mol $dm^{-3}$ NaOH, $CH_2Cl_2$, −15 to 0 °C, 20 min; iv, $Bu_4NF$, THF, 3 h (TBDPS = *tert*-butyldiphenylsilyl)

butane-1,4-diol (Scheme 1). After thio radical attack on the vinyl epoxide, the resulting oxyl-radical is favourably placed to abstract hydrogen from the carbon α to the ether oxygen *via* a six-membered transition state. This mimics hydrogen abstraction from the positions adjacent to an oxygen atom in the deoxyribose of DNA.

Photolysis of diphenyl disulfide (giving phenylthio radicals) and compound **3** in benzene did indeed, after addition to the epoxide, effect the desired hydrogen abstraction (Scheme 2).

The presence of the resulting radical **5** was proved *via* standard 5-*exo* cyclisations A and B onto the two available double bonds giving **7** (5.1%) and **8** (15.5%), respectively as the major products. Cyclisation A followed by a 1,6-H shift, loss of PhS• and tautomerism of the enol formed gave ketone **6**, observed in trace amounts; addition of thiophenol or PhS• to **6** gave ketone **7**. Formation of radical **5** proves that the allyloxyl radicals easily abstract hydrogen from carbon atoms adjacent to oxygen as in deoxyribose, and hence should be able to effect damage or cleavage of DNA by this mechanism.

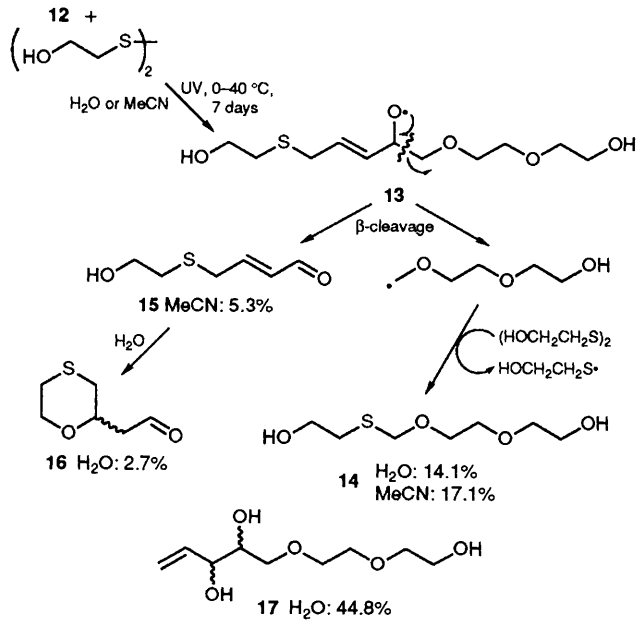
To model oxyl-radical addition onto the π-bonds of DNA bases, compound **9** was synthesised in two steps from pent-4-en-1-ol. Addition of allyloxyl radicals onto the simple double bond,[11] proved to be quite facile. Generation of thio radicals from thiophenol with (*A*) azoisobutyronitrile (AIBN) and heat or (*B*) oxygen at room temperature, in the presence of **9**, gave smooth 5-*exo* cyclisation onto the double bond, producing mainly the substituted tetrahydrofuran **10** and some of the quenched alcohol **11** (Scheme 3).

Thus, the two mechanisms of DNA attack have been confirmed in organic solvents. However, the desired radical-induced epoxide activation must be achievable at ambient temperature and in water to be biologically useful. The water-soluble compound **12**, synthesised in four steps from triethylene glycol (Scheme 4), was selected as the substrate for this reaction.

Extended photolysis of **12** with 2-hydroxyethyl disulfide in water gave the oxyl-radical **13**, whose presence was proved by a characteristic[12] β-cleavage reaction giving diol **14** and aldehyde **16**, formed by Michael cyclisation of **15**, as well as triol **17** formed by hydrolysis of the epoxide. (Scheme 5)† (Performing the reaction in acetonitrile allowed isolation of **14** and **15**). This proves that simple vinyl epoxides can indeed undergo radical activation in water.

In conclusion, these studies have shown that allyloxyl radicals produced from vinyl epoxides have the capacity to cause DNA damage or cleavage, mimicking •OH radical radiation damage. These results led us to synthesise a vinyl

† Note that the long reaction time arises due to the weak UV absorption of the alkyl disulfide.




**Scheme 5**

epoxide attached to a DNA intercalator, which was tested for its DNA-cleaving properties.[13]

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