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Radicals and the Birth and Death of DNA

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Abstract: Radicals have important functions in the enzymatic synthesis of deoxyribonucleotides and their destruction by antibiotics. The chemical basis for these reactions is that radicals dramatically speed up ionic reactions. Nature has developed methods to generate these radicals under mild conditions in water as solvent. The 'catalytic' effect of these reactive intermediates is described.

Keywords: Bleomycin · DNA · Enzymes · Radicals · Ribonucleotide reductase

1. Introduction

Radicals play an important role in biological systems [1]. Among the targets for radical attack are nucleotides as well as their polymers, DNA and RNA. This attack leads to reactive intermediates with radical centers at the carbohydrates or the heterocyclic bases of the nucleic acids. We have studied these radicals by synthesis of their precursors and selective generation of the reactive intermediates. Of special interest are the 3'- and 4'-nucleotide radicals because they are involved in the birth and the death of DNA.

As shown in Scheme 1, the building blocks for DNA, deoxyribonucleotides **3**, are formed in a radical deoxygenation reaction of ribonucleotides **1**. The biosynthesis is catalyzed by the enzyme ribonucleotide reductase, which generates 3'-ribonucleotide radical **2** as a reactive intermediate. With the help of kinases and polymerases these deoxyribonucleotides **3** give birth to double-stranded DNA, which is a very stable molecule. Nevertheless, its structure is destroyed under radical attack, and certain antibiotics like bleomycin or OH• radicals (formed during oxidative stress) cause the death of DNA [1]. One of the intermediates involved in these reactions is the 4'-DNA radical **5**, which leads to strand cleavage even in the absence of O_2.

2. Generation of the Radicals

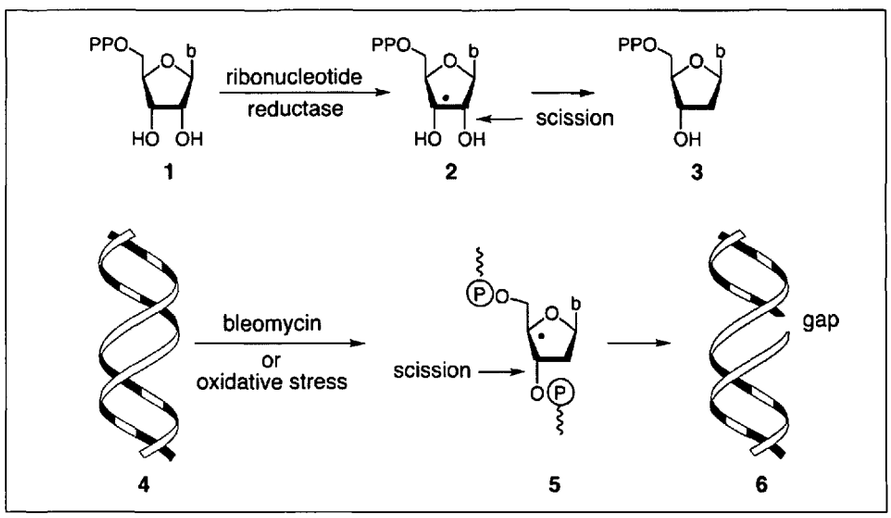
We have synthesized acyl selenide **11** and pivaloyl ketone **16** as precursors for the 3'-and 4'-nucleotide radicals, respectively [2][3] (Scheme 2).

From **11**, the 3'-radical **17** can be generated *via* tributyltin radicals, whereas **16** was first incorporated into double stranded **18**, which leads to the 4'-radical **19** upon photolysis (Scheme 3).

The chemistry of these radicals was studied in order to understand the action of the enzyme ribonucleotide reductase and of the antibiotic bleomycin.

3. Enzymatic Synthesis of Deoxyribose

It is known, especially from the experiments of J. Stubbe and W.A. van der Donck [4], that the first step of the enzymatic deoxygenation of ribonucleotides is the homolytic cleavage of their 3'-C,H-bond by a thiyl radical (**20**→**21**). This is a surprising reaction, because thiols are normally used to trap carbon-centered radicals, which corresponds to the reverse reaction **21**→**20**. Our model experiments have solved this apparent contra-



Scheme 1. Radicals as decisive reactive intermediates in the formation of deoxyribonucleotides and their destruction in the DNA.

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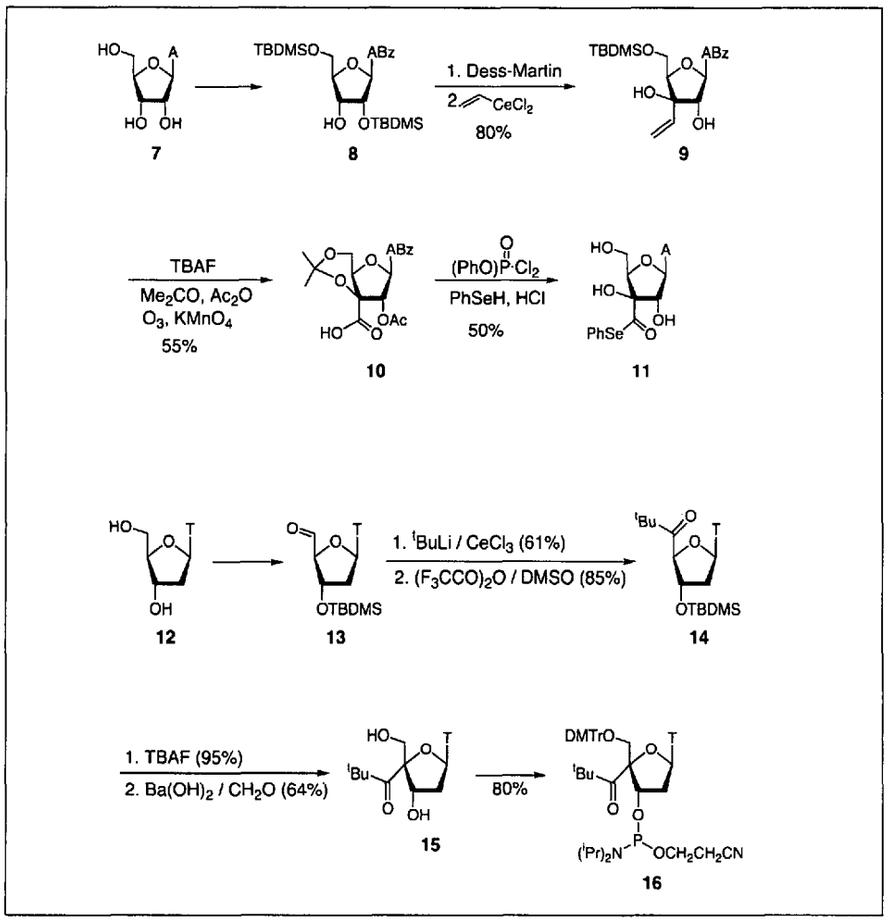
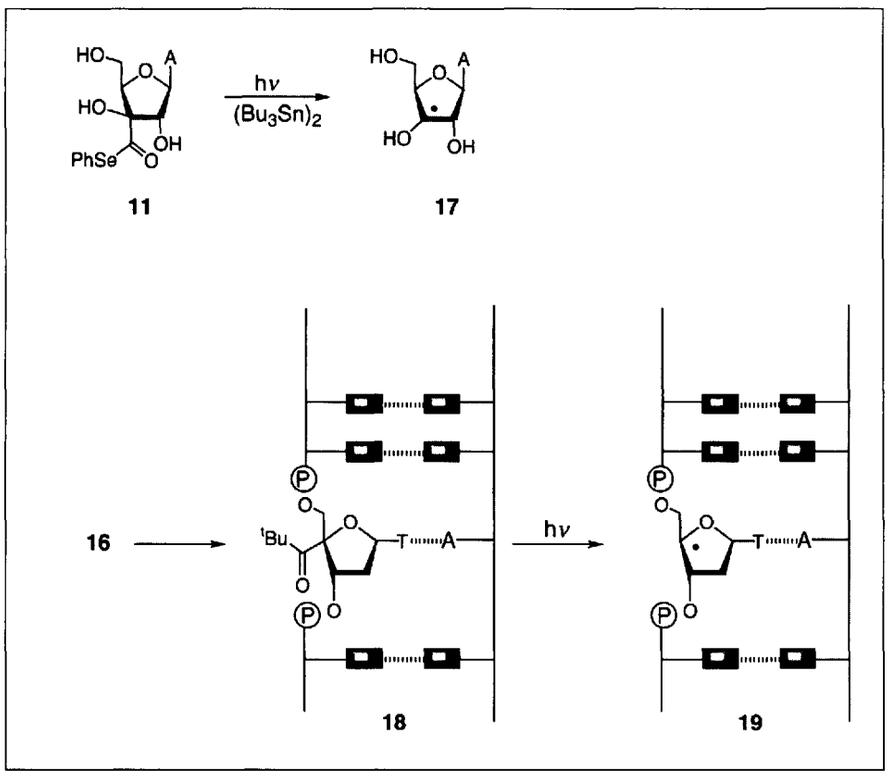


Scheme 2. Synthesis of the radical precursors **11** and **16**.



Scheme 3. Generation of the 3'-ribonucleoside radical **17** and the 4'-DNA radical **19**.

diction. It turned out that the deoxygenation of a radical like **21** is generally base catalyzed [2]. The slowest step of this process is the deprotonation (**21**→**22**), which is followed by an irreversible β-C,O-bond cleavage (**22**→**23**). As a result of these fast subsequent reaction steps even very small equilibrium concentrations of radical **21** can lead to an efficient deoxygenation reaction (Scheme 4).

The glutamic acid at position 441 plays the role of the general base catalyst in the enzyme. Its reaction leads to the protonated carboxylic acid in **23**, which subsequently catalyzes the reduction of ketone **24** [2]. Unprotonated ketones cannot be reduced by a disulfide radical anion (formed by the H-atom abstraction step **23**→**24**), but a reduction is easily feasible after protonation of the ketone. Thus, our model studies suggest an enzyme mechanism where acid/base catalysis is decisive for the chemical reactions (Scheme 5). Trapping of the 3'-radical **25** by cystein 439 completes the synthesis of the deoxyribonucleotide.

In support of these conclusions, X-ray studies of Uhlin and Eklund have shown that glutamic acid 441 is in an appropriate position to act as the acid/base catalyst [5] (Fig. 1).

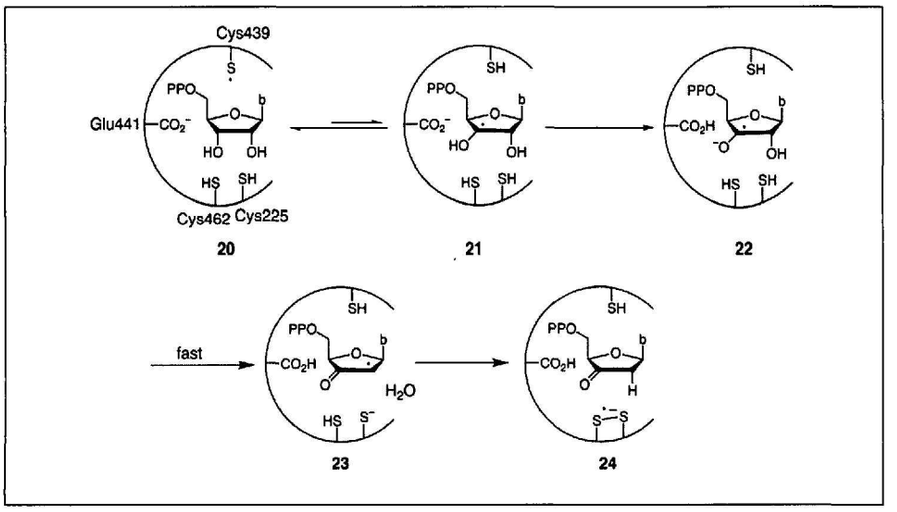
4. Radical Induced DNA Strand Cleavage

The antibiotic bleomycin generates 4'-DNA radical **19** after binding as a metal/oxygen complex to DNA and subsequent H-atom abstraction [6]. We have formed this 4'-nucleotide radical by photolysis of ketone **18** and studied its chemistry [7]. Depending upon the conditions, radical **19** leads to products **26–28** (Scheme 6).

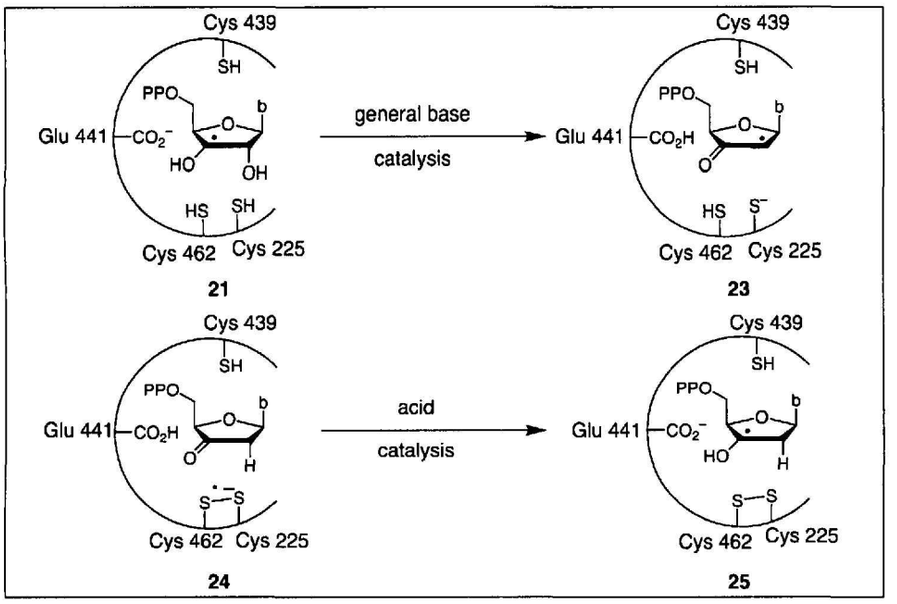
In the presence of metal oxides radical **19** is oxidized to the cation, which is trapped by water and yields ketoaldehyde **26** [8].

Aerobic conditions generate peroxyl radical **32** which leads to hydroperoxide **33** in the presence of H-donors [9]. If the hydroperoxide is situated within the DNA strand, a Criegee rearrangement occurs, whereas a hydroperoxide at the end of the strand (R^2 = H) undergoes a Grob fragmentation [9]. Subsequent elimination and/or hydrolysis leads to glycolate **27** (Scheme 7).

The chemically most interesting reaction is the O_2-independent cleavage of the 4'-DNA radical. In very detailed studies using kinetic experiments [10], chem-



Scheme 4. Schematic reaction scheme for the enzymatic deoxygenation of ribonucleotide **20**. The decisive amino acids are glutamic acid (position 441) and cystein (position 225, 439, 462).



Scheme 5. Base and acid catalyzed deoxygenation (**21**→**23**) and reduction (**24**→**25**) steps during the enzymatic synthesis of deoxyribonucleotides.

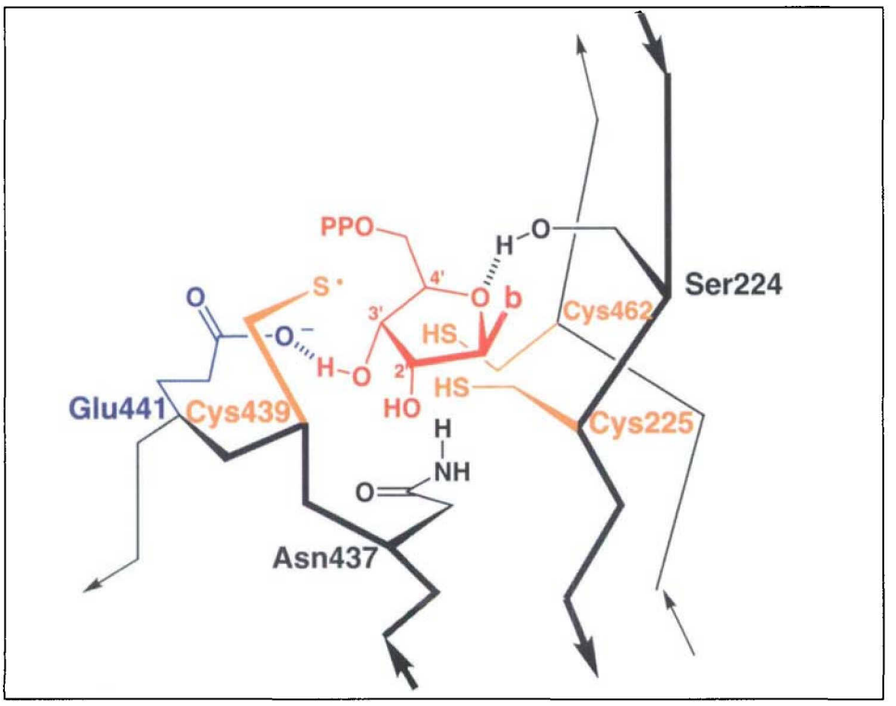
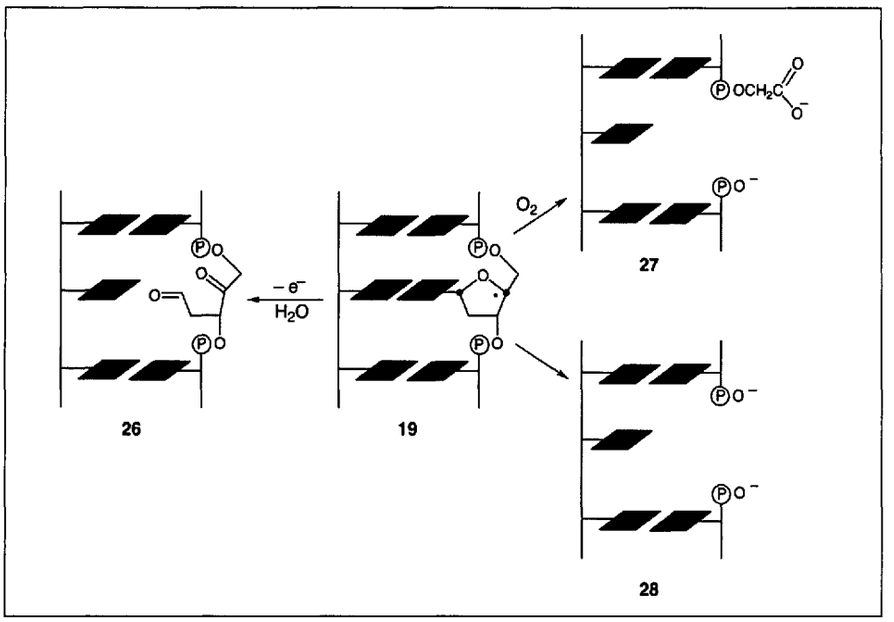
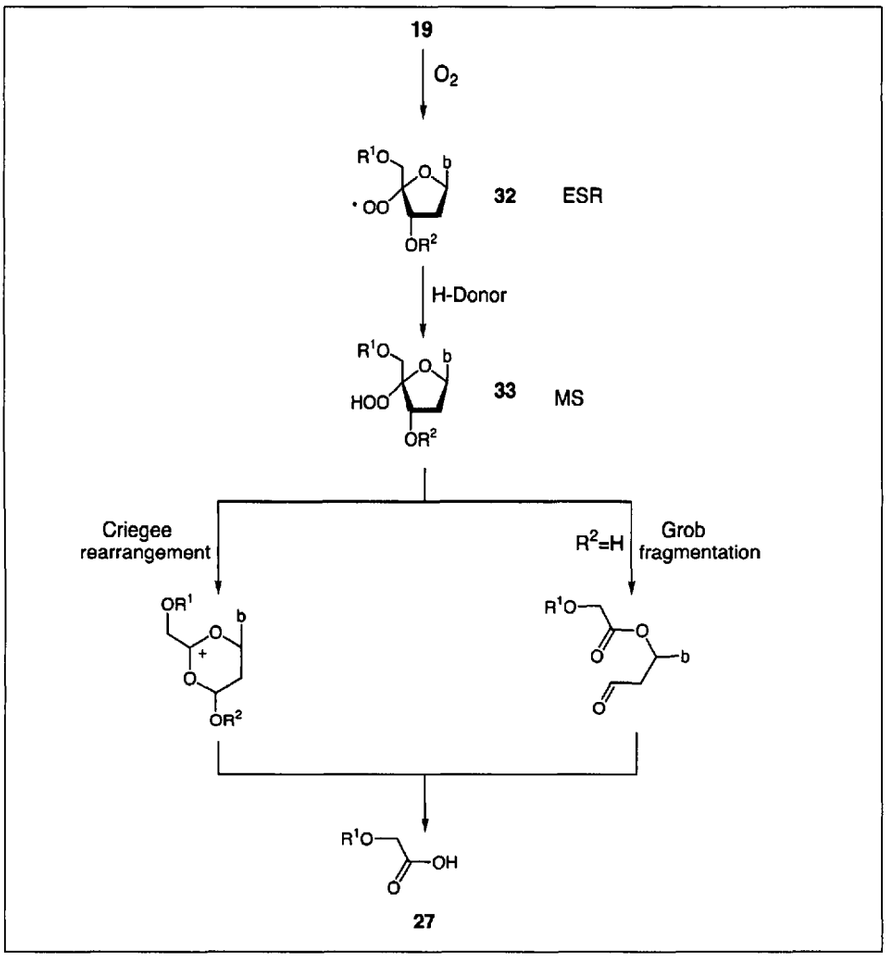


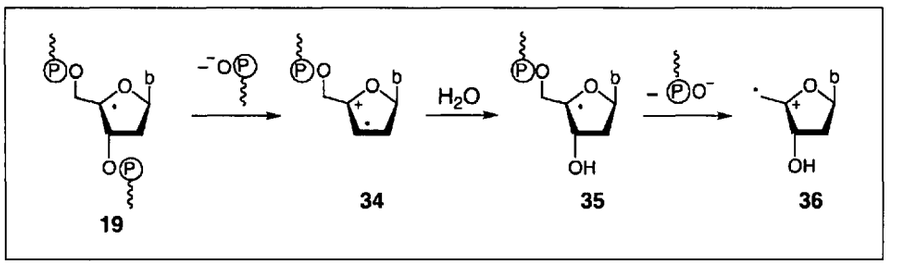
Fig. 1. Orientation of the ribonucleotide in the active site of ribonucleotide reductase.



Scheme 6. Reaction products of the 4'-DNA radical in the presence of oxidizing metal oxides (**19**→**26**), O_2 (**19**→**27**), or in the absence of radical traps (**19**→**28**).



Scheme 7. Mechanism for the formation of glycolate **27** from 4'-DNA radical **19** under aerobic conditions.



Scheme 8. Mechanism for the strand cleavage of 4'-DNA radical **19** under anaerobic conditions.

ical trapping reactions [10,11], photocurrent measurements [12], spectroscopic observations [13], as well as quantum chemical calculations [11], the existence of an enol ether radical cation **34** as crucial intermediate of this cleavage process was proven (Scheme 8).

The fast heterolytic cleavage reaction of radical **19** demonstrates that radical centers are very efficient neighboring groups for ionic reactions [14]. Radical **19** is hydrolyzed (**19**→**35**) at least 10^{10} times faster than the corresponding phosphate without a radical center at the 4'-position. The driving force is the mesomeric stabilization of radical cation **34** and the solvation energy [11]. We have utilized this neighboring group effect for the development of new photocleavable linkers [15].

Another spin-off is our observation that radical cation **34** oxidizes the guanine bases of DNA [16]. This induces long-distance charge transfer through DNA that we are studying in detail. We have been able to show that long-distance charge transport through DNA is possible and that it occurs in a multistep hopping process [17].

Strand cleavage of the 4'-DNA radical **19** can be prevented by H-atom donors. Interestingly, the H-atom comes mainly from the minor groove; therefore, we assume that the 4'-DNA radical has the conformation of a σ-radical **37** (Fig. 2), which has a lifetime of milliseconds in the absence of radical traps [18].

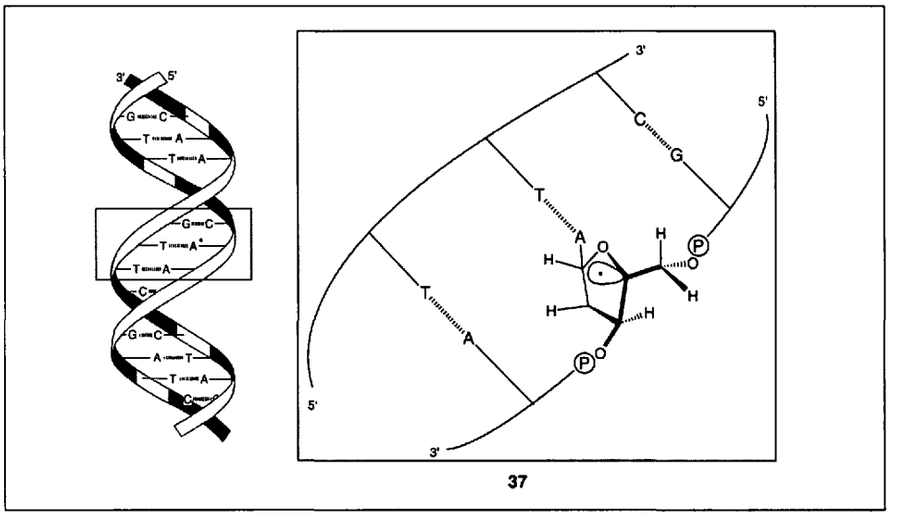


Fig. 2. Configuration of the 4'-DNA radical in double strands.

5. Enzymatic Synthesis of DNA

During the enzymatic polymerization of deoxyribonucleotides, the growing DNA strand is in close contact to specific areas of the enzymes, and we have studied this reaction using the 4'-substituted nucleotides, the precursors of the 4'-radicals (Fig. 3).

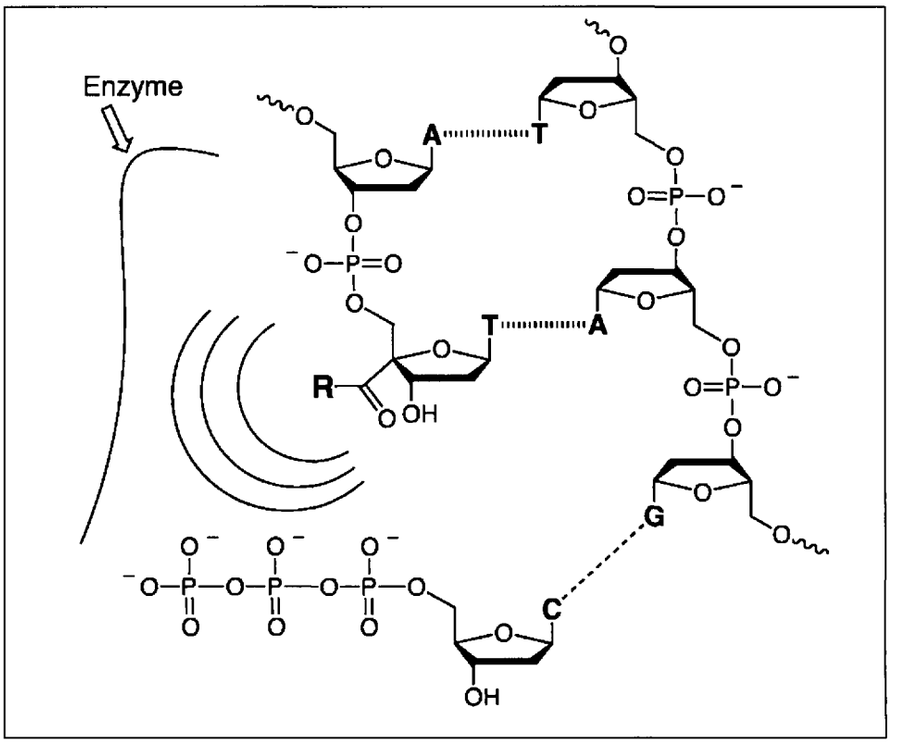


Fig. 3. Interaction between a 4'-acyl group at the nucleotide and the enzyme during DNA double strand synthesis.

It turns out that these modified nucleotides are excellent probes to determine the contact sites between the growing DNA strand and the enzyme. The rate of each polymerization step can be measured, and a site of close contact between enzyme and DNA is recognized by its slow chain elongation [19] (Fig. 4).

6. Conclusion

The language of chemistry uses molecules as its basic units and can help us to understand biological events. But, during these studies new insights into chemical reactions can also be gained. Examination of the radical-induced 'birth' and 'death' of DNA has led to the surprising chemical result that radicals are very efficient neighboring groups for ionic reactions.

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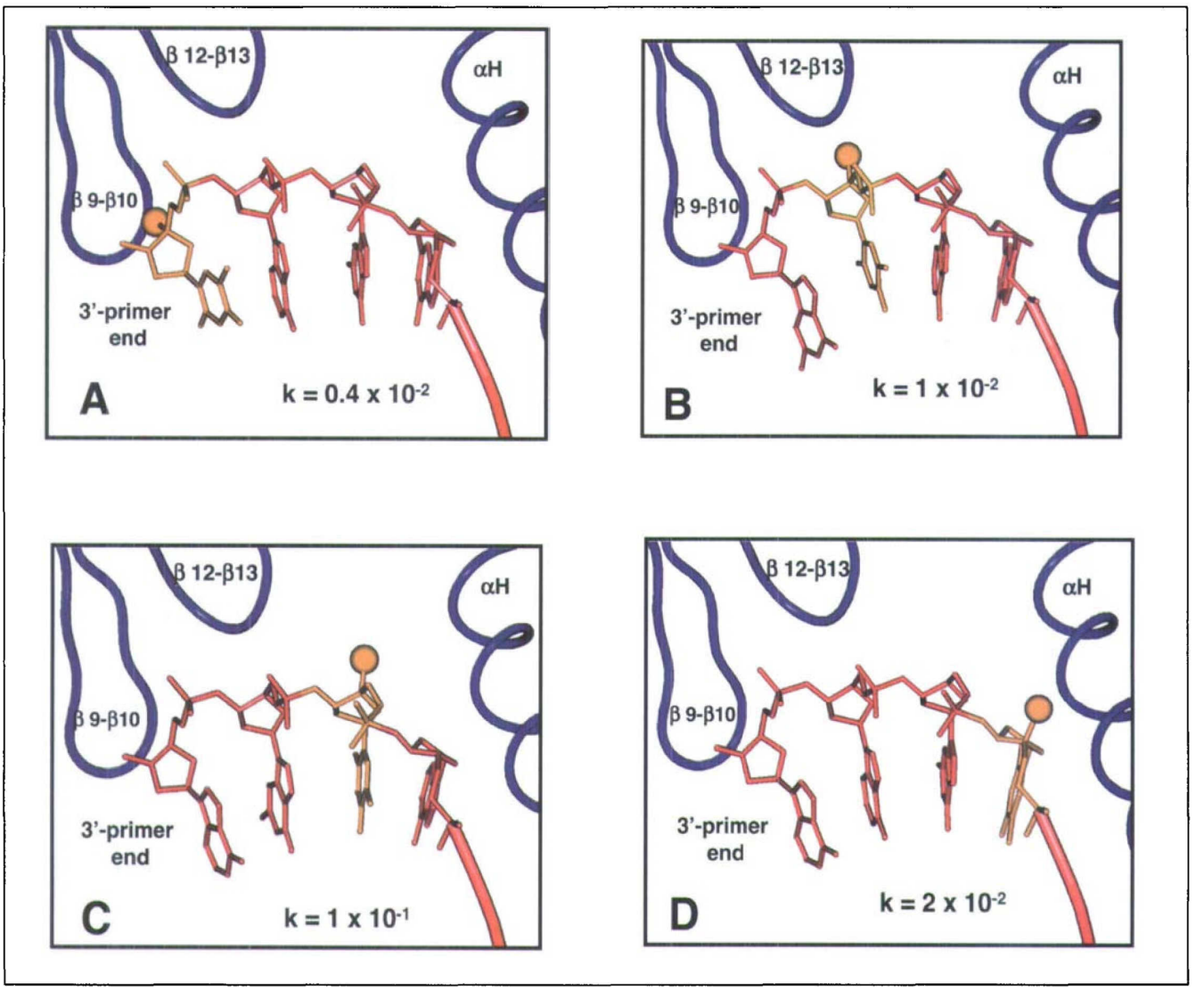

Fig. 4. Migration of the growing DNA strand (red), carrying a 4'-substituted nucleotide (yellow), through the active site of the enzyme (red). The different rates of the chain elongation steps show the different interactions between the substituent and the enzyme at the different positions.

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