

University of Canterbury

## End of Year Examination 2008

Prescription Number(s):	CHEM 405 BCHM 410
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Paper Title:	Bioorganic Chemistry
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Time Allowed: TWO HOURS

Number of pages: SIX

Answer **THREE** questions out of **FOUR**.

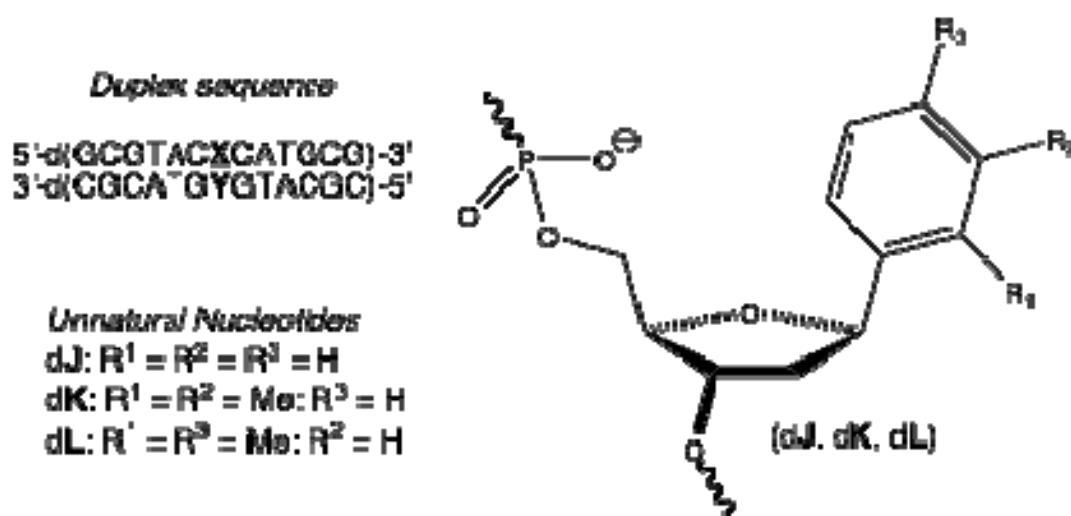
**TURN OVER**



1. Give a concise account of the way that the structural features of nucleotide bases affect the stability and selectivity of base-pairing in duplex DNA structures and how the factors involved can be investigated experimentally. Illustrate your account with specific examples, including nucleotides incorporating natural and synthetic bases.

Either as part of your account, or separately, comment on the melting temperature data,  $T_m$ , in the Table below concerning base-pair stability between oligonucleotides incorporating unnatural bases (from *J. Am. Chem. Soc.*, 2004, **126**, 14419).

Table:  $T_m$  values ( $^{\circ}\text{C}$ ) for selected synthetic oligonucleotide duplexes



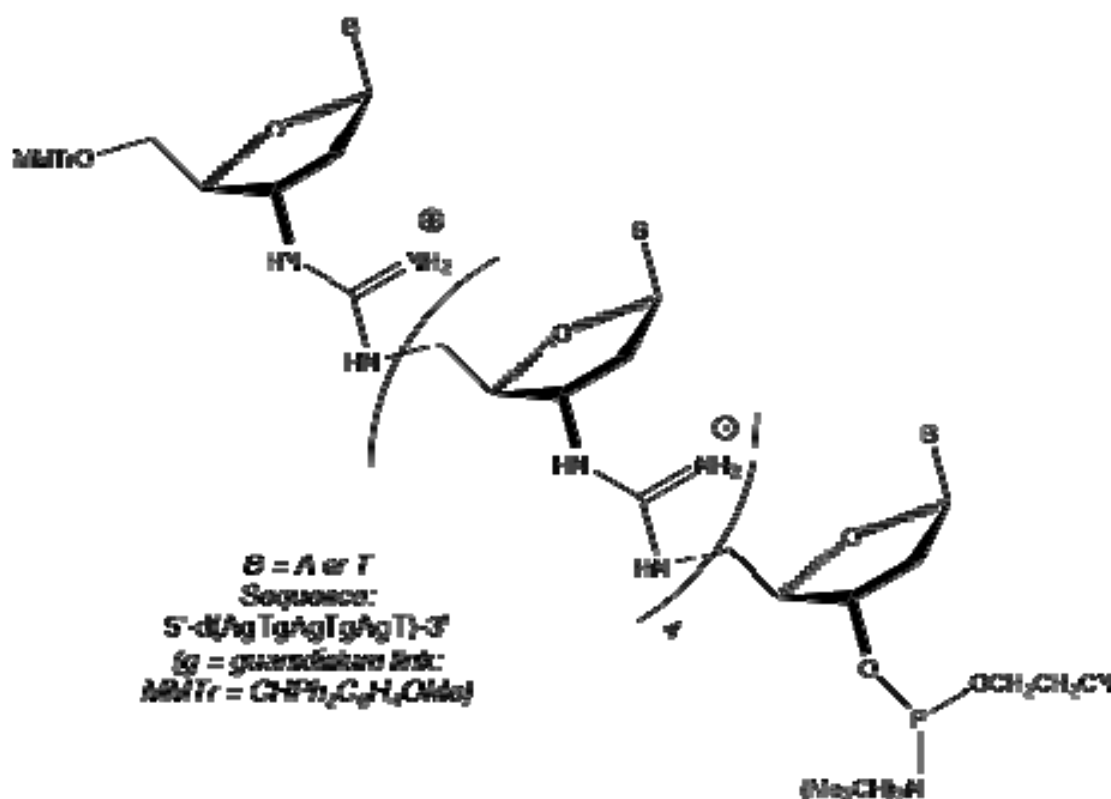
X	Y	$T_m$
dA	dT	59.2
dJ	dJ	52.8
dJ	dC	45.0
dJ	dG	49.1
dJ	dT	47.6
dJ	dA	49.2
dJ	dL	54.6
dK	dL	58.1
dL	dL	57.3

( $T_m$  values determined in 10 mM  $\text{MgCl}_2$ , 100 mM  $\text{NaCl}$ , pH 7.0)

**TURN OVER**

2. Briefly discuss the way in which automated DNA synthesis has been adapted to produce oligonucleotides incorporating analogues with modified sugar phosphates; and the information about the role of the sugar phosphate backbone in duplex formation that has been generated by these studies. Your account should be illustrated with specific examples.

A hexameric oligonucleotide analogue, 5'-d(AgTgAgTgAgT)-3', with guanidinium links has been prepared and incorporated into a larger oligonucleotide(I) (*Bioorg. Med. Chem. Lett.*, 2008, **18**, 3488). The melting temperatures,  $T_m$ , of duplexes of the target DNA sequence (III) with (I) and a DNA control (II) are given below. Either as part of your account, or separately, comment on this information and its significance.



Oligonucleotides evaluated (p = phosphodiester link; g = guanidinium link):

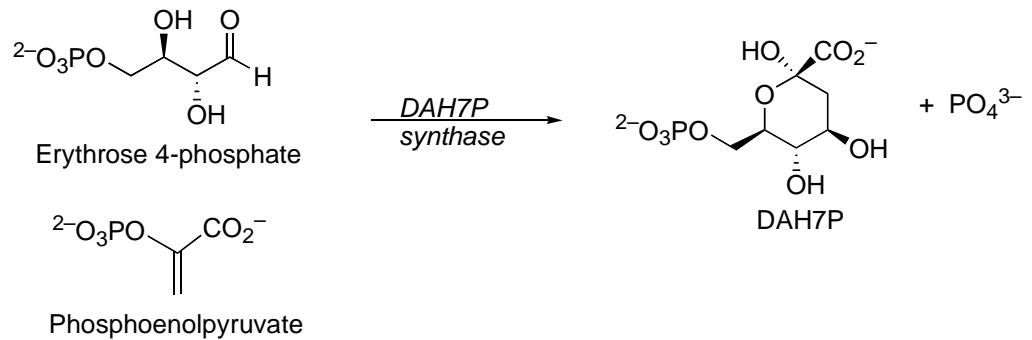
- 5'-d(CpGpCpApCpGpCpAgTgAgTgAgTpGpTpGpGpCpGpC)-3' I  
 5'-d(CpGpCpApCpGpCpApTpApTpApTpGpTpGpGpCpGpC)-3' II  
 3'-d(GpCpGpTpGpCpGpTpApTpApTpApCpApCpCpGpCpG)-5' III

Melting temperature data

Duplex I::III II::III

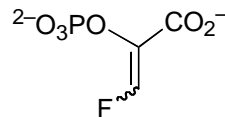
$T_m$ (°C) 75.0 48.3

3. 3-Deoxy-D-*arabino*-heptulose phosphate (DAH7P) synthase catalyses the reaction between phosphoenolpyruvate (PEP) and erythrose 4-phosphate (E4P) to form DAH7P.



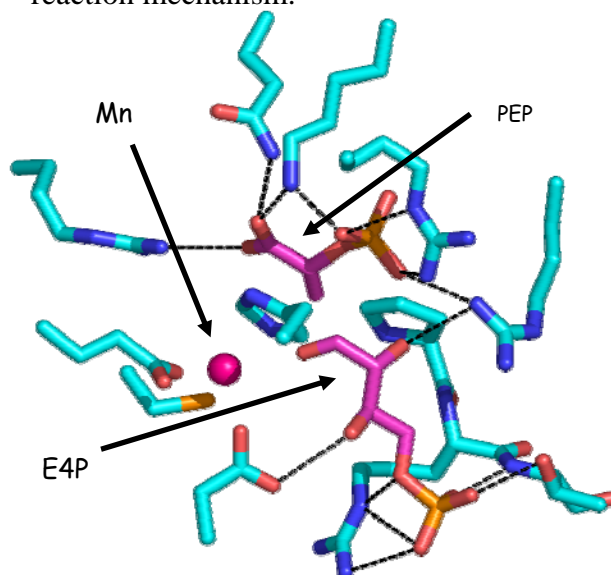
Outline the mechanism of this reaction, and use this mechanism and the figure below to discuss the following:

- The use of sequence alignments for identifying the key catalytic residues that are important for catalysis.
- How 3-fluorophosphoenolpyruvate was used as a substrate analogue for PEP to determine the facial selectivity of the enzyme-catalysed reaction.



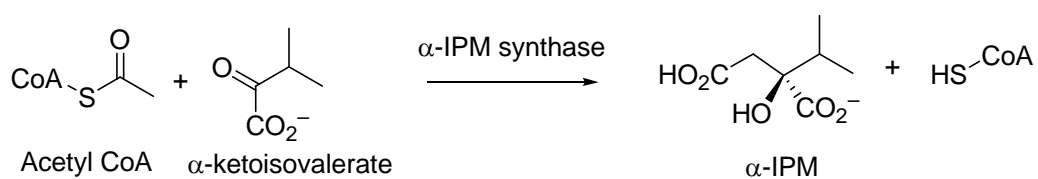
3-Fluorophosphoenolpyruvate

- How isotopic labelling studies can be used to help elucidate the enzymatic reaction mechanism.



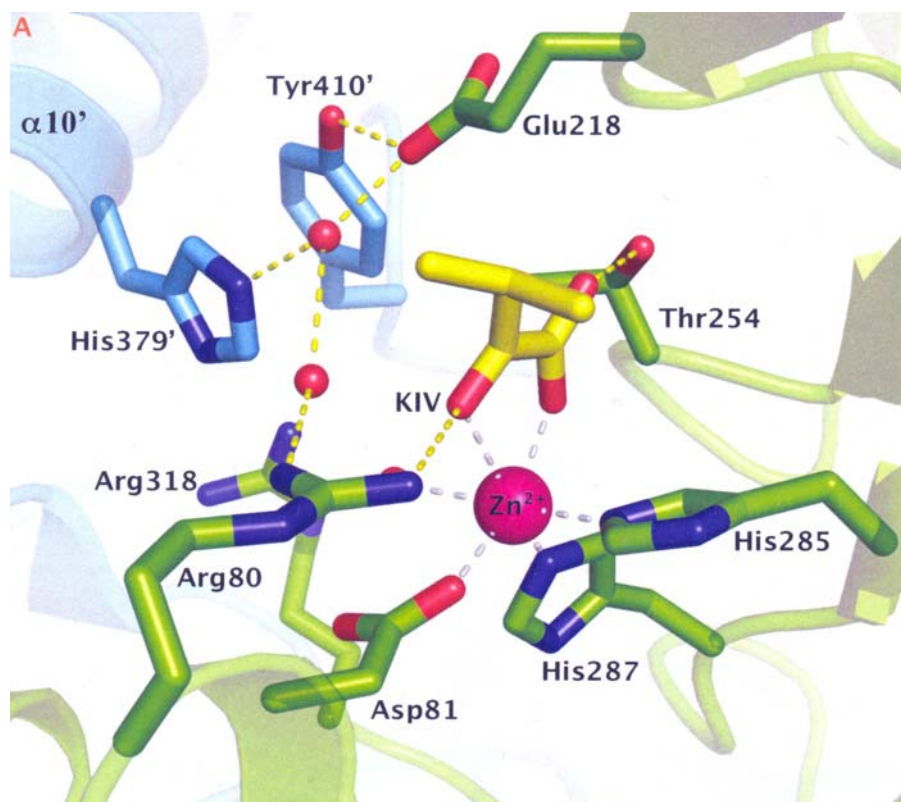
**Fig:** Active site of *Pyrococcus furiosus* DAH7P synthase

4. The first committed step in the formation of leucine is the reaction between  $\alpha$ -ketoisovalerate and acetyl CoA catalysed by  $\alpha$ -isopropyl malate ( $\alpha$ -IPM) synthase.



Outline the mechanism of this reaction, and use this mechanism and the figure below to discuss the following:

- The role of the metal ion in the enzyme-catalysed reaction mechanism.
- How determining the kinetic mechanism of the reaction can assist in studying the reaction mechanism.
- How determining the structure of the protein in complexes with substrates can help elucidate the reaction mechanism.



**Fig:** Active site of *Mycobacterium tuberculosis*  $\alpha$ -isopropylmalate synthase

**END OF PAPER**