

University of Canterbury

End of Year Examinations 2009

Prescription Number(s):	CHEM 405 BCHM 410
Paper Title:	Bio-organic Chemistry

Time Allowed: TWO HOURS

Number of pages: SIX

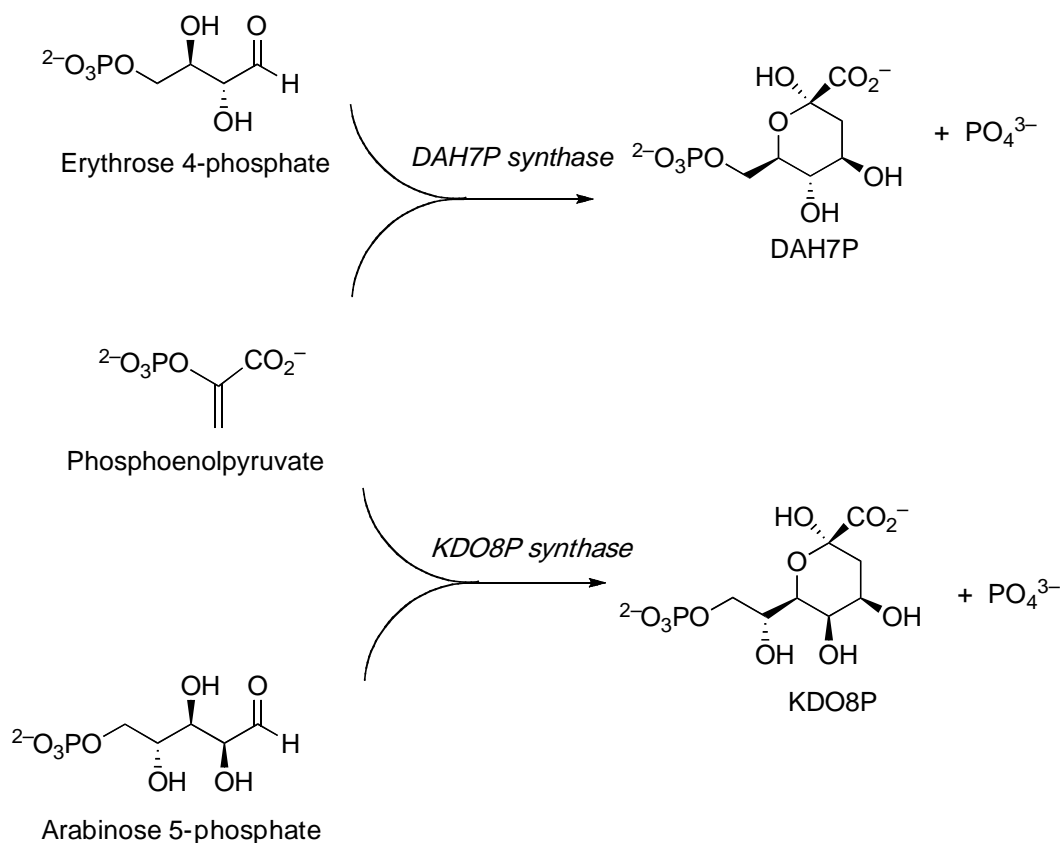
Answer **BOTH** questions.

Question 1 is worth **60%** of the total mark.

Question 2 is worth **40%** of the total marks

1. (This question is worth 60% of the total mark.)

3-Deoxy-D-*arabino*-heptulosonate 7-phosphate (DAH7P) synthase and 3-deoxy-D-*manno*-octulosonate 8-phosphate (KDO8P) synthase catalyse similar reactions in different biosynthetic pathways using a common substrate, phosphoenolpyruvate (PEP):



- (a) Outline the mechanism of the DAH7P synthase reaction, and use this mechanism to account for the absolute requirement for a divalent metal ion for enzymic activity.
- (b) Explain how using labelled PEP and PEP analogues has helped elucidate the mechanistic details and the reaction stereochemistry of the DAH7P synthase reaction.

Question 1 continued on following page

Question 1 continued on following page

- (c) The activity of most DAH7P synthases is controlled by the presence of aromatic amino acids, phenylalanine, tyrosine and tryptophan. Explain generally how this control of enzymic activity is mediated, and how different strategies are used by DAH7P synthases from different sources.

- (d) Explain how substrate specificity analysis of DAH7P synthase and KDO8P synthase with respect to the aldose phosphate substrate has been important for understanding the similarities and differences between the two enzyme-catalysed reactions.

- (e) Explain the debate over the identity of the nucleophilic water in KDO8P synthase. Discuss how resolution of this is important for a detailed understanding of the similarities and differences between KDO8P synthase and DAH7P synthase.

2. (This question is worth 40% of the total mark.)

Write a critical essay describing how synthetic nucleotide analogues have informed our understanding of the structure and function of nucleic acids. In your essay you should include a discussion of what we have learned about the structural features of nucleic acid duplexes that underpin their role as carriers of genetic information; how it is possible to create duplexes that are more stable than naturally occurring ds-DNA; and why this information is technologically useful.

Your account should be illustrated with a wide range of specific examples and include a detailed analysis of the information below (taken from *J. Amer. Chem. Soc.*, 2008, **130**, 4886).

Nucleoside analogue (**X**) (shown on the next page) was converted to a dimethoxytrityl-protected phosphoramidite derivative and incorporated into synthetic 2'-deoxy-oligonucleotides using an automated DNA synthesizer. Duplex formation and the stability of duplexes formed by modified oligonucleotides against complementary ssRNA and ssDNA were examined by UV melting experiments (T_m measurement), and the results were compared with those obtained with natural oligonucleotides. Representative results are shown in the **Table**.

Table:

Oligonucleotide	Target	T_m ($^{\circ}\text{C}$)
d(GCGTTTTTTGCT)	D	50
d(GCGTTXTTTGCT)	D	49
d(GCGXXXXXXXXGCT)	D	61
d(GCGTTTTTTGCT)	R1	45
d(GCGTTXTTTGCT)	R1	50
d(GCGTTXTTTGCT)	R2	37
d(GCGTTXTTTGCT)	R3	48
d(GCGTTXTTTGCT)	R4	34
d(GCGXXXXXXXXGCT)	R1	80

Target ssDNA: D = 5'-d(AGCAAAAACGC)-3'.

Target ssRNA: R1 – R4 = 5'-r(AGCAAAYAACGC)-3';

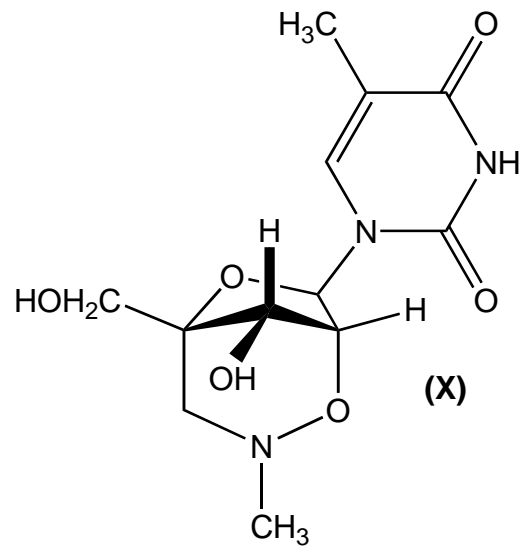
R1, Y = A; R2, Y = U; R3, Y = G; R4, Y = C

Conditions: 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl; strand concentration 4 μM .

Question 2 continued on following page

TURN OVER

Question 2 continued



END OF PAPER