Master's Thesis Engineering Technology

2019-2020

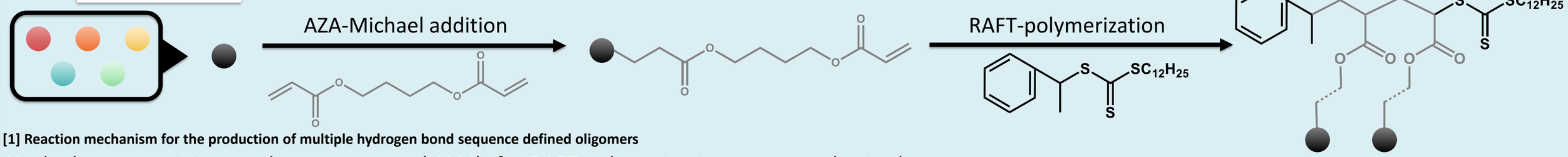
Base pairing analysis of nucleobase functionalized monomers

Pieterjan Broekx

Master of Chemical Engineering Technology

Introduction Selective base pairing between nucleobases, implemented into monomers or synthetic sequence defined oligomers, is a field of research that has remained fairly unexplored until recently. This master's thesis is among one of the first researches attempting to elucidate and characterize the base pairing of these DNA-building blocks, implemented into monomers and synthetic oligomers. Among various possible analysis techniques, regular ^{1}H-NMR and NOE-NMR were successfully used for this characterization.

Synthesis



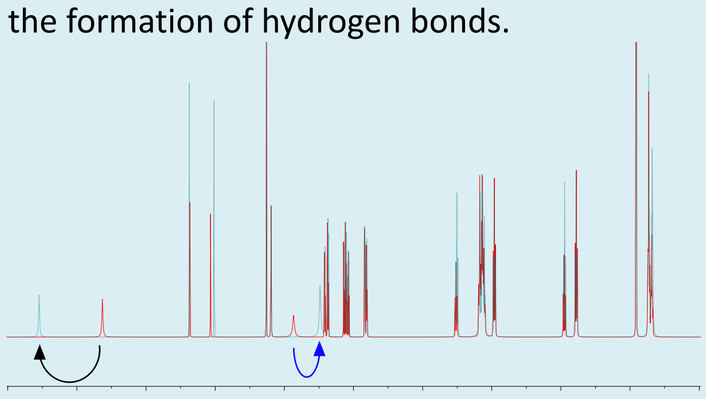
[1] Reaction mechanism for the production of multiple hydrogen bond sequence defined oligomers

Nucleobase containing acrylate monomers (NAM), for RAFT-polymerization, were synthesized by derivatization of the nucleobases with a diacrylate by means of aza-Michael addition. Only for guanine the Michael addition was performed on 2-amino-6-chloropurine followed by a nucleophilic substitution with $HCOOH/H_2O$. The sequence defined oligomers were synthesized by RAFT-Polymerization with 2-dodecyl-1-phenylethyl trithiocarbonate as chain transfer agent.

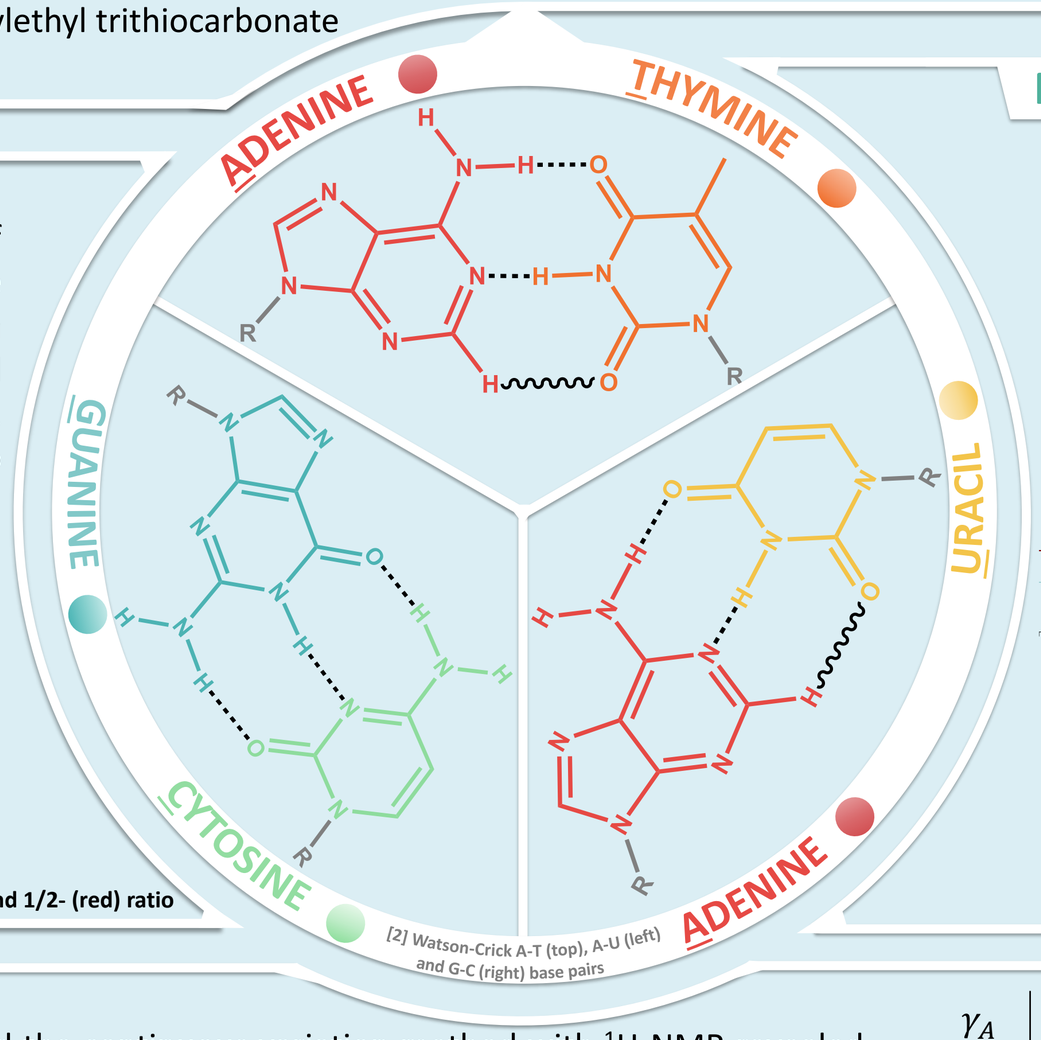
By tuning the [CTA]/[NAM]-ratio, oligomers containing one to four insertions were synthesized and isolated. An oligomer containing two NAM is a result of the procedure displayed above.

^{1}H-NMR

Regular ^{1}H-NMR enables examination of hydrogen bonding due to "deshielding". As the fraction of a proton participating in hydrogen bonding increases/decreases, the observed shift of this proton increases/decreases as well. Therefore, change in shift is directly related to the formation of hydrogen bonds.



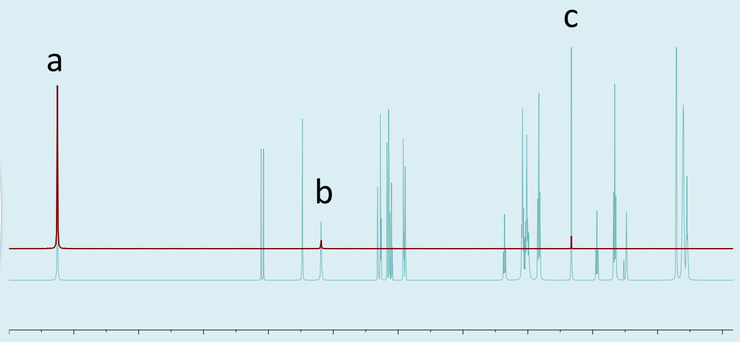
[3] ^{1}H-NMR spectrum of mixtures containing A and T in a 1/1- (blue) and 1/2- (red) ratio



[2] Watson-Crick A-T (top), A-U (left) and G-C (right) base pairs

NOE-NMR

Due to the nuclear Overhauser effect proximity of protons to other protons can be examined. 1D NOE experiments can therefore be used to examine which nucleobases form base pairs.



[4] ^{1}H-NMR spectrum (bleu) and NOE-spectrum (red) of a mixture containing AAM and TAM

This 1D NOE-spectrum proves that the proton of an adenine monomer (H*, a) is in close proximity to the proton of a thymine monomer (H*, b) when both are mixed in DMSO (c).

Results

Stoichiometry of base pairing → NOE-NMR and the continuous variation method with ^{1}H-NMR revealed that base pairing stoichiometry (X) in NAM is dependant on the concentration ratios (γ) of the NAM.

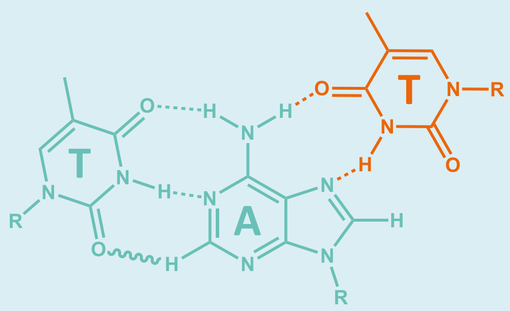
Temperature → Job plots revealed that the association constant is temperature-dependant. Due to the special stoichiometry of base pairing, a complex method is required to determine the actual K_a-values.

Solvent

→ NOE analysis revealed that a non-polar solvent causes selectivity in base pairing to seem absent. Increasing polarity implied increasing selectivity based on analysis of the deshielding effect.

→ NOE- and H^1-NMR analysis revealed that base pairing still occurs in the polar solvent DMSO.

γ_A	0.5	0.25
γ_T	0.5	0.75
X	1:1 T:A	1:3 T:A:T
	✓	✗



[5] Desired 1:1-stoichiometry (blue) and undesired 1:2-stoichiometry (orange + blue) base pairing

Conclusion In this research the expected 1:1-stoichiometry of base pairing has proven to be environmentally dependant. In order to obtain the desired base pairing between NAM or nucleobase containing oligomers, in the desired 1:1-stoichiometry, characterizing and optimizing influences of the following factors on base pairing will be crucial: temperature, solvent composition, concentration ratios and chain length.

Prospects By characterization and optimization of previous mentioned parameters, formation of Watson-Crick base pairs in a 1:1-stoichiometry might be promoted. When selectivity is obtained, template based polymerization and affinity separation can be examined. This could then resolve the current low yield problem of RAFT-polymerization of NAM. Finally, when this problem is resolved, application based research with nucleobase containing sequence defined oligomers can be executed.

Supervisors / Cosupervisors: Prof. dr. T. Junkers, Prof. dr. L. Thomassen, Prof. dr. J. Lievens, Drs. ing. L. Maes

PRD POLYMER REACTION DESIGN

UHASSELT

KU LEUVEN