FEROCENOLIRANJE CITOZINA I C5-SUPSTITUIRANIH URACILA: REAKTIVNOST I REGIOSELAKTIVNOST
FERROCENOLATION OF CYTOSINE AND C5-SUBSTITUTED URACILS: REACTIVITY AND REGIOSELECTIVITY

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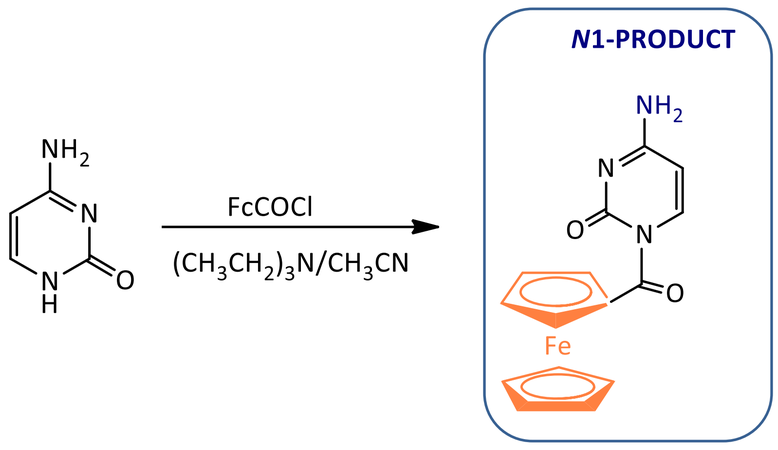
Introduction

Pyrimidine nucleobases, such as uracil and cytosine, are important building blocks of nucleic acids and valuable targets in medicinal chemistry. Ferrocene, owing to its unique redox properties, chemical stability, and biocompatibility, is a promising structural motif for the development of modified nucleobase derivatives. Previous studies established efficient conditions for the N-ferrocenoylation of uracil and C5-substituted uracil derivatives and showed that the electronic nature of C5 substituents significantly affects the reactivity and regioselectivity of uracil ferrocenoylation. [1, 2] The results further indicated that, in addition to the choice of solvent and deprotonating base, the electronic properties of the substituents play a crucial role in determining the reaction outcome, providing a basis for extending these studies to other C5-substituted uracil derivatives. [3, 4] In this study, ferrocenoylation of cytosine and 5-substituted uracil derivatives was investigated under previously optimized conditions, employing triethylamine as the deprotection base and acetonitrile as the reaction solvent, in order to evaluate the influence of electron-donating and electron-withdrawing C5 substituents on the reaction outcome and regioselectivity. The structures of the resulting products were elucidated by spectroscopic methods.

Ferrocenoylation of cytosine



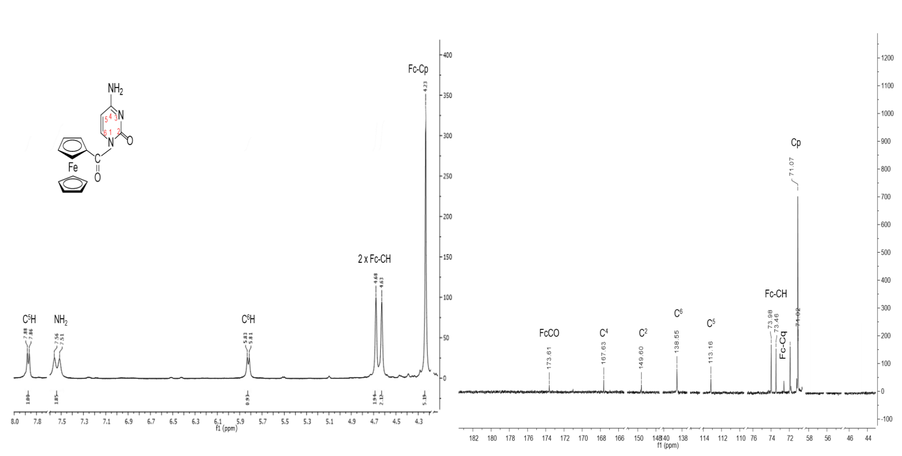
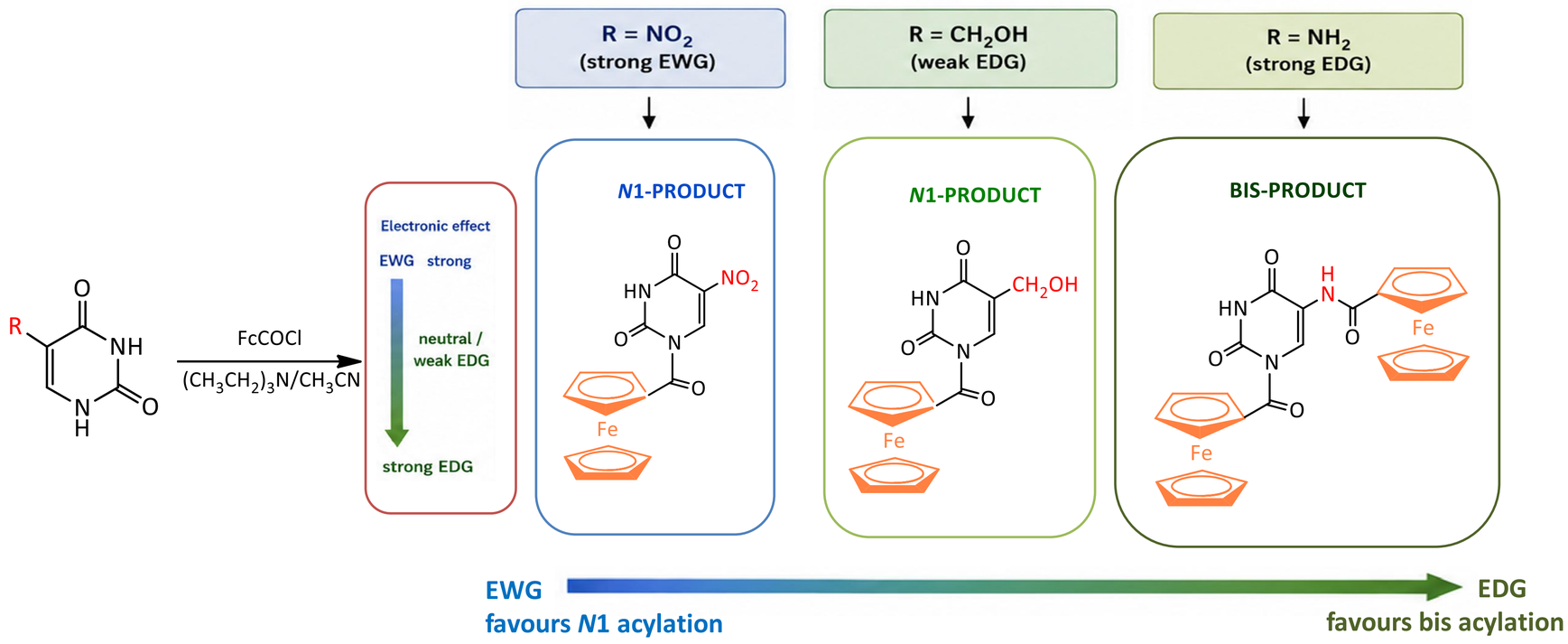
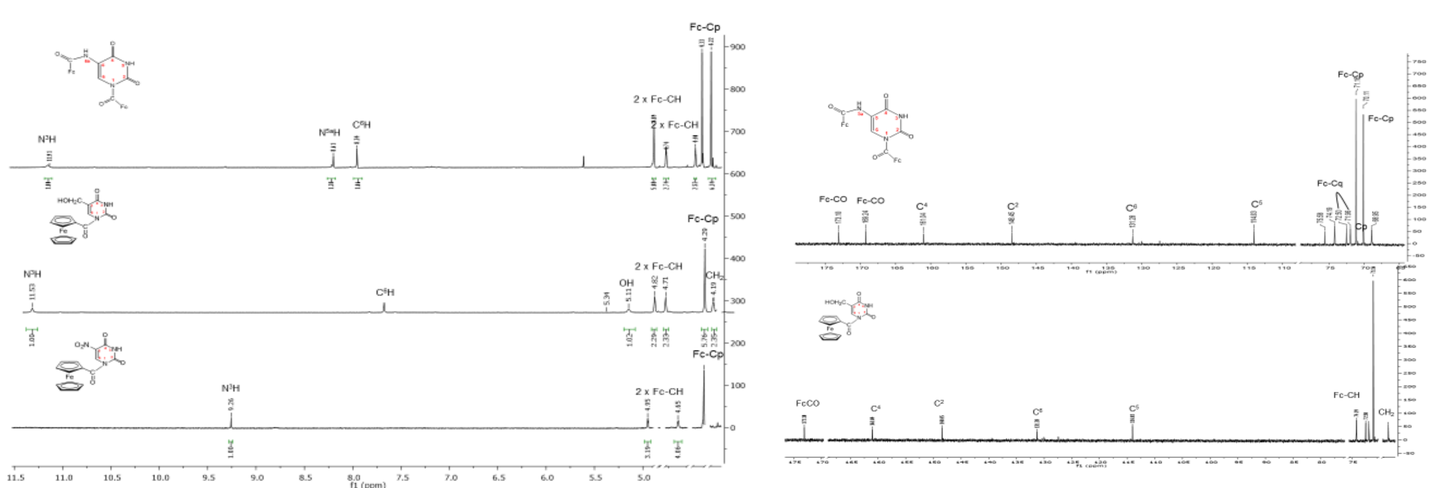


Figure 1. ^{1}H and ^{13}C NMR spectra of *N*1-ferrocenoylcytosine

- ✓ Under the optimized reaction conditions, *N*1-ferrocenoylcytosine is formed exclusively as the sole reaction product.
- ✓ The highest yield of the bioconjugate was achieved with deprotection at 50 °C, followed by the coupling reaction at 0 °C.

Effect of C5-Substituted Uracil Derivatives on the Outcome of Ferrocene Acylation





- ✓ The difference in reactivity between uracil and its 5-substituted derivatives is attributed to the electronic effects of the substituents.
- ✓ EWG or weak EDG groups (NO_2, CH_2OH) favour *N*1-products.
- ✓ The strong EDG group (NH_2) promotes additional functionalization of the exocyclic amino group, resulting in bis-products.
- ✓ Based on the chemical shifts in the ^{1}H and ^{13}C NMR spectra, the product structures were confirmed: *N*1-products contain one ferrocene unit attached at *N*1, while bis-product contain two ferrocene units attached to *N*1 and the exocyclic NH_2 group at C5

Figure 2. ^{1}H and ^{13}C NMR spectra of *N*1-products and *N*1/*N*5-bisproduct

Conclusions

- ➢ Cytosine undergoes preferential *N*1-ferrocenoylation under the investigated reaction conditions, with the optimized conditions affording exclusively the *N*1-ferrocenoylated product. The different reactivity of cytosine compared with uracil may be attributed to its lower acidity (pK_a 12.2 vs 9.5), which makes deprotonation more difficult.
- ➢ Base, solvent, and temperature are key to the efficient and selective ferrocenoylation of cytosine and require further optimisation.
- ➢ Ferrocenoylation of 5-substituted uracils is strongly influenced by the electronic nature of the C5 substituent, which affects both the reactivity and regioselectivity of the reaction.
- ➢ Electron-withdrawing NO_2 and CH_2OH substituent selectively afford *N*1-ferrocenoylated products under all investigated reaction conditions, indicating a pronounced preference for acylation at the *N*1-position.
- ➢ In contrast, the electron-donating NH_2 substituent promotes additional acylation, leading to bis-ferrocenoylation with ferrocenoyl groups introduced at the *N*1-position and at the exocyclic amino group.
- ➢ These results demonstrate that increasing electron-donating character of the C5 substituent promotes additional acylation and significantly affects the regioselectivity of ferrocenoylation.
- ➢ Spectroscopic analysis, including NMR and IR spectroscopy, confirmed the structures of thebioconjugates obtained and unambiguously established the ferrocenoylation sites.

References

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