寄稿論文

Furan Photo-Oxidation Based Click Reactions: Labeling, Crosslinking and Conjugation of Peptides and Oligonucleotides under Physiological Conditions

Annemieke Madder

Organic and Biomimetic Chemistry Research Group,
Department of Organic and Macromolecular Chemistry, Ghent University, Belgium

Keywords: Furan oxidation, Photosensitizer, Oligonucleotides, DNA to Protein crosslinking, Peptide labeling

Site-specific chemical modification of proteins and nucleic acids is crucial for understanding protein and nucleic acid structure and interactions as well as providing insights into cellular events. We here report on a simple, efficient and versatile procedure for furan-oxidation mediated modification of biomolecules. Furan derivatives are commercially available under a variety of forms thus allowing straightforward incorporation of the furan moiety during solid phase synthesis.[1] Once incorporated into a peptide or nucleic acid, furan can be considered a caged electrophile which can be uncovered by selective activation through oxidation (Figure 1).

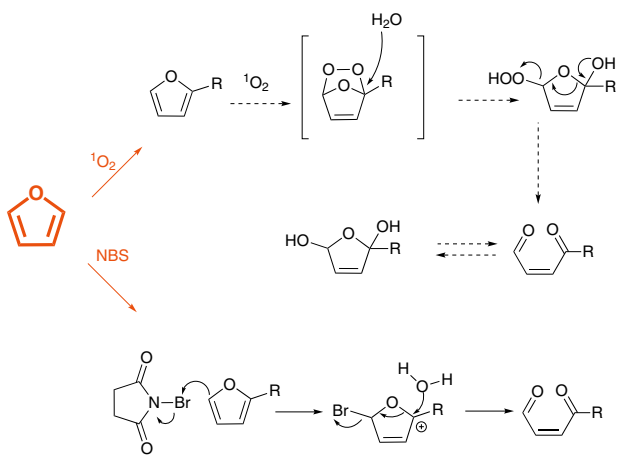


Figure 1. Furan oxidation by NBS or singlet oxygen treatment

Furan-containing peptides and oligonucleotides can be subjected to mild oxidative conditions (NBS or air/light/photosensitiser) so that the reactive electrophilic species are generated. These reactive intermediates can be intercepted by various nucleophiles to form stable conjugates.

Scope:

I. Furan-mediated Nucleic Acid Interstrand Crosslinking[2]

Easily accessible furan modified nucleosides, a commercially available photosensitizer, and visible light irradiation constitute the necessary tools to achieve selective duplex interstrand cross-linking of furan modified oligonucleotides. The crosslink mechanism involves formation of a covalent link through reaction with exocyclic amino functionalities at the corresponding position in the complementary strand (Figure 2). Both DNA[3] as well as RNA targets[4] can be covalently trapped in this way using furan-modified oligonucleotide probes.

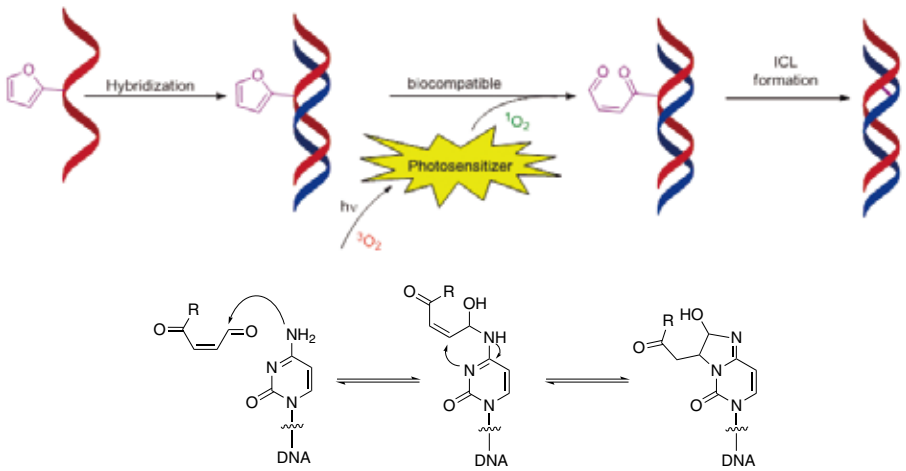


Figure 2. Principle and mechanism of furan-oxidation mediated interstrand crosslinking

II. DNA to Protein Crosslinking

The methodology has also been further extended for its bidirectional use in protein – nucleic acid cross-linking, modifying protein or nucleic acid respectively to react with the unmodified counterpart as target (Figure 3).[5] As the cross-link reaction is highly distance dependent, mapping of interaction surfaces is within reach. Understanding the remarkable selectivity and affinity of nucleic acid binding proteins for their targets (DNA and RNA) in the complex cellular environment, which is central in the regulation and execution of biological processes, is a major challenge driven by the ambition to design novel drugs that can compete in such interactions.

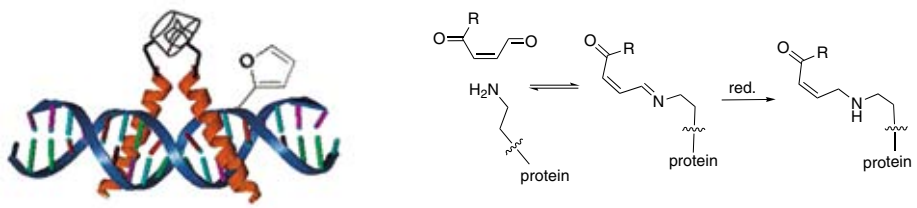


Figure 3. Principle and mechanism of furan-oxidation mediated DNA to protein crosslinking

III. Solid Phase-based Peptide Labeling

Furan-oxidation was also used as a method for the labeling of solid-phase bound peptides by generation of a reactive aldehyde in the sequence. Hereby, the non-natural *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-3-(2-furyl)-L-alanine can be incorporated into a peptide and subsequently converted into a 4-oxo-enal moiety by selective oxidation of the furan moiety. Next, a simple reductive amination allows introducing the desired fluorophore.[6] The incorporated furan moiety further enables another orthogonal strategy for highly selective labeling. The furan diene has shown to be a useful partner in Diels–Alder reactions with commercially available maleimides as dienophile. The more recently described 1,2,4-triazole-3,5-diones present an excellent alternative.[7]

IV. Singlet Oxygen ($^1O_2$) Mediated Furan-based Peptide Labeling in Physiological Aqueous Solutions

Completely deprotected furan-containing peptides, following selective furan-oxidation in aqueous solution can also be intercepted by α-effect nucleophiles (such as hydrazine derivatives of dyes or fluorescent labels) to form stable conjugates. Incorporation of nucleophilic fluorophores through a cascade reaction sequence, leads to the efficient construction of site-selectively labeled fluorescent peptides.[8] This reaction can be used for the site specific labeling of peptides and proteins and can be carried out in aqueous solution.

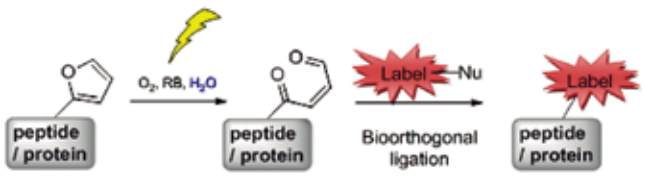


Figure 4. Furan-based peptide labeling strategy initiated by singlet oxygen

References

[1] *N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-3-(2-furyl)-L-alanine, TCI product number F1013; 2-Furylboronic Acid, TCI product number F0394; 3-(2-Furyl)acrylic Acid, TCI product number F0084.

[2] L. L. G. Carrette, E. Gyssels, A. Madder, in *Current Protocols in Nucleic Acid Chemistry*, John Wiley & Sons, New York, **2013**, 54: 5.12.1.

[3] a) K. Stevens, A. Madder, *Nucleic Acids Res.* **2009**, *37*, 1555. b) M. Op de Beeck, A. Madder, *J. Am. Chem. Soc.* **2011**, *133*, 796. c) M. Op de Beeck, A. Madder, *J. Am. Chem. Soc.* **2012**, *134*, 10737.

[4] L. L. G. Carrette, E. Gyssels, J. Loncke, A. Madder, *Org. Biomol. Chem.* **2014**, *12*, 931.

[5] L. L. G. Carrette, T. Morii, A. Madder, *Bioconjugate Chem.* **2013**, *24*, 2008.

[6] A. Deceuninck, A. Madder, *Chem. Commun.* **2009**, 340.

[7] K. Hoogewijs, D. Buyst, J. M. Winne, J. C. Martins, A. Madder, *Chem. Commun.* **2013**, *49*, 2927.

[8] E. Antonatou, K. Hoogewijs, D. Kalaitzakis, A. Baudot, G. Vassilikogiannakis, A. Madder, *Chem. Eur. J.* **2016**, *22*, 8457. DOI: http://dx.doi.org/10.1002/chem.201601113.

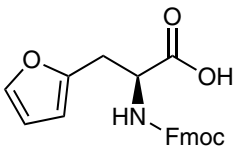
執筆者紹介

Annemieke Madder
Prof. Dr.

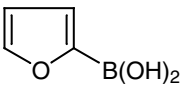
Organic and Biomimetic Chemistry Research Group, Department of Organic and Macromolecular Chemistry, Ghent University

Annemieke Madder started chemistry studies at Ghent University in 1988. After undergraduate thesis work at the University of Santiago de Compostela in Spain, she graduated in June 1992. In February 1997, she obtained her Ph.D. in organic chemistry from Ghent University. After postdoctoral stays in the laboratory of Prof. Dr. C. Gennari at the University of Milan and in the research group of Prof. Dr. R. Strömberg at the Karolinska Institute in Stockholm, she returned to Ghent and obtained a position as a Lecturer in 2002. In 2014, she was promoted to a Full Professor at the Department of Organic and Macromolecular Chemistry. Currently she is heading the Organic and Biomimetic Chemistry Research Group specialized in the design and synthesis of modified peptides and nucleic acids and methods for their conjugation and labeling.

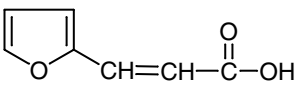
TCI 関連製品



N-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-3-(2-furyl)-L-alanine
200mg, 1g
[F1013]



2-Furylboronic Acid
1g, 5g
[F0394]



3-(2-Furyl)acrylic Acid
10g, 25g
[F0084]