Investigation of Molecular Recognition Processes of Glycopeptides and Peptides using Photoreactive alkaloids

Ion Neda[1,3], Catalin V. Maftei[1,3], Elena Maftei[1,3], M. Heiko Franz[2,3], Corina Macarie[3], Ionel Balcu[3]

[1] Institut für Anorganische und Analytische Chemie, Technische Universität Carola Wilhelmina, Hagenring 30, D-38106 Braunschweig, Germany;
[2] InnoChemTech GmbH, Hagenring 30, D-38106 Braunschweig, Germany;
[3] Institutul National de Cercetare Dezvoltare pentru Electrochimie si Materie Condensata Str. Dr. A. Paunescu Podeanu Nr. 144, Ro-300569 Timisoara, Romania.
* franz@innochemtech.de, tel.: 0531 391 5391

Introduction

Photorective peptides are used in photoaffinity labeling to study biomolecular interactions. Photoreactive peptides are peptides modified with a photophore, a photolabile group. Irradiation of these peptides generates a highly reactive intermediate, usually a carbene or nitrene, which will react with an acceptor molecule to form an intermolecular covalent bond [1].

[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]arenes were introduced as photophores by Brunner et al. [2] to provide a more favourable carbene than the one produced from diazoesters or from aryldiazirines.

In our study we are interested in using a modified enantiomeric pure quinine-based alkaloid fragment, Quincorine, with a [3-(Trifluoromethyl)-3*H*-diazirin-3-yl]arenes photoreactive motif and see if the selectivity is preserved. Lindel *et al*. investigated the chemical selectivity [3]. They found that for Tyr-Val-dipeptide the *O*-benzylation of the phenol-moiety is favoured against *C*-benzylation of it or other benzylations (Fig. 1).

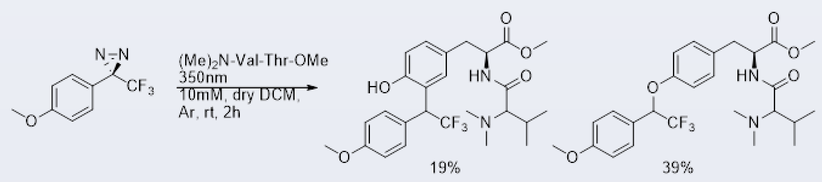


Figure 1. Moving on to biological motifs

General idea of photoaffinity labeling of a ligand-contact site

The idea of photoaffinity labelling is that a small molecule carrying a photophore binds to a macromolecule, e.g. pepetide. After the small photophore coordinates to the contact side the solution is irridated and the photophore generated a covalent bond. After cleavage of the macromolecule the unit can by identified at which photophore coordinates (Fig 2).

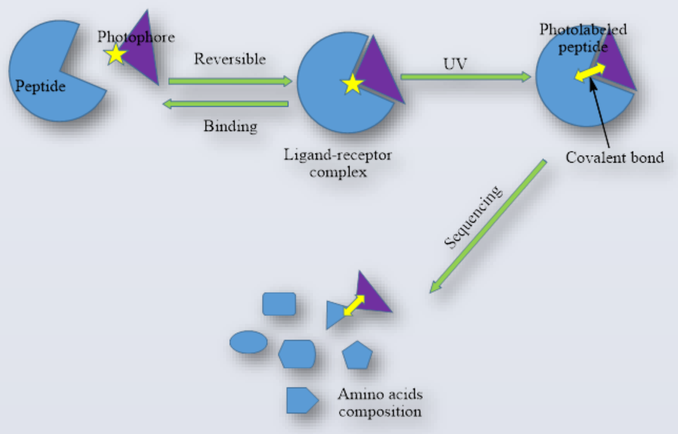
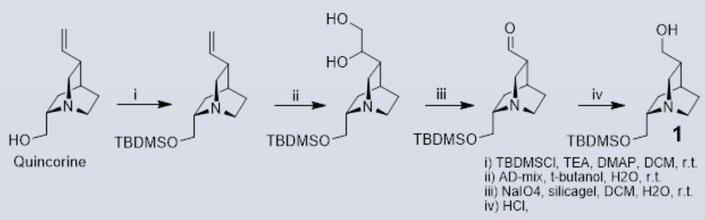


Figure 2. Illustration of photoaffinity labeling and identification

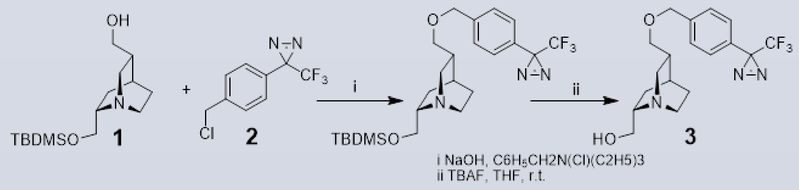
a. Synthesis of the functionalised QCI with [3-(Trifluoromethyl)-3*H*-diazirin-3-yl]arenes

The coordinating side of Quincorine (QCI) is the tertiary amine in combination with the hydroxy-group. The double bond should be used to connect the photophore. Therefore the hydroxyl group has to be protected first. Afterwards was the double bond transferred in a second hydroxyl group. The modified QCI **1** was coupled to the [3-(Trifluoromethyl)-3*H*-diazirin-3-yl]arene **2** (Scheme 1) [4].

1. Synthesis of protected QCI



2. Introduction of the photophore

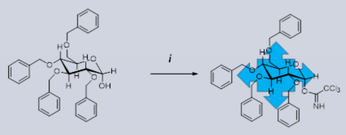


Scheme 1. Modification of QCI 1 and coupling with diazirine 2

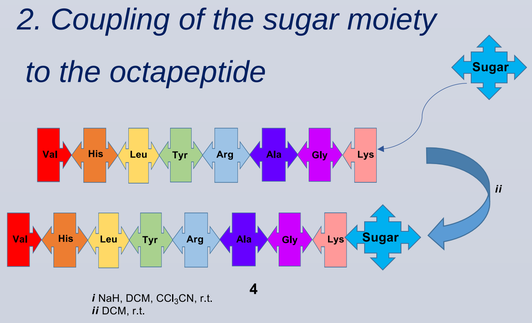
b. Synthesis of the peptide

As target we choose the glucopeptide **4**. This was synthesised starting from the octapeptide Val-His-Leu-Tyr-Arg-Ala-Gly-Lys (scheme 2) [5][6].

1. Activation of the Tetrabenzyl-glucose



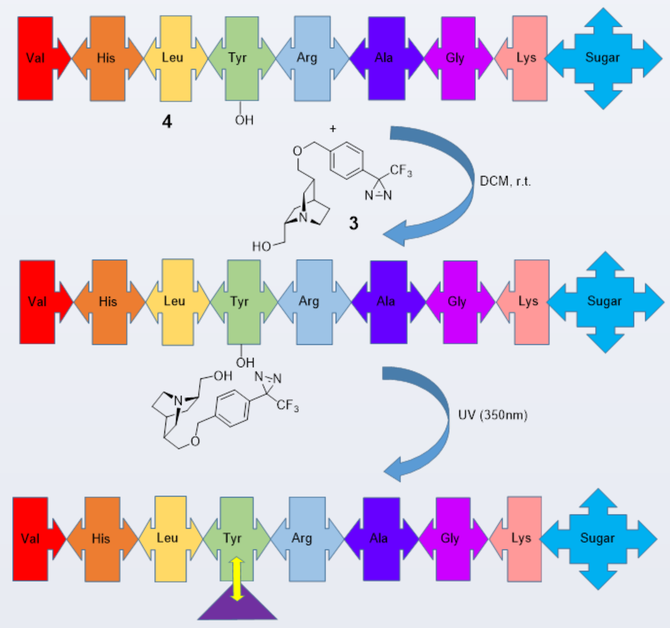
2. Coupling of the sugar moiety to the octapeptide



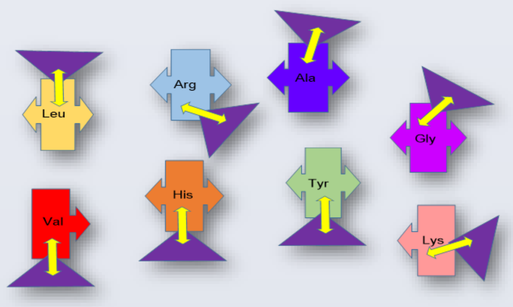
Scheme 2. Synthesis of glucopeptide 4

c. Site-specific incorporation of the functionalised QCI with [3-(Trifluoromethyl)-3*H*-diazirin-3-yl]arenes into peptide

The glucopeptide was dissolved together with the QCI-photophore in Dichloromethane and stirred for 30 min. After that time the solution was irridated with UV-light (350nm).



d. Fragments identification



The glucopeptide linked covalent to the photophore was cleaved in the single amino acids and the supposed amino acids linked to the photophore. This mixture was analysed by mass spectroscopy (electro spray; ESI-MS). The calculated masses for the amino acids linked to the former diazirin are given in table 1. The measured masses were compared with this masses [7].

Figure 3. Amino acids composition

Results

Fragment	*Calculated Mass*	*Found Mass*	*Intensity*
	[g/mol]	[u]	
Val-Linker-QCI	458,2392	459,2462	trace
His-Linker-QCI	496,2279	497,2367	micro
Leu-Linker-QCI	472,2549	473,2619	trace
Tyr-Linker-QCI	522,2342	523,2411	major
Arg-Linker-QCI	515,2719	516,2790	trace
Ala-Linker-QCI	430,2079	-	n.d.
Gly-Linker-QCI	416,1923	-	n.d.
Lys-Linker-QCI	487,2658	488,2729	trace
Sugar	683,1608	-	n.d.

One of the main results is that that the “Tyr-Linker-QCI” fragment has the major signal. Further signals related with Tyr have been detected: the Na^+-adduct ($M+Na^+$ = 545,2234), one dipeptide (Leu-Tyr-Linker-QCI; $M'+H^+$ = 636,3255) and the dimer ($(Tyr(Linker\text{-}QCI)_2$; $2*M+H^+$ = 1027,4650). Also no signals of coupling with the sugar unit have been not observed.

Table 1. Calculated and measured masses of amino acid-linker-QCI derivatives

Conclusions

The functionalised QCI with [3-(Trifluoromethyl)-3*H*-diazirin-3-yl]arenes, used as a photophore, under UV showed prebonding to the structure of Tyrosine unit. That means that it can be used for targeting, a method complementary with other approaches of the structural biology. In this way new strategies can be opened for drug discovery and development

References

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[7] For comparison we synthesised Tyr-”Linker”-QCI, Arg-”Linker”-QCI, Lys-”Linker”-QCI and His-”Linker”-QCI. A equimolar mixture gave similar intensities for the substances.

Acknowledgements

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