Introduction
Photoreactive 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].

In our study we are interested in using a modified enantiomeric pure quinine-based alkaid fragment. Quincone, with a [3-(Trifluoromethyl)-3H-diazirin-3-yl]aren is photoreactive motif and see if the selectivity is preserved. Lindel et al. investigated the chemical selectivity [2]. They found that for Tyr-Val-dipeptide the C- benzylation of the phenol-moiety is favored against C-benzylation of it or other benzylations (Fig. 1).

General idea of photoaffinity labeling of a ligand-contact site
The idea of photoaffinity labeling is that a small molecule carrying a photophore binds to a macromolecule, e.g. peptide. After the small photophore coordinates to the contact side the solution is irradiated and the photophore generated a covalent bond. After cleavage of the macromolecule the unit can be identified at which photophore coordinates (Fig 2).

Figure 2. Illustration of photoaffinity labeling and identification

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

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

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

Results

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

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Calculated Mass</th>
<th>Found Mass</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val-Linker-QCI</td>
<td>458.2392</td>
<td>459.2462</td>
<td>trace</td>
</tr>
<tr>
<td>His-Linker-QCI</td>
<td>496.2379</td>
<td>497.2367</td>
<td>micro</td>
</tr>
<tr>
<td>Leu-Linker-QCI</td>
<td>472.2349</td>
<td>473.2319</td>
<td>trace</td>
</tr>
<tr>
<td>Tyr-Linker-QCI</td>
<td>533.2341</td>
<td>533.2341</td>
<td>major</td>
</tr>
<tr>
<td>Arg-Linker-QCI</td>
<td>515.2379</td>
<td>516.2370</td>
<td>trace</td>
</tr>
<tr>
<td>Ala-Linker-QCI</td>
<td>430.2379</td>
<td>-</td>
<td>n.d.</td>
</tr>
<tr>
<td>Lys-Linker-QCI</td>
<td>487.2058</td>
<td>488.2729</td>
<td>trace</td>
</tr>
<tr>
<td>Sugar</td>
<td>683.1608</td>
<td>-</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

One of the main results is 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+= 636.3458) and the dimer (Tyr-Linker-QCi2; M+2H+= 1027.4600). Also no signals of coupling with the sugar unit have been observed.

Conclusions
The functionalised QCI with [3-(Trifluoromethyl)-3H-diazirin-3-yl]aren, 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
[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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