DEVELOPMENT OF BIOCONJUGATES AND THEIR MODUL CONSTRUCTS FOR TARGETED THERAPY OF CANCERS WITH HIGH MORTALITY

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Synthesis of drug-peptide conjugates using bifunctional spacers

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Introduction

The main challenge of the drug delivery concept in cancer therapy is to transport a sufficient amount of the cytotoxic agent to the diseased site(s) while minimizing their exposure to healthy tissues. To achieve this, one main strategy is the application of peptide – drug conjugates (PDCs).[1]

PDCs are nowadays an emerging class of prodrugs, formed through the covalent attachment of a specific peptide sequence to a drug *via* linker(s). PDCs usually consist of a cytotoxic agent (drug), a tumor homing peptide (carrier and targeting moiety) and a labile or enzyme cleavable linker between them. The utilization of peptides in drug delivery systems has many advantages, as they allow for the incorporation of a great degree of functionality into PDCs (*e.g.* improving solubility, tumor selectivity, special metabolism). This versatility would allow an arbitrary combination of targeting peptides, linkers and drug molecules to develop personalized cancer therapeutics upon selecting a tumor homing peptide that will be most appropriate for the type of cancer needed.[2] In addition, peptide sequences can be selected according to the required physicochemical properties or the characteristic groups necessary for the conjugation with the therapeutic payload. It follows, however, that the different conjugation methods and functional groups used to form PDCs from the three building blocks (carrier, linker and drug), needs to be compatible with each other.

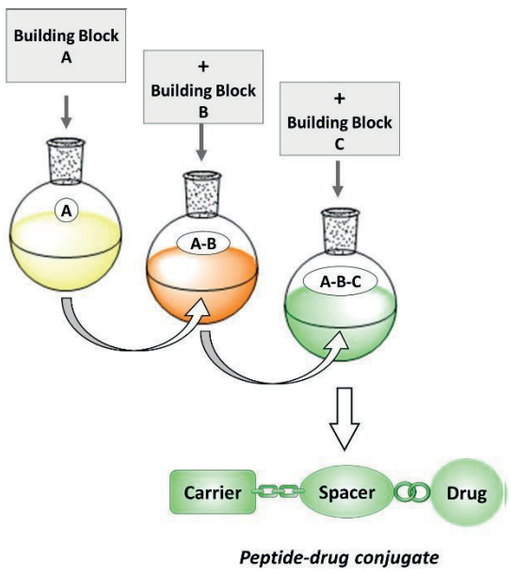

Figure 1. Sequential one-pot reaction

To this end, we set up a model system in which the side-by-side applicability of the most commonly used conjugation reactions can be investigated. Our main focus was to develop so-

called “sequential one-pot” combinations, as this would be the most efficient way to build PDC libraries (Figure 1).

Our model carrier was GnRH-III (<EHWSHDWKPG-NH_2), a well-known tumor homing peptide.[3] GnRH-III also gave us the advantage of examining the conjugation steps in the presence of delicate amino acids, as the peptide contains oxidation and alkylation sensitive histidine and tryptophan. For linker, the GFLG tetrapeptide has been chosen, as a widely used Cathepsin B sensitive spacer,[4] which is also poorly water soluble, thus, the problem of sparingly soluble sequences also had to be addressed. Two clinically used chemotherapeutic drugs were selected as our model drug molecules: daunomycin (Dau) and methotrexate (Mtx) as they allow a different kind of conjugation.[5,6]

For the selection of the conjugation techniques, three aspects were considered: it must occur frequently in the literature, must be compatible with each other and should be easily performed with minimal laboratory background. Thus, the oxime-ligation, thioether- and peptide-bond formations were chosen.

Results

Two different derivatives of GnRH-III were prepared, modified on ε-amino group of ^{3}Lys with chloroacetic acid or acetylcysteine (Figure 2, *C1* and *C2*), which allowed the formation of thioether bond with the spacers containing maleimidohexanoic acid (Mal-Hx) (*S5, S7*), chloroacetyl (Cl-Ac) group (*S4, S6*) or cysteine (*S1, S2, S3*), accordingly.

The conjugation to the drug molecules could happen *via* amide (*Mtx* and *Dau1*) or oxime bond formation (*Dau2*), therefore the spacer sequences contained a free amine (^{3}Lys side chain: *S4, S5*) or succinic acid (Suc) (*S1*) or aminooxyacetic acid (Aoa) (*S4, S5, S6, S7*). This palette of compounds allowed us to study and optimize the sequential one-pot formation of PDCs not only in the order of carrier + spacer→ carrier-spacer+ drug, but also the other way around, spacer + drug→ spacer-drug + carrier. As the aminooxy-functional group is sensitive to acylation, the combination of oxime and amide conjugation methods were not studied. Therefore, two other combinations were investigated in depth.

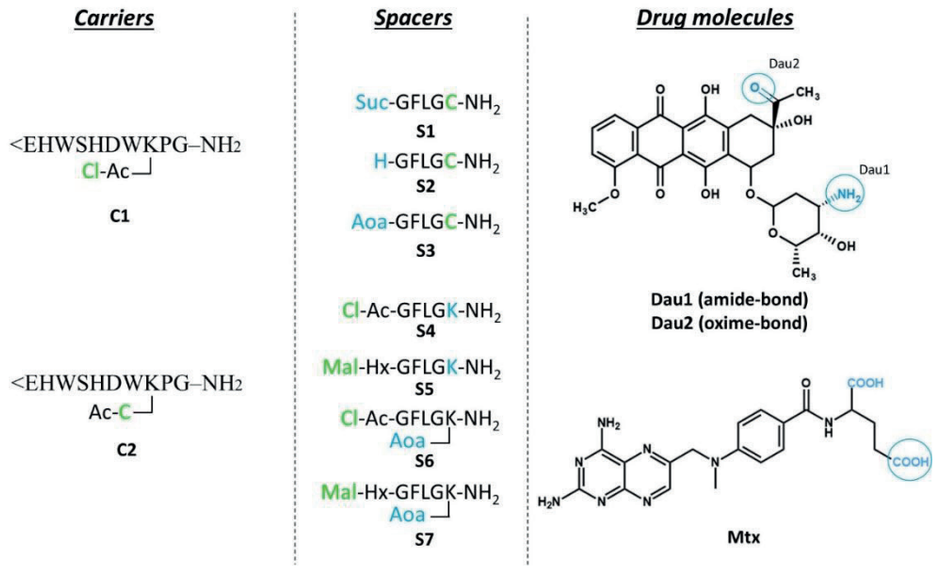

Figure 2. Prepared carrier and spacer molecules and the conjugated chemotherapeutic drugs

Compatibility of amide- and thioether-bond

The peptide-bond formation between the drugs and spacers were carried out in solution, in the presence of PyBOP and DIPEA. Under this alkaline conditions maleimido and chloroacetyl groups, and also daunomycin quickly degraded, therefore it was concluded that ***S1-Dau1, S4-Mtx, S5-Mtx*** cannot be synthesised.

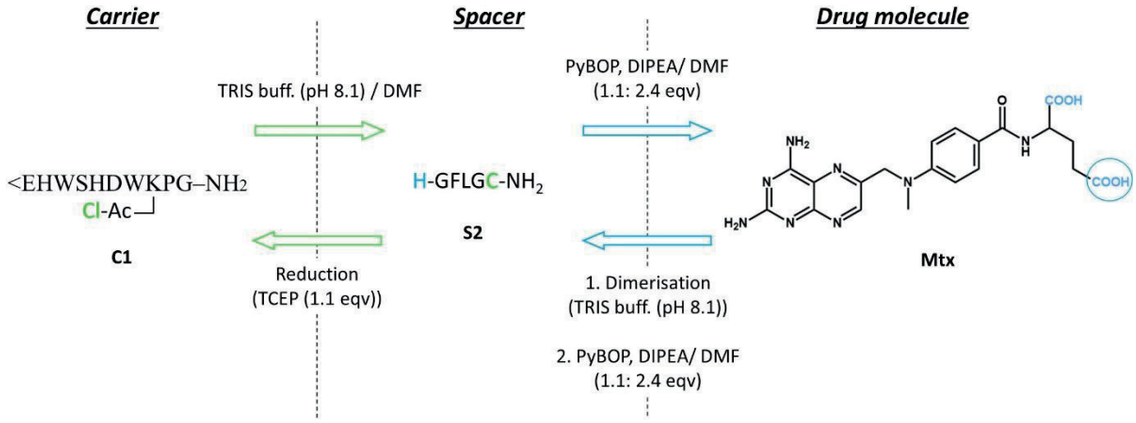

Figure 3. Sequential amide and thioether bond formation

Nevertheless, the two conjugation types could be compatible with each other, even as the first step is the amide bond formation (*Figure 3*). As *S*-acylation is a more reactive pathway compared to *N*-acylation the free thiol group of the **S2** spacer had to be masked

temporarily by dimerization through a disulfide bridge. This was followed by the peptide bond formation with Mtx (***Mtx-S2-S2-Mtx***) and after the reduction of the disulfide bridge with TCEP, the thioether bonded product could be readily synthesised (***C1-S2-Mtx***). Our experimental results also confirmed that a less complicated pathway is when the first conjugation step is the thioether formation, followed by the peptide bond (***C1+S2→ C1-S2+Mtx → C1-S2-Mtx***; Figure 3).

Compatibility of oxime and thioether bond

It can be said that the two types of conjugation (oxime and thioether) methods are compatible, unfortunately, as it turned out, our model system was not entirely suitable to investigate it in depth. Thioether formation requires alkaline media and the reaction usually takes 16 h to be totally completed.

Under these conditions, as it was earlier already mentioned, daunomycin quickly decomposes in minutes. Therefore, in our case, sequential one-pot reactions could be carried out only if the first conjugation step is the thioether-bond formation. Under such circumstances **C1-S3-Dau2**, **C2-S6-Dau2** and **C2-S7-Dau2** were effortlessly synthesised in aqueous media only by setting the pH (Figure 4).

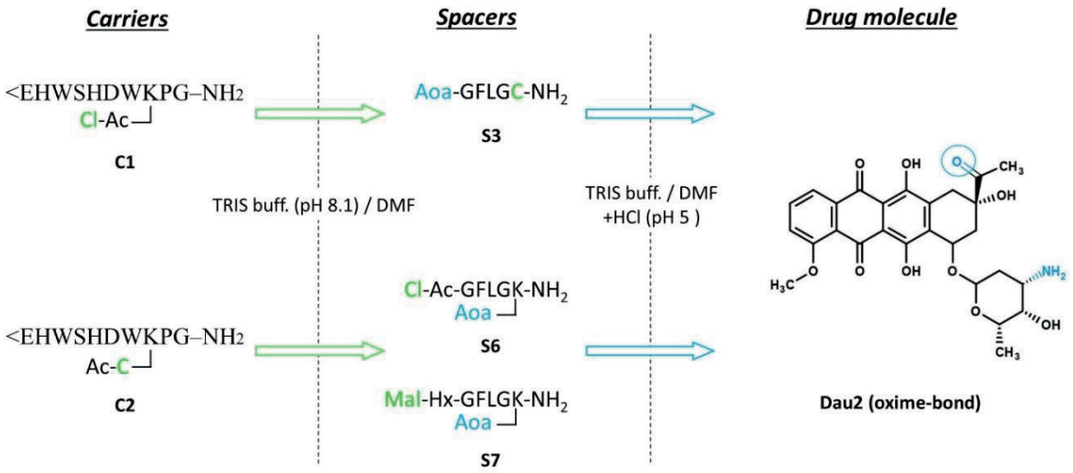

Figure 4. Sequential oxime and thioether bond formation

In summary, our investigation showed that oxime-thioether and amide-thioether conjugations can be used effectively and easily in sequential order for the development of PDC libraries. Further plans include the investigation of other conjugation methods (*e.g.* click-reaction) and their compatibility with each other.

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