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A novel molecular platform for co-delivery of monomethyl auristatin E and ispinesib to prostate cancer cells

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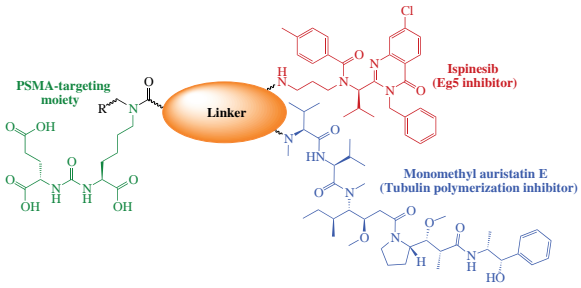
A conjugate for co-delivery of ispinesib and monomethyl auristatin E was prepared using methods of peptide synthesis and azide–alkyne cycloaddition. Cytotoxicity studies of the mentioned drug pair were performed. *In vitro* studies of the synthesized bimodal conjugate were conducted on prostate cancer cell lines.



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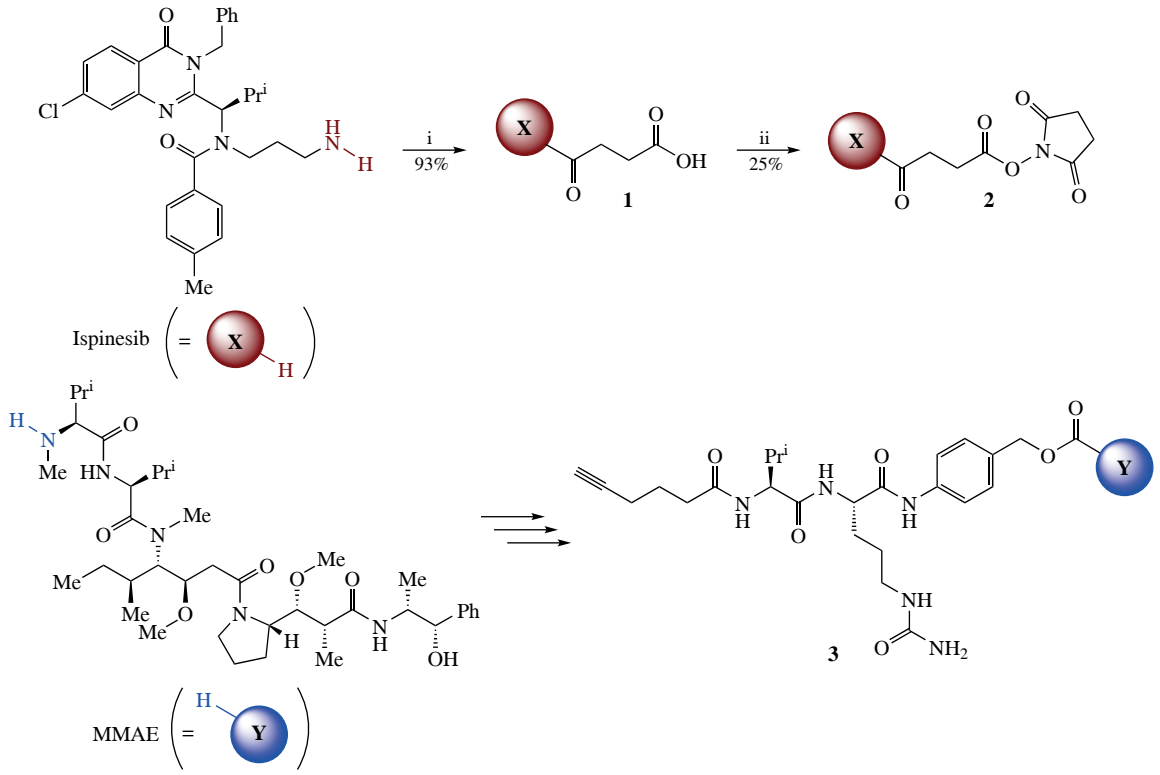
Prostate cancer (PCa) is the second most commonly diagnosed cancer among men.[1] In recent years, approaches based on the targeted delivery of diagnostic and therapeutic agents to PCa cells using low molecular weight ligands of prostate specific membrane antigen (PSMA) have been actively developed.[2–7] This approach is quite promising due to the overexpression of PSMA in tumor tissues compared to healthy tissues.[8] Another promising approach in the treatment of PCa is the use of a combination of different therapeutic agents to achieve a synergistic effect.[9–11] This approach utilizes both high molecular weight[12,13] and low molecular weight delivery systems,[14] including PSMA-targeted delivery systems. The combination of these two approaches may, on the one hand, reduce the non-specific toxicity of a number of drugs compared to the unconjugated form (*e.g.* for monomethyl auristatin E, MMAE). On the other hand, the use of a combination of two therapeutic agents with different mechanisms of action may enhance the efficacy of drugs that have not shown sufficient efficacy individually, *e.g.* inhibitors of the kinesin spindle protein (Eg5, kinesin 5) such as ispinesib.[15–17]

The aim of this work was to investigate the efficacy of the combination of the kinesin 5 inhibitor ispinesib and the tubulin polymerization inhibitor monomethyl auristatin E, to synthesize a bimodal conjugate of PSMA ligand with these therapeutic agents and to study its cytotoxicity. To accomplish the conjugation with the vector fragment, the therapeutic agents had to be pre-modified. Ispinesib was acylated with succinic anhydride to obtain derivative **1**, which was further reacted with *N*-hydroxysuccinimide to obtain compound **2** (Scheme 1). Monomethyl auristatin E (MMAE) was modified with a cathepsin-cleavable linker containing a 5-hexynoic acid residue according to the described procedure[5] to give compound **3**.

Currently, various approaches to the preparation of PSMA ligands have been documented. For a number of vector molecules, total synthesis of protected ligands on a solid-phase carrier is possible.[18] However, this approach has a number of limitations and is not suitable for the preparation of ligands with a lysine residue in the linker structure. An approach combining solid-phase and liquid-phase methods is better suited for the preparation of such compounds. An urea-based DCL ligand obtained by the previously described methodology[14] was chosen as the vector platform (full description of the synthesis, synthetic schemes and characterization of the products are outlined in Online Supplementary Materials). The choice of this ligand is based on its relatively high affinity to PSMA [K_i(ligand) = 1.3 ± 0.3 nM against K_i([Ga^{68}]-PSMA-11) = 12 ± 3 nM].[14,19] It should also be noted that based on a monomodal analog lacking lysine residue in the peptide linker fragment (IC_{50} = 9 ± 3 nM),[3] a series of conjugates with various therapeutic agents have been prepared and investigated.[5,20,21]

The preparation of the target bimodal conjugate **4** (Figure 1) was carried out according to the reported synthetic protocol.[14] In the first step, an azide–alkyne cycloaddition reaction between alkyne **3** and the corresponding azide was carried out (see Online Supplementary Materials), after which the $(CH_2)_4NH_2$ part of the obtained compound was introduced into acylation with NHS ester **2**. The overall yield of **4** upon two conjugation steps was 36%, which was higher than that for the previously



Scheme 1 *Reagents and conditions*: i, succinic anhydride, DIPEA, DMF; ii, NHS, EDC·HCl, Et_3N, DMF.

described enzalutamide/MMAE drug combination analog (10%).[14]

In the first phase of the *in vitro* studies, free drug combination was evaluated against breast adenocarcenoma (MCF7) and lung adenocarcinoma (A549) cell lines as the tumor models (Table 1). Also, cell lines HEK293T (culture of normal human embryonic kidney cells) and VA13 (culture of normal human lung cells) were tested as negative controls. To date, a number of studies on

Table 1 CC_{50} values obtained in different cell lines for individual MMAE and ispinesib as well as their equimolar mixture.

Compound	CC_{50}/nM			
	VA13	MCF7	A549	HEK293T
Ispinesib	19±4	34±11	5±1	0.56±0.09
MMAE	29±10	8±2	1.6±0.2	0.29±0.04
Combination	19±8	7±2	1.5±0.3	0.32±0.04

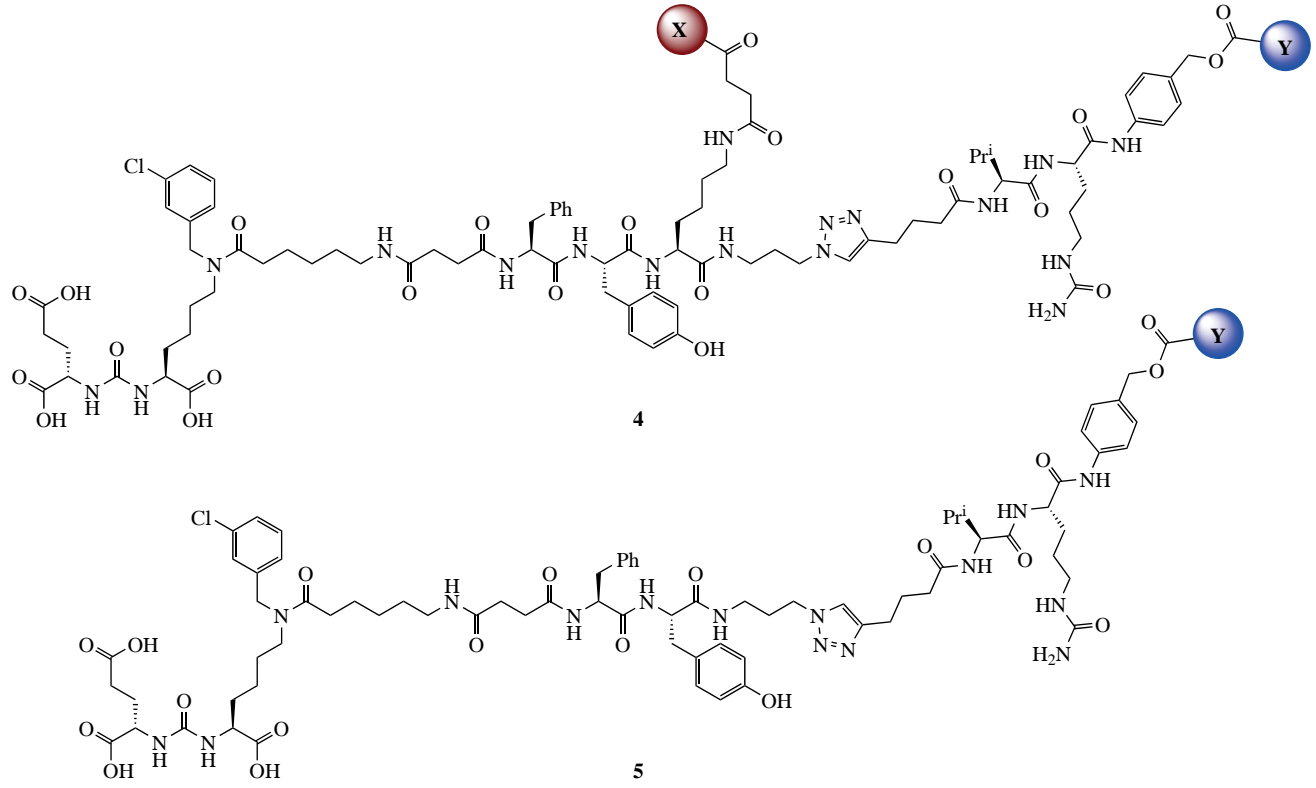


Figure 1 Structure of bimodal conjugate **4** and comparison conjugate **5**. Structures of **X** (residue of ispinesib) and **Y** (residue of MMAE) are presented in Scheme 1.

Table 2 CC_{50} values and selectivity indices (SI) obtained for MMAE, ispinesib, conjugate **5** and compound **4**.

Compound	CC_{50}/nM				SI		
	PC-3	LNCaP	22Rv1	VA13	VA13/PC-3	VA13/LNCaP	VA13/22Rv1
Ispinesib	2.3 ± 0.2	0.2 ± 0.1	0.6 ± 0.2	5 ± 3	2.3	33.3	9.7
MMAE	1.2 ± 0.4	2.1 ± 0.8	1.8 ± 0.4	3 ± 2	2.5	1.4	1.6
5	54 ± 14	55 ± 40	80 ± 31	~240	4.4	4.3	3.0
4	4814 ± 4432	2029 ± 1672	1915 ± 1772	~33333	6.9	16.4	17.4

the use of ispinesib in combination with other therapeutic agents have been published,[17,22,23] however no data on its use in combination with tubulin polymerization inhibitors (*e.g.*, MMAE) have been presented. Therefore, an *in vitro* experiment was performed to evaluate the cytotoxicity of free preparations of MMAE and ispinesib (see Scheme 1) and their equimolar mixture. The obtained CC_{50} values are presented in Table 1 while these data in graphical form can be found in the Online Supplementary Materials (Figure S1). In all cell lines, the tested combination of ispinesib and MMAE demonstrated CC_{50} values comparable to those for MMAE, which suggests that there is no antagonism between the drugs at their equimolar ratios.

The subsequent *in vitro* cytotoxicity studies were performed on the obtained bimodal conjugate **4**, individual drugs MMAE and ispinesib, and mono-conjugate **5** described in the literature (see Figure 1).[5] Testing was performed on PCa cell lines with different levels of PSMA expression (PC-3 is non-expressing, LNCaP and 22Rv1 are expressing) and on lung fibroblast cell line VA13 as the negative control. The CC_{50} values and selectivity indices are given in Table 2.

As can be seen from the presented data, compound **4** showed moderate selectivity against PSMA-expressing tumor models (SI for VA13/LNCaP is 16.4; SI for VA13/22Rv1 is 17.4), which, however, can be explained by the selective mechanism of action of the drugs themselves, on which the conjugate is based (SI of ispinesib for VA13/LNCaP is 33.3). Also, conjugate **4** is significantly inferior in toxicity to both free drugs and monomodal conjugate of PSMA ligand with MMAE **5**. At the same time, it should be mentioned that compounds of similar structure, while being inferior in CC_{50} to free drugs in *in vitro* experiments, were shown to exhibit rather high efficiency in *in vivo* experiments. Thus, monomodal conjugate **5** demonstrated an order of magnitude lower cytotoxicity *in vitro* (CC_{50} of **5** for 22Rv1 of 29 ± 2 nM *vs*. CC_{50} of docetaxel for 22Rv1 of 3.3 ± 0.5 nM). At the same time, in the *in vivo* experiment on xenograft model, conjugate **5** (dosage 0.3 mg kg^{-1}) demonstrated tumor growth inhibition (TGI) of 85–70% during the whole experiment, which is comparable to that for docetaxel (dosage 10 mg kg^{-1}; TGI was 87–84%).[5] Thus, conjugate **4** may reveal greater efficacy in an *in vivo* experiment, which is a purpose for further studies.

To summarize, a co-delivery system of monomethyl auristatin E and ispinesib based on the ligand of prostatic specific membrane antigen was synthesized. Synthetic approaches allowing one to obtain the target conjugate with higher yields compared to those described earlier were developed. The obtained bimodal conjugate **4** was tested for *in vitro* cytotoxicity on PCa cell lines. The *in vitro* experiment on cytotoxic activity of the MMAE/ispinesib drug combination showed that there was no antagonism between the drugs at equimolar ratio. The methodology developed in this work can be used to produce new bimodal conjugates with other combinations of therapeutic agents for co-delivery to PSMA.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7661.

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