New-Generation Self-Immolative Spacers for the Release of Anticancer Drugs from ADCs

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Introduction

- The targeted delivery of cytotoxic agents via antibody-drug conjugates (ADCs) has become a highly attractive method for treating various types of cancers. From a structural viewpoint, traditional ADCs consist of a monoclonal antibody (mAb) linked to a cytotoxic agent via a protease-cleavable peptide linker and a self-immolative (SI) spacer.
- In recent years, the significant success of this emerging technology in different types of cancers has stimulated the global effort to find different ADC components and new connections between them, in order to improve the pharmacokinetic properties of the whole ADC construct. For instance, a fast self-immolation mechanism of a proline-based SI spacer (Figure 1) for the release of alcohol payloads from carbamate prodrugs has been investigated through successful new drug-linker syntheses and conjugation on engineered mAb.
- In this communication, I will describe the structure and activation kinetics of new-generation self-immolative spacers, designed to release hydroxy-bearing drugs. Upon synthesis of the linker-spacer-payload modules and their conjugation to a model antibody, the ADCs were incubated in the presence of a protease, and the metabolites were analyzed by LC-MS.

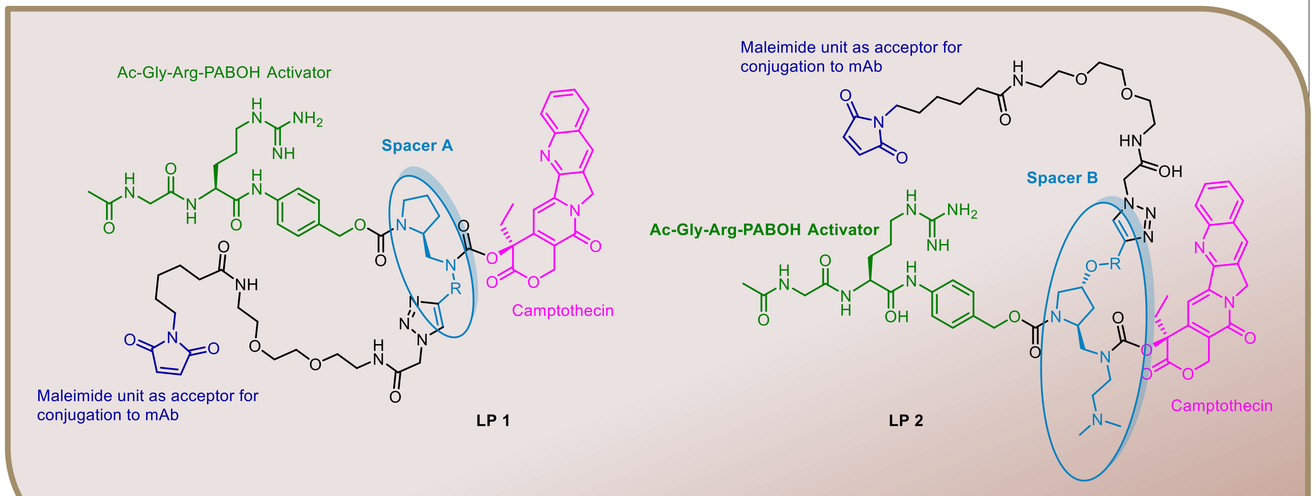
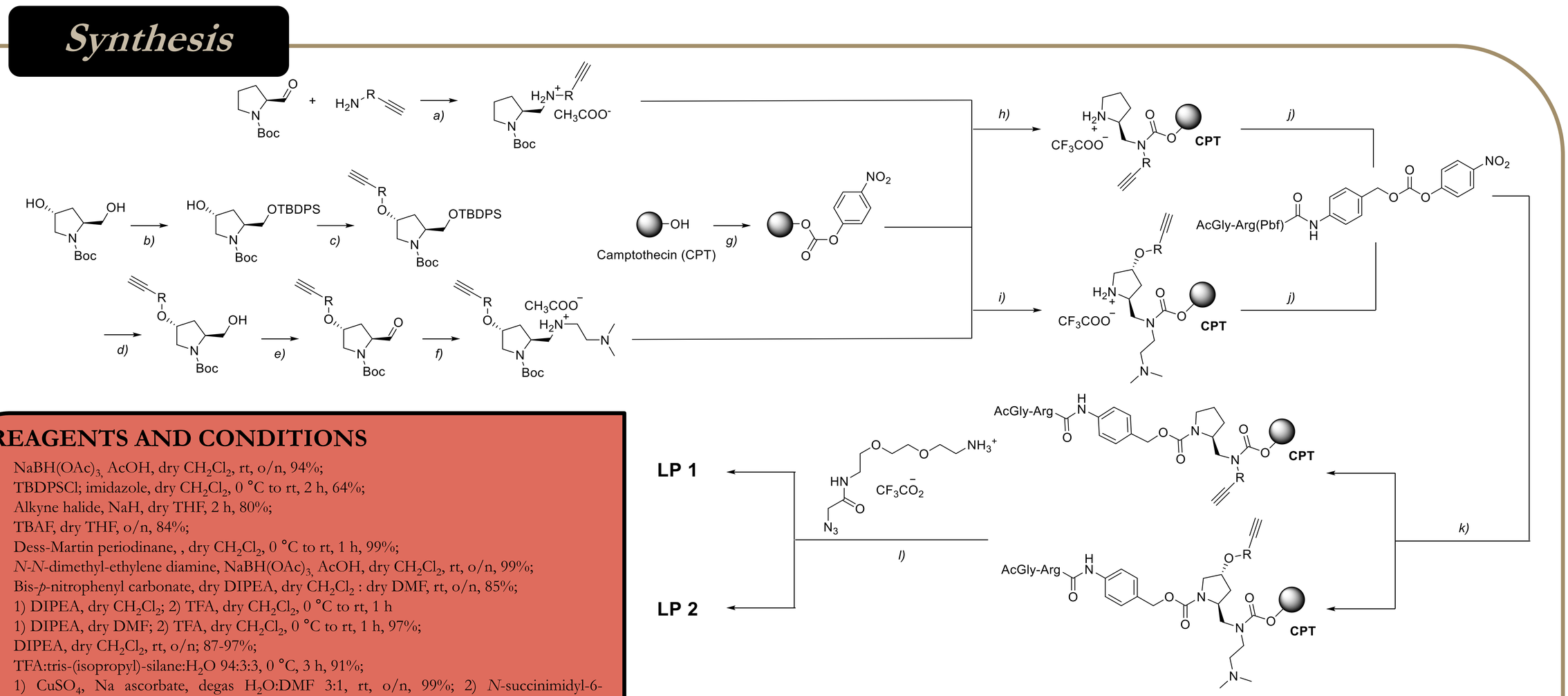


Figure 1. New modules of linker-payload with two different proline SI-spacers.

Synthesis

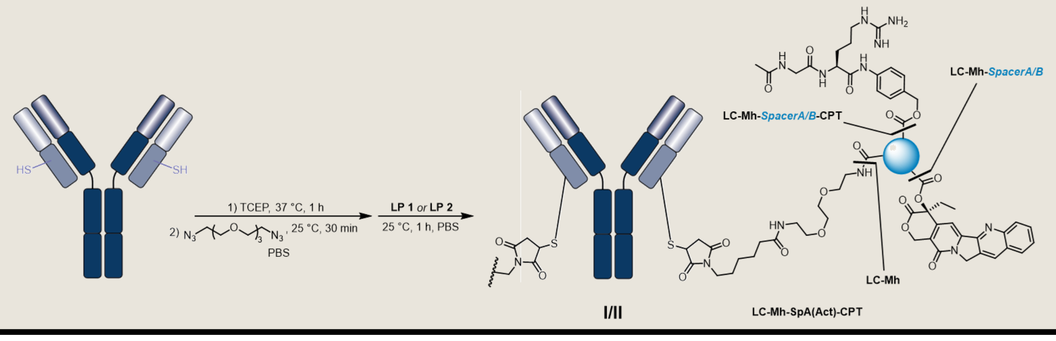


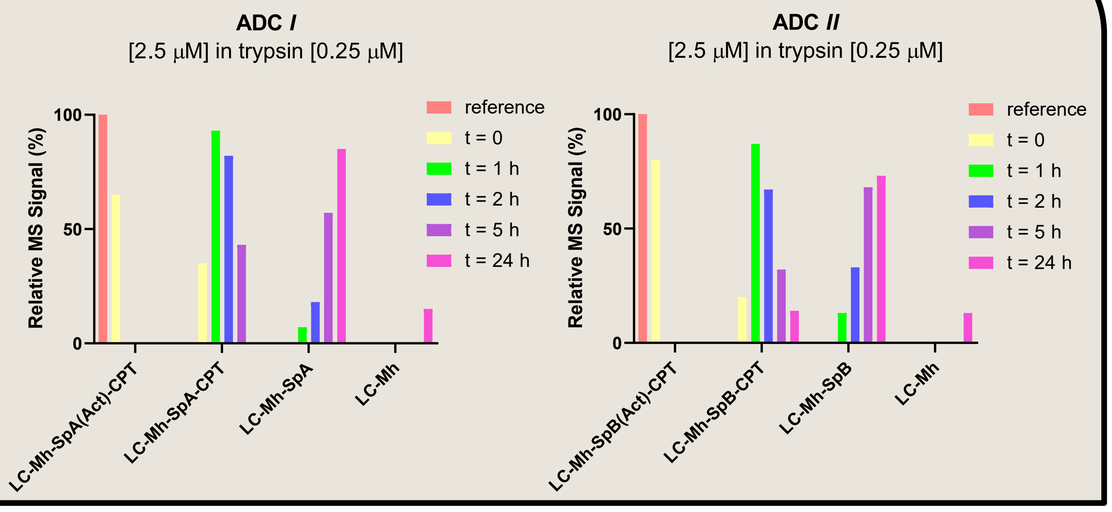
REAGENTS AND CONDITIONS

a) $NaBH(OAc)_3$, AcOH, dry CH_2Cl_2, rt, o/n, 94%;
b) TBDPSCl; imidazole, dry CH_2Cl_2, 0 °C to rt, 2 h, 64%;
c) Alkyne halide, NaH, dry THF, 2 h, 80%;
d) TBAF, dry THF, o/n, 84%;
e) Dess-Martin periodinane, , dry CH_2Cl_2, 0 °C to rt, 1 h, 99%;
f) *N-N*-dimethyl-ethylene diamine, $NaBH(OAc)_3$, AcOH, dry CH_2Cl_2, rt, o/n, 99%;
g) Bis-*p*-nitrophenyl carbonate, dry DIPEA, dry CH_2Cl_2 : dry DMF, rt, o/n, 85%;
h) 1) DIPEA, dry CH_2Cl_2; 2) TFA, dry CH_2Cl_2, 0 °C to rt, 1 h
i) 1) DIPEA, dry DMF; 2) TFA, dry CH_2Cl_2, 0 °C to rt, 1 h, 97%;
j) DIPEA, dry CH_2Cl_2, rt, o/n; 87-97%;
k) TFA:tris-(isopropyl)-silane:H_2O 94:3:3, 0 °C, 3 h, 91%;
l) 1) $CuSO_4$, Na ascorbate, degas H_2O:DMF 3:1, rt, o/n, 99%; 2) *N*-succinimidyl-6-maleimido-esanoic acid, DIPEA, dry DMF, rt, o/n; ***LP 1*** = 63%, ***LP2*** = 81%.

Bioconjugation and Release Tests

- As a model mAb for the stability evaluation of our LP modules, we opted for a chimeric antibody (clone 4M5.3) specific for fluorescein, engineered for site-specific conjugation at the C-terminal Cys of the LCs.
- After bioconjugating the two LPs to the mutant mAb, we conducted preliminary tests to monitor the release mechanism of the payload over time through incubation at 37 °C of the ADC ***I*** and ***II*** in a solution of trypsin, a human protease specific to diverse peptide bonds.
- The different aliquots taken at five short time points (0, 1 h, 2 h, 5 h, and 24 h) were purified via affinity chromatography featuring a FITC-labelled resin, and the fragments were analyzed with LC-MS.





Conclusions

In summary, the system worked in both cases: enzymatic cleavage occurred correctly even with trifunctional spacers directly attached to the antibody, the drug release can be easily monitored in LC-MS, and the release rates are consistent with what is already present in literature; plus, we can infer that ADC ***II*** is faster than ADC ***I*** at least for the first time points.

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