Beyond SMCC: Uniform Isothiocyanate-Peptide Bioconjugates

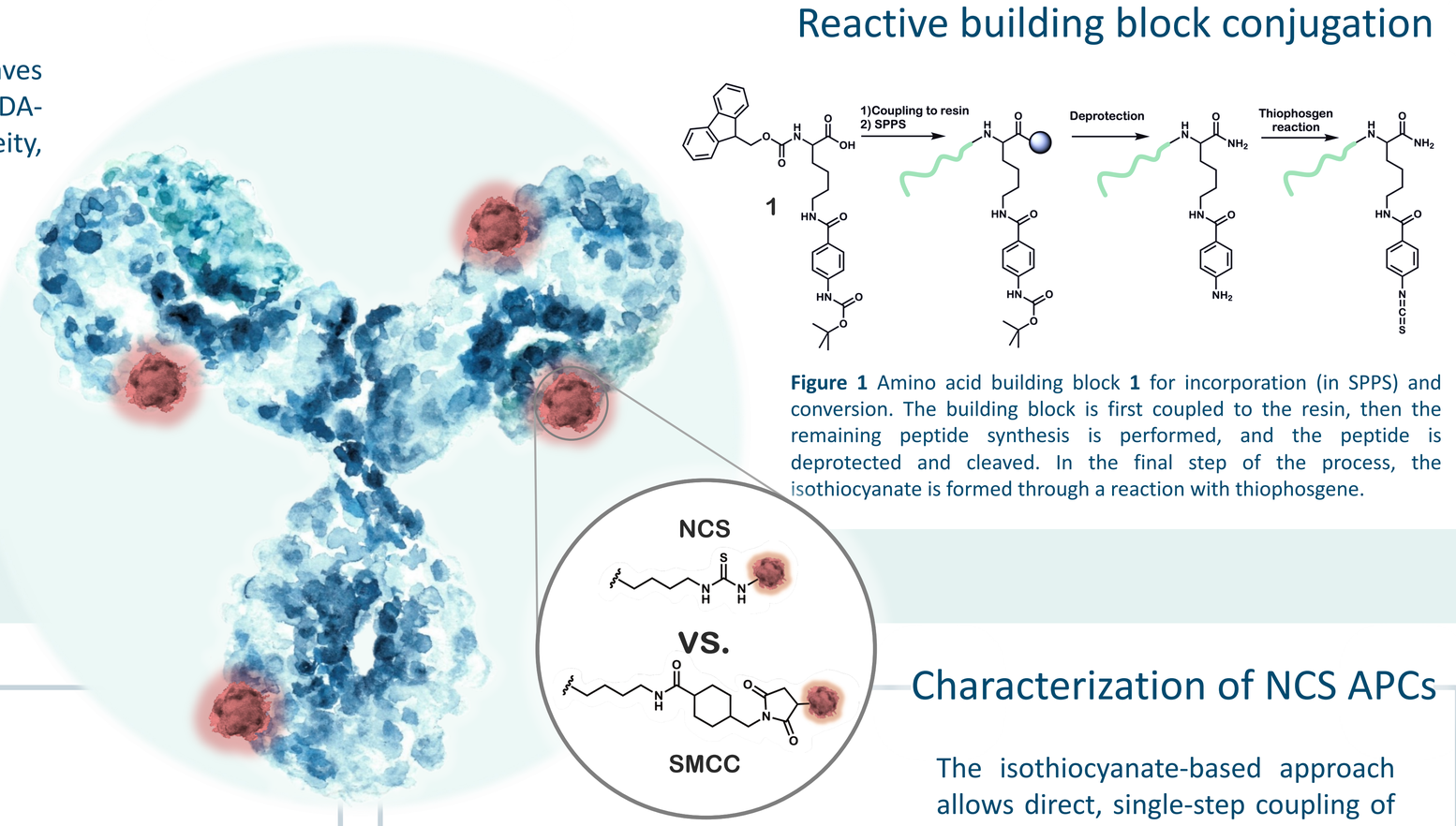
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Motivation and Objective

Conventional SMCC-based antibody conjugation often leaves unreacted or crosslinked linker residues, as seen in the FDA-approved ADC Kadcyla®, leading to structural heterogeneity, incomplete reactions, and reduced reproducibility.[1,2]
To address these challenges, we developed a direct, single-step isothiocyanate-based conjugation approach that allows stochastic attachment of peptides or chelator-peptides to antibodies. This streamlined. method enhances uniformity and stability while minimizing structural alterations and simplifying radioimmunoconjugate synthesis.

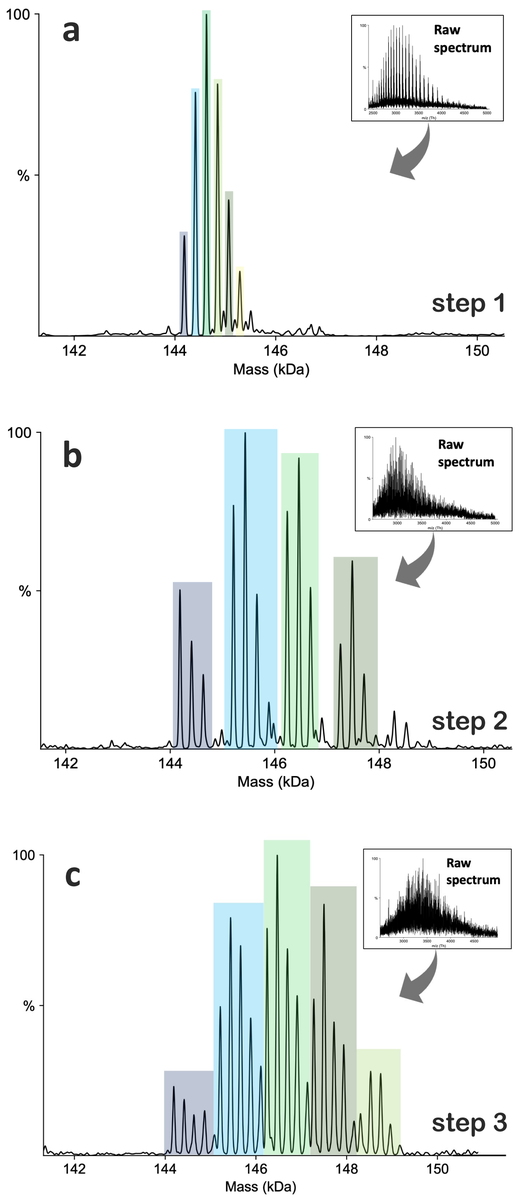


Reactive building block conjugation

Figure 1 Amino acid building block **1** for incorporation (in SPPS) and conversion. The building block is first coupled to the resin, then the remaining peptide synthesis is performed, and the peptide is deprotected and cleaved. In the final step of the process, the isothiocyanate is formed through a reaction with thiophosgene.

Results

Characterization of SMCC APCs



Mass spectrometric characterization of SMCC-based antibody-peptide conjugates (APCs) revealed incomplete coupling and significant product heterogeneity. Despite the use of an excess of thiol-peptide after the initial SMCC conjugation (**Figure 2a**), unreacted or crosslinked maleimidocaproyl (MCC) residues persisted, leading to several minor peaks observed in the deconvoluted spectra (**Figure 2b**). Optimization of reaction parameters, including incubation time and purification, resulted in only slight improvements in peptide-antibody ratio and reproducibility. Additional experiments with N-acetylcysteine confirmed that most MCC groups became chemically inactive, likely through intra- and inter-molecular crosslinking rather than hydrolysis.[1,2] The subsequent DOTA coupling step (**Figure 2c**) further increased conjugate complexity, generating mixed species with varying degrees of peptide and chelator substitution.

Figure 2 (a) Multi-charged *m/z* profiles (right corner) and deconvoluted spectrum of conjugation step 1 (SMCC conjugation) after deglycosylation using Rapid PNGase F. Colors represent the different MCC species. (b) Multi-charged *m/z* profiles (right corner) and deconvoluted spectrum of conjugation step 2 (coupling of SH-peptide to the MCC-antibody) after deglycosylation using Rapid PNGase F. Colors indicates the same number of coupled peptides. (c) Multi-charged *m/z* profiles (right corner) and deconvoluted spectrum of conjugation step 3 (DOTA conjugation to the peptide-MCC-antibody) after deglycosylation using Rapid PNGase F. Colors indicate the same number of coupled peptides.

Characterization of NCS APCs

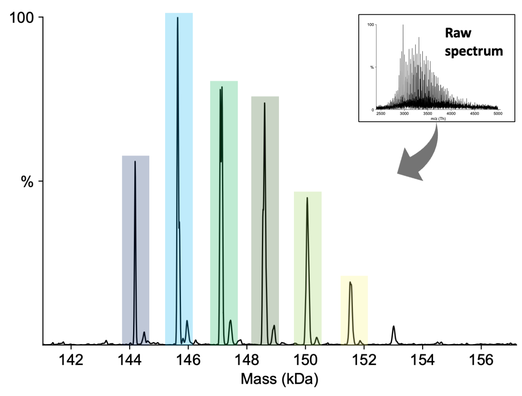


Figure 4 Multi-charged *m/z* profiles (right corner) and deconvoluted spectrum of NCS-peptide-mAb conjugate following deglycosylation with Rapid PNGase F. Colors indicate the different PAR assignment (0-5).

The isothiocyanate-based approach allows direct, single-step coupling of DOTA-NCS-peptides to antibodies under mild basic conditions (pH 9, 24 h). In contrast to the multi-step SMCC method, purification involves only one ultrafiltration step, resulting in reproducible conjugation efficiency across experiments. Mass showed distinct and well-resolved peaks for each peptide-to-antibody ratio, confirming uniform conjugation without side reactions or residual linker species (**Figure 4**). The resulting conjugates displayed high structural integrity and uniform modification, providing a reliable platform for subsequent radiolabelling.[3,4]

Conjugation steps

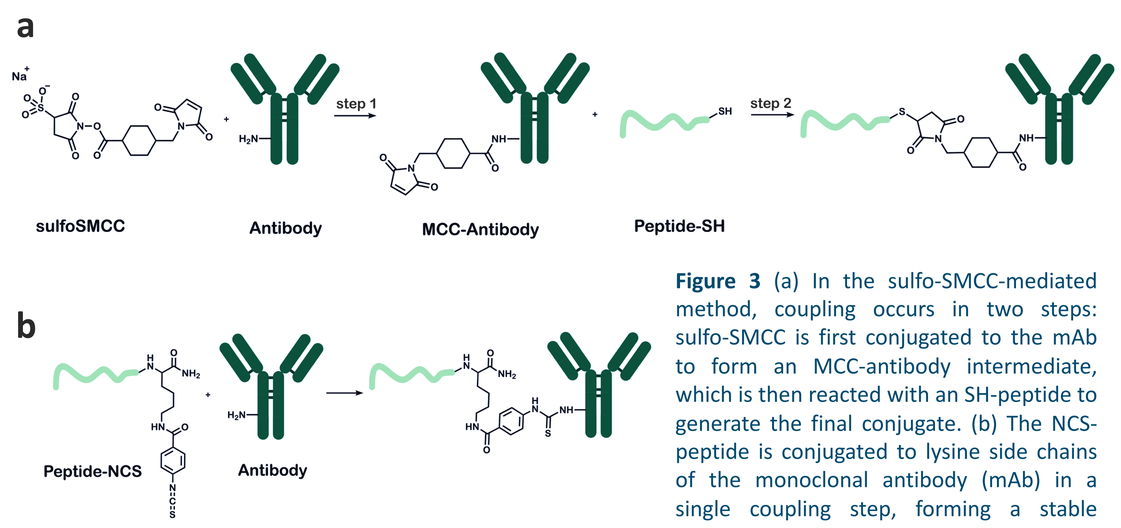


Figure 3 (a) In the sulfo-SMCC-mediated method, coupling occurs in two steps: sulfo-SMCC is first conjugated to the mAb to form an MCC-antibody intermediate, which is then reacted with an SH-peptide to generate the final conjugate. (b) The NCS-peptide is conjugated to lysine side chains of the monoclonal antibody (mAb) in a single coupling step, forming a stable thiourea bond.

Summary

Isothiocyanate-mediated peptide conjugation using our amino acid building block offers an efficient, one-step alternative to SMCC-based methods. This approach enables non-site-specific antibody attachment while preserving structural integrity and producing uniform, stable conjugates suitable for diverse applications.

CONTACT

References

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