An Optimized Protocol for the Synthesis of Peptides Containing trans-Cyclooctene and Bicyclononyne Dienophiles as Useful Multifunctional Bioorthogonal Probes

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Abstract: Despite the great advances in solid-phase peptide synthesis (SPPS), the incorporation of certain functional groups into peptide sequences is restricted by the compatibility of the building blocks with conditions used during SPPS. In particular, the introduction of highly reactive groups used in modern bioorthogonal reactions into peptides remains elusive. Here, we present an optimized synthetic protocol enabling installation of two strained dienophiles, trans-cyclooctene (TCO) and bicyclononyne (BCN), into different peptide sequences. The two groups enable fast and modular post-synthetic functionalization of peptides, as we demonstrate in preparation of peptide-peptide and peptide-drug conjugates. Due to the excellent biocompatibility, the click-functionalization of the peptides can be performed directly in live cells. We further show that the introduction of both clickable groups into peptides enables construction of smart, multifunctional probes that can streamline complex chemical biology experiments such as visualization and pull-down of metabolically labeled glycoconjugates. The presented strategy will find utility in construction of peptides for diverse applications, where high reactivity, efficiency and biocompatibility of the modification step is critical.

Introduction

Peptides represent a unique class of macromolecules with wide range of properties, functions and applications.[1] It is the great structural diversity on one hand, and synthetic accessibility on the other, which make peptides an attractive functional modality that in many aspects combines the advantages of small molecules and larger biomolecules. Indeed, the last decades have witnessed a huge increase in the popularity of peptides as therapeutics,[2] diagnostics,[3] delivery agents[4] and chemical biology probes.[5] Many of these conjugates require the attachment of additional functional moieties to the peptide backbone to fulfill their function. Owing to the chemical complexity of peptides, the development of methods enabling their selective functionalization is not trivial. The most straightforward method for construction of functionalized peptides is based on the use of modified building blocks, unnatural amino acids, during standard SPPS. The obvious requirement for the success of this approach is the compatibility of the modified building blocks with SPPS. In this regard, the most challenging part of the synthesis involves the deprotection/cleavage steps, which require the use of cocktails containing as much as 95 % trifluoroacetic acid (TFA). The synthetic or commercial availability of modified building blocks, which are usually used in an excess, can be also a limiting factor. Another approach for synthesis of functional peptides is based on the attachment of the functional moiety after the synthesis of the peptide backbone. This post-synthetic modification approach takes advantage of the unique reactivity of functional groups present on natural amino acids[6] or, alternatively, involves a two-step process where an artificial reactive group, compatible with SPPS, is introduced into the sequence first, and is then used for the functionalization by selective chemical click reaction in the second step.[7]

Among the bioorthogonal click reactions available,[8] the strain-promoted alkyn-azide cycloaddition (SPAAC)[9] and the inverse electron-demand Diels-Alder reaction (iEDDA)[10] of 1,2,4,5-tetrazines with strained dienophiles are especially valued due to high reaction rates, good biocompatibility and no need for additional catalysis. For application of SPAAC in peptide chemistry, the main strategy is based on the introduction of the azide moiety during SPPS.[11] Examples of peptides functionalized with different cyclooctyne moieties also exist.[12] The strained dienophile/dipolarophile was in most of the cases installed via active ester chemistry on the N-terminus or single lysine residue within the peptide sequence. A general method for the de novo synthesis of a bicyclononyne amino acid (BCN-AA) has been published, however, the compound was not used in SPPS.[13] Recently, a copper-protection strategy was devised...
to enable incorporation of dibenzycyclooctyne (DBCO) moiety into peptides.\cite{14}

The application of iEDDA for peptide modification is relatively scarce. The use of tetrazine-containing amino acids during standard Fmoc-based SPPS turned out to be problematic due to incompatibility of the modified building blocks with the coupling step or Fmoc removal.\cite{15} Therefore, attachment of tetrazine carboxylic acids to the N-terminus or lysine side chains was employed instead.\cite{16} Similarly to cyclooctynes, selective incorporation of trans-cyclooctene (TCO) dienophiles into peptides was achieved only via post-synthetic modification of fully deprotected peptides containing a single lysine residue using the respective TCO active esters.\cite{17}

Owing to the high reactivity of cyclooctynes and TCOs,\cite{18} which is naturally associated with reduced chemical stability, it is not surprising that compatibility of these groups with standard SPPS is problematic. However, the ability to introduce these functional groups into peptide sequence would greatly expand the application potential of functionalized peptides. Especially appealing would be the possibility to perform selective functionalization of peptides directly in living systems, which is difficult to achieve using currently available methods.

Toward this aim, we herein present an optimized synthetic protocol that enables incorporation of bicyclononyne and trans-cyclooctene moieties, or even their combination, into synthetic peptides at any selected positions within the amino acid (AA) sequence (Figure 1). With the goal to stick to conditions of standard Fmoc SPPS and to make the procedure broadly applicable, we devised a method based on selective post-synthetic modification of orthogonally protected peptide backbones and the use of NHS active esters, which are commercially available or synthetically easily accessible. A set of diverse (multi)click-functionalized peptides of biological interest have been efficiently prepared. Using model sequences, we finally demonstrate that the TCO- and BCN-containing peptides can be employed in the construction of peptide-drug, peptide-peptide conjugates and as multifunctional chemical biology probes.

**General considerations**

In our initial experiments, we focused on the stability of BCN and TCO moieties under acidic cleavage conditions traditionally used for removal of the amino acid side chain protecting groups and/or peptide cleavage from the resin. We synthesized two model Fmoc-protected amino acids containing BCN and TCO groups (1 and 2 in Figure 1) and incubated them in cleavage cocktails containing various concentrations of TFA. Stability of the amino acids was determined by HPLC-MS (see Supporting Information, Section 4, Figure S1). These experiments showed that both the BCN- and TCO-containing amino acids quickly decompose under acidic conditions, which makes them unsuitable for the standard SPPS.

Based on these results, we devised an alternative approach which consists of a post-synthetic modification of lysine residues within the peptide sequence using BCN and TCO active esters and a sequence of selective deprotection steps. To achieve this goal, we had to face several challenges (Scheme 1). First, we needed to find two to three orthogonal protecting groups (including the Fmoc group present at N\textsubscript{\alpha} of standard AAs) that will survive the acidic conditions required for the side chain deprotection. Second, these orthogonal protecting groups must be susceptible to deprotection under conditions compatible with the labile BCN and TCO moieties. Third, the final steps of the synthesis, N-terminal Fmoc deprotection,
removal of the last lysine-protecting group and cleavage of the peptide from the resin, must be also performed under conditions compatible with the BCN and TCO groups.

Results and discussion

Compatibility among the different functionalities and reaction conditions

Considering these challenges, the first experiments were focused on the evaluation of stability of the reactive and sensitive TCO and BCN groups under different conditions used for PGs removal, as well as on probing the orthogonality among the proposed PGs. Four suitable PGs compatible with standard FmocSPPS were selected: tert-butyloxy carbonyl (Boc), allyloxy carbonyl (Alloc), tetrachlorophthalimide (TCP) and trifluoroacetyl (tfa).

Boc (PG1) would be the first PG removed along with the side chain deprotection of the supported peptide in the presence of TFA, followed by the Pd-catalyzed removal of Alloc (PG2), leaving only Fmoc and PG3 attached to the peptide. TCP and tfa groups can be removed under basic conditions, therefore, in principle, they can be deprotected separately or together with Fmoc removal from the N-terminus, or during the final cleavage from a base-labile solid support. To investigate the compatibility of the groups, we prepared a series of model compounds containing BCN, TCO and selected PGs, including commercially available building blocks (Nα-Fmoc-Ne-Boc-Lys, Nα-Fmoc-Ne-Alloc-Lys, TCO-OH, BCN-OH) as well as short peptides attached to a solid support (Figure 1).

The model compounds were subjected to the corresponding reaction conditions to assess both stability and deprotection efficiency, allowing us to reveal the puzzle of compatibilities among the clickable groups and the amino-PGs (Table 1, for experiment details see Supporting Information, Section 4.b–d).

In the case of TCO and BCN, both groups were stable toward basic conditions (piperidine, NaOH, DBU) required for Fmoc and tfa removal as well as toward nucleophilic ethylenediamine (EDA) required for TCP removal. Interestingly, the use of hydrazine (H₂NNH₂·H₂O) for TCP deprotection was not suitable as both clickable groups were affected, mainly by reduction (see Supporting Information, Section 4.c). During Alloc deprotection using Pd(0), the TCO group remained untouched when nucleophilic scavengers such as morpholine or dimethyl barbituric acid (DBA) were present, while other scavengers (SiHPr₃, Me₂NB₃) led to decomposition of the clickable group (reduction was observed, among other side reactions). On the other hand, the BCN group was not stable toward Pd(0) (partial reduction), regardless of the nature of the scavenger. After successfully assessing the stability of the TCO and BCN groups under these conditions, we next moved on to evaluate the stability of the PGs, as well as their efficient removal.

As shown in Table 1, the selected PGs were compatible with the proposed synthetic strategy except for TCP, which showed
several limitations. Removal of TCP with EDA partially deprotected the Fmoc group as well, which is not an issue if both groups are detached at the final step from the solid support.

3.2. Identification of Suitable Conditions

Based on HPLC-MS results, the side chain deprotection in the presence of TFA without further experimentation, we were able to identify suitable conditions and found that, for TCP-containing substrates, piperidine can be replaced by DBU for the Fmoc deprotection[23] and dimethylbarbituric acid[21] is the most suitable scavenger during Alloc elimination.

### Synthesis of peptides containing the clickable groups

With the optimized conditions in hand, we next synthesized peptides containing a single clickable group. We chose the neuropilin-binding peptide (NRP),[24] mitochondrial-targeting peptide,[20] and a random AA sequence as test systems (Scheme 2a). The introduction of a single clickable group is straightforward and requires only a Boc-protected lysine residue, which after deprotection, can be used to attach the clickable group via standard active ester chemistry. An important consideration for the success of this strategy is the correct selection of the resin linker, which enables efficient AAs side chain deprotection in the presence of TFA without premature cleavage of the peptide from the polymer. For this purpose, the 4-hydroxymethylbenzoic acid linked HypoGel resin (HG-HMBA) was selected, and the optimized final cleavage of peptides was carried out with 0.1 M aq. NaOH:dioxane (1:2). Importantly, NaOH-promoted Fmoc deprotection was recently developed as a green alternative to the commonly used TFA:[26] only hydrolyzed product was detected by LC/MS, (g) for complete removal it is necessary to use an excess of reagent: higher concentration (0.2 M) and extended reaction time; (h) for short periods of time (5–10 min) only partial Fmoc removal was observed.

### Table 1. Compatibilities of clickable and protecting groups under different reaction conditions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Conditions</th>
<th>TCO</th>
<th>BCN</th>
<th>Boc</th>
<th>Alloc</th>
<th>TCP</th>
<th>Tfa</th>
<th>Fmoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFA</td>
<td>OK</td>
<td>X(^{[b]})</td>
<td>X(^{[b]})</td>
<td>Removed</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
</tr>
<tr>
<td>Pd + morpholine or DBA</td>
<td>OK</td>
<td>X</td>
<td>–</td>
<td>Removed</td>
<td>OK (only with DBA)</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
</tr>
<tr>
<td>Pd + SiHPr(_3) or Me(_3)NBH(_3)</td>
<td>X(^{[b]})</td>
<td>X(^{[b]})</td>
<td>–</td>
<td>Removed</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>OK</td>
</tr>
<tr>
<td>EDA</td>
<td>OK</td>
<td>OK</td>
<td>–</td>
<td>–</td>
<td>Removed</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Piperidine</td>
<td>OK</td>
<td>OK</td>
<td>OK(^{[e]})</td>
<td>OK</td>
<td>X Part. transform.</td>
<td>OK</td>
<td>–</td>
<td>Removed</td>
</tr>
<tr>
<td>DBU</td>
<td>–</td>
<td>–</td>
<td>OK(^{[e]})</td>
<td>OK(^{[e]})</td>
<td>OK</td>
<td>–</td>
<td>–</td>
<td>Removed</td>
</tr>
<tr>
<td>NaOH aq.</td>
<td>OK</td>
<td>OK</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>X(^{[i]})</td>
<td>–</td>
<td>Removed</td>
</tr>
</tbody>
</table>

\(^{[a]}\) Reaction conditions: TFA (trifluoroacetic acid): TFA:DCM (9:1) or TFA:H\(_2\)O:TIPS (95:2.5:2.5); Pd + scavenger: Pd(PPh\(_3\))\(_2\), (0.3 equiv.), scavengers: morpholine (12 equiv.) or N\(_2\)N-dimethylbarbituric acid (DBA) (12 equiv.) or SiHPr\(_3\) (TIPS, 2–20 equiv.) or Me\(_3\)NBH\(_3\) (2–20 equiv.), DCM, rt, 30 min, 2 cycles; EDA: ethylenediamine:DMF (1:200), 50 °C, 60 min; Piperidine: piperidine 20% in DMF, rt, 10–60 min; DBU: DBU 2%, DMF, rt, 5 min; NaOH: NaOH aq. 0.1 M, rt, 60 min. Abbreviations and notes: X: not stable; Part. removed: partially removed; Part. transform.: partially transformed; –: not investigated; [b] decomposition; [c] reduced and isomerized products were detected; [d] mostly decomposition, reduced products were detected; [e] data from literature; [f] only hydrolyzed product was detected by LC/MS; [g] for complete removal it is necessary to use an excess of reagent: higher concentration (0.2 M) and extended reaction time; [h] for short periods of time (5–10 min) only partial Fmoc removal was observed.
terminus after Fmoc deprotection. Final cleavage with NaOH yielded the desired double functionalized peptide Pep4-TCO-BCN.

To confirm that the TCO and BCN groups remain amenable to further derivatization, we labeled the synthesized peptides with diphenyltetrazine (diPhTz) (Supporting Information, Section 5.a.). Both groups are known to react in the inverse electron-demand Diels-Alder (iEDDA) reaction with tetrazines. HPLC-MS analysis of the reaction mixtures revealed that the clickable groups were available for the reaction, confirming that our synthetic strategy leads to formation of fully functional TCO- and BCN-containing peptides. This validation of click reactivity was used throughout the whole study (see Supporting Information for details of each particular modified peptide).

Encouraged by these results, we moved on to exploit the synthetic strategy for introduction of the TCO and BCN groups onto two different lysine residues within a peptide backbone. To achieve this, a second lysine residue with an orthogonal PG, Nα-Fmoc-Nε-Alloc-Lys, was introduced during the SPPS. After TFA-mediated side chain deprotection, a TCO-NHS active ester was coupled with the free Lys residue (derived from the Lys (Boc) AA). Selective and TCO-compatible removal of the Alloc group by Pd catalysis, followed by reaction with BCN-NHS enabled the introduction of the second BCN clickable group (Scheme 3a). This strategy yielded peptides functionalized with the two orthogonal click handles (Pep5-TCO-BCN, Pep6-TCO-BCN) in good total yields (15–17 %) after HPLC purification. During these experiments, we found that it is important to extensively wash the solid support after the Pd-catalyzed Alloc removal with a solution of sodium N,N-diethylthiocarbamate to remove traces of palladium. Otherwise, the BCN coupling step was problematic.

This synthetic strategy also allows the preparation of TCO-modified peptides containing free lysine residues in the sequence. By simply ending the synthesis after the Alloc deprotection, we prepared a TCO-functionalized KDEL peptide motif in excellent total yield (82 %, Pep7-TCO) (Scheme 3b).

Unfortunately, this combination of PGs is not compatible with the BCN moiety, which is affected by the palladium complex (Table 1). To achieve incorporation of BCN, we used trifluoroacetamide (tfa)-protected lysine (Nα-Fmoc-Nε-tfa-Lys) instead of Nα-Fmoc-Nε-Alloc-Lys. In this case, the tfa PG was conveniently removed together with the final cleavage of the peptide from the solid support in one step, as proved by successful synthesis of peptide Pep8-BCN (Scheme 3c).

The generation of peptides containing two orthogonal clickable handles, in combination with free lysine residues is the most challenging goal to achieve. For this purpose, three orthogonally protected Lys AAs are required. Boc and Alloc PGs were used in combination with TCP or tfa PGs, which can be removed with ethylenediamine (EDA) or NaOH, respectively. As a proof of concept, we decided to prepare the TCO, BCN-double modified FLAG tag peptide epitope (Scheme 4). To investigate which strategy is better, we prepared the FLAG peptide using either TCP or tfa as the third protecting group. To ensure efficient recognition of the epitope by the anti-FLAG antibody,
we spatially separated the FLAG peptide sequence by a short PEG linker from the two lysine residues used for TCO and BCN attachment. While the TCP group strategy required removal of the Fmoc group with DBU, the use of the tfa PG allowed classical piperidine Fmoc deprotection and tfa removal directly during the peptide backbone release from the resin. The final step required a higher concentration of NaOH (0.2 M) and longer incubation time to achieve complete deprotection and peptide cleavage, possibly due to the presence of four aspartic acid residues in the FLAG tag sequence. In general, the synthetic route based on the tfa-protection was more straightforward with respect to manipulation, monitoring of the entire synthesis and final purification. In addition, the total yield of the final clickable group-derivatized FLAG peptides (Pep9-TCO-BCN) was higher, 15% total yield over 26 steps compared to 17% total yield over 25 steps with the TCP group strategy. The synthetic strategy used to prepare TCO and BCN-containing FLAG tag epitope is shown in Scheme 4.
BCN) was notably different depending on the PG used. The strategy based on the use of tfa afforded the final peptide in 9% total isolated yield, while the TCP group-based strategy in only 0.3%.

Application examples of peptides containing the clickable groups

The main goal of this study was to develop an efficient strategy enabling the construction of synthetic peptides containing the highly reactive TCO and BCN dienophiles. Such peptides could find numerous applications that include synthesis of peptide-peptide conjugates, peptide-drug conjugates or various smart chemical biology probes. To demonstrate the utility of the developed methodology in the context of such applications, we carried out several experiments. First, we prepared a tetrazine-containing FLAG peptide (Pep10-Tz, by incorporation of a tetrazine-containing Lys13, see Supporting Information, Section 2) and selected two TCO- and BCN-presenting peptides (Pep1-TCO and Pep3-BCN) as the coupling partners in a model experiment for the construction of peptide-peptide conjugates. (Scheme 5a, for experimental details see Supporting Information, Section 6.a.). The reaction was performed using stoichiometric amounts of the reaction partners and was in both cases finished within 15 minutes at 1 mM concentration, highlighting the great efficiency of the iEDDA reaction. The same two peptides Pep1-TCO and Pep3-BCN, were next conjugated with a derivative of the chemotherapy drug docetaxel, Docetaxel-Tz. As in the previous case, the reaction proceeded cleanly and both peptide-drug conjugates were formed within 15 minutes. (Scheme 5b, for experimental details see Supporting Information, Section 6.b.)

The orthogonally double clickable group-containing peptides allow for more challenging applications. The BCN group also efficiently reacts with azides in the strain-promoted alkyne-azide cycloaddition. Therefore, peptides containing the TCO and BCN groups could be used for sequential double functionalization by the SPAAC and iEDDA reactions with for example bioactive molecules, targeting molecules, fluorescent tags or biophysical probes. To show that such an approach is viable, we used the FLAG tag peptide Pep9-TCO-BCN and conjugated it first at the BCN-moiety using an azido derivative of sialic acid (Sia-N3). Subsequent reaction of the remaining TCO group with...
a fluorogenic coumarin tetrazine probe to yield the fluorescently-labeled glycopeptide Pep9-FL-Sia (Scheme 6, for experimental details see Supporting Information, Section 6.c.). In addition to the high reaction rate, the excellent biocompatibility is another valuable attribute of SPAAC and iEDDA reactions as it allows to perform the conjugations in/on living systems. To demonstrate the utility of the peptides for such application, we incubated live prostate adenocarcinoma cell line (PC3) expressing neuropilin with the TCO-containing neuropilin-targeting peptide Pep2-TCO. Subsequent addition of the fluorogenic coumarin tetrazine probe confirmed that the TCO group on Pep2-TCO is available for functionalization in the cells (see Supporting Information, Section 6.d., Figure S7).

Studies of more complex biological questions often require the use of multiple probes in sequential experiments. An example is the incorporation of unnatural carbohydrates for metabolic glycoengineering. These experiments use metabolic enzymes to install artificial reporter groups onto glycoconjugates of cells, where the reporter group is used for subsequent visualization or pull-down of the glycoconjugates. Typically, such experiments require the use of two different labeling probes. One fluorophore for the visualization and one for the

Figure 2. A) Pep9-TCO-BCN is a multifunctional probe that can be used for labeling and pull-down of metabolically azidated glycoconjugates on live cells. B) Schematic presentation of the experiment. C) Images from confocal microscopy showing live U2OS cells grown in the presence of AcManNAz (or without as control experiment) and labeled with Pep9-TCO-BCN, followed by Tz-Cy3 and anti-FLAG antibody. D) Overlay histogram showing the fluorescence intensity of control (gray) and Cy3 (pink) or FLAG (green) labeled cells analyzed by a flow cytometer. E) SDS-PAGE of glycoproteins from serum-starved (red) and control (green) U2OS cells, labeled with two fluorescent dyes and immunoprecipitated from the pooled lysate using anti-FLAG antibody. Lane 1-control cell lysate, 2-serum starved cell lysate, 3-sample from both lysates mixed in 1:1 ratio, 4-sample from the lysate after pull down of FLAG-labeled proteins, 5-sample from glycoproteins enriched on beads with anti-FLAG antibody. Arrows point to examples of bands showing different expression/glycosylation between the two conditions. MWM—molecular weight marker.
pull-down. Our peptide probes containing two groups with orthogonal reactivity could significantly simplify the experimental workflow in such cases. We decided to examine if the FLAG tag Pep9-TCO-BCN could be used for such application (Figure 2A). We metabolically incorporated azide groups into glycoconjugates of live cells by the use of peracetylated N-azidocetylmannosamine (AcManNAz). The cells were subsequently treated with Pep9-TCO-BCN to covalently attach the peptide to the cell surface glycoconjugates via SPAAC reaction between the incorporated azide groups and the BCN moieties on the peptide. We then used the TCO group to visualize the tagged glycoconjugates on the cells by reaction with Tz–Cy3 fluorophore. The presence of the FLAG tag epitope was confirmed in a sandwich assay using anti-FLAG antibody and fluorescently-labeled secondary anti-mouse antibody (Figure 2B, C, D and Supporting Information Section 6.d.). Importantly, control experiments with cells without prior AcManNAz metabolic labeling did not show the labeling pattern. To investigate if Pep9-TCO-BCN could be used in similar experiments for tracing changes in glycoprotein expression of cells grown under different conditions, we cultivated U2OS cells in medium containing 10% serum or 0.1% serum (starved). We labeled the cells first with Pep9-TCO-BCN and two different Tz dyes (Tz-FA488 for cells grown in 10% serum and Tz–Cy5 for 0.1% serum). After cell lysis, we used the FLAG tag to pull-down the glycoproteins by anti-FLAG antibody beads and analyzed the proteins by SDS-PAGE. Differences in the composition of glycoproteins after FLAG-tag enrichment were clearly visible by overlaying the two channels in the fluorescence images (Figure 2E). These experiments clearly illustrate the potential of the multifunctional peptide probes accessible by the presented synthetic protocol to streamline complex chemical biology experiments.

Conclusions

In summary, we report an optimized modular protocol for the synthesis of peptides containing the highly reactive TCO and BCN dienophiles. Because these moieties are not compatible with standard SPPS, the presented strategy is based on the post-synthetic attachment of the groups via commercially available NHS active esters to lysine residues that are generated on the peptide backbone via selective deprotection steps. The optimized synthetic strategy enables the introduction of these strained groups, or their combination, into virtually any position within the amino acid sequence as we illustrate by the synthesis of a set of mono- and double-clickable group-functionalized peptides of biological interest. We show that the TCO- and BCN-containing peptides can be used for efficient construction of peptide-peptide conjugates and for preparation of peptide-drug conjugates. Due to the high reaction rate and excellent biocompatibility, the TCO- and BCN-modified peptides can serve as useful chemical biology probes as we demonstrate by fluorescent labeling and pull-down experiments of metabolically engineered cell-surface glycoconjugates. The developed strategy allows for construction of versatile peptides that could find utility as next generation therapeutics, bioimaging agents and as multifunctional probes for chemical biology research.

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Conflict of Interest

The authors declare no competing interests.

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