Tetrahedron 70 (2014) 5122–5127

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Development of a fluoride-responsive amide bond cleavage device that is potentially applicable to a traceable linker

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ARTICLE INFO

Article history:
Received 28 April 2014
Received in revised form 29 May 2014
Accepted 31 May 2014
Available online 6 June 2014

Keywords:
Cleavable linker
Fluoride-responsive
Traceable linker
Stimulus-responsive amino acid

ABSTRACT

A fluoride-responsive (FR) amino acid that induces amide bond cleavage upon the addition of a fluoride was developed, and it was applied to an FR traceable linker. By the use of an alkyne-containing peptide as a model of an alkynylated target protein of a bioactive compound, introduction of the FR traceable linker onto the peptide was achieved. Subsequent fluoride-induced cleavage of the linker followed by labeling of the released peptide derivative was also conducted to examine the potential applicability of the FR traceable linker to the enrichment and labeling of alkynylated target molecules.

1. Introduction

A wide variety of molecules including natural products, peptides, and synthetic small compounds exhibit their biological activities through specific interactions with target biomacromolecules. Proteins including enzymes, receptors, and ion channels represent the major group of these targets. Identification of unknown protein targets that interact with biologically active ligands has become indispensable in the fields of chemical biology and drug development; however, this research approach is time-consuming and laborious. The target identification comprises a sequence of processes: (1) fishing a target using a biologically active ligand as bait; (2) enrichment of the hooked target; and (3) sequence analysis of the target by Edman degradation or mass spectrometry (MS).[1] For the first step, photo-affinity labeling, which allows bait to be covalently bounded to the corresponding target upon photo-irradiation has significant use, because of the potential applicability to low affinity ligand-target pairs.[1a,b,2] The hooked target is then linked with a biotinylated linker molecule for facile purification by streptavidin beads using the biotin–streptavidin interaction.[1,3] The immobilized target is subsequently released from the beads for sequence analysis by attenuating the biotin–streptavidin interaction. The high affinity of the biotin–streptavidin interaction (K_d=10^{-15} M),[4] however, hampers liberation of the target from the beads. An alternative to liberate the target is the use of a cleavable linker between the bait and biotin.[5] This approach enables efficient elution of the target protein from the beads via the linker cleavage, but contamination owing to the presence of non-target proteins sometimes hampers identification of the target.[6] The cleavage under mild conditions and generation of an orthogonal functional group not seen in proteins, therefore, has been desired in this procedure. The orthogonal functional group enables chemoselective labeling of the target protein by an isotopic or fluorescent tag. That facilitates discrimination of the target from contaminated proteins by MS using isotopic tag or SDS-PAGE using fluorescent tag.

We previously developed a traceable linker as an advanced cleavable linker that enables selective labeling of the target protein after elution from the streptavidin beads via the linker cleavage

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http://dx.doi.org/10.1016/j.tet.2014.05.110
0040-4020/

(Fig. 1a).[7,8] A key component of the traceable linker is a stimulus-responsive amino acid that possesses a stimulus-removable protective group (PG) on the phenolic hydroxyl group (Fig. 1b).[9] The stimulus-responsive amino acid induces amide bond cleavage after stimulus-induced removal of the PG and subsequent lactonization of a trimethyl lock moiety.[10] In a previous report, the traceable linker composed of a thiol-responsive amino acid, in which the PG is a *p*-nitrobenzenesulfonyl (*p*Ns) group, was presented (Fig. 1a). The traceable linker was introduced onto an alkynylated protein by click chemistry and then adsorbed onto the streptavidin beads. Subsequent addition of a thiol triggered the cleavage of the linker to release the protein possessing an aminooxy group. Since the aminooxy group can react with an aldehyde chemoselectively,[11] the eluted target protein was selectively labeled with an aldehyde-containing labeling reagent even when contaminated non-target proteins co-existed. As the thiol was used as the cleavage inducer in this system, it is preferable to remove endogenous thiols, such as glutathione before the use of the thiol-responsive traceable linker.[12] To avoid the risk of unintentional cleavage of the traceable linker, in this study, we have developed a fluoride-responsive (FR) traceable linker, because there are few fluoride ions present in a living body.[13]

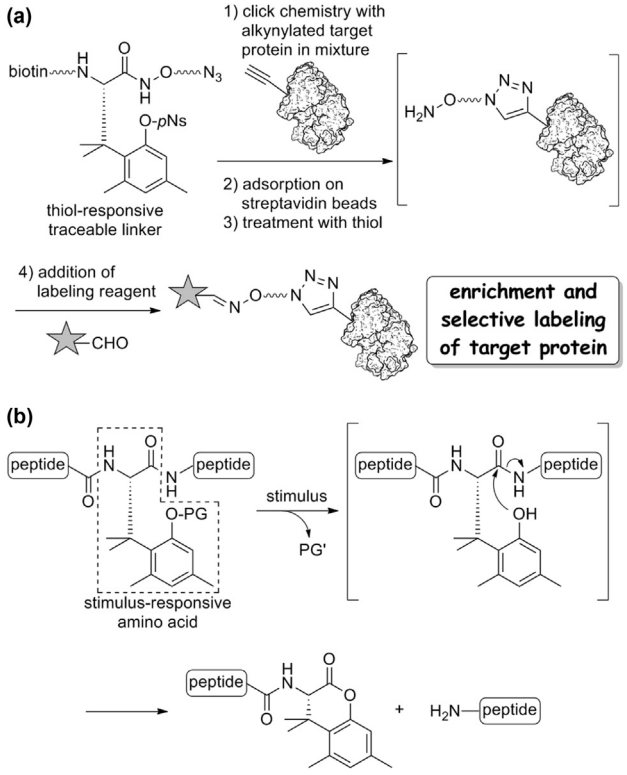
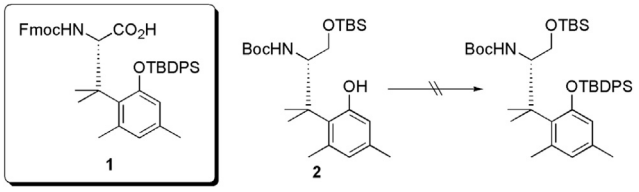


Fig. 1. Molecular design. (a) Purification and selective labeling of an alkynylated protein using a thiol-responsive traceable linker (*p*Ns: *p*-nitrobenzenesulfonyl group). (b) A stimulus-responsive amide bond cleavage system (PG: protective group i.e., removable by appropriate stimulus).

2. Results and discussion

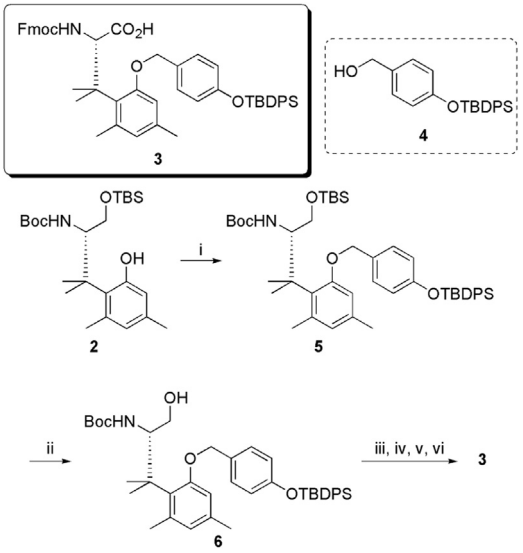
2.1. Synthesis of an FR amino acid

Preparation of the FR amino acid possessing a fluoride-removable protective group as the PG of the stimulus-responsive amino acid was attempted. A *tert*-butyldiphenylsilyl (TBDPS) group was chosen as the fluoride-removable PG because acid treatment is unavoidable for the synthesis of the traceable linker and the TBDPS group is relatively acid tolerant compared with other trisubstituted silyl protections.[14] In this study, Fmoc protected derivatives were designed for Fmoc-based solid phase peptide synthesis (Fmoc SPPS). We first attempted to synthesize silyl ether **1**, but introduction of the TBDPS group onto the phenolic hydroxyl group of **2**[15] did not proceed (Fig. 2). In these reactions, recovery of the starting material, removal of the Boc group and/or removal of the *tert*-butyldimethylsilyl (TBS) group were observed. We speculated that the direct introduction of the TBDPS group onto the phenol is sterically unfavorable and steric crowding around the phenol was observed in an energy minimized structure of substrate **2** using an MM2 calculation (Fig. S1). Therefore, preparation of **3** possessing a sterically less demanding siloxybenzyl unit, which can be removed via fluoride-induced cleavage of the silyl group followed by release of the quinone methide, onto the phenol was next examined (Scheme 1).[16] Starting from phenol **2**, it was subjected to the modified Mitsunobu reaction with the TBDPS derivative **4**[17] using *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (TMAD).[18] The TBS group of **5** was then removed under acidic conditions to yield alcohol **6**. After stepwise oxidation of the alcohol of **6**, the Boc group was removed by the use of Ohfune's protocol,[19] because cleavage of the *p*-siloxybenzyl group was observed when trifluoroacetic acid (TFA) or hydrogen chloride was employed. The obtained amine was finally protected with an Fmoc group to yield FR amino acid **3**.



Entry	Attempted reaction conditions
1	TBDPSCl, imidazole, DMF
2	TBDPSCl, imidazole, DMF, 60 °C
3	TBDPSOTf, 2,6-lutidine, CH_2Cl_2
4	TBDPSCl, LiHMDS, THF
5	TBDPSCl, NaH, DMF
6	TBDPSCl, $AgNO_3$, DMF
7	TBDPSCl, $AgNO_3$, pyridine, 60 °C

Fig. 2. Synthetic attempts to prepare FR amino acid **1** (LiHMDS: lithium hexamethyldisilazide; TBDPSOTf *tert*-butyldiphenylsilyl trifluoromethanesulfonate).



Scheme 1. Reagents and conditions: (i) **4**, TMAD, *n*-Bu_3P, toluene, 98%; (ii) AcOH, THF, H_2O, quant.; (iii) oxalyl chloride, DMSO, Et_3N, THF; (iv) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-BuOH, acetone, H_2O; (v) *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), 2,6-lutidine, CH_2Cl_2; (vi) FmocOSu, Na_2CO_3, acetonitrile (MeCN), H_2O, 43% (four steps).