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A 4-OTBS Benzyl-based Protective Group for Carboxylic Acids

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ABSTRACT

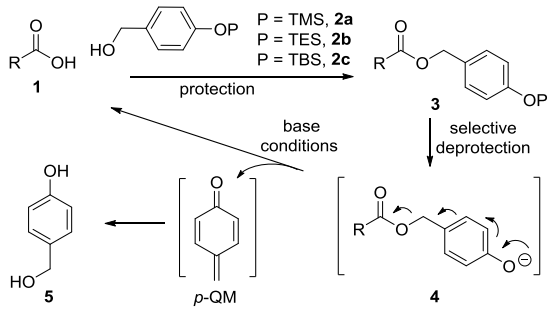
Reported herein is a novel 4-OTBS benzyl-based protective group for carboxylic acids. This protective group can be removed in the presence of TBAF or TFA with high efficiency, which makes it compatible with base-sensitive or acid-sensitive substrates. With this protective group, a near-infrared fluorogenic probe for the detection of γ-glutamyltranspeptidase activities was readily prepared.

Introduction

Carboxylic acid is among the most commonly encountered functional groups in natural products and biologically interesting molecules. Due to the high reactivity, carboxylic acids are normally protected in multiple-step synthesis, mostly in form of ester,[1] such as allyl, benzyl, *tert*-butyl, trimethylsilylethyl, and diphenylmethyl ester. Photolabile protecting groups,[2] for instance, 2,5-dimethylphenacyl,[3] 1-[2-(2-hydroxyalkyl)phenyl]ethanone,[4] aminocoumarin,[5] 3-hydroxy -2-naphthalenemethanol,[6] N-alkyl-4-picolinium (NAP),[7] 2-(1'-hydroxyethyl)-anthraquinone,[8] 3-aminobenzyl,[9] α-carboxy-6-nitroveratryl,[10] and benzoin-derived desyl (Dsy),[11] are another type of compelling protective groups for carboxylic acid because of the unique deprotection strategy. Very recently, Fang reported the use of dM-Dim for protection of carboxylic acid, which allowed selectively deprotection under nearly neutral oxidative conditions.[12] Almost at the same time, an azulene-based protection of carboxylic acids was developed by the Harvey lab, in which, interestingly, the removal of this protection group can be monitored by the change of color.[13] Nevertheless, in spite of great success of these protection strategies, novel protection group for carboxylic acids that is orthogonal to other types of protective groups is still of high demand given the vast variety of chemical transformations involved carboxylic acids in organic synthesis. Herein, we wish to report a 4-OTBS benzyl group for the protection of carboxylic acids, which can be readily introduced and removed in nearly quantitative yields.

Benzyl is one of the most common protective groups for carboxylic acids and the removal of benzyl to regenerate carboxylic acids usually involves hydrogenolysis in the presence of transition metals (e.g., Pd, Pt). This deprotection strategy, though highly efficient, may be less compatible with other hydrogenation-sensitive functional groups, for instance, nitro, carboxybenzyl (Cbz), and olefin.[1a, 1c] To avoid the use of hydrogenolysis and thus make it orthogonal to hydrogenation-sensitive substrates, we envisaged the introduction of a caged hydroxyl on the *para*-position of benzyl might allow releasing protective group in a different manner: once the hydroxyl group is uncaged the resulting 4-hydroxyl benzyl ester **4** will undergo a spontaneously 1, 6-elimination to yield free carboxylic acid **1** and *para*-quinone methide (*p*-QM) under base conditions (Scheme 1). And the highly active *p*-QM may be further attacked by water to form 4-hydroxyl benzyl alcohol (**5**). As a result, we may manipulate the deprotection specificity of carboxylic acid by the selection of proper protective group for 4-hydroxyl group.

Silyl ethers, such as trimethylsilyl (TMS), triethylsilyl (TES), *tert*-butyldimethylsilyl (TBS), and *tert*-butyldiphenylsilyl (TBDPS), are a type of well-studied protective groups for hydroxyl. And stability of these protective groups increases in this order TBDPS>TBS>TES> TMS.[1a, 14] Proper selection of reaction conditions allows removal of these protecting groups selectively.



Scheme 1. Protection of carboxylic acid with caged 4-hydroxyl benzyl.

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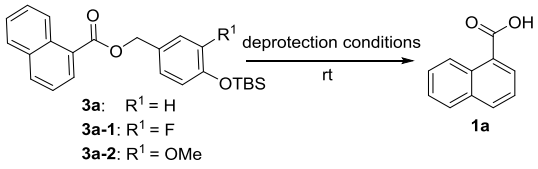
Table 1. Optimization of deprotection conditions[a]



3a: R^1 = H
3a-1: R^1 = F
3a-2: R^1 = OMe

Entry	Substrate	F^- source (equiv.)	Solvent	Time (h)	Yield (%)[b]
1	**3a**	NaF (2)	PBS:THF = 1:2	2	<5 (14)[c]
2	**3a**	$(NH_4)HF_2$ (1)	PBS:THF = 1:2	2	<5 (12)[c]
3	**3a**	Py•HF (2)	PBS:THF = 1:2	2	<5
4	**3a**	Et_3N•3HF (0.67)	PBS:THF = 1:2	2	<5
5	**3a**	KHF_2 (1)	PBS:THF = 1:2	2	<5
6	**3a**	TBAF (2)	PBS:THF = 1:2	2	68
7	**3a**	TBAF (2)	DMF:THF = 1:2	2	75
8	**3a**	TBAF (2)	MeOH:THF = 1:2	2	74
9	**3a**	TBAF (2)	MeCN:THF = 1:2	2	76
10	**3a**	TBAF (2)	THF	2	76
11	**3a**	TBAF (3)	THF	2	81
12	**3a**	TBAF (5)	THF	2	93
13	**3a**	TBAF (8)	THF	0.5	95
14	**3a**	TBAF (10)	THF	0.5	99
15	**3a-1**	TBAF (10)	THF	0.5	97
16	**3a-2**	TBAF (10)	THF	0.5	98

[a] Unless otherwise noted, a mixture of **3** (33 μmol) and fluoride reagent in indicated solvent (0.1 mL) were stirred at room temperature. [b] Determined by RP-HPLC analysis on C18 column. [c] Used $NaHCO_3$ aqueous solution instead of PBS as co-solvent.

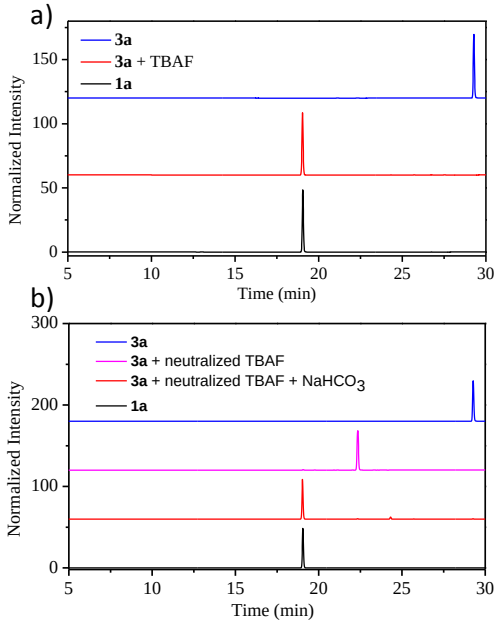
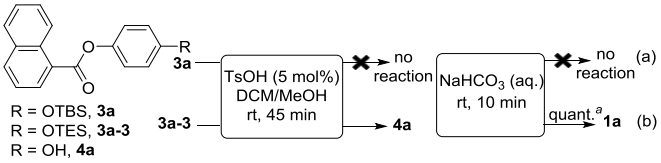


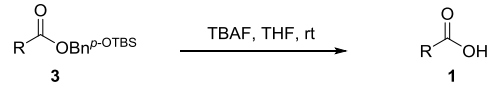
Figure 1. Deprotection of **3a** by TBAF monitored by HPLC. a) HPLC trace of reaction mixture of **3a** and TBAF (red line) in THF for 0.5 h at 310 nm. b) HPLC trace of reaction mixture of **3a** and neutralized TBAF (pH 7) before (magenta line) and after treatment with $NaHCO_3$ (aq.) (red line). See Supplementary Information for details.

To test the feasibility of our hypothesis, we first investigated the use of 4-OTBS benzyl as the protective group for carboxylic acids. Experimentally, we selected 4-OTBS benzyl 1-naphthoic ester (**3a**) as model substrate, partially due to its characteristic UV-Vis absorbance at 310 nm which allows easily monitoring reaction process with HPLC.

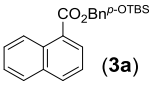
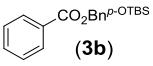
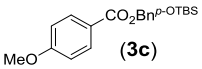
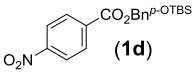
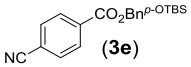
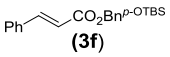
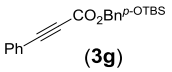
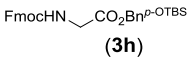
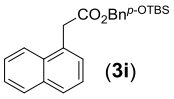


Scheme 2. Selective deprotection of substituted benzyl esters. **3a** or **3a-3** (24 μmol) and TsOH (5 mol%) in DCM/MeOH (1:1, 0.42 mL) were stirred at rt for 45 min, followed by the addition of NaHCO3 (aq., 0.42 mL). [a] Determined by HPLC analysis.

Table 2. Deprotection of *p*-OTBS benzyl esters.[a]



3 **1**

Entry	Ester	Acid	Yield (%)[b]
1	 (**3a**)	**1a**	95
2	 (**3b**)	**1b**	93
3	 (**3c**)	**1c**	98
4	 (**1d**)	**1d**	98
5	 (**3e**)	**1e**	99
6	 (**3f**)	**1f**	96
7	 (**3g**)	**1g**	97
8[b]	 (**3h**)	**1h**	93[c] (97[d])
9[b]	 (**3i**)	**1i**	73 (94[d])

[a] Unless otherwise noted, **3** (0.28 mmol) was treated with TBAF (1.0 M in THF, 2.8 mL) for 0.5 h at rt. [b] Isolated yield. [c] Mixed with pre-neutralized TBAF (pH 7) for 0.5 h followed by the treatment of $NaHCO_3$ (aq.). [d] Conducted with a mixture of TFA:DCM (1:1).

Fluoride ion has been reported as efficient TBS-deprotection agent.[1a] Therefore, a number of fluoride sources, including sodium fluoride, ammonium hydrogen difluoride, pyridine hydrogen fluoride, triethylamine trihydrofluoride, potassium bifluoride, and tetrabutylammonium fluoride (TBAF), were initially examined and a mixture of phosphate buffered saline (pH = 7.4) and tetrahydrofuran (THF) was used as solvent (Table 1). To our surprise, the use of sodium fluoride, ammonium hydrogen difluoride, trimethylamine trihydrofluoide, and potassium bifluoride, did not lead to the formation of desired free carboxylic acid **1a** but remained basically unreacted, which might be resulted from the poor solubility of these fluoride reagents in THF (entries 1, 2, 4, and 5). Replacing PBS with an aqueous solution of sodium bicarbonate as co-solvent slightly promoted the deprotection process, affording desired free acid in less than 15% yield. Pyridine hydrogen fluoride seemed to be more efficient to remove TBS-protecting group; about 40% of starting material had been converted to corresponding 4-hydroxyl benzyl ester (**4a**) but only trace amount of **1a** was detected (entry 3). Fortunately, TBAF, the typical reagent for TBS-deprotection, was

proven to be also efficient in the removal of 4-OTBS benzyl, affording **1a** in 68% yield (entry 6).

Encouraged these results, we further performed solvent screening for this deprotection reaction and it turned out this process was compatible with a number of organic solvents, including *N, N*-dimethyl formamide (DMF), methanol, acetonitrile, and THF (entries 7-9). On the other hand, increasing the amount of TBAF could further accelerate this reaction (entries 10-14). As shown in Figure 1a, 10 equivalents of TBAF led to the completion of deprotection within 0.5 h and exclusively formed free acid **1a** (based on the HPLC analysis of the reaction mixture). Moreover, from the reaction mixture, the adduct of QM and water, 4-hydroxy benzyl alcohol (**5**), was identified by the HPLC (Figure 1S) and LC-MS analysis, which is in line with our initial hypothesis. Further investigation revealed that the substituents on the phenyl ring of benzyl, for example, fluoro (electron withdrawing group, **3a-1**) and methoxyl (electron donating group, **3a-2**), had little effect on this process; all of these substrates gave practically identical results.

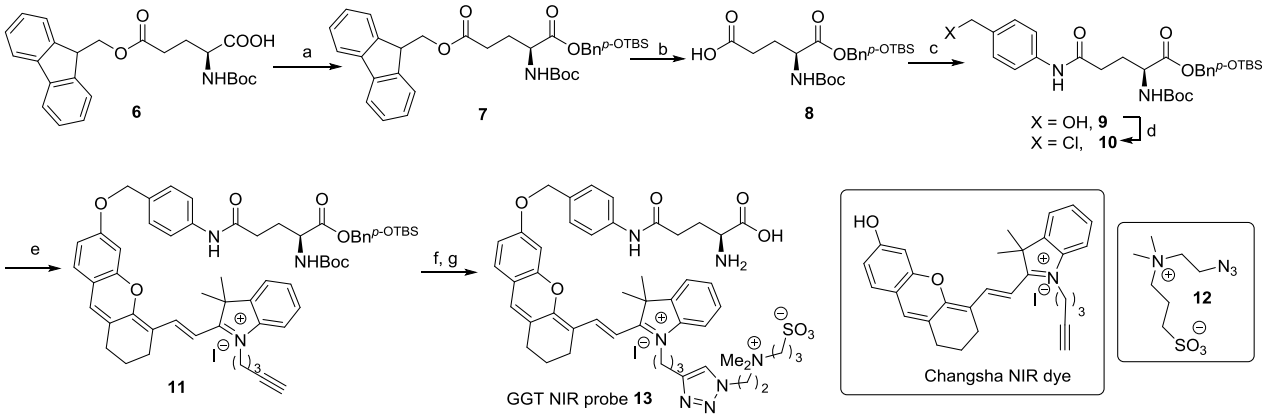
To gain more information on the TBAF-mediated deprotection process, we incubated **3a** and pre-neutralized TBAF (pH = 7) for 30 minutes. HPLC analysis of the reaction mixture indicated that all of the starting material **3a** disappeared, leaving the corresponding 4-hydroxyl benzyl ester (**4a**) as the only product (Figure 1b). This ester was not stable upon addition of sodium bicarbonate aqueous solution; it underwent a 1,6-elimination and converted to free acid **1a** completely within a short period of time. The successful removal of 4-OTBS benzyl with neutralized TBAF makes it orthogonal to base-sensitive functional group.

These results support our initial design that the removal of 4-OTBS benzyl group is a two-step process and the use of weak base can promote the hydrolysis of 4-hydroxyl benzyl ester. Therefore, besides TBS, other hydroxyl-protective groups, such as TMS, TBDPS, and allyl, could also be used in the protection of carboxylic acids, and thus renders versatile deprotection selectivities, which is particularly useful in the synthesis of carboxylic acid-involving compounds with multiple functional groups. For instance, 4-OTBS benzyl protected acid (**3a**) was inert to catalytic amount of *p*-toluenesulfonic acid while 4-OTES benzyl acid (**3a-3**) was labile to such condition,[15] yielding 4-OH benzyl ester **4a** exclusively. The addition of sodium bicarbonate aqueous solution led to the formation of free acid **1a** in quantitative yield (Scheme 2 and Figure 3S).

Electron-rich benzyl group (e.g., *p*-methoxybenzyl) might be sensitive to strong acid.[1a] To test whether strong acid conditions could be used as an alternative way to remove the 4-OTBS benzyl group, we treated **3a** with a 1:1 mixture of trifluoroacetic acid (TFA) and dichloromethane (DCM) at room temperature. HPLC analysis (Figure 2S) revealed that all of the starting material had been exclusively converted to **1a** within 5 minutes (> 99% yield based on HPLC), indicating acid condition could be an alternative manner for the deprotection of 4-OTBS benzyl.

As a protective group, both the protection and deprotection process are crucial to the overall synthetic efficiency. We identified the use of *N*-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydro chloride (EDCI) as coupling reagent along with 1.1 equivalents of 4-Dimethylaminopyridine (DMAP) provided optimal yield of 4-OTBS benzyl ester.[1a, 16] Under such reaction conditions, a number of esters, including aromatic (**3a-3e**) and aliphatic carboxylic esters (**3h**, **3i**), as well as α, β-unsaturated carboxylic esters (**3f**, **3g**), were prepared in excellent yields (94-98%, Scheme 1S).

Having demonstrated our protection strategy for carboxylic acid,



Scheme 2. Preparation of GGT NIR probe **13**. (a) **2c**, EDCI, DMAP, DCM, 2.5 h, 97%; (b) Piperidine, DMF, 15 min; (c) 4-aminobenzyl alcohol, EDCI, DMF, 1h, 70% from **7**; (d) $SOCl_2$, DMF, 0 °C, 1 h; (e) Changsha NIR dye, $KHCO_3$, 18-C-6, KI, DMF, 2 h, 52% from **9**; (f) TFA:TIPS:DCM = 45:5:50, 2 h; (g) **12**, $CuSO_4$, THPTA, Vc, H_2O:DMSO = 1:1, 5 h, 41% from **11**.

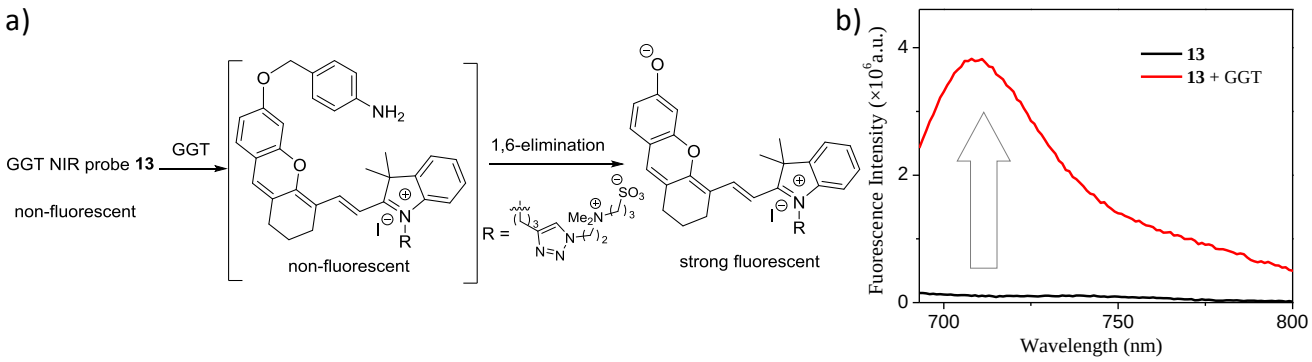


Figure 2. a) GGT-mediated cleavage of probe **13** and turn-on of fluorescence; b) Fluorescent spectra of probe **13** before (black line) and after incubation with GGT (red line). λ_{ex} = 682 nm.

we next sought to explore the scope of this deprotection process. As shown in Table 2, TBAF was highly efficient in removal of 4-OTBS benzyl protection for all aromatic esters with up to 99% yield, regardless of the electron properties of substituents on the aromatic rings. Moreover, cinnamic ester **3f** and phenylpropioloic ester **3g** were also deprotected smoothly in the presence of TBAF, releasing corresponding free acids in excellent yields (96% and 97%, respectively) within 0.5 h. 4-OTBS benzyl *N*-Fmoc glycine ester (**3h**) seemed to be incompatible with TBAF and led to deprotection of both 4-OTBS benzyl and *N*-Fmoc, which is likely due to strong basicity of TBAF.[17] Fortunately, the treatment of **3h** with neutralized TBAF helped to prevent the deprotection of Fmoc. Simply workup with sodium bicarbonate aqueous solution afforded free acid in 93% yield (entry 8). In the meantime, deprotection of **3h** was also efficient in acid conditions; Fmoc-protected glycine **1h** could be obtained in 97% yield upon incubation **3h** with a 1:1 mixture of TFA and DCM for only 5 minutes in room temperature. Under acid conditions, the deprotection of 4-OTBS benzyl naphthalene acetic ester went smoothly, affording desired free acid **1i** in 94% yield, though, to our surprise, the TBAF-mediated deprotection resulted in only moderate yield (73%).

γ-Glutamyltranspeptidase (GGT)[18] is a cell surface-bound protease, which catalyzes hydrolysis of γ-glutamyl bond of glutathione, as well other γ-glutamyl compounds. This enzyme takes part in cysteine homoeostasis and cellular glutathione, both of which are closely related to a variety of physiological and pathological processes, such as diabetes, asthma and cancer. Accurate visualization activities of GGT is of high importance for disease diagnosis at early stage. Activatable fluorescent probes, especially near-infrared (NIR) fluorescent probes, are particularly useful for the detection of GGT activities *in vivo*.

GGT probes[19] are mostly derivatives of glutamate, whose synthesis usually involved protection of free carboxylic acid. Herein, to further demonstrate the usability of our carboxylic acid protection approach, we applied it in the synthesis of fully functionalized GGT NIR-fluorogenic probe **13**.

As shown in Scheme 2, the synthesis started from coupling commercially available protected glutamate **6** and 4-OTBS benzyl alcohol, resulting **7** in 97% yield. The Fmoc protective group of **7** was then selectively removed by treatment of 10% piperidine in DMF, followed by coupling with 4-aminebenzyl alcohol to afford **9** in 70% for two steps. These results indicated the 4-OTBS benzyl protected carboxylic acid is stable enough in 10% of piperidine in DMF (typical conditions for the deprotection of Fmoc) and thus makes such protective group compatible with Fmoc protection. Chlorination of **9** and subsequent nucleophilic substitution by the alkyne-containing Changsha NIR fluorophore yielded key intermediate **11** in 52% yield (two steps). The Boc and 4-OTBS benzyl protective groups were removed simultaneously in the presence of a mixture of TFA, triisopropylsilane (TIPS) and DCM (45:5:50). The final probe was obtained after a CuAAC click reaction.[20]

As exhibited in Figure 2, probe **13** is basically non-fluorescent. However, upon incubation with GGT in PBS, strong fluorescent signal at 708 nm was detected, demonstrating its potential in detection of GGT activities. Further application of this probe in biological system is still under investigation.

In summary, we have developed a novel 4-OTBS benzyl-based protective group for carboxylic acids. This protective group can be readily removed in the presence of TBAF or TFA, which makes it compatible with base-sensitive or acid-sensitive substrates. The merits of this protective group are highlighted by the excellent installation and deprotection yields. The usability of this protective group was further demonstrated by the efficient synthesis of γ-glutamyltrans-peptidase NIR probe **13**. Moreover, mechanism studies have confirmed the TBAF-mediated deprotection of 4-OTBS benzyl ester undergoes 4-hydroxylbenzyl ester intermediate followed by a 1,6-elimination in base conditions. This allows the use of versatile hydroxyl-protective groups to cage 4-hydroxylbenzyl and thus control the selectivity of carboxylic acid deprotection, which is particularly useful in the synthesis of multi-functional molecules.

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Supplementary data

Experimental procedures, characterizations, ^{1}H NMR and ^{13}C NMR spectra, HPLC traces are available in the online version.

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Zhijun Fang, Yuyao Li and Hexin Xie*

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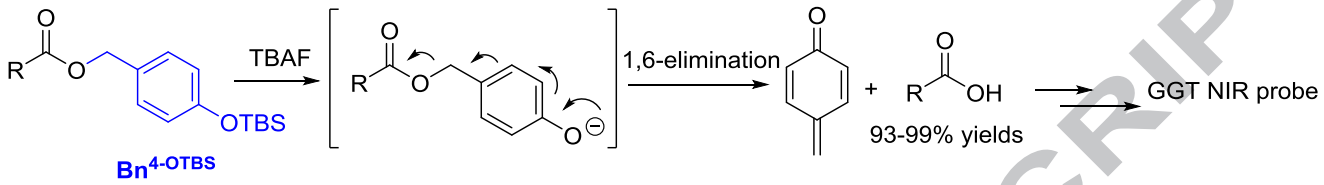

$Bn^{4\text{-OTBS}}$

Research highlights

- Excellent protection and deprotection yields.
- Compatible with base-sensitive or acid-sensitive substrates.
- High selectivity