Understanding Tetrahydropyranyl as a Protecting Group in Peptide Chemistry

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1. Introduction

The formation of a peptide bond requires the reaction between an activated carboxylic acid of an amino acid—typically an active ester—and the amino function of another amino acid.[1, 2] To avoid side reactivity, the remaining functional groups of each amino acid have to be conveniently protected.[3] In solid-phase peptide synthesis (SPPS), the carboxylic acid of the reacting amine is usually attached to the solid support. Furthermore, it is noted that protection of amino acid side chains is often required in SPPS due to the presence of a wide range of reactive functional groups along a peptide sequence. Thus, during peptide elongation, various types of protecting groups might co-exist.[4]

In the area of peptide synthesis, protecting groups[5] are divided into three main classes: 1) permanent protecting groups, which are removed at the end of the synthetic process. These comprise mainly side-chain protecting groups and the C-terminal protecting group, which, in the case of SPPS, is effectively the linker to the solid support; 2) temporary protecting groups, which are removed after each synthetic step and include mainly those protecting the α-amino groups; and 3) semi-permanent protecting groups, which are removed in the middle of the synthetic process to perform a reaction that requires the presence of other protecting groups, such as for the formation of branched[6–8] or cyclic peptides.[9, 10]

For SPPS, amino acid protecting groups should be: 1) easily introduced, 2) stable during peptide sequence elongation, and 3) easily and orthogonally removed. To measure the balance between stability and instability of the protecting groups, orthogonal strategies represent the best choice in the sense that two protecting groups belong to independent chemical classes and consequently they can be removed by different chemistry.[10] Furthermore, the two groups can be removed in any order. Orthogonal protection schemes are milder because the selective deprotection is governed by alternative cleavage mechanisms rather than by reaction rates. This is perfectly implemented if Nα-(9-fluorenlymethyloxycarbonyl) (Fmoc) amino acids are used in combination with acid-labile side-chain protecting groups, as well as a C-terminal amino acid that is anchored to a resin. Whereas the Fmoc group is removed after each coupling step by repetitive treatment with piperidine/DMF (1:4), the side-chain protecting groups and C-terminal peptide cleavage is achieved using acid treatment, typically with trifluoroacetic acid (TFA). These acid-labile protecting groups are based on benzyl-derived electron-donating groups or derivatives of tertiary alcohols. In this regard, tert-butyl (tBu) and trityl (Trt) are the most commonly used protecting groups. In order to trap the carbocation formed, these derivatives are removed in acidic conditions in the presence of scavengers.[4]

In peptide chemistry, little attention has been paid to the tetrahydropyranyl (Thp) group, which has otherwise been recognized as a useful protecting group for alcohols in organic synthesis.[11] Historically, the extensive contribution of Paul to the chemistry of pyran and furan rings attributes him with the discovery of Thp as a hydroxyl protecting group (Paul et al., in 1934, were credited for the discovery of pyran and furan rings, and Thp was not used for –OH protection until then; however, Anderson et al., in 1948, used them for hydroxyl protection). In 1934, Paul observed that 2-methoxytetrahydropyran was ob-
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Professor Fernando Albericio developed his academic career in Europe, USA, Latin America, and presently in Africa. His major research interests cover practically all aspects of peptide synthesis and combinatorial chemistry methodologies, as well as the synthesis of peptides and small molecules with therapeutic activities, especially against tuberculosis, infectious diseases, and cancer. His research group is also involved in the development of new strategies for drug delivery. He is deeply involved in the development of the third mission of the University: the transference of knowledge and technology to society.
tained after adding methanol to 3,4-dihydro-2H-pyran (DHP) in the presence of HCl. Later, Woods and Kramer studied the addition of various alcohols to the dihydropyran ring in greater depth and also postulated the stability of these tetrahydropropyran-acetal functionalities under basic conditions. However, the description of Thp as an alcohol-protecting group was not investigated until 1948, when Parham and Anderson reported the conversion of alcohols to acetals and their easy cleavage in mildly acidic aqueous conditions with the regeneration of the hydroxyl group.

The Thp group offers versatile protection of hydroxyl functional groups (alcohols and sometimes phenols) during multi-step organic synthesis. Thp ethers are attractive because of the low cost of DHP, its easy incorporation, the stability of the resulting Thp derivatives in various reaction conditions, and ease of removal under mildly acidic conditions (Scheme 1). Thp has the advantage over benzyl-based protecting groups, such as Trt, diphenylmethyl (Dpm), 4-methoxyphenyl(diphenylmethyl (methoxytrityl, Mmt) or benzyloxymethyl (Bom), because it lacks aromaticity and offers better solubility. In addition to protecting alcohols and sometimes phenols during multi-step organic synthesis, Thp affects the inter-/in-chain interactions during peptide elongation, which might compromise the purity of the final product.

In view of the aforementioned observations, here we provide a concise analysis of the Thp protection of various amino acids functionalities (NH, SH, OH and COOH) and its application to peptide synthesis (Figure 1). This Review is organized according to the functional group protected. For each case, methods for the introduction and removal of the protecting groups, as well as the scope and limitations, are discussed.

2. Thp Protection of the NH Group (Tryptophan)

2.1. General

The dihydropyranylation of nitrogen-based functional groups in SPPS has not been extensively studied. The reversible binding of purines through one of the nitrogen atoms of imidazole has been reported, as well as through the indolic nitrogen, for the synthesis of 2,3-disubstituted indoles. Additionally, Cheng and Hii reported the solution-phase synthesis of -alkylaminotetrahydrofurans, as well as -alkylamino- and -arylaminotetrahydrofurans under neutral pH conditions by a hydroamination reaction catalyzed by palladium thiocyanate complexes. The authors concluded that the -aminotetrahydrofuran ring underwent rapid reversible ring-opening in aqueous solution, as revealed in studies on N-glycosidic bond lability in aqueous media with N-glucosamines.

More recently, Nicolás et al. used the dihydropyran-yl linker to anchor the indolic nitrogen of the amino acid Trp to a solid support for the synthesis of Trp-containing diketopiperazines. Furthermore, they determined the conditions required for Thp removal, which is achieved using a strong acid (TFA) in the presence of a carbocation scavenger, as discussed below.

2.2. Introduction of the Protecting Group

The protocol for alkylamine tetrahydropropyranylation described by Cheng and Hii considered only the use of dialkylamines and aniline for the hydroamination reaction catalyzed by palladium thiocyanate complexes over long reaction times (>12 h) and high temperature (70–100 °C).

The introduction of a tetrahydropropyran moiety into primary amines is challenging, and our lab attempted the protection of Phe with Thp. For this purpose, we used various commercially available Pd catalysts and also prepared the catalyst that the aforementioned authors demonstrated to be the most efficient for the N-Thp linkage, that is, K2Pd(SCN)4. However, we were unable to introduce an N-Thp group into Phe using Pd chemistry under these conditions.

Interestingly, the introduction of the Thp moiety at the indolic nitrogen of Trp was more successful and has a greater impact on SPPS. The protocol described by Nicolás et al. involved the reaction of Fmoc-Trp-OR (R = Allyl or Me) with DHP using pyridinium p-toluenesulfonate (PPTS) as catalyst and 1,2-dichloroethane (DCE) as solvent at 70 °C for 8 h. A yield of 80% was achieved for Fmoc-Trp(Thp)-OMe and 69% for Fmoc-Trp(Thp)-OAllyl. Furthermore, they also anchored a Trp derivative on Ellman resin through its side chain using PPTS as a catalyst in anhydrous DCE under microwave conditions for 2 h at 120 °C. The authors realized that, in order to attach Trp to a tetrahydropropyran-functionalized resin through its indole ring, the -amino and carboxyl functions had to be protected.
with groups compatible with the conditions necessary for the formation of the hemiaminal linkage and peptide chain elongation.\textsuperscript{[22]}

In order to amplify the use of Thp as a protecting group for SPPS, and taking into account the side-chain anchoring of Trp, we attempted to protect the indole side chain of Fmoc-Trp-OH with Thp (see Scheme 1). The protocol followed involved reaction of Fmoc-Trp-OH with DHP using p-toluenesulfonic acid (PTSA) as catalyst. The mixture was left to react at room temperature for 2.5 h and then washed with water and brine. Purification by column chromatography on silica gel using CH\textsubscript{2}Cl\textsubscript{2}/MeOH (96:4) as eluent yielded the protected Fmoc-Trp(Thp)-OH (82% yield). Although the carboxylic acid reacts with DHP in a similar way, the corresponding hemiacetal ester was not stable to the aqueous work-up, so Thp was cleaved from the carboxyl group. Thus, it can be concluded that Fmoc-Trp-OH can be side-chain protected with Thp without prior protection of the carboxyl group (Scheme 2).

### 2.3. Removal of the Protecting Group

The reported conditions for the cleavage of the hemiaminal bond between the indolic nitrogen and the Thp moiety of Ellman resin involve a mixture of TFA/1,3-dimethoxybenzene/CH\textsubscript{2}Cl\textsubscript{2} (5:10:85) at room temperature for 1 h.\textsuperscript{[22]} In addition, we have studied the conditions of cleavage of Thp from Fmoc-Trp(Thp)-OH using various concentrations of TFA in the presence of scavengers (see Table 1). A comparison was made between Fmoc-Trp(Boc)-OH and Fmoc-Trp(Thp)-OH to test lability under different acidic conditions (Table 1). In this regard, the cleavage cocktail containing TFA/H\textsubscript{2}O/CH\textsubscript{2}Cl\textsubscript{2} (10:2:88) was the best choice, achieving nearly 90% deprotection within 1 h of treatment for Fmoc-Trp(Thp)-OH (Table 1, entries 11–13). In contrast, only 69% deprotection was achieved for Fmoc-Trp(Boc)-OH (Table 1, entries 4–6). However, even at a higher concentration of TFA (60%), complete cleavage of Thp could not be achieved.

### 2.4. Peptide Elongation

As mentioned above, Nicolás et al. used a tetrahydropryan moiety to perform the synthesis of diketopiperazines using SPPS. They demonstrated that the N-Thp bond is compatible with the steps of SPPS: protecting group introduction and elimination, amino acid coupling, and peptide cleavage.\textsuperscript{[22]}

In order to expand the scope of this work, we introduced Fmoc-Trp(Thp)-OH smoothly into several model peptides. However, the removal of Thp from Thp-protected Trp-containing peptides did not take place quantitatively under the cleavage conditions tested. After several trials, we concluded that Thp does not prevent alkylation of the indole during TFA cleavage, because this is the main side reaction to be avoided in peptide synthesis.\textsuperscript{[23, 24]}

### 3. Thp Protection of the Thiol Group (Cysteine)

#### 3.1. General

Protection of the thiol group is crucial for many fields of organic chemistry research, particularly in peptide and protein synthesis. Table 1 presents the results of the acid lability studies of Fmoc-Trp(Thp)-OH using various concentrations of TFA in the presence of scavengers (see Table 1). A comparison was made between Fmoc-Trp(Boc)-OH and Fmoc-Trp(Thp)-OH under different acidic conditions (Table 1). In this regard, the cleavage cocktail containing TFA/H\textsubscript{2}O/CH\textsubscript{2}Cl\textsubscript{2} (10:2:88) was the best choice, achieving nearly 90% deprotection within 1 h of treatment for Fmoc-Trp(Thp)-OH (Table 1, entries 11–13). In contrast, only 69% deprotection was achieved for Fmoc-Trp(Boc)-OH (Table 1, entries 4–6). However, even at a higher concentration of TFA (60%), complete cleavage of Thp could not be achieved.

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syntheses, which often involve the use of Cys.\textsuperscript{[24]} Thp has also been found to be efficient and stable for the protection of thiol groups. Parham and DeLaitsch studied and compared the pyranylation of hydroxyl and thiol groups, observing higher reactivity for pyranylation of hydroxyl groups and less stability for the resulting tetrahydropyran acetal than for thioacetals.\textsuperscript{[14, 26]} In 1958, Holland and Cohen addressed the introduction of DHP into Cys for the protection of the thiol group for the synthesis of insulin peptides in solution.\textsuperscript{[27]}

Although thiol groups are protected most widely as thioethers, especially Trt-, Dpm-, and benzyl-like groups, developed in the labs of Yajima and Nishiuchi, S,O-acetal groups, such as Bom for Boc chemistry\textsuperscript{[28]} and more recently methoxybenzoxymethyl (MBom) for Fmoc chemistry,\textsuperscript{[29]} have also been used. However, these derivatives can result in a very low level of racemization, hinder Bom and MBom synthesis, and hamper the quality of the final product because formaldehyde is formed as a side product during cleavage and is accompanied by concomitant hydroxymethylation.\textsuperscript{[30]} With the same idea of exploiting the S,O-acetal protecting group concept, we introduced Thp as a Cys-protecting group for SPPS.\textsuperscript{[31]} We demonstrated that the use of Thp resulted in lower racemization levels than the conventional protecting groups for Cys (Trt, Dpm and S-tBu), either if using Thp in the peptide sequence or as a C-terminal Cys-protecting group.\textsuperscript{[31]}

Additionally, the solubility of Thp-protected peptides was improved with respect to strategies involving the commonly used hindered aromatic protecting groups. Furthermore, Thp is suitable for protecting N-terminal Cys residues, and, in comparison to other protecting groups, it does not lead to formulation upon removal of the protecting group.\textsuperscript{[32]} Our results revealed that Thp is a useful protecting group for Cys if applied to the Fmoc/tBu strategy for SPPS.

### 3.2. Introduction of the Protecting Group

Thp-protected Cys can be prepared from Cys and DHP in the presence of BF\textsubscript{3}-Et\textsubscript{2}O using Et\textsubscript{2}O as the solvent at room temperature for 30 min\textsuperscript{[33]} or by reaction with DHP in an acidic medium.\textsuperscript{[27]} However, we have recently demonstrated that the most convenient method for protecting Fmoc-Cys-OH with Thp is in the presence of PTSA in CH\textsubscript{2}Cl\textsubscript{2} for 60 min at room temperature,\textsuperscript{[31]} as shown in Scheme 3.

#### 3.3. Removal of the Protecting Group

The methods reported in the early literature described the removal of Thp based on the use of H\textsubscript{2}SO\textsubscript{4}, Lewis acids, aqueous acetic acid, Selectfluor, or PPTS in alcoholic solvent.\textsuperscript{[34–37]} We demonstrated that a convenient method for Thp removal involves the use of > 10% TFA in the presence of scavengers such as water and triisopropylsilane (TIS). Therefore, the conventional cleavage conditions used in SPPS, such as TFA/TIS/CH\textsubscript{2}Cl\textsubscript{2} (10:2.5:87.5), TFA/TIS/H\textsubscript{2}O (95:2.5:2.5), and 0.1 N HCl in HFIP–TIS (99:1) will ensure complete elimination of Thp in short treatments.\textsuperscript{[38]}

A comparison was made between the protected amino acids Fmoc-Cys(Thp)-OH and Fmoc-Ser(Thp)-OH to test elimination conditions in acidic media. Table 2 shows a comparison of the lability of Fmoc-Ser(Thp)-OH and Fmoc-Cys(Thp)-OH in aqueous solutions. It was concluded from the data that Fmoc-Cys(Thp)-OH is more stable compared to Fmoc-Ser(Thp)-OH under moderately acidic aqueous conditions (Table 2, entries 1–5).

During studies on the removal of Thp, we observed that the thioacetal in Fmoc-Cys(Thp)-OH remained stable to mildly acidic conditions, whereas the acetal in Fmoc-Ser(Thp)-OH underwent smooth cleavage. Accordingly, a hydrolysis study at pH 4.8 was carried out by HPLC over 3 days (Figure 2).

### Table 2. Study of Thp removal from Fmoc-Cys(Thp)-OH and Fmoc-Ser(Thp)-OH.\textsuperscript{[35]}

| Entry | Compound | Cocktail composition | Reaction time [h] | Deprotected amino acid [%] |
|-------|----------|----------------------|-------------------|---------------------------|
| 1     | Fmoc-Cys(Thp)-OH | 100 mM MES\textsuperscript{[27]} (pH 4.8) | 48 | 0 |
| 2     | Fmoc-Ser(Thp)-OH | 0.1 mM MES, 1.5% TIS (pH 4.8) | 48 | 0 |
| 3     | Fmoc-Cys(Thp)-OH | 100 mM MES, 137 mM NaCl, 2.7 mM KCl (pH 4.8) | 48 | 0 |
| 4     | Fmoc-Ser(Thp)-OH | 100 mM MES, 1.5% TIS (pH 4.8) | 48 | 0 |
| 5     | Fmoc-Cys(Thp)-OH | 100 mM MES, 1.5% TIS (pH 4.8) | 48 | 0 |
| 6     | Fmoc-Cys(Thp)-OH | PBS; 10 mM PO\textsubscript{4}\textsuperscript{3–}, 2.7 mM KCl, 137 mM NaCl (pH 7.4) | 120 | 0 |
| 7     | Fmoc-Ser(Thp)-OH | PBS; 10 mM PO\textsubscript{4}\textsuperscript{3–}, 2.7 mM KCl, 137 mM NaCl (pH 7.4) | 120 | 0 |

\textsuperscript{[a]} 2-(N-Morpholino)ethanesulfonic acid.
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highly relevant for the protection of unnatural ω-hy-

amino acids, which are difficult to synthesize, such as 

the allo-Thr derivatives and other β-hydroxy amino acids that naturally occur as cyclodepsipeptides. 

4. Thp Protection of the Hydroxy Group (Serine and Threonine)

4.1. General

The use of Thp as a protecting group for Ser and Thr was ten- 

tatively addressed due to the generation of a new stereocenter 

that complicated the isolation of intermediates. Nevertheless, 

some successful examples of Thp protection in the solution 

phase have been reported, including the preparation of the 

iron-chelating siderophores enterobactin and salmoche-

lin, a cyclic trimer of l-Ser, as well as for the syntheses of 

cyclic depsipeptides, oxazoles, oxazolidin-5-ones and 

analogues of 7-keto-8-aminopelargonic acid vitamers. 

Furthermore, the O-Thp, N-Fmoc protected version of Thr was 

used as part of interesting SPPS strategies described by Krish-

namoorthy et al. and Iijima et al. for the total syntheses of 

callipeltin B and (4-) -antimycin A, respectively. 

In addition to the abovementioned use as a protecting 

group, the Thp moiety was also studied as a cleavable linker for SPPS. Thompson and Ellman introduced the use of a DHP- 

functionalized support. The use of Ellman resin provides a 

general and straightforward method for attachment of pri-

mary and secondary alcohols through the base-stable tetrahy-

dropropyran ethylene linkage. Furthermore, this resin has proved 

convenient for the synthesis of a wide range of hydroxyl-con-

taining organic compounds. Accordingly, Ellman resin has found application in the synthesis of alcohol-containing 

peptides.

4.2. Introduction of the Protecting Group

The general method for preparing Thp ethers is by reaction 

of the hydroxyl group with DHP in the presence of appropriate 

catalysts. The examples described for hydroxyl group protection 

include the use of hydrochloric acid, PTSA, BF3·Et2O, PPTS, 

electrogenerated acid (EG acid), Amberlyst H-15, Nafion-H, montmorillonite clay K-10, H-Y zeolite, Spanish sepiolite clay, and dicyanoketene ethylene. However, only a few studies have addressed the use of Thp as a protecting 

group of Ser and Thr for SPPS. Few examples can be found 

in the literature describing the introduction of Thp as a protecting 

group for these amino acids. The protocols most widely 

used involve the introduction of DHP either in the presence of 
PPTS at 60 °C for 16 h or PTSA at room temperature for 30– 

60 min using CH3Cl or DCE as the solvent. For instance, Fmoc-Thr(Thp)-OAllyl was synthesized by adding DHP followed by PPTS (2.5 mol%) in DCE. The reaction was then stirred for 

12 h at 60 °C. Finally, removal of the allyl group afforded 

Fmoc-Thr(Thp)-OH as the key intermediate for the synthesis of callipeltin B.

Nevertheless, we have demonstrated that the protection of 

the carboxylic acid group is not necessary for the preparation of 

Fmoc-Thr/Ser(Thp)-OH. Although the carboxylic acid reacts with DHP similarly to hydroxyl groups, the corresponding hemi-

acetal ester was found to be unstable to aqueous work-up, 

generating the free carboxylic acid. Thus it can be concluded that Fmoc-Thr/Ser-OH can be side-chain protected without extra protection of the carboxyl group (Scheme 4). This conclu-

sion is highly relevant for the protection of unnatural ω-hy-

droxy amino acids, which are difficult to synthesize, such as 

the allo-Thr derivatives and other β-hydroxy amino acids that naturally occur as cyclodepsipeptides.

Furthermore, we have also demonstrated that the use of PTSA as a catalyst is more convenient and efficient than PPTS. Accordingly, PPTS has been reported as a catalyst for pyryla-

tion, a process that involves heating the reaction mixture to almost 60 °C for 10–12 h. However, compared to PPTS, PTSA is more efficient as the rate of the reaction increases. The side-chain anchoring of protected Thr and Ser and amino alcohols 

to Ellman resin has been achieved using PPTS as catalyst in 

DCE for 16 h at 60–80 °C. Furthermore, we have demonstrated that the hydroxyl group can also be introduced onto 

Ellman resin using PTSA as catalyst in THF for 30 min at room temperature.

4.3. Removal of the Protecting Group

It has been reported that Ser and Thr hydroxyl side chains 

protected with Thp are deprotected under acidic conditions. In this regard, HCl, TFA-containing cocktails (1, 2 or 95% TFA), TsOH or PPTS in alcoholic solvents have been reported to cleave the Thp group. Accordingly, we have recently confirmed that Thp removal can be achieved using 2% TFA in CH3Cl in the presence of scavengers. Unexpectedly, it was found that Thp protection of the Thr hydroxyl side chain is slightly more labile than in the case of Ser. We also showed that the Thp protecting group of the Ser hydroxyl can
be partially removed by acidic aqueous solutions (see Table 2). This latter observation might be interesting for the application of this protecting group for the preparation of bioconjugates. It was also found that cleavage of a peptide from Ellman resin can be accomplished using the conditions mentioned above.

5. Thp Protection of a Phenol (Tyrosine)

5.1. General

Although successful protection of different kinds of phenols with the Thp group has been described in acidic media,[13, 14, 15, 26–30] the O-Thp side-chain protection of Tyr has not been studied extensively, and, to the best of our knowledge, Thp-protected Tyr has not been used in SPPS. The introduction of Thp into the side chain of Tyr was first described by Iselin and Schwyzer[31] using a trace of HCl as a catalyst. Shortly after, Thp protection of Tyr was attempted for the controlled synthesis of peptides in aqueous media using ε-amino acid N-carboxyanhydrides,[32, 33] and also for the preparation of water-soluble random L-Tyr-containing copolymers with Nε-(3-hydroxypropyl)-L-glutamine.[34] Moreover, the Tyr phenolic hydroxyl was protected with Thp for the synthesis of Tyr-containing compounds or analogues philanthoxatin,[35] a potent inhibitor of L-glutamate and nicotinic acetylcholine receptors, and related macrocycles of tirofiban, an anti-platelet aggregation agent.[36] As Thp protection had not been addressed in detail for SPPS, we decided to initiate a study to investigate the scope and limitations of the reaction.

5.2. Introduction of the Protecting Group

Similar to protection of the alcohol, and as discussed above, Thp ether formation at the phenolic group in Tyr is achieved through the reaction of the phenolic hydroxyl group with DHP in the presence of acid catalysts. Accordingly, Thp-protected Tyr has been reported with N-carboxybenzyl (N-Cbz)[30, 31] or N-(para-toluene sulfonyl) (N-Ts)[32] groups but not in the N-Fmoc form. In this regard, our lab attempted to introduce Thp as a protecting group into the side chain of Tyr through the reaction of Fmoc-Tyr-OH with DHP using PTSA as catalyst and CH2Cl2 as solvent at room temperature for 35 min (Scheme 5). After completion of the reaction, followed by aqueous work-up and silica gel purification, Fmoc-Tyr(Thp)-OH was obtained in 63% yield. It was difficult to monitor the purity using HPLC and also molecular mass through LC–MS as cleavage of Thp (> 60% yield) occurred due to the presence of 0.1% TFA in the mobile phase. This demonstrates the lability of Thp if it is used as a protecting group for the phenolic side chain of Tyr.

5.3. Removal of the Protecting Group

Similar to Ser and Thr, Thp in Fmoc-Tyr(Thp)-OH is removed by acid treatment. The conditions described in the literature involve the use of TFA/CHCl3 (1:2) at room temperature for 5 h[69] or PPTS in CH2Cl2/MeOH (1:1).[70] In our experiments, Thp removal from the phenolic side chain of Fmoc-protected Tyr was observed under HPLC and LC–MS conditions, thereby clearly indicating the high acid sensitivity of the phenolic Thp ether.

6. Thp Protection of the Carboxyl Group

6.1. General

Initially, the Thp group was used to activate carboxylic acids in order to achieve amide bonds. However, compared with other active esters, Thp esters are not highly suited for synthetic purposes because they are easily hydrolyzed.[60] Reports on Thp protection for carboxyl protection are rare. In 1958, Holland and Cohen reported that the use of one equivalent of DHP in acidic medium resulted in blocking the carboxyl group in preference to the SH group. They reported that DHP, used in excess, reacted with N-protected Cys to protect both SH and COOH functional groups, and the resulting compound was resistant to mild alkaline saponification.[25] Additionally, Pappo[26] and Bernady[27] exploited the protection of the carboxyl function of prostaglandins with Thp. Using a peptidic scaffold, Wipf and Kim[28] protected the C-terminal Phe with Thp for the convergent total synthesis of cyclotheonamide A. However, in 2001, Holden and co-workers[29] attempted to protect carboxylic acids with Thp as an intermediate for the preparation of pilocarpine derivatives. They explained that use of Thp to protect the carboxyl group was problematic due to its instability at room temperature and its decomposition on silica during purification.

6.2. Introduction of the Protecting Group

As described for other functional groups, the introduction of Thp is achieved by treating the corresponding carboxylic acid with DHP in the presence of a catalytic amount of acid, such as PTSA·H2O,[27] methanesulfonic acid,[64, 65] HCl,[27, 60] or PPTS.[73] We recently protected the carboxylic acid group of several Nα-protected amino acids with Thp (Table 3). The amino acids studied were treated with DHP in the presence of a catalytic amount of PTSA at room temperature. The reaction time varied from 10 to 30 min depending on the amino acids (Scheme 6 and Table 3). Fmoc-Gly-OH was successfully protected using Thp. The crude product was purified by silica gel column chromatography after aqueous work-up. However, undesired removal of Thp from other amino acids was observed during aqueous work-up. This could be attributed to the high lability of the hemiacetal esters, as previously described.[60, 64, 73] Therefore, after monitoring the reaction to completion by...
7. Conclusions

Here we have reviewed the use of Thp as a protecting group in SPPS. Although the early literature describes several methods based on rather strong conditions for the introduction of the Thp group, there is now consensus that the use of PTSA as catalyst is more amenable in terms of reaction rate and purity of the final product. Thus, the reaction time using PTSA varies from 10 min to 3 h, depending upon the functional group protected, whereas that of PPTS involves longer heating times (nearly 12 h). Furthermore, it has been demonstrated that Thp is a useful group for protecting the side-chain functions of Ser, Thr and Cys and thus is compatible with the Fmoc/ToBu SPPS strategy. However, Thp has been demonstrated to be useful for the protection of neither amines, due to difficulty of introduction, nor for the carboxyl group, due to the extremely high acid lability of the hemiacetal ester. Due to the high acid lability of Thp-protected carboxylic acids, hydroxyl- and thiol-containing amino acids can be conveniently protected leaving the carboxyl group free. This observation reflects the superiority of Thp over other acid-labile protecting groups such as Bu and Trt, the introduction of which into the side-chain of hydroxyl-bearing amino acids first requires protection of the carboxyl group.

The solid-phase-supported version of Thp—Ellman resin—has proved to be suitable for side-anchoring of Ser/Thr and Trp. However, the use of Thp for protecting the indole moiety of Trp is not optimal. It is envisaged that Ellman resin will also be useful for thiol anchoring.

Due to its low cost, ease of installation, general stability to most non-acidic reagents, lower tendency for side reactions, better solubility and the ease with which it is removed, we conclude that Thp will find application for the synthesis of Ser-, Thr- or Cys-containing peptides.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] C. A. Montalbetti, V. Falque, Tetrahedron 2005, 61, 10827–10852.
[2] A. El-Faham, F. Albericio, Chem. Rev. 2011, 111, 6557–6602.
