Organic LETTERS

Letter

pubs.acs.org/OrgLett

Tetrahydropyranyl, a Nonaromatic Acid-Labile Cys Protecting Group for Fmoc Peptide Chemistry

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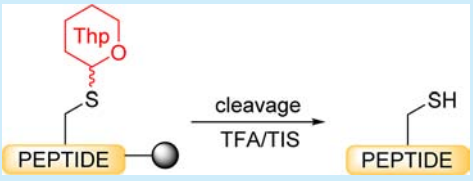
Supporting Information

ABSTRACT: Tetrahydropyranyl (Thp), which exploits the concept of being an *S,O*-acetal nonaromatic protecting group for cysteine, has been shown to be superior to Trt, Dpm, Acm, and S*t*Bu in solid-phase peptide synthesis using the Fmoc/*t*Bu strategy. Thus, Cys racemization and C-terminal 3-(1-piperidinyl)-alanine formation were minimized when the Cys was protected with Thp. This nonaromatic protecting group also improved the solubility of Cys-containing protected peptides.



Although solid-phase peptide synthesis (SPPS) is highly efficient,[1] amino acid racemization is still an issue and measures for its minimization are needed. Of the repertoire of natural amino acids, Cys is the most problematic due to its tendency to lose integrity. Furthermore, it has been shown that the degree of racemization depends on the protection of the β-thiol group, which can modulate the acidity of the α-proton.[2] Associated with the same phenomenon, the SPPS of C-terminal Cys-containing acid peptides can be contaminated with C-terminal 3-(1-piperidinyl)alanine derivatives, which are formed through β-elimination, followed by piperidine addition.[3] Consequently, an interesting attempt to avoid this side reaction has been done.[4]

While the most used thiol protection is in the form of thioethers,[5] the groups of Yajima and Nishiuchi developed *S,O*-acetal protecting groups, such as the benzyloxymethyl (Bom) for Boc chemistry[6] and 4-methoxybenzyloxymethyl (MBom) for Fmoc chemistry.[7] Bom and MBom, whose syntheses are not straightforward, result in a very low level of racemization; however, their use can hamper the quality of the final product because formaldehyde is formed as a side product during cleavage and is accompanied by concomitant hydroxymethylation.

With the same idea of exploiting the *S,O*-acetal protecting group concept, we introduced tetrahydropyranyl (Thp) as a Cys protecting group for SPPS. Thp has an advantage over benzyl-based protecting groups (Trt, Dmp, Mmt, Bom) in that it lacks aromaticity. In addition to producing more protected hydrophobic peptides, the use of bulky aromatic protecting groups in SPPS affects to the inter/intrachain interaction during peptide elongation and therefore jeopardizes the purity of the final product.[8,9]

The Thp group has been widely used as a hydroxyl protecting group due to the stability of the acetal derivative toward strongly basic conditions, organometallics, hydrides, and acylating and alkylating reagents. Thp elimination is usually performed in acidic media through the hydrolysis or alcoholysis of the acetal bond but also through various Lewis acids.[10,11]

To amplify the methodological spectrum of peptide synthesis, especially for Cys-containing molecules, and taking into account that the *S,O*-acetal is an excellent choice as a Cys protecting group, we present Thp as a nonaromatic Cys protecting group for the Fmoc/*t*Bu strategy in SPPS. Although Thp was introduced by Holland et al.[12] as a Cys protecting group a half-century ago for the synthesis in solution of insulin peptides, to date, it has not been used in Fmoc chemistry.

The main drawback of using Thp in organic synthesis is the formation of a new stereocenter that leads to diastereomeric mixtures; however, when used as a protecting group, the chiral center that forms is temporary. Consequently, the formation of the stereocenter is not a shortcoming.

Fmoc-Cys(Thp)-OH (**2**) was synthesized in good yield by means of an acid-catalyzed reaction between Fmoc-Cys-OH (**1**) and the versatile vinyl ether dihydropyran, using *p*-toluene sulfonic acid as catalyst and dichloromethane (DCM) as solvent (see Scheme 1).

To determine the acid stability of Thp as a protecting group, **2** was studied under a range of acidolytic conditions (see Table 1).

Thp remained stable in mild acidic conditions (entries 1−3). On the contrary, the acid lability of the Thp group was strongly

Received: February 11, 2015
Published: March 12, 2015

DOI: 10.1021/acs.orglett.5b00444
Org. Lett. 2015, 17, 1680−1683

Scheme 1. Synthesis of Fmoc-Cys(Thp)-OH (2)

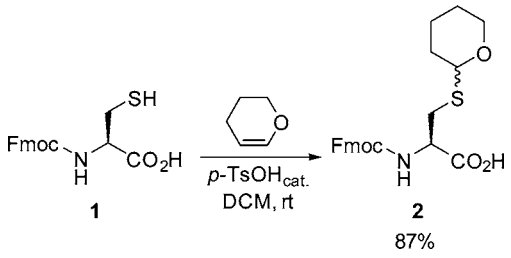


Table 1. Acid Lability Studies of Fmoc-Cys(Thp)-OH (2)

	"cocktail"			
entry	composition	ratio (%)[a]	reaction time	deprotected Cys, **1** (%)
1	MES[b]/H_2O	2:98	48 h	0
2	MES[b]/TIS/H_2O	2:1.5:96.5	48 h	0
3	TFE[c]/AcOH/DCM	20:20:60	48 h	4.7
4	TFA[d]/H_2O/DCM	1:1:98	48 h	28.5
5	TFA/H_2O/DCM	10:1:89	48 h	93.7
6	TFA/DCM	1:99	24 h	27.4
7	TFA/DCM	10:90	24 h	89.0
8	TFA/TIS/DCM	1:1.5:97.5	2 h	98.5
9	TFA/TIS/DCM	10:1.5:88.5	5 min	>99.0
10	HCl/dioxane	12:88	2 h	>99.0
11	HCl/TIS/dioxane	12:1.5:86.5	2 h	>99.0

[a]% w/v for MES (entries 1 and 2) and % v/v for the others (entries 3−11). [b]MES = 2-(*N*-morpholino)ethanesulfonic acid. [c]TFE = trifluoroethanol. [d]TFA = trifluoroacetic acid.

increased by the presence of triisopropylsilane (TIS) (entries 4, 6 vs 8 and 5, 7 vs 9) (Table 1). Interestingly, HCl in dioxane (4 M) removed Thp. Therefore, conventional cleavage conditions such as TFA/TIS/DCM (10:2.5:87.5), TFA/TIS/H_2O (95:2.5:2.5), and 0.1 N HCl/HFIP-TIS (99:1)[13] will ensure complete elimination of Thp in short treatments.

To determine a broader compatibility of the Cys(Thp)-containing peptide with continuous piperidine treatments and coupling conditions, Fmoc-Ala-Cys(Thp)-Leu-X (X = NH_2, **3a**; X = OH, **4a**) was prepared by SPPS using Sieber amide **3a** and 2-chlorotrityl chloride (2-CTC) (**4a**) resins as solid supports (Figure 1). Moreover, the performance of Thp as a Cys protecting group was studied by comparison of the synthesis of **3a** and **4a** with several Fmoc-Ala-Cys(PG)-Leu-X synthesized using Cys protecting groups (PG): Trt (**3b**,**c** and **4b**,**c**), Dpm (**3d**), and Acm (**4d**) (Figure 1).[2] To incorporate the protected Cys derivatives, a 5 min preactivation procedure using Fmoc-Cys(PG)-OH/DIPCDI/Oxyma Pure (2 equiv of each compound) in dimethylformamide (DMF) for 90 min at room temperature was used, which is described to minimize the racemization.[14]

All synthesized tripeptides (**3a**−**e**, **4a**−**c**) were cleaved from the resin using low TFA concentrations [TFA/DCM (1:99)] and analyzed by reverse phase high-performance liquid chromatography (RP-HPLC). The chromatograms for the C-terminal amides (**3a**−**e**, Figure 2) revealed that the Thp group was stable during the tripeptide elongation and also during cleavage from the resin. Similar behavior was observed for C-terminal acid tripeptides (**4a**−**c**; see Supporting Information). The RP-HPLC profiles also corroborate that the use of Thp as a thiol protecting group (**3a**) rendered a more soluble tripeptide, in comparison to that achieved with the commonly

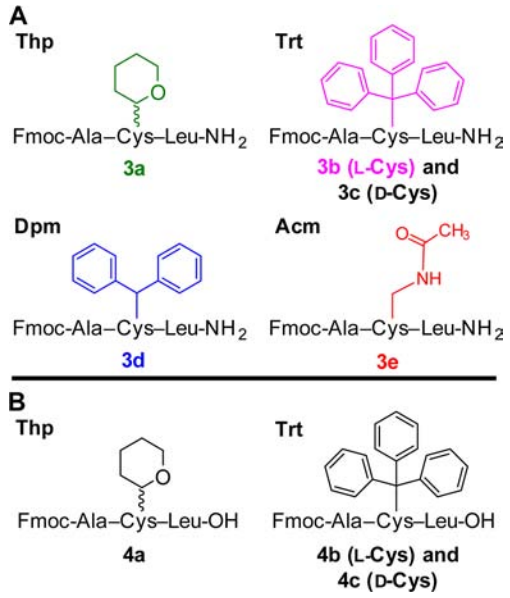


Figure 1. Fully protected synthesized Cys-containing tripeptides on (A) Sieber amide resin and (B) 2-chlorotrityl chloride resin.

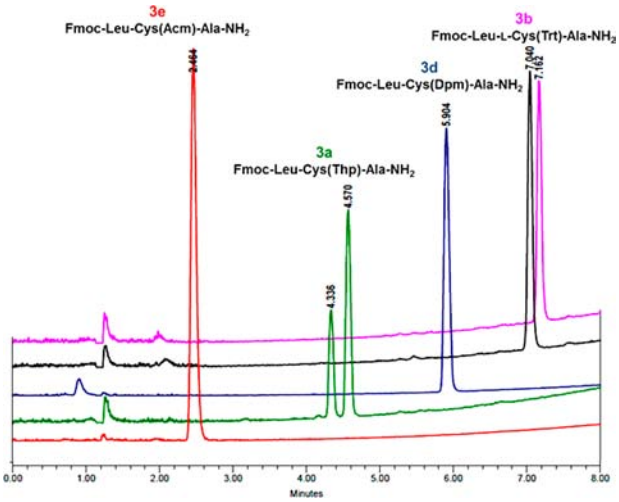


Figure 2. RP-HPLC profiles of the synthesized C-terminal amide tripeptides **3a**−**e**. Linear gradient H_2O/MeCN (50:50 to 0:100) over 8 min.

used Trt (**3b**,**c**) and Dpm (**3d**) groups. This parameter is relevant during the synthesis of Cys-containing protected peptides.

Furthermore, as expected given the particularity of Thp, a mixture of diastereoisomers was observed in the isolated tripeptide cleaved under low TFA concentrations (Figure 3A). Nevertheless, using higher concentrations of TFA in the presence of scavenger [TFA/TIS/DCM (95:2.5:2.5)] as cleavage conditions, only a single pure product (**3**) was obtained (Figure 3B).

Fmoc removal followed by peptide cleavage with TFA/TIS/DCM (95:1.5:1.5) yielded H-Ala-Cys-Leu-X tripeptides (X = NH_2, **3g**; X = OH, **4e**). When Thp was used (**3a**), an inappreciable level of racemization ([D-peptide/L-peptide] × 100 = 0.74%) compared with Trt (**3b**, 3.3%) and Dpm groups (**3d**, 6.8%) was observed. The model tripeptides were also synthesized under the same coupling conditions, by using Rink

DOI: 10.1021/acs.orglett.5b00444
Org. Lett. 2015, 17, 1680−1683

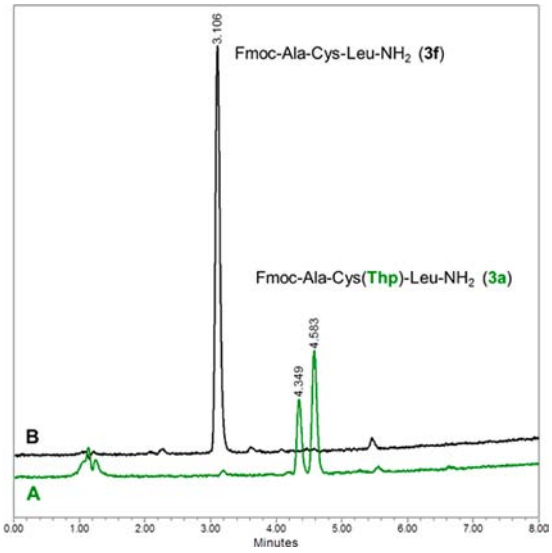


Figure 3. RP-HPLC profiles of (A) Fmoc-Ala-Cys(Thp)-Leu-NH_2 (**3a**) and (B) Fmoc-Ala-Cys-Leu-NH_2 (**3**), using TFA/DCM (1:99) and TFA/TIS/DCM (95:2.5:2.5) as cleavage conditions, respectively. Linear gradient H_2O/MeCN (50:50 to 0:100) over 8 min.

amide resin, which yielded similar crude products and low racemization levels: **3a** (0.45%), **3b** (2.3%), and **3d** (1.6%).

As mentioned above, the synthesis of C-terminal Cys-containing acid peptides showed increased difficulty due to racemization and formation of 3-(1-piperidinyl)alanine derivatives in response to the repetitive piperidine treatments for Fmoc elimination.[15]

To determine the racemization level of the C-terminal cysteine, Boc-Ala-Leu-Cys(PG)-O-resin (PG = Thp or Trt) was synthesized; both peptides were immobilized in 2-CTC and Wang resins.

Peptidyl resins were exposed to piperidine/DMF (1:4) (standard basic conditions for Fmoc removal in SPPS), and then a small amount was collected after 2, 4, 6, and 24 h. Each sample was cleaved with TFA/TIS/H_2O (95:2.5:2.5), and the tripeptide crude products were then analyzed by RP-HPLC to determine racemization. The racemization level was determined by the analysis of the area under the curve (AUC) corresponding to H-Ala-Leu-L-Cys-OH (**5a**) and H-Ala-Leu-D-Cys-OH (**5b**) present in the tripeptides synthesized in the two resins (Figure 4).

As reported in similar studies,[15] the racemization ratio for Wang was higher than that for 2-CTC resin. Moreover, for both resins, a greater racemization level was obtained when the Trt group was used to protect C-terminal Cys, and therefore, the racemization of the Cys-containing Thp group in the C-terminal was considerably lower than the same Trt-protected tripeptide.

Furthermore, the formation of the Ala-Leu-[3-(1-piperidinyl)alanine]-OH (**5d**) was also detected, and its extension was monitored for the SPPS of C-terminal Cys tripeptides **5a** and **5c** in both Wang and 2-CTC resins (Figure 5).[3]

In addition, the alkylation of the thiol group with a *p*-hydroxybenzyl (**5e**,**f**) $[M + 107]^+$ was detected in the SPPS conducted on Wang resin under standard cleavage conditions [TFA/TIS/H_2O (95:2.5:2.5)]. This side product resulted from the undesired cleavage of the Wang linker.[16−19] The formation of this side product was reduced using TFA/TIS/DMB

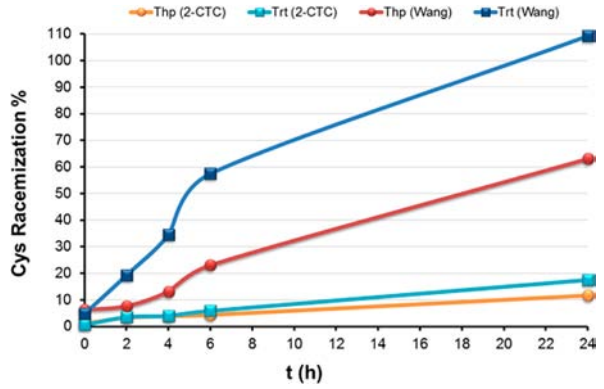


Figure 4. C-terminal cysteine tripeptide racemization studies for Boc-Ala-Leu-Cys(PG)-O-resin (2-CTC and Wang resins), using piperidine/DMF (1:4) for 24 h. Cys racemization ratio was defined as (AUC of H-Ala-Leu-D-Cys-OH [**5b**]/AUC of H-Ala-Leu-L-Cys-OH [**5a**]) × 100.

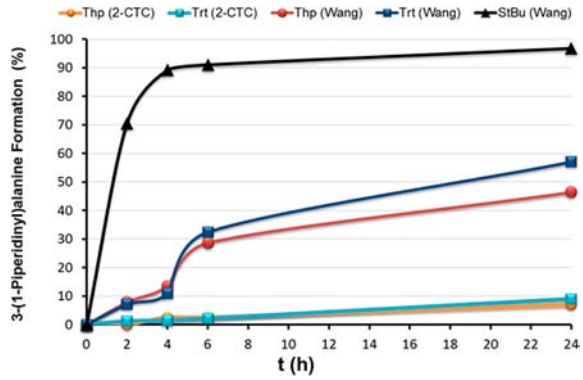


Figure 5. Formation of Ala-Leu-[3-(1-piperidinyl)alanine]-OH (**5d**) during the piperidine/DMF treatment of Boc-Ala-Leu-Cys(PG)-O-resin. Percentage of **5d** was calculated considering the AUC of **5d**, and D/L-tripeptides in the crude product analyzed **5a**,**b**.

(92.5:2.5:5) as the cleavage cocktail (Scheme 2, products from the cleavage from Wang resin).

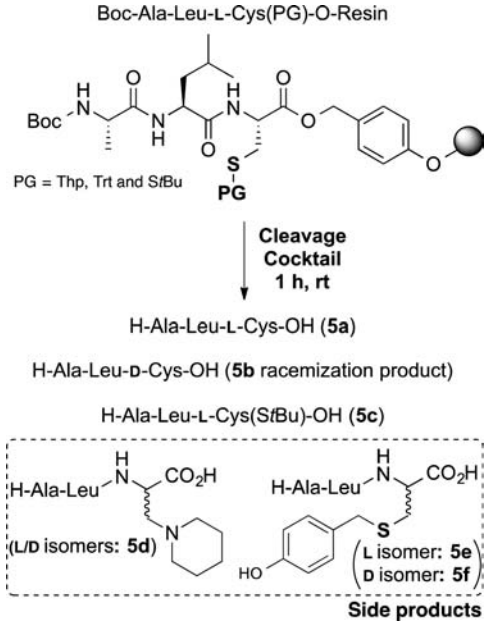
As expected, when *t*Bu was used as a Cys protecting group, the product from the cleavage reaction was the thiol (S*t*Bu)-protected C-terminal Cys tripeptide (**5c**).[20]

Comparing the results between Boc-Ala-Leu-Cys(PG)-OH, with the protecting groups of Thp, Trt, and S*t*Bu, we conclude that Thp presented a C-terminal Cys racemization lower than that of the tripeptide in which Trt was used as the protecting group (Figure 4). Furthermore, less formation of adducts **5d** was detected when Thp was used as the C-terminal Cys protecting group (Figure 5).

In summary, the use of the Thp group for cysteine in SPPS allowed racemization levels lower than that of conventional protecting groups for Cys (Trt, Dpm, and S*t*Bu), with Thp in a peptide sequence (**3a** and **4a**, <1%) or as C-terminal Cys protecting group (**5a**, <6%). In addition, fewer side products, such as the 3-(1-piperidinyl)alanine adducts, were detected during the synthesis of C-terminal Cys carboxylic acid peptides. Furthermore, the solubility of Thp-protected peptides was improved with respect to strategies involving the commonly used hindered aromatic protecting groups. Our results reveal Thp as a useful protecting group for Cys when applied to the Fmoc/*t*Bu strategy in SPPS. Due to lower racemization levels, decrease of side reactions, better solubility, and its atom

DOI: 10.1021/acs.orglett.5b00444
Org. Lett. 2015, 17, 1680−1683

Scheme 2. Obtained C-Terminal Cys Tripeptides from Wang Resins 5a–c and Their Side Products 5d–f



economy, Thp will be particularly useful for the effective synthesis of natural Cys-rich peptides.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods, experimental section, compound characterization, and RP-HPLC chromatograms and their analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

I.R-T. thanks the Generalitat de Catalunya for a predoctoral fellowship. This work was funded in part by the following: the CICYT (CTQ2012-30930), the Generalitat de Catalunya (2014 SGR 137), and the Institute for Research in Biomedicine Barcelona (IRB Barcelona) (Spain); the National Research Foundation and the University of KwaZulu Natal (South Africa); and SENESCYT (Ecuador).

■ REFERENCES

(1) Liu, H.; Boudreau, M. A.; Zheng, J.; Whittal, R. M.; Austin, P.; Roskelley, C. D.; Roberge, M.; Andersen, R. J.; Vederas, J. C. *J. Am. Chem. Soc.* **2010**, *132*, 1486.
(2) Hibino, H.; Miki, Y.; Nishiuchi, Y. *J. Pept. Sci.* **2014**, *20*, 30.
(3) Lukszo, J.; Patterson, D.; Albericio, F.; Kates, S. *Lett. Pept. Sci.* **1996**, *3*, 157.
(4) Huang, Z.; Derksen, D. J.; Vederas, J. C. *Org. Lett.* **2010**, *12*, 2282.
(5) Góngora-Benítez, M.; Mendive-Tapia, L.; Ramos-Tomillero, I.; Breman, A. C.; Tulla-Puche, J.; Albericio, F. *Org. Lett.* **2012**, *14*, 5472.
(6) Otaka, A.; Morimoto, H.; Fujii, N.; Koide, T.; Funakoshi, S.; Yajima, H. *Chem. Pharm. Bull.* **1989**, *37*, 526.
(7) Hibino, H.; Nishiuchi, Y. *Org. Lett.* **2012**, *14*, 1926.
(8) Isidro-Llobet, A.; Alvarez, M.; Albericio, F. *Chem. Rev.* **2009**, *109*, 2455.
(9) Postma, T. M.; Albericio, F. *Eur. J. Org. Chem.* **2014**, *2014*, 3519.
(10) Liu, J.; Wong, C.-H. *Tetrahedron Lett.* **2002**, *43*, 4037.
(11) Stephens, J. R.; Butler, P. L.; Clow, C. H.; Oswald, M. C.; Smith, R. C.; Mohan, R. S. *Eur. J. Org. Chem.* **2003**, *19*, 3827.
(12) Holland, G. F.; Cohen, L. A. *J. Am. Chem. Soc.* **1958**, *80*, 3765.
(13) Palladino, P.; Stetsenko, D. *Org. Lett.* **2012**, *14*, 6346.
(14) Subirós-Funosas, R.; Prohens, R.; Barbas, R.; El-Faham, A.; Albericio, F. *Chemistry* **2009**, *15*, 9394.
(15) Fujiwara, Y.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1994**, *42*, 724.
(16) Castro, V.; Rodriguez, H.; Albericio, F. *Org. Lett.* **2013**, *15*, 246.
(17) Stathopoulos, P.; Papas, S.; Pappas, C.; Mousis, V.; Sayyad, N.; Theodorou, V.; Tzakos, A. G.; Tsikaris, V. *Amino Acids* **2013**, *44*, 1357.
(18) Stathopoulos, P.; Papas, S.; Tsikaris, V. *J. Pept. Sci.* **2006**, *12*, 227.
(19) King, D. S.; Fields, C. G.; Fields, G. B. *Int. J. Pept. Protein Res.* **2009**, *36*, 255.
(20) McCurdy, S. N. *Pept. Res.* **1989**, *2*, 147.

DOI: 10.1021/acs.orglett.5b00444
Org. Lett. 2015, 17, 1680–1683