# A practical solution-phase synthesis of an antagonistic peptide of TNF-α based on hydrophobic tag strategy†

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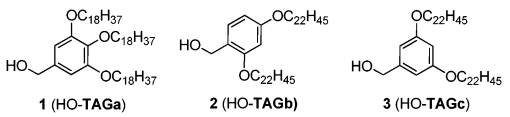
**A simple *acid-resistant* hydrophobic tag, which can be removed rapidly in a single-step procedure after overall peptide synthesis, has been developed to accomplish practical solution-phase synthesis of a 15-mer antagonistic peptide of TNF-α (A-TNF-α). Hydrophobically tagged peptides can be separated as precipitates at each step by addition of a polar organic solvent.**

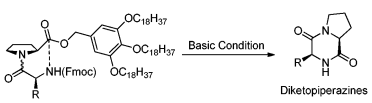
Since the pioneering work of Merrifield, peptide synthesis based on solid-phase techniques has been established to realize a practical synthesis for both naturally occurring and artificial peptides, and has also become essential for automated synthesis and combinatorial chemistry.[1–12] The great advantage of solid-phase synthesis is the facile separation processes, requiring only filtration. In this context, a wide variety of effective protection/deprotection methods were developed to elaborate target sequences. Alternatively, tag-assisted solution-phase synthesis has also been demonstrated to be an efficient approach, especially for multi-step reactions.[13–21] In this case, high reaction rate and rapid reaction monitoring are both attained. The integration of solid-phase separation and solution-phase synthesis should facilitate highly practical peptide synthesis.

Previously, we developed a solution-phase reaction system based on a simple hydrophobic tag, 3,4,5-trioctadecyloxybenzyl alcohol (HO-**TAGa**) **1**, that could easily be used in multi-step reactions (Scheme 1).[22–27] In particular, **1** can serve as an effective protecting group for the peptide C-terminus and as a phase-tag that can selectively be separated from surplus reagents, including amino acids (AAs) and condensing agents, in polar reaction solutions. However, C-terminal benzyl esters are often hydrolyzed under acidic conditions. Thus, Boc-chemistry could not be applied to this system. This drawback is crucial when proline is placed at the C-terminus (or next to the C-terminus), because the formation of diketopiperazines, an intramolecular cyclized by-product, takes place immediately under the basic conditions required for N-terminal Fmoc group deprotection (Scheme 2). This troublesome process can be prevented under acidic conditions because the nucleophilicity of the N-terminus should be significantly decreased by protonation. Therefore, an *acid-resistant* hydrophobic tag would allow for the use of both Boc- and Fmoc-chemistry, extending the potential uses of hydrophobic tag-based peptide synthesis. We herein describe the development of a new hydrophobic tag for peptide synthesis that can be applied in both Boc- and Fmoc-chemistry. Furthermore, hydrophobically tagged peptides can be effectively separated as precipitates by addition of a polar organic solvent. The tag can also be removed in a single step procedure after overall peptide synthesis (Scheme 3). We chose a peptide possessing a C-terminal prolyl residue, *i.e.*, antagonist of tumor necrosis factor-α[28] (A-TNF-α), as the model compound to demonstrate our strategy.

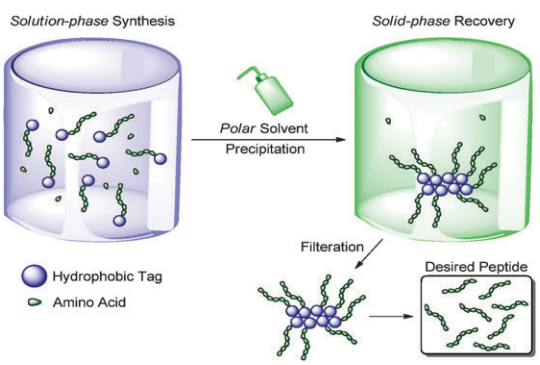



**Scheme 1** Structures of hydrophobic tags (HO-**TAGa–c**) **1–3** used in this study.

The present work began with the preparation of 2,4-didocosyloxybenzyl alcohol (HO-**TAGb**) **2** and 3,5-didocosyloxybenzyl alcohol (HO-**TAGc**) **3**. To evaluate the stability of the hydrophobic tags **1–3** against the acidic conditions used for N-terminal Boc group deprotection, N-Boc-leucine was then introduced to **1–3** using diisopropylcarbodiimide (DIC) in the presence of a catalytic amount of dimethylaminopyridine (DMAP) to form hydrophobically tagged N-Boc-leucines (N-Boc-Leu-O-**TAGa–c**) **4–6** quantitatively (Scheme 4). When




**Scheme 2** Formation of diketopiperazines from N-Fmoc-AA-Pro-O-**TAGa** under basic conditions required for N-terminal Fmoc group deprotection.




**Scheme 3** General concept of the hydrophobic tag strategy.

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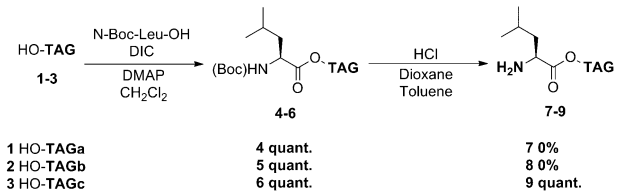
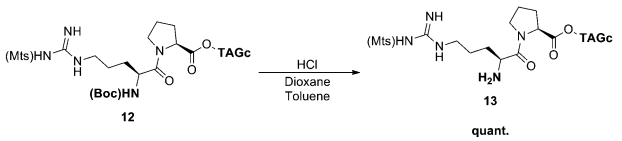



**Scheme 4** Evaluation of the stability of HO-**TAGa–c** (**1–3**) against the acidic conditions used for N-terminal Boc group deprotection.

**4–6** were subjected to N-terminal Boc group deprotection conditions, **6** afforded deprotected product **9** quantitatively, whereas deprotected products **7** and **8** were not obtained. Hydrolyzed leucine **S1** was recovered from the reaction mixture, along with decomposition products of **1**, by acid treatment of **4** (Scheme S1 in supporting information). On the other hand, acid treatment of **5** resulted in the quantitative formation of red-colored resorcinarene **S2** (Scheme S2 in supporting information). These results indicated that the C-terminal benzyl esters of **4** and **5** were hydrolyzed under the acidic conditions required for N-terminal Boc group deprotection, affording the corresponding benzyl cations stabilized by *ortho*- and/or *para*-alkoxy groups, which then decomposed and cyclized. Meanwhile, hydrolysis of the C-terminal benzyl ester of **6** was not observed even when **6** was stirred for 3 days under acidic conditions required for N-terminal Boc group deprotection, clearly suggesting that **3** functioned as an *acid-resistant* hydrophobic tag that will enable the incorporation of Boc-chemistry. It should also be emphasized that quantitative amounts of both **6** (Fig. S1 in supporting information) and **9** (Fig. S2 in supporting information) could be isolated with excellent purities by addition of acetonitrile.

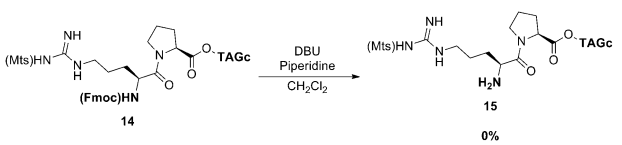
With the *acid-resistant* hydrophobic tag **3** in hand, we then turned our attention to the synthesis of A-TNF-α. Initially, N-Fmoc-proline was introduced to **3** using DIC in the presence of a catalytic amount of DMAP. After the completion of the reaction, an excess amount of acetonitrile was added to the reaction mixture to precipitate hydrophobically tagged N-Fmoc-proline (N-Fmoc-Pro-O-**TAGc**) **10** quantitatively (Scheme S3 and Fig. S3 in supporting information). Compound **10** was then stirred under basic conditions consisting of 1,3-diazabicyclo[5.4.0]undec-7-ene (DBU) and piperidine to deprotect the N-terminal Fmoc group, which was then precipitated by the addition of acetonitrile to give a quantitative amount of deprotected product **11** (Scheme S4 in supporting information). Through careful examination, the Mts group was found to be a suitable protective group for the arginine side chain. Thus, N-Boc-Arg(Mts)-OH was introduced to **11** to give hydrophobically tagged N-Boc-dipeptide [N-Boc-Arg(Mts)-Pro-O-**TAGc**] **12** quantitatively as a precipitate by the addition of an excess amount of acetonitrile (Scheme S5 and Fig. S3 in supporting information). It was also found that the use of *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxy-1*H*-benzotriazole (HOBt) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) was more effective after the second introduction of an amino acid. Compound **12** was then stirred under acidic conditions to deprotect the N-terminal Boc group, which was then precipitated by the addition of acetonitrile to give a quantitative



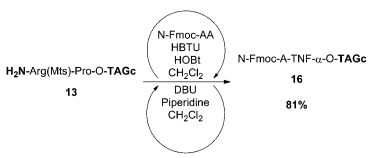

**Scheme 5** N-Boc group deprotection of N-Boc-Arg(Mts)-Pro-O-**TAGc** (**12**).

amount of deprotected product **13** with excellent purity (Scheme 5). Cleavage of the C-terminal benzyl ester of **12** was not observed, while no deprotected product **13** was obtained through N-terminal Fmoc group deprotection of hydrophobically tagged N-Fmoc-peptide [N-Fmoc-Arg(Mts)-Pro-O-**TAGc**] **14** under basic conditions, and only **3** was recovered as a precipitate (Scheme 6 and Fig. S4 in supporting information). Based on these results, repeated peptide elongation with N-Fmoc-AAs [except the last, N-Boc-Asp(*t*Bu)-OH], deprotection in solution-phase, and separation by precipitation were performed using HBTU and HOBt in the presence of DIPEA to afford hydrophobically tagged N-Boc-A-TNF-α (N-Fmoc-A-TNF-α-O-**TAGc**) **15** in 81% isolated yield over 25 steps (Scheme 7). Finally, deprotection of compound **15** was achieved under two different types of acidic conditions to give deprotected compound, A-TNF-α, **16** in 86% isolated yield in two steps with excellent purity (Scheme 8 and Fig. S6 in supporting information).

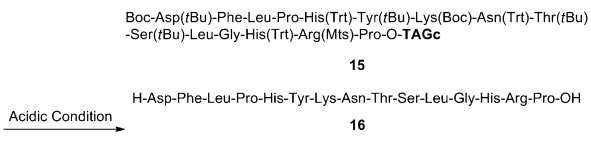
In conclusion, we have successfully developed a simple *acid-resistant* hydrophobic tag, which can rapidly be removed in a single step procedure after overall peptide synthesis, to accomplish practical solution-phase synthesis of A-TNF-α. This strategy could effectively incorporate both Boc- and Fmoc-chemistry and the products could readily be separated as precipitates at each step by addition of a polar organic solvent, which allows for elaboration of target sequences in peptide synthesis.




**Scheme 6** Basic deprotection of N-Fmoc-Arg(Mts)-Pro-O-**TAGb** (**14**).




**Scheme 7** Synthesis of N-Boc-A-TNF-α-O-**TAGc** (**15**).




**Scheme 8** Acidic deprotection of N-Boc-A-TNF-α-O-**TAGc** (**16**).

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