oNBS−SPPS: A New Method for Solid-Phase Peptide Synthesis

Stephen C. Miller* and Thomas S. Scanlan

Departments of Pharmaceutical Chemistry and Cellular and Molecular Pharmacology
University of California
San Francisco, California 94143-0446

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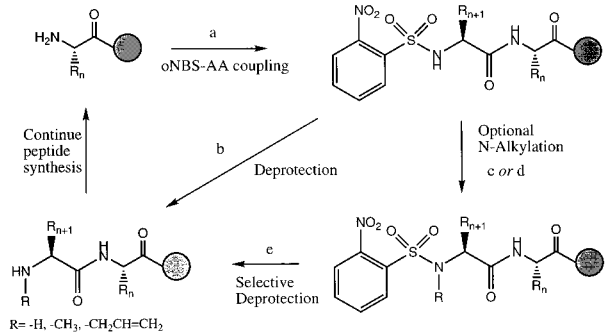
Since the advent of solid-phase peptide synthesis (SPPS),[1] many different protecting group strategies have been employed for the side chains and amino termini of peptides. The Boc protecting group scheme,[2] where the temporary Boc amino protecting group is labile to trifluoroacetic acid and the side chain protection is cleaved by anhydrous HF, is still in wide use but has been replaced in many laboratories by Fmoc−SPPS.[3] Advantages of Fmoc−SPPS include milder reagents, ability to monitor Fmoc deprotection by UV, and in general, higher yields of desired peptide. However, it is considerably more expensive than Boc−SPPS. Here, we introduce a method for SPPS based on a new α-amino protecting group that retains compatibility with Fmoc−SPPS and offers several additional advantages.

The *o*-nitrobenzenesulfonyl group (oNBS), first described by Fukuyama et al.,[4] offers a number of exemplary features that support its use as a temporary amino protecting group: (1) deprotection of oNBS-protected peptides releases a yellow chromophore which allows simple visual confirmation of deprotection, (2) unlike Fmoc−SPPS, oNBS−SPPS (Scheme 1) allows optional and *selective* N-methylation of the oNBS-protected nitrogen during peptide synthesis,[5] (3) oNBS amino acid chlorides can be used to couple to extremely hindered amines on a solid support where the analogous Fmoc amino acid chlorides fail to couple well, and (4) the reagent necessary to synthesize oNBS amino acids, 2-nitrobenzenesulfonyl chloride, is commercially available and considerably cheaper than Fmoc-Cl.

Recently, we reported a method for the selective methylation of peptides on solid support.[5] Our scheme required the deprotection of a support-bound Fmoc-protected peptide and its subsequent reprotection with the oNBS group. These two steps could be eliminated if the amino acid of interest was originally incorporated as an oNBS amino acid. We have synthesized a number of oNBS amino acids using the Schotten−Baumann procedure[6] and have found their coupling to proceed cleanly using standard Fmoc coupling procedures. For example, the coupling of oNBS−Leu and oNBS−Arg(Pmc), respectively, to the resin-bound peptides LRN and GAP with the peptide coupling agent

Scheme 1. Flexibility in oNBS−SPPS[a]



[a] Key: (a) 4 equiv of oNBS−AA-OH, 3.8 equiv of HBTU, 0.4 M NMM/DMF, 20 min; (b) 0.5 M PhSH, 2 equiv of K_2CO_3 per PhSH, DMF, 10 min; (c) 4 equiv of methyl 4-nitrobenzenesulfonate, 3 equiv of MTBD, DMA, 30 min (ref 5); (d) 15 equiv of allyl methyl carbonate, 10 mol % Pd_2dba_3−$CHCl_3$, 80 mol % Ph_3P, THF, 2 h; (e) 10 equiv of 2-mercaptoethanol, 5 equiv of DBU, DMF, 30 min (ref 5).

HBTU for 20 min in 0.4 M NMM/DMF gave oNBS−LLRN and oNBS−RGAP and showed no impurities by HPLC. Subsequent Pd(0)-catalyzed allylation of oNBS−LLRN was achieved in 98% yield,[7] and methylation of oNBS−RGAP was virtually quantitative.[8] Deprotection was carried out as reported,[5] giving the respective N-alkylated peptides in high yields. Peptide synthesis could then be continued using either oNBS or Fmoc amino acids.

We were interested in determining whether oNBS amino acid derivatives could be used, like Fmoc amino acids, in the general synthesis of *unalkylated* peptide sequences. Thus, we attempted the automated solid-phase synthesis of the thrombin-receptor agonist peptide amide, SFLLRN, on 0.05 mmol scale using oNBS amino acids.[9] Deprotection of oNBS-protected amino acid derivatives with 2-mercaptoethanol is selective for the N-alkylated derivatives,[5] but thiophenol readily cleaves unalkylated oNBS derivatives. Thus, our original attempts at oNBS−SPPS utilized a 5% solution of thiophenol in DMF with a number of different soluble bases in place of the 20% piperidine/DMF solution used for Fmoc deprotection. Solutions of thiophenol and base are particularly prone to air oxidation, and thus, all manipulations were performed under inert atmosphere. Deprotection was easily followed by simple visual inspection of the released yellow chromophore. Use of secondary and tertiary amines, such as piperidine and triethylamine, yielded SFLLRN peptides in 80−85% purity, but deprotection was slow, requiring 45−60 min. The use of potassium carbonate as base similarly resulted in peptides of 85% purity, but led to dramatically faster deprotection times, allowing complete oNBS cleavage in less than 10 min.[10] For comparison purposes, SFLLRN synthesized under the same conditions on the same instrument using Fmoc−SPPS gave a peptide of 91% purity. The impurities for both syntheses are mostly identical by reverse-phase HPLC (Figure 1) and MALDI-MS. Although not all of the impurities have been identified, their presence in both peptide products suggests that they are not artifacts of the amino protection group chemistry. Purification of the peptides by reverse-phase HPLC and subsequent lyo-

(1) (a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149. (b) Abbreviations used in the text: Boc, *tert*-butyloxycarbonyl; Fmoc, 9-fluorenylmethyloxycarbonyl; oNBS, *o*-nitrobenzenesulfonyl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; OtBu, *O*-*tert*-butoxy; Trt, trityl; HBTU, *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DMF, dimethylformamide; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; NMM, *N*-methylmorpholine; HPLC, high-performance liquid chromotography; TFA, trifluoroacetic acid. Amino acids are referred to using their standard three-letter codes as monomers, and one-letter codes in peptides. All peptides are C-terminal amides.

(2) Merrifield, R. B. *Biochemistry* **1964**, *3*, 1385.

(3) For a review, see: Fields, G. B.; Noble R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161−214.

(4) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373−6374.

(5) Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1997**, *119*, 2301−2302.

(6) Milne, H. B.; Peng, C.-H. *J. Am. Chem. Soc.* **1957**, *79*, 639−644. The following oNBS amino acids were prepared: Ser(OtBu), Leu, Phe, Arg(Pmc), and Asn(Trt). They were isolated as solids except Ser(OtBu), which was a viscous oil. All compounds gave satisfactory NMR and MS.

(7) Allylation performed with 15 equiv of allyl methyl carbonate, 10 mol % tris(dibenzylideneacetone)dipalladium chloroform adduct, and 80 mol % triphenylphosphine in tetrahydrofuran for 2 h under argon.

(8) Methylation carried out as described (see ref 5), except that dimethylacetamide (DMA) was substituted for DMF, which eliminated any arginine-modified impurities.

(9) Using Rink Amide MBHA resin. See ref 5.

(10) Two equivalents of K_2CO_3 relative to thiophenol were thoroughly shaken with the 5% solution of thiophenol in DMF, and the K_2CO_3 was allowed to sediment. Thus, the deprotection solution applied to the peptide resin was completely homogeneous and presumed to contain soluble potassium thiophenolate.

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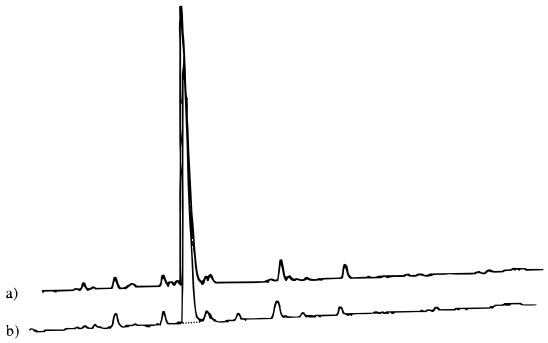

Figure 1. The C-terminal peptide amide SFLLRN synthesized by (a) Fmoc−SPPS and (b) oNBS−SPPS. Reverse-phase HPLC with 10−50% acetonitrile/water gradient, 0.1% TFA, UV detection (215 nm).

philization yielded pure (>99%) peptides in 50% (oNBS) and 62% (Fmoc) overall yields.[11]

In our attempts to develop methods that allow routine N-alkylation of peptides on solid-support, we have been using oNBS-protected amino acids for the selective N-allylation of peptides using Pd(0) chemistry. In addition to the conformational effects of such a modification, an allyl group serves as a useful chemical functionality, allowing subsequent selective chemical modification of the peptide in a combinatorial fashion. Particularly interesting and applicable chemistries include ozonolysis,[12] [3 + 2] cycloaddition,[13] and ring-closing metathesis.[14] However, peptide coupling to *N*-allyl amino acids is considerably more difficult than coupling to *N*-methyl amino acids. Using the peptide *N*-allyl-LLRN, synthesized as described above, we were able to achieve only 5% coupling with Fmoc−Phe using Carpino's coupling reagent HATU.[15] The use of the highly reactive acid chloride, Fmoc−Phe-Cl, fared only slightly better, giving 16.5% coupling in dichloromethane with collidine as base. The failure of Fmoc acid chlorides to couple to such hindered amines on solid-phase is presumably due to competing oxazolone formation.[16]

The inability of oNBS-protected amino acid chlorides to form oxazolones suggested that they may prove more suitable than the Fmoc amino acid chlorides. However, α-sulfonyl-protected amino acid chlorides are known to decompose under basic conditions.[17] While decomposition is surely competing, we found that treatment with oNBS−Phe-Cl for 15 min gave 75% coupling to the N-allylated peptide, a considerable improvement over Fmoc−Phe-Cl.[18,19] Repeat coupling with oNBS−Phe-Cl and elaboration of the peptide using oNBS−SPPS as described yielded SF(*N*-allyl)LLRN in 75% purity and 35% overall isolated yield after HPLC purification.[20]

We have shown that oNBS amino acids can be used in automated solid-phase peptide synthesis and are compatible with the side-chain protection used in Fmoc−SPPS. The deprotection of oNBS amino acids can be followed spectrophotometrically, like Fmoc, with the additional advantage that the released chromophore is visible to the naked eye. Unlike Fmoc amino acids, oNBS amino acids can be selectively N-alkylated on solid-support prior to deprotection. Coupling to highly hindered sequences, such as N-allylated peptides, can be achieved with oNBS amino acid chlorides in yields superior to those achievable with Fmoc amino acid chlorides, presumably due to the inability of oNBS amino acids to form oxazolones. This also suggests that oNBS amino acids may be less prone to racemization, as the primary mechanism of racemization for most amino acids is via an oxazolone intermediate. Finally, the requisite reagent for synthesizing oNBS amino acids, 2-nitrobenzenesulfonyl chloride, is more than 10-fold cheaper on a molar basis than Fmoc-Cl.[21] Under the currently outlined conditions, Fmoc−SPPS gives peptides of slightly higher purity and yield than oNBS−SPPS, and it remains to be seen how well oNBS−SPPS will fare with longer and more varied peptides. However, the current data and the compatible nature of Fmoc−SPPS and oNBS−SPPS support the use of oNBS−SPPS when N-alkylation is desired and when difficult coupling reactions are anticipated.

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Supporting Information Available: A detailed experimental procedure, NMR of the oNBS amino acids, and HPLC and MS for the synthesized peptides (25 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(11) Based on the manufacturer's stated resin substitution. Yield calculated for the di(trifluoroacetate) salt.

(12) Sylvain, C.; Wagner, A.; Mioskowski, C. *Tetrahedron Lett.* **1997**, *38*, 1043−1044.
(13) Pei, Y. H.; Moos, W. H. *Tetrahedron Lett.* **1994**, *35*, 5825−5828.
(14) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606−9614.
(15) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.
(16) Carpino, L. A.; Chao, H. G.; Beyermann, M.; Bienert, M. *J. Org. Chem.* **1991**, *56*, 2635−2642.

(17) Decomposition has been reported to occur in aqueous sodium hydroxide. For a review of sulfonyl protecting groups in peptide synthesis, see: Rudinger, J. In *The Chemistry of Polypeptides*; Katsoyannis, P. G., Ed.; Plenum Press: New York, 1973; pp 87−123.

(18) For both Fmoc and oNBS amino acid chlorides, we used 4 equiv of acid chloride with 4 equiv of collidine as base in dichloromethane. Use of stronger bases or DMF as solvent proved detrimental to coupling yields for both derivatives. Coupling yields are based on reverse-phase HPLC (215 nm).

(19) For a recent report of solution-based coupling of sulfonamide-protected amino acid chlorides with similarly positive results, see: Vedejs, E.; Lin, S.; Klapars, A.; Wang, J. *J. Am. Chem. Soc.* **1996**, *118*, 9796−9797.

(20) The peptide sequence and site of allylation were confirmed by collision-induced dissociation (CID) tandem mass spectrometry.

(21) Based on the Aldrich 1996−97 catalog, where 2-nitrobenzenesulfonyl chloride is $122/mol, and 9-fluorenylmethyloxycarbonyl chloride is $1875/mol, or greater than 15 times as expensive.