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Amino acid bromides: their utilization for difficult couplings in solid-phase peptide synthesis

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Abstract—Use of *N*-protected-α-amino acid bromides for facile solid-phase synthesis of peptides (SPPS) containing extremely sterically hindered non-proteinogenic amino acids is presented. Amino acid bromides (Aaa-Br), generated in situ, were used for the synthesis of long chain homopeptides containing up to eight successive α-MeVal or Aib residues. SPPS of a heteropeptide containing a very bulky amino acid building block is also described. The choice of suitable *N*-protections is discussed.

Recently, we have described a new coupling method for the solution-synthesis of particularly difficult peptide bonds, using *N*-protected amino acid bromides.[1] These Aaa-Br can be easily generated in situ under very mild conditions with 1-bromo-*N*,*N*-1-trimethyl-1-propenylamine, a reagent introduced by Ghosez et al. for the synthesis of carboxylic acid bromides.[2,3] Use of this procedure greatly increased the speed of sterically hindered couplings, that is, with $C^{\alpha,\alpha}$-dialkyl and N^{α}-alkylamino acids, which are characterized by the low reactivity of the amino terminus; moreover, in some cases, it made possible coupling when all other known methods failed. We could demonstrate the high efficiency of the new method, building up a sequence of eight residues of L-α-MeVal, one of the most sterically demanding $C^{\alpha,\alpha}$-dialkylamino acids, using the corresponding azidocarboxylic acid bromides.[4] To further assess the utility of Aaa-Br, we sought to extend their use to the solid-phase synthesis of long chains of particularly sterically demanding oligopeptides.

In this letter, we report on the successful application of Aaa-Br method to the solid-phase synthesis of the two octahomopeptides H-(L-α-MeVal)$_8$OH **1** and H-(Aib)$_8$-OH **2**. The synthesis of tetrapeptide **3**, containing an extremely bulky building block, is also described, as a representative example of a product only obtainable via Aaa-Br. The common Wang resin was used for SPPS of the two homopeptides **1** and **2**, while we started from Fmoc-Gly-Sasrin™(Bachem)-® for heterotetrapeptide **3**.

In Scheme 1, the synthesis of the two homopeptide-octamers is described; the results were similar for both compounds.

For each coupling step, a solution of 3 equiv of the α-azido acid bromide in dichloromethane (prepared immediately before use as previously described)[4] was added to the resin together with 6 equiv of collidine, left under stirring at ambient temperature for 1.5 h, followed by a second loading, when necessary. Disappearance of the substrate was monitored by cleavage of a single bead and subsequent HPLC analysis. The reduction of the azido group was performed with the complex Sn(II)/PhSH/Et_3N in DMF (prepared from $SnCl_2$+PhSH+Et_3N immediately before use)[5,10] followed by 2 N NaOH, and was quantitative within 30 min. The first attack, coupling/deprotection sequence, to the Wang resin proceeded with 90% efficiency after two loadings. Yields were quantitative in the successive steps until formation of the tripeptide, then varied from 85% to 75% for the tetramer up to the hexamer, and from 70% to 60% for the hepta- and octamer, respectively; for the two latter sequences, an additional loading was

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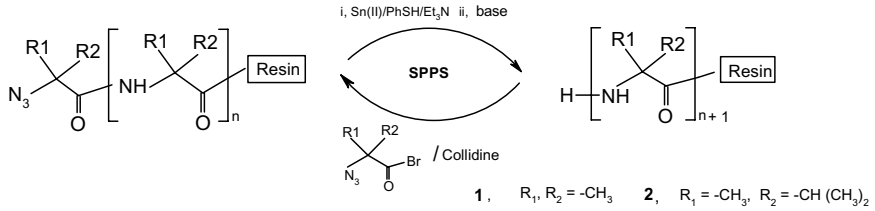
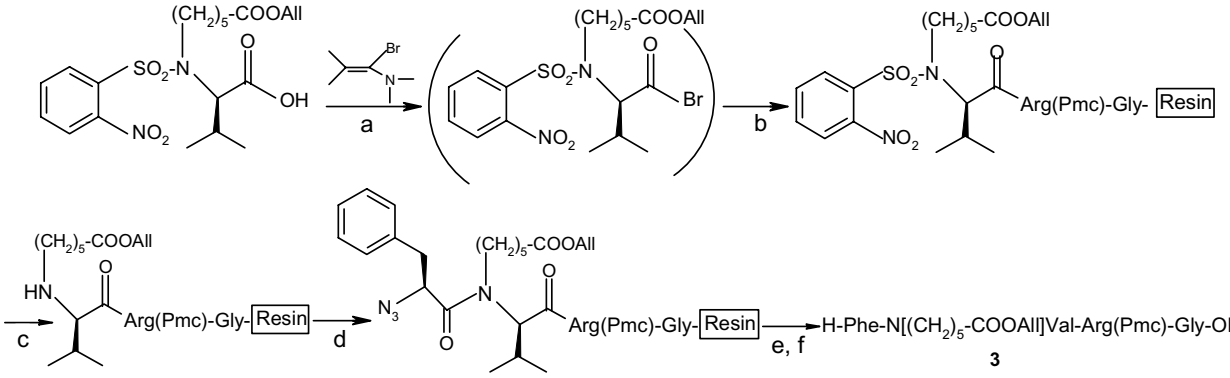


Scheme 1.



Scheme 2. Reagents and conditions: (a) bromoenamine 1.4 equiv, DCM, rt, Argon, 10 min; (b) H-Arg(Pmc)-Gly-Resin, sym-collidine 12 equiv, AaBr 3 equiv, DCM, rt, 2 h; (c) 2-mercaptoethanol 2.0 equiv, DBU 10 equiv, Argon, 1 h; (d) 2-azido-3-phenylpropionic acid bromide 5 equiv, collidine 20 equiv, DCM, rt, 2 h; (e) $SnCl_2$ 10 equiv, PhSH 40 equiv, Et_3N 30 equiv, DMF, 1 h, 2 N NaOH; (f) 1% TFA, DCM.

necessary. After cleavage from the resin with 50% trifluoroacetic acid in dichloromethane and purification by preparative HPLC, overall yields of 20% resulted for both octapeptides. Yields were not optimized; improvement might be achieved by warming the resin and/or prolonging the coupling time, especially for the longer sequences. The structures were confirmed by ^{1}H NMR and MALDI-TOF-MS.

When the *N*-2-nitrobenzenesulfonyl protected amino acid bromides (*o*-Nbs-Aaa-Br) were used for the synthesis of the same long sequences as described in Scheme 1, yields were very poor, because of the bulky *N*-protecting group. Nevertheless, *o*-Nbs-Aaa-Br proved to be useful in the SPPS synthesis of very difficult heteropeptides such as **3**, exemplified in Scheme 2. In fact, this synthesis was tried by us, without or with limited success, using either common condensing agents (TBTU/HOBT) or *N*-protected-Aaa-Cl (generated in situ according to the method proposed by Gilon),[6] but afforded peptide bonds with quantitative yields when *o*-Nbs-Aaa-Br were used.

The product was characterized by ^{1}NMR, ESI-MS and RP-HPLC for stereochemical purity.

A drawback with the amino acid bromides is the use of a limited number of *N*-protecting groups; among compatible groups, we have experimented phthaloyl-(Pht),[1] arylsulfonyl,[4,7] dithiasuccinoyl (Dts),† 1,1-dimethylthiomethylene [$(MeS)_2$–C═],† and azide as precursor of the amino function; the latter clearly proved to be the most convenient, because of its small size and stability, as already proved for Aaa-Cl by several authors.[8–11]

However, the hazard in the use of azides represents a problem for industrial scale-up. Thus, when possible, other protecting groups compatible with bromide activation, such as *o*-Nbs, can be introduced.

In conclusion, we have shown herein that amino acid bromides are versatile reagents for solid phase peptide synthesis and that the reactivities obtained are superior even to those of Fischer's amino acid chlorides, which still represent the most active acylating reagents. A further advantage of the overactivated bromides is to avoid the use of strong bases during the coupling, which inevitably gives rise to racemization. As the scope and generality of high-throughput synthesis advances, there is a need to incorporate virtually any residue into a peptide chain; however, synthetic difficulties severely limit the attainment of certain peptide bond formation. In these cases, utilization of Aaa-Br offers a valid alternative over existing procedures.

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† Unpublished results.

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