N^{α}-Alloc temporary protection in solid-phase peptide synthesis. The use of amine–borane complexes as allyl group scavengers

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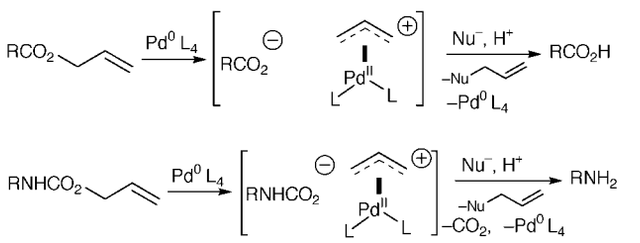
The use of a combination of amine–borane complexes and soluble palladium catalyst allows the fast deprotection of allyl carbamates under near-to-neutral conditions and without any side-formation of allylamine. Preliminary experiments indicate that palladium catalyst–amine–borane systems seem ideally suited for removal of N^{α}-Alloc terminal groups during solid phase peptide synthesis according to the N^{α}-Alloc temporary protection strategy.

Introduction

Two main strategies are in current use in solid phase peptide synthesis (SPPS).[1,2] The Merrifield strategy, historically the first one, is based on graduated acid lability and utilizes the Boc group for "temporary" protection of the N^{α}-amino function. The Boc group is generally removed with trifluoroacetic acid (30–50%) in dichloromethane. "Permanent" side-chain protection usually involves benzylic or related groups that are cleaved with strong acids, typically liquid hydrogen fluoride or trifluoromethanesulfonic acid. The second strategy, based on orthogonality, makes use of Carpino's base labile Fmoc (fluoren-9-ylmethoxycarbonyl) group for temporary protection of the N^{α}-amino function. The Fmoc group is typically removed by piperidine in DMF. Permanent side-chain protection usually relies on the use of base-stable acid-labile *tert*-butylic or related groups which are cleaved in trifluoroacetic acid or other media of similar acidity. Despite its cost, the second strategy tends more and more to supersede the first one in many laboratories, as it involves milder reagents and generally leads to peptides of higher purity and in higher yields.[3]

Despite its mild conditions however, the Fmoc/*t*-Bu strategy still may entail procedures too harsh for the synthesis of extremely fragile peptides and there is a constant need for the development of protecting groups which can be removed under neutral conditions. Towards this end, allylic protecting groups, which have been introduced first by Kunz and co-workers in the field of peptide chemistry,[4] seem well-suited.[5] Allylic groups, namely the allyl (All) group for protection of carboxylic acids and the allyloxycarbonyl (Alloc) group for protection of amines may be readily removed through palladium catalysed transfer of the allyl entity to various nucleophilic species as represented in Scheme 1. *tert*-Butylic groups are not affected by these procedures. Neither are the Fmoc or the Fm (fluoren-9-ylmethyl) groups, provided that the nucleophilic allyl group scavenger chosen for palladium catalysed removal of allylic protecting groups is of sufficiently low basicity. As to the allyl and allyloxycarbonyl groups, they are, conversely, sufficiently robust to stand the basic conditions of Fmoc removal and the acid conditions of Boc removal. This orthogonality between allyl-based protecting groups and *t*-Bu- and Fm-based protecting groups has given rise, in the recent years, to a number of useful

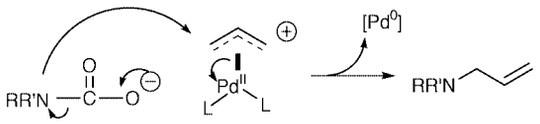


Scheme 1 Palladium catalysed deprotection of allyl carboxylates and carbamates.

applications,[5] especially in the field of peptide synthesis. In particular, semi-permanent protection by the All or the Alloc groups of *α*-carboxy or N^{α}-amino groups and of various side-chain functionalities has been frequently used for the synthesis of cyclic or branched peptides and in glycopeptide chemistry. Allylic linkers have been devised and used in SPPS.[5,6] Recently, we showed[7] that advantage could be taken of the neutral conditions involved in the tandem deprotection–coupling of N^{α}-Alloc amino acids with N^{α}-Fmoc amino acid fluorides in the presence of palladium catalyst and phenylsilane as the allyl group scavenger, to suppress, in cases where this side reaction may be troublesome, the formation of diketopiperazine during SPPS. Meanwhile, apart from a synthesis of substance P published in 1987,[8] we are not aware of examples, in the literature, of the use of the allyloxycarbonyl group in a repetitive way for temporary N^{α}-amino protection in SPPS.[9] We report here on such a utilization, based on a new deprotection system involving amine–borane complexes as allyl group scavengers.

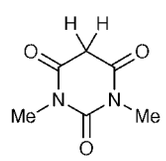
Nucleophilic species which may, in principle, be used in palladium-catalysed removal of allylic protecting groups are many and include oxygen, nitrogen, carbon and sulfur nucleophiles, as well as various hydride donors.[5] Yet, if the deprotection procedure is to be used in a repetitive manner, only quite reactive allyl group scavengers can be considered, in order to get fast and very selective reactions. Of particular concern is the possible formation, during deprotection of allyl carbamates, of allylamines as side products,[5] which, in the present case, cannot be tolerated even to the extent of a few percent. This side-formation of allylamine may result not only from competition between the newly deprotected amine and the nucleophilic allyl group scavenger for trapping of the π-allyl entity, but also from direct intramolecular decarboxylative condensation within the carbamato-π-allylic intermediate[10] as represented in Scheme 2.

In the aforementioned synthesis of substance P according to the N^{α}-Alloc temporary protection strategy,[8] cleavage was effected by the hydrostannolytic procedure which utilizes tributyltin hydride as the allyl group scavenger. This procedure had been selected essentially for two reasons: firstly, the hydrostannolytic procedure is extremely fast, being complete within



Scheme 2 Decarboxylative rearrangement of carbamato π-allylic intermediate into allylamine.

a few minutes and therefore reducing the risk of allylamine formation; secondly, the reaction is very selective since tributyltin hydride is non-basic and is, at room temperature, inert towards almost any reducible function which does not interact with soluble palladium catalysts. With this method, substance P was obtained in moderate, non-optimized 25% yield. Two drawbacks are however associated with the use of tributyltin hydride: the first one is that tributyltin hydride is not easy to handle and, in particular, shows a propensity, in the presence of various agents including palladium catalyst, to decompose into hexabutyldistannane and dihydrogen;[8] the second one is associated with the toxicity of tin compounds and the difficulty often encountered in their complete elimination from the desired end products (this last difficulty being however minimized in the case of synthesis on solid supports). With the aim of developing alternative tin-free methods well-suited for repetitive Alloc removal, we decided to investigate other allyl group scavengers and to first test their ability to deprotect allyl carbamates without side-formation of allyl amines. Given the additional requirement of operating under conditions as close to neutrality as possible, we chose to investigate the use of scavengers whose nucleophilicity is unveiled only through deprotonation, itself induced by palladium cleavage of the allyl group of carbamates and the subsequent formation of amines. Such pronucleophilic species include Kunz's *N*,*N*′-dimethylbarbituric acid[11] (NDMBA, **1**) and Genêt's thiosalicylic acid[12] (TSA, **2**). We chose also to investigate the use of pseudometallic hydrides, *i.e*. borohydrides (BH_4^-) initially proposed by Zhu and Beugelmans,[13] phenylsilane ($PhSiH_3$) already used by ourselves,[14] and several hitherto untested amine–borane complexes including $NH_3{\cdot}BH_3$, $(Me)_2NH{\cdot}BH_3$, *t*-Bu-$NH_2{\cdot}BH_3$, $(Me)_3N{\cdot}BH_3$ and $Py{\cdot}BH_3$. Indeed, such amine–borane complexes[15] seemed attractive for several reasons: they are non-basic and decomposed only slowly even in relatively strong acidic medium; as reducing agents they are quite inert at room temperature towards functional groups usually encountered in peptide chemistry. At the same time, as suggested by the fact that hydroboration by related species such as catecholborane is catalysed by many transition metal complexes,[16] it could be reasonably expected that their hydride-donor ability towards palladium π-allyl complexes would be very high; finally, the expected ultimate by-products of reaction (volatile amines and boric acid) should not interfere significantly in the purification steps.



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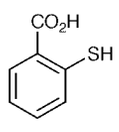

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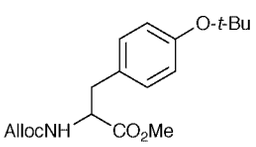

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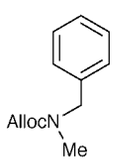

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Table 1 Palladium-catalysed deprotection of the Alloc derivative of *N*-methylbenzylamine **4** in the presence of various allyl group scavengers

	Yield (%)	
Allyl group scavenger	H–N(Me)CH_2Ph[a]	All–N(Me)CH_2Ph[a]
NDMBA (**1**)	100	0
TSA (**2**)	100	0
$PhSiH_3$	95	5
$Bu_4N^+BH_4^-$	60	40
$NH_3{\cdot}BH_3$	99.6 to 100	0 to 0.4
$Me_2NH{\cdot}BH_3$	100	0
t-Bu-$NH_2{\cdot}BH_3$	96 to 97	3 to 4
$Me_3N{\cdot}BH_3$	0	100
$Py{\cdot}BH_3$	0	100

[a] As determined by capillary GC (detection limits ≤ 0.1%) after proper calibration. In all cases, the measurements were made after complete deprotection of starting Alloc derivative of methylbenzylamine.

Results and discussion

Deprotection of model allyl carbamates and other allyl-protected functions in solution

In order to operate a selection among all the aforementioned potentially useful allyl group scavengers, their ability to deprotect allyl carbamates without side-formation of allylamines was tested in solution on two model Alloc derivatives: the Alloc derivative of *O*-*tert*-butyltyrosine methyl ester **3** was chosen as a mimic of the N^{α}-A terminal group of a peptide; the Alloc derivative of *N*-methylbenzylamine **4** was chosen on the grounds that, like other Alloc derivatives of secondary amines, it shows a much higher propensity than Alloc derivatives of primary amines to lead to allylamine[17] in the presence of palladium catalyst and therefore constitutes a more severe and discriminating test.

All reactions were conducted in dichloromethane at room temperature, using 3 to 5 mol% of $Pd(PPh_3)_4$ as the catalyst and two *molar* equivalents of allyl group scavengers. The initial concentration in Alloc derivative was in all cases 0.15 M. The reactions were monitored by coupled gas chromatography–mass spectroscopy (detection limits ≤ 0.1%) on aliquot portions after short filtration through silica gel. The final products of reactions were analysed in the same way. Concerning this last point, all scavengers used in the deprotection of **3**, namely NDMBA (**1**), TSA (**2**), $PhSiH_3$, Bu_4N^+ BH_4^-, $NH_3{\cdot}BH_3$, $Me_2NH{\cdot}BH_3$ and *t*-$BuNH_2{\cdot}BH_3$, were found to lead selectively to the deprotected non-allylated amine. Only in the case of tetrabutylammonium borohydride and $PhSiH_3$ could *traces* (<1%) of *N*-allyl derivative be detected.

As anticipated, and illustrated in Table 1, the second test proved to be more discriminating. The pronucleophilic scavengers NDMBA and TSA, and the amine borane complexes $H_3N{\cdot}BH_3$ and $Me_2NH{\cdot}BH_3$ were found to lead selectively to *N*-methylbenzylamine. Meanwhile, increasing formation of *N*-allyl-*N*-methylbenzylamine was observed in the series *t*-$BuNH_2{\cdot}BH_3 \leq PhSiH_3 < BH_4^-$. As to the experiments with $Py{\cdot}BH_3$ and $Me_3N{\cdot}BH_3$ (which had not been tested in the deprotection of **3**), they were found to lead exclusively to the *N*-allylated amine. Clearly, the tertiary amine–borane complexes do not act at all as allyl group scavengers.

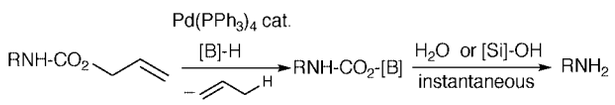
From the above experiments and in regard to selectivity, the pronucleophilic species NDMBA and TSA on the one hand, and the amine–borane complexes $H_3N{\cdot}BH_3$ and $Me_2NH{\cdot}BH_3$ on the other hand appear to be the best-suited scavengers for the palladium-catalysed removal of *N*-Alloc groups. In regard to reactivity however, the amine–borane complexes which lead to complete deprotection in less than 10 min are much superior to the pronucleophilic species for which reaction times between

Table 2 Palladium-catalysed deprotection of allylic side-chain protecting group with the Me_2HN-BH_3–$Pd(PPh_3)_4$ system[a]

Protected derivative	Deprotected compound	Isolated yield (%)
Fmoc-Lys(Alloc)-OMe	Fmoc-Lys-OMe	88
Fmoc-Tyr(All)-OMe	Fmoc-Tyr-OMe	88
Fmoc-Asp(OAll)-OMe	Fmoc-Asp-OMe	75
Boc-Tyr(All)-OMe	Boc-Tyr-OMe	90

[a] The deprotection reactions were run in dichloromethane at room temperature using 2 molar equivalents of Me_2HN-BH_3 and 5 mol% of $Pd(PPh_3)_4$. The initial concentration of allylic substrate was 0.1 M.

30 and 90 min are needed to reach completion. Therefore, only the two amine–borane complexes $H_3N{\cdot}BH_3$ and $Me_2NH{\cdot}BH_3$ were eventually selected as deprotecting agents for our further SPPS study. It should be noted that, in a way similar to what is observed with other pseudometallic hydrides such as tributyltin hydride[8] or phenylsilane,[14,18] the deprotection of *N*-Alloc derivatives with amine–borane complexes does not lead to the free amines but to the corresponding boron carbamates which may be characterized by IR spectroscopy. For instance, treatment of **4** with $Me_2NH{\cdot}BH_3$ in the presence of palladium catalyst results in a shift of the carbonyl absorption from 1705 cm^{-1} to 1692 cm^{-1} (in dichloromethane solution). The boron carbamates are readily hydrolysed and decarboxylated upon exposure for instance to small amounts of water or silica (Scheme 3).



Scheme 3 Palladium catalysed deprotection of allyl carbamates in the presence of boranes.

Before testing the efficiency of amine–borane complexes as allyl group scavenger in SPPS according to an N^{α}-Alloc temporary protection strategy, their reactivity towards several tryptophan derivatives which may be ranged among the most reducible entities that may be encountered in peptide synthesis was examined. We thus found that Ac-Trp-OMe and Fmoc-Trp(Boc)-OMe are left unchanged (RMN, TLC) after exposure[19] at room temperature for 48 h to $H_3N{\cdot}BH_3$ or $Me_2NH{\cdot}BH_3$ either in the absence or the presence (5 mol%) of $Pd(PPh_3)_4$. In the absence of catalyst, the more sensitive *N*-formylindolyl derivative Boc-Trp(CHO)-OMe does not react with $Me_2NH{\cdot}BH_3$ but is slowly reduced by $H_3N{\cdot}BH_3$ with a half-reaction time of *ca*. 24 h to the corresponding *N*-hydroxymethylindolyl derivative Boc-Trp(CH_2OH)-OMe. The presence of palladium catalyst (5 mol%) accelerates the reaction and half-conversion of Boc-Trp(CHO)-OMe into Boc-Trp(CH_2OH)-OMe occurs within a few hours even with $Me_2NH{\cdot}BH_3$.

The deprotection, in solution, of amino acid derivatives with allylic protection on their side-chain functionalities was also briefly investigated on four examples: Fmoc-Lys(Alloc)-OMe, Fmoc-Tyr(All)-OMe, Boc-Tyr(All)-OMe and Fmoc-Asp(OAll)-OMe. In all cases, complete and selective deprotection was achieved within 10 min at room temperature, and, as shown in Table 2 the deprotected compounds were isolated in satisfactory yields after purification by column chromatography. The easy deprotection of allyl ethers of tyrosine is particularly worthy of note and confirms the fact that pseudometallic hydrides (Bu_3SnH,[8,20] sodium or lithium borohydrides,[13] $PhSiH_3$,[14] and amine–boranes complexes) seem the best allyl group scavengers in such case.

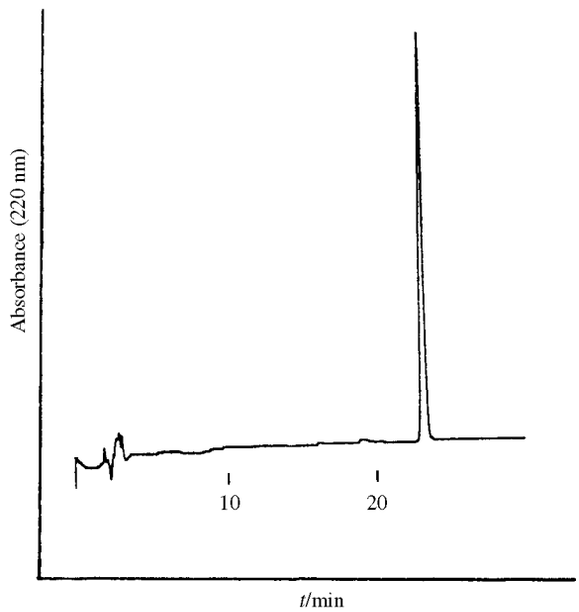


Fig. 1 HPLC of crude Tyr-Ala-Gly-Phe-Leu-NH_2 synthesised from Alloc amino acid pentafluorophenyl esters. Allylic deprotection method: $Pd(PPh_3)_4$ (10%), borane–ammonia complex (BH_3–NH_3) (6 equivalents). HPLC conditions: Nucleosil C18 (250 × 4 mm) column; eluents: A; 0.045% TFA in water; B, 0.036% TFA in CH_3CN; gradient: linear from 10% B to 40% in 30 min, then 40% B to 100% in 10 min; flow rate: 1 mL min^{-1}.

SPPS using N^{α}-Alloc amino acids and palladium-catalysed Alloc group removal in the presence of ammonia– or dimethylamine–borane complexes

The N^{α}-Alloc amino acids for use in SPPS were prepared as previously described.[7] Although these derivatives are obtained in the form of oils, they can be crystallised, purified and stored as their dicyclohexylammonium salts. Alternatively, we have found that the pentafluorophenyl esters are also crystalline compounds, and therefore these esters can constitute a convenient method of storage of N^{α}-Alloc amino acid derivatives, which as active species can be used directly for synthetic purpose. These derivatives, namely Alloc-Gly-OPfp, Alloc-Ala-OPfp, Alloc-Leu-OPfp, Alloc-Phe-OPfp and Alloc-Tyr-(*t*-Bu)-OPfp were obtained in good yields (80–95%) by reaction of the N^{α}-Alloc amino acids with pentafluorophenyl trifluoroacetate in the presence of pyridine in DMF, according to the general procedure of Green and Berman.[21] To evaluate the efficacy of N^{α}-Alloc protection in SPPS, the oligopeptide-amides Tyr-Ala-Gly-Phe-Leu-NH_2 and Lys-Gly-Phe-Leu-Glu-Glu-Val-NH_2 were synthesized on a PAL-PS-resin. The Boc group was used for side-chain protection of the Lys residue and the *t*-Bu group for side-chain protection of the Tyr and Glu residues. Coupling reactions were carried out either with free N^{α}-Alloc amino acids (obtained after liberation from their dicyclohexylammonium salts with 0.5 M aqueous sulfuric acid and extraction with EtOAc)[22] in the presence of diisopropylcarbodiimide (DIPCDI) and HOBt in DMF or with their pentafluorophenyl esters in the presence of HOBt in DMF. The removal of the N^{α}-Alloc groups was accomplished under an argon atmosphere in dichloromethane at room temperature with 10 mol% (based on the substitution of the resin) of $Pd(PPh_3)_4$ and 6 equiv. of $H_3N{\cdot}BH_3$ or $Me_2NH{\cdot}BH_3$. The amine borane complex was *first* added to the resin under agitation, followed, a few min later, by the catalyst. After 10 min, the Pd solution was drained and the resin was washed with DCM (3 × 30 s) and the deprotection procedure was once repeated. Before next coupling, the resin was washed sucessively with DCM (8 × 30 s), TFA 0.2%–DCM (2 × 60 s), DCM (5 × 30 s), DIEA 5%–DCM (3 × 60 s) and DCM (5 × 30 s).

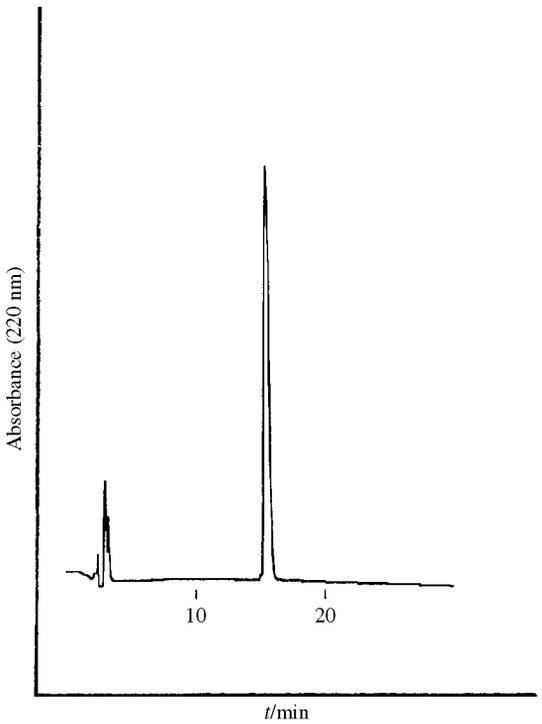


Fig. 2 HPLC of crude Lys-Gly-Phe-Leu-Glu-Glu-Val-NH_2 synthesised from carboxy-free Alloc amino acids. Allylic deprotection method: $Pd(PPh_3)_4$ (10%), borane–dimethylamine complex (BH_3–$NHMe_2$) (6 equivalents). HPLC conditions: same as in Fig. 1.

After removal of the last N^{α}-Alloc group and washing of the resin, the peptidic chain was cleaved from the support and the side-chain functionalities were deprotected by treatment with trifluoroacetic acid–water 95:5. The HPLC profiles of the crude peptides thus obtained after precipitation with Et_2O were all satisfactory. Two of them are reproduced in Figs. 1 and 2. All peptides showed an amino acid analysis of the acid hydrolysed in agreement with the expected results and a correct MS (FAB and MALDI-TOF) with the absence of signals corresponding to allyl peptides. This confirms results found in solution that amine back-alkylation is not a side-reaction when amine–borane complexes are used as allyl group scavengers in the removal of Alloc protecting groups.

In conclusion, the preliminary results exposed in this communication indicate that SPPS under near-to-neutral conditions appears possible using N^{α}-Alloc protection and its removal through palladium catalysed cleavage in the presence of amine–borane complexes. Work is in progress to apply this methodology to the synthesis of more complex and highly base-labile peptides or pseudopeptides.

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