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Deprotection of *N*-Alloc amines by Pd(0)/DABCO—an efficient method for in situ peptide coupling of labile amino acids

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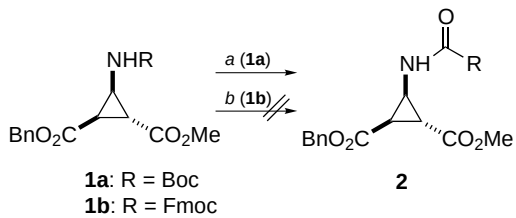
Abstract—A highly efficient *one-pot* deprotection/peptide coupling protocol of *N*-Alloc amino acids with activated *N*-Boc or *N*-Fmoc amino acids was developed in solution and on solid phase. DABCO was found to be especially effective for the deprotection of the *N*-Alloc group, resulting in short reaction times (10–20 min) and allowing the coupling of amino acids that are unstable in unprotected forms.

The synthesis of amides by coupling of a carboxylic acid with an amine is well established, most notably in peptide synthesis. Commonly, a carboxylic acid is activated by appropriate reagents in situ and is then reacted with an unprotected amine.[1] However, if the amine is difficult to prepare or even unstable in unprotected form, a technique which allows its in situ generation and coupling with a carboxylic acid is required. For example, β-aminocyclopropane carboxylic acids[2] (β-ACCs), e.g. **1**,[3] have been recognized recently as conformationally rigid amino acids for the synthesis of peptides.[4,5] Since donor–acceptor 1,2-disubstituted cyclopropanes rapidly undergo ring opening,[6] derivatives of **1** are only stable if an electron-withdrawing group protects the amino functionality.[7] We were able to couple **1a** with amino acids via its ammonium chloride salt (Scheme 1);[5b] however, this strategy is not compatible with acid-sensitive groups that are commonly encountered as protecting groups of amino acid side chains. Moreover, the protocol requires saturated HCl gas in ethyl acetate, which is also not amenable to automated solid-phase synthesis.

Consequently, we were looking for a milder method that would allow in situ deprotection and coupling of amines with carboxylic acids. An attractive choice would have been a strategy based on the Fmoc group, taking into account its great importance for peptide synthesis. However, deprotection of Fmoc by base in the presence of activated carboxylic acids is inherently problematic and, indeed, we were unsuccessful when trying to convert derivative **1b** to **2** under various conditions for Fmoc cleavage (TBAF, piperidine, morpholine); only ring opening products of the cyclopropane moiety could be obtained (Scheme 1).

We then turned our attention to the *N*-Alloc protecting group which is known to be readily cleaved by catalytic amounts of palladium(0) in the presence of a nucleophile.[8] Among the allyl scavengers that have been already used in the context of peptide chemistry for *N*-Alloc cleavage,[9] metal hydrides[10] and especially phenylsilane introduced by Guibé[11] seemed to be most promising for our purpose, since it has already been successfully employed for domino deprotection/coupling reactions with active esters of amino acids, giving excellent results. In situ activation of the amino acid to be coupled is also possible, as was shown by Hiemstra and Speckamp[10a,b] or Zhu,[10c] respectively. However, all examples reported so far use *N*-Alloc amino acids that



Scheme 1. (a) (i) **1a**, HCl/EtOAc, (ii) *N*-Boc-XXa, EDC, HOBt, 76–86%; (b) **1b**, base (TBAF, piperidine, or morpholine), RCOX (X = Cl, OBt)→decomposition.

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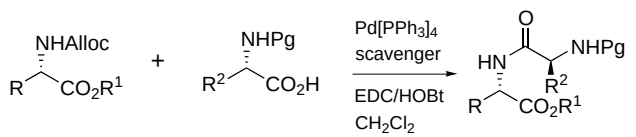
are stable in *N*-unprotected forms and, therefore, do not require an in situ-coupling protocol.

On the other hand, morpholine, pyrrolidine or piperidine have been shown to be also highly effective for the deprotection of the Alloc group in peptide synthesis,[12] having the distinct advantage of hydrolytic stability—especially in the presence of transition metals—and lower cost in comparison to phenylsilane. Although the presence of a secondary amine should not be compatible with amino acids that are Fmoc protected at the N-terminus or activated with a carbodiimide/HOBt[13] at the C-terminus, we envisioned that a tertiary amine such as DABCO[13] or pyridine would either not interfere with the amino acid or could even aid its activation. Moreover, these reagents should offer similar operational advantages like secondary amines, and indeed, pyridine, triethylamine, or *N*-methylmorpholine have been used as allyl scavengers before,[8] albeit not in a one-pot peptide-coupling protocol.

Using *N*-Alloc-alanine benzylester (**3a**) and *N*-Alloc-valine methylester (**3b**) as model compounds, we first tested if pyridine or DABCO could be used for *N*-Alloc deprotection in the presence of an activated carboxylic acid to ultimately achieve peptide coupling (Scheme 2 and Table 1). Indeed, the dipeptides **5** were obtained in high yields (94–99%) using pyridine/$Pd[PPh_3]_4$ as the deprotecting agent in the presence of *N*-protected amino acids activated by EDC/HOBt.[13] *N*-Boc- and especially *N*-Fmoc-protected amino acids are suitable coupling partners. Nevertheless, the reactions proceeded slowly (entry 1) making 20 mol% of palladium catalyst necessary to stay within acceptable reaction times (entries 2–4).



3a: R=Me, R^1=Bn
3b: R=^{i}Pr, R^1=Me

4a: R^2=Me, Pg=Boc
4b: R^2=Bn, Pg=Boc
4c: R^2=Me, Pg=Fmoc

5

Scheme 2. One-pot peptide coupling of amino acids **3** and **4** (cf. Table 1).

Using DABCO instead of pyridine, however, dramatically accelerated the deprotection/coupling sequence, giving rise to **5** after a reaction time of only 10–20 min when 10 mol% of palladium(0) were employed (87–99% yields, entries 5–8).[14] Most notably, under these reaction conditions *N*-Fmoc-protected amino acids are again tolerated as coupling partners (entry 8), so that the synthesis of peptide fragments suitable for Fmoc strategy becomes possible. Moreover, the protection of carboxylic acids as benzyl esters is possible (entries 1–3, 5, and 6), since the latter functionality is not cleaved by Pd(0) using the protocol described here. With both protocols, little ($\leq$2%) or no racemization was observed in the coupling step.

While the examples given so far have only been advantageous from an operational point of view due to the convenience of a one-pot deprotection/coupling process, a more challenging task from a synthetic point of view is the coupling of β-ACC derivatives, such as **6**, being unstable in the *N*-unprotected form.

Gratifyingly, both protocols were also applicable for the coupling of **6** (Table 2); the dipeptides **7** could be obtained in high yields (92–97%). Again the use of DABCO resulted in considerable shortened reaction times, but another advantage of this procedure in comparison to the use of pyridine became apparent: although ring opening of the in situ-generated cyclopropylamino carboxylic acid could be suppressed in both cases, epimerization of the stereocenter bearing the amino group was observed to a substantial extent (up to 20%) when pyridine was employed. In contrast, epimerization was minimal (<3%) when DABCO was used, indicating that this reagent is not responsible solely for the cleavage of the allyl group but might also activate the HOBt ester of the amino acid used as the coupling partner.

Using phenyl silane as allyl scavenger, the coupling of **6** with *N*-Fmoc-alanine not only afforded a good yield (90%) of **7** (entry 7), but also 5% of the ring-opening product, which made the purification of the dipeptide difficult.

We were also able to apply the in situ-coupling protocol to solid-phase synthesis, thereby demonstrating that the building block **8** can be used as an unnatural amino

Table 1. One-pot peptide coupling of amino acids **3** and **4** (cf. Scheme 2)

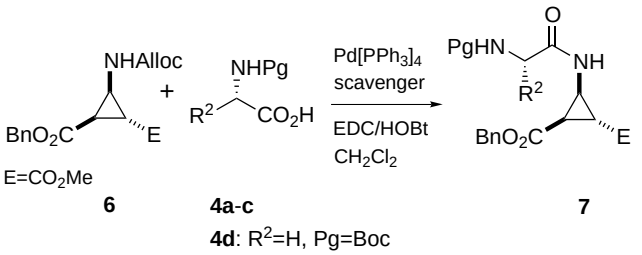
Entry	Amino acids[a]		Scavenger[b]	$Pd(PPh_3)_4$ (mol%)	Time (min)	Yield (%)
1	**3a**	**4a**	Py	10	900	94
2	**3a**	**4b**	Py	20	90	99
3	**3a**	**4c**	Py	20	90	94
4	**3b**	**4c**	Py	20	90	98
5	**3a**	**4a**	DABCO	10	10	99
6	**3a**	**4b**	DABCO	10	10	99
7	**3b**	**4a**	DABCO	10	20	97
8	**3b**	**4c**	DABCO	10	20	87

[a] 3 equiv. **4a**,**4b** or 1.5 equiv. **4c**, respectively, were employed.
[b] 20 equiv. pyridine or 5 equiv. DABCO were employed.

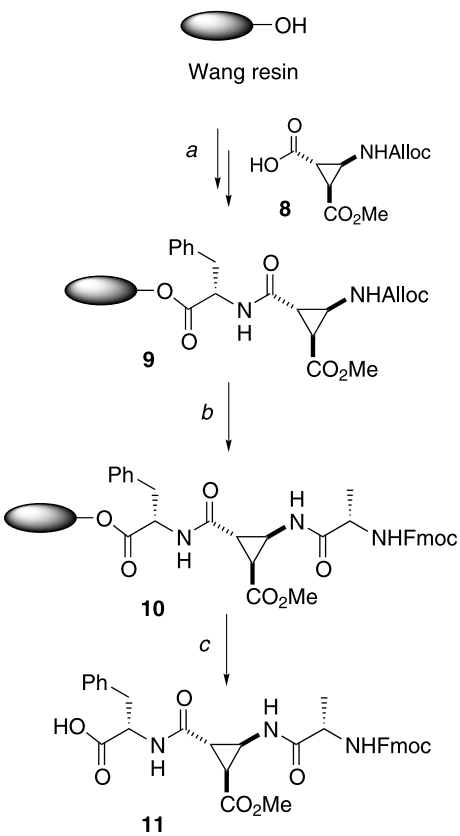
Table 2. One-pot peptide coupling of amino acids **4** and **6** (cf. Scheme 3)

Entry	Amino acid[a]	Scavenger[b]	$Pd(PPh_3)_4$ (mol%)	Time (min)	Yield (%)
1	**4a**	Py	20	900	93
2	**4d**	Py	10	120	95
3	**4a**	DABCO	10	15	96
4	**4b**	DABCO	10	15	93
5	**4c**	DABCO	10	15	92
6	**4d**	DABCO	10	15	97
7	**4c**	$PhSiH_3$	10	15	90[c]

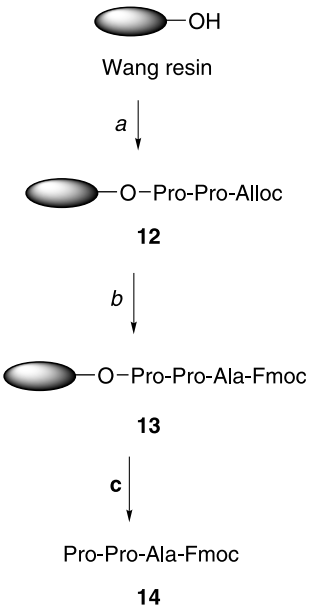
[a] 3 equiv. **4a**,**4b** or 1.5 equiv. **4c**, respectively, were employed.
[b] 10 equiv. pyridine or 5 equiv. DABCO or $PhSiH_3$ were employed.
[c] Contaminated with 5% of the ring-opening product of **6**.



Scheme 3. One-pot peptide coupling of amino acids **4** and **6** (cf. Table 2).



Scheme 4. (a) (i) Fmoc-PheOH, DIC, HOBt, DMF, (ii) piperidine, DMF, (iii) **8**, DIC, HOBt, DMF; (b) $Pd[PPh_3]_4$, DABCO *or* $PhSiH_3$ (12 equiv.), Fmoc-Ala-OH, EDC, HOBt, CH_2Cl_2, 2 h, rt; (c) TFA, CH_2Cl_2 2:1; isolated yield based on loading of Fmoc-Phe-OH to the resin: 85% (DABCO), 83% ($PhSiH_3$).



Scheme 5. (a) (i) Fmoc-Pro-OH, DIC, HOBt, DMF, (ii) piperidine, DMF, (iii) Alloc-Pro-OH, DIC, HOBt, DMF; (b) $Pd[PPh_3]_4$, DABCO *or* $PhSiH_3$ (12 equiv.), Fmoc-Ala-OH, EDC, HOBt, CH_2Cl_2, 2 h, rt; (c) TFA, CH_2Cl_2 2:1; isolated yield based on loading of Phe to the resin: 99% (DABCO), 99% ($PhSiH_3$).

acid in automated peptide synthesis (Scheme 4). Using standard DIC/HOBt[13] peptide-coupling techniques, dipeptide **9** was synthesized on a Wang™ resin with an Advanced Chem Tech ACT-90 synthesizer. Palladium(0)-catalyzed deprotection with DABCO or phenylsilane in the presence of Fmoc-Ala-OH resulted in the resin-bound peptide **10**. Upon cleavage from the resin with trifluoroacetic acid, tripeptide **11** was formed without any detectable epimerization in 83–85% isolated yield. While we chose to add $PhSiH_3$ manually[15] just prior to the Alloc-deprotection/coupling step to insure its integrity, we used a solution of DABCO in CH_2Cl_2 without special precaution other than carrying out the reaction under a nitrogen atmosphere within the setup of an automated peptide synthesizer.

Finally, the peptide protocols described here also allowed the linear coupling of amino acids on solid phase between the second and third position. This transformation is particularly difficult to accomplish

since intramolecular diketopiperazine (DKP) formation with concurrent cleavage from the resin, being initiated by the free N-terminus of the peptide, is often encountered.[16] The Alloc tandem deprotection/coupling strategy was already successfully applied to suppress DKP formation.[11b] We demonstrate here the application of this strategy in combination with the in situ activation of the amino acid towards dipeptides containing proline residues, which are especially prone to DKP formation. Gratifyingly, even the *N*-Alloc-protected Pro-Pro dipeptide **12** smoothly underwent in situ coupling with Fmoc-Ala either with DABCO or $PhSiH_3$ acting as allyl scavenger.[17] After cleavage from the resin, **14** was obtained in quantitative yield (Scheme 5).

In conclusion, a new variant of the tandem deprotection/coupling strategy for the synthesis of peptides using *N*-Alloc-protected amino acids has been developed, which seems to be especially useful for amino acids and peptide fragments that are labile in the *N*-unprotected form.

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13. *Abbreviations*: DABCO, 1,8-diazabicyclo[2.2.2]cyclooctane; EDC, 1-(3(dimethylamino)propyl)-3-ethylcarbodiimide; DIC, diisopropylcarbodiimide.
14. Representative procedure: All solvents were dried by standard laboratory methods and degassed with nitrogen prior to use. The *N*-Boc or *N*-Fmoc amino acid **4** (3 equiv.) was activated by stirring in CH_2Cl_2 with EDC (3 equiv.) and HOBt (3 equiv.) for 1 h at 0°C and 1 h at rt under a nitrogen atmosphere. The palladium(0) catalyst (10–20 mol%) and, subsequently, a solution of the *N*-Alloc amino acid **3** or **6** (0.4 mmol, 1 equiv.) in CH_2Cl_2 were added. Finally, DABCO (5 equiv.) was added in one portion and the reaction mixture was stirred for the indicated time. Extractive work-up of the organic layer (saturated $NaHCO_3$, 1N $KHSO_4$, and saturated $NaHCO_3$), drying ($MgSO_4$), concentration and purification of the residue on silica gel afforded **5** or **7** (Tables 1 and 2).
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17. One referee raised the important question whether DABCO can be rigorously defined as an allyl scavenger, since the fate of the allyl group remained unclear. It was suggested that the allyl group might end up as allyl ether of HOBt or even as an *N*-allyl derivative of HOBt. This intriguing speculation implied that DABCO could act simply as a catalyst. We tested this hypothesis by coupling **6** with benzoic acid using our general procedure (see Ref. 14, activation with DIC) but only in the presence of 10 mol% DABCO. Unfortunately, we were able to obtain very little of the expected coupling product. Therefore, we must conclude at this point that the allylammonium salt of DABCO is formed and that the latter indeed serves as the primary allyl scavenger.