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An efficient method for the synthesis of phenacyl ester-protected dipeptides using neutral alumina-supported sodium carbonate 'Na_2CO_3/n-Al_2O_3'

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In the synthesis of dipeptides (Boc-AA^1-AA^2-OPac: AA^1 and AA^2 represent amino acids) protected by phenacyl (Pac) ester, amines and solid bases as the base for the conversion of the trifluoroacetic acid (TFA) salt of the amino component (TFA·H-AA^2-OPac) into the corresponding free amino component (H-AA^2-OPac) were examined. The synthesis of a dipeptide (Boc-Ala-Gly-OPac) using amines for the conversion afforded an unsatisfactory yield with by-products. On the other hand, the use of neutral alumina-supported Na_2CO_3 (Na_2CO_3/n-Al_2O_3) as a solid base for the conversion provided the dipeptide in a quantitative yield without by-products. The application of Na_2CO_3/n-Al_2O_3 to the synthesis of some dipeptides protected by Pac ester gave the desired peptides in excellent yields. Copyright © 2013 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: neutral alumina-supported sodium carbonate; solid base; phenacyl ester; peptide synthesis; amine

Introduction

Phenacyl (Pac) esters offer a useful means by which to protect the carboxy groups of amino acids and other organic compounds. They are easily and selectively removed by special conditions, such as zinc powder in acetic acid [1], sodium thiophenoxide [2], and photolysis [3], without affecting other protective groups such as *t*-butoxycarbonyl (Boc) and ethyl ester (OEt), which are eliminated by treatment with acid or base, respectively. Additionally, a magnesium reduction with acetic acid has been reported for the cleavage of Pac esters [4]. Because of these unique means of deprotection, Pac esters have been successfully applied to the protection of carboxy groups during peptide and other organic syntheses [5,6]. Also, it is an advantage that N-protected amino acid Pac esters are easy to handle because they crystallize easily. When a Pac ester-protected dipeptide (Boc-AA^1-AA^2-OPac: AA^1 and AA^2 represent the carboxy and amino component amino acids, respectively) is synthesized by a solution-phase method, the trifluoroacetic acid (TFA) salt of the amino component (TFA·H-AA^2-OPac) derived from Boc-AA^2-OPac is usually converted in advance into the corresponding free amino component (H-AA^2-OPac) by treatment with amines such as triethylamine (Et_3N), *N*-methylmorpholine (NMM), and *N*,*N*-diisopropylethylamine (DIEA) in an organic solvent. Next, the desired peptide (Boc-AA^1-AA^2-OPac) is synthesized by adding a solution containing the produced H-AA^2-OPac to a solution including the carboxy component (Boc-AA^1-OH). In our previous synthesis of Pac ester-protected peptides, the use of amines in the conversion occasionally brought about unsatisfactory yields of the desired peptides [6]. It was suggested to the authors that such lower yields are affected by the length of time that the H-AA^2-OPac produced by the treatment with an amine in an organic solvent exists in those solution. Hence, in order to improve the yields, the use of solid bases in the synthesis of dipeptides using Pac ester-protected amino acids was examined. If a solid base exists in the reaction solution, it is assumed that TFA·H-AA^2-OPac is converted into H-AA^2-OPac on the solid base, and the resulting H-AA^2-OPac will couple *in situ* with a carboxy component which dissolves in the same solution. The use of solid bases will therefore shorten the time in which H-AA^2-OPac exists in the solution and should improve the coupling yields using Pac esters. In this paper, we report on the effectiveness of neutral alumina-supported Na_2CO_3 (Na_2CO_3/n-Al_2O_3) as a solid base for the synthesis of dipeptides that are protected by Pac esters (Scheme 1).

Results and Discussions

The effect of various solid bases and amines on the conversion of TFA·H-Gly-OPac [7] into H-Gly-OPac was examined by the coupling between Boc-Ala-OH and TFA·H-Gly-OPac in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hy

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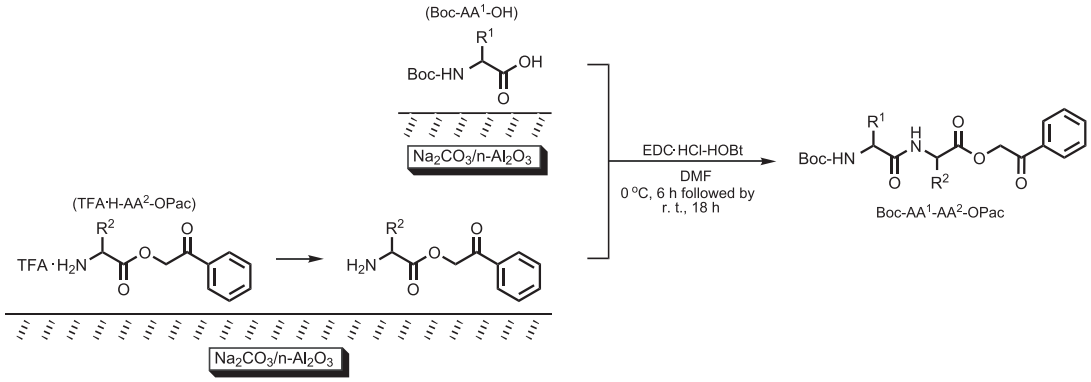

Scheme 1. Synthetic method of dipeptides protected by Pac ester using Na_2CO_3/n-Al_2O_3 as a solid base. AA^1 and AA^2 represent the carboxy and amino component amino acids, respectively. R^1 and R^2 represent the side chains of amino acids. Reagents and conditions: TFA·H-AA^2-OPac (1.00 equiv), Na_2CO_3/n-Al_2O_3 0.530 g (Na_2CO_3 0.500 equiv for TFA·H-AA^2-OPac), Boc-AA^1-OH (1.10 equiv for TFA·H-AA^2-OPac), EDC·HCl (1.20 equiv for Boc-AA^1-OH), HOBt (1.50 equiv for Boc-AA^1-OH), DMF (4.0 ml).

drochloride (EDC·HCl)-1-hydroxybenzotoriazole (HOBt) in *N*,*N*-dimethylformamide (DMF) at 0 °C for 6.0 h followed by room temperature (r. t.) for 18 h. These results are shown in Table 1. The coupling using powdered Na_2CO_3 (0.500 mmol) as a solid base gave Boc-Ala-Gly-OPac (**1**) in an 83% yield. The use of neutral alumina (n-Al_2O_3, 0.480 g) afforded **1** in a 78% yield. Also, the coupling using a mixture of powdered Na_2CO_3 (0.500 mmol) and n-Al_2O_3 (0.480 g) (Na_2CO_3: n-Al_2O_3 = 1 : 10 weight ratio) gave **1** in a much higher yield of 96%. These results suggest that the coexistence of Na_2CO_3 and n-Al_2O_3 in the reaction system is necessary to produce **1** in a high yield. On this basis, the coupling using neutral alumina-supported 10% Na_2CO_3 (Na_2CO_3/n-Al_2O_3) [8] as a solid base yielded **1** in a quantitative yield of 99%. On the other hand, the couplings employing the amines such as Et_3N, NMM, and DIEA gave **1** in an 87%, 85%, and a 71% yield, respectively. This shows that Na_2CO_3/n-Al_2O_3 is a very effective base compared with the amines for the coupling using the Pac ester-protected amino acids. Although the results of Table 1 also suggested that the n-Al_2O_3 itself acts as base, the insufficient conversion to H-AA^2-OPac of TFA·H-AA^2-OPac will result in a lowering of coupling yields. Therefore, subsequent experiments were performed with an equivalent amount of sodium carbonate to TFA·H-AA^2-OPac. The specific rotation data of **1** obtained by the couplings employing Na_2CO_3/Al_2O_3 and general Et_3N were nearly identical values (Table 1).

Table 1. Effect of various bases in the synthesis of Boc-Ala-Gly-OPac (**1**) by the coupling of Boc-Ala-OH with TFA·H-Gly-OPac[a]

Bases	Yield/%
	Boc-Ala-Gly-OPac (**1**)
Na_2CO_3/n-Al_2O_3	99[b]
Powdered Na_2CO_3 + n-Al_2O_3	96
Powdered Na_2CO_3	83
n-Al_2O_3	78
Et_3N	87[c]
NMM	85
DIEA	71

[a]Yield was determined by RP-HPLC using an internal standard sample. Reagents and conditions: TFA·H-Gly-OPac (1.00 mmol), Boc-Ala-OH (1.10 mmol), EDC·HCl (1.30 mmol), HOBt (1.70 mmol); bases: Na_2CO_3/n-Al_2O_3 (0.533 g, Na_2CO_3: 0.500 mmol), powdered Na_2CO_3 (0.500 mmol) + n-Al_2O_3 (0.480 g), powdered Na_2CO_3 (0.500 mmol), n-Al_2O_3 (0.480 g), Et_3N (1.00 mmol), NMM (1.00 mmol), DIEA (1.00 mmol); reaction temperature and time: 0 °C for 6.0 h followed by r. t. for 18 h.
[b]$[\alpha]_D^{27}$ 28.4 (MeOH, c = 1.00).
[c]$[\alpha]_D^{27}$ 28.3 (MeOH, c = 1.00).

In order to further clarify the effectiveness, each solution extracted by ethyl acetate (EtOAc) after the couplings between Boc-Ala-OH and TFA·H-Gly-OPac using both Na_2CO_3/n-Al_2O_3 and Et_3N bases were analyzed by reversed phase HPLC (RP-HPLC), as shown in Figure 1. The RP-HPLC profile after the coupling using Na_2CO_3/n-Al_2O_3 showed only one peak (**a**) corresponding to **1** without peaks of by-products [Figure 1 (A)]. In contrast, the RP-HPLC profile after the coupling using Et_3N showed peaks corresponding to by-products, **b** and **c**, along with **a** [Figure 1(B)]. These RP-HPLC profiles strongly indicate that Na_2CO_3/n-Al_2O_3 is preferable to Et_3N for the coupling employing the Pac ester-protected amino acids. In addition, the RP-HPLC results show that Na_2CO_3/n-Al_2O_3 is a very favorable base for inhibiting the formation of by-products. The by-products, **b** and **c**, in Figure 1(B) were isolated, and their respective structures were characterized by mass spectrometry (MS). Each by-product was additionally confirmed by direct comparison with authentic samples synthesized independently. By these analyses, **b** and **c** were identified as Boc-Ala-OPac and Boc-Ala-Gly-Gly-OPac, respectively. On the basis of these results, it was suggested that the Pac ester in H-Gly-OPac is hydrolyzed into glycine and 2-hydroxyacetophenone by water, which will be included in the commercial grade solvent (DMF), and then **b** and **c** are formed by the couplings among the degradation products and a small excess carboxy component (Boc-Ala-OH). In the synthesis of **1**, an intramolecular cyclized by-product was not observed [9,10].

Table 2 shows the results using Na_2CO_3/n-Al_2O_3 in various couplings between Boc-AA^1-OH and TFA·H-AA^2-OPac in the presence of EDC·HCl-HOBt in DMF at 0 °C for 6.0 h followed by r. t. for 18 h. For the couplings of Boc-Ala-OH and TFA·H-AA^2-OPac (AA^2 = Gly, Ala, Leu, and Val), the products (Boc-Ala-AA^2-OPac) were obtained in high yields of 99%, 87%, 89%, and 85%, respectively. The coupling between Boc-Ala-OH and TFA·H-Ile-OPac using EDC·HCl (1.30 mmol) produced Boc-Ala-Ile-OPac in an unsatisfactory yield of 75%. By increasing the amount of EDC·HCl to 1.65 mmol, the yield increased to 91%.

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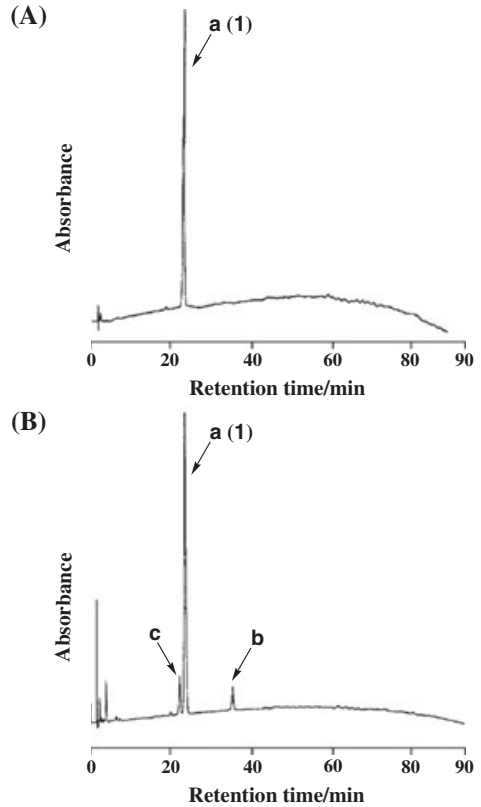

Figure 1. HPLC profiles of each solution extracted by EtOAc after couplings used (A) Na_2CO_3/Al_2O_3 and (B) Et_3N in the synthesis of Boc-Ala-Gly-OPac (**1**). Peak **a**: **1**, peak **b**: Boc-Ala-OPac, peak **c**: Boc-Ala-Gly-Gly-OPac. Elution conditions: column, Waters μ-Bondasphere 5 μm C_{18}-300 Å (3.9 × 150 mm); eluent, 14–68% CH_3CN/H_2O-0.1% TFA (v/v/v); running conditions, 90 min linear gradient; flow rate, 1.0 ml/min; detection, 210 nm.

Table 2. Yield of the couplings of carboxy components, Boc-AA^1-OH, with amino components, TFA·H-AA^2-OPac, using Na_2CO_3/n-Al_2O_3[a]

Products	Boc-AA^1-OH	TFA·H-AA^2-OPac	Yield/%	
	AA^1	AA^2	Na_2CO_3/n-Al_2O_3[b]	Et_3N[c]
Boc-Ala-Gly-OPac (**1**)	Ala	Gly	99	87
Boc-Ala-Ala-OPac	Ala	Ala	87	80
Boc-Ala-Leu-OPac	Ala	Leu	89	73
Boc-Ala-Val-OPac	Ala	Val	85	83
Boc-Ala-Ile-OPac	Ala	Ile	75	62
Boc-Ala-Ile-OPac	Ala	Ile	91[d]	73[d]
Boc-Ala-Phe-OPac	Ala	Phe	70	65
Boc-Ala-Phe-OPac	Ala	Phe	70[d]	—
Boc-Gly-Gly-OPac	Gly	Gly	99	87
Boc-Pro-Gly-OPac	Pro	Gly	99	84

[a]Yield was determined by RP-HPLC using an internal standard sample. Reagents and conditions: Boc-Ala-OH (1.10 mmol), TFA·H-Gly-OPac (1.00 mmol), EDC·HCl (1.30 mmol), HOBt (1.70 mmol), DMF (2.0 ml), reaction temperature and time: 0 °C for 6.0 h followed by r. t. for 18 h.
[b]Na_2CO_3/n-Al_2O_3 of 0.533 g was used.
[c]Et_3N of 1.00 mmol was employed.
[d]EDC·HCl of 1.65 mmol was used.

The improvement in the yield will be attributed to couple with Boc-Ala-OH before the decomposition of H-Ile-OPac through the addition of excess EDC·HCl. The coupling of Boc-Ala-OH and TFA·H-Phe-OPac with EDC·HCl (1.30 mmol) also gave Boc-Ala-Phe-OPac in an unsatisfactory yield of 70%. However, the yield was 70% despite using a higher excess level of EDC·HCl (1.65 mmol). This result suggests that H-Phe-OPac is liable to sustain the degradation. The coupling between Boc-AA^1-OH (AA^1 = Gly and Pro) and TFA·H-Gly-OPac afforded Boc-Gly-Gly-OPac and Boc-Pro-Gly-OPac in a quantitative yield of 99%. On the other hand, as can be seen from Table 2, the synthetic yields of the dipeptides (Boc-AA^1-AA^2-OPac) using Et_3N were 62–87%. These couplings using Et_3N gave low yields of 2–18% compared with those employing Na_2CO_3/n-Al_2O_3. These results showed that Na_2CO_3/n-Al_2O_3 is an excellent base compared with Et_3N for the synthesis of the Pac ester-protected dipeptides. Although Pac esters are utilized in the synthesis of peptide fragments, the lowering of the synthetic yields employing Et_3N is disadvantageous in terms of cost. Accordingly, the present method using Na_2CO_3/n-Al_2O_3 should extend the possibility for the use of Pac esters in the synthesis of peptide fragments.

As a scope for this method, we think that the present system using Na_2CO_3/n-Al_2O_3 will be applicable to the syntheses of N-protected-dipeptide-esters employing different acid salts of amino acid esters (X·H-AA^2-OY: X represents acids such as TFA, hydrogen chloride, and *p*-toluenesulfonic acid, etc.; Y represents Pac, Et, and benzyl, etc.). However, as a limitation of this method, we think that the system may not contribute toward controlling the racemization of the proline Pac ester during the synthesis of Boc-AA^1-Pro-OPac.

Conclusions

The application of the Na_2CO_3/n-Al_2O_3 in the synthesis of dipeptides that are protected by a Pac ester depresses the side reactions and produces the desired dipeptides in high yields. The superior yields will be due to couple with a carboxy component (Boc-AA^1-OH) *in situ* before a free amino component (H-AA^2-OPac) generated on the Na_2CO_3/n-Al_2O_3 decompose, and these results will support the assumption mentioned in the introduction.

Materials and Methods

All reagents and solvents were used in commercial grade. The amino acids used, with the exception of glycine, are of the L-configuration. Melting points were measured with a Type B-540 (BUCHI, Flawil, Switzerland). RP-HPLC was performed on a PU-2080 Plus system (JASCO, Tokyo, Japan) with a Waters μ-Bondasphere column (C_{18}, 5 μm, and 300 Å; 3.9 I.D. × 150 mm) using a flow rate of 1.0 ml/min and the following solvent systems: 0.1% TFA in 5% CH_3CN/H_2O and 0.1% TFA in 95% CH_3CN/H_2O. Yields were determined by RP-HPLC results at 210 nm. Fast atom bombardment (FAB) mass spectra were recorded using a JMS-DX 303 spectrometer (JEOL, Tokyo, Japan). Optical rotations were determined with a DIP-360 digital type polarimeter (JASCO, Tokyo, Japan).

Preparation of Na_2CO_3/n-Al_2O_3

To sodium carbonate (5.0 g) dissolved in water (80 ml), neutral alumina (n-Al_2O_3; ICN Biomedical N-super 1, particle size 63–200 μm; 45 g) was added. After the mixture was stirred for 30 min at r. t., the water was removed by evaporation. The residual solid was dried at 150 °C for 6.0 h under *vacuo* to give Na_2CO_3/n-Al_2O_3.

Typical Coupling Procedure Using Na_2CO_3/n-Al_2O_3; Boc-Ala-Gly-OPac (**1**)

To a solution of Boc-Ala-OH (0.208 g, 1.10 mmol) in DMF (2.0 ml), HOBt (0.223 g, 1.65 mmol) and EDC·HCl (0.253 g, 1.32 mmol) were added at 0 °C. To the mixture was added Na_2CO_3/n-Al_2O_3 (0.533 g; Na_2CO_3 0.500 mmol), immediately followed by a solution of TFA·H-Gly-OPac (1.00 mmol) dissolved in DMF (2.0 ml) at r. t. The mixture was stirred at 0 °C for 6.0 h and then at r. t. for 18 h. To the mixture was then added DMF (10 ml) and Boc-Phe-OPac as the internal standard reagent. The solution was applied to an RP-HPLC column under the conditions described in the succeeding text. From the RP-HPLC result, the yield of **1** was determined using a calibration curve with a straight line plotted against three different concentration ratios of Boc-Ala-Gly-OPac and Boc-Phe-OPac. Yield 99%; mp 102.0–102.4 °C; MS (FAB): *m/z* $[M+H]^+$ calculated for $C_{18}H_{25}O_6N_2$: 365, found: 365; $[M+Na]^+$ calculated for $C_{18}H_{24}O_6N_2Na$: 387, found: 387; $[\alpha]_D^{27}$ 28.4 (MeOH, $c = 1.00$). Retention time: Boc-Ala-Gly-OPac 9.9 min. RP-HPLC conditions: solvent, 23–46% CH_3CN/H_2O-0.1% TFA (v/v/v); linear gradient, 30 min.

Typical Coupling Procedure Using Amines (Et_3N); Boc-Ala-Gly-OPac (**1**)

TFA·H-Gly-OPac (1.00 mmol) was dissolved in DMF (2.0 ml) at r. t., and then the solution was mixed with Et_3N (1.00 mmol) at 0 °C. The solution was added to another solution containing Boc-Ala-OH (1.10 mmol), HOBt (1.65 mmol), and EDC·HCl (1.32 mmol) in DMF (2.0 ml) at 0 °C. The mixture was stirred at 0 °C for 6.0 h and then at r. t. for 18 h. The yield of **1** was determined by RP-HPLC by using an internal standard sample. Yield 85%; $[\alpha]_D^{27}$ 28.3 (MeOH, $c = 1.00$).

Boc-Ala-OPac (b)

MS (FAB) *m/z* $[M+H]^+$ calculated for $C_{16}H_{22}O_5N$: 308, found: 308; $[M+Na]^+$ calculated for $C_{16}H_{21}O_5NNa$: 330, found: 330.

Boc-Ala-Gly-Gly-OPac (c)

MS (FAB) *m/z* $[M+H]^+$ calculated for $C_{20}H_{28}O_7N_3$: 422, found: 422; $[M+Na]^+$ calculated for $C_{20}H_{27}O_7N_3Na$: 444, found: 444.

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