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One-pot protection and activation of amino acids using pentafluorophenyl carbonates

Ramesh Ramapanicker, Nasir Baig Rashid Baig, Kavita De and Srinivasan Chandrasekaran*

Protection of the amino group and activation of the carboxylic acid groups are the most important steps associated with any peptide synthesis protocol; hence, a one-pot process to achieve these is highly desirable. A possible strategy is to use pentafluorophenyl carbonates to simultaneously protect the amino group as a carbamate derivative and activate the carboxylic acid group as a pentafluorophenyl ester. A detailed study is carried out to understand the scope and limitations of this method using five different pentaflurophenyl carbonates. The efficiency of these one-pot reactions depends largely on the nature of the pentafluorophenyl carbonates and also on the nature of the amino acids. Electron deficient and sterically less demanding carbonates reacted faster than the others, whereas amino acids with longer aliphatic side chains gave better yields than more polar amino acids.

Supporting information may be found in the online version of this article

Keywords: carbonates; protecting groups; amino acids; one-pot reactions; peptide synthesis; active esters

Introduction

The efficiency and simplicity of peptide synthesis relies largely on the strategies used for protecting the α-amino group and the activation of the carboxylic acid group prior to peptide coupling. The above two steps are undoubtedly the most important in any form of peptide synthesis. The protection of the amino group is most commonly achieved by preparing a carbamate derivative of the amine [1]. Although the use of various coupling reagents is the method of choice for the activation of the carboxylic acid group, preformed active esters, especially pentafluorophenyl esters [1,2] are frequently used for achieving slow and reliable coupling reactions with minimum side reactions [1]. It is a definite advantage over existing strategies if the protection of the amino group and the activation of the carboxylic acid group of amino acids can be achieved in one step.

The use of pentafluorophenyl esters and carbonates as reagents for achieving the simultaneous protection and activation of amino acids has existed as a possible option. Encouraged by the early efforts of Suto and Gayo using pentafluorophenyl trifluoroacetate (TfaOPfp) [3] and Rao *et al*. using *N*-triluoroacetoxysuccinimide (TfaOSu) [4] for the simultaneous protection and activation of amino acids, we had examined the utility of propargyl pentafluorophenyl carbonate (PocOPfp, **1**) [5] as a reagent for the simultaneous urethane protection and carboxy activation of amino acids [6]. We had achieved better and more useful results using **1**, as our attempts involved the protection of the amino groups as easily removable propargyloxycarbonyl (Poc) derivatives [7].

Results and Discussion

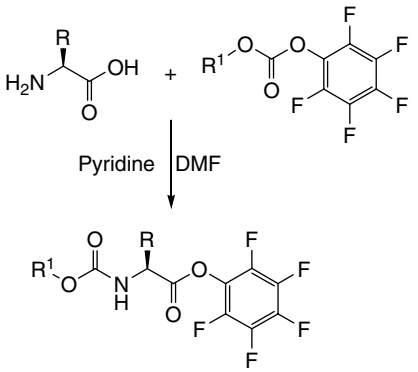
Apart from these isolated reports, the generality of the methodology was never evaluated and its practical implications were never addressed. Herein, we report a detailed study on the one-pot protection and activation of amino acids using various pentafluorophenyl carbonates (Scheme 1). The efficiency of these reactions with respect to the nature of the carbonates is analyzed as an effort to understand the scope and limitations of this methodology. Treating amino acids with PocOPfp (2.1 equiv) resulted in the protection of the amino group as a Poc derivative and activation of the carboxyl group as a pentafluorophenyl (Pfp) ester [6]. In our studies using PocOPfp (**1**), we had used the conditions reported by Suto and Gayo [3] (pyridine as the base and DMF as the solvent) for carrying out these reactions. 3-amino benzoic acid (**2**) on treatment with **1** under the above conditions yielded the corresponding *N*-protected active ester (**3**) in 92% yield [6].

Taking this particular example as a standard we examined the usefulness of a few other commonly used organic bases such as triethylamine, DMAP, *N*-methylmorpholine, DBU, etc. for effecting this reaction. It was observed that although the expected product **3** was obtained, the *N*-Poc propargyl ester derivative (**4**) [6] was also formed in these reactions (Scheme 2). However, the formation of the propargyl ester, **4** was not observed when pyridine (2 equiv) was used as the base in DMF. The formation of **4** on using stronger bases for the reaction could be due to the base catalyzed degradation of the active anhydride, **5** (Scheme 2) [8].

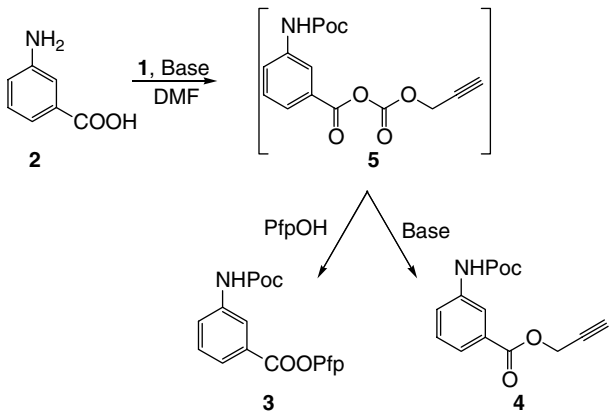
The efforts to carry out this reaction using solvents other than DMF were unsuccessful as the amino acids were completely insoluble in most of the other organic solvents. Using DMSO as the

* *Correspondence to: Srinivasan Chandrasekaran, Department of Organic Chemistry, Indian Institute of Science, Bangalore-560012, India. E-mail: scn@orgchem.iisc.ernet.in*

Department of Organic Chemistry, Indian Institute of Science, Bangalore-560012, India



Scheme 1. Simultaneous protection and activation of amino acids using pentafluorophenyl carbonates.



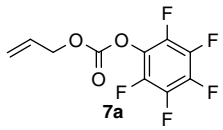
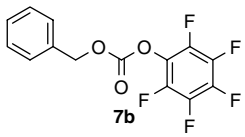
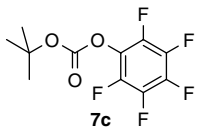
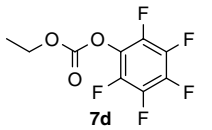
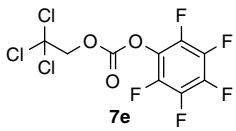
Scheme 2. Reaction of PocOPfp (**1**) with 3-amino benzoic acid (**2**) in the presence of strong bases.

solvent resulted in the reaction yielding **3** in 87% yield. Although the reaction could be carried out with pyridine as the solvent rather than as a stoichiometric base, the reaction yielded **4** (55%) as the major product. All these results suggested that pyridine in DMF as used by Suto and Gayo [3] could be the best base-solvent combination for the simultaneous protection and activation of amino acids using pentafluorophenyl carbonates.

It was then expected that treating amino acids with any pentafluorophenyl carbonate (pyridine, DMF, 0 °C – rt) will protect the amino group as the corresponding carbamate and activate the carboxylic acid group as a pentafluorophenyl ester. We decided to carry out the studies using alkyl pentafluorophenyl carbonates, which will introduce the most commonly used carbamate protecting groups onto the amino group. Suto and Gayo had used FmocOPfp (9-fluorenylmethyl pentafluorophenyl carbonate) for the simultaneous protection and activation of anthranilic acid (**6**), but required 6 equiv of the reagent and 2 days for the reaction to go to completion [3]. We achieved the simultaneous protection and activation of **6** with 2.1 equiv of PocOPfp (**1**) in 3 h [6]. The comparison of the reactivities of FmocOPfp and **1** towards **6** suggested that the former is not a suitable reagent for this methodology and was not selected for our studies reported here.

We synthesized five different pentafluorophenyl carbonates namely; allyl pentafluorophenyl carbonate (AlocOPfp, **7a**), benzyl pentafluorophenyl carbonate (CbzOPfp, **7b**), *tert*-butyl pentafluorophenyl carbonate (BocOPfp, **7c**), ethyl pentafluorophenyl carbonate (EocOPfp, **7d**) and trichloroethyl pentafluorophenyl carbonate (TrocOPfp, **7e**) for our studies. BocOPfp (**7c**) was prepared from Boc_2O and pentafluorophenol in the presence of DMAP (CH_2Cl_2, 0 °C, 5 h) whereas the remaining four carbonates were prepared from the corresponding chloroformates and pentafluorophenol (CH_2Cl_2, NMM, 0 °C, 5 h). The pentafluorophenyl carbonates **7a-e** were obtained in excellent yields (Table 1) and are stable under ambient conditions for several days.

Table 1. Synthesis of pentafluorophenyl carbonates

Entry	Pentafluorophenyl carbonates	Reaction conditions	Yield (%)[a]
1	 **7a**	AlocCl, PfpOH, NMM CH_2Cl_2, 0 °C, 5 h	97
2	 **7b**	CbzCl, PfpOH, NMM CH_2Cl_2, 0 °C, 5 h	93
3	 **7c**	Boc_2O, PfpOH, DMAP CH_2Cl_2, 0 °C, 5 h	85
4	 **7d**	EocCl, PfpOH, NMM CH_2Cl_2, 0 °C, 5 h	98
5	 **7e**	TrocCl, PfpOH, NMM CH_2Cl_2, 0 °C, 5 h	97

[a] Yields correspond to those of the pure compounds isolated after column chromatography.

We started our experiments using AlocOPfp (**7a**), which we assumed would give results similar to those obtained using PocOPfp due to the similarities in their structure. Reactions were carried out with a few of the most commonly used α-amino acids by treating them with **7a** and pyridine (DMF, 0 °C to rt, 4–24 h). The reactions were continued till the complete disappearance of the carbonate **7a** in TLC or for 24 h. The yields of the *N*-Aloc protected active amino esters (**8a–g**, Table 2: column 4) were similar to those obtained with PocOPfp (**1**) [6]. The results were in line with our expectations and confirmed that the similarities in the structures of the pentafluorophenyl carbonates can give similar results for the one-pot protection and activation of amino acids using them. The yields were in general high for amino acids bearing longer aliphatic side chains. The best result was obtained for proline (84%, column 4: entry 1) whereas, alanine yielded the least (45%, column 4: entry 5) among the amino acids studied.

The experiments were then carried out with CbzOPfp (**7b**) by treating it with a few α-amino acids under the same conditions (DMF, pyridine, 0 °C to rt, 4–24 h). Although all the amino acids studied yielded the corresponding *N*-Cbz protected active amino esters (**9a–f**), the yields (Table 2: column 5) were poorer than

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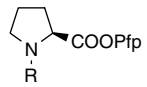
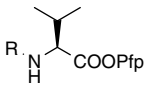
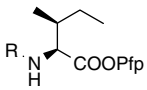
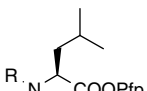
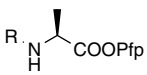
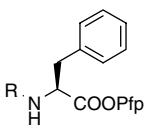
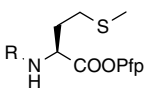
Table 2. One-pot protection and activation of amino acids using pentafluorophenyl carbonates

Entry	Amino acid	*N*-protected Pfp ester	R is Aloc	R is Cbz	R is Boc	R is Eoc	R is Troc
			8a	9a	10a	11a	12a
1	H-Pro-OH		Time = 4 h Yield = 84%	Time = 4 h Yield = 83%	Time = 24 h Yield = 55%	Time = 10 h Yield = 65%	Time = 3 h Yield = 90%
			8b	9b	10b	11b	12b
2	H-Val-OH		Time = 5 h Yield = 72%	Time = 8 h Yield = 75%	Time = 24 h Yield = 24%	Time = 24 h Yield = 45%	Time = 3 h Yield = 87%
			8c	9c	10c	11c	12c
3	H-Ile-OH		Time = 5 h Yield = 74%	Time = 8 h Yield = 73%	Time = 24 h Yield = 35%	Time = 24 h Yield = 52%	Time = 3 h Yield = 85%
			8d	9d	10d	11d	12d
4	H-Leu-OH		Time = 9 h Yield = 73%	Time = 24 h Yield = 31%	Time = 24 h Yield = 20%	Time = 24 h Yield = 24%	Time = 4 h Yield = 85%
			8e	9e	10e	11e	12e
5	H-Ala-OH		Time = 24 h Yield = 45%	Time = 24 h Yield = 30%	Time = 24 h Yield = 12%	Time = 24 h Yield = 22%	Time = 8 h Yield = 70%
			8f	9f	10f		
6	H-Phe-OH		Time = 24 h Yield = 53%	Time = 24 h Yield = 45%	Time = 24 h Yield = 0%	–	–
			8g				
7	H-Met-OH		Time = 24 h Yield = 50%	–	–	–	–

Yields correspond to those of the pure compounds isolated after column chromatography.

those obtained with AlocOPfp (**7a**). As with the results obtained using **7a**, the reaction with proline was the best (83%, column 5: entry 1) and alanine was the worst (30%, column 5: entry 5), and the yields for amino acids bearing longer aliphatic chains were better (entries 1–3). However, it was quite surprising to note that leucine gave a poor yield of the product (31%, column 5: entry 4) in this reaction whereas with AlocOPfp (**7a**), the yield of the product (**8d**) from leucine was comparable to those of proline, valine and isoleucine.

Our efforts to use BocOPfp (**7c**), for protection and activation of amino acids in one-pot were disappointing. We assumed the use of **7c** in these reactions will be of greater importance as the products, *N*-Boc protected active amino esters would be more useful in conventional peptide synthesis. However, when various α-amino acids were treated with **7c** (DMF, pyridine, 0 °C to rt, 24 h), the yields of the products (**10a–f**) were quite insignificant (Table 2: column 6). None of the amino acids other than proline (column 6: entry 1) reacted to give the *N*-Boc active ester in good yield. No product could be isolated from the reaction of phenylalanine with **7c** even after 24 h (column 6: entry 6).

The lower reactivity of amino acids with CbzOPfp (**7b**) and BocOPfp (**7c**) compared to their reactivity with AlocOPfp (**7a**) and PocOPfp (**1**) was thought to be due to the steric bulk of the pentafluorophenyl carbonates **7b** and **7c**. We assumed that this can be proved by checking the reactivity of ethyl pentafluorophenyl carbonate (EocOPfp, **7d**) towards amino acids under the conditions studied (DMF, pyridine, 0 °C to rt, 10–24 h). EocOPfp (**7d**) being sterically the least demanding was expected to give the best results. However, when EocOPfp was treated with a few amino acids, the results were surprising. Although the yields of the *N*-Eoc protected active esters (**11a–e**, Table 2: column7) were better than those obtained with BocOPfp (**7c**), they were not as good as those with AlocOPfp (**7a**), CbzOPfp (**7b**) or PocOPfp (**1**). These observations are a clear indication that the steric hindrance of the pentafluorophenyl carbonates is not the only factor that affects the yields of these one-pot reactions.

We then studied the reactivity of trichloroethyl pentafluorophenyl carbonate (TrocOPfp, **7e**) with amino acids assuming that its reactivity will offer a direct comparison between the roles of steric bulk and electronic effects leading to increased reactivity. The results obtained on treating amino acids with **7e** (DMF, pyridine, 0 °C to rt, 24 h) were the best and the *N*-Troc protected active amino esters (**12a–e**) were isolated in excellent yields (Table 2: column 8). The electron deficient nature of **7e** resulted in better reactivity with amino acids even though it is sterically bulkier than EocOPfp (**7d**).

A comparison of the results (Table 2) obtained for the one-pot protection and activation of amino acids with the five different pentafluorophenyl carbonates (**7a–e**) confirms that the effectiveness of the methodology is largely dependent on the nature of the pentafluorophenyl carbonates. The reactivity of AlocOPfp (**7a**) towards amino acids was very much similar to that of PocOPfp (**1**) and the yields and reaction times are comparable (Column 4). The reduced reactivity of CbzOPfp (**7b**) with leucine, alanine and phenylalanine (Column 5) could be attributed to the increased steric bulk of **7b** when compared with **1** and **7a**. The

ineffectiveness of sterically bulky pentafluorophenyl carbonates in these reactions is well demonstrated with the reaction of BocOPfp (**7c**), which did not react completely, even after stirring for 24 h with different amino acids (Column 6). EocOPfp (**7d**), which is sterically the least bulky among the pentafluorophenyl carbonates studied was not a very useful reagent for this transformation. The faster reaction of TrocOPfp (**7e**) with amino acids despite being sterically bulky indicates the positive effect of electron deficiency of the pentafluorophenyl carbonates in this methodology. All the amino acids reacted faster and yielded the *N*-protected active esters in better yields with **7e** (Column 8) than with the other pentafluorophenyl carbonates studied (**7a–d**).

Notably, there is a difference in the reactivity of different amino acids with pentafluorophenyl carbonates, which is mostly independent of the nature of the pentafluorophenyl carbonate. We have observed that amino acids bearing longer aliphatic side chains react better and the results are consistent with the results reported earlier using PocOPfp (**1**) [6]. Proline, valine and isoleucine were the most reactive amino acids whereas alanine and phenylalanine reacted poorly. The results could be accounted based on the solubility of amino acids in a mixture of DMF and pyridine. None of the amino acids completely dissolved in the reaction mixture within the first 30 min. In low yielding reactions (examples: the reaction between alanine and BocOPfp, leucine and CbzOPfp etc.) a considerable amount of the amino acid was found undissolved even after 24 h.

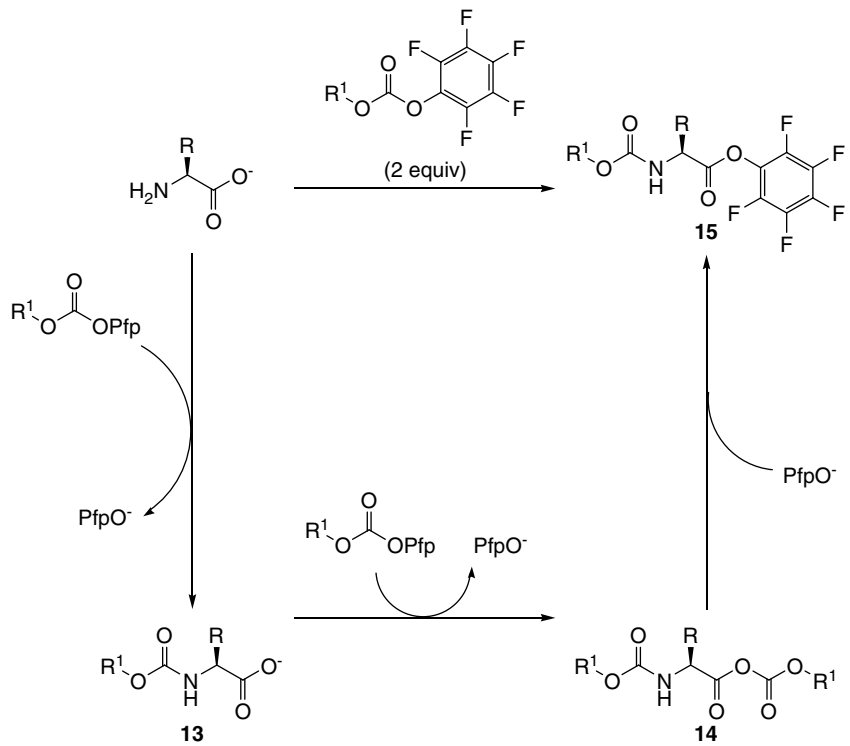
The protection and activation of the amino acids involve a series of reactions (Scheme 3). The process is initiated by a reaction between the dissolved fraction of the amino acid and the pentafluorophenyl carbonate resulting in the protection of the amino group. The *N*-protected amino acid (**13**) then reacts with another equivalent of the pentafluorophenyl carbonate to give the active anhydride (**14**), which reacts with the liberated pentafluorophenolate anion to give the *N*-protected active ester (**15**). We assume that the first step involving the dissolution of the amino acid and protection of the amino group should be the slowest step and should account for the slow rate of the reaction in the case of alanine, which is least soluble in a mixture of DMF and pyridine.

In order to substantiate our arguments, it was essential to prove that the second and third step leading to the formation of the active anhydride (**14**) and the *N*-protected active ester (**15**) are fast and occurs readily after the formation of the *N*-protected amino acid (**13**). Towards this we treated Boc-Ala-OH (**16**) with different pentafluorophenyl carbonates (DMF, Pyridine, 0–28 °C) to examine the efficiency of these carbonates to activate the *N*-protected amino acids as the corresponding pentafluorophenyl esters (Scheme 4, Table 3).

It was observed that all the pentafluorophenyl carbonates studied were efficient reagents for the activation of *N*-protected amino acids as the corresponding pentafluorophenyl esters. Although the rate of this activation reaction depended on the nature of the pentafluorophenyl carbonate used, Boc-Ala-OPfp (**10e**) could be isolated in excellent yields from all the reactions. The results confirm a fast and efficient activation of the *N*-protected amino acids to pentafluorophenyl esters. Therefore, the inefficiency of one-pot protection and activation of certain amino acids can be accounted based on the relatively poor solubility of such amino acids leading to a slow rate of *N*-protection.

Conclusion

In conclusion, we have studied the one-pot protection and activation of amino acids using various pentafluorophenyl carbonates in detail. Reaction times and the yields of the



Scheme 3. Various steps involved in the simultaneous protection and activation of amino acids using pentafluorophenyl carbonates.

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Scheme 4. Activation of Boc-Ala-OH using various pentafluorophenyl carbonates.

Table 3. Activation of Boc-Ala-OH using various pentafluorophenyl carbonates

Entry	Pfp carbonate	Time (h)	Yield (%) of **10e**[a]
1	**1**	2	87
2	**7a**	2	90
3	**7b**	3	93
4	**7c**	5	82
5	**7d**	2	88
6	**7e**	0.5	90

[a] Yields correspond to that of the pure compound isolated after column chromatography.

N-protected active amino esters obtained largely depend on the nature of the pentafluorophenyl carbonates and are also dependent on the nature of the amino acids. Steric bulk of the pentafluorophenyl carbonates had adverse effect on these reactions while electron deficiency of the carbonates resulted in better reactivity. BocOPfp which is sterically bulky gave very poor yield of the products; while TrocOPfp, though sterically hindered gave the best yields probably due to the increased electron deficiency. Amino acids with longer aliphatic side chains reacted better than the others, which is attributed to the increased solubility of those amino acids in the reaction mixture. All the carbonates studied were found to be excellent reagents for the activation of *N*-protected amino acids as the corresponding pentafluorophenyl carbonates. Our studies provide a direct account of the applicability of one-pot protection and activation of amino acids using active carbonates in general and pentafluorophenyl carbonates in particular. Based on our studies, we believe that the methodology could be used to good effect, while preparing specifically protected active esters of hydrophobic unnatural amino acids.

Experimental Section

All reagents were purchased from commercial sources and were used without further treatment. Melting points were recorded on Buchi 510 melting apparatus. Optical rotations were measured with a 1-cm cell (concentration c given in g/100 ml, solvent) on a Perkin Elmer Polarimeter at 25 °C. ^{1}H (300 MHz), ^{13}C NMR (75 MHz) and ^{19}F (282 MHz) were recorded on a JEOL 300 MHz spectrometer. Chemical shifts are reported in parts per million downfield from the internal reference, tetramethylsilane for ^{1}H and ^{13}C NMR and those for ^{19}F NMR are reported with respect to the external standard CF_3COOH. The chemical shift values for the pentafluorophenyl residues are not listed as they are highly split due to coupling with the flourines. IR spectra were recorded on a JASCO FTIR spectrophotometer. High-resolution mass spectra were recorded on a Micromass QTOF ESI MS instrument.

General Procedure for the Preparation of Pentafluorophenyl Carbonates (7a–e)

The chloroformate (10 mmol) was added to a stirred solution of pentafluorophenol (1.84 g, 10 mmol) in dichloromethane (30 ml) at 0 °C. The solution was stirred for 10 min and *N*-methyl morpholine (1.1 ml, 10 mmol) was added dropwise over a period of 15 min. (BocOPfp, 7c was prepared by using DMAP instead of NMM and the workup was done with saturated citric acid solution instead of HCl). After 5 h the reaction mixture was diluted with dichloromethane (50 ml) and washed with 0.5 N HCl (20 ml), water (2 × 20 ml) and brine solution (20 ml). The organic solution containing the pentafluorophenyl carbonate was dried over anhydrous Na_2SO_4 and was purified by column chromatography (silica gel, 100–200 mesh), eluting with 3% solution of ethyl acetate in hexane.

AlocOPfp, 7a

Colorless oil; Yield: 97%; FTIR (Neat): 1791 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.92–6.06 (m, 1H), 4.79–5.49 (m, 2H), 4.79–4.82 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 151.1, 130.1, 120.3, 70.8; ^{19}F NMR (282 MHz, $CDCl_3$): δ = −41.8 (2F), −46.2 (1F), −50.8 (2F); High-resolution ESMS (m/z): Calculated for $C_{10}H_5F_5O_3$ + Na: 291.0057; Observed: 291.0061.

CbzOPfp, 7b

White Crystalline solid; Melting point: 55 °C; Yield: 93%; FTIR (Neat): 1789 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34–7.41 (m, 5H), 5.30 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 151.3, 133.8, 129.1, 128.7, 128.5, 72.0; ^{19}F NMR (282 MHz, $CDCl_3$): δ = −41.6 (2F), −46.1 (1F), −50.6 (2F); High resolution ESMS (m/z): Calculated for $C_{14}H_7F_5O_3$ + Na: 341.0213; Observed: 341.0208.

BocOPfp, 7c

Colorless oil; Yield: 85%; FTIR (Neat): 1791 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.58 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 149.4, 86.7, 27.3; ^{19}F NMR (282 MHz, $CDCl_3$): δ = −41.9 (2F), −46.7 (1F), −50.9 (2F); High-resolution ESMS (m/z): Calculated for $C_{11}H_9F_5O_3$ + Na: 307.0370; Observed 307.0367.

EocOPfp, 7d

Colorless oil; Yield: 98%; FTIR (Neat): 1790 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.40 (q, J = 7.8 Hz, 2H), 1.43 (t, J = 7.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 151.2, 66.8, 13.8; ^{19}F NMR (282 MHz, $CDCl_3$): δ = −42.1 (2F), −46.6 (1F), −51.1 (2F); High resolution ESMS (m/z): Calculated for $C_9H_5F_5O_3$ + Na: 279.0057; Observed: 279.0065.

TrocOPfp, 7e

White crystalline solid; Melting point: 43 °C; Yield: 97%; FTIR (Neat): 1791 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.93 (s, 2H), ^{13}C NMR (75MHz, $CDCl_3$): δ = 150.6, 93.4, 78.1; ^{19}F NMR (282 MHz, $CDCl_3$): δ = −41.3 (2F), −44.8 (1F), −49.8 (2F); High-resolution ESMS (m/z): Calculated for $C_9H_2Cl_3F_5O_3$ + Na: 380.8888; Observed: 380.8884.

General Procedure for the Simultaneous Protection and Activation of Amino Acids using Pentafluorophenyl Carbonates

A suspension of the amino acid (1.1 mmol) and the pentafluorophenyl carbonate (2 mmol) in DMF (2 ml) was cooled to 0 °C and pyridine (0.177 ml, 2.2 mmol) was added to it dropwise. The reaction mixture was allowed to attain room temperature (28 °C) and was stirred till the disappearance of the starting materials or for 24 h. The reaction mixture was then diluted with dichloromethane (30 ml) and washed with saturated citric acid solution (10 ml), water (2 × 10 ml) and brine (10 ml). The resulting solution was dried over Na_2SO_4 and concentrated under vacuum and the products were purified by column chromatography (silica gel, 100–200 mesh) eluting with 5–15% solution of ethyl acetate in hexane.

The use of bases other than pyridine or the use of pyridine as a solvent in the reaction of 3-aminobenzoic acid (**2**) with PocOPfp (**1**) resulted in the formation of the propargyl ester, **4**[6] along with the expected active ester, **3**.

Aloc-Pro-OPfp, 8a

Isolated as a mixture of two rotamers. Colorless oil; Yield: 84%; $[\alpha]_D$: −42 (C = 1, methanol); FTIR (Neat): 3093 (br), 1720 (s), 1682 (s); ^{1}H NMR (300MHz, $CDCl_3$): δ = 5.84–6.02 (m, 1H), 5.19–5.36 (m, 2H), 4.55–4.74 (m, 3H), 3.51–3.73 (m, 2H), 2.36–2.54 (m, 1H), 2.14–2.31 (m, 1H), 1.99–2.12 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.0, 168.7, 154.6, 153.9, 132.6, 132.3, 117.8, 117.6, 66.4, 66.3, 58.8, 58.4, 46.8, 46.4, 31.2, 30.1, 24.4, 23.5; ^{19}F NMR (282 MHz, $CDCl_3$): δ = −40.8, −41.5, −45.9, −46.2, −50.4, −50.7; High resolution ESMS (m/z): Calculated for $C_{13}H_{12}F_5NO_4$ + Na: 388.0584; Observed: 388.0580.

Cbz-Val-OPfp, 9b

White solid; mp: 50 °C, Yield: 75%; $[\alpha]_D$: −10 (C = 1, methanol); FTIR (Neat): 3329 (br), 1783 (s), 1711 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35 (bs, 5H), 5.41 (d, J = 9.3 Hz, 1H), 5.15 (s, 2H), 4.68 (dd, J_1 = 8.9 Hz, J_2 = 4.5 Hz, 1H), 2.34–2.42 (m, 1H), 1.09 (d, J = 7.2 Hz, 3H), 1.02 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.4, 156.4, 135.7, 128.5, 128.3, 128.1, 67.6, 59.0, 31.1, 18.8, 17.2; ^{19}F NMR (282 MHz, $CDCl_3$): δ = −40.4 (2F), −45.5 (1F), −50.3 (2F); High-resolution ESMS (m/z): Calculated for $C_{19}H_{16}F_5NO_4$ + Na: 440.0897; Observed: 440.0890.

Boc-Ile-OPfp, 10c

Colorless oil; Yield: 35%; $[\alpha]_D$: −5 (C = 1, methanol); FTIR (Neat): 3330 (br), 1784 (s), 1714 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.04 (d, J = 9 Hz, 1H), 4.61 (dd, J_1 = 9 Hz, J_2 = 4.8 Hz, 1H), 2.04 (bs, 1H), 1.57 (s, 9H), 1.22–1.31 (m, 2H), 1.07 (d, J = 6.6 Hz, 3H), 0.969 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.7, 155.3, 86.3, 58.1, 37.8, 27.4, 24.9, 15.4, 11.5; ^{19}F NMR (282 MHz, $CDCl_3$): δ = −41.7 (2F), −46.6 (1F), −50.8 (2F); High-resolution ESMS (m/z): Calculated for $C_{17}H_{20}F_5NO_4$ + Na: 420.1210; Observed: 420.1207.

Eoc-Leu-OPfp, 11d

Colorless oil; Yield: 24%; $[\alpha]_D$: −18 (C = 1, methanol); FTIR (Neat): 3324 (br), 1795 (s), 1699 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.08 (bd, J = 7.8 Hz, 1H), 4.68–4.75 (m, 1H), 4.17 (q, J = 6.9 Hz, 2H), 1.67–1.87 (m, 3H), 1.27 (t, J = 6.9 Hz, 3H), 1.02 (d, J = 6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.5, 156.0, 61.6, 52.3, 41.2, 24.8, 22.8, 21.6, 14.4; ^{19}F (282 MHz, $CDCl_3$): δ = −40.6 (2F), −45.8 (1F), −50.4 (2F); High resolution ESMS (m/z): Calculated for $C_{15}H_{16}F_5NO_4$ + Na: 392.2737; Observed: 392.2741.

Troc-Ala-OPfp, 12e

Colorless oil; Yield: 70%: $[\alpha]_D$: −16 (C = 1, methanol); FTIR (Neat): 3336 (br), 1795 (s), 1735 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.62 (bd, J = 7.8 Hz, 1H), 4.70–4.83 (m, 3H), 1.68 (d, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.0, 153.8, 95.1, 74.8, 49.7, 18.1; ^{19}F NMR (282 MHz, $CDCl_3$): δ = −40.9 (2F), −45.3 (1F), −50.1 (2F); High-resolution ESMS (m/z): Calculated for $C_{12}H_7Cl_3F_5NO_4$ + Na: 451.9259; Observed: 451.9254.

Procedure for the activation of Boc-Ala-OH (16) with pentafluorohenyl carbonates

A solution of the Boc-Ala-OH (0.189 g, 1 mmol) and the pentafluorophenyl carbonate (1.1 mmol) in DMF (2 ml) was cooled to 0 °C. Pyridine (0.88 ml, 1.1 mmol) was added to this solution drop wise while stirring it magnetically. The reaction mixture was stirred for 3 h and was diluted with dichloromethane (30 ml). It is then washed with saturated citric acid solution (10 ml), water (2 × 10 ml) and brine solution (10 ml) and dried over anhydrous Na_2SO_4. The active ester Boc-Ala-OPfp (**10e**) was then purified by column chromatography (silica gel, 100–200 mesh) eluting with 5% solution of ethyl acetate in hexane.

Boc-Ala-OPfp, 10e

White solid; mp: 83 °C (reported: 85–86 °C); Yield: 82–93%; $[\alpha]_D$: −10 (c = 1, MeOH), FTIR (Neat): 3341 (br), 1795 (s), 1717 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.09 (bs, 1H), 4.64–4.69 (m, 1H), 1.59 (d, J = 7.5 Hz, 3H); 1.47 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.8, 154.9, 80.5, 49.1, 28.2, 18.1; ^{19}F NMR (282 MHz, $CDCl_3$): δ = −40.9 (2F), −45.5 (1F), −50.2 (2F); High-resolution ESMS (m/z): Calculated for $C_{14}H_{14}F_5NO_4$ + Na: 378.2471; Observed: 378.2468.

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Supporting Information

Supporting Information includes detailed experimental procedures and complete characterization data for all the compounds reported in this article.

References

1 (a) Bodanszky M. *Principles of Peptide Synthesis*, 3rd edn, Springer: Verlag, Berlin, 1993; (b) Williams PL. *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC Press: Boca Raton, Florida, 1997.
2 Kisfaludy L, Schön I. Preparation and applications of pentafluorophenyl esters of 9-fluorenylmethyloxycarbonyl amino acids for peptide synthesis. *Synthesis* 1983; 325–327.
3 Gayo LM, Suto MJ. Use of pentafluorophenyl esters for one-pot protection/activation of amino and thiol carboxylic acids. *Tetrahedron Lett* 1996; **37**: 4915–4918.
4 Rao TS, Nampalli S, Sekher P, Kumar S. TFA-NHS as bifunctional protecting agent: simultaneous protection and activation of amino carboxylic acids. *Tetrahedron Lett* 2002; **43**: 7793–7795.

5 Bhat RG, Kerouredan E, Porheil E, Chandrasekaran S. Synthesis and applications of propargyl pentafluorophenyl carbonate for peptide synthesis. *Tetrahedron Lett.* 2002; **43**: 2467–2469.
6 Ramesh R, Rajasekaran S, Gupta R, Chandrasekaran S. Simultaneous protection and activation of amino acids using propargyl pentafluorophenyl carbonate. *Org. Lett.* 2006; **8**: 1933–1936.
7 Deprotection of *N*-propargyloxycarbonyl derivatives using benzyltriethylammonium tetrathiomolybdate is reported: (a) Sinha S, Ilankumaran P, Chandrasekaran S. The prop-2-ynyloxy carbonyl function (POC): A new amino-protecting group removable from sulfur-containing peptides by ultrasonic irradiation with tetrathiomolybdate under mild and neutral conditions. *Tetrahedron Lett*. 1999; **40**: 771–774; (b) Bhat RG, Sinha S, Chandrasekaran S. Propargyloxycarbonyl (Poc) amino acid chlorides as efficient coupling reagents for the synthesis of 100% diastereopure peptides and resin bound tetrathiomolybdate as an effective deblocking agent for the Poc group. *Chem. Commun.* 2002; 812–813.
8 Kim S, Lee JI, Kim YC. A simple and mild esterification method for carboxylic acids using mixed carboxylic-carbonic anhydrides. *J. Org. Chem.* 1985; **50**: 560–565.

