Oxime-Based Carbonates as Useful Reagents for Both
N-Protection and Peptide Coupling

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Abstract: We have demonstrated that oxime-based mixed carbonates are very effective reagents for both N-protection and peptide coupling.

Keywords: oxime; additives; N-protection; activation; coupling; peptide

1. Introduction

Peptides are increasingly gaining recognition as potential bioactive ingredients in the pharmaceutical industry [1–3]. Peptide synthesis depends on the strategies used for protecting the α-amino group and for activating the carboxylic acid group prior to peptide coupling. The two main classes [4–6] of carboxylic acid group activation methods are: (i) those that require in situ activation of the carboxylic
acid and (ii) those that require an activated species that has previously been prepared (usually from an \textit{in situ} activation step), isolated, purified, and characterized.

The amino group is most commonly protected by preparing the corresponding carbamate derivative. Despite the vast number of reagents reported to date for introducing the protecting group into the \(N\)-terminal amino group, there is still no universally active species capable of providing optimal protecting group introduction.

The traditional chloroformate strategy is an extremely powerful approach, providing fast amino group protection [7–9]. Nevertheless, in some cases, presence of the free carboxylic acid group can interfere with the reaction and lead to formation of byproducts such as dipeptides and even tripeptides (Scheme 1) [10].

Scheme 1. Mechanism for the formation side products (dipeptides and tripeptides) during the protection of amino acids with haloformates.

Since these side-reactions are associated with the quality of the leaving group, the less reactive species such as the dicarbonates 2 (Figure 1) [11–14] and the succinimidocarbonates 3 (Figure 1) [15,16] have previously been proposed as alternatives to the chloride 1 (Figure 1). Use of the azide derivatives 4 (Figure 1), [17,18] has also been proposed as an alternative for the chloride to prepare the \(N\)-protection of amino acids, but the explosive nature of azides precludes their use in large-scale synthesis. Moreover, several other approaches, based on the use of other less reactive species such as the 1,2,2,2-tetrachloroethyl [19,20], the 5-norbornene-2,3-dicarboximido [21], the pentafluorophenyl [22–24], and the 1-hydroxybenzotriazole [14,25,26] mixed carbonates 5–8 (Figure 1), have been proposed.

Recently, ethyl 2-cyano-2-(hydroxyimino)acetate (OxymaPure\textsuperscript{®}, 12a) has been tested as an additive for use in the carbodimide approach for the formation of peptide bonds [27]. OxymaPure\textsuperscript{®} and its uronium-based phosphonium coupling reagents displayed a remarkable capacity to inhibit racemization, together with impressive coupling efficiency, in both automated and manual synthesis, superior to those of 12d and which has recently been reported to exhibit explosive properties [26] at least comparable to those of HOAt uronium-based phosphonium coupling reagents [28–34].
Later, we reported a series of Fmoc/Alloc-oxime carbonate reagents which are easy to prepare, stable, and highly reactive crystalline materials that afford nearly pure Fmoc/Alloc-amino acids in high yields. Among the Fmoc-oxime carbonates that we evaluated for the preparation of Fmoc/Alloc-Gly-OH, the N-hydroxypicolinimidoyl cyanide derivative 9 (Figure 1) gave the best results [35]. More recently, our research group reported the cyanoacetamide-based oximes 10 (Figure 1), which show unusual ability to afford Fmoc-protected amino acids in high yield, high purity and at lower cost relative to compound 9 [36].

Figure 1. Structure of carbonates derivatives.

Herein, we extended our studies for the synthesis of a new family of carbonate derivatives based on OxymaPure®, which are easy to prepare, stable, and have shown high efficiency in N-protection as well as peptide coupling.

2. Results and Discussion

2.1. Preparation of Carbonate Derivatives

The carbonate derivatives 13 were readily prepared by reacting ethyloxycarbonyl chloride (11) with an oxime (compounds 12a or 12b), N-hydroxy-2-pyridinone (12c) or benzotriazole derivatives (HOBt, 12d or 6-Cl-HOBt, 12f) in the presence of sodium carbonate in DCM/H2O (3:2) as solvent at 0 °C, with stirring at this temperature for 2 h (Scheme 2). After subsequent workup followed by isolation and recrystallization from CH2Cl2/hexane, ethyl 2-cyano-2-(ethoxycarbonyloxyimino)acetate (13a), (ethoxycarbonyloxy)carbonimidoyl dicyanide (13b), ethyl 2-oxopyridin-1(2H)-yl carbonate (13c), 1H-benzo[d][1,2,3]triazol-1-yl ethyl carbonate (13d), and 6-chloro-1H-benzo[d][1,2,3]triazol-1-yl ethyl carbonate (13f), were prepared in 46–78% yield (Table 1).
Scheme 2. Preparation of the carbonate derivatives 13.

![Diagram showing Scheme 2]

(A) = Na₂CO₃, DCM/H₂O, 0 °C → rt
(B) = KOH, CH₃CN, 0 °C → rt

Table 1. Yield, m.p. and elemental analysis of the carbonate derivatives 13.

| Compd. | Method | Yield (%) | m.p. (°C) | Elemental Analysis: Calculated (Found) |
|--------|--------|-----------|-----------|---------------------------------------|
|        |        |           |           | C                   | H                  | N                   |
| 13a    | A      | 78        | 44–45     | 44.86 (45.08)       | 4.71 (4.63)        | 13.08 (13.17)       |
| 13b    | A      | 69        | oily      | 43.12 (43.25)       | 3.02 (2.89)        | 25.14 (25.33)       |
| 13c    | A      | 76        | 64–67     | 52.46 (52.21)       | 4.95 (5.16)        | 7.65 (7.91)         |
| 13d    | A      | 52        | 138–139   | 52.17 (51.96)       | 4.38 (4.54)        | 20.28 (20.49)       |
| 13e *  | B      | 77        | 133–135   | 46.16 (45.88)       | 3.87 (4.14)        | 26.91 (27.19)       |
| 13f    | A      | 76        | 144–145   | 44.74 (44.53)       | 3.34 (3.61)        | 17.39 (17.18)       |

* 13e was prepared by reacting ethyloxycarbonyl chloride 11 with HOAt 12e in the presence of anhydrous potassium hydroxide (1 equivalent) in acetonitrile as solvent at 0 °C.

Three different oxime carbonate derivatives: ethyl 2-cyano (isobutoxycarbonyloxyimino)acetate (17), ethyl 2-(allyloxycarbonyloximin o)-2-cyanoacetate (18) and ethyl 2-(benzyloxycarbonyloximino)-2-cyanoacetate (19) were prepared by reacting the corresponding chloroformates 14–16 and OxymaPure® (12a) in the presence of sodium carbonate in DCM/H₂O (3:2) as solvent at 0 °C (Scheme 3). After subsequent workup followed by isolation and recrystallization from CH₂Cl₂/hexane, the corresponding oxime carbonates 17–19 were obtained in 87–94% yield (Table 2).

Scheme 3. Preparation of oximinocarbonate derivatives.

![Diagram showing Scheme 3]
Table 2. Yield, m.p. and elemental analysis of the oximinocarbonate derivatives 17–19.

| Product | Yield (%) | m.p. (°C) | Elemental Analysis: Calculated (Found) |
|---------|-----------|-----------|---------------------------------------|
|         |           |           | C          | H          | N          |
| 17      | 93        | 59–60     | 49.58 (49.81) | 5.83 (5.57) | 11.56 (11.74) |
| 18      | 94        | Oily      | 47.79 (47.93) | 4.46 (4.61) | 12.39 (12.58) |
| 19      | 87        | 99–100    | 56.52 (56.23) | 4.38 (4.62) | 10.14 (10.41) |

2.2. Preparation of 4-(Ethoxycarbonylamino)benzoic Acid

To study the reactivity of prepared carbonate derivatives 13a–f and their utility for the preparation of the N-protected amino acids, we initiated our studies with 4-aminobenzoic acid (20), which on treatment with the previously synthesized carbonate derivatives 13a–f in a homogenous acetone/aqueous solvent mixture in the presence of sodium carbonate with stirring overnight at room temperature, provides the product 21. Samples of 4-(ethoxycarbonylamino)benzoic acid (21) were obtained from the different carbonate derivatives after removing the unreacted starting carbonate by extracting with ether and acidifying the aqueous layer with 1N HCl (Scheme 4). The purity of the product 21 was determined after injection onto reverse-phase HPLC are shown in Table 3.

![Scheme 4. N-protection of 4-aminobenzoic acid using carbonate derivatives 13a–f.](image)

Table 3. Yield %, m.p., purity % of 4-(ethoxycarbonylamino)benzoic acid 21.

| Carbonate | Yield (%) | m.p. (°C) | Purity * (%) |
|-----------|-----------|-----------|--------------|
| 13a       | 43        | 198–200   | 95.3         |
| 13b       | 38        | 199–201   | 88.5         |
| 13c       | 59        | 198–200   | 100          |
| 13d       | 11        | 184–192   | 77.1         |
| 13e       | 31        | 198–202   | 97.0         |
| 13f       | 42        | 190–195   | 83.6         |

* The purity was determined by HPLC using the following Conditions: detection at 220 nm (Waters 996 PDA detector); Sunfire C18 column (3.5 μm 4.6 × 100 mm); linear gradient over 14 min (10 to 100% CH3CN in H2O/0.1% TFA); flow rate 1.0 mL/min. *tR [4-(ethoxycarbonylamino)benzoic acid] = 4.18 min.

Table 3 showed that, 1H-benzo[d][1,2,3]triazol-1-yl ethyl carbonate (13d) provided the lowest purity (77.1%) and yield (11%) of all the ethoxycarbonyl carbonate derivatives. (Ethoxycarbonyloxy) carbonimidoyl dicyanide (13b) and 6-chloro-1H-benzo[d][1,2,3]triazol-1-yl ethyl carbonate (13f) provided moderate levels of purity (88.5% and 83.6%) and yield (38% and 42%). Ethyl 2-cyano-2-(ethoxycarbonyloxyimino)acetate (13a) and 3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl ethyl carbonate (13e) provided high purity (95.3% and 97.0%, respectively) and moderate yield (43% and 31%), while,
ethyl 2-oxopyridin-1(2H)-yl carbonate (13c) provided the highest yield (59%) with excellent purity (100%) as indicated from the HPLC traces.

2.3. HPLC Study of the Rate of Formation of the Active Ester

Before attempting simultaneous protection and activation of amino acids, we tried to prepare active esters of N-protected amino acids using the carbonate derivatives 13a, 17, 18, and 19 to ensure that these compounds can activate carboxylic acids by forming the corresponding active ester for different Fmoc-amino acids. The reaction of Fmoc-amino acids with oxime carbonate derivatives was monitored by HPLC to study the rate of formation of the active ester. Aliquots (5 µL) of the reaction mixture were taken, diluted with acetonitrile, and then analyzed by HPLC. Follow-up samples were studied at intervals of time 30 min and 1, 2, 4 and 24 h pre-activation. This enabled us to determine the optimum pre-activation time for each carbonate reagent, as excessively long times could lead to greater formation of alkyl or aryl esters.

2.3.1. The Rate of Formation of the Active Ester of Fmoc-Val-OH Using Oxime Carbonate Derivatives

Mixing of Fmoc-Val-OH 22 with the oxime carbonate reagents EtocOXY 13a, iBuocOXY 17, AllocOXY 18 and ZOXY 19 in the presence of pyridine in DMF, we observed maximum levels of the active ester was formed at 4 h for 13a, 2 h for 17, 30 min for 18 and 1 h for 19. Whereas the alkyl or aryl esters 26 started to be formed after 2 and 1 h in case of EtocOXY 13a and iBuocOXY 17, respectively, and after half an hour in case of AllocOXY 18 and ZOXY 19 respectively. Therefore, the optimum pre-activation time should not exceed more than 2 and 1 h in case of EtocOXY 13a and iBuocOXY 17, respectively, and should be less than half an hour in case of both AllocOXY 18 and ZOXY 19 systems. Best results for formation of the active ester were obtained with the oxime carbonate 17 and 18, while the oxime carbonates 13a and 19 gave high yield of the alkyl ester (Tables 4–7).

Table 4. The rate of formation of the active ester of Fmoc-Val-OH 22 using ethyl 2-cyano-2-(ethoxycarbonyloxyimino)acetate (13a).

| Pre-activation time (hr) | Oxyma 12a | Fmoc-Val-OH 22 | Active ester 27 | Ethyl ester |
|-------------------------|------------|----------------|----------------|------------|
| ½                       | 7.2        | 40.6           | 37.8           | n/a        |
| 1                       | 3.7        | 48.0           | 39.8           | n/a        |
| 2                       | 2.3        | 29.5           | 61.7           | n/a        |
| 4                       | 3.5        | 18.7           | 61.1           | 13.2       |
| 24                      | 19.1       | 17.4           | 15.0           | 48.5       |

Table 5. The rate of formation of the active ester of Fmoc-Val-OH 22 using ethyl 2-cyano-2-(isobutoxycarbonyloxyimino)acetate (17).

| Pre-activation time (hr) | Oxyma 12a | Fmoc-Val-OH 22 | Active ester 27 | Isobutyl ester |
|-------------------------|------------|----------------|----------------|---------------|
| ½                       | 3.1        | 54.7           | 33.6           | n/a           |
| 1                       | 0.9        | 45.9           | 44.3           | n/a           |
| 2                       | 1.4        | 37.2           | 54.8           | 1.9           |
| 4                       | 2.6        | 27.3           | 63.5           | 6.7           |
| 24                      | 16.8       | 16.0           | 21.9           | 45.3          |
2.3.2. The Rate of Formation of the Active Ester of Fmoc-Phe-OH Using Oxime Carbonate Derivatives

Due to the best results obtained from the previous example with the oxime carbonate derivatives 17 and 18, Fmoc-Phe-OH 23 was tested with iBuOXY 17 and AllocOXY 18 under the same conditions used in the previous example. From the results obtained from HPLC monitoring, the maximum levels of the active ester 27 are formed from the oxime carbonate derivatives 17 and 18 at 1 and 2 h, respectively; while the alkyl esters 26 appeared after 30 min and 1 hour, respectively. Thus, the pre-activation time in both systems should not exceed more than 30 min (Tables 8 and 9).

Table 6. The rate of formation of the active ester of Fmoc-Val-OH 22 using ethyl 2-(allyloxy carbonyloxyimino)-2-cyanoacetate (18).

| Pre-activation time (hr) | Oxyma 12a | Fmoc-Val-OH 22 | Active ester 27 | Allyl ester |
|-------------------------|-----------|----------------|-----------------|-------------|
| ½                       | n/a       | 9.4            | 63.9            | 10.8        |
| 1                       | n/a       | 14.2           | 58.1            | 3.7         |
| 2                       | n/a       | 5.7            | 73.0            | 6.1         |
| 4                       | 23.6      | 2.8            | 55.9            | n/a         |

Table 7. The rate of formation of the active ester of Fmoc-Val-OH 22 using ethyl 2-(benzylloxy carbonyloxyimino)-2-cyanoacetate (19).

| Pre-activation time (hr) | Oxyma 12a | Fmoc-Val-OH 22 | Active ester 27 | Benzyl ester |
|-------------------------|-----------|----------------|-----------------|--------------|
| ½                       | 1.5       | 40.4           | 52.5            | n/a          |
| 1                       | 3.4       | 28.1           | 53.7            | 7.5          |
| 2                       | 6.9       | 19.0           | 48.4            | 13.1         |
| 4                       | 11.6      | 14.1           | 32.2            | 20.2         |
| 24                      | 22.1      | 14.1           | 2.1             | 31.4         |

Table 8. The rate of formation of the active ester of Fmoc-Phe-OH 23 using ethyl 2-cyano-2-(isobutoxycarbonyloxyimino)acetate (17).

| Pre-activation time (hr) | Oxyma 12a | Fmoc-Phe-OH 23 | Active ester 27 | Isobutyl ester |
|-------------------------|-----------|----------------|-----------------|---------------|
| ½                       | n/a       | 28.3           | 43.0            | 14.7          |
| 1                       | 0.8       | 15.8           | 72.0            | 8.4           |
| 2                       | 0.5       | 19.5           | 58.2            | 6.1           |
| 4                       | 0.8       | 16.2           | 63.3            | 1.5           |
| 24                      | 3.5       | 21.4           | 52.7            | 22.0          |

Table 9. The rate of formation of the active ester of Fmoc-Phe-OH 23 using ethyl 2-(allyloxy carbonyloxyimino)-2-cyanoacetate (18).

| Pre-activation time (hr) | Oxyma 12a | Fmoc-Phe-OH 23 | Active ester 27 | Allyl ester |
|-------------------------|-----------|----------------|-----------------|-------------|
| ½                       | 4.7       | 24.7           | 58.56           |             |
| 1                       | 4.6       | 22.3           | 65.9            | 0.4         |
| 2                       | 5.5       | 12.6           | 74.6            | 0.6         |
| 4                       | 5.8       | 19.2           | 68.3            | 0.4         |
| 24                      | 13.5      | 4.6            | 53.4            | 25.4        |
2.3.3. The Rate of Formation of the Active Ester of Fmoc-Pro-OH Using Oxime Carbonate Derivatives

To ensure that we will get the same results with the oxime carbonate derivatives 17 and 18, further study was performed with the more sterically hindered amino acid Fmoc-Pro-OH 24 using the two carbonate derivatives 1BuocOXY 17 and AllocOXY 18. From the results obtained by HPLC monitoring, we observed the maximum levels of the active ester 27 after 30 min and 2 h, respectively; while the alkyl esters 26 appeared after 30 min and 1 hour, respectively. Thus, the pre-activation time in both systems should not exceed more than 30 min, which in agreement with the previous results (Tables 10 and 11).

Table 10. The rate of formation of the active ester of Fmoc-Pro-OH 24 using ethyl 2-Cyano-2-(isobutoxycarbonyloxyimino)acetate (17).

| Pre-activation time (hr) | Oxyma 12a | Fmoc-Pro-OH 24 | Active ester 27 | Isobutyl ester |
|-------------------------|-----------|----------------|-----------------|---------------|
| ½                       | 0.2       | 13.4           | 59.8            | 5.0           |
| 1                       | 1.6       | 47.4           | 34.1            | 6.7           |
| 2                       | 1.3       | 30.7           | 53.9            | 3.1           |
| 4                       | 1.6       | 30.8           | 55.8            | 3.8           |
| 24                      | 17.8      | 75.9           | 6.3             | n/a           |

Table 11. The rate of formation of the active ester of Fmoc-Pro-OH 24 using ethyl 2-(allyloxycarbonyloxyimino)-2-cyanoacetate (18).

| Pre-activation time (hr) | Oxyma 12a | Fmoc-Pro-OH 24 | Active ester 27 | Allyl ester |
|-------------------------|-----------|----------------|-----------------|------------|
| ½                       | 1.4       | 13.2           | 65.7            | n/a        |
| 1                       | 1.8       | 13.3           | 70.3            | 2.8        |
| 2                       | 3.0       | 15.2           | 70.6            | 2.7        |
| 4                       | 13.8      | 58.0           | 24.3            | 3.9        |
| 24                      | 19.4      | 46.7           | 18.7            | 15.2       |

The results obtained from Tables 4–11 may indicate that, the activation of carboxylic acid group of the amino acid using oximinocarbonate derivatives may proceed through a mixed anhydride-type intermediate 25, which may react with the oxyma anion to afford the active ester 27 (Scheme 5). Its decarboxylation will afford alkyl or aryl esters 26, which is a rather slow step. All intermediate stages of the reaction are run at low temperature to prevent side reactions.

Scheme 5. Mechanism of the formation of the oxime active ester using oxyme carbonates.
2.4. Synthesis of Dipeptide Fmoc-Val-Ala-OMe

As an initial model to examine the reactivity of the oxime carbonate 13a, 17, 18 and 19 as coupling reagents, these reagents were examined in the stepwise coupling of a previously studied model system [29] Fmoc-Val-Ala-OMe 28. The pre-activation time was determined for each of these coupling reagents according to the previous studies obtained from HPLC for the rate of formation of active ester. All coupling reactions were performed in the presence of 2 equiv. pyridine as a base and in DMF as solvent. The results for each coupling reagent are given in Table 12.

Table 12. Coupling of Fmoc-Val-OH with H-Ala-OMe 28 using different oxime carbonate derivatives.

| Coupling reagent | Pre-activation time (hr) | m.p. (°C) | Yield (%) | HPLC Purity (%) |
|------------------|--------------------------|-----------|-----------|-----------------|
| EtocOXY 13a      | 2                        | 195–196   | 77        | 100             |
| 'BuocOXY 17      | 1                        | 192–193   | 54        | 96.1            |
| AllocOXY 18      | ½                        | 194–195   | 75        | 98.7            |
| ZOXY 19          | ½                        | 194–195   | 64        | 97.7            |

The purity of the dipeptide 28 was determined by HPLC, and was found to be 96.1 to 100% at \( t_R \) [Fmoc-Val-Ala-OMe] = 8.32 min. From Table 12, the highest yield and purity was obtained with EtocOXY 13a, while the lowest yield and purity was obtained with 'BuocOXY 17 and ZOXY 19. While, the AllocOXY 18 had moderate yield and purity. None of the D,L-isomer was detected by HPLC or NMR spectra in all the cases, but the impurities were related to the alkyl ester and oxime.

3. Experimental

3.1. Materials

The solvents used were of HPLC reagent grade. Melting points were determined with a Mel-Temp apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 series Fourier transform instrument as KBr pellets. Nuclear Magnetic resonance spectra (\(^1\)H-NMR and \(^13\)C-NMR spectra) were recorded on a JOEL 500 MHz and on a Mercury 400 MHz spectrometer with chemical shift values reported in \( \delta \) units (ppm) relative to an internal standard. Elemental analyses were performed on Perkin-Elmer 2400 elemental analyzer, and the values found were within ±0.3% of the theoretical values. Follow-up of the reactions and checks of the purity of the compounds was done by TLC on silica gel-protected aluminum sheets (Type 60 GF254, Merck, Barcelona, Spain) and the spots were detected by exposure to UV-lamp at \( \lambda \) 254 nm for a few seconds. The compounds were named using ChemDraw Ultra version 11, CambridgeSoft Corporation (Cambridge, MA, USA).

3.2. General Method for Preparation of Ethyloxycarbonyl Derivatives 13(a–d, f)

A solution of ethyloxycarbonyl chloride (11, 0.95 mL, 10 mmol) in CH\(_2\)Cl\(_2\) (30 mL) was added slowly to a solution of sodium carbonate (2.12 g, 20 mmol) and 10 mmol of oximes (12a, 12b), 1-hydroxypyridin-2(1H)-one (12c), or benzotriazole derivatives (12d or 12f) in H\(_2\)O (20 mL) with stirring at 0 °C. The resulting clear mixture was stirred at 0°C for 30 min and then at room temperature for 2 h. After dilution with CH\(_2\)Cl\(_2\) (50 mL), the organic phase was collected and washed with water.
and saturated aqueous NaCl (30 mL), dried over anhydrous Na₂SO₄ and then filtered, and the solvent was then removed on a rotary evaporator. The residue was recrystallized from CH₂Cl₂/hexane to give the ethyloxycarbonyl derivatives 13(a–d, f).

**Ethyl 2-cyano-2-(ethyloxycarbonyloxyimino)acetate (13a).** The product was obtained as white crystals (1.67 g; 78.17% yield) (m.p. 44–45 °C). IR (KBr): 2241 (w, CN), 1812 (s, CO), 1741 (s, CO, ester) cm⁻¹. ¹H-NMR (CDCl₃):  δ 1.39–1.41 (m, 6H, 2 CH₃), 4.43–4.47 (m, 4H, 2 CH₂). ¹³C-NMR (CDCl₃): δ 14.14, 14.26, 64.76, 67.33, 106.67, 131.03, 150.87, 156.81. Elemental analysis Calcd for C₈H₁₀N₂O₅: C, 44.86; H, 4.71; N, 13.08. Found: C, 45.08; H, 4.63; N, 13.17.

**(Ethoxycarbonyloxy)carbonimidoyl dicyanide (13b).** The product was obtained as an oil at room temperature (1.15 g; 68.89% yield). IR (KBr): 2248 (w, CN), 1811 (s, CO, ester) cm⁻¹. ¹H-NMR (CDCl₃):  δ 1.43 (t, 3H, 3  J = 7.2 Hz, CH₃), 4.49 (q, 2H, 3  J = 7.2 Hz, CH₂). ¹³C-NMR (CDCl₃): δ 14.18, 68.32, 104.81, 108.03, 114.44, 149.70. Elemental analysis: Calcd for C₆H₅N₃O₃: C, 43.12; H, 3.02; N, 25.14. Found: C, 43.25; H, 2.89; N, 25.33.

**Ethyl 2-oxopyridin-1(2H)-yl carbonate (13c).** The product was obtained as white crystals (1.38 g; 75.47% yield) (m.p. 64–67 °C). IR (KBr): 1792 (s, CO), 1668 (s, CO, amidic) cm⁻¹. ¹H-NMR (CDCl₃):  δ 1.42 (t, 3H, 3  J = 7.2 Hz, CH₃), 4.42 (q, 2H, 3  J = 7.2 Hz, CH₂), 6.20 (td, 1H, 3  J = 6.8 Hz, 4  J = 1.6 Hz, Py-H), 6.72–6.74 (m, 1H, Py-H), 7.36 (td, 1H, 3  J = 6.8 Hz, 4  J = 2 Hz, Py-H), 7.46 (dd, 1H, 3  J = 6.8 Hz, 4  J = 2 Hz, Py-H). ¹³C-NMR (CDCl₃): δ 14.25, 67.53, 105.29, 123.18, 135.14, 139.69, 152.45, 157.31.

**1H-Benz[d][1,2,3]triazol-1-yl ethyl carbonate (13d).** The product was obtained as white crystals (1.07 g; 51.84% yield) (m.p. 138–139 °C). IR (KBr): 1751 (s, CO) cm⁻¹. ¹H-NMR (CDCl₃):  δ 1.53 (t, 3H, 3  J = 7.2 Hz, CH₃), 4.63 (q, 2H, 3  J = 7.2 Hz, CH₂), 7.56 (td, 1H, 3  J = 8.4 Hz, 4  J = 0.8 Hz, Ar-H), 7.78 (td, 1H, 3  J = 8.4 Hz, 4  J = 1.2 Hz, Ar-H), 8.00 (d, 1H, 3  J = 8.4 Hz, Ar-H), 8.21 (d, 1H, 3  J = 8.4 Hz, Ar-H). ¹³C-NMR (CDCl₃): δ = 14.42, 65.67, 115.27, 115.88, 126.46, 132.91, 133.54, 147.52. Elemental analysis: Calcd for C₉H₉N₃O₃: C, 52.17; H, 4.38; N, 20.28. Found: C, 51.96; H, 4.54; N, 20.49.

**6-Chloro-1H-benzo[d][1,2,3]triazol-1-yl ethyl carbonate (13f).** The product was obtained as white crystals (1.81 g; 75.67% yield) (m.p. 144–145 °C). IR (KBr): 1743 (s, CO) cm⁻¹. ¹H-NMR (CDCl₃):  δ 1.53 (t, 3H, 3  J = 7.2 Hz, CH₃), 4.62 (q, 2H, 3  J = 7.2 Hz, CH₂), 7.72 (dd, 1H, 3  J = 8.8 Hz, 4  J = 2 Hz, Ar-H), 8.00 (d, 1H, 3  J = 8.4 Hz, Ar-H), 8.16 (d, 1H, 3  J = 8.8 Hz, Ar-H). ¹³C-NMR (CDCl₃): δ = 14.42, 65.99, 115.70, 116.32, 132.19, 132.81, 133.78, 147.32. Elemental analysis: Calcd for C₉H₈ClN₃O₃: C, 44.74; H, 3.34; N, 17.39. Found: C, 44.53; H, 3.61; N, 17.18.

### 3.3. 3H-[1,2,3]Triazolo[4,5-b]pyridin-3-yl Ethyl Carbonate (13e)

A solution of HOAt (12e, 0.68 g, 5 mmol) and anhydrous potassium hydroxide (0.3 g, 5.5 mmol) in acetonitrile (5 mL) was cooled to 0 °C. A solution of ethyloxycarbonyl chloride (11, 0.47 mL, 5 mmol) in acetonitrile (5 mL) was slowly added dropwise for 30 min to the solution as it was stirred magnetically. The resulting clear mixture was stirred at room temperature overnight. It was then filtered, and the solvent was removed with a rotary evaporator. The residue was recrystallized from CH₂Cl₂/hexane to give 3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl ethyl carbonate (13e). The product was
obtained as 0.8 g (77.14% yield) of white crystals (m.p. 133–135 °C). IR (KBr): 1744 (s, CO) cm⁻¹. ¹H-NMR (DMSO): δ 1.05 (t, 3H, 3J = 7.2 Hz, CH₃), 3.43 (q, 2H, 3J = 7.2 Hz, CH₂), 7.49–7.53 (m, 1H, Ar-H), 8.52–8.55 (m, 1H, Ar-H). ¹³C-NMR (DMSO): δ 18.47, 55.94, 120.62, 124.09, 134.52, 139.50, 151.01. Elemental analysis: Calcd for C₈H₈N₄O₃: C, 46.16; H, 3.87; N, 26.91. Found: C, 45.88; H, 4.14; N, 27.19.

3.4. General Method for Preparation of Oxime Carbonate Derivatives 17–19

A solution of chloroformate (10 mmol) [isobutoxycarbonyl chloride (14), allyloxy carbonyl chloride (15) or benzyloxy carbonyl chloride (16)] in CH₂Cl₂ (30 mL) was added slowly to a solution (10 mmol) of oxima 12a and sodium carbonate (2.12 g, 20 mmol) in H₂O (20 mL) with stirring at 0 °C. The resulting clear mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. After dilution with CH₂Cl₂ (50 mL), the organic phase was collected, washed with water and saturated aqueous NaCl (30 mL), and then dried over anhydrous MgSO₄. It was then filtered, and the solvent was removed with a rotary evaporator. The residue was recrystallized from CH₂Cl₂/hexane to give oxime carbonate derivatives 17–19.

Ethyl 2-cyano-2-(isobutoxycarbonyloxyimino)acetate (17). The product was obtained as a white solid (2.42 g; 93% yield) (m.p. 59–60 °C). IR (KBr): 1814 (s, CO), 1758 (s, CO, ester) cm⁻¹. ¹H-NMR (CDCl₃): δ 1.00 (d, J = 6.8 Hz, 6H, 2 CH₃), 1.42 (t, J = 7.2 Hz, 3H, CH₃), 2.06–2.13 (m, 1H, CH), 4.17 (d, J = 6.4 Hz, 2H, CH₂), 4.50 (q, J = 7.2 Hz, 2H, CH₂). ¹³C-NMR (CDCl₃): δ 14.16, 18.89, 27.95, 64.75, 106.70, 130.97, 151.10, 156.84. Elemental analysis: Calcd for C₁₀H₁₄N₂O₅: C, 49.58; H, 5.83; N, 11.56. Found: C, 49.81; H, 5.57; N, 11.74. The purity of 17 was determined after injection onto reverse-phase HPLC. Conditions: detection at 254 nm Waters 996 PDA detector, Sunfire C₁₈ column 3.5 µm 4.6 × 100 mm, linear gradient over 14 min of 10 to 100% CH₃CN in H₂O/0.1% TFA, flow rate 1.0 mL/min. tᵣ [ethyl 2-cyano-2-(isobutoxycarbonyloxyimino)acetate] = 7.38 min; purity 100%.

Ethyl 2-(allyloxy carbonyloxyimino)-2-cyanoacetate (18). The product was obtained as an oily substance that solidified in the refrigerator (2.26 g; 94% yield) (m.p. 59–60 °C). IR (KBr): 2211 (w, CN), 1809 (s, CO), 1758 (s, CO, ester) cm⁻¹. ¹H-NMR (CDCl₃): δ 1.42 (t, 3H, 3J = 7.2 Hz, CH₃), 4.48 (q, 2H, 3J = 7.2 Hz, CH₂), 4.85–4.87 (m, 2H, CH₂), 5.39–5.51 (m, 2H, CH₂), 5.96–6.03 (m, 1H, CH). ¹³C-NMR (CDCl₃): δ 14.11, 64.78, 71.23, 106.62, 121.27, 130.07, 131.24, 150.74, 156.73. Elemental analysis: Calcd for C₉H₁₀N₂O₅: C, 47.79; H, 4.46; N, 12.39. Found: C, 47.79; H, 4.46; N, 12.39. The purity of 18 was determined after injection onto reverse-phase HPLC. Conditions: detection at 254 nm Waters 996 PDA detector, Sunfire C₁₈ column 3.5 µm 4.6 × 100 mm, linear gradient over 14 min of 10 to 100% CH₃CN in H₂O/0.1% TFA, flow rate 1.0 mL/min. tᵣ [ethyl 2-(allyloxy carbonyloxyimino)-2-cyanoacetate] = 6.69 min; purity 100%.

Ethyl 2-(benzyloxy carbonyloxyimino)-2-cyanoacetate (19). The product was obtained as white crystals (2.76 g; 87% yield) (m.p. 99–100 °C). IR (KBr): 1802 (s, CO), 1743 (s, CO, ester) cm⁻¹. ¹H-NMR (CDCl₃): δ 1.41 (t, 3H, 3J = 7.2 Hz, CH₃), 4.47 (q, 2H, 3J = 7.2 Hz, CH₂), 5.38 (s, 2H, CH₂), 7.40–7.44 (m, 5H, Ar-H). ¹³C-NMR (CDCl₃): δ 14.17, 64.80, 72.57, 106.63, 129.06, 129.23, 129.63, 131.23, 133.59, 151.00, 156.76. Elemental analysis: Calcd for C₁₃H₁₂N₂O₅: C, 56.52; H, 4.38; N, 10.14.
Found: C, 56.23; H, 4.62; N, 10.41. The purity of 19 was determined after injection onto reverse-phase HPLC. Conditions: detection at 254 nm Waters 996 PDA detector, Sunfire C18 column 3.5 µm 4.6 × 100 mm, linear gradient over 14 min of 10 to 100% CH3CN in H2O/0.1% TFA, flow rate 1.0 mL/min. tR [ethyl 2-(benzyloxycarbonyloxyimino)-2-cyanoacetate] = 7.31 min; purity 100%.

3.5. Synthesis of 4-(Ethoxycarbonylamino)benzoic Acid (21)

A solution of ethyl oxycarbonyl derivative 13(a–f) (1 mmol) in acetone (10 mL) was added dropwise to a stirring solution of 4-aminobenzoic acid 20 (0.14 g, 1 mmol) and sodium carbonate (0.32 g, 3 mmol) in acetone (20 mL) and H2O (10 mL). After stirring overnight, the reaction mixture was concentrated under reduced pressure, and then extracted with CH2Cl2 (20 mL) to remove the unreacted ethyl oxycarbonyl derivatives. The reaction mixture was acidified with 1 N HCl (detected with Congo red litmus paper) to give a white solid, which was filtered, washed with water several times, dried and then recrystallized (ethyl acetate/n-hexane) to give a white solid. The purity of 21 was determined by reverse-phase HPLC. Conditions: detection at 220 nm (Waters 996 PDA detector); Sunfire C18 column (3.5 µm 4.6 × 100 mm); linear gradient over 14 min (10 to 100% CH3CN in H2O/0.1% TFA); flow rate 1.0 mL/min. tR [4-(ethoxycarbonylamino)benzoic acid] = 4.18 min. IR (KBr): 3334 (w, NH), 3400–2500 (br, OH, acid), 1704 (s, CO, acidic), 1686 (s, CON) cm⁻¹. 1H-NMR (DMSO): δ 1.24 (t, 3H, 3J = 7.2 Hz, CH3), 4.13 (q, 2H, 3J = 7.2 Hz, CH2), 7.54 (d, 2H, 3J = 8.4 Hz, Ar-H), 7.82 (d, 2H, 3J = 8.4 Hz, Ar-H), 9.93 (s, 1H, NH). 13C-NMR (DMSO): δ 15.11, 61.11, 117.91, 124.91, 131.05, 144.12, 153.00, 167.63.

3.6. HPLC Study of the Rate of Formation of Active Esters

3.6.1. The Rate of Formation of the Active Ester of Fmoc-Val-OH 22 Using Oxime Carbonate Derivatives 13a, 17–19

A solution of Fmoc-Val-OH 22 (0.0423 g, 0.125 mmol) and the oxime carbonate derivatives 13a, 17, 18 or 19 (0.125 mmol) was dissolved in DMF (2 mL) in the presence of pyridine (20 µL). The reaction was monitored by HPLC. Aliquots (5 µL) were taken from the reaction mixture, diluted with ACN and detected by HPLC. Follow-ups were done at 30 min and at 1, 2, 4 and 24 h pre-activation. The percentages of OxymaPure® 12a, Fmoc-Val-OH 22, active ester 27, and alkyl or aryl ester 26 were determined by HPLC analysis of the diluted reaction mixture. Conditions: detection at 254 nm (Waters 996 PDA detector); Sunfire C18 column (3.5 µm 4.6 × 100 mm); linear gradient over 14 min (10 to 100% CH3CN in H2O/0.1% TFA); flow rate 1.0 mL/min. tR [active ester] = 8.4 min. The percentages of OxymaPure® 12a, Fmoc-Val-OH 22, active ester 27, and alkyl or aryl esters 26 are shown in Tables 4–7.

3.6.2. The Rate of Formation of the Active Ester of Fmoc-Phe-OH 23 Using Oxime Carbonate Derivatives 17 or 18

A solution of Fmoc-Phe-OH 23 (0.0483 g, 0.125 mmol) and oxime carbonate derivative 17 or 18 (0.125 mmol) was dissolved in DMF (2 mL) in the presence of pyridine (20 µL). The reaction was monitored by HPLC. Aliquots (5 µL) were taken from the reaction mixture, diluted with ACN and
detected by HPLC. Follow-ups were done at 30 min and at 1, 2, 4 and 24 h pre-activation. The percentages of OxymaPure® (12a), Fmoc-Phe-OH 23, active ester 27 and alkyl ester 26 were determined by HPLC analysis of the diluted reaction mixture. Conditions: detection at 254 nm (Waters 996 PDA detector); Sunfire C18 column (3.5 µm 4.6 × 100 mm); linear gradient over 14 min (10 to 100% CH3CN in H2O/0.1% TFA); flow rate 1.0 mL/min. tR [active ester] = 8.4 min. The percentages of OxymaPure® (12a), Fmoc-Phe-OH 23, active ester 27 and alkyl esters 26 are shown in Tables 8 and 9.

3.6.3. The Rate of Formation of the Active Ester of Fmoc-Pro-OH 24 Using Oxime Carbonate Derivatives 17 or 18

A solution of Fmoc-Pro-OH 24 (0.0421 g, 0.125 mmol) and oxime carbonate derivative 17 or 18 (0.125 mmol) was dissolved in DMF (2 mL) in the presence of pyridine (20 µL). The reaction was monitored by HPLC. Aliquots (5 µL) were taken from the reaction mixture, diluted with ACN and analyzed by HPLC. Follow-ups were done at 30 min and at 1, 2, 4 and 24 h pre-activation. The percentages of OxymaPure® (12a), Fmoc-Pro-OH 24, active ester 27 and alkyl ester 26 were determined by HPLC analysis of the diluted reaction mixture. Conditions: detection at 254 nm Waters 996 PDA detector, Sunfire C18 column 3.5 µm 4.6 × 100 mm, linear gradient over 14 min of 10 to 100% CH3CN in H2O/0.1% TFA, flow rate 1.0 mL/min. tR [active ester] = 8.2 min. The percent of Oxyma 12a, Fmoc-Pro-OH 24, active ester 27 and alkyl esters 26 are shown in Tables 10 and 11.

3.7. General Method for the Synthesis of Dipeptide Fmoc-Val-Ala-OMe 28

A solution of Fmoc-Val-OH 22 (0.339 g, 1 mmol) and the appropriate coupling reagent (1 mmol) in DMF (2 mL) was cooled to 0 °C and treated dropwise with pyridine (0.088 mL, 1.1 mmol). The reaction mixture was stirred for pre-activation at different times, depending on the conditions of the entry studied, and then treated with a solution of H-Ala-OMe.HCl (0.139 g, 1 mmol) and pyridine (0.088 mL, 1.1 mmol) in DMF (1 mL). The reaction mixture was stirred overnight. After dilution with 25 mL of ethyl acetate, the organic phase was washed with 5% citric acid (3 × 15 mL), saturated aq. NaHCO3 (3 × 15 mL) and saturated aq. NaCl (3 × 15 mL), and then dried over anhydrous Na2SO4 and filtered. The solvent was removed with a rotary evaporator, and the residue was recrystallized from CH2Cl2/hexane to give the dipeptide Fmoc-Val-Ala-OMe 28. The purity of 28 was by reverse-phase HPLC. Conditions: detection at 220 nm (Agilent 1200 PDA detector); Eclipse plus C18 column (3.5 µm 4.6 × 100 mm); linear gradient over 14 min (10 to 100% CH3CN in H2O/0.1% TFA); flow rate 1.0 mL/min. tR LL [Fmoc-Val-Ala-OMe] = 8.32 min. The results of coupling of Fmoc-Val-OH with H-Ala-OMe using different oximinocarbonate derivatives are shown in Table 12. 1H-NMR (CDCl3): δ 0.94–0.98 (m, 6H, 2CH3), 1.40 (d, 3H, 3J = 6.9 Hz, CH3), 2.10–2.11 (m, 1H, CH), 3.73 (s, 3H, CH3), 3.90–4.00 (m, 1H, CH), 4.17–4.21 (m, 1H, CH), 4.34–4.43 (m, 2H, CH2), 4.55–4.58 (m, 1H, CH), 5.47–5.51 (m, 1H, NH), 6.42–6.45 (m, 1H, NH), 7.25–7.76 (m, 8H, Ar-H).

4. Conclusions

Protection of the amino group and activation of the carboxylic acid groups are the most important steps associated with peptide synthesis. A possible strategy is to use oxime carbonate derivatives to
simultaneously protect the amino group as a carbamate derivative and activate the carboxylic acid
group as an active oxime ester was performed. A detailed study is carried out to understand the scope
and limitations of this method using different oxime carbonate derivatives. The efficiency of these
derivatives depends on the nature of oxime carbonates and also on the nature of the amino acids. From
our studies we determined that the new family of oximes are useful reagents for both $N$-protection and
activation of the protected amino acid. As a final conclusion from our studies, the 'BuocOXY
compound 17 and AllocOXY compound 18 both give the best results for formation of the active ester
with less alkyl ester formation, while the EtocOXY compound 13a gave the best results for the coupling
step. The ZOXY reagent 19 might be not useful in either the activation or coupling steps.

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*Sample Availability:* Samples of the most part of the compounds are available from the authors.

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