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Oxime Carbonates: Novel Reagents for the Introduction of Fmoc and Alloc Protecting Groups, Free of Side Reactions

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Fmoc and Alloc protecting groups represent a consistent alternative to classical Boc protection in peptide chemistry. The former was established in the last decades as the α-amino protecting group of choice, whereas the latter allows a fully orthogonal protection strategy with Fmoc and Boc. Usually, the introduction of the Fmoc and Alloc moieties takes place through their halogenoformates, azides, or activated carbonates. This rather simple reaction is accompanied by several side reactions, specially the formation of Fmoc/Alloc dipeptides and even tripeptides. The present work describes new promising Fmoc/Alloc-oxime reagents, which are easy to prepare, stable, and highly reactive crystalline materials that afford almost contaminant-free Fmoc/Alloc-amino acids in high yields by following a conventional procedure. Amongst the Fmoc-oxime derivatives, the *N*-hydroxypicolinimidoyl cyanide derivative (*N*-{[(9H-fluoren-9-yl)methoxy]carbonyloxy}picolinimidoyl cyanide) gave the best results for the preparation of Fmoc-Gly-OH, which is the most predisposed to give side reactions. The same Alloc-oxime analogue afforded the preparation of Alloc-Gly-OH in good yield, purity, and extremely low dipeptide formation, as analyzed by reverse-phase HPLC and NMR spectroscopy.

Introduction

An appropriate choice of the protection strategy is essential to the success of peptide[1] synthesis, conditioning the yield and purity of the desired product. To meet that purpose, several protecting groups have been proposed that offer a wide range of removal conditions, enabling the required orthogonality.[2,3] 9-Fluorenylmethyloxycarbonyl amino acids (Fmoc-amino acids) are potentially amongst the most versatile intermediates for peptide synthesis, especially when used in conjunction with acid-labile side-chain protecting groups, being considered as a milder alternative to the more classical Boc strategy.[4,5] The allyloxycarbonyl (Alloc) group has also been proposed for the preparation of *N*-urethane blocked amino acids, successfully allowing the synthesis of antitumoral peptides when used as temporary α-amino protecting group.[6,7] Due to its stability to general basic and acidic conditions, it introduces orthogonality towards Boc and Fmoc, being of special interest as side-chain protection in the synthesis of cyclic peptides, when the so-called three dimensional protection strategy is used.[8] Its removal can be easily and selectively achieved with tetrakis(triphenylphosphane)palladium(0) and a suitable nucleophile/scavenger.[9,10]

The use of chloroformates represents the most potent strategy for the *N*-protection with Fmoc and Alloc moieties.[11] Nevertheless, it has been reported that the high reactivity of these chlorides, such as Fmoc-Cl (**1**) or Alloc-Cl (**2**), might lead to the formation of amino-acid-based byproducts, including the protection of bulky residues, that become inserted into the peptide chain.[12–16] As result of this detrimental side reaction, considerable formation of Fmoc/Alloc-dipeptides and tripeptides (1–20%) has been observed, as established by HPLC, amino acid analysis, NMR spectroscopy, and the synthesis of a number of standards.[13] A previous "in situ" bis-trimethylsilylation step of protection of the residue, before conducting the reaction with the chloroformate, can prevent the formation of such byproducts.[15,17] In fact, Fmoc/Alloc-oligopeptides are likely formed through the intermediary formation of relatively stable mixed anhydrides when highly reactive chloroformates are used (Scheme 1, path B).[18,19]

The drawbacks associated to these powerful reagents prompted the evaluation of less reactive approaches for the introduction of *N*-blocking groups, like the 1,2,2,2-tetrachloroethyl,[20,21] 5-norbornene-2,3-dicarboximido,[22] pentafluorophenyl,[23] symmetrical pyrocarbonates,[24] and

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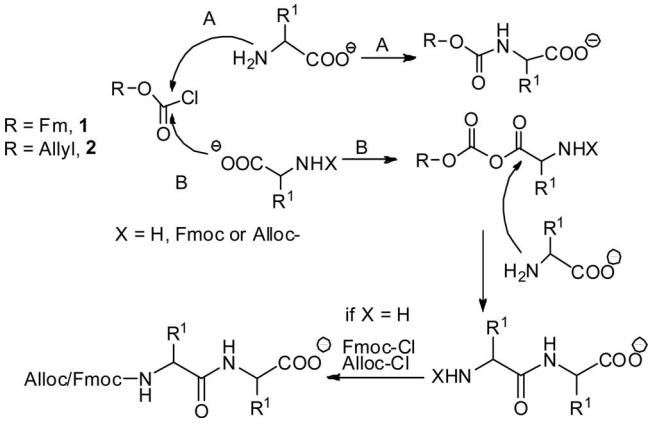
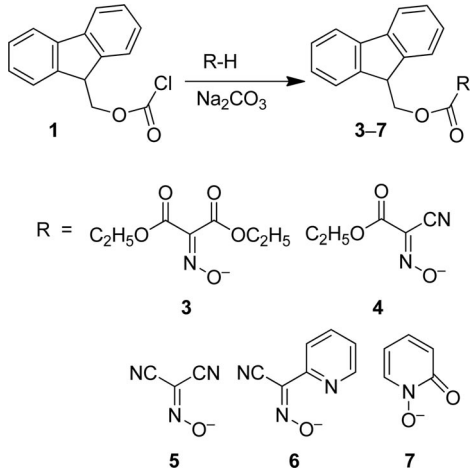


Scheme 1. Side reaction during Fmoc protection.



Scheme 2. Synthesis of Fmoc-oxime and *N*-hydroxypyridinone derivatives.

2-MBt (2-mercaptobenzothiazole),[25] although only the hydroxysuccinimido (Su) carbonate found general acceptance.[13–16,26,27] Nonetheless, the formation of up to 0.4% of some amino-acid-based byproducts like Fmoc/Alloc-β-Ala-OH and Fmoc/Alloc-β-Ala-AA-OH can occur when using this reagent for α-amino protection.[28] A detailed discussion of the mechanism of this side reaction has been proposed by Isidro-Llobet et al.[25] Azide derivatives, which can be used as solids after isolation from the chloroformates[12,29] or formed in situ before reacting with the amino acid,[30] have also been proposed as an alternative pathway to the N-protection of amino acids, although its explosive nature compromises its use in large-scale synthesis.

Our group has recently been interested in the search of a new family of *N*-hydroxy-containing compounds as replacements for 1-hydroxybenzotriazole (HOBt) and its derivatives. Some ketoximes, bearing various electron-withdrawing substituents were considered for that purpose, as they are rather stable compared to oximes with hydrogen atoms in the α-carbon position,[31] and they also contain a highly acidic *N*-hydroxy moiety, which results in an excellent leaving group. A deep study on ethyl 2-cyano-2-hydroxyiminoacetate (Oxyma) was undertaken, showing a high reduction of racemization and coupling efficiency in hindered sequences when this additive was employed.[32–35] Therefore, this oxime can be converted into promising carbonate-type reagents for the introduction of Fmoc and Alloc.

Results and Discussion

The suitability of these oximes as leaving groups in carbonate-type reagents for Fmoc/Alloc introduction was first tested in the synthesis and evaluation of Fmoc derivatives. Therefore, preparation of Fmoc-oximes **3**–**6** and (9*H*-fluoren-9-yl)methyl-2-oxopyridin-1(2*H*)-yl carbonate (**7**) was readily achieved by reaction of the corresponding oximes or *N*-hydroxy-2-pyridinone (HOPO)[36] with Fmoc-Cl (**1**) in the presence of sodium carbonate, following a well-reported method described for other reagents (Scheme 2).[26]

The Fmoc-introducing reagents were afforded by this method in high yields (>80% in all analogues synthesized) and purity, according to the found elemental analysis, compared to the calculated values (Table 1). Fmoc-oximes **3**–**6** and Fmoc-hydroxypyridinone **7** were obtained as white solids after recrystallization in CH_2Cl_2/hexane, except for diethylcarboxylate analogue **3**, which was an oil at room temperature, and therefore, its handling was tedious in comparison to the rest of the derivatives.

Table 1. Yield, m.p., and elemental analysis of the protecting groups.

	Yield	M.p.	Elemental analysis calculated (found)		
	[%]	[°C]	C	H	N
3	84	–[a]	64.23 (64.44)	5.14 (5.30)	3.40 (3.63)
4	91	174–175	65.93 (66.14)	4.43 (4.70)	7.69 (7.91)
5	87	150–151	68.14 (68.39)	3.49 (3.61)	13.24 (13.48)
6	93	165–166	71.54 (71.77)	4.09 (4.32)	11.38 (11.53)
7	89	195–196	72.06 (72.31)	4.54 (4.68)	4.20 (4.41)

[a] Ca. 20 °C; oil at room temperature.

Due to its size, H-Gly-OH is one of the amino acids that becomes most contaminated during its α-amino protection. When Fmoc-Gly-OH was synthesized by using Fmoc-Cl (**1**) in dioxane/aqueous sodium carbonate following the standard procedure described in the literature, the product was contaminated with a major (10–20%) amount of Fmoc-Gly-Gly-OH, as found by TLC.[13,18,19] For this reason, this residue has been chosen as a model for carrying out a careful in-depth study. The reaction was carried out overnight in water/dioxane, in the presence of sodium hydrogen carbonate.[37] In general, Fmoc-oxime reagents were quite stable to competitive hydrolysis by NaOH, Na_2CO_3, or triethylamine in the solvent mixture at room temperature. Compound **3** (diethylcarboxylate derivative) dissolves only partly in a solvent water mixture at an initial stage of the reaction, but the mixture usually becomes homogeneous within 1 h, whereas in the case of **6** (cyanopyridyl deriva-

tive) the reaction becomes homogeneous after 10–15 min. The results obtained in the preparation of Fmoc-Gly-OH with reagents **3**–**7** are summarized in Table 2.

Table 2. Yield of Fmoc-Gly-OH, m.p., formation of byproduct Fmoc-Gly-Gly-OH.

	Yield [%] Fmoc-Gly-OH	M.p. [°C][a]	Purity [%] Fmoc-Gly-OH[b]	Fmoc-Gly-Gly-OH [%][b]
3	82.9	166–167	99.7	0.17
4	92.1	164–165	99.4	0.57
5	48.5	158–159	99.7	0.22
6	91.6	165–166	99.9	0.01
7	92.8	166–167	99.5	0.41

[a] Authentic m.p. of Fmoc-Gly-OH = 166–167 °C. [b] HPLC analysis of the crude product.

Table 2 showed, after HPLC analysis of the crude Fmoc-Gly-OH obtained, that dipeptide formation with analogues **3**–**7** took place in the range 0.01–0.57% (Figure 1; the presence of the Fmoc-Gly-Gly-OH dipeptide in the crude mixture was identified after co-injection with a pure sample synthesized in solid-phase) with purities higher than 99% in all cases, confirming the first hypothesis about the reactivity of oxime derivatives. The most reactive oxime-based reagent **4** gave the highest percentage of the dipeptide (0.57%) and the least reactive one (i.e., cyanopyridyl, **6**) gave the best results (0.01%). Diester **3** and dicyano **5** also gave a very similar performance (0.17 and 0.22%, respectively). Yields obtained were reasonably high in general, with the exception of dicyano analogue **5**. The results obtained show that the Fmoc derivative of HOPO (**7**) is not recommended due to the formation of a considerable amount of Fmoc-dipeptide.

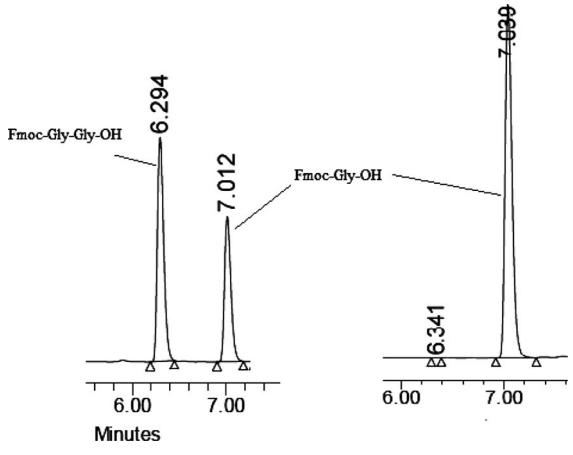
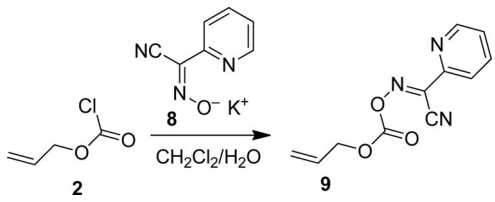


Figure 1. Fmoc-Gly-OH from **6** and co-injection with an authentic sample of Fmoc-Gly-Gly-OH.

To extend this methodology to other *N*-urethane-type protecting groups, the introduction of the Alloc moiety to the H-Gly-OH α-amino group was studied (allyloxime carbonates have been used in the past for allylation of active methylene compounds using Pd catalysis).[38] Considering the outstanding performance of cyanopyridyl reagent **6** in the Fmoc protection compared to other derivatives, only this analogue was selected to carry out the experiments regarding Alloc. The formation of the Alloc-cyanopyridyloxime reagent was accomplished by using the potassium salt of the oxime **8**, previously isolated by treatment with KOH in ethanol, and Alloc-Cl **2** (Scheme 3). This modified procedure should enhance the reaction rate over the use of the oxime plus addition of a base. Indeed, the reaction was complete in 3 h, affording Alloc-cyanopyridyloxime **9** as a pale-brownish solid after recrystallization (CH_2Cl_2/hexane) in 81% yield and high purity (>99%), as determined by HPLC and ^{1}H NMR spectroscopy.



Scheme 3. Synthesis of Alloc-cyanopyridyloxime reagent.

The performance of novel Alloc-oxime reagent **9** in preventing Alloc-based dipeptide formation was evaluated in the *N*-protection of H-Gly-OH. In comparison with Fmoc-introducing reagents, the Alloc-based analogues usually give rise to higher dimerization percentages as a result of the considerably lower steric hindrance of the allyl group than the fluorenylmethyl one, which favors this unwanted side reaction. The α-amino protection was complete after 1 h in 1%Na_2CO_3/dioxane at controlled pH 8, which was found to have great impact on the yield obtained. By using this methodology, Alloc-Gly-OH was afforded in excellent yield (83%) and purity (99.7%), as determined by HPLC and ^{1}H NMR spectroscopic analysis. The percentage of dipeptide in the crude mixture, although higher than in the analogue Fmoc-protection with compound **6**, was extremely low (0.02%), almost undetectable. This peptidic byproduct was identified after co-injection with a pure dipeptide reference, synthesized in the solid-phase by using the same Alloc-Gly-OH crude (Figure 2).

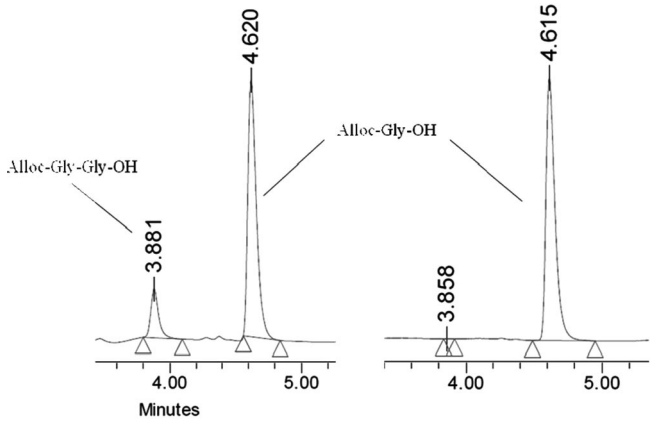


Figure 2. Alloc-Gly-OH obtained with Alloc-oxime **9** and co-injection with an authentic sample of Alloc-Gly-Gly-OH.

Conclusions

The use of the novel Fmoc-cyanopyridyloxime **6** and Alloc-cyanopyridyloxime **9** allows the preparation of Fmoc-amino acids and Alloc-amino acids free of side-products. Thus, Fmoc-Gly-Gly-OH and Alloc-Gly-Gly-OH dipeptides are formed in a negligible amount, and the absence of a succinimide moiety precludes the formation of β-alanine derivatives. Furthermore, the higher amount of dipeptide obtained with the cyanoethylester oxime (Oxyma) confirms our previous findings that this leaving group is the best substitution for hydroxybenzotriazole derivatives.[32,33] Interestingly and without studying the formation of dipeptides, Itoh and co-workers proposed a similar oxime, the phenyl one, for the introduction of the Boc group.[31]

Experimental Section

Materials: The solvents used were of HPLC reagent grade. Melting points were determined with a Mel-Temp apparatus. Magnetic resonance spectra (^{1}H NMR and ^{13}C NMR spectra) were recorded with a Joel 500 MHz (Fmoc-oxime experiments) and with a Mercury 400 MHz (Alloc-oxime experiments) spectrometer with chemical shift values reported in δ units (ppm) relative to an internal standard. Elemental analyses were performed with a 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), and the spots were detected by exposure to UV light at λ = 254 nm for a few seconds. The compounds were named by using ChemDraw Ultra version 11, Cambridge Soft Corporation. Exact masses were determined with a Waters Synapt HDMS mass spectrometer (ESI positive polarity, W analyzer, 3000 V capillary voltage, 150 and 100 °C desolvation and source temperature, 40 V sample cone, 100–1500 *m*/*z*) by introducing the sample by direct infusion. HPLC analysis was undertaken by using a reverse-phase Waters 2695 HPLC separation module, coupled to a Waters 2998 PDA UV detector, processing the chromatograms with Empower software. Separation was accomplished by using a Waters SunFire C_{18} (3.5 μ, 4.6 × 100 mm) column and linear gradients of solvent A [0.045% trifluoroacetic acid (TFA) in H_2O] in solvent B (0.036% TFA in CH_3CN) with flow = 1.0 mL min^{-1}. The mass of peptide materials was detected by using a HPLC-PDA system as the above described, coupled to a Waters Micromass ZQ mass detector, with MassLynx 4.1 software.

General Method for the Preparation of Fmoc-Oxime Derivatives 3–7: A solution of 9-fluorenylmethyloxycarbonyl chloride (10 mmol) in CH_2Cl_2 (30 mL) was added slowly to a solution of oxime or 1-hydroxypyridin-2(1*H*)-one (10 mmol) and sodium carbonate (20 mmol) in H_2O (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_2Cl_2 (50 mL), the organic phase was collected and washed with water and saturated aqueous NaCl (30 mL) and then dried with Na_2SO_4 (anhydrous). After filtering, the solvent was removed under reduced pressure, and the residue was recrystallized (CH_2Cl_2/hexane) to give Fmoc derivatives **3**–**7**.

Diethyl 2-{[(9*H*-Fluoren-9-yl)methoxy]carbonyloxyimino}malonate (3): The product was obtained as an oil at room temperature (m.p. about 20 °C) in 84% yield (3.45 g). ^{1}H NMR ($CDCl_3$): δ = 1.30–1.46 (m, 6 H, 2 CH_3), 4.29–4.50 (m, 5 H, CH, 2 CH_2), 4.55 (d, J = 7.7 Hz, 2 H, CH_2), 7.31 (t, J = 6.9 Hz, 2 H, Ar-H), 7.38–7.43 (m, 2 H, Ar-H), 7.60 (t, J = 7.7 Hz, 2 H, Ar-H), 7.77 (d, J = 7.7 Hz, 2 H, Ar-H) ppm. ^{13}C NMR: δ = 14.04, 14.08, 46.51, 62.57, 62.74, 71.68, 106.71, 120.13, 120.27, 124.87, 127.69, 128.22, 141.41, 142.82, 144.37, 149.50, 151.95, 158.86, 160.22, 160.82 ppm. $C_{22}H_{21}NO_7$ (411.40): calcd. C 64.23, H 5.14, N 3.40; found C 64.44, H 5.30, N 3.63.

Ethyl 2-{[(9H-Fluoren-9-yl)methoxy]carbonyloxyimino}-2-cyanoacetate (4): The product was obtained as a white solid (m.p. 174–175 °C) in 91% yield (3.31 g). ^{1}H NMR ($CDCl_3$): δ = 1.43 (t, J = 6.9 Hz, 3 H, CH_3), 4.35 (t, J = 6.9 Hz, 1 H, CH), 4.49 (q, J = 6.9 Hz, 2 H, CH_2), 4.64 (d, J = 6.9 Hz, 2 H, CH_2), 7.35, 7.44 (2 t, J = 7.7 Hz, 4 H, Ar-H), 7.63, 7.80 (2 d, J = 7.7 Hz, 4 H, Ar-H) ppm. ^{13}C NMR: δ = 14.09, 46.44, 64.79, 72.62, 106.56, 120.38, 125.23, 127.51, 128.37, 131.23, 141.43, 142.50, 150.86, 156.65 ppm. $C_{20}H_{16}N_2O_5$ (364.35): calcd. C 65.93, H 4.43, N 7.69; found C 66.14, H 4.70, N 7.91.

{[(9*H*-Fluoren-9-yl)methoxy]carbonyloxy}carbonimidoyl Dicyanide (5): The product was obtained as a white solid (m.p. 150–151 °C) in 87% yield (2.76 g). ^{1}H NMR ($CDCl_3$): δ = 4.28–4.35 (m, 1 H, CH), 4.54, 4.62 (2 d, J = 7.7 Hz, 2 H, CH_2), 7.32–7.36 (m, 2 H, Ar-H), 7.41–7.45 (m, 2 H, Ar-H), 7.59 (t, J = 7.7 Hz, 2 H, Ar-H), 7.78 (t, J = 6.9 Hz, 2 H, Ar-H) ppm. ^{13}C NMR: δ = 46.41, 72.60, 106.25, 120.35, 120.39, 125.15, 127.47, 127.52, 128.34, 128.40, 132.63, 141.43, 142.44, 142.49, 150.85, 151.09, 157.36 ppm. $C_{18}H_{11}N_3O_3$ (317.30): calcd. C 68.14, H 3.49, N 13.24; found C 68.39, H 3.61, N 13.48.

***N*-{[(9*H*-Fluoren-9-yl)methoxy]carbonyloxy}picolinimidoyl Cyanide (6):** The product was obtained as a white solid (m.p. 165–166 °C) in 93% yield (3.43 g). ^{1}H NMR ($CDCl_3$): δ = 4.38 (t, J = 7.7 Hz, 1 H, CH), 4.62 (d, J = 7.7 Hz, 2 H, CH_2), 7.36, 7.44 (2 t, J = 6.9 Hz, 4 H, Ar-H), 7.48–7.51 (m, 1 H, Ar-H), 7.66, 7.79 (2 d, J = 7.7 Hz, 4 H, Ar-H), 7.84 (t, J = 6.9 Hz, 1 H, Ar-H), 8.14 (d, J = 8.4 Hz, 1 H, Ar-H), 8.79 (d, J = 4.6 Hz, 1 H, Ar-H) ppm. ^{13}C NMR: δ = 46.53, 72.00, 107.94, 120.34, 122.08, 125.32, 126.89, 127.48, 128.29, 137.35, 139.67, 141.43, 142.78, 147.16, 150.46, 151.97 ppm. $C_{22}H_{15}N_3O_3$ (369.37): calcd. C 71.54, H 4.09, N 11.38; found C 71.77, H 4.32, N 11.53.

(9*H*-Fluoren-9-yl)methyl 2-oxopyridin-1(2*H*)-yl Carbonate (7): The product was obtained as a white solid (m.p. 195–196 °C) in 89% yield (2.96 g). ^{1}H NMR ($CDCl_3$): δ = 4.37 (t, J = 7.7 Hz, 1 H, CH), 4.61 (d, J = 7.7 Hz, 2 H, CH_2), 6.20 (t, J = 6.9 Hz, 1 H, Py-H), 6.75 (t, J = 9.2 Hz, 1 H, Py-H), 7.33–7.44 (m, 6 H, Ar-H), 7.64 (d, J = 7.7 Hz, 2 H, Ar-H), 7.78 (d, J = 7.7 Hz, 2 H, Ar-H) ppm. ^{13}C NMR: δ = 46.51, 72.76, 105.27, 120.28, 123.20, 125.33, 127.45, 128.26, 134.89, 139.64, 141.43, 142.67, 152.40, 157.18 ppm. $C_{20}H_{15}NO_4$ (333.34): calcd. C 72.06, H 4.54, N 4.20; found C 72.31, H 4.68, N 4.41.

***N*-(9-Fluorenylmethyloxycarbonyl)glycine (Fmoc-Gly-OH):** A solution of Fmoc-OX derivative **3**–**7** (20 mmol) in acetone (100 mL) was added dropwise to a stirred solution of glycine (20 mmol) and $NaHCO_3$ (50 mmol) in water (100 mL). After stirring overnight, the reaction mixture was concentrated under reduced pressure and then extracted with CH_2Cl_2 (50 mL) to remove the unreacted Fmoc-OX derivatives. After cooling, the reaction mixture was acidified with 10% HCl to congo red litmus paper to give a white solid, which was filtered and washed with water several times, dried, and recrystallized (ethyl acetate/*n*-hexane) to give a white solid [m.p. 166–167 °C, authentic commercial sample m.p. 166–167 °C (Table 2)]. The purity of Fmoc-Gly-OH was determined by injection of 10 μL of a sample prepared from Fmoc-Gly-OH in acetonitrile onto HPLC by using the following conditions: linear

gradient 10 to 90% of solvent A in solvent B over 8 min; PDA detection at 254 nm. t_R (Fmoc-Gly-OH) = 7.03 min and co-injection with an authentic sample of Fmoc-Gly-OH.

***N*-(9-Fluorenylmethyloxycarbonyl)glycinylglycine (Fmoc-Gly-Gly-OH):** The synthesis was carried out in a plastic syringe, attached to a vacuum manifold so as to effect rapid removal of reagents and solvent. The 2-chlorotritylchloride resin with a loading 1.55 mmol/g (1 g) was washed with CH_2Cl_2 (3 × 50 mL), and then acylated with Fmoc-Gly-OH (2 mmol) and *N*,*N*-diisopropylethylamine (DIEA, 14 mmol) in CH_2Cl_2 (10 mL). Then, the reaction mixture was stirred slowly for 1 min and let to couple for 15 min. Extra DIEA (6 mmol) was added, and the resin was stirred and left to stand with stirring from time to time for 45 min. MeOH (1 mL) was added to the resin, which was stirred for 5 min and then left to stand for an extra 5 min. The resin was filtered and then washed with CH_2Cl_2 and *N*,*N*-dimethylformamide (DMF, 2 × 50 mL each), and then deblocked by 20% piperidine in DMF for 7 min, washed with DMF, CH_2Cl_2, and DMF (2 × 50 mL each), and then coupled with the next Fmoc-Gly-OH (3 mmol), *N*,*N*-diisopropylcarbodiimide (DIC, 3 mmol), and HOBt (3 mmol) for 1 h. The dipeptide was cleaved from the resin by TFA/CH_2Cl_2 (2%, 50 mL) at room temperature for 5 min and then washed with extra solution of TFA/CH_2Cl_2 (2%, 20 mL). TFA and CH_2Cl_2 were removed in vacuo, and the crude dipeptide was precipitated with ether. The precipitate was collected and washed with ether (2×) and then dried under vacuum. The purity (99.7%) was determined by HPLC analysis by using a linear gradient 10 to 90% of solvent A in solvent B over 8 min; PDA detection at 254 nm. t_R (Fmoc-Gly-Gly-OH) = 6.29 min.

***N*-(Allyloxycarbonyloxy)picolinimidoyl Cyanide (9):** A solution of allyl chloroformate (**2**, 12 mmol) in CH_2Cl_2 (35 mL) was slowly added to a solution of the potassium salt of *N*-hydroxypicolinimidoyl cyanide (**8**, 12 mmol)[33] in H_2O (40 mL) with stirring at 0 °C. The resulting biphasic mixture was vigorously stirred at 0 °C for 30 min and then at room temperature for 3 h. After dilution with CH_2Cl_2 (50 mL), the organic phase was collected, washed with water (2 × 50 mL) and saturated aqueous NaCl (2 × 50 mL), and dried with anhydrous Mg_2SO_4. After filtering, the solvent was removed under reduced pressure, and the residue was recrystallized (CH_2Cl_2/hexane) to give desired Alloc-oxime compound **9** as a pale brownish solid (m.p. 102–103 °C) in 81% yield (2.24 g). ^{1}H NMR ($CDCl_3$): δ = 4.85–4.86 (dt, 2 H, CH_2), 5.38–5.40 (m, 1 H, CH_2), 5.46–5.50 (m, 1 H, CH_2), 5.97–6.07 (m, 1 H, CH), 7.47–7.50 (m, 1 H, Py-H), 7.82–7.86 (m, 1 H, Py-H), 8.12–8.15 (m, 1 H, Py-H), 8.77–8.79 (m, 1 H, Py-H) ppm. ^{13}C NMR (CCl_3): δ = 70.62, 107.98, 120.85, 122.16, 126.88, 130.48, 137.39, 139.63, 147.34, 150.52, 151.89 ppm. The purity of **9** was also determined after injection onto reverse-phase HPLC by using the following conditions: linear gradient 5 to 100% of solvent A in solvent B over 8 min; PDA detection at 220 nm. t_R [*N*-(allyloxycarbonyloxy)picolinimidoyl cyanide] = 6.84 min. A sample for exact mass determination was prepared, dissolving in $CHCl_3$ and diluting 1 to 100 in H_2O/MeOH (1:1): m/z = 254.0546 $[M + Na]^+$.

***N*-(Allyloxycarbonyl)glycine (Alloc-Gly-OH):** A solution of **9** (2.16 mmol) in dioxane (7 mL) was added dropwise to a stirred solution of H-Gly-OH (2.37 mmol) in 1% aqueous Na_2CO_3 (7 mL). The pH of the reaction mixture was controlled at 8 with addition of 10% aqueous Na_2CO_3. After stirring the reaction mixture for 1 h at room temperature, H_2O (50 mL) was added. The resulting mixture was acidified to pH 7 by addition of 2% aqueous HCl, and the white solid *N*-hydroxypicolinimidoyl cyanide was filtered. To remove the remaining oxime, the aqueous layer was washed with CH_2Cl_2 (3 × 60 mL) at pH 7 and pH 6.5. The aqueous layer was acidified to pH 1–2, and the product was extracted with AcOEt (4 × 60 mL). The organic layer was dried with Na_2SO_4 and filtered, and the solvent was removed under reduced pressure to render a yellow oil in 83% yield (284.1 mg). The purity of Alloc-Gly-OH was determined by injection onto reverse-phase HPLC by using the following conditions: linear gradient 5 to 40% of solvent A in solvent B over 8 min; PDA detection at 220 nm. t_R (Alloc-Gly-OH) = 4.61 min, t_R (Alloc-Gly-Gly-OH) = 3.86 min. The dipeptide was identified after co-injection with a pure sample, solid-phase synthesized by using the Alloc-Gly-OH prepared with this method. ^{1}H NMR spectroscopy showed a purity above 99%. ^{1}H NMR (CD_3COCD_3): δ = 3.88 (d, J = 6.2 Hz, 2 H, CH_2), 4.53 (dt, 2 H, CH_2), 5.14–5.17 (m, 1 H, CH_2), 5.28–5.32 (m, 1 H, CH_2), 5.89–5.94 (m, 1 H, CH), 6.53 (br. s, 1 H, NH) ppm.

***N*-(Allyloxycarbonyl)glycinylglycine (Alloc-Gly-Gly-OH):** The synthesis was carried out in a plastic syringe, attached to a vacuum manifold so as to effect rapid removal of reagents and solvent. The 2-chlorotritylchloride resin (500 mg, loading = 1.55 mmol/g) was swelled in CH_2Cl_2 (3 × 1 mL), conditioned in DMF (5 × 1 mL), and then acylated with Fmoc-Gly-OH (0.3 mmol, 1 equiv.) and DIEA (3 equiv.) in CH_2Cl_2 (0.8 mL). The reaction mixture was manually stirred for 10 min and then extra DIEA (7 equiv.) was added. The resin was left to stand for 45 min more with stirring. MeOH (0.4 mL) was added to the resin and this was manually stirred for 15 min. The resin was filtered and washed with CH_2Cl_2 (10 × 1 mL) and DMF (10 × 1 mL). After deblocking by treatment with 20% piperidine in DMF (1 + 5 + 5 min) and quantification of Fmoc, the new loading was calculated (0.57 mmol/g). This H-Gly-resin (101.6 mg) was washed with DMF, CH_2Cl_2, and DMF (10 × 1 mL each), and it was acylated by using a 0.4 M solution of the Alloc-Gly-OH prepared following the above-mentioned method (3 equiv.) and Oxyma (3 equiv.), preactivated with DIC (3 equiv.) for 3 min and left to stand for 1 h with stirring. The dipeptide was cleaved from the resin with 5% TFA/CH_2Cl_2 (1 mL, 5 × 5 min) at room temperature, washing the resin with CH_2Cl_2. The solvent and TFA were removed under an atmosphere of nitrogen, and the crude dipeptide was precipitated by adding cold Et_2O (3 × 3 mL). The resulting crude was lyophilized in H_2O (3 mL), obtaining a white solid powder in 92% yield (11.5 mg). The purity (>99%) was determined by HPLC analysis by using a linear gradient 5 to 100% of solvent A in solvent B over 8 min; PDA detection at 220 nm. t_R (Alloc-Gly-Gly-OH) = 3.39 min. HPLC-MS showed the expected mass for the dipeptide: m/z = $[M + H]^+$ = 217.04.

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