

PHTHALIMIDYL DERIVATES USED AT PEPTIDE BONDS FORMATION

IOAN GROZESCU, ADRIANA BEBESELEA^a, ADINA SEGNEANU^{*}

National Institute for Research and Development in Electrochemistry and Condensed Matter, Timisoara, Condensed Matter Department, P. Andronescu Street, 300254, Timisoara, Romania,

^aFaculty of Economics, Ioan Slavici University of Timisoara, 144 Dr. A. Paunescu-Podeanu Street, 300587 Timisoara, Romania

Dipeptide synthesis through an original method using an N-protected amino acid with a mixed carbonate phtalimidyl was investigated. Applications of this carbonates type at amino acid protecting is relatively less studied although present a similar reactivity succinimidyl derivatives, having the advantage that allows quantitative recovery of *N*-hydroxyphtalimide after reaction. The performed study has led to the formation of peptide bond with good efficiencies, demonstrating the applicability of these intermediates in the synthesis of peptides, compounds with biological activity used as an intermediate in the pharmaceutical industry.

(Received August 28, 2012; Accepted October 30, 2012)

Keywords: dipeptide, amino acid, carbonate phtalimidyl, dicyclohexylcarbodiimide

1. Introduction

From the great variety of types of protective groups used in peptide synthesis, are preferred those that allow deprotection in mild cleavage conditions without inducing racemisation. The use of *N*-phtalimidyl asymmetrical carbonates for the protection of amine moieties from primary amines and amino acids in peptide synthesis is too less studied, although it is known their high reactivity due to nucleofuge character of the corresponding group [2-8].

The possibilities to introduce the phtalimidyl derivates as *N*-protecting groups for peptide bonds are interesting because these are mild reagents, the co product, hidroxi phtalimide, is easy to recover and ready to be reuse. The literature revealed few methods for the peptide bonds formation, in which the *N*-protecting are used with a mixed carbonate of succinimidyl (benzyl-*N*-succinimidylcarbonate) and in the phase coupling is used dicyclohexylcarbodiimide. [5-11]. The coupling reaction occurs between the *N*-protected amino acid (*Z*-Gly), the functionalized amino acid and in presence of diciclohexylcarbodiimide (DCC) and of a tertiary amine, triethylamine (TEA). It uses a small excess of DCC and amine (molar ratio *N*-protected amino acid: functionalized amino acid : DCC : TEA = 1:1:1.1:1.1). The reaction is carried out in an organic solvent (dichloromethane) at low temperature (-18° C) [6].

The paper investigates the synthesis of a dipeptide in similar conditions using an amino acid *N*-protected with an asymmetrical phtalimidyl carbonate.

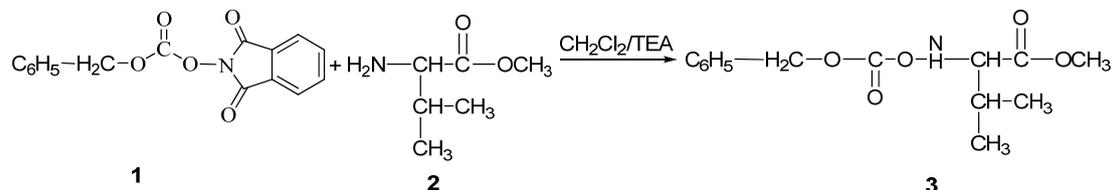
2. Results and discussion

Because in the literature there are few informations about phtalimidyl derivates in peptide synthesis, was developed a two stage synthetic route for peptide bond formation. First, for the *N*-protection of the amino moiety from amino acid was necessary to investigate the protecting reaction by alkoxy-carbonylation at nitrogen nucleophiles of aminoacid-type in order to find the

^{*} Corresponding author: s_adinaelena@yahoo.com

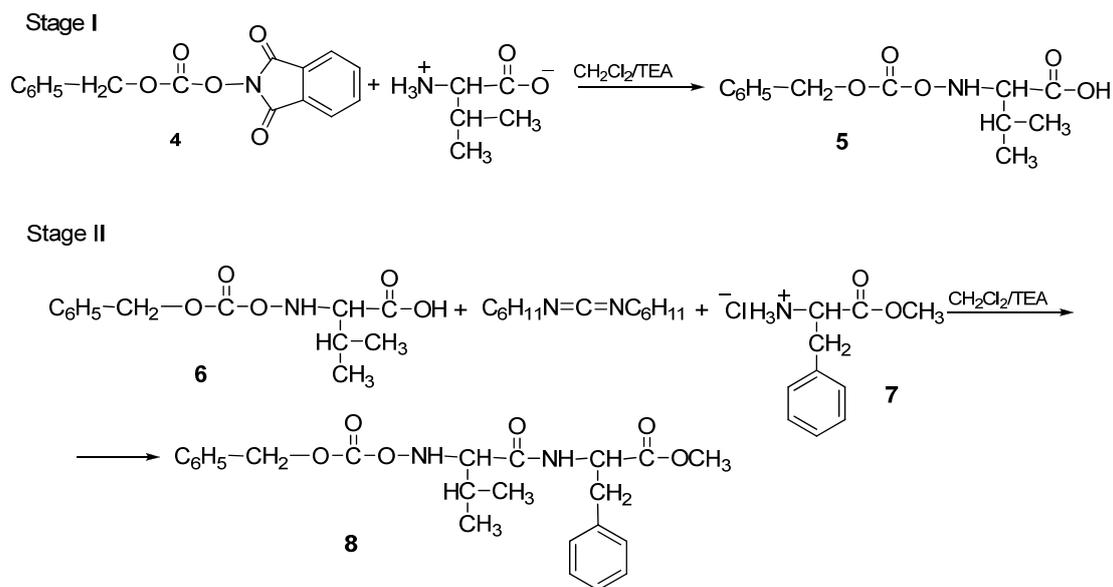
optimal reaction conditions (reaction time, solvent). The successive experimental determinations have proved that the optimal molar ratio carbonate: amino acid : amine = 1:1:3, and the reaction time was 15 hours.

Starting from benzyl-*N*-phthalimidyl carbonate (**1**) and *L*- valine methyl ester, (**2**) in the presence of triethylamine and dichloromethane as solvent, was obtained *N*-benzyloxycarbonyl-*L*-valine methyl ester (**3**, **Scheme 1**) in good yield (78.5%). TLC indicated complete consumption of the starting material after 15 h at 25 °C with obviously no significant formation of side products.



Scheme 1. Alkoxycarbonylation reaction of L- valine methyl ester.

The formation of peptide bond was achieved in two stage synthetic procedure (**Scheme 2**). In the first stage it was obtained the *N*-benzyloxycarbonyl-*L*-valine (**5**) starting from benzyl-*N*-phthalimidyl carbonate (**4**) and *L*- valine, in presence of TEA and dichloromethane as solvent. In the stage two, the compound **4**, *N*-protected aminoacid was coupled with a second aminoacid, *O*-protected using DCC. The dipeptide was prepared by coupling benzyl-*N*-phthalimidyl carbonate and Δ -phenylalanine methyl ester hydrochloride (**6**) with dicyclohexylcarbodiimide and triethylamine in molar ratio of 1:1:1:1 and dichloromethane as solvent.



Scheme 2. The dipeptide synthesis

The final product, *N*-benzyloxycarbonyl-*L*-valine- Δ -phenylalanine methyl ester hydrochloride (**8**) was achieved in good yield (75%). Also, the H^1 -NMR-spectra of compound **3** revealed the presence of unreacted benzyl-*N*-phthalimidylcarbonate around 25%. (signal from $\delta=7.7 \div 7.8$ ppm corresponding to aromatic protons of *N*-phthalimidyl). This problem is probably due to the use of solvents without further purification.

3. Experimental section

General Methods

All used reagents and solvents were purchased from commercial sources (Sigma-Aldrich) and used without a further purification. Reactions were monitored by thin layer chromatography,

plates were coated with 0.2 mm of silica gel 60 F254 (Merck) and were visualized by UV irradiation (254 nm).

Melting points:

Melting points were performed on Boetius Carl Zeiss Jena device.

Infrared spectra of solid compounds were recorded in KBr pellets on FT-IR spectrometer Vertex 70 Bruker, in the range of 400–4,000 cm^{-1} .

The **NMR spectra** were recorded on a Bruker DPX at 200 MHz in DMSO- d_6 , with TMS as reference. The values of coupling constants are normal for vicinal couplings (CH-CH, CH-NH): 6.5–7 Hz.

Detailed IR, MS, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra are available from the authors.

THE ALCOXYCARBONILATION REACTION

At benzyl-*N*-phtalimidylcarbonate (0,507 mmoli) in 10 ml dichloromethane (CH_2Cl_2) is added *L*-valine methyl ester (0,507 mmoli). In the reaction mixture is added in portions triethylamine (0,2 ml, 1,521 mmoli) with stirring. Stirring was done for 15 hours at room temperature. After that the vacuum solvent removal the obtained residue was purified and obtained was dissolved in ethyl acetate (15 mL) and the solution was three times washed with 20% citric acid solution (5 mL), 5% NaHCO_3 solution (5 mL) and saturated NaCl solution (5 mL). The organic phase was dried (MgSO_4) and concentrated to dryness.

N-benzyloxycarbonyl-*L*-valine methyl ester (**3**)

$^1\text{H-RMN}$ (DMSO- D_6): 8.3t, 7,82q, 7,59m, 7,48m, 7,3m, 5.3d, 3,8d, 3.5t, 2.21m, 0,9q.

$^{13}\text{C-RMN}$ (DMSO- D_6): 171,7(C_{10}), 155,9(C_1), 137,5(C_3), 128,9(C_5), 128,7(C_6), 127,6(C_4), 71.9 (C_7), 71.5(C_2), 59,5(C_{11}), 33,3(C_{10}), 19.3(C_9).

SYNTHESIS OF DIPEPTIDE

Stage I

At a solution of benzyl-*N*-phtalimidylcarbonate (0.43 g, 1.461 mmol) in 15 ml dichloromethane is added *L*-valine (0.171 g, 1.461 mmol) and triethylamine (0.4 ml, 2.923 mmol). The reaction mixture was stirred at room temperature for 15 h. The completion of the reaction was followed by thin layer chromatography (TLC), using as eluent a mixture of butanol: acetic acid: water 2:1:1. The solvent is evaporated in vacuum and the residue is repeated with dichloromethane (5 ml) and washed with water (10 ml). Aqueous layer is washed with dichloromethane (10 ml). Aqueous layer is acidified at pH 2–2.5 with 0.1 N HCl, and the organic layer was washed with ethyl acetate until the complete discoloration. After that the vacuum solvent removal, the residue was crystallized from ethyl acetate-hexane. The white solid (0.288 g, $\eta = 78.5\%$).

N-benzyloxycarbonyl-*L*-valine (**5**)

Mp: 65°C

IR (KBr, cm^{-1}): 3149i, 3096s, 3035s, 1854s, 1789m, 1737i, 1710i, 1608m.

$^1\text{H-RMN}$ (DMSO- D_6): 10.8s, 7,82q, 7,59m, 7,48m, 7,3m, 5.3d, 3,8d, 3.5t, 2.21m, 0,9q.

$^{13}\text{C-RMN}$ (DMSO- D_6): 171,7(C_{10}), 155,9(C_1), 137,5(C_3), 128,9(C_5), 128,7(C_6), 127,6(C_4), 71.9 (C_7), 71.5(C_2), 33,3(C_{10}), 19.3(C_9).

Stage II

N-(benzyloxycarbonyl) valine (0.152 g, 0.606 mmol) in dichloromethane 10 ml was added methyl ester hydrochloride of phenylalanine (0.131 g, 0.606 mmol) and triethylamine (TEA) (0.08 ml, 0.606 mmol). The reaction mixture was cooled at 0°C with stirring and then was added dicyclohexylcarbodiimide (DCC) (0.125 g, 0.606 mmol). The reaction mass was cooled at a temperature of -18°C for 48 h. After dicyclohexyl urea filtering, the filtrate was dissolved in dichloromethane (4 ml) then washed with water (4 ml). Organic layer was washed with a solution of 5% sodium bicarbonate (2 x 4 ml), water (4 ml) and saturated solution of sodium chloride (2 x 4 ml), and then are dried on anhydrous Na_2SO_4 . The organic phase was dried (MgSO_4) and concentrated to dryness. The result residue was recrystallized from anhydrous ethyl ether. The final product, a pale yellow solid (0.183 g, $\eta = 70.34\%$) was characterized by TLC, IR and NRM spectroscopy.

TLC: (BuOH: CH_3COOH : H_2O eluent) (Rf %): 0.35 for dipeptide, 0.32 for *L*-valine and 0.57 for phenylalanine.

IR (KBr, cm^{-1}): 3324i, 3062s, 3031s, 1789u, 1746i, 1729i, 1697m, 1643m, 1627m, 1606s.

¹H-RMN (DMSO-D₆): 8,37t, 8,15d, 7,82q, 7,59m, 5,05d, 4,51d, 3,93t, 3,66s, 3,1d, 1,93q, 1,70d, 1,01q, 0,9q.

¹³C-RMN(DMSO-D₆): 171,7(C₁₂), 155,9(C₁), 137,5(C₃), 134,8(C₁₅), 128,9(C₁₆), 128,7(C₁₇), 128,2(C₅), 128,1(C₄), 127,6(C₁₈), 126,4(C₆), 65,4(C₇), 59,5(C₁₁), 51,6(C₁₃), 33,3(C₂).

4. Conclusions

The present study reports the successful application of asymmetrical phthalimidyl carbonates at *N*-protection of aminoacids for the peptide synthesis.

It has been developed a convenient two stage synthetic procedure for peptide bonds formation using *N*-protected amino acids with mixed carbonated of *N*-phthalimidyl (benzyl-*N*-phthalimidyl carbonate). The analysis of the final product showed it was obtained Val-Phe dipeptide protected at amino group with benzyloxycarbonyl group and with *O*-protected carboxyl group in the form of methyl ester. But, this is contaminated by the presence of unreacted benzyl-*N*-phthalimidylcarbonate about 25%.

Despite the estimated slightly lower reactivity of the phthalimidyl moiety mentioned, we found no further evidence in the literature of its use in any kind of protecting strategy and peptide bond formation leading to the opinion that an investigation of an amine protecting group based on the phthalimidyl derivative might turn out as a promising endeavor.

Acknowledgments

This research was supported by the grants from “IMMUNOMODULANTE FLUORO-GLYCOPEPTIDE MOLECULAR ARCHITECTURES”- code PN-II-ID-PCE-2011-3-0856, Contract number: C 341/5.10.2011.

Refereces

- [1] Manske R. H. F., Benzyl Phthalimide, Organic Syntheses, Coll. **2**, 83 (1943); **12**, 10 (1932);
- [2] Norbert Seward, Hans-Dieter Jakubke, Peptide: Chemistry and Biology, Wiley-VCH Verlag GmbH&Co., Weinheim, **2002**;
- [3] Pillai, R.V.N., Photoremovable Protecting Groups in Organic Synthesis, Synthesis, International Journal of Methods in Synthetic Organic Chemistry, (1), p. 1-26 (1980).
- [4] Sakakibara, S., Chemical Synthesis of Proteins in Solution, Biopolymers (Peptide Science) John Wiley&Sons Inc, **51**, 279 (1999).
- [5] Hidetomo I., Keisuke K., Yoshio I., Polymides derived from bis-*N*-hydroxyimides. III. Polyimide-carbonates and polyimide-urethanes synthesized from bischlorformate of *N,N'*-dihydroxypyromellitic diimide, Journal of Polymer Science, Polymer Chemistry Edition, **19**, 1855 (1981).
- [6] Shiina, I., Kawakita, Y., A new method for the synthesis of carboxamides and peptides using 1,1'-carbonyldioxydi[2(1H)-pyridone] (CDOP) in the absence of basic promoters, Tetrahedron, **44**, 1951 (2002).
- [7] Zimmerman, J. E., Anderson, G.W., J. Am. Chem. Soc., **89**(26), 7151 (1967).
- [8] Golkowski M., Ziegler T., The 2-(Triphenylsilyl)ethoxycarbonyl- (“Tpscoc”-) Group: A New Silicon-Based, Fluoride Cleavable Oxycarbonyl Protecting Group Highly Orthogonal to the Boc-, Fmoc- and Cbz-Groups, Molecules **16**, 4695 (2011).
- [9] Dahiya R., Kumar A., Yadav R., Synthesis and Biological Activity of Peptide Derivatives of Iodoquinazolinones/Nitroimidazoles, Molecules, **13**, 958-976 (2008).
- [10] Colthup, N.B., Daly, L.D., Wiberley, S.E., Introduction in Infrared and Raman Spectroscopy, Academic Press, New York and London, 254, 1964.
- [11] Silverstein, R.M., Bassler, C.G., Morrell, T.C., Spectrometric Identification of organic Compounds, fifth edition, J. Wiley&Sons Inc., 729–814, 1991.