New series of asymmetrical carbonates used in peptide synthesis

A.-E. SEGNEANU, M. MILEA[a], I. GROZESCU[*]
National Institute of Research & Development for Electrochemistry and Condensed Matter – INCEMC Timisoara, Romania, 300569, 144 Aurel Paunescu Podeanu
[a]*University „Politehnica" P-ta Victoriei nr.1, Timisoara, Romania*

In many preparations of delicate organic compounds, some specific parts of their molecules cannot survive the required reagents or chemical environments. Then, these parts, or groups, must be protected. The organic reactive carbonates are used especially as alkoxycarbonyl-type (carbamate) groups for protection of the amino function in amino acids. These protection groups are widely used in peptide synthesis because of their tendency to suppress racemisation of optically active centers. This paper presents the synthesis of new asymmetrical reactive carbonates with leaving group from the corresponding symmetrical carbonates and different type of alcohols, by an original procedure, as an alternative to the traditional method which employs toxic and dangerous reagents (phosgene and its chlorinated derivatives), as well as the applicability of these derivatives to the synthesis of bioactive compounds with many utilizations in pharmaceutics and food industries. The synthesis of asymmetrical primary-, secondary- and tertiary-alkyl phtalimidyl carbonates from *N,N'*-diphtamidylcarbonates (DPC) can be performed in one step reaction and with good yields. For the structure elucidations of final products was used the yield of these reactive carbonates depends on the alcohols reactivity, on the hindering factor and on the stability of the final product.

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1. Introduction

The global interest in sustainable chemical development (green chemistry) imposed the establishing and adoption of measures for the reduction of the negative effects of chemical compounds on the environment and human health. Thus, the resolution regarding the synthesis of products as a result of molecular transformations, which imply low energy consumption, reduction of the formation of waste and the use of solvents and toxic or dangerous reagents, was adopted in the European Union in 2003 [1].

Within the current orientation of organic synthesis and, in general, of chemistry towards to the sustainable chemical development (green chemistry), to the use and expansion of non-polluting chemical technologies, and towards to the environmental protection by the replacement of chemical toxic compounds, the use of the phosgene derivatives in the carbonylation reactions has been avoided. The development of a new reagents and techniques, the synthesis of the smallest peptides, have received considerable attention recently because the need for green, economic, robust, scalable and reliable processes for organic synthesis applications in the pharmaceutical and fine chemical industries.

The need to identify and develop new organic reactive carbonates is very important in fine organic synthesis because these compounds present extremely many applications especially in the peptide synthesis.

In the peptides synthesis, alkoxycarbonyl amino-protecting group (carbamate) from a symmetric carbonate and primary, secondary or tertiary alcohols are used. The use of some mixed phtalimidyl carbonates is an alternative option for the *N*-protecting amino acids. The protection of functional groups is directly accomplished by converting the particular group into a stable derivative, from which the original group can be regenerated without affecting the synthesized molecule. A good yield for the deprotecting step is mandatory [5].

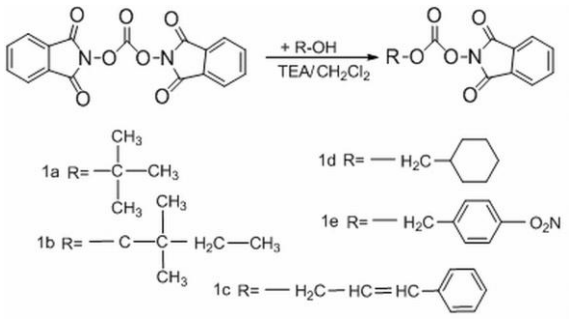
Organic reactive carbonates are interesting target since their conventional production involves the use of toxic phosgene [9]. These compounds have numerous potential applications for synthesis of different important organic molecules (functionalized carbonates, pharmaceutical and cosmetic intermediaries, lubrificants, solvents etc). Carbonates production by a clean process, possesses properties of non-toxicity and biodegradability, make these compounds as true green reagent to be used in syntheses that prevent pollution at the source.

Organic carbonates are considered an option for classical synthetic ways which takes place in the presence of toxic compounds [4,6].

The coproduct, dioxide is nontoxic, an advantage over phosgene and carbon monoxide, and easy to handle and to store, but much less reactive [2]. However, the implementation of safer technologies justifies the assessment of chemical reactions based on carbon dioxide as it can be viewed as a renewable raw material.

The preparation of some new asymmetrical reactive carbonate with leaving groups (tert-buthyl-*N*-phtalimidylcarbonate (a), 2-methyl-2-buthyl-*N*-

phtalimidylcarbonate (b), cinnamyl-*N*-phtalimidylcarbonate (c), cyclohexyl-methyl-*N*-phtalimidylcarbonate (d), 4-nitro-benzyl-*N*-phtalimidyl-carbonate (e)) by an original procedure will be investigated.



Scheme 1. Reaction for obtainainig asymmetrical carbonates.

2. Experimental

2.1. Materials

All reagents were purchased from chemical suppliers and used without further purification.

N,N'-diphtalimidylcarbonate was synthesized according Ref 8 and 9.

2.2. Synthesis of asymmetrical carbonates

To a solution of alcohol (1.52 mmols) in CH_2Cl_2 (10 ml), diphtalimidyl carbonate (1.52 mmols) and triethylamine (TEA) (1.52 mmols) were added by drops. The reaction mixture is maintained under stirring at room temperature for 24 hours, and then is washed with citric acid (5% excess to the amine stoichiometric ratio) and the unreacted *N*-hydroxiphtalymide was then filtered. The filtrate is washed with saturated NaCl solution. The organic layer is dried on $MgSO_4$, filtered and then the solvent evaporated. The residue is recrystallized from $CHCl_3$/heptanes.

2.3. Characterisation

2.3.1. FT-IR spectroscopy

The *IR spectra* of solid compounds were recorded in KBr pellets and the reaction monitoring was carried out in thermostatic silicon cells of 0.137 mm thickness on a Jasco FT/IR-Vertex 70 instrument.

2.3.2. Mass spectroscopy

Mass spectrometry was conducted on a High Capacity Ion Trap Ultra (HCT Ultra, PTM discovery) mass spectrometer from Bruker Daltonics, Bremen, Germany. HCT MS is interfaced to a PC running the CompassTM 1.2 integrated software package, which include the HystarTM 3.2.37 and Esquire 6.1.512 modules for instrument controlling and chromatogram/spectrum acquisition, and Data Analysis 3.4.179 portal for storing the ion chromatograms and processing the MS data.

To identify and analysis the purity of the final products, samples were dissolved in 1.5 ml HPLC grade methanol. In this way, the samples were carried significant evidence. The samples were maintained at room temperature for 24 hours, and then were evaporated in Speed Vac Concentrator (Thermo Electron Corporation, Milford, MA USA).

Infusion into mass spectrometer (ESI HCT Ultra, Bruker Daltonics, Germany) was performed by the robot NanoMate (Advion Biosciences, UK). The robot is an automatic injection device electrospray chip. 20µl of sample was pipetted into microtitre plate wells, containing 96 holes.

Then the robot's software was chosen to test wells and was given command of infusion through silicon chip (chip contains 400 holes) in the mass spectrometer. It was measured in positive ionization technique, the amount of gas (nebuliser) of 50 psi and temperature spectrometer source 200^{o}C. Robot parameters were set as follows: pipette robotic arm to aspirate 10 ml of sample and 2 ml of air, gas pressure was 0.30 psi, and the voltage of 1.40 kV (low voltage it was chosen to prevent fragmentation at source).

2.3.3. RMN spectroscopy

The *^{1}H-NMR and ^{13}C-NMR* were recorded on a Bruker DPX at 200 MHz in DMSO-*d6*, 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}H-NMR and ^{13}C-NMR spectra are available from the authors.

3. Results and disscutions

First, starting from the preparation of mixed succinimidyl carbonates according Ref 8 and 9 was investigated the most appropriate conditions for the synthesis of phtalimidyl asymmetrical carbonates in heterogeneous medium using different types of alcohols (Scheme 1). It was demonstrated that symmetrical carbonate (*N*,*N*-succinimidylcarbonate) can react efficiently with alcohols and amine (TEA) in the molar ratio carbonate: alcohol: amine = 1,1:1:1,1. The reactions we carried out in acetonitrile at room temperature for 4 hours.

The procedure for the preparation of a new series of asymmetrical carbonates starting to phtalimidyl carbonate and different types of alcohols, used in peptide synthesis, was investigated.

It was found that under this same reaction conditions, the mixed carbonates were obtained in very low yield and major product was *N*-hidroxyphtalimide result from symmetrical carbonates decomposition. It was has shown that is necessary to change the reaction medium with another solvent in which the *N,N`*-diphtalimidyl-carbonate is insoluble. To facilitate the isolation and removal of the coproduct, (*N*-hidroxy-phtalimide) was chosen as reaction medium dichloromethane. To increase the yields of was necessary to determinate the optimal conditions (reaction time, molar ratio) of these types of mixed carbonates by FT- IR spectroscopy. Thus, it was developed a chemoselective, convenient and efficient synthetic procedure for preparation of phtalimidyl asymmetrical carbonates in good yields.

It was prepared five asymmetric carbonates from *N,N`*-diphtalimidylcarbonate and alcohols (benzyl and aliphatic type with electron attractive groups) and TEA in a molar ratio 1:1:1. The reactions occurs in a heterogeneous medium, at room temperature (25°C) due to the low solubility of the symmetric carbonate (*N,N`*-diphtalimidyl-carbonate) in dichlormethane.

The new asymmetrical reactive phtalimidylcarbonates with leaving groups (tert-buthyl-*N*-phtalimidylcarbonate (a), 2-methyl-2-buthyl-*N*-phtalimidylcarbonate (b), cinnamyl-*N*-phtalimidylcarbonate (c), cyclohexylmethyl-*N*-phtalimidylcarbonate (d), 4-nitro-benzyl-*N*-phtalimidyl-carbonate (e)) were analyzed by FT-IR, mass spectrometry, ^{1}H-NMR and ^{13}C-NMR.

Tert-buthyl-N-phtalimidylcarbonate - **1a**, white solid, 70,8%, IR ν(cm^{-1}): 1734.1(C=O); 1761.1(C=O); 1774.6(C=O); 1795.8(C=O); 1805.4(C=O); 1807.3(C=O); 1851.7(C=O); ^{1}H-NMR(CDCl3, DMSO): δ 0.85(m); 3.8(s); 7.87(m); ^{13}C-NMR(CDCl3, DMSO): 25.4(C3).; 54.6; 123.7; 131.5; 132.2(C3); 153.6(C2); 161(C1); MS (EI) m/z: 89.6; 114.4; 123.3; 157.3; 213.2; 229.2; 245.2; 263.2 [Calc. for $C_{13}H_{13}NO_5$: requires M, 263.13. Found: 263.2 (M+)].

2-Methyl-2-buthyl-N-phtalimidylcarbonate - **1b**, white solid, 63,2%; IR ν(cm^{-1}): 1701.7(C=O); 1720(C=O); 1734.3(C=O); 1759.4(C=O); 1770.7; 1788(C=O);1799.6(C=O); 1817(C=O); ^{1}H-NMR (CDCl3, DMSO): δ 0.88(t); 1.2(d); 7.88(m); ^{13}C-NMR(CDCl3, DMSO): 25.4(C3).; 123.7; 132.5; 153.6; 161; MS (EI) m/z143.3; 157.2; 171.2; 220.2; 233.2; 250.2; 277.2, 279.2, 289.2 [Calc. for $C_{15}H_{15}NO_5$: requires M, 289.13. Found: 289.2 (M+)].

Cinnamyl-N-phtalimidylcarbonate - **1c**, white solid, 74.1%; IR ν(cm^{-1}): 1734.1(C=O); 1763(C=O); 1772.6(C=O); 1784.2(C=O); 1838.2(C=O); 1849.8(C=O); 1871(C=O); ^{1}H-NMR(CDCl3, DMSO): δ1.23(s); 2.4(d); 3.8(d); 5.25(d); 7.16(m); 7.85(m); ^{13}C-NMR(CDCl3, DMSO): 21.3(C3).; 54.6; 123.3; 125.7; 129; 132; 138.4; 153.6; 167; MS (EI) m/z114.3; 158.2; 190.1; 229.1; 265.3; 279.2; 293.2; 312.3; 323.1[Calc. for $C_{18}H_{13}NO_5$: requires M, 323,13. Found: 323,1 (M+)].

Cyclohexylmethyl-N-phtalimidylcarbonate -**1d**, white solid, 60.1%; IR ν(cm^{-1}): 1734.7(C=O); 1761.2(C=O); 1774.6(C=O); 1803.5(C=O); 1807.3(C=O); ^{1}H-NMR(CDCl3, DMSO): δ 0.9(t); 1.4(m); 1.52(m); 1.61(m); 3.8(d); 7.9(m); ^{13}C-NMR(CDCl3, DMSO): 25.5(C3).; 26.1; 31.3; 34.2; 54.6; 123.7; 129.1; 132.2; 153.6; 161; MS (EI) m/z114.4; 160.2; 207.2; 235.2; 250.3; 263.2; 279.2; 300.3; 303.2[Calc. for $C_{16}H_{17}NO_5$: requires M, 303.13. Found: 303.2 (M+)].

4-Nitro-benzyl-N-phtalimidylcarbonate -**1e**, white solid, 68.5%; IR ν(cm^{-1}): 1737.9(C=O), 1751.4(C=O); 1768.8(C=O); 1770.7(C=O); 1788(C=O); 1817(C=O); 1821(C=O); 1822.8(C=O), 1834.3(C=O); ^{1}H-NMR(CDCl3, DMSO): δ2.34(m); 3.05(d); 3.8(d); 7.11(d); 7.85(m); ^{13}C-NMR(CDCl3, DMSO): 41.3; 124; 129; 135.4; 138.3; 156; 169.7; MS (EI) m/z265.3; 279.2; 292.3; 337.4; 342.2[Calc. for $C_{16}H_{10}N_2O_7$: requires M, 342.13. Found: 342,2 (M+)].

4. Conclusion

The optimal conditions (reaction time, molar ratio) of these types of mixed carbonates were determinate by FT-IR spectroscopy. It was developed a convenient synthesis procedure for the one step preparation of a new series of asymmetrical carbonates from *N,N'*-diphtalimidyl-carbonate and different aromatic and aliphatic alcohols in good yields (60-70.8%). The reactive organic carbonates yield depends on alcohol reactivity, hindering factor and stability of final product.

This synthetic procedure represents an easier and eco-friendly alternative for preparation of new key intermediaries use for protection of amino group from amines and amino-acids and than in peptide synthesis, compounds with biological activity, useful in pharmaceutical industry.

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References

[1] P. T. Anastas, J. C. Warner, Green Chemistry: Theory and Practice, Oxford University Press, New York (1998).
[2] M. Aresta, E. Quaranta, Carbon Dioxide: A Substitute for Phosgene, CHEMTECH **27**, 32 (1997).
[3] A. K. Ghost, T. T. Duong, S. P. McKee, W. J. Thompson, Tetrahedron Lett., **32** (20), 2781 (1992).
[4] M. A. Pacheco, C. L. Marshall. Review of Dimethyl Carbonate Manufacture and its Characteristics as a Fuel Additive, Energy & Fuels, **11**, 2 (1997).
[5] R. V. N. Pillai, Photoremovable Protecting Groups in Organic Synthesis, Synthesis, International Journal of Methods in Synthetic Organic Chemistry, **1**, 1-2 (1980).
[6] F. Rivetti, The Role of Dimethyl Carbonate in the Replacement of Hazardous Chemicals, C. R. Acad. Sci. Paris Série IIC Chimie, **3**, 497 (2000).
[7] A. E. Segneanu, M. Milea, M. Simon, C. Csunderlik, Revista de Chimie, **58** (6), 542 (2007).

[8] A. E. Segneanu, Ed. Politehnica Timisoara, seria 2: Chimie, nr. **1** (2007).
[9] A. G. Shaikh, S. Sivaram, Organic Carbonates, American Chemical Society, Chemical Reviews, **96**, 3, 951 (1996).
[10] P. Tundo, New Developments in Dimethyl Carbonate Chemistry, Pure and Applied Chemistry, **73**, 1117 (2001).
[11] A. E. Segneanu, I. Balcu, M. C. Mirica, M. I. Iorga, M. Milea, Z. Urmosi, Environmental Engineering and Management Journal, **8**(4), 797 (2009).
[12] A. E. Segneanu, M. Milea, I. Grozescu, Optoelectron. Adv. Mater. – Rapid Commun. **6**(1-2), 197 (2012).

*Corresponding author: ioangrozescu@gmail.com