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One-Pot Formation of Succinimidyl Esters by the System Chlorophosphate / Hydroxysuccinimide / Base

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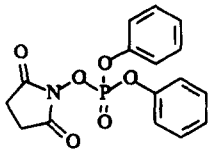
Abstract: Succinimidyl esters of various carboxylic acids are formed in high yield at ambient to slightly elevated temperature by the system chlorophosphate / hydroxysuccinimide / base.

The literature is replete with "coupling reagents" proposed for the effective formation of peptide bonds as a crucial step in the formation or elongation of peptides. In pursuit of high coupling yields or simple application many "miraculous reagents"[1] have been developed, irrespective of their toxicity, ease of preparation or cost effectiveness. Among the reagents producing "active esters", such as N-hydroxysuccinimidyl or N-hydroxy-norborn-5-ene-2,3-dicarboximidyl esters as reactive intermediates in peptide coupling, the corresponding diphenyl phosphate esters, succinimidyl diphenyl phosphate (**1**) and norborn-5-ene-2,3-dicarboximidyl diphenyl phosphate (**2**) have been proposed.[2, 3]



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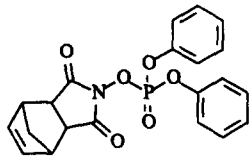

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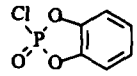

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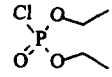

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Figure 1

They enlarge the concept of "reactive group transfer" from phosphoric to carboxylic acid derivatives with earlier examples in the formation of acyl azides via phosphoryl azide derivatives[4], acyl imidazolides via phosphoryl imidazolides[5] or, most trivially, the formation of acid chlorides via phosphoryl chlorides.

Compound **1** is prepared from diphenyl chlorophosphate and N-hydroxysuccinimide[2], both readily available starting materials. The use of **1** in peptide coupling reactions reportedly avoids racemization. This property makes it an attractive coupling agent in principle, but it has only limited shelf-life and is sensitive to moisture. Therefore it has found only few applications in peptide coupling reactions.[6]

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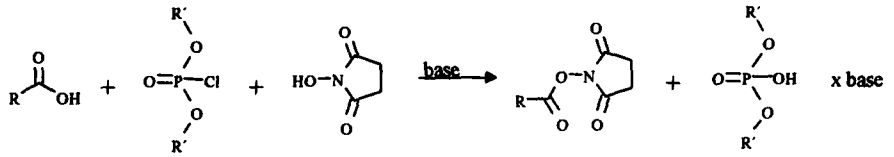
We studied the development of industrially feasible, cost-effective peptide coupling methods and found that SDPP as a succinimidyl ester forming reagent need not be separately synthesized and isolated. It can be prepared in situ from diphenyl chlorophosphate and N-hydroxysuccinimide in presence of an organic or inorganic base. Thus in a reaction mixture containing a carboxylic acid, N-hydroxysuccinimide and a base in appropriate proportions, the succinimidyl ester of the carboxylic acid is formed upon addition of a chlorophosphate in nearly quantitative yield, with the corresponding phosphate salt of the employed base as single by-product.



Scheme 1: Formation of succinimidyl esters. R' = Et, Ph; for R see Figure 2.

The reaction has several advantages: It can be performed at ambient to slightly elevated temperatures. In contrast to DCC-coupling reactions, no solid by-product is formed. The workup procedure usually consists of an extraction of the organic phase, (which contains the succinimidyl ester) with sodium bicarbonate solution to remove the phosphate. The succinimidyl ester can be further purified or isolated, or its solution can be used in the subsequent coupling step.

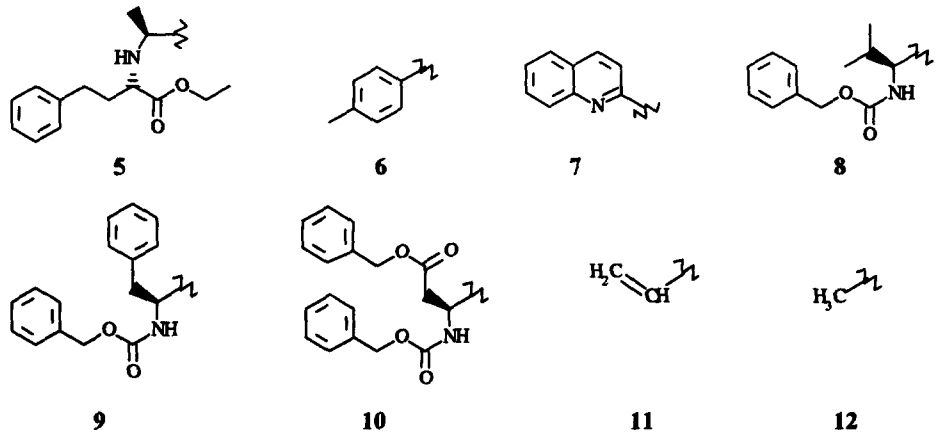


Figure 2: Structures of carboxylic acids under investigation: R in R-COOH

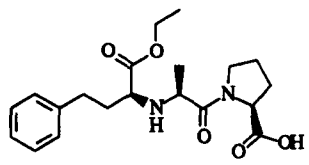
The reaction is applicable to a wide variety of carboxylic acids and finds limitations mainly where the active ester formed is unstable due to further reactive groups present in the molecule[7]. Accordingly, amino acids have to be protected by usual methods prior to their use in the reaction. A variety of phosphate esters can be employed to transfer the succinimidyl group. Thereby the reactivity of the system can be fine-tuned to meet

the requirements of the carboxylic acid to be transformed. Phenylene chlorophosphate (2-chloro-benzo-1,3,2-dioxaphospholen-2-oxide) (**3**) proved to be more reactive than diphenyl chlorophosphate, but its use led to the formation of equilibrium mixtures between succinimidyl ester and acyl phosphate, similar to the equilibrium between acyl imidazolide and acyl phosphate as described by Cramer[5]. Diethyl chlorophosphate (**4**) is equally reactive, having the advantage of producing no phenol in waste streams. Depending on the nature of the carboxylic acid, we found sodium bicarbonate, triethylamine or N-methylmorpholine as suitable bases. Figure 2 gives an overview over the carboxylic acids employed in the reaction as outlined in the Scheme 1. Table 1 depicts some conditions of succinimidyl ester formation.

Possible by-products of the reaction (unchanged carboxylic acid, succinimidyl phosphate and the phosphate salt of the base) are removed by base extraction during workup, and therefore the method is especially useful if applied in combination with the following step, usually an amide or ester formation in the same pot. Examples of products are given in Figure 3, conditions of acylations in Table 2.

Table 1. Conditions of Succinimidyl Ester Formation

Comp.	Time (h)	Temp. (°C)	Solvent	Stoichiometry: NHS / Phosphate / Base (Acid=1)	Meth.	Yield (%)	Remarks
5	1	50	Me_2CO	1 / 1.2 / 4.1 ($NaHCO_3$)	A	86	used without further purification
5	1.5	50	AcOEt	1.2 / 1.4 / 4.1 ($NaHCO_3$)	A	80	Acid added as last component
6	1	r.t.	AcOEt	1.05 / 1.05 / 2.5 (NEt_3)	B	94	raw material extracted with *t* BuOMe
6	3	r.t.	AcOEt	1.2 / 1 / 2.5 (NEt_3)	D	60	
7	20	r.t.	Me_2CO	1.1 / 1.2 / 2.5 (NEt_3)	C	91	
7	16	r.t.	MeCN	1.2 / 1.2 / 3.3 ($NaHCO_3$)	A	70	recrystallized from CH_2Cl_2 / heptane
8	23	r.t.	AcOEt	1.1 / 1.2 / 2.5 (NEt_3)	B	76	recrystallized from CH_2Cl_2 / *i* Pr_2O
9	2	50	AcOEt	1.05 / 1.5 / 4.0 ($NaHCO_3$)	A	54	extracted with *t* BuOMe
10	24	r.t.	AcOEt	1.1 / 1.2 / 2.5 (NEt_3)	B	72	recrystallized from *i* Pr_2O
11	24 +2	r.t. 50	Me_2CO	1.1 / 1.2 / 4.0 ($NaHCO_3$)	A	65	extracted with *i* Pr_2O
12	16	45-55	AcOEt	1.1 / 1.2 / 2.5 (NEt_3)	B	70	extracted with *i* PrOH



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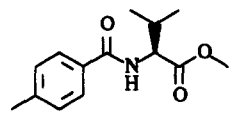

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Figure 3: Products of acylations using succinimidyl esters

Table 2: Conditions of Acylation

Comp.	Time (h)	Temp. (°C)	Solvent	Stoichiometry: Succinimidyl ester / Amino acid / Base	Yield (%)	Remarks
13	16	r.t.	EtOH	1 / 2 / 4 (L-proline / NEt_3)	93	*in situ* transformation into maleate possible
14	1.5 +3.5	r.t 80	AcOEt	1 / 1 / 2 (L-valine methyl ester / NEt_3)	55	one-pot reaction starting from 4-toluic acid

Given the known high efficiency of acylations by succinimide esters, one might think of repetitive procedures of succinimide ester formation and e.g. peptide coupling, using the hydroxy succinimide liberated in the coupling reaction for the next succinimidyl ester formation, thus saving the comparatively high costs of N-hydroxysuccinimide.

EXPERIMENTAL:

General procedures. Melting points were determined on a Reichert Thermovar melting point microscope and are uncorrected. All NMR spectra were recorded on a Bruker AM 300 spectrometer and were referred to TMS. All reagents were obtained from commercial sources and were used as acquired.

Syntheses of succinimidyl esters. Method A. 3.3-4.1 Equivalents of sodium bicarbonate are suspended in solvent. Then one equivalent of acid and 1.0-1.2 equivalents of N-hydroxysuccinimide are added. The reaction mixture is stirred at ambient temperature or 40-50 °C. After addition of 1.0-1.2 equivalents of diphenyl chlorophosphate the mixture is kept at that temperature for 1-24 h. After completion of the reaction the mixture is extracted with sodium bicarbonate solution and washed several times with deionized water. The organic layer is separated and dried over sodium sulfate. Finally the solvent is distilled off in vacuo. If water soluble solvents such as acetone are used, the solvent has to be changed before aqueous workup.

Syntheses of succinimidyl esters. Method B. In general the same as method A. 2.2-2.5 Equivalents of triethylamine are used instead of 3.3-4.1 equivalents of sodium bicarbonate.

Syntheses of succinimidyl esters. Method C. In case of very insoluble succinimide esters like that of quinaldic acid a reaction in acetone and a non-aqueuos workup are preferred. The reaction itself is the same as in method B. 1.0 Equivalents of quinaldic acid and 1.0-1.2 equivalents of N-hydroxysuccinimide are suspended in acetone. After addition of 2.2-2.5 equivalents of triethylamine and 1.0-1.2 equivalents of diphenyl chlorophosphate the reaction mixture is stirred at ambient temperature or 40-50 °C for 1-24 h. After completion of the reaction the acetone is distilled off. The residue is stirred in ethanol for 0.5-1 h, separated by filtration and dried in vacuo.

Syntheses of succinimidyl esters. Method D. In general the same as method B. 1.2 Equivalents of diethyl chlorophosphate are used instead of diphenyl chlorophosphate.

Succinimidyl ester of (S,S)-N-(1-ethoxycarbonyl-3-phenylpropyl)alanine **(5)**. Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.28 (t, 3H), 1.56 (d, 3H), 1.80-2.10 (m, 3H), 2.70-2.80 (m, 2H), 2.80 (s, 4H), 3.46 (dd, 1H), 3.74 (q, 1H), 4.21 (q, 2H), 7.14-7.32 (m, 5H) ppm.

Succinimidyl ester of 4-toluic acid **(6)**. Mp 176-180 °C (dec.); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.42 (s, 3H), 2.87 (s, 4H), 7.44 (d, 2H), 7.97 (d, 2H) ppm.

Succinimidyl ester of quinaldic acid **(7)**. Mp 193-195 °C (dec.); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.97 (s, 4H), 7.85 (t, 1H), 7.99 (t, 1H), 8.21 (d, 1H), 8.27 (d, 2H), 8.75 (d, 1H) ppm.

Succinimidyl ester of N-benzyloxycarbonyl-L-valine **(8)**. Mp 117-120 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0,98 (d, 6H), 2.16 (m, 1H), 2.79 (s, 4H), 4.32 (dd, 1H), 5.05 (s, 2H), 7.32 (m, 5H), 8.00 (d, 1H) ppm.

Succinimidyl ester of N-benzyloxycarbonyl-L-phenylalanine **(9)**. Mp 135-139 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.85 (s, 4H), 3.04 (dd, 1H), 3.26 (dd, 1H), 4.72 (m, 1H), 5.05 (s, 2H), 7.20-7.40 (m, 10H), 7.80 (d, 1H) ppm.

Succinimidyl ester of N-benzyloxycarbonyl-L-aspartic acid β-benzyl ester **(10)**. Mp 82-84 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.83 (s, 4H), 3.11-3.33 (m, 2H), 4.63 (m, 1H), 5.08 (s, 2H), 5.18 (s, 2H), 7.30-7.40 (m, 10H), 8.01 (d, 1H) ppm.

Succinimidyl ester of acrylic acid **(11)**. Mp 63-68 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.86 (s, 4H), 6.34-6.72 (m, 3H) ppm.

(S,S,S)-N-((1-Ethoxycarbonyl-3-phenylpropyl)alanyl)proline **(13)**. 5.65 g (0.015 mol) of the succinimidyl ester of **5** were dissolved in 45 ml ethanol. Within 5 min a solution of 3.4 g (0.029 mol) of L-proline in 5.65 ml of water was added, followed by the addition of 8.3 ml (0.060 mol) triethylamine within 15 min. In the course of this the temperature rose from 22 to 30 °C. The solution was then stirred at room temperature overnight and the ethanol was removed in a rotary evaporator at 40 °C and 20 mbar. The residue was dissolved in 17 ml of water and then extracted two times with 55 ml each and once with 25 ml of methyl tert-butyl ether . The three ether phases were combined and concentrated in a rotary evaporator at 40°C and 20mbar. The aqueous phase of pH 6.3 was adjusted to pH 2.8 using 6 ml of 2M sulfuric acid. 1.7 g of sodium sulfate p.a. (0.7 M based on H_2O) were then added. The mixture was then extracted three times with 80 ml each and twice with 30 ml each of ethyl acetate. The combined ethyl acetate phases were dried using sodium sulfate and concentrated in a rotary evaporator at 40 °C and 20 mbar to yield 5.25 g (92.9%) of **13.** Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$) Spectrum recorded at ambient temperature shows 2 conformers in ratio 2.5:1; δ = 1.25 (t, 3H), 1.36, 1.45 (2d, Σ = 3H), 1.80-2.25 (m, 6H), 2.66-2.81 (m, 2H), 3.40-3.81, 3.98-4.26 (2m, Σ = 6H), 4.42-4.50 (m, 1H), 7.17-7.27 (m, 5H), 8.42 (s, 2H + H_20) ppm.

N-Toluoyl-L-valine methyl ester **(14)**. 2.42 g (0.021 mol) of N-hydroxysuccinimide, 2.72 g (0.021 mol) of 4-toluic acid and 4.05 g (0.040 mol) triethylamine were initially introduced in 20 ml of ethyl acetate and the

suspension was stirred at room temperature. A solution of 5.64 g (0.021 mol) of diphenyl chlorophosphate in 40 ml of ethyl acetate was added dropwise to this suspension in the course of 20 min, the reaction mixture warming to about 35 °C. To complete the reaction, it was stirred at room temperature for a further 1 h. The reaction mixture was then diluted with 50 ml of ethyl acetate and washed with 50 ml each of water, 2N hydrochloric acid, saturated $NaHCO_3$ solution and water again. The organic phase was separated and treated with 4.05 g (0.040 mol) of triethylamine and 2.62 g (0.020 mol) of L-valine methyl ester. The reaction mixture was stirred at room temperature for 1.5 h and then heated under reflux for 3.5 h. After stirring at room temperature overnight, an oily, heavier second phase was separated off and the ethyl acetate phase was washed with 50 ml of each water, saturated $NaHCO_3$ solution and a further three times with 50 ml each of water. After removing the diluent at 50 °C in vacuo, 3.41 g of crude product remained. 3.01 g of this were washed by stirring with 10 ml of methyl tert-butyl ether for 1 h. After filtration and drying of the residue in vacuo at 50 °C, 2.42 g (55%) of white, crystalline **14** were obtained. Mp 98 - 100 °C; ^1H NMR (300 MHz, DMSO-d_6): δ = 0.98-1.05 (m, 6H), 2.19-2.30 (m, 1H), 2.41 (s, 3H), 3.71 (s, 3H), 4.33-4.38 (m, 1H), 7.33 (d, 2H), 7.86 (d, 2H), 8.53 (d, 1H) ppm.

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