Synthesis of Antimicrobial *N*-Phthaloyl-alanyl-derived Amidophosphates and Triazoles

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Dedicated to Professor Richard Neidlein on the occasion of his 77th birthday

N-Phthaloyl-alanylazide reacts smoothly with trialkyl phosphites producing the corresponding α-aminophosphates. With dialkyl hydrogenphosphonates in the presence of benzoyl peroxide, amidophosphates were the isolated products whereas the oxoaziridin-1-yl-phosphonic diamide was preferentially provided from the reaction of the azide with tris(dimethylamino)phosphine. The azide was also allowed to react with α-keto-, α-ethoxycarbonyl- and α-cyanomethylenetriphenylphosphorane to give the corresponding linear disubstituted 1,2,3-triazoles. Screening results of antibiotic potency for the products were discussed in terms of structure-activity relationship (SAR), and an attempt was made to define the structural features for lead compounds.

Key words: α-Aminophosphates, Amidophosphates, Disubstituted 1,2,3-Triazoles, *N*-Phthaloyl-alanylazide

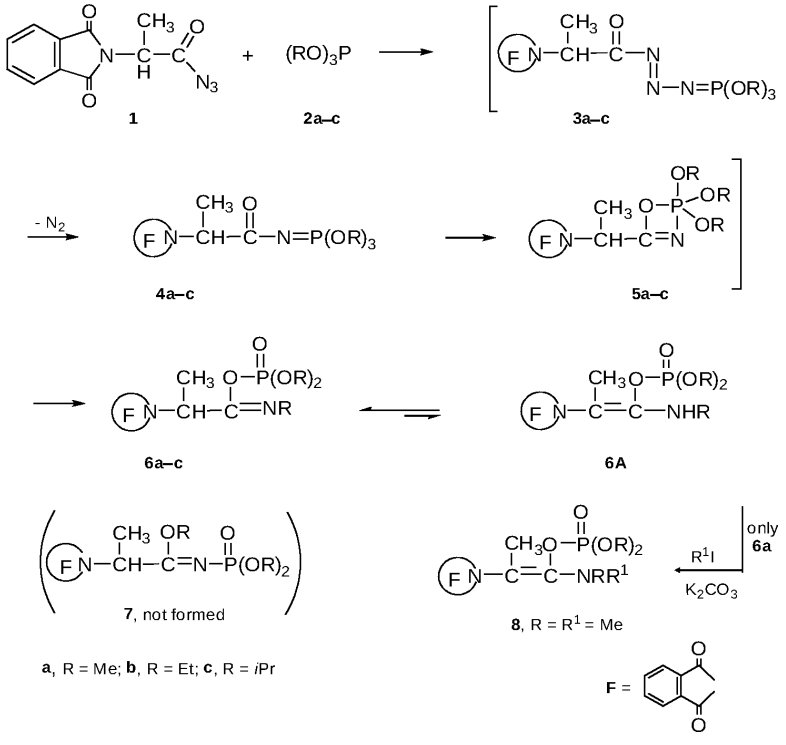
Introduction

Phthalimides constitute a class of compounds which has attracted considerable attention in heterocyclic chemistry [1, 2]. In effect, phthalimide derivatives have demonstrated significant and potential biological activities in agricultural [3, 4] and in medicinal chemistry [5, 6]. With the aim to develop expanded applications of phthalimides, very recently [7] we elaborated an efficient one-pot procedure for the synthesis of α- and β-phosphono-substituted phthalimides with antibiotic activity. The method was based on the reaction of phosphorus(III) reagents with 2-methoxy- and 2-anilino-isoindole-1,3 (2*H*)-dione. In our continuing development of phosphono-substituted phthalimides for application purposes, we now apply trivalent and pentavalent phosphorus reagents to 2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propionylazide (**1**, also known as *N*-phthaloyl-alanylazide). Trialkyl phosphites **2a** – **c**, dialkyl hydrogenphosphonates **12a** – **c** and tris(dimethylamino)phosphine (**16**) were the applied phosphorus(III) reagents whereas triphenylalkylidenephosphoranes **18** and **20a**, **b** were the phosphorus(V) reagents. The reactions studied and the products obtained are depicted in Schemes 1 – 6.

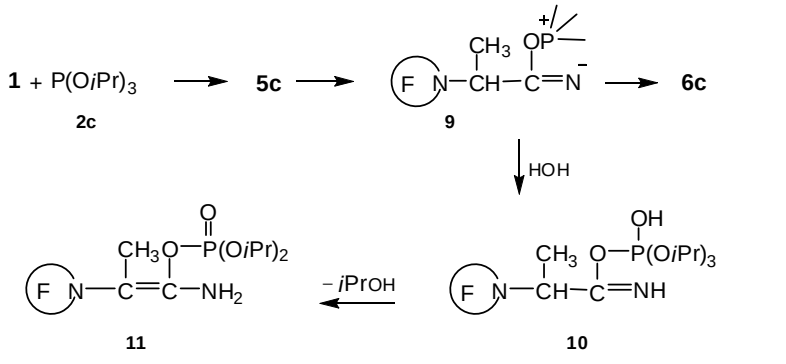
Results and Discussion

*Reaction of N-phthaloyl-alanylazide (**1**) with trialkyl phosphites **2a** – **c***

In the presence of an excess of trimethyl phosphite (**2a**), the reaction with azide **1** led to the formation of the substituted imidoylphosphate **6a** in 72 % yield. Obviously, the mechanism of condensation of the azide **1** with **2a** involved the formation of the Staudinger phosphoranimine intermediate **4a** that arose from the denitrogenation of the initially formed triazenylidene phosphorane **3a** [8, 9]. Subsequent ring closure followed by ring opening [10], and rearrangement (tautomeric conversion) [8] through alkyl group shift led to the phosphate product **6a** (Scheme 1). The composition and the structure of **6a** – **c** are based on the recorded elemental analyses, molecular weight determinations (MS), and spectroscopic data [11, 12]. Compound **6a** showed a ^{31}P NMR chemical shift around δ = 3.8 ppm, which indicates the presence of a P–O linkage in the molecule, and readily eliminates a structure like **7** for which a signal at $\delta \approx 11 - 16$ ppm for P=N (or P–N) would be expected. The IR spectrum of **6a** revealed the absence of the stretching vibration bands at 1708 and 2200 cm^{-1} related to the carbonyl



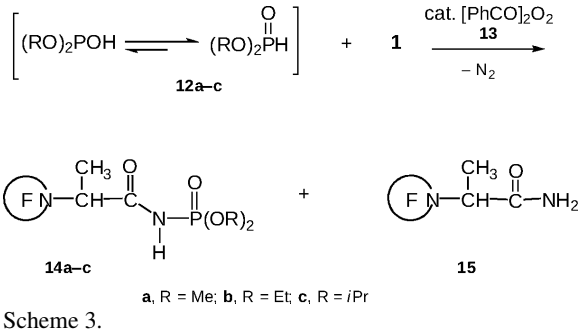
Scheme 1.



Scheme 2.

and the azido group, respectively, present in the IR spectrum of the azide **1**, and showed strong absorption bands at 1580 assigned to the imide (C=N) stretching, and at 1778 and 1728 cm^{-1} assigned to a coupled C=O vibration of cyclic imides. In the ^1H NMR spectrum ($CDCl_3$) of **6a** the exocyclic methine proton, present in the spectrum of **1** at δ = 4.78 ppm (q), was displayed at δ = 5.17 (dq) ppm. The deshielding is clearly due to the phosphorus entity. Although the physical and spectroscopic data indicated that **6** existed in the imino form, the stable amino structure **6A** clearly could not be overlooked. Treatment of **6a** with methyl iodide in acetone, containing potassium carbonate, gave the expected *N,N*-dialkylated product **8** in 80 % yield.

In a similar way, the reaction product of **1** with triethyl phosphite (**2b**) was assigned the analogous structure **6b** (74 % yield). Conversely, treatment of **1** with triisopropyl phosphite (**2c**) afforded a mixture of the expected analog **6c** together with the vinyl phosphate **11** (Scheme 2). This behavior is not unexpected since the bulky isopropyl group would impede the Arbusov reaction. It appears that a partial hydrolysis at the stage of the dipolar intermediate **9** has occurred to give the final product **6c** along with **11** *via* the intermediate **10**. It is known that lengthening or branching the alkyl rad-



Scheme 3.

icals in trialkyl phosphites results in reduction of their migration aptitude [13].

*Reactions of **1** with dialkyl hydrogenphosphonates **12a – c***

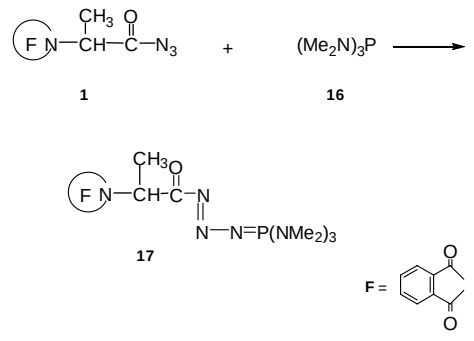
The behavior of **1** toward **12a – c** was studied next, and the products obtained were those depicted in Scheme 3. However, the reaction between **1** and **12** proceeded only when a catalytic amount of benzoyl peroxide (**13**) was present in the medium [15, 16] to yield the respective amido-phosphates **14a – c** along with the known [14] phthaloyl-DL-α-alanylamide (**15**) (Scheme 3). The reaction products **14a – c** were obtained as colorless crystals in $\sim$ 62 % yield. Satisfactory elemental analyses and molecular weight determinations (MS) confirmed structure **14**. ^{31}P NMR signals of **14a – c** were found at $\delta = \sim$ 14 ppm [17]. The ^{1}H NMR spectrum ($CDCl_3$) of **14b** showed among others the NH proton at δ = 6.32 ppm.

*Reaction of **1** with Tris(dimethylamino)phosphine (**16**)*

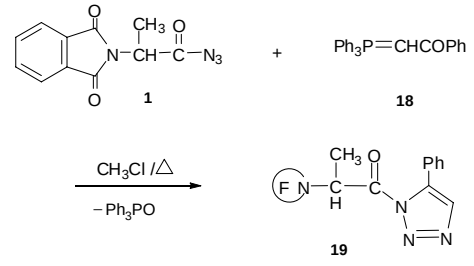
Addition of an excess of tris(dimethylamino)phosphine (**16**) in dry tetrahydrofuran to the carbonyl azide **1** in dry THF at 20 °C led to the precipitation of the triazenylidene-phosphorane **17** (88 % yield). A ^{31}P NMR signal was observed at δ = 40.3 ppm. Correct elemental analysis and expected ^{1}H, ^{13}C NMR data also confirmed the assigned structure (Scheme 4).

*Reactions of N-phthaloyl-alanylazide (**1**) with alkylidenephosphoranes*

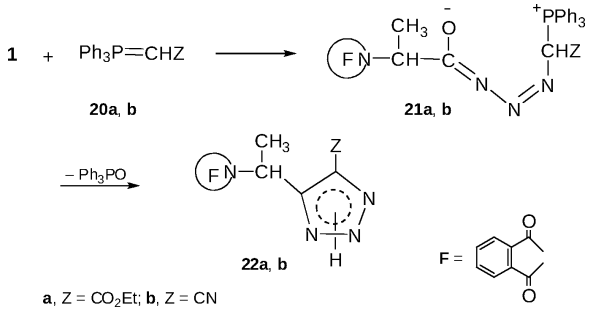
The present study was extended to investigate the application of P(V) reagents namely, alkylidenephosphoranes, to the azide **1**, in order to prepare as yet unknown 1-(*N*-phthaloyl-alanyl)-1,2,3-triazoles. Thus, the reaction of **1** with benzoylmethylenetriphenylphosphorane (**18**) was carried out in boiling chloroform for 36 h to furnish the 1,5-disubstituted 1,2,3-triazole **19** in moderate 42 % yield *via* 1,3-cycloaddition [18 – 20]. Triphenylphosphine oxide was also isolated from the reaction medium. However, when the reaction was carried out in dimethylformamide (DMF) solution, and the mixture was heated in a microwave oven for 20 min, product **19** was obtained in 90 % yield (Scheme 5). The triazole structure **19** was deduced from analytical and spectroscopic data.



Scheme 4.



Scheme 5.



Scheme 6.

The reaction of α-ethoxycarbonyl- (**20a**) and α-cyanomethylenetriphenylphosphorane (**20b**), however, did not proceed in this way, but gave the triazoles **22a** or **22b** in quantitative yields (Scheme 6). Conceivably an addition of the ylides **20a**, **20b** to the terminal nitrogen of the azide takes place (Staudinger reaction), followed by an aza-Wittig reaction to give the

Table 1. Antibacterial activity data of **6a – c**, **14a**, **b**, **19** and **22a**, **b**.

Compound	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
6a	22[a] (5.85)[b]	27 (5.85)	20 (5.85)	23 (5.85)
6b	18 (5.85)	27 (5.85)	23 (5.85)	21 (5.85)
6c	–	15 (12.5)	20 (12.5)	–
14a	21 (5.85)	25 (5.85)	20 (5.85)	25 (5.85)
14b	22 (5.85)	26 (5.85)	24 (5.85)	22 (5.85)
19	19 (8.25)	18 (25)	13 (25)	9 (25)
22a	12 (12.5)	15 (12.5)	12 (25)	19 (8.5)
22b	14 (12.5)	14 (12.5)	12 (12.5)	13 (12)
Standard[c]	22 (5.85)	28 (5.85)	24 (5.85)	25 (5.85)

[a] Indicates the diameter of zone of inhibition; [b] minimum inhibitory concentration values (MIC, μg mL^{-1}), [c] Amoxicillin is used as standard.

Table 2. Antifungal activity data of compounds **6a – c**, **14a**, **b**, **19** and **22a**, **b**.

Comp.	*A. niger*	*C. albicans*	*A. fumigatus*	*P. marneffei*
6a	25[a] (5.85)[b]	23 (5.85)	22 (5.85)	22 (5.85)
6b	22 (8.4)	21 (6.25)	25 (6.25)	25
6c	20 (5.85)	21 (6.25)	23 (8.8)	19 (8.8)
14a	27 (5.85)	23 (5.85)	25 (5.85)	22 (5.85)
14b	28 (5.85)	25 (5.85)	26 (5.85)	28 (5.85)
19	18 (12.5)	12 (25)	–	–
22a	9 (22)	14 (13)	–	–
22b	10 (22)	18 (8.8)	20 (5.85)	10 (12.5)
Standard[c]	25 (5.85)	21 (5.85)	23 (5.85)	22 (5.85)

[a] Indicates the diameter of zone of inhibition; [b] minimum inhibitory concentration values (MIC, μg mL^{-1}); [c] Fluconazole is used as standard.

4,5-disubstituted triazoles **22a**, **b** *via* the intermediates **21a**, **b**, and extrusion of TPPO.

Formulas **22a** and **22b** are consistent with analytical and spectroscopic data. It is noteworthy that the position of the N-H hydrogen atom in the *N*-unsubstituted triazoles such as structure **22** has been the subject of a contradictory discussion [21] (Scheme 6).

Pharmacological screening

Antibacterial activity

A selection of the newly prepared compounds were screened for their antibacterial activity against *Escherichia coli*, *Staphylococus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (recultured) bacterial strains by the disc diffusion method [22, 23]. The antibacterial screening data shown in Table 1 indicate that compounds **6a**, **6b**, **14a**, and **14b** exhibit good antibacterial activity against all tested bacterial strains almost equivalent to that of the standard drug Amoxicilline.

Antifungal activity

Selected compounds were screened for their antifungal activity against *Aspergillus niger*, *Candida albicans*, *Aspergillus fumigatus*, and *Pencillium marneffei* (recultured) by the agar diffusion method [23]. The diameter of zone of inhibition and minimum inhibitory concentration values are given in Table 2. The results revealed that compounds **6a**, **14a** and **14b** show significant antifungal activity. Other tested compounds, however, showed moderate activity as compared to that of the standard drug Fluconazole.

Summary and Conclusion

To summarize, we have reported an efficient access to a series of phthalimidoyl-phosphorus derivatives and linear disubstituted *vic*-triazoles that may complement those existing in the literature. This sequence included a number of α-amino-phosphate, amidophosphate and triazenylphosphine derivatives from the reactions of the carbonyl azide **1** with different types of P(III) reagents. In the second part, disubstituted triazole derivatives were obtained from the reactions of **1** with phosphorus ylides. The latter reactions fit into the general class of concerted 1,3-dipolar reactions of azides to dipolarophiles [18 – 20]. Furthermore, the antimicrobial screening studies revealed that compounds with a phosphorus moiety (phosphate, phosphazane, or phosphoryl diamide) showed excellent activity. Finally, the results of the present investigation reflect the versatility of the phosphorus reagents and of the azido group as well.

Experimental Section

General remarks

Melting points were measured on an Electrothermal melting point apparatus. The IR spectra were recorded on a Perkin Elmer 317 Grating IR spectrophotometer, using KBr pellets. The ^{1}H and ^{13}C NMR spectra were measured on a Jeol E.C.A. 500 MHz instrument using $SiMe_4$ as internal standard. The ^{31}P NMR spectra were recorded with the same instrument, relative to external H_3PO_4 (85 %). The mass spectra were performed on a Jeol JMS-A X 500 spectrometer. Elemental analyses were carried out at the Microanalysis Laboratory, Cairo University, Cairo, Egypt. All reactions were performed under argon. The appropriate precautions in handling moisture-sensitive compounds were considered. Solvents were dried by standard techniques. TLC: Merck 0.2 mm silica gel 60 F154 aluminum plates. Column chromatography (CC): silica gel (silica gel 60 mesh, particle size 0.2 – 0.5 mm; E. Merck, Darmstadt). The substrate 2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propionyl-azide (**1**) was prepared according to the reported method [24].

*General procedure for the reaction of **1** with trialkyl phosphites **2a – c***

A mixture of *N*-phthaloyl-alanylazide (**1**) (0.7 g, 2.87 mmol) and a trialkyl phosphite **2a – c** (4 mL) was heated at 100 °C, in absence of a solvent for ≈ 15 h (TLC). The excess of the phosphite was removed under vacuum, and then the residue was washed several times with light petroleum (40 – 60 °C), and crystallized from the proper solvent to give compounds **6a**, **6b** or **6c** and **11**, respectively.

*(1Z)-2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N-methylpropanimidoyl dimethyl phosphate (**6a**)*

Straw-yellow prisms. Yield: 700 mg (72 %). M. p. 153 – 155 °C (from acetone/ether). – IR (film): ν = 1778, 1728 (C(1,3)=O), 1580 (C=N), 1256 (P=O), 1044 (P-O-C) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.55 (d, J = 6.8 Hz, 3 H, C-Me), 3.18 (s, 3 H, N-Me), 3.79 (d, ^3J = 12.4 Hz, 6 H, POMe), 5.17 (m dq, 1 H, *H*CMe), 7.76, 7.88 (2 × d, J = 4.4 Hz, 4 H, H-Ph). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 14.6 (*Me*-C), 41.6 (d, ^3J = 10.5 Hz, *C*HMe), 42.4 (NMe), 54.3 (d, ^2J = 12.8 Hz, MeOP), 124.3, 134.6, 135.3 (all C-Ph), 165.5 (C(1,3)=O), 174.6 (d, ^2J = 18.4 Hz, C=N). – ^{31}P NMR (500 MHz, $CDCl_3$, H_3PO_4): δ = 3.8 ppm. – MS (EI, 70 eV): *m/z* (%) = 340 (22) $[M]^+$, 325 (9), 310 (31), 295 (44), 280 (100), 185 (30), 171 (83), 146 (80), 110 (54), 104 (41), 77 (56). – $C_{14}H_{17}N_2O_6P$ (340.3): calcd. C 49.4, H 5.0, N 8.2, P 9.1; found C 49.5, H 4.9, N 8.1, P 9.2.

*(1Z)-2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N-ethylpropanimidoyl diethyl phosphate (**6b**)*

Straw-yellow needles. Yield: 810 mg, 74 %. M. p. 135 – 137 °C (from CH_2Cl_2). – IR (film): ν = 1782, 1720 (C(1,3)=O), 1587 (C=N), 1265 (P=O), 1100 (P-O-C) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.88 (t, J = 6.6 Hz, 3 H, H_3C-CN), 1.22 (dt, J = 6.6 Hz, ^4J = 4.8 Hz, 6 H, H_3C-COP), 1.57 (d, J = 6.8 Hz, 3 H, H_3C-C), 3.65 (q, J = 6.6 Hz, H_2CN), 4.08 (dq, J = 6.6 Hz, ^3J = 5.2 Hz, 4 H, H_2COP), 5.17 (dq, J = 6.8 Hz, ^4J = 4.2 Hz, 1 H, *H*CMe), 7.68, 7.78 (2 × d, J = 7.4 Hz, 4 H, H-Ar). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 15.5 (*C*H_3CN), 15.8 (*C*H_3CH), 16.3 (*C*H_3COP), 38.4 (CH_2N), 42.6 (d, ^3J = 8.4 Hz, *C*H-Me), 64.7 (d, ^2J = 16.8 Hz, CH_2OP), 124.2, 133.4, 135.7 (all C-Ar), 166.5 (C(1,3)=O), 173.4 (d, ^2J = 18.5 Hz, C=N). – ^{31}P NMR (500 MHz, $CDCl_3$, H_3PO_4): δ = −2.4 ppm. – MS (EI, 70 eV): *m/z* (%) = 382 (9) $[M]^+$, 338 (18), 309 (31), 280 (100), 185 (16), 174 (96), 146 (90), 110 (52), 104 (28), 77 (54). – $C_{17}H_{23}N_2O_6P$ (382.4): calcd. C 53.4, H 6.0, N 7.3, P 8.1; found C 53.3, H 6.1, N 7.2, P 8.2.

Compounds **6c** and **11** were fractionally crystallized from the reaction of **1** with **2c**:

*(1Z)-2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N-isopropylpropanimidoyl diisopropyl phosphate (**6c**)*

Yellow crystals. Yield: 400 mg, 36 %. M. p. 161 – 162 °C (from MeCN). – IR (film): ν = 1774, 1724 (C(1,3)=O), 1575 (C=N), 1262 (P=O), 1088 (P-O-C) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.96 (d, J = 7.2 Hz, 6 H, *iso*-$(H_3C)_2$-C-N), 1.28, 1.13 (2 × dd, J = 6.6 Hz, ^{4} J= 5.7 Hz, 12 H, *iso*-$(H_3C)_2$-COP), 1.57 (d, J = 6.8 Hz, 3 H, H_3C-C), 3.87 (sept, J = 7.2 Hz, 1 H, HC-N), 4.12 (d sept, ^3J = 13.2 Hz, 2 2 H, HCOP), 4.94 (dq, J = 6.6 Hz, 1 H, *H*C-Me), 7.76, 7.88 (2 × d, J = 4.4 Hz, 4 H, H-Ar). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 15.8 (*C*H_3C), 23.7 (*C*H_3C-N), 24.5 (*C*H_3COP), 42.6 (d, ^3J = 14.8 Hz, *C*H-Me), 43.5 (CHN), 72.3 (d, ^2J = 26.8 Hz, CHOP), 124.2, 133.4, 135.7 (all C-Ph), 165.8 (C(1,3)=O), 170.4 (d, ^2J = 16.8 Hz, C=N). – ^{31}P NMR (500 MHz, $CDCl_3$, H_3PO_4): δ = 4.3 ppm. – MS (EI, 70 eV): *m/z* (%) = 425 (48) $[M+1]^+$, 366 (13), 323 (10), 281 (34), 257 (100), 174 (28), 147 (33), 111 (24), 104 (14), 76 (15). – $C_{20}H_{29}N_2O_6P$ (424.4): calcd. C 56.6, H 6.8, N 6.6, P 7.3; found C 56.7, H 6.9, N 6.5, P 7.2.

*(1E)-1-Amino-2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-prop-1-en-1-yl diisopropyl phosphate (**11**)*

Yellow crystals. Yield: 360 mg (33 %). M. p. 161 – 162 °C (from MeCN). – IR (film): ν = 3355 – 3173 (NH_2), 1782, 1730 (C(1,3)=O), 1613 (C=C), 1228 (P=O, bonded), 1080 (P-O-C) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.28, 1.34 (2 × dd, J = 6.6 Hz, ^4J = 5.5 Hz, 12 H, *iso*-$(H_3C)_2$-COP), 2.37 (s, 3 H, H_3C-C), 4.42 (d-sept, ^3J = 12.9 Hz, 2 H, HCOP), 6.13, 6.34 (br 2s, 2 × 1H, H_2N), 7.76, 7.88 (2 × d, J = 4.4 Hz, 4 H, H-Ar). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 22.7 (*C*H_3C=), 24.8 (*iso*-(*C*H_3)$_2$COP), 72.7 (d, ^2J = 28.6 Hz, HCOP), 100.4 (d, ^3J = 12.6 Hz, *C*(Me)=C), 125.6, 130.4, 133.2 (all C-Ar), 165.8 (C(1,3)=O), 182.4 (d, ^2J = 28.6 Hz, =COP). – ^{31}P NMR (500 MHz, $CDCl_3$, H_3PO_4): δ = 4.3 ppm. – MS (EI, 70 eV): *m/z* (%) = 381 (17) $[M–1]^+$, 380 (20) $[M–2]^+$, 366 (19), 323 (21), 281 (43), 257 (100), 174 (58), 147 (33), 111 (20), 104 (34), 76 (42). – $C_{17}H_{23}N_2O_6P$ (382.4): calcd. C 53.4, H 6.0, N 7.3, P 8.1; found C 53.5, H 6.1, N 7.2, P 8.0.

*Methylation of the phosphate **6a***

A mixture of the phosphate **6a** (0.5 g, 1.47 mmol), methyl iodide (3.0 mL), and anhyd. K_2CO_3 (2 g) in dry acetone (50 mL) was refluxed for 2 h and then filtered while hot. After evaporation of the volatile materials in vacuum, the residue was triturated with light petroleum ether, and then allowed to cool in an ice-chest. The solid obtained was recrystallized from aqueous ethanol to give **8**.

*(1E)-1(Dimethylamino)-2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)prop-1-en-1-yl dimethyl phosphate (**8**)*

Colorless needles. Yield: 416 mg (80 %). M. p. 118 – 120 °C. – IR (film): ν = 1778, 1728 (C(1,3)=O), 1622 (C=C, exocyclic), 1268 (P=O), 1050 (P-O-C) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.57 (s, 3 H, H_3CC), 2.98, 3.01 (2 × s, 2 × 3 H, $(H_3C)_2N$), 3.85 (d, ^3J = 12.5 Hz, 6 H, H_3COP), 7.77, 7.90 (2 × d, J = 4.4 Hz, 4 H, H-Ar). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 12.3 (CH_3C), 41.6, 41.8 ($N(CH_3)_2$), 54.3 (d, ^2J = 8.3 Hz, CH_3OP), 93.8 (d, ^3J = 10.3 Hz, *C*-Me), 124.3, 131.6, 133.3 (all C-Ar), 162.5 (C(1,3)=O), 166.2 (d, ^2J = 28.3 Hz, =COP). – ^{31}P NMR (500 MHz, $CDCl_3$, H_3PO_4): δ = 4.8 ppm. – MS (EI, 70 eV): *m/z* (%) = 354 (15) $[M]^+$, 325 (21), 310 (23), 295 (46), 280 (100), 185 (29), 171 (81), 146 (82), 116 (54), 104 (40), 77 (52). – $C_{15}H_{19}N_2O_6P$ (354.3): calcd. C 50.8, H 5.4, N 7.9, P 8.7; found C 50.7, H 5.5, N 7.8, P 8.8.

*General procedure for the reaction of **1** with dialkyl hydrogenphosphonates **12a** – **c***

A mixture of **1** (0.8 g, 3.28 mmol) and 6 mmol of dimethyl (**12a**), diethyl (**12b**) or diisopropyl phosphonate (**12c**) was refluxed in benzene (20 mL) containing a catalytic amount of benzoyl peroxide (**13**) for ≈ 4 h (TLC). After cooling, the colorless material that precipitated was collected, crystallized, and identified as phthaloyl-DL-α-alanylamide (**15**, 12 %). M. p. 214 – 216 °C (from ethanol) (lit. [14]: 214 – 215.5 °C). The filtrate was evaporated under reduced pressure, and then the residue was crystallized from the proper solvent to give the corresponding amidophosphates **14a**, **14b** or **14c**.

*Dimethyl [2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-propanoyl]amidophosphate (**14a**)*

Colorless crystals. Yield: 620 mg (58 %). M. p. 164 – 167 °C (from acetone). – IR (film): ν = 3320 (NH), 1777, 1732 (C(1,3)=O), 1708 (C=O, amide), 1232 (P=O, bonded), 1048 (POC), 978 (N-P) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.55 (d, J = 6.8 Hz, 3 H, H_3C-C), 3.79 (d, ^3J = 12.4 Hz, 6 H, H_3COP), 5.17 (q, J = 6.8 Hz, 1 H, *H*CMe), 6.28 (br, 1 H, HN), 7.76, 7.88 (2 × d, J = 4.4 Hz, 4 H, H-Ar). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 17.6 (CH_3-C), 52.3 (d, ^2J = 21.0 Hz, CH_3OP), 54.3 (d, ^3J = 12.3 Hz, *C*H-C), 123.7, 134.5, 135.3 (all C-Ar), 164.5 (d, ^2J = 10.8 Hz, C=O, amide), 168.4 (C(1,3)=O). – ^{31}P NMR (500 MHz, $CDCl_3$, H_3PO_4): δ = 14.3 ppm. – MS (EI, 70 eV): *m/z* (%) = 325 (18) $[M–1]^+$, 311 (31), 281 (44), 202 (68), 174 (100), 146 (80), 110 (54), 104 (49) 77 (33). – $C_{13}H_{15}N_2O_6P$ (326.2): calcd. C 47.8, H 4.6, N 8.6, P 9.5; found C 47.7, H 4.7, N 8.7, P 9.4.

*Diethyl [2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-propanoyl]amidophosphate (**14b**)*

Colorless needles. Yield: 720 mg (62 %). M. p. 155 – 157 °C (from CH_2Cl_2). – IR (film): ν = 3280 (NH), 1780, 1730 (C(1,3)=O), 1700 (C=O, amide), 1224 (P=O, bonded), 1077 (POC), 970 (N-P) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.22 (dt, J = 6.6 Hz, ^4J = 4.8 Hz, 6 H, H_3C-COP), 1.57 (d, J = 6.8 Hz, 3 H, H_3C-C), 4.08 (dq, J = 6.6 Hz, ^3J = 5.2 Hz, 4 H, H_2COP), 5.17 (dq, J = 6.8 Hz, ^4J = 4.2 Hz, 1 H, *H*C-Me), 6.32 (br, 1 H, HN), 7.68, 7.78 (2 × d, J = 7.4 Hz, 4 H, H-Ar). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 15.6 (CH_3COP), 17.8 (CH_3C), 54.3 (*C*H-Me), 60.6 (d, ^2J = 16.8 Hz, CH_2OP), 123.8, 133.4, 134.7 (all C-Ar), 164.5 (d, ^2J = 24.2 Hz, C=O, amide), 166.7 (C(1,3)=O). – ^{31}P NMR (500 MHz, $CDCl_3$, H_3PO_4): δ = 14.6 ppm. – MS (EI, 70 eV): *m/z* (%) = 353 (10) $[M–1]^+$, 339 (15), 311 (11), 202 (42), 174 (100), 146 (58), 110 (37), 104 (55), 77 (43). – $C_{15}H_{19}N_2O_6P$ (354.3): calcd. C 50.8, H 5.4, N 7.9, P 8.7; found C 50.7, H 5.5, N 7.8, P 8.8.

*Diisopropyl [2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-propanoyl]amidophosphate (**14c**)*

Colorless crystals. Yield: 800 mg (64 %). M. p. 176 – 178 °C (from EtOH). – IR (film): ν = 3342 (NH), 1782, 1728 (C(1,3)=O), 1705 (C=O, amide), 1235 (P=O, bonded), 1105 (POC), 974 (N-P) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.98, 1.14 (2 × dd, J = 6.6 Hz, ^4J = 5.4 Hz, 12 H, *iso*-$(H_3C)_2$-COP), 1.57 (d, J = 6.8 Hz, 3 H, H_3C-C), 4.12 (d-sept, J = 6.6 Hz, ^3J = 8.9 Hz, 2 H, HCOP), 4.94 (q, J = 6.6 Hz, 1 H, *H*C-Me), 6.24 (br, 1 H, HN), 7.67, 7.78 (2 × d, J = 4.4 Hz, 4 H, H-Ar). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 12.8 (CH_3COP), 17.8 (CH_3-C), 54.7 (*C*H-Me), 69.8 (d, ^2J = 11.8 Hz, CHOP), 123.2, 133.4, 135.7 (all C-Ar), 164.5 (d, ^2J = 22.2, C=O, amide), 166.4 (C(1,3)=O). – ^{31}P NMR (500 MHz, $CDCl_3$, H_3PO_4): δ = 14.3 ppm. – MS (EI, 70 eV): *m/z* (%) = 381 (22) $[M–1]^+$, 367 (18), 334 (36), 306 (18), 263 (14), 202 (48), 174 (100), 146 (55), 110 (23), 104 (44), 77 (43). – $C_{17}H_{23}N_2O_6P$ (382.4): calcd. C 53.4, H 6.0, N 7.3, P 8.1; found C 53.3, H 6.1, N 7.2, P 8.2.

*Reaction of **1** with tris(dimethylamino)phosphine (**16**)*

Trisaminophosphine **16** (6 mL, 4.5 mmol) in super-dry tetrahydrofuran (THF) (5 mL) was added dropwise to the carbonyl azide **1** (0.5 g, 2.1 mmol) in THF (5 mL), and the reaction mixture was stirred at r. t. for ≈ 10 h (TLC). After cooling, the precipitated material was collected and dried to give compound **17**.

*3-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)propanoyl]-2-triazenylidene-tris-(dimethylamino)phosphorane (**17**)*

Pale yellow material (stable for *ca.* two weeks in a refrigerator). Yield: (88 %). M. p. 208 – 211 °C. – IR (film): ν =

1774, 1730 (C(1,3)=O), 1696 (C=O), 1355 (P=N), 1335, 860 [P(N(Me_2)$_3$] cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.55 (d, J = 4.8 Hz, 3 H, H_3C-C), 2.45, 2.63 (2d, J =10.8 Hz, 18 H, $(H_3C)_2$N-P), 5.98 (q, J = 4.8 Hz, 1 H, HC-C), 7.77, 7.85 (2d, J = 4.8 Hz, 4 H, H-Ar). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 16.8 (CH_3-C), 37.4 (d, ^2J = 30.6 Hz, {$(CH_3)_2N$}$_3$P], 44.4 (*C*-Me), 124.3, 130.4, 134.7 (all C-Ar), 150,4, 166.4 (C(1,3)=O), 179.8 (C=O). – ^{31}P NMR (500 MHz, $CDCl_3$, H_3PO_4): δ = 40.3 ppm. – MS (EI, 70 eV): *m/z* (%) = 407 (15) $[M]^+$, 392 (25) [M–Me]$^+$, 379 (56) [M–N_2 or M–CO]$^+$, 191 (36) $[C_6H_{18}N_5P]^+$, 173 (100) $[M–C_8H_{21}N_6OP]^+$, 146 (82) $[M–C_9H_{22}N_6OP]^+$, 116 (35), 104 (23), 77 (56). – $C_{17}H_{26}N_7O_3P$ (407.4): calcd. C 50.1, H 6.4, N 24.1, P 7.6; found C 50.2, H 6.3, N 24.2, P 7.5.

*Reaction of **1** with alkylidenephosphoranes*

Azide **1** (0.8 g, 3.28 mmol) and benzoylmethylenetriphenylphosphorane (**18**) (1.3 g, 3.5 mmol) in dry chloroform (20 mL) were boiled under reflux for 36 h. After removing the solvent, the residue was chromatographed on silica gel to afford the triazole **19** (42 %). Next, the azide **1** (0.8 g, 3.28 mmol) and 3.5 mmol of benzoyl- (**18**), ethoxycarbonyl- (**20a**) or cyanomethylenetriphenylphosphorane (**20b**) in dry dimethylformamide (DMF) (8 mL) in a Pyrex glass beaker were heated under microwave irradiation for 20 – 25 min. After the completion of the reaction (TLC analysis), the volatile materials were evaporated under reduced pressure to dryness. The resulting residue was chromatographed on silica gel (*n*-hexane/AcOEt) to give the triazoles **19**, **22a** and **22b**, respectively. Triphenylphosphine oxide was also isolated from the above four reactions, eluent: *n*-hexane/AcOEt (2 : 3, v/v).

*2-[1-Methyl-2-oxo-2-(5-phenyl-1H-1,2,3-triazol-1-yl)ethyl]-1H-isoindol-1,3(2H)-dione (**19**)*

Eluent: *n*-hexane/AcOEt (1 : 1, v/v). Yield: 1.0 g (90 %). M. p. 200 – 202 °C (from $CHCl_3$). – IR (film): ν = 1782, 1732 (C(1,3)=O), 1705 (C=O, amide), 1180 (triazole) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.83 (d, J = 6.6 Hz, 3 H, H_3C-C), 5.83 (q, J = 6.6 Hz, 1 H, HC-C), 6.18 (s, 1H, C(4)H-triazole), 7.51 – 7.88, 8.05 – 8.29 (2 × m, 9 H, H-Ar, H-Ph). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 17.9 (CH_3CH), 56.8 (*C*H-Me), 122.3, 123.6, 124.3, 125.4, 131.4, 133.7, 134.3, 134.6 (all C-Ar, C-Ph), 137.2 (C(4), triazole), 163.8 (C=O), 166.2 (C(1,3)=O). – MS (EI, 70 eV): *m/z* (%) = 346 (18) $[M]^+$, 345 (14), 331 (33), 318 (26), 303 (41), 146 (48), 116 (84), 104 (52), 77 (36). – $C_{19}H_{14}N_4O_3$ (346.3): calcd. C 65.9, H 4.1, N 16.1; found C 65.8, H 4.2, N 16.0.

*Ethyl 5-[1-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-2H-1,2,3-triazole-4-carboxylate (**22a**)*

Eluent: *n*-hexane/AcOEt (1 : 1, v/v). Yield: 900 mg (88 %). M. p. 168 – 170 °C (from CH_2Cl_2). – IR (film): ν = 3452 (NH), 1754, 1720 (C(1,3)=O), 1718 (C=O, ester), 1182 (triazole) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.23 (t, J = 7.4 Hz, 3 H, H_3CCO), 1.83 (d, J = 6.6 Hz, 3 H, H_3CC), 3.34 (q, J = 7.4 Hz, 2 H, H_2CO), 6.14 (q, J = 6.6 Hz, 1 H, HC-C), 7.86, 7.95 (2 × d, J = 4.8 Hz, 4 H, H-Ar), 8.48 (s, 1H, HN). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 13.4 (CH_3C), 14.6 (CH_3CO), 46.8 (*C*H-Me), 60.7 (CH_2O), 125.4, 134.4, 135.7 (all C-Ar), 138.2 (C(4), triazole), 151.1 (C(5)-triazole), 163.8 (C=O), 164.2 (C(1,3)=O). – MS (EI, 70 eV): *m/z* (%) = 314 (10) $[M]^+$, 313 (13), 299 (13), 285 (18), 270 (36), 242 (100), 146 (76), 116 (54), 104 (40), 77 (46). – $C_{15}H_{14}N_4O_4$ (314.3): calcd. C 57.3, H 4.5, N 17.8; found C 57.4, H 4.4, N 17.8.

*5-[1-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-2H-1,2,3-triazole-4-carbonitrile (**22b**)*

Eluent: *n*-hexane/AcOEt (1 : 1, v/v). Yield: 740 mg (85 %). M. p. 193 – 195 °C (from MeCN). – IR (film): ν = 3452 (NH), 2218 (CN), 1756, 1732 (C(1,3)=O), 1186 (triazole) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.82 (d, J = 6.6 Hz, 3 H, H_3C-C), 5.24 (q, J = 6.6 Hz, 1 H, HCMe), 7.98, 8.08 (2 × d, J = 4.8 Hz, 4 H, H-Ar), 8.67 (s, 1H, HN). – ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 14.7 (CH_3CH), 46.8 (*C*H-Me), 108.7 (*C*-CN), 124.3 (CN), 124.3, 131.4, 133.7, 134.6 (all C-Ar), 137.2 (C(5), triazole), 166.2 (C(1,3)=O). – MS (EI, 70 eV): *m/z* (%) = 267 (36) $[M]^+$, 266 (32), 251 (29), 241 (31), 225 (45), 147 (100), 146 (88), 116 (68), 104 (36), 77 (25). – $C_{13}H_9N_5O_2$ (267.2): calcd. C 58.4, H 3.4, N 26.2; found C 58.3, H 3.5, N 26.1.

Antibacterial assay

The prepared compounds were screened for their activity against four bacterial strains by the disc diffusion method. A standard inoculum ($1 – 2 \times 10^7$ c. f. u. mL 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 8.32 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile disc previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37 °C. The inhibition zones were measured and compared with the controls. The minimum inhibitory concentration (MIC) was determined by the broth dilution technique. The nutrient broth, which contained a logarithmic serially twofold diluted amount of test compound and controls were inoculated with approximately 5×10^5 c. f. u. mL of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C, and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was re-

garded as minimum inhibitory concentration (MIC). Amoxicillin was used as the standard drug.

Antifungal assay

The prepared compounds were screened for their activity against four fungal strains strains by the agar diffusion method. *Sabbouraud* agar media were prepared by dissolving 1 g peptone, 4 g D-glucose, and 2 g agar in 100 mL distilled water, and adjusting the pH to 5.7 using buffer. Normal saline was used to make a suspension of spores of the fungal strain for lawning. A loopful of a particular fungal strain was transferred to 3 mL saline to get a suspension of the corresponding species. 20 mL of the agar medium was poured into each Petri dish. Excess of suspension was decanted, and the plates were dried by placing them in an incubator at 37 °C. Using an agar punch, wells were made, and each well was labeled. A control was also prepared in four wells and maintained at 37 °C for 3 – 4 days. The inhibition zone diameters were measured and compared with those of the controls. The nutrient broth, which contained logarithmic serially twofold diluted amounts of the tested compound and control were inoculated with approximately $1.6 - 6 \times 10^4$ c. f. u. mL of the activity fungal strains. The cultures were incubated for 48 h at 35 °C, and the growth was monitored visually. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as the minimum inhibitory concentration (MIC). Fluconazol was used as the standard drug.

[1] M. B. Winstead, H. W. Heine, *J. Am. Chem. Soc*. **1955**, *77*, 1913 – 1914; A. F. Fahmy, *Arkivoc* **2006**, *7*, 395 – 415; M. K. Hargreaves, J. G. Pritchard, H. R. Dave, *Chem. Rev*. **1970**, *70*, 439 – 469; Y. J. Chun, J. H. Park, G. M. Oh, S. I. Hong, Y. J. Kim, *Synthesis* **1994**, 909 – 910; P. G. Baraldi, M. Guarneri, F. Moroder, G. P. Pollini, D. Simoni, *Synthesis* **1982**, 653 – 655.

[2] M. N. J. Khan, *J. Org. Chem*. **1996**, *61*, 8063 – 8068.

[3] C. Fest, K. J. Schmidt, *The Chemistry of Organophosphorus Pesticides*, Springer-Verlag, Berlin, **1973**.

[4] N. N. Melnikov in *Chemistry of Pesticides*, (eds.: F. A. Gunther, J. D. Gunther), Springer-Verlag, New York, **1971**.

[5] D. P. A. Laboratori Baldacci, **1984**, *Jpn. Pat*. JP 59 46,268; *Chem. Abstr*. **1984**, *101*, 54922.

[6] E. Valencia, I. Weiss, S. Fidous, A. J. Freyer, M. Shamma, A. Urzua, V. Fayardo, *Tetrahedron* **1984**, *40*, 3957 – 4962; H. A. Priestap, *Phytochemistry* **1985**, *24/44*, 849 – 852.

[7] W. M. Abdou, M. A. I. Salem, R. E. Khidre, *Eur. J. Med. Chem*. **2009**, submitted.

[8] Y. G. Gololobov, I. N. Zhmurova, L. F. Kasukhin, *Tetrahedron* **1981**, *17*, 437 – 472; B. C. Challis, J. A. Challis, J. N. Iley, *J. Chem. Soc., Perkin Trans 2* **1978**, 813 – 818; M. I. Kabachnik, V. A. Gilyarov, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk* **1961**, 816 – 818.

[9] N. K. El-Din, *Phosphorus, Sulfur and Silicon* **1995**, *102*, 25 – 29; L. S. Boulos, N. K. El-Din, *Tetrahedron* **1993**, *49*, 3871 – 3878.

[10] E. P. Kyba, D. C. Alexander, *J. Chem. Soc., Chem. Comm*. **1977**, 934 – 935; J. Vicente, M. T. Chicote, J. Fernandez-Baeza, F. J. Lahoz, J. A. Lopez, *J. Inorg. Chem*. **1991**, *30*, 3617 – 3620; E. Ciganek, *J. Org. Chem*. **1970**, *35*, 3631 – 3636; M. Yamakawa, T. Kubota, Y. Terui, T. Honma, Y. Tada, *Bull. Chem. Soc. Jpn*. **1978**, *51*, 3059 – 3060.

[11] R. M. Silverstein, F. X. W. Webster, D. J. Kiemle, *Spectrometric Identification of Organic Compounds* (7th ed.), John Wiley & Sons, New York **2005**.

[12] G. C. Levy, R. L. Lichter, G. L. Nelson, *Carbon-13 Nuclear Magnetic Resonance Spectroscopy* (2nd ed.), Wiley Interscience, New York **1980**.

[13] V. A. Gilyarov, R. V. Kudryavtsev, M. I. Kabachnik, *Zh. Obshch. Khim*. **1966**, *36*, 708 – 715.

[14] I. Fratta, *US* 66-545540 19660427, **1969**.

[15] F. Ramirez, S. Dershowitz, *J. Org. Chem*. **1957**, *22*, 1282 – 1283; F. Ramirez, N. McKelvie, *J. Am. Chem. Soc*. **1957**, *79*, 5829 – 5830; F. Ramirez, S. Dershowitz, *J. Am. Chem. Soc*. **1956**, *78*, 5614 – 5622.

[16] R. L. McConnell, H. W. Coover, Jr., *J. Am. Chem. Soc*. **1957**, *79*, 1961 – 1969; R. F. Moore, W. A. Waters, *J. Chem. Soc*. **1953**, 238 – 240.

[17] M. M. Crutchfield, O. H. Dungan, J. H. Letcher, V. Mark, J. R. Van Wazer, in *Topics in Phosphorus Chemistry*, Vol. 5 (eds.: M. Grayson and E. J. Griffith), Wiley Interscience, New York, **1967**, chapter 4.

[18] V. Cesare, T. M. Lyons, I. Lengyel, *Synthesis* **2002**, 1716 – 1720; J. Elguero, R. Jacquier, *Tetrahedron Lett*. **1969**, 495 – 498; J. I. Cadogan, N. J. Stewart, N. J. Tweddle, *J. Chem. Soc., Chem. Comm*. **1978**, 182 – 183.

[19] P. Ykman, G. L'Abbe, G. Smets, *Tetrahedron* **1971**, *27*, 5623 – 5629; P. Ykman, G. L'Abbe, G. Smets, *Tetrahedron Lett*. **1970**, 5225 – 5228; P. H. Lambert, M. Vaultier, R. Carrié, *J. Org. Chem*. **1985**, *50*, 5352 – 5356; L. S. Boulos, N. K. El-din, *Tetrahedron* **1993**, *49*, 3871 – 3878.

[20] P. Ykman, G. L'Abbe, G. Smets, *Tetrahedron* **1971**, *27*, 845 – 849.

[21] L. Birkofer, P. Wegner, *Chem. Ber*. **1967**, *100*, 3485 – 3494.

[22] A. H. Collins, *Microbiological Methods* (2nd ed.), Butterworth, London, **1976**.

[23] Z. K. Khan, Proc. Int. Workshop UNIDO-CDRI, *in vitro* and *in vivo* Screening Techniques for Bioactivity Screening and Evaluation, **1997**, pp. 210 – 220.

[24] S. A. Essawy, A. H. A. El-Aleem, S. G. Donia, R. N. Metwally, *Pol. J. Chem*. **1991**, *65*, 1243 – 1250.