

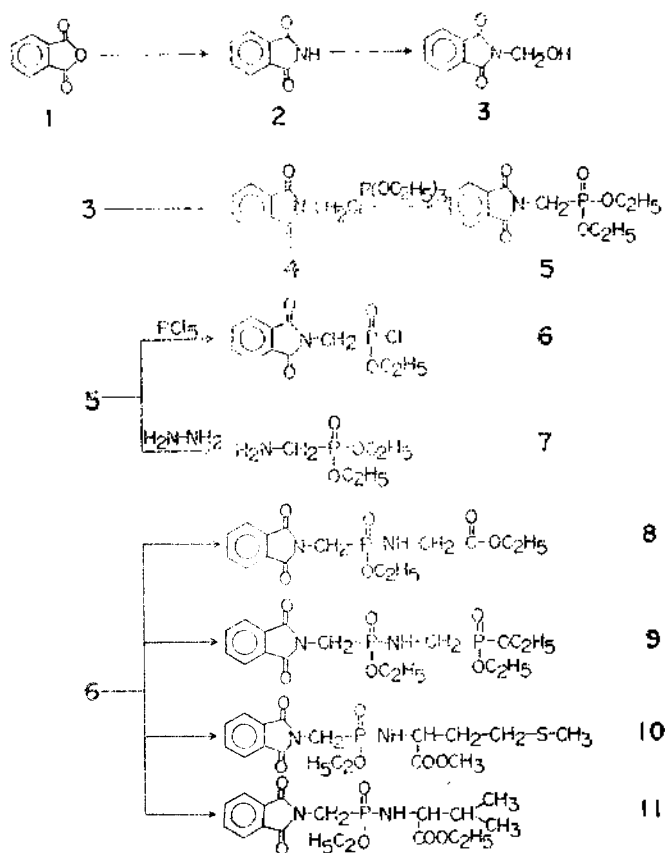


So far the authors have published a series of papers on the syntheses of peptides containing aminophosphonic acids as well as phosphonamides.<sup>1-7</sup>

This paper is reporting the syntheses of new 9 compounds containing phosphonamido group and particularly the compound which has phosphonamido group at C-6 position of 6-APA expecting to improve the antimicrobial activity of this compound against gram-negative bacteria in further study.

### Discussion

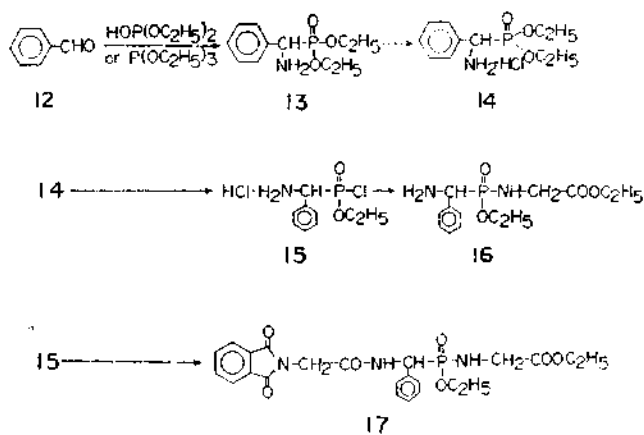
The following scheme 1 has been applied to the syntheses of some phosphonamides containing diethyl aminomethylphosphonate and diethyl DL-1-aminobenzylphosphonate.



Scheme 1.

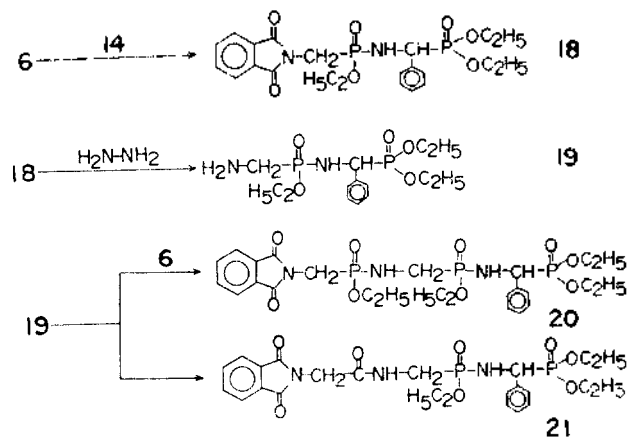
Phosphonamide 8 was prepared by the reaction of chloridate 6 and glycine ethyl ester hydrochloride; Phosphonamide 9, by the reaction of chloridate 6 and compound 7; phosphonamide 10, by the reaction of chloridate 6 and L-(+)-methionine methyl ester hydrochloride and also phosphonamide 11, by the reaction of chloridate 6 and L-(+)-valine ethyl ester hydrochloride.

The IR spectra and melting point of 8 and 9 showed good accordance with the literature listed.<sup>8-9</sup> The IR spectra of 8, 9, 10 and 11 showed the characteristic absorption band of P-NH- at 3200, 3150, 3240 and 3150  $\text{cm}^{-1}$  respectively. The specific rotations of 10 and 11 were found to be  $[\alpha]_{\text{D}}^{23} = +2.25$  and  $[\alpha]_{\text{D}}^{23} = +2.5$  in chloroform respectively.



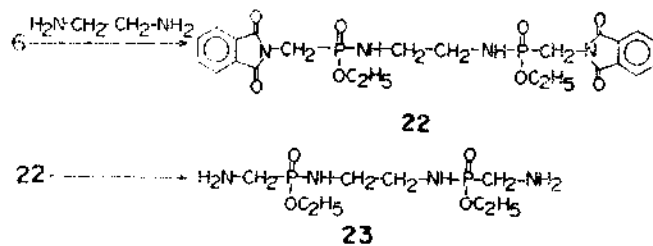
Scheme 2.

Compound 15 was prepared from compound 14 by a procedure similar to that reported by Penetta.<sup>10</sup> The phosphonamide 17 was obtained in 38% yield from compound 16 with positive ninhydrin test.



Scheme 3.

Compound 19 was obtained by the dephthalation of compound 18. The IR spectrum of the compound did not show the characteristic absorption band of phthalyl C=O at 1780 and 1720  $\text{cm}^{-1}$  which was observed in compound 18. The phosphonamide 20 and 21 was prepared from compound 19 in 24% and 13% yield respectively.

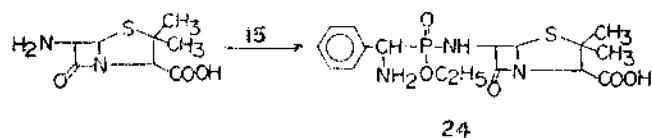


Scheme 4.

The compound 22 was prepared by the reaction of ethylene diamine and chloridate 6 in 80.7% yield. Diamine 23 was obtained in a oily state by the dephthalation of compound 22.

22. It is expected that diamine **23** can be used as the monomer of a polyamide.

The IR spectra of all above phosphonamides showed the characteristic absorption band of P-NH-at 3100-3250  $\text{cm}^{-1}$ .



Scheme 5.

The compound **24** was prepared from chloridate **15** by the similar method used in the synthesis of 6-sulfobenzylpenicillin.<sup>11</sup> The IR spectrum of the compound also showed the characteristic band P-NH-at 3200  $\text{cm}^{-1}$ .

It has been reported by Green<sup>12</sup> that the IR spectrum of  $\beta$ -lactam antibiotics show characteristic absorption band of C=O in  $\beta$ -lactam ring at 1790-1770  $\text{cm}^{-1}$ . Morin and coworkers<sup>13</sup> have proposed that the higher IR frequency of the  $\beta$ -lactam C=O is, the better the antimicrobial activity is. Since the IR spectra of compound **24** showed the higher absorption band of  $\beta$ -lactam C=O at 1797  $\text{cm}^{-1}$  as compared with that of ampicillin at 1760  $\text{cm}^{-1}$ , we expect compound **24** to have considerable high antimicrobial activity in further study.

## Experimental

1. *Synthesis of Starting Materials.* Diethyl N-phosphonylmethylphthalimide **5** was prepared from N-chloromethyl phthalimide **4**<sup>14</sup> adapting the procedure of Imoto and his coworkers: mp 65-67°C (lit.<sup>18</sup> mp 68°C). Glycine ethyl ester hydrochloride was obtained from glycine: mp 143-144°C (lit.<sup>16</sup> 144°C). Methionine methyl ester hydrochloride and Valine ethyl ester hydrochloride were synthesized from L-methionine and L-valine by the same procedure which we used for glycine ethyl ester hydrochloride: mp 149-151°C (lit.<sup>17</sup> 151-153°C),  $[\alpha]_{\text{D}}^{25} = +20.85$  (C=4, H<sub>2</sub>O) and 100-102°C (lit.<sup>18</sup> 102-104°C),  $[\alpha]_{\text{D}}^{25} = +6.8$  (C=4, H<sub>2</sub>O) respectively. Ethyl phthalimidomethylphosphonochloridate **6** was prepared from compound **5** by the same method used by Imoto and his coworkers; mp 90-92°C (lit.<sup>19</sup> 92-94°C). Diethyl DL-1-aminobenzylphosphonate hydrochloride **14** (ABP. HCl) was prepared from benzaldehyde and diethyl phosphite<sup>20</sup> or triethyl phosphite<sup>6</sup>; mp 160°C (lit.<sup>21</sup> 159-160°C).

2. *Synthesis of Phosphonamide 8.* Glycine ethyl ester hydrochloride (0.93g, 0.0066 mol) was suspended in 16ml of chloroform and the suspension was cooled to 0-5°C in ice water bath. Three ml of triethylamine was added into the suspension and mixture was stirred for 30 min. The mixture was slowly added to the solution of compound **6** (1.9g) which were dissolved in 4ml of tetrahydrofuran.

The reaction mixture was stirred at room temperature for 10 h. The final solution was filtered to remove triethylamine hydrochloride (E<sub>3</sub>N·HCl). The filtrate was concentrated by a rotary evaporator under the reduced pressure of water

aspirator and then the residue was dissolved in 16ml of chloroform. The solution was washed repeatedly with 10ml of aqueous saturated sodium bicarbonate solution and water. The organic layer was dried over drierite for overnight. After filtering the drierite, the filtrate was evaporated under the reduced pressure of water aspirator. To the residue 20ml of ethyl ether was added and then the solution was allowed to stand at room temperature. The resulting white crystals of **8** were obtained in 85% (2.0g) yield. The compound was recrystallized from ether-tetrahydrofuran mixture: mp. 130-132°C (lit.<sup>22</sup> 132-133°C).

IR (KBr): 3450 (N-H), 3200 (P-NH-), 1770, 1710 (phthalyl C=O), 1220 (P=O), 1070  $\text{cm}^{-1}$  (P-OEt)

3. *Synthesis of Phosphonamide 9.* Diethyl aminomethylphosphonate (1.2g, 0.0066 mol) was dissolved in 16ml of chloroform and the solution was cooled to 0-5°C in a ice water bath. Two ml of triethylamine was added into this solution and the mixture was treated as described above for the preparation of **8**. The resulting white crystals of **9** were obtained in 55% (1.6g); mp 105-108°C (lit.<sup>9</sup> 108-109°C)

IR (KBr): 3500 (N-H), 3150 (P-NH-), 1780, 1720 (phthalyl C=O), 1210 (P=O), 1030  $\text{cm}^{-1}$  (P-OEt)

4. *Synthesis of Phosphonamide 10.* L-Methionine methylester hydrochloride (7.9g) was suspended in 90ml of chloroform and the suspension was cooled to 0-5°C in a ice-water bath. To the suspension was added 22ml of triethylamine and the mixture was stirred for 30 min. To the reaction mixture a solution of compound **6** in 24ml of tetrahydrofuran was slowly added over 30 min. The reaction mixture was treated by the same procedure as used in the preparation of compound **8**. Finally the compound **10** was obtained in 40% (6.6g) yield; mp 120-122°C. The specific rotation of this compound was found to be  $[\alpha]_{\text{D}}^{25} = +2.25^\circ$  (C=4, CHCl<sub>3</sub>)

IR (KBr): 3520 (N-H), 3240 (P-NH-), 1775, 1715 (phthalyl C=O), 1215 (P=O), 1055  $\text{cm}^{-1}$  (P-OEt)

Anal. Calcd for C<sub>17</sub>H<sub>23</sub>N<sub>2</sub>O<sub>6</sub>PS: N, 6.76%; P, 7.49%  
Found: N, 6.58%; P, 7.40%

5. *Synthesis of Phosphonamide 11.* Compound **11** was prepared from L-valine ethyl ester hydrochloride (7.2g) by adopting the same procedure as used in the preparation of compound **10**. The compound **11** was obtained in 35% (5.5g); mp 104-106°C. The specific rotation of the compound was  $[\alpha]_{\text{D}}^{25} = +2.5^\circ$  (C=4, CHCl<sub>3</sub>)

IR (KBr): 3450 (N-H), 3150 (P-NH-), 1780, 1720 (phthalyl C=O), 1210 (P=O), 1060  $\text{cm}^{-1}$  (P-OEt).

Anal. Calcd for C<sub>10</sub>H<sub>23</sub>N<sub>2</sub>O<sub>4</sub>P: N, 10.53%; P, 11.7%  
Found: N, 10.4%; P, 11.5%

6. *Ethyl Aminobenzylphosphonochloridate Hydrochloride 15.* Diethyl DL-1-aminobenzylphosphonate hydrochloride (5.1g) was suspended in 15ml of benzene and to the suspension was added a phosphorous pentachloride (4.0g). After the mixture had been stirred for 15h at room temperature, it was allowed to cool to room temperature and was concentrated by rotary evaporator under the reduced pressure of water aspirator. The resulting white semisolid was suspended in 10ml of tetrahydrofuran and the suspension was used in following reaction.

**7. Synthesis of Phosphonamide 16.** Glycine ethyl ester hydrochloride (2.6g) was suspended in 16ml of chloroform and the suspension was cooled to 0–5°C in a ice-water bath. Sixteen ml of triethylamine was added to the suspension and was stirred for 30 min. at room temperature. The reaction mixture was mixed slowly with the suspension of compound **15** at room temperature. Then, the mixture was filtered to remove the resulting triethylamine hydrochloride, and the filtrate was concentrated by rotary evaporator under the reduced pressure of water aspirator. The resulting residue was taken up in 15ml of chloroform and the solution was washed repeatedly with each 10ml of saturated sodium bicarbonate solution and also water. The organic layer was dried over drierite for overnight. After filtering the drierite, the filtrate was evaporated under the reduced pressure of water aspirator. The resulting residue was dissolved 10ml of ethyl alcohol and the solution was treated by activated charcoal. After filtering the charcoal, the solvent was removed by rotary evaporator under the reduced pressure of water aspirator. The resulting syrup of **16** was obtained in 39 % (2.2g) yield and gave positive ninhydrin test.

IR (KBr): 3400 (N–H), 3250 (P–NH–), 1750 (ester C=O), 1220 (P=O), 1040cm<sup>-1</sup> (P–OEt)

**8. Synthesis of Phosphonamide 17.** A mixture of **16** (2.3g) phthalylglycine (0.77g) and dicyclohexylcarbodiimide (1.6g) in 15ml of tetrahydrofuran, was prepared and it was stirred for 10h. Further 0.8ml of acetic acid and 1.6ml of water were added to it and again was stirred for 2h at room temperature. The resulting precipitate was filtered, and the filtrate was concentrated as before. The residue was taken up in 15ml of chloroform and was washed with 8ml of water, 8ml of aqueous saturated sodium bicarbonate solution and 2ml of water. The organic layer was dried over drierite for overnight and the solvent was removed by rotary evaporator under the reduced pressure of water aspirator. The residue was dissolved in the least amount of benzene and the solution was allowed to stand in a refrigerator for overnight. The resulting white crystals of **21** were obtained in 38.2 % (0.7g) yield: mp 174–176 °C.

IR (KBr) 3350 (N–H), 3070 (P–NH–), 1780, 1720 (phthalyl C=O), 1650 (peptide C=O), 1240 (P=O), 1080cm<sup>-1</sup> (P–OEt)

*Anal.* Calcd for C<sub>23</sub>H<sub>26</sub>N<sub>3</sub>O<sub>7</sub>P; N, 8.62 %; P, 6.37 %  
Found: N, 8.4 %; P, 6.3 %

**9. Synthesis of Phosphonamide 18.** Diethyl DL–1-aminobenzylphosphonate hydrochloride (11.1g) was suspended in 90ml of chloroform and the suspension was cooled to 0–5 °C in a ice-water bath. To the suspension 22ml of triethylamine was added and the mixture was stirred for 30 min. A solution of **6** (11.4g) in 24ml of tetrahydrofuran was mixed with the above reaction mixture. After stirring the mixture for 10h at room temperature, it was further treated by the procedure of experiment **2**. Compound **18** was obtained in white crystalline form having 61 % (12g) yield: mp 162–164 °C.

IR (KBr): 3400 (N–H), 3200 (P–NH–), 1780, 1720 (phthalyl C=O), 1200 (P=O), 1070 (P–OEt)

*Anal.* Calcd for C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O<sub>7</sub>P<sub>2</sub>; N, 5.7 %; P, 12.6 %

Found: N, 5.6 %; P, 12.4 %

**10. Synthesis of Phosphonamide 19.** Compound **18** (5.3g) in 20ml of ethyl alcohol was mixed with 1.1ml of hydrazine (100 %), and the mixture was stirred for overnight at room temperature. The resulting precipitate was filtered, and the filtrate was concentrated by rotary evaporator under the reduced pressure of water aspirator. The residue was treated according to the same procedure as in experiment **7** to give the syrup of **19** in 77.5 % (3g) yield. The syrup gave positive ninhydrin test.

IR (KBr): 3400 (N–H), 3240 (P–NH), 1220 (P=O), 1045cm<sup>-1</sup> (P–OEt)

**11. Synthesis of Phosphonamide 20.** Compound **19** (3.6g) was suspended in 25ml of tetrahydrofuran and the suspension was cooled to 0–5°C in a ice-water bath. To the suspension was added 3ml of triethylamine and the mixture was stirred for 30min. To this reaction mixture compound **6** (2.9g) in 5ml of tetrahydrofuran was slowly added. The mixture was stirred for 10h at room temperature and was filtered to remove the resulting triethylamine hydrochloride. The filtrate was treated further by the procedure of experiment **2**. Compound **21** was obtained in 13.1 % (0.8g) yield; mp 204–206 °C.

IR (KBr): 3250 (N–H), 3250 (P–NH–), 1780, 1720 (phthalyl C=O), 1230 (P=O), 1040cm<sup>-1</sup> (P–OEt)

*Anal.* Calcd for C<sub>25</sub>H<sub>36</sub>N<sub>3</sub>O<sub>9</sub>P<sub>3</sub>; N, 6.83 %; P, 15.1 %  
Found: N, 6.7 %; P, 15.4 %

**12. Synthesis of Phosphonamide 21.** A solution of **19** (3g), phthalylglycine (1.7g) and dicyclohexylcarbodiimide (DCC) (2g) in 20ml of tetrahydrofuran was stirred for 10h. The reaction mixture was treated with the mixed solution of 1.6ml of acetic acid and 2.4ml of water. Then, the mixture was stirred for additional 2h, and the resulting precipitate was filtered. The filtrate was concentrated by rotary evaporator under reduced pressure of water aspirator and the residue was taken up in 20ml of chloroform. The solution was washed repeatedly with about each 10ml of water, aqueous saturated sodium bicarbonate solution and again water. The organic layer was dried over drierite for overnight, and the drierite was filtered, and the filtrate was concentrated by rotary evaporator under the reduced pressure of water aspirator. The residue was dissolved in the least amount of ethyl ether and the solution was allowed to stand for 2 days. White crystalline compound **20** was obtained in 24.2 % (1.1g) yield: mp 190–192 °C.

IR (KBr): 3350 (N–H), 3250 (P–NH–), 1770, 1710 (phthalyl C=O), 1630 (peptide C=O), 1190 (P=O), 1020cm<sup>-1</sup> (P–OEt)

*Anal.* Calcd for C<sub>24</sub>H<sub>31</sub>N<sub>3</sub>O<sub>8</sub>P<sub>2</sub>; N, 7.62 %; P, 11.25 %  
Found: N, 7.4 %; P, 11.1 %

**13. Synthesis of Phosphonamide 22.** A solution of ethylene diamine (0.44ml) in 32ml of chloroform was cooled to 0–5°C, and mixed with triethylamine (6ml). After stirring for 30min, it was mixed slowly with a solution of ethyl phthaliminomethylchloridate (3.8g) in 6ml of tetrahydrofuran. The mixture was stirred for 10h and filtered to remove the resulting triethylamine hydrochloride. The filtrate was concentrated by rotary evaporator under the reduced pressure of water

aspirator. The residue was dissolved in 30ml of chloroform, and the solution was washed with each 30ml of water, aqueous saturated sodium bicarbonate solution and water. The organic layer was dried over drierite for overnight and the solvent was removed by rotary evaporator under the reduced pressure of water aspirator. The residue was dissolved in 50ml of ethyl ether allowed to stand in the refrigerator for overnight. Compound **22** was obtained in white crystalline form in 80.7 % (3g) yield: mp 196–199 °C.

IR (KBr): 3450 (N–H), 3200 (P–NH–), 1780, 1720 (phthalyl C=O), 1200 (P=O), 1050cm<sup>-1</sup> (P–OEt)

**14. Synthesis of Diamine 23.** To the suspension of **22** in 8ml of ethyl alcohol was added a hydrazine hydrate (100 %, 2.6g), and the mixture was stirred for 10h followed by 2h of reflux. After cooling it to room temperature, the resulting precipitate so obtained was filtered.

The filtrate was concentrated by rotary evaporator under the reduced pressure of water aspirator. The light yellowish viscous oil was obtained in 72.8 % (0.22g) yield and gave positive ninhydrin test.

IR (KBr): 3400 (N–H), 3150 (P–NH–), 1210 (P=O) 1040cm<sup>-1</sup> (P–OEt)

**15. 6-(Ethyl DL-1-aminobenzylphosphonyl) aminopenicillanic acid 24.** To a suspension of 6-aminopenicillanic acid (6-APA, 3.95g) in 33ml of water slowly added sodium bicarbonate (5.4g), and the resulting mixture was cooled to 5°C. Dropwise a solution of compound **15** obtained from experiment **6** has added to this. The mixture was allowed to stand at 0–5°C for 60min. Then, the organic layer was separated and the pH of aqueous layer was adjusted by a dilute hydrochloric acid solution. The aqueous mixture was allowed to stand at 0°C for overnight. The resulting white crystals were obtained by filtering and washing with acetone. The white crystals of compound **24** were dried. The yield of the compound was obtained in 13.3 % (1g) yield and decomposed at 200 °C.

IR (KBr): 3450 (N–H), 3200 (P–NH–), 2050 (–NH<sub>3</sub><sup>+</sup>), 1797 (β-lactam C=O), 1330 (P=O), 1140cm<sup>-1</sup> (P–OEt)

Anal. Calcd for C<sub>17</sub>H<sub>24</sub>N<sub>3</sub>O<sub>5</sub>PS: N, 10.17 %; P, 7.51 %  
Found: N, 9.96 %; P, 7.3 %

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