

Synthesis and antimicrobial activity of 2,10-dichloro-6-substituted amino acid ester-12*H*-dibenzo[d,g][1,3,2]dioxaphosphocin-6-oxides

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A new class of 2,10-dichloro-6-substituted amino acid ester-12*H*-dibenzo[d,g][1,3,2]dioxaphosphocin-6-oxides have been synthesized in good yields via the condensation of 2,10-dichloro-6-chloro-12*H*-dibenzo[d,g][1,3,2]dioxaphosphocin with various amino acid esters in the presence of triethylamine. The title compounds were characterized by elemental analysis, IRS, NMR (¹H, ¹³C and ³¹P) and mass spectral studies and found to exhibit moderate antimicrobial activity.

Key words: Dioxaphosphocin-6-oxides, amino acid esters, spectral studies, antimicrobial activity.

INTRODUCTION

Phosphoramidate compounds substituted with amino acid esters are important class of rationally designated therapeutics especially with antineoplastic properties. Phosphate triester derivatives of nucleotides have been prepared as the membrane-soluble prodrug of the bio-active nucleotides and were found to contain good activity against HIV-1 in-vitro [1]. The aryloxyphosphoramidates were found to exhibit enhanced activity against HIV-1 and HIV-2 in cellular culture, compared to their parent ddN's with full retention of activity in thymidine kinase – deficient cell lines [2,3]. This type of nucleosides have been shown to be potent inhibitors of HIV and to display reduced toxicity in progenitor cells [4-6]. Exhaustive modifications to the amino acid moiety in aryloxyphosphoramidate have established L-alanine to be optimal for antiviral activity [7]. Hence synthesis of the title compounds were contemplated and accomplished. They have been characterised by elemental analysis, IRS, multi NMR and mass spectral analysis.

EXPERIMENTAL

Melting points were determined in open capillary tubes on a Mel-Temp apparatus and were uncorrected. IR spectra (ν_{\max} in cm^{-1}) were recorded in KBr pellets on Perkin Elmer 1000 unit. The ¹H, ¹³C and ³¹P NMR spectra were recorded on Varian Gemini 300 and Varian AM X 400 MHz NMR spectrometer operating at 300 or 400 MHz for ¹H, 75.46 or

100.57 MHz for ¹³C and 121.7 MHz for ³¹P. All the compounds were dissolved in DMSO-d₆ and chemical shifts were referenced to TMS (¹H and ¹³C) and 85% H₃PO₄ (³¹P). Micro analytical data were obtained from Central Drug Research Institute, Lucknow, India.

RESULT AND DISCUSSION

The synthetic route (Scheme 1) involves the cyclocondensation of 5,5'-dichloro-2,2'-dihydroxy biphenyl methane (**1**) with phosphorus oxychloride at 0°C under inert and anhydrous conditions in dry toluene to afford the 2,10-dichloro-6-chloro-12*H*-dibenzo[d,g][1,3,2]dioxaphosphocin-6-oxide (**3**) and its subsequent condensation with various amino acid esters in the presence of triethylamine in dry tetrahydrofuran at room temperature. The final products were purified by column chromatography using hexane, ethyl acetate as step gradient mixtures as eluents.

IR spectra of **4a-i** showed absorption bands at 3383–3333, 1759–1733 and 1241–1219 cm^{-1} for NH [8], C=O and P=O [9, 10], respectively (Table 1). Their ¹H NMR spectra gave complex multiplets at δ 6.72–7.91 for aromatic protons (Table 2). The splitting pattern of bridged methylene protons showed their non-equivalence [11]. One of the bridging protons appeared as doublet of doublet in the region 4.21–4.41 (dd, ² $J_{\text{HH}} = 12.9$ – 13.9 Hz and ⁵ $J_{\text{PH}} = 3.5$ – 4.3 Hz) due to the germinal coupling with the another bridging proton and long range coupling with the phosphorus. Second bridging proton resonated as doublet in the region δ 3.57–3.65 (² $J_{\text{HH}} = 13.6$ – 14.9 Hz) due to germinal coupling with

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germinal proton and its arrangement masked it from coupling with phosphorus.

The dioxaphosphocin ring in all these compounds appeared to exist in boat like configuration, in which one of the bridging protons protruded away from the phosphorus. The NH proton resonated as a broad singlet in the region δ 3.82–5.43.

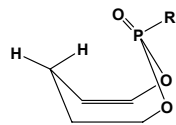
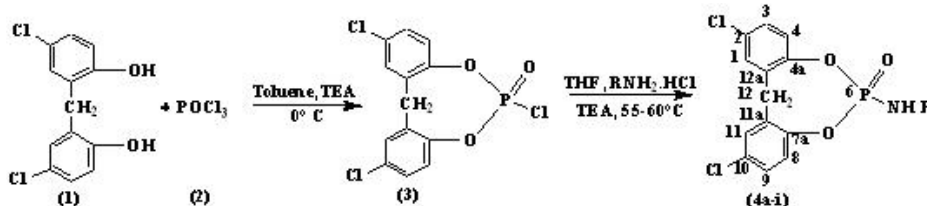


Table 1. Infrared spectral data of compounds **4a–i**.

Comp.	IR band maximum, cm^{-1}		
	N–H	C=O	P=O
4a	3351	1751	1231
4b	3374	1736	1238
4c	3333	1749	1230
4d	3371	1742	1231
4e	3342	1752	1239
4f	3356	1746	1224
4g	3364	1759	1241
4h	3383	1733	1237
4i	3341	1742	1219



Compound	R	Compound	R
4a	$-\text{CH}_2\text{COOCH}_3$	4f	$-\text{CHCOOCH}_3$ $ \text{CH}_2$ $ \text{CH}(\text{CH}_3)_2$
4b	$-\text{CH}_2\text{COOCH}_2\text{CH}_3$	4g	$-\text{CHCOOCH}_3$ $ \text{CHCH}_3$ $ \text{CH}_2\text{CH}_3$
4c	$-\text{CHCOOCH}_3$ $ \text{CH}_3$	4h	$-\text{CHCOOCH}_3$ $ \text{CH}_2\text{CH}_3$
4d	$-\text{CHCOOCH}_3$ $ \text{C}_6\text{H}_5$	4i	$-\text{CHCOOCH}_3$ $ \text{CH}_2\text{Cl}$
4e	$-\text{C}_6\text{H}_4(\text{H}_3\text{COOC})$		

Scheme 1.

Table 2. ^1H NMR spectral data^{a,b} of compounds **4a–i**.

Comp.	Ar-H	CH ₂	N-H	Amino acid ester
4a	6.82–7.74 (m, 6H)	4.33 (dd, $J = 13.2, 3.9$ Hz), 3.61 (d, $J = 13.5$ Hz)	3.92 (brs, 1H)	4.47 (s, 3H, OCH ₃), 3.87 (s, 2H, CH ₂)
4b	6.89–7.86 (m, 6H)	4.25 (dd, $J = 13.4, 3.5$ Hz), 3.63 (d, $J = 13.8$ Hz)	4.12 (brs, 1H)	4.39 (q, $J = 7.1$ Hz, 3H, OCH ₂), 3.79 (s, 2H, CH ₂), 3.12 (t, $J = 6.9$ Hz, 3H, CH ₃)
4c	6.72–7.79 (m, 6H)	4.21 (dd, $J = 13.7, 4.1$ Hz), 3.65 (d, $J = 13.9$ Hz)	4.62 (s, 1H)	4.29 (s, 3H, OCH ₃), 3.40 (m, 1H, CH), 1.41 (d, $J = 7.9$ Hz, 3H, CH–CH ₃)
4d	7.02–7.93 (m, 11H)	4.23 (dd, $J = 12.9, 3.5$ Hz), 3.61 (d, $J = 13.2$ Hz)	4.45 (s, 1H)	4.79 (s, 1H, CH), 4.40 (s, 3H, OCH ₃)
4e	6.97–7.82 (m, 10H)	4.22 (dd, $J = 13.2, 3.6$ Hz), 3.64 (d, $J = 13.2$ Hz)	5.43 (s, 1H)	4.11 (s, 3H, OCH ₃)
4f	6.93–7.91 (m, 6H)	4.24 (dd, $J = 13.5, 4.2$ Hz), 3.59 (d, $J = 14.2$ Hz)	-	4.32 (s, 3H, OCH ₃), 3.41 (t, 1H, –CH–CH ₂), 1.78–1.73 (m, 2H, –CH–CH ₂ –CH(CH ₃) ₂), 1.51–1.45 (m, 1H, –CH–CH ₂ –CH(CH ₃) ₂), 1.09–0.96 (m, 6H)
4g	7.03–7.88 (m, 6H)	4.31 (dd, $J = 13.9, 3.7$ Hz), 3.57 (d, $J = 13.8$ Hz)	5.13 (s, 1H)	4.19 (s, 3H, OCH ₃), 3.58–3.52 (m, 1H, –CH–CH(CH ₃)–CH ₂ CH ₃), 2.21–2.25 (m, 1H, –CH–CH(CH ₃)CH ₂ CH ₃), 1.51–1.48 (m, 2H, CHCH(CH ₃)CH ₂ CH ₃), 1.09–0.94 (m, 6H)
4h	7.01–7.74 (m, 6H)	4.41 (dd, $J = 13.2, 4.3$ Hz), 3.61 (d, $J = 13.6$ Hz)	3.83 (brs, 1H)	4.24 (s, 3H, OCH ₃), 3.51 (m, 1H, CH(COOCH ₃)CH ₂), 2.08–2.02 (m, 2H, CH ₂ –CH ₃), 1.02 (t, $J = 7.4$ Hz, 3H, CH ₂ –CH ₃)
4i	6.82–7.71 (m, 6H)	4.29 (dd, $J = 13.5, 3.7$ Hz), 3.59 (d, $J = 13.5$ Hz)	5.04 (s, 1H)	4.23 (s, 3H, OCH ₃), 4.10–3.91 (m, 2H, CH ₂ Cl), 3.58 (s, 1H, CH)

a - Chemical shifts are in ppm from TMS and coupling constants (J) in Hz are given in parenthesis; b - Recorded in DMSO- d_6 .

Their ¹³C chemical shifts were interpreted based on comparison of basic units, present in them. Because of the symmetrical nature of dibenzophosphocin moiety only seven ¹³C signals were observed for thirteen carbons (Table 3). The oxygen bearing C (4a) and C (7a) atoms gave signals in the down field 149.97–152.41 ppm as a doublet (²J_{POC} = 6.9–7.3 Hz). The doublets at δ 131.72–132.98 (d, J = 3.7–4.5 Hz) were assigned to the C-11a and C-12a atoms. The chemical shifts in the region 130.98–131.92 ppm were assigned to chlorine bearing C-2 and C-10 atoms. The carbonyl carbon of amino acid ester moiety appeared at δ 169.49–176.2.

³¹P NMR chemical shifts [12] for all the title compounds were observed in the region 2.13–8.19 ppm (Table 4). The compounds **4a** and **4b** exhibited molecular ion peaks at their respective and relative molecular ion weights in their mass spectrum (Table 5).

Antimicrobial activity

Compounds **4a–i** were screened with respect to

Table 4. Synthetic, elemental and ³¹P NMR spectral data of compounds **4a–i**.

Comp.	M. p., °C	Yield, %	Molecular Formula	Elemental analysis, % Found (Calc.)		³¹ P NMR, δ
				C	H	
				4a	162–164	
4b	171–173	76	C ₁₇ H ₁₆ NO ₅ Cl ₂ P	49.21 (49.06)	3.98 (3.87)	6.82
4c	149–151	75	C ₁₇ H ₁₆ NO ₅ Cl ₂ P	49.19 (49.06)	3.99 (3.87)	7.81
4d	197–199	71	C ₂₂ H ₁₈ NO ₅ Cl ₂ P	55.39 (55.25)	3.91 (3.79)	7.99
4e	133–135	69	C ₂₁ H ₁₆ NO ₅ Cl ₂ P	55.48 (54.33)	3.61 (3.47)	8.19
4f	204–206	77	C ₂₀ H ₂₂ NO ₅ Cl ₂ P	52.57 (52.42)	4.97 (4.84)	2.13
4g	153–155	74	C ₂₀ H ₂₂ NO ₅ Cl ₂ P	52.59 (52.42)	5.01 (4.84)	4.22
4h	187–189	79	C ₁₈ H ₁₈ NO ₅ Cl ₂ P	50.39 (50.25)	4.38 (4.22)	2.20
4i	146–148	73	C ₁₇ H ₁₅ NO ₅ Cl ₃ P	45.42 (45.31)	3.46 (3.35)	5.44

Table 5. Mass spectral data of compounds **4a** and **4c**.

Compd.	m/z (%)
6	404 [9, M ⁺ +2], 402 [28, M ⁺ *], 369 (11), 358 (24), 343 (22), 325 (17), 314 (27), 268 (19)
7	418 [9, M ⁺ +2], 416 [31, M ⁺ *], 383 (17), 372 (16), 343 (21), 339 (19), 314 (21), 268 (16), 140 (32)

their antibacterial activity against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) by the disc-diffusion method [13, 14] in nutrient agar medium at various concentrations (250, 500 ppm) in dimethyl formamide (DMF). These solutions were added to each filter disc and DMF was used as control. The plates were incubated at 35°C and examined for zone of inhibition around each disc after 24 h. The results were compared with the activity of the standard antibiotic Penicillin (250 ppm) (Table 6).

The antifungal activity of the synthesized compounds was evaluated against *Curvularia lunata* and *Aspergillus niger* at different concentrations (250, 500 ppm) and Griseofulvin was used as the reference compound. Fungal cultures were grown on potato dextrose broth at 25°C and spore suspension was adjusted to 10⁵ spore/mL. Most of the compounds showed moderate activity against bacteria and high activity against fungi (Table 7).

Table 6. Antibacterial activity of compounds **4a–i**.

Comp.	Zone of inhibition			
	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
	250 ^a	500 ^a	250 ^a	500 ^a
4a	8	13	12	17
4b	7	11	14	19
4c	7	12	13	18
4d	6	11	15	18
4e	8	11	16	25
4f	7	12	14	22
4g	6	12	13	20
4h	5	10	13	19
4i	6	12	14	20
Penicillin ^b	12		24	

a - Concentration in ppm; b - Standard reference.

Table 7. Antifungal activity of compounds **4a–i**.

Comp.	Zone of inhibition			
	<i>Curvularia lunata</i>		<i>Asperigillus niger</i>	
	250 ^a	500 ^a	250 ^a	500 ^a
4a	16	27	20	27
4b	14	21	16	20
4c	16	23	19	25
4d	16	22	22	29
4e	14	20	19	26
4f	18	28	23	31
4g	15	27	13	17
4h	15	26	21	29
4i	16	21	15	20
Griseofulvin ^b	23		26	

a - Concentration in ppm; b - Standard reference.

Table 3. ^{13}C NMR spectral data^{a,b} of compounds **4a–i**.

Comp.	C(1/11)	C(2/10)	C(3/9)	C(4/8)	C(4a/7a)	C(11a/12a)	C(12)	α	β	γ	Ar-H				C=O	OCH ₂ / OCH ₃	CH ₃
											C-1'	C-2'/6'	C-3'/5'	C-4'			
4a	129.38	131.84	128.62	124.50	150.14 [d, <i>J</i> = 7.1 Hz]	132.98 [d, <i>J</i> = 3.7 Hz]	33.41	54.89 [d, <i>J</i> = 126.2 Hz]	–	–	–	–	–	–	174.90	52.61	–
4b	129.48	131.85	128.67	124.53	150.16 [d, <i>J</i> = 7.2 Hz]	132.87 [d, <i>J</i> = 4.5 Hz]	33.48	54.70 [d, <i>J</i> = 126.2 Hz]	–	–	–	–	–	–	172.91	–	13.22
4c	129.51	131.89	128.61	124.51	150.15 [d, <i>J</i> = 6.8 Hz]	132.85 [d, <i>J</i> = 4.5 Hz]	33.39	54.79 [d, <i>J</i> = 126.2 Hz]	23.23	–	–	–	–	–	172.61	56.92	–
4d	129.11	131.67	128.32	124.47	149.97 [d, <i>J</i> = 7.0 Hz]	132.52 [d, <i>J</i> = 4.1 Hz]	33.12	54.21 [d, <i>J</i> = 126.2 Hz]	–	–	135.21	128.92	127.89	125.4	169.49	56.63	–
4e	129.69	131.53	128.11	124.82	151.31 [d, <i>J</i> = 7.3 Hz]	131.99 [d, <i>J</i> = 4.0 Hz]	34.09	–	–	–	149.62	116.8	132.9	118.12	179.32	53.91	–
4f	129.12	131.92	127.92	124.03	152.11 [d, <i>J</i> = 7.1 Hz]	132.91 [d, <i>J</i> = 4.3 Hz]	34.12	54.13 [d, <i>J</i> = 121.3 Hz]	26.3	15.7 13.3(γ')	–	–	–	–	171.34	54.32	–
4g	128.97	131.62	128.41	124.53	151.31 [d, <i>J</i> = 6.9 Hz]	132.17 [d, <i>J</i> = 3.9 Hz]	33.79	55.03 [d, <i>J</i> = 126.7 Hz]	28.4 14.6(β')	16.2 13.9 (β')	–	–	–	–	172.37	52.31	–
4h	129.32	130.98	128.17	124.32	152.41 [d, <i>J</i> = 7.0 Hz]	131.72 [d, <i>J</i> = 4.0 Hz]	35.31	53.97 [d, <i>J</i> = 120.3 Hz]	29.3	14.1	–	–	–	–	176.20	51.20	–
4i	129.82	131.03	128.47	124.55	150.75 [d, <i>J</i> = 6.9 Hz]	132.11 [d, <i>J</i> = 4.5 Hz]	33.72	55.07 [d, <i>J</i> = 123.7 Hz]	9.3	–	–	–	–	–	173.11	51.37	–

a - Chemical shifts are in ppm from TMS and coupling constants (*J*) in Hz are given in parenthesis; b - Recorded in DMSO-*d*⁶.

Synthesis of 2,10-dichloro-6-glycine methyl ester-
12H-dibenzo[d,g][1,3,2]dioxaphosphocin-6-oxid
4a. General Procedure

To a well stirred solution of 5,5'-dichloro-2,2'-dihydroxy biphenyl methane(**1**, 1.34 g, 0.005 mole) and triethylamine (1.01 g, 0.01 mole) in dry toluene (25 mL) phosphorous oxychloride (0.466 g, 0.005 mole) in dry toluene (15 mL) was added at 0°C. After the addition, the temperature of the reaction mixture was raised slowly and kept at 55–60°C for 2 hours. Completion of the reaction was monitored by TLC analysis. After cooling down to room temperature it was filtered to remove triethylamine hydrochloride and evaporated in rotary evaporator to obtain concentrated solution of 2,10-dichloro-6-chloro-12H-dibenzo[d,g][1,3,2]dioxaphosphocin-6-oxide (**3**), which is used for the next step without any further purification.

To the concentrated solution of compound **3** (1.748 g, 0.005 mole) and triethylamine (1.01 g, 0.01 mole) in dry tetrahydrofuran (30 mL), a solution of L-glycine methyl ester (0.698 g, 0.005 mole) was added at room temperature. Progress of the reaction was monitored by TLC analysis. Triethylamine hydrochloride was filtered and the filtrate was evaporated in rotary evaporator. Finally, the residue was purified by column chromatography by using hexane and ethyl acetate mixtures as eluents to yield 1.63 g (72%) m.p. 162–164°C.

Analogous compounds were prepared by adopting the same procedure.

SUMMARY

A new class of phosphonides substituted with amino acid esters having good anti-microbial activity were synthesized conveniently in good yields.

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REFERENCES

1. L. I. Wiebe, E. E. Knaus, *Adv. Drug Delivery Rev.*, **39**, 63 (1999).
2. J. Balzarini, J. Kruining, O. Wedggood, C. Pannecouque, S. Aquaro, C. F. Perno, L. Naesen, M. Wifuroun, R. Heijtkink, E. Declercq, C. McGuigan, *FEBS Lett.*, **410**, 324 (1997).
3. C. McGuigan, D. Cahard, H. H. Sheeka, E. De Clercq, J. Balzarini, *Bioorg. Med. Chem. Lett.*, **6**, 1183 (1996).
4. C. McGuigan, D. Cahard, H. H. Sheeka, E. De Clercq, J. Balzarini, *J. Med. Chem.*, **39**, 1748 (1996).
5. C. McGuigan, H. W. Tsang, D. Cahard, S. Turner, S. Velazquez, A. Salgado, L. Bidois, L. Naesens, E. De Clercq, J. Velazquez, *Antiviral Res.*, **35**, 195 (1997)
6. J. Balzarini, G. J. Kang, M. Dala, P. Herdewijn, E. De Clercq, S. Broder, D. G. Johns, *Mol. Pharmacol.*, **32**, 162 (1987).
7. M. Baba, R. Pauwels, P. Hardewijn, E. De Clercq, J. Desmyter, M. Vandeputte, *Biochem. Biophys. Res. Commun.*, **142**, 128 (1987).
8. M. M. Mansuri, M. J. M. Hitchcock, R. A. Buroker, C. L. Bergman, I. Ghazzouli, J. V. Desdrio, J. E. Starrett, R. Z. Jr. Sterzycki, J. C. Martin, *Antimicrob. Agents Chemother.*, **34**, 637(1990).
9. W. Kemp, *Organic Spectroscopy*, Third Edition, Plgrave Publishers Ltd. New York, 1991, p. 83-86.
10. K. A. Kumar, C. S. Reddy, C. D. Reddy, *Phosphorus, Sulfur Silicon*, **177**, 1745 (2000).
11. K. A. Kumar, C. S. Reddy, L. N. Prasad Rao, C. N. Raju, *Heterocyclic Commun.*, **7**, 253 (2001).
12. B. S. Kumar, A. U. Ravi Sankar, C. S. Reddy, S. K. Nayak, C. N. Raju, *Arkivoc*, **13**, 155 (2007).
13. J. C. Vincent, H. W. Vincent, *Proc. Soc. Enpt. Biol. Med.*, **55**, 162 (1944).
14. H. J. Benson, *Microbiological applications*, 5th Edn., W. C. Brown Publications, Boston, 1990.

SYNTHESIS AND АСИНТЕЗ И АНТИМИКРОБНА АКТИВНОСТ НА 2,10-ДИХЛОРО-6-ЗАМЕСТЕНИ С ЕСТЕРИ НА АМИНОКИСЕЛИНИ-12H-ДИБЕНЗО[d,g][1,3,2]ДИОКСА-ФОСФОЦИН-6-ОКСИДИ

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(Резюме)

Синтезирани са с добър добив нов клас 2,10-дихлор-6-заместени с естери на аминокиселини-12H-добензо[d,g][1,3,2]ди-оксафосфоцин-6-оксиди чрез кондензация на 12H-добензо[d,g][1,3,2]диоксафосфоцин с различни естери на аминокиселини в присъствие на триетиламин. Съединенията са охарактеризирани с елементарен анализ, ИЧС, ЯМР (¹H, ¹³C и ³¹P) и масспектроскопия и показват умерена антимикробна активност.