Diamide Analogues of Phosphatidyl Choline as Potential Anti-AIDS Agents

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Acylation of 3-tetrahydropyran-2-yloxypropane-1,2-diamine with dodecanoyl chloride (lauroyl chloride) and hexadecanoyl chloride (palmitoyl chloride) followed by *O*-deprotection afforded racemic diamide analogues of 1,2-diglycerides which were converted by sequential treatment with 2-chloro-1,3,2-dioxaphospholan-2-one and trimethylamine into the corresponding diamide analogues of phosphatidyl choline. One of these compounds shows moderate anti-HIV activity.

As part of a previous study into potential antagonists of protein kinase C (PKC),[1,2] we prepared a short-chain diamide analogue of a 1,2-diacylglycerol, 2,3-dioctanamidopropan-1-ol (1,2-dideoxy-1,2-dioctanamidoglycerol) **1**. In an anti-HIV screen[3] this

1 $R^1 = CO(CH_2)_6Me$, $R^2 = H$
2 $R^1 = H$, $R^2 = H$
3 $R^1 = CO(CH_2)_{10}Me$, $R^2 = H$
4 $R^1 = CO(CH_2)_{14}Me$, $R^2 = H$
5 $R^1 = CO(CH_2)_{10}Me$, $R^2 = P(O)OCH_2CH_2\overset{+}{N}Me_3$ (O^-)
6 $R^1 = CO(CH_2)_{14}Me$, $R^2 = P(O)OCH_2CH_2\overset{+}{N}Me_3$ (O^-)

7 R = H
8 $R = CO(CH_2)_{10}Me$
9 $R = CO(CH_2)_{14}Me$

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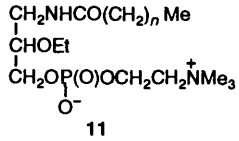

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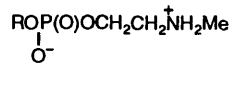

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compound showed slight activity, and as a result of a report by Piantadosi and co-workers[4] that certain phosphatidyl choline analogues possessed reasonable activity against HIV-1, we were encouraged to prepare new, hitherto uninvestigated, analogues of this type, based on 2,3-diaminopropan-1-ol **2**, for tests against HIV.

3-Tetrahydropyran-2-yloxypropane-1,2-diamine **7** is readily prepared[5] from 2,3-dibromopropan-1-ol‡ by reaction with 3,4-dihydropyran, azide displacement on the product and subsequent reduction of the diazide. Acylation of **7** with dodecanoyl chloride gave material with a wide melting range that afforded, through recrystallisation and chromatography, a compound **A** with a sharp melting point (108–109 °C) that gave an elemental analysis, and IR and NMR spectra consistent with the expected structure **8**. Column chromatography of the combined mother liquors and recrystallisation of the main fraction gave a second sharp melting (76–77 °C) material **B** also having the expected elemental analysis and physical characteristics for **8**. Although **A** and **B** gave indistinguishable solution IR spectra they could not be interconverted by recrystallisation of one in the presence of the other, ruling out dimorphism as a possible explanation for isolation of the two products. Since on hydrolysis they gave the same compound (see below) it is clear that **A** and **B** are diastereoisomers resulting from the chiral centres at C-2 in both the glycerol and the tetrahydropyranyl moieties.

Heating a solution of either **A** or **B** in aqueous methanol in the presence of toluene-*p*-sulfonic acid led to loss of the tetrahydropyranyl protecting group and formation of the same compound, m.p. 106–107 °C. Thus, the melting point of each hydrolysis product was not depressed on admixture with the other, their IR and NMR spectra were indistinguishable, and the elemental analyses of both were in agreement with the product being 2,3-di(dodecanamido)propan-1-ol **3**.

The conversion of an alcohol into its phosphocholine derivative is usually carried out in one of two ways.[6] The first involves[4,7] reaction of the alcohol with β-bromoethyl phosphorodichloridate followed by hydrolysis and then nucleophilic displacement of bromide ion by trimethylamine. In the second and apparently preferable procedure,[8] the alcohol is treated with 2-chloro-1,3,2-dioxaphospholan-2-one (ethylene chlorophosphate)[9] **10** in benzene in the presence of triethylamine, the triethylammonium chloride so formed is removed by filtration, and the crude cyclic phosphate obtained on evaporation of the benzene solution is treated, in acetonitrile, with trimethylamine under pressure at 65 °C, or in *N*,*N*-dimethylformamide[10] at room temperature.§ Although this second technique is efficacious, we found it operationally tedious to remove by filtration triethylammonium chloride and to exchange the solvent under the strictly anhydrous conditions required. We have simplified the procedure, therefore, by carrying out the whole reaction in acetonitrile, and by using trimethylamine as both base and reagent. Thus, a suspension of **3** in acetonitrile containing trimethylamine and dissolved **10** was placed in a glass pressure vessel contained in a steel outer-container and heated at 65 °C for 16 h, then stored at 20 °C for 48 h. The product obtained on removal of the solvent was subjected to column chromatography to afford the required phosphocholine derivative **5** as a non-crystalline solid in 45% yield.

A similar sequence of reactions was performed on the diamine **7** using hexadecanoyl chloride as the acylating agent. As in the previous case, this reaction afforded, through crystallisation and then column chromatography, two diastereoisomeric products, (**C** and **D**), which in this case had m.p. 110–112 and 84–85 °C, and which both had an elemental analyses and spectral properties in agreement with the expected structure 1,2-di-

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‡ Since this starting material is racemic, all of the tetrahydropyran-2-yloxypropane [*O*-(tetrahydropyran-2-yl)glycerol] derivatives described in this paper are a mixture of four stereoisomers, which exist as two pairs of enantiomers. In this paper, reference to a 'diastereoisomer' of a tetrahydropyran-2-yloxypropane derivative is taken to mean a mixture of one pair of enantiomers. Clearly, all compounds lacking the tetrahydropyran-2-yl protecting group are racemic.

§ Ring opening of the cyclic phosphate is accelerated significantly in the presence of trimethylsilyl triflate.[11]

(hexadecanamido)-3-tetrahydropyran-2-yloxypropane **9**. Since on hydrolysis they both gave the same compound **4**, and their interconversion could not be achieved by recrystallisation with appropriate seeding, we surmise that **C** and **D** also are diastereoisomers resulting from the two chiral centres in the molecules.*

Conversion of **4** into the phosphocholine derivative **6** was performed as described for the transformation of **3** into **5**.

Compounds **5** and **6** were submitted for anti-HIV tests against HIV-1 IIIB in infected cell (C8166) cultures. Although **6** proved to be inactive, compound **5** showed moderate activity and reduced virus progeny in infected cells by 99% at 50 μmol dm^{-3}. This apparent dependence of anti-HIV activity on chain length of the amido groups appears to be significant in view of the observation[4] that in a series of racemic 3-amido-2-ethoxypropylphosphocholines **11** the chain length of the amido group may affect antiviral activity and toxicity. Thus, a comparison of compounds with amido groups of 16, 18 and 20 carbons, respectively, showed that the one with the octadecanamido group had the highest differential selectivity as measured by the ratio of the concentration required to inhibit cell growth by 50% to the concentration required to inhibit HIV-1 plaque formation by 50%. Further, it is noteworthy that in a series of alkylphospho *N*-methyl ethylamines **12**, activity against HIV-1 in C8166 T-lymphoblastoid cells was strongly dependent on chain length of the alkyl group.[12] Thus, the hexyl- and dodecyl-containing compounds were relatively inactive compared with the octadecyl and oleyl containing compounds. Further investigation into structure–activity relationships in these phosphocholine derivatives of 2,3-amidopropan-1-ol seems warranted.

Experimental

^1H NMR spectra were recorded in [^2H]chloroform or [$^2H_4$]-methanol (internal Me_4Si) at 270 MHz with a JEOL EX-90 spectrometer. *J*-Values are given in Hz. TLC and column chromatography was performed on silica gel (Machery–Nagel, SIL G-25UV$_{254}$ and Silica Gel 60 (Merck, 70–230 mesh), respectively. After TLC, phosphocholine derivatives were developed with a molybdic acid spray.[13] Organic solutions were dried over anhydrous sodium sulfate.

Preparation of the Diastereoisomers of 1,2-*Di*(*dodecanamido*)-3-*tetrahydropyran*-2-*yloxypropane* **8**.—A solution of 3-tetrahydropyran-2-yloxypropane-1,2-diamine **7** (2.48 g, 14.2 mmol), prepared[5] from the corresponding diazide, and dodecanoyl chloride (6.59 g, 30.1 mmol) in pyridine (10 cm^3) was stored for 12 h at room temp. Water (0.2 cm^3) was then added followed after 20 min by saturated aqueous sodium hydrogen carbonate (15 cm^3), with stirring. The mixture was extracted with dichloromethane (3 × 20 cm^3) and the combined extracts were washed with aqueous sodium hydrogen carbonate (25 cm^3) then water (2 × 20 cm^3). The dried organic phase was concentrated and pyridine removed by co-evaporation with toluene to afford, as a white solid, a mixture of diastereoisomers (7.74 g) which on recrystallisation from acetone gave material (4.03 g), m.p. 72–80 °C. Further recrystallisation from acetone gave material (1.79 g), m.p. 102–103 °C (with prior softening) which on column chromatography with ethyl acetate–light petroleum (1:4 v/v) gave one diastereoisomer **A** of **8** m.p. 108–109 °C (Found: 71.55; H, 11.9; N, 5.1. $C_{32}H_{62}N_2O_4$ requires C, 71.3; H, 11.6; N, 5.2%); ν_{max}(Nujol)/cm^{-1} 3280 (br, NH) and 1640 (CO); $\delta_H(CDCl_3)$ 0.88 (6 H, t, *J* 6.8, 2 × Me), 1.10–1.90 (42 H, complex, 21 × CH_2), 2.10–2.30 (4 H, m, 2 × CH_2CO), 3.10–4.20 (7 H, complex, 2 × CH_2O, CH_2N, CHN), 4.50 (1 H, m, OCHO) and 6.28–6.76 (2 H, br m, 2 × NH).

The mother liquor was concentrated and the residue subjected to column chromatography firstly with ethyl acetate–light petroleum (1:2 v/v) to afford a small amount (0.27 g) of unidentified material, and then with dichloromethane–methanol (9:1 v/v) to give the second diastereoisomer **B** of **8** which, after recrystallisation from acetone, had m.p. 76–77 °C (Found: C, 71.4; H, 11.8; N, 5.1. $C_{32}H_{62}N_2O_4$ requires C, 71.3; H, 11.6; N, 5.2%); ν_{max}(Nujol)/cm^{-1} 3300 (br, NH) and 1640 (CO); $\delta_H(CDCl_3)$ 0.88 (6 H, t, *J* 6.8, 2 × Me), 1.10–2.00 (42 H, complex, 21 × CH_2), 2.10–2.30 (4 H, m, 2 × CH_2CO), 3.14–3.94 (7 H, complex, 2 × CH_2O, CH_2N, CHN), 4.50 (1 H, m, OCHO) and 6.10–6.50 (2 H, br m, 2 × NH).

The two diastereoisomers gave indistinguishable solution (CH_2Cl_2) IR spectra, could not be separated by TLC, and, as expected, could not be interconverted by recrystallisation in the presence of the other.

Preparation of 2,3-*Di*(*dodecanamido*)*propan*-1-*ol* **3**.—(*a*) *From diastereoisomer* **A**. A solution of **A** (0.31 g) in methanol (40 cm^3) containing water (0.2 cm^3) and toluene-*p*-sulfonic acid (0.03 g) was heated at 50 °C for 2 h. TLC (ethyl acetate) indicated formation of a product with R_f 0.4 which was less mobile than the starting material (R_f 0.6). After neutralisation with aqueous sodium hydrogen carbonate the mixture was concentrated to give a solid which was dissolved in dichloromethane (20 cm^3) and this solution was then washed with water (2 × 20 cm^3), dried, and concentrated to give the *title compound* **3** (0.24 g, 92%), m.p. 106–107 °C (Found: C, 71.5; H, 12.1; N, 5.9. $C_{27}H_{54}N_2O_3$ requires C, 71.3; H, 12.0; N, 6.2%); ν_{max}(Nujol)/cm^{-1} 3640 (br, OH), 3300 (NH) and 1640 (CO); $\delta_H(CDCl_3)$ 0.88 (6 H, t, *J* 6.8, 2 × Me), 1.26 (32 H, br s, 16 × CH_2), 1.61 (4 H, br s, 2 × CH_2CH_2CO), 2.20 (4 H, m, 2 × CH_2CO), 3.15–3.95 (5 H, complex, CH_2O, CH_2N, CHN) and 6.41 and 6.55 (2 × 1 H, 2 × m, 2 × NH).

(*b*) *From diastereoisomer* **B**. Hydrolysis of **B** (0.46 g) in methanol (50 cm^3) containing water (0.24 cm^3) and toluene-*p*-sulfonic acid (0.025 g) as described for **A** afforded **3** (0.39 g, 99%), m.p. 106–107 °C (Found: C, 71.7; H, 12.3; N, 5.95. $C_{27}H_{54}N_2O_3$ requires C, 71.3; H, 12.0; N, 6.2%). The mixed m.p. of the two samples of **3** was 106–108 °C and they had indistinguishable IR and ^1H NMR spectra.

2,3-*Di*(*dodecanamido*)*propylphosphocholine* **5**.—To a suspension of compound **3** (0.227 g, 0.5 mmol) in acetonitrile (20 cm^3) was added trimethylamine (1.5 cm^3, 16.7 mmol) followed by 2-chloro-1,3,2-dioxaphospholan-2-one **10** (0.06 cm^3, 0.66 mmol). The mixture was placed in a glass pressure vessel enclosed in a stainless steel container † and the latter was heated in an oil bath at 65 °C for 16 h, then stored at 20 °C for 48 h. Methanol (20 cm^3) was then added to the reaction mixture which caused dissolution of a precipitate which had formed. Concentration of this solution gave a solid which was dissolved in a minimum amount of methanol (*ca.* 5 cm^3) and was thereby introduced onto a silica gel column (2.5 × 10 cm) which was eluted with dichloromethane–methanol, initially with a solvent ratio of 1:2 v/v and gradually changing to 1:4 v/v. Fractions containing the required product [R_f 0.17 in dichloromethane–methanol, 1:2 (v/v)] were combined and concentrated to afford, as a non-crystalline solid, the title compound **5** (0.14 g, 45%), *m/z* (FAB MS) 620.4761 (MH^+, $C_{32}H_{67}N_3O_6P$); ν_{max}(Nujol)/

* A by-product isolated in this preparation proved to be 1,5-di(hexadecanoyloxy)pentane which, we presume, arose by acylation of pentane-1,5-diol produced by reduction of tetrahydropyran-2-ol present as an impurity in one preparation of the diazido precursor of **7**.

† The steel container has a small hole drilled through it to vent the contents should the glass vessel fail.

cm^{-1} 1240 (P=O) and 1070 ($POCH_2$); $\delta_H(CD_3OD)$ 0.90 (6 H, t, *J* 6.6, 2 × Me), 1.29 (32 H, br s, 16 × CH_2), 1.60 (4 H, br s, 2 × CH_2CH_2CO), 2.18 (4 H, m, 2 × CH_2CO), 3.23 (9 H, s, Me_3N^+) and 3.24–4.35 [9 H, complex, $NCH_2CH(NR)CH_2OPO_3CH_2CH_2$].

Preparation of the Diastereoisomers of 1,2-*Di(hexadecanamido)*-3-*tetrahydropyran*-2-*yloxypropane* **9**.—Diamine **7** (2.04 g, 11.7 mmol) was acylated with hexadecanoyl chloride (7.43 g, 27 mmol) in pyridine (10 cm^3) as described for the preparation of **8**. After addition of saturated aqueous sodium hydrogen carbonate to the reaction mixture the latter was extracted with dichloromethane (3 × 80 cm^3). Storage of the combined extracts at 20 °C for 12 h induced crystallisation of a single diastereoisomer, **C**, of **9** (0.375 g, 5%), m.p. 110–112 °C (Found: C, 73.8; H, 12.4; N, 4.1. $C_{40}H_{78}N_2O_4$ requires C, 73.8; H, 12.1; N, 4.3%); ν_{max}(Nujol)/cm^{-1} 3280 (br, NH) and 1640 (CO); $\delta_H(CDCl_3)$ 0.88 (6 H, t, *J* 6.7, 2 × Me), 1.15–1.95 (58 H, complex, 29 × CH_2), 2.10–2.30 (4 H, m, 2 × CH_2CO), 3.18–4.22 (7 H, complex, 2 × CH_2O, CH_2N, CHN), 4.52 (1 H, m, OCHO) and 6.10–6.75 (2 H, br m, 2 × NH). The mother liquors were concentrated to dryness and crystallised from acetone–methanol to give material (4.87 g), m.p. 47–85 °C.* Column chromatography of this material with ethyl acetate–light petroleum (1:2 v/v) first gave 1,5-di(hexadecanoyloxy)-pentane (1.67 g), m.p. 48–49 °C, mixed m.p. with an authentic sample 48–49 °C. Further elution with dichloromethane–methanol (9:1 v/v) gave, after fractions containing mixtures, the second stereoisomer **D** of **9** (2.14 g), m.p. 84–85 °C (Found: C, 73.9; H, 12.35; N, 4.1. $C_{40}H_{78}N_2O_4$ requires C, 73.8; H, 12.1; N, 4.3%); ν_{max}(Nujol)/cm^{-1} 3300 (br, NH) and 1642 (CO); $\delta_H(CDCl_3)$ 0.88 (6 H, t, *J* 6.6, 2 × Me), 1.15–1.90 (58 H, complex, 29 × CH_2), 2.10–2.30 (4 H, m, 2 × CH_2CO), 3.15–3.95 (7 H, complex, 2 × CH_2O, CH_2N, CHN), 4.50 (1 H, m, OCHO) and 6.30–6.78 (2 H, br m, 2 × NH). Compounds **C** and **D** gave similar solution (CH_2Cl_2) IR spectra, and were not interconverted if recrystallised from acetone–methanol in the presence of the other.

Preparation of 2,3-*Di(hexadecanamido)propan*-1-*ol* **4**.—(*a*) *From diastereoisomer* **C**. Hydrolysis of **C** (0.217 g) in a similar manner to that reported for preparation of **3**, followed by cooling of the methanolic solution, gave directly compound **4** (0.17 g, 90%), m.p. 110–112.5 °C (Found: C, 73.9; H, 12.6; N, 4.6. $C_{35}H_{70}N_2O_3$ requires C, 74.1; H, 12.45; N, 4.9%); ν_{max}(Nujol)/cm^{-1} 3600–3120 (br, OH), 3300 (NH) and 1640 (CO); $\delta_H(CDCl_3)$ 0.88 (6 H, t, *J* 6.9, 2 × Me), 1.25 (48 H, br s, 24 × CH_2), 1.62 (4 H, br s, 2 × CH_2CH_2CO), 2.20 (4 H, m, 2 × CH_2CO), 3.16–3.95 (5 H, complex, CH_2O, CH_2N, CHN) and 6.37 (2 H, m, 2 × NH).

(*b*) *From diastereoisomer* **D**. Hydrolysis of **D** (0.378 g) in a similar manner to that described for **C** afforded, on direct crystallisation from the cooled methanolic solution, compound **4** (0.299 g, 91%), m.p. 111–112 °C (Found: C, 74.1; H, 12.6; N, 4.6. $C_{35}H_{70}N_2O_3$ requires C, 74.1; H, 12.45; N, 4.9%). The mixed m.p. of the two samples of **4** was 110–112 °C and they had indistinguishable IR and ^1H NMR spectra.

* By column chromatography (ethyl acetate–light petroleum 1:4 → 1:2 v/v) of a second crop of crystals, material (0.54 g), m.p. 46–47 °C, was isolated which was shown by comparison of its IR and ^1H NMR spectra with those of an authentic sample to be 1,5-di(hexadecanoyloxy)pentane.

2,3-*Di(hexadecanamido)propylphosphocholine* **6**.—A suspension of compound **4** (0.139 g, 0.244 mmol) in acetonitrile (25 cm^3) was treated with trimethylamine (2 cm^3, 22.2 mmol) and 2-chloro-1,3,2-dioxaphospholan-2-one **10** (0.03 cm^3, 0.33 mmol) in a glass pressure vessel as described for the preparation of the propylphosphocholine **5**. Methanol (25 cm^3) was then added to the reaction mixture, a small amount of remaining solid was removed by filtration, and the filtrate was concentrated to give a solid which was subjected to column chromatography with dichloromethane–methanol, initially 1:1 v/v changing to 1:3 v/v. Combination and concentration of the fractions containing the required product [R_f 0.17 in dichloromethane–methanol 1:2 (v/v)] afforded, as a non-crystalline solid, compound **6** (0.065, g, 37%), *m/z* (FAB MS) 732.6044 (MH^+, $C_{40}H_{83}N_3O_6P$); ν_{max}(Nujol)/cm^{-1} 1240 (P=O) and 1070 ($POCH_2$); $\delta_H(CD_3OD)$ 0.89 (6 H, t, *J* 6.9, 2 × Me), 1.29 (48 H, br s, 24 × CH_2), 1.60 (4 H, br s, 2 × CH_2CH_2CO), 2.18 (4 H, m, 2 × CH_2CO), 3.22 (9 H, s, Me_3N^+) and 3.30–4.32 [9 H, complex, $NCH_2CH(NR)CH_2OPO_3CH_2CH_2$].

1,5-*Di(hexadecanoyloxy)pentane*.—Acylation of pentane-1,5-diol with hexadecanoyl chloride in pyridine in the usual way and crystallisation of the product from methanol gave the title compound, m.p. 48–49 °C (Found: C, 76.5; H, 12.7. $C_{37}H_{72}O_4$ requires C, 76.5; H, 12.5%).

Acknowledgements

This work was supported with funds from the People's Republic of China (to C. J.), the University of East Anglia, and the British Technology Group. We thank the SERC Mass Spectrometry Service Centre at Swansea for determination of the high resolution mass spectra, Dr. N. Mahmood of the MRC Collaborative Centre for conducting the anti-HIV experiments and Dr. M. J. Cook for helpful discussion.

References

1 J. C. Briggs, A. P. Dawson, I. Gibson, A. H. Haines, J. Hook, A. Lloyd, S. Meiners and R. J. K. Taylor, *BioMed. Chem. Lett.*, 1992, **2**, 127.
2 J. C. Briggs, A. H. Haines, R. J. K. Taylor, A. P. Dawson, I. Gibson, J. Hook, A. Lloyd and S. Meiners, *Carbohydr. Res.*, 1992, **234**, 23.
3 J. C. Briggs, A. H. Haines and R. J. K. Taylor, unpublished results.
4 K. L. Meyer, C. J. Marasco, Jr., S. L. Morris-Natschke, K. S. Ishaq, C. Piantadosi and L. S. Kucera, *J. Med. Chem.*, 1991, **34**, 1377.
5 M. S. Okamoto and E. K. Barefield, *Inorg. Chem.*, 1974, **13**, 2611.
6 See, for example, S. K. Bhatia and J. Hajdu, *Synthesis*, 1989, 16.
7 P. P. M. Bonsen, G. H. de Haas, W. A. Pieterson and L. L. M. van Deenen, *Biochem. Biophys. Acta*, 1972, **270**, 364.
8 N. S. Chandrakumar and J. Hajdu, *J. Org. Chem.*, 1982, **47**, 2144.
9 N. T. Thuong and P. Chabrier, *Bull. Soc. Chim. Fr.*, 1974, 667.
10 U. T. Kim, S. K. Bhatia and J. Hajdu, *Tetrahedron Lett.*, 1991, **32**, 6521.
11 T. R. Gadek, *Tetrahedron Lett.*, 1989, **30**, 915.
12 C. McGuigan, T. J. O'Connor, B. Swords and D. Kinchington, *AIDS*, 1991, **5**, 1536.
13 J. C. Dittmer and R. L. Lester, *J. Lipid Res.*, 1964, **5**, 126.

Paper 3/03664B
Received 28*th June* 1993
Accepted 13*th July* 1993