Pergamon

Tetrahedron Letters 40 (1999) 8985–8988

TETRAHEDRON LETTERS

A Versatile Synthesis of Tetraester Polyamine Lipids for Gene Transfection

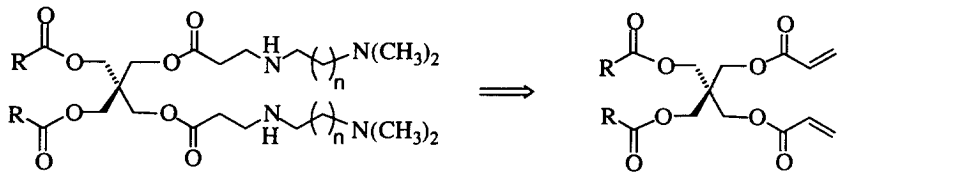
Mike E. Lizarzaburu, Mark J. Kurth,* and Michael H. Nantz*

Department of Chemistry, One Shields Avenue
University of California, Davis CA 95616

Received 6 August 1999; revised 4 October 1999; accepted 7 October 1999

Abstract: The preparation of polyamine lipids via Michael addition of diamines to tetraester bisacrylates is described. A new pentaerythritol protection strategy enabled the synthesis of a bis(*p*-methoxybenzyl)diether diol which was used to prepare the saturated and unsaturated diester cores.
© 1999 Elsevier Science Ltd. All rights reserved.

The inability to efficiently introduce foreign DNA into eukaryotic cells continues to be a considerable obstacle for the advancement of gene therapy and is prompting the need to find more active non-viral transfection reagents.[1] Systematic examinations of lipid architecture have focused on the influence of the polar domain and hydrophobic domain on transfection activity.[2] Since polyamine headgroups have shown the ability to efficiently bind and package DNA, the incorporation of polyamines into the polar domains of transfection lipids has emerged as powerful means to improve gene transfer activity.[3] We disclose herein a method for the incorporation of polyamine headgroups onto a tetraester lipid framework that was shown to have reduced cytotoxicity in DNA transfection experiments.[4] Our method to prepare the representative polyamine lipids **1-4** relies on a convenient synthesis of bisacrylates **5** and **6**. A novel bis-protection of pentaerythritol was developed to make these bisacrylates readily available.



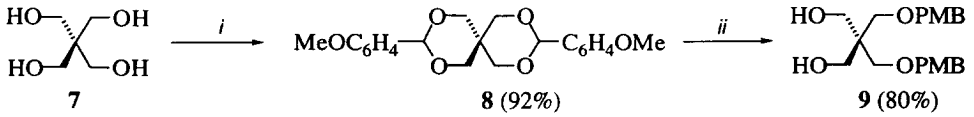
1: R = $CH_2(CH_2)_{11}CH_3$, n = 1
2: R = $CH_2(CH_2)_{11}CH_3$, n = 2
3: R = (*Z*)-$CH_2(CH_2)_6CH{=}CH(CH_2)_7CH_3$, n = 1
4: R = (*Z*)-$CH_2(CH_2)_6CH{=}CH(CH_2)_7CH_3$, n = 2

5: R = $CH_2(CH_2)_{11}CH_3$
6: R = (*Z*)-$CH_2(CH_2)_6CH{=}CH(CH_2)_7CH_3$

The key step in the synthesis of the target polyamine lipids involves the Michael addition of a diamine to bisacrylate **5** or **6**. A convenient synthesis of the diester precursors to **5** and **6**, however, proved to be the more challenging aspect of this work as was encountered during construction of analogous pentaerythritol tetraesters.[4] Efficient bisesterfication of penterythritol is inherently difficult due to problems of over-esterification. The di- and trioleoyl esters of penterythritol are particularly difficult to separate using column chromatography. Indeed, our best direct bisesterification of pentaerythritol to obtain the dioleoyl derivative proceeded in 30% yield.[4] We addressed this problem by extending a strategy first reported by Issidoridies and coworkers who demonstrated that pentaerythritol diacetals can be reductively cleaved to

0040-4039/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(99)01934-6

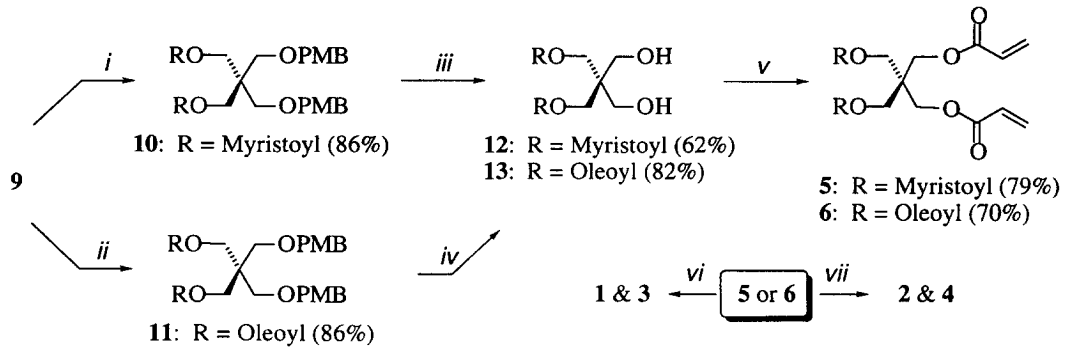
afford diol diethers.[5] Recently we disclosed a multigram synthesis of the dimyristoyl (C14, saturated) derivative of pentaerythritol utilizing a dibenzyl ether intermediate.[6] However, using this protocol, we were unable to synthesize unsaturated diester derivatives since palladium-catalyzed hydrogenolysis of the benzyl groups is not compatible with unsaturation. We anticipated that bis(*p*-methoxybenzyl) (PMB) diether diol **9** (Scheme 1) could easily be obtained by the reductive cleavage of bis(*p*-methoxybenzylidene) **8** and would have general utility for the synthesis of both the saturated and unsaturated diesters of pentaerythritol.



Scheme 1. *i.* *p*-$CH_3OC_6H_4CHO$ (2 eq), cat. TsOH, C_6H_6, reflux, 12 h; *ii.* DIBAL-H (5 eq), hexane-CH_2Cl_2, 0 °C to rt, 22 h.

Upon cooling to room temperature, bis(benzylidene) **8** crystallized from the reaction of **7** as outlined in **Scheme 1** and was suitably pure for further reaction. Reductive cleavage of the diacetal functionality was effected by addition of excess diisobutylaluminum hydride (DIBAL-H). The bis(PMB)diether diol **9** was isolated in 74% (from **7**) after recrystalization from *n*-butanol.

Bisesterification of diol **9** using either a saturated or unsaturated fatty acid was performed using standard procedures. A dimyristoyl analog was prepared by addition of a stoichiometric amount of the myristoyl chloride giving diether diester **10** after a filtration through a bed of silica gel (CH_2Cl_2 eluent). Introduction of an unsaturated side chain was smoothly accomplished by addition of the mixed anhydride of oleic acid, prepared *in-situ* by reaction of oleic acid with pivaloyl chloride.[7] Diester **11** required purification using SiO_2 column chromatography (15:85 EtOAc:hexanes).



Scheme 2. *i.* $CH_3(CH_2)_{12}C(O)Cl$ (2.2 eq), Et_3N, cat. DMAP, CH_2Cl_2, 0 °C to rt, 4h; *ii.* a. $(CH_3)_3CC(O)Cl$ (2.1 eq.), (Z)-$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$ (2.1 eq), Et_3N, cat. DMAP, CH_2Cl_2, 0 °C to rt, 1h; b. **9**, 4h; *iii.* 10% Pd/C, H_2, EtOAc, 24h; *iv.* $(NH_4)_2Ce(NO_3)_6$ (6 eq), CH_3CN:H_2O:CH_2Cl_2 9:1:3, rt, 40 min; *v.* $H_2C{=}CHC(O)Cl$ (3.0 eq), Et_3N (3 eq), cat. DMAP, CH_2Cl_2, 0 °C to rt, 24h; *vi.* N,N'-dimethylethylenediamine (20 eq), THF, rt, 2 h; vii. 3-(dimethylamino)propylamine (20 eq), THF, rt, 2h.

The PMB protecting groups in **10** were removed by catalytic hydrogenolysis to produce diester diol **12.** Removal of the PMB protecting groups in **11** proved to be challenging. Standard DDQ deprotection procedures produced large amounts of the corresponding mono(benzylidene) and mono(benzoate) byproducts.[8] Treatment of **11** with Me_2BBr was also ineffective due principally to complications of product isolation arising from strong chelation of boron to the 1,3-diol.[9] Fortunately, treatment of **11** with cerric ammonium nitrate (CAN) resulted in PMB ether cleavage to give diol

13.[10] The bis(PMB)-deprotection proceeds smoothly using 6 equivalents of CAN at room temperature to afford **13** in 82% yield after chromatography. Diester diols **12** and **13** were esterified by addition of acryloyl chloride to give bisacrylates **5** and **6.** Finally, the desired polyamine lipids were prepared by Michael addition of either N,N-dimethylethylenediamine (20 eq) or 3-(dimethylamino)-propylamine (20 eq) to bisacrylate **5** or **6** giving compounds **1 - 4** in 83-96% yield.[11, 12]

The possibility of a retro-Michael reaction potentially would limit the application of polyamines **1-4** in gene transfection experiments. However, we were gratified to find that the polyamines were stable to column chromatography (SiO_2; 20:80 MeOH:CH_2Cl_2, 0.5% Et_3N) and to liposome formulation conditions (hydration at 8 mM, vortex mixing, warming to 50 °C with sonication). Spectral examination of the polyamines recovered after liposome formulation revealed only slight (~5%) decomposition arising from ester hydrolysis.[13] We found no evidence to suggest facile retro-Michael decomposition. Studies on DNA complexation and delivery using compounds **1-4** are underway.

In conclusion, we have disclosed methodology that permits the flexible synthesis of polyamine lipids wherein the polar and hydrophobic domains are linked via a biocompatible tetraester core. Furthermore, the reductive cleavage of bis(*p*-methoxybenzylidene) acetals may find general use in the selective bisprotection of other tetrols.

Acknowledgment. Funding from the Cystic Fibrosis Foundation and the National Science Foundation is gratefully acknowledged. We thank Mr. Edmund Niedzinski and Mr. Ryan Jones for preliminary studies.

References and notes:

1 (a) Bally, M. B.; Harvie, P.; Wong, F. M. P.; Kong, S.; Wasan, E. K.; Reimer, D. L. *Adv. Drug Delivery Rev.* **1999**, *38*, 291. (b) A. D. Miller, *Angew. Chem. Int. Ed.* **1998**, *37*, 1768.

2 (a) Lee, R. E.; Marshall, J.; Siegel, C. S.; Jiang, C.; Yew, N. S.; Nichols, M.; Nietupski, J. B.; Ziegler, R. J.; Lane, M. B.; Wang, K.X.; Wan, N.C.; Scheule, R.K.; Harris, D.J.; Smith, A.E.; Cheng, S.H. *Human Gene Therapy* **1996**, 7, 1701. (b) Bennett, M. J.; Aberle, A. M.; Balasubramaniam, R. P.; Malone, J. G.; Malone, R. W.; Nantz, M. H. *J. Med. Chem.* **1997**, *40*, 4069.

3 (a) Behr, J.P.; Demeneix, B.; Loeffler, J.P.; Perez-Mutul, J. *Proc. Natl. Acad. Sci., USA* **1991**, *86*, 6982. (b) Remy, J.S.; Sirlin, C.; Vierling, P.; Behr, J.P. *Bioconjugate Chem.* **1994**, *5*, 647. (c) Byk, G.; Frederic, M.; Scherman, D. *Tetrahedron Lett.* **1997**, *38*, 3219. (e) Geall, A. J.; Blagbrough, I. S. *Tetrahedron Lett.* **1998**, *39*, 443.

4 Aberle, A. M.; Tablin, F.; Zhu, J.; Walker, N. J.; Gruenert, C. D.; Nantz, M. H. *Biochemistry* **1998**, *37*, 6533.

5 Abdun-Nur, A. R.; Issidoridies, C. H. *J. Org. Chem.* **1962**, *27*, 67.

6 Lizarzburu, M. E.; Jones, R. M.; Nantz, M. H.; Kurth, M. J. *Org. Prep. Proc. Int.* **1999**, *31*, 0000.

7 Leplawy, M. T.; Jones, D. S.; Kenner, G. W.; Sheppard, R. C. *Tetrahedron* **1960**, *11*, 39.

8 Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 889.

9 Srisiri, W.; Lamparski, H.G.; O'Brien D.F. *J. Org. Chem.* **1996**, *61*, 5911.

10 Johansson, R.; Samuelsson, B. *J. Chem. Soc., Chem. Commun.* **1984**, *108*, 201.

11 For an analogous reaction, see: Pastor, S. D.; Togni, A. *Tetrahedron Lett.* **1990**, *31*, 839.

12 Spectral and analytical data for new compounds: **1**: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, *J* = 6.8 Hz, 6 H), 1.23 (m, 40 H), 1.56 (m, 4 H), 2.22 (s, 12H) 2.27 (t, *J* = 7.5 Hz, 4 H), 2.42 (t, *J* = 6.2 Hz, 4 H), 2.53 (t, *J* = 6.6 Hz, 4 H), 2.70 (t, *J* = 6.2 Hz, 4 H), 2.88 (t, *J* = 6.6 Hz, 4 H), 4.08 (s, 4 H), 4.11 (s, 4 H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.7, 29.0-29.5 (5 signals), 31.8, 34.0, 34.4, 34.4, 41.9, 44.9, 45.3, 46.8, 61.8, 62.1, 171.9, 173,1; HRMS ($C_{47}H_{93}O_8N_4$, MH^+) calcd, 841.6993; found, 841.7039.

2: ^{1}H NMR ($CDCl_3$) δ 0.87 (t, *J* = 6.3 Hz, 6 H), 1.25 (m, 40 H), 1.59 (m, 4 H), 1.70 (m, 4H), 2.25 (s, 12H), 2.30 (m, 4 H), 2.38 (m, 4 H), 2.58 (t, *J* = 6.2 Hz, 4 H), 2.71 (t, *J* = 6.3 Hz, 4 H), 2.90 (m, 4 H), 4.10 (s, 4 H), 4.12 (s, 4 H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.8, 27.4, 29.1-29.6 (5 signals), 31.8, 34.0, 34.2, 42.0, 44.8, 45.4, 48.2, 57.9, 61.9, 62.1, 172.0, 173,1; HRMS ($C_{49}H_{97}O_8N_4$, MH^+) calcd, 869.7306; found, 869.7312.

3: ^{1}H NMR ($CDCl_3$) δ 0.87 (t, *J* = 6.0 Hz, 6 H), 1.26 (m, 20 H), 1.29 (m, 20 H), 1.56 (m, 4 H), 2.00 (M, 8 H), 2.24 (s, 12H), 2.29 (t, *J* = 7.7 Hz, 4 H), 2.44 (t, *J* = 6.3 Hz, 4 H), 2.54 (t, *J* = 6.5 Hz, 4 H), 2.71 (t, *J* = 6.0 Hz, 4 H), 2.90 (t, *J* = 6.5 Hz, 4 H), 4.10 (s, 4 H), 4.13 (s, 4 H), 5.33 (M, 4H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.7, 27.41(2 signals), 29.0-29.6 (5 signals), 31.8, 33.9, 34.3, 41.9, 46.7, 58.4, 61.8, 62.1, 129.6, 129.9, 171.9, 173.0; HRMS ($C_{55}H_{105}O_8N_4$, MH^+) calcd, 949.7932; found, 949.7904.

4: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, *J* = 6.5 Hz, 6 H), 1.24 (m, 20 H), 1.27 (m, 20 H), 1.57 (m, 4 H), 1.65 (m, 4 H), 1.98 (M, 8 H), 2.20 (s, 12 H), 2.29 (m 8 H), 2.52 (t, *J* = 6.5 Hz, 4 H), 2.65 (t, *J* = 6.9 Hz, 4 H), 2.86 (t, *J* = 6.3 Hz, 4 H), 4.08 (s, 4 H), 4.10 (s, 4 H), 5.30 (M, 4 H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.7, 27.1, 29.1-29.6 (5 signals), 31.8, 33.9, 34.3, 41.9, 44.8, 45.4, 48.1, 57.9, 61.8, 62.0, 129.6, 129.9, 172.0, 173.0; HRMS ($C_{57}H_{109}O_8N_4$) calcd, 977.8245; found, 977.8279.

5: ^{1}H NMR ($CDCl_3$) δ 0.87 (t, *J* = 6.5 Hz, 6 H), 1.25 (m, 40 H), 1.59 (m, 4 H), 2.30 (t, *J* = 6.9 Hz, 4 H), 4.16 (s, 4 H), 4.24 (s, 4 H), 5.87 (dd, *J* = 10.5, 1.1 Hz, 2H), 6.10 (dd, *J* = 17.3, 6.41 (dd, *J* = 10.4 , 9 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.7, 29.0-29.5 (5 signals), 31.8, 34.0, 41.9, 62.1, 62.4, 127.6, 131.5, 165.3, 173,1; Anal. calcd for $C_{57}H_{92}O_8$: C, 70.44 H, 10.31. Found: C, 70.39; H, 10.31.

6: ^{1}H NMR ($CDCl_3$) δ 0.87 (t, *J* = 6.0 Hz, 6 H), 1.26 (m, 20 H), 1.28 (m, 20 H), 1.59 (m, 4 H), 1.99 (m, 8 H), 2.30 (t, *J* = 7.5 Hz, 4 H), 4.16 (s, 4 H), 4.24 (s, 4 H), 5.36 (m, 4 H) 5.86 (d, *J* = 10.5 Hz, 2 H), 6.10 (dd, *J* = 17.4, 10.5 Hz, 2 H), 6.41 (d, *J* = 17.4 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 13.9, 22.5, 24.7, 27.0 (2 signals), 28.9 -29.6 (6 signals), 31.7, 33.8, 41.9, 62.1, 62.4, 127.5, 129.5, 129.7, 131.3, 165.2, 172.9; Anal. calcd for $C_{57}H_{92}O_8$: C, 73.02; H, 10.43. Found: C, 72.47; H, 10.36.

8: ^{1}H NMR ($CDCl_3$) δ 3.64 (d, *J* = 11.4 Hz, 2 H), 3.81 (s, 6 H), 3.84 (m, 4 H), 4.85 (dd, *J* = 11.4 Hz, 0.9 Hz, 2 H), 5.41 (s, 2 H), 6.90 (d, *J* = 8.9 Hz, 4 H), 7.41 (d, *J* = 8.9 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 32.3, 55.2, 70.5, 71.0, 102.1, 113.5, 127.3, 130.4, 160.0. *Anal.* Calcd for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50. Found: C, 67.49; H, 6.52.

9: ^{1}H NMR ($CDCl_3$) δ 2.60 (s, 2 H, O*H*), 3.52 (s, 4 H), 3.65 (s, 4 H), 3.80 (s, 6 H), 4.42 (s, 4 H), 6.87 (d, *J* = 8.4 Hz, 4 H), 7.20 (d, *J* = 8.4 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 44.6, 54.9, 64.3, 70.9, 73.0, 113.6, 128.9, 129.9, 159.0; Anal. calcd for $C_{21}H_{28}O_6$: C, 67.00; H, 7.50. Found C, 66.92; H, 7.49.

10: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.5 Hz, 6 H), 1.25 (m, 40 H), 1.54 (m, 4 H), 2.21 (t, *J* = 7.5 Hz, 4 H), 3.41 (s, 4 H), 3.79 (s, 4 H), 4.12 (s, 4 H), 4.38 (s, 4 H), 6.84 (d, *J* = 8.4 Hz, 4 H), 7.18 (d, *J* = 8.4 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 24.9, 29.1-29.6 (5 signals), 31.9, 34.2, 55.1, 62.9, 68.2,72.9, 113.6, 129.0, 130.3, 159.1, 173.3. Anal. Calcd for $C_{49}H_{80}O_8$: C, 73.82; H, 10.12. Found: C, 73.77; H, 10.14.

11: ^{1}H NMR ($CDCl_3$) δ 0.86 (t, *J* = 6.8 Hz, 6 H), 1.27 (m, 40 H), 1.54 (m, 4 H), 2.01 (m, 8 H), 2.21 (t, *J* = 7.5 Hz, 4 H), 3.42 (s, 4 H), 3.79 (s, 6 H), 4.12 (s, 4 H), 4.38 (s, 4 H), 5.33 (m, 4 H), 6.84 (d, *J* = 8.7 Hz, 4 H), 7.18 (d, *J* = 8.4 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.8, 27.1, 29.1-29.6 (5 signals), 31.8, 34.1, 43.4, 55.1, 62.9, 68.2, 72.9, 113.5, 128.9, 129.6, 129.9, 130.2, 159.0, 173.2. Anal. calcd for $C_{57}H_{92}O_8$: C, 75.62; H, 10.24. Found C, 75.54; H, 10.26.

13 Ester hydrolysis is suggested by the emergence of ^{1}H NMR signals at δ 3.53-3.57, presumably corresponding to hydroxymethyl groups (e.g., as in precursors **12** and **13**).