SYNTHESIS OF TETRAPYRROLE NITROGEN MUSTARDS WITH POTENTIAL ANTI-TUMOR ACTIVITIES

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Abstract - Eight porphyrin nitrogen mustards and six meso-tetraphenylporphin nitrogen mustards were synthesized. Most of the compounds possess both the chemotherapeutic and photochemotherapeutic effects on tumor.

INTRODUCTION

Lack of selectivity between normal and tumor tissues greatly limited the use and efficiency of chemotherapeutic drugs in clinical tumor therapy. With the development of laser medicine and technology in light-conducting fiber and endoscope, photodynamic therapy (PDT) obtained regulatory approvals in the United States and elsewhere[1,2] to cure malignent tumors in skin, esophagi, bronchi and bladder. PDT has achieved selective tumoricidal effects without many of the serious side effects of conventional therapy in a variety of tumors, coming closer to the cancer treatment paradigm of the selective destruction of tumor tissue without disruption of normal tissue function.[3]

Hematoporphyrin derivatives(HpD) are porphyrin complex, some components of which can retain in skin and result in the cutaneous photosensitivity which would last for 6 to 8 weeks after the treatment.[4,5] We focused our attention on both the alleviation of patient' s suffering from damages of normal tissues in chemotherapy and the elimination of cutaneous photosensitivity as well as the enhancement of the depth of tumor necrosis, which is generally not more than 8-10 nm even under the maximum safe light dose in PDT. Thus, we synthesized a series of new compounds tetrapyrrole nitrogen mustards by introducing the alkylating agent nitrogen mustard into the periphery of porphyrins and meso-tetraphenyl porphins.[6]

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Biological experiments showed that the photosensitizing abilities of some porphin nitrogen mustards were higher than those of HpD.[6] Most of the compounds possess both the chemotherapeutic and photochemotherapeutic effects on animal tumor as well as human cancer cells *in vitro*.[6] The concentration of porphyrin methyl ether nitrogen mustard($\mathbf{I_2}$) was over 40 times higher in tumor than that in liver at a given time post drug administration. It was also shown that $\mathbf{I_2}$ could both inhibit the synthesis of DNA (as shown by the value of counts per minute after being treated with ^{3}H-thymodeoxyriboside) and destroy the cell membrane (as shown by the increasing of the activity of lactic acid dehydration enzyme in the culture medium); the inhibiting or destructive activity enhanced with the increase of light dose.[7] These biological experimental results will be reported in relating journals in detail.

RESULTS AND DISCUSSION

Porphyrin nitrogen mustards, namely, 2,7,12,18-tetramethyl-13,17-di[3-*N*,*N*-di(2-chloroethyl)-aminopropyl]porphin ($\mathbf{I_1}$) and 2,7,12,18-tetramethyl-3,8-di(1-alkyloxyethyl)-13,17-bis[3-*N*,*N*-di(2-chloroethyl)aminopropyl]porphin ($\mathbf{I_{2\text{-}8}}$) were synthesized from the starting material hemin(**1**). Preparations of deuteroporphyrin IX and 3,8-di(1-bromoethyl)deuteroporphyrin IX(**4**) were carried out following the reported procedures.[8]

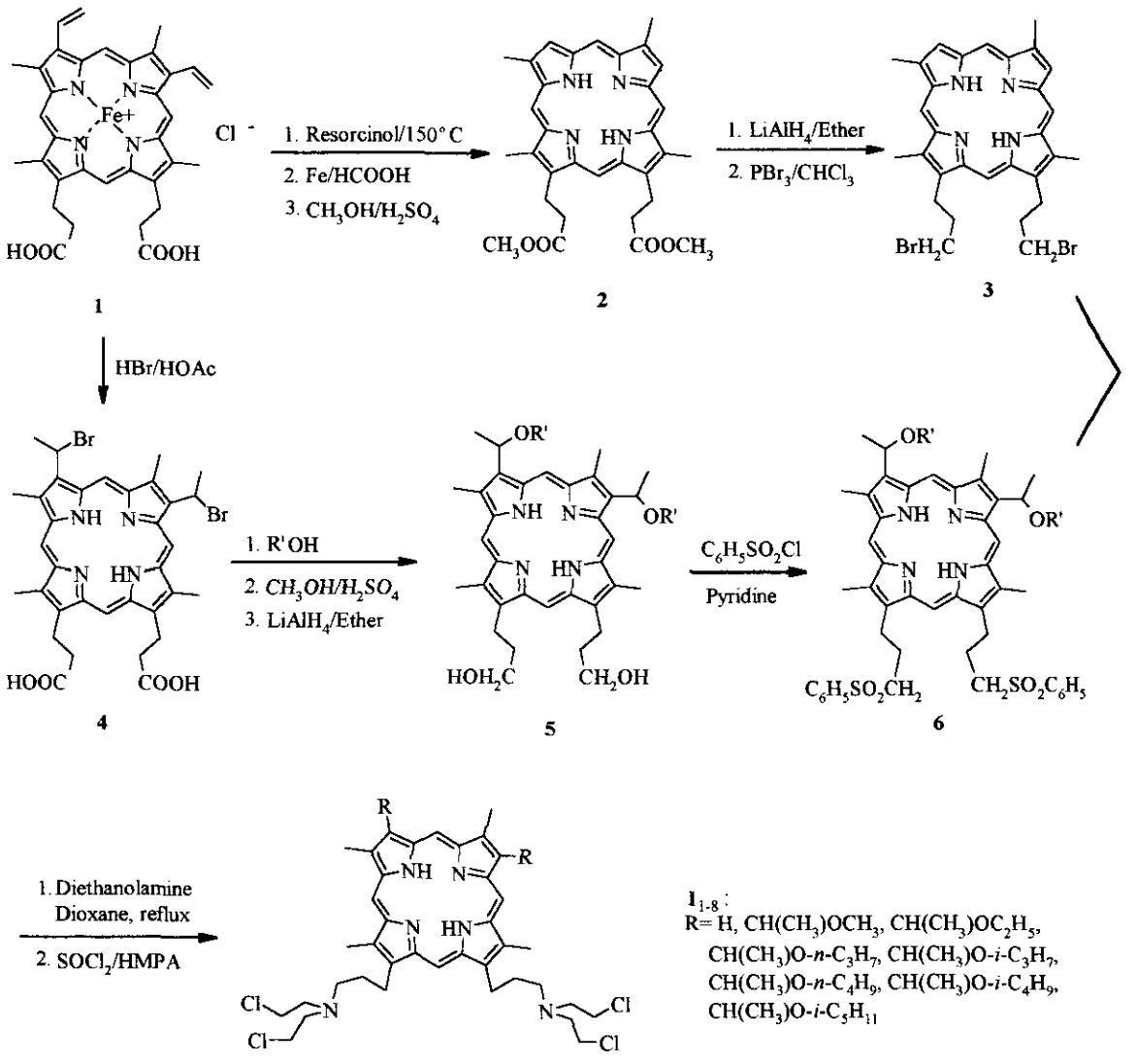
Deuteroporphyrin IX dimethyl ester(**2**) was prepared by esterification of deuteroporphyrin IX in methanol in the presence of H_2SO_4. Reduction of the ester (**2**) with $LiAlH_4$ in ether at room temperature gave the diol. Porphyrin-nitrogen mustard($\mathbf{I_1}$) was obtained by treating the diol with PBr_3 in chloroform followed by refluxing with diethanolamine in dioxane and chlorination with $SOCl_2$ in HMPA. The overall yield was about 15% (based on hemin).

4 reacted with different alcohols to give hematoporphyrin IX 3,8-diethers which were esterified in methanol catalyzed by H_2SO_4(*5%, molar ratio*) and then reduced with $LiAlH_4$ to give the corresponding diol(**5**). Porphyrin-nitrogen mustards($\mathbf{I_{2\text{-}8}}$) were prepared through the reaction of **5** with benzenesulfonyl chloride and pyridine followed by refluxing with diethanolamine in dioxane and chlorination with $SOCl_2$ in HMPA (Scheme **1**). The mean overall yield of $\mathbf{I_{2\text{-}8}}$ was 13% (based on **4**).

We found porphins with 1-alkyloxyethyl group at positions-3,8 can be decomposed by PBr_3, so benzenesulfonyl chloride and pyridine was used in place of PBr_3 in Scheme **1**. When R was replaced by vinyl- or 1-[(2-alkyloxyethyl)oxy]ethyl groups, porphins with [3-*N*,*N*-di(2-hydroxyethyl)]aminopropyl groups at positions-13,17 also could be destroyed completely by $SOCl_2$ in HMPA.

3-Chloromethyl-4-methoxybenzaldehyde(**7**) was prepared by reaction of anisaldehyde with formaldehyde catalyzed by hydrochloride. 1 Equivalent of **7** was condensed with 3 equivalent of substituted benzaldehydes and pyrrole in chloroform catalyzed by BF_3-$(C_2H_5)_2O$ [9] at room temperature. The product

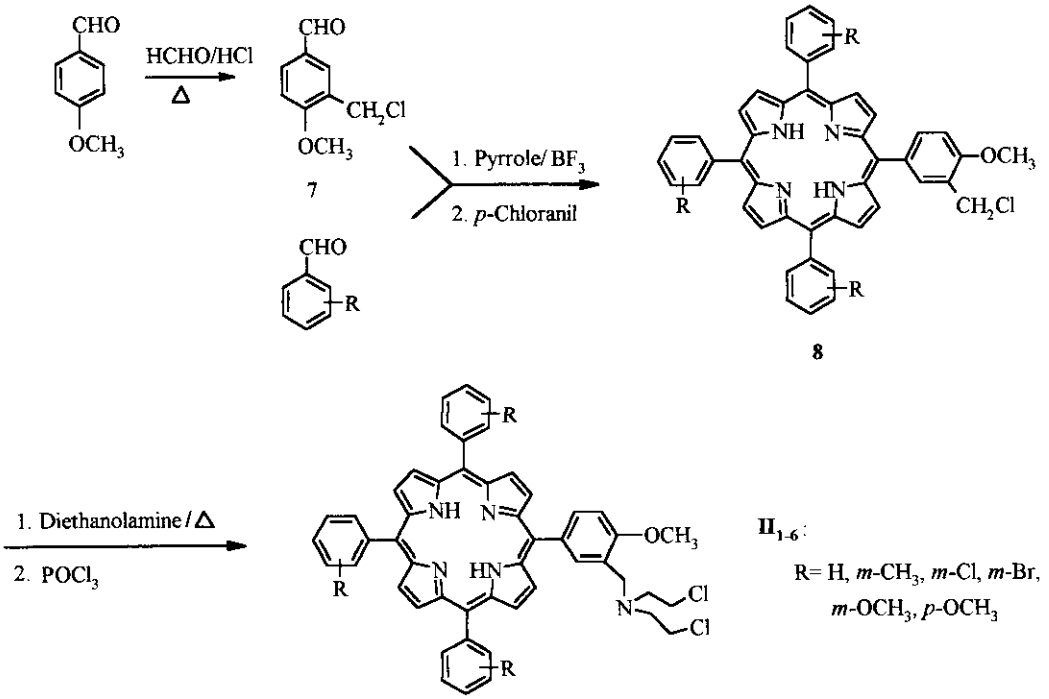
was then oxidized with tetrachloro-1,4-benzoquinone at 60°C to give 5-(3-chloromethyl-4-methoxy)phenyl-10,15,20-tri(substituted phenyl)porphins(**8**). **8** reacted with diethanolamine and $POCl_3$, in turn, to give meso-tetra(substituted phenyl)porphin nitrogen mustards, namely, 5-[3-*N*,*N*-di(2-chloroethyl)aminomethyl-4-methoxy]phenyl-10,15,20-tri(substituted phenyl)porphin($\mathbf{II}_{1\text{-}6}$) (Scheme **2**). The mean yield was 3.8%.



Scheme 1

Organic acids such as propanoic acid and trifluoroacetic acid as solvent could catalyze the condensation of pyrrole with benzaldehyde without liable functional group under reflux.[10] BF_3-$(C_2H_5)_2O$ was used in this experiment in place of organic acid in $CHCl_3$ at room temperature to avoid the coupling reaction between

benzyl chloride and pyrrole. The color of the reaction mixture was orange red at the beginning, then gradually turned into purple. Yellow precipitate yielded immediately when benzaldehyde wass replaced by *p*-nitrobenzaldehyde or *o*-nitrobenzaldehyde. UV/Vis spectrum showed that this precipitate had not the characteristic absorption of meso-tetraphenyl substituted porphin at about 410 nm, so meso-tetraphenylporphin nitrogen mustards with nitro- groups at benzene periphery could not be synthesized in this method. The five types of porphins yielded after condensation and oxidation were separated with thin layer chromatography for their similar polarity.



Scheme 2

We failed to obtain chlorin nitrogen mustards derived from chlorophyll which also have the photodynamic effect on tumor. When chlorophyll derivatives with diethanolamine group at the periphery of chlorin reacted with thionyl chloride, the color of reaction mixture turned into yellow. ^{1}H-NMR spectrum of the products showed that the characteristic peaks of chlorophyll derivatives disappeared, so the π-conjugated system of chlorophyll derivatives must be destroyed, which resulted in the vanishment of photodynamic effects on tumor. $POCl_3$ could destroy porphyrins with natural structure, while it could turn diethanolamine adhered to meso-tetraphenyl porphin into nitrogen mustard, so meso-tetraphenyl porphin was more stable than porphyrins with natural structure which was also more stable than chlorophyll derivatives.

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11. Typical experimental procedures for compound ($\mathbf{I}_1$):

Deuteroporphyrin IX dimethyl ester (2).

98% Sulfuric acid (25 mL) was added to a solution of deuteroporphyrin IX (30 g, 58.8 mmol) in methanol (500 mL). The reaction mixture was stirred at rt for 8 h . H_2O (1 L) and sodium acetate (10 g, 122 mmol) were added to the reaction mixture. The precipitate was collected, dried at 60 °C for 8 h under vacuum and then chromatographed on silica gel eluting with chloroform-methanol (10 : 0.5, v/v) to afford **2** (29.5 g, 93.4%) as a purple solid (mp 226-227 °C). IR (KBr): 1737 (s, C=O), 1437 (m, Ar), 1160 (m, C-O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ ppm): -3.96 (s, 2H, 2×pyrrole-NH), 3.27 (t, 4H, J = 6.0 Hz, 2× 13,17 -CH_2-), 3.60-3.72(m, 18H, 4× Ar-CH_3, 2× -$COOCH_3$), 4.39(t, 4H, J = 7.5 Hz, 2×-CH_2COO-), 9.06(s, 2H, 2×β-pyrrole-H), 9.98, 10.03, 10.04, 10.07(4s, 4H, 4×Ar-H). *m/z* (FAB MS): 540(M^++2, 100), 538(M^+, 30).

2,7,12,18-Tetramethyl-13,17-di(3-hydroxypropyl)porphin.

To a solution of **2** (20.0 g, 37.2 mmol) in anhydrous ether (1 L) was added $LiAlH_4$(2 g, 54 mmol). The mixture was stirred at rt for 20 min. Ethyl acetate (10 mL) was added dropwise into the mixture to decompose the excess $LiAlH_4$. The precipitate was filtered off and the filtrate was concentrated. The residue was chromatographed on silica gel eluting with chloroform-methanol (10:1, v/v) to afford a purple solid (mp 182-183 °C, 15.0 g, 83.7 %). IR (KBr): 1437 (m, Ar), 1059 (m, C-O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ ppm): -3.25 (s, 2H, 2× pyrrole-NH), 1.21(s, 2H, 2×-OH), 2.67(m, 4H, 2×$C\underline{H}_2CH_2OH$), 3.45, 3.54, 3.55, 3.61 (4s, 12H, 4×Ar-CH_3), 4.15(t, 4H, J = 3.8 Hz, 2×-$C\underline{H}_2OH$), 4.34(t, 4H, J = 3.0 Hz, 2×Ar-CH_2-), 9.22, 9.23 (2s, 2H, 2×β-pyrrole-H), 10.28, 10.31, 1076 (3s, 4H, 4×Ar-H) . *m/z* (FAB MS): 482 (M^+,100).

2,7,12,18-Tetramethyl-13,17-di(3-bromopropyl)porphin (3).

To a solution of 2,7,12,18-tetramethyl-13,17-di(3-hydroxypropyl)porphin (10.0 g, 20.7 mmol) in chloroform (600 mL) stirred at 0°C was added phosphorus tribromide (10 mL, 105.6 mmol) dropwise. The

mixture was stirred at 0 °C for 2 h before being washed with H_2O (2 L×3). The chloroform layer was dried over anhydrous sodium sulfate (100 g) and evaporated. The residue was chromatographed on silica gel eluting with chloroform to give **3** as a purple solid(mp 193-194 °C, 6.8 g, 54.2%). IR (KBr) : 1431 (m, Ar)cm^{-1}. *m/z* (FAB MS): 606 (M^+, 28).

2,7,12,18-Tetramethyl-13,17-di[3-*N*,*N*-di(2-chloroethyl)aminopropyl]porphin (I_1).

A mixture of **3** (5.0 g, 8.25 mmol), diethanolamine (50 mL, 523 mmol) and 1,4-dioxane (500 mL) was refluxed with stirring for 8 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel with chloroform-methanol-triethylamine (10:1:1, v/v/v) to afford a solid (4.1 g). To a solution of the solid in chloroform (300 mL) was added dropwise a solution of thionyl chloride (5 mL, 69.3 mmol) in HMPA(30 mL) with stirring. The mixture was refluxed for 1 h, then washed twice with H_2O and dried over anhydrous sodium sulfate (50 g).. The solution was evaporated to give a residue, which was chromatographed on silica gel eluting with chloroform-methanol (10:0.5, v/v) to afford $\mathbf{I_1}$ (2.57 g, 42.8%) as a purple solid(mp 152-153 °C). The overall yield was 15% (based on hemin). IR (KBr): 1443 (m, Ar), 1106 (m, C-N) cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ ppm): -4.10 (s, 2H 2× pyrrole-NH), 2.19-2.24 (m, 4H, 2×-C$\underline{H_2}$CH_2N<), 2.74-2.83 (m, 12H, 2×-C$\underline{H_2}$N(C$\underline{H_2}$ -)$_2$), 3.41-3.63 (m, 20H, 4×-CH_2Cl, 4×Ar-CH_3), 3.85 (t, 4H, J=6.6 Hz, 2×Ar-CH_2-), 8.95 (s, 2H, β-pyrrole-H) 9.74, 9.88, 9.93 (3s, 4H, 4×Ar-H). *m/z* (FAB MS): 728 (M^+, 23).

12. Typical experimental procedures for compound ($\mathbf{II_1}$):

To 700 mL of chloroform were added redistilled pyrrole (0.55 mL, 8 mol), benzaldehyde (0.6 mL, 6 mmol), **7** (0.37 g, 2 mmol) and trifluoroboride-ether solution(0.33 mL, 2.75 mmol). The reaction mixture was stirred at rt for 13 h. Triethylamine (0.5 mL, 3.5 mmol) and tetrachloro-1,4-benzoquinone(1.5 g, 6.25 mmol) were added. The resulting solution was heated at 60 °C for 1 h. The reaction mixture was evaporated to give a residue, which was extracted with 300 mL of ether. The ether layer was concentrated and the residue was chromatographed on silica gel eluting with chloroform-methanol(10:1, v/v) to afford 350 mg of purple solids. **2** (91 mg) was separated from the solid by thin layer chromatography developing with hexane-chloroform(2:1, v/v), 8.75%. *m/z* (FAB MS): 692 (M^+, 93).

The solid mentioned above was dissolved in DMSO (50 mL). To this solution was added 5 mL(52.3 mmol) of diethanolamine and the resulting mixture was stirred at 115°C for 8 h. The solution was evaporated under reduced pressure to remove the solvent. The residue was dissolved in 50 mL of chloroform. 5 mL (54.6 mmol)of phosphorus oxychloride was added to the solution. The mixture was stirred at rt for 2 h. 100 mL of saturated solution of sodium bicarbonate was added to wash the chloroform solution. The organic layer was dried with anhydrous magnesium sulfate and evaporated to give a residue, which was chromatographed on silica gel eluting with chloroform-methanol(10:1.5, v/v) to afford $\mathbf{II_1}$ as a purple solid

(mp 172-173 °C, 52 mg, 3.9 % calculated on the basis of 3-chloromethyl anisaldehyde). IR (KBr): 3318(w, NH), 1596, 1472, 1350(m, C=C), 1253(s, C-O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ ppm): -2.77(s, 2H, -NH), 3.08(t, 4H, J = 7.5 Hz, $-NCH_2$), 3.57(t, 4H, J=7.5 Hz, $-CH_2Cl$), 4.06(s, 2H, 3' $-CH_2N<$), 4.12(s, 3H, $-OCH_3$), 7.75 (m, 10H, 3',4',5'-H), 8.22(m, 8H, 2',6'-H), 8.84 and 8.86(2s, 8H, β-pyrrolic H). *m/z* (FAB MS): 797[M^+, 15].

13. IR, ^{1}H-NMR (300 MHz, $CDCl_3$), and MS (FAB) data of selected compounds:

Compound (**I_2**). IR (KBr): 1447 (m, Ar), 1112 (m, C-O).1090 (m, C-N) cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ ppm): -3.69 (s, 2H, 2×pyrrole-NH), 2.27 (d, 6H, J = 6.6 Hz, 2×-CH(OMe)C<u>H</u>$_3$), 2.38 (m, 4H, 2×-C<u>H</u>$_2$CH$_2$N<), 2.90 - 2.97 (m, 12H, 2×-$CH_2N(CH_2-)_2$), 3.44 - 3.50 (m, 8H, 4×-CH_2Cl), 3.51-3.74 (m, 18H, 2×-OCH_3, 4×Ar-CH_3), 4.04 (t, 4H, J=6.4 Hz, 2×Ar-CH_2-), 6.04-6.08 (m, 2H, 2×Ar-CH<), 10.00, 10.09, 10.52, 10.56 (4s, 4H, 4×Ar-H). *m/z* (FAB MS): 844 (M^+,18). Compound (**I_5**): IR (KBr): 1447 (m, Ar), 1159 (m, C-O).1081 (m, C-N) cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ ppm): -3.70 (s, 2H 2× pyrrole-NH), 1.32 (m, 12H, 2×-OCH(C<u>H</u>$_3$)$_2$), 2.25 (m, 6H, 2×-CH(OR)C<u>H</u>$_3$), 2.46 (m, 4H, 2×-C<u>H</u>$_2$CH$_2$N<), 2.96 (m, 12H, 2×-C<u>H</u>$_2$N(C<u>H</u>$_2$ -)$_2$), 3.50 (m, 8H, 4×-CH_2Cl), 3.64, 3.67, 3.69, 3.71 (4s, 14H, 4×Ar-CH_3, 2×-OCH<), 4.11 (m, 4H, 2×Ar-CH_2 -), 6.24 (m, 2H, 2×Ar-CH<), 10.01, 10.11, 10.69, 10.70 (4s, 4H, 4×Ar-H). *m/z*(FAB MS): 901 (M+1,90), 900 (M^+,70). Compound (**II_6**). IR (KBr): 3320(w, NH), 1606,1509,1350 (s, C=C), 1247(s, C-O) cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ ppm): -2.74(s, 2H, -NH), 3.07(t, 4H, J=7.2 Hz, $-NCH_2$), 3.57(t, 4H, J=7.2 Hz, $-CH_2Cl$), 4.03(s, 2H, 3' $-CH_2N<$), 4.10(s, 12H, 4' $-OCH_3$), 7.24(m, 8H, 3'-H), 8.11(m, 8H, 2'-H), 8.85 (m, 8H, β-pyrrolic H). *m/z* (FAB MS): 887[M^+, 94].

Received, 22nd April, 1998