Monofunctional Electrophilic and Nucleophilic Derivatives of *meso*-Tetraphenylporphyrin for Attachment to Peptides

Susan E. Matthews, Colin W. Pouton and Michael D. Threadgill*

School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath UK BA2 7AY
E-mail: m.d.threadgill@bath.ac.uk

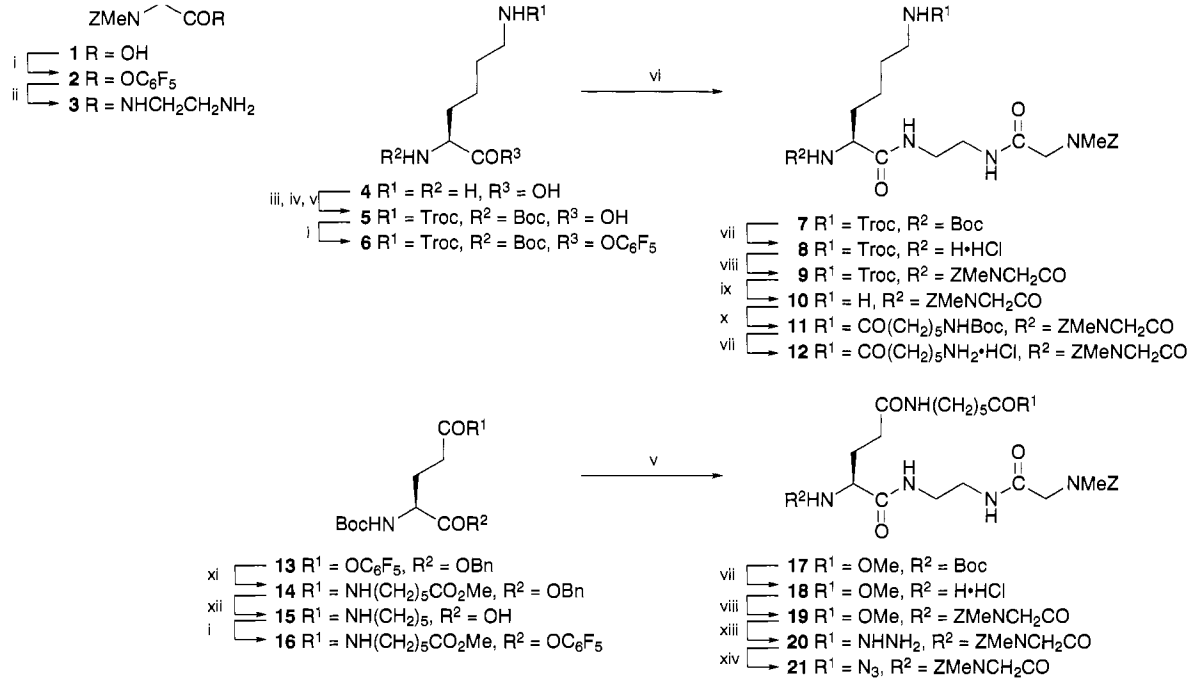
4-Nitrophenyl *N*-[4-(10,15,20-triphenylporphyrin-5-yl)phenyl]carbamate and 5-[4-(*N*-glycylamino)phenyl]-10,15,20-triphenylporphyrin have been synthesised from a readily prepared monofunctionalised porphyrin; they couple efficiently with the side-chains of extended lysyl and glutamyl peptide derivatives, respectively.

Porphyrins alone or linked to polymers and other targetting moieties have important roles in diagnosis and therapy of cancer. For example, the so-called 'haematoporphyrin derivative' and other porphyrins act as photosensitisers for conversion of triplet oxygen to singlet oxygen in photodynamic therapy[1] and porphyrins are known to accumulate selectively in some types of tumour tissue,[2] leading to prospects of their use as targetting groups. Porphyrinatomanganese complexes are used as contrast-enhancing agents in magnetic resonance imaging (MRI), owing to their high molar relaxivities in aqueous solution.[3] Attachment of metalloporphyrin and other agents to a soluble polymer enhances molar relaxivity and thus effectiveness as a MRI contrast agent.[4]

Significant difficulty in preparing mono-functionalised porphyrins in a controlled manner is caused by the fact that the most readily available naturally-occurring porphyrins, such as protoporphyrin IX and mesoporphyrin II, carry more than one identical electrophilic or nucleophilic functional group. The classical Adler–Rothemund[5] procedure for synthesis of *meso*-tetraarylporphyrins from arylaldehydes and pyrrole proceeds in <20% yield and mono-Ar-functionalised porphyrins are only obtained in very low yields by separation of statistical mixtures of porphyrins formed from mixtures of aldehydes,[6] whereas the recently-reported Heck coupling[7] needs several synthetic steps to prepare the starting 5,15-diphenyl-10-iodoporphyrin. To obviate these problems of synthesis of mono-Ar-substituted tetraphenylporphyrins, Kruper *et al.*[8] developed an efficient mononitration and subsequent reduction of the readily-available *meso*-tetraphenylporphyrin. We now report our exploitation of this weakly nucleophilic amine in generating reactive electrophilic and nucleophilic monofunctional porphyrins for attachment to side-chain extended α,ω-bis(methylamino) peptides. Polymers derived from the latter will be of use in MRI.

α,ω-Bis[benzyloxycarbonyl(methyl)amino] peptides with carboxylic acid derivatives and primary amines in side-chains of the same length were built up as shown in Scheme 1. *N*-(Benzyloxcarbonyl)sarcosine **1**[9] was converted to its pentafluorophenyl (PFP) active ester **2**† and this was added to a 20-fold excess of ethane-1,2-diamine to set up the protected sarcosine aminoethylamide **3**† as the sequence inverting unit for the C-termini of the peptides.

Orthogonal protection of the α- and ε-amines of L-lysine **4** was required for elaboration of the peptide chain and of the side-chain. This was achieved by complexation with copper(II), selective acylation of the ε-amine with 2,2,2-trichloroethyl chloroformate, decomplexation and acylation of the α-amine with di-*tert*-butyl dicarbonate in a two-phase system, in a modification of the method of Yajima *et al.*[10] The resulting BocLys(Troc)OH **5**† was converted to the PFP active ester **6**,† prior to coupling with **3** to afford the fully orthogonally



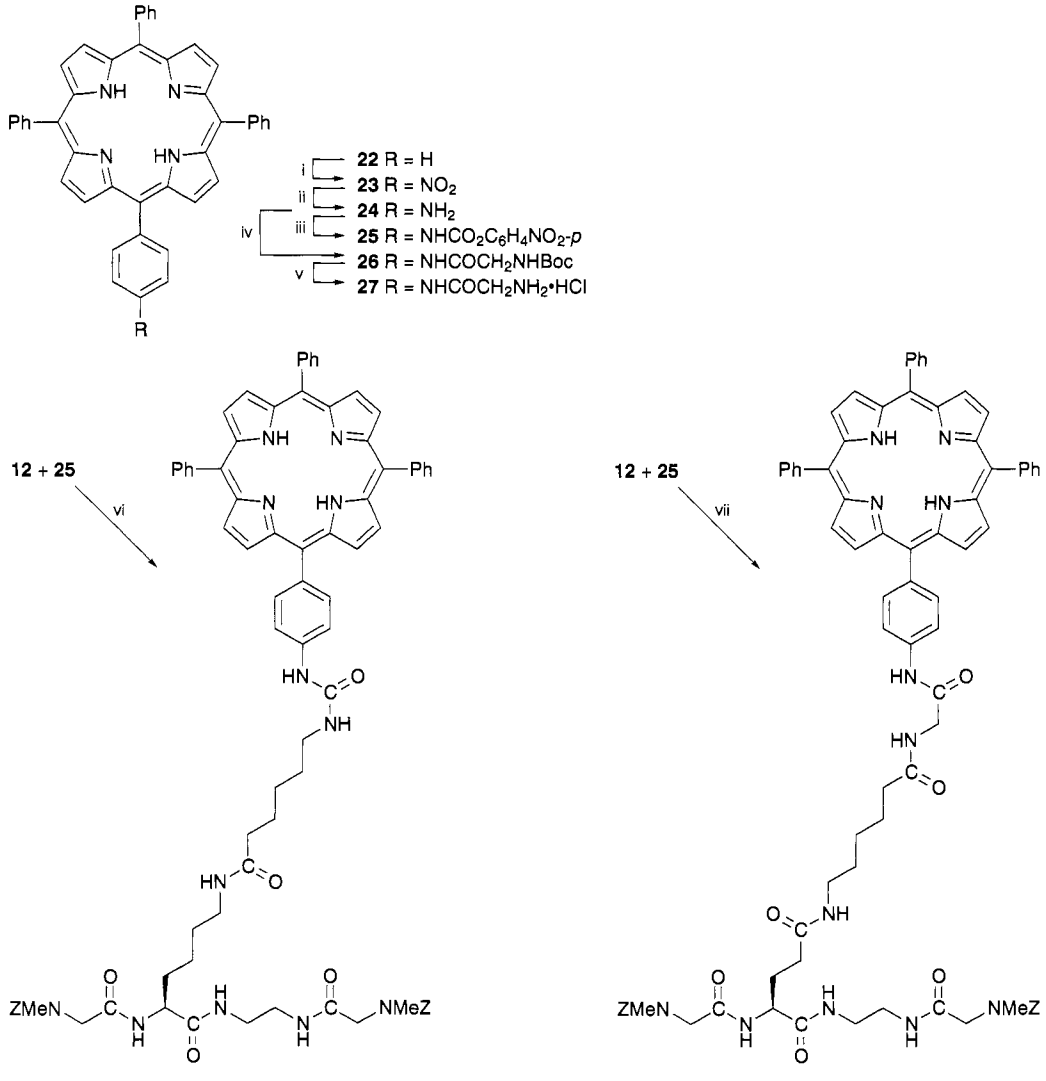
Scheme 1 Synthesis of extended sequence-inverted peptides **12** and **21**. Troc = 2,2,2-trichloroethoxycarbonyl. *Reagents and conditions*:†† i, C_6F_5OH, DCC, EtOAc, 0 °C, 20 h, 90–95%; ii, $H_2NCH_2CH_2NH_2$ (20 × excess), CH_2Cl_2, 3 h, 82%; iii, $CuCO_3$, H_2O, reflux, 3 h, then TrocCl, Na_2CO_3, H_2O, 0 °C, 20 h; iv, $Na^+{}_2$ $EDTA^{2-}$, H_2O, reflux, 2 h; v, Boc_2O, Et_3N, H_2O, dioxan, 3 d, 58% from **4**; vi, **3**, $Pr^i{}_2NEt$, CH_2Cl_2, 85%; vii, HCl, CH_2Cl_2, 1 h, quant.; viii, **2**, $Pr^i{}_2NEt$, DMAP, CH_2Cl_2, 4 d, 87%; ix, Zn, MeOH, reflux, 5 h, 83%; x, $BocNH(CH_2)_5CO_2C_6F_5$, $Pr^i{}_2NEt$, DMAP, CH_2Cl_2, 6 d, 55%; xi, $H_2N(CH_2)_5CO_2Me$·HCl, $Pr^i{}_2NEt$, DMAP, CH_2Cl_2, 7 d, 89%; xii, H_2, Pd/C, tetrahydrofuran, 3 h, quant; xiii, $N_2H_4·H_2O$, MeOH, 40 °C, 8 h, quant; xiv, Bu^tONO, DMF, THF, dioxan, HCl, −20 °C, 50 min, then $Pr^i{}_2NEt$, −60 °C (this solution was taken forward for reaction with **27**, Scheme 2).

protected inverted-sequence peptide **7**.† The peptide chain was completed by acidic removal of the Boc group, giving the salt **8**,† and acylation with one further equivalent of **2**. With the inverted sequence of the peptide now complete, attention was turned to extension of the length of the side-chain of **9**.† The ε-amine **10**† was revealed by selective reductive removal of the Troc group (zinc dust in methanol). Acylation with pentafluorophenyl 6-(*tert*-butoxycarbonylamino)hexanoate[11] gave the orthogonally protected peptide derivative **11**.† Deprotection (HCl) afforded the target inverted-sequence peptide **12**† with the extended primary amine side-chain for coupling with an appropriate monofunctional porphyrin electrophile.

In the assembly of a corresponding inverted-sequence peptide with an extended activated carboxylic acid side-chain, the extension was performed prior to construction of the peptide to avoid problems of formation of pyroglutamates. Acylation of the spacer unit, methyl 6-aminohexanoate, with Boc glutamic acid α-benzyl ester γ-PFP ester **13**[12] gave the extended derivative **14**.† The α-carboxylic acid **15**† was revealed by selective hydrogenolysis of the benzyl ester. In a series of steps similar to those used for building the inverted-sequence lysine peptide, this carboxylic acid was activated as the PFP ester **16**† and coupled with the sequence-inverting unit **3** to afford **17**.† Again, selective acidolytic deprotection revealed the Glu α-amine **18**. Coupling with **3** afforded the target inverted-sequence peptide **19**† with the extended carboxylic acid side-chain protected as the methyl ester. This ester resisted selective hydrolysis under both basic and acidic conditions but succumbed to hydrazinolysis, giving the hydrazide **20**.† From this, the acyl azide **21**†‡ was formed by reaction with *tert*-butyl nitrite under acidic conditions, other nitrosating agents (*e.g.* sodium nitrite) being either inefficient or destructive. This provides an active acylating function for reaction with an appropriate porphyrin nucleophile.

The monoaminophenylporphyrin **24** was prepared in 46% overall yield in two steps from *meso*-tetraphenylporphyrin **22**, in a modification of the method of Kruper *et al.* (Scheme 2).[8] The corresponding isocyanate would represent a potent electrophile for reaction with the extended lysine derivative **12** but **24** reacted slowly with phosgene, giving mainly the corresponding *N,N'*-bis(tetraphenylporphyrinyl)urea. However, the amine **24** was acylated smoothly§ by 4-nitrophenyl chloroformate, giving the carbamate **25**, a synthon for the required isocyanate. Treatment of **25** under mildly basic conditions generated the isocyanate which coupled *in situ*§ with the extended lysine derivative **12**, giving the protected porphyrinyl peptide derivative **28**¶ in good yield.

The arylamine **24** was found to be a remarkably weak nucleophile, reacting with succinic anhydride only after a prolonged period at elevated temperature and not reacting with



Scheme 2 Activation of tetraphenylporphyrinamine **24** as an electrophile and as a nucleophile; coupling with extended sequence-inverted peptides **12** and **21**. *Reagents and conditions*:†† i, fuming HNO_3, $CHCl_3$, 5 h, 55%; ii, $SnCl_2$, conc. aq. HCl, 80 °C, 2 h, 84%; iii, 4-nitrophenyl chloroformate, Pr^i_2NEt, $CHCl_3$, 20 h, then chromatography, 67%; iv, $BocGlyOC_6F_5$, Pr^i_2NEt, DMAP, $CHCl_3$, 46 h, 95%; v, HCl, CH_2Cl_2, 1 h, quant; vi, Pr^i_2NEt, DMAP, CH_2Cl_2, 20 h, 82%; vii; Pr^i_2NEt, DMAP, $CHCl_3$, 2 h, 58%.

peptide active esters or with the acyl azide **21**. Much greater nucleophilicity is required for efficient coupling with peptide derivatives under mild conditions. To introduce a primary aliphatic amine as a more potent nucleophile, the arylamine was acylated by treatment‖ with a two-fold excess of the PFP active ester of N-Boc-glycine at 40 °C, forming **26**. The primary aliphatic amine **27** was revealed by deprotection‖ with hydrogen chloride. This more reactive nucleophile then coupled efficiently‖ with the extended peptide derivative acyl azide **21**, giving the porphyrinyl peptide derivative **29**.**

The monoaminotetraphenylporphyrin **24** is thus demonstrated to be a readily accessible monofunctionalised porphyrin which can be converted straightforwardly into a reactive electrophile and a reactive unhindered nucleophile which should have general utility in controlled attachment of porphyrins to peptides, polymers and other molecules.

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Footnotes

† All novel compounds were characterised by ^{1}H NMR and by FAB MS and were shown to be pure by TLC. Target compounds and major intermediates were also characterised by high resolution FAB MS.

‡ Acyl azide **21** was prepared and used without purification, to avoid Curtius rearrangement.

§ *Method*: Compound **24** (5.70 g, 9.25 mmol) was stirred with 4-nitrophenyl chloroformate (1.86 g, 9.25 mmol) and Pr^i_2NEt (1.19 g, 9.25 mmol) in $CHCl_3$ (50 cm^3) for 20 h. Chromatography gave **25** (4.85 g, 67%) as a purple glass. Compound **12** (385 mg, 530 μmol) and **25** (520 mg, 670 μmol) were stirred with Pr^i_2NEt (205 mg, 1.6 mmol) and DMAP (10 mg) in CH_2Cl_2 (10 cm^3) for 20 h. Chromatography gave **28** (593 mg, 82%) as a purple glass.

¶ *Spectroscopic data* for **28**: δ ($CDCl_3$) −2.78 (2 H, s, porphyrin 21, 23-H_2), 1.2–1.7 (10 H, m, Lys β, γ-H_4 + $NCH_2CH_2CH_2CH_2CH_2CO$), 2.12 (2 H, br, CH_2CH_2CO), 2.93 (3 H, s, NCH_3), 2.99 (3 H, s, NCH_3), 3.0–3.3 (8 H, Lys ε-H_2 + $NCH_2CH_2CH_2$ + NCH_2CH_2N), 3.77 (2 H, m, Sar-H_2), 3.86 (2 H, m, Sar'-H_2), 4.36 (1 H, m, Lys α-H), 5.06 (3 H, s) and 5.07 (1 H, s) (2 × $PhCH_2O$), 5.75 (1 H, br, NH), 5.83 (1 H, br, NH), 6.39 (1 H, br, NH), 6.57 (1 H, br, NH), 6.94 (1 H, br, NH), 7.24 (10 H, br s, 2 × benzyloxy Ph-H_5), 7.37 (1 H, br, NH), 7.69 (11 H, m, 3 × porphyrin-Ph 3,4,5-H_3 + porphyrin-C_6H_4N 2,6-H_2), 8.06 (2 H, d, *J* 8.4 Hz, porphyrin-C_6H_4N 3,5-H_2), 8.15 (6 H, m, 3 × porphyrin-Ph 2,6-H_2), 8.79 (2 H, d *J* 4.7 Hz, porphyrin 3,7-H_2), 8.82 (4 H, s, porphyrin 12,13,17,18-H_4), 8.87 (2 H, d, *J* 5.1 Hz, porphyrin 2,8-H_2); *m/z* (FAB) 1367.6384 (M + H) ($C_{81}H_{83}N_{12}O_9$ requires 1367.6406).

‖ *Method*: Compound **24** (5.00 g, 8.16 mmol) was stirred with Boc-$GlyOC_6F_5$ (5.58 g, 16.3 mmol), Pr^i_2NEt (2.32 g, 18.0 mmol) and DMAP (50 mg) in $CHCl_3$ (100 cm^3) for 46 h at 40 °C. Chromatography gave **26** (6.00 g, 95%) as a purple glass. This compound (2.24 g, 2.9 mmol) was treated with excess HCl in CH_2Cl_2 (100 cm^3) for 1 h. The solvent and excess reagent were evaporated to give **27** (2.10 g, quantitative). *tert*-Butyl nitrite (0.22 cm^3) in THF (1.75 cm^3) was added to **21** (1.50 mg, 2.07 mmol) in DMF (3.0 cm^3) and HCl in 1,4-dioxan (4.0 mol dm^{-3}, 1.86 cm^3) at −20 °C. The mixture was stirred for 2 h. Pr^i_2NEt (1.06 g) was added at −60 °C, followed by **27** (2.09 g, 2.9 mmol) and Pr^i_2NEt (1.12 g, 18.7 mmol) in $CHCl_3$ (30 cm^3). The mixture was stirred for 2 h. Chromatography gave **29** (1.64 g, 58%) as a purple glass.

** *Spectroscopic data* for **29**: δ_H ($CDCl_3$) −2.75 (2 H, porphyrin 21,23-H_2), 0.89 (2 H, m, $NCH_2CH_2CH_2CH_2CH_2CO$), 1.25–1.65 (6 H, m, $NCH_2CH_2CH_2CH_2CH_2CO$ + Glu β-H_2), 1.9–2.4 (4 H, Glu γ-H_2 + $CH_2CH_2CH_2CO$), 3.0–3.1 (8 H, m, $NCH_2CH_2CH_2$ + 2 × NCH_3), 3.35 (4 H, br, NCH_2CH_2N), 3.85–4.05 (4 H, m, 2 × Sar-H_2), 4.15–4.25 (2 H, m, Gly-H_2), 4.42 (1 H, m, Glu α-H), 5.13 (4 H, br s, 2 × $PhCH_2O$), 7.25–7.33 (14 H, m, 4 × NH + 2 × benzyloxy Ph-H_5), 7.70–7.76 (11 H, m, 3 × porphyrin-Ph 3,4,5-H_3 + porphyrin-C_6H_4N 2,6-H_2), 7.96 (2 H, m, 2 × NH), 8.12 (2 H, d, *J* 8.2 Hz, porphyrin-C_6H_4N 3,5-H_2), 8.17–8.23 (6 H, m, 3 × porphyrin-Ph 2,6-H_2), 8.84 (8 H, br s, porphyrin 2,3,7,8,12,13,17,18-H_8); *m/z* (FAB) 1381.6184 (M + H) ($C_{81}H_{81}N_{12}O_{10}$ requires 1381.6199).

†† Reactions took place at ambient temperature, unless otherwise stated.

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