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[19] Compound **1** was obtained as a minor product in the standard etioporphyrin I synthesis. Apparently an ethyl group was replaced by bromine during the formation of the dipyrrylmethene precursor under harsh bromination conditions. Being less basic, **1** can be separated from etioporphyrin by acid extractions, for example, 20 g of **1** from 3 kg of etioporphyrin. Other Heck reactions with homologous β-bromoporphyrins have been reported, see H. Ali, J. E. van Lier, *Tetrahedron* **1994**, *50*, 11933.

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[21] **2**: 76% yield. UV/Vis (CH_2Cl_2): $\lambda_{max} = 401$, 533, 574 nm; ^{1}H NMR (300 Hz, $CDCl_3$, 25 °C): $\delta = 1.78$ (s, 12H; CH_3), 1.80 (m, 9H; CH_3), 3.44, 3.50, 3.64, 3.96 (s, 3H each; CH_3), 3.98 (m, 6H; CH_2), 9.60, 9.64, 9.90, 10.76 (s, 1H each; *meso*-H); FAB HRMS for $C_{36}H_{43}BN_4O_2Zn$: calcd: 638.27712; found: 638.27705.

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[23] **3a**: 62% yield. UV/Vis (CH_2Cl_2): $\lambda_{max} = 403$, 415, 534, 579 nm; ^{1}H (300 Hz, $CDCl_3$, 25 °C): $\delta = 1.74$ (t, $^3J(H,H) = 7.2$ Hz, 6H; CH_3), 1.90 (t, $^3J(H,H) = 7.2$ Hz, 6H; CH_3), 2.02 (t, $^3J(H,H) = 7.2$ Hz, 6H; CH_3), 2.88, 3.65, 3.74, 3.92 (s, 6H each; CH_3), 4.20 (m, 12H; CH_2), 10.06, 10.14, 10.22, 10.50 (s, 2H each; *meso*-H); FAB HRMS for $C_{60}H_{62}N_8Zn_2$: calcd: 1022.36804; found: 1022.36765; elemental analysis (%) for $C_{60}H_{62}N_8Zn$: calcd: C 70.24, H 6.09, N 10.92; found: C 70.32, H 6.12, N 11.03.

[24] Crystallographic data: $C_{63}H_{74}N_8O_3Zn_2$ from CH_3OH/CH_2Cl_2, $M_r = 1122.04$, monoclinic, space group $C2/c$, $a = 14.58560(10)$, $b = 21.5321(3)$, $c = 19.6353(3)$ Å, $\beta = 104.5520(10)°$, $V = 5968.81(13)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.249$ g cm^{-3}, $F(000) = 2368$, $\lambda(Mo_{K\alpha}) = 0.7107$ Å, crystal dimensions $0.5 \times 0.25 \times 0.10$ mm^3. A total of 8840 reflections were collected at -90 °C using a Siemens diffractometer equipped with a CCD detector in the θ range of 1.72 to 20.00°, of which 2788 were unique ($R_{int} = 0.0570$). The structure was solved by the Patterson heavy atom method in conjunction with standard difference Fourier techniques. Hydrogen atoms were placed in calculated positions using a standard riding model and were refined isotropically. A methanol solvent molecule was found to be disordered and was modeled by standard procedures. The largest peak and hole in the difference map were 0.957 and -0.424 e Å^{-3}, respectively. The least-squares refinement converged normally giving residuals of $R = 0.0749$ and $wR^2 = 0.1983$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-133672. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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[29] Standard exciton considerations predict that B_y should blue-shift and B_x should red-shift about the unperturbed *B* band. In **3a**, both transitions red-shift relative to the *B* band of the monomer. The same situation occurs for the *meso*–β and the *meso*–*meso* bis-porphyrins of ref. [16b)]. These red shifts indicate that the exciton coupling in porphyrins linked by a single bond is augmented by other factors; one likely factor is electronic coupling between porphyrin subunits across the C–C bond.

[30] **3b–e**: **3b**, 91% yield. UV/Vis (CH_2Cl_2): $\lambda_{max} = 402$, 530, 570 nm; ^{1}H NMR (300 Hz, $CDCl_3$, 25 °C): $\delta = 1.73$ (m, 9H; CH_3), 3.39, 3.41, 3.50, 3.64 (s, 3H each; CH_3), 3.86 (m, 6H; CH_2), 7.70–8.22 (m, 5H; Ar–H), 9.41, 9.53, 9.74, 9.84 (s, 1H each; *meso*-H); FAB HRMS for $C_{36}H_{36}N_4Zn$: calcd: 588.2223; found: 588.2230. **3c**, 93% yield. UV/Vis (CH_2Cl_2): $\lambda_{max} = 402$, 533, 569 nm; ^{1}H NMR (300 Hz, $CDCl_3$, 25 °C): $\delta = 1.75$ (m, 9H; CH_3), 2.72 (s, 3H; CH_3), 3.39 (s, 6H; CH_3), 3.44, 3.66 (s, 3H each; CH_3), 3.86 (m, 6H; CH_2), 7.68, 8.08 (dd, $^3J(H,H) = 14.5$ Hz, 2H; Ar–H), 9.40, 9.50, 9.73, 9.84 (s, 1H each; *meso*-H); FAB HRMS for $C_{37}H_{36}N_4OZn$: calcd: 602.23879; found: 602.23908. **3d**, 88% yield. UV/Vis (CH_2Cl_2): $\lambda_{max} = 404$, 534, 570 nm; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 1.74$ (t, $^3J(H,H) = 7.5$ Hz, 3H; CH_3), 1.85 (t, $^3J(H,H) = 7.3$ Hz, 3H; CH_3), 1.92 (t, $^3J(H,H) = 7.5$ Hz, 3H; CH_3), 3.07, 3.41, 3.56, 3.65 (s, 3H each; CH_3), 4.08 (m, 6H; CH_2), 7.17 (m, 2H; Ar–H), 7.53 (m, 2H; Ar–H), 7.81 (d, $^3J(H,H) = 8.8$ Hz, 2H; Ar–H), 8.30 (d, $^3J(H,H) = 8.9$ Hz, 2H; Ar–H), 8.86 (s, 1H; Ar–H), 9.48, 10.00, 10.06, 10.34 (s, 1H each, *meso*-H); FAB HRMS for $C_{44}H_{40}N_4Zn$: calcd: 688.25444; found: 688.25432. **3e**, 82% yield. UV/Vis (CH_2Cl_2): $\lambda_{max} = 406$, 533, 574 nm; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 1.72$ (m, 9H; CH_3), 3.36 (s, 3H; CH_3), 3.40 (s, 6H; CH_3), 3.59 (s, 3H; CH_3), 3.84 (m, 6H; CH_2), 8.34 (dd, $^3J(H,H) = 14.9$ Hz, 2H; Ar–H), 9.43, 9.50 (s, 1H each; *meso*-H), 9.62 (s, 2H; *meso*-H), 10.35 (s, 1H; CHO); FAB HRMS for $C_{37}H_{36}N_4OZn$: calcd: 616.21806; found: 616.21786.

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One-Pot Synthesis of Antigen-Bearing, Lysine-Based Cluster Mannosides Using Two Orthogonal Chemoselective Ligation Reactions**

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Dendritic cells (DCs) are well-recognized for playing a crucial role in the control of immunity. These professional antigen-presenting cells act both as initiators and modulators

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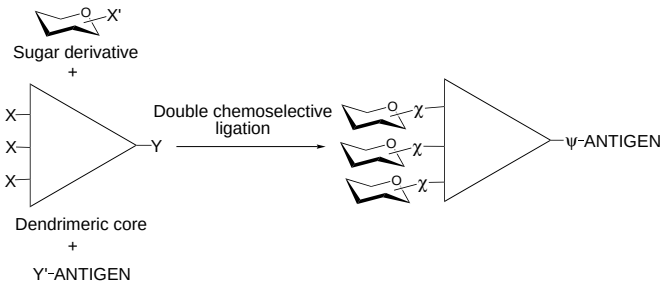
of the immune response.[1] In particular, they are able to stimulate T-lymphocyte response by presenting antigens bound to major histocompatibility complex class II molecules.[2] DCs utilize at least two distinct mechanisms to accomplish antigen uptake, one involves internalization by fluid-phase mediated macropinocytosis and the other follows association with a mannose receptor.[3, 4] In fact, mannosylation of peptide or protein antigens results in a 200 – 10 000 fold enhanced potency for the stimulation of peptide-specific T-cell clones, when compared with their non-mannosylated peptides.[4] This suggests that mannosylation of antigens leads to selective targeting and a subsequent superior presentation by DCs, which may improve the efficacy of vaccines and permit the use of lower doses. The mannose receptor preferentially selects microorganisms or molecules possessing several sugar residues such as D-mannose, *N*-acetyl-D-glucosamine, or L-fucose,[5] which is consistent with the cluster effect.[6] Thus, the targeting of the mannose receptor requires the preparation of multivalent ligands, such as cluster glycosides or glycodendrimers.[7]

The sole antigen-bearing cluster mannosides prepared to date have been synthesized following a recurrent strategy.[4] Thus, we have sought to develop a convergent approach which, in addition to improved yields and purities, would allow us to vary independently the nature of the glycosides, antigens, and dendrimeric cores, giving access to a large number of constructs for the rapid optimization of the antigen presentation (Scheme 1). Our modular strategy relies on: a) the synthesis of dendrimeric cores bearing two sets of

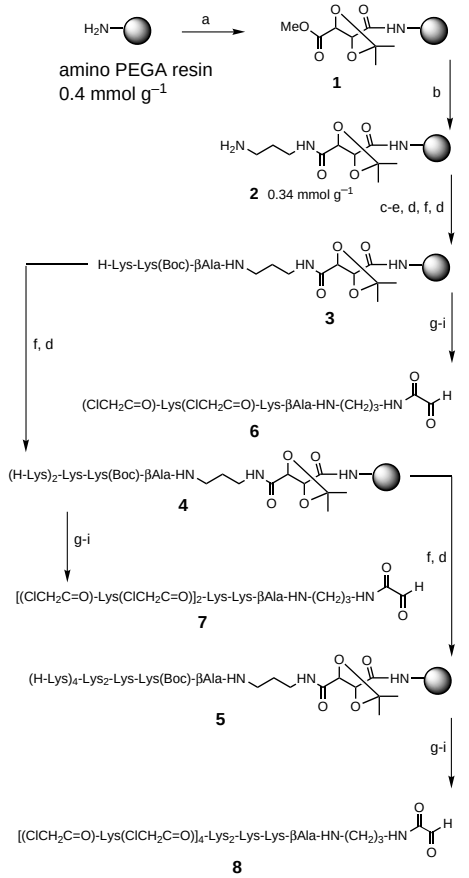


Scheme 1. Double ligation strategy to antigen-associated lycodendrimers. X = $ClCH_2CO-$, X′ = HS−, Y = CHOCO−, Y′ = NH_2-NH-, χ = $-SCH_2CO-$, ψ = =N−NH−.

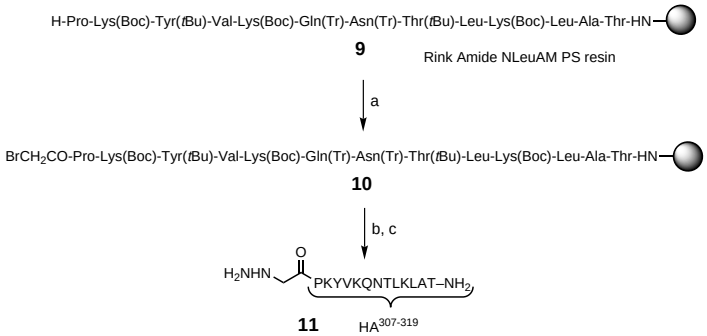
chemocompatible functional groups; b) the derivatization of mannosides and antigens with complementary functional groups; c) the assembly of the purified, fully deprotected fragments in a one-pot procedure using two successive orthogonal chemoselective ligation reactions.

The chemoselective ligation, which refers to the coupling of fully deprotected molecular fragments through two mutually and uniquely reactive functional groups in an aqueous environment, has found broad applications.[8] Despite the potential usefulness of assembling macromolecules using a double ligation reaction, Kent's preparation of the cMyc-Max factor, a heterodimeric transcription factor, provides a unique example where two successive oxime and thioester ligations were performed in a one-pot procedure, to the best of our knowledge.[9] We decided to examine the reaction of *N*-chloroacetylated L-lysinyl cores,[10] modified with a glyoxylyl function, with *N*-terminal hydrazinopeptide antigens and 2-thioethyl α,D-mannopyranoside **13**[11] using combined hydrazone/thioether ligation.

Thus, di-, tetra-, and octavalent lysinyl cores **6**, **7**, and **8**, whose lysine ε-amino group near the C-terminus was left for further derivatization with a fluorescent probe,[12] have been prepared with the Fmoc/*tert*-butyl solid phase peptide strategy (SPPS, Scheme 2).[13] The solid support was a polyethylene glycol – dimethylacrylamide copolymer resin (PEGA), derivatized with a novel (+)-dimethyl 2,3-*O*-isopropylidene-D-tartrate-based linker.[14] This commercially available tartrate derivative was first partially saponified and then coupled to the amino resin. The intermediate **1** was further transacylated using 1,3-diaminopropane to give **2**. The following steps



Scheme 2. Synthesis of glyoxylyl *N*-(chloroacetyl)lysinyl cores. a) Dimethyl 2,3-*O*-isopropylidene-D-tartrate (46 equiv), H_2O (4 equiv), DBU (4 equiv), RT, 1 h then addition to amino resin (1 equiv), BOP (4 equiv), DMF, RT, 40 min; b) 1,3-diaminopropane (7.8 equiv), DMF, RT, 1 h; c) Fmoc-β-Ala-OH (4 equiv), HBTU/HOBt/*i*PrNEt$_2$ (4:4:12 equiv), NMP, 40 min; d) piperidine/NMP 20:80, 20 min; e) Fmoc-L-Lys(Boc)-OH (4 equiv), HBTU/HOBt/*i*PrNEt$_2$ (4:4:12 equiv), NMP, 40 min; f) Fmoc-L-Lys(Fmoc)-OH (4 equiv/NH_2), HBTU/HOBt/*i*PrNEt$_2$ (4:4:12 equiv/NH_2), NMP, 40 min; g) $ClCH_2COOH$ (8 equiv/NH_2), DIC (4 equiv/NH_2), DMF, RT, 1 h; h) TFA/anisole/H_2O 95:2.5:2.5, RT, 2 h; i) $NaIO_4$ (6 equiv), AcOH/H_2O 1:2.4, RT, 2 min; 18, 31 and 23.5% overall yields for compounds **6**, **7** and **8**, respectively. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, RT = room temperature, BOP = 1-benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate, Fmoc = 9-fluorenylmethoxycarbonyl, HBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxy-1*H*-benzotriazole, NMP = *N*-methylpyrrolidone, Boc = *tert*-butoxycarbonyl, DIC = diisopropylcarbodiimide, TFA = trifluoroacetic acid.



Scheme 3. Preparation of the hydrazine-modified antigen **11**. a) Bromoacetic acid (8 equiv), DIC (4 equiv), DMF, 20 min then added to peptidyl resin, DMF, RT, 1 h; b) $BocNHNH_2$ (4 equiv), *i*$PrNEt_2$ (6 equiv), DMF, RT, overnight; c) TFA/anisole/H_2O 95:2.5:2.5, RT, 3 h, 40%.

comprised conventional SPPS to furnish peptidyl resins **3**–**5**, the simultaneous removal of the Boc and isopropylidene protecting groups, and finally a solid phase periodic oxidation allowing both separation of the product from the solid support and the formation of the glyoxylyl group. The cores were obtained in 18–31% yield, after several washings of the resin with H_2O and RP-HPLC purification.

The synthesis of an α-hydrazinoacetyl modified epitope, $HA^{307–319}$, was undertaken as shown in Scheme 3. Compound **11** was obtained with the Fmoc/*tert*-butyl strategy;[15] acylation of peptidyl resin **9** at the N-terminus with bromoacetic acid and then displacement of the bromine with commercial *tert*-butylcarbazate to install the hydrazine moiety[16] gave compound **10**. Hydrazinoantigen **11** was finally obtained in 40% overall yield as sole product following deprotection and cleavage from the resin by acidic treatment.

Having all the building blocks to hand, we next examined their ability to give the target molecules using a one-pot double orthogonal reaction (Scheme 4). Compound **6** was coupled with hydrazinoantigen **11** in DMF/citrate phosphate buffer (pH 5.2)[17] to furnish cleanly the corresponding hydrazone fragment (as detected by RP-HPLC). The chloroacetyl moieties were reacted with the mannosyl derivative **13** at pH 8–8.5,[18] to give construct **14** in 43% isolated yield. Cores **7** and **8** were reacted similarly to give the antigen-bearing clusters **15** and **17** in 40 and 18% yields, respectively (Figures 1 and 2). Compound **17** was obtained in a much lower yield compared with the first two generations, as indicated by the complex RP-HPLC trace obtained following the double ligation (Figure 3d). The mannose derivative **13** was found to react easily with simple *N*-chloroacetylated lysinyl trees.[11] Thus, we envisioned performing the thioetherification at once, since the glyoxylyl group should be stable and, therefore, not interfere during the substitution reaction. As expected, the thioetherification proceeded smoothly to give intermediate **16** (Figure 3a), which was further treated with **11** (Figures 3b and 3c). The presence of the desired tree **17**, together with reformed disulfide **12**, was detected almost exclusively in the crude mixture by RP-HPLC, which contrasted with the profile observed when the alternative route was applied (compare Figures 3c and 3d). We were actually able to obtain the fully assembled construct **17** in a considerably improved yield (51%). Following semipreparative RP-HPLC purification, all

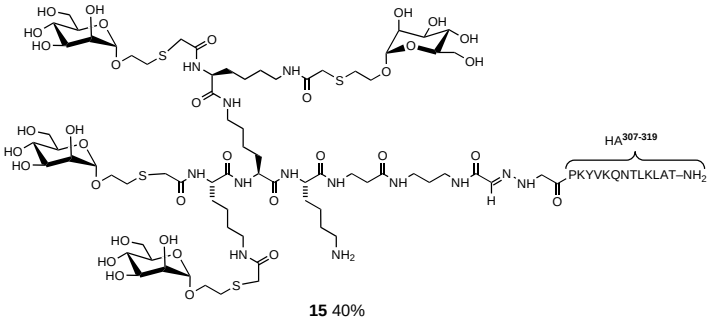
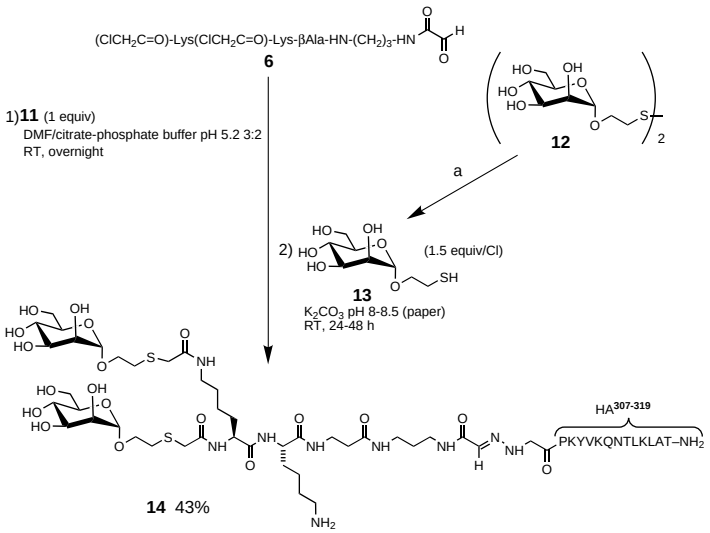


Figure 1. Product of orthogonal hydrazone/thioether ligation reaction between glyoxylyl *N*-(chloroacetyl)lysinyl core **7**, the hydrazinopeptide antigen **11**, and the thio-sugar derivative **13**.



Scheme 4. Orthogonal hydrazone/thioether ligation reaction between glyoxylyl *N*-(chloroacetyl)lysinyl core **6**, the hydrazinopeptide antigen **11**, and the thio-sugar derivative **13**. a) *n*Bu_3P (1 equiv), *n*PrOH/H_2O 1:1, RT, overnight.

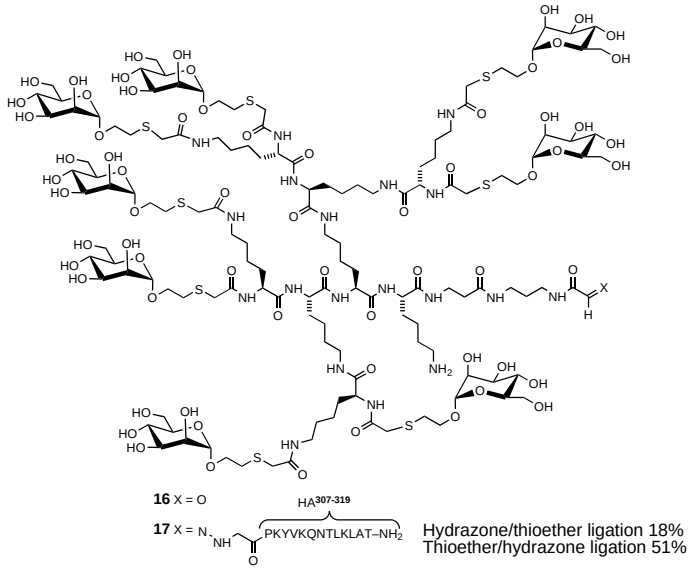


Figure 2. Product of orthogonal hydrazone/thioether ligation reaction between glyoxylyl *N*-(chloroacetyl)lysinyl core **8**, the thio-sugar derivative **13**, and the hydrazinopeptide antigen **11**.

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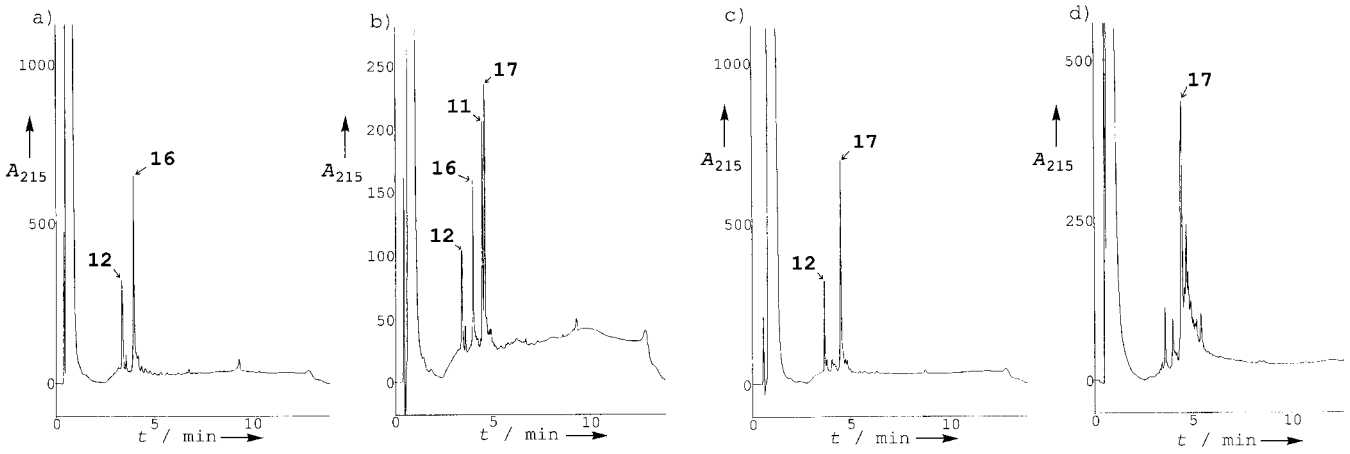

Figure 3. RP-HPLC chromatograms of crude mixtures from: a) the thioether ligation of core **8** with **13** after 36 h; b) and c) the second ligation with hydrazinoantigen **11** after 30 min and overnight, respectively; d) sequential hydrazine/thioether ligation to give **17**. Chromatographic conditions: TSK gel (TosoHaas) C18 (110 Å, 2 μm, 4.6 × 50 mm). Flow rate 2 mL min^{-1}, 50 °C. Buffer A: 0.05 % aqueous TFA; buffer B: 0.05 % TFA in CH_3CN/H_2O 80:20. Gradient: 0 % B for 5 min, 0 – 100 % B over 10 min, 100 % B for 1 min, 0 % B for 2 min.

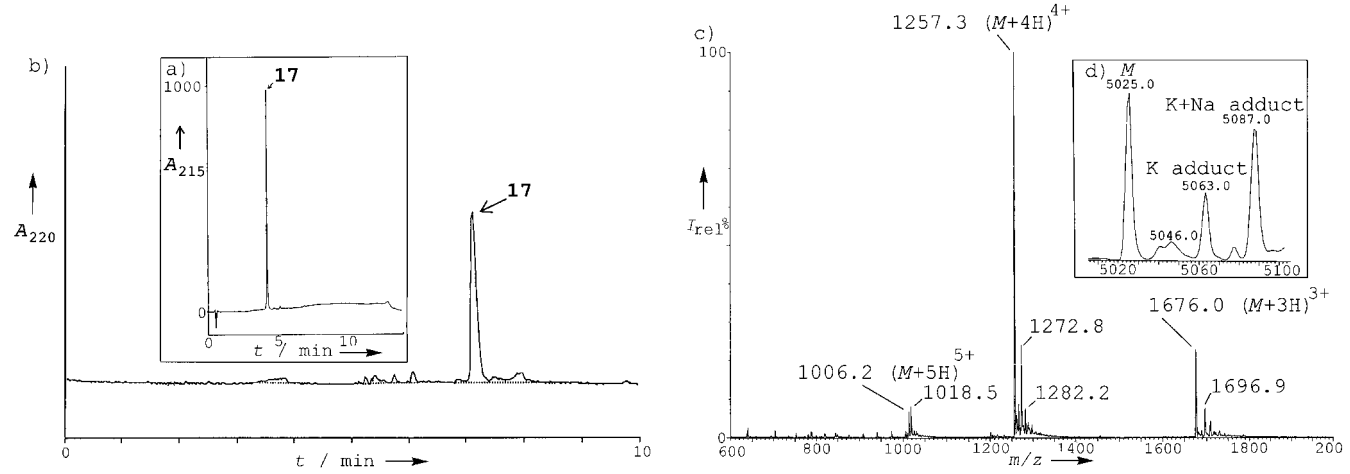

Figure 4. a) RP-HPLC chromatogram of pure compound **17**. b) CZE profile of compound **17**. Conditions: 75 μm × 500 mm fused silica capillary, with a 20 mA current and a 30 kV field in an Applied Biosystems Model 270A-HT system (Foster City, USA); separation was performed at 50 °C using a 50 mM sodium borate (pH 9) migration buffer. c) Positive ESI-MS of compound **17**. Flow rate 5 μL min^{-1} at a concentration of 5 pmol mL^{-1} in 0.2 % HCO_2H in CH_3CN/H_2O 50:50. d) ESI-MS true mass scale of compound **17**.

compounds were characterized by electrospray ionization mass spectrometry (ESI-MS) and capillary zone electrophoresis (CZE) as illustrated for **17** (Figure 4).

Experimental Section

Synthesis of **17** with the thioether/hydrazone ligations procedure: Compound **12** (5.75 mg, 6 equiv) was dissolved in *n*PrOH/H_2O (1:1, 500 μL) and treated with *n*Bu_3P (3.0 μL, 6 equiv) at room temperature overnight under nitrogen. The reaction was monitored by RP-HLPC. The mixture was concentrated under reduced pressure for 20 min and then, dissolved in H_2O (50 μL) under N_2. Compound **8** (3.9 mg, 2 μmol) was dissolved in DMF (600 μL) and added to this solution. The apparent pH was adjusted to 8.0 – 8.5 by addition of solid potassium carbonate. The mixture was stirred for 36 h at room temperature and monitored by RP-HPLC. Citrate/phosphate buffer (350 μL) and modified $HA^{307-319}$ peptide **11** (4.05 mg, 1 equiv) were then added to the mixture and the pH adjusted to 5.2 by addition of aqueous 1N HCl. The mixture was further stirred for 12 h at room temperature, diluted with H_2O, frozen and lyophilized. Compound **17** (5.46 mg, 51 %) was obtained as a white powder following RP-HPLC purification and lyophilization.

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The Tetraaza[1.1.1.1]*m*,*p*,*m*,*p*-cyclophane Dication: A Triplet Diradical Having Two *m*-Phenylenediamine Radical Cations Linked by Twisted Benzenes**

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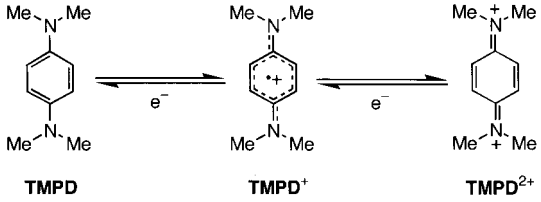
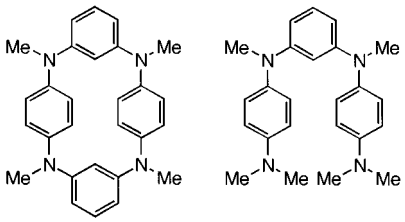
Many kinds of cyclophanes have been synthesized so far for the purpose of investigating the peculiar electronic and spectroscopic properties, which result from the interesting stereochemistry, as well as the effective binding properties for use as host molecules in molecular recognition chemistry.[1] Although carbon is the most common bridging element in useful macrocyclic compounds such as porphyrins[2] and calixarenes,[3] other elements such as nitrogen, oxygen, silicon, phosphorus, and sulfur can be used to obtain additional chemical and physical properties. Indeed, over the past few years, a considerable number of studies have been devoted to the syntheses of various heteroatom-bridged [1_n]cyclophanes.[4]

We are interested in aza-bridged cyclophanes[5] in connection with the chemistry of high-spin organic molecules; several cyclophane-based high-spin molecules have been prepared to date.[6] Chemically and thermally stable polyradicals often contain heteroatoms that serve as spin-bearing sites[7] and are connected to each other through *m*-phenylene units, which have proven to be effective ferromagnetic couplers.[8] For example, a *p*-phenylenediamine (PD), such as *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD; Scheme 1), can be readily converted by one-electron oxidation into a stable semiquinone radical (Wurster's blue), in contrast to the instability of ammonium radicals. Therefore, the use of this type of radical as a spin-bearing unit is pertinent to the exploitation of novel high-spin organic molecules.[9]



Scheme 1. Conversion of TMPD into the stable semiquinone radical (Wurster's blue) as well as the dication.

When PD is to be employed as a spin-bearing site, **1** is a promising precursor of high-spin azacyclophanes with the smallest ring size possible. In addition, **1** can be regarded as *m*-phenylene-tethered version of **2**. We have already reported



the spin preference of $\mathbf{2}^{2+}$ and also pointed out the presence of several conformational isomers.[10] On the other hand, **1** is anticipated to retain only one conformation in which the two PD moieties are juxtaposed. Such a conformation is not feasible for **2** because of steric repulsion between the methyl groups. Here, we report on the synthesis, structure, and redox properties of cyclophane **1**. Furthermore, the electronic structure of its dication is described on the basis of EPR and quantum-chemical studies.

Palladium-catalyzed condensation of *m*-dibromobenzene with *N*,*N*′-dimethyl-*p*-phenylenediamine gives the desired **1** in an abysmal yield of 0.28 %[11] (Scheme 2), but in a one-pot reaction. The UV/Vis spectrum of **1** in cyclohexane measured at room temperature shows two absorption maxima at $\lambda_{max} =$ 236 and 302 nm, which are slightly blue-shifted to those of

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