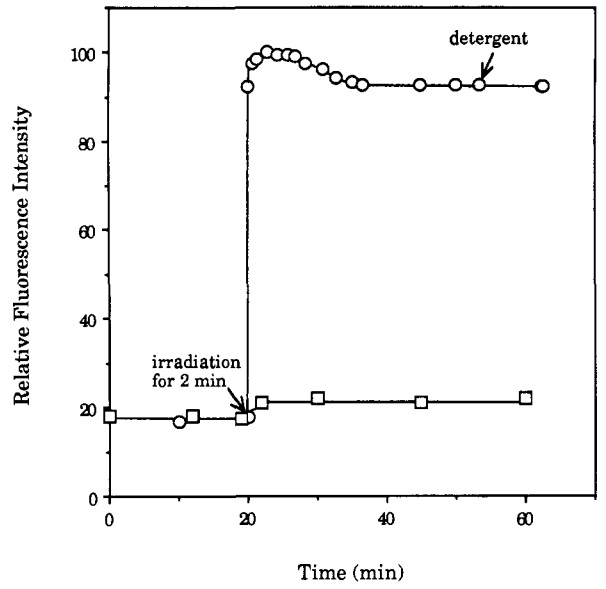

Figure 3. Fluorescence intensity of calcein-loaded suspensions of **2c** suspended in 1.6 mM **3a** (1 mM Tris buffer, 100 mM NaCl); (○), PEAA 0.2 mg/mL; (□), polymer-free control. The initial pH of sample was 7.7, the final pH 6.69. Arrows indicate times of irradiation and detergent addition.

Figure 1 shows the time course of acidification upon irradiation of suspensions of **2a** and **2b** prepared in 4 mM aqueous solutions of **3b**.[5] In the absence of PEAA, pH decreases nearly linearly with irradiation time, from an initial value of 7.4 to a value of 5.7 in 10 min under these irradiation conditions. Addition of PEAA increases the buffer capacity of the solution and slows the rate of pH depression; nevertheless, acidification is sufficient to drive the vesicle-to-micelle transition, as shown by an abrupt loss of the turbidity of the suspension (Figure 2).[6]

Figure 3 confirms that photoinduced acidification of PC suspensions can be used to effect release of vesicle contents.[7] Calcein was used as the marker dye, and release is reported as an increase in fluorescence emission as the dye is diluted into the extravesicular space.[8] As shown in Figure 3, the initial vesicle preparation (at pH 7.7) is stable with respect to leakage, but irradiation for 2 min results in quantitative release of marker. Irradiation for shorter times can be used to effect partial release of contents, as shown by a further increment in fluorescence emission upon addition of lytic concentrations of Triton X-100.

Acknowledgment. This work was supported by U.S. Army Research Office Grant DAAL03-88-K-0038.

(4) 3,3′-Dicarboxydiphenyliodonium disulfate (mp 162–4 °C) and hexafluorophosphate (mp 173–8 °C) were prepared by the method of Beringer and co-workers (Beringer, F. M.; Drexler, M.; Gindler, E. M.; Lumpkin, C. C. *J. Am. Chem. Soc.* **1953**, *75*, 2705).

(5) A solution of 15 mg of **2** in 3 mL of $CHCl_3$ was evaporated to dryness in a long-necked, round-bottomed flask on a rotary evaporator. After drying under vacuum for ca. 6 h, the lipid film was hydrated with 15 mL of 10 mM Tris buffer containing 50 mM NaCl and 0.01% NaN_3 with vortex agitation at a temperature slightly above the lipid melting transition. Rates of pH depression of aqueous solutions of iodonium salts (4 mM) in the absence and in the presence of PEAA (0.7 mg/mL) were measured in quartz cells of 0.4-cm inner diameter upon irradiation at 30 °C in a Rayonet minireactor (Southern New England Ultraviolet Co.) equipped with a merry-go-round sample holder and four 254-nm RPR mercury lamps. The sample cells were periodically withdrawn and shielded from the light, and pH was measured immediately. The sample of PEAA used in this work was of number-average molecular weight ca. 2×10^4 (Schroeder, U. K. O.; Tirrell, D. A. *Macromolecules* **1989**, *22*, 765) and is nearly atactic (Seki, K.; Tirrell, D. A. *Macromolecules* **1984**, *17*, 1692).

(6) For measurement of suspension turbidity, solutions of PEAA (0.7 mg/mL), 3,3′-dicarboxydiphenyliodonium hexafluorophosphate (4 mM), and lipid (0.7 mg/mL) in 10 mM Tris buffer were irradiated as described above. The sample cells were periodically withdrawn, shielded from the light, and kept in a constant-temperature bath (at 24 °C for **2a**, 30 °C for **2b**) to give a total of 30 min of incubation time. Optical density at 600 nm and pH were then measured at 25 ± 0.2 °C.

(7) Calcein-loaded vesicles of **2c** were prepared by sonication of a 15 mg/mL lipid suspension in a 250 mM solution of the dye in 1 mM Tris containing 100 mM NaCl. Small vesicles were isolated by gel filtration on Sepharose CL-4B. Lipid concentration was determined by the method of Charles and Stewart (Charles, J.; Stewart, M. *Anal. Biochem.* **1980**, *104*, 10) and adjusted to 0.04 mg/mL. PEAA was added to a concentration of 0.2 mg/mL and **3b** to a concentration of 1.6 mM. A 2-mL aliquot of the suspension was irradiated for 2 min in a quartz cuvette with a 254-nm Pen-Ray UVP lamp (4500 mW/cm² at 2.5 cm). Fluorescence intensity at 520 nm was recorded immediately on a Perkin-Elmer MPF-66 spectrometer thermostated at 25 °C (excitation at 495 nm; 5-nm slit widths). Maximum fluorescence intensity was obtained by addition of 0.05 mL of a 15% solution of Triton X-100.

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Multiring Interlocked Systems: Structure Elucidation by Electrospray Mass Spectrometry

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Interlocked rings (catenanes) have been a synthetic challenge in chemistry for decades.[1,2] They have experienced a revival of interest since new synthetic strategies have been developed based either on the three-dimensional template effect of a transition metal[3] or on preorganized donor–acceptor stacked arrangements.[4]

Systems consisting of several interlocked cycles may lead to long multilink chains (olympics), molecular collar chains, etc. and are thus especially appealing. The template synthesis of catenands (coordinating interlocked rings) opens the door to an infinity of topologically novel molecular systems, and in particular, it allows relatively easy synthesis of [3]-catenands[5,6] consisting of two peripheral rings separately interlocked to a central cycle.

The most efficient procedure relies on a cyclodimerization reaction, as represented in Figure 1. Besides the expected cyclodimer ($n = 1$), a substantial proportion of higher homologues may also be found.

Applying the strategy of Figure 1 to coordinating fragments bearing terminal acetylenic functions and using copper(I) as templating species, we prepared two series of cyclooligomeric compounds. The first family contains 30-membered peripheral rings interlocked to a large central cycle, whereas the second one contains 27-membered peripheral rings. Unexpectedly, we observed in both series the formation of higher homologues ranging from $n = 1$ to $n = 5$.

The present report will be restricted to the description of the various catenates consisting of up to six peripheral 27-membered rings interlocked to a large central cycle (up to 132 atoms). The various compounds studied are represented in Figure 2.[7]

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(7) **1**, **2**, and $[1,2,Cu]^+$-BF_4^- were prepared as previously reported.[6,9] Chromatographic separation (silica; CH_2Cl_2 and small amounts of CH_3OH as eluent) afforded 3^{2+} (23%), 4^{3+} (23%), and 5^{4+} (10%) as pure isolated BF_4^- salts. These compounds are deep red glassy solids. They display very similar ^1H NMR properties and have identical elemental analyses. They can thus only be distinguished from one another by mass spectrometry. In addition, a mixture of 5^{4+}, 6^{5+}, and 7^{6+} (6%, 8%, and 5%, respectively) was also obtained. The yields indicated in ref 9 for 3^{2+} and 4^{3+} are erroneous due to the rigorously identical ^1H NMR spectra of these two compounds.

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Table I. ESMS Characterization of [3]- to [7]-Catenates[a]

compound	calcd molec wt, Da	rel proportn[b] in the crude mixture	characteristic ions[e]
$[3^{2+},2BF_4^-]$	2222.9	23%[c]	1024.4 $[3]^{2+}$; 2135.9 $[3^{2+},BF_4^-]^+$
$[4^{3+},3BF_4^-]$	3333.3	23%[c]	1024.3 $[4]^{3+}$; 1579.8 $[4^{3+},BF_4^-]^{2+}$
$[5^{4+},4BF_4^-]$	4444.7	16%[c]	1024.4 $[5]^{4+}$; 1395.0 $[5^{4+},BF_4^-]^{3+}$; 2136.6 $[5^{4+},2BF_4^-]^{2+}$
$[6^{5+},5BF_4^-]$	5557.2	8%[d]	1024.6 $[6]^{5+}$; 1302.6 $[6^{5+},BF_4^-]^{4+}$; 1765.7 $[6^{5+},2BF_4^-]^{3+}$
$[7^{6+},6BF_4^-]$	6668.6	5%[d]	1024.6 $[7]^{6+}$; 1246.7 $[7^{6+},BF_4^-]^{5+}$; 1580.7 $[7^{6+},2BF_4^-]^{4+}$; 2136.9 $[7^{6+},3BF_4^-]^{3+}$

[a] Each compound is named by the number of interlocking rings of which it consists. [b] The fraction of each component was obtained by adding the amount of product isolated pure to that determined on a mixture of catenates. [c] Isolated compound. [d] Nonseparated mixture. [e] The measured mass of each ion is in excellent agreement with the calculated mass ($\Delta M = \pm 0.3$ Da).

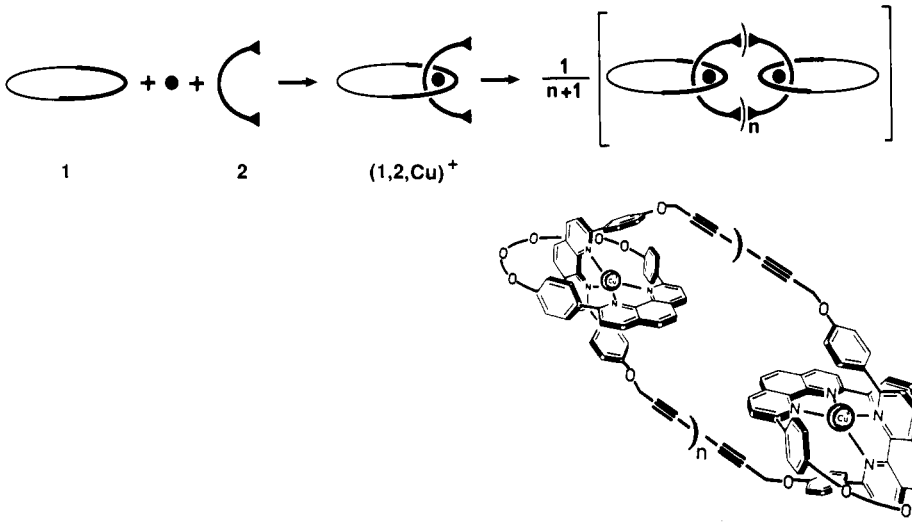

Figure 1. Template synthesis of multiring catenates. The gathering transition metal (Cu^+) is represented by a black dot, and the coordinating fragments are drawn in thick lines. The triangles represent the terminal functions (—C≡CH) to be dimerized to —C≡CC≡C—. The systems obtained (3^{2+}, 4^{3+}, 5^{4+}, ..., for n = 1, 2, 3, ..., respectively) consist of $n + 1$ peripheral rings separately interlocked to a central ring built during the cyclooligomerization reaction (acetylenic coupling).

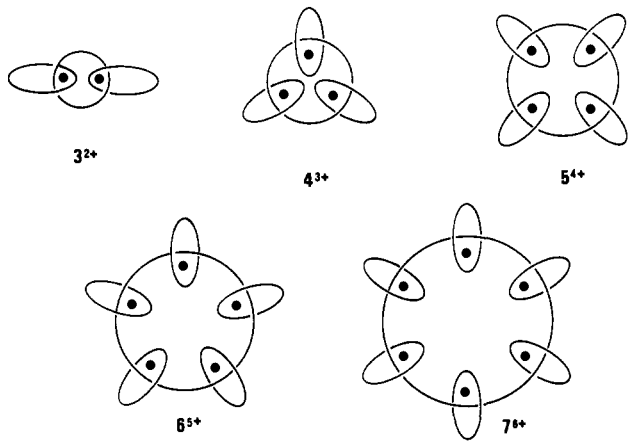

Figure 2. Multicatenates 3^{2+}, 4^{3+}, 5^{4+}, 6^{5+}, and 7^{6+}.

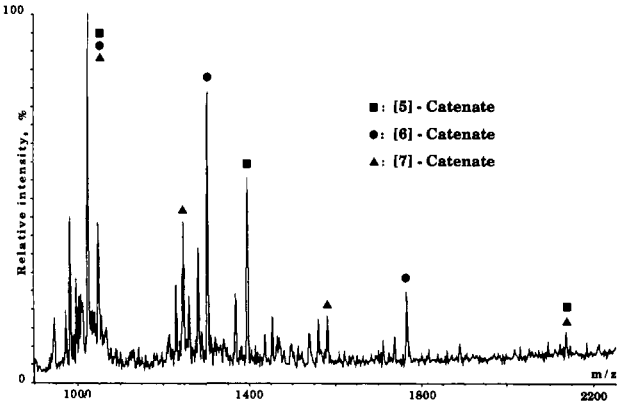

Figure 3. ESMS spectrum of a mixture of catenates (mostly 5^{4+}, 6^{5+}, and 7^{6+} as their BF_4^- salts). All unlabeled peaks correspond to fragmentations or traces of [8]-catenate. ESMS was performed on a VG BIO-Q quadrupole mass spectrometer with a mass range of 4000. The electrostatic spray ion source was operated at atmospheric pressure at 4000 V with an extraction cone voltage (V_c) value of 80 V. The samples were dissolved in dichloromethane. Ten microliters of a 50 pmol/μL solution was introduced into the ion source at a flow rate of 2 μL/min.

Identification of these multiring systems could be achieved thanks to electrospray mass spectrometry (ESMS), recently used for protein characterization. Indeed, since FABMS has a tendency to be less efficient for compounds larger than 3 or 4 kDa, ESMS was used instead to explore the possible formation of large homologues. In the case of proteins the ionization is obtained by multiple protonation or multiple sodium adducts,[8] but for the coordination compounds studied here the ionization is obtained by the loss of counterions. Since each monomeric unit bears one positive charge (Cu^+), it is clear that all compounds formed will appear in an ESMS spectrum at the same m/z value when all counterions are lost (m/z = 1024.6 in the series described here). To calculate the molecular weight of the different homologues of the series, it is therefore necessary to observe at least one ion with a reduced state of charge and therefore containing one or several BF_4^-.

In Table I are collected relevant mass spectrometry data as well as the relative proportions of the various catenates either isolated pure or obtained as mixtures. Figure 3 shows a representative MS spectrum taken on a mixture of catenates.

The synthetic procedure previously described for [3]-catenates[6,9] leads to surprisingly good yields of higher cyclooligomers. Indeed, Glaser's oxidative coupling applied to the synthesis of porphy-

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rin-containing macrocycles has recently been shown to also afford very large rings.[10]

Acknowledgment. We thank the CNRS and the Conseil Général de la Région Alsace for their financial supports.

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An Experimental Demonstration of the Stereochemistry of Enzymic Cyclization of 2,3-Oxidosqualene to the Protosterol System, Forerunner of Lanosterol and Cholesterol

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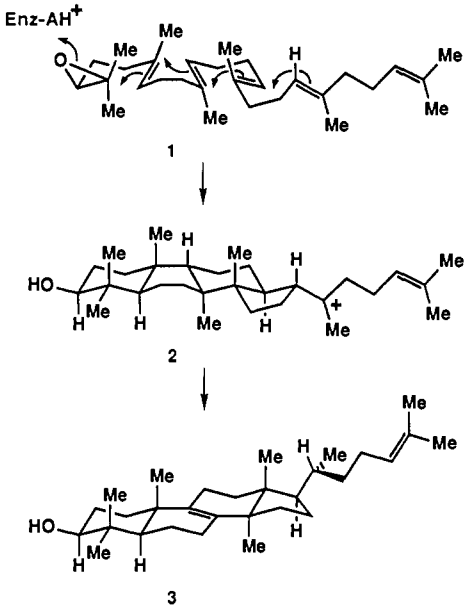
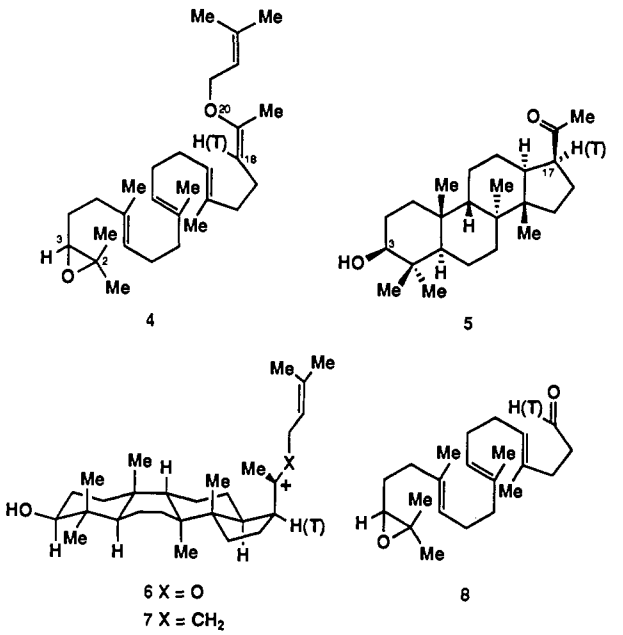
The biosynthesis of sterols is generally thought to be initiated by the enzymic cyclization of 2,3-oxidosqualene[1] (**1**) to the cationic protosterol **2**[2] (or equivalent), which undergoes a series of suprafacial 1,2-shifts leading, after proton loss, to lanosterol (**3**).



A problem associated with this scheme is that a rotation about the C(17)–C(20) bond of 120° is required prior to H migration from C(17) to C(20) in order to produce the natural *R* configuration at C(20), whereas only a 60° rotation (counterclockwise when viewed down the C(17)–C(20) axis) would lead, after H shift, to the unnatural *S* configuration at C(20). Perhaps because of this difficulty, it has been proposed that the initial tetracyclic intermediate is not cation **2** but an equivalent that results from covalent attachment of some nucleophile from the cyclase enzyme, often referred to as an "X group", to the *re* face of C(20) during the closure of ring D.[3,4] Reported herein is a demonstration that the closure of ring D during sterol biosynthesis produces a protosterol with a 17β-oriented side chain rather than the 17α arrangement previously assumed (**2** or its X-group equivalent).[2,3] The experimental evidence includes the bioconversion of an analogue of 2,3-oxidosqualene to a protosterol,[5] which was identified by comparison with totally synthetic material.[6]

The (±)-20-oxa analogue of 2,3-oxidosqualene (**4**) and the 18-tritio form of **4** were synthesized as described below and incubated with sterol-free microsomal protein from bakers' yeast (*Saccharomyces cerevisiae*)[7,8] at pH 6.2 (0.5 M phosphate buffer containing 0.3% Triton X-100) at 23 °C for 40 h. The product of cyclization was isolated by removal of water in vacuum, addition of tetrahydrofuran (THF), drying, evaporation, and chromatography on silica gel, in a 1:1 ether–hexane fraction. Although the principal product is chromatographically similar to ergosterol (TLC R_f 0.27, silica gel, 1:1 ether–hexane), it was not this yeast sterol, but the 17β-acetyl protostane derivative, **5** (56% of the theoretical yield).[9] Biosynthetic **5** was converted to the 3-*tert*-butyldimethylsilyl (TBS) ether (TBSCl, imidazole, DMF, 35 °C, 6 h) and compared with totally synthetic material, prepared as described below. The biosynthetic and synthetic 3-TBS ethers of **5**, mp 200–202 °C (undepressed on admixture), $[\alpha]^{23}_D$ +65.8° (c = 0.6, $CHCl_3$), were identical in all respects, including TLC mobility, 500-MHz ^1H and 125-MHz ^{13}C NMR spectra, and infrared and high-resolution mass spectra. Treatment of biosynthetic **5** TBS ether with 1% KOH in 1:1:1 THF–CH_3OH–H_2O at 50 °C for 2 h effected complete isomerization to the more stable C(17) diastereomer (17α-acetyl), which was identical with a totally synthetic standard by ^1H NMR, IR, HRMS, and TLC comparison.[10]



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(8) Type II bakers' yeast (Sigma) was washed and suspended in pH 7 phosphate buffer, lysed by two passages through a French pressure cell, and centrifuged at 10000*g* to afford a supernatant, which was centrifuged at 100000 g. The microsomal pellet was resuspended in pH 6.2, 0.025 M phosphate buffer containing 2% Triton X-100 and centrifuged at 100000 g, and the protein in the supernatant was freed of sterols by using a hydroxylapatite column.

(9) In contrast the 18,19(*Z*)-isomer of **4** is not converted to sterol products by the cyclase under the above-described conditions (which afford a >70% yield of lanosterol from 2,3-oxidosqualene).

(10) During the 17β → 17α epimerization, radioactivity was removed from the 17-tritiated TBS ether of **5** produced biosynthetically from 18-tritiated **4**.

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