Steroids in Molecular Recognition

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Contents

I. Introduction

Steroids are found in all eucaryotic organisms and display a great variety of different biological functions.[1] The most frequent steroid is the highly lipophilic cholesterol (**1**, Figure 1) which is metabolized to the bile acids in the liver and also serves as starting material for the synthesis of steroid hormones. Its incorporation into cell membranes influences their fluidity and prevents the development of crystalline substructures.[2] It is also involved in the regulation of gene transcription and protein degradation.[3,4] Cholesterol transport within as well as to cells is highly regulated, and there exist at least three kinds of cholesterol transport mechanisms within the cell.[5] Cholesterol is delivered either exogenously by way of food uptake or is synthesized endogenously in the endoplasmatic reticulum.[2,6−8] Transport of highly water-insoluble exogenous cholesterol or its esters[9] is mediated by a delivery system consisting of very low density (VLDL), low density (LDL), and high density (HDL) lipoproteins, and the partitioning of the steroid between these supramolecular transport systems is delicately balanced.[6−8,10−13] Cholesterol is a major component of atherosclerotic plaque deposits in atherosclerosis, one of the most frequent causes of death in industrialized countries where diet is too rich in the steroid.

The other members of the steroid family adopt equally important biological functions. For digestion to proceed in the intestine, bile acids such as cholic acid (**2**, Figure 1) are complexing and dispersing water-insoluble fats, fatty acids, and other lipids by forming micelles. Steroid hormones are subdivided into progestins, mineralocorticoids, glucocorticoids, androgens, and estrogens, depending on their function.[14−16] The progestin progesterone (**3**), synthesized in the ovarian cells, is besides estradiol the most important female sex hormone. Its function is the preparation of the uterine endometrium for the implantation of the fertilized egg and the maintenance of pregnancy. The mineralocorticoid aldosterone (**4**), which is in equilibrium with hemiacetal **5**, is synthesized in the adrenal cortex. In case of low Na^+ concentration or excessively low blood pressure, it is released from the adrenal cortex in the kidney. It directly influences the Na^+ concentration and indirectly regulates the amount of water in cells. In situations of stress or inflammation, the glucocorticoid hydrocortisone (**6**) is abundant. It induces the conversion of proteins to carbohydrates; thus its function is opposite to that of insulin. Hydrocortisone also has a complex suppressant effect on the immune system. Easy oxidation of the 11β-hydroxy group leads to cortisone (**7**). This molecule is less active than **6**, but since the oxidation is reversible, **7** is equipotent. Important male sex hormones are the androgens testosterone (**8**) and stanolone (5α-dihydrotestosterone, **9**) which are responsible for the development of male characteristics, while the estrogens estradiol (**10**) and estrone (**11**) control the growth of female sex characteristics.

Via blood stream, the steroid hormones reach their target cells, where they activate the transcriptional machinery in a mechanism which seems to be similar for all steroid hormones. Since the highly lipophilic compounds are almost insoluble in water, they are transported by globulins, glycoproteins, and albumin.[17] Before entering the cell by diffusion, they are

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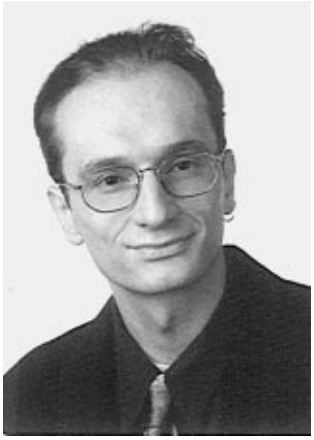
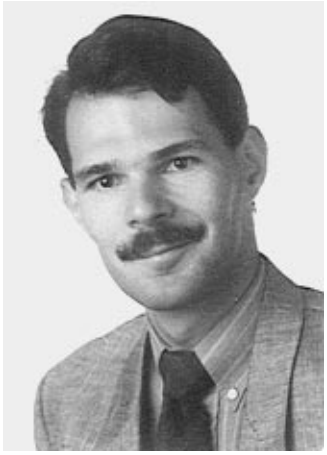


Peter Wallimann, born in 1965 in Lucerne, Switzerland, studied chemistry at the University of Zurich, where he received his degree in 1992 under the supervision of Wolf-Dieter Woggon. Subsequently, he joined the research group of François Diederich at the ETH Zurich. In his doctoral work in supramolecular organic chemistry, he developed and investigated both conventional and dendridic-functionalized artificial steroid receptors for aqueous solution. In 1997, a postdoctoral project is planned with Daniel Kemp at MIT, Boston.

Thomas Marti, born in 1970, grew up in Schaffhausen, Switzerland, where he also received his schooling. In 1990 he embarked on a degree in chemistry at the ETH Zürich, and subsequently received his diploma in 1994. Currently he is in his second year as a Ph.D. student in the group of Professor F. Diederich; his research interests are the selective binding of steroids, especially estradiol, through rational design and synthesis of cyclophane hosts.

released from the transport protein. As a part of the signal transduction chain, they bind to a receptor in the nucleus or the cytoplasm.[18−20] The steroid receptor class is part of a receptor superfamily which further contains thyroid hormone, vitamin D, and retinoic acid receptors.[21] Ligand binding alters the conformation of the receptor, leading to dimerization which is necessary for association to the hormone response element of the target gene. The receptor binding to DNA occurs mainly *via* two zinc fingers of the protein inserting into the major groove.[22] Details of the steroid hormone-mediated signal induction and transcription processes are rapidly becoming revealed.[18−22] Several nongenomical effects (*i.e.* effects not related to transcription) of steroid hormones are also known.[23]

At the heart of all the mentioned biological processes lies the molecular recognition of the various steroids. A profound molecular level understanding of the principles governing steroid complexation and



Andreas Fürer, born in Berne, Switzerland, in 1969, studied chemistry at the University of Berne, where he received his diploma under the supervision of the late Professor Rolf Scheffold, in 1994. In the same year he joined the group of Professor François Diederich where he is currently doing his Ph.D. studies. His main interests are in bioorganic chemistry, especially the synthesis of receptors for cholesterol.

François Diederich, born in Ettelbruck, Luxembourg, in 1952, studied chemistry at the University of Heidelberg and received his doctoral degree under the supervision of Heinz A. Staab in Heidelberg in 1979. After postdoctoral work with Orville L. Chapman at UCLA from 1979 to 1981, he became a research associate at the Max-Planck-Institute for medical research in Heidelberg where he completed his habilitation in 1985. Subsequently, he joined the faculty at UCLA where he advanced in 1987 to associate and in 1989 to full professor or organic chemistry. Since April 1992, he has been professor of organic chemistry at the ETH Zürich. The general theme of his research is the design, preparation, and study of functional molecular architecture. In bioorganic chemistry, he develops receptors for small biomolecules, supramolecular enzyme-like catalysts, dendritic models for globular proteins, and nonpeptidic lead compounds for medicinal chemistry. At the center of materials-oriented research are new allotropes of carbon, the covalent chemistry of fullerenes, fullerene chirality, acyclic and macrocyclic acetylenic molecular scaffolding, and new carbon-rich polymers with unusual electronic and optical properties.

transport could open new perspectives for biomedical research, and such reasoning induced the writing of this review. Studies with small synthetic hosts should ideally complement the biological investigations with large protein receptors and supramolecular transporting assemblies in enhancing the insight into steroid recognition processes, ultimately showing ways to potentially new therapeutical approaches. Established therapies against high cholesterol levels are bile acid sequestration by synthetic polymers such as cholestyramin, and inhibition of the enzymes HMG-CoA reductase or squalene cyclase, which are required for endogenous cholesterol synthesis.[6,7,11] New approaches based on detailed molecular recog-

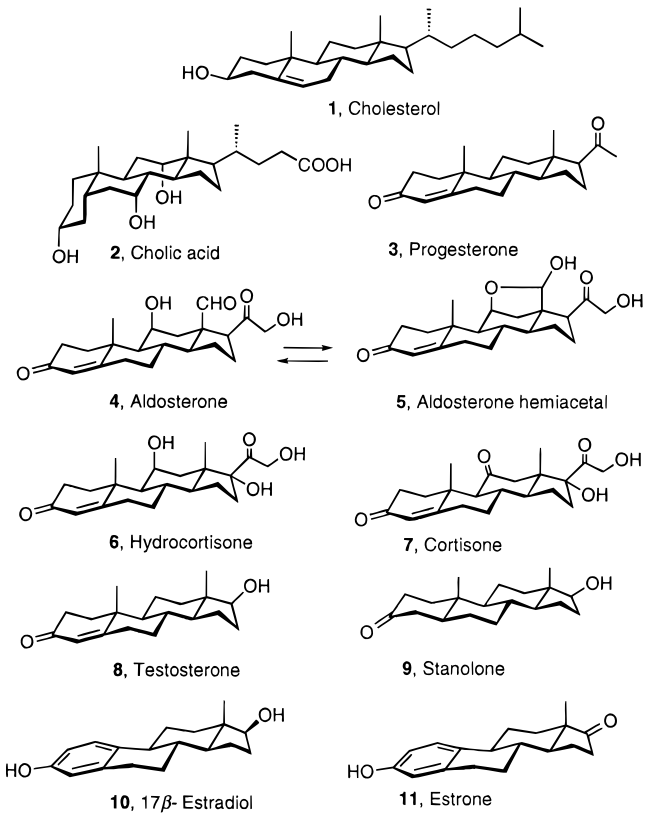


Figure 1. Representatives of the most important classes of steroids.

nition insights could be the development of selective artificial receptors and solubilizing agents for cholesterol or of compounds altering the partitioning of cholesterol between VLDL, LDL, and HDL lipoproteins. Investigations into selective binding mechanisms of steroid hormones could contribute to the rational development of methods to interfere with the signal induction and transcriptional machinery involving the steroid hormone receptor family. Tightly binding receptors with slow exchange kinetics[24,25] could be used as hormone deposits and slow-release systems.

The review starts with an analysis of biological steroid recognition on the basis of X-ray crystal structure analyses, since knowledge gained from natural systems should assist and fertilize the design of synthetic receptors. In the next section, the steroid complexation properties of natural and modified cyclodextrins are summarized. These receptors have been known for a long time and have proven to be useful in a wide range of applications such as solubility mediation, transport, and stabilization of steroids by inclusion complexation. The following section presents progress with cyclophanes, a more recent class of synthetic hosts for steroids which form inclusion complexes with apolar substrates mainly through aromatic-aliphatic contacts. Finally, the last section discusses receptors which take advantage of the chirality and rigid shape of steroids in the formation of preorganized binding sites with convergent functional groups for recognition.

II. X-ray Studies of Biological Steroid Receptors

Although supramolecular structures and assemblies in the solid state[26] may differ markedly from solution structures, an examination of natural substrate−receptor interactions on a crystallographic basis[27−34] could help to improve the understanding of molecular recognition principles[25,35−44] applicable to the design of novel synthetic steroid receptors and nonproteinogenic artificial enzymes.[45−48] A still limited, but growing number of three-dimensional structures of steroid-recognizing proteins, enzymes, and antibodies, have become available over the past decade.[49−73] The first well-resolved single crystal X-ray structure was solved for uteroglobin, a small progesterone-binding protein of unknown function.[49,50] Further protein structures were obtained by both X-ray[51−53] and NMR[54−57] analyses of fragments of the nuclear hormone receptor family.[20,58,59] Also known are a number of crystal structures of enzymes from bacteria,[60−64] fungi,[65] and mammals,[66,67] and of some antibody fragments raised against a sex hormone[68−71] and a cardiac glycoside.[72,73] Of the above-mentioned structures, a few have been obtained for both the free and the complexed receptors[61−65,68−73] at resolutions between 1.8 and 2.7 Å and are particularly interesting with respect to investigations of steroid molecular recognition principles. In the following discussion, we shall restrict ourselves mainly to systems, which allow a close examination of steroid−receptor interactions in the binding site.

A. Steroid Binding Antibodies

1. The DB3 Antibody

In 1993, Wilson and co-workers published the refined three-dimensional structure of an antigen-binding fragment (Fab) of the monoclonal antibody DB3 both in its uncomplexed state and bound to progesterone (**3**).[70] DB3 was raised in mice against an 11α-substituted progesterone bovine serum−albumin conjugate and shares the typical immunoglobulin fold.[74,75] The Fab fragment is responsible for steroid recognition in the complementarity determining regions (CDRs) and shows high affinity not only for progesterone (**3**) but also for the structurally differing hormones **12**−**15** (Figure 2), with relative binding affinities (IC_{50}) in the nanomolar range (Table 1). The antibody's ability to bind a diversity of substrates with similar affinity, referred to as cross-reactivity, has been explained by an induced-fit mechanism for substrate recognition.[76−78] In addition to the progesterone−Fab complex, the authors also crystallized and analyzed the four corresponding complexes with the haptens **12**−**15**[71] to shed light on how such cross-reactive steroids bind to a single receptor. The steroidal guests differ in their substitution pattern as well as in their A ring geometry. For example, compound **13**, 5α-pregnane-3β-ol-20-one hemisuccinate, displays a *trans*-fused A ring and possesses a roughly planar fully saturated A/B/C/D ring system. In contrast, progesterone (**3**) and progesterone-11α-ol hemisuccinate (**12**) both share a partially unsaturated A ring which deviates by about 45° from a mean molecular plane comprising rings B/C/D. Compounds **14** and **15**, aetiochol-

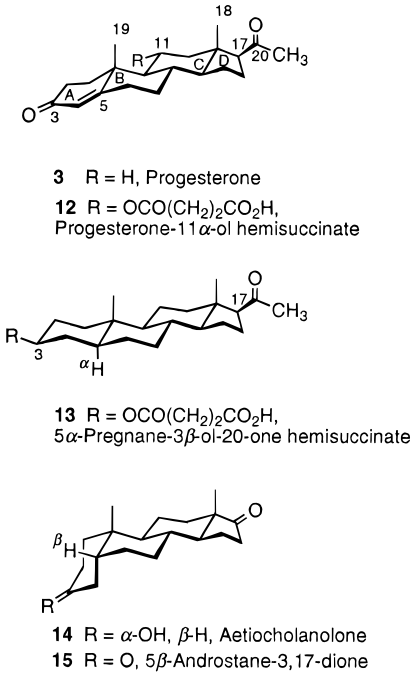


Figure 2. Progesterone (**3**) and steroids **12**−**15** form complexes with the Fab of monoclonal antibody DB3, which were characterized by X-ray crystallography.

Table 1. Comparison of the Binding Characteristics for Five DB3 Fab−Steroid Complexes

	steroid				
	3	**12**	**13**	**14**	**15**
IC_{50} (nM)	1	0.36	2	21	8
orientation[a]	*syn*	*syn*	*syn*	*anti*	*anti*
buried ligand surface (Å^2)	240	291	288	227	223
ligand buried (%)	89	81	82	91	88
van der Waals contacts[b]	59	68	58	27	19
H-bonds	2	2	4	2	2

[a] According to Figure 3. [b] Cut-off: 4.1 Å.

anolone and 5β-androstane-3,17-dione, show even greater structural differences, with their *cis*-fused A rings being almost perpendicular to the rest of the molecule.

The crystallographic analysis of the progesterone-complexed Fab revealed a narrow, hydrophobic, and highly complementary binding site, lined by several aromatic amino acid side chains in a perpendicular, edge-to-face orientation.[70] In this complex, 89% or 240 Å^2 of the progesterone surface area is buried in the cavity-type recognition site, leading to 59 intermolecular van der Waals contacts within a distance of 4.1 Å (Table 1). The D ring is buried deepest and points to the bottom of the P1 pocket, mainly made up by the aromatic side chains of Tyr^{H97} and Phe^{H100b} (Figure 3a). The B and C rings in the P2 pocket are sandwiched between two indole rings, with their β-face covered by Trp^{H50} and their α-face by Trp^{H100} (*syn* orientation). The steroid is further recognized and oriented by two H-bonds (distance O···X 3.1 Å, angle O···H−X ∼152°) between the keto groups at C(3) and C(20) and His^{L27d} and Asn^{H35}, respectively. A comparison between the complexed and uncomplexed Fab crystal structures displayed only small movements in most of the antibody's main chain and

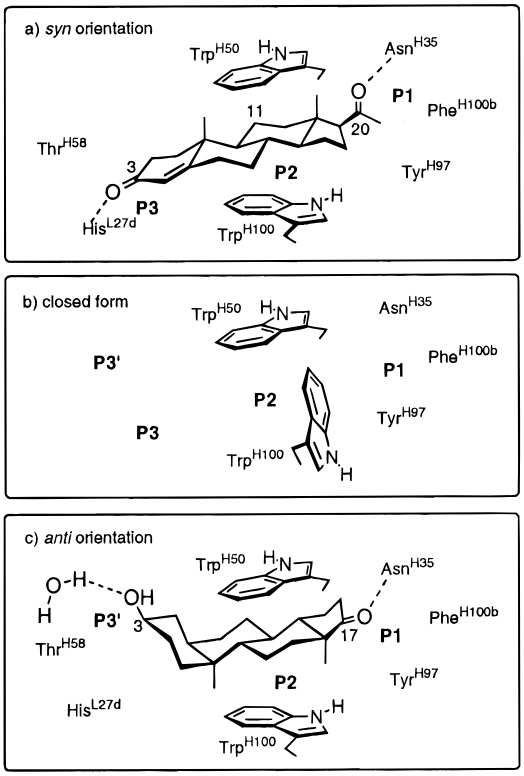


Figure 3. Simplified representation of the complexes of DB3−Fab with progesterone (**3**) in a *syn* mode (a) and with aetiocholanolone (**14**) in an *anti* mode (c). In the free Fab fragment, the indole ring of Trp^{H100} occupies the P1 pocket in an edge-to-face orientation with respect to the indole moiety of Trp^{H50} (b).

the amino acid side chain residues. However, in the unbound Fab the indole ring of Trp^{H100} is moved by 5.5 Å into the P1 pocket adopting an edge-to-face orientation with respect to the indol ring of Trp^{H50} (Figure 3b).

The X-ray crystal structures of the corresponding complexes with steroids **12**−**15** revealed a second docking orientation which explains the observed cross-reactivity.[71] Compounds **12** and **13** bind in a mode similar to the one observed for progesterone (**3**) with the axial Me groups pointing towards Trp^{H50}. In contrast, the two more sterically demanding 5β-steroids **14** and **15** are bound in an opposite orientation, with their β-faces pointing toward Trp^{H100} (*anti* orientation, Figure 3c). A net rotation of the indole ring of Trp^{H100} by 25° accounts for the variable positions of the steroidal B, C, and D rings for the different haptens. Furthermore, compounds **14** and **15** occupy with their tilted A rings the alternative small pocket P3′ while their flattened counterparts **3**, **12**, and **13** use the P3 pocket instead. Nevertheless, the overall shape of the antigen binding site is highly conserved and only small changes in the CDR are necessary to modulate complementarity for all five haptens.

Table 1 summarizes the features of the five Fab−steroid complexes. In all cases, 80−90% of the steroidal surface is buried within the antibody's cavity corresponding to a total area of 220−290 Å^2. Interestingly, the *syn*-oriented and most active compounds **3**, **12**, and **13** make more than twice as much van der Waals contacts than the *anti*-bound haptens

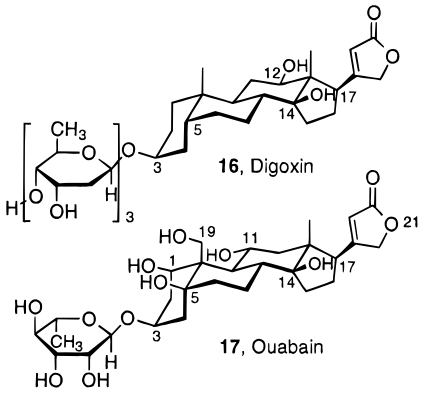


Figure 4. Digoxin (**16**) and ouabain (**17**) form complexes with the Fab of monoclonal antibodies 26−10 and 40−50, respectively, which were characterized by X-ray crystallography.

14 and **15**. This can be rationalized by the fact that the DB3 antibody was originally raised against a conjugate of **12**, which is structurally more similar to **3** and **13** than to **14** and **15**. In all cases, the Asn^{H35} residue in the P1 compartment forms one H-bond with the keto functions at C(17) or C(20). Also, with the exception of **13**, a second H-bond is formed with the O-atoms at C(3) either by His^{L27d} in the P3 pocket, or with an oriented water molecule near Thr^{H58} in the P3′ pocket. The hemisuccinate chain in compound **13** makes three H-bonds, two with its peripheral carboxylate to a valine and a histidine, and another with its ester carbonyl group to His^{L27d}. The corresponding hemisuccinate side chain in compound **12**, however, is solvated by bulk water since it is located near the entrance of the cavity binding site.

2. The 26–10 and 40–50 Antibodies

In 1993 and 1995, Sheriff and co-workers published the three-dimensional structures of two further monoclonal antibody fragments, Fab 26−10 and Fab 40−50, obtained from digoxin-immunized mice.[72,73] Digoxin (**16**, Figure 4) is a cardiac glycoside that consists of both a HO-substituted 5β,14α-steroidal aglycon with an unsaturated γ-lactone at C(17) and a trigitoxose moiety at C(3). Antibody 26−10 exhibits high affinity for digoxin and related congeners, such as ouabain (**17**), with associations constants K_a up to 10^{10} L mol^{-1}. The X-ray crystal structures of Fab 26−10, with and without bound digoxin,[72] and of Fab 40−50, with and without ouabain,[73] were solved and refined to 2.7 Å resolution.

Digoxin penetrates an increasingly hydrophobic cleft of Fab 26−10 in the CDR with its D ring buried deepest. The rotation about the bond between C(17) and the lactone ring is highly restricted. In contrast, the sugar moiety is disordered, fully solvent-exposed, and does not contribute to the binding at all. Also, the polar β-HO groups at C(12) and C(14) are both solvated. Aromatic amino acid side chains again seem to play an important role in steroid binding and account for 60% of the 61 observed van der Waals contacts between digoxin and the receptor. Also, the steroidal skeleton is sandwiched between several aromatic units, in this case Tyr^{H33}, Tyr^{H50}, and Trp^{H100}, comparable to the DB3−progesterone complex (Figure 3a). Most strikingly however, Fab 26−10 does not form any H-bonds with digoxin, despite several polar groups on the steroidal skeleton.

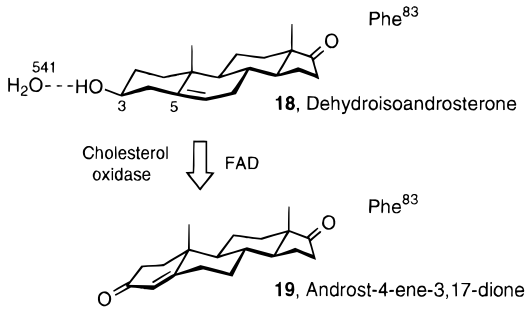


Figure 5. Conversion of dehydroisoandrosterone (**18**) to androst-4-ene-3,17-dione (**19**) by cholesterol oxidase from *Brevibactericum sterolicum*.

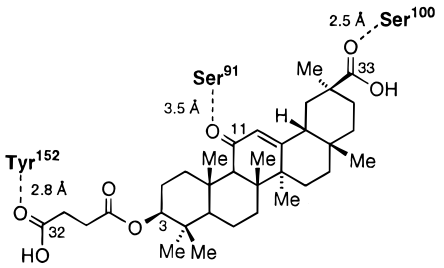
In the complex of Fab 40−50 with ouabain (**17**), the hapten is inserted into a 17 Å deep groove with the D ring tightly fitting a 6 × 7 Å wide apolar pocket. One H-bond is formed between O(21) and His^{H35}, placing the lactone ring in an orientation which is flipped by 180° as compared to the orientation of this ring in the Fab 26−10 complex with digoxin. A second H-bond is formed between C(14)−OH and the C═O group of Ser^{L91}. Here, 76% (348 Å^2) of the total surface of ouabain is buried within the Fab, and again, 60% of the van der Waals contacts are made with aromatic amino acid side chains of Fab 40−50. The rhamnose sugar moiety and most of the steroidal HO groups remain solvated. The shape complementarity between the Fabs and steroids **16** and **17** is reasonably good, although not as perfect as between the DB3 binding site and compounds **3** and **12**−**15**. As in the case of the DB3−anti-progesterone antibody, no significant structural changes are observed between the bound and unbound forms of the two receptors.

B. Steroid-Binding Enzymes

1. Cholesterol Oxidase

Blow and co-workers determined the three-dimensional structure of cholesterol oxidase,[61,62] a flavin−adenine−dinucleotide (FAD)-dependent, catabolic enzyme from *Brevibactericum sterolicum*. The enzyme transforms cholesterol (**1**) and a variety of similar Δ^5-3β-hydroxysteroids like dehydroisoandrosterone (**18**) into the corresponding Δ^4-3-keto compounds such as androst-4-ene-3,17-dione (**19**) by an oxidation−isomerization reaction sequence (Figure 5). The high-resolution X-ray crystal structure of cholesterol oxidase, as a complex with **18** and FAD, provided insight into the mode of binding and catalysis.[62] The enzyme consists of two major domains responsible for the recognition of the cofactor and the steroidal substrate at their interface.

Dehydroisoandrosterone (**18**) is bound in an internal, 11 Å long hydrophobic cavity completely sealed from bulk solvent. A loop formed by the amino acid residues 70−90 blocks the entrance with the phenyl ring of Phe^{83} pointing inside. Major apolar contacts in the binding pocket are established between **18** and Pro^{76}, Val^{77}, Phe^{83}, Pro^{344}, Leu^{375}, and Tyr^{446}. In the absence of the substrate, the active site is occupied



20, Carbenoxolone

Figure 6. H-bonding interactions between bacterial 3α,-20β-hydroxysteroid dehydrogenase and the glucocorticoid-type inhibitor carbenoxolone (**20**).

by an ordered cluster of 13 H_2O molecules of which 12 get expelled upon complexation. The remaining H_2O[541] is multiply H-bonded in a polar network composed of the steroidal HO group at C(3), parts of the cofactor, Asn[485], and His[447] (X···Y distances of 2.8−3.2 Å in X···H−Y). No second H-bond is observed between the enzyme and the keto group of the steroid. Interestingly, while **18** is small enough to fit fully into the binding pocket of cholesterol oxidase, modeling studies show that the C_8H_{17} side chain of cholesterol (**1**) itself cannot be accommodated together with the steroidal ring system and probably protrudes from the cavity. Nevertheless, owing to its enhanced hydrophobic character, cholesterol binds stronger to the oxidase than **18**.

2. Dehydrogenases

Three more X-ray crystal structures of related, $NADP^+$-dependent enzymes with oxidoreductase properties were solved recently. Lewis and co-workers determined the three-dimensional structure of rat liver 3α-hydroxysteroid/dihydrodiol dehydrogenase,[66] and Ghosh *et al.* published the structures of two other, quite similar enzymes, human 17β-hydroxysteroid dehydrogenase[67] and bacterial 3α,20β-hydroxysteroid dehydrogenase,[63] which was cocrystallized with a steroidal inhibitor.[64] In all cases, the receptors display hydrophobic clefts or cavities either partly or mainly shaped by aromatic amino acid side chains. For example, in the extreme case of 3α-hydroxysteroid/dihydrodiol dehydrogenase, the steroid binding site is aligned by only one aliphatic (Leu[54]) and five aromatic amino acid residues (Tyr[55], Trp[86], Phe[118], Phe[129], and Tyr[216]).

3α,20β-Hydroxysteroid dehydrogenase possesses four analogous active sites at the interface of four corresponding monomeric units.[63] In the complex with carbenoxolone (**20**, Figure 6), a glucocorticoid-type inhibitor, the four binding sites in the crystal are unequally occupied by 100, 95, 54, and 36%, respectively.[64] Such a distribution and the interfacial arrangement indicate a possible cooperative effect in steroid recognition. Furthermore, since the dehydrogenase provides only one particular catalytic center per monomer unit, the steroidal substrates must be able to orient themselves in two ways in order to get oxidized or reduced both at C(3) and C(20). In the complex of 3α,20β-hydroxysteroid dehydrogenase with **20**, the inhibitor is anchored at both ends by two short H-bonds (Figure 6). The keto function at C(33) forms one H-bond to the Ser[100] HO group, and the O atom at C(32) of the peripheral hemisuccinate chain makes contact with the HO group of Tyr[152], thereby disabling the cofactor to approach the active site properly. An additional, weaker contact is also observed between the C=O group at C(11) and Ser[91], whereas the rest of the molecule displays extensive apolar interactions with the receptor.

C. Steroid-Binding Proteins

Uteroglobin is a small progesterone (**3**) binding protein (15.8 kD) from rabbit uterus with unclear function. An X-ray crystal structure was first solved in 1980[49] and subsequently refined to high resolution (1.34 Å), unfortunately in the absence of a substrate.[50] The main characteristics of the uteroglobin amino acid sequence is the lack of Trp and the small number of Tyr and Phe moieties. The protein consists of two identical, α-helical monomer chains each comprised of 70 amino acids and, in its oxidized form, is linked by two disulfide bridges. Despite ligand exchange taking place only in the protein's reduced state involving four free cystein residues, uteroglobin in its oxidized state was shown to contain a central hydrophobic cavity of 15.6 × 9.0 Å size, filled with 14 disordered H_2O molecules. Computer modeling studies proved good surface complementarity between the central core and progesterone (**3**), and two H-bonding interactions with Tyr[21] and Tyr[21′] were proposed for anchoring the substrate.

Further partial structures of steroid binding proteins of the nuclear hormone receptor family were obtained for both a rat glucocorticoid receptor[51] and a human estrogen receptor fragment.[52,53] The structures were solved in the presence of bound consensus oligonucleotides for the investigation of protein−DNA rather than protein−steroid interactions and, therefore, are outside the scope of this work.

D. Conclusions

The X-ray structural data gained for seven antibody−steroid complexes can be summarized as follows: Antigen-binding fragments (Fabs) sharing the immunoglobulin fold recognize steroids in apolar grooves or pockets between the variable light and heavy chains V_L and V_H in the complementarity determining regions (CDRs). Comparison of both complexed and uncomplexed receptors reveals highly preorganized binding sites. Only small structural changes in the antibody are necessary to respond to different, cross-reactive haptens. Hydrophobic desolvation[36,79] and dispersion interactions are the major driving forces for the associations which are characterized by high host−guest shape complementarity and the burial of large apolar regions of the substrates in the order of 220−350 Å^2. Usually, 75−90% of the steroid surface makes extensive van der Waals contacts with the receptor. Thereby, the antibody's aromatic amino acid side chains (Trp, Tyr, Phe) account for 50−60% of the apolar contacts with the ligand. These aromatic residues are often aligned in an edge-to-face geometry,[80−83] a well-known motive, recently explained as a more dispersive[84,85] rather than an electrostatic phenomenon.[86,87] An

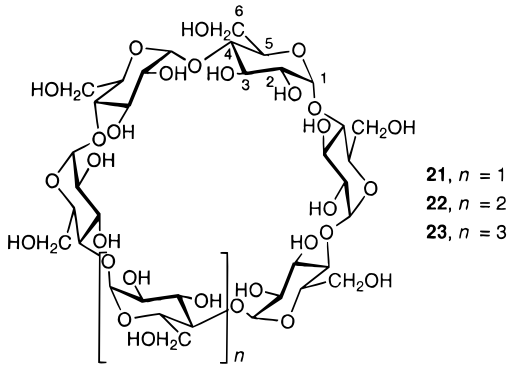


Figure 7. Structures of the most abundant α- (**21**), *β*- (**22**), and *γ*-cyclodextrins (**23**).

amazingly small number of H-bonds is found in the Fab−steroid complexes. Polar substrate substituents are either oriented into bulk water or remain at least partially solvated. However, in highly apolar complementary regions, H-bonding interactions may occur and help to orient the haptens, thereby fine-tuning specificity. It has not yet been determined to whether the desolvation of large apolar surfaces in the binding of steroids by antibodies results in a significant favorable entropic contribution to the free energy of complexation.

In case of the enzymatic receptors, steroids are usually bound in apolar regions at the interface of two or more monomeric subunits. In some cases, aromatic amino acid side chains seem to be less important for the stabilization of the complexes, as compared to the antibody recognition processes, and certain substrates are not too tightly bound and can orient in different ways. Nevertheless, binding is mainly achieved by the formation of numerous van der Waals contacts, as well as the expulsion of water molecules from the recognition site. As in the antibody complexes, usually one or two H-bonds help to orient the substrate, thereby directing important functional groups into the catalytic site.

Generally speaking, all these observations give rise to a conception in which enzymes, unlike antibodies or proteins, are selected for transition-state stabilization rather than for tight substrate or product binding.[88,89] Too close a fit would make the catalysts prone to competitive inhibition and reduced turn-over rates. Since binding strength is not directly related to catalytic activity, cross-reactive enzymes can still be highly selective with regards to catalysis. Unfortunately, there is very limited kinetic data available for steroid-transforming enzymes.[90−93]

III. Steroid Complexation by Cyclodextrins

A. General Structural Features and Physical Properties of Cyclodextrins

Cyclodextrins are formed by enzymatic degradation of starch and were first isolated by Villiers in 1891.[94] The most abundant of these cyclic oligomers are composed of six (α, **21**), seven (*β*, **22**), or eight (*γ*, **23**) α-D-glucopyranose rings connected by 1,4′-*O*-glycosidic bonds (Figure 7).[95] Cyclodextrins are moderately toxic; the lethal dose LD_{50} of *β*-cyclodextrin in mice was found to be 0.2−0.5 g kg^{-1}.[96] Their most interesting feature lies in the ability to form inclusion complexes in solution and in the solid state with a great diversity of molecules and ions.[95,97,98] First comprehensive studies of inclusion complexation by cyclodextrins were performed in the late 1940s to early 1950s by Friedrich Cramer as part of his habilitation thesis.[99]

Table 2. Solubilities of α-, *β*-, and *γ*-Cyclodextrin in Water (Room Temperature)[104] and Solvation Enthalpies and Entropies As Determined from Dissolution Studies[103]

cyclodextrin	solubility in water: g/100 mL	solubility in water: mol L^{-1}	solvation enthalpy: $\Delta H°$ (kcal mol^{-1})	solvation entropy: $\Delta S°$ (cal K^{-1} mol^{-1})
α (**21**)	14.5	0.15	7.67	13.8
β (**22**)	1.85	0.016	8.31	11.7
γ (**23**)	23.2	0.18	7.73	14.7

It was shown by X-ray crystallography that cyclodextrins possess a toroidal shape.[100,101] The conformation of the glucopyranose units is always a $^4C_1$ chair which, in the smaller cyclic oligomers **21** and **22**, is slightly distorted in order to close the macrocyclic frame.[102] For steric reasons, all glucopyranose rings adopt a nearly perpendicular orientation to the mean molecular plane and rotation about the axis passing through C(1) and C(4) is not possible; thus, the cavities in cyclodextrins are highly preorganized. All primary HO groups line the narrower and all secondary HO groups the wider rim of the toroidal macrocycle, thereby interacting with bulk solvent and providing significant solubility in protic environments. Interestingly, α- and *γ*-cyclodextrins show much larger water solubility than the *β*-homolog (Table 2).[103,104] The cyclodextrin cavity is lined by the inward pointing H-atoms at C(3) and C(5) and the glycosidic oxygen atoms. Since all HO groups with high solvation requirements point outward, the cavity is lipophilic in character, and both hydrophobic desolvation and London dispersion forces provide the major driving force for inclusion complexation of suitably sized apolar molecules in water. The diameters of the cone-shaped binding sites range from 4.7 to 5.3 Å in α-, from 6.0 to 6.5 Å in *β*-, and from 7.5 to 8.3 Å in *γ*-cyclodextrin.[105] The depth of their cavities amounts to *ca.* 7.9−8.0 Å, as estimated from Corey-Pauling-Koltun (CPK) models.[100]

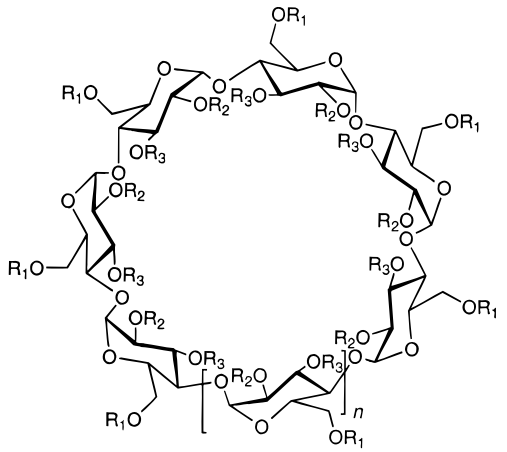
B. Cyclodextrins as Receptors for Organic and Inorganic Substrates

1. General Features

In the absence of synthetic hosts prior to the late 1960s,[35,106,107] cyclodextrins were the only compounds which, similar to biological receptors, displayed the ability to form molecular complexes with many guest molecules in aqueous solution. Following the pioneering work of Cramer,[99] the ability of cyclodextrins to form inclusion complexes, to differentiate in binding between enantiomeric substrates, and to act as artificial enzymes[45−47] by catalyzing reactions of bound guests attracted the interest of many scientists. A variety of intermolecular interactions and solvation effects were proposed to explain the stability of the inclusion complexes formed with a great

diversity of substrates, ranging from neutral and charged organic molecules to inorganic ions.[100,108] Hydrophobic desolvation and van der Waals interactions are among the most important driving forces for cyclodextrin complexation besides H-bonding between polar groups of the substrate and the OH groups of the macrocycles. Upon guest inclusion, water molecules ("high energy water"[100,108]) are released from the cavity into the bulk which provides a favorable enthalpic driving force by gain of solvent cohesive energy. A further increase in solvent cohesive energy occurs when lipophilic substrates are removed from their bulk solvent cage into the cyclodextrin cavity.[79,109] In addition, entropy is gained by desolvation of the complementary surfaces of host and guest upon association. Calorimetric studies with various substrates such as *p*-nitrophenol derivatives showed that the formation of tight inclusion complexes by α-cyclodextrins is often an enthalpically driven process following an isoequilibrium (isokinetic) relationship, with a significant fraction of the enthalpic gain being compensated by a negative entropic term.[110] Thereby, a large number of close van der Waals contacts are established at the expense of degrees of freedom of the two binding partners.[111,112] In contrast, inclusion complexation of the same substrates in the wider *β*-cyclodextrin cavity was proven to be more entropically driven. Since in the latter case the host−guest fit is loose, the gain in entropy by desolvation of the complementary surfaces of host and guest is not greatly reduced by the loss in degrees of freedom upon association of the binding partners. Also, less van der Waals contacts are established in such a loose complex which leads to an overall smaller change in enthalpy. Inclusion complexation by cyclodextrins and their alkylated derivatives such as permethylated *β*-cyclodextrin (**24**, Figure 8) is also observed in organic solvents.[98,113] Usually, substrates are axially included, with their long axis in direction to the C_n- axis ($n = 6-8$) which passes through the cyclodextrin cavity perpendicular to the mean molecular plane. In most cases, rotation of the complexed guests about this axis is rapid on the NMR time scale.

Substrate specificity is determined by the dimensions of the cyclodextrin cavity. α-Cyclodextrin (**21**) is too small for full encapsulation of a benzene ring. However, benzene derivatives such as *p*-iodoaniline form complexes with **21** in water. The substrate penetrates the cavity from its wider cavity side, leading to full inclusion of the highly polarizable iodine atom and parts of the aromatic ring, as was observed in the X-ray crystal structure of the complex.[114] As a result of partial aromatic ring inclusion, intermolecular contacts shorter than 3.0 Å were observed between C(3)−*H* or C(5)−*H* of the host and some aromatic C atoms in the X-ray crystal structure of the complex between α-cyclodextrin and *p*-nitrophenol.[115,116] Similar C−H···π interactions are frequently observed in the X-ray crystal structures of proteins and their complexes.[115] In the X-ray crystal structures of the complexes between α-cyclodextrin and *p*-nitrophenol or *p*-hydroxybenzoic acid, distortions of the macrocycle to an elliptical shape are observed.[116] In these complexes, the NO_2 and COOH



	n	R_1	R_2	R_3
24	1	CH_3	CH_3	CH_3
25	1	$CH_2CHOHCH_3$	H	H
26	1	CH_3	CH_3	H
35	1	$CH_2CHOHCH_2OH$	H	H
36	1	CH_2CH_2OH	H	H
37	1	$CH_2CH_2CH_2OH$	H	H
38	1	$COCH_3$	H	H
39	1	$COCH_2CH_3$	H	H
40	1	$COCH_2CH_2CH_3$	H	H
41	1	H or $C_4H_8SO_3Na$	H or $C_4H_8SO_3Na$	H or $C_4H_8SO_3Na$
42	1	H or SO_3Na	H or SO_3Na	H or SO_3Na
43	2	$CH_2CHOHCH_3$	H	H

Figure 8. Modified *β*- and *γ*-cyclodextrins in complexes with steroids for which association constants for 1:1 host−guest stoichiometry have been reported (Table 4).

substituents of the guests, respectively, are pointing into the cavity. This inclusion mode is inverted in the complex of permethylated α-cyclodextrin with *p*-nitrophenol, which shows the hydroxy group enclosed in the ellipsoidally distorted cavity.[117]

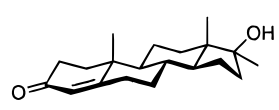
Crystalline *β*-cyclodextrin (**22**) is solvated by either 11 or 12 water molecules.[118,119] The H-bonding patterns in these crystals were determined by neutron diffraction studies[118] and also simulated by molecular dynamics calculations.[120] This combined approach revealed that the orientations of the H-bonds were not well defined thus producing "flip-flop" H-bonding chains. Inclusion complexes of *β*-cyclodextrin may be formed with benzene derivatives carrying bulky substituents like *p*-*tert*-butylphenol[108] and, even better, by cylindrically shaped molecules such as steroids, adamantane,[98] or ferrocene[121] derivatives. The latter compounds are much more complementary in shape to the toroidal cavity than flat aromatic rings.

Finally, *γ*-cyclodextrin (**23**) crystallizes from aqueous solution with 17 water molecules.[122] Its wide cavity can readily accommodate polycyclic aromatic hydrocarbons such as pyrene or benzo[*a*]pyrene as well as steroids. The complexes of the latter are discussed in the following section.

2. Cyclodextrins as Hosts for Natural and Synthetic Steroids

One of the major applications of cyclodextrins in chemistry and pharmacology is the solubilization of

Table 3. Enhancement of Steroid Solubility in a 0.075 M (10g/100 mL) Aqueous Solution of "Dimethyl β-Cyclodextrin" (26) at 25 °C[127]



27, Methyltestosterone (17β-hydroxy-17α-methylandrost-4-en-3-one)

29, 3β,17α,21-Triacetoxypregn-5-en-20-one

28, Nortestosterone (17β-hydroxy-estr-4-en-3-one)

30, 21-Acetoxy-3β,17α-dihydroxypregn-5-en-20-one

steroid	S_1, solubility in water (mg/mL)	S_2, solubility in 0.075 M aqueous **26** (mg/mL)	enhancement factor (S_2/S_1)
progesterone (**3**)	0.016	13.0	812
hydrocortisone (**6**)	0.33	23	56
digoxin (**16**)	0.27	22.2	81
methyltestosterone (**27**)	0.071	13.7	193
nortestosterone (**28**)	0.31	14.7	47
3β,17α,21-triacetoxypregn-5-en-20-one (**29**)	0.01	10.2	1025
21-acetoxy-3β,17α-dihydroxypregn-5-en-20-one (**30**)	0.008	9.1	1137

steroids which, due to their hydrophobic cyclic framework, are highly insoluble in water. Upon complexation by cyclodextrins, steroid solubility increases strongly. The most widely used cyclodextrin derivative for this purpose is "(2-hydroxypropyl)-β-cyclodextrin" (**25**, Figure 8), a β-cyclodextrin derivative in which the primary OH groups are converted into 2-hydroxypropyl ethers.[123−126] Table 3 shows the solubility enhancement of steroids **3**, **6**, **16**, and **27**−**30** in water mediated through complexation by "dimethyl-β-cyclodextrin" (**26**), a modified β-cyclodextrin in which the primary and the secondary OH groups at C(2) are transformed into methyl ethers.[127] Steroid solubilization has also been achieved by using cyclodextrin polymers.[128−130]

Complexation and solubilization by cyclodextrins makes steroids available for a wide field of pharmaceutical applications. Oral,[131−135] sublingual,[136] buccal,[137] intravenous and intracerebral,[138] or transdermal[139−142] administrations have been made possible by complexation. Eyedrops bearing the steroidal drug dexamethasone (**31**, Figure 11) have been prepared using a cyclodextrin as the solubilizing agent.[143]

The chemical stability of delicate steroidal drugs under physiological conditions may be improved by complexation with cyclodextrins. Steroid esters, such as betamethasone-17-valerate (**32**, Figure 11), are sensitive to intramolecular transesterification.[144] The rate of this undesired rearrangement was successfully retarded by complexation with γ-cyclodextrin (**23**) or "dimethyl-β-cyclodextrin" (**26**).[145] On the other hand, addition of β-cyclodextrin to a solution of **32** enhanced the rate of transesterification whereas α-cyclodextrin showed no effect. Furthermore, inclusion complexation by β-cyclodextrin derivatives was shown to enhance the chemical stability of the steroidal drugs digitoxin (**33**) and proscillaridin (**34**) (Figure 11) to acidic pH at 37 °C.[146]

Information on the stoichiometry and stability of the inclusion complexes of cyclodextrins with steroidal guests was obtained by several methods.[147] The most widely used assay is the determination of phase solubility diagrams. Thereby, solubility measurements are carried out by adding an excess of steroid to aqueous solutions containing various concentrations of cyclodextrins followed by equilibration for several days. An aliquot is centrifuged and filtered, diluted with ethanol/water 1:1 (v/v), and analyzed spectrophotometrically or by HPLC methods.[139] A typical phase solubility diagram obtained by such measurements is shown in Figure 9 for progesterone (**3**) and γ-cyclodextrin (**23**).[147]

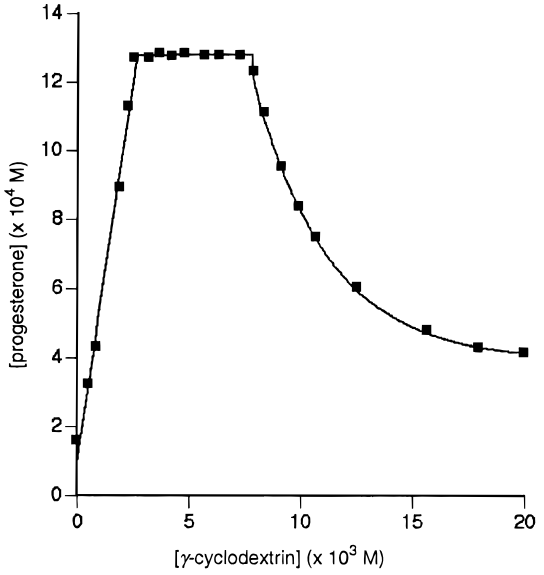


Figure 9. Phase solubility diagram of progesterone (**3**) and γ-cyclodextrin in water at 25 °C.[147] The association constant for 1:1 host−guest complexation can be calculated from the initial straight line portion of the curve.

As can be seen from the diagram, the solubility of the steroid increases linearly with the amount of added cyclodextrin until a critical concentration is reached. A sudden change in the slope of the curve indicates the formation of higher complexes with a cyclodextrin−steroid stoichiometry higher than 1:1.

Such associations usually are less soluble and precipitate from the solution. The association constants for 1:1 host−guest complexes can be calculated from the initial straight line portion of the phase solubility curve, following the equation of Higuchi and Connors (eq 1).[148]

$$K_a = \frac{\text{slope}}{\text{intercept } (1 - \text{slope})} \quad (1)$$

An alternative method was used by Andersen and Bundgaard[145] who calculated the association constants for the complexes formed between cyclodextrins and betamethasone-17-valerate (**32**) from kinetic data of the steroid transesterification reaction in the absence and presence of the receptor. In eq 2, k_{obs} is the observed rearrangement rate, k_1 the rearrangement rate of free steroid, k_2 the corresponding rate of bound steroid, $[CD]_t$ the total cyclodextrin concentration, and K_a the association constant.

$$k_{obs} - k_1 = -\frac{(k_{obs} - k_1)}{K_a[CD]_t} + (k_2 - k_1) \quad (2)$$

Agnus *et al.* reported direct determinations of association constants from high-performance liquid chromatography (HPLC) with cyclodextrins added to the eluent.[149] For the calculation of K_a values, eq 3 was used in which k' and k_o' represent the capacity factors in the presence and absence of cyclodextrin and $[CD]_t$ the total concentration of the receptor in the mobile phase.[150]

$$1/k' = 1/k_o' + \frac{K_a[CD]_t}{k_o'} \quad (3)$$

Only limited information about complex geometries is obtained from electronic absorption and emission,[151,152] circular dichroism, and NMR spectra. A study by Yamasaki and co-workers[147] revealed bathochromic shifts of the positive and negative Cotton effects in the circular dichroism spectrum of hydrocortisone (**6**) when bound to β-cyclodextrin (**22**). The UV spectrum showed a decrease in the intensity of the $\pi-\pi^*$ transition of the enone chromophore in **6** at $\lambda = 250$ nm. The origin of these spectral changes was rationalized by the location of the steroidal chromophore in the hydrophobic cavity of the host.

In contrast to the studies with cyclophane receptors described in section IV, ^{1}H NMR binding assays are only of limited value in providing thermodynamic and structural information in cyclodextrin−steroid complexation. In the absence of anisotropic effects resulting from aromatic ring currents in both receptors and aliphatic steroids, the observed *c*omplexation-*i*nduced changes in chemical *s*hift (CISs) of protons of both binding partners are very small. Thus, upon complexation of hydrocortisone (**6**) by β-cyclodextrin,[147] the internal protons H-C(3) and H-C(5) of the receptor, which point into the cavity, showed a nonspecific maximum upfield shift of −0.042 ppm at [host] = 4.0 mM and [guest] = 1.1 mM. At the same time, the guest protons H-C(4), $H_3C(18)$, and $H_3C(19)$ shifted downfield by a maximum of +0.142 ppm. The downfield shifts of H-C(4) and $H_3C(19)$ were taken as evidence for inclusion of the steroidal A ring into the receptor cavity. ^{1}H spin−lattice relaxation time measurements[147,153] showed decreasing relaxation times of the substrate protons upon complexation, especially of the proton at C(4), thus confirming the above-mentioned inclusion mode.

The solid complexes obtained by precipitation from aqueous solutions containing both cyclodextrins and steroids[147] were further analyzed by X-ray diffractometry, IR spectroscopy, and differential thermal analysis (DTA). Besides powder X-ray diffractometry,[154] DTA has proven useful in distinguishing between precipitated mixtures of cyclodextrins and steroids and real inclusion complexes.[147] For example upon melting, testosterone shows an endothermic peak at 155 °C. This peak is not present in a coprecipitate of the steroid with β- or γ-cyclodextrin, which is taken as evidence for complex formation in the solid state. IR spectra measured for cyclodextrin complexes of hydrocortisone (**6**) showed inclusion-induced changes in shape and location (up to 12 cm^{-1} to higher energy) of the C=O stretching band of the guest.[147] Stoichiometry and geometry of precipitated complexes may obviously be quite different from those of the host−guest associations that are prevailing in the liquid phase.

Table 4 shows the association constants K_a for a variety of cyclodextrin−steroid complexes for which 1:1 host−guest stoichiometry in solution was reported. The structures of the parent α-, β-, and γ-cyclodextrins (**21**−**23**) are shown in Figure 7, those of the modified β- and γ-cyclodextrins **24**−**26** and **35**−**43** without appending chromophores in Figure 8, those of β- and γ-cyclodextrin−chromophore conjugates **44**−**54** in Figure 10. The structures of the steroidal substrates **1**−**3**, **6**−**8**, **31**−**34**, and **55**−**90** are shown in Figure 11 and those of the fluorescent derivatives **91**−**93** in Figure 12. Generally, the self-aggregation tendency of receptors and/or substrates has not been considered in these studies. Only one research group reported quantitative measurements of the aggregation behavior of steroidal substrates.[155] Since cyclodextrins tend to form aggregates,[156,157] it is not surprising that the stoichiometry of cyclodextrin−steroid complexes varies and is often higher in receptor, with 2:1,[147,158,159] 3:2,[147] or 3:1[160] host−guest complexes being formed at elevated cyclodextrin concentrations.

Receptor size is crucial to the recognition of substrates. The smallest, α-cyclodextrin (**21**), forms much less stable complexes (entries 1−26) with steroids than the larger β- and γ-cyclodextrins **22** and **23** and their derivatives (entries 27−245). An aliphatic steroidal ring does not fit into the toroidal binding site of **21,** and complexation presumably occurs only through docking of the substrate with partial inclusion of one, preferentially unsaturated ring, or by incorporation of some smaller steroidal side chains into the cyclodextrin cavity. Correspondingly, no defined complexes of α-cyclodextrins with steroids have been precipitated out of aqueous solution, while solid-state complexes of the β- and γ-derivatives with various host−steroid stoichiometry

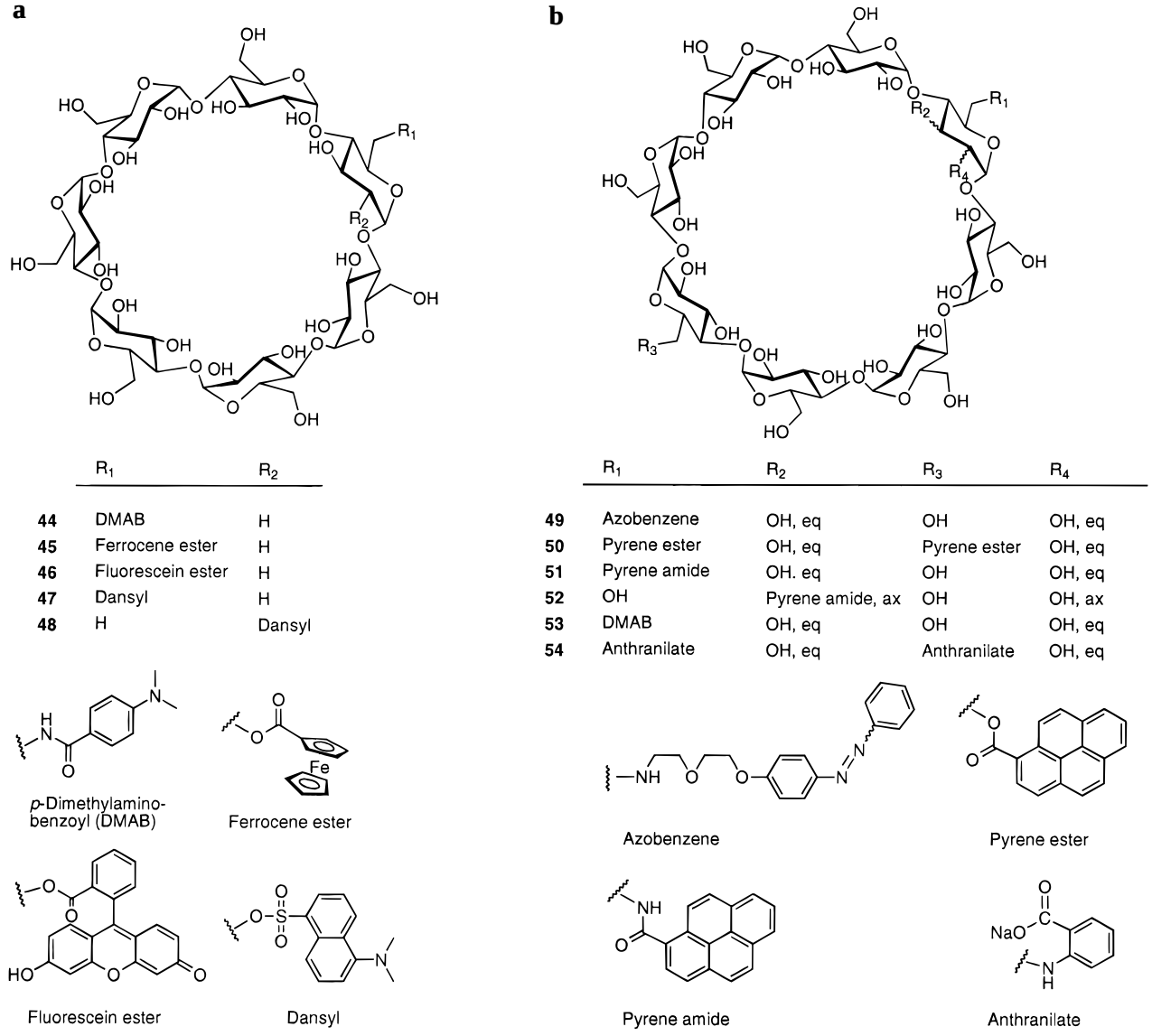


Figure 10. β- (a) and γ-Cyclodextrin−chromophore conjugates (b) in complexes with steroids for which association constants for 1:1 host−guest stoichiometry have been reported (Table 4).

have been isolated and studied by both X-ray powder diffraction[132,134,141,147,161,169,187] and DTA.[147,158,162,173,188,189]

Steroid complexation by cyclodextrins is most efficient in pure water, and the addition of alcoholic cosolvents lowers the association strength (entries 32−38, 49−50, and 81−85),[149,165] as is generally observed for apolar association processes.[36,190,191] With increasing temperature, stability constants of the cyclodextrin−steroid complexes usually decrease,[162,175] although such effects are often quite small which is indicative of a small complexation entropy (entries 144−148, 162−166, and 193−197). In general, the literature lacks thermodynamic measurements and only few determinations of complexation enthalpies and entropies have been reported. From van't Hoff plots of variable-temperature phase solubility measurements, the thermodynamic quantities shown in Table 5 were obtained for complexes of hydrocortisone butyrate (**56**) with various cyclodextrins.[162] The steroid is bound in an enthalpically driven way by the receptors, with only small changes in entropy accompanying the complexation processes (entries 11−13, 58, 61, 62, 112, 115, 116, 162, 165, and 166).

The stability of the complexes formed by functionalized cyclodextrins tends to correlate with the hydrophobicity of the added substituents.[168] Complexes formed with testosterone (**8**), digitoxin (**33**), and prednisolone (**62**) showed a modest increase in stability in the series of substituted cyclodextrins **35** < **36** ≤ **22** < **25** < **37** < **26** (for digitoxin (**33**), see entries 55, 149, 161, 172, 177, and 179). Interestingly, permethylated β-cyclodextrin (**24**) forms a weaker complex with digitoxin (**33**) than β-cyclodextrin (**22**) itself (entries 55 and 135). However, sulfobutylated β-cyclodextrin **41** forms a much stronger cholesterol complex than the corresponding sulfated derivative **42**, a result that confirms the expected influence on K_a of increasing the hydrophobic binding surface of the receptor (entries 190 and 191).[163]

For β- and γ-cyclodextrin complexes, association strength increases with decreasing steroid solubility and with increasing Hansch log P_{oct} values, *i.e.* the octanol−water partition factor.[192−194] Steric host−guest complementarity is of course essential for inclusion complexation. Thus, α-cyclodextrin tends to form more stable complexes with doubly unsaturated 3-ketosteroids which possess a relatively flat A

Table 4. Association Constants Determined for 1:1 Complexes of Cyclodextrin Receptors (CD) with Steroidal Substrates

entry	CD	steroid	K_a (L mol^{-1})	solvent	T (°C)	method[a]	ref(s)
1	**21**	**3**	145	H_2O	25	sol	147
2	**21**	**6**	57	H_2O	25	sol	147
3	**21**	**7**	63	H_2O	25	sol	147
4	**21**	**8**	134	H_2O	25	sol	147
5	**21**	**16**	180	H_2O	25	sol	134
6	**21**	**31**	169	H_2O	25	sol	147
7	**21**	**32**	302	H_2O	25	sol	147
8	**21**	**33**	290	H_2O	25	sol	134
9	**21**	**34**	110	H_2O	25	sol	169
10	**21**	**55**	88	H_2O	25	sol	147
11	**21**	**56**	321	H_2O	20	sol	162
12	**21**	**56**	282	H_2O	30	sol	162
13	**21**	**56**	249	H_2O	37	sol	162
14	**21**	**59**	86	H_2O	25	sol	147
15	**21**	**61**	960	H_2O	25	sol	132
16	**21**	**62**	298	H_2O	25	sol	147
17	**21**	**63**	274	H_2O	25	sol	147
18	**21**	**64**	121	H_2O	25	sol	147
19	**21**	**65**	256	H_2O	25	sol	147
20	**21**	**66**	300	H_2O	25	sol	147
21	**21**	**67**	223	H_2O	25	sol	147
22	**21**	**68**	316	H_2O	25	sol	147
23	**21**	**69**	489	H_2O	25	sol	147
24	**21**	**70**	297	H_2O	25	sol	147
25	**21**	**71**	354	H_2O	25	sol	147
26	**21**	**73**	400	H_2O	25	sol	134
27	**22**	**1**	16 100	H_2O	25	sol	163
28	**22**	**2**	1 488	D_2O	21	NMR	155
29	**22**	**2**	3 150	H_2O	25	therm	164
30	**22**	**3**	13 300	H_2O	25	sol	147
31	**22**	**3**	24 705	H_2O	30	sol	158
32	**22**	**3**	347	H_2O/MeOH 55:45	20	HPLC	149
33	**22**	**3**	240	H_2O/MeOH 53:47	20	HPLC	149
34	**22**	**3**	261	H_2O/MeOH 50:50	20	HPLC	149
35	**22**	**3**	216	H_2O/MeOH 47:53	20	HPLC	149
36	**22**	**3**	165	H_2O/MeOH 45:55	20	HPLC	149
37	**22**	**3**	103	H_2O/MeOH 35:65	20	HPLC	165
38	**22**	**3**	50	H_2O/MeOH 25:75	20	HPLC	165
39	**22**	**6**	1 720	H_2O	25	sol	147
40	**22**	**6**	4 170	H_2O	25	sol	128, 129
41	**22**	**6**	3 000	D_2O	29	NMR	166
42	**22**	**6**	2 683	H_2O	30	sol	158
43	**22**	**7**	3 352	H_2O	20	sol	167
44	**22**	**7**	2 300	H_2O	25	sol	147
45	**22**	**7**	2 632	H_2O	30	sol	158
46	**22**	**8**	7 540	H_2O	25	sol	147
47	**22**	**8**	7 000	H_2O	25	sol	168
48	**22**	**8**	5 058	H_2O	30	sol	158
49	**22**	**8**	2 200	H_2O/MeOH 35:65	20	HPLC	165
50	**22**	**8**	780	H_2O/MeOH 25:75	20	HPLC	165
51	**22**	**16**	11 200	H_2O	25	sol	134
52	**22**	**31**	4 660	H_2O	25	sol	147
53	**22**	**32**	2 990	H_2O	25	sol	147
54	**22**	**32**	1 200	H_2O[b]	24	kin	145
55	**22**	**33**	17 000	H_2O	25	sol	134,146,168
56	**22**	**34**	4 200	H_2O	25	sol	169
57	**22**	**55**	3 250	H_2O	25	sol	147
58	**22**	**56**	2 688	H_2O	20	sol	162
59	**22**	**56**	1 691	H_2O	25	CD	162
60	**22**	**56**	1 456	H_2O	25	UV	162
61	**22**	**56**	1 782	H_2O	30	sol	162
62	**22**	**56**	1 442	H_2O	37	sol	162
63	**22**	**57**	7 211	H_2O	20	sol	167
64	**22**	**58**	952	H_2O/EtOH 85:15	20	sol	167
65	**22**	**59**	4 150	H_2O	25	sol	147
66	**22**	**61**	27 500	H_2O	25	sol	132
67	**22**	**62**	3 600	H_2O	25	sol	147
68	**22**	**62**	1 600	H_2O	25	sol	168
69	**22**	**62**	2 000	D_2O	29	NMR	166
70	**22**	**63**	5 770	H_2O	25	sol	147
71	**22**	**64**	2 370	H_2O	25	sol	147
72	**22**	**65**	3 230	H_2O	25	sol	147
73	**22**	**66**	3 530	H_2O	25	sol	147
74	**22**	**67**	5 420	H_2O	25	sol	147

Table 4 (Continued)

entry	CD	steroid	K_a (L mol^{-1})	solvent	T (°C)	method[a]	ref(s)
75	**22**	**68**	9 560	H_2O	25	sol	147
76	**22**	**69**	2 540	H_2O	25	sol	147
77	**22**	**70**	3 000	H_2O	25	sol	147
78	**22**	**71**	1 120	H_2O	25	sol	147
79	**22**	**72**	24 900	H_2O[c]	37	sol	146
80	**22**	**73**	11 400	H_2O	25	sol	134
81	**22**	**75**	953	H_2O/MeOH 55:45	20	HPLC	149
82	**22**	**75**	670	H_2O/MeOH 53:47	20	HPLC	149
83	**22**	**75**	635	H_2O/MeOH 50:50	20	HPLC	149
84	**22**	**75**	540	H_2O/MeOH 47:53	20	HPLC	149
85	**22**	**75**	345	H_2O/MeOH 45:55	20	HPLC	149
86	**22**	**80**	23 000	H_2O	25	therm	164
87	**22**	**81**	32 000	H_2O	25	therm	164
88	**22**	**83**	88	D_2O[d]	21	NMR	155
89	**22**	**84**	2 400	H_2O	25	therm	164
90	**22**	**85**	336	D_2O	21	NMR	155
91	**22**	**85**	1 950	H_2O	25	therm	164
92	**22**	**86**	371	D_2O	21	NMR	155
93	**22**	**86**	32 000	H_2O	25	therm	164
94	**22**	**87**	26 000	H_2O	25	therm	164
95	**22**	**88**	2 600	H_2O	25	therm	164
96	**22**	**89**	110 000	H_2O	25	therm	164
97	**22**	**90**	81 000	H_2O	25	therm	164
98	**22**	**92**	2 500	H_2O	20	fluor	202,203
99	**22**	**93**	2 300	H_2O	20	fluor	202,203
100	**23**	**2**	362	D_2O	21	NMR	155
101	**23**	**3**	24 000	H_2O	25	sol	147
102	**23**	**6**	2 240	H_2O	25	sol	147
103	**23**	**7**	2 170	H_2O	25	sol	147
104	**23**	**8**	16 500	H_2O	25	sol	147
105	**23**	**16**	12 200	H_2O	25	sol	134
106	**23**	**31**	26 600	H_2O	25	sol	147
107	**23**	**32**	9 850	H_2O	25	sol	147
108	**23**	**32**	12 000	H_2O[b]	24	kin	145
109	**23**	**33**	63 600	H_2O	25	sol	134
110	**23**	**34**	4 900	H_2O	25	sol	169
111	**23**	**55**	2 270	H_2O	25	sol	147
112	**23**	**56**	3 297	H_2O	20	sol	162
113	**23**	**56**	1 483	H_2O	25	UV	162
114	**23**	**56**	2 037	H_2O	25	CD	162
115	**23**	**56**	2 561	H_2O	30	sol	162
116	**23**	**56**	2 067	H_2O	37	sol	162
117	**23**	**59**	2 470	H_2O	25	sol	147
118	**23**	**61**	7 600	H_2O	25	sol	132
119	**23**	**62**	3 240	H_2O	25	sol	147
120	**23**	**63**	3 880	H_2O	25	sol	147
121	**23**	**64**	9 920	H_2O	25	sol	147
122	**23**	**65**	26 100	H_2O	25	sol	147
123	**23**	**66**	12 100	H_2O	25	sol	147
124	**23**	**67**	21 600	H_2O	25	sol	147
125	**23**	**68**	37 300	H_2O	25	sol	147
126	**23**	**69**	8 310	H_2O	25	sol	147
127	**23**	**70**	31 900	H_2O	25	sol	147
128	**23**	**71**	6 300	H_2O	25	sol	147
129	**23**	**73**	13 600	H_2O	25	sol	134
130	**23**	**85**	210	D_2O	21	NMR	155
131	**23**	**86**	239	D_2O	21	NMR	155
132	**23**	**91**	6 700	H_2O	20	fluor	202,203
133	**23**	**92**	5 000	H_2O	20	fluor	202,203
134	**23**	**93**	3 800	H_2O	20	fluor	202,203
135	**24**	**33**	5 600	H_2O	25	sol	146
136	**25**	**3**	17 000	H_2O	25	sol	170
137	**25**[e]	**6**	1 000	H_2O	23	sol	171
138	**25**[e]	**6**	900	H_2O	r.t.	sol	172
139	**25**	**8**	13 000	H_2O	21−23	sol	173
140	**25**	**8**	12 000	H_2O	25	sol	168,170
141	**25**	**16**	7 300	H_2O	25	sol	170
142	**25**	**31**	1 230	H_2O	23	sol	174
143	**25**	**31**	1 550	H_2O[f]	23	sol	174
144	**25**	**31**	890	H_2O	23	sol	175
145	**25**	**31**	840	H_2O	30	sol	175
146	**25**	**31**	770	H_2O	40	sol	175
147	**25**	**31**	660	H_2O	50	sol	175
148	**25**	**31**	590	H_2O	60	sol	175
149	**25**	**33**	18 000	H_2O	25	sol	146,168,170

Table 4 (Continued)

entry	CD	steroid	K_a (L mol^{-1})	solvent	T (°C)	method[a]	ref(s)
150[g]	**25**	**60**	263	H_2O[h]	80	kin	176
151	**25**	**62**	1 800	H_2O	25	sol	168,170
152[g]	**25**	**74**	333	H_2O[h]	80	kin	176
153	**25**	**75**	9 000	H_2O	r.t.	sol	177
154	**25**	**76**	4 760	H_2O	r.t.	sol	177
155	**25**	**78**	2 000	H_2O	25	sol	139
156	**26**	**3**	55 000	H_2O	25	sol	170
157	**26**	**6**	5 910	H_2O	25	sol	128,129
158	**26**	**8**	29 000	H_2O	25	sol	168,170
159	**26**	**16**	37 000	H_2O	25	sol	170
160	**26**	**32**	7 800	H_2O	24	kin	145
161	**26**	**33**	84 000	H_2O	25	sol	146,168,170
162	**26**	**56**	8 293	H_2O	20	sol	162
163	**26**	**56**	6 039	H_2O	25	CD	162
164	**26**	**56**	4 834	H_2O	25	UV	162
165	**26**	**56**	6 122	H_2O	30	sol	162
166	**26**	**56**	5 273	H_2O	37	sol	162
167[i]	**26**	**60**	1 610	H_2O[h]	80	kin	176
168	**26**	**62**	7 000	H_2O	25	sol	168,170
169	**26**	**72**	117 000	H_2O[c]	37	sol	146
170[i]	**26**	**74**	2 680	H_2O[h]	80	kin	176
171	**35**	**8**	5 200	H_2O	25	sol	168
172	**35**	**33**	14 000	H_2O	25	sol	168
173	**35**	**62**	760	H_2O	25	sol	168
174	**36**	**3**	7 500	H_2O	25	sol	170
175	**36**	**8**	5 100	H_2O	25	sol	168,170
176	**36**	**16**	5 600	H_2O	25	sol	170
177	**36**	**33**	17 000	H_2O	25	sol	146,168,170
178	**36**	**62**	820	H_2O	25	sol	168,170
179	**37**	**33**	20 000	H_2O	25	sol	168
180	**37**	**62**	2 000	H_2O	25	sol	168
181	**38**	**3**	41 546	H_2O	30	sol	159
182	**38**	**6**	3920	H_2O	30	sol	159
183	**38**	**7**	4 574	H_2O	30	sol	159
184	**38**	**8**	16 529	H_2O	30	sol	159
185	**39**	**3**	50 137	H_2O	30	sol	159
186	**39**	**6**	5 630	H_2O	30	sol	159
187	**39**	**7**	5 769	H_2O	30	sol	159
188	**39**	**8**	18 255	H_2O	30	sol	159
189	**40**	**3**	53 880	H_2O	30	sol	159
190	**41**[j]	**1**	11 100	H_2O	25	sol	163
191	**42**[k]	**1**	190	H_2O	25	sol	163
192	**43**	**8**	11 000	H_2O	21−23	sol	173
193	**43**	**31**	6 100	H_2O	23	sol	175
194	**43**	**31**	5 400	H_2O	30	sol	175
195	**43**	**31**	5 090	H_2O	40	sol	175
196	**43**	**31**	4 750	H_2O	50	sol	175
197	**43**	**31**	4 000	H_2O	60	sol	175
198	**44**	**2**	27 000	H_2O	25	fluor	178,179
199	**44**	**80**	51 000	H_2O	25	fluor	178,179
200	**44**	**81**	178 000	H_2O	25	fluor	178,179
201	**44**	**82**	47 000	H_2O	25	fluor	178,179
202	**44**	**83**	158 000	H_2O	25	fluor	178,179
203	**45**	**80**	2 960	H_2O/ethylene glycol 80:20	25	CD	180
204	**45**	**81**	3 420	H_2O/ethylene glycol 80:20	25	CD	180
205	**46**	**81**	6 300	H_2O[l]	25	VIS	181
206	**46**	**81**	680 000	H_2O[c]	25	VIS	181
207	**47**	**2**	1 300	H_2O	25	fluor	182
208	**47**	**80**	64 800	H_2O	25	fluor	182
209	**47**	**81**	1 651 000	H_2O	25	fluor	182
210	**47**	**82**	2 760	H_2O	25	fluor	182
211	**47**	**83**	5 740 000	H_2O	25	fluor	182
212	**48**	**2**	2 920	H_2O	25	fluor	182
213	**48**	**80**	155 000	H_2O	25	fluor	182
214	**48**	**81**	1 050 000	H_2O	25	fluor	182
215	**48**	**82**	6 290	H_2O	25	fluor	182
216	**48**	**83**	3 934 000	H_2O	25	fluor	182
217	**49**	**2**	1 330/800[m]	H_2O	25	CD	183
218	**49**	**80**	13 100/5 290[m]	H_2O	25	CD	183
219	**49**	**81**	27 400/11 400[m]	H_2O	25	CD	183
220	**49**	**82**	5 770/3 790[m]	H_2O	25	CD	183
221	**49**	**83**	>200 000/17 000[m]	H_2O	25	CD	183
222	**50**[n]	**83**	420 000	H_2O/Me_2SO 70:30	25	fluor	184
223	**50**[o]	**83**	1 100 000	H_2O/Me_2SO 70:30	25	CD	184
224	**50**[p]	**83**	5 000 000	H_2O/Me_2SO 70:30	25	fluor	184

Table 4 (Continued)

entry	CD	steroid	K_a (L mol^{-1})	solvent	T (°C)	method[a]	ref(s)
225	**50**[p]	**83**	3 700 000	H_2O/Me_2SO 70:30	25	CD	184
226	**50**[q]	**83**	400 000	H_2O/Me_2SO 70:30	25	fluor	184
227	**50**[q]	**83**	370 000	H_2O/Me_2SO 70:30	25	CD	184
228	**51**	**80**	29 000	H_2O	25	fluor	185
229	**51**	**81**	26 000	H_2O	25	fluor	185
230	**51**	**82**	18 000	H_2O	25	fluor	185
231	**52**	**80**	92 000	H_2O	25	fluor	185
232	**52**	**82**	106 000	H_2O	25	fluor	185
233	**52**	**83**	4 000 000	H_2O	25	fluor	185
234	**53**	**2**	4 100	H_2O	25	fluor	179
235	**53**	**80**	58 000	H_2O	25	fluor	179
236	**53**	**81**	35 000	H_2O	25	fluor	179
237	**53**	**82**	22 000	H_2O	25	fluor	179
238	**53**	**83**	84 000	H_2O	25	fluor	179
239	**54**	**2**	15 000	H_2O	25	fluor	186
240	**54**	**77**	26 000	H_2O	25	fluor	186
241	**54**	**79**	190 000	H_2O	25	fluor	186
242	**54**	**80**	78 000	H_2O	25	fluor	186
243	**54**	**81**	95 000	H_2O	25	fluor	186
244	**54**	**82**	76 000	H_2O	25	fluor	186
245	**54**	**83**	1 400 000	H_2O	25	fluor	186

[a] Abbreviations: sol, solubility method; NMR, ^{1}H NMR titrations; HPLC, high-performance liquid chromatography; kin, kinetic measurements; CD, circular dichroism measurements; UV, UV absorption spectroscopy; therm, microcalorimetry; VIS, VIS absorption spectroscopy; fluor, fluorescence spectroscopy. [b] pH 9, borate buffer. [c] pH 1.2. [d] p[H + D] 11−12. [e] Molar substitution: 0.6 (average number of propylene oxide molecules that have reacted with one glucopyranose unit). [f] Contains 0.1% hydroxypropyl methylcellulose. [g] Molar substitution 0.9. [h] pH 7.4, phosphate buffer, 0.14 M; ionic strength 0.5. [i] Degree of substitution 1.8. [j] Degree of substitution 3.5. [k] Degree of substitution 10.7. [l] pH 9.3, 0.5 M carbonate buffer. [m] First entry, *trans* form; second entry, *cis* form. [n] Chromophores attached to glucose units 1 and 2. [o] Chromophores attached to glucose units 1 and 3. [p] Chromophores attached to glucose units 1 and 4. [q] Chromophores attached to glucose units 1 and 5.

Table 5. Thermodynamic Parameters from van't Hoff Analysis of Variable-Temperature Phase Solubility Studies for the Complexation of Hydrocortisone Butyrate (56) by Various Cyclodextrins[162]

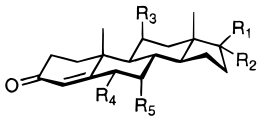
cyclo-dextrin	K_a (30 °C) (L mol^{-1})	ΔG (30 °C) (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (30 °C) cal mol^{-1} K^{-1}
21	282	−3.40	−2.84	+1.83
22	1 782	−4.51	−5.59	−3.58
23	2 561	−4.73	−4.92	−0.63
26	6 122	−5.25	−4.81	+1.46

ring small enough to partially penetrate the cavity, as compared to substrates possessing only one C(4)−C(5) double bond. Fully saturated tetracyclic steroidal frameworks do not fit at all into the cavity of this smallest cyclodextrin. Attempts to correlate these and other factors for the prediction of stability constants have been made by QSAR (*q*uantitative *s*tructure−*a*ctivity *r*elationship) calculations.[195,196]

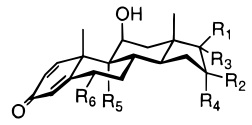
Strong binding of steroids could lead to potential medical applications. Cholesterol (**1**) forms a stable complex with β-cyclodextrin (K_a = 16100 L mol^{-1}, entry 27).[163] Rothblat and co-workers showed a high efficiency of cyclodextrins in stimulating cellular cholesterol efflux.[197] However, *in vivo* studies in rats showed that oral application of β-cyclodextrin derivatives had only marginal effects on the *de novo* biosynthesis of cholesterol and that chronic administration in mice did not significantly change the total cholesterol plasma levels.[198]

The structural insight into cyclodextrin−steroid complex geometries is rather limited since most of the binding assays that have been carried out (Table 4) are phase solubility experiments. No X-ray crystal structures of such complexes have been published to date and only a few NMR studies in liquid phase have been reported. From ^{13}C NMR investigations, Szejtli and co-workers[127,188] concluded that, cholecalciferol (vitamin D_3, **94**, Figure 13) is encapsulated in a 2:1 complex by two molecules of the β-cyclodextrin derivative **26** with simultaneous inclusion of the A ring and the side chain.[199] In another ^{1}H NMR investigation, a downfield shift of the steroidal resonance H-C(4) of hydrocortisone butyrate (**56**) complexed by β-cyclodextrin (**22**) suggested that the steroidal A ring is included in the cavity, while complexation of the same substrate by γ-cyclodextrin led to nonspecific shifts of the steroidal proton resonances.[147,162]

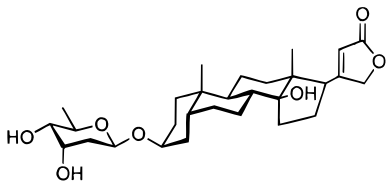
Further structural and thermodynamic insight into cyclodextrin−steroid complexation was obtained from studies with bile acids.[200] By ^{1}H NMR titrations in D_2O, Brown and co-workers[155] investigated the complexation of the four bile acids cholic (**2**), lithocholic (**83**), glycocholic (**85**), and glycochenodeoxycholic acid (**86**) by β- and γ-cyclodextrins (Table 4, entries 28, 88, 90, 92, 100, 130, and 131). Their experimentally observed CISs suggested a preferred inclusion of the charged carboxylate side chain by the smaller β-cyclodextrin, whereas they indicated a preferential encapsulation of the more hydrophobic tetracyclic steroidal frame into the larger cavity of γ-cyclodextrin. In another study,[164] inclusion complexation of bile acids by β-cyclodextrin was indicated by significant downfield shifts of the cyclodextrin protons H-C(3), H-C(5), and H-C(6) while protons H-C(1), H-C(2), and H-C(4) located at the exterior were only weakly affected. The downfield shifts of the intracavity protons of the receptor can be explained by van der Waals proximity contacts with the included substrate.[201] For cholic acid (**2**), the data suggested an inclusion mode in which the $H_3C(18)$ and $H_3C(21)$ groups were incorporated in the cavity with the



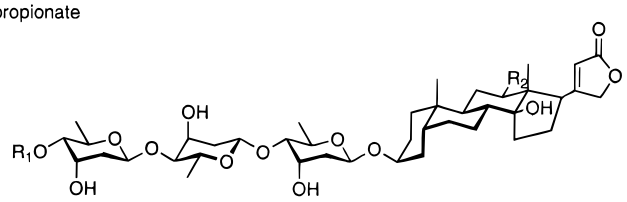
Steroid	R_1	R_2	R_3	R_4	R_5
3, Progesterone	Ac	H	H	H	H
6, Hydrocortisone	$COCH_2OH$	OH	OH	H	H
7, Cortisone	$COCH_2OH$	OH	=O	H	H
8, Testosterone	OH	H	H	H	H
55, Hydrocortisone-21-acetate	$COCH_2OAc$	OH	OH	H	H
56, Hydrocortisone-17-butyrate	$COCH_2OH$	$OCOC_3H_7$	OH	H	H
57, Corticosterone	$COCH_2OH$	H	OH	H	H
58, Deoxycorticosterone	$COCH_2OH$	H	H	H	H
59, Cortisone-21-acetate	$COCH_2OAc$	OH	=O	H	H
60, Medroxyprogesterone acetate	Ac	OAc	H	CH_3	H
61, Spironolactone	$-OCO(CH_2)_2-$		H	H	SAc



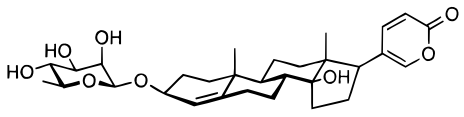
Steroid	R_1	R_2	R_3	R_4	R_5	R_6
31, Dexamethasone	$COCH_2OH$	H	OH	CH_3	F	H
32, Betamethasone-17-valerate	$COCH_2OH$	CH_3	$OCOC_4H_9$	H	F	H
62, Prednisolone	$COCH_2OH$	H	OH	H	H	H
63, Prednisolone-21- acetate	$COCH_2OAc$	H	OH	H	H	H
64, Triamcinolone	$COCH_2OH$	H	OH	OH	F	H
65, Triamcinolone acetonide	$COCH_2OH$	H	$-OC(CH_3)_2O-$		F	H
66, Triamcinolone diacetate	$COCH_2OAc$	H	OH	OAc	F	H
67, Betamethasone	$COCH_2OH$	CH_3	OH	H	F	H
68, Dexamethasone-21-acetate	$COCH_2OAc$	H	OH	CH_3	F	H
69, Paramethasone	$COCH_2OH$	H	OH	CH_3	H	F
70, Fluocinolone acetonide	$COCH_2OH$	H	$-OC(CH_3)_2O-$		F	F
71, Beclomethasone-17,21-dipropionate	$COCH_2OCOEt$	CH_3	OCOEt	H	Cl	H



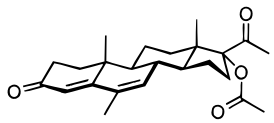
72,Digitoxigenin-monodigitoxoside



	R_1	R_2
33, Digitoxin	H	H
16, Digoxin	H	OH
73, Methyldigoxin	CH_3	OH

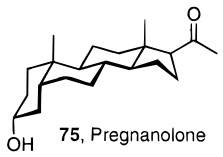


34, Proscillaridin



74, Megestrol acetate

1, Cholesterol

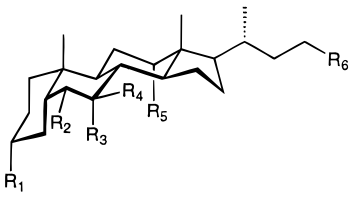


75, Pregnanolone

76, Pregnenolone

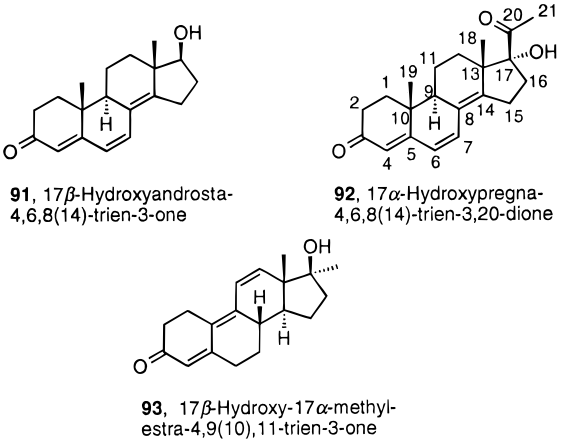
77, Dehydroepiandrosterone

78, 17α-Ethynylestradiol



Steroid	R_1	R_2	R_3	R_4	R_5	R_6
2, Cholic Acid	OH	H	OH	H	OH	COOH
79, Hyodeoxycholic acid	OH	OH	H	H	H	COOH
80, Chenodeoxycholic acid	OH	H	OH	H	H	COOH
81, Ursodeoxycholic acid	OH	H	H	OH	H	COOH
82, Deoxycholic acid	OH	H	H	H	OH	COOH
83, Lithocholic acid	OH	H	H	H	H	COOH
84, Dehydrocholic acid	=O	H	=O		=O	COOH
85, Glycocholic acid	OH	H	OH	H	OH	$CONHCH_2COOH$
86, Glycochenodeoxycholic acid	OH	H	OH	H	H	$CONHCH_2COOH$
87, Glycoursodeoxycholic acid	OH	H	H	OH	H	$CONHCH_2COOH$
88, Taurocholate	OH	H	OH	H	OH	$CONHCH_2CH_2SO_3Na$
89, Taurochenodeoxycholate	OH	H	OH	H	H	$CONHCH_2CH_2SO_3Na$
90, Tauroursodeoxycholate	OH	H	H	OH	OH	$CONHCH_2CH_2SO_3Na$

Figure 11. Steroids in cyclodextrin complexes for which association constants for 1:1 host−guest stoichiometry have been reported (Table 4).

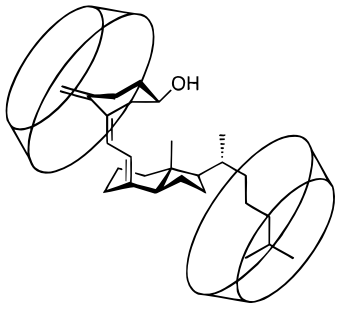


91, 17β-Hydroxyandrosta-4,6,8(14)-trien-3-one

92, 17α-Hydroxypregna-4,6,8(14)-trien-3,20-dione

93, 17β-Hydroxy-17α-methyl-estra-4,9(10),11-trien-3-one

Figure 12. Fluorescent steroids for the examination of protein−steroid and cyclodextrin−steroid interactions, described by Kempfle *et al.*[202,203]



94, Cholecalciferol (Vitamin D_3)

Figure 13. Schematic representation of the complexation of cholecalciferol (vitamin D_3, **94**) in the cavities of two molecules of β-cyclodextrin derivative **26**.

primary HO side of the receptor being in contact with the steroidal D ring.

Microcalorimetric measurements[164] showed that the stability of complexes formed by β-cyclodextrin (**22**) increased in the series glycocholic acid (**85**) ≈ dehydrocholic acid (**84**) ≈ taurocholic acid (**88**) < cholic acid (**2**) < chenodeoxycholic acid (**80**) ≈ glycoursodeoxycholic acid (**87**) < ursodeoxycholic acid (**81**) ≈ glycochenodeoxycholic acid (**86**) < tauroursodeoxycholic acid (**90**) < taurochenodeoxycholic acid (**89**) (Table 4, entries 29, 86, 87, 89, 91, and 93−97).

A fluorescence assay, which had been previously developed by Kempfle *et al.* to study protein−steroid interactions, was also applied to determine the stability of cyclodextrin complexes.[202,203] In this assay, the quenching of the emission of fluorescent steroids **91**−**93** (Figure 12) upon transfer from the more polar aqueous phase into the less polar receptor binding site is evaluated. It was found that, within the concentration limits of the assay, steroids **91**−**93** did not undergo stable association with α-cyclodextrin (**21**) while β- and γ-cyclodextrins (**22** and **23**) underwent complex formation with 1:1 (Table 4, entries 98, 99, and 132−134) or 1:2 host−guest stoichiometry.

3. Applications of Cyclodextrins to Steroid Sensorics and Separations

Ueno and co-workers developed several cyclodextrin sensory systems for the selective detection of

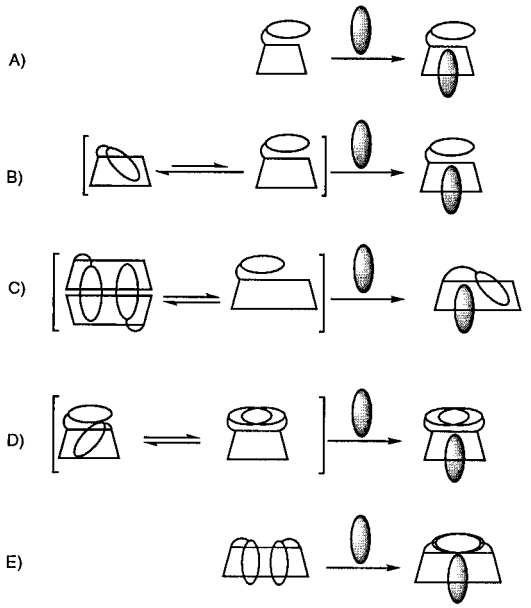


Figure 14. Proposed modes of steroid complexation by fluorescent chemosensors: (A) α-cyclodextrin−chromophore conjugates; (B) β-cyclodextrin−chromophore conjugates; (C) γ-cyclodextrin−chromophore conjugates; (D and E) β- and γ-cyclodextrin−bis-chromophore conjugates. Symbols: dark oval, guests; white oval, fluorescent chromophore; trapezoid, cyclodextrin.

steroids, monoterpenes, monocyclic unsaturated alcohols, and other lipophilic molecules. These systems consist of one or two[204] cyclodextrins with appended chromophores that change their fluorescence behavior upon substrate inclusion. Fluorescence binding assays with receptors **44** and **47**−**54** and bile acid substrates were carried out in H_2O, H_2O/Me_2SO, and H_2O/ethylene glycol mixtures, and the measured stability constants are shown in Table 4 (entries 198−202, and 207−245).[178,179,182−186] As a general trend, an increase in complex stability with increasing lipophilicity of the bile acid alicyclic core was observed. The association constants usually increased in the series: cholic acid (**2**) < deoxycholic acid (**82**) < chenodeoxycholic acid (**80**) < ursodeoxycholic acid (**81**) < lithocholic acid (**83**). A comparison between steroid complexes formed by β-cyclodextrin (Table 4, entries 28−29, and 86−97) and the β-cyclodextrin−chromophore conjugates **44**−**48** (entries 198−216) shows that the latter associations give much higher stability constants. Apparently, the costs for displacement of the appended chromophores by the steroids are not very high. Rather, the chromophores seem to contribute significantly to the stability of the steroid inclusion complexes by extending the apolar binding site and providing additional host−guest interactions.

Several recognition mechanisms have been proposed to explain the function of these fluorescent chemosensors (Figure 14).[152,179,186] Since the cavity in α-cyclodextrin is too narrow for chromophore incorporation, the latter only acts as a hydrophobic cap (Figure 14A).[179] Upon steroid binding by the β-cyclodextrin derivatives **47** and **48**, the substrate displaces the dansyl chromophore from the cavity into the more polar solvent (Figure 14B), and this change in environmental polarity translates into a reduction of fluorescence quantum yield as well as

into a bathochromic shift in the emission wavelength.[182] γ-Cyclodextrin−pyrene conjugates, such as **52**, tend to dimerize in the free state (Figure 14C).[152,185] In contrast, in the host−guest complexes the chromophore may either act as a hydrophobic cap or remain in the large cavity, enhancing complex stability by a kind of induced fit mechanism.[205] Similarly, bis-chromophore-β- and γ-cyclodextrin conjugates, *e.g.* **50**, can orient their fluorescent groups either into or atop the cavity for further interactions with the encapsulated guest (Figures 14D and 14E[206]). Additionally, photoswitchable receptors, *e.g.* **49**, with an azobenzene moiety attached were prepared and tested for binding affinity.[183] In each case, the *trans*-azobenzene conjugate was a better binder than the corresponding *cis* derivative (entries 217−221, Table 4).

Cyclodextrins have also been used as additives in the mobile phase and as the stationary phase in HPLC separations of steroids.[165,207] Agnus *et al.*[149] separated pregnanolone (**75**) and progesterone (**3**) on a reversed phase column, using indirect photodetection, decreasing the retention time by addition of β-cyclodextrin to the mobile phase. Lamparczyk *et al.*[208] developed a chromatographic procedure for the simultaneous determination of β-estriol (**95**), estrone (**11**), and 17β-estradiol (**10**) from human urine using reversed-phase techniques with β-cyclodextrin added to the mobile phase. They also examined the temperature dependence of the capacity factors of 17α-(**96**) and 17β-estradiol (**10**) and found that the addition of cyclodextrin affects the separation only below 50 °C.[209]

Steroid	R_1	R_2	R_3
10, β-Estradiol	OH	H	H
11, Estrone	=O		H
95, β-Estriol	OH	H	OH
96, α-Estradiol	H	OH	H

C. Conclusions

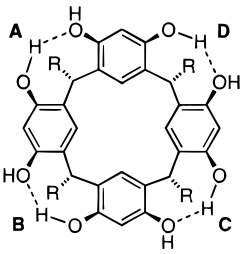
Being readily available, the natural cyclodextrins and their derivatives have been studied extensively as receptors for steroids. Many of these investigations make use of the significant complexation-induced enhancement of steroid solubility in aqueous solutions, and target pharmaceutical or sensory applications.[210] Although numerous binding studies by various methods have been described and a wide variety of stability constants for 1:1 cyclodextrin−steroid complexes measured (Table 4), insight into the thermodynamics of the associations and the structures of the complexes formed in solution and the solid state remains limited. In general, the cavity of α-cyclodextrin is too small for sizeable encapsulation of steroidal guests. Therefore, the binding affinity of α-cyclodextrin for steroids is much weaker than that of the larger β- and γ-cyclodextrins. The latter presumably form axial inclusion complexes, incorporating preferentially the more apolar moieties of the steroidal substrates. Binding affinity correlates well with the lipophilicity of receptors and substrates, which gives rise to a conception in which hydrophobic desolvation of the complementary surfaces of host and guest as well as dispersion interactions between the binding partners represent the most important driving forces for complexation. A limited set of data suggests that cyclodextrin-steroid complexation in water is characterized thermodynamically by a favorable negative complexation enthalpy, whereas the entropic terms are quite small, with either positive or negative sign.

IV. Synthetic Receptors for Steroids

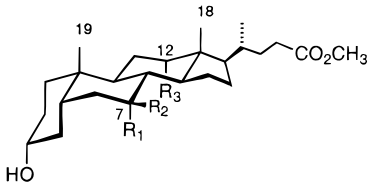
Although the development of synthetic, highly selective steroid receptors could open up interesting perspectives in medicinal chemistry,[12] such as new steroid transport and delivery systems,[211−213] supramolecular steroid chemistry is only in its infancy. The large number and diversity of steroids requires a profound understanding of their molecular recognition principles in order to discriminate between derivatives with quite similar structures yet very different biological properties. Except for cyclodextrins, supramolecular steroid chemistry today is quite away from potential applications and technologies, and the design and construction guidelines for efficient synthetic steroid receptors are just starting to be explored. The X-ray crystal structures of steroid complexes with antibodies, protein receptors, and enzymes (section II) suggest that the large apolar steroidal tetracyclic ring system is best bound in deep, highly preorganized apolar cavities lined by aromatic rings. To generate steroid selectivity, convergent functionality should be appropriately positioned within the binding site to form H-bonds to the polar groups of the substrate. While the cyclodextrins are known to rather unspecifically complex a broad range of apolar substrates including steroids (section III), the first artificial hosts for steroids were only reported some 10 years ago. Since then, a very limited number of novel synthetic steroid receptors have appeared in the literature.[214−229] Except for some steroid-recognizing polymers[230,232] obtained by molecular imprinting,[231] so far all artificial receptors have been based on cyclophanes[39,233−235] (including calixarenes[236−238]), a major class of macrocyclic hosts comprising bridged aromatic systems. Thereby, a few macrocycles have been shown to recognize polar steroids by means of multiple H-bonding in both apolar organic solvents[214−216] and in the solid state,[217] while the others bind in aqueous or alcoholic solutions,[218−229] taking advantage of hydrophobic desolvation and dispersion forces besides electrostatic donor−acceptor interactions.[239−242]

A. Steroid Complexation by Synthetic Receptors in Apolar Solvents and in the Solid State

Aoyama and co-workers investigated the supramolecular properties of resorcin[4]arene **97** (Figure 15) which forms complexes with a variety of steroids in $CHCl_3$.[214,215] The rigid macrocycle is cone-shaped with a narrower apolar lower and a larger, polar upper rim. The structure is preorganized and sta-



97, R = $C_{11}H_{23}$

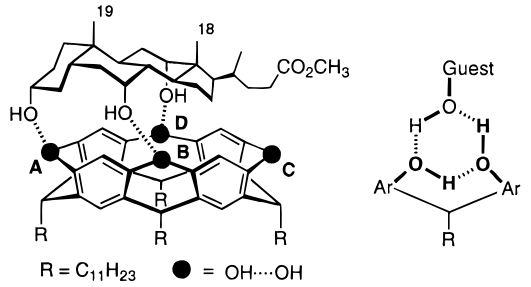


Steroid	R_1	R_2	R_3	K_a (l mol^{-1})
98, Methyl cholate	OH	H	OH	690
99, Methyl chenodeoxycholate	OH	H	H	270
100, Methyl deoxycholate	H	H	OH	120
101, Methyl ursodeoxycholate	H	OH	H	59
102, Methyl lithocholate	H	H	H	9

Figure 15. Structure of the resorcin[4]arene receptor **97** and the bile acid derivatives **98**−**102**. The association constants for the corresponding 1:1 complexes were determined at 298 K by induced circular dichroism titrations in $CHCl_3$.

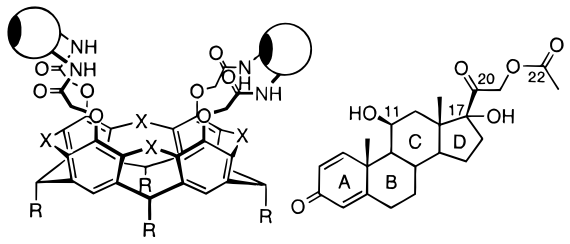
bilized by four intramolecular H-bonds between the eight HO groups at the upper rim which gives rise to the four symmetrical H-bonding sites **A**−**D**. The four bridging C(sp^3) atoms in the skeleton of the lower rim carry each an axially oriented long alkyl chain enhancing solubility.

The complexation of the bile acid methyl esters **98**−**102** by **97** was readily followed by induced circular dichroism.[214] The stability of the complexes decreases from methyl cholate (**98**), to methyl chenodeoxycholate (**99**), to methyl deoxycholate (**100**), to methyl ursodeoxycholate (**101**), and to methyl lithocholate (**102**) with the association constant K_a varying from 690 to 9 L mol^{-1} (Figure 15). Thus the receptor shows high specificity and, for instance, differentiates by $\Delta(\Delta G) = 0.9$ kcal mol^{-1} between the two epimers **99** (K_a = 270 L mol^{-1}) and **101** (K_a = 59 L mol^{-1}). Since association strength increases continuously with the number of α-oriented steroidal HO groups, the authors propose multipoint H-bonding as the major driving force for association as shown in Figure 16 for the 1:1 complex between **97** and methyl cholate (**98**). Probably, three of the four H-bonding sites in **97** stabilize the complex by forming a total of three six-membered ring host−guest H-bonding networks. In this recognition model, all the polar groups of the substrate bind in a parallel fashion; therefore, it is not too surprising that efficient association is restricted to the α-face of the steroid. ^{1}H NMR binding studies further supported this assumption. The incorporation of organic residues into cavities of calixarenes and cyclophanes causes pronounced upfield changes in the chemical shift of guest protons, if these are located in the shielding region of aromatic rings.[243−246] In the complexes between **97** and **98**, the protons of the axial methyl groups $H_3C(18)$ and H_3C-



R = $C_{11}H_{23}$ ● = OH···OH

Figure 16. Tripodal interaction between receptor **97** with the α-face of methyl cholate (**98**). The four H-bonding sites of the host are shown in black. Two adjacent HO groups of the resorcinarene can form a six-membered H-bonding network with each HO group of the guest.



103 (*endo-exo*) **63**, Prednisolone-21-acetate

R = $C_{11}H_{23}$, X = O-CH_2-O

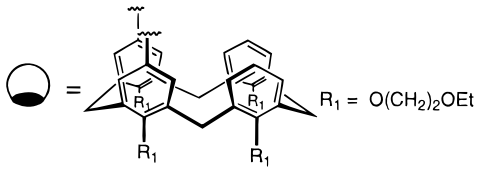


Figure 17. Schematic representation of the *endo-exo* isomer of the doubly calixarene-bridged resorcin[4]arene receptor **103**. The two calixarene moieties are believed to favorably orient the amide H-bonding sites rather than to participate in the binding to prednisolone-21-acetate (**63**).

(19) did not show any CISs (*c*omplexation-*i*nduced changes in ^{1}H NMR chemical *s*hifts), whereas some of the steroidal ring protons (presumably on the α-side) displayed upfield shifts as large as 0.4 ppm.

Receptor **97** was also tested for the complexation of cholesterol and derivatives.[215] However, association of these highly apolar monoalcohols was found to be very weak ($K_a \leq 11$ L mol^{-1} in $CHCl_3$) which underlines the importance of multipoint host−guest interactions.

Reinhoudt and co-workers investigated the steroid-binding properties of another resorcin[4]arene host (**103**) doubly extended by two calix[4]arene moieties.[216] The coupling reaction between two identical calixarenes and a central resorcinarene afforded three isomers in which the upper rims of the two calixarenes are oriented inward (*endo-endo*), outward (*exo-exo*), or in- and outward (*endo-exo*, Figure 17). Computer modeling suggested that the four amide groups in **103** provide four H-bonding sites for association with HO−C(11), HO−C(17), O=C(20), and O=C(22) of prednisolone-21-acetate (**63**). As a consequence of such H-bonding, the acetyl CH_3 group of the substrate would be buried inside the central resorcinarene cavity and undergo stabilizing C−H···π-interactions[215,247] with the aromatic rings. As a matter of fact, this is a common motif found in the X-ray crystal structures of molecular complexes

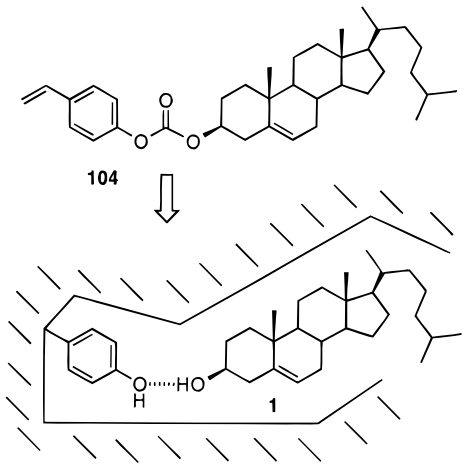


Figure 18. Cholesteryl 4-vinylphenyl carbonate (**104**) was used for the construction of steroid-recognizing polymers. After copolymerization with a cross-linking agent, the steroidal template was hydrolyzed off under loss of CO_2 leading to shape-complementary cavities suitable for binding cholesterol (**1**).

formed by calixarenes.[248−252] The association constant for the complex between **103** and **63** was determined by ^{1}H NMR titration in $CDCl_3$ as K_a = 830 L mol^{-1}. The two other receptor isomers revealed similar affinities for **63** with K_a = 430 L mol^{-1} measured for the complex of the *endo-endo* and K_a = 530 L mol^{-1} for the complex of the *exo-exo* receptor. Steroids structurally related to **63**, but lacking the acetyl group or one of the two HO groups on rings C and D, did not show any affinity for **103** in the concentration range considered. The authors showed that the two calixarenes at the upper rim of the resorcinarene cavity did not directly participate in steroid binding; rather they take an important role in preorganizing the H-bonding sites of the amide linkers in a favorable way for interactions with the substrate.

Parini *et al.* found evidence for calixarene−steroid complex formation in the solid state, based on both time-dependent Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC).[217] Upon grinding mixtures of different Δ^4-3-ketosteroids with *p*-(*tert*-butyl)[6]- and *p*-(*tert*-butyl)-[8]calixarene, the crystal structure of the guests apparently breaks up. From the changes in IR frequency and the thermal behavior of the crystalline mixtures, the authors deduced that the steroidal C(3)-carbonyl group is transferred into a more hydrophobic environment, while the HO substituents of the steroid are involved in forming much tighter H-bonds.

A different approach to steroid binding was reported by Whitcombe *et al.*[230] Recognition-site functionality was introduced into a polymer by means of molecular imprinting technique.[231] Cholesteryl 4-vinylphenyl carbonate (**104**) served as a styrene-like monomeric template and was copolymerized together with a cross-linking agent (Figure 18). Once the templating cholesterol (**1**) was hydrolyzed off, the polymeric material was found to selectively bind this steroid, owing to shape complementarity and the formation of a H-bond with HO-C(3) of the substrate. An association constant of K_a = 1700 L mol^{-1} was

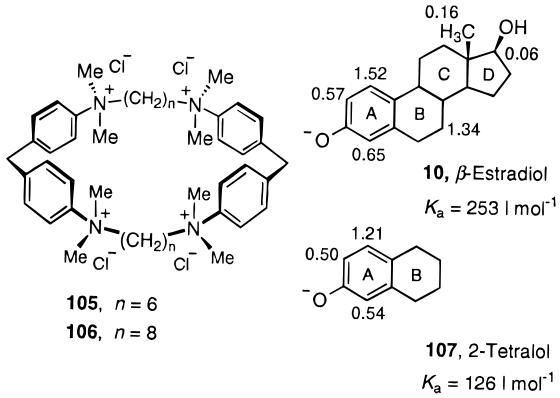


Figure 19. The tetraazonia[*n*.1.*n*.1]paracyclophanes **105** and **106** are hosts for deprotonated β-estradiol (**10**) and 2-tetralol (**107**) in basic D_2O/CD_3OD 3:2. The upfield complexation-induced changes in ^{1}H NMR chemical shifts of the guest proton resonances indicate that the steroid is included preferentially by rings A and B rather than by rings C and D in the cavity of **105**. Shown are the stability constants for the complexes formed by **105**.

calculated for 1:1 complexation of cholesterol in hexane which indicates high structural complementarity within the pores of such steroid-recognizing polymers. Also by molecular imprinting,[232] Mosbach and co-workers prepared efficient polymeric receptors for corticosteroids.[232a]

B. Steroid Receptors for Aqueous Solutions

In the early 1980s, paracyclophanes[233−235] became popular for their arene-binding properties in aqueous solution.[253−257] For this reason, some of the initial studies on steroid recognition focused on the complexation of the aromatic A ring of estrogens.

Kumar and Schneider[218] studied the steroid-binding properties of the tetraazonia[*n*.1.*n*.1]paracyclophanes **105** and **106** (Figure 19) in aqueous solution. The smaller receptor **105** was found to be superior in complexing β-estradiol (**10**) in both its neutral and ionized form. ^{1}H and ^{13}C NMR binding titrations in binary aqueous solvent mixtures at various pHs revealed that complexation of deprotonated **10**, *i.e.* the phenoxide form, was more effective by one order of magnitude (in K_a) compared to the inclusion of the corresponding neutral phenolic form. Upon changing from strongly basic to neutral solution, the association constant for the 1:1 complex between **105** and **10** decreases from 253 to 21 L mol^{-1} in D_2O/CD_3OD 3:2. This implicates that not only hydrophobic desolvation (*i.e.* gain in entropy by release of surface water into the bulk and gain in enthalpy through increase of solvent cohesive interactions) and dispersion interactions, but also ion-pairing effects stabilize the complex of the phenoxide form. For both deprotonated β-estradiol (**10**) and 2-tetralol (**107**), the CISs of the guest resonances (Figure 19) support complexation geometries in which the A and B rings preferentially occupy the cyclophane cavity, whereas the bulkier C and D rings of the steroid remain mostly in solution. The inclusion of the aromatic A ring into the cavity lined by four quaternary ammonium ions is clearly sterically preferred over the incorporation of a fully saturated cyclohexane ring. It also should provide a better host−guest ion-pairing geometry.

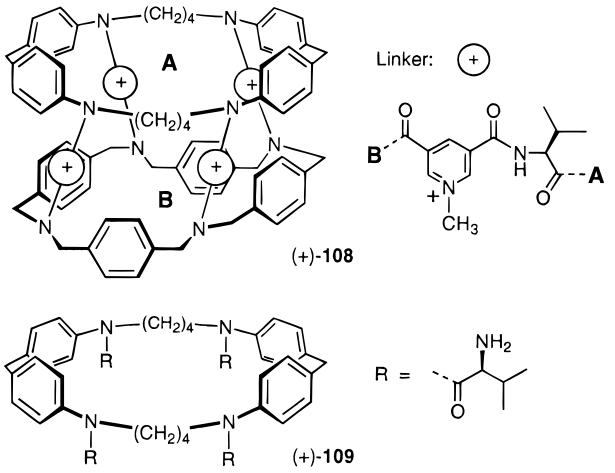


Figure 20. The cage-type macrocyclic receptor (+)-**108** built by bridging cyclophanes **A** and **B** by four chiral, L-valine-derived spacers and reference compound (+)-**109**.

Host	K_a (l mol^{-1}) 10	95	96
(+)-**108**	760	360	460
(−)-**108**	700	520	1300
(+)-**109**	620	360	540
(−)-**109**	610	340	600

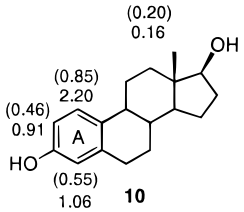


Figure 21. Association constants for the 1:1 complexes between receptors **108** and **109** with estrogens in D_2O/CD_3OD 3:1 (T = 300 K). Also shown are the upfield changes in ^{1}H NMR chemical shift at saturation binding observed for the protons of β-estradiol (**10**) when complexed to (+)-**109** or (+)-**108** (numbers in parentheses).

Furthermore, aromatic−aromatic[36,191] as well as onium cation−π interactions[44,258−260] may additionally stabilize the complex formed upon incorporation of the aromatic A ring.

Chiral, cage-type receptors for the inclusion complexation of aromatic substrates, including aromatic steroids, were prepared by Murakami *et al.* (Figure 20).[45,219,220] The enantiomeric macrocycles (+)- and (−)-**108** are composed of [6.1.6.1]- (**A**) and [3.3.3.3]-paracyclophane (**B**) substructures bridged by four chiral spacers comprising both *N*-methylpyridinium and either D- or L-valine residues. Circular dichroism spectra and computer model examinations indicated that the chirality of the bridges provides an overall helical twist to the receptors. For comparison, the macromonocyclic reference compounds (+)- and (−)-**109** were also studied.

^{1}H NMR titrations in D_2O/CD_3OD 3:1 afforded comparable stability constants for the complexation of β-estradiol (**10**) by (+)-**108** and (+)-**109**, respectively (Figure 21). However, markedly smaller upfield CISs were observed for the aromatic guest resonances in the complex with (+)-**108** than in the complex with (+)-**109**, which could indicate a less deep penetration of the A ring into the shielding interior cage of (+)-**108** than into the cavity of cyclophane (+)-**109**. Estriol (**95**) also forms complexes of similar stability with both receptors. A modest enantioselectivity was observed with cage receptor (−)-**108** which formed a more stable complex with α-estradiol (**96**) than (+)-**108**. However, the cavities of both receptors were found to be too narrow for the inclusion of nonaromatic steroids such as testosterone.

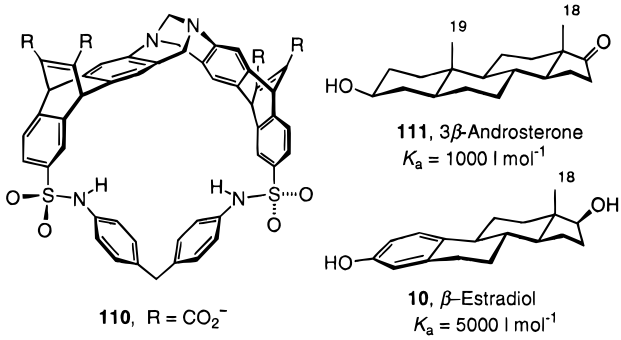


Figure 22. Chiral macrocycle **110** for the shape-selective inclusion complexation of 3β-androsterone (**111**) and β-estradiol (**10**) in D_2O/CD_3OD 1:1 (T = 298 K).

Another chiral, highly preorganized cyclophane receptor was prepared in enantiomerically pure form by Wilcox and co-workers.[221] Macrocycle **110** consists of two bridged 9,10-dihydroanthracenes connected by a Tröger's base-like structure[261] on one side and closed on the other by a diphenylmethane moiety (Figure 22). The receptor was developed for the selective inclusion complexation of flat alicyclic substrates in aqueous solution. The authors found that conformationally locked cyclohexane guests bearing an axial methyl group or a similarly sized substituent are too bulky for full encapsulation. The observed shape-exclusion phenomenon was applied to the selective complexation of β-estradiol (**10**) over 3β-androsterone (**111**).[222] ^{1}H NMR binding titrations in D_2O/CD_3OD 1:1 (T = 298 K) proved that the additional H_3C(19) group, which is present only in the androgene (K_a = 1000 L mol^{-1}), reduces the binding affinity by a factor of 5 compared to the sterically less demanding estrogene (K_a = 5000 L mol^{-1}). Studies within a series of cyclohexane derivatives afforded similar selectivities and, therefore, the observed differentiation is mainly attributable to steric rather than electronic factors.

The first cyclophane receptor shaped for the complexation of fully aliphatic steroids was reported by Koga and co-workers.[223,224] The typical diphenylmethane moiety present in most arene-binding hosts at that time was replaced by the wider naphthylphenylmethane spacer to form tetraazoniacyclophane **112** (Figure 23), yielding a nearly rectangular cavity with parallel, face-to-face oriented aromatic walls. The four ammonium groups provide good solubility in protic solvents and, at the same time, keep the binding site open by means of charge repulsion. ^{1}H NMR binding studies in D_2O revealed that **112** is capable of recognizing a variety of large aliphatic guests including the sodium salt of deoxycholic acid (**82**).

The first systematic study of synthetic steroid receptors was undertaken by Diederich and co-workers who, over the course of the past few years, investigated several cyclophanes[225−229] for the complexation of a variety of steroidal substrates. Initial investigations[225,226] focused on the two cyclophanes **113** and **114** which share the bis(naphthylphenylmethane) skeleton of receptor **112** introduced by Koga and co-workers. While the endocyclic am-

Table 6. Association Constants (K_a, L mol^{-1}), Calculated Complexation-Induced Changes in Chemical Shift at Saturation Binding ($\Delta\delta_{sat}$), Enthalpic ($\Delta H°$) and Entropic ($T\Delta S°$) Contributions to the Free Enthalpy of Complexation ($\Delta G°$) for the 1:1 Inclusion Complexes Formed by Different Steroids and Cyclophane Receptor 113 in D_2O/CD_3OD 1:1, As Determined by Variable-Temperature ^1H NMR Titrations and van't Hoff Linear Regression Analysis

steroid	K_a [a] (L mol^{-1})	$-\Delta G$ [a] (kcal mol^{-1})	$\Delta\delta_{sat}$ (ppm) $H_3C(19)$	$\Delta\delta_{sat}$ (ppm) $H_3C(18)$	$-\Delta G°$ [b] (kcal mol^{-1})	$-\Delta H°$ (kcal mol^{-1})	$+T\Delta S°$ [b] (kcal mol^{-1})
2[c]	150	2.9	−0.73	−0.56			
82[c]	250	3.2	−0.76	−0.66			
80[c]	800	3.9	−0.47	−1.40			
81[c]	1 750	4.4	−0.30	−1.49	4.1	13.5	−9.4
83[c]	7 100	5.2	−0.55	−1.49	5.1	8.7	−3.6
6	1 100	4.1	−1.44	−0.26	3.8	12.6	−8.8
7	1 500	4.3	−1.48	−0.30	4.0	13.7	−9.7
8	3 500	4.8	−1.48	−0.43	4.7	12.0	−7.3

[a] Determined at 293 K.[225] [b] Determined at 298 K.[226] [c] In the presence of 0.1 M Na_2CO_3.

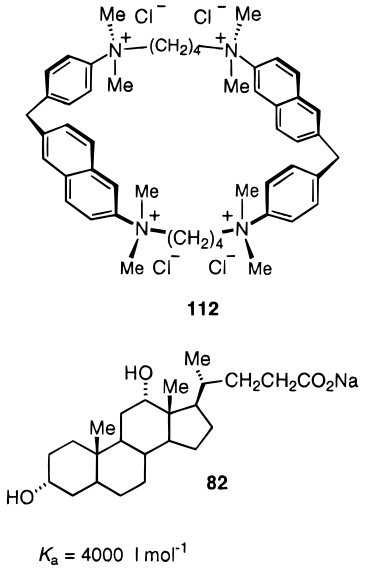
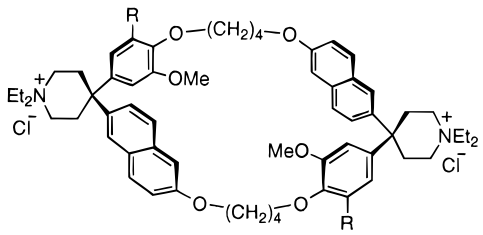


Figure 23. The first water-soluble cyclophane receptor (**112**) for the inclusion complexation of aliphatic steroids such as the sodium salt of deoxycholic acid (**82**) in D_2O.[223,224]

monium groups of the latter were replaced by ether O atoms for rendering the binding sites more lipophilic, water solubility was preserved by the introduction of peripheral ammonium groups. Furthermore, MeO substituents were introduced in the phenyl rings because they are known to raise the critical aggregation concentration of similar hosts in aqueous solution[191,262,263] and further deepen the cyclophane cavity.



113, R = OMe
114, R = $CH_2NHCOCH_2\overset{+}{N}Me_2Et\ Cl^-$

In order to avoid self-aggregation of both host and guest during investigations of stoichiometric inclusion complexation, initial ^1H NMR titrations were run in D_2O solutions containing CD_3OD as cosolvent. Upon addition of **113** to a solution of different bile acids (**2** and **80**−**83**, Figure 11) and steroid hormones (**6**−**8**, Figure 1) in D_2O/CD_3OD 1:1, the upfield CISs of the steroid methyl groups $H_3C(18)$ and $H_3C(19)$ were monitored (Table 6). The large changes in chemical shift not only provided evidence for inclusion complexation but also permitted to calculate the association constants of the formed 1:1 complexes by means of nonlinear least-squares curve fitting.

As can be seen from Table 6, binding affinity generally decreases with increasing number of polar substituents in the guests, revealing high selectivity in some cases. For example, lithocholic acid (**83**), the least substituted steroid in this series, binds to **113** by 2 kcal mol^{-1} stronger than deoxycholic acid (**82**) which only differs structurally by the presence of an additional 12α-HO group at ring C. The affinity sequence **2** (cholic acid) < **82** (deoxycholic acid) < **80** (chenodeoxycholic acid) < **81** (ursodeoxycholic acid) < **83** (lithocholic acid) observed with **113** (Table 6) is identical to that observed with many cyclodextrin receptors (see entries 212−216, Table 4). It can therefore be concluded, that similar host−guest interactions, namely hydrophobic desolvation and dispersion forces, are responsible for steroid complexation in aqueous solution by the two different classes of (aromatic) cyclophane and (nonaromatic) cyclodextrin receptors. In the case of the resorcinarene receptor **97**, a similar, but inverse selectivity to that shown in Table 6 had been observed for the complexation of the corresponding methyl ester derivatives of bile acids **2** and **80**−**83** in chloroform.[214] While in organic solution these guests are recognized by the formation of multiple H-bonds, in aqueous solution the desolvation of polar functional groups upon complexation is mainly responsible for selectivity effects. Carlson and Jorgensen showed by Monte Carlo liquid-phase simulations[264] that the complex between **113** and cholic acid (**2**) is strongly disfavored compared to the complexes of steroids lacking the 12α-HO group, since the latter becomes deeply buried inside the cyclophane cavity and cannot be stabilized neither by the apolar host nor by solvation. Similar conclusions had previously been drawn from Corey−Pauling−Koltun (CPK) molecular model examinations.[225] The difference in stability of 2 kcal mol^{-1} between the complexes formed by lithocholic acid (**83**) and deoxycholic acid (**82**) with host **113** provides an impressive example for a binding selectivity in aqueous solution that does not originate from differences

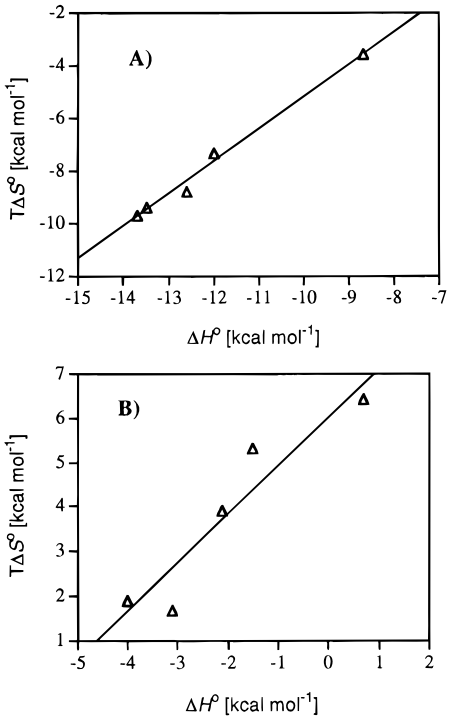


Figure 24. Enthalpy−entropy compensation in D_2O/CD_3OD 1:1 for the complexation between receptor **113** and the steroids listed in Table 6 (A), and in D_2O between receptor **114** and the steroids listed in Table 7 (B).

in attractive host−guest interactions but rather from energetically unfavorable, complexation-induced polar functional group desolvation.

Variable-temperature ^1H NMR titrations[226] provided insight into the driving force for steroid inclusion complexation with **113** (Table 6). In D_2O/CD_3OD 1:1, the association is strongly enthalpy driven ($-\Delta H°$ = 8.7−13.7 kcal mol^{-1}) and the enthalpic driving force is compensated by unfavorable entropic terms ($-T\Delta S°$ = 3.6−9.7 kcal mol^{-1}). The plot of the entropic *vs* enthalpic contributions to the free enthalpy of complexation, ΔG, yields a strong isoequilibrium relationship (Figure 24A), referred to as enthalpy−entropy compensation, a well-known phenomenon observed in molecular association processes.[36,265−271] A gain in solvent cohesive and dispersion-type interactions upon transferring the large apolar steroid from the bulk solvent into the receptor cavity are believed to be responsible for such enthalpically controlled tight complexation.[36,190,271] Upon steroid inclusion, protic solvent molecules in the apolar receptor cavity and around the complementary substrate surface are transferred back into bulk solvent where they will be more stabilized by H-bonding interactions, resulting in a gain in cohesive energy. One can also consider that creating a cavity in a protic solvent to accommodate a free apolar substrate is energetically unfavorable. Correspondingly, cohesive energy is gained back when the substrate is transferred into the receptor binding site and, as a result, solvent may reoccupy the room previously taken by the substrate.[79,109,272] Dispersion energy is gained in a tight apolar complexation process in water since the polarizability of CH, CH_2, and CH_3 groups is higher than that of OH groups. Upon complexation, van der Waals contacts between hydrocarbon surface and the less polarizable solvent

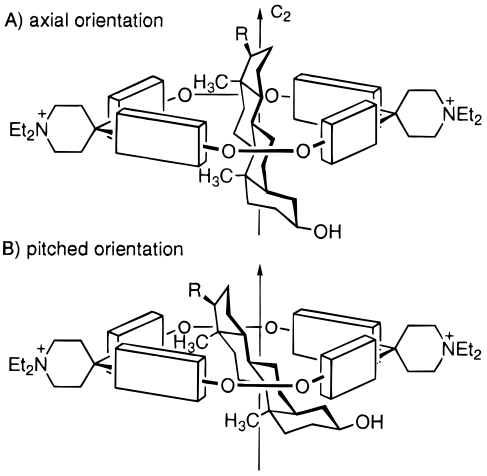


Figure 25. Representation of two possible binding patterns for the inclusion complexation of steroids by cyclophane **113**.

molecules are replaced by energetically more favorable contacts between the highly polarizable hydrocarbon surfaces of host and guest.[36,190,271,273] Similar driving forces for tight apolar complexation have been previously discussed to explain the enthalpically driven complexation of aromatic substrates by cyclophane receptors.[36]

Two preferred inclusion geometries can be proposed for the steroid complexes of **113**, based on the observed CISs of the guest proton resonances and computer modeling. Assuming axial-type inclusion, *i.e.* a binding pattern in which the substrate moves along the central C_2 axis of the host perpendicular to the mean plane of the macrocycle, host−guest association will be most favorable if the apolar steroid rings are buried deeply in the cyclophane cavity while the polar groups orient into solution (Figure 25A). According to the calculated upfield CISs at saturation binding ($\Delta\delta_{sat}$) shown in Table 6, the $H_3C(19)$ and $H_3C(18)$ groups of cholic (**2**) and deoxycholic acid (**82**) interact almost equally with the aromatic cavity walls of **113** ($-\Delta\delta_{sat}$ = 0.6−0.8 ppm). Therefore, the substrates may be complexed both with their B and parts of their C rings preferentially. In such a geometry, the carboxylate and the 3α-HO group remain fully solvent exposed while the 7α- and 12α-HO groups must become at least partially desolvated, which accounts for the reduced binding affinities in these cases. On the other hand, chenodeoxycholic (**80**), ursodeoxycholic (**81**), and lithocholic (**83**) acids display especially large upfield CISs for their $H_3C(18)$ resonance ($-\Delta\delta_{sat}$ = 1.4−1.5 ppm). In an axial steroid−cyclophane complex, this would give rise to a binding pattern in which the more apolar steroidal C and D rings are buried deepest. In contrast, hydrocortisone (**6**), cortisone (**7**), and testosterone (**8**) are preferentially included with their A and B rings, as revealed by the particularly large upfield CISs of their $H_3C(19)$ proton resonance ($-\Delta\delta_{sat}$ = 1.4−1.5 ppm). However, the computational investigations by Carlson and Jorgensen[264] showed that a second complexation geometry must also be allowed for, in which the substrate can adopt a more pitched rather than an axial orientation (Figure 25B).

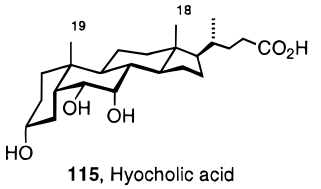
The improved solubility of cyclophane **114** as compared to **113** made it possible to perform ^1H NMR

Table 7. Association Constants (K_a, L mol^{-1}), Enthalpic ($\Delta H°$) and Entropic ($T\Delta S°$) Contributions to the Free Enthalpy of Complexation (ΔG) for the 1:1 Inclusion Complexes Formed by Different Steroids and Cyclophane Receptor 114 in Pure Water Containing 0.01 M Na_2CO_3, As Determined by Microcalorimetric and ^{1}H NMR Titrations

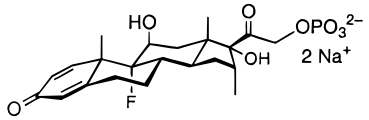
steroid	K_a [a] (L mol^{-1})	$-\Delta G°$ [b] (kcal mol^{-1})	$-\Delta H°$ (kcal mol^{-1})	$+T\Delta S°$ (kcal mol^{-1})	method[a]
84	3 000	4.8	3.1	1.7	A
115	13 500	5.7	−0.7	6.4	A
81	18 900	5.9	4.0	1.9	A
116	23 200	6.0	2.1	3.9	A
116	24 900	6.0			B
86	37 300	6.3			B
79	100 000	6.8	1.5	5.3	A

[a] A: Microcalorimetric titration in H_2O at 298 K. [b] B: 500 MHz ^{1}H NMR titration in D_2O at 298 K.

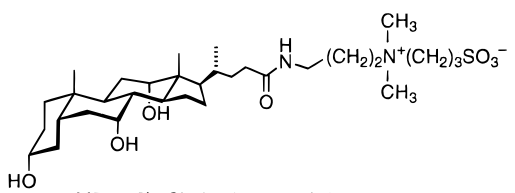
and microcalorimetric titrations[274] in pure water (containing 0.01 M Na_2CO_3) with steroidal substrates that possess elevated critical micelle concentrations[275] such as ursodeoxycholic (**81**), hyocholic (**115**), hyodeoxycholic (**79**), glycochenodeoxycholic (**86**), dehydrocholic acid (**84**), as well as disodium dexamethasone-21-phosphate (**116**). Upon changing the solvent from D_2O/CD_3OD 1:1 to pure water, the free energy of complexation increases by *ca.* 2 kcal mol^{-1}; a finding previously obtained for several cyclophane−arene complexes.[36,191,276] While **113** was found to be the better receptor for neutral steroids, cyclophane **114** with its four quaternary ammonium residues was shown to be more efficient in binding anionic guests (Table 7). As expected from the results obtained with **113**, the stability of the complexes formed by receptor **114** is related to both the number and the orientation of the functional groups in the substrates. For example, highly substituted dehydrocholic (**84**) and hyocholic acid (**115**) show the least affinity for **114**, and their complexes are destabilized by 1−2 kcal mol^{-1} relative to those formed by hyodeoxycholic acid (**79**).



115, Hyocholic acid



116, Disodium dexamethasone-21-phosphate



117, 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate

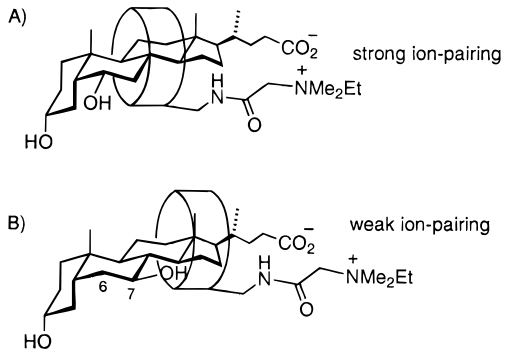
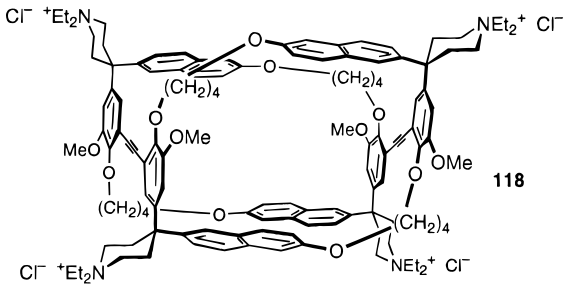


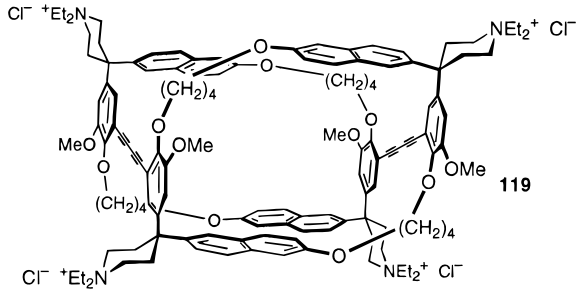
Figure 26. Representation of two possible inclusion geometries for the complexes between receptor **114** (schematically shown) and hyodeoxycholate (**79**, A) and ursodeoxycholate (**81**, B). To avoid unfavorable inclusion and desolvation of the equatorial 7α-HO group, **81** probably adopts a complex geometry which does not allow efficient ion pairing with the pendant ammonium side arms of the receptor.

There is evidence that the complexes of cyclophane **114** (Table 7) are additionally stabilized by ion pairing between one or even two of the ammonium groups attached by linkers to the phenyl rings and the negatively charged side chains of the substrates that are present in basic aqueous solution. Firstly, the zwitterionic steroid **117** with an ammonium side chain on its D ring is not bound at all by the tetraammonium host due to charge repulsion.[226] Secondly, anionic steroid inclusion by **114** in water was found to be accompanied by a favorable change in entropy as compared to the complexation by **113** in D_2O/CD_3OD 1:1. One contribution to such a favorable entropic driving force in pure water could be the increased hydrophobic effect,[79] *i.e.* the entropically more favorable desolvation of the apolar surfaces of host and guest. However, another contribution, which we believe is dominating, is the entropy gain due to the release of ordered water molecules from the solvation shells of the ionic centers of the binding partners that undergo ion pairing in the complex. Such an entropically favorable desolvation[277] is absent in the complexation by **113** which lacks the cationic side chains at the phenyl rings. Table 7 shows that the association between **114** and hyodeoxycholate (**79**) is mainly entropically controlled ($\Delta H° = -1.5$ kcal mol^{-1}, $T\Delta S° = 5.3$ kcal mol^{-1}), while the complex formation between **114** and ursodeoxycholate (**81**) is more enthalpically governed ($\Delta H° = -4.0$ kcal mol^{-1}, $T\Delta S° = 1.9$ kcal mol^{-1}). At first glance, this result is very surprising since the two substrates differ only in the position of their equatorial HO group on the B ring. However, differences in ion pairing, as a result of different complex geometries, could account for the change in the thermodynamic driving force. As can be seen from CPK model examinations, the carboxylate side chain of hyodeoxycholate (**79**) can interact with one of the pendant ammonium groups of the host if both the B and C rings are complexed (Figure 26A). A similar interaction is possibly prevented in the corresponding complex of ursodeoxycholate (**81**). Here, the 7α-HO group of the guest should enforce a different complexation geometry, hindering the charged groups to approach close enough for the formation of a strong, partially desolvated salt bridge.

Ion pairing in addition to apolar binding interactions has been previously shown to lead to high selectivities in the complexation of aromatic substrates by cyclophane receptors.[38,244,278] Complexation of anionic steroids (Table 7) by receptor **114** is again characterized by a characteristic isoequilibrium relationship (Figure 24B). In this case, an increase in entropy during complexation is compensated by an increasingly less negative enthalpy.

Cyclophanes **113** and **114**, similar to the cyclodextrins, possess cavities that are at best capable of including one or possibly two rings of the tetracyclic steroidal skeleton. In order to create deep cavities capable of full incorporation of a steroid, Diederich and co-workers connected two [6.1.6.1]paracyclophanes similar to **113** and **114** by means of acetylenic linkers to give the D_2-symmetrical macrotricyclic receptors **118** and **119**.[227,228] The smaller cyclophane **118** incorporates two ethynediyl bridges and possesses an 11 Å deep and 8 × 11 Å wide cavity, while the bis(butadiyndiyl) bridged receptor **119** provides an enlarged pocket of 9 × 12 Å width and 13 Å depth according to computer modeling studies.[228] In section II.B, it was shown that cholesterol oxidase binds substrates like dehydroisoandrosterone (**18**) in a pocket of comparable depth (11 Å) to that of the macrotricyclic receptors **118** and **119**.





In initial experiments with the new receptors, solubilization of cholesterol (**1**), progesterone (**3**), and testosterone (**8**) was studied using solid−liquid extraction techniques. Cholesterol was best solubilized by the smaller receptor **118**. A 1 mM solution of **118** in D_2O enhanced the solubility of cholesterol by a factor of 190 from 4.7 μM[279] to 0.85 mM. Extraction of solid cholesterol with a 1 mM solution of **119** only provided a 0.42 mM solution of the steroid which corresponds to a complexation-mediated increase in solubility by a factor of 90. From these solid−liquid extraction data, association constants for the 1:1 complexes formed in the aqueous phase were calculated to be in the range of $K_a = 10^5-10^6$ L mol^{-1} (Table 8).[278] The 1:1 stoichiometry of the complexes was supported by computer-modeling studies.[280]

Table 8. Association Constants K_a (L mol^{-1}) and Binding Free Energies ΔG (kcal mol^{-1}) for 1:1 Steroid Complexes of 118 and 119 As Determined at 295 K by Solid−Liquid Extraction in D_2O[a]

steroid	receptor	K_a (L mol^{-1})	ΔG (kcal mol^{-1})
cholesterol (**1**)	**118**	1.1×10^6	−8.2
cholesterol (**1**)	**119**	1.5×10^5	−7.1
progesterone (**3**)	**119**	1.5×10^5	−7.1
testosterone (**8**)	**118**	6.8×10^4	−6.5

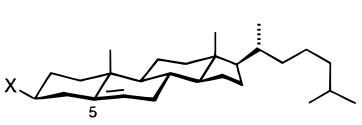
[a] Reproducibility of $\Delta G \pm 0.4$ kcal mol^{-1}

A comparative and more accurate investigation of the steroid-binding properties of **118** and **119** was conducted by ^{1}H NMR binding titrations in CD_3OD where, unlike in water, most steroids are readily soluble and do not form aggregates. Although the change in solvent polarity from water to methanol reduces the complexation free energy by 3−4 kcal mol^{-1}, receptors **118** and **119** form remarkably stable complexes with many steroids in pure methanol (Table 9). In these complexes, the steroid is axially incorporated in the cavity, *i.e.* its longest axis extends in the direction of the C_2 axis passing through the cavity perpendicular to the mean molecular plane of each of the two bridged [6.1.6.1]paracyclophanes. The most striking observations of the ^{1}H NMR binding studies can be summarized as follows.

(i) Differences in the binding patterns seen with **118** and **119** are determined by the different cavity widths. The narrower cavity of **118** prefers encapsulation of steroids that possess a double bond in their B rings which therefore are flattened relative to their fully aliphatic analogs. Receptor **118** is quite selective for cholesterol and its derivatives such as cholesteryl acetate (**120**) or 5-cholestene (**121**) (entries 1−3 compared to entry 4, Table 9) as well as for testosterone (**8**, entry 8). In contrast, a fully aliphatic steroid such as cholestane fits well into the wider cavity of **119** (entry 15, Table 9).

(ii) In addition to differences in shape, functional group desolvation upon inclusion complexation is responsible for selectivity effects. For example, cholesteryl acetate (**120**) is bound stronger by 0.5−0.6 kcal mol^{-1} by both receptors as compared to cholesterol (**1**) (entries 1, 12 *vs* 3, 14). Also, progesterone (**3**) binds more strongly to **119** than testosterone (**8**) by 1.6 kcal mol^{-1} (entries 17, 19). On the one hand, these findings can be rationalized by the fact that the acetyl groups in **120** and **3** are probably less solvated than the corresponding HO groups in **1** and **8**, respectively. On the other hand, C−H$\cdots\pi$ interactions between the aromatic cavity walls of the receptor and the acetyl groups may further stabilize the more apolar derivatives over the HO substituted one. Similar interactions could also explain the high stability of the complex formed by **119** and pregnelonone acetate (**124**). Additionally, the depth of the cavity should also influence the degree to which desolvation of peripheral functional groups occurs. Thus, the better binding of testosterone (**8**) to **118** (entry 8) as compared to **119** (entry 19) may both be due to the different cavity width as well as to the greater depth of the binding site in **119**, leading to more complete steroid encapsulation and greater functional group desolvation.

Table 9. Association Constants (K_a, L mol^{-1}, reproducibility ± 10%) and Binding Free Energies $\Delta G°$ (kcal mol^{-1}) from ^{1}H NMR Titrations for 1:1 Steroid Complexes Formed by Receptors 118 and 119 in CD_3OD at 298 K[a]



X	
1, Cholesterol	OH
120, Cholesteryl acetate	$OCOCH_3$
121, 5-Cholestene	H

R	
122, 5α-Cholestane	$CH(CH_3)(CH_2)_3CH(CH_3)_2$
123, 5α-Androstane	H

124, Pregnenolone acetate

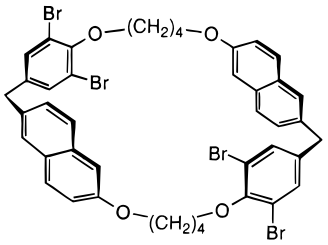
entry	receptor	steroid	K_a (L mol^{-1})	$-\Delta G°$ (kcal mol^{-1})	$\Delta\delta_{sat}$ (ppm): $H_3C(18)$
1	**118**	**120**, cholesteryl acetate	4 755	5.0	−1.95
2	**118**	**121**, 5-cholestene	3 190	4.8	−1.19
3	**118**	**1**, cholesterol	1 535	4.4	−1.70
4	**118**	**122**, 5α-cholestane	865	4.0	−1.57
5	**118**	**123**, 5α-androstane	500	3.7	−1.74
6	**118**	**6**, hydrocortisone	110	2.8	−0.60
7	**118**	**7**, cortisone	150	3.0	−2.16
8	**118**	**8**, testosterone	2 090	4.5	−1.69
9	**118**	**10**, β-estradiol	390	3.5	−2.04
10	**118**	**80**, chenodeoxycholic acid		no significant binding	
11	**118**	**83**, lithocholic acid	305	3.4	0.57
12	**119**	**120**, cholesteryl acetate	2 300	4.6	−1.33
13	**119**	**121**, 5-cholestene	2 300	4.6	−1.20
14	**119**	**1**, cholesterol	900	4.1	−0.97
15	**119**	**122**, 5α-cholestane	2 700	4.7	−1.10
16	**119**	**123**, 5α-androstane	370	3.5	−1.16
17	**119**	**3**, progesterone	2 600	4.7	−1.63
18	**119**	**124**, pregnenolone acetate	2 100	4.5	−1.68
19	**119**	**8**, testosterone	200	3.1	−1.13
20	**119**	**10**, β-estradiol	170	3.0	−1.10

[a] Also shown are the CISs at saturation binding $\Delta\delta_{sat}$ of the $H_3C(18)$ protons, which were evaluated in the titration.

(iii) The side chain of cholesterol and related steroids has a remarkable effect on the stability of the complexes formed by receptor **119** with its 13 Å deep binding site. For instance, 5α-cholestane (**122**) forms a much more stable complex ($\Delta(\Delta G°) = 1.2$ kcal mol^{-1}) than 5α-androstane (**123**) which lacks the isoprenoidal side chain (entries 15 and 16, Table 9). Diagnostic upfield CISs of the H_3C resonances in the side chain of **122** upon complexation with **119** provide experimental evidence for the additional incorporation of that flexible part of the guest in the host cavity. In contrast, the less deep (11 Å) cavity of **118** is not efficient in incorporating the isoprenoidal side chain and 5α-cholestane (**122**) and 5α-androstane (**123**) form complexes of similar stability (entries 4 and 5).

(iv) Finally, both cyclophane receptors **118** and **119** discriminate between aliphatic and aromatic guests, the corresponding complexes with β-estradiol (**10**) being weak in comparison with those of most aliphatic guests (entries 9 and 20, Table 9).

Recently, the first X-ray crystal structure of a naphthylphenylmethane-shaped cyclophane (**125**) became available, which proved that these receptors provide highly preorganized, nearly rectangular cavities with a parallel, face-to-face orientation of the two naphthalene and the two benzene rings, respectively.[229] In a 1:2 inclusion complex with toluene, the cavity dimensions were found to be 8.3 × 11.4 Å (distances between face-to-face aromatic walls). Cyclophane **125** forms crystalline inclusion complexes with a variety of solvent molecules such as 1,2-dichloroethane, benzene, toluene, or *p*-xylene which fill infinite molecular channels formed by stacking macrocycles.[281]



125

Tetrabromocyclophane **125** served as a precursor for the construction of tetracarboxylic acid **126**, which

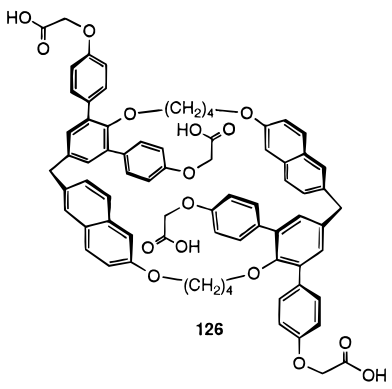
Table 10. Association Constants K_a (L mol^{-1}) and Complexation Free Enthalpies $\Delta G°$ (kcal mol^{-1}) for Dendrophane−Steroid Complexes in Borate-Buffered D_2O (pD 10.5)/CD_3OD 1:1 (v/v) at 298 K[a]

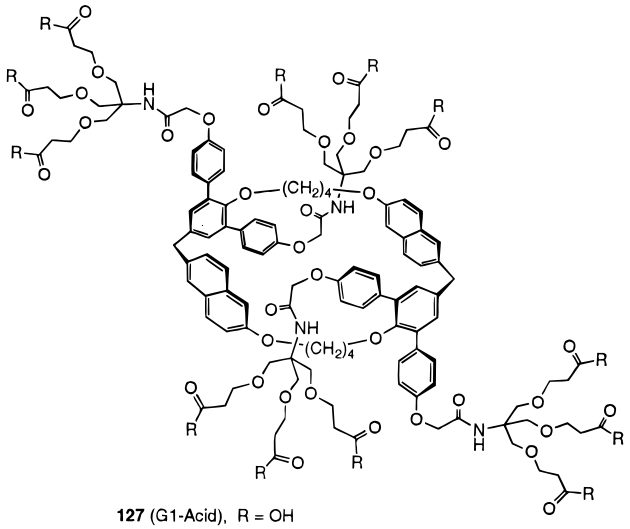
receptor	steroid	K_a (L mol^{-1})	$\Delta G°$ [b] (kcal mol^{-1})	$\Delta\delta_{sat}$ (ppm) $H_3C(19)$	$H_3C(18)$
126[c]	**3**	1 520	−4.3	*d*	
126	**8**	1 300	−4.2	*d*	
126	**7**	380	−3.5	*d*	
126	**83**	270	−3.3	*d*	
126	**6**	80	−2.6	*d*	
126	**79**	40	−2.2	*d*	
126	**8**	1 350	-4.3	−0.81	−0.24
127	**8**	700	−3.9	−0.97	−0.25
128	**8**	750	−3.9	−1.60	−0.35
129	**8**	1 100	−4.2	−1.33	−0.30

[a] Also shown are the CISs calculated for saturation binding ($\Delta\delta_{sat}$) for the resonances of $H_3C(19)$ and $H_3C(18)$ of the bound steroid.[229] [b] Uncertainties in $\Delta G° \pm 0.1$ kcal mol^{-1}. [c] Linear van't Hoff regression analysis of variable-temperature ^{1}H NMR titrations yielded $\Delta H° = -5.0$ kcal mol^{-1} and $T\Delta S° = -0.8$ kcal mol^{-1}. [d] Host signals were followed ($\Delta\delta_{sat} = 0.16-0.50$ ppm).

was subsequently used as the core of steroid-binding dendritic receptors.[229] Before the water-soluble *dendr*itic cyclo*phanes* (dendrophanes)[282] **127**−**129** of first to third generation were constructed, following known dendrimer techniques,[283−287] the steroid-recognizing properties of the core receptor **126** were explored in ^{1}H NMR titrations conducted in borate-buffered D_2O (pD 10.5)/CD_3OD 1:1 (Table 10). Similar to cyclophane **113**, receptor **126** discriminates between steroids of different polarity: complexation strength decreases from progesterone (**3**), to testosterone (**8**), to cortisone (**7**), to lithocholic acid (**83**), to hydrocortisone (**6**), and to hyodeoxycholic acid (**79**). The stability of the inclusion complexes is lowered by increasing steroid polarity and by electrostatic repulsion, if the substrates also possess carboxylate residues.

The third-generation dendrophane **129** ($C_{858}H_{1372}N_{52}O_{432}$, Chart 1) has a molecular weight of 19 328 D and carries 108 peripheral carboxylate groups. All three dendrophanes **127**−**129** were investigated for their steroid binding properties and were found to form 1:1 complexes of comparable stability to that of core cyclophane **126** in borate-buffered D_2O (pD 10.5)/CD_3OD 1:1 (Table 10). Apparently, the deeply buried cyclophane recognition site remains open and accessible within the dendritic shells, and hydrophobic collapse does not occur in polar solvents. The observed CISs of the steroidal methyl group ^{1}H NMR resonances clearly showed that complexation occurred within the cyclophane core in a 1:1 stoichiometry rather than in a statistic way within the dendritic shell;[288] the data also provided evidence for a generation-dependent conformational change in the steroid−dendrophanes complexes. Furthermore, the host−guest exchange kinetics remained fast even for the generation three dendrophane, which possesses a globular structure of about 20 Å radius: a finding of some importance with respect to the future construction of artificial proteins and enzymes.[45−48]





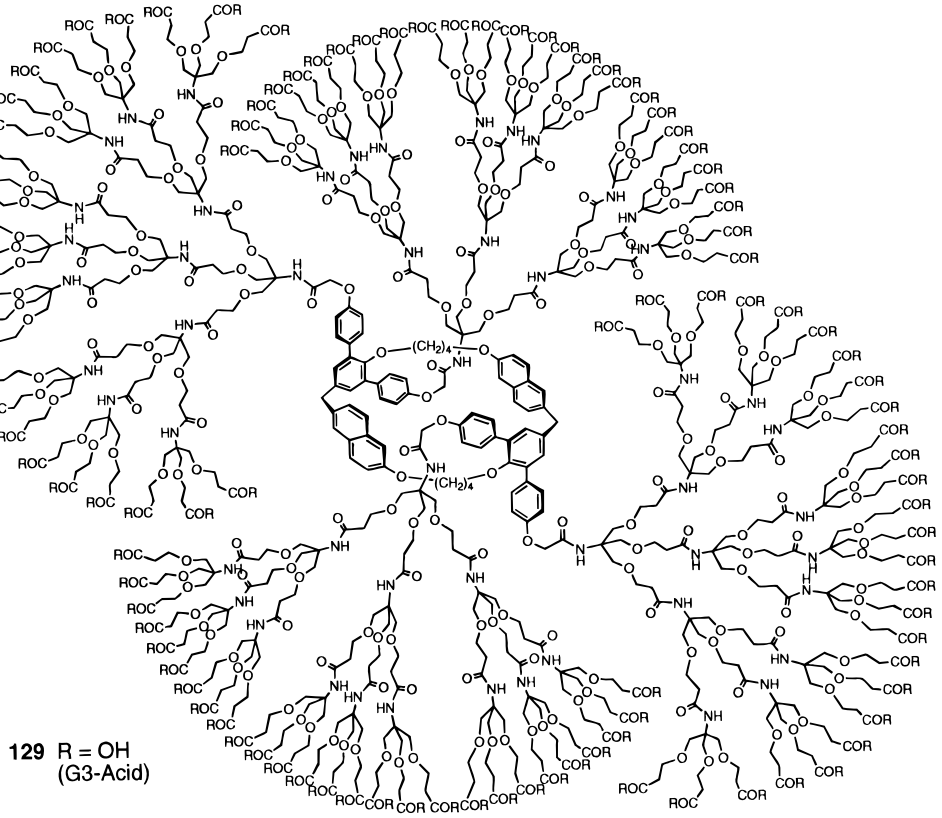
127 (G1-Acid), R = OH

128 (G2-Acid), R = $NHC[CH_2O(CH_2)_2CO_2H]_3$

C. Conclusions

Water-soluble cyclophane receptors shaped by naphthylphenylmethane units are efficient binders of steroids in aqueous solutions. Complex stoichiometry and stability are conveniently assayed in ^{1}H NMR binding titrations by taking advantage of the complexation-induced changes in chemical shift (CISs) that are observed when the steroidal substrate is incorporated into the aromatic cavity of the binding site. The major driving forces for inclusion complexation are entropically favorable hydrophobic desolvation, enthalpically favorable increase in solvent cohesive energy, as well as in dispersion interactions. As in other molecular association processes, complexation thermodynamics are characterized by strong enthalpy−entropy compensation. In general, the more hydrophobic the steroid core, the more stable the complexes formed. Significant selectivities can result from ion pairing interactions (in case of receptor **114**), from unfavorable desolvation of functional groups of the steroid upon incorporation into the apolar cavity binding site (in case of receptor **113**), and from shape differences as subtle as the presence or absence of a double bond in the steroidal B ring (in case of receptors **118** and **119**). The latter macrotricyclic receptors efficiently solubilize steroids such as cholesterol in water. With their highly preorganized, 11 (**118**) and 13 Å (**119**) deep apolar cavities, they can fully include all four rings A, B, C, and D of the steroidal substrate and, in case of **119**, even provide space for incorporation of at least parts

Chart 1. Compound 129



of the isoprenoidal side chain of cholesterol derivatives. Thus, **118** and **119** resemble most closely some of the deep apolar pockets found in steroid-binding antibodies, proteins, and enzymes (section II). In the extension of steroid binding cyclophanes to dendrophanes, the most amazing result was the observation that complexation of testosterone by the globular third-generation receptor **129**, with *ca*. 4 nm diameter, occurs still fast on the ^{1}H NMR time scale. This opens up exciting perspectives for catalytically active, steroid-converting dendrophanes: catalytic mechanisms must be very efficient before dissociation rates from the catalytic cyclophane core will become rate determining. The development of highly specific, tight-binding steroid receptors could provide new strategies for interfering with biologically important steroids *in vivo* and potentially lead to a new class of pharmacological agents. Receptors specific for cholesterol may offer an alternative pharmacological strategy for the dissolution of cholesterol deposits such as those in atherosclerotic plaque. Many of the pharmacological objectives, previously targeted exclusively with cyclodextrins (section III), might be targeted in the future with tailor-made synthetic receptors, which are tunable in their properties by design and molecular construction.

V. Steroid-Based Synthetic Receptors

Steroids offer several attractive features to be used as building blocks in the construction of molecular receptors. In fact, as will be shown, some of the highly functionalized steroids act themselves as molecular receptors. One distinct advantage is their high degree of preorganization. When inserted into a receptor, the rigid tetracyclic steroid frame enforces a preorganized binding site and provides a high degree of conformational homogeneity, known to be crucial to selective molecular recognition. Furthermore, substituted steroids such as the natural and modified bile acids can be viewed as rigid molecular surfaces from which functional groups, capable of H-bonding interactions or ion pairing diverge in a stereochemically well-defined way. Although crystalline bile acid inclusion complexes have been known since the past century, the application of steroids to the construction of molecular receptors that function in the liquid phase has efficiently been pursued only during the past decade. Whereas steroids have been used as substrates in the studies described in the past three sections, they act as receptors or receptor fragments in the systems described in this final section. Only by exploring the function and properties of steroids as both hosts and guests, the impact of these compounds in molecular recognition will be revealed.

A. Steroid-Based Receptors for Molecular Recognition in the Liquid Phase

One of the first developments of steroid-based receptors was described by Guthrie *et al.*[289−294] By starting from corticosterone,[290] a catalytic steroid was prepared with an appended imidazolyl group, which was able to catalyze both ester hydrolysis[291] and β-elimination[292] reactions of substrates with good leaving groups in aqueous solutions. The tweezer-like molecule **130** (Figure 27) was found to be more efficient in binding and catalysis[293] than monomeric steroids, as a result of increased hydrophobic interactions and a better defined association mode.[294] The

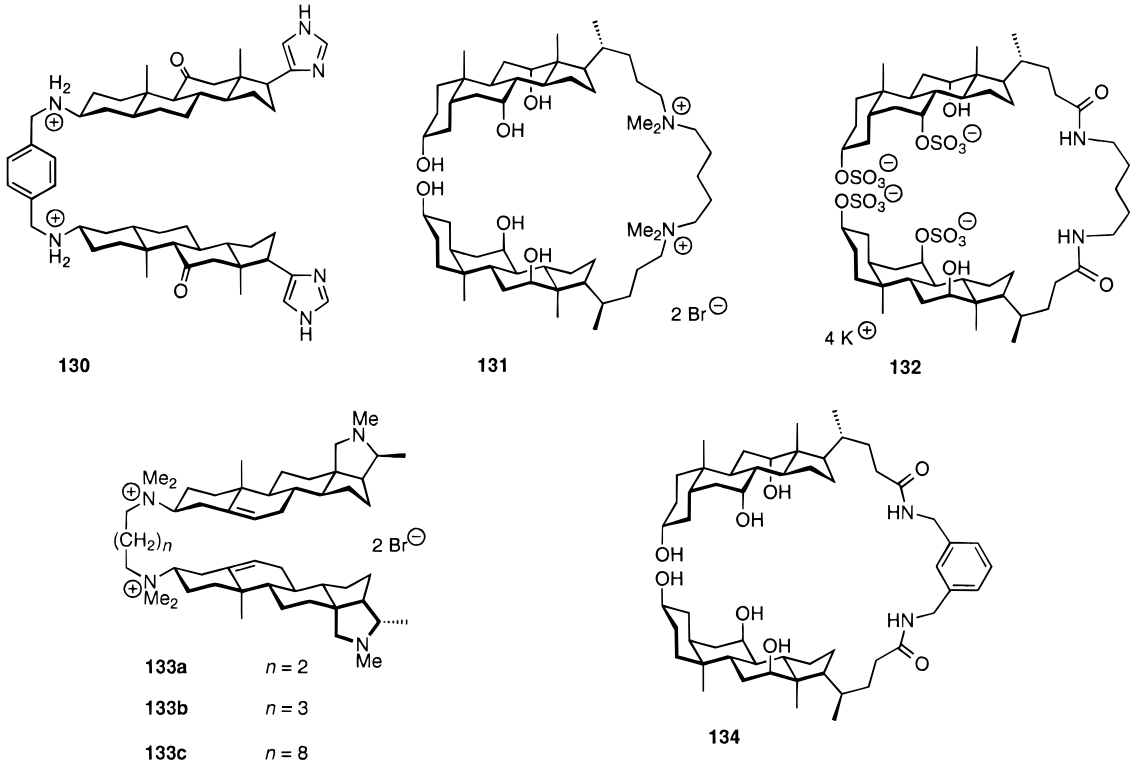


Figure 27. Tweezer-type steroid receptors and catalysts synthesized by Guthrie *et al.* (**130**),[289−294] McKenna *et al.* (**131**−**133**),[295] and Burrows *et al.* (**134**).[297−299]

latter type of bis-steroid receptor had previously been developed by McKenna *et al.* in order to find potential enzyme mimics.[295] The resulting podands **131**−**133** (Figure 27) are able to solubilize perylene (water solubility $<2 \times 10^{-9}$ mol L^{-1})[278,296] quite efficiently in water, without the formation of micelles.

More detailed investigations on the conformations and binding properties of receptor **134**, another cholic acid-based system, were carried out by Burrows and co-workers.[297−299] NMR studies showed conformation dependence on both temperature and solvent. The authors proposed two conformational preferences: a more closed form as shown schematically in Figure 27, which is stabilized by intramolecular H-bonding, and an open, freely rotating form. In apolar solvents such as $CDCl_3$, the spectra obtained indicated a dynamic exchange between these two forms with an energy barrier of 14 kcal mol^{-1} (coalescence at 29 °C at 300 MHz). Such an assumption was supported by the fact that addition of more competitive, H-bond-forming solvents such as CD_3OD favored free rotation even at low temperatures. Receptor **134** showed association with *O*(1)-pentyl glucopyranoside at 56 °C in $CDCl_3$,[298] however, no binding constants were measured. Computational studies using macrocyclic model compounds for **134** and inositol as a model for the monosaccharide suggested that the guest is bound by H-bonding interactions with the convergent polar functional groups in the pseudo-macrocyclic cavity.[299]

Polyamines such as putrescine, spermidine, and spermine are known for their DNA binding affinity. Also, steroidal amines such as squalamine (**135**), a natural product isolated from sharks, and the squalamine mimic **136**[300] are of considerable interest as antibiotics (Figure 28). In qualitative binding assays, the protonated bis-steroidal tetraamine **137**[301] required a lower concentration than simple polyamines or model compounds for 50% ethidium bromide displacement from its DNA complex.

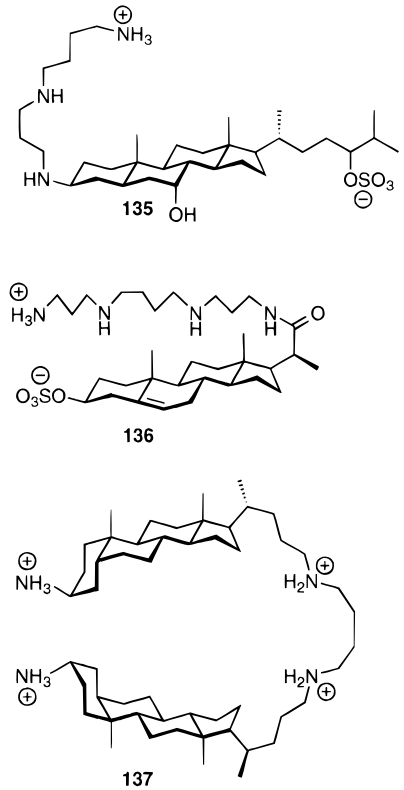
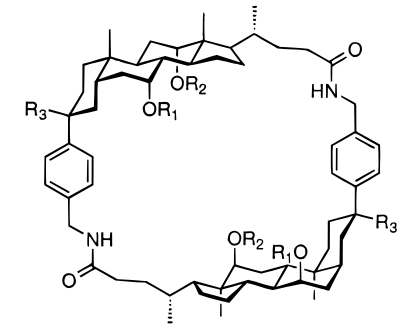
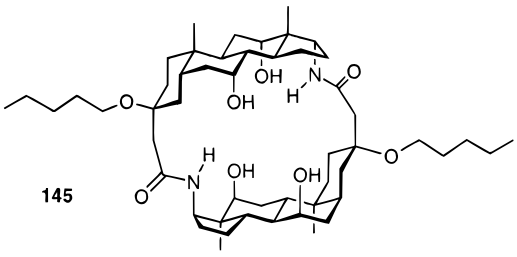


Figure 28. Steroidal amines squalamine (**135**) isolated from sharks, the squalamine model compound **136**, and the DNA complexing bis-steroid **137**.

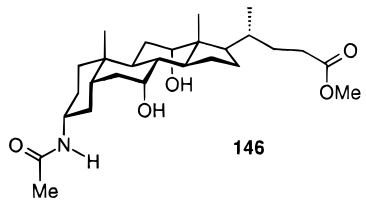
The rigid, L-shaped[302] steroidal nucleus of cholic acid has served as a versatile molecular frame for



	R_1	R_2	R_3
138	H	H	H
139	H	$PhCH_2$	H
140	Ac	H	H
141	Ac	Ac	H
142	PhCO	PhCO	H
143	Ac	$PhCH_2$	H
144	Ac	Ac	$CH(CN)_2$



145



146

Figure 29. Cholaphanes **138**−**144**, steroid-based cryptand **145**, and comparison compound **146** prepared by Davis and co-workers.[305−315]

the construction of macrocyclic receptors[303,304] named cholaphanes, and designed for the complexation of polar substrates in apolar solvents via H-bond formation.[305−315] In this work by Davis and co-workers, advantage is taken from the fact that cholic acid possesses two faces: the highly apolar β-face and the functionalized α-face with three HO groups, which can be readily transformed into other functionality.[304,305−307] In cholaphane **138**, the β-face is located at the exterior, providing solubility in apolar solvents while the α-face, with two HO groups, positions H-bond donor and acceptor centers within the cavity (Figure 29). Analogs with either partially (**139** and **140**) or fully protected (**141**[308]−**143**) HO groups were also synthesized.[309,310] Inversion of the function of the cholaphane, toward hydrophobic binding in aqueous solution, was achieved by attachment of polar groups to the exterior face and full protection of the HO groups in the cavity.[311] The corresponding macrocycle **144**, which is less flexible than **138**−**143**, formed a crystalline complex incorporating two molecules of tetrahydrofuran in its cavity.[312]

Table 11. Association Constants K_a (L mol^{-1}) and Binding Free Energies $\Delta G°$ (kcal mol^{-1}) Determined by ^{1}H NMR Binding Titrations for the Complexes Formed between Alkyl Glycosides and Cholaphanes 138 and 139 in $CDCl_3$ (T = 298 K)

receptor	substrate	K_a (L mol^{-1})	$\Delta G°$ (kcal mol^{-1})
138	*O*(1)-octyl α-D-glucopyranoside	580	−3.8
138	*O*(1)-octyl β-D-glucopyranoside	3 100	−4.8
138	*O*(1)-octyl α-L-glucopyranoside	1 030	−4.1
138	*O*(1)-octyl β-L-glucopyranoside	1 000	−4.1
138	*O*(1)-dodecyl β-D-glucopyranoside	1 740	−4.4
139	*O*(1)-dodecyl β-D-glucopyranoside	700	−3.9

Table 12. Association Constants K_a (L mol^{-1}) and Binding Free Energies $\Delta G°$ (kcal mol^{-1}) Determined by ^{1}H NMR Titrations at 298 K for the Complexes Formed by Cholaphane 145 and Comparison Compound 146 with Tetrabutylammonium Halides in $CDCl_3$

receptor	substrate	K_a (L mol^{-1})	$\Delta G°$ (kcal mol^{-1})
145	$Bu_4N^+F^-$	3 220 ± 350	−4.8
145	$Bu_4N^+Cl^-$	990 ± 80	−4.1
145	$Bu_4N^+Br^-$	250 ± 20	−3.3
146	$Bu_4N^+Cl^-$	53 ± 2	−2.3
146	$Bu_4N^+Br^-$	36 ± 1	−2.1

The unprotected cholaphane **138** is an efficient receptor for alkyl glucosides in $CDCl_3$. The substrates bind in the cavity of **138** by forming intermolecular H-bonds between their HO residues and the convergent HO and NH groups of the receptor. Binding was detected by following the downfield CIS of the N*H* resonance of the host. As a result of the high preorganization of the chiral macrocycle, both a remarkable diastereo- and enantioselectivity was observed (Table 11).[313] Also, upon HO group protection, the association strength decreases: benzylated cholaphane **139** forms weaker complexes than unprotected **138**.[314]

Steroid-based cryptand **145** as well as comparison compound **146** recognize halide anions in $CDCl_3$.[315] The stability of the 1:1 complexes with **145** decreases in the sequence $F^- > Cl^- > Br^-$ (Table 12). Expectedly, the complexes of the nonmacrocyclic receptor are much less stable than those formed by the macrocycle. According to computer modeling and evaluation of CISs in the ^{1}H NMR spectra of the corresponding complexes, **145** encapsulates the halide anions in a cryptate-like fashion.[35] The complexes are particularly stabilized by ionic H-bonds between the halide anion and the N−H groups of the receptor; additional stabilization arises from H-bonds between the enclosed substrate and the HO groups of **145** or **146**.

Another interesting series of macrocyclic receptors was obtained by Bonar-Law and Sanders,[316−319] who combined the properties of steroids with those of metalloporphyrins. The zinc(II) derivative **147** was designed for the complexation of a variety of nitrogen bases via both metal−amine and H-bonding interactions (Figure 30). In compound **147**, each cap consists of two cholic acid moieties linked in a macrocyclic fashion by two ester bridges. Association constants for complexes of **147** were determined and compared to those measured for complexes formed by a simple, noncapped zinc porphyrin. In $CDCl_3$ it

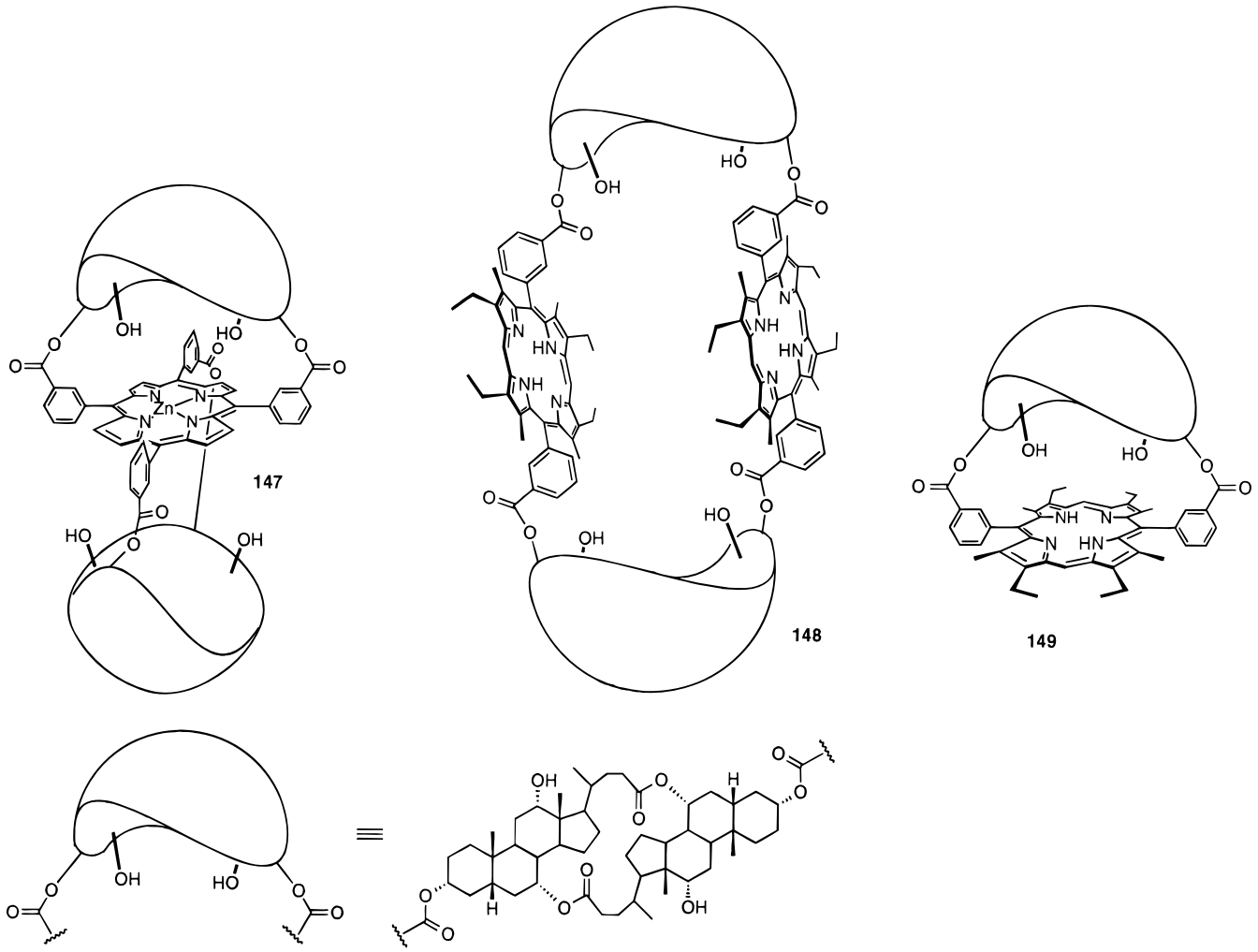


Figure 30. Steroid-capped porphyrins for complexation of amines and monosaccharides *via* H-bonding and metal center coordination.

was found that nitrogen bases, such as 4-hydroxypyridine, which can undergo both metal−amine and H-bonding interactions, formed a more stable complex with **147** than with the simple metalloporphyrin. The opposite binding behavior was observed for pyridine derivatives lacking additional polar functional groups. The strongest association with **147** was observed with purine as a guest ($K_a = 2.1 \times 10^6$ L mol^{-1} in $CHCl_3$, $T = 293$ K).[317] Receptor **147** was also solubilized in water by incorporation into micelles formed by sodium dodecyl sulfate (SDS) with the expectation that the amine substrates could now be bound in aqueous solution.[316] The catalytic properties of **147** were examined in transacylation reactions in chloroform. Upon addition of 2 equiv of 4-(*N*,*N*-dimethylamino)pyridine and an excess of the mixed anhydride formed from 3-carboxypyridine and 2,6-dichlorobenzoic acid, rapid acylation of one HO group of **147** was observed. The phenomenon was not observed with the analogous Zn-free system, suggesting that pyridine−zinc(II) association is responsible for positioning the active species in close proximity and favorable orientation to the reacting HO group, thereby lowering the activation free energy of the transacylation process.[317]

The large receptor **$Zn_2$148** was found to strongly bind to substrates capable of interacting in a ditopic mode with both zinc(II) centers. Accordingly, formation of highly stable complexes with 4,4′-bipyridine ($K_a = 2.5 \times 10^6$ L mol^{-1}, $T = 295$ K) and 1,4-diazabicyclo[2.2.2.]octane (DABCO, $K_a = 8.0 \times 10^7$ L mol^{-1}) was observed in CH_2Cl_2.[318]

Steroid-capped porphyrins such as **149** and metalated **Zn149** are suitable for polyol and carbohydrate recognition.[319] Table 13 shows the thermodynamic data for the 1:1 inclusion complexation of alkyl pyranosides in CH_2Cl_2, for which both modest anomeric and enantioselectivity were observed. A comparison between the affinities to **149** and **Zn149** showed that substrate coordination to the Lewis acidic metal ion center hardly influences the association processes. Most of the complexation strength appears to be derived from H-bonding to the HO groups of the steroidal cap as well as from dispersion interactions in the cavity binding site. Presumably, the cavity is too large for the pyranoside substrates; thus contacts to both HO groups and the metal center cannot be well established. However, complexation is strengthened upon addition of stoichiometric amounts of water or methanol, which cobind in the cavity, thus making it geometrically more complementary to the shape of the sugars.

Cyclic oligomers of cholic acid, the so-called cyclocholates, with the HO groups transformed into methoxyethoxymethyl (MEM) ethers, were prepared for alkali metal ion complexation in organic solvents

Table 13. Binding Free Energies $\Delta G°$ (kcal mol^{-1}) determined by UV/vis and/or ^{1}H NMR Binding Titrations at Constant Carbohydrate Concentration for the Complexes Formed between Various Pyranosides and the Capped Porphyrins 149 and Zn149 in the Presence and Absence of Defined, Small Amounts of Added H_2O or MeOH (T = 295 K)

			$\Delta G°$ (kcal mol^{-1})		
receptor	substrate	solvent	dry	+H_2O	+MeOH
149	*O*(1)-decyl α-D-mannopyranoside	CH_2Cl_2	−5.26		
149	*O*(1)-octyl β-D-glucopyranoside	CH_2Cl_2	−3.90		
149	*O*(1)-octyl α-D-glucopyranoside	CH_2Cl_2	−3.47		
Zn149	*O*(1)-decyl β-D-mannopyranoside	CH_2Cl_2	−5.02	−5.77 (95)[a]	−5.43 (125)
Zn149	*O*(1)-decyl α-D-mannopyranoside	CH_2Cl_2	−5.19	−5.53 (90)	−5.79 (125)
Zn149	*O*(1)-decyl α-D-mannopyranoside	CCl_4	−8.40	−8.76 (6)	−9.33 (12)
Zn149	*O*(1)-octyl β-D-glucopyranoside	$CHCl_3$	−4.69	−5.81 (50)	
Zn149	*O*(1)-octyl β-D-glucopyranoside	CH_2Cl_2	−4.28	−5.17 (90)	−4.64 (125)
Zn149	*O*(1)-octyl α-D-glucopyranoside	CH_2Cl_2	−3.99		
Zn149	*O*(1)-octyl α-L-glucopyranoside	CH_2Cl_2	−3.25		
Zn149	*O*(1)-decyl β-D-galactopyranoside	CH_2Cl_2	−3.11		
Zn149	*O*(1)-decyl α-D-galactopyranoside	CH_2Cl_2	−3.64		

[a] The numbers in parentheses represent the concentration of added H_2O or MeOH (mmol L^{-1}).

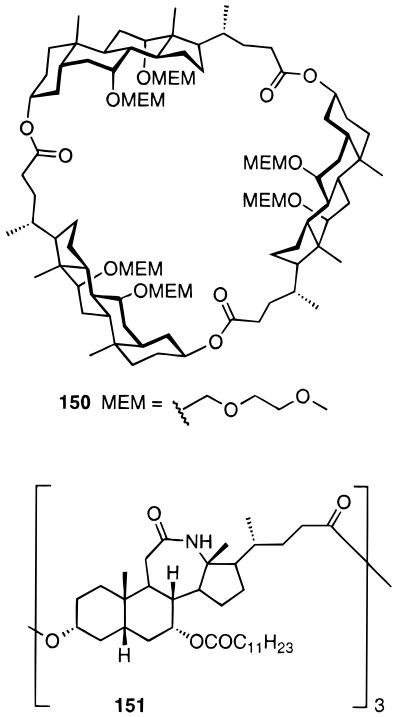


Figure 31. Cyclic oligomers **150** and **151** of cholic acid derivatives, named "cyclocholates".[320−322]

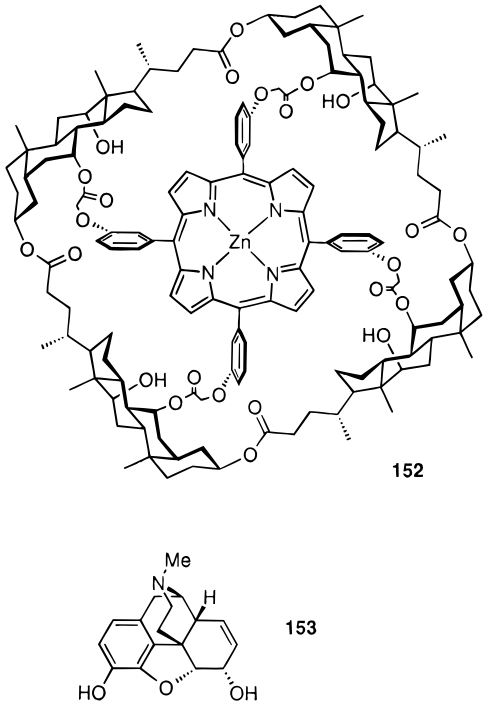


Figure 32. Natural (−)-morphine (**153**) is an excellent substrate for the molecular bowl-type receptor **152**.[324,325]

such as chloroform.[320,321] In macrocycle **150** (Figure 31) and larger oligomers, with MEM or other acyclic oligoether functions attached, the steroid moieties act as semirigid spacers with podand function. Association constants for alkali metal ion binding to **150** varied between K_a = 700 and 4300 L mol^{-1} in wet $CDCl_3$ at T = 292−295 K, with no peak selectivity being observed.[320] Cyclocholate **151** was synthesized via a Beckmann rearrangement and showed a remarkable tendency to dimerize in CCl_4 (dimerization constant of 30 000 L mol^{-1}).[322] The authors suggest that this high association tendency is due to intermolecular H-bonding between the amide groups of two macrorings.

Capping a cyclocholate by a porphyrin led to the molecular bowl **152** (Figure 32) which is suitable for the recognition of (−)-morphine (**153**) and its derivatives in CH_2Cl_2 at 293 K.[323] Host−guest binding occurs by a combination of both metal−ligand and H-bonding interactions, with the main contribution to the association free energy coming from H-bonding. This was clearly demonstrated in comparative studies with **153** and protected derivatives, whereas natural (−)-morphine (**153**) binds with an association constant K_a of 230 000 L mol^{-1}, codeine (morphine monomethyl ether) gives K_a = 13 000 L mol^{-1}, and the corresponding dimethyl ether only bound with K_a = 240 L mol^{-1}.[324] A high degree of chiral recognition was observed: natural (−)-morphine binds by 2.2 kcal mol^{-1} better to the optically active molecular bowl than the enantiomeric (+)-morphine.[325]

In a combinatorial approach to optimized receptors for pentapeptidic ^{5}Leu enkephalin derivatives, Still and co-workers[326,327] used the tetracyclic frame of steroids as rigid spacers[328] which position two peptidic side chains at a defined mutual distance and orientation (Figure 33). Libraries of receptors **154** and **155**, immobilized to polymer beads, were prepared by variation of the amino acids (AA) in the two

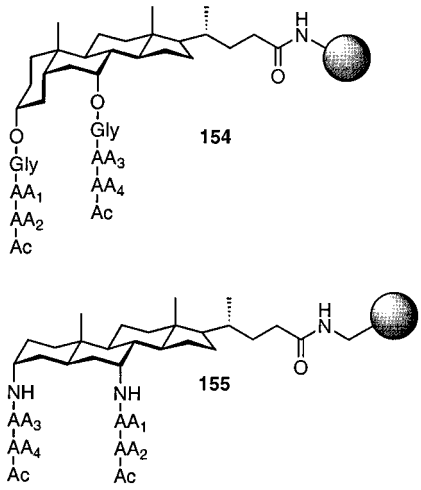


Figure 33. Steroids used as rigid backbones for peptide chains in a combinatorial approach by Still and co-workers to selective receptors for ^{5}Leu enkephalin derivatives.[326,327] The gray circles symbolize the polymer beads on which the receptors are immobilized. AA_1−AA_4 are amino acid residues.

peptide side chains. Screening the libraries demonstrated sequence-specific binding of *N*-acylated ^{5}Leu enkephalin methyl ester by **155** in $CDCl_3$, with the most effective association occurring for AA_1 = L-Asn, AA_2 = D-Asn, and AA_3 = D-Phe.

B. Steroids as Hosts in Supramolecular Assemblies

With their large lipophilic surface, steroids and in particular cholesterol derivatives[329] can associate under the formation of supramolecular assemblies such as vesicles. Hydrogel nanoparticles of 20−30 nm diameter form in water by self-assembly of polymeric amphiphiles such as cholesterol-bearing pullulan, a polysaccharide.[330] The size and density of the hydrogel nanoparticles is controlled by the amount of cholesterol attached to the carbohydrate, and the structure is greatly determined by domains of associated cholesterol. These polymeric self-aggregates bind to a variety of hydrophobic substances, particularly proteins such as α-chymotrypsin or bovin serum albumin. Upon complexation, the thermal stability of these proteins is greatly enhanced.

Gokel and co-workers prepared the lariat ethers[331,332] **156** and **157** (Figure 34) by linking hydrophobic 3-cholestanol or cholesterol to crown ethers.[333−335] The amphiphilic compound **157a** was shown to form vesicles in aqueous solutions.[336] Other vesicles, including redox-switchable molecular aggregates, were obtained by attachment of a ferrocenyl group to cholesterol and derivatives.[337,338]

Crown ether appended cholesterol derivatives (Figure 35)[339,340] may form liquid crystals and have been investigated by Shinkai and co-workers as artificial ion channels. A 1:1 mixture of **158a** and **158b** displayed liquid crystalline behavior at room temperature. Upon immobilization on a polymeric support, an ion channel with good selectivity for K^+ ions was obtained.[341−343] A related liquid crystalline system was formed from **158b**[344] mixed with cholesteryl nonaoate and 3-chloro-5-cholestene and acted as a chromophoric sensor.[344] The system responded

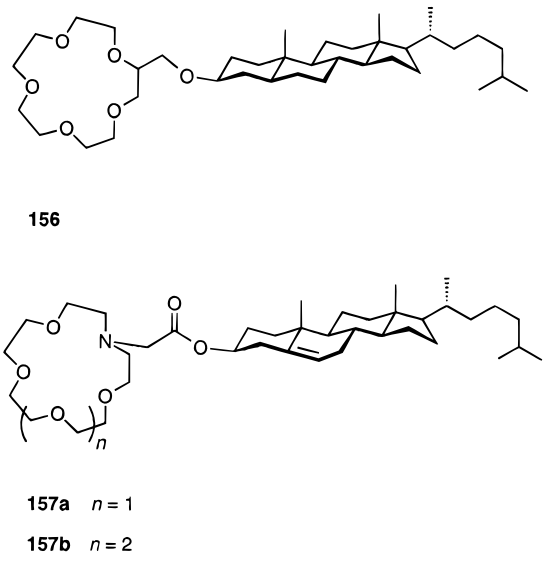


Figure 34. Steroidal lariat ethers prepared by Gokel and co-workers.[331−336]

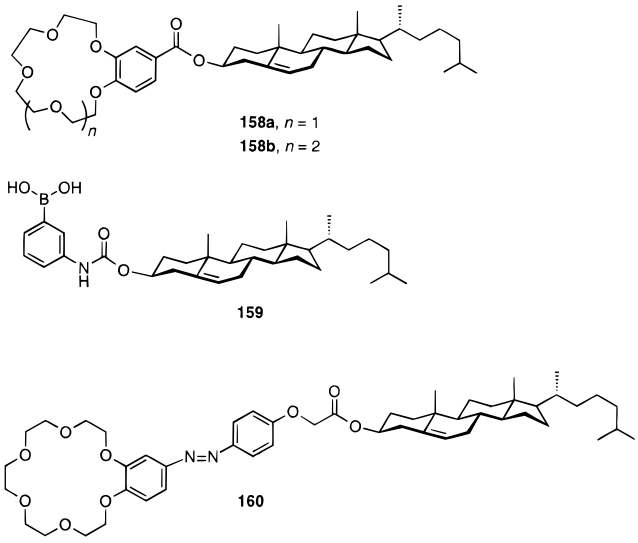


Figure 35. Cholesterol derivatives for ion binding (**158a/b**), for pyranoside recognition (**159**), and for photoswitchable gels (**160**), prepared by Shinkai and co-workers.[342−352]

by a change in color to the differential complexation of the enantiomers of substrates such as phenylalanine methyl ester hydrochloride[344] or alkali mandelates[345] which bind with their cationic centers to the crown ether portion. Compound **158b** was also used as an enantiomerically selective sensor at the air−water interface.[346]

Chiral recognition of monosaccharides was observed in a cholesteric liquid crystalline membrane containing the cholesterol−boronic acid conjugate **159**. Pyranose and furanose substrates were shown to react with the boronic acid residue of **159** under formation of a cyclic boronate ester. The membrane altered its color upon treatment with the guest molecules, depending on their chirality.[347] Chiral discrimination of monosaccharides was also achieved in monolayers at the air−water interface, composed of **159**.[348]

The photoswitchable cholesterol−crown ether conjugate **160** and related compounds containing an azobenzene moiety[349,350] were found to act as gelators

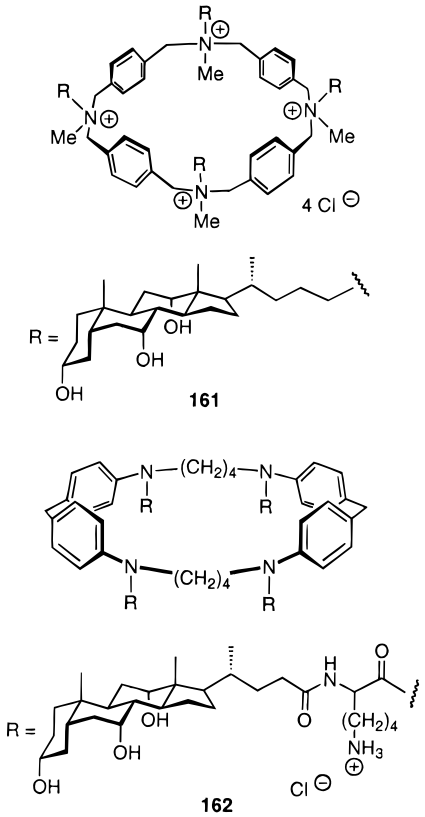


Figure 36. Steroid appended cyclophanes **161** and **162** for membrane insertion and hydrophobic complexation.[353−356]

of several organic solvents.[351] The CD spectra of these gels changed significantly upon irradiation, which induces *trans* → *cis* isomerization of the azobenzene chromophore.[352]

The steroid-appended water-soluble cyclophanes **161** and **162** (Figure 36) were prepared by Murakami and co-workers.[353,354] Binding of the fluorescent probe 8-anilinonaphthalene-1-sulfonate (ANS) to **161** was observed in aqueous solution ($K_a = 3.3 \times 10^5$ L mol^{-1})[355] and also in the presence of synthetic bilayer membranes in which the cyclophane was incorporated.[356]

Groves and Neumann reported the regioselective epoxidation[357] and hydroxylation[358] of steroids in water catalyzed by membrane spanning Fe(III) or Mn(III) porphyrins, respectively, in the presence of oxygen-transfer agents such as iodosylbenzene or sodium periodate.[359] The cytochrome P-450 mimics insert into membranes with the aid of four cholesterol residues that are attached to the four *meso*-phenyl rings of the metalloporphyrin. The cholesterol residues all arrange on one face of the porphyrin and confine a binding site for steroids such as cholesterol. The substrates are trapped and expose selected bonds to the reactive higher valent metal−oxo species which regioselectively transfers the O atom. A related system was able to bind the electron transfer protein cytochrome *c* at a membrane surface.[360]

Guest selectivity in micellar inclusion processes is usually not high, but it is noteworthy that cholic acid micelles (**2**) bind fatty esters more weakly than micelles of deoxycholic (**82**) or chenodeoxycholic acid (**80**).[361] The bile acid micelles accelerate the hydrolysis of fatty acid substrates by enhancing their solubility and through catalytic mechanisms such as proximity between reactive groups, reduced micropolarity, and possibly acid−base catalysis. Interestingly, a micellar system composed of chenodeoxycholic acid was found to assist dissolution of gallstones.[361] Cholate micelles have also been explored as chiral microreactors for the enantioselective reduction of ketones by $NaBH_4$, but the enantiomeric excesses obtained were low.[362]

C. Steroid Clathrates

Steroids, particularly deoxycholic acid (**82**) are able to form crystalline inclusion complexes (clathrates). Systems of the deoxycholic acid type, also called choleic acids, were described in the last century.[363] The structures of these inclusion complexes, however, were only elucidated during the past decades, after X-ray crystal structure analysis became available. In this review, we shall restrict ourselves to a brief and more general description of clathrate-forming steroids with regard to structure and interesting function and application. A complete overview would lie far beyond the scope of this work.

As mentioned above, the most frequently used host for steroidal solid-state inclusion complexes is deoxycholic acid (**82**). Depending on the guest, **82** forms three principal types of clathrates of orthorhombic, tetragonal, or hexagonal structure.[363] Thereby, the most common crystal structure is orthorhombic. As shown in Figure 37, the guest molecules are packed in channels located between antiparallel bilayers of head-to-tail arranged host molecules and the crystal packing is stabilized by intermolecular H-bonding networks between the steroidal HO and COOH groups. In the tetragonal and hexagonal structures, the hosts either also form bilayers (tetragonal) or are helically arranged (hexagonal). In both types, guest inclusion also occurs within channels running through the crystal lattice.

Usually, the host/guest ratio increases with the length of the guest. Guests in a deoxycholic acid lattice can be as diverse as aliphatic, alicyclic, and aromatic hydrocarbons, alcohols, ketones, fatty acids, esters, ethers, phenols, azo dyes, nitriles, peroxides, and even ferrocene. The inclusion complexes are often formed by slow evaporation of an ethanolic solution containing host and guest.[363,364]

Apocholic acid (**163**) differs from deoxycholic acid (**22**) only by a double bond between C(8) and C(14) and forms also channel inclusion complexes, mostly with hydrocarbons and ketones. In many studies, solid-state complexes of apocholic acid were prepared for comparison with the clathrates of deoxycholic acid in order to explore on the molecular level the preference for formation of particular crystal structures.[364−366]

Although cholic acid (**2**) is very similar to deoxycholic acid (**82**), only a few inclusion complexes of this bile acid had been discussed prior to the middle of the 1980s, whereas deoxycholic acid clathrates have been known since the last century.[367] More recently however, a large number of guests such as alcohols, carboxylic acids, esters, ketones, aldehydes, ethers, amines, and others have also been shown to form clathrates with cholic acid and the structures of these inclusion complexes have been characterized.[367]

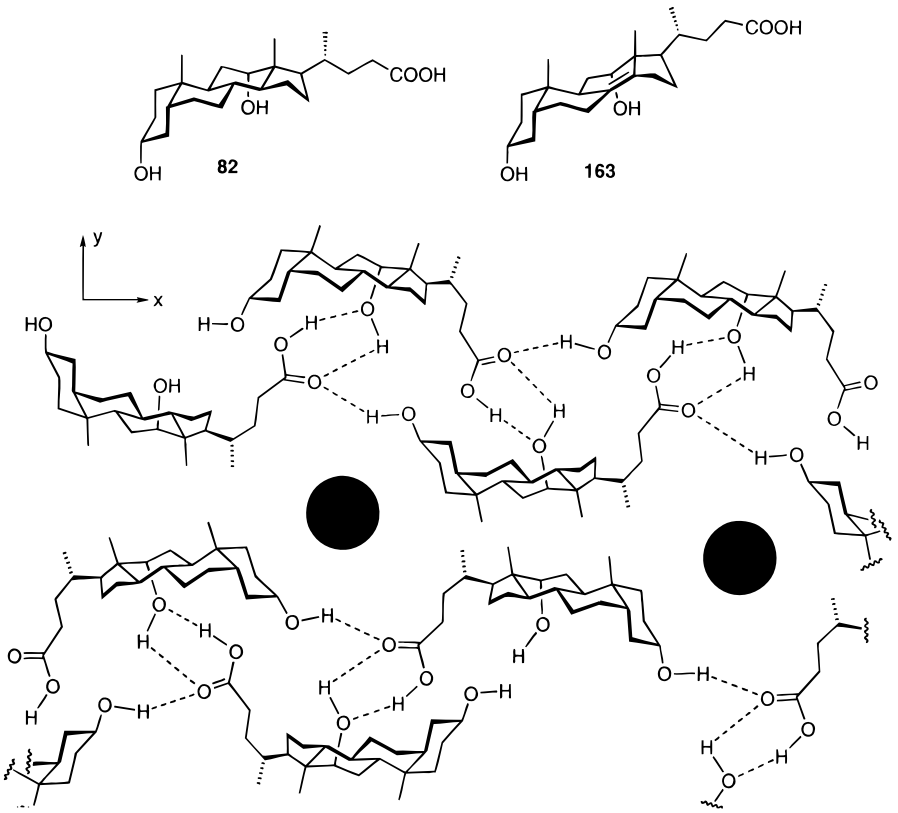


Figure 37. Deoxycholic acid (**82**) and apocholic acid (**163**) form clathrates with a variety of guests. Shown is schematically an orthorhombic structure viewed in the *x*,*y* plane. Guest molecules (black circles), such as acetophenone, are enclosed in infinite channels extending along the *z* axis. These channels are located between antiparallel bilayers formed by a head-to-tail arrangement of the host molecules. Also shown is the H-bonding pattern (dashed lines) between host molecules in one bilayer.

A substantial difference between the inclusion properties of deoxycholic acid (**82**) and cholic acid (**2**) is their differential ability to form clathrates with hydrocarbons such as 1,3-butadienes when recrystallized from ethanol. Whereas under such conditions deoxycholic acid forms a hydrocarbon clathrate, cholic acid clearly prefers to include the alcoholic solvent molecules. The packing in the latter inclusion complexes is also of the bilayer type, but since the arrangement of the host molecules is head-to-head, the bilayers are less densely packed and more flexible compared to deoxycholic acid. This might be a reason for the observed inclusion of either ethanol or acetophenone in the cholic acid lattice, which was clearly demonstrated by X-ray diffraction.[367,368] Since the crystal structures of cholic acid and its ethanol and acetophenone clathrates were known, the formation of binary complexes by crystal-to-crystal transformation starting from pure host crystals in a guest solution could be shown by X-ray diffraction and SEM (scanning electron micrography) in addition to IR spectroscopy. Although the structures of the lattices changed during the solid-state transformation from pure cholic acid to the binary complexes, the crystallinity of the latter was fully maintained.

Not much is known about the inclusion complex forming abilities of other steroids. The X-ray crystal structures of cholesterol hydrate and the clathrates with ethanol and other guests have been described.[369] Also, formation and characterization of clathrates between sitosterol (**164**), stigmasterol (**165**), and ergosterol (**166**) and various guests were reported. Because these steroids only possess one HO group, they cannot form H-bonding networks. These apolar compounds arrange in bilayers but do not undergo channel formation.

164, Sitosterol

165, Stigmasterol

166, Ergosterol

Since deoxycholic acid clathrates were known for a long time, several applications have been investigated. Sobotka and Goldberg[370] found that enantioselective enclathration by deoxycholic acid (**82**) allowed the resolution of racemates. Deoxycholic acid was also successfully tested for dissolving other steroids like testosterone or cortisone acetate which are poorly soluble in water.[371] In the pH range of gastric acids, the inclusion complexes formed by **82** and the insoluble steroids are stable, but under

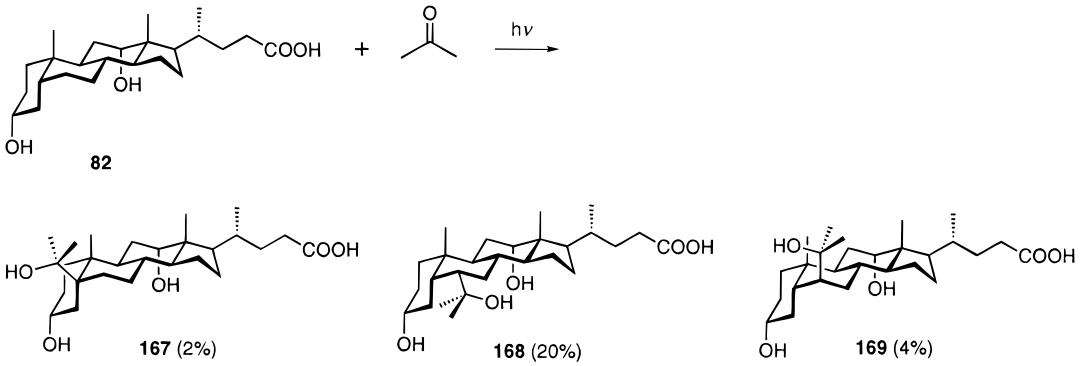


Figure 38. The topochemically controlled photoaddition in the clathrate formed by deoxycholic acid (**82**) and acetone yields three diastereoisomeric products due to three different positions taken by the enclathrated acetone molecules.

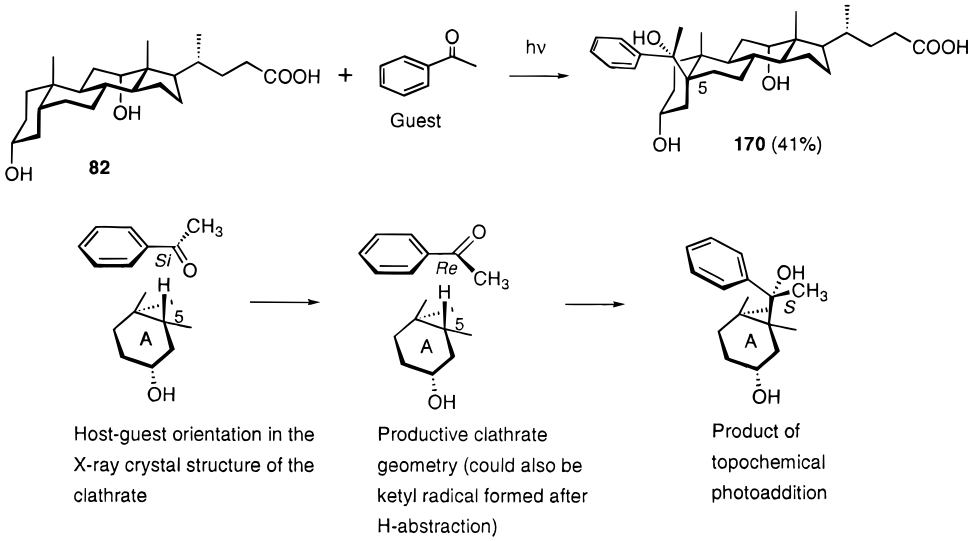


Figure 39. Photoaddition of acetophenone enclathrated by deoxycholic acid (**82**) yields one single diastereoisomeric product resulting from attack at the *Si* face of the ketone, which requires a rotation 180° of the acetyl group from its original position seen in the X-ray crystal structure of the inclusion complex.

neutral or slightly basic conditions, the guests are released.[372,373] Schlenk *et al.*[374] showed that clathrate formation by deoxycholic acid-protected air-sensitive guests, such as linolenic acid or vitamin A palmitate from autoxidation.

Crystalline inclusion complexes, in which the guests occupy well-defined positions in the host lattice, enable highly specific, topochemical reactions, which mimic those of highly oriented substrates in enzymatic conversions. The group of Leiserowitz and Lahav at the Weizmann Institute tested deoxycholic (**82**) and apocholic acid (**163**) for this purpose. Upon irradiation, enclathrated acetone for instance was shown to regioselectively add to **82** via H abstraction and radical recombination (Figure 38).[365,366]

The deoxycholic acid−acetone clathrate, with a 10:6 stoichiometry, forms orthorhombic crystals, which are also seen in the other systems discussed below. Since it is possible to distinguish between three crystallographically different acetone molecules included in the lattice channels, three different diastereoisomeric products were obtained in the topochemical photoreaction, with the reaction occurring at positions $C(5)_\beta$ (**167**), $C(6)_\alpha$ (**168**), and $C(6)_\beta$ (**169**). The same reaction was carried out with apocholic acid (**163**) as host, but no product was formed. Apparently, the different enclathration of the guests in **163** compared to **82** is less favorable for the photoaddition to occur.[365,366]

Since the reaction in **82** proceeds with high regioselectivity, it was performed with acetophenone as guest in order to explore whether attack at the ketone group would lead to formation of an enantiomerically pure product (Figure 39). In the crystal of the channel-type inclusion complex between **82** and acetophenone with 5:2 host−guest stoichiometry, the equatorial proton H_{eq}-C(5) is located close to the *Si* face of the carbonyl group of the guest. Indeed, upon irradiation, photoaddition occurred at C(5) under formation of a single diastereoisomer (**170**). However, the product was derived from attack at the *Re* face of the ketone and the newly generated stereogenic center at C(5) of the steroid had the *S* configuration. This was explained by a net rotation by 180° of the acetyl group or the intermediate ketyl radical in the guest before addition to the host.[365,366,375]

An analogous photoaddition was also carried out with several propiophenones. In the reaction of **82** with α-chloropropiophenone, one product with *R* configuration at the new stereogenic steroid C(5) atom was obtained and only traces of the corresponding diastereoisomer with *S* configuration at C(5) were found. Photoaddition is thus possible with or without rotation of the acetyl group or the intermediate ketyl

radical depending on the steric host−guest interactions in the reaction site.[376,377]

Besides host−guest, guest−guest reactions such as polymerizations are also possible in channel-type inclusion complexes. After studying the inclusion polymerization of enclathrated 1,3-dienes in urea channel-type clathrates, analogous reactions were also tried with a variety of conjugated monomers in the channels of deoxycholic acid.[364,378] Suitable guest monomers for inclusion polymerization are buta-1,3-diene and derivatives such as 2,3-dimethylbuta-1,3-diene. Deoxycholic acid clathrates of these compounds were either obtained by cocrystallization or, more successfully, by guest exchange. Polymerization was initiated by γ-irradiation. Since these clathrates are stable up to 100 °C, the conversion could be further improved at elevated temperature. After completion of the polymerization reaction, the host crystals were dissolved and removed from the formed insoluble polymer. Monitoring the polymerization process by electron spin resonance (ESR) spectroscopy showed that the radical intermediates formed had long lifetimes, because enclathration protects them from bimolecular reaction except with the neighboring monomers in the crystal. In general, the shape of the synthesized polymers only depends on the monomer packing and alignment in the clathrate crystal and not on the reactivity of the monomers. Thus, inclusion polymerization of 2,3-dimethylbuta-1,3-diene only provided the 1,4-*trans* product. Larger monomers like 2,4-dimethylpenta-1,3-diene are less suitable for inclusion polymerization in deoxycholic acid (**82**) compared to apocholic acid (**163**), because the channel size is larger in the latter steroid host lattice.[364] Acetylenes can also be polymerized in channel inclusion complexes formed with **82** and **163**.[364] Asymmetric polymerization was also tested, but enantiomeric excesses were rather low.[364]

D. Conclusions

The diverse content of section V on steroid-based receptors in molecular recognition reflects the rich variety of functions that steroids may adopt in biotic and abiotic systems. A combination of interesting properties has already led to a rich application of steroids as receptors or as building blocks for systems that bind and/or catalyze. These properties are the following: (i) A wide range of functionalized steroids are naturally and commercially available, and further functional group transformations have been worked out over the past decades. (ii) With their rigid tetracyclic frames, steroidal building blocks provide a high degree of preorganization to a recognition site. (iii) Many steroids, in particular the bile acids, can be viewed as highly functionalized amphiphilic surfaces with a lipophilic β-face and a more polar α-face bearing functional groups that are converging in clefts or macrocyclic receptor frames for interaction with a bound substrate. Many of the steroid-based macrocyclic receptors, such as the cholaphanes or cyclocholates take advantage of the distinct amphiphilic character of the rigid steroidal frame: The lipophilic phase is oriented outward into organic solutions and the functional groups converge into the macrocyclic cavity and interact with suitable substrates via H-bonding interactions. (iv) The functional groups on the α-face can be transformed into more expanded recognition elements such as the oligopeptides in the combinatorial receptor libraries prepared by Still and co-workers. These receptors possess an overall U-type shape with the side chain at C(17) enabling facile immobilization on solid support. (v) With their rigid expanded shapes, cholic acid derivatives, in particular, form defined channel-type crystal lattices in which a variety of substrates are included under clathrate formation. These channels have not only been exploited for solid-state recognition but also as reaction vessels that provide topochemical control to conversions of included substrates. (vi) Finally, steroid-based receptors further benefit from the inherent chirality of the steroids, which, combined with conformational homogeneity, leads to high enantioselectivity in many binding processes studied in solution and in the solid state.

VI. Perspectives

This review clearly documents that interest in the molecular recognition properties of steroids is growing strongly and rapidly. A combination of unique structural and functional properties makes them ideal building blocks for construction of molecular receptors (section V). Following several decades during which cyclodextrins were the only receptors available for steroid complexation in the liquid phase (section III), powerful synthetic hosts are now emerging that show high selectivity in binding steroids in aqueous solution (section IV). Together with the increasing structural information obtained from X-ray investigations on biological steroid complexes (section II), the studies with artificial receptors are rapidly enhancing our understanding of the principles governing selective molecular recognition of steroids. Further insight into steroid interaction modes is being gained from investigations with steroid-based receptors (section V). Besides this fundamental interest in steroid molecular recognition principles, pharmaceutical applications of steroid binding and solubilization by synthetic hosts are emerging. Steroidal drug stabilization, enhanced steroid transport and delivery, and improved formulations and oral administrations could result as benefits from the development of these artificial binders. Receptors specific for cholesterol may offer an alternative pharmacological strategy for the dissolution of cholesterol deposits such as those in atherosclerotic plaque or in gallstones. Alternatively, they could provide a way of altering the partitioning of cholesterol between the various mammalial supramolecular transport and delivery systems consisting of lipoproteins. An even more fascinating perspective is the possibility to interfere in a rational way with the binding of steroid hormones to their natural receptors and therefore to interfere with steroid hormone-mediated signal transduction processes. The rational development of steroid mimetics could lead to compounds which permit one to selectively enhance or reduce the association of natural receptors to the hormone response elements of the target gene. Finally, steroid analytics, sensorics, and

separations will continue to benefit from the developments reported in this review.[379,380]

VII. Acknowledgments

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