Imprinted Polymers as Protecting Groups for Regioselective Modification of Polyfunctional Substrates

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Abstract: Imprinted polymers were prepared using a functional monomer derived from boronophthalide and a number of steroid templates bearing spatially separated hydroxyl groups. The cooperative nature of the binding interaction was demonstrated in polymers imprinted with androst-5-ene-3β,17β-diol and its structural analogues. The stoichiometry and kinetics of binding were probed using IR spectroscopy, selective solvent extractions, and chemical modification experiments. The feasibility of using imprinted polymers as reusable protecting groups was established by the regioselective acylation of trihydroxysteroids bound to polymers imprinted with structurally related diols. In polymers prepared with *tert*-butyl ester templates "matched" to the substrate, regioselectivities as high as 23.1:1 (24-acetate:3-acetate) in the monoester products (65% of recovered material) were seen. In the "unmatched" case, the ratio fell to 5.4:1; however, in functionally identical control polymers, imprinted with ethylene glycol, the regioselectivity was completely reversed (<1:100), and only poor yields of monoesters (13%) were obtained.

Introduction

Molecular imprinting is a methodology for the introduction of selective recognition sites into highly cross-linked polymeric matrixes via template-directed assembly of functionalized monomers in a polymer-forming mixture. This enables the positioning of complementary functionality in discrete cavities in the polymer, with the precise spatial arrangement of functional groups to provide specific interactions with the template on rebinding.[1] Over the past few years, imprinting has been successfully applied to the preparation of polymers with selectivity to a wide range of natural products such as amino acids[2] and short peptides,[3] monosaccharides and their derivatives,[4] nucleotide bases,[5,6] and steroids,[7,8] as well as numerous drugs[9] and pollutants.[10] Most of the research has focused on the synthesis of polymers capable of resolving racemic mixtures,[11] but other applications, e.g., in solid-phase extraction,[12] imprinted polymer-supported reagents,[13] catalysis,[14] sensors,[15] removal of undesirable components from complex mixtures,[16]

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controlling crystallization,[17] and separation and concentration of proteins[18] and microorganisms,[19] have also been reported.

The formation of a template−monomer complex in the polymerization mixture can be accomplished either by covalent attachment of monomers to the template using appropriate chemistry or by noncovalent complexation in situ. However, regardless of the methodology employed, the selectivity of the resulting materials is dependent on a number of contributing factors, i.e., interactions between template/ligand and polymer-forming components,[20] the density and rigidity of the polymer matrix as determined by the degree and chemical nature of cross-linkers,[21] and perhaps most importantly, the interaction between functional groups in the polymer's recognition site with those of the ligand. Indeed, in most cases it is charge interactions, strong hydrogen bonding and coordination with metals, and the formation of a labile covalent bond that are the main contributors to the overall energy of binding. Consequently, the polymer's ability to discriminate between the target and its structural analogues is largely determined by the cumulative effect of individual interactions in terms of free energies or, in other words, by the cooperativity between functional groups in the recognition site.

Cooperative interactions, especially in covalently imprinted polymers, have been extensively investigated, notably by the groups of Shea and Wulff and co-workers (see reviews[1b,d]). It has been conclusively shown that the specificity of imprinted sites is increased dramatically when two functional groups rather than one are introduced into the polymer's recognition site, in terms of the ability of polymers imprinted with a single enantiomer to resolve racemic mixtures in chromatography.[22] The effects of additional functional groups in the template molecule[23] and the structures of polymerizable boronic acids[24] on the selectivity of binding have also been studied in some detail. Furthermore, Shea and Sasaki[25] have established, in a comprehensive FT-IR and solid-state NMR study, that rebinding of bifunctional ligands leads to the formation of two covalent bonds in a high proportion of bifunctional sites, as might be expected considering the chemistry initially employed to prepare the imprinted polymers. Although some unresolved issues with regard to the kinetics of binding, e.g., the relative rates of reaction between the first and the second pairs of functional groups in the imprinted sites, still remain, it is generally accepted that the binding of ligands to imprinted polymers is driven by the reconstitution of the same multiple interactions in the sites which were engaged in the assembly of template−polymer complexes. However, if this is the case, then it should be feasible to produce imprinted polymers capable of "holding" the ligand in place by interacting with some of the functional groups while leaving other groups on the ligand accessible for selective chemical modification. This approach, where imprinted polymers are effectively used as protecting groups for regioselective synthesis, is rather different from other "synthetic" applications reported for imprinted polymers, for example their use as catalysts,[14] supported reagents,[13] and "microreactors" for chiral synthesis.[26,27]

In this paper we describe the interaction of a series of homologous sterols with their respective imprinted polymers containing one or two boronic acid residues in the recognition sites. The covalent method of imprinting was chosen as the most suitable for the purpose of this investigation, as it does not require functional monomers to be present in the polymerization mixture in excess. This was important for achieving the incorporation of functionality exclusively into the recognition sites to enhance the selectivity in chemical modification experiments on polymer-bound ligands. Sterols were selected as model templates on account of their biomedical importance[28] and because numerous structural analogues are commercially available and/or can be synthesized according to published procedures. Also, the regioselective modification of individual hydroxyl groups in sterols relies on the extensive use of protecting groups and is notoriously difficult to accomplish. Hence, it was an ideal model system for testing the utility of imprinted materials as protecting groups in polymer-directed regioselective synthesis.

Results and Discussion

For the purpose of this study, two methods for imprinting compounds such as sterols with single or spatially separated hydroxyl groups were suitable.[29] One relies on the formation

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(29) The noncovalent strategy has been used successfully for imprinting steroids (see refs 7 and 8). However, it was not suitable for our investigation owing to the necessity of working with an excess of functional monomer, which would inevitably lead to the incorporation of some functional groups "outside" the imprints, thus complicating any investigation of cooperative interactions in the recognition sites.

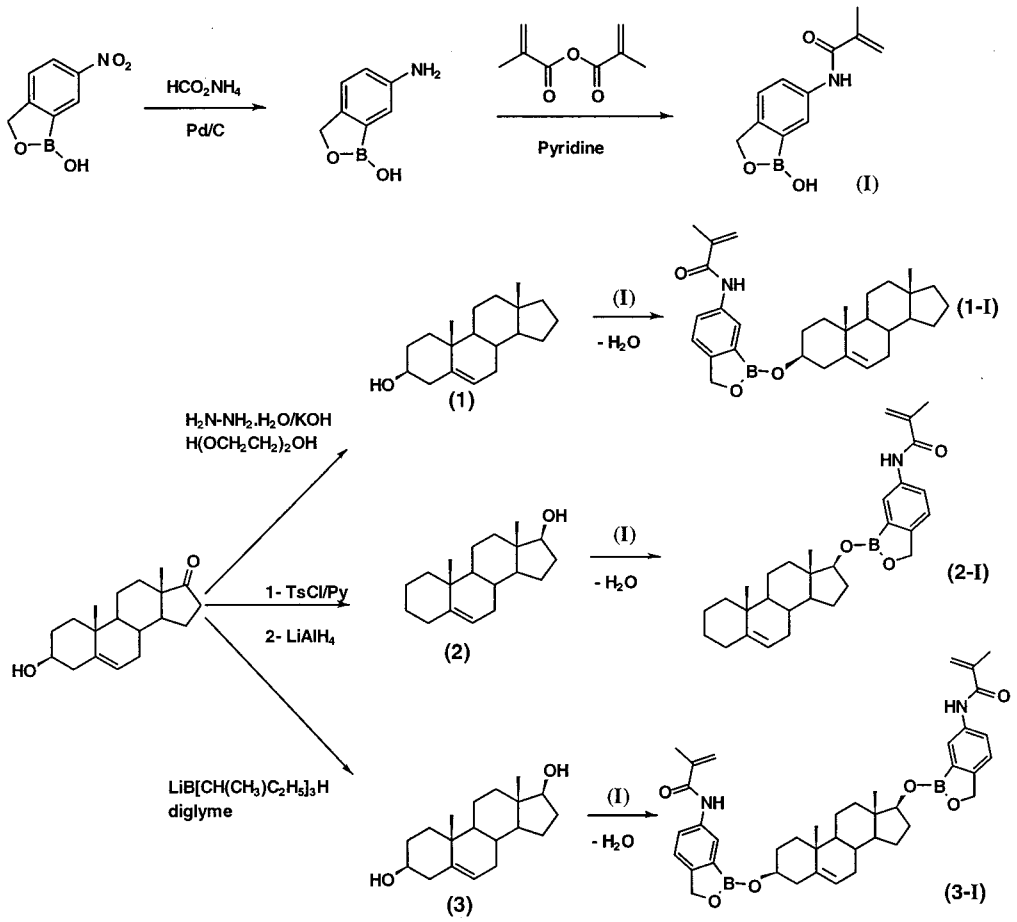

Figure 1. Synthesis of polymerizable sterol-boronophthalide complexes.

of labile boronate esters; this was introduced and extensively studied with glycoside templates by Wulff and co-workers.[21,22,30] The other is the sacrificial spacer approach developed in our laboratory.[31] Although both allow the introduction of functional groups exclusively into the polymer's recognition sites, the former methodology was selected as much stronger binding of ligands, in absolute terms, was expected in this case (more energy is required for breaking the boronate ester bond compared to a single hydrogen bond). This was perceived to be an advantage in the context of this investigation over noncovalent and sacrificial spacer methods because relatively little "leakage" of polymer-bound ligands was expected during chemical modification experiments. Boronophthalide (**I**) was employed as a functional monomer rather than the more commonly used 4-vinylphenyl boronic acid in order to ensure that each polymerizable moiety could bind to only a single hydroxyl group. This monomer was synthesized in two steps from 5-nitroboronophthalide[32] (Figure 1), using a variant of the method of Dederichs.[33] Initial experiments were performed on a set of polymers imprinted with androst-5-ene derivatives **1**−**3** bearing either a single hydroxyl group at the 3β or 17β positions (**1** and **2**, respectively) or hydroxy substituents at both positions (**3**), as these compounds were easily accessible from dehydroisoandrosterone. Boronate esters of each sterol with **I** were prepared and characterized by NMR spectroscopy;[34] all were highly moisture-sensitive solids and thus were prepared and polymerized in situ.[35]

The polymers **P1**, **P2**, and **P3** (Table 1) were prepared by thermal polymerization of boronate-sterol esters (**1-I**, **2-I**, and **3-I**, respectively) at template loadings of 2.5 mol % with divinylbenzene (DVB) as the cross-linker in chloroform. "Non-imprinted" polymers were synthesized using the mono- and bis-boronophthalide esters of methanol and ethyleneglycol (polymers **PNI-1** and **PNI-2**) rather than free **I** to overcome the low solubility of the latter in the polymerization mixture. After polymerization, the materials were ground to a fine powder,[36] and the template was removed by Soxhlet extraction with aqueous ethanol. The polymers were dried and shaken with sterol solutions in the presence of CaH_2, used as a mild drying agent,[37] to assess their binding properties.

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(34) A preliminary ^{1}H NMR assignment of these complexes has been published (Smith, C. R.; Whitcombe, M. J.; Vulfson, E. N. In *Separations for Biotechnology 3*; Pyle, D. L., Ed.: Royal Society of Chemistry: Cambridge, UK, 1994; pp 482−488).

(35) ^{1}H NMR spectra of the polymerization mixture in $CDCl_3$, monitoring the 3α and 17α protons at 4.2 and 4.3 ppm, respectively, indicated that the complexes remained intact up to the point of gelation, when signal broadening rendered spectra difficult to interpret. IR spectra of the polymers prior to washing did not display free hydroxyl bands, further suggesting that the boronophthalide esters were not decomposed under the polymerization conditions.

(36) Ground polymers were not subject to any particle sizing. In view of the slow kinetics of binding and the fact that polymers were used at equilibrium, it was not considered necessary.

Table 1. Composition and Characterization Data for DVB-Based Polymers[a]

polymer	template	yield, %[b]	BET surface area, m^2/g	template recovery, %	boronophthalide groups,[d] μmol/g
P1	**1**	93	27	92	168
P2	**2**	94	35	95	173
P3	**3**	91	16	90	330
P4	**7**	89	212	91	329
P5	**8**	94	240	90	328
PNI-1	MeOH	93	5	nd[c]	182[e]
PNI-2	$HOCH_2CH_2OH$	85	111	nd[c]	362[e]

[a] Polymers were prepared with 55% technical grade divinylbenzene at 2.5 mol % template loading and polymerized thermally at 60 °C (see Experimental Section for details). [b] Yield of polymer after drying, before template removal. [c] Not determined. [d] Calculated from template recovery. [e] Calculated assuming all template was removed.

Extensive binding studies with all the combinations of templates and polymers yielded several conclusions. First, polymers imprinted with androst-5-ene-3β-ol (**1**) and androst-5-ene-17β-ol (**2**), when challenged with these two ligands, showed little discrimination (less than 10%) in binding. Similar results were obtained with polymers synthesized with different cross-linking monomers[38] (EDGMA and trimethylolpropane-trimethacrylate (TMPTMA)) and at template loadings from 2 to 5 mol %. Second, less than 2% binding of sterols was detected with control polymers prepared in DVB and the methacrylates (EGDMA and TMPTMA) in the absence of boronophthalide. This experiment proved that the binding observed was due to interaction between the boronophthalide moiety and the hydroxyl groups of the ligand. A further comparison between polymers imprinted with boronophthalide esters of **1**, **2**, and a short-chain primary alcohol (**PNI-1)** was carried out. Imprinted polymers (**P1** and **P2**) showed 14% and 12% higher uptake, respectively, of the sterols compared to **PNI-1**, suggesting that, at least in this case, the binding of sterols by only one point was insufficient to engender significant specificity.

We then turned to the polymer imprinted with androst-5-ene-3β,17β-diol (**P3**) and investigated the binding of template **3** as well as that of ligands **1** and **2**. In accordance with earlier studies,[39] the introduction of the second functional group in the recognition site of the imprinted polymer had a very pronounced effect on the selectivity of binding. It is evident from Figure 2a that the uptake of **3** was much better than that of **1** and **2** (85% uptake compared to 40−50%), clearly suggesting a high degree of cooperativity between the boronophthalide residues in the site. The same pattern of binding was observed in chloroform and two other solvents, THF and ethyl acetate.[40] Furthermore, the uptake of the diol **3** to polymers imprinted with **1** and **2** and to a polymer "imprinted" with ethyleneglycol (**PNI-2**) was far inferior (up to 50% reduction in binding; see Figure 2b), which is significant as the ethyleneglycol-imprinted polymer

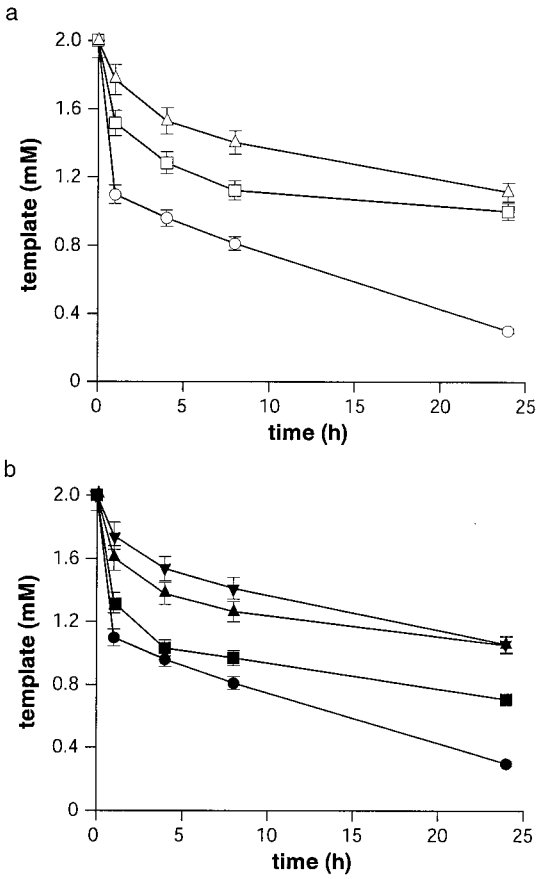

Figure 2. Uptake of sterols by imprinted polymers. (a) Uptake of androst-5-ene-3β,17β-diol (○), androst-5-ene-3β-ol (□), and androst-5-ene-17β-ol (△) by androst-5-ene-3β,17β-diol-imprinted polymer in $CHCl_3$. (b**)** Uptake of androst-5-ene-3β,17β-diol by androst-5-ene-3β,-17β-diol-imprinted (●), androst-5-ene-3β-ol-imprinted (■), androst-5-ene-17β-ol-imprinted (▲), and ethyleneglycol-imprinted (▼) polymers in $CHCl_3$.

was prepared with exactly the same template loading as **P3** and differed only in the spatial distribution of boronophthalide functionality in the matrix. Data shown are for DVB polymers **P3** and **PNI-2**, **P1**, **P2**, and **P3** at 2.5 mol % template loading, but similar results were also obtained at template loadings of 5 mol % and in EGDMA and TRIM polymers.[41]

Inevitably, some binding of **3** to nonimprinted boronophthalide-containing polymers **PNI-1** and **PNI-2** was observed in all the experiments because, regardless of the distribution of **I**, the formation of ester bonds between steroid hydroxyls and boronophthalide residues should have occurred whenever the groups could come into close enough proximity to react. It appeared, therefore, that a cooperative interaction between at least two functional groups in the polymer sites was required for specific binding, with the "pocket" itself playing a lesser

(37) In the absence of CaH_2, uptake of templates was exceedingly slow. No binding of sterol to drying agent alone was observed.

(38) Previous reports (Wulff, G.; Vietmeier, J.; Poll, H. G. *Makromol. Chem.* **1987**, *188***,** 731−740) have suggested that EGDMA-based imprinted polymers were superior in performance to those made with DVB, due possibly to the greater flexibility of the polymer chains, thus allowing facile removal and rebinding of templates. However, in this work, no problems were encountered in template removal with DVB polymers, which was essentially quantitative in all cases, and in uptake experiments these polymers performed as well as those prepared with EGDMA.

(39) The improvement in selectivity engendered by the introduction of a second binding group in the imprinted site has been well-documented by Wulff, (see ref 1d, pp 1819 and references therein).

(40) The best uptake of templates was shown in the solvent used for polymerization, chloroform, which accords well with previous observations (Spivak, D.; Gilmore, M. A.; Shea, K. J. *J. Am. Chem. Soc*. **1997**, *119*, 4388−4393), suggesting that the imprinted site returns to its original conformation only if swollen by the correct solvent. Nevertheless, some binding of templates was observed in tetrahydrofuran and ethyl acetate, with the pattern of sterol binding being rather similar to that seen in chloroform.

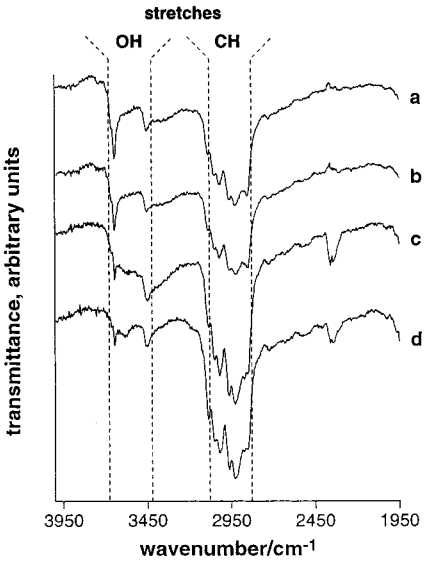

Figure 3. Infrared spectra of androst-5-ene-3β,17β-diol (**3**) and polymer **P3** (a) before reflux, (b) after 1 h reflux, (c) after 24 h reflux, and (d) after 36 h reflux

role in recognition. It should also be stressed that it was possible to achieve virtually quantitative binding (over 90% mol equiv relative to boronophthalide) of the sterols to imprinted polymers by carrying out the experiments under more vigorous dehydrating conditions, to favor a shift of the equilibrium toward esterified products. However, the results of these latter experiments showed that the binding of the sterols to the polymers was surprisingly slow, compared to the rate of the same boronophthalide−sterol reactions in solution. Was this because of hindered access to recognition sites within the matrix or due to some unanticipated chemical reason? To shed some light on the actual mechanism, the binding in DVB-based polymers was studied by FT-IR (Figure 3), following hydroxyl absorptions between 3400 and 3650 cm^{-1}.

Examination of diagnostic OH stretch absorptions at 3644 and 3450 cm^{-1} (due to the steroid and boronophthalide hydroxyl moieties) for imprinted polymers loaded with ligands after 1 and 36 h under reflux (>90% uptake in both cases) revealed a significant difference between the two samples: a large proportion of hydroxyl groups still remained free after 1 h, despite the presence of the ligand in the active site. Even after prolonged reflux (36 h), with azeotropic removal of water via CaH_2 in a Soxhlet thimble suspended above the solution, complete suppression of the hydroxyl absorption was not achieved, in contrast to solution-state reactions between boronophthalides and the respective sterols, which were complete as indicated by ^{1}H NMR within 30 min at reflux.[42] This behavior, in terms of rebinding at both points in a bifunctional imprint site, has certain similarities to the results of Shea and Sasaki, who observed low reaction rates for bis-ketalization in binding sites even of relatively small diketone templates.[43] However, attempts to accelerate boronate ester formation by adding electron donors such as pyridine and methylpiperidine failed to effect any improvement. Thus, although the initial binding of the sterol to the polymer was rapid, covalent template−polymer bonds were formed extremely slowly.[44]

To provide further evidence for slow formation of covalent bonds in the polymer binding sites, a series of "washing" experiments was carried out. We reasoned that, if the sterols were not bound in the sites covalently, it would be possible to remove them by washing the polymer with an appropriate anhydrous aprotic solvent. Subsequent washes with a hydroxylic solvent such as methanol would then release the covalently bound fraction of the ligand, thus allowing quantitative comparison. Indeed, it was found that only 50% of androst-5-ene-3β,17β-diol (**3**) was bound covalently after 1 h reflux in chloroform (>90% overall binding), and this increased to 80% after 36 h. Qualitatively similar results (30% covalently bound after 1 h, 70% after 36 h) were obtained with mono-alcohols **1** and **2**, although the proportion of covalently bound ligand was somewhat lower. This was to be expected because the formation of just one ester bond in the case of **3** would be sufficient to prevent the release of this ligand by an aprotic solvent wash.

To verify further the formation of covalent bond(s) at the recognition site, chemical modification of the polymer-bound sterol was carried out. Clearly, no hydroxyl groups would be free to react if cooperative covalent binding of the androst-5-ene-3β,17β-diol (**3**) in the recognition site of the polymer occurred. In contrast, binding of diol **3** to a mono-alcohol -imprinted polymer should result in boronate ester formation at one end of the molecule, with the remaining hydroxyl accessible to derivatization. Accordingly, **3** was bound to polymers **P1**, **P2**, and **P3** (imprinted with androst-5-ene-3β-ol, androst-5-ene-17β-ol, and androst-5-ene-3β,17β-diol, respectively), with stoichiometries chosen such that the excess of steroid hydroxyl relative to boronophthalide was the same in each case. The sterol was reacted with the polymer for at least 72 h under reflux to ensure sufficient time for the formation of covalent bonds (sterol concentration in solution and hydroxyl IR absorption on the polymers were monitored until no further change occurred), and exhaustive extraction with an aprotic solvent was carried out to remove unbound **3**. The polymer−sterol complex was then refluxed with a large excess of acetic anhydride and pyridine.[45] After a second extraction stage to remove excess reagents, the polymer was washed with THF/

(41) The increase in levels of template loading from 2.5 and 5 mol % for DVB polymers (and for 2−5 mol % imprinted polymers prepared from EGDMA and TRIM) did not lead to a difference in the selectivity of ligand binding, as might be anticipated if the sites became less isolated. Clearly, if the sites were not sufficiently isolated, the difunctional template **3** might "bridge" boronophthalide residues from adjacent sites, and the level of uptake would have decreased relative to that of **1** and **2**. In addition, if the sites were "poorly defined", reaction of excess monoalcohol **1** and diol **3**, respectively, with androst-5-ene-3β,17β-diol-imprinted polymer should lead to substantially higher level of binding of **1** as compared to **3** (i.e., two one-point bindings of the mono-alcohol as opposed to one two-point binding of the diol). In fact, only 1 mol of androst-5-ene-3β-ol bound per bifunctional site, suggesting that binding of androst-5-ene-3β-ol in the cavity rendered the second residue of boronophthalide completely unreactive, presumably due to steric shielding: once one molecule was bound, a second could not enter the site.

(42) The formation of the boronophthalide-sterol esters was followed by IR and NMR: representative spectra for starting materials and the product of reaction between **3** and **I** are appended as Supporting Information.

(43) Shea and Sasaki, in their detailed study (ref 25) of acetal-forming reaction between benzylic alcohols and diketones, also observed that, despite extensive one-point binding taking place after 24 h in the imprinting sites, even after a further 24 h reaction time only 60% of the difunctional template was bound covalently at both positions. The authors suggested that this was largely due to a rate-limiting segmented chain motion of the polymer backbone supporting the binding groups: this reasoning should apply in our case, too.

(44) The presence of a residual broadened OH stretching band at 3550 cm^{-1}, even after 36 h reflux, may indicate that a number of sterol hydroxyl groups were attached to the polymer via hydrogen bonds rather than as esters: however, the low intensity of this band suggests that this number was small.

(45) Under the reactions conditions used (300-fold excess of acetic anhydride in pyridine/$CHCl_3$ at reflux), both the 3β and 17β hydroxyl groups were rapidly acylated in solution in the absence of polymer. The same acylation procedure carried out on polymer **P3** prior to hydrolysis (i.e., with androst-5-ene-3β,17β-diol remaining bound to the polymer) yielded only the unmodified diol in the protic wash fraction.

Table 2. Modification of Androst-5-ene-3β,17β-diol (**3**) on Imprinted Polymers

polymer	yield[a] of **3** (μmol)	yield[a] of mono-acetates (μmol)	ratio of diol:mono-acetate[b]
P1	9.0	3.2	2.8:1
P2	5.2	4.4	1.2:1
P3	20.7	0.6	34.5:1

[a] Yields are for products bound to polymers and recovered from the protic wash fraction. Results are averaged from at least duplicate measurements (typical variation $\pm$0.2 μmol). [b] Determined by GC analysis..

methanol/water, and the products were recovered by conventional workup. Although the major component was unchanged starting material **3** in all cases (Table 2), up to 40% mono-acetates[46] were observed in the products from **3** bound to polymers **P1** and **P2**. By contrast, less than 3% mono-acetate was recovered from the reaction products of diol **3** bound to polymer **P3**. This strongly supported the conclusion that most of the diol was covalently attached at both "ends" in the diol-imprinted polymer.[47] The two mono-acetates (identified by TLC and GC from authentic chemically prepared standards) were formed in similar quantities, indicating that discrimination between ends of the molecule was poor. The same pattern of products was observed independent of the solvent used for sterol loading or modification and for template loadings of 2.5−5 mol %.[48]

Having established that the two boronophthalide residues were acting cooperatively in the binding site of polymer **P3** and that covalent boronate ester bonds with the 3β and 17β hydroxyls of **3** were re-formed (albeit after long reaction times and drastic conditions), we proceeded to investigate the feasibility of using imprinted polymers as "protecting groups" for regioselective chemical modification.[49] To this end, the polymer imprinted with androst-5-ene-3β,17β-diol (**P3**) was reacted with the trihydroxysteroid androst-5-ene-3β,11β,17β-triol (**4**), and modification with acetic anhydride (*Figure 4*) was carried out as described above. Although the majority of product (90%) recovered in the protic solvent wash under these conditions was unmodified triol, NMR analysis suggested the formation of a small amount (5−10% by integration) of the 11-acetoxy derivative, as judged by a peak at 5.25 ppm in the ^{1}H NMR spectrum, assigned to the geminal 11α proton.[50] The low degree of modification of the 11β-hydroxyl group was perhaps not too surprising, given that it is strongly hindered by the angular methyl groups at C10 and C13 and was further shielded by the polymer backbone. However, the ratio of 11β-acetoxy to 3β-acetoxy products was at least 1:1 by integration, and no evidence for modification at the 17β position was obtained, whereas in solution the 3β,17β-diacetate was readily formed, while large excesses of reagents and long reaction times were required in order to achieve complete acylation of all three hydroxyl groups on **4**.[51] It was concluded that the low reactivity of the target group, combined perhaps with poor accessibility of imprinted sites to relatively bulky acetic anhydride/pyridine complexes, was responsible for the poor yield. As **4** was the only trihydroxyandrost-5-ene available and the quantities were insufficient for a more detailed investigation,[52] we decided to adopt another sterol-based system, with the particular aim of addressing the issue of reagent accessibility.

Owing to the difficulties inherent in modification of many sterols (and their high costs), we employed more readily available and functionally amenable hydroxy steroids in further investigations. The isomeric bile compounds deoxycholic acid (**5**) and chenodeoxycholic acid (**6**) were chosen for their accessible chemistry and availability in gram quantities. To imprint these sterols, the carboxylic acid groups were first converted to *tert*-butyl esters (**7** and **8**) and (via the triols **9** and **10**) the trityl ethers (**11** and **12**), to prevent the formation of mixed boronic anhydrides with **I** during the imprinting stage and, at the same time, to create an extra pocket to facilitate access by acylating reagents (Figure 5).

Polymers were prepared with both *tert*-butyldeoxycholate- and *tert*-butylchenodeoxycholate-bis(boronophthalide) esters[53] in chloroform at template loadings of 2.5 and 5 mol %. The cross-linkers used were DVB (both 80% and 55% tech grades), EGDMA, and TMPTMA. Almost quantitative (>90%) template removal was achieved for *tert*-butyl esters from DVB 55 polymers, but the recovery fell to 70−80% for polymers prepared at higher cross-linking density, as well as for those imprinted with bulkier trityl ether derivatives. In the experiments described below, DVB 55 materials polymerized at 2.5 mol % template loading were used (polymers **P4** and **P5**, Table 1), although qualitatively the same results were obtained for modifications carried out on EGDMA- and TMPTMA-based polymers.

For the modification experiments, isomeric trihydroxysterols were prepared by reduction of the acid functionality of deoxycholic acid and chenodeoxycholic acid using $LiAlH_4$ in THF, which generated the corresponding deoxycholan-24-ol (**9**) and chenodeoxycholan-24-ol (**10**). The triols were refluxed with imprinted polymers in chloroform under dehydrating conditions as carried out for the androstene templates. After removal of unbound template by a chloroform wash,[54] the attached sterols were reacted with excess acetic anhydride and pyridine at reflux[55] and washed successively with aprotic and hydroxylic solvents as before. Two sets of reactions were carried out: modifications of deoxycholan-24-ol (**9**) or chenodeoxycholan-24-ol (**10**) attached to *tert*-butyldeoxycholate-imprinted polymer

(46) Although the two isomeric mono-acetates were resolvable by TLC, GC analysis under a range of conditions failed to effect baseline separation.

(47) As a control, monomer−template complexes **1-I**, **2-I**, and **3-I** were prepared in $CDCl_3$ and subjected to the same acetylating conditions as **3** bound to polymers **P1**, **P2**, and **P3**. ^{1}H NMR (monitoring the 3α and 17α protons at 4.2 and 4.3 ppm, respectively) indicated that the complexes did not break down under these conditions, and no acetylation of the steroid hydroxyl groups took place while esterified with boronophthalide.

(48) This provided further evidence for site isolation: reaction of androst-5-ene-3β,17β-diol (added in excess) with **P3** (250 mg, theoretical boronophthalide content 366 μmol/g) led to the binding of 46 $\pm$ 2 μmol (188 $\pm$ 8 μmol/g) of sterol, corresponding to 100−104% occupation of available binding sites (based on theoretical content and template removal, respectively) or 0.51−0.52 mol of sterol/mol of available boronophthalide group rather than the theoretical value of 0.5. If this was a "coincidence" with the ligand forming "random" covalent bonds in bridging sites, acylation of the polymer bound diol should have yielded a significant amount of monoacetates, rather than the 3% observed.

(49) For a discussion of polymeric supports used as protecting groups, see: Hodge, P. In *Syntheses and Separations Using Functional Polymers*; Sherrington, D. C., Hodge, P., Eds.; John Wiley & Sons: Chichester, 1988; pp 43−122.

(50) No previous NMR data were available for this compound; however, the 11α-proton in a structurally similar 11-acetoxyandrost-5-ene sterol was reported with a chemical shift of 5.2−5.3 ppm (Moon, S.; Stuhmiller, L. M.; Chadha, R. K.; McMorris, T. C. *Tetrahedron* **1990**, *46*, 2287−2306).

(51) Conditions for acylation were 300-fold excess of acetic anhydride in pyridine/$CHCl_3$ at reflux for 4 h.

(52) Androst-5-ene-3β,11β,17β-triol was formerly obtainable in milligram quantities from Sigma, but production was discontinued before the end of the project.

(53) Both compounds were prepared and polymerized in situ to prevent hydrolysis. ^{1}H NMR spectroscopy, following proton resonances at C3β (4.5 ppm) and C7β (4.8 ppm) or C12β (4.7 ppm), verified the complete formation of the complexes.

(54) The removal of adsorbed template in this way ensured that the product distribution reflected only modifications of templates bound at or in the sites.

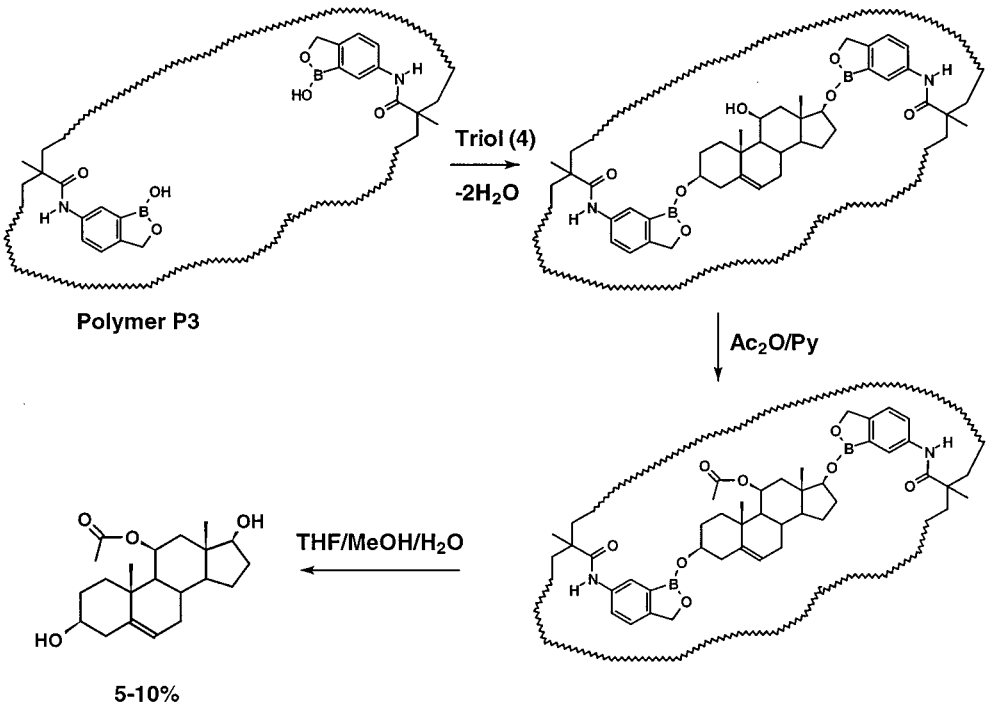

Figure 4. Modification of androst-5-ene-3β,11β,17β-triol (**4**) on polymer **P3**.

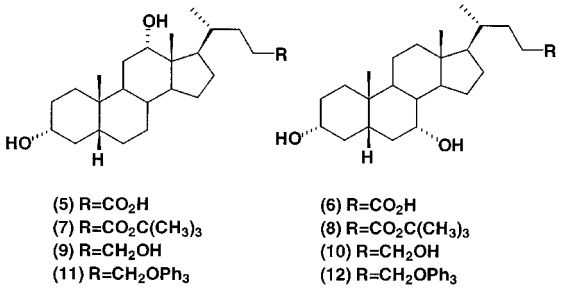

Figure 5. Structures of bile salt derivatives.

P4, and chenodeoxycholan-24-ol (**10**) or deoxycholan-24-ol (**9**) bound to *tert*-butylchenodeoxycholate-imprinted polymer **P5**. In this way, the polymer−sterol complexes were classed as "matched" or "unmatched", dependent on whether the template was of the correct theoretical fit to the polymer site, as illustrated in Figure 6 for *tert*-butyldeoxycholate-imprinted polymer **P4**.

It is evident from Figure 6 that binding of a triol in the "correct" mode in the matched sites should lead to the formation of two ester bonds between the boronophthalide residues and hydroxyl groups at C3 and C12 (or C3 and C7 for the case of polymer **P5**). This would leave the primary 24-hydroxyl group available for modification. However, binding of the sterol to the unmatched site can only result in the formation of a single ester bond, most likely at the more reactive 3- or 24-positions.[56] Thus, if the binding in the matched sites was 100% correct, modification with acetic anhydride would give a single product, i.e., the 24-acetoxysterol. Similarly, one should expect the formation of a mixture of monoesters[57] in the unmatched sites.

In accordance with this prediction, we found (Table 3) that the major component (up to 95% of acetylated products; >50% of total) recovered by the protic solvent wash from the matched sites for both trihydroxysterols was the 24-acetoxy derivative (any 24-acetoxy derivatives formed in solution or not bound covalently to the polymer would have been removed in the aprotic solvent wash, and therefore should have been discarded prior to analysis). This confirmed that most of the ligand was, indeed, bound in the "correct" orientation. However, a significant amount of unreacted material (40−45%) was also recovered, which indicated poor access of acylpyridinium intermediates once a molecule of sterol was in place. The use of bulkier trityl ethers as templates or alternative methacrylate cross-linkers did not lead to noticeable improvements in yields. The fact that acetylation took place predominantly at C24 in the matched cases suggested that an efficient "molecular sorting" must have taken place in the imprinted sites during template loading, because one might expect the 24-hydroxyl moiety to be the first to react with boronophthalide residues, as it is a primary hydroxyl on a flexible chain. This would lead to a kinetic preference for the "wrong" orientation. However, the correct mode of binding should be thermodynamically more stable due to the formation of two ester bonds as opposed to one, and the regioselectivity of modification provided evidence for this manner of binding. The relative proportions of 3-acetoxy products were also indicative of a better fit in the "matched" sites. For both sets of templates, the ratio of 24-acetoxy to 3-acetoxy products was much lower (4:1 rather than 20:1) in the "unmatched" sterol−polymer complexes, indicating that, where the template could not fit exactly, it bound via the most reactive, i.e., the 24-hydroxyl group, leaving secondary hydroxyls accessible for modification. As a further test of this hypothesis, acetylation of deoxycholan-24-ol (**9**) bound to the ethyleneglycol-imprinted polymer **PNI-2** was carried out. In this

(55) Under the reactions conditions used (300-fold excess of acetic anhydride in pyridine/$CHCl_3$ at reflux), in solution in the absence of polymer all the steroid hydroxyl groups were acylated. Thus, any difference in the pattern of acetate products was due to the effect of the polymer imprint site, rather than that of the intrinsic reactivity of the sterols.

(56) The differences in the reactivity of the various hydroxyl groups on the cholic acid framework have been considered thoroughly elsewhere (Baker, J. F.; Blickenstaff, R. T. *J. Org. Chem.* **1975**, *40*, 1579) (and by Bonar-Law et al. in ref 57); however, in this case, binding of the sterol to the polymer was more likely to occur via the reactive primary hydroxyl at C24 rather than the secondary hydroxyl groups at C3, C7, and C12.

(57) The formation of diesters might also be expected for "unmatched" template−polymer complexes, but none were observed, due possibly to the difficulties in fitting two acetate groups into the sites.

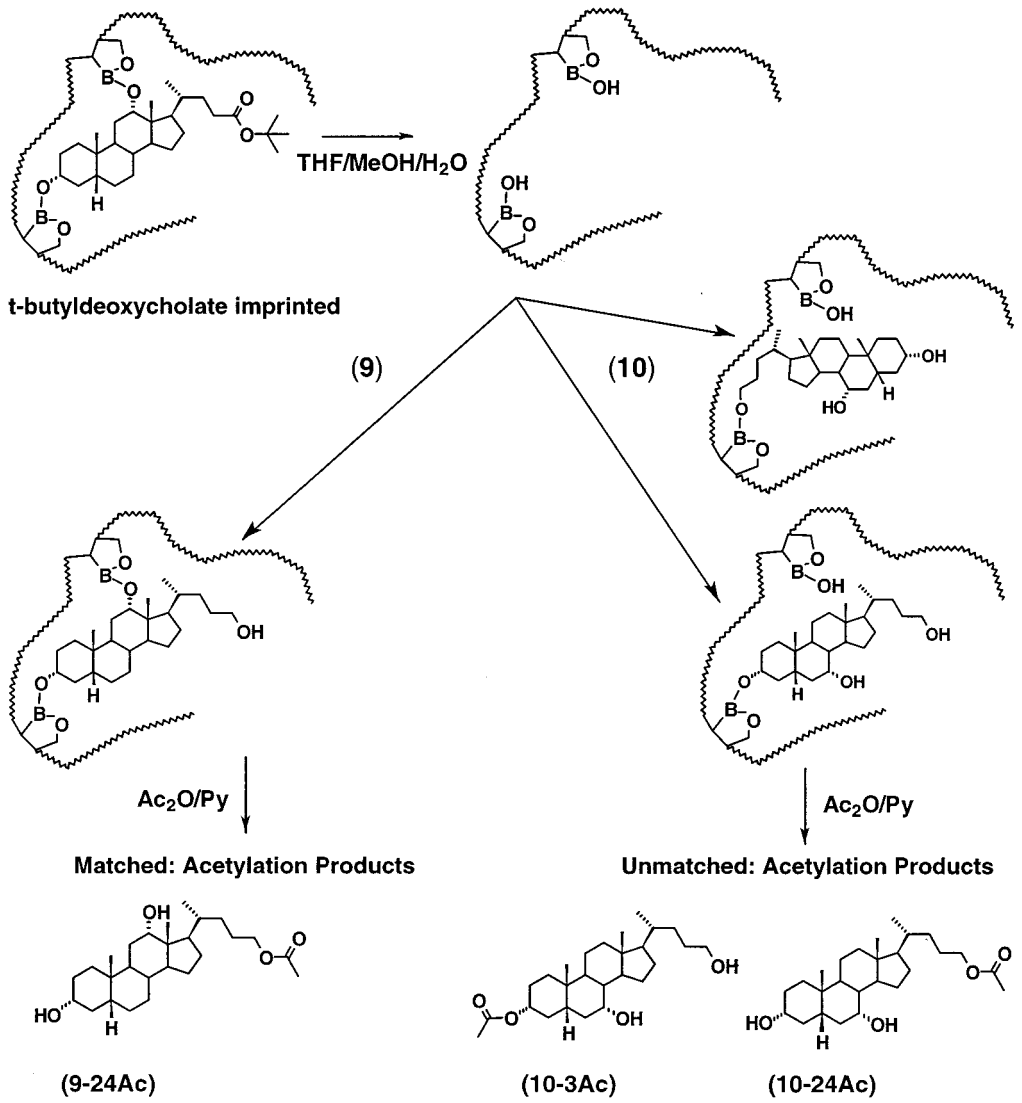

Figure 6. Modification of triols **9** and **10** on polymer **P4**.

Table 3. Modification of Sterols **9** and **10** on Imprinted Polymers

polymer[a]	sterol	sterol bound, μmol[b]	fraction unmodified, %	fraction mono-acetates, %	ratio 24-acetoxy:3-acetoxy
P4 (matched)	9	29.0	36	64	10.5:1
P5 (matched)	10	26.1	35	65	23.1:1
P5 (unmatched)	9	25.5	49	51	5.4:1
PNI-2	9	8.3	87	13	<1:100

[a] "Matched" refers to deoxycholan-24-ol (**9**) bound to *tert*-butyldeoxycholate-imprinted polymer or chenodeoxycholan-24-ol (**10**) bound to *tert*-butylchenodeoxycholate-imprinted polymer. "Unmatched" refers to deoxycholan-24-ol bound to *tert*-butylchenodeoxycholate-imprinted polymer. [b] Yield quoted is for the total amount of sterol and acetylated derivatives recovered from the polymer after the protic solvent wash. Results are averaged from at least duplicate measurements (typical variation in sterol bound, $\pm$0.15 μmol).

case, almost no 24-acetoxy product was formed, with the predominant component being unreacted **9** and the remainder (~13%) shown by GC to be the 3-acetoxydeoxycholan-24-ol. This suggested a more random binding process, with attachment to the polymer occurring primarily via the 24-hydroxyl position. Virtually no modification at the 7- or 12-positions was observed[58] in any of the above cases, probably due to much lower reactivity of these hydroxyls[56] compared to C3 and C24 and/or hindered access to the acetylating reagents in the sites.

(58) Characterization of reaction products was based on NMR assignments. See: (a) Bonar-Law, R. P.; Davis, A. P. *Tetrahedron* **1993**, *49*, 9845−9854. (b) Bonar-Law, R. P.; Davis, A. P.; Dorgan, B. J. *Tetrahedron* **1993**, *49*, 9855−9866 and references therein.

Conclusions

We have shown that the incorporation of two boronophthalide residues into the polymer's recognition site results in cooperative interactions with target dihydroxytemplates. This is evident from the higher uptake of the diol **3** compared to that of monohydroxyandrost-5-enes (**1** and **2**) by **P3** as well as from the experiments on the comparative binding of **3** to other polymers (**P1**, **P2**, and **PNI-2**). This conclusion is also supported by the results of the acylation experiments, where low amounts (<3%) of monoester products were observed in the modification of **3** loaded on **P3** compared to **P1** and **P2**. These data, combined with other observations (FT-IR and washing the loaded polymers with THF/MeOH/H_2O), strongly suggest that both (rather than

one) covalent bonds were re-formed in the majority of recognition sites on binding. The same conclusion can be drawn from the results of acetylation of polymer-bound androst-5-ene-3β,-11β,17β-triol (**4**) and (cheno)deoxycholan-24-ol (**9** and **10**). In the former case, the formation of the sterically hindered 11-acetoxy product was predicted and, indeed, observed, while in the latter experiment the difference in the product distribution obtained can best be explained by preferential binding of the matched ligands in the correct orientation to allow the formation of two boronate ester bonds.

Another question which arises from this investigation is the scope for using imprinted polymers as protecting groups for regioselective modifications of multifunctional compounds.[59] Our results, as well as those of others using imprinted polymer binding sites as "microreactors",[26,27] suggest that this is feasible, although several issues must be resolved to make it attractive from a practical standpoint. First, it seems that access of reagents to the imprinted sites is hampered by the high density of the cross-linked polymeric network. The creation of a special pocket for the reagent may alleviate the problem to a certain extent but is unlikely to solve it completely. Presumably, using polymers with significantly lower degrees of cross-linking would be beneficial and, provided that very strong, preferably covalent, interactions between the functionality of the target and polymer's recognition site are employed, there is no reason this cannot be achieved. The second, and perhaps more elegant, approach would be to have imprinted sites positioned exclusively on the surface of polymeric particles or films, and we are currently studying this possibility. Among potential applications of this approach, one would envisage imprinted polymer-directed regioselective modifications of complex natural products or drugs such as, for example, taxol[60] and the synthesis of combinatorial libraries. The latter is especially attractive because the polymer can be used for a number of different modifications of the same functional group in a target molecule and, in most cases, very small quantities of compounds are required for the evaluation of biological activity.

Experimental Section

General Methods. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL EX 270 Fourier transform spectrometer at 67.8 (^{13}C) and 270.05 MHz (^{1}H). All chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS). Chemical shift values (δ) and peak assignments were determined by a combination of one-dimensional ^{1}H and ^{13}C NMR spectroscopic analysis (including DEPT 90 and DEPT 135) and two-dimensional ^{1}H$-^{1}$H homonuclear COSY and ^{1}H$-^{13}$C heteronuclear COSY experiments. IR spectra were recorded on a Perkin-Elmer 1600 series spectrometer by the diffuse reflectance method using KBr as dispersant. Negative fast atom bombardment mass spectrometry (FAB-MS) spectra were obtained on a Kratos MS9/50TC spectrometer, using Xenon at 5−8 keV. Accurate mass measurements were recorded at 1.0 mamu resolution, using PEG 600 ions as reference. GC analysis was undertaken on a Hewlett-Packard 5890 gas chromatograph equipped with FID. Trimethylsilyl derivatives (1 μL), prepared by adding a sample to a 1:1 mixture of bis(trimethylsilyl)trifluoroacetamide (BSTFA):pyridine at 50 °C for 15 min, were applied to a BPX-5 capillary column using helium as the carrier gas. The oven temperature gradient began at 50 °C and increased at 5 °C/min to 120 °C, followed by 10 °C/min to 250 °C, and 4 °C/min up to 275 °C. For analysis of cholic acid derivatives, the column was held at 275 °C for a further 10 min. Thin-layer chromatography (TLC) was used for the qualitative analysis of sterol derivatives, using silica gel plates and hexane/ethyl acetate/methanol (55:40:5 v/v) as the eluent. TLC plates were developed by spraying with phosphomolybdic acid and heating at 100 °C for 1 min. Flash chromatography was performed using Silica gel 60 (230−400 mesh) from Aldrich. All standard reagents were purchased from Aldrich or BDH and used as received. Solvents used for chromatography were purchased from Fisher Scientific and were at least HPLC grade. Anhydrous solvents were prepared by standard methods.[61]

Starting Materials. Dehydroisoandrosterone, androst-5-ene-3β,11β,-17β-triol, deoxycholic acid, and chenodeoxycholic acid were purchased from Aldrich. Androst-5-ene-3β-ol (**1**) was prepared by reduction of dehydroisoandrosterone with hydrazine hydrate/KOH/diethyleneglycol and recrystallized from ethyl acetate to constant melting point 133 °C (lit.[62] mp 135 °C). Other spectroscopic data were consistent with the desired product.[63] Androst-5-ene-17β-ol (**2**) was synthesized in two steps by tosylation of the 3-hydroxyl group with *p*-toluene sulfonyl chloride in $CHCl_3$/pyridine, followed by reduction with $LiAlH_4$ and successive crystallization from hexane and ethyl acetate: melting point 156 °C (lit.[64] mp 158−165). Androst-5-ene-3β,17β-ol (**3**) was prepared by direct reduction of dehydroisoandrosterone with L-Selectride (lithium tri(*sec*-butyl)borohydride, Aldrich) in diglyme. Spectroscopic data were in accordance with literature values.[65] 3β-Acetoxyandrost-5-ene-17β-ol was purchased from Sigma and used as received. 17β-Acetoxy-androst-5-ene-3β-ol was prepared by literature methods[66] and recrystallized from hexane/acetone.

5-Aminoboronophthalide. From 5-nitroboronophthalide, prepared by the method of Lennarz and Snyder,[32] the unstable amino derivative was synthesized by reduction with palladium/carbon and ammonium formate.[67] (See Supporting Information for full details.)

Preparation of (5-Methacryloylamino)boronophthalide (I). A variation of the procedure of Dederich[33] was followed. Spectroscopic and analytical data were in accordance with the desired structure. (See Supporting Information for full details.)

Preparation of *tert*-Butyl-3α,12α-dihydroxy-5β-cholan-24-oate (*tert*-Butyldeoxycholate) (7) and *tert*-Butyl-3α,7α-dihydroxy-5β-cholan-24-oate (*tert*-Butylchenodeoxycholate) (8). The procedure followed for both **7** and **8** was a modification of that used by Bonar-Law et al.[68] for the preparation of *tert*-butylcholate. (See Supporting Information for full details.)

***tert*-Butyl-3α,12α-dihydroxy-5β-cholan-24-oate (*tert*-Butyldeoxycholate) (7).** The crude compound yield was 19 g, and it was shown by NMR to be the desired compound. TLC indicated only one component, but crystallization from nonpolar solvents could not be effected. Slow crystallization from water/EtOH yielded the pure compound: mp 72−76 °C. Overall yield was 18.4 g, (82%). NMR assignments were based on those by Bonar-Law and Davis for cholic acid derivatives.[58]

^{1}H NMR (δ/ppm, $CDCl_3$): 0.61 (3H, s, 18-Me), 0.84 (3H, s, 19-Me), 0.89 (3H, d, J = 4 Hz, 21-Me), 1.37 (9H, s, C-(CH_3)$_3$), 2.15 (1H, m, J = 13 Hz, CH_2CO$_2$-*t*-Bu), 2.22 (1H, m, J = 12 Hz, CH_2CO$_2$-*t*-Bu), 3.52 (1H, m, tt, J = 11, 4 Hz, 3β-H), 3.92 (1H, t, J = 4 Hz, 12β-H).

^{13}C NMR (δ/ppm, $CDCl_3$): 12.60 (C18), 17.16 (C21), 23.15 (C19), 28.02 (C-(*C*H$_3$)$_3$), 71.50 (C3), 72.96 (C12), 79.78 (O-*C*(CH$_3$)$_3$), 173.60 (C24).

(59) Byström et al. (ref 13) successfully demonstrated that regioselective modifications could be mediated by imprinted polymers: in this case, reduction of steroidal ketones using $LiAlH_4$ introduced into imprinted sites after template removal. This is, however, different from carrying out regioselective modifications with added reagents using imprinted polymers as "self-assembled" protecting groups.

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IR (ν_{max}/cm^{-1}, KBr): 3388 (O−H stretch), 2934 (C−H stretch), 1728 (C═O stretch), 1450 (CH_3 asymm bend).

Mass spectra EI (*m*/*z*, relative intensity): 448.4 (M^+, 0.1), 391.30 ($M - C(CH_3)_3$, 11.3), 373.31 (−OH, 49), 356.30 ($-H_2O$, 88), 273.29 (steroid ring backbone, 100).

Accurate mass: calculated for $C_{28}H_{44}O_4$, 448.3552; found, 448.3599.

***tert*-Butyl-3α,7α-dihydroxy-5*β*-cholan-24-oate (*tert*-Butylchenodeoxycholate) (8).** TLC (CH_2Cl_2/EtOAc 1:1) indicated the presence of two components, the first at R_f 0.3 (shown to be the desired product by NMR) and a faster moving component (R_f 0.75), which was not identified. Column chromatography on silica gel (CH_2Cl_2/EtOAc) followed by evaporation of solvent yielded the *tert*-butylchenodeoxycholate as a colorless foam, which was crystallized from water/MeOH and again from hexane: mp 76−80 °C. Overall yield after column chromatography was 13.9 g (62%).

^{1}H NMR (δ/ppm, $CDCl_3$): 0.59 (3H, s, 18-Me), 0.83 (3H, s, 19-Me), 0.86 (3H, d, J = 4 Hz, 21-Me), 1.37 (9H, s, C-(CH_3)$_3$), 2.10 (1H, m, J = 13 Hz, CH_2CO_2-*t*-Bu), 2.17 (1H, m, J = 10 Hz, CH_2CO_2-*t*-Bu), 3.40 (1H, m, tt, J = 11, 4 Hz, 3β-H), 3.78 (1H, q, J = 4 Hz, 7β-H).

^{13}C NMR (δ/ppm, $CDCl_3$): 11.72 (C18), 18.24 (C21), 22.73 (C19), 28.07 (C-(CH_3)$_3$), 68.43 (C7), 71.92 (C3), 79.86 (O-C(CH_3)$_3$), 173.69 (C24).

IR (ν_{max}/cm^{-1}, KBr): 3380 (O−H stretch), 2920 (C−H stretch), 1726 (C═O stretch), 1450 (CH_3 asymm bend).

Mass spectra EI (*m*/*z*, relative intensity): 448.3615 (M^+, 0.2), 392.43 ($MH - C(CH_3)_3$), 12.6), 374.42 (−OH, 55), 356.41 ($-H_2O$, 100).

Accurate mass: calculated for $C_{28}H_{44}O_4$, 448.3552; found, 448.3615.

Preparation of 3α,12α,24-Trihydroxy-5*β*-cholane (Deoxycholan-24-ol) (9) and 3α,7α,24-Trihydroxy-5*β*-cholane (Chenodeoxycholan-24-ol) (10). A solution of deoxycholic acid or chenodeoxycholic acid (3.92 g, 10 mmol) in dry THF (350 mL) was stirred rapidly as lithium tetrahydroaluminate (2.5 g, excess) was added carefully in small portions under nitrogen. The reaction was continued overnight at room temperature before the suspension was poured carefully onto a mixture of cooled ethyl acetate (400 mL) and methanol (50 mL). The suspension was filtered, and the filtrate was shaken with 1 M HCl (3 × 100 mL) and water (3 × 50 mL). The residual solids were washed with aqueous methanol (4 × 100 mL) and ethyl acetate (250 mL) to extract any adsorbed organics. The combined organic layers were then washed with 1 M NaOH (500 mL) and water (2 × 500 mL) before drying over ($MgSO_4$) and removal of solvent in vacuo.

3α,12α,24-Trihydroxy-5*β*-cholane (9). The resultant white solid (3.02 g, 80%) was pure by TLC (hexane/ethyl acetate/methanol 30:20:5 v/v). Crystallization from hexane/ethyl acetate yielded colorless plates: mp 134−136 °C (lit.[69] mp 122−124 °C). Spectroscopic and other analytical data were in agreement with literature values.

3α,7α,24-Trihydroxy-5*β*-cholane (10). The product was shown by TLC to contain a small amount of starting material and two faster-moving components. Column chromatography (silica gel, elution with ethyl acetate) yielded the desired compound (2.87 g, 75%) as colorless needles:mp 148−152 °C (lit.[70] mp 150 °C). Analytical data were in accordance with the expected structure.

Preparation of 3α,12α-Dihydroxy-5*β*-cholan-24-oxytriphenylmethane (11) and 3α,7α-Dihydroxy-5*β*-cholan-24-oxytriphenylmethane (12). A solution of deoxycholan-24-ol (**9**) or chenodeoxycholan-24-ol (**10**) (378 mg, 1 mmol) in dry pyridine (20 mL) was stirred rapidly under nitrogen as triphenylmethyl chloride (750 mg, excess) was added in one portion. The reaction was continued overnight at room temperature before the solvent was removed in vacuo. The resultant oily solid was purified by column chromatography (silica; elution with $CHCl_3$ and $CHCl_3$/MeOH (10:1)).

3α,12α-Dihydroxy-5*β*-cholan-24-oxytriphenylmethane (11). The yield of product was 526 mg (85%) and was shown by TLC to consist of one component. Crystallization from hexane/ethyl acetate yielded colorless needles: mp 188−189 °C.

^{1}H NMR (δ/ppm, $CDCl_3$): 0.56 (3H, s, 18-Me), 0.82 (3H, s, 19-Me), 0.86 (3H, d, J = 6 Hz, 21-Me), 2.93 (2H, m, CH_2-O-CPh_3,), 3.51 (1H, m, tt, J = 11, 4 Hz, 3β-H), 3.90 (1H, t, J = 4 Hz, 12β-H), 7.15, (10H, m, J = 5, 5 Hz, ArCH), 7.34 (5H, m, J = 5, 5 Hz, ArCH).

^{13}C NMR (δ/ppm, $CDCl_3$): 12.65 (C18), 17.56 (C21), 23.08 (C19), 63.99 (C24), 71.52 (C3), 73.70 (C12), 86.14 (O-CPh$_3$), 126.38, 127.57, 128.57 (Ar-CH), 144.40 (Ar-C).

IR: (ν_{max}/cm^{-1}, KBr): 3565, 3401 (O−H stretch), 3048 (ArC−H stretch), 2920 (C−H stretch), 1595 (ArC═C stretch).

Mass spectra EI (*m*/*z*, relative intensity): 620.4 (M^+, 0.1), 602.5 ($M - H_2O$, 0.2), 543.5 (−Ph, 2.1), 243.2 (CPh_3), 100).

Accurate mass: calculated for $C_{43}H_{56}O_3$, 620.4229; found, 620.4231.

3α,7α-Dihydroxy-5*β*-cholan-24-oxytriphenylmethane (12). The yield of product was 520 mg (84%), and it was shown (TLC) to consist of one component. Crystallization from hexane/ethyl acetate yielded colorless plates: mp 84−86 °C.

^{1}H NMR (δ/ppm, $CDCl_3$): 0.56 (3H, s, 18-Me), 0.83 (3H, s, 19-Me), 0.88 (3H, d, J = 6 Hz, 21-Me), 2.95 (2H, m, CH_2-O-CPh_3,), 3.44 (1H, m, tt, J = 11, 4 Hz, 3β-H), 3.79 (1H, q, J = 4, 4 Hz, 7β-H), 7.18, (10H, m, J = 5, 5 Hz, ArCH), 7.32 (5H, m, J = 5, 5 Hz, ArCH).

^{13}C NMR (δ/ppm, $CDCl_3$): 11.77 (C18), 18.62 (C21), 22.77 (C19), 64.13 (C24), 68.57 (C7), 72.02 (C3), 86.20 (O-CPh$_3$), 126.77, 127.67, 128.68 (Ar-CH), 144.53 (Ar-C).

IR (ν_{max}/cm^{-1}, KBr): 3378 (O−H stretch), 3046 (ArC−H stretch), 2930 (C−H stretch), 1596 (ArC═C stretch).

Mass spectra EI (*m*/*z*, relative intensity): 620.4 (M^+, 0.3), 543.2 (−Ph, 4.2), 243.0 (CPh_3), 100).

Accurate mass: calculated for $C_{43}H_{56}O_3$, 620.4229; found, 620.4237.

Preparation of Sterol-Boronophthalide-Imprinted Polymers. A standard method was used for the synthesis of all the imprinted polymers in this study: details of polymer composition are given in Table 1.

A mixture of sterol (0.25 mmol) and (5-methacryloylamino)-boronophthalide (54.25 mg, 0.25 mmol per sterol hydroxyl) in $CHCl_3$ (2 mL) was heated to reflux in a tube fitted with a suspended CaH_2 drying thimble. Removal of water by azeotropic distillation through the drying agent resulted in the production of a clear solution. Dry divinylbenzene, 55% (1.38 mL = 1.2675 g, 9.75 mmol), was added and reflux maintained for a further 60 min before the addition of AIBN (10 mg). The solution was degassed three times with freeze−thaw cycles before polymerization at 60 °C for 24 h. Removal of solvent in vacuo yielded a polymer which was ground to a fine powder in an agate mortar on a Fritsch Pulverisette "O" grinding mill and extracted with chloroform, followed by aqueous ethanol in a Soxhlet apparatus for 12 h. The polymer was dried at 80 °C in vacuo for 24 h prior to use.

Batch Binding Experiments of Androst-5-ene Derivatives to Polymers. Solutions of steroid ligand (2 mL, 2mM) in $CHCl_3$ were shaken with imprinted polymers (40 mg) and CaH_2 (10 mg) for 24 h. Aliquots (100 μL) were taken at intervals and added to BSTFA/pyridine (1:1 v/v, 100 μL); after 30 min, ethyl acetate (500 μL) was added to each aliquot for GC analysis. Where filtration was necessary, aliquots were increased to 150 μL (sample and BSTFA). To correct for adsorption of template to the dehydrating reagent, control samples were prepared with template solutions and CaH_2 (10 mg) without polymer. Peak areas were normalized to tetradecane internal standard (5 μL mL^{-1}). Results shown are the average of at least two measurements.

Binding of Androst-5-ene Derivatives to Polymers: Elevated Temperature Studies. A solution of template (10−100 mg) in $CHCl_3$ (5.0 mL) was refluxed with imprinted polymer (250 mg) under a dry nitrogen atmosphere in a tube with a suspended Soxhlet thimble containing CaH_2 as drying agent. Aliquots (100 μL) were taken at intervals and added to BSTFA/pyridine (1:1 v/v, 100μL), followed by addition of ethyl acetate (500μL) as above. Peak areas were normalized to tetradecane internal standard (5 μL mL^{-1}). For solvent wash studies to probe template binding, after a set time (1−36 h), the polymers were washed with dry chloroform (3 × 10 mL, fraction 1) and MeOH/THF/H_2O (3 × 10 mL, fraction 2). For analysis, the solvent was removed from each fraction and the yield of recovered template recorded either gravimetrically or by GC.

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Polymer-Directed Synthesis. (a) Modification of Androst-5-ene-3,-17-diol (3).

A solution of **3** (100 mg, 0.35 mmol) in $CHCl_3$ (5.0 mL) was refluxed with imprinted polymer (250 mg) for 72 h under a dry nitrogen atmosphere using CaH_2 as drying agent in a thimble suspended above the solution. After the template loading was finished, the polymers were washed with dry $CHCl_3$ (5 × 10 mL) and dried in vacuo. Dry $CHCl_3$ (5.0 mL) was added to the template−polymer complex, and the contents were brought to reflux under a dry nitrogen atmosphere before acetic anhydride (2 mL) and pyridine (2.5 mL) were added. After reaction was complete (12 h, reflux), the polymers were again washed with $CHCl_3$ (5 × 10 mL, into methanol (10 mL)) and aqueous THF/methanol (5 × 10 mL), collecting each fraction separately. Following solvent removal, ethyl acetate (5.0 mL) was added and the product composition determined by GC as above.

(b) Modification of 3α,12α,24-Trihydroxy-5*β*-cholane (9) and 3α,7α,24-Trihydroxy-5*β*-cholane (10). A solution of **9** (100 mg, 0.26 mmol) or **10** (100 mg, 0.26 mmol) in $CHCl_3$ (5.0 mL) was refluxed with imprinted polymer **P4** or **P5** (250 mg) for 72 h under a dry nitrogen atmosphere using CaH_2 as drying agent in a thimble suspended above the solution. After the template loading was finished, the polymers were washed with dry $CHCl_3$ (5 × 10 mL) and dried in vacuo. Dry $CHCl_3$ (5.0 mL) was added to the template−polymer complex, and the contents were brought to reflux under a dry nitrogen atmosphere before acetic anhydride (2 mL) and pyridine (2.5 mL) were added. After reaction was complete (12 h, reflux), the polymers were again washed with $CHCl_3$ (5 × 10 mL, into methanol (10 mL)) and aqueous THF/methanol (5 × 10 mL), collecting each fraction separately. Following solvent removal, ethyl acetate (5.0 mL) was added and the product composition again determined by GC.

Preparation of Reference 3α,12α,24-Trihydroxy-5*β*-cholane and 3α,7α,24-Trihydroxy-5*β*-cholane Acetates. A solution of trihydroxy-5*β*-cholane **9** or **10** (378 mg, 1 mmol) and DMAP (4 mg) in dry pyridine (0.5 mL) and dry $CHCl_3$ (5 mL) was vigorously stirred as acetyl chloride (240 mg = 217 μL) was added rapidly. The reaction was continued overnight before the contents were poured onto ice/HCl. Extraction of the aqueous layer with ethyl acetate (3 × 50 mL), washing of the organic layer with aqueous acid (3 × 50 mL), aqueous base (NH_4OH, 3 × 50 mL), and water (3 × 50 mL), and drying over $MgSO_4$ yielded, after removal of solvent, a white solid in each case. Flash chromatography ($CH_2Cl_2 \gg CH_2Cl_2$/EtOAc/MeOH (10:2:1) was carried out for both sets of sterol derivatives, and the products were identified by NMR.

3α,12α,24-Tris(acetoxy)-5*β*-cholane (13). Crystallization from aqueous ethanol yielded colorless plates: mp 75−78 °C (lit.[71] mp 79.5−80.5).

^{1}H NMR (δ/ppm, $CDCl_3$): 0.66 (3H, s, 18-Me), 0.74 (3H, d, 21-Me), 0.84 (3H, s, 19-Me), 1.97 (3H, s, C*H*$_3$C═O), 1.98 (3H, s, C*H*$_3$C═O), 2.04 (3H, s, C*H*$_3$C═O), 3.95 (2H, m, C*H*$_2$OAc), 4.62 (1H, tt, J = 11, 5 Hz, 3β H), 5.02 (1H, m, 12β H).

^{13}C NMR (δ/ppm, $CDCl_3$): 12.40 (C18), 17.70 (C21), 20.95 (24-OCO*C*H$_3$), 21.33 (12-OCO*C*H$_3$), 21.40 (3-O-CO*C*H$_3$), 23.03 (C19), 64.89 (C24), 74.13 (C3), 75.86 (C12), 170.42 (12-O*C*OCH$_3$), 170.49 (3-O*C*OCH$_3$), 171.14 (24-O*C*OCH$_3$).

IR (ν_{max}/cm^{-1}, KBr): 2931, 2848 (C−H stretch) 1731 (C═O stretch), 1449 (CH_3 asymm bend), 1241 (C−O acetate stretch).

Mass spectra EI (*m*/*z*, relative intensity): 444.490 (M − CH_3CO_2H, 1.3), 384.4 (M − 2CH_3CO_2H, 65), 255.0 (steroid backbone).

3α,24-Bis(acetoxy)-12α-hydroxy-5*β*-cholane (14). Spectroscopic and other data accorded with previous data for this compound; however, as reported by Hammann and Habermehl,[72] attempted crystallization from a range of solvents gave only a viscous gum.

^{1}H NMR (δ/ppm, $CDCl_3$): 0.66 (3H, s, 18-Me), 0.88 (3H, s, 19-Me), 0.95 (3H, d, 21-Me), 1.99 (3H, s, C*H*$_3$C═O), 2.01 (3H, s, C*H*$_3$C═O), 3.96 (3H, overlying m, C*H*$_2$OAc, 12β H), 4.62 (1H, tt, J = 11, 5 Hz, 3β H).

^{13}C NMR (δ/ppm, $CDCl_3$): 12.65 (C18), 17.5 (C21), 20.95 (24-OCO*C*H$_3$), 21.38 (3-O-CO*C*H$_3$), 23.04 (C19), 64.94 (C24), 73.03 (C12), 74.22 (C3), 170.66 (3-O*C*OCH$_3$), 171.19 (24-O*C*OCH$_3$).

IR (ν_{max}/cm^{-1}, KBr): 3550 (O−H stretch), 2931, 2860 (C−H stretch) 1731 (C═O stretch), 1445 (CH_3 asymm bend), 1243 (C−O acetate stretch).

Mass spectra EI (*m*/*z*, relative intensity): 462.3361 (M$^+$, 0.4), 444.4 (M − H_2O, 5.0), 402.3 (M − CH_3CO_2H, 27), 384.0 (−H_2O, 75), 255.0 (steroid backbone).

3α-Acetoxy-12α,24-dihydroxy-5*β*-cholane (15). Crystallization from hexane/ethyl acetate yielded colorless plates: mp 34−42 °C.

^{1}H NMR (δ/ppm, $CDCl_3$): 0.62 (3H, s, 18-Me), 0.85 (3H, s, 19-Me), 0.92 (3H, d, 21-Me), 1.96 (3H, s, C*H*$_3$C═O), 3.55 (3H, s overlying m, C24 C*H*$_2$-OH), 3.94 (1H, m, 12β H), 4.62 (1H, tt, J = 11, 5 Hz, 3β H).

^{13}C NMR (δ/ppm, $CDCl_3$): 12.56 (C18), 17.51 (C21), 21.28 (3-O-CO*C*H$_3$), 23.02 (C19), 63.53 (C24), 73.02 (C12), 74.09 (C3), 170.0 (3-O*C*OCH$_3$).

IR (ν_{max}/cm^{-1}, KBr): 3450 (br O−H stretch), 2931, 2860 (C−H stretch) 1737 (C═O stretch), 1448 (CH_3 asymm bend), 1244 (C−O acetate stretch).

Mass spectra EI (*m*/*z*, relative intensity): 420.3258 (M$^+$, 0.1), 402.2 (M − H_2O, 2.2), 384.2 (M − 2H_2O, 9.1) 360.2 (M − CH_3CO_2H, 10.9), 342.2 (−H_2O, 31.1), 255.2 (steroid backbone).

24-Acetoxy-3α,12α-dihydroxy-5*β*-cholane (16). Crystallization from hexane/ethyl acetate yielded colorless plates. A broad melting range was observed (82−116 °C), indicative of a mesophase.

^{1}H NMR (δ/ppm, $CDCl_3$): 0.61 (3H, s, 18-Me), 0.84 (3H, s, 19-Me), 0.91 (3H, d, 21-Me), 1.97 (3H, s, C*H*$_3$C═O), 3.54 (1H, tt, J = 11, 5 Hz, 3β H), 3.96 (3H, overlying m, C*H*$_2$OAc, 12β H).

^{13}C NMR (δ/ppm, $CDCl_3$): 12.56 (C18), 17.34 (C21), 20.98 (24-O-CO*C*H$_3$), 23.00 (C19), 64.93 (C24), 71.41 (C12), 72.93 (C3), 171.17 (24-O*C*OCH$_3$).

IR (ν_{max}/cm^{-1}, KBr): 3500 (br O−H stretch), 2930, 2860 (C−H stretch) 1735 (C═O stretch), 1450 (CH_3 asymm bend), 1245 (C−O acetate stretch).

Mass spectra EI (*m*/*z*, relative intensity): 420.3232 (M$^+$, 0.1), 402.4 (M − H_2O, 16.0), 384.4 (M − 2H_2O, 53.9) 360.4 (M − CH_3CO_2H, 2.7), 342.4 (−H_2O, 18), 273.8, 255.4 (steroid backbone, 83.8, 100).

3α,7α,24-Tris(acetoxy)-5*β*-cholane (17). Crystallization from aqueous ethanol yielded colorless plates: mp 92−98 °C.

^{1}H NMR (δ/ppm, $CDCl_3$): 0.68 (3H, s, 18-Me), 0.82 (3H, d, 21-Me), 0.86 (3H, s, 19-Me), 1.97 (3H, s, C*H*$_3$C═O), 1.98 (3H, s, C*H*$_3$C═O), 2.04 (3H, s, C*H*$_3$C═O), 3.95 (2H, m, C*H*$_2$OAc), 4.61 (1H, tt, J = 11, 5 Hz, 3β H), 4.82 (1H, q, J = 4, 4 Hz, 7β H).

^{13}C NMR (δ/ppm, $CDCl_3$): 11.61 (C18), 18.45 (C21), 20.95 (24-OCO*C*H$_3$), 21.42 (7-OCO*C*H$_3$), 21.52 (3-O-CO*C*H$_3$), 23.00 (C19), 64.96 (C24), 71.16 (C7), 74.08 (C3), 170.39 (7-O*C*OCH$_3$), 170.57 (3-O*C*OCH$_3$), 171.14 (24-O*C*OCH$_3$).

IR (ν_{max}/cm^{-1}, KBr): 2931, 2848 (C−H stretch) 1731 (C═O stretch), 1449 (CH_3 asymm bend), 1238 (C−O acetate stretch).

Mass spectra EI (*m*/*z*, relative intensity): 504 3441 (M$^+$, 0.1%) 444.5 (M − CH_3CO_2H, 6.2), 384.4 (M − 2CH_3CO_2H, 100).

3α,24-Bis(acetoxy)-7α-hydroxy-5*β*-cholane (18). Crystallization from hexane/ethyl acetate yielded colorless needles: mp 84−88 °C.

^{1}H NMR (δ/ppm, $CDCl_3$): 0.60 (3H, s, 18-Me), 0.86 (3H, s, 19-Me), 0.92 (3H, d, 21-Me), 1.93 (3H, s, C*H*$_3$C═O), 1.96 (3H, s, C*H*$_3$C═O), 3.79 (1H, q, J = 3, 3 Hz, 7β H), 3.96 (2H, m, C*H*$_2$OAc), (1H, tt, J = 11, 5 Hz, 3β H).

^{13}C NMR (δ/ppm, $CDCl_3$): 11.61 (C18), 18.44 (C21), 20.75 (24-OCO*C*H$_3$), 21.18 (3-O-CO*C*H$_3$), 23.00 (C19), 64.91 (C24), 68.45 (C7), 74.32 (C3), 170.60 (3-O*C*OCH$_3$), 171.10 (24-O*C*OCH$_3$).

IR (ν_{max}/cm^{-1}, KBr): 3550 (O−H stretch), 2920, 2860 (C−H stretch) 1726 (C═O stretch), 1449 (CH_3 asymm bend), 1243 (C−O acetate stretch).

Mass spectra EI (*m*/*z*, relative intensity): 462.3361 (M$^+$, 4.8), 444.2 (M − H_2O, 9.5), 402.2 (M − CH_3CO_2H, 13.1), 384.2 (−H_2O, 100).

3α-Acetoxy-7α,24-dihydroxy-5*β*-cholane (19). Attempted crystallization from hexane/ethyl acetate yielded a colorless oil which could not be crystallized.

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^{1}H NMR (δ/ppm, $CDCl_3$): 0.60 (3H, s, 18-Me), 0.85 (3H, s, 19-Me), 0.89 (3H, d, 21-Me), 1.95 (3H, s, C*H*$_3$C═O), 3.55 (2H, m, C24 C*H*$_2$-OH), 3.79 (1H, q, $J = 3$, 3 Hz, 7β H), 4.52 (1H, tt, $J = 12$, 5 Hz, 3β H).

^{13}C NMR (δ/ppm, $CDCl_3$): 11.75 (C18), 18.60 (C21), 20.54 (3-O-CO*C*H$_3$), 22.72 (C19), 63.56 (C24), 68.82 (C7), 74.36 (C3), 170.80 (3-O*C*OCH$_3$).

IR (ν_{max}/cm^{-1}, KBr): 3400 (br O−H stretch), 2931, 2861 (C−H stretch) 1731 (C═O stretch), 1449 (CH_3 asymm bend), 1245 (C−O acetate stretch).

Mass spectra EI (*m*/*z*, relative intensity): 420.3232 (M^+, 0.1), 402.4 ($M - H_2O$, 9.5), 384.4 ($M - 2H_2O$, 13.6) 360.4 ($M - CH_3CO_2H$, 12.7), 342.4 ($-H_2O$, 100).

24-Acetoxy-3α,7α-dihydroxy-5*β*-cholane (20). Crystallization from hexane/ethyl acetate yielded colorless plates: mp 48−68 °C. Analytical data were in agreement with the expected structure.[73]

^{1}H NMR (δ/ppm, $CDCl_3$): 0.59 (3H, s, 18-Me), 0.85 (3H, s, 19-Me), 0.89 (3H, d, 21-Me), 1.98 (3H, s, C*H*$_3$C═O), 3.50 (1H, tt, $J = 11$, 5 Hz, 3β H), 3.79 (1H, q, $J = 3$, 3 Hz, 7β H), 3.96 (2H, m, C*H*$_2$-OAc).

^{13}C NMR (δ/ppm, $CDCl_3$): 11.66 (C18), 18.43 (C21), 20.87 (24-O-CO*C*H$_3$), 23.10 (C19), 65.37 (C24), 68.32 (C7), 71.68 (C3), 171.21 (24-O*C*OCH$_3$).

IR (ν_{max}/cm^{-1}, KBr): 3450 (br O−H stretch), 2930, 2860 (C−H stretch) 1735 (C═O stretch), 1450 (CH_3 asymm bend), 1245 (C−O acetate stretch).

Mass spectra EI (*m*/*z*, relative intensity): 420.3253 (M^+, 3.5), 402.4 ($M - H_2O$, 28.1), 384.4 ($M - 2H_2O$, 100).

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Supporting Information Available: IR and ^{1}H NMR spectra and experimental details of the synthesis of 5-aminoboronophthalide, 5-methacryloylaminoboronophthalide, *tert*-butyldecoxycholate, and *tert*-butylchenodeoxycholate (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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