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Practical Synthesis of 3β-Amino-5-cholestene and Related 3β-Halides Involving *i*-Steroid and Retro-*i*-Steroid Rearrangements

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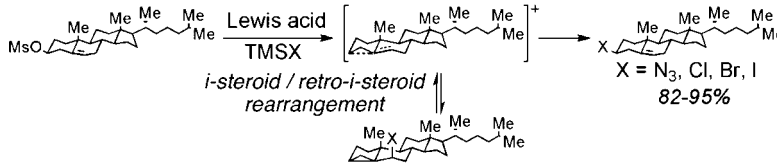
ABSTRACT



Derivatives of 3β-amino-5-cholestene (3β-cholesterylamine) are of substantial interest as cellular probes and have potential medicinal applications. However, existing syntheses of 3β-amino-5-cholestene are of limited preparative utility. We report here a practical method for the stereoselective preparation of 3β-amino-5-cholestene, 3β-chloro-5-cholestene, 3β-bromo-5-cholestene, and 3β-iodo-5-cholestene from inexpensive cholesterol. A sequential *i*-steroid/retro-*i*-steroid rearrangement promoted by boron trifluoride etherate and trimethylsilyl azide converted cholest-5-en-3β-ol methanesulfonate to 3β-azido-cholest-5-ene with retention of configuration in 93% yield.

Synthetic mimics of cholesterol represent important molecular tools in the fields of bioorganic/medicinal chemistry and chemical biology. Cholesterol derivatives have been used to facilitate the delivery of siRNA,[1] enhance DNA transfection,[2] probe cellular membrane subdomains,[3] and have been proposed for tumor targeting applications.[4] The cationic cholesterol mimic 3β-amino-5-cholestene (3β-cholesterylamine) is of particular interest because of its high affinity for phospholipid membranes.[5] Derivatives of 3β-amino-5-cholestene have been used to construct photoaffinity probes,[6] and related aminosteroids,[7] including squalamine,[8,9] exhibit antimicrobial activity. *N*-Alkyl derivatives of 3β-amino-5-cholestene can insert into plasma membranes of living mammalian cells and cycle between the cell surface and early/recycling endosomes, mimicking the membrane trafficking of many cell surface receptors.[10] These compounds are under investigation as tools for drug delivery[11] and when

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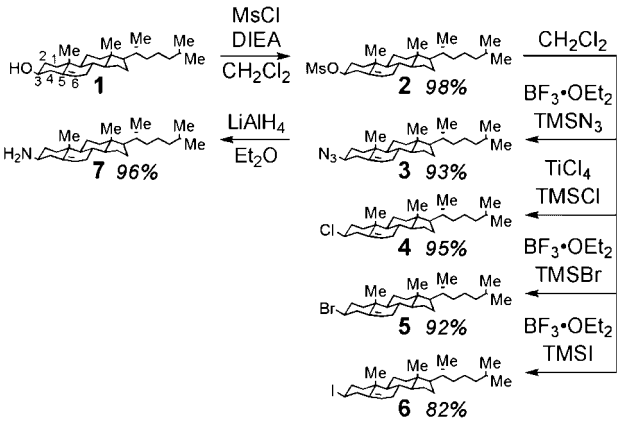
linked to endosome disruptive peptides[12] can deliver molecules into the cytosol and nucleus of mammalian cells.

Windaus[13] was the first to report that reaction of cholesteryl chloride with ammonia, or reduction of cholestenone oxime, provides 3-substituted cholesterylamines in modest yields. More recently, stereoselective syntheses of 3β-azido-5-cholestene, an important precursor to 3β-amino-5-cholestene, have involved conversion of cholesterol to epicholesterol followed by a Mitsunobu reaction with hydrazoic acid[5,14] or treatment of 6β-methoxy-3α,5-cyclo-5α-cholestane and related compounds with hydrazoic acid.[6,15,16] However, because of participation of the homoallylic double bond at the C5 position of the steroid, substitution reactions of cholesterol and derivatives can suffer from poor stereoselectivity, elimination, and rearrangement.[17−21] These complications limit existing synthetic methods to small-scale preparation of 3β-azido-5-cholestene. To overcome this limitation, we report here a practical and efficient two-step method for the synthesis of 3β-azido-5-cholestene from cholesterol. As shown in Scheme 1, this new approach,

Scheme 1. Optimized Synthesis of 3β-Amino-5-cholestene (**7**) and Related 3β-Derivatives (**2**−**6**) from Cholesterol (**1**)[a]



[a] Yields represent reactions on a 1 mmol scale.

involving conversion of cholesterol (**1**) to the mesylate (**2**), followed by treatment with trimethylsilyl azide ($TMSN_3$) and boron trifluoride etherate (BF_3•OEt_2) in dichloromethane, allows preparation of multigram quantities of pure 3β-azido-5-cholestene (**3**) in high yield. Subsequent reduction of 3β-azido-5-cholestene (**3**) with $LiAlH_4$[14] provided 3β-amino-5-cholestene (**7**) in 96% yield (1 mmol scale). Alternatively, **7** could be obtained in 89% yield by hydrogenation of **3** in THF over 10% palladium on carbon. The high efficiency of each step of this synthesis allowed the preparation of **3** on a 100 g scale (see the Supporting Information for details).

We additionally examined the utility of other TMS derivatives for preparation of 3β-substituted cholestenes. Correspondingly, as shown in Scheme 1, 3β-chloro-5-cholestene (**4**), 3β-bromo-5-cholestene (**5**), and 3β-iodo-5-cholestene (**6**) were synthesized in high yield from Lewis acids and cognate TMS compounds. This approach is particularly useful for preparation of compounds **5** and **6** due to their high susceptibility to elimination reactions.

Our rationale for investigating this approach was inspired in part by reports[22,23] of the use of $TMSN_3$ and Lewis acids in neighboring group-assisted glycosylation reactions that proceed with overall retention of configuration. Moreover, as elucidated by Shoppee[21] and Winstein,[24,25] cholesterol and reactive derivatives have been shown to undergo solvolysis with retention of 3β-configuration via the involvement of a nonclassical carbocation that is formed by neighboring participation of the homoallylic alkene. This homoallylic carbocation reacts rapidly at the C6 position of the steroid to afford 6β-substituted-3α,5-cyclosteroids through a process termed the *i*-steroid rearrangement. Slower reaction of this cation at the C3 position yields cholesteryl 3β-derivatives.[26,27] On the basis of these precedents, we hypothesized that $TMSN_3$ and Lewis acids might efficiently convert **2** into **3** with retention of configuration.

Table 1 lists our investigation of the effects of different Lewis acids and $TMSN_3$ on the conversion of **2** to **3**. Among the Lewis acids investigated, the addition of 2 equiv of BF_3•OEt_2 at ambient temperature (22 °C) for 2 h proved optimal, providing **3** in 93% yield. In addition to azide **3**, $SnCl_4$, $TiCl_4$, and $AlCl_3$ generated 3β-chloro-cholest-5-ene (**4**) as a major byproduct. Reaction of fluoride derived from BF_3•OEt_2 with $TMSN_3$ is presumably involved in the production of the nucleophilic azide, and the high stability of TMSF likely prevents formation of byproducts compared to the chlorinated Lewis acids. In control experiments, studies of the corresponding 3α-mesylate (**8**) and the dihydro analogue (**9**) revealed that both the 3β-configuration of **2** and the C5 alkene were required for reaction of **2** with BF_3•OEt_2/$TMSN_3$. These results are consistent with previous studies of solvolytic rate enhancements of cholesterol deriva-

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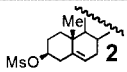
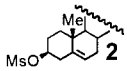
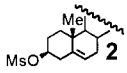
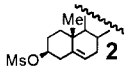
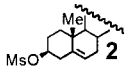
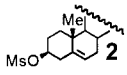
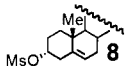
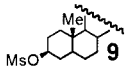
Table 1. Effects of Lewis Acid and Substrate Structure on Yield of 3β-Azido-cholest-5-ene (**3**) from **2**, **8**, and **9**[a]

entry	substrate	Lewis acid	reaction conditions	yield (%)
1		$SnCl_4$ (1 equiv)	-20 °C, 1 h to 22 °C, 2 h	54 (**3**); 36 (**4**)
2		$TiCl_4$ (1 equiv)	-20 °C, 1 h to 22 °C, 2 h	21 (**3**); 64 (**4**)
3		$AlCl_3$ (1 equiv)	22 °C, 1 h	57 (**3**); 34 (**4**)
4		TMSOTf (1 equiv)	22 °C, 12 h	complex mixture
5		BF_3•OEt_2 (1 equiv)	22 °C, 12 h	68 (**3**); 19 (**2**)
6		BF_3•OEt_2 (2 equiv)	22 °C, 2 h	93 (**3**)
7		BF_3•OEt_2 (2 equiv)	22 °C, 2 h	NR
8		BF_3•OEt_2 (2 equiv)	22 °C, 2 h	NR

[a] Reactions were run on a 1 mmol scale in anhydrous CH_2Cl_2 containing $TMSN_3$ (1.1 equiv). In general, reaction conditions were optimized to maximize the yield of **3**, with products isolated by column chromatography (hexane eluent). For entries 1−3, the yields of the major byproduct, 3β-chloro-cholest-5-ene (**4**), are additionally listed. Recovery of starting material (**2**) is additionally shown in entry 5. NR: no reaction observed.

tives that compared the corresponding unsaturated and saturated substrates and that examined the stereospecificity of related reactions.[21,28] These results indicate that the homoallylic double bond of **2** is a key participant in the outcome of this reaction.

The effect of the leaving group on the reactivity of 3β-cholesteryl derivatives was probed as listed in Table 2. The less reactive tosylate (**10**) similarly furnished **3** in excellent yield but required a larger excess of BF_3•OEt_2 (5 equiv) and a longer reaction time than the mesylate (**2**). Interestingly, whereas BF_3•OEt_2 had little effect on conversion of 3β-chloro-5-cholestene (**4**), $SnCl_4$ converted 50% of this compound to the azide (**3**). Substrates bearing poorer leaving groups, such as **11** and **1**, were unreactive.

The nature of the solvent was also found to play a critical role in this reaction. Benzene could be substituted for dichloromethane (or chloroform) without appreciably diminishing the yield of **3** from **2** (92%) but required additional equivalents of BF_3•OEt_2 (5 equiv) and a longer reaction time (12 h) for completion of reaction. No reaction was observed in solvents bearing heteroatoms that function as Lewis bases, including tetrahydrofuran, acetone, diethyl ether, or DMF, further emphasizing the role of the Lewis acid in activating the leaving group. Poor solubility of **2** precluded evaluation of reactivity in hexane, acetonitrile, or DMSO.

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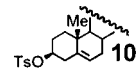
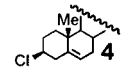
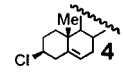
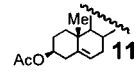
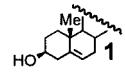
Table 2. Effects of Leaving Group on Yield of 3β-Azido-cholest-5-ene (**3**)[a]

entry	substrate	Lewis acid	reaction conditions	yield (%)
1		BF_3•OEt_2 (5 equiv)	22 °C, 3 h	93 (**3**)
2		$SnCl_4$ (1 equiv)	-20 °C, 1 h to 22 °C, 2 h	50 (**3**); 45 (**4**)
3		BF_3•OEt_2 (2 equiv)	22 °C, 2 h	trace
4		BF_3•OEt_2 (2 equiv)	22 °C, 2 h	NR
5		BF_3•OEt_2 (2 equiv)	22 °C, 2 h	NR

[a] Reactions were run on a 1 mmol scale in anhydrous CH_2Cl_2 containing $TMSN_3$ (1.1 equiv). Recovery of starting material (**4**) is additionally shown in entry 2. NR: no reaction observed.

Because the conversion of **2** to **3** proceeded rapidly at ambient temperature in the presence of BF_3•OEt_2 and $TMSN_3$, the course of the reaction could be readily followed by ^{1}H NMR. As shown in Figure 1 (panel A), the acquisition of

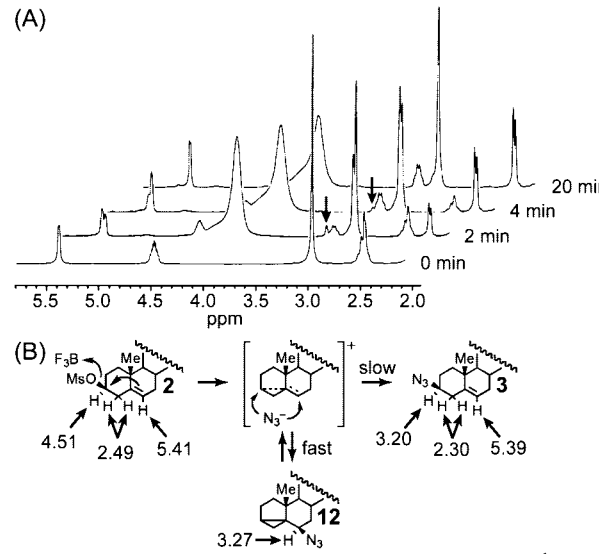


Figure 1. Examination of the conversion of **2** to **3** by ^{1}H NMR (400 MHz). Panel A: ^{1}H NMR spectra of **2** before (bottom spectrum) and at selected time points after the addition of $TMSN_3$ (1 equiv) and BF_3•OEt_2 (2 equiv) to **2** (0.5 mmol) in $CDCl_3$ (0.5 mL). The arrows point to signals identified as the C6 proton of 6β-azido-3α,5-cyclo-5α-cholestane (**12**). Panel B: Observed chemical shifts of **2**, **12**, and **3** and the proposed mechanism of reaction.

^{1}H NMR spectra at different time points allowed clear observation of the disappearance of signals of starting material and the emergence of product. Importantly, these experiments detected the transient appearance of a signal at

3.27 ppm (observed at 2 and 4 min) that did not correspond to the starting material (**2**) or product (**3**) but rather could be assigned as the C6 proton of 6β-azido-3α,5-cyclo-5α-cholestane (**12**), a structure that was further supported by distinctive cyclopropyl signals observed at 0.53 ppm (shown in the Supporting Information).[19] These data are consistent with generation of the *i*-steroid intermediate **12** derived from attack of azide on C6 of the homoallylic cation as shown in Figure 1, panel B.

The NMR data shown in Figure 1, panel A, in conjunction with the necessity of both 3β-stereochemistry of the mesylate (**2**) and the presence of the homoallylic double bond (Table 1) suggest that **2** is initially converted by the Lewis acid to a homoallylic carbocation that rapidly undergoes the *i*-steroid rearrangement and retro-*i*-steroid rearrangement shown in Figure 1, panel B. This mechanism is consistent with pioneering studies of related solvolysis reactions by Winstein.[24,25] Winstein demonstrated[25] that attack at the C6 carbon of cholesterol-derived homoallylic carbocations is kinetically favored, and the structure of the homoallylic cation engenders formation of the 6β-product over the 6α-product. Although the attack of nucleophiles at C3 of the homoallylic carbocation is slower than C6, this addition occurs to form the thermodyamic product with exclusively β-stereochemistry. This stereochemical outcome is a result of the nonclassical carbocation forming a partial bond between C5 and C3 only on the α face of the steroid.

To further support the idea that the *i*-steroid rearrangement product can lead to **3**, we prepared 6β-azido-3α,5-cyclo-5α-cholestane (**12**) by treatment of **2** with NaN_3 in refluxing methanol, a modification of the method of Freiberg.[19] Importantly, treatment of **12** with $BF_3{\cdot}OEt_2$ (2 equiv) and $TMSN_3$ (1 equiv) resulted in clean conversion to **3** within 2 h at ambient temperature (data shown in the Supporting Information). In contrast, treatment of **12** with $TMSN_3$ alone or $TMSN_3$ and TBAF to generate the azide ion did not result in conversion to **3**, indicating that the Lewis acid is required to promote the retro-*i*-steriod rearrangment. Treatment of **12** with excess $BF_3{\cdot}OEt_2$ (2 equiv) resulted in formation of **3** within 10 min. However, byproducts were associated with treatment of **12** with $BF_3{\cdot}OEt_2$ in the absence of $TMSN_3$. Correspondingly, the addition of 2 equiv of $BF_3{\cdot}OEt_2$ and 1.1 equiv of $TMSN_3$ to **2** proved to be optimal to both generate the homoallylic cation and convert **12** to **3** in high yield.

In summary, we developed a novel and highly efficient method for the synthesis of 3β-amino-5-cholestene and related halides. This method, which involves regiospecific and sterospecific *i*-steroid and retro-*i*-steroid rearrangements, is amenable to large-scale preparation of these compounds from inexpensive cholesterol. This approach may also be useful for the synthesis of other steroid derivatives bearing similar π-systems.

Acknowledgment. We thank the National Institutes of Health (CA83831) and the University of Kansas Cancer Center for financial support. This manuscript is dedicated to Professor David A. Lightner on the occasion of his retirement from the University of Nevada, Reno.

Supporting Information Available: Experimental procedures and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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