Published in final edited form as:

Org Lett. 2009 February 5; 11(3): 567–570. doi:10.1021/ol802343z.

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

Qi Sun¹, Sutang Cai², and Blake R. Peterson^{3,*}

¹Integrative Biosciences Graduate Program, The Pennsylvania State University, University Park

²Chemistry Graduate Program, The Pennsylvania State University, University Park

³Department of Medicinal Chemistry, The University of Kansas, Lawrence, KS, 66045

Abstract

Derivatives of 3 β -amino-5-cholestene (3 β -cholesterylamine) are of substantial interest as cellular probes and have potential medicinal applications. However, existing syntheses of 3 β -amino-5-cholestene are of limited preparative utility. We report here a practical method for the stereoselective preparation of 3 β -amino-5-cholestene, 3 β -chloro-5-cholestene, 3 β -bromo-5-cholestene, and 3 β -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-3 β -ol methanesulfonate to 3 β -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 3 β -amino-5-cholestene (3 β -cholesterylamine) is of particular interest because of its high affinity for phospholipid membranes.⁵ Derivatives of 3 β -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 3 β -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 linked to endosome disruptive peptides¹² can deliver molecules into the cytosol and nucleus of mammalian cells.

Windaus¹³ 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 3 β -azido-5-cholestene, an important precursor to 3 β -amino-5-cholestene, have involved conversion of cholesterol to epicholesterol followed by a Mitsunobu reaction with hydrazoic acid^{5,14} or treatment of 6 β -methoxy-3 α ,5-cyclo-5 α -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 3 β -azido-5-cholestene. To overcome this limitation, we report here a practical and efficient two step method for the synthesis of 3 β -azido-5-cholestene from cholesterol. As shown in Scheme 1, this new approach, involving conversion of cholesterol (**1**) to the mesylate (**2**), followed by treatment with trimethylsilyl azide (TMSN₃) and boron trifluoride etherate (BF₃•OEt₂) in dichloromethane, allows preparation of multi-gram quantities of pure 3 β -azido-5-cholestene (**3**) in high yield. Subsequent reduction of 3 β -azido-5-cholestene (**3**) with LiAlH₄¹⁴ provided 3 β -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-gram scale (see the Supporting Information for details).

We additionally examined the utility of other TMS derivatives for preparation of 3 β -substituted cholestenes. Correspondingly, as shown in Scheme 1, 3 β -chloro-5-cholestene (**4**), 3 β -bromo-5-cholestene (**5**), and 3 β -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 TMSN₃ and Lewis acids in neighboring group-assisted glycosylation reactions that proceed with overall retention of configuration. Moreover, as elucidated by Shoppee²¹ and Winstein,^{24,25} cholesterol and reactive derivatives have been shown to undergo solvolysis with retention of 3 β -configuration via the involvement of a non-classical 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 6 β -substituted-3 α ,5-cyclosteroids through a process termed the *i*-steroid rearrangement. Slower reaction of this cation at the C3 position yields cholesteryl 3 β -derivatives.^{26,27} Based on these precedents, we hypothesized that TMSN₃ 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 TMSN₃ on the conversion of **2** to **3**. Among the Lewis acids investigated, the addition of two equivalents of BF₃•OEt₂ at ambient temperature (22 °C) for 2 hours proved optimal, providing **3** in 93% yield. In addition to azide **3**, SnCl₄, TiCl₄, and AlCl₃ generated 3 β -chloro-cholest-5-ene (**4**) as a major byproduct. Reaction of fluoride derived from BF₃•OEt₂ with TMSN₃ 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 3 α -mesylate (**8**) and the dihydro analogue (**9**) revealed that both the 3 β -configuration of **2** and the C5 alkene were required for reaction of **2** with BF₃•OEt₂ / TMSN₃. These results are consistent with previous studies of solvolytic rate enhancements of cholesterol derivatives 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 3 β -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 BF₃•OEt₂ (5 equiv) and a longer reaction time than the mesylate (**2**). Interestingly, whereas BF₃•OEt₂ had little effect on conversion of 3 β -chloro-5-cholestene (**4**), SnCl₄ 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 $\text{BF}_3 \cdot \text{OEt}_2$ (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.

Because the conversion of **2** to **3** proceeded rapidly at ambient temperature in the presence of $\text{BF}_3 \cdot \text{OEt}_2$ and TMSN_3 , the course of the reaction could be readily followed by ^1H NMR. As shown in Figure 1 (panel A), the acquisition of ^1H 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 minutes) that did not correspond to the starting material (**2**) or product (**3**), but rather could be assigned as the C6 proton of 6 β -azido-3 α ,5-cyclo-5 α -cholestane (**12**), a structure that was further supported by distinctive cyclopropyl signals observed at 0.53 ppm (shown in the Supporting Information).¹⁹ This data is 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 3 β -stereochemistry of the mesylate (**2**) and the presence of the homoallylic double bond (Table 1) suggests 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 demonstrated²⁵ 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 6 β -product over the 6 α -product. Although the attack of nucleophiles at C3 of the homoallylic carbocation is slower than C6, this addition occurs to form the thermodynamic product with exclusively β -stereochemistry. This stereochemical outcome is a result of the non-classical carbocation forming a partial bond between C5 and C3 only on the alpha face of the steroid.

To further support the idea that the *i*-steroid rearrangement product can lead to **3**, we prepared 6 β -azido-3 α ,5-cyclo-5 α -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 $\text{BF}_3 \cdot \text{OEt}_2$ (2 equiv) and TMSN_3 (1 equiv) resulted in clean conversion to **3** within 2 hours 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*-steroid rearrangement. Treatment of **12** with excess $\text{BF}_3 \cdot \text{OEt}_2$ (2 equiv) resulted in formation of **3** within 10 minutes. However, byproducts were associated with treatment of **12** with $\text{BF}_3 \cdot \text{OEt}_2$ in the absence of TMSN_3 . Correspondingly, the addition of two equivalents of $\text{BF}_3 \cdot \text{OEt}_2$ 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 3 β -amino-5-cholestene and related halides. This method, which involves regiospecific and stereospecific *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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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.

References

1. Wolfrum C, Shi S, Jayaprakash KN, Jayaraman M, Wang G, Pandey RK, Rajeev KG, Nakayama T, Charrise K, Ndungo EM, Zimmermann T, Kotliansky V, Manoharan M, Stoffel M. *Nat. Biotechnol* 2007;25:1149–1157. [PubMed: 17873866]
2. Pitard B, Oudrhiri N, Vigneron JP, Hauchecorne M, Aguerre O, Toury R, Airiau M, Ramasawmy R, Scherman D, Crouzet J, Lehn JM, Lehn P. *Proc. Natl. Acad. Sci. U.S.A* 1999;96:2621–2626. [PubMed: 10077560]
3. Sato SB, Ishii K, Makino A, Iwabuchi K, Yamaji-Hasegawa A, Senoh Y, Nagaoka I, Sakuraba H, Kobayashi T. *J. Biol. Chem* 2004;279:23790–23796. [PubMed: 15026415]
4. Firestone RA. *Bioconj. Chem* 1994;5:105–113. [PubMed: 8031872]
5. Kan CC, Yan J, Bittman R. *Biochemistry* 1992;31:1866–1874. [PubMed: 1737039]
6. Spencer TA, Wang P, Li D, Russel JS, Blank DH, Huuskonen J, Fielding PE, Fielding CJ. *J. Lipid Res* 2004;45:1510–1518. [PubMed: 15175357]
7. Salmi C, Loncle C, Vidal N, Laget M, Letourneux Y, Brunel JM. *Lett. Drug Design Discov* 2008;5:169–172.
8. Moore KS, Wehrli S, Roder H, Rogers M, Forrest JN Jr, McCrimmon D, Zasloff M. *Proc. Natl. Acad. Sci. U.S.A* 1993;90:1354–1358. [PubMed: 8433993]
9. Salmi C, Loncle C, Vidal N, Letourneux Y, Fantini J, Maresca M, Taieb N, Pages JM, Brunel JM. *PLoS ONE* 2008;3:e2765. [PubMed: 18648511]
10. Peterson BR. *Org. Biomol. Chem* 2005;3:3607–3612. [PubMed: 16211095]
11. Boonyarattanakalin S, Hu J, Dykstra-Rummel SA, August A, Peterson BR. *J. Am. Chem. Soc* 2007;129:268–269. [PubMed: 17212394]
12. Sun Q, Cai S, Peterson BR. *J. Am. Chem. Soc* 2008;130:10064–10065. [PubMed: 18613675]
13. Windaus A, Adamla J. *Berichte Der Deutschen Chemischen Gesellschaft* 1911;44:3051–3058.
14. Boonyarattanakalin S, Martin SE, Dykstra SA, Peterson BR. *J. Am. Chem. Soc* 2004;126:16379–16386. [PubMed: 15600339]
15. Patel MS, Peal WJ. *J. Chem. Soc* 1963:1544–1546.
16. Jarreau FX, Khuonghuu Q, Goutarel R. *Bull. Soc. Chim. France* 1963;8–9:1861–1865.
17. Corey EJ, Nicolaou KC, Shibasaki M, Machida Y, Shiner CS. *Tetrahedron Lett* 1975;16:3183–3186.
18. Aneja R, Davies AP, Knaggs JA. *Tetrahedron Lett* 1975;16:1033–1036.
19. Freiberg LA. *J. Org. Chem* 1965;30:2476–2479.
20. Haworth RD, Lunts LHC, McKenna J. *J. Chem. Soc* 1955:986–991.
21. Shoppee CW, Summers GHR. *J. Chem. Soc* 1952:3361–3374.
22. Stimac A, Kobe J. *Carbohydr. Res* 2000;324:149–160. [PubMed: 10724529]
23. El Akri K, Bougrin K, Balzarini J, Faraj A, Benhida R. *Bioorg. Med. Chem. Lett* 2007;17:6656–6659. [PubMed: 17931862]
24. Kosower EM, Winstein S. *J. Am. Chem. Soc* 1956;48:4347–4354.
25. Winstein S, Kosower EM. *J. Am. Chem. Soc* 1959;81:4399–4408.
26. Galynker I, Still WC. *Tetrahedron Lett* 1982;23:4461–4464.
27. Koen MJ, Leguyader F, Motherwell WB. *J. Chem. Soc. Chem. Comm* 1995:1241–1242.
28. Story, PR.; Clark, BC. *Carbonium Ions*. Olah, GA.; Schleyer, PvR, editors. Vol. Volume III. Wiley and Sons; 1972. p. 1007–1016.

Figure 1.

Examination of the conversion of **2** to **3** by ^1H NMR (400 MHz). Panel A: ^1H NMR spectra of **2** before (bottom spectrum) and at selected time points after the addition of TMSN_3 (1 equiv) and $\text{BF}_3\cdot\text{OEt}_2$ (2 equiv) to **2** (0.5 mmol) in CDCl_3 (0.5 mL). The arrows point to signals identified as the C6 proton of 6 β -azido-3 α ,5-cyclo-5 α -cholestane (**12**). Panel B: Observed chemical shifts of **2**, **12**, and **3** and the proposed mechanism of reaction.

Scheme 1.

Optimized synthesis of 3β-amino-5-cholestene (**7**) and related 3β derivatives (**2-6**) from cholesterol (**1**). Yields represent reactions on a 1 mmol scale.

Table 1Effects of Lewis acid and substrate structure on yield of 3 β -azido-cholest-5-ene (**3**) from **2**, **8**, and **9**.

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

Reactions were run on a 1 mmol scale in anhydrous CH₂Cl₂ containing TMSN₃ (1.1 equiv). For entries 1–4, 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, 3 β -chloro-cholest-5-ene (**4**), are additionally listed. Recovery of starting material (**2**) is additionally shown in entry 5. NR: no reaction observed.

Table 2Effects of leaving group on yield of 3 β -azido-cholest-5-ene (**3**)

entry	substrate	Lewis acid	reaction conditions	yield (%)
1	10	BF ₃ •OEt ₂ (5 equiv)	22 °C, 3 h	92
2	4	SnCl ₄ (1 equiv)	−20 °C, 1 h to 22 °C, 2 h	50 (3); 45 (4)
3	4	BF ₃ •OEt ₂ (2 equiv)	22 °C, 2 h	trace
4	11	BF ₃ •OEt ₂ (2 equiv)	22 °C, 2 h	NR
5	1	BF ₃ •OEt ₂ (2 equiv)	22 °C, 2 h	NR

Reactions were run on a 1 mmol scale in anhydrous CH₂Cl₂ containing TMSN₃ (1.1 equiv). Recovery of starting material (**4**) is additionally shown in entry 2. NR: no reaction observed.