Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 5189–5192

The synthesis of facial amphiphile 3α,7α-diaminocholestane

Sharaf Nawaz Khan, Nam-Ju Cho and Hong-Seok Kim*

Department of Applied Chemistry, Kyungpook National University, Daegu 702-701, Republic of Korea

Received 6 April 2007; revised 25 May 2007; accepted 28 May 2007
Available online 2 June 2007

Abstract—The facial amphiphile 3α,7α-diaminocholestane **3** was synthesized from 3β-acetoxy-7-ketocholestane **1** through a stepwise reductive amination. The reductive amination of **1** with NH_4OAc in the presence of $NaBH_3CN$, and protection with Boc_2O yielded 7α-(*tert*-butyloxycarbonyl)-aminocholestane **4** in 86% yield. The reductive amination of **6**, which was obtained from **4** after hydrolysis and subsequent oxidation, with NH_4OT_f in the presence of $NaBH(OEh)_3$ provided **3** in 75% yield after protection with Boc_2O.

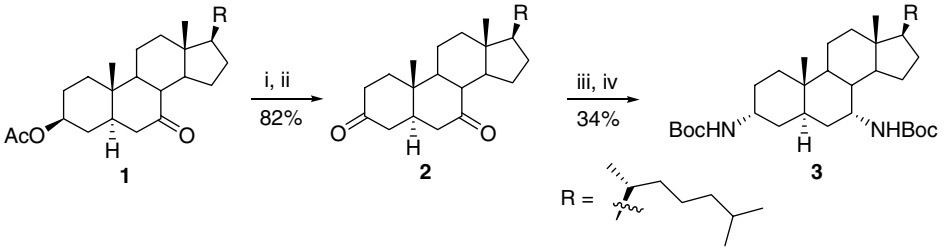
1. Introduction

Hydrophilic and hydrophobic equivalents constituting facial amphiphilic molecules supported by molecular modeling experiments are shown to sensitize cell membranes leading to cell death.[1] These molecules are promising antibiotics against microbes.[2] Steroid-based facial amphiphiles are advantageous because hydrophobic steroid molecules provide a rigid framework, and hydrophilic axial functionality to the molecules serves as an appendage.[3] Non-steroidal facial amphiphiles and 'tripod amphiphiles' have also been reported.[4] Facial amphiphiles are distinguished for their ion transport and membrane selectivity,[5] and they are utilized to synthesize molecular receptors especially in anion recognition.[6,7] The introduction of facial amino groups to steroid molecules increases hydrophilicity and offers flexibility, which can be manipulated for further modifications. Cholic acid based receptors 'cholapods', reported by Davis et al., facilitate the transport of chloride ions across membranes,[6] and are widely exploited for the synthesis of facial amphiphiles to introduce axial triamino-functionality at C3–C7–C12. Cholic acid possesses a 5β AB cis architecture and has a C–O bond angled at ~70° for C3–C7–C12, while for allocholic acid a 5α AB trans configuration is ~90°.[8] Receptors synthesized with 5α AB trans geometry provide some extra space between C3 and C7 and exhibit improved binding constants for anions.[8] The preferred method to introduce an amino group into keto-steroids is by oximation and subsequent reduction,[6,7] although the less familiar reductive amination (RA) is also known.[9] Previously, cholesteryl acetate was converted to 7α-aminocholesterol via a two-step reaction: the introduction of the azide group at the 7α position, by treatment with $(CH_3)_3SiN_3$ in the presence of DDQ, and the subsequent reduction with $LiAlH_4$, afforded 7α-aminocholesterol in 36% yield. 7β-Aminocholesterol was prepared through the oximation of 7-ketocholesteryl acetate and subsequent reduction with DIBAH in 27% yield.[10] Common obstacles for the preparation of aminosteroids include multi-step syntheses, and the low overall yield carves out a niche for considerable improvements. We have described a highly stereoselective procedure for 3α-aminosteroid from 3-ketosteroid[11] and in a follow-up, we report a high-yielding and stereoselective procedure that synthesizes 7α-aminocholestane and 3α,7α-diaminocholestane.

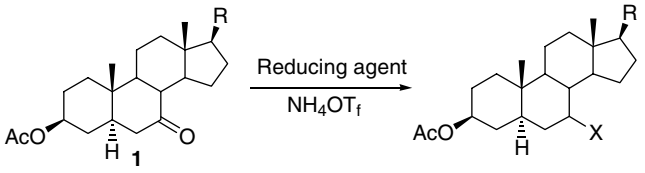
To synthesize the facial amphiphile **3**, first we performed a one step RA of diketone **2** as shown in Scheme 1. The direct RA of **2** with $NaBH_3CN$ in the presence of NH_4OAc generated **3** in a low yield (34%) and a mixture of α and β isomers of 3,7-dihydroxycholestane as side products. The RA of 3-ketosteroid with $NaBH_3CN$ in the presence of NH_4OAc produced preferentially 3β-isomer.[11] With other reducing reagent such as $NaBH_3(OAc)$, RA of diketone **2** did not produce any desired product **3**. Consequently, we investigated the sequential stepwise procedure to improve the yield and stereoselectivity of 3α,7α-diaminocholestane **3** starting from readily available compound **1**. The RA of **1** with NH_4OT_f, in the presence of reducing agents, gave a mixture of 7-hydroxy and 7-amino compounds, as shown in

Keywords: Stereoselective; Facial amphiphile; Reductive amination; 3α,7α-Diaminocholestane.
* Corresponding author. Tel.: +82 53 9505588; fax: +82 53 9506594; e-mail: kimhs@knu.ac.kr

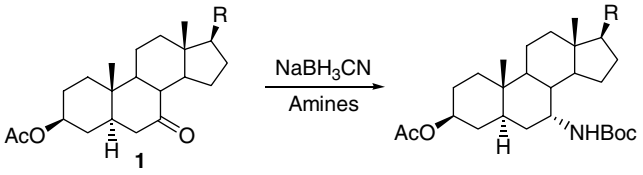
0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.05.157



Scheme 1. The RA of 3,7-diketocholestane. Reagents and conditions: (i) KOH/EtOH; (ii) PCC, CH_2Cl_2; (iii) NH_4OAc, $NaBH_3CN$, MeOH/THF = 1:1; (iv) Boc_2O, MeOH.



Scheme 2. The RA of 3β-acetoxy-7-ketocholestane (**1**) with various reducing agents.

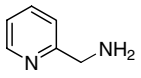


Scheme 3. The RA of 3β-acetoxy-7-ketocholestane (**1**) with various amines.

Scheme 2, and the results are summarized in Table 1. 7-Ketosteroid (1 equiv) and 10 equiv of NH_4OT_f with 2 equiv of $NaBH(OAc)_3$, in THF at room temperature, produced a 1:1 mixture of 7-hydroxy and 7-amino compounds in a low yield. In contrast to the RA of 3-ketosteroid, the RA of 7-ketosteroid requires large quantities of ammonia precursors. By increasing the amount of NH_4OT_f to 30 equiv, in a refluxing THF for 24 h, a mixture of 7-amino and 7-hydroxy compounds with a ratio of 10:1 in 86% yield was provided (Table 1, entry 1). In order to reduce the formation of the 7-hydroxy compound, other reducing agents were tested. With $NaBH_3CN$ in a mixed solvent (THF/MeOH, 1:1) at room temperature for 18 h, 7-NH_2/7-OH with a ratio of 7:3 in 78% yield was generated. When the reactions were carried out with other reducing agents, such as $NaBH_2(OAc)_2$, $NaBH_3(OAc)$, and picoline borane in THF, the 7-amino product was not formed.

To avoid drastic conditions we used $NaBH_3CN$ at room temperature, which resulted in 7-aminocholestane as shown in Scheme 3 and Table 2. To ease product separation, a formed primary amine was protected with Boc_2O in methanol. The RA of **1** with 30 equiv of NH_4OAc, and 2 equiv of $NaBH_3CN$ at room temperature for 5 h provided 7α isomer in 86% yield. With HCO_2NH_4, the reaction gave a similar yield, but the diastereoselectivity of the product decreased compared to that of NH_4OAc and the side product, which was hydrolyzed at 3β-acetate, was formed. Among the tested ammonia sources, NH_4OAc, HCO_2NH_4, NH_4Cl, NH_4OT_f, and $CF_3CO_2NH_4$, NH_4OAc provided the best results and gave the highest yield with a high diastereoselectivity toward the 7α isomer including a short reaction time. The general applicability of the RA methodology with other amines gave satisfactory results, for example, *n*-butyl amine, 2-aminoethanol,

Table 2. The RA of 7-ketocholestane (**1**) with $NaBH_3CN$ in the presence of various amines[a]

Entry	Amines (30 equiv)	Time (h)	Yield (%)	Configuration
1	$CH_3CO_2NH_4$	5	86	7α
2	HCO_2NH_4	5	85	7α
3	NH_4OT_f	18	78	7α
4	$CF_3CO_2NH_4$	48	48	7α
5	NH_4Cl	72	—	—
6	*n*-$BuNH_2$	8	75	7α
7	$HOCH_2CH_2NH_2$	48	82	7α
8	Boc-spermidine	24	65	7α
9	$C_6H_5NH_2$	72	—	—
10	$C_6H_5CH_2NH_2$	24	62	7α
11	$C_6H_5CH_2CH_2NH_2$	24	71	7α
12		48	56	7α

[a] Reactions were carried out in a THF/MeOH = 1:1.

Table 1. The RA of 7-ketocholestane (**1**) with NH_4OT_f in the presence of various reducing agents

Entry	Reducing agent (2 equiv)	Time (h)	Temperature (°C)	X = NH_2/OH	Configuration	Yield (%)
1	$NaBH(OAc)_3$	24	Reflux	10:1	7α	86
2	$NaBH_3CN$	18	25	7:3	7α	78
3	$NaBH_2(OAc)_2$[a]	48	Reflux	—	—	Trace
4	$NaBH_3(OAc)$[b]	48	Reflux	—	—	Trace
5	Picoline borane	48	25	—	—	0

[a] CH_3COOH (2 equiv) + $NaBH_4$ (1 equiv).
[b] CH_3COOH (1 equiv) + $NaBH_4$ (1 equiv).

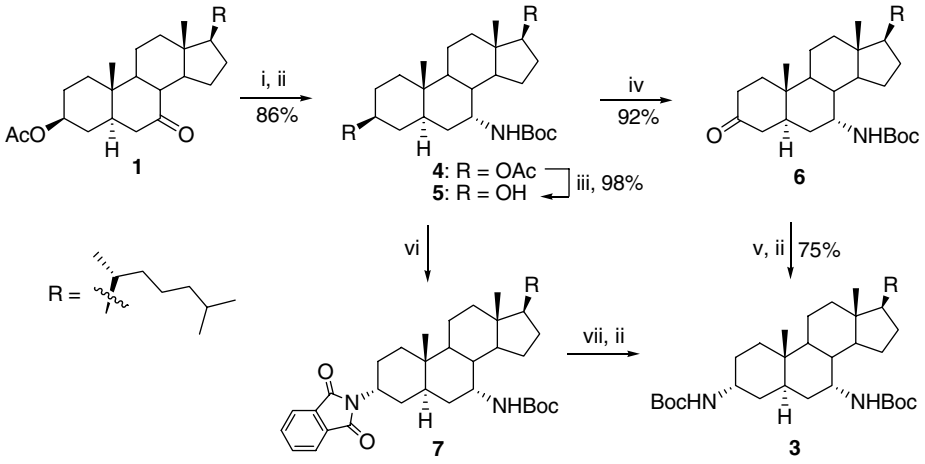
Boc-spermidine, benzylamine, phenethylamine, and 2-aminomethyl pyridine gave their corresponding amino products in moderate to good yields (Table 2, entries 6–12). But they required longer reaction times than $CH_3CO_2NH_4$. NH_4Cl (entry 5) and aniline (entry 9) did not produce any amino compound even after 72 h.

The stereochemistry of 7α-aminosteroid (**4**) was determined based on the R_f value and the chemical shifts of 7α-NH and 7-H protons in the ^1H NMR as well as the chemical shifts of C7 and C-13 in the ^{13}C NMR spectrum.[12] Further relative stereochemistry was confirmed by data obtained from COSY, HETCO, DEPT and comparisons with the similar published structure.[13] The IR spectrum of **4** showed characteristic N–H stretching, acetate and carbamate carbonyl bands at 3323, 1735 and 1686 cm^{-1}, respectively.

The second direct RA of **6** was carried out, as shown in Scheme 4, and the results are summarized in Table 3. The RA of **6**, which was obtained from **4** after hydrolysis and subsequent oxidation, with $NaBH(OAc)_3$ in the presence of NH_4OT_f, gave **3** as a mixture of a 3α/3β-isomer in 69% yield. High diastereoselectivity was achieved by modifying $NaBH_4$ with different carboxylic acids, in the synthesis of squalamine analogues.[11] Thus, in order to improve selectivity toward the 3α isomer, various acyloxyborohydrides were prepared from $NaBH_4$ and the bulky carboxylic acids. It had been observed that due to the increase in the bulkiness of the carboxylic acid, stereoselectivity was biased toward the 3α isomer. $NaBH_4$ (1.0 equiv) and carboxylic acid (3.0 equiv) generated in situ sodium tris(acyloxy)borohydride [$NaBH(OCOR)_3$].[14,15] A reagent prepared with 2-ethylhexanoic acid (Eh) improved stereoselectivity and the reaction rate also increased; it provided 3α/3β with a ratio of 9:1 in 85% yield. Isovaleric acid (Iv) provided similar results as Eh, however, Iv caused a bad odor when the reagent was prepared. $NaBH_3CN$ exhibited more selectivity toward the 3β isomer in a lower yield (55%).[16]

The ^1H NMR of **3** showed a 3α-NH proton of an α isomer (**3**) at δ 4.72 and a 3β-NH proton of a 3β isomer at δ 4.35. In the ^{13}C NMR spectrum of **3**, two carbamate carbons appeared at δ 155.7 and 147.1, and *tert*-butyloxy carbons appeared at δ 85.5 and 79.5. The IR spectrum of **3** showed two characteristic carbamate N–H stretching bands at 3460 and 3366 cm^{-1} and carbonyl stretching bands at 1718 and 1699 cm^{-1}. To examine the 3α stereochemistry, the 3β-hydroxy-7α-(*tert*-butyloxycarbonyl) aminosteroid (**5**) was transformed under Mitsunobu conditions to **7**. Treatment of **5** with diethyl azodicarboxylate (DEAD), PPh_3, and phthalimide produced **7** followed by hydrazinolysis with H_2NNH_2 in refluxing ethanol, and protection with Boc_2O provided the 3α isomer. The spectroscopic data of the obtained 3α isomer were identical with **3**.

In conclusion, we have developed a method, which will be useful in the synthesis of aminosteroids, with high stereoselectivity. The one step RA of 3,7-diketocholestane (**2**) with $NaBH_3CN$ provided **3** in 34% yield, but the sequential RA of 7-ketosteroid (**1**) with $NaBH_3CN$, followed by RA of resulting 3-ketosteroid (**6**) with NaB-



Scheme 4. The synthesis of 3α,7α-bis(*tert*-butyloxycarbonyl)amino-5α-cholestane **3**. Reagents and conditions: (i) NH_4OAc, $NaBH_3CN$, MeOH/THF = 1:1; (ii) Boc_2O, MeOH; (iii) KOH/EtOH; (iv) PCC, CH_2Cl_2; (v) NH_4OTf, $NaBH(OEh)_3$, THF; (vi) DEAD, PPh_3, phthalimide, THF; (vii) H_2NNH_2, EtOH.

Table 3. The RA of 3-keto-7α-aminocholestane (**6**) with various reducing agents

Entry	Amines	Reagent	Solvent (THF/MeOH)	Time (h)	Product ratio (3α:3β)	Yield (%)
1	NH_4OT_f	$NaBH(OAc)_3$ (2 equiv)	1:0	1	7:3	69
2	NH_4OT_f	$NaBH(OEh)_3$ (2 equiv)	1:0	1	9:1	85
3	NH_4OT_f	$NaBH(OIv)_3$ (2 equiv)	1:0	2	9:1	79
4	NH_4OAc	$NaBH_3CN$ (1 equiv)	1:1	0.5	4:6	55

$H(OEh)_3$, yielded the desired product **3** in 58% yield. The synthesis of facial amphiphile 3α,7α-diaminocholestane **3** from 3β-acetoxy-7-ketocholestane (**1**) was achieved in total yields of 58% via two direct RAs.

Acknowledgments

This research was supported by the Kyungpook National University Fund, 2004, and the BK 21.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.05.157.

References and notes

1. Li, C.; Peters, A. S.; Meredith, E. L.; Allman, G. W.; Savage, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 2961.
2. (a) Chen, W. H.; Shao, X. B.; Moellering, R.; Wennersten, C.; Regen, S. L. *Bioconjugate Chem.* **2006**, *17*, 1582; (b) Ding, B.; Guan, Q.; Walsh, J. P.; Boswell, J. S.; Winter, T. W.; Winter, E. S.; Boyd, S. S.; Li, C.; Savage, P. B. *J. Med. Chem.* **2002**, *45*, 663; (c) Guan, Q.; Li, C.; Schmidt, E. J.; Boswell, J. S.; Walsh, J. P.; Allman, G. W.; Savage, P. B. *Org. Lett.* **2000**, *2*, 2837.
3. (a) Savage, P. B.; Li, C.; Taotafa, U.; Ding, B.; Guan, Q. *FEMS Microbiol. Lett.* **2002**, *217*, 1; (b) Taotafa, U.; McMullin, D. B.; Lee, S. C.; Hansen, L. D.; Savage, P. B. *Org. Lett.* **2000**, *2*, 4117.
4. (a) Menger, F. M.; Sorrells, J. L. *J. Am. Chem. Soc.* **2006**, *128*, 4960; (b) McQuade, T. D.; Quinn, M. A.; Yu, S. M.; Polans, A. S.; Krebs, M. P.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2000**, *39*, 758.
5. (a) Bandyopadhyay, P.; Janout, V.; Zhang, L.; Regen, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 7691; (b) Janout, V.; Lanier, M.; Regen, S. L. *J. Am. Chem. Soc.* **1997**, *119*, 640.
6. (a) Clare, J. P.; Ayling, A. J.; Joos, J. B.; Sisson, A. L.; Magro, G.; Pérez-Payán, M. N.; Lambert, T. N.; Shukla, R.; Smith, B. D.; Davis, A. P. *J. Am. Chem. Soc.* **2005**, *127*, 10739; (b) McNally, B. A.; Koulov, A. V.; Smith, B. D.; Joos, J. B.; Davis, A. P. *Chem. Commun.* **2005**, 1087; (c) Del-Amo, V.; Siracusa, L.; Markidis, T.; Baragaña, B.; Bhattarai, K. M.; Galobardes, M.; Naredo, G.; Pérez-Payán, M. N.; Davis, A. P. *Org. Biomol. Chem.* **2004**, *2*, 3320; (d) Koulov, A. V.; Lambert, T. N.; Jain, M.; Boon, J. M.; Smith, B. D.; Li, H. Y.; Sheppard, D. N.; Joos, J. B.; Clare, J. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2003**, *42*, 4931; (e) Lambert, T. N.; Boon, J. M.; Smith, B. D.; Pérez-Payán, M. N.; Davis, A. P. *J. Am. Chem. Soc.* **2002**, *124*, 5276; (f) Ayling, A. J.; Broderick, S.; Clare, J. P.; Davis, A. P.; Pérez-Payán, M. N.; Lahtinen, M.; Nissinen, J. J.; Rissanen, K. *Chem. Eur. J.* **2002**, *8*, 2197; (g) Siracusa, L.; Hurley, F. M.; Dresen, S.; Lawless, L. J.; Pérez-Payán, M. N.; Davis, A. P. *Org. Lett.* **2002**, *4*, 4639.
7. (a) Zhou, X.-T.; Rehman, A.-U.; Li, C.; Savage, P. B. *Org. Lett.* **2000**, *2*, 3015; (b) Barry, J. F.; Davis, A. P.; Pérez-Payán, M. N.; Elsegood, M. R. J.; Jackson, R. F. W.; Gennari, C.; Piarulli, U.; Gude, M. *Tetrahedron Lett.* **1999**, *40*, 2849; (c) Davis, A. P.; Pérez-Payán, M. N. *Synlett* **1999**, 991; (d) Li, C.; Rehman, A.; Dalley, N. K.; Savage, P. B. *Tetrahedron Lett.* **1999**, *40*, 1861; (e) Broderick, S.; Davis, A. P.; Williams, R. P. *Tetrahedron Lett.* **1998**, *39*, 6083.
8. Bhattarai, K. M.; Del-Amo, V.; Magro, G.; Sisson, A. L.; Joos, J. B.; Charmant, J. P. H.; Kantacha, A.; Davis, A. P. *Chem. Commun.* **2006**, 2335.
9. Cheng, Y.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1996**, *118*, 1813.
10. Choucair, B.; Dherbomez, M.; Roussakis, C.; El Kihel, L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4213.
11. Khan, S. N.; Bae, S. Y.; Kim, H.-S. *Tetrahedron Lett.* **2005**, *46*, 7675.
12. See Supplementary data.
13. (a) El Kihel, L.; Choucair, B.; Dherbomez, M.; Letourneux, Y. *Eur. J. Org. Chem.* **2002**, 4075; (b) Fouace, S.; El Kihel, L.; Dherbomez, M.; Letourneux, Y. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3011.
14. Gribble, G. W. *Chem. Soc. Rev.* **1998**, *27*, 395.
15. Burks, J. E.; Espinosa, L.; LaBell, E. S.; McGill, J. M.; Ritter, A. R.; Speakman, J. L.; Williams, M. A. *Org. Process Res. Dev.* **1997**, *1*, 198.
16. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.