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Synthesis and comparative spectroscopic analysis of two chenodeoxycholic acid (CDCA) derivatives with closely related 7α-ester moieties

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ABSTRACT

3α-Hydroxyl-7α-(4-pentenoyloxy)-5β-cholanoic acid (**5**) has been synthesized in four step reactions starting from CDCA. The serendipitous synthesis of methyl 3α-(ethoxycarbonyloxy)-7α-(allyloxycarbonyloxy)-5β-cholanoate (**7**) has led us to compare the spectroscopic difference of the 7α-(allyloxycarbonyloxy) group versus the 7α-(4-pentenoyloxy) group. The molecular structures of these compounds were confirmed by X-ray crystallography. Methyl 3α-(ethoxycarbonyloxy)-7α-(allyloxycarbonyloxy)-5β-cholanoate was obtained by a method, which may prove useful in the synthesis of ^{14}C-labeled derivatives for metabolic studies.

1. Introduction

In recent years, there has been increased interest in developing prodrugs to target various transporters. Some peptides and oral drugs have been attached to bile acids to give prodrugs to improve their bioavailability.[1] Acyclovir which is an antiviral drug with a poor intestinal permeability has been linked to acidic group of C-24 of CDCA (chenodeoxycholic acid) to give a prodrug to target hASBT (the human apical sodium-dependent bile acid transporter). The oral administration of this prodrug showed that the bioavailability of acyclovir ester was enhanced two times compared to that of acyclovir alone.[2,3] The use of peptide drugs is limited by their poor oral absorption and enzymatic hydrolysis in GI tract. Drug substrates conjugated to a compound which can be transported efficiently by intestinal carrier system is one of the practical strategies to overcome the drawback of peptide drugs. Swaan et al.[4] attached a peptide moiety to the 24-carboxylic acid group of cholic acid using automatic peptide synthesizer. This resulted in increased intestinal absorption and metabolic stability. Based on the concept that hASBT is the major transporter of bile acids, designing bile acid conjugates represents a strategy for drug delivery. Though ^{3}H-labeled taurocholic acid has been used to study the transportation mechanism of hASBT,[5,6] the mechanism of interaction of hASBT with bile acids is still not fully understood. In our group, work has been focused on the selective etherization and esterification of sterically hindered 7α-hydroxy group of CDCA to make some novel CDCA derivatives as moieties for eventual construction of macro-cyclomers, which represent another venue for drug delivery systems based on host/guest interactions. We have serendipitously synthesized a CDCA derivative (**7**) with ambient CO_2 that offers a pathway to make ^{14}C-labeled bile acid derivatives, which can be used to explore the transport mechanism of hASBT through labeling with $^{14}CO_2$. In addition, for most bile acid based prodrugs, the drug substrate is usually covalently coupled to C24 or C3-position, with little effort directed toward attaching drugs to C7-position.[7] The selective functionalization of 7α-OH group to give the novel derivative **5** could inspire the development of prodrugs with drug conjugated at C7 and further study their effectiveness and bioavailability.

2. Results and discussion

The synthesis of compound **5** is shown in Scheme 1. The carboxylic acid on CDCA (**1**) was methyl esterified to give ester **2**.[8] Then, the hydroxyl group on 3α-position of ester **2** was selectively protected by reacting with ethyl chloroformate in dried pyridine at −20 °C to form 7α-monohydroxyl ester **3,** using the previous published conditions.[9] Ester **4** was produced by reacting 7α-hydroxyl group of **3** with 4-pentenoic acid, 2,6-dichlorobenzoyl chloride, and DMAP via Yamaguchi reaction.[10] Careful saponification of ester **4** gave the desired 3α-hydroxy-7α-(4-pentenoyloxy)-5β-cholanoic acid (**5**), which is an important intermediate for further macrolactonization and ring-closing metathesis by using Grubbs' catalyst.[11]

^{1}H NMR and ^{13}C NMR assignments of the important functional groups of **5** were identified by applying ^{1}H, decoupled ^{13}C, 2D H–H COSY and coupling ^{13}C NMR techniques (cf. with Supplementary data).

The synthesis and proposed formation of ester **7** are described in Scheme 2. The route for synthesizing methyl 3α-(ethoxycarbonyloxy)-7α-(allyloxy)-5β-cholanoate (**8**) was designed by silylation

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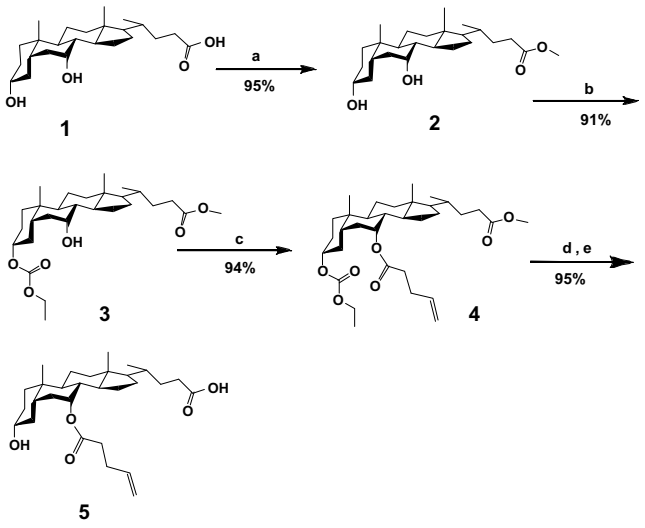
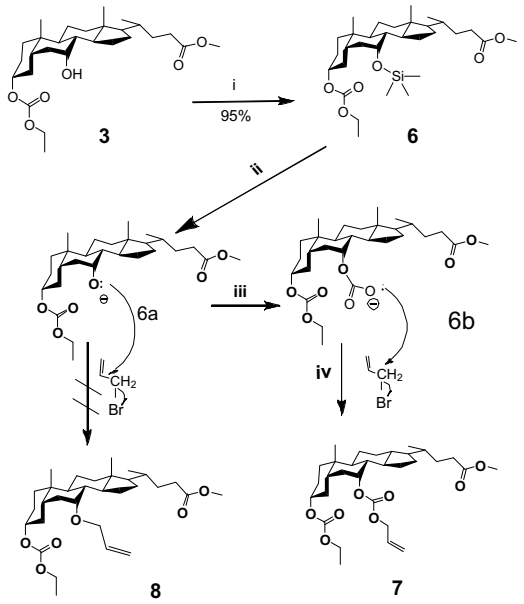


Scheme 1. Synthesis of **5**. Reagents and conditions: (a) AcCl, CH_3OH, at 0 °C, 1 h; (b) ClCOOEt, py at −20 °C, 1 h; (c) 2,6-dichlorobenzoyl chloride, 4-pentenoic acid, Et_3N, DMAP, rt, 24 h; (d) K_2CO_3, CH_3OH, THF, reflux, 12 h; (e) 3 M HCl.



Scheme 2. Synthesis and proposed formation of **7**. Reagents and conditions: (i) TMCS, imidazole, THF, rt, 12 h; (ii) TBAF, THF, rt, 10 h; (iii) CO_2 in the air; (iv) allyl bromide, rt, 6 h.

of 7α-OH on 7α-monohydroxyl ester **3** with TMCS and imidazole in THF,[12] followed by dried TBAF desilylation of **6** to presumably produce an alkoxide intermediate, which undergoes S_N2 with allyl bromide to afford allyl ether.[13] The results of mass spectroscopy further informed us that the molecular weight of this compound (562.3492) is 44 mass units higher than that of anticipated product (**8**) with MW (518.36). The 44 mass units difference is consistent with CO_2 insert between alkoxide ion and allyl group. X-ray crystallography proved that CO_2 is really involved in this reaction. Apparently, the compound was methyl 3α-(ethoxycarbonyloxy)-7α-(allyloxycarbonyloxy)-5β-cholanoate (**7**) instead of methyl 3α-(ethoxycarbonyloxy)-7α-(allyloxy)-5β-cholanoate (**8**) synthesized by following the designed route.

The serendipitous synthesis of compound **7** suggests that the steric hindrance of alkoxide ion on **6a** impedes its direct reaction with allyl bromide compared to the more acidic CO_2. The alkoxide ion thus reacts with CO_2, which is more acidic and smaller to form more extended, less sterically hindered carboxylate anion **6b** which can rapidly attack allyl bromide via a typical S_N2 mechanism to afford compound **7**. We have tried to synthesize **8** by rigorous exclusion of atmospheric CO_2. When reaction was carried out either under N_2 gas or in sealed glassware with degassed reaction mixture, we failed to get even a little **8**. This indicates that it may not be possible to obtain **8** by using 7α-O^- attached on steroid skeleton to attack allyl bromide via S_N2 mechanism due to steric hindrance of 7α-position, where 7α-O^- is located in the concave face of steroid skeleton. Since TBAF is very hygroscopic, it has been difficult to remove water molecules completely from it. The yield of compound **7** is only 30%. Large amount of product **3** was recovered due to the presence of even trace amount of water in reaction system. Synthesis of **7** has to be proceeded with THF freshly distilled from sodium and potassium alloy and anhydrous TBAF, which is dried by following procedure in the literature.[14] The use of TBAT and cesium fluoride which are available in anhydrous form failed to deprotect the silyl group on **6**, probably, due to the steric bulk of TBAT and the low solubility of CsF in THF, Although TBAT and CsF are very useful as a good source to provide dry F^-.[15–18]

Single-crystal structures of **5** and **7** were determined by X-ray diffraction as shown in Figures 1 and 2.[19] The crystal of **5** is orthorhombic (*P* 21 21 21 space group), and 7α-OH has been replaced by 4-pentenoyloxy group, therefore, there is no hydrogen bond involved in 7α-position, which makes the hydrogen-bonding network of **5** less complex than that of CDCA single-crystal structure.[20] The 3α-hydroxyl and 24-carboxylic acid groups provide two hydrogen-bonding sites, and each site forms two hydrogen bonds with other two molecules. Each molecule **5** is interconnected with four molecules via two different hydrogen bonds ($O_1 \rightarrow O_4$: 2.73 Å, $O_5 \rightarrow O_1$: 2.61 Å) in Figure 1a. O_4 on carboxylic acid group is hydrogen bond acceptor, and O_5–H of carboxylic acid is hydrogen bond donor, but O_1 on 3α-OH group acts as both hydrogen bond donor and acceptor. The distance of hydrogen bond between H_{10} and O_4 is 1.94 Å, and between H_{50} and O_1 is 1.85 Å as shown in Figure 1a. The crystal structure of compound **7** is monoclinic (C_2) and tightly compacted and arrayed by extensive intermolecular van der Waals interactions as shown in Figure 2. The distances of $H_4 \cdots H_{19A}$, $O_6 \cdots H_{6A}$, $O_1 \cdots H_{19}$, and $H_{31C} \cdots O_7$ are 2.36 Å, 2.36 Å, 2.54 Å, 2.64 Å, respectively, as shown in Figure 2a. These intermolecular hydrophobic interactions are a significant driving force to make compound **7** form a single crystal (Fig. 2) even though the 3α-OH, 7α-OH, and 24-carboxylic acid functional groups are replaced by three different ones. There are distinctive differences in the ethenyl moieties belonging to the 4-pentenoyl (**5**) and allyloxycarbonyl (**7**) groups. In the ^1H NMR and ^{13}C NMR spectra, both the methylene and the methine protons and carbons of the latter are more deshielded due to the influence of the extra oxygen. In the X-ray crystal structures, the carbonyl moiety in the former is directed toward, and in the latter away from the steroid skeleton. Also, the carbonyl moiety tends to be *anti* to the 7β-hydrogen in the former and *syn* in the latter.

In summary, the structures of **5** and **7** have been fully characterized by ^1H NMR, ^{13}C NMR, HRFAB, and X-ray crystallography. Further efforts will be focused on improving the yield of **7** and on applying this method to other compounds with hydroxyl functional groups. A novel method has evolved for attaching allyloxycarbonyloxy group to 7α-position via first deprotecting silyl group, then introducing CO_2, and finally adding allyl bromide into reaction system. This route may find application in ^{14}C-labeled substrates of drugs via $^{14}CO_2$. Compound **7** and its derivatives will

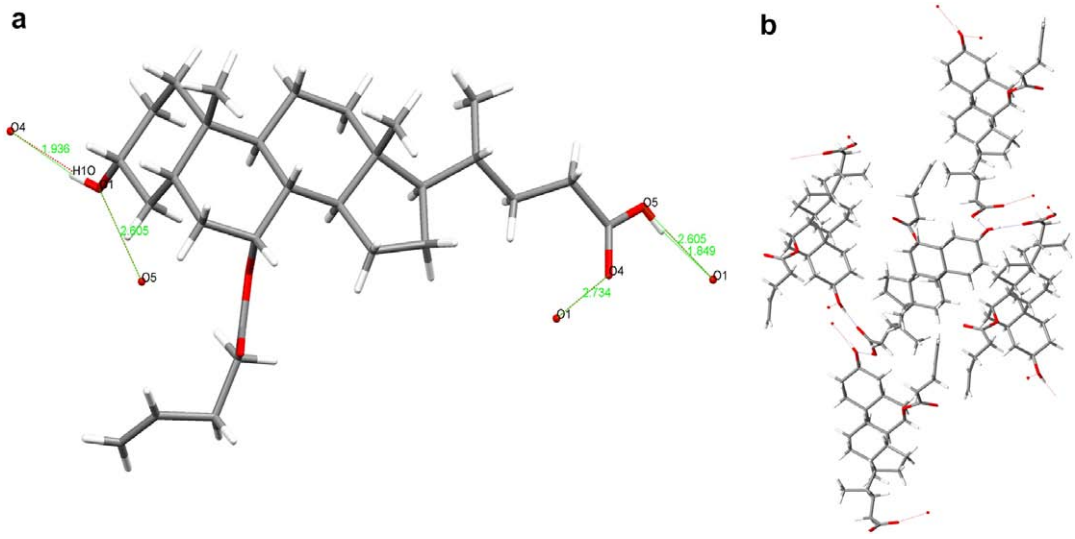


Figure 1. Single-crystal X-ray structure of **5**. (a) Distances (in Å) of the hydrogen bonds observed in **5**. (b) Top view of hydrogen bond network formed by five molecules of **5**.

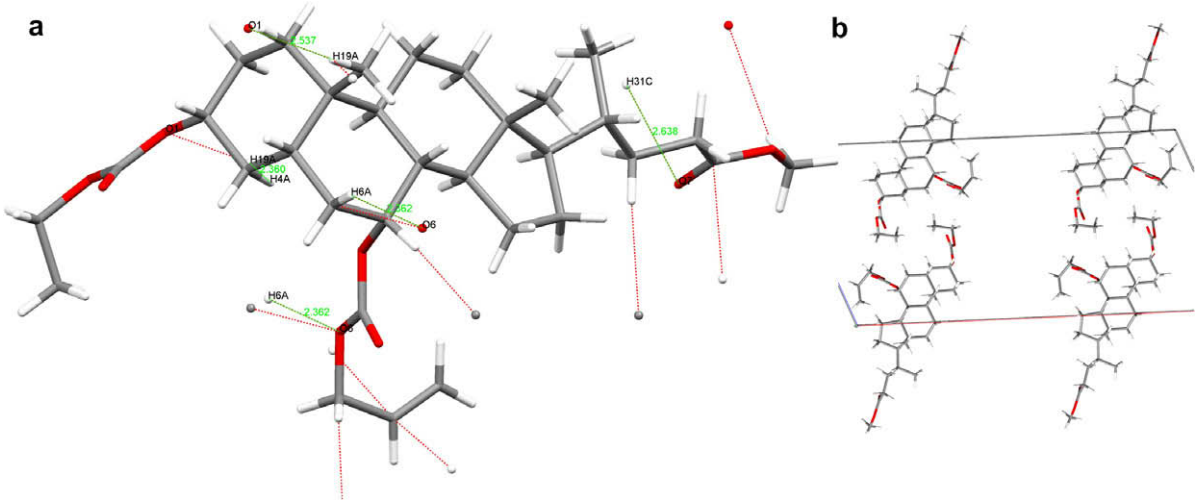


Figure 2. Single-crystal X-ray structure of **7**. (a) Distances (in Å) of intermolecular interaction observed in the **7**. (b) Top view of unit cell and packing of **7** from *b*-axis.

have a lot of potential applications in the study of the metabolic mechanism of drugs in human's liver.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.11.052.

References and notes

1. Sievanen, E. *Molecules* **2007**, *12*, 1859–1889.
2. Tolle-Sander, S.; Lentz, K. A.; Maeda, D. Y.; Coop, A.; Polli, J. E. *Mol. Pharmaceut.* **2004**, *1*, 40–48.
3. Balakrishnan, A.; Polli, J. E. *Mol. Pharmaceut.* **2006**, *3*, 223–230.
4. Swaan, P. W.; Hillgren, K. M.; Szoka, F. C., Jr.; Oie, S. *Bioconj. Chem.* **1997**, *8*, 520–525.
5. Alrefai, W. A.; Sarwar, Z.; Tyagi, S.; Saksena, S.; Dudeja, P. K.; Gill, R. K. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2005**, *288*, G978–G985.
6. Weinman, S. A.; Carruth, M. W.; Dawson, P. A. *J. Biol. Chem.* **1998**, *273*, 34691–34695.
7. Kramer, W.; Schuber, G.; Bickel, M.; Hoffmann, A.; Baringhaus, K. H., et al *Falk Symp.* **1993**, *68*, 161–176.
8. Gao, H.; Dias, J. R. *Eur. J. Org. Chem.* **1998**, 719–724.
9. Gao, H.; Dias, J. R. *Org. Prep. Proced. Int.* **1999**, *31*, 145–166.
10. Dhimitruka, I.; SantaLucia, J., Jr. *Org. Lett.* **2006**, *8*, 47–50.
11. Lee, C. W.; Choi, T.-L.; Grubbs, R. H. *J. Am. Chem. Soc.* **2002**, *124*, 3224–3225.
12. Li, Y.; Dias, J. R. *Synthesis* **1997**, 425.
13. Marzinzik, A. L.; Felder, E. R. *Molecules* **1997**, *2*, 17–30.
14. Wiles, C.; Watts, S.; Haswell, S. J.; Villar, E. P. *Lab On a Chip* **2001**, 100.
15. Aggarwal, V. K.; Alonso, E.; Ferrara, M.; Spey, S. E. *J. Org. Chem.* **2002**, *67*, 2335–2344.
16. Pilcher, A. S.; DeShong, P. *J. Org. Chem.* **1996**, *61*, 6901.
17. Poissonnet, G.; Theret-Bettiol, M.-H.; Dodd, R. H. *J. Org. Chem.* **1996**, *61*, 2273–2282.
18. Fraser, C. L.; Anastasi, N. R.; Lamba, J. J. S. *J. Org. Chem.* **1997**, *62*, 9314–9317.
19. Cambridge Crystallographic Data Centre: Allocated the deposition numbers CCDC 701461 and CCDC 701462.
20. Alvarez, M.; Jover, A.; Carrazana, J.; Meijide, F.; Soto, V. H.; Vázquez Tato, J. *Steroids* **2007**, *72*, 535–544.