Lipophilic X-ray contrast agents diatrizoyl double esters with cholesterol and cholic acid

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Dedicated to Professor Kjell Undheim on the Occasion of his 70th. birthday
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Abstract
Potential biodegradable and organ-specific X-ray contrast agents have been prepared by linking diatrizoic acid to cholesterol and cholic acid, respectively, via a spacer group containing a carbonate double ester. In vitro experiments revealed that the cholic acid esters were enzymatically degraded in the presence of pig liver esterase, while the double esters with cholesterol appeared to be stable, possibly due to low solubility in water.

Keywords: X-Ray contrast agents, diatrizoic acid, cholesterol, cholic acid, double ester carbonates, pig liver esterase

Introduction
X-ray contrast agents for parenteral use today are triiodinated benzene derivatives with hydrophilic substituents to secure high water-solubility. These agents have similar pharmacokinetic properties such as extracellular distribution and renal elimination.1,2 During the past there has been considerable interest in organospecific contrast agents; e.g. agents for liver imaging.1,3 Various approaches have been evaluated to target contrast agents to the liver: liposome encapsulated water-soluble iodinated agents,4 iodinated fat emulsions,5 solid particles,6 and iodinated cholesterol derivatives.7-9 Long residual time in the liver and other organ systems is a major problem with these agents.
Bile acid drug conjugates, e.g. the anticancer drug chlorambucil,\textsuperscript{10,11} are potential liver-specific targeting drugs. So-called double esters are frequently used in prodrug design because of their high affinity for unspecific esterases.\textsuperscript{12} The antibiotic pivampicillin is a typical example.\textsuperscript{13} Double esters have also been evaluated in particulate contrast agents.\textsuperscript{6}

Chloroethoxycarbonyl chloride has been extensively used to prepare linkers in contrast agent such as gas generating polymers.\textsuperscript{14-16} The preparation of potential biodegradable conjugates between diatrizoic acid and the steroids cholesterol and cholic acid, respectively, and evaluation of their degradability \textit{in vitro}, constitutes the subject of the present report.

**Results and Discussion**

**Synthesis**
Epimeric cholest-5-en-3\textbeta-yl 1(R,S)-chloroethyl carbonates (2) were prepared in quantitative yield by esterification of cholesterol (I) with (±)-1-chloroethoxycarbonyl chloride according to Dang \textit{et al.},\textsuperscript{17} cf. Scheme 1. The epimeric double esters 3 were subsequently prepared in 66% yield by esterification of diatrizoic acid as its tetrabutylammonium salt with the chlorocarbonates 2 in DMF, in the presence of catalytic amounts of potassium iodide.

\textit{t}-Butyl cholate (5) was prepared as described by Bonar-Law\textsuperscript{18} who first reacted cholic acid (4) with trifluoroacetic anhydride (TFAA) to the corresponding C-24 mixed anhydride and with the C-3 and C-7 hydroxyl groups trifluoromethyl acetylated, \textit{cf.} Scheme 2. This mixed anhydride was then treated with \textit{t}-butanol followed by hydrolysis of the C-3 and C-7 trifluoromethyl acetyl groups with aqueous ammonia furnishing \textit{t}-butyl cholate (5).

The C-3 position of \textit{t}-butyl cholate (5) was selectively\textsuperscript{19-21} \textit{O}-acylated with (±)-1-chloroethoxycarbonyl chloride to the chlorocarbonate esters 6 in 85% yield. Subsequent esterification of the chlorocarbonates 6 with tetrabutylammonium diatrizoate in DMF using potassium iodide as catalyst, yielded the \textit{t}-butyl protected esters 7 in 79% yield. Deprotection with TFA furnished the epimeric cholic acid conjugates 8 in 95% yield.
Scheme 1.  a: (±)-1-Chloroethoxycarbonyl chloride, pyridine, CH₂Cl₂; b: Diatrizoic acid as Bu₄N⁺ salt, KI, DMF
Enzymatic degradation

Degradation studies with pig liver esterase revealed that the cholic acid conjugates 8 was a good substrate for the enzyme. The half life of the two epimeric esters 8 was found to be less than three hours.

The cholesterol derived double esters 3 appeared to be stable in similar degradation studies. The stability might be due to its low solubility in aqueous solutions.
Experimental Section

General Procedures. $^1$H NMR spectra were recorded at 200, 300, and 500 MHz using Varian Gemini 200, Varian XL 300 (manual), and Bruker Avance DRX 500 spectrometers. $^{13}$C NMR spectra were recorded at 50, 75 and 125 MHz using the above mentioned spectrometers. Assignments of NMR shifts have been limited to the diagnostically important parts, e.g. the contrast agent segment and the spacer (= the 1-chloro-ethoxycarbonate portion). Similarly, we report only assigned $^{13}$C NMR shifts for selected parts of the molecules comprising the contrast agent segment, the spacer and the resonances of C$_3$, C$_7$, C$_{12}$, C$_{21}$ in the steroid moieties. Additional resolved resonances are listed without assignments. Presented assignments for $^1$H - and $^{13}$C NMR spectra are based on published data for relevant compounds.$^{22-24}$ Melting points were measured with a Reichert melting point microscope and are uncorrected. FAB and EI spectra were obtained using a Trio-2 Mass Spectrometer, VG Biotech Ltd. The EI spectra were recorded using 70 electronvolt ionizing voltage. Electrospray MS was obtained using a Bruker Apex 4.7 instrument. Elemental analyses were performed by Ilse Beetz, Kronach, Germany. TLC analyses were performed on silica gel plates (Merck 4500).

Cholest-5-en-3β-yl 1(R,S)-chloroethyl carbonates (2). Cholest-5-en-3β-yl 1(R,S)-chlo-roethyl carbonates (2) were prepared in quantitative yield as described by Dang et al.$^{17}$

Cholest-5-en-3β-yl 1(R,S)-(3,5-diacetamido-2,4,6-triodobenzoyloxy)ethyl carbonates (3). Diatrizoic acid tetrabutylammonium salt (1.73 g, 2.0 mmol) was added to a solution of cholest-5-en-3β-yl 1(R,S)-chloroethyl carbonates (2, 1.00 g, 2.0 mmol) and potassium iodide (0.04 g, 0.24 mmol) in DMF (30 ml) and stirred for 3 h at 60 °C. The mixture was cooled and diluted with CHCl$_3$ (150 ml). The organic phase was washed with saturated aqueous NaHCO$_3$ (4 x 150 ml), water (2 x 150 ml) and dried over Na$_2$SO$_4$. The solvent was removed in vacuo and the product purified by flash chromatography on silica gel (5% MeOH/CHCl$_3$) furnishing the carbonates 3 as a colourless solid (1.41 g, 66%). Mp. 221 - 222 °C (dec.); R$_f$ 0.38 (5% MeOH/CHCl$_3$); $^1$H NMR (500 MHz, DMSO-d$_6$): $\delta$ 0.64 (3 H, s, C-18(H$_3$)), 0.83 (6 H, d, C-26/27(H$_3$)), 0.88 (3 H, d, C-21(H$_3$)), 0.96 (3 H, s, C-19(H$_3$)), 1.64 (3 H, d), 2.00 (6 H, s, CH$_3$CONH-), 4.39 (1 H, m), 5.35 (1 H, m), 6.90 (1H, m), 10.04 (2 H, d, NH); $^{13}$C NMR (125 MHz, DMSO-d$_6$): $\delta$ 12.3 (C-18), 19.2 (C-21), 19.5 (C-19), 23.9 (C-39, C-41), 27.8 (C-30), 36.7 (C-10), 37.0 (C-1) 78.8 (C-3), 79.8 (C-29), 97.0 (C-33, C-37), 109.7 (C-35), 123.1 (C-6), 139.8 (C-5), 145.4 (C-32), 146.2 (C34, C-36), 152.2 (C-28), 166.1 (C-38, C-40), 168.3 (C-31). Anal. found (C) 45.75, (N) 2.87, (H) 5.27; Calcd. for C$_{41}$H$_{57}$N$_2$O$_7$I$_3$ (C) 46.00, (H) 5.37, (N) 2.62.

$\alpha$-[1(R,S)-Chloroethyl carbonate] t-butyl 7 α 12 α -dihydroxy-5β-cholan-24-oates (6). ($\pm$)-1-Chloroethoxycarbonyl chloride (0.40 g, 2.8 mmol) was added dropwise to a cooled (0 °C)
solution of t-butyl 3α,7α,12α-trihydroxy-5β-cholan-24-oate (5) (= t-butyl cholate\textsuperscript{18}, 1.00 g, 2.2 mmol) and 4-N,N-dimethylaminopyridine (0.36 g, 2.9 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (15 ml). The mixture was stirred for 24 h at room temperature and refluxed for 20 min. The solution was cooled, diluted with CH\textsubscript{2}Cl\textsubscript{2} (100 ml), and washed with saturated aqueous CuSO\textsubscript{4} (100 ml x 4), saturated aqueous NaHCO\textsubscript{3} (100 ml x 2), and brine (100 ml). The organic phase was dried over MgSO\textsubscript{4}. The solvent was removed under reduced pressure and the product purified by column chromatography on alumina (grade 1, 0.5% MeOH/CHCl\textsubscript{3}) giving the carbonates 6 as a solid (1.04 g, 85%). Mp. 76 - 80 °C; R\textsubscript{f} 0.66 (5% MeOH/CHCl\textsubscript{3}); $^1$H NMR (300 MHz, CD\textsubscript{2}Cl\textsubscript{2}): δ 0.67 (3 H, s, C-18(H\textsubscript{3})), 0.92 (3 H, s, C-21(H\textsubscript{3})), 0.96 (3 H, s, C-21(H\textsubscript{3})), 1.42 (9 H, s, 1.69 (3 H, d, 5.73 Hz), 3.84 (1 H, m, C-7(H)), 3.98 (1 H, m, C-12(H)), 4.47 (1 H, m, C-3(H)), 6.30 (1 H, q, 6.49 Hz); $^{13}$C NMR (75 MHz, CD\textsubscript{2}Cl\textsubscript{2}): δ 12.6 (C-18), 17.5 (C-21), 22.5 (C-19), 23.5 (C-15), 25.5 (C31), 26.9 (C-9), 28.2 (C-16, C-26, C-27, C-28), 28.5 (C-11) 31.3 (C-2, C-23), 32.8 (C22), 35.0 (C-6, C-10), 35.6 (C-1, C-20), 39.8 (C-4, C-8), 41.6 (C-5), 42.2 (C-14), 46.8 (C13), 47.6 (C-17), 68.5 (C-7), 73.3 (C-12), 80.0 (C-25, C-30), 84.9 (C-3) 154.4 (C-29), 174.6 (C-24). Anal. found (C) 65.24, (H) 9.34; Calcd. for C\textsubscript{31}H\textsubscript{51}O\textsubscript{7}Cl (C) 65.19, (H) 9.00.

3α-[1(R,S)-3,5-Diacetamido-2,4,6-triiodobenzoyloxy]ethyl carbonate| t-butyl 7 α, 12 α - dihydroxy-5β-cholan-24-oates (7). Diatrizoic acid tetrabutylammonium salt (0.99 g, 1.2 mmol) was added to a solution of 3α-[1(R,S)-chloroethyl carbonate] t-butyl 7α,12α-dihydroxy-5β-cholan-24-oates (6, 0.87 g, 1.4 mmol) and KI (0.02 g, 0.12 mmol) in DMF (20 ml). The mixture was kept at 60 °C for 3h, cooled, and diluted with CHCl\textsubscript{3} (100 ml). The CHCl\textsubscript{3}-solution was washed with saturated aqueous NaHCO\textsubscript{3} (4 x 100 ml) and water (2 x 100 ml). The organic phase was dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated to dryness in vacuo. The product was purified by column chromatography on alumina (grade 1, 3% MeOH/CHCl\textsubscript{3}) furnishing the carbonates 7 as a solid (0.88 g, 79%). Mp. 196-201 °C (dec.); R\textsubscript{f} 0.15 (5% MeOH/CHCl\textsubscript{3}); $^1$H NMR (200 MHz, CD\textsubscript{3}CO\textsubscript{2}H): δ 0.66 (3 H, s, C-18(H\textsubscript{3})), 0.89 (3 H, s, C-19(H\textsubscript{3})), 1.13 (3 H, d, C-21(H\textsubscript{3})), 1.42 (9 H, s, 1.69 (3 H, d, 5.73 Hz), 3.81 (1 H, m, C-7(H)), 3.95 (1 H, m, C-12(H)), 4.43 (1 H, m, C-3(H)), 7.34 (1 H, m), 8.85 (2 H, m, NH), $^{13}$C NMR (50 MHz, DMSO-d\textsubscript{6}): δ 23.8 (C-40, C-42), 27.3 (C31), 28.7 (C-26, C-27, C-28), 68.8 (C-7), 73.6 (C-12), 80.5 (C-3), 94.0 (C-30), 96.4 (C34, C-38), 108.6 (C-36), 145.0 (C-37), 145.1 (C-35), 147.1 (C-33), 153.2 (C-29), 167.2 (C-32), 170.4 (C-41) ; MS (FAB): m/z 1171 [M + Na\textsuperscript{+}], 1153 [M + Na - H2O\textsuperscript{+}], 1045 [M + Na + H – I\textsuperscript{+}]. Anal. found (C) 43.75, (H) 5.22, (N) 2.55; Calcd. for C\textsubscript{42}H\textsubscript{59}N\textsubscript{2}O\textsubscript{11}I\textsubscript{3} (C) 43.92, (H) 5.18, (N) 2.44.

(7 α,12 α-Dihydroxy-5β-cholan-24-oic acid 3α-yl) 1(R,S)-(3,5-diacetamido-2,4,6-triiodobenzoyloxy)ethyl carbonates (8). TFA (1.5 ml) was added to a cooled solution (0 °C) of 3α-[1(R,S)-(3,5-diacetamido-2,4,6-triiodobenzoyloxy)ethyl carbonate] t-butyl 7α, 12α-dihydroxy-5β-cholan-24-oates (7, 0.20 g, 0.18 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (5 ml). The solution was stirred for 45 min at room temperature, diluted with ethyl acetate (50 ml), and washed with water (3 x
50 ml). The organic phase was dried over Na$_2$SO$_4$, concentrated to dryness in vacuo, and the product 7 purified by flash chromatography on silica gel (20% MeOH/CHCl$_3$). The colourless product (0.18 g, 95%) decomposed at 217-220 °C; Rf 0.42 (20% MeOH/CHCl$_3$); $^1$H NMR (500 MHz, DMSO-d$_6$): $\delta$ 0.59 (3 H, s, C-18(H$_3$)), 0.86 (3 H, s, C-19(H$_3$)), 0.92 (3 H, d, 7.39 Hz, C-21(H$_3$)), 1.62 (3 H, d, 4.99 Hz), 2.02 (6 H, s, CH$_3$CONH-), 3.81 (1 H, m, C-12(H)), 4.23 (1 H, m, C-7(H)), 4.44 (1 H, m, C-3(H)), 6.9 (1 H, q, 3.16 Hz), 10.13 (2 H, d); $^{13}$C NMR (125 MHz, DMSO-d$_6$): $\delta$ 21.1 (C-36, C-38), 28.2 (C-27), 68.2 (C-7), 72.7 (C-12), 81.2 (C-3, C-26), 98.5 (C-30, C-34), 110.9 (C32), 147.1 (C-31, C-33), 153.6 (C-29), 159.8 (C-25), 167.4 (C-28, C-35, C-37); MS (electrospray; MeOH/H$_2$O) m/z: 1093.0635 [M + H]$^+$; calcd. for C$_{38}$H$_{52}$N$_2$O$_{11}$I$_3$: 1093.0699.

**Stability of the diatrizoyl double esters 3 and 8 in esterase solutions.** The conjugates 8 (0.10 g, 0.09 mmol) were added to a solution of pig liver esterase (0.8 ml, Sigma E 3128) in ammonium carbonate buffer (500 ml). The solution was shaken at 37 °C for 12 h, and the reaction monitored by TLC. TLC revealed that the major part of the double ester (7) hydrolyzed during the first 3 h. No conjugates could be detected after 12 h. The mixture was freeze dried, and the residue purified by flash chromatography (silica gel, CHCl$_3$ :MeOH = 4 : 1). Cholic acid and diatrizoic acid were identified by $^1$H NMR and TLC.

The yield of cholic acid was 98%. Hydrolysis was not observed in the absence of esterase.

The cholesterol derived double esters 3 did not hydrolyze when treated under similar conditions.
References and Notes