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The Synthesis of Branched Steroidal Prodrugs of Nitrogen Mustard for Antitumor Targeting via Reconstituted LDL

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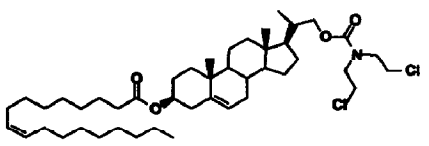
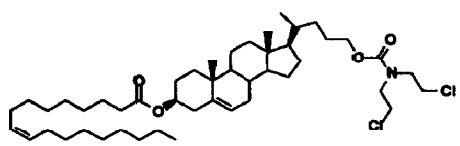
Abstract Bis and tris nitrogen mustard oleoyl-steroid carbamates were synthesized from commercially available cholenic acids for antitumor drug targeting via the LDL pathway. The tris-mustards were prepared through triester intermediates made from selective alkylation of the dianion of t-butyl 3-(diethyl malonyl)-propionate **10** with steroid iodides **8**.

In order to optimize delivery of cytotoxics to cancer cells, increasing attention is being given to drug transport vehicles that either selectively localize at sites on tumor cells, such as monoclonal antibodies,[1] or are more quickly taken up by fast growing tumor cells in the course of their accelerated metabolism. One of the latter is low density lipoprotein (LDL) which, as the most important exogenous source of cholesterol, has been shown to be cleared rapidly by a number of aggressive tumor lines.[2] Moreover, studies have demonstrated an inverse correlation between LDL cholesterol levels and degree of malignant disease.[3] In order to take advantage of the tumor cell's affinity for LDL particles, which are essentially micellular bodies surrounding a core of cholesterol esters and into which a targeting protein (apoprotein B) has been inserted, they have been loaded with hydrophobic drugs using various methods.[4] In most cases the drug is not well retained in the reconstituted LDL (rLDL) and it slowly leaks out. The best retention occurs when the drug is attached to a fatty acid-steroid anchor (**1** and **2**). The two steroid prodrugs of nitrogen mustard, **1** and **2**, reconstituted well into LDL and the resulting rLDL possessed activity against transformed CHO cells at protein concentrations of 10 μg/mL (cytostatic) and 2 μg/mL (cytotoxic), respectively.[4d]



1



2

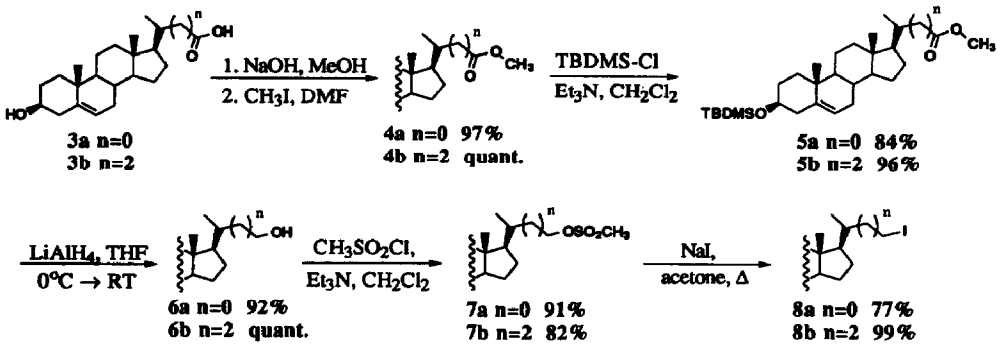
We wanted to improve the potency of rLDL by increasing the number of drug molecules on each carrier without lowering hydrophobicity as this might compromise retention in the LDL core. Therefore, carbon chain

branching was indicated. In addition, we wanted the drugs to be held as far as possible from the sterically congested steroid since we believe that the origin of the greater potency of **2** lies in the greater accessibility of the carbamate to lysosomal esterases or proteases.[4d]

Synthesis[5]

Beginning with two commercially available steroid acids, 22, 23-bisnor-5-cholenic acid-3β-ol **3a** and 5-cholenic acid-3β-ol **3b**, which differ by two methylene units in the length of their D-ring side chains, we formed the methyl esters **4** by treatment of the sodium salts with methyl iodide in DMF. The A-ring hydroxyl group was then protected as the t-butyldimethylsilyl (TBDMS) ether and the methyl ester reduced to the alcohol **6** with lithium aluminum hydride. Formation of the mesylate **7** and subsequent displacement with iodide in refluxing acetone proceeded smoothly to give **8**.

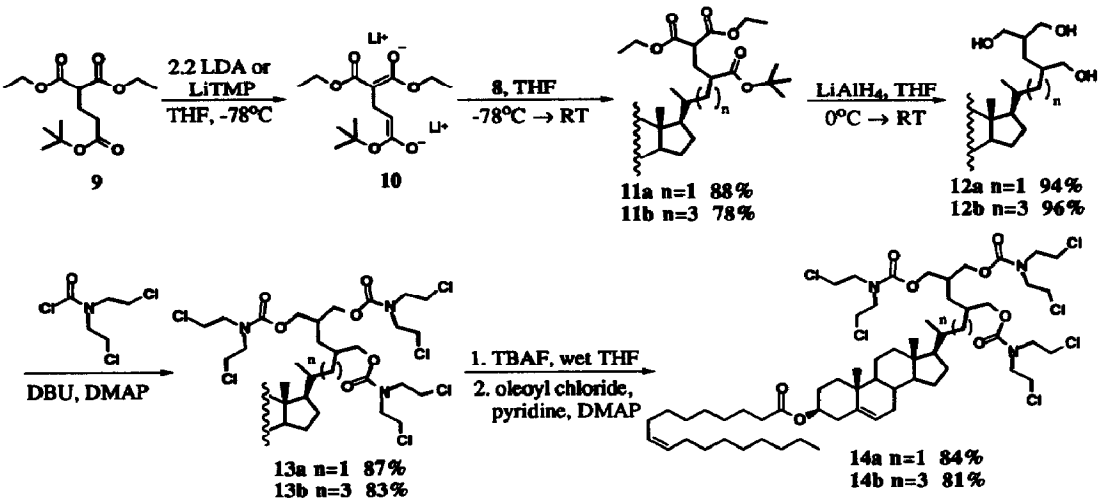
Scheme 1



t-Butyl 3-(diethyl malonyl) propionate **9**[6] in THF was treated with 2.2 equivalents of freshly prepared LDA at -78°C. After 20 min. the steroid iodide **8** in THF was added via syringe to the dilithium salt **10** and the mixture was allowed to slowly warm to room temperature as the dry ice-acetone bath melted. Normal aqueous workup after stirring overnight gave a thick oil which was chromatographed on silica, eluting with 6% ethyl acetate/hexane. The product (**11**), in both cases a waxy, white solid, was formed in high yield. It is significant that displacement of the sterically crowded iodide **8a** was unimpeded. No products resulting from addition of the malonyl carbon, or from base-promoted elimination were observed. Reduction of the triesters **11** to the triols **12** with 1M $LiAlH_4$ in THF proceeded in high yield without the need for chromatography.[7]

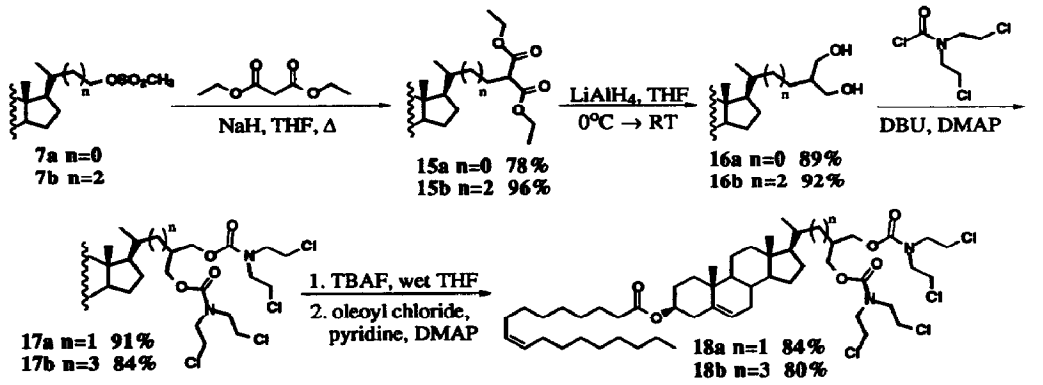
The nitrogen mustard carbamates **13** were formed by reaction of **12** with bis-(2-chloroethyl)aminochloroformate (NMCOCl) (7 equiv.) in the presence of diazabicycloundecene (DBU) (7 equiv.) and 6-dimethylaminopyridine (DMAP) (15-20%) in methylene chloride. Triethylamine is not effective as a replacement for DBU; nor will reaction occur with only DBU or DMAP. Removal of the TBDMS protecting group worked smoothly as long as some water was present to potentiate the basicity of tetrabutylammonium fluoride (TBAF), and the resulting alcohols were cleanly acylated with oleoyl chloride/pyridine/DMAP to give the tris-nitrogen mustard prodrugs (**14**).[8]

Scheme 2



The bis-nitrogen mustard LDL compounds were prepared in a similar manner as shown in scheme 3. Mesylates **7** were displaced with the sodium salt of diethyl malonate in THF at reflux in high yield. Reduction of the diesters **15** with $LiAlH_4$ provided diols **16** which were carried on as described above for triols **12** to give the target compounds **18**.[9]

Scheme 3



Compounds **14** and **18** were reconstituted into LDL according to the method of Krieger[4d] and the rLDL was tested against two groups of transformed CHO cells: one which expresses the LDL receptor normally, and one which displays extremely low levels of expression. Bis-nitrogen mustards **14** proved to be twice as potent as **2** while the tris-mustards **18**, unexpectedly, lacked significant activity. Full biological results will be reported elsewhere.

Acknowledgements

We thank Professor Monty Krieger for carrying out LDL reconstitution and cytotoxicity assays.

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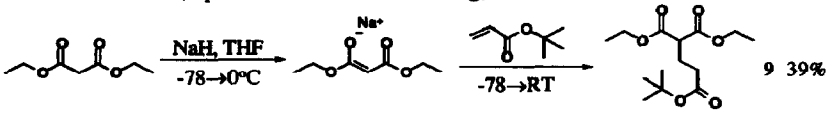
5. All new compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, mass spec. and either microanalysis or high resolution mass spec.

6. Triester **9** is easily prepared in large quantities by alkylation of t-butyl acrylate with the sodium salt of diethyl malonate and distillation (b.p. 105-110°C/0.02 mm Hg).



7. For **11a**: ^{1}H-NMR ($CDCl_3$) δ 0.02 (6H, s, Si-CH_3), 0.61 & 0.64 (each 3H, s, C-18 & C-19 CH_3), 0.82-2.42 (23H, steroid CH & CH_2), 0.85 (9H, s, Si-t-Bu), 0.97 (3H, d, C-21 CH_3), 1.22 and 1.27 (6H, 2xt, Et CH_3), 1.42 (9H, s, O-t-Bu), 2.05 (1H, m, C-23 CH), 3.34 (1H, 2xABq, malonyl CH), 3.43 (1H, m, C-3 CH), 4.19 (4H, m, Et CH_2), 5.29 (1H, d, C-6 CH); ^{13}C-NMR (selected) δ 61.9 (Et CH_2), 73.3 (C-3), 81.0 (t-Bu O-C), 121.9 (C-6), 142.2 (C-5), 170.5 (2 peaks, Et ester CO), 175.4 & 176.3 (t-Bu ester CO); Mass spec. (FAB) 716 $(MH)^+$, 659 $(M\text{-t-Bu})^+$, 643 $(M\text{-t-BuO})^+$; Exact mass calc. for $C_{42}H_{71}O_7Si$: 715.4969; Found: 715.4973. For **11b**: Mass spec. (FAB) 744 $MH)^+$, 688 $(M\text{-t-Bu})^+$, 671 $(M\text{-t-BuO})^+$; Exact mass calc. for $C_{44}H_{75}O_7Si$: 743.5282; Found: 743.5285.

8. For **14a**: ^{1}H-NMR ($CDCl_3$) δ 0.63 & 0.97 (each 3H, s, C-18 & C-19 CH_3), 0.85 (3H, t, oleoyl CH_3), 0.89 (3H, d, C-21 CH_3), 1.22 & 1.28 (16H, brs, oleoyl CH_2), 1.54, 1.79 & 1.96 (8H, m, allylic CH_2), 1.89 & 2.10 (each 1H, m, side chain CH), 2.23 (2H, t, CO-CH_2), 3.61 (24H, m, N-CH_2 & Cl-CH_2), 4.04 (6H, m, O-CH_2), 4.57 (1H, m, C-3 CH), 5.30 (3H, m, vinyl CH); ^{13}C-NMR (selected) δ 42.0 & 42.7 (Cl-CH_2), 50.8 & 51.4 (N-CH_2), 65.6, 65.9 & 68.0 (O-CH_2), 74.1 (C-3), 123.2 (C-6), 130.5 & 130.8 (oleoyl vinyl), 140.2 (C-5), 158.4 & 158.7 (N-CO), 174.1 (oleoyl CO); Mass spec. (FAB) 1239 $(M+Na)^+$; Exact mass calc. for $C_{61}H_{102}N_3O_8Cl_6$: 1214.5798, Found: 1214.5770. For **14b**: Mass spec. (FAB) 1269 $(M+Na)^+$; Exact mass calc. for $C_{63}H_{106}N_3O_8Cl_6$: 1242.6111, Found: 1242.6081.

9. For **18a**: Mass spec. (FAB) 1013 $(M+Na)^+$; Exact mass calc. for $C_{53}H_{89}N_2O_6Cl_4$: 989.5475, Found: 989.5453. For **18b**: Mass spec. (FAB) 1039 $(M+Na)^+$, 1055 $(M+K)^+$; Exact mass calc. for $C_{55}H_{93}N_2O_6Cl_4$: 1017.5788, Found: 1017.5754.

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