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SYNTHESIS OF BILE ACID - DRUG CONJUGATES: POTENTIAL DRUG - SHUTTLES FOR LIVER SPECIFIC TARGETING

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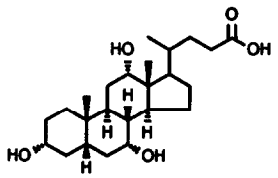
Abstract: *Cholic acid (1) has been coupled via ω-aminoalkoxy spacer at C-3 to chlorambucil (3), HR 780 (4) and oxaproline peptide (5). Drug conjugates 9, 11, 18 exhibit strong affinity to specific bile acid transport systems.*

Tissue selective drug delivery is highly desired to minimize toxicity and unwanted side effects of drugs and their metabolites due to systemic circulation. Site specific molecular recognition seems to be the most straightforward way for realization.[1] For specific delivery of drugs to the liver and the biliary system the endogenous bile acid transport system is an attractive candidate.[2] Bile acids fulfill various physiological functions during digestion and resorption of fat and cholesterol. Passive diffusion and active transport processes take care of nearly total reabsorption during intestinal passage. The occurence of specific Na^+-dependent bile acid transport systems of high capacity in the sinusoidal membrane of hepatocytes and brush border membrane of ileal enterocytes should guarantee high specificity and large capacity.[3] We wish to report the synthesis of bile acid drug conjugates **2** that are susceptible to proteins of bile acid transport systems.



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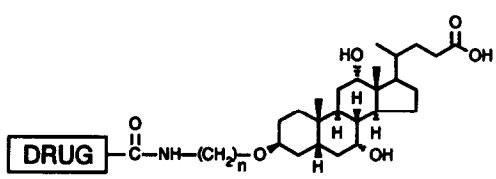

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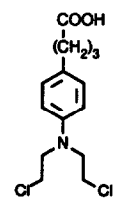
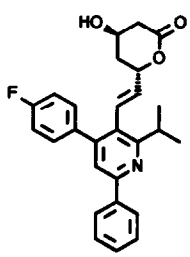
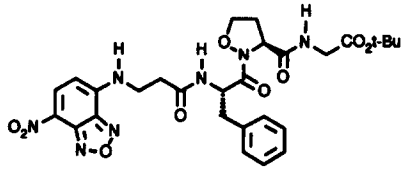


3 Chlorambucil



4 HR 780



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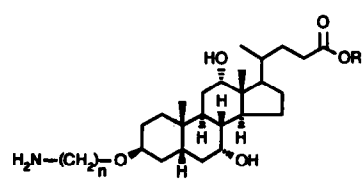
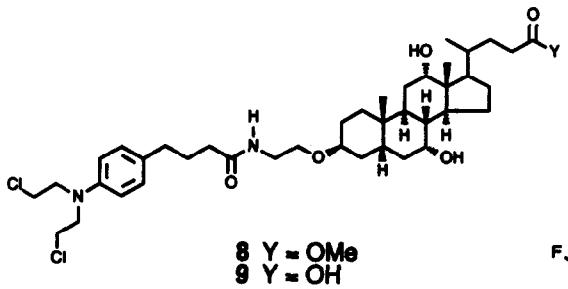
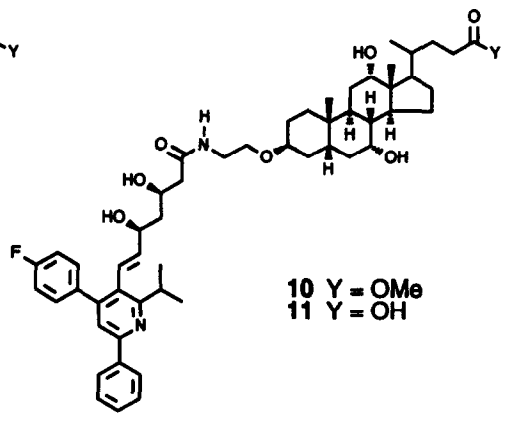


6 n = 2, R = Me
7 n = 5, R = t-Bu

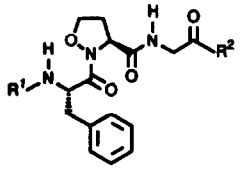
An amide bond has been chosen for the connection of drug and bile acid to guarantee sufficient hydrolytic stability of **2** during recognition and transport. Three structurally unrelated agents, chlorambucil (**3**), an alkylating cytostatic agent, HR 780 (**4**),[4] an inhibitor of HMG-CoA reductase, and oxaproline peptide **5**, an inhibitor of prolyl-4-hydroxylase[5] have been chosen for conjugation to bile acids.



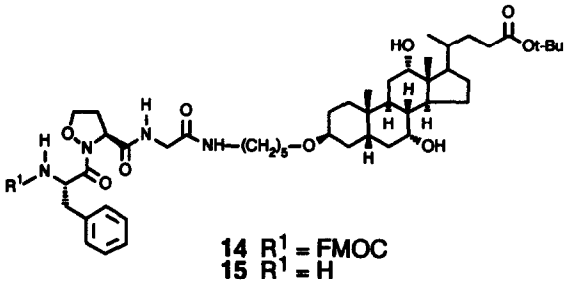
8 Y = OMe
9 Y = OH



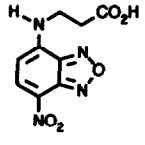
10 Y = OMe
11 Y = OH



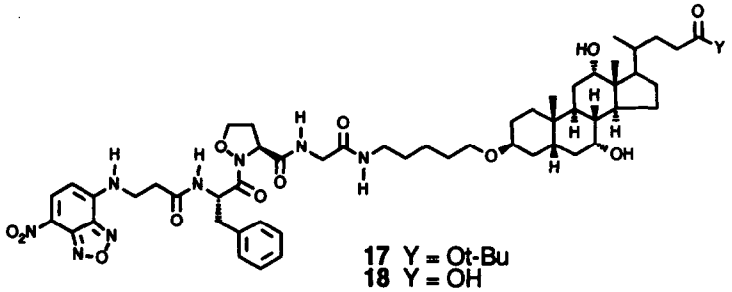
12 R^1 = FMOC, R^2 = Ot-Bu
13 R^1 = FMOC, R^2 = OH



14 R^1 = FMOC
15 R^1 = H



16



17 Y = Ot-Bu
18 Y = OH

For the synthesis of **9** chlorambucil (Sigma) was reacted with 1.0 equiv. ethyl chloroformate in the presence of 20 equiv. triethylamine in THF at 0°C for 15 min. Subsequent addition of 1.0 equiv. amine **6**[6] at 0°C and reaction at room temperature for 30 min provided **8** after chromatography (silica gel, ethyl acetate; ethyl acetate/methanol = 9:1) in 81% yield. Hydrolysis of **8** with 2.0 equiv. aqueous sodium hydroxide in ethanol for 3 h at room temperature gave chlorambucil-bile acid conjugate **9**[7] quantitatively.

For the synthesis of **11** HR 780 (**4**)[4] was refluxed with 1.0 equiv. amine **6** in the presence of 20 equiv. triethylamine and 1.0 equiv. 4-dimethylaminopyridine in THF for 48 h. **10** was obtained in 70% yield after chromatography (silica gel, ethyl acetate/methanol = 19:1). Hydrolysis of ester **10** using 7.0 equiv. aqueous sodium hydroxide in ethanol at room temperature for 12 h and careful chromatography (chloroform/methanol = 85:15) gave HR 780-bile acid conjugate **11**[7] in 85% yield.

For the synthesis of **18** neither peptide **5**[8] nor its corresponding free acid could be coupled directly to amine **7**[6] in acceptable yield. Therefore, 9-fluorenylmethoxycarbonyl (FMOC) protected oxaproline peptide

13[9] served as starting material. **13** was reacted with 1.0 equiv. amine **7** in the presence of 1.0 equiv. N,N'-dicyclohexylcarbodiimide (DCC), 1.1 equiv. 1-hydroxybenzotriazole (HOBT) and 3.0 equiv. N-ethylmorpholine (NEM) in DMF at room temperature for 16 h to give **14** in 67% yield after chromatography (silica gel, dichloromethane/methanol = 20:1). Deprotection of the amino group with an excess of piperidine in DMF at room temperature for 18 h provided **15** quantitatively after chromatographic purification (silica gel, dichloromethane/methanol = 40:1, 20:1, 9:1). **15** was coupled with the fluorescent β-alanine derivative **16**[10] using 1.0 equiv. DCC, 1.1 equiv. HOBT and 3.0 equiv. NEM in DMF at room temperature for 16 h. **17** was obtained in 44% yield after chromatography (silica gel, dichloromethane/methanol = 20:1). Removal of the t-butylester of **17** (trifluoroacetic acid/dichloromethane = 1:1, room temperature) had to be monitored carefully by tlc. Peptide-bile acid conjugate **18**[7] was obtained in 58% yield after chromatography (silica gel, dichloromethane/methanol = 20:1, 9:1).

Interaction of **9**, **11** and **18** with the specific bile acid transport system was studied by inhibition of Na^+-dependent [^{3}H]-tauro cholate uptake into ileal brush border membrane vesicles (rabbit).[14] Bile acid-drug conjugates **9**, **11** and **18** are recognized by the specific ileal bile acid transport system with IC_{50} values comparable to natural bile acids (17μM, cholic acid): 41μM (**9**), 2.3μM (**11**) and 100μM (**18**). These data confirm that the characteristic molecular features for interaction with the proteins of the bile acid transport system are preserved in the bile acid part of **9**, **11** and **18**. More generally, bile acid drug conjugates **2** might be useful as drug shuttles for liver specific targeting.

Detailed biological studies regarding transport of drugs in comparsion to parent compounds will be published elsewhere.[2]

Acknowledgments: We wish to thank Dr. Fehlhaber and Dr. Kogler for analytical support, F. Girbig, U. Gutjahr and S. Kowalewski for performing biological experiments. We are also grateful to Dr. S. Henke for helpful discussions on oxaproline peptide chemistry.

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7. Characteristic analytical data:

9 amorphous solid, mp 60-70°C; ^{1}H-NMR 270 MHz ($CDCl_3$) δ 0.70 (s, 3 H), 0.91 (s, 3 H), 1.00 (d, J = 6 Hz, 3 H), 1.10 - 2.50 (m, 30 H), 2.57 (t, J = 7.2 Hz, 2 H), 3.41 (m, 4 H), 3.51 - 3.74 (m, 9 H), 3.86 (m, 1 H), 4.00 (m, 1 H), 5.84 (broad, 1 H), 6.61 (d, J = 8.0 Hz, 2 H), 7.06 (d, J = 8.0 Hz, 2 H);

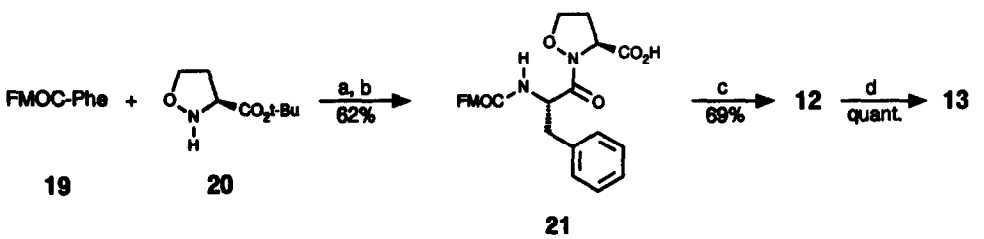
11 amorphous solid, mp 85-90°C; ^{1}H-NMR 270 MHz ($CDCl_3$) δ 0.69 (s, 3 H), 0.91 (s, 3 H), 0.98 (d, J = 5.2 Hz, 3 H), 1.34 (d, J = 7.2 Hz, 6 H), 1.05 - 2.50 (m, 29 H), 3.42 (m, 4 H), 3,56 (m, 1 H), 3.84 (m, 1 H), 3.98 (m, 1 H), 4.15 (m, 1 H), 4.40 (m, 1 H), 5.38 (dd, J_1 = 16 Hz, J_2 = 6 Hz, 1 H), 6.38 (broad, 1 H), 6.60 (d, J = 16 Hz, 1 H), 7.08 (m, 2 H), 7.25 - 7.50 (m, 6 H), 8.10 (m, 2 H);

12 amorphous solid, mp 72-78°C; ^{1}H-NMR 270 MHz ($CDCl_3$) δ 1.49 (s, 9 H); 2.55 (bs, 1 H), 2.78 (m, 1 H); 3.16 (m, 2 H), 3.88 (d, J = 5 Hz, 2 H), 3.95 (m, 1 H), 4.20 (t, J = 6 Hz, 2 H), 4.31 (t, J = 6 Hz, 1 H), 4.41 (dd, J_1 = 10 Hz, J_2 = 8 Hz, 1 H), 4.85 (bt, J = 7 Hz, 1 H), 5.12 (bq, J = 6 Hz, 1 H), 5.45 (d, J = 8 Hz, 1 H), 6.79 (bs, 1 H), 7.17 - 7.35 (m, 7 H), 7.41 (t, J = 6 Hz, 2 H), 7.57 (dd, J_1 = 8 Hz, J_2 = 2 Hz, 2 H), 7.75 (d, J = 8 Hz, 2 H);

18 amorphous solid, mp 160-165°C; ^{1}H-NMR 270 MHz (CD_3OD) δ 0.69 (s, 3 H), 0.88 (s, 3 H), 1.00 (d, J = 6 Hz, 3 H), 1.25 - 2.00 (m), 2.10 - 2.50 (m), 2.60 (bs, 1 H), 2.98 (m, 2 H), 3.18 (t, J = 6 Hz, 2 H), 3.50 (m, 2 H), 3.75 - 3.95 (m), 4.10 (m, 1 H), 4.22 (d, J = 6 Hz, 1 H), 4.55 (m, 1 H), 4.70 (m, 1 H), 5.50 (s, 1 H), 6.44 (d, J = 10 Hz, 1 H), 7.25 (m, 5 H), 7.73 (m, 1 H), 8.55 (d, J = 10 Hz, 1 H).

8. **5** can be obtained from **12**[9] in 83% yield after deprotection (piperidine/DMF) and coupling with **16**[10] (DCC).

9. **13** can easily be prepared starting from FMOC-L-phenylalanine **19** and **20**[11] using routine methodology.



(a) DCC, HOBT, NEM, DMF; (b) CF_3CO_2H, CH_2Cl_2; (c) tert.-butylglycinate dibenzenesulfimide salt, TOTU[12], NEM, DMF; (d) CF_3CO_2H, CH_2Cl_2

10. **16**, mp 192-196°C, was prepared from β-alanine and 7-chloro-4-nitrobenz-2-oxa-1,3-diazole in ethanol in the presence of sodium acetate, according to ref. 13.

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