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CARBOHYDRATE MODIFICATIONS IN THE SPIROSTANE CELLOBIOSIDE CHOLESTEROL ABSORPTION INHIBITOR SERIES

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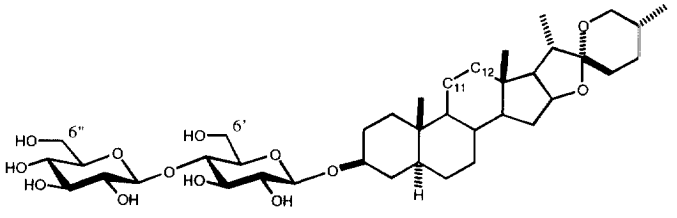
Abstract: Cholesterol absorption inhibition remains an attractive approach for the treatment of hypercholesterolemia. We have continued our SAR development in the spirostanyl cellobioside class of agents seeking a greater understanding of the role carbamoyl substitution has on the potency in this series. In this regard, a series of differentially substituted carbamate analogs were made with and without deoxygenations. From this study, it was determined that the minimal requirements for optimal potency was a lone carbamate at C4" and deoxygenation at the C6" position.

Introduction

Steroidal glycosides have been endowed with a wide range of biological activity including antifungal,[1,2] antitumor,[3] and antiviral,[4,5] properties. With the notable exception of digitalis, medicinal application of these agents is hampered by very low oral bioavailability. We therefore were intrigued by the reports of Kintya[6] and more recently, Malinow[7-9] which reported the cholesterol lowering properties of certain spirostane glycosides. Particularly noteworthy was the proposed mechanism of cholesterol lowering, that is cholesterol absorption inhibition in the intestinal lumen, since it removed the requirement for systemic exposure to drug. We recently described a series of spirostanyl glycoside cholesterol absorption inhibitors which included pamaqueside (**1**, CP-148,623).[10] It was subsequently discovered that conversion of certain hydroxyl groups on the cellobiose to carbamates resulted in potency improvements as large as two orders of magnitude.[11] We assumed that these agents were acting through a purely physicochemical mechanism in the intestinal lumen and



1 C_{11} = carbonyl, C_{12} = CH_2
2 C_{11} = CH_2; C_{12} = carbonyl

such dramatic SAR was surprising. In this communication, our efforts directed at further defining the requirements necessary for the high potency of these analogs are described. Specifically, additional carbamate substitution patterns as well as deoxygenated analogs are reported.

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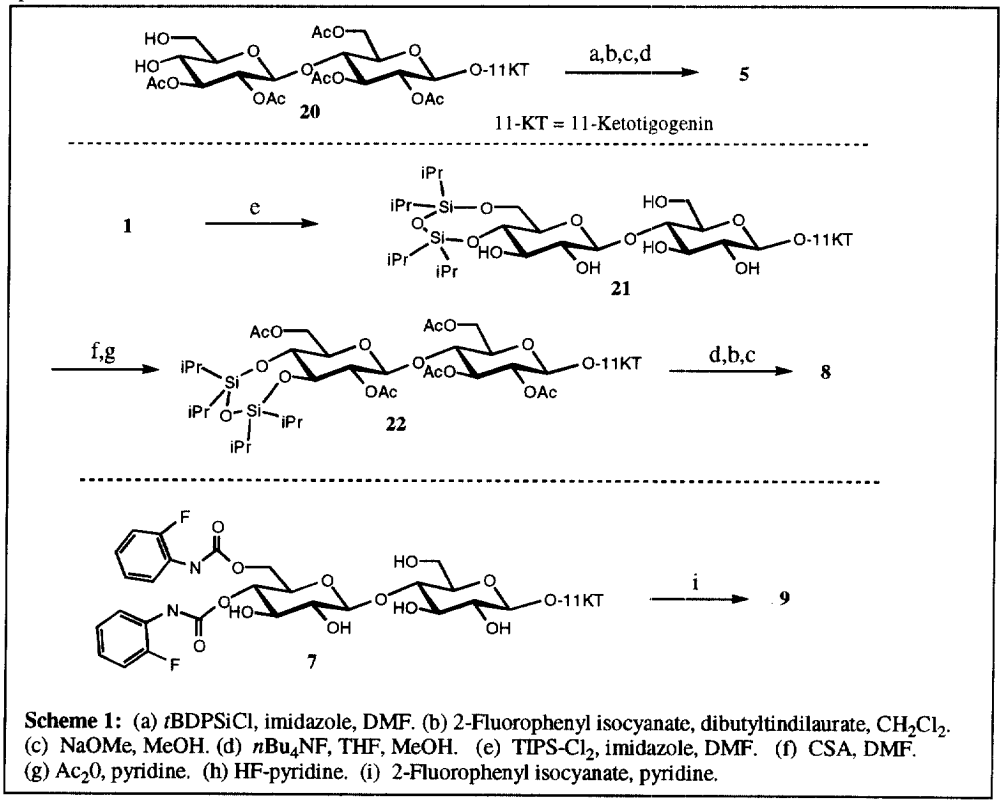
Chemistry

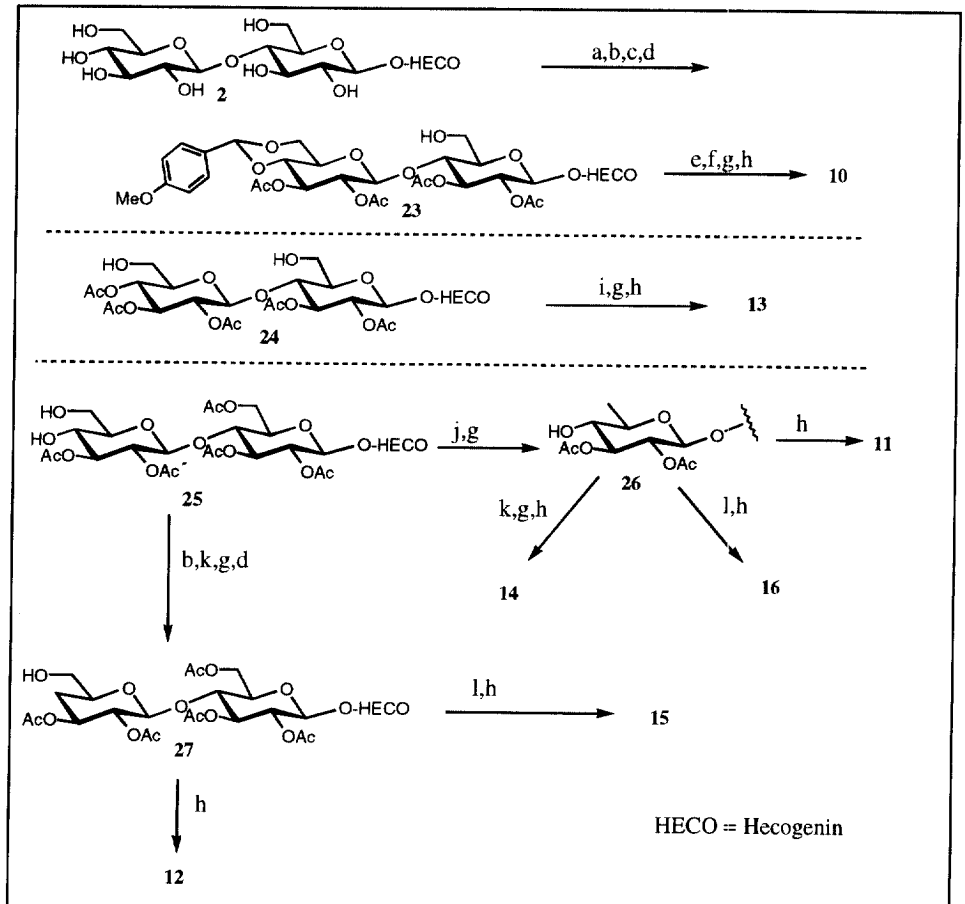
The syntheses of the carbamate substituted analogs listed in Table 1 are shown in Scheme 1.[12] The 11-ketotigogenin steroid was used in each case. In general, the chemistry involved the use of protecting groups which allowed for the selective preparation of intermediates containing uniquely freed hydroxyl groups. These alcohols were then converted to carbamates using an isocyanate. 2-Fluorophenyl isocyanate was used since 2-fluorophenyl substitution was previously shown to provide optimal potency. Compounds **3**, **4**, **6**, and **7** were described in the earlier paper.[11] Analog **5**, which contains a carbamate at the 4" position, was prepared from diol **20**. Selective silylation of the primary alcohol using TBDPSiCl and imidazole in DMF was followed by carbamate formation and deprotection to furnish **5**. Silicon-based protecting group chemistry was also crucial for the preparation of the 3",4" dicarbamate **8**. Thus, treatment of **1** with TIPS-Cl_2 and imidazole in DMF afforded the initial kinetic product **21**. Acid catalyzed isomerization[13] and acylation gave the fully protected intermediate **22**. Siloxane deprotection, carbamate formation through the reaction with 2-fluorophenyl isocyanate under tin catalysis, and final acetate cleavage afforded **8**. The sole tricarbamate **9** was synthesized in one step from **7**.



Scheme 1: (a) *t*BDPSiCl, imidazole, DMF. (b) 2-Fluorophenyl isocyanate, dibutyltindilaurate, CH_2Cl_2. (c) NaOMe, MeOH. (d) nBu_4NF, THF, MeOH. (e) TIPS-Cl_2, imidazole, DMF. (f) CSA, DMF. (g) Ac_20, pyridine. (h) HF-pyridine. (i) 2-Fluorophenyl isocyanate, pyridine.

The deoxy analogs in Table 2 were prepared from the corresponding halo or thiocarbamate derivatives through the action of tri-*n*-butyl tin hydride as shown in Scheme 2. The 6' deoxy analog **10** was prepared from compound **2**.[14] The 4" and 6" hydroxyl groups were first protected as a 4-methoxy benzylidene acetal. The lone primary alcohol at 6' was silylated and the remaining hydroxyl groups were acylated. Silyl deprotection gave compound **23**. The single free hydroxyl group was mesylated and converted to the bromide using LiBr in

DMF. Under these conditions the benzylidene acetal was also cleaved. The bromide was reduced with tin hydride and the acetates were hydrolyzed to provide analog **10**. The dideoxy analog **13** was prepared in a straightforward manner from diol **24** by the three step sequence of iodination, radical dehalogenation, and deprotection. The remaining analogs were all derived from the 4",6" diol **25**. Selective bromination (CBr_4) and dehalogenation with nBu$_3$SnH afforded the intermediate **26**. Sodium methoxide deprotection furnished analog **11**. The 4" hydroxyl group in **26** could be removed by radical deoxygenation of the corresponding thiocarbamate (carbonyl diimidazole, Et_3N). Deprotection with NaOMe afforded the dideoxy analog **14**. Compound **26** could also be carbamoylated using 2-fluorophenyl isocyanate and CuCl[15] and after standard deacylation, gave **16**. Selective protection of the primary hydroxyl group in **25** (TBSCl, imidazole) allowed for the removal of the alcohol at 4". Fluoride mediated desilyation gave the intermediate **27**. Through the same sequence of reactions as described for **26**, analogs **12** and **15** were in hand.

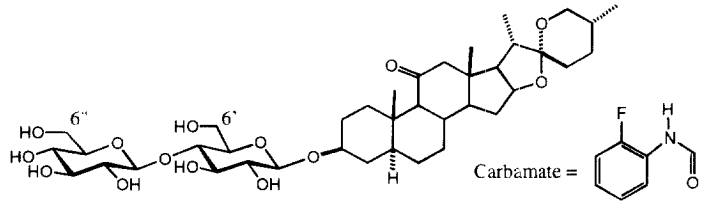


Scheme 2: (a) Anisaldehyde dimethylacetal, CSA, DCE. (b) TBSCl, imidazole, DMF. (c) Ac_2O, pyridine, CH_2Cl_2. (d) tBu$_4$NF, THF. (e) MsCl, Et_3N, CH_2Cl_2. (f) LiBr, DMF. (g) nBu$_3$SnH, AIBN, toluene, reflux. (h) NaOMe, MeOH. (i) I_2, PPh_3, imidazole, toluene. (j) CBr_4, Ph_3P, CH_2Cl_2. (k) Thiocarbonyl diimidazole, Et_3N, CH_2Cl_2. (l) 2-Fluorophenyl isocyanate,CuCl, DMF.

Results

In our earlier account, we prepared several positional carbamate isomers and their activities were measured in an acute model of cholesterol absorption inhibition in the hamster.[16] Table 1 expands on this SAR with a series of mono, di, and tri-carbamate analogs. We examined every position that could be readily accessed through protecting group chemistry, namely the 3", 4", 6', and 6" positions. Of the mono carbamates, there is a slight preference for the 4" position over the 6", with both analogs (**4**, **5**) showing at least a ten-fold improvement in activity over the parent compound **1**. However, substitution at 6' (compound **3**) is apparently detrimental to activity and is the only analog in the series less active than **1**. Three dicarbamates were made, with the most potent being the 4",6" isomer **7**, which has an ED_{50} of 0.025 mg/kg. The comparison of compounds **5** and **8** suggests that substitution at 3" has no effect. Compound **6** was the least active at 0.8 mg/kg further exemplifying the negative effect of the carbamate at 6'(~8-fold loss in potency). A similar loss in activity was registered for the tricarbamate **9** relative to compound **7**. These data suggest that carbamate substitution at both the 6" and 4" each provide significant potency enhancements, with substitution at both positions required for maximal benefit.

Table 1

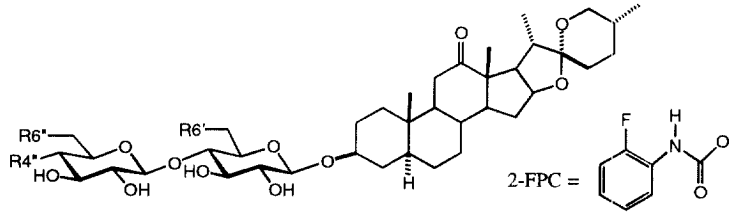


Compound	Carbamate Position(s)	ED_{50} mg/kg	Rel. Potency
1	None	2.0	80
3	6'	7.0	280
4	6"	0.2	8
5	4"	0.1	4
6	6',6"	0.8	32
7	4",6"	0.025	1
8	3",4"	0.1	4
9	6',4",6"	0.2	8

It is clear from Table 1 that the presence of carbamates on the cellobiose can greatly influence the potency. However, it is unclear how these functional groups elicit this effect particularly in the absence of any known biological receptor. We chose to use additional SAR to give us a better understanding of the minimum requirements for obtaining highly potent compounds. Toward this end, a series of deoxygenated derivatives with and without 2-fluorophenyl carbamate substituents were prepared and tested in the standard in vivo hamster model[11] (Table 2). These analogs were made using the hecogenin steroid which in general are about three-fold less potent than the corresponding 11-ketotigogenin analogs. Although less potent, hecogenin is also less costly as it is isolated in large quantities from plants and is the precursor to 11-ketotigogenin.[17] Compounds **17-19**, the hecogenin analogs of **4**, **5**, and **7**, respectively, are included in Table 2 for reference. Two of the mono-deoxy derivatives showed a slight improvement in potency (**10** and **11**) while the 4" deoxy

analog **12** was slightly less active. Two dideoxy cellobiosides were prepared (**13, 14**) and both resulted in a two to three-fold potency enhancement. We wanted to see if we could augment these effects by combining the deoxy series with the carbamate series. This strategy led to compounds **15** and **16**, which contain a 2-fluorophenyl carbamate at the 6" and 4" positions, respectively, while the alternate position was deoxygenated. As expected, large potency advances were registered with compound **16** being the most active (ED_{50} of 0.03 mg/kg). This result is in contrast to the data from Table 1 showing that both the 4" and 6" positions needed to be carbamoylated to reach this level of potency. In comparing compounds **18** and **16**, one sees a five fold improvement in potency, which is in the same range as that seen for compound **11** vs. **2**. It is possible that the presence of the hydroxyl group at 6" influences the conformation of the carbohydrates in a detrimental manner. This effect may be relieved upon deoxygenation or carbamoylation. Thus in the deoxy series, highly potent analogs such as **16** can be generated with only one carbamate substituent.

Table 2



Cmpd	R6'	R6"	R4"	ED_{50} mg/kg	Rel. Potency
2	OH	OH	OH	10	333
10	H	OH	OH	5.0	167
11	OH	H	OH	3.2	107
12	OH	OH	H	13	433
13	H	H	OH	5.0	167
14	OH	H	H	3.0	100
15	OH	2-FPC	H	0.15	5
16	OH	H	2-FPC	0.03	1
17	OH	2-FPC	OH	0.3	10
18	OH	OH	2-FPC	0.15	5
19	OH	2-FPC	2-FPC	0.07	2.3

Summary

We have previously shown that carbamoylation of the C4" and C6" positions of spirostanyl cellobiosides results in a marked improvement in cholesterol absorption inhibition activity. The present study served to further define the structural requirements necessary to achieve this level of potency. We examined analogs with one, two, and three carbamate groups at various positions on the cellobiose. In addition, mono and di-deoxygenated derivatives were prepared, with and without a carbamate substituent. The data generated showed that potency comparable to the 4",6" dicarbamate series could be achieved with a single 2-fluorophenyl carbamate substituent at C4" as long as the C6" position was deoxygenated.

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