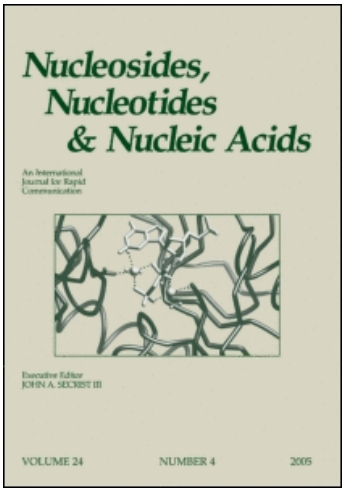

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SYNTHESIS OF 5'-*C*-METHYL-D-ALLO- & L-TALO-RIBONUCLEOSIDE 3'-*O*-PHOSPHORAMIDITES & THEIR INCORPORATION INTO HAMMERHEAD RIBOZYMES

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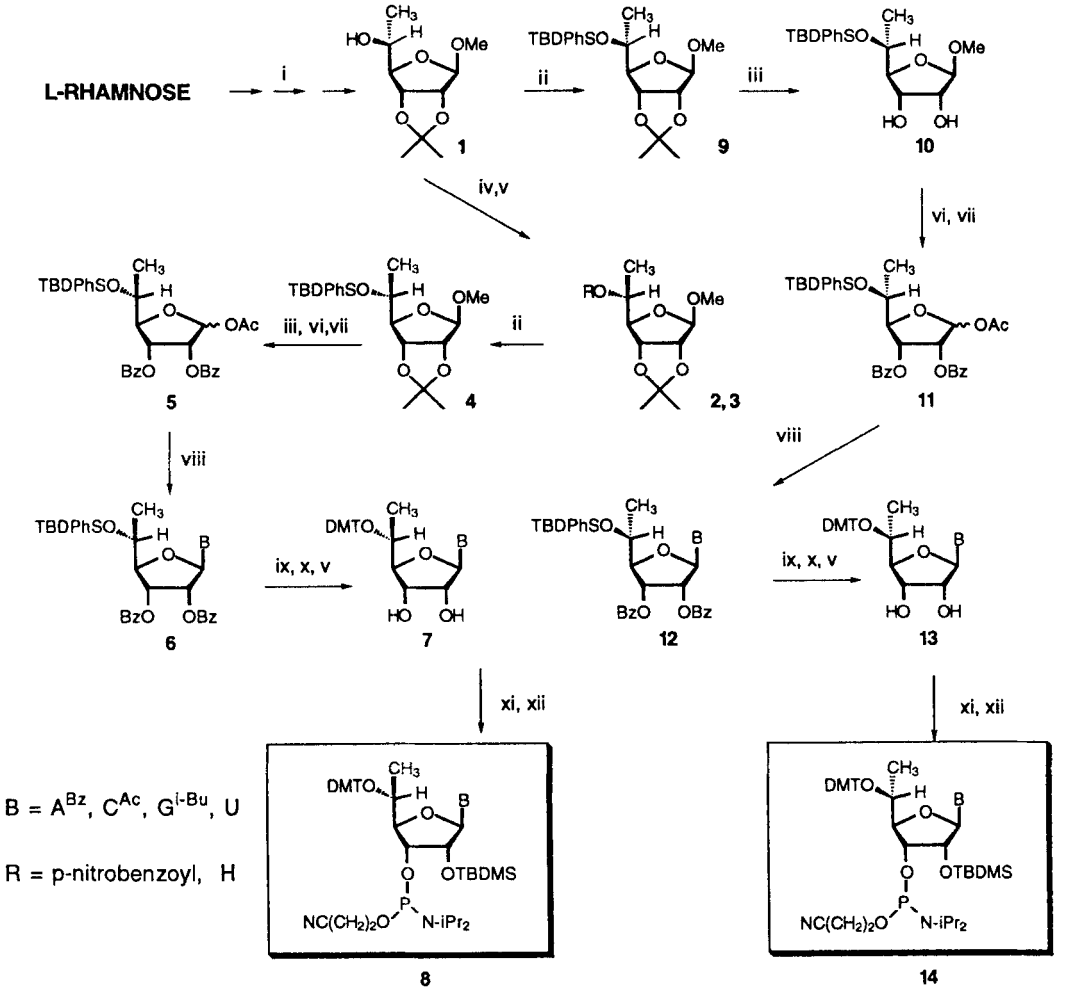
Abstract: 5'-*C*-Methyl-D-allo & L-talo-ribonucleoside 3'-*O*-phosphoramidites were prepared from L-rhamnose in 13 and 15 steps respectively. Incorporation of L-talo residues in the hammerhead ribozyme and the resulting activity and stability of the modified ribozymes is described.

The highly sequence-specific endoribonuclease activity of hammerhead ribozymes suggests their use as therapeutic agents for the inhibition of gene expression.[1] As a part of our studies on the molecular mechanism of action of hammerhead ribozymes we were interested in the effect of the incorporation of 5'-*C*-methyl nucleotides in a hammerhead ribozyme model sequence. To date, the incorporation of 5'-*C*-Me nucleosides into oligomers was limited to dimer synthesis using a phosphodiester methodology[2] or enzymatic polymerization of 5'-*O*-triphosphates.[3] The synthesis of 5'-*C*-Me nucleoside 3'-*O*-phosphoramidites allows the application of these structurally interesting compounds to oligonucleotide structure-function studies.

In the synthesis of 5'-*C*-Me nucleoside 3'-*O*-phosphoramidites the major challenge is to design a protection strategy that allows for the discrimination of the three secondary hydroxyl groups of the protected 5'-*C*-Me nucleosides. After several unsuccessful attempts to use base-labile protecting groups for the exocyclic 5-OH of isopropylidene derivative **1**, we chose the *t*-butyldiphenylsilyl group for the selective protection and deprotection of this hydroxyl group. Commercially available L-rhamnose was converted, in three steps,[4,5] to isopropylidene derivative **1**. Mitsunobu inversion[6] at the 5-position of D-allo derivative **1** with 4-nitrobenzoic acid gave L-talo product **2** (R = *p*-nitrobenzoyl) in 80% yield. Compound **2** was deprotected to give talo-furanoside **3** (R = H). Subsequent introduction of a *t*-butyldiphenylsilyl group in the presence of $AgNO_3$,[7] resulted in the formation of the 5-*t*-butyldiphenylsilyl ether **4** in 80% yield. The isopropylidene group in



Reagents and Conditions: *i)* Ref. 1 & 2; *ii)* *t*-butyldiphenylsilyl chloride, $AgNO_3$/DMF; *iii)* CF_3COOH-H_2O-dioxane (2:1:1), 0 °C, 2 h; *iv)* *p*-nitrobenzoic acid, PPh_3, DEAD/dioxane, RT, 16 h; *v)* NaOMe/MeOH; *vi)* BzCl/Pyr; *vii)* AcOH-Ac_2O-H_2SO_4/EtOAc, 0 °C, 2 h; *viii)* silylated nucleobase, $CF_3SO_3SiMe_3$/MeCN; *ix)* TBAF/THF; *x)* DMT-Cl, $AgNO_3$, *sym*-collidine/CH_2Cl_2; *xi)* TBDMS-Cl, $AgNO_3$, Pyr/THF; *xii)* 2-cyanoethyl-*N,N*-diisopropylchloro-phosphoramidite, DIPEA/CH_2Cl_2.

FIGURE 1

Synthesis of 5'-*C*-Methyl-D-Allo- & L-Talo-Ribonucleoside 3'-*O*-Phosphoramidites

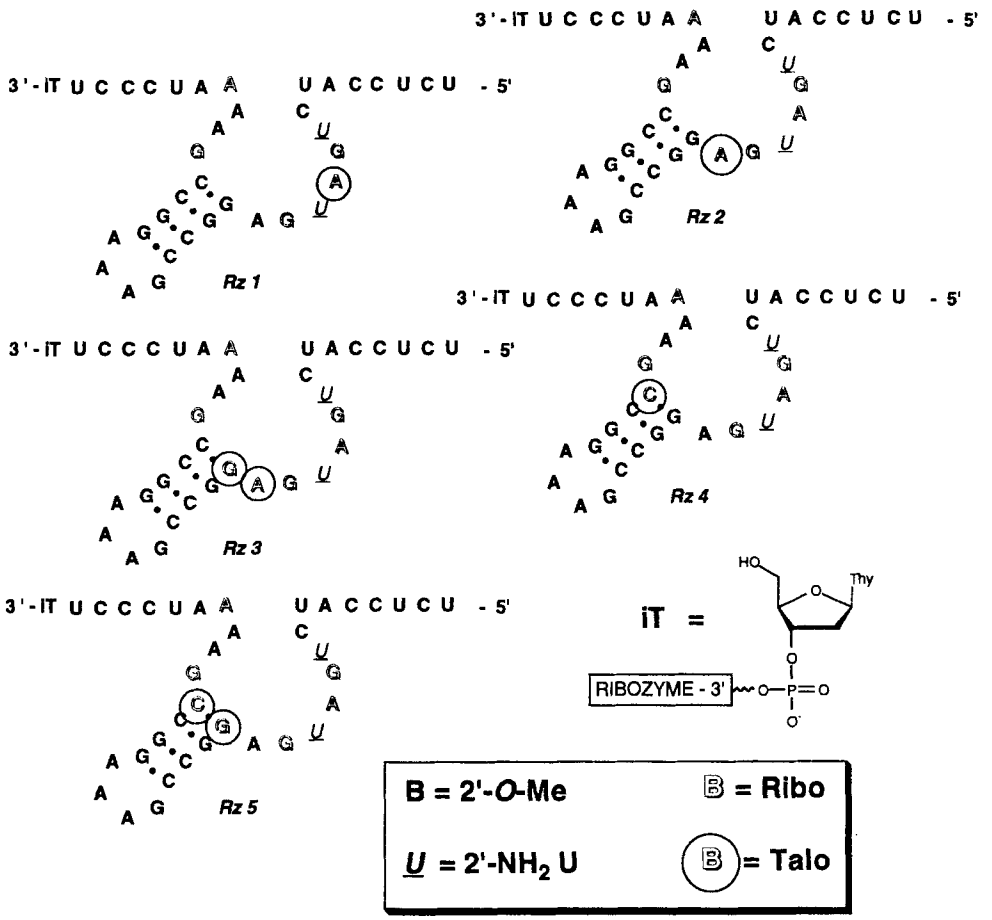


FIGURE 2

Hammerhead Ribozymes Containing 5'-*C*-Methyl Nucleoside Modifications

compound **4** could be selectively hydrolyzed in the presence of the *t*-butyldiphenylsilyl group by CF_3COOH/H_2O/dioxane (2:1:1) at 0 °C. Then, without separation, the reaction mixture was treated with BzCl. Following mild acetolysis,[8] the glycosylation synthon **5** was obtained in an overall yield of 60%.

Vorbrüggen glycosylation[9] of nucleobases with **5** led to the corresponding nucleosides **6** in 50-90% yield. The protected L-talo nucleosides **6** were desilylated, dimethoxytritylated in the presence $AgNO_3$ and *sym.*-collidine, and debenzoylated to give key synthons **7**. Dimethoxytrityl derivatives **7** were converted to the corresponding phosphoramidites **8** using standard methods. Analogously to the L-talo series, the D-allo

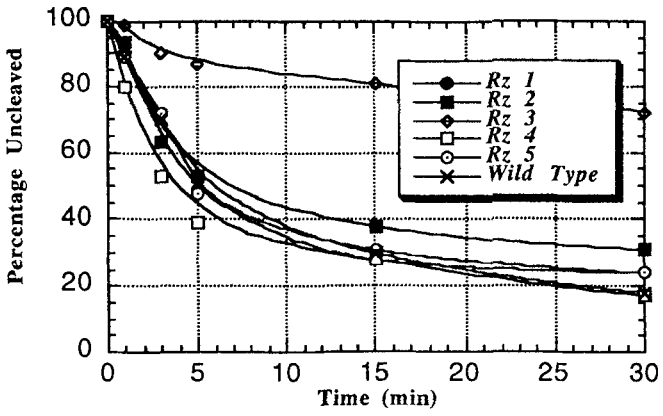


FIGURE 3

Cleavage Activity Of The Ribozymes Containing 5'-*C*-Me-L-Talo Ribonucleoside Modifications

phosphoramidites **14** were obtained from furanoside **9** *via* synthons **11** and **12**. The L-talo phosphoramidites were incorporated into hammerhead ribozymes by standard solid phase RNA synthesis[10] with increased detritylation and coupling times.

We demonstrated recently[11] that a generic hammerhead motif consisting of iT at the 3'-end, 5 ribonucleotides (G5, A6, G8, G12, A15.1), 2'-NH_2-U (U4 & U7) and 2'-*O*-Me nucleotides (other 31 residues) has almost wild-type (WT) activity and increased stability in human serum. Attempts to introduce additional 2'-*O*-Me residues in the "5 ribo-motif" were detrimental to catalytic activity, indicating that other modifications should be developed to stabilize these positions. 5'-*C*-Me-L-talo nucleotides are promising alternatives since their dinucleoside monophosphates have increased nuclease stability[12] and the corresponding nucleosides conformationally resemble natural ribonucleosides.[13]

We introduced 5'-*C*-Me-L-talo nucleotides at positions A6, A9, A9 + G10, C11.1 and C11.1 + G10, ***Rzs 1-5*** shown in Figure 2. ***Rz 3*** demonstrated low catalytic activity, whereas ***Rzs 1, 2, 4*** and ***5*** had almost WT activity (Figure 3). We also modified positions G5 and G8, which resulted in the loss of catalytic activity. The stability of ***Rzs 1-5*** was tested in human serum, ***Rzs 1-3*** showed stability close to, or slightly higher than, the stable generic ribozyme (*vide supra*). In the case of ***Rzs 4*** and ***5*** some degradation products were observed corresponding to cleavage at position C11.1. The effect was more pronounced for ***Rz 5***.

A complete systematic investigation of the incorporation of 5'-*C*-Me-nucleotides into, and their influence on catalytic activity and nuclease resistance of, hammerhead ribozymes is in progress.

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