Evaluation of GalNAc assemblies to enable hepatic siRNA delivery and robust gene silencing

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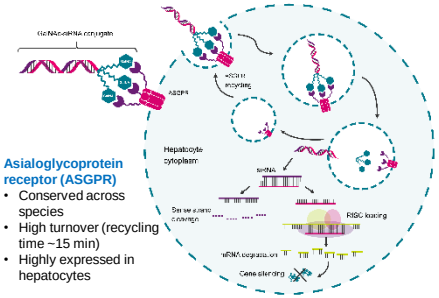
AXOLABS
Nucleic Acid Therapeutics by LGC

Introduction

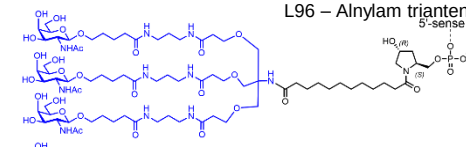
Multivalent N-acetylgalactosamine (GalNAc) ligand conjugation to nucleic acids therapeutics (NATs) such as short interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs) improves the intracellular delivery of these drugs to hepatocytes *in vivo*.[1] The high affinity of GalNAc for the asialoglycoprotein receptor (ASGPR) combined with receptor properties, such as high expression level and fast recycling rates on one hand and improvements in the chemical modification strategy of NATs on the other hand laid the foundation for potent and durable gene silencing activity. These advancements fuelled numerous clinical trials in recent years and led to 4 GalNAc conjugated siRNA drug approvals by the FDA and more are expected to follow[2]. The present investigation aims to establish a structure activity relation (SAR) of GalNAc conjugates[3], their orientation – (clustered vs dispersed), their site-selectivity within siRNA duplexes (3' vs 5' terminus) and the evaluation of their consequent ASGPR binding and RNAi activity *in vitro* and *in vivo*. In that context synthetically accessible GalNAc-structures that can readily be incorporated/conjugated, is a critical point for our consideration



Asialoglycoprotein receptor (ASGPR)
- Conserved across species
- High turnover (recycling time ~15 min)
- Highly expressed in hepatocytes

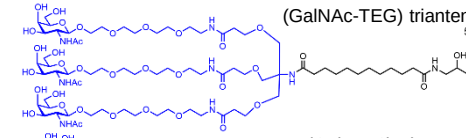
GalNAc – ligand design refinement

L96 – Alnylam triantennary GalNAc cluster[4]
5'-sense str



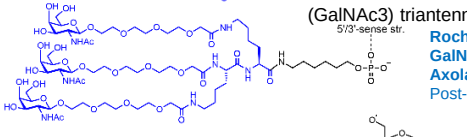
- **commercially available (IP!)**
- *receptor specificity*
- *metabolic stability*
- *safety and CMC*

(GalNAc-TEG) triantennary GalNAc cluster[5]
5'-Sense str.



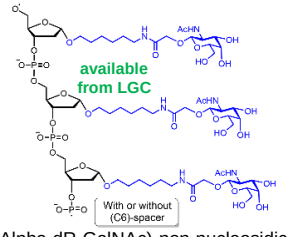
commercially available

(GalNAc3) triantennary GalNAc cluster[6]
5'/3'-sense str.



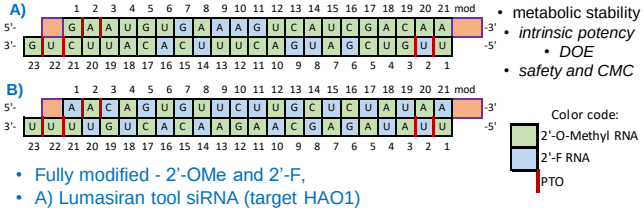
Roche, Arrowhead GalNAc, also used by Axolabs;
Post-synthetic conjugation

- commercially available GalNAc-units c.f. L96-Alnylam GalNAc
- influence of ligand position, proximity and steric factors on silencing activity
- evaluation of non-nucleosidic monovalent GalNAc units - trimeric as well as trivalent cluster-like (branching-linker) orientation through SPOS
- potentially streamline manufacturing and hence reduce cost of goods



(Alpha-dR-GalNAc) non-nucleosidic monomer

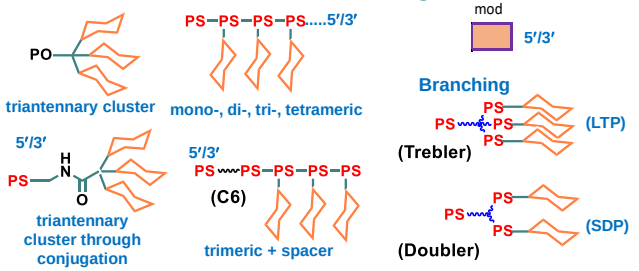
Target and Sequence design



- metabolic stability
- *intrinsic potency*
  - *DOE*
- *safety and CMC*

- Fully modified - 2'-OMe and 2'-F,
- A) Lumasiran tool siRNA (target HAO1)
  - cross reactive in human and mouse
  - 'advanced ESC' DV-18 21/23 pattern – reduced 2'-F content
- B) mTTR siRNA (target TTR expressed in mouse)
  - 'ESC' 21/23 pattern
- 2 X PS present at either termini (except for GalNAc-Cluster)

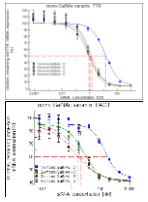
Schematics – GalNAc design



Mouse TTR and HAO1 – *in vitro* evaluation

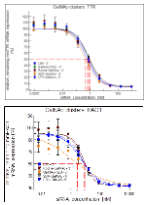
Incubation of primary mouse hepatocytes with the different GalNAc-conjugates in a dose-response experiment

Monomeric GalNAc (1 to 4, 5'/3')



	mTTR		mHAO1	
description	IC_{50} [nM]	max. inhib. [%]	IC_{50} [nM]	max. inhib. [%]
1x-mono-GalNAc - 5'	15.553	95.7	302.624	82.5
2x-mono-GalNAc - 5'	1.093	96.4	12.169	88.6
3x-mono-GalNAc - 5'	0.981	96.3	3.271	87.6
4x-mono-GalNAc - 5'	1.466	96.4	5.417	88.6
3x-mono-GalNAc - 3'	1.245	94.5	8.407	87.1
4x-mono-GalNAc - 3'	1.578	95.2	8.240	87.5
3x-C6-mono-GalNAc - 5'	0.796	96.9	5.348	88.1
3x-C6-mono-GalNAc - 3'	1.061	95.5	9.948	88.0
L96 - 3'	0.606	94.8	9.179	85.6

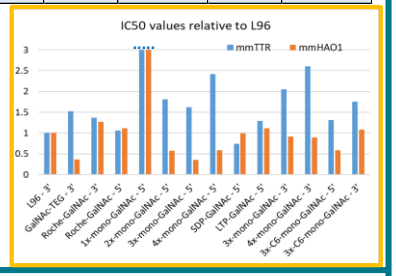
Cluster based GalNAc units



	mTTR		mHAO1	
description	IC_{50} [nM]	max. inhib. [%]	IC_{50} [nM]	max. inhib. [%]
GalNAc-TEG - 3'	0.921	94.9	5.285	87.0
Roche-GalNAc - 3'	0.830	95.2	11.661	86.7
Roche-GalNAc - 5'	0.645	96.2	10.190	88.5
SDP-GalNAc - 5'	0.450	95.7	9.077	86.2
LTP-GalNAc - 5'	0.785	95.9	10.276	87.5
L96 - 3'	0.606	94.8	9.179	85.6

Observation

All GalNAc-modifications perform closely similar to L96-cluster - mediating excellent siRNA uptake in a dose-dependent manner.
Exception: single mono-GalNAc variant has a significantly reduced internalisation ability.



Conclusion

The present study outlines SAR considerations, synthesis strategies and provides efficacy data in primary hepatocytes for the different GalNAc displays studied. Potency of the various GalNAc displays follows the trend mono << di <= tri ~ tetra. Potencies of clustered monomeric GalNAc units (SPOS) were high, resembled triantennary clusters and were closely similar to the L96 industry standard. Based on these *in vitro* data the mono-GalNAc phosphoramidite and solid support building blocks offered by LGC are highly viable options to design GalNAc ligands enabling functional delivery of NATs. *In vivo* studies, stability assessment and duration of effect (DOE) evaluation is currently ongoing.

Ref.:1. *J. Am. Chem. Soc.* **2014**, *136*, 16958. 2. *J. Clin. Invest.* **2019**; *129*, 915. 3. *J. Med. Chem.* **2016**, *59*, 2718. 4. US Patent WO2016055601A1. 5. *Bioconjugate Chem.* **2018**, *29*, 2478. 6. US Patent 8,426,554 B2.