Design, Syntheses and Biological Evaluation of Inositol-based Molecular Transporters

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I. Objective

In this research project, a series of organic molecules employing *myo*- and *scyllo*-inositol as the core structure are to be designed and synthesized according to known facts about the membrane-penetration efficiency. These compounds, mainly due to poly-guanidinium groups which demonstrated high efficiency in peptide cases, are expected to work as molecular transporters which may facilitate the membrane-crossing of some drugs which, by themselves are too polar to go through the cell membrane.

Starting from *myo*-inositol, molecular transporters with different chain length or varied substructures were designed as follows (Fig. 1 & Fig. 2).

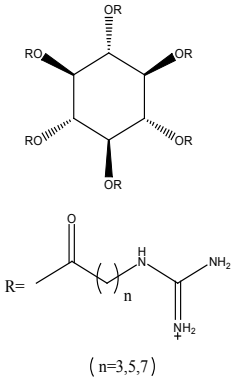

Fig. 1 *scyllo*-inositol based molecular transporters with 6 guanidinium groups (G6)

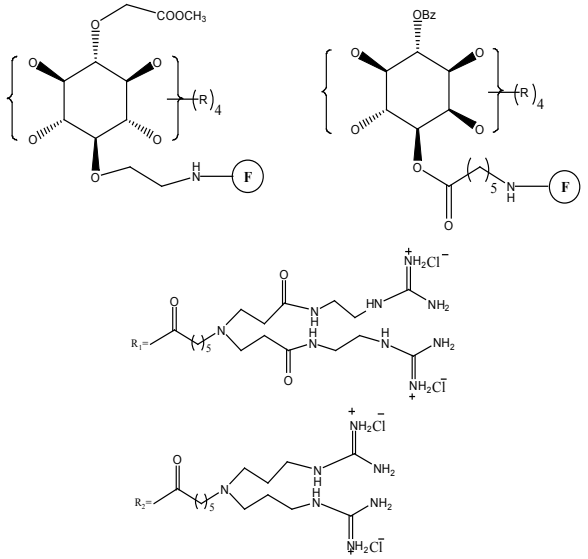

Fig. 2 *myo*- & *scyllo*-inositol based molecular transporters with 8 guanidinium groups (G8)

II. Background

Many drugs, drug candidates, and probe molecules fail because of cellular uptake problem, as efficient delivery of therapeutic compounds into cells in vivo can be achieved only when the molecules are small—typically less than 600 daltons.[1]

To circumvent these problems, the physical properties of the agent must be optimized to achieve acceptable levels of passive entry into cells, a process often requiring the synthesis and evaluation of numerous analogues. An emerging and highly effective alternative strategy involves conjugation of the agent to a molecular transporter allowing agents with a wide range of physical properties to enter cells.[2]

Recently, peptides derived from HIV Tat[3,4] and from Antennapedia[5] have been demonstrated to be able to enter membrane and used as transporters of otherwise difficult to deliver agents.

Futaki examined membrane permeabilities into mouse macrophage RAW264.7 cell of various peptides having a plurality of arginine residues and a fluorescent tag attached. The result revealed that a peptide having several arginine residues shows a permeability similar to that of Tat protein (AA 49-57), and an oligomer having eight (8) arginine residues is most effective in enhancing the transportation of molecules attached thereto across a biological membrane.[6] (Fig. 3).

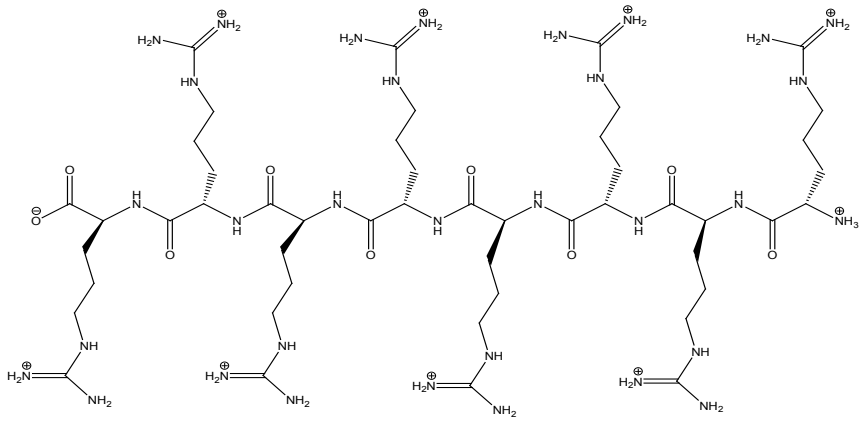

Fig. 3

Wender prepared a series of analogues of $Tat_{49\text{-}57}$ and studied their cellular uptake efficiency by flow cytometry. Study indicated that the cationic residue of $Tat_{49\text{-}57}$ play a principle role in its uptake process, and guanidinium groups play a greater role in cellular internalization rather than charge and backbone structure.[7]

Several points have been cleared up on the basis of the translocation efficiency of molecular transporters. The guanidinium head group is essential for transportation and poly guanidine peptides with different lengths and flexibility in side chains showed greater uptake efficiency. It has also been proved that increasing the flexibility of the side chains might allow a greater number of guanidinium groups to contact simultaneously a biological membrane or receptors.

However, such polyarginine peptides or peptoid molecules have the problem of rapid metabolism and elimination through the liver and kidney and *in vivo* toxicity liability.[7,8] Therefore, peptoids and poly carbamate—guanidinium rich oligomers are the alternative choices than Tat- sequence for cellular transportation. More recently, dendrimers, β-oligoarginines and γ-peptides are among other alternatives. Still all the variants are prone to rapid metabolism and have a processing cost problem.

In this research project, we are going to prepare a novel class of cell-penetrating transporters based on inositol derivatives as a core unit. A plurality of positively charged guanidinium groups are to be attached to the inositol core through various numbers of methylene spacer or different branches, and fluorescence probe is incorporated either through covalent bonding or

ionic complex. One significant advantage of inositol backbone is that no toxic metabolite is expected to be produced in vivo degradation.

The synthesized molecular transporters are expected to penetrate cell membrane with high efficiency and facilitate the cellular uptake of bioactive molecules which by themselves are not able to.

The ability to enable or enhance the uptake of agents into cells or tissue can have major implications in the development of new drugs or in the resurrection of previously non-viable drug candidates.

III. Research Plan

1. Design and Syntheses of Inositol-based Molecular Transporters

While reported arginine rich oligomer or peptoid molecular transporters employ unnatural back bone, in this research, we are going to take inositol, which is universal in biosystems, as the core structure to prepare guanidinium inositol derivatives which are expected to facilitate the translocation of bioactive molecules.

1.1 Synthesis of *scyllo*-inositol derivative from *myo*-inositol

Scyllo-inositol with all equatorial hydroxyl groups has been chosen as synthetic core unit, since it represents the simplest isomer in terms of symmetry. From *myo*-inositol, which is the most abundant inositol in nature, *scyllo*-inositol derivative is to be prepared after protection/deprotection and Mitsunobu conversion (Fig. 4).

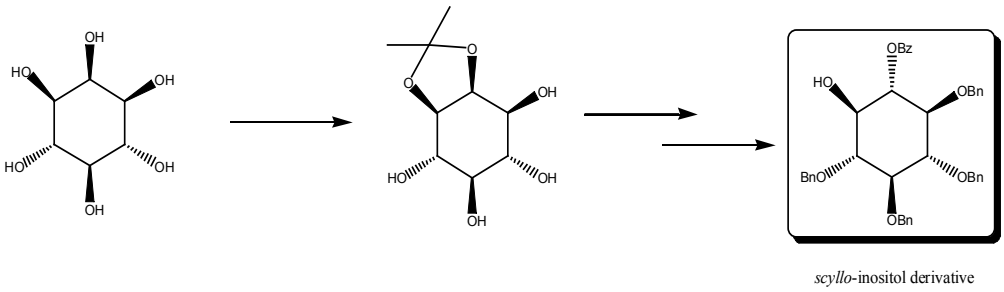

Fig. 4 Preparation of *scyllo*-inositol derivative from *myo*-inositol

1.2 Synthesis of *scyllo*-inositol based molecular transporters with 6 guanidinium groups (***scyllo*-G6**)

With the above prepared *scyllo*-inositol derivative as the starting point, *scyllo*-inositol based molecular transporters with 6 guanidinium groups are to be prepared after deprotection and EDC-mediated ester coupling (Fig. 5).

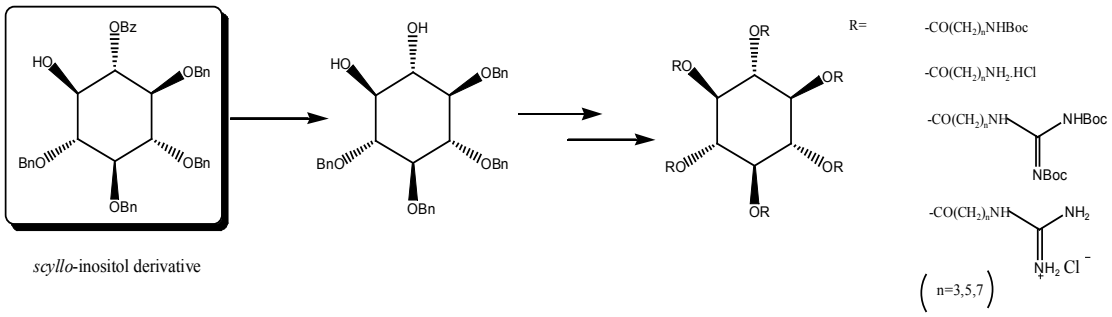

Fig. 5 Preparation of *scyllo*-inositol based molecular transporters with 6 guanidinium groups

1.3 Synthesis of *scyllo*-inositol based molecular transporters with 8 guanidinium groups (***scyllo*-G8**)

In this part, *scyllo*-inositol core structure is to be modified to contain two handles, one is for fluorescence labeling and the other as a free handle available for potential cargo/drug attachment. Side chains containing two guanidinium groups with different substructure are to be prepared in advance and attached to the core structure (Fig. 6).

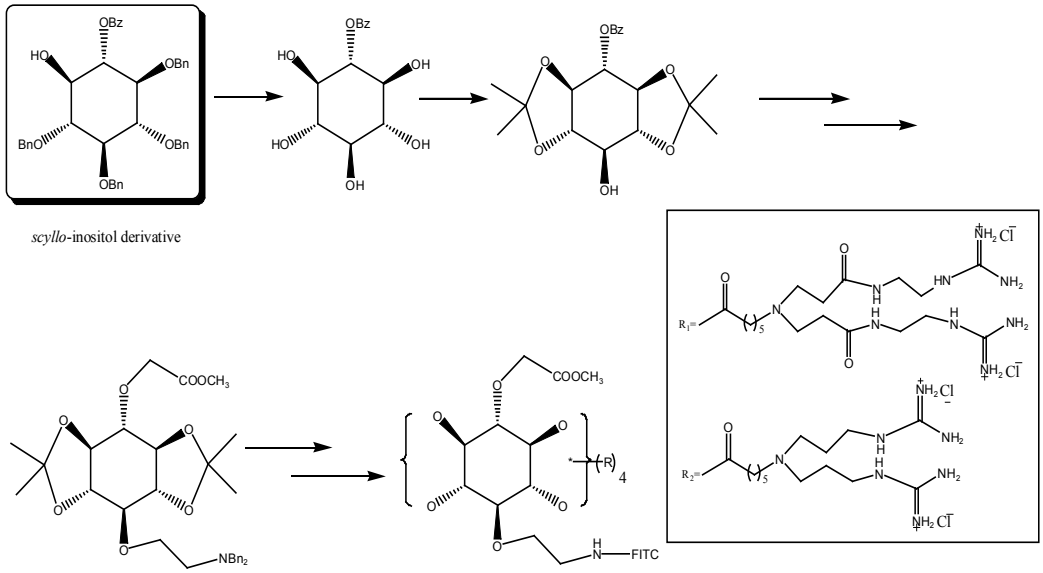

Fig. 6 Preparation of *scyllo*-inositol based molecular transporters with 8 guanidinium groups

1.4 Synthesis of *myo*-inositol based molecular transporters with 8 guanidinium groups (***myo*-G8**)

Similar to *scyllo*-G8 case, this series compounds have one handle fluorescence labeled and the other available for cargo/drug attachment. They are to be made from *myo*-inositol through careful protection/deprotection, attachment of one handle for fluorescence probe and EDC-mediated ester coupling with pre-made side branches (Fig. 7).

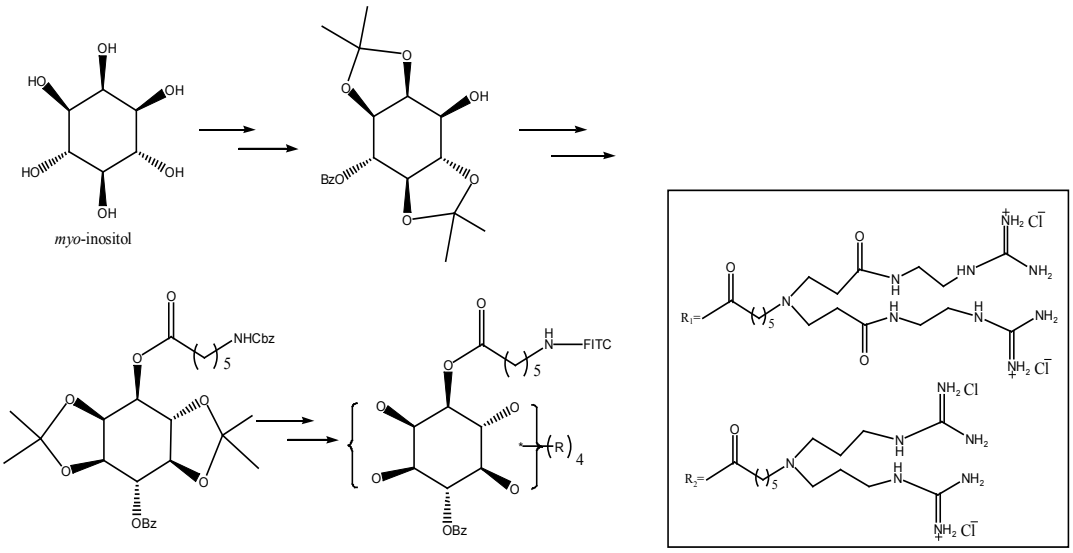

Fig. 7 Preparation of *myo*-inositol based molecular transporters with 8 guanidinium groups

2. Biological Evaluation (Bioassay) of Inositol-based Molecular Transporters

In this thesis, two categories of molecular transporters are to be synthesized. *scyllo*-Inositol based molecular transporters with 6 guanidinium groups do not contain fluorescence probe, while those with 8 guanidinium groups, both *myo*- and *scyllo*-inositol based, have fluorescence probe covalently attached.

The membrane-penetration effects of all synthesized molecular transporters are to be examined by Confocal Laser Scanning Microscopy (CLSM).[9] Compounds of the first category are to be mixed with some fluorescence probe to form ionic complex before confocal experiments.

IV. References

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