Improving Cryopreservation of Hematopoietic Stem and Progenitor Cells with *N*-Aryl-D-Gluconamides as Ice Recrystallization Inhibitors

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What are HSPCs?

The purpose of this experiment is to improve the storage of hematopoietic stem and progenitor cells (HSPCs) for long periods of time. HSPCs found in umbilical cord blood (UCB) can be used to combat many hematological and non-hematological diseases, such as metabolic disorders and hematological cancers. Furthermore, HSPCs are becoming an integral part of emerging cellular regenerative therapies for numerous diseases, including neurological disorders and diabetes.[1] Due to the increasing usefulness of HSPC products as life-saving treatments, the proper storage of them is extremely important.

While long-term storage at sub-zero temperatures help preserve HSPCs, cryopreservation also damages and reduces function of the cells post-thaw.[2] Cryoprotectants help reduce this damage, but the most commonly used cryoprotectant, dimethyl sulfoxide (DMSO), is toxic and fails to reduce the cryoinjury[2] from ice recrystallization, a process where large ice crystals form from smaller ones. Therefore, the Ben lab is currently trying to combat the cryodamage through the use of ice recrystallization inhibitors (IRIs).

This specific experiment tests the IRI activity of two previously synthesized compounds which have recently shown to be effective at preserving the functionality of HSPC post-thaw,[3] 4-methoxyphenyl-D-gluconamide (**1**) and 2-fluorophenyl-D-gluconamide (**2**), at two concentrations (11 and 5.5 mM), along with investigating how a 1:1 combination of these two compounds affects IRI activity (both at 5.5 mM). The experiment also involves the synthesis and IRI analysis of a new compound, 2-fluoro-4-methoxyphenyl-D-gluconamide (**3**). Gluconamide **3** incorporates the key structural features present in IRI-active compounds **1** and **2.**

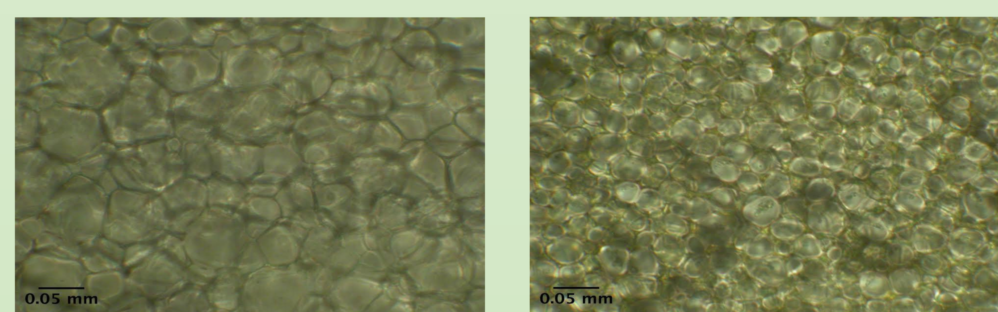
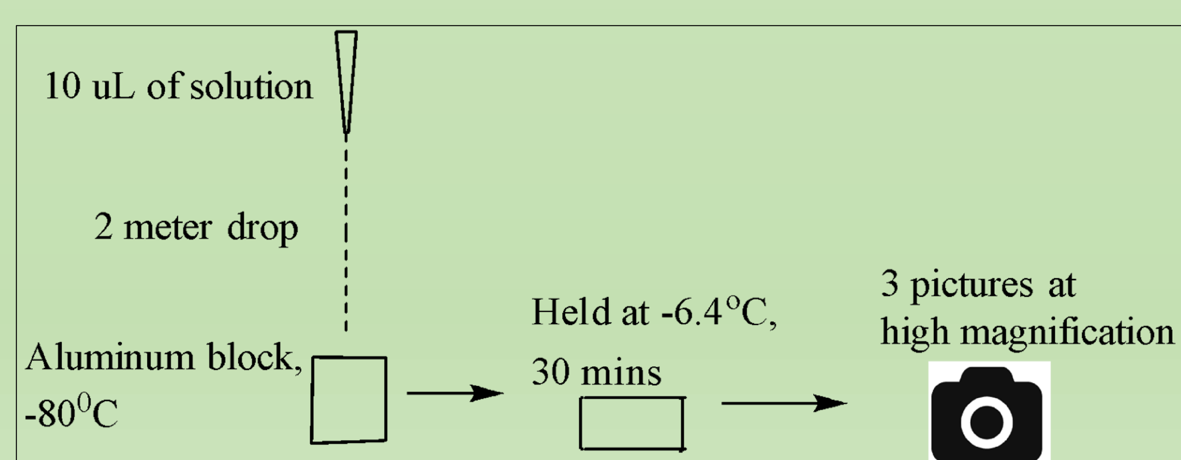


Figure 1: Images of ice crystals without (left) and with an ice recrystallization inhibitor (right).[4]

Splat-Cooling Assay:[5]

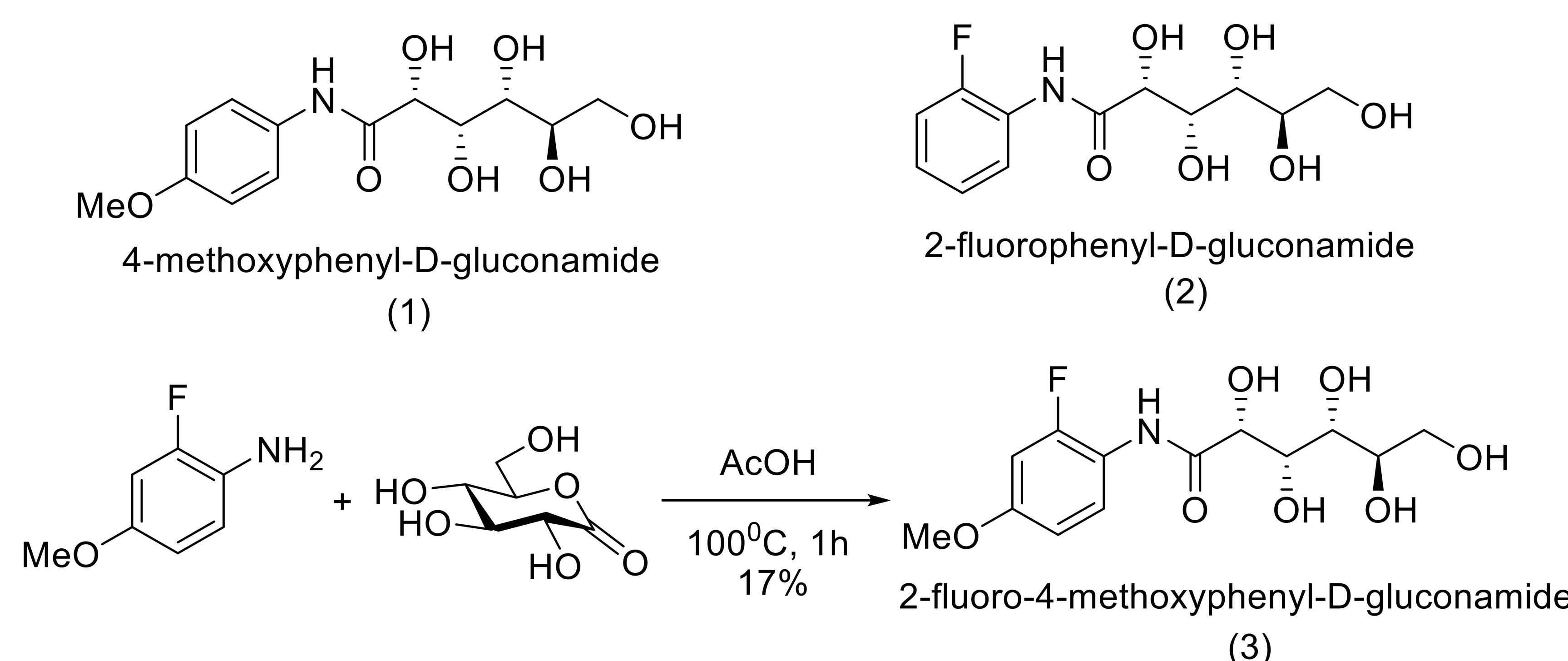
This assay is designed to determine the extent of ice recrystallization occurring in the presence of the *N*-aryl-D-gluconamides. Ice wafers are formed from the compound of interest dissolved in phosphate-buffered saline, PBS.

- 10 μL of the solution is dropped onto an aluminum block cooled to -80 °C which instantly forms an ice wafer.
- The ice wafer is kept at -6.4 °C for a 30-minute annealing period. This was found to be the best conditions for potential ice recrystallization.
- Pictures are taken with a camera fitted to a microscope and later analyzed to find the average area of the ice crystals. The mean grain size (MGS) determined for the compound is relative to PBS, the positive control for ice recrystallization.



Scheme 1: Splat-cooling assay used to determine IRI activity of carbohydrate-based small molecules.[5]

Ice Recrystallization Inhibitors in this study:



Scheme 2: Structures of **1** and **2** and synthesis of *N*-aryl-D-gluconamide **3**. Compounds **2** and **3** were synthesized under similar conditions.[3]

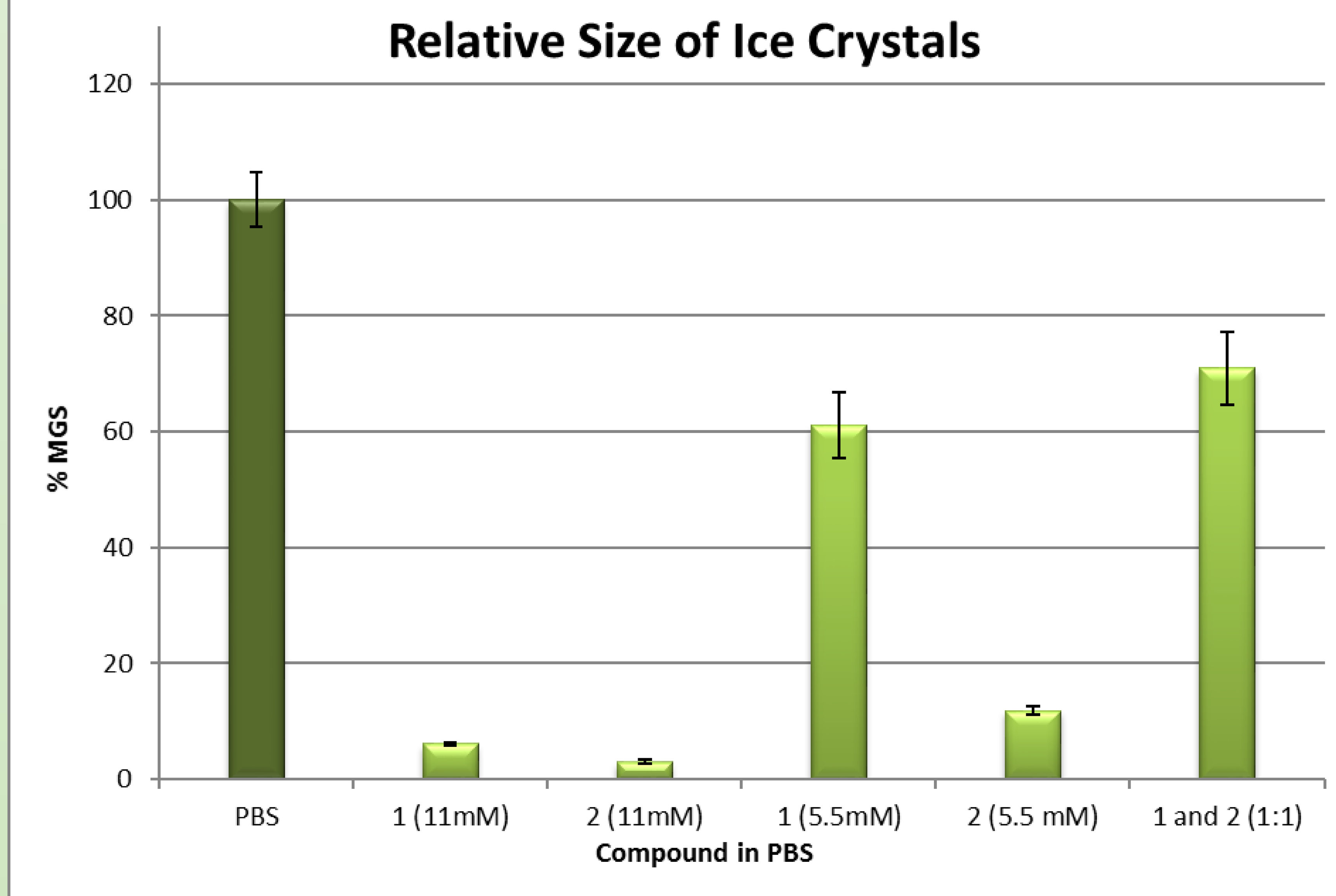


Figure 2: IRI activity of gluconamides **1** and **2** represented as % MGS (mean grain size) of ice crystals relative to PBS. Values represent the average of three runs ± % SEM. The 1:1 solution contained 5.5 mM of both **1** and **2**. The value for **2** at 11 mM was obtained from reference 3.

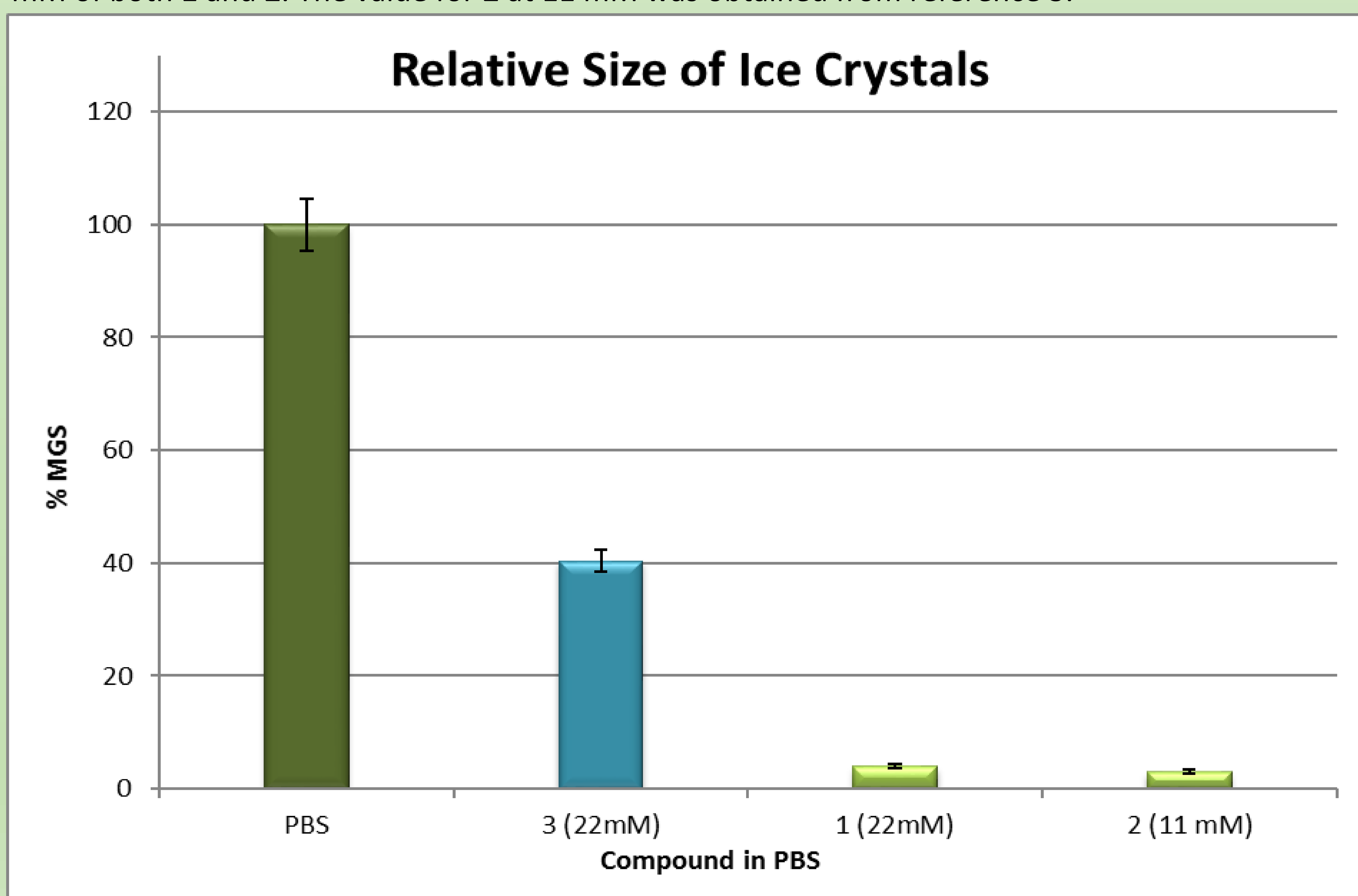


Figure 3: IRI activity of gluconamides at 22 mM represented as % MGS (mean grain size) of ice crystals relative to PBS. Values represent the average of three runs ± % SEM. The values for **1** and **2** were obtained from reference 3.

Conclusions:

- Gluconamide **2** was a more effective IRI than **1** at both concentrations tested, albeit only slightly at 11 mM (figure 2).
- Interestingly, the IRI activity obtained for the 1:1 solution of **1** and **2** indicates that the two compounds together are less effective at inhibiting ice recrystallization than on their own (at the same concentration).
- Gluconamide **3** was a less effective IRI than either of the other two compounds (figure 3).
- These results contradict the hypothesis that combining two key elements of the parent molecules would maintain or increase IRI activity. This further indicates the complexity of the inhibition process.

Future work:

- Optimize the reaction conditions for the synthesis of gluconamide **3**.
- Develop a dose-response curve and IC_{50} value for compound **3** to see if it is more effective at higher concentrations.
- Cryopreserve HSPCs with **3** to analyze its ability to act as a cryoprotectant. The cells will be frozen with the compounds, thawed, and then put in a culture for a number of weeks to test viability and function post-thaw. If these *in vitro* analyses show promising results, **3** should be tested *in vivo*.
- Testing combinations of **1** and **2** at different ratios to see the resulting IRI activity. The fact that the IRI activity obtained for the 1:1 mixture of **1** and **2** mimics the less active compound is unexpected. Further investigation is warranted, and includes repetition of the splat-cooling assay and analyzing the consistency of the IRI activity throughout the ice wafer.

References:

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2. Baust, J. M. *Cell Preserv. Technol*. **2002**, *1*, 17.
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4. Adam, M. K. *et al. RSC Adv*., **2016**, 6, 39240.
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Acknowledgements: