STEM Games 2018 - Science Arena

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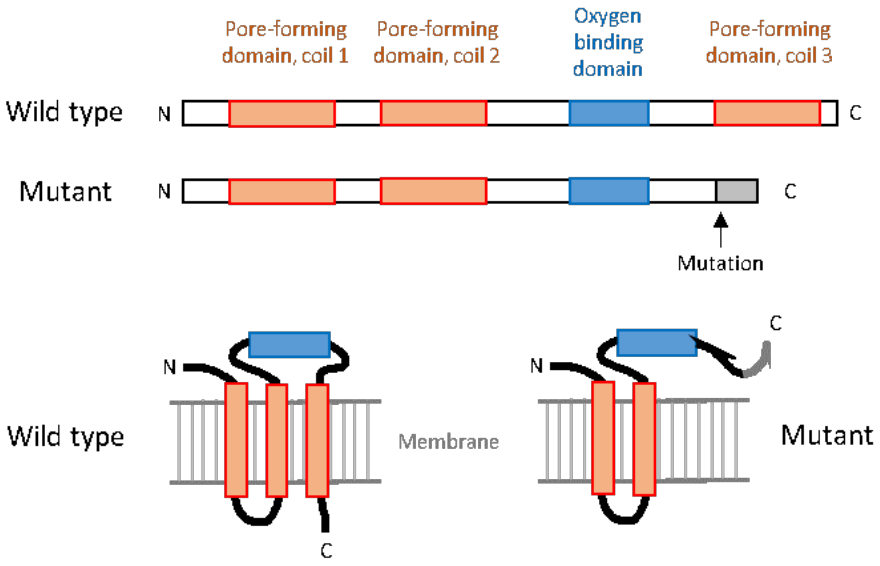
Day 1 - Molecular Biology

1.1 Introduction

These tasks concern a rare (and fictional) respiratory disease, known as Intermittent Systemic Tiredness with Respiratory Anomalies (ISTRA) syndrome, which your research group is studying. ISTRA syndrome is a genetic condition known to be caused because of difficulties in oxygen diffusion across the membranes of the alveoli and into the blood. This leads to symptoms of mild, but persistent, hypoxia.

Your recent research has shown that in most patients, ISTRA syndrome is caused by an inherited mutation in the (also fictional) Putative Oxygen Recycling and Exchange Channel (POREC) gene. The POREC gene encodes a protein, PoreC, which aids in oxygen exchange across the alveoli membranes. This mutation is fatal if two copies of the mutant gene are inherited, while individuals with one wild-type POREC gene and one mutant POREC gene survive but develop ISTRA syndrome.

PoreC is a 426 amino acid long protein, but the mutation causes a frameshift at amino acid 353. As a result of the frameshift, the protein loses its final 73 amino acids, and instead has 12 non-specific amino acids followed by a stop codon. PoreC is a transmembrane channel protein, and both the wild-type and mutant form are found in the epithelial membrane. However, while the mutant form can bind to oxygen, it cannot transfer the oxygen into the cell. The mutant PoreC proteins therefore compete for oxygen molecules with the wild-type proteins, leading to less oxygen successfully crossing the membrane.



Schematic of the PoreC protein, it its wild type and mutant forms. Top: Linear diagram. Bottom: The folding of the protein when it is inserted into the membrane.

Your group have now designed a set of chemical compounds that should bind specifically to the mutant form of PoreC, and stop it from binding to oxygen. If this is successful, then they should

be usable as a drug, administered in gaseous form through inhalation, to treat ISTRA syndrome. But first you need to test your potential drugs in vivo.

1.2 Task

You must design a transgenic animal that can be used as a model of ISTRA syndrome.

In order to test the compound, you will need a transgenic animal which mimics as closely as possible, the genetic mutation found in ISTRA patients. This could involve adding synthetic genes, or deleting or modifying existing ones. The choice of animal and of genetic change is up to you. You will then have to select (from your own knowledge or reading the literature) a standard biological system by which the change can be introduced to the animal. This needs to be practical and appropriate for the transgenic animal you wish to produce. Finally, a schematic should be provided of the genetic construct that you would use for the task.
You must provide:

- the species of animal to be used, with brief justification of why it is appropriate
- details of exactly what genes you would introduce/mutate/remove, with brief justification
- the system through which you would perform these alterations, with brief justification
- a simple schematic of the genetic construct that will be used for this process, indicating the major elements

Day 2 - Organic Synthesis

2.1 Introduction

Your transgenic animal is now being produced. Meanwhile you have been performing in vitro tests on your various chemical compounds, in order to find our which binds most efficiently to the mutant PoreC protein. These were supplied to you by a collaboration partner, initially only with labels, no chemical structures.

After much analysis, you have determined that one compound, HR52440, appears to bind very strongly to the mutant form of PoreC, but not to the wild-type protein. It therefore appears to be the most promising novel drug treatment for ISTRA syndrome. Now you need to begin producing more of it for use in your animal experiments, and hopefully in patients!
Your collaboration partner has sent you a description of how HR52440 was produced:

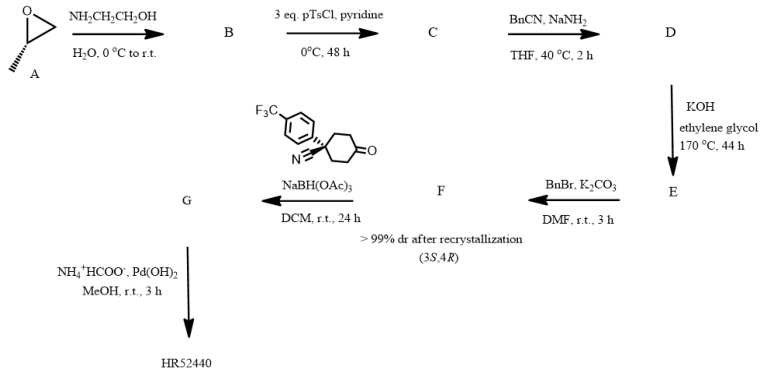


Figure 1: Route of synthesis for compound **HR52440**

Unfortunately, it was found that solid **HR52440** is of low bioavailability in test subjects, due to very poor solubility in intestinal fluids.

You must determine the structure of **HR52440**. In the scheme above, compound A is the starting material, while compound **HR52440** is your potential drug. Starting with compound A, optically active epoxide, work your way through the synthetic scheme and fill in the missing structures.
Additional analysis of intermediates and final product is given:

- ^{1}H NMR spectrum of compound **B** in d6-DMSO shows three wide peaks (each corresponding to one hydrogen atom), which are not present in 1H NMR spectrum of compound **B** in D2O.

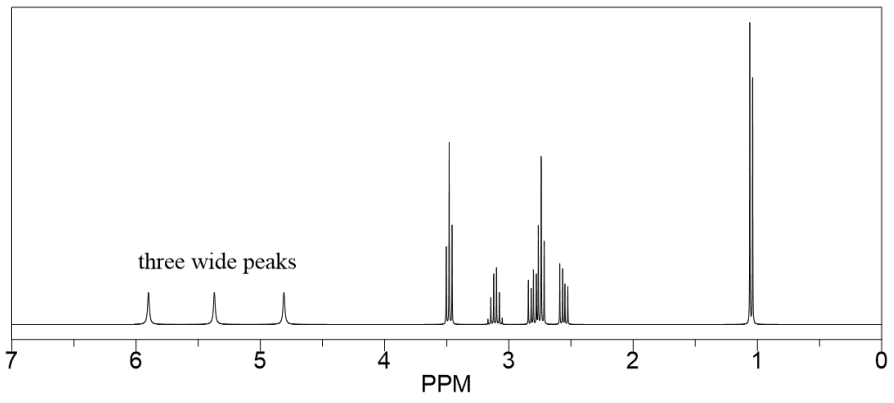


Figure 2: ^{1}H NMR spectrum of compound B in d6-DMSO

- Compound **D** was crystallized and characterized by X-ray diffraction. It was found that unit cell of compound **D** is:

$$a = 8.74 \text{ Å}, b = 9.12 \text{ Å}, c = 11.56 \text{ Å}, \alpha = \beta = \gamma = 90, Z = 2$$

  Density of compound **D** is 1277 kg m^{-3}.

- FTIR spectra of compound **D** revealed medium-strong absorption band at 2235 cm^{-1}
- Compounds **D** and **E** were obtained as a 1:1 diastereomeric mixture.
- Recrystallization of compound **E** only slightly improved diastereomeric ratio (diastereomeric purity).
- Compound **E** readily forms salts with strong inorganic bases.
- Elemental analysis of compound **E**: C 64.32%, H 6.21%, N 3.75%, S 8.59%
- Compound **F** was obtained by recrystallization of crude product isolated from reaction mixture. Recrystallization provided a good diastereomeric ratio (over 99% dr) and a good yield. Compound F has (3*S*, 4*R*) absolute configuration.
- Chemical formula for compound **G** is $C_{34}H_{35}F_3N_2O_2$.
- Conversion of **F** to **G** is via unsaturated intermediate.
- Final compound, **HR52440**, is an amphiprotic compound with 3 chiral centers.

2.2 Task

1. Provide chemical structures for each intermediate stage (compounds **B-G**) and your final drug (**HR52440**). Justify your solution for each compound.
2. Suggest and explain isolation and purification procedures for compounds **B**, **E** and **HR52440** from their respective reaction mixtures.
3. Write a detailed and well commented mechanism for conversion of **C** to **D**.
4. Suggest and explain possible ways to improve bioavailability of **HR52440**.

Day 3 - Biomedicine

3.1 Introduction

Following successful trials of your compound, **HR52440** in transgenic animals, you are now working toward beginning clinical trials. Initial tests have shown **HR52440** to be safe for use in humans, and so you now want to perform a pilot study in ISTRA syndrome patients. As a reminder, these patients experience reduced transfer of oxygen into the epidermal cells of their lungs, due a frameshift mutation in the *POREC* gene, and so experience symptoms relating to mild, persistent hypoxia.

The initial pilot study will involve 10 patients, 5 of which will be given your compound, and 5 of which will receive a placebo. Additionally, 5 healthy control individuals will be given the placebo. At the beginning of the trial, and once per month after that, each participant (patient or control) will attend the clinic to be tested. They will remain in the clinic for approximately one hour. This will be enough time to perform one test on the patient themselves, and to collect material (blood, skin sample, etc.) for one laboratory test. After their fourth visit (three months after the trial began), you will use the data from these experiments to determine if the drug, **HR52440** is being effective.

3.2 Task

You must design two experimental assays to determine if **HR52440** is effective in patients.

The first is to be applied to the patient while they are in the clinic. The test must be possible to perform in less than an hour, and in a hospital. You will have access to any reagents and equipment that could reasonably be expected to be found in a well-equipped hospital.

For the second experiment, you must select a type of tissue sample (e.g. blood, skin sample) to take from the patient, and devise a biochemical or cell biology assay by which you could assess the effects of the drug later in a laboratory. Invasive procedures (e.g. surgery) to collect the sample are not allowed, however a small amount of discomfort for the patient, not requiring anaesthetic, is allowed if it will provide access to data that cannot be gained more easily. You can assume that you have access to reagents and equipment that could be expected to be found in a well-equipped biochemistry and/or cell biology laboratory. Only a single sample type may be taken and your experiment must be based on a single experimental approach, however it is possible to collect multiple sets of data.

For the first experiment, you must provide:

- a diagram of the experimental apparatus
- a protocol for the experiment
- details of how the data would be analysed

For the second experiment, you must provide:

- details of the type of clinical sample, with justification
- a protocol for the experiment
- details of how the data would be analysed

In both tests, the data obtained must be quantitative, so that data from patients given drugs or the placebo can be compared. It is up to you to determine how you would assess the success of the drug treatment; however this could include determination of oxygen uptake, function of the *PoreC* protein, or more general physiological consequences of hypoxia.