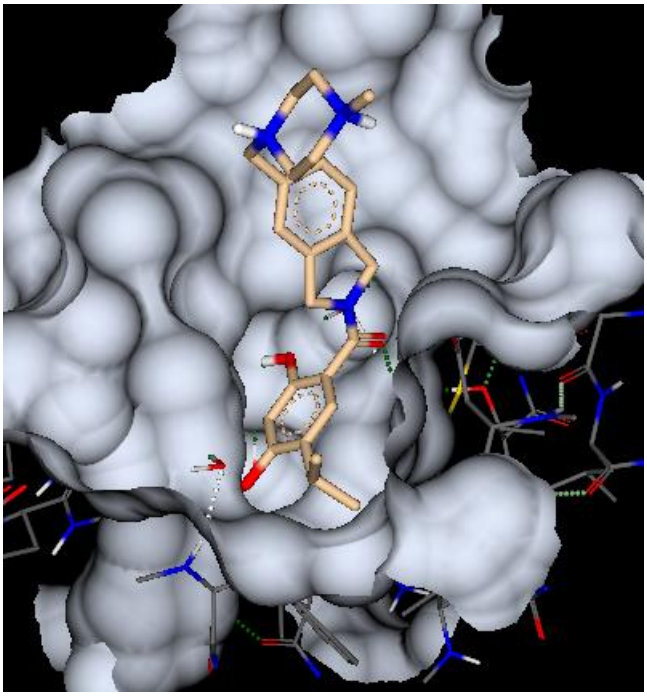
Worked Example:

R-group Clipping of Reagents for Library Enumeration

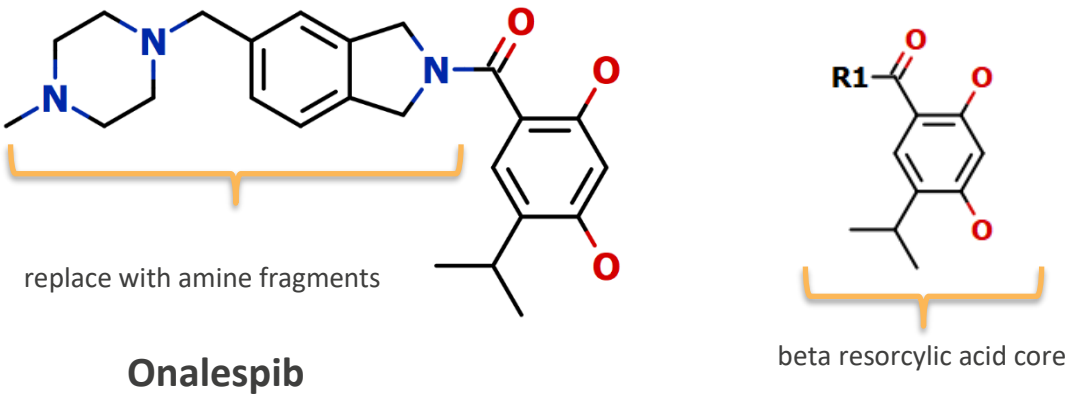
This example explores some of the challenges typically encountered in scaffold-based library design, in particular the task of creating reagent fragments (clipping) for use in scaffold-based library enumeration. Using StarDrop's R-group clipping tool, we will quickly transform chemical building blocks into their corresponding substituents, ready to enumerate a virtual library in StarDrop's Nova module.



The crystal structure on the right (PDB 2XJX) shows the binding site of Heat Shock Protein 90 (HSP90) with Onalespib as the co-crystallised ligand. Onalespib is a selective, potent HSP90 inhibitor that displays a long duration of anti-tumour activity. The beta resorcinol group forms a tight hydrogen bond network in the binding site, but the 5-(piperazin-1-ylmethyl)-isoindoline does not form any strong interactions with the protein.

We will prepare a data set of virtual compounds based on an amide coupling reaction with a beta resorcylic acid core and commercially available secondary amines. The resulting amide library will be suitable for further analysis and prioritisation in StarDrop, including multi-parameter optimisation using ADME property calculations and Probabilistic Scoring. The virtual compounds can also be

investigated further using StarDrop's Pose and Affinity modules to generate 3D conformations and assess their binding affinity. Alternatively, the compounds can be submitted to any other docking software via StarDrop's Pose Generation Interface to explore binding site interactions.

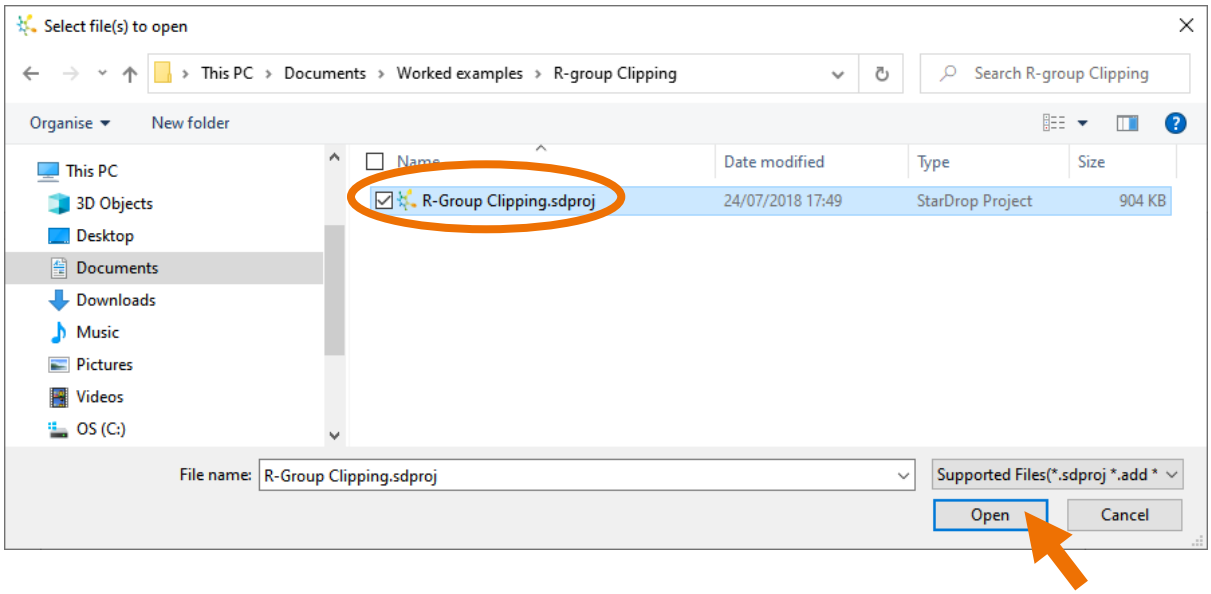


Onalespib

Step-by-step instructions for all the features you will need to use in StarDrop are provided, along with screenshots and examples of the output you are likely to generate. If you have any questions, please feel free to contact stardrop-support@optibrium.com.

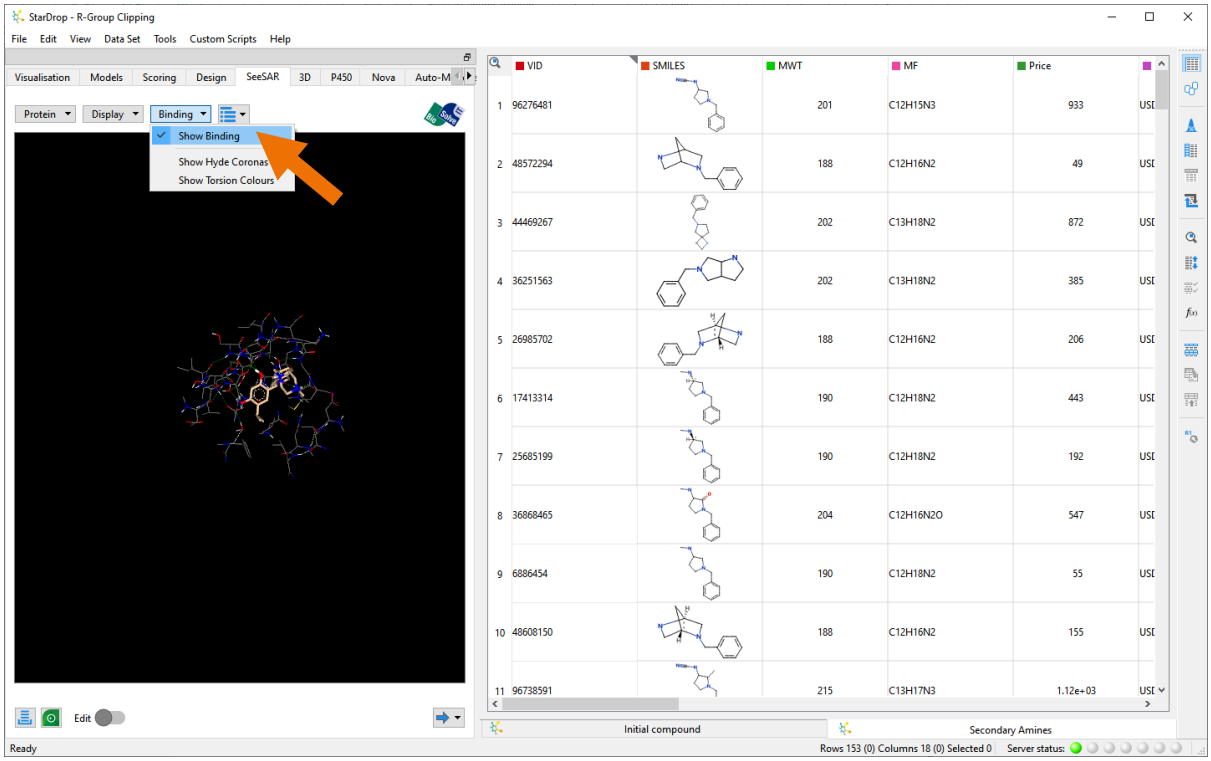
Exercise

- In StarDrop, open the file **R-group Clipping.sdproj** by selecting **Open** from the **File** menu.

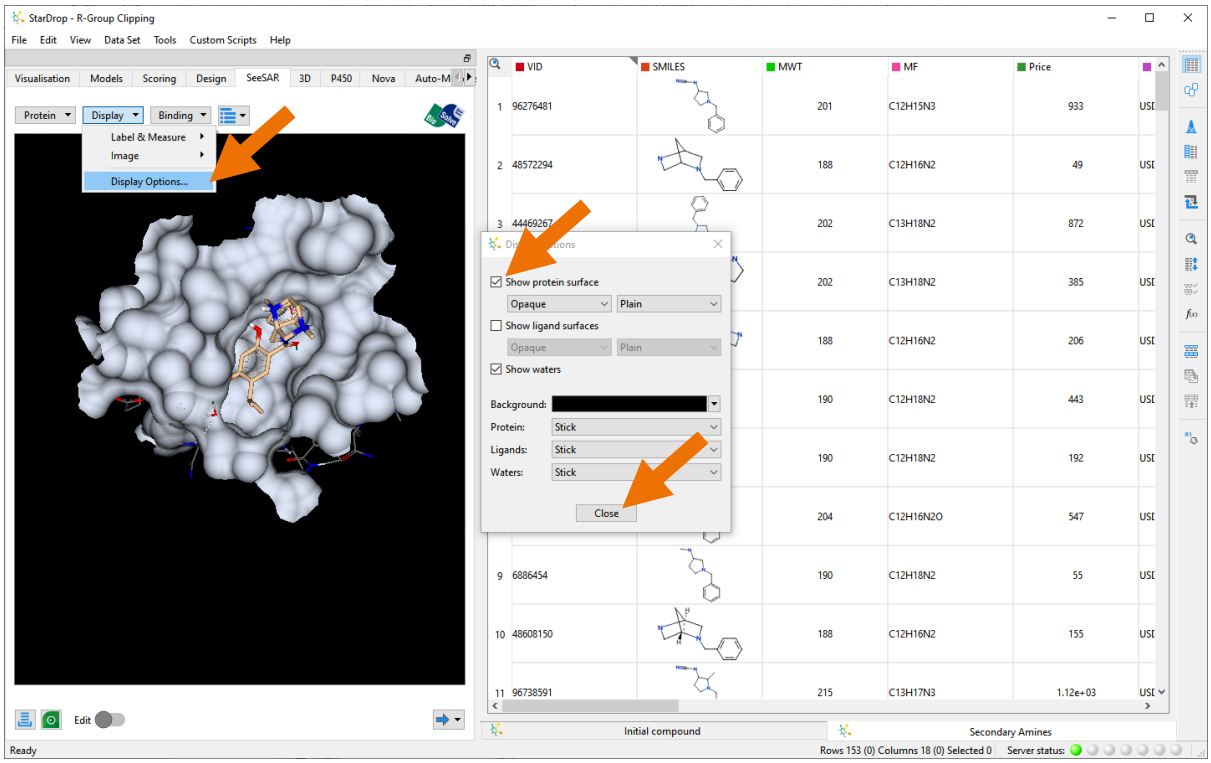


On the left, in the SeeSAR area, the protein HSP90 is displayed with its secondary structure, and the co-crystallised ligand, Onalespib. To better understand the binding mode of Onalespib, we can explore the binding site.

- In the SeeSAR area, select **Show Binding** from the **Binding** menu above the protein to change the view of the protein to focus on the binding pocket.



- To show the binding site surface, select **Display Options** from the **Display** menu at the top of the SeeSAR area.
- Select **Show protein surface** from the **Display Options** dialogue box and then click the **Close** button to close the dialogue box.



Hint: Using the mouse, you can interact with the view of the protein:

- Use the mouse-wheel to zoom in and out
- Use the left mouse button and drag to rotate the view
- Use the right mouse button and drag to pan the view

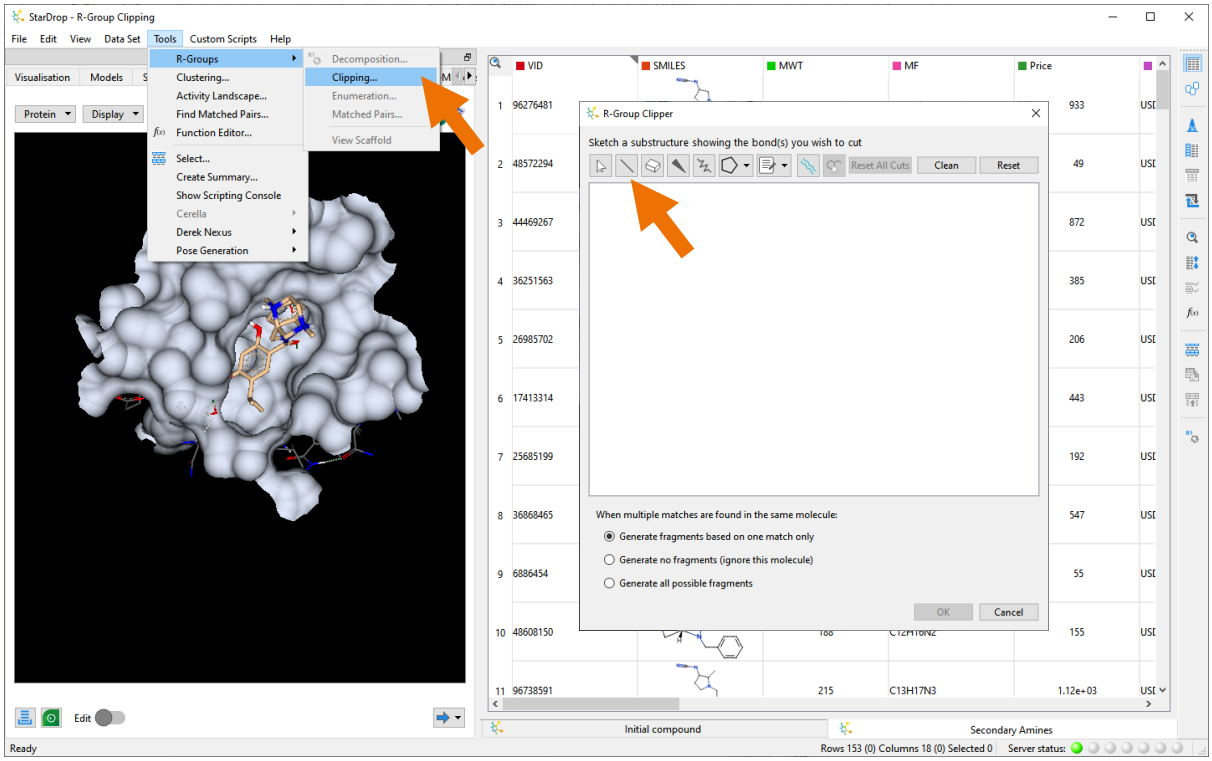
Note: Hydrogen bond interactions are indicated by either green or white dashed lines depending on the strength of the interaction, with green being stronger.

On the right, we have included in this project a data set containing 153 secondary amine structures and their associated meta-data, which were retrieved directly from eMolecules. To learn more about querying and retrieving information on eMolecules compounds directly from StarDrop, please visit:

https://www.optibrium.com/community/videos/introduction-to-stardrop-modules-and-features/357-stardropemolecules

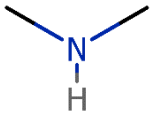
The first step in creating the virtual library is to clip the secondary amine reagents into R-group fragments that we can use to enumerate the new library.

- Open the **R-Group Clipper** dialogue by selecting **R-Groups** from the **Tools** menu and choose **Clipping**.



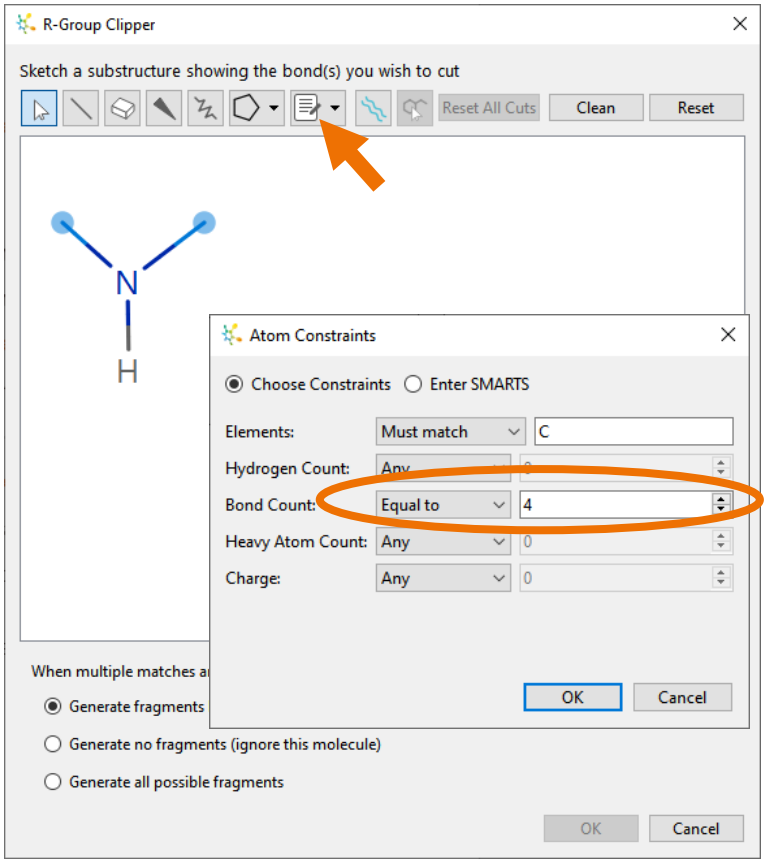
In the R-Group Clipper, we can sketch a substructure that defines how compounds in the data set should be clipped. In this case, we will sketch the secondary amine and impose some bond and atom constraints to limit the fragment set to only cyclic, aliphatic, secondary amines.

- In the sketch area, use the **Bond** tool  to sketch a simple dimethyl amine molecule.



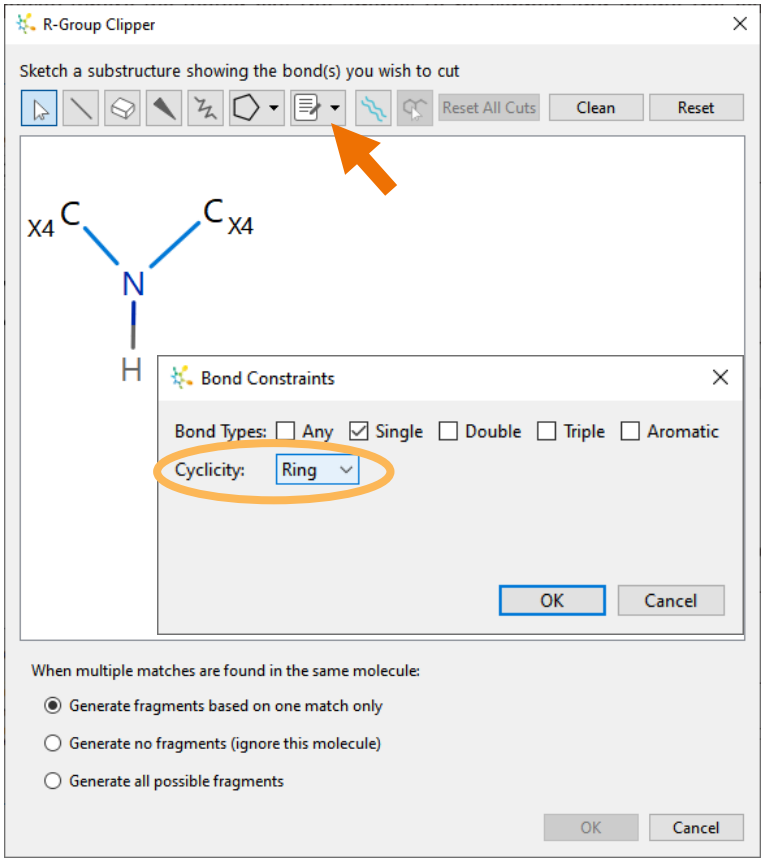
Hint: To specify an element, hover over an atom and type the element symbol, in this case, “N” and “H”.

- To add some atom constraints to the two carbon atoms, first select them both by pressing the **CTRL** key while using the **Selection** tool  .

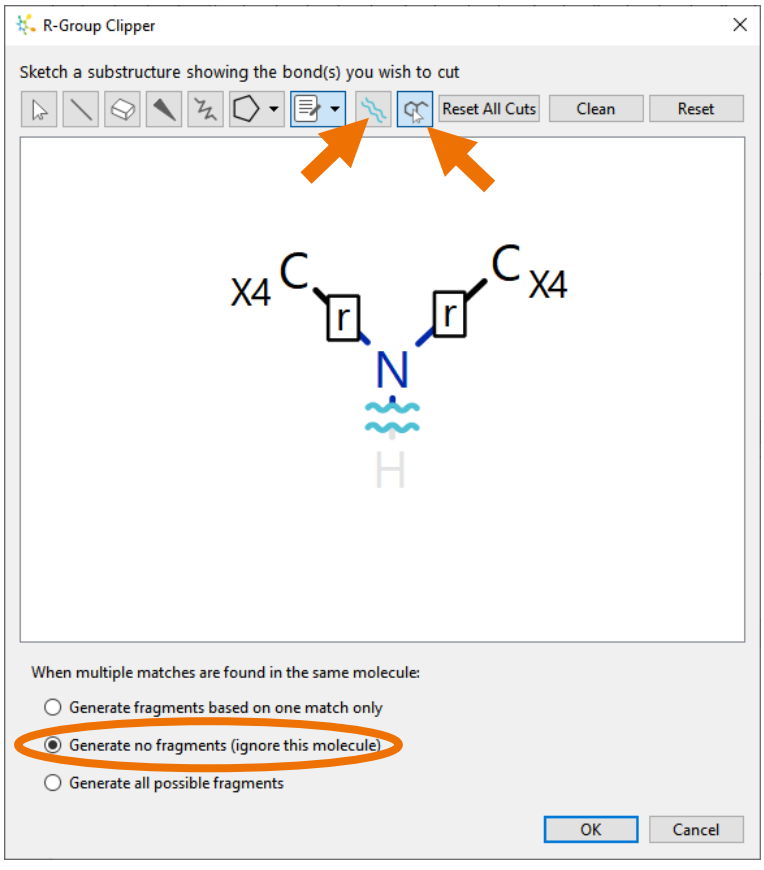


- Click on the **Constraints** menu  and choose **Edit Atom Constraints** to display the **Atom Constraints** dialogue.
- Specify that each carbon atom’s bond count should be **Equal to 4** and click the **OK** button.
- To add some bond constraints, first select the two N-C bonds by pressing the **CTRL** key while using the **Selection** tool  .

- Choose **Edit Bond Constraints** from the **Constraints** menu  to display the **Bond Constraints** dialogue.
- Select **Ring** from the **Cyclicity options** to specify that these bonds must be single bonds that are part of a ring.
- Click the **OK** button.



Now we need to specify where we would like the amines to be clipped and define the excluded fragment.



- Select the **Cut** button  and click on the N-H bond to clip this bond.
- Select the **Choose** button  and then click on the Hydrogen to exclude it from the generated fragments.

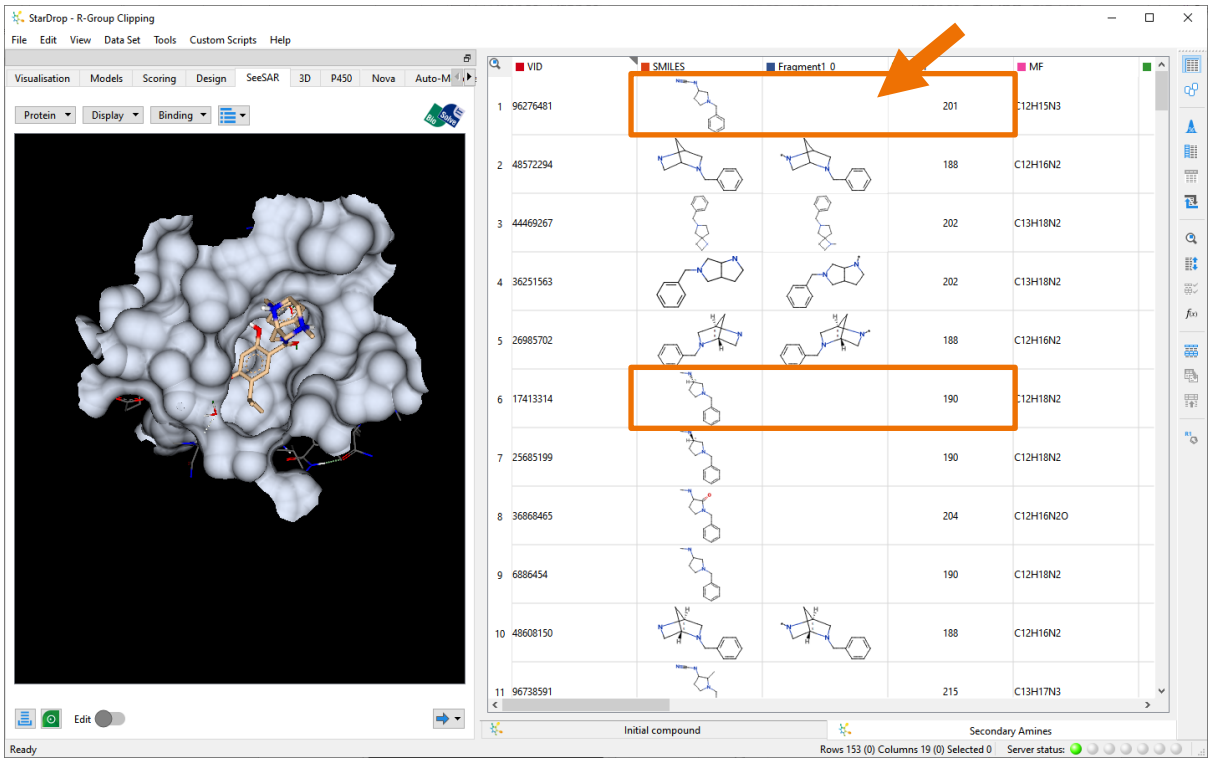
When comparing molecules in the data set with the specified substructure to determine where to clip them, it is possible that multiple matches might be found within the same molecule. At the bottom of the **R-Group Clipper** dialogue, you

can specify what should happen when this occurs and, in this example, we will ignore these secondary amines and generate no fragments when multiple fragments are possible.

- Select the option to **Generate no fragments (ignore this molecule)** and click the **OK** button**.**

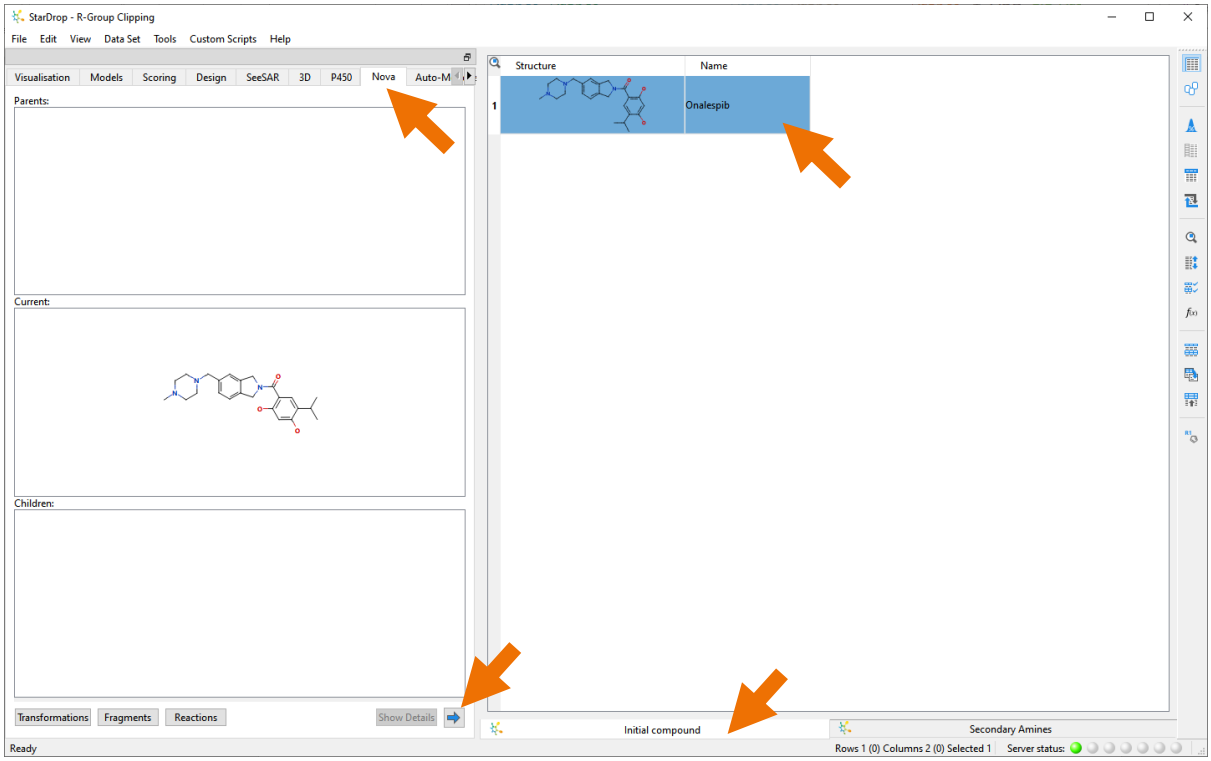
The fragments will be generated in a new column called **Fragment1_0** as shown in the screenshot below with a * indicating the attachment point.

Note: Some rows will not contain a fragment due to the exclusion criteria we specified. Examples are highlighted in the screenshot below.



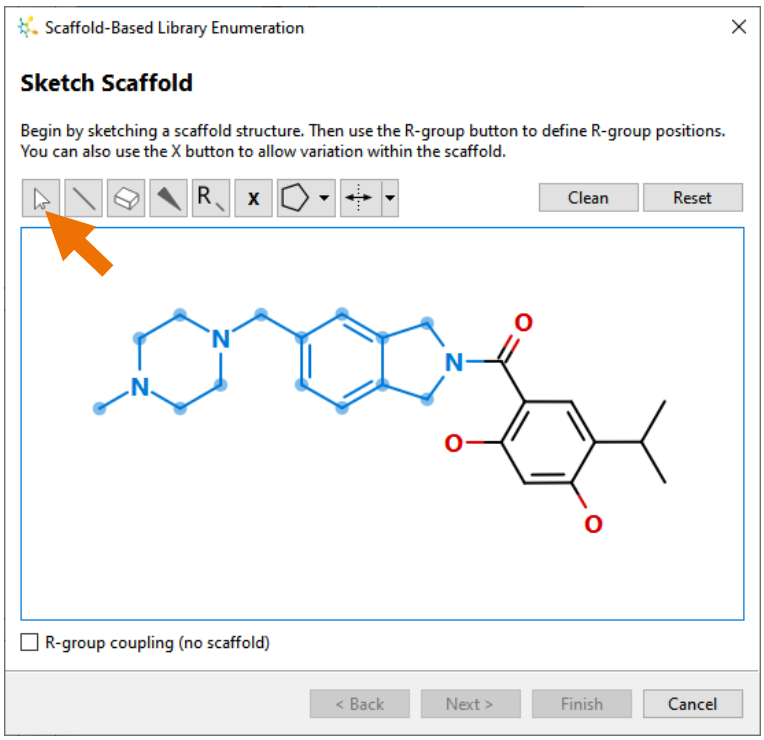
Using this set of fragments, we can now enumerate an amide library using a resorcylic acid scaffold derived from Onalespib. The structure of Onalespib is available in the **Initial Compound** data set, which is also part of this project.

- Click on the **Initial Compound** data set tab and select the row containing Onalespib.
- Click on the **Nova** tab.

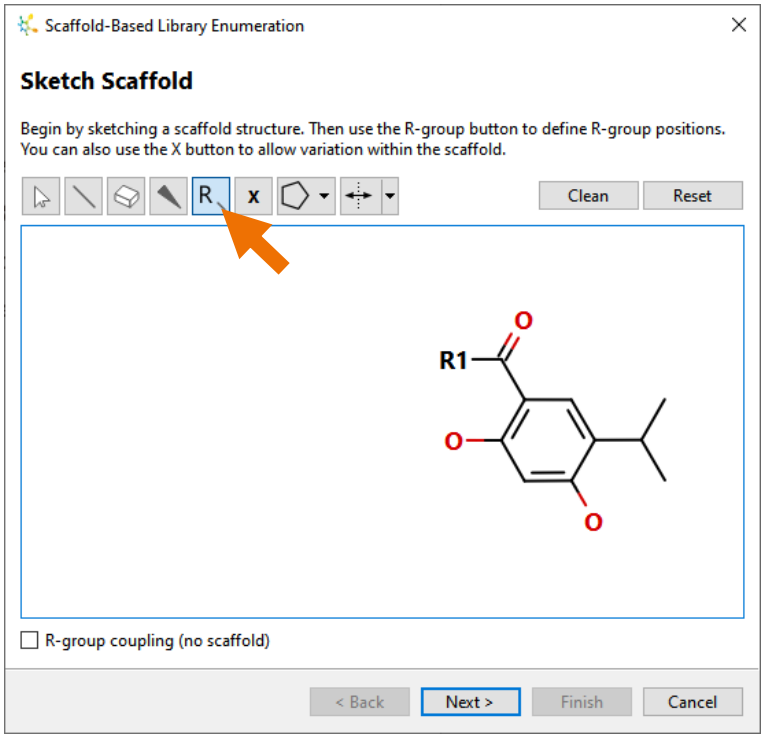


- Click  at the bottom of the Nova area to start the enumeration. In the wizard that appears, select **Scaffold-Based Library Enumeration** and click the **Next** button.

The **Sketch Scaffold** page will be shown containing Onalespib. If desired, we could sketch a new scaffold by clicking the **Reset** button, but in this case, we'll edit the displayed compound to create the scaffold for our new library.



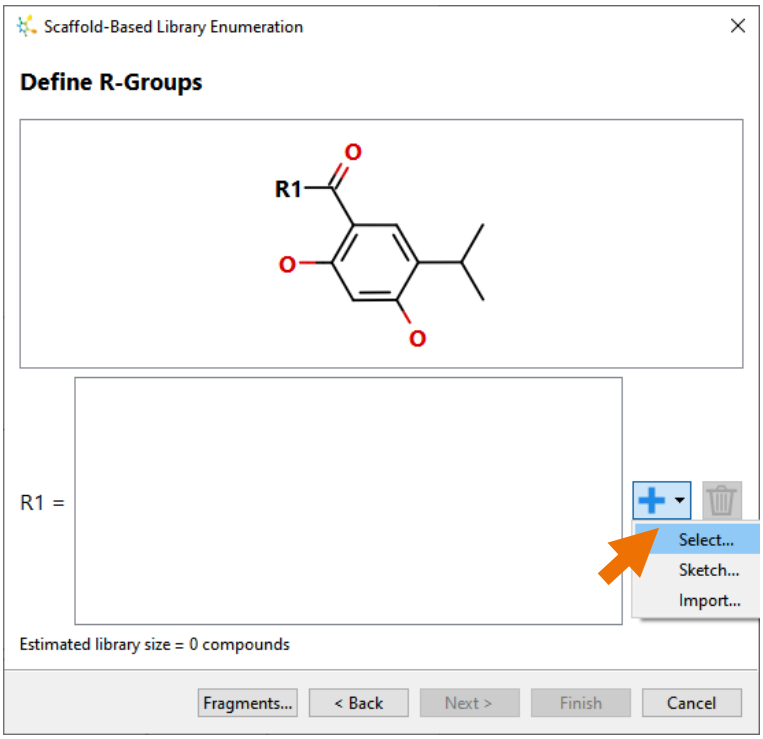
- Use the **Select** tool  to lasso the amine portion of the molecule.
- Click the **Delete** key to delete the highlighted atoms.



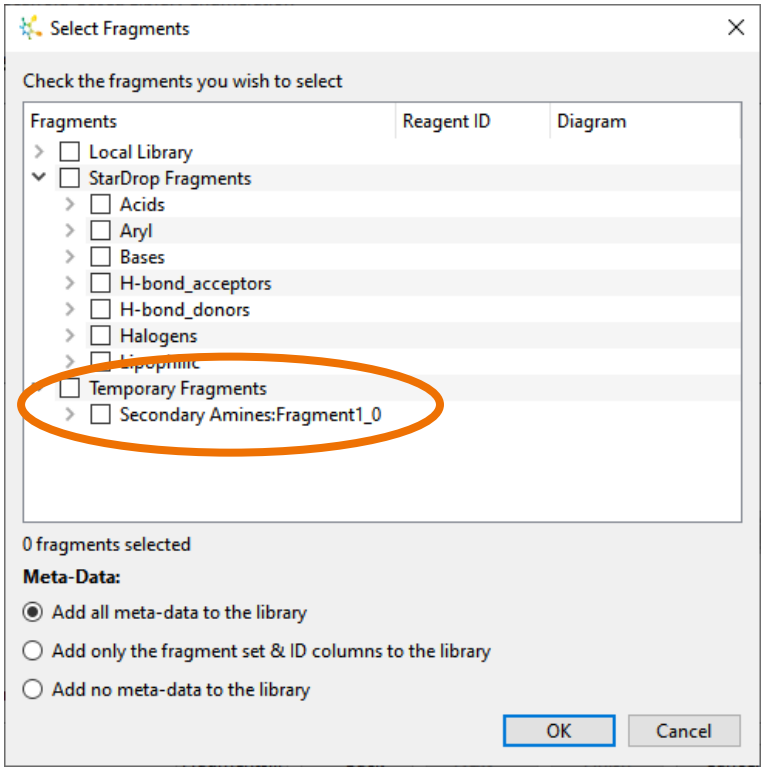
- Use the **R-group** tool  to add an R-group by clicking on the atom to which it should be connected.
- Click the **Next** button.

The **Define R-groups** page is displayed. Here we will define the list of secondary amine fragments to use in the enumeration.

- Click the **Add** button  next to **R1** and choose **Select** to open the library of predefined substituent groups.



In the fragment library, you will see all the fragments that have been previously saved. The fragments derived from the R-group clipping of the amine library are available at the bottom of the list. They are listed as "Temporary Fragments" because they are from one of the project data sets and have not explicitly been added to the library for future use in other StarDrop projects.

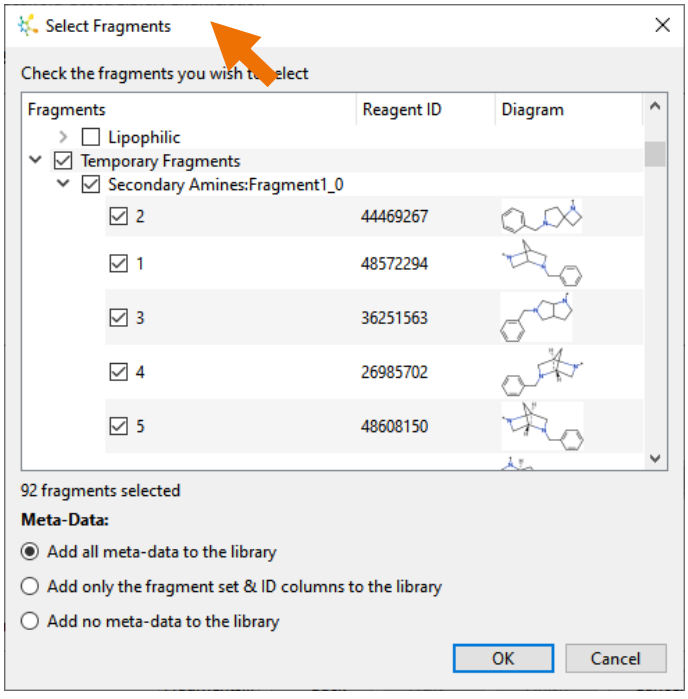


Hint: To add a set of fragments to the library permanently, right-click on the fragment column header in the data set and choose Add Data Set to Fragment Library from the menu.

- Tick the box next to **Secondary Amines:Fragment1_0** to select these fragments.

The **Meta-Data** options enable you to specify what, if any, data from the fragment library are added to the new series data set.

- Select the **Add all meta-data to the library** option and click the **OK** button.

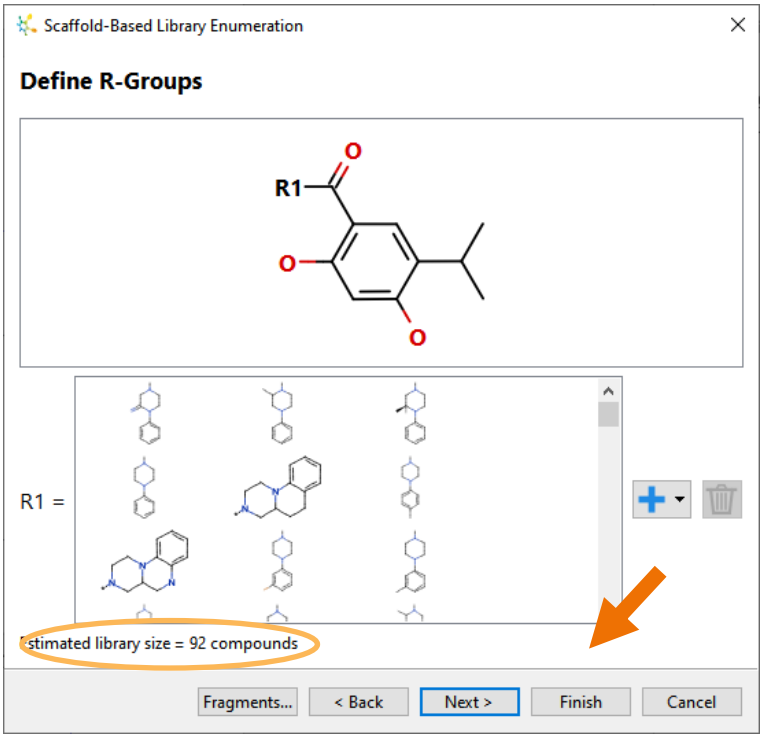


Note that with this selection, the columns of information imported from eMolecules will be added to the enumerated library, making it easy to see which reagents are required for each of the virtual compounds.

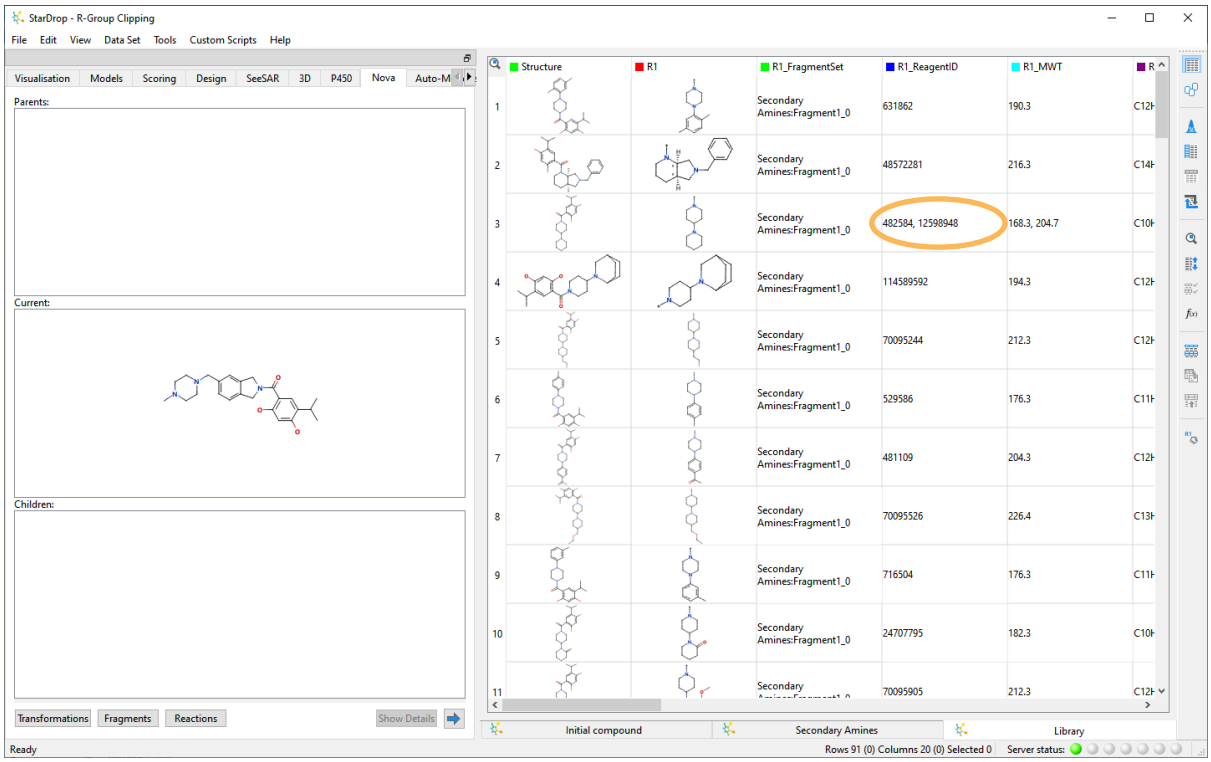
The fragments selected will be shown next to R1. If we wish to add further fragments, we can do so by clicking the **Add** button  again, but in this case, we will only use the fragments we already have.

The estimated library size is 92 compounds.

- Click the **Finish** button.



A new data set will be added to the project called **Library**. It contains 91 structures along with all the reagent meta-data from eMolecules.



Note: Two of the fragments generated during the clipping process were duplicates, originating from different starting amines resulting in there being 91 unique enumerated compounds. The meta-data for both fragments have been preserved for this compound, as highlighted above.

Hint: Scroll to the right to see additional meta-data from eMolecules including the hyperlinks to the reagents in the eMolecules web site for convenient reagent ordering. Note that if you select columns and/or rows then StarDrop's right-click menu will enable you to copy & paste multiple IDs into an ordering system.

The data set can now be used with all StarDrop's capabilities for optimising and selecting compounds.

To see how the SeeSAR Pose module can be used within StarDrop to generate 3D poses for this library, enabling further assessment and design, take a look at the following example:

https://www.optibrium.com/videos/stardrops-seesar-pose-module/

The SeeSAR Affinity module can also be used to assess these compound's binding affinities. Take a look at the following worked example:

https://www.optibrium.com/videos/stardrops-seesar-affinity-module/

If you are using alternative docking software, then this library can also be evaluated by docking in the HSP90 binding site using StarDrop's Pose Generation Interface to provide seamless integration with docking models from 3rd party platforms. Whilst this is beyond the scope of this exercise, if you would like to learn more about the Pose Generation Interface, please visit the following link in our online community videos:

https://www.optibrium.com/videos/pose-generation-interface/