Application Note

Automated synthesis planning and execution

With SYNTHIA™ Retrosynthesis Software and Synple Automated Synthesizer

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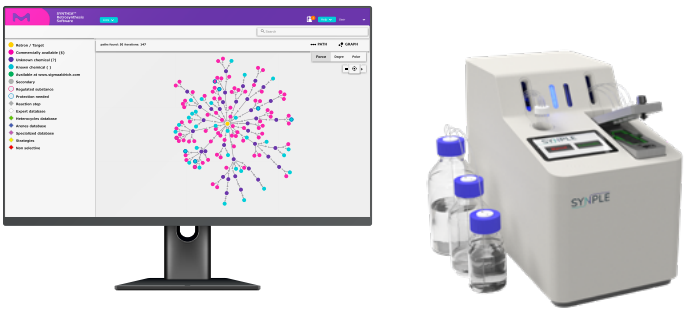
Automated synthesis planning and execution with SYNTHIA™ retrosynthesis software and Synple Automated Synthesizer

Part 1: Custom search configuration in SYNTHIA™ enables Synple-executable routes

Introduction

Despite significant advances in recent decades, synthetic chemistry remains a highly challenging field. Synthesis of novel molecules with unique properties is an essential tool in drug discovery, but remains a time-consuming endeavor that can often be a bottleneck to the overall process. Highly trained synthetic chemists commit a significant amount of time and consideration to route scouting and the optimization of reaction conditions to access novel structures. This reliance on human expertise introduces the potential for errors and biases and is subject to limits in scalability and efficiency. With an increasing demand for novel molecules with specific properties, there is a pressing need to streamline the synthesis process.



SYNTHIA™ Retrosynthesis Software application displaying the graph view from the search results of a target molecule (left) and Synple™ automated synthesizer shown with solvent reservoirs (right).

Enabling technologies

Fortunately, advancements in technology and automation are now revolutionizing synthetic chemistry. Today, the emergence of robotic systems and enabling tools, machine learning algorithms, and virtual screening methods are beginning to help accelerate the discovery and synthesis of new molecules. These technologies offer the potential to not only reduce the time and effort required to generate a solution for a given synthesis but also minimize the likelihood of errors. By combining the power of automation to standardize chemical synthesis with a data-driven AI approach to rapidly analyze huge data sets and predict new outcomes, researchers can explore a broader chemical space in significantly less time.

One example of a technology aiding synthesis design is SYNTHIA™ retrosynthesis software, a powerful tool for organic chemists to design synthetic routes. It employs expert-coded rules to predict feasible routes from commercially available starting materials and can accelerate the route design process by offering chemists a customized set of ranked potential routes to any given target. From the synthesis automation perspective, Synple's automated cartridge-based synthesis technology is an easy to use, enabling tool that can carry out many of the chemical reactions commonly used in discovery chemistry. By using the device and cartridges, a chemist can initiate and complete the reaction, work up and product isolation process at the touch of a button to yield significant time and cost savings.

In this case study, we present not only how these two new technologies can accelerate the design and synthesis processes, but also how interfacing them can make this combined process even more productive. This combination is enabled via the implementation of a specific set of search criteria in SYNTHIA™ which allows it to identify routes that can be effected in an automated fashion using the Synple platform.

SYNTHIA™ search configurations

SYNTHIA™ provides different configurations for conducting searches, which are aimed at identifying routes suited to specific applications (Fig. 1). In addition to the preset search options, users can create a custom search configuration to meet the specific needs of their project. In this study, we compared 2 of the preset search configurations, General and Discovery, with a third custom configuration, Synple, that promotes reactions that can be executed using the Synple automated synthesis system.

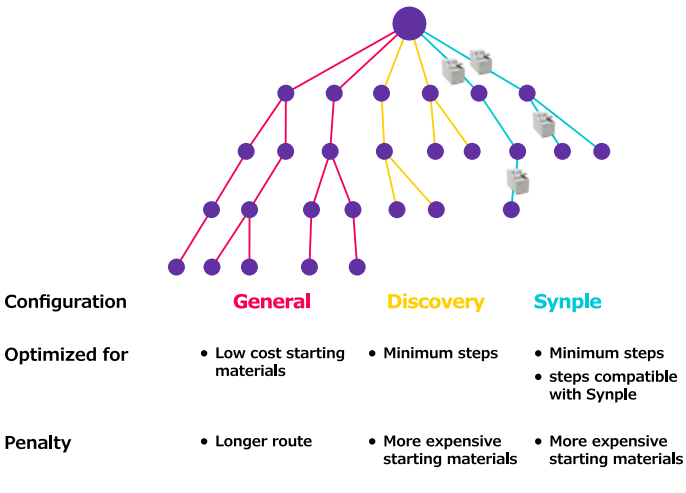


Figure 1. SYNTHIA™ Search Configurations. General balances number of steps with cost of starting materials. Discovery prioritizes shorter routes over inexpensive starting materials to meet the needs of discovery chemists who need to quickly access small amounts of their target. Synple is a custom configuration based on the Discovery setting that prioritizes reactions that can be executed using the Synple automated synthesis system.

Configuration comparisons

A test set of 10 representative molecules, selected from known drug candidates and a drug-like library was used to assess the output of the different search configurations (**Figure 2**). All ten molecules were uploaded to SYNTHIA™ as a single file using the Batch Retrosynthesis Module. The batch was run with each of the different search configurations, which were selected from a drop-down list during the search set-up.

The retrosynthetic analyses took between 9-16 minutes to complete for each of the targets. The top ranked pathway for each configuration was given a score for the total number of synthetic steps (including any required protection/deprotection) and the number of steps that could be executed using Synple (Table 1).

As expected, the *General* configuration typically identified retrosynthetic pathways with the highest number of steps using lower cost starting materials, while the Discovery and *Synple* configurations prioritized pathways with fewer steps. A closer analysis of the top pathways from the latter two configurations revealed two key differences:

1) While the Discovery configuration often used more challenging or exotic reactions, the Synple configuration returned pathways with steps mostly using routine transformations that could also be carried out using the Synple synthesis system

2) Since the Synple configuration was designed to promote protection/deprotections available as Synple cartridges (e.g. Boc), these routes were more likely to utilize commercially available protected starting materials and included exact deprotection steps. Routes from the *Discovery* configuration were more likely to include general protection/deprotection steps from unprotected starting materials, allowing the user to choose from a list of compatible protecting group options.

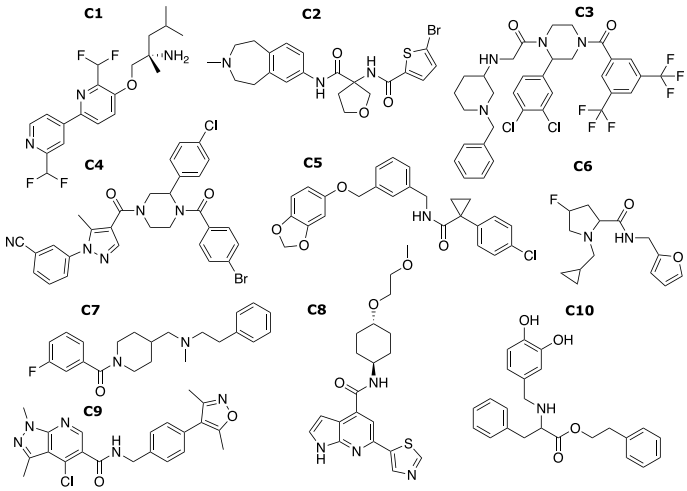


Figure 2. Compounds used in batch. C1. AAK1 inhibitor,[1] C2. Antithrombotic agent[2], C3. Neurokinin antagonist[3], C4. eIF4A3 inhibitor[4], C5-7. Library Compounds[5], C8. CD38 inhibitor[6], C9. GR antagonist[7], C10. Library Compound.

These trends are exemplified in analysis of the results for **C3**, a Neurokinin antagonist candidate.[3] The General configuration proposes a 6-step route starting from less expensive starting materials and including only one step that could be automated using the Synple system (Scheme 1). The top routes for the *Discovery* and *Synple* configurations both contain 5 total steps, but only 1 can be potentially automated using the Discovery route while all 5 can be automated in the Synple route. Additionally, the *Synple* route finds a Boc-protected starting material and includes the Boc-deprotection as an explicit step, while the Discovery route starts from an unprotected starting material and allows the user to decide which protecting group to use from a list of compatible options. The more common synthetic transformations proposed in the Synple route may also be more appealing to the chemist by reducing the risk of failure and necessity for optimization.

It is important to note that while the retrosynthetic analysis may be conducted using the Synple configuration, the user is still free to pick from 50 possible routes containing both manual and automatable steps. For example, the shortest route to get to C8 involved 2 Synple steps and 1 manual step, highlighting a case where the most optimal route combines the power of both the chemist and the machine. By offering the freedom to make target molecules using both automated and manual chemistry, SYNTHIA™ affords chemists the flexibility to automate the easy steps and focus their skills and effort on key non-automatable synthetic steps.

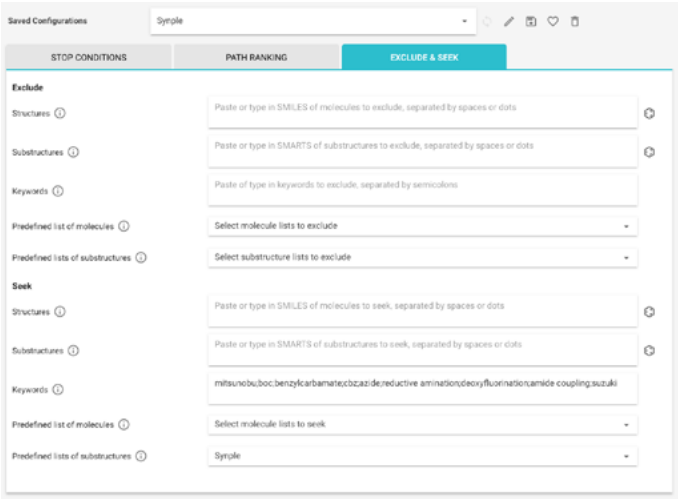


Figure 3. Synple customization using Seek Function to prioritize keywords and a list of substructures related to Synple-enabled reaction

An additional time saving feature in SYNTHIA™ is the prediction of the route based on commercially available compounds with a direct link to the commercial sources. This eliminates the need for additional searches to find price and available of starting materials and allows the user to confidently choose routes knowing the required compounds will be accessible.

Table 1. Number of total synthetic steps/Number of steps compatible with Synple for each batch molecule with the different search configurations

	C1	C2	C3	C4	C5
General	7/0	7/1	6/1	6/1	2/2
Discovery	4/1	3/0	5/1	3/3	2/2
Synple	4/2	4/3	5/5	3/3	2/2

	C6	C7	C8	C9	C10
General	2/0	2/0	8/1	2/2	2/1
Discovery	2/0	2/0	3/2	1/1	2/1
Synple	2/2	3/2	3/2	1/1	2/1

Synthetic execution

In order to test the synthetic routes recommended by SYNTHIA™ and demonstrate the power of interfacing SYNTHIA™ and Synple synthesis automation, one of the test molecules was selected for synthesis using the routes recommended by the Discovery configuration (manual synthesis) and the *w* configuration (automated synthesis).

The synthesis of **C6** via the Discovery route required a total of six hours "hands-on" working time (not including the reaction time while stirring). The first alkylation step required additional scouting for different conditions due to reactivity of the alkyl halide. Using the route suggested by the Synple configuration, only one hour of working time was required to set up the two steps on the Synple machine followed by evaporation of the solvent after the automated reaction.

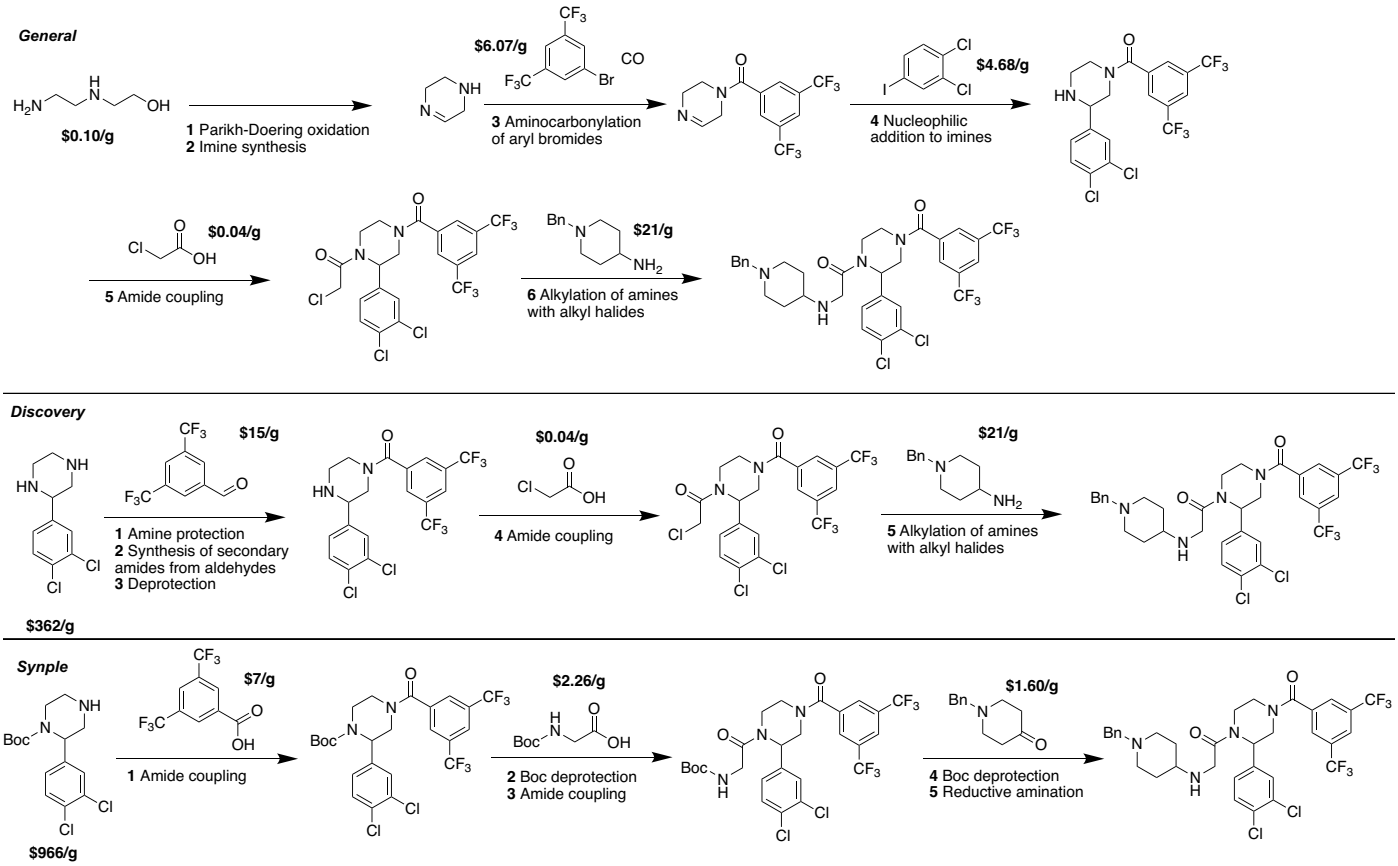
Both routes performed roughly equally well in terms of overall yield (49% vs 46%). However, the product from the Synple route was pure enough with 90% that no additional purification was required.

We demonstrated here the quick and efficient synthesis of the proposed SYNTHIA™ computed routes both in a traditional and automated way. Using the complete workflow, C6 could be accessed in only a couple of hours of working time without any drawback compared to the traditional approach.

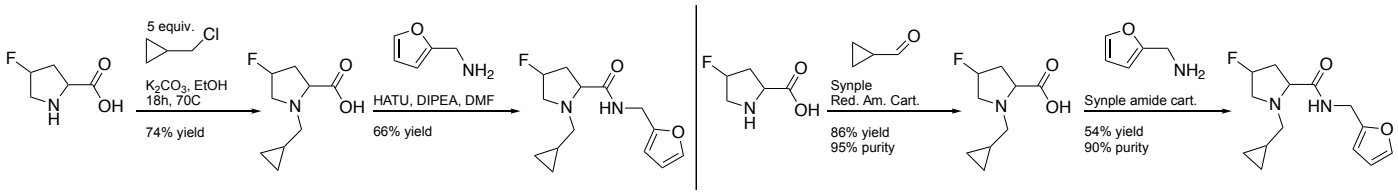
Summary

The application of a customized set of search parameters in SYNTHIA™ can be used to easily plan efficient routes to target molecules that can be quickly executed using Synple automated synthesis technology. This allows the chemist to save manual time required for route planning and searching for appropriate starting materials as well as in bench execution, ultimately accelerating the drug discovery process.

Given the ease with which the Synple automatable chemistry search configuration was implemented in SYNTHIA™, it is possible to extend this application to prioritize any subset of reactions that a specific user would preferentially like to use for their project. As such, SYNTHIA™ has the potential to serve as a powerful tool for a uniquely customized and focused retrosynthetic analysis.



Scheme 1. Proposed routes for C3 with each search configuration



Scheme 2. Manual and automated syntheses of C6 using SYNTHIA proposed routes

Product List	SKU#
Building Blocks	
N-(2-Hydroxyethyl)ethylenediamine	127582
1,3-Bis(trifluoromethyl)-5-bromobenzene	290157
Carbon monoxide	295116
3,4-Dichloroiodobenzene	541753
Chloroacetic acid	402923
3,5-Bis(trifluoromethyl)benzaldehyde)	290130
Boc-Gly-OH	15420
Tert-butyl-2-(3,4-dichlorophenyl)piperazine-1-carboxylate	ENAH3050A613
3,5-Bis(trifluoromethyl) benzoic acid	232882
1-Benzyl-4-piperidone	B29806
4-fluoropyrrolidine-2-carboxylic acid	ENAH3045A016
(Chloromethyl)cyclopropane	184667
Furfurylamine	F20009
Cyclopropanecarboxaldehyde	272213
Synple	
Synple 2 Automated Synthesizer	SYNPLE-SC002
Reagent Cartridge - Reductive Amination	SYNPLE-R001
Reagent Cartridge - Amide Formation	SYNPLE-A011

Synthesis Details

Discovery-Manual Route to C6:

Step 1: (2S,4S)-4-Fluoroproline-2-carboxylic acid (68.6 mg, 0.50 mmol, 1.0 equiv.) and potassium carbonate (86.4 mg, 0.625 mmol, 1.25 equiv.) were suspended in EtOH (2 mL). (Chloromethyl)-cyclopropane (0.231 mL, 2.50 mmol, 5.0 equiv.) was added and suspension was stirred at 70°C overnight. The suspension was cooled down to room temperature, filtered on Celite®, then the solid was washed with EtOH. The filtrate was concentrated to yield crude product as a white solid, which was directly used for next step (74% conversion calculated from the crude NMR).

Step 2: To a suspension of crude acid from Step 1 in CH_2Cl_2 (2.5 mL) were added in sequence furfurylamine (0.044 mL, 0.50 mmol, 1.0 equiv.), triethylamine (0.070 mL, 0.50 mmol, 1.0 equiv.), EDC hydrochloride (105.4 mg, 0.55 mmol, 1.1 equiv.) and Cl-HOBt (113.1 mg, 0.55 mmol, 1.1 equiv.). The resulting suspension was stirred at room temperature overnight, then solvent was evaporated, residue taken up with EtOAc and washed with dil. $NaHCO_3$, water and brine. Crude material was purified by flash chromatography (CH_2Cl_2/MeOH gradient 0% → 20%) to afford pure compound **C6** as a pale-yellow oil (65.6 mg, 49% yield over two steps).

Synple-Automated route to C6

Step 1: A 40 mL glass vial was loaded with (2S,4S)-4-Fluoroproline-2-carboxylic acid (68.6 mg, 0.50 mmol, 1.0 equiv.) and cyclopropanecarboxaldehyde (0.075 mL, 1.00 mmol, 2.0 equiv.) and a magnetic stir bar, then CH_2Cl_2 (4 mL) and 1,1,1,3,3,3-hexafluoroisopropanol (1 mL) were added to dissolve the starting materials and the vial was connected to the Synple machine. A freshly packed "reductive amination" capsule was inserted in the machine. The "reductive amination – A2" sequence was loaded and the sequence was started. At the end of the sequence, evaporation of the resulting solution afforded the product as a white crystalline solid (80.5 mg, 86% yield).

Step 2: A 40 mL glass vial was loaded with product from Step 1 (80.5 mg, 0.43 mmol, 1.1 equiv.), furfurylamine (0.035 mL, 0.39 mmol, 1.0 equiv.) and a magnetic stir bar, then CH_2Cl_2 (2 mL) and EtOH (2 mL) were added to dissolve the starting materials and the vial was connected to the Synple machine. A freshly packed "amide coupling" capsule was inserted in the machine. The "Amide coupling" sequence was loaded and the sequence was started. At the end of the sequence, evaporation of the resulting solution afforded crude compound C6 as a yellow oil with ~90% purity. Product could be further purified by flash chromatography for analysis purposes (CH_2Cl_2/MeOH gradient 0% → 20%) to afford pure compound **C6** as a pale-yellow oil (52.9 mg, 51% yield).

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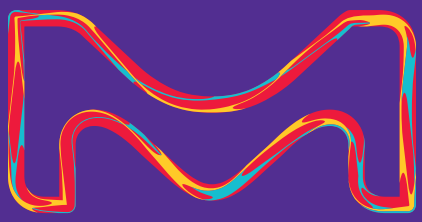

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