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Application Note Part 2

Automated synthesis planning and execution

With SYNTHIA™ Retrosynthesis Software and Synple Automated Synthesizer

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Part 2: Analysis of time-savings using automated technologies compared with traditional methods

Introduction

Enabling technologies such as SYNTHIA™ retrosynthesis software for route planning and Synple's automated cartridge-based synthesis technology can be used to streamline the drug discovery process by accelerating synthesis design and execution.

In Part 1, we introduced how the two platforms can be used together through a custom set of search parameters in SYNTHIA™ that allows it to plan routes that can be executed using Synple's automated synthesizer. Herein, we will present a detailed comparison of the syntheses of two compounds using automated technology or traditional methods to highlight the time-savings enabled by the use of SYNTHIA™ and Synple together.

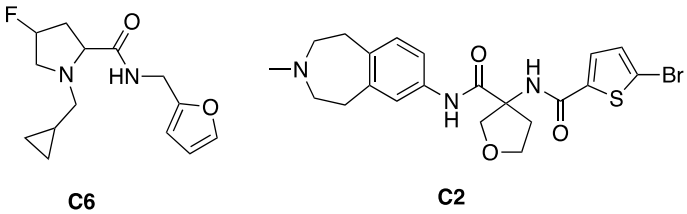
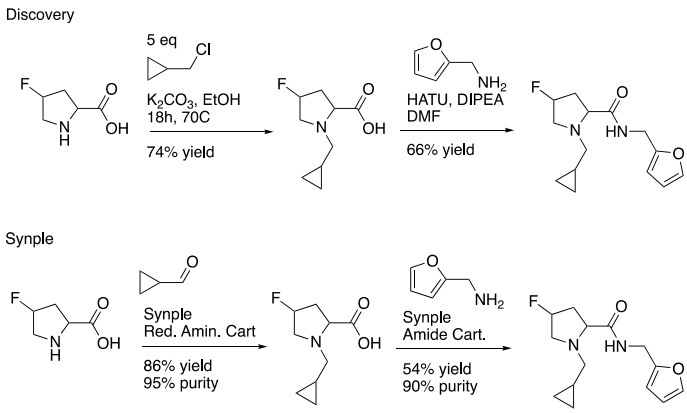


Figure 1. Compounds synthesized manually and using Synple.

Synthesis of C6

The first compound selected **(C6)**, a fluoroproline-based compound, was part of an earlier collaboration of Synple. In the resulting publication1, it was shown that the compound can be made rapidly with the Synple instrument in 3 steps. When submitted to SYNTHIA™ using the preset *Discovery* search configuration, the software was able to suggest a 2-step route consisting of an alkylation followed by an amide coupling. Using the custom search configuration that was developed to promote Synple-automatable reactions, SYNTHIA™ was able to propose an alternative 2-step route starting with a reductive amination instead of the alkylation reaction that is not available using the Synple platform.



Scheme 1. Synthesis routes of C6 calculated by SYNTHIA executed manually and with Synple automation

The first route based on the *Discovery* configuration was executed via typical synthesis in round-bottom flasks. Alkylation of the amine proved challenging under basic conditions due to the facile elimination of the halide. A reaction screening was performed in order to identify the appropriate base. It was discovered that the optimal conditions were potassium carbonate with 5 equivalents of the halide. For the second step, a short literature search was performed to find the best coupling reagent for these substrates. Using HATU yielded the desired compound after a short purification step.

The second route based on the *Synple* search configuration was carried out exclusively on the Synple platform. Execution of the first reaction required first filling the solvent reservoirs, setting up the reaction on the automated synthesizer and then evaporating the solvent from the final product. All together this required only a few minutes of manual work. The second step was set up in the same manner, and the resulting target compound did not require any additional purification.

Both the routes proposed by SYNTHIA™ reduced the total step count to get to the target over the original route. The route proposed using the *Discovery* configuration of SYNTHIA™ required 7.25 hours of manual labor to execute using traditional batch synthesis in a round-bottom flask (Table 1). This did not include waiting for delivery of the starting materials or reaction time. The same compound was obtained via the *Synple* configuration route with only 30 minutes of manual labor and a similar yield as the manually executed route. Using the SYNTHIA™-Synple combination saved 6.75 hours of manual labor, which a chemist could instead spend on other high-value activities.

Table 1. Manual time to execute SYNTHIA™-planned syntheses of compound **C6** using either Traditional or Synple-enabled workflow. Time is given in 'hands-on' working time, not including reaction time

	Traditional	Synple
Step 1	Alkylation	Reductive Amination
Literature Search	0.75 h	0 h
Reaction Optimization	4.5 h	0 h
Reaction Set-up	0.25 h	0.25 h
Work-up	0.5 h	0 h
Purification	0 h	0 h
Step 2	Amide Coupling	Amide Coupling
Literature Search	0.5 h	0 h
Reaction set-up	0.5 h	0.25 h
Work-up	0.5 h	0 h
Purification	1.0 h	0 h
Total Working Time	**7.25 h** (49% yield)	**0.5 h** (46% yield)

In addition to the laboratory execution, there are other time-consuming tasks that are important to consider when analyzing the time savings gained by using tools such as SYNTHIA™ and Synple to accelerate research. These include things like literature searches for reaction conditions and vendor searches for commercial compounds. To consider these non-lab tasks, we analyzed the total working time required to access **C6** via three different approaches: a) Traditional synthesis workflow using neither SYNTHIA™ nor Synple, b) Route planning using SYNTHIA™ but traditional lab execution, and c) Combination of route planning using SYNTHIA™ and automated execution with Synple. This is summarized in Table 2.

To determine the planning time required to design the synthesis, two chemists independently planned routes to the target molecule – one using only traditional literature searching tools and the other using SYNTHIA™ in addition to traditional tools. The first approach via traditional literature search alone required about 2 hours of the chemist's time, followed by an additional 1 hour to identify and order starting materials and related chemicals from various vendors. The second approach, which included using SYNTHIA™ to plan the routes, shortened the time for the literature search to 1.25 hours. Since the overall route had already been proposed by the software, only searches for reaction conditions were necessary. SYNTHIA™ allows for commercially available starting materials to be viewed directly within the platform. As a result, the time to select and procure starting materials was significantly reduced by 45 minutes. In the third approach using SYNTHIA™ and Synple, the overall time was further reduced since the reaction conditions were already programmed in Synple and no additional research by the chemist was necessary. Considering all of the required labor together, SYNTHIA™ was able to reduce the manual time to get to the purified compound by 24%, while the combination of SYNTHIA™ and the Synple platform was able to reduce the manual time by 93% over a traditional workflow.

Table 2. Overall time required to access **C6**

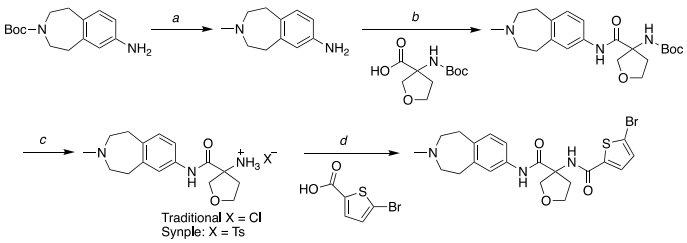
	Literature search	Material preparation/ Ordering	Synthesis	Total
Full traditional workflow	2 h	1 h	8.5 h*	11.5 h
SYNTHIA™+ traditional synthesis	1.25 h	0.25 h	7.25 h	8.75 h **-24%**
SYNTHIA™ + Synple	0 h	0.25 h	0.5 h	0.75 h **-93%**

*Estimated based on 3-step published path[1]

Synthesis of C2

A second target **C2** was selected for synthesis representing a more complex scaffold. This compound was patented as an antithrombotic agent and was synthesized in 6 steps[2].

The target was run on SYNTHIA™ using both the *Discovery* configuration and the *Synple* configuration. The routes proposed using the *Discovery* configuration employed several complex steps, while the *Synple* configuration proposed a straightforward 4-step route. Because of this, the *Synple* route was chosen to be executed using both traditional and automated synthesis workflows (Scheme 2).



Scheme 2. Synthetic route for **C2** as proposed by SYNTHIA™'s *Synple* configuration. Conditions for traditional execution: a. $LiAlH_4$, THF, 5h, 60°C, 71%; b. HATU, NMM, DMF, 18h, 65°C, 61%; c. HCl, MeOH, rt, 2h, quant; d. TsCl, NMM, MeCN, 5h, rt, 74%. Conditions for automated execution with Synple: b. Amide coupling capsule, MeCN, rt, 16h, 57%; c. Boc-cleavage capsule, DME, rt, 2 h, quant.; d. Amide coupling capsule, MeCN, rt, 16h, 36%.

Because the carbamate reduction step (Scheme 2, a) was not available on Synple, this step had to be executed manually and resulted in a 71% yield. The subsequent amide coupling and Boc deprotection steps gave similar yields for both traditional and automated methods. The last step was an amide coupling which generated the product in a 74% yield when executed manually. For the automated execution, the intermediate amine had to be pre-treated to remove the salt before running the Synple method. This required additional time and contributed to the lower 36% yield for this step. This is not completely unsurprising considering the Synple cartridge system is designed to provide quick access to compounds with a wide substrate scope, but is not necessarily optimized to deliver the highest yield for a particular substrate.

The route proposed by SYNTHIA™ and executed using the traditional workflow was able to provide **C2** in 32% overall yield with 8 hours of total working time. The automated route took only 5.5 hours of manual working time, albeit yielding slightly less material. The majority of the time spent on the synthesis was due to the manual first step. This demonstrates that introducing Synple automation can save time even in a partially automated, hybrid workflow.

Table 3. Hands-on time to execute SYNTHIA™-planned syntheses of compound **C2** using either Traditional or Synple-enabled workflow. Time is given in 'hands-on' working time, not including reaction time

	Traditional	Synple
Step 1: Reduction		
Literature Search	0.5 h	Step not available on Synple
Reaction Set-up	0.75 h	
Work-up	0.5 h	
Purification	0 h	
Step 2: Amide Coupling		
Literature Search	0.25 h	0 h
Reaction Set-up	0.75 h	0.25 h
Work-up	0.5 h	0 h
Purification	0 h	0 h
Step 3: Boc Deprotection		
Literature Search	0 h	0 h
Reaction Set-up	0.25 h	0.25 h
Work-up	0.75 h	0 h
Purification	0 h	0 h
Step 4: Amide Coupling		
Literature Search	0.5 h	0 h
Reaction Set-up	1.0 h	0.75 h*
Work-up	0.5 h	0 h
Purification	1.0 h	1.0 h
Total Working Time	**8h** (32% yield)	**5.5h** (15% yield)

*includes pretreatment of amine

The same analysis was performed for **C2** that was done for **C6** to consider the total working time for each method including any additional time required for literature searches and material ordering (Table 4). In this case, using SYNTHIA™ was able to cut down 6.5 hours of work and Synple another 3.25 hours of work, resulting in a total 61% reduction in working time using the combined technologies.

Table 4. Overall time required to access **C2**

	Literature search	Material preparation/ Ordering	Synthesis	Total
Full traditional workflow	2.5 h	1.5 h	12 h*	16 h
SYNTHIA™+ traditional synthesis	1.25 h	0.25 h	8.0 h	0.5 h **-40%**
SYNTHIA™ + Synple	0.5 h	0.25 h	5.5 h	6.25 h **-61%**

*Estimated based on 6-step published path[2]

Summary

In both cases, the use of SYNTHIA™ and Synple was able to reduce the overall time required to access the target molecules. In the case of **C6,** the use of SYNTHIA™ alone created a modest time-savings of 24%, but the combination of SYNTHIA™and Synple reduced over 90% of the time required to obtain the final molecule. In the case of the more complex target, **C2**, the use of SYNTHIA™ resulted in a more significant 40% time-savings. Although only 3 of the 4 steps were able to be automated, using Synple for the synthesis execution reduced another 21% of the overall working time.

In summary, we were able to show significant time-savings by combining SYNTHIA™ and Synple. This study has determined that each platform separately can have varying potential for time optimization. Using the combination of technologies enhances the synthesis workflow and allows the chemist to spend time on other high-value activities.

Material List	
4-fluoropyrrolidine-2-carboxylic acid	ENAH3045A016
(Chloromethyl)cyclopropane	184667
Furfurylamine	F20009
Cyclopropanecarboxaldehyde	272213
HATU	445460
7-Amino-1,2,4,5-tetrahydro-3-Boc-3-benzazepine-3-carboxylic acid	SY3432447954
3-(Boc-amino)tetrahydrofuran-3-carboxylic acid	SY3432448312
5-Bromo-2-thiophenecarboxylic acid	467944
Synple 2 Automated Synthesizer	SYNPLE-SC002
Reagent Cartdige – Reductive Amination	SYNPLE-R001
Reagent Cartridge – Amide Formation	SYNPLE-A011
Reagent Cartridge – Boc deprotection	SYNPLE-B011

References

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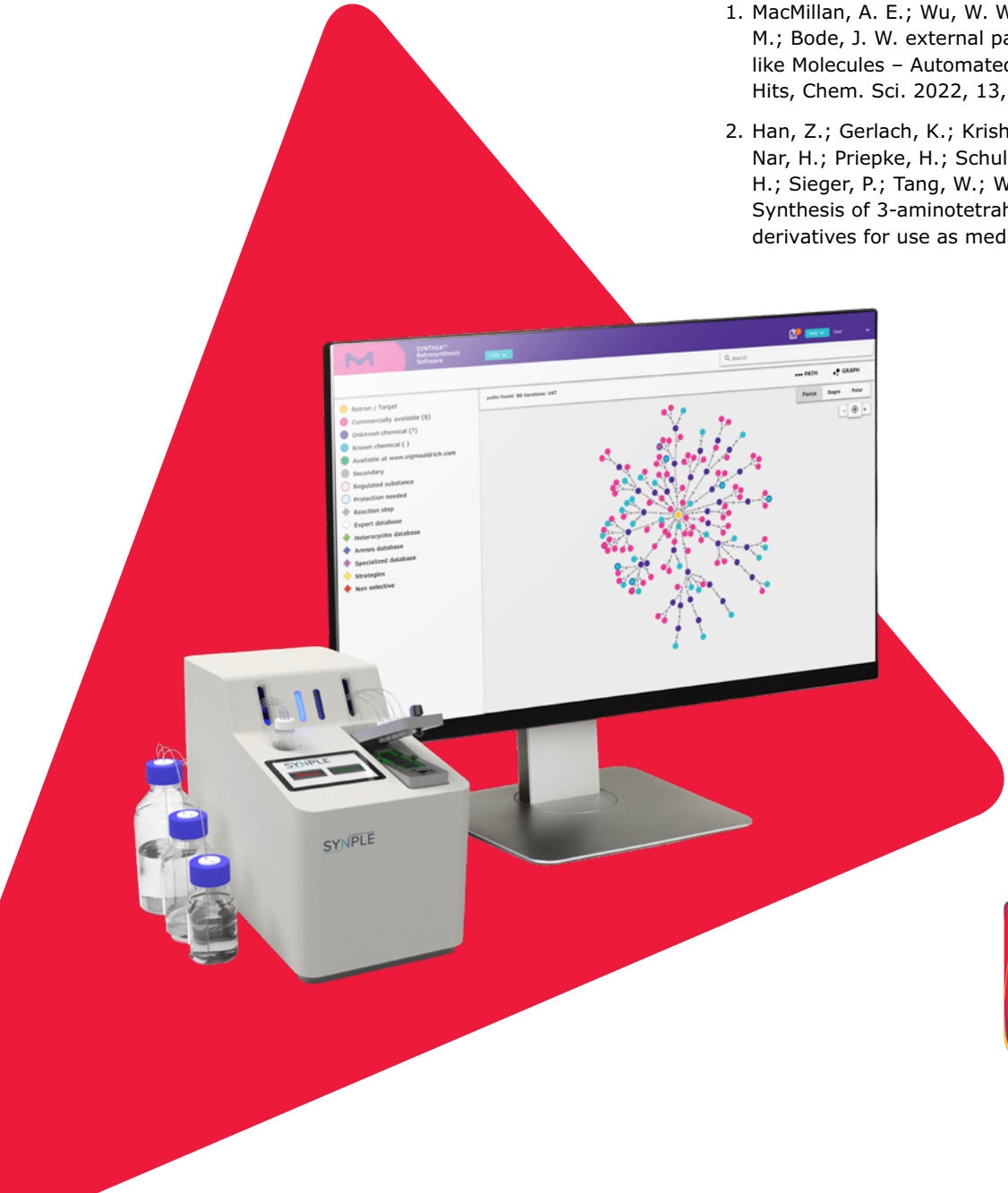
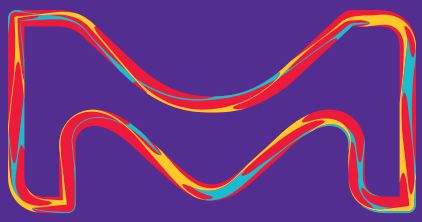
2. Han, Z.; Gerlach, K.; Krishnamurthy, D.; Matthes, B.; Nar, H.; Priepke, H.; Schuler-Metz, A.; Senanayake, C. H.; Sieger, P.; Tang, W.; Wienen, W.; Xu, Y.; Yee, N. K. Synthesis of 3-aminotetrahydrofuran-3-carboxylic acid derivatives for use as medicaments. WO2008080891, 2007.





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