Chemo-enzymatic flow synthesis of the histone deacetylase inhibitor vorinostat

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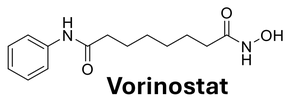
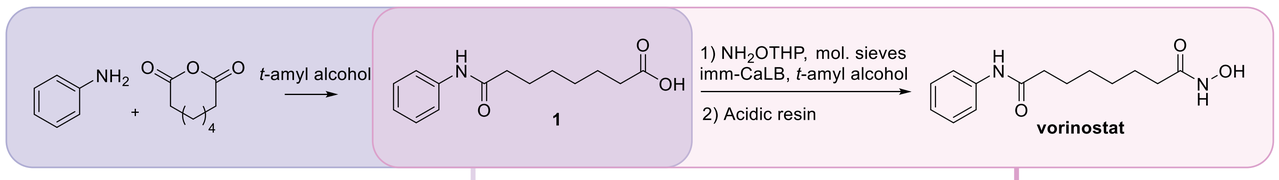
01 Aim of the project


Vorinostat

The anticancer agent **vorinostat** (also known as suberoylanilide hydroxamic acid, SAHA,) is one of the only three histone deacetylase inhibitors (HDAC), together with romidepsin and belinostat, approved by FDA and available on the market for the treatment of patients with **cutaneous T-cell lymphoma**. [1] In 2025 the global vorinostat market is projected to continue expanding due to the increasing incidence of cancer and to the development of combination therapies.

Considering our interest in hydroxamic acid-based HDAC inhibitors and previous synthetic methodologies, we explored the feasibility of a **lipase-mediated biocatalytic approach** for the synthesis of vorinostat using *Candida antarctica* lipase B (CaLB) as biocatalyst.[2,3] Starting from suberic anhydride a **two-step chemoenzymatic** protocol has been established and optimized for the preparation of vorinostat, both in batch and continuous conditions.



02 Preliminary batch studies

A panel of organic solvents was evaluated for the preparation of the **key intermediate suberanoyl acid**, commercially available though very expensive. Suberic anhydride, obtained by dehydration of commercially available suberic acid *via* boiling acetic anhydride, was suspended or dissolved in the selected solvent (Table 1) at 0.4 M concentration and a slight excess of aniline (1.2 eq) was added dropwise. Notably, the use of the green solvent *t*-amyl alcohol resulted in the shortest reaction time (**1 h**), the highest recovered yield (**90 %**), without requiring any co-solvent.

Table 1.

Solvent	Time (min)	Greenness	Appearance	Isolated yield (%)
THF	30		Suspension	94
2-MeTHF	30		Suspension	75
TMO	60		Suspension	78
t-amyl alcohol	60		Suspension	90
dioxane	30		Solution	55
toluene	60		Suspension	60
2-MeTHF/NMP (10% v/v)	60		Solution	91
toluene/NBP (10% v/v)	60		Solution	84

O-tetrahydropyran hydroxylamine (**NH_2OTHP**), a protected derivative of hydroxylamine, was selected as **nucleophile**, since no conversion into vorinostat was observed using hydroxylamine hydrochloride were performed in *t*-amyl alcohol and dioxane in presence of TEA or potassium hydroxide. Different solvent systems were investigated (Table 2).

Table 2.

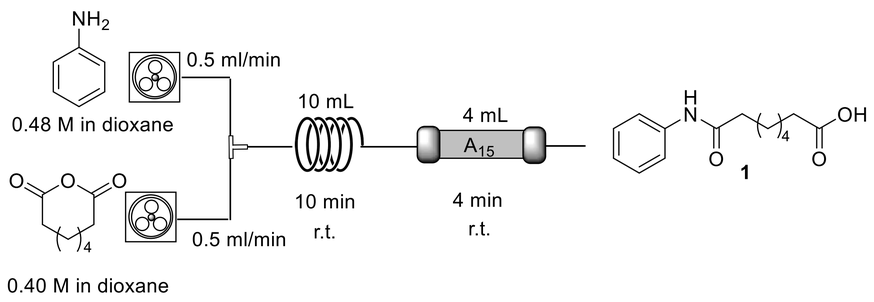
Entry	Solvent	Time (h)	Isolated yield (%)[a]
1	*t*-amyl alcohol/DMSO	16	79
2	dioxane	24	48
3	2-MeTHF/DMSO	16	62
4	toluene/acetonitrile	1.5	60
5	toluene/NBP[b]	24	43
6	2-MeTHF/NMP[b]	16	57

Exploiting the best set of reaction conditions (i.e. 0.1 M, 2 eq of hydroxylamine at 70 °C), disappearance of the starting material was observed after **16 h** for most of the reported mixtures, except for 10% acetonitrile in toluene. A clear solution was obtained only when dioxane was used with no need of co-solvents. In the other cases, NMP or NBP favored the obtainment of a homogeneous solution of the starting material.
The best result in terms of isolated yield was achieved in presence of **10% DMSO in *t*-amyl alcoho**l (Entry 1, **79% yield**). Pure hydroxamate was finally deprotected from the THP moiety and converted to hydroxamic acid vorinostat by acid treatment. A **strong acid resin** (Si-propylsulfonic acid) was employed. This approach allowed us **to remove the THP protecting group**, **catching** at the same time **the excess of hydroxylamine**. In these conditions, hydroxamate was completely converted into vorinostat in **30 min**, while the resin has been recovered, washed and reactivated, ready to be used again for the same purpose.

03 Continuous process optimization

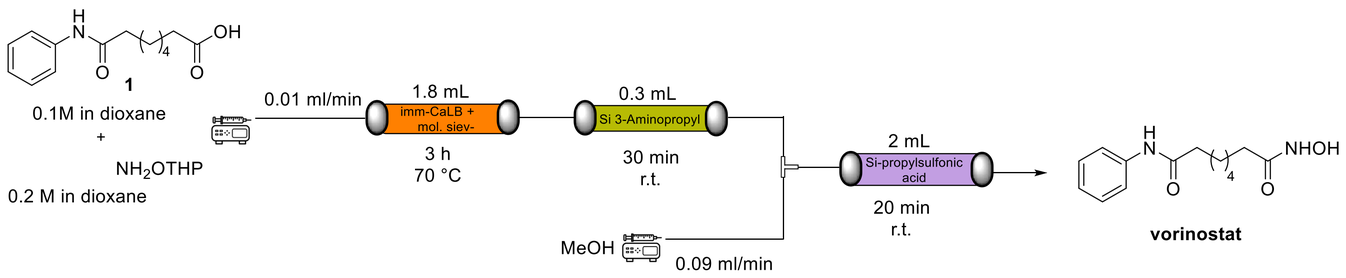
t-Amyl alcohol and toluene/acetonitrile emerged as the best performing solvents, giving fine **suspension mixtures**. However, formation of sediments in the coil was observed even using an oscillator, whereas the PBD reactor blocked the reagents acting as a physical filter. Consequently, for the flow protocol we selected **dioxane**, in view of its ability to efficiently dissolve reagents and products.

Considering the fast aniline acylation in batch conditions, short residence times were taken into consideration (i.e. 5–15 min). Using 1.2 eq of aniline the complete consumption of the anhydride was observed in only **10 min at room temperature** (vs 30 min batch) thanks to the more efficient mass transfer in continuous flow reactor.



The resulting crude, in which suberanoyl acid and the excess of aniline were present, was then purified in-line passing through a glass column reactor packed with acidic resin **Amberlyst® 15 to catch the aromatic amine and readily protonate the desired carboxylic acid**. Pure **compound 1** was collected after the removal of the solvent under reduced pressure

Using the optimized molar ratio between compound **1** and NH_2OTHP (1:2), the same concentration (0.1 M in dioxane) and the same temperature (70 °C) of the batch protocol, different residence time were evaluated. The reaction reached **75% conversion** into the desired product in **3 h** (entry 2) versus 24 h in batch.



Entry	Residence Time (h)	Concentration (M)	HPLC Conversion (%)
1	1	0.1	18
2	3	0.1	75
3	3	0.05	77
4	6	0.05	82

In-line purification was designed: to trap the unreacted suberanoyl acid a basic scavenger has been employed, followed by an acidic ion scavenger to catch the exceeding *O*-THP hydroxylamine while deprotecting hydroxamate.

Different basic scavengers were evaluated showing **3-aminopropyl functionalized silica** able to catch suberanoyl acid in **30 minutes**. Similarly, for the *O*-THP deprotection both silica-based and styrene-divinylbenzene-based sulfonic acid resins were evaluated. **Si-propylsulfonic** acid resulted to be the best option in terms of reaction time and purity of vorinostat .

Biocatalyst stability in the reaction conditions was assessed. The conversion into the desired product was stable around 75% for the first two cycles, then slightly reduced to 52% and definitely dropped to 38% with the fifth reaction performed.

04 Conclusions

Here are described our recent efforts to the synthesis of vorinostat exploiting greener and more sustainable reaction conditions. The protocol was successfully transferred to a continuous flow reactor, thus minimizing reaction time and solvent waste, and enhancing the system automation. Moreover, it represents one of the few examples in literature reporting a multistep chemoenzymatic synthesis in flow.
In particular, the whole process took place in **16 hours and 30 minutes** plus the time required by work up procedures. Subsequently, the protocol has then been transferred to continuous reactor, allowing not only the design of tailor-made in-line purification procedures, but also to reduce the **overall time to 4 hours**. The described approaches could pave the way for a more convenient and eco-friendly preparation of hydroxamic acids of pharmaceutical interest.[4]

References:

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