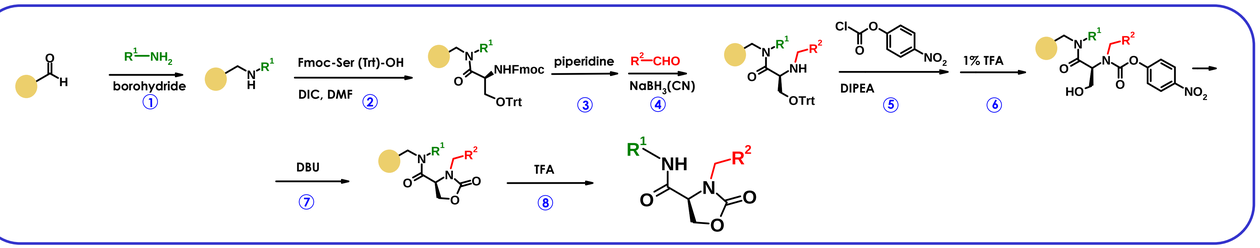
Combinatorial synthesis of oxazolidin-2-ones using Fmoc-Ser(Trt)-OH, a library with high degree of diversity

Marek Kořínek, Václav Šťastný and Miroslav Havránek
RE&D VUFB, Prague, Czech Republic

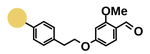
Abstract

Fmoc-Ser(Trt)-OH was used for solid phase synthesis of core structure of the library of compounds, which fulfill requirements as hit-like compounds (with potential antimicrobial activity). The synthetic sequence consists of loading of amines, acylation with Fmoc-Ser(Trt)-OH, reductive alkylation with various aldehydes, Trt deprotection, cyclization to oxazolidinone and cleavage. Optimization studies were carried out during the development of the synthesis, in particular of loading of amines, deprotection of Trt and cleavage from the resin. The stress was put on the development of robust reaction which enable high degree of diversity and a careful selection of building blocks was performed as well. Combinatorial synthesis of 800 compounds was performed on ACT organic synthesizer. All compounds were purified on LC/MS using SunFire (Waters) column for the separation of majority of compounds and Synergi Polar RP (Phenomenex) for more polar compounds. The initial studies towards thiazolidinones and other derivatives were made.



Resin choice

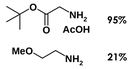
Novabiochem methoxy benzaldehyde resin



Stable for O-Trt deprotection (1% TFA),
Cleavage of product with 50 % TFA

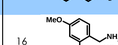
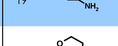
1st step: Reductive amination

Literature conditions[1] loading:
$NaBH_3CN$, 1 vol. % AcOH, DMF



Optimized conditions
$NaBH(OAc)_3$, 10 *vol.*% AcOH, DMF

Table 1. Diverse building blocks and their loadings obtained by using optimized procedure

Entry	Amine	Loading	Entry	Amine	Loading	Entry	Amine	Loading
1	CH_3NH_2 HCl	80%	8	NH_4OAc*	20%	15		97%
2		46%	9		94%	16		~100%
3		82%	10		~100%	17		98%
4		95%	11		95%	18		92%
5		98%	12		98%	19		75%
6		~100%	13		~100%	20		86%
7		88%	14		~100%	21		~100%

Reaction conditions: 10 equiv. of amine, 10 equiv. $NaBH(OAc)_3$, 10 vol.% AcOH, DMF, 6 hr., rt. Only 5 equiv. of amine and borohydride was used in the library synthesis. The loading was estimated by using HPLC calibration method with biphenyl as internal standard and Fmoc release products at 275 nm after acylation with Fmoc-amino acid. *-not included in the library synthesis. Entry 16: Synthon of free aminogroup.

2nd and 3rd step: Acylation and Fmoc deprotection

Fmoc-Ser(Trt)-OH (3 equiv.), DIC (2 equiv.), DMF overnight (reliable method using as little acid as possible)

Piperidine (20% in DMF), 2 x 15 min. Released fluorenyl product was used for loading determination.

[1] Novabichem catalog

[2] Szardenings,A.K.; Burkoth,T.S.; Look,G.C.; Campbell, D.A. *J. Org. Chem.* **1996**, *61*, 6720

4th step: Reductive alkylation

Literature procedure:[2] Aldehyde (10-20 equiv.) in TMOF (trimethyl orthoformate), 30 min. then $NaBH_3CN$ in TMOF, 1 vol.% AcOH, 10 min.

Large excess of aldehyde, overalkyation with some aldehydes , e.g.

Less reagent → no complete reaction with some aldehydes, e.g.

Our procedure: Resin swelled in DCM, aldehyde (5 equiv.) in TMOF, 2 hr. Resin drained, add $NaBH_3CN$ in TMOF, 1 vol.% AcOH, 1 hr.

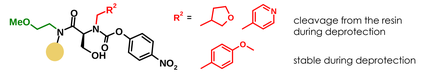
5th step: Acylation with 4-nitrophenyl chloroformate

0.5 M solution of 4-nitrophenyl chloroformate (5 equiv.) in THF/DCM and DIPEA (4.5 equiv.)

6th step: O-Trt deprotection

1% TFA in dichloroethane (+3% triisopropyl silane) – 2 x 10 min. - not complete removal of Trt in case of several compounds

Increase to 1.5 % TFA
- deprotection complete



Final conditions: 1% TFA in dichloroethane (+3% triisopropylsilane) – 3 x 10 min.

7th step: Cyclization

0.3 M DBU in DMF (3 x 30 min.)

8th step: Cleavage

50-90% TFA in 1,2-dichloroethane (DCE) in the presence of triisopropylsilane (TIS)

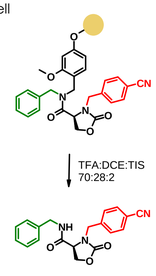
Optimization: The best is 70% TFA

Problem: Cleavage of the compound having amino group gives low yield.

- Higher conc. of TFA (90%)	low yield, resin doesn't swell
- H_2O as a scavenger (instead of TIS)	lower yield
- Increase of temperature	no effect
- Longer reaction time	see Table 2

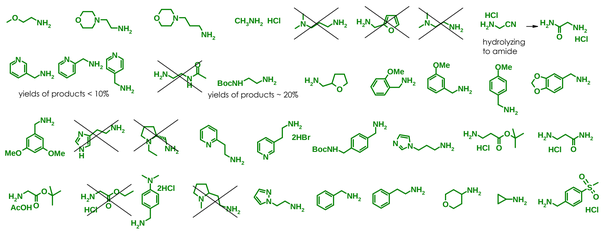
Table 2. Yield of cleavage dependence on time. Resin bound compound 0.066 mmol, TFA:DCE:TIS 70:28:2

Entry	Cleavage time	Preparative yield
1	4 h	5.8 mg (28%)
2	16 h	10.0 mg (50%)
3	40h	16.7 mg (83%)

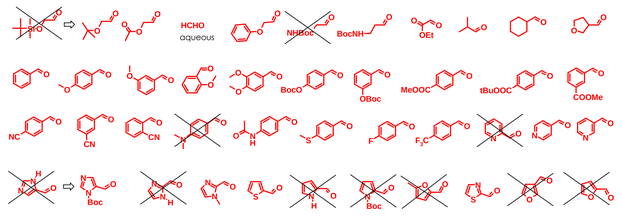


Building blocks

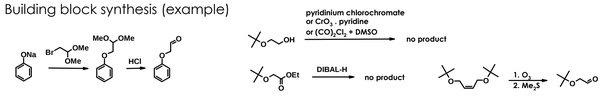
Amines



Aldehydes



Building block synthesis (example)



Conclusion

The library of oxazolidinones was synthesized on solid phase.

29 Amines (out of 38) and 32 aldehydes (out of 41) with various functional group were used.

All compounds were purified and their quality was checked by re-analyses.

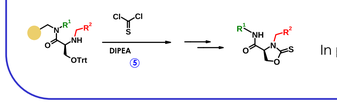
739 Compounds (80% of theory 29 x 32 = 928) with high diversity were obtained in 0.5 - 15 mg amounts.

MW 201-300 ... 145 compounds
MW 300-400 ... 554 compounds MW ≤350 ... 459 compounds
MW 401-456 ... 41 compounds

RE&D VÚFB

Thiazolidinones

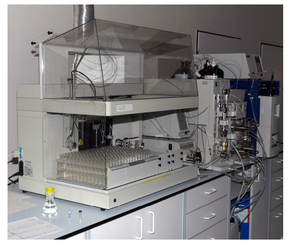
Oxazolidinethiones



Library synthesis

Resin: 2-(4-Formyl-3-methoxyphenoxy) ethyl polystyrene HL (Novabiochem), loading 1.1 mmol/g; 60 mg (0.066 mmol) in one reaction well

ACT organic synthesizer (96 wells per batch); 1 batch was performed in two weeks.

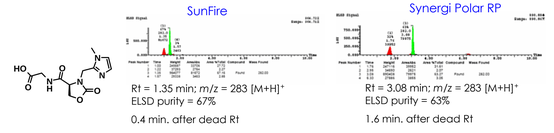


Purification

Crude products in TFA were transferred into 96 well plate, TFA was evaporated, compounds were dissolved in DMF/MeOH/aqueous NH_4OAc (5:4:1), filtered through 96 filter plate into well plate and analyzed.

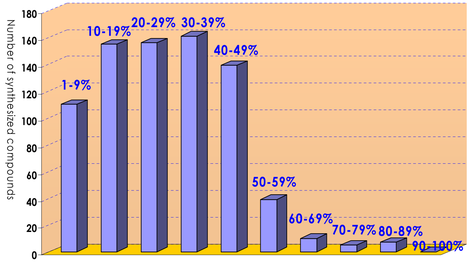
LC/MS : Waters autopurification station

Column: SunFire 4.6 x 100 mm (Waters) or Synergi Polar RP 4.6 x 150 mm (Phenomenex); H_2O:MeCN 95:5→ 5:95 (+ 0.1% HCOOH), 1 ml/min



All mixtures containing desired compound (m/z) were purified using automatic preparative LC/MS. Collected fractions were reanalyzed to check the purity and concentrated in *vacuo* on parallel evaporator (Savant Speed Vac), transferred into bar-coded vials (MeOH) and weighed.

Yield distribution in the library of oxazolidinones



havranek@readvufb.cz, http://www.readvufb.cz