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COMBINATORIAL SYNTHESIS OF NIKKOMYCIN ANALOGUES ON SOLID SUPPORT

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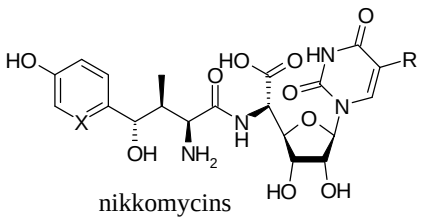
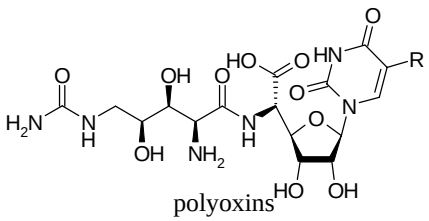
Abstract—Synthesis of nikkomycin analogue library by combinatorial synthesis on solid support and evaluation of their enzyme inhibitory activity against *Candida albicans* chitin synthases are described.

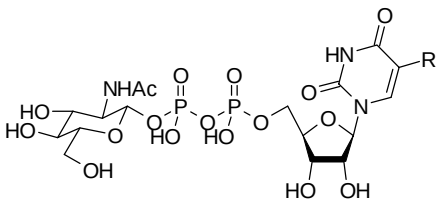
Chitin is a liner polymer of repeating *N*-acetylglucosamine units linked in a β-1,4-fasion. It is found in the cell wall of most fungi. Because this polymer does not exist in mammals, compounds which interfere the synthesis of chitin could be useful as a therapy of mycoses. Nikkomycins and polyoxins are naturally-occurring peptidyl nucleoside antibiotics. These natural products are selective and competitive inhibitors of fungal chitin synthases.[1] The structural similarity between these compounds and UDP-*N*-acetylglucosamine (UDP-GlcNAc), a substrate for chitin synthases, was thought to be responsible for their biological activity. They are, however, only weakly active against mammalian pathogenic fungi , e.g. *Candida albicans*,[2] presumably due to their hydrolytic liability and inefficient permeability to fungal cell wall.



nikkomycins
(R=H, CH_2OH, Me; X=N, CH)



polyoxins
(R=H, CH_2OH, CO_2H)



UDP GlcNAc

The polyoxins and nikkomycins enter the fungal cell *via* a dipeptide transport pathway.[3] Considerable research has been conducted to produce analogues having the ability to penetrate fungal cell wall by

modification of the amino terminal moiety or simplification of the complex structures, but the results were still unsatisfactory. [4]

Combinatorial chemistry and parallel synthesis in pharmaceutical research are an emerging technology that would dramatically reduce the time and cost associated with the development of new drugs.[5] Among various combinatorial chemistry strategies, that would dramatically reduce the time and cost associated with the development of new drugs.[5] Among various combinatorial chemistry strategies, multi-component reaction is especially attractive because a large number of compounds with a common core structure can be synthesized in one step by combination of each building blocks in combinatorial way.[6] Ugi reaction is a four-component condensation with an amine, an aldehyde, a carboxylic acid, and an isocyanide in a single transformation to yield an α-aminoacid derivative (**1**) (*Scheme 1*).[7]

Scheme 1. Ugi reaction

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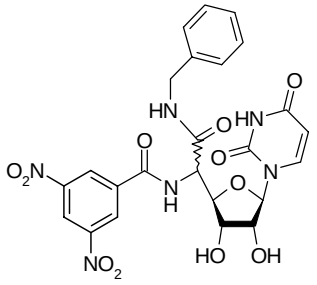
R. W. Armstrong[6] and A. M. M. Mjalli[8] have recently reported the combinatorial synthesis using the Ugi reaction on solid support.

Here we wish to report the synthesis of nikkomycin analogue library (**4**) using the Ugi reaction in which an amino component is on the solid support (*Scheme 2*).[9] We chose Rink amide resin [10] as the solid support because i) the product can be readily cleaved from the resin under relatively mild conditions, under which the acetal group in the product can be removed at the same time, ii) the desired secondary amides can be obtained after releasing the products from the Rink amide resin. The presence of the NH group at C-5' position is reported to be critical for chitin synthases inhibitory activity.[4] The aldehyde (**2**) was easily synthesized from uridine in 2 steps.[11] We first investigated the optimal solvent systems for Ugi reaction of **1** on the solid support by use of 3,5-dinitrobenzoic acid as a mixture of carboxylic acid component and benzyl isocyanide as an isocyanide in *Scheme 2.* The Ugi reaction proceeded smoothly by stirring the or 48 h at room temperature to give the compound (**3**) (R^1: 3,5-dinitrophenyl, R^2: benzyl). The desired product (**5**) was obtained after cleavage of the product from the resin with 10% hydrogen chloride in MeOH at room temperature, followed by HPLC purification. The best result was achieved when pyridine was used as a co-solvent (*Table 1).* As reported by A. M. M. Mjalli pyridine was essential as a co-solvent, a buffer of the reaction mixture and a stabilizer of isocyanide.[12] DMF-pyridine-MeOH (1:1:1) was found to be the most efficient solvent system for this reaction.

Table 1. Solvent system for Ugi reaction on solid support.

Solvents	Yield of compound **5** (%)
CH_2Cl_2-MeOH (1:1)	54
DMF-MeOH (1:1)	43
$CHCl_3$-Py-MeOH (1:1:1)	84
DMF-Py-MeOH (1:1:1)	96



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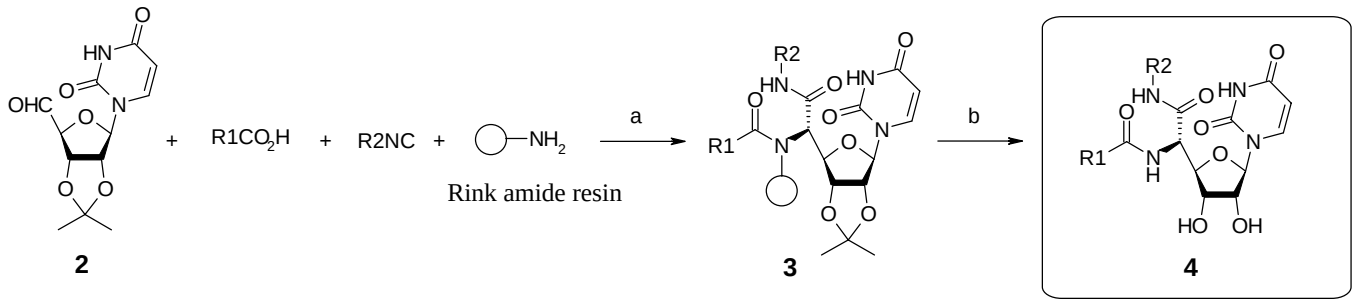
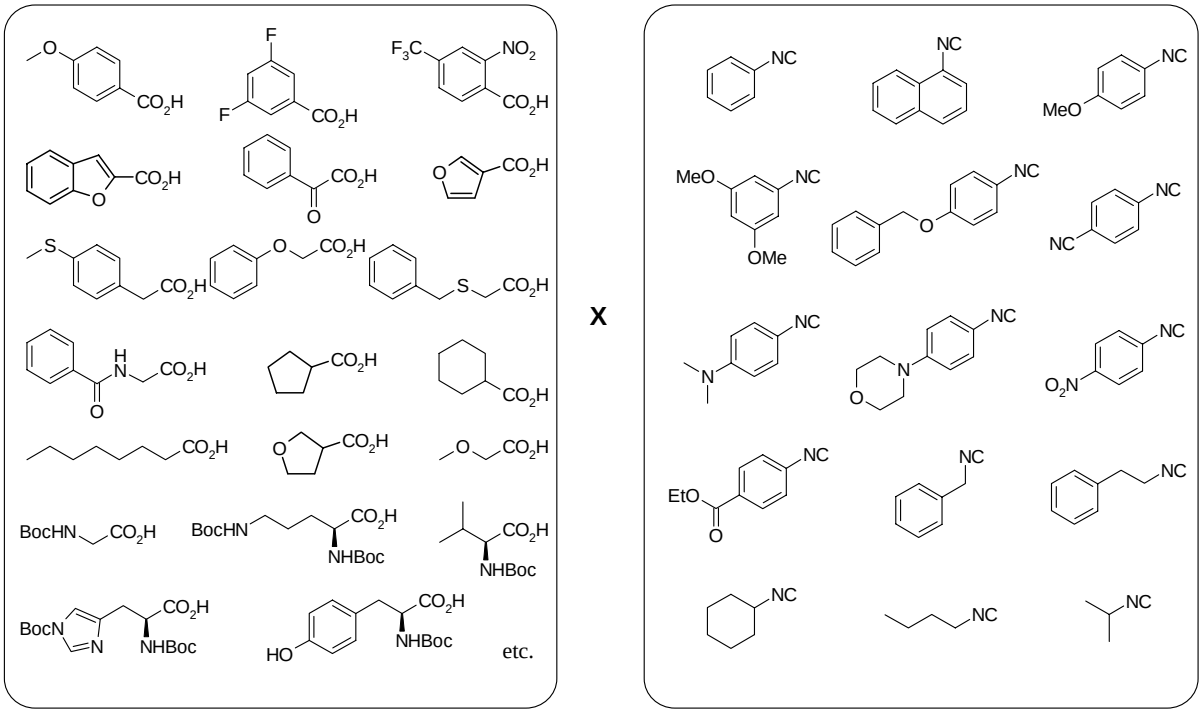
Scheme 2. Combinatorial synthesis of nikkomycin derivatives using Ugi reaction on solid support.
(a) DMF-Py-MeOH (1:1:1), rt, 48 h; (b) 10% HCl-MeOH, rt, 30 min



For the construction of the nikkomycin analogue library, we used 59 carboxylic acids and 15 isocyanides, thus we carried out 885 reactions in totals. Example of carboxylic acids and isocyanides is shown in *Scheme 3*.

Scheme 3. Example of building blocks for the Ugi reaction.

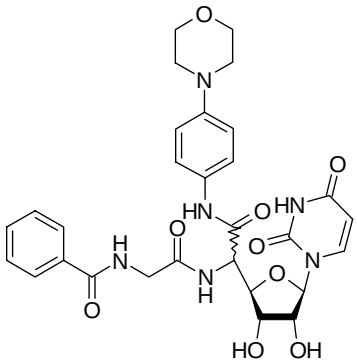


59 carboxylic acids **15 isocyanides**

Construction of the library was carried out by the synthesizer (Advanced ChemTech 496 MOS synthesizer[13]). After the cleavage of the product from the resin, the filtrate was concentrated under reduced pressure. The presence of the desired product (**4**) in the reaction mixture was confirmed by LC-MS. The crude samples that contain the desired products (450 samples) were tested for the enzyme inhibitory activity against *Candida albicans* chitin synthase 1. Among them, 246 samples exhibited more than 50% inhibition against *Candida albicans* chitin synthase 1 at the concentration of 10 μg/mL. To confirm the structure of the active component in the crude product, we re-synthesized the corresponding compounds and purified them by HPLC. Each Ugi product was obtained as a mixture of diastereomers at C-5' position. The IC_{50} values of the purified compounds (**6**, **7** and **8**) against *Candida albicans* chitin synthase 1 were determined to be 6.07 μM, 15.0 μM, and 16.8 μM respectively (*Table 2*). They were as potent as nikkomycin Z in the enzyme inhibitory activity against *Candida albicans* chitin synthase 1. Among these three compounds, only the compound (**6**) showed inhibitory activity against *Candida albicans* chitin synthase 2 (IC_{50} = 4.78 μM, *Table 2*).

Table 2. Enzyme inhibitory activity of nikkomycin analogues.

Compounds	Inhibition against *Candida albicans* chitin synthase 1 IC_{50}, μM	Inhibition against *Candida albicans* chitin synthase 2 IC_{50}, μM
6	6.07	4.78
7	15.0	inactive
8	16.8	inactive
Nikkomycin Z	9.49	0.06



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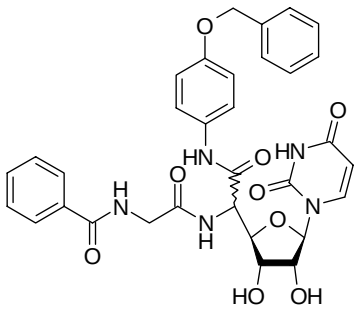

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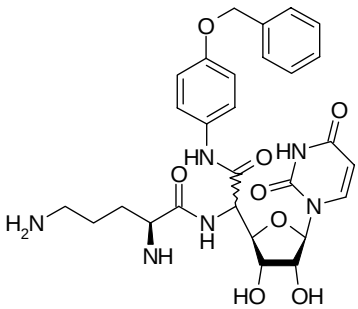

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Typical reaction conditions for Ugi reaction on solid support and cleavage: Rink amide resin (52 mg; 0.14 mmol/g loading) was swelled with 0.1 mL of MeOH, 0.1 mL of pyridine and 0.1 mL of DMF. To the reaction mixture 0.1 mL of aldehyde **1**(0.3 M in MeOH), 0.1 mL of carboxylic acid (0.3 M in DMF), and 0.1 mL of isocyanide (0.3 M in pyridine) were added successively. After the solution was shaken on

an orbital shaker for 48 h at room temperature, the resin was washed with DMF, MeOH, dichloromethane, and MeOH. Each solvent addition was approximately 1 mL, and each washing was repeated 3 times. The resin was then dried in vacuo. 0.6 mL of 10% hydrogen chloride in MeOH was added the reaction vessel. The reaction vessel was incubated at room temperature for 30 min, after which the reaction mixture was filtered and the resin was washed with 0.5 mL of MeOH for 3 times, and the collected solvent was evaporated under reduced pressure to give the crude product.

In conclusion, we developed a synthetic procedure for the preparation of nikkomycin analogues using Ugi reaction on solid support. The procedure was applied to the preparation of the combinatorial library, which resulted in the discovery of compound (**6**) having a simpler structure than nikkomycin Z. Further modification study using this technique is in due course.

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